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Abstract

The importance of Leucine-Rich Repeat Kinase 2 (LRRK2) to mature neurons is well-established, since mutations in *PARK8*, the gene encoding LRRK2, are the most common known cause of Parkinson's disease. Nonetheless, despite the LRRK2 knockout mouse having no overt neurodevelopmental defect, numerous lines of *in vitro* data point towards a central role for this protein in neurogenesis. Roles for LRRK2 have been described in many key processes, including neurite outgrowth and the regulation of microtubule dynamics. Moreover, LRRK2 has been implicated in cell cycle control, suggesting additional roles in neurogenesis that precede terminal differentiation. However, we contend that the suggested function of LRRK2 as a scaffolding protein at the heart of numerous Wnt signaling cascades provides the most tantalizing link to neurogenesis in the developing brain. Numerous lines of evidence show a critical requirement for multiple Wnt pathways in the development of certain brain regions, not least the dopaminergic neurons of the ventral mid-brain. In conclusion, these observations indicate a function of LRRK2 as a subtle yet critical mediator of the action of Wnt ligands on developing neurons. We suggest that LRRK2 loss- or gain-of-function are likely modifiers of developmental phenotypes seen in animal models of Wnt signaling deregulation, a hypothesis that can be tested by cross-breeding relevant genetically modified experimental strains.

Leucine-rich repeat kinase 2: LRRK2

Leucine-rich repeat kinase 2 (LRRK2) is a protein that has been the subject of extensive research in recent years. This interest stems from the identification of LRRK2 as the product of the human *PARK8* gene, previously implicated as the cause of a familial form of Parkinson's disease in genetic linkage studies (Paisán-Ruíz *et al.*, 2004; Zimprich *et al.*, 2004). Furthering interest in LRRK2, *PARK8* has subsequently been associated with cancer, leprosy and Crohn's disease (Hassin-Baer *et al.*, 2009; Van Limbergen *et al.*, 2009; Zhang *et al.*, 2009). The importance of LRRK2 to these diseases will not be discussed further as the relevance to neuronal biology is limited. Nonetheless, the association of a single gene with four distinct medical conditions serves to highlight the complexity of LRRK2 function.

Parkinson's disease is a currently incurable late-onset neurodegenerative disorder with increasing public health implications in an aging population (Gasser, 2010). Therefore uncovering the molecular events causing this condition with the ultimate aim of identifying therapeutic targets for disease modifying treatment has gained in importance. The *PARK* genes mutated in patients with familial Parkinson's disease represent an obvious starting point for this research. Although *PARK8* is just one of more than a dozen loci linked to Parkinson's disease, certain lines of evidence indicate that LRRK2 is of special relevance. Globally *PARK8* mutations are estimated to contribute to 1-5% of Parkinson's disease cases, which represents the greatest contribution from any known genetic or environmental cause (Kumari & Tan, 2009). In some populations, most notably North African Berbers, *PARK8* mutations are very common and account for as much as two-fifths of all Parkinson's disease cases (Jasinska-Myga *et al.*, 2010; Lesage *et al.*, 2005). Importantly, patients with *PARK8* mutations exhibit symptoms that are clinically indistinguishable from the more common idiopathic form of Parkinson's disease, while observed post-mortem brain pathologies are also largely identical (Zimprich *et al.*, 2004). Thus, it seems likely that LRRK2 also plays a role in an as yet undetermined process that is deregulated very early in the pathogenesis of idiopathic Parkinson's disease (Berwick & Harvey, 2011; Kumari & Tan, 2009).

From a biochemical perspective, there are two immediate observations to be made about LRRK2. First, LRRK2 is a large (2527 amino acid) protein, containing multiple protein-protein interaction domains (Fig 1). Unsurprisingly, a vast number of interaction partners have been reported and LRRK2 has been suggested to function primarily as scaffolding protein (Berwick & Harvey, 2011; Lewis & Manzoni, 2012). Indeed, the breadth of reported interactors is so wide that LRRK2 probably functions in a number of distinct multi-protein complexes. Second, LRRK2 contains two separate enzymatic activities: serine-threonine phosphorylation (kinase activity) and guanine triphosphate hydrolysis (GTPase activity). Understandably, this has lead to the suggestion of alternative roles for LRRK2 as a 'conventional' signaling protein, either functioning as a protein kinase or in an analogous manner to small GTPases such as Ras or Rac (Berwick & Harvey, 2011).

A detailed review of LRRK2 function is beyond the scope of this article, but what is most important to stress is that the function of LRRK2 remains unclear and in many cases is controversial. For example, eight years of research have failed to find a reproducible kinase

substrate other than LRRK2 itself, while there is still no agreement on whether the GTPase activity controls kinase activity or vice versa. It seems probable therefore that these functions are interdependent. In any case, the enzymatic activities of LRRK2 are certainly of some importance in the physiological and pathological function of this protein. To date all described *PARK8* mutations clearly segregating with Parkinson's disease cause changes in the GTPase or kinase domains, but not in any of the protein-protein interaction domains (Fig. 1). Over-expression of LRRK2 in cultured cells and transgenic animals has been widely reported to cause cytotoxicity (Biosa *et al.*, 2012; Greggio *et al.*, 2006; Iaccarino *et al.*, 2007; Smith *et al.*, 2006; Stafa *et al.*, 2012; West *et al.*, 2007; Xiong *et al.*, 2010). Whether this observation reflects an artifactual effect of overexpressing this large protein is unclear. Nonetheless, it is now generally accepted that this observed cytotoxicity is enhanced by *PARK8* mutations but ameliorated by loss of LRRK2 kinase or GTPase activity. In light of this, much work has been predicated on the idea that pathological effects of *PARK8* mutations require LRRK2 kinase activity, and in consequence a great deal of effort has gone into developing a pharmacological inhibitor of LRRK2 kinase activity (Ray & Liu, 2012). However, although the common G2019S mutation has been reproducibly shown to elicit increased LRRK2 kinase activity this mutation appears unique: all other mutations segregating with Parkinson's disease have no reproducible effect on kinase activity (Greggio & Cookson, 2009). Furthermore, the G2385R mutation, a risk factor within Asian populations, has been reported to have decreased kinase activity (Rudenko *et al.*, 2012). Thus LRRK2 kinase activity appears to regulate the function of this protein, but whether increased kinase activity is responsible for pathogenesis in *PARK8* patients remains to be established. In conclusion, we favor a model where LRRK2 functions primarily as a scaffold that nucleates multiple protein complexes, but where protein function is nonetheless dependent on LRRK2 kinase and GTPase activities.

Despite considerable disagreement about LRRK2 function at the biochemical level, cell biological and transgenic animal studies have allowed advances to be made. In the following section we review aspects of LRRK2 biology where there are sufficient data to paint an overall picture that is beyond dispute, even if specific details are controversial or not yet known. We value the importance of work performed in lower organisms and will mention data obtained from these systems where pertinent, however this review will focus on mammalian data. This distinction is justified since mammals express two LRRK2 proteins, LRRK1 and LRRK2, which despite strong similarities in sequence and structure appear to have contrasting functions. Lower organisms, in particular *Drosophila melanogaster* and *Caenorhabditis elegans*, encode a single LRRK protein, which is thus the ortholog of both LRRK1 and LRRK2. As we outline, there is sufficient evidence in existence to make roles for LRRK2 in three processes that underlie neurogenesis beyond debate. These are roles in synaptic and endosomal vesicle trafficking, macroautophagy and regulation of microtubule dynamics are illustrated in Figure 2. Furthermore, an effect of LRRK2 on neurite outgrowth – a direct measure of the latter stages of neurogenesis – is extremely well supported. Coupled with growing evidence of roles in adult neurogenesis and in proliferation we contend that these data make a strong case for a central role for LRRK2 in multiple stages of neurogenesis.

Roles for LRRK2 in functions underlying neurogenesis

Regulated membrane trafficking events underlie many key processes involved in neurogenesis. These include requirements for endocytosis for the proper function of neurogenic signaling pathways, such as Notch and Wnt cascades, and membrane receptors involved in axonal outgrowth (Blitzer & Nusse, 2006; Le Borgne, 2006; Winckler & Yap, 2011). Importantly, roles for LRRK2 in membrane trafficking events are supported by data extending from early reports placing LRRK2 protein on cellular membranes (Biskup *et al.*, 2006; Hatano *et al.*, 2007) to evidence of vacuolation in LRRK2 knockout mouse kidney cells (Tong *et al.*, 2012). Intriguingly, the kinase activity of LRRK2 appears to be enhanced at membranes (Berger *et al.*, 2010), while the distribution of LRRK2 between membrane and cytosolic fractions can be regulated by extracellular stimuli (Berwick & Harvey, 2012a). These observations suggest that LRRK2 plays an active role in membrane trafficking events, and is not simply present as a by-stander.

The precise membranous compartments and/or vesicles LRRK2 inhabits remains contentious since many have been suggested, however membrane compartments involved in two cellular processes – pre-synaptic vesicle trafficking and macroautophagy – stand prominent. Evidence of a role for LRRK2 in the trafficking of pre-synaptic vesicles comes from multiple experimental techniques. These include localization of LRRK2 to vesicles in synaptic terminals by confocal and electron microscopy (Piccoli *et al.*, 2011; Xiong *et al.*, 2010), demonstrable electrophysiological defects following knock-down or over-expression of LRRK2 in cultured neurons (Piccoli *et al.*, 2011; Shin *et al.*, 2008), and biochemical interaction and co-localization of LRRK2 with the early endosomal marker Rab5b (Shin *et al.*, 2008). The function of LRRK2 in pre-synaptic vesicular compartments remains to be determined, although a recent report that the LRRK2 homolog in *Drosophila* phosphorylates endophilinA proteins is promising (Matta *et al.*, 2012).

A wealth of data implicate LRRK2 in macroautophagy, the process by which damaged organelles and protein aggregates are 'consumed' by engulfment by membranous autophagosomes that subsequently fuse with lysosomes (Codogno *et al.*, 2012). Macroautophagy is traditionally considered a mechanism of cellular homeostasis and regulated cell death. However, neural development is especially dependent on this process, most likely for mediating the extensive physical remodeling required (Cecconi *et al.*, 2007; Cecconi & Levine, 2008). Studies performed using mouse models of LRRK2 dysfunction have been particularly revealing, since multiple observations show impaired macroautophagy in the kidney of *Lrrk2* null animals (Herzig *et al.*, 2011; Tong *et al.*, 2012; Tong *et al.*, 2010). These data include increased numbers of lysosomes and related structures, and an accumulation of the macroautophagy substrates p62 protein and lipofuscin granules. Much of this kidney phenotype appears to be replicated in transgenic mice that over-express LRRK2 containing an artificial kinase-inactivating mutation (Herzig *et al.*, 2011). In agreement with these observations, over-expression of LRRK2 impacts upon the autophagic pathway in HEK293 cells (Alegre-Abarrategui *et al.*, 2009; Gómez-Suaga *et al.*, 2012). Importantly, published data also support a role for LRRK2 in macroautophagic processes in the central nervous system. Most notably, brains from aged transgenic mice that over-express human

LRRK2 variants containing either the R1441C or G2019S *PARK8* mutations have an accumulation of autophagic vacuoles (Ramonet *et al.*, 2011). This observation appears to be corroborated *in vitro*, where over-expression of G2019S has similar effects in cultured primary neurons or differentiated SH-SY5Y cells (MacLeod *et al.*, 2006; Plowey *et al.*, 2008).

Studies in *Drosophila* suggest another connection between LRRK2 and macroautophagy: via interaction with the small GTPase Rab7 (Dodson *et al.*, 2012). Rab7 is well established as a key regulator of the fusion step between macroautophagic organelles and lysosomes (Codogno *et al.*, 2012). If this observation can be replicated in mammalian systems it would be a fascinating result, since Rab7 is also involved in the latter stages of endocytosis, mediating late endosomal maturation and fusion with the lysosome (Wang *et al.*, 2011). As mentioned above, LRRK2 has also been reported to interact with Rab5b (Shin *et al.*, 2008). Since Rab5 proteins link pre-synaptic vesicle trafficking with early stages of endocytosis and Rab7 links autophagy with late-stage endocytosis, these observations together connect LRRK2 with the entire endocytic pathway.

