Module 3

Ion Channels

Synopsis

Ion channels have two main signalling functions: either they can generate second messengers or they can function as effectors by responding to such messengers. Their role in signal generation is mainly centred on the Ca^{2+} signalling pathway, which has a large number of Ca^{2+} entry channels and internal Ca^{2+} release channels, both of which contribute to the generation of Ca^{2+} signals.

Ion channels are also important effectors in that they mediate the action of different intracellular signalling pathways. There are a large number of K^+ channels and many of these function in different aspects of cell signalling. The voltage-dependent K^+ (K_V) channels regulate membrane potential and excitability. The inward rectifier K^+ (K_{ir}) channel family has a number of important groups of channels such as the G protein-gated inward rectifier K^+ (GIRK) channels and the ATP-sensitive K^+ (K_{ATP}) channels. The two-pore domain K^+ (K_{2P}) channels are responsible for the large background K^+ current. Some of the actions of Ca^{2+} are carried out by Ca^{2+}-sensitive K^+ channels and Ca^{2+}-sensitive Cl^- channels. The latter are members of a large group of chloride channels and transporters with multiple functions.

There is a large family of ATP-binding cassette (ABC) transporters some of which have a signalling role in that they extrude signalling components from the cell. One of the ABC transporters is the cystic fibrosis transmembrane conductance regulator (CFTR) that conducts anions (Cl^- and HCO_3^-) and contributes to the osmotic gradient for the parallel flow of water in various transporting epithelia. Many of these epithelia also express aquaporins, which are water channels that increase the flux of water during fluid secretion or absorption. The cation channel of sperm (CatSper) is sensitive to intracellular pH and has a vital role to play in maintaining sperm hyperactivity prior to fertilization. Finally, there are hyperpolarizing-activated cyclic nucleotide-gated (HCN) channels that play an important role in various pacemaker mechanisms both in the heart and nervous system.

Hemichannels provide membrane channels responsible for releasing ions and messengers from cells. On the other hand, gap junctions provide an intercellular pathway to transfer ions and messengers between cells.

Mechanosensitive channels that open in response to membrane deformation have multiple functions especially in sensory systems such as touch and hair cell mechanoelectrical transduction.

A large number of genetic diseases have been traced to mutations in various channels. For example, mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) channel is the cause of cystic fibrosis. Many of the other channelopathies have been linked to changes in the channels that gate either the entry or the release of Ca^{2+}.

Ca^{2+} entry channels

Ca^{2+} entering the cell from the outside is a major source of signal Ca^{2+}. The entry process is driven by the large electrochemical gradient that exists across the plasma membrane. In addition to the inside of the cell being negative with respect to the outside, it also has a very low concentration of Ca^{2+} (100 nM) compared with the 2 mM level present in plasma. Despite this very large driving force for Ca^{2+} entry, cells are remarkably impermeable to Ca^{2+} under resting conditions. However, they draw upon this external reservoir as a source of signal Ca^{2+} by activating a variety of entry channels with widely different properties. There are **voltage-operated channels (VOCs)**, found predominantly in excitable cells, that generate the large and rapid entry of Ca^{2+} to control fast cellular processes, such as muscle contraction and neurotransmitter release at synaptic endings. At present, we know most about these channels that open in response to a change in voltage.

There are many other Ca^{2+} entry channels that are activated by a wide range of external signals. There is a large category of **agonist-operated channels (AOCs)** that are defined on the basis of how they are activated. In

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addition, there are many other channel types such, as the **thermosensors**, and **mechanosensitive channels** (stretch-activated channels), that are sensitive to a diverse array of stimuli. Many of these channels belong to the very large **transient receptor potential (TRP) ion channel family**, which tend to have lower conductances and thus can operate over much longer timescales without swamping the cell with too much Ca^{2+}. Members of this TRP family are particularly important in controlling slower cellular processes, such as smooth muscle contractility and cell proliferation. The **mechanism of Ca^{2+} oscillations**, which are often responsible for activating these longer-term processes, depends upon the entry of Ca^{2+} through the AOC entry channels.

Voltage-operated channels (VOCs)

As their name implies, the voltage-operated channels (VOCs) that introduce Ca^{2+} into the cell are regulated by a change in the voltage that exists across the plasma membrane. In general, such channels are closed when the membrane is hyperpolarized, but open upon depolarization. The large number of VOCs has been divided into three families: Ca_V1, Ca_V2 and Ca_V3. The voltage-operated channel (VOC) terminology and classification of these three families has proved difficult, and here I have adopted the lettering system that is used most commonly. The Ca_V1 family of L-type channels contains four L-type channels that produce the long-lasting Ca^{2+} currents found in skeletal muscle, heart, neurons and endocrine cells. The Ca_V2 family of N-type, P/Q-type and R-type channels contains the N-type channels, the P/Q-type channels and the R-type channels that are found mainly in the nervous system, where they function in synaptic transmission. The Ca_V3 family of T-type channels contains three T-type channels, so called because of their transient kinetics. These properties of the T-type channels seem to suit them for special functions generating pacemaker channel currents in the sinoatrial node, stimulus–secretion coupling in adrenal glomerulosa cells, neuronal rhythmicity, sperm acrosome reaction and cell proliferation.

The overall voltage-operated channel (VOC) structure is similar for all the family members in that it consists of a multimeric complex. The major component is the pore-forming α_1 subunit coupled together with a variable number of voltage-operated channel (VOC)-associated subunits (α_2, β, δ and γ). The voltage-operated channel (VOC) properties are designed to produce a range of Ca^{2+} entry signals. In addition to the subunit interactions that make up these channels, some of the VOCs interact directly with downstream targets. For example, the L-type channels in skeletal muscle are coupled directly to the ryanodine receptor type 1 (RYR1) subunits to trigger release from the sarcoplasmic reticulum (SR). Similarly, the N-type and P/Q-type channels in neurons interact directly with the soluble *N*-ethylmaleimide-sensitive fusion protein-attachment protein receptor (SNARE) proteins associated with synaptic vesicles. This intimate protein–protein interaction with their downstream targets greatly increases the speed of response of these VOCs, which are some of the fastest information transfer mechanisms in biology. Analysis of voltage-operated channel (VOC) distribution and function illustrates how they have many roles to play in regulating the activity of both excitable and non-excitable cells. A variety of mechanisms, including Ca^{2+} itself, phosphorylation and G proteins, contribute to the modulation of the $Ca_V1.1$ L-type channel, the modulation of $Ca_V1.2$ L-type channel from heart muscle and the modulation of the Ca_V2 family of N-type and P/Q-type channels.

Mutations within these VOCs can lead to a variety of inherited diseases known collectively as channelopathies:

- Episodic ataxia type 2
- Familial hemiplegic migraine (FHM)
- Spinocerebellar ataxia type 6 (SCA6)
- Spinocerebellar ataxia type 12 (SCA12)
- Timothy syndrome

Voltage-operated channel (VOC) terminology and classification

There are many different voltage-operated channels (VOCs) that open to produce a range of Ca^{2+} signals in response to membrane depolarization. These can be small, highly localized pulses resulting from brief channel openings or they can be much larger signals that develop when channels remain open for longer periods. The terminology and classification of VOCs has gradually evolved as more and more channels were discovered. The current classification has separated the channels into three families (Module 3: Table VOC classification). The Ca_V1 and the Ca_V2 families require large membrane depolarizations and thus are referred to as high-voltage-activated channels, whereas the Ca_V3 family has low-voltage-activated channels in that they respond to much smaller fluctuations in membrane potential. The different family members also have widely different channel properties, some of which tend to merge with each other (e.g. their conductances and activation thresholds), thus making it difficult to classify individual channels on the basis of such biophysical properties alone.

Apart from their molecular properties, the best way of distinguishing channels is to use pharmacological criteria. Many of the channels are very sensitive to specific toxins and drugs. This specific sensitivity reflects the fact that voltage-operated channel (VOC) structure is highly variable in that these channels are made up of different molecular components encoded by different genes.

Voltage-operated channel (VOC) structure

α_1 subunit

The voltage-operated channels (VOCs) are multimeric complexes consisting of a pore-forming subunit (the α_1 subunit) (Module 3: Table VOC classification) together with a number of voltage-operated channel (VOC)-associated subunits (α_2, β, δ and γ) (Module 3: Figure VOC subunits). Ten genes code for the α_1 subunit (Module 3: Table VOC classification). Further structural and functional diversity is achieved through alternative splicing. Although all of the channels have an α_1 subunit,

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Module 3: | Table VOC classification
Summary of the terminology, classification and properties of voltage-operated channels (VOCs).

VOC	Subunit and gene	Pharmacology	Tissue distribution	Conductance (pS)	Activation threshold (mV)
Ca_V1 family					
$Ca_V1.1$ L-type	α_{1S} subunit	Dihydropyridine	Skeletal muscle and CNS	11-25	~ −30
$Ca_V1.2$ L-type	α_{1C} subunit *CACNA1C*	Dihydropyridine	Heart, CNS and endocrine cells	11-25	~ −30
$Ca_V1.3$ L-type	α_{1D} subunit	Dihydropyridine	CNS and endocrine cells	11-25	~ −30
$Ca_V1.4$ L-type	α_{1F} subunit		Retina	11-25	~ −30
Ca_V2 family					
$Ca_V2.1$ P/Q-type	α_{1A} subunit	ω-Agatoxin	CNS, neuromuscular junction	10-20	~ −30
$Ca_V2.2$ N-type	α_{1B} subunit	ω-Conotoxin	CNS, neuromuscular junction	16	~ −30
$Ca_V2.3$ R-type	α_{1E} subunit	SNX-482	CNS, neuromuscular junction	12	~ −30
Ca_V3 family					
$Ca_V3.1$ T-type	α_{1G} subunit	Kurotoxin	CNS, heart, smooth muscle	7	~ −60 to −70
$Ca_V3.2$ T-type	α_{1H} subunit	Kurotoxin, Mibefradil	CNS, heart, liver, kidney, zona glomerulosa	5	~ −60 to −70
$Ca_V3.3$ T-type	α_{1L} subunit		CNS	11	~ −60 to −70

Voltage-operated channels (VOCs) consist of three families: the Ca_V1 family of L-type channels, the Ca_V2 family of N-type, P/Q-type and R-type channels, and the Ca_V3 family of T-type channels. CNS, central nervous system.

the channels vary with regard to which types of associated subunits they have. For example, the γ subunit seems to be associated predominantly with the L-type channels. The large cytoplasmic loop on some α_1 subunits functions to link channels to synaptic vesicles (Module 3: Figure Ca_V2 channel family) or the ryanodine receptor (Module 3: Figure $Ca_V1.1$ L-type channel)

The subtle variations of VOC structure become apparent when one considers the molecular organization of the different channels such as the $Ca_V1.1$ L-type skeletal muscle channel (Module 3: Figure $Ca_V1.1$ L-type channel) or the $Ca_V1.2$ L-type cardiac channel (Module 3: Figure $Ca_V1.2$ L-type channel).

Voltage-operated channel (VOC)-associated subunits

The ancillary subunits (α_2, β_{1-4}, δ and γ) that are associated with the large α_1 subunit function to enhance channel diversity. For example, the β **subunit** can increase peak current amplitude and can alter the activation and inactivation properties.

γ subunit

At least eight putative γ subunits have been identified, but the function of most of them remains to be established. Most information exists for the γ_1 subunit, which is expressed exclusively in skeletal muscle, where it appears to function by reducing the passage of Ca^{2+} through the L-type channel.

The γ_2, γ_3 and γ_4 subunits are found exclusively in the brain. A γ_7 subunit is also found in brain, where it has an unusual function of limiting the expression of the $Ca_V2.2$ N-type channels.

β subunit

There are four β subunits (β_{1-4}) that are coded for by the cytoplasmic Ca^{2+} channel β subunits (CACNBs) genes (*CACNB1–4*), which are a sub-family of the membrane-associated guanylate kinase (MAGUK) scaffolding proteins (Module 6: Figure MAGUKs). These β subunits function to regulate both the trafficking and the activity of the α_1 subunits of voltage-operated Ca^{2+} channels. All four of the β subunit isoforms can associate with the L-, N- and PQ-type channels and this greatly increases the plasticity of Ca^{2+} channel function.

The GK domain binds to an α1-interaction domain (AID) on the I-II cytoplasmic loop of the α subunit (Module 3: Figure $Ca_V1.1$ L-type channel). This interaction also masks an endoplasmic reticulum retention signal to facilitate the transport of the α subunit to the plasma membrane.

A mutation of the *CACNB2* gene that codes for the β2 subunit of the $Ca_V1.2$ channel is a schizophrenia-associated gene and has been linked to bipolar disorder and other psychiatric disorders.

Voltage-operated channel (VOC) properties

A variety of biophysical parameters are used to define the way the channel opens (activation), how many ions flow through the pore (conductance), and the process of channel closing (deactivation), which is related to a process of inactivation that determines the likelihood of the channel reopening (Module 3: Figure VOC properties). These different parameters vary considerably between the channel types and thus provide the versatility that enables these voltage-operated channels (VOCs) to regulate many different physiological processes.

Channel properties can be monitored using a patch electrode, which isolates a small area of the membrane containing a single channel, thus making it possible to study how the channel opens and closes when the membrane is depolarized. During the activation process, the channel suddenly switches from a closed to an open state and begins to conduct Ca^{2+}, which forms a plume in the immediate vicinity of the channel mouth (Module 3: Figure VOC properties). This elementary Ca^{2+} event has now been visualized for the $Ca_V1.2$ L-type channel in cardiac cells, where it has been called a sparklet (Module 3: Figure Ca^{2+} sparklet). After a variable open time, the channel closes (deactivates), and the plume begins to dissipate as the Ca^{2+} diffuses into the cytoplasm. During deactivation, the channel undergoes a variable inactivation process, which determines when the channel will reopen. For those

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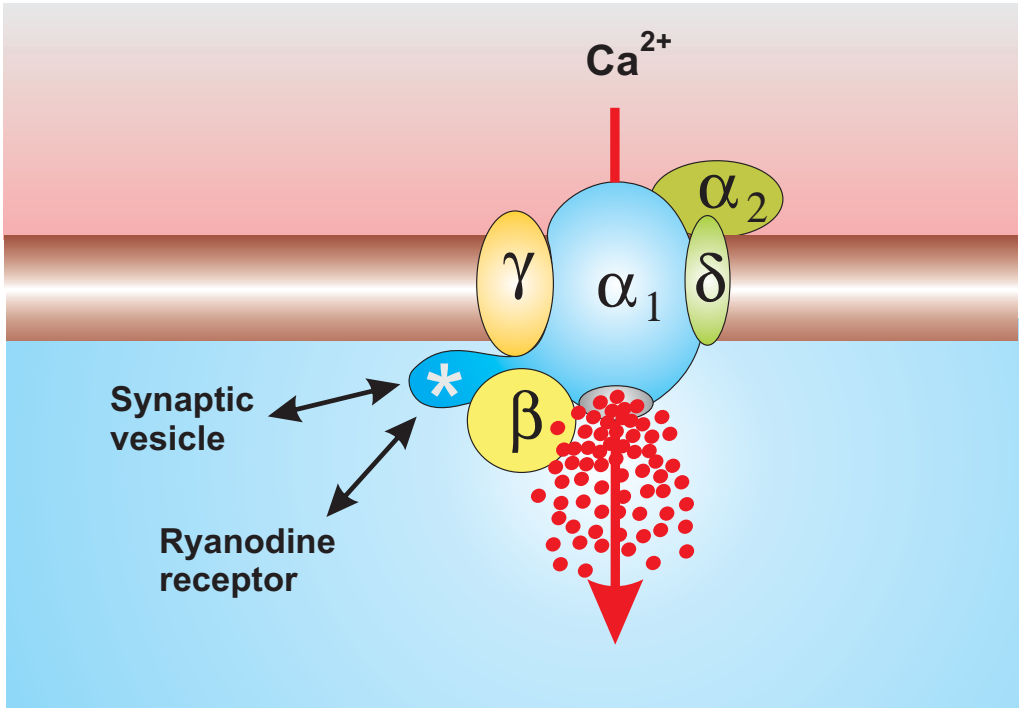
Module 3: | Figure VOC subunits



Structural organization of the VOC subunits.

The channel complex is based on the large α_1 subunit, which contains the pore region, voltage sensor and binding sites for Ca^{2+} and drugs such as dihydropyridine (DHP). The large cytoplasmic loop (*) on some α_1 subunits function to link channels to synaptic vesicles or the type 1 ryanodine receptor (RYR1) of skeletal muscle. The extracellular α_2 subunit has numerous glycosylation sites and is linked through a disulphide bond to a δ subunit that has a single transmembrane region. The variable γ subunit is another transmembrane protein. Finally, there is an intracellular β subunit, which seems to alter the function of different channels and is the target of some of the modulatory signalling pathways (e.g. Module 3: Figure $Ca_V1.1$ L-type channel).

channels such as the L-type channels that inactivate slowly, the channel opens and closes repeatedly.

Voltage-operated channel (VOC) distribution and function

Voltage-operated channels (VOCs) are found predominantly in excitable cells such as muscle cells (skeletal, cardiac and smooth muscle cells), neurons, and neuroendocrine and endocrine cells (Module 3: Table VOC classification). However, certain non-excitable cells also express these voltage-sensitive channels, especially the low-voltage-activated T-type channels.

The Ca_V1 family of L-type channels are widely expressed in muscle, neuronal and endocrine cells. The Ca_V2 family of N-type, P/Q-type and R-type channels are expressed primarily in neurons, where they have an important role in regulating the release of transmitters from synaptic endings. The Ca_V3 family of T-type channels is widespread in both excitable and non-excitable cells, and is of particular interest because these channels have been implicated in cell proliferation.

Individual cells can express more than one channel type, and this is especially the case in neurons, where there is marked regional separation of different channels. For example, the P/Q- and N-type channels are concentrated in the synaptic endings, where they control presynaptic events responsible for the release of synaptic vesicles, whereas the L-type channels are located in the proximal dendrites and cell body, where they can generate Ca^{2+} signals used to induce neuronal gene transcription.

Ca_V1 family of L-type channels

The Ca_V1 family of L-type channels are widely expressed in muscle, neuronal and endocrine cells. In muscle, they function in the process of excitation–contraction coupling. One of the important properties of these channels is that they inactivate slowly, which means that they remain responsive to repeated or maintained depolarization and thus play a significant role in processes such as muscle contraction, hormone secretion and gene activation, where Ca^{2+} signals are required over long time periods.

The L-type channels are high-voltage-activated channels in that the potential has to depolarize to approximately −30 mV before the channel opens. Once activated, the L-channels have a high conductance (~25 pS). It is calculated that the channel conducts about 1000 Ca^{2+}/ms.

There are four different L-type channels coded by different α_1 subunits, which have different tissue distributions and functions (Module 3: Table VOC classification). The $Ca_V1.1$ L-type channels are located primarily in skeletal muscle. The $Ca_V1.2$ L-type channels are found in heart, smooth muscle and neurons.

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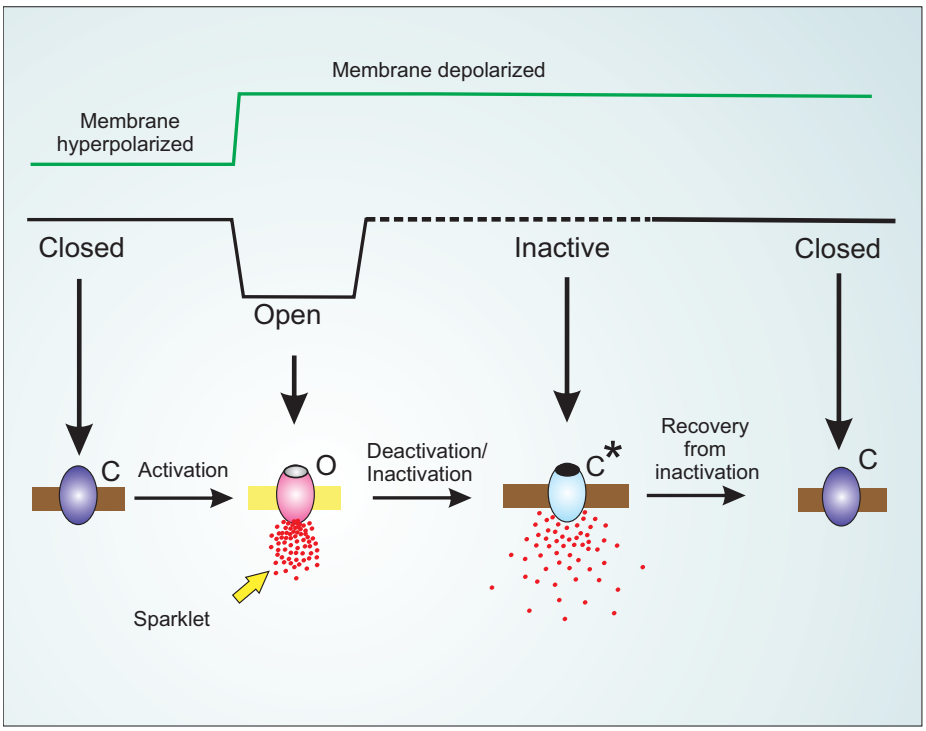
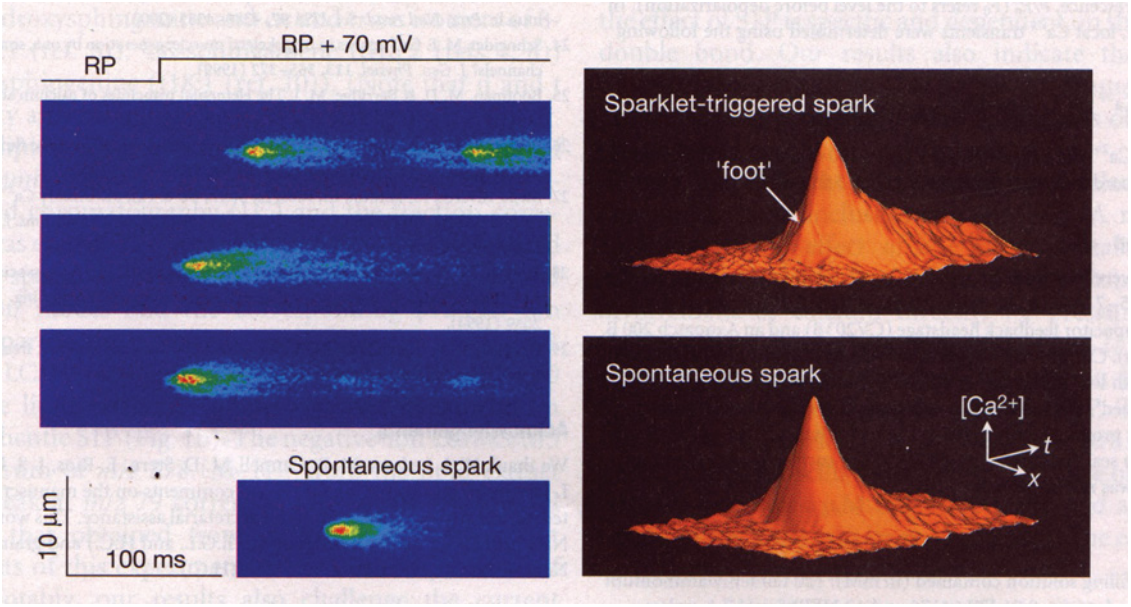
Module 3: | Figure VOC properties



Gating properties of a typical voltage-operated channel (VOC).
When hyperpolarized, the voltage-operated channel (VOC) is closed (C). Depolarization induces an activation process and the channel opens (O), and Ca^{2+} enters the cytosol to form a plume of Ca^{2+}. Upon deactivation/inactivation, the channel closes (deactivation), and the plume of Ca^{2+} dissipates by diffusion into the cytosol. After deactivation the channel is closed, but is in an inactive state (C*). After a variable period of inactivation (broken line), the channel recovers and is ready to open again. The plume of Ca^{2+} that forms around the mouth of the open channel has been visualized in cardiac cells and is called a sparklet (Module 3: Figure Ca^{2+} sparklet).

Module 3: | Figure Ca^{2+} sparklet



Visualization of the elementary events of Ca^{2+} signalling in a ventricular heart cell.
The three top traces on the left are spatiotemporal records of the Ca^{2+} events recorded in rat ventricular cells when the resting potential (RP) is depolarized by 70 mV. Opening of the $Ca_V1.2$ L-type channel is responsible for the small sparklet at the front of the comet-shaped spark. The spontaneous spark at the bottom lacks a sparklet. The surface plots on the right also reveal the small sparklet represented as a 'foot' (arrow) at the front of the spark. The spontaneous spark at the bottom lacks such a foot. Reproduced by permission from Macmillan Publishers Ltd: *Nature*, Wang, S.-Q., Song, L.-S., Lakatta, E.G. and Cheng, H. (2001) Ca^{2+} signaling between single L-type Ca^{2+} channels and ryanodine receptors in heart cells, 410:592-596. Copyright (2001); http://www.nature.com; see Wang et al. 2001.

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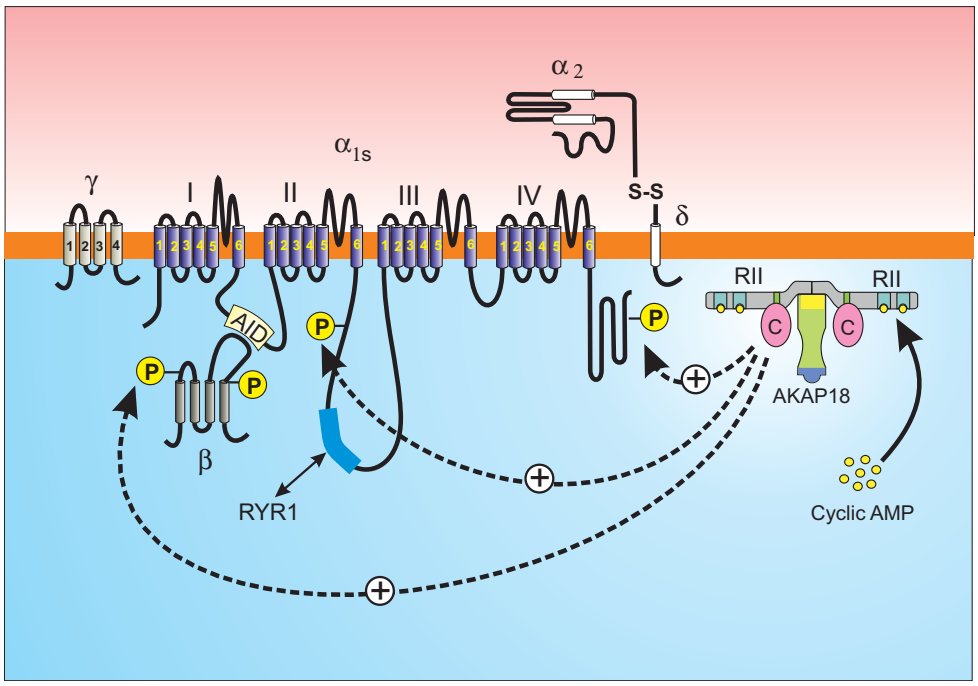
Module 3: | Figure $Ca_V1.1$ L-type channel



Molecular organization of the $Ca_V1.1$ L-type channel from skeletal muscle.
The pore-forming α_{1S} subunit is made up four repeated domains (I-IV) each containing six transmembrane segments (S1-S6). Located between segments S5 and S6 is an additional loop that embeds itself in the membrane to form the channel pore. The intracellular β subunit, which binds strongly to an α1-interaction domain (AID) on the cytosolic loop between domains I and II, has four α-helices, but no transmembrane regions. A region of the cytoplasmic loop between domains II and III (marked in blue) interacts with the ryanodine receptor type 1 (RYR1). The γ subunit is embedded in the membrane through four transmembrane segments. Finally, there is an α_2 subunit, which is extracellular and remains associated with the receptor complex through its linkage by a disulphide bond to the δ subunit, which is embedded in the membrane through a single transmembrane segment.

The $Ca_V1.3$ L-type channels are found in neurons, such as the dopaminergic (DA) neurons located in the substantia nigra pars compacta (SNc), and endocrine cells. The $Ca_V1.4$ L-type channels are located in the retina.

$Ca_V1.1$ L-type channel

The molecular structure of the subunits of the skeletal muscle $Ca_V1.1$ L-type channel reveals the presence of extracellular, intracellular and integral membrane components (Module 3: Figure $Ca_V1.1$ L-type channel). The primary function of these channels is to trigger the contraction of skeletal muscle. Somewhat paradoxically, the channel exerts this stimulatory effect without gating Ca^{2+} across the sarcolemma. Instead, the channel acts indirectly by activating the type 1 ryanodine receptors (RYR1s) to release Ca^{2+} from the sarcoplasmic reticulum (Module 7: Figure skeletal muscle E-C coupling). Upon membrane depolarization, the L-type channel undergoes a conformational change that is transferred to the ryanodine receptor type 1 (RYR1) through a direct protein–protein interaction. This conformational coupling mechanism ensures that membrane depolarization results in a very rapid activation of the RYR1s to release the Ca^{2+} signal that activates contraction. The tight coupling between these two proteins is evident from the fact that repolarizing the membrane results in RYR1 being rapidly switched off.

Mutations of this $Ca_V1.1$ L-type channel are one of the causes of hypokalaemic periodic paralysis (HPP).

The regions on the $Ca_V1.1$ L-type channel and the RYR1 that interact with each other are beginning to be defined. A short 45-amino-acid stretch (residues 720–765) of the cytoplasmic loop lying between the II and III transmembrane regions of the α_{1S} subunit (marked in blue in Module 3: Figure $Ca_V1.1$ L-type channel) interacts with a region on the cytoplasmic region of RYR1. What is remarkable about this process of conformational coupling is that the small α_{1S} subunit embedded in the sarcolemma is able to induce a conformational change in the very much larger RYR1 that is transmitted through to the other side of the molecule to open the channel in the sarcoplasmic reticulum (Module 3: Figure L-type channel/RYR1 complex). Through these molecular interactions, these two proteins are tightly tied together, as is evident by the fact that information can flow in both directions. During the anterograde flow, the L-channel induces a conformational change in the RYR1. However, there also are indications for a retrograde flow of information in that the RYR1 can act to influence the L-channel.

Modulation of the $Ca_V1.1$ L-type channel

The $Ca_V1.1$ L-type channel is modulated by cyclic AMP acting through protein kinase A (PKA), which is associated with the channel through its attachment to A-kinase-anchoring protein 18 kDa (AKAP18) (Module 3: Figure $Ca_V1.1$ L-type channel). The action of cyclic AMP is very fast (occurring within 50 ms) and depends on the

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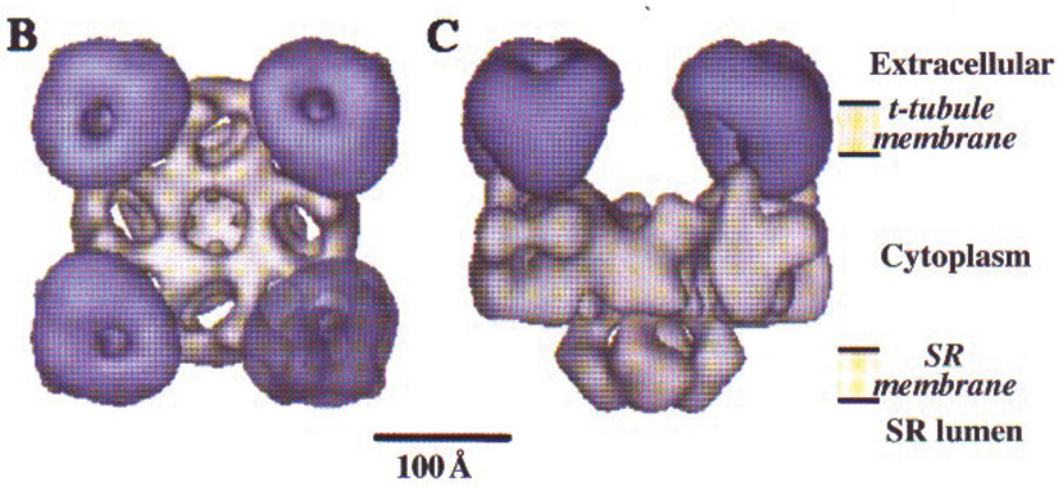
Module 3: | Figure L-type channel/RYR1 complex



A model of the skeletal muscle excitation-contraction coupling domain.
(B) When viewed from the top, the four L-type channels (blue) are shown sitting on top of the large type 1 ryanodine receptor (RYR1) (grey). (C) In a side view, two of the Ca^{2+} channels that are embedded in the T-tubule membrane interact with the top of the large cytoplasmic head of the RYR1. The neck of the RYR1 is embedded in the SR membrane. Reproduced, with permission, from Serysheva, I.I., Ludtke, S.J., Baker, M.R., Chiu, W. and Hamilton, S.L. (2002) Structure of the voltage-gated L-type Ca^{2+} channel by electron cryomicroscopy. Proc. Natl. Acad. Sci. U.S.A. 99:10370-10375. Copyright (2002) National Academy of Sciences, U.S.A.; see Serysheva et al. 2002.

PKA being tightly associated with the channel through AKAP18, which interacts with the C-terminal domain of the $Ca_V1.1$ L-type channel through a novel leucine zipper. AKAP18 is a member of the A-kinase-anchoring proteins (AKAPs), which function as scaffolds to bring PKA close to its substrate [Module 2: Figure protein kinase A (PKA)]. In this case, the substrate is a Ca^{2+} channel and the catalytic subunit of PKA phosphorylates two sites on the α_{1S} subunit Ser-687 on the II–III loop and Ser-1854 in the C-terminus (Module 3: Figure $Ca_V1.1$ L-type channel). In addition, PKA can also phosphorylate Ser-182 and Thr-205 on the β subunit. The precise contribution of these different phosphorylation sites to the increase in channel activity is not clear.

$Ca_V1.2$ L-type channel

The $Ca_V1.2$ L-type channels (Module 3: Figure $Ca_V1.2$ L-type channel) are located in heart, smooth muscle and neurons. Unlike the $Ca_V1.1$ L-type channels in skeletal muscle that function by coupling directly to ryanodine receptor type 1 (RYR1), these $Ca_V1.2$ L-type channels function by gating Ca^{2+}. There are multiple isoforms of these $Ca_V1.2$ L-type channels, and there are clear differences between those expressed in the heart and neurons.

In heart cells, the $Ca_V1.2$ L-type channels play a central role in the process of excitation–contraction coupling by providing the brief pulse of trigger Ca^{2+} that is responsible for activating the underlying type 2 ryanodine receptors (RYR2s) through the process of Ca^{2+}-induced Ca^{2+} release (CICR) (Module 7: Figure ventricular cell E-C coupling). The initial brief trigger pulse has been called a sparklet and can be clearly seen at the leading edge of the larger Ca^{2+} spark (Module 3: Figure Ca^{2+} sparklet).

A gain-of-function mutation of the *CACNA1C* gene that codes for the α_{1C} subunit of the $Ca_V1.2$ channel causes Timothy syndrome, which is a multisystem disorder characterized by cardiac arrhythmia, developmental abnormalities such as syndactyly, immune deficiency and behavioural abnormalities that resemble autism. *CACNA1C* is also a schizophrenia-associated gene and has been linked to bipolar disorder and other psychiatric disorders.

Modulation of $Ca_V1.2$ L-type channel from heart muscle

The Ca^{2+} current flowing through the Ca_V 1.2 L-type channel from heart muscle is subject to a number of modulatory signals (Module 3: Figure $Ca_V1.2$ L-type channel). The most dominant modulation is mediated by Ca^{2+}, which inactivates channel gating. This autoregulation of the channel is mediated by calmodulin (CaM). This inactivation process occurs within milliseconds in response to the plume of Ca^{2+} that builds up around the mouth of the channel. The channel is able to respond so quickly to Ca^{2+} by having a resident calmodulin molecule attached to an IQ site on the C-terminus. Apo-calmodulin may be tethered to two sites, where it has separate functions. At rest, it displays slow inactivation, but when the C-terminal binds to the nearby CaM effector sequence (i.e. the IQ motif), the braking is removed and the typical Ca^{2+}-dependent inactivation takes over.

The Ca_V 1.2 L-type channel from the heart is also regulated by cyclic AMP. The current flowing through the channel in response to depolarization is increased by β-adrenergic stimulation. There is no change in conductance, but there is an increase both in the probability of channel opening and in the time the channel remains open,

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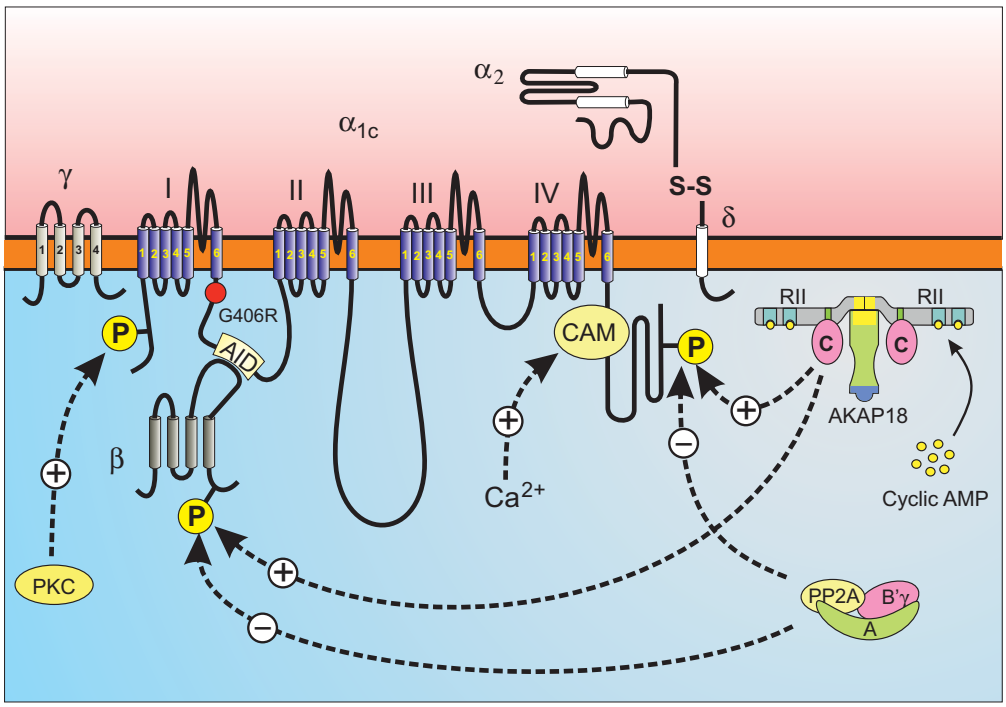
Module 3: | Figure $Ca_V1.2$ L-type channel



Molecular organization of the $Ca_V1.2$ L-type channel from heart muscle.
The essential features of the channel are similar to those described for the $Ca_V1.1$ L-type channel from skeletal muscle (Module 3: Figure $Ca_V1.1$ L-type channel). A major difference concerns the modulatory mechanisms. The channel is modulated by protein kinase A (PKA) associated with A-kinase-anchoring protein of 18 kDa (AKAP18), which phosphorylates sites on both the α_{1C} and β subunits. Protein kinase C (PKC) phosphorylates a site in the N-terminal region of the α_{1C} subunit. The Ca^{2+}-dependent inactivation of the channel is mediated by a resident calmodulin (CaM) bound to the C-terminal region. The red circle indicates the position of Gly-406 that is replaced by arginine in Timothy syndrome.

i.e. there is a delay in the process of deactivation that shuts the channel.

The mode of action of cyclic AMP is still not certain. The α_{1C} subunit is phosphorylated on Ser-1928. Phosphorylation of the β subunit may also contribute to this modulation. As for the $Ca_V1.1$ L-type channel in skeletal muscle, the cyclic AMP-dependent phosphorylation of the Ca_V 1.2 L-type channel from heart appears to be mediated by A-kinase-anchoring protein of 18 kDa (AKAP18), an AKAP isoform that is expressed in the heart. Protein phosphatase 2A (PP2A) reverses the protein kinase A (PKA)-induced phosphorylation events. PP2A is directed to this channel by a B'γ subunit, which is one of the regulatory subunits of PP2A (Module 5: Figure PP2A holoenzyme).

The $Ca_V1.2$ L-type channels in heart cells are inhibited by protein kinase C (PKC), which has no effect on the isoform expressed in neurons. This inhibitory effect on the $Ca_V1.2$ L-type channels in the heart is mediated by phosphorylation of two threonine residues [positions 27 and 31, located in the N-terminus (Module 3: Figure $Ca_V1.2$ L-type channel)].

Ca_V2 family of N-type, P/Q-type and R-type channels

The Ca_V2 family of voltage-operated channels (VOCs) are located primarily in neurons. The $Ca_V2.1$ P/Q-type channels and the $Ca_V2.2$ N-type channels function to release transmitters, with the former acting predominantly at central synapses, whereas the latter act at both central and peripheral synapses. Their main function is to trigger the release of neurotransmitters at synaptic endings.

Less is known about the $Ca_V2.3$ R-type channels. Modulation of the Ca_V2 family of N-type and P/Q-type channels is regulated by similar mechanisms involving an autoregulation by Ca^{2+} and inhibition through a G protein-regulated mechanism.

$Ca_V2.1$ P/Q-type channels

The $Ca_V2.1$ P/Q-type channels are characterized by having an α_{1A} subunit that is acutely sensitive to ω-agatoxin IVA (Module 3: Table VOC classification). The P- and Q-type channels will be treated together because they appear to use the same α_{1A} subunit and have very similar properties and tissue distributions. Their primary function is to trigger the release of transmitter from central synapses. The sequence of events that occur during synaptic transmission are extremely rapid (Module 10: Figure kinetics of neurotransmission). Information is transferred from one neuron to the next in less than 2 ms and only a small fraction of this time (approximately 200 μs) is used up during the depolarization-induced release of neurotransmitter. Therefore, within 200 μs of the action potential invading the synaptic ending, these voltage-operated channels (VOCs) open to initiate the influx of Ca^{2+} that activates the Ca^{2+}-dependent exocytosis (Module 4: Figure Ca^{2+}-induced membrane fusion). To achieve this

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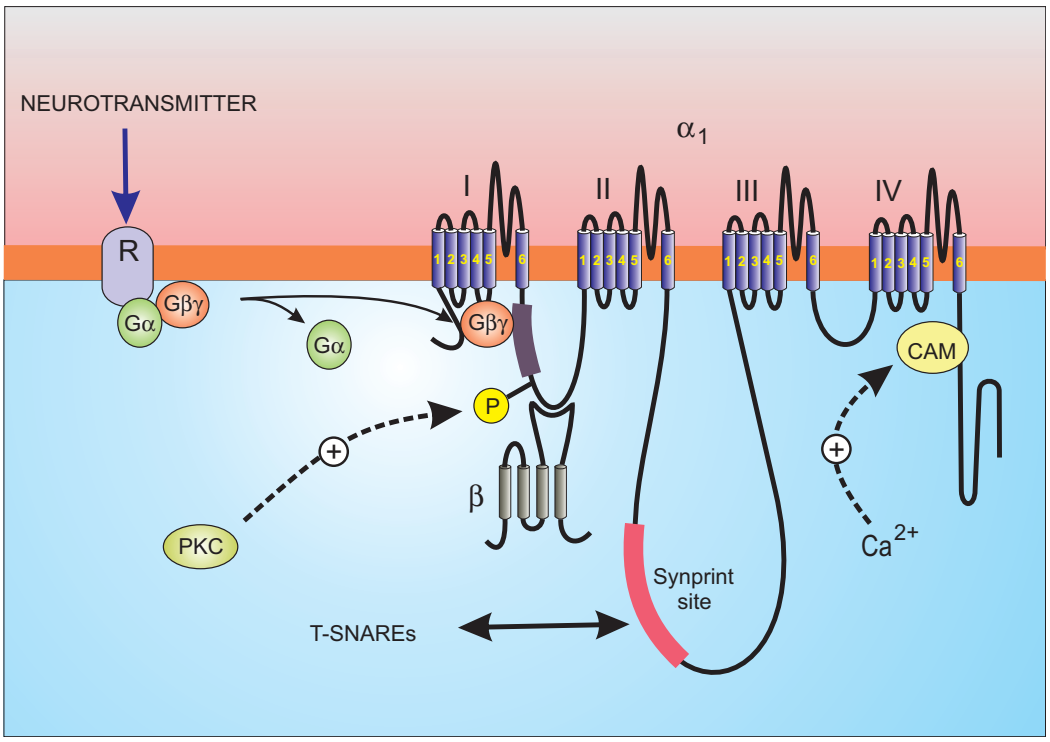
Module 3: | Figure Ca_V2 channel family



Structural organization of the Ca_V2 family of channels (N-type, P/Q-type and R-type) summarizing the sites where various signalling pathways act to control channel function.
The α_1 channel subunit resembles that for the other voltage-operated channels (VOCs). The main difference lies in the specializations that enable these channels to interact with the exocytotic machinery. The synprint site on the long cytoplasmic loop between the II and III membrane regions is responsible for binding to target soluble *N*-ethylmaleimide-sensitive fusion protein-attachment protein receptors (t-SNAREs) [syntaxin and 25 kDa synaptosome-associated protein (SNAP-25)] (Module 4: Figure Ca^{2+}-induced membrane fusion).

very rapid signalling event, the Ca_V2 family of VOCs are anchored to the exocytotic machinery, thus ensuring that the Ca^{2+} entering through the channel can activate membrane fusion with a minimum of delay. This close proximity also ensures that the channels can deliver the high Ca^{2+} concentrations that are necessary to trigger exocytosis. In addition to the anterograde signal (information flowing from the channel to the exocytotic machinery), there is also a retrograde signal whereby the exocytotic machinery modulates channel gating. [This two-way flow of information is very similar to that found for the association between the L-type channel and the ryanodine receptor type 1 (RYR1).] The interactions between these two proteins therefore appear to have two separate functions: anchoring and channel modulation.

The Ca_V2 family of channels has a similar molecular organization to that of other VOCs (Module 3: Figure Ca_V2 channel family). The main differences lie in the molecular components that enable these channels to anchor themselves to the exocytotic machinery and to respond to various modulatory signals. Like the N-type channels, the P/Q-type channels have a synprint region (synaptic protein interaction region) located in the cytoplasmic loop between domains II and III that is responsible for anchoring the P/Q-type channel to the target soluble *N*-ethylmaleimide-sensitive fusion protein-attachment protein receptor (t-SNARE) proteins that contribute to the exocytotic machinery (Module 4: Figure Ca^{2+}-induced membrane fusion).

The P/Q-like channel has multiple isoforms, some of which arise through alternative splicing in the synprint region. These isoforms bind to different components of the exocytotic machinery. For example, the BI isoform can bind to both syntaxin and 25 kDa synaptosome-associated protein (SNAP-25), whereas the rbA isoform seems to bind just to SNAP-25.

Mutations in the α_{1A} subunit of these P/Q channels are responsible for a number of genotypic human disorders such as episodic ataxia type 2, spinocerebellar ataxia type 6 (SCA6) and familial hemiplegic migraine (FHM).

Antibodies to this P/Q channel are responsible for Lambert-Eaton myasthenic syndrome.

