An ERcentric view of Parkinson's disease

Gabriela Mercado[1,2], Pamela Valdés[1,2], and Claudio Hetz[1,2,3,4]

[1] Biomedical Neuroscience Institute, Faculty of Medicine, University of Chile, Santiago, Chile
[2] Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, Santiago, Chile
[3] Neurounion Biomedical Foundation, Santiago, Chile
[4] Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA, USA

Parkinson's disease (PD) is the second most common neurodegenerative disease and is characterized by the selective loss of dopaminergic neurons of the substantia nigra pars compacta and the accumulation of intracellular inclusions containing α-synuclein (αSyn). Growing evidence from studies in human PD brain, in addition to genetic and toxicological models, indicates that endoplasmic reticulum (ER) stress is a common feature of the disease and contributes to neurodegeneration. Recent reports place ER dysfunction as an early component of PD pathogenesis, and in this article we review the impact of ER stress in PD models and discuss the multiple mechanisms underlying the perturbation of secretory pathway function. Possible therapeutic strategies to mitigate ER stress in the context of PD are also discussed.

Parkinson's disease

PD is an irreversible and progressive neurodegenerative disorder that impairs movement control. It is characterized by the appearance of several motor symptoms including rigidity, resting tremor, bradykinesia (see Glossary), and postural instability. The pathological hallmarks underlying the clinical manifestation of the disease are generated, in part, because of the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). The presence of intracellular inclusions, termed Lewy bodies (LBs), is a histopathological feature of the disease [1]; fibrillar aggregates of post-translationally modified (ubiquitinated, phosphorylated, and/or *S*-nitrosylated) αSyn constitute a major component of these protein deposits [2,3]. PD is the second most common age-related neurodegenerative disease, affecting 1% of the population over 60 years of age [4]. Aging is the major risk factor for developing PD, and its incidence increases with age: PD affects 0.6% of the population who are 65–69 years old and 2.6% of people between 85 and 89 years of age [4].

Neuronal loss in PD results in a severe and gradual depletion of dopamine content in the striatum, a phenomena that is responsible for the motor symptoms [1]. The SNpc neurons form the nigrostriatal dopaminergic circuit, which controls voluntary movements via the release of dopamine by these cells. High levels of dopamine promote motor activity, whereas low levels demand greater effort for any given movement [5]. The net consequence of dopamine depletion in PD is hypokinesia, an overall reduction of motor outputs. Levodopa is a palliative treatment for PD that increases overall dopamine levels, but it can produce excessive neuronal activity, generating dyskinesia. There is no cure for PD, but surgery, medications, and a proper multidisciplinary treatment provide temporal relief from disease symptoms. Despite

Glossary

Akinesia: the inability to initiate movement due to difficulty selecting and/or activating motor programs in the central nervous system. This disease sign is a result of a severe decrease in dopaminergic neuron activity.
Bradykinesia: the symptom of slow movements. Instead of being a slowness in initiation (akinesia), bradykinesia describes an alteration in the execution of movements.
Endoplasmic reticulum-associated degradation (ERAD): a protein-degradation pathway that targets misfolded proteins from the ER to the cytosol followed by ubiquitination and subsequent degradation mediated by the proteasome.
ER stress: the cellular condition involving the accumulation of misfolded/unfolded proteins at the ER. ER stress can be triggered by perturbations in protein maturation, disrupted ER calcium homeostasis, altered redox metabolism, high demand for protein folding and secretion, and expression of mutant proteins, among other stimuli. ER stress activates UPR stress sensors to adapt to stress or trigger apoptosis of irreversibly-damaged cells.
Hypokinesia: the decreased bodily movement observed in PD patients; hypokinesia is associated with basal ganglia alterations. Hypokinesia describes a spectrum of disorders including akinesia, bradykinesia, freezing rigidity, and postural instability
Macroautophagy: a catabolic process involved in the degradation of cellular components including protein aggregates and organelles through the lysosomal pathway.
MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine): a PD-inducing neurotoxin precursor that crosses the blood–brain barrier and is metabolized/oxidized by glial cells to 1-methyl-4-phenylpyridinium (MPP+). MPP+ is then released and taken up by dopaminergic neurons via dopamine transporters. MPP+ inhibits mitochondrial complex I, triggering oxidative stress and eventual cell death.
Parkinson's disease (PD)-inducing neurotoxins: a group of chemical compounds that induce Parkinsonism. These toxins trigger the selective death of dopaminergic neurons of the substantia nigra pars compacta, the main brain region affected in PD. Since their discovery as a cause of sporadic PD, these compounds are widely used as toxicological models of the disease in monkeys, rats, and mice. This group of compounds includes 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and the pesticides rotenone and paraquat.
Ubiquitin proteasome system (UPS): the major pathway of non-lysosomal proteolysis of intracellular proteins. The central event in this system is the covalent linkage of ubiquitin to targeted proteins, which are then recognized by the 26S proteasome for proteolytic degradation in the cytosol.
Unfolded protein response (UPR): a complex and integrated signal-transduction pathway that is activated in response to an accumulation of unfolded or misfolded proteins at the ER lumen. The UPR mediates the adaptation to protein-folding stress or the elimination of nonfunctional cells by apoptosis.

Corresponding author: Hetz, C. (chetz@med.uchile.cl, chetz@hsph.harvard.edu).
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intensive investigation, current treatments are palliative and are mostly focused on improving the quality of life of the patient – they do not stop the neurodegeneration in the brain.

Understanding the mechanisms involved in the selective neuronal vulnerability of dopaminergic neurons in PD is a primary subject of study in the field. Several pathogenic processes are proposed to contribute to dopaminergic neuron loss in PD, including oxidative stress, impaired calcium homeostasis, mitochondrial dysfunction, altered ER-to-Golgi trafficking, and altered mitophagy and proteasome function, among other events. Although more than 90% of PD cases are sporadic, mutations in several genes, the *PARK* loci, have been shown to trigger the development of familial parkinsonian syndromes. These inherited mutations only account for 5% of the total PD cases [6]. For example, mutations in the leucine-rich repeat serine/threonine kinase 2 gene (*LRRK2*/*PARK8*) occur in 1% to 2% of all PD cases, representing the most common known genetic cause of the disease. The most studied PD-related gene is αSyn (*SNCA*/*PARK1)*, in which point mutations (Ala53Thr, Ala30Pro and Glu46Lys), as well as duplication and triplication of the gene, cause autosomal-dominant PD [6]. LBs are enriched in misfolded αSyn, and for many years it was proposed that fibrillar and aggregated forms of the protein are the pathogenic species. However, recent evidence indicates that small oligomeric forms of αSyn are highly diffusible and neurotoxic. Moreover, cell-to-cell transfer and propagation of αSyn is emerging as a key event in the pathology, as demonstrated in cellular and animal models of PD (see below) in addition to human postmortem studies [7]. Mutation of the E3 ubiquitin ligase Parkin gene, *PARK2*, is the single most common genetic cause of recessive PD and could represent about 40% of cases with onset before the age of 40 years. Loss-of-function mutations in the Parkin locus cause high vulnerability to cellular stress, and the accumulation of one of its substrates, Pael-R, resulting in dopaminergic neuron loss [1]. Mutations in the mitochondrial serine/threonine protein kinase gene *PINK1/PARK6* may increase the susceptibility of cells to oxidative stress and apoptosis, and together with Parkin has been implicated in mitophagy [8].