In neurons endocytic vesicles can be trafficked over huge distances, particularly in axons. The importance of microtubules for this process is well described (Perlson *et al.*, 2010). It is therefore interesting that an association between LRRK2 and microtubules has been reported by a large number of groups (Biskup *et al.*, 2006; Dzamko *et al.*, 2010; Gandhi *et al.*, 2008; Gillardon, 2009a; Gillardon, 2009b; Gloeckner *et al.*, 2006; Kawakami *et al.*, 2012; Kett *et al.*, 2012; Sancho *et al.*, 2009; Sheng *et al.*, 2012). This physical association appears highly relevant to neurogenesis as LRRK2 has been reported to co-localize with microtubules within growth cones (Sancho *et al.*, 2009).

The nature of the LRRK2-microtubule interaction is still unresolved. For example there are conflicting reports about whether inhibition of LRRK2 kinase activity promotes (Dzamko *et al.*, 2010) or weakens (Kett *et al.*, 2012) the association. However, a role for LRRK2 in microtubule dynamics seems beyond dispute. Certain clues suggest LRRK2 may affect the stability of microtubules. LRRK2 has been reported to enhance the polymerization of bovine tubulin in the presence of microtubule associated proteins (MAPs) *in vitro* (Gillardon, 2009b). In addition, LRRK2 has been linked to canonical Wnt signaling (Berwick & Harvey, 2012a; Lin *et al.*, 2010; Sancho *et al.*, 2009), which is well described as a modulator of the microtubule cytoskeleton in neurons (Salinas, 2007). Perhaps most strikingly though, numerous lines of *in vivo* data implicate LRRK2 in modulating the function of the MAP tau, best known for its role in Alzheimer's disease (Taymans & Cookson, 2010). Post-mortem analysis of Parkinson's disease brains carrying Y1699C, G2019S or I2020T *PARK8* mutations have been reported to display 'tau pathology' in a number of cases (Khan *et al.*, 2005; Rajput *et al.*, 2006; Ujiie *et al.*, 2012; Zimprich *et al.*, 2004). Tau hyperphosphorylation has also been reported in brains from transgenic mice over-expressing LRRK2 with the G2019S or R1441G mutations (Li *et al.*, 2009; Melrose *et al.*, 2010), while *Lrrk2* knockout has been reported to decrease tau phosphorylation (Gillardon, 2009b) although others have been unable to replicate this observation (Hinkle *et al.*, 2012). G2019S

LRRK2 has also been reported to promote tau phosphorylation in Drosophila (Lin *et al.*, 2010). Mechanistically, the details linking LRRK2 to tau phosphorylation are lacking but one might predict the interaction between LRRK2 and microtubules would bring LRRK2 and tau into proximity. Indeed, a recent report suggests this may be the case (Kawakami *et al.*, 2012). Whether LRRK2 phosphorylates tau directly remains unclear with one supporting direct phosphorylation (Kawakami *et al.*, 2012), and another suggesting that phosphorylation is performed by glycogen synthase kinase 3 (GSK3) (Lin *et al.*, 2010), a reported LRRK2 interactor (Berwick & Harvey, 2012a; Lin *et al.*, 2010). These reports are not necessarily in conflict, since the experimental systems used are different and tau contains over 80 reported phosphorylation sites (http://cnr.iop.kcl.ac.uk/hangerlab/tautable). However, the possibility that phosphorylation is via GSK3 is intriguing, since this kinase has been implicated in the control of multiple MAPs besides tau, such as *adenomatous polyposis coli* (Zhou *et al.*, 2004) and collapsin response mediator protein 2 (Cole *et al.*, 2004). Therefore, it is plausible that the control of microtubule dynamics by LRRK2 involves the modulation of a multitude of MAPs and takes place at a variety of microtubule sites, not just those regulated by tau in axons.

Parallel to a role for LRRK2 on microtubules, LRRK2 has also been connected to the actin cytoskeleton via the regulation of ERM (Ezrin-Radixin-Moesin) protein phosphorylation. ERM proteins are three homologous proteins involved in anchoring actin filaments to the plasma membrane (Mangeat *et al.*, 1999). Phosphorylation of ERM proteins is believed to induce a conformational change resulting in an open 'active' shape (Mangeat *et al.*, 1999). Two groups have found a positive correlation between LRRK2 levels and ERM protein phosphorylation (Jaleel *et al.*, 2007; Parisiadou *et al.*, 2009), that is most likely mediated by an indirect regulation (Nichols *et al.*, 2009). Importantly, ERM proteins are essential for growth cone morphology and motility (Paglini *et al.*, 1998), thus indicating that LRRK2 also impacts upon neurogenesis through ERM proteins. The connection between LRRK2 and the actin cytoskeleton is strengthened by a mass spectrometry study which found endogenous LRRK2 in HEK293 cells to associate with actin and a number of proteins known to modulate the actin cytoskeleton (Meixner *et al.*, 2011). In light of these observations, it is interesting to speculate about a role for LRRK2 in coordinating neuronal microtubule as well as actin networks.

Thus, there are several lines of evidence for the importance of LRRK2 in a number of processes that underlie neurogenesis, but what evidence *directly* supports a requirement for LRRK2 in neurogenesis? Importantly, a comprehensive study in mouse embryos found a spatio-temporal LRRK2 mRNA expression pattern that is highly consistent with a key role for LRRK2 in neurogenesis (Zechel *et al.*, 2010). Using *in situ* hybridization, LRRK2 mRNA expression was detected as early as day E10.5 in the developing central nervous system, with transcript detectable throughout the cortex by day E12.5. Crucially, the authors describe embryonic expression of Lrrk2 as being most prominent in brain regions with 'high proliferative and migratory activity, as well as sites of differentiation and cell death' (Zechel *et al.*, 2010). These include the ventricular and subventricular zones of the telencephalon, in agreement with a previous report investigating older mice (Melrose *et al.*, 2007). Lrrk2 was

also found to be expressed in neural stem cells isolated from the dentate gyrus or striatal subventricular zone of E18.5 or adult mice. Thus LRRK2 expression patterns are consistent with a role in neurogenesis throughout life.

It is thus unsurprising that a growing body of experimental data show defects in neurogenesis caused by altered LRRK2 function. Experiments in cell and animal models utilized LRRK2 knock-down or knockout, over-expression of wild-type LRRK2, familial LRRK2 mutants and artificial kinase or GTPase dead mutants to measure predominantly neurite outgrowth and reported changes in length, number and branching of neurites *in vitro* and in brain slices. Most reports agree that the over-expression of familial LRRK2 mutants elicit decreased neurite length (Biosa *et al.*, 2012; Chan *et al.*, 2011; Cherra *et al.*, 2012; Cho *et al.*, 2012; Dächsel *et al.*, 2010; Heo *et al.*, 2010; Lin *et al.*, 2010; MacLeod *et al.*, 2006; Maekawa *et al.*, 2012; Parisiadou *et al.*, 2009; Plowey *et al.*, 2008; Ramonet *et al.*, 2011; Sheng *et al.*, 2012; Stafa *et al.*, 2012; Winner *et al.*, 2011). Over-expression of wild-type LRRK2 was generally found to have no effect, or to cause mild neurite shortening, either reaching statistical significance or remaining as a trend. In contrast, loss of LRRK2 appears to have the opposite effect, allowing longer and more branched neurites to develop (Dächsel *et al.*, 2010; Heo *et al.*, 2010; MacLeod *et al.*, 2006; Parisiadou *et al.*, 2009; Paus *et al.*, 2012; Stafa *et al.*, 2012), although not all studies are in agreement (Gillardon, 2009b; Meixner *et al.*, 2011). In general, these studies provide overwhelming evidence that the level of LRRK2 expression impacts upon neuritogenesis as predicted from the LRRK2 expression pattern throughout brain development.

Finally, we also note two publications investigating a role for LRRK2 in adult neurogenesis (Paus *et al.*, 2012; Winner *et al.*, 2011). These studies are of special relevance to Parkinson's disease, where impaired adult neurogenesis has been implicated in the development of non-motor symptoms (Marxreiter *et al.*, 2012). A study in mice over-expressing the G2019S LRRK2 mutant reported decreased proliferation in the dentate gyrus and subventricular zones associated with decreased dendritic length and branching, and decreased cell survival (Winner *et al.*, 2011). Another study by Paus and colleagues looked at adult neurogenesis in the dentate gyrus of *Lrrk2* knockout mice (Paus *et al.*, 2012). As might be predicted, increased dendritic length and neurite arborisation was observed. No defects in cell proliferation or survival were found however, although loss of Lrrk2 led to a greater number of doublecortin positive cells (Paus *et al.*, 2012).

Thus a wealth of data implicates LRRK2 as a central player in the latter stages of neurogenesis – in particular neurite outgrowth and synaptogenenesis – possibly via a combination of modulating vesicle trafficking and cytoskeleton dynamics. Nonetheless, emerging evidence also links LRRK2 to earlier stages of neuronal development, prior to cell cycle exit (Winner *et al.*, 2011), supported by evidence of effects of LRRK2 on proliferation (Liu *et al.*, 2012; Milosevic *et al.*, 2009) and carcinogenesis (Hassin-Baer *et al.*, 2009). Thus, LRRK2 might play a role from early mitotic neuronal precursors to terminal differentiation. As outlined above, we would suggest that LRRK2 is likely to function as a scaffolding protein in a number of distinct complexes, some of these important in neurogenesis.

Nonetheless, two crucial questions remain: what is upstream of these complexes, and how are they regulated?

A role for LRRK2 in Wnt signaling

Wnt (wingless/Int) signaling pathways constitute a family of highly conserved signal transduction cascades that have long been established as master regulators of animal development (Freese *et al.*, 2010). The relevance of these pathways to neurogenesis is beyond doubt and will not be reviewed in detail in this article. Nonetheless the reader should be aware that a growing body of data also implicates Wnt signaling in the function of mature, post-mitotic neurons (Inestrosa & Arenas, 2010). Moreover, deregulated Wnt signaling pathways are suggested pathomechanisms for a number of neurological conditions, including Alzheimer's disease, Parkinson's disease, autism and schizophrenia (Berwick & Harvey, 2012b; De Ferrari & Moon, 2006; Inestrosa & Toledo, 2008). Thus Wnt cascades can be considered essential for the central nervous system at all stages of life.

Wnt ligands themselves are secreted glycoproteins that bind to the extracellular domains of frizzled receptors on the plasma membrane of target cells. Signaling specificity is achieved in part through the large repertoire of Wnt ligands and frizzled receptors expressed in higher organisms, but also through the involvement of co-receptors. In the case of the canonical Wnt pathway, these receptors are low density lipoprotein receptor-like proteins 5 and 6 (LRP5/6), which have also been reported to bind Wnt ligands at the cell surface. Upon binding of Wnt ligands to frizzled receptors and associated co-receptors the signal is relayed across the membrane, resulting in the activation of one or more intracellular cascades. Wnt signaling pathways relevant to neurogenesis and/or the function of mature neurons are depicted in Figure 3.

The best-described Wnt signaling cascade is the canonical Wnt pathway. This signaling mechanism ultimately results in the activation and nuclear recruitment of β-catenin protein, leading to the modulation of downstream target genes. In consequence, this pathway is sometimes referred to as the Wnt-β-catenin pathway. Canonical Wnt signaling is an unusual signaling mechanism as several events take place in the absence of a stimulus. In particular, β-catenin is sequestered into an inhibitory cytosolic complex known as the β-catenin destruction complex. Here, β-catenin is phosphorylated by GSK3 (the same protein implicated in tau phosphorylation). β-catenin phosphorylation results in the targeting of β-catenin for degradation by the proteosome. Therefore, in the absence of canonical Wnt pathway activators, β-catenin is continually degraded, and consequently unable to accumulate in the nucleus to regulate gene expression. Binding of Wnt ligand to frizzled receptors and LRP5/6 results in the recruitment of cytosolic dishevelled (DVL) proteins (key intermediates of most Wnt signaling branches) to the plasma membrane. Via interaction with key components of the β-catenin destruction complex such as Axin, DVL proteins cause the subsequent relocalization of the β-catenin destruction complex to the same juxtamembrane site. This elicits the penultimate stage of canonical Wnt signaling, the inhibition of β-catenin phosphorylation, which allows β-catenin to become proteasome resistant and thus accumulate

throughout the cell. However, the complexity of this mechanism is emerging. A requirement for the internalization of the cell membrane associated protein complex containing Wnt ligand, frizzled receptor, LRP5/6, DVL proteins and the β-catenin destruction complex into the endosomal system is now widely accepted. This internalized signaling complex passes through the endosomal system where it continues to signal from the cytosolic face of intracellular membranes (the so-called 'signalosome' hypothesis). Finally, the signalosomes are sequestered from the cytosol into multi-vesicular bodies. Traditionally, by analogy to growth factor signaling pathways, this has been considered the termination step in Wnt signaling. However, recent data suggest that the sequestration of signalosomes into multi-vesicular bodies constitutes a final 'signal activation' mechanism, since this leads to the removal of the canonical Wnt signaling pool of GSK3 from the cytosol (Dobrowolski & De Robertis, 2012).

