Review Article

Rab GTPases in Parkinson's disease: a primer

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Parkinson's disease is a prominent and debilitating movement disorder characterized by the death of vulnerable neurons which share a set of structural and physiological properties. Over the recent years, increasing evidence indicates that Rab GTPases can directly as well as indirectly contribute to the cellular alterations leading to PD. Rab GTPases are master regulators of intracellular membrane trafficking events, and alterations in certain membrane trafficking steps can be particularly disruptive to vulnerable neurons. Here, we describe current knowledge on the direct links between altered Rab protein function and PD pathomechanisms.

Introduction

Parkinson's disease (PD) is the most common neurodegenerative movement disorder which affects over 10 million people worldwide. It is clinically characterized by symptoms such as resting tremor, bradykinesia, rigidity and postural instability. These motor symptoms are due to the loss of dopaminergic neurons in the substantia nigra pars compacta. However, not all dopaminergic neurons are affected in PD, and cell death is also observed in certain non-dopaminergic neurons in select brain areas [1–3]. The selective vulnerability of these cells is thought to be due to a combination of features including long highly branched axons with a very large number of transmitter release sites, combined with their distinctive physiological characteristics. Neurons susceptible to cell death in PD share tonic autonomous pacemaker activity, cytosolic calcium oscillations and low intrinsic calcium buffering capability. This is associated with calcium entry into mitochondria to stimulate oxidative phosphorylation and the production of ATP. Whilst this assures that bioenergetic needs are met, it also makes these cells particularly vulnerable. On one hand, stimulation of oxidative phosphorylation leads to increased production of reactive oxygen species (ROS), which compromises mitochondrial function and increases the need for mitochondrial quality control and the autophagic clearance of damaged mitochondria. On the other hand, high mitochondrial calcium concentrations can trigger permeability transition pore opening and apoptosis, and high cytosolic calcium concentrations impair proper autophagic-lysosomal degradation and thus the clearance of misfolded proteins and damaged organelles [1–3]. Such cell-autonomous mechanisms associated with increased vulnerability are further influenced by non cell-autonomous mechanisms, including inappropriate trophic support provided by various glial cells and detrimental effects related to inflammatory events (Figure 1).

Around 10% of PD cases are due to mutations in a set of *PARK* genes, providing us with an opportunity to understand disease mechanisms and identify therapeutic approaches aimed at decreasing neuronal loss. Conceptually, the gene products associated with familial PD may perform the same function(s) in all neuronal and even non-neuronal cell types. However, and if involved in one of the cellular processes implicated in the selective vulnerability as outlined above, their dysfunction will become particularly detrimental to the neurons which die in PD. Various observations support this possibility. For example, mutations in *PRKN* or *PINK1* cause autosomal-recessive PD, and both proteins are widely expressed throughout the body and in neurons and glia across the brain, even though their loss-of-function is associated with the selective death of vulnerable neurons [4,5]. Similarly, mutations in *LRRK2* are the most common cause of autosomal-dominant PD and cause loss of vulnerable neurons, even though LRRK2 is highly expressed in lung, kidney and immune cells, and to a lower degree in many neurons throughout the brain [6–8].
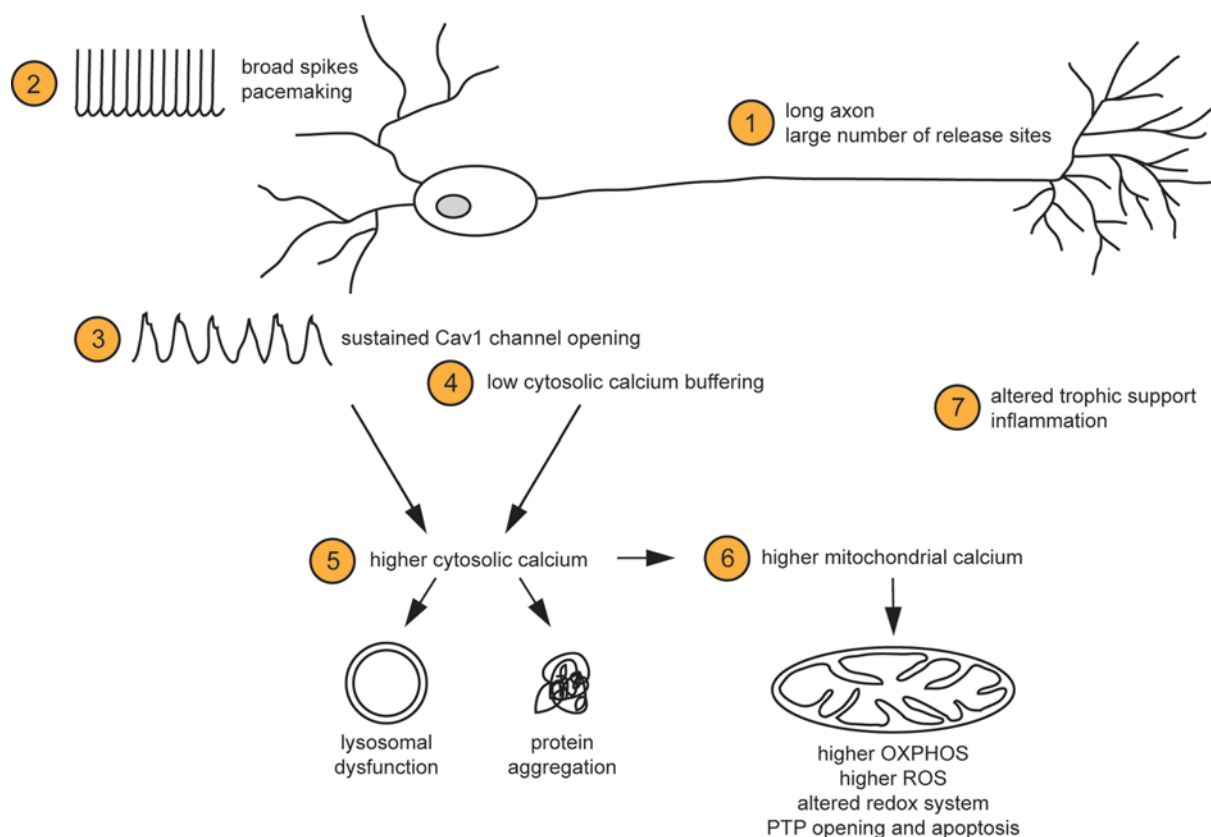
Figure 1. Selective vulnerability of neurons in PD
Schematics of traits common to vulnerable neurons. (1) Neurons have long and highly branched axons with a very large number of transmitter release sites, posing a burden on the appropriate transport of organelles such as mitochondria and autophagosomes to/from the axon. (2) Neurons have broad action potentials and autonomous pacemaking activity, spiking on their own without any excitatory synaptic input. (3) This slow spiking causes large oscillations in intracellular calcium concentrations due to the involvement of Cav1 calcium channels. (4) Vulnerable neurons express low levels of calcium-buffering proteins. (5) The cytosolic calcium oscillations and low intrinsic calcium buffering capability lead to increased cytosolic calcium which impairs lysosomal function and poses a burden on the proper turnover of aggregated proteins and the autophagic clearance of damaged organelles. (6) Increased cytosolic calcium/calcium oscillations are a signal for bioenergetic need, which is relayed by calcium entry into mitochondria to stimulate oxidative phosphorylation (OXPHOS) for increased ATP production. This can lead to increased production of reactive oxygen species (ROS) and alterations in the mitochondrial redox system. Mitochondrial damage poses a burden on mitochondrial quality control systems such as mitophagy and can lead to permeability transition pore (PTP) opening and apoptosis. (7) Additional non cell-autonomous mechanisms such as lack of trophic support or inflammatory insults negatively impact on the health of already vulnerable neurons.

