domain of the parkin gene [15]. Furthermore, an NMR analysis has revealed binding between the ubiquitin-like domain of parkin and the Rpn 10 subunit of the 26S proteasome [16], strongly suggesting the link between Parkin and the UPS (Figure 9.1).

9.2.2
Proteasome-independent Role of Parkin

Polyubiquitin chains are formed through distinct types of linkages using one of the seven internal lysine residues (K6, K11, K27, K29, K33, K48 and K63) within the previous ubiquitin molecule [17].

Recently, parkin was shown to be a dual function ubiqutin ligase that mediates both K48- and K63-linked polyubiquitination [18]. K48-linked polyubiquitin chain, the best characterized form of polyubiquitin, leads the proteins to degradation via 26S proteasomes, constituting the ubiquitin–proteasome system as mentioned before. On the other hand, K63-linked chains act as proteasome-independent signals in several different cellular pathways [17]. Dual specificity seems to be determined by the E2 enzymes that parkin recruits. In the assembly of a K63-linked polyubiquitin chain, parkin interacts with the UbcH13/Uev1a heterodimer [18]. Parkin mediates K63-linked, proteasome-independent ubiquitination of its substrate synphilin 1 [19]. Although K63-linked ubiqitination is implicated in inclusion body formation, further study is required to clarify its physiological relevance [20].

9.2.3
Multiple Monoubiquitination is Mediated by Parkin

Surprisingly, two recent reports have shown that Parkin mediates multiple monoubiquitination *in vitro* [21, 22]. Both reports have demonstrated that the second RING finger is responsible for E3 activity in an *in-vitro* ubiquitination assay, where bacterially-produced recombinant Parkin was used. Previous findings that mutations in regions other than the second RING finger showed reduced E3 activity *in vivo* might be ascribed to their insolubility and sequestration [23–26]. Parkin itself as well as maltose binding protein (MBP) connected to Parkin as a pseudosubstrate and p38 as a substrate, have been shown to be monoubiquitinated *in vitro* and *in vivo* respectively. In contrast to a previous report that Parkin accelerates polyubiquitin chain formation [18], Parkin has been shown to mediate monoubiquitination in concert with Ubc13 as well as Ubc7 or Ubc H7 under pure *in vitro* conditions [21, 22]. These results suggest that Parkin may mediate monoubiquitination regardless of its partner E2s.

A recent report showed that Parkin mediates monoubiquitination of an adaptor protein Eps15 with two ubiquitin-interacting motifs (UIMs) [27]. Eps15 interacts with and positively regulates the endocytosis of ubiquitinated epithelial growth factor receptor (EGFR). Parkin-mediated ubiquitination of Eps15 inhibits its ability to bind with and promote endocytosis of EGFR, resulting in suppression of EGFR

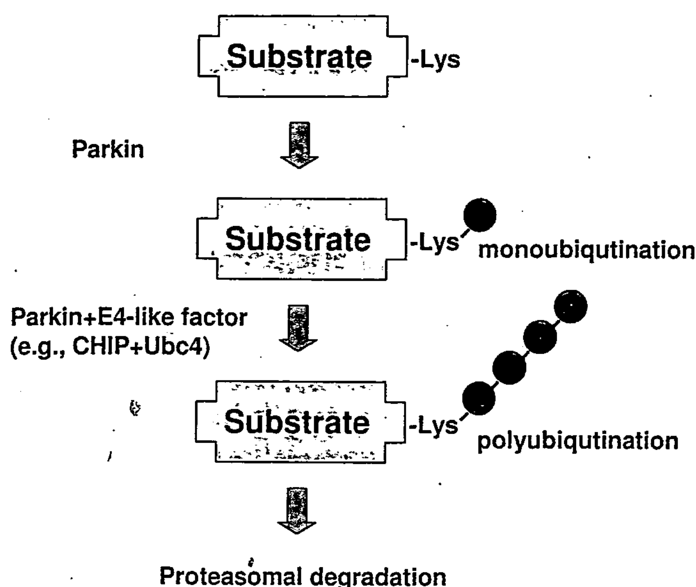

Fig. 9.2. Hypothetical two-step ubiquitination of Parkin substrates. At the first step, Parkin monoubiqutinates its substrate. Then, E4-like factors promote the elongation of polyubiquitin chain on the substrate molecules, thereby targeting the substrates to 26S proteasome. CHIP and its partner E2 Ubc4 are candidates for such E4-like molecules [28].

internalization and degradation, and promoting phosphoinositide 3-kinase (PI(3)K)-Akt signaling. Since Akt plays an important role in neuronal survival, this proteasome-independent function of Parkin may explain some aspects of neurodegeneration.

On the other hand, monoubiquitination may lead to proteasomal degradation. A previous report showed that the carboxy-terminus of Hsc70-interacting protein (CHIP), a U-box motif containing E3 protein, together with Ubc4, serves as an E4-like protein and cooperates with Parkin to form polyubiquitin chains [28]. Given the presence of E4-like factor, monoubiquitination catalyzed by Parkin may eventually target the proteins to degradation via the 26S proteasome (Figure 9.2). Whether Parkin can mediate both monoubiquitination and polyubiquitination should be re-examined and clarified in light of the recent findings.

9.2.4
Modulators of Parkin E3 Activity

Parkin is a component of a high molecular weight complex located in cells and the function of parkin seems to be modulated by its binding partners [28, 29]. Two parkin-associated proteins have been shown to promote the elimination of Pael-R by parkin: CHIP and Hsp70 [28].

CHIP contains a U-box motif, which is structurally similar to the RING-finger motif and exhibits U box-dependent E3 activity [30–32]. On the other hand, CHIP has been shown to downregulate chaperone ATPase activity [33]. Moreover, CHIP

has been shown to ubiquitinate improperly-folded protein in a chaperone-dependent manner [32]. When bound to parkin however, CHIP markedly enhances parkin-mediated ubiquitination of Pael-R *in vitro* [28]. Consistent with this observation, overexpression of CHIP accelerates Pael-R degradation in cultured cells, leading to a marked reduction in the steady-state level of Pael-R protein.

In contrast to CHIP, Hsp70 has been observed to inhibit ubiquitination of Pael-R *in vitro* and to increase levels of the soluble form of Pael-R *in vivo*, presumably by facilitating the proper folding of Pael-R. Moreover, Hsp70 inhibits CHIP-mediated degradation of soluble and probably functional Pael-R, so that only insoluble aggregates of the receptor are removed.

It has also been shown that bcl-2-associated athanogene 5 (BAG5), a BAG-family member, directly interacts with parkin and the chaperone Hsp70. BAG5, similar to CHIP, downregulates chaperone ATPase activity. Within this complex, BAG5 inhibits both parkin E3 ubiquitin ligase activity and Hsp70-mediated refolding of misfolded proteins. BAG5 enhances parkin sequestration within protein aggregates and attenuates parkin-dependent preservation of proteasome function [34].

Two binding partners of Parkin, 14-3-3 eta and Nrdp1/FLRF are also found to be negative regulators of Parkin E3 activity [35, 36].

9.3 Substrates of Parkin

9.3.1 Parkin Substrates and their Recognition Mechanisms

Although Parkin may mediate diverse forms of ubiquitination, it is likely that some, but not all, are involved in the UPS. Given that Parkin targets its ubiquitinated substrates to the 26S proteasome, Parkin dysfunction should lead to the accumulation of its substrate. Accumulation of toxic substrate(s) of Parkin (substrate-X) due to loss of parkin E3 activity or disruption of the parkin–proteasomal interaction in AR-JP patients with a genetic defect of parkin, should result in the development of dopaminergic neurodegeneration.

Based on this hypothesis, the identification of such toxic substrate(s) is the key to understanding the molecular mechanisms underlying AR-JP.

To date, 13 proteins have been identified as substrates of parkin [37]: CDCrel-1 , synaptotagmin XI [38], synphilin-1 [39], glycosylated α-synuclein [40], α/β-tubulin [41], the p38 subunit of an aminoacyl-tRNA synthetase (ARS) complex [42], *P*arkin-*a*ssociated *e*ndothelin receptor-like *r*eceptor (Pael-R) [43], the expanded form of polyglutamine [44], and cyclin E [45], SEPT5_v2/CDCrel-2 [46], misfolded dopamine transporter [47], far upstream element-binding protein 1 [48], RanBP2 [49] and Eps15 [27] (Table 9.1). It has been shown that the unmodified form of α-synuclein, a major component of Lewy body, is not a substrate for Parkin [39].

Although there are no apparent common properties among Parkin substrates, it has been noted that several different substrates are found within Lewy bodies.