Data from our laboratory has strongly implicated LRRK2 in multiple aspects of the canonical Wnt pathway. This work arose from a yeast-two-hybrid screen identifying interactors of the Roc and/or COR domains of LRRK2 (Sancho *et al.*, 2009). Since Roc and COR domains are expressed together throughout nature (Marín *et al.*, 2008), it is reasonable to consider both domains part of a single functional unit conferring GTPase activity that was termed the RocCOR tandem domain. Using this LRRK2 RocCOR tandem domain as bait, the yeast-two-hybrid screen returned cDNAs encoding the human dishevelled proteins DVL2 and DVL3 as potential interactors. Subsequent assays confirmed a direct interaction between the LRRK2 RocCOR domain and all three human DVL proteins, with the interaction site mapped to the dishevelled-Egl10-pleckstrin (DEP) domain of DVL1-3 (Sancho *et al.*, 2009). Co-immunoprecipitation experiments confirmed that LRRK2 associates with DVL proteins in mammalian cells, while confocal microscopy revealed a striking recruitment of LRRK2 into polymeric DVL structures that are induced by over-expression of these proteins (Sancho *et al.*, 2009).

Since DVL proteins are essential intermediates of all major branches of Wnt signaling (Fig. 3), our work opened the possibility that LRRK2 may function in multiple Wnt cascades. However, follow-up studies focused on the canonical Wnt pathway, which can be assayed easily using TOPflash assays (Veeman *et al.*, 2003). TOPflash assays are luciferase-based reporter assays that quantitatively determine the level of β-catenin-mediated transcriptional activity in cells. Importantly, TOPflash assays revealed the LRRK2-DVL protein interaction to be functional as well as physical, since co-transfection of LRRK2 protein with any of the three human DVL proteins resulted in an enhancement of DVL-driven canonical Wnt activity (Berwick & Harvey, 2012a). This effect required the kinase and GTPase activities of LRRK2 and was increased further by targeting LRRK2 (and presumably, therefore, the LRRK2-DVL interaction) to membranes. Interestingly, Wnt3a treatment was found to increase the amount of endogenous LRRK2 present in membrane fractions of HEK293 cells (Berwick & Harvey, 2012a). Since the activation of canonical Wnt signaling takes place at intracellular membranes – consistent with the signalosome hypothesis – this lead us to investigate whether LRRK2 might physically interact with Wnt signaling receptors. Using a combination of confocal microscopy and co-immunoprecipitation LRRK2 was discovered to associate with

LRP6, but not frizzled-1, frizzled-4 or frizzled-5. Yeast-two-hybrid assays confirmed that the interaction was direct and, similar to interaction with DVL proteins, involved the LRRK2 RocCOR tandem domain (Berwick & Harvey, 2012a). These data are consistent with a role for LRRK2 in the activation of canonical Wnt signaling bringing DVL proteins to cellular membranes.

However, somewhat counter-intuitively, knock-down of LRRK2 was also found to potentiate DVL-driven TOPflash activity (Berwick & Harvey, 2012a). A similar effect was observed on basal and Wnt3a-driven β-catenin activity. In this regard knockdown of LRRK2 mimicked knockdown of AXIN1, an established component of the β-catenin destruction complex. Loss of AXIN1 is well known to disrupt the β-catenin destruction complex thereby compromising β-catenin degradation and leading to an increase in basal canonical Wnt activity. We therefore wondered whether loss of LRRK2 might compromise an inhibitory role for LRRK2 in the β-catenin destruction complex. Consistent with this, co-immumoprecipitation of endogenous protein from mouse brain revealed Lrrk2 to exist in complex with multiple components of the β-catenin destruction complex, including GSK3 and β-catenin. Taken together, these results suggest a role for LRRK2 as a scaffold in canonical Wnt signaling. In the basal state, LRRK2 functions as part of the cytosolic β-catenin destruction complex and loss of LRRK2 compromises this role, leading to disruption of the complex and pathway activation. Following stimulation of cells with Wnt ligand, LRRK2 is recruited to cellular membranes. Here, via interaction with DVL proteins, the β-catenin destruction complex and LRP6, LRRK2 assists in the formation of Wnt signalosomes, enhancing the Wnt signal activity.

Our data are supported by work from other laboratories. Most notably, supporting the idea that LRRK2 associates with the β-catenin destruction complex, an interaction between LRRK2 and GSK3 has been reported in *Drosophila* (Lin *et al.*, 2010). While this report does not investigate which cellular GSK3 pool associates with LRRK2 we note that the interaction modulated tau phosphorylation. Since Wnt signaling is well known to regulate the phosphorylation of tau by GSK3 (Hooper *et al.*, 2008), this would suggest that the GSK3 pool found to bind LRRK2 by Lin and co-workers could indeed represent the same Wnt-responsive fraction identified in our study (Berwick & Harvey, 2012a; Lin *et al.*, 2010). Perhaps most interesting in this report, the LRRK2-GSK3 interaction was shown to be enhanced by the G2019S *PARK8* mutation (Lin *et al.*, 2010). Curiously, the strength of the LRRK2 interaction with both DVL proteins and LRP6 was also affected by *PARK8* mutations (Berwick & Harvey, 2012a; Sancho *et al.*, 2009). Unsurprisingly therefore, all investigated *PARK8* mutations decreased the capacity of LRRK2 to enhance the β-catenin activation elicited by DVL proteins (Berwick & Harvey, 2012a). This observation has obvious implications for the pathogenesis of Parkinson's disease, where perturbed Wnt signaling has already been suggested as a candidate pathomechanism (Berwick & Harvey, 2012b). However, as we outline below, decreased canonical Wnt signaling associated with familial *PARK8* mutations suggests that transgenic LRRK2 animal models of Parkinson's disease might present with discrete developmental phenotypes associated with Wnt dysfunction.

In addition to studies linking LRRK2 to Wnt signaling by protein-protein interaction strong circumstantial evidence from transcriptomics studies support this notion. In particular, an investigation into the effect of LRRK2 knockdown in human SH-SY5Y cells found mRNA species encoding a number of Wnt signaling proteins to be altered (Häbig *et al.*, 2008). As it is well described that many Wnt signaling components are regulated at the transcriptional level by pathway activation, knockdown of LRRK2 would be expected to alter expression of other Wnt signaling proteins. Data from *C.elegans* also support this observation, with mRNA transcripts encoding Wnt signaling proteins being described as 'coregulated with LRRK2' (Ferree *et al.*, 2011).

Further support, albeit indirect, of a role for LRRK2 in Wnt signaling comes from studies investigating altered gene expression in animal models of Parkinson's disease. These investigations used a variety of different neurotoxins to elicit dopaminergic cell death resulting in Parkinsonian-like motor phenotypes. L'Episcopo and colleagues reported increased *Wnt1* gene expression as well as deregulated *Fzd1* and β-catenin expression in the ventral midbrain of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated mice (L'Episcopo *et al.*, 2011). These experiments support the idea that canonical Wnt signal activation via increased *Wnt1* expression in astrocytes is neuroprotective (L'Episcopo *et al.*, 2011). Another study used 6-hydroxydopamine (6-OHDA) to induce dopaminergic cell death in rats resulting in increased expression of the Wnt signal inhibitor Dickkopf-1 (Dkk1) (Dun *et al.*, 2012). Both models are in agreement with a neuroprotective role for Wnt signaling, since treatment with Dkk1 exacerbated toxic effects, whilst GSK3 inhibition was found to be protective (L'Episcopo *et al.*, 2011; Dun *et al.*, 2012). An unbiased genome-wide RNAseq approach in mice treated with a variety of pesticides showed altered expression of mRNAs encoding Wnt signaling components in ventral midbrain and striatum (Gollamudi *et al.*, 2012). Exposure to pesticides is a well known environment risk factor for Parkinson's disease, further suggesting that dysregulated Wnt signaling might be a common mechanism underlying dopaminergic cell death in Parkinson's disease. Expression studies in human Parkinson's disease brains have not been as conclusive, although it is important to note that altered expression of Wnt pathway genes has been reported in women but not in men (Cantuti-Castelvetri *et al.*, 2007). These studies need to take into account that the brains analysed are usually from individuals with symptomatic Parkinson's disease reflecting the loss of the majority of dopaminergic neurons in the substantia nigra. Therefore gene expression changes are likely no longer reflective of the initial underlying etiology. However, in addition to work on LRRK2 outlined above, other clues from genetic causes of Parkinson's disease are consistent with altered Wnt signaling. Most notably Parkin, the product of the *PARK2* gene, has been reported to inhibit canonical Wnt signaling (Rawal *et al.*, 2009), whilst the transcription factor Nurr1, which has been strongly linked to Parkinson's disease, is regulated by β-catenin (Jankovic *et al.*, 2005; Kitagawa *et al.*, 2007). Finally, it is not just Parkinson's disease-related genes that have been associated with Wnt signaling; Wnt signaling genes themselves have been linked to risk of Parkinson's disease. In particular, GSK3β has been suggested to modify disease risk in two studies (Kalinderi *et al.*, 2011; Kwok *et al.*, 2005) although a third failed to find any affect (Wider *et al.*, 2011).

In summary therefore, LRRK2 binds three central Wnt signaling components (Berwick & Harvey, 2012a; Lin *et al.*, 2010; Sancho *et al.*, 2009), while loss of LRRK2 and pathogenic *PARK8* mutations impact upon the activity of the canonical Wnt pathway (Berwick & Harvey, 2012a). In addition, connections between LRRK2 and Wnt cascades are strengthened by a number of studies supporting a role for dysregulated Wnt signaling in the early stage of Parkinson's disease. As outlined above, there is overwhelming evidence for a central function for LRRK2 in neurogenesis. Combining these ideas, we postulate a specific role for LRRK2 in Wnt-mediated neurogenesis. In the final section of this article we will elaborate on this and suggest experimental approaches to test our hypothesis.

LRRK2 as a major player in Wnt-mediated neuronal differentiation?

It is beyond dispute that Wnt ligands represent potent morphogens required for numerous aspects of neurogenesis, in particular to the development of dopaminergic neurons of the ventral midbrain (Brault *et al.*, 2001; Carpenter *et al.*, 2010; Castelo-Branco *et al.*, 2010; Castelo-Branco *et al.*, 2004; Castelo-Branco *et al.*, 2003; Parish *et al.*, 2008). Degeneration of these neurons underlies the typical motor symptoms associated with Parkinson's disease (Berwick & Harvey, 2012b). In this context, deregulated Wnt signaling caused by *PARK8* mutations might cause subtle defects in establishing neuronal circuitries, leaving these dopaminergic neurons more vulnerable to additional insults important for the pathogenesis of Parkinson's disease. In the remainder of this review we describe roles for Wnt signaling pathways in modulating the same neurogenic events that were reported to be influenced by LRRK2. Combined with evidence of a function for LRRK2 as a Wnt signaling scaffold, this further supports the idea that LRRK2 is a central player in Wnt-mediated neurogenesis.