However, parkin and PINK1 are important for mitophagy, and LRRK2 is known to regulate a variety of events including autophagic-lysosomal degradation, and these processes are especially critical for vulnerable neurons which are already functioning at a dangerously high level of bioenergetic and proteostatic needs, further aggravated by alpha-synuclein pathology [1–3]. Whilst such complex structural and physiological features of vulnerable neurons are not easily recapitulated when working with cellular disease models, they need to be taken into account when investigating mechanistic aspects underlying PD pathogenesis.

Several *PARK* genes function in membrane trafficking pathways, and Rab GTPases play a critical role in most intracellular membrane trafficking events including mitophagy and autophagic-lysosomal degradation. Therefore, it is perhaps not surprising that many Rabs have been linked to mechanisms underlying PD pathogenesis. However, it has become a challenge to disentangle cause versus effect, which is especially important when considering potential therapeutic interventions. Membrane trafficking alterations may be causal for PD, occuring as a direct consequence of a disease mutation or a post-translational modification of a Rab or a Rab regulatory protein. Alternatively, Rab-driven
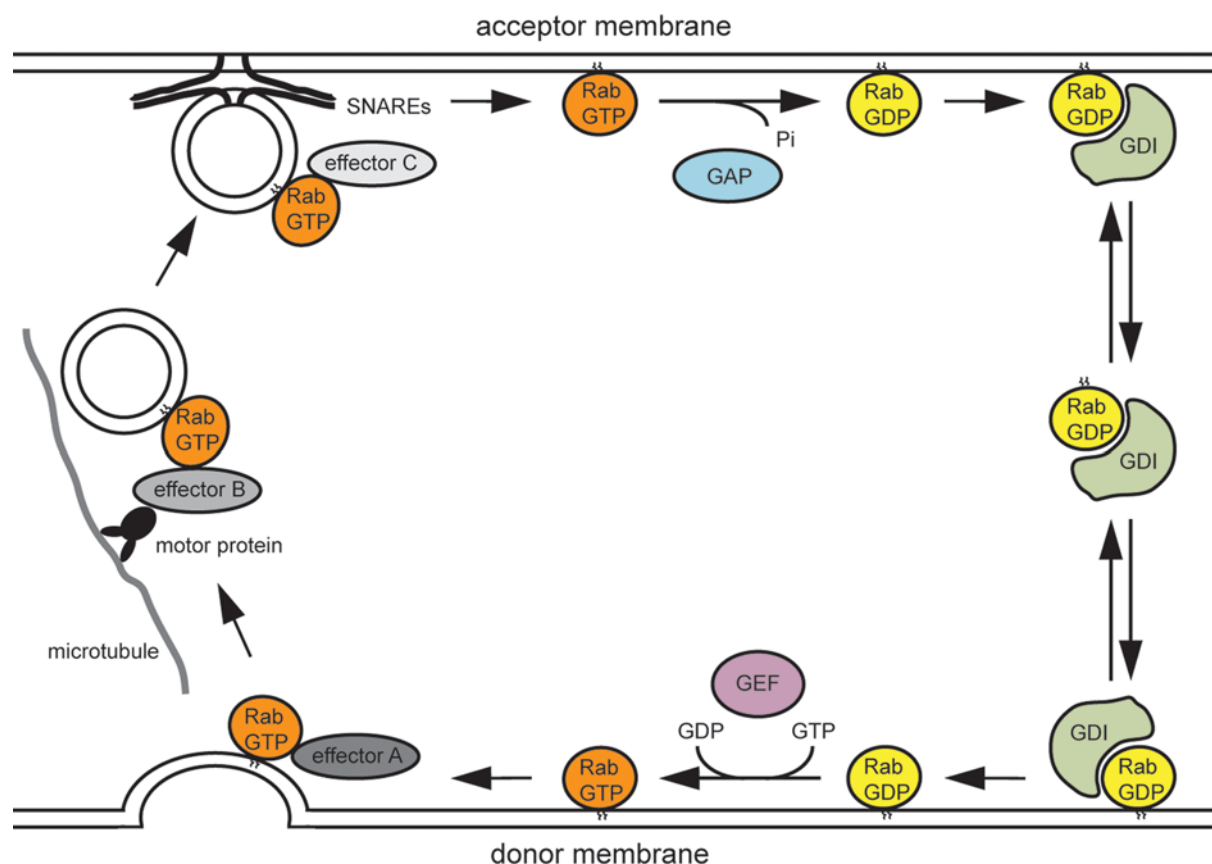
Figure 2. The Rab GTPase cycle