The accumulation of misfolded proteins in the brain is a salient feature of most common neurodegenerative diseases including Alzheimer's disease, amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD), in addition to PD. These diseases are now classified as protein misfolding disorders (PMDs) [7]. Although the neuronal populations and specific neurological functions affected in PMDs are diverse, converging evidence suggests that alterations in the function of the ER are a common and salient feature of these diseases [9]. Although there is no consensus about possible common molecular events underlying sporadic and genetic forms of PD, novel evidence from different laboratories indicates a central contribution of ER stress to PD. In this review we discuss different models that explain the occurrence of ER stress in PD and summarize the data uncovering how this pathway might contribute to dopaminergic neuron loss. Therapeutic strategies to attenuate ER stress levels in a disease context are also discussed.

The unfolded protein response (UPR)

The homeostasis of the ER can be altered by a series of conditions including calcium depletion from its lumen, oxidative stress, and mutations in proteins that traffic through the secretory pathway, among other events. All of these perturbations can result in disruption of the folding process in the ER, leading to the accumulation of misfolded/unfolded proteins (ER stress). ER stress activates the UPR, a complex signal-transduction pathway that mediates cellular adaptation to restore ER homeostasis (reviewed in [10]). Under chronic ER stress the UPR triggers cell death by apoptosis, eliminating damaged cells.

In mammalian cells, the UPR is initiated by the activation of three distinct types of stress sensors located at the ER membrane: double-stranded RNA-activated protein kinase-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring kinase 1α (IRE1α). The mechanism of stress sensing by these proteins is poorly understood, but one of the most widely accepted models involves the recognition of unfolded proteins by the ER chaperone Grp78/Bip, leading to its dissociation from the sensors and the release of a repressive interaction. Once activated, these stress sensors transduce information about protein-folding status at the ER to the nucleus by controlling the expression of specific downstream transcription factors. Through this mechanism, the UPR upregulates a variety of target genes with functions in almost every aspect of the secretory pathway (Figure 1) [10].

IRE1α is a serine–threonine kinase and endoribonuclease that, upon activation, initiates the unconventional splicing of the mRNA encoding the transcription factor X-box binding protein-1 (XBP1). This splicing event shifts the coding reading frame and leads to the expression of a more stable and active transcription factor, termed XBP1s. XBP1s regulates a subset of UPR target genes related to folding, ER/Golgi biogenesis, and ER-associated degradation (ERAD) [10]. IRE1α also signals to the cytosol by binding adapter proteins, which triggers the activation of alarm pathways (i.e., JNK, ASK1, and NF-κB). These signaling events impact diverse processes such as autophagy, apoptosis, and inflammatory responses. In addition, IRE1α can selectively degrade mRNAs encoding for proteins that are predicted to be difficult to fold and microRNAs [10].

ATF6 is a membrane-spanning protein localized to the ER. Upon dissociation from Bip, ATF6 traffics to the Golgi and undergoes subsequent proteolytic processing to release the cytosolic domain, ATF6f, an active transcription factor. Cytosolic ATF6f is then imported into the nucleus and can induce expression of protein quality control genes, either independently of or synergistically with XBP1s.

Activated PERK phosphorylates eukaryotic initiation factor 2α (eIF2 α), resulting in a general attenuation of protein translation, which is one mechanism that decreases the overload of proteins at the ER. eIF2α phosphorylation allows the specific translation of activating transcription factor 4 (ATF4), which upregulates many important genes that function in redox control, metabolism, and folding

