

Module 12

Signalling Defects and Disease

Synopsis

A large number of diseases are caused by defects in signalling pathways. The nature of these defects and how they are induced varies enormously. Pathogenic organisms and viruses, many of which can interfere with signalling events, can cause some of these defects. There are other diseases that can be traced to defects in the function of cell signalling pathways. The concept of signalsome remodelling and disease provides a framework for considering how defects in signalling pathways can result in disease. It is convenient to separate these defects into phenotypic remodelling of the signalsome and genotypic remodelling of the signalsome. Most of the serious diseases in humans, such as hypertension, heart disease, diabetes and many forms of mental illness, seem to arise from subtle phenotypic modifications of signalling pathways. Such phenotypic remodelling alters the behaviour of cells so that their normal functions are subverted, leading to disease. Since it has proved difficult to clearly establish this relationship between signalsome remodelling and disease, there has been relatively little progress in designing effective treatments.

Genotypic modifications resulting from either somatic mutations or germline mutations have been somewhat easier to diagnose, but have also proved difficult to treat as witnessed by the failure of many of the gene therapy strategies. Clearly, there is an urgent need to understand more about all of these disease states in order to design better therapies. The enormous redundancy built into cell signalling mechanisms offers many opportunities for discovering new ways of correcting many disease states. The reversal of Ca^{2+} -dependent neurodegeneration is an example where such a strategy could provide novel therapies for treating some of the major neural diseases in humans such as Alzheimer's disease and Parkinson's disease.

Pathogenic organisms and viruses

A number of pathogenic organisms and viruses exert their deleterious effects by modifying the signalling processes operating in specific cell types:

- Bacillary dysentery is caused by *Shigella flexneri*, which acts by interfering with the $\text{PtdIns}4,5\text{P}_2$ regulation of actin remodelling and is also able to activate the $\text{PtdIns}3$ -kinase signalling pathway. A similar mechanism is employed by *Salmonella enterica* serotype Typhimurium.
- Cholera is caused by the Gram-negative bacterium *Vibrio cholerae*, which secretes the cholera toxin (CT) that causes severe water loss, vomiting and muscle cramps. CT activates the cyclic AMP signalling pathway (Module 2: Figure cyclic AMP signalling).
- Chlamydial diseases caused by *Chlamydia trachomatis* also survive by inhibiting the phagocytic processes of macrophages.

- Listeriosis is caused by *Listeria monocytogenes*, which is a Gram-positive pathogen that survives in macrophages by escaping through the phagolysosome membrane.
- Peptic ulcers are caused by an excessive production of acid caused by infection of the stomach with *Helicobacter pylori*.
- Tuberculosis is an example where the pathogen (*Mycobacterium tuberculosis*) survives by switching off the phagosome maturation process that macrophages use to kill infectious organisms.

Bacillary dysentery

Shigella flexneri, which causes bacillary dysentery, modifies some of the host cell signalling pathways to facilitate their entry and to boost their virulence. *S. flexneri* injects the host cell with an effector protein IpgD, which is a phosphoinositide phosphatase that hydrolyses $\text{PtdIns}4,5\text{P}_2$ to $\text{PtdIns}5\text{P}$. Entry of the pathogen into the host is facilitated by the decline in the level of $\text{PtdIns}4,5\text{P}_2$ because of the disruption in the normal $\text{PtdIns}4,5\text{P}_2$ regulation of actin remodelling. In addition, the $\text{PtdIns}5\text{P}$ formed by the hydrolysis may also play a role by activating the

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PtdIns5P signalling cassette, which can regulate a number of host cell functions. In particular, PtdIns5P can activate the class IA PtdIns 3-kinase to activate the PtdIns 3-kinase signalling pathway, and one effect of this will be to promote host survival by preventing apoptosis. The hormonal modulation of apoptosis is strongly regulated by the PtdIns 3-kinase signalling pathway.

Cholera

Cholera is characterized by severe water loss, vomiting and muscle cramps. It is caused by infection with the Gram-negative bacterium *Vibrio cholerae*, which secretes cholera toxin (CT) that can activate the cyclic AMP signalling pathway (Module 2: Figure cyclic AMP signalling). CT is composed of a catalytic A subunit and five B subunits. The latter attach the toxin to the surface of the cell, where they function as membrane-penetration subunits that inject the catalytic subunit into the cell. The toxin interacts with a G_{M1} ganglioside on the cell surface of intestinal epithelial cells, and this enables the A subunit to enter the cell, where it stimulates fluid secretion by activating cyclic AMP formation (Module 7: Figure intestinal secretion). The catalytic A subunit catalyses the transfer of ADP-ribose from NAD to an arginine group on the α subunit of G_s (Module 2: Figure cyclic AMP signalling). This ADP ribosylation inhibits the ability of G_s to hydrolyse GTP, which means that this G protein is locked in its active configuration and thus maintains a persistent activation of cyclic AMP and intestinal secretion.

Listeriosis

After bacteria have been engulfed, they are normally destroyed by a process of phagosome maturation. In the case of the Gram-positive pathogen *Listeria monocytogenes*, however, the pathogen survives the attention of macrophages by escaping through the membrane of the phagolysosome to enter the cytoplasm where they can replicate and then go on to infect other cells. The bacterial virulence factor listeriolysin O (LLO) seems to be critical in that it can oligomerize to form pores in host membranes. One of the functions of these pores is to provide a leak pathway for proteins that prevents the acidification of the phagosome that is necessary for it to fuse with the lysosomes. In order to lyse the phagosomal membrane to gain access to the cytoplasm, LLO must be reduced and this is done by making use of the γ -interferon-inducible lysosome thiol reductase (GILT), which is a host enzyme located in the phagosome. The phagosome membrane has NADPH oxidase and nitrogen oxide synthase that generate the antimicrobial agents reactive oxygen species (ROS) and reactive nitrogen species (RNS) respectively. GILT neutralizes the action of ROS and RNS thus enabling *Listeria* to escape into the cytoplasm.

Peptic ulcers

Peptic ulcers are caused by an excessive production of acid that damages the gastric mucosa. One of the main causes of ulcers is infection of the stomach with *Helicobacter pylori*. This Gram-negative spiral bacterium colonizes the stom-

ach by developing a number of adaptations that enables it to cope with the highly acidic environment. The bacterium attaches itself to the surface of the epithelial cells through a variety of adhesion proteins. Marshall and Warren received the Nobel Prize in Physiology and Medicine in 2005 for uncovering the link between *H. pylori* and the onset of peptic ulcer disease. It is now known that most duodenal ulcers and approximately 70% of gastric ulcers are caused by this bacterial infection. In addition, most stomach cancers are associated with infections of *H. pylori*. Eradication of the infection with drugs has proved to be a highly effective means of curing peptic ulcers.

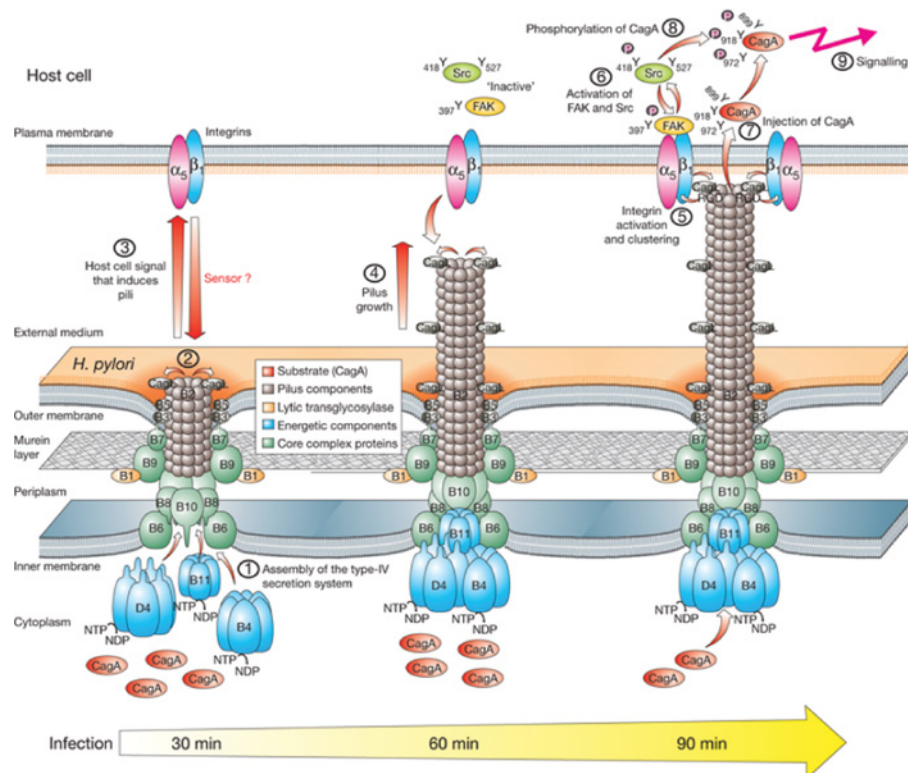
H. pylori has an elaborate 'nano-syringe' that it uses to inject proteins such as the cytotoxin-associated gene A (CagA) directly into the host cell (Module 12: Figure *H. pylori* nano-syringe). At the tip of this syringe is the protein cytotoxin-associated gene A ligand (CagL) that has RGD motifs that enables it to bind to the $\alpha_5\beta_1$ integrin complex found on the surface of the host cell surface (Module 1: Figure integrin receptor). The CagL thus functions as a stimulus to activate integrin signalling that then creates the conditions for both the injection and the activation of the oncoprotein cytotoxin-associated gene A (CagA).

Once it enters the epithelial cells, CagA interacts with resident regulatory proteins to alter both cell structure and cell fate. It disrupts tight junctions by associating with proteins such as ZO-1, JAM and the partitioning-defective 1 (PAR1)/microtubule affinity-regulating kinase (MARK) family of serine-threonine kinases. The resulting junctional and polarity defects result in ulcerations, inflammation (gastritis) and can lead to gastric carcinogenesis. An increase in proliferation is facilitated by the ability of CagA to induce various signalling pathways to stimulate transcription factors such as NF- κ B, NFAT and SRF.

Just how *H. pylori* increases in acid secretion that causes ulcers is still being worked out. There is increasing evidence to show that the bacterium somehow alters the activity of the cells that regulate the release of gastrin. For example, there is an increase in the resting rate of gastrin release by the G cells located in the antrum of the stomach (Module 7: Figure stomach structure). In addition, there is a decrease in the activity of the D cells that release somatostatin, which is part of a negative-feedback loop that operates in the control of parietal cell secretion (Module 7: Figure HCl secretion).

Tuberculosis

Mycobacterium tuberculosis evades the rapid inflammatory responses used to attack foreign pathogens as part of the innate immune system. Once such pathogens are taken up into the phagosome, they are killed when hydrolytic enzymes are added during phagosome maturation (Module 4: Figure phagosome maturation). *M. tuberculosis* has developed various mechanisms to manipulate the host's signalling pathways enabling it to survive and proliferate. In one mechanism, the bacterium subverts phagosome maturation by somehow switching off the Ca^{2+} signals that

Module 12: | Figure *H. pylori* nano-syringeType IV secretion system of *H. pylori*.

H. pylori assemble a secretion apparatus that has 32 proteins some of which produce a long tube (pilus) that has a cytotoxin-associated gene A ligand (CagL) protein with an RGD motif that makes contact with and activates the $\alpha_5\beta_1$ integrin complex on the surface of the host cell. This binding to the $\alpha_5\beta_1$ integrin complex initiates the activation of host cell signalling components that play a role in the injection and activation of the cancer-associated protein CagA. Reproduced from Kwok et al 2007.

are responsible for driving the maturation process. This inhibition seems to depend on lipoarabinomannan (LAM), which is one of the pathogen-associated molecular patterns (PAMPs), released from mycobacteria such as *M. tuberculosis* (Module 11: Figure formation and action of PAMPs). This LAM markedly reduces the Ca^{2+} signals that normally occur during the maturation processes. It seems that the LAM, presumably acting through Toll-like receptor 2 (TLR2), interferes with the phospholipase D (PLD) signalling pathway responsible for generating the Ca^{2+} -mobilizing messenger sphingosine 1-phosphate (S1P) (Module 4: Figure phagosome maturation). However, these pathogenic mycobacteria can be killed by macrophages if ATP activates an alternative Ca^{2+} signalling system through the entry of external Ca^{2+} following activation of P2X7 receptors (Module 3: Table receptor-operated channel toolkit).

M. tuberculosis has approximately 17 genes coding for adenylyl cyclase and it uses one of these, the *Rv0386* isoform, to manipulate the macrophage cyclic AMP signalling pathway. The cyclic AMP generated by *Rv0386* diffuses out of the phagosome to enter the host's cytoplasm where it activates the cyclic AMP pathway to increase the formation and secretion of the pro-inflammatory cytokine tumour necrosis factor α (TNF α). The resulting inflammatory response enhances the lung disease associated with tuberculosis.

Chlamydial diseases

The obligate intracellular bacterium *Chlamydia trachomatis* causes infection in both the eye and the genital tract that can result in blindness and female infertility. This bacterium can survive within macrophages by inhibiting the phagosome maturation process, during which the phagosome fuses with the lysosome (Module 4: Figure phagosome maturation).

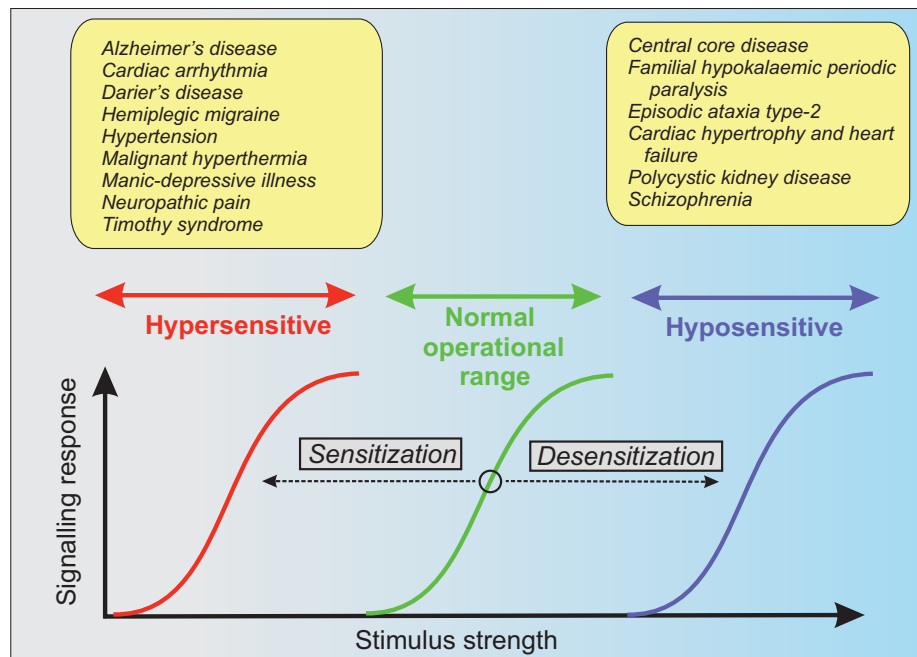
Hepatitis

The human hepatitis C virus encodes a non-structural protein 5A (NS5A), which appears to disturb Ca^{2+} homeostasis, resulting in an increase in reactive oxygen species (ROS) in the mitochondria and the translocation of nuclear factor κB (NF- κB) and signal transducer and activator of transcription 3 (STAT 3) into the nucleus. These signalling systems may thus contribute to the pathogenesis associated with liver disease.

Signalsome remodelling and disease

All differentiated cells have a cell-type-specific signalsome, which functions to create the normal output signals used by each cell to control its particular function (Module 1: Figure remodelling the signalsome). Disease states can result from a remodelling of the signalsome resulting

Module 12: | Figure signalsome remodelling



Relationship between stimulus strength and signalling responses for normal and remodelled signalsomes.

The signalsome that makes up each signalling system has a normal operational range (green curve) over which it responds to changes in stimulus strength with a characteristic dose-response curve. If the signalsome is remodelled, this sensitivity will change such that the signalsome becomes either sensitized (red curve) or desensitized (blue curve). Such shifts in sensitivity may be responsible for various disease states.

from either phenotypic remodelling of the signalsome or genotypic remodelling of the signalsome.

One way of considering the relationship between signalsome remodelling and disease is to consider how signalsomes respond to changes in stimulus strength. For each signalsome, there will be a normal operational range over which the cell responds to an increase in stimulus strength (Module 12: Figure signalsome remodelling). When components of the signalsome are remodelled, the operational range over which the signalling system operates will be altered to become either hyper- or hypo-sensitive, resulting in a number of disease states (Module 12: Figure signalsome remodelling).

Phenotypic remodelling of the signalsome

During development, a process of signalsome expression results in the appearance of cell type-specific signalsomes to create the normal output signals used to control particular cellular functions (Module 8: Figure signalsome expression). These cell-specific signalsomes are maintained by ongoing transcriptional processes, and this signalsome stability is essential for the normal operation of the cell. Indeed, such remodelling has many beneficial effects:

- A good example is the increased force of contraction of the heart that occurs during physical exertion. This inotropic response is achieved through **modulation of ventricular Ca^{2+} signals**. This is achieved through re-

versible phosphorylation of key Ca^{2+} signalling elements that enables the heart cells to generate larger Ca^{2+} signals.

- The synaptic plasticity evident during short-term memory results from a Ca^{2+} -dependent phosphorylation of the α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor in the synaptic spine (Module 10: Figure coincident detection).
- An example of how a change in the expression of signalling components can remodel the signalsome occurs during cell proliferation of lymphocytes. Activation of the immediate early genes in G_1 switches on the expression of interleukin 2 (IL-2) signalling components responsible for an autocrine loop to complete the switch from G_1 into S (Module 9: Figure T cell signalling map).
- In slow-twitch skeletal muscle fibres, nuclear factor of activated T cells (NFAT) maintains the differentiated state and its inhibition can result in skeletal myofibre conversion. This is an example of remodelling, where different skeletal muscle phenotypes undergo conversions as part of an adaptive response to changes in load (e.g. exercise training).
- During pregnancy, the uterine smooth muscle cells undergo a number of changes to their signalling systems that result in the activation of a uterus smooth muscle cell membrane oscillator that begins to generate the electrical signals responsible for driving the strong contractions during labour.

In all of these examples, a change in the signalling phenotype is a normal response to the way the cells go about their particular functions. However, there are examples where such adaptive normal phenotypic remodelling breaks down to produce abnormal signalsomes that generate inappropriate output signals, leading to many of the major diseases in humans (Module 12: Figure phenotypic and genotypic remodelling).

An important aspect of such phenotypic remodelling is that it is potentially reversible, which explains why so many drug companies are searching for drugs such as receptor blockers and protein kinase inhibitors that will operate to correct such phenotypic modifications. For example, the reversal of Ca^{2+} -dependent neurodegeneration could provide a strategy for treating major neural diseases such as Alzheimer's disease and Parkinson's disease. Many of the major diseases of man may result from abnormal phenotypic remodelling of the signalsome:

- Ageing
- Age-related macular degeneration (AMD)
- Alzheimer's disease
- Asthma
- Atopic dermatitis (AD)
- Attention deficit hyperactivity disorder (ADHD)
- Bipolar disorder (BD)
- Chronic obstructive pulmonary disease (COPD)
- Cirrhosis of the liver
- Cushing's syndrome
- Diabetes
- Diabetes insipidus (DI)
- Diabetic nephropathy
- Diarrhoea
- Drug addiction
- Ejaculatory dysfunction
- Endotoxic shock
- End-stage renal disease (ESRD)
- Epilepsy
- Erectile dysfunction
- Heart disease
- Humoral hypercalcaemia of malignancy (HHM)
- Hypertension
- Irritable bowel syndrome
- Manic-depressive illness
- Metabolic syndrome
- Migraine
- Multiple sclerosis (MS)
- Narcolepsy
- Nausea
- Obesity
- Osteoporosis
- Pain
- Pancreatitis
- Parkinson's disease
- Primary hyperparathyroidism
- Secondary hyperparathyroidism
- Manic-depressive illness
- Premature labour
- Rheumatoid arthritis
- Schizophrenia

- Sudden infant death syndrome (SIDS)
- Zollinger–Ellison syndrome

Ageing

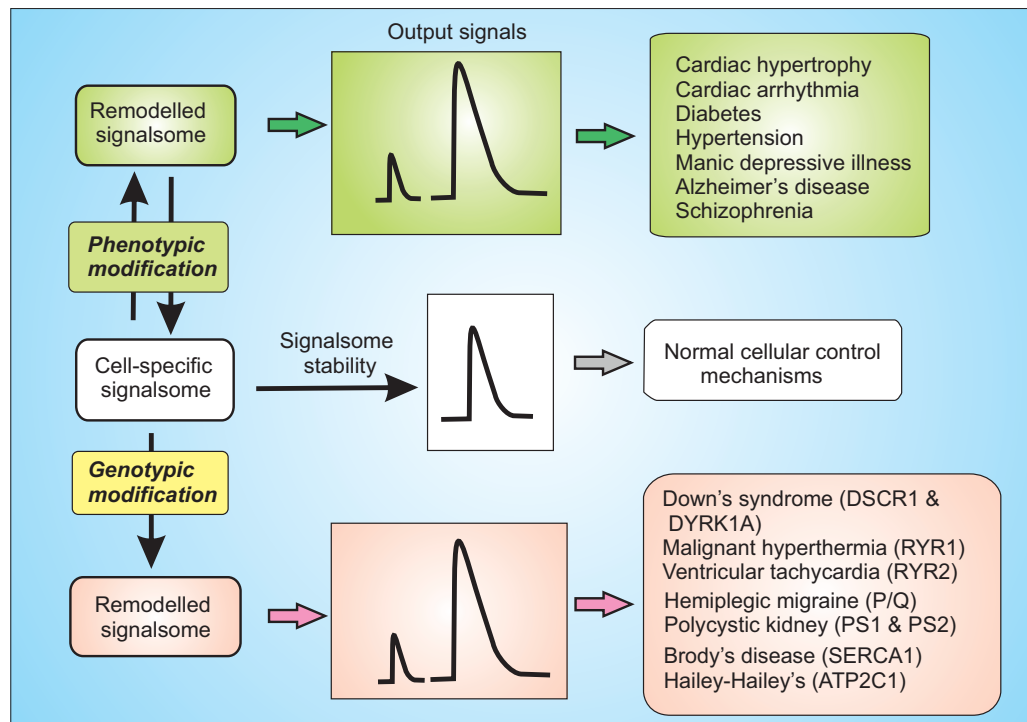
The ageing process, which is still not properly understood, seems to be driven by a large number of processes and factors many of which are clearly linked together (Module 12: Figure ageing mechanisms). The aim here is to describe some of these putative ageing processes and how they are related in order to try to identify what might be the primary underlying cause(s) of ageing.

The following working hypothesis, which provides a framework to bring together much of the current information, proposes that ageing is caused by a subtle and gradual decline in the transcriptional mechanisms responsible for phenotypic stability with a particular emphasis on signalsome stability. It is argued that this gradual phenotypic drift is caused by a decline in the quality of the transcriptional mechanisms responsible for maintaining the cellular processes and signalling mechanisms that maintain each specific cellular phenotype. For example, muscle cells continue to contract while skin cells continue to produce epidermis, but the quality of these processes declines over time. The fact that this gradual phenotypic erosion occurs simultaneously in cells within different tissues suggests that there may be some commonality in the mechanism driving the ageing process, which is the holy grail of ageing research. However, the consequence of some putative ageing process focused on an alteration in gene transcription will manifest itself differently depending on the pre-existing phenotypic landscape of the specific cell types in different tissues. Because of this, the relationship between phenotypic reprogramming and ageing will be described in some specific cell types in order to try to determine whether there are any common elements that might reveal the nature of the ageing process.

The hypothesis that a central feature of ageing is some process that induces a qualitative change in gene transcription can link together many of the proposed ageing processes. For example, there is considerable evidence that calorie restriction (CR) can extend lifespan and the quality of life in diverse organisms. CR may act by altering the expression of the genes that control both the pathways responsible for energy metabolism and the antioxidant and detoxifying systems that reduce reactive oxygen species (ROS) (Module 12: Figure ageing mechanisms). The latter is the basis of the ROS hypothesis of ageing that considers that ageing is caused by the accumulated damage caused by a build-up of ROS.

The link between the availability of nutrients and the changes in gene transcription that control energy and ROS metabolism seems to depend on the sirtuins. Sirtuins may also play an important role in the relationship between mitochondrial dysfunction and ageing. The contribution of sirtuins may depend on the link between genome instability and ageing, which is also played out through a change in gene transcription. Epigenetic changes in ageing can also account for the alterations in transcription. There

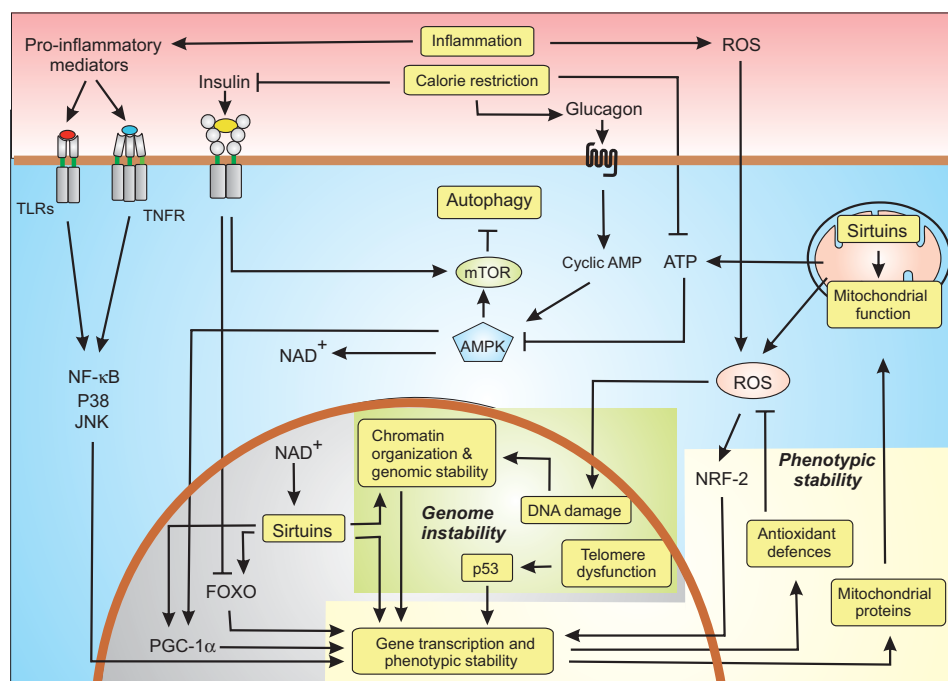
Module 12: | Figure phenotypic and genotypic remodelling



Phenotypic and genotypic remodelling of cell-specific signalsome.

Each cell-specific signalsome is designed to generate a typical output signal in order to carry out its particular cellular control mechanisms. This signalsome can be remodelled through phenotypic (top) or genotypic (bottom) modifications to produce output signals that are either too strong or too weak. Such modified signalsomes are responsible for many disease states.

Module 12: | Figure ageing mechanisms



Ageing mechanisms.

Many of the processes that have been implicated in the ageing process are linked to each other through a complex web of interactions. Many of these act by regulating gene transcription to bring about a change in phenotypic stability. Many of these changes alter the expression levels of mitochondrial proteins and antioxidant defences.

is a well-established link between cell signalling mechanisms and ageing, particularly with regard to changes in Ca^{2+} signalling pathways and in the insulin signalling pathway that uses FOXO to regulate gene transcription. A change in signalling pathways may also contribute to the link between inflammation and ageing, which appears to be mediated through the nuclear factor κB (NF- κB) signalling pathway.

All of the above mechanisms have an impact on gene transcription and could potentially alter the stability of many different cellular phenotypes. A decline in the maintenance of energy metabolism and antioxidant defences appear to be two of the main defects that develop as a consequence of the decline in gene transcription and phenotypic stability (Module 12: Figure ageing mechanisms). An intriguing aspect of these two processes is that they both contribute to the formation and metabolism of reactive oxygen species (ROS), which has been strongly implicated in the ageing process. The slow ageing of long-lived dwarf mice seems to be related to their ability to resist multiple forms of stress many of which depend on an elevation of ROS. An alteration in mitochondrial energy metabolism coupled to a reduction of antioxidant defences may act together to increase ROS formation and may be one of the universal drivers of ageing.

The mitochondrial dysfunction and an accompanying dysregulation of Ca^{2+} may also contribute to some of the neurodegenerative diseases associated with ageing, such as Alzheimer's disease (AD) and Parkinson's disease.

Calorie restriction (CR)

Calorie restriction (CR) can extend the lifespan of many different organisms ranging from yeast to mammals. In the latter case, the evidence is somewhat problematical especially in the case of humans. What is not in doubt, however, is that CR provides for a much more healthy life and appears to retard many of the age-related disorders and diseases such as metabolic syndrome and insulin resistance, diabetes, obesity, liver steatosis, cancer, autoimmune diseases and perhaps neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. The way in which CR results in these beneficial effects has provided valuable insights into the ageing process. In the ageing hypothesis outlined earlier, it was proposed that ageing is a consequence of a subtle and gradual decline in the transcriptional mechanisms responsible for phenotypic stability. Perhaps the most important aspect to emerge from studies on CR is that this age-associated functional deterioration may arise as a consequence of phenotypic reprogramming that results in alterations in metabolic homeostasis that is particularly related to the control of transcriptional processes necessary for the maintenance of energy metabolism and antioxidant defences (Module 12: Figure ageing mechanisms).

Maintenance of energy metabolism and antioxidant defences

Deterioration in the mechanisms responsible for regulating both mitochondrial energy metabolism and antioxidant defences, which are intimately connected to each

other, is a major component of the ageing process. Much of the information on this topic has emerged from studying calorie restriction (CR). A decline in calorie intake acts on both endocrine and intracellular signalling pathways that combine to maintain the transcriptional network responsible for maintaining energy metabolism and antioxidant defences (Module 12: Figure ageing mechanisms). A decline in blood glucose levels following CR will trigger the endocrine mechanisms responsible for controlling the metabolic energy network that maintains whole-body energy homeostasis (Module 7: Figure metabolic energy network).

With regard to the endocrine mechanisms, the decline in blood glucose influences the release of hormones such as insulin and glucagon. There will be a decline in the release of insulin and this may be very significant because there is considerable evidence to show that a decrease in the insulin signalling pathway can reduce the ageing process and enhance longevity. On the other hand, low glucose levels will result in stimulation of the glucagon-secreting α -cells (Module 7: Figure α -cell signalling) to release glucagon, which then acts to mobilize glucose from liver cells (Step 11 in Module 7: Figure metabolic energy network). Glucagon acts through the cyclic AMP signalling pathway to stimulate the hydrolysis of glycogen (Module 7: Figure liver cell signalling). In addition, an increase in cyclic AMP also provides a strong stimulus to the AMPK signalling pathway (Module 2: Figure AMPK control of metabolism) and this can have a major influence on gene transcription pathways by stimulating the sirtuins as described below.

Another important aspect of CR is how it influences intracellular signalling mechanisms with much attention being focused on the sirtuins that may be one of the links between the decline in energy consumption and the alteration in the transcriptional events that maintain phenotypic stability (Module 12: Figure ageing mechanisms). Sirtuin activity is enhanced through two mechanisms. There is an increased expression of the sirtuins and their activity may also be enhanced by an increase in the level of NAD^+ that invariably occurs during CR and depends on the activation of the AMP signalling pathway (Module 2: Figure AMPK control of metabolism).

The sirtuins are part of an energy-sensing system that functions to maintain energy metabolism and antioxidant defences by modulating a number of cellular processes. For example, SIRT1 modulates the activity of transcription factors such as the peroxisome-proliferator-activated receptors (PPAR α , PPAR δ and PPAR γ), PPAR γ coactivator-1 α (PGC-1 α), forkhead box O (FOXO1, FOXO3a and FOXO4) factors and nuclear factor κB (NF- κB) (Module 12: Figure ageing mechanisms). The activity of PGC-1 α is regulated by reversible acetylation: it is acetylated by general control of amino-acid synthesis (GCN5) and is deacetylated and activated by SIRT1 (Module 2: Figure AMPK control of metabolism). In addition, mitochondrial sirtuins, such as SIRT3 and SIRT4, have a major influence on mitochondrial function (Module 5: Figure mitochondrial Ca^{2+} signalling). The overall effect of these different actions is to alter the way energy is

produced: there is a reduction in glycolysis and the TCA cycle, whereas energy derived from gluconeogenesis and the breakdown of protein and lipid is enhanced. These alterations may be part of a stress response to nutrient deprivation to maintain a supply of glucose for the brain. These metabolic changes, which vary depending on the pre-existing phenotype, are a key feature of the relationship between phenotypic reprogramming and ageing that occurs in specific cell types.

Cell signalling mechanisms and ageing

During ageing, there is a marked deterioration in Ca^{2+} signalling that is usually manifested as a gradual increase in the resting level of Ca^{2+} , which is very similar to that described in the calcium hypothesis of Alzheimer's disease. This elevation in Ca^{2+} levels has a number of consequences. For example, binding of Ca^{2+} to the MARCKS protein causes it to dissociate from the membrane and this may account for the decrease in the membrane levels of PtdIns4,5P_2 , which can have a number of consequences as the PtdIns4,5P_2 signalling cassette has multiple functions (Module 2: Figure PtdIns4,5P_2 signalling). In neurons, it may contribute to the decline in learning and memory that occurs during ageing.

Phenotypic reprogramming and ageing

In the following sections, the relationship between phenotypic instability and the ageing processes will be examined in specific cell types. Deterioration in the maintenance of energy metabolism and antioxidant defences is a prominent feature of the ageing process in many different cell types. Just how these phenotypic conversions are carried out in specific cell types may throw considerable light on the nature of the ageing process

- Phenotypic reprogramming of liver cells
- Phenotypic reprogramming of skeletal muscle
- Phenotypic reprogramming of white fat cells

Phenotypic reprogramming of liver cells

A clue to what might be happening during ageing in liver cells has emerged from the fact that calorie restriction (CR) induces a number of marked changes in the quality of its metabolic phenotype that concerns energy metabolism. For example, during energy deprivation, there is a shift towards an increase in liver cell gluconeogenesis that plays a major role in producing glucose using lactate and fatty acids as substrates (Module 7: Figure glycogenolysis and gluconeogenesis). SIRT1 plays a role in maintaining the activity of $\text{PGC-1}\alpha$ (Module 4: Figure $\text{PGC-1}\alpha$ gene activation), which is a major component of the transcriptional events that drive gluconeogenesis (Module 7: Figure liver cell signalling). The $\text{PGC-1}\alpha$ signalling cascade, which is a component of the ageing process (Module 12: Figure ageing mechanisms), can also increase fatty acid oxidation and inhibit glycogenolysis. This remodelling of the metabolic pathways enables liver cells to adjust their substrate usage to synthesize glucose from fatty acids and lactate under low nutrient conditions.

Phenotypic reprogramming of skeletal muscle

The phenotypic reprogramming of skeletal muscle is sensitive to a variety of changes including calorie restriction (CR) and exercise that orchestrate changes in both energy metabolism and skeletal myofibre conversion that clearly reveal the nature of the transcriptional events that drive these phenotypic conversions. For example, in response to prolonged stimulation, as occurs during intense exercise, there are changes in the expression of numerous genes resulting in an increase in mitochondrial biogenesis, oxidative capacity and a conversion of the type of muscle fibres such that fast-twitch type II fibres are switched to type I slow-twitch fibres. A similar conversion to type I fibres has also been observed in mice lacking the *CISD2* gene and this seems to be associated with an increase in cytosolic Ca^{2+} levels.

The elevation in Ca^{2+} responsible for excitation-contraction (E-C) coupling in skeletal muscle (Module 7: Figure skeletal muscle E-C coupling) during intense exercise is responsible for activating myocyte enhancer factor-2 (MEF2), which is normally inactivated in an inhibitory complex containing factors such as histone deacetylase (HDAC), Cabin1 and the co-repressor switch independent (SIN3) (Module 4: Figure MEF2 activation). Activation of CaMKIV phosphorylates HDAC resulting in its export from the nucleus, whereas activation of calcineurin (CaN) results in dephosphorylation and activation of MEF2. The Ca^{2+} /CaM complex binds to Cabin1 to release the inhibitory Cabin1-SIN3 complex. After these inhibitory elements have been removed, MEF2 can bind to its co-activators p300, CREB and NFAT. The type I fibres preferentially use fatty acid metabolism as an energy source and this oxidative mechanism depends on the activation of the MEF2 promoter to increase the transcription of $\text{PGC-1}\alpha$ (Module 4: Figure $\text{PGC-1}\alpha$ gene activation) that then induces mitochondrial biogenesis and fatty acid oxidation.

Phenotypic reprogramming of white fat cells

The metabolism of white fat cells is poised between anabolism and catabolism, depending on the energy requirements of the organism. There is a tendency for fat accumulation to increase during ageing and age-related obesity that markedly increases the risk of developing metabolic syndromes and white fat cell insulin resistance. Some of the beneficial effects of calorie restriction (CR) are related to a reduction in the fat content of the white fat cells. During fasting or calorie restriction, the white fat cell mobilizes its triacylglycerol store through a process of lipolysis to release free fatty acids (FFAs) and glycerol into the blood (Module 7: Figure lipolysis and lipogenesis). During lipogenesis, FFAs are removed from the blood and converted back into triacylglycerol to reconstitute the lipid reservoir (Module 7: Figure white fat cells). The switch from lipogenesis to lipolysis is orchestrated, at least in part, by an increase in the activity of sirtuins that occurs during fasting. SIRT1 acts to inhibit lipogenesis by acting through peroxisome-proliferator-activated receptor γ (PPAR γ) and its cofactors NcoR and SMRT to switch off a whole host of genes that function in lipogenesis

(Module 4: Figure PPAR γ activation). The onset of age-related obesity and diabetes may result from a decline in the activity of such control mechanism that regulate the balance between lipolysis and lipogenesis

Mitochondrial dysfunction and ageing

Mitochondrial dysfunction appears to be a contributory factor in ageing and in many of the diseases associated with the ageing process. Since components of the mitochondria are constantly turning over, stability is maintained through a renewal system that is very sensitive to the prevailing energy state of the cell that depends on a variety of transcriptional signalling pathways (Module 12: Figure ageing mechanisms). A complex web of transcriptional processes maintains mitochondrial phenotypic stability by activating mitochondrial biogenesis (Module 5: Figure mitochondrial biogenesis). A key element of the mechanism responsible for mitochondrial stability is the functional communication between the nucleus and the mitochondria. A key element of this communication is the provision of key mitochondrial components responsible for oxidative phosphorylation that are encoded in the nuclear genome. Disruption of this nuclear-mitochondrial communication resulting from a decline in the nuclear level of NAD⁺ may contribute to the onset of ageing. This NAD⁺ acts on SIRT1 to deacetylate HIF-1 that normally acts to inhibit mitochondrial genes that function in oxidative phosphorylation (Module 5: Figure mitochondrial biogenesis). In addition, SIRT1 also activates peroxisome-proliferator-activated receptor γ (PPAR γ) coactivator-1 α (PGC-1 α), which is key regulator of many of the genes that function in mitochondrial biogenesis. High levels of NAD⁺ are thus essential for maintaining the transcription of the essential components of mitochondrial metabolism. A decline in the level of nuclear NAD⁺ may be a contributory factor for the onset of mitochondrial dysfunction that occurs during ageing.

Another important factor that contributes to mitochondrial dysfunction appears to be the gradual accumulation of oxidative damage to the DNA, protein and lipid components of the mitochondria. Mitochondria are particularly susceptible to such damage because the mitochondrial reactive oxygen species (ROS) formation mechanism is continuously producing reactive oxygen species (ROS) that result from a leakage of electrons from the mitochondrial electron transport chain (Module 2: Figure sites of ROS formation). Despite the fact that mitochondria possess an impressive array of antioxidant mechanisms to counteract this continuous ROS onslaught, oxidative damage does occur and can accumulate progressively to the point that mitochondrial function begins to be compromised and can tip over into a vicious pathological cycle. For example, oxidative damage begins to reduce electron transport and the resulting reduction in oxidative phosphorylation and mitochondrial membrane potential enhances ROS formation that then causes further oxidative damage and so on. An important component of this oxidative damage is the peroxidation and inactivation of cardiolipin (CL), which plays an important role in promoting mitochondrial respiration (Module 5: Figure cardiolipin). In both

heart and brain, there is a progressive loss of CL during ageing. In addition, ageing is also associated with a decline in the levels of melatonin that plays an important antioxidant role to prevent oxidative damage to CL.

There is also a progressive accumulation of ROS-induced mutations in mitochondrial DNA that will begin to contribute to mitochondrial dysfunction as the resulting defects in the respiratory enzymes coded for by this resident DNA will gradually begin to erode mitochondrial respiration. The mitochondrial generation of ROS is exacerbated by a decline in the expression of the antioxidant defences, which is an important component of the ROS hypothesis of ageing.

ROS hypothesis of ageing

As part of their normal metabolic and cell signalling mechanisms, cells are constantly producing reactive oxygen species (ROS) and an active ROS messenger action is an important aspect of normal cell signalling pathways. It is important therefore, that ROS are maintained at the levels necessary for signalling and do not rise to the levels that cause cell damage. The danger of such excessive ROS is exacerbated by inflammation and a variety of environmental factors such as smoking and sunlight. There is an elaborate system of ROS metabolism that provides the antioxidant defence against abnormal elevation in ROS levels and is a key component of the maintenance of energy metabolism and antioxidant defences. Ageing and many age-related pathological conditions such as neurodegeneration (Alzheimer's disease and Parkinson's disease), cancer, cardiovascular diseases and diabetes appear to be associated with a redox imbalance. Resveratrol, which is a naturally occurring antioxidant found in grapes and cocoa, appears to have anti-ageing effects and may also provide protection against the deleterious effects of obesity and diabetes.

The basic tenant of the ROS hypothesis considers that ageing is caused by the gradual accumulation of oxidative damage to macromolecules such as DNA, proteins and lipids. For example, damage to the DNA and the proteins that modify chromatin contributes to genomic instability and ageing that will gradually accumulate to bring about a decline in phenotypic stability (Module 12: Figure ageing mechanisms). Many of the anti-ageing benefits of calorie restriction (CR) and a decrease in the insulin signalling pathway may depend on a reduction in redox stress that may arise through elevation of antioxidant mechanisms. A major player in maintaining these antioxidant defences is nuclear factor erythroid 2-related factor 2 (NRF-2) (Module 4: Figure NRF-2 antioxidant function).

A decline in the effectiveness of these signalling networks responsible for the metabolic and antioxidant adjustments to cope with such cell stresses may be a major factor in ageing. A decline in ROS metabolism is a major feature of the relationship between mitochondrial dysregulation and ageing.

Inflammation and ageing

Ageing is often associated by the onset of a low-grade inflammatory environment. This inflammatory response can

arise through activation of either the innate or adaptive immune responses. Just what induces these inflammatory responses in tissues that are ageing normally is not immediately obvious. This inflammation, which is usually induced by resident macrophages in peripheral tissues or the microglia in the brain, is associated with the formation of an 'inflammatory soup' containing many proinflammatory mediators and toxic factors such as reactive oxygen species (ROS) that will feed directly into the various mechanisms that contribute to the ageing process (Module 12: Figure ageing mechanisms). The pro-inflammatory mediators such as tumour necrosis factor α (TNF α) and cytokines such as interleukin-1, will act through the TNF α receptors and Toll-like receptors (TLRs) respectively to relay information through either the nuclear factor κ B (NF- κ B) signalling pathway or the MAPK signalling pathway (p38 and JNK).

This low-grade inflammation is not always deleterious and can often be beneficial because a two-way communication between immune cells and neighbouring tissue cells can often be beneficial in that it may help to maintain phenotypic stability. This cellular dialogue is nicely exemplified by the multiple actions of the nuclear factor κ B (NF- κ B) signalling pathway that can promote the expression of both proinflammatory cytokines and immunoregulators (Module 2: Figure Toll receptor signalling). Another example occurs in the brain, where the inflammatory cytokine tumour necrosis factor α (TNF α) can maintain the expression of signalling components such as the inositol 1,4,5-trisphosphate (InsP₃) receptor and the anti-apoptotic protein Bcl-2.

This fine balance between inflammation being both beneficial and deleterious begins to tip towards the latter during the onset of various diseases. The following examples illustrate how enhanced local inflammatory responses are thought to contribute to the onset and progression of age-related diseases:

- Inflammation in Alzheimer's disease
- Inflammation in Parkinson's disease

Genome instability and ageing

Instability of the genome seems to play a major role in the ageing process. A number of different changes have been identified ranging from macroscopic changes in the chromosomal organization within the nucleus and gene transcription all the way through to the epigenetic changes in ageing that result from molecular modification of DNA and its associated histones. With regard to the former, there is a precise organization of the chromatin such that the non-coding heterochromatin tends to be localized at the periphery attached to the nuclear lamina, whereas euchromatin representing the decondensed, transcriptionally active genes is found within the interior of the nucleus where the euchromatin parts of different chromosomes come together to form transcription factories (Module 12: Figure chromatin organization).

A significant aspect of this chromatin architecture is the linker of nucleoskeleton and cytoskeleton (LINK) complex that not only tethers the chromosomes to the inner

nuclear membrane (INM), but it also provides a link to the actin in the cytoplasm. Beginning from the cytoplasmic side, the protein nesprin is attached to actin at one end while its other end is embedded in the outer nuclear membrane (ONM) (Module 12: Figure chromatin organization). Within the lumen of the nuclear envelope, nesprin associates with SUN1, which is embedded in the inner nuclear membrane (INM). The N-terminal region is in the lumen of the nuclear envelope whereas the C-terminal region projects into the nucleus where it makes contact with the chromosomes and the nuclear lamina. The Nesprin-SUN1 complex thus provides a direct connection between the cytoskeleton and the nuclear lamina. Emerin, embedded in the INM extends into the nucleus where it interacts with SUN1, the nuclear lamina, barrier-to-autointegration factor (BAF) and chromosomes.

The lamin B receptor (LBR), which is an integral membrane protein located in the INM, binds to heterochromatin by interacting with the core histones (H3/H4). LBR can also bind to members of the heterochromatin protein 1 (HP1) family that has multiple nuclear functions.

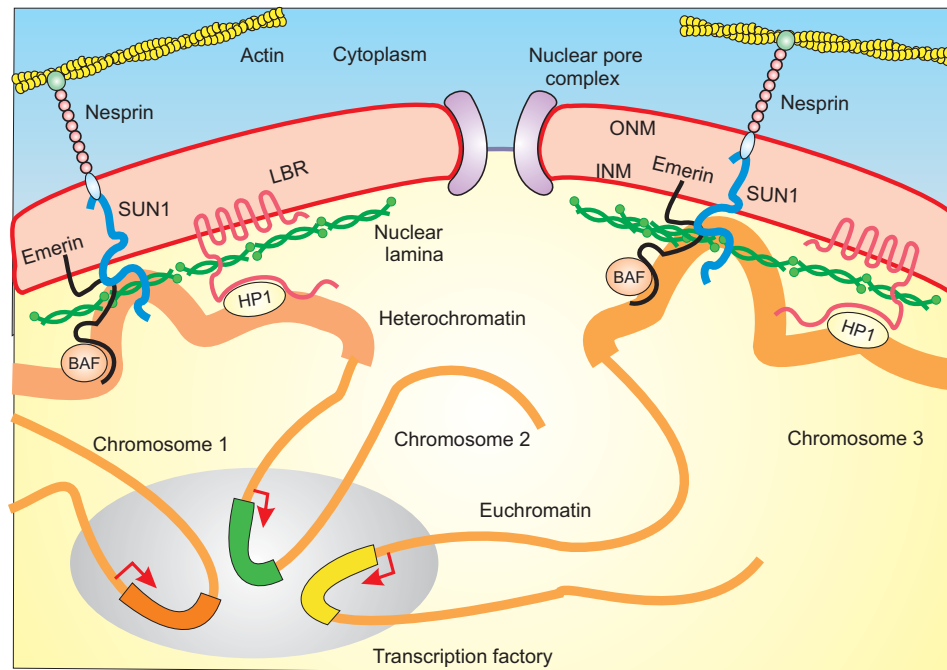
An important component of the LINK complex that functions in chromatin organization is the nuclear lamina. Mutations in some of the components of this nuclear lamina and its associated proteins have been linked to a number of laminopathies and various progeria syndromes.

Nuclear lamina

The nuclear lamina, which plays a central role in maintaining the architecture of the nucleus and chromosome function, is a highly-ordered structure composed of type V intermediate filament proteins called lamins (Module 12: Figure chromatin organization). There are three lamin genes (*LMNA*, *LMNB1* and *LMNB2*): the *LMNA* gene encodes lamin A and lamin C that are present in most differentiated cells. *LMNB1* (encodes LMNB1) and *LMNB2* (encodes LMNB2) are also found in most cells, whereas *LMNB3* is restricted to germ cells. These lamins have a highly conserved central α -helical coiled-coil (CC) domain and polymerize with each other to form a meshwork of fibres located on the inner aspect of the nuclear membrane. This nuclear lamina is part of the linker of nucleoskeleton and cytoskeleton (LINK) complex that functions to maintain the mechanical stability of the nucleus and also has a critical role in controlling genome stability, gene expression and chromosome replication.

A significant feature of this architectural role of the nuclear lamina is how it organizes the structure of chromatin in that the non-coding heterochromatin tends to be localized at the periphery attached to the lamina, whereas euchromatin representing the decondensed, transcriptionally active genes is found within the interior of the nucleus. The phenotypic stability of each specific cell type may depend on the distribution of active genes in the interior and the silent genes tightly bound to the nuclear lamina. Such a spatial re-organization of the genome may thus contribute to genome instability and ageing.

Mutations in certain components of the LINK complex give rise to a number of laminopathies. A number of these laminopathies have been linked to various premature

Module 12: | Figure chromatin organization**Chromatin organization.**

The two types of chromatin are spatially segregated in the nucleus. The heterochromatin is associated with a linker of nucleoskeleton and cytoskeleton (LINK) complex, which is a scaffolding network consisting of proteins such as the lamins that constitute the nuclear lamina, lamin B receptor (LBR), heterochromatin protein 1 (HP1), emerin, barrier-to-autointegration factor (BAF) and SUN1. The SUN1 is connected to the actin-binding protein nesprin. The euchromatin chromosomal regions extend into the nucleus where genes from different chromosomes (e.g. chromosomes 1-3) come together in a localized region, so called transcription factories, where protein expression is optimized by sharing various transcription machinery components.

ageing syndromes, which have also been referred to as progeroid syndromes that are characterized by the symptoms of old age that appear in young children. There is early hair loss, skeletal abnormalities, atherosclerosis and cardiovascular abnormalities resulting in premature death. Examples of such diseases are Hutchinson–Gilford progeria syndrome (HGPS) and Werner syndrome. All this evidence strongly supports the notion that genomic instability may be a major factor in the ageing process.

Laminopathies

Laminopathies arise through mutations of components of the nuclear lamina that bring about the genome instability and ageing (Module 12: Figure chromatin organization) that gives rise to a range of disorders. These disorders can influence different cellular systems. The first recognized laminopathy was Emery–Dreifuss muscular dystrophy (EDMD), which is characterized by progressive skeletal muscle weakness and wasting. A key feature is the dilated cardiomyopathy including early conduction system abnormalities, which is the main life-threatening aspect of this syndrome. EDMD is caused by mutations in the *LMNA* gene that encodes the lamin A and lamin C that are key components of the nuclear lamina. One of the *LMNA* mutations gives rise to a truncated version of lamin A known as progerin. The accumulation of progerin in the nucleus results in a change in nuclear morphology and impairs nuclear function, causing various laminopathies and has also been linked to normal ageing. Mutations in

emerin have also been linked to X-linked recessive Emery–Dreifuss muscular dystrophy.

A curious feature of *LMNA* mutations is that they can give rise to a number of different disorders. In addition to the EDMD mentioned above, mutations in *LMNA* have also been linked to the following disorders:

- Dunnigan-type familial partial lipodystrophy, which is an autosomal-dominantly inherited disorder characterized by a loss of adipose tissue that can lead on to insulin resistance and diabetes mellitus.
- Charcot–Marie–Tooth type 2 disorder type 2B1, which is an autosomal-recessive peripheral neuropathy.
- Hutchinson–Gilford progeria syndrome (HGPS), which is an autosomal-dominant syndrome that is characterized by accelerated ageing.

Mutations in the Barrier-to-autointegration factor 1 (*BANF1*) gene that codes for barrier-to-autointegration factor (BAF), which is a component of the LINK complex (Module 12: Figure chromatin organization), has been linked to Néstor–Guillermo progeria syndrome (NGPS).

Epigenetic changes in ageing

Epigenetic modifications based on histone acetylation may contribute to the phenotypic changes that occur during ageing by reducing transcriptional activity. This trend is enhanced in various neurodegenerative diseases where excessive activation of both HDAC2 and HDAC4 reduces

neuronal gene transcription (Module 10: Figure neuronal gene transcription). Inhibitors such as sodium butyrate and suberoylanilide hydroxamic acid (SAHA) can reduce the activity of the class 1a HDACs. These HDAC inhibitors have been successfully used to improve some of the symptoms of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS).

Age-related macular degeneration (AMD)

Age-related macular degeneration (AMD), which is the leading cause of blindness, results from damage to the retina causing a loss of vision. The change occurs in the macula, which is located in the centre of the retina and is responsible for visual acuity. There are two forms of AMD: wet and dry AMD. The more common form is dry AMD, which is caused by atrophy of the photoreceptors (rods and cones) in the central part of the eye. The resulting cellular debris called *drusen* builds up between the retina and the choroid. In general, it is less likely to cause a severe loss of vision. On the other hand, wet AMD is far more serious resulting in irreversible damage and vision loss. In this case, the damage is caused by choroidal neovascularization where the blood vessels in the macula behind the retina begin to grow abnormally. As these new vessels are very fragile they tend to rupture and leak blood that raises the macula and damages the retina.

The angiogenesis responsible for promoting the growth of blood vessels is driven by vascular endothelial growth factor (VEGF). There has been some success in treating wet AMD using drugs that inhibit VEGF. There also are indications that the chemokine receptor CCR3, which is located on the choroidal endothelial cells, might play an important role in promoting the growth of choroidal vessels. The retinal pigmented epithelium may release the chemokines eotaxin-1 (CCL11), eotaxin-2 (CCL24) and eotaxin-3 (CCL26) that activate CCR3 (Module 1: Figure chemokines).

Stargardt disease is a common form of inherited juvenile macular degeneration.

Alzheimer's disease

There are two forms of Alzheimer's disease (AD): sporadic Alzheimer's disease (AD) and Familial Alzheimer's disease (FAD). The latter, which closely resembles the sporadic form of AD, develops much earlier in life. Many of the genes that have been linked to both familial and sporadic AD function either in the formation and release of the amyloid protein or in the signalling pathways that are disrupted in this disease.

AD is a progressive neurodegenerative disease characterized by the appearance of neurofibrillary tangles (NFTs) and amyloid fibrils and plaques. The plaques build up outside nerve terminals whereas the neurofibrillary tangles accumulate within the neurons. These fibrils and plaques arise through two separate biochemical changes that have provided important information about the cause of AD.

The tangles are composed of the protein tau, which normally functions to stabilize microtubules within neuronal axons. The enzyme glycogen synthase kinase-3 (GSK-3)

may play a role in the formation of both the tangles and the plaques. It may promote the tangles by phosphorylating the protein tau, which functions in the assembly and maintenance of microtubules. Under pathological conditions, kinases such as GSK-3 hyperphosphorylate tau causing it to dissociate from the microtubules and then to aggregate into tangles (Module 12: Figure amyloid plaques and tangles). Once tau is removed from microtubules, they dissociate, thus interfering with the process of axonal transport. The reason why these tau protein tangles cause neuronal defects is not clear and they may not be responsible for the onset of AD. However, mutations in tau that result in tangles similar to those seen in AD appear to be responsible for frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17).

More information is available on the nature of the plaques that build up in the brain during the development of AD (Module 12: Figure amyloid cascade hypothesis). These plaques are formed by the polymerization of the β -amyloid ($A\beta$) protein, which is derived from the β -amyloid precursor protein (APP) that is hydrolysed by β -secretase and the γ -secretase complex. The idea is that AD arises when the amount or the nature of this amyloid is abnormally altered to bring about neuronal cell death.

The notion that β -amyloid ($A\beta$) processing might be the cause of AD has been supported by the fact that mutations in some of the components of the amyloid pathway, such as APP and the presenilin-1 and presenilin-2 (PS1 and PS2) enzymes that process APP, are responsible for autosomal-dominant early-onset familial Alzheimer's disease (FAD). The gene for APP is also carried on the extra chromosome in Down's syndrome, and this may account for the early plaque formation and onset of dementia associated with this disease. The risk of developing AD is markedly increased in individuals that inherit the ApoE4 isoform of apolipoprotein E (ApoE), which functions to carry lipids around the brain.

To understand how AD develops, it is therefore necessary to consider two related aspects. First, what is the nature of the change in amyloid formation that is the basis of the amyloid cascade hypothesis? This hypothesis must include a description of how early onset AD is induced by mutations in APP, the presenilins and apoE4. The second question is to consider how this change in amyloid formation brings about the massive neuronal cell death that causes AD. One possibility is the calcium hypothesis of Alzheimer's disease. It is argued that there is calcium-induced memory loss in Alzheimer's disease. Another possibility is that there is a process of astrocyte-induced neuronal death. There also appears to be an important relationship between changes in acetylation and Alzheimer's disease.

Familial Alzheimer's disease (FAD) and genetic risk factors

The genetic factors responsible Alzheimer's disease (AD) have emerged through the analysis of individuals with either Familial Alzheimer's disease (FAD) or sporadic AD.

Familial AD is an early-onset form of Alzheimer's disease (AD) that is caused by mutations in proteins that code for a range of different proteins as summarized below:

- Apolipoprotein E (ApoE) has a major role in influencing how the amyloid β is formed and hydrolysed (Module 12: Figure amyloid cascade hypothesis).
- β -Amyloid precursor protein (APP), is the precursor protein that is cleaved to produce the β -amyloid ($A\beta$) protein (Module 12: Figure amyloid cascade hypothesis).
- Presenilins PS1 and PS2 mutations have been linked to early-onset Familial AD since they alter the processing of APP to produce more of the neurotoxic amyloid β 42 ($A\beta_{42}$) protein that causes neurodegeneration (See step 5 in Module 12: Figure amyloid cascade hypothesis).

A number of genes have emerged from the analysis of individuals that have developed sporadic AD:

- Clusterin (CLU) is an apolipoprotein that may have an action similar to that of ApoE. CLU may bind to amyloid β ($A\beta$) to enhance its clearance from the brain.
- Complement component (C3b/C4b) receptor 1 (CR1) may act by binding to complement fragment C3b to enhance the clearance of $A\beta$.
- Phosphatidylinositol-binding clathrin assembly protein (PICALM), which is also known as clathrin assembly lymphoid-myeloid leukaemia (CALM), plays an important role in endocytosis that functions in the trafficking of both neurotransmitters and their receptors. In neurons, PICALM functions to regulate the trafficking of synaptobrevin, also known as vesicle-associated membrane protein 2 (VAMP2), which is a v-SNARE that is part of the exocytotic machinery that functions in synaptic vesicle exocytosis (Module 4: Figure Ca^{2+} -induced membrane fusion).
- Triggering receptor expressed in myeloid cells 2 (TREM-2) functions as a negative regulator of innate immunity. A variant of TREM-2, which reduces its anti-inflammatory function, is associated with a markedly increased risk of developing AD (Module 12: Figure Inflammation and Alzheimer's disease).
- Phospholipase D3 (PLD3), which plays a role in the processing and release of the amyloid protein, has been identified as a significant risk factor in AD.

Analysing the function of these proteins has greatly facilitated our understanding of sporadic AD. The amyloid cascade hypothesis describes how APP and the presenilins function to release the β amyloid proteins responsible for neurodegeneration (Module 12: Figure amyloid cascade hypothesis). The calcium hypothesis of Alzheimer's disease and the inflammation in Alzheimer's disease sections describe how many of these proteins contribute to loss of memory and subsequent neuronal cell death.

Amyloid cascade hypothesis

The basis of the amyloid cascade hypothesis is that AD is caused by an alteration in the normal processing of the β -amyloid ($A\beta$) protein. The following description concerns the various pathways responsible for forming and

processing the amyloids (Module 12: Figure amyloid cascade hypothesis):

1. The β -amyloid precursor protein (APP) is synthesized and transferred to the plasma membrane by the endoplasmic reticulum-Golgi secretory pathway.
2. The function of APP in the membrane is still uncertain, but it has been implicated in the regulation of neuronal migration and synaptic plasticity. With regard to the latter, much of the processing of APP is carried out in the region of the synapses. APP can bind to F-spondin located in the extracellular matrix.

Non-amyloidogenic pathways

3. The APP in the membrane is processed in two ways, which are known as the non-amyloidogenic pathways because they do not give rise to the β amyloids. First, APP is cleaved by α -secretases such as ADAM-10 and ADAM-17 (Module 1: Table ADAM proteases) resulting in the shedding of the soluble APP α (sAPP α) and the C-terminal fragment α (CTF α) that is retained in the membrane. The CTF α can then be hydrolysed by presenilin-1 (PS1), which is a component of the γ -secretase complex resulting in release of APP intracellular domain (AICD). The APP intracellular domain (AICD) functions as a transcription factor (as described below). Secondly, the APP that associates with various low-density lipoprotein receptors (LDLRs), such as LR11 (also known as SorLA and SORL1), can be internalized and enters the recycling endosomes and thus can be returned to the membrane. These two non-amyloidogenic pathways do not result in the release of the β -amyloids.