In the cytosol, inactive GDP-bound Rab proteins are bound to GDI (GDP dissociation inhibitor). GDI extracts the Rab proteins, and also transiently inserts them into different membranes. When inserted into the appropriate donor membrane, inactive Rabs can be activated by a specific GEF (guanine nucleotide exchange factor), which exchanges GDP for GTP. The active GTP-bound Rab protein can then interact with its effectors, allowing for vesicle budding (effector A), vesicle transport (effector B) or vesicle docking (effector C) at the acceptor membrane for subsequent SNARE-mediated membrane fusion. At the acceptor membrane, the GTP-bound Rab is inactivated by a specific GTPase activating protein (GAP), and the GDP-bound inactive Rab protein is subsequently extracted from the acceptor membrane by GDI.

membrane trafficking alterations may be a secondary consequence of another disease-causing event which is driving pathology. Finally, Rab-mediated alterations may merely reflect an inconsequential correlate, or may actually be protective. Therefore, and given recent and comprehensive reviews on the links between Rabs, membrane trafficking and neurodegeneration [9–13], we will focus only on evidence for the direct links between aberrant Rab function and PD pathogenesis.

The Rab GTPase cycle

The Rab GTPase family in humans comprises more than 70 evolutionarily highly conserved members. They are stably geranylgeranylated at their C-termini and localized to the cytoplasmic surface of vesicles and membranes where they function as master regulators of membrane trafficking events including vesicle budding, tethering, transport and docking to allow for subsequent SNARE-mediated membrane fusion [14–16]. Distinct Rabs serve as markers for distinct subcellular organelles, and they act as molecular switches by cycling between an active, GTP-bound and an inactive, GDP-bound state. In the cytosol, they are bound by GDI (GDP dissociation inhibitor), which is required to insert as well as extract the Rabs from the membrane (Figure 2). Upon membrane insertion, and given their slow GDP dissociation rate, Rabs need to be activated by specific GEFs (guanine nucleotide exchange factors) which exchange GDP for GTP. This causes a conformational change and allows the Rabs to interact with specific effector proteins to catalyze downstream events such as vesicle trafficking (e.g., via motor proteins) or membrane fusion (e.g., via SNARE

proteins). The Rabs have weak intrinsic GTPase activity, and the concentration of cytosolic GTP is 10-fold higher than that of GDP. Therefore, Rab inactivation is aided by GTPase activating proteins (GAPs), and GDP-bound inactive Rabs are subsequently extracted from membranes by GDI [14–16] (Figure 2). Some GEFs and GAPs are specific to a single Rab, whilst others have multiple Rab substrates. Almost all GEFs and GAPs have multiple domains including protein–protein or protein–lipid interaction domains. These domains serve as localization signals and/or scaffolds for the formation of protein complexes which allow for the translocation to a specific compartment of the cell where the particular Rab is located [17,18]. In some cases, GEF interaction can occur with an upstream GTP-bound Rab protein, allowing for so-called Rab cascades [19]. Such intricate regulation of Rab activation/inactivation by GEFs and GAPs therefore allows for the spatiotemporal coordination and directionality of all membrane trafficking events.