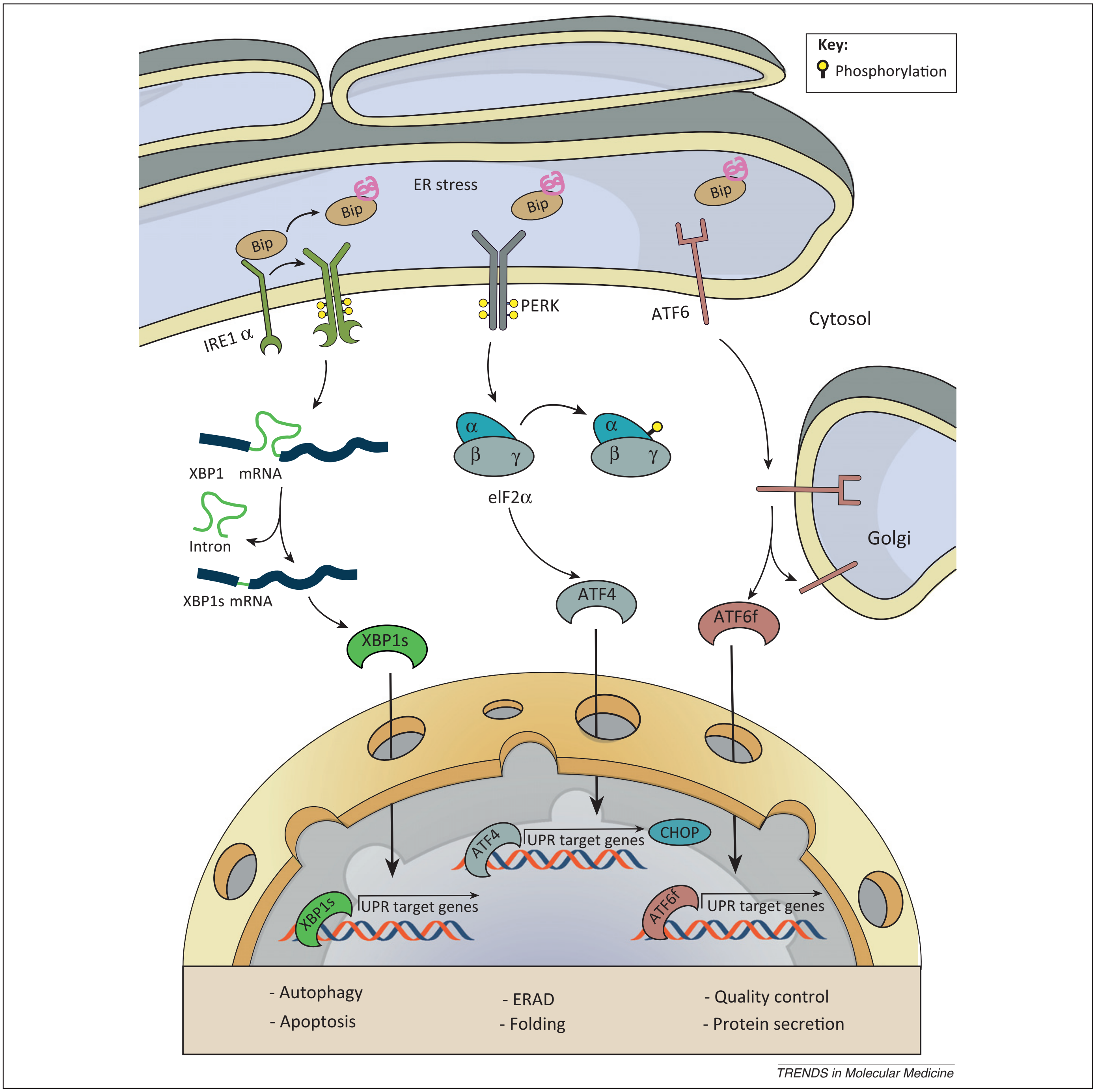

Figure 1. The unfolded protein response (UPR). Accumulation of misfolded proteins at the endoplasmic reticulum (ER) lumen triggers an adaptive stress response known as the UPR that is mediated by three types of ER stress sensors: IRE1α, PERK, and ATF6. In cells undergoing ER stress, IRE1α dimerizes and autophosphorylates, leading to the activation of its endoribonuclease activity at the cytosolic domain. Active IRE1α processes the mRNA encoding XBP1, a transcription factor that upregulates many essential UPR genes. In addition, activation of PERK decreases the general protein synthesis rate through phosphorylation of the initiation factor eIF2α. eIF2α phosphorylation increases translation of the ATF4 mRNA, which encodes a transcription factor that induces the expression of genes involved in amino acid metabolism, antioxidant responses, apoptosis, and autophagy. ATF6 is a type II ER transmembrane protein encoding a bZIP transcription factor in its cytosolic domain and that localizes to the ER in unstressed cells. Upon the induction of ER stress, ATF6 is processed at the Golgi apparatus, releasing its cytosolic domain which then translocates to the nucleus where it increases the expression of some ER chaperones and ER-associated degradation (ERAD)-related genes.

(Figure 1). Under persistent or severe ER stress, ATF4 contributes to the induction of cell death by controlling the transcription of pro-apoptotic BCL-2 family members including PUMA and BIM, in addition to GADD34 and CHOP. Thus, the UPR is a global stress network that integrates information about the intensity and kinetics of protein misfolding at the ER, controlling the decision on cell fate through a variety of complementary mechanisms.

ER stress in PD: cause or consequence?

A few reports have revealed ER stress in human tissue derived from PD patients (Figure 2). The first study describing UPR activation in PD post-mortem tissue described immunoreactivity for phosphorylated PERK and eIF2α in dopaminergic neurons of the SNpc [11], and the neurons presenting activated PERK were positive for αSyn inclusions [11]. Other studies demonstrated that the ER