Table 9.1. The reported substrates of parkin

Protein	Biological function	Lewy body
O-glycosylated α-synuclein	Septin family protein with unknown function	–
CDCrel-1	Isoform of α-synuclein with unknown function	N.D.
(Misfolded) Pae1 receptor	Orphan G-protein coupled receptor	+
p38 subunit of the aminoacyl-tRNA synthetase	Role in protein biosynthesis	+
Synaptotagmin XI	Regulation of exocytosis of neurotransmitters	+
Expanded polyglutamine(polyQ) proteins	Aberrant proteins responsible for polyQ diseases	–
α/β-Tubulins	Microtubule proteins	+
Synphilin-1	α-Synuclein-binding protein	+
Cyclin E	Cell cycle regulation of mitotic cells; unknown function in neurons	N.D.
SEPT5_v2/CDCrel-2	SEPT5_v2 is highly homologous with CDCrel-1	N.D.
Misfolded dopamine transporter	Regulation of dopamine uptake	N.D.
Far upstream element-binding protein-1	A binding partner of p38	N.D.
RanBP2	Small ubiquitin-related modifier (SUMO) E3 ligase family protein	N.D.
EPS15	Adaptor protein with ubiquitin-interacting motifs (UIMs)	N.D.

N.D., not detected.

Considering that the components of Lewy bodies consist of misfolded proteins, molecular chaperones and proteasome subunits, it is likely that Parkin ubiquitinates a subset of misfolded proteins. Consistent with this idea, an expanded form of polyglutamine, which is a causative agent of polyglutamine diseases such as Huntington's disease, has been identified as a parkin substrate [44]. Moreover, there is evidence to suggest that binding between parkin and polyglutamine is mediated by Hsp70, which is known to be a binding partner of Parkin. Hsp70-mediated substrate recognition explains the diversity of substrate specificity observed with parkin (Figure 9.3).

Among the various substrate molecules discussed above, the Pael receptor (Pael-R), CDC-rel1, cyclin E, synphilin-1, and the p38 subunit of aminoacyl tRNA synthetase have either been shown or suggested to promote cell death under certain conditions, and so represent the proteins which are most likely relevant to neurodegeneration in AR-JP.

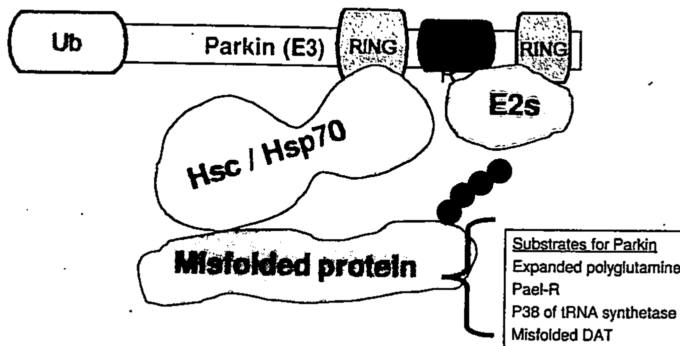

Fig. 9.3. Parkin may recognize a subset of misfolded proteins through Hsc/Hsp70. Parkin interacts with Hsc/Hsp70 through its first RING domain [28]. Parkin may recognize misfolded proteins including Pael-R, and p38 by using Hsc/Hsp70 as a substrate recognition subunit.

9.3.2
The Link between Substrate Accumulation and Cell Death: Pael-R

9.3.2.1 Pael-R and Endoplasmic Reticulum Stress

Pael-R is a putative G-protein-coupled orphan receptor, which is highly expressed in the central nervous system, especially in the substantia nigra [43, 50, 51]. Although the physiological function of Pael-R is implicated in dopamine metabolism, its ligand has yet to be identified [52]. It has been shown that misfolded Pael-R was ubiquitinated by parkin at the level of the endoplasmic reticulum and the disturbance of Pael-R degradation leads to ER stress-induced cell death.

The endoplasmic reticulum (ER) functions as a quality control regulator of membrane and secretory proteins [53]. Newly synthesized secretory proteins are transported to the ER. Inside its lumen, ER chaperones such as BiP/GRP78 bind to these newly synthesized proteins to facilitate their proper folding. After this, proteins enter the conventional secretory pathway. Proteins that are not properly folded are transported back to the cytosol where they are degraded via ubiquitin-proteasomal degradation, a process known as endoplasmic reticulum-associated degradation (ERAD) [54] (Figure 9.4). It has been shown that parkin is an ERAD-related E3 and that Pael-R is a substrate. When insoluble misfolded Pael-R is accumulated in the cells by the inhibition of the proteasome, Pael-R is first accumulated in the ER and then forms a special type of aggregate, known as an aggresome, in the cytoplasm [55]. As these aggresomes form, the cells undergo apoptosis, demonstrating cell death due to the accumulation of Pael-R.

Pael-R-induced cell death was assumed to be mediated by ER stress. Abnormal accumulation of unfolded protein in the ER is a major threat to cell viability, a phenomenon known as ER stress or unfolded protein stress. Cells attempt to adapt to ER stress in several different ways, including transcriptional upregulation of ER chaperones, and suppression of translation. These cellular responses are

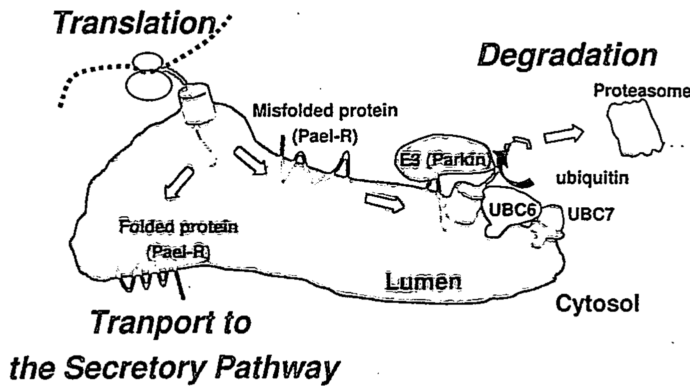

Fig. 9.4. Endoplasmic reticulum-associated degradation (ERAD). ERAD is a protein degradation system for unfolded secretory and membrane proteins. Improperly folded Pael-R is subject to ERAD, and parkin is an E3 involved in ERAD.

collectively known as unfolded protein responses (UPR) [53]. However, when the burden of accumulated protein exceeds these protective mechanisms, cells undergo a death process accompanied by the activation of JNK and caspases as well as upregulation of CHOP [56, 67].

Consistent with the idea that accumulation of misfolded Pael-R contributes to the pathogenesis of AR-JP, the level of detergent-insoluble Pael-R was elevated in the brains of AR-JP patients [43].

9.3.2.2 Pael-R Overexpressing Animals and Dopaminergic Neurodegeneration

The Drosophila model for AR-JP was created by overexpression of Pael-R [58]. When Pael-R was expressed in dopaminergic neurons in Drosophila, the number of dopaminergic neurons observed within the dorsomedial cluster fell to about 50% of that observed in control flies at 40 days of age. Equal numbers of dopaminergic neurons were observed in younger Pael-R and control flies, indicating that the observed cell loss was due to neurodegeneration occurring after birth. Moreover, even when Pael-R expression was driven by a pan-neuronal promoter, only dopaminergic neurons underwent degeneration. This suggests that dopaminergic neurons are selectively vulnerable to Pael-R toxicity.

A recent report showed that Pael-R overexpression in the substantia nigra of mouse brain through adenoviral vectors, resulted in induction of ER stress followed by dopaminergic neuronal death [59]. Pael-R-induced cell death was greatly enhanced in the parkin-deficient mouse and was suppressed by the overexpression of an ER chaperone, ORP150. Moreover, when the animal was pretreated with dopamine synthesis inhibitor, dopaminergic neuronal death was significantly attenuated, indicating that dopamine enhances Pael-R toxicity. It has been reported that dopamine covalently modifies and functionally inactivates Parkin [60]. Although the relationship between Pael-R toxicity and dopamine is still obscure, cellular protective mechanisms against Pael-R toxicity other than Parkin, might also be inactivated by dopamine.

9.3.3
The Link between Substrate Accumulation and Cell Death: CDC-rel1, Synphilin-1, Cyclin E and p38

When CDC-rel1 was introduced into the striatum and the substantia nigra of rat brain by using adeno-associated viral vectors, only dopaminergic cells in the substantia nigra underwent cell death [61]. Since the reduction of dopamine levels by pharmacological treatment alleviated nigral cell death and CDC-rel1 overexpression in PC12 cells decreased the extracellular dopamine level, the accumulation of dopamine by CDC-rel1-mediated exocytosis inhibition is thought to contribute to dopaminergic neuron-selective cell death.

Synphilin-1 is an α-synuclein-interacting protein that promotes the formation of Lewy body-like inclusions in cultured cells [62]. Parkin mediates K63-linked polyubiquitinanation of synphilin-1, apparently contributing to inclusion formation by α-synuclein and synphilin-1 in cultured cells [19]. Moreover, parkin can protect against the toxicity induced by α-synuclein plus synphilin-1 overexpression following proteasome inhibition [39].