Evidence that Wnt ligands are major regulators of synaptic vesicle trafficking and synaptogenesis is accumulating (Farías *et al.*, 2010; Inestrosa & Arenas, 2010). Published data support pre-synaptic and post-synaptic effects of multiple branches of Wnt signaling. Mammalian pre-synaptic development appears particularly dependent on Wnt-7a, an agonist of the canonical Wnt pathway (Farías *et al.*, 2010). This Wnt ligand appears to be required for normal expression of the pre-synaptic vesicle protein, Synapsin 1, in the developing mouse brain (Hall *et al.*, 2000), with similar effects seen in mature neurons (Farías *et al.*, 2007). Treatment of cultured neurons with dickkopf 1, an LRP5/6 antagonist, has confirmed this pre-synaptic effect of Wnt7a is through the canonical pathway (Davis *et al.*, 2008). Curiously though, data from a number of laboratories suggest this effect is independent of transcription (Farías *et al.*, 2010). This observation has led to the cascade by which Wnt7a modulates pre-synaptic and axonal (see below) function being described as a 'divergent' canonical cascade (Ciani *et al.*, 2004). Interestingly, LRRK2 is not just a key Wnt signaling protein interacting with LRP6 but was also found to interact with synapsin-1 and play a role in synaptic vesicle trafficking (Piccoli et al, 2011). The above evidence supports the idea of a Wnt7a-induced LRRK2-mediated canonical Wnt pathway with a direct transcriptionally independent effect on synapse formation and maintenance.

Both LRRK2 and Parkinson's disease pathogenesis have been linked to macroautophagy. In addition, there is also evidence consistent with the idea that macroautophagy is modulated by

Wnt ligands. Strikingly, knock-down of β-catenin alone appears sufficient to induce macroautophagy in carcinoma cells (Chang *et al.*, 2012). Correspondingly, acute treatment of hippocampal neurons with the β-catenin agonist 2-amino-4-[3,4-(methylenedioxy)benzyl-amino]-6-(3-methoxyphenyl)pyrimidine (Liu *et al.*, 2005) was found to reduce oxygen-glucose deprivation induced macroautophagy (Wang *et al.*, 2012). Taken together, these observations suggest β-catenin to be a negative regulator of macroautophagy. In addition, GSK3 activity almost certainly impacts upon macroautophagy. GSK3 has recently been reported to phosphorylate TIP60, a histone acetyl transferase required for induction of macroautophagy. Mutation of the reported phosphorylation site to an alanine residue is sufficient to prevent growth factor deprivation-induced macroautophagy (Lin *et al.*, 2012). In agreement with this, intranasal treatment with a GSK3 inhibitor peptide was reported to result in decreased autophagy and increased lysosomal acidification in brains from an Alzheimer's disease transgenic mouse model (Avrahami *et al.*, 2013), with similar results obtained *in vitro*. These data predict a model where canonical Wnt pathway activation – resulting in GSK3 inhibition and β-catenin accumulation – would lead to decreased macroautophagy. However, the events do not appear straightforward, since GSK3 inhibition in a neuroblastoma cell line has been reported to induce increased lysosomal biogenesis, leading to increased macroautophagic flux (Parr *et al.*, 2012). There are numerous reasons for this potential discrepancy, for example cell lines and treatments used, however it is perhaps more relevant to observe that these studies are at very early stages. More pertinently still, none look at the regulation of macroautophagy during neural differentiation, where one would expect the requirements placed on the autophagic machinery of developing neurons to be more subtle than under conditions of stress. In conclusion, even though evidence supports the importance for LRRK2 and canonical Wnt signaling in macroautophagy, the specific signal transduction cascade, especially during neuronal differentiation, requires further investigation.

Wnt signaling is well-known to influence the dynamic instability of the microtubule cytoskeleton (Salinas, 2007). Multiple proteins involved in both the canonical and non-canonical Wnt pathway have been reported to affect microtubule stability, whilst GSK3 phosphorylates a variety of microtubule associated proteins (Cole *et al.*, 2004; Kim *et al.*, 2011; Salinas, 2007; Zhou *et al.*, 2004). Modulation of microtubule structures by Wnt pathways appear common to multiple cell types, for example Wnt ligands appear to be key regulators of mitotic spindles (Walston *et al.*, 2004). In light of the above-described presynaptic function of LRRK2 and Wnt7a, it is important to emphasize that a large number of studies showing Wnt-mediated regulation of microtubules have used axon outgrowth as the model system. Much of this work was initiated by the observation that Wnt7a elicits axonal spreading and branching in cultured cerebellar granule cells (Lucas & Salinas, 1997), with corroborating data soon obtained *in vivo* (Hall *et al.*, 2000). This effect is mimicked by GSK3 inhibitors and likely involves inhibition of phosphorylation of the microtubule associated protein MAP1B (Lucas *et al.*, 1998; Lucas & Salinas, 1997). Additional mechanisms involved in Wnt-mediated control of axonal microtubules include the identification of the β-catenin destruction complex members *adenomatous polypsis coli* (APC) and AXIN1 as microtubule binding proteins in axons (Ciani *et al.*, 2004; Purro *et al.*, 2008). The precise details are still being elucidated, but it is fair to assume that, via

interaction with microtubules, APC and AXIN1 create a spatially controlled signaling mechanism, specific to growing axons and growth cones. Importantly, LRRK2 also interacts with microtubules, induces hyperphosphrylation of the axonal MAP tau (Biskup *et al.*, 2006; Dzamko *et al.*, 2010; Gandhi *et al.*, 2008; Gillardon, 2009a; Gillardon, 2009b; Gloeckner *et al.*, 2006; Kawakami *et al.*, 2012; Kett *et al.*, 2012; Sancho *et al.*, 2009; Sheng *et al.*, 2012), interacts with components of the β-catenin destruction complex (Berwick & Harvey, 2012a; Lin *et al.*, 2010) and co-localizes with DVL1 to neurites in cell culture models at early stages of differentiation (Sancho et al, 2009). This further supports the idea of a LRRK2 mediated Wnt signaling pathway important during neuronal differentiation.

Above we have established good evidence for roles of LRRK2 and Wnt signaling in the regulation of pre-synaptic vesicle trafficking and microtubule dynamics, processes crucial for axonal outgrowth and synpatogenesis. While the evidence of a role for LRRK2 in macroautophagy is overwhelming, data supporting a role for Wnt signaling in modulating this process are more circumstantial. Nonetheless, the hypothesis that LRRK2 might function specifically in Wnt-mediated neuritogenesis is plausible, especially for the latter stages of neurogenesis. But what about the earlier stages? Here, the role for Wnt signaling is beyond doubt. For example, treatment with Wnt1, which activates the canonical Wnt pathway, causes expansion of ventral midbrain precursors (Castelo-Branco *et al.*, 2003). Conversely, loss of *Wnt1* in mice leads to a complete failure of mid- and hind-brain precursors to expand, leading to a near absence of these brain regions (Thomas & Capecchi, 1990). Similarly, Wnt3a (another canonical pathway agonist) secreted by hippocampal astrocytes has been shown to be essential for adult neurogenesis in the dentate gyrus (Lie *et al.*, 2005). By contrast evidence of a function for LRRK2 in the proliferation of neural precursors and in adult neurogenesis are promising but at an early stage (Milosevic *et al.*, 2009; Winner *et al.*, 2011).

In conclusion, there is a remarkable degree of overlap between the effects of Wnt signaling and LRRK2 on neurogenesis. The importance of LRRK2 in canonical Wnt signaling further supports the notion of a specific function for LRRK2 in Wnt-mediated neurogenesis. This hypothesis can be investigated by crossing the relevant transgenic animals with known defects in Wnt-mediated neuronal differentiation with LRRK2 transgenics looking for an enhancement or rescue of phenotype. Of course, such experiments come with the usual important caveats associated with using animal models. For example, long non-coding RNAs are known to be poorly conserved between species making their study in model organisms of questionable relevance to humans (Pang *et al.*, 2006). Human-specifc transcriptional networks have also been reported in the brain (Konopka *et al.*, 2012). However at the level of protein function, conservation across species is usually very high, and thus, even though the data should be treated with caution, crossing of transgenic animal models is a justifiable approach. Indeed, this strategy has proven particularly useful for unveiling milder neurodevelopmental phenotypes. For example, crossing of *Wnt7a* and *Dvl1* knockout mice allowed a requirement for these genes in the development of cerebellar glomerular rosettes to be uncovered (Ahmad-Annuar *et al.*, 2006). As *Lrrk2* knockout and familial *PARK8* mutant transgenic mice likely represent models of subtly increased and decreased canonical Wnt

signaling, respectively, crossing of these lines with *Wnt7a* and/or *Dvl1* knockout animals would be of great interest.

References

[1] A. Ahmad-Annuar, L. Ciani, I. Simeonidis, J. Herreros, N.B. Fredj, S.B. Rosso, A. Hall, S. Brickley, and P.C. Salinas (2006). Signaling across the synapse: a role for Wnt and Dishevelled in presynaptic assembly and neurotransmitter release. *J Cell Biol* 174, 127-39.

[2] J. Alegre-Abarrategui, H. Christian, M.M. Lufino, R. Mutihac, L.L. Venda, O. Ansorge, and R. Wade-Martins (2009). LRRK2 regulates autophagic activity and localizes to specific membrane microdomains in a novel human genomic reporter cellular model. *Hum Mol Genet* 18, 4022-34.

[3] L. Avrahami, D. Farfara, M. Shaham-Kol, R. Vassar, D. Frenkel, and H. Eldar-Finkelman (2013). Inhibition of glycogen synthase kinase-3 ameliorates β-amyloid pathology and restores lysosomal acidification and mammalian target of rapamycin activity in the Alzheimer disease mouse model: in vivo and in vitro studies. *J Biol Chem* 288, 1295-306.

[4] Z. Berger, K.A. Smith, and M.J. Lavoie (2010). Membrane localization of LRRK2 is associated with increased formation of the highly active LRRK2 dimer and changes in its phosphorylation. *Biochemistry* 49, 5511-23.

[5] D.C. Berwick, and K. Harvey (2011). LRRK2 signaling pathways: the key to unlocking neurodegeneration? *Trends Cell Biol* 21, 257-65.

[6] D.C. Berwick, and K. Harvey (2012a). LRRK2 functions as a Wnt signaling scaffold, bridging cytosolic proteins and membrane-localized LRP6. *Hum Mol Genet* 21, 4966-79.

[7] D.C. Berwick, and K. Harvey (2012b). The importance of Wnt signalling for neurodegeneration in Parkinson's disease. *Biochem Soc Trans* 40, 1123-8.

[8] A. Biosa, A. Trancikova, L. Civiero, L. Glauser, L. Bubacco, E. Greggio, and D.J. Moore (2012). GTPase Activity Regulates Kinase Activity and Cellular Phenotypes of Parkinson's Disease-Associated LRRK2. *Hum Mol Genet*.

[9] S. Biskup, D.J. Moore, F. Celsi, S. Higashi, A.B. West, S.A. Andrabi, K. Kurkinen, S.W. Yu, J.M. Savitt, H.J. Waldvogel, R.L. Faull, P.C. Emson, R. Torp, O.P. Ottersen, T.M. Dawson, and V.L. Dawson (2006). Localization of LRRK2 to membranous and vesicular structures in mammalian brain. *Ann Neurol* 60, 557-69.

[10] J.T. Blitzer, and R. Nusse (2006). A critical role for endocytosis in Wnt signaling. *BMC Cell Biol* 7, 28.

[11] V. Brault, R. Moore, S. Kutsch, M. Ishibashi, D.H. Rowitch, A.P. McMahon, L. Sommer, O. Boussadia, and R. Kemler (2001). Inactivation of the beta-catenin gene by Wnt1-Cre-mediated deletion results in dramatic brain malformation and failure of craniofacial development. *Development* 128, 1253-64.

[12] I. Cantuti-Castelvetri, C. Keller-McGandy, B. Bouzou, G. Asteris, T.W. Clark, M.P. Frosch, and D.G. Standaert (2007). Effects of gender on nigral gene expression and parkinson disease. *Neurobiol Dis* 26, 606-14.

[13] A.C. Carpenter, S. Rao, J.M. Wells, K. Campbell, and R.A. Lang (2010). Generation of mice with a conditional null allele for Wntless. *Genesis* 48, 554-8.