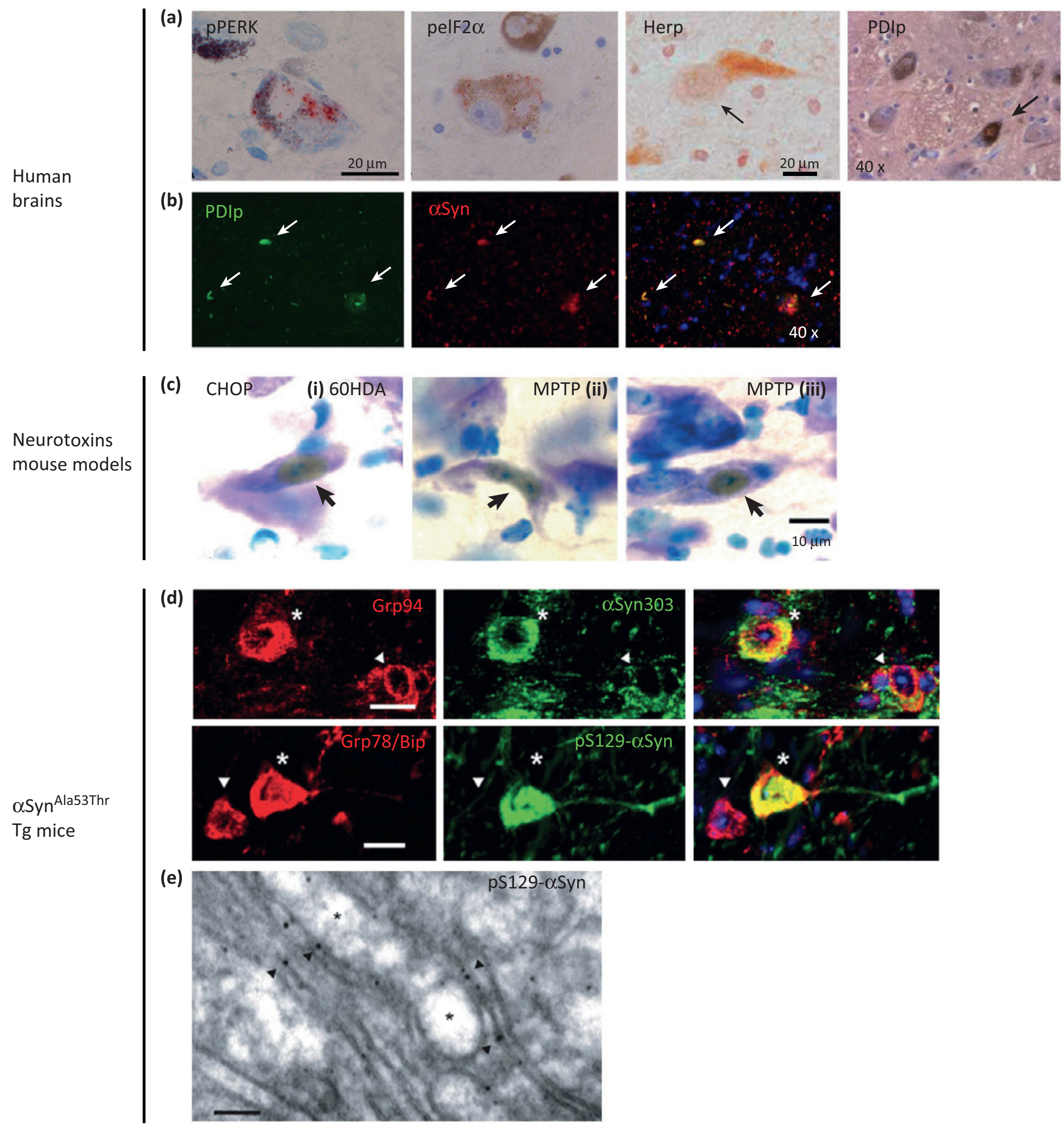

Figure 2. ER stress markers in human Parkinson's disease (PD) brain and animal models of the disease. **(a)** Immunohistochemical detection of phosphorylated-PERK (pPERK), phosphorylated-eIF2 (peIF2α), Herp, and PDIp in dopaminergic neurons from the substantia nigra pars compacta (SNpc) of individuals with PD. Arrows indicate dopaminergic neurons positive for the staining. **(b)** Coexpression of α-synuclein (αSyn; red) and PDIp (green) in Lewy bodies (LB) of the SNpc in a PD brain is shown. The yellow staining shows areas where both proteins are found. The typical LB structures are shown with arrows. **(c)** Immunohistochemical detection of CHOP in the SNpc of adult mice (i) following intrastriatal injection of the toxin 6-OHDA. CHOP expression is also observed in SNpc neurons following injection of the neurotoxin precursor MPTP in adult mice by either (ii) chronic or (iii) acute regimens. **(d)** Expression of Grp94 or Grp78/Bip in cells presenting abnormal αSyn deposition in the brainstem and cortex of end-stage αSyn^{A53T}-transgenic (Tg) mice. Sections were double-immunostained for Grp94/αSyn303 or for Grp94/pS129-αSyn. Nuclei were stained with DAPI. **(e)** Immunogold staining for pS129-αSyn and electron microscopy analysis of spinal cord sections from end-stage αSyn^{A53T} Tg mice. All images were adapted, with permission, from [11,12,14,19,42].

stress-responsive proteins Herp, Bip, and pPDI are upregulated in the SNpc of PD patients [12–14] and colocalize with LBs [12,14]. Although these reports suggest that ER stress occurs in affected neurons in PD brains, general characterization of ER stress markers in the available literature is still very poor, and proximal signaling components (i.e., ATF6, XBP1, IRE1α, etc.) remain to be properly measured.

The mechanisms leading to ER stress in PD and the actual impact of the UPR on the degeneration cascade in the disease are just starting to be uncovered. In this section we summarize the evidence linking ER stress to the pathophysiology of the disease and discuss the most recent data arguing in favor of a functional role for the UPR in PD. Special emphasis is given to mechanistic aspects that explain the occurrence of ER stress in PD.

(i) *Genetic models of PD*

Activation of the UPR is recapitulated in cellular models of PD by the overexpression of mutant αSyn, which leads to the occurrence of chronic ER stress responses associated with neurotoxicity (reviewed in [9]). In addition, ER stress may feed back to enhance αSyn aggregation [15], suggesting a vicious cycle between ER stress and αSyn aggregation as has been indicated for other PMDs (see below) [16]. ER stress markers are upregulated in the brain of αSyn transgenic mice, including the expression of Bip, XBP1, CHOP, and ATF4 [17–19]. The stress marker Bip is also present in complexes with αSyn oligomers both *in vivo* and *in vitro* [20].

Two recent studies by Michael Lee's group have described in more detail the consequences of αSyn accumulation at the ER [19,20]. Kinetic studies have shown that the presence of toxic αSyn oligomers at the ER correlates with the occurrence of ER stress and disease progression in mutant αSyn transgenic mice. More importantly, αSyn accumulation at the ER is also observed in post-mortem human brain tissue from PD patients [19]. Unexpectedly, only a subset of ER stress markers are induced early in the disease, specifically in the brain areas showing αSyn-related pathology [19,20]. The physical interaction of αSyn and ER chaperones had been observed previously, but this time the interaction was mapped to ER-enriched fractions [19]. Thus, these two recent studies place the ER as the possible site of generation and accumulation of αSyn neurotoxic species, and suggest a possible pathogenic mechanism by which abnormal αSyn conformers sequester important ER chaperones, leading to impaired ER folding and chronic stress.

Susan Linquist's group has identified an additional mechanism that contributes to the induction of ER stress by αSyn. Using a screening system in yeast, they have discovered defects in ER-to-Golgi trafficking upon αSyn expression [21]. A functional relationship between αSyn and the small GTPase Ypt1p in yeast, and αSyn and the mammalian ortholog Rab1, has been proposed in which impaired vesicle transport from the ER triggers the accumulation of immature proteins in this compartment [21,22]. Overexpression of Rab1 and some of its homologs rescues dopaminergic neuron loss induced by αSyn overexpression [21]. ER–Golgi trafficking defects were also shown to affect mitochondrial functioning [23], a central aspect of PD pathogenesis.

Post-translational modification of αSyn can also affect its aggregation and toxicity. UPR activation by αSyn overexpression is partially dependent on phosphorylation at serine 129 (pS129) [19,24] and *S*-nitrosylation [19]. Finally, a third mechanism may contribute to ER stress induced by mutant αSyn expression. Using a cell-culture model of PD, the functional upregulation of the ER stress marker gene, the homocysteine-induced ER protein, Herp, has been studied upon αSyn overexpression [17]. In this study, the induction of ER stress was linked to disrupted ER calcium homeostasis, which was possibly explained by the Herp-dependent degradation of calcium channels (IP3R and RyR) through ERAD [17]. Alterations in ER calcium levels may then feed back to enhance αSyn aggregation and the engagement of chronic ER stress responses [17]. A similar pattern of ER calcium channel disruption and stress marker expression was observed in the brains of αSyn transgenic mice [17] and individuals with PD [13]. All of this evidence indicates that the direct disruption of ER function by αSyn expression is a primary and early event in PD pathogenesis.

Mutations in the serine/threonine kinase gene *LRRK2* are the most frequent genetic defect identified in PD patients, and LRRK2 partially localizes to the ER in dopaminergic neurons of PD patients [25]. Although the function of LRRK2 remains a matter of constant debate, studies in *C. elegans* demonstrated that expression of wild type LRKK2 protects dopaminergic neurons against neurotoxicity induced by either 6-OHDA or human αSyn [26]. LRRK2-mediated neuroprotection involves the upregulation of Bip through p38 signaling [26]. *C. elegans* lacking the LRRK2 homolog develop spontaneous neurodegeneration and hyper-susceptibility to experimental ER stress, a phenotype reverted in a background lacking the worm homolog of the mitochondrial serine/threonine kinase PINK1 [27]. Despite these interesting reports, the possible contribution of ER stress to mutant LRKK2 pathogenesis in mammalian cells has not yet been addressed.