Amyloidogenic pathways

4. APP can also enter an amyloidogenic pathway where it is processed to release the β -amyloids responsible for AD. Internalized APP that ends up in the late endosomes undergoes different processing events that begins by its hydrolysis by the enzyme β -secretase, which is also known as β -site APP-cleaving enzyme (BACE), that sheds the N-terminal sAPP β region leaving the C-terminal fragment β (CTF β) in the membrane (Module 12: Figure APP processing). As outlined below, both fragments (sAPP β and CTF β) have been implicated in the neurodegeneration responsible for AD.
5. CTF β is hydrolysed by the γ -secretase complex that contains the presenilin enzymes, either the PS1 or PS2 isoforms. This γ -secretase cleaves CTF β at two sites to yield either amyloid β 40 ($A\beta_{40}$) or amyloid β 42 ($A\beta_{42}$), which are released to the inside of the vesicle, and the APP intracellular domain (AICD) that is released to the cytoplasm. The amyloids are transported and released to the surface via the constitutive secretory pathway, whereas the AICD enters the nucleus where it functions as a transcription factor.
6. The amyloid β ($A\beta$) monomers that are released to the outside of the neuron have two fates (Module 12: Figure amyloid cascade hypothesis). They can be destroyed by the insulin-degrading enzyme (IDE), which

is released by the microglia. The microglia also plays a role in removing the amyloid plaques and fibrils by a process of phagocytosis (see step 3 in Module 7: Figure microglia interactions). The fibrils that are taken up by the microglia are hydrolysed by neprilysin (NEP).

7. Apolipoprotein E (ApoE) has a major role in influencing how the amyloid β is formed and hydrolysed (Module 12: Figure amyloid cascade hypothesis). ApoE is the main apolipoprotein in the brain where it functions to distribute lipids between the glial cells and neurons. ApoE is synthesized by the astrocytes and microglia and once it is released, the ABCA1 transporter functions in its lipidation. As the ApoE loads up with lipid, it changes its conformation and this is important with regard to its ability to bind to the amyloids. ApoE can influence the amyloid processing in two ways. First, it can increase the degradation of the β amyloids by functioning as a chaperone to shepherd them to the insulin-degrading enzyme (IDE). Secondly, the apoE can bind to various members of the low-density lipoprotein receptor (LDLR) family that control the endocytosis of lipoproteins such as apoE. Some of these LDLRs such as LR11 (also known as SorLA and SORL1) interact with APP to influence its subsequent processing once it enters the endocytic pathways. In particular, LR11 may direct APP to the recycling endosome and thus redirects it back to the surface and prevents it from being hydrolysed by the β -secretase pathway. In this way, ApoE protects neurons by reducing both the formation of the A β monomers and by enhancing their degradation.

The importance of ApoE in reducing the build up of amyloids is emphasized by the fact that a polymorphism of the *APOE* gene markedly increases the susceptibility of developing AD. The *APOE* gene encodes three isoforms ApoE2, ApoE3 and ApoE4. The risk of developing AD is markedly increased in individuals that inherit the ApoE4 isoform. On the basis of the mechanisms outlined in Module 12: Figure amyloid cascade hypothesis, it would seem that this isoform enhances the onset of AD by increasing the conversion of APP into the amyloids and by reducing their clearance by the degradation pathway.

8. One of the continuing mysteries surrounding AD concerns the way the formation of the β -amyloid peptides results in a devastating cognitive decline. While much previous attention focused on the plaques, there is increasing evidence that the peptides themselves may have a role to play. One of the difficulties with studying this topic is that these β -amyloid peptides can aggregate to form complexes of different sizes, starting with dimers and oligomers and then proceeding progressively to larger complexes such as protofibrils and then the large plaques. Recent evidence has found that an oligomer of approximately 12 A β_{42} monomers might be responsible for the pathological changes in neuronal function that lead to memory loss.
9. The pathological effects of the A β oligomer might be mediated by the cellular prion protein (PrP^C) (for fur-

ther details see Module 12: Figure amyloids and Ca²⁺ signalling).

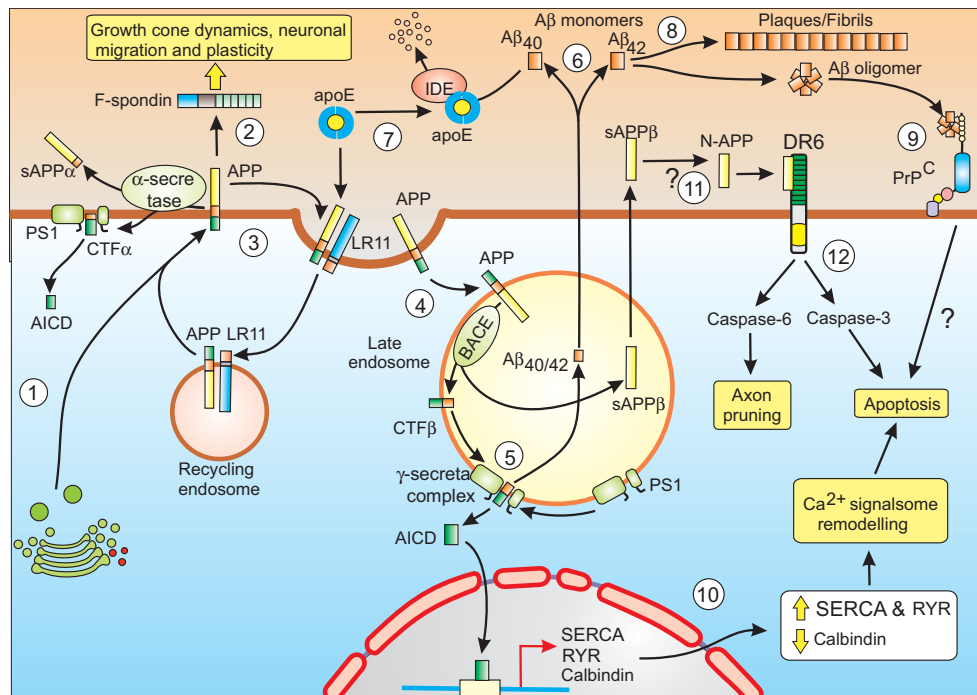
10. Formation of the transcription factor AICD may result in a significant remodelling of the Ca²⁺ signalling system. There are indications that it might promote the expression of Ca²⁺ signalling components such as the SERCA pump, the ryanodine receptor (RYR) and possibly the Ca²⁺ buffer calbindin D-28k (CB). This remodelling of the Ca²⁺ signalsome will result in hypersensitivity of the Ca²⁺ signalling system and is the basis of the calcium hypothesis of Alzheimer's disease (Module 12: Figure amyloids and Ca²⁺ signalling).
11. The N-terminal sAPP β that is formed when APP is hydrolysed by BACE (see step 4 above) may also play a signalling role during both normal and pathological conditions. The sAPP β is cleaved by an unknown enzyme to form an N-terminal APP (N-APP) fragment that may be a ligand for the orphan DR6 receptor. DR6 is a member of the superfamily of tumour necrosis factor (TNF) receptors (TNF-Rs). One of the functions of such receptors is to stimulate apoptosis by activating caspases (Module 11: Figure TNF α apoptotic signalling). DR6, which is strongly expressed in neurons, appears to have a similar role, but its action varies between different regions of the neuron. DR6 located on the axon responds to N-APP by activating caspase 6 that functions to control axonal pruning during development. On the other hand, DR6 at the soma activates caspase 3 that then triggers apoptosis. An increase in the amyloidogenic pathway resulting in an increase in the formation of N-APP may thus contribute to the increase in cell death associated with Alzheimer's disease.

The main tenant of the amyloid cascade hypothesis is that an alteration in the processing of APP, which results in an accumulation of β amyloids, is responsible for the onset of AD. The next aspect to consider is how this change in amyloid processing results in the neurodegeneration that leads to dementia. The calcium hypothesis of Alzheimer's disease suggests that the change in amyloid processing alters the operation of the Ca²⁺ signalling pathways resulting in apoptosis and neurodegeneration. This new focus on the β -amyloid peptides is of interest, because these soluble peptides may also play a role in astrocyte-induced neuronal death that will contribute to neuronal cell death.

Calcium hypothesis of Alzheimer's disease

The main feature of the calcium hypothesis of Alzheimer's disease is that an increase in the amyloidogenic pathway (Module 12: Figure amyloid cascade hypothesis), which results in the release of the soluble amyloids, brings about changes in neuronal Ca²⁺ signalling pathways that initially induce a decline in memory and later neuronal cell death (Module 12: Figure amyloids and Ca²⁺ signalling). There is a bidirectional relationship between Ca²⁺ signalling and the amyloidogenic pathway. While the amyloids stimulate an increase in Ca²⁺, the latter can stimulate the metabolism of APP. This link between Ca²⁺ and amyloid formation may also explain the regional variation in the deposition of amyloid that is greatest in those areas of the brain where

Module 12: | Figure amyloid cascade hypothesis



Non-amyloidogenic and amyloidogenic pathways.

The amyloid cascade hypothesis proposes that Alzheimer's disease is caused by an increase in the formation of the amyloid β peptides $A\beta_{40}$ and $A\beta_{42}$. In the non-amyloidogenic pathway, the amyloid precursor protein (APP) is either recycled through the endosomal pathway or is cleaved by α -secretase. During the amyloidogenic pathway, APP enters the late endosome where it is cleaved to form the amyloid β peptides ($A\beta_{40}$ and $A\beta_{42}$) and the APP intracellular domain (AICD). These products may act to enhance Ca^{2+} signalling (Module 12: Figure amyloids and Ca^{2+} signalling). Details of how BACE and the γ -secretase complex hydrolyse APP to form amyloid β peptides and the APP intracellular domain (AICD) is shown in Module 12: Figure APP processing.

neurons are most active and will be experiencing more prolonged elevations in Ca^{2+} . Such a two-way interaction between these two processes introduces an element of positive feedback that might be critical for the onset and progression of both AD and FAD. In addition, it raises an interesting question as to which one of these processes is the primary cause of AD.

The Ca^{2+} signals responsible for controlling both presynaptic events and postsynaptic events depend on a number of neuronal Ca^{2+} entry and release channels. One of the major changes in Ca^{2+} signalling that occurs during AD appears to be an increase in the amount of Ca^{2+} being released from the internal stores. An example of this can be seen in the Ca^{2+} signals recorded from neurons that express genes that alter amyloid processing (Module 12: Figure neuronal Ca^{2+} signals in AD mice). The APP and tau mutations (APPTau) used in this study had no effect in that the responses to either the photolysis of $InsP_3$ or the induction of action potentials were similar to those seen in the non-transgenic control (NonTg). However, the presence of the presenilin 1 mutation (PS1) present by itself (PS1_{K1}) or together with APP and tau (3xTg-AD) caused a large increase in the amount of Ca^{2+} released by $InsP_3$, whereas the response to depolarization was not changed. These mouse models of AD, which are consistent with many other studies, suggest that the changes in Ca^{2+} signalling result from an increase in the amount of

Ca^{2+} being released from the internal stores. One reason for this might be the increase in both the connectivity and the function of the mitochondrial-associated ER membranes (MAMs) (Module 5: Figure mitochondrial-associated ER membranes). This link between MAMs and Alzheimer's disease resulting from a hypersensitivity of the Ca^{2+} signalling pathway illustrates how AD is an example of signalsome remodelling causing disease (Module 12: Figure signalsome remodelling).

There are a number of mechanisms whereby mutations that induce changes in amyloid processing might bring about this up-regulation of the neuronal Ca^{2+} releasing mechanisms as illustrated in Module 12: Figure amyloids and Ca^{2+} signalling.

Recent evidence has suggested that the cellular prion protein (PrP^C), which is tethered to the outside of the membrane through a glycosyl phosphatidylinositol (GPI) anchor, functions as an amyloid β receptor and may thus carry out some of the pathological actions of $A\beta_{42}$. These $A\beta$ oligomers, which are thought to be the active components responsible for AD, may act through a number of other mechanisms to increase the level of Ca^{2+} . They can be inserted into the membrane to form channels or they enhance Ca^{2+} entry through various receptor-operated channels such as the NMDA receptors (NMDARs). There also is evidence that the $A\beta$ oligomers can activate the calcium-sensitive receptor (CaR) to increase the level of

inositol 1,4,5-trisphosphate (InsP₃) that will increase the release of Ca²⁺ from the internal store (Module 12: Figure amyloids and Ca²⁺ signalling). The activity of the CaR is regulated by RGS4, which is reduced in the brain in Alzheimer's disease (AD) and could thus contribute to the increase in the CaR-induced formation of InsP₃.

The calcium homeostasis modulator 1 (CALHM1), which promotes the entry of external Ca²⁺, may also have a specific role in regulating amyloid metabolism. Of particular interest was the observation that a polymorphism in the *CALHM1* gene, which reduced Ca²⁺ entry, increased amyloid formation.

The Ca²⁺ that enters the cell through these amyloid-dependent mechanisms is then pumped into the endoplasmic reticulum by the sarco-endoplasmic reticulum ATPase (SERCA) pump. An increase in the luminal level of Ca²⁺ will serve to increase the amount of Ca²⁺ being released from the internal stores. The level of Ca²⁺ within the lumen of the endoplasmic reticulum is regulated by the balance between the activity of these SERCA pumps and passive leak pathways. The channels responsible for this leak remain to be properly characterized, but there is increasing evidence that presenilins may function as such a leak channel. The level of Ca²⁺ within the lumen and the amount of Ca²⁺ being released in response to InsP₃ is markedly reduced in cells that over express PS1. Conversely, the mutated forms of PS1 that give rise to early-onset familial Alzheimer's disease (FAD) reduce the passive leak resulting in enhanced Ca²⁺ signals. Similarly, the calcium homeostasis modulator 1 (CALHM1), which is expressed in both the plasma membrane and the ER, may also provide such a leak. Polymorphisms in the *CALHM1* gene, which reduced Ca²⁺ permeability, might act like the mutated PS1 to increase store loading and hence Ca²⁺ signalling.

In addition to enhancing the Ca²⁺ content of the ER, the mutated presenilins can also increase Ca²⁺ signalling by remodelling the Ca²⁺ signalling system (Module 12: Figure amyloid cascade hypothesis). While in the ER, the presenilin holoenzyme controls the passive leak of Ca²⁺ and thus increases Ca²⁺ signals as described above. However, when these presenilins are processed and enter the endosomes and Golgi, they contribute to the γ -secretase complex where they increase the formation of the amyloid β 42 (A β ₄₂) (see step 5 in Module 12: Figure amyloid cascade hypothesis) and they also release the APP intracellular domain (AICD), which is a transcription factor that acts to enhance the expression of both the SERCA pump and the ryanodine receptor (RyR). An increase in the SERCA pump, which will enhance the Ca²⁺ content of the Ca²⁺ store, together with the increase in the number of RyRs will greatly enhance the amount of Ca²⁺ being released in response to those receptors that act through InsP₃.

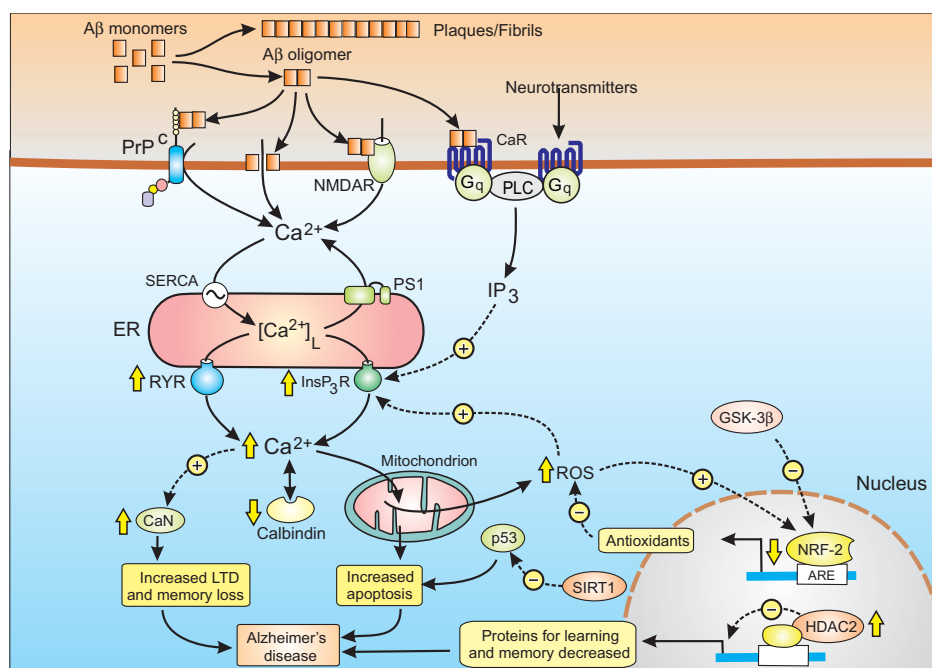
Indeed, there appears to be a marked increase in the activity of the type 1 InsP₃ receptors (InsP₃R1s) in AD and this could arise through a number of processes such as the increase in luminal Ca²⁺ described above or through changes that occur from the increase in inflammation in Alzheimer's disease (Module 12: Figure inflammation in Alzheimer's disease). Inflammation increases the forma-

tion of reactive oxygen species (ROS), which are known to be an InsP₃R agonist. The TNF α formed during inflammatory responses may act to increase the expression of the InsP₃Rs (See step 6 in Module 12: Figure inflammation in Alzheimer's disease). Such remodelling of the Ca²⁺ signalling system may thus be a significant step in the progression of AD. This hypersensitivity of Ca²⁺ signalling in AD is an example of signalsome remodelling causing disease (Module 12: Figure signalsome remodelling).

It has been known for some time that during normal ageing there are gradual changes in certain Ca²⁺ signalling components that increase neuron vulnerability to the stimuli that induce cell death. For example, there is a gradual decline in the level of the Ca²⁺ buffer calbindin D-28k (CB) that normally functions to restrict the amplitude of Ca²⁺ signals. A decline in this buffer may also be one of the consequences of AD because mice expressing mutant APP that have markedly increased levels of amyloid β 42 (A β ₄₂) also display a decline in the level of calbindin D-28k (CB) especially in the dentate gyrus region of the hippocampus, which functions in learning and memory. Just how metabolism of mutant APP results in the change in the expression of calbindin D-28k (CB) is not clear, but it may depend on the formation of the APP intracellular domain (AICD) that occurs when mutant APP is hydrolysed (see step 5 in Module 12: Figure amyloid cascade hypothesis).

Neurotransmitters activate metabotropic receptors on neurons and this often results in the synaptic stimulation of global Ca²⁺ signals as occurs in neocortical neurons (Module 10: Figure neocortical Ca²⁺ wave). The Ca²⁺ signal that initiates in the synaptic region spreads down the dendrites and into the soma through the regenerative process of Ca²⁺-induced Ca²⁺ release (CICR) whereby the InsP₃ receptors activate each other (Module 10: Figure somatic and dendritic Ca²⁺ waves). In the case where neurons also express RyRs, the Ca²⁺ signal generated by InsP₃ can also be amplified by recruiting RyRs. Indeed, the Ca²⁺ signals induced by the photolysis of InsP₃ shown in Module 12: Figure neuronal Ca²⁺ signals in AD mice had a large contribution from the RyRs.

As a result of the increased output of Ca²⁺ due to the hypersensitivity of the Ca²⁺ signalling system described earlier (Module 12: Figure amyloids and Ca²⁺ signalling), the release of Ca²⁺ from the internal stores is much larger than normal and this can have two serious consequences. First, it will decrease the synaptic plasticity responsible for learning and memory. Secondly, the excess Ca²⁺ will activate the mitochondria to initiate the intrinsic pathway of Ca²⁺-induced apoptosis. The uptake of Ca²⁺ by the mitochondria stimulates the release of cytochrome c that then triggers the onset of apoptosis (Module 5: Figure ER/mitochondrial shuttle). The death of neurons is particularly evident in the basal forebrain where the cortical cholinergic neurons play a role in the cognitive processes of memory and attention. The selective vulnerability of these cholinergic neurons is exacerbated by the age-dependent decline in the expression of the calbindin D-28k (CB) that normally buffers cells against the deleterious effects of excess Ca²⁺. A similar decline in this buffer occurs during

Module 12: | Figure amyloids and Ca^{2+} signalling**Amyloids and Ca^{2+} signalling.**

The calcium hypothesis of Alzheimer's disease suggests that alterations in amyloid processing results in the formation of A β oligomers that bring about an overall increase in Ca^{2+} signalling that then induces memory loss, neuronal apoptosis and the onset of dementia. The way in which the amyloidogenic pathway functions to remodel the Ca^{2+} signalling system is described in Module 12: Figure amyloid cascade hypothesis.

the onset of AD. It is noteworthy that in Parkinson's disease, a subpopulation of dopaminergic neurons that express CB survive the neurodegeneration characteristic of this disease.

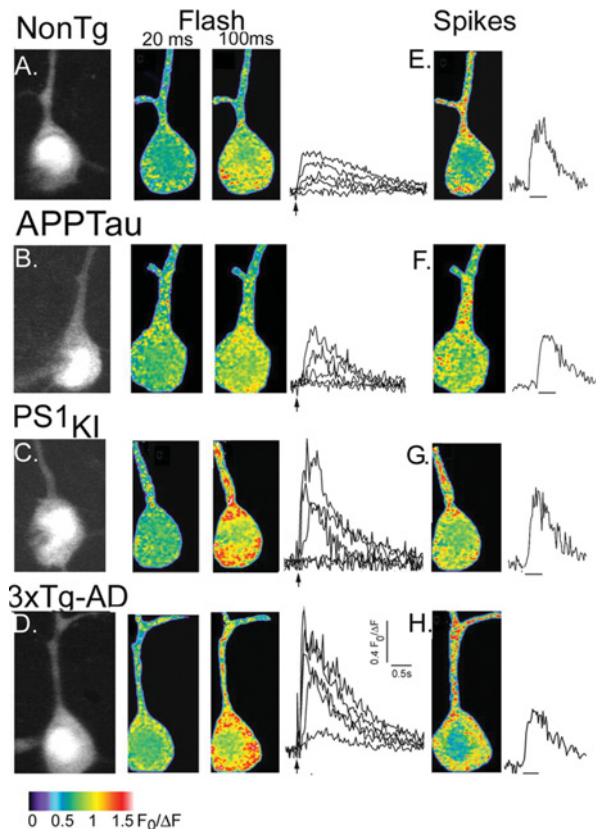
In mouse models of AD, reversal of Ca^{2+} -dependent neurodegeneration has been achieved by a variety of agents that inhibit either the generation or the mode of action of neuronal Ca^{2+} signals (Module 12: Figure reversal of Ca^{2+} signalling in neurodegeneration).

Reversal of Ca^{2+} -dependent neurodegeneration

If a persistent elevation in Ca^{2+} is responsible for the early memory defects and subsequent neuronal cell death, it should be possible to reverse this Ca^{2+} -dependent neurodegeneration by reducing Ca^{2+} back to its resting levels. There already are a number of examples indicating that such a reversal can be achieved and suggests that novel therapies could be developed to normalize Ca^{2+} signalling pathways to reverse the consequences of neurodegeneration. Such treatments must be fairly subtle and need to concentrate on methods that will lower the resting level of Ca^{2+} , without interfering with the normal Ca^{2+} signalling pathways. In the case of Alzheimer's disease, there already is some evidence that the deleterious effects of excess Ca^{2+} can be reversed by adjusting either the levels of Ca^{2+} or its downstream signalling events using treatments such as Li^+ , Bcl-2, dantrolene, FK506 and mitoQ (Module 12: Figure reversal of Ca^{2+} signalling in neurodegeneration). A relationship between vitamin D and Ca^{2+} regulation in neurodegeneration may be of particular significance.

There are indications that Li^+ may reduce the risk of developing Alzheimer's disease. In the case of bipolar disorder, its action may depend on its ability to reduce the activity of the $\text{InsP}_3/\text{Ca}^{2+}$ signalling pathway as formulated by the inositol depletion hypothesis (Module 12: Figure inositol depletion hypothesis) and exactly the same mechanism could explain its protective effect in AD. Li^+ would act to reduce the formation of InsP_3 and this would reduce the amount of Ca^{2+} being released from the internal store. Another possibility is that Li^+ might act to inhibit the glycogen synthase kinase-3 β (GSK-3 β), which inhibits the nuclear factor erythroid 2 related factor 2 (NRF-2) responsible for maintaining antioxidant defences (Module 4: Figure NRF-2 antioxidant function). In animal models of AD, the expression and activation of GSK-3 β is enhanced and this could account for the reduction in NRF-2 function.

Another way of reducing neuronal Ca^{2+} signalling is to inhibit its release from the internal store. Some of this release of Ca^{2+} can be regulated through inositol 1,4,5-trisphosphate receptor (InsP_3R) modulation using a mechanism based on the Bcl-2 superfamily control of Ca^{2+} signalling. When expressed in a mouse model of AD, the anti-apoptotic factor Bcl-2 was able to improve cognition and prevented neuronal apoptosis. This is consistent with the calcium hypothesis of AD because Bcl-2 is known to bind to the InsP_3R to reversibly inhibit InsP_3 -dependent channel opening (Module 12: Figure reversal of Ca^{2+} signalling in neurodegeneration). AD symptoms in mouse models can also be reduced by dantrolene that acts to inhibit release of Ca^{2+} through the ryanodine receptors (RYRs). If

Module 12: | neuronal Ca^{2+} signals in AD mice **Ca^{2+} signals in neurons from Alzheimer's disease mouse models.**

Ca^{2+} signals were induced either by the photolysis of caged InsP_3 (arrows in A-D) or by a depolarizing current (bars in E-H) that induced a train of action potentials. A and E, non-transgenic controls (NonTg); B and F, transgenic mouse neurons carrying mutations in APP and tau (APPTau); C and G, transgenic mouse neurons carrying mutations in presenilin 1 (PS1_{KI}); D and H, transgenic mouse neurons carrying mutations in APP, tau and presenilin 1 (3xTG-AD). Reproduced from Stutzmann, G.E., Smith, I., Caccamo, A., Oddo, S., LaFerla, F.M. and Parker, I. (2006) Enhanced ryanodine receptor recruitment contributes to Ca^{2+} disruptions in young, adult and aged Alzheimer's disease mice. *J. Neurosci.* 26:5180-5189. Copyright (2006), with permission from the Society of Neuroscience; see Stutzmann et al. 2006.

such a mechanism operates in neurons, a reduction in the release of Ca^{2+} from the internal store and the subsequent decline in the level of Ca^{2+} would support the notion that the up-regulation of Ca^{2+} signalling is responsible for driving memory loss in AD.

An increase in the formation of reactive oxygen species (ROS), which are known to be InsP_3 R agonists, contributes to the elevation in intracellular Ca^{2+} . One of the sources of ROS are the mitochondria and inhibition of this ROS formation by a mitochondrial-targeted antioxidant MitoQ prevents the cognitive decline in a transgenic mouse model of AD (Module 12: Figure reversal of Ca^{2+} signalling in neurodegeneration).

Another way of counteracting the elevation of Ca^{2+} is to reduce its ability to activate its downstream targets such as the calcineurin (CaN) that is responsible for increasing the process of long-term depression (LTD) that is postulated to cause the early stage memory loss in AD

(Module 12: Figure reversal of Ca^{2+} signalling in neurodegeneration). The level of CaN was found to be elevated in aged rats and in an APP transgenic mouse model of AD that display defects in cognition. In the case of the transgenic mouse, the defects in cognition could be reversed by FK506, which is an inhibitor of CaN.

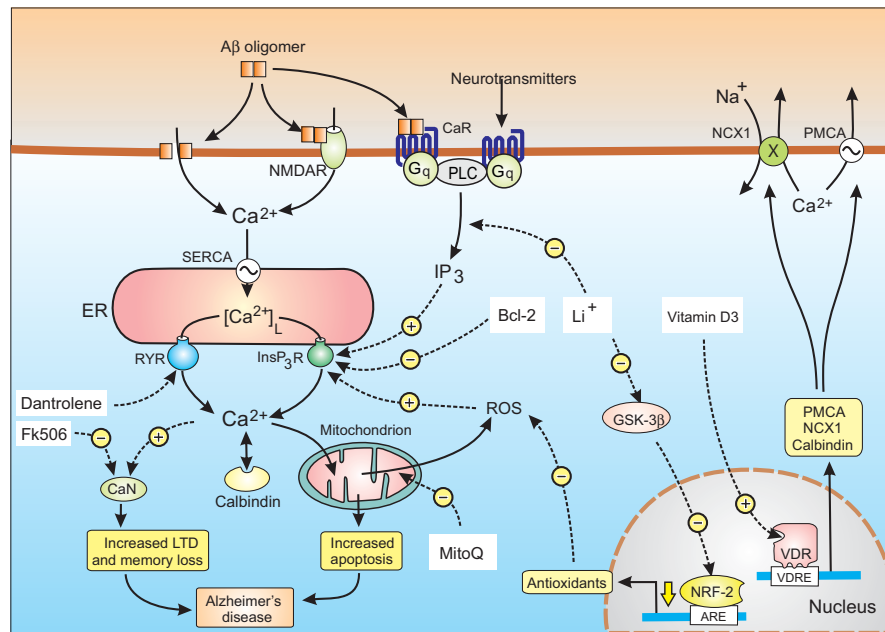
These inhibitory effects of Li^+ , Bcl-2 and FK506 on neurodegeneration not only support the idea that up-regulation of Ca^{2+} may be responsible for the onset of AD, but they also provide proof of concept that this debilitating neurodegenerative disease could be alleviated by treatments targeted at neuronal Ca^{2+} signalling pathways. The relationship between vitamin D and Ca^{2+} regulation in neurodegeneration suggests another potential therapeutic strategy to reduce the symptoms of a number of neural diseases.

Vitamin D and Ca^{2+} regulation in neurodegeneration

There are an increasing number of studies indicating that a deficiency in vitamin D may contribute to the onset of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and multiple sclerosis. With regard to AD, the decline in cognition that occurs normally in older adults may also be linked to vitamin D deficiency. Enhanced dietary vitamin D intake lowers the risk of developing AD in a study of older women. Since both AD and PD seem to be caused by abnormal elevations in Ca^{2+} , I shall develop the notion that the deleterious effect of vitamin D deficiency may be explained by an alteration in its normal role in regulating intracellular Ca^{2+} homeostasis.

The brain possesses all the enzyme responsible for both vitamin D formation and metabolism (vitamin D3 25-hydroxylase and 25-hydroxyvitamin D3-1 α -hydroxylase and vitamin D3 25-hydroxylase) (Module 7: Figure vitamin D metabolism). Neurons also express the vitamin D receptor (VDR) and VDR polymorphisms have been associated with Parkinson's disease and AD and have been linked to an age-related decline in cognition and the incidence of depressive symptoms. The VDR is strongly expressed in the dopaminergic neurons in the substantia nigra (Module 10: Figure basal ganglia), which are particularly vulnerable to Ca^{2+} stress in that they have to deal with repetitive surges in Ca^{2+} every few seconds during the operation of the dopaminergic neuronal pacemaker mechanism (Module 10: Figure tonic oscillation in DA neurons). The neurotoxic effect of 6-hydroxydopamine, which seems to depend on an increase in Ca^{2+} and reactive oxygen species (ROS), is reduced by vitamin D in rats. Vitamin D may alleviate the deleterious effects of ROS by increasing expression of γ -glutamyl transpeptidase that synthesizes the redox buffer glutathione (GSH).

All the evidence outlined above indicates that vitamin D has a significant protective role in the brain by helping to maintain both Ca^{2+} and ROS homeostasis. Such an action is consistent with the fact that vitamin D can regulate the expression of those Ca^{2+} signalling toolkit components responsible for reducing Ca^{2+} levels (Module 12: Figure reversal of Ca^{2+} signalling in neurodegeneration). For example, vitamin D stimulates the expression of the

Module 12: | Figure reversal of calcium signalling in neurodegeneration**Reversal of calcium signalling in neurodegeneration.**

The neurodegeneration in mouse models of Alzheimer's disease (AD) can be reversed by a variety of agents (shown in the white boxes). Consistent with the calcium hypothesis of AD, many of these agents act to reduce the abnormal elevation in intracellular calcium that is proposed to be the cause of memory loss and increased apoptosis.

plasma membrane Ca^{2+} ATPase (PMCA), the $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger (NCX) and Ca^{2+} buffers such as calbindin (CB) and parvalbumin. Neuronal levels of CB are known to be reduced in AD. In addition to enhancing these mechanisms for lowering the level of Ca^{2+} , vitamin D can curb the influx of external Ca^{2+} by reducing the expression of L-type voltage-sensitive channels.

In summary, any reduction in vitamin D levels will result in elevated neuronal Ca^{2+} levels and this could account for a number of neurodegenerative diseases. There is every reason to suspect that vitamin D could prove efficacious in many neural diseases such as AD and PD that are driven by a dysregulation of Ca^{2+} signalling.

The cancer drug bexarotene acts by stimulating RXR and may thus increase the activity of the vitamin D receptor (VDR) (Module 7: Figure vitamin D receptor activation). Such an action might explain the reported beneficial effects on both Parkinson's disease and AD, but these findings are somewhat controversial.

Calcium-induced memory loss in Alzheimer's disease

One of the interesting features of the calcium hypothesis of Alzheimer's disease is the idea that remodelling neuronal Ca^{2+} signalling may disrupt the relationship between Ca^{2+} and synaptic plasticity (Module 10: Figure Ca^{2+} -induced synaptic plasticity) that is responsible for learning and memory long before the onset of neuronal cell death. The appearance of amyloid oligomers or the acute application of such oligomers has been correlated with a decline in learning mechanisms such as long-term potentiation (LTP) or long-term depression (LTD). Furthermore, these acute effects can be reversed by A β antibodies. The following

LTD hypothesis of Alzheimer's disease suggests that this amyloid-dependent remodelling of the Ca^{2+} signalling system may continuously activate the process of LTD that is used to erase memories.

The formation and storage of memories during the day depend on brief high concentration (approximately 1000 nM) spikes of Ca^{2+} that activate the process of long-term potentiation (LTP) (Module 12: Figure LTD hypothesis of Alzheimer's disease). Information placed in this temporary memory is then uploaded and consolidated in more permanent memory stores during certain phases of sleep. During another phase of sleep, smaller elevation in Ca^{2+} to approximately 300 nM activates a process of long-term depression (LTD) that then erases information from the temporary memory store. On the basis of the calcium hypothesis of Alzheimer's disease discussed earlier, the specific proposal is that the abnormal amyloid metabolism in AD results in a permanent elevation in the resting level of Ca^{2+} into the 300 nM range that then quickly erases these memories from the temporary memory store before they can be consolidated. This hypothesis thus focuses attention on how the amyloid-dependent remodelling of Ca^{2+} signalling may disrupt learning and memory by permanently activating LTD.

Since LTD is driven by relatively small elevations in Ca^{2+} , a small amyloid-dependent up-regulation of Ca^{2+} signalling will selectively enhance LTD to continuously erase any memories initiated by LTP. Since one of the mechanisms for inducing LTD is the activation of metabotropic glutamatergic receptors that generate InsP_3 to release Ca^{2+} from internal stores, the enhanced memory loss seen in Alzheimer's disease could well be explained by

an increased activation of these InsP_3 receptors. In effect, the change in Ca^{2+} signalling in Alzheimer's disease may switch the brain from a system of memory storage to one of memory loss. In mouse models of AD, a reversal of Ca^{2+} -dependent neurodegeneration has been achieved by reducing Ca^{2+} back to its resting levels.

This LTD mechanism, which erases putative memories, appears to depend on activation of calcineurin (CaN). For example, CaN activates the removal of both NMDA and AMPA receptors by a process of endocytosis (Module 10: Figure Ca^{2+} -induced synaptic plasticity). In aged rats and in APP transgenic mice, which show defects in cognition, there is an up-regulation of CaN. The increased expression of Down's syndrome critical region 1 (DSCR1) in Alzheimer's disease patients may reflect the elevation of CaN and Ca^{2+} , which are known activators of DSCR1 transcription (Module 4: Figure NFAT control of Ca^{2+} signalling toolkit). Spatial memory is impaired in DSCR1^{-/-} animals, thus strengthening the idea that an abnormal activation of CaN may disrupt the processes of learning and memory. The associative learning disorder in transgenic mice could be reversed by treatment with the CaN inhibitor FK506 (Module 12: Figure reversal of Ca^{2+} signalling in neurodegeneration), thus supporting the notion that an increase in the activity of CaN and the inappropriate activation of LTD might be one of the main consequences of remodelling the Ca^{2+} signalling system in the early phase of Alzheimer's disease.

The ER stress response in neurodegenerative diseases may contribute to the loss of memory in AD. An alteration in ER Ca^{2+} levels that is described in the calcium hypothesis of Alzheimer's disease (AD) together with the processing of the amyloid proteins may induce endoplasmic reticulum (ER) stress signalling (Module 2: Figure ER stress signalling). One consequence of ER stress is the activation of PERK, which phosphorylates and inactivates the eukaryotic initiation factor eIF-2 α . The resulting decline in the neuronal protein synthesis is required for memory consolidation. Some of this memory consolidation may take place at the individual spines where a spine-specific Ca^{2+} signal is responsible for triggering protein synthesis at the polyribosome located at the base of each spine (step 8 in Module 10: Figure Ca^{2+} -induced synaptic plasticity).

As the disease progresses, the elevation of Ca^{2+} begins to rise further to a point where it will activate apoptosis resulting in the neuronal cell loss responsible for the final stages of dementia.

Autophagy and Alzheimer's disease

A marked feature of AD is a decline in autophagy, which seems to be associated with an increase in the activity of the mammalian target of rapamycin (mTOR) (Module 12: Figure autophagy and Alzheimer's disease). The InsP_3R is known to play a role in autophagy by assembling a complex containing regulators such as Beclin-1, Bcl-2 and hVps34 (Module 11: Figure autophagy). The level of Beclin-1, which is a key component of the autophagy complex, is known to be reduced in AD. The decline in autophagy in AD may be related to an increase in the level of

InsP_3 that disrupts the autophagic complex by binding to the InsP_3R . The drug Li^+ , which is known to reduce the risk of developing AD, can reduce this inhibitory effect by lowering the level of InsP_3 as outlined in the inositol depletion hypothesis. Autophagy may also be reduced in AD by the elevated levels of Ca^{2+} that can disrupt the complex by activating hVps34. This activation of hVps34 may also account for the increase in mTOR that could explain the decline of autophagy in AD. The cognitive decline in mouse models of AD is reduced by rapamycin, which inhibits the activity of mTOR. Another role for mTOR is to phosphorylate Tau to increase its pathological role in AD. The elevation of Ca^{2+} can also stimulate CaMKK2 to increase the activity of AMP kinase (AMPK) that then enhances the phosphorylation of Tau thus contributing to the symptoms of AD.

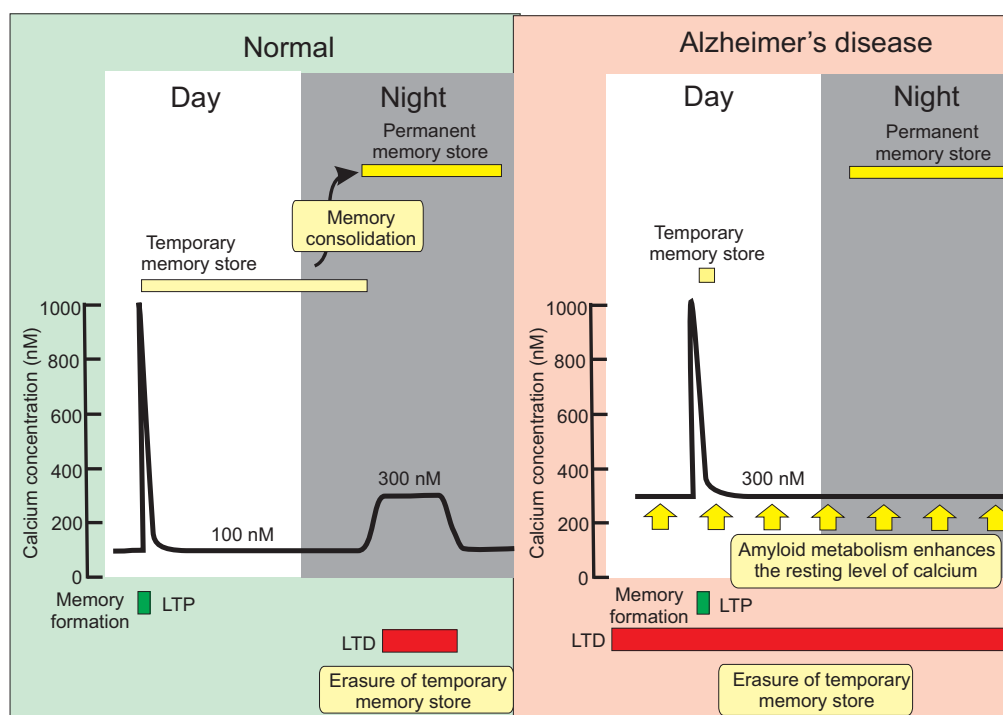
Acetylation and Alzheimer's disease

There are some marked changes in protein acetylation that may contribute to both the defects in memory and also the increase in neuronal cell death that occurs in Alzheimer's disease. With regard to memory loss, there are indications that an epigenetic inhibition of the transcription of the proteins that function in learning and memory is brought about by an increase in the activity of histone deacetylase 2 (HDAC2) (Module 12: Figure amyloids and Ca^{2+} signalling).

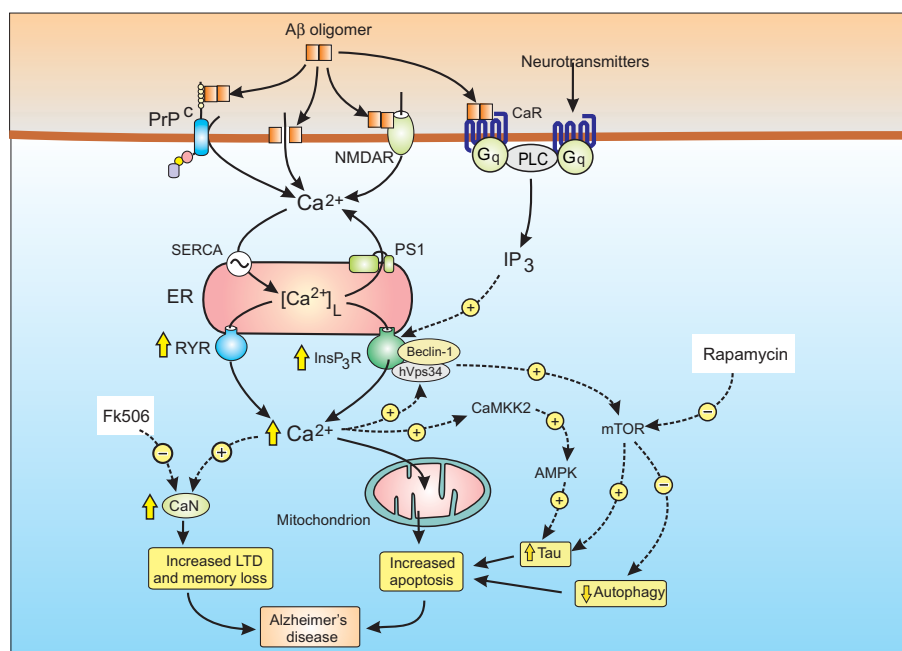
Inflammation in Alzheimer's disease

The amyloid cascade hypothesis proposes that the development of Alzheimer's disease (AD) is driven by the accumulation of amyloid β ($\text{A}\beta$) that is released during the amyloidogenic pathway (Module 12: Figure amyloid cascade hypothesis). There is increasing evidence to support the calcium hypothesis of Alzheimer's disease that suggests that the build-up of $\text{A}\beta$ then acts to alter Ca^{2+} signalling pathways to bring about the loss of memory and neuronal cell death that characterizes AD (Module 12: Figure amyloids and Ca^{2+} signalling). This effect on Ca^{2+} signalling is enhanced by local inflammatory responses driven primarily by the neighbouring microglial cells and, to a lesser extent, by the astrocytes. The amyloid β ($\text{A}\beta$), which is responsible for AD, may be considered as a pathogenic neuron-derived factor that has a number of actions as outlined in the following sequence of events (Module 12: Figure Inflammation and Alzheimer's disease):

1. The amyloid β ($\text{A}\beta$) oligomers that accumulate outside the diseased neuron can act on the neurons to bring about elevations in Ca^{2+} as described more fully in the calcium hypothesis of Alzheimer's disease (Module 12: Figure amyloids and Ca^{2+} signalling). They can be inserted into the membrane to form channels or they can activate the calcium-sensitive receptor (CaR) to increase the level of inositol 1,4,5-trisphosphate (InsP_3). The CaR is coupled to phospholipase C through the G protein G_q , which is inhibited by the regulator of G protein signalling 4 (RGS4). The level of RGS4 is reduced in the human AD brain and this may further enhance the generation of InsP_3 . In addition, $\text{A}\beta$ can act

Module 12: | Figure LTD hypothesis of Alzheimer's disease**LTD hypothesis of Alzheimer's disease.**

During the day, brief high concentration (1000 nM) spikes of Ca^{2+} are responsible for activating long-term potentiation (LTP) that form memories that are held in a temporary memory store. At night, these temporary memories are consolidated following their transfer to a permanent memory store during sleep. The memories in the temporary store are then erased by a period of intermediate elevation of Ca^{2+} (approximately 300 nM). In Alzheimer's disease, amyloid metabolism results in a permanent elevation of Ca^{2+} into this intermediate range that continuously erases memories from the temporary memory store soon after they are formed. Memories can still be formed by brief high-intensity spikes of Ca^{2+} , but the persistent amyloid-dependent elevation of Ca^{2+} erases these temporary memories before they can be transferred to the permanent memory store.

Module 12: | Figure autophagy and Alzheimer's disease**Autophagy and Alzheimer's disease.**

The elevation in intracellular Ca^{2+} reduces autophagy and increases Tau that then contributes to the onset of apoptosis.

on the neighbouring microglia and astrocytes to trigger inflammatory responses.

2. The InsP_3 acts on the InsP_3 receptors (InsP_3Rs) on the endoplasmic reticulum (ER) to release Ca^{2+} , which can result in a persistent elevation in the resting level of Ca^{2+} . In the early stages of AD, the characteristic loss of memory may be driven by this persistent elevation in the level of Ca^{2+} that activates long-term depression (LTD). As the disease progresses, the elevation of Ca^{2+} will begin to activate apoptosis (Module 12: Figure Inflammation and Alzheimer's disease).
3. This dysregulation of neuronal Ca^{2+} signalling seems to be exacerbated by $\text{A}\beta$ -induced neuroinflammation that occurs during AD. $\text{A}\beta$ -induced Ca^{2+} signals can enhance microglial inflammatory responses by increasing the release of cytokines and reactive oxygen species (ROS). The $\text{A}\beta$ also acts through Ca^{2+} -sensing receptors (CaRs) to produce InsP_3 that then releases Ca^{2+} from internal stores. Depletion of these stores then triggers store-operated Ca^{2+} entry through the Orai1 channel that is maintained by the hyperpolarization induced by the calcium-activated potassium channel KCa3.1 . Microglial-dependent neurotoxicity could be reduced *in vivo* by inhibiting these KCa3.1 channels with triaryl methane-34 (TRAM-34), thus emphasizing the significance of Ca^{2+} in regulating neuroinflammation.
4. One of the consequences of the $\text{A}\beta$ -dependent elevation of microglial Ca^{2+} is the activation of the inflammasome. The oligomers that are taken up in the phagosome vesicles enter the cytosol to increase NLRP3 activity resulting in stimulation of caspase 1 that cleaves pro-interleukin-1 β (IL-1 β) to form IL-1 β . The inflammasome inhibits phagocytic clearance of $\text{A}\beta$ and has been strongly implicated in AD (Module 12: Figure Inflammation and Alzheimer's disease). The triggering receptor expressed in myeloid cells 2 (TREM-2), which functions as a negative regulator of innate immunity, suppresses the ability of the microglia to release inflammatory mediators such as $\text{TNF}\alpha$. TREM2 is a transmembrane glycoprotein that associates with DNAX-activating protein 12 (DAP12). A variant of TREM2, which reduces the anti-inflammatory role of TREM2, is associated with a markedly increased risk of developing AD.
5. The microglia, which are sensitive to many different stimuli (Module 7: Figure microglia interactions), also respond to amyloid β ($\text{A}\beta$) that acts through the toll-like receptors (TLRs). Polymorphisms in the toll-like receptors (TLR-2 and TLR-4) receptors have been associated with an increased susceptibility and progression of AD. Activation of the TLR-2/4 receptors, which can have both beneficial and deleterious actions, is coupled to two important functions. Firstly, they activate the $\text{NF-}\kappa\text{B}$ signalling pathway that are coupled to a number of pro-inflammatory mediators such as tumour necrosis factor α ($\text{TNF}\alpha$), interleukin-1 β (IL-1 β) and reactive oxygen species (ROS), all of which can have marked deleterious effects

on neuronal functions. Secondly, they can also have a beneficial effect because they stimulate phagocytosis that removes and destroys amyloid β ($\text{A}\beta$). The monophosphoryl lipid A (MPL) has an interesting property of being able to promote this phagocytic mechanism and thus may prove to be an effective treatment for AD (Module 12: Figure Inflammation and Alzheimer's disease). Omega-3 fatty acids can also enhance phagocytosis of $\text{A}\beta$ to reduce the formation of pro-inflammatory cytokines.

6. The inflammatory response in the microglia can have a marked effect on a number of neural functions. The $\text{TNF}\alpha$ released from the microglia can bind to the tumour necrosis factor (TNF) receptor (TNF-R) to contribute to neuronal cell death by activating apoptosis (Module 11: Figure $\text{TNF}\alpha$ apoptotic signalling). The $\text{TNF}\alpha$ can also activate the JNK signalling pathway and this may have an impact on the dysregulation of Ca^{2+} signalling by promoting the expression of the InsP_3Rs . The JNK phosphorylates the transcription factor specificity protein 1 (Sp1) that acts to increase the expression of the InsP_3Rs (Module 12: Figure Inflammation and Alzheimer's disease). Both $\text{TNF}\alpha$ and IL-1 β have also been shown to reduce the electrophysiological correlates of memory formation such as long-term potentiation (LTP) and long-term depression (LTD), but how this achieved is not clear.

Another effect of the microglial inflammatory response is to increase the inhibitory activity of Methyl-CpG-binding protein 2 (MeCP2), which is a transcriptional repressor that functions in gene silencing (Module 4: Figure MeCP2 activation). The MeCP2 represses the expression of neuroligin 1, which has an important role in regulating synaptic plasticity by binding together the pre- and postsynaptic endings (Module 10: Figure postsynaptic density).

7. The exogenous ROS, which diffuses into the neuron, will combine with the ROS being generated by the mitochondria. In AD, there is a decrease in the level of mortalin that results in mitochondrial dysfunction and increased ROS formation. The increase in ROS levels will alter the redox balance by increasing the proportion of glutathione (GSH) that exists in the GSSG form. One consequence of this increase in ROS is to induce redox signalling effects on Ca^{2+} signalling that include both an increase in the activity of the sensitivity of the InsP_3Rs and the plasma membrane Ca^{2+} -ATPase (PMCA). These two effects of ROS will enhance the resting level of Ca^{2+} that is responsible for inducing AD as formulated by the calcium hypothesis of Alzheimer's disease (Module 12: Figure amyloids and Ca^{2+} signalling).

The elevation of ROS, which increases the resting level of Ca^{2+} , will potentially set up a positive-feedback system in that the excess Ca^{2+} will increase mitochondrial ROS formation. Inhibition of ROS formation by a mitochondrial-targeted antioxidant MitoQ prevents the cognitive decline in a transgenic mouse model of AD (Module 12: Figure reversal of Ca^{2+} signalling in neurodegeneration).

8. The astrocytes also play a role in the onset of AD. The mechanism of astrocyte-induced neuronal death has been postulated to depend on amyloid β ($A\beta$) activating an increase in ROS formation (Module 12: Figure astrocyte-induced neuronal death). In the original proposal, this formation of ROS was thought to occur through an increase in Ca^{2+} entry, but it is just as likely to have occurred through the activation of an inflammatory response similar to that taking place in the microglia. Whatever the mechanism turns out to be, an increase in ROS formation in the astrocytes will decrease the level of the antioxidant glutathione (GSH) and this will have serious repercussions for the neuron since it receives its GSH from the astrocytes. A decrease in neuronal GSH levels will enable ROS to have a greater impact on Ca^{2+} levels as outlined above.
9. There is considerable evidence for a relationship between vitamin D and Ca^{2+} regulation in neurodegeneration. Vitamin D3 has at least two actions. Firstly, it can act to dampen down the inflammatory responses and may do so by reducing the formation of $TNF\alpha$. Secondly, vitamin D3 can act through the vitamin D3 receptor (VDR) to promote the expression of proteins that act to lower the level of intracellular Ca^{2+} (Module 12: Figure inflammation and Alzheimer's disease). Expression of the vitamin D receptor (VDR) is reduced in the hippocampus of AD patients and VDR polymorphisms have been identified as risk factors for AD. There is evidence that $A\beta$ acts to both reduce the expression of VDR while increasing the expression of the $Cav2.1$ Ca^{2+} channel.

Administration of vitamin D3 can reverse many of the changes induced by $A\beta$. In cultured primary neurons, vitamin D3 acts to increase the expression of the VDR and it reduces the expression of the $Cav1.2$ L-type Ca^{2+} channel. In the intestine, vitamin D3 is known to increase the expression of proteins such as the PMCA, NCX1 and Ca^{2+} buffers such as calbindin (CB) and parvalbumin. It seems reasonable to propose that vitamin D may reduce the risk of AD by promoting the expression of those proteins, such as PMCA and NCX1 that act to lower the level of intracellular Ca^{2+} . Vitamin D can also have a beneficial effect by dampening down the microglial inflammatory responses to reduce the formation of $TNF\alpha$.

In summary, the buildup of $A\beta$ oligomers during the onset of AD has a profound effect on the activity of the local community of cells in the brain. The inflammatory response in both the microglia and astrocytes contribute to the dysregulation of neural Ca^{2+} signalling that seems to be one of the major factors in the development of AD. It is argued that in the early stages of AD, this alteration in signalling is manifest as a persistent elevation of the resting level of Ca^{2+} that results in memories acquired during the wake period being rapidly erased before they can be consolidated during sleep. Vitamin D3 may play a critical role in memory retention by regulating the expression of the Ca^{2+} components necessary to maintain low resting levels of Ca^{2+} .

Astrocyte-induced neuronal death

There have been many reports linking a change in Ca^{2+} homeostasis and the pathogenesis of AD. However, the nature of the signalling events has never been clearly demonstrated. The calcium hypothesis of Alzheimer's disease attempts to relate how a change in amyloid processing leads to neuronal cell death. A new hypothesis has emerged recently that incorporates the astrocytes as key players in the link between the accumulation of β -amyloid peptides and neuronal cell death (Module 12: Figure astrocyte-induced neuronal death). It is argued that the increase in the level of β -amyloid peptides acts on the astrocytes to induce an influx of external Ca^{2+} that results in an increase in the production of reactive oxygen species (ROS) by the mitochondria. An increase in the latter causes a decrease in the level of glutathione (GSH), which is the major redox buffer in cells that protects them against apoptosis. The level of GSH will also fall in the neurons, because they receive their GSH from the astrocytes. Since there is an important role for redox signalling in apoptosis, this decline in GSH will make neurons very susceptible to apoptosis.

A role for oxidative stress in AD is supported by the fact that there appears to be a decrease in the activity of nuclear factor erythroid 2-related factor 2 (NRF-2), which is a key transcription factor that functions to maintain antioxidant defences both in the neurons and astrocytes. In the case of astrocytes, the decline in GSH described above could be explained by a decrease in the ability of NRF-2 to maintain the expression of the enzymes that produce GSH (Module 4: Figure NRF-2 antioxidant function).

α -Secretase

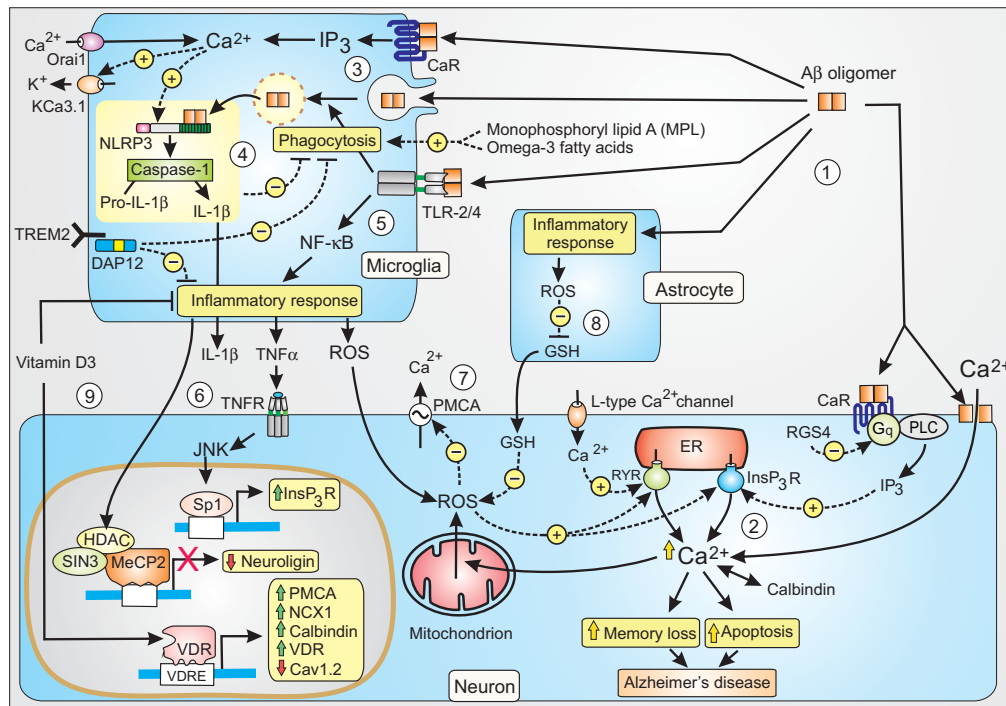
α -Secretase activity depends on ADAM proteases such as ADAM-10 and ADAM-17 (Module 1: Table ADAM proteases) that act on transmembrane proteins such as the β -amyloid precursor protein (APP) resulting in the shedding of the soluble $APP\alpha$ (sAPP α), whereas the C-terminal fragment α (CTF α) is retained in the membrane (see step 3 in Module 12: Figure amyloid cascade hypothesis).

β -Secretase

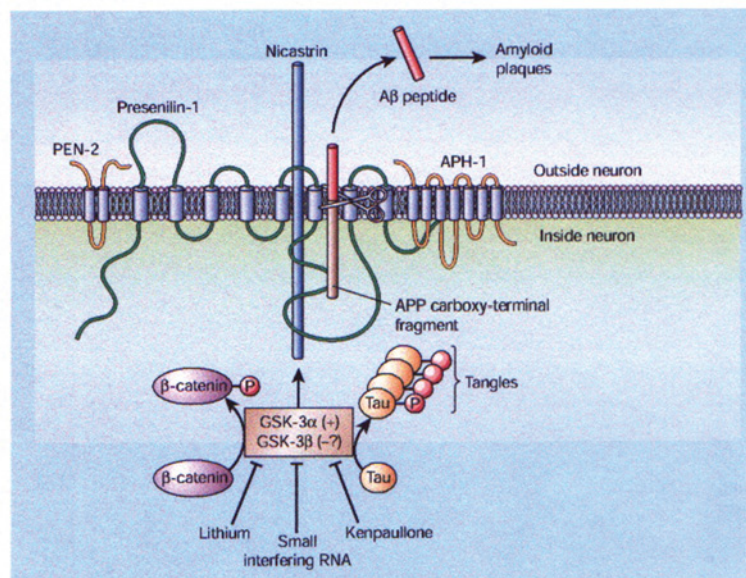
This enzyme, which is also known as β -site APP-cleaving enzyme (BACE), initiates the processing of the β -amyloid precursor protein (APP) that results in the formation of the amyloid proteins that are implicated in Alzheimer's disease (AD) (Module 12: Figure amyloid cascade hypothesis).

γ -Secretase complex

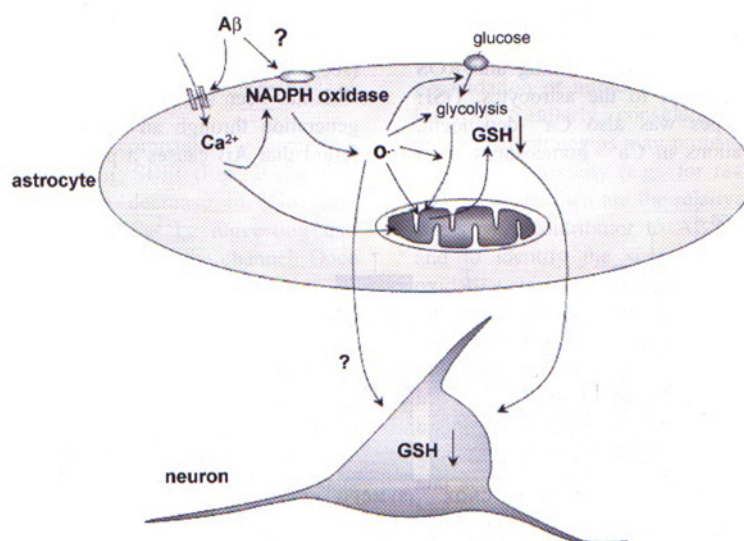
The γ -secretase complex has a number of functions. It acts on Notch to release the Notch intracellular domain (NICD) during the operation of the Notch signalling pathway (Module 2: Figure Notch signalling). Its other major function is to cleave the β -amyloid precursor protein (APP) to release components such as the β -amyloid peptides and the APP intracellular domain (AICD) (see step 5 in Module 12: Figure amyloid cascade hypothesis). The enzyme complex is composed of a number of proteins that are embedded in the membrane (Module 12: Figure APP processing). The enzymic component consists of presenilin 1 or 2 (PS1 or PS2) and this is associated

Module 12: | Figure Inflammation and Alzheimer's disease**Inflammation and Alzheimer's disease.**

Inflammation seems to play an important role in the development of Alzheimer's disease, which is triggered by the accumulation of amyloid β ($\text{A}\beta$), which aggregates into oligomers that have a number of actions. The $\text{A}\beta$ can act directly on the neurons to bring about the elevations in Ca^{2+} that have been linked to the initial phase of memory loss and the subsequent increase in apoptosis that characterizes the development of AD. The $\text{A}\beta$ can also induce inflammatory responses in the neighbouring microglia and astrocytes that activate processes that influence this dysregulation of Ca^{2+} signalling. Vitamin D3 can alleviate AD development by inhibiting the inflammatory responses and by increasing the expression of processes that reduce the elevation of Ca^{2+} within neurons. See the text for further details of all these processes.

