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Synaptic Protein Alterations in Parkinson's Disease

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Abstract Alterations occur within distal neuronal compartments, including axons and synapses, during the course of neurodegenerative diseases such as Parkinson's disease (PD). These changes could hold important implications for the functioning of neural networks, especially since research studies have shown a loss of dendritic spines locating to medium spiny projection neurons and impaired axonal transport in PD-affected brains. However, despite ever-increasing awareness of the vulnerability of synapses and axons, inadequate understanding of the independent mechanisms regulating non-somatic neurodegeneration prevails. This has resulted in limited therapeutic strategies capable of targeting these distinct cellular compartments. Deregulated protein synthesis, folding and degrading proteins, and protein quality-control systems have repeatedly been linked with morphological and functional alterations of synapses in the PD-affected brains. Here, we review current understanding concerning the proteins involved in structural and functional changes that affect synaptic contact-points in PD. The collection of studies discussed emphasizes the need for developing therapeutics aimed at deregulated protein synthesis and degradation pathways operating at axonal and dendritic synapses for preserving "normal" circuitry and function, for as long as possible.

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Abbreviations

ACh	Acetylcholine
SNCA [gene]	α-Synuclein
αSYN [protein]	α-Synuclein
BACE1	β-Site amyloid precursor cleaving enzyme
ßSYN [protein]	ß-Synuclein
AD	Alzheimer's disease
Aß	Amyloid-ß
APP	Amyloid precursor protein
CSPα	Cysteine-string protein α
DLBD	Diffuse Lewy body disease
DA	Dopamine
EF1A	Elongation factor 1-alpha
FRET	Förster resonance energy transfer
GST	Glutathione *S*-transferase
Hsp	Heat shock protein
LBs	Lewy bodies
LRRK2	Leucine-rich repeat kinase 2
LRP1	Lipoprotein receptor-related protein 1
LC-MS/MS	Liquid chromatography tandem mass spectrometry
MEF2	Myocyte enhancer factor 2
MSNs	Medium spiny neurones
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NSF	*N*-ethylmaleimide sensitive factor
PINK1	Phosphatase and tensin homologue-induced kinase1
PolyQ	Polyglutamine
PKC	Putative protein kinase C

5-HT	Serotonin
6-OHDA	6-Hydroxydopamine
PrP	Prion protein
5-HT	Serotonin
SN	Substantia nigra
UCH-L1	Ubiquitin carboxyl terminal hydrolase-L1

Introduction

Synapses form the basic currency of information exchange between neurons and also underlie the basic physiological function of neuronal circuits. Electron microscopic images have revealed the principal structural components that comprise the synapse (Fig. 1). These include the presynaptic bouton that contains the synthetic machinery for synthesizing neurotransmitters, the synaptic vesicles for neurotransmitter storage and release via the presynaptic membrane into the synaptic cleft, whilst the postsynaptic membrane contains the target receptors which activate upon specific binding to a neurotransmitter protein. In the majority of neurotransmitter systems, the presynaptic bouton contains sophisticated reuptake mechanisms and enzymes, for removing excess neurotransmitter particles from the cleft, thus terminating the neural response. The system relies heavily on mitochondria for generating the necessary cellular energy to drive processes and on intact protein synthetic/metabolic machinery for maintaining functional integrity.

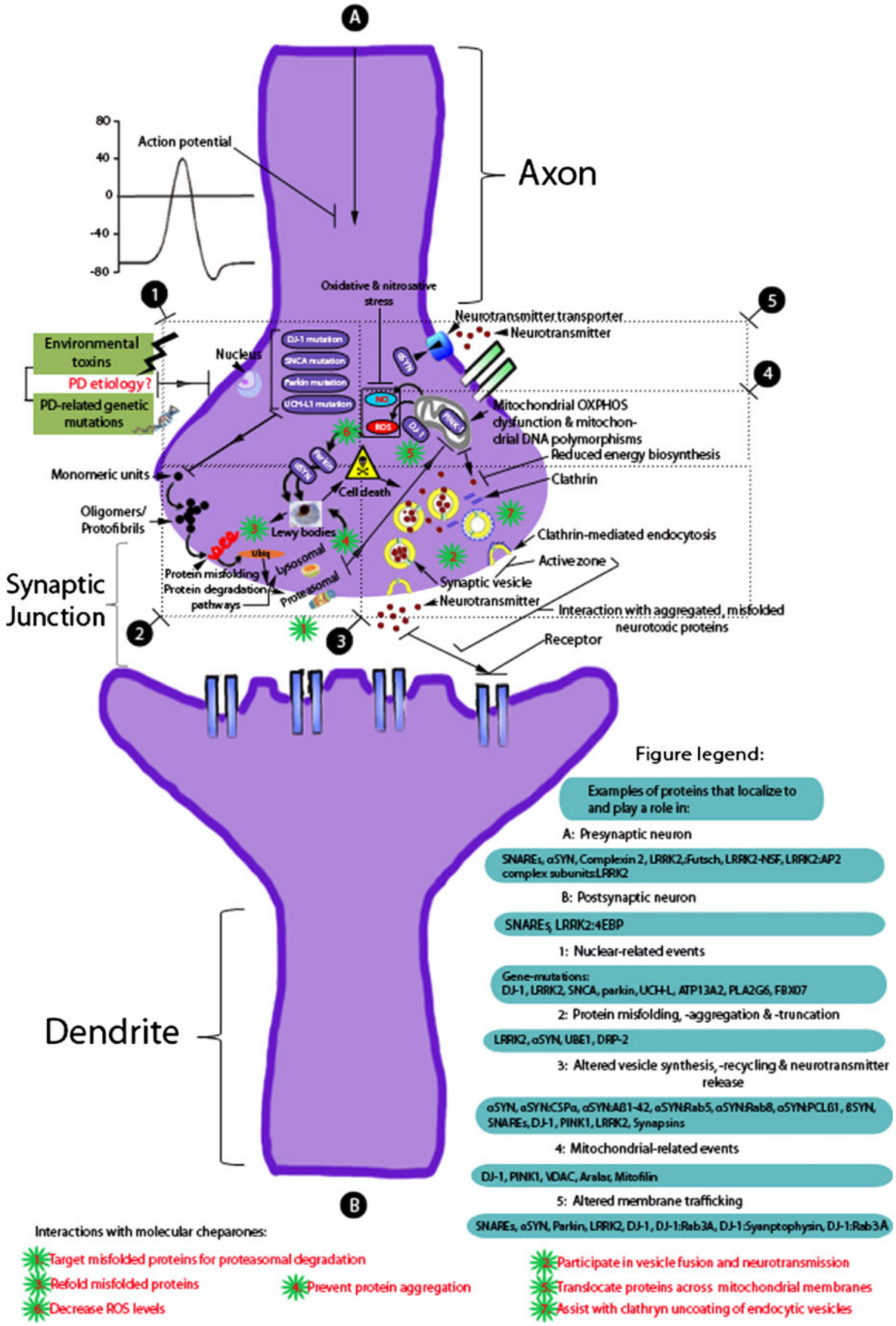
In considering the vast complexity of processes and components that comprise correct synaptic function and assembly, it is reasonable to assume that pathologies affecting synapses and resulting in their eventual failure may manifest as clinically recognizable disease. Insights into how neurodegenerative processes in disease impact on synaptic integrity are key for developing therapies to halt neurodegenerative processes in diseases, such as Alzheimer's disease (AD), Huntington's chorea and Parkinson's disease (PD).

Synaptic Protein Aberrations in Neurodegenerative Disease

Recent studies highlighted the important role that non-somatic neuronal compartments might play in the onset and progression of neurodegeneration [1, 2]. This includes synaptic dysfunction that may result from deregulated protein synthesis and protein–protein interactions, operating at all steps that comprise communication involving extracellular signalling. In essence, these involve six steps: (1) synthesis and (2) release of the signalling molecule by the signalling cell, (3) transport of the signal to the target cell, (4) detection of the signal by a specific receptor protein within the postsynaptic membrane, (5) a change in cellular metabolism, function or development triggered by the receptor-signal complex, and (6) removal of the signal, which often terminates the cellular response. For these, the presynaptic machinery regulates the synthesis, packaging and secretion of transmitter, whilst the postsynaptic apparatus detects the transmitter and propagates the signal. These processes are depicted in Fig. 1, whilst also providing a reference to the PD-linked genes identified thus far which, when mutated, could hold important implications for synaptic-related events. The accompanying figure provides an illustrated overview of how PD-related pathology (i.e. genetic mutations, known and unknown environmentally derived toxin exposure) could impact on the normal synaptic machinery, comprising of a presynaptic neuron with its various synaptic elements (i.e. synaptic vesicles for neurotransmitter storage, release and recycling, mitochondria for generating the processes underlying normal synapse function and cellular processes for handling misformed and aggregated proteins). Various proteins linked to PD pathology are indicated on the right-side legend. However, since compilation of the figure was based on current research as to such protein's role in normal synaptic physiology and when affected by PD, we can only place their gross neuronal (pre- vs. postsynaptic) and microscopic location (i.e. mitochondrial, cytosolic, presynaptic membrane) according to recent research results, as discussed in this review. The green stars indicate chaperone activity, which have been implicated in various PD-related pathological events. These provide a rationale for improved understanding of the role of synaptic proteins in PD, since information on such processes and the integral protein role players involved provides a feasible means for designing therapeutic strategies for improving PD symptoms.

AD pathology is characterized by substantial synaptic loss, demonstrated by immunohistochemical synaptic markers that label synaptophysin to stain for presynaptic vesicles. This synaptic loss was revealed to provide the best indicator for cognitive impairment, including dementia in AD cases [3, 4]. Although the molecular mechanism underlying this phenomenon remains to be better explained by future investigations, current evidence suggests that soluble β amyloid protein (Aβ), which can exist as oligomers or aggregate into amyloid plaques, is involved in the synaptic loss/deficits. This includes a study describing that in AD brains, insoluble Aβ peptides reduce levels of putative protein kinase C (PKC), regarded as important for synaptogenesis and neurotransmitter release [5], whilst also inhibiting its activation [6, 7].

Amyloid precursor protein (APP), from which Aβ peptides derive, is transported to presynaptic terminals [8, 9]. The precise physiological roles of APP within the synapses


A
Action potential
80
40
0
-40
-80
Axon
Oxidative & nitrosative stress
Neurotransmitter transporter
Neurotransmitter
Environmental toxins
PD etiology?
PD-related genetic mutations
Nucleus
DJ-1 mutation
SNCA mutation
Parkin mutation
UCH-L1 mutation
ROS
Mitochondrial OXPHOS dysfunction & mitochondrial DNA polymorphisms
Reduced energy biosynthesis
Clathrin
Monomeric units
Cell death
Oligomers/ Protofibrils
Lewy bodies
Clathrin-mediated endocytosis
Active zone
Synaptic vesicle
Neurotransmitter
Interaction with aggregated, misfolded neurotoxic proteins
Receptor
Protein misfolding
Protein degradation pathways
Ubiq
Lysosomal
Proteasomal
Synaptic Junction
Dendrite
B
Figure legend:
Examples of proteins that localize to and play a role in:
A: Presynaptic neuron
SNAREs, αSYN, Complexin 2, LRRK2,:Futsch, LRRK2-NSF, LRRK2:AP2 complex subunits:LRRK2
B: Postsynaptic neuron
SNAREs, LRRK2:4EBP
1: Nuclear-related events
Gene-mutations: DJ-1, LRRK2, SNCA, parkin, UCH-L, ATP13A2, PLA2G6, FBX07
2: Protein misfolding, -aggregation & -truncation
LRRK2, αSYN, UBE1, DRP-2
3: Altered vesicle synthesis, -recycling & neurotransmitter release
αSYN, αSYN:CSPα, αSYN:Aß1-42, αSYN:Rab5, αSYN:Rab8, αSYN:PCLß1, ßSYN, SNAREs, DJ-1, PINK1, LRRK2, Synapsins
4: Mitochondrial-related events
DJ-1, PINK1, VDAC, Aralar, Mitofilin
5: Altered membrane trafficking
SNAREs, αSYN, Parkin, LRRK2, DJ-1, DJ-1:Rab3A, DJ-1:Synaptophysin, DJ-1:Rab3A
Interactions with molecular cheparones:
Target misfolded proteins for proteasomal degradation
Refold misfolded proteins
Decrease ROS levels
Prevent protein aggregation
Participate in vesicle fusion and neurotransmission
Translocate proteins across mitochondrial membranes
Assist with clathryn uncoating of endocytic vesicles

are receiving increasing focus from AD research scientists due to the finding that spine loss occurs in hippocampal neurons in transgenic mice expressing human mutant APP [10]. The Aβ cascade hypothesis, which states that Aβ accumulation is the principal pathological event leading to neurodegeneration in the AD brain [11], has received support from studies performed on transgenic mice that express familial mutations of human APP. In particular, animals produced essential aspects of the disease, including amyloid plaques, deficits in performing cognitive tasks and abnormalities affecting the mechanisms underlying synaptic plasticity, resulting in adverse effects on learning and memory [12]. However, this line of investigation has been compromised by reports that such animals seldom develop neurofibrillary tangles and exhibit little synaptic and neuronal loss, which serve as pathological hallmarks of AD, as reviewed by Gotz and colleagues [13]. Related to this, Guisti-Rodriguez and colleagues [14] recently examined the role of Aβ elevation in the CK-p25 inducible mouse model of neurodegeneration, which recapitulate many hallmark features of AD, including progressive neuronal loss, elevated Aβ levels, tau pathology, cognitive dysfunction and impaired synaptic plasticity [15, 16]. In particular, the study evaluated the impact of p25 overexpression on a background of normalized Aβ levels in the CK-p25 mouse, due to Cdk5 being a proline-directed serine/threonine kinase whose activation by the p25 protein has been implicated in a number of neurodegenerative disorders, possibly by upregulating expression and activity of the β-site amyloid precursor cleaving enzyme (BACE1), for initiating the sequential cleavage of APP and resulting in intraneuronal accumulation of Aß peptides [9, 17, 18]. The study demonstrated that partial genetic deletion of BACE1 and concomitant increases in p25/Cdk5 activity served to restore Aβ levels in the CK-p25 mouse to those of wild-type mice and were accompanied by a rescue of synaptic and cognitive deficits.

