ISSN 0006-2979, Biochemistry (Moscow), 2006, Vol. 71, No. 8, pp. 851-860. © Pleiades Publishing, Inc., 2006.
Published in Russian in Biokhimiya, 2006, Vol. 71, No. 8, pp. 1050-1061.

REVIEW

Ubiquitin-Protein Ligase Parkin and Its Role in the Development of Parkinson's Disease

O. A. Buneeva and A. E. Medvedev*

Institute of Biomedical Chemistry, Russian Academy of Medical Sciences, Pogodinskaya ul. 10, 119121 Moscow, Russia; fax: (495) 245-0857; E-mail: alexei.medvedev@ibmc.msk.ru

Received April 11, 2006

Abstract—Parkin is a protein encoded by the corresponding parkin gene. It exhibits ubiquitin-protein ligase activity. In this review, we analyze domain structure, substrate specificity, subcellular localization of parkin, and regulation of its activity. Then we discuss data on the effects of various mutations in the *parkin* gene on structure and functions of this protein and results obtained with *parkin* knock-out animals. Better understanding of parkin biochemistry, its compartmentalization, functions, and altered functions would help the development of new approaches for the treatment of both inherited and sporadic cases of Parkinson's disease.

DOI: 10.1134/S0006297906080050

Key words: parkin, Parkinson's disease, proteasome, ubiquitination, ubiquitin-protein ligase, mitochondria

Parkinson's disease (PD), originally described by James Parkinson in 1817, is one of the most common and very widespread neurodegenerative diseases in humans. About 1% of the population over 65 and 4-5% of the population over 85 year old suffers from this disease [1, 2]. Typical symptoms of PD include bradykinesia, tremor, muscle rigidity, and total decrease in mobility. Appearance of these symptoms is attributed to selective loss of dopaminergic neurons in the *substantia nigra*; this results in critical decrease in striatal dopamine [3]. Replacement therapy with dopamine precursors usually has palliative effect on manifestation of movement disorders, and in most cases PD gradually progresses up to death of patients [4].

Although clinical manifestations and neuropathological causes of various forms of PD are quite similar, this is rather heterogeneous disease. Most cases are obviously sporadic, but genetic predisposition to PD also exists. Development of PD caused by some inherited genetic defect represents less than 10% of all cases of PD [5]. However, identification of genes responsible for the development of this disease may give new insights into pathogenesis and also into investigation of putative role of the expressed proteins [4, 6, 7]. The *parkin* gene identified by Japanese scientists in 1998 is one of these genes. Its protein product is involved in proteasome degradation of intracellular proteins.

There are diagnostic signs of PD: dystrophic neuritis also known as Lewy neuritis and characteristic eosinophilic oval inclusions, Lewy bodies, found in cytoplasm [8]. The Lewy bodies are detected in remaining neurons of *substantia nigra*, brain cortex neurons, and forebrain basal ganglia [9]. The Lewy bodies contain protein fibrils of 10-15 nm in diameter surrounding lipoprotein core formed by granules and filaments. The major constituent of these bodies is the presynaptic protein α-synuclein. Lewy bodies are immunoreactive with respect to ubiquitin, a protein playing the key role in processes of proteolytic degradation of intracellular proteins. It is possible that formation of cytoplasmic protein aggregates associated with damaged ubiquitin–proteasome system is the cause underlying the appearance of Lewy bodies and neuritis [3].

Abbreviations: CDCrel-1) cell division control-related protein; CHIP) C-terminus Hsp 70-interacting protein; EPR) endoplasmic reticulum; Hsp 70) heat shock protein 70 kD; IBR) in-between RING-site; MAO) monoamine oxidase; Pael-R) parkin-associated endothelial-like receptor; PD) Parkinson's disease; RING) really interesting gene; UBL-domain) ubiquitin-like domain; UCL-L1) ubiquitin carboxy-terminal hydrolase L1; UPD-domain) unique parkin domain.

* To whom correspondence should be addressed.

STRUCTURE AND FUNCTIONING OF PROTEASOMES

Proteasomes are complex supramolecular structures performing most of the nonlysosomal energy-dependent proteolysis (Fig. 1). These multisubunit complexes have been found in cytoplasm, perinuclear space, and nucleus of all eukaryotic cells [10].

In proteasomes polyubiquitinated proteins undergo proteolytic cleavage into peptides and amino acids; polyubiquitin chain is cleaved off and released ubiquitin molecules may be then involved into the next ubiquitination cycle.

26S proteasome contains two regulatory 19S complexes, which cap catalytic 20S core proteasome; the latter consists of subunits exhibiting proteolytic activity and also structural and regulatory subunits possessing ATPase activity [10]. 20S proteasome is composed of 28 distinct subunits, which form four stacked heptameric rings. They form a hollow cylinder structure where proteolysis occurs [11, 12]. 20S proteasomes contain more than ten types of various subunits, which are subdivided into two families, α- and β-subunits. The external rings are formed by α-subunits, whereas the internal rings are formed by β-subunits. *In vitro* experiments revealed that active sites of each of seven β-subunits are characterized by different primary substrate specificity: chymotrypsin-, trypsin-, and caspase-like active sites. All these active sites contain catalytic N-terminal threonine residues [13].

In cytoplasm, proteasomes are associated with centrosomes, cytoskeletal network, and the outer surface of endoplasmic reticulum (EPR); in the nucleus they have identified in nucleoplasm but not in the nucleolus [12]. When proteasome activity is impaired, they form aggresome-like structures in these cell compartments and provoke apoptosis [14].

The ubiquitin–proteasome system plays a crucial role in maintenance of cell viability and normal functioning because proteasomes are involved into ubiquitin-dependent degradation of most cell proteins [15, 16]. Only membrane and extracellular proteins undergo lysosomal degradation after endocytosis [10]. Proteasomes are involved in degradation of abnormal (misfolded and/or mutant) proteins. Proteins damaged by toxins, heavy metal ions, heat shock, or oxidative stress also undergo proteasome degradation provided that their native structure cannot be restored by chaperones [12]. Thus, the ubiquitin–proteasome system is as important as chaperone families in protection of eukaryotic cells against accumulation of insoluble aggregates of defective proteins [10].

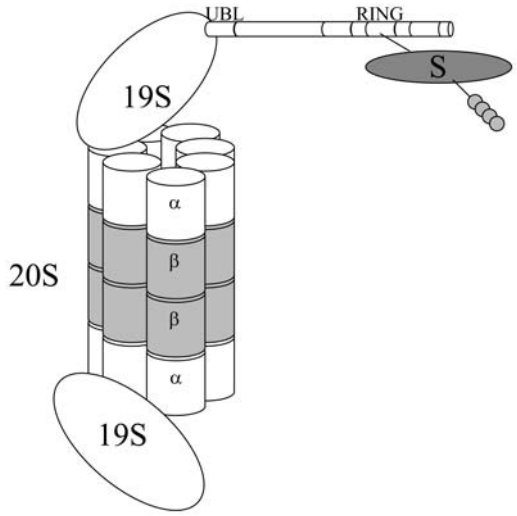

Fig. 1. Proteasome structure and its interaction with parkin. 20S core proteasome is shown as a cylinder structure. (Proteolysis occurs in the inner chamber of this cylinder.) 20S proteasome is made up of four stacked rings, each of which contains seven subunits. The external and internal rings are formed by α- and β-subunits, respectively. At both ends the 20S proteasome is capped by two multisubunit regulatory 19S complexes, and together they form 26S proteasome. Polyubiquitinated substrates interact with parkin within the RING-finger site whereas 19S proteasome complex interacts with parkin at its UBL-domain (see text). Parkin is shown as a horizontal cylinder separated into domains; dark oval and small closed circles designate substrate and ubiquitin residues, respectively.