Cyclin E has been implicated in glutamate-induced neuronal death, since it is accumulated in primary neuronal cultures in response to glutamanergic excitotoxin kainate. Interestingly, parkin overexpression inhibits the accumulation of cyclin E and cell death induced by kainate treatment, whereas RNAi-mediated parkin downregulation showed the opposite effects. The mechanism underlying cyclin E-induced cell death is not clear.

The p38 subunit plays an essential role in the *in vivo* assembly of the ARS complex [63]. When overexpressed in dopaminergic neuroblastoma cells, it forms aggresomes and induces cell death by unknown mechanisms. Parkin promotes the formation of ubiquitinated p38-positive inclusion bodies and suppresses the p38-induced cell toxicity [42]. It has been noted that only p38 is shown to be upregulated by 15% in the ventral midbrain of the parkin-null mouse among all the substrates identified [48]. The role of p38 in dopaminergic neuronal death should be validated in animal models in the future.

9.4
The Animal Models of AR-JP

To establish animal models of AR-JP, parkin gene deletion mutants for Drosophila and mouse were created [64–69]. However, the phenotypes of parkin-null mutant animals are very different from those of AR-JP patients.

9.4.1
Drosophila Model of AR-JP

The parkin gene deletion mutant Drosophila are small in size, have a short life span and become vulnerable to oxidative stress [64, 65]. The most remarkable

phenotypes of the mutant fly are apoptotic muscle degeneration and disturbances in spermatogenesis, which result in locomotor dysfunction and male sterility respectively [64, 65]. Ultrastructural analysis revealed abnormal mitochondrial morphology in both muscle and sperm [64]. On the other hand, the number of dopaminergic neurons was not reduced, although shrinkage of the cell bodies and decreased tyrosine hydroxylase immunostaining in proximal dendrites of dopaminergic neurons were observed [64].

9.4.2 Parkin-null Drosophila and Drosophila

Mutations in the PTEN-induced putative kinase 1(PINK1) are responsible for the autosomal recessive form of familial Parkinson's disease termed PARK6 [70]. PINK1 is a putative mitochondrial protein kinase, whose function is totally unknown. Three recent reports have shown that deletion of Drosophila PINK1 leads to an almost identical phenotype to that of Parkin-deficient Drosophila, i.e. it produces mitochondrial dysfunction resulting in male sterility, apoptotic muscle degeneration, and moderate loss of dopaminergic neurons [71–73]. Interestingly, the disease phenotype of PINK1-deleted Drosophila was rescued by Parkin overexpression, but not vice versa, suggesting that Parkin functions downstream of PINK1.

9.4.3 Mouse Model of AR-JP

Parkin knockout mice, in which exon 3 or 7 is deleted, have been described by three different groups [66–68]. Dopaminergic neuronal loss was not observed in any of the reports, even in aged mice. However, regarding the parkin exon-3 deletion mutant mouse, the dopamine level in the limbic system was elevated and the level of dopamine transporters was lowered according to one report, while the extracellular dopamine concentration in the striatum was increased according to a second report [66, 67]. These changes are accompanied by behavioral or electrophysiological alterations. In addition, in the mutant mouse reported by Goldberg et al., mild mitochondrial dysfunction and mild increase of oxidative stress were observed [74].

On the other hand, in exon-7-deleted parkin mutant mice, abnormalities in the nigrostriatal dopaminergic system were not detected [68]. However, noradrenergic neurons in the locus coeruleus were decreased by 20% in 70% of the total number in mice. These mild noradrenergic neuronal losses were detected as early as 2 months after birth and do not appear to progress with further aging. Consistent with this neuronal loss, the level of noradrenalin in the brain and spinal cord was reduced, accompanied by a significant reduction in the noradrenalin-dependent startle response.

In contrast to these reports, a recent extensive analysis of parkin exon-2 deletion mutant mouse revealed that the behavioral profile and cathecholamine levels in

the brain were not different from those of control mice [69]. Moreover these mutant mice were not more sensitive to 6-hydroxydopamine or methamphetamine neurotoxicity, indicating that Parkin-deficient mice are not a robust model of parkinsonism [75].

9.4.4
The Problems with Animal Models of AR-JP

The differences between parkin-deficient fly and mouse models may be explained by the difference in the endogenous substrates or the presence of redundant pathways dealing with parkin substrates in mice. Drosophila and human parkin shows a similar cell protective effect against human Pael-R- and alpha-synuclein-mediated toxicities, suggesting that the substrates are conserved to some extent [58, 76, 77]. It is particularly important to investigate whether the relationship between Parkin and PINK1 is conserved in mice and humans.

The reason why dopaminergic cell loss does not occur in the parkin knockout mouse may be due to the existence of redundant ubiquitination pathways in mice. For example, Pael-R is known to be ubiquitinated by an ER-resident E3, Hrd1 as well as by Parkin [78]. In addition, the absence of dopaminergic cell loss can also probably be attributed to the relatively short lifespan of mice (2–3 years), which would not provide enough time for the toxic substrates to accumulate in concentrations sufficient to cause cell death.

It has been noted that disturbances of the nigrostriatal system, which may represent the early signs of neurodegeneration, are suggested to occur in two exon 3-deletion mutant mice. However, the individual key findings in these papers are not in accord and no dopaminergic phenotype was detected in the exon 7- and exon 2-deletion mutant mice with respect to parkin [68, 69]. Some of the discrepancies in the detection of mild phenotypes might be caused by the different techniques employed or differences in the genetic backgrounds of the mice. Taking these possibilities into consideration, detailed and careful comparison of the phenotypes of these different parkin knockout mice should be carried to identify the real and reproducible phenotype.

9.5
Future Directions

Seven years have passed since parkin was identified as a ubiquitin ligase, and since then 13 different molecules have been isolated as parkin substrates. Some of the substrate molecules appear to explain the pathogenetic mechanisms underlying AR-JP. However, proof of accumulation of known substrates in the parkin knockout mouse brain has not been obtained except for p38, probably because of the relatively short lifespan of the mouse. So, what then is the next step?

One of the potentially promising approaches is to examine whether the nigral dopaminergic neurons in parkin-deficient mice are vulnerable to a specific stress

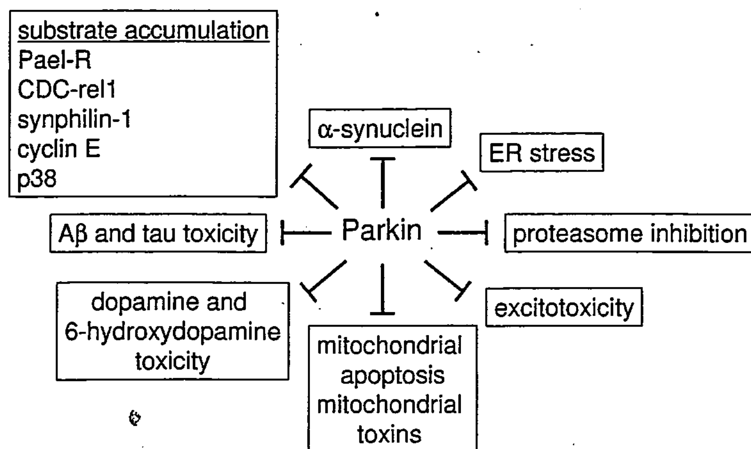

Fig. 9.5. Parkin protects cells from various stresses.

or overexpression of a specific substrate using virus vector or transgenic approaches, as has been applied to Pael-R [59]. On one hand, it is possible that the disease-causing substrate(s) has not been identified and further efforts to identify such a substrate(s) will also be important.

On the other hand, parkin appears to have cell-protective functions against various stresses (Figure 9.5). According to the reports to date, Parkin protects cells against ER stress [13], proteasomal inhibition [77], excitotoxicity [45], ceramide-induced mitochondrial apoptosis [79], mitochondrial toxins [80], intracellular Aβ [80], tau [81, 82], dopamine or 6-hydoxydopamine toxicity [47, 83] and α-synuclein-induced cell death [58, 76, 77, 84]. It is intriguing to ask whether clearance/sequestration of certain parkin substrate(s) contributes to such cell protective effects. Whether the pathways to neurodegeneration caused by parkin mutations are multiple or not should be clarified in the future.

References

1 Savitt, J.M., Dawson, V.L. and Dawson, T.M. (2006). Diagnosis and treatment of Parkinson disease: molecules to medicine. *J Clin Invest* 116, 1744–1754.
2 Eriksen, J.L., et al. (2003). Caught in the act: alpha-synuclein is the culprit in Parkinson's disease. *Neuron* 40, 453–456.
3 Dauer, W. and Przedborski, S. (2003). Parkinson's disease: mechanisms and models. *Neuron* 39, 889–909.
4 Dawson, T.M. and Dawson, V.L. (2003). Rare genetic mutations shed light on the pathogenesis of Parkinson disease. *J Clin Invest* 111, 145–151.
5 Lucking, C.B., et al. (2000). Association between early-onset Parkinson's disease and mutations in the parkin gene. *N Engl J Med* 342, 1560–1567.
6 Ishikawa, A. and Tsuji, S. (1996). Clinical analysis of 17 patients in 12 Japanese families with autosomal-recessive type juvenile parkinsonism. *Neurology* 47, 160–166.
7 Takahashi, H., et al. (1994). Familial juvenile parkinsonism: clinical and pathologic study in a family. *Neurology* 44, 437–441.
8 Kitada, T., et al. (1998). Mutations in the parkin gene cause autosomal recessive

juvenile parkinsonism. *Nature* 392, 605–608.

