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The Ubiquitin Proteasome System in Neurodegenerative Diseases: Sometimes the Chicken, Sometimes the Egg

Review

Aaron Ciechanover[1,*] and Patrik Brundin[2,*]
[1]Department of Biochemistry and
The Rappaport Family Institute for Research
in the Medical Sciences
The Bruce Rappaport Faculty of Medicine
Technion-Israel Institute of Technology
Haifa 31096
Israel
[2]Section for Neuronal Survival
Wallenberg Neuroscience Center
Biomedical Center (BMC)
Department of Physiological Sciences
Lund University
S-221 84 Lund
Sweden

The ubiquitin-proteasome system targets numerous cellular proteins for degradation. In addition, modifications by ubiquitin-like proteins as well as proteins containing ubiquitin-interacting and -associated motifs modulate many others. This tightly controlled process involves multiple specific and general enzymes of the system as well as many modifying and ancillary proteins. Thus, it is not surprising that ubiquitin-mediated degradation/processing/modification regulates a broad array of basic cellular processes. Moreover, aberrations in the system have been implicated, either as a primary cause or secondary consequence, in the pathogenesis of both inherited and acquired neurodegenerative diseases. Recent findings indicate that the system is involved in the pathogenesis of Parkinson's, Alzheimer's, Huntington's, and Prion diseases as well as amyotrophic lateral sclerosis. This raises hopes for a better understanding of the pathogenetic mechanisms involved in these diseases and for the development of novel, mechanism-based therapeutic modalities.

Introduction

Degradation of intracellular proteins via the ubiquitin-proteasome system (UPS) is a highly complex, temporally controlled, and tightly regulated process that plays major roles in a variety of basic cellular processes. Targeting of protein to degradation via the UPS involves two discrete and successive steps: (1) conjugation of multiple ubiquitin moieties to the substrate to generate the polyubiquitin degradation signal and (2) destruction of the tagged protein by the downstream 26S proteasome complex with release of free and reusable ubiquitin, a reaction catalyzed by deubiquitinating enzymes (DUBs). With the multitude of substrates targeted and the myriad processes involved, it is not surprising that aberrations in the pathway have been implicated in the pathogenesis of many diseases, including certain malignancies, disorders of the immune and inflammatory response, and neurodegeneration. While inactivation of a major enzyme, such as E1, is obviously lethal, mutations in enzymes or in recognition motifs in substrates that do not affect vital pathways or that affect the involved process only partially result in a broad array of phenotypes. Likewise, acquired changes in the activity of the system can also evolve into certain pathologies. The pathological states associated with the ubiquitin system can be generally classified into two groups: (1) those that result from loss of function—mutations in a ubiquitin system enzyme or target substrate that result in stabilization of certain proteins, and (2) those that result from gain of function—abnormal or accelerated degradation of the protein target. Recent findings have linked aberrations in the ubiquitin system to neurodegenerative disorders. In some cases, such as mutations in the ubiquitin ligase Parkin that results in Autosomal Recessive Juvenile Parkinsonism (AR-JP), the pathogenetic linkage between the aberration in the system and the resulting disease is direct (the egg, according to one view). In many other cases, however, it appears that the aggregated, disease-specific proteins that characterize many of these disorders inhibit the activity of the UPS. Here, inhibition of the system may play a secondary role in the pathogenetic process (the chicken). In addition to Parkinson's disease, this review discusses Alzheimer's disease, Prion diseases, familial amyotrophic lateral sclerosis (ALS) with mutations in the *SOD1* gene, and polyglutamine expansion disorders. In each case, the potential role of the UPS is critically discussed, with an emphasis given to whether the observed changes are primary or whether they are secondary phenomena resulting from another underlying cause.

*Correspondence: c_tzachy@netvision.net.il (A.C.), patrik.brundin@neurol.lu.se (P.B.)