UBIQUITIN AND ITS ROLE IN PROTEASOME DEGRADATION OF PROTEINS

Ubiquitin is one of the key components of this proteolytic system. This protein of molecular mass of 8 kD consists of 76 amino acid residues; it has been found in all eukaryotic cells. Besides involvement in proteolysis, ubiquitination of various protein targets is crucial for many intracellular processes including regulation of gene expression, regulation of cell cycle and division, response to stress, DNA repair, protein import into mitochondria, ribosome assembly, apoptosis, etc. [17]. However, the role of ubiquitin in protein degradation is far better investigated and understood [7, 18].

The involvement of ubiquitin in proteasome degradation of proteins consists in their targeting by covalent attachment of a polyubiquitin chain; this contrasts with protein monoubiquitination unrelated to proteolytic degradation processes [12]. The polyubiquitination process proceeds in three steps, which sequentially involve different enzymes (Fig. 2). Initially, a ubiquitin-activating enzyme E1 binds and activates ubiquitin by forming a high-energy thioester intermediate with the C-terminal glycine using ATP. Ubiquitin is then transferred from E1 to a ubiquitin-conjugating enzyme E2 via a *trans*(thio)esterification reaction. The final step involves an E3 ubiquitin-protein ligase, which catalyzes ubiquitin

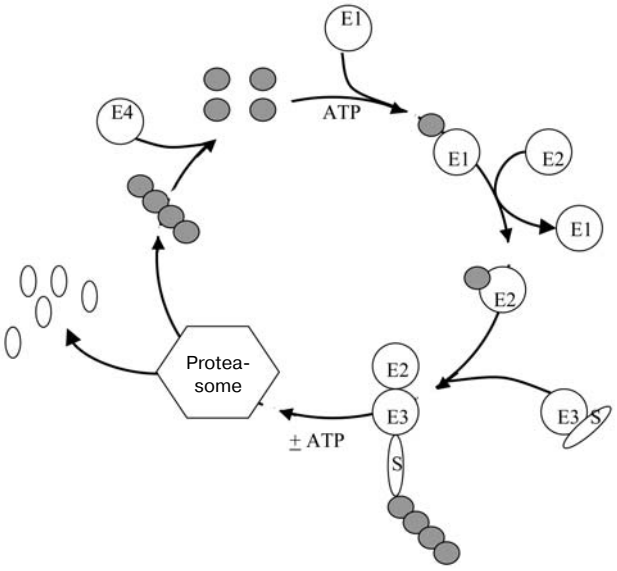

Fig. 2. Ubiquitin and proteasome. Closed circles show ubiquitin residues, a large oval is ubiquitinated substrate S, and small ovals designate peptides, the products of proteolytic degradation. Open circles show enzymes involved in the ubiquitination process: E1 is ubiquitin-activating enzyme; E2 is ubiquitin-conjugating enzyme; E3 is ubiquitin-protein ligase; E4 is ubiquitin carboxyhydrolase, which cleaves the polyubiquitin chain into ubiquitin monomers, which can be subsequently reused.

transfer to an ε-amino group of a lysine residue of the protein target. Activated ubiquitin molecules are then covalently added to Lys48 of the previously attached ubiquitin, thus forming the polyubiquitin chain. Effective labeling of a protein target (for its recognition by the proteasome) requires attachment of at least four ubiquitin molecules [12].

Initially ubiquitin-tagged protein substrates bind to 19S regulatory complex using their polyubiquitinated chains. This structure contains ATPases, which unfold globular proteins and transfer them to 20S proteasome. When such activated protein is further subjected to degradation, its polyubiquitinated chain is released and cleaved in 19S proteasome by ubiquitin carboxyhydrolase; this results in formation of free ubiquitin monomer molecules, which can be used in a new cycle [10].

Certain evidence exists that substrate specificity of the ubiquitin system is determined by E3 or by E3 in combination with E2 attached to it [19]. Mammalian cells contain hundreds of various E3; together with specific E2, they are involved in ubiquitination of various proteins [10]. Although E3 functioning is well described, little is known about selective recognition of certain unfolded or damaged proteins.

Thus, there is unquestioned evidence for the role of ubiquitin-dependent degradation of cytosolic proteins in regulation of various intracellular processes.

UBIQUITIN INVOLVEMENT IN METABOLISM OF MITOCHONDRIAL PROTEINS

In contrast to the well-established role of ubiquitin in metabolism of cytosolic proteins, little is known about ubiquitin involvement in metabolism of mitochondrial proteins, although ubiquitin protein aggregates were also found in these organelles [20]. Brain mitochondria have intrinsic ubiquitin-conjugating systems [21, 22], but concrete ubiquitinated proteins have not been identified yet.

McCauley's group revealed that insertion of newly synthesized molecules of monoamine oxidases (MAO) A and B into the outer mitochondrial membranes required ubiquitin and ATP [23, 24]. In the central nervous system, these mitochondrial enzymes play the key role in catabolism of major monoamine neurotransmitters; impairments in their turnover have been found in many neurodegenerative diseases including PD [25-28].

We found the incorporation of exogenous ubiquitin into brain mitochondria *in vitro* was accompanied by increased sensitivity of MAOs to proteolytic degradation [29]. Earlier studies of our laboratory also revealed that pathological conditions characterized by activation of oxidative processes and increase formation of ubiquitin conjugates in mitochondria cause oxidative modification of MAOs accompanied by increased sensitivity to trypsinolysis [30-32]. Taken together these results suggest that mitochondrial MAOs are putative targets for ubiquitin-dependent degradation.

INHERITED FORMS OF PARKINSONISM AND THEIR ROLE FOR CHARACTERIZATION OF PATHOGENESIS OF THIS DISEASE

In 2000, Shimura and coworkers found that the protein product of *parkin*, the causative gene of autosomal recessive juvenile form of PD, exhibits ubiquitin ligase activity [33]. This gave a key for better understanding of the role of impairments of the ubiquitin–proteasome system in the pathogenesis of PD.