In contrast to αSyn and LRRK2, a few unconnected studies suggest that other genes linked to PD can alter ER function. Very little *in vivo* validation is available, and most of the observations that have been described remain to be confirmed in other experimental settings. For example, it has been suggested that the E3 ubiquitin ligase Parkin/PARK2 and the ubiquitin carboxyl-terminal hydrolase UCHL-1/PARK5 participate in the ubiquitin and proteasome system (UPS). Given that the UPS is an essential component of the ERAD pathway, it is feasible that Parkin or UCHL-1 mutations generate ER stress. In a cell-culture model, Parkin overexpression has been shown to reduce ER stress caused by the expression of a polyglutamine peptide [28]. Interestingly, Parkin expression is upregulated by ER stress; ATF4 controls its levels through direct binding to the promoter region [29]. In addition, the subcellular distribution of Parkin is altered by ER stress [30]. However, the functional connection between Parkin and ER stress has not been established directly. On the other hand, downregulation of DJ-1/PARK7 enhances the susceptibility of cells to ER stress, as well as other cell-death stimuli [31]. Expression of Parkin-associated endothelin receptor-like receptor Pael-R, a substrate of ubiquitin ligase Parkin, induces ER stress and neurodegeneration in the SNpc of mice [32,33], and the effects of Pael-R on ER stress are enhanced by Parkin deficiency [33]. Finally, mutations identified in a Chilean family in *ATP13A2/PARK9*, encoding a lysosomal type 5 P-type ATPase, cause a rare form of early-onset parkinsonism. Mutation of ATP13A2 leads to its retention at the ER, triggering chronic ER stress and cell death [34]. Taken together, perturbation of ER homeostasis is emerging as a common pathological event triggered by genes linked to PD. The mechanisms of action for individual mutant PD genes may involve diverse pathways, but they may culminate in a final related outcome that includes pathogenic ER stress.

(ii) *Toxicological models of PD*

A decade ago, two pioneer gene expression profile analyses identified the UPR as the major signature engaged by PD-inducing neurotoxins in cell culture [35,36]. The authors used neurotoxins that trigger PD, including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA), and the pesticide rotenone, showing a clear activation of the PERK and IRE1α pathways [35,36]. These findings have been confirmed by many other groups using toxicological models of PD (reviewed in [9]). More importantly, targeting UPR components through genetic manipulation has a clear impact on the survival of dopaminergic neurons upon exposure to PD-inducing neurotoxins (see next section).

At the mechanistic level, mitochondrial dysfunction and oxidative stress may cause the ER stress triggered by PD-related neurotoxins due to oxidative damage to ER proteins [37]. Interestingly, Stuart Lipton's group has observed the *S*-nitrosylation of an essential ER foldase, protein disulfide isomerase (PDI), in both post-mortem tissue derived from PD patients and cellular models of the disease [38]. This oxidative modification inhibits PDI activity, triggering ER stress and possibly cell death [39]. Exposure of cells to rotenone also results in the *S*-nitrosylation of PDI

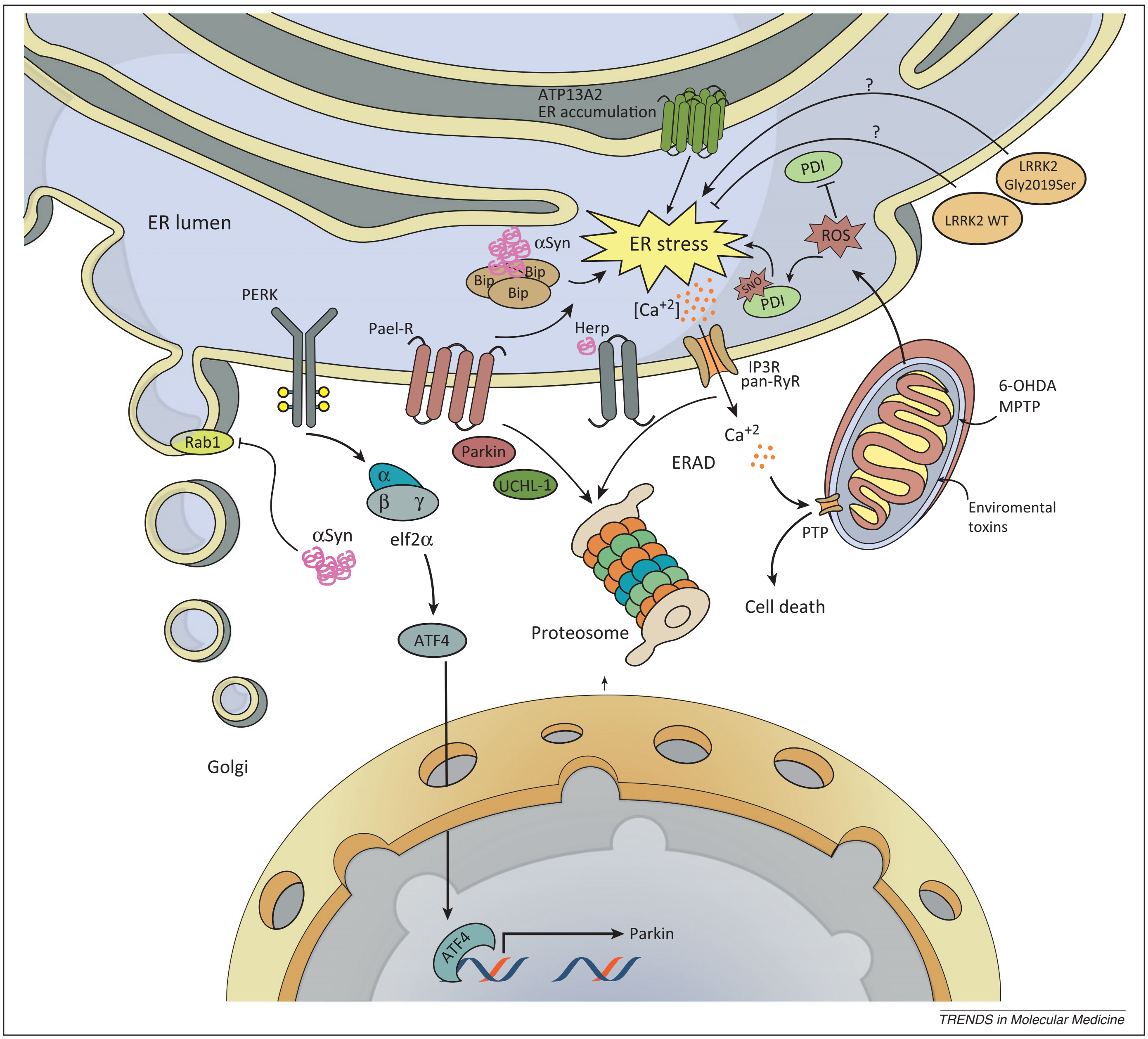

Figure 3. Mechanisms underlying the induction of endoplasmic reticulum (ER) stress in Parkinson's disease (PD). The figure summarizes different pathological events observed in PD models that trigger ER stress including: α-synuclein (αSyn) accumulation at the ER and interaction with the ER chaperone Bip, local oxidative stress and *S*-nitrosylation of PDI, altered ER calcium homeostasis by Herp-mediated degradation of IP3R and pan-RyR, αSyn inhibition of ER to Golgi trafficking, altered ERAD (endoplasmic reticulum-associated degradation), and accumulation of mutant LRRK2 or ATP13A2, among other indicated mechanisms.