Module 12: | Figure amyloid plaques and tangles**Amyloid processing and the formation of plaques.**

The amyloid precursor protein (APP) is cleaved to form a C-terminal fragment that is embedded in the neuronal plasma membrane. This fragment is cleaved by a complex, which contains presenilin-1, Apha-1, PEN-2 and nicastrin, to release β -amyloid peptide ($\text{A}\beta$) that then goes on to form external plaques. Internal tangles are formed by the polymerization of hyperphosphorylated tau proteins. Glycogen synthase kinase-3 (GSK-3) seems to play a critical role in the formation of plaques and tangles. Reproduced by permission from Macmillan Publishers Ltd: *Nature*, De Strooper, B. and Woodgett, J. (2003) Mental plaque removal. 423:392-393. Copyright (2003); <http://www.nature.com>; see De Strooper and Woodgett 2003.

Module 12: | Figure astrocyte-induced neuronal death**Astrocyte-induced neuronal cell death hypothesis.**

The hypothesis is that β -amyloid peptides ($A\beta$) acting on astrocytes bring about an increase in Ca^{2+} entry and may also activate NADPH oxidase to generate superoxide ($O_2^{\bullet-}$) that then severely depletes the astrocyte level of GSH, with a corresponding decrease in neuronal GSH because neurons receive their GSH from astrocytes. This decline in neuronal GSH will greatly increase their sensitivity to oxidation-induced cell death. Reproduced from *Biochim. Biophys. Acta*, Vol. 1742, Abramov, A.Y., Canevari, L. and Duchen, M.R., Calcium signals induced by amyloid β peptide and their consequences in neurons and astrocytes in culture, pp. 81-87. Copyright (2004), with permission from Elsevier; see Abramov et al. 2004.

with APH1 (APH1a or APH1b), PEN2 and nicastrin. The names for APH1 (Anterior pharynx defective 1) and PEN2 (Presenilin enhancer-2 gene) were derived from studies in *C. elegans*.

Presenilins

There are two presenilins (PS1 and PS2), which are widely expressed especially in the brain, and are integral membrane proteins located in the endoplasmic reticulum, plasma membrane and the endosomes where they appear to have two separate functions. One function is to act as proteases within the γ -secretase complex (Module 12: Figure APP processing). The other is to function as an ion channel to control the leak of Ca^{2+} across the endoplasmic reticulum (Module 12: Figure amyloids and Ca^{2+} signalling). These two functions seem to depend on the complex processing of the presenilins. Following its synthesis, the holoprotein is embedded within the lumen of the endoplasmic reticulum. The holoenzyme, which consists of eight transmembrane (TM) regions, functions as a leak channel that allows Ca^{2+} to flow from the lumen back into the cytoplasm (see step 3 in Module 12: Figure amyloids and Ca^{2+} signalling).

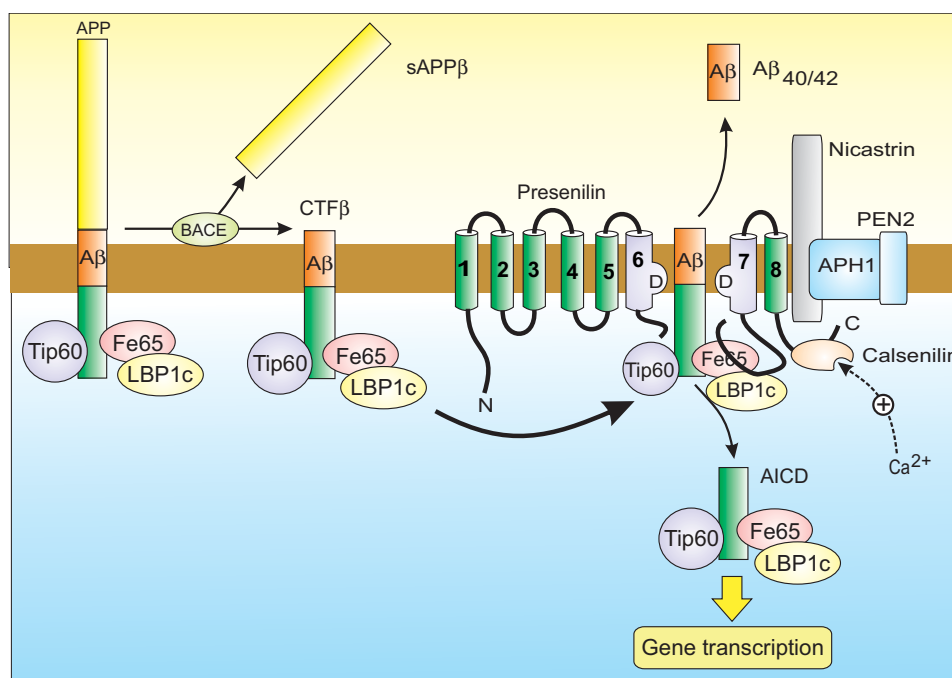
The other function of the presenilins is to contribute protease activity to the γ -secretase complex. Before functioning as a protease, the holoenzyme undergoes endoproteolysis that cleaves the cytosolic loop between TM6 and TM7. The two fragments remain associated with each other and then associates with nicastrin, APH1 and PEN2 to form the γ -secretase complex (Module 12: Figure APP processing). This enzyme complex then leaves the ER and passes to the endosomal compartments and the plasma membrane where it processes integral membrane proteins

such as Notch during the operation of the Notch signalling pathway (Module 2: Figure Notch signalling) or the β -amyloid precursor protein (APP) (Module 12: Figure amyloid cascade hypothesis).

The neuronal Ca^{2+} sensor (NCS) protein KChIP3/DREAM/calsenilin has been implicated in the control of these different functions of presenilin. In its calsenilin guise, it associates with the C-terminal tail of presenilin (Module 12: Figure APP processing). In general, the calsenilin seems to enhance the deleterious effects of presenilin by increasing the amount of Ca^{2+} being released from the ER or by increasing the activity of the γ -secretase complex that releases the β -amyloid peptides. A similar role may also be performed by calcium and integrin-binding protein 1 (CIB1), which interacts with presenilin 2.

Mutations in the two isoforms of PS (PS1 and PS2), which cause early-onset familial Alzheimer's disease (FAD), alter the processing of APP to produce more of the neurotoxic amyloid β 42 ($A\beta_{42}$) protein that causes neurodegeneration. APP is normally processed to the amyloid β 40 ($A\beta_{40}$) form whereas the PS1 and PS2 mutation increases the proportion of $A\beta_{42}$. It also decreases the production of the neuroprotective secreted form of APP (sAPP α). There are indications that sAPP α can stabilise neuronal Ca^{2+} homeostasis that may depend on controlling the expression of genes involved in Ca^{2+} signalling, such as calbindin-D28k. Cells carrying mutations in PS1 were very sensitive to mitochondrial toxins, leading to increased Ca^{2+} levels and the formation of reactive oxygen species (ROS). If the level of Ca^{2+} was suppressed, the generation of ROS and apoptosis decreased. This is of some interest, considering the positive-feedback loop that

Module 12: | Figure APP processing

**Processing of amyloid precursor protein (APP) by the γ-secretase complex.**

APP is cleaved first by BACE to release sAPPβ leaving behind the CTFβ, which is the precursor that is cleaved by the γ-secretase complex. The enzymic component of this complex is presenilin, which has two fragments: the N-terminal fragment (NTF) that has six transmembrane (TM) domains and the C-terminal fragment (CTF) that has two TMs. TM 6 and 7 have intramembrane aspartate (D) residues that are part of the catalytic mechanism that cuts CTFβ in the middle of its intramembrane region to release β-amyloid (Aβ) to one side and APP intracellular domain (AICD) to the other side. The AICD together with various associated factors functions to activate gene transcription (for further details see Module 12: Figure amyloid cascade hypothesis).

exists between ROS and Ca²⁺ signalling (Module 2: Figure ROS effects on Ca²⁺ signalling).

β-Amyloid precursor protein (APP)

The β-amyloid precursor protein (APP) is the integral membrane protein that is the precursor that is cleaved either by α-secretase or β-secretase to release sAPPα and sAPPβ respectively (Module 12: Figure amyloid cascade hypothesis). Following the cleavage of APP by β-secretase, the CTF β fragment that remains in the membrane is cleaved by the γ-secretase complex to release the β-amyloids (Module 12: Figure APP processing). Formation of amyloid β 42 (Aβ₄₂) is thought to be responsible for inducing the neurodegenerative changes that cause Alzheimer's disease (AD)

Asthma

Asthma develops as a result of changes in the airways caused by inflammation, bronchial hyperresponsiveness and obstruction of the airways by excessive contraction of the airway smooth muscle cells (SMCs) and also to bronchial wall thickening due to hypertrophy of these SMCs. Contraction of these SMCs can be activated by a number of stimuli such as acetylcholine (ACh) and 5-hydroxytryptamine (5-HT) (Module 7: Figure bronchiole-arteriole contraction). The onset of asthma has also been linked to the formation of thymic stromal lymphopoietin (TSLP).

Such alterations in contractility may arise through changes in the operation of the smooth muscle cell cytosolic oscillator responsible for controlling the contractile state of the airway muscle cells (Module 7: Figure SMC cytosolic oscillator). An important regulator of airway smooth muscle cell contractility is the bronchodilator hydrogen sulfide (H₂S). A decline in the levels of H₂S may thus contribute to the onset of asthma. Other contributory factors might be a change in the activities of PDE4D or the Rho signalling mechanism that acts to regulate the Ca²⁺ sensitivity of the myosin light chain kinase (MLCK). Inhalation of the β₂-adrenergic agonist albuterol is used in asthma to relax the airway smooth muscle cells. The albuterol appears to relax the muscle by reducing the frequency of the Ca²⁺ oscillations that drive contraction and it also reduces the Ca²⁺ sensitivity of the contractile apparatus.

Airway smooth muscle proliferation resulting in increased mass can cause severe asthma and obstructive pulmonary disease. Diverse stimuli (e.g. growth factors, contractile agonists, cytokines and extracellular matrix proteins) can stimulate smooth muscle cell proliferation. An increase in the expression of the transient receptor potential 1 (TRPC1) channel may contribute to bronchial constriction and SMC proliferation.

The expression of the Ca²⁺-sensitive Cl⁻ channel 1 (CLCA1) is up-regulated in patients with asthma. It is located in the bronchial epithelial cells (EPC in

Module 7: Figure lung morphology) and particularly in the goblet cells, which produce mucus, where it may contribute to the onset of asthma through its mucus-inducing activity.

Glucocorticoids such as prednisone, dexamethasone and hydrocortisone that act through the glucocorticoid receptor GR) are used to treat asthma.

Atopic dermatitis (AD)

Atopic dermatitis (AD) is an inflammatory disorder characterized by chronic itching of the skin. Many patients suffering from AD will go on to develop asthma and this progression has been referred to as the “atopic march”. One of the factors that seems to be responsible for AD is the thymic stromal lymphopoietin (TSLP), which is a cytokine released from the keratinocytes in the skin. The TSLP then acts on sensory neurons in the skin to activate Itch (Module 10: Figure Itch signal transduction mechanism).

Attention deficit hyperactivity disorder (ADHD)

Attention deficit hyperactivity disorder (ADHD), which is sometimes referred to as hyperkinetic disorder, is characterized by lack of attention often associated with hyperactivity. ADHD begins early in childhood and can continue to the beginning of the teenage years. The cause of ADHD is unknown, but could result from a decrease in the activity of the transmitters released from the ascending arousal system that stimulate the tonic excitatory drive that controls brain rhythms (Module 10: Figure tonic excitatory drive and neuronal rhythms).

The drugs used to treat ADHD, such as methylphenidate (MPH) (Ritalin) and dexamfetamine, are often referred to as stimulants. The MPH acts to improve transmitters such as dopamine (DA) and norepinephrine (NE) by blocking the transporters responsible for the reuptake of these transmitters. For example, the dopamine transporters (DATs) are Na⁺-coupled transport proteins located on the presynaptic terminal that act to rapidly reduce the extracellular dopamine. A vesicular monoamine transporter 2 (VMAT2; SLC18A2) is then responsible for transporting dopamine from the cytoplasm back into vesicles. Such psychostimulants are particularly active in enhancing DA and NE signalling in the prefrontal cortex (PFC).

Chronic obstructive pulmonary disease (COPD)

Chronic obstructive pulmonary disease (COPD) is caused by a narrowing of tubes in the lung that reduces the flow of air resulting in a shortness of breath. It is caused by a severe inflammatory response of the lung to noxious particles or gases and the most common risk factor is cigarette smoking. There is a decrease in the level of nuclear factor erythroid 2-related factor 2 (NRF-2) in the alveolar macrophages of patients with COPD. NRF-2 is a stress-sensing transcription factor that responds to reactive oxygen species (ROS) by enhancing the expression of antioxidant defences (Module 4: Figure NRF-2 antioxidant function). The reduction in NRF-2 would thus account for the decrease in glutathione (GSH) and the increase

in lipid peroxidation in the lung of patients with COPD. These observations indicate that a reduction in antioxidant defences and the resulting increase in oxidative stress is a prominent feature of COPD.

A decline in the level of the bronchodilator hydrogen sulfide (H₂S) may also contribute to the pathogenesis of COPD.

Cirrhosis of the liver

Liver cirrhosis is induced by a range of damaging agents such as alcohol abuse, viral hepatitis and various toxins. One of the earliest stages of cirrhosis is the fibrogenesis induced by activated hepatic stellate cells (Module 7: Figure hepatic stellate cells). The activity of the hepatic stellate cells is curtailed by the processes of senescence, which may help to reduce the onset of cirrhosis.

Cushing's syndrome

Cushing's syndrome is an endocrine disorder that is caused by the excessive production of cortisol that is synthesized and released from the zona fasciculata cells of the adrenal cortex (Module 7: Figure adrenal gland). The syndrome is characterized by multiple symptoms such as rapid weight gain with an unusual distribution in the trunk (central obesity), face ('moon face') and along the collar bone and back causing a 'buffalo hump'. Other symptoms include increased sweating, dilations of the capillaries, atrophy of the skin, which bruises easily, and females can develop a male facial pattern of hair growth (hirsutism). There may also be psychological disorders such as depression and anxiety.

Diabetes

Diabetes is characterized by an excessive excretion of urine. The most common form is diabetes mellitus, which is caused by a disturbance in insulin function that results in an inability to metabolize glucose. There is a large increase in the plasma level of glucose that, if unchecked, can lead to serious complications such as renal failure, blindness and limb amputation. One of the spectacular successes of modern medicine has been the control of diabetes by administering purified insulin.

Diabetes mellitus is a complex disorder that can have a number of causes. Type 1 diabetes, which is also known as insulin-dependent diabetes mellitus, is caused by an autoimmune destruction of the insulin-secreting β -cells responsible for synthesizing and releasing insulin (Module 7: Figure β -cell signalling). This type of diabetes often appears early in life and has been referred to as juvenile onset diabetes.

Type 2 diabetes, also known as non-insulin-dependent diabetes mellitus, usually develops after the age of 40 and is caused by a combination of both insulin resistance and insulin deficiency. The incidence of Type 2 diabetes is strongly correlated with obesity. The connection between obesity and the onset of diabetes is proving difficult to unravel, because it appears to involve subtle changes in different components of the metabolic energy network (Module 7: Figure metabolic energy network). However,

there is a clear link between insulin resistance and a decline in the activity of the insulin-secreting β -cells (Module 12: Figure insulin resistance).

There are a number of inherited disorders that can lead to hyperglycaemia and some of these have been identified as components of the mechanisms for either insulin secretion or insulin action.

Mutations related to insulin release and biosynthesis:

- Mutation of β -cells hexokinase IV (glucokinase), which detects external glucose (Module 7: Figure β -cell signalling), reduces insulin release
- Mutations in the Kir6.2 K^+ channel or the ABCC8 sulphonylurea receptor, which constitute the ATP-sensitive K^+ (K_{ATP}) channels, reduce the ability of ATP to depolarize the membrane sufficiently to trigger the release of insulin. Mutations in ABCC8 have been identified in many cases of neonatal diabetes.
- Mutations of the transcription factor hepatocyte nuclear factor 4 α (HNF4 α) contribute to diabetes by reducing the synthesis of insulin (Module 7: Figure β -cell signalling).

Mutations related to the action of insulin:

- In skeletal muscle, the insulin control of skeletal muscle glycogen metabolism is reduced by a nonsense mutation in the Rab GAP called Tbc1d4, which reduces the translocation of GLUT4 to the plasma membrane of skeletal muscle (Module 7: Figure skeletal muscle E-C coupling), causing insulin resistance.

A mutation in calpain 10 has been linked to Type 2 diabetes. A polymorphism in the promoter region of the gene encoding uncoupling protein 2 (UCP2), which results in an increased expression of this coupling protein, results in defective insulin secretion and has been linked to the onset of Type 2 diabetes.

Diabetes insipidus (DI)

Diabetes insipidus (DI) is caused by a phenotypic remodelling resulting in a change in the production or release of the vasopressin responsible for vasopressin-induced antidiuresis (Module 7: Figure collecting duct function). Nephrogenic diabetes insipidus (NDI) is caused by a failure of the kidney tubule to respond to vasopressin through mutations in either the vasopressin receptor or the aquaporins.

Diabetic nephropathy

Diabetic nephropathy is one of the kidney diseases that can progress to end-stage renal disease (ESRD). As its name implies, diabetic nephropathy is caused by diabetes and is exacerbated by the hypertension that often accompanies diabetes. It is estimated that approximately 30% of Type 2 diabetic patients go on to develop ESRD. The nephropathy that develops during diabetes develops slowly over a number of years through various stages:

- 1–5 years; the first indications of kidney disease appear, characterized by an increase in glomerular filtration rate

that is associated with an increase in mesangial cell proliferation.

- 5–15 years; the appearance of microalbuminuria is one of the first signs of kidney disease and result from a severe disruption in the function of the glomerulus.
- After 20 years, the onset of macroalbuminuria indicates that ESRD has occurred and this requires either dialysis or kidney transplantation.

This gradual deterioration in glomerular filtration function seems to depend on alterations in many of the functions normally carried out by the mesangial cells (Module 7: Figure mesangial cell). This is an example of mesangioproliferative glomerulonephritis. The alteration in mesangial cell function may result from a disruption in the signalling cross-talk that occurs between the Smad signalling pathway and the PtdIns 3-kinase signalling pathway orchestrated by miR-192. The early signs of disease are caused by an abnormal increase in mesangial cell proliferation, which is associated with an increase in glomerular filtration rate. This is then followed by glomerulosclerosis, which is caused by a change in the way the mesangial cells function to control the extracellular matrix (ECM) that forms the renal filter. Formation and degradation of the ECM, which is controlled by transforming growth factor β 1 (TGF- β 1) operating through the Smad signalling pathway, is normally well balanced, but during diabetic nephropathy, there is a marked sclerosis resulting from an increase in ECM formation. This increase in the deposition of the ECM seems to result from a decrease in the expression of various matrix metalloproteinases (MMPs) such as MMP-2 and MMP-9 (Module 1: Table MMPs and their inhibitors). In addition, the degradation of the ECM is reduced further by an increase in the expression of the tissue inhibitors of metalloproteinases (TIMPs), which act to inhibit the activity of MMPs (Module 1: Figure MMP activation and function). It is this build-up of the ECM that causes the sclerosis of the renal filter that is a feature of the gradual deterioration of the glomerulus that culminates in ESRD.

Diarrhoea

Diarrhoea is caused by an increase in the water content of the faeces, which develops when the normal balance between fluid absorption and secretion is tipped in favour of secretion. This reversal of the normal water fluxes can occur either in the small intestine or in the colon or in both. If the small intestine increases its rate of secretion and reduces its rate of absorption, the amount of fluid delivered to the colon increases from 2.5 to approximately 6.5 litres. Since the latter has a maximal absorptive capacity of 4.0 litres, there will be a large increase in the water content of the faeces, resulting in diarrhoea. The severity of this water loss is enhanced if the colon is also switched from net absorption to net secretion.

A number of factors can activate this hypersecretion of fluid. Small intestine fluid secretion is activated by stimuli such as acetylcholine (ACh) and vasoactive intestinal polypeptide (VIP) released from the enteric nervous system (Module 7: Figure small intestine). Any increase in the

release of these secretagogues will strongly activate fluid secretion (Module 7: Figure intestinal secretion). Such a mechanism accounts for the diarrhoea caused by bacterial infections. For example, the massive water loss during cholera is caused by activation of the cyclic AMP signalling pathway. Similarly, certain strains of *Escherichia coli* secrete the STa toxin that increases intestinal secretion by activating the cyclic GMP signalling pathway by stimulating the particulate guanylyl cyclase C (pGC-C) receptor that is normally activated by guanylin.

Drug addiction

Serious societal problems result from self-administration of addictive drugs such as cocaine, morphine and Δ -tetrahydrocannabinol, which seem to act by modulating the activity of certain neurotransmitter signalling pathways. The mesolimbic dopaminergic pathway appears to be particularly important. Dopamine acting together with glutamate seems to play an important role in inducing a change in the neural circuitry so that individuals learn to anticipate a reward. The gene transcription that brings about the neural plasticity responsible for long-term addiction seems to depend on the protein phosphatase 1 (PP1) inhibitor dopamine and cyclic AMP-regulated phosphoprotein of apparent molecular mass 32 kDa (DARPP-32), which acts as a node to integrate the dopamine and glutamate signalling pathways (Module 10: Figure medium spiny neuron signalling).

Ejaculatory dysfunction

There are two major types of ejaculatory dysfunction. First, there is a complete failure of ejaculation that can have multiple causes. Secondly, there is retrograde ejaculation that results from relaxation of the internal urethral sphincter (Module 7: Figure urinogenital tract) allowing the ejaculate to pass into the urine. Abnormal ejaculation often arises as a side effect of treatments using α_1 -adrenoreceptor antagonists. This has led to the suggestion that changes in the function of the vas deferens smooth muscle cells, which depend upon α_1 -adrenoreceptor signalling pathways (Module 7: Figure vas deferens activation), may explain some forms of ejaculatory disorder.

Endotoxic shock

During inflammatory responses, release of endotoxins from the surface of bacteria can result in general vasodilation of blood vessels throughout the circulatory system. The decrease in blood pressure means that blood is diverted away from essential organs such as the brain and heart, and this hypoperfusion can be fatal. One of the causes of this decrease in blood pressure is an increase in the production of nitric oxide (NO) resulting from an up-regulation of inducible nitric oxide synthase (iNOS) in endothelial and smooth muscle cells by lipopolysaccharide (LPS) and various cytokines.

End-stage renal disease (ESRD)

The major cause of end-stage renal disease (ESRD) is a failure in the glomerular filter resulting in proteinuria. The causes for such kidney failure are complex and are often

a secondary consequence of other systemic diseases, such as diabetes, hypertension, lupus and infections. Diabetic nephropathy is an example of one of these diseases that can progress to ESRD.

Epilepsy

Patients suffering from epilepsy appear to have alterations in a number of components that function in the regulation of neuronal excitability. For example intractable epilepsy has been linked to a reduction in the level of the K^+ - Cl^- cotransporter 2 (KCC2) that function to regulate the intracellular level of Cl^- . There also are reduced levels of one of the K^+ -channel-interacting proteins (KChIPs), which act to enhance the current flowing through K^+ channels. Defects in the $K_v7.3$ K^+ channel, which is a component of the PtdIns4,5P₂ regulation of ion channels and exchangers, has been linked to benign neonatal convulsions. The phosphoinositide signalling pathway may also play a role because epileptic seizures are a feature of mice where the phospholipase C β (PLC β) has been deleted. Human temporal lobe epilepsy can be induced by kainate, and mice lacking the GluR6 subunit of kainate receptors are less susceptible to such kainate-induced seizures.

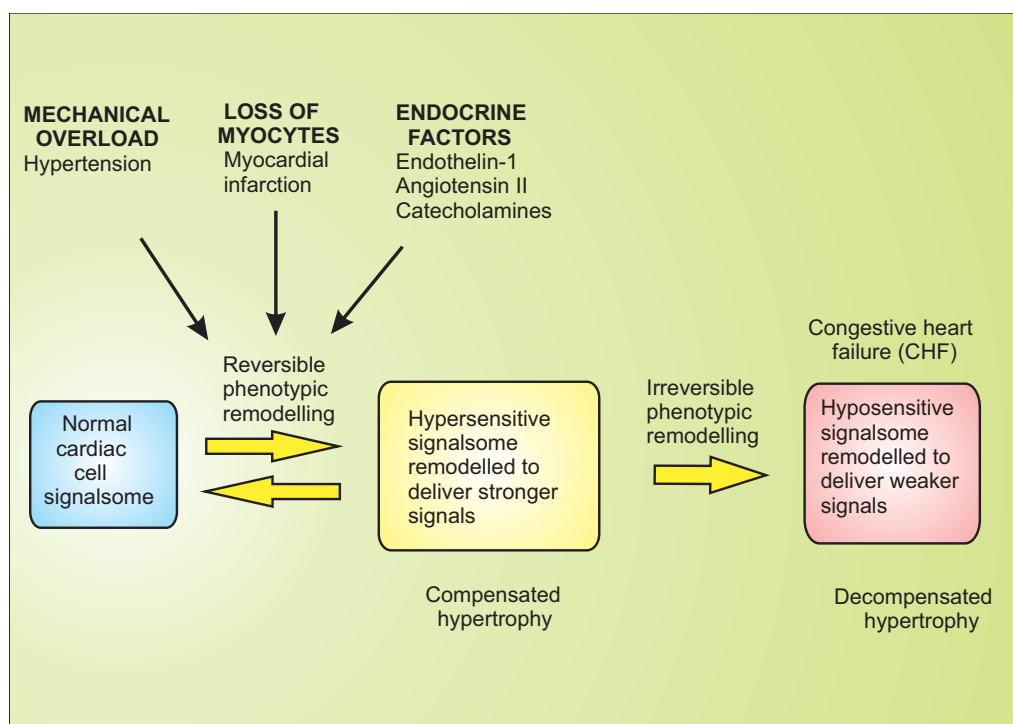
Erectile dysfunction

The National Institute of Health Consensus Statement in 1993 defined male erectile dysfunction as the “inability to achieve or maintain an erection adequate for sexual satisfaction”. Emotional, medical and physical factors can contribute to this dysfunction. Erectile dysfunction is often associated with other medical conditions such as hypertension, diabetes, hypercholesterolaemia and obesity. Penile erection is a neurovascular process orchestrated by a whole range of stimuli (Module 7: Figure corpus cavernosum). In many cases, the dysfunction seems to result from a defect in the operation of the nitric oxide (NO)/cyclic GMP signalling pathway responsible for controlling the smooth muscle relaxation necessary for an effective erection. PDE5 inhibitors such as Viagra, which act by inhibiting the hydrolysis of cyclic GMP, have proved very effective in treating erectile dysfunction.

Another potential target is the smooth muscle Rho/Rho kinase signalling pathway that serves to increase the Ca^{2+} sensitivity of the contractile machinery. Enhanced erectile responses have been obtained using inhibitors of Rho kinase.

Heart disease

Heart disease encompasses a number of pathological states characterized by a decrease in the ability of the heart to perform its role of pumping blood around the body. This dysfunction, often referred to as congestive heart failure (CHF), is a major cause of human morbidity and mortality. CHF has a complex pathology that can be induced by multiple factors (Module 12: Figure remodelling the cardiac signalsome), many of which appear to act by increasing the workload of the heart. For example, the increase in blood pressure that occurs during hypertension causes an increased mechanical load on the heart. The

Module 12: | Figure remodelling the cardiac signalsome

Congestive heart failure (CHF) results from a two-step remodelling of the cardiac signalsome.

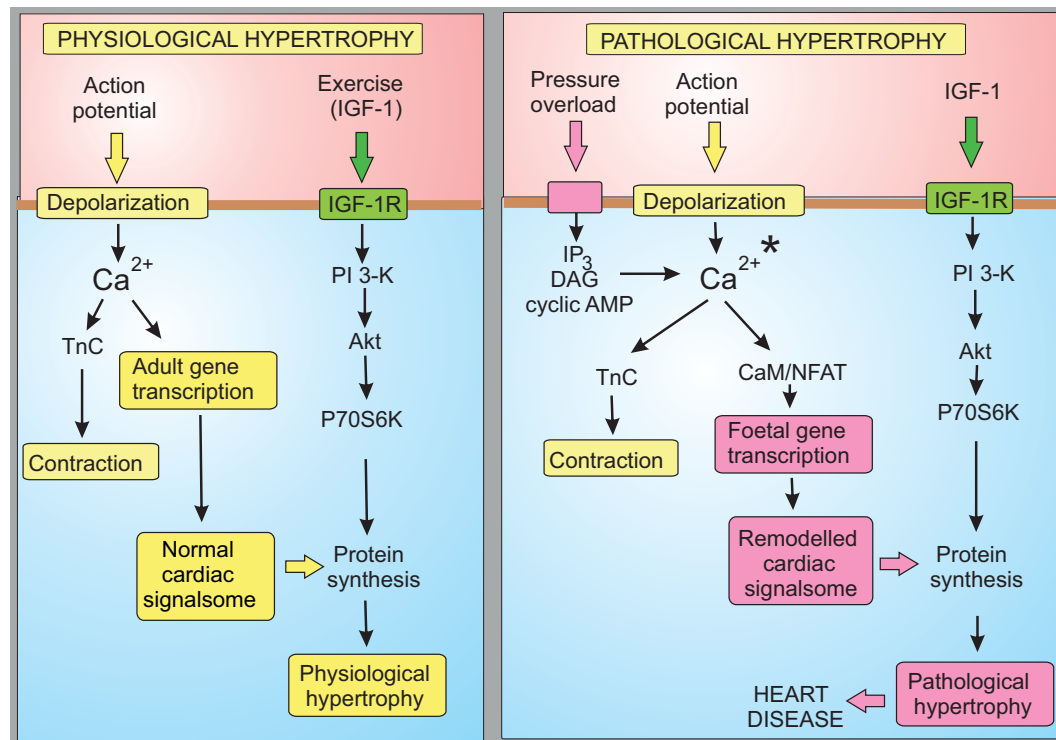
Hypertrophic conditions, such as mechanical overload, loss of myocytes and endocrine factors, bring about a remodelling of the cardiac signalsome that begins with compensated hypertrophy, which is then followed by an irreversible step to produce the decompensated hypertrophy characteristic of congestive heart failure (CHF).

onset of heart disease is also one of the complications that arise from obesity. CHF can develop after ischaemic injury, where a myocardial infarction has removed many of the functioning myocytes so that the remaining cells have to work harder to maintain the pump. The onset of CHF can also be induced by arrhythmias and by endocrine disorders, which result in an increased circulation of hormones such as endothelin-1, angiotensin II and catecholamines.

Before considering the pathological consequences further, it is necessary to draw a distinction between physiological and pathological hypertrophy (Module 12: Figure physiological and pathological hypertrophy). It is important to stress that an enlargement of the heart can be a normal response to an increase in workload, as occurs in athletes that take regular exercise. This physiological hypertrophy is driven by an increase in the release of insulin-like growth factor I (IGF-I), which acts through the PtdIns 3-kinase signalling pathway to increase protein synthesis using the adult template of messenger RNAs that define the adult cardiac signalsome. The Ca^{2+} transients in the normal functioning heart not only activate contraction, they may also be responsible for signalsome stability by maintaining the transcription of the adult genes through a processes of Ca^{2+} -induced transcription of Ca^{2+} signalling components (Module 4: Figure signalsome transcription). However, under pathological conditions when the heart is subjected to persist-

ent pressure overload, the Ca^{2+} signal may change so that it begins to activate foetal genes (Module 12: Figure physiological and pathological hypertrophy). In the following sections, I shall develop the hypothesis that these foetal genes then bring about a phenotypic remodelling of the cardiac signalsome that then causes heart disease.

The first adaptation that occurs to these pathophysiological stresses is an increase in the size of the heart (Module 12: Figure remodelling the cardiac signalsome). This cardiac hypertrophy is a compensatory mechanism resulting from a reversible phenotypic remodelling process, because the heart will return to its original size if the abnormal inputs are reduced. However, if the stresses persist, this compensated hypertrophy shifts to the more irreversible state of congestive heart failure (CHF). Both cardiac hypertrophy and CHF are examples of phenotypic remodelling of the signalsome, resulting in cardiac cells with altered physiological properties. During hypertrophy, the remodelled signalsome gives rise to a cell that is capable of generating stronger contractions, whereas during CHF, the signalsome delivers weaker signals, due primarily to a decrease in the activity of the Ca^{2+} signalling system (Module 12: Figure CSQ-induced hypertrophy). In particular, there appears to be a major defect in the cycling of Ca^{2+} across the sarcoplasmic reticulum (SR) due to a decrease in the activity of the sarco/endo-plasmic reticulum Ca^{2+} -ATPase (SERCA) pump.

Module 12: | Figure physiological and pathological hypertrophy**Signalling pathways in physiological and pathophysiological cardiac hypertrophy.**

In physiological hypertrophy, action potentials generate a Ca^{2+} signal that controls contraction and may also stabilize the normal cardiac signalsome by maintaining the adult genes. The insulin-like growth factor 1 (IGF-1) that is produced during exercise uses this adult template when it activates protein synthesis, acting through the PtdIns 3-kinase (PI 3-K) signalling pathway. During pathological hypertrophy, pressure overload activates signalling pathways that alter the nature of the Ca^{2+} signal (*), which activates a foetal set of genes that results in a remodelled cardiac signalsome that results in heart disease.

It is important to make the distinction between these two phases, i.e. the early stage of compensatory hypertrophy and the more terminal phase of CHF. Some important clues as to the mechanism of cardiac hypertrophy and CHF have come from the analysis of monogenic disorders that can lead to familial cardiomyopathies, which are a typical example of genotypic remodelling of the signalsome. The two main examples are familial hypertrophic cardiomyopathy and familial dilated cardiomyopathy. Many of these familial cardiomyopathies have been traced to mutations in different components of the contractile and cytoskeletal elements of the cardiac cell. This genotypic remodelling of the cardiac signalsome generates phenotypes that are remarkably similar to those induced by pressure overload, suggesting that all of these mechanisms feed into common signalling pathways. The challenge is to find out how all of this information is integrated and, in particular, to understand the nature of the transition from compensated to decompensated hypertrophy (Module 12: Figure remodelling the cardiac signalsome).

A heart disease working hypothesis attempts to provide a conceptual framework to understand the nature of cardiac hypertrophy and how it switches into CHF. Since there is strong evidence that an alteration in Ca^{2+} signalling has a central role to play in the induction and modulation of hypertrophy, Ca^{2+} features significantly in this heart disease hypothesis.

Heart disease working hypothesis

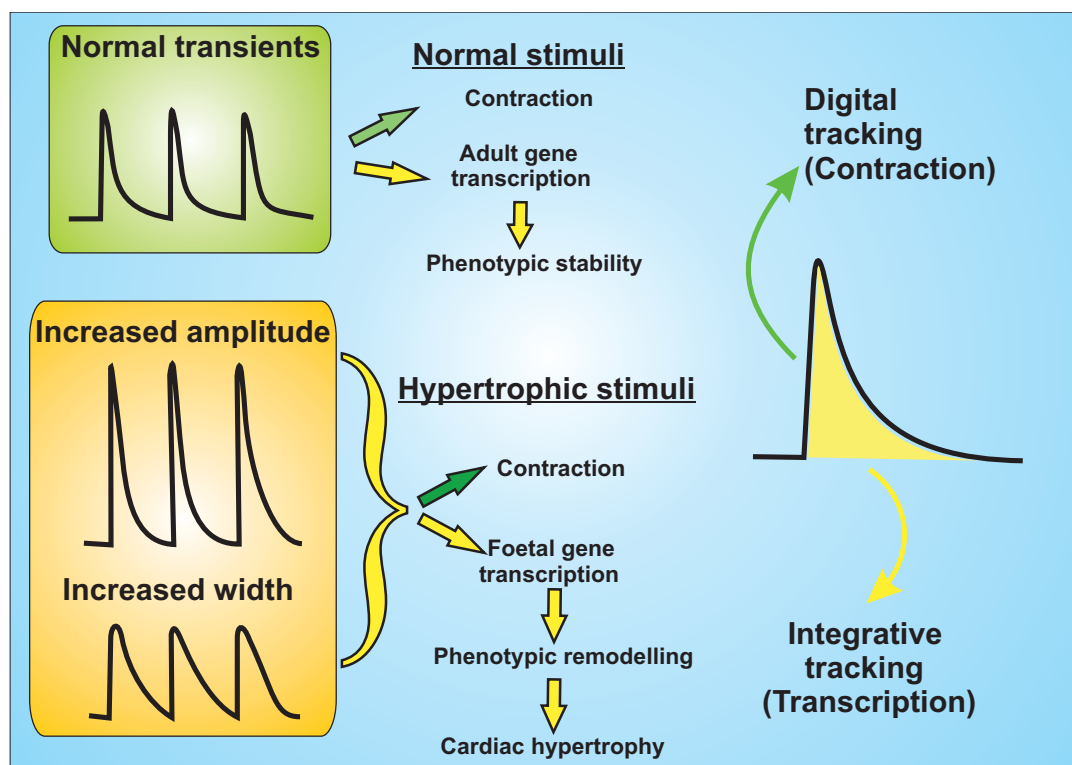
"The study of complex heart diseases has always been a vexing task, akin to solving a 1000-part jigsaw puzzle with few obvious connecting pieces."

Chien (1999)

In addition to highlighting the complexity of the problem of trying to unravel the causes of hypertrophy, Chien has also drawn attention to the lack of "connecting pieces". The following working hypothesis attempts to provide the beginning of such a connection by suggesting a possible link between the two remodelling phases of cardiac hypertrophy and the subsequent progression to congestive heart failure (CHF) (Module 12: Figure remodelling the cardiac signalsome):

Phase 1: reversible phenotypic remodelling of cardiac hypertrophy

The initial adaptive response is driven mainly by extrinsic factors, most of which are linked to the pressure overload imposed on the heart. These factors act through a variety of signalling mechanisms to switch transcription from adult genes to foetal genes. The central tenet is that modification of the shape of the Ca^{2+} transient, an increase in either its amplitude or its width, is one of the primary signals for the phenotypic remodelling (Module 12: Figure hypertrophy working hypothesis). Increasing both the size of the heart and the amplitude of the Ca^{2+} signals enhances

Module 12: | Figure hypertrophy working hypothesis

A hypothesis concerning the role of Ca^{2+} transients in cardiac hypertrophy.

The normal transients drive both contraction and the transcription of adult genes to maintain phenotypic stability. Under conditions that induce hypertrophy, the modified Ca^{2+} transients (increase in amplitude or width) are such that they can induce both contraction and the activation of foetal genes that bring about the phenotypic remodelling that leads to cardiac hypertrophy.

the strength of each heartbeat. It is the up-regulation of the Ca^{2+} signalling pathway that may provide the connection to Phase 2.

Phase 2: irreversible phenotypic remodelling of congestive heart failure (CHF)

While Phase 1 is driven primarily by extrinsic factors, the transition to congestive heart failure (CHF) may depend upon the intrinsic control mechanisms responsible for maintaining the phenotypic stability of the cardiac signalsome. This more speculative aspect of the working hypothesis proposes that the increase in Ca^{2+} signalling that occurs during Phase 1 triggers a progressive down-regulation of the cardiac signalsome so that it fails to deliver the strong Ca^{2+} pulses necessary to maintain the cardiac pump cycle.

An important aspect of this working hypothesis is that it draws attention to the fact that there are two types of phenotypic remodelling. Firstly, there are the normal feedback processes associated with enhancing the effectiveness of the heart cell as it tries to adapt to an increased workload. During this first phase, there is an increase in Ca^{2+} signalling, with much of it being driven by the endocrine factors that act through either cyclic AMP or the phosphoinositide signalling pathways that are responsible for the first phase of phenotypic remodelling that leads to cardiac hypertrophy (Module 12: Figure hypertrophy working hypothesis). However, the fact that the signalsome is

being driven hard to deliver larger contractions may be responsible for driving a second process of signalsome remodelling. In particular, the enhanced Ca^{2+} signalling activity may be responsible for the down-regulation of its own signalling pathway. There is lots of evidence to show that Ca^{2+} can exert a strong feedback control of the transcription of Ca^{2+} signalling components (Module 4: Figure NFAT control of Ca^{2+} signalling toolkit). This may well be happening in the heart, because the application of diltiazem to reduce the level of the normal Ca^{2+} transients ameliorates some of the changes in Ca^{2+} signalling components.

Cardiac hypertrophy

The first phase of hypertrophy is not a disease state. Indeed, this compensatory process is beneficial in that it helps the heart to adapt to an increased workload. However, if this pressure overload persists, the compensatory mechanism switches into a more pathological state, leading to heart failure and sudden heart death (Module 12: Figure remodelling the cardiac signalsome). The heart disease working hypothesis suggests that the onset of disease occurs through two closely related steps. Here, we consider Phase 1, which concentrates on the signalling pathways of compensatory hypertrophy, where the challenge is to understand the processes responsible for integrating the various hypertrophic stimuli (mechanical load, loss of

myocytes and endocrine factors) into the signalling pathways that induce the transcriptional events responsible for hypertrophy.

Signalling pathways of compensatory hypertrophy

A major problem with trying to understand cardiac hypertrophy is the fact that the heart is not quiescent, but contracts regularly, driven by periodic Ca^{2+} signals that flood through the cytoplasm and nucleus every time the heart contracts. The extrinsic factors that drive this early hypertrophic response (e.g. mechanical load, loss of myocytes and endocrine factors) are thought to act through Ca^{2+} , and thus have to do so against this background of repetitive Ca^{2+} pulses. How do cardiac cells that normally are receiving a pulse of Ca^{2+} every few seconds to activate contraction avoid triggering a hypertrophic response? A possible answer may lie in the properties of the individual spikes. The hypothesis is that there is a subtle difference in the frequency, amplitude or width of the Ca^{2+} transients, and this may be sufficient to begin to induce the transcription of the foetal genes responsible for hypertrophy (Module 12: Figure hypertrophy working hypothesis).

In addition to Ca^{2+} signalling, a number of other pathways have been implicated in inducing this first phase of compensatory hypertrophy (Module 12: Figure hypertrophy signalling mechanisms). A role for mitogen-activated protein kinase (MAPK) signalling in cardiac hypertrophy and PtdIns 3-kinase signalling in cardiac hypertrophy are also important control mechanisms operating in tandem with the Ca^{2+} signalling mechanism. What is remarkable about these hypertrophic signalling pathways is just how similar they are to the growth factor-mediated signalling pathways that drive cell proliferation (Module 9: Figure growth factor signalling). This analogy becomes all the more interesting following observations that components of the cell cycle regulatory proteins seem to play a role in hypertrophy. The activity of the cyclin D/cyclin-dependent kinase 4 (CDK4) complex is increased during hypertrophy, and the CDK inhibitor $\text{p16}^{\text{INK4A}}$ can inhibit endothelin-induced cardiac hypertrophy. However, instead of controlling cell proliferation, these cell cycle signalling pathways contribute to the transcriptional remodelling responsible for cardiac compensatory hypertrophy.

Although these other signalling systems play a role in hypertrophy, there is a general consensus that Ca^{2+} signalling and cardiac hypertrophy is a central feature of the complex mechanisms that drive cardiac hypertrophy.

Ca^{2+} signalling and cardiac hypertrophy

Perturbations of Ca^{2+} signalling are a central feature of the development of cardiac hypertrophy. The cardiac Ca^{2+} signal depends upon a large number of interconnected processes (Module 7: Figure ventricular Ca^{2+} signalling). Experimental alterations in the expression levels of different components of the Ca^{2+} signalling system result in the development of hypertrophy that often progresses to congestive heart failure (CHF). For example, altering the expression of Ca^{2+} signalling components such

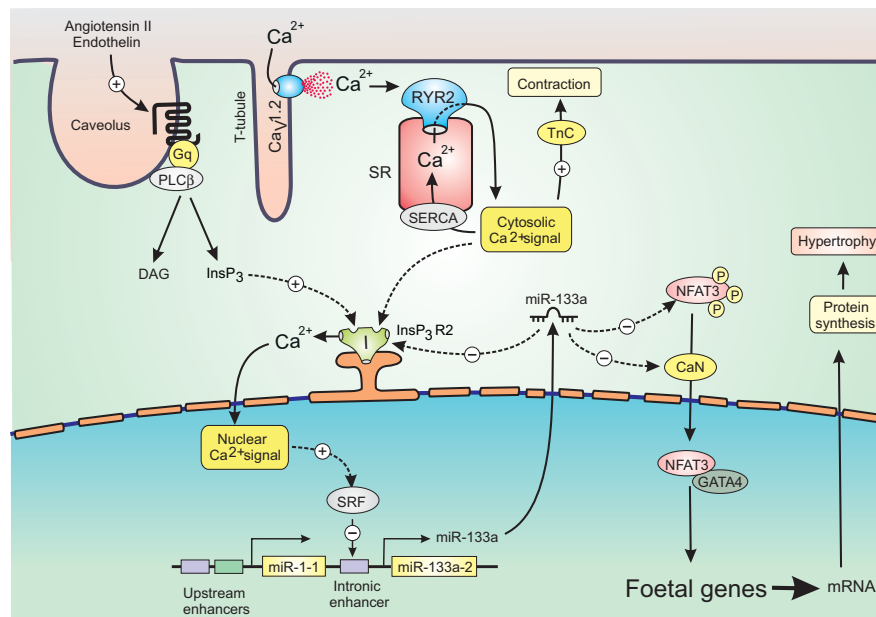
as angiotensin type 1 receptor, $\text{G}\alpha_q$, triadin 1, junctin, calsequestrin (CSQ), phospholamban (PLN), type 2 InsP_3 receptors (InsP_3Rs), calcineurin (CaN) and nuclear factor of activated T cells 3 (NFAT3) all result in hypertrophy. Expression of some of these components is regulated co-ordinately by miR-133a (Module 12: Figure miRNA and cardiac hypertrophy). The Ca^{2+} released by the InsP_3Rs enters the nucleus where it interacts with the serum response factor (SRF) to inhibit transcription of miR-133a to set up a positive-feedback loop that will strongly enhance the development of hypertrophy.

The significance of the phosphoinositide signalling pathway is evident from the observation that the expression of an active form of $\text{G}\alpha_q$ in heart can lead to hypertrophy. Conversely, when $\text{G}\alpha_q$ is absent in transgenic mice, there is no hypertrophy in response to a pressure overload. Just how this signalling system operates is unclear. An obvious possibility is for the inositol 1,4,5-trisphosphate (InsP_3) to contribute to a local Ca^{2+} signal within the nucleus as part of the cardiac histone deacetylase (HDAC) shuttle. However, another suggestion is that $\text{Ins}1,4\text{P}_2$ might play a role because its level is increased during hypertrophy and an increase in the expression of the inositol polyphosphate 1-phosphatase that hydrolyses this inositol phosphate to InsP_4 (Step 2 in Module 2: Figure inositol phosphate metabolism) exerts an anti-hypertrophic response.

Another critical signalling component that influences hypertrophy is the sarco/endo-plasmic reticulum Ca^{2+} -ATPase 2a (SERCA2a) pump on the sarcoplasmic reticulum (SR) that is responsible for the uptake and storage of Ca^{2+} (Step 6 in Module 7: Figure ventricular Ca^{2+} signalling). The critical importance of a change in SERCA2a pump activity, for both the onset of compensatory hypertrophy and the subsequent onset of congestive heart failure (CHF), has been confirmed by experiments on transgenic mice that manipulate the role of the adrenergic pathway, PLN and CSQ in controlling the Ca^{2+} pumping activity and the level of Ca^{2+} within the SR:

- Chronic stimulation with isoprenaline (isoproterenol) is one of the most effective ways of inducing cardiac hypertrophy. For example, isoprenaline treatment was used to produce hypertrophy when studying the effect of a mutation in glycogen synthase kinase-3 β (GSK-3 β) that will be described below (Module 12: Figure GSK-3 β and cardiac hypertrophy). Similarly, over-expression of the β_2 adrenergic receptor can cause hypertrophy, and this leads to a severe impairment of cardiac performance. The β_2 adrenergic receptor increases the level of cyclic AMP, which then phosphorylates phospholamban (PLN) and prevents it from inhibiting the SERCA pump (Step 7 in Module 7: Figure ventricular Ca^{2+} signalling). The latter can then increase the load of Ca^{2+} within the SR, which has the effect of increasing the amplitude of the Ca^{2+} transients, and this may be responsible for driving Phase 1 of the heart disease working hypothesis. This hypertrophy, which is driven by excessive signalling through the cyclic AMP signalling pathway, was prevented when PLN was

Module 12: | Figure miRNA and cardiac hypertrophy



Regulation of hypertrophy by miRNA.

Expression of the type 2 inositol 1,4,5-trisphosphate receptor (InsP₃R2), calcineurin (CaN) and the nuclear factor of activated T cells 3 (NFAT3) is regulated by miR-133a. Under normal conditions, the miR-133a restrains the expression of these components. During hypertrophic stimuli that activate the InsP₃R2s, there is an increase in nuclear Ca²⁺ that acts through serum response factor (SRF) to reduce the expression of miR-133a and this reduces its inhibitory effect on these components and results in activation of the transcriptional events that give rise to hypertrophy. For further details concerning this activation of hypertrophy see Module 12: Figure hypertrophy signalling mechanisms.

removed. The increased Ca²⁺ signalling during Phase 1 may then result in the adaptive down-regulation of the SERCA pump that occurs in Phase 2 to cause CHF. In fact, CHF occurs in transgenic mice that express a mutant form of PLN, which acts as a superinhibitor of SERCA2a.

- The heart failure phenotype that develops in mice lacking the cytoskeletal muscle-specific LIM protein (MLP) (Module 12: Figure cardiac contractile elements) can be corrected by ablating phospholamban. The MLP-deficient mice have characteristically weak Ca²⁺ responses that return to normal when the inhibitory effect of phospholamban is removed (Module 12: Figure MLP^{-/-} Ca²⁺ responses).
- Overexpression of calsequestrin (CSQ), which buffers Ca²⁺ within the lumen of the SR (see Step 8 in Module 7: Figure ventricular Ca²⁺ signalling), results in CHF, with an aggressive phenotype that develops rapidly. The increase in CSQ will have the effect of reducing the amount of releasable Ca²⁺, and this may be responsible for the weak signals characteristic of the failing heart (Module 12: Figure CSQ-induced hypertrophy). An interesting consequence of this overexpression of calsequestrin and the decline in Ca²⁺ signalling is an up-regulation of the Ca²⁺ release proteins [ryanodine receptor 2 (RYR2), junctin and triadin], and this is explored more fully in the section on signalsome stability. The severe hypertrophy caused by the overexpression of CSQ can be rescued by various manoeuvres designed to enhance the Ca²⁺ capacity of the endoplasmic reticulum (ER). For example, ablation of PLN, which has

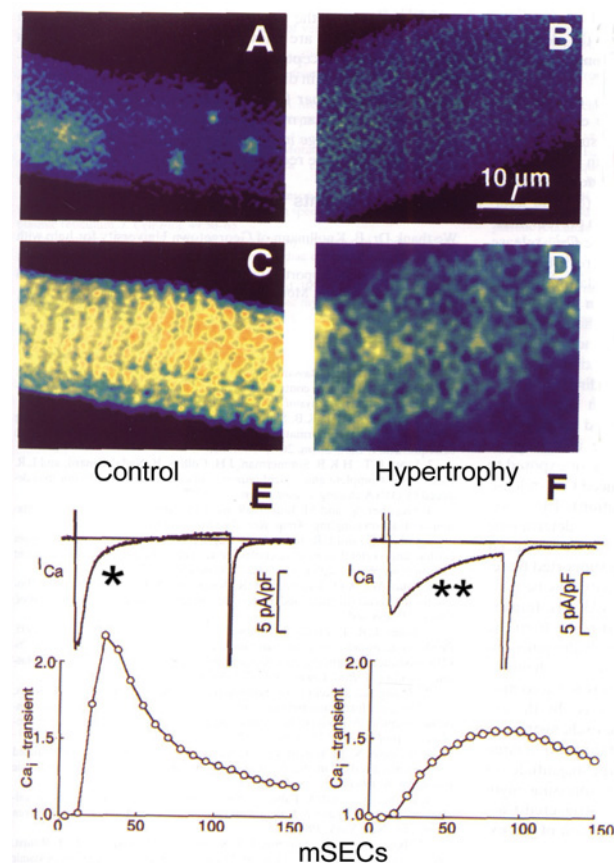
the effect of enhancing the SERCA pump, restored the Ca²⁺ signalling capacity of the SR and was able to reverse some of the transcriptional events associated with hypertrophy. The cardiomyopathy that develops in the mice that overexpress CSQ can also be delayed by inhibiting the β -adrenergic receptor kinase 1 (β ARK1) responsible for down-regulating the cyclic AMP signalling pathway that regulates PLN (Step 7 in Module 7: Figure ventricular Ca²⁺ signalling). This manoeuvre will increase SERCA2a pump activity, which will be able to pump more Ca²⁺ into the SR, thereby negating the increased buffering of CSQ.

All of these modifications of the cardiac Ca²⁺ signalsome are dependent upon alterations in the expression levels of Ca²⁺ signalling components that result from changes in cardiac gene transcription.

Cardiac gene transcription

One of the characteristics of cardiac hypertrophy is the process of de-differentiation, in that hypertrophic stimuli activate a programme of foetal cardiac gene transcription (Module 12: Figure hypertrophy working hypothesis). There is considerable evidence to indicate that Ca²⁺ signalling plays a major role in switching on the transcription of the set of foetal genes to bring about the phenotypic remodelling that occurs during hypertrophy. However, the heart receives continuous pulses of Ca²⁺ to drive contraction under normal conditions without initiating a change in transcription. So what is it about the hypertrophic Ca²⁺ signals that initiate the remodelling of cardiac gene transcription? The heart disease working hypothesis proposes

Module 12: | Figure CSQ-induced hypertrophy

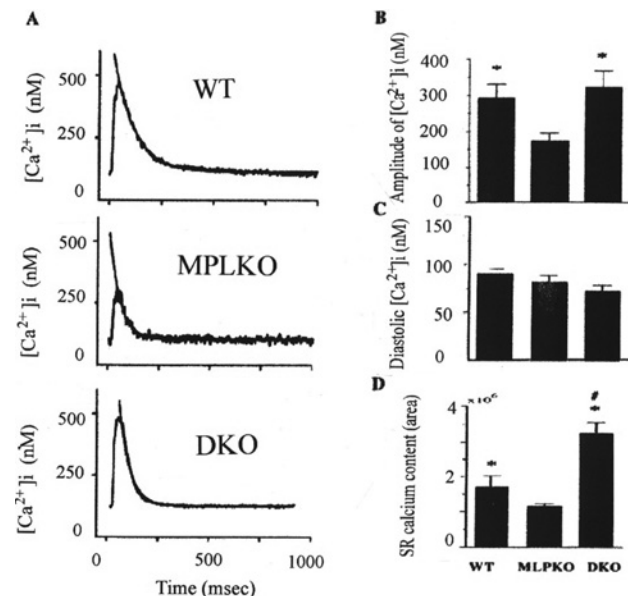


Comparison of Ca^{2+} release patterns in control (A, C and E) and hypertrophic (B, D and F) cardiac myocytes overexpressing calsequestrin (CSQ).

The distribution of Ca^{2+} at rest (-70 mV; A and B) and after stimulation (depolarization to 0 mV; C and D) clearly shows that the control cells display Ca^{2+} sparks at rest (A) and a large global signal upon stimulation (C). By contrast, the hypertrophic myocyte displayed no sparks (B), and the signal following activation was much smaller and had a blotchy appearance (D). Note that the Ca^{2+} current (I_{Ca}) that flows into the cell is larger and inactivates quicker (E, *) than that in the hypertrophic cell (F, **). The lower traces indicate that the Ca^{2+} transient in control cells is larger and sharper than that recorded in hypertrophic cells. Reproduced, with permission, from Jones, L.R., Suzuki, Y.S., Wang, W., Kobayashi, V.R., Ramesh, V., Franzini-Armstrong, C., Cleeman, L. and Morad, M. (1998) Regulation of Ca^{2+} signaling in transgenic mouse cardiac myocytes overexpressing calsequestrin. *J. Clin. Invest.* 101:1385-1393; see Jones et al. 1998.

that the normal periodic global Ca^{2+} signals that flood the cytoplasm, including the nucleus, control both contraction and the transcription of the adult genes responsible for phenotypic stability (Module 12: Figure hypertrophy working hypothesis). However, subtle changes in the characteristics of the individual Ca^{2+} transients (e.g. increases in amplitude or width) induced by hypertrophic stimuli may then be sufficient to activate the foetal transcriptional events that induce the phenotypic remodelling that leads to hypertrophy.

There is some evidence to support the notion that the properties of the Ca^{2+} transients in cardiac cells undergoing cardiac hypertrophy are altered. For example, hypertrophy develops in transgenic mice containing a truncated $\text{K}_{\text{V}}4.2$ channel that results in a prolongation of the cardiac

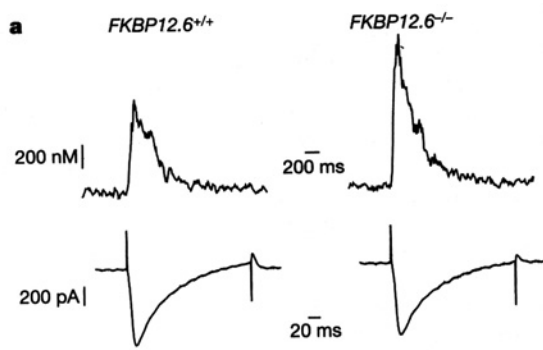
Module 12: | Figure $\text{MLP}^{-/-}$ Ca^{2+} responses

Reduced Ca^{2+} responses in muscle-specific LIM protein (MLP) $^{-/-}$ mice.

A. Ca^{2+} responses in muscle-specific LIM protein (MLP) $^{-/-}$ mice (MLPKO) had a much reduced Ca^{2+} transient, which was restored to normal if phospholamban was also removed. In the double knockout (DKO), the rate of recovery was increased. B. The statistics reveal that there was no change in the diastolic Ca^{2+} level, but there was a marked reduction in the Ca^{2+} content of the sarcoplasmic reticulum (SR). When phospholamban was removed, the Ca^{2+} content of the store was increased above that of the wild-type (WT), and this increased availability of Ca^{2+} was able to compensate for the effect of removing MLP. Reproduced from *Cell*, Vol. 99, Minamisawa, S., Hoshijima, M., Chu, G., Ward, C.A., Frank, K., Gu, Y., Martone, M.E., Wang, Y., Ross, J., Kranias, E.G., Giles, W.R. and Chien, K.R., Chronic phospholamban-sarcoplasmic reticulum calcium ATPase interaction is the critical calcium cycling defect in dilated cardiomyopathy, pp. 313-322. Copyright (1999), with permission from Elsevier; see Minamisawa et al. 1999.

action potential with an associated increase in the influx of Ca^{2+} . An increase in the amplitude of Ca^{2+} transients has been recorded in hypertrophic cardiac cells following deletion of FK506-binding protein 12.6 (FKBP12.6) (Module 12: Figure FKBP12.6 deletion induces hypertrophy). By contrast, the width of transients was increased in cells taken from the hypertrophic heart of transgenic mice that overexpress triadin 1 (Module 12: Figure Ca^{2+} in triadin 1-overexpressing mice). An interesting aspect of these triadin 1-overexpressing mice was the compensatory changes in the other proteins of the signalling complex. There was a down-regulation of junctin and ryanodine receptor 2 (RYR2), but an up-regulation of the L-type Ca^{2+} channel, which accounts for a marked change in the property of the action potential, resulting in an increase in the amount of Ca^{2+} entering the cell during each transient. Not only was there an increase in the current density, but also the action potential was prolonged. Again, there appears to be a correlation between a change in the Ca^{2+} transient and the onset of hypertrophy.

The heart disease working hypothesis is also consistent with recent studies on nitric oxide (NO)/cyclic GMP and cardiac hypertrophy. The nitric oxide/cyclic GMP

Module 12: | Figure FKBP12.6 deletion induces hypertrophy

Deletion of FK506-binding protein 12.6 (FKBP12.6) results in an increase in the amplitude of the Ca^{2+} transient.

In transgenic mice where the FK506-binding protein 12.6 (FKBP12.6) protein has been deleted, the onset of hypertrophy is associated with a marked increase in the amplitude of the Ca^{2+} transient. This increase in amplitude results from an increase in the amount of Ca^{2+} released from the sarcoplasmic reticulum (SR). As shown in the lower current traces, there was no change in the influx of Ca^{2+} across the plasma membrane, indicating that the increase in amplitude is due to the ryanodine receptor 2 (RyR2) channels opening for longer to increase the amount of released Ca^{2+} . Of particular interest was the observation that the hypertrophy occurred only in the males. The females appear to be protected by oestrogen, because they did develop hypertrophy when treated with tamoxifen, an oestrogen receptor antagonist. Reproduced by permission from Macmillan Publishers Ltd: *Nature*, Xin, H.-B., Senbonmatsu, T., Cheng, D.-S., Wang, Y.-X., Copello, J.A., Ji, G.-J., Collier, M.L., Deng, K.-Y., Jeyakumar, L.H., Magnuson, M.A., Inagami, T., Kotlikoff, M.I. and Fleischer, S. (2002) Oestrogen protects FKBP12.6 null mice from cardiac hypertrophy. 416:334-338. Copyright (2002); <http://www.nature.com>; see Xin et al. 2002.

signalling pathway acts to reduce the level of Ca^{2+} signalling in cardiac cells, and this may explain how it can protect against hypertrophy.

All of the evidence described above is consistent with the idea that Ca^{2+} transients may carry information both to regulate contraction and for remodelling cardiac gene transcription to induce hypertrophy (Module 12: Figure hypertrophy working hypothesis). This is an example of how information can be encoded in Ca^{2+} transients through amplitude or width modulation (Module 6: Figure encoding oscillatory information).