9 Marin, I., et al. (2004). Parkin and relatives: the RBR family of ubiquitin ligases. *Physiol Genomics* 17, 253–263.

10 Joazeiro, C.A. and Weissman, A.M. (2000). RING finger proteins: mediators of ubiquitin ligase activity. *Cell* 102, 549–552.

11 Koegl, M., et al. (1999). A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly. *Cell* 96, 635–644.

12 Shimura, H., et al. (2000). Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. *Nat Genet* 25, 302–305.

13 Imai, Y., Soda, M. and Takahashi, R. (2000). Parkin suppresses unfolded protein stress-induced cell death through its E3 ubiquitin-protein ligase activity. *J Biol Chem* 275, 35661–35664.

14 Zhang, Y., et al. (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 USA* 97, 13354–13359.

15 Mata, I.F., Lockhart, P.J. and Farrer, M.J. (2004). Parkin genetics: one model for Parkinson's disease. *Hum Mol Genet* 13 Spec No 1: R127–R133.

16 Sakata, E., et al. 2003. Parkin binds the Rpn10 subunit of 26S proteasomes through its ubiquitin-like domain. *EMBO Rep* 4, 301–306.

17 Pickart, C.M. and Fushman, D. (2004). Polyubiquitin chains: polymeric protein signals. *Curr Opin Chem Biol* 8, 610–616.

18 Doss-Pepe, E.W., Chen, L. and Madura, K. (2005). Alpha-synuclein and parkin contribute to the assembly of ubiquitin lysine 63-linked multiubiquitin chains. *J Biol Chem* 280, 16619–16624.

19 Lim, K.L., et al. (2005). Parkin mediates nonclassical, proteasomal-independent ubiquitination of synphilin-1: implications for Lewy body formation. *J Neurosci* 25, 2002–2009.

20 Lim, K.L., Dawson, V.L. and Dawson, T.M. (2006). Parkin-mediated lysine 63-linked polyubiquitination: a link to protein inclusions formation in Parkinson's and other conformational diseases? *Neurobiol Aging* 27, 524–529.

21 Matsuda, N., et al. (2006). Diverse effects of pathogenic mutations of Parkin that catalyze multiple monoubiquitylation *in vitro*. *J Biol Chem* 281, 3204–3209.

22 Hampe, C., et al. (2006). Biochemical analysis of Parkinson's disease-causing variants of Parkin, an E3 ubiquitin-protein ligase with monoubiquitylation capacity. *Hum Mol Genet* 15, 2059–2075.

23 Gu, W.J., et al. (2003). The C289G and C418R missense mutations cause rapid sequestration of human Parkin into insoluble aggregates. *Neurobiol Dis* 14, 357–364.

24 Henn, I.H., et al. (2005). Pathogenic mutations inactivate parkin by distinct mechanisms. *J Neurochem* 92, 114–122.

25 Wang, C., et al. (2005). Alterations in the solubility and intracellular localization of parkin by several familial Parkinson's disease-linked point mutations. *J Neurochem* 93, 422–431.

26 Sriram, S.R., et al. (2005). Familial-associated mutations differentially disrupt the solubility, localization, binding and ubiquitination properties of parkin. *Hum Mol Genet* 14, 2571–2586.

27 Fallon, L., et al. (2006). A regulated interaction with the UIM protein Eps15 implicates parkin in EGF receptor trafficking and PI(3)K-Akt signalling. *Nat Cell Biol* 8, 834–842.

28 Imai, Y., et al. (2002). CHIP is associated with Parkin, a gene responsible for familial Parkinson's disease, and enhances its ubiquitin ligase activity. *Mol Cell* 10, 55–67.

29 Winklhofer, K.F., et al. (2003). Inactivation of parkin by oxidative stress and C-terminal truncations: a protective role of molecular chaperones. *J Biol Chem* 278, 47199–47208.

30 Hatakeyama, S., et al. (2001). U box proteins as a new family of ubiquitin-protein ligases. *J Biol Chem* 276, 33111–33120.

31 Jiang, J., et al. (2001). CHIP is a U-box-dependent E3 ubiquitin ligase: identification of Hsc70 as a target for ubiquitylation. *J Biol Chem* 276, 42938–42944.

32 Murata, S., et al. (2001). CHIP is a chaperone-dependent E3 ligase that ubiquitylates unfolded protein. *EMBO Rep* 2, 1133–1138.

33 Ballinger, C.A., et al. (1999). Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. *Mol Cell Biol* 19, 4535–4545.

34 Kalia, S.K., et al. (2004). BAG5 inhibits parkin and enhances dopaminergic neuron degeneration. *Neuron* 44, 931–945.

35 Sato, S., et al. (2006). 14-3-3eta is a novel regulator of parkin ubiquitin ligase. *EMBO J* 25, 211–221.

36 Zhong, L., et al. (2005). RING finger ubiquitin-protein isopeptide ligase Nrdp1/FLRF regulates parkin stability and activity. *J Biol Chem* 280, 9425–9430.

37 Imai, Y. and Takahashi, R. (2004). How do Parkin mutations result in neurodegeneration? *Curr Opin Neurobiol* 14, 384–389.

38 Huynh, D.P., et al. (2003). The autosomal recessive juvenile Parkinson disease gene product, parkin, interacts with and ubiquitinates synaptotagmin XI. *Hum Mol Genet* 12, 2587–2597.

39 Chung, K.K., et al. (2001). Parkin ubiquitinates the alpha-synuclein-interacting protein, synphilin-1: implications for Lewy-body formation in Parkinson disease. *Nat Med* 7, 1144–1150.

40 Shimura, H., et al. (2001). Ubiquitination of a new form of alpha-synuclein by parkin from human brain: implications for Parkinson's disease. *Science* 293, 263–269.

41 Ren, Y., Zhao, J. and Feng, J. (2003). Parkin binds to alpha/beta tubulin and increases their ubiquitination and degradation. *J Neurosci* 23, 3316–3324.

42 Corti, O., et al. (2003). The p38 subunit of the aminoacyl-tRNA synthetase complex is a Parkin substrate: linking protein biosynthesis and neurodegeneration. *Hum Mol Genet* 12, 1427–1437.

43 Imai, Y., et al. (2001). An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin. *Cell* 105, 891–902.

44 Tsai, Y.C., et al. (2003). Parkin facilitates the elimination of expanded polyglutamine proteins and leads to preservation of proteasome function. *J Biol Chem* 278, 22044–22055.

45 Staropoli, J.F., et al. (2003). Parkin is a component of an SCF-like ubiquitin ligase complex and protects postmitotic neurons from kainate excitotoxicity. *Neuron* 37, 735–749.

46 Choi, P., et al. (2003). SEPT5_v2 is a parkin-binding protein. *Brain Res Mol Brain Res* 117, 179–189.

47 Jiang, H., et al. (2004). Parkin protects human dopaminergic neuroblastoma cells against dopamine-induced apoptosis. *Hum Mol Genet* 13, 1745–1754.

48 Ko, H.S., et al. (2006). Identification of far upstream element-binding protein-1 as an authentic Parkin substrate. *J Biol Chem* 281, 16193–16196.

49 Um, J.W., et al. (2006). Parkin ubiquitinates and promotes the degradation of RanBP2. *J Biol Chem* 281, 3595–3603.

50 Zeng, Z., et al. (1997). A novel endothelin receptor type-B-like gene enriched in the brain. *Biochem Biophys Res Commun* 233, 559–567.

51 Donohue, P.J., et al. (1998). A human gene encodes a putative G protein-coupled receptor highly expressed in the central nervous system. *Brain Res Mol Brain Res* 54, 152–160.

52 Marazziti, D., et al. (2004). Altered dopamine signaling and MPTP resistance in mice lacking the Parkinson's disease-associated GPR37/parkin-associated endothelin-like receptor. *Proc Natl Acad Sci USA* 101, 10189–10194.

53 Mori, K. (2000). Tripartite management of unfolded proteins in the endoplasmic reticulum. *Cell* 101, 451–454.

54 Meusser, B., et al. (2005). ERAD: the long road to destruction. *Nat Cell Biol* 7, 766–772.

55 Johnston, J.A., Ward, C.L. and Kopito, R.R. (1998). Aggresomes: a cellular response to

misfolded proteins. *J Cell Biol* **143**, 1883–1898.