[38]. Thus, studies in pharmacological models of PD, which resemble the most common sporadic forms of the disease, also involve chronic ER stress in dopaminergic neurons due to direct alteration of the protein-folding machinery (Figure 3).

Possible strategies for targeting ER proteostasis in PD

Recent evidence from both toxicological and genetic models of PD indicates that activation of the UPR has a beneficial effect on the survival of dopaminergic neurons. In this section we discuss recent findings demonstrating a functional contribution of ER stress to PD-mediated neurodegeneration (Table 1).

Animal models for only a few UPR components have been used in PD studies, but the results are striking. For example, the accumulation of ubiquitin-positive inclusions and the loss of dopaminergic neurons induced by MPTP is enhanced in ATF6α-deficient animals [40,41], suggesting that activation of the UPR has an important adaptive function to maintain protein homeostasis in this model. Although ATF6 is not essential for the development and survival of dopaminergic neurons in mice, this stress sensor controls the levels of Bip, ERAD components, and promotes astroglial activation under resting conditions in dopaminergic neurons [40,41]. Similarly, deletion of the gene encoding the proapoptotic factor CHOP protects dopaminergic neurons against exposure to 6-OHDA and MPTP in different experimental settings (Figure 2) [42]. A recent report indicated that loss-of-function of the nonspecific cation channel TRPC1 in mice increases ER stress levels and dopaminergic neuron loss upon exposure to MPTP [13]. Although a direct connection between TRPC1 and ER stress is unclear, this protein has been linked to the regulation of ER calcium content.

The potential therapeutic value of targeting the UPR in PD has been explored using gene therapy. Delivery of the XBP1s transgene into the striatum through stereotaxic injection using recombinant adenoviruses protected dopaminergic neurons against MPTP-induced degeneration [43]. A more thorough study has recently been performed in a rat model of PD, where both adeno-associated viruses (AAV) expressing the ER chaperone Bip and αSyn were coinjected directly into the SNpc [44]. Bip overexpression significantly diminished αSyn toxicity and improved motor performance, probably because of reduced ER stress levels [44]. These two studies provide the first proof of concept in favor of a positive impact of manipulating the UPR in adult animals in the context of PD. Of note, we have recently tested an AAV-mediated gene therapy to deliver XBP1s in mouse models of Huntington's disease [45] and spinal cord injury [46], observing positive effects in alleviating disease features. Gene therapy to attenuate ER stress may be tested in the clinic in the near future because there are at least five clinical trials being performed in PD patients using the brain delivery of AAVs to test novel therapeutic approaches [47].

Pharmacological targeting of ER stress is currently employed in several different disease contexts. Salubrinal is a small compound that enhances eIF2α phosphorylation by inhibiting its phosphatase PP1 [48]. Salubrinal partially protects cells against apoptosis in cellular models of PD [49], and treatment of mice with salubrinal delays disease onset and attenuates motor deficits in a rat model of PD based on αSyn overexpression [19]. However, salubrinal treatment did not protect dopaminergic neurons from degeneration [19]. Similar effects were observed in mutant αSyn transgenic mice, and were associated with increased expression of UPR target genes in the brain including *Bip/GRP78*. Unexpectedly, salubrinal administration reduced the accumulation of αSyn in ER-enriched fractions [19]. Bip expression is also enhanced in mice treated with the methoxyflavone tangeretin, a UPR activating compound, and pre-treating with methoxyflavone reduces dopaminergic neuron loss triggered by acute or chronic exposure to MPTP [41,50]. Interestingly, flavonols induce IRE1 activation in yeast, possibly by binding to an allosteric site [51].

Chemical chaperones have been widely used to attenuate ER stress levels in several neurodegenerative diseases [9]. 4-Phenylbutyrate (4-PBA), a well-described chemical chaperone, protects animals against αSyn-mediated neurodegeneration [52,53]. Similarly, the chemical chaperone tauroursodeoxycholic acid (TUDCA) increases neuronal survival in MPTP-treated rats [54,55]. However, the possible attenuation of ER stress in these experiments was not determined. A dibenzoylmethane derivative has also been shown to protect neurons against 6-OHDA, correlating with decreased ER stress levels [56]. In summary, a growing body of evidence suggests the promising therapeutic potential of manipulating the UPR and ER stress levels in PD (Figure 4). Many interesting novel compounds are

Table 1. Funtional studies linking ER stress with PD

Main target	Method	PD model	Effect	Refs
XBP1s	• Adenoviral-mediated delivery	MPTP	Decreased dopaminergic neuron loss	[43]
ATF6	• ATF6α *knock-out* mice	MPTP	Increased ubiquitin positive inclusions Increased dopaminergic neuron loss	[40,41]
BiP	• AAV-mediated delivery	AAV αSyn	Reduced dopaminergic neurons loss Improved motor performance	[44]
eIF2α	• Salubrinal treatment	αSyn^{A53T} Tg	Increased life span Reduced αSyn accumulation at the ER	[19]
	• Salubrinal treatment	AAV αSyn	Improved motor performance	[19]
Strategy	**Method[a]**	**PD model**	**Effect**	**Refs**
Non-lethal ER stress	• Tm feeding in flies	6-OHDA	Improved climbing ability and dopaminergic neurons survival	[58]
	• Tm i.p. injections in mice	6-OHDA	Reduced dopaminergic neurons loss	[58]

[a]Tm, Tunicamycin; i.p., Intraperitoneal.