The Ubiquitin-Proteasome Pathway

Degradation of a protein via the ubiquitin-proteasome pathway involves two discrete and successive steps: (1) tagging of the substrate by covalent attachment of multiple ubiquitin molecules to synthesize the polyubiquitin chain proteolytic signal and (2) degradation of the tagged protein by the 26S proteasome complex with release of free and reusable ubiquitin catalyzed by ubiquitin-recycling enzymes (DUBs; for a scheme of the ubiquitin system, see Figure 1).

Conjugation of ubiquitin, a highly evolutionarily conserved 76 residue polypeptide, to the protein substrate proceeds via a three-step cascade mechanism. Initially, the ubiquitin-activating enzyme E1 activates ubiquitin in an ATP-requiring reaction to generate a high-energy thiol ester intermediate, E1-S∼ubiquitin, where ubiquitin is bound to an internal E1 Cys residue. One of several E2 enzymes (ubiquitin-carrier proteins or Ubiquitin-Conjugating enzymes [UBCs]) transfers the activated ubiquitin moiety from E1, via an additional high-energy thiol ester intermediate, E2-S∼ubiquitin, to the substrate that is specifically bound to an E3, a member of the ubiquitin-protein ligase family of proteins. Several distinct families of E3 enzymes have been described. For the Homologous to the E6-AP C Terminus (HECT) domain E3s, the

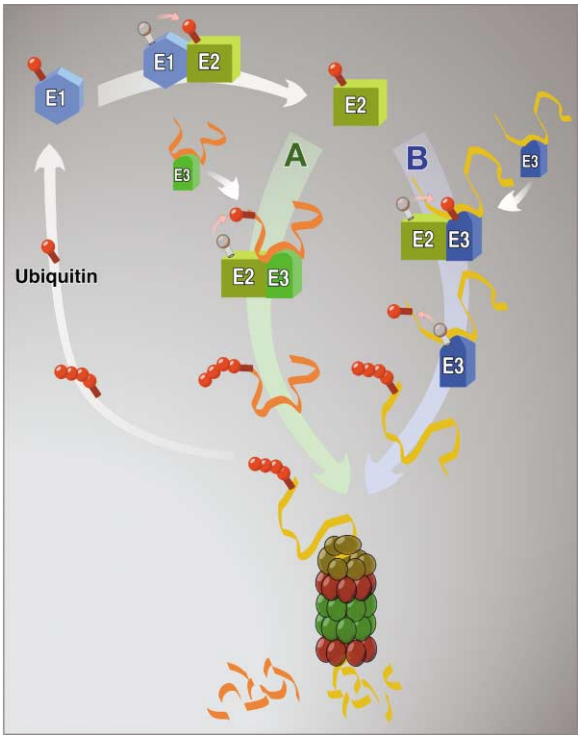

Figure 1. The Ubiquitin-Proteasome System

Ubiquitin is first activated by the ubiquitin-activating enzyme E1, to a high-energy thiol ester intermediate. It is then transferred, still as a high-energy intermediate, to a member of the ubiquitin-carrier proteins family of enzymes, E2 (known also as a ubiquitin-conjugating enzyme [UBC]). From E2, it can be transferred directly to the substrate that is bound specifically to a member of the ubiquitin ligase family of proteins, E3 (A). This occurs when the E3 belongs to the RING finger family of ligases. In the case of an Homologous to the E6-AP C Terminus (HECT) domain-containing ligase (B), the activated ubiquitin moiety is transferred first to the E3, to generate yet another high-energy thiol ester intermediate, before it is transferred to the E3-bound target substrate. Additional ubiquitin moieties are added successively to the previously conjugated one in a similar mechanism to generate a polyubiquitin chain. The polyubiquitinated substrate binds specifically to the 26S proteasome complex: the substrate is degraded to short peptides, and free and reusable ubiquitin is released via the activity of deubiquitinating enzymes.

