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Two-Pore Channels and Parkinson's Disease: Where's the Link?

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Abstract

Two-pore channels are endolysosomal Ca^{2+} release channels involved in proper trafficking to and from those organelles. They are the likely targets for the Ca^{2+} -mobilizing messenger NAADP, and are further regulated by a variety of mechanisms including phosphoinositide levels and Rab proteins. As discussed here, recent studies highlight a role for these channels in the pathomechanism(s) underlying Parkinson's disease, with important implications for possible alternative treatment strategies.

Keywords

TPCN1; TPCN2; NAADP; Parkinson's Disease; LRRK2; Rab Proteins; Calcium; Iron

Introduction

Endosomes and lysosomes play crucial roles for many aspects of cellular physiology including signalling, protein transport and digestion of macromolecules. Endolysosomal trafficking is a highly regulated process whereby cargoes are delivered to their respective destinations. For example, macromolecules can be sorted for degradation by the fusion of autophagosomes or phagosomes with lysosomes, which contain acidic hydrolases required for degradation. Cell surface receptors can be endocytosed into early endosomes, and from there can be targeted for degradation after reaching the lysosome via the late endosome. Alternatively, receptors can be recycled back to the cell surface either directly from early endosomes or via trafficking through recycling endosomes. Late endosomes/lysosomes receive newly synthesized hydrolytic enzymes from the trans-Golgi network (TGN), and can fuse with the plasma membrane to mediate plasma membrane repair/secretion of lysosomal content via a process termed lysosomal exocytosis. Finally, lysosome-related organelles (LROs) are a heterogeneous set of organelles derived from the endolysosomal system and usually contain lysosomal proteins. They exist in various specialized cells such as lytic granules in cytotoxic T lymphocytes, dense granules in platelets, melanosomes in melanocytes, osteoclast granules in osteoclasts and lamellar bodies in lung epithelial type II cells (Maxfield and McGraw, 2004; Luzio et al., 2007; Raposo et al., 2007).

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Interfering with proper endolysosomal trafficking underlies a variety of lysosomal storage diseases which in most cases are associated with neurodegeneration (Platt et al., 2012). Conversely, a variety of neurodegenerative diseases such as Alzheimer's disease, amyotrophic lateral sclerosis and Parkinson's disease (PD) seem to involve endolysosomal deficits as an underlying molecular mechanism (Nixon, 2013; Abeliovich and Gitler, 2016). Therefore, a detailed understanding of the endolysosomal system has become crucial towards developing therapies able to restore normal cellular function for a range of neurodegenerative diseases with worldwide socioeconomic impact.

Two-pore channels (TPCNs) form a subfamily of the voltage-gated ion channel superfamily, with two distinct gene products expressed in humans (Patel and Cai, 2015). They are localized to endosomes, lysosomes and lysosome-related organelles (LROs), suggesting that they may contribute to the proper functioning of the endolysosomal system. Recent studies have revealed that these channels may be intimately linked to at least some pathomechanism(s) underlying PD.

Regulators of Endolysosomal Trafficking

Early endosomes, recycling endosomes, late endosomes and lysosomes are characterized by differences in the protein and lipid composition of their membranes as well as by their intraluminal content, which together contribute to the proper trafficking through the endolysosomal system (Fig. 1). The actual fusion event between two lipid bilayers is mediated by distinct soluble N-ethylmaleimidesensitive fusion protein (NSF) attachment protein receptors (SNAREs). SNARE complexes form a highly stable four-helix bundle mediated by three Q-SNAREs on one membrane and one R-SNARE on the opposing membrane (Südhof and Rothman, 2009). A variety of SNARE proteins have been described across the endolysosomal system (Kümmel and Ungermann, 2014; Luzio et al., 2009), including the R-SNARE VAMP4 on early endosomes and VAMP7 on lysosomes, the Q-SNAREs syntaxin 6/13 on early endosomes, syntaxins 7/8 on early/late endosomes, or syntaxins 12/16 on recycling endosomes, respectively (Fig. 1). Membrane fusion within the endolysosomal system also seems to require an increase in juxta-organellar Ca^{2+} mediated by Ca^{2+} release channels and sensed by Ca^{2+} -sensing proteins, such as synaptotagmin VII on lysosomes or synaptotagmin IX on recycling endosomes, respectively (Luzio et al., 2007).