Other evidence showed that deletion of a major lipoprotein receptor, low-density lipoprotein receptor-related protein 1 (LRP1), results in brain lipid metabolism deficits and correlates with progressive, age-dependent dendritic spine degeneration, synapse loss, neuroinflammation, memory loss and eventual neurodegeneration [19].

Huntington's chorea manifests due to expression of mutant forms of the ubiquitous protein huntingtin, containing a pathologically expanded polyglutamine (polyQ) repeats at the N-terminal region of this ubiquitously expressed protein. This results in degeneration with concomitant synaptic dysfunction of neurons located in the striatum and cerebral cortex, resulting in motor dysfunction and dementia [20]. Use of a *Drosophila* HD model reported enhanced neurotransmitter release, resulting in neurodegeneration in neuromuscular synapses expressing mutant htt [21]. However, in order to discern primary synaptic changes from changes resulting from neurodegeneration, Rozas and colleagues [22] made use of a transgenic mouse model of HD to show that expanded htt increases synaptic release at vertebrate motor nerve terminals.

Prion diseases are neurodegenerative disorders caused by transmissible particles, composing of a pathogenic conformation of a host-encoded glycoprotein called the prion protein, which forms a normal constituent of cell membranes [23]. This group of diseases affects both animals and humans and includes Creutzfeldt–Jakob disease and Gerstmann–Sträussler–Scheinker in humans, bovine spongiform encephalopathy also known as "mad cow disease" affecting cattle, chronic wasting disease seen in mule deer and elk, and scrapie that manifests in sheep [24]. The view of AD has undergone a remarkable shift in recent times, from the once-held perspective entailing diffuse errors in protein folding, arising autonomously throughout a tissue due to a pathologic stressor, to one where AD pathogenesis entails a seed of pathologically misfolded protein, yielding a template to catalyze misfolding of the native protein [25]. A series of studies using homogenates taken from human AD brains, and therefore containing Aβ, or a mouse model of AD, provided support to the growing contention that Aβ holds "infectious" properties, similar to prion protein (PrP), by revealing the ability of pathological Aβ to "seed" in naïve mouse models [26–28].

It has been proposed that in PD-affected brains, alpha-synuclein (αSYN), a small, intrinsically unstructured

◀ **Fig. 1** Protein mishandling as a prominent feature of synaptic pathology seen in PD. An action potential is evoked in the presynaptic neuron (**a**), allowing for the release of neurotransmitter into the synaptic junction, and postsynaptic neuronal (**b**) changes. To prevent chronic stimulation of receptors locating to the postsynaptic membrane, excess neurotransmitter molecules are either recycled or removed through cellular and molecular machinery. Several proteins are involved in maintaining this basic flow of activities at synaptic level, whilst changes affecting such proteins can disrupt synaptic transmission, resulting in symptoms indicative of PD. Such changes are illustrated in the figure and include postsynthesis proteins that may acquire different misfolded conformations and may form dimers, oligomers and, in later stages, amyloid fibrils. These may sequester into inclusion bodies, such as LBs. Mitochondrial dysfunction, oxidative stress and protein mishandling have been tightly interconnected in this hypothesized pathogenic cascade and are indicated as modifiers in the figure. Additional possible interactors have been omitted for clarity. The *dotted outline boxes* describe various cellular and molecular events associated with PD pathology, along with examples of proteins involved as role players in such processes. For example, *dotted outline box 3* indicates disruptions affecting normal vesicle synthesis, recycling and neurotransmitter release, all being recognized as processes that are likely to underlie aspects of parkinsonism. Immediately below follows a summarized list of proteins identified by various studies thus far that have implicated proteins such as the presynaptic protein αSYN in disrupting such processes, as well as work that highlighted a possible role for altered relations between αSYN and various other synaptic proteins, including CSPα, Aß1-42, Rab5, Rab8 and PCLß1

neuronal protein, found enriched in presynaptic nerve terminals and which appears to fulfil a role in regulating synaptic function recycling [29, 30], transfers intercellularly to, in the process, recruit and aggregate protein in recipient cells in a prion-like manner. This was evidenced by substantial analyses performed on autopsied material taken from PD patients, which showed that LBs first emerge in the brain stem and olfactory bulb prior to the appearance of motor symptoms, followed by gradual spreading throughout the brain [31]. The concept of a prion-like propagation as an underlying mechanism for PD pathology gained considerable support from postmortem analyses performed on PD patients who had received embryonic neuron grafts approximately a decade before patients eventually died. Taken together, the studies all showed that pathological αSYN-immunoreactivity appears not only in the patient's natural brain tissue but also in the grafted neurons [32–34]. Although the precise means by which the transfer of αSYN to neighbouring neurons might occur remains enigmatic, a plausible suggestion is that this may involve tunnelling nanotubes, F-actin extensions which could play a vital part in cell signalling [35]. In fact, this mechanism has already been demonstrated to operate for transferring PrP [36].

Numerous reports highlight pre- and postsynaptic dysfunction that involve proteins that extend beyond the prion perspective, suggested to explain PD pathogenesis, with such proteins that have been implicated in various neuronal cell types in both PD patients and in animal models used for replicating aspects of the human disease [37, 38]. The synaptic pathology associated with PD provides an opportunity for therapeutic intervention, through restoration of neurotransmitter tone. In this regard, how synaptic proteins and protein–protein interactions might contribute towards the onset and progression of the disease phenotype remains an important area for ongoing research. The aim of the current review is to advocate for a shift in the current conceptual view of the treatment of PD from a restoration of extracellular neurotransmitter depletion to replacement of lost striatal synapses by using the numerous examples drawn from the growing literature on the subject.

Parkinson's Disease: Pathogenesis

PD is the most common movement disorder [39], second most common only in the broad spectrum of diseases that make up the neurodegenerative diseases, which also includes AD. The primary motor dysfunction phenotype of PD consists of bradykinesia, tremor and rigidity. Postural instability, gait and balance anomalies develop in the later stages of the disease [40]. The primary motor features of the disease are mainly due to progressive degeneration of dopaminergic neurons which originate in the substantia nigra (SN) and project to the caudate and putamen, with a subsequent decline in caudate–putamen dopamine (DA) content. Insights resulting from this observation have led to the introduction of DA replacement therapy. However, chronic treatment with such pharmacological agents (e.g. L-DOPA) associates with motor complications that mar the clinical benefit of the drug. Non-dopaminergic neuronal degeneration has also been reported in multiple sites in the PD-affected brain and associates mainly with non-motor symptoms, including dementia, sleep disturbances, autonomic dysfunction, severe anxiety, mood swings, concentration difficulties and fatigue, amongst other difficulties [41, 42]. These symptoms exert considerable impact on the quality of life of both patient and caregiver [43] and are believed to result from dysfunctions affecting neuron types such as those that synthesize, store and release noradrenaline, serotonin (5-HT), acetylcholine (ACh) and glutamate. Such insights have served to direct research efforts for ascertaining the efficacy of non-dopaminergic drugs aimed at manipulating PD symptoms beyond the damaged dopaminergic system [44–48].

PD is believed to stem from multifactorial origins, with exposure to pesticides, advancing age and an inherent genetic vulnerability for developing the disease comprising the most prominent risk factors for sustaining PD [49]. Mitochondrial dysfunction has been detected in multiple tissues taken from individuals diagnosed with sporadic PD. The first description of mitochondrial dysfunction in idiopathic PD was narrowed down to a deficiency of complex I activity in the SN [50–52]. This initial report was soon followed by reports of mitochondrial dysfunction seen in skeletal muscle, platelets and lymphoblasts in some PD patients [53, 54]. Although other studies have reported mitochondrial defects in the frontal cortex of PD patients [55], such claims await substantiation.

These include convincing reports that make reference to energy deficits, enhanced production of free-radical species and concomitant oxidative stress [56] and altered regulation of macro-autophagy and selective mitophagic clearance of damaged mitochondria as potential primary generators of PD pathology [57–59]. Neurotoxins such as rotenone [60, 61] and MPTP [62, 63], used for mimicking aspects of human PD in animals to a remarkably accurate extent, act by inhibiting complex I of the mitochondrial electron transport chain, locating to the lipid bilayer of the inner mitochondrial membrane. Results showing that certain PD-associated gene products, especially from studies performed on PINK1 and parkin, exert either direct or indirect adverse impacts on mitochondrial integrity provide further compelling evidence for a strong causal relation between mitochondrial dysfunction and concomitant oxidative stress and parkinsonism [64–66]. This includes molecular analysis studies which revealed that PINK1 serves as a tumour

suppressor, composing of an NH_2-terminal mitochondrial targeting signal motif, a putative transmembrane region and a serine-threonine kinase domain, with certain mutations affecting PINK1 that can destabilize the protein, whereas others may decrease kinase activity [67].

However, the exact extent to which mitochondrial-related events function either independently or in synergy to contribute towards PD pathology remains to be determined [49].

A change in synaptic physiology, brought on by neurodegenerative disease, evokes homeostatic shifts in the ratio of mobile to stationary mitochondria, coordination of this relation being critical to ensure optimal neuronal function [68]. Understanding mitochondrial events operating within synapses, such as to provide energy to ensure effective transport of synaptic vesicles, neurotransmission and to buffer excess Ca^{2+} at synapses, promises to contribute towards a viable potential therapy for PD.

Emerging evidence also implicates incomplete proteolysis by the ubiquitin–proteasome system for controlling the degradation of accumulated, misfolded, mutant and oxidatively damaged cellular proteins in PD etiopathogenesis [69]. This has been demonstrated using varieties of dopaminergic neurotoxins applied to in vitro and in vivo models that recapitulate some of the key features of PD by inhibiting proteasomal function [70]. Apart from the clues that such studies provide for a role played by environmental factors in incomplete proteolysis in PD, genetic factors also appear to contribute to proteolytic machinery failure [71–74]. However, the degree to which each factor contributes to the initiation and progression of PD remains to be determined.

Although the vast majority of PD cases present sporadically, approximately 7% of all PD cases result from a monogenic cause [75]. Sequence or copy number variants in several genes have been identified, supporting a causal association with familial forms of PD. These include α-synuclein (*SNCA*), *parkin*, ubiquitin carboxyl terminal hydrolase-L1 (*UCH-L1)*, *DJ-1* (*PARK7*), leucine-rich repeat kinase 2 (*LRRK2)*, *PTEN* (phosphatase and tensin homologue)-induced kinase1 (*PINK1*) [76, 77] and in ATP13A, encoding for a lysosomal type 5 P-type ATPase [78]. For a recent overview and up-to-date information on the genetic-molecular foundations of familial PD, see the reviews by Hardy et al. [79] and Schulman and colleagues [80].

Others have shown a homozygous mutation in *PLA2G6* on chromosome 22 [81], whilst mutations affecting *FBX07*, the product being an F-box protein, result in PARK15 and associate with autosomal recessive, early onset parkinsonian pyramidal syndrome [82, 83]. Mutations have also been detected in the gene encoding the Htra2/Omi protein and in *GIGYF2* encoding the Grb10-interacting GYF protein 2; both of which correlate significantly with autosomal-dominant PD [84–86].