The next question to consider is how this information might be decoded into a change in gene transcription. The answer may lie in the processes of integrative tracking, whereby each transient causes a small change in some dynamic process that then switches to a new equilibrium position (Module 6: Figure decoding oscillatory information). In the case of the cardiac cell, digital tracking may regulate contraction, whereas integrative tracking may enable the same transient to bring about a change in gene transcription (Module 12: Figure decoding cardiac Ca^{2+} spikes). The integrative process may operate through the cardiac nuclear factor of activated T cells (NFAT) shuttle and the cardiac histone deacetylase (HDAC) shuttle.

Cardiac nuclear factor of activated T cells (NFAT) shuttle

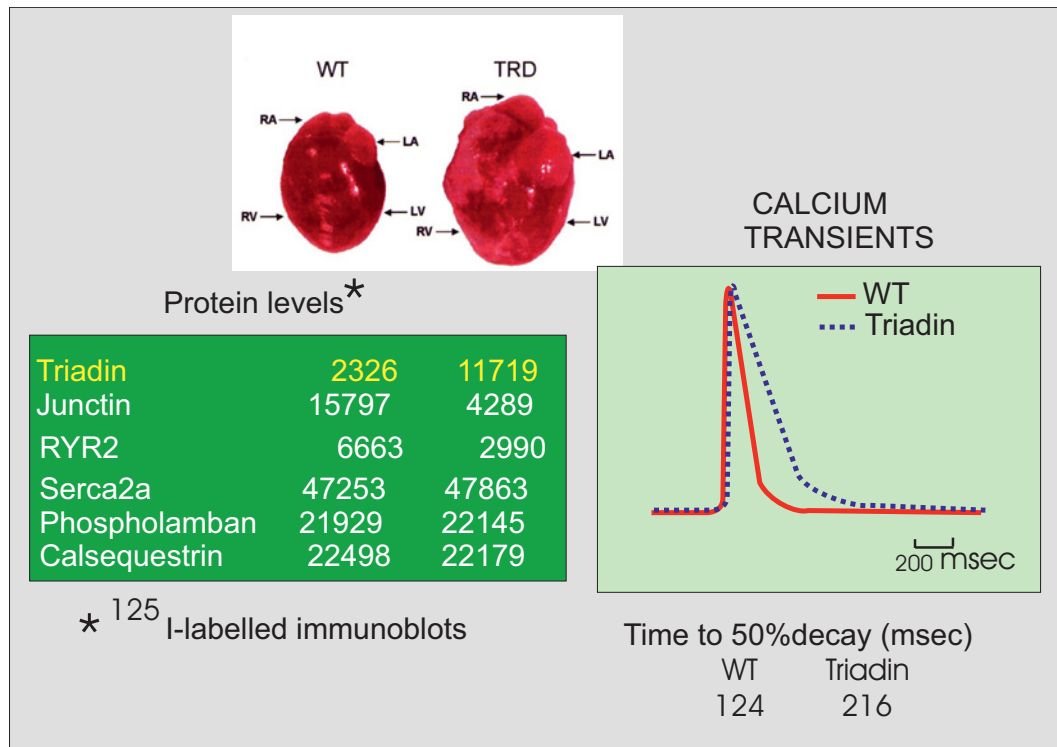
One of the targets of Ca^{2+} signalling during hypertrophy is calcineurin (CaN), which acts to dephosphorylate the nuclear factor of activated T cells (NFAT) (Module 4: Figure NFAT activation). There is considerable genetic evidence for CaN having a role in hypertrophy. Mice expressing constitutively active CaN result in cardiac enlargement and sudden death. Alternatively, hypertrophy is reduced by introducing various calcineurin inhibitors, such as cyclosporin A (CsA) and Cain/Cabin. During hypertrophy, there is an increased expression of calcium and integrin binding protein 1 (CIB1) and this may locate CaN in specific locations where it can respond to the Ca^{2+} signals that trigger activation of NFAT. Hypertrophy develops in mice overexpressing activated CaN, whereas ablation of the *CaNA β* gene prevents the onset of hypertrophy induced by pressure overload, angiotensin II or isoprenaline (isoproterenol) infusion. The activation of CaN initiates the hypertrophic process by dephosphorylating NFAT3, which is one of the five available NFAT genes that interacts with GATA4 (a cardiac-specific zinc-finger transcription factor) to induce gene transcription (Module 12: Figure hypertrophy signalling mechanisms). The active CaN dephosphorylates phosphorylated NFAT3 to NFAT3, which enters the nucleus to begin transcription. There are various NFAT kinases within the nucleus that will phosphorylate NFAT, thereby inactivating it by driving it back into the cytosol, thus setting up the dynamic process that constitutes the NFAT shuttle. One of these is glycogen synthase kinase-3 (GSK-3), which carries out one of the functions of PtdIns 3-kinase signalling in cardiac hypertrophy.

Cardiac histone deacetylase (HDAC) shuttle

The histone deacetylase (HDAC) shuttle is another system that functions to induce cardiac hypertrophy. The HDACs remove acetyl groups from histones causing chromatin condensation and a cessation of transcription. The myocyte enhancer factor-2 (MEF2) is one of the HDAC-sensitive genes (Module 4: Figure MEF2 activation) that is activated during cardiac hypertrophy. This activation, which depends on the class II HDAC4 being exported from the nucleus, is controlled by two separate mechanisms: a Ca^{2+} -sensitive mechanism and a redox-sensitive mechanisms.

The Ca^{2+} -sensitive mechanism depends on Ca^{2+} stimulating the Ca^{2+} /calmodulin-dependent protein kinase II δ (CaMKII δ) and/or IV (CaMKIV) that phosphorylate HDAC4 causing it to leave the nucleus (Module 12: Figure hypertrophy signalling mechanisms). Once the phosphorylated HDAC enters the cytosol, it can be dephosphorylated to return to the nucleus to set up a shuttle that might be influenced by the frequency or the shape of the Ca^{2+} transients (Module 12: Figure hypertrophy signalling mechanisms). This might represent another mechanism for decoding information in the Ca^{2+} transients.

It seems that this shuttle does not operate during the Ca^{2+} transients that control contraction under normal conditions. However, upon treatment with endothelin-1, which acts by increasing inositol 1,4,5-trisphosphate

Module 12: | Figure Ca^{2+} in triadin-1-overexpressing mice**Cardiac hypertrophy in triadin 1-overexpressing mice.**

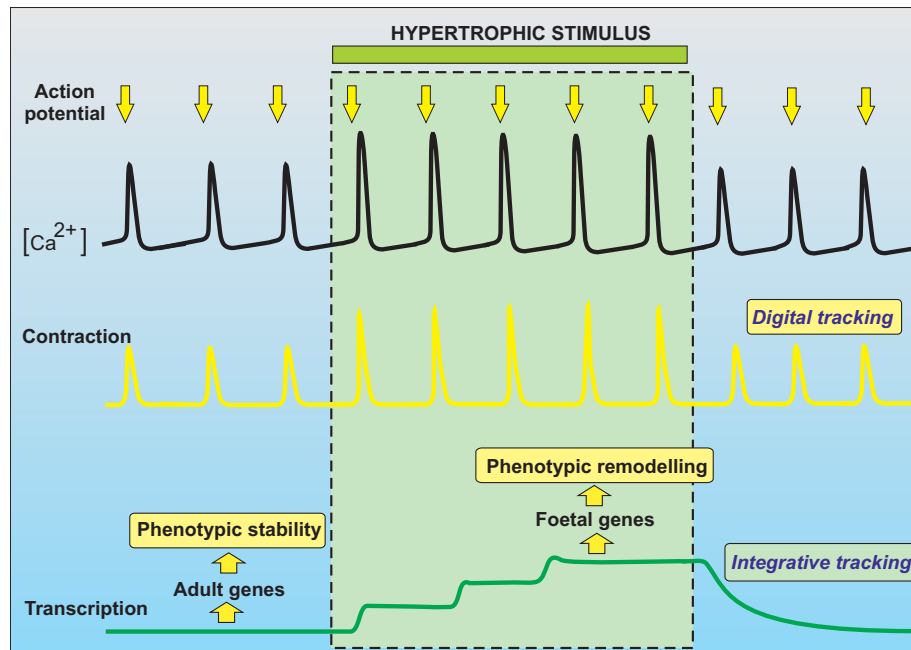
Triadin 1 is located within the sarcoplasmic reticulum (SR) and associates with the type 2 ryanodine receptor (RYR2), junctin and calsequestrin (CSQ). In the triadin 1 (TRD)-overexpressing mouse, there was a large increase in the protein level of triadin 1, with a compensatory decrease in the levels of RYR2 and junctin. The major change with regard to the Ca^{2+} transient was a considerable prolongation of the Ca^{2+} transient in the triadin 1 $^{-/-}$ mouse (blue dotted trace) compared with the wild-type (WT) (red trace). When integrated over time, the average intracellular Ca^{2+} level will be higher in the triadin 1-overexpressing myocytes, and this may be the signal that results in hypertrophy. Reproduced from Kirchhefer, U., Neumann, J., Baba, H.A., Begrow, F., Kobayashi, Y.M., Reinke, Y., Schmitz, W. and Jones, L.R. (2001) Cardiac hypertrophy and impaired relaxation in transgenic mice overexpressing triadin 1. *J. Biol. Chem.* 276:4142-4149, with permission from the American Society for Biochemistry and Molecular Biology; see Kirchhefer et al. 2001.

(InsP_3), there is a rapid translocation of HDAC5 from the nucleus into the cytoplasm. Since there are type 2 InsP_3 receptors ($\text{InsP}_3\text{R}_2\text{s}$) located in the perinuclear region, it has been suggested that InsP_3 induces a localized release of Ca^{2+} that invades the nucleus to stimulate the phosphorylation of HDAC5. However, this InsP_3 -dependent nuclear Ca^{2+} signal has not been seen. An alternative possibility, which is consistent with the heart disease working hypothesis, is that the $\text{InsP}_3\text{R}_2\text{s}$ in the vicinity of the nucleus amplify the individual Ca^{2+} transient originating from the type II ryanodine receptors (RYR2s) to provide a larger or longer-lasting Ca^{2+} signal capable of carrying out the HDAC5 phosphorylation. Such a mechanism depends on the fact that release of Ca^{2+} by InsP_3Rs depends on both InsP_3 and Ca^{2+} (Module 3: Figure InsP_3R activation). The idea is that endothelin-1 will act to provide a background elevation of InsP_3 , which in itself is not sufficient to release Ca^{2+} . However, the Ca^{2+} transient that drives contraction will provide the Ca^{2+} such that the InsP_3Rs will boost the local Ca^{2+} signal in the nucleus to activate the HDAC shuttle (Module 12: Figure hypertrophy signalling mechanisms). In effect, the InsP_3Rs may function as coincident detectors to integrate information coming from the endothelin-1 receptors (InsP_3) and in-

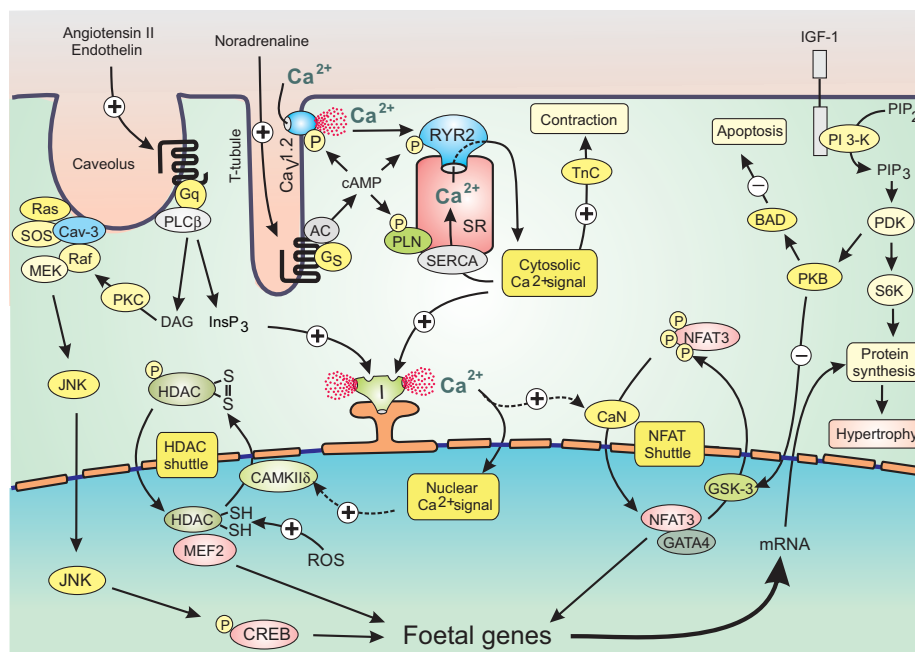
formation from the Ca^{2+} transient to create a larger local nuclear Ca^{2+} signal capable of driving HDAC5 phosphorylation. The amplitude of the InsP_3 -dependent Ca^{2+} signals may be enhanced by the neuronal Ca^{2+} sensor-1 (NCS-1), which interacts with the InsP_3Rs . The level of NCS-1 increases in hypertrophic hearts and thus provides a positive feed-forward signal to reinforce the local Ca^{2+} signals that drive the transcriptional processes responsible for hypertrophy.

A redox-sensitive pathway may also function to export HDAC4 from the nucleus. HDAC4 has two cysteine residues (Cys-667 and Cys-669) that can be oxidized by reactive oxygen species (ROS) to form an intramolecular disulfide bond and the resulting conformational change results in the oxidized HDAC4 being exported from the nucleus. This disulfide bond can be reduced by thioredoxin 1 (TRX1) operating in conjunction with a heat shock protein DnaJb5 and Trx1-binding protein-2 (TBP-2) that allows the reduced HDAC4 to be imported back into the nucleus.

While it is clear that a network of signalling systems is driving the onset of hypertrophy, it seems that Ca^{2+} is the major player. An important feature of the heart disease working hypothesis is that the increase in the activity of

Module 12: | Figure decoding cardiac Ca^{2+} spikes**Information decoding in ventricular cardiac cells.**

A central feature of the heart disease working hypothesis is that the repetitive Ca^{2+} transients convey information to both contraction (digital tracking) and transcription (integrative tracking). In the absence of hypertrophic stimuli, the Ca^{2+} transients drive contraction and maintain the level of transcription of adult genes responsible for phenotypic stability. During hypertrophic stimuli, there are changes in the nature of the transient (e.g. an increase in amplitude as shown here), which is faithfully translated through digital tracking into an increase in the strength of each contraction. In addition, the increase in transient amplitude may switch on expression of the foetal genes responsible for remodelling the signalsome.

Module 12: | Figure hypertrophy signalling mechanisms**Remodelling the cardiac signalsome during compensatory hypertrophy.**

A number of signalling pathways have been implicated in the activation of the transcription factors responsible for switching on the foetal genes that remodel the signalsome. One important action of Ca^{2+} is to stimulate the calcineurin (CaN)/nuclear factor of activated T cells (NFAT) pathway. Ca^{2+} released from inositol 1,4,5-trisphosphate (InsP_3) receptors (I) in the vicinity of the nucleus has been implicated in controlling the histone deacetylase (HDAC) shuttle. The mitogen-activated protein kinase (MAPK) pathway may contribute to hypertrophy by activating the transcription factor cyclic AMP response element-binding protein (CREB). The PtdIns 3-kinase signalling pathway has several functions: it can inhibit apoptosis, it facilitates the NFAT shuttle by inhibiting glycogen synthase kinase-3 (GSK-3), and it stimulates protein synthesis, which contributes to the increase in size of the heart.

Ca^{2+} signalling during this initial hypertrophic response may be one of the primary causes for switching on the subsequent down-regulation of the Ca^{2+} signalling system that characterizes the progression into congestive heart failure (CHF).

Mitogen-activated protein kinase (MAPK) signalling in cardiac hypertrophy

Studies with transgenic mice have emphasized the importance of the mitogen-activated protein kinase (MAPK) signalling pathway. For example, expression of active MAPK/extracellular-signal-regulated kinase (ERK) kinase 1 (MEK1) results in the development of hypertrophy, which fails to go on to the next stage of congestive heart failure (CHF). This is consistent with the heart disease working hypothesis, because the MAPK pathway can induce the up-regulation of gene transcription without the need to increase the Ca^{2+} signalling that is proposed to induce the next phase of irreversible hypertrophy. Of the three MAPK signalling pathways (Module 2: Figure MAPK signalling), it seems that the c-Jun N-terminal kinase (JNK) pathway is of major significance (Module 12: Figure hypertrophy signalling mechanisms).

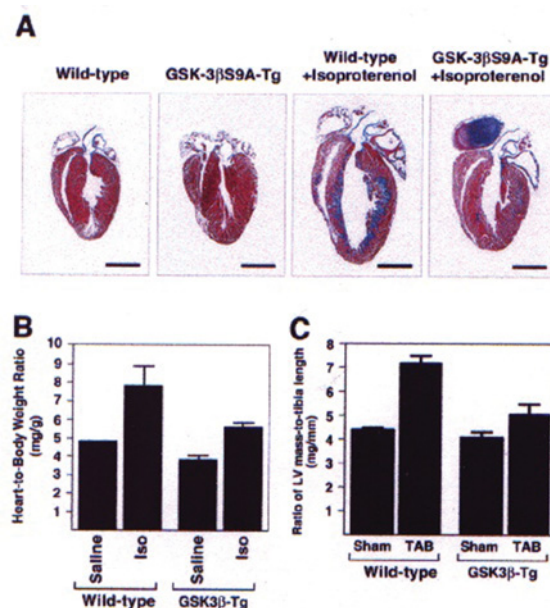
The G protein-coupled receptors (GPCRs) that can induce hypertrophy (e.g. endothelin and angiotensin II receptors) can activate JNK signalling through various pathways, including PtdIns 3-kinase and through the G protein subunit $\text{G}_{12/13}$ (Module 2: Figure JNK signalling).

The action of MAPK signalling in the heart seems to be concentrated at the caveolae, which occur on the surface of cardiac cells (Module 6: Figure cardiac caveolae). Such caveolae are associated with a number of signalling pathways (Module 6: Figure caveolae molecular organization). The development of hypertrophy in caveolin-3 knockout mice seems to depend on hyperactivation of the MAPK cascade. The removal of caveolin-3 results in the disappearance of caveolae and a decline in the normal signalling function of caveolae. One of the functions of caveolin is to exert a tonic inhibition of the MAPK cascade. When this inhibition is removed, there is an up-regulation of MAPK signalling, and this seems to contribute to hypertrophy.

PtdIns 3-kinase signalling in cardiac hypertrophy

The PtdIns 3-kinase signalling pathway can promote cardiac hypertrophy at several levels. It can facilitate the programme of foetal cardiac gene transcription by removing the inhibitory influence of glycogen synthase kinase-3 β (GSK-3 β), thereby keeping nuclear factor of activated T cells (NFAT) active by preventing its migration out of the nucleus (Module 12: Figure hypertrophy signalling mechanisms). GSK-3 is inactivated following phosphorylation of Ser-9 by protein kinase B (PKB). When dephosphorylated under resting conditions, the enzyme is active and can keep NFAT phosphorylated. Transgenic mice that express a constitutively active GSK-3 (the Ser-9 inactivating site was mutated to alanine) can greatly reduce the hypertrophic response induced by stimuli such as chronic β -adrenergic stimulation (Module 12: Figure GSK-3 β and cardiac hypertrophy) and pressure overload.

Module 12: | Figure GSK-3 β and cardiac hypertrophy



Constitutively active glycogen synthase kinase 3 β (GSK-3 β) prevents isoprenaline (isoproterenol)-induced hypertrophy.

A. The histological sections reveal that mice expressing a constitutively active glycogen synthase kinase 3 β (GSK-3 β) suppressed the hypertrophy induced by chronic treatment with isoprenaline (isoproterenol) in the ventricles, but not in the atria. B. This protective effect is clearly evident in this statistical analysis measuring the heart-to-body weight ratios. C. A similar effect of constitutively active GSK-3 β was also seen when hypertrophy was induced by thoracic aortic banding (TAB). Reproduced from Antos, C.L., McKinsey, T.A., Frey, N., Kutsche, W., McAnally, J., Shelton, J.M., Richardson, J.A., Hill, J.A. and Olson, E.N. (2002) Activated glycogen synthase-3 β suppresses cardiac hypertrophy *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.* 99:907-912. Copyright (2002) National Academy of Sciences, U.S.A.; see Antos et al. 2002.

Another function of PtdIns 3-kinase signalling is to alleviate the risk of apoptosis, which will be heightened during the enhanced Ca^{2+} signalling that accompanies the onset of hypertrophy (Module 12: Figure hypertrophy signalling mechanisms). Through its ability to enhance protein synthesis, this signalling pathway will also help to carry out the expression of the proteins that are up-regulated during hypertrophy.

Nitric oxide (NO)/cyclic GMP and cardiac hypertrophy

The nitric oxide (NO)/cyclic GMP signalling pathway functions as a negative regulator of hypertrophy by inhibiting Ca^{2+} signalling. Procedures that increase the formation of NO act through the second messenger cyclic GMP to reduce the amount of Ca^{2+} entering through the L-type Ca^{2+} channels. There also are indications that cyclic GMP may also reduce the activation of myocyte enhancer factor-2 (MEF2), which is a key regulator of foetal gene transcription (Module 12: Figure hypertrophy signalling mechanisms).

Inhibition of cardiac hypertrophy by NO/cyclic GMP signalling was also observed when endothelial nitric oxide synthase (eNOS) was overexpressed in the vascular endothelium, indicating that it is the presence of NO that reverses or alleviates the hypertrophic signals.

Congestive heart failure (CHF)

Congestive heart failure (CHF) results from the irreversible remodelling events that result in the weak signals characteristic of a hyposensitive signalsome (Module 12: Figure remodelling the cardiac signalsome). While the changes in signalling that occur during compensatory cardiac hypertrophy clearly involve changes in Ca^{2+} signalling, there is not much evidence that the Ca^{2+} signalsome has been substantially remodelled. An important feature of Phase 2 of the heart disease working hypothesis is that the cardiac Ca^{2+} signalsome undergoes irreversible phenotypic remodelling, resulting in a severe down-regulation of Ca^{2+} signalling characteristic of CHF. It is proposed that this down-regulation is a direct consequence of the homeostatic mechanisms whereby Ca^{2+} maintains signalsome stability by controlling the expression of its signalling components. There are numerous examples whereby Ca^{2+} regulates the expression of components of the Ca^{2+} signalsome (Module 4: Figure NFAT control of Ca^{2+} signalling toolkit). When Ca^{2+} signalling is excessive, as occurs during hypertrophy, the Ca^{2+} signalling system may be turned down by reducing the activity of various components of the cardiac signalsome:

- The sarco/endo-plasmic reticulum Ca^{2+} -ATPase (SERCA) pump. The most noticeable change that occurs in the signalsome during CHF is a dramatic decline in the activity of the SERCA pump (Step 6 in Module 7: Figure ventricular Ca^{2+} signalling). This decline is caused by a decrease in its mRNA and protein expression level. Sumoylation of SERCA markedly enhances its stability by preventing its proteasomal degradation. The reduced ability to pump Ca^{2+} means that the level of Ca^{2+} within the sarcoplasmic reticulum (SR) will be below normal, and this explains the marked reduction in Ca^{2+} transient amplitude in the failing heart (Module 12: Figure CSQ-induced hypertrophy). *SUMO-1* gene transfer to increase the level of cardiac SUMO1 has proved to be an effective therapy in animal models of CHF.
- The adrenergic/phospholamban modulatory mechanism. A decline in Ca^{2+} signalling is exacerbated by a decrease in the level of cyclic AMP caused by a marked down-regulation of β -adrenergic receptor signalling in the failing heart. Catecholamines contribute to the hypertrophic response by activating the cyclic AMP pathway that phosphorylates both phospholamban (PLN) and the ryanodine receptor 2 (RYR2) (Step 7 in Module 7: Figure ventricular Ca^{2+} signalling). In its phosphorylated state, PLN cannot inhibit SERCA2a, thus allowing the latter to enhance Ca^{2+} signalling by increasing the amount of stored Ca^{2+} . However, when the β -adrenergic receptor is down-regulated, all of the PLN will be dephosphorylated, resulting in inhibition of the SERCA2a pump (State 5 in Module 5: Figure phospholamban mode of action).
- In the failing heart, the phosphatases normally associated with RYR2 are lost, which means that it remains phosphorylated and this alters its gating properties, making it less effective and leaky, thus contrib-

uting to the decline in the Ca^{2+} content of the SR. The change in the adrenergic system is thus a critical event in the transition between the two forms of hypertrophy, and this may depend upon an overexpression of transforming growth factor- β (TGF- β), which acts by stimulating the Smad signalling pathway (Module 2: Figure TGF- β R activation). There is increased expression of TGF- β 1 during the transition from stable hypertrophy to heart failure. TGF- β 1 may act to increase β -adrenergic signalling, and thus contribute to the excessive catecholamine stimulation during the critical transition phase.

- The $\text{Na}^+/\text{Ca}^{2+}$ exchanger. The activity of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger is increased during CHF. This exacerbates the failing Ca^{2+} signalling system by extruding too much Ca^{2+} from the heart cells.

In summary, the remodelling events that occur during CHF have an impact on the Ca^{2+} signalling system so that it fails to deliver the transients necessary to drive strong contractions. Down-regulation of the SERCA2a pump is particularly important. The expression of SERCA2a is decreased, the inactivation of the adrenergic modulatory mechanism increases the inhibition of the pump, and the increase in the $\text{Na}^+/\text{Ca}^{2+}$ exchanger will reduce the access of the SERCA pump to Ca^{2+} . All of these processes conspire to reduce the capacity of the SR to pump Ca^{2+} , resulting in an increase in the diastolic Ca^{2+} concentration and a marked decrease in the systolic Ca^{2+} transient. These changes are directly attributable to a decline in the level of Ca^{2+} within the lumen of the endoplasmic reticulum (ER). The basic hypothesis is therefore that the central role of an increase in Ca^{2+} signalling during the early phase of hypertrophy sets the stage for the severe down-regulation of the signalling system that characterizes CHF.

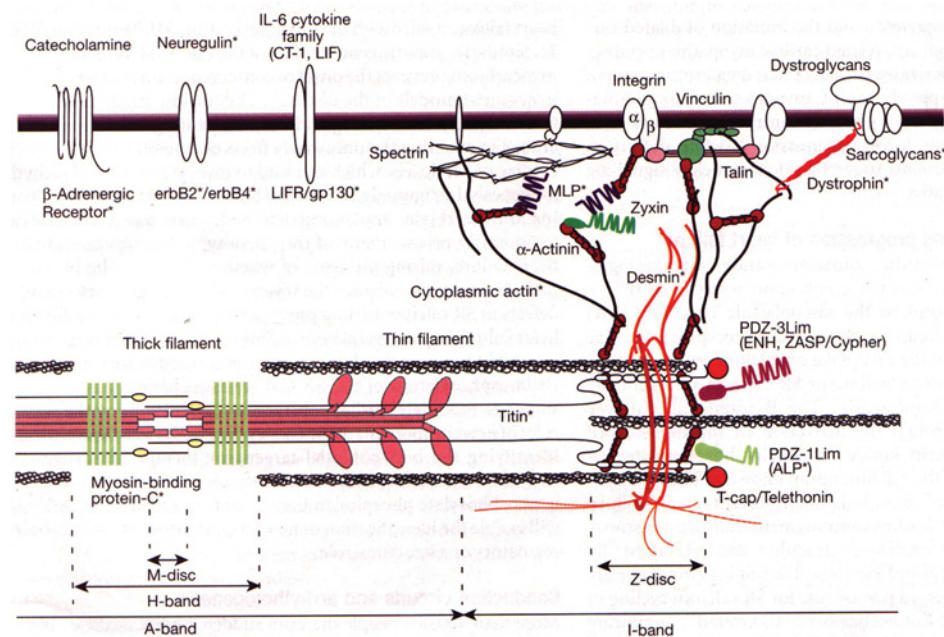
Familial cardiomyopathies

Considerable attention has been focused on the contractile and cytoskeletal elements of the cardiac cell following the realization that a number of cardiomyopathies result from mutations in the genes that code for components of the mechanotransduction system of the heart. The two types of familial cardiomyopathies have very different characteristics. Familial hypertrophic cardiomyopathy results in asymmetrical ventricular hypertrophy, with a high incidence of sudden heart death in young adults. On the other hand, dilated cardiomyopathy is characterized by cardiac dilation with much reduced systolic function, resulting in heart failure.

Contractile and cytoskeletal elements of the cardiac cell

The organization of the contractile and cytoskeletal elements plays a critical role in the development of cardiac disease. Mutations in these structural elements might lead to cardiomyopathies by interfering with the intricate relationship that exists between the contractile and cytoskeletal components (Module 12: Figure cardiac contractile elements). A significant aspect of this relationship is that the contractile elements, made up primarily of actin and myosin and associated proteins (titin, etc.), are

Module 12: | Figure cardiac contractile elements



Structural organization of the contractile and cytoskeletal elements of cardiac cells.

The asterisks identify proteins that can lead to hypertrophy when they are mutated. Reproduced by permission from Macmillan Publishers Ltd: *Nature*, Chien, K.R. (2000) Genomic circuits and the integrative biology of cardiac diseases. 407:227-232. Copyright (2000); <http://www.nature.com>; see Chien 2000.

connected to the plasma membrane through a complex meshwork of cytoskeletal proteins. The Z-disc is particularly important in that it is the point where the contractile components connect to the cytoskeletal elements. The latter are attached to the plasma membrane through a number of elements, including integrins, dystroglycans, sarcoglycans and dystrophin that represent a mechanism whereby the structural elements can link into various signalling pathways that might play a role in mediating some of the hypertrophic signals.

The fact that hypertrophy develops when mutations occur in either the contractile or cytoskeletal elements suggests that the cell is responding to some defect in either the generation of force or the way in which this force is being transmitted. Mutations in various components may alter the effectiveness of the cytoskeletal/contractile system, and this may resemble what happens when the heart is subjected to a pressure overload. Good examples of this are the mutations that occur in the troponin system. For example, a number of the mutations in troponin T and troponin I that cause familial hypertrophic cardiomyopathy result in an increase in the Ca^{2+} -sensitivity of the contractile system. On the other hand, a deletion mutation of troponin T, which causes dilated cardiomyopathy, has the opposite effect of decreasing the Ca^{2+} -sensitivity of contraction. These positive and negative changes in sensitivity will alter the mechanical output of the heart, and this causes different forms of hypertrophy, thus stressing the complexity of the interacting control systems.

One way of linking the state of contractility with the hypertrophic changes might be through a change in the mechanical stresses on the heart. For example, mechanical

stress can stimulate phospholipase C (PLC) activity and Ca^{2+} levels in neonatal rat cardiomyocytes. Some of the effects of stretch might be mediated through the activation of stretch-activated Ca^{2+} channels. What is clear is that the hypertrophy that results from genetic alterations in the mechanical system seems to be mediated through the same signalling systems that occur during other hypertrophic stimuli. Evidence for this has come from genetic complementation studies, where the hypertrophy that develops following ablation of the muscle-specific LIM domain cytoskeletal protein (MLP) (Module 12: Figure cardiac contractile elements) can be corrected by knocking out phospholamban. The MLP-deficient myocytes had the typical reduced Ca^{2+} signalling response that was restored to normal when phospholamban was removed (Module 12: Figure MLP^{-/-} Ca^{2+} responses).

Familial hypertrophic cardiomyopathy

Many types of familial hypertrophic cardiomyopathy result from mutations of the sarcomeric contractile proteins (e.g. β -myosin heavy chain, troponin T, troponin I, α -tropomyosin, myosin-binding protein C, myosin essential light chain, myosin regulatory light chain and titin). Changes in the Ca^{2+} signalling system may result from these alterations in the contractile and cytoskeletal elements of the cardiac cell.

Dilated cardiomyopathy

Familial dilated cardiomyopathy often is associated with mutations of various proteins associated either with the cytoskeletal elements (e.g. dystrophin, actin, desmin and δ -sarcoglycan) or with signalling elements. With regard to the latter, mutations of phospholamban (PLN) have been

identified in two cases of human dilated cardiomyopathy. In one case, there is an arginine-to-cysteine missense mutation at residue 9 (R9C) (Module 5: Figure phospholamban and sarcolipin), which blocked the ability of protein kinase A (PKA) to remove this inhibitor, thus resulting in a permanently depressed sarco/endo-plasmic reticulum Ca^{2+} -ATPase 2a (SERCA2a) pump. There was no change in the rate of Ca^{2+} release, but the recovery phase was delayed, resulting in a broadening of the Ca^{2+} transient. In keeping with the heart disease working hypothesis, this change in the shape of the transient may provide the signal to trigger the hypertrophic response. In another example, a termination codon replaces Leu-39 (L39stop), resulting in a human null mutation. In the absence of this normal inhibitory protein, the SERCA2a pump operates at a continuously high rate (State 4 in Module 5: Figure phospholamban mode of action). Again, it is reasonable to propose that the continuous presentation of high-amplitude Ca^{2+} transients would provide the signal to initiate premature dilated cardiomyopathy and cardiac failure.

Humoral hypercalcaemia of malignancy (HHM)

Humoral hypercalcaemia of malignancy (HHM) is a form of hypercalcaemia that results from malignant tumours releasing hormones/cytokines that then activate bone resorption. In most cases, the hormone that is released is parathyroid hormone (PTH)-related peptide (PTHrP). The degradation of bone results in the release of Ca^{2+} and various growth factors [e.g. platelet-derived growth factor (PDGF), and insulin-like growth factor (IGF) I and II]. These various factors then act on the cancer cells to release more PTHrP, which thus sets up a positive amplification system. A part of this positive loop is the ability of Ca^{2+} to act on the Ca^{2+} -sensing receptor (CaR) to increase the release of PTHrP.

Inflammatory Bowel Disease (IBD)

This intestinal disorder is caused by chronic inflammation of the bowel caused by the release of inflammatory mediators such as tumour necrosis factor (TNF), IL-6, IL-8 and MCP-1 that are induced by the activation of TREM-1 receptors located on macrophages in the lamina propria. The two most common forms of IBD are Crohn's disease and ulcerative colitis.

Irritable bowel syndrome

Irritable bowel syndrome is a disorder of the intestinal tract that is characterized by changes in motility, secretion and visceral sensations, including abdominal pain. In many cases, there is diarrhoea or constipation, which seems to result in some alteration in the small intestine neural and endocrine system that controls the balance between fluid absorption and secretion. Considerable attention is beginning to focus on the possibility that some of these symptoms may be caused by an alteration in the 5-hydroxytryptamine (5-HT) signalling systems (Module 7: Figure small intestine). This 5-HT, which is released from the enterochromaffin cells, plays a central role in regulating both the motility of the intestine and its secretion of fluid. For example, if the secretion of 5-HT or its subsequent

actions to activate the enteric nervous system is set too high, the intestine will begin to secrete fluid, resulting in both diarrhoea-predominant irritable bowel syndrome and an increase in intestinal motility. Conversely, a decrease in the operation of the 5-HT signalling system will reduce secretion of fluid, resulting in constipation.

Migraine

Migraine is characterized by very severe and long-lasting headaches. There is an intense throbbing pain at the front or on one side of the head often associated with an enhanced sensitivity to light. A curious feature of migraine is that the onset of the attack is often preceded by an aura, which consists of an altered sensory experience, usually a visual or olfactory disturbance. There is increasing evidence that migraine might be caused by alterations in ion channel activity. In the case of familial hemiplegic migraine (FHM), there are mutations in the $\text{Ca}_v2.1$ P/Q-type channel (Module 3: Figure Ca_v2 channel family). In another case of familial migraine, there are mutations in TRESK, which is a member of the two-pore domain K^+ (K_{2P}) channels (Module 3: Table two-pore domain K^+ channels) that carry much of the background K^+ current that regulates neuronal membrane potentials.

Migraine has also been linked to the gene that encodes casein kinase I δ (CKI δ), which is also responsible for familial advanced sleep phase syndrome (FASPS).

Multiple sclerosis (MS)

Multiple sclerosis (MS) is an inflammatory demyelinating disease that affects the central nervous system (CNS). The incidence of MS is approximately 1% and is most common in women and in Caucasians. It usually occurs between the ages of 20–40. The initial attacks, which are often mild and transient, begin with a loss of sensation in the arms legs and face, a partial loss of vision, weakness and problems with balance and walking.

The cause of MS is not known, but it has all the hallmarks of an autoimmune disease that results in damage to the oligodendrocytes that are responsible for forming the myelin sheath, which enables neurones to conduct action potentials. In keeping with the immunological explanation, the idea is that a breach in the blood–brain barrier enables T cells to enter the CNS where they recognize the oligodendrocytes as foreign cells and this initiates an autoimmune inflammatory response. Processes that enhance the entry of T cells will exacerbate this immune response. T cell chemotaxis is controlled by chemokines such as CCL4 and CCL5 that act through the CCR5 receptor, which are G protein-coupled receptors (GPCRs). The sensitivity of these GPCRs is controlled by a family of G protein receptor kinases (GRKs) (Module 1: Figure homologous desensitization). One of the isoforms is GRK2, which is particularly effective in T cells. It is of interest, therefore, to find that mice that have a partially defective GRK2, which would thus heighten T cell chemotaxis, were found to be more sensitive to experimental autoimmune encephalomyelitis, which is a model for multiple sclerosis.

The levels of IGF-binding protein 2 (IGFBP-2) is increased in MS and this could reduce the neuroprotective

and myelinogenetic actions of insulin-like growth factor I (IGF-I).

There is increasing evidence that exposure to light is an important risk factor in MS and other neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. For example, the incidence of MS is lowest at the equator and increases progressively as one moves towards the poles. Such evidence has led to the realization that a deficiency in vitamin D may contribute to neurological diseases such as MS. Evidence is growing for links between vitamin D and Ca^{2+} regulation in neurodegeneration and for vitamin D control of inflammation. The latter is particularly significant in MS. In a mouse model of MS [autoimmune encephalomyelitis (EAE)], administration of a single dose of calcitriol followed by Vitamin D supplementation was found to improve the inflammatory symptoms typical of MS. This vitamin D control of inflammation seems to depend on the selective regulation of CD4^{+} T lymphocytes.

An exception to the inverse relationship between exposure to sunlight and the incidence of MS has been uncovered in Sardinia. A large proportion of the population in this Mediterranean island suffer from MS. Furthermore, they have normal high levels of Vitamin D, which thus challenged the hypothesis of a link between sunlight exposure and MS. However, subsequent studies revealed that the MS patients in Sardinia have reduced expression of the *Ifng* gene that encodes Interferon- γ (IFN- γ). The action of Vitamin D is markedly affected by this reduction in *Ifng* gene activity, because IFN- γ plays a role in the expression of the Vitamin D receptor (VDR).

Narcolepsy

Narcolepsy is characterized by excessive daytime sleepiness and can result in a sudden onset of sleep during normal periods of wakefulness. Many cases of narcolepsy are associated with a marked decline in the expression of the sleep-inducing peptide orexin that functions in the orexinergic arousal system.

Nausea

Noxious chemicals in the lumen of the intestine can cause nausea and vomiting. This also occurs in response to many of the drugs used in cancer chemotherapy such as cisplatin. This adverse reaction has been traced to the stimulation of 5-hydroxytryptamine (5-HT) release by the enterochromaffin cells located in the mucosa of the small intestine (Module 7: Figure small intestine). The 5-HT release from these cells acts on the 5-HT type 3 (5-HT₃) receptors on the endings of vagal afferents that conduct the messages to the brain that induce nausea. The anti-emetic drug ondansetron is an effective 5-HT₃ receptor antagonist.

Primary hyperparathyroidism

Primary hyperparathyroidism is a disease of the parathyroid gland, which functions in parathyroid hormone (PTH) synthesis and release (Module 7: Figure PTH secretion). This disease state is usually caused by a parathyroid gland adenoma. The cancerous cells release

excessive amounts of PTH that results in a chronic elevation of blood Ca^{2+} (hypercalcaemia). Loss of Ca^{2+} from the bones cause bone decalcification, and the excess Ca^{2+} in the blood causes the development of kidney stones, headache and mental confusion. Under severe conditions, this can lead to convulsions and coma.

Secondary hyperparathyroidism

Secondary hyperparathyroidism is a disease of the parathyroid gland, which functions in parathyroid hormone (PTH) synthesis and release (Module 7: Figure PTH secretion). In contrast with primary hyperparathyroidism, which is a disease of the parathyroid gland, this secondary form is driven by disease states outside the gland. A common cause is disease of the kidney. Much of the Ca^{2+} filtered by the glomeruli is reabsorbed by the tubules (Module 7: Figure Ca^{2+} homeostasis). However, during kidney disease this Ca^{2+} reabsorption by the kidney is compromised, resulting in a large net loss of Ca^{2+} and a fall in the level of plasma Ca^{2+} . This hypocalcaemia then stimulates the excessive secretion of PTH, which contributes to parathyroid gland hyperplasia that further enhances the release of PTH leading to secondary hyperparathyroidism.

Another cause of secondary parathyroidism is vitamin deficiency. Vitamin D metabolism gives rise to 1,25-dihydroxyvitamin D₃ [$1,25(\text{OH})_2\text{D}_3$]. One of the functions of $1,25(\text{OH})_2\text{D}_3$ is to inhibit the synthesis of PTH (Module 7: Figure PTH secretion). If vitamin D is limiting, the decline in the level of $1,25(\text{OH})_2\text{D}_3$ removes this inhibition and the resulting increase in PTH results in hyperparathyroidism.

One of the complications of secondary hyperparathyroidism is the development of osteoporosis.

Hypertension

Hypertension is a complex condition manifested as an increase in blood pressure that can be caused by many factors, including obesity. The increase in blood pressure, which is usually taken as a resting diastolic pressure above 90 mmHg, can result from an increase in either blood volume or the force exerted on this fluid by the vascular smooth muscle cells surrounding the blood vessels. With regard to blood volume control, the main determinants are the mechanisms that contribute to blood Na^{+} regulation (Module 7: Figure blood pressure control). Most of this regulation occurs in the kidney that has the role of reabsorbing most of the Na^{+} that is filtered during the process of urine formation. It has been estimated that approximately "1.2 kg of cooking salt" are filtered each day (Kleta and Bockenhauer 2006). In order to ensure that blood [Na^{+}] remains normal, 99% of the filtered Na^{+} has to be reabsorbed. Hypertension occurs if the amount being reabsorbed is set too high. Support for the notion that alterations in kidney salt retention are a major cause of both hypertension and hypotension has come from the analysis of heritable human diseases:

- Gordon's disease results from mutations in the WNK protein kinases that result from excessive reabsorption

by the distal convoluted tubule (DCT) (Module 7: Figure kidney salt reabsorption).

- Mutations in the $\text{Na}^+\text{-Cl}^-$ cotransporter (NCC) or the $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter 2 (NKCC2) that reduce salt reabsorption cause hypotension as found in Gitelman's disease and type I Bartter's disease respectively.
- Polymorphic variants of GRK4, which is one of the G protein receptor kinases (GRKs) that control receptor desensitization (Module 1: Figure homologous desensitization), result in hypertension. The constitutive activation of GRK4 desensitizes the D1 dopamine receptors resulting in a decrease in Na^+ secretion.
- A gain-of-function polymorphism of CLC-Kb, which functions in salt reabsorption by the kidney (Module 7: Figure kidney salt reabsorption), results in a predisposition to hypertension.

Another cause of hypertension is an increase in vascular smooth muscle cell tone and this might be due to a decrease in the operation of the nitric oxide (NO)/cyclic GMP and smooth muscle relaxation mechanism (Module 7: Figure smooth muscle cell cyclic GMP signalling). Such a link is strengthened by the observation that endothelial nitric oxide synthase (eNOS)^{-/-} mice are hypertensive. One suggestion is that the availability of NO may be reduced by an increase in the production of superoxide ($\text{O}_2^{\bullet-}$), which diverts NO away from the cyclic GMP signalling pathway by converting it into peroxynitrate (ONOO^-).

Another suggestion is that smooth muscle tone might be disrupted by an alteration in the generation of smooth muscle cell Ca^{2+} sparks. In an angiotensin II-induced hypertension mouse model, there was a decrease in the expression of the β_1 -subunit that regulates the Ca^{2+} -sensitivity of the large-conductance (BK) channels responsible for the hyperpolarization of the membrane that induces smooth muscle relaxation (Module 7: Figure smooth muscle cell spark). In mice where the β_1 subunits have been knocked out, the incidence of sparks is greatly reduced (Module 3: Figure smooth muscle cell Ca^{2+} sparks). The knockout animal becomes hypertensive because the hyperpolarization necessary to reduce muscle tone is reduced.

Another major cause of hypertension is an alteration in blood Na^+ regulation, where the renin-angiotensin system (RAS) is set too high and the resulting increase in the level of angiotensin II results in an increase in blood volume (Module 7: Figure blood pressure control). Many of the current anti-hypertensive treatments are directed towards a reduction in this control system. For example the angiotensin-converting enzyme (ACE) inhibitors block the terminal step in angiotensin II formation (Step 5 in Module 7: Figure blood pressure control) while the angiotensin II type 1 (AT_1) receptor blockers inhibit the multiple actions of this hormone (Steps 6 and 8–11 in Module 7: Figure blood pressure control).

The continuous elevation of blood pressure during hypertension can result in a number of serious pathologies, including heart disease, resulting from cardiac hypertrophy, cerebral haemorrhage, myocardial infarction, stroke, kidney failure and retinopathy. The drugs used

to treat hypertension (e.g. α -blockers, β -blockers, Ca^{2+} channel antagonists and ACE inhibitors) clearly indicate how important alterations in Ca^{2+} signalling pathways are in the aetiology of hypertension.

An increase in Ca^{2+} entry may also be feature of hypertension. Entry through store-operated channels (SOCs) appears to be greater in lymphocytes from African Americans than in Caucasians, and this may predispose the latter to hypertension. In spontaneous hypertensive rat (SHR) cells, there was increased basal and augmented angiotensin II-induced Ca^{2+} release, which may also result in an enhanced entry of external Ca^{2+} .

Liddle's disease is a salt-sensitive form of hypertension.

Manic-depressive illness (bipolar disorder)

Over 2 million people in the UK are diagnosed as suffering from depression each year. Almost half of these have the more severe disorder of manic-depressive illness also known as bipolar disorder (BPD), which is characterized by extreme mood swings. During the manic phase, patients lose contact with reality and can experience hallucinations and euphoria. The latter is often associated with very grand and overoptimistic ideas. They are often irritable, and this may arise through an inability or unwillingness to sleep.

Alterations in sleep patterns are also experienced during the depressive phase, during which patients awake feeling tired and unrefreshed. They appear to lack energy and have a persistent low mood.

Untreated manic-depressive illness causes severe social problems. Sufferers are often unable to maintain personal relationships, leading to breakdown of marriages, unemployment and homelessness.

Just what triggers a manic-depressive episode is not clear, but its onset is often associated with a period of severe stress. Even less is known about the nature of the neuronal changes responsible for this profound change in behaviour. Changes in neural signalling are a feature of two of the hypotheses to explain the nature of this disease and how it is controlled by antidepressants. The neurogenesis hypothesis considers that manic-depressive illness is caused by a stress-induced modification of neurogenesis, resulting in a decrease in hippocampal circuitry that can be corrected by antidepressants. The inositol depletion hypothesis considers that the disease arises through overactive phosphoinositide signalling pathways that are corrected by drugs such as Li^+ and valproate. These two hypotheses are not mutually exclusive, and it might be useful to consider their amalgamation into a unified hypothesis. For example, some of the defects predicted by the inositol depletion hypothesis, such as the overactive phosphoinositide signalling pathways, might result in alterations in neurogenesis.

Bcl-2 gene single nucleotide polymorphisms (SNPs) have been associated with a risk of developing manic depressive illness. These SNPs reduce the expression of Bcl-2, which could have two important consequences. First, a decrease in the level of this anti-apoptotic factor could lead to an increase in apoptosis, which would be consistent with the neurogenesis hypothesis. Secondly, a decrease in Bcl-2 will reduce its inhibitory effect on InsP_3 -induced Ca^{2+}

release (Module 5: Figure ER/mitochondrial shuttle) and will lead to an elevation in the level of Ca^{2+} and this would be consistent with the **inositol-depletion hypothesis**.

A critical aspect of any hypothesis on manic-depressive illness is the long time it takes for antidepressants to work. Even though many of these antidepressants reach their targets very quickly, it takes 2–3 weeks before a change in behaviour occurs.

Neurogenesis hypothesis

The neurogenesis hypothesis considers that a decrease in neurogenesis causes the onset of manic-depressive illness. The volume of the hippocampus in patients suffering from this disease is much reduced. It is known that the onset of this disease is often triggered by a period of stress that is also known to reduce the volume of the hippocampus. Approximately 50% of depressed patients have defects in the regulation of the hypothalamic–pituitary–adrenal (PHA) axis, which results in a decrease in cortisol levels that reduces neurogenesis. Some of the symptoms of the disease may arise from a decrease in hippocampal circuitry because this region provides inputs to the prefrontal cortex, cingulate cortex and amygdala, which contribute to the altered mood and emotions of depression. A defective hippocampus is also consistent with the fact that depressed patients display cognitive defects.

Another essential feature of this hypothesis is that many antidepressant therapies result in an increase in neurogenesis to restore the hippocampus to its normal volume. Patients with depression have low serum levels of brain-derived neurotrophic factor (BDNF), which seems to play a role in regulating neurogenesis. Antidepressants cause an increase in the expression of BDNF. The antidepressants, which alter behaviour and restore the volume of the hippocampus, include those that act on the function of neurotransmitters, as well as those such as Li^+ and valproate that can manipulate inositol metabolism. The only exception is transcranial magnetic stimulation (TMS), which has no effect on neurogenesis, but has some antidepressant effects. However, proponents of the neurogenesis hypothesis argue that the antidepressant effects are not sufficiently robust to provide the exception that will disprove the hypothesis.

An important aspect of the neurogenesis hypothesis is that it can explain the long time lag for antidepressants to work. Because the process of neurogenesis is relatively slow, the recovery of hippocampal volume following antidepressant treatment takes several weeks to occur, exactly in line with the time it takes to see any behavioural improvements.

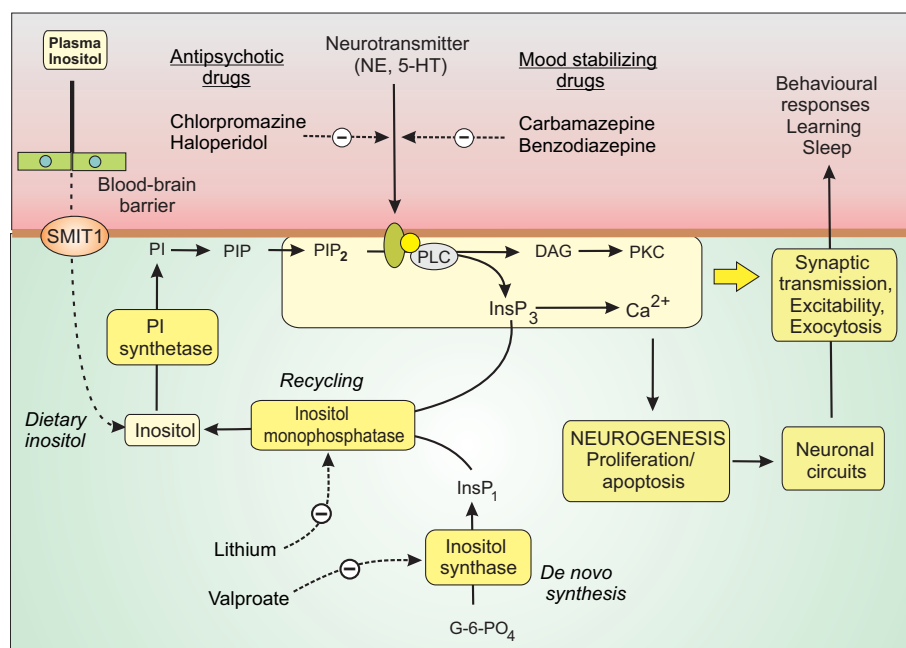
Inositol depletion hypothesis

A clue to the possible cause of the disease can be gleaned from both the symptoms of the disease and the mode of action of some of the drugs currently in use to control manic-depressive illness (Module 12: Figure Li^+ action). Many of these, such as the antipsychotic drugs (e.g. chlorpromazine and haloperidol) and some of the mood-stabilizing drugs, appear to act outside the cell to influence the action of various neurotransmitters, such as noradrenaline

(norepinephrine) and 5-hydroxytryptamine (5-HT). The fact that these two transmitters are known to act through metabotropic receptors, some of which operate through inositol lipid hydrolysis, suggests that this signalling pathway may play a role in manic-depressive illness. This possibility is strengthened by the fact that two of the major mood-stabilizing drugs (i.e. Li^+ and valproate) also appear to act by modifying this signalling pathway. The inositol depletion hypothesis sets out to explain how Li^+ and valproate may act to control manic-depressive illness by reducing the supply of inositol.

The inositol depletion hypothesis emerged from the observation that Li^+ is a potent inhibitor of the inositol monophosphatase responsible for hydrolysing inositol monophosphates (Ins4P, Ins1P and Ins3P) to free inositol (Steps 3 in Module 2: Figure inositol phosphate metabolism). By inhibiting the formation of inositol, Li^+ chokes off the supply of the free inositol required to resynthesize the PtdIns necessary to provide the PtdIns4,5P₂ required for the signalling system (Module 2: Figure InsP₃/DAG recycling). This inositol depletion hypothesis was strengthened when it was discovered that valproate has a similar action in that it too will deplete internal inositol by inhibiting the inositol synthase responsible for the *de novo* synthesis of inositol from glucose 6-phosphate (Module 12: Figure Li^+ action).

A corollary of the inositol depletion hypothesis is that manic-depressive illness is caused by a phenotypic remodelling of the signalsome resulting in excessive elevation of the neuronal phosphoinositide signalling pathway (Module 12: Figure inositol depletion hypothesis). The consequence of this change will depend on whether this increase in signalling occurs in either the excitatory or inhibitory neurons. Therefore, the new concept is that changes in the activity of either the excitatory or inhibitory neurons result in subtle alterations in the neuronal circuits that control behaviour. The basic idea is that the periodic switching between depression and mania, which is a characteristic feature of BPD, is caused by an alteration in the excitation-inhibition (E-I) balance that controls neuronal activity (Module 10: Figure tonic excitatory drive and neuronal rhythms). During the generation of brain rhythms, it is essential for the excitatory and inhibitory neurons to be activated equally. In effect, the tonic excitatory drive that determines the pacemaker potential for each neuronal type must be balanced. The idea that BPD is caused by such an E-I imbalance is supported by the observation that many of the transmitters (e.g. ACh, DA and 5-HT), which have been linked to this disease, activate the tonic excitatory drive using a variety of signalling mechanisms (Module 10: Figure tonic excitatory drive and neuronal rhythms). For example, ACh acts through M1 receptors to stimulate PtdIns4,5P₂ hydrolysis, which contributes to depolarization by decreasing the M current while promoting the Ca^{2+} -activated non-selective cation current. The most effective mood-stabilizing drugs, such as lithium (Li^+) and valproate, act by modulating downstream intracellular signalling pathways that generate this tonic excitatory drive that regulates the membrane excitability that controls mood. An imbalance in the activity of these

Module 12: | Figure Li^+ action

The inositol depletion hypothesis for the control of manic-depressive illness by mood-stabilizing drugs such as Li^+ and valproate.

The basis of this hypothesis is that Li^+ and valproate modify the signalling pathways associated with the phosphoinositide lipids, which have many functions within the nervous system. The idea is that manic-depressive illness may arise when this signalling pathway is overactive. Li^+ and valproate may function to reduce the activity of this pathway by reducing the supply of inositol used to replenish the membrane supply of inositol lipids. Neurons are uniquely sensitive to Li^+ and valproate because they are denied access to plasma inositol, which fails to cross the blood-brain barrier.

signalling pathways that regulate the tonic drive may thus be one of the causes of BPD. Such a mechanism explains why Li^+ is effective in treating both mania and depression. If the E-I imbalance is such that the tonic excitatory drive to the excitatory neurons is over-active then this could account for mania, whereas an overactive inhibitory system would explain depression.

The inositol depletion hypothesis has a number of interesting features that may provide new insights into both the mode of action of these drugs and the nature of the neural signalling defect that may be responsible for manic-depressive illness:

1. The central feature of the inositol depletion hypothesis is that Li^+ and valproate act to inhibit the supply of inositol required to maintain the inositol lipid signalling pathway. Cells in the periphery have access to dietary inositol that circulates in the plasma and is taken up by the sodium-dependent *myo*-inositol cotransporter-1 (SMIT1) (Module 2: Figure InsP₃/DAG recycling). However, this source is denied to neurons closeted behind the blood-brain barrier, which is relatively impermeable to inositol (Module 12: Figure Li^+ action). Therefore, since neurons rely on recycling and *de novo* synthesis, they are uniquely sensitive to Li^+ and valproate. Conversely, cells in the periphery are protected against inositol depletion induced by these two drugs by taking up inositol from the plasma.
2. The inhibition of the inositol monophosphatase by Li^+ occurs through an uncompetitive mechanism that has a very unusual consequence with regard to its drug action

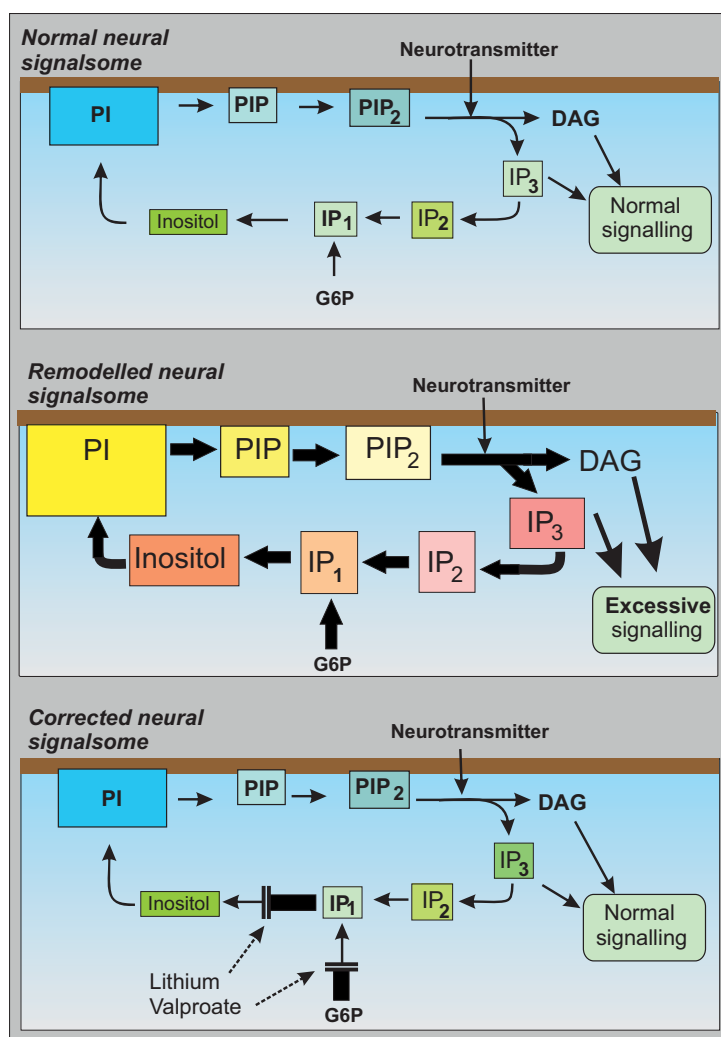
(Module 12: Figure uncompetitive inhibition). What this means therapeutically is that Li^+ will have little action when signalling pathways are operating normally, but will become increasingly efficacious the more abnormal signalling becomes (Module 12: Figure Li^+ increases IP₁ formation). Li^+ is the perfect homeostatic drug. If the system is operating normally, Li^+ has no effect. Once the system becomes overactive, it begins to exert its therapeutic action, where its efficacy is tailored to the severity of the disease state.

3. How does the inositol depletion hypothesis account for the long time lag for antidepressants to work? The depletion of inositol is thought to correct overactive signalling by suppressing the membrane levels of the inositol lipids. Since cells have a large reservoir of inositol lipid, which may be increased further during manic-depressive illness, it may take a long time for the lipids to be reduced to more normal operational levels. In addition, the signalling components whose expression was altered during the phenotypic modification may take time to be remodelled back to their normal levels (Module 12: Figure remodelling the neural signalsome). Finally, if the increase in phosphoinositide signalling has effects on neurogenesis, the delay may depend on the recovery of the hippocampal circuits, as discussed for the neurogenesis hypothesis.

Obesity

Obesity is not a disease in the strict sense, but has been included here because it brings with it a number of

Module 12: | Figure inositol depletion hypothesis

**Inositol depletion hypothesis.**

A key component of the inositol depletion hypothesis is that manic-depressive illness is caused by a remodelling of the neural signalsome, resulting in excessive signalling by the phosphoinositide signalling pathway. When operating normally (top panel), neurotransmitters induce a small hydrolysis of PtdIns(4,5)P₂ to give normal levels of inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG). The former is then recycled back to inositol, which is used to resynthesize PtdIns (PI). In manic depression, it is argued that a phenotypic modification has resulted in a remodelled neural signalsome, which is set too high and delivers excessive signals (middle panel). This remodelled signalsome is corrected by Li⁺ or valproate that both act to reduce the supply of the inositol required to resynthesize PI (bottom panel). Through this depletion of inositol, the overactive phosphoinositide signalling pathway returns to its normal operational level.