Before the actual membrane fusion event, a variety of pre-fusion steps assure fidelity of trafficking across the endolysosomal system, and they are mediated by Rab proteins, Rab effectors, tethering factors and phosphoinositides (PIPs) (Di Paolo and De Camilli, 2006; Hutagalung and Novick, 2011; Jean and Kiger, 2012). Rab proteins are small GTPases which in their active, GTP-bound form are able to recruit specific effector proteins to define organelle identity, trafficking specificity and vesicle motility. Rab proteins localized across the endolysosomal system include Rab5 on early endosomes, Rab7 on late endosomes/lysosomes or Rab8 and Rab11 involved in vesicular transport to and from recycling endosomes (Fig. 1). Targeting Rab proteins to their correct membrane compartments and regulation of their activity is a complex process also involving PIPs (Di Paolo and De Camilli, 2006). PI(3)P is a crucial determinant of early endosome membrane identity and

required for almost every aspect of endosomal membrane trafficking and fusion, including autophagy and phagocytosis. It is generated from PI either by VPS34, or by sequential dephosphorylation of PI(3,4,5)P3 by inositol phosphatases specific for the 5 and 4 positions. PI(3)P is then phosphorylated at the 5 position by the Fab1/PIKfyve kinase, which is localized to late endosomes/lysosomes and generates PI(3,5)P2 (Fig. 1). Whilst generally of low abundance, microdomains of PI(3,5)P2 are thought to be important in regulating a variety of events along the late endocytic pathway including trafficking from late endosomes to lysosomes or autophagosome-lysosome fusion, and PI(3,5)P2 is important for late endosomal/lysosomal vesicular fission events as well (Jean and Kiger, 2012). Dephosphorylation of PI(3,5)P2 can be brought about by various phosphatases at the 5 position as well as by myotubularin/myotubularin-related protein, a 3-phosphatase (Di Paolo and De Camilli 2006). Interestingly, mutations in PI(3,5)P2-metabolizing enzymes cause a variety of neurodegenerative diseases including amyotrophic lateral sclerosis and Charcot-Marie-Tooth disease (Bolino et al., 2000; Chow et al., 2007; Chow et al., 2009), and mutations in the polyphosphoinositide phosphatase synaptojanin 1 have recently been associated with autosomal recessive, early-onset Parkinsonism (Quadri et al., 2013), highlighting a mechanistic link between abnormal PIP metabolism and PD.

The above-mentioned components important for trafficking through the endolysosomal system undergo additional complex and mutual regulation in various ways. For example, SNAREs can regulate Rab effectors and Ca^{2+} channels, and PIPs can modulate SNAREs as well as Ca^{2+} release channels (Shen et al., 2011). In addition, there is intricate crosstalk between PIPs and Rab GTPases. For example, PIPs regulate Rab GTPase activity by recruiting and modulating the activity of guanine nucleotide exchange factors (GEFs) or GTPase activating proteins (GAPs) which are known to stimulate Rab GTP loading or GTP hydrolysis, respectively. PIPs also often function as co-receptors with Rab proteins to recruit effectors to specific membrane compartments. Finally, several PIP-metabolizing enzymes have been described as Rab protein effectors. Such positive and negative feedback loops between Rab proteins and PIPs are thought to allow for the establishment of precise identity codes amongst membranes and membrane domains, and help define vesicular identity to assure proper directionality in membrane trafficking (Di Paolo and De Camilli, 2006; Jean and Kiger, 2012).