ubiquitin is transferred once again from the E2 enzyme to an active site Cys residue on the E3 to generate a third high-energy thiol ester intermediate, ubiquitin-S∼E3, prior to its transfer to the ligase bound substrate. On the other hand, RING finger-containing E3s catalyze, most probably, direct transfer of the activated ubiquitin moiety from E2 to the E3 bound substrate. Members of other families of E3s, such as the U-Box- or PHD domain-containing ligases, act most probably in a similar manner to that of the RING finger ligases. E3s catalyze the last step in the conjugation process: covalent attachment of ubiquitin to the substrate. The first ubiquitin moiety is generally transferred to an ϵ-NH_2 group of an internal Lys residue in the substrate to generate a covalent isopeptide bond. In some cases, however, it is conjugated linearly to the free NH_2 group at the N-terminal residue. By successively adding additional activated ubiquitin moieties to internal Lys residues on the previously conjugated ubiquitin molecule, a polyubiquitin chain is synthesized. The degradation signal that is recognized by the 26S proteasome complex is made of a Lys48 polyubiquitin chain. Conjugation to other Lys residues, Lys63 for example, serves nonproteolytic functions of the system, such as activation of transcription.

It appears that E3s play a key role in the ubiquitin-mediated proteolytic cascade since they serve as the specific substrate recognition factors of the system. Approximately 1000 different E3s have been identified in the human genome, based on specific, commonly shared structural motifs, such as the RING finger. In some cases, proteins are modified by a single ubiquitin moiety. In many of these cases, the modification leads to targeting of the tagged substrate for degradation in the lysosome/vacuole. It should be noted that modification by a single ubiquitin moiety is catalyzed via an identical mechanism and set of enzymes to those that catalyze polyubiquitination. In some other cases, the first ubiquitin moiety is conjugated to the substrate by one E3, while chain elongation is catalyzed by a different ligase, often termed E4.

Degradation of polyubiquitinated substrates is carried out by a large protease complex, the 26S proteasome that does not generally recognize nonmodified substrates. In one established and exceptional case, however, that of the polyamine synthesizing enzyme ornithine decarboxylase (ODC), the proteasome recognizes and degrades the substrate following its association with another protein, antizyme, without prior ubiquitination. The proteasome is a large, multicatalytic protease that degrades polyubiquitinated proteins to small peptides. It is composed of two subcomplexes: a 20S core particle (CP) that carries out the catalytic activity and a regulatory 19S regulatory particle (RP). The 20S CP is a barrel-shaped structure composed of four stacked rings: two identical outer α rings and two identical inner β rings. The eukaryotic α and β rings are each composed of seven distinct subunits, giving the 20S complex the general structure of $\alpha_{1\text{-}7}\beta_{1\text{-}7}\beta_{1\text{-}7}\alpha_{1\text{-}7}$. The catalytic sites are localized to some of the β subunits. The population of proteasomes in any given cell may be heterogeneous. Most of the 20S complexes are capped by two 19S complexes, one on each extremity; however, they may have a 19S subcomplex on one side and a different complex, e.g., PA28, on the other. This polar organization can secure unidirectionality in the flow of substrates, and also, according to certain studies, generation of preferred peptide products such as antigenic epitopes that are trimmed properly to fit recognition by the MHC class I molecules and the cytotoxic T cell. One important function of the 19S RP is to recognize ubiquitinated proteins and other potential substrates of the proteasome. Two ubiquitin binding subunits of the 19S RP have been identified, Rpn10 (S5a in mammalian cells) and Rpt5 (S6′); however, their essential role and mode of action have not been discerned. A second function of the 19S RP is to open an orifice in the α ring that will allow entry of the substrate into the proteolytic chamber. Also, since a folded protein would not be able to fit through the narrow proteasomal channel, it is assumed that the 19S particle unfolds substrates and inserts them into the 20S CP. Both the channel opening function and the unfolding of the substrate require meta-

bolic energy, and indeed, the 19S RP contains six different ATPase subunits. Following degradation of the substrate, short peptides are released along with free and reusable ubiquitin. The peptides are further degraded by cytosolic amino- and carboxypeptidases, although a small fraction of them are transported across the endoplasmic reticulum (ER) membrane, bind to MHC class I molecules, and are presented to cytoxic T cells. Proteasomal degradation does not always generate peptides. In some cases, the proteasome processes the ubiquitinated substrate precisely, releasing a truncated product. In the case of the NF-κB transcriptional regulator, active subunits are generated from longer inactive precursors.