[14] G. Castelo-Branco, E.R. Andersson, E. Minina, K.M. Sousa, D. Ribeiro, C. Kokubu, K. Imai, N. Prakash, W. Wurst, and E. Arenas (2010). Delayed dopaminergic neuron differentiation in Lrp6 mutant mice. *Dev Dyn* 239, 211-21.

[15] G. Castelo-Branco, N. Rawal, and E. Arenas (2004). GSK-3beta inhibition/beta-catenin stabilization in ventral midbrain precursors increases differentiation into dopamine neurons. *J Cell Sci* 117, 5731-7.

[16] G. Castelo-Branco, J. Wagner, F.J. Rodriguez, J. Kele, K. Sousa, N. Rawal, H.A. Pasolli, E. Fuchs, J. Kitajewski, and E. Arenas (2003). Differential regulation of midbrain dopaminergic neuron development by Wnt-1, Wnt-3a, and Wnt-5a. *Proc Natl Acad Sci U S A* 100, 12747-52.

[17] F. Cecconi, S. Di Bartolomeo, R. Nardacci, C. Fuoco, M. Corazzari, L. Giunta, A. Romagnoli, A. Stoykova, K. Chowdhury, G.M. Fimia, and M. Piacentini (2007). A novel role for autophagy in neurodevelopment. *Autophagy* 3, 506-8.

[18] F. Cecconi, and B. Levine (2008). The role of autophagy in mammalian development: cell makeover rather than cell death. *Dev Cell* 15, 344-57.

[19] D. Chan, A. Citro, J.M. Cordy, G.C. Shen, and B. Wolozin (2011). Rac1 protein rescues neurite retraction caused by G2019S leucine-rich repeat kinase 2 (LRRK2). *J Biol Chem* 286, 16140-9.

[20] H.W. Chang, Y.S. Lee, H.Y. Nam, M. Han, H.J. Kim, S.Y. Moon, Hyesungjeon, J.J. Park, T.E. Carey, S.E. Chang, S.W. Kim, and S.Y. Kim (2012). Knockdown of β-catenin controls both apoptotic and autophagic cell death through LKB1/AMPK signaling in head and neck squamous cell carcinoma cell lines. *Cell Signal*.

[21] S.J. Cherra, E. Steer, A.M. Gusdon, K. Kiselyov, and C.T. Chu (2012). Mutant LRRK2 Elicits Calcium Imbalance and Depletion of Dendritic Mitochondria in Neurons. *Am J Pathol*.

[22] H.J. Cho, G. Liu, S.M. Jin, L. Parisiadou, C. Xie, J. Yu, L. Sun, B. Ma, J. Ding, R. Vancraenenbroeck, E. Lobbestael, V. Baekelandt, J.M. Taymans, P. He, J.C. Troncoso, Y. Shen, and H. Cai (2012). MicroRNA-205 regulates the expression of Parkinson's disease-related leucine-rich repeat kinase 2 protein. *Hum Mol Genet*.

[23] L. Ciani, O. Krylova, M.J. Smalley, T.C. Dale, and P.C. Salinas (2004). A divergent canonical WNT-signaling pathway regulates microtubule dynamics: dishevelled signals locally to stabilize microtubules. *J Cell Biol* 164, 243-53.

[24] P. Codogno, M. Mehrpour, and T. Proikas-Cezanne (2012). Canonical and non-canonical autophagy: variations on a common theme of self-eating? *Nat Rev Mol Cell Biol* 13, 7-12.

[25] A.R. Cole, A. Knebel, N.A. Morrice, L.A. Robertson, A.J. Irving, C.N. Connolly, and C. Sutherland (2004). GSK-3 phosphorylation of the Alzheimer epitope within collapsin response mediator proteins regulates axon elongation in primary neurons. *J Biol Chem* 279, 50176-80.

[26] E.K. Davis, Y. Zou, and A. Ghosh (2008). Wnts acting through canonical and noncanonical signaling pathways exert opposite effects on hippocampal synapse formation. *Neural Dev* 3, 32.

[27] G.V. De Ferrari, and R.T. Moon (2006). The ups and downs of Wnt signaling in prevalent neurological disorders. *Oncogene* 25, 7545-53.

[28] R. Dobrowolski, and E.M. De Robertis (2012). Endocytic control of growth factor signalling: multivesicular bodies as signalling organelles. *Nat Rev Mol Cell Biol* 13, 53-60.

[29] M.W. Dodson, T. Zhang, C. Jiang, S. Chen, and M. Guo (2012). Roles of the Drosophila LRRK2 homolog in Rab7-dependent lysosomal positioning. *Hum Mol Genet* 21, 1350-63.

[30] Y. Dun, G. Li, Y. Yang, Z. Xiong, M. Feng, M. Wang, Y. Zhang, J. Xiang, and R. Ma (2012). Inhibition of the canonical Wnt pathway by Dickkopf-1 contributes to the neurodegeneration in 6-OHDA-lesioned rats. *Neurosci Lett* 525, 83-8.

[31] N. Dzamko, M. Deak, F. Hentati, A.D. Reith, A.R. Prescott, D.R. Alessi, and R.J. Nichols (2010). Inhibition of LRRK2 kinase activity leads to dephosphorylation of Ser(910)/Ser(935), disruption of 14-3-3 binding and altered cytoplasmic localization. *Biochem J* 430, 405-13.

[32] J.C. Dächsel, B. Behrouz, M. Yue, J.E. Beevers, H.L. Melrose, and M.J. Farrer (2010). A comparative study of Lrrk2 function in primary neuronal cultures. *Parkinsonism Relat Disord* 16, 650-5.

[33] G.G. Farías, J.A. Godoy, W. Cerpa, L. Varela-Nallar, and N.C. Inestrosa (2010). Wnt signaling modulates pre- and postsynaptic maturation: therapeutic considerations. *Dev Dyn* 239, 94-101.

[34] G.G. Farías, A.S. Vallés, M. Colombres, J.A. Godoy, E.M. Toledo, R.J. Lukas, F.J. Barrantes, and N.C. Inestrosa (2007). Wnt-7a induces presynaptic colocalization of alpha 7-nicotinic acetylcholine receptors and adenomatous polyposis coli in hippocampal neurons. *J Neurosci* 27, 5313-25.

[35] A. Ferree, M. Guillily, H. Li, K. Smith, A. Takashima, R. Squillace, M. Weigele, J.J. Collins, and B. Wolozin (2011). Regulation of Physiologic Actions of LRRK2: Focus on Autophagy. *Neurodegener Dis*.

[36] J.L. Freese, D. Pino, and S.J. Pleasure (2010). Wnt signaling in development and disease. *Neurobiol Dis* 38, 148-53.

[37] P.N. Gandhi, X. Wang, X. Zhu, S.G. Chen, and A.L. Wilson-Delfosse (2008). The Roc domain of leucine-rich repeat kinase 2 is sufficient for interaction with microtubules. *J Neurosci Res* 86, 1711-20.

[38] T. Gasser (2010). Identifying PD-causing genes and genetic susceptibility factors: current approaches and future prospects. *Prog Brain Res* 183, 3-20.

[39] F. Gillardon (2009a). Interaction of elongation factor 1-alpha with leucine-rich repeat kinase 2 impairs kinase activity and microtubule bundling in vitro. *Neuroscience* 163, 533-9.

[40] F. Gillardon (2009b). Leucine-rich repeat kinase 2 phosphorylates brain tubulin-beta isoforms and modulates microtubule stability--a point of convergence in parkinsonian neurodegeneration? *J Neurochem* 110, 1514-22.

[41] C.J. Gloeckner, N. Kinkl, A. Schumacher, R.J. Braun, E. O'Neill, T. Meitinger, W. Kolch, H. Prokisch, and M. Ueffing (2006). The Parkinson disease causing LRRK2 mutation I2020T is associated with increased kinase activity. *Hum Mol Genet* 15, 223-32.

[42] S. Gollamudi, A. Johri, N.Y. Calingasan, L. Yang, O. Elemento, and M.F. Beal (2012). Concordant signaling pathways produced by pesticide exposure in mice correspond to pathways identified in human Parkinson's disease. *PLoS One* 7, e36191.

[43] K. Gotthardt, M. Weyand, A. Kortholt, P.J. Van Haastert, and A. Wittinghofer (2008). Structure of the Roc-COR domain tandem of C. tepidum, a prokaryotic homologue of the human LRRK2 Parkinson kinase. *EMBO J* 27, 2352.

[44] E. Greggio, and M.R. Cookson (2009). Leucine-rich repeat kinase 2 mutations and Parkinson's disease: three questions. *ASN Neuro* 1.

[45] E. Greggio, S. Jain, A. Kingsbury, R. Bandopadhyay, P. Lewis, A. Kaganovich, M.P. van der Brug, A. Beilina, J. Blackinton, K.J. Thomas, R. Ahmad, D.W. Miller, S. Kesavapany, A. Singleton, A. Lees, R.J. Harvey, K. Harvey, and M.R. Cookson (2006). Kinase activity is required for the toxic effects of mutant LRRK2/dardarin. *Neurobiol Dis* 23, 329-41.

[46] E. Greggio, I. Zambrano, A. Kaganovich, A. Beilina, J.M. Taymans, V. Daniëls, P. Lewis, S. Jain, J. Ding, A. Syed, K.J. Thomas, V. Baekelandt, and M.R. Cookson (2008). The Parkinson disease-associated leucine-rich repeat kinase 2 (LRRK2) is a dimer that undergoes intramolecular autophosphorylation. *J Biol Chem* 283, 16906-14.

[47] P. Gómez-Suaga, B. Luzón-Toro, D. Churamani, L. Zhang, D. Bloor-Young, S. Patel, P.G. Woodman, G.C. Churchill, and S. Hilfiker (2012). Leucine-rich repeat kinase 2 regulates autophagy through a calcium-dependent pathway involving NAADP. *Hum Mol Genet* 21, 511-25.

[48] A.C. Hall, F.R. Lucas, and P.C. Salinas (2000). Axonal remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling. *Cell* 100, 525-35.

[49] S. Hassin-Baer, Y. Laitman, E. Azizi, I. Molchadski, G. Galore-Haskel, F. Barak, O.S. Cohen, and E. Friedman (2009). The leucine rich repeat kinase 2 (LRRK2) G2019S substitution mutation. Association with Parkinson disease, malignant melanoma and prevalence in ethnic groups in Israel. *J Neurol* 256, 483-7.

[50] T. Hatano, S. Kubo, S. Imai, M. Maeda, K. Ishikawa, Y. Mizuno, and N. Hattori (2007). Leucine-rich repeat kinase 2 associates with lipid rafts. *Hum Mol Genet* 16, 678-90.

[51] H.Y. Heo, K.S. Kim, and W. Seol (2010). Coordinate Regulation of Neurite Outgrowth by LRRK2 and Its Interactor, Rab5. *Exp Neurobiol* 19, 97-105.

[52] M.C. Herzig, C. Kolly, E. Persohn, D. Theil, T. Schweizer, T. Hafner, C. Stemmelen, T.J. Troxler, P. Schmid, S. Danner, C.R. Schnell, M. Mueller, B. Kinzel, A. Grevot, F. Bolognani, M. Stirn, R.R. Kuhn, K. Kaupmann, P.H. van der Putten, G. Rovelli, and D.R. Shimshek (2011). LRRK2 protein levels are determined by kinase function and are crucial for kidney and lung homeostasis in mice. *Hum Mol Genet* 20, 4209-23.

[53] K.M. Hinkle, M. Yue, B. Behrouz, J.C. Dächsel, S.J. Lincoln, E.E. Bowles, J.E. Beevers, B. Dugger, B. Winner, I. Prots, C.B. Kent, K. Nishioka, W.L. Lin, D.W. Dickson, C.J. Janus, M.J. Farrer, and H.L. Melrose (2012). LRRK2 knockout mice have an intact dopaminergic system but display alterations in exploratory and motor co-ordination behaviors. *Mol Neurodegener* 7, 25.

[54] C. Hooper, R. Killick, and S. Lovestone (2008). The GSK3 hypothesis of Alzheimer's disease. *J Neurochem* 104, 1433-9.