$Ca_V2.2$ N-type channels

The $Ca_V2.2$ N-type channels are characterized by having an α_{1B} subunit that is acutely sensitive to ω-conotoxin GVIA toxin (Module 3: Table VOC classification). Its primary role is to mediate the rapid release of neurotransmitter at neuronal synaptic endings. The molecular organization of the N-type channels resembles those of the other family members (Module 3: Figure Ca_V2 channel family). The important differences concern the special components that anchor the channels to the exocytotic machinery

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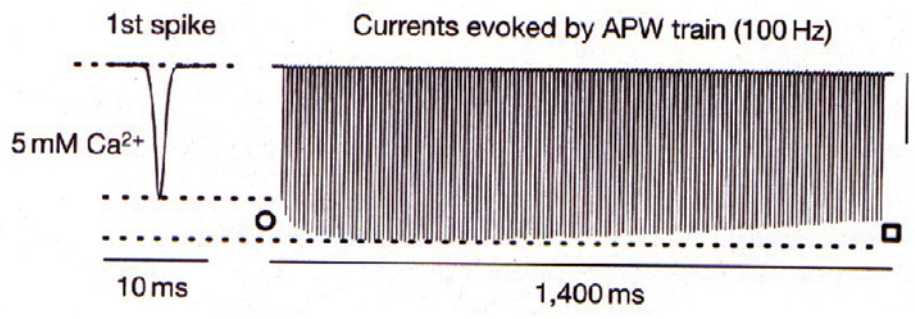
Module 3: | Figure P/Q-type channel facilitation



Facilitation of the P/Q-type channel induced by repetitive stimulation.

An action potential waveform (APW) train administered at 100 Hz induced a long series of repetitive Ca^{2+} currents. The circle illustrates the process of facilitation, i.e. the gradual increase in the amplitude of the Ca^{2+} current spikes, which was followed later by inactivation (square). Reproduced by permission from Macmillan Publishers Ltd: *Nature*, DeMaria, C.D., Soong, T.W., Alseikhan, B.A., Alvania, R.S. and Yue, D.T. (2001) Calmodulin bifurcates the local Ca^{2+} signal that modulates P/Q-type Ca^{2+} channels. 411:484-489. Copyright (2001); http://www.nature.com; see Demaria et al. 2001.

and the regions that mediate the modulation of channel activity.

N-type channels, like the P/Q-type channels, are anchored to specific components of the exocytotic machinery (Module 4: Figure Ca^{2+}-induced membrane fusion). The major proteins that contribute to exocytosis can be divided into those that carry out the fusion process [e.g. the soluble *N*-ethylmaleimide-sensitive fusion protein-attachment protein receptor (SNARE) proteins] and those that mediate the stimulatory action of Ca^{2+} (e.g. synaptotagmin). All the evidence suggests that the N-type channels are interacting with the target SNARE (t-SNARE) proteins, particularly with syntaxin 1A and with 25 kDa synaptosome-associated protein (SNAP-25). The region of the cytoplasmic loop between domains II and III, referred to as the synprint region (synaptic protein interaction region), is responsible for anchoring the N-type channel to these SNARE proteins (Module 3: Figure Ca_V2 channel family). In the case of syntaxin 1A, the synprint region attaches to the H3 region located close to the transmembrane domain. The latter region also seems to be responsible for the modulatory effect of syntaxin 1A; the corresponding binding site on the N-type channel has not been determined, but is likely to be the cytoplasmic loop between domains I and II.

The fact that the anchoring and modulatory aspects are regulated by separate molecular interactions may have important implications for channel function. One suggestion is that, when the vesicle is primed ready for release, the modulatory site on syntaxin is buried in the SNARE complex, thus freeing up the N-channel to provide the Ca^{2+} signal for release. Once release occurs and the core complex dissociates, syntaxin and SNAP-25 can once again inhibit the channel until they are incorporated into another docked vesicle, whereupon the channel will once again be free to trigger another cycle of release.

$Ca_V2.3$ R-type channels

The R-type channels, which are strongly inhibited by a tarantula venom (SNX-482), are expressed in regions of the brain that control pain transmission (e.g. dorsal root ganglion and dorsal horn of the spinal cord). They are also strongly expressed in the amygdala where they may play a role in fear responses.

Transient expression of R-type channels may play a role in myelinogenesis.

Modulation of the Ca_V2 family of N-type and P/Q-type channels

The Ca_V2 family of N-type and P/Q-type channels are modulated through a number of mechanisms, such as autoregulation through Ca^{2+} or inhibition by various neurotransmitter receptors through a G protein-dependent mechanism (Module 3: Figure Ca_V2 channel family).

Ca^{2+}-dependent modulation

The Ca_V2 family, like some of the L-type channels, are sensitive to Ca^{2+} which can induce both facilitation and inactivation. The former is observed during repetitive stimulation during which there is a gradual increase in the current carried during each spike (Module 3: Figure P/Q channel facilitation). This facilitation is Ca^{2+}-specific because it does not occur when Ba^{2+} replaces external Ca^{2+}. The inactivation phenomenon occurs during the course of each spike and is particularly evident during a maintained depolarization when the current decays progressively (Module 3: Figure Ca^{2+} channel inactivation). Although both phenomena depend on Ca^{2+}, the mechanisms seem to be different in that the inactivation is more sensitive to Ca^{2+} concentration buffering. The Ca^{2+} sensor appears to be calmodulin, but others have been implicated such as the neuronal Ca^{2+} sensor-1 (NCS-1).

The IQ-like domain of the α_{1A} channel has calmodulin (CaM) prebound and is thus able to respond quickly to the feedback effects of Ca^{2+} on both facilitation and inactivation. CaM induces facilitation when Ca^{2+} is bound to its C-terminal lobe, whereas binding to the N-terminal lobe causes inactivation. This indicates that CaM may exert lobe-specific Ca^{2+} signalling, with the C-terminal lobe responding to the localized spike-like component of Ca^{2+}

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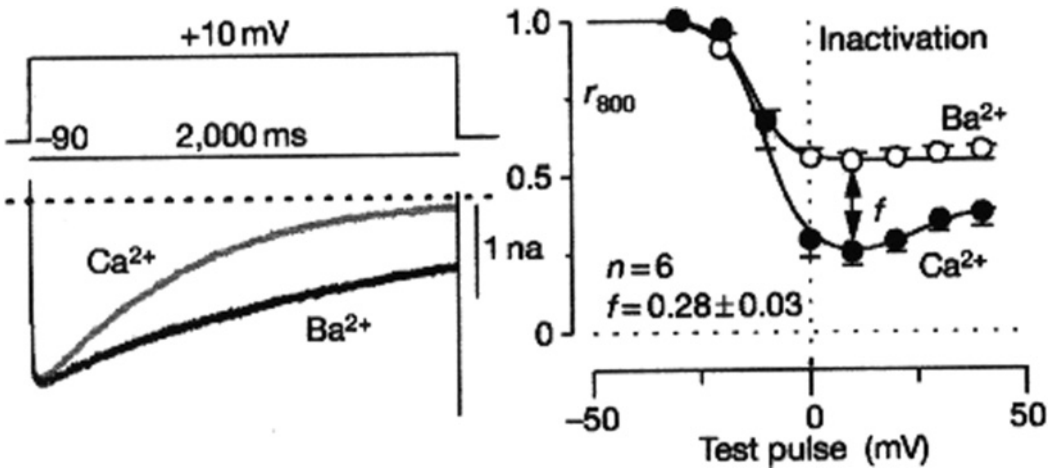
Module 3: | Figure Ca^{2+} channel inactivation



Inactivation of P/Q-type channels.

In response to a maintained depolarization, the Ca^{2+} current inactivated almost completely in about 2 s. When Ca^{2+} was replaced by Ba^{2+}, the current declined much more slowly, indicating that Ca^{2+} plays a role in inactivating the channels. The graph on the right illustrates how the degree of inactivation varies with the voltage of the test pulse, which provides further evidence to support a role for Ca^{2+} in inactivation. Reproduced by permission from Macmillan Publishers Ltd: *Nature*, DeMaria, C.D., Soong, T.W., Alseikhan, B.A., Alvania, R.S. and Yue, D.T. (2001) Calmodulin bifurcates the local Ca^{2+} signal that modulates P/Q-type Ca^{2+} channels. 411:484-489. Copyright (2001); http://www.nature.com; see Demaria et al. 2001.

signals, whereas the N-terminal lobe may respond to more global aspects.

G protein-dependent modulation

These Ca_V2 channels are characterized by being sensitive to modulation by neurotransmitter receptors that act by inhibiting their gating activity, leading to a reduction in neurotransmission. The N-type channels appear to be more sensitive to such modulation than the P/Q-type channels. The modulatory neurotransmitter receptors act through heterotrimeric G proteins, which are dissociated to release the Gα and Gβγ subunits. It is the Gβγ subunit that interacts with the α_1 subunit to inhibit channel gating by increasing their voltage dependence and by lowering the rate of activation. The Gβγ subunit seems to exert its inhibitory effect by binding to the I–II loop (Module 3: Figure Ca_V2 channel family). In addition, the Gβγ subunit may also bind to sites on the N- and C-termini.

This G protein-dependent modulatory mechanism has been described in a number of systems:

- In chromaffin cells, the purinergic P2Y and opiate-μ and opiate-δ receptors inhibit the predominant P/Q-type channels that trigger the release of adrenaline (Module 7: Figure chromaffin cell secretion).
- The endocannabinoid retrograde signalling mechanism uses Gβγ subunits to inhibit the Ca^{2+} entry mechanisms (Module 10: Figure endocannabinoid retrograde signalling) to regulate transmitter release from both excitatory and inhibitory nerve endings.

PKC-dependent modulation

The N-type channel is phosphorylated by protein kinase Cε (PKCε), which is one of the novel PKCs (nPKCs) (Module 2: Figure PKC structure and activation). Enigma homologue (ENH) is a PKC-binding protein that serves to couple PKCε to the N-type channel. This PKCε/ENH/N-type channel complex functions to potentiate channel activity.

Ca_V3 family of T-type channels

The Ca_V3 family of T-type channels is widely distributed in both excitable and non-excitable cells. There are three T-type channels that differ from the other two channel families in two main respects. They have lower conductances and they are activated at much lower voltages (Module 3: Table VOC classification). Despite the lower conductances, they can pass large amounts of Ca^{2+} because they operate at more negative voltages, where the electrochemical driving force for Ca^{2+} is high. These T-type channels display the fastest inactivation kinetics among all the Ca^{2+} channel subtypes. The rapid inactivation prevents cells from being overloaded with Ca^{2+}, and such channels are often found in cells that have to be active for long periods. These T-type channels function in a variety of cellular processes:

- Pacemaker activity of the sinoatrial node pacemaker cells (Module 7: Figure cardiac pacemaker).
- T-type channels contribute to neuronal rhythmicity.
- T-type channels contribute to stimulus–secretion coupling in adrenal glomerulosa cells.
- Entry of Ca^{2+} through T-type channels functions in the sperm acrosome reaction (Module 8: Figure ZP3-induced Ca^{2+} signals).
- The $Ca_V3.2$ T-type channel containing the α_{1H} subunit functions in myoblast fusion during skeletal muscle terminal differentiation.
- $Ca_V3.2$ T-type channels are used to generate the prolonged Ca^{2+} signal required to activate aldosterone

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secretion from zona glomerulosa cells (Module 7: Figure glomerulosa cell signalling).
- Smooth muscle cells express T type channels that have been implicated in the pacemaker activity of uterine smooth muscle cells (Module 7: Figure uterus activation) and the cytosolic Ca^{2+} oscillator in vascular and airway smooth muscle (Module 7: Figure SMC cytosolic oscillator).

Sodium channels

There are a number of voltage-gated sodium channels ($Na_V1.1$ to $Na_V1.9$). The channels consist of a pore-forming α subunit, which is associated with auxiliary β subunits (β1 to β4) that modify the gating properties of the α subunit. The auxiliary β subunits also play an important role in the localization of the channel through their interaction with the intracellular cytoskeleton and various adhesion molecules.

These Na_V channels are responsible for initiating and propagating action potentials in excitable cells such as neurons, muscle and neuroendocrine cells. These channels are also expressed in various sensory organs such as the $Na_V1.2$ channels in the type III presynaptic taste receptor cells (Module 10: Figure taste receptor cells) and the $Na_V1.7$ channels in the sensory neurons that function in nociception (Module 10: Figure nociception).

The pore-forming α subunits are encoded by the *SCN1A–SCN5A* and *SCN8A–SCN11A* genes, whereas the β-subunits are encoded by the *SCN1B–SCN4B* genes. Mutations in some of these genes have been linked to a number of syndromes:

- *SCNA1* has been linked to Dravet syndrome.
- *SCNA2* has been linked to autism spectrum disorders (ASDs).
- *SCNA9*, which codes for the $Na_V1.7$ channel, has been linked to erythermalgia.

Neuronal rhythmicity

Some brain rhythms are generated by a membrane oscillator. An example of such a neuronal rhythm is the synchronized bursts of action potentials that occur in thalamocortical cells during slow-wave sleep. The thalamus is the major gateway for signals travelling to the cerebral cortex. During slow-wave sleep, this thalamocortical network begins to oscillate rhythmically at a frequency of 0.5–4 Hz. During this rhythmic behaviour, the individual neurons display a Ca^{2+} oscillation brought about by the periodic activation of T-type channels, which is an essential component of the membrane oscillator. The other essential component is a hyperpolarizing-activated cyclic nucleotide-gated channel (HCN). The latter produces a hyperpolarization-activated current (I_h) that has a very similar role to play in neuronal rhythmicity as I_f does in generating the pacemaker activity of the sinoatrial node (Module 7: Figure cardiac pacemaker). Activation of the T-type channels depolarizes the membrane and this gives rise to a burst of Na^+ action potentials. These large action potentials inactivate both the T-type channel current ($I_{Ca,T}$) and I_h, causing the membrane to hyperpolarize. As the membrane hyperpolarizes, the I_h will once again reactivate and will begin to depolarize the membrane to the point when the $I_{Ca,T}$ switches on again and the whole cycle repeats itself to set up regular oscillations.

Stimulus–secretion coupling in adrenal glomerulosa cells

T-type channels in adrenal zona glomerulosa cells mediate control of aldosterone secretion by angiotensin II (AngII) (Module 7: Figure glomerulosa cell signalling). At physiological concentrations, AngII induces a small membrane depolarization just sufficient to activate these T-type channels. At higher concentrations, the depolarization is sufficient to recruit L-type channels.

Modulation of the Ca_V3 family of T-type channels

The activity of Ca_V3 family members are regulated by G protein-coupled receptors (GPCRs). For example, dopamine acting through D_1 receptors results in inhibition of T-type channels. In the case of the α_{1H} channel, this inhibition is carried out specifically by $\beta_2\gamma_2$ subunits acting on the intracellular loops that connect the transmembrane domains II and III.

Agonist-operated channels (AOCs)

Agonists arriving at the cell surface can activate the entry of Ca^{2+} through a number of mechanisms, as illustrated in **Module 3: Figure Ca^{2+} entry mechanisms**:

1. In the case of the receptor-operated channels (ROCs), agonists have a direct action by binding to the channel, as occurs for the neurotransmitters acetylcholine, glutamate, 5-hydroxytryptamine (5-HT) and ATP. Receptors that produce second messengers, such as cyclic AMP, diacylglycerol (DAG), inositol 1,4,5-trisphosphate ($InsP_3$) and arachidonic acid (AA), activate second messenger-operated channels (SMOCs):
2. Those receptors that produce cyclic AMP can activate the cyclic nucleotide-gated channels (CNGCs) that operate in sensory systems (visual, olfactory and gustatory signalling pathways).
3. DAG acting within the plane of the membrane stimulates an entry channel that appears to be transient receptor potential canonical 6 (TRPC6).
4. AA activates an arachidonic acid-regulated Ca^{2+} (ARC) channel. The action of $InsP_3$ is somewhat more problematic and two mechanisms have been proposed:
5. $InsP_3$ may act indirectly to open store-operated channels (SOCs) by releasing Ca^{2+} from the endoplasmic reticulum (ER). As the store empties, the decrease in Ca^{2+} is detected by the ER Ca^{2+} sensor STIM that activates the SOCs through conformational coupling mechanism as outlined in Module 3: Figure conformational coupling hypothesis.

Another important group of channels are found in the large **transient receptor potential (TRP) ion channel family**, which tend to have lower conductances and thus can operate over much longer time scales without swamping the cell with too much Ca^{2+}. Members of this TRP

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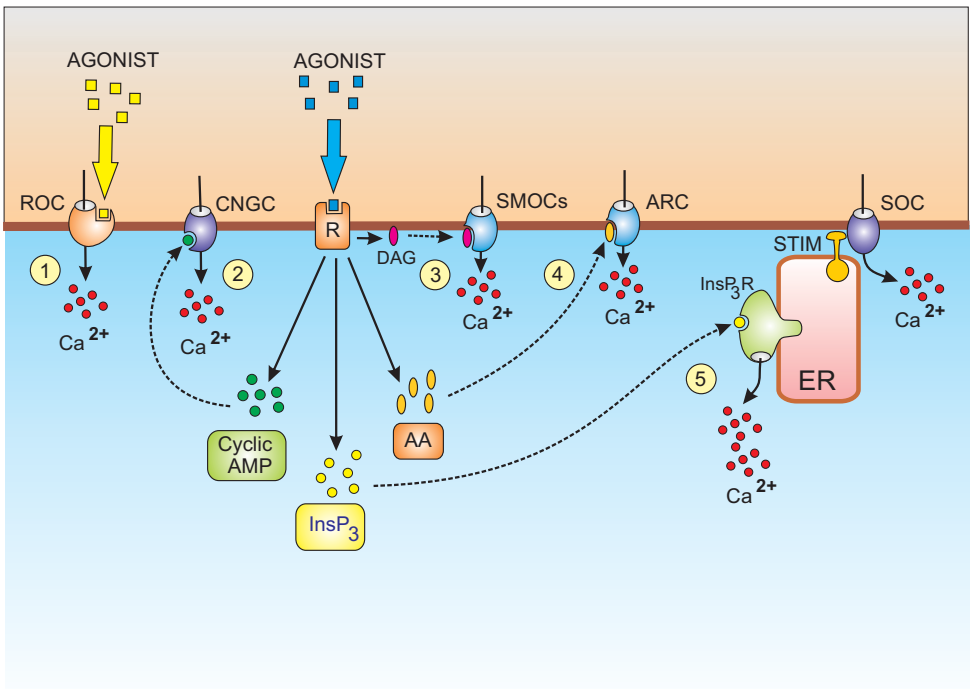
Module 3: | Figure Ca^{2+} entry mechanisms



Entry of Ca^{2+} through agonist-operated channels.

Entry of external Ca^{2+} can be activated by stimuli arriving at the cell surface or by signals generated within the cell. The most direct mechanism occurs in the receptor-operated channels (ROCs), where the agonist binds directly to the ion channel. Agonists can also activate entry indirectly by recruiting various internal signalling pathways to activate second messenger-operated channels (SMOCs) or a store-operated channel (SOC) as described in the text.

family are particularly important in controlling slower cellular processes such as sensory perception, smooth muscle contractility and cell proliferation. Very often these channels are essential for maintaining the Ca^{2+} oscillations responsible for driving these longer-term processes.

Receptor-operated channels (ROCs)

These receptor-operated channels (ROCs) are multimeric ion channels that are gated by external agonists such as neurotransmitters and hormones. They have two important signalling functions. Firstly, by gating ions (e.g. Na^+, K^+, Cl^- and Ca^{2+}), they alter membrane potential and are essential for controlling excitable cells such as neurons and muscle cells. In addition, many of the channels are permeable to Ca^{2+} and this represents an important mechanism for generating this second messenger, particularly in excitable cells. There are three main groups of ROCs (Module 3: Table receptor-operated channel toolkit):

- Cysteine-loop receptors:
  - Nicotinic acetylcholine receptors (nAChRs)
  - 5-Hydroxytryptamine 3 (5-HT_3) receptors
  - γ-Aminobutyric acid (GABA) receptors
  - Glycine receptors (GlyRs)
- Glutamate receptors:
  - α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors
  - *N*-methyl-D-aspartate (NMDA) receptors
  - Kainate receptors
- ATP-sensitive P2X receptors

Many of these channel families consist of multiple subunits which are mixed and matched to create channels with different properties.

Cysteine-loop receptors

Nicotinic acetylcholine receptors (nAChRs)

Cholinergic neurons play an important role in regulating both central and peripheral processes. The acetylcholine (ACh) they release can act either on muscarinic receptors, which are coupled to various second messenger pathways, or on ionotropic nicotinic acetylcholine receptors (nAChRs). The nAChRs are potently blocked by hexamethonium. One of the primary actions of nAChRs is to transmit signals at the neuromuscular junction or at synapses in the nervous system, but they also have functions on non-excitable cells. The nAChRs take their name from the fact that the drug nicotine can mimic the action of ACh in opening these channels. There are two main types of nAChRs. There are the nAChRs responsible for excitation–contraction coupling in skeletal muscle and the neuronal nAChRs. Nicotine, which is the main psychoactive ingredient in tobacco, acts on these neuronal receptors located both in the central and peripheral nervous systems.

nAChRs can transmit information via two complementary signalling mechanisms:

- Nicotinic acetylcholine receptor (nAChR) signalling through membrane depolarization

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Module 3: | Table receptor-operated channel toolkit
Receptor-operated channel toolkit.

Component	Comments
CYSTEINE-LOOP RECEPTORS	
Nicotinic acetylcholine receptors (nAChRs)	
α1	Muscle subunit
α2-α9	Neuronal subunits
β1-β4, δ, ε, γ	
5-Hydroxytryptamine type 3 (5-HT_3) receptor	
GABA receptors	
$GABA_A$	Pentameric hetero-oligomers consisting of mixture of α, β, γ subunits
$GABA_B$	The $GABA_B$ receptor is a G protein-coupled receptor (see Module 1: Table G protein-coupled receptors)
$GABA_C$	
Glycine receptors (GlycRs)	
α1-α3	
β	
GLUTAMATE RECEPTORS	
AMPA receptor	
GluR1-GluR4	
NMDA receptors	
NR1	This subunit is obligatory and is combined with the NR2 or NR3 subunits to form functional NMDA receptors
NR2A-NR2D	
NR3A-NR3B	
Kainate receptors	
GluR5-GluR7	
KA1-KA2	
P2X RECEPTORS	
P2X1	See Module 11: Figure platelet activation and Module 7: Figure vas deferens activation
P2X2	See Module 10: Figure carotid body chemoreception
P2X3	See Module 10: Figure inflammatory soup
P2X4	
P2X5	
P2X6	
P2X7	See Module 11: Figure macrophage signalling

- Nicotinic acetylcholine receptor (nAChR) signalling through activation of Ca^{2+} signalling

Nicotinic acetylcholine receptor (nAChR) signalling through membrane depolarization

Since nicotinic acetylcholine receptors (nAChRs) gate the inward flow of positive ions such as Na^+ and Ca^{2+}, they depolarize membranes, and this is responsible for activating various potential-dependent processes:

- Excitation–contraction coupling in skeletal muscle is triggered by the depolarization induced by nAChRs at the neuromuscular junction (Module 7: Figure skeletal muscle E-C coupling).
- nAChRs function in mossy fibre presynaptic Ca^{2+} release (Module 10: Figure mossy fibre presynaptic release).
- nAChRs are responsible for fast synaptic transmission in ganglionic neurons by triggering a process of excitation–secretion coupling at certain neuronal synapses.
- nAChRs are responsible for the membrane depolarization that triggers excitation–secretion coupling in chromaffin cells (Module 7: Figure chromaffin cell secretion).
- Acetylcholine released from glomus cells in the carotid body act on nAChRs to excite the afferent nerve endings to send information back to the brain during O_2 sensing (Module 10: Figure carotid body chemoreception).

Nicotinic acetylcholine receptor (nAChR) signalling through activation of Ca^{2+} signalling

Since many of the nicotinic acetylcholine receptors (nAChRs) have a considerable Ca^{2+} permeability, they can introduce Ca^{2+} into cells to carry out a variety of signalling functions in both excitable and non-excitable cells:

- nAChRs are expressed on macrophages where they function to attenuate systemic inflammatory responses by inhibiting the release of tumour necrosis factor (TNF) (Module 11: Figure macrophage signalling) This inhibitory process is a critical component of the cholinergic anti-inflammatory pathway.
- Presynaptic nAChRs can modulate the release of transmitters such as acetylcholine, glutamate, noradrenaline dopamine and γ-aminobutyric acid (GABA). These presynaptic facilitatory nAChRs are located on the synaptic endings of both central and peripheral neurons. In the case of mossy fibres in the hippocampus, nicotine increased the frequency of miniature excitatory postsynaptic potentials (mEPSPs) (Module 10: Figure mossy fibre presynaptic release). It has been proposed that nicotine facilitates release by inducing the entry of external Ca^{2+} that then loads up the store so that it begins to trigger Ca^{2+} sparks that appear to be responsible for increasing transmitter release (Module 10: Figure mossy fibre presynaptic Ca^{2+} release). Presynaptic nAChRs are found on the nerve terminals that innervate the vas deferens and the inhibitory interneurons in the hippocampus (Module 10: Figure gamma oscillatory mechanisms). A decrease in the expression of nAChRs have been found in patients with schizophrenia.
- nAChRs are located on both T and B cells, where they may act to impair activation processes, and this could account for the immune depression found in smokers.
- nAChRs located at the postsynaptic region on hair cells generate a microdomain of Ca^{2+} (region 4 in Module 10: Figure hair cell) that functions in somatic electromotility.

Structure and function of nicotinic acetylcholine receptors (nAChRs)

Nicotinic acetylcholine receptors (nAChRs), which belong to the cysteine-loop family (Module 3: Table receptor-operated channel toolkit), have a pentameric organization assembled from a number of different subunits. The way in which these are put together gives rise to a wide range of different channels. For example, the subunits that muscle nAChRs have are α1α1βγδ, while the neuronal forms have highly variable stoichiometries many of which remain to be established. These different subunit com-

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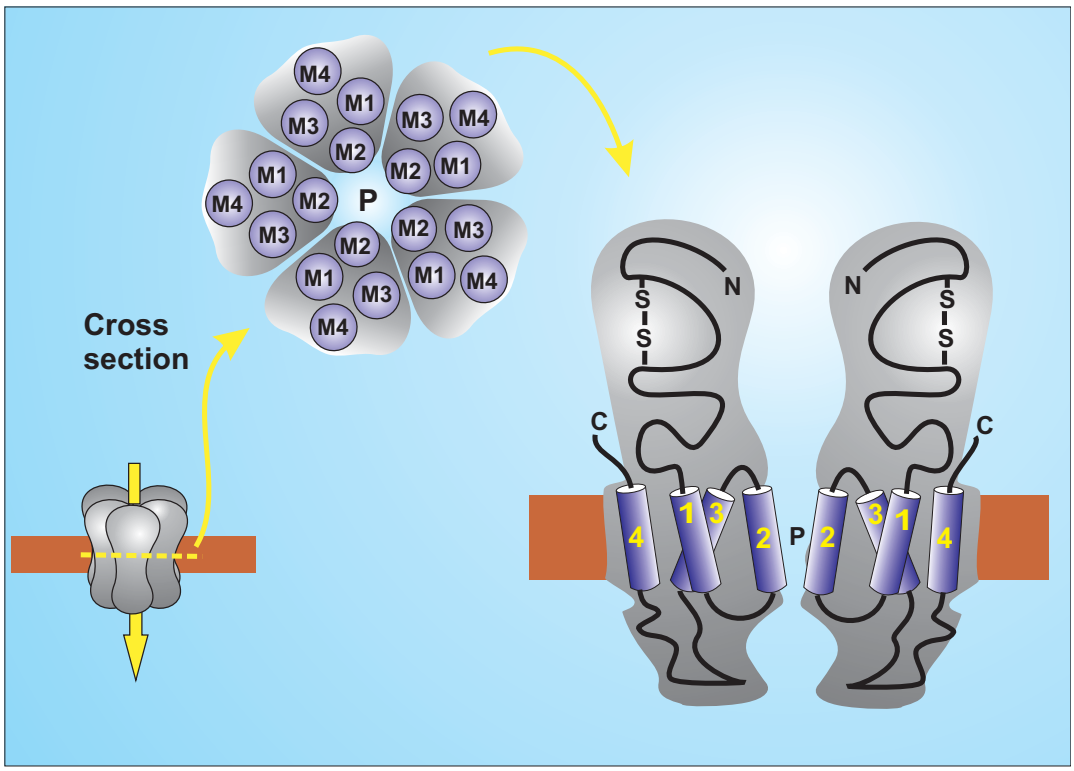
Module 3: | Figure nicotinic acetylcholine receptor



Structural organization of the nicotinic acetylcholine receptor.
The nicotinic acetylcholine receptor (nAChR) is a typical cysteine-loop receptor. It is a pentamer that contains at least two α subunits together with different combinations of the other subunits. A cross section of the channel taken at the level of the membrane reveals that each subunit is made of four membrane-spanning segments (M1-M4), with M2 facing the pore (P). The organization of two subunits in longitudinal section (shown at the bottom) illustrates the way in which the M2 segment forms the pore. The N-terminal region that extends to the outside contains the cysteine loop. The way in which the acetylcholine binds may act to induce a movement of the M2 segments to open the pore (Module 3: Figure nicotinic acetylcholine receptor gating).

binations have an important consequence of altering the relative permeabilities to Ca^{2+} and Na^{+}, which can be compared by measuring the fractional current (P_f) carried by Ca^{2+}. For example, the homomeric α7 neuronal nAChRs have a P_f of approximately 12%, which is considerably larger than that of many of the other receptors.

Each of the five subunits that make up the pentameric structure has four membrane-spanning segments (M1–M4) so that segment M2 lines the pore (Module 3: Figure nicotinic acetylcholine receptor). Structural studies have begun to reveal how the binding of acetylcholine opens the pore (Module 3: Figure nicotinic acetylcholine receptor gating).

5-Hydroxytryptamine type 3 (5-HT_3) receptor

The 5-hydroxytryptamine type 3 (5-HT_3) receptor is a typical receptor-operated channel that gates Ca^{2+}. One of their locations is on the vagal afferents in the gut (Module 7: Figure small intestine). Their activation causes nausea and vomiting, particularly during cancer treatment by radiation and chemotherapy. The 5-HT_3 receptors may also play a role in activating Itch in the histamine-sensitive Itch neurons (Module 10: Figure Itch signal transduction mechanism).

γ-Aminobutyric acid (GABA) receptors

γ-Aminobutyric acid (GABA) receptors are mainly expressed in the brain, where they respond to the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and are responsible for neuronal inhibition. These GABA receptors gate Cl^{-}, which thus hyperpolarizes the membrane to induce the inhibitory responses. The channel is a hetero-oligomer made up from five different subunits encoded by different genes (α, β, γ, and δ). The main $GABA_A$ receptor subtype has a subunit composition of 2α, 2β and 1γ. These $GABA_A$ receptors are targets for a number of psychoactive drugs, such as the benzodiazepines and barbiturates. They are also the targets for many anaesthetics.

Anaesthetics

Many anaesthetics act by either enhancing the activity of inhibitory receptors or to inhibit excitatory receptors. For example, chloroform, diethyl ether, halothane and isoflurane act by increasing the activity of $GABA_A$ receptors that inhibit neuronal activity. In the case of isoflurane, this action is indirect because this anaesthetic stimulates the activity of the VLPO neurons that normally release GABA to promote sleep (Module 10: Figure sleep/wake cycle regulation). These same anaesthetics are also potent activators of TREK channels. Pentobarbital inhibits $GABA_A$ receptors but has no effect on TREK channels. Conversely,

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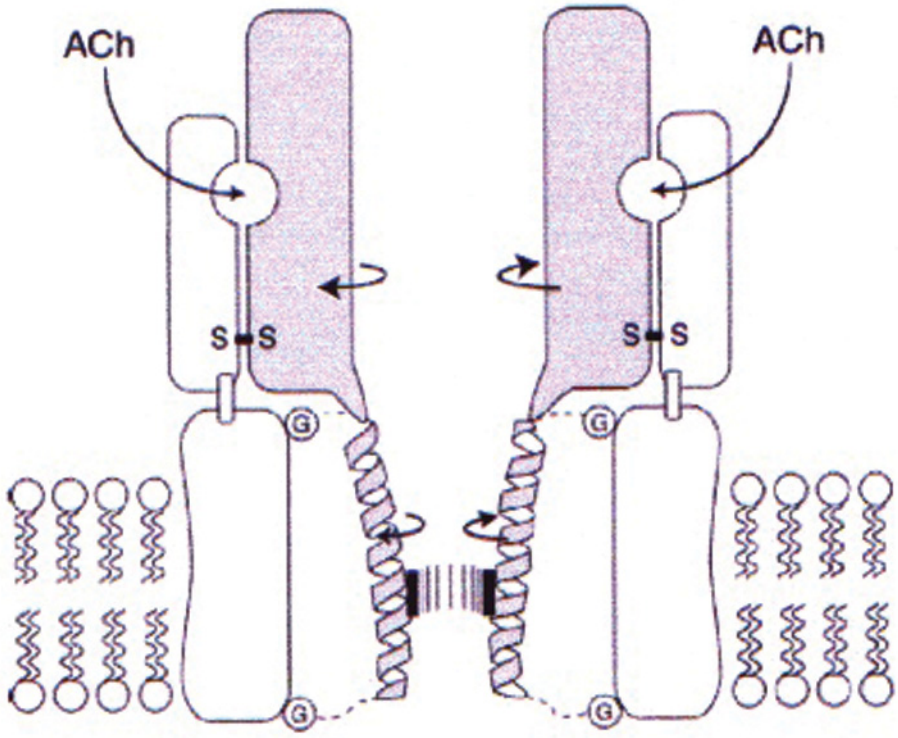
Module 3: | Figure nicotinic acetylcholine receptor gating



Proposed gating mechanism of the nicotinic acetylcholine receptor.
In this model, the binding of acetylcholine is thought to rotate the α subunits, which is transmitted to the gate in the plane of the membrane. These rotations are possible because these moving parts are isolated from the outer parts of the channel by flexible loops that contain glycine residues (G). The disulphide bond (S-S) also provides a pivot for the ligand-binding domain. Reproduced by permission from Macmillan Publishers Ltd: *Nature*, Miyazawa, A., Fujiyoshi, Y. and Unwin, N. (2003) Structure and gating mechanism of the acetylcholine receptor pore. 423:949-955. Copyright (2003); http://www.nature.com; see Miyazawa et al. 2003.

TREK channels are the targets for some anaesthetics such as chloral hydrate, N_2O, xenon and cyclopropane that have no effect on $GABA_A$ receptors.

Glycine receptors (GlyRs)

Glycine receptors (GlyRs) are ligand-gated Cl^- channels, which usually induce a membrane hyperpolarization and thus act to inhibit neural activity. However, they can also cause depolarization if cells have large amounts of Cl^- such that the Cl^- equilibrium potential is more positive than the resting potential. As for the other cysteine-loop receptors, the GlyR channel is a pentamer composed of α and β subunits. There are examples of channels being formed from α subunits alone or from both α and β subunits. In the latter case, the heterotrimeric receptor has a 3α + 2β composition. During foetal development, the receptor is mainly the α2 homomer, but in adulthood, this switches to predominantly the α1β heteromer. A characteristic feature of these GlyRs is that they are inhibited by strychnine.

GlyRs are found in many brain regions (e.g. spinal cord, pons, thalamus and hypothalamus), but they appear to be absent from higher brain regions. They have been implicated in motor reflex and pain sensory pathways in the spinal cord. Glycine is an inhibitory neurotransmitter in the retina, which has many GlyRs that have different subunits expressed in a clearly defined spatial pattern. For example, the bipolar and ganglion cells mainly express α1 subunits, whereas the amacrine cells have α2 subunits. GlyRs are also found on spermatozoa, where they may function in the acrosome reaction (Module 8: Figure ZP3-induced Ca^{2+} signals).

Glutamate receptors

Glutamate receptors are primarily located in the brain, where they function in neurotransmission by responding to the neurotransmitter glutamate. The tetrameric structure can have different subunits to create channels with subtly different properties. One of the differences is in the glutamate-binding site, which is sufficiently different to be distinguished by molecules resembling glutamate. Indeed, the three families take their names from the drugs that activate them:

- α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) activates the AMPA receptors.
- Kainic acid activates the kainate receptors.
- *N*-Methyl-D-aspartate (NMDA) activates the NMDA receptors.

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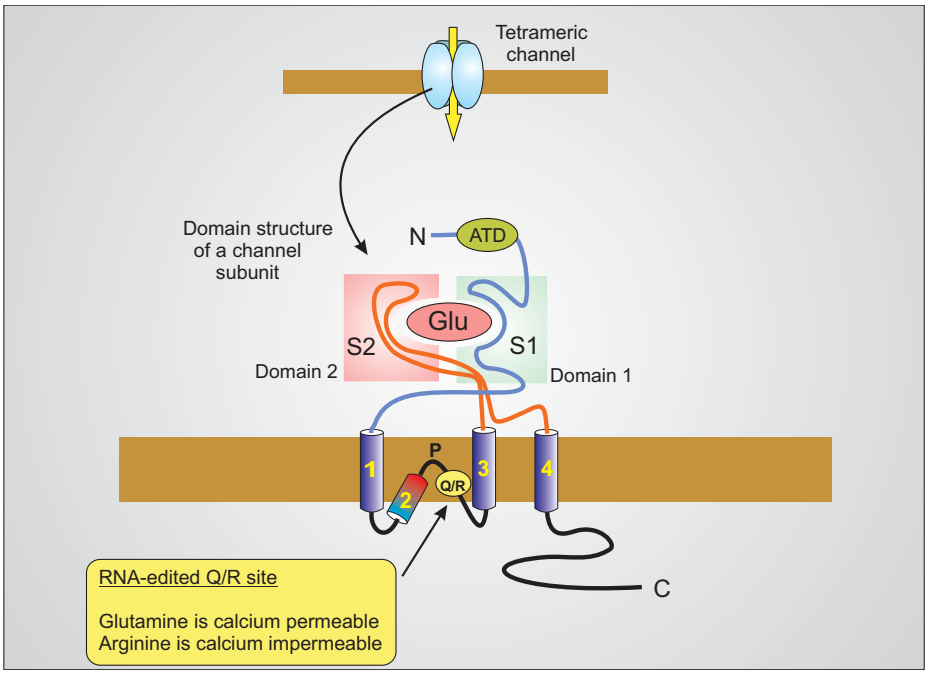
Module 3: | Figure ionotropic glutamate receptor



Structural organization of a typical glutamate receptor.
All three of the ionotropic glutamate receptor subtypes [α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), *N*-methyl-D-aspartate (NMDA) and kainate receptors] have the same structural organization. The functional receptor is a tetramer formed by four homologous subunits.

In addition to having different glutamate-binding sites, these receptors also have different gating properties tuned to their specific functions in neural signalling. The AMPA receptors and kainate receptors activate rapidly, but desensitize within milliseconds, and are thus ideally suited for the rapid transmission of information between neurons during information processing in the brain (Module 10: Figure kinetics of neurotransmission). By contrast, the NMDA receptor activates slowly, and also inactivates slowly and incompletely. They are ideally suited for a role in synaptic plasticity because they provide a prolonged influx of the Ca^{2+} during the sustained glutamate release that activates long-term potentiation (LTP).

All of the ionotropic glutamate receptors have a similar structural organization (Module 3: Figure ionotropic glutamate receptor). The channel is formed by the membrane-spanning domains 1, 3 and 4 together with the pore-forming (P) domain 2. The latter has a Q/R editing site that determines the Ca^{2+} permeability of the subunits. Those subunits that have a glutamine residue are permeable to Ca^{2+}, but if this is changed to an arginine residue, permeability to Ca^{2+} is lost. The glutamate-binding site is formed by an interaction between Domain 1 (blue) and Domain 2 (red). The former is formed by the S1 region of the N-terminus, whereas Domain 2 is formed by S2, which is a part of the long linker region between membrane-spanning domains 3 and 4. An N-terminal domain (ATD) is located at the N-terminus. The C-terminus, which is of variable length, extends into the cytoplasm. In the case of the AMPA receptor, this C-terminus has sites that are phosphorylated by protein kinase A (PKA) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) during long-term potentiation (Module 3: Figure AMPA receptor phosphorylation).

α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors

The α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor is one of the main glutamate receptors located in neuronal excitatory synapses. There are four subunits (GluR1–GluR4) that form the functional heterotrimeric channel that has different properties depending on the subunit combinations. Those AMPA receptors that lack a GluR2 subunit gate Na^+ and K^+, whereas those that have GluR2 gate Ca^{2+}.

One of the main functions of AMPA receptors is to respond to glutamate during synaptic transmission to produce rapid excitatory postsynaptic potentials (Module 10: Figure kinetics of neurotransmission). Astrocytes, which are part of the tripartite synapse, also have AMPA receptors that gate Ca^{2+} in response to the glutamate spilling out from the synaptic cleft (Module 7: Figure astrocyte tripartite synapse).

These subunits have long C-terminal cytoplasmic regions that interact with a variety of signalling and scaffolding components located in the postsynaptic density (PSD) (Module 10: Figure postsynaptic density). One of these is glutamate receptor-interacting protein (GRIP), which is

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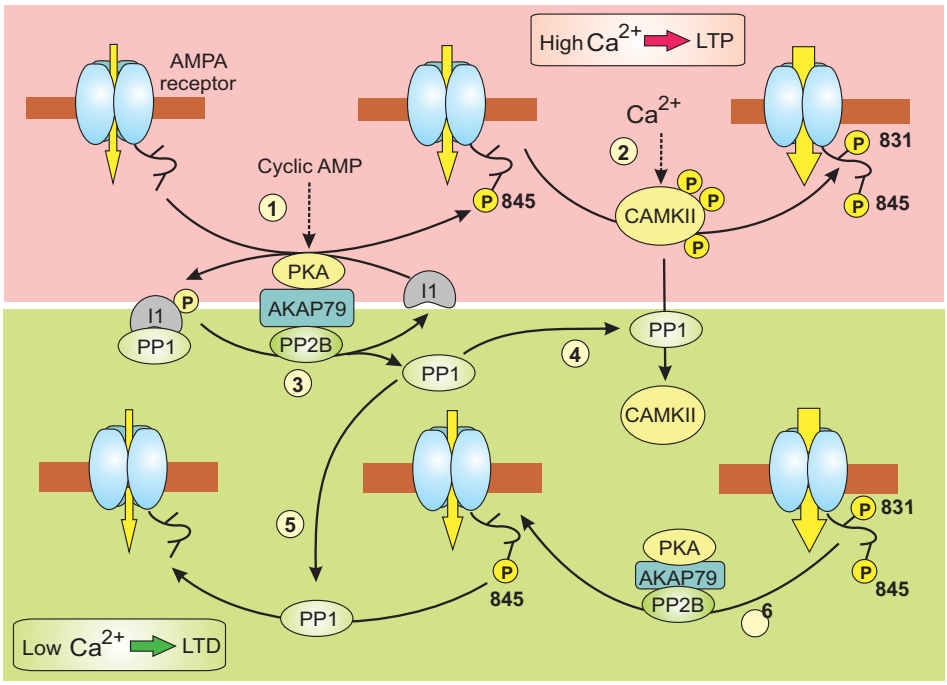
Module 3: | Figure AMPA receptor phosphorylation



Phosphorylation of the C-terminal cytoplasmic tail of the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor modulates channel function during synaptic plasticity.

The channel properties of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors are modified by a collection of kinases and phosphatases that control the phosphorylation status of two serine residues (Ser-831 and Ser-845) through a series of reactions that depend on both cyclic AMP and Ca^{2+} signalling. High levels of Ca^{2+} that induce long-term potentiation (LTP) promote phosphorylation (top panel), whereas lower levels of Ca^{2+} favour the dephosphorylation events that lead to long-term depression (LTD).

distributed throughout the neuron and may play an important role in the trafficking of AMPA receptors from their sites of synthesis in the soma to the peripheral synaptic regions. The GluR1 C-terminal region binds to the scaffolding protein synapse-associated protein 97 (SAP97), which is associated with A-kinase-anchoring protein of 79 kDa (AKAP79). The latter is associated with enzymes such as protein kinase A (PKA) and protein phosphatase 2A (PP2A), which play an important role in regulating the activity of AMPA receptors. AMPA receptors are also associated with Stargazin, which is an integral membrane protein that functions in the trafficking of AMPA receptors. Stargazin is linked to PSD-95, which is associated with the AKAP Yotiao that binds both PKA and PP1. Through these associations with the AKAPs, the AMPA receptor comes into close association with PKA and the phosphatases PP2A and PP1, which are responsible for changes in the phosphorylation of the AMPA receptor during receptor recycling and synaptic plasticity.

There is an important role for Ca^{2+} in regulating receptor phosphorylation as indicated in the steps shown in Module 3: Figure AMPA receptor phosphorylation:

1. High levels of Ca^{2+} activate CaM-stimulated adenylyl cyclases to increase the level of cyclic AMP, which stimulates PKA to phosphorylate Ser-845. In addition, PKA phosphorylates inhibitor 1 (I1), which assists the phosphorylation process by inactivating PP1.
2. High levels of Ca^{2+} also activate Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), which phosphorylates Ser-831 to increase the unitary conductance of the AMPA receptor. In addition, CaMKII also acts to increase the insertion of new AMPA receptors into the synapse (Module 10: Figure Ca^{2+}-induced synaptic plasticity).
3. At low levels of Ca^{2+}, calcineurin (PP2B) acts to dephosphorylate I1, which then activates PP1.
4. PP1 inactivates CaMKII.
5. PP1 dephosphorylates Ser-845.
6. PP2B dephosphorylates Ser-831.

Many of these proteins are associated with each other in the PSD (Module 10: Figure postsynaptic density).