Intraneuronal inclusion bodies (Lewy bodies (LBs) and Lewy neurites) serve as the major histopathological hallmark of PD when found present in the SN of postmortem cases [31]. These are composed of aggregates of normal, misfolded and truncated proteins, the most prominent of these being aggregate-prone alpha-synuclein (αSYN), which locates to the presynaptic neuronal compartment [87] (Fig. 1). In PD, αSYN is reported to be abnormally glycated, phosphorylated, nitrated and oxidized; displays abnormal crystallographic structure and solubility; and is prone to forming aggregates and insoluble fibrils [88–95]. The biochemical mechanisms responsible for these post-translational changes that results in aggregation, to ultimately form proteinacious inclusion bodies, remain a highly active area of research.

Proteins Affecting Neurotransmitter Synthesis and Release in PD

Neurotransmitter release has evolved as a specialized form of membrane trafficking in neurons, a process that is calcium-regulated and is responsible for extremely rapid network communications through the central nervous system. Several proteins have been deemed central to the exocytotic apparatus' functioning and include the Sec18/N-ethylmaleimide-sensitive fusion protein (NSF) homologues, Sec17/soluble NSF attachment proteins (SNAPs), SNAP receptors (SNAREs, an acronym derived from SNAP (*S*oluble *N*-ethylmaleimide sensitive fusion *A*ttachment *P*rotein) *RE*ceptor), Sec1/Munc18 homologues (SM proteins) and small GTPases of the Rab protein family. In addition, proteins such as synaptotagmin 1, Munc13-1 and the complexins have been deemed crucial for Ca^{2+}-triggered exocytosis, being specialized for the tight spatial and temporal regulation of neurotransmitter release.

The PD-linked proteins αSYN, parkin and LRRK2 localize to synaptic membranes whilst also associating with membrane trafficking [96–100]. Insights into how such proteins may assist in membrane transport functions at the synapses are only beginning to emerge.

Several experimental reports support the notion that formation of αSYN amorphous, granular and/or fibrillar aggregates may result in neuronal dysfunction, potentially by obstructing normal cellular trafficking and/or trapping cellular components in inappropriate locations [71, 101]. This pathology has been observed in synapses, axons, dendrites and neuronal cell somata.

Rieker and colleagues [102] examined transgenic mice expressing the wild-type mouse αSYN ortholog, carrying a threonine at position 53, similar to the PD-linked A53T human mutant version. The group reported that neuromuscular synapses in long white matter tracts of the spinal cord

showed signs of presynaptic degeneration, although less pronounced as reported previously in lines expressing human αSYN [103].

αSYN's presynaptic-specific location was suggested to play a role in mobilizing synaptic vesicles [104]. The functions relate to how, in healthy brain tissue, NACP/αSYN is transferred to the synaptic terminals, where it gets assembled on the surface of the synaptic vesicle. In this regard, recent evidence reported that small oligomer aggregates of αSYN accumulate at the presynaptic membrane, triggering toxic stimuli that lead to synapse degeneration during progressive PD [105]. These different mechanisms of cellular dysfunction may not be mutually exclusive. For understanding how NACP/αSYN is involved in the synaptic pathology seen in PD, it is imperative to enhance our knowledge of proteins that co-localize with this molecule.

In a highly informative series of experiments, Chandra and co-workers revealed that wild-type αSYN acts as a molecular chaperone by assisting in the folding and refolding of SNARE protein complexes, crucial for neurotransmitter release into the synaptic cleft following the arrival of action potentials at the synaptic terminal, for vesicle recycling and to ensure synaptic integrity. The authors also tested for possible interactions between αSYN and cysteine-string protein α (CSPα, also known as DnaJ5 and heat shock protein (Hsp)40), a presynaptic molecular chaperone, and a member of a family of conserved small proteins (167-249 amino acid residues in length) found abundantly within synapses. The strategic location of CSPα, on the cytoplasmic face of presynaptic vesicles, contributes to its crucial role for maintaining the integrity of synaptic nerve terminals [106]. Critical functions assigned to CSPα include folding and refolding synaptic proteins that have proved critical for neurotransmitter release, vesicle recycling and synaptic integrity [107].

The study by Chandra and colleagues showed that transgenic expression of αSYN abolishes the lethal phenotype created by deletion of CSPα in mice, manifesting as widespread age-dependent neurodegeneration, including defects in synaptic transmission which could account for the impaired motor performance seen in these mice. Furthermore, transgenic expression of human A53T mutant αSYN completely abolished the lethality of CSPα knock-out mice.

These results are indicative of a physiological role for αSYN in protecting nerve terminals against injury, whilst the study also revealed that transgenic αSYN was only capable of exerting this physiological effect when binding to phospholipid membranes. This finding was affirmed by results which showed that knock-out of endogenous synucleins produces contrasting results by accelerating the lethality of the CSPα deletion.

The authors also revealed an intricate protein–protein interaction between CSPα and αSYN for controlling the appropriate SNARE complex assembly. This was demonstrated by the result that overexpressing *SNCA* restored SNARE complex levels whilst suppressing presynaptic degeneration, motor dysfunction and eventual death of CSPα-null mice.

This compelling report on the physiology of αSYN in vivo raises several speculations as to how aberrant activity of this protein might lead to neurodegeneration in PD and other synucleinopathies. A key question emerging from this observation in light of the suggested function for LB formation—to provide a cellular protective response against misfolded or abnormal proteins—is whether an aberrant chaperone activity of αSYN could interfere with synaptic integrity. Chaperones specifically recognize misfolded proteins whilst serving as key specificity factors for other cellular defences, such as proteolysis by the proteasome and autophagy. The suggestion that native αSYN provides a cellular defence mechanism against toxic protein conformers for counteracting PD pathogenesis therefore highlights the therapeutic potential in drugs designed to upregulate the molecular chaperon activities of αSYN in brain tissues or for promoting the conditions that could restore the efficiency of the cellular defence decreases in stressed or aging neurons, leading to neuroinflammation, apoptosis and tissue loss. On the other hand, recent work revealed that various small Hsps bind weakly to and affect the fibrillization of wild-type and disease-associated mutant αSYN by inhibiting or slowing down the protein's aggregation [108]. Yet, this effect on intracellular αSYN fibrillization was not consistently seen for all small Hsps, with some rather promoting aggregation. Hence, findings emerging from this study advocate for efforts to optimize the interaction between chaperones such as small Hsps and αSYN as an interesting target for therapeutic intervention in the pathogenesis of α-synucleinopathies such as PD. In this regard, other documented work supports the possibility that enhanced function of chaperone proteins, including Hsp70 and TorsinA, a protein with homology to yeast Hsp104, can alter normal and abnormal αSYN function [109–112].

Related to this, the finding by Chandra and co-workers that altered CSPα function may contribute to PD, since CSPα naturally protects against αSYN toxicity, misfolding and aggregation, where all of which may contribute towards the synaptic degeneration seen in PD, suggests CSPα to be considered as a promising therapeutic approach for treating PD. However, further studies will be necessary to substantiate the validity of such claims.

Shin and colleagues [113] made use of a yeast two-hybrid screen to identify Rab5b as an LRRK2-interacting protein partner. Subcellular fractionation and immunocytochemical analyses furthermore revealed that a fraction of both proteins co-localize in synaptic vesicles. The authors also showed that either overexpression or knockdown of endogenous LRRK2 in primary neuronal cells significantly

impaired synaptic vesicle endocytosis, which was rescued by co-expressing functional Rab5b protein. Taken together, the results of this study were the first to show that LRRK2 may be a critical interaction partner of Rab5b, with this interaction serving to modulate endocytosis of synaptic vesicles.

Sophisticated transport and delivery systems are required for proteins expressed within mammalian cells to allow them to reach their correct intracellular locations. This depends on a constant turnover of vesicles, which relies on synaptic vesicles located in presynaptic nerve terminals, for storing, transporting and releasing neurotransmitter content into the synaptic cleft in a highly coordinated manner. This step is preceded by an increase in the level of Ca^{2+}, resulting in the vesicles fusing with the presynaptic membrane at the active zones in the terminals [114, 115]. Following the fusion events, new synaptic vesicles form via endocytosis, which then enter the nerve terminals to get refilled with neurotransmitter for reuse [116].

Piccoli and co-workers demonstrated an interaction effect between LRRK2 and several presynaptic proteins [117]. The authors explored pre- and postsynaptic properties of cortical neurons following LRRK2 silencing. In particular, the authors demonstrated that LRRK2 depletion affects the mobility and transportation of vesicles, vesicle dynamics in the synaptic bouton and their distribution in presynaptic pools locating to synapses. Utilization of co-immunoprecipitation and glutathione *S*-transferase pull-down assays furthermore identified several LRRK2 interacting proteins, including *N*-ethylmaleimide sensitive factor (NSF), AP-2 complex subunits, SV2A, synapsing, syntaxin 1 and actin; all of which have previously been described as key elements of presynaptic vesicle trafficking [118].

αSYN expresses predominantly in the CNS, localizing to neuronal presynaptic terminals [119, 120], where it regulates synaptic vesicle formation and neurotransmitter release [121]. Nemani and co-workers [122] explored potential mechanisms underlying the reported association between overexpressed wild-type human αSYN and membrane trafficking defects. The authors reported that even modest αSYN overexpression, within the range predicted for gene multiplication, but in the absence of overt toxicity, markedly reduced neurotransmitter release. Imaging of the synaptic vesicle cycle elucidated on a possible mechanism for this by revealing a reduction in the size of the vesicle pool, reduced synaptic vesicle density at the active zone and defective reclustering of synaptic vesicles following endocytosis. Reduced levels of synapsins seen in transgenic αSYN overexpressing mice was suggested as a potential mechanism to explain the apparent control that αSYN exerts over synaptic vesicle clustering and the size of the recycling pool. This multigene family of neuron-specific phosphoproteins locates to synaptic vesicles and is able to interact in vitro with lipid and protein components of synaptic vesicles and with various cytoskeletal proteins, including actin [123]. The result implies that rather than the physiological effects of αSYN overexpression reflecting a loss of synapsins, both seem to influence related processes involved in vesicle mobilization [122]. These findings also implicate that heightened levels of αSYN produce defects in synaptic vesicle recycling, which may precede overt PD-related neuropathology. Furthermore, the work also demonstrated impairments in glutamate and DA release in this transgenic model, supporting a prediction that neural network dysfunction leads to further pathology. However, this postulation did not receive experimental support from use of a knockout model of wild-type human αSYN. Conversely, increasing αSYN expression 2–3-fold was shown to inhibit neurotransmitter release in glutamatergic hippocampal pyramidal neurons and mesencephalic dopaminergic neurons. ßSYN overexpression was shown to be capable of inhibiting this neurotransmitter release, suggesting for a dual control over this function with αSYN.

The study also revealed that αSYN overexpression reduces the size of the synaptic vesicle pool and prevents synaptic vesicle clustering. Other work reported a link between αSYN and Rab3a [124], a protein involved in synaptic vesicle trafficking, and between αSYN–rabphilin interaction. Previous work suggested that Rab3a fulfils non-essential functions [125, 126]. However, the observation of synaptic morphological changes in *Caenorhabditis elegans* rab3-null mutants, including fewer synaptic vesicles, especially near the active zone, but more synaptic vesicles at ectopic sites [125], suggests that transport of synaptic vesicles at nerve terminals is impaired in the absence of rab3. Rabphilin is a peripheral membrane protein present in synaptic vesicles, which binds selectively to the GTP-form of rab3a and rab3c [127]. A functional role for rabphilin in exocytosis was suggested by the finding that overexpression of rabphilin in chromaffin cells enhances transmitter release significantly [128]. Since αSYN, rab3a and rabphilin participate in the docking and fusion of synaptic vesicles, the result emerging from the study by Dalfó and colleagues [124], to demonstrate decreased binding of rab3a to rabphilin and increased binding of rab3a to αSYN aggregates, suggests that neurotransmitter exocytosis may be impaired in LB diseases such as PD.

Other work by the same group revealed an altered relationship between αSYN and Rab5, and αSYN and Rab8 in transgenic mice expressing wild-type and mutant human A30P in the *SNCA* gene, deemed causal in familial PD. The mice showed a progressive parkinsonian motor phenotype, accompanied by accumulation of αSYN in the soma and neurites, with accompanying CNS gliosis [129]. The αSYN interactions were not regarded as being dependent on the level of Rab protein used in the assay. Rather, αSYN

interactions with Rab proteins were seemingly due to mutant αSYN instead. Since Rab3a, Rab5 and Rab8 fulfil important roles in synaptic vesicle trafficking and exocytosis at the synapse, vesicle endocytosis and trans-Golgi transport, the results from this suggest that such functions may be impaired in PD patients harbouring the A30P *SNCA* mutation.