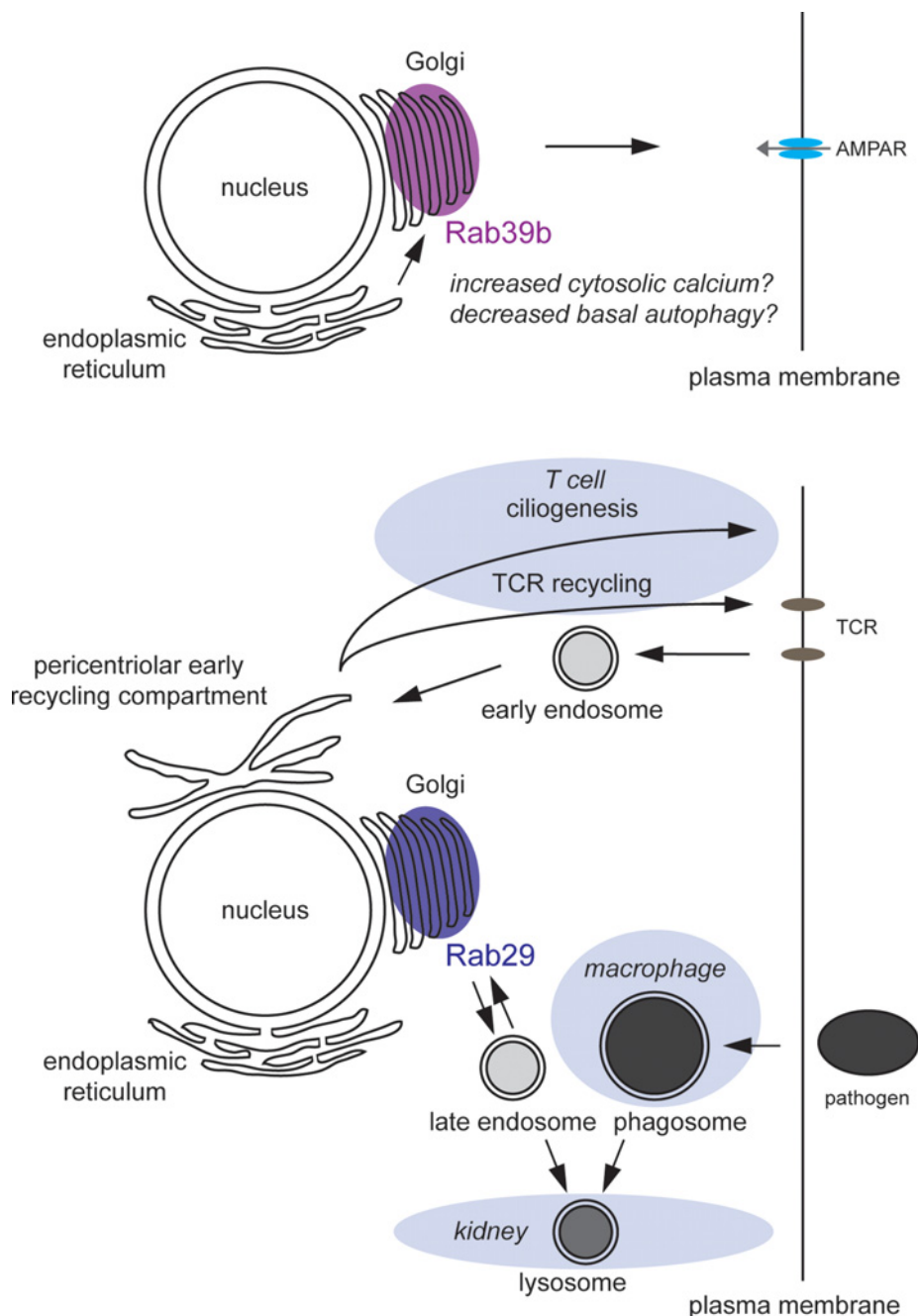## Mutations in Rab39b cause atypical PD

The most compelling evidence for the deregulation of Rab proteins as a primary cause of PD comes from studies of Rab39b [20,21]. Mutations in Rab39b cause X-linked intellectual disability associated with autism, epilepsy and macrocephaly, as well as rare early-onset PD with classical clinical symptoms, Lewy body pathology and dopaminergic cell loss in the substantia nigra [22–24]. Atypical PD-causing mutations in Rab39b generally result in the loss of protein expression [20,21]. Rab39b is enriched in the brain, and *in situ* hybridization studies indicate that expression may be neuron-specific [25]. Rab39b is highly expressed in the hippocampus, cortex and substantia nigra [25,26], raising the possibility that it may be important to maintain dopaminergic cell health in a cell-autonomous manner.

The function of this brain-enriched Rab protein remains unclear. When expressed in various cell types, Rab39b localizes to the ER and Golgi complex [22,25,27], suggesting a role in anterograde secretory transport. Consistent with this notion, Rab39b and its downstream effector PICK1 (protein interacting with C-kinase 1) have been reported to regulate ER-Golgi trafficking and surface expression of the AMPA receptor (AMPAR) subunit GluA2 in hippocampal and cortical neurons [28,29]. The composition of AMPAR subunits determines its channel properties, and the loss of Rab39b skews AMPAR composition toward more calcium-permeable, GluA2-lacking forms. This alters synaptic activity and may contribute to the cognitive dysfunctions associated with Rab39b mutations [28,29]. It will be interesting to determine whether Rab39b, possibly in conjunction with PICK1, may regulate ER-Golgi trafficking and surface expression of receptors also in dopaminergic neurons. Such altered trafficking may culminate in increased neuronal vulnerability, possibly in a calcium-mediated manner (Figure 3).

Apart from intellectual disability-related behaviour, Rab39b knockout mice also recapitulate the macrocephaly phenotype observed in humans, which is due to enhanced proliferation and delayed differentiation of neural progenitor cells (NPCs) and likely mediated by hyperactivation of the PI3K-AKT-mTOR pathway [30]. Rab39b deficiency also compromises autophagic flux at basal levels [31] which may relate to increased mTOR activity, or the involvement of C9orf72, which functions as a GEF for both Rab8a and Rab39b [32–34]. However, Rab39b knockout mice do not show dopaminergic neuronal cell loss. This is also the case for the vast majority of genetic mouse models of PD and may be due to the lack of aging-related cellular senescence processes in rodent models, as compared with non-human primates and humans [35,36]. Therefore, further studies are warranted to understand the role of Rab39b as relevant to PD pathogenesis.

Rab29 variants modulate risk for PD

Rab29 (also called Rab7L1) is a gene within the *PARK16* locus. Extensive genome-wide association studies have nominated both protective and risk variants associated with Rab29 as highly significant contributors to PD risk [37–41]. Rab29 is expressed in many cell types and tissues, with highest expression in macrophages and spleen, but hardly detectable levels in brain [42]. When expressed in cells, Rab29 localizes to the Golgi complex [43–46], and it may also localize to axonal autophagosomes [47]. Knockdown of Rab29 was initially reported to trigger fragmentation of the trans-Golgi complex [48], but subsequent studies could not recapitulate these findings [42,49–51], indicating that the presence of Rab29 is not required for the structural integrity of the Golgi complex. Rather, Rab29 seems to participate in various intracellular membrane trafficking events (Figure 3). Whilst not required for endolysosomal trafficking [50], Rab29 may contribute to retromer-mediated trafficking of the mannose-6-phosphate receptor which carries newly synthesized lysosomal enzymes to late endosomes and then needs to be shuttled back to the Golgi complex [51,52]. In T cells, Rab29 is required in the endocytic recycling of the T-cell antigen receptor which is crucial for proper immune synapse assembly [49]. The same endosomal trafficking pathways which regulate immune synapse assembly are also involved in vesicle targeting to the primary cilium [53]. Indeed, Rab29 depletion also causes deficits in ciliogenesis, perhaps via altered interactions with Rab8 and Rab11, both of which are important for proper ciliary trafficking [49]. Finally, in macrophages which display high Rab29 protein levels, Rab29 can be recruited away from