[55] K. Häbig, M. Walter, S. Poths, O. Riess, and M. Bonin (2008). RNA interference of LRRK2-microarray expression analysis of a Parkinson's disease key player. *Neurogenetics* 9, 83-94.

[56] C. Iaccarino, C. Crosio, C. Vitale, G. Sanna, M.T. Carrì, and P. Barone (2007). Apoptotic mechanisms in mutant LRRK2-mediated cell death. *Hum Mol Genet* 16, 1319-26.

[57] N.C. Inestrosa, and E. Arenas (2010). Emerging roles of Wnts in the adult nervous system. *Nat Rev Neurosci* 11, 77-86.

[58] N.C. Inestrosa, and E.M. Toledo (2008). The role of Wnt signaling in neuronal dysfunction in Alzheimer's Disease. *Mol Neurodegener* 3, 9.

[59] M. Jaleel, R.J. Nichols, M. Deak, D.G. Campbell, F. Gillardon, A. Knebel, and D.R. Alessi (2007). LRRK2 phosphorylates moesin at threonine-558: characterization of how Parkinson's disease mutants affect kinase activity. *Biochem J* 405, 307-17.

[60] J. Jankovic, S. Chen, and W.D. Le (2005). The role of Nurr1 in the development of dopaminergic neurons and Parkinson's disease. *Prog Neurobiol* 77, 128-38.

[61] B. Jasinska-Myga, J. Kachergus, C. Vilariño-Güell, C. Wider, A.I. Soto-Ortolaza, M. Kefi, L.T. Middleton, L. Ishihara-Paul, R.A. Gibson, R. Amouri, S.B. Yahmed, S.B. Sassi, M. Zouari, G. El Euch, O.A. Ross, F. Hentati, and M.J. Farrer (2010). Comprehensive sequencing of the LRRK2 gene in patients with familial Parkinson's disease from North Africa. *Mov Disord* 25, 2052-8.

[62] N.D. Jorgensen, Y. Peng, C.C. Ho, H.J. Rideout, D. Petrey, P. Liu, and W.T. Dauer (2009). The WD40 domain is required for LRRK2 neurotoxicity. *PLoS One* 4, e8463.

[63] K. Kalinderi, L. Fidani, Z. Katsarou, J. Clarimón, S. Bostantjopoulou, and A. Kotsis (2011). GSK3β polymorphisms, MAPT H1 haplotype and Parkinson's disease in a Greek cohort. *Neurobiol Aging* 32, 546.e1-5.

[64] F. Kawakami, T. Yabata, E. Ohta, T. Maekawa, N. Shimada, M. Suzuki, H. Maruyama, T. Ichikawa, and F. Obata (2012). LRRK2 phosphorylates tubulin-associated tau but not the free molecule: LRRK2-mediated regulation of the tau-tubulin association and neurite outgrowth. *PLoS One* 7, e30834.

[65] L.R. Kett, D. Boassa, C.C. Ho, H.J. Rideout, J. Hu, M. Terada, M. Ellisman, and W.T. Dauer (2012). LRRK2 Parkinson disease mutations enhance its microtubule association. *Hum Mol Genet* 21, 890-9.

[66] N.L. Khan, S. Jain, J.M. Lynch, N. Pavese, P. Abou-Sleiman, J.L. Holton, D.G. Healy, W.P. Gilks, M.G. Sweeney, M. Ganguly, V. Gibbons, S. Gandhi, J. Vaughan, L.H. Eunson, R. Katzenschlager, J. Gayton, G. Lennox, T. Revesz, D. Nicholl, K.P. Bhatia, N. Quinn, D. Brooks,

A.J. Lees, M.B. Davis, P. Piccini, A.B. Singleton, and N.W. Wood (2005). Mutations in the gene LRRK2 encoding dardarin (PARK8) cause familial Parkinson's disease: clinical, pathological, olfactory and functional imaging and genetic data. *Brain* 128, 2786-96.

[67] Y.T. Kim, E.M. Hur, W.D. Snider, and F.Q. Zhou (2011). Role of GSK3 Signaling in Neuronal Morphogenesis. *Front Mol Neurosci* 4, 48.

[68] H. Kitagawa, W.J. Ray, H. Glantschnig, P.V. Nantermet, Y. Yu, C.T. Leu, S. Harada, S. Kato, and L.P. Freedman (2007). A regulatory circuit mediating convergence between Nurr1 transcriptional regulation and Wnt signaling. *Mol Cell Biol* 27, 7486-96.

[69] G. Konopka, T. Friedrich, J. Davis-Turak, K. Winden, M.C. Oldham, F. Gao, L. Chen, G.Z. Wang, R. Luo, T.M. Preuss, and D.H. Geschwind (2012). Human-specific transcriptional networks in the brain. *Neuron* 75, 601-17.

[70] U. Kumari, and E.K. Tan (2009). LRRK2 in Parkinson's disease: genetic and clinical studies from patients. *FEBS J* 276, 6455-63.

[71] J.B. Kwok, M. Hallupp, C.T. Loy, D.K. Chan, J. Woo, G.D. Mellick, D.D. Buchanan, P.A. Silburn, G.M. Halliday, and P.R. Schofield (2005). GSK3B polymorphisms alter transcription and splicing in Parkinson's disease. *Ann Neurol* 58, 829-39.

[72] F. L'Episcopo, C. Tirolo, N. Testa, S. Caniglia, M.C. Morale, C. Cossetti, P. D'Adamo, E. Zardini, L. Andreoni, A.E. Ihekwaba, P.A. Serra, D. Franciotta, G. Martino, S. Pluchino, and B. Marchetti (2011). Reactive astrocytes and Wnt/β-catenin signaling link nigrostriatal injury to repair in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. *Neurobiol Dis* 41, 508-27.

[73] R. Le Borgne (2006). Regulation of Notch signalling by endocytosis and endosomal sorting. *Curr Opin Cell Biol* 18, 213-22.

[74] S. Lesage, P. Ibanez, E. Lohmann, P. Pollak, F. Tison, M. Tazir, A.L. Leutenegger, J. Guimaraes, A.M. Bonnet, Y. Agid, A. Dürr, A. Brice, and F.P.s.D.G.S. Group (2005). G2019S LRRK2 mutation in French and North African families with Parkinson's disease. *Ann Neurol* 58, 784-7.

[75] P.A. Lewis, and C. Manzoni (2012). LRRK2 and human disease: a complicated question or a question of complexes? *Sci Signal* 5, pe2.

[76] Y. Li, W. Liu, T.F. Oo, L. Wang, Y. Tang, V. Jackson-Lewis, C. Zhou, K. Geghman, M. Bogdanov, S. Przedborski, M.F. Beal, R.E. Burke, and C. Li (2009). Mutant LRRK2(R1441G) BAC transgenic mice recapitulate cardinal features of Parkinson's disease. *Nat Neurosci* 12, 826-8.

[77] D.C. Lie, S.A. Colamarino, H.J. Song, L. Désiré, H. Mira, A. Consiglio, E.S. Lein, S. Jessberger, H. Lansford, A.R. Dearie, and F.H. Gage (2005). Wnt signalling regulates adult hippocampal neurogenesis. *Nature* 437, 1370-5.

[78] C.H. Lin, P.I. Tsai, R.M. Wu, and C.T. Chien (2010). LRRK2 G2019S mutation induces dendrite degeneration through mislocalization and phosphorylation of tau by recruiting autoactivated GSK3ß. *J Neurosci* 30, 13138-49.

[79] S.Y. Lin, T.Y. Li, Q. Liu, C. Zhang, X. Li, Y. Chen, S.M. Zhang, G. Lian, K. Ruan, Z. Wang, C.S. Zhang, K.Y. Chien, J. Wu, Q. Li, J. Han, and S.C. Lin (2012). GSK3-TIP60-ULK1 signaling pathway links growth factor deprivation to autophagy. *Science* 336, 477-81.

[80] G.H. Liu, J. Qu, K. Suzuki, E. Nivet, M. Li, N. Montserrat, F. Yi, X. Xu, S. Ruiz, W. Zhang, U. Wagner, A. Kim, B. Ren, Y. Li, A. Goebl, J. Kim, R.D. Soligalla, I. Dubova, J. Thompson, J. Yates, C.R. Esteban, I. Sancho-Martinez, and J.C. Izpisua Belmonte (2012). Progressive degeneration of human neural stem cells caused by pathogenic LRRK2. *Nature* 491, 603-7.

[81] J. Liu, X. Wu, B. Mitchell, C. Kintner, S. Ding, and P.G. Schultz (2005). A small-molecule agonist of the Wnt signaling pathway. *Angew Chem Int Ed Engl* 44, 1987-90.

[82] F.R. Lucas, R.G. Goold, P.R. Gordon-Weeks, and P.C. Salinas (1998). Inhibition of GSK-3beta leading to the loss of phosphorylated MAP-1B is an early event in axonal remodelling induced by WNT-7a or lithium. *J Cell Sci* 111 (Pt 10), 1351-61.

[83] F.R. Lucas, and P.C. Salinas (1997). WNT-7a induces axonal remodeling and increases synapsin I levels in cerebellar neurons. *Dev Biol* 192, 31-44.

[84] D. MacLeod, J. Dowman, R. Hammond, T. Leete, K. Inoue, and A. Abeliovich (2006). The familial Parkinsonism gene LRRK2 regulates neurite process morphology. *Neuron* 52, 587-93.

[85] T. Maekawa, S. Mori, Y. Sasaki, T. Miyajima, S. Azuma, E. Ohta, and F. Obata (2012). The I2020T Leucine-rich repeat kinase 2 transgenic mouse exhibits impaired locomotive ability accompanied by dopaminergic neuron abnormalities. *Mol Neurodegener* 7, 15.

[86] P. Mangeat, C. Roy, and M. Martin (1999). ERM proteins in cell adhesion and membrane dynamics. *Trends Cell Biol* 9, 187-92.

[87] F. Marxreiter, M. Regensburger, and J. Winkler (2012). Adult neurogenesis in Parkinson's disease. *Cell Mol Life Sci*.

[88] I. Marín, W.N. van Egmond, and P.J. van Haastert (2008). The Roco protein family: a functional perspective. *FASEB J* 22, 3103-10.

[89] S. Matta, K. Van Kolen, R. da Cunha, G. van den Bogaart, W. Mandemakers, K. Miskiewicz, P.J. De Bock, V.A. Morais, S. Vilain, D. Haddad, L. Delbroek, J. Swerts, L. Chávez-Gutiérrez, G. Esposito, G. Daneels, E. Karran, M. Holt, K. Gevaert, D.W. Moechars, B. De Strooper, and P. Verstreken (2012). LRRK2 controls an EndoA phosphorylation cycle in synaptic endocytosis. *Neuron* 75, 1008-21.

[90] A. Meixner, K. Boldt, M. Van Troys, M. Askenazi, C.J. Gloeckner, M. Bauer, J.A. Marto, C. Ampe, N. Kinkl, and M. Ueffing (2011). A QUICK screen for Lrrk2 interaction partners--leucine-rich repeat kinase 2 is involved in actin cytoskeleton dynamics. *Mol Cell Proteomics* 10, M110.001172.

[91] H.L. Melrose, J.C. Dächsel, B. Behrouz, S.J. Lincoln, M. Yue, K.M. Hinkle, C.B. Kent, E. Korvatska, J.P. Taylor, L. Witten, Y.Q. Liang, J.E. Beevers, M. Boules, B.N. Dugger, V.A. Serna, A. Gaukhman, X. Yu, M. Castanedes-Casey, A.T. Braithwaite, S. Ogholikhan, N. Yu, D. Bass, G. Tyndall, G.D. Schellenberg, D.W. Dickson, C. Janus, and M.J. Farrer (2010). Impaired dopaminergic neurotransmission and microtubule-associated protein tau alterations in human LRRK2 transgenic mice. *Neurobiol Dis* 40, 503-17.