Moreover, altered interactions were suggested between αSYN and phospholipase C (PLCβ1), which acts as a signalling step to metabotropic glutamate receptors [130]. The study revealed reduced levels of PLCβ1 in the frontal cortex taken from the postmortem brains of diffuse Lewy body disease (DLBD) patients. This degenerative disease of the nervous system involves the brain stem, diencephalic nuclei and cerebral cortex and associates with abnormal αSYN aggregation and widespread formation of LBs and Lewy neurites. The study also affirmed αSYN/PLCß1 binding in control patients and decreased αSYN/PLCß1 binding in DLBD patients. Additionally, the study showed that αSYN aggregation, as seen in DLBD, produces an inhibitory effect on transmitter release by cortical glutamatergic synapses to highlight glutamatergic receptors as putative pharmacological targets in DLBD. It remains to be seen whether such an abnormal protein interaction, with consequences on glutamatergic transmission, is present also in other synucleinpathic diseases such as PD.

Another protein that showed an interaction effect with overexpressed αSYN was complexin 2. Similar to αSYN, complexin 2 localized to presynaptic nerve terminals and showed a significantly reduced level of detection in tissue taken from transgenic mice overexpressing αSYN. This coincided with a proposed role for αSYN to act as a chaperone to the SNARE proteins, since a change in complexin levels has been associated with the step immediately preceding fusion with the plasma membrane [131]. The authors discounted altered SNARE protein function from contributing towards the defect seen in synaptic vesicle mobilization and reduced clustering of synaptic vesicles near the active zone, as this study reported here [132]. However, the investigators proposed that the reduction in complexin 2 seen in the transgenic mice may reflect a gain in normal function for αSYN.

In summary, the study served to illustrate a potential mechanism that may underlie the observation that even modest overexpression of αSYN can significantly elevate the risk of forming synucleinopathies and becoming symptomatic of PD. A further implication of this work is that due to the fact that αSYN is diffusely expressed throughout the brain, it is reasonable to assume that its overexpression is not limited to a particular cell phenotype, hence impacting on the function of a widespread array of brain circuits.

A study by the Spillantini group [133] utilized a previously characterized transgenic mouse line called αSYN(1-120), which revealed a novel gain of toxic function of αSYN at the synaptic level. Here, the cells expressed carboxy-terminally truncated human *SNCA*(1-120) under control of the rat tyrosine hydroxylase promoter and with a mouse *SNCA*-null background. Truncation of αSYN is regarded of clinical importance, with the biochemical process that is known to enhance the formation of cellular aggregates containing granular and filamentous αSYN in dopaminergic neurons of the SN in mice, similar to what was seen in PD patients [134].

The model used by the Spillantini group accurately reflects early pathological events in human PD by showing, in addition to the biochemical changes mentioned previously, that animals also showed reduced striatal DA levels and motor function abnormalities. Members of the SNARE protein superfamily are primarily responsible for mediating vesicle fusion with the membrane to evoke exocytosis of cellular transport vesicles. The SNARE proteins were proposed to account for the specificity of membrane fusion, and they are directly responsible for executing fusion by forming a tight complex (the SNARE or core complex) to thereby bring the synaptic vesicle and plasma membranes together.

The study [133] contributes to current understanding concerning the early pathological changes affecting the parkinsonian brain by demonstrating that the expression of truncated or full-length αSYN results in an age-dependent redistribution of the SNARE proteins SNAP-25, syntaxin-1 and synaptobrevin-2 at synapses, based within the striatum. This result was clinically verified in idiopathic and familial PD (the A53T mutation in *SNCA*) case tissue, revealing that SNARE proteins also accumulate in the striatum of human patients.

The release of DA was monitored by microdialysis to investigate whether the accumulation of αSYN and SNARE proteins associates with synaptic failure. Results revealed that basal levels of DA were reduced in transgenic mice compared to controls in an age-dependent manner, indicating that progressive altered exocytosis and eventual synaptic failure associate with increased SNARE redistribution [133].

Although infrequently seen, PD-linked mutations in the *PINK1* gene associate with several molecular and cellular deficits [135]. These include disrupted mitochondrial quality control and disposal [136], altered mitochondrial morphology and reduced ATP production, whilst overexpressed wild-type PINK1 served to promote mitochondrial loss [102]. These mitochondrial anomalies associated with significantly impaired DA release [137], contributing to the motor deficits seen in patients harbouring this type of mutation. Neuronal synaptic compartments are the site at which demands placed on mitochondrial functions, such as for energy supply and the buffering of intracellular Ca^{2+}, are especially significant [138]. *PINK1*-deficient *drosophilae*

displayed severely affected synaptic physiology, revealed as inadequate mobilization of the reserve pool of synaptic vesicles [139]. This deficit was rescued by applying ATP to the synapse. The study serves to corroborate the critical importance of PINK1 for regulating energy homeostasis under increased metabolic demand at neuronal sites (synapses) that may be particularly vulnerable against the degenerative processes operating in neurodegenerative disease.

Mutations in the PARK7, alias *DJ-1* gene, are the least common of the known causes of autosomal recessive early onset parkinsonism (~1% of early onset PD), and it was first identified as a PD-linked causative gene in a Dutch and Italian family [140]. In particular, affected members of the Dutch family carried a homozygous deletion of *PARK7* exons 1–5, whilst Italian patients were homozygous carriers of a missense mutation (L166P). Since then, various *DJ-1* missense mutations in coding and promoter regions, frameshift and splice site mutations, as well as exonic deletions, have also been reported to be present in some PD patients [141–146]. However, other studies have been less optimistic and have detected no or extremely low occurrence of *DJ-1* mutations in the PD populations screened for [147–153].

Although predominantly being a cytosolic protein, DJ-1 has also been detected subcellularly in the mitochondria in vitro [154, 155] and in vivo [155]. The distribution to mitochondria seemingly increases under conditions of oxidative stress when DJ-1 redistributes to the outer mitochondrial membranes [156, 157]. However, the conflicting finding that endogenous DJ-1 associates with the intermembrane space and the matrix, but was absent from the mitochondrial membranes [155], emphasizes the need for additional studies to clarify the association in terms of location and function between DJ-1 and mitochondria in health and disease. Exogenously applied DJ-1 has also been found present in the cytosol, nucleus and microsomes [140, 154, 157, 158].

Cellular functions assigned to DJ-1 so far include serving as a molecular chaperon [159] and a transcriptional regulator [160–163], as well as protecting cells against oxidative stress [157, 158]. It was demonstrated that endogenously present DJ-1 locates to presynaptic terminals of striatal axons and dendrites [164], revealing molecular interaction with membranes in cultured cells [165]. This result suggests for a functional role by endogenous DJ-1 in dopaminergic neurotransmission. In this regard, the group showed through the use of immunohistochemistry that DJ-1 localized, in part, to the synaptic cytosol, vesicles and membranes which comprise the synaptic terminals in the mouse brain. Furthermore, DJ-1 co-localized with synaptic vesicle proteins, including synaptophysin and Rab3A, whilst, in living cells, Förster resonance energy transfer (FRET) analysis revealed that a fraction of DJ-1 interacts directly with synaptophysin. The study revealed that DJ-1 demonstrated a similar distribution pattern than Rab proteins which, in turn, form part of a monomeric GTPase family. The study also revealed that DJ-1 partly co-localizes with Rab3A at synaptic terminals, a member of the Rab proteins, which play functional roles in regulating aspects of membrane trafficking [166]. This finding, along with a previous report that *DJ-1* knockout mice exhibited altered synaptic functions, including loss of sensitivity to the inhibitory effects of D_2 autoreceptor stimulation [167], suggests for a potentially critical role by DJ-1 for controlling vesicular trafficking during neurotransmitter exocytosis, a process which involves sequential associations between various synaptic proteins. Although the precise participatory role that DJ-1 might fulfil in synaptic vesicle transport and, ultimately, in regulating neurotransmitter release remains to be ascertained through future experimental work, the authors of this paper postulated that DJ-1's molecular chaperone activity on synaptic vesicles may serve to regulate vesicular fusion, a process central to exocytosis. Taken together, the study's confirmation of an association between DJ-1, a mutation in the encoding gene which associates with familial PD, and synaptic vesicles contributes to understanding the pathological mechanisms underlying *PARK7*-linked PD.

Proteins Affecting Chemical Synaptic Transmission in PD

Following the release of neurotransmitter molecules into the synaptic cleft, the molecule diffuses within microseconds across the cleft to reversibly bind to and activate specific receptors located to the postsynaptic cell membrane. Such neurotransmitter–receptor site interactions cause specific ion channels to open on the postsynaptic membrane, triggering ionic fluxes which either depolarize or hyperpolarize the membrane. Whereas excitatory synapses depolarize postsynaptic membranes, inhibitory synapses hyperpolarize them.

Work by Hummel and colleagues [168] contributed to understanding the molecular mechanisms underlying synaptic transmission defects seen in PD patients harbouring *LRRK2* mutations, revealing that *Drosophila* LRRK2 phosphorylates and negatively regulates the function of the protein Futsch at the presynaptic level. This result is potentially significant since the Futsch protein has previously been shown to be fundamentally important for regulating axonal and dendritic growth during embryogenesis and for synaptic morphogenesis during larval neuromuscular junction development [168, 169]. In addition, the work showed that the previously characterized *Drosophila* LRRK substrate and translational repressor 4E-BP [170] functionally interact with LRRK2 postsynaptically [168]. Given the similarity of the *Drosophila* neuromuscular junction synapse to

mammalian excitatory glutamatergic synapses, the results from the study by Hummel and colleagues [168] suggest synaptic dysfunction to be a primary effect of LRKK2-linked PD, and hence, holds clinical importance.

With symptoms indicative of severe parkinsonism only presenting after at least a 90% depletion of striatal DA levels, evidence has been sought for adaptive strategies within this system, capable of maintaining function despite extensive neuronal loss during the presymptomatic phase of PD [171]. Processes postulated to contribute to this phenomenon include increased DA synthesis and release from remaining terminals, and reduced DA clearance from extracellular fluid [172]. Compelling evidence in support of such adaptive changes in DA synthesis and uptake was seen in rat brains, lesioned by injecting the experimental neurotoxin 6-hydroxydopamine (6-OHDA) [173–176]. In addition, in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated parkinsonian monkeys, the remaining corticostriatal and thalamostriatal axo-spinous synapses were seen to undergo complex ultrastructural remodelling, suggesting for increased synaptic activity [177].

αSYN was shown to be specifically upregulated in a discrete population of presynaptic terminals of songbirds during a period of song acquisition [119], suggesting for a role in synaptic plasticity. A modulatory role for αSYN in brain plasticity was further emphasized by electron microscopic studies revealing that NACP/αSYN associates with the synaptic vesicles of the presynaptic terminals [104], similar to that seen for synaptophysin [178]. However, the inconsistent results obtained for the effects of αSYN in terms of synaptic plasticity in health and in pathological conditions such as PD call for more elaborate experiments for investigating the complexity of the proteins involved in regulating synaptic plasticity.

Dynamic pre- and postsynaptic adaptations operate within the inhibitory GABAergic (expressing γ-aminobutyric acid) medium spiny neurones (MSNs), which project towards the striatum that serves as the main input nucleus of the basal ganglia. This follows the significant loss of dendritic spines on MSNs during PD, which results from striatal DA denervation. The complexity of this arborization continues with the formation of glutamatergic synapses that form on spines that stud the dendrites of MSNs [179]. This pyramidal neuron type provides input from the cerebral cortex, carrying information with regards to sensory and motor function and motivational state; all of which integrate to guide the striatal control of thought and movement [180].

By utilizing a culture model of the striatum, Tian and colleagues [181] recently revealed a novel version of striatal homeostatic plasticity, thereby providing the outline of a molecular mechanism for activity-dependent synaptic scaling, by which activity may control synaptic strength [182]. The authors reported a near 50% down-regulation of synaptic connectivity for glutamatergic neurons in striatopallidal MSNs, following sustained depolarization of co-cultures of cerebral cortex and in a transgenic striatum model, which mimics elevated activity. This result correlated with previous findings obtained from using animal models of PD [183]. This loss seemed dependent upon the participation of two proteins, the Ca^{2+}-dependent protein phosphatase calcineurin and myocyte enhancer factor 2 (MEF2), in an upregulated state. This result is interesting in light of previous studies which showed that an upregulated level of MEF2 increased expression of *Nur77* and *Arc*; both of which are known for promoting down-regulation of glutamatergic synapses [184]. It remains for future work to enhance understanding of how participating protein alterations in striatal MSNs contribute to PD symptomology, with such insights that promise to significantly enhance therapeutic options available to PD patients.

Proteins for Synapse Formation, Assembly and Structural Maintenance in PD

Additional functions required by neurons for effective synaptic transmission include synapse formation and assembly of specialized pre- and postsynaptic structures that allow for coupling of synaptic vesicle fusion to sites of postsynaptic receptor clustering. Moreover, to ensure effective synaptic maintenance, it was suggested that axon and synapse loss seen in neurodegenerative disease may be predisposed by inefficient/interrupted delivery of maintenance materials that originate from the cell body and are transported via the axon to its distal ends [1].