56 Nakagawa, T., et al. (2000). Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. *Nature* **403**, 98–103.

57 Urano, F., et al. (2000). Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* **287**, 664–666.

58 Yang, Y., et al. (2003). Parkin suppresses dopaminergic neuron-selective neurotoxicity induced by Pael-R in Drosophila. *Neuron* **37**, 911–924.

59 Kitao, Y., et al. (2007). Pael receptor induces death of dopaminergic neurons in the substantia nigra via endoplasmic reticulum stress and dopamine toxicity, which is enhanced under condition of Parkin inactivation. *Hum Mol Genet.* **16**, 50–60.

60 LaVoie, M.J., et al. (2005). Dopamine covalently modifies and functionally inactivates parkin. *Nat Med* **11**, 1214–1221.

61 Dong, Z., et al. (2003). Dopamine-dependent neurodegeneration in rats induced by viral vector-mediated overexpression of the parkin target protein, CDCrel-1. *Proc Natl Acad Sci USA* **100**, 12438–12443.

62 Engelender, S., et al. (1999). Synphilin-1 associates with alpha-synuclein and promotes the formation of cytosolic inclusions. *Nat Genet* **22**, 110–114.

63 Quevillon, S., et al. (1999). Macromolecular assemblage of aminoacyl-tRNA synthetases: identification of protein–protein interactions and characterization of a core protein. *J Mol Biol* **285**, 183–195.

64 Greene, J.C., et al. (2003). Mitochondrial pathology and apoptotic muscle degeneration in Drosophila parkin mutants. *Proc Natl Acad Sci USA* **100**, 4078–4083.

65 Pesah, Y., et al. (2004). Drosophila parkin mutants have decreased mass and cell size and increased sensitivity to oxygen radical stress. *Development* **131**, 2183–2194.

66 Goldberg, M.S., et al. (2003). Parkin-deficient mice exhibit nigrostriatal deficits but not loss of dopaminergic neurons. *J Biol Chem* **278**, 43628–43635.

67 Itier, J.M., et al. (2003). Parkin gene inactivation alters behaviour and dopamine neurotransmission in the mouse. *Hum Mol Genet* **12**, 2277–2291.

68 Von Coelln, R., et al. (2004). Loss of locus coeruleus neurons and reduced startle in parkin null mice. *Proc Natl Acad Sci USA* **101**, 10744–10749.

69 Perez, F.A. and Palmiter, R.D. (2005). Parkin-deficient mice are not a robust model of parkinsonism. *Proc Natl Acad Sci USA* **102**, 2174–2179.

70 Valente, E.M., et al. (2004). Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* **304**, 1158–1160.

71 Clark, I.E., et al. (2006). Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature* **441**, 1162–1166.

72 Park, J., et al. (2006). Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. *Nature* **441**, 1157–1161.

73 Yang, Y., et al. (2006). Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of Drosophila Pink1 is rescued by Parkin. *Proc Natl Acad Sci USA* **103**, 10793–10798.

74 Palacino, J.J., et al. (2004). Mitochondrial dysfunction and oxidative damage in parkin-deficient mice. *J Biol Chem* **279**, 18614–18622.

75 Perez, F.A., Curtis, W.R. and Palmiter, R.D. (2005). Parkin-deficient mice are not more sensitive to 6-hydroxydopamine or methamphetamine neurotoxicity. *BMC Neurosci* **6**, 71.

76 Haywood, A.F. and Staveley, B.E. (2004). Parkin counteracts symptoms in a Drosophila model of Parkinson's disease. *BMC Neurosci* **5**, 14.

77 Petrucelli, L., et al. (2002). Parkin protects against the toxicity associated with mutant alpha-synuclein: proteasome dysfunction selectively affects catecholaminergic neurons. *Neuron* **36**, 1007–1019.

78 Omura, T., et al. (2006). A ubiquitin ligase HRD1 promotes the degradation of Pael

receptor, a substrate of Parkin. *J Neurochem.* 99, 1456–1469.

79 Darios, F., et al. (2003). Parkin prevents mitochondrial swelling and cytochrome c release in mitochondria-dependent cell death. *Hum Mol Genet* 12, 517–526.

80 Rosen, K.M., et al. (2006). Parkin protects against mitochondrial toxins and beta-amyloid accumulation in skeletal muscle cells. *J Biol Chem* **281**, 12809–12816.

81 Klein, R.L., et al. (2006). Parkin is protective for substantia nigra dopamine neurons in a tau gene transfer neurodegeneration model. *Neurosci Lett* **401**, 130–135.

82 Menendez, J., et al. (2006). Suppression of Parkin enhances nigrostriatal and motor neuron lesion in mice over-expressing human-mutated tau protein. *Hum Mol Genet* **15**, 2045–2058.

83 Vercammen, L., et al. (2006). Parkin protects against neurotoxicity in the 6-hydroxydopamine rat model for Parkinson's disease. *Mol Ther* **14**, 716–723.

84 Lo Bianco, C., et al. (2004). Lentiviral vector delivery of parkin prevents dopaminergic degeneration in an alpha-synuclein rat model of Parkinson's disease. *Proc Natl Acad Sci USA* **101**, 17510–17515.

ANTIOXIDANTS & REDOX SIGNALING
Volume 9, Numbers 5, 2007
© Mary Ann Liebert, Inc.
DOI: 10.1089/ars.2006.1524

Forum Review

Expanding Insights on the Involvement of Endoplasmic Reticulum Stress in Parkinson's Disease

HUA–QIN WANG and RYOSUKE TAKAHASHI

ABSTRACT

Parkinson's disease (PD) is the second most common neurodegenerative disease characterized by selective loss of dopaminergic neurons and the presence of Lewy bodies. The pathogenesis of PD remains incompletely understood. Environmental factors, oxidative damage, misfolded protein aggregates, ubiquitin–proteasome system impairment, and mitochondrial dysfunction might all be involved. Recent studies point to activation of endoplasmic reticulum (ER) stress-mediated cell death linked to PD. Accumulation of unfolded and/or misfolded proteins in the ER lumen induces ER stress. To withstand such potentially lethal conditions, intracellular signaling pathways collectively termed the unfolded protein responses (UPR) are activated. The UPR include translational attenuation, induction of ER resident chaperones, and degradation of misfolded proteins through the ER-associated degradation. In case of severe and/or prolonged ER stress, cellular signals leading to cell death are activated. Accumulating evidence suggests that ER stress induced by aberrant protein degradation is implicated in PD. Here the authors review the emerging role of ER stress in PD and related disorders, and highlight current knowledge in this field that may reveal novel insight into disease mechanisms and help to provide novel avenues to potential therapies. *Antioxid. Redox Signal.* 9, 553–561.

INTRODUCTION

PARKINSON'S DISEASE (PD) is the most common neurodegenerative movement disorder among elderly people. The classical symptoms of the disease include rigidity, resting tremor, bradykinesia, and postural instability. The pathological hallmarks underlying the clinical phenotypes are characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), together with the presence of intraneuronal inclusions termed Lewy bodies (7). Although the molecular mechanisms underlying neurodegeneration remain elusive, its pathogenesis begins to be considered as a multifactorial cascade of deleterious factors. Mitochondrial dysfunction, protein aggregation, impairment of the ubiquitin–proteasome system (UPS), and activation of the stress kinase signaling pathways have been supposed to be involved in the pathogenesis of PD. Recently, emerging lines of evidence from familial forms of PD, coupled with those findings from toxin-induced PD models, raise the possibility of widespread involvement of unfolded protein responses [UPR, also known as endoplasmic reticulum (ER) stress responses], the term given to an imbalance between the cellular demand for ER function and ER capacity (2, 43, 44), in the pathogenesis of this disease.

Neuronal loss in both familial and sporadic forms of neurodegenerative disorders is often accompanied by formation of inclusion bodies and aggregation of misfolded proteins (45). Upregulation of ER stress markers has been observed in postmortem brain tissues and cell culture models of many neurodegenerative diseases including PD, Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and expanded polyglutamine diseases such as Huntington's disease and spinocerebellar ataxias (4, 26). Several chaperones ameliorate the accumulation of misfolded proteins triggered by oxidative or nitrosative stress, or of mutated gene products (26, 40, 58). The hypothesis that ER dysfunction plays an important role in the development of dopaminergic neuronal

Department of Neurology, Kyoto University Graduate School of Medicine, Kyoto, Japan.

loss in PD has recently been put forward by observations that parkin has been associated with ER stress-induced cell death. Mutations in the *PARK2* gene coding for parkin cause autosomal recessive juvenile Parkinsonism (AR-JP), the most common form of familial PD. This review summarizes new observations implying that impairment of ER functioning is a common denominator of neuronal death in PD.