The GluRA2 subunit is also phosphorylated on Tyr-876. Dephosphorylation of this group by a protein tyrosine phosphatase may enable it to interact with BRAG2, which is a GEF that activates Arf6 to facilitate the endocytosis of AMPA receptors during Ca^{2+}-induced synaptic plasticity (see step 4 in Module 10: Figure Ca^{2+}-induced synaptic plasticity)

N-methyl-d-aspartate (NMDA) receptors

The *N*-methyl-D-aspartate (NMDA) receptor is a typical receptor-operated channel (ROC) that responds to the neurotransmitter glutamate (Module 3: Figure NMDA receptor). It has an important function in neuronal coincident detection during the memory acquisition phase

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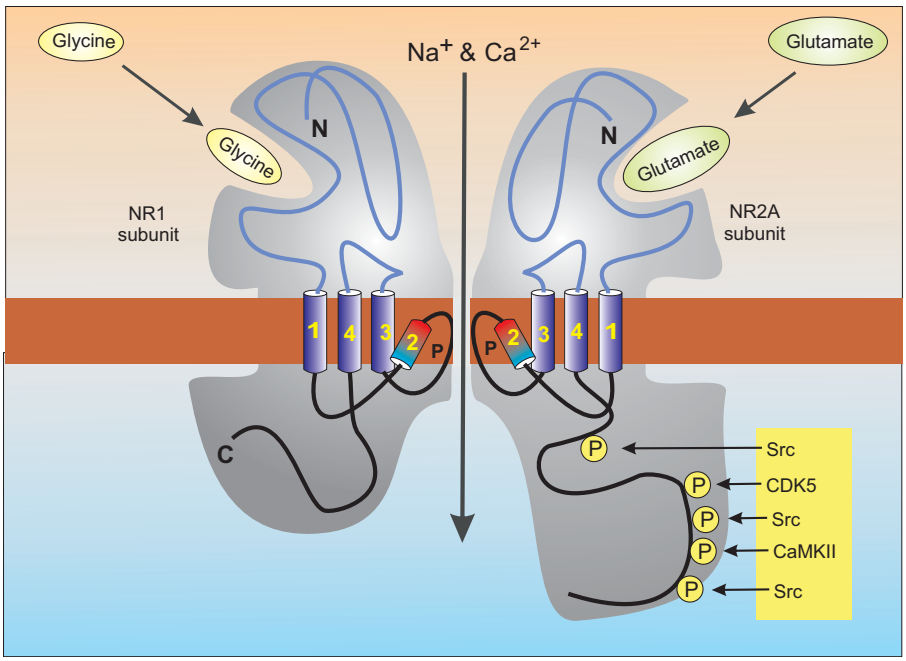
Module 3: | Figure NMDA receptor



Structural organization of the *N*-methyl-D-aspartate (NMDA) receptor.
The *N*-methyl-D-aspartate (NMDA) receptor is usually a tetramer composed of NR1 subunits and a variable number of NR2A-NR2D subunits. The organization is similar to that of other ionotropic glutamate receptors (see Module 3: Figure ionotropic glutamate receptor). The NR2 subunits have long C-terminal tails with a number of phosphorylation sites. As shown here for the NR2A, these sites are phosphorylated by a number of kinases that regulate the activity of the NMDA receptors.

of learning (Module 10: Figure coincidence detectors) because it gates the Ca^{2+} responsible for the changes in synaptic plasticity. Its role as a coincident detector depends on the fact that it requires two separate conditions before it will open. Firstly, the membrane must be depolarized, and this removes Mg^{2+} that normally blocks the channel at resting membrane potentials. Secondly, glutamate has to bind to the receptor to stimulate channel opening.

Like the other glutamate receptors, the channel is a heteromeric complex composed of a single NMDA receptor subunit 1 (NR1), which is essential for channel function, and four NR2 subunits (NR2A–NR2D) (Module 3: Figure NMDA receptor).

The NR2a subunit acts as the agonist binding site for glutamate.

The NR1 subunit is encoded by the *GRIN1* gene, whereas the NR2A-NR2D subunits are encoded by the *GRIN2A*, *GRIN2B*, *GRIN2C* and *GRIN2D*. This receptor is the predominant excitatory neurotransmitter receptor in the mammalian brain.

Channel opening depends on both glutamate and glycine. Glycine binds to the NR1 subunit, whereas glutamate interacts with the NR2 subunit. The latter also determines both the Mg^{2+} dependency and the properties of the channel. For example, the receptors that have the NR2B subunit remain open for longer than those with NR2A. What this means with regard to the memory acquisition phase is that the NR2B-containing receptors have a longer phase of activation during which they can respond to the other signal of depolarization, thereby increasing the window during which neuronal coincident detection can occur. Mice that have an increased expression of the NR2B subunit have an enhanced ability to learn, thus raising the possibility of a genetic enhancement of learning and memory.

The activity of the NMDA receptor is regulated by protein phosphorylation. It is associated with various protein kinases [protein kinase A (PKA) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII)] and protein phosphatases [protein phosphatase 1 (PP1)]. PKA and PP1 are associated with the channel through the scaffolding protein Yotiao [a splice variant of A-kinase-anchoring protein of 350 kDa (AKAP350)] that binds to the cytoplasmic region of the NR1 subunit (Module 10: Figure postsynaptic density). The mutated form of the huntingtin protein, which causes Huntington's disease, appears to alter the interaction between Yotiao and the NR1A/NR2B channel complex.

There is a strong correlation between NMDA receptor hypofunction and schizophrenia.

The *GRIN2B* gene has been implicated in autism spectrum disorders (ASDs).

Kainate receptors

Kainate receptors are one of the subtypes of the ionotropic glutamate receptor family (Module 3: Table receptor-operated channel toolkit). They are distinguished from other members of the family by being sensitive to kainic acid. They have a distinct set of subunits (GluR5–GluR7 and KA1–KA2). These subunits have the same tetrameric organization as the other glutamate receptors (Module 3: Figure ionotropic glutamate receptor). Some of the subunits (GluR5, GluR6 and KA2) have the Q/R editing site

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in the P loop that determines their single channel conductance and permeability to Ca^{2+}. The five subunits can be assembled in different combinations to give a wide range of channels with different properties.

Kainate receptors are widely distributed throughout the brain (cortex, striatum, hippocampus and cerebellum). There have been many studies on the hippocampus where kainate receptors have both pre- and post-synaptic effects. For example, at the Schaffer collaterals, it acts presynaptically to reduce transmitter release. Conversely, it has a postsynaptic function at the mossy fibre synapse on to the CA3 neurons. Kainate receptors have been implicated in epilepsy.

P2X receptors

The ionotropic P2X receptors are channels that respond to extracellular ATP to induce membrane depolarization and an influx of Ca^{2+}. They are found on many cell types where they can mediate rapid responses, such as fast excitatory synaptic transmission between neurons, to much slower responses such as the regulation of cell growth and blood coagulation. There are seven P2X subunits that all have the same basic topology of two transmembrane regions connected by a large extracellular loop with the N- and C-terminal regions extending into the cytoplasm (Module 3: Figure P2X receptor structure). Functional channels are made from homo- or hetero-trimers, which is consistent with the fact that channel opening depends on the binding of three glycine molecules. The subunit composition of the channels determines their biophysical properties, and this is particularly evident with regard to their rates of inactivation. For example, the P2X3 channels inactivate, whereas the P2X7 channels, which have been implicated in growth control, remain active for long periods.

ATP released from glomus cells in the carotid body act on P2X2 receptors to excite the afferent nerve endings to send information back to the brain during O_2 sensing (Module 10: Figure carotid body chemoreception). P2X1 receptors provide an influx of external Ca^{2+} that contributes to blood platelet activation (Module 11: Figure platelet activation) and stimulation of the vas deferens (Module 7: Figure vas deferens activation). The P2X7 receptor provide an influx of external Ca^{2+} that contributes to macrophage activation (Module 11: Figure macrophage signalling).

Second messenger-operated channels (SMOCs)

There are a number of second messenger-operated channels (SMOCs) that are controlled by messengers coming from inside the cell (Module 3: Figure Ca^{2+} entry mechanisms). Important examples of these are the cyclic nucleotide-gated channels (CNGCs), the arachidonic acid-regulated Ca^{2+} (ARC) channel and the diacylglycerol (DAG)-sensitive transient receptor potential canoncical 6 (TRPC6) channel.

Cyclic nucleotide-gated channels (CNGCs)

Cyclic nucleotide-gated channels (CNGCs), which conduct Ca^{2+} and, to a lesser extent, Na^+, play an important role in sensory transduction of visual, olfactory and gustatory signals. However, the CNGCs are expressed in other cell types within the brain, testis and kidney. There are six mammalian CNGC genes, which fall into two subgroups: CNGA and CNGB (Module 3: Figure cyclic nucleotide-gated channels). The CNGA1–CNGA3 subunits form channels, whereas the CNGA4 and two CNGB subunits have a more modulatory function. The N-terminal region of the modulatory subunits has an IQ-type calmodulin (CaM)-binding site, which is able to bind apocalmodulin (i.e. CaM that is not bound to Ca^{2+}). Since the CaM is already in place, Ca^{2+} can act very quickly as part of a negative-feedback loop to inactivate the channels.

The function channel is a heterotetramer containing different combinations of the A and B subunits, as illustrated in Module 3: Figure cyclic nucleotide-gated channels. These CNGCs are particularly important in the function of a number of sensory cells:

- In retinal rods, the channel is composed of a heterotetramer containing three CNGA1 subunits and one CNGB1 subunit. The channel responds to cyclic GMP to maintain the dark current (Module 10: Figure phototransduction overview).
- In olfactory cilia, the channel has two CNGA2 subunits, one CNGA4 subunit and one CNGB1b subunit. The channel opens in response to cyclic AMP formed during transduction of olfactory stimuli (Module 10: Figure olfaction).

Patients with retinitis pigmentosa carry mutations in CNGA1 and CNGB1. Achromatopsia (colour blindness) is caused by loss of CNGA3 and CNGB3.

Arachidonic acid-regulated Ca^{2+} (ARC) channel

The arachidonic acid-regulated Ca^{2+} (ARC) channel is a highly selective Ca^{2+} channel. These ARC channels have been identified in both parotid and pancreatic acinar cells. The suggestion is that these channels are activated by the arachidonic acid (AA) released by the agonist-dependent activation of phospholipase A_2 (PLA_2). The sensitivity of ARC to arachidonic acid depends on its phosphorylation by protein kinase A (PKA). Conversely, the channel is inactivated when this phosphate group is removed by calcineurin (CaN). Both PKA and CaN may associate with the channel through an A-kinase-anchoring protein (AKAP).

Store-operated channels (SOCs)

Much of the Ca^{2+} used for signalling is released from the store held within the endoplasmic reticulum (ER). Because the latter has a finite capacity, its signalling function depends on mechanisms to ensure that the store remains topped up. Much of this ER Ca^{2+} homoeostasis depends upon the sarco/endo-plasmic reticulum Ca^{2+}-ATPase (SERCA) pumps that recycle the released Ca^{2+}. However, there are always some losses to the outside, resulting in store depletion, which will not only result in a decline in signalling capacity, but also trigger the endoplasmic reticulum (ER) stress signalling pathways. To

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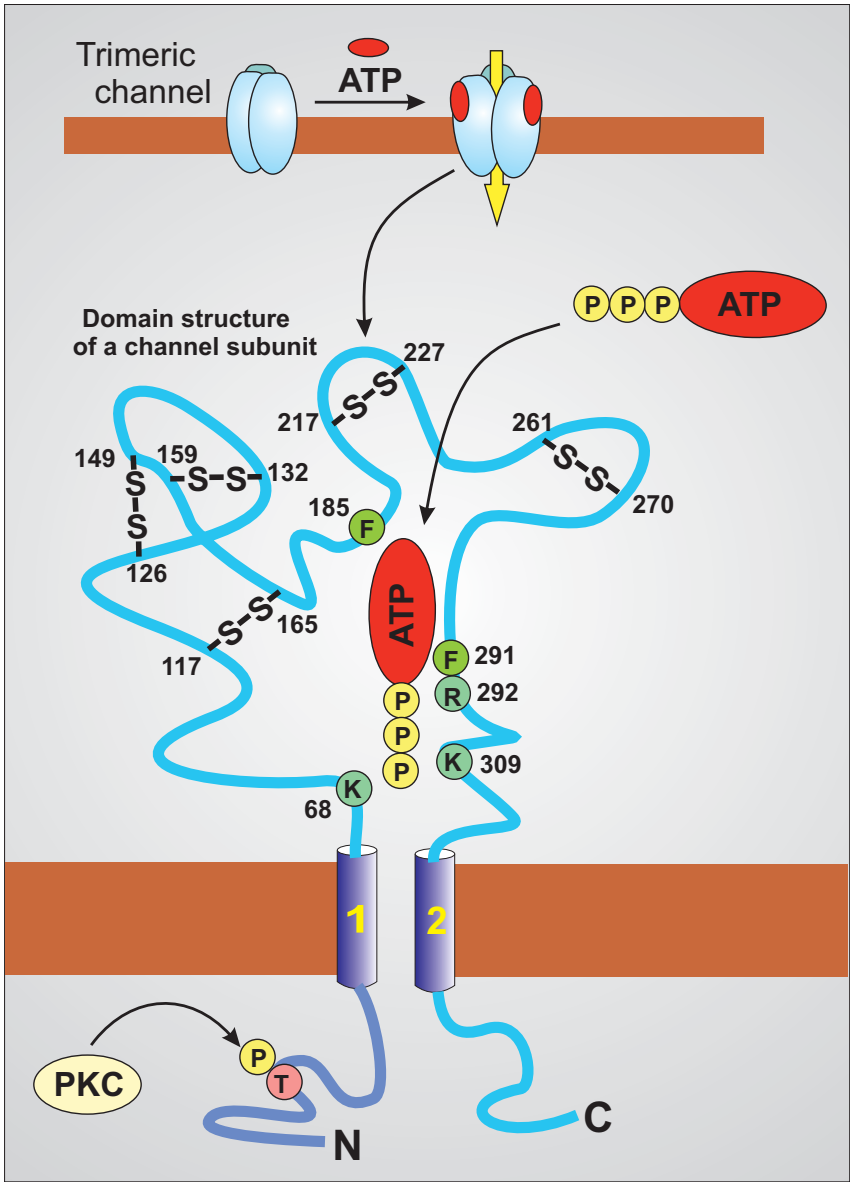
Module 3: | Figure P2X receptor structure



Structural organization of the human P2X1 receptor.
The P2X receptor has two transmembrane domains with the N- and C-terminal regions extending into the cytoplasm. There is an extensive extracellular loop that contains the ATP-binding site that has both hydrophobic residues (Phe-185 and Phe-291) that bind to the adenine ring and charged residues (Lys-68, Arg-292 and Lys-309) that interact with the phosphates. The structural integrity of the extracellular domain is maintained by a number of disulphide bonds. The functional P2X channel is a trimeric structure. The N-terminal region contains a threonine that is phosphorylated by protein kinase C (PKC), which may enhance channel activity by reducing the rate of desensitization. This regulation through PKC may play an important role in nociception (Module 10: Figure nociception).

guard against the deleterious effects of Ca^{2+} store depletion, the cell employs store-operated channels (SOCs) that open in response to store emptying to ensure a constancy of its internal Ca^{2+} store. This entry mechanism has also been referred to as capacitative Ca^{2+} entry (CCE) to draw attention to the fact that it functions in an analogous way to an electrical capacitor. When the store is full, there is little entry of Ca^{2+} through the SOCs, but as soon as the store content of Ca^{2+} declines, entry begins (Module 3: Figure capacitative Ca^{2+} entry). One of the main functions of this entry mechanism is therefore to maintain the internal store of Ca^{2+}. However, it can also function as a source of signal Ca^{2+}, especially under conditions where Ca^{2+} signalling has to be maintained over a prolonged period, as occurs during the stimulation of cell proliferation. Despite the importance of this entry pathway, the mechanism of store-operated channel (SOC) activation is still not known. The way in which the empty ER store signals to the channels in the plasma membrane is still a matter of considerable debate.

Electrophysiological studies have revealed that there may be different types of SOCs that vary in their Ca^{2+} selectivity. The classical example of a SOC is the Ca^{2+} release-activated Ca^{2+} (CRAC) channel found in

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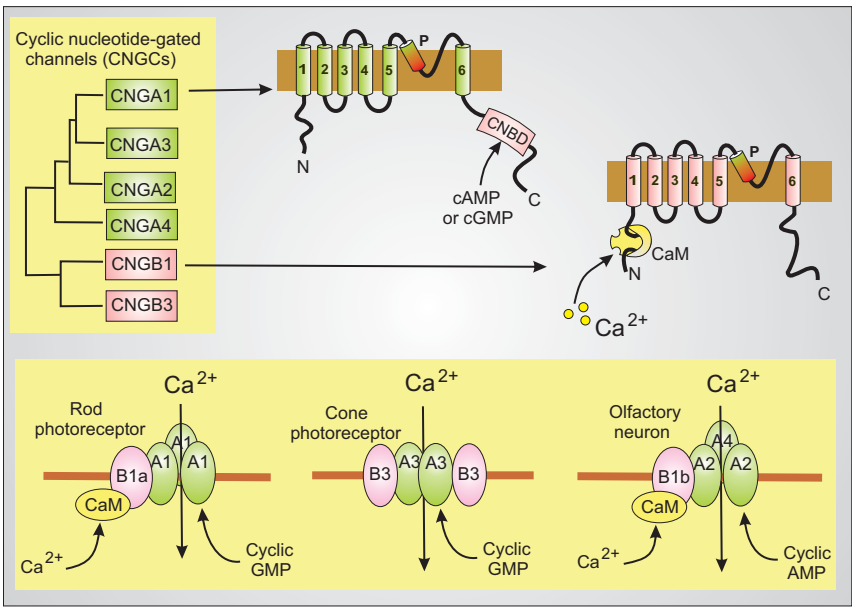
Module 3: | Figure cyclic nucleotide-gated channels



The cyclic nucleotide-gated channel (CNGC) family.
There are six members of the cyclic nucleotide-gated channel (CNGC) family: four α subunits, which form the pores, and two β subunits that have more of a modulatory role. They all have six transmembrane (TM) domains with both the N- and C-termini facing the cytoplasm. A pore region lies between TM5 and TM6. The examples shown at the bottom illustrate how the functional channels found in different sensory cells are heterotetramers made up of different subunit combinations. The C-terminal domain contains the cyclic nucleotide-binding domain (CNBD), whereas the N-terminal region of the CNGB1 subunit contains a binding site for calmodulin (CaM).

lymphocytes, which has a very high selectivity for Ca^{2+} and a very low conductance. The Orai1 protein has been identified as this CRAC channel, whereas some of the other SOCs appear to be members of the transient receptor potential (TRP) ion channel family.

An increase in the activity of SOCs may contribute to hypertension.

Ca^{2+} release-activated Ca^{2+} (CRAC) channel

The Ca^{2+} release-activated Ca^{2+} (CRAC) channel that is expressed in T lymphocytes (Module 9: Figure T cell Ca^{2+} signalling) is a classical store-operated channel (SOC) that is opened by store depletion. Like other SOCs, the CRAC current (I_{CRAC}) can be activated by depleting the internal store of Ca^{2+} by inhibiting the sarco/endo-plasmic reticulum Ca^{2+}-ATPase (SERCA) pumps with thapsigargin. The striking properties of these CRAC channels is their high selectivity for Ca^{2+} over Na^{+} (1000 to 1) and their small unitary conductance (10–20 fS), which is below the sensitivity of single channel current measurements.

The I_{CRAC} current in T cells is carried by Orai1 channels. T cells expressing an Arg-91 to tryptophan mutation of Orai1 are unable to sustain Ca^{2+} signalling and this causes severe combined immune deficiency (SCID). Patients with SCID have a defect in the Ca^{2+} entry mechanism that results in severely reduced Ca^{2+} signals, as shown in Module 3: Figure Ca^{2+} signalling in SCID:

(a) Activation of the T cell receptor occurred through anti-CD3 followed by cross-linking with anti-IgG (g-α-m).

(b) The Ca^{2+} ionophore ionomycin (iono) activated store-operated entry by releasing Ca^{2+} from the internal store.

(c) Thapsigargin (TG), which blocks the SERCA pump on the internal stores, caused a small elevation in all of the cells; upon re-addition of Ca^{2+}, there was a large increase in all the control cells, but no change in cells from the SCID patient.

(d) This is a repeat of (c), except that valinomycin was added to ensure that the membrane was hyperpolarized.

(e) Cells treated as in (c) were imaged at different points during the experiment: immediately before addition of TG (left panels), 100 s after addition of TG [centre panels; note that both the control cells and the patient's (Pat) cells showed a small elevation of Ca^{2+}], and 5 min after the re-addition of Ca^{2+} (right panels; most of the control cells displayed strong Ca^{2+} signals, whereas there were no responses in the Pat cells).

Orai

The gene responsible for severe combined immune deficiency (SCID) syndrome has been cloned and has been called Orai1 based on a word from Greek mythology. Orai are the 'keepers of the gates of heaven'. The Oria1 channel is equivalent to the Ca^{2+} release-activated Ca^{2+} (CRAC) channel that drives the T cell receptor (TCR) Ca^{2+} signalling process responsible for activating T cell proliferation (Module 9: Figure T cell Ca^{2+} signalling).There are three Orai genes coding for Orai1, Orai2 and Orai3. Most information is available for Orai1 which is a component

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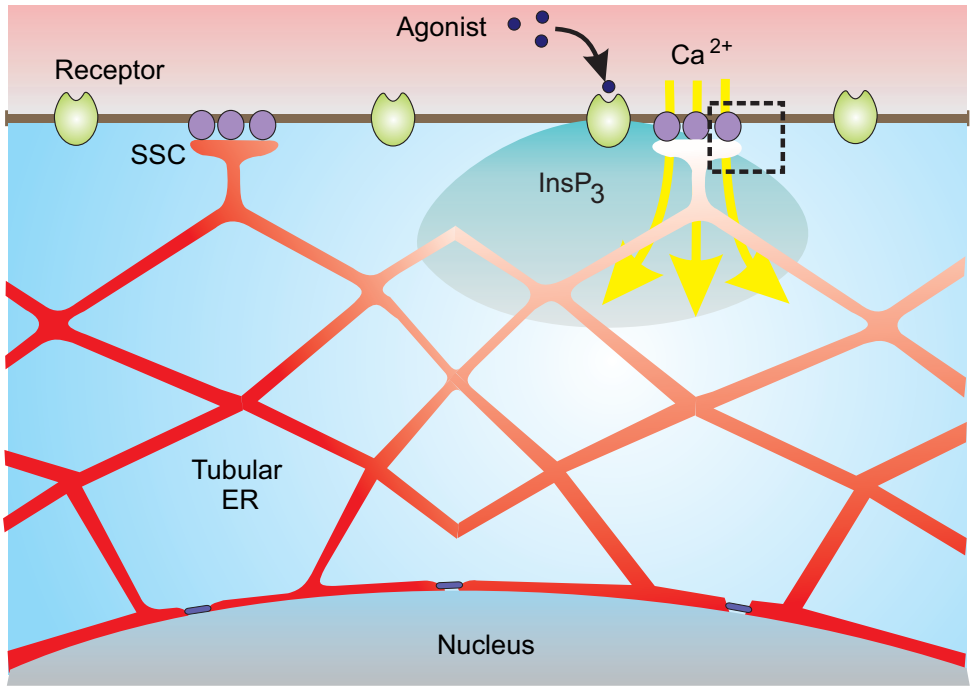
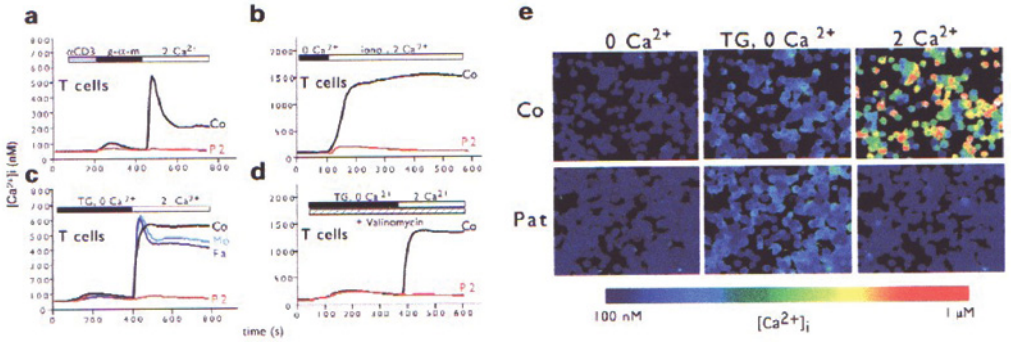
Module 3: | Figure capacitative Ca^{2+} entry



Capacitative Ca^{2+} entry activated by depletion of Ca^{2+} in localized regions of the endoplasmic reticulum (ER) close to the plasma membrane.
The hypothesis outlined in this figure suggests that capacitative Ca^{2+} entry occurs at localized regions where there is close apposition of the endoplasmic reticulum (ER) and plasma membrane. The ER shown in red is a network of tubules connected to the nuclear membrane. Short arms of this ER network come into contact with the plasma membrane to form subsurface cisternae (SSC). The narrow gap between the SSC and the plasma membrane is the site where the signal from the empty ER is transferred to the store-operated channels (SOCs). Agonists acting on cell-surface receptors (green) in the plasma membrane stimulate phospholipase C (PLC) to produce a microdomain of inositol 1,4,5-trisphosphate ($InsP_3$) (mauve), which functions to deplete Ca^{2+} in localized SSC. The latter then send a message to the SOCs in the plasma membrane to promote entry. This signal might occur through a protein-protein interaction, as outlined in the conformational coupling hypothesis. The box outlines the region where this mechanism might be located, as illustrated in Module 3: Figure conformational coupling hypothesis.

Module 3: | Figure Ca^{2+} signalling in SCID



Defective Ca^{2+} signalling in the T cells of a patient suffering from severe combined immune deficiency (SCID).
The Ca^{2+} shown in panels a and b represent the responses of T cell populations taken from a patient (P2), normal control T cells (Co) or T cells from the patient's mother (Mo) or father (Fa). The different treatments identified store-operated channels (SOCs) as the likely site of the defect in Ca^{2+} signalling. The cells were treated in Ca^{2+}-free medium to deplete the stores and the degree of store-operated entry was then tested by re-adding Ca^{2+}. For all the treatments, the entry of Ca^{2+} was almost completely absent from the cells from the SCID patient (red traces). Reproduced by permission from Macmillan Publishers Ltd: *Nat. Immunol.*, Feske, S., Giltnane, J., Dolmetsch, R., Staudt, L.M. and Rao, A. (2001) Gene regulation mediated by calcium signals in T lymphocytes. Vol. 2, pages 316-324. Copyright (2001); http://www.nature.com/ni; see Feske et al. 2001.

of the mechanism of store-operated channel (SOC) activation. It contains four transmembrane segments with the N- and C-terminal regions facing the cytoplasm (Module 3: Figure SOC signalling components). The C-terminal region contains the Arg-91to tryptophan mutation responsible for SCID. A coiled-coil region located towards the end of the C-terminal region may play a role in the putative conformational-coupling processes that operates during the mechanism of store-operated channel (SOC) activation (Module 3: Figure STIM-induced Ca^{2+} entry).

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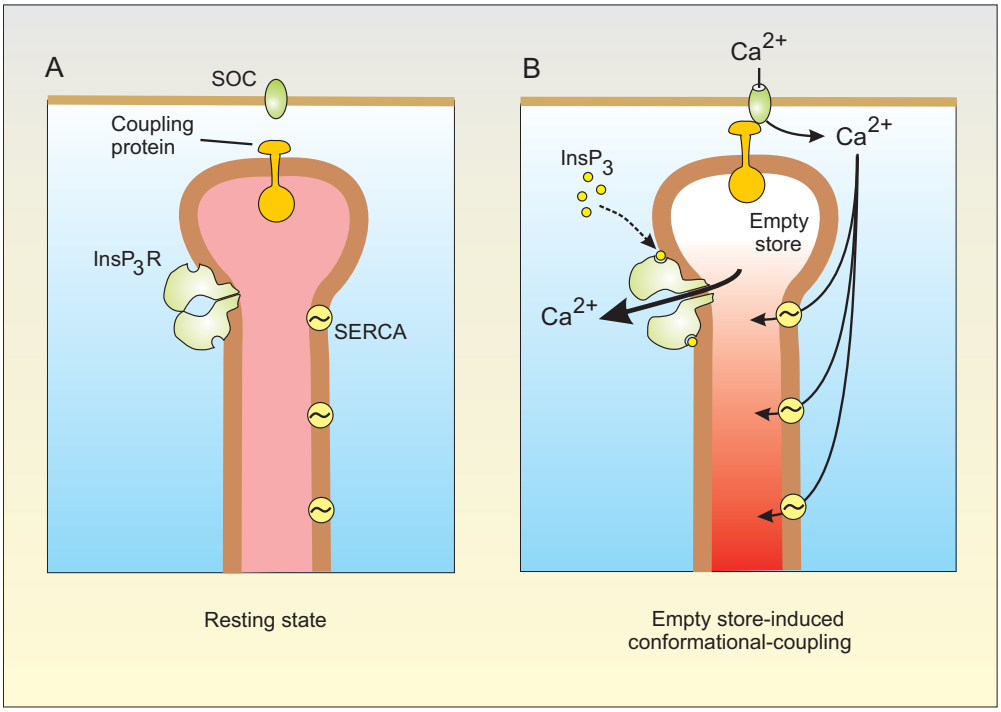
Module 3: | Figure conformational coupling hypothesis



A conformational coupling hypothesis of capacitative Ca^{2+} entry.
The conformational coupling hypothesis proposes that there is an interaction between the store-operated channel (SOC) in the plasma membrane and some coupling protein that detects Ca^{2+} depletion of the endoplasmic reticulum (ER). A. In the resting state when the ER is full, there is no entry. B. When receptors are activated to produce $InsP_3$, this activates $InsP_3Rs$ to induce a local depletion of ER, which then activates the coupling protein to induce a conformational change that is responsible for switching on entry through the SOC channels. For further details of some of the proposed coupling mechanisms, see Module 3: Figure STIM-induced Ca^{2+} entry.

Orai1 has a specific role in activating Ca^{2+}-sensitive adenylyl cyclases such as AC8. The latter is located close to the site of Ca^{2+} entry by being bound directly to Orai1.

Mechanism of store-operated channel (SOC) activation

The nature of the signal that emanates from the endoplasmic reticulum (ER) to activate store-operated channels (SOCs) is still a matter of some debate. One of the important questions to consider is whether or not the whole of the ER is involved in SOC activation. This seems unlikely, because depletion of the ER Ca^{2+} can trigger stress signalling pathways by seriously interfering with the processes of protein synthesis and packaging. There is growing evidence that a small specialized region of the ER closely associated with the plasma membrane regulates the entry of Ca^{2+} (Module 3: Figure capacitative Ca^{2+} entry). The major unsolved problem concerns the mechanism whereby the empty store sends a message to open the SOC in the plasma membrane.

Some of the proposed mechanisms include the generation of a Ca^{2+} influx factor (CIF), exocytosis of vesicles containing SOCs, or a conformational coupling mechanism whereby a protein in the ER interact directly with the SOC in the plasma membrane (Module 3: Figure conformational coupling hypothesis). The latter mechanism resembles that found in skeletal muscle, where L-type voltage-operated channels (VOCs) in the plasma membrane interact directly with the ryanodine receptor type 1 (RYR1) channels in the sarcoplasmic reticulum (Mechanism 4 in Module 2: Figure Ca^{2+} modules). A similar conformational coupling mechanism has been proposed to explain how empty ER stores can stimulate entry through SOCs in the plasma membrane (Module 3: Figure conformational coupling hypothesis). In this latter case, information is flowing in the opposite direction, i.e. the signal travels from the ER to the plasma membrane. When the store is emptied, following the activation of the inositol 1,4,5-trisphosphate ($InsP_3$) receptors ($InsP_3Rs$), the coupling protein is activated to stimulate entry through the SOC channels. This Ca^{2+} can be used for signalling and it is pumped into the ER by the sarco/endo-plasmic reticulum Ca^{2+}-ATPase (SERCA) pumps to replenish the ER store.

Some of the main molecular components of this putative conformational coupling mechanism have been identified (Module 3: Figure SOC signalling components). The classical example of a SOC is the Ca^{2+} release-activated Ca^{2+} (CRAC) channel, which has been identified as the Orai1 protein. There also are indications that there may be a number of other SOCs coded for by various members of the transient receptor potential (TRP) ion channel family such as TRP canonical 1 (TRPC1), 4 (TRPC4) and TRP vanilloid 6 (TRPV6).

The coupling protein in the ER that senses store emptying and then relays information to the SOCs, has been identified as the stromal interaction molecule 1 (STIM1),

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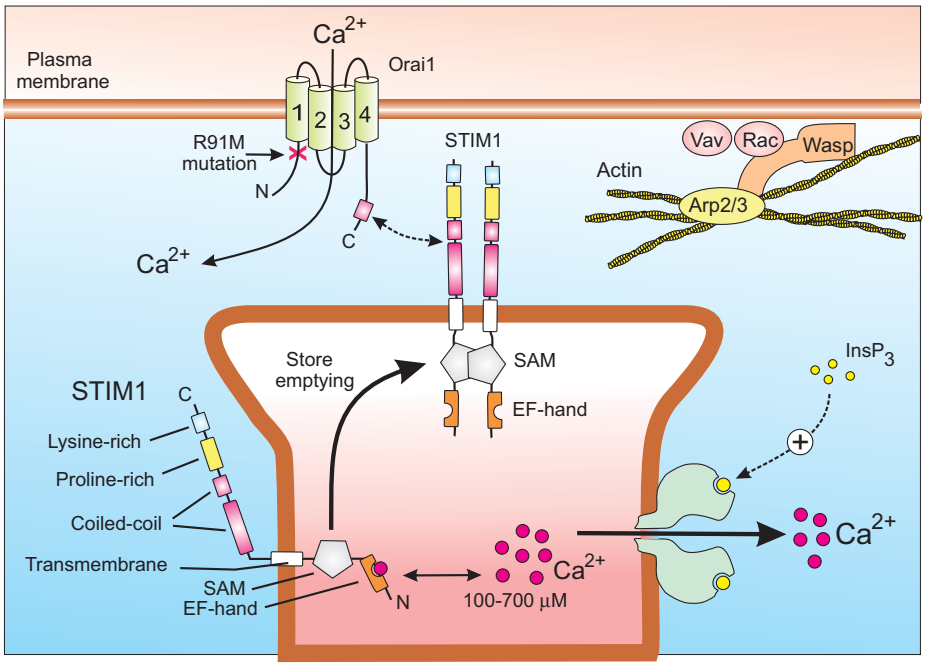
Module 3: | Figure SOC signalling components



Molecular components of store-operated (SOC) Ca^{2+} entry.
The best characterized store-operated entry channel is Orai1, which has four transmembrane regions with the N- and C-termini facing the membrane where they participate in the conformation-coupling process. The STIM1 protein located in the endoplasmic reticulum (ER) has an EF-hand domain that monitors the level of Ca^{2+} within the ER lumen. The cytoplasmic domain of STIM1 has a variety of domains that may function in conformational coupling. In response to an increase in inositol 1,4,5-trisphosphate ($InsP_3$) the store empties and STIM1 migrates towards the ER regions facing the plasma membrane where it initiates Ca^{2+} entry. Some of the proposed coupling mechanisms are described in Module 3: Figure STIM-induced Ca^{2+} entry.

which is located within the ER and has a single EF-hand that might function to sense Ca^{2+} (Module 3: Figure SOC signalling components). If STIM is knocked out, there is a dramatic decline in store-operated entry, indicating that this protein plays a direct role in the coupling mechanism. Under resting conditions when the store is full of Ca^{2+} (100–700 μM), STIM1 appears to be distributed throughout the ER. Upon store emptying, Ca^{2+} is no longer bound to the EF-hand of STIM, which then moves to the surface to aggregate at sites of close contact between the ER and the plasma membrane and is thus ideally situated to act as a sensor for conformational coupling (Module 3: Figure STIM-induced Ca^{2+} entry).

Two ways have been proposed for how STIM in the ER induces opening of the Oria1 channel in the plasma membrane. One mechanism proposes that information is passed through a direct interaction between the cytoplasmic domain of STIM and the cytoplasmic regions of Orai1. However both STIM1 and Oria1 are relatively small molecules and it seems that their cytosolic regions may not be large enough to span the gap (15–20 nM) between the ER and the plasma membrane. Another proposal is that there is an accessory protein that functions as an intermediary in the interaction between Orai1 and STIM1 (Module 3: Figure STIM-induced Ca^{2+} entry). This accessory protein remains to be identified, but there are suggestions that either the inositol 1,4,5-trisphosphate ($InsP_3$) receptor ($InsP_3R$) or the ryanodine receptor (RYR) may function as such a coupling protein. Some of the most direct evidence for conformational coupling has come from studies showing a functional interaction between TRPC3 and $InsP_3R1$. In excised patches, Ca^{2+} entry through the TRPC3 channels was lost, but could be restored by the direct addition of $InsP_3R1$. However, there are arguments against a role for the $InsP_3Rs$ functioning in conformational coupling because DT40 cells that lack all three $InsP_3R$ isoforms still reveal SOC-mediated entry. Such observations may suggest a possible role for the RYRs, which have been shown to couple to TRP channels. The DT40 cells that lack $InsP_3Rs$ were found to express both the RYR1 and RYR3. Such an involvement of the RYRs would mean that this release channel might play a pivotal role in both the release of internal Ca^{2+} (as occurs in skeletal muscle) and in the entry of external Ca^{2+}. Indeed, there is some evidence to show that both mechanisms may coexist in skeletal muscle, where the SOCs may function in long-term Ca^{2+} homoeostasis to overcome fatigue during intensive exercise. The SOC may couple to the RYR1 through mitsugumin 29 (MG29), which is a synaptophysin-family-related protein positioned within the junctional space between the plasma membrane and the sarcoplasmic reticulum (SR).

The conformational coupling hypothesis depends upon the ER making close contact with the plasma membrane so that information can be relayed between proteins located in the two apposed membranes. Many of the components that function in store-operated entry are associated with the caveolae (Module 6: Figure caveolae organization), which have been suggested as a possible site for this

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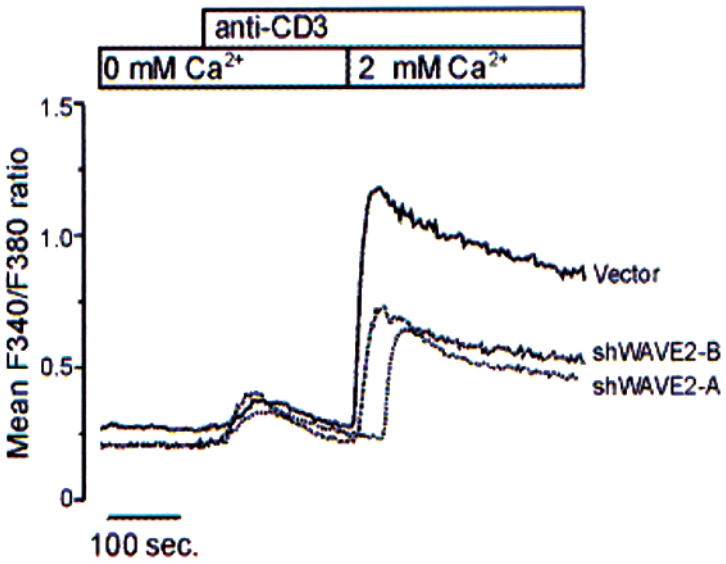
Module 3: | Figure WAVE2 effects on Ca^{2+} entry



Regulation of Ca^{2+} entry in Jurkat T cells.

Jurkat T cells were transfected with vector or with two shWAVE2 vectors. After incubation for 72 h, cells were treated first with anti-CD3 in a Ca^{2+}-free medium. All three groups gave a similar transient, indicating that the release of internal Ca^{2+} was normal. However, on addition of 2 mM Ca^{2+}, the two cells where Wiskott-Aldrich syndrome protein (WASP) verprolin homologous 2 (WAVE2) had been suppressed showed much reduced Ca^{2+} entry responses. Reproduced from Nolz, J.C., Gomez, T.S., Zhu, P., Li, S., Medeiros, R.B., Shimizu, Y., Burkhardt, J.K., Freedman, B.D. and Billadeau, D.D. (2006) The WAVE2 complex regulates actin cytoskeletal reorganization and CRAC-mediated calcium entry during T cell activation. Curr. Biol. 16:24-34. Copyright (2006), with permission from Elsevier; see Nolz et al. 2006.

entry process. There are numerous examples of the ER making intimate contact with the caveolae, as described for smooth muscle (Module 6: Figure smooth muscle caveolae).

Another important structural component for entry are various cytoskeletal components such as actin and the Septins, which may function to form and/or maintain the structural integrity of this complex (Module 3: Figure STIM-induced Ca^{2+} entry).

Recent studies on proteins that function in actin remodelling, such as Wiskott-Aldrich syndrome protein (WASP) verprolin homologous (WAVE), can profoundly influence store-operated Ca^{2+} entry (Module 3: Figure WAVE2 effects on Ca^{2+} entry). In the case of T cells, activation of the T cell receptor (TCR) relays information out to a number of signalling pathways. One of these is the activation of Vav, which seems to act through Rac and WAVE2 to remodel the actin cytoskeleton, which seems to have a role in regulating the entry of Ca^{2+} through the CRAC channel (Module 3: Figure STIM-induced Ca^{2+} entry).

Actin polymerization controlled by the monomeric G protein Rho has also been implicated in store-operated Ca^{2+} entry in endothelial cells (Module 7: Figure endothelial cell contraction).

The Septins also play a major role in facilitating the formation and stabilization of the STIM1/Orai 1 interaction responsible for store-operated Ca^{2+} entry.

Stromal interaction molecule (STIM)

Stromal interaction molecule (STIM) is a type I transmembrane protein that is located in the endoplasmic reticulum (ER) (Module 3: Figure SOC signalling components). The N-terminal region, which is located within the lumen of the ER, has a SAM domain and an unpaired Ca^{2+}-binding EF-hand motif. When Ca^{2+} is bound to this EF-hand, STIM1 is inactive. Upon store emptying, Ca^{2+} leaves the EF-hand and STIM1 undergoes a conformational change enabling it to migrate to the ER-plasma membrane contact sites. The individual STIM molecules interact with each other, perhaps through their SAM domains, to form complexes that then activate Orai1 channels to induce Ca^{2+} entry. Further evidence supporting a role for STIM as the Ca^{2+} sensor controlling entry has come from mutating the EF-hand so that it no longer binds Ca^{2+}. The mutated protein turns out to be constitutively active in that it induces entry without store emptying, indicating that it has assumed a conformational change resembling that which occurs normally during store emptying.The cytoplasmic C-terminal region has two coiled-coil (CC) domains that enables STIM to oligomerize and also may function in the interaction with the C-terminal CC domains in Orai1. There also is a proline-rich region and a lysine-rich region. STIM has attracted considerable attention as a putative sensor of store emptying as described in the mechanism of store-operated channel (SOC) activation (Module 3: Figure STIM-induced Ca^{2+} entry).

Transient receptor potential (TRP) ion channel family

The mammalian transient receptor potential (TRP) ion channel family takes its name from the *Drosophila* TRP channel located within the rhabdomeres of the

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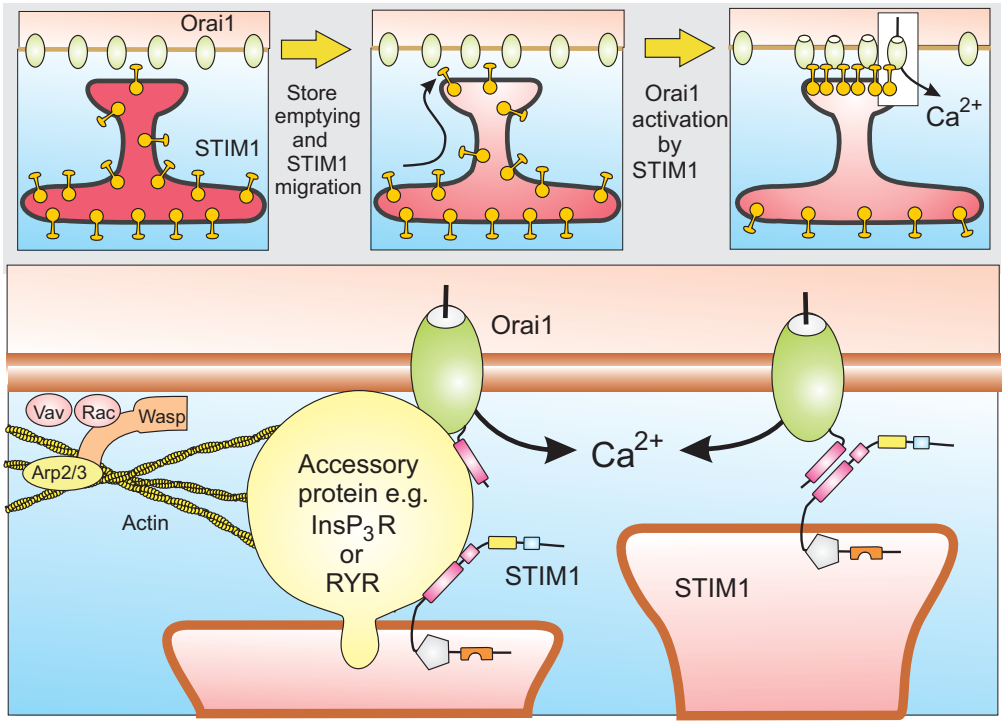
Module 3: | Figure STIM-induced Ca^{2+} entry



Proposed function of stromal interaction molecule (STIM) in store-operated Ca^{2+} entry in T cells.

The top panels illustrate how the entry of Ca^{2+} depends upon activation of a store-operated channel such as Orai1. When the store empties, the stromal interaction molecule 1 (STIM1) migrates towards regions of the endoplasmic reticulum where it functions to open the Orai1 channels (white box). The models at the bottom show two possible mechanisms for the conformational coupling mechanism. It may occur through a direct interaction between Orai1 and STIM1, perhaps involving the coiled-coil domains (red boxes). Since these proteins are relatively small, such an interaction would require close apposition of the two membranes. Alternatively, STIM1 may exert its effects through an accessory protein such as the inositol 1,4,5-trisphosphate receptor ($InsP_3R$) or through a ryanodine receptor (RYR). Formation of such coupling complexes appear to depend upon cytoskeletal remodelling driven by Wiskott-Aldrich syndrome protein (WASP) verprolin homologous 2 (WAVE2) and controlled through the monomeric G proteins Vav and Rac.

photoreceptor. In response to light, the process of visual transduction depends upon rhodopsin activating phospholipase C (PLC) to hydrolyse PtdIns4,5P_2, which then acts through mechanisms that remain to be described to open the Ca^{2+}-permeable TRP channels responsible for the depolarization of the photoreceptors during the light response. A search for TRP homologues in other species has revealed a surprising number of related channels. For example, the *Caenorhabditis elegans* genome contains 13 genes homologous with the *Drosophila* TRP. Likewise, the mammalian TRP ion channel family, which is considered in more detail below, contains up to 23 different genes. Despite the large number of members, the structural organization of the transient receptor potential (TRP) ion channel family is remarkably similar. On the basis of their homologies, the large family of mammalian TRPs has been divided into six subfamilies (Module 3: Figure TRP channel phylogeny). This phylogenetic tree illustrates the relationships between the major canonical transient receptor potential (TRPC) family, the vanilloid transient receptor potential (TRPV) family and the melastatin-related transient receptor potential (TRPM) family. In addition to these three families, there are smaller groups such as the polycystins, which belong to the TRPP subfamily. The mucolipin transient receptor potential (TRPML) family contains the mucolipins. There is a single gene coding for TRPA1, which is a multitasking channel capable of sensing pain, temperature and mechanical deformation.

The remarkable aspect of this large family is just how many of them are expressed in the brain, where their functional role is still largely unknown.

Structural organization of the transient receptor potential (TRP) ion channel family

One of the characteristics of the transient receptor potential (TRP) ion channel family is that they share the same basic structural organization (Module 3: Figure TRP channel family). The arrangement of having six transmembrane segments with a pore loop located between segments 5 and 6 is identical with the organization of one of the four repeated units of the voltage-operated channels (VOCs), as exemplified by the $Ca_V1.1$ L-type channel (Module 3: Figure $Ca_V1.1$ L-type channel). In the case of the VOCs, the four units are all connected together to form a functional channel. It seems that there is some special requirement to have four units, because the functional TRP channels also depend upon the oligomerization of four of the separate units shown in Module 3: Figure TRP channel family.