Recently several chromosomal loci concealing candidate genes for PD have been identified; their mutations cause the development of PD. It has reliably been determined that mutations of five different genes (α-synuclein gene, *DJ-1*, *PINK1*, *LRRK2*, and parkin genes) cause inherited forms of parkinsonism [34-41]. Although mutations in the first three genes of this group are rather rare, their investigation may provide better understanding of common molecular mechanisms responsible for death of dopaminergic neurons in different forms of PD [42-44]. For example, mutations in the gene encoding presynaptic protein α-synuclein recognized in several families with PD resulted in significant progress in our understanding of the nature of pathological formation of Lewy bodies [45-49]. α-Synuclein was named for the place of its local-

ization: it was originally discovered in synapses and nuclei of electric eel [38]. This small protein involved in regulation of synaptic vesicle turnover [50] readily polymerizes *in vitro*; this is accompanied by formation of fibrils of 10 nm. Bundles of these fibrils represent a basis of peripheral filaments of Lewy bodies, which may also include ubiquitin, neurofilaments, and various proteasome elements [51]. Point mutation in the α-synuclein gene (substitution of G209 for A) sharply increased polymerization capacity of the protein product of this gene and inclusion body formation, a characteristic sign of PD, occurred. It remains unclear whether Lewy bodies are neurotoxic and cause neuron degeneration or their formation represents a protective mechanism, separating abnormal proteins from the "normal" cell content. The hypothesis of a protector role of aggregate formation from toxic soluble proteins has many supporters [52]. This hypothesis is also supported by observations that parkin mutations may cause the development of PD without Lewy body pathology [53]. It should be noted that mutations of the α-synuclein gene can cause not only PD but also other central nervous system disorders such as variants of Alzheimer's disease, multiple system atrophy, and other diseases, which are termed α-synucleinopathias [54].

In contrast to the above considered mutations, the mutations of *LRRK2* and parkin gene are rather frequent and their discovery has great clinical importance because gene testing may soon become possible. Mutations in *LRRK2* [34] and in the gene encoding ubiquitin carboxyhydrolase (ubiquitin carboxy-terminal hydrolase L1, UCL-L1) cause development of late-onset PD, whereas mutations in the parkin gene (with a few exceptions [36]) have been found in cases of idiopathic (sporadic) and inherited juvenile PD. Mutations of *UCL-L1* were described in two members of a German family with autosomal-dominant form of PD [49]. Mutations in the parkin gene are associated with autosomal-recessive juvenile parkinsonism. (In reality, the term "juvenile" is inappropriate, because only a small percent of cases is characterized by onset during the first decades of life, in most cases symptoms of PD appear between ages from 40 to 60.) Besides symptoms typical for PD, autosomal recessive juvenile parkinsonism is characterized by some characteristic symptoms: foot dystonia, slow progression of disease, and effectiveness of Levadopa treatment increasing dopamine level. These patients are characterized by degeneration of dopaminergic neurons in *substantia nigra* and *locus coeruleus* and lack of Lewy bodies [12, 55] (with one exception [53]).

Parkin, a gene implicated in autosomal recessive juvenile parkinsonism, is located on the long arm of chromosome 6 (6q25.2-q27). By positional cloning in a Japanese patient with a microdeletion involving marker D6S305, which is closely linked to autosomal recessive juvenile parkinsonism, a complementary DNA clone was isolated in 1998 in the Mizuno laboratory. It encoded a protein of 465 amino acid residues with moderate similarity to ubiquitin at the N-terminus and a RING-finger motif at the C-terminus [56]. The gene spans more than 500 kb and has 12 exons, five of which (exons 3-7) have been deleted in the patient. Four other Japanese patients with autosomal recessive juvenile parkinsonism from three unrelated families have a deletion affecting exon 4 alone. A 4.5-kb transcript that is expressed in many human tissues but is abundant in the brain, including the *substantia nigra*, is shorter in brain tissue from one of the groups of exon-4-deleted patients. Since mutations in the newly identified gene appear to be responsible for the pathogenesis of autosomal recessive juvenile parkinsonism, the authors named the protein product as "parkin" [56]. Subsequently mutations of the parkin gene have been found in individuals from families with inherited early-onset parkinsonism all over the world. Various deletions and point mutations in the parkin gene have been observed in more than 50% of patients with autosomal recessive juvenile parkinsonism [12, 57].

STRUCTURE AND FUNCTIONS OF PARKIN

Parkin, a protein of molecular mass of 52 kD, consists of 465 amino acid residues. Its N-terminal domain structurally related to ubiquitin is known as the UBL (ubiquitin like) domain. The C-terminal half of parkin comprises a specific arrangement of three zinc-finger domains: two RING fingers flank a domain known as the cysteine-rich in-between RING (IBR) domain (Fig. 3) [57]. Certain RING cysteine and histidine residues coordinate a structurally important zinc atom [58]. The median fragment contains cleavage sites for the pro-apoptotic caspases 1 and 8 [59] and also cysteine-rich UPD domain [57, 60]. The extreme C-terminus of parkin binds to the calcium/calmodulin-dependent serine protein kinase, thereby promoting targeting for the synaptic transport of parkin. All of these structural elements are essential for the functional integrity of parkin, because point mutations cluster in the UBL, UPD, RING–IBR–RING, and the C-terminus [57].

Parkin is ubiquitin-protein ligase (E3), which operates in cooperation with ubiquitin-conjugating enzymes (E2) UbcH7 and UbcH8 [59, 61]. Structures of RING and IBR are typical for E3 ligases. RING–IBR–RING domain contains binding sites for substrates and E2 carrying activated ubiquitin; it is involved in regeneration of E2 component in the ubiquitination mechanism [38, 62]. UBL domain is involved in proteasome organization required for association of polyubiquitinated substrates with the ubiquitin–proteasome system [63]. Mechanisms responsible for involvement of parkin in initial ubiquitination of substrates remain unclear due to incomplete knowledge of parkin structure. Other E3 ligases contain-

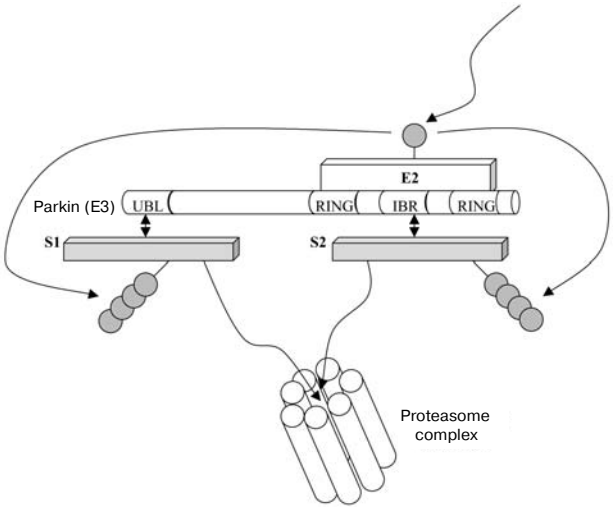

Fig. 3. Parkin and ubiquitin–proteasome system (modification of [4]). Parkin (E3) is a link between ubiquitinated substrates (S1 and S2) and E2, which binds to RING finger site. Various parkin sites can recognize various substrates. For example, UBL domain binds α-synuclein isoform, αSp22 – S1, whereas RING finger site interacts with CDCrel-1, Pael-receptor, and synphilin-1 (these proteins are defined as S2). Ubiquitin residues (designated with closed circles) are transferred from E1 to E2 and, finally, to substrate, thus labeling it for proteasome degradation.

ing similar RING domains act as scaffolds that bind to E2s and substrates simultaneously, bringing into close proximity ubiquitin bound to E2 and lysine residues of protein substrate. These E3 ligases do not form thioester derivative, but they follow similar reaction mechanism and can undergo self-ubiquitination as parkin [38].

Some authors believe that in contrast to wild type parkin the mutant parkin gene protein products lack ubiquitin-protein ligase (E3) activity in the case of autosomal recessive juvenile parkinsonism [33, 64, 65]. Others suggest that low enzymatic activity of parkin rather than total loss of biological functions causes onset of symptoms of autosomal recessive juvenile parkinsonism: in patients with inherited form of this disease mutant parkin exhibited poor binding of UbcH7 and UbcH8 and low E3 ubiquitin-protein ligase activity [12].