In spite of the major progress that has been made in elucidating the mode of action of the UPS, major problems have remained unsolved. For example, how does the system achieve its high specificity and selectivity? Why are certain proteins extremely stable in the cell, while others are extremely short-lived? Why are certain proteins degraded only at a particular time point during the cell cycle or only following specific extracellular stimuli, yet are stable under most other conditions? It appears that specificity of the ubiquitin system is determined by two distinct and unrelated groups of proteins: (1) E3s and (2) ancillary proteins. First, within the ubiquitin system, substrates must be specifically recognized by an appropriate E3 as a prerequisite for their ubiquitination. In most cases, substrates are not recognized in a constitutive manner, and in some cases, they are not recognized directly by the E3. In many cases, the substrate must undergo a certain change, e.g., posttranslational modification such as phosphorylation, that renders it susceptible for recognition. In some instances, the E3 must be "switched on" by undergoing posttranslational modification in order to yield an active form that recognizes the substrate. The stability of additional proteins depends on association with ancillary proteins, such as molecular chaperones, that act as recognition elements in *trans* and serve as a link to the appropriate ligase. Others, such as certain transcription factors, have to dissociate from the specific DNA sequence to which they bind in order to be recognized by the system. Stability of yet other proteins depends on hetero- or homooligomerization: only the monomers are degraded. Thus, in addition to the E3s themselves, modifying enzymes (such as kinases), ancillary proteins, or DNA sequences to which substrates bind also play an important role in the recognition process.

Ubiquitin-mediated proteolysis of a variety of cellular proteins plays an important role in many basic cellular processes. Among these are regulation of cell cycle and division, differentiation and development, involvement in the cellular response to stress and extracellular effectors, morphogenesis of neuronal networks, modulation of cell surface receptors, ion channels and the secretory pathway, DNA repair, transcriptional regulation, transcriptional silencing, long-term memory, circadian rhythms, regulation of the immune and inflammatory responses, and biogenesis of organelles. The list of cellular proteins that are targeted by ubiquitin is growing rapidly. Among them are cell cycle regulators such as cyclins, cyclin-dependent kinase inhibitors and proteins involved in sister chromatid separation, tumor suppressors, and transcriptional activators and their inhibitors. Cell surface receptors and ER proteins are also targeted by the system. Finally, mutated and denatured/misfolded proteins are recognized specifically and are removed efficiently. In this capacity, the UPS plays a key role in the cellular quality control and defense mechanisms.

The UPS can be regulated at the level of ubiquitination or at the level of proteasome activity. Since conjugation and proteasomal degradation are required for numerous cellular functions, regulation must be delicately and specifically tuned. In a few cases, general rather than specific components of the system can be modulated by physiological signals. For example, upregulation of the pathway is observed during massive degradation of skeletal muscle proteins that occurs under normal fasting but also under pathological conditions such as cancer-induced cachexia, severe sepsis, metabolic acidosis, or following denervation. In most cases, however, regulation is specific, and the target substrates are recognized by specific ligases that bind to defined motifs. The targeting motif can be a single amino acid residue (e.g., the N-terminal residue), a sequence (the Destruction box in cyclins), or a domain (a hydrophobic patch, for example) that is not normally exposed. In other cases, the motif is generated posttranslationally, e.g., phosphorylation that occurs in response to external signals. Phosphorylation can occur either on the substrate or the ligase. The phosphorylated site in the substrate can serve as the ligase-anchoring motif [such as the phosphorylated domain—$DS^{32}(P)GLDS^{36}(P)$—in IκBα that serves to bind the βTrCP ligase]. As for phosphorylation of the ligase, it is not known whether it activates the enzyme to a form that now recognizes the substrate(s) or whether it is involved directly in substrate recognition/binding.