Figure 3. Membrane trafficking steps regulated by PD-linked Rab39b and Rab29

Top: Rab39b is highly expressed in brain including neurons in the substantia nigra, and atypical PD-causing mutations are consistent with a loss-of-function phenotype. When expressed in cells, Rab39b localizes to the Golgi complex. In hippocampal and cortical neurons, Rab39b is required for the proper anterograde secretory trafficking and surface expression of the AMPA receptor subunit GluA2 (AMPAR, blue). Similar trafficking deficits of GluA2 in Rab39b-mutant dopaminergic neurons may enhance neuronal vulnerability due to an increase in cytosolic calcium and/or due to impaired autophagic flux. Bottom: Rab29 is highly expressed in macrophages and other non-neuronal tissues, with very low levels in brain. It remains unclear whether PD risk variants are associated with increased or decreased Rab29 protein levels. When expressed in cells, Rab29 localizes to the Golgi complex. Rab29 regulates retromer-mediated trafficking of the mannose 6-phosphate receptor between the Golgi and late endosomes. In the kidney, Rab29 is required for proper lysosomal functioning. In T cells, Rab29 is required for endocytic recycling of the T-cell antigen receptor (TCR) (brown) and immune synapse assembly, as well as for ciliogenesis. In macrophages, Rab29 is required for phagosome maturation and/or trafficking. Therefore, Rab29 seems to be a multifunctional Rab protein able to regulate distinct trafficking events in a cell type-specific manner, perhaps able to contribute to PD risk in a non cell-autonomous manner.

the Golgi complex on to pathogen-containing organelles where it functions in their trafficking and/or maturation processes [54,55].

Human genetic data suggest that single-nucleotide polymorphisms (SNP) within the *PARK16* locus can positively or negatively influence PD risk [37,56]. Some of these SNPs are found in the putative Rab29 promoter region and are predicted to alter transcription factor binding sites [56], which may have positive or negative effects on mRNA and thus protein levels, possibly in a context-dependent manner [57]. One SNP in the putative Rab29 promoter region which is associated with reduced PD risk correlates with reduced Rab29 RNA levels [43], which would indicate that decreased Rab29 levels are protective against PD. In contrast, another SNP associated with increased PD risk was proposed to cause decreased Rab29 levels [51], which would indicate that decreased Rab29 levels increase PD risk. Therefore, and to understand the precise link between Rab29 protein levels and PD risk, it will be critical to determine how distinct PD risk-related promoter polymorphisms affect Rab29 protein levels.

The link between Rab29 and LRRK2

Understanding the involvement of Rab29 in PD has been further complicated by its reported cross-talk with LRRK2, a protein kinase implicated in both familial and sporadic PD [58]. There is currently little evidence that LRRK2 phosphorylates Rab29, even though phosphorylation clearly occurs in overexpression studies [45,62]. There exist epistatic interactions between polymorphisms in the *LRRK2* and *Rab29* genes which influence PD risk [51,59]. In addition, studies in *Caenorhabditis elegans* show that homologues of these two proteins act coordinately to regulate axon morphology [60]. Whilst knockout of Rab29, LRRK2 or both is not associated with any brain pathology [60,61], double-knockout of Rab29 and LRRK2 causes non-additive lysosomal defects and pathology in the kidney [60], consistent with these two proteins acting in converging pathways.

At the protein level, LRRK2 has been shown to directly interact with Rab29 [43,51]. When highly overexpressed in cells, Rab29 is able to recruit LRRK2 to the Golgi complex, which is associated with LRRK2 activation and the phosphorylation of its downstream kinase substrates [44–46,62]. Such Rab29-mediated LRRK2 activation occurs independently of the identity of the membrane compartment, as shown when artificially targeting Rab29 to other intracellular membranes [63]. Compounds which induce lysosomal damage cause the recruitment of LRRK2 to damaged lysosomes, even though the involvement of Rab29 in this process remains controversial [42,64,65]. Importantly though, recent studies show that moderate overexpression of Rab29 or endogenous Rab29 do not stimulate the LRRK2 kinase activity [42]. This is consistent with our previous data showing that endocytic trafficking defects mediated by pathogenic LRRK2 are rescued upon moderate, but not high expression of Rab29 [50]. Such moderate Rab29 expression may also underlie the rescue of reduced neurite process length and lysosome swelling in neurons expressing pathogenic LRRK2 [51]. Thus, current cell biological data are consistent with the possibility that Rab29 and LRRK2 function in similar intracellular membrane trafficking events, even though Rab29 does not seem to be a physiological regulator of the kinase activity of wild-type or pathogenic LRRK2 [42].

Rab proteins as LRRK2 kinase substrates

Autosomal-dominant point mutations in LRRK2 are the most common cause of familial PD, and coding and non-coding variants increase risk for sporadic PD [58]. All pathogenic LRRK2 mutations increase the kinase activity [66]. Moreover, increased LRRK2 kinase activity is observed in familial PD cases due to mutations in vps35 [67], and possibly in sporadic PD cases as well [68,69]. Therefore, LRRK2 kinase inhibitors may hold great therapeutic potential [70] and currently are in various stages of clinical development.

Phosphoproteomics analyses performed in MEFs (murine embryonic fibroblasts) and immune-stimulated PBMCs (peripheral blood mononuclear cells) have identified a small subset of Rab GTPases as endogenous LRRK2 substrates, with Rab10 being the top hit in both cases [66,71]. Systematic analyses have identified additional Rab proteins which serve as endogenous LRRK2 kinase substrates including Rab3, Rab8, Rab12, Rab35 and Rab43 [72], even though most studies to date have focused on Rab10.