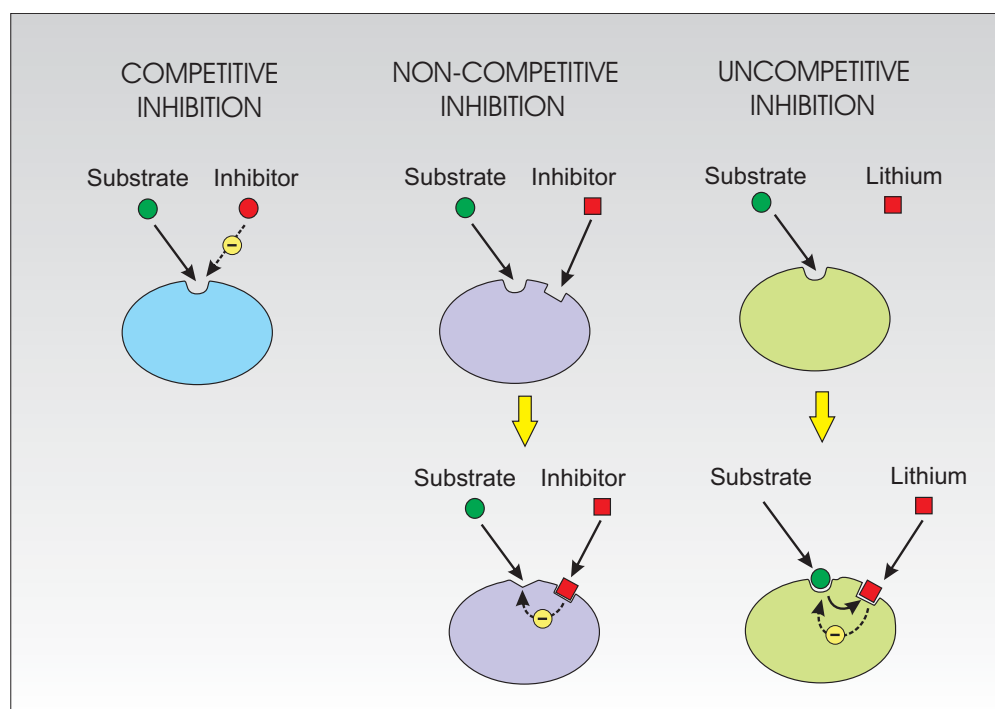
associated medical problems and has become one of the major health problems in the Western world.

Control of energy homeostasis is at the heart of the current obesity crisis. Obesity develops due to an imbalance in the metabolic energy network. (Module 7: Figure metabolic energy network) In particular, an excessive intake in calories reverses the normal balance that favours energy consumption over energy storage, resulting in a massive increase in lipid synthesis, and the white fat cells expand both in size and numbers to accommodate the large build-up of fat droplets. The ready availability of highly palatable energy-rich fatty food together with a decline in physical activity has conspired to increase the incidence of obesity. Within the human population, there is a natural variation in body weight. It is likely that those individuals that become obese may have defects in the homeostatic mechanisms that control food intake and body weight (Module 7: Figure control of food intake).

For some reason there are alterations in the signalling mechanisms that control the balance between the feeding and satiety centres that determine food intake. This view is supported by the finding that some of the rare genetic mutations that have been linked to human obesity are located in components of the control system that regulates food intake:

- Severe obesity results from either leptin deficiency or through defects in the leptin receptor Ob-Rb.
- Early-onset obesity develops in individuals carrying mutations in the gene that encodes the pro-opiomelanocortin (POMC) precursor used to form the α -MSH released from the POMC/CART neurons

Module 12: | Figure uncompetitive inhibition



Different types of inhibitory action.

Inhibitors can act in different ways. In competitive inhibition, they compete with a substrate for the same binding site. In non-competitive inhibition, the inhibitor binds to a separate site to induce a conformational change that inhibits the substrate-binding site. Finally, there is uncompetitive inhibition, where the binding site for the inhibitor appears only when the substrate is bound. This uncompetitive mechanism, which is the way that Li^+ works, introduces a usage-dependent aspect to the inhibition. At low substrate concentrations, there will be little enzyme-substrate interaction and there will be little inhibition by Li^+ , but as the substrate concentration increases, there will be a corresponding and progressive increase in its inhibitory action.

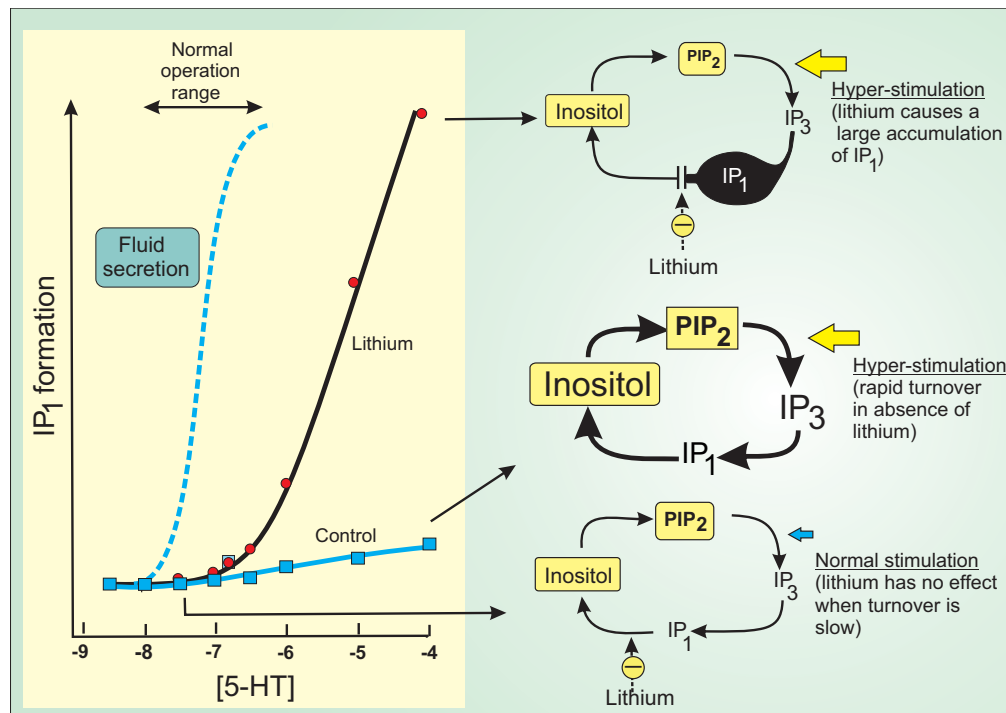
in the hypothalamus (Step 7 in Module 7: Figure control of food intake).

- Early-onset obesity develops in individuals carrying a mutation in the melanocortin receptor MC4R that carries out the action of the α -MSH released from the POMC/CART neurons in the hypothalamus (Step 7 in Module 7: Figure control of food intake).
- Haploinsufficiency of the *Sim1* gene, which codes for the transcription factor single-minded 1 (Sim1) that is activated by the MC4Rs on the second order neurons that relay information to the satiety centre (Step 7 in Module 7: Figure control of food intake), causes hyperphagic obesity.
- A susceptibility to obesity has been linked to an allele of the Rab GAP Tbc1d1, which reduces the insulin-sensitive GLUT4 translocation to the plasma membrane during the insulin control of skeletal muscle glycogen metabolism (see Module 7: Figure skeletal muscle E-C coupling).

The neurotransmitter 5-hydroxytryptamine (5-HT) is a potent suppressor of appetite. Some of its actions occur in the arcuate nucleus (ARC) where it simultaneously can inhibit the orexigenic NPY/AgRP neurons while activating the POMC/CART neurons (Module 7: Figure control of food intake). Analogues of 5-HT have been used to treat obesity.

Dysregulation of the endocannabinoid signalling system occurs during obesity and may contribute to some of the metabolic abnormalities, such as insulin resistance and the onset of Type 2 diabetes. In obesity or following feeding on high-fat diets, there is an up-regulation of this endocannabinoid signalling pathway, including an increase in the expression of CB1 receptors, which can have effects both centrally and in the periphery. With regard to the former, an increase in signalling through CB1 receptors in the hypothalamus can alter the balance between the orexigenic and anorexigenic neurons that control food intake and body weight (Module 7: Figure control of food intake). The endocannabinoid stimulation of appetite is associated with a decrease in the firing rate of the anorexigenic POMC/CART neurons. The peripheral effects of the increase in endocannabinoid activity is proposed to be the induction of insulin resistance in tissues such as the white fat cells, skeletal muscle and kidney cells (Module 12: Figure insulin resistance), which then leads on to the development of Type 2 diabetes.

The switch towards energy storage as fat during the onset of obesity is exacerbated by the positive-feedback loop that free fatty acids (FFAs) exert on the process of lipogenesis (i.e. Step 7 in Module 7: Figure metabolic energy network). This positive-feedback loop is based on the ability of fatty acids to stimulate the peroxisome-proliferator-activated receptor γ (PPAR γ), which up-regulates the components responsible for the uptake of FFAs and

Module 12: | Figure Li^+ increases IP_1 formation **Li^+ enhances the accumulation of inositol monophosphate (IP_1).**

As a consequence of its uncompetitive action, Li^+ exerts little inhibitory effect when the signalling pathway is operating within its normal operational range, but becomes increasingly effective as the signalling pathway is hyperstimulated. The graph on the left illustrates the accumulation of inositol monophosphate (IP_1) in the blowfly salivary gland in response to 5-hydroxytryptamine (5-HT) in the presence or absence of 1 mM Li^+ . The physiological response of fluid secretion is included to show that the gland goes from a quiescent to a fully active state between 1×10^{-8} and 1×10^{-7} M 5-HT. In the absence of Li^+ , there is very little accumulation of IP_1 , even at very high 5-HT concentrations. In the presence of Li^+ , however, there is a large accumulation of IP_1 , which begins to rise exponentially when the 5-HT concentration increases above 1×10^{-7} M. What is revealing is that over the normal operational range of 5-HT concentration that regulates fluid secretion, there was almost no effect of Li^+ on IP_1 accumulation. It was only when the glands were hyperstimulated that Li^+ began to inhibit the inositol monophosphatase, resulting in large accumulations of IP_1 .

their conversion into triacylglycerol. This strong positive-feedback loop is responsible for the onset of obesity, and is an example of phenotypic remodelling, which can lead to a number of serious disease states.

In addition to driving the increased rate of lipid synthesis, the activated $\text{PPAR}\gamma$ also stimulates white fat cell differentiation that results in the appearance of new fat cells, which add to the development of obesity.

Obesity is the cause of a whole host of medical problems; many of these are metabolic defects that fall under the umbrella of metabolic syndrome:

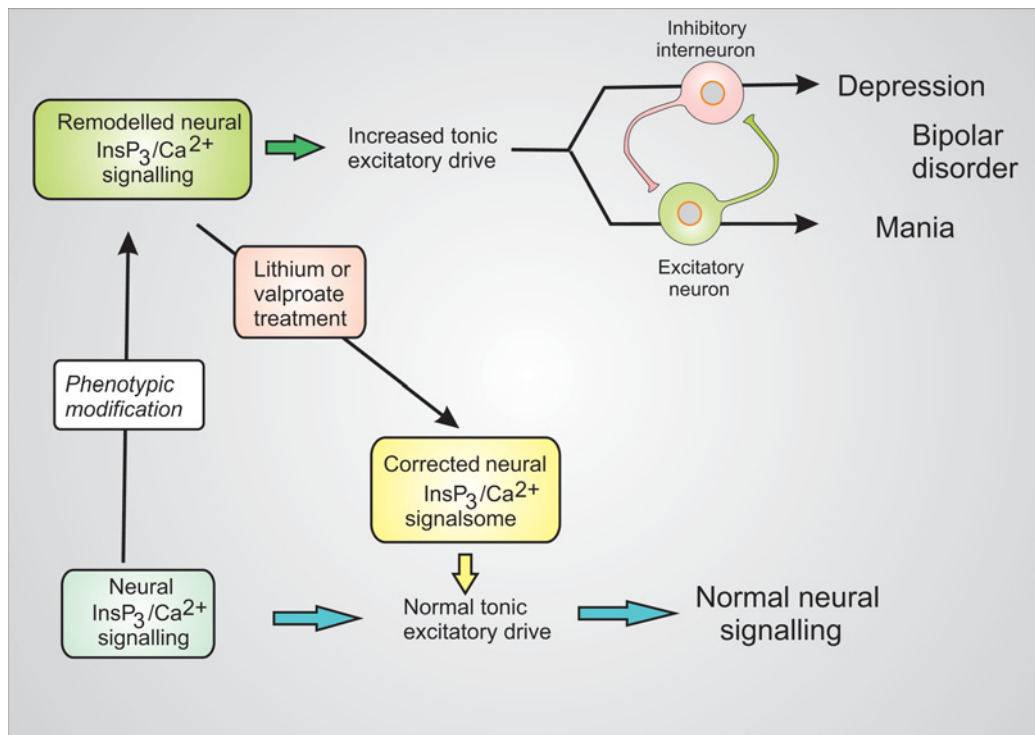
- Hypertension
- Heart disease
- Inflammatory hepatocellular adenomas (IHCAAs)
- Diabetes
- Atherosclerosis
- Blindness resulting from age-related macular degeneration, cataracts, glaucoma and diabetic retinopathy.

Obesity increases the risk of complications in pregnancy caused by a cholesterol-dependent increase in the activity of large-conductance (BK) channels that then reduces the activity of the uterus smooth muscle cell membrane oscillator that drive contractions during labour.

Metabolic syndrome

Metabolic syndrome, which is often brought on as a consequence of obesity, is a general term used to describe a combination of metabolic disorders such as dyslipidaemia (low HDL, high LDL and triglycerides), hypertension, high fasting glucose, insulin resistance and non-alcoholic fatty liver disease (NAFLD). These metabolic disorders greatly enhance the risk of developing Type 2 diabetes, atherosclerosis and heart disease. The problem with trying to understand this complex syndrome is the fact that it involves most of the major players that participate in the metabolic energy network (Module 7: Figure metabolic energy network). Energy derived from food intake enters the plasma from the intestine and is then distributed to cells involved in energy consumption, while any excess is dealt with by cells that function in energy storage. Under normal homeostatic conditions, the amount of energy stored in the white fat cells and muscle is not excessive and is readily mobilized in time of need. When food intake is excessive, the persistent storage of excess energy begins to overwhelm the energy storage cells, particularly the white fat cells. A decline in the ability of the white fat cells to store energy is exacerbated by the onset of insulin resistance, and the resulting elevation in plasma levels of fatty acids and glucose precipitates many of the disorders that fall under the umbrella of metabolic syndrome.

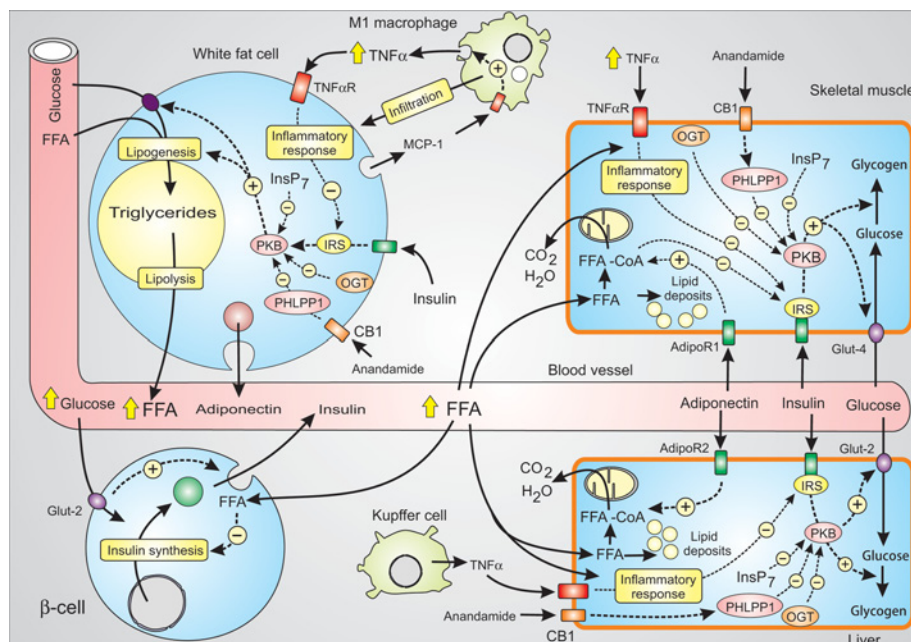
Module 12: | Figure remodelling the neural signalsome



Li^+ remodels the neural inositol 1,4,5-trisphosphate (InsP_3)/ Ca^{2+} signalsome.

The inositol depletion hypothesis suggests that the neural inositol 1,4,5-trisphosphate (InsP_3)/ Ca^{2+} signalsome that is responsible for normal neural signalling is remodelled so that it induces excessive metabotropic receptor signalling that leads to manic-depressive illness. Li^+ and valproate correct the signalsome, such that it can return to transmitting normal InsP_3 / Ca^{2+} signalling. The time taken to remodel this signalling system may account for the long time lag for Li^+ to exert its effects on manic-depressive illness.

Module 12: | Figure insulin resistance



Insulin resistance.

A build-up of free fatty acids (FFAs) initiates insulin resistance through a number of mechanisms that all impinge on changes in the insulin signalling pathway in the various cell types that play a role in energy storage, such as the white fat cells, skeletal muscle and liver. An inability to cope with the build-up of glucose in the blood places great pressure on the β -cells that often collapse resulting in the onset of diabetes. See the text for details of the proposed mechanisms that links excessive energy consumption to insulin resistance.

Insulin resistance

A major cause of metabolic syndrome is insulin resistance, which is often associated with obesity. As the term insulin resistance implies, there is a decline in the ability of the energy-storing cells to respond to insulin by storing energy either as triglycerides (white fat cells) or as glycogen (skeletal muscle and liver) (see steps 4, 7 and 8 in Module 7: Figure metabolic energy network). The inevitable rise in glucose places a great strain on the β -cells that attempt to adapt by producing more insulin, but this adaptive mechanism often fails, resulting in Type 2 diabetes. At the heart of the strong relationship between obesity and diabetes is the process of insulin resistance, which is still poorly understood. The problem is to understand how the build-up of fatty acids triggers a reduction in the activity of the insulin receptor (Module 2: Figure insulin receptor). Insulin resistance depends on a decline in some aspect of the PtdIns 3-kinase signalling pathway, which is used by cells to activate a number of metabolic events. A number of mechanisms have been proposed to explain how the build-up of fatty acids triggers the decline in insulin signalling, such as the onset of an inflammatory response, enhanced endocannabinoid signalling or enhanced O-linked β -N-acetylglucosamine (O-GlcNAc) transferase (OGT) activity (Module 12: Figure insulin resistance). Inhibition of protein kinase B (PKB), also known as Akt, by InsP₇ has also been implicated as a possible mechanism for insulin resistance.

The onset of insulin resistance and diabetes depends on functional interactions between a number of cell types (Module 12: Figure insulin resistance):

- White fat cell insulin resistance
- Skeletal muscle insulin resistance
- Liver cell insulin resistance
- β -Cell dysfunction during insulin resistance

White fat cell insulin resistance

The white fat cells are particularly important in initiating the events that result in the onset of insulin resistance because they play a key role in regulating the level of free fatty acids (FFAs). The FFAs and glucose are taken up by the white fat cells and converted into triglycerides that are stored in lipid droplets (Module 7: Figure white fat cells). This process of lipogenesis is enhanced by insulin, which will be elevated when the high levels of glucose stimulate the β cells to release this hormone. If the store of lipid becomes excessive, as occurs during obesity, the white fat cells are less able to cope with the supply of fatty acids that then begin to increase in the plasma and appear to be a major factor for the development of insulin resistance both within the fat cells and also in muscle and liver cells (Module 12: Figure insulin resistance).

Some of the metabolic disorders associated with insulin resistance may be caused by a low-grade inflammatory state. This is particularly the case in obesity where insulin resistance may arise from pro-inflammatory factors produced within adipose tissue both by the white fat cells and particularly by the M1 macrophages. This inflammatory hypothesis suggests that the build-up of fatty acids in cells

initiates a stress response that results in the formation of inflammatory mediators that can alter insulin signalling in a number of cells (Module 12: Figure insulin resistance).

In the case of the white fat cells, FFA accumulation results in the release of pro-inflammatory factors such as monocyte chemoattractant protein-1 (MCP-1). MCP-1 is a chemokine that is released by the fat cells and acts on the chemokine receptor CCR2 on M1 macrophages to increase their infiltration into adipose tissue where they begin to release tumour necrosis factor α (TNF α). The TNF α acts on the TNF α receptor (TNF α R) to set up an inflammatory response that contributes to insulin resistance by reducing the ability of the white fat cells to respond to insulin.

Another proposed mechanism for insulin resistance invokes a role for the endocannabinoids, such as anandamide, which act through CB1 receptors (Module 1: Figure endocannabinoids). In obesity or following feeding on high-fat diets, there is an up-regulation of this endocannabinoid signalling pathway including an increase in the expression of CB1 receptors. Just how this signalling system induces insulin resistance is not clear but it seems to depend on the modulation of the pleckstrin homology domain leucine-rich repeats protein phosphatase 1 (PHLPP1) that inhibits the enzyme PKB, which is a key component of the PtdIns 3-kinase signalling pathway responsible for mediating the actions of insulin (Module 12: Figure insulin resistance).

The signalling components of the insulin receptor are also the target for another proposed mechanism based on enhanced O-linked β -N-acetylglucosamine (O-GlcNAc) transferase (OGT) activity that carries out a protein glycosylation reaction that inhibits the activity of both protein kinase B (PKB) and the insulin receptor substrate (IRS). The idea is that increases in glucose and FFAs activate OGT that then acts to inhibit both PKB and IRS thus reducing both carbohydrate and lipid metabolism not only in white fat cells but also in muscle and liver cells (Module 12: Figure insulin resistance). An elevation of InsP₇, which also acts by inhibiting PKB, may also contribute to insulin resistance.

The resulting decrease in lipogenesis leads to a build-up of plasma fatty acids and this is enhanced by the white fat cells switching from lipid storage to the export of lipid as a result of an increase in lipolysis. The high levels of plasma FFAs is then thought to play a direct role in inducing both skeletal muscle insulin resistance and liver cell insulin resistance.

Skeletal muscle insulin resistance

The enhanced lipid load in skeletal muscle, which is manifest as a build-up of lipid droplets, is thought to contribute to insulin resistance through mechanisms that are still being worked out. One possibility is that the enhanced formation of fatty acyl-CoA and diacylglycerol (DAG) may result in the activation of protein kinase θ (PKC θ) that then phosphorylates the insulin receptor substrate (IRS). Another possibility is that the FFAs may enhance the inflammatory response that is activated by various cytokines.

The two effects of FFAs, the build-up of internal lipid and the enhancement of the inflammatory response is

thought to be responsible for reducing the activity of the insulin receptor signalling pathway responsible for the insulin control of skeletal muscle glycogen synthesis (Module 7: Figure skeletal muscle E-C coupling). Most of the actions of the insulin receptor are relayed through the insulin receptor substrate (IRS) to activate processes such as glucose entry and glycogen synthesis that function to store excess glucose as glycogen (Module 2: Figure insulin receptor). This anti-diabetic action of insulin is inhibited by serine/threonine phosphorylation carried out by kinases such as JNK (activated as part of the inflammatory response) and protein kinase θ (PKC θ) that is activated by the build up of fatty acyl-CoA. The resulting insulin resistance means that skeletal muscle cannot convert glucose into glycogen, resulting in hyperglycaemia.

Some of the other proposals to explain insulin resistance in skeletal muscle include the endocannabinoid signalling hypothesis, the protein glycosylation hypothesis, the elevation of InsP₇ as described earlier in the section on white fat cell insulin resistance or the activation of mitsugumin 53 which is an ER ubiquitin ligase that seems to function by targeting both the insulin receptor and the insulin receptor substrate (IRS) for ubiquitin-dependent degradation.

Mitsugumin 53 (MG53)

Mitsugumin 53 (MG53) belongs to the tripartite motif-containing (TRIM) family, which is expressed specifically in muscle (cardiac and skeletal). MG53 has been implicated in cardiac preconditioning, muscle membrane repair and in skeletal muscle insulin resistance. In the latter case, MG53 function by targeting both the insulin receptor and the insulin receptor substrate (IRS) for ubiquitin-dependent degradation.

Liver cell insulin resistance

The large concentrations of FFAs circulating in the blood also has a major deleterious effect on the metabolic role for the liver cell signalling mechanisms in regulating blood glucose. As in skeletal muscle, there appears to be a low-grade inflammatory response induced by the Kupffer cells (Module 7: Figure liver cell structure) that release TNF α . The latter may then set up an inflammatory response in the liver cells similar to that proposed for skeletal muscle insulin resistance. As for skeletal muscle, the elevated levels of FFAs and the resulting formation of diacylglycerol (DAG) results in the activation of protein kinase C ϵ (PKC ϵ) that phosphorylates the insulin receptor substrate (IRS) to prevent it from carrying out the action of insulin, and this will contribute to the build-up of blood glucose that occurs when insulin resistance develops in liver and skeletal muscle.

Some of the other proposals to explain insulin resistance in liver cells include the endocannabinoid signalling system, protein glycosylation or the elevation of InsP₇ as described earlier in the section on white fat cell insulin resistance.

β -Cell dysfunction during insulin resistance

The inability of skeletal muscle and liver to take up and store glucose as glycogen results in an increase in the plasma level of glucose. Initially, the insulin-secreting

β -cell responds to the hyperglycaemia by increasing the production of insulin, but with time the β -cells become 'exhausted' resulting in a dramatic decline in insulin secretion and the onset of Type 2 diabetes. The increase in FFAs may also contribute to the decline in insulin secretion by inhibiting insulin synthesis and secretion.

Osteoporosis

Osteoporosis is characterized by a decrease in bone mass and deterioration in the microarchitecture that results in bone fragility and a high susceptibility to fractures. There are a number of causes for this change in bone structure, and one of the commonest is the decrease in oestrogen in postmenopausal women. One of the functions of oestrogen is to suppress cytokines such as interleukin-1 (IL-1) and tumour necrosis factor (TNF), which act to promote osteoclastogenesis. Osteoporosis is also caused by a decrease in mechanical loading or as a complication of secondary hyperparathyroidism.

The gradual loss of bone that causes osteoporosis results from a shift in the normal bone cell coupling mechanism that maintains a balance between bone formation by the osteoblasts and bone resorption by the osteoclasts (Module 7: Figure bone cells).

Pain

There are two forms of pain. Acute pain is a physiological response to injury or inflammation that functions to prevent further injury. The other is chronic pain that can have different manifestations:

- Erythralgia
- Congenital insensitivity to pain
- Hyperalgesia
- Allodynia
- Neuropathic pain

Pancreatitis

There are two primary forms of pancreatitis, acute that begins suddenly and lasts for a few days or chronic that persists for many years. One of the major causes of pancreatitis is chronic alcohol abuse. Excess ethanol reduces the activity of the alcohol dehydrogenase (ADH) that is responsible for the oxidative metabolism of ethanol and results in an increase in the non-oxidative metabolism of ethanol to fatty acid ethyl esters (FAEEs). It is these non-oxidative metabolites of ethanol that have been implicated in the pathogenesis of various alcoholic diseases such as liver injury and pancreatitis. To understand how these FAEEs act to damage the pancreas it is necessary to consider the structure and function of the exocrine pancreas.

The primary function of the exocrine pancreas is to secrete digestive enzymes. The exocrine pancreatic acinar cells, which synthesize and release these enzymes, have a profuse rough endoplasmic reticulum (ER) and large accumulations of zymogen granules (Module 7: Figure exocrine pancreas). Most of the rough ER that synthesizes proteins is located in the basal region, but there are thin strands that percolate down into the apical region, where they play a critical role in producing the localized Ca²⁺ signals that

regulate both exocytosis and fluid secretion (Module 7: Figure control of pancreatic secretion). The FAEs that have been implicated in pancreatitis act by stimulating the type 2 and type 3 inositol 1,4,5- P_3 receptors (InsP₃Rs) on both the ER and the acidic store resulting in the intracellular activation of the trypsin stored in the zymogen granules. The activation of digestive enzyme within the cell then initiates a process of autodigestion resulting in the necrosis that characterizes pancreatitis.

Parkinson's disease (PD)

Parkinson's disease (PD) is a neurodegenerative disease characterized by muscular rigidity, poor balance, tremor and bradykinesia that is caused by the degeneration and death of the dopaminergic (DA) neurons located in the substantia nigra pars compacta (SNc), which is part of the dopaminergic arousal system (Module 10: Figure sleep/wake cycle regulation). It is not clear why this small group of neurons is so uniquely vulnerable, whereas the closely related DA neurons in the ventral tegmental area (VTA) do not die during PD. The puzzle is to determine why these SNc neurons are so vulnerable.

The single most important risk factor for Parkinson's disease (PD) is ageing. In addition to this age-dependent sporadic onset, there are familial forms of PD and many of these have been linked to mutations in genes that fall into two main groups: the autosomal-dominant genes such as α -synuclein (α -syn) and a number of autosomal recessive genes that influence mitochondrial function such as *PINK1*, *Parkin* and *DJ-1*. Another reason for suspecting the mitochondria has come from postmortem studies of the SNc cells in sporadic PD patients where defects have been described in mitochondrial complex I and this seems to result from the damage caused by mitochondrial reactive oxygen species (ROS) formation. Increased inflammation in Parkinson's disease is thought to increase the severity of the disease.

Much attention is now focused on what makes these SNc neurons so susceptible to mitochondrial damage resulting in the death of this subset of neurons. There is considerable support for a calcium and ROS hypothesis of Parkinson's disease. A strategy based on the reversal of Ca^{2+} -dependent neurodegeneration could provide novel therapies for treating PD.

Calcium and ROS hypothesis of Parkinson's disease

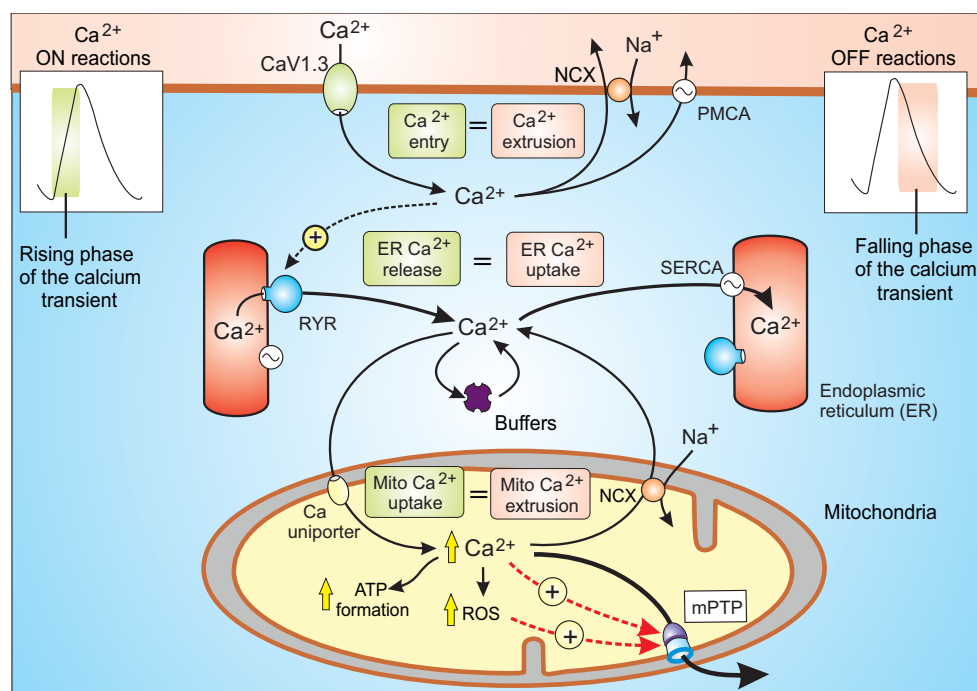
The basis of this calcium and ROS hypothesis of Parkinson's disease is that there is a dysregulation of Ca^{2+} homeostasis in the substantia nigra pars compacta (SNc) dopaminergic neurons that has an impact on the mitochondria to increase the formation of reactive oxygen species (ROS) creating oxidative stress and neuronal cell death. The dopaminergic (DA) neuronal pacemaker mechanisms of these SNc neurons generates regular pulses of Ca^{2+} every second that depend on both the entry and release of internal Ca^{2+} (Module 10: Figure tonic oscillation in DA neurons). During each transient, the Ca^{2+} ON reactions causes the Ca^{2+} to rise and this is followed by the onset of the Ca^{2+} OFF reactions that brings the concentration back to its resting level (Module 12: Figure calcium

homeostasis in DA neurons). For homeostasis to occur, these two reactions have to be balanced. The entry of Ca^{2+} through the Cav1.3 channels is balanced by extrusion of Ca^{2+} by the plasma membrane Ca^{2+} -ATPase (PMCA) and the Na^+/Ca^{2+} exchanger (NCX). Likewise, the amount of Ca^{2+} released from the endoplasmic reticulum (ER) by the ryanodine receptors (RYRs) during the rising phase is balanced by an equivalent amount of Ca^{2+} being pumped back into the ER by the sarco/endoplasmic Ca^{2+} -ATPase (SERCA) during the falling phase of the transient.

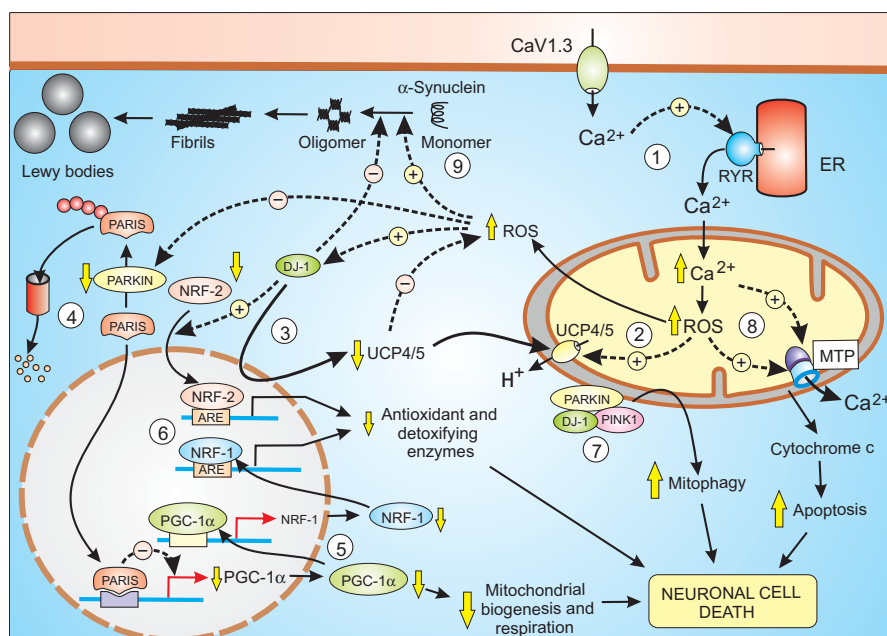
Each time the cytosolic level of Ca^{2+} rises during the course of a transient, Ca^{2+} is handled by the cytosolic buffers and mitochondria. The buffers play a relatively minor role because their concentration in these SNc dopaminergic neurons is low relative to other neurons. Much less is known about the dynamics of mitochondrial Ca^{2+} handling during the course of each transient. When the mitochondrion is fully hyperpolarized, the amount of Ca^{2+} taken up during each transient may exceed that being extruded during the recovery period resulting in an increase in the concentration of Ca^{2+} within the mitochondrial matrix. This elevation of Ca^{2+} may be part of a normal regulatory mechanism to increase the rate of oxidative phosphorylation for the mitochondrial generation of ATP (Module 5: Figure mitochondrial Ca^{2+} signalling) to maintain various neuronal functions. However, this increase in Ca^{2+} necessary to enhance mitochondrial energy metabolism comes with a price because it is usually associated with an increase in mitochondrial ROS formation (Module 12: Figure calcium homeostasis in DA neurons). The SNc neurons appear to balance this need for enhanced energy production and pathological ROS formation by ensuring that the level of Ca^{2+} does not rise too high (for further details see point 8 in dopaminergic pacemaker mechanisms) and by enhancing the antioxidant mechanism responsible for ROS metabolism. Temporary opening of the mPTP may be an important mechanism for maintaining mitochondrial Ca^{2+} homeostasis (Module 5: Figure mitochondrial flickers).

There is a sense that mitochondrial Ca^{2+} and ROS regulation in these SNc neurons is teetering on the verge of oxidative stress that can readily tip over into a pathological decline in neural activity and neuronal cell death characteristic of Parkinson's disease (PD). Support for this calcium and ROS hypothesis of PD has emerged from the finding that many PD susceptibility genes function in the mechanism that regulate mitochondrial Ca^{2+} and ROS dynamics with far reaching effects on processes throughout the cell as outlined below (Module 12: Figure signalling pathways in Parkinson's disease):

1. The Cav1.3 channels gate the entry of external Ca^{2+} that then activates the RYRs to release cytosolic Ca^{2+} from the endoplasmic reticulum (ER). Some of this cytosolic Ca^{2+} is taken up by the mitochondria where it can activate formation of both ATP and ROS (for details see Module 10: Figure tonic oscillation in DA neurons).
2. Pathological elevations of Ca^{2+} and ROS seem to be prevented by a protective mechanism that depends on

Module 12: | Figure calcium homeostasis in DA neurons**Dopaminergic neuronal calcium homeostasis.**

The Ca^{2+} ON reactions (shown in green) responsible for the rising phase of each transient are balanced by the OFF reactions responsible for the falling phase (for details see Module 10: Figure tonic oscillation in DA neurons). Both the cytosolic buffers and the mitochondria play a role in regulating Ca^{2+} dynamics during the course of each transient. Parkinson's disease may be caused by a dysregulation of this Ca^{2+} network that seems to have a major impact on mitochondrial metabolism resulting in excessive reactive oxygen species (ROS) formation.

Module 12: | Figure signalling pathways in Parkinson's disease**Parkinson's disease (PD) signalling defects in dopaminergic neurons.**

The signalling mechanisms of dopaminergic neurons located in the substantia nigra pars compacta (SNc) undergo numerous changes (as illustrated by the yellow arrows) that arise either spontaneously, as occurs in sporadic PD or they are induced by mutations of genes as occurs in familial PD. Many of these changes result in a reduction in mitochondrial function that is the main cause of neuronal cell death.

the ROS stimulating the opening of the uncoupling protein 4 (UCP4) and uncoupling protein 5 (UCP5) channels to leak H^+ that will depolarize the inner mitochondrial membrane potential thus reducing respiration and the entry of Ca^{2+} (for further details see point 8 in dopaminergic pacemaker mechanisms) (Module 10: Figure tonic oscillation in DA neurons).

3. The protein DJ-1 is a redox-sensitive protein that facilitates antioxidant defences in a number of ways. It can promote the migration of the redox-sensitive transcription factor nuclear factor erythroid 2-related factor 2 (NRF-2) into the nucleus where it activates the expression of many antioxidant and detoxifying enzymes (Module 4: Figure NRF-2 antioxidant function). In animal models of PD, the expression of NRF-2 is reduced, which could contribute to the elevation in ROS. The activation of glycogen synthase kinase-3 β (GSK-3 β) is enhanced and this could account for the reduction in NRF-2 function.

DJ-1 also enters the nucleus to increase the expression of the UCP4 and UCP5 channels. A decrease in the activity of DJ-1 caused by an autosomal-recessive mutation has been linked to a familial form of AD. Also, there are indications that DJ-1 can act as a chaperone to inhibit the aggregation of α -synuclein (α -syn).

Inhibition of the HDACs by phenylbutyrate has been shown to alleviate some of the symptoms of PD by increasing the expression of DJ-1.

4. Another redox-sensitive protein that is mutated in AD is the E3 ubiquitin ligase Parkin. One of the functions of Parkin is to ubiquitinate Parkin interacting substrate (PARIS) that is then subjected to proteasomal degradation. The activity of Parkin is reduced by ROS allowing PARIS to accumulate and enter the nucleus where it acts as a potent repressor of peroxisome-proliferator-activated receptor γ (PPAR γ) coactivator-1 α (PGC-1 α), which can regulate a number of different processes. In addition, Parkin can contribute to genotoxic stress activation of NF- κ B signalling, which increases the expression of optic atrophy 1 (OPA1) (Module 2: Figure NF- κ B activation). The OPA1 helps maintain healthy mitochondria by stabilizing the cristae to prevent the loss of cytochrome *c* and subsequent apoptosis (Module 5: Figure OPA1 and mitochondrial cristae remodelling).
5. One of the actions of PGC-1 α is to stimulate the expression of proteins involved in mitochondrial biogenesis and respiration. A decrease in the activity of this system will contribute to the onset of neuronal cell death. Another action of PGC-1 α is to stimulate the expression of the transcription factor nuclear response factor-1 (NRF-1).
6. NRF-1, like NRF-2 described earlier, is responsible for maintaining the levels of both antioxidants and detoxifying enzymes that protect against the deleterious effects of ROS. One of the enzymes it induces is GSH peroxidase (GPx) that uses the reducing power of glutathione (GSH) to convert H_2O_2 into water (Module 2: Figure H_2O_2 metabolism). A decrease in

the activity of these systems, which can be traced back to a ROS-dependent decline in the activity of Parkin and DJ-1, will contribute to the onset of the neuronal cell death that occurs during Parkinson's disease.

7. Another of the autosomal-recessive genes that has been implicated in familial PD is *PINK1* that codes for PTEN-induced putative kinase 1 (PINK1). Dissipation of the membrane potential in dysfunctional mitochondria results in the recruitment of PINK1, Parkin and DJ-1 and this complex then remodels the OMM that marks out such impaired mitochondria for removal by mitophagy. This quality control mechanism may help to protect cells by identifying and removing defective mitochondria.
8. Under conditions where the protective mechanisms are not able to regulate the formation of Ca^{2+} and ROS, these two will reach levels that will begin to activate the mitochondrial permeability transition pore (mPTP), which can have two consequences. Under normal conditions there appears to be a relationship between the temporary opening of the mPTP and mitochondrial Ca^{2+} homeostasis. Periodic opening of the mPTP results in mitochondrial flickers during which Ca^{2+} and ROS are released from the mitochondria (Module 5: Figure mitochondrial flickers). Failure of this safety valve mechanism results in a permanent opening of the mPTP, the release of cytochrome *c* and the activation of apoptosis (Module 5: Figure ER/mitochondrial shuttle) as is thought to occur in the later stages of PD when SNc neurons begin to die.
9. Mutations or overexpression of α -synuclein (α -syn) have been identified in PD but how they induce the degeneration of the substantia nigra pars compacta (SNc) neurons is still not clear. The elevation in ROS levels that occur in the early stages of PD may be responsible for converting α -synuclein (α -syn) from its normal monomeric form into the oligomers and fibres that end up in the Lewy bodies that are a characteristic feature of the degenerating SNc neurons.

Neurons may be protected from the deleterious effects of α -syn by its lysosomal degradation that seems to depend on the operation of the ALP pathway (see step 11 in Module 4: Figure membrane and protein trafficking).

Polymorphisms of hVps41, which is a key component of this trafficking pathway, increases the susceptibility of developing Parkinson's disease.

Inflammation in Parkinson's disease

Parkinson's disease (PD) is characterized by a build-up of α -synuclein (α -syn), particularly the oligomeric forms that can be released from oligodendrites and can activate microglial cells in the substantia nigra pars compacta (SNc). The microglia respond to α -synuclein by inducing the release of pro-inflammatory mediators such as tumour necrosis factor α (TNF α), interleukin-1 β (IL-1 β) and reactive oxygen species (ROS) (Module 7: Figure microglia interactions) that could contribute to the death of the resident dopaminergic (DA) neurons. Polymorphisms of the TNF α gene that result in enhanced levels of this cy-

tokine increase the risk of developing sporadic PD. The α -synuclein acts through Toll-like receptor 4 (TLR-4), which operate through the NF- κ B signalling pathway that has a number of actions such as the formation of the cytotoxic factors mentioned above. Another action of this signalling pathway is to inhibit the expression of Parkin, which will result in an increase in the levels of PARIS and a subsequent inhibition of antioxidant formation thus creating greater stress on the DA neurons and provides further evidence for the calcium and ROS hypothesis of Parkinson's disease (Module 12: Figure signalling pathways in Parkinson's disease).

α -Synuclein (α -syn)

α -Synuclein (α -syn) is located in neurons throughout the brain. Its function is still not clear, but it appears to play a role in membrane processes such as synaptic vesicle recycling and lipid interactions. It has come to prominence because some familial forms of Parkinson's disease (PD) seem to result from either mutations or overexpression of α -syn. Just how mutations in α -syn induce the degeneration of the dopaminergic neurons of the substantia nigra pars compacta (SNc), which is part of the dopaminergic arousal system, is still not clear. The mutated α -syn forms toxic oligomers and fibrils that aggregate and are a major component of the Lewy bodies, which are a pathological hallmark of diseased neurons in PD (Module 12: Figure signalling pathways in Parkinson's disease). It has also been suggested that the oligomers may combine to form pores in the plasma membrane to allow Ca^{2+} to enter the cell to overwhelm and damage the mitochondria.

Mitochondrial reactive oxygen species (ROS) formation may contribute to α -syn aggregation during the development of sporadic PD.

Leucine-rich repeat kinase (LRRK)

Mammals have two homologues of leucine-rich repeat kinase (LRRK1 and LRRK2). Particular attention has focused on LRRK2 because gain-of-function mutations of this kinase are a strong risk factor for Parkinson's disease (PD). LRRK2 is widely expressed and is a multidomain protein. The central region of the protein has the kinase domain that lies next to a region that might function in GTP-binding and hydrolysis, which consists of two domains: a GTP binding Ras of complex (ROC) and a carboxy-terminal of ROC (COR) domain. On either side of this central catalytic domain there are various protein-protein interaction domains such as ankyrin repeats, leucine-rich repeats and WD40 domains. Most of the PD mutations are located in the central GTPase and kinase domains. LRRK2 functions as a dimer and there are indications that the kinase domain can phosphorylate the ROC domain.

One of the possible functions of LRRK2 is to regulate neurite growth during development.

Premature labour

The critical phase of labour is the activation of the endogenous electrical pacemaker activity that drives the periodic contractions of the uterus necessary to expel the foetus. There are serious consequences for the foetus if

labour begins before development is complete. Premature labour is a major cause of neonatal morbidity and children born prematurely are often disabled and may face long-term health problems. There is an urgent need to develop better ways of treating this early onset of labour. One of the problems is that relatively little is known about the nature of the membrane oscillator that drives labour. A uterus smooth muscle cell membrane oscillator model has been developed that attempts to integrate some of the major signalling components that control uterus contractility during labour (Module 7: Figure uterus activation). A remarkable feature of uterine smooth muscle cells is that they undergo a phenotypic remodelling of their signalsome. There is a rapid switch from relative quiescence to a cycle of strong contractions during labour. For example, there is an increase in the expression of PDE4B, which decreases the effect of tocolytic agents such as β -adrenergic agents that use cyclic AMP to inhibit uterine contractions. There is an increase in the expression of the oxytocin receptors that act by inducing and accelerating contractions. The expression of the ryanodine receptor 2 (RYR2) is up-regulated to facilitate the process of excitation-contraction coupling, whereas the Na^+ - K^+ -ATPase pump activity declines and this helps to accelerate the uterus smooth muscle cell membrane oscillator. It would seem, therefore, that premature labour may result if the timing of these changes in the signalsome is advanced such that uterine excitability is enhanced before term.

Rheumatoid arthritis

Rheumatoid arthritis is a complex disease that is likely to have many causes. One cause is the onset of chronic inflammatory responses. There also is considerable interest in the role of the matrix metalloproteinases (MMPs) that play a critical role in tissue remodelling. Release of MMPs by fibroblast-like synoviocytes (FLSs) that produce synovial fluid may be controlled by thioredoxin (Trx), which functions in the recovery of oxidation-sensitive processes (Module 2: Figure recovery of protein oxidation). The extracellular Trx may reduce a disulphide bond on the TRPC5 channels that are used to activate FLS secretion. There are indications that concentrations of Trx are highly elevated during rheumatoid arthritis.

Enhanced inflammatory responses may also occur as a result of the decreased levels of GRK2 and GRK6, which are members of a family of G protein receptor kinases (GRKs) that act to desensitize G protein-coupled receptors (GPCRs).

Schizophrenia

Schizophrenia is a severe psychiatric condition that affects about 1% of the human population. It has a number of characteristic features. There are both positive symptoms, such as hallucinations and paranoia, and negative symptoms (poor attention, decline in social interactions and lack of motivation). One of the most consistent features is a decline in cognition. Alterations in these higher-order brain functions seem to depend on changes in brain rhythms. Multiple genetic susceptibility elements have also been identified and many of these have been included in

the following hypothesis that attempts to integrate much of this genetic and physiological evidence into a unifying concept that has the following key elements:

- Brain rhythms and schizophrenia: schizophrenia is caused by subtle changes in the brain rhythms responsible for driving processes such as perception, memory and consciousness.
- Inhibitory interneuronal defects in schizophrenia: these changes in brain rhythms are caused by defects in fast-spiking inhibitory interneurons that occur during development or can be induced in the adult brain by factors such as drug use.
- NMDA receptor hypofunction in schizophrenia: a decrease in the activity of NMDA receptors results in Ca^{2+} -dependent phenotypic remodelling of the inhibitory interneurons to reduce release of the inhibitory neurotransmitter GABA that controls oscillatory activity.
- Redox signalling and schizophrenia: the function of the inhibitory interneurons is particularly sensitive to redox balance and an increase in oxidative stress may contribute to hypofunction of the NMDA receptors.
- Schizophrenia-associated gene mutations: a large number of mutations have been linked to schizophrenia and the challenge is to determine how alterations in these genes contribute to the symptoms of this disease.

Brain rhythms and schizophrenia

Most of the symptoms of schizophrenia can be explained by alterations in the brain rhythms responsible for the global integration of information processing during sensory stimulation, attention selection and working memory. With regard to the latter, there is a loss of the executive control that operates working memory, which depends on storing bits of information for short periods in order to control a particular train of thought or a behavioural response. The assembly of these different bits of information, which are drawn from memories stored in different brain regions such as the hippocampus and cortex, depends on the activity of these brain regions being synchronized. The increase in such synchronization during the operation of working memory is one of the changes that occur in schizophrenia. Brain imaging studies have identified the dorsolateral prefrontal cortex (DLPFC) as one of the brain regions where such deficits occur. The operation of working memory depends on the pyramidal neurons of the DLPFC firing in a sustained and synchronous manner in the gamma frequency range of approximately 40 Hz. Such oscillatory activity is a characteristic feature of the neural circuits in many regions in the brain (Module 10: Figure brain circuitry and rhythms). An important feature of the neuronal oscillations in any one brain region is that they are often synchronized with those in other regions. For example, hippocampal theta oscillations can modulate the phase of the neocortical oscillation so that their theta oscillations are in phase with each other. This brain rhythm synchronization is impaired in schizophrenia. The fact that the adult patterns of brain rhythms emerge gradually during the end of adolescence may explain why the defects

in the neural circuitry responsible for the symptoms of schizophrenia begin to emerge at this time. These alterations in brain rhythms are caused by inhibitory interneuronal defects in schizophrenia.

Inhibitory interneuronal defects in schizophrenia

Brain rhythms are generated by network oscillators based on the neural circuits located primarily within the hippocampus and cortex. A typical example of such an oscillator is the hippocampal gamma oscillation, which illustrates the role of fast-spiking interneurons (Module 10: Figure gamma oscillatory mechanisms). As part of the network oscillator, these inhibitory interneurons receive an excitatory input based on glutamate that acts on AMPA receptors, NMDA receptors and glutamatergic receptors. The interneurons are excited by this glutamatergic input to release GABA, which then inhibits the pyramidal neurons to complete the oscillatory cycle. The operation of this oscillator depends on a precise synaptic communication between the pyramidal neurons and the inhibitory interneurons. Abnormal brain development occurring early during gestation results in the onset of schizophrenia later in life. Indeed, such children often display cognitive and behavioural impairments before they go on to develop schizophrenia. The early alterations in neural circuitry seem to result from viral infections during pregnancy or obstetric complications, or are due to the influence of the various genetic abnormalities that have been associated with schizophrenia. Some of these genes function in GABAergic neurotransmission, which is consistent with the notion that schizophrenia results from defects in the operation of the inhibitory interneurons. This possibility has been strengthened by the observation that NMDA receptor antagonists, such as phencyclidine (PCP) and ketamine, can induce schizophrenic symptoms in healthy adults. Such observations have focused attention on a role for NMDA receptor hypofunction in schizophrenia.

NMDA receptor hypofunction and schizophrenia

Hypofunction of the NMDA receptors on the inhibitory interneurons appears to be one of the primary causes of schizophrenia and also offers an explanation for the action of many of the genes that have been associated with schizophrenia. The inhibitory interneuronal defects in schizophrenia caused by a decline in the NMDA receptor signalling pathway is an example of signalsome remodelling and disease (Module 12: Figure signalsome remodelling). In the interneuron, the NMDA receptor channel consists of NR1 and NR2A subunits. The NR2A subunit has the glutamate-binding site whereas NR1 responds to D-serine and glycine, which function as co-agonists to activate channel opening (Module 12: Figure schizophrenia). A reduction in the level of D-serine has been reported in the blood and cerebrospinal fluid (CSF) of individuals with schizophrenia. Such reductions may have been caused by mutations in D amino acid oxidase activator (DAOA), which functions to enhance the D amino acid oxidase (DAO) that metabolizes D-serine.

Activation of the NMDA receptor is not only responsible for providing some of the excitatory postsynaptic

potentials (EPSPs) that establish gamma oscillations (Module 10: Figure gamma oscillatory mechanisms), but they also appear to play a central role in maintaining the GABAergic phenotypic, which enables these interneurons to carry out their inhibitory role as part of the membrane oscillator that drives gamma oscillations. As outlined below, multiple causes have been implicated in the subtle modifications of the GABAergic interneuron phenotype that reduces their responsiveness to glutamate operating through the NMDA receptor and distorts the mechanism for generating brain oscillations (Module 12: Figure schizophrenia):

1. The *neuregulin-1* gene encodes neuregulin-1 (NRG1), which is a stimulus for the EGF receptor family (Module 1: Figure EGF stimuli and receptors). NRG1 has been associated with schizophrenia and this is of potential interest because ErbB regulates the expression of various signalling components that are related to NMDA receptor function. It regulates expression of the NMDA receptor itself, the scaffolding protein PSD95 that links the NMDA receptor to nNOS (Module 10: Figure postsynaptic density) and the nicotinic acetylcholine receptor (nAChR), which regulates the release of glutamate from the presynaptic endings. The NRG1/ErbB signalling pathway may thus contribute to establishing the GABAergic phenotype of the inhibitory interneurons.
2. The expression of brain-derived neurotrophic factor (BDNF) and its receptor TrkB are reduced in schizophrenia and this may have a major impact on the function of the inhibitory interneurons. The signalling pathways induced by TrkB receptors control the expression of a number of the components that could account for the phenotypic alterations that disrupt the function of the inhibitory interneurons, particularly during early development. The TrkB receptors are typical protein tyrosine kinase-linked receptors (PTKRs) that are coupled to a number of signalling pathways (Module 1: Figure tyrosine kinase-linked receptors). One of these is the mitogen-activated protein kinase (MAPK) signalling pathway that uses Erk1/2 to activate the transcription factor CREB (Module 4: Figure CREB activation). TrkB receptors also activate the PtdIns 3-kinase signalling pathway, which also functions in controlling gene transcription by removing the inhibitory action of FOXO (Module 4: Figure FOXO control mechanisms).
The signalling pathways used by the TrkB receptors are known to play an important role in the function of Ca^{2+} in synaptic plasticity (Module 10: Figure Ca^{2+} -induced synaptic plasticity) and may thus contribute to the defects in cognition that occurs in schizophrenia. This possibility is strengthened by the observation that disrupted in schizophrenia (DISC1), can interact with glycogen synthesis kinase 3β (GSK3 β) to inhibit its ability to activate the transcription factor β -catenin (Module 12: Figure schizophrenia). The activity of GSK3 β is controlled by phosphorylation through PKB/Akt, which is the downstream target of the PtdIns 3-kinase pathway. Both PKB/Akt and DISC1 have been implicated in schizophrenia.
3. One of the functions of NMDA receptors in adult life is to maintain the GABAergic phenotype, which seems to depend on the entry of external Ca^{2+} that then acts through CaMKIV in the nucleus to phosphorylate the transcription factor CREB to control the expression of signalling components similar to those activated by the TrkB receptors. Hypofunction of the NMDA receptors results in a reduction in the expression of a number of the components that are known to be reduced and can thus account for the following phenotypic alterations of the interneurons in schizophrenia.
4. There is a decrease in the expression of glutamic acid decarboxylase 67 (GAD67) that synthesizes the inhibitory transmitter GABA. This GABA is packaged into vesicles that are transported to the presynaptic terminals where they are released as part of the network oscillatory mechanism. A reduction in the level of GAD67 and the resulting decreased availability of GABA is one of the most characteristic features of schizophrenia. The transport of GABA-containing vesicles down the axon to the presynaptic ending is mediated by the dynein motor, which travels down microtubules (Module 4: Figure dynein). One of the genes mutated in schizophrenia is disrupted in schizophrenia 1 (DISC1), which functions as a dynein adaptor. The mutations in DISC1 will thus disrupt multiple neuronal processes such as mitosis, neuronal migration, neurite formation and axon elongation, as well as the transport of GABA vesicles. As outlined in the next point, some of the phenotypic changes that have been recorded in the interneurons seem to be compensatory responses caused by the primary defect in the expression of GAD67.

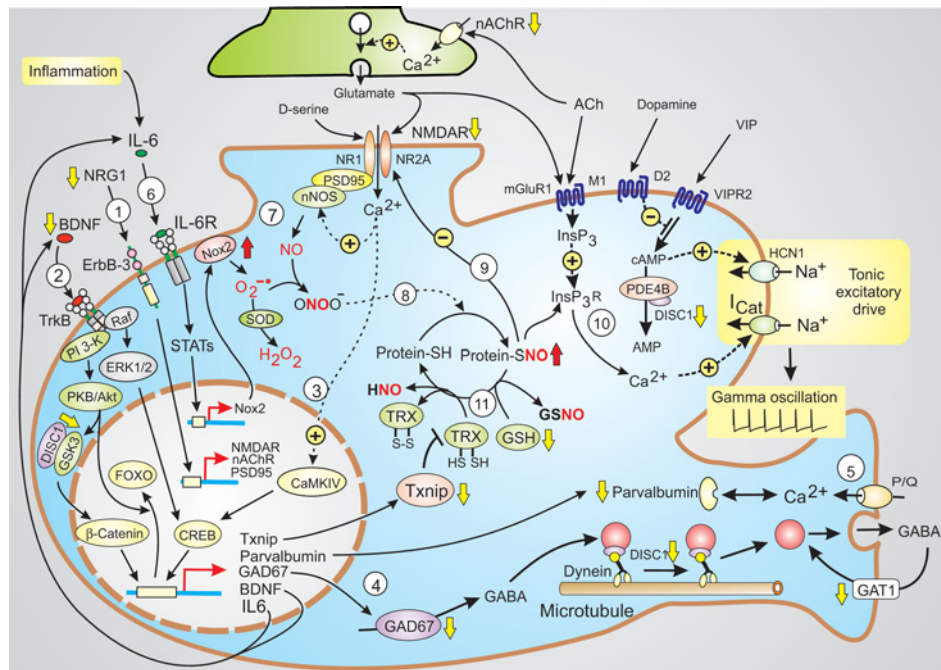
5. The release of GABA is controlled by a brief pulse of Ca^{2+} resulting from the entry of Ca^{2+} through $\text{Ca}_v2.1\text{P/Q}$ type channels, which are located close to the exocytotic vesicles in order to increase the speed of the inhibitory response. This pulse of signal Ca^{2+} is rapidly buffered by parvalbumin, which thus reduces the process of facilitation that depends on the build-up of Ca^{2+} during synaptic activity. In schizophrenia, there is a reduction in the level of parvalbumin and this will enhance facilitation and may be a compensatory response to the reduction in GABA levels caused by the decrease in GAD67. Another compensatory mechanism depends on a decrease in the expression of the GABA membrane transporter 1 (GAT1), which is located in the presynaptic ending where it functions to return GABA back into the interneuron. A reduction in this removal mechanism will help to enhance the activity of the reduced amount of GABA being released in schizophrenia. Another compensatory mechanism concerns the makeup of the GABA_A receptors on the pyramidal cells. These receptors are pentameric heterooligomers formed by mixing together different subunits (Module 3: Table receptor-operated channel toolkit). In schizophrenia, the proportion of the $\alpha 2$ -subunit, which is the predominant α -subunit expressed in the pyramidal neurons, is greatly increased. This may contribute to the compensatory mechanisms because these $\alpha 2$ -subunits have a high affinity and faster activation times that will enhance the activity of the reduced amounts of GABA being released from the interneurons.