Endolysosomal structures also display distinct subcellular positioning which is important for their proper functioning. Lysosomes are localized either peripherally or are clustered in a perinuclear area dependent on cellular nutrient status (Korolchuk et al., 2011), whilst lysosomes undergoing lysosomal exocytosis for plasma membrane repair seem to be localized peripherally in a manner dependent on Rab3a (Encarnaço et al., 2016). Early endosomes are generally peripheral structures, whilst recycling endosomes are located deeper inside the cell around the microtubule-organizing center and involved not only in endocytic, but also exocytic vesicle trafficking events (van IJendoorn, 2006). Finally, the different endocytic compartments display distinct intraluminal pH, with early endosomes (pH 5.9–6), late endosomes (pH 5.0–6.0) and lysosomes (pH 5.0–5.5) acidic, LROs generally displaying an acidic pH as well, but recycling endosomes (pH 6.4–6.5) somewhat more alkaline in comparison (van IJendoorn, 2006; Raposo et al., 2007).

Endolysosomal Ca^{2+} and Ca^{2+} Release Channels

Endosomes, lysosomes and LROs contain significant amounts of the intracellular signaling ion Ca^{2+} (Patel and Muallem, 2011). Endolysosomal Ca^{2+} can be mobilized from these stores, and Ca^{2+} -induced Ca^{2+} release from the ER can then generate physiologically relevant and spatiotemporally complex Ca^{2+} signals (Cancela, et al., 1999; Churchill et al., 2002; Galione, 2015). Importantly, apart from regulating global Ca^{2+} signaling, vesicular fusion events along the endolysosomal system also depend on luminal Ca^{2+} release from these organelles (Luzio et al., 2007).

A variety of endolysosomal Ca^{2+} channels have been described, including members of the transient receptor potential (TRP) ion channel family, ATP-gated ionotropic receptors (P2X) and TPCNs (Patel and Cai, 2015). TPCNs are largely ubiquitously expressed, with TPCN1 more broadly distributed across the endolysosomal system, and TPCN2 more prominent in lysosomes (Fig. 1) (Calcraft et al., 2009; Brailoiu et al., 2009; Patel and Cai, 2015) as well as in various LROs (Davis et al., 2012; Ambrosio et al., 2015; Ambrosio et al., 2016). Interestingly, TPCN2 has also been described to regulate vesicular trafficking dependent on recycling endosomes, even though it currently remains unknown whether this correlates with a localization of the channel to those organelles (Ruas et al., 2010). TPCNs can conduct distinct ions including Ca^{2+} , Na^+ , H^+ and Fe^{2+} (Wang et al., 2012; Cang et al., 2013; Jha et al., 2014; Patel, 2015; Fernández et al., 2016; Penny et al., 2016). Their conductance is further modulated by $\text{PI}(3,5)\text{P}_2$ as well as by nicotinic acid adenine dinucleotide phosphate (NAADP), the most potent Ca^{2+} -mobilizing messenger known to date (Patel, 2015). Whilst NAADP has been reported to also activate select TRP channels and ryanodine receptors (Lee et al., 2015; Gerasimenko et al., 2015), most data are consistent with NAADP most prominently regulating the Ca^{2+} conductance of TPCN channels (Ruas et al., 2015). Thus, NAADP and select antagonists (Naylor et al., 2009) allow for molecular dissection of the role of TPCNs in endolysosomal functioning.

Pathogenic Events Underlying PD

PD is an age-related neurodegenerative movement disorder with unknown etiology, characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, and by the presence of intracellular protein aggregates rich in α -synuclein, which are called Lewy bodies, in surviving neurons. The molecular and cellular mechanisms underlying the disease have largely remained unknown. The majority of PD cases appear to be idiopathic, with age as major risk factor, and environmental insults and genetic predisposition seem to play a contributing role as well. Studies into the sporadic forms of PD have centered on abnormal protein degradation, toxic protein accumulation and cellular alterations leading to oxidative stress. However, over the past years, studies into genetic forms of the disease have greatly helped in deciphering the possible mechanisms underlying cellular demise. Autosomal-dominant missense mutations in LRRK2 comprise the most common monogenic form of PD, which clinically and pathologically resembles the sporadic form of the disease. Furthermore, genomewide association studies have shown that variations in the LRRK2 gene comprise an important risk factor for sporadic PD. These findings suggest that LRRK2 plays a role for the entire PD disease spectrum, which allows

for the establishment of well-defined cellular and animal models to define disease mechanisms (Gómez-Suaga et al., 2014; Cookson, 2016; Abeliovich and Gitler, 2016).