ER STRESS AND ER STRESS RESPONSES

Besides calcium storage and signaling, a central function of the ER is quality control for membrane or secretory proteins, which comprise nearly one-third of all cellular proteins (29). The importance of the ER for normal cell function is highlighted by the observation that blocking of the protein folding or processing reactions can be lethal for cells. Indeed, in various cases such as depletion of ER calcium stores, blocking the proteasome that is required for degradation of unfolded proteins, or genetic mutations resulting in proteins that cannot be properly folded, the ER functions are impaired and unfolded proteins accumulate in the ER. Accumulation of unfolded proteins in the ER is a severe form of stress that will induce apoptosis if ER function cannot be restored. To cope with conditions associated with impairment of ER function, cells activate highly conserved stress response, the UPR (2, 43, 44). The main purpose of UPR is to remove aberrant substrates and restore the ER to an efficiently operating maturation compartment. The UPR pathway functions as a tripartite signal that comprises (i) inhibition of general translation to attenuate the load of proteins to the ER, (ii) transcriptional activation of ER chaperones to increase protein folding and processing capacity; (iii) activation of ER-associated degradation (ERAD) to promote degradation of terminally misfolded proteins. However, when the ER stress is severe or prolonged, the cells eventually activate apoptotic signals, leading to cell death (5, 29) (Fig. 1).

Cells have developed two pathways for removing unfolded proteins from the lumen of the ER, increasing folding capacity through upregulation of ER chaperones (Fig. 2A) and promoting degradation of terminally misfolded proteins through activation of ERAD (Fig. 2B). The ERAD pathway is

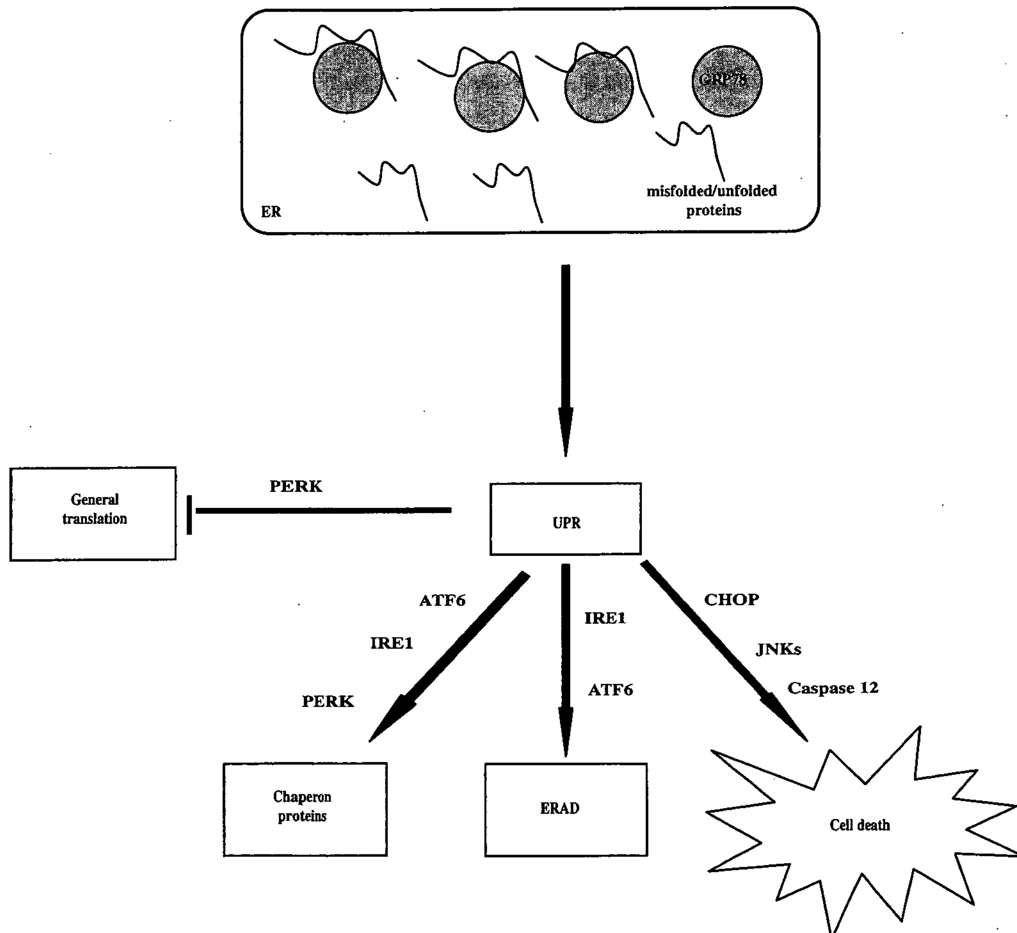

FIG. 1. The tripartite unfolded protein response. Three primary transducers of the unfolded protein response (UPR) signal, known as ATF6, IRE1, and PERK, seek to relieve ER stress through suppression of translational initiation, increased folding capacity of ER, and degradation of terminally misfolded proteins until the aberrations have been alleviated. However, severe or prolonged ER stress eventually activates apoptotic pathway.

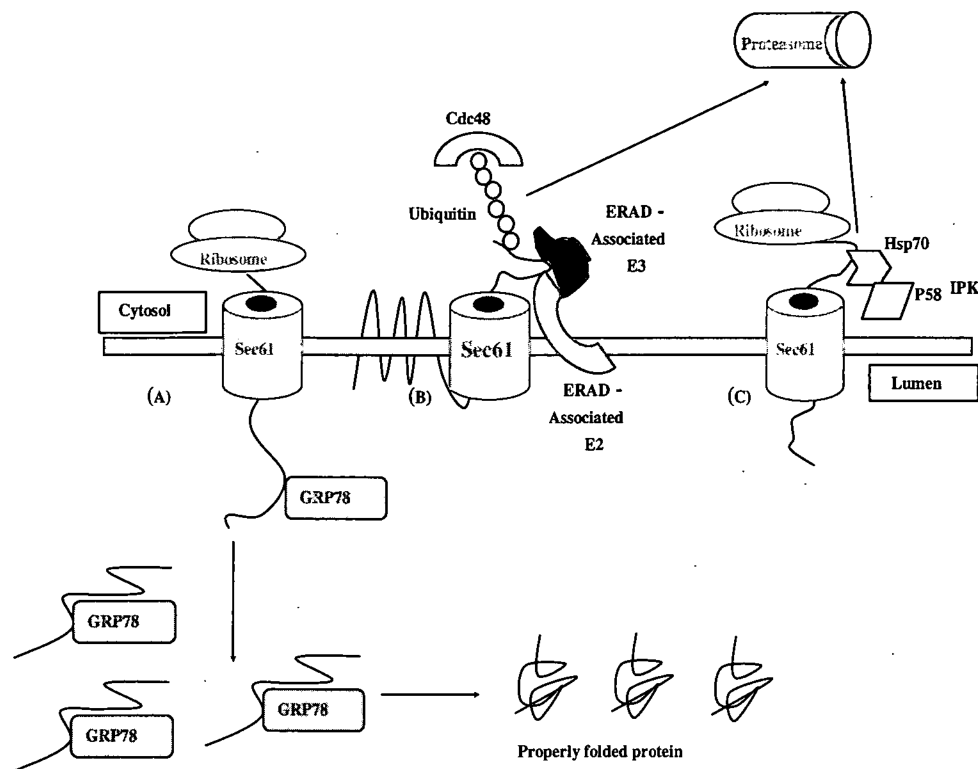

FIG. 2. The pathways related to clearance of unfolded proteins upon ER stress. Under ER stress conditions, cells develop three pathways to clear unfolded proteins in the ER. **(A)** ER chaperones such as GRP78 are upregulated to facilitate proper substrate folding. **(B)** Activation of ER associated degradation (ERAD)-mediated degradation of terminally unfolded proteins. **(C)** Cotranslational degradation of newly synthesized proteins trapped in the Sec61 translocon to decrease load burden to the ER.

characterized by the polyubiquitination and subsequent degradation of misfolded proteins (36–38). With the aid of the cytosolic AAA-ATPase p97/Cdc48, the misfolded ER protein is extruded through the ER membrane conduit Sec61, where it is then polyubiquitinated and delivered to the proteasome for degradation in the cytosol (Fig. 2B).

A recent study has revealed a new layer in the UPR pathway that permits the cotranslocational degradation of secretory proteins involving P58^{IPK}/DNAJC3, which collaborates with cytosolic chaperone networks and appears to assist in the cotranslational/translocational degradation of nascent polypeptide chains that are stalled in ER translocons (Fig. 2C). This function diminishes the biosynthetic burden on the ER by degrading proteins at a stage earlier than previously envisioned. This protective effect might reflect a reduction in protein flux into the stressed ER's lumen. Alternatively, intervention early in the protein biogenesis by P58^{IPK}/DNAJC3 might allow the maturation and quality control machinery to focus its attenuation on the pre-existing improperly folded proteins that triggered the initial UPR signal (34).