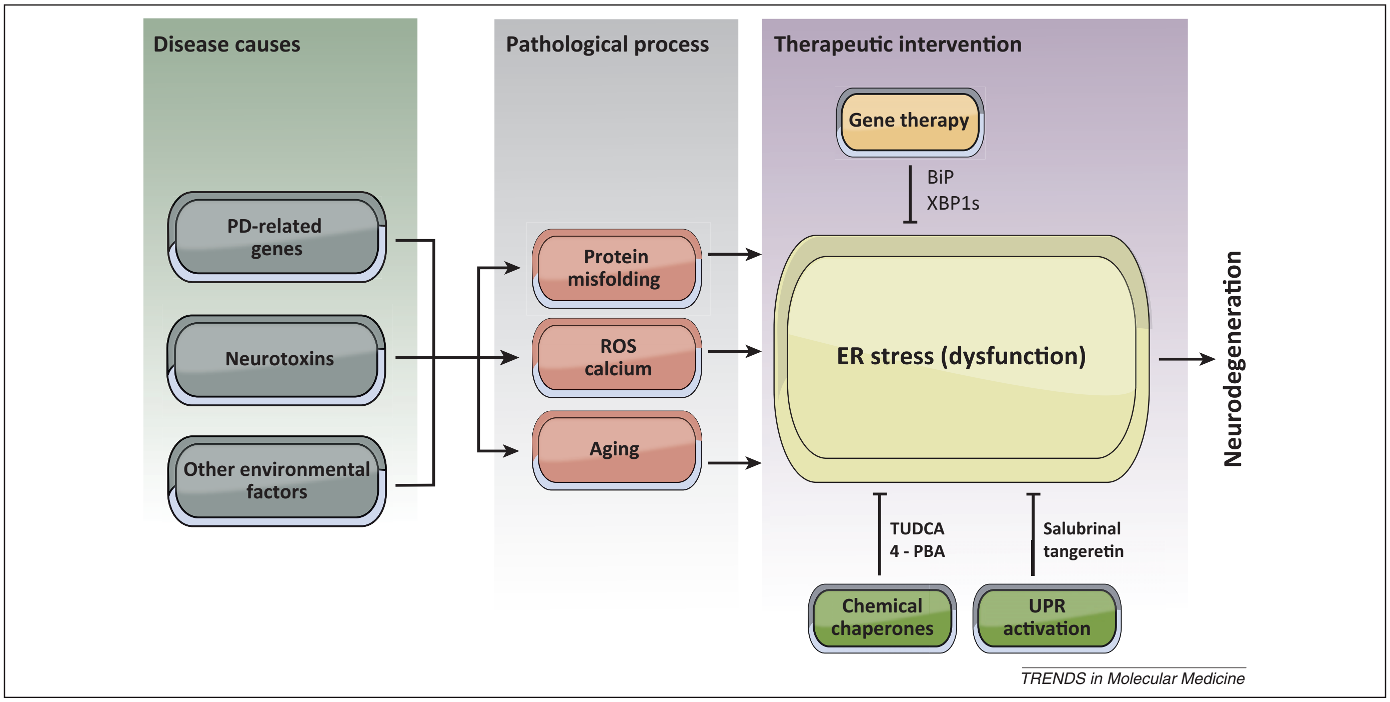

Figure 4. Therapeutic targets to interfere with endoplasmic reticulum (ER) stress in Parkinson's disease (PD). A synopsis of the neurodegenerative cascade in PD including disease causes, pathological process, and the possible therapeutic intervention points at the level of ER stress to modify disease onset and progression is shown using gene therapy and pharmacological approaches. 4-PBA, 4-phenylbutyrate; ROS, reactive oxygen species; TUDCA, tauroursodeoxycholic acid.

available to target other components of the UPR, such as inhibitors of IRE1α, PDI, or JNK [57], representing interesting tools to validate the impact of ER stress in the context of PD.

Hormesis: a protective role of mild ER stress in PD?

Given the dual role of the UPR in maintaining cell viability and the engagement of cell death, it is predicted that low levels of ER stress during the early stages of PD may actually protect dopaminergic neurons against proteostasis defects. This hypothesis has recently been tested in an elegant study by the Mollereau group [58]. Treatment of mouse and fly models of PD with low concentrations of the ER stress agent tunicamycin increases neuronal survival in genetic and pharmacological models of the disease [58]. In these experiments tunicamycin triggered a preconditioning effect in which sublethal levels of ER stress selectively engaged adaptive UPR signaling events involving the expression of XBP1s in the brain (adaptive signal) but not the proapoptotic factor CHOP [58]. Similar studies from the same group have shown that mild ER stress protects against neurodegeneration [59]. Interestingly, nonlethal ER stress enhances autophagy, a prosurvival pathway known to protect against neurodegeneration in most PMDs. Using cell-culture models and *in vivo* genetic manipulation of *Drosophila melanogaster*, the authors demonstrated that ER stress-induced autophagy protects against neurodegeneration [58]. A similar phenomenon has been suggested by our group to operate in ALS and HD models *in vivo*. In these models targeting the UPR shifts the protein homeostasis network toward increasing autophagy, which provides neuroprotection [60,61].

The idea that low levels of stress may actually protect against a subsequent injury is a known concept in the toxicology field. Preconditioning treatments have also been applied in the context of brain ischemia–reperfusion and neurodegeneration [62]. Hormesis (from ancient Greek *hormáein* 'to set in motion, impel, urge on') is the concept of favorable biological responses upon exposure to low levels of toxins and other stressors [63]. Conditions that stimulate hormesis engage adaptive stress signaling that shifts the homeostasis network and renders cells resistant against a high dose of the same stimulus. Thus, pharmacological or gene therapy strategies to stimulate hormesis in the context of PD are an interesting concept for future therapeutic development.

Cell-to-cell transfer of αSyn: a vicious stress cycle?

An emerging field of study in PD and other PMDs is the mechanism behind the cell-to-cell transfer of misfolded proteins as a disease propagation mechanism [7]. In the case of PD, αSyn secretion increases under various stress conditions that alter protein homeostasis [64,65]. Extracellular αSyn is also neurotoxic and may enhance the aggregation process of endogenous αSyn through a seeding process, contributing to the formation of LB-like inclusions [66,67]. Interestingly, extracellular exposure of cells to αSyn oligomers triggers ER stress [68], and pharmacological ER stress enhances αSyn aggregation [15,17]. Based on this evidence, we propose a speculative model whereby a vicious cycle operates in PD: αSyn accumulation triggers ER stress, and this pathological phenomenon then feeds back in a cyclical manner to further enhance αSyn aggregation (Figure 5).