A number of synaptic adhesion proteins were suggested to function in synapse formation and assembly. In one study, it was revealed that LRRK2 plays an important role in regulating synaptic morphogenesis through distinct substrate proteins locating to presynaptic and postsynaptic compartments [170]. On the presynaptic side, LRRK2 seemingly formed a complex with tubulin and the microtubule-associated 1B (MAP1B) homolog Futsch, with actin and microtubule cytoskeleton dynamics that play a crucial role in the formation of the nervous system, by regulating fundamental processes such as axonal guidance and synaptogenesis.

In other work, Imai and colleagues [185] investigated how altered LRRK2 kinase activity affects synapse structure and function, eventually resulting in neurodegeneration. The study made use of immunohistochemical and neurochemical analysis to reveal that endogenous expression of a single orthologue of human LRRK2 is ubiquitously expressed in the *Drosophila* brain. Double labelling further showed that it is expressed in DA neurons. The immunohistochemical approach revealed that *Drosophila* LRRK transgenic flies expressing either wild-type or mutant *Drosophila* LRRK proteins carrying point mutations found in human PD

patients localized to vesicular structures that co-stain with endosomal markers whilst partially overlapping with synaptic vesicle markers. The investigators found functional changes in the transgenic flies expressing mutant *Drosophila* LRRK2, showing significantly reduced levels of brain DA levels, when compared to wild-type or control flies. In contrast, DA content was elevated in LRRK2 knock-out flies. The study investigated whether this physiological change was due to a LRRK2-based regulatory mechanism, resulting in differential maintenance of dopaminergic neurons. Analysis of young (10-day-old) flies revealed no difference in dopaminergic neuron numbers compared to normal controls. However, a significant reduction in the number of dopaminergic neurons was detected in the brains of aged (60-day-old) flies expressing pathogenic LRRK2. Other findings emerging from this work include that expression of a kinase-dead form (3KD) of *Drosophila* LRRK or wild-type *Drosophila* LRRK exerted no significant effect on DA neuron number and that both young and aged *LRRK* knock-out flies retained healthy dopaminergic neurons. These results, taken together, led the authors to suggest that LRRK2's negative regulation of DA content is likely to be due to changes in DA storage or metabolism.

Gillardon [186] revealed that recombinant human LRRK2 preferentially binds and then phosphorylates bovine brain derived tubulin-beta. The phosphorylation effect was shown to be enhanced three-fold when LRRK2 contained the G2019S mutation. LRRK2 harbouring this mutation has repeatedly been shown to associate with enhanced kinase activity, which could, in turn, mediate degeneration of transfected neurons in vitro [187–189]. The study also made use of tandem mass spectrometry to identify Thr107 as comprising the phosphorylation site, which was shown to be highly conserved between tubulin-beta family members and those belonging to different family members. Of interest was the finding that in vitro co-incubation of bovine brain tubulins with LRRK2 increased microtubule stability in the presence of microtubule-associated proteins, which may explain the reduction in neurite length in LRRK2-deficient cultured neurons. Taken together, these findings suggest that LRRK2(G2019S)-induced neurodegeneration in parkinsonian brains may, at least in part, be mediated by enhanced tubulin phosphorylation in the presence of microtubule-associated proteins. One such protein is Tau, which did not appear to be directly phosphorylated by LRRK2 in vitro in this study [186]. However, previous work showed that increased Tau phosphorylation and aggregation is present in LRRK2 overexpressing neurons and in brain autopsy samples taken from LRRK2 mutation carriers [190]. This suggests that alterations in the phosphorylation levels of neuron-specific Tau might indicate for LRRK2 dysregulation affecting cytoskeletal structure, which may contribute to decreased neurite outgrowth.

In other work, Gillardon [191] provided insights into the regulators/effectors that might contribute to the pathophysiological functions of LRRK2. Specifically, the study showed that human LRRK2 interacts with elongation factor 1-alpha (EF1A) in vivo. The PD-linked LRRK2(G2019S) mutation did not affect the binding of EF1A. The study's findings further suggest that interaction between LRRK2 and EF1A may reciprocally modulate their physiological function. In this regard, co-incubation with recombinant EF1A significantly reduced the kinase activity of LRRK2.

Since LRRK2 localizes to microtubules, but not to the actin cytoskeleton within cells [187, 192], whilst EFiA was previously shown to promote in vitro stabilization/bundling [193], the study also explored the effects of an EF1A–LRRK2 interaction in microtubule dynamics [191]. Results showed that EF1A promoted microtubule cytoskeleton assembly, which, conversely, was impaired in the presence of LRRK2. It remains for further work to establish whether a disturbance of EF1A-mediated cytoskeletal stability by LRRK2 might ultimately contribute to the accumulation of tubulin and EF1A within intraneuronal LBs, the pathological hallmark of PD.

Understanding the mechanisms underlying non-somatic degeneration has received a major breakthrough from the numerous studies that have taken advantage of the slow Wallerian degeneration (*Wld*^{s}) mutation, which selectively protects synapses and axons against a wide variety of disease-related degeneration-inducing stimuli [194, 195]. Wishart and colleagues performed a differential proteomics analysis of synaptic proteins in *Wld*^{s} and wild-type mice [196]. For this, isolated nerve terminals (synaptosomes) were extracted from the striatum, a brain region that undergoes substantial degeneration during progressive PD. Sixteen proteins were identified whose expression levels were significantly altered in *Wld*^{s} synapses, compared to those in age- and sex-matched wild-type controls. Prominent changes in level of expression were detected in eight proteins, including VDAC1, Aralar1 and mitofilin, known to localize to mitochondria, suggesting that altered mitochondria-related responses to neurodegenerative stimuli may be critically important in the *Wld*^{s} neuroprotective phenotype. Synaptic terminals have an exceedingly high demand for energy and calcium homeostasis, which are fulfilled by having evolved specialized mechanisms. To fulfil the requirement at the synapses, stationary mitochondria are in place to serve as local energy production plants that produce ATP. Additionally, axonal mitochondria maintain local Ca^{2+} homeostasis at presynaptic boutons.

Additional, non-mitochondrial proteins that showed altered expression levels included the ubiquitin-activating enzyme UBE1 and DRP-2, providing experimental support that the ubiquitin–proteasome pathway and NAD-associated pathways may be required to elicit the full *Wld*^{s} phenotype. In essence, since Wld^s was seen to ameliorate axonopathy in

models of various neurodegenerative conditions, including PD [145], the study by Wishart and colleagues [196] served to identify several proteins to target therapeutically, in human, neurodegenerative disease.

Concluding Remarks

In general, studies converge to emphasize the central importance of synapse loss in neurodegeneration and the central role that certain synaptic proteins fulfil in the neuropathology of neurodegenerative diseases. However, several issues remain to be elucidated, i.e. whether synapse loss precedes or follows neuron cell death. Despite accumulating evidence to conclusively suggest the former, with synapse loss having been observed to precede neuron loss in early AD [197], further experimental work is called for to establish whether this phenomenon is also present in PD and in other neurodegenerative diseases to elucidate potential common mechanisms relevant to synaptic loss and neurodegeneration.

A related issue for experimental exploration is to establish the relationship of the function of particular synaptic proteins to synapse loss or neuronal loss, i.e. does dysfunction of synaptic proteins lead to synapse loss or neuron cell death? Further issues remaining to be resolved relate to the processing of such identified synaptic proteins during synaptic plasticity or synapse loss and their normal function in the synapse, whilst ultimately understanding such processes might offer a new paradigm for understanding and ultimately treating PD.

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References

1. Coleman M (2005) Axon degeneration mechanisms: commonality amid diversity. Nat Rev Neurosci 6:889–898
2. Wishart TM, Parson SH, Gillingwater TH (2006) Synaptic vulnerability in neurodegenerative disease. J Neuropathol Exp Neurol 65:733–739
3. Masliah E, Mallory M, Hansen L, DeTeresa R, Terry RD (1993) Quantitative synaptic alterations in the human neocortex during normal aging. Neurology 43:192–197
4. Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R (1991) Physical basis of cognitive alterations in Alzheimer's disease: synaptic loss is the major correlate of cognitive impairment. Ann Neurol 30:572–580
5. Shirai Y, Adachi N, Saito N (2008) Protein kinase Cepsilon: function in neurons. FEBS J 275:3988–3994
6. Favit A, Grimaldi M, Nelson TJ, Alkon DL (1998) Alzheimer's-specific effects of soluble beta-amyloid on protein kinase C-alpha and -gamma degradation in human fibroblasts. Proc Natl Acad Sci U S A 95:5562–5567
7. Lee W, Boo JH, Jung MW, Park SD, Kim YH, Kim SU, Mook-Jung I (2004) Amyloid beta peptide directly inhibits PKC activation. Mol Cell Neurosci 26:222–231
8. Masters CL, Simms G, Weinman NA, Maulthaup G, McDonald BL, Beyreuther K (1985) Amyloid plaque core protein in Alzheimer's disease and Downs syndrome. Proc Natl Acad Sci U S A 82:4245–4249
9. Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S et al (1999) Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 286:735–741
10. Lanz TA, Carter DB, Merchant KM (2003) Dendritic spine loss in the hippocampus of young PDAPP and Tg2576 mice and its prevention by the ApoE2 genotype. Neurobiol Dis 13:246–253
11. Karran E, Mercken M, De Strooper B (2011) The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. Nat Rev Drug Discov 10:698–712
12. Higgins GA, Jacobsen H (2003) Transgenic mouse models of Alzheimer's disease: phenotype and application. Behav Pharmacol 14:419–438
13. Gotz J, Streffer JR, David D, Schild A, Hoerndli F, Pennanen L, Kurosinski P, Chen F (2004) Transgenic animal models of Alzheimer's disease and related disorders: histopathology, behavior and therapy. Mol Psychiatry 9:664–683
14. Guisti-Rodriguez P, Gao J, Gräff J, Rei D, Soda T, Tsai LH (2011) Synaptic deficits are rescued in the p25/Cdk5 model of neurodegeneration by the reduction of ß-secretase (BACE1). J Neurosci 31:15751–15756
15. Cruz JC, Tseng HC, Goldman JA, Shih H, Tsai LH (2003) Aberrant Cdk5 activation by p25 triggers pathological events leading to neurodegeneration and neurofibrillary tangles. Neuron 40:471–483
16. Fischer A, Sananbenesi F, Pang PT, Lu B, Tsai LH (2005) Opposing roles of transient and prolonged expression of p25 in synaptic plasticity and hippocampus-dependent memory. Neuron 48:825–838
17. Wen Y, Yu WH, Maloney B, Bailey J, Ma J, Marié I, Maurin T, Wang L et al (2008) Transcriptional regulation of [beta]-secretase by p25/Cdk5 leads to enhanced amyloidogenic processing. Neuron 57:680–690
18. Cruz JC, Kim D, Moy LY, Dobbin MM, Sun X, Bronson RT, Tsai LH (2006) p25/cyclin-dependent kinase 5 induces production and intraneuronal accumulation of amyloid beta in vivo. J Neurosci 26:10536–10541
19. Liu Q, Trotter J, Zhang J, Peters MM, Cheng H, Bao J, Han X, Weeber EJ, Bu G (2010) Neuronal LRP1 knockout in adult mice leads to impaired brain lipid metabolism and progressive, age-dependent synapse loss and neurodegeneration. J Neurosci 30:17068–17078
20. The Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat is expanded and unstable on Huntington's disease chromosomes. Cell 72:971–983
21. Romero E, Cha GH, Verstreken P, Ly CV, Hughes RE, Bellen HJ, Botas J (2008) Suppression of neurodegeneration and increased neurotransmission caused by expanded full-length accumulating in the cytoplasm. Neuron 57:27–40
22. Rozas JL, Gómez-Sánchez L, Tomas-Zápico C, Lucas JJ, Fernández-Chacón R (2011) Increased neurotransmitter release at the neuromuscular junction in a mouse model of polyglutamine disease. J Neurosci 31:1106–1113
23. Prusiner SB (1982) Novel proteinaceous infectious particles cause scrapie. Science 216:136–344
24. Johnson RT (2005) Prion diseases. Lancet 4:635–642