ER STRESS IN FAMILIAL FORMS OF PD

As in most cases of PD, the degeneration is idiopathic, the etiology of the disease remains unknown. The recent identification of genetic mutations in familial cases of PD has advanced our understanding of the molecular mechanisms that cause the neurodegeneration. So far, six PD-associated genes have been identified.

Autosomal dominant forms of PD

Three rare missense mutations in the α-*synuclein* gene (A30P, E46K, and A53T) cause autosomal dominant familial PD (23, 39). Although the function of α-synuclein is still unclear, the discoveries that α-synuclein is a main component of Lewy bodies (48) and that its overexpression and gene triplication can cause neurodegeneration (1, 46) suggest that abnormalities of α-synuclein might be crucial for the pathogenesis of both familial and sporadic forms of PD. α-Synuclein transgenic mouse or *Drosophila* at least partially recapitulated PD phenotype including α-synuclein positive aggregate formation, although no obvious dopaminergic neuronal loss was observed in transgenic mice (12, 49). Lentivirus-mediated overproduction of α-synuclein in rat substantia led to significant cell death (27). Leucine-rich repeat kinase 2 (LRRK2) has recently been added to the list of genes that are implicated in autosomal dominant PD (35, 59). LRRK2 is a GTP/GDP-regulated protein kinase, and increased kinase activity appears to be implicated in neurodegeneration (47). Another gene, ubiquitin carboxyl-terminal esterase L1 (UCHL1), has been associated with the dominantly inherited disease, but the genetic evidence for its pathogenicity is not established since only a single mutation with low penetrance has been identified in one family (39).

Autosomal recessive forms of PD

Three recessive forms of parkinsonism have been identified, including mutations in the genes that encode parkin, DJ1, and PTEN-induced kinase 1 (PINK1).

Mutations in the *parkin* gene were originally discovered from the linkage study of Japanese AR-JP families, the most frequent type of familial PD (20). Thereafter its mutations have been found worldwide. Parkin is a 465 amino acid protein characterized by a ubiquitin-like domain at its NH2-terminus, as well as two RING (really interesting new gene) finger domains flanking a domain known as the IBR (in-between RING) at its COOH-terminus (RING–IBR–RING). Like many other proteins containing a RING domain, parkin has been found to function as an ubiquitin ligase (E3) (Fig. 3). E3s are part of the cellular machinery that tags proteins with ubiquitin, thereby targeting them for degradation by the proteasome. The UPS plays a major role in many vital cellular processes, and its dysfunction has been implicated in the pathogenesis of neurodegenerative disorders including sporadic PD. Parkin mutants associated with AR-JP reduce or abolish its E3 activity. Therefore, the most straightforward mechanism by which the dysfunction of parkin would cause neurodegeneration is accumulation of some neurotoxic substrate protein(s), which leads to dysfunction and eventually the death of susceptible neurons.

Mutations in *PINK1* were initially identified in three large consanguineous families with autosomal recessive forms of PD (52). Mutations in *PINK1* have differential effects on protein stability, localization, and kinase activity (3). As the kinase domain is the hot spot of mutations, disruption of the kinase activity is the most probable disease mechanism. Although functional data are limited, wild-type PINK1 protected neurons from mitochondrial dysfunction and apoptosis induced by oxidative stress (11), supporting an involvement of mitochondria in the pathogenesis.

A third gene linked to recessively inherited albeit rare PD is *DJ-1* (6). DJ1 has been assigned various functions, but perhaps the most relevant function in terms of the pathogenesis of PD is its potential role in oxidative stress response, either as a redox sensor or antioxidant protein (8).

In this review, rather than attempting to overview the entire picture, we focus on potential involvement of ER stress in this disease according to published data.

ER stress in α-synuclein-associated PD

In a *Drosophila* model of PD engineered to express wild-type and mutant α-synuclein, expression of molecular chaperone heat shock protein 70 (Hsp70) prevented dopaminergic cell loss mediated by accumulation of α-synuclein (1). However, Hsp70 is not directly activated in the UPR. A recent study provided direct evidence indicating the implication of ER stress in α-synuclein-mediated cell death (47). In a mammalian cell culture model, induction of the expression of A53T α-synuclein induced ER stress, as evidenced by the elevation in expression of CHOP and GRP78, increased phosphorylation of eIF2α, and activation of caspase-12. Furthermore, decrease of eukaryotic initiation factor 2α (eIF2α) phosphorylation by inhibitor, or knockdown of caspase-12 levels by RNA interference partially protected against cell death (47), indicating that ER stress at least partially contribute to A53T α-synuclein-induced cell death. Overexpression of mutant forms of α-synuclein in cultured neuronal cells leads to decrease in proteasome activity (51). The mechanism underlying mutant α-synuclein-induced impairment of proteasome activity remains to be identified. α-Synuclein is reported to be degraded through several different pathways including macroautophagy, chaperone-mediated autophagy, and proteasome (10, 50, 53). Since α-synuclein interacts with a subunit of proteasome regulatory complexes (15), it is possible that mutant α-synuclein directly affects the proteasome complex. It is of interest that an important means of removing misfolded proteins from the ER is their degradation by proteasomes. In addition, it has been reported that partial inhibition of the proteasome activity by poly-Q was sufficient to cause ER stress in primary neurons (33). Therefore, ER stress observed in overexpression of mutant forms

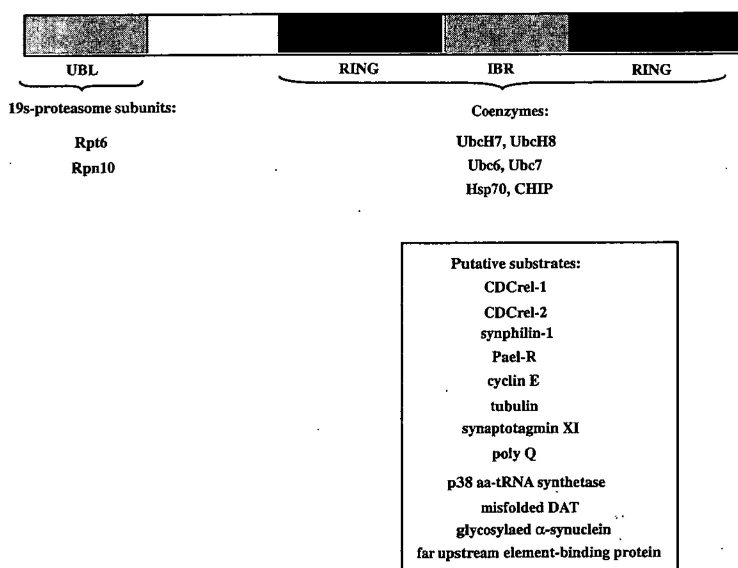

FIG. 3. Modular structure of parkin. Parkin has a modular structure, containing a ubiquitin-like (UBL) domain at the amino-terminus and two real interesting new gene (RING) fingers at its carboxy-terminus. In addition, an in-between RING (IBR) domain is inserted in the middle portion between two RING finger motifs. The two RINGs and IBR are named as a RING box. Furthermore, the linker region is located between UBL and RING box. The UBL binds to 19S proteasome subunits, and the RING–IBR–RING domain binds to specific co-enzymes and substrates (except for glycosylated α-synuclein, which binds to the UBL domain).

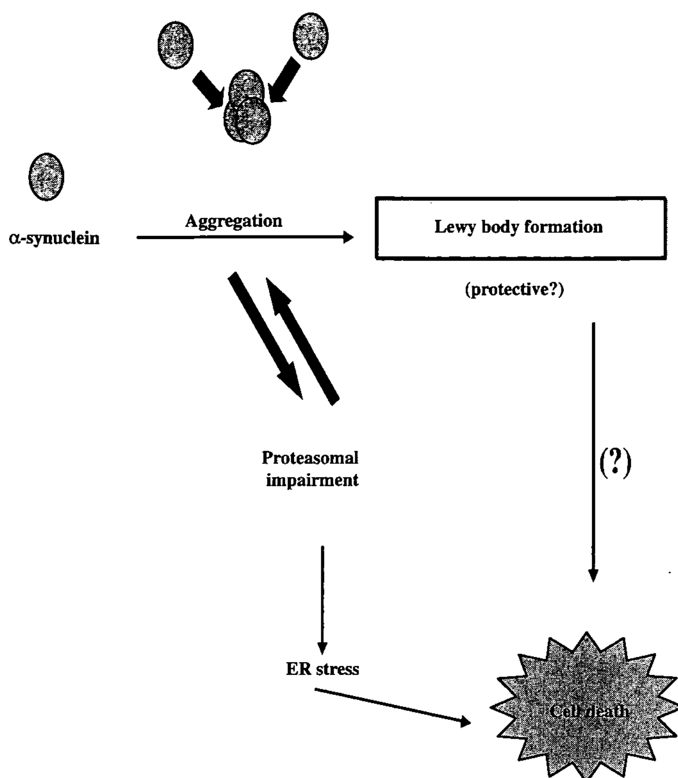

FIG. 4. Hypothetical mechanism of ER stress-mediated cell death induced by α-synuclein. The insidious loop feedback between α-synuclein aggregation and proteasomal impairment induces ER stress, which at least in part contributes to α-synuclein-mediated cell death.

of α-synuclein in cultured neuronal cells possibly derive from disruption of proteasome activity (Fig. 4).