Mouse tissue extracts display high levels of LRRK2 in lung, kidney and spleen but low levels in brain [6,73]. Accordingly, LRRK2-mediated Rab10 phosphorylation is most readily detected in lung, kidney and spleen and is regulated by pathogenic LRRK2 and kinase inhibitors to varying degrees [6,42]. However, there are tissue-specific differences in phosphorylation preference, with Rab10 the most prominent LRRK2 kinase substrate in the lung, but not in spleen or kidney [73]. In brain, phospho-Rab10 levels are very low [6,42,73], and when assayed from whole brain extracts, only Rab12 phosphorylation levels are detectably regulated by pathogenic LRRK2 and kinase inhibitors [73,74]. Thus, current data suggest that LRRK2 preferentially phosphorylates distinct Rabs in distinct tissues, perhaps reflecting differences in the relative abundance of the different Rabs, or due to other currently unknown mechanisms.
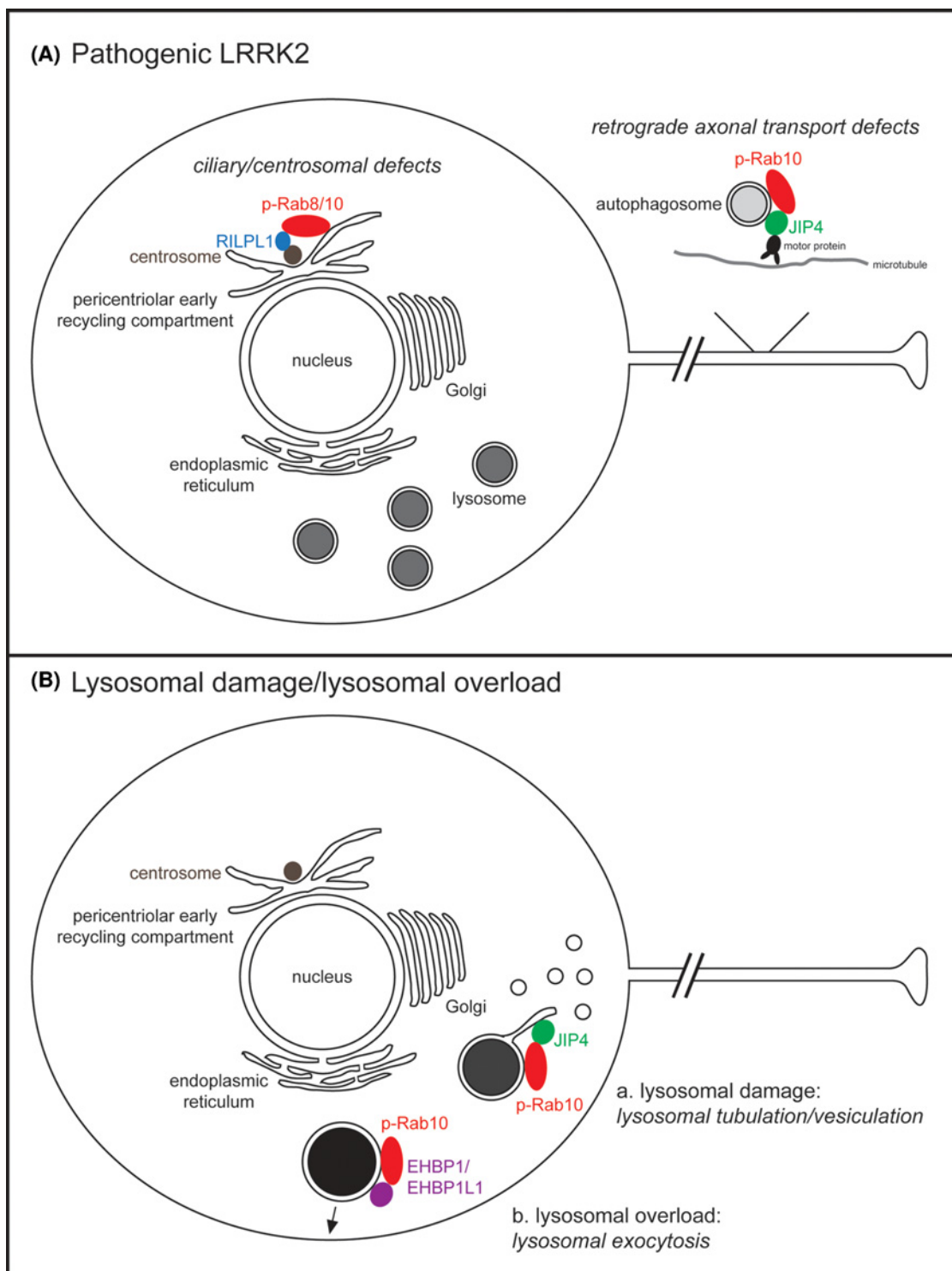
Figure 4. LRRK2-mediated Rab phosphorylation and possible PD pathomechanisms

(**A**) Pathogenic LRRK2 phosphorylates Rab8 and Rab10 which are normally localized to the pericentriolar early recycling compartment. The phospho-Rab8/10 proteins bind to centrosome-localized RILPL1, which causes centrosomal/ciliary defects. In the axon, pathogenic LRRK2 causes accumulation of phospho-Rab10 on autophagosomes, which is followed by the recruitment of JIP4 and activation of kinesin, thereby interfering with the proper retrograde trafficking of autophagosomes. (**B**) Lysosomal damage or lysosomal overload, both disease-relevant triggers, cause activation of wildtype LRRK2. Lysosomal damage causes lysosomal accumulation of phospho-Rab10 and JIP4, leading to the vesiculation of lysosomal membranes. Lysosomal overload causes lysosomal accumulation of phospho-Rab10 and EHBP1/EHBP1L1, leading to lysosomal exocytosis.