25. Guest WC, Silverman JM, Pokrishevsky E, O'Neill MA, Grad LI, Cashman NR (2011) Generalization of the prion hypothesis to other neurodegenerative diseases: an imperfect fit. J Toxicol Environ Health 74:22–24
26. Eisele YS, Bolmont T, Heikenwalder M, Langer F, Jacobson LH, Yan ZX, Roth K, Aguzzi A et al (2010) Induction of cerebral beta-amyloidosis: intracerebral versus systemic Abeta inoculation. Proc Natl Acad Sci U S A 106:12926–12931
27. Kane MD, Lipinski WJ, Callahan MJ, Bian F, Durham RA, Schwartz RD, Roher AE, Walker LC (2000) Evidence for seeding of beta-amyloid by intracerebral infusion of Alzheimer brain extracts in beta-amyloid precursor protein-transgenic mice. J Neurosci 20:3606–3611
28. Meyer-Luehmann M, Coomaraswam J, Bolmont T, Kaeser S, Schaefer C, Kilger E, Neuenschwander A, Abramowski D et al (2006) Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. Science 313:1781–1784
29. Burré J, Sharma M, Tsetsenis T, Buchman V, Etherton MR, Südhof TC (2010) Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. Science 329:1663–1667
30. Chandra S, Fornai F, Kwon H-B, Yazdani U, Atasoy D, Liu X, Hammer RE, Battaglia G, German DC, Castillo PE et al (2004) Double-knockout mice for alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. Science 329:1663–1667
31. Braak H, Del Tredici K (2008) Invited article: nervous system pathology in sporadic Parkinson disease. Neurology 70:1916–1925
32. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW (2008) Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. Nat Med 14:504–506
33. Kurowska Z, Englund E, Widnerd H, Lindvalle O, Lia J-Y, Brundin P (2011) Signs of degeneration in 12–22 year old grafts of mesencephalic dopamine neurons in patients with Parkinson's disease. J Park Dis:83–92
34. Li J-Y, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, Lashley T, Quinn NP, Rehncrona S, Björklund A et al (2008) Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. Nat Med 14:501–503
35. Gerdes H-H, Bukoreshtliev NV, Barroso JFV (2007) Tunnelling nanotubes: a new route for the exchange of components between animal cells. FEBS Lett 581:2194–2201
36. Gousset K, Schiff E, Langevin C, Marijanovic Z, Caputo A, Browman DT, Chenouard N, de Chaumont F, Martino A, Enninga J et al (2009) Prions hijack tunnelling nanotubes for intercellular spread. Nat Cell Biol 11:328–336
37. Plowey ED, Chu CT (2011) Synaptic dysfunction in genetic models of Parkinson's disease: a role for autophagy? 43:60–67
38. Bagetta V, Ghiglieri V, Sgobio C, Calabresi P, Picconi B (2010) Synaptic dysfunction in Parkinson's disease. Biochem Soc Trans 38:493–497
39. Wirdefeldt K, Adami H-O, Cole P, Trichopoulos D, Mandel J (2011) Epidemiology and etiology of Parkinson's disease: a review of the evidence. Eur J Epidemiol 26:S1–S58
40. Ehringer H, Hornykiewcz O (1960) Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behaviour in disease of the extrapyramidal system. Klin Wochenschr 38:1236–1239
41. Chaudhuri KR, Healy DG, Schapira AH, National Institute for Clinical Excellence (2006) Non-motor symptoms of Parkinson's disease: diagnosis and management. Lancet Neurol 5:235–245
42. Chaudhuri KR, Odin P (2010) The challenge of non-motor symptoms in Parkinson's disease. Prog Brain Res 184:325–341
43. Poewe W (2008) Non-motor symptoms in Parkinson's disease. Eur J Neurol 15:14–20
44. Kanda T, Jackson MJ, Smith LA, Pearce RK, Nakamura J, Kase H, Kuwana Y, Jenner P (2000) Combined use of the adenosine A (2A) antagonist KW-6002 with L-DOPA or with selective D1 or D2 dopamine agonists increases anti-parkinsonian activity but not dyskinesia in MPTP-treated monkeys. Exp Neurol 162:321–327
45. Bibbiani F, Oh JD, Chase TN (2001) Serotonin 5-HT1A agonist improves motor complications in rodent and primate parkinsonian models. Neurology 57:1829–1834
46. Savola JM, Hill M, Engstrom M, Merivuori H, Wurster S, McGuire SG, Fox SH, Crossman AR, Brotchie JM (2003) Fipamezole (JP-1730) is a potent alpha2 adrenergic receptor antagonist that reduces levodopa-induced dyskinesia in the MPTP-lesioned primate model of Parkinson's disease. Mov Disord 18:872–883
47. Fox SH, Lang AE, Brotchie JM (2006) Translation of nondopaminergic treatments for levodopa-induced dyskinesia from MPTP-lesioned nonhuman primates to phase IIa clinical studies: keys to success and roads to failure. Mov Disord 21:1578–1594
48. Gregoire L, Samadi P, Graham J, Bedard PJ, Bartoszyk GD, Di Paolo T (2009) Low doses of sarizotan reduce dyskinesias and maintain antiparkinsonian efficacy of L-Dopa in parkinsonian monkeys. Parkinsonism Relat Disord 15:445–452
49. Pienaar IS, Dexter D, Burkhard P (2010) Mitochondrial proteomics as a selective tool for unravelling Parkinson's disease pathogenesis. Expert Rev Prot 7:205–226
50. Schapira AH, Cooper JM, Dexter D, Jenner P, Clark JB, Marsden CD (1989) Mitochondrial complex I deficiency in Parkinson's disease. Lancet 1:1269
51. Schapira AH, Cooper JM, Dexter D, Clark JB, Jenner P, Marsden CD (1990) Mitochondrial complex I deficiency in Parkinson's disease. J Neurochem 54:823–827
52. Schapira AH, Mann VM, Cooper JM, Dexter D, Daniel SE, Jenner P, Clark JB, Marsden CD (1990) Anatomic and disease specificity of NADH CoQ1 reductase (complex I) deficiency in Parkinson's disease. J Neurochem 55:2142–2145
53. Schapira AH (2008) Mitochondria in the aetiology and pathogenesis of Parkinson's disease. Lancet Neurol 7:97–109
54. Schapira AH, Gegg M (2011) Mitochondrial contribution to Parkinson's disease pathogenesis. Parkinsons Dis 2011:159–160
55. Parker WD, Parks JK, Swerdlow RH (2008) Complex I deficiency in Parkinson's disease frontal cortex. Brain Res 1189:215–218
56. Lin MT, Beal MF (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443:787–795
57. Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC (2003) Alpha-synuclein is degraded by both autophagy and the proteasome. J Biol Chem 278:25009–25013
58. Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D (2004) Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. Science 305:1292–1295
59. Vogiatzi T, Xilouri M, Vekrellis K, Stefanis L (2008) Wild type alpha-synuclein is degraded by chaperone-mediated autophagy and macroautophagy in neuronal cells. J Biol Chem 283:23542–23556
60. Greenamyre JT, Betarbet R, Sherer TB (2003) The rotenone model of Parkinson's disease: genes, environment and mitochondria. Parkinsonism Relat Disord 9:S59–S64
61. Panov A, Dikalov S, Shalbuyeva N, Taylor G, Sherer T, Greenamyre JT (2005) Rotenone model of Parkinson disease: multiple brain mitochondria dysfunctions after short-term systemic rotenone intoxication. J Biol Chem 280:42026–42035
62. Przedborski S, Vila M (2003) The 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine mouse model: a tool to explore the pathogenesis of Parkinson's disease. Ann N Y Acad Sci 991:189–198

63. Przedborski S, Tieu K, Perier C, Vila M (2004) MPTP as a mitochondrial neurotoxic model of Parkinson's disease. J Bioenerg Biomembr 36:375–379
64. Bogaerts V, Theuns J, Broeckhoven C (2008) Genetic findings in Parkinson's disease and translation into treatment: a leading role for mitochondria? Genes Brain Behav 7:129–151
65. Pilsl A, Winklhofer KF (2011) Parkin, PINK1 and mitochondrial integrity: emerging concepts of mitochondrial dysfunction in Parkinson's disease. Acta Neuropathol (in press)
66. Abramov AY, Gegg M, Grunewald A, Wood NW, Klein C, Schapira AH (2011) Bioenergetic consequences of PINK1 mutations in Parkinson's disease. PLoS One 6:e25622
67. Beilina A, Van Der Brug M, Ahmad R, Kesavapany S, Miller DW, Petsko GA, Cookson MR (2005) Mutations in PTEN-induced putative kinase 1 associated with recessive parkinsonism have differential effects on protein stability. Proc Natl Acad Sci U S A 102:5703–5708
68. Cai Q, Davis ML, Sheng ZH (2011) Regulation of axonal mitochondrial transport and its impact on synaptic transmission. Neurosci Res 70:9–15
69. McNaught KS, Shasidharan P, Perl DP, Jenner P, Olanow CW (2002) Aggresome-related biogenesis of Lewy bodies. Eur J Neurosci 16:2136–2148
70. Xie W, Li X, Li C, Zhu W, Jankovic J, Le W (2010) Proteasome inhibition modelling nigral neuron degeneration in Parkinson's disease. J Neurochem 115:188–199
71. Dawson TM, Dawson VL (2003) Rare genetic mutations shed light on the pathogenesis of Parkinson disease. J Clin Invest 111:145–151
72. Upadhya SC, Hegde AN (2003) A potential proteasome-interacting motif within the ubiquitin-like domain of parkin and other proteins. Trends Biochem Sci 28:280–283
73. Zhang Y, Gao J, Chung KK, Huang H, Dawson VL, Dawson TM (2000) Parkin functions as an E2-dependent ubiquitin-protein ligase and promotes the degradation of the synaptic vesicle associated protein, CDCrel-1. Proc Natl Acad Sci U S A 97:13354–13359
74. Liu Y, Fallon L, Lashuel HA, Liu Z, Lansbury PT Jr (2002) The UCH-L1 gene encodes two opposing enzymatic activities that affect alpha-synuclein degradation and Parkinson's disease susceptibility. Cell 111:209–218
75. Martin I, Dawson VL, Dawson TM (2011) Recent advances in the genetics of Parkinson's disease. Annu Rev Genomics Hum Genet 12:301–325
76. Hatano T, Kubo S, Sato S, Hattori N (2009) Pathogenesis of familial Parkinson's disease: new insights based on monogenic forms of Parkinson's disease. J Neurochem 111:1075–1093
77. Crosiers D, Theuns J, Cras P, Van Broeckhoven C (2011) Parkinson disease: insights in clinical, genetic and pathological features of monogenic disease subtypes. J Chem Neuroanat 42:131–141
78. Ramirez A, Heimbach A, Grundemann J, Stiller B, Hampshire D, Cid LP, Goebel I, Mubaidin AF et al (2006) Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. Nat Genet 38:1184–1191
79. Hardy J, Lewis P, Revesz T, Lees A, Paisan-Ruiz C (2009) The genetics of Parkinson's disease: a critical review. Curr Opin Genet Dev 19:254–265
80. Shulman JM, De Jager PL, Feany MB (2011) Parkinson's disease: genetics and pathogenesis. Annu Rev Patol 6:193–222
81. Paisan-Ruiz C, Bhatia KP, Li A, Hernandez D, Davis M, Wood NW, Hardy J, Houlden H et al (2009) Characterisation of PLA2G6 as a locus for dystonia-parkinsonism. Ann Neurol 65:19–23
82. Shojaee S, Sina F, Banihosseini SS, Kazemi MH, Kalhor R, Shahidi GA, Fakhrai-Rad H, Ronaghi M et al (2008) Genome-wide linkage analysis of a parkinsonian-pyramidal syndrome pedigree by 500 K SNP arrays. Am J Hum Genet 82:1375–1384
83. Di Fonzo A, Dekker MC, Montagna P, Baruzzi A, Yonova EH, Correia Guedes L, Szczerbinska A, Zhao T et al (2009) FBXO7 mutations cause autosomal recessive, early-onset parkinsonian-pyramidal syndrome. Neurology 72:240–245
84. Pankratz N, Nichols WC, Uniacke SK, Halter C, Rudolph A, Shults C, Conneally PM, Foroud T, Parkinson Study Group (2003) Significant linkage of Parkinson disease to chromosome 2q36-37. Am J Hum Genet 72:1053–1057
85. Strauss KM, Martins LM, Plun-Favreau H, Marx FP, Kautzmann S, Berg D, Gasser T, Wszolek Z et al (2005) Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease. Hum Mol Genet 14:2099–2111
86. Lautier C, Goldwurm S, Dürr A, Giovannone B, Tsiaras WG, Pezzoli G, Brice A, Smith RJ (2008) Mutations in the GIGYF2 (TNRC15) gene at the PARK11 locus in familial Parkinson disease. Am J Hum Genet 82:822–833
87. Spillantini MG, Schmidt ML, Lee VMY, Trojanowski JQ, Jales R, Goedert M (1997) Alpha-synuclein in Lewy bodies. Nature 6645:839–840
88. Baba M, Nakajo S, Tu PH, Tomita T, Nakay K, Lee VM, Trojanowski JQ, Iwatsubo T (1998) Aggregation of α-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. Am J Pathol 152:879–884
89. Duda JE, Giasson BI, Chen Q, Gur TL, Hurtig HI, Stern MB, Gollomp SM, Ischiropoulos H, Lee VM, Trojanowski JQ (2000) Widespread nitration of pathological inclusions in neurodegenerative synucleinopathies. Am J Pathol 157:1439–1445
90. Giasson BI, Duda JE, Murray IV, Chen Q, Souza JM, Hurtig HI, Ischiropoulos H, Trojanowski JQ et al (2000) Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. Science 290:985–989
91. Fujiwara H, Hasegawa M, Dohmae N, Kawashima A, Masliah E, Goldberg MS, Shen J, Takio K et al (2002) Alpha-synuclein is phosphorylated in synucleinopathy lesions. Nat Cell Biol 4:160–164
92. Anderson JP, Walker DE, Goldstein JM, de Laat R, Banducci K, Caccavello RJ, Barbour R, Huang J et al (2006) Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. J Biol Chem 281:29739–29752
93. Danielson SR, Held JM, Schilling B, Oo M, Gibson BW, Andersen JK (2009) Preferentially increased nitration of alpha-synuclein at tyrosine-39 in a cellular oxidative model of Parkinson's disease. Anal Chem 81:7823–7828
94. Shavali S, Combs CK, Ebadi M (2006) Reactive macrophages increase oxidative stress and alpha-synuclein nitration during death of dopaminergic neuronal cells in co-culture: relevance to Parkinson's disease. Neurochem Res 31:85–94
95. Castellani R, Smith MA, Richey PL, Perry G (1996) Glycoxidation and oxidative stress in Parkinson disease and diffuse Lewy body disease. Brain Res 737:195–200
96. Abeliovich A, Schmitz Y, Fariñas I, Choi-Lundberg D, Ho WH, Castillo PE, Shinsky N, Verdugo JM et al (2000) Mice lacking alpha-synuclein display functional deficits in the nigrostraiatal dopamine system. Neuron 25:239–252
97. Fallon L, Moreau F, Croft BG, Labib N, Gu WJ, Fon EA (2002) Parkin and CASK/LIN-2 associate via a PDZ-mediated interaction and are co-localized in lipid rafts and post-synaptic densities in brain. J Biol Chem 277:486–491
98. Hatano T, Kubo S, Imai S, Maeda M, Ishikawa K, Mizuno Y, Hattori N (2007) Leucine-rich repeat kinase 2 associates with lipid rafts. Hum Mol Genet 16:678–690