ER stress in parkin-associated PD

Evidence supporting the involvement of ER stress in parkin mutations-induced cell death is more potent. Parkin is an E3 enzyme that interacts with Hsp70 and CHIP and plays a general role in protein degradation during ER stress (17–19). In line with this notion, cognate E2 (ubiquitin conjugating enzyme) partners of parkin include Ubc6 and Ubc7, which are ER-associated E2s involved in ERAD, indicating parkin is a component of ERAD machinery. It is easily conceivable that disruption of parkin function directly leads to ER stress, since ERAD and ER stress are coordinately regulated and deletion of ERAD components results in ER stress (13).

Given that accumulation of the substrate of parkin might play a key role in the neurodegenerative process, identification of parkin substrates has therefore been a major focus of many laboratories working on parkin. Typically, one expects an E3 to be highly specific for one or possibly a small number of substrates. Unexpectedly, a large number of putative parkin substrates have been reported (Fig. 3). Interestingly, several parkin substrates are misfolded or aggregation-prone proteins and are components of Lewy bodies. Considering that misfolded proteins, associated molecular chaperones, and proteasomal subunits are accumulated in Lewy bodies, the substrates of parkin may represent a subset of misfolded proteins. The C-terminus of Hsc70 interaction protein (CHIP), a U-box containing E3, has been shown to recognize misfolded protein through the heat-shock protein Hsp70 and is proposed to be a "quality control E3" that is contributed to the clearance of misfolded proteins (31). Given that parkin also binds to Hsp70, parkin may have a similar function to CHIP in dealing with misfolded proteins.

Among these substrates of parkin, one of the best-characterized parkin substrates, Pael-R underscores the ER stress-mediated cell death in the pathogenesis of AR-JP (18). Pael-R is a multipass G protein-coupled transmembrane protein with homology to the endothelin receptor type B, the function of which is unknown. Folding of Pael-R is a formidable challenge to cells. When overexpressed in cultured cells, Pael-R tends to become unfolded and insoluble; at the early stage of Pael-R accumulation, ER chaperones showed transcriptional upregulation, indicating that accumulation of Pael-R actually induced ER stress. Interestingly, CHIP serves as a cofactor of parkin. When Pael-R misfolding exceeds the cellular chaperone capacity, CHIP is upregulated, which sequesters Hsp70 and facilitates parkin-mediated ubiquitination of Pael-R (17). Under these conditions, parkin apparently acts as part of the ERAD machinery, utilizing the ER associated E2 enzymes Ubc6 and Ubc7 as the collaborating partners.

The UPR induces upregulation of parkin mRNA per se, and cells overexpressing parkin, but not mutant parkins found in AR-JP patients, are particularly resistant to unfolded protein-induced cell death (19). Furthermore, when astrocytes and neurons were exposed to conditions associated with ER stress, parkin protein levels were upregulated in astrocytes

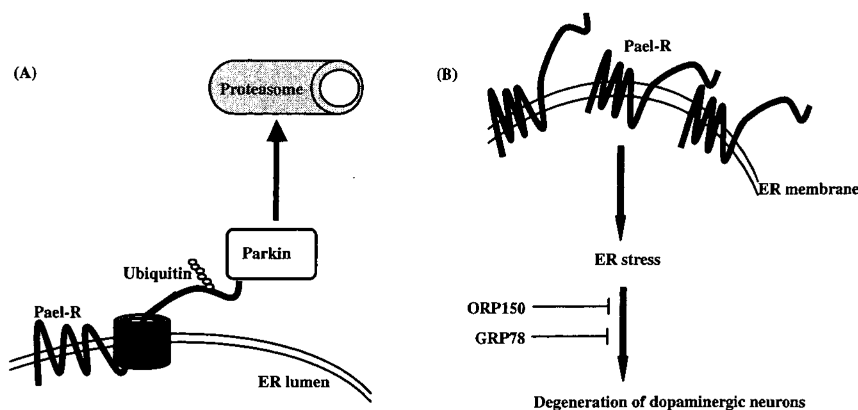

FIG. 5. Implication of ER stress in Pael-R-induced neuronal degeneration. (A) Pael-R is a difficult protein to be folded, and parkin ubiquitinates misfolded Pael-R and facilitates its degradation by UPS. (B) When dysfunction of parkin or overexpression of Pael-R, unfolded Pael-R accumulates in the ER and causes ER stress. ER chaperones, GRP78 and ORP150, suppress Pael-R-induced neuronal degeneration, possibly through enhancing the folding capacity of the ER.

but not in neurons (25). In the brain, Pael-R is primarily expressed in oligodendrocytes and shows little expression in neurons, except for a few distinct subpopulations of neurons, including hippocampal neurons and dopaminergic neurons in the SNpc. This implies that dopaminergic neurons of patients suffering from AR-JP are less well protected from neurotoxicity arising under conditions of Pael-R accumulation-induced ER stress. Thus, the inability of neurons to respond to ER stress by activating the expression of parkin and distributional pattern of Pael-R may contribute to the high vulnerability of dopaminergic neuronal cells. Using a transgenic *Drosophila* expressing human Pael-R, Yang *et al.* (55) found that this fly model revealed the age-dependent selective degeneration of dopaminergic neurons in spite of pan-neuronal expression of Pael-R. This Pael-R mediated dopaminergic neuronal loss was suppressed by the coexpression of human parkin and exacerbated by knockdown of endogenous parkin in the *Drosophila* by RNA interference. Recent *in vivo* observations in mice further highlights the important role of ER stress in Pael-R-mediated toxicity (21). Adenovirus-mediated overexpression of Pael-R in dopaminergic neurons induced ER stress and degeneration. This Pael-R-mediated neuronal death was suppressed by increased GRP78 or oxygen regulated protein 150 (ORP150), whereas cell death was exacerbated by downregulation of parkin or ORP150 (Fig. 5). Furthermore, a complicated interplay between ER stress and dopamine toxicity might present a mechanism underlying Pael-R-induced selective dopaminergic neuronal death, as evidenced by a neuroprotective effect of a tyrosine hydroxylase (TH) inhibitor (21).

ER STRESS IN TOXIN-INDUCED PD MODELS

Mitochondria toxins, 6-OHDA, rotenone, and MPP^+, are believed to contribute to dopaminergic neuronal death. These reagents can promote the generation of reactive oxygen species (ROS) via the inhibition of mitochondrial complex I or their oxidative function (14). Using functional genomics approaches to identify transcriptional alterations, numerous changes in genes associated with UPR were identified (42). Notably, a major target of the UPR pathway, the transcription factor CHOP, was dramatically upregulated by these reagents, as well as numerous markers of UPR including GRP78, splicing of XBP1, PERK, and the JNKs pathway. The assumption that ER dysfunction may play a role in the pathological process resulting in PD is corroborated by the observation that exposing cells to 6-OHDA, rotenone, or MPP^+, which are cellular models mimicking pathological disturbances associated with PD, induces a striking increase in transcripts associated with UPR (16, 42, 54). A number of reports have shown that both proteasome inhibition and ROS can trigger ER stress-mediated cell death pathways. One possible mechanism of ER stress induced by these mitochondria toxins is that accumulation of damaged oxidized proteins by the effects of these reagents on mitochondrial respiration causes ER stress (13). Alternatively, oxidative stress can directly compromise proteasomal components (41). However, the oxidative stress caused by the effects of these agents on mitochondrial respiration may not be totally attributable since a nonselective oxidant does not trigger ER stress. In addition, neurons lacking expression of PERK are defective in ER response and are significantly more sensitive to the death-promoting effects of PD mimetics (42). Thus, not only do mitochondria toxins provoke ER stress, but neurons lacking the capacity to deal with this by inducing an appropriate UPR are at greater risk of death, suggesting that ER stress is likely to play a causative role in neuronal cell death induced by these mitochondria toxins. Coupled with evidence from familial forms of PD, the induction of UPR and ER stress in these generally used neurotoxin models raise the possibility of widespread involvement of ER stress-mediated cell death in the pathogenesis of PD and other related disorders (Fig 6).

CROSSTALK OF ER STRESS WITH OXIDATIVE STRESS IN PD

PD has been closely associated with oxidative stress and mitochondrial dysfunction. In addition, dopaminergic neurons are particularly subjected to increased oxidative stress due to production of free radicals during dopamine autooxidation and dopamine metabolism (24). ER stress is intricately connected to oxidative stress. As described above, oxidative stress can directly or indirectly induce ER stress (13, 41). Evidence is also accumulating for a converse