Redox signalling and schizophrenia

The fast spiking inhibitory interneurons in the cortex seem to be particularly sensitive to the redox state of the brain. An increase in the oxidation state of these inhibitory neurons has been linked to the onset of schizophrenia. Of particular interest is the fact that dysregulation of the redox signalling pathway may provide an explanation for the developmental origins of schizophrenia because there appears to be a link between maternal viral infection during gestation and the incidence of this disease in the offspring. In rat models that reproduce this phenomenon, the developmental defects induced by such inflammatory responses are caused by activation of redox signalling. An increase in oxidation has a detrimental effect on the phenotypic stability and function of the inhibitory interneurons particularly with regard to the operation of the NMDA receptor as outlined in Module 12: Figure schizophrenia:

6. During viral infections, there is an increase in interleukin-6 (IL-6), which has a prominent role in activating the redox signalling pathway. IL-6 acts through the JAK/STAT signalling pathway and this may explain its ability to increase the expression of Nox2, which is one of the NADPH oxidases responsible for generating superoxide ($\text{O}_2^{\bullet -}$) (Module 2: Figure plasma membrane ROS formation).
7. Both the superoxide ($\text{O}_2^{\bullet -}$) and the hydrogen peroxide (H_2O_2), which is formed from $\text{O}_2^{\bullet -}$ by superoxide dismutase (SOD), can function as second messengers during the operation of the reactive oxygen species (ROS) signalling pathway (Module 2: Figure summary of redox signalling). These reactive oxygen species ($\text{O}_2^{\bullet -}$ and H_2O_2) can also react with nitric oxide (NO) to form peroxynitrite (ONOO^-), which is very much more reactive than the two parent molecules. The NMDA receptor is particularly important in generating NO because it is physically linked to the nNOS in the post-synaptic density through the scaffolding protein PSD95 (Module 10: Figure postsynaptic density). The nNOS is thus ideally positioned to respond to the pulses of Ca^{2+} , which enter through the NMDA receptor, to generate NO that then interacts rapidly with $\text{O}_2^{\bullet -}$ to form ONOO^- (Module 12: Figure schizophrenia).
8. The ONOO^- then acts through the reactive nitrogen species (RNS) signalling pathway that depends on the nitrosylation reaction to oxidize various key proteins (Module 2: Figure NO and cGMP signalling).
9. Nitrosylation of the NR2A subunit at Cys-399 inhibits channel open probability and thus reduces the ability of the NMDA receptor to generate Ca^{2+} signals and this has a number of consequences. Since there is a relationship between Ca^{2+} and synaptic plasticity (Module 10: Figure Ca^{2+} -induced synaptic plasticity), this oxidation-induced reduction in NMDA receptor function may have a direct bearing on the cognitive defects in schizophrenia. Another action of Ca^{2+} is to enhance gene transcription by activating nuclear CaMKIV. The constant input of Ca^{2+} coming from the repetitive activation of the NMDA receptor, as part of the membrane oscillator, may be responsible for the signalsome stability of the interneuronal phenotype by activating the transcription factor CREB (see step 3 in Module 12: Figure schizophrenia).
10. The activity of the inhibitory interneurons may also be influenced by nitrosylation of other proteins, such as those that have been implicated in the tonic excitatory drive responsible for generating brain rhythms as described for the hippocampal gamma oscillation (Module 10: Figure gamma oscillatory mechanisms). The cholinergic system, which plays an important role in providing this tonic drive (Module 12: Figure schizophrenia), has two roles. First, acetylcholine can facilitate the release of glutamate as it does in the case of the mossy fibre presynaptic Ca^{2+} release mechanism where acetylcholine acting through ionotropic nicotinic acetylcholine receptors (nAChRs) enhances the release of glutamate (Module 10: Figure mossy fibre presynaptic release). In patients with schizophrenia, the expression of nAChRs is reduced and this could contribute to hypofunction of glutamatergic signalling in the interneurons. Secondly, ACh also acts through M1 receptors to increase the formation of InsP_3 , which may act through Ca^{2+} to enhance the tonic drive. Nitrosylation of the InsP_3 receptor (InsP_3) can enhance the amount of Ca^{2+} being released from the internal store and this may contribute to the mechanisms responsible for the excitatory drive. Similarly, nitrosylation and inactivation of potassium channels may help to increase

Module 12: | Figure schizophrenia



Functional modulation of GABAergic interneurons in schizophrenia

This Figure summarizes some of the signalling mechanisms of the fast-spiking GABAergic interneurons that are thought to be altered in schizophrenia. Some components have reduced activity (yellow arrows), whereas others are increased (red arrows), usually as part of a compensatory mechanism. See the text for further details.

the depolarization necessary to drive the oscillatory mechanism.

11. The role of enhanced oxidation in driving the phenotypic alterations of the inhibitory interneurons is also consistent with studies on the changes in the antioxidant mechanisms that have been described in schizophrenia. The denitrosylation reaction, which functions to reverse the nitrosylation reactions, is carried out by two main antioxidants: glutathione (GSH) and thioredoxin (TRX) (Module 2: Figure NO and cGMP signalling).

In schizophrenia, gene polymorphisms have been described in the enzymes responsible for the synthesis of GSH, such as the GCL catalytic subunit (GCLC) and a GCL modifier subunit (GCLM), that combine to form the glutamate cysteine ligase (GCL) responsible for one of the steps of GSH synthesis. Such defects in GSH synthesis may explain the decreased GSH levels found in schizophrenia. Such a decline in the level of GSH may contribute to the decline in NMDA receptor function that reduces the activity of the fast-spiking interneurons (Module 12: Figure schizophrenia).

The NMDA receptor plays an important role in regulating the redox state by reducing the expression of the thioredoxin-interacting protein (Txnip), which binds to Trx and inhibits its participation in the denitrosylation reaction. Hypofunction of the NMDA receptor may thus contribute to the increased redox signalling that occurs in schizophrenia.

Schizophrenia-associated gene mutations

Large numbers of gene mutations have been identified that have been linked to psychiatric disorders such as schizophrenia. The way in which alterations in some of these genes contribute to this neural disease is outlined below:

- The *MIR137* gene, which encodes the microRNA miR-137, has been implicated in neurogenesis and neuronal maturation. Some of the targets of miR-137 are *CACNA1C*, *CSMD1*, *C10orf26* and *TCF4*
- Disrupted in schizophrenia 1 (*DISC1*) is a rare but highly penetrant mutation.
- *TCF4* is a transcription factor that regulates neuronal progenitor cell development.
- The *CACNA1C* gene encodes the α_{1C} subunit of the $\text{Ca}_v1.2$ L-type channels (Module 3: Figure $\text{Ca}_v1.2$ L-type channel). Mutations in this gene have also been linked to bipolar disorder and to Timothy's syndrome.
- The *CACNB2* gene codes for the β_2 subunit, which is one of the cytoplasmic Ca^{2+} channel β subunits (*CACNBs*) that modulate the activity of the $\text{Ca}_v1.2$ L-type channels (Module 3: Figure $\text{Ca}_v1.2$ L-type channel).

Sudden infant death syndrome (SIDS)

The cause of sudden infant death syndrome (SIDS) is still being investigated. It seems likely that it may be caused by a defect in the regulatory mechanism that operates on the respiratory centre to control breathing (Module 10: Figure breathing control). During hypoxia, the ac-

cumulation of CO_2/H^+ activates the respiratory centre to increase the rate of breathing including a gasping response. In SIDS, there appears to be a decrease in the 5-hydroxytryptamine (5-HT) receptors that contribute to the regulation of breathing.

Zollinger–Ellison syndrome

Zollinger–Ellison syndrome, which is associated with significant gastric hyperplasia is caused by an increase in the formation and release of the hormone gastrin by the G cells in the antrum of the stomach (Module 7: Figure stomach structure).

Genotypic remodelling of the signalsome

Genotypic remodelling of the signalsome is another important cause of disease (Module 12: Figure phenotypic and genotypic remodelling). There are two ways in which the genotype can be remodelled. There are somatic mutations, where a gene in a single cell undergoes a mutation that may profoundly alter the setup of the signalsome. A good example of this is cancer, where somatic mutations of different components of the growth factor-mediated signalling pathways that normally function to control cell fate during the cell cycle (Module 12: Figure cell cycle network and cancer) give rise to a signalsome that drives proliferation continuously, leading to the formation of tumours.

The second form of genotypic remodelling results from germline mutations that are passed on from one generation to the next and are responsible for inherited diseases. Many of these are channelopathies associated with defects in ion channels.

Examples of such inherited disease states that are associated with such genotypic remodelling of the signalsome include:

- Achromatopsia
- Acute periodic paralysis
- Acute promyelocytic leukaemia (APL)
- Albinism
 - Oculocutaneous albinism type 1 (OCA1)
 - Oculocutaneous albinism type 2 (OCA2)
 - Oculocutaneous albinism type 3 (OCA3)
- Alport syndrome, mental retardation, midface hyperplasia and elliptocytosis (AMME)
- Amyotrophic lateral sclerosis (ALS)
- Anderman's disease
- Andersen-Tawil syndrome
- Angelman syndrome (AS)
- Arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2)
- Ataxia telangiectasia (AT) syndrome
- Autism spectrum disorders (ASD)
- Autosomal dominant hypocalcaemia (ADH)
- Bardet-Biedl syndrome
- Bartter's disease
- Bernard-Soulier syndrome
- Brody disease
- Bruton's type X-linked agammaglobulinaemia

- Cancer
- Catecholergic polymorphic ventricular tachycardia (CPVT)
- Central core disease (CCD)
- Centronuclear myopathy (CNM)
- Charcot-Marie-Tooth disease
 - Charcot-Marie-Tooth disease 2A
 - Charcot-Marie-Tooth disease 2B
 - Charcot-Marie-Tooth disease 4B
 - Charcot-Marie-Tooth disease 4J
- Choroideremia
- Concurrent generalized epilepsy with paroxysmal dyskinesia
- Congenital chloride diarrhoea
- Congenital hyperinsulinism of infancy
- Congenital insensitivity to pain
- Congenital lipoid adrenal hyperplasia
- Cowden's disease
- Craniofrontonasal syndrome (CFNS)
- Cystic fibrosis (CF)
- Darier's disease
- Dent's disease
- Dominant nonsyndromic deafness type 2 (DFNA2)
- Down's syndrome
- Dravet syndrome
- Dubin-Johnson syndrome
- Early-onset obesity
- Emery-Dreifuss muscular dystrophy
- Episodic ataxia type 2
- Erythralgia
- Familial adenomatous polyposis (FAP)
- Familial advanced sleep phase syndrome (FASPS)
- Familial Alzheimer's disease (FAD)
- Familial amyloidosis
- Familial cardiomyopathies
- Familial episodic pain syndrome
- Familial expansile osteolysis
- Familial hemiplegic migraine
- Familial hemophagocytic lymphohistiocytosis type 3 (FHL3)
- Familial hypocalciuric hypercalcaemia (FHH)
- Familial exudative vitreoretinopathy (FEVR)
- Familial focal segmental glomerulosclerosis (FSGS)
- Fanconi anaemia
- Fragile X syndrome (FXS)
- Francois-Neetens mouchette fleck corneal dystrophy (CFD)
- Frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17)
- Gitelman's disease
- Glanzmann's thrombasthenia
- Glaucoma
- Glycogen storage disease
- Gordon's disease
- Gorlin's syndrome
- Griscelli syndrome (GS)
- Hailey-Hailey disease
- Hearing loss
- Hemorrhagic telangiectasia-2 (HHT2)

- Hereditary sensory and autonomic neuropathies (HSAN)
- High bone mass syndrome (HBM)
- Huntington's disease
- Huntington's disease-like 2 (HDL2)
- Hutchinson–Gilford progeria syndrome (HGPS)
- Hyper-IgE syndromes (HIES)
- Hypogonadotropic hypogonadism
- Hypokalaemic periodic paralysis (HPP)
- Hypomagnesaemia hypercalciuria syndrome
- Hypomagnesaemia with secondary hypocalcaemia
- Idiopathic infantile hypercalcemia
- Infertility
- Inflammatory hepatocellular adenomas (IHCA)
- Kindler syndrome
- Lambert–Eaton myasthenic syndrome
- Leber congenital amaurosis (LCA)
- Liddle's disease
- Limb girdle muscular dystrophy type 2A
- Lissencephaly
- Long QT syndrome
- Lowe's oculocerebrorenal (OCRL) syndrome
- Malignant hyperthermia
- MALT lymphomas
- Martsolf syndrome
- Medulloblastomas
- Mental retardation
- Microcephaly
- Microvillus inclusion disease
- Miller–Dieker syndrome
- MORM syndrome
- Mucopolidosis
- Muscular dystrophy
- Myotonia congenita
- Neonatal severe hyperparathyroidism (NSHPT)
- Nephrogenic diabetes insipidus (NDI)
- Néstor–Guillermo progeria syndrome (NGPS)
- Neurofibromatosis type 1
- Neurofibromatosis type 2
- Niemann–Pick disease
- Optic atrophy 1 (OPA1)
- Osteopetrosis
- Osteoporosis pseudoglioma (OPPG)
- Peutz–Jeghers syndrome
- Piebaldism
- Polycystic kidney disease
- Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOS)
- Posterior polymorphous corneal dystrophy-3 (PPCD)
- Prader–Willi syndrome (PWS)
- Progressive familial intrahepatic cholestasis type III (PFIC3)
- Psychiatric disorders
- Respiratory distress syndrome (RDS)
- Retinitis pigmentosa
- Rett syndrome
- Rubinstein–Taybi syndrome (RTS)
- Scott syndrome
- Severe combined immune deficiency (SCID)
- Severe psychomotor retardation
- Sezary's syndrome
- Sitosterolaemia
- Spinocerebellar ataxia type 6 (SCA6)
- Spinocerebellar ataxia type 12 (SCA12)
- Stargardt disease
- Tangier syndrome
- Timothy syndrome
- TNF-receptor-associated periodic febrile syndrome (TRAPs)
- Tuberous sclerosis
- Usher syndrome
- Van Buchem disease
- Waardenburg syndrome
 - Waardenburg syndrome 1 (WS1)
 - Waardenburg syndrome 2a (WS2a)
 - Waardenburg syndrome 3 (WS3)
 - Waardenburg syndrome 4 (WS4)
- Warburg Micro syndrome
- Werner syndrome
- Wiskott–Aldrich syndrome
- Wolf–Hirschhorn syndrome (WHS)
- Wolfram syndrome (WFS)
- X-linked adrenoleukodystrophy (X-ALD)
- X-linked lymphoproliferative syndrome (XLP)
- X-linked mental retardation
- X-linked recessive Emery–Dreifus muscular dystrophy
- X-linked recessive myotubular myopathy
- X-linked severe combined immune deficiency (X-SCID)

Achromatopsia

Achromatopsia or colour blindness is caused by loss of the cyclic nucleotide-gated channel (CNGC) subunit CNGA3 or the modulatory subunit CNGB3.

Acute periodic paralysis

Acute periodic paralysis is a skeletal muscle disorder characterized by defects in a number of ion channels including those that conduct K^+ . Some of these defects are caused by mutations in MiRP2, which is one of the minimal K^+ (MinK) channel subunits (Module 3: Figure K^+ channel domains).

Albinism

Albinism is a classical example of an inborn error of metabolism that occurs with a frequency of 1:20,000. It is characterized by mutations in the enzymes that synthesize melanin during the process of melanogenesis (Module 7: Figure melanogenesis). The resulting hypopigmentation occurs in the skin, hair and eyes. The number of melanocytes remains the same, but mutations in the enzymes that synthesize melanin are responsible for different forms of albinism such as oculocutaneous albinism type 1 (OCA1), oculocutaneous albinism type 2 (OCA2) and oculocutaneous albinism type 3 (OCA3).

Oculocutaneous albinism type 1 (OCA1)

Oculocutaneous albinism type 1 (OCA1) is characterized by an absence of melanin in the hair and skin. The

inability to tan greatly increases the risk of developing skin cancer. OCA1 is caused by a mutation in the enzyme tyrosinase (TYR) that is responsible for converting tyrosine into 3,4-dihydroxyphenylalanine during the process of melanogenesis (Module 7: Figure melanogenesis).

Oculocutaneous albinism type 2 (OCA2)

Oculocutaneous albinism type 2 (OCA2) is caused by a mutation in the human P homologue of the mouse pink-eyed dilution gene (*p*), which encodes a melanosome protein that may function to transport tyrosine into the developing melanosome (Step 7 in Module 7: Figure melanogenesis).

Oculocutaneous albinism type 3 (OCA3)

Oculocutaneous albinism type 3 (OCA3), which is characterized by the appearance of reddish-brown skin and ginger hair, is particularly evident in African populations. OCA3 is caused by a mutation in the tyrosinase-related protein 1 (TYRP1) that contributes to the process of melanogenesis (Module 7: Figure melanogenesis).

Alport syndrome, mental retardation, midface hyperplasia and elliptocytosis (AMME)

Alport syndrome, mental retardation, midface hyperplasia and elliptocytosis (AMME) is caused by a mutation in MinK-related protein 4 (MiRP4), which is an integral membrane protein that has a single transmembrane (TM) domain (Module 3: Figure K^+ channel domains). MiRP4 functions as an auxiliary subunit to modulate the activity of voltage-dependent K^+ (K_v) channels.

Amyotrophic lateral sclerosis (ALS)

The neurodegenerative disease amyotrophic lateral sclerosis (ALS) is characterized by degeneration of the motor neurons in the brainstem, spinal cord and motor cortex. The resulting symptoms are muscle weakness, gradual atrophy and fasciculation. Degeneration also occurs in other brain regions, including astrogliosis. Most ALS cases are sporadic, but approximately 10% of the cases are familial. A possible cause has emerged from studying these familial form of ALS, where mutations have been traced to different regions of the oxidant scavenger superoxide dismutase 1 (SOD1), which functions in reactive oxygen species (ROS) metabolism during redox signalling (Module 2: Figure plasma membrane ROS formation). ALS is also associated with a decrease in the level of nuclear factor erythroid 2-related factor 2 (NRF-2), which is a stress-sensing transcription factor that responds to reactive oxygen species (ROS) by enhancing the cells antioxidant defences. A subsequent increase in oxidative stress will contribute to neuronal cell death.

Mutations in the lipid phosphatase Sac3/Fig4, which functions in the PtdIns3,5P₂ signalling cassette (Module 2: Figure PIKfyve activation) and in the FIP2/optineurin gene, have also been linked to ALS.

The level of SIRT1 is elevated in mouse models of ALS and this might be an adaptive response to reduce the stress of this disease particularly by increasing the activity of peroxisome-proliferator-activated receptor γ

(PPAR γ) coactivator-1 α (PGC-1 α) that up-regulates antioxidant defences as it also does in Parkinson's disease (PD) (Module 12: Figure signalling pathways in Parkinson's disease). SIRT1 also deacetylates p53 and this reduces its ability to induce apoptosis.

There is an increase in expression of S100B in ALS and this may be linked to neurodegeneration.

Anderman's disease

Anderman's disease is caused by mutations in *SLC12A6* that encodes the K^+ -Cl⁻ cotransporter 3 (KCC3). This is a complex disease characterized by motor and sensory neuropathy that is linked to agenesis of the corpus callosum. There are defects in development and neurodegeneration occurs resulting in mental retardation and paraplegia.

Andersen-Tawil syndrome

Andersen-Tawil syndrome is characterized by ventricular arrhythmias, periodic paralysis of skeletal muscle and dysmorphic features. It is caused by a defect in the action potential mechanism responsible for membrane repolarization that results from a loss-of-function mutation in the KCNJ2 gene that codes for the $K_{ir}2.1$ channel (Module 3: Table inward rectifier K^+ (K_{ir}) channel). This is one of the inward rectifier K^+ (K_{ir}) channel family that provides the I_{K1} current that contributes to repolarization of the action potential (Module 7: Figure cardiac action potential).

Angelman syndrome (AS)

Angelman syndrome is a neurodevelopmental syndrome resulting from an imprinting disorder of genes on chromosome 15q11-q13. The defects in neural developmental result in a propensity for seizures, ataxia, dysmorphic facial features, speech difficulties, severe mental retardation and problems in relating to others. The *Ube3a* gene within the 15q11-13 chromosomal region has been identified as the genetic locus for AS. The *Ube3a* gene codes for an E6-AP ubiquitin ligase that functions in protein degradation. The decline in cognition, which is associated with AS, may be caused by a decrease in the activity of CaMKII that functions in synaptic plasticity (Module 10: Figure Ca^{2+} -induced synaptic plasticity). One of the functions of E6-AP is to regulate the activity of Arc, which has been implicated in the process of endocytosis that removes AMPA receptors from the spine surface (see step 4 in Module 10: Figure Ca^{2+} -induced synaptic plasticity). Arc may also act by interfering with the role of BDNF that functions in activating the protein synthesis required for memory consolidation (see step 7 in Module 10: Figure Ca^{2+} -induced synaptic plasticity).

This clinical phenotype shows considerable overlap with that found in Rett syndrome. Indeed, some AS patients that do not show obvious 15q11-13 abnormalities do have mutations in methyl-CpG-binding protein 2 (MECP2), which is primarily responsible for Rett syndrome.

Arrhythmogenic ventricular cardiomyopathy type 2 (ARVD2)

Mutations in the ryanodine receptor 2 (RYR2), which is expressed in the heart, is the cause of this arrhythmia that causes sudden heart death in children and young adults. The cause of the arrhythmia is very similar to that of another sudden death syndrome called catecholaminergic polymorphic ventricular tachycardia (CPVT). Unlike CPVT, however, ARVD2 usually progresses to a morphological change in the right ventricle, where the wall of the ventricle is infiltrated with fibrous and fatty tissue.

The mutations causing ARVD2 occur in hot spots on RYR2 (Module 12: Figure RYR mutations). Although the mutations causing ARVD2 and CPVT overlap each other, they do tend to cluster in different regions. See the section on catecholaminergic polymorphic ventricular tachycardia (CPVT) for details of how these mutations may lead to arrhythmias.

Ataxia telangiectasia (AT) syndrome

Ataxia telangiectasia (AT) syndrome, which is characterized by brain damage and a high incidence of lymphoid cancers, results from a mutation in the protein kinase ataxia telangiectasia mutated (ATM) that responds to ionizing radiation to phosphorylate and activate the tumour suppressor p53 (Module 4: Figure p53 function).

Autism spectrum disorders (ASD)

Autism spectrum disorders (ASD) encompass a range of conditions that include autism, Asperger's syndrome, pervasive developmental disorder not otherwise specified (PDD-NOS) and Rett syndrome. The term spectrum is included to reflect the fact that the symptoms of ASD are very varied and can range from mild to severe. Approximately 1% of children have an ASD, which is more prevalent in boys than girls. These are neurodevelopmental disorders characterized by deficits in social communication manifested as a lack of understanding and awareness of how other people feel. Often there are problems with language and difficulties in initiating and maintaining conversations.

These disorders begin in early childhood and persist into adulthood. Attempts to understand ASD is complicated by the considerable genetic and phenotypic heterogeneity displayed by the different syndromes. However, there is a general consensus that these ASD syndromes are neurodevelopmental disorders that owe their origins to defects in the way the nervous system develops and this has been particularly well characterized in classical autism.

During brain development, the embryonic cells destined to become neuronal cells begin to proliferate rapidly within the first few weeks to generate large numbers of neuronal progenitor cells (Module 8: Figure brain development). These progenitors become connected together through gap junctions and begin to generate Ca^{2+} signals that initiate at a pacemaker cell and then spread out to neighbouring cells to produce large hubs. Neuronal precursor cells within these hubs then begin to differentiate into excitatory (green cells) and inhibitory neurons (red cells) as evident by the axonal and dendritic sprouting. As these neurons differentiate further they begin to form

synapses with each other to create the neural circuits that begin functioning at birth and continue to mature for many months in the postnatal period.

There is increasing evidence that ASD is the result of defects in the way that these different processes of brain development occur both *in utero* and during the postnatal period. As outlined below, evidence for this neural development hypothesis is supported by the fact that many of the genes that have been linked to ASD, and particularly to autism, code for proteins that are important components of the structure and function of neural assemblies such as synapse organization, glutamate receptor function, neuronal proliferation, differentiation and migration:

Synapse organization

- **Shank.** The Shank proteins, which are a key component of the postsynaptic density (PSD) signalling elements, function as master organizers in that they act as scaffolding proteins that bring together many of the major components that function in the dendritic spines (Module 10: Figure postsynaptic density). The likelihood that defects in these Shank proteins are responsible for some forms of ASD is enhanced by the fact they are directly connected to the neuroligin proteins that have also been linked to ASD.
- **Neuroligin and Neurexins.** The neuroligin family of adhesion proteins, which are located in the spines, associate with the neurexins located in the presynaptic membrane and this interaction is essential for both synapse formation and maintenance (see step 5 in Module 10: Figure postsynaptic density). Mutations in the genes coding for NL-1, NL-3, NL-4 and neurexin-1 have been associated with ASD. For example, X-linked familial autism has been linked to mutations in two neuroligin genes (*NLGN3* and *NLGN4*). Alterations in the expression of these neuroligins may alter the excitatory-to-inhibitory ratio and this could contribute to the onset of ASD. It is essential for there to be an excitation-inhibition (E-I) balance in order to maintain the processes that generate brain rhythms such as the network gamma oscillation mechanism.
- **Voltage-gated sodium channel (Nav1.2).** The *SCN2A* gene that codes for the $\text{Na}_v1.2$ sodium channel is strongly associated with ASD. Alterations in the properties of this channel would seriously interfere with brain function as it would alter the way neurons generate and propagate action potentials to communicate with each other.

Glutamate receptor function

- **NMDA receptor 2B (NR2B) subunit.** The NR2B subunit of the *N*-methyl-D-aspartate (NMDA) receptor is encoded by the *GRIN2B* gene, which has been linked to ASD. These NMDA receptors have a central role in the relationship between Ca^{2+} and synaptic plasticity during the process of memory formation (Module 10: Figure Ca^{2+} -induced synaptic plasticity). In addition to its role in ASD, there also is a strong correlation between NMDA receptor hypofunction and schizophrenia.

- Kainate receptor subunit GluR6. The *GRIK2* gene, which encodes the GluR6 subunit of kainate receptors, is a candidate gene for ASD.
- Synaptic Ras GTPase activating protein 1 (SYNGAP1). SYNGAP1, which is a Ca^{2+} -sensitive Ras GTPase-activating protein (Module 2: Figure Ras signalling), is an important component of the postsynaptic signalling pathway in excitatory neuronal spines where it acts downstream of the NMDA receptor (Module 10: Figure Ca^{2+} -induced synaptic plasticity). It acts as an inhibitory regulator of the Ras signalling pathway and has an important role in regulating synaptic functions such as memory formation. Mutations in SYNGAP1 have been linked to both ASD and the closely related syndrome intellectual disability (ID).

The way in which mutations might result in ASD and ID has emerged recently in a mouse model with a pathogenic mutation in the *SYNGAP1* gene (Clement et al 2012):

“...a mouse model of human SYNGAP1 haploinsufficiency had glutamatergic synapses that matured at an accelerated rate during the first few weeks of neonatal development. Loss of this essential glutamatergic synapse repressor dramatically disrupted E/I balance in neural networks that support cognition and behaviour and these effects were linked to life-long intellectual disability. These studies provide a neurophysiological mechanism linking abnormal glutamatergic synapse maturation during development to enduring abnormalities in behaviours indicative of neurodevelopmental disorders.”

This study has emphasized how important the excitation-inhibitory (E-I) balance is in order to maintain the processes that generate brain rhythms such as the network gamma oscillation mechanism. A similar E-I imbalance as a result of the neurologically mutations described earlier.

Neuronal proliferation, differentiation and migration

- Dual-specificity tyrosine-phosphorylation regulated kinase 1A (DYRK1A). This serine/threonine kinase, which functions in early brain development and continues to operate in the adult brain, has multiple functions both in the nucleus and in the cytoplasm. Within the nucleus, it is responsible for phosphorylating the transcription factor NFAT to promote its export from the nucleus (Module 4: Figure NFAT control of Ca^{2+} signalling toolkit). Through such actions, DYRK1A functions to control the processes of neuronal proliferation, differentiation and maturation that are critical phases of brain development.

DYRK1A also phosphorylates various endocytic accessory proteins that function during membrane invagination and scission during the process of endocytosis (Module 4: Figure scission of endocytic vesicles) and this contributes to memory because endocytosis is responsible for memory erasure (see step 5 in Module 10: Figure Ca^{2+} -induced synaptic plasticity)

- T-brain 1 (TBR1) transcription factor. The TBR1 transcription factor functions early in embryonic brain de-

velopment (Module 8: Figure brain development) to control the differentiation and migration of neurons. It acts together with the calcium/calmodulin-dependent serine protein kinase (CASK) to regulate the transcription of *GRIN2B*, which encodes the NR2B subunit of the N-methyl-d-aspartate (NMDA) receptor as described above. TBR1 also controls some other candidate ASD genes such as *RELN* and the autism susceptibility candidate 2 (*AUTS2*). The *RELN* gene encodes the extracellular matrix glycoprotein reelin that plays a role in neuronal migration and also contributes to dendritic and spine formation.

- β -Catenin. One of the main functions of β -catenin is to act as a transcription factor that is activated by the canonical Wnt/ β -catenin pathway (Module 2: Figure Wnt canonical pathway). β -Catenin is encoded by the *CTNNB1* gene, which is one of the genes that has been linked to ASD. Alterations in the expression of β -catenin will have a serious effect on neural development because the Wnt/ β -catenin signalling pathway plays a prominent role in regulating proliferation, neuronal specification, migration and synapse formation.
- Chromodomain helicase DNA binding protein 8 (CHD8). CHD8 is a DNA helicase that functions in chromatin remodelling. It acts to inhibit signalling by the canonical Wnt/ β -catenin pathway by binding to β -catenin (Module 2: Figure Wnt canonical pathway). The role of CHD8 in ASD probably depends on this inhibition of Wnt signalling that plays a pivotal role in the embryonic developmental process responsible for brain development.
- Katanin. The katanin protein, which is also known as katanin p60 subunit A-like 2, is a microtubule severing protein that is encoded by the *KATNAL2* gene that has been implicated in ASD. It acts by inducing ATP-dependent internal breaks in microtubules. It is strongly expressed in neurons and any alteration in its function will induce a rapid reorganization of the microtubules with serious consequences for neuronal function and particularly axon growth.

Autism

Autism, which is one of the autism spectrum disorders (ASD), is characterized by deficits in social communication often manifested as a lack of understanding and awareness of how other people feel. Often, there are problems with language, learning difficulties and below-average levels of intelligence. Like other examples of ASD, autism is a neurodevelopmental disorder that begins early in the embryo at the start of brain development (Module 8: Figure brain development). Autism is a disease of synaptic transmission. Many of the mutations that have been linked to autism seem to have a function in either the structural components of the synapse or in the various signalling pathways that control how information is passed across from the pre- to the post-synaptic ending. The defective processing may also include defects in mechanisms such as protein synthesis that are responsible for information storage during learning and memory.

The way in which various genetic modifications result in the development of autism is described in the section on ASD.

Autosomal dominant hypocalcaemia (ADH)

Autosomal dominant hypocalcaemia (ADH) is an autosomal dominant disease caused by a gain of function mutation of the *CaR* gene that encodes the Ca^{2+} -sensing receptor (CaR). The CaR becomes more sensitive, which displaces the set-point of Ca^{2+} -regulated parathyroid hormone (PTH) secretion response to the left (see Module 7: Figure PTH secretion for the normal position of the response curve). Individuals are usually asymptomatic, but they display mild hypocalcaemia due to the depressed level of PTH.

Bardet-Biedl syndrome

Bardet-Biedl syndrome is a genetically heterogeneous disorder caused by mutations in 14 different genes, which all seem to function in the trafficking of proteins for ciliogenesis. Defects in the function of the primary cilium has many manifestations such as retinal degeneration, renal abnormalities, postaxial polydactyly, truncal obesity, learning abnormalities and hypogonadism.

Barth syndrome

Barth syndrome is an X-linked disorder caused by mutations in the gene *TFZ*, which codes for the enzyme tafazzin (TFZ) that synthesizes the lipid cardiolipin (CL) that plays a critical role in facilitating many mitochondrial functions (Module 5: Figure cardiolipin). This syndrome is characterized by cardiac and skeletal myopathy, growth delay and neutropenia.

Bartter's disease

Bartter's disease is a complex disease characterized by hypotension, hypokalaemia and metabolic acidosis. It is caused by mutations in at least five different genes that are linked together through their participation in salt reabsorption by the thick ascending loop of Henle (TALH) (Module 7: Figure kidney salt reabsorption). Mutations in the different genes give rise to the different types of Bartter's disease:

- Type I Bartter's disease is caused by mutations in *SLCA12A1* that encodes the $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter (NKCC2) that transports Na^+ and Cl^- from the tubule lumen into the TALH cells.
- Type II Bartter's disease is caused by mutations in the ROMK channel responsible for recycling K^+ across the TALH cell apical membrane in order to maintain the operation of NKCC2.
- Type III Bartter's disease is caused by mutations in the CLC-Kb channel responsible for the efflux of Cl^- across the basolateral membrane (Module 7: Figure kidney salt reabsorption), which functions to keep the intracellular $[\text{Cl}^-]$ at the low level necessary for the continued operation of NKCC2.
- Type IV Bartter's disease is caused by mutations in Barttin, which is a regulatory subunit of CLC-Ka and

CLC-Kb channels. In the TALH cells, it functions as a chaperone to direct the CLC-Kb channels to the basolateral membrane (Module 7: Figure kidney salt reabsorption). Patients with type IV also display sensorineural deafness due to a defect in CLC-Ka trafficking in various cells of the ear (marginal cells of the stria vascularis and dark cells in the vestibular organ) that function in K^+ secretion into the endolymph (Module 10: Figure inner ear).

- Type V Bartter's disease has been linked to gain-in-function mutations in the Ca^{2+} -sensing receptor (CaR), which is strongly expressed on the basolateral membrane of the TALH cells (Module 7: Figure kidney salt reabsorption). As a consequence of the gain-in-function mutations, the CaR responds to low levels of extracellular Ca^{2+} resulting in inhibition of salt reabsorption.

Bernard-Soulier syndrome

Bernard-Soulier syndrome is an autosomal dominant disorder that is characterized by increased bleeding times and impaired platelet responses. There also is a thrombocytopenia that gives rise to giant platelets. This developmental and bleeding disorder is caused by mutations in components of the glycoprotein (GP) 1b-GPIX-GPV complex, which is the receptor on blood platelets that recognizes von Willebrand factor (vWF) (Module 11: Figure platelet activation). The mutations are found in GPIb α , GPIb β and GPIX.

Brody disease

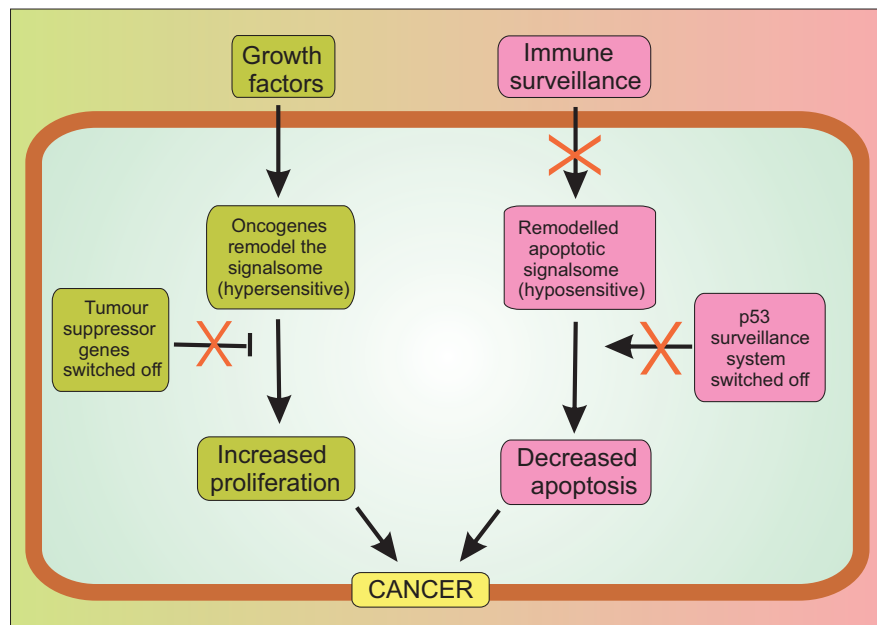
Brody disease is a skeletal muscle disorder that is characterized by stiffness and cramp brought on by prolonged Ca^{2+} elevation and a slowing of relaxation. The defect results from mutations in the sarco/endo-plasmic reticulum Ca^{2+} -ATPase 1 (SERCA1) pump, which is expressed in fast-twitch muscle (Module 5: Table Ca^{2+} pumping toolkit). SERCA1 returns Ca^{2+} to the sarcoplasmic reticulum during the recovery phase (Step 5 in Module 7: Figure skeletal muscle E-C coupling).

Bruton's type X-linked agammaglobulinaemia

Bruton's type X-linked agammaglobulinaemia results from an X-linked mutation in the pleckstrin homology (PH) domain of Bruton's tyrosine kinase (Btk), which is one of the Tec tyrosine kinase family that functions in signal transduction in B cells (Module 2: Figure ROS effects on Ca^{2+} signalling) and in mast cells (Module 11: Figure Fc ϵ RI mast cell signalling).

Cancer

One of the most feared diseases in humans is cancer. It strikes at any age without warning and has proved extremely difficult to cure. One reason for this is that cancer is a multistep process that depends upon the accumulation of approximately four to seven different mutations (Module 12: Figure the cancer signalsome). Cancer is thus an example of a genotypic remodelling of the signalsome that depends upon a gradual acquisition of multiple muta-

Module 12: | Figure the cancer signalsome**Summary of the major genotypic remodelling processes that result in cancer.**

There are two major aspect of the remodelling process. The proliferative signalling pathways are switched on, while the apoptotic pathway is switched off. Oncogenes are derived from the normal stimulatory elements of proliferative signalling pathways (proto-oncogenes) that become constitutively active. In effect, these components of the signalsome become hypersensitive to increase cell proliferation. In addition, tumour suppressor genes that normally function to inhibit proliferation are switched off. The apoptotic signalsome is remodelled so that it becomes hyposensitive by switching off the p53 surveillance system. In addition, the cells also escape from the immune surveillance system that normally kills cancer cells. The increase in cell proliferation and the decrease in apoptosis are the hallmarks of cancer cells.

tions in different signalling components that belong to three major signalling systems. Firstly, oncogenes remodel the proliferative signalling pathways, which become constitutively active and drive cells into repeated rounds of cell division. Secondly, there are mutations in the tumour suppressors, which are inhibitory components that normally act to inhibit proliferative signalling pathways. Thirdly, there are mutations in the signalling system that detect aberrant cells and remove them from the cell cycle by inducing either senescence or apoptosis. Cancer depends on enhanced proliferation and decreased apoptosis. In order for a cancer cell to develop, it has to evade both the normal immune surveillance system and the internal control mechanisms that remove most cancer cells before they proliferate to form tumours. Therefore cancer arises from different combinations of activated oncogenes, inactivated tumour suppressor genes and the development of various anti-apoptotic mechanisms, which explains why therapeutic strategies have proven to be so difficult to devise, because cancer is a multifactorial disease and no two cancers are the same. Nevertheless, there clearly are well-defined cancer cell phenotypes that have proved very important in providing clues as to how a typical cancer cell develops.

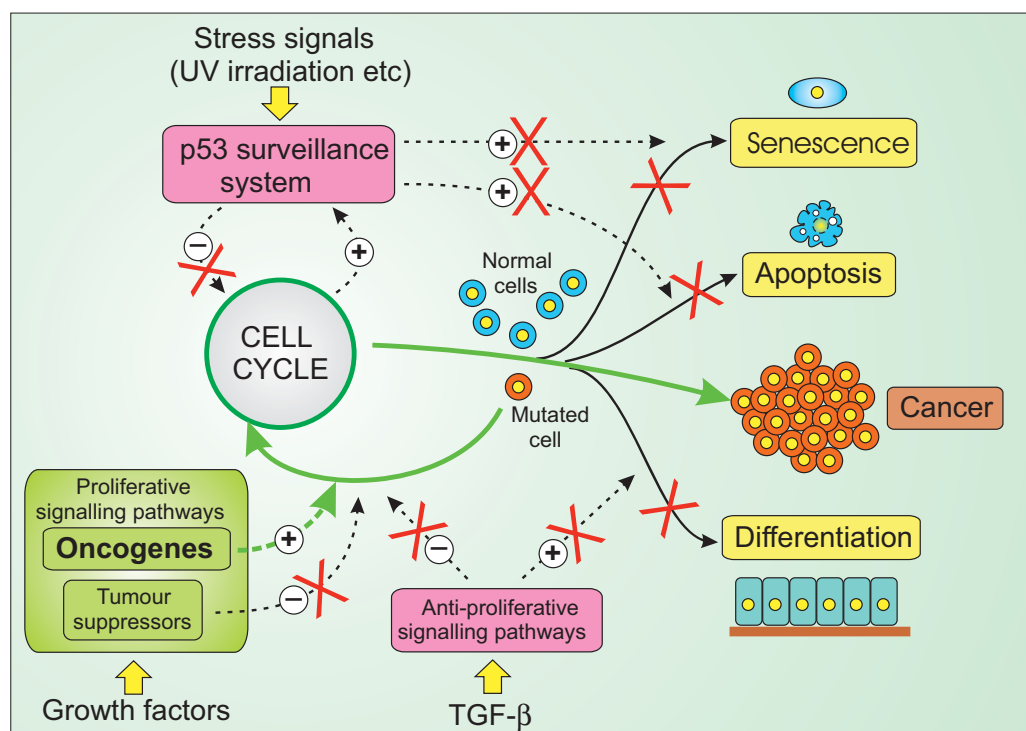
When this tight regulation on proliferation and apoptosis breaks down, the aberrant cell begins to proliferate and forms a large clone of cells that we recognize as a cancerous growth. Cells remain as a tight mass when the modifications are benign, but once they become malignant they migrate away to invade other regions of the body with disastrous consequences.

The tumour cell microenvironment plays a special role in not only maintaining the tumour but may also contribute to the onset of metastasis. An important aspect of this microenvironment is the activation of tumour angiogenesis to provide the tumour with a supply of blood. A relationship between inflammation and cancer may also be a significant feature of the microenvironment in many tumours. A significant aspect of this inflammatory connection is the relationship between chemokines and cancer, which has revealed that chemokines such as CXCL12 and its receptor CXCR4 (Module 1: Figure chemokines) may play a critical role in the establishment of secondary tumours during metastasis.

Cancer depends on enhanced proliferation and decreased apoptosis

The development of a cancer cell occurs through a series of discrete steps that gradually alter the normal signalling pathways that operate on the cell cycle network to control both cell proliferation and cell fate (primarily apoptosis). These signalling systems not only determine whether or not a cell should divide, but also decide on what happens to the two daughter cells once they exit the cell cycle after completing mitosis (Module 9: Figure cell cycle network). Emergence of a cancer cell signalsome depends on two major changes: there must be an increase in cell proliferation and a decrease in apoptosis (Module 12: Figure the cancer signalsome).

A predisposition to cancer occurs when DNA is damaged by defects in non-homologous end-joining and ho-

Module 12: | Figure cell cycle network and cancer

Multiple alterations of the signalling systems that control the cell cycle lead to the development of cancer.

Multiple genetic modifications occur during the development of a cancer cell. The mutated cell enters the cell cycle repeatedly to form a cancer. The proto-oncogenes that provide the positive signals to drive cells into the cell cycle are mutated into constitutively active oncogenes, whereas the tumour suppressors and the anti-proliferative signalling pathways are inactivated. In cancer, the cell-fate pathways of senescence, apoptosis and differentiation are also switched off by inactivation of the p53 surveillance system, thus leaving the developing cancer cells with no other option than to continue proliferating.

ologous recombination, damage of checkpoint controls or telomere maintenance that result in genomic instability. Cancer cells begin to emerge when this genomic instability results in the accumulation of mutations in proto-oncogenes and tumour suppressors (Module 12: Figure cell cycle network and cancer). The signalling systems that drive cell proliferation have to be switched on, and the systems that decide whether cells should senesce, die or differentiate have to be switched off. For example, one of the essential steps in the development of cancer is the expression of telomerase, which thus allows the emerging cancer cell to escape replicative senescence (Module 11: Figure senescence).

Cancer is a multistep process because a number of signalling systems have to be altered in order to achieve both the increased growth potential and to switch off alternative non-proliferating cell fates.

Cancer is a multistep process

Almost all cancers are clonal in that they descend from a single abnormal cell that has undergone the multistep genetic modifications that enable it both to proliferate and to switch off other cell fates, such as differentiation, senescence and apoptosis. This transformation of cells with multiple potential fates into a cell that has a single-track proliferative fate depends upon multiple mutations dis-

persed throughout the many control systems that regulate the cell cycle network (Module 9: Figure cell cycle network). It has been estimated that between four and seven mutations are necessary for a cancer cell to develop. These multistep genetic changes occur relatively slowly as the cell gradually transforms into a cancer cell. Normal cell proliferation depends upon a number of positive and negative signalling systems that operate on the cell cycle network. The proliferative signalling pathways exert both positive and negative control of the cell cycle. The positive signals are supplied by the proto-oncogenes, whereas the inhibitory signals come from the tumour promoters and by the anti-proliferative signalling pathways, such as those supplied by transforming growth factor β (TGF- β). During the development of cancer, mutations occur in both these positive and negative signalling systems (Module 12: Figure cell cycle network and cancer). Mutations of the proto-oncogenes convert them into oncogenes that are constitutively activated to stimulate the cell cycle independently of growth factors. Inactivation of tumour suppressors removes their negative effects on cell cycle progression. In addition, the anti-proliferative signalling systems based on TGF- β are often switched off, which also helps to enhance the proliferative drive, as occurs in colorectal cancer (CRC), which provides a good example of how a typical cancer develops through the gradual acquisition of multiple mutations.

Activation of proliferation alone will not lead to cancer, because the internal surveillance system mainly operated by p53 detects cells with abnormal growth potential and shunts them off towards either senescence or p53-induced apoptosis. The important role of p53 in tumour suppression is evident by the fact that many cancers carry mutations that inactivate p53. In addition to promoting apoptosis, p53 also regulates senescence, which also has to be evaded for cancers to develop. A prerequisite for cancer to develop is the expression of telomerase that enables the cell to evade replicative senescence, whereas inactivation of the tumour suppressors such as p16, E2F/retinoblastoma protein (Rb) and p53 prevents the cells from entering stress-induced senescence (Module 11: Figure senescence).

The onset of cancer thus depends upon multiple steps, many of which are due to the activation of oncogenes together with inactivation of tumour suppressors.

Oncogenes

Trying to understand cancer is a daunting task, because each cancer cell type carries a different set of mutations distributed throughout the many signalling systems that control cell proliferation and cell fate (Module 9: Figure proliferation signalling network). For example, they can occur at the level of the growth factors, receptors, transducing elements and signalling cassettes, and in the transcription and cell cycle elements that activate DNA synthesis. So far, approximately 100 proto-oncogenes and tumour suppressor genes have been identified.

Here, we consider some of the main examples of signalling components (proto-oncogenes) that can be altered to become oncogenes. This will illustrate how such oncogenes are distributed throughout many signalling pathways, and how they are altered to contribute to the development of a cancerous cell.

Oncogenic growth factors

An example of an oncogenic growth factor is *sis*, which codes for the potent mitogenic agent platelet-derived growth factor (PDGF), which activates the PDGF receptor to activate many of the signalling pathways that control cell proliferation (Module 1: Figure PDGFR activation).

Some cancers, such as small cell lung carcinoma (SCLC), seem to arise through an uncontrolled release of mitogenic peptides such as bombesin, gastrin-releasing peptide, neuromedin B, bradykinin, cholestokinin (CCK), galanin, neurotensin and vasopressin. In effect, the cells are trapped within an autocrine loop, in that they are kept growing by continuously secreting their own growth factors. Many of these growth factors are likely to act through G protein-coupled receptors (GPCRs), which is consistent with the observation that many human cancers have mutations in these GPCRs.

Oncogenic growth factor receptors

A number of growth factor receptors become oncogenic, usually following various truncations.

c-ErbB2

ErbB2 is one of the epidermal growth factor (EGF) receptors (Module 1: Figure EGF stimuli and receptors). A

truncated version of this ErbB2 receptor is found in mammary carcinoma. The antibody Herceptin[®], which is being used to treat breast cancer, inhibits ErbB2.

c-Fms

Fms is a truncated version of colony-stimulating receptor-1 (CSF-1) receptor, which has been identified in a number of cancer cells.

These two examples are tyrosine kinase-linked receptors that become constitutively active in that they begin to activate the various downstream signalling cassettes independently of an external signal.

c-KIT

c-KIT, which is the receptor for stem cell factor (SCF), is a proto-oncogene that is mutated in many gastrointestinal stromal tumours and in acute myelogenous leukaemia.

Trk

Trk is an oncogenic growth factor receptor that is formed by the fusion of the first seven exons of tropomyosin to the transmembrane and cytoplasmic domains of the TrkA receptor. *Trk* has been identified in thyroid papillary carcinomas and in colon carcinoma.

Oncogenic signalling transducers

A number of oncogenes have been identified that act downstream of growth factor receptors by constitutively activating various proliferative signalling pathways (Module 9: Figure proliferation signalling network):

BCR-ABL oncogene

The BCR-ABL is a fusion protein resulting from a translocation that fuses sequences from the B cell receptor (BCR) gene with the *c-abl* gene. The latter encodes the non-receptor protein tyrosine Abl, which regulates a large number of cellular processes (Module 1: Figure Abl signalling). This BCR-ABL oncogene is responsible for chronic myelogenous leukaemia (CML). The drug Gleevec, which inhibits the tyrosine kinase activity of Abl has proved to be an effective anticancer agent.

Ras

Ras is a 21 kDa GTP-binding protein, which participates in a number of Ras signalling mechanisms (Module 2: Figure Ras signalling). One of the pathways activated by Ras is the mitogen-activated protein kinase (MAPK) signalling pathway (Module 2: Figure ERK signalling). Oncogenic *ras* has mutations in codons 12, 13 and 16 that interfere with its ability to hydrolyse GTP, which means that this signal transducer remains in its active state and signals continuously to the MAPK pathway to stimulate entry into the cell cycle.

PtdIns 3-kinase signalling and cancer

The type IA PtdIns 3-kinase (PtdIns 3-K) is overexpressed in some types of ovarian cancers and is strongly activated by the BCR-ABL oncogene in chronic myelogenous leukaemia (CML). There also is a high incidence of mutations in the *PIK3CA* gene, which encodes the p110 α subunit of this kinase. Many of these mutations are clustered in three hot spots at Glu-542, Glu-545 and His-1047. These are gain-of-function mutations, which imply that some can-

cers may arise from an excessive activation of the PtdIns 3-kinase signalling pathway. The p110 α subunit has also been identified in v-p3k, which is a viral oncogene present in avian sarcoma virus.

Increased levels of protein kinase B (PKB), which has also been identified as a retroviral oncoprotein, have been found in some ovarian, breast and thyroid cancers.

Ca²⁺ signalling and cancer

A perturbation in Ca²⁺ signalling may be responsible for the development of some cancers. In mice, the loss of one Atp2a2 allele, which reduces expression of the sarco/endo-plasmic reticulum Ca²⁺-ATPase 2 (SERCA2) pump, results in the development of various squamous cell tumours in a number of regions (oesophagus, oral mucosa, tongue and skin). This is an important observation, because it illustrates that a perturbation in Ca²⁺ signalling can be an initiating event in cancer. Such SERCA2 haploinsufficiency is a feature of Darier's disease.

The Ca²⁺-binding protein S100B inhibits the activity of p53, thus reducing its tumour suppressor role, and therefore contributes to the onset and progression of cancer.

Oncogenic transcription factors

Transcription factors such as Myc and β -catenin, which play a role in the early events of proliferative signalling (Module 9: Figure proliferation signalling network), have very significant oncogenic potential and have been identified in many cancers.

Myc dysregulation and cancer development

One of the most important oncogenic transcription factors is Myc, which is frequently overexpressed in tumours. In many of these tumours, there is a decrease in the level of miR-145, which is known to regulate Myc expression. Its oncogenic potential is heightened by the fact that it not only controls the transcription of many of the components responsible for cell growth and proliferation (Module 4: Figure Myc as a gene activator), but also acts to switch off some of the major cyclin-dependent kinase (CDK) inhibitors, such as p15 and p21 (Module 4: Figure Myc as a gene repressor). In pancreatic cancer cells, there is increased expression of nuclear factor of activated T cells c1 (NFATc1), which is known to activate the expression of Myc.

β -Catenin as an oncogene

The transcription factor β -catenin is a major oncogene in many human cancers. It normally functions in the canonical Wnt/ β -catenin pathway, where it is activated in the cytoplasm and then translocates into the nucleus to activate genes that play a role in cell proliferation (Module 2: Figure Wnt canonical pathway). One of the genes activated by β -catenin is the oncogene Myc, which thus places these two transcription factors at the very centre of the proliferative signalling pathways (Module 9: Figure proliferation signalling network).

β -Catenin is one of the transcription factors that are often activated in colon cancer (Module 12: Figure colon cancer).

Oncogenic cell cycle signalling components

Some components of the cell cycle signalling system have been implicated as oncogenes. In most cases, the transformation seems to depend upon an increase in expression levels, but there are instances of mutations, as described for cyclin D.

Cyclin D

Cyclin D1, which is the first cyclin that appears in cells during the onset of proliferative signalling (Module 9: Figure proliferation signalling network), is often overexpressed in many human cancers. The resulting up-regulation of the cyclin-dependent kinase 4 (CDK4) results in hyperphosphorylation and inactivation of the retinoblastoma (Rb) tumour suppressor protein (Module 9: Figure cyclin D/CDK action).

Mouse double minute-2 (MDM2)

Mouse double minute-2 (MDM2) is considered to be an oncogene because it causes tumours when overexpressed in cells. MDM2 is an ubiquitin ligase that targets the transcription factor p53 for degradation (Module 4: Figure p53 function). MDM2 also functions in the receptor down-regulation of G protein-coupled receptors (GPCRs) (Module 1: Figure homologous desensitization) and in the inactivation of the protein acetylase TIP60.

The turnover of MDM2 is regulated by an autoubiquitination process that targets it for destruction by the ubiquitin-proteasome system (Module 1: Figure ubiquitin-proteasome system). The ubiquitin-specific protease Usp7/HAUSP stabilizes the level of MDM2 by reversing this autoubiquitination.

Tumour suppressors

Many cancers arise through alterations of tumour suppressor genes, which normally function as negative regulators of cell growth (Module 9: Figure cell cycle network). Classical examples of such tumour suppressors are p53, the retinoblastoma (Rb) gene, APC and the CDK inhibitors such as p21 (highlighted in red in Module 9: Figure proliferation signalling network). The following are examples of tumour suppressors that are inactivated during the development of cancer:

- Adenomatous polyposis coli (APC)
- Alternative reading frame (ARF)
- Breast cancer 1 (BRCA1)
- Breast cancer 2 (BRCA2)
- CYLD
- LKB1
- Melastatin
- Merlin
- Oncostatin (OSM)
- Patched receptors (PTC1 and PTC2)
- p53
- p16^{INK4A}
- Promyelocytic leukaemia (PML)
- Protein phosphatase 2A (PP2A)
- PTEN
- SMADs
- Retinoblastoma susceptibility gene (Rb)

Breast cancer 1 (BRCA1)

Breast cancer 1 (BRCA1) is a tumour suppressor that has a number of functions including the repair of DNA damage and gene transcription. BRCA1 can directly bind to DNA especially at sites of DNA damage. For example, it binds to phosphorylated H2AX and thus associates with the flanking chromatin during the G₁ checkpoint signalling to DNA double-strand breaks (DSBs) (Module 9: Figure G₁ checkpoint signalling). BRCA1 is also a component of complex 2 in the Fanconi anaemia/BRCA pathway (Module 9: Figure Fanconi anaemia pathway).

Breast cancer 2 (BRCA2)

Breast cancer 2 (BRCA2) is a tumour suppressor that functions in the repair of DNA damage. BRCA2 is a member of the Fanconi anaemia complementation group (FANC) that functions in the Fanconi anaemia/BRCA pathway (Module 9: Figure Fanconi anaemia pathway). The BRCA2 protein is the same as the D1 protein that is part of complex 2. Mutations in D1/BRCA2 greatly increase the risk of developing breast cancer because the defect in DNA repair mechanisms results in a gradual accumulation of mutations of other genes that code for signalling components that control cell proliferation.

CYLD

CYLD binds to NEMO and inhibits the subsequent phosphorylation of I κ B [inhibitor of nuclear factor κ B (NF- κ B)] as part of the TNF α signalling pathway (Module 2: Figure NF- κ B activation).

p53 and cancer

The transcription factor p53 is one of the most frequently mutated genes in cancer. The function of p53 in tumour suppression is to remove cancer cells as soon as they begin to emerge. It functions as a checkpoint regulator in that it can shut down the cell cycle when DNA is damaged, or it can induce cells to senesce or enter apoptosis (Module 9: Figure cell cycle network). However, when this highly effective p53 surveillance system is inactivated by mutation, these newly formed cancer cells survive and begin to grow by clonal expansion to form tumours (Module 12: Figure cell cycle network and cancer).

The mutations that contribute to cancer are clustered around the DNA-binding domain of p53 (Module 4: Figure p53 domains). Indeed, the *TP53* gene is one of the most commonly mutated genes in human cancers, with more than 50% of human tumours carrying a deletion or mutation of p53. Of particular importance for the survival of these cancer cells is to have the p53-induced apoptosis mechanism switched off so as to evade apoptosis (Module 12: Figure cell cycle network and cancer).

The anticancer role of p53 is clearly evident from studies on p53^{-/-} mice that are highly susceptible to developing tumours. In humans, a rare inherited cancer predisposition known as Li-Fraumeni syndrome results from having a single copy of the *TP53* gene. Inactivation of p53 often occurs in the final transformation of colonoepithelial cells to form colorectal cancer (Module 12: Figure colon cancer).

The E6 protein of the oncogenic papilloma virus types 16 and 18 binds to p53 and stimulates its degradation through the ubiquitin-dependent protease.

Promyelocytic leukaemia (PML)

The promyelocytic leukaemia (PML) protein, which exists in multiple isoforms originating by alternative splicing, is found in both the cytoplasm and nucleus. In the latter case, PML is an essential component of the PML nuclear bodies (PML NBs). Each nucleus contains 10–30 such PML NBs, which have been implicated in a number of functions such as gene transcription, DNA repair, cell cycle regulation, apoptosis and cell stress. Sumoylation of PML plays a central role in the formation of the PML NBs, which disassemble during mitosis and reform during interphase. PML contains both consensus SUMO acceptor sites such as Ψ KxE SUMO and the SUMO-interacting/binding motif (SIM/SBM) that enables it to interact both with itself and with many of the other proteins found within the PML NB such as Sp100 and Daxx.

One of the locations of PML in the cytoplasm is the mitochondrial-associated ER membranes (MAMs) where it functions within a complex that regulates the activity of the inositol 1,4,5-trisphosphate receptors (InsP₃Rs) (Module 5: Figure mitochondrial-associated ER membranes).

Acute promyelocytic leukaemia (APL) is caused by disruption of the *PML* gene.

Rb

The product of the retinoblastoma susceptibility gene (*Rb*) is one of the pocket proteins, which act as a typical tumour suppressor to inhibit the activity of the E2F transcription factors (Module 9: Figure cyclin D/CDK action). *Rb* is inactivated in many human cancers.

The function of *Rb* in oncogenesis first became evident when it was discovered that *Rb* formed oligomeric complexes with various viral oncoproteins, such as the large T antigen [the simian virus 40 (SV40) oncogene product], adenovirus E1A and E1B, and human papilloma viral proteins E6 and E7.

Rb appears to play a role by binding to and inactivating the non-receptor protein tyrosine kinase Abl located within the nucleus (Module 1: Figure Abl signalling).

Patched (PTC)

Patched receptors (PTC1 and PTC2) function as tumour suppressors because they act to inhibit the seven-membrane-spanning protein smoothened (SMO) that acts as the Hedgehog transducer of the Hedgehog signalling pathway. When PTC is inactivated, the SMO receptors can activate a signalling pathway (Module 2: Figure Hedgehog signalling pathway) that functions to control cell proliferation in many cell types.

Mutations in PTC have been linked to basal cell carcinomas, medulloblastomas, gliomas and rhabdomyosarcomas in muscle cells. An inherited mutation in PTC is responsible for Gorlin's syndrome.

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN)

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is an inositol polyphosphate 3-phosphatase that inactivates the potent PtdIns3,4,5P₃ lipid second messenger that functions in the PtdIns 3-kinase signalling pathway (Module 2: Figure PtdIns 3-kinase signalling). This tumour suppressor is frequently mutated in a variety of cancers, such as prostate cancer, melanoma, glioblastoma and endometrial carcinoma.

Alternative reading frame (ARF)

Alternative reading frame (ARF) protein (also known as p14^{ARF} or p19^{ARF} for the mouse homologue) functions to control the stability of p53 by acting through the p53 ubiquitination and degradation pathway. It acts to stabilize p53 by retaining it in the nucleus, where it escapes the protein degradation pathways in the cytoplasm (Module 4: Figure p53 function). The ARF protein is encoded by the *p16^{INK4A}/ARF* locus, which thus produces two tumour suppressors, ARF and p16^{INK4A}. Mutations of this locus have been found in many cancers, and particularly in skin cancers such as squamous cell carcinomas (SCCs). The expression of ARF is activated by Myc (Module 4: Figure Myc as a gene activator).

p16^{INK4A}

p16^{INK4A}, is one of the cyclin-dependent kinase (CDK) inhibitors, which shares the same coding locus as alternative reading frame (ARF), is inactivated in numerous cancers. It is significant that p16^{INK4A}, cyclin D1 and retinoblastoma (Rb), all of which have potent oncogenic activity, all function in a common pathway that is critical at the time of the restriction point when the cell becomes irreversibly committed to begin DNA synthesis. At an early stage in cancer development, an increase in the expression of p16^{INK4A} plays an important role in shunting aberrant cells into stress-induced senescence (Module 11: Figure senescence).

p16^{INK4A} has also been implicated in the signalling pathways of compensatory hypertrophy.

Adenomatous polyposis coli (APC)

The adenomatous polyposis coli (APC) protein is a potent tumour suppressor that is frequently mutated in cancer cells, and particularly in those that develop within the intestine. APC functions in the Wnt signalling pathway, where it is part of the complex that degrades the transcription factor β -catenin (Module 2: Figure Wnt signalling pathway). When APC is mutated, there is a decrease in the breakdown of β -catenin, which builds up in the cytoplasm and then enters the nucleus to initiate the transcriptional events responsible for initiating cell proliferation. Mutations in APC are usually the first mutations that occur during the gradual progression towards colorectal cancer (CRC) (Module 12: Figure colon cancer).

Germline mutations of APC are responsible for familial adenomatous polyposis (FAP).

Merlin

Merlin (moesine-zonula occludens-like protein) regulates cell proliferation through a number of actions. It is a key com-

ponent of the hippo signalling pathway (Module 2: Figure hippo signalling pathway). It also acts to inhibit an early step in the Ras signalling mechanism. It appears to inhibit the early activation step when Ras.GDP is converted into Ras.GTP (Module 2: Figure Ras signalling). Merlin is inactive as a tumour suppressor when it is phosphorylated on Ser-518. Its activation depends on it being dephosphorylated by the δ isoform of protein phosphatase 1 (PP1 δ) associated with the myosin/actin target in subunit MYPT-1 (Module 5: Table PP1 regulatory, targeting and inhibitory subunits). This MYPT-1/PP1 δ myosin phosphatase is, in turn, inhibited by a protein kinase C-potentiated inhibitor (CPI-17).