[92] H.L. Melrose, C.B. Kent, J.P. Taylor, J.C. Dachsel, K.M. Hinkle, S.J. Lincoln, S.S. Mok, J.G. Culvenor, C.L. Masters, G.M. Tyndall, D.I. Bass, Z. Ahmed, C.A. Andorfer, O.A. Ross, Z.K. Wszolek, A. Delldonne, D.W. Dickson, and M.J. Farrer (2007). A comparative analysis of leucine-rich repeat kinase 2 (Lrrk2) expression in mouse brain and Lewy body disease. *Neuroscience* 147, 1047-58.

[93] J. Milosevic, S.C. Schwarz, V. Ogunlade, A.K. Meyer, A. Storch, and J. Schwarz (2009). Emerging role of LRRK2 in human neural progenitor cell cycle progression, survival and differentiation. *Mol Neurodegener* 4, 25.

[94] R.J. Nichols, N. Dzamko, J.E. Hutti, L.C. Cantley, M. Deak, J. Moran, P. Bamborough, A.D. Reith, and D.R. Alessi (2009). Substrate specificity and inhibitors of LRRK2, a protein kinase mutated in Parkinson's disease. *Biochem J* 424, 47-60.

[95] G. Paglini, P. Kunda, S. Quiroga, K. Kosik, and A. Cáceres (1998). Suppression of radixin and moesin alters growth cone morphology, motility, and process formation in primary cultured neurons. *J Cell Biol* 143, 443-55.

[96] C. Paisán-Ruíz, S. Jain, E.W. Evans, W.P. Gilks, J. Simón, M. van der Brug, A. López de Munain, S. Aparicio, A.M. Gil, N. Khan, J. Johnson, J.R. Martinez, D. Nicholl, I.M. Carrera, A.S. Pena, R. de Silva, A. Lees, J.F. Martí-Massó, J. Pérez-Tur, N.W. Wood, and A.B. Singleton (2004). Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron* 44, 595-600.

[97] K.C. Pang, M.C. Frith, and J.S. Mattick (2006). Rapid evolution of noncoding RNAs: lack of conservation does not mean lack of function. *Trends Genet* 22, 1-5.

[98] C.L. Parish, G. Castelo-Branco, N. Rawal, J. Tonnesen, A.T. Sorensen, C. Salto, M. Kokaia, O. Lindvall, and E. Arenas (2008). Wnt5a-treated midbrain neural stem cells improve dopamine cell replacement therapy in parkinsonian mice. *J Clin Invest* 118, 149-60.

[99] L. Parisiadou, C. Xie, H.J. Cho, X. Lin, X.L. Gu, C.X. Long, E. Lobbestael, V. Baekelandt, J.M. Taymans, L. Sun, and H. Cai (2009). Phosphorylation of ezrin/radixin/moesin proteins by LRRK2 promotes the rearrangement of actin cytoskeleton in neuronal morphogenesis. *J Neurosci* 29, 13971-80.

[100] C. Parr, R. Carzaniga, S.M. Gentleman, F. Van Leuven, J. Walter, and M. Sastre (2012). Glycogen synthase kinase 3 inhibition promotes lysosomal biogenesis and autophagic degradation of the amyloid-β precursor protein. *Mol Cell Biol* 32, 4410-8.

[101] M. Paus, Z. Kohl, N.M. Ben Abdallah, D. Galter, F. Gillardon, and J. Winkler (2012). Enhanced dendritogenesis and axogenesis in hippocampal neuroblasts of LRRK2 knockout mice. *Brain Res*.

[102] E. Perlson, S. Maday, M.M. Fu, A.J. Moughamian, and E.L. Holzbaur (2010). Retrograde axonal transport: pathways to cell death? *Trends Neurosci* 33, 335-44.

[103] G. Piccoli, S.B. Condliffe, M. Bauer, F. Giesert, K. Boldt, S. De Astis, A. Meixner, H. Sarioglu, D.M. Vogt-Weisenhorn, W. Wurst, C.J. Gloeckner, M. Matteoli, C. Sala, and M. Ueffing (2011). LRRK2 controls synaptic vesicle storage and mobilization within the recycling pool. *J Neurosci* 31, 2225-37.

[104] E.D. Plowey, S.J. Cherra, Y.J. Liu, and C.T. Chu (2008). Role of autophagy in G2019S-LRRK2-associated neurite shortening in differentiated SH-SY5Y cells. *J Neurochem* 105, 1048-56.

[105] S.A. Purro, L. Ciani, M. Hoyos-Flight, E. Stamatakou, E. Siomou, and P.C. Salinas (2008). Wnt regulates axon behavior through changes in microtubule growth directionality: a new role for adenomatous polyposis coli. *J Neurosci* 28, 8644-54.

[106] A. Rajput, D.W. Dickson, C.A. Robinson, O.A. Ross, J.C. Dächsel, S.J. Lincoln, S.A. Cobb, M.L. Rajput, and M.J. Farrer (2006). Parkinsonism, Lrrk2 G2019S, and tau neuropathology. *Neurology* 67, 1506-8.

[107] D. Ramonet, J.P. Daher, B.M. Lin, K. Stafa, J. Kim, R. Banerjee, M. Westerlund, O. Pletnikova, L. Glauser, L. Yang, Y. Liu, D.A. Swing, M.F. Beal, J.C. Troncoso, J.M. McCaffery, N.A. Jenkins, N.G. Copeland, D. Galter, B. Thomas, M.K. Lee, T.M. Dawson, V.L. Dawson, and D.J. Moore (2011). Dopaminergic neuronal loss, reduced neurite complexity and autophagic abnormalities in transgenic mice expressing G2019S mutant LRRK2. *PLoS One* 6, e18568.

[108] N. Rawal, O. Corti, P. Sacchetti, H. Ardilla-Osorio, B. Sehat, A. Brice, and E. Arenas (2009). Parkin protects dopaminergic neurons from excessive Wnt/beta-catenin signaling. *Biochem Biophys Res Commun* 388, 473-8.

[109] S. Ray, and M. Liu (2012). Current understanding of LRRK2 in Parkinson's disease: biochemical and structural features and inhibitor design. *Future Med Chem* 4, 1701-13.

[110] I.N. Rudenko, A. Kaganovich, D.N. Hauser, A. Beylina, R. Chia, J. Ding, D. Maric, H. Jaffe, and M.R. Cookson (2012). The G2385R variant of leucine-rich repeat kinase 2 associated with Parkinson's disease is a partial loss-of-function mutation. *Biochem J* 446, 99-111.

[111] P.C. Salinas (2007). Modulation of the microtubule cytoskeleton: a role for a divergent canonical Wnt pathway. *Trends Cell Biol* 17, 333-42.

[112] R.M. Sancho, B.M. Law, and K. Harvey (2009). Mutations in the LRRK2 Roc-COR tandem domain link Parkinson's disease to Wnt signalling pathways. *Hum Mol Genet* 18, 3955-68.

[113] Z. Sheng, S. Zhang, D. Bustos, T. Kleinheinz, C.E. Le Pichon, S.L. Dominguez, H.O. Solanoy, J. Drummond, X. Zhang, X. Ding, F. Cai, Q. Song, X. Li, Z. Yue, M.P. van der Brug, D.J. Burdick, J. Gunzner-Toste, H. Chen, X. Liu, A.A. Estrada, Z.K. Sweeney, K. Scearce-Levie, J.G. Moffat, D.S. Kirkpatrick, and H. Zhu (2012). Ser1292 Autophosphorylation Is an Indicator of LRRK2 Kinase Activity and Contributes to the Cellular Effects of PD Mutations. *Sci Transl Med* 4, 164ra161.

[114] N. Shin, H. Jeong, J. Kwon, H.Y. Heo, J.J. Kwon, H.J. Yun, C.H. Kim, B.S. Han, Y. Tong, J. Shen, T. Hatano, N. Hattori, K.S. Kim, S. Chang, and W. Seol (2008). LRRK2 regulates synaptic vesicle endocytosis. *Exp Cell Res* 314, 2055-65.

[115] W.W. Smith, Z. Pei, H. Jiang, V.L. Dawson, T.M. Dawson, and C.A. Ross (2006). Kinase activity of mutant LRRK2 mediates neuronal toxicity. *Nat Neurosci* 9, 1231-3.

[116] K. Stafa, A. Trancikova, P.J. Webber, L. Glauser, A.B. West, and D.J. Moore (2012). GTPase activity and neuronal toxicity of Parkinson's disease-associated LRRK2 is regulated by ArfGAP1. *PLoS Genet* 8, e1002526.

[117] J.M. Taymans, and M.R. Cookson (2010). Mechanisms in dominant parkinsonism: The toxic triangle of LRRK2, alpha-synuclein, and tau. *Bioessays* 32, 227-35.

[118] K.R. Thomas, and M.R. Capecchi (1990). Targeted disruption of the murine int-1 proto-oncogene resulting in severe abnormalities in midbrain and cerebellar development. *Nature* 346, 847-50.

[119] Y. Tong, E. Giaime, H. Yamaguchi, T. Ichimura, Y. Liu, H. Si, H. Cai, J.V. Bonventre, and J. Shen (2012). Loss of leucine-rich repeat kinase 2 causes age-dependent bi-phasic alterations of the autophagy pathway. *Mol Neurodegener* 7, 2.

[120] Y. Tong, H. Yamaguchi, E. Giaime, S. Boyle, R. Kopan, R.J. Kelleher, and J. Shen (2010). Loss of leucine-rich repeat kinase 2 causes impairment of protein degradation pathways, accumulation of alpha-synuclein, and apoptotic cell death in aged mice. *Proc Natl Acad Sci U S A* 107, 9879-84.

[121] S. Ujiie, T. Hatano, S. Kubo, S. Imai, S. Sato, T. Uchihara, S. Yagishita, K. Hasegawa, H. Kowa, F. Sakai, and N. Hattori (2012). LRRK2 I2020T mutation is associated with tau pathology. *Parkinsonism Relat Disord* 18, 819-23.

[122] J. Van Limbergen, D.C. Wilson, and J. Satsangi (2009). The genetics of Crohn's disease. *Annu Rev Genomics Hum Genet* 10, 89-116.

[123] M.T. Veeman, D.C. Slusarski, A. Kaykas, S.H. Louie, and R.T. Moon (2003). Zebrafish prickle, a modulator of noncanonical Wnt/Fz signaling, regulates gastrulation movements. *Curr Biol* 13, 680-5.

[124] T. Walston, C. Tuskey, L. Edgar, N. Hawkins, G. Ellis, B. Bowerman, W. Wood, and J. Hardin (2004). Multiple Wnt signaling pathways converge to orient the mitotic spindle in early C. elegans embryos. *Dev Cell* 7, 831-41.

[125] S. Wang, Z.Z. Chong, Y.C. Shang, and K. Maiese (2012). WISP1 (CCN4) autoregulates its expression and nuclear trafficking of β-catenin during oxidant stress with limited effects upon neuronal autophagy. *Curr Neurovasc Res* 9, 91-101.

[126] T. Wang, Z. Ming, W. Xiaochun, and W. Hong (2011). Rab7: role of its protein interaction cascades in endo-lysosomal traffic. *Cell Signal* 23, 516-21.

[127] A.B. West, D.J. Moore, C. Choi, S.A. Andrabi, X. Li, D. Dikeman, S. Biskup, Z. Zhang, K.L. Lim, V.L. Dawson, and T.M. Dawson (2007). Parkinson's disease-associated mutations in LRRK2 link enhanced GTP-binding and kinase activities to neuronal toxicity. *Hum Mol Genet* 16, 223-32.

[128] C. Wider, C. Vilariño-Güell, M.G. Heckman, B. Jasinska-Myga, A.I. Ortolaza-Soto, N.N. Diehl, J.E. Crook, S.A. Cobb, J.A. Bacon, J.O. Aasly, J.M. Gibson, T. Lynch, R.J. Uitti, Z.K. Wszolek, M.J. Farrer, and O.A. Ross (2011). SNCA, MAPT, and GSK3B in Parkinson disease: a gene-gene interaction study. *Eur J Neurol* 18, 876-81.

[129] B. Winckler, and C.C. Yap (2011). Endocytosis and endosomes at the crossroads of regulating trafficking of axon outgrowth-modifying receptors. *Traffic* 12, 1099-108.