99. Kahle PJ, Neumann M, Ozmen L, Muller V, Jacobsen H, Schindzielorz A, Okochi M, Leimer U et al (2000) Subcellular localization of wild-type and Parkinson's disease-associated mutant alpha-synuclein in human and transgenic mouse brain. J Neurosci 20:6365–6373
100. Kubo SI, Kitami T, Noda S, Shimura H, Uchiyama Y, Asakawa S, Minoshima S, Shimizu N et al (2001) Parkin is associated with cellular vesicles. J Neurochem 78:42–54
101. Cookson MR (2005) The biochemistry of Parkinson's disease. Annu Rev Biochem 74:29–52
102. Rieker C, Kumlesh KD, Lehnhoff K, Barbieri S, Ksiazek I, Kauffmann S, Danner S, Schnell H et al (2011) Neuropathology in mice expressing mouse alpha-synuclein. PLoS One 6:e24834
103. Van der Putten H, Wiederhold KH, Probst A, Barbieri S, Mistl C, Danner S, Kauffmann S, Hofele K et al (2000) Neuropathology in mice expressing human alpha-synuclein. J Neurosci 20:6021–6029
104. Iwai A (2000) Properties of NACP/α-synuclein and its role in Alzhemer's disease. Biochim Biophys Acta 1502:95–109
105. Kazantsev AG, Kolchonsky AM (2008) Central role of alpha-synuclein oligomers in neurodegeneration in Parkinson's disease. Arch Neurol 65:1577–1581
106. Dawson-Scully K, Lin Y, Imad M, Zhang J, Marin L, Horne JA, Meinertzhagen IA, Karunanithi S et al (2007) Morphological and functional effects of altered cysteine string protein at the Drosophila larval neuromuscular junction. Synapse 61:1–16
107. Fernandez-Chacon R, Wolfel M, Nishimune H, Tabares L, Schmitz F, Castellano-Munoz M, Rosenmund C, Montesinos ML et al (2004) The synaptic vesicle protein CSP alpha prevents presynaptic degeneration. Neuron 42:237–251
108. Bruinsma IB, Bruggink KA, Kinast K, Versleijen AA, Segers-Nolten IM, Subramaniam V, Kuiperij HB, Boelens W et al (2011) Inhibition of α-synuclein aggregation by small heat shock proteins. Proteins 79:2956–2967
109. Auluck PK, Chan HY, Trojanowski JQ, Lee VM, Bonini NM (2002) Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson's disease. Science 295:865–868
110. Klucken J, Shin Y, Masliah E, Hyman BT, McLean PJ (2004) Hsp70 reduces alpha-synuclein aggregation and toxicity. J Biol Chem 279:25497–25502
111. Cantuti-Castelvetri I, Klucken J, Ingelsson M, Ramasamy K, McLean PJ, Frosch MP, Hyman BT, Standaert DG (2005) Alpha-synuclein and chaperones in dementia with Lewy bodies. J Neuropathol Exp Neurol 64:1058–1066
112. McLean PJ, Kawamata H, Shariff S, Hewett J, Sharma N, Ueda K, Beakefield XO, Hyman BT (2002) TorsinA and heat shock proteins act as molecular chaperones: suppression of alpha-synuclein aggregation. J Neurochem 83:846–854
113. Shin N, Jeong H, Kwon J, Heo HY, Kwon JJ, Yun HJ, Kim CH, Han BS et al (2008) LRRK2 regulates synaptic vesicle endocytosis. Exp Cell Res 314:2055–2065
114. Couteaux R, Pécot-Dechavassine M (1970) Synaptic vesicles and pouches at the level of "active zone" of the neuromuscular junction. C R Acad Sci Hebd Seances Acad Sci D 271:2346–2349
115. Südhof TC (2004) The synaptic vesicle cycle. Annu Rev Neurosci 27:509–547
116. Kavalali ET (2006) Synaptic vesicle reuse and its implications. Neuroscientist 12:57–66
117. Piccoli G, Condliffe SB, Bauer M, Giesert F, De Astis S, Meixner A, Sarioqlu H, Vogt-Weisenhorn DM et al (2011) LRRK2 controls synaptic vesicle storage and mobilization within the recycling pool. J Neurosci 31:2225–2237
118. Takamori S, Holt M, Stenius K, Lemke EA, Grønborg M, Riedel D, Urlaub H, Schenck S et al (2006) Molecular anatomy of a trafficking organelle. Cell 127:831–846
119. George JH, Jin H, Woods WS, Clayton DF (1995) Characterization of a novel protein regulated during the critical period for song learning in the zebra finch. Neuron 15:361–372
120. Schultz-Schaeffer WJ (2010) The synaptic pathology of alpha-synuclein aggregation in dementia with Lewy bodies, Parkinson's disease and Parkinson's disease dementia. Acta Neuropathol 120:131–143
121. Cabin DE, Shimazu K, Murphy D, Cole NB, Gottschalk W, McIlwain KL, Orrison B, Chen A et al (2002) Synaptic vesicle depletion correlates with attenuated synaptic responses to prolonged repetitive stimulation in mice lacking alpha-synuclein. J Neurosci 22:8797–8807
122. Nemani VM, Berge V, Nakamura K, Onoa B, Lee MK, Chaudhry FA, Nicoll RA, Edwards RH (2010) Increased expression of alpha-synuclein reduces neurotransmitter release by inhibiting synaptic vesicle reclustering after endocytosis. Neuron 65:66–79
123. Hilfiker S, Pieribone VA, Czernik AJ, Kao HT, Augustine GJ, Greengard P (1999) Synapsins are regulators of neurotransmitter release. Philos Trans R Soc Lond B Biol Sci 354:269–279
124. Dalfó E, Barrachina M, Rosa JL, Ambrosio S, Ferrer I (2004) Abnormal alpha-synuclein interactions with rab3a and rabphilin in diffuse Lewy body disease. Neurobiol Dis 16:92–97
125. Nonet ML, Stauton JE, Kilgard MP, Fergestad T, Hartwieg E, Horvitz HR, Jorgersen EM, Meyer BJ (1997) Caenorhabditis elegans rab-3 mutant synapses exhibit impaired function and are partially depleted of vesicles. J Neurosci 17:8061–8063
126. Geppert M, Bolshakov VY, Siegelbaum SA, Takei K, De Camilli P, Hammer RE, Südhof TC (1994) The role of Rab3A in neurotransmitter release. Nature 369:493–497
127. Li C, Takei K, Geppert M, Daniell L, Stenius K, Chapman ER, Jahn R, De Camilli P et al (1994) Synaptic targeting of rabphilin-3A, a synaptic vesicle Ca^{2+}/phospholipid-binding protein, depends on Rab3A/C. Neuron 13:885–898
128. Chung SH, Takai Y, Holz RW (1995) Evidence that the rab3a-binding protein, rabphilin 3a, enhances regulated secretion. Studies in adrenal cromaffin cells. J Biol Chem 270:16714–16719
129. Dalfó E, Gómez-Isla T, Rosa JL, Nieto Bodelón M, Cuadrado Tejedor M, Barrachina M, Ambrosio S, Ferrer I (2004) Abnormal alpha-synuclein interactions with Rab proteins in alpha-synuclein A30P transgenic mice. J Neuropathol Exp Neurol 63:302–313
130. Dalfó E, Albasanz JL, Martin M, Ferrer I (2004) Abnormal metabotropic glutamate receptor expression and signaling in the cerebral cortex in diffuse Lewy body disease is associated with irregular alpha-synuclein/phospholipase C (PLCbeta1) interactions. Brain Pathol 14:388–398
131. Südhof TC, Rothman JE (2009) Membrane fusion: grappling with SNARE and SM proteins. Science 323:474–477
132. Chandra S, Gallardo G, Fernandez-Chacon R, Schluter OM, Sudhof TC (2005) Alpha-synuclein cooperates with CSP-alpha in preventing neurodegeneration. Cell 123:383–396
133. Garcia-Reitböck P, Anichtchik O, Belluci A, Lovino M, Ballini C, Fineberg E, Ghetti B, Della Corte L et al (2010) SNARE protein redistribution and synaptic failure in a transgenic mouse model of Parkinson's disease. Brain 133:2032–2044
134. Liu CW, Giasson BI, Lewis KA, Lee VM, DeMartino GN, Thomas PJ (2005) A precipitating role for truncated α-synuclein and the proteasome in α-synuclein aggregation: implications for pathogenesis of Parkinson disease. J Biol Chem 280:22670–22678
135. Lees AJ, Hardy J, Revesz T (2009) Parkinson's disease. Lancet 373:2055–2066
136. Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, Shen J, Cookson MR, Youle RJ (2010) PINK1 is selectively stabilized on impaired mitochondria to activate parkin. PLoS Biol 8:1000298