Merlin is coded for by the neurofibromatosis type 2 gene *Nf2*. Mutations in *Nf2* result in the autosomal dominant disorder neurofibromatosis type 2, which increases the likelihood of developing tumours.

Protein phosphatase 2A (PP2A)

The protein phosphatase 2A (PP2A) is considered as a tumour suppressor because it plays an important role in reversing many of the phosphorylation events that occur in the proliferative signalling pathways. Protein phosphatase 2A (PP2A) functions include inhibitory effects on Myc, β -catenin and the mitogen-activated protein kinase (MAPK) signalling pathway (Module 9: Figure proliferation signalling network). The functional relationship between protein phosphatase 2A (PP2A) and tumour suppression depends upon a number of modifications or interactions with tumour viruses that inhibit its activity (Module 5: Figure PP2A modifications and cancer).

Smads

Smads are the intracellular transducers of the Smad signalling pathway. Smad2 and Smad4 are tumour suppressors that contribute to the transforming growth factor β (TGF- β) inhibition of cell proliferation, where they have two main functions (Module 9: Figure proliferation signalling network). One function is to inhibit the activity of Myc, and the other is to activate transcription of the cyclin-dependent kinase (CDK) inhibitor p15, which is opposed by the action of the proto-oncogene Myc (Module 4: Figure Myc as a gene repressor). Smad4 mutations were first identified as DPC4 (deleted in pancreatic carcinoma locus 4), because approximately 50% of pancreatic cancers have such mutations. However, similar mutations have also been found in other tumours, such as in colorectal cancer (CRC) (Module 12: Figure colon cancer).

Juvenile polyposis syndromes (JPSs) are caused by germline mutations in the transcription factor Smad4.

Melastatin

Melastatin, which is also known as TRPM1, is a member of the melastatin-related TRP family. A decrease in its expression level occurs during the metastatic phase of malignant melanoma.

LKB1

LKB1, which functions in the AMP signalling pathway, plays a role as a tumour suppressor because it can inhibit

cell growth control by regulating the way target of rapamycin (TOR) controls protein synthesis (Module 9: Figure target of rapamycin). Mutation of LKB1 is responsible for Peutz–Jeghers syndrome.

Tuberous sclerosis 1 and 2 (TSC1/2)

The tuberous sclerosis 1 and 2 (TSC1/2) genes are considered to be tumour promoters because they play a critical role in regulating cell growth control by co-ordinating many of the inputs that regulate the control of protein synthesis by the protein kinase target of rapamycin (TOR). The genes encoding TSC1/2 are inactivated in the autosomal dominant disorder tuberous sclerosis.

Tumour cell microenvironment

In order to grow efficiently, cancers depend on a microenvironment composed of stromal cells such as the blood vessels, fibroblasts, immune cells and epithelial cells. Cancer cells play an active role in ensuring the active support of the surrounding stromal cells. One mechanism is to promote an effective blood supply through a process of tumour angiogenesis. Another mechanism depends on the cancer cells releasing hedgehog that then acts in a paracrine manner to stimulate the hedgehog signalling pathway in neighbouring stromal cells to enhance their supporting roles in providing both an extracellular matrix and essential growth factors such as insulin-like growth factor (IGF) and Wnt.

Tumour angiogenesis

One of the major factors controlling tumour angiogenesis is vascular endothelial growth factor-A (VEGF-A), which stimulates the proliferation of endothelial cells during angiogenesis (Module 9: Figure VEGF induced proliferation). One of the actions of VEGF-A is to stimulate phospholipase C γ (PLC γ) to initiate both the release of internal Ca²⁺ and the entry of external Ca²⁺. The latter seems to be particularly important in that angiogenesis could be blocked by carboxyamidotriazole (CAI), which inhibits the entry mechanism and has been suggested to function as a tumour static agent.

In colon cancer cells, the expression of VEGF is induced through insulin-like growth factor 1 (IGF-1) and depends upon the up-regulation of hypoxia-inducible factor-1 (HIF-1). HIF-1 is normally increased during hypoxia through an inhibition of its ubiquitination and degradation. However, IGF-1 acts through the PtdIns 3-kinase and mitogen-activated protein kinase (MAPK) signalling pathways.

The S100A4 Ca²⁺-binding protein may also act as an angiogenic factor. Its overexpression in gastric cancer has proved to be a reliable diagnostic tool in assessing metastatic potential.

Inflammation and cancer

Inflammation can play an important role in cancer progression. It has been known for sometime that there is an increased risk of cancer in inflammatory bowel disease. How this might occur is beginning to emerge through studies on the development of benign tumours such as in-

flammatory hepatocellular adenomas (IHCA), which are driven by constitutively active mutations in gp130, which is a subunit common to a number of cytokine receptors (Module 1: Figure type I cytokine receptors). Inflammation and tumour metastasis is particularly important during the final stages of tissue invasion when inflammatory responses mediated by macrophages and haematopoietic progenitors create an inflammatory environment that promotes tumour metastasis.

Infertility

Human male infertility has been linked to a reduction in the expression of phospholipase C ζ (PLC ζ) or to point mutations in the catalytic Y domain of PLC ζ . The mutated PLC ζ was incapable of triggering the Ca²⁺ transients responsible for the activation of fertilization (Module 8: Figure mammalian fertilization). Wild-type sperm PLC ζ protein was capable of triggering normal fertilization in the presence of the mutated protein thus indicating a potential therapy for such cases of male infertility.

Inflammatory hepatocellular adenomas (IHCA)

Inflammatory hepatocellular adenomas (IHCA) are particularly prevalent in women with obesity or following excessive alcohol use. These benign tumours have an increased expression of typical inflammatory proteins that are part of the JAK/STAT signalling pathway such as JAK2, STAT1-3 and their downstream targets such as suppressor of cytokine signalling proteins (SOCS3), CRP, SAA2, SPINK1 and FBG. Many of these IHCA were found to have a mutation in gp130, which is one of the subunits used by a number of cytokine receptors (Module 1: Figure type I cytokine receptors). Most of the mutations lie in the cytokine-receptor homology region (CHR) where it interacts with the interleukin-6 (IL-6) α -subunits (IL-6R α). These mutated IL-6 receptors are constitutively active and can stimulate an inflammatory response in the absence of IL-6.

The gp130 receptor subunit may thus be considered to be an oncogene for benign tumours. The presence of such somatic mutations may then contribute to the development of inflammatory hepatocellular carcinomas following the acquisitions of mutations in other oncogenic targets such as β -catenin.

Chemokines and cancer

Many cancer cells express chemokine receptors that can markedly enhance tumour development by increasing angiogenesis, proliferation and survival and they also contribute to metastasis. The CXCR4 receptor, which responds to CXCL12 [also known as stromal-derived factor-1 (SDF-1)] (Module 1: Figure chemokines), is strongly expressed on many human cancers. Many of the tissues where cancer cells take up residence during metastasis, such as bone, liver, lymph nodes and lung, have stromal cells and endothelial cells that express and release CXCL12 that will thus act as a chemoattractant to direct cancer cells towards these tissues. In T cell acute lymphoblastic

leukaemia (T-ALL), the transformed T cells have an increased expression of the CCR7 chemokine receptor that responds to the chemokine CCL19 released from brain venules near the site where the T-cells enter the brain.

The expression of both CXCL12 and its receptor CXCR4 are regulated by hypoxia-inducible factor (HIF) (Module 4: Figure HIF activation) and this may be particularly relevant to metastasis and the development of cancer cell malignancy. In a developing mass of cancer cells, where oxygen is limiting, the activation of HIF will lead to an increase in the expression of the chemotactic CXCR4 receptors that will enhance their metastatic potential. These CXCR4 receptors seek out CXCL12-enriched regions that are ideal for establishing secondary tumours.

Cancer cell phenotypes

The emergence of cancer cells is a somewhat stochastic process dependent upon multiple mutations that either activate oncogenes or inactivate tumour suppressors. One of the difficulties in trying to find a cure for this disease is that there are a large number of cancer cell phenotypes. Despite the stochastic nature of the genetic changes that occur, there are some clearly identified phenotypes where certain mutations occur at much higher frequencies than one might expect from a purely stochastic process. These phenotypes are usually found in cancers that develop from cells that have a high rate of proliferation, such as the somatic stem cells.

In long-lived organisms such as humans, cell proliferation is essential for tissue maintenance and repair. This is particularly so for those epithelia that face harsh environments, such as the intestine and skin, where damaged cells are constantly being replaced from a resident population of stem cells. Many of these stem cells are nurtured and maintained within a specialized niche, where they are controlled by specific signalling pathways. Mutations in these specific signalling pathways tend to predominate in those cancers that emerge from particular tissues. For example, mutations of components of the Wnt signalling pathway, such as APC and β -catenin, are particularly evident in colorectal cancer (CRC). Another example is skin cancer, where there is a high incidence of mutations in components of the Hedgehog signalling pathway.

- Acute promyelocytic leukaemia (APL)
- Chronic lymphocytic leukaemia (CLL)
- Chronic myelogenous leukaemia (CML)
- Colorectal cancer (CRC)
- Diffuse large B cell lymphoma (DLBCL)
- Familial adenomatous polyposis (FAP)
- Juvenile polyposis syndromes (JPSs)
- Li-Fraumeni syndrome
- MALT lymphomas
- Myeloid neoplasms
- Neuroblastoma
- Pancreatic cancer
- Prostate cancer
- Renal cell carcinoma (RCC)
- Skin cancer
- Stomach cancer

- T cell acute lymphoblastic leukaemia (T-ALL)
- Uveal melanoma

Acute promyelocytic leukaemia (APL)

Acute promyelocytic leukaemia (APL) is caused by a translocation of the *PML* tumour suppressor gene resulting in its fusion to the retinoic acid receptor α gene (*RAR α*) to form the oncogenic *PML-RAR α* chimera. The normal promyelocytic leukaemia (*PML*) protein functions in the assembly and function of *PML* nuclear bodies (*PML* NBs).

Chronic lymphocytic leukaemia (CLL)

Chronic lymphocytic leukaemia (CLL), which affects B-cells, is the most frequent adult leukaemia. Unlike most other types of leukaemia, the malignant CLL cells are mostly immature non-dividing cells that survive for long periods. The CLLs gradually build-up in the lymph nodes where they begin to physically interfere with the immune system by crowding out the normal operation of immune cells that function in processes such as B-cell differentiation in the lymph node (Module 8: Figure germinal centre). In keeping with their prolonged survival, the CLL cells overexpress the anti-apoptotic factor Bcl-2. The increased Bcl-2 may result from the down-regulation or deletion of miR-15 and miR-16 that normally act to repress the expression of Bcl-2.

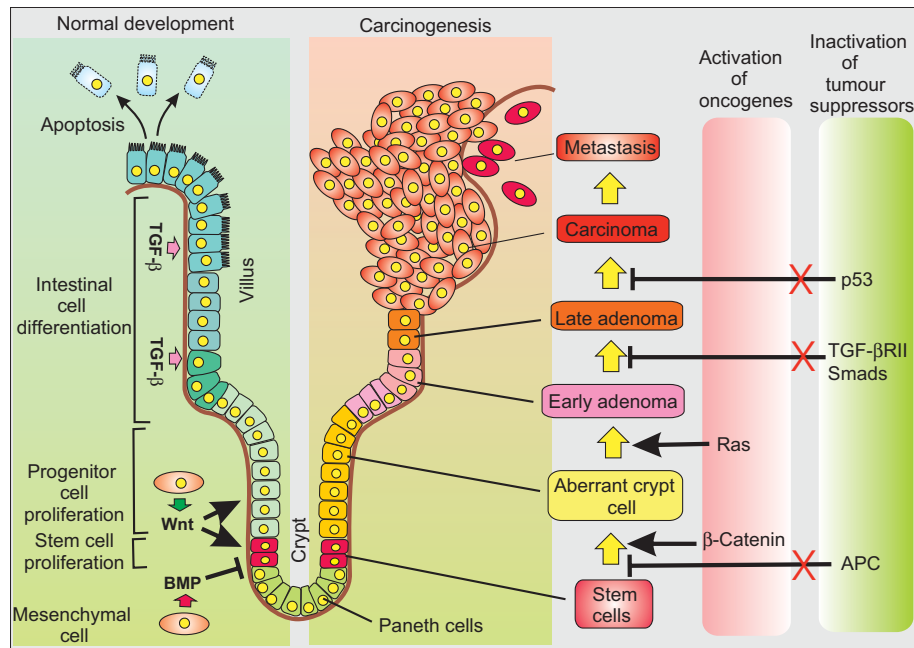
Chronic myelogenous leukaemia (CML)

This form of leukaemia results from the BCR-ABL oncogene. BCR-ABL is a hybrid protein, which is formed by fusion of parts of the *BCR* gene and *Abl* gene to produce a tyrosine kinase with unique signalling properties. One of its important actions is to activate the class IA PtdIns 3-kinase (PtdIns 3-K) that produces the lipid messenger PtdIns3,4,5P₃ that activates the PtdIns 3-kinase signalling pathway (Module 2: Figure PtdIns 3-kinase signalling). This pathway not only functions to drive cell proliferation, but also switches off apoptosis, which are two of the hallmarks of cancer. The BCR portion of this oncogene contains tyrosine residues that recruit growth factor receptor-binding protein 2 (Grb2) thus enabling this oncogene to plug into the mitogen-activated protein kinase (MAPK) signalling pathway.

Colorectal cancer (CRC)

The development of colorectal cancer (CRC) illustrates the way that cancer is a multistep process. The intestinal epithelium is constantly renewing itself by recapitulating the process of the differentiation of intestinal cells that first occurs when the intestine develops. This developmental process continues in adult life to replace the large number of intestinal cells that die each day. The intestinal cell production line begins with an asymmetrical division of the stem cells located at the base of the crypt. One of the cells remains as a stem cell, whereas the other moves off to become a progenitor cell. Clonal expansion of this progenitor cell produces the large number of cells that then go on to differentiate into epithelial intestinal cells (left-hand part of Module 12: Figure colon cancer). Despite the fact that the intestine generates approximately 100 billion cells each

Module 12: | Figure colon cancer



Progressive development of a typical colon cancer.

The normal development and differentiation of the intestinal cell is shown on the left. Approximately four stem cells located in the crypt proliferate relatively slowly to create the progenitor cells that then divide rapidly to produce the large population of cells that differentiate into intestinal cells as they migrate up the villus. The proliferation and maintenance of the crypt cells is regulated by Wnt, which probably comes from the mesenchymal cells. As the proliferating progenitor cells move up the crypt they come into contact with transforming growth factor β (TGF- β), which switches off proliferation and promotes intestinal cell differentiation. The oncogenic events that occur during the development of colon cancer are shown on the right, together with some of the prominent mutations that activate oncogenes and inactivate the tumour suppressors. Initially, a benign adenoma develops, which can then transform into a carcinoma with the potential for metastasis. (Adapted from information taken from Giles et al. 2003 and Mishra et al. 2005.)

day, the system is remarkably robust and there are very few errors. However, this carefully orchestrated process of cell proliferation and differentiation can be subverted by mutations that switch the progenitor cells into cancer cells. This reprogramming of the progenitor cells' signalling system is not immediate, but occurs gradually through a number of stages as the cells acquire more and more transforming mutations. These mutations depend upon the activation of oncogenes and the inactivation of tumour suppressors (the right-hand part of Module 12: Figure colon cancer).

An important feature of colon cancers is that many of the oncogenic mutations are found within the signalling pathways that normally control the proliferation and maintenance of the crypt stem cells. The Wnt signalling pathway is particularly important, and by far the majority of all colonic cancers have mutations in this pathway. For example, mutations in adenomatous polyposis coli (APC), which is a tumour suppressor that functions in the Wnt pathway (Module 2: Figure Wnt canonical pathway), occur at a particularly high frequency (85%). The progression towards malignancy depends upon the gradual accumulation of mutations, and it seems likely that inactivation of APC and the oncogenic activation of β -catenin and Ras are early steps in the initial aberrant crypt cell. In addition, mutations in the transforming growth factor β (TGF- β) receptor and the downstream Smads switch

off the anti-proliferative function of the Smad signalling pathway, which enable the cells to go on growing (Module 12: Figure cell cycle network and cancer). Cells with these mutations acquire a distinct growth advantage, and a clone of modified cells begins to emerge to form an adenomatous polyp (Module 12: Figure colon cancer). Approximately 50% of the adult population will develop such polyps by the age of 70 years. Such polyps are an important step towards the formation of a cancer cell because they have the genetic instability to introduce further mutations, such as those in p53 that drive the cells towards an early adenoma that can then progress towards a carcinoma and malignancy.

Diffuse large B cell lymphoma (DLBCL)

There are three main types of diffuse large B cell lymphoma (DLBCL): activated B cell-like (ABC), germinal centre B cell-like (GBC) and primary mediastinal B cell lymphoma (PMBL). The ABC subtype, which is the least curable, seems to depend on a constitutive activation of the Toll receptor signalling pathway caused by mutations in the MyD88 that functions as an adaptor protein (Module 2: Figure Toll receptor signalling). The resulting increase in the NF- κ B signalling pathway enhances the survival of these lymphoma cells.

Familial adenomatous polyposis (FAP)

Familial adenomatous polyposis (FAP) is an inherited autosomal dominant condition that arises through a loss of one of the alleles that code for the tumour suppressor adenomatous polyposis coli (APC). It results in the formation of multiple adenomas found most frequently in the colon and rectum, but tumours can also occur in the thyroid and brain. It is a highly penetrant cancer-predisposition syndrome, because one of the APC alleles is inactivated and cancers develop when mutations arise in the remaining allele. The progression of the disease is similar to that described for the onset of colorectal cancer (CRC).

Juvenile polyposis syndromes (JPSs)

Juvenile polyposis syndromes (JPSs) is caused by a germline mutation in *Smad4*, which functions in the Smad signalling pathway that mediate the anti-proliferative signalling pathway activated by the transforming growth factor β (TGF- β) superfamily (Module 9: Figure proliferation signalling network).

Li–Fraumeni syndrome

Li–Fraumeni syndrome is an inheritable disease characterized by having a high risk of developing cancer. The germline mutation results a single copy of the *TP53* gene that codes for the tumour suppressor p53. A central role for p53 in tumour suppression means that any mutation that inactivates the normal allele will result in the appearance of tumours.

MALT lymphomas

Mucosa-associated lymphoid tissue protein (MALT) lymphomas are caused by a number of genetic changes. One of these is a t(11;18)(q21;q21) chromosomal translocation of the *MALT1* gene to the genomic locus of the inhibitor of apoptosis gene *cIAP* to give an *API2-MLT* fusion gene that has been linked to MALT lymphomas. Mutations in the *Bcl10* gene have also been linked to MALT lymphomas. Both MALT and *Bcl10* function as scaffolding proteins to activate the NF- κ B signalling pathway (Module 9: Figure TCR signalling).

Myeloid neoplasms

Myeloid neoplasms are a heterogeneous group of cancers of one or more different cell types. A subset of these patients has mutations in the gene that codes for Cbl, which functions in the Cbl down-regulation of signalling components (Module 1: Figure receptor down-regulation).

Neuroblastomas

Approximately 15% of all childhood cancer deaths are caused by neuroblastoma, which are some of the most intractable paediatric cancers. In some cases, a genetic predisposition results from germ line mutations. In addition to such familial cases, neuroblastomas can also arise from somatic mutations. Amplification of *MYCN* is one of the significant genetic changes found in many of these cancers. Another major neuroblastoma-predisposition gene is *ALK*, which codes for the anaplastic lymphoma kinase

(ALK) receptor. Many of the cancer-inducing mutations occur in the cytoplasmic kinase domain. Unlike the normal ALK receptor, the mutated ALK is hyperphosphorylated resulting in an increased tyrosine kinase activity enabling it to phosphorylate downstream targets such as STAT3, PKB and ERK1/2 all of which function in cell proliferation.

Pancreatic cancer

Many pancreatic cancer cells overexpress the Ca^{2+} -sensitive transcription factor nuclear factor of activated T cells c1 (NFATc1), which may stimulate cell proliferation by driving Myc dysregulation and cancer development.

Prostate cancer

Prostate cancer begins with an early stage, which depends on androgens for growth and survival, and then progresses to an androgen-insensitive stage that is difficult to control. This transformation to an androgen-insensitive cell represents an alteration in its signalling systems characterized by a switch in emphasis from enhanced proliferation to an up-regulation of the processes that lead to inhibition. The way in which apoptosis is suppressed may vary. In some cases, the PtdIns 3-kinase signalling pathway is important, whereas in other cases an overexpression of *Bcl-X_L* antagonizes the pro-apoptotic factor Bad.

Some of the prostate cancers appear to have alterations in their Ca^{2+} signalling pathways. Human prostate cancer cell growth can be inhibited by blocking Ca^{2+} entry. Release and emptying the internal store initiates apoptosis in prostate cancer cells, and this may be significant because apoptosis inhibition rather than enhanced proliferation occurs in prostate cancers. Activation of apoptosis by store depletion could be prevented by the anti-apoptotic protein *Bcl-X_L*.

The vanilloid transient receptor potential 6 (TRPV6) channel is highly expressed in human prostatic carcinoma cells. It appears to be linked to the progression of the disease. Expression of this channel was suppressed by androgen, and was induced by an androgen antagonist. Melastatin-related transient receptor potential 8 (TRPM8) is another Ca^{2+} -permeable channel that is strongly up-regulated in malignant prostate cancer cells and in a number of other primary tumours of breast, colon, lung and skin origins.

Renal cell carcinoma (RCC)

Renal cell carcinoma (RCC) displays a high degree of organ-specific metastasis, which accounts for the mortality and morbidity associated with these tumours. The invasiveness has been related to the expression of the chemokine receptor CXCR4, which responds to the chemokine stromal-derived factor-1 (SDF-1) that is produced by organs such as bone, lung, liver and lymph nodes.

Skin cancer

Cancer of the skin is one of the commonest forms of human cancer. The high proliferative rate of skin cells, together with the fact that they often are subjected to damaging levels of UV irradiation, makes them particularly susceptible to oncogenic mutations. Such UV-induced cell stress

causes DNA damage and the acquisition of mutations that result in various forms of skin cancer, such as squamous cell carcinoma (SCC), basal cell carcinoma (BCC) and melanomas. The commonest form of malignancy is BCC, which accounts for 90% of all skin cancers. A high predisposition to develop skin cancer is found in a variety of disorders, such as Gorlin's syndrome, Bazex's syndrome, xeroderma pigmentosum and albinism. Gorlin's syndrome is particularly revealing, because it results from a mutation in patched (PTC), which is a tumour suppressor that operates in the Hedgehog signalling pathway (Module 2: Figure Hedgehog signalling pathway). Mutations in the Hedgehog signalling pathway, which play a prominent role in regulating the proliferation of keratinocytes, are particularly prominent in BCCs.

Stomach cancer

Most forms of stomach cancer are associated with the bacterium *Helicobacter pylori*, which is also the main cause of stomach ulcers. The way in which the bacterial oncoprotein cytotoxin-associated gene A (CagA) is injected into epithelial cells and activated is described in the section on peptic ulcers (Module 12: *H. pylori* nano-syringe).

T cell acute lymphoblastic leukaemia (T-ALL)

T cell acute lymphoblastic leukaemia (T-ALL) occurs in many children and adolescents. It is a very common childhood cancer representing approximately 30% of all paediatric cancers. In most of the T-ALL patients, the T-cells have a genetic alteration in the Notch signalling pathway that not only results in rapid proliferation, but it also creates a clone of cells that have an enhanced ability to infiltrate the brain. One consequence of up-regulation of Notch signalling is an increase in the expression of the CCR7 chemokine receptor, which responds to the chemokine CCL19 released from brain venules near the site where the T-cells enter the brain.

Uveal melanoma

As its name suggests, uveal melanoma is a cancer found in the uvea (choroid, ciliary body and iris). The cancer arises from the melanocytes that are responsible for the colour of the eye. Most of these uveal melanomas have mutations in *CNAQ* and *GNA11*, which encode $G_{\alpha q}$ and the closely related $G_{\alpha 11}$, respectively. These are members of the heterotrimeric G proteins that play an important role in controlling many different signalling pathways (Module 2: Figure heterotrimeric G protein signalling). In particular, they play a central role in growth factor signalling pathways (Module 9: Figure growth factor signalling).

Catecholamine polymorphic ventricular tachycardia (CPVT)

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is caused by mutations in the ryanodine receptor 2 (RYR2), which is responsible for releasing Ca^{2+} to control cardiac contraction (Module 7: Figure ventricular cell E-C coupling). CPVT results in a form of sudden heart death in children and young adults. It closely resembles another sudden death syndrome called arrhythmogenic

right ventricular cardiomyopathy type 2 (ARVD2). Mutations for both syndromes occur in the same three regions where mutations causing malignant hyperthermia (MH) and central core disease (CCD) are located (Module 12: Figure RYR mutations). In the case of CPVT and ARVD2, the mutations tend to cluster in different locations, and this may explain why ARVD2 has a slightly different phenotype in that it usually progresses to a morphological change in the right ventricle where the wall is infiltrated with fibrous and fatty tissue.

The arrhythmogenic effects of CPVT and ARVD2 can lie latent, and the heart appears to be normal. However, arrhythmias suddenly appear during periods of emotional and physical stress. An important triggering signal appears to be an increase in β -adrenergic stimulation. It seems that these mutations result in an increase in the sensitivity of the ryanodine receptor 2 (RYR2) channels to the stimulatory effect of Ca^{2+} . The result is that the more sensitive RYR2 channels begin to release Ca^{2+} during diastole and this then triggers an inward flow of current to cause a delayed after-depolarization (DAD). If this DAD is large enough to reach the threshold to trigger an action potential, this will result in the catastrophic arrhythmias that result in sudden heart death.

CPVT can also result from mutations in calsequestrin (CSQ). These mutations were found to reduce the buffering capacity of CSQ, and the resulting increase in the luminal load of Ca^{2+} will enhance the sensitivity of the RYR2, leading to the inappropriate release of Ca^{2+} as just described for the CPVT and ARVD2 mutations.

Central core disease (CCD)

Central core disease (CCD), which is an autosomal dominant myopathy, is caused by a dysregulation of skeletal muscle Ca^{2+} homeostasis resulting from mutations in the type 1 ryanodine receptor (RYR1). The disease takes its name from the finding that the central areas of the skeletal muscle have a core where the mitochondria have disappeared and the contractile filaments are disorganized. The disease is often associated with muscle weakness and delayed muscle development. Some of the mutations in the RYR1 are also found in patients suffering from malignant hyperthermia (MH). Indeed, there are some patients that can show symptoms of both diseases. In the case of CCD, most of the mutations tend to cluster in the C-terminal region (Module 12: Figure RYR mutations). Just how these RYR1 mutations result in the morphological changes in the core region are still being debated. One idea is that the RYR1 mutation increases the leak of Ca^{2+} , which then overwhelms and destroys the central mitochondria that seem less able to cope with the Ca^{2+} overload.

The way in which the leak may occur through a disruption of the interaction between regions 1 and 2 of RYR1 is described in the section on malignant hyperthermia (MH).

Centronuclear myopathy (CNM)

Centronuclear myopathy (CNM) is a severe congenital muscular disease that is caused by the internalization and centralization of the micronuclei. This disorder has been

associated with missense mutations of the myotubularin MTMR14, which hydrolyses PtdIns3P that functions in the PtdIns3P signalling cassette to control both endosome vesicle fusion to early endosomes (Module 4: Figure endosome vesicle fusion) and autophagy (Module 11: Figure autophagy signalling mechanisms).

Charcot-Marie-Tooth disease

Charcot-Marie-Tooth disease consists of a heterogeneous group of inherited peripheral neuropathies where there are defects in sensory, motor or autonomic neurons. There are two main types of disease state: those where there is decreased nerve conduction velocities caused by prominent demyelination resulting from mutations in myelin-specific proteins. In the second type, disease is caused by axonal degeneration resulting from genetic mutations in components of various cellular processes and particularly those responsible for mitochondrial function and axonal transport pathways.

Charcot-Marie-Tooth disease 2A

Charcot-Marie-Tooth disease 2A is an inherited peripheral sensorimotor neuropathy that is characterized by axonal degeneration. It is caused by mutations in mitofusin 2 (MFN2). One of the functions of MFN2 is to tether the endoplasmic reticulum to the mitochondria to ensure a functional coupling between these two organelles during the operation of the endoplasmic reticulum (ER)/mitochondrial Ca^{2+} shuttle (Module 5: Figure ER/mitochondrial shuttle).

Charcot-Marie-Tooth disease 2A is also caused by a point mutation in the ATP-binding site of the motor domain of the kinesin-3 motor protein KIF1B β that is responsible for transporting mitochondria (Module 4: Figure kinesin cargo transport in neurons).

Charcot-Marie-Tooth disease type 2B

Charcot-Marie-Tooth disease type 2B is characterized by loss of pain sensation resulting in recurrent ulcers, deformities and frequent need for amputation of the lower limbs. As such, CMT2B is alternatively classified as an ulcero-mutilating neuropathy. Mutations in Rab7A have been associated with this neuropathy.

Charcot-Marie-Tooth type 2 disorder type 2B1 has been linked to mutations in the *LMNA* gene that codes for lamin A and lamin C that contribute to the nuclear lamina (Module 12: Figure chromatin organization).

Charcot-Marie-Tooth disease 4B

Charcot-Marie-Tooth is a demyelinating neuropathy caused by a mutation in MTMR2, which belongs to the myotubularin family of 3-phosphatases that dephosphorylates PtdIns3P. The PtdIns3P signalling cassette functions in the regulation of membrane trafficking and may also regulate the activity of the intermediate-conductance (IK) channel.

Charcot-Marie-Tooth disease 4J

Charcot-Marie-Tooth disease 4J, which is characterized by asymmetric neural degeneration, is caused by mutation

in the lipid phosphatase Sac3/Fig4 that functions in the PtdIns3,5P₂ signalling cassette (Module 2: Figure PIKfyve activation).

Choroideremia

Choroideremia is an X-linked disease that results in blindness caused by degeneration of the retinal pigment. This degeneration, which seems to result from a defect in the transport of the melanosomes that normally protect the retinal pigment epithelium from the harmful exposure of light, is caused by a mutation in the Rab export protein 1 (REP1). This REP1 facilitates the geranylgeranylation of the Rab proteins that are essential for them to function in the Rab signalling mechanism (Module 2: Figure Rab signalling). In the case of the retinal pigment epithelium, it is Rab27A that is responsible for this melanosome transport (Module 4: Figure myosin motor).

Concurrent generalized epilepsy with paroxysmal dyskinesia

Concurrent generalized epilepsy with paroxysmal dyskinesia is caused by a single missense mutation in the gene for the large-conductance (BK) channels (Module 3: Figure K⁺ channel domains). The mutation is located in the region of the regulator of conductance for K⁺ (RCK).

Congenital chloride diarrhoea

Congenital chloride diarrhoea is caused by mutations in the down-regulated in colonic adenoma (DRA) protein that is thought to be the Cl[−]/OH[−] exchanger that contributes to fluid absorption by the colon (Module 7: Figure colon function).

Congenital hyperinsulinism of infancy

Congenital hyperinsulinism of infancy is an early-onset disease that presents itself at birth or during the first year of life. It is characterized by a continuous up-regulation of insulin. It develops as a result of mutations in several of the components that function in the control of insulin release, such as the enzyme glucokinase and the two subunits of the ATP-sensitive K⁺ (K_{ATP}) channel: Kir6.2 and SUR1. The most common mutations occur in the SUR1 subunit, which is the regulatory subunit of the K_{ATP} channel.

Congenital insensitivity to pain

An inability to sense pain can prove serious particularly in childhood when infants are susceptible to permanent injury as they fail to perceive illness or injuries. They are also incapable of learning pain avoiding behaviours. This congenital insensitivity is caused by the loss of function of the SCN9A gene that codes for the Nav1.7 channel that function in pain perception (Module 10: Figure nociception). Conversely, mutations that increase the sensitivity of this gene are responsible for erythralgia.

Congenital lipid adrenal hyperplasia

Congenital lipid adrenal hyperplasia is caused by a mutation in the steroidogenic acute regulator (StAR), which appears to be the Ca²⁺-sensitive step responsible for reg-

ulating the synthesis of aldosterone in zona glomerulosa cells (Module 7: Figure glomerulosa cell signalling).

Cowden's disease

Germline mutations of phosphatase and tensin homologue deleted on chromosome 10 (PTEN) lead to Cowden's disease characterized by multiple hamartomatous lesions of the skin, mucous membranes, thyroid and breast.

Craniofrontonasal syndrome (CFNS)

Craniofrontonasal syndrome (CFNS) is an X-linked disorder characterized by hypertelorism, a central nasal groove and squint. In addition to the craniofacial abnormalities, there also are thoracic and limb defects. These skeletal abnormalities that occur during development have been linked to mutations in the gene *EFNB1* that encodes ephrin-B1, which is one of the components of the ephrin (Eph) receptor signalling system (Module 1: Figure Eph receptor signalling).

Cystic fibrosis (CF)

Cystic fibrosis (CF) results from a defect in the cystic fibrosis transmembrane regulator (CFTR) channel (Module 3: Figure CFTR channel) that contributes to fluid secretion in a number of epithelia. Mutations in this channel, which decrease the flow of ions and water, result in dehydrated mucous that blocks the ducts in the lung that affects breathing and can result in lethal bacterial infections. Multiple mutations of the CFTR gene have been identified and they disrupt channel function in many different ways:

- Some mutations terminate transcription causing truncated unstable transcripts
- Missense mutations such as $\Delta F508$ -CFTR result in protein misfolding and degradation
- Some mutations reduce channel function by either interfering with the ATP-dependent activation mechanism or by altering the gating mechanism
- Alterations in the alternative splicing mechanism reduces the amount of functional protein

In the airway epithelia in the lung, there are two Cl^- channels that play a critical role in establishing the ionic gradient that drives water movement. One of these is the CFTR channel, which is defective in CF, while the other is a Ca^{2+} -sensitive Cl^- channel (CLCA). There are indications that the latter might be up-regulated to partially alleviate some of the symptoms of CF.

The infertility that is associated with CF may arise through a decrease in the transport of HCO_3^- into the fluid in the genital tract. This HCO_3^- activates capacitation, which is the maturation process that makes the sperm competent to fertilize the egg.

Darier's disease

Darier's disease is a genetic skin disease, which is caused by a null mutation in one copy of the *ATP2A2* gene that codes for the sarco/endo-plasmic reticulum Ca^{2+} -ATPase 2 (SERCA2) pump. This autosomal dominant skin dis-

order results in multiple keratotic papules in the seborrheic regions of the body.

Dent's disease

Dent's disease is familial X-linked proximal tubule syndrome that is characterized by proteinuria, hypercalciuria, formation of kidney stones and renal failure. It is caused by mutations of the CLC-5 chloride transporter (Module 3: Table CLC chloride channel and transporter components). The symptoms of Dent's disease may result from a defect in the acidification or renal endocytic vesicles.

Mutations in oculocerebrorenal syndrome of Lowe (OCRL) have also been identified in a subset of patients with Dent's disease.

Dominant nonsyndromic deafness type 2 (DFNA2)

Dominant nonsyndromic deafness type 2 (DFNA2) is caused by mutations in the delayed rectifier $\text{K}_{\text{v}}7.4$ that controls K^+ efflux from outer hair cells.

Down's syndrome

Down's syndrome is a complex pathology characterized by altered craniofacial morphology, cardiac defects and mental retardation. Since brain development in Down's syndrome is characterized by a decreased number of neurons, there has been considerable interest in trying to determine why the neurons die by apoptosis. The dementia that occurs in later life resembles Alzheimer's disease. Down's syndrome occurs in about 1 in 700 births and is caused by trisomy of chromosome 21. The region of chromosomal triplication results in a 1.5-fold increase in approximately 230 genes, and the hunt has been on to identify those that might be causally linked to Down's syndrome. There is much interest in the fact that this region has the gene for the amyloid peptide precursor responsible for Alzheimer's disease. Other interesting candidates are the genes that encode Down's syndrome critical region 1 (DSCR1), which may link Down's syndrome and calcineurin inhibition, and dual-specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A) that may link Down's syndrome and nuclear factor of activated T cells (NFAT). Considerable information has been derived from the trisomy 16 mouse, which is a model of human trisomy 21 or Down's syndrome.

One of the striking features of Down's syndrome is the significant suppression of solid tumours: mortality was less than 10% of that in the normal population. This reduced cancer risk seems to depend on the increased expression of DSCR1 and DYRK1A that function by inhibiting angiogenesis, thus reducing the blood supply to tumours. During angiogenesis, the activation of endothelial proliferation by vascular endothelial growth factor (VEGF) depends on the enhanced activation of the transcription factor NFAT (Module 9: Figure VEGF-induced proliferation).

Because of the nature of the genetic modification, it is likely that the disease results from the overexpression of genes located on chromosome 21. Some of these genes

code for proteins that are associated with various signalling pathways:

Down's syndrome and redox signalling

Considerable attention has focused on the possibility that Down's syndrome may result from overactive redox signalling. In particular, the onset of Down's syndrome may result from the role of redox signalling in apoptosis. Chromosome 21 is known to encode superoxide dismutase 1 (SOD1), which is known to be overexpressed in the brains of Down's syndrome patients, and this may result in an increase in the production of hydrogen peroxide (H_2O_2) that may be responsible for neuronal cell death. Indeed, Down's syndrome neurons produce large amounts of reactive oxygen species (ROS). Cultured hippocampal neurons from the trisomy 16 mouse have an augmented cell death, which is enhanced by decreasing the glutathione (GSH) that normally counteracts the increase in ROS.

Down's syndrome and inositol metabolism

The availability of inositol in the brain may exert an important influence on neural signalling mechanisms. Inositol distribution might be altered in Down's syndrome, possibly due to overexpression of the Na^+ / myo -inositol co-transporter gene, which is located on chromosome 21, resulting in an abnormal accumulation of inositol in the cerebrospinal fluid (CSF). If the increase in inositol export decreases the intracellular level, this will severely reduce the various phosphoinositide signalling pathways, such as the PtdIns 3-kinase signalling cassette, which is known to promote cell survival and could thus account for the increases in apoptosis.

Down's syndrome and calcineurin inhibition

The brains of Down's syndrome patients overexpress a protein encoded by a Down's syndrome critical region 1 (DSCR1) gene, which is an inhibitor of calcineurin (CaN) (Module 4: Figure NFAT control of Ca^{2+} signalling toolkit).

Down's syndrome and nuclear factor of activated T cells (NFAT)

Many cellular control mechanisms including activation of the immune response and a whole host of development processes depend upon nuclear factor of activated T cells (NFAT) function. The operation of this Ca^{2+} -activated transcription factor depends on a NFAT shuttle whereby phosphorylated cytosolic NFAT is activated by calcineurin (CaN) and is imported into the nucleus. Within the nucleus, NFAT activates many different genes some of which code for proteins that function in Ca^{2+} signalling (Module 4: Figure NFAT control of Ca^{2+} signalling toolkit). Two of the genes within the region of chromosomal triplication code for proteins that control this shuttle. As described above, Down's syndrome critical region 1 (DSCR1) gene is an inhibitor of calcineurin, whereas dual-specificity tyrosine-phosphorylation regulated kinase 1A (DYRK1A) is a protein kinase responsible for inactivating NFAT by facilitating its export from the nucleus. The 1.5-fold increase in the expression of these

two genes would combine to reduce the activity of NFAT because DSCR1 would inhibit its import, while DYRK1A would enhance its export. Since NFAT has such a central role in so many developmental processes, a reduction in the activity of NFAT may well account for many of the symptoms associated with Down's syndrome. For example, the reduction in NFAT activity can inhibit the ability of VEGF to increase angiogenesis (Module 9: Figure VEGF-induced proliferation).

Down's syndrome and endocytosis

One of the genes located on the trisomy region on chromosome 21 is the dual-specificity tyrosine-phosphorylation regulated kinase 1A (DYRK1A), which can phosphorylate a number of the endocytic accessory proteins such as dynamin, amphiphysin and synaptojanin 1 (SJ1) that function in membrane invagination and scission (Module 4: Figure scission of endocytic vesicles). As a result of the gene imbalance, the expression of DYRK1A will be increased resulting in increased phosphorylation and hence inhibition of these accessory proteins. A reduction in endocytosis could contribute to the complex Down's syndrome phenotype.

The *ITSN1* gene, which codes for the scaffolding protein intersectin 1 (ITSN1), is also located on the trisomy region and may contribute to the syndrome because of its role in endocytosis.

Dravet syndrome

Dravet syndrome, which is also known as severe myoclonic epilepsy of infancy (SMEI), is a severe form of intractable epilepsy that begins early in infancy. It is characterized by frequent febrile seizures. The condition affects development resulting in poor language and motor skills.

Mutations in the *SCN1A* gene that encodes the $Na_v1.1$ sodium channel have been linked to this syndrome.

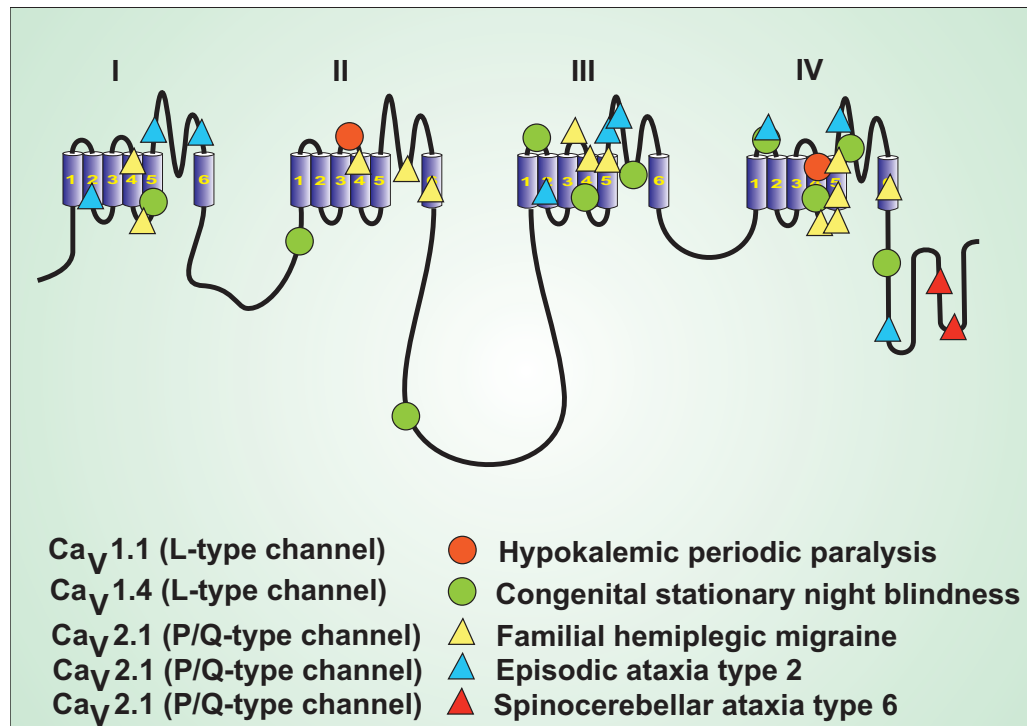
Dubin-Johnson syndrome

Dubin-Johnson syndrome is an autosomal recessive metabolic disorder caused by a defect in the secretion of conjugated bilirubin by the hepatocytes. The secretory defect is caused by a mutation in the gene that codes for ABCC2 organic anion transporter [also known as the multidrug resistance protein 2 (MRP2)] that is responsible for the ATP-dependent transport of certain organic anions across the canalicular membrane of the hepatocyte. As a result, pigments accumulate in the lysosomes causing the liver to turn black.

Early-onset obesity

Mutations in components of the signalling pathways that function in the control of food intake and body weight have been linked to early-onset obesity. These mutations have been located in the following genes:

- One of the genes encodes the pro-opiomelanocortin (POMC) precursor used to form the α -MSH released from the POMC/CART neurons in the hypothalamus (step 7 in Module 7: Figure control of food intake).

Module 12: | Figure channelopathies

The mutations responsible for a variety of hereditary Ca²⁺ channelopathies have been located within the α subunit of different voltage-operated channels (VOCs).

Voltage-operated channels (VOCs) have an α subunit made up of four repeated domains (I-IV), each of which has six transmembrane segments. Mutations either in these transmembrane domains or in the loops that connect these different transmembrane domains are responsible for many channelopathies.

- Early-onset obesity also develops in individuals carrying a mutation in the melanocortin receptor MC4R, which carries out the action of the α -MSH released from the POMC/CART neurons in the hypothalamus (step 7 in Module 7: Figure control of food intake)

Episodic ataxia type 2

Episodic ataxia type 2 is an autosomal dominant neurological disease that results from spontaneous mutations of the Ca_V2.1 P/Q-type channel α_1 subunit (Module 3: Figure Ca_V2 channel family). The mutations have been mapped to a number of different regions on the α_1 subunit (Module 12: Figure channelopathies). These are loss-of-function mutations that cause a change in the firing patterns of cerebellar Purkinje cells that control motor co-ordination. Stress and exertion are the main triggers that bring on the ataxia, which is characterized by loss of limb co-ordination and an unsteady gait.

Erythralgia

Erythralgia is characterized by a greatly heightened sense of temperature such that mild warmth is perceived as severe burning sensations. This is caused by mutations in the SCN9A gene that codes for the α -subunit of the sodium channel Na_V1.7, which amplifies the generator potentials that occur during pain perception (Module 10: Figure nociception).

Conversely, loss-of-function mutations of the same gene results in congenital insensitivity to pain.

Familial advanced sleep phase syndrome (FASPS)

Familial advanced sleep phase syndrome (FASPS), which is characterized by early sleep times and early morning awakening, is an inherited dominant disorder of the circadian clock. It is caused by mutations in either the casein kinase I-binding site on the clock gene PER or in the casein kinase I ϵ (CKI ϵ) that reduce its ability to phosphorylate and degrade the clock component PER (Module 6: Figure circadian clock molecular mechanism). This sleep syndrome is also caused by mutations in the gene that encodes casein kinase I δ (CKI δ), which can also cause severe migraines.

Familial amyloidosis

Amyloidosis is a condition that develops when normal soluble proteins become insoluble and begin to deposit in the extracellular space where they begin to disrupt the functions of various tissue such as the eyes, nerves, skin and kidneys. The symptoms vary depending on where the deposits occur. Familial amyloidosis results in mutations in specific genes. One of these inherited genes is the Ca²⁺-binding protein gelsolin, which functions in actin remodelling (Module 4: Figure actin remodelling). The gelsolin mutation, which is located in the type-2

calcium-binding site of domain G2, results in the formation of peptide fragments that then aggregate to form the amyloid deposits that disrupt the functions of many different tissues.

Familial episodic pain syndrome

Familial episodic pain syndrome (FEPS) is characterized by episodic upper body pain that can be triggered by various conditions such as stress, cold or fasting. It is caused by an autosomal dominant missense mutation of the TRPA1 channel.

Familial expansile osteolysis

Familial expansile osteolysis is a bone disorder characterized by focal areas of increased bone remodelling that has been linked to mutations of the *TNFRSF11A* gene that encodes the receptor activator of nuclear factor κ B (NF- κ B) ligand (RANKL) receptor (RANK), which plays a critical role in osteoclastogenesis. The differentiation of the bone resorbing osteoclasts is critical dependent on the activation of RANK (Module 8: Figure osteoclast differentiation).

Familial hemiplegic migraine (FHM)

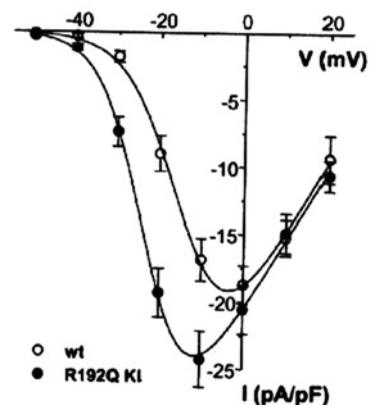
Familial hemiplegic migraine (FHM) is a somewhat rare autosomal dominant form of migraine. It may help to provide insights into the causes of normal migraine. More than half of the families with FHM have missense mutations in the gene that codes for the α_{1A} subunit of the $\text{Ca}_v2.1$ P/Q-type channel (Module 3: Figure Ca_v2 channel family). The mutations have been mapped to a number of points on the α_{1A} subunit (Module 12: Figure channelopathies). This is the voltage-operated channel (VOC) family that is responsible for Ca^{2+} -induced transmitter release (Module 4: Figure Ca^{2+} -induced membrane fusion). These channels are expressed in those regions of the brain that have been implicated in the generation of migraine. The mutations have complex effects in that they reduce the number of channels that are inserted in the membrane. However, this decrease in channel density is counteracted by a shift in the activation curve, indicating that there is a marked increase in the open probability (Module 12: Figure $\text{Ca}_v2.1$ current densities). This is thus a gain-of-function mutation, resulting in an increase in the influx of Ca^{2+} over a larger range of densities. This suggests that migraines may result from an increased excitability of the channels that release excitatory transmitters such as glutamate.

Some families that display FHM have mutations in the α_2 subunit of the Na^+/K^+ -ATPase, which is expressed in high levels in astrocytes. A loss of the Na^+/K^+ -ATPase would prevent the astrocytes from removing K^+ from the vicinity of neurons, and this would increase neuronal excitability and therefore have an effect similar to that of the gain-of-function $\text{Ca}_v2.1$ mutation.

Familial hemophagocytic lymphohistiocytosis type 3 (FHL3)

Familial hemophagocytic lymphohistiocytosis type 3 is caused by a defect in hMunc13-4, which acts as an effector

Module 12: | Figure $\text{Ca}_v2.1$ current densities



Current densities of wild-type and mutant $\text{Ca}_v2.1$ P/Q-type channels. These plots show the current produced by wild-type (wt) and R192Q mutant channels, which are the cause of familial hemiplegic migraine. Reproduced from *Neuron*, Vol. 41, van den Maagdenberg, A.M.J.M., Pietrobon, D., Pizzorusso, T., Kaja, S., Broos, L.A.M., Cesetti, T., van de Ven, R.C.G., Tottene, A., van der Kaa, J., Plomp, J.J., Frants, R.R. and Ferrari, M.D., A *Cacna1a* knockin migraine mouse model with increased susceptibility to cortical spreading depression, pp. 701-710. Copyright (2004), with permission from Elsevier; see van den Maagdenberg et al. 2004.

for Rab27A to induce the maturation of the perforin-containing T cell granules at the immunological synapse.

Familial hypocalciuric hypercalcaemia (FHH)

Familial hypocalciuric hypercalcaemia (FHH) occurs in individuals that have a loss of function mutation in one copy of the *CaR* gene that codes for the Ca^{2+} -sensing receptor (CaR). The condition resembles primary hyperparathyroidism, but in FHH, individuals are usually asymptomatic. FHH may thus contribute to the onset of hyperparathyroidism.

Familial exudative vitreoretinopathy (FEVR)

Familial exudative vitreoretinopathy (FEVR) is an ocular disorder characterized by a failure of the peripheral retinal vascularization, which is caused by a mutation of the Fz4 receptor that operates in the Wnt/ Ca^{2+} signalling pathway (Module 2: Figure Wnt signalling pathways).

Familial focal segmental glomerulosclerosis (FSGS)

Familial focal segmental glomerulosclerosis (FSGS) is an end-stage renal disease caused by mutations of the canonical transient receptor potential 6 (TRPC6) gene, which encodes a diacylglycerol (DAG)-sensitive Ca^{2+} channel. Approximately 20% of patients on dialysis suffer from FSGS. This syndrome is characterized by abnormalities in the glomerulus (podocytes and slit diaphragm), resulting in proteinuria and renal insufficiency leading to renal failure. It is a gain-of-function mutation, and the resulting increase in Ca^{2+} entry may contribute to the morphological changes in the glomerulus.

Fanconi anaemia

Fanconi anemia is a rare autosomal X-linked disorder. Patients have different types of leukaemia and tumours in the head, neck, skin and reproductive organs. Also, there is failure of the bone marrow. Chromosomal instability and a heightened sensitivity to cross-linking agents results in a marked susceptibility to cancer. Genetic analysis has revealed that the disorder can arise from mutations in at least 12 genes, which have been called the Fanconi anaemia complementation group (FANC). These FANC proteins function in the Fanconi anaemia/BRCA pathway that is activated in response to DNA cross-links when the replication fork stalls during DNA synthesis (Module 9: Figure Fanconi anaemia pathway).

François-Neetens Mouchetée fleck corneal dystrophy (CFD)

François-Neetens Mouchetée fleck corneal dystrophy (CFD) is an autosomal dominant syndrome characterized by large numbers of white flecks distributed throughout the stroma of the cornea. These corneal flecks result from large vacuoles that are responsible for the swelling of the keratinocytes. This syndrome is caused by mutations in the lipid kinase PIKfyve that functions in the PtdIns3,5P₂ signalling cassette (Module 2: Figure PIKfyve activation). Most of the mutations are located in the Cpn_60TCP1 domain, which enables this kinase to interact with other members of the functional complex.

Fragile X syndrome (FXS)

Fragile X syndrome (FXS) is one of the main causes of mental retardation and is characterized by dysmorphic feature, seizures and behavioural problems. FXS has been linked to a mutation in the *fragile X mental retardation protein 1 (FMRP1)* gene, which occurs with a frequency of 1 in 4000 males and 1 in 8000 females. Many FXS males have symptoms that resemble autism. The fragile X mental retardation protein 1 (FMRP1) protein, which is expressed widely particularly in the brain and testis, may function to regulate the changes in synaptic plasticity responsible for learning and memory. In FXS there is a marked increase in both the number of spines, which are abnormally long and thin and appear to lack the features normally associated with functional synapses.

The main mutational change is an expansion of a non-coding CGG repeat located in the 5' untranslated region. This region normally has 6–55 repeats but this increases to 200 or more in FXS. This expansion results in an increased methylation of the 5' regulatory region resulting in a decline in the expression of FMRP1. Just how this protein normally functions is still being worked out and two of the suggested proposals are shown in Module 10: Figure Ca²⁺-induced synaptic plasticity. There are indications that FMRP1 binds to mRNA through two KH domains and this may then suppress the local neuronal protein synthesis that occurs on the polyribosomes associated with each spine. In the absence of this inhibition, there may be an abnormal increase in protein synthesis leading to the changes in spine morphology associated with FXS. Another proposal is that FMRP1 inhibits the

p21-activated kinase (PAK), which functions in actin remodelling (Module 10: Figure Ca²⁺-induced synaptic plasticity). A decline in FMRP1 would result in enhanced actin polymerization, which would be consistent with the elongated spines found in FXS.

Frontotemporal dementia (FTD)

In frontotemporal dementia (FTD), atrophy of the frontal and temporal lobes result in multiple pathologies that include loss of language with marked changes in both personality and social interactions. Mutations in a number of proteins: microtubule-associated tau protein (MAPT), valosin-containing protein (VCP), chromatin modifying protein 2B (CHMP2B), tau and progranulin (PGRN) have been linked to FTD. Mutations in tau have been linked to Frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17). Such subtle FTD phenotypes seem to depend on which mutation has occurred and also on the type of inclusions found within the neurons. These inclusions are made of protein aggregates such as the tau-positive inclusions and the ubiquitin-positive cytoplasmic inclusions some of which consist of TAR-DNA binding protein 43 (TDP-43).

The GRN mutations result in haploinsufficiency as evident by the 50% reduction in the amount of released progranulin (PGRN). Since the inflammatory response of microglial cells is normally inhibited by PGRN, its decline may result in enhanced inflammation and the neuronal cell death that occurs in FTD.

Frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17)

Frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) is an autosomal dominant neurodegenerative disorder that is caused by a mutation in the protein tau. This disorder is characterized by changes in behaviour and personality that are often accompanied by motor and cognitive defects.

Gitelman's disease

Gitelman's disease is an autosomal recessive disorder caused by inactivating mutations of *SLC12A3* that encodes the Na⁺-Cl⁻ cotransporter (NCC). The hypokalaemia, hypomagnesaemia, hypocalciuria and metabolic acidosis that characterize this disorder are caused by a decrease in the activity of this cotransporter in the distal convoluted tubule (DCT) cell (Module 7: Figure kidney salt reabsorption). The symptoms of Gitelman's disease closely resemble those of Bartter's disease except for one major difference. In Bartter's disease there is hypercalciuria whereas in Gitelman's there is hypocalciuria.

Glanzmann's thrombasthenia

Glanzmann's thrombasthenia is one of the commonest of the platelet diseases. It is an autosomal recessive bleeding dyscrasia that has been linked to inactivating mutations of the β_3 -integrin subunit that functions in α_{IIb}/β_3 integrin receptor signalling during blood platelet activation (Module 11: Figure platelet activation).

Glaucoma

Glaucoma is a major cause of permanent blindness that results from a gradual loss of retinal ganglion cells. The commonest form of this disease is primary open angle glaucoma (POAG), which is age-related and frequently associated with elevated intraocular pressure. The latter is controlled by a balance between the production of aqueous humour and its outflow from the anterior chamber. POAG is a polygenic disease and causal mutations have been found in myocilin (MYOC), FIP2 [also known as optineurin (OPTN)] and WD40-repeat36 (WDR36). It is still not clear why mutations in these glaucoma genes lead to blindness. FIP2/optineurin is known to link myosin VI to various cargoes at the Golgi.

Glaucoma has also been described in patients suffering from posterior polymorphous corneal dystrophy (PPCD).

Glycogen storage disease

Glycogen storage disease is caused by a mutation of the γ 3-subunit of the AMP-activated protein kinase (AMPK) (Module 2: Figure AMPK structure).

Gordon's disease

Gordon's disease, which is also known as familial hyperkalaemic hypertension (FHHT) or pseudohypoaldosteronism type II (PHAII), is an autosomal dominant disease characterized by hypertension that is associated with hyperkalaemia and hyperchloraemic metabolic acidosis. There also are low levels of renin caused by depression of the renin-angiotensin system (RAS). All these symptoms have been traced to mutations in at least three genes. Two of these genes have been identified as members of the WNK protein kinase family that function in the signalling network that controls salt reabsorption by the distal convoluted tubule (DCT) (Module 7: Figure kidney salt reabsorption). One of these genes is WNK1 where the mutations result from deletion of the first intron causing an increase in the expression of this kinase. The other gene is WNK4 that has missense mutations in the coding sequence located outside the kinase domain resulting in a loss of function. Since WNK4 normally acts to inhibit the expression of the $\text{Na}^+\text{-Cl}^-$ cotransporter (NCC) by promoting its internalization, this mutation will increase the activity of salt reabsorption. The gain-of-function mutation of WNK1 ends up having a similar effect because it normally acts to inhibit WNK4. Therefore an increase in WNK1 activity will reduce WNK4 resulting in enhanced NCC activity.

In many respects, the symptoms of Gordon's disease are a mirror image of Gitelman's disease. In the latter case, the disease results from inactivating mutations of *SLC12A3* that encodes the $\text{Na}^+\text{-Cl}^-$ cotransporter (NCC) resulting in a decrease in salt reabsorption and hypotension. By contrast, the hypertension of Gordon's disease is caused by an increase in salt reabsorption.

Gorlin's syndrome

Gorlin's syndrome, which is also known as nevoid basal cell carcinoma syndrome (NBCC), results from a mutation in the gene for the tumour suppressor protein patched

(PTC) that functions in the Hedgehog signalling pathway (Module 2: Figure Hedgehog signalling pathway). The phenotype of Gorlin's syndrome reflects the main functions of the Hedgehog signalling pathway in controlling both development and cell proliferation. There are developmental abnormalities and a predisposition to skin cancers such as basal cell carcinomas.

Griscelli syndrome (GS)

Griscelli syndrome (GS) is a complex syndrome characterized by severe neurological disorders (mental retardation, epilepsy and ataxia) and is sometimes also associated with partial albinism. There are three forms of Griscelli syndrome (GS) caused by mutations in three genes that are functionally related to the transport function of the motor protein myosin Va (see panel C in Module 4: Figure myosin motor):

- Griscelli syndrome 1 (GS1) is caused by mutations in the *MYO5A* gene that codes for the molecular motor myosin Va. The severe neurological disorders associated specifically with GS1 may be caused by a defect in the transport of InsP_3 receptors into the dendritic spines.
- Griscelli syndrome 2 (GS2) does not display the severe neurological defects seen in GS1, but they do show the albinism, and there also is severe immunodeficiency caused by a decline in the exocytosis of cytotoxic granules from neutrophils and T-cells. These defects are caused by mutations in the Rab27a, which couples the myosin Va motor to various vesicles, such as the melanosomes and the cytotoxic granules released from cytotoxic T lymphocytes (CTLs) and natural killer (NK).
- Griscelli syndrome 3 (GS3) does not display the severe neurological defects seen in GS1, but they do show the albinism that is caused by mutations in the adaptor protein melanophilin, which connects myosin Va to Rab27a (see panel C in Module 4: Figure myosin motor).

Hailey-Hailey disease

Hailey-Hailey disease is an autosomal dominant skin disease characterized by skin erosions, particularly on the neck and in intertriginous regions such as the groin, axillae and submammary gland. These lesions can progress to squamous cell carcinomas. This disease is caused by mutations that inactivate one of the *ATP2C1* alleles that encode the secretory-pathway Ca^{2+} -ATPase (SPCA) that pumps Ca^{2+} into the Golgi. Just how a decrease in SPCA1 pump activity results in the skin lesions is not fully known, but it seems to depend upon a change in Ca^{2+} regulation within the keratinocytes. There appears to be a decrease in the Ca^{2+} concentration within the lumen of the Golgi, and this could lead to alterations in the processing of proteins, and particularly of the adhesion molecules that may play a role in cell-cell adhesion.

Hearing loss

A progressive loss of hearing is very common, especially as a consequence of aging, and can have many causes. A clue to how this might occur has been uncovered by the discovery of a human mutation in the type 2 isoform of the

plasma membrane Ca^{2+} -ATPase (PMCA2). This PMCA2 isoform extrudes Ca^{2+} from the hair cells during the hair cell mechanoelectrical transduction process responsible for hearing (Module 10: Figure tip link). When expressed in cultured cells, the mutated PMCA2 had a reduced pump capacity and this may explain why the operation of the hair cells is compromised resulting in progressive hearing loss.

