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9

The Molecular Pathway to Neurodegeneration in Parkin-Related Parkinsonism

Ryosuke Takahashi

9.1

Introduction

Parkinson's disease (PD) is the most common neurodegenerative disease of the motor system amongst elderly people. The prevalence of PD is approximately 1% of people by the age of 70 years [1]. PD is characterized by a progressive loss of dopaminergic neurons in the pars compacta of the substantia nigra accompanied by the formation of Lewy bodies. Lewy bodies are intra-neuronal fibrillary inclusions mainly composed of α -synuclein [2]. They are regarded as the hallmark of idiopathic PD. Loss of neurons within the pars compacta of the substantia nigra causes progressive motor disturbances, classically tremor, rigidity, bradykinesia and postural instability. To date, there is no known effective therapy to prevent or retard neurodegeneration as a result of PD [1, 3].

Most cases of PD develop sporadically, however, fewer than 10% of cases are familial and presumably inherited [4]. Autosomal recessive juvenile parkinsonism (AR-JP) accounts for approximately 50% of cases of early-onset familial PD in affected European families [5]. It is characterized by several unique features, including young age of onset (usually under 40 years of age), dystonia, and a marked response to dopamine. The neuropathological hallmark of AR-JP is selective degeneration of dopaminergic neurons in the substantia nigra zona compacta, similar to that observed in the idiopathic form of PD. However, AR-JP is not usually associated with Lewy bodies [6, 7].

Mutations in the parkin gene are responsible for AR-JP [8]. In this chapter, the role of parkin in the ubiquitin-proteasome system will be focused and discussed in light of recent findings.

9.2

Parkin is an E3 Ubiquitin Ligase

9.2.1

Parkin and the Ubiquitin-Proteasome System

Parkin is a 465-amino acid protein characterized by a ubiquitin-like domain at its NH₂-terminus, as well as two RING-finger motifs and an IBR (in-between RING fingers) motif at its COOH terminus (RING-IBR-RING or RBR domain) [9]. The RING domain has been shown to be a feature of ubiquitin ligase involved in the ubiquitination reaction [10]. Polyubiquitination involves a sequence of reactions performed by ubiquitin-activating (E1), ubiquitin-conjugating (E2) and ubiquitin ligating (E3) enzymes. E3 interacts with specific substrate(s) and facilitates the formation of covalent bonds between the COOH terminus of ubiquitin and ϵ -lysine, either on a target protein or on the last ubiquitin of a protein-bound polyubiquitin chain in concert with its partner E2s. Yeast protein UFD2 is a multi-ubiquitin chain elongation factor, also called E4, required for efficient multi-ubiquitination of a substrate [11]. Polyubiquitin chains are thought to be potent targeting signals for the degradation of proteins within 26S proteasomes.

Several groups have shown that wild-type parkin is an E3 ubiquitin ligase [12–14] (Figure 9.1). Parkin ubiquitinates substrate proteins or itself in concert with E2s, such as UbcH7, UbcH8, Ubc6 and Ubc7 [12–14]. Moreover, several AR-JP-related missense mutations have been identified in the ubiquitin-like

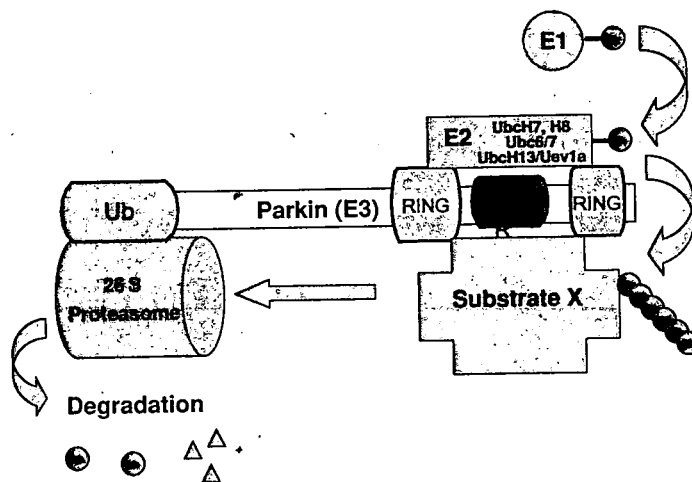


Fig. 9.1. Function of parkin in the ubiquitin proteasomal pathway. Parkin is an E3 ubiquitin ligase that recognizes substrate X and promotes ubiquitination in adjunct with two other ubiquitination enzymes, E1 and E2. Polyubiquitinated substrate X is recognized and degraded by the 26S proteasome. The

N-terminal ubiquitin-like domain and the C-terminal RING-IBR-RING domain of parkin serve as recruitment domains for 26S proteasome and E2 enzymes, respectively. Some of the known substrates of parkin associate with its RING-IBR-RING domain.