Similar to sporadic PD, pathogenic LRRK2-mediated events seem to converge on altered endolysosomal functioning leading to impaired autophagic clearance of damaged organelles and aggregated proteins, and alterations in intracellular calcium homeostasis, both known to be crucial for the proper functioning of dopaminergic neurons (Gómez-Suaga et al., 2014; Cookson, 2016; Abeliovich and Gitler, 2016; Surmeier et al., 2017). Whilst the precise mechanism(s) by which LRRK2 causes such deficits remain to be properly deciphered, recent studies imply an involvement of TPCN channels in this process.

PD and Aberrant TPCN Functioning

Whilst there is currently no evidence that mutations in TPCNs are associated with familial forms of PD, a recent transcriptomic meta-analysis and network analysis of blood microarrays from patients with PD identified several deregulated genes including nicotinamide phosphoribosyltransferase (NAMPT) and TPCN2 (Santiago et al., 2016). Interestingly, NAMPT is involved in NAD biosynthesis which serves as a precursor for NAADP, suggesting that both TPCN2 and its biological activator NAADP may be altered in sporadic PD patients.

TPCNs are expressed in dopaminergic neurons of the substantia nigra (Lutas et al., 2016), and additional links between TPCNs and PD have recently emerged. Pathogenic LRRK2 was found to cause defects in autophagic flux associated with a decrease in the number of acidic, functional lysosomes, without a change in total lysosome number. These alterations were mimicked by NAADP and reverted by Ned19, an NAADP antagonist (Naylor et al., 2009), as well as by overexpression of dominant-negative TPCN2 (Gómez-Suaga et al., 2012). In agreement with those data, overexpression of TPCN2 has been found to inhibit autophagosome-lysosome fusion via alkalinizing lysosomal pH (Lu et al., 2013), suggesting that increased TPCN2 functioning may underlie the autophagic deficits associated with pathogenic LRRK2. Similarly, aberrant lysosomal morphology was described in fibroblasts from PD patients carrying LRRK2 mutations as compared to healthy controls, and such altered morphology was reversed by molecular silencing of TPCN2 or by NAADP antagonists (Hockey et al., 2015). NAADP stimulation was further shown to cause increased global Ca^{2+} signals in fibroblasts from LRRK2-PD patients as compared to controls. The lysosomal morphology alterations were reversed by acute Ca^{2+} chelation, suggesting that the observed enlargement of lysosomes may be due to excessive TPCN2-mediated and Ca^{2+} -dependent fusion events amongst these organelles (Hockey et al., 2015). Altogether, these data are consistent with the idea that pathogenic LRRK2 increases TPCN2 functioning, and thus that regulating TPCN channel activity (Rahman et al., 2014), or generally modulating intracellular Ca^{2+} levels (Rivero-Ríos et al., 2016; Surmeier et al., 2017) may be a promising therapeutic strategy for at least LRRK2-related PD.

The precise downstream cellular consequences of the altered lysosomal morphology for endomembrane trafficking and lysosomal functioning remain unclear. The enlarged endolysosomal structures may display deficits in proper tubulation and lysosome

reformation, a process recently shown to be dependent on TRPML1, another endolysosomal Ca^{2+} channel (Li et al., 2016), with downstream consequences for a variety of vesicular trafficking events to and from lysosomes. Furthermore, the increase in endolysosomal pH (Gomez-Suaga et al., 2012) is expected to cause deficiencies in proper lysosomal degradation, as the activities of the lysosomal hydrolases depend on an adequately acidic environment. A proper acidic luminal environment may additionally contribute to the appropriate directional movement and maturation of organelles along the endolysosomal pathway (Hu et al., 2015).