PROTEIN SUBSTRATES OF PARKIN

Now there is convincing evidence that parkin ubiquitinates various substrates, thus targeting them for proteasomal degradation. Parkin deficiency or its mutations cause accumulation of neurotoxic proteins accompanied by onset of symptoms of parkinsonism [38, 57, 64]. Although molecular mechanisms of these processes are not well understood, the searches driven by this hypothesis have led to the discovery of aggregation-prone protein substrates of parkin.

Cell division control-related protein-1 (CDCrel-1) was the first identified substrate of parkin. CDCrel-1 is a synaptic vesicle polypeptide of molecular mass of about 44 kD [65]. It belongs to the septin family, which also includes GTPases involved in cytokinesis. CDCrel-1 is suggested to be involved into regulation of synaptic vesicles release [66], but its role in dopamine release remains unclear. It is possible that parkin mutations influence this function of CDCrel-1, making a decisive contribution to the development of PD symptoms [7].

Parkin-associated endothelial-like receptor (Pael-R), a transmembrane polypeptide of EPR coupled to G-protein, is one of the best-studied parkin substrates. Overexpression of this receptor in cells is accompanied by impairments of its folding, forming insoluble aggregates causing cell death as the result of so-called unfolded protein reaction (UPR) [67]. UPR is a mechanism triggering various processes of EPR response to stress such as stimulation of chaperone biosynthesis in response to increased content of unfolded or mutant proteins. (The UPR reaction is similar to the heat shock response of cytosolic chaperones homologous to EPR chaperones [10].) Parkin ubiquitinates Pael-R and promotes its degradation by means of two ubiquitin-conjugating enzymes E2, Ubc6 and Ubc7, located in EPR. Especially high concentrations of Pael-R have been found in neurons containing tyrosine hydroxylase, the key enzyme of catecholamine biosynthesis. In brain of patients with autosomal recessive juvenile parkinsonism, accumulation of insoluble aggregates of Pael-R was found [7, 68, 69].

Synphilin-1 is the other protein undergoing parkin-dependent ubiquitination [80]. Functions of synphilin-1 remain unknown, but it has been identified and cloned as the α-synuclein-interacting protein [71]. Synphilin-1 (as well as CDCrel-1) is a synaptic vesicle protein, which has also been found in Lewy bodies [71, 72]. In cell culture, coexpression of synphilin-1 with α-synuclein resulted in formation of Lewy-body-like aggregates containing both these proteins; in the presence of parkin a significant proportion of these aggregates underwent ubiquitination [71]. The fact that patients with mutant parkin lack Lewy bodies has lead to suggestion that pathogenetic mechanisms induced by mutations in the parkin gene differ from those responsible for development of sporadic PD and PD induced by mutations in α-synuclein [73, 74]. However, the interaction of synphilin-1 with α-synuclein and parkin suggests the existence of common mechanisms of the development of different forms of PD, related to both parkin mutations and defective α-synuclein. It is possible that parkin is directly involved in ubiquitination of such components of Lewy bodies as synphilin-1. Moreover, parkin isolation within such inclusions may result in the loss of its functions. In the absence of parkin the proteins of Lewy bodies would not undergo ubiquitination, and this accelerates formation of Lewy bodies [18, 73]. This hypothesis is consistent with the observa-

tion that in patients with inherited form of PD mutant parkins cannot ubiquitinate synphilin-1.

Farrer and coworkers described a patient with mutation *R275W* in one parkin allele and deletion of 40 base pairs in exon 3 (Ex3Δ40) in the other allele; in this patient, Lewy bodies typical for PD were observed. The parkin mutation *R275W* causes a decrease (but not total loss) of catalytic activity [70]. The authors concluded that this mutation represents an exception, which confirms the rule: parkin requirement for formation of Lewy bodies. These pathological inclusions contribute to neuronal death by sequestering parkin and thus "depriving" cells of its activity required for degradation of specific proteins [18].

Using immunological methods, Shimura et al. identified in healthy human brain the O-glycosylated isoform of α-synuclein (αSp22) [61]. Mutant parkins from patients with inherited form of PD did not bind αSp22: in *in vitro* experiments αSp22 underwent ubiquitination by normal but not by mutant parkin. However, the presence of αSp22 in normal brain is still questioned, because the experiments by Shimura et al. have not been confirmed by other laboratories. It should also be mentioned that such type of complex glycosylation is not typical for cytoplasmic protein. Usually a component of brain neurons, non-glycosylated α-synuclein is not a parkin substrate, because *in vivo* and *in vitro* parkin does not bind and does not ubiquitinate this protein [61, 70].

It has recently been demonstrated that the p38 subunit of aminoacyl-tRNA synthetase complex, involved in biogenesis of brain proteins, undergoes ubiquitination by parkin [75]. Overexpression of p38 in COS7 cells resulted in formation of aggresome-like inclusions in which parkin was systematically sequestered. In the human dopaminergic neuroblastoma-derived SH-SY5Y cell line, parkin promoted the formation of ubiquitinated p38-positive inclusions. Moreover, the overexpression of p38 in SH-SY5Y cells caused significant cell death against which parkin provided protection. This suggests that p38 plays a role in the pathogenesis of PD.

Synaptotagmin XI found both in Lewy bodies and healthy neurons of *substantia nigra* is another synaptic protein substrate for ubiquitination by parkin [76]. Being located in neurotransmitter secretory granules, it is involved in exocytosis stimulated by calcium ions. In the case of parkin inactivation, synaptotagmin cannot be ubiquitinated and this is the reason underlying impairments of dopamine release in *parkin* mutant mice.

In a multisubunit ligase complex parkin ubiquitinates cyclin E, which is involved in physiological regulation of the cell cycle. Parkin deficiency results in accumulation of cyclin E in postmitotic neurons treated with exogenous toxicant, cainate, and development of apoptosis. Parkin protects neurons (including dopaminergic neurons) pretreated with this toxin against cyclin accumulation and prevents apoptosis [77].

Parkin also ubiquitinates microtubular proteins, α- and β-tubulins [78]. Misfolded tubulin monomers are highly toxic [79] and so have to be ubiquitinated for subsequent proteolytic degradation. There are other parkin substrates: septins SEPT5_ v2/CDCrel-2, which are homologous to CDCrel-1 [80], and also polyglutamine proteins (polyQ) [81]. Accumulation of aberrant polyglutamine proteins in cells is typical not only for PD, but also for some other neurodegenerative diseases such as Huntington's disease and spinocerebellar ataxias. Tsai and coworkers revealed that parkin is involved in elimination of these aggregation-prone cytosolic polypeptides. Ataxin 3 containing a polyglutamine chain (of 79 glutamine residues) causes cytosolic stress accompanied by protein misfolding. This triggers regulatory mechanisms mediated by the chaperone known as 70 kD heat shock protein (Hsp 70). (This process is similar to that of endoplasmic stress involving Pael-R.) When the number of polyglutamine proteins exceeds chaperone capacities of Hsp 70, the latter binds to parkin interacting with its RING–IBR–RING-domain. Parkin ubiquitinates a chaperone-bound polyglutamine protein and targets it for proteasome degradation [81]. Direct binding of UBL domain of parkin to regulatory 19S proteasome accelerates delivery of polyglutamine substrates to proteasome [63, 81].