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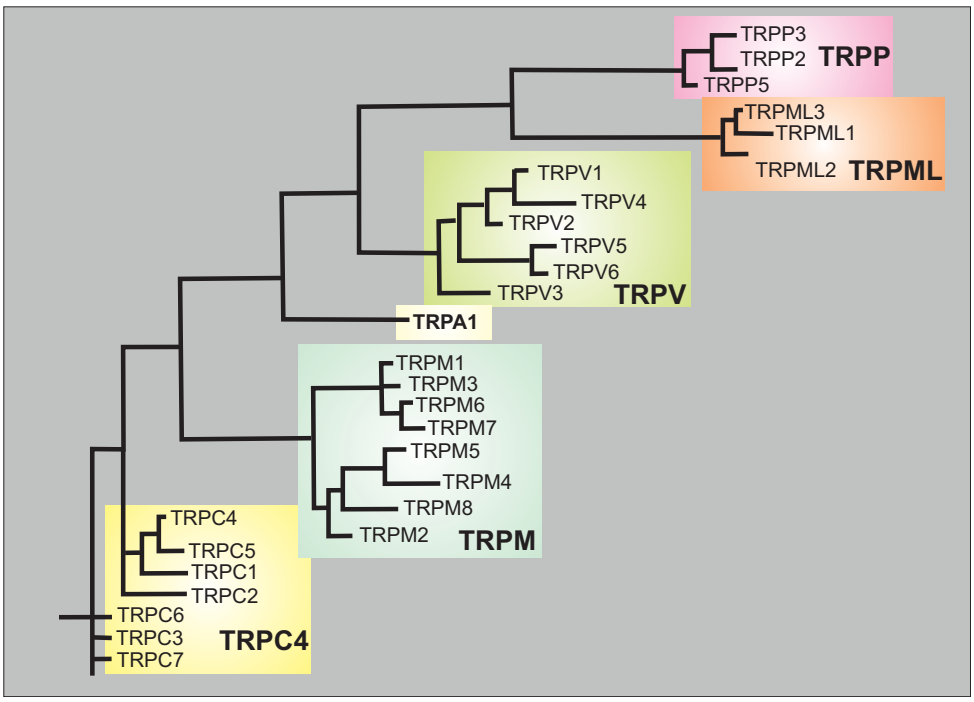
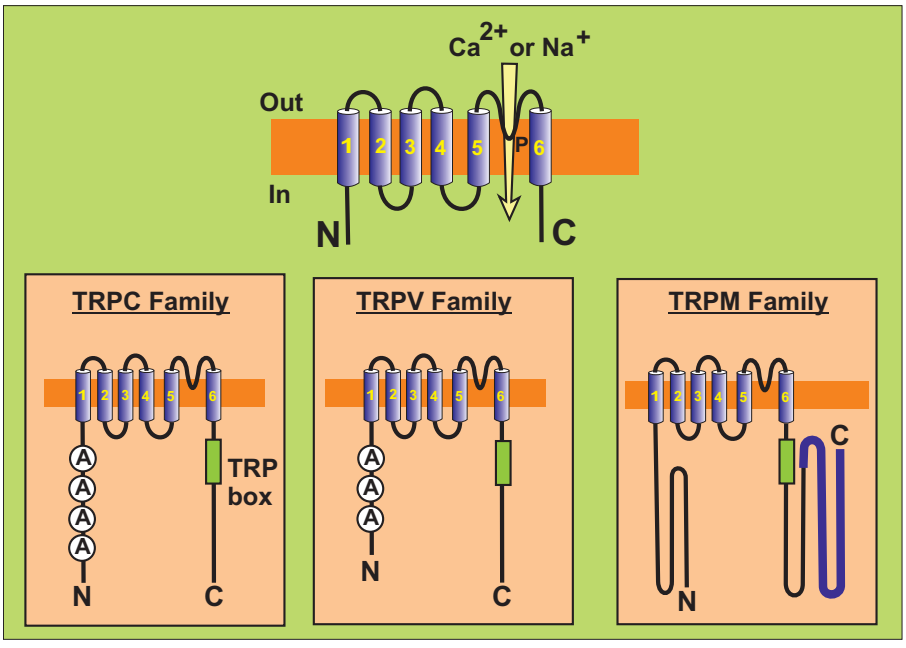
Module 3: | Figure TRP channel phylogeny



Phylogenetic tree of the TRP ion channel family.
Reproduced, with permission, from Nilius, B., Voets, T. and Peters, J. (2005) TRP channels in disease. Science STKE, re8. Copyright (2005) American Association for the Advancement of Science; http://www.sciencemag.org; see Nilius et al. 2005.

Module 3: | Figure TRP channel family



Structural organization of the transient receptor potential (TRP) ion channel family.
All the transient receptor potential (TRP) family members have the same basic structure (see the top panel) consisting of six transmembrane domains with the N- and C-termini located in the cytoplasm. Between the fifth and sixth transmembrane domains, which are highly conserved among all the homologues, there is a re-entrant pore loop (P). Most of the differences between the three families are found within the N- and C-terminal regions. The canonical TRP (TRPC) and vanilloid TRP (TRPV) family members are fairly similar in that they contain three or four ankyrin repeats (A). The melastatin-related TRP (TRPM) family is characterized by having very long N- and C-termini. The latter contains enzymatic activity (blue region) in some of the channels, e.g. TRPM2 has an ADP-ribose pyrophosphatase, whereas TRPM7 contains an atypical protein kinase.

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Since individual cells can express different members of the TRP ion channel family, the individual units can form tetramers as either homo- or hetero-multimers. There already is evidence for heteromultimers forming between TRPC1 and TRPC5 in the brain, and also between TRPC1 and TRPC4 in the adrenal cortex. The fact that heteromultimerization can occur has greatly complicated attempts to characterize the properties of these entry mechanisms. This problem has particularly bedevilled the large number of expression studies because the expressed channel units may have formed heteromultimers with endogenous units. Despite these difficulties, there has been considerable progress in characterizing the properties and functions of the different members of the canonical transient receptor potential (TRPC) family, the vanilloid transient receptor potential (TRPV) family and the melastatin-related transient receptor potential (TRPM) family.

Canonical transient receptor potential (TRPC) family

The canonical transient receptor potential (TRPC) family can be divided into four functional groups:

- TRPC1, TRPC2, TRPC3 and TRPC6
- TRPC7
- TRPC4
- TRPC5

Some of these channels may be responsible for the increased leak of Ca^{2+} that may play a role in the onset of muscular dystrophy. These subfamilies share both sequence homology and some functional properties:

TRPC1

The TRPC1 channel remains somewhat enigmatic. Despite being widely expressed in the brain and in many other tissues, its precise function and control mechanism remain somewhat elusive. The fact that it is so widespread and has a propensity for oligomerizing with other channel units (e.g. TRPC4 and TRPC5) may have complicated attempts to define its properties. There are suggestions that it might contribute to store-operated channels (SOCs), but there also are indications that it might be a mechanosensitive channel regulated by membrane stretch that could thus play a role in touch. Such a mechanosensitive function is also consistent with the finding that TRPC1 can interact with dystrophin and an overactive action of this Ca^{2+} entry channel may contribute to the increased $[Ca^{2+}]$ observed in muscular dystrophy.

One of its functions is to provide the Ca^{2+} signal responsible for smooth muscle cell proliferation where it may contribute to the onset of asthma. In neuronal synapses, TRPC1 may be responsible for the postsynaptic changes in membrane potential that develop following stimulation of the metabotropic glutamate receptor 1 (mGluR1). In this case, TRPC1 is tightly coupled to mGluR1 through the Homer protein (Module 10: Figure postsynaptic density).

In endothelial cells, TRPC1 plays a role in regulating microvascular permeability (Module 7: Figure endothelial cell contraction). TRPC1 is also one of the channels found in caveolae (Module 6: Figure caveolae organization).

TRPC2

In humans, *TRPC2* is a pseudogene, but in other species, the protein forms functional channels located in the vomeronasal organ, brain, heart and in sperm. In the latter case, it contributes to the entry of Ca^{2+} during the acrosome reaction, where it contributes to ZP3-induced Ca^{2+} signalling (Module 8: Figure ZP3-induced Ca^{2+} signals). In mice, the TRPC2 found in the vomeronasal organ functions in the pheromone response during sex discrimination. When the gene is deleted, the mutant males are more docile and fail to initiate aggressive attacks on intruder males. Although their sexual behaviour is mostly normal, they do vigorously mount other males.

There is some evidence to indicate that TRPC2 is activated by store depletion.

TRPC3

The TRPC3 channels are located in the brain and in heart muscle. The high levels found in central nervous system (CNS) neurons seem to be associated with entry of Ca^{2+} induced by brain-derived neurotrophic factor (BDNF) acting through **TrkB receptors**. These channels appear to be non-selective with a conductance of about 60 pS. They are also found in T cells, where they appear to contribute to the entry of Ca^{2+} responsible for T cell activation.

Just how these channels are activated is still somewhat of a mystery, with evidence being presented for both store depletion and diacylglycerol (DAG) activation mechanisms. With regard to the former, there is some experimental evidence that they may participate in the process of conformational coupling through interactions with either the ryanodine receptors (RYRs) or inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$).

Entry of Ca^{2+} through the TRPC3 channels is inhibited following phosphorylation by cyclic GMP-dependent protein kinase (cGK).

The expression of TRPC3 is increased through the nuclear factor of activated T cells (NFAT) transcriptional mechanism (Module 4: Figure NFAT control of Ca^{2+} signalling toolkit). It seems that such an induction of TRPC3 channels during the neural control of differentiation may provide the inward flux of Ca^{2+} responsible for skeletal muscle cell remodelling. In cerebellar Purkinje cells, TRPC3 channel opening generates a slow excitatory postsynaptic potential (EPSP) (Module 10: Figure synaptic signalling mechanisms). The TRPC3 channel may contribute to the entry of Ca^{2+} that maintains the smooth muscle cell cytosolic oscillator (Module 7: Figure SMC cytosolic oscillator), which controls contractility in vascular smooth muscle cells, airway smooth muscle cells and corpus cavernosum smooth muscle cells.

TRPC4

The TRPC4 channel is located in the brain, adrenal cortex, endothelial cells, testis and placenta. There is strong evidence from both the adrenal cortex and endothelial cells that TRPC4 is activated by store depletion. The channels

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display inward rectification and are strongly selective for Ca^{2+}, properties normally associated with the classical Ca^{2+} release-activated Ca^{2+} (CRAC) channel.

There are other indications that TRPC4 is activated directly by receptors and not by store depletion, as has been described for the channels expressed on the dendrites of thalamic interneurons (Module 10: Figure dendritic GABA release). Release of γ-aminobutyric acid (GABA) from the F2 terminals on these interneurons is controlled by extrathalamic input from glutamatergic, cholinergic and serotonergic neurons. In the case of the latter, the 5-hydroxytryptamine (5-HT) acts through 5-HT_2 receptors that are coupled through G_q to stimulate the hydrolysis of PtdIns4,5P_2 to generate InsP_3 and diacylglycerol (DAG). As part of this signalling pathway, the TRPC4 channels are activated to induce the entry of Ca^{2+} that triggers the release of GABA.

A possible clue as to how the channels are controlled is the presence of a PDZ-binding motif (VTTRL) located in the C-terminal region, which may determine its localization to the plasma membrane. Such a motif may explain the reported association between TRPC4 and either the Na^+/H^+ exchange regulatory factor or phospholipase Cβ1 (PLCβ1). The action of TRPC4 may thus depend upon its association with other signalling components.

There is some indication that TRPC4 may contribute to arachidonic acid-induced entry.

TRPC5

The are a number of similarities between TRPC5 and its closely related subfamily member TRPC4. TRPC5 also has a PDZ domain, enabling it to associate with other proteins. An unusual property of TRPC5 is that it appears to be regulated by extracellular thioredoxin (Trx), which functions in the recovery of oxidation-sensitive processes (Module 2: Figure recovery of protein oxidation). TRPC5, like the closely related TRPC4 and TRPC1, have two conserved cysteine residues near the glutamic acid residue 543. These cysteine residues form an intra-subunit disulphide bond that inhibits channel opening. Reduced Trx acting from the outside removes this disulphide bond resulting in an increase in channel opening. This control mechanism may be important in regulating the activity of TRPC5 in fibroblast-like synoviocytes (FLS) that secrete synovial fluid and thus may have implications for rheumatoid arthritis. One of the functions of FLS cells is to release matrix metalloproteinases (MMPs) such as MMP-2 and MMP-9.

TRPC5 channels, which are located in multiple neuronal cell types, are activated by intracellular Ca^{2+} and may thus contribute to the non-selective cation currents that play a role in the regulation of neuronal excitability. For example, they could contribute to the I_{CaN} inward current that contributes to the slow oscillation mechanism that occurs during sleep (Module 10: Figure slow oscillation mechanism).

TRPC5 channels are expressed in the growth cones of hippocampal neurons, where they function to regulate growth cone morphology and neurite extension.

TRPC6

The TRPC6 channel has a fairly wide distribution (brain, smooth muscle cells, blood platelets, heart, kidney and lung). It conducts both univalent and bivalent cations with a preference for Ca^{2+} over Na^+. It is activated by G protein-coupled receptors (GPCRs). For example, TRPC6 is the α_1-adrenergic-activated Ca^{2+} channel in smooth muscle cells. It appears not to be activated by store depletion, but by diacylglycerol (DAG) through a direct mechanism independent of protein kinase C (PKC) (Mechanism 2 in Module 3: Figure Ca^{2+} entry mechanisms). In addition, like some other TRP channels, the activity of TRPC6 is markedly inhibited by PtdIns4,5P_2 and is thus an example of PtdIns4,5P_2 regulation of ion channels and exchangers (Module 2: Figure PtdIns4,5P_2 regulation of TRP channels). TRPC6 contributes inward current during the mechanotransduction process responsible for myogenic vasoconstriction.

Mutations in TRPC6 are the cause of familial focal segmental glomerulosclerosis (FSGS), which is an end-stage renal disease. There also are suggestions that TRPC6 may play a role in idiopathic pulmonary arterial hypertension by contributing to the proliferation of arterial smooth muscle cells.

TRPC7

The TRPC7 channel closely resembles TRPC6 in being sensitive to diacylglycerol (DAG). This TRPC7 channel may contribute to the entry of Ca^{2+} that maintains the smooth muscle cell cytosolic oscillator (Module 7: Figure SMC cytosolic oscillator), which controls contractility in vascular smooth muscle cells, airway smooth muscle cells and corpus cavernosum smooth muscle cells.

Vanilloid transient receptor potential (TRPV) family

The overall structure of the vanilloid transient receptor potential (TRPV) family resembles that of the canonical transient receptor potential (TRPC) family (Module 3: Figure TRP channel family). They typically have three ankyrin repeats in the N-terminal region.

The TRPV family consists of three groups:

- TRPV1 and TRPV2, which are both chemosensors (vanilloid compounds) and thermosensors.
- TRPV4
- TRPV5 and TRPV6, function as epithelial Ca^{2+} transport channels, but are also known to function as highly selective entry channels during Ca^{2+} signalling.

TRPV1

The TRPV1 channel is found on peripheral sensory neurons and also within the brain and spinal cord, where they function in temperature sensing and possibly also as mechanosensitive channels for touch. It is a Ca^{2+}-permeable channel that senses noxious stimuli. Its distinctive characteristic is that it can respond to multiple stimuli such as chemicals (e.g. capsaicin, resinferatoxin, olvanil and zingerone), heat and inflammatory mediators such as the eicosanoid 12-(*S*-hydroperoxyeicosatetraenoic

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acid [12-(*S*)-HPETE] and leukotriene B_4. TRPV1 is also activated by anandamide (Module 1: Figure anandamide). In medium spiny neurons (MSNs) and dentate gyrus neurons, this activation of TRPV1 by anandamide induces the process of long-term depression (LTD).

TRPV1 was first detected as the channel that responds to capsaicin, which is the active ingredient in hot chilli peppers. It was subsequently found to also respond to moderate temperatures, i.e. above 43°C (Module 3: Figure temperature sensing). It is thus a key component of a battery of hot- and cold-sensitive channels that constitute the thermosensors that enable us to detect temperature. The opening of TRPV1 channels also has a role in histamine-sensitive Itch neurons (Module 10: Figure Itch signal transduction mechanism).

These TRPV1 channels are activated by three vanillotoxins (VaTx1–VaTx3) isolated from the venom of a tarantula.

The TRPV1 channels are particularly important in the process of nociception. They are polymodal receptors that can respond to H^+, irritants such as capsaicin and noxious temperatures (Module 10: Figure nociception). In addition to responding to capsaicin, TRPV1 can also be sensitized by agents such as bradykinin and nerve growth factor (NGF) that are released at sites of tissue injury and serve to increase the sensation of pain that characterizes hyperalgesia. This hyperalgesic effect of bradykinin and NGF seems to be mediated by the hydrolysis of PtdIns4,5P_2, which normally acts to inhibit TRPV1 (Module 2: Figure PtdIns4,5P_2 regulation of TRP channels). Consistent with such a mechanism, the TRPV1 channel exists as a ternary complex together with the TrkA receptor and phospholipase Cγ (PLCγ).

In the urinary bladder, TRPV1 is found both in the afferent nerve terminals and in the epithelial cells lining the bladder lumen. It seems to contribute to the stretch-evoked release of ATP that controls bladder function. It may contribute to some bladder diseases.

TRPV1 can be activated by the satiety factor oleoylethanolamide (OEA), but its action seems to require prior phosphorylation of the channel by protein kinase C (PKC).

TRPV2

TRPV2 resembles TRPV1 in that it functions in temperature sensing, and is also sensitive to heat, but operates at higher temperatures, i.e. at 50°C (Module 3: Figure temperature sensing). Given its function as one of the thermosensors, it is located on sensory neurons, but is also located in other brain regions.

TRPV3

TRPV3 is another temperature-sensing channel that is strongly expressed in skin, tongue and in various neuronal regions (dorsal root ganglia, trigeminal ganglion, spinal cord and brain). It reports temperatures in the 34–40°C range (Module 3: Figure temperature sensing) and thus enables the skin to detect warmth. The channel is capable of responding to relatively small innocuous increases in temperature, and thus functions together with TRPV4 to span the gap between the low-temperature recorder TRPM8 and the hot recorder TRPV1.

TRPV3 seems to play a role in providing Ca^{2+} signals that promote the proliferation and differentiation of keratinocytes during skin development.

TRPV3 is unusual in that it displays a marked sensitization in response to repetitive heat challenges.

TRPV4

The TRPV4 channel, which has some homology with TRPV1 and TRPV2, seems to function in both temperature sensing and osmosensing. With regard to the former, it detects warmth in the 27–40°C range (Module 3: Figure temperature sensing). It is found in the distal nephron of the kidney, where it may carry out its osmosensing function to detect dilute environments. These TRPV4 channels may also be mechanosensitive channels that respond to changes in membrane tension induced by fluctuations in osmotic pressure. This osmosensing mechanism may occur indirectly through the generation of a lipid messenger. Cell swelling seems to activate phospholipase A_2 (PLA_2) to release arachidonic acid (AA) that is then metabolized by cytochrome P450 epoxygenase to form 5',6'-EET. TRPV4 is activated by both AA and 5',6'-EET suggesting that its osmosensing ability depends indirectly on the formation of these lipid intermediates.

TRPV4 channels that are strongly expressed in airway epithelial cells, liver, heart, brain and keratinocytes. In the case of the latter, these channels have been implicated in pain perception in that they may respond to increases in temperature by releasing ATP, which then activates the sensory neurons (Module 10: Figure inflammatory soup).

Within the brain, TRPV4 is found in many neuronal cell types and is also expressed in the lateral ventricle ependymal cells, in auditory hair cells and in sensory cells of the trigeminal ganglion. The channel is activated when cells are placed in a dilute medium that causes cell swelling and may thus function in osmoreception. TRPV4 located in the hypothalamic structures seems to function in the integration of both thermal and osmotic information. Osmosensitive neurons are located in circumventricular organs, such as the organum vasculosum of the lamina terminalis (OVLT). Neurons in these areas innervate the magnocellular neurons, which synthesize vasopressin (VP) [antidiuretic hormone (ADH)], that are located in the supraoptic and paraventricular nucleus of the hypothalamus.

TRPV4 channels are activated by anandamide, and this has led to the suggestion that these channels might be regulated by endocannabinoids and arachidonic acid.

These channels are unusual in being activated by increases in intracellular Ca^{2+} acting through calmodulin (CaM) binding to a C-terminal CaM-binding domain.

TRPV5

The TRPV5 channel was originally known as the epithelial Ca^{2+} channel 1 (ECaC1), which is located in the kidney, small intestine and placenta. Its properties are very similar to TRPV6 (also known as ECaC2). These two channels are coded for by two genes that lie next to each other on

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human chromosome 7q35, indicating that they resulted from gene duplication. TRPV5 has a very high selectivity for Ca^{2+} (P_{Ca}/P_{Na} >100) and displays strong inward rectification (i.e. there is a strong inward current with very little outward current). It functions in the transport of Ca^{2+} across epithelia especially in distal kidney tubules.

TRPV5 appears to be constitutively active and will continue to transport Ca^{2+} as long as the internal concentration of Ca^{2+} remains low. However, a small elevation in intracellular Ca^{2+} will rapidly inactivate the channel (half maximal inhibition 200 nM). In the case of Ca^{2+} reabsorption by the kidney, a close association between TRPV5 and the Ca^{2+} buffer calbindin D-28k (CB) reduces this inactivation and thus facilitates the transcellular flux of Ca^{2+} (Module 7: Figure kidney Ca^{2+} reabsorption).

Expression of the *TRPV5* gene is regulated by the Vitamin D control of Ca^{2+} homoeostasis.

TRPV6

The TRPV6 channel was originally called the epithelial Ca^{2+} channel (ECaC2). It was first cloned from the duodenum, where it functions in transcellular transport of Ca^{2+}. However, it is now known to have a much more widespread distribution (placenta, kidney, salivary gland and prostate). It has biophysical properties resembling those of TRPV5, it shows strong inward rectification and also shows high selectivity for Ca^{2+} (P_{Ca}/P_{Na} >100). Like the TRPV5 channel, TRPV6 is strongly inhibited by an increase in intracellular Ca^{2+}.

TRPV6 is one of the few transient receptor potential (TRP) channels that have been shown to bind calmodulin (CaM), which seems to play a role in the Ca^{2+}-dependent inactivation. In the case of Ca^{2+} reabsorption by the intestine, a close association between TRPV6 and the Ca^{2+} buffer calbindin D-9k may reduce this inactivation and thus facilitate the transcellular flux of Ca^{2+} (Module 7: Figure intestinal Ca^{2+} reabsorption).

TRPV6 channels are highly expressed in human prostate cancer cells and appear to be linked to the development of malignancy. They are particularly evident in metastatic and androgen-insensitive prostatic lesions. TRPV6 is also expressed in other forms of cancer, including breast, thyroid, colon and ovarian cancer.

Expression of the *TRPV6* gene is regulated by the Vitamin D control of Ca^{2+} homoeostasis.

Melastatin-related transient receptor potential (TRPM) family

The melastatin-related transient receptor potential (TRPM) family is characterized by having very long N- and C-termini (Module 3: Figure TRP channel family). Some members of this family (TRPM2, TRPM6 and TRPM7) are distinctive in that they function as both channels and enzymes.

TRPM1

The TRPM1 protein is found in the eye and in melanocytes. TRPM1 appears to function as a tumour suppressor protein and is also known as melastatin. Whether or not it functions as a channel and how it is controlled remain to be determined.

TRPM2

TRPM2 is a Ca^{2+}-permeable non-selective channel that is expressed in the brain and placenta. Unlike many of the other TRP channels, it has a linear current–voltage relationship with a conductance of 60 pS. It is a bifunctional protein in that it combines its channel function with an enzymatic function located in the C-terminal region (Module 3: Figure TRP channel family). The latter contains a Nudix box sequence that is found in enzymes that hydrolyse nucleoside diphosphates. This part of the channel contains an ADP-ribose pyrophosphatase that hydrolyses ADP-ribose (ADPR), a breakdown product of the Ca^{2+}-mobilizing second messenger cyclic ADP-ribose (cADPR) (Module 2: Figure cADPR/NAADP function).

TRPM2 is activated by adenine dinucleotides such as ADPR and is also sensitive to low levels of H_2O_2 and may thus contribute to redox signalling. The channel is located in the plasma membrane where it can induce the influx of external Ca^{2+} and it can also release Ca^{2+} from a lysosomal compartment.

There are some indications that TRPM2 may play some role in the development of manic-depressive illness.

TRPM3

TRPM3 undergoes alternative splicing to produce channels with different permeabilities to divalent cations. There is a suggestion that one of TRPM3 variants might be activated by sphingosine, which is formed by the sphingomyelin signalling pathway.

TRPM4

TRPM4, like TRPM5, is unusual in that is has limited permeability for Ca^{2+}; instead, these channels conduct univalent cations. A new member of the TRPM4 family, TRPM4b, codes for a Ca^{2+}-sensitive cation channel (gates Na^+ and K^+), which may play a regulatory role by adjusting membrane potential during Ca^{2+} signalling. The Ca^{2+} oscillations in lymphocytes (Module 9: Figure T cell Ca^{2+} oscillations) appear to be modulated by TRPM4, which by depolarizing the membrane, probably acts to reduce the driving force for Ca^{2+} entry. This TRPM4 channel desensitizes rapidly to an increase in intracellular Ca^{2+} and this seems to depend on a decrease in the level of $PtdIns4,5P_2$, which acts by shifting the voltage dependence of TRPM4 activation towards negative potentials. The removal of $PtdIns4,5P_2$ dramatically reduces the sensitivity of the channel to the stimulatory effect of Ca^{2+}. This is an example of the $PtdIns4,5P_2$ regulation of ion channels and exchangers (Module 2: Figure $PtdIns4,5P_2$ regulation of TRP channels).

The sensitivity of the channel to Ca^{2+}, both activation and inactivation, would be ideally suited for its proposed role as a pacemaker channel. It has been implicated in the control of breathing in the medullary respiratory pacemaker mechanism (Module 10: Figure respiratory pacemaker mechanism) and in the uterus smooth muscle cell membrane oscillator where it could contribute a

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depolarizing inward current during the pacemaker depolarization (Module 7: Figure uterus activation).

TRPM5

TRPM5 has a fairly wide distribution. It occurs in taste receptor cells, but has also been found in the small intestine, liver, lungs, testis and brain. This channel has a 23 pS conductance and appears to be a Ca^{2+}-activated univalent cation channel. The EC_{50} for Ca^{2+} is approximately 30 μM. It thus has properties resembling the TRPM4b isoform of the TRPM4 channel. These Ca^{2+}-activated univalent cation channels are widely distributed in both excitable and non-excitable cells, where they function in cardiomyocytes and in the firing of pyramidal cells in the cortex, and have also been implicated in the generation of the slow oscillations of thalamocortical neurons during sleep. TRPM5 has also been implicated in the membrane oscillator that controls breathing in the medullary respiratory pacemaker mechanism (Module 10: Figure respiratory pacemaker mechanism) and in the uterus smooth muscle cell membrane oscillator where it contributes a depolarizing inward current during the pacemaker depolarization.

TRPM6

TRPM6 has a high permeability to Mg^{2+} and seems to function primarily in the intestine and kidney.

Mutations of TRPM6 cause hypomagnesaemia with secondary hypocalcaemia.

TRPM7

The TRPM7 channel is widely expressed (brain, heart, liver, kidney and lung). TRPM7 is responsible for producing the Ca^{2+} flickers that form the Ca^{2+} signalling microdomains and chemotactic orientation mechanism that may control neutrophil chemotaxis (Module 11: Figure neutrophil chemotactic signalling).

Like TRPM2, the TRPM7 channel is bifunctional in that it is both a channel and an enzyme. In the latter case, the enzyme is an atypical serine/threonine protein kinase with homology with an MHCK/EEF2α family of protein kinases whose activity is essential for channel activity. Just how this kinase activity contributes to channel activity is unknown. Similarly, the control of channel opening is also a mystery, but there have been a number of suggestions:

- A possible clue to TRPM7 activation is its ability to associate with the C2 domain of phospholipase Cβ1 (PLCβ1). Activation of the latter results in inhibition of the channel. This inhibition seems to depend upon the hydrolysis of PtdIns4,5P_2, which appears to be a key regulator of the TRPM7 channel (Module 2: Figure PtdIns4,5P_2 regulation of TRP channels). Such a mechanism would resemble that of the TRPV1 channel, which has a similar association with PLC. However, the activation of PLC to hydrolyse PtdIns4,5P_2 seems to have opposite effects on these two channels: it helps to open TRPV1, but closes TRPM7. This an example of the PtdIns4,5P_2 regulation of ion channels and exchangers.
- The channel may be regulated by physiological levels of Mg-ATP. The channel is activated when the Mg-ATP level falls below 1 mM.
- The channel may function in the uptake of trace metal ions such as Zn^{2+}, Mn^{2+} and Co^{2+}.

TRPM8

TRPM8 has a high permeability to Ca^{2+}, and the current displays strong inward rectification. It is one of the voltage-dependent cold-sensing channels and is located in a subset of sensory neurons such as those in the dorsal root ganglia and trigeminal ganglia that function in temperature sensing by responding to temperature and noxious stimuli. Within these sensory neurons it is a cold- and menthol-sensitive receptor that functions as one of the thermosensors that transduce cold stimuli. The channel is activated by temperatures in the 15–22°C range (Module 3: Figure temperature sensing). Just as capsaicin can modulate the temperature sensitivity of the 'hot' TRP channels (TRPV1 and TRPV2), menthol functions as an allosteric modulator in that it shifts the activation threshold of the channel to less cold temperatures. TRPM8 is also strongly activated by icilin and eucalyptol.

The channel is desensitized by the entry of external Ca^{2+} that seems to act by stimulating the Ca^{2+}-sensitive phospholipase Cδ (PLCδ), which hydrolyses PtdIns 4,5P_2 resulting in channel inactivation (Module 2: Figure PtdIns4,5P_2 regulation of TRP channels). The PtdIns4,5P_2 seems to bind to positively charged residues in the TRP domain. The removal of PtdIns4,5P_2 shifts the voltage dependence of channel activation to more positive potentials leading to desensitization.

The TRPM8 channel was originally identified in prostate cancer cells, but is also found in many other tumours. Its ability to gate Ca^{2+} may contribute to its pathological role in prostate cancer.

Mucolipin transient receptor potential (TRPML) family

Members of the mucolipin transient receptor potential (TRPML) family (TRPML1–TRPML3) are unusual in that they are not present in the plasma membrane, but are located on internal vesicles. Most attention has focused on TRPML1.

TRPML1

TRPML1 is a cation-specific channel located in the endolysosomal compartment, which appears to be regulated by the PtdIns3,5P_2 signalling cassette (see step 10 in Module 4: Figure membrane and protein trafficking). One of its functions is to transfer Fe^{2+} from the lumen of these internal organelles in to the cytoplasm. It also appears to have a role in endosomal acidification to control the final fusion event between the late endosome and the lysosome. It has been suggested that TRPML1 could act by releasing Ca^{2+} from the late endosome to form a microdomain to trigger fusion with the lysosome. Such an example of this final fusion event occurs during phagocytosis to form the phagolysosome (Module 4: Figure phagosome maturation).

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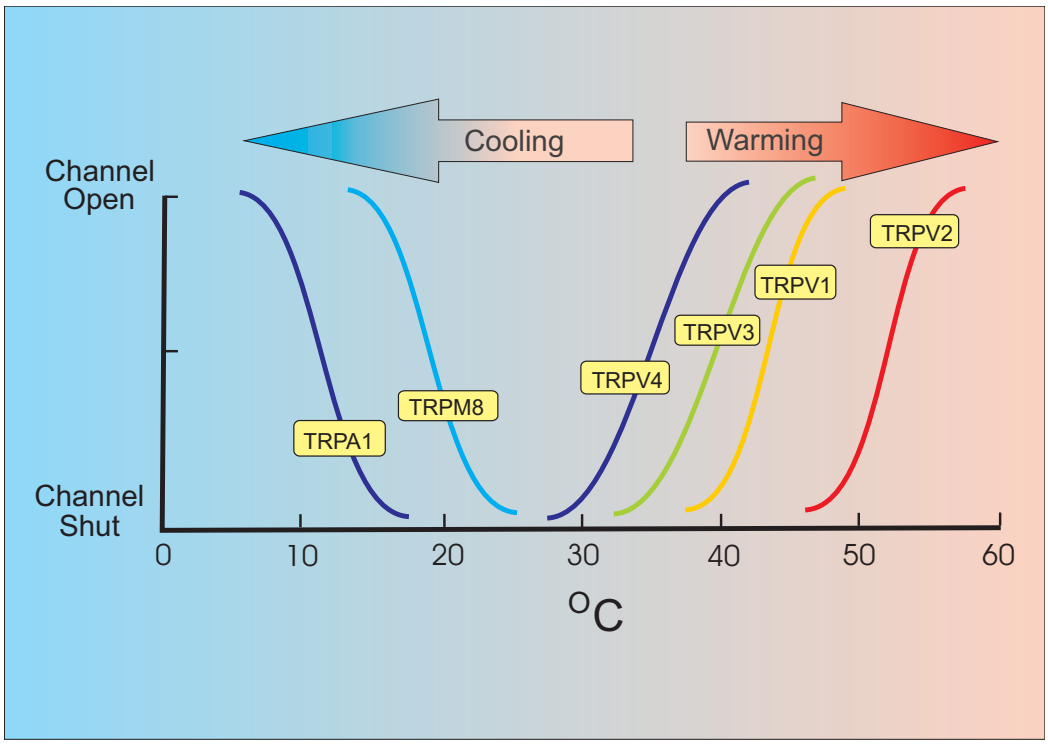
Module 3: | Figure temperature sensing



The function of transient receptor potential (TRP) channels in temperature sensing by sensory neurons.
The nerve endings of sensory neurons contain different isoforms of the transient receptor potential (TRP) ion channel family that function as thermosensors that respond to either cooling or warming. As the channels open in response to changes in temperature they gate cations such as Ca^{2+} and the resulting depolarization is sufficient to excite the sensory neuron such that it fires and sends action potentials back to the brain.

Mutation of the gene *mcoln1*, which codes for TRPML1, causes mucolipidosis type IV (MLIV).

TRPA1

The TRPA1 family contains a single member. It is expressed in hair cells, where it may function in hair cell mechanoelectrical transduction (Module 10: Figure tip link). The large number of ankyrin repeats located in the N-terminal tail may function as a gating spring. It is also expressed in sensory neurons, where it responds to a number of pungent components such as isothiocyanates (found in horseradish), allicin (found in garlic) and cinnamaldehyde (found in cinnamon). There also are indications that TRPA1 may contribute to the hypersensitivity to touch that develops during inflammation of the skin and plays an important role in histamine-insensitive Itch neurons (Module 10: Figure Itch signal transduction mechanism).

There also are reports that it can respond to cold. TRPA1 is located in dorsal root ganglion (DRG) neurons that function in temperature sensing. It is co-expressed with TRPV1, which detects heat. By contrast, TRPA1 senses cold and begins to be activated at about −17°C, which means that it operates at temperatures lower than that of the other cold-sensing channel TRPM8 (Module 3: Figure temperature sensing).

TRPA1 thus appears to be a multipurpose sensor functioning in mechanosensation, temperature and pain sensing. Mutations of TRPA1 have been linked to familial episodic pain syndrome (FEPS).

Temperature sensing

The peripheral nervous system is responsible for detecting a range of different sensations, such as touch, pain and temperature. The dorsal root ganglion (DRG) neurons of the peripheral nervous system are the major neuronal cell type responsible for detecting these different modalities. The cell bodies of these DRG neurons are located in the DRG and the sensory part of the neuron runs out into the skin, where the endings are specialized to detect various stimuli, such as variations in temperature. Such sensory information is then relayed back to the dorsal horn of the spinal cord en route to the brain. The signalling problem is to understand how the thermosensors located in the sensory nerve endings in the surface of the skin can detect changes in temperature.

Thermosensors

The thermosensors, which belong to the transient receptor potential (TRP) ion channel family, have the unique property of being sensitive to changes in temperature (Module 3: Figure temperature sensing). The different channel members are divided into the cold sensors TRPA1 and TRPM8, the warm sensors TRPV3 and TRPV4 and the hot sensors TRPV1 and TRPV2.

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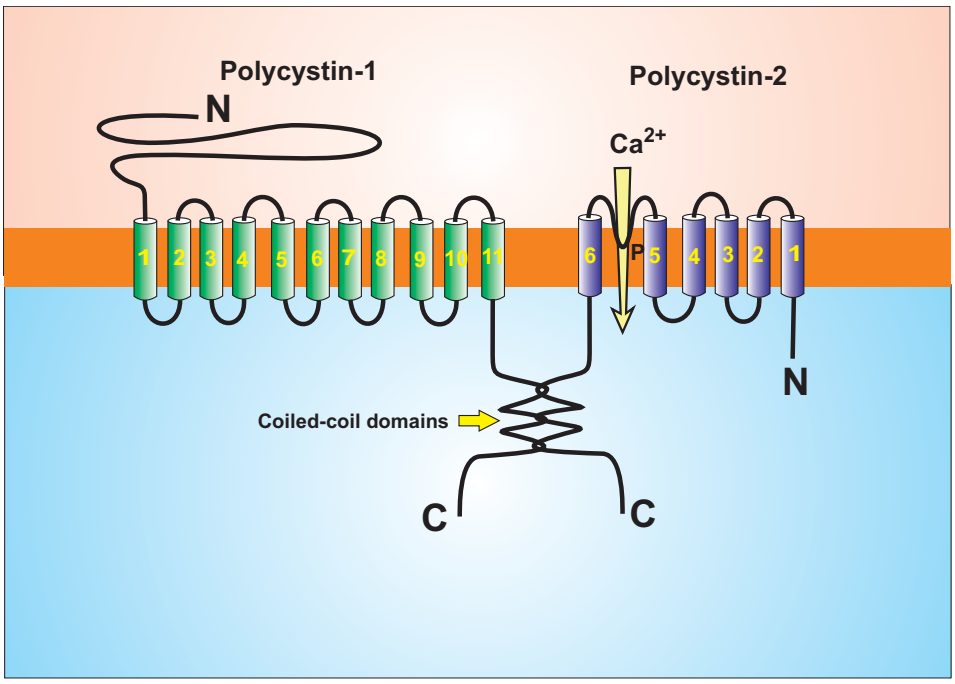
Module 3: | Figure polycystin domain structure



The domain structure of polycystin-1 and polycystin-2.
Polycystin-1 is a membrane-bound protein that has a large extracellular N-terminus, 11 transmembrane domains and a cytosolic C-terminus that contains a coiled-coil domain. Polycystin-2 has a domain structure resembling that of the transient receptor potential (TRP) ion channel family (Module 3: Figure TRP channel family) in that it has six transmembrane domains with cytosolic N- and C-termini. The C-terminus has a coiled-coil domain that enables it to interact with the similar domain on polycystin-1.

Polycystins

The polycystins are the membrane proteins encoded by the genes that are mutated in polycystic kidney disease. The autosomal dominant polycystic kidney disease genes 1 and 2 (*PKD1* and *PKD2*) encode membrane bound polycystin-1 and polycystin-2 (TRPP2) respectively (Module 3: Figure polycystin domain structure). One of the important locations of the polycystins is on the primary cilium, which is present on most cells. Usually each cell has a single cilium. Such primary cilia function in mechanotransduction signalling pathways that detect fluid flow. The primary cilium functions in mechanotransduction in kidney cells to detect fluid flow (Module 3: Figure flow-induced Ca^{2+} signals), and this might be used to couple the rate of ion transport to the flow rate within the nephron. Another important example of fluid flow detection occurs in the nodal region of the developing embryo, where it functions to determine left-right asymmetry.

Polycystin-1

Polycystin-1 is a membrane-bound protein of uncertain function. It seems to be located in the plasma membrane, where its large extracellular N-terminus has a number of potential adhesion and protein–protein interaction domains, which is consistent with the suggestion that polycystin-1 may function as a receptor mediating cell–cell or cell–matrix interactions. It also plays a critical role in interacting with, and perhaps controlling, the channel properties of polycystin-2. Together, these two proteins seem to function as a mechanosensory system capable of detecting fluid flow by an increase in the level of Ca^{2+} (Module 3: Figure flow-induced Ca^{2+} signals).

Polycystin-2

Polycystin-2 (PKD2 or TRPP2) is an integral membrane protein with an amino acid sequence that suggests that it is related to the transient receptor potential (TRP) ion channel family. Indeed, its domain structure (Module 3: Figure polycystin domain structure) also resembles that of the TRP family (Module 3: Figure TRP channel family). Polycystin-2 is highly concentrated on the primary cilium. Polycystin-2 seems to function as a Ca^{2+} channel and its activity may depend upon its interaction with polycystin-1. The two proteins interact through their C-terminal coiled-coil domains to form a functional unit capable of gating both monovalent cations (e.g. Na^+) and Ca^{2+}, with a permeability to Ca^{2+} somewhat higher (6-fold) than that of Na^+.

One of the problems with trying to understand the normal function of polycystin-2 concerns its localization in the cell. There is evidence of it operating both in the plasma membrane and within the endoplasmic reticulum (Module 3: Figure polycystin channel location). The activity of polycystin-2 may be regulated by mammalian diaphanous-related formin 1 (mDia1), which is one of the downstream effectors of the Rho signalling mechanisms. Under resting conditions, Dia1 binds to polycystin-2 and

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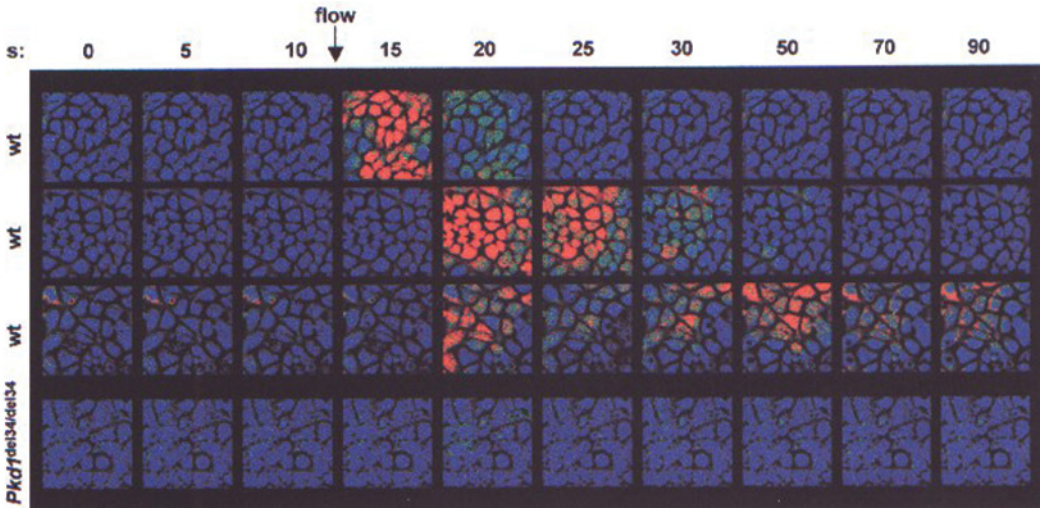
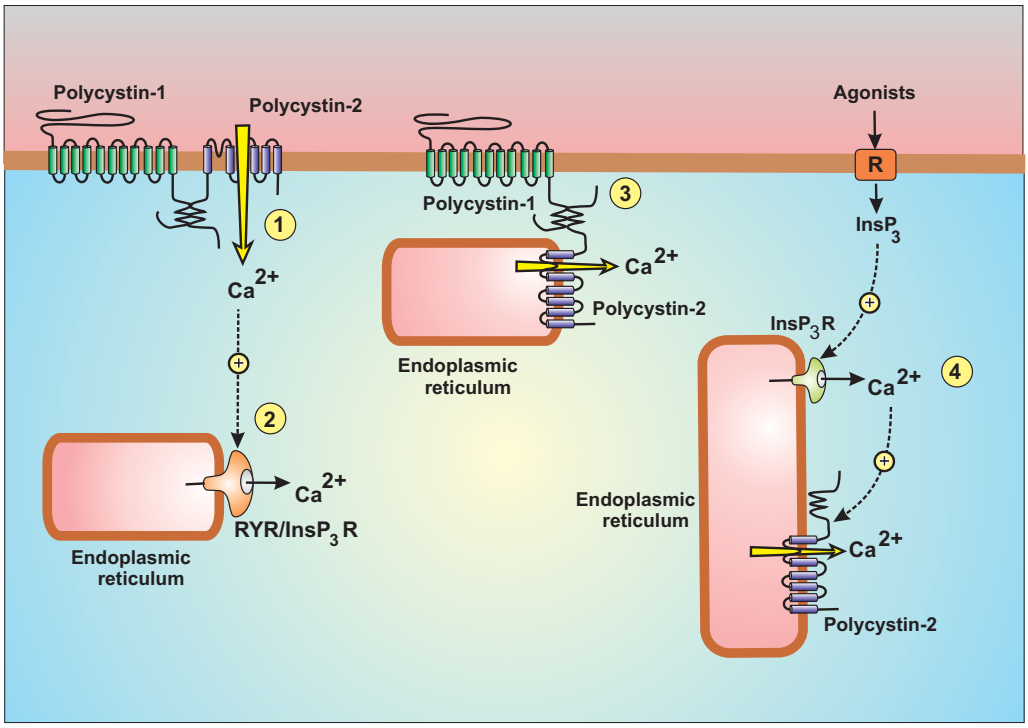
Module 3: | Figure flow-induced Ca^{2+} signals



Flow-induced Ca^{2+} signals in kidney epithelia cells.

Wild-type kidney epithelial cells in culture responded to fluid flow over their surface by an increase in Ca^{2+} that occurred either immediately (top row of panels) or after a 5 s delay (upper and lower centre rows of panels). Cells carrying a mutation in *PKD1* failed to respond to fluid flow (bottom row of panels). Reproduced by permission from Macmillan Publishers Ltd: *Nature Genetics*, Nauli, S.N., Alenghat, F.J., Luo, Y., Williams, E., Vassilev, P., Li, X., Elia, A.E.H., Lu, W., Brown, E.M., Quinn, S.J., Ingbar, D.E. and Zhou, J. (2003) Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. 33:129-137. Copyright (2003); http://www.nature.com/ng/; see Nauli et al. 2003).

Module 3: | Figure polycystin channel location



Proposed locations and functions of polycystin-2.

There are suggestions of polycystin-2 functioning in different cellular locations:

1. Polycystin-2 associated with polycystin-1 gates Ca^{2+} entry across the plasma membrane.
2. In the case of mechanosensitive cilia in kidney cells, the primary entry of external Ca^{2+} is amplified by releasing further Ca^{2+} from the endoplasmic reticulum either through ryanodine receptors (RYRs) or through inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$).
3. There is a suggestion that polycystin-1 located in the plasma membrane may be associated with, and regulates the release of internal Ca^{2+} by, polycystin-2 located in the endoplasmic reticulum.
4. Polycystin-2 located in the endoplasmic reticulum may be stimulated to release Ca^{2+} through a Ca^{2+}-induced Ca^{2+} release (CICR) mechanism using the Ca^{2+} released through $InsP_3$.