In metazoans, the mechanism of Ca^{2+} uptake into acidic organelles has been difficult to dissect, but seems to be mediated by a variety of mechanisms which may or may not require endolysosomal acidification (López et al., 2005; Demaegd et al., 2013; Melchionda et al., 2016; Garrity et al., 2016). Therefore, the observed alkalization of lysosomal pH may not necessarily cause altered intraluminal Ca^{2+} content. Rather, it may be a reflection of enhanced TPCN2 functioning, as it is known that NAADP-mediated Ca^{2+} release causes a concomitant increase in endolysosomal pH (Morgan and Galione, 2007).

How LRRK2 may cause enhanced TPCN2 functioning remains unknown as well. Since the observed cellular effects are dependent on LRRK2 kinase activity (Gómez-Suaga et al., 2012; Hockey et al., 2015), they must be associated with alterations in the phosphorylation of select LRRK2 kinase substrates. TPCN2 has been shown to be regulated by various kinases (Cang et al., 2013; Jha et al., 2014) and is able to interact with LRRK2 (Gómez-Suaga et al., 2012), but whether kinase-mediated effects are via direct phosphorylation of the channel remains to be determined.

LRRK2 may impact upon the modulators of TPCN2 activity, and the recent report of LRRK2-mediated phosphorylation of synaptojanin I (Islam et al., 2016) raises the possibility that pathogenic LRRK2 may regulate TPCN2 channel functioning through increasing PI(3,5)P2 levels. TPCN2 has also been shown to interact with a variety of Rab proteins including Rab1, Rab5, Rab7, Rab8, Rab10, Rab11, Rab14 and Rab21, various SNARE proteins including syntaxins 6, 7, 8, 16 and 18, as well as VAMP7 (Fig. 1) (Lin-Moshier et al., 2014; Grimm et al., 2014). Some of those interactions may occur during the biosynthetic trafficking of TPCN2, such as interactions with Rab1, localized to ER exit sites and the pre-Golgi intermediate compartment, or interactions with syntaxin 18, localized mainly at the ER (Stenmark, 2009). Other reported interactors such as Rab8 and Rab11, or syntaxin 12/16, play roles in the endocytic recycling compartment. Whilst not acidic in nature, this compartment has been shown to contain Ca^{2+} as well as TRPML2 (Shen et al., 2011), raising the possibility that TPCN2 may also play a role in vesicular trafficking to and from the recycling compartment (Ruas et al., 2010). Finally, the TPCN2 interactor Rab14 is known to be involved in the recycling pathway between the Golgi and endosomal compartments, whilst other interactors such as Rab7, VAMP7, syntaxins 7 and 8 are localized to late endosomes and lysosomes, thus overlapping with the most prominent steady-state localization of TPCN2 (Fig. 1).

Analysis of the regulation of TPCN2 functioning by the late endosomal/lysosomal protein Rab7 revealed that the N-terminus of TPCN2 is important, with a triple-mutation in the N-

terminus displaying reduced interaction and reduced channel activity (Lin-Moshier et al., 2014). In addition, a competitive nucleotide binding inhibitor of Rab7 (Agola et al., 2012) decreased TPCN2 functioning, altogether suggesting that TPCN2 is modulated by Rab7 activity.

LRRK2 has been reported to cause deficits in endolysosomal trafficking associated with decreased Rab7 activity when assayed either from transfected cells or from fibroblasts of mutant LRRK2 PD patients as compared to controls (Gómez-Suaga et al., 2014). At first glance, such decreased Rab7 activity would be expected to decrease TPCN2 function, contrary to what is observed in pathogenic LRRK2-expressing cells (Gómez-Suaga et al., 2014; Hockey et al., 2015). However, a recent study reported that Ca^{2+} release through the endolysosomal P2X_A channel causes downregulation of Rab11a activity and vacuole fusion, and this was mediated by a Ca^{2+} -dependent increase in Rab11a GAP activity (Parkinson et al., 2014). These data suggest a model whereby Rab proteins, when in their active form, signal the correct membrane apposition to endolysosomal Ca^{2+} release channels which, upon Ca^{2+} release and membrane fusion, inactivate the respective Rab protein to correctly terminate the process. In this context, it is tempting to speculate that even though capable to be modulated by Rab7, the enhanced TPCN2 activity in the presence of pathogenic LRRK2 may cause a concomitant Ca^{2+} -mediated decrease in overall Rab7 activity.