REGULATION OF PARKIN ACTIVITY. PROTEASOME SYSTEM AND CHAPERONES AS TWO COMPONENTS OF A COMMON CELLULAR SYSTEM RESPONSIBLE FOR ELIMINATION OF MISFOLDED PROTEINS

Besides studies of effects of various mutations on the structure and functions of parkin, certain attempts have been made for elucidation of regulation of its activity. Mutations may cause attenuation of parkin interaction with other proteins, for example with CHIP, C-terminus Hsp 70-interacting protein [82]. Imai and coworkers demonstrated that CHIP binds to the parkin gene and this increases ubiquitin ligase activity. CHIP, Hsp 70, parkin, and Pael-R form a complex *in vivo* and *in vitro* [82]. EPR stress was accompanied by 3-fold increase in CHIP content in this complex. CHIP promotes dissociation of Hsp 70 from parkin and Pael-R and thus provokes parkin-dependent ubiquitination of Pael-R. Moreover, *in vitro* CHIP facilitates parkin-dependent ubiquitination of Pael-R in the absence of Hsp 70. CHIP also increases parkin-dependent inhibition of cell death induced by Pael-R. All these results suggest that CHIP is an E4-like protein regulating E3 activity of parkin [82]. Murata and coworkers demonstrated that CHIP is a chaperone-dependent E3 ligase ubiquitinating unfolded proteins [83].

Such dual role of chaperones, which not only prevent protein aggregation and cause refolding, but also promote ubiquitination and rapid degradation of defect

proteins, is very important for cells. Besides chaperone Hsp 70, a similar role has also been demonstrated for chaperone Hsp 40. Interestingly, ATPases of 26S proteasome not only promote proteolytic degradation, they also exhibit chaperone-like activity and may prevent aggregation of proteins; they may also act as promoters of refolding of denatured proteins [10].

Yamamoto and coworkers revealed the role of post-translational modification of parkin essential for regulation of its activity. In experiments on human embryonic cell culture HEK293 and human neuroblastoma SH-SY5Y cells, they found parkin phosphorylation; certain protein kinases caused parkin phosphorylation at serine residues 101, 131, and 136 (within median site) and 296 and 378 (within RING–IBR–RING-domain). Protein misfolding in cells reduced parkin phosphorylation, and non-phosphorylated parkin exhibited increased capacity for self-ubiquitination. Some authors believe that complex regulation of parkin phosphorylation degree may be involved in cell response to stress by increasing the content of unfolded proteins [60].

PARKIN LOCALIZATION IN VARIOUS CELL COMPARTMENTS: PARKIN AND MOLECULAR MECHANISMS UNDERLYING MITOCHONDRIAL DYSFUNCTIONS IN PARKINSON'S DISEASE

Characterizing some substrates of parkin, we have already mentioned its role in prevention of accumulation of defective protein deposits in EPR and cytosol.

Parkin has been found in mitochondria [84], and some authors suggest that impairments of mitochondrial functions may play the key role in the pathogenesis of PD [38, 85]. There is experimental evidence supporting this hypothesis.

Studies of brain autopsy material obtained from PD patients revealed damage of mitochondria [86, 87]. Mitochondrial Complex I inhibitors (1-methyl-4-phenyl-1,2,4,6-tetrahydropyridine (MPTP), rotenone, paraquat) cause the development of parkinsonian symptoms in man and various experimental models [88, 89].

Darios et al. [90] reported that parkin overproduction protected PC12 cells against ceramide-induced death by decreasing mitochondrial swelling and release of cytochrome *c* from them.

In vivo experiments using animals mutant in the parkin gene do not contradict the hypothesis on the role of mitochondrial dysfunctions in the pathogenesis of PD. These studies employed mutant *Drosophila* [91, 92] and mice [87, 93-95]. *Drosophila* mutants were phenotypically different from wild type animals; they were also characterized by reduced longevity, movement disorders, and male sterility; the latter was due to defects of spermatid individualization at later stages of spermatogenesis. Pathology of mitochondria of flight muscle cells and spermatids was a typical morphological sign of these mutants; this was the putative reason for apoptotic death of corresponding cells, which require large energy resources for normal functioning [85]. Such tissue-specific effect may suggest that similar mitochondrial dysfunctions may cause selective neuronal death in the autosomal recessive form of juvenile parkinsonism [91].

Paradoxically, mice lacking parkin were viable and with one exception [96] were characterized by normal brain morphology without characteristic inhibition of *substantia nigra* neurons. However, there was increased level of extracellular dopamine in the *striatum* of these mice. Parkin deficient mice were also characterized by impairments of mitochondrial respiration and decreased resistance to oxidative stress typical for PD [94]. Employment of proteomic techniques revealed in the ventral part of midbrain of these mice the altered ratio of various mitochondrial respiratory chain proteins and also some proteins protecting against oxidative stress.

Taken together, these results suggest that parkin promotes degradation of mitochondrial protein substrates required for normal functioning of brain dopaminergic neurons. However, nature of these substrates remains unknown.

CONCLUSION

The discovery that mutations of five different genes (α-synuclein gene, *DJ-1*, *PINK1*, *LRRK2*, and parkin gene) cause inherited forms of parkinsonism has made the revolutionary break in the history of studies of mechanisms underlying the development of PD, which has always been thought to be the neurodegenerative disease unrelated to heredity [97]. Although these mutations occur in a small group of the population (members of several families) they give a tool for investigation of functioning of certain genes and reveal important aspects of pathogenesis not only of "family" parkinsonism but also more common sporadic cases of this disease.

The parkin gene was the first discovered gene that is responsible for manifestation of recessive PD [56]. Some authors believe that 50% of cases of inherited forms of PD are determined by mutations of this gene [98]. It was originally discovered in patients with juvenile parkinsonism; however, certain evidence now exists that *parkin* mutations also cause PD in older people as well [97]. The autosomal recessive juvenile form of PD is characterized by slow progression of disease and some specific symptoms (e.g., dystonia) and also lack of Lewy bodies (with one exception [53]). Similar clinical symptoms are also typical for other forms of parkinsonism (e.g., MPTP-induced parkinsonism) [99]. Many authors believe that autosomal recessive parkinsonism is the phenocopy of sporadic PD [38]. In any case, *parkin* is the most intensively investigated gene.

The protein product of this gene, parkin, exhibits ubiquitin-protein ligase activity and plays a key role in the ubiquitin mediated proteasome degradation of damaged or mutant proteins [33, 65]. Discovery of domain structure provided a better understanding of details of its functioning [63]. Analysis of parkin gene mutations revealed targets of its protein product (the neurotoxic substrates) and some mechanisms of its control [38, 100].

Identification of subcellular localization of parkin, employment of cell models, and mutant animals lacking parkin activity clarified some aspects of molecular mechanisms underlying development of PD [12, 38]. Phenotypic changes common for all experimental animal models consist in changes of mitochondrial morphology and impairments of mitochondrial functions [86, 90]. This is consistent with *in vitro* experiments in which parkin specifically prevented cytochrome *c* release from mitochondria and apoptotic cell death [94].