[130] B. Winner, H.L. Melrose, C. Zhao, K.M. Hinkle, M. Yue, C. Kent, A.T. Braithwaite, S. Ogholikhan, R. Aigner, J. Winkler, M.J. Farrer, and F.H. Gage (2011). Adult neurogenesis and neurite outgrowth are impaired in LRRK2 G2019S mice. *Neurobiol Dis* 41, 706-16.

[131] Y. Xiong, C.E. Coombes, A. Kilaru, X. Li, A.D. Gitler, W.J. Bowers, V.L. Dawson, T.M. Dawson, and D.J. Moore (2010). GTPase activity plays a key role in the pathobiology of LRRK2. *PLoS Genet* 6, e1000902.

[132] S. Zechel, A. Meinhardt, K. Unsicker, and O. von Bohlen Und Halbach (2010). Expression of leucine-rich-repeat-kinase 2 (LRRK2) during embryonic development. *Int J Dev Neurosci* 28, 391-9.

[133] F.R. Zhang, W. Huang, S.M. Chen, L.D. Sun, H. Liu, Y. Li, Y. Cui, X.X. Yan, H.T. Yang, R.D. Yang, T.S. Chu, C. Zhang, L. Zhang, J.W. Han, G.Q. Yu, C. Quan, Y.X. Yu, Z. Zhang, B.Q. Shi, L.H. Zhang, H. Cheng, C.Y. Wang, Y. Lin, H.F. Zheng, X.A. Fu, X.B. Zuo, Q. Wang, H. Long, Y.P. Sun, Y.L. Cheng, H.Q. Tian, F.S. Zhou, H.X. Liu, W.S. Lu, S.M. He, W.L. Du, M. Shen, Q.Y. Jin, Y. Wang, H.Q. Low, T. Erwin, N.H. Yang, J.Y. Li, X. Zhao, Y.L. Jiao, L.G. Mao, G. Yin, Z.X. Jiang, X.D. Wang, J.P. Yu, Z.H. Hu, C.H. Gong, Y.Q. Liu, R.Y. Liu, D.M. Wang, D. Wei, J.X. Liu, W.K. Cao, H.Z. Cao, Y.P. Li, W.G. Yan, S.Y. Wei, K.J. Wang, M.L. Hibberd, S. Yang, X.J. Zhang, and J.J. Liu (2009). Genomewide association study of leprosy. *N Engl J Med* 361, 2609-18.

[134] F.Q. Zhou, J. Zhou, S. Dedhar, Y.H. Wu, and W.D. Snider (2004). NGF-induced axon growth is mediated by localized inactivation of GSK-3beta and functions of the microtubule plus end binding protein APC. *Neuron* 42, 897-912.

[135] A. Zimprich, S. Biskup, P. Leitner, P. Lichtner, M. Farrer, S. Lincoln, J. Kachergus, M. Hulihan, R.J. Uitti, D.B. Calne, A.J. Stoessl, R.F. Pfeiffer, N. Patenge, I.C. Carbajal, P. Vieregge, F. Asmus, B. Müller-Myhsok, D.W. Dickson, T. Meitinger, T.M. Strom, Z.K. Wszolek, and T. Gasser (2004). Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron* 44, 601-7.

Figure Legends

Figure 1. Domain Structure of LRRK2. Domains are color-coded according to function: those implicated in protein-protein interaction are depicted in blue; domains involved in GTPase function are green; and the kinase domain red. Here, LRRK2 is depicted as a dimer, although LRRK2 also exists as monomers and in higher molecular weight complexes (Greggio *et al.*, 2008). Dimerization is likely to be mediated by the COR and/or WD40 domains (double-headed arrows). COR domains are established as dimerization devices in ROCO proteins (Gotthardt *et al.*, 2008), whilst ablation of the WD40 domain has been reported to disrupt LRRK2 dimerization (Jorgensen *et al.*, 2009). The location of pathological mutations proven to segregate with Parkinson's disease are shown with asterisks and bold font. Although only considered a risk factor, the G2385R mutation is also depicted with a plus sign, since this mutation is mentioned in the main text and is very frequent amongst Asian populations. Abbreviations: ARM, armadillo repeat; ANK, ankyrin repeat; LRR, leucine-rich repeat; Roc, ras of complex proteins; COR, c-terminal of Roc; Kin, kinase.

Figure 2. LRRK2 Regulates Cell Biological Functions Important for Neurogenesis. Several lines of evidence support roles for LRRK2 in microtubule function, endocytosis/vesicle trafficking and autophagy. LRRK2 is likely to impact upon neurite outgrowth and the latter stages of neurogenesis through direct association with membrane structures and microtubules and/or regulation of signaling pathways. In addition, LRRK2 has been implicated in proliferation and may therefore also govern early stages of neurogenesis. The interaction between microtubule function, vesicle trafficking, autophagy and proliferation and the canonical Wnt pathway is depicted but other pathways are likely to play additional roles.

Figure 3. Overview of Wnt Signaling Pathways. The three major branches of Wnt signaling – the canonical, planar cell polarity (PCP), and Wnt-Ca^{2+} – pathways are illustrated. Note that in growing neurites a further branch has been reported, the so-called divergent canonical pathway, which impacts upon microtubule stability. Abbreviations: APC, adenomatous polyposis coli; CaN, calcineurin; CK1, casein kinase 1; DAAM, dishevelled-associated activator of morphogenesis; DVL, dishevelled; FzR,

frizzled receptor; GSK3, glycogen synthase kinase 3; JNK, c-Jun n-terminal kinase; LRP5/6, low-density lipoprotein receptor-related protein 5/6; NFAT, nuclear factor of activated T-cells; PKC, protein kinase C; PLC, phospholipase C; ROCK, Rho-associated protein kinase.
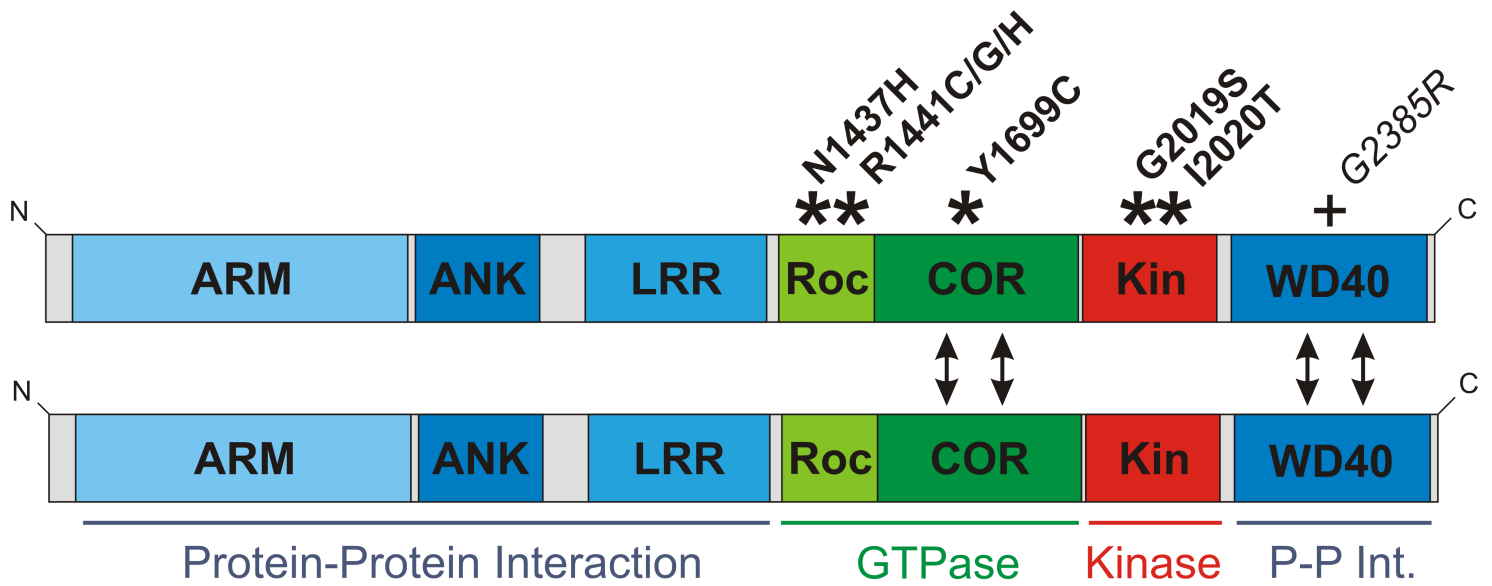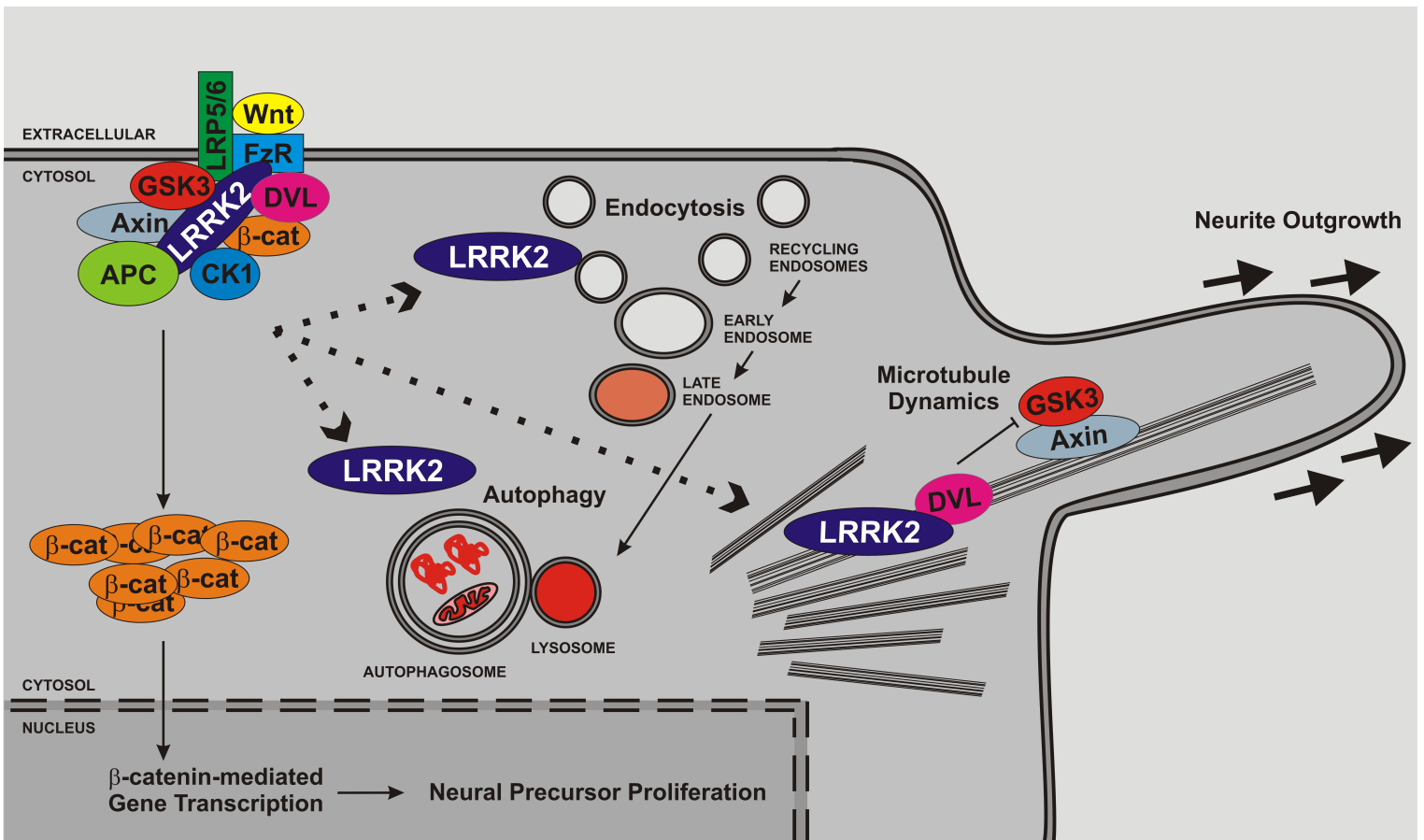
Figure 1.TIF

LRRK2 Domain Structure

Figure 2.TIF

Figure 3.TIF