A recent phosphoproteomics screen has identified a subset of Rab proteins as bona fide LRRK2 kinase substrates (Steger et al., 2016). Rab7 was not identified as LRRK2 kinase substrate, again suggesting that the LRRK2-mediated effects on Rab7 activity may be indirect. However, a set of Rab proteins were identified as LRRK2 kinase substrates including Rab8 and Rab10 (Fig. 1). Phosphorylation of these Rab proteins caused decreased interaction with GDI1/2 as well as with GEFs, suggesting that phosphorylated Rab proteins may accumulate in membranes in their inactive, GDP-bound forms (Steger et al., 2016). Therefore, further studies are warranted to determine the role for TPCN2 in trafficking to and from the recycling compartment, its regulation by Rab8 and/or Rab10, and possible alterations in TPCN2 activity upon LRRK2-mediated phosphorylation of these Rab proteins.

Additional scenarios by which LRRK2 kinase activity may impact upon TPCN2 functioning are possible as well. For example, LRRK2 has been shown to phosphorylate NSF, causing an increased rate of SNARE complex disassembly (Belluzzi et al., 2016), which may in turn regulate TPCN2 functioning via differential interactions with the reported SNARE proteins. Finally, TPCN2 channels have been reported to be permeable to iron as well (Fernández et al., 2016). Iron is an important transition metal playing a crucial role for many chemical reactions, but can also cause hydroxyl radical production via the Fenton reaction, leading to oxidative stress and eventual cellular demise. During healthy ageing, iron is known to accumulate especially in the substantia nigra, and a further increase in iron accumulation is observed in brains of PD patients as compared to healthy aged-matched controls (Ward et al., 2014). Thus, aberrant conductance of TPCN2 channels may contribute to iron-associated cellular alterations and cell death of neurons in the substantia nigra in sporadic PD patients.

At least with respect to pharmacological approaches targeting LRRK2-related PD, special attention to its role in peripheral tissues is warranted as well. LRRK2 is highly expressed in

some peripheral tissues such as lung, and genetic or pharmacological kinase inhibition has been shown to lead to alterations in lamellar body surfactant secretion in alveolar type II epithelial cells (Baptista et al., 2013; Miklavc et al., 2014; Fuji et al., 2015). Since lamellar bodies are another type of LROs, and it will be interesting to determine whether they contain TPCN2 channels, and if so, whether the reported safety liability issues around LRRK2 kinase inhibition can be reverted by selectively targeting TPCN2 channel activity in peripheral tissues.

Conclusions and Outlook

There are several emerging links between aberrant TPCN2 channel functioning and pathomechanism(s) underlying PD. Whilst the precise downstream cellular consequences of altered TPCN2 functioning remain to be further dissected, they seem to converge on alterations in endolysosomal trafficking and autophagy, both processes known to be affected in PD. Alterations in TPCN2 channel activity may occur by a variety of mechanisms, including phosphorylation-mediated changes of the channel itself, or of PIPs or proteins which modulate channel functioning such as Rab proteins. Indeed, apart from the LRRK2-mediated phosphorylation and concomitant inactivation of a subset of Rab proteins which may contribute to a PD-specific phenotype, other links between Rab proteins and PD have emerged as well. For example, both Rab7L1 and Rab39b have been genetically linked to PD (MacLeod et al., 2013; Beilina et al., 2014; Lesage et al., 2015), and Rab1, Rab3, Rab8 and Rab11 are known to modulate α -synuclein-mediated aggregation and toxicity in cellular and animal models of PD (Cooper et al., 2006; Gitler et al., 2008; Breda et al., 2015). Whilst the precise Rab proteins able to regulate TPCN2 channel functioning in the distinct subcellular compartments require further investigation, currently available data highlight the possibility that targeting the activity of endolysosomal TPCN2 channels may provide alternative therapeutic strategies against neurodegeneration underlying PD.