However, in spite of significant progress in our knowledge of the causes underlying selective dopaminergic cell death associated with PD, there are many problems that still require further investigation. The hypothesis that dopaminergic neurons are particularly prone to mitochondrial damage is also supported by experiments with other proteins (PINK1 and DJ-1) [38]. Mutations of genes encoding these proteins also cause recessive parkinsonism [44, 101]. If we take into consideration protein-ligase activity of parkin, death of neurons lacking this protein suggests impairments of proteasome functioning [102-104]. Indeed, there is certain experimental evidence *in vitro* that proteasome inhibitors selectively affect catecholaminergic neurons [102].

In a recent review on biochemistry of PD, M. Cookson considers three possible modes of interactions between mitochondrial and proteasome mechanisms underlying onset of parkinsonism [38]. It is possible that each of them leads to death of certain neuronal cells irrespectively to each other. The other mode of neuronal death might require combination of both pathways operating independently on early stages but interacting at a certain (later) stage. The third, the simplest mode, implies the existence of a common metabolic pathway, in which all three genes (*PINK1*, *DJ-1*, and *parkin*) are its markers.

It is difficult to separate mitochondrial and proteasome damages of cells. Proteasome inhibitors increase sensitivity of catecholaminergic neurons to rotenone and MPTP *in vitro* [103]. Inhibitors of mitochondrial Complex I cause the decrease in proteasome activity possibly due to sensitivity of ubiquitin-proteasome system to deficit of ATP or due to oxidation or both these reasons [103]. Some evidence also exists that proteasome inhibitors cause mitochondrial damage [105]. Both these interdependent processes (impairments of mitochondrial and proteasome functioning) cause cell death.

Elucidation of the role of parkin and protein products of other recessive genes involved in protection of cells against accumulation of toxic protein aggregates requires further studies. Their success may culminate in the development of new therapeutic strategies not only for symptomatic treatment of rare inherited forms of parkinsonism, but also in the cases of common sporadic form of Parkinson's disease.

This work was partially supported by grants from the Russian Foundation for Basic Research (No. 06-04-48355-a) and The Welcome Trust (072381/Z/03/Z).

REFERENCES

1. Lang, A. E., and Lozano, A. M. (1998) *N. Engl. J. Med.*, **339**, 1044-1053.
2. Lang, A. E., and Lozano, A. M. (1998) *N. Engl. J. Med.*, **339**, 1130-1143.
3. Forno, L. S. (1996) *J. Neuropathol. Exp. Neurol.*, **55**, 259-272.
4. Giasson, B. I., and Lee, V. M.-Y. (2001) *Neuron*, **31**, 885-888.
5. Gasser, T. (2001) *J. Neurol.*, **248**, 833-840.
6. Dawson, T., Mandir, A., and Lee, M. (2002) *Neuron*, **35**, 219-222.
7. Dawson, T. M., and Dawson, V. L. (2003) *J. Clin. Invest.*, **111**, 145-151.
8. Goedert, M. (2001) *Nat. Rev. Neurosci.*, **2**, 492-501.
9. Braak, H., Braak, E., Yilmazer, D., Schultz, C., de Vos, R. A., and Jansen, E. N. (1995) *J. Neural Transm.*, **46** (Suppl.), 15-31.
10. Sherman, M. Y., and Goldberg, A. L. (2001) *Neuron*, **29**, 15-32.
11. DeMartino, G. N., and Slauter, C. A. (1999) *J. Biol. Chem.*, **274**, 22123-22126.
12. Betarbet, R., Sherer, T. B., and Greenamyre, J. T. (2005) *Exp. Neurol.*, **191**, S17-S27.
13. Rotanova, T. V. (2001) *Vopr. Med. Khim.*, **47**, 3-19.
14. Wojcik, C., and DeMartino, G. N. (2003) *Int. J. Biochem. Cell. Biol.*, **35**, 579-589.
15. Coux, O., Tanaka, K., and Goldberg, A. L. (1996) *Annu. Rev. Biochem.*, **65**, 801-847.
16. Baumeister, W., Walz, J., Zuhl, F., and Seemuller, E. (1998) *Cell*, **92**, 367-380.
17. Ciechanover, A. (1994) *Biol. Chem. Hoppe-Seyler*, **375**, 565-581.
18. Chung, K. K., Dawson, V. L., and Dawson, T. M. (2001) *Trends Neurosci.*, **24** (Suppl.), S7-S14.
19. Weissman, A. M. (2001) *Nat. Rev. Mol. Cell Biol.*, **2**, 169-178.
20. Hu, B. R., Martone, M. E., Jones, Y. Z., and Liu, C. L. (2000) *J. Neurosci.*, **20**, 3191-3199.
21. Adamo, A. M., Pasquini, L. A., Moreno, M. B., Oteiza, P. I., Soto, E. F., and Pasquini, J. M. (1999) *J. Neurosci. Res.*, **55**, 523-531.
22. Mangani, M., Serafini, G., Antonelli, A., Malatesta, M., and Gazzanelli, G. (1991) *J. Biol. Chem.*, **266**, 21018-21024.
23. Zhuang, Z., and McCauley, R. (1989) *J. Biol. Chem.*, **264**, 14594-14596.
24. Zhuang, Z., Marks, B., and McCauley, R. (1992) *J. Biol. Chem.*, **267**, 591-596.