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 ubiquitin 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 ubiquitination 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

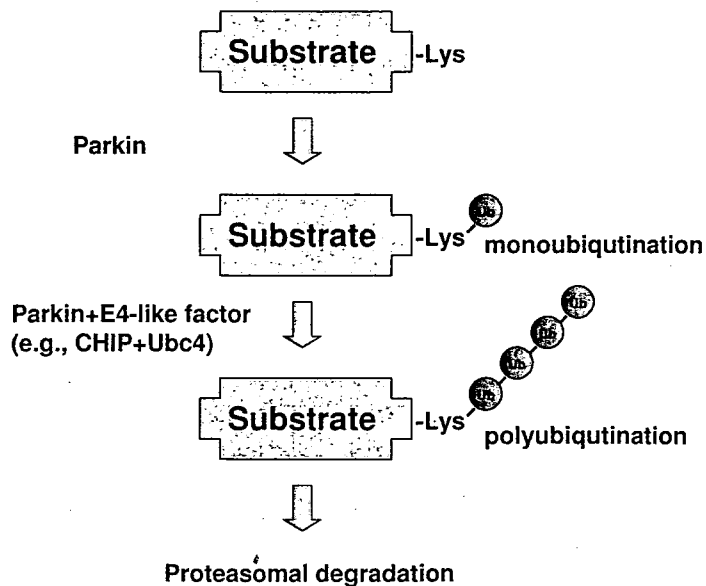


Fig. 9.2. Hypothetical two-step ubiquitination of Parkin substrates. At the first step, Parkin monoubiquitinates its substrate. Then, E4-like factors promote the elongation of polyubiquitin chain on the substrate molecules, thereby targeting the substrates to 26S proteasome. CHIP and its partner E2 Ubc4 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], Parkin-associated endothelin receptor-like receptor (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 \otimes	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.

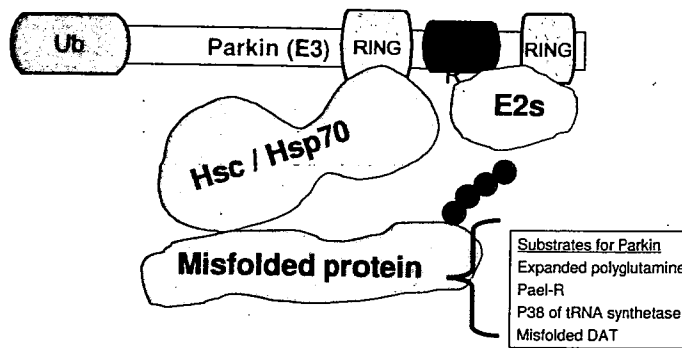


Fig. 9.3. Parkin may recognize a subset of misfolded proteins through Hsc/Hsp70. Parkin interacts with Hsc/Hsp70 through its first RING domain [28]. Parkin may recognize misfolded proteins including Pael-R, and p38 by using Hsc/Hsp70 as a substrate recognition subunit.

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

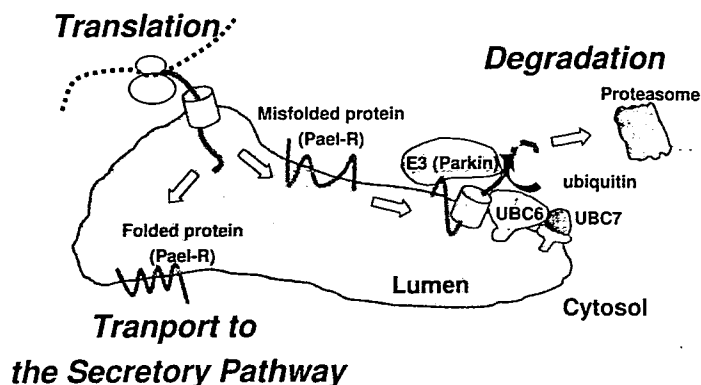


Fig. 9.4. Endoplasmic reticulum-associated degradation (ERAD). ERAD is a protein degradation system for unfolded secretory and membrane proteins. Improperly folded Pael-R is subject to ERAD, and parkin is an E3 involved in ERAD.

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 polyubiquitination 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 glutamatergic 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 catecholamine 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

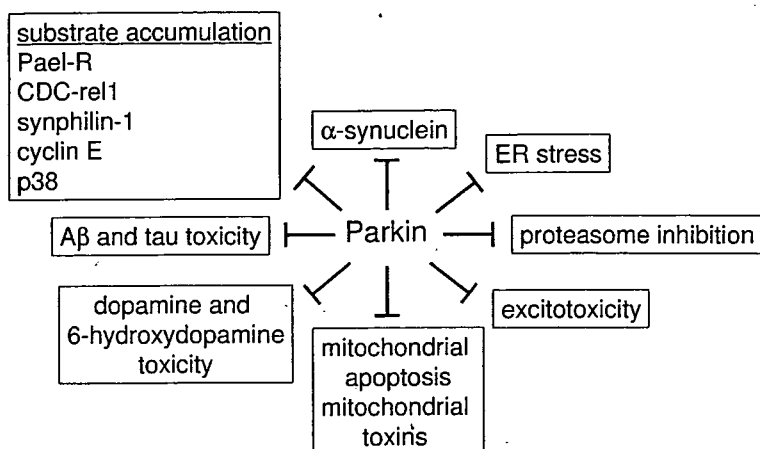


Fig. 9.5. Parkin protects cells from various stresses.

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 A β [80], tau [81, 82], dopamine or 6-hydroxydopamine 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.

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