It is not known if the source of αSyn accumulated at the ER originates in an intrinsic manner – in other words it derives from the cells where the accumulation is observed – or if the αSyn results from internalization processes. Of note, αSyn secretion to the extracellular space is not prevented by ER-to-Golgi trafficking inhibitors [64,65]. For

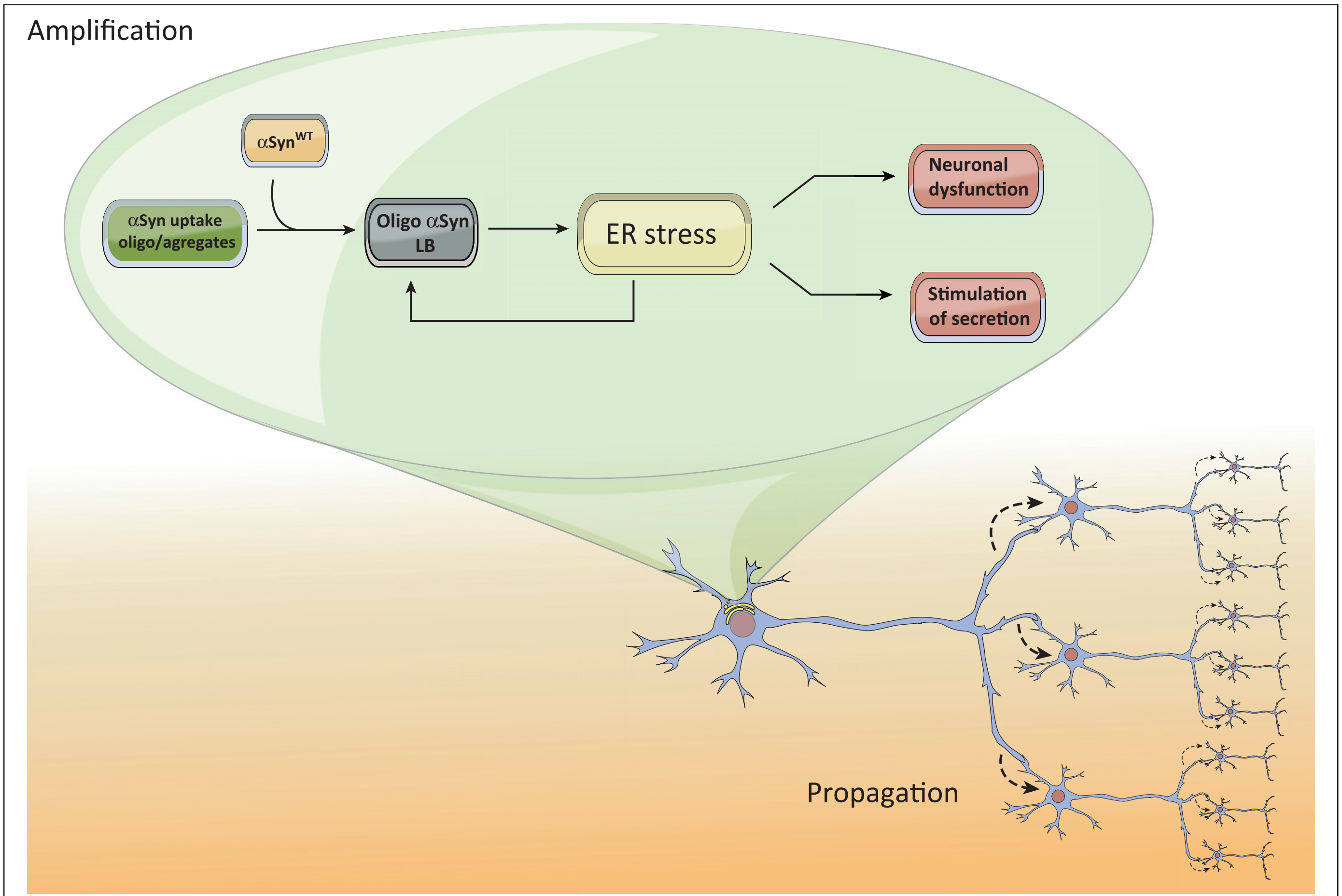

Figure 5. The vicious cycle of α-synuclein (αSyn) accumulation: disease amplification and propagation in PD. The uptake of αSyn aggregates and/or oligomers contributes to the accumulation of Lewy bodies (LB). αSyn accumulation triggers endoplasmic reticulum (ER) stress and neuronal dysfunction. In addition to stress stimulation, αSyn secretion, and cell-to-cell transfer, ER stress feeds back to enhance αSyn aggregation. Internalized αSyn then induces the pathological amplification of its aggregation by interacting with wild type αSyn (αSyn^{WT}) in the recipient cell as a disease mechanism of propagation.

this reason, it may be possible that the accumulation of αSyn at the ER lumen involves its uptake from the extracellular space and retrograde transport to the ER. This model has been extensively described for some bacterial toxins [69] which also trigger ER stress in mammalian cells. In agreement with this idea, internalized αSyn has been observed in microsomal fractions [70]. Strategies to attenuate this vicious cycle (Figure 5) offer additional points for disease intervention including (i) αSyn secretion, (ii) cell reception, (iii) incorporation, and (iv) ER stress amplification. More studies are needed to address the mechanisms and pathogenesis of cell-to-cell transfer of αSyn.

Concluding remarks

In this review we discuss in detail the most recent evidence linking disturbances of ER function to PD pathogenesis and note many interesting, complementary aspects underlying the impact of ER stress on the disease process. Predicting the contribution of UPR signaling to PD is theoretically complex because of the dual role of the pathway in cell survival and cell death. This concept may be particularly relevant during early presymptomatic stages of the disease when neuronal death could be prevented. In this disease phase, low and transient levels of ER stress may even protect dopaminergic neurons through a hormesis mechanism, delaying the appearance of disease signs. In the symptomatic phase, ER stress may be a chronic process associated with irreversible cell damage and neurodegenerative processes. Recently, important findings from studies in genetic and toxicological models of PD favor ER stress as part of the disease mechanism, and the first studies are now available that provide proof of concept for a positive effect of targeting UPR components in a disease context.

The UPR has a central role in supporting the function of specialized secretory cells, where high demand for protein folding and secretion engages this pathway as a survival mechanism. In addition to this classical concept of physiological ER stress, the UPR is relevant in several processes including cell differentiation, immunity, lipid and cholesterol synthesis, and energy metabolism [71]. Until now, it is not known if the UPR has a physiological function in the motor and cognitive functions of the brain, and most of the studies addressing the impact of this signaling network in the nervous system focus on disease conditions. Interestingly, a few studies have shown that manipulating components of the dopaminergic circuit triggers spontaneous ER stress, as observed in knockout mice for the dopamine receptor D2 [72]. Moreover, gene expression profile analysis of animals treated with methamphetamine (which stimulates dopamine-mediated neurotransmission) [73], or of cells treated with dopamine [74], reveals that ER stress is a major transcriptional signature. Understanding the possible impact of ER stress and the UPR on the activity of dopaminergic neurons is an important step toward associating this pathway with the selective neuronal vulnerability observed in PD. Of note, the occurrence of ER stress has been shown to underlay the differential vulnerability of motoneuron cells in ALS mouse models [75]. Given that specific patterns of calcium signals are associated with the physiology of susceptible neurons in the SNpc, it will be interesting to test whether these characteristics of dopaminergic neurons impact upon ER physiology and their high susceptibility to ER stress.

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