25. Mandel, S., Weinreb, O., Amit, T., and Youdim, M. B. (2005) *Brain Res. Rev.*, **48**, 379-387.
26. Youdim, M. B., Maruyama, W., and Naomi, M. (2005) *Drugs Today (Barc)*, **41**, 369-391.
27. Riederer, P., Lachenmayer, L., and Laux, G. (2004) *Curr. Med. Chem.*, **11**, 2033-2043.
28. Birks, J., and Flicker, L. (2003) *Cochrane Database Syst. Rev.*, CD000442.
29. Buneeva, O. A., Medvedeva, M. V., and Medvedev, A. E. (1999) *Neurobiology*, **7**, 257-261.
30. Medvedev, A. E., and Gorkin, V. Z. (1994) *Int. J. Devel. Neurosci.*, **12**, 151-155.
31. Medvedev, A. E., Kirkel, A., Kamyshanskaya, N., and Gorkin, V. (1993) *Int. J. Biochem.*, **25**, 1791-1799.
32. Medvedev, A. E., and Tipton, K. F. (1997) *Vopr. Med. Khim.*, **43**, 471-481.
33. Shimura, H., Hattori, N., Kubo, S., Mizuno, Y., Asakawa, S., Minoshima, S., Shimizu, N., Iwai, K., Chiba, T., Tanaka, K., and Suzuki, T. (2000) *Nat. Genet.*, **25**, 302-305.
34. Bonifati, V. (2005) *Minerva Med.*, **96**, 175-186.
35. Dawson, T. M., and Dawson, V. L. (2003) *J. Clin. Invest.*, **111**, 145-151.
36. Klein, C., Pramstaller, P. P., Kis, B., Page, C. C., Kann, M., Leung, J., Woodward, H., Castellan, C. C., Schrerer, M., Vieregge, P., Breakfield, X. O., Kramer, P. L., and Ozelius, L. J. (2000) *Ann. Neurol.*, **48**, 65-71.
37. Ramsden, D. B., Parsons, R. B., Ho, S. I., and Waring, R. H. (2001) *J. Clin. Pathol. Mol. Pathol.*, **54**, 369-380.
38. Cookson, M. R. (2005) *Ann. Rev. Biochem.*, **74**, 29-52.
39. Gasser, T., Muller-Myhsok, B., Wszolek, Z. K., Oehlmann, R., Calne, D. B., Bonifati, V., Bereznai, B., Fabrizio, E., Vieregge, P., and Horstmann, R. D. (1998) *Natl. Genet.*, **18**, 262-265.
40. Funayama, M., Hasegawa, K., Kowa, H., Saito, M., Tsuji, S., and Obata, F. (2002) *Ann. Neurol.*, **51**, 296-301.
41. Wszolek, Z. K., Pfeiffer, R. F., Tsuboi, Y., Uitti, R. J., McComb, R. D., Stoessl, A. J., Strongosky, A. J., Zimprich, A., Muller-Myhsok, B., Farrer, M. J., Gasser, T., Calne, D. B., and Dickson, D. W. (2004) *Neurology*, **62**, 1619-1622.
42. Canet-Aviles, R. M., Wilson, M. A., Miller, D. W., Ahmad, R., McLendon, C., Bandyopadhyay, S., Baptista, M. J., Ringe, D., Prtsko, G. A., and Cookson, M. R. (2004) *Proc. Natl. Acad. Sci. USA*, **101**, 9103-9108.
43. Yokota, T., Sugawara, K., Ito, K., Takahashi, R., Ariga, H., and Mizusawa, H. (2003) *Biochem. Biophys. Res. Commun.*, **312**, 1342-1348.
44. Valente, E. M., Abou-Sleiman, P. M., Caputo, V., Muqit, M. M., Harvey, K., Gispet, S., Ali, Z., DelTurco, D., Bentivoglio, A. R., Healy, D. G., Albanese, A., Nussbaum, R., Gonzalez-Maldonado, R., Deller, T., Salvi, S., Cortelli, P., Gilks, W. P., Latchman, D. S., Harvey, R. J., Dallapiccola, B., Auburger, G., and Wood, N. W. (2004) *Science*, **304**, 1158-1160.
45. Polymeropoulos, M. H., Goelbe, L. I., Johnson, W. G., Ide, S. E., di Iorio, G., Sanges, G., Stenroos, E. S., Pho, L. T., Schaffer, A. A., Lazzarini, A. M., Nussbaum, R. L., and Duvoisin, R. C. (1996) *Science*, **274**, 1197-1199.
46. Kruger, R., Kuhn, W., Muller, T., Woitalla, D., Graeber, M., Kosel, S., Przuntek, H., Epplen, J. T., Schols, L., and Riess, O. (1998) *Nat. Genet.*, **18**, 106-108.
47. Zarranz, J. J., Alegre, J., Gomez-Esteban, J. C., Lezcano, E., Ros, R., Ampuero, I., Vidal, L., Hoenicka, J., Rodriguez, O., Atares, B., Llorens, V., Gomez Tortosa, E., del Ser, T., Munoz, D. G., and de Yebenes, J. G. (2004) *Ann. Neurol.*, **55**, 164-173.
48. Singleton, A. B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R., Lincoln, S., Crawley, A., Hanson, M., Maraganore, D., Adler, C., Cookson, M. R., Muenter, M., Baptista, M., Miller, D., Blancato, J., Hardy, J., and Gwinn-Hardy, K. (2003) *Science*, **302**, 841.
49. Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E. S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, A. M., Lazzarini, A. M., Duvoisin, R. S., di Iorio, G., Golbe, L. I., and Nussbaum, R. L. (1997) *Science*, **276**, 2045-2047.
50. Clayton, D. F., and George, J. M. (1998) *Trends Neurosci.*, **21**, 249-254.
51. McNaught, K. S., Olanow, C. W., Halliwell, B., Isacson, O., and Jenner, P. (2001) *Nat. Rev. Neurosci.*, **2**, 589-594.
52. Ciechanover, A., and Brundin, P. (2003) *Neuron*, **40**, 427-446.
53. Farrer, M., Chan, P., Chen, R., Tan, L., Lincoln, S., Hernandez, D., Forno, L., Gwinn-Hardy, K., Petrucelli, L., Hussey, J., Singleton, A., Tanner, C., Hardy, J., and Langston, J. W. (2001) *Ann. Neurol.*, **50**, 293-300.
54. Duda, J. E., Lee, V. M.-Y., and Trojanovski, J. Q. (2000) *J. Neurosci. Res.*, **61**, 121-127.
55. Mizuno, Y., Hattori, N., and Matsumine, H. (1998) *J. Neurochem.*, **71**, 893-902.
56. Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y., and Shimizu, N. (1998) *Nature*, **392**, 605-608.
57. Kahle, P. J., and Haass, C. (2004) *EMBO Rep.*, **5**, 681-685.
58. Marin, I., and Ferrus, A. (2002) *Mol. Biol. Evol.*, **19**, 2039-2050.
59. Kahns, S., Kalai, M., Jakobsen, L. D., Clark, B. F. C., Vandenabeele, P., and Jensen, P. H. (2003) *J. Biol. Chem.*, **78**, 23376-23380.
60. Yamamoto, A., Friedlein, A., Imai, Y., Takahashi, R., Kalle, P. J., and Haass, C. (2005) *J. Biol. Chem.*, **280**, 3390-3399.
61. Shimura, H., Schlossmacher, M. G., Hattori, N., Frosch, M. P., Trockenbacher, A., Schneider, R., Muzino, Y., Kosik, K. S., and Selkoe, D. J. (2001) *Science*, **293**, 263-269.
62. Upadhia, S. C., and Hegde, A. N. (2003) *Trends Biochem. Sci.*, **28**, 280-283.
63. Sacata, E., Yamaguchi, Y., Kurimoto, E., Kikuchi, J., Yokoyama, S., Yamada, S., Kawahara, H., Yokosawa, H., Hattori, N., Mizuno, Y., Tanaka, K., and Kato, K. (2003) *EMBO Rep.*, **4**, 301-306.
64. Imai, Y., Soda, M., and Takahashi, R. (2000) *J. Biol. Chem.*, **275**, 35661-35664.
65. Zhang, Y., Gao, J., Chung, K. K., Huang, H., Dawson, V. L., and Dawson, T. M. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 13354-13359.
66. Beites, C. L., Xie, H., Bowser, R., and Trimble, W. S. (1999) *Nat. Neurosci.*, **2**, 434-439.
67. Imai, Y., Soda, M., Inoue, H., Hattori, N., Mizuno, Y., and Takahashi, R. (2001) *Cell*, **105**, 891-902.
68. Murakami, T., Shoji, M., Imai, Y., Inoue, H., Kawarabayashi, T., Matsubara, E., Harigaya, Y., Sasaki,