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Biographies

Pilar Rivero-Ríos received her degree in Biology from the University of Granada in 2012, and is currently studying for a Ph.D. in the laboratory of Dr. Sabine Hilfiker in the IPBLN-CSIC (Granada), which is expected to be completed in October 2018. She studies the effects of LRRK2, the main determinant of inherited Parkinson's disease, on intracellular

membrane trafficking, focusing on the link between LRRK2, Rab proteins and endolysosomal calcium channels.

Belén Fernández received her degree in Biochemistry in 2005, and her Ph.D. in Chemistry from the University of Granada in 2009. She joined Hilfiker's laboratory to work on cellular mechanisms underlying neurodegeneration as a postdoctoral fellow funded by a prestigious "Juan de la Cierva" fellowship, and then pursued further postdoctoral work to expand on her expertise in chemistry at the University of Granada. She is currently a senior postdoctoral associate in Hilfiker's laboratory, and her main research focus is on understanding the link between iron dyshomeostasis and endolysosomal dysfunction underlying familial forms of Parkinson's disease.

Jesús Madero-Pérez received his degree in Biochemistry in 2011 and his master's degree in Regenerative Biomedicine in 2012, both from the University of Granada. He is currently finishing his Ph.D. studies on Parkinson's disease in Sabine Hilfiker's laboratory at the Spanish National Research Council (CSIC) and the University of Granada. His work during the Ph.D. has focused on elucidating the cellular and molecular mechanism underlying Parkinson's disease caused by LRRK2 mutations, also using iPSC-derived neurons as cellular model systems.

María Romo Lozano received her degree in Biology from the University of Granada in 2015, and her Master in Regenerative Biomedicine in 2016 from the same University. She is currently a Ph.D. student at the IPBLN, CSIC, in Granada, in Hilfiker's laboratory. Her project is centered on understanding the role of pathogenic LRRK2 and two-pore channels

in retromer-mediated protein trafficking, which may underlie at least some forms of familial Parkinson's disease.

Sabine Hilfiker received her M.S. from the University of Basel, Switzerland in 1992 and her Ph.D. in molecular neuroscience from the Rockefeller University, New York in 1998, under the supervision of Paul Greengard, Nobel Laureate 2000 in Medicine or Physiology. Following a short postdoctoral appointment at the Rockefeller University, Dr. Hilfiker was awarded a BBSRC David Phillips Research Fellowship to join the Faculty of Life Sciences at the University of Manchester, UK in 2000. Since 2008, she is a Principal Investigator in the CSIC (Spanish National Research Council) in Spain. Her laboratory is interested in understanding the molecular and cellular pathways underlying Parkinson's disease. She is recipient of the first Research Prize of the Spanish Federation for Parkinson's Disease (2008), a member of the LRRK2 Biology consortium of the Michael J. Fox Foundation (USA), and has published widely on cellular mechanisms underlying LRRK2-related Parkinson's disease, with recent links highlighting the importance of endolysosomal calcium channels in this disease.

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Figure 1.

Schematics of different endocytic compartments and the distribution of PIPs and TPCN2-associated proteins. TPCN2-reported interactors are in bold. Interactors which also have been reported to be substrates for the kinase activity of LRRK2 are shaded in blue. The endocytic recycling compartment may contain TPCN2, and several of the TPCN2 interactors and LRRK2 kinase substrates are localized to this compartment. Luminal Ca^{2+} concentration in the different compartments is estimated to be in the micromolar to millimolar range, whilst Ca^{2+} concentration in the recycling compartment remains to be precisely determined. Note that regulation of TPCN2 by PI(3,5)P₂ is thought to occur in the late endosome and lysosome, but not in the endocytic recycling compartment which is devoid of this PIP. For further information see text.