Both enzymes and substrates of the ubiquitin system have been found to be modified by UBiquitin-Like (UBL) proteins. Modification by UBLs occurs only once. In the case of enzymes, modification affects their activity. For example, modification of the Cullin component of the SCF E3 complexes by the ubiquitin-like protein NEDD8 increases the affinity of the ligases to the E2 component of the conjugation machinery. Skp1, Cullin, F-Box proteins (SCFs) are ubiquitin ligase complexes composed of shared, common components (Skp1, Cullin, and also a RING finger protein) and a unique, substrate-recognizing F-Box protein. They are mostly involved in the recognition of phosphorylated substrates. In the case of substrates, modification can affect their availability to the ubiquitination/degradation machinery and, consequently, their cellular stability. For example, in the case of IκBα, the inhibitor of the transcriptional regulator NF-κB, modification by SUMO-1 was shown to protect the substrate from ubiquitination. In a completely different case, SUMOylation of RanGAP1 is necessary for targeting of the protein to its subcellular destination in the nuclear pore complex. Modification by UBLs requires E1, E2, and possibly an E3 that use a similar catalytic mechanism to that used by the enzymes that catalyze ubiquitination. Yet, they are different enzymes, and the two sets are not interchangeable.

A recently discovered group of proteins are those that carry a UBL domain, such as the ubiquitin ligase Parkin,

and proteins that have a Ubiquitin Interacting Motif (UIM) or a UBiquitin-Associated (UBA) domain. The UBL domain that is part of other proteins cannot be conjugated, as it lacks a free, C-terminal Arg-Gly-Gly domain. The UBL domain in these proteins serves, most probably, to facilitate interaction with other ubiquitin binding proteins of the system, such as the proteasome. This interaction may enhance the efficiency of the proteolytic process by bringing together different components of the system. The UIM- and UBA-containing proteins serve, most probably, as recognition elements in *trans*, binding ubiquitinated proteins and linking them to other elements of the system. Thus, Rad23 and Dsk2 in yeast are believed to bind polyubiquitinated substrates in the cytosol and target them to the proteasome. Again, such linking elements may increase the efficiency of the proteolytic process.

(For recent reviews on ubiquitination, the proteasome, and ubiquitin-like proteins, see Adams, 2003; Di Fiore et al., 2003; Glickman and Ciechanover, 2002; Hilt and Wolf, 2000; Joazeiro and Weissman, 2000; Pickart, 2001; Verger et al., 2003; Voges et al., 1999; Weissman, 2001).