Hemorrhagic telangiectasia-2 (HHT2)

Hemorrhagic telangiectasia-2 (HHT2) is a vascular dysplasia characterized by dilated and leaky capillaries that is caused by loss-of-function mutations in the gene that codes for activin receptor-like kinase 1 (ALK1), which belongs to the TGF- β signalling receptor family.

Hereditary sensory and autonomic neuropathies (HSAN)

Hereditary sensory and autonomic neuropathies (HSAN), which is also known as familial dysautonomia, are characterized by the loss of sensation for pain and temperature, the absence of fungiform tongue papillae, excessive sweating and alacrima. It is an autosomal recessive disease that has been classified into five types.

HSAN type 1, which is characterized by lancinating pain, has been linked to mutations in serine palmitoyl transferase (SPT), a rate-limiting step in the sphingolipid synthesis during the generation and function of ceramide and sphingosine 1-phosphate (S1P) (see Step 1 in Module 2: Figure sphingomyelin signalling).

HSAN type II includes patients with Charcot-Marie-Tooth disease 2B that is caused by mutations in Rab7A. The overlap between the type I and type II syndromes may arise because by Rab7 plays a role in both the endocytosis and transport of sphingolipids.

High bone mass syndrome (HBM)

High bone mass syndrome (HBM) is caused by gain-of-function mutations in the LRP5 gene, which binds to the Wnt antagonist Dickkopf1 (DKK1) (Module 2: Figure Wnt canonical pathway). The syndrome is characterized by high bone density that appears in adolescents and persists into adulthood. An increase in the activity of LRP5, which is a co-receptor in the canonical Wnt/ β -catenin pathway, may have two actions. First, it will increase the proliferation and differentiation of the osteoblasts required for bone formation (Module 7: Figure osteoblast function). Secondly, the LRP5 defect may decrease the formation and release of 5-HT by the enterochromaffin cells and this too will increase bone formation since 5-HT acts to inhibit the osteoblasts.

Huntington's disease

Huntington's disease is an autosomal dominant neurological disease, which usually strikes in mid-life, resulting in a progressive intellectual decline leading to death after 10–15 years. In addition, the disease is associated with involuntary twisting and wriggling movements that resemble a dance-like motion that gave rise to its original name chorea. The cause of the disease has been traced to a 350 kDa cyto-

plasmic protein called huntingtin (Htt), which undergoes a polyglutamine expansion in the N-terminus (Htt^{exp}).

The neuropathology of the disease is characterized by a selective loss of neurons in the striatum, and particularly the medium spiny neurons. The loss of these neurons in Huntington's disease may occur through alterations in Ca^{2+} signalling caused by the modified Htt^{exp}. The normal Htt protein forms a ternary complex with an Htt-associated protein-1A (HAP1A) and the type 1 inositol 1,4,5-trisphosphate receptor (InsP₃R1). Htt^{exp} binds very strongly to InsP₃R1 and enhances its sensitivity to InsP₃, thus giving larger Ca^{2+} signals. Htt may also regulate Ca^{2+} signalling by inhibiting entry through the *N*-methyl-D-aspartate (NMDA) receptor. Htt^{exp} is less effective at binding the postsynaptic density 95 (PSD95)/NR1A/NR2B complex, and this can result in a greater entry of Ca^{2+} .

Huntington's disease-like 2 (HDL2)

Huntington's disease-like 2 (HDL2) is a progressive neurodegenerative disorder that resembles Huntington's disease. HDL2 is characterized by dementia, psychiatric symptoms and abnormal movements. It is caused by mutations in junctophilin 3 (JP3) that may function to maintain the subsurface cisternae (SSCs) that are located on the soma of neurons (Module 10: Figure neuronal structure).

Hutchinson-Gilford progeria syndrome (HGPS)

Hutchinson-Gilford progeria syndrome (HGPS) is a premature ageing syndrome characterized by premature hair loss, atherosclerosis and reduced joint mobility. This is caused by a mutation in the *LMNA* gene that encodes lamin A and lamin C, which are key components of the nuclear lamina, and result in disruption of the normal nuclear architecture (Module 12: Figure chromatin organization) that is essential to maintain the expression patterns that maintain the phenotypic stability of differentiated cells.

Hyper-IgE syndromes (HIES)

Hyper-IgE syndrome (HIES), which is also known as Job's syndrome, is a rare primary immunodeficiency characterized by recurring sinopulmonary and cutaneous viral infections together with elevated levels of serum IgE. Most cases are sporadic, but autosomal dominant and autosomal recessive forms have been described. Mutations in STAT3, which operates in the signal transducers and activators of transcription (STATs) activation cascade (Module 2: Figure JAK/STAT function), have been linked to the autosomal dominant form of HIES. Loss-of-function mutations in dedicator of cytokinesis 8 (DOCK8), which inhibits lymphocyte function, such as B-cell differentiation in the lymph node (Module 8: Figure B cell maturation signalling), have also been linked to HIES.

Hypogonadotropic hypogonadism

Hypogonadotropic hypogonadism is caused by low plasma levels of luteinising hormone (LH), follicle-stimulating hormone (FSH) and the sex steroids that are components of the hormonal system that controls

reproduction (Module 10: kisspeptin neuronal circuit). The decrease in this hormonal system results in under-developed gonads and sterility. One of the causes has been linked to mutation in the GPR54 receptor located on the gonadotropin-releasing hormone (GnRH) neurons that function to release GnRH (Module 10: Figure GnRH neuron).

Hypokalaemic periodic paralysis (HPP)

Hypokalaemic periodic paralysis (HPP) is an autosomal disorder that can be caused by mutations in different channels. In the case of HPP-1, the mutation is in the α_1 subunit of $\text{Ca}_v1.1$ L-type channel. The disorder is characterized by skeletal muscle weakness or paralysis, and is associated with hypokalaemia. Some of the symptoms can be relieved by K^+ supplementation. The position of two of the mutations is shown in Module 12: Figure channelopathies. The result of these mutations is to prolong the action potential, resulting in Na^+ channel inactivation and a general loss of membrane excitability.

Hypomagnesaemia hypercalciuria syndrome

Patients with hypomagnesaemia hypercalciuria syndrome have impaired Ca^{2+} reabsorption and this may arise through mutations in paracellin 1 (PCLN-1). PCLN-1 is located in the tight junctions between cells in the thick ascending loop of Henle (TALH) where it maintains the paracellular pathway responsible for Ca^{2+} reabsorption by the kidney (Module 7: Figure kidney Ca^{2+} reabsorption).

Hypomagnesaemia with secondary hypocalcaemia

Hypomagnesaemia with secondary hypocalcaemia is an autosomal recessive disease that is caused by a loss-of-function mutation in melastatin-related transient receptor potential 6 (TRPM6), which is a member of the melastatin-related transient receptor potential (TRPM) family of ion channels (Module 3: Figure TRP channel family). This rather rare disorder is characterized by low plasma levels of Mg^{2+} caused by a decrease in Mg^{2+} absorption across the intestine and reabsorption across the collecting ducts. The decrease in Mg^{2+} causes seizures and muscle spasms.

Idiopathic infantile hypercalcaemia

Idiopathic infantile hypercalcaemia is characterized by severe hypercalcaemia that was particularly prevalent when vitamin D supplementation is excessive. In addition to the increased Ca^{2+} levels, there is vomiting, dehydration and nephrocalcinosis. This syndrome is also linked to loss-of-function mutations in *CYP24A1* that codes for the enzyme $1\alpha,25\text{-(OH)}_2\text{D}_3$ 24-hydroxylase that inactivates the vitamin D hormone $1,25\text{(OH)}_2\text{D}_3$ (Module 7: Figure vitamin D metabolism).

Kindler syndrome

Kindler syndrome is a somewhat rare autosomal-recessive epidermal defect characterized by blistering, fragile skin and abnormal pigmentation that also carries an increased cancer risk. It is thought to arise from a mutation in one

of the kindlin isoforms (kindlin-1), which function in the formation of focal adhesion complexes (Module 6: Figure integrin signalling).

Lambert-Eaton myasthenic syndrome

Lambert-Eaton myasthenic syndrome is an autosomal Ca^{2+} channelopathy where antibodies against the P/Q-type down-regulate these channels in autonomic neurons. The immunogens are likely to be the S5–S6 linker regions within the III and IV domains that face the extracellular space (Module 3: Figure Ca_v2 channel family).

Leber congenital amaurosis (LCA)

Leber congenital amaurosis (LCA) is a severe early-onset retinopathy characterized by complete blindness within one year of birth. There is a marked decrease in the expression of the phosphodiesterase PDE6 enzyme that plays a central role in phototransduction (Module 10: Figure phototransduction overview). The defective PDE6 is caused by a mutation in the aryl hydrocarbon-interacting protein-like 1 (AIPL1), which functions as chaperone during PDE6 biosynthesis.

Liddle's disease

Liddle's disease, which is an autosomal dominant disorder, is a salt-sensitive form of hypertension caused by excessive Na^+ absorption by the distal convoluted tubule (DCT) and collecting ducts of the kidney (Module 7: Figure kidney tubule function). This is caused by mutations in the proline-rich regions of the epithelial Na^+ channel (ENaC) responsible for absorbing Na^+ from the lumen of the tubule. The mutation prevents the normal turnover of the channel that then accumulates in the membrane to bring about the enhanced rate of Na^+ reabsorption that result in hypertension.

Limb girdle muscular dystrophy type 2A

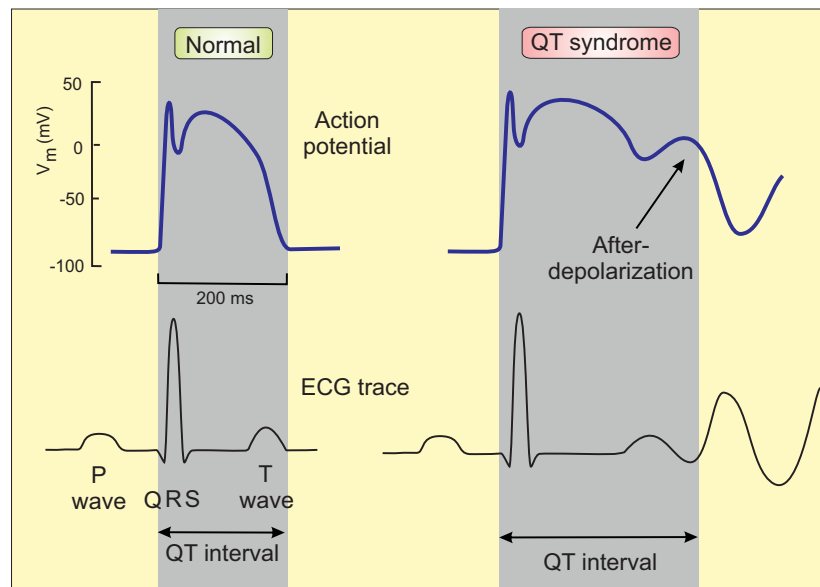
Limb girdle muscular dystrophies are a heterogeneous group of diseases that have been traced to mutations in a number of different genes. They are characterized by atrophy of the muscles of the pelvis and shoulder. The type 2A form has been traced to a mutation in the Ca^{2+} -activated protease calpain 3.

Lissencephaly

Lissencephaly is characterized by severe malfunction of the brain and is associated with mental retardation and epilepsy. The brain has a smooth cerebral surface due to the absence of the usual gyri and sulci that result from a defect in the process of neuronal mitosis and neuronal cell migration. There is a haploinsufficiency due to a deletion at human chromosome 17p13.3. The neuronal defect seems to be caused by the loss of the *LIS1* gene and this was confirmed by the identification of a point mutation in a patient with lissencephaly. The *LIS1* gene encodes lissencephaly (*LIS1*), which functions to connect dynein to the kinetocore by interacting with centromere protein F (CENPF) and ZW10 (Module 4: Figure dynein).

A more severe form of lissencephaly is Miller-Dieker syndrome (MDS), where the brain defects are associated

Module 12: | Figure QT syndrome

**Long QT syndrome.**

The normal action potential and electrocardiogram (ECG) is shown on the left. In the case of the long QT syndrome heart (shown on the right), the action potential duration is prolonged and often leads to an after depolarization that can trigger premature action potentials in neighbouring cells resulting in typical ventricular arrhythmias that show up as irregular waves in the ECG trace.

with craniofacial dysmorphisms. Children with MDS are also severely retarded; they suffer from epilepsy and this condition is often fatal in early childhood. In addition to defects in *LIS1*, there may be mutations in other genes such as 14-3-3 ϵ .

Long QT syndrome

Long QT is characterized by cardiac arrhythmias that can be recorded in electrocardiograms (Module 12: Figure QT syndrome). The fluctuations in the ECG trace reflect the large ionic currents generated by the heart during a typical contraction. The P wave reflects the atrial activation process that is then followed by ventricular activation with the QRS sequence reflecting the onset of the action potential. Finally, the T wave reports the repolarization of the action potential. Therefore the time between onset of the action potential (Q) and its termination (T) is the QT interval, which is thus a measure of the duration of the action potential.

In the case of long QT syndrome (shown on the right), the action potential duration is prolonged and this often leads to an after depolarization that can trigger premature action potentials in neighbouring cells resulting in typical ventricular arrhythmias that show up as irregular waves in the ECG trace. This prolongation of the action potential is caused by mutations in the channels that are responsible for the repolarization processes (Module 7: Figure cardiac action potential). Mutations in either the $K_v7.1$ channel or its auxiliary subunit $MinK\beta$ (Table 3: Figure voltage-dependent K^+ channels) cause Jervell and Lange-Nielsen long QT syndrome. An autosomal dominant Romano-Ward long QT syndrome results from mutations in *KCNQ1* that

codes for the $K_v11.1$ channel or in the *KCNE2* gene that codes for the auxiliary subunit *MiRP1*.

A prolongation of the QT interval is also a feature of Timothy syndrome.

Lowe's oculocerebrorenal (OCRL) syndrome

Lowe's oculocerebrorenal (OCRL) syndrome is caused by a deficiency in oculocerebrorenal syndrome of Lowe (OCRL), which is an inositol polyphosphate 5-phosphatase that can remove the 5-phosphate from either of the inositol phosphates ($Ins1,4,5P_3$ or $Ins1,3,4,5P_4$; see Step 1 in Module 2: Figure inositol phosphate metabolism) or the phosphoinositides $PtdIns4,5P_2$ and $PtdIns3,4,5P_3$ (Module 2: Figure phosphoinositide metabolism). Deficiency of this enzyme causes a multisystem disorder resulting in cataract, mental retardation and renal Fanconi syndrome.

Malignant hyperthermia (MH)

Malignant hyperthermia (MH) is an autosomal dominant hypermetabolic disorder of skeletal muscle caused by mutations in the ryanodine receptor 1 (RYR1). Patients with this syndrome function normally, and the first indication that they are carrying a RYR1 mutation is revealed if they are given an inhalation anaesthetic, such as halothane, or depolarizing muscle relaxants. These pharmacological agents trigger the rapid onset of muscle rigidity, arrhythmias, respiratory and metabolic acidosis, which is accompanied by a rapid increase in body temperature that is fatal unless drugs such as dantrolene are administered to inhibit the RYR1s. All of these symptoms are caused by an excessive release of Ca^{2+} in skeletal muscle cells, and the increase in heat results from the enormous

consumption of ATP that is used up trying to restore normal Ca^{2+} homeostasis.

The mutated RYR1s have a dramatic increase in their sensitivity to Ca^{2+} , which is somehow induced by volatile anaesthetics. The basis of this hypersensitivity is still being worked out. Indeed, a study of these mutant receptors is beginning to throw some light on the activation mechanisms of these RYRs. A clue to this action has come from mapping the location of the mutations. Most RYR1 mutations are due to single nucleotide changes, but some MH-susceptible families have a three base pair deletion that results in the loss of a conserved glutamic acid residue at position 2347. When these mutations were mapped, they were found to congregate in three hot spots: one is in an N-terminal region (hot spot 1: Cys-35 to Arg-614), another is in a central region (hot spot 2: Asp-2129 to Arg-2458), and the third is a C-terminal region (hot spot 3: Ile-3916 to Ala-4942) (Module 12: Figure RYR mutations). It has been suggested that regions 1 and 2 may interact with each other to form a 'domain switch' that is responsible for channel opening. When these regions are zipped together, the channel is closed, but is unzipped during channel activation. The mutations that cluster in these regions may weaken the closed zip configuration, thus increasing the leak of Ca^{2+} and the hypersensitivity of the mutated channels. The drug dantrolene, which is an effective RYR1 inhibitor and is used to alleviate the symptoms of malignant hyperthermia, may act to stabilize the domain switch.

Medulloblastomas

Medulloblastomas, which are thought to originate from cerebellar granule progenitor cells that fail to differentiate, is a highly malignant brain tumour. One of the causes for tumour formation has been linked to miR-324-5p, which regulates the transcription factor GLI1 that functions in the Hedgehog signalling pathway (Module 2: Figure Hedgehog signalling pathway). These medulloblastomas have also been described in Gorlin's syndrome, which is also caused by mutations operating in the Hedgehog signalling pathway.

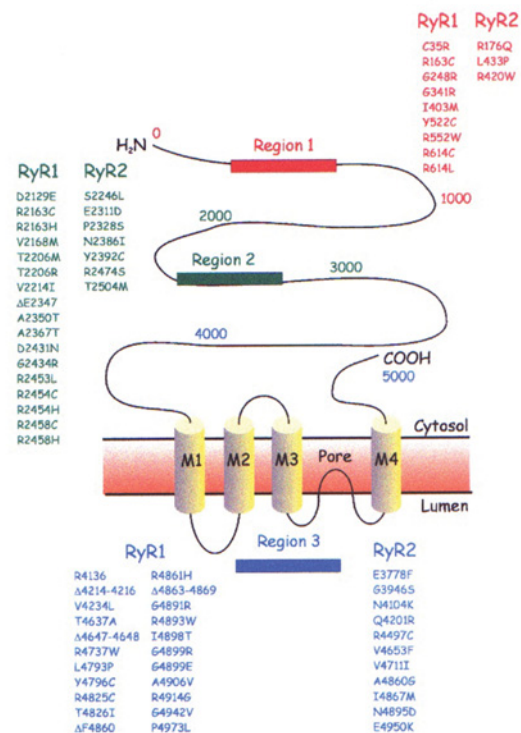
Martsof syndrome

Martsof syndrome is an autosomal recessive disorder, which is a milder form of Warburg Micro syndrome. There is mild mental retardation, congenital cataract and hypogonadism. It is caused by mutation in RabGAP2, which is a subunit of RabGAP1 that functions in the Rab signalling mechanism (Module 2: Rab signalling).

Mental retardation

Mental retardation is characterized by a significant reduction in cognitive abilities that usually appears before the age of 18. This is a complex condition with multiple causes. A number of genes have been linked to this syndrome. In the case of X-linked mental retardation, these genes are located on the X chromosome. Another specific example is fragile X syndrome (FXS). However, chromosomal abnormalities and single gene mutations can occur on other chromosomes.

Module 12: | Figure RYR mutations



Ryanodine receptor (RYR) mutations map to three hot spots.

Mutations of ryanodine receptors type 1 and 2 (RYR1 and RYR2) cause various disease such as malignant hyperthermia (MH), central core disease (CCD), catecholergic polymorphic ventricular tachycardia (CPVT) and arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). These mutations are located in three hot spots (regions 1-3). Reproduced from *Biochem. Biophys. Res. Commun.*, Vol. 322, Brini, M., Ryanodine receptor defects in muscle genetic diseases, pp. 1245-1255. Copyright (2004), with permission from Elsevier; see Brini 2004.

The challenge for the future is to characterize the mutated proteins and to determine how they alter neuronal functions. For example, Birk Barel mental retardation that has characteristic hypotonia and dysmorphism has been linked to a genomically imprinted gene on chromosome 8 that has been identified as TASK-3. This is a dominant-negative mutation that completely inhibits channel activity.

Another of the mutated proteins is Oligophrenin-1 (OPHN1), which is a Rho GAP that functions in the Rho signalling pathway. OPHN1 appears to have an important role in the maturation and stabilization of neuronal AMPA receptors and is thus crucial for the developmental of functional synapses. Defects in synaptic formation will lead to a decrease in glutamatergic neurotransmission and could thus account for mental retardation.

Mutations in dedicator of cytokinesis (DOCK8) have also been linked to mental retardation. Since DOCK8 is thought to regulate the organization of filamentous actin, this fits with observations on X-linked mental retardation where mutations in GDP dissociation inhibitor 1 (GDI1) may also influence the actin cytoskeleton.

Microcephaly

Microcephaly is an autosomal recessive disease that appears to depend upon the mutation of abnormal spindle protein-like microcephaly associated (ASPM), which functions in assembly of the spindle during nuclear envelope breakdown and spindle assembly. Since the phenotype depends upon defects in the brain, it is possible that ASPM may play some role in neurogenesis.

Microvillus inclusion disease

Microvillus inclusion disease results from the absence of microvilli on intestinal cells. It is characterized by malnutrition and diarrhoea. This disease seems to be caused by a defect in the trafficking of proteins to the apical surface and has been linked to mutations in the motor protein myosin Vb. This disease may also be caused by a defect in the transcriptional regulation of Rab8A.

MORM syndrome

MORM syndrome is an autosomal-recessive disorder that closely resembles Bardet–Biedl syndrome. MORM is characterized by mental retardation, retinal dystrophy, truncal obesity and micropenis.

MORM syndrome has been linked to mutations in the INNP5E gene that encodes the inositol polyphosphate 5E-phosphatase (INPP5E) that removes a phosphate from the 5-position of PtdIns3,4,5P₃ and PtdIns4,5P₃ to form PtdIns3,4P₂ and PtdIns4P respectively (See step 12 in Module 2: Figure phosphoinositide metabolism). The alteration in lipid metabolism seriously interferes with the role of the primary cilium in regulating development and a variety of cellular functions, which accounts for the multiple defects in MORM syndrome.

Mucopolidosis

Mutation of TRPML1, which is a member of the mucolipin transient receptor potential (TRPML) family of ion channels, causes mucopolidosis type IV (MLIV). This is a recessive lysosomal storage disease that results in neurodegeneration, leading to severe psychomotor retardation, degeneration of the retina and a deficiency of iron. The mutation seems to have two effects. First, it interferes with the ability of TRPML1 to trigger endosome–lysosome fusion, resulting in large accumulations of dense bodies resembling lysosomes. Secondly, the mutation also results in a defect in the ability of TRPML1 to transfer Fe²⁺ from the lumen of the endolysosomal compartment in to the cytoplasm.

Muscular dystrophy

Muscular dystrophy is caused by the absence of dystrophin, a cytoskeletal protein associated with the inner surface of the plasma membrane in muscle cells (Module 12: Figure cardiac contractile elements). Muscle degeneration may occur due to localized elevation of Ca²⁺ within the subsarcolemmal space. In human Duchenne muscular dystrophy myotubes, there appears to be some alteration in the transmembrane Ca²⁺ transport system, and there also is a much higher density of the inositol 1,4,5-trisphosphate (InsP₃) receptors.

In Mdx mice, which are an animal model for the human disease, there appears to be a Ca²⁺ influx through ‘leak channels’, which have not been defined, that results in an increased rate of proteolysis. There is some evidence that increased entry may be coming through various members of canonical transient receptor potential (TRPC) ion channel family (i.e. TRPC1, TRPC4 and TRPC6). If these channels were repressed in Mdx fibres, the leak was reduced to one-tenth of its normal level. This increased leak may cause an increase in the concentration of Ca²⁺ in the lumen of the sarcoplasmic reticulum (SR). This increased load within the lumen of the SR may result in greater Ca²⁺ fluxes through the mitochondria, which may be potential targets for impaired Ca²⁺ homeostasis in muscular dystrophy.

Myotonia congenita

Myotonia congenita is a neuromuscular disorder that is caused by mutations in the gene *CLCN1* that codes for the CLC-1 chloride channel expressed in skeletal muscle. Thomsen’s myotonia, in which CLC-1 mutation is inherited in a dominant fashion, is less severe than Becker myotonia where the inheritance is recessive. The myotonia results from an inability of the mutated CLC-1 to function properly in muscle relaxation and this causes muscle contraction and stiffness.

Neonatal severe hyperparathyroidism (NSHPT)

Neonatal severe hyperparathyroidism (NSHPT) is an autosomal dominant disease that appears when individuals have loss of function mutations in both copies of the *CaR* gene that codes for the Ca²⁺-sensing receptor (CaR). The symptoms associated with NSHPT appear early in life and are typical of hyperparathyroidism such as hypercalcaemia, decreased mineralization and abnormal development of the skeleton with frequent fractures.

Nephrogenic diabetes insipidus (NDI)

Nephrogenic diabetes insipidus (NDI) results from a failure of vasopressin-induced antidiuresis by the kidney collecting ducts (Module 7: Figure collecting duct function). For example, NDI results from mutations on the vasopressin V₂ receptor gene, which is located on the X chromosome. It can also be caused by autosomal recessive defects in the aquaporin 2 (AQP2) gene. Many of these mutations have been mapped to the B and E loops that form the aqueous pore (Module 3: Figure aquaporin structure). Some of the other mutations also result in trafficking defects that prevent the AQP2-containing vesicles from fusing with the apical membrane during the action of vasopressin (Module 7: Figure collecting duct function).

Néstor-Guillermo progeria syndrome (NGPS)

Néstor-Guillermo progeria syndrome (NGPS) is a progeria syndrome that has many similarities to Hutchinson–Gilford progeria syndrome. NGPS has been linked to mutations in the Barrier-to-autointegration factor 1 (*BANF1*) gene that codes for barrier-to-autointegration factor (BAF). This protein

plays a role in nuclear assembly by acting to attach heterochromatin to the inner nuclear membrane and this enables the euchromatin to organize into transcription factories (Module 12: Figure chromatin organization).

Neurofibromatosis type 1

Neurofibromatosis type 1 (NF1) is an autosomal dominantly inherited disorder that is caused by the loss of the tumour suppressor neurofibromin (encoded by the *NF1* gene), which is a GTPase-activating protein that acts to suppress the activity of the proto-oncogene Ras (Module 2: Figure Ras signalling). NF1 may thus be considered as a tumour suppressor.

A neurofibromatosis type 1-like syndrome (NFLS), which has characteristics resembling NF1, except for the absence of neurofibromas, is caused by mutations in the gene that codes for SPRED1.

Both NF1 and NFLS, which are characterized by hyperactivation of the MAP kinase signalling pathway and the PtdIns 3-kinase signalling pathway, are highly associated with macrocephaly, learning disabilities, mental retardation and autism. Such neural defects are not surprising given the important role of these signalling pathways in the relationship between Ca^{2+} and synaptic plasticity (Module 10: Figure Ca^{2+} -induced synaptic plasticity).

Neurofibromatosis type 2

Neurofibromatosis type 2 is an autosomal dominant inherited disorder that resembles the type 1 above, but, in this case, it is caused by mutations in the tumour suppressor merlin. The latter acts by reducing the activation of Ras. In addition, Merlin has a role in the hippo signalling pathway (Module 2: Figure hippo signalling pathway).

Niemann–Pick disease

The acidic isoform of sphingomyelinases (SMases), which function in the sphingomyelin signalling pathway (Module 2: Figure sphingomyelin signalling), is defective in patients with Niemann–Pick disease.

Optic atrophy 1 (OPA1)

Mutations in the optic atrophy 1 (OPA1) gene is a major factor in the development of optic atrophy 1, which is a dominantly inherited neuropathy that is characterized by a progressive loss of visual acuity caused by a degeneration of the optic nerve and retinal ganglion cells.

Osteopetrosis

Osteopetrosis is a rare inherited disorder characterized by an increase in bone density that results from a change in the normal bone cell coupling mechanism that normally maintains the balance between bone formation and resorption. There appears to be a defect in osteoclast activation that is responsible for bone resorption and the formation of the bone marrow. The change in architecture resulting from the increase in bone density means that the bones are less able to cope with normal strains and thus break easily. An important aspect of osteoclast activation is the insertion of lysosomal transporters (V type H^{+} -ATPase and the chloride transporter CLC-7) in to the ruffled membrane that

faces the resorption pit (see Step 2 in Module 7: Figure osteoclast function). Petrositis can be caused by mutations in the different components responsible for acid secretion such as the V type H^{+} -ATPase, CLC-7 and Ostm1.

Osteoporosis pseudoglioma (OPPG)

Osteoporosis pseudoglioma (OPPG) syndrome is caused by loss-of-function mutations in the LRP5 gene, which binds to the Wnt antagonist Dickkopf1 (DKK1) (Module 2: Figure Wnt canonical pathway). The syndrome is characterized by low bone density with an increased likelihood of fractures. A decrease in the activity of LRP5, which is a co-receptor in the canonical Wnt/ β -catenin pathway, may have two actions. First, it will reduce the proliferation and differentiation of the osteoblasts required for bone formation (Module 7: Figure osteoblast function). Secondly, the LRP5 defect may enhance the formation and release of 5-HT by the enterochromaffin cells and this too will decrease bone formation.

Peutz–Jeghers syndrome

Peutz–Jeghers syndrome is a familial colorectal polyp disorder that is caused by inactivation of LKB1, which is the enzyme that functions to control cell growth by regulating cellular energy status (Module 2: Figure AMPK control of metabolism). Patients suffering from this syndrome are prone to early-onset cancers, not only in the colon, but also in other tissues as well.

Piebaldism

Piebaldism is an autosomal depigmentary disorder characterized by a white forelock and depigmented areas of the skin. In some cases, there can be deafness without pigmentary changes suggesting an overlap with Waardenburg syndrome. Piebaldism has been linked to mutations in c-KIT and stem cell factor (SCF) that function in one of the signalling pathways that control both melanocyte development and melanogenesis (Module 7: Figure melanogenesis).

Polycystic kidney disease

Autosomal dominant polycystic kidney disease (ADPKD) is one of the commonest inherited human diseases, affecting more than 1 in 1000. Its main manifestation is the appearance of large fluid-filled cysts in the kidney, resulting in disruption of kidney function and severe renal failure. Such cysts can also be found in other organs, such as the pancreas and liver. This is a typical example of genotypic remodelling of the signalsome in that the disease results from mutations in two genes, polycystic kidney disease 1 and 2 (PKD1 and PKD2). Approximately 85% of the ADPKD families carry mutations in PKD1, whereas the remaining 15% have a mutated PKD2. The fact that the polycystic disease symptoms are identical for mutations in either gene is consistent with current ideas of how these two proteins are functionally linked together (Module 3: Figure polycystin domain structure). The curious aspect of this disease is that in themselves the PKD1 or PKD2 mutations do not cause disease, which develops when the corresponding normal allele acquires a second hit somatic

mutation. The resulting cell that then has mutations in both PKD1 and PKD2 begins to proliferate rapidly to form a clone; they lose their normal structural relationship to neighbouring cells and begin to form huge cysts that disrupt kidney function. This cellular recessive mechanism is somewhat reminiscent of the mechanism of cancer development, which also depends on a multi-hit mutational mechanism. The fact that disease develops through a somatic mutation in individual cells explains the random appearance of cysts throughout the organ and their increased appearance with age.

The PKD1 and PKD2 genes that are mutated in ADPKD families encode the polycystins, which are membrane proteins called polycystin-1 and polycystin-2 respectively. These polycystins, which are located on primary cilia, gate Ca^{2+} and appear to play a role in mechanotransduction in kidney cells. There is considerable debate about how these membrane proteins might function to transport Ca^{2+} (Module 3: Figure polycystin channel location). There is also little information on how mutations of these polycystins induced the cell proliferation that leads to ADPKD. One interesting hypothesis suggests that the channel mutations result in a decline in Ca^{2+} signalling, which then triggers a phenotypic switch in the mitogen-activated protein kinase (MAPK) signalosome (Module 12: Figure polycystins and polycystic kidney disease).

The MAPK signalling pathway activates cell proliferation by initiating the cell cycle process whereby cyclin E controls G_1 progression and DNA synthesis. Roscovitine, which is a potent inhibitor of the cyclin E/cyclin-dependent kinase 2 (CDK2) complex, is effective in arresting cystic disease in mouse models of polycystic kidney disease.

Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOS)

Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOS), which is also known as Nasu–Hakola disease, is a recessively inherited disease characterized by abnormalities in both bone and the central nervous system. With regard to the former, patients develop large bony cysts in the spongy bone and may also develop arthritis, osteoporosis and fractures. There are multiple neuropsychiatric symptoms including frontal lobe syndrome and dementia. PLOS has been linked to mutations in the gene *TYROBP*, which codes for the DNAX-activating protein 12 (DAP12). One of the functions of DAP12 is as an adaptor protein for the triggering receptor expressed in myeloid cells (TREM-2). Indeed, some families that have PLOS symptoms carry mutations in TREM2. The TREM2/DAP12 combination generates the Ca^{2+} signals that contribute to the development of the bone resorbing osteoclasts (Module 8: Figure osteoclast differentiation). In addition, TREM2/DAP12 is located on the microglia in the brain where they act to suppress inflammatory responses (Module 7: Figure microglia interactions) and this may account for the frontal dementia, which is a characteristic feature of this syndrome.

Posterior polymorphous corneal dystrophy-3 (PPCD)

Posterior polymorphous corneal dystrophy (PPCD) is a dominantly inherited condition that is characterized by changes in the properties of Descemet's membrane and the corneal endothelium. The corneal changes, which usually develop slowly, can lead to blindness due to loss of corneal transparency resulting from a thickening of Descemet's membrane and transformation of the corneal endothelial cells. In some cases, PPCD can include glaucoma. PPCD has been linked to mutations in the ZEB1 gene, which regulate the expression of E-cadherin.

Prader-Willi syndrome (PWS)

Prader-Willi syndrome (PWS) is a complex genetic disease that depends on an imprinting disorder resulting in loss of paternal genes on chromosome 15 in region q11–13. There are a number of symptoms including mental retardation, hyperphagia resulting in obesity, short stature and muscular hypotonia. The high level of the gut hormone ghrelin, which functions to increase appetite in the control of food intake and body weight, has attracted much interest as a possible cause of the hyperphagia.

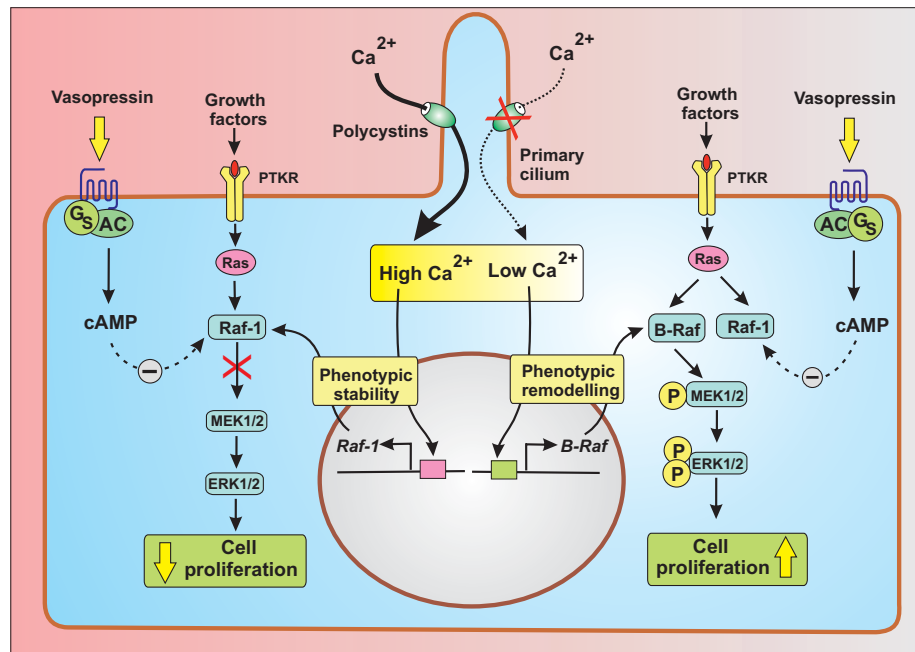
Progressive familial intrahepatic cholestasis type III (PFIC3)

Progressive familial intrahepatic cholestasis type III (PFIC3) is a chronic autosomal recessive disorder characterized by hepatomegaly, cholestasis and hepatic fibrosis that can lead to end-stage liver disease. Phospholipid levels in the bile are very low and this results in damage to the bile duct by bile-acids. PFIC3 is caused by defects in ABCB4, which is one of the ATP-binding cassette (ABC) transporters (Module 3: Table ABC transporters).

Psychiatric disorders

There are a number of psychiatric disorders such as autism spectrum disorder, attention deficit-hyperactivity disorder (ADHD), bipolar disorder, major depressive disorder and schizophrenia that are closely related to each other. The similarities between these different disorders have been highlighted by the finding that certain genetic risk factors are shared between these disorders. In particular, mutations in the $\text{Ca}_v1.2$ L-type channels, suggests that alterations in voltage-dependent Ca^{2+} signalling might be a feature of many of these disorders. The *CACNA1C* codes for the $\alpha1c$ subunits whereas the *CACNB2* codes for $\beta2$ that is one of the auxiliary β -subunits of the voltage-operated $\text{Ca}_v1.2$ channel (Module 3: Figure $\text{Ca}_v1.2$ L-type channel).

Individuals that are carrying the rs1006737 single-nucleotide *CACNA1C* polymorphism display a reduction in hippocampal activation and episodic memory recall. There is also a disruption in the coupling between the left and right regions of the hippocampus that seems to be important for memory recall. There was also an increased risk of developing depression and other psychopathological traits such as anxiety, neuroticism and obsessive-compulsive behaviour. These studies on humans are supported by studies on knockout mice where deletion of the $\text{Ca}_v1.2$ channel has little effect on memory acquisition,

Module 12: | Figure polycystins and polycystic kidney disease**Mitogen-activated protein kinase (MAPK) remodelling hypothesis of polycystic kidney disease**

Polycystic kidney disease results from mutations of the polycystins that are located at the cilium, where they appear to gate Ca^{2+} into the cytoplasm. It is proposed that this constant influx of Ca^{2+} is responsible for the phenotypic stability of the mitogen-activated protein kinase (MAPK) signalling pathway by maintaining the transcription of Raf-1, which is sensitive to inhibition by cyclic AMP. When the polycystins are mutated, a reduction in the level of Ca^{2+} results in a phenotypic switch such that B-Raf is expressed in addition to Raf-1. Since the former is insensitive to inhibition by cyclic AMP, the MAPK pathway is now able to transmit proliferative signals into the nucleus, and the mutated cell begins the cell division cycles that produce the cysts that characterize polycystic kidney disease.

which depends on the activation of NMDARs (see step 1 in Module 10: Figure Ca^{2+} -induced synaptic plasticity), but markedly affects the processes of memory consolidation that depends on Cav1.2 channel-dependent activation of neuronal protein synthesis and neuronal gene transcription.

Respiratory distress syndrome (RDS)

Respiratory distress syndrome (RDS) is seen in premature infants where there is a defect in the production of the surfactants necessary to expand the lung alveoli. One of the causes of RDS is mutations in ABCA3, which is one of the ATP-binding cassette (ABC) transporters (Module 3: Table ABC transporters).

Retinitis pigmentosa

Retinitis pigmentosa is caused by mutations in CNGA1 and CNGB1, which are members of the cyclic nucleotide-gated channels (CNGCs) that function in visual transduction in rod photoreceptors (Module 3: Figure cyclic nucleotide-gated channels). Retinitis pigmentosa also occurs in Usher syndrome.

Rett syndrome

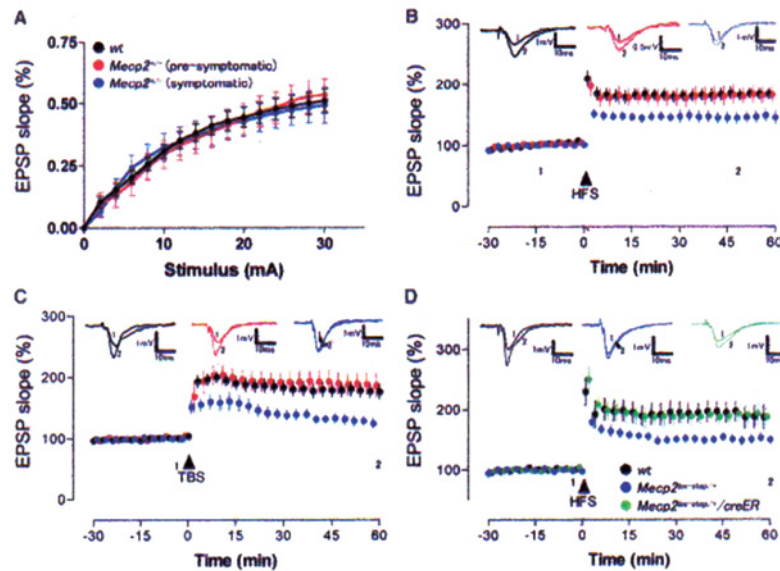
The majority of patients with human X-linked Rett syndrome are girls that carry a mutation in the gene for the methyl-CpG-binding protein 2 (MECP2) transcriptional repressor (Module 4: Figure MeCP2 activation). They have a profound neurological disorder characterized by arrested

neurological development and defects in memory. Normally found in 1 in 10,000 girls. Development appears to proceed normally up to 6–18 months but is gradually replaced by the loss of acquired skills and the development of a whole range of symptoms, including characteristic hand wringing, loss of social skills and the onset of behaviour that resembles that seen in autism spectrum disorders (ASD). Social and language skills decline and there is a lack of eye contact while their faces remain expressionless. Seizures begin to occur and can develop into intractable epilepsy.

There also are problems with breathing that becomes irregular with frequent episodes of apnoea. Rett syndrome is characterized by a reduction in the cerebrospinal fluid level of substance P (SP), which plays a critical role in modulating the respiratory centre responsible for generating breathing rhythms (Module 10: Figure breathing control). When SP is applied to this centre located in the medulla, there is an increase in firing rate of the respiratory network (Module 10: Figure SP and respiration).

One of the difficulties in trying to understand how mutations in MeCP2 can give rise to these many different symptoms is compounded by the considerable variability in the Rett phenotype. In some cases, the symptoms are rather mild with a late onset whereas in the most severe cases the symptoms begin to appear very early without the usual period of normal development seen in classical cases of the syndrome. Much of this variability probably depends on the fact that many different MeCP2

Module 12: | Figure LTP in Rett mice



Inhibition and recovery of long-term potentiation (LTP) in a mouse model of Rett syndrome.

Mice containing a transgene expressing a fusion between Cre recombinase and a modified oestrogen receptor enabled MeCP2 to be reduced and then reintroduced. (A) This stimulus response curve illustrates that synaptic transmission was normal in control, pre-symptomatic and symptomatic animals. (B and C) LTP induced by either high-frequency stimulation or a theta burst was reduced in symptomatic animals (blue points) but not in presymptomatic animals. (D) When MeCP2 levels were restored, LTP returned to normal (green points). Reproduced from Guy et al (2007)

mutations have been identified including missense, non-sense, truncations and null alleles that may influence target gene expression in different ways.

In most cases of Rett, the effects of the MeCP2 mutation only become apparent after the brain has developed. Most of the neural connections appear to be in place as indicated by the fact that early infant behaviour is normal. The appearance of symptoms around 6–18 months coincides with the time when the brain needs to process information and, perhaps more importantly, to store sensory information necessary to respond to the outside world and to create social interactions. In this aspect, it resembles autism with which it shares many similarities. As proposed for autism, it seems likely that MeCP2 mutations result in a defect in the mechanisms of synaptic plasticity necessary for learning and storing information in synaptic endings. This is certainly consistent with the fact that the brains of Rett patients are 10–30% smaller and the neurons have fewer of the spines that function in synaptic plasticity. Also consistent with this view is that long-term-potential (LTP) is reduced in a mouse model of Rett whereas it is enhanced in mice carrying an extra copy of MeCP2. These synaptic changes are correlated with corresponding down- or up-regulation of excitatory glutamatergic synapses.

Recent studies on such mice carrying the MeCP2 mutation have raised the possibility that the neurological defects might be reversed if the defective gene could be replaced with the normal allele. In an elegant experiment on mice, Rett-like symptoms were induced following inactivation of MeCPs, but this phenotype was reversed back to normal when the gene was switched on again. Of particular interest was the observation that the reduction in LTP following

MeCP2 inactivation (Panels B and C) was also reversed (Panel D in Module 12: Figure LTP in Rett mice). There was no change in the level of synaptic transmission (Panel A) indicating that information was being transferred across the synapse normally, but the ability to undergo changes in synaptic plasticity required to store information was impaired.

Rubinstein-Taybi syndrome (RTS)

Rubinstein-Taybi syndrome (RTS), which is also known as broad thumb-hallux syndrome, is characterized by a broadening of the thumbs and large toes and by a short stature and distinctive facial features. In addition, there is mental retardation, learning difficulties and an increased risk of developing cancer. This condition is caused by mutations in the CREB-binding protein (CBP) or its closely related paralogue p300.

Scott syndrome

Scott syndrome is a disorder of blood platelets that results from a defect in the ability of Ca^{2+} to activate the ATP-binding cassette (ABC) transporter ABCA1, which transports phosphatidylserine (PS) from the inner to outer leaflet of the plasma membrane (Step 7 in Module 11: Figure platelet activation). A mutation of transmembrane protein 16F (TMEM16F), which also has phospholipid scrambling activity, has also been linked to Scott syndrome.

Severe combined immune deficiency (SCID)

Severe combined immune deficiency (SCID) has been traced in some patients to a defect in the Ca^{2+} entry mechanism that delivers the prolonged Ca^{2+} signal responsible

for activating T lymphocyte proliferation (Module 3: Figure Ca^{2+} signalling in SCID). This entry is carried out by the Ca^{2+} release-activated Ca^{2+} (CRAC) channel, which is now known to be the Orai1 protein. SCID is caused by an Arg-91 to tryptophan mutation located in the C-terminal region of Orai1.

Severe psychomotor retardation

Severe psychomotor retardation is characterized by a variety of neurological abnormalities that seem to result from delays in a number of developmental processes. These developmental defects have been linked to mutations in the gene that codes for the monocarboxylate transporter 8 (MCT8), which functions as a thyroid hormone transporter. Such an action is illustrated in the control of thyrotrophs (see step 5 in Module 10: Figure thyrotroph regulation).

Sezary's syndrome

Sezary's syndrome is a T cell lymphoma characterized by a generalized exfoliative erythroderma. There are indications that it may be caused by changes in the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) function in growth and development.

Sitosterolaemia

Sitosterolaemia is a somewhat rare autosomal recessive disease that is caused by an increase in the intestinal absorption of plant sterols. There is also a decrease in the hepatic excretion of sterols into the bile causing elevated concentrations in plasma phytosterols. These changes in sterol transport results in the presence of tendon xanthomas and premature atherosclerosis. The defect is caused by loss-of-function mutations in either ABCG5 or ABCG8, which are ATP-binding cassette (ABC) transporters that play a role in the trafficking of sterols through intestinal and liver cells.

Spinocerebellar ataxia type 6 (SCA6)

A mutation in the α_{1A} subunit of the $\text{Ca}_v2.1$ P/Q channel (Module 12: Figure channelopathies) caused by a small expansion of a CAG repeat at the 3' end of the coding region of *CACNA1A* results in spinocerebellar ataxia type 6 (SCA6). Since the channel appears to gate normally, the defect seems to result from an overexpression of the channel.

Spinocerebellar ataxia type 12 (SCA12)

This form of autosomal dominant spinocerebellar ataxia is caused by a mutation of protein phosphatase 2A (PP2A) arising from expansion of a CAG trinucleotide repeat of the B β gene, which codes for one of the regulatory subunits of the PP2A holoenzyme (Module 5: Figure PP2A holoenzyme).

Spinocerebellar ataxia type 12 (SCA12)

Spinocerebellar ataxia type 15 (SCA15), which progresses very slowly, is characterized by a slow progressive gait, limb ataxia, upper limb postural tremor, mild hyper-

reflexia, gaze-evoked nystagmus and impaired vestibulo-ocular reflex gain.

The only gene known to be associated with SCA15 is *ITPR1*, which codes for the type 1 inositol 1,4,5-trisphosphate receptor (InsP₃R1) that functions to release Ca^{2+} from the endoplasmic reticulum (Module 2: Figure Ca^{2+} signalling toolkit).

Stargardt disease

Stargardt disease is a common form of inherited juvenile macular degeneration. There is a progressive loss of vision caused by the death of photoreceptors in the central region of the retina called the maculae. It is caused by mutations in *ABCA4*, which is one of the ATP-binding cassette (ABC) transporters (Module 3: Table ABC transporters).

Tangier syndrome

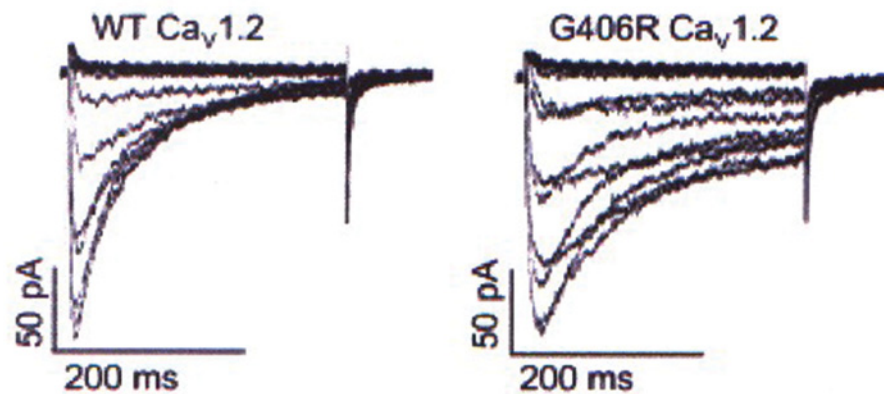
Tangier syndrome is characterized by a large build up of cholesterol in tissues such as the liver, tonsils, spleen and arteries, which leads to coronary artery diseases, peripheral neuropathy and muscle wasting. Cholesterol accumulation is caused by a mutation in *ABCA1*, which is one of the ATP-binding cassette (ABC) transporters (Module 3: Table ABC transporters). It is responsible for removing cholesterol from peripheral tissues and this results in very low levels of plasma high-density lipoprotein (HDL).

Timothy syndrome

Timothy syndrome is a multisystem disorder resulting from a mutation of the α_{1C} subunit of $\text{Ca}_v1.2$ L-type channel responsible for gating Ca^{2+} in a number of cells, including the heart, neurons and smooth muscle. The G406R mutation (Module 3: Figure $\text{Ca}_v1.2$ L-type channel) results in a decrease in the inactivation mechanism that normally acts to switch off the channels, which thus results in a prolongation of Ca^{2+} entry (Module 12: Figure $\text{Ca}_v1.2$ L-type channel inactivation). In the heart, such prolongation of the action potential greatly prolongs the QT interval (Module 12: Figure QT syndrome) and is one of the causes of the cardiac arrhythmias. In addition, the enhanced entry of Ca^{2+} will result in Ca^{2+} overloading, and this might account for many of the other defects associated with Timothy syndrome.

The fact that an alteration in the gating properties of this Ca^{2+} channel can have multiple effects attests to the importance of Ca^{2+} in both development and normal cell functions. With regard to development, the $\text{Ca}_v1.2$ L-type channel is expressed in cells of the apical ectoderm ridge of developing digits, and the excess loading with Ca^{2+} resulting from the G406R mutation may induce cell death, resulting in syndactyly.

In addition to these effects on the heart, individuals carrying the G406R mutation also display other abnormalities such as syndactyly, immune deficiency and behavioural defects resembling autism. With regard to the latter, it would be interesting to know whether the defective channel has caused developmental defects in the nervous system.

Module 12: | Figure $\text{Ca}_v1.2$ L-type channel inactivation**A reduction in $\text{Ca}_v1.2$ L-type channel inactivation in Timothy syndrome.**

The collection of traces records the increase in Ca^{2+} current in response to a range of voltage pulses. A. In the wild-type (WT) channels, the voltage-dependent increase in current inactivates rapidly. B. In the G406R channels, the degree of inactivation is much reduced, and the entry current persists at an elevated level for the duration of the depolarization. This maintained entry of Ca^{2+} will overload the cell and may cause some of the defects of Timothy syndrome. Reproduced from *Cell*, Vol. 119, Splawaski, I., Timothy, K.W., Sharpe, L.M., Decher, N., Kumar, P., Bloise, R., Napolitano, C., Schwartz, P.J., Joseph, R.M., Condouris, K., Tager-Flusberg, H., Priori, S.G., Sanguinetti, M.C. and Keating, M.T., $\text{Ca}_v1.2$ calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism, pp. 19-31. Copyright (2004), with permission from Elsevier; see Splawaski et al. 2004.

TNF-receptor-associated periodic febrile syndrome (TRAPS)

TNF-receptor-associated periodic febrile syndrome (TRAPS) is a hereditary syndrome characterized by recurrent fever attacks and localized inflammation. TRAPS is caused by a dominant-negative mutation in the cleavage site of the tumour necrosis factor α (TNF α) receptor, which prevents its down-regulation by the ADAM family of proteases responsible for its ectodomain shedding.

Tuberous sclerosis

This cancer syndrome is characterized by a predisposition to form hamartomatous polyps. It is caused by inactivation of tuberous sclerosis 1 and 2 (TSC1/2), which function as tumour suppressors in that they control cell proliferation by regulating the way the target of rapamycin (TOR) controls protein synthesis (Module 9: Figure target of rapamycin signalling).

Usher syndrome

Usher syndrome (USH) is a complex hereditary disease that causes both deafness and blindness with an incidence of about 1 in 25000. It has been divided into three clinical categories (USH1, USH2 and USH3) depending on the severity of the hearing impairment and the age when the retinitis pigmentosa that causes blindness begins. The most severe form is USH1 characterized by severe congenital deafness and an early onset of blindness. USH2 symptoms are milder and the moderate hearing impairment remains stable with a later onset of visual impairment. USH3 patients develop deafness progressively with an associated blindness developing at any age.

The mutations responsible for USH1 have been mapped to seven different loci (USH1A–USH1G) and five of the genes have been identified:

- *USH1B* was found to code myosin VIIa.
- *USH1C* encodes harmonin, which appears to be a scaffolding protein containing three PDZ domains and two coiled-coil domains.
- *USH1D* encodes cadherin-23, which is one of the classical cadherin proteins that functions in hearing. Cadherin-23 forms the helical tip link filament that connects the ends of stereocilia on hair cells (Module 10: Figure tip link).
- *USH1F* encodes protocadherin-15, which appears to have two functions on the stereocilia located on the tips of the hair cells in the cochlea (Module 10: Figure hair cell). Firstly, it contributes to the lateral links that tie the stereocilia together. Secondly, it functions as part of the attachment sites for the cadherin-23 tip link that functions in mechanotransduction process (Module 10: Figure tip link).
- *USH1G* encodes a protein called Sans, which contains three ankyrin domains and a sterile alpha motif (SAM), suggesting that it may function as a scaffolding protein.

Van Buchem disease

Van Buchem disease is characterized by excessive bone formation is caused by a mutation in the sclerostin (SOST) gene, which is one of the inhibitors of Wnt signalling (Module 2: Figure Wnt canonical pathway).

Waardenburg syndrome

Waardenburg syndrome (WS) is characterized by defects in both pigmentation and hearing, which have been linked to mutations of the signalling mechanisms responsible for the activation of the microphthalmia-associated transcription factor (MITF) (Module 7: Figure melanogenesis). Four main phenotypes have been recognized: Waardenburg syndrome 1 (WS1) (mutations in PAX3),

Waardenburg syndrome 2a (WS2a) (mutations in *MITF*), Waardenburg syndrome 3 (WS3) (mutations in *Pax3*) and Waardenburg syndrome 4 (WS4) [mutations in *SOX10*, endothelin-3 (ET-3) and its receptor ET_B].

Waardenburg syndrome 1 (WS1)

Waardenburg syndrome 1 (WS1) has the usual defects in pigmentation and hearing but these are coupled to craniofacial deformities. This phenotype is caused by mutations in the transcription factor *PAX3*, which functions in melanogenesis by contributing to the activation of the microphthalmia-associated transcription factor (*MITF*) that functions in melanogenesis (Module 7: Figure melanogenesis).

Waardenburg syndrome 2a (WS2a)

Waardenburg syndrome 2a (WS2a) has the typical defects in pigmentation and hearing that characterize this syndrome. This phenotype is caused by mutations in the microphthalmia-associated transcription factor (*MITF*) that functions in melanogenesis (Module 7: Figure melanogenesis).

Waardenburg syndrome 3 (WS3)

Waardenburg syndrome 3 (WS3) has the usual defects in pigmentation and hearing but these are coupled to limb deformities. This phenotype resembles that in WS1 and is caused by a different mutation in the same transcription factor paired box 3 (*Pax3*), which functions in melanogenesis by contributing to the activation of the microphthalmia-associated transcription factor (*MITF*) that functions in melanogenesis (Module 7: Figure melanogenesis).

Waardenburg syndrome (WS4)

Waardenburg syndrome 4 (WS4) has the usual defects in pigmentation and hearing. This phenotype resembles that in WS1 and is caused by mutations in *SOX10*, endothelin-3 (ET-3) and its receptor ET_B, which function in melanogenesis by contributing to the activation of the microphthalmia-associated transcription factor (*MITF*) that functions in melanogenesis (Module 7: Figure melanogenesis).

Warburg Micro syndrome

Warburg Micro syndrome is an autosomal recessive disorder, which is characterized by neurodevelopmental defects with mental retardation and spastic cerebral palsy, ocular defects and hypothalamic hypogonadism. This syndrome is caused by mutations in the *RabGAP1* subunit, which is a *Rab3* GAP that functions in the *Rab* signalling mechanism (Module 2: *Rab* signalling). A milder form of this syndrome is known as Martsolf syndrome.

Wiskott-Aldrich syndrome

This syndrome is caused by mutations in the Wiskott-Aldrich syndrome protein (*WASP*), which is an X-linked human disease with defects in blood platelets and lymphocytes characterized by bleeding and recurrent infections. The defect in lymphocytes may result from dis-

ruption of the T cell cytoskeletal reorganization responsible for assembling the immunological synapse (Module 9: Figure T cell actin scaffold).

Wolf-Hirschhorn syndrome (WHS)

Wolf-Hirschhorn syndrome (WHS) is a complex disorder caused mainly by defects in early development that result in cranio-facial malformation, growth delays, cardiac defects and learning disability. The phenotypic variability of WHS suggests that it may be caused by defects in a number of genes. One of these genes might encode the H3K36me3-specific histone methyltransferase Wolf-Hirschhorn syndrome candidate 1 (*WHSC1*).

Wolfram syndrome (WFS)

Wolfram syndrome (WFS) is a somewhat rare autosomal-recessive neurodegenerative disease characterized by multiple genetic defects including diabetes, optic atrophy and vision loss, neurodegeneration and psychiatric illness. Given the multiple pathological features, the syndrome has been divided into separate groups, which reflects the way that different symptoms are caused by mutations in different genes.

The WS1 group have mutations in the *WFS1* gene that codes for wolframin, which is part of the endoplasmic reticulum (ER) stress signalling pathway (for details see step 3 in Module 2: Figure ER stress signalling).

The WS2 group have been linked to mutations in the *CISD2* gene that codes for CDGSH iron sulfur domain 2 (*CISD2*). There is a single missense mutation (G to C at nucleotide 109) that results in a truncated protein lacking the CDGSH cytosolic domain and this results in an alteration in the relationship between Ca²⁺ signalling and autophagy (Module 11: Figure autophagy).

Werner syndrome

Werner syndrome (WS) is an example of the link between genome instability and ageing. WS is a premature ageing disease that is characterized by an early onset of atherosclerosis, diabetes and the appearance of aged skin. WS is caused by mutations in *WRN*, which is the gene that encodes RecQ DNA helicases that function to maintain genome stability by removing DNA recombination intermediates and stabilizing replication forks.

X-linked adrenoleukodystrophy (X-ALD)

X-linked adrenoleukodystrophy (X-ALD), which is a severe neurological disorder characterized by peripheral adrenal insufficiency (Addison's disease), demyelination, mental deterioration and cortical blindness, is caused by mutations in *ABCD1*, which is one of the ATP-binding cassette (ABC) transporters (Module 3: Table ABC transporters).

X-linked lymphoproliferative syndrome (XLP)

Many cases of X-linked lymphoproliferative syndrome (XLP), which is a severe primary immunodeficiency, are caused by mutations in the gene *Src* homology 2 (SH2) domain protein 1A (*SH2D1A*) that codes for SLAM-associated protein (SAM). A characteristic feature

of XLP is a severe susceptibility to Epstein–Barr virus (EBV) infections and this can lead to severe or fatal infectious mononucleosis, B-cell lymphomas and lymphoproliferation. Hypogammaglobulinaemia is also a feature of this syndrome

X-linked mental retardation

X-linked mental retardation is a form of mental retardation that results from mutations in various genes located on the X chromosome:

- One of these is the interleukin-1 receptor accessory protein-like (IL1RAPL) protein. IL1RAPL interacts with neuronal Ca^{2+} sensor-1 (NCS-1), which carries out a number of Ca^{2+} -dependent functions in neurons.
- Mutations in the gene encoding the transcriptional repressor methyl-CpG-binding protein 2 (MeCP2) has also been implicated in this syndrome.
- A small group of patients with X-linked non-specific mental retardation have mutations in the gene for GDP dissociation inhibitor 1 (GDI1), which is particularly abundant in the brain where it functions in the Rab membrane cycle that is part of the Rab signalling mechanism (Module 2: Figure Rab signalling). Since Rab plays a role in membrane recycling in brain synapses, the symptoms of this disease may arise from aberrant synaptic transmission.

X-linked recessive myotubular myopathy

X-linked recessive myotubular myopathy is caused by a mutation in MTM1, which belongs to a group of 3-phosphatases called the myotubularins that dephosphorylates PtdIns3P. The PtdIns3P signalling cassette functions in the regulation of membrane trafficking and may also regulate the activity of the intermediate-conductance (IK) channel.

X-linked severe combined immune deficiency (X-SCID)

X-linked severe combined immune deficiency (X-SCID) results from mutations in the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signalling pathway. These mutations alter the way that Janus kinase (JAK)/signal transducer and activator of transcription (STAT) function in the control of growth and development.

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