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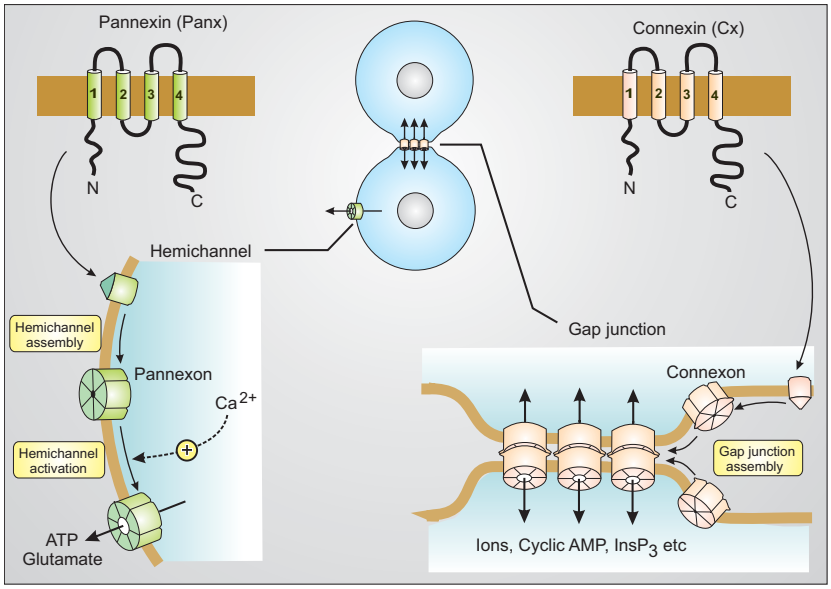
Module 3: | Figure hemichannels and gap junctions



Hemichannels and gap junctions.

Members of the connexin and pannexin families are membrane proteins that have similar transmembrane topologies with four transmembrane domains with the N- and C-termini located in the cytoplasm. Six of the pannexin subunits come together to form pannexons, whereas a similar assembly of connexins is known as a connexon. Pannexons and connexons form membrane channels with different functions. The pannexon, which is also known as a hemichannel, is activated by Ca^{2+} to release components from the cytosol such as ATP and glutamate. On the other hand, the connexons from neighbouring cells interact with each other to form gap junctions that act as intercellular channels permeable to ions and second messengers such as cyclic AMP and $InsP_3$.

prevents channel opening. This inhibition is removed by Rho/GTP to allow channel opening.

Gap junctions

Gap junctions are low-resistance channels that provide an intercellular pathway for ions and small molecules to flow from one cell to the next (Module 1: Figure cell communication). Such a direct line of communication is very fast and is particularly important in excitable cells such as the heart where gap junctions are critical for spreading action potentials to the cardiac cells to drive each heart beat. Gap junctions are formed from the large family (21 genes in humans) of connexins (Cxs) that are distinguished from each other by their molecular mass, which is indicated by the number following the Cx abbreviation. For example, Cx43 is the main gap junction protein in astrocytes (Module 7: Figure astrocyte structure). The connexins have four transmembrane domains with the C- and N-termini facing the cytoplasm (Module 3: Figure hemichannels and gap junctions). Six of these subunits come together to form a connexon, which is also known as a hemichannel as it is half of the gap junctional channel. As a hemichannel, the connexon is essentially impermeable, but when two connexons from neighbouring cells interact with each other, they form a fully functional intercellular channel capable of passing ions and second messengers such as cyclic AMP and $InsP_3$.

Hemichannels

Hemichannels located in the plasma membrane, which have a high unitary conductance of approximately 500 pS, function to release components from the cell (Module 3: Figure hemichannels and gap junctions). Some of these such as ATP and glutamate have important signalling functions. These hemichannels are formed from members of the pannexin (Panx) protein family that has three members (Panx1, Panx2 and Panx3). The membrane topology of the pannexins (Panxs) is similar to that of the connexons (Cxs) that make up the gap junctions. While the Panxs have been shown to form gap junction-like structures, they appear to function normally as hemichannels to regulate the release of important autocrine and paracrine mediators.

It is the Panx1 subunit that is primarily responsible for forming these hemichannels. Six of the Panx1 subunits aggregate to form a pannexon that is the functional hemichannel, which opens in response to an elevation of intracellular Ca^{2+} to trigger the release of glutamate and ATP. The release of such mediators plays an import role in regulating a number of physiological processes:

- Release of ATP and glutamate from hemichannels may function in neuronal-astrocyte communication (Module 7: Figure astrocyte tripartite synapse).
- Hemichannels may function in the propagation of intercellular Ca^{2+} waves as shown in mechanism B in Module 6: Figure intercellular Ca^{2+} waves.
- Release of ATP by hemichannels operates in the processes of signal transduction in type II taste receptor cells (Module 10: Figure taste receptor cells).
- During neutrophil chemotaxis, ATP released through hemichannels in the region of the pseudopod can

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feedback in an autocrine manner to activate receptors at the front and thus contribute to chemotaxis by amplification of early polarity signalling (Module 11: Figure neutrophil chemotaxis).
- Activation of Panx1 by caspase 3 results in the release of ATP that functions as a 'find-me' signal to attract phagocytes during apoptosis.

Mechanotransduction

Mechanotransduction is a process whereby cells transduce mechanical stimuli into biochemical or electrical signals. Most attention has focused on the notion the detection and transduction process might be carried out by mechanosensitive channels. However, it is not clear yet whether these channels detect mechanical deformation directly or whether they respond to signals coming from some other mechanotransducing mechanism. The latter has been suggested as the mechanotransducing mechanism operating during myogenic vasoconstriction of vascular smooth muscle cells.

Mechanosensitive channels

Mechanosensitive channels, which have also been referred to as stretch-activated cation channels (SACCs), are thought to function in mechanotransduction by opening in response to mechanical force. Such channels have been implicated in certain sensory processes such as hearing, touch, osmosensing and myogenic vasoconstriction. They may also function to detect shear stress in endothelial cells. In all these examples, mechanical deformation of the membrane provides the stimulus to open these mechanosensitive channels. The identity and opening mechanisms of these channels is still being worked out. A number of mechanisms have been proposed to explain how such channels might sense mechanical forces:

1. Channels may sense tension developed within the plane of the bilayer as might occur when the membrane is stretched.
2. Channels may be opened by forces applied through protein links attached either to the extracellular matrix or the cytoskeleton. Such a mechanism is thought to operate during hair cell mechanoelectrical transduction when hair cell tip links pull on the mechanosensitive channels that gate Ca^{2+} (Module 10: Figure tip link).
3. Mechanical force may be sensed by a separate non-channel mechanism that then generates a 'messenger' to open channels. Such a mechanism may operate in the process of myogenic vasoconstriction.

It is clear from the above that there is considerable uncertainty as to the transduction processes responsible for converting mechanical force into changes in membrane potential. The following are examples of putative mechanosensitive channels:

- Piezo
- TRPA1
- TRPC1
- TRPM7
- TRPV1
- TRPV4
- TREK

Piezo

Piezos are putative components of a novel mechanosensitive cation channel system. There are two genes, *piezo1* and *piezo2* that code for large membrane proteins that seem to have an organization similar to that of voltage-sensitive Na^+ channels in that there are 24 transmembrane regions arranged into four groups, but there is no obvious pore domain. At present it is not clear whether they form the mechanosensitive channel itself or is an essential subunit of such a channel. There are indications that piezo2 may play a role in somatosensory neurons.

K^+ channels

There are a number of K^+ channels that are regulated by various signalling pathways, which either open or close the channels to cause membrane hyperpolarization or depolarization respectively (Module 3: Figure K^+ channel domains). Examples of the former are the Ca^{2+}-sensitive K^+ channels that function in both excitable and non-excitable cells and the G protein-activated inwardly rectifying K^+ (GIRK) channels that are located in cardiac pacemaking cells, atrial myocytes and in various neurons and endocrine cells. On the other hand, the ATP-sensitive (K_{ATP}) channels are closed by stimuli that raise the intracellular level of the metabolic messenger ATP.

The function of many of these K^+ channels is modulated by a number of different K^+ channel auxillary subunits (Module 3: Table K^+ channel auxillary subunits).

Voltage-dependent K^+ (K_v) channels

There is a large family of voltage-dependent K^+ (K_v) channels (Module 3: Table voltage-dependent K^+ channels). There are 40 genes that code for the pore-forming α-subunits. In addition, there is a diverse family of cytoplasmic auxiliary subunits that code for the $K_v\beta$-subunits (4 genes) and the KChIPs (4 genes) that modulate the activity of the α-subunits. These α-subunits have six α-helical transmembrane segments with the pore-forming loop (P) located between segments 5 and 6, while the main voltage-sensing component is located in segment 4. Four of these α-subunits come together to form a functional channel. One of the main functions of these K_v channels is to repolarize the membrane during the action potentials in excitable cells. An example of such a repolarization function is found during the course of the ventricular cell action potential (Module 7: Figure cardiac action potential).

The following examples illustrate the role of these K_v channels in the control of some specific cellular processes:

- $K_v1.3$
- $K_v4.2$
- $K_v7.1$

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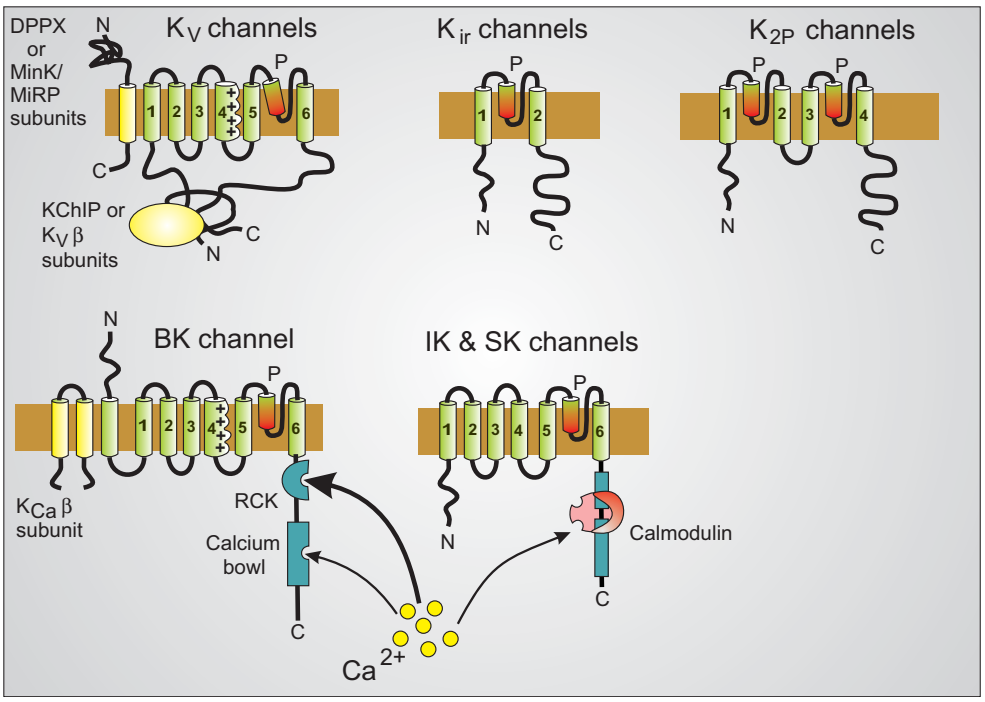
Module 3: | Figure K^+ channel domains



The domain structure of K^+ channels.
Like many other ion channels, the α subunits of the K^+ channels have a variable number of both the transmembrane (TM) domains (shown in green) and pore-forming loops (P). The latter forms the K^+ permeable channel. The two-pore K^+ channels (K_{2P}) have two P loops. The voltage sensor in K_v and BK channels is located in TM4. The K^+ channels are modulated by a number of auxiliary subunits (shown in yellow) that are either cytoplasmic (KChIP or $K_v\beta$ subunits) or integral membrane proteins (DPPX, the MinK/MiRP and the $K_{Ca}\beta$ subunits). In BK channels, there is a high-affinity Ca^{2+}-binding site called the 'calcium bowl' and a low-affinity site called the regulator of conductance for K^+ (RCK) located close to TM6. By contrast, the Ca^{2+}-sensitivity of the intermediate-conductance (IK) and small-conductance (SK) channels depends upon a resident calmodulin molecule.

- $K_V7.2$ and $K_V7.3$
- $K_v11.1$

$K_v1.3$

The $K_v1.3$ channel is a member of the family of voltage-dependent K^+ (K_v) channels (Module 3: Table voltage-dependent K^+ channels). It is a delayed rectifier that is widely expressed in both excitable and non-excitable cells. One of the functions of $K_v1.3$ is to maintain membrane potential and to promote T cell receptor (TCR) Ca^{2+} signalling (Module 9: Figure T cell Ca^{2+} signalling) and the activation of blood platelets (Module 11: Figure platelet activation).

$K_v4.2$

The $K_v4.2$ channel is a member of the family of voltage-dependent K^+ (K_v) channels (Module 3: Table voltage-dependent K^+ channels). One of its functions is in the cardiac action potential where it provides the transient outward current (I_{to}) responsible for the notch that immediately follows the rising phase (Module 7: Figure cardiac action potential). It also functions as the fast inactivating A-type current in neurons. In CA1 hippocampal neurons it reduces the back-propagating action potentials. The functional channel depends on its association with KChIP and DPPX, which are K^+ channel auxiliary subunits (Module 3: Table K^+ channel auxiliary subunits).

$K_v7.1$

The $K_v7.1$ channel is a member of the family of voltage-dependent K^+ (K_v) channels (Module 3: Table voltage-dependent K^+ channels). It generates a slow delayed rectifier current that has a number of functions. In the heart, the $K_v7.1$ channel associates with the MinKβ auxiliary subunit (encoded by the KCNE1 gene) to produce the I_{ks} that functions to repolarize the cardiac action potential (Module 7: Figure cardiac action potential). It also functions in the recycling of K^+ at the stria vascularis in the inner ear. This channel is also expressed in zona glomerulosa cells (Module 7: Figure glomerulosa cell signalling).

Mutations in either the $K_v7.1$ channel or its auxiliary subunit MinK cause congenital long QT syndrome.

$K_V7.2$ and $K_V7.3$

The $K_v7.2$ and $K_v7.3$ are delayed rectifier channels that contribute to the M current to regulate neuronal excitability. These channels are activated by the $PtdIns4,5P_2$ regulation of ion channels and exchangers signalling pathway to provide the tonic excitatory drive that enhances neuronal activity (Module 10: Figure tonic excitatory drive and neuronal rhythms).

Alterations in $K_v7.3$ have been implicated in a form of epilepsy known as benign familial neonatal convulsions.

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Module 3: | Table Voltage-dependent K^+ channels
Voltage-dependent K^+ (K_v) channels.

K_v channel	Gene	Comments
K_v1.1	KCNA1	Delayed rectifier that maintains membrane potential and excitability in neurons, cardiac and skeletal muscle cells
K_v1.2	KCNA2	Delayed rectifier that maintains membrane potential and excitability in neurons, cardiac and smooth muscle cells
K_v1.3	KCNA3	Delayed rectifier that maintains membrane potential and promotes Ca^{2+} signalling in T cells (Module 9: Figure T cell Ca^{2+} signalling) and blood platelets (Module 11: Figure platelet activation)
K_v1.4	KCNA4	Fast inactivating A-type current, functions in neuronal afterpolarization
K_v1.5	KCNA5	Ultrarapid-activating K^+ current (IK_{ur}) in heart
K_v1.6	KCNA6	Delayed rectifier that maintains membrane potential in neurons
K_v1.7	KCNA7	Delayed rectifier generates currents resembling the ultrarapid-activating K^+ current (IK_{ur})
K_v1.8	KCNA10	Delayed rectifier, regulates membrane potential in renal proximal tubules
K_v2.1	KCNB1	Delayed rectifier that maintains membrane potential in neurons and muscle cells
K_v2.2	KCNB2	Delayed rectifier that maintains membrane potential and excitability in neurons
K_v3.1	KCNC1	Delayed rectifier that functions in fast-spiking by regulating duration of action potentials in presynaptic terminals
K_v3.2	KCNC2	Delayed rectifier that functions in fast-spiking by GABAergic interneurons
K_v3.3	KCNC3	A-type voltage-dependent K^+ channel in neurons
K_v3.4	KCNC4	A-type fast-inactivating located in neurons, parathyroid, pancreatic acinar cells and skeletal muscle. Colocalizes with MiRP2
K_v4.1	KCND1	A-type K^+ current expressed in multiple cell types. Function channel depends on the auxiliary KChIP subunits
Kv4.2	KCND2	A-type K^+ current responsible for the I_{to} (notch phase) of the cardiac cell action potential (Module 7: Figure cardiac action potential) and reduces the back-propagating action potentials in CA1 hippocampal neurons
Kv4.3	KCND3	A-type K^+ current responsible for the Ito (notch phase) in cardiac cells
K_v5.1	KCNF1	No channel function, but may act to modify members of the K_v2 family of channels
K_v6.1	KCNG1	No channel function but may act to suppress members of the K_v2 family of channels
K_v6.2	KCNG2	No channel function but may act to modify members of the K_v2 family of channels
K_v6.3	KCNG3	No channel function but may act to modify members of the K_v2 family of channels
K_v6.4	KCNG4	No channel function but may act to modify members of the K_v2.1 channels
K_v7.1	KCNQ1	Delayed rectifier that is responsible for I_{ks} current that functions to repolarize the cardiac action potential (Module 7: Figure cardiac action potential). Mutations cause congenital long QT syndrome
K_v7.2	KCNQ2	Delayed rectifier that contributes to the M current that regulates neuronal excitability (Module 10: Figure tonic excitatory drive and neuronal rhythms)
K_v7.3	KCNQ3	Delayed rectifier that contributes to the M current that regulates neuronal excitability (Module 10: Figure tonic excitatory drive and neuronal rhythms). May contribute to benign familial neonatal convulsions
K_v7.4	KCNQ4	Delayed rectifier that controls K^+ efflux from outer hair cells. KCNQ4 mutations cause autosomal dominant nonsyndromic deafness type 2 (DFNA2)
K_v7.5	KCNQ5	Delayed rectifier that contributes to the M current that regulates neuronal excitability
K_v8.1	KCNV1	Also classified as K_v2.3. Has no channel function but functions to suppress members of the K_v2 family of channels
K_v8.2	KCNV2	No channel function but may act to suppress members of the K_v2 family of channels in multiple cell types
K_v9.1	KCNS1	No channel function but may act to modulate members of the K_v2 family of channels
K_v9.2	KCNS2	No channel function but may act to modulate members of the K_v2 family of channels
K_v9.3	KCNS3	No channel function but may act to modulate members of the K_v2 family of channels to regulate membrane potential in pulmonary artery myocytes
K_v10.1	KCNH1	Delayed rectifier related to the *Drosophila* channel *ether-a-go-go.* Controls cell cycle and/or cell proliferation
K_v10.2	KCNH5	Outward-rectifying, non-inactivating K^+ channel
K_v11.1	KCNH2	Also known as human *ether-a-go-go*-related gene (hERG) responsible for cardiac I_{kr} current that functions to repolarize the action potential (Module 7: Figure cardiac action potential). Mutations cause autosomal dominant long QT syndrome 2
K_v11.2	KCNH6	K_v channel of unknown function
K_v11.3	KCNH7	K_v channel of unknown function
K_v12.1	KCNH8	Slowly activating and deactivating K_v channel
K_v12.2	KCNH3	K_v channel of unknown function
K_v12.3	KCNH4	Slowly activating Kv channel

Much of the information used to compile this table was taken from Gutman et al. 2005.

K_V7.4

This delayed rectifier controls K^+ efflux from outer hair cells. Mutations in KCNQ4, which encode K_V7.4, cause autosomal dominant nonsyndromic deafness type 2 (DFNA2).

K_v11.1

The K_v11.1 channel, which is also known as the human *ether-a-go-go* (hERG) channel, is a member of the family of voltage-dependent K^+ (K_v) channels (Module 3: Table voltage-dependent K^+ channels). It generates a rapid delayed rectifier K^+ current (I_{kr}) that has a number of functions. In the heart, the I_{kr} functions to repolarize the cardiac action potential (Module 7: Figure cardiac action potential).

The K_v11.1 channel is an example of the PtdIns4,5P_2 regulation of ion channels (Module 2: Figure PtdIns4,5P_2 regulation of K^+ channels).

Mutations in KCNQ1 that codes for the K_v11.1 channel cause congenital long QT syndrome.

K^+ channel auxiliary subunits

The pore-forming α subunits of many of the K^+ channels are associated with various auxiliary subunits (Module 3: Table K^+ channel auxiliary subunits) that function in both

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their trafficking to the membrane and in their channel gating properties. Some of the auxiliary subunits are cytoplasmic proteins that associate with the cytoplasmic regions of the channel proteins, whereas others are integral membrane proteins that are in close contact with the channels (Module 3: Figure K^+ channel domains). There are a number of these auxiliary subunits:

- $K_v\beta$ subunits
- K^+-channel-interacting protein (KChIP)
- Dipeptidyl-peptidase-like protein (DPPX)
- Minimal K^+ (MinK) channel subunits
- Sulphonylurea receptor (SUR) subunits
- $K_{Ca}\beta$ subunits

$K_v\beta$ subunits

The $K_v\beta1$–4 subunits are cytoplasmic proteins that associate with members of the large family of voltage-dependent K^+ (K_v) channels (Module 3: Figure K^+ channel domains). They have multiple functions. They can help in the trafficking of the pore-forming $K_v\alpha$ subunit to the plasma membrane. They also have a role in bringing about rapid inactivation of the functional channel. The $K_v\beta$ subunits form a tetramer that associates with the cytoplasmic N-terminal domain of the $K_v\alpha$ subunit.

Dipeptidyl-peptidase-like protein (DPPX)

Dipeptidyl-peptidase-like protein (DPPX) is a K^+ channel auxiliary subunit that functions to modulate the activity of the voltage-dependent K^+ (K_v) channels (Module 3: Figure K^+ channel domains). For example it contributes to the activity of the $K_v4.2$ channels.

Minimal K^+ (MinK) channel subunits

The minimal K^+ (MinK) and the four MinK-related proteins (MiRP1–4) are integral membrane proteins that have a single transmembrane (TM) domain (Module 3: Figure K^+ channel domains). They function as auxiliary subunits to modulate the activity of various K^+ channels. MinK modulates the activity of the $K_v7.1$ channel responsible for producing the I_{ks} current that functions to repolarize the cardiac action potential (Module 7: Figure cardiac action potential). Mutations in MinK cause congenital long QT syndrome. MiRP1 can interact with a number of the voltage-dependent K^+ (K_v) channels such as $K_v7.1$ and the $K_v11.1$, which is also known as the human *ether-a-go-go* (hERG) channel.

Mutations in the KCNE2 gene that codes for the auxiliary subunit MiRP1 cause congenital long QT syndrome. MiRP2 colocalizes with $K_v3.4$ in skeletal muscle. The KCNE3 gene, which codes for MiRP2, is mutated in acute periodic paralysis. MiRP4 is mutated in Alport syndrome, mental retardation, midface hyperplasia and elliptocytosis (AMME).

Sulphonylurea receptor (SUR) subunits

The sulphonylurea receptor-1 (SUR1) subunit is an integral component of the ATP-sensitive (K_{ATP}) channels. SUR1, which is one of the ATP-binding cassette proteins that is encoded by ABCC8, functions to regulate the $K_{ir}6.1$ and $K_{ir}6.2$ channels (Module 3: Table K^+ channel auxillary subunits).

$K_{Ca}\beta$ subunits

There are four $K_{Ca}\beta$ auxiliary subunits ($K_{Ca}\beta1$–4) (Module 3: Table K^+ channel auxiliary subunits). Each has two transmembrane (TM) domains with the C- and N-termini facing the cytoplasm (Module 3: Figure K^+ channel domains). These subunits, which function to modulate the activity of the α-subunit of the large-conductance (BK) channels, have different properties.

The $K_{Ca}\beta1$ subunit operates by changing the Ca^{2+}-dependence of the pore-forming α-subunit. In mice where the $\beta1$ subunits have been knocked out, the incidence of the large BK-dependent hyperpolarizing responses to Ca^{2+} 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. Expression of the $\beta1$ subunits may thus be an important determinant of hypertension.

The $K_{Ca}\beta2$ subunit functions like the $K_{Ca}\beta1$ subunit to change the Ca^{2+}-dependence of the pore-forming α-subunit

The $K_{Ca}\beta3$ subunit alters the gating properties of the α-subunit by providing an inactivation domain and by blocking the external pore through cysteine cross-linking.

The $K_{Ca}\beta4$ subunit modulates the α-subunit through a phosphorylation-dependent mechanism.

Inward rectifier K^+ (K_{ir}) channels

The inward rectifier K^+ (K_{ir}) channel family consist of 15 members (Module 3: Table inward rectifier K^+ (K_{ir}) channel). The pore-forming subunit has two transmembrane domains and one pore-forming loop and four of these subunits are thought to come together to form the functional channel. They are called inward rectifiers because they conduct K^+ better in an inward rather than an outward direction. Such an inward flow occurs when the membrane potential is more negative than the equilibrium potential for K^+ (E_K).This influx is prevented at more positive potentials by Mg^{2+} and polyamines such as spermine blocking the channel. As a result of this unusual property, the K_{ir} channels function to set the resting potential close to E_K.

These K_{ir} channels have a number of prominent signalling functions:

- $K_{ir}1.1$
- $K_{ir}2.1$
- $K_{ir}3$
- $K_{ir}4.1$
- $K_{ir}6$

$K_{ir}1.1$

The $K_{ir}1.1$, which is also known as the renal outer medullary K^+ (ROMK) channel transports K^+ across the luminal membrane of the thick ascending loop of Henle (TALH) (Module 7: Figure kidney salt reabsorption). $K_{ir}1.1$ is also expressed on the apical membrane of type I glial-like cells located in the taste buds where it may

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Module 3: | Table K^+ channel auxiliary subunits
K^+ channel auxiliary subunits

K^+ channel auxiliary subunits	Gene	Comments
Auxiliary subunits of K_v and K_{ir} channels		
$K_v\beta1$		
$K_v\beta2$		
$K_v\beta3$		
$K_v\beta4$		
KChIP1		
KChIP2		
KChIP3		
KChIP4		
DPPX		Dipeptidyl-peptidase-like protein. Associates with $K_v4.2$ channels
MinK	KCNE1	Minimal K^+ (MinK) protein associates with K_v 7.1 to form a functional channel. Mutations in MinK cause long QT syndrome 2
MiRP1	KCNE2	Mink-related protein 1 (MiRP1) is an auxiliary subunit that appears to associate with $K_v11.1$. Mutations in MiRP1 cause long QT syndrome 2
MiRP2	KCNE3	
MiRP3	KCNE4	
MiRP4	KCNE5	
SUR1		Sulphonylurea receptor-1 associates with $K_{ir}6.2$ to form a functional K_{ATP} channel
Auxiliary subunits of Ca^{2+}-sensitive K^+ (K_{Ca}) channels		Integral membrane proteins associated with the α subunit of K_{Ca} channels (Module 3: Figure K^+ channel domains)
$K_{Ca}\beta1$	KCNMB1	
$K_{Ca}\beta2$	KCNMB2	
$K_{Ca}\beta3$	KCNMB3	
$K_{Ca}\beta4$	KCNMB4	

Module 3: | Table inward rectifier K^+ (K_{ir}) channels
Inward rectifier K^+ (K_{ir}) channel family.

Inward rectifier K^+ (K_{ir}) channel family	Gene	Comments
$K_{ir}1.1$	KCNJ1	Also known as ROMK that transports K^+ in the kidney (Module 7: Figure kidney salt reabsorption). Mutated in Bartter's disease
$K_{ir}2.1$	KCNJ2	Provides the I_{K1} current that holds the cardiac resting potential in its hyperpolarized state (Module 7: Figure cardiac action potential)
$K_{ir}2.2$		
$K_{ir}2.3$		
$K_{ir}2.4$		
G protein-gated inward rectifier K^+ (GIRK) channels		Also known as K_G channels that are activated by $G_{\beta\gamma}$ subunits
$K_{ir}3.1$ (GIRK1)		Function in acetylcholine-dependent slowing of the cardiac pacemaker
$K_{ir}3.2$ (GIRK2)		Expressed in neurons
$K_{ir}3.3$ (GIRK3)		
$K_{ir}3.4$ (GIRK4)		Expressed in cardiac cells
$K_{ir}4.1$		Glial-specific channel that functions in the spatial buffering of K^+ in the CNS (Module 7: Figure astrocyte structure)
$K_{ir}5.1$		Do not form functional homomeric channels but can participate with $K_{ir}4.1$ to form functional channels in glial cells
ATP-sensitive K^+ (K_{ATP}) channels		Functions in insulin secretion by insulin-secreting β-cells (Module 7: Figure β-cell signalling)
$K_{ir}6.1$	KCNJ8	Function to couple energy metabolism to electrical activity in the plasma membrane
$K_{ir}6.2$	KCNJ11	

function in K^+ recycling (Module 10: Figure taste receptor cells).

Mutations in the gene (KCNJ1) that encodes $K_{ir}1.1$ cause Type II Barrter's disease.

$K_{ir}2.1$

The channel provides the I_{K1} current that holds the cardiac resting potential in its hyperpolarized state (Module 7: Figure cardiac action potential). Expression of $K_{ir}2.1$ is modulated by miR-1 during the differentiation of cardiac cells (Module 8: Figure cardiac development). Mutation of the gene that encodes $K_{ir}2.1$ causes Andersen-Tawil syndrome.

$K_{ir}3$

The K_{ir} family constitute the G protein-gated inward rectifier K^+ (K_G) channels, which are also known as GIRK channels that regulate the membrane potential in many excitable cells.

$K_{ir}4.1$

$K_{ir}4.1$ belongs to the inward rectifier K^+ (K_{ir}) channel family. It is a glial-specific channel that functions in the spatial buffering of K^+ by astrocytes in the nervous system. It provides a 'K^+ siphoning' system that takes K^+ away from active neuronal sites and releases it near the blood vessels (Module 7: Figure astrocyte structure). A decrease in the expression of $K_{ir}4.1$ in satellite glial cells may be one of the causes of neuropathic pain.

$K_{ir}6$

The two $K_{ir}6$ channels make up the ATP-sensitive K^+ (K_{ATP}) channels that function to couple energy metabolism to electrical activity in the plasma membrane as occurs in insulin-secreting β-cells (Module 7: Figure β-cell signalling).

Mutations in $K_{ir}6.2$ have been linked to diabetes.

Two-pore domain potassium channels (K_{2P})

The membrane potential of cells is dominated by its high permeability to K^+. There is a high background K^+ current and much of this is carried by a family of two-pore domain K^+ (K_{2P}) channels (Module 3: Table two-pore domain K^+ channels). The functional channel is usually a homodimer. Each subunit has an unusual structure in that there are four transmembrane domains and two pore

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loop (P) domains (Module 3: Figure K^+ channel domains). Within the homodimer, the four P domains come together to form the selectivity filter that resembles the structure found in other K^+ channels (Module 3: Figure K^+ channel structure).

The K_{2P} family is divided into six subfamilies, which are mainly distinguished on the basis of how they are activated:

- TWIK
- TREK
- TASK
- TALK
- THIK
- TRESK

TWIK

The tandem of P domains in a weak inward rectifying K^+ (TWIK) channel subfamily, which are a subfamily of the large family of two-pore domain K^+ (K_{2P}) channels, has three members: TWIK-1, TWIK-2 and KCNK7 (Module 3: Table two-pore domain K^+ channels).

TWIK-1 is expressed in the brain, heart, lung and kidney. In the case of kidney, TWIK-1 may contribute to the recycling of K^+ in the thick ascending limb of the loop of Henle (TALH) (Module 7: Figure kidney salt reabsorption). This channel has also been located on intracellular membranes where it might function in membrane and protein trafficking.

The TWIK-1 channel is down-regulated in various tumours (ovarian cancer, melanomas and glioblastomas). A possible role in the regulation of tumour growth is supported by the finding that TWIK-1 expression is increased by TAp73, which is a member of the p53 tumour suppressor family.

The KCNK7 member of this subfamily is unusual in that the C-terminal tail has an EF-hand suggesting that it mught be sensitive to elevations in cytosolic Ca^{2+}.

TREK

The TWIK-related K^+ (TREK) channels, which are a subfamily of the large family of two-pore domain K^+ (K_{2P}) channels, has three members: TREK-1, TREK-2 and TWIK-related arachidonic acid activated K^+ (TRAAK) channel. These channels are strongly expressed in the brain, but are also found in other tissues. This TREK/TRAAK subfamily are mechanosensitive channels in that the channel opens when the membrane is stretched and closes during membrane shrinkage. The mechanosensitive region resides in a stretch of the molecule that extends from the terminal region of transmembrane segment 4 to include part of the long C-terminus that extends into the cytoplasm (Module 3: Figure K^+ channel domains). In conjunction with their mechanosensitivity, this channel subfamily is sensitive to arachidonic acid (AA) and to other polyunsaturated fatty acids (PUFAs), such as docosahexenoic acid, linolenic and linoleic acid. One action of these PUFAs, which bind to a site on the C-terminal tail, is to enhance channel sensitivity to mechanical stimuli. The C-terminal tail is also the target of various anaesthetics that activate these channels to bring about membrane hyperpolarization.

The TREK-1 channel is somewhat unusual compared with other members of the family of two-pore domain K^+ (K_{2P}) channels in that it displays outward rectification and has some degree of voltage dependence. Following membrane depolarization, the channel opens quickly and inactivates very slowly, which is in keeping with its role as a background leak channel.

Activity of the TREK-1 and TREK-2 channels is regulated by a number of mechanisms. The channel is sensitive to intracellular acidification with the H^+ sensor located on Glu-306 in the C-terminal tail. These channels are also regulated by G protein-coupled receptors (GPCRs) that are coupled to the cyclic AMP signalling pathway. In the case of TREK-1, AKAP150 is tightly associated with the C-terminal tail and positions protein kinase A (PKA) such that it can phosphorylate Ser-333, resulting in a decrease in channel activity. The diacylglycerol (DAG)/protein kinase C (PKC) signalling cassette can also reduce the activity of TREK-1 and TREK-2 by phosphorylating Ser-300 and Ser-359 respectively. The same serine residues on these two channels can also by phosphorylated by AMP-activated protein kinase (AMPK), which thus provides a mechanism for the metabolic state of the cell to control membrane potential. In TREK-1, activation of the cyclic GMP signalling pathway by nitric oxide (NO) results in phosphorylation of Ser-351 and a decrease in channel activity.

TREK channels have been implicated in the control of a wide range of cellular functions such as organizing the growth cone during brain morphogenesis, relaxation of gastrointestinal, bladder and uterine smooth muscle cells, dilatation of cerebral arteries, and aldosterone secretion by the zona glomerulosa cells in the adrenal cortex (Module 7: Figure glomerulosa cell signalling).

TASK

The TWIK-related, acid-sensitive K^+ (TASK) channels, which belong to a subfamily of the large family of two-pore domain K^+ (K_{2P}) channels, has three members: TASK-1, TASK-3 and TASK-5 (Module 3: Table two-pore domain K^+ channels). As their name imples, these channels are very sensitive to external pH with the H^+ sensor located on the extracellular loop that connects TM1 to TM2 (Module 3: Figure K^+ channel domains).

One of the functions of TASK is to regulate aldosterone secretion by the zona glomerulosa cells in the adrenal cortex (Module 7: Figure glomerulosa cell signalling). The TASK-1 and TASK-3 channels are inhibited by receptors such as angiotensin II that act by hydrolysing PtdIns4,5P_2. A similar inhibitory action might control the TASK channels that are expressed in a number of neuronal cells such as hypoglossal motoneurons, respiratory neurons and thalamocortical neurons. The TASK-2 channel has an important role in facilitating the reabsorption of Na^+ and HCO_3^- across the proximal convoluted tubule (PCT) (Module 7: Figure proximal tubule function).

The role of these channels in regulating the background K^+ current and hence the resting membrane potential may

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explain how a dominant-negative mutation of TASK-3 results in mental retardation.

TALK

The TWIK-related alkaline pH-activated K^+ (TALK) channels, which belong to a subfamily of the large family of two-pore domain K^+ (K_{2P}) channels, has three members: TASK-2, TALK-1 and TALK-2 (Module 3: Table two-pore domain K^+ channels). A characteristic feature of these channels is their activation following alkalinization of the external medium in the pH 7.5–10 range. The sensitivity to external pH resides in basic amino acids located in the second pore loop (P) located between TM3 and TM4 (Module 3: Figure K^+ channel domains).

THIK

The tandem pore domain halothane-inhibited K^+ (THIK) channels, which are a subfamily of the large family of two-pore domain K^+ (K_{2P}) channels, has two members: THIK-1 and THIK-2 (Module 3: Table two-pore domain K^+ channels). These two channels, which behave as typical background K^+ channels, are fairly widely distributed and are characterized by being sensitive to halothane. Their function remains to be determined.

TRESK

The TWIK-related spinal cord K^+ (TRESK) channel is the single member of a subfamily of the two-pore domain K^+ (K_{2P}) channels. This TRESK channel is particularly prevalent in neurons, but has also been identified in testis. It has an unusual gating behaviour in that there are 'square wave-like openings' when the membrane is depolarized, but this changes to bursts of very brief openings when the membrane is hyperpolarized. Unlike other K_{2P} channels, the TRESK channel is activated by receptors that act through the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette. Channel opening depends on Ca^{2+} activating calcineurin (CaN), which is closely associated with the cytoplasmic loop that links TM2 to TM3 (Module 3: Figure K^+ channel domains). The binding of CaN, which depends on an elevation of Ca^{2+}, dephosphorylates Ser-276 resulting in an increase in channel opening.

Genetic analysis of familial migraine has identified mutations in TRESK as a possible cause for the high incidence of migraine in some families.

Module 3: | Table two-pore domain potassium channels (K_{2P})
Two-pore domain potassium channel ($K2_P$) family.

Two-pore domain potassium channels (K_{2P}) (Module 3: Figure K^+) (channel domains)	Gene	This large family of channels, which provide background leak K^+ currents, are divided into six subfamilies
TWIK-1 (K_{2P}1.1)	KCNK1	Tandem of P domains in a weak inward rectifying K^+ (TWIK) channels
TWIK-2 (K_{2P}6.1)	KCNK6	
K_{2P}7.1	KCNK7	
TREK-1 (K_{2P}2)	KCNK2	TWIK-related K^+ channel 1
TREK-2 ($K_2$10)	KCNK10	TWIK-related K^+ channel 2
TRAAK (K_{2P}4)	KCNK4	TWIK-related arachidonic acid-activated K^+ channel
TASK-1 (K_{2P}3)	KCNK3	TWIK-related, acid-sensitive K^+ channel regulates K^+ efflux in adrenal glomerulosa cells (Module 7: Figure glomerulosa cell signalling)
TASK-3 (K_{2P}9)	KCNK9	
TASK-5 (K_{2P}15)	KCNK15	
TASK-2	KCNK5	TWIK-related alkaline pH-activated K^+ channel
TALK-1	KCNK16	
TALK-2	KCNK17	
THIK-1	KCNK13	Tandem pore domain halothane-inhibited K^+ channel
THIK-2	KCNK12	
TRESK (K_{2P}18.1)	KCCK18	TWIK-related spinal cord K^+ channel. Mutation of TRESK has been linked to migraine

This large family of channels provide the large background K^+ current characteristic of the plasma membrane of all cells. Information for this Table was taken from Enyedi and Czirjak (2010).

Ca^{2+}-sensitive K^+ channels

Many cells have K^+ channels that are activated by Ca^{2+}. These Ca^{2+}-sensitive K^+ channels are a heterogeneous family of channels whose opening is activated by an increase in intracellular Ca^{2+} resulting in hyperpolarization of the membrane. These channels are particularly important in excitable cells, where they function to repolarize the membrane following Ca^{2+}-dependent action potentials in neurons and smooth muscle cells. In these excitable cells, these channels regulate neuronal excitability by adjusting interspike frequencies and they contribute to neurosecretion, pacemaking activities and smooth muscle tone. However, these channels are equally important in non-excitable cells, where a link has been established between K^+ channels and cell proliferation. To carry out these different functions, cells have access to a number of such channels with different properties that are divided into three main families:

- Large-conductance (BK) channels
- Intermediate-conductance (IK) channels
- Small-conductance (SK) channels

The K^+ channel structure is similar for all these families, especially in the organization of the permeation pathway, but they differ in the way that Ca^{2+} activates the gating process.

Large-conductance (BK) channels

BK channels (K_{Ca}1.1) have a large conductance (~250 pS) and are activated in a highly synergistic manner by both Ca^{2+} and membrane depolarization. When the channels open, the rapid efflux of K^+ hyperpolarizes the membrane potential, which then shuts off any voltage-dependent Na^+ and Ca^{2+} channels. This ability to regulate both membrane excitability and the influx of Ca^{2+} is used to control many processes:

- Smooth muscle Ca^{2+} signalling (Module 7: Figure smooth muscle cell E-C coupling). The BK channel

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contributes to the uterine smooth muscle membrane oscillator (Module 7: Figure uterus activation). An increase in cholesterol associated with obesity appears to enhance BK channels and the resulting increase in the quiescent state of the uterus can lead to the risk of complications in pregnancy.

- The BK channel is one of the protein substrates that are phosphorylated by cyclic GMP-dependent protein kinase (cGK) in smooth muscle cells (Module 7: Figure smooth muscle cell cGMP signalling), where it plays an important role during the corpus cavernosum relaxation required for penile erection (Module 7: Figure corpus cavernosum).
- One of the main functions of these BK channels is to modulate neuronal excitability by facilitating the repolarization of action potentials.
- The BK channel in the endfoot of astrocytes releases K^+ to control smooth muscle contractility in cerebral arterioles (Module 7: Figure astrocyte endfoot signalling).
- Activation of BK channels contributes to the salivary gland control mechanisms that regulate fluid secretion by salivary glands (Module 7: Figure salivary gland secretion).
- BK channels increases the efflux of K^+ from mesangial cells to hyperpolarize the membrane as part of a negative-feedback loop (Module 7: Figure mesangial cell).

These BK channels are unusual with regard to their sensitivity to Ca^{2+} in that they can respond to a wide range of Ca^{2+} concentrations (Module 3: Table properties of Ca^{2+}-sensitive K^+ channels). They have high-affinity sites capable of responding to global Ca^{2+} signals. They also have low-affinity sites that can respond to more local signals, such as the relatively high levels found near the opening of both Ca^{2+} entry and release channels, which explains their close association with various types of Ca^{2+} channels. For example, BK channels are located within 30 nm of the N-type voltage-operated channels (VOCs). BK channels can also be activated by smooth muscle cell Ca^{2+} sparks that are generated by ryanodine receptors (RYRs) (Module 7: Figure smooth muscle cell cGMP signalling). The low sensitivity to Ca^{2+} of these BK channels is thus matched to the high concentrations of Ca^{2+} that exist in the microdomains close to the vicinity of such Ca^{2+} channels. Since such spark sites appear to be restricted to an area with a diameter of about 500 nm, it seems that the BK channels are concentrated at such sites where they are exposed to Ca^{2+} concentrations of the order of 10 μM.

Like many other ion channels, there are transmembrane (TM) domains with a pore-forming loop (P) between TM5 and TM6 (Module 3: Figure K^+ channel domains). The large-conductance (BK) channels are unusual in that they have an extra TM domain in front of TM1, which means that the N-terminal region faces the outside. These BK channels are also unusual in that they are voltage-sensitive and the voltage sensor is thought to be in TM4. It is in the C-terminal region, which contains the regulator of K^+ conductance (RCK), where the main difference between the channels exists. In BK channels, there is a high-affinity Ca^{2+}-binding site called the 'calcium bowl' that has a domain containing five consecutive aspartic acid residues. The low-affinity RCK site is located close to TM6. By contrast, the Ca^{2+}-sensitivity of the intermediate-conductance (IK) and small-conductance (SK) channels depends upon a resident calmodulin molecule.

The activity of the BK channels is also regulated by a family of K_{Ca} β subunits, which belong to a group of K^+ channel auxiliary subunits (Module 3: Table K^+ channel auxillary subunits). In smooth muscle cells, these pore-forming α-subunits are associated with accessory β1 subunits, which act to increase the Ca^{2+}-sensitivity of the BK channels. In effect, these β1 subunits enhance the coupling between the RYRs and the BK channels. Expression of these β1 subunits is regulated by Ca^{2+} acting through the transcription factor NFAT3, which is an example of how Ca^{2+} can regulate the expression of Ca^{2+} signalling components (Module 4: Figure NFAT control of Ca^{2+} signalling toolkit). In mice where the β1 subunits have been knocked out, the incidence of the large BK-dependent hyperpolarizing responses to Ca^{2+} 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. It is concluded that expression of the β1 subunits may be an important determinant of hypertension.

Concurrent generalized epilepsy with paroxysmal dyskinesia is caused by a single missense mutation located in the region of the regulator of conductance for K^+ (RCK).

There are indications that other signalling pathways can modulate the BK channels. For example, in smooth muscle cells, the BK channel appears to be associated with the tyrosine kinase c-Src. In response to agonist stimulation, the c-Src phosphorylates the BK channel to cause channel inhibition and vasoconstriction.

Intermediate-conductance (IK) channels

The IK channel ($K_{Ca}3.1$), which has a conductance of 20--80 pS, is expressed mainly in peripheral tissues such as those of the haematopoietic system, colon, placenta, lung and pancreas. The $K_{Ca}3.1$ channel in red blood cells was the first Ca^{2+}-sensitive K^+ channel to be identified and it has been implicated in a wide range of cell functions, including vasodilation of the microvasculature, K^+ flux across endothelial cells of brain capillaries and the phagocytic activity of neutrophils. $K_{Ca}3.1$ is of primary importance in the relationship between K^+ channels and cell proliferation. In the latter case, a human *hIKCa1* gene encodes the channel found in T cells, which is responsible for the hyperpolarization that is required to keep Ca^{2+} flowing into the cell through the I_{CRAC} channels (Module 9: Figure T cell Ca^{2+} signalling).