The Ubiquitin System and Pathogenesis of Neurodegeneration

Accumulation of ubiquitin conjugates and/or inclusion bodies associated with ubiquitin, proteasome, and certain disease-characteristic proteins have been reported in a broad array of chronic neurodegenerative diseases, such as the neurofibrillary tangles of Alzheimer's disease (AD), brainstem Lewy bodies (LBs) (the neuropathological hallmark in Parkinson's disease [PD]), Bunina bodies in Amyotrophic Lateral Sclerosis (ALS), and nuclear inclusions in CAG repeat expansion (polyglutamine/Q extension) disorders such as Huntington's disease, Spinocerebellar Ataxias (SCAs), and Spinal and Bulbar Muscular Atrophy (SBMA; Kennedy's disease) (reviewed recently by Alves-Rodrigues et al., 1998; Sherman and Goldberg, 2001) (Figure 2). As discussed later, a direct linkage between an aberration in the ubiquitin system and the resulting pathology has been established in certain rare cases of PD or AD. However, in most cases, and especially in the sporadic, late-onset diseases, such a direct linkage does not appear to exist. One factor that complicates the foundation of such a link is the realization that many of these diseases, e.g., AD and PD, can no longer be considered etiopathological entities, but rather syndromes with different etiologies. In these cases, accumulation of ubiquitin conjugates in LBs may be secondary and reflect failed attempts by the UPS to remove the damaged/abnormal proteins. While the initial hypothesis was that inclusion bodies are generated because of the inherent tendency of the abnormal proteins to associate with one another and aggregate, it is now thought that the process may be more complex and involves active cellular machineries, such as generation of specific reversible aggregate concentrates (aggresomes) (Johnston et al., 1998) or movement of the proteasome to specific subcellular sites where proteolysis of abnormal proteins may occur (Fabunmi et al., 2000). The pathogenetic significance of these aggregates has remained enigmatic. Recent findings demonstrate that soluble aggregated proteins can inhibit the ubiquitin system (Bence et al., 2001). Yet, an emerging concept is that the sequestration of the aggregated proteins from the cytosol and nucleoplasm and their concentration in defined inclusion bodies separates them from sensitive cellular machineries, such as transcriptional apparati. Therefore, the inclusion bodies may be protective, and it is the soluble fraction of the aggregated proteins that is toxic. In any event, the appearance of the inclusion bodies that contain the aggregated, disease-specific proteins has emerged as a common but poorly understood mechanistic theme in neurodegenerative disorders.

Parkinson's Disease

The case of PD highlights the complexity of the involvement of the UPS in the pathogenesis of neurodegeneration. In PD, the main neuropathological feature is the progressive death of neurons in the substantia nigra pars compacta with resulting loss of dopaminergic innervation of the striatum. This causes a gradual development of akinesia, rigidity, and tremor. In the vast majority of patients, some of the remaining nigral dopaminergic neurons exhibit aggregated proteins in the form of cytoplasmic LB inclusions. Several apparently independent aberrations linked to defects in the UPS have been described in various rare forms of hereditary PD. These rare cases shed light on basic pathogenetic mechanisms that maybe relevant also to the most prevalent, late-onset and sporadic cases of PD (for a recent review on the subject, see Giasson and Lee, 2003).

Parkin Mutations in Autosomal-Recessive Parkinson's Disease

An important player in the pathogenesis of PD is Parkin (*PARK2*). Parkin is a 465 amino acid residue, ~52 kDa protein with a UBL domain at the N-terminal region and two RING finger motifs at the carboxy-terminal region. Various deletion and point mutations in the gene have been found in ~50% of patients with AR-JP (known also as Autosomal Recessive Parkinson's Disease [ARPD]), one of the most common familial forms of PD (Kitada et al., 1998). Interestingly, with a few exceptions, AR-JP is characterized by a lack of LBs. Later studies have identified Parkin as a ubiquitin-protein ligase that acts along with the ubiquitin-conjugating enzymes UbcH7 and UbcH8 (Imai et al., 2000; Shimura et al., 2001). The RING finger domain is involved, most probably, in recruitment of the E2 component of the ubiquitination machinery, while the UBL can serve as a proteasome binding motif (Upadhya and Hegde, 2003), thus facilitating transfer of the polyubiquitinated substrate(s) to the degrading machinery. Indeed, it has been reported that Parkin associates with the RPN10 (S5a) subunit of the 26S proteasome (Sakata et al., 2003). The binding to the proteasome may facilitate transfer of the conjugates to the proteolytic machinery. Interestingly, mutation at R42 abrogates the binding of Parkin to the proteasome, suggesting that it can cause AR-JP. Recent findings have demonstrated that Parkin is a component of an SCF-like E3 complex along with the F-box/WD repeat protein hSel-10 and Cullin 1 (Staropoli et al., 2003). This finding, if further corroborated, has far reaching implications. From this study, it appears that Parkin serves as a common, RING finger-containing component shared by a