A.,Takahashi, R., and Abe, K. (2004) *Ann. Neurol.*, **55**, 439-442.
69. Yang, Y., Nishimura, I., Imai, Y., Takahashi, R., and Lu, B. (2003) *Neuron*, **37**, 911-924.
70. Chung, K. K., Zhang, Y., Lim, K. L., Tanaka, Y., Huang, H., Gao, J., Ross, C. A., Dawson, V. L., and Dawson, T. M. (2001) *Nat. Med.*, **7**, 1144-1150.
71. Engelender, S., Kaminsky, Z., Guo, X., Sharp, A. H., Amaravi, R. K., Kleiderlein, J. J., Margolis, R. L., Troncoso, J. C., Lanahan, A. A., Worley, P. F., Dawson, V. L., Dawson, T. M., and Ross, C. A. (1999) *Nat. Genet.*, **22**, 110-114.
72. Wakabayashi, K., Engelender, S., Yoshimoto, M., Tsuji, S., Ross, C. A., and Takahashi, H. (2000) *Ann. Neurol.*, **47**, 521-523.
73. Dawson, T. M. (2000) *Cell*, **101**, 115-118.
74. Golbe, L. I., and Mouradian, M. M. (2004) *Ann. Neurol.*, **55**, 153-156.
75. Corti, O., Hampe, C., Koutnikova, H., Darios, F., Jacquier, S., Prigent, A., Robinson, J. C., Pradier, L., Ruberg, M., Mirande, M., Hirsch, E., Rooney, T., Fournier, A., and Brice, A. (2003) *Hum. Mol. Genet.*, **12**, 1427-1437.
76. Huynn, D. P., Scoles, D. R., Nguyen, D., and Pulst, S. M. (2003) *Hum. Mol. Genet.*, **12**, 2587-2597.
77. Staropoli, J. F., McDermott, C., Martinat, C., Schulman, B., Demirava, E., and Abelovich, A. (2003) *Neuron*, **37**, 735-749.
78. Ren, Y., Zhao, J., and Feng, J. (2003) *J. Neurosci.*, **23**, 3316-3324.
79. Burke, D., Gasdaska, P., and Hartwell, L. (1989) *Mol. Cell Biol.*, **9**, 1049-1059.
80. Imai, Y., and Takahashi, R. (2004) *Curr. Opin. Neurobiol.*, **14**, 384-389.
81. Tsai, Y. C., Fishman, P. S., Thakor, N. V., and Oyler, G. A. (2003) *J. Biol. Chem.*, **278**, 22044-22055.
82. Imai, Y., Soda, M., Hatakeyama, S., Akagi, T., Hashikawa, T., Nakayama, K. I., and Takahashi, R. (2002) *Mol. Cell*, **10**, 55-67.
83. Murata, S., Minami, Y., Minami, M., Chiba, T., and Tanaka, K. (2001) *EMBO Rep.*, **2**, 1133-1138.
84. Mouatt-Prigent, A., Muriel, M. P., Gu, W. J., El Hachimi, K. H., Lucking, C. B., Brice, A., and Hirsch, E. C. (2004) *J. Neural Transm.*, **111**, 1209-1218.
85. Shen, J., and Cookson, M. R. (2004) *Neuron*, **43**, 301-304.
86. Beal, M. F. (2003) *Ann. N. Y. Acad. Sci.*, **991**, 120-131.
87. Jenner, P., and Olanow, C. W. (1998) *Ann. Neurol.*, **44**, S72-S84.
88. Betarbet, R., Sherer, T. B., MacKenzie, G., Garcia-Osuna, M., Panov, A. V., and Greenamyre, J. T. (2000) *Nat. Neurosci.*, **3**, 1301-1306.
89. Dauer, W., and Przedborski, S. (2003) *Neuron*, **39**, 889-909.
90. Darios, F., Corti, O., Lucking, C. B., Hampe C., Muriel, M. P., Abbas, N., Gu, W. J., Hirsch, E. C., Rooney, T., Ruberg, M., and Brice, A. (2003) *Hum. Mol. Genet.*, **12**, 517-526.
91. Greene, J. C., Whitworth, A. J., Kuo, I., Andrews, L. A., Feany, M. B., and Pallanck, L. J. (2003) *Proc. Natl. Acad. Sci. USA*, **100**, 4078-4083.
92. Pesah, Y., Pham, T., Burgess, H., Middlebrooks, B., Verstreken, P., Zhou, Y., Harding, M., Bellen, H., and Mardon, G. (2004) *Development*, **131**, 2183-2194.
93. Goldberg, M. S., Fleming, S. M., Palacino, J. J., Cepeda, C., Lam, H. A., Bhatnagar, A., Meloni, E. G., Wu, N., Ackerson, L. C., Klapstein, G. P., Galjendiran, M., Roth, B. L., Chesselet, M. F., Maidment, N. T., Levine, M. S., and Shen, J. (2003) *J. Biol. Chem.*, **278**, 43628-43635.
94. Palacino, J. J., Sagi, D., Goldberg, M. S., Krauss, S., Motz, C., Wacker, M., Klose, J., and Shen, J. (2004) *J. Biol. Chem.*, **279**, 18614-18622.
95. Itier, J. M., Ibanes, P., Mena, M. A., Abbas, N., Cohen-Salmon, C., Bohme, G. A., Laville, M., Pratt, J., Corti, O., Pradier, L., Ret, G., Joubert, C., Periquet, M., Araujo, F., Negroni, J., Casarejos, M. J., Canals, S., Solano, R., Serrano, A., Gallego, E., Sanchez, M., Denefle, P., Benavides, J., Tremp, G., Rooney, T. A., Brice, A., and Garcia de Yebenes, J. (2003) *Hum. Mol. Genet.*, **12**, 2277-2291.
96. Von Coelln, R., Thomas, B., Savitt, J. M., Lim, K. L., Sasaki, M., Hess, E. J., Dawson, V. L., and Dawson, T. M. (2004) *Proc. Natl. Acad. Sci. USA*, **101**, 10744-10749.
97. West, A. B., and Maidment, N. T. (2004) *Hum. Genet.*, **114**, 327-336.
98. Mata, I. F., Lockhart, P. J., and Farrer, M. J. (2004) *Hum. Mol. Genet.*, **13 (**Suppl. 1**)**, R127-R133.
99. Langston, J. W. (1996) *Neurology*, **47**, S153-S160.
100. Tanaka, K., Suzuki, T., Hattori, N., and Mizuno, Y. (2004) *Biochim. Biophys. Acta*, **1695**, 226-238.
101. Bonifati, V., Rizzu, P., van Baren, M. J., Schaap, O., Breedveld, G. J., Krieger, E., Dekker, M. C., Squitieri, F., Ibanez, P., Joosse, M., van Dongen, J. W., Vanacore, N., van Swieten, J. C., Brice, A., Meco, G., van Duijn, C. M., Oostra, B. A., and Heutink, P. (2003) *Science*, **299**, 256-259.
102. Petrucelli, L., O'Farrell, C., Lockhart, P. J., Baptista, M., Kehoe, K., Vink, L., Choi, P., Wolozin, B., Farrer, M., Hardy, J., and Cookson, M. R. (2002) *Neuron*, **36**, 1007-1019.
103. Hoglinger, G. U., Carrard, G., Michel, P. P., Medja, F., Lombes, A., Ruberg, M., Friguuet, B., and Hirsch, E. C. (2003) *J. Neurochem.*, **86**, 1297-1307.
104. McNaught, K. S. P., Perl, D. B., Brownell, A.-L., and Olanow, C. W. (2004) *Ann. Neurol.*, **56**, 149-162.
105. Sullivan, P. G., Dragicevic, N. B., Deng, J.-H., Bai, Y., Dimayuga, E., Ding, Q., Chen, Q., Bruce-Keller, A. J., and Keller, J. N. (2004) *J. Biol. Chem.*, **279**, 20699-20707.