In comparison with the large-conductance (BK) channels, $K_{Ca}3.1$ is much more sensitive to Ca^{2+} (Module 3: Table properties of Ca^{2+}-sensitive K^+ channels) and can thus respond to the global level of Ca^{2+}. This high affinity for Ca^{2+} depends upon four resident calmodulin molecules tightly bound to the cytoplasmic tails of the four

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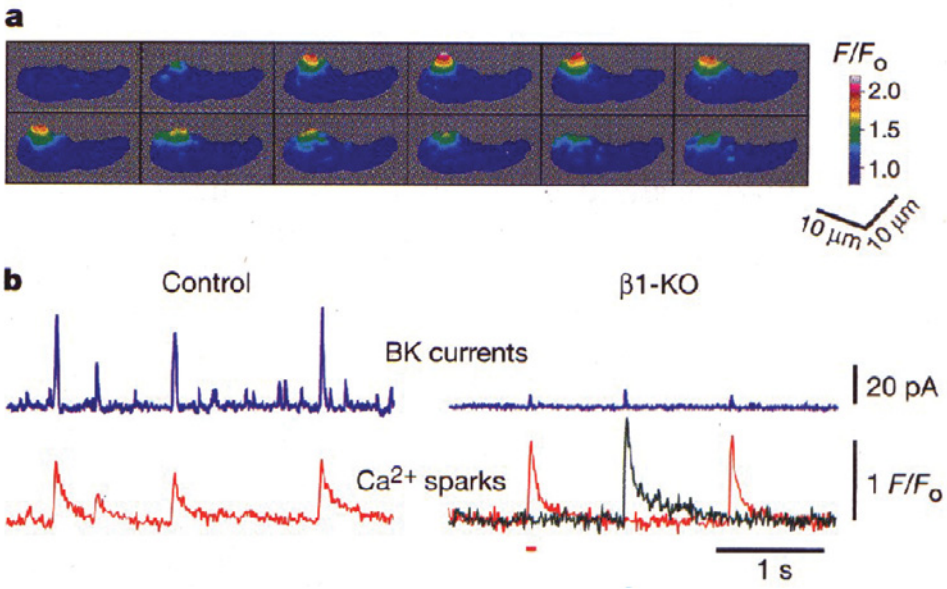
Module 3: | Figure smooth muscle cell Ca^{2+} sparks



Effect of deleting the β1 subunit of the large-conducatance (BK) channel on spark frequency in smooth muscle cells.

a. Images of a smooth muscle cell from a β1 knockout mouse (β1-KO) taken every 8.33 ms to show the generation and recovery of a Ca^{2+} spark. b. Simultaneous measurements of large-conducatance (BK) currents (shown in blue) and Ca^{2+} sparks (red) in a control cell and a $\beta1^{-/-}$ (β1-KO) cell, which had two spark sites (red and black). Note how the sparks in the β1-KO cell had very little effect on the BK current in comparison with the control cells, where each spark produced a large current response. Reproduced by permission from Macmillan Publishers Ltd: *Nature*, Brenner, R., Peréz, G.J., Bonev, A.D., Eckman, D.M., Kosek, J.C., Wiler, S.W., Patterson, A.J., Nelson, M.T. and Aldrich, R.W. (2000) Vasoregulation by the β1 subunit of the calcium-activated potassium channel. Vol. 407, pp. 870-876. Copyright (2000); http://www.nature.com; Brenner et al. 2000.

Module 3: | Table properties of Ca^{2+}-sensitive K^+ channels

K^+ channel	Genes	Inhibitors	Voltage sensitivity	Ca^{2+} sensitivity	Ca^{2+} sensor
BK					
$K_{Ca}1.1$	KCNMA1	Tetraethylammonium, charybdotoxin, iberiotoxin	Yes	1-10 μM high affinity; 100 μM low affinity	Ca^{2+} bowl
SK					
$K_{Ca}2.1$ (SK1)	KCNN1	Apamin, scyllatoxin	No	100-400 nM	Calmodulin
$K_{Ca}2.2$ (SK2)	KCNN2				
$K_{Ca}2.3$ (SK3)	KCNN3				
IK					
$K_{Ca}3.1$	KCNN4	Charybdotoxin, clortrimazole	No	300 nM	Calmodulin

pore-forming α-subunits (Module 3: Figure IK/SK channel opening). Before the channel can open, Ca^{2+} must bind to each of the calmodulins to induce the co-operative conformational change that opens the gate, which explains why this process has a Hill coefficient of 4. This Ca^{2+}-induced gating process resembles that which has been described for the small-conductance (SK) channels. The fact that calmodulin is prebound to its effector enables the channels to respond to Ca^{2+} very quickly.

The PtdIns3P signalling cassette may play a role in regulating the activity of $K_{Ca}3.1$. If this signalling lipid is hydrolysed by MTMR6, which is one of the myotubularins, there is a decrease in the activity of the Ca^{2+} activated channel.

Small-conductance (SK) channels

SK Ca^{2+}-activated K^+ channels, which have a conductance of 5–15 pS, are particularly important in neurons where they regulate neuronal excitability. For example, they function to inhibit cell firing by causing a membrane after-hyperpolarization (AHP), as has been described in CA1 hippocampal neurons (Module 10: Figure neuronal SK channel function). There are three SK family members (Module 3: Table properties of Ca^{2+}-sensitive K^+ channels). SK channels are located 50–150 nM from the L-type voltage-operated channel (VOC), and this close association accounts for their rapid activation.

SK channels play an important role in regulating the membrane potential of hair cells during the process of somatic electromotility. Acetylcholine released from the main efferent input to the cochlea terminates on the outer hair cells, where it acts through nicotinic acetylcholine receptors (nAChRs) to gate Ca^{2+} that then activate the SK channels to produce a rapid membrane hyperpolarization.

K^+ channel structure

Considerable progress has been made in determining the structural organization of K^+ channels. They all share a

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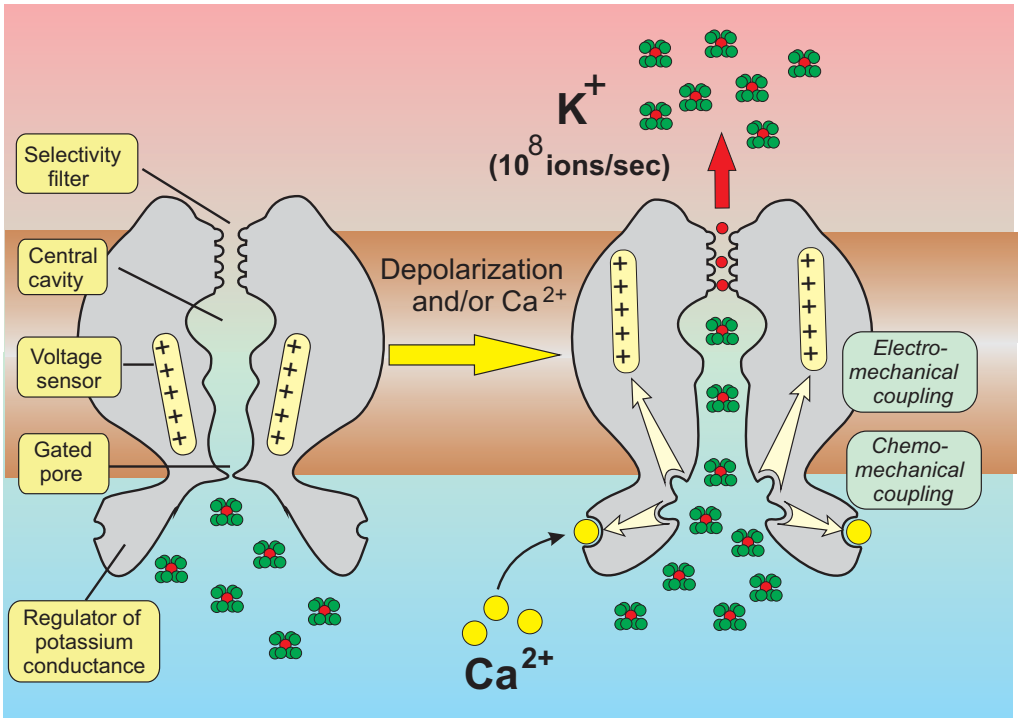
Module 3: | Figure K^+ channel organization



Functional organization of Ca^{2+}-sensitive K^+ channels.

The closed channel (shown on the left) opens in response to membrane depolarization and/or an increase in internal Ca^{2+} to allow K^+ to flow out of the cell at a rate of 10^8 ions/s. Depolarization is detected by a voltage sensor that opens the pore through an electromechanical coupling process. Ca^{2+} acts through binding sites located on a region called the regulator of K^+ conductance to open the pore through a chemomechanical coupling process. Once these activation processes open the gated pore, K^+ enters the central cavity where it gains access to the selectivity filter. The latter provides a K^+-specific filter, which allows K^+ to pass through with a high degree of selectivity. As K^+ ions (red dots) pass through the filter, they lose their water of hydration (green dots). An animated version of this figure is available.

number of similarities, particularly with regard to the permeation and channel gating mechanisms. These channels have a number of distinct domains (selectivity filter, central cavity, voltage sensor, gated pore and regulator of K^+ conductance) that perform different functions (Module 3: Figure K^+ channel organization). The molecular components of these domains have now been defined (Module 3: Figure K^+ channel structure).

Before describing how these domains co-operate to gate K^+ ions, it is important to consider the remarkable permeation properties of these channels. In order to have an impact on the membrane potential, which is their primary function, the channels must conduct a large number of K^+ ions (i.e. 10^8 ions/s) while maintaining a high degree of selectivity. This balance between the need for high flux rates and high selectivity is achieved through the operation of a very precise selectivity filter (Module 3: Figure K^+ channel organization). Another very important property of these channels is that they can be rapidly switched on and off by stimuli such as depolarization and/or an increase in the intracellular level of Ca^{2+}.

The molecular organization of the K^+ channel domains has begun to define how they function (Module 3: Figure K^+ channel domains). Each of the subunits that make up the tetrameric K^+ channels have six transmembrane domains, except for the large-conductance (BK) channels that have an extra transmembrane (TM) domain at the N-terminus. The Ca^{2+}-sensitive regions are located at the C-terminus. The way in which Ca^{2+} functions in BK channel opening (Module 3: Figure BK channel opening) is very different to how it operates in intermediate-conductance (IK) and small-conductance (SK) channel opening (Module 3: Figure IK/SK channel opening).

Large-conductance (BK) channel opening

A critical aspect of large-conductance (BK) channel opening is that it is activated in a synergistic manner by both depolarization and by an increase in intracellular Ca^{2+} (Module 3: Figure BK channel opening). The ability of the BK channel to respond to a wide range of Ca^{2+} concentrations seems to depend on it having different sensors. The low-affinity sites are located on the Ca^{2+} bowl, whereas the regulator of conductance for K^+ (RCK) site detects the high-affinity signals (Module 3: Figure K^+ channel domains). Just how Ca^{2+} acts is uncertain because there is evidence that sensitivity to Ca^{2+} is retained even when the C-terminal region containing the bowl is removed. In the case of smooth muscle cells, an accessory β1 subunit is responsible for regulating the Ca^{2+}-sensitivity of the BK channels.

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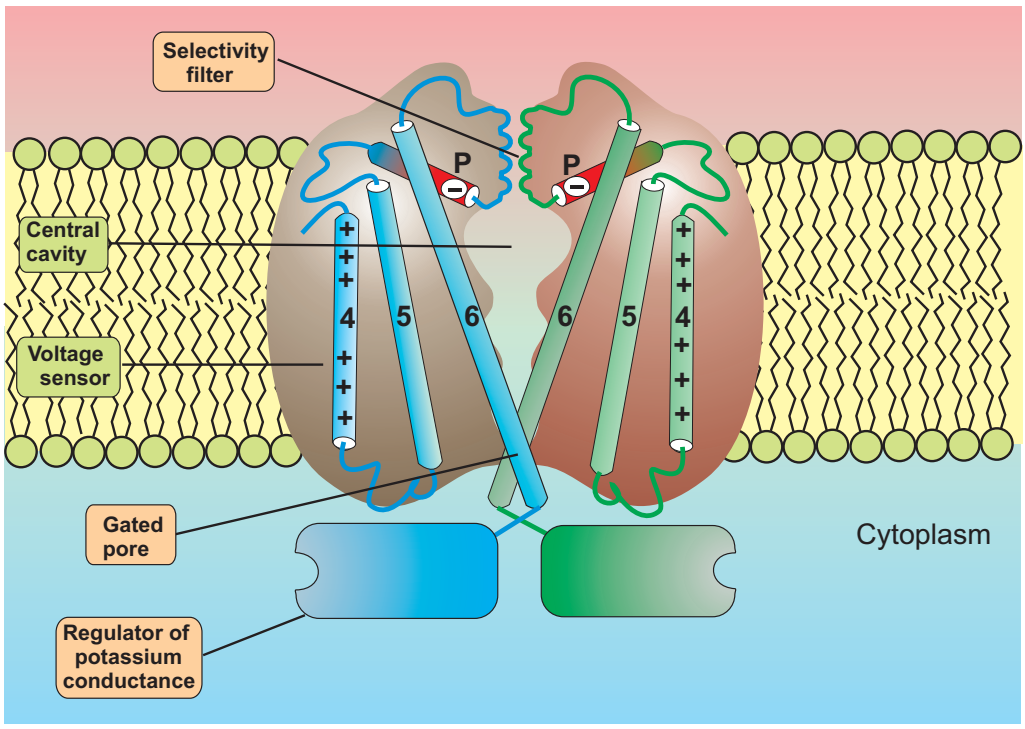
Module 3: | Figure K^+ channel structure



The structural organization of Ca^{2+}-sensitive K^+ channels.

This figure illustrates the molecular organization of two apposing subunits (shown in blue and green). Of key importance is transmembrane (TM) domain 6, which is organized in the form of an inverted tepee. At the top, it forms a cradle that supports the pore-forming loop (P), whose helix is orientated with the negative (carbonyl) end of its dipole (shaded in red) facing inwards towards the pore. At the other end (facing the cytoplasm), the TM6 domains of the different subunits converge on each other to form the gated pore. The three-dimensional organization is best appreciated by looking at stereo views of the channel (Module 3: Figure K^+ channel stereo views). An important part of this gate region is a kink in TM6, where it connects to the regulator of K^+ conductance domain.

Intermediate-conductance (IK) and small-conductance (SK) channel opening

The intermediate-conductance (IK) and small-conductance (SK) channels have a similar Ca^{2+}-dependent opening mechanism that is controlled by a resident calmodulin (CaM) molecule tightly bound to the C-terminal tails of these K^+ channels (Module 3: Figure IK/SK channel opening).

G protein-gated inwardly rectifying K^+ (GIRK) channels

The G protein-gated inwardly rectifying K^+ (GIRK) channels play a critical role in modulating excitability in both the heart and brain. There are four GIRKs (GIRK1–GIRK4) (Module 3: Table inward rectifier K^+ (K_{ir}) channel), which are usually found as heterotetramers. They are activated by neurotransmitters such as acetylcholine, dopamine, serotonin, opioids, somatostatin, adenosine and GABA which act through the pertussis toxin-sensitive heterotrimeric G proteins $G_{\alpha i}$ and $G_{\alpha o}$ (Module 2: Figure heterotrimeric G protein signalling). It is the $\beta\gamma$ subunit released from these G proteins that is responsible for activating the GIRKs. The GIRKs have multiple functions:

- GIRKs function in the acetylcholine-dependent slowing of the sinoatrial node pacemaker (Module 7: Figure cardiac pacemaker).
- In neurons, a number of transmitters can activate GIRKs to hyperpolarize the membrane giving rise to slow inhibitory postsynaptic potentials that reduces neuronal activity as has been described during the synchronization of oxytocin neurons (Module 10: Figure oxytocin neurons)

Regulators of G protein signalling (RGS) proteins may play a role in this regulation. At rest, these RGS proteins appear to be inhibited by PtdIns3,4,5P_3, but this inhibition is removed during depolarization when Ca^{2+}/calmodulin (CaM) binds to the RGS protein, and this results in closure of the GIRK channel. The GIRKs can also be modulated by phosphorylation with PKA enhancing channel activity, whereas PKC has an inhibitory effect.

ATP-sensitive K^+ (K_{ATP}) channels

ATP-sensitive K^+ (K_{ATP}) channels are regulated by the metabolic messenger adenosine triphosphate (ATP). These K_{ATP} channels are expressed in many different cells, where they function to couple energy metabolism to electrical activity in the plasma membrane (Module 2: Figure metabolic messengers). Since these channels are closed by ATP, an increase in metabolism that increases the level of ATP shuts off these K_{ATP} channels, leading to membrane depolarization. Conversely, a fall in ATP opens these channels and the membrane hyperpolarizes.

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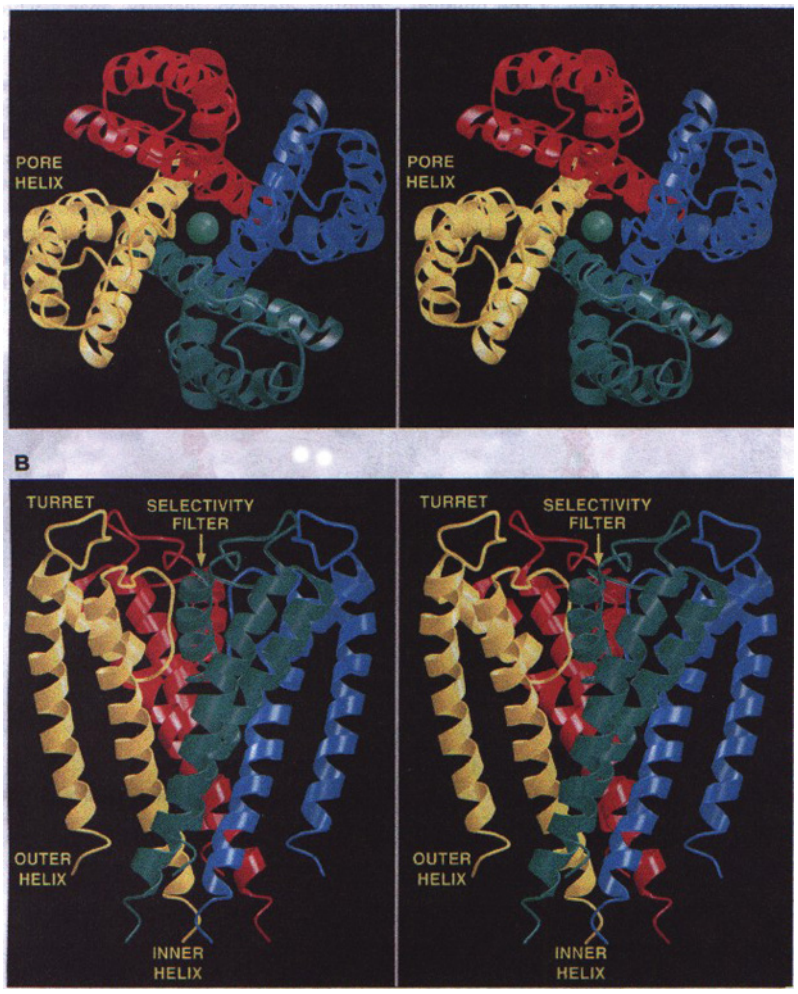
Module 3: | Figure K^+ channel stereo views



Stereo views of K^+ channel viewed from the extracellular side (top) or from the side of the channel.
Reproduced, with permission, from Doyle, D.A., Cabral, J.M., Pfuetzner, R.A., Kuo, A., Gulbis, J.M., Cohen, S.L., Chait, B.T. and MacKinnon, R. (1998) The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. Science 280:69-77. Copyright (1998) American Association for the Advancement of Science; see Doyle et al. 1998).

The K_{ATP} channel is made up of pore-forming $K_{ir}6$ subunits and regulatory sulphonylurea receptor-1 (SUR1) subunits. The functional channel is an octamer composed of four $K_{ir}6$ subunits that form the K^+ channel, which is surrounded by four SUR1 subunits. There are two subunits of inwardly rectifying $K_{ir}6$ subunits: $K_{ir}6.1$, which seems to be restricted to smooth muscle, and $K_{ir}6.2$ that is expressed more widely. An example of the latter is the insulin-secreting β cell (Module 7: Figure β-cell signalling). In the case of arterial smooth muscle, the $K_{ir}6.1$ subunit of the K_{ATP} channel is located in caveolae (Module 6: Figure caveolae organization).

Control of channel opening is complicated because ATP and ADP can be both stimulatory and inhibitory. ATP can stimulate the channel by binding to a site on the SUR1 subunit, and this effect depends on Mg^{2+}. Conversely, ATP acting on the $K_{ir}6$ subunits inhibits the channel through a process independent of Mg^{2+}. Under normal conditions in the cell, the dominant effect of ATP is to close the channel, whereas Mg-ADP acts to reverse this inhibition. The K_{ATP} channel is also sensitive to hydrogen sulfide (H_2S).

K_{ATP} channels function in the control of a number of different cell types:

- Insulin secretion by insulin-secreting β-cells (Module 7: Figure β-cell signalling)
- Stimulation of hypothalamic neurons to increase the autonomic input to the glucagon-secreting α-cells
- Secretion of glucagon-like peptide 1 (GLP-1) from L-cells
- Contribution to the tone of vascular smooth muscle cells

Mutations in either of the K_{ATP} channel subunits causes congenital hyperinsulinism of infancy.

Hyperpolarizing-activated cyclic nucleotide-gated (HCN) channels

A small family of hyperpolarizing-activated cyclic nucleotide-gated (HCN) channels, which are found in a number of excitable cells, play a particularly important role in generating pacemaker activity. As the name suggests, the channels are activated by membrane

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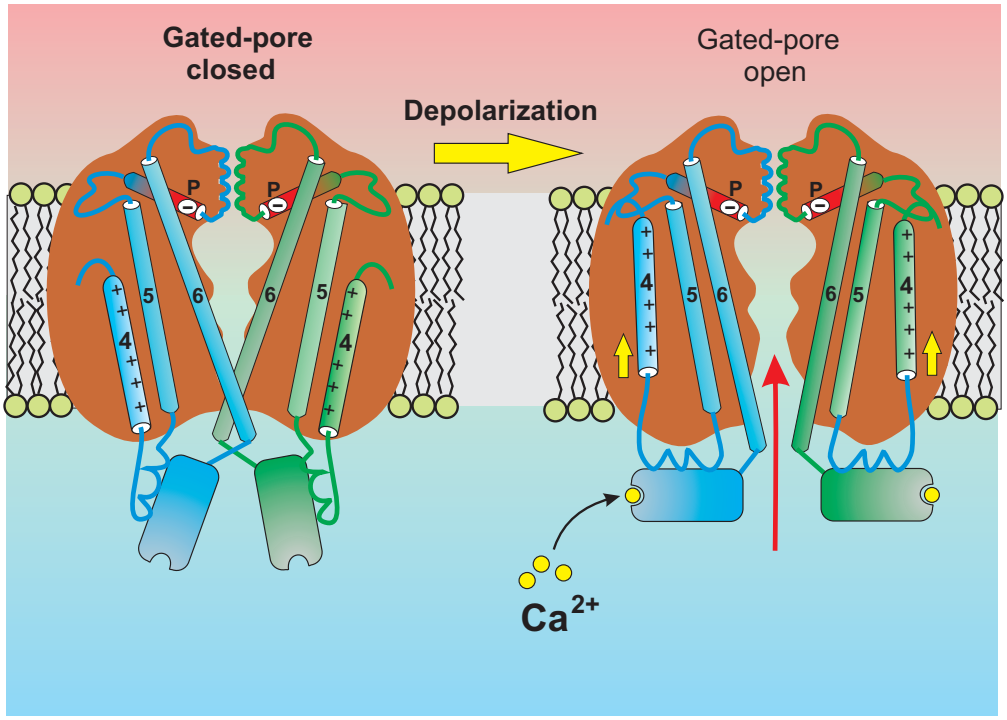
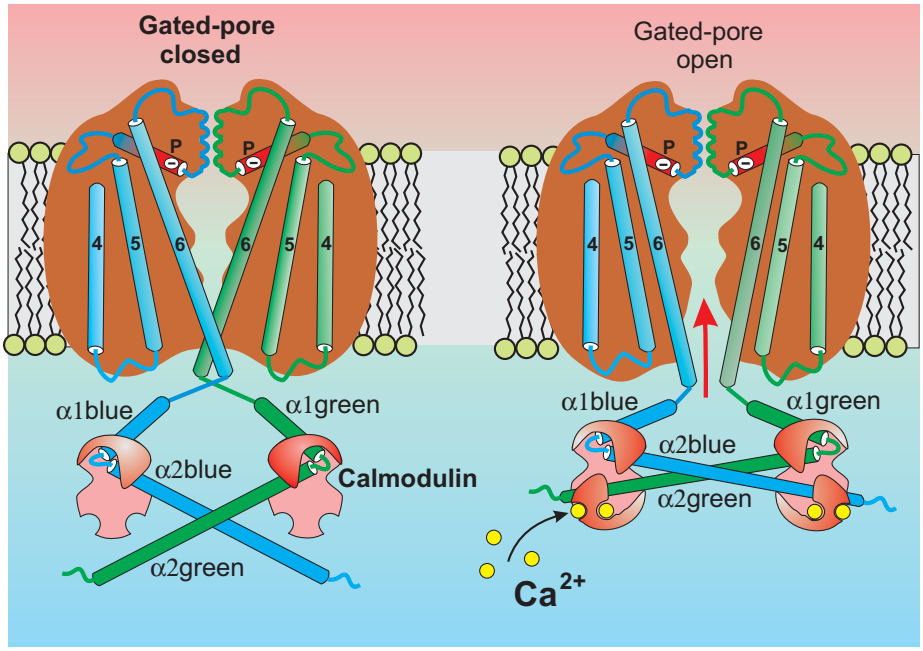
Module 3: | Figure BK channel opening



Large-conductance (BK) channel opening by both membrane depolarization (electromechanical coupling) and by intracellular Ca^{2+} (chemo-mechanical coupling).
The opening mechanism seems to depend upon a displacement of transmembrane domain 6 (TM6) brought about by a movement of the regulator of K^+ conductance, which is attached to the end of TM6. The nature of the conformational changes that occur following the movement of the voltage sensor (yellow arrow) or the displacement of the regulator following the binding of Ca^{2+} remains to be determined.

Module 3: | Figure IK/SK channel opening



Intermediate-conductance (IK) and small-conductance (SK) channel opening by chemomechanical coupling induced by Ca^{2+} binding to calmodulin.
This figure shows two of the four α-subunits, each of which is controlled by a resident calmodulin molecule. In the closed position shown on the left, the α1 helix of each channel subunit is bound by the C lobe of the calmodulin molecules. When Ca^{2+} binds to the two EF-hand regions of the N lobe of calmodulin, a conformational change occurs that pulls in the α2 helix of a neighbouring subunit to form a dimer with the α2 blue helix that lies antiparallel to the α2 green helix. The formation of this complex then opens the pore by pulling on the transmembrane 6 domains. The structure of this complex is illustrated in Module 3: Figure CaM/SK channel complex.

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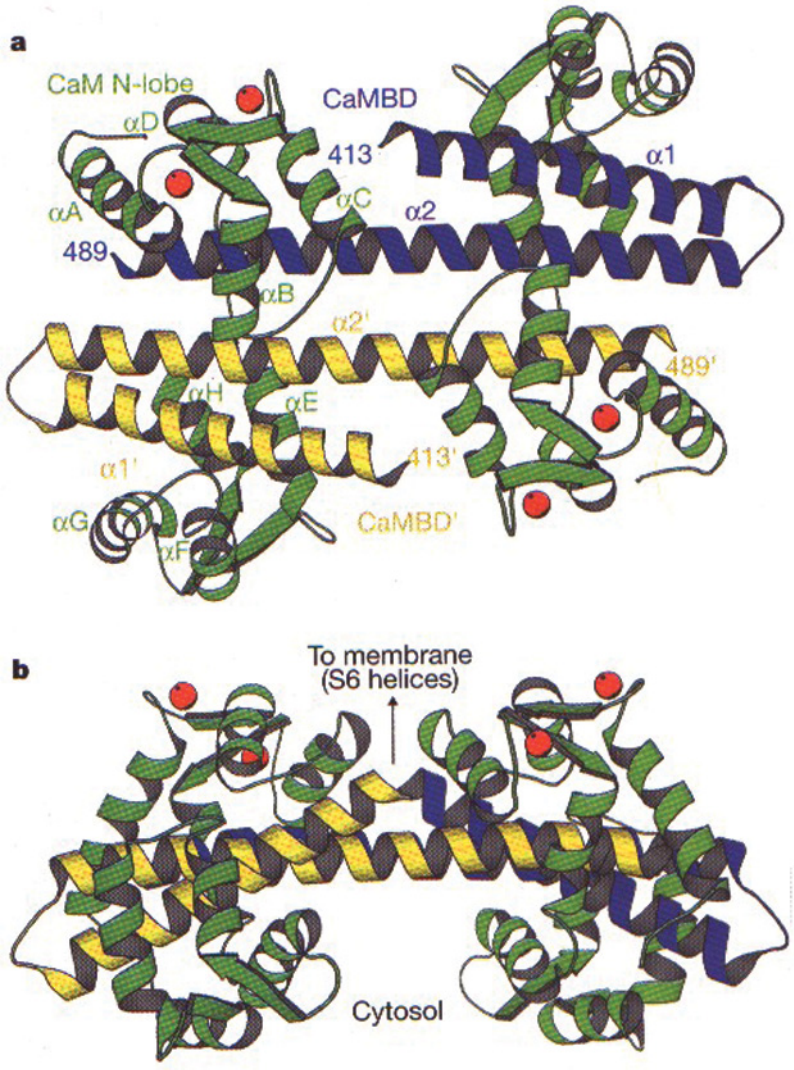
Module 3: | Figure CaM/SK channel complex



Structure of the complex formed between calmodulin and the C-terminal region of the small-conductance (SK) channel.

a. The two calmodulin (CaM) molecules (green) have two Ca^{2+} ions (red) bound to their N lobes, which wrap around the $\alpha 2$ helices of neighbouring subunits (shown in blue and yellow). b. A side view of the complex illustrates where the $\alpha 1$ helices connect to the transmembrane S6 helices, as shown diagrammatically in Module 3: Figure IK/SK channel opening. Reproduced by permission from Macmillan Publishers Ltd: *Nature*, Schumacher, M.A., Rivard, A.F., Bächinger, H.P. and Adelman, J.P. (2001) Structure of the gating domain of a Ca^{2+}-activated K^+ channel complexed with Ca^{2+}/calmodulin. 410:1120-1124. Copyright (2001); http://www.nature.com; see Schumacher et al. 2001.

hyperpolarization and are usually switched on when action potentials repolarize back to a hyperpolarized state. The domain structure reveals six transmembrane (TM) domains with a pore loop located between TM5 and TM6 (Module 3: Figure HCN channels). The different family members differ with regard to their speed of activation, with HCN1 being the fastest, followed by HCN2, HCN3 and HCN4. The functional channel is usually a tetramer, and they conduct mainly Na^+ with small amounts of K^+.

The channels have a cyclic nucleotide-binding domain (CNBD), which has a 10-fold higher affinity for cyclic AMP over cyclic GMP. The binding of cyclic AMP does not activate the channel, but it alters the open probability by shifting the activation curve towards more depolarizing potentials.

The channels open in response to membrane hyperpolarization and the voltage sensor is located in TM4, which has a large number of positive charges. The entry of Na^+ produces an inward current that has been given different names I_h, I_f and I_q (where h, f and q stand for hyperpolarizing, funny and queer respectively). In the case of the cardiac pacemaker in the sinoatrial node, it is known as I_f, which plays a critical role in providing the inward current that depolarizes the membrane during the pacemaker depolarization (Module 7: Figure cardiac pacemaker). The ability of sympathetic stimulation to accelerate the pacemaker depends upon cyclic AMP binding to the channel to enhance I_f. By contrast, acetylcholine acting on muscarinic receptors has the opposite effect.

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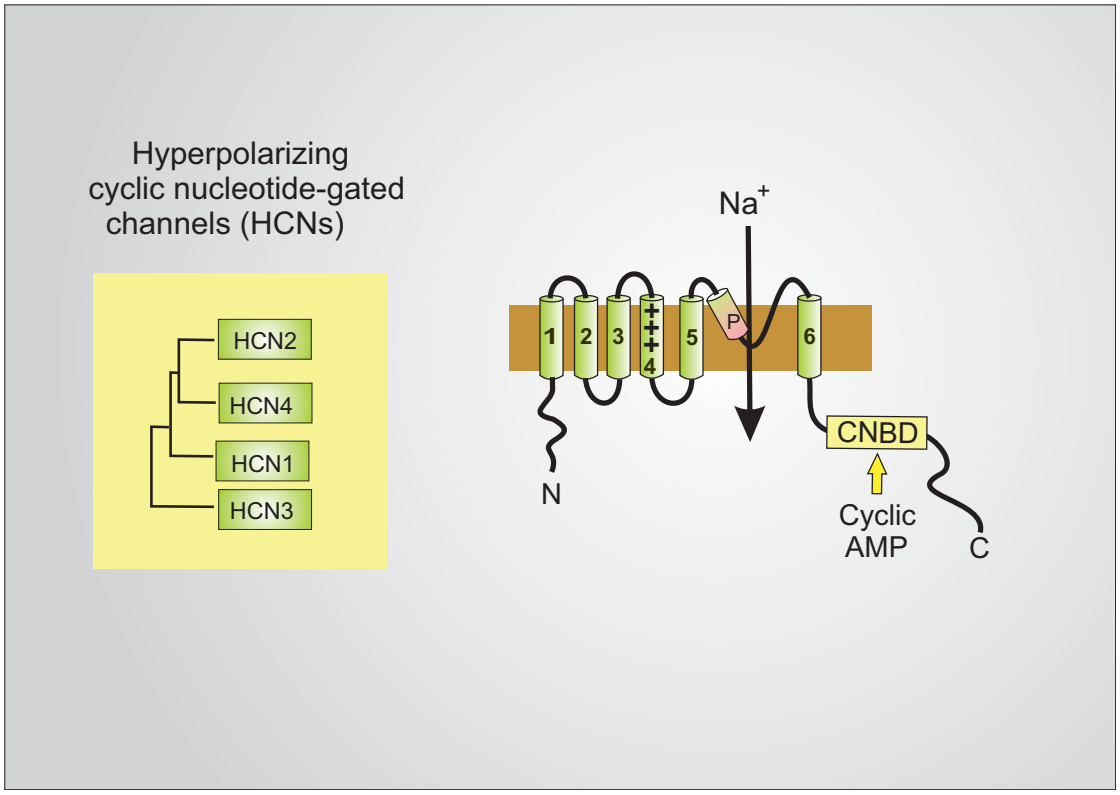
Module 3: | Figure HCN channels



Hyperpolarizing-activated cyclic nucleotide-gated (HCN) channels.
There are four members of the hyperpolarizing-activated cyclic nucleotide-gated (HCN) channel family. Each subunit has six transmembrane (TM) domains, with a pore-forming loop located between TM5 and TM6. The voltage sensor is located in TM4, which has a large number of positive charges that sense the change in voltage to bring about the conformational change that opens the channel pore. The C-terminal region that faces the cytoplasm has the cyclic nucleotide-binding domain (CNBD) that responds to cyclic AMP.

Chloride channels and transporters

The major anion in cells is chloride (Cl^-), which is transported across both the plasma membrane and intracellular organelle membranes by a number of ion channels and transporters. These channels/transporters have two main functions. First, they carry charge and thus contribute to the regulation of membrane potential and thus cell excitability. Secondly, the bulk flow of Cl^- contributes to cell volume regulation and the flow of ions and water across transporting epithelial. From a signalling perspective, attention has focused on those channels and transporters that contribute to the control of cell function:

- Ca^{2+}-sensitive Cl^- channels (CLCAs)
- Cation-chloride cotransporters
- CLC chloride channels and transporters
- Cystic fibrosis transmembrane conductance regulator (CFTR)
- GABA receptors
- Glycine receptors (GlyRs)

Ca^{2+}-sensitive Cl^- channels (CLCAs)

Many cells express (CLCAs) that respond to an increase in intracellular Ca^{2+} by activating a Cl^- current that is either inward or outward, depending on the Cl^- equilibrium potential. The gating of the CLCAs seems to require the same β-subunit normally associated with Ca^{2+}-sensitive large-conductance (BK) channels. The Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) seems to play a role in regulating some of these channel types. The inhibition of CLCAs by $Ins3,4,5,6P_4$ seems to be acting through this CaMKII-dependent pathway.

The genes coding for these CLCAs are still being identified. It has been proposed that a family of transmembrane proteins (TMEM16) may function as Ca^{2+}-sensitive Cl^- channels.

This Ca^{2+}-activated flux of Cl^- contributes to a number of cellular processes:

- CLCAs are localized to the apical membrane of pancreatic acinar cells (Module 7: Figure control of pancreatic secretion) and salivary gland (Module 7: Figure salivary gland secretion), where they respond to Ca^{2+} spikes in the apical pole to release Cl^- into the lumen as part of the fluid secretory process.
- The activation of these Cl^- channels function in olfaction by amplifying the signal transduction mechanism (Module 10: Figure olfaction).
- In mesangial cells, activation of a CLCA provides the depolarization necessary to activate the voltage-operated channels (VOCs) that drive contraction to

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regulate blood flow through the glomerulus (Module 7: Figure mesangial cell).
- The CLCAs operate in the smooth muscle cell cytosolic oscillator (Module 7: Figure SMC cytosolic oscillator), which controls contractility in vascular smooth muscle cells, airway smooth muscle cells and corpus cavernosum smooth muscle cells.
- In sweat glands, CLCAs contribute to fluid secretion (Module 7: Figure sweat gland function).

CLCAs have attracted a lot of interest as a possible way of compensating for some of the channel defects associated with cystic fibrosis (CF). In CF, there are mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), which mediates control over Na^+ and Cl^- channels. The severity of CF pathology can vary, and this may depend upon the expression of a non-CFTR-mediated Cl^- conductance. Likely candidates are the CLCAs.

The CLCA1 channel is strongly up-regulated, and is correlated with mucous production in bronchial epithelial cells of patients with asthma.

Transmembrane protein (TMEM16) family

There are ten members of the transmembrane protein (TMEM16) family: TMEM16a to TMEM16h, TP53i5 (tumour promoter 53 inducible protein 5) and TMEM16k. These membrane proteins have eight transmembrane domains with the N- and C-termini facing the cytoplasm. The ten members of this family have also been called ANO1--10 on the basis that they are anionic channels with eight transmembrane domains.

TMEM16a, which codes for Ano1, and TMEM16b appear to function as Ca^{2+}-activated Cl^- channels. In the case of Ano1, the activation by Ca^{2+} occurs independently of Calmodulin (CaM) or phosphorylation.

TMEM16F functions as a phospholipid scramblase, which reduces the asymmetry of membrane lipids such as phosphatidylserine (PS) that is flipped from the inner to the outer membrane. The activity of this scramblase is particularly evident in blood platelets (Module 11: Figure platelet activation).

A mutation of TMEM16F, which results in premature termination of this protein, has been identified in a patient with Scott syndrome.

CLC chloride channels and transporters

The family of CLC chloride channels and transporters has nine members (Module 3: Table CCL chloride channel and transporter components). Four of these function within the plasma membrane as channels (CLC-1, CLC-2, CLC-Ka and CLC-Kb), while the remainder (CLC-3, CLC-4, CLC-5, CLC-6 and CLC-7) are transporters located in membranes surrounding internal organelles such as the endosomes, lysosomes and synaptic vesicles where they may operate as Cl^--H^+ exchangers. These CLC channels/transporters appear to function as dimers with each subunit having a permeation pathway thus forming a 'double-barrelled channel'. The trafficking and function of CLC-Ka and CLC-Kb are regulated by the β-subunit barttin. Similarly, the CLC-7 tranporter found in lysosomes is regulated and stabilized by the β-subunit Ostm1.

CLC-1

CLC-1 is expressed in skeletal muscle where it functions to stabilize membrane potential by providing the background Cl^- conductance g_{Cl}. It is a voltage-sensitive channel in that it opens on membrane depolarization and contributes to membrane repolarization following an action potential. Using Cl^- for repolarization instead of K^+ may avoid the problem of clogging up the T-tubules with K^+ that would depolarize the membrane. Mutations of CLC-1 are the cause of different types of myotonia congenita such as Thomsen's disease and Becker-type myotonia.

CLC-2

CLC-2 is expressed widely in tissues such as heart, epithelia, neurons and glia. It is an inward rectifying Cl^- channel. Unlike CLC-1, which is activated by depolarization, CLC-2 is sensitive to hyperpolarization and can also be activated by cell swelling and external pH. There are indications that CLC-2 might play a role in cell volume regulation.

The CLC-2 expressed in neurons and glia might operate to regulate the concentration of ions in the narrow extracellular spaces. In the colon, CLC-2 is located in the basolateral membrane where it may contribute to Cl^- reabsorption (Module 7:Figure colon function).

CLC-K

The two CLC-K isoforms belong to the CLC chloride channel and transporter family (Module 3: Table CLC chloride channel and transporter components). There are two CLC-K channels, CLC-Ka and CLC-Kb, that have a close sequence identity and their genes lie close to each other on chromosome 1p36 suggesting that they emerged through gene duplication. They are expressed in the kidney and in the cochlea. These channels are particularly suited to provide large transepithelial fluxes of Cl^- over a wide range of membrane potentials. In the inner ear, both CLC-K isoforms are expressed in the stria vascularis that secretes K^+ and Cl^- into the scala media (Module 10: Figure inner ear). In the kidney, CLC-Ka is expressed mainly in the thin limb of the loop of Henle, whereas the CLC-Kb is located in the thick ascending limb of the loop of Henle (TALH) and in the distal convoluted tubule and collecting duct. CLC-Kb functions in Na^+ and Cl^- reabsorption (Module 7: Figure kidney salt reabsorption).

Mutations of CLC-Kb are responsible for Bartter's disease type III. A predisposition to hypertension results from a CLC-Kb polymorphism.

CCL-3

CLC-3 is a member of the homology group of CLC channels, which includes CLC-4 and CLC-5, that are expressed on membranes of the endocytic pathway (Module 3: Table CLC chloride channel and transporter components). CLC-3 is slightly unusual because it is also expressed on the membranes of synaptic vesicles. The function of

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CLC-3 is still being worked out, but most evidence is compatible with it functioning as a Cl^--H^+ exchanger.

CLC-4

CLC-4, which is homologous with CLC-3 and CLC-5, is widely expressed and located within endosomes (Module 3: Table CLC chloride channel and transporter components). Like its close relatives, it appears to carry out Cl^--H^+ exchange.

CLC-5

CLC-5, which is homologous with CLC-3 and CLC-4, is expressed mainly in epithelia such as the kidney and intestine where it is located on endosomes where it colocalizes with the V-type H^+-ATPase (Module 3: Table CLC chloride channel and transporter components). Like its close relatives, CLC-5 appears to carry out an electrogenic Cl^--H^+ exchange and may thus function in the acidification of renal endocytic vesicles

The CLCN5 gene, which is located on the X-chromosome, is mutated in the inherited disorder Dent's disease found mainly in males.

CLC-6

CLC-6 is widely expressed in tissues such as the brain, testis and kidney. It appears to be located on the late endosomes and may be important for lysosomal function.

CLC-7

CLC-7 is approx. 45% homologous with CLC-6 and is mainly confined to the lysosome where it appears to function as a Cl^--H^+ exchanger. CLC-7 is highly expressed in osteoclasts where it is inserted into the ruffled membrane that faces the resorption pit (Module7: Figure osteoclast function).

Some patients with osteopetrosis have mutations in the *CLCN7* gene that codes for CLC-7.

Barttin

Barttin is a β-subunit that regulates the trafficking and function of CLC-Ka and CLC-Kb (Module 7: Figure kidney salt reabsorption). Barttin, which is the product of the *BSND* gene, has two transmembrane domains with the N- and C-termini facing the cytoplasm.

Mutations of barttin are responsible for Bartter's disease type IV.

Ostm1

Ostm1 is an auxiliary β-subunit that regulates the trafficking and function of CLC-7. Ostm1 may function to enhance the stability of CLC-7, which unlike other CLC proteins is not glycosylated and is thus susceptible to the hydrolytic activity of lysosomal enzymes. However, CLC-7 may be protected through its close association with Ostm1, which is highly glycosylated. Ostm1 is expressed in osteoclasts where it is responsible for CLC-7 insertion into the ruffled membrane that faces the resorption pit (Module7: Figure osteoclast function).

Some forms of osteopetrosis are caused by mutations in the *OSTM1* gene that codes for Ostm1.

Module 3: | Table CLC chloride channel and transporter components
Components of the CCL chloride channel and transporter toolkit.

CLC chloride channels and transporters	Comments
CLC-1	Expressed in skeletal muscle. Functions as a channel to stabilize membrane potential.
CLC-2	Expressed widely. Epithelial transport channel.
CLC-Ka	Expressed in kidney tubules and inner ear. Epithelial transport channel regulated by barttin.
CLC-Kb	Expressed in kidney tubules and inner ear. Epithelial transport channel regulated by barttin (see Module 7: Figure kidney salt reabsorption).
CLC-3	Widely expressed. Acidification of synaptic vesicles and endosomes.
CLC-4	Widely expressed. Acidification of endosomes.
CLC-5	Kidney and intestine. Acidification of endosomes.
CLC-6	Widely expressed. Acidification of endosomes.
CLC-7	Widely expressed. Acidification of endosomes and acid secretion by osteoclasts (see Module 7: Figure osteoclast function).
CLC regulators	
Barttin	β-subunit that controls trafficking and function of CLC-Ka and CLC-Kb (see Module 7: Figure kidney salt reabsorption).
Ostm1	β-subunit that controls the stability of CLC-7 (see Module 7: Figure kidney salt reabsorption).

Information for this Table was taken from Jentsch (2008).

ATP-binding cassette (ABC) transporters

The superfamily of ATP-binding cassette (ABC) transporters first came to be noticed through their ability to reduce the activity of various anticancer drugs by actively pumping them out of the cell. They were originally called multidrug resistance proteins (MRPs). It soon became apparent that these belonged to a superfamily and were subsequently renamed the ABC transporters (Module 3: Table ABC transporters). This name reflects the fact that they use the energy of ATP to extrude a wide range of anions (e.g. chloride, amino acids, sugars, peptides, phospholipids and vitamins) from the cell. The human genome contains 48 ABC transporters that have been subdivided into seven subfamilies (A–G).

These transporters have three main domain structures that vary in the number of transmembrane domains (TMDs) and nucleotide-binding domains (NBDs) (Module 3: Figure ABC transporters). Some of the transporters have 17 transmembrane (TM) α-helices grouped in to three TMDs (see A). The first has five TMs and the other two have six each and these are separated by a large cytoplasmic loop that has the first NBD. The other NBD is located in the C-terminus. Another group of transporters has a similar structure except that they lack the first two TMs (see B in Module 3: Figure ABC transporters). As shown in the lower figure, the NBD is made up of two Warker motifs that flank the ABC signature motif shown in yellow. These NBDs are responsible for binding and hydrolysing ATP to provide the energy necessary to drive the different transport processes. It is now thought that the two NBD

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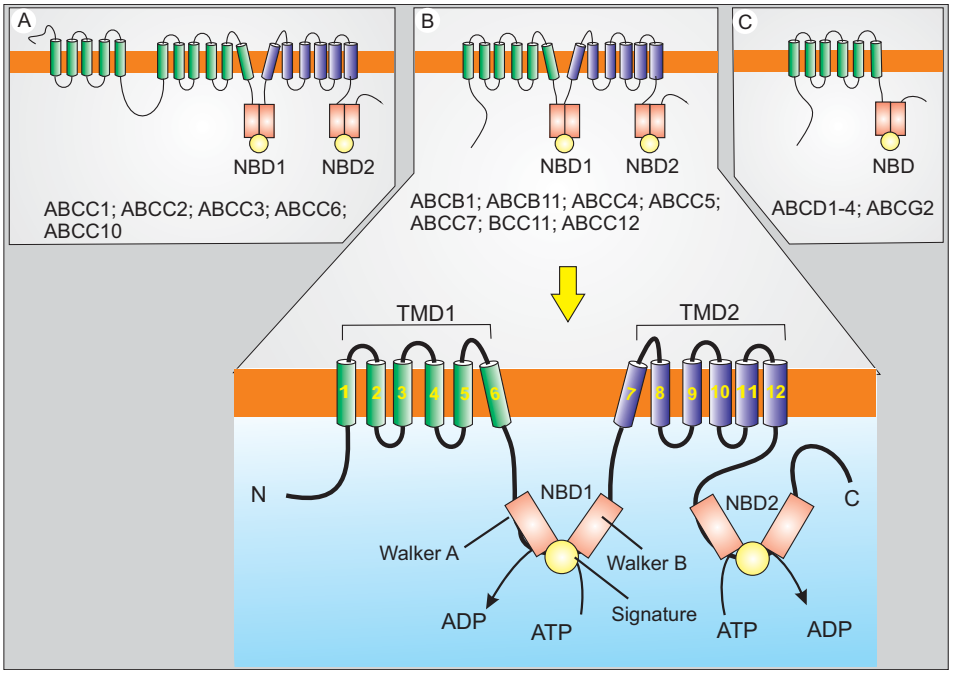
Module 3: | Figure ABC transporters



Domain structure of ABC transporters.
The ATP-binding cassette (ABC) transporters have different domain structures. A. There are three transmembrane domains (TMBs) and two nucleotide-binding domains (NBDs). B. These transporters have two TMBs and two NBDs, which are shown in more detail in the lower figure. C. Some members of the family are half-transporters that have a single TMB and a single NBD.

subunits interact with each as is shown for ABCC7, which is the cystic fibrosis transmembrane conductance regulator (CFTR) (Module 3: Figure CFTR channel). The last group comprise half-transporters in that they have a single TMD and a single NBD (see C in Module 3: Figure ABC transporters). In order to form a functional transporter, these half-transporters have to form either homo- or heterodimers by interacting with other half-transporters.

In addition to their pharmacological action in extruding xenobiotics such as anticancer drugs, these transporters have important signalling functions through their ability to pump a variety of signalling and related molecules across the plasma membrane. Members of this family have cell-specific expression patterns suggesting that they may have distinct functions in different cell types as illustrated below.

ABCA1

ABCA1 is a lipid transporter that is highly expressed in adrenal gland, uterus and liver. In the liver , ABCA1 functions in lipid transport with a special role in transferring cholesterol from the plasma membrane to the lipid acceptor apolipoprotein-A1 (apoA-I). It also functions to transfer lipids to apolipoprotein E (ApoE), which is synthesized and released by the glial cells, to transfer lipids around the brain. Another function for ABCA1 is to transport phosphatidylserine (PS) across the plasma membrane of blood platelets (Step 7 in Module 11: Figure platelet activation).

Mutations in ABCA1 may be responsible for Tangier syndrome and Scott syndrome.

ABCA2

ABCA2 is expressed mainly in oligodendrocytes where it appears to be located primarily in intracellular membranes where it seems to play a role in lipid trafficking for the synthesis of myelin.

ABCA3

ABCA3 is expressed in a number of tissues (brain, thyroid and testis) and particularly in the lung where it functions in lipid transport to produce the surfactant that accumulates in the lamellar bodies of the type II lung alveolar epithelial cells. Mutations in ABCA3 leads to respiratory distress syndrome (RDS) in newborn infants.

ABCA4

ABCA4 is mainly expressed in the retina where it functions to transport retinyldiene phospholipids between the rod outer segments. Stargardt disease is caused by mutations in ABCA4.

ABCB4

ABCB4 is a lipid transporter that functions to move phosphatidylcholine (PC) across membranes such as the canalicular membrane of hepatocytes. Progressive familial intrahepatic cholestasis type III (PFIC3) is caused by a defect in this transporter.

ABCC1

One of the functions of this transporter is to extrude sphingosine 1-phosphate (S1P) during the operation of

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the sphingomyelin signalling pathway (Module 2: Figure sphingomyelin signalling).

ABCC2

ABCC2 is an 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. Mutations in ABCC2 cause Dubin-Johnson syndrome.

ABCC4

This versatile transporter, which is expressed in a number of cells such as alveolar epithelial cells, kidney and intestinal cells, can transport a number of signalling molecules such as cyclic nucleotides, eicosanoids and conjugated steroids. For example, it has been identified as the cyclic AMP efflux transporter, which is one of the OFF mechanisms for the cyclic AMP signalling pathway (Module 2: Figure cyclic AMP signalling).

ABCC7

ABCC7 is the cystic fibrosis transmembrane conductance regulator (CFTR) channel, which conducts the anions (Cl^- and HCO_3^-) that contribute to the osmotic gradient for the parallel flow of water in epithelia (Module 3: Figure CFTR channel). CFTR functions in small intestine fluid secretion (Module 7: Figure intestinal secretion). It has a similar function in the colon (Module 7: Figure colon function) and in epithelial cells in the uterus during capacitation.

A mutation in the CFTR channel is the cause of cystic fibrosis (CF).

ABCC8

ABCC8 encodes the sulphonylurea receptor-1 (SUR1), which is a subunit of the ATP-sensitive K^+ (K_{ATP}) channel that functions in insulin release and biogenesis (Module 7: Figure β-cell signalling).

ABCC8 mutations cause neonatal diabetes.

ABCD1

ABCD1 belongs to those ABC transporters that are known as half-transporters as they contain only six transmembrane domains and a single nucleotide-binding domain (NBD). In order to function it has to form either homodimers or it teams up with other members of this D subfamily (Module 3: Table ABC transporters) to form heterodimers. All four members of this family seem to function intracellularly where they are located in peroxisomes where they transport very-long-chain fatty acids. Mutations in ABCD1 causes X-linked adrenoleukodystrophy (X-ALD).

ABCG5

ABCG5 plays a role in the trafficking of sterols through intestinal and liver cells. Mutations in ABCG5 causes sitosterolaemia.

ABCG8

ABCG8 plays a role in the trafficking of sterols through intestinal and liver cells. Mutations in ABCG8 causes sitosterolaemia.

Cystic fibrosis transmembrane conductance regulator (CFTR)

Epithelia that line the airways, intestinal tract and ducts in the pancreas, sweat glands and vas deferens express the cystic fibrosis transmembrane conductance regulator (CFTR) channel, which conducts anions (Cl^- and HCO_3^-) that contribute to the osmotic gradient for the parallel flow of water. CFTR is also known as ABCC7 and belongs to a large family of ATP-binding cassette (ABC) transporters. However, it is the only member of this superfamily that can function as an ion channel.

The channel is constructed from a single polypeptide. The N-terminal region is cytosolic and is connected to the first membrane-spanning helix of the first transmembrane domain (TMD1) (Module 3: Figure CFTR channel organization). TMD1 has six α-helices, and helix 6 is thought to contribute to the formation of the pore. The cytoplasmic side of helix 6 is connected to the first nucleotide-binding domain (NBD1), and this is then followed by the regulatory (R) domain, which has phosphorylation sites for both protein kinase A (PKA) and protein kinase C (PKC). This R domain then connects with helix 12 of the second transmembrane domain (TMB2). Helix 12 connects to NBD2, and the molecule ends with a short C-terminal tail. The transport function of CFTR seems to depend on the Na^+/H^+ exchanger regulatory factor isoform-1 (NHERF1), which functions as a scaffold to assemble a macromolecular signalling complex. Interactions between NBD1 and NBD2 play a critical role in channel gating (Module 3: Figure CFTR channel).

Channel opening depends upon the binding of ATP, and the subsequent hydrolysis of ATP closes the channel (Module 3: Figure CFTR channel). These actions of ATP are carried out by NBD1 and NBD2 that use the energy derived from ATP hydrolysis to drive the channel between its open and closed configurations. The NBD1/NBD2 complex has two homologous nucleotide-interacting motifs that bind two molecules of ATP. At one of the sites, the ATP appears to remain in place, while at the other, ATP comes on and off to drive channel gating. When this second ATP binds, it brings the two molecules together, and this induces a conformational change that is transmitted up to the membrane helices to induce pore opening. NBD2 has the ATPase activity to remove the terminal phosphate from ATP and this hydrolysis disrupts the tight heterotrimeric interaction between NBD1 and NBD2, resulting in channel closure and the release of ADP.

The ATP-dependent opening and closing of the CFTR channel is controlled by the R domain, which is phosphorylated by protein kinase A (PKA). In the resting state, the channel has a very low open probability (< 0.003), but this increases about 100-fold following protein kinase A (PKA) phosphorylation of the R domain (Module 3: Figure CFTR gating). The latter can also be phosphorylated by protein kinase C (PKC), but this causes little activation

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Module 3: | Table ABC transporters
The human ATP-binding cassette (ABC) transporter toolkit.

ATP-binding cassette (ABC) transporters	Comments
A subfamily	
ABCA1	Functions to transfer cholesterol from the plasma membrane to the lipid acceptor apolipoprotein-A1 (apoA-I). Also transports phosphatidylserine (PS) in platelets (see Module 11: Figure platelet activation)
ABCA2	Expressed in oligodendrocytes where it functions in lipid trafficking for the synthesis of myelin
ABCA3	Expressed mainly in type II lung alveolar epithelial cells to produce the surfactants
ABCA4	Expressed in the retina to transport retinyldiene phospholipids between the rod outer segments.
ABCA5	
ABCA6	
ABCA7	
ABCA8	
ABCA9	
ABCA10	
ABCA12	
ABCA13	
B subfamily	
ABCB1	
ABCB2	
ABCB3	
ABCB4	Transports phosphatidylcholine (PC) across membranes such as the canalicular membrane of hepatocytes
ABCB5	
ABCB6	
ABCB7	
ABCB8	
ABCB9	
ABCB10	
ABCB11	
C subfamily	
ABCC1	Extrudes S1P during the sphingomyelin signalling pathway (see Module 2: Figure sphingomyelin signalling).
ABCC2	Transports certain organic anions across the canalicular membrane of the hepatocyte. Also known as multidrug resistance protein 2 (MRP2)
ABCC3	
ABCC4 (MRP4)	Functions to pump cyclic AMP out of cells (see Module 2: Figure cyclic AMP signalling)
ABCC5	
ABCC6	
ABCC7	The cystic fibrosis transmembrane conductance regulator (CFTR) channel that functions in fluid transport (see Module 7: Figure intestinal secretion)
ABCC8	Encodes the sulphonylurea receptor-1 (SUR1), a component of the K_{ATP} channel in beta cells (Module 7: Figure β-cell signalling)
ABCC9	
ABCC10	
ABCB11	
ABCB1	
ABCB2	
ABCB3	
ABCB4	
ABCB5	
ABCB6	

Module 3: | Table Continued

ATP-binding cassette (ABC) transporters	Comments
ABCB7	
ABCB8	
ABCB9	
ABCB10	
ABCB11	
ABCC12	
ABCC13	
D subfamily	
ABCD1	Located in peroxisomes to transport very-long-chain fatty acids
ABCD2	
ABCD3	
ABCD4	
E subfamily	
ABCE1	
F subfamily	
ABCF1	
ABCF2	
ABCF3	
G subfamily	
ABCG1	
ABCG2	
ABCG3	
ABCG4	
ABCG5	Transports sterols in intestinal and liver cells
ABCG8	Transports sterols in intestinal and liver cells

although it might be a prerequisite for subsequent phosphorylation by PKA. There are up to eight serine residues that can be phosphorylated by PKA, and it seems that the degree of channel gating can vary, depending on how many of these are phosphorylated. Just how this phosphorylation of R enables ATP to begin the cycle of channel opening and closing is unclear, but it may depend on the R domain interacting with the N-terminal tail.

The CFTR has many functions to control ion and water transport across epithelia:

- Small intestine fluid secretion depends on the flow of Cl^- into the lumen to provide the electrical gradient for a parallel flow of Na^+, and this provides the osmotic gradient for a parallel flow of water (Module 7: Figure intestinal secretion). A similar mechanism operates in the colon to regulate both absorption and secretion (Module 7: Figure colon function).
- Epithelial cells in the uterus and oviduct produce the HCO_3^-, which enters the genital tract through the CFTR during capacitation.
- Mutations in the CFTR channel causes cystic fibrosis (CF).

Cation-chloride cotransporters

The electroneutral cation-chloride cotransporters move chloride across the plasma membrane together with cations (Na^+ and K^+). The driving force for Cl^- movement is the electrochemical gradient for either Na^+ or K^+ depending on the carrier. The main carriers belong to the solute carrier (SLC) family:

- *SLC12A1* encodes the Na^+-K^+-$2Cl^-$ cotransporter 2 (NKCC2)

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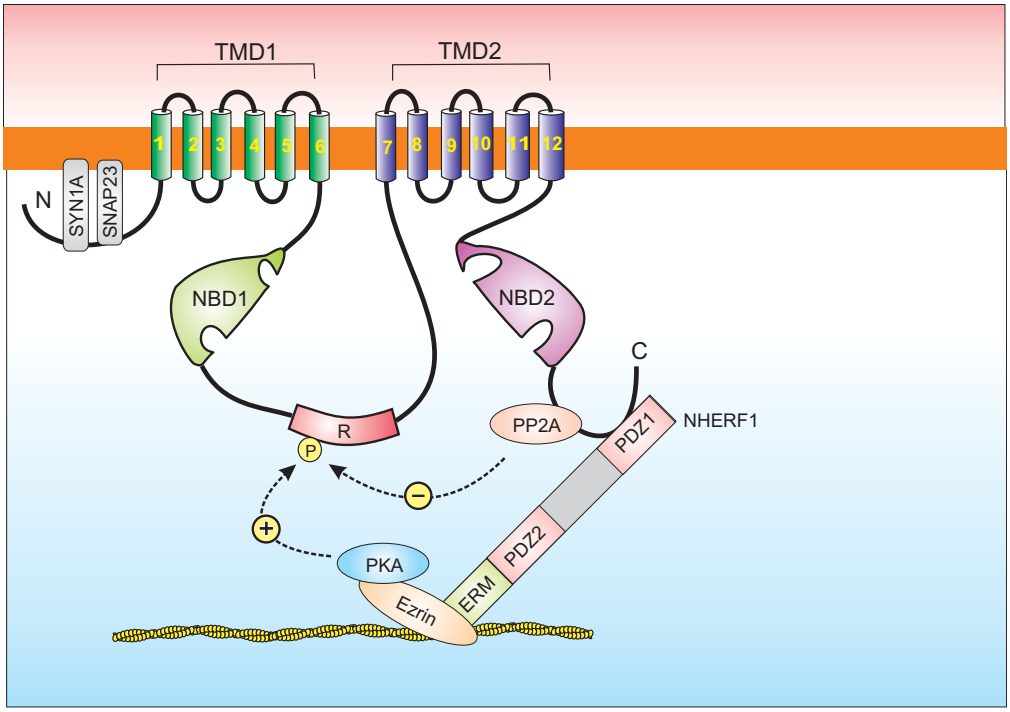
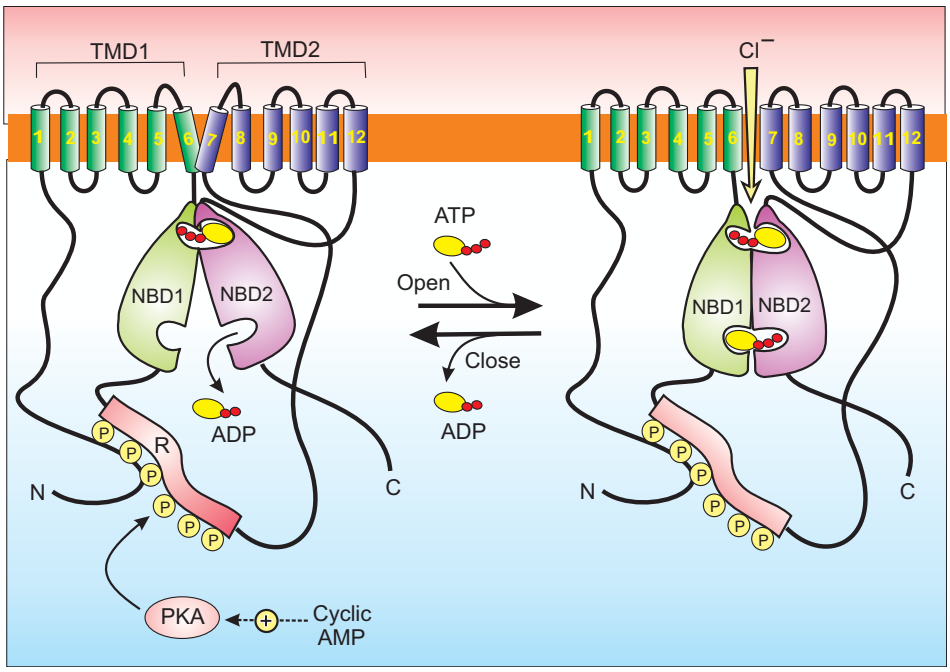
Module 3: | Figure CFTR channel organization



CFTR channel organization.
The cystic fibrosis transmembrane conductance regulator (CFTR) channel is a single polypeptide that has two transmembrane domains (TMB1 and TMB2). The large cytoplasmic loop connecting transmembrane helix 6 and 7 has the first nucleotide-binding domain (NBD1) and a regulatory (R) domain. The C-terminal tail has the second NBD2. The Na^+/H^+ exchanger regulatory factor isoform-1 (NHERF1) functions as a scaffold to assemble a macromolecular signalling complex that regulates the transport function of CFTR. The operation and control of CFTR is described in Module 3: Figure CFTR channel.

Module 3: | Figure CFTR channel



Structure and function of the cystic fibrosis transmembrane conductance regulator (CFTR) channel.
The cystic fibrosis transmembrane conductance regulator (CFTR) channel is a single polypeptide that conducts anions, mainly Cl^- and HCO_3^-. The channel opens when the nucleotide-binding domains 1 and 2 (NBD1, NBD2) undergo a conformational change when they bind ATP. The channels close when NBD2 hydrolyses ATP to ADP. This ATP-dependent switching between open and closed configurations depends upon the phosphorylation of a regulatory (R) domain by protein kinase A (PKA)

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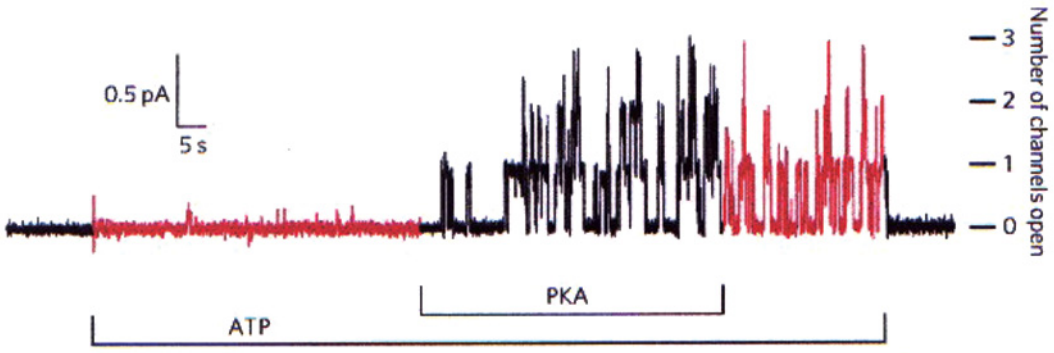
Module 3: | Figure CFTR gating



Control of cystic fibrosis transmembrane conductance regulator (CFTR) channel gating by protein kinase A (PKA).
This trace records the Cl^- current resulting from the opening of individual cystic fibrosis transmembrane conductance regulator (CFTR) channels in a patch of membrane. Addition of ATP had no effect, but channel activity began soon after the addition of protein kinase A (PKA), which acts by phosphorylating the regulatory domain. Reproduced by permission from Macmillan Publishers Ltd: Gadsby, D., Vergani, P. and Csanády, L. (2006) The ABC protein turned chloride channel whose failure causes cystic fibrosis. 440:477-483. Copyright (2006); http://www.nature.com; see Gadsby et al. 2006.

- *SLC12A2* encodes the Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1)
- *SLC12A3* encodes the Na^+-Cl^- cotransporter (NCC)
- *SLC12A4* encodes the K^+-Cl^- cotransporter 1 (KCC1)
- *SLC12A5* encodes the K^+-Cl^- cotransporter 2 (KCC2)
- *SLC12A6* encodes the K^+-Cl^- cotransporter 3 (KCC3)
- *SLC12A7* encodes the K^+-Cl^- cotransporter 4 (KCC4)

Members of the SLC family perform a multitude of functions including cell volume regulation, control of neuronal excitability and they are responsible for many of the ionic fluxes that drive epithelial fluid secretion and absorption. The activity of these cotransporters is controlled by the WNK protein kinase family.

Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1)

The Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1), which is a large protein (1212 amino acids) encoded by *SLC12A2* located on chromosome 5q23, is expressed in many different cells. In the case of epithelial cells, it is found on the basolateral membrane as occurs in the intestine and kidney. NKCC1 is thought to operate as a homodimer. Like other members of the SLC, NKCC1 has 12 hydrophobic transmembrane (TM) α-helices with the N- and C-terminal regions located in the cytoplasm (Module 3: Figure cation chloride cotransporters). TM7 and TM8 are connected by a long extracellular hydrophilic loop that has a variable number of glycosylation sites. In the case of NKCC1, the N-terminal region has three threonine residues (Thr-184, Thr-189 and Thr-202) that can be phosphorylated by Ste-related proline-alanine-rich kinase (SPAK) and the related oxidative stress response 1 (OSR1). Phosphorylation of the Thr-189 residue seems to be particularly important for regulating the activity of NKCC1. These phosphates are removed by protein phosphatase 1 (PP1). This phosphatase and the kinases associate with NKCC1 through a SPAK-binding motif and a PP1-binding motif that overlap each other.

The entry of Cl^- through NKCC1 has multiple functions:

- NKCC1 functions in cell volume regulation that occurs when cells face changes in osmolarity.
- NKCC1 provides the influx of Cl^- across the basolateral membrane that is then transported into the lumen across the apical membrane during small intestine fluid secretion (Module 7: Figure intestinal secretion), fluid secretion by the colon (Module 7: Figure colon function), the salivary gland control mechanism (Module 7: Figure salivary gland secretion) and fluid secretion by exocrine pancreatic acinar cells (Module 7: Figure control of pancreatic secretion).
- The neuronal excitability of immature neurons depends on NKCC1, which functions to maintain a high internal concentration of this ion. The resulting Cl^- equilibrium potential is such that GABA gives depolarizing rather than the hyperpolarizing responses of adult neurons.
- Fluid secretion during spermatogenesis depends on NKCC1.

Na^+-K^+-$2Cl^-$ cotransporter 2 (NKCC2)

The Na^+-K^+-$2Cl^-$ cotransporter 2 (NKCC2) is a large protein (1099 amino acids) encoded by *SLC12A1* located on chromosome 15. Its expression is restricted to the thick ascending loop of Henle (TALH), which functions to dilute the urine by reabsorbing Na^+ and Cl^- (Module 7: Figure kidney tubule function). NKCC2 has a similar domain structure to NKCC1 (Module 3: Figure cation chloride cotransporters).

The expression of NKCC2 in the macula densa cells, which is part of the TALH, functions in the sensing of salt concentration in the tubule lumen as part of the tubuloglomerular feedback (TGF) mechanism that respond to an increase in the concentration of Na^+ and Cl^- in the lumen of the TALH by releasing ATP (Module 7: Figure macula densa).

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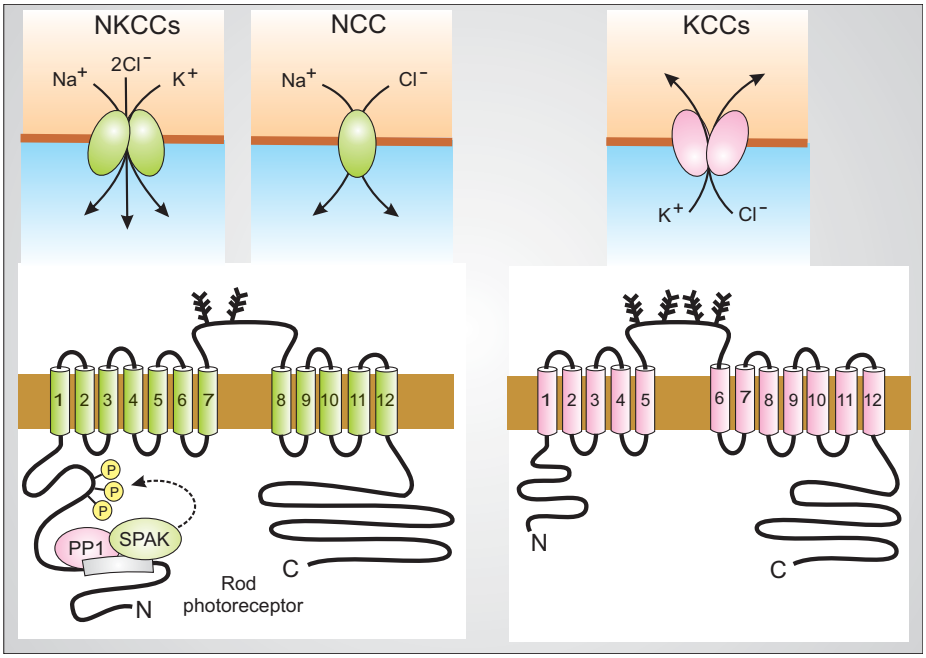
Module 3: | Figure cation chloride cotransporters



Structure and function of cation-chloride cotransporters.
There are three main types of cation-chloride cotransporters. The Na^+-K^+-Cl^- cotransporters (NKCCs) and the Na^+-Cl^- cotransporter (NCC) move Cl^- into the cell whereas the K^+-Cl^- cotransporters (KCCs) move Cl^- to the outside. They all have a similar domain structure with subtle variations. For example the NKCCs and NCC have a large extracellular hydrophilic loop between transmembrane (TM) α-helices 7 and 8 whereas in the KCCs it is found between TM5 and TM6.

Mutations in NKCC2 are responsible for the loss of salt and hypotension found in Type I Barrter's disease.

Na^+-Cl^- cotransporter (NCC)

The Na^+-Cl^- cotransporter (NCC) is a protein of 1021 amino acids that is encoded by *SLC12A3* located on human chromosome 16q13. NCC is thought to function as a monomer and has a domain structure similar to that of the two NKCC cotransporters (Module 3: Figure cation chloride cotransporters). This cotransporter is expressed mainly in the distal convoluted tubule (DCT) where it plays a major role in Cl^- reabsorption (Module 7: Figure kidney salt reabsorption).

Mutations in NCC have been linked to Gitelman's disease.

K^+-Cl^- cotransporter 1 (KCC1)

The four K^+-Cl^- cotransporters (KCCs) function to remove K^+ and Cl^- from cells (Module 3: Figure cation chloride cotransporters). The KCC1 isoform, which is encoded by *SLC12A4* on chromosome 16q22, has a ubiquitous distribution and seems to function in cell volume regulation acting in the opposite direction to that of Na^+-K^+-Cl^- cotransporter 1 (NKCC1). While the latter functions in the regulatory volume increase that occurs when cells face an increase in external osmolarity, KCC1 functions to decrease cell volume by allowing K^+ and Cl^- to leave the cell. This activation of KCC1 by hypotonicity depends on its dephosphorylation by protein phosphatase 1 (PP1) (Module 3: Figure cell volume regulation). The topology of the KCC isoforms resemble that of the NKCC cotransporters except that the hydrophilic loop, which has four glycosylation sites, is connected to TM5 and TM6.

K^+-Cl^- cotransporter 2 (KCC2)

The K^+-Cl^- cotransporter 2 (KCC2), which has 1116 amino acids and is encoded by *SCL12A5* located on 20q13, is a neuron-specific gene. The *SCL12A5* gene has a neuronal-restrictive silencing element (NRSE) that ensures its expression is restricted to neurons.

KCC2 functions together with Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1) and the K^+-Cl^- cotransporter 3 (KCC3) to regulate neuronal excitability by adjusting the intracellular concentration of Cl^-, which is one of the determinants of neuronal excitability. In embryonic neurons, $[Cl^-]$ is set to a high level through the operation of NKCC1, but when neurons mature, expression of the latter declines and is replaced by KCC2 that removes Cl^-. This switch in the expression of these cotransporters means that transmitter receptors such as the γ-aminobutyric acid (GABA) receptors cause depolarization in immature neurons but hyperpolarization in adult neurons.

The expression of KCC2 is regulated by a number of stimuli. Brain-derived neurotrophic factor (BDNF) and neurotrophin appears to enhance excitability by reducing the expression of KCC2, whereas GABA acting through the $GABA_A$ receptor generates Ca^{2+} signals that reduces excitability as found in adult neurons.

Intractable epilepsy occurs when the expression of KCC2 is reduced.

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K^+-Cl^- cotransporter 3 (KCC3)

The K^+-Cl^- cotransporter 3 (KCC3), which has 1150 amino acids and is encoded by *SCL12A6* located on 15q13-14, is expressed fairly widely (liver, brain, muscle, lung, heart and kidney).

Mutations in KCC3 have been linked to Anderman's disease.

K^+-Cl^- cotransporter 4 (KCC4)

The K^+-Cl^- cotransporter 4 (KCC4), which is encoded by *SCL12A7* located on 5p15,3, is expressed mainly in kidney and heart and to a lesser extent in brain. It also functions in the inner ear.

WNK protein kinase

The WNK protein kinases are a family of serine-threonine kinases that function in ion homoeostasis by controlling ion movements and particularly those carried out by the cation-chloride cotransporters. In the kidney, WNKs control the balance between NaCl reabsorption and K^+ secretion that occurs in the distal regions of the nephron.

WNK stands for With no lysine (k), which indicates that it lacks the catalytic lysine residue in subdomain II that is present in most protein kinases. In the case of WNK, the lysine required for ATP binding is in subdomain I. There are four human WNKs, which are encoded by *PRKWNW1*, *PRKWNW2*, *PRKWNW3* and *PRKWNW4*, that closely resemble each other especially in the N-terminal region that has the highly conserved kinase domain:

- WNK1
- WNK2
- WNK3
- WNK4

Mutations in WNK1 and WNK 4 are responsible for Gordon's disease.

WNK1

WNK1 is found in a number of cell types and is particularly strongly expressed in the distal convoluted tubule (DCT) and cortical collecting ducts of the kidney tubule. A truncated version of WNK1, which is catalytically inactive, is located specifically in the DCT and hence has been called kidney specific WNK1 (KS-WNK1). KS-WNK1 functions as an inhibitor of WNK1. WNK1 controls the activity of the Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1), Na^+-Cl^- cotransporter (NCC), renal outer medullary K^+ (ROMK) channel ($K_{ir}1.1$) and may also regulate some of the vanilloid transient receptor potential (TRPV) family (TRPV4 and TRPV5). WNK1 functions in cell volume regulation and also contributes to the signalling network that controls NaCl reabsorption in the DCT (Module 7: Figure kidney salt reabsorption).

WNK2

There is little information on the distribution and function of WNK2.

WNK3

WNK3 is highly expressed in brain and kidney. In the latter case, it is expressed along the entire tubule. It seems to be particularly important in responding to changes in cell volume and/or [Cl^-] during processes such as the transport of ions across the kidney tubule and in cell volume regulation. It has the ability to enhance the cotransporters that bring chloride into the cell while inhibiting those responsible for Cl^- efflux and this is central for the process of cell volume regulation (Module 3: Figure cell volume regulation). The expression of WNK3 in neurons closely tracks that of K^+-Cl^- cotransporter 2 (KCC2) indicating that it may contribute to the shift in cotransporter function responsible for changes in neuronal excitability.

WNK4

WNK4 is found in many different epithelial cells but predominately in the distal convoluted tubule (DCT) and cortical collecting ducts of the kidney (Module 7: Figure kidney salt reabsorption). It is found mainly in the cytoplasm, but also associates with the tight junctions where it phosphorylates claudin to increase the paracellular flux of Cl^-. One of the main actions of WNK4 is to inhibit both the Na^+-Cl^- cotransporter (NCC) and the renal outer medullary K^+ (ROMK) channel. These inhibitory effects seem to be mediated by reducing the expression of these transporters in the apical membrane by promoting their internalization by the phosphorylation of synaptotagmin 2. WNK4 also functions in cell volume regulation.

Cell volume regulation

Regulation of cell volume depends on the activity of members of the cation-chloride cotransporter family (Module 3: Figure cation chloride cotransporters). The Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1) plays a major role in increasing cell volume by bringing Cl^- into the cell whereas the K^+-Cl^- cotransporter 1 (KCC1) leaches Cl^- out of the cell resulting in cell volume decreases (Module 3: Figure cell volume regulation). There has been a longstanding question concerning how the two opposing cotransporters are regulated to bring about these changes in cell volume. While NKCC1 is activated upon phosphorylation of the N-terminal region (Module 3: Figure cation chloride cotransporters), KCC1 is activated by dephosphorylation through protein phosphatase 1 (PP1). It is likely therefore, that cell volume regulation will depend on protein kinases and phosphatases that determine these phosphorylation states. There is increasing evidence that the WNK protein kinases may function as the 'sensors' to monitor either the intracellular [Cl^-] or cell volume or both (Module 3: Figure cell volume regulation). A decline in [Cl^-] or cell volume activates both WNK1 and WNK3 and these seem to be responsible for initiating the phosphorylation cascades that control the activity of the cotransporters. WNK1 appears to act through WNK4 and the Ste-related proline-alanine-rich kinase (SPAK), which is then responsible for phosphorylating and activating Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1), which brings Cl^- into the cell and this then increases cell volume. The WNK3 can also

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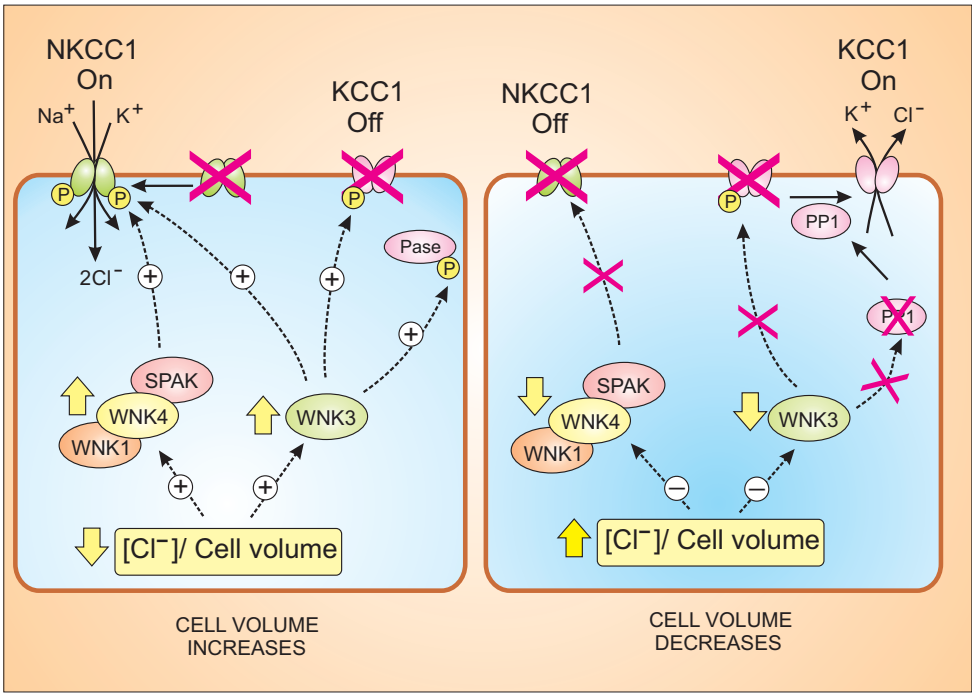
Module 3: | Figure cell volume regulation



Cell volume regulation model.
This model attempts to show how the cation-chloride exchangers such as Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1) and K^+-Cl^- cotransporter 1 (KCC1) are activated or inhibited to bring about cell volume increases and decreases. The With no lysine (k) (WNK) kinases and the Ste-related proline-alanine-rich (SPAK) kinase form a signalling network to regulate the cotransporters.

phosphorylate NKCC1 and KCC1. Phosphorylation of KCC1 serves to inhibit its activity and thus contributes to the accumulation of internal Cl^-. In addition, WNK3 may also phosphorylate PP1 that prevents it from dephosphorylating and activating KCC1.

These various phosphorylation events are reversed when the internal [Cl^-] or cell volume is high, which seems to inactivate WNK1 and WNK4 (Module 3: Figure cation chloride cotransporters). The absence of kinase activity results in dephosphorylation and inactivation of NKCC1 whereas KCC1 is activated. The dephosphorylation of KCC1 may be facilitated by an increase in protein phosphatase activity. The increase in KCC1 and the decrease in NKCC1 results in a loss of Cl^-, resulting in a decrease in [Cl^-] and cell volume. The mechanism of cell volume regulation illustrated in Module 3: Figure cell volume regulation is still somewhat speculative but it does show some of the potential players and how they might interact with each other.

Cell volume regulation may also be regulated by CLC-2, which is sensitive to cell swelling.

Aquaporins

The aquaporins are a family of membrane channels that conduct water and related molecules (Module 3: Table aquaporin family). The family can be divided into two main groups: the aquaporins that are permeable to water only, and the aquaglyceroporins that are permeable to water to varying extents and also have a high permeability to other solutes, notably glycerol. However, the primary function of most of the aquaporins is to conduct water across membranes, and this is particularly evident in the case of fluid-transporting epithelia. For example, in the case of the collecting duct cells in the kidney, aquaporin 2 (AQP2) transports water into the cell, whereas AQP3 and AQP4 enable water to leave the cell across the basolateral membrane (Module 7: collecting duct function). Aquaporin 7 is strongly expressed in white fat cells where it functions to transport glycerol out of the cell following lipolysis (Module 7: Figure lipolysis and lipogenesis).

The functional aquaporin channel is a tetramer (Module 3: Figure aquaporin structure). Each subunit is a functional channel composed of a single 28 kDa polypeptide that consists of an internal repeat. Each repeat has three transmembrane domains (shown in green and blue) that are connected together through loop C. Each repeat has the signature motif Asn-Pro-Ala (NPA) located in loops B and E, which meet and overlap with each other in the middle of the membrane to form the aqueous pore.

The C-terminal tail of AQP2 contains Ser-256, which is the target of cyclic AMP-dependent phosphorylation responsible for the translocation of AQP2-containing vesicles to the apical plasma membrane of the kidney collecting duct cells (Module 7: collecting duct function).Vasopressin acts by increasing the shuttling of aquaporin-containing vesicles to the apical membrane.

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Module 3: | Table aquaporin family
Distribution of aquaporins.

Aquaporin (AQP)	Comments
AQP0	Located in the plasma membrane of the lens
AQP1	Widely distributed (red blood cells, kidney proximal tubule, lung, brain and eye)
AQP2	Apical membrane of kidney collecting ducts (Module 7: collecting duct function)
AQP3	Basolateral membrane of kidney collecting ducts (Module 7: collecting duct function), skin, lung eye and colon; channel also conducts glycerol and urea
AQP4	Basolateral membrane of kidney collecting ducts (Module 7: collecting duct function), brain, muscle, lung stomach and small intestine
AQP5	Apical membrane of salivary gland, lacrimal gland, sweat gland and cornea
AQP6	Confined to intracellular vesicles on type A intercalated cells in kidney collecting duct; channel also conducts anions (NO_3^- Cl^-)
AQP7	Located in plasma membrane of adipose tissue, kidney and testis; channel also conducts glycerol, urea and arsenite
AQP8	Testis, kidney, liver pancreas, small intestine and colon
AQP9	Liver, leukocytes, brain and testis; channel has a low permeability to water, but conducts glycerol, urea and arsenite
AQP10	Intracellular vesicles in small intestine; channel has a low permeability to water, but conducts glycerol and urea

Information reproduced from Table 1 in King et al. 2004.

Aquaporin water permeability is inhibited reversibly by $HgCl_2$; this mercurial sensitivity depends upon Cys-189 in the E loop.

Some types of nephrogenic diabetes insipidus (NDI) is caused by mutations in the *AQP2* gene.

Ca^{2+} release channels

Release from internal stores represents a major source of signal Ca^{2+} for many cells. The major Ca^{2+} store is located in the extensive endoplasmic reticulum (ER) network that extends throughout the cell. In muscle, parts of this network have been specialized to form the sarcoplasmic reticulum (SR). There are also stores in the Golgi and within lysosomal organelles that can be accessed by different Ca^{2+} release channels that are specially adapted to release Ca^{2+} in response to various stimuli, such as direct protein–protein interactions or through a variety of Ca^{2+}-mobilizing second messenger systems, such as Ca^{2+} itself acting through a process of Ca^{2+}-induced Ca^{2+} release (CICR), the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette, nicotinic acid-adenine dinucleotide phosphate (NAADP) signalling and cyclic ADP-ribose (cADPR) signalling. These different mechanisms for mobilizing Ca^{2+} act through two main families of Ca^{2+}-sensitive channels: the ryanodine receptors (RYRs) and the inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$) that are located on the ER and SR membranes. A critical feature of these channels is that they are activated by Ca^{2+} that is derived either from the outside through voltage-operated channels (VOCs) or receptor-operated channels (ROCs) or from neighbouring receptors, as occurs when these release channels excite each other to create intracellular Ca^{2+} waves (Module 2: Figure Ca^{2+}-induced Ca^{2+} release). The Ca^{2+}-sensitivity of the release channels is determined by cytosolic second messengers (e.g. $InsP_3$ and cADPR) or by the concentration of Ca^{2+} within the lumen of the ER/SR. This luminal regulation of Ca^{2+} release channels is still somewhat contentious, but may turn out to be one of the key elements in regulating the function of these release channels.

Ryanodine receptors (RYRs)

Ryanodine receptors (RYRs) function to release Ca^{2+} from internal stores. There are three different RYR isoforms: RYR1, RYR2 and RYR3. They take their name from the fact that they bind strongly to the alkaloid ryanodine that stimulates RYRs at low concentrations, but becomes inhibitory at higher doses. The three isoforms share approximately 65% amino acid sequence identity and are encoded by separate genes. These RYRs are very large proteins containing more that 5000 amino acids. Functional channels are formed when four subunits associate to form homotetramers. About one-fifth of this large complex forms the channel pore embedded in the endoplasmic reticulum (ER)/sarcoplasmic reticulum (SR) membrane, whereas the remaining four-fifths forms a large cytoplasmic head approximately 20 nm in diameter that can span the gap between the plasma membrane and the ER/SR membranes, as occurs during excitation–contraction coupling in skeletal muscle.

Little is known about ryanodine receptor 3 (RYR3), even though it is widely distributed. Most attention has focused on ryanodine receptor 1 (RYR1) and ryanodine receptor 2 (RYR2). While these two receptors have a similar structure, they are regulated through different mechanisms (Module 2: Figure Ca^{2+} modules). The RYR1s are activated through a conformational coupling mechanism, whereas the RYR2s are activated by Ca^{2+}.

Mutations in RYRs cause a number of pathologies. RYR1 mutations are responsible for malignant hyperthermia (MH) and central core disease (CCD). A number of RYR2 mutations have been linked to catecholamine polymorphic ventricular tachycardia (CPVT) and a closely related syndrome, arrhythmogenic ventricular cardiomyopathy type 2 (ARVD2).

Ryanodine receptor 1 (RYR1)

The ryanodine receptor 1 (RYR1) is expressed mainly in skeletal muscle, but has also been described in certain neurons such as the cerebellar Purkinje cells. The main function of RYR1 is in excitation-contraction (E-C) coupling in skeletal muscle (Steps 3 and 4 in Module 7: Figure skeletal muscle E-C coupling). The process of excitation--contraction (E-C) coupling depends upon the $Ca_V1.1$ L–type channel in the T-tubule membrane making direct contact with RYR1 located in the underlying sarcoplasmic reticulum (SR) membrane at the triadic junctions (Module 3: Figure L-type channel/RYR1 complex). The gap between the SR and the T-tubule membrane is spanned by the large cytoplasmic head of the RYR1, which makes contact with

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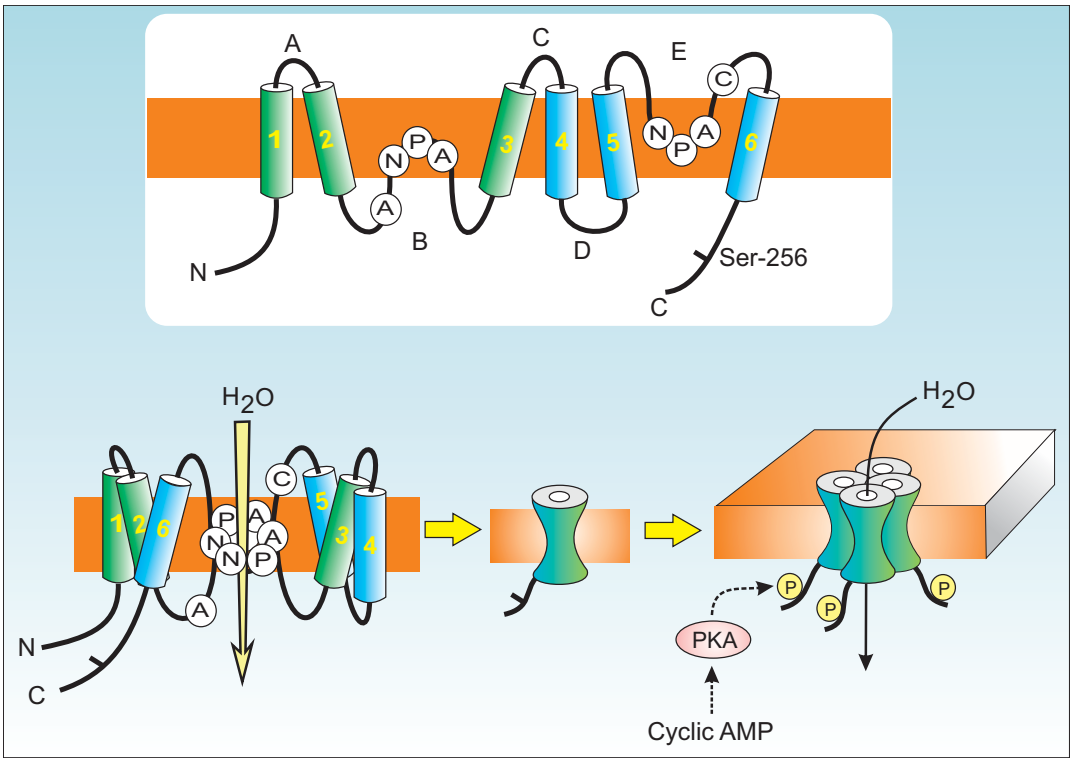
Module 3: | Figure aquaporin structure



Aquaporin structure and functional organization.
The domain structure of a typical aquaporin is shown at the top. These domains come together to form one of the subunits (bottom left), which associate as tetramers to form a functional water channel (bottom right). Cyclic AMP-dependent phosphorylation of Ser-256 on the cytoplasmic C-terminal tail plays a critical role in the translocation of aquaporin-containing vesicles to the plasma membrane during the action of vasopressin (Module 7: collecting duct function).

four L-type channels. Depolarization of the T-tubule induces a conformational change in the L-type Ca^{2+} channels that then act to open the RYR1 channel through a direct protein–protein interaction. The molecular determinants of this interaction are now being identified. The loop linking domains II and III of the α1 subunit of the L-type channel (Module 3: Figure $Ca_V1.1$ L-type channel) binds to residues 1837 and 2168 of the RYR1.