Studies in peripheral cells including MEFs and various human patient-derived cells have consistently shown prominent Rab10 phosphorylation which is modulated by LRRK2 kinase inhibitors [73,75–77]. Importantly, recent quantitative mass spectrometry-based assays have revealed an increase in phospho-Rab10 levels in neutrophils from LRRK2-PD patients as compared with healthy controls [78], indicating that the phosphorylation status of Rab10 can be used as readout for increased LRRK2 kinase activity in clinical settings. Our studies have also described cell biological assays in patient-derived peripheral cells which are able to stratify PD patients with elevated LRRK2 kinase activity who may benefit from LRRK2-related therapeutics [77]. However, LRRK2 does not prominently phosphorylate the different Rab substrates. For example, the stoichiometry of LRRK2-mediated Rab10 phosphorylation in various cell lines and in healthy patient-derived neutrophils is around 1% [66,78–80] and increases to approximately 2–5% in the presence of pathogenic LRRK2, respectively [78,79,81]. Whilst it remains possible that such stoichiometry may be higher in disease-relevant cell types, this raises the issue of how such a small change in phospho-Rab protein levels may cause PD in a dominant fashion.

LRRK2 phosphorylates the Rabs on an evolutionarily conserved residue in a region called the switch II domain, which changes conformation upon nucleotide binding and is critical for the interaction of the Rabs with multiple regulatory and effector proteins [14,15]. Whilst phospho-mimetic Rab mutants are non-functional and cytosolic when expressed in cells [82–84], studies using phosphorylated Rab8 have shown that the LRRK2-mediated phosphorylation of the switch II domain interferes with the interaction of Rab8 with GDI and with various previously described effector proteins [72]. In addition, Rab8 and Rab10 need to be membrane-associated to be phosphorylated by LRRK2 [45,83]. Therefore, the LRRK2-mediated phosphorylation of Rab8/10 proteins is predicted to lead to their accumulation at the membrane as unable to be extracted by GDI, and at the same time also unable to interact with their respective downstream effector proteins. Such loss-of-function type mechanism may impair the trafficking steps the various Rab proteins are involved in. Consistent with this prediction, we have shown that endolysosomal trafficking and endocytic recycling deficits due to pathogenic LRRK2 expression are mimicked by transient knockdown of either Rab8 or Rab10 and are rescued upon LRRK2 kinase inhibition or upon expression of active Rab8 or Rab10, respectively [50,85,86]. It will be important in the future to determine how interfering with Rab3, Rab12, Rab35 or Rab43 may affect various intracellular trafficking steps, and how that may relate to possible PD pathomechanisms.

Surprisingly, phosphorylated Rab8 and Rab10 not only loose the ability to interact with regulatory and effector proteins but also gain the ability to interact with a small set of novel proteins [72,87]. For example, both phospho-Rab8 and phospho-Rab10 gain the ability to interact with RILPL1, RILPL2, JIP3 and JIP4 [72,87]. It is currently unclear which of these nascent interactions are the most prominent ones, and under which conditions and in which cell types they occur. It is also unknown whether Rab3, Rab12, Rab35 or Rab43 gain the ability to interact with certain novel proteins upon their phosphorylation by LRRK2. In either case, current findings highlight the possibility that these novel phospho-Rab interactions may cause cellular alterations in a dominant fashion to trigger LRRK2-mediated pathogenesis.

The consequences of phospho-Rab8/10 binding to RILPL1 have been most thoroughly characterized. In MEF cells, copy number proteomics has revealed that a cell contains around 1.3 million copies of Rab10 and around 50,000 copies of RILPL1 [73]. Therefore, and taking into account the above-mentioned stoichiometry of Rab phosphorylation, pathogenic LRRK2 may profoundly affect the extent to which RILPL1 is complexed with phospho-Rabs. RILPL1 is a poorly characterized protein which regulates ciliogenesis [88]. The binding of phospho-Rab8/10 to centrosome-localized RILPL1 causes a decrease in primary ciliogenesis in various cell types *in vitro* as well as in the intact mouse brain [72,83,84,89], which is at least in part due to the impaired centrosomal recruitment of tau tubulin kinase 2 (TTBK2), a step essential for ciliogenesis initiation [90,91]. We have shown that apart from ciliogenesis, the same phospho-Rab8/10-RILPL1 interaction also causes centrosomal cohesion deficits in dividing cells including in patient-derived peripheral cells [77,82,83]. Whilst it is established that the centrosomal cohesion and ciliogenesis deficits are a direct consequence of enhanced LRRK2 kinase activity and are mediated by the centrosomal accumulation of phospho-Rab8/10 bound to RILPL1, the pathophysiological significance of those alterations remains unclear. One postulated mechanism relates to the observation of ciliary deficits in cholinergic neurons in the striatum, which is expected to disrupt their ability to send neuroprotective signals back to dopaminergic neurons in the substantia nigra [84,92], but other cell-autonomous and non cell-autonomous mechanism(s) are possible as well [89].

JIP3 and JIP4 are adaptor proteins which interact with microtubule motors. They are bidirectional adaptors, able to interact with both anterograde (microtubule plus-end) kinesins as well as with retrograde (microtubule minus-end) dynein/dynactin. Thus, they can act as bidirectional switches to target cargo dependent on cellular cues and demand [93]. Both JIP3 and JIP4 have overlapping functions in promoting axonal transport of lysosomes [94,95]. Interestingly, a recent study indicates that pathogenic LRRK2 causes accumulation of phospho-Rab10 on axonal autophagosomes

which is followed by enhanced recruitment of JIP4 and activation of kinesin [47]. This causes a tug of war between autophagosome motor proteins to disrupt appropriate axonal transport, which will impair appropriate autophagic degradation [47] and will be particularly damaging to vulnerable neurons with their long, highly branched axons and increased needs for autophagic and mitophagic turnover. Recent studies also indicate that pathogenic LRRK2 impairs basal mitophagy in a kinase-mediated manner [96], and the putative link between impaired mitophagy and Rab10 phosphorylation merits further investigation.