137. Kitada T, Pisani A, Porter DR, Yamaguchi H, Tscherter A, Martella G, Bonsi P, Zhang C et al (2007) Impaired dopamine release and synaptic plasticity in the striatum of PINK1-deficient mice. Proc Natl Acad Sci U S A 104:11441–11446
138. Shepherd GM, Harris KM (1998) Three-dimensional structure and composition of CA3–>CA1 axons in rat hippocampal slices: implications for presynaptic connectivity and compartmentalization. J Neurosci 18:8300–8310
139. Morais VA, Verstreken P, Roethig A, Smet J, Snellinx A, Vanbrabant M, Haddad D, Frezza C et al (2009) Parkinson's disease mutations in PINK1 result in decreased Complex I activity and deficient synaptic function. EMBO Mol Med 1:99–111
140. Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, Dekker MC, Squitieri F et al (2003) Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science 299:256–259
141. Abou-Sleiman PM, Healy DG, Quinn N, Lees AJ, Wood NW (2003) The role of pathogenic DJ-1 mutations in Parkinson's disease. Ann Neurol 54:283–286
142. Djarmati A, Hedrich K, Svetel M, Schafer N, Juric V, Vukosavic S, Hering R, Riess O et al (2004) Detection of parkin (PARK2) and DJ1 (PARK7) mutations in early-onset Parkinson disease: parkin mutation frequency depends on ethnic origin of patients. Hum Mutat 23:525
143. Hague S, Rogaeva E, Hernandez D, Gulick C, Singleton A, Hanson M, Johnson J, Weiser R et al (2003) Early-onset Parkinson's disease caused by a compound heterozygous DJ-1 mutation. Ann Neurol 5(4):271–274
144. Hering R, Strauss KM, Tao X, Bauer A, Woitalla D, Mietz EM, Petrovic S, Bauer P et al (2004) Novel homozygous pE64D mutation in DJ1 in early onset Parkinson disease (PARK7). Hum Mutat 24:321–329
145. Macedo MG, Verbaan D, Fang Y, van Rooden SM, Visser M, Anar B, Uras A, Groen JL et al (2009) Genotypic and phenotypic characteristics of Dutch patients with early onset Parkinson's disease. Mov Disord 24:196–203
146. Tarantino P, Civitelli D, Annesi F, De Marco EV, Rocca FE, Pugliese P, Nicoletti G, Carrideo S et al (2009) Compound heterozygosity in DJ-1 gene non-coding portion related to parkinsonism. Parkinsonism Relat Disord 15:324–326
147. Clark LN, Afridi S, Mejia-Santana H, Harris J, Louis ED, Cote LJ, Andrews H, Singleton A et al (2004) Analysis of an early-onset Parkinson's disease cohort for DJ-1 mutations. Mov Disord 19:796–800
148. Hedrich K, Djarmati A, Schafer N, Hering R, Wellenbrock C, Weiss PH, Hilker R, Vieregge P et al (2004) DJ-1 (PARK7) mutations are less frequent than parkin (PARK2) mutations in early-onset Parkinson disease. Neurology 62:389–394
149. Ibanez P, De Michele G, Bonifati V, Lohmann E, Thobois S, Pollak P, Agid Y, Heutink P et al (2003) Screening for DJ-1 mutations in early onset autosomal recessive parkinsonism. Neurology 61:1429–1431
150. Lockhart PJ, Lincoln S, Hulihan M, Kachergus J, Wilkes K, Bisceglio G, Mash DC, Farrer MJ (2004) DJ-1 mutations are a rare cause of recessively inherited early onset parkinsonism mediated by loss of protein function. J Med Genet 41:e22
151. Mellick GD, Siebert GA, Funayama M, Buchanan DD, Li Y, Imamichi Y, Yoshino H, Silburn PA et al (2009) Screening PARK genes for mutations in early-onset Parkinson's disease patients from Queensland, Australia. Parkinsonism Relat Disord 15:105–109
152. Pankratz N, Pauciulo MW, Elsaesser VE, Marek DK, Halter CA, Wojcieszek J, Rudolph A, Shults CW et al (2006) Mutations in DJ-1 are rare in familial Parkinson disease. Neurosci Lett 408:209–213
153. Tan EK, Tan C, Zhao Y, Yew K, Shen H, Chandran VR, Teoh ML, Yih Y et al (2004) Genetic analysis of DJ-1 in a cohort Parkinson's disease patients of different ethnicity. Neurosci Lett 367:109–112
154. Miller DW, Ahmad R, Hague S, Baptista MJ, Canet-Aviles R, McLendon C, Carter DM, Zhu PP et al (2003) L166P mutant DJ-1, causative for recessive Parkinson's disease, is degraded through the ubiquitin–proteasome system. J Biol Chem 278:36588–36595
155. Zhang L, Shimoji M, Thomas B, Moore DJ, Yu SW, Marupudi NI, Torp R, Torgner IA et al (2005) Mitochondrial localization of the Parkinson's disease related protein DJ-1: implications for pathogenesis. Hum Mol Genet 14:2063–2073
156. Blackinton J, Lakshminarasimhan M, Thomas KJ, Ahmad R, Greggio E, Raza AS, Cookson MR, Wilson MA (2009) Formation of a stabilized cysteine sulfinic acid is critical for the mitochondrial function of the parkinsonism protein DJ-1. J Biol Chem 284:6476–6485
157. Canet-Aviles RM, Wilson MA, Miller DW, Ahmad R, McLendon C, Bandyopadhyay S, Baptista MJ, Ringe D et al (2004) The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization. Proc Natl Acad Sci U S A 101:9103–9108
158. Taira T, Iguchi-Ariga SM, Ariga H (2004) Co-localization with DJ-1 is essential for the androgen receptor to exert its transcription activity that has been impaired by androgen antagonists. Biol Pharm Bull 27:574–577
159. Lee SJ, Kim SJ, Kim IK, Ko J, Jeong CS, Kim GH, Park C, Kang SO et al (2003) Crystal structures of human DJ-1 and *Escherichia coli* Hsp31, which share an evolutionarily conserved domain. J Biol Chem 278:44552–44559
160. Kim RH, Peters M, Jang Y, Shi W, Pintilie M, Fletcher GC, DeLuca C, Liepa J et al (2005) DJ-1, a novel regulator of the tumor suppressor PTEN. Cancer Cell 7:263–273
161. Niki T, Takahashi-Niki K, Taira T, Iguchi-Ariga SM, Ariga H (2003) DJBP: a novel DJ-1 binding protein, negatively regulates the androgen receptor by recruiting histone deacetylase complex, and DJ-1 antagonizes this inhibition by abrogation of this complex. Mol Cancer Res 1:247–261
162. Shinbo Y, Taira T, Niki T, Iguchi-Ariga SM, Ariga H (2005) DJ-1 restores p53 transcriptional activity inhibited by Topors/p53BP3. Int J Oncol 26:641–648
163. Takahashi K, Taira T, Niki T, Seino C, Iguchi-Ariga SM, Ariga H (2001) DJ-1 positively regulates the androgen receptor by impairing the binding of PIASx alpha to the receptor. J Biol Chem 276:37556–37563
164. Olzmann JA, Bordelon JR, Muly EC, Rees HD, Levey AI, Li L, Chin LS (2007) Selective enrichment of DJ-1 protein in primate striatal neuronal processes: implications for Parkinson's disease. J Comp Neurol 500:585–599
165. Usami Y, Hatano T, Imai S, Kubo S-I, Sato S, Saiki S, Fujioka Y, Ohba Y et al (2011) DJ-1 associates with synaptic membranes. Neurobiol Dis 43:651–662
166. Harald Stenmark VMO (2011) The Rab GTPase family. Genome Biol 2:1-3007.1–1-3007.7
167. Goldberg MS, Pisani A, Haburcak M, Vortherms TA, Kitada T, Costa C, Tong Y, Martella G et al (2005) Nigrostraital dopaminergic deficits and hypokinesia caused by inactivation of the familial parkinsonism-linked gene DJ-1. Neuron 45:489–496
168. Hummel T, Krukkert K, Roos J, Davis G, Klämbt C (2000) Drosophila Futsch/22C10 is a MAP1B-like protein required for dendritic and axonal development. Neuron 26:357–370
169. Roos J, Hummel T, Ng N, Klämbt C, Davis GW (2000) Drosophila Futsch regulates synaptic microtubule organization and is necessary for synaptic growth. Neuron 26:371–382

170. Lee S, Liu H-P, Lin W-Y, Guo H, Lu B (2010) LRRK2 kinase regulates synaptic morphology through distinct substrates at the presynaptic and postsynaptic compartments of the Drosophila neuromuscular junction. J Neurosci 30:16959–16969
171. Hornykiewicz O, Kish SJ (1986) Biochemical pathophysiology of Parkinson's disease. In: Yahr MD, Bergmann KJ (eds) Advances in neurology. Raven Press, New York, pp 19–34
172. Zigmond MJ, Abercrombie ED, Grace AA, Stricker EM (1990) Compensations after lesions of central dopaminergic neurons: some clinical and basic implications. TINS 13:290–295
173. Altar CA, Marien MR, Marshall JF (1987) Time course of adaptations in dopamine biosynthesis, metabolism, and release following nigrostriatal lesions: implications for behavioral recovery from brain injury. J Neurochem 48:390–399
174. Onn SP, Berger TW, Stricker EM, Zigmond MJ (1986) Effects of intraventricular 6-hydroxydopamine on the dopaminergic innervation of striatum: histochemical and neurochemical analysis. Brain Res 376:8–19
175. Wolf ME, Zigmond MJ, Kapatos G (1989) Tyrosine hydroxylase content of residual striatal dopamine nerve terminals following 6-hydroxydopamine administration: a flow cytometric study. J Neurochem 53:879–885
176. Zigmond MJ, Acheson AL, Stachowiak MK, Stricker EM (1984) Neurochemical compensation after nigrostriatal bundle injury in an animal model of preclinical parkinsonisms. Arch Neurol 41:856–861
177. Raju DV, Ahern TH, Shah DJ, Wright TM, Standaert DG, Hall RA, Smith Y (2008) Differential synaptic plasticity of the corticostriatal and thalamostriatal systems in an MPTP-treated monkey model of parkinsonism. Eur J Neurosci 27:1647–1658
178. Wiedenmann B, Franke WW (1985) Identification and localization of synaptophysin, an integral membrane glycoprotein of Mr 38 000 characteristic of presynaptic vesicles. Cell 41:1017–1028
179. Bolam J, Hanley J, Booth P (2000) Synaptic organisation of the basal ganglia. J Anat 196:527–542
180. Graybiel A (1990) Neurotransmitters and neuromodulators in the basal ganglia. Trends Neurosci 13:244–254
181. Tian X, Kai L, Hockberger PE, Wokosin DL, Surmeier DJ (2010) MEF-3 regulates activity-dependent spine loss in striatopallidal medium spiny neurons. Mol Cell Neurosci 44:94–108
182. Turrigiano GG (2008) The self-tuning neuron: synaptic scaling of excitatory synapses. Cell 135:422–435
183. Day M, Wang Z, Ding J, An X, Ingham CA, Shering AF, Wokosin D, Ilijic E et al (2006) Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. Nat Neurosci 9:251–259
184. Steward O, Wallace CS, Lyford GL, Worley PF (1998) Synaptic activation causes the mRNA for the IEG Arc to localize selectively near activated postsynaptic sites on dendrites. Neuron 21:741–751
185. Imai Y, Gehrke S, Wang HQ, Takahashi R, Hasegawa K, Oota E, Lu B (2008) Phosphorylation of 4E-BP by LRRK2 affects the maintenance of dopaminergic neurons in Drosophila. EMBO J 27:2432–2443
186. Gillardon F (2009) Leucine-rich repeat kinase 2 phosphorylates brain tubulin-beta isoforms and modulates microtubule stability: a point of convergence in parkinsonian neurodegeneration? J Neurochem 110:1514–1522
187. Gloeckner CJ, Kinkl N, Schumacher A, Braun RJ, O'Neill E, Meitinger T, Kolch W, Prokisch H et al (2006) The Parkinson disease causing LRRK2 mutation I2020T is associated with increased kinase activity. Hum Mol Genet 15:223–232
188. Smith WW, Pei Z, Jiang H, Dawson VL, Dawson TM, Ross CA (2006) Kinase activity of mutant LRRK2 mediates neuronal toxicity. Nat Neurosci 9:1231–1233
189. Jaleel M, Nichols RJ, Deak M, Campbell DG, Gillardon F, Knebel A, Alessi DR (2007) LRRK2 phosphorylates moesin at threonine-558: characterization of how Parkinson's disease mutants affect kinase activity. Biochem J 405:307–317
190. MacLeod D, Dowman J, Hammond R, Leete T, Inoue K, Abeliovich A (2006) The familial parkinsonism gene LRRK2 regulates neurite process morphology. Neuron 52:587–593
191. Gillardon F (2009) Interaction of elongation factor 1-alpha with leucinerich repeat kinase 2 impairs kinase activity and microtubule bundling in vitro. Neuroscience 163:533–539
192. Biskup S, Moore DJ, Celsi F, Higashi S, West AB, Andrabi SA, Kurkinen K, Yu S-W et al (2006) Localization of LRRK2 to membranous and vesicular structures in mammalian brain. Ann Neurol 60:557–569
193. Moore RC, Durso NA, Cyr RJ (1998) Elongation factor-1alpha stabilizes microtubules in a calcium/calmodulin-dependent manner. Cell Motil Cytoskeleton 41:168–180
194. Wang MS, Fang G, Culver DG, Davis AA, Rich MM, Glass JD (2001) The Wld^s protein protects against axonal degeneration: a model of gene therapy for peripheral neuropathy. Ann Neurol 50:773–779
195. Sajadi A, Schneider BL, Aebischer P (2004) Wlds-mediated protection of dopaminergic fibers in an animal model of Parkinson disease. Curr Biol 14:326–330
196. Wishart TM, Paterson JM, Short DM, Meredith S, Robertson KA, Sutherland C, Cousin MA, Dutia MB et al (2007) Differential proteomics analysis of synaptic proteins identifies potential cellular targets and protein mediators of synaptic neuroprotection conferred by the slow Wallerian degeneration (Wld^s) gene. Mol Cell Proteomics 6:1318–1330
197. Lue LF, Kuo YM, Roher AE, Brachova L, Shen Y, Sue L, Beach T, Kurth JH et al (1999) Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. Am J Pathol 155:853–862