The function of RYR1 appears to be regulated by the FK506-binding protein (FKBP) 12 (FKBP12). Each RYR1 monomer subunit within the tetramer binds a single FKBP12 molecule and this can be displaced by FK506 or rapamycin, which results in an increase in the probability of channel opening. FKBP12 appears to function by stabilizing the closed state of the channel.

Ryanodine receptor 2 (RYR2)

Ryanodine receptor 2 (RYR2) is one of the major Ca^{2+} release channels that functions in a large number of cell types, such as cardiac muscle, smooth muscle, many neuronal cells and adrenal chromaffin cells. Like the other RYRs, the functional channel is a homotetramer, with the C-terminal transmembrane (TM) domains embedding the subunits in the endoplasmic reticulum (ER)/sarcoplasmic reticulum (SR) membrane and the large N-terminal region forming a bulbous head that functions as a scaffolding protein that binds a large number of regulatory components (Module 3: Figure ryanodine receptor structure). There is some debate as to the exact number of TM domains, but there seems to be some consensus that there may be six such domains with a pore (P) loop situated between TM5 and TM6. The C-terminal region is short in contrast with the very large N-terminal region, which is decorated with a large number of proteins that function to modulate channel activity.

The cyclic AMP signalling pathway has been implicated in the modulation of RYR2 activity (Module 2: Figure cyclic AMP signalling). The cyclic AMP effector protein kinase A (PKA) is attached to residues 3003–3039 of RYR2 through the muscle A-kinase-anchoring protein (mAKAP). The PKA attached to RYR2 acts to phosphorylate Ser-2809. Dephosphorylation is carried out by phosphatases that are also attached to this channel. Protein phosphatase 1 (PP1) is linked to RYR2 between residues 554 and 588 through spinophilin, which is one of its targeting subunits (Module 5: Table PP1 regulatory, targeting and inhibitory subunits). Protein phosphatase 2A (PP2A) is linked to RYR2 between residues 1603 and 1631 through PR130, which is one of the B'' regulatory subunits (Module 5: Table PP2A subunits).

The RYR2 channel binds FK506-binding protein (FKBP) 12.6 (FKBP12.6) by binding to a site between residues 2416 and 2430. This association with RYR2 can be disrupted by both FK506 and rapamycin, and results in the appearance of subconductance states. FKBP12

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appears to act by stabilizing the closed state of RYR2 by co-ordinating the activity of the four subunits that make up the functional tetramer. There is also an indication that FKBP12 may contribute to a process of coupled gating whereby the individual tetramers interact with each other to open in unison.

Another important aspect of RYR2 is the luminal regulation of Ca^{2+} release channels. This sensitivity to luminal Ca^{2+} depends on its interaction with three proteins. Two of these proteins, junctin and triadin, are integral proteins embedded in the SR membrane (Module 3: Figure ryanodine receptor structure). Their C-terminal tails, which extend into the lumen of the SR, interact with calsequestrin (CSQ), which is the major Ca^{2+} buffer within the lumen. A remarkable aspect of junctin, triadin and RYR2 is that their expression is tightly co-ordinated. This emerged from transgenic studies, where the level of triadin was increased and resulted in a down-regulation of both junctin and RYR2 (Module 12: Figure Ca^{2+} in triadin-1-overexpressing mice). Junctin and triadin thus draw CSQ into a complex with the RYR2, and may provide a mechanism whereby the level of luminal Ca^{2+} can adjust the Ca^{2+}-sensitivity of RYR2.

The function of RYR2 has been studied most extensively in the case of cardiac muscle, where it creates the Ca^{2+} signals that drive contraction of both the ventricular cells and atrial cells. RYR2 is a Ca^{2+}-sensitive channel that functions in the processes of Ca^{2+}-induced Ca^{2+} release (CICR). One of the primary modes of activation is by Ca^{2+} entering the cell through voltage-operated Ca^{2+} channels (Mechanism 5 in Module 2: Figure Ca^{2+} modules). The mode of activation and function of RYR2 varies between cell types:

- In ventricular cells, the release of Ca^{2+} from the junctional complex creates a Ca^{2+} spark (Module 7: Figure ventricular cell E-C coupling). Electrical recruitment of the individual sparks from the 10000 junctional complexes in a ventricular cell is then responsible for contraction (Module 7: Figure ventricular and atrial cell kinetics).
- In atrial cells, RYR2s near the membrane are activated by Ca^{2+} entry, as described above for ventricular cells. In addition, this spark at the cell surface ignites an intracellular wave by stimulating RYR2s located on the parallel strands of the SR that extend into the cell (Module 7: Figure ventricular and atrial cell kinetics).
- In the spines of hippocampal CA1 neurons, RYR2 is activated by Ca^{2+} entry from either the voltage-operated channel or from the *N-methyl-D-aspartate (NMDA) receptor* (Module 10: Figure synaptic signalling mechanisms).
- The RYR2 channel, which is up-regulated during pregnancy, facilitates excitation–contraction coupling in the uterus (Module 7: Figure uterus activation).

Ryanodine receptor 3 (RYR3)

Not much is known about ryanodine receptor 3 (RYR3). In the case of the Interstitial cells of Cajal (ICC), it may play a role in the initiation of the ICC cytosolic Ca^{2+} oscillator (Module 7: Figure ICC pacemaker).

Inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$)

The inositol 1,4,5-trisphosphate ($InsP_3$) and Ca^{2+} signalling system functions in a large number of cellular control processes. This signalling pathway depends on the stimulation of phospholipase C (PLC) that hydrolyses the precursor lipid $PtdIns4,5P_2$ to produce both diacylglycerol (DAG) and $InsP_3$ (Module 2: Figure $InsP_3$ and DAG formation). The $InsP_3$ that is released into the cytoplasm then functions as a second messenger to mobilize Ca^{2+} from the endoplasmic reticulum (ER) by acting on $InsP_3$ receptors ($InsP_3Rs$) (Mechanism 6 in Module 2: Figure Ca^{2+} modules).

There are three different $InsP_3Rs$ ($InsP_3R1$, $InsP_3R2$ and $InsP_3R3$), which have fairly similar primary structures and properties. However, they are sufficiently different for cells to express them in different proportions and in different cellular locations. In the following discussion, they will be considered together because there is not enough information at present to warrant separate descriptions. Four subunits come together to form functional channels, and the similarity between the three isoforms is reflected in the fact that they can form both homo- and hetero-tetramers (Module 3: Figure $InsP_3R$ regulation).

The domain structure of one of the subunits reveals that the receptor is organized into three main regions (Module 3: Figure $InsP_3R$ structure). The transmembrane (TM) and pore (P) domain embeds the receptor in the endoplasmic reticulum. There are six TM domains, with the pore loop located between TM5 and TM6. The luminal loops of the pore are glycosylated. There is a very long N-terminal region that has an $InsP_3R$-binding domain at the end, which is connected to TM1 by the modulatory domain. This modulatory loop has two important functions. Firstly, it has to transmit the conformational change induced by the binding of $InsP_3$ and Ca^{2+} down to the transmembrane region to open the pore. Secondly, it is the site where many of the modulators act to alter the release of Ca^{2+} (Module 3: Figure $InsP_3R$ regulation).

The primary regulators of the $InsP_3R$ are the Ca^{2+}-mobilizing second messenger $InsP_3$ and Ca^{2+}. The way in which these two messengers co-operate with each other is complicated, but the basic mechanism is that $InsP_3$ binds to the $InsP_3$-binding domain to induce a conformational change which sensitizes a Ca^{2+}-binding site, and it is the subsequent binding of Ca^{2+} that then acts to open the channel (Module 3: Figure $InsP_3R$ activation). The effect of Ca^{2+} on the $InsP_3R$ is bimodal; it is stimulatory at low levels of Ca^{2+}, but then becomes inhibitory at concentrations above about 300 nM. This dual control of the $InsP_3R$ by both $InsP_3$ and Ca^{2+} is central to its multiple functions in cell signalling. It is central to the mechanism of Ca^{2+} oscillations (Module 6: Figure Ca^{2+} oscillation model). The fact that the opening of the $InsP_3R$ depends upon the presence of both $InsP_3R$ and Ca^{2+} has suggested a possible role as a neuronal coincident detector (Module 10: Figure coincidence detectors).

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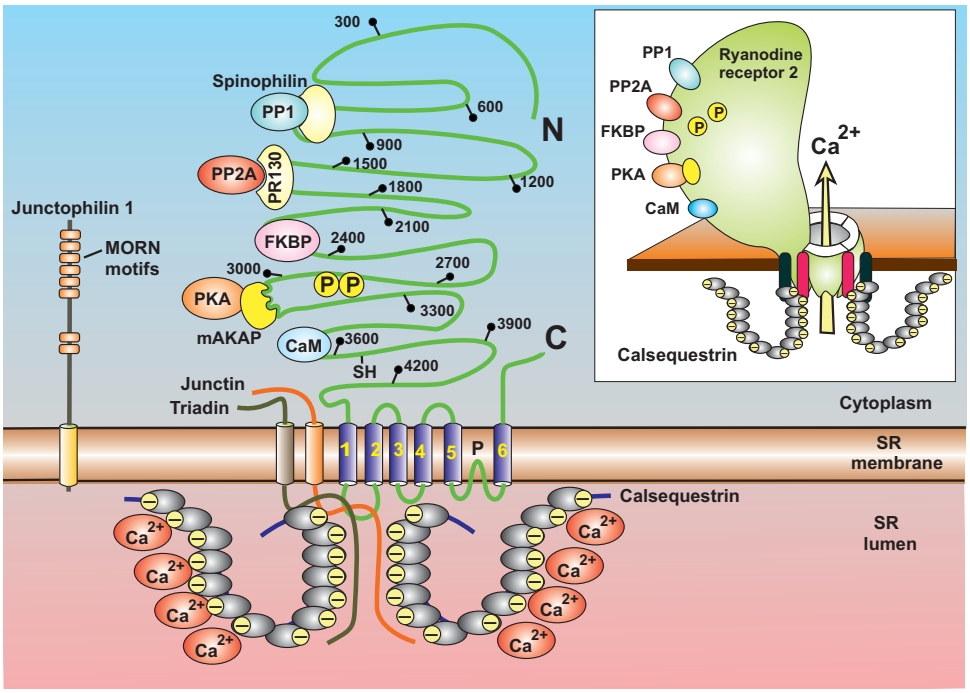
Module 3: | Figure ryanodine receptor structure



Domain structure of the type 2 ryanodine receptor (RYR2).
RYR2 is a tetramer. In the C-terminal region of each subunit there are transmembrane domains that form the channel by embedding in the membrane. The very large N-terminal region forms a bulbous head that functions as a scaffold to bind a large number of regulatory components. The loops within the lumen interact with junctin and triadin, which also associate with the Ca^{2+}-binding protein calsequestrin.

In addition to $InsP_3$ and Ca^{2+}, there are a large number of other physiological and pharmacological regulators that are able to modulate the activity of the $InsP_3Rs$ (Module 3: Figure $InsP_3R$ regulation).

The expression of $InsP_3Rs$ is regulated through a number of signalling pathways operating at both the transcriptional and post-transcriptional levels. $InsP_3R$ transcription is regulated by the nuclear factor of activated T cells (NFAT), which is part of a signalsome stability network (Module 4: Figure NFAT control of Ca^{2+} signalling toolkit). In the case of neurons, transcription of $InsP_3R1$ is induced in response to tumour necrosis factor α (TNFα) during inflammation in Alzheimer's disease (AD) (Module 12: Figure inflammation in Alzheimer's disease).

At the post-transcriptional level, expression of the type 2 $InsP_3R$ is regulated by two different mechanisms. Firstly, miR-133a acts through a mechanism that plays a significant role in the regulation of Ca^{2+} signalling and cardiac hypertrophy (Module 12: Figure miRNA and cardiac hypertrophy). Secondly, the $InsP_3R$ pre-mRNA undergoes alternative splicing by the U2 snRNA spliceosomal complex that is regulated by both calcium homoeostasis endoplasmic reticulum protein (CHERP) and apoptosis-linked gene 2 (ALG-2).

Apoptosis-linked gene 2 (ALG-2)

Apoptosis-linked gene 2 (ALG-2) is a penta-EF-hand Ca^{2+}-binding protein and is a component of the Ca^{2+} signaling toolkit (Module 2: Table Ca^{2+} signalling toolkit).

One of the functions of ALG-2 is to co-operate with the calcium homoeostasis endoplasmic reticulum protein (CHERP) to regulate alternative splicing of the $InsP_3R1$ Ca^{2+} release channel.

The ALG-2 may also function in the Ca^{2+}-dependent regulatory step to modify the membrane events related to the COPII-mediated transport from ER to Golgi (Module 4: Figure COPII-coated vesicles).

Calcium homoeostasis endoplasmic reticulum protein (CHERP)

As its name implies, the calcium homoeostasis endoplasmic reticulum protein (CHERP) has been implicated in the regulation of Ca^{2+} release by both the ryanodine receptor (RYR) and inositol 1,4,5-trisphosphate ($InsP_3$) receptor ($InsP_3R$) families. However, there is some degree of uncertainty as to how this regulation of release is achieved. There is evidence that CHERP may control release by binding directly to the channels. An alternative view is that CHERP acts more indirectly by controlling the expression of these channels as has been described for the $InsP_3Rs$. The pre-mRNA of these $InsP_3Rs$ undergoes alternative splicing by the U2 snRNA spliceosomal complex that is regulated by CHERP. This post-transcriptional regulation by CHERP seems to occur in co-operation with apoptosis-linked gene 2 (ALG-2).

Mutations in the *ITPR1* gene, which codes for $InsP_3R1$, causes spinocerebellar ataxia type 15 (SCA15).

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Inositol 1,4,5-trisphosphate receptor ($InsP_3R$) agonists

In addition to $InsP_3$ itself, there are a number of other endogenous and pharmacological $InsP_3R$ agonists (Module 3: Figure $InsP_3R$ regulation):

- The fungal product adenophostin is 100-fold more active than inositol 1,4,5-trisphosphate ($InsP_3$). It activates the receptor by binding to the $InsP_3$-binding site.
- The $InsP_3$ receptor ($InsP_3R$) is sensitive to reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), which may act by oxidizing the two highly conserved cysteine residues located in the C-terminal region of the molecule. The oxidizing agent thimerosal can also activate the $InsP_3R$, and when added to intact cells, it can faithfully reproduce the action of normal stimuli such as the fertilization of mouse oocytes (Module 2: Figure thimerosal-induced Ca^{2+} signalling).
- The luminal regulation of Ca^{2+} release channels applies to the $InsP_3R$ and is an integral part of the mechanism of Ca^{2+} oscillations (Module 6: Figure Ca^{2+} oscillation model).
- ATP binds to a site in the modulatory domain where it potentiates the effect of $InsP_3$. This stimulatory effect of ATP could provide a mechanism for shaping Ca^{2+} signals to the metabolic state of the cell.

Inositol 1,4,5-trisphosphate receptor ($InsP_3R$) modulation

The activity of the inositol 1,4,5-trisphosphate receptor ($InsP_3R$) is modulated by a number of mechanisms. The assembly of this release channel in the ER membrane is regulated by GRP78. The subsequent function of the channel is modulated by protein phosphorylation driven by a number of signalling pathways (Module 3: Figure $InsP_3R$ regulation). The effects of phosphorylation are complex because it can result in either an increase or a decrease in $InsP_3$-induced Ca^{2+} release. In the parotid gland, acetylcholine-evoked secretion is potentiated by agents that elevate cyclic AMP, which acts by enhancing the degree of Ca^{2+} signalling by phosphorylating the $InsP_3R$ (Module 7: Figure salivary gland secretion). Similar effects occur in liver cells, where both cyclic AMP and cyclic GMP enhance the phosphorylation of the $InsP_3R1$ and increase the release of Ca^{2+}.

The cyclic AMP-dependent modulation of the $InsP_3R$ is carefully regulated by a large number of signalling components. A major component is Bcl-2, which serves to bind both DARPP-32 and calcineurin (CaN) (Module 3: Figure cyclic AMP modulation of the $InsP_3R$). In response to cyclic AMP, protein kinase A (PKA) phosphorylates both DARPP-32 and the $InsP_3R$. The phosphorylation of Ser-1755 greatly sensitizes the $InsP_3R$, whereas the phosphorylated DARPP-32 enables it to inhibit the protein phosphatase 1 (PP1) thus preventing desensitization. In response to $InsP_3$, the sensitized $InsP_3R$ gives rise to a large release of Ca^{2+}. Some of this Ca^{2+} feeds back to stimulate calcineurin (CaN) that then dephosphorylates DARPP-32 thus removing its inhibition of PP1. The PP1 then dephosphorylates the $InsP_3R$ resulting in a much reduced Ca^{2+} release. This negative-feedback loop operating through Bcl-2, DARPP-32, CaN and PP1 may function to prevent excessive elevations of Ca^{2+}.

There are other cell types where phosphorylation of the receptor results in a decrease in release. For example, phosphorylation of the $InsP_3R$ in vascular smooth muscle cells by cyclic GMP-dependent kinase (cGK) on Ser-1755 causes a rapid inhibition of Ca^{2+} mobilization. This action of cyclic GMP plays a role in relaxation of smooth muscle in response to nitric oxide (NO) (Module 7: Figure smooth muscle cell cyclic GMP signalling). This action of cyclic GMP is mediated by an $InsP_3R$-associated cyclic GMP kinase substrate (IRAG), which is phosphorylated by cGK in smooth muscle cells and is an essential component for the regulation of $InsP_3$-induced Ca^{2+} release. In other cells, such as megakaryocytes and their progeny the blood platelets, the formation of cyclic AMP can inhibit $InsP_3$-induced Ca^{2+} release (Module 11: Figure platelet activation). In platelets, α_{2A}-adrenergic receptor stimulation lowers the level of cyclic AMP, and this relieves the cyclic AMP-mediated suppression of the $InsP_3R$ and results in the release of Ca^{2+}.

Phosphorylation of the $InsP_3R$ by protein kinase B (PKB) functions to inhibit $InsP_3$-induced Ca^{2+} release and this could provide a possible mechanism for the inhibition of Ca^{2+}-dependent glycogenolysis by insulin in liver cells (Module 7: Figure liver cell signalling).

The Bcl-2 superfamily control of Ca^{2+} signalling depends on Bcl-2 binding to the $InsP_3R$ to reduce Ca^{2+} release during the operation of the endoplasmic reticulum (ER)/mitochondrial shuttle (Module 5: Figure ER/mitochondrial shuttle). This action of Bcl-2 seems to be facilitated by CICD2 and this may play a role in Ca^{2+} signalling and autophagy (Module 11: Figure autophagy). In addition, the Bcl-2 binds to DARPP-32 and calcineurin (CaN) that operate a negative-feedback loop as described earlier (Module 3: Figure cyclic AMP modulation of the $InsP_3R$).

The calcium hypothesis of Alzheimer's disease (AD) suggests that memory loss may be caused by an elevation of the resting level of Ca^{2+} and some of this may result from an increase in $InsP_3$-dependent Ca^{2+} release (Module 12: Figure amyloids and Ca^{2+} signalling). The ability of Bcl-2 to reduce the symptoms of Alzheimer's disease may be explained by its ability to reduce the release of Ca^{2+} by inhibiting $InsP_3$ receptors.

The Ca^{2+}-binding protein 1 (CaBP1) acts to reduce $InsP_3$-induced Ca^{2+} release by interfering with the gating mechanism of the $InsP_3R$. NCS-1 has also been implicated as a modulator of $InsP_3R$ function in that it acts to promote the release of Ca^{2+} from the endoplasmic reticulum (Module 3: Figure $InsP_3R$ regulation). This of interest in that there is an up-regulation of NCS-1 in the prefrontal cortex in patients with schizophrenia and bipolar disorders.

Inositol 1,4,5-trisphosphate receptor ($InsP_3R$) antagonists

There are a number of $InsP_3R$ antagonists operating from both the cytoplasmic and luminal sides (Module 3: Figure $InsP_3R$ regulation):

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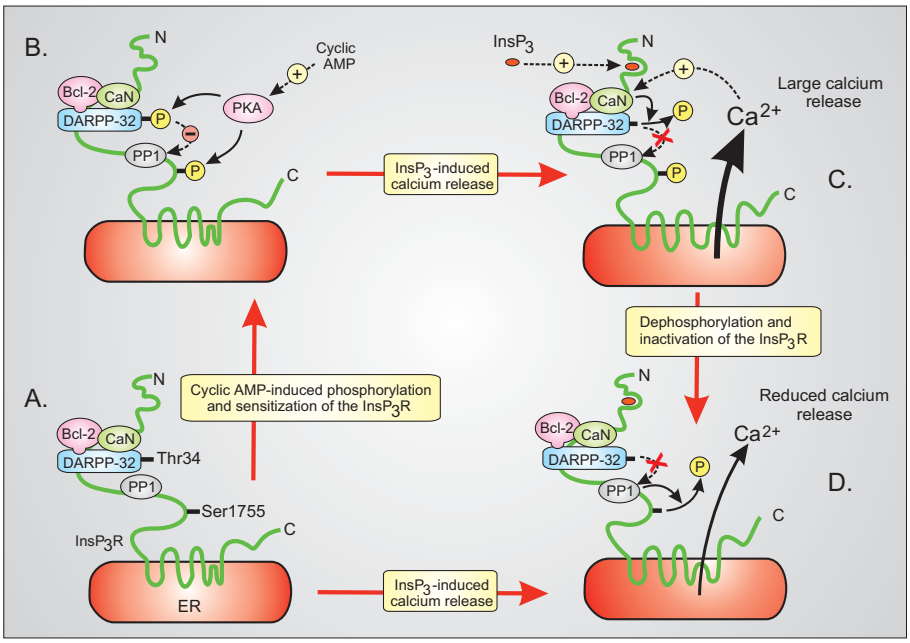
Module 3: | Figure cyclic AMP modulation of the $InsP_3R$



Cyclic AMP modulation of the $InsP_3R$.
The $InsP_3R$ binds a number of regulatory components that regulate its modulation by cyclic AMP-induced phosphorylation. A. Resting state. B. Cyclic AMP activates protein kinase A (PKA) to phosphorylate both DARPP-32, which enables it to inhibit the protein phosphatase 1 (PP1) thus preventing desensitization, and the $InsP_3R$, which greatly sensitizes the latter. C. In response to $InsP_3$ there is a large Ca^{2+} release. Some of this Ca^{2+} feeds back to stimulate calcineurin (CaN) that then dephosphorylates DARPP-32 thus removing its inhibition of PP1. D. The PP1 dephosphorylates the $InsP_3R$ resulting in a much reduced Ca^{2+} release. This Figure was redrawn from a figure in Chan et al. (2014).

- The $InsP_3R$-binding protein released with inositol 1,4,5-trisphosphate (IRBIT) is a cytoplasmic protein that acts to adjust the sensitivity of the $InsP_3R$ by masking the $InsP_3$-binding site. Once IRBIT is phosphorylated, it can compete with $InsP_3$ for the $InsP_3R$-binding site.
- Xestospongins are potent membrane-permeant blockers of the inositol 1,4,5-trisphosphate receptor ($InsP_3R$). However, they can also inhibit the endoplasmic reticulum (ER) Ca^{2+} pump and this may explain some of its inhibitory action on $InsP_3$-induced Ca^{2+} release.
- Caffeine is an effective permeant and a reversible inhibitor of $InsP_3$-induced Ca^{2+} release. Caffeine may act by competing with ATP, which is an essential co-activator of $InsP_3R$. The inhibitory effect can be overcome by increasing the concentration of $InsP_3$.
- 2-Aminoethoxydiphenyl borate (APB) is a membrane-permeant inhibitor of the $InsP_3R$. While it clearly is not completely specific and can have effects on both Ca^{2+} pumps and various membrane entry channels, it has proved to be an effective tool for inhibiting Ca^{2+} release.
- Hydrogen sulfide (H_2S) may act by diffusing into the ER lumen where it reduces a disulfide bond on a luminal loop of the $InsP_3R$ resulting in a decrease in the release of Ca^{2+}. Such an action may be particularly important in regulating the contraction of airway smooth muscle cells.
- The endoplasmic reticulum resident protein 44 (ERp44) may act to reduce $InsP_3R$ activity by binding to the luminal loop between TM5 and TM6. A similar function may also be performed by endoplasmic reticulum oxidoreductin-1 alpha (ERO-1α). Ero1α also interacts with the $InsP_3R$ and potentiates the release of Ca^{2+} during ER stress.

$InsP_3R$-binding protein released with inositol 1,4,5-trisphosphate (IRBIT)

$InsP_3R$-binding protein released with inositol 1,4,5-trisphosphate (IRBIT) was first identified as an inositol 1,4,5-trisphosphate receptor ($InsP_3R$) antagonist that is capable of competing with $InsP_3$ for the $InsP_3R$-binding site (Module 3: Figure $InsP_3R$ regulation). The inhibitory activity of IRBIT is activated following its phosphorylation by casein kinase 1 (CK1). This phosphorylation is reversed by protein phosphatase 1 (PP1).

In addition to regulating the release of Ca^{2+} from the endoplasmic reticulum (ER), IRBIT may also regulate the secretion of HCO_3^- by the exocrine pancreatic centroacinar cells.

Luminal regulation of Ca^{2+} release channels

The concentration of Ca^{2+} within the lumen of the endoplasmic reticulum (ER) or sarcoplasmic reticulum (SR) seems to play an important role in regulating the activity of both ryanodine receptors (RYRs) and inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$). The main consequence of this "luminal effect" is to adjust the sensitivity of these two release channels to the stimulatory effect of Ca^{2+}. One of the consequences of this change in sensitivity is the

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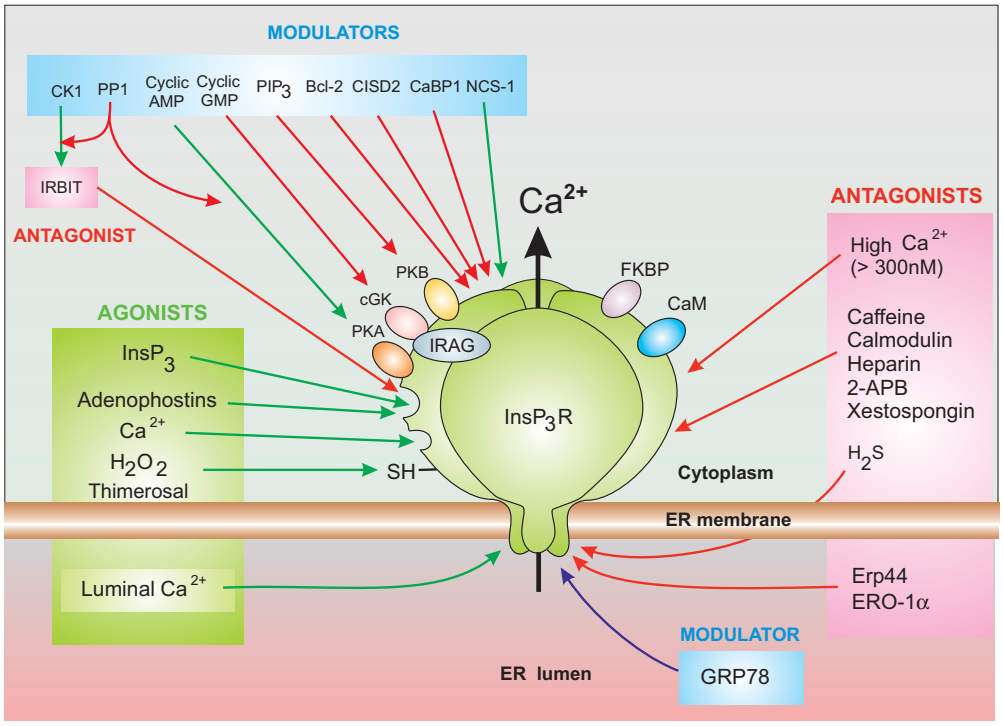
Module 3: | Figure $InsP_3R$ regulation



Regulation of the inositol 1,4,5-trisphosphate receptor ($InsP_3R$).

Summary of the physiological and pharmacological factors that regulate Ca^{2+} release by the inositol 1,4,5-trisphosphate receptor ($InsP_3R$). The primary agonists are Ca^{2+} and $InsP_3$. Adenophostin mimics the action of $InsP_3$. The receptor is also sensitive to reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and this action can be mimicked by thimerosal. The luminal level of Ca^{2+} also seems to be important for regulating the sensitivity of the $InsP_3R$ (Module 3: Figure $InsP_3R$ activation). A number of modulators can influence activity either positively (green arrows) or negatively (red arrows). Some of these phosphorylations are reversed by protein phosphatase 1 (PP1). Channel activity is modulated by phosphorylation through various signalling pathways using messengers such as cyclic AMP, cyclic GMP and PtdIns3,4,5P_3 (PIP_3). High levels of Ca^{2+} inhibit the receptor. There also are a range of pharmacological agents that have been used to inhibit release of Ca^{2+} by the $InsP_3R$.

marked increase in Ca^{2+} sparks that occur in cardiac and smooth muscle cells when the internal store becomes overloaded with Ca^{2+}. Although there is compelling physiological evidence for such a sensitizing role, there has been considerable debate as to the site where this luminal Ca^{2+} exerts its action. One suggestion is that the Ca^{2+} leaks through the channel to exert its effect by acting on Ca^{2+}-binding sites located on the cytoplasmic side of the channel. However, there also is evidence that Ca^{2+} acts from within the lumen.

In the case of the RYRs, the Ca^{2+}-binding protein calsequestrin (CSQ) may function as the sensor that acts through triadin and junctin to alter the Ca^{2+}-sensitivity of the RYRs (Module 3: Figure ryanodine receptor structure). However, luminal sensitivity is maintained in mice after CSQ has been deleted. It appears that the RYRs are directly sensitive to Ca^{2+} located in the lumen. There now is evidence for a luminal Ca^{2+} sensor in both the RYRs and $InsP_3Rs$ that is located close to the Ca^{2+} gate formed by the helix bundle crossing region that faces the internal pore and is thus freely accessible to Ca^{2+} in the lumen (see the asterisk in Module 3: Figure $InsP_3R$ activation).

This sensitivity to Ca^{2+} levels within the lumen has important functional consequences for the operation of these two Ca^{2+} release channels. In the case of the RYRs, the fall in the level of Ca^{2+} in the junctional SR, which results in a blink (Module 7: Figure ventricular cell Ca^{2+} blink), may play a critical role in the inactivation of ventricular type 2 ryanodine receptors (RYR2s). As the Ca^{2+} refills the junctional zone, the RYRs rapidly resensitize to Ca^{2+} and are thus able to function again to create the Ca^{2+} signal for the next heartbeat. A very similar desensitization/resensitization process may function as part of the mechanism of Ca^{2+} oscillations. Release of Ca^{2+} by the $InsP_3Rs$ depends upon an interplay between cytosolic $InsP_3$ and Ca^{2+} and the luminal level of Ca^{2+} (Module 3: Figure $InsP_3R$ regulation). It is the periodic loading of the lumen with Ca^{2+} that functions to sensitize the $InsP_3Rs$ to release the regular pulses of Ca^{2+} that make up the Ca^{2+} oscillations that feature so significantly in so many cellular control processes.

Triadin

Triadin is one of the proteins located in the junctional zone of muscle (skeletal and cardiac). It has a single transmembrane domain embedded in the sarcoplasmic reticulum (SR) with a short N-terminal cytoplasmic segment and a larger, highly charged segment in the lumen. In skeletal muscle, this luminal domain is thought to interact with both calsequestrin and the ryanodine receptor

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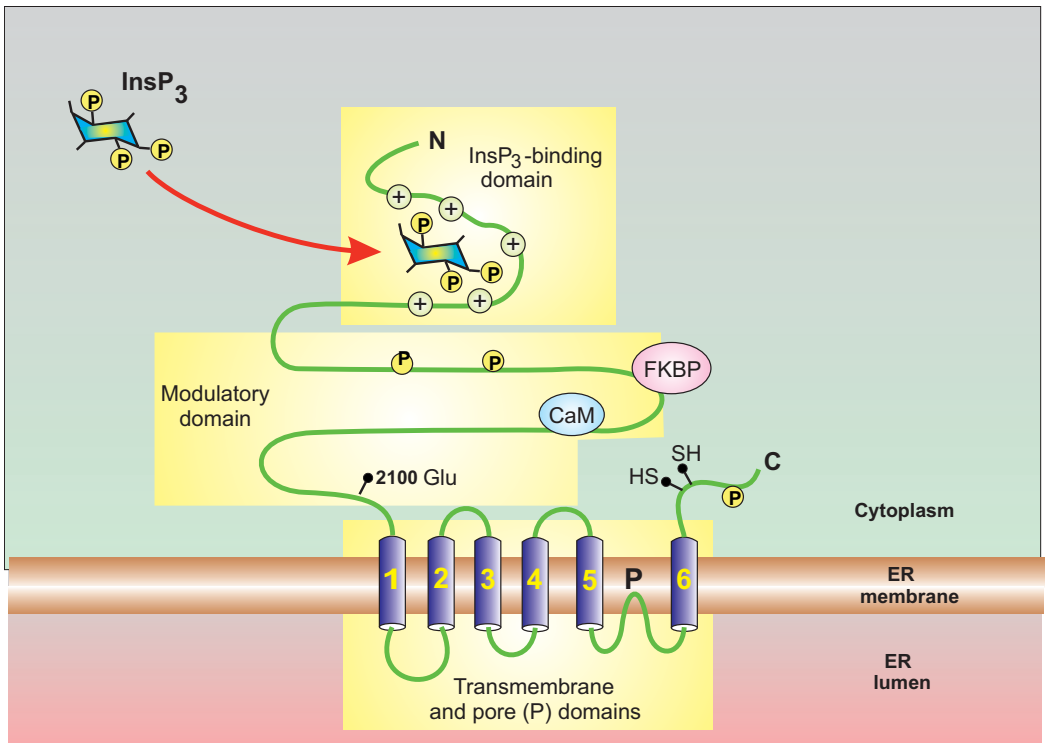
Module 3: | Figure $InsP_3R$ structure



Structural organization of the inositol 1,4,5-trisphosphate ($InsP_3$) receptor.
The inositol 1,4,5-trisphosphate receptor ($InsP_3R$) can be divided into three functional domains (see yellow boxes). The transmembrane and pore domain has six transmembrane (TM) domains with a pore (P) domain located between TM5 and TM6. There is a short C-terminal tail containing two highly conserved thiol groups. There is a very long N-terminal region that comprises an $InsP_3R$-binding domain that is connected to TM1 by a modulatory domain. The way in which these subunits are thought to interact to form a functional channel is illustrated in Module 3: Figure $InsP_3R$ activation.

type 1 (RYR1). Cardiac triadin 1 is a shorter, alternatively spliced version of the triadin gene. The dihydropyridine receptor (DHPR), RYR and triadin co-localize in atrial and ventricular muscle cells (Module 3: Figure ryanodine receptor structure). In both skeletal and cardiac muscle, the triadin seems to play some role in regulating the release properties of the RYRs in the SR.

Overexpression of triadin 1 in mice results in cardiac hypertrophy (Module 12: Figure Ca^{2+} in triadin-1-overexpressing mice).

Junctin

Junctin is an intermediary protein that functions together with triadin to regulate the interaction between calsequestrin (CSQ) and RYR1. It is a transmembrane protein located in the sarcoplasmic reticulum that binds to both CSQ and RYR1 (Module 3: Figure ryanodine receptor structure).

Sorcin

Sorcin is a 22 kDa Ca^{2+}-binding protein which appears to associate with the ryanodine receptor type 2 (RYR2) and to regulate excitation–contraction coupling in the heart. An interesting feature of sorcin is that its expression in fibroblasts results in the expression of RYRs. Sorcin is a penta-EF-hand protein that acts to reduce Ca^{2+} release, and this inhibition is removed following its phosphorylation by protein kinase A (PKA). It may thus play a role as an inhibitor of the positive-feedback loop of Ca^{2+}-induced Ca^{2+} release (CICR). As Ca^{2+} builds up in the vicinity of the RYR2, it binds to sorcin to induce a conformational change that allows it to associate with the RYR2 to inhibit its activity. In effect, it acts as a regulator of the 'gain' of excitation–contraction coupling.

Annexin A7 binds to sorcin and may thus participate in the regulation of excitation–contraction coupling.

Junctophilin (JP)

A family of four junctophilins function to hold together the plasma membrane and the underlying endoplasmic/sarcoplasmic reticulum (ER/SR) at various junctional complexes. The C-terminal region of JP has a transmembrane domain that anchors the protein to the ER/SR membrane (Module 3: ryanodine receptor structure). The N-terminal region has eight MORN motifs, which consist of 14 residues that are thought to bind to phospholipids in the plasma membrane. The junctophilins function in cell signalling by maintaining the structural integrity of junctional complexes where information is transmitted in both directions between the plasma membrane and the ER/SR membrane.

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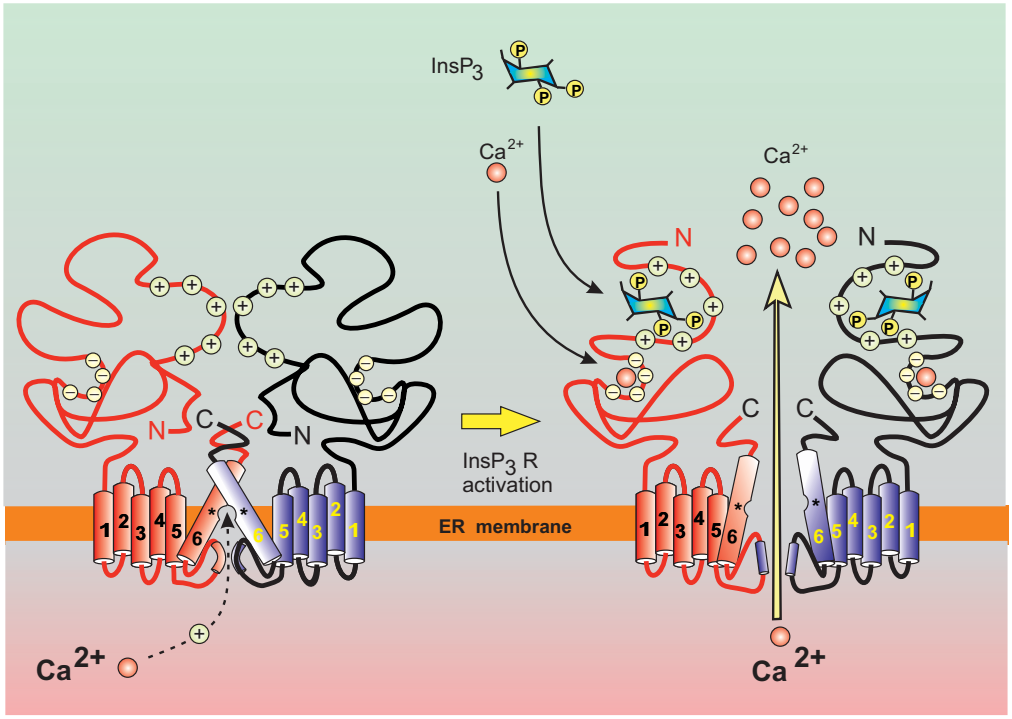
Module 3: | Figure $InsP_3R$ activation



Proposed activation of the inositol 1,4,5-trisphosphate receptor ($InsP_3R$) by the co-operative action of $InsP_3$ and Ca^{2+}.
In this figure, two of the channel subunits (coloured red and blue) that make up the tetramer are shown with their pore regions coming together to form the channel. In the resting state (shown on the left), the pore may be held in a closed state by the N-terminal region of one receptor interacting with the C-terminal region of a neighbouring receptor. Upon binding first $InsP_3$ and then Ca^{2+}, there is a marked conformational change that removes this N-terminal/C-terminal interaction and the gate region opens to allow Ca^{2+} to flow out of the endoplasmic reticulum (ER) into the cytoplasm. This drawing is based on a model shown in Figure 5 of Taylor et al. 2004. Reproduced from *Trends Biochem. Sci.*, Vol. 29, Taylor, C.W., da Fonseca, P.C.A. and Morris, E.P, IP_3 receptors: the search for structure, pages 210-219. Copyright (2004), with permission from Elsevier. The position of the luminal Ca^{2+} sensor (indicated by an asterisk) is postulated to lie close to the helix bundle crossing region that constitutes the gate, based on information in Chen et al. (2014).

Junctophilin 1 (JP1)

Junctophilin 1 (JP1), which is also known as mitsugumin 72 (MG72), maintains the structural integrity of the triadic junction in skeletal muscle. This junction is responsible for the rapid communication between the $Ca_V1.1$ L-type channels and the type 1 ryanodine receptor (RYR1) during excitation-contraction (E-C) coupling in skeletal muscle cells (see step 3 in Module 7: Figure skeletal muscle E-C coupling).

Junctophilin 2 (JP2)

Junctophilin 2 (JP2) is the cardiac member of the junctophilin (JP) family. It functions to organize the diadic junction in cardiac cells and plays a critical role in the process of ventricular cell excitation-contraction (E-C) coupling.

Mutations in JP2 have been linked to cardiac hypertrophy.

Junctophilin 3 (JP3)

Junctophilin 3 (JP3) together with junctophilin 4 (JP4) are expressed fairly widely and appear to be particularly important in neurons where they contribute to the formation of the subsurface cisternae (SSC), which are neuronal junctional complexes that are particularly evident in the soma (Module 10: Figure neuronal structure). These SSCs may play an important role in regulating neuronal excitability by controlling the activity of the Ca^{2+}-sensitive K^+ channels that give rise to the slow after-hyperpolarizing potential (sAHP) that follows either single or trains of action potentials (Module 10: Figure neuronal SK channel function). In addition to being opened by Ca^{2+} that enters through voltage-operated Ca^{2+} channels, these K^+ channels can also be activated by Ca^{2+} released from the internal stores. The close proximity of the ER to the plasma membrane at these subsurface cisternae (SSCs) enables Ca^{2+} released from the ryanodine receptors (RYRs) to control membrane excitability by activating K^+ channels in the plasma membrane.

Mutations in JP3 causes Huntington's disease-like 2 (HDL2).

Calsequestrin (CSQ)

Calsequestrin (CSQ) is located within the sarcoplasmic reticulum (SR) of cardiac, skeletal and smooth muscle cells and has also been described in other locations such as the brain (cerebellum). CSQ functions as a high-capacity, low-affinity buffer for Ca^{2+}. The buffering property of CSQ

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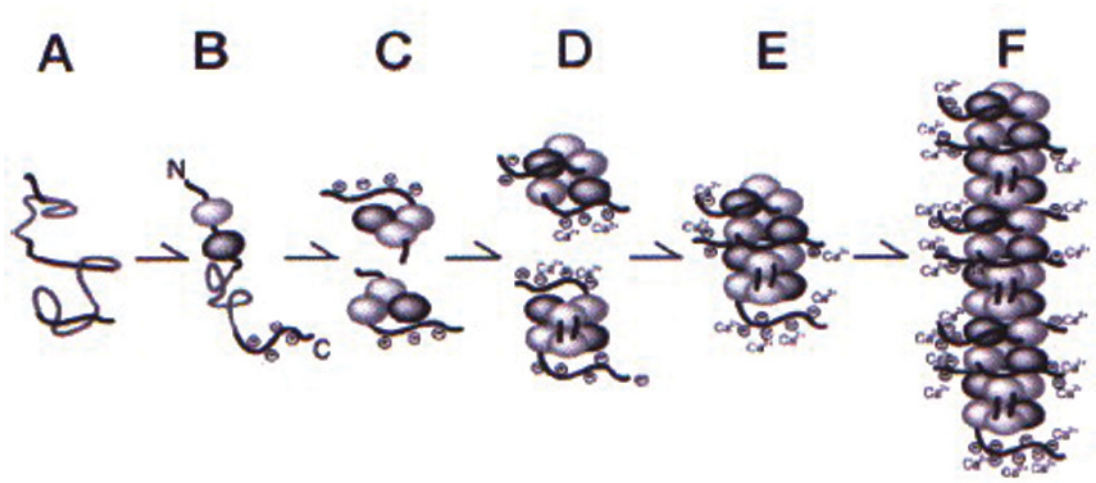
Module 3: | Figure CSQ polymerization



A model of calsequestrin polymerization to form Ca^{2+}-decorated fibrils.

A. In the absence of Ca^{2+}, the unfolded monomer is in an extended state because of the charge repulsion of its many negatively charged amino acids. B and C. As Ca^{2+} begins to bind, the charge repulsion is shielded, and the protein begins to fold up into its characteristic tertiary structure. D. The monomers then form front-to-front dimers. E. The dimers then form front-to-back tetramers. F. The tetramers then polymerize further to form the long fibrils. Reproduced from Figure 6 in Park, H., Wu, S., Dunker, A.K. and Kang, C. (2003) Polymerization of calsequestrin. Implications for Ca^{2+} regulation. J. Biol. Chem. 278:16176-16182, with permission from the American Society for Biochemistry and Molecular Biology; see Park et al. 2003.

enables it to store large amounts of Ca^{2+} that are immediately available for release to regulate muscle contraction. This role in release is not passive because CSQ is associated with the ryanodine receptor (RYR) through a complex that includes the luminal regions of both triadin and junctin (Module 3: Figure ryanodine receptor structure).

There are two genes that encode cardiac and skeletal muscle isoforms of CSQ. CSQ is a linear polymer, which has a large proportion of the amino acids aspartate or glutamate that are responsible for the negative charges that bind large amounts of Ca^{2+}. The skeletal muscle isoform binds approximately 40 Ca^{2+} ions, which is almost twice as many as its cardiac counterpart even though both have a similar content of negatively charged amino acids. The ability of CSQ to bind large amounts of Ca^{2+} induces large conformational changes that result in its polymerization into long fibres, which may constitute the fibrous array observed in electron micrographs of the junctional zone. The way in which these polymers are formed depends upon sequential processes of Ca^{2+}-induced protein folding to form monomers that then combine to form long polymers (Module 3: Figure CSQ polymerization).

Ca^{2+} has two important functions in this linear polymer. Firstly, it plays a role as the counter-ion that brings about the conformational changes that result in polymerization. Secondly, a large amount of Ca^{2+} that is bound to the polyanionic C-terminal tails can dissociate rapidly to provide a readily releasable pool of Ca^{2+} in the immediate vicinity of RYRs. The fact that these linear polymers are covered in a linear film of exchangeable Ca^{2+} ions would provide a mechanism of surface diffusion to explain the process of tunnelling, whereby Ca^{2+} ions can rapidly diffuse through the lumen of the endoplasmic reticulum.

Polymorphism of the human CSQ gene can cause catecholaminergic polymorphic ventricular tachycardia (CPVT).

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