All above-mentioned studies analyzed pathogenic LRRK2-mediated changes under physiological, non-stimulated conditions. However, several stimuli can cause wild-type LRRK2 recruitment and activation, followed by phospho-Rab accumulation at distinct intracellular locations. For example, induction of lysosomal damage or lysosomal overload both cause recruitment of LRRK2 to damaged lysosomes, which is followed by lysosomal phospho-Rab10 accumulation. Lysosomal damage causes JIP4 recruitment and the formation of tubular structures and vesicles lacking lysosomal identity, whilst lysosomal overload causes EHBP1/EHBP1L1 recruitment and triggers lysosomal exocytosis [64,97]. The reported differences in such LRRK2-mediated lysosomal outcomes require further investigation but may depend on cell type and/or the type of lysosome-damaging agent employed. In addition, pathogens are known to recruit LRRK2 to phagosomal membranes in macrophages, causing phagosomal phospho-Rab8/10 accumulation which may impair phagosomal maturation processes [98–100].

In summary, currently available data indicate that in the cell body and under non-stressed cellular conditions, pathogenic LRRK2 phosphorylates Rab8 and Rab10 and causes their RILPL1-mediated accumulation at the centrosome, followed by ciliary and centrosomal defects. In the presence of a trigger such as lysosomal damage, wild-type LRRK2 is recruited to damaged lysosomes by a currently unknown mechanism, where it causes the accumulation of phospho-Rab10 and recruitment of JIP4, which results in the tubulation/vesiculation of the lysosome [97], with far-reaching implications for proper lysosomal functioning as relevant for PD pathogenesis (Figure 4). It will be interesting to determine whether the pRab10/JIP4 complex acts through kinesin-mediated trafficking defects similar to those described for axonal autophagosomal cargoes. Similarly, alterations in motor binding to a phospho-Rab10/JIP4 complex at the phagosome and the resulting impaired phagocytic clearance may underlie some of the reported roles of LRRK2 in pathogen infection control [101].

The differential binding of phospho-Rab8/10 to either RILPL1 or JIP4 is likely explained by differences in the subcellular localization of the LRRK2-mediated phosphorylation event. Under non-stimulated conditions in the cell soma, the Rabs may be preferentially phosphorylated by pathogenic LRRK2 in their appropriate intracellular compartments. Since both Rab8 and Rab10 are localized to the early endocytic recycling compartment which is in direct contact with the centrosome [102], their proximity to RILPL1 would favor formation of a phospho-Rab/RILPL1 complex at the centrosome (Figure 4). In contrast, when phospho-Rabs accumulate on axonal autophagosomes, on damaged lysosomes or on phagosomes away from the centrosome, they would preferentially engage in forming a phospho-Rab/JIP4 complex. Thus, one can envision a scenario whereby pathogenic LRRK2 causes ciliary and centrosomal alterations mediated by the phospho-Rab/RILPL1 complex in the cell body, and autophagosomal transport deficits mediated by the phospho-Rab/JIP4 complex in the axon, both of which may negatively impact upon the viability of vulnerable dopaminergic neurons. Conversely, various disease-relevant triggers may activate wild-type LRRK2 and cause accumulation of a phospho-Rab/JIP4 complex on damaged lysosomes or possibly on other damaged intracellular organelles, leading to their dysfunction via inappropriate trafficking mediated by microtubule-dependent vesicular motors. In this manner, both pathogenic LRRK2 and aberrant wild-type LRRK2 activation may contribute to familial and sporadic PD in a phospho-Rab-mediated manner, albeit via distinct mechanisms (Figure 4).

Concluding remarks

Due to the heterogeneity of causal elements underlying disease pathogenesis, treatment of PD is expected to become more personalized in the future. Genetic forms of PD provide a window towards our mechanistic understanding of disease pathogenesis in the context of selective cellular vulnerability. Aberrant Rab GTPase function can cause atypical PD (Rab39b) or regulate PD risk (Rab29), and phosphorylation of a small subset of Rab GTPases may relay aberrant LRRK2 kinase signaling with familial and sporadic PD. Understanding the precise steps modulated by these PD-relevant Rab proteins may aid in the design and refinement of novel PD treatment approaches.

Summary

- A subset of Rab proteins are directly implicated in the mechanisms underlying PD pathogenesis.

- Atypical PD-causing mutations in Rab39b alter anterograde secretory transport and basal autophagy which may particularly affect vulnerable neurons.

- PD risk is modulated by Rab29, which regulates distinct intracellular trafficking events in distinct cell types.

- The PD kinase LRRK2 phosphorylates a subset of Rab proteins including Rab8 and Rab10, which causes their accumulation in distinct subcellular compartments.

- Enhanced LRRK2 kinase activity and phospho-Rab accumulation may cause PD-relevant cellular alterations in a context-dependent manner.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution

All authors contributed to the manuscript.

Abbreviations

AKT, Serine/threonine protein kinase B; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AMPAR, AMPA receptor; ATP, adenosine triphosphate; Cav1, Cav1.3 L-type calcium channel; ER, endoplasmic reticulum; GAP, GTPase activating protein; GDI, GDP dissociation inhibitor; GDP, guanosine diphosphate; GEF, guanine nucleotide exchange factor; GluA2, glutamate receptor ionotropic AMPA 2; GTP, guanosine triphosphate; iPSC, induced pluripotent stem cell; JIP, JNK-interacting protein; LRRK2, leucine-rich repeat kinase 2; MEF, murine embryonic fibroblast; mRNA, messenger RNA; mTOR, mammalian target of rapamycin; NPC, neural progenitor cell; OXPHOS, oxidative phosphorylation; PBMC, peripheral blood mononuclear cell; PD, Parkinson's disease; PBMC, peripheral blood mononuclear cell; PD, Parkinson's disease; PI3K, phosphatidylinositol 3-kinase; PICK1, protein interacting with C-kinase 1; PINK1, PTEN-induced putative kinase 1; PTP, permeability transition pore; Rab, Ras-associated binding protein; RILPL1, Rab-interacting lysosomal protein-like 1; ROS, reactive oxygen species; SNARE, SNAP (soluble NSF attachment protein) receptor; SNP, single-nucleotide polymorphism; TCR, T-cell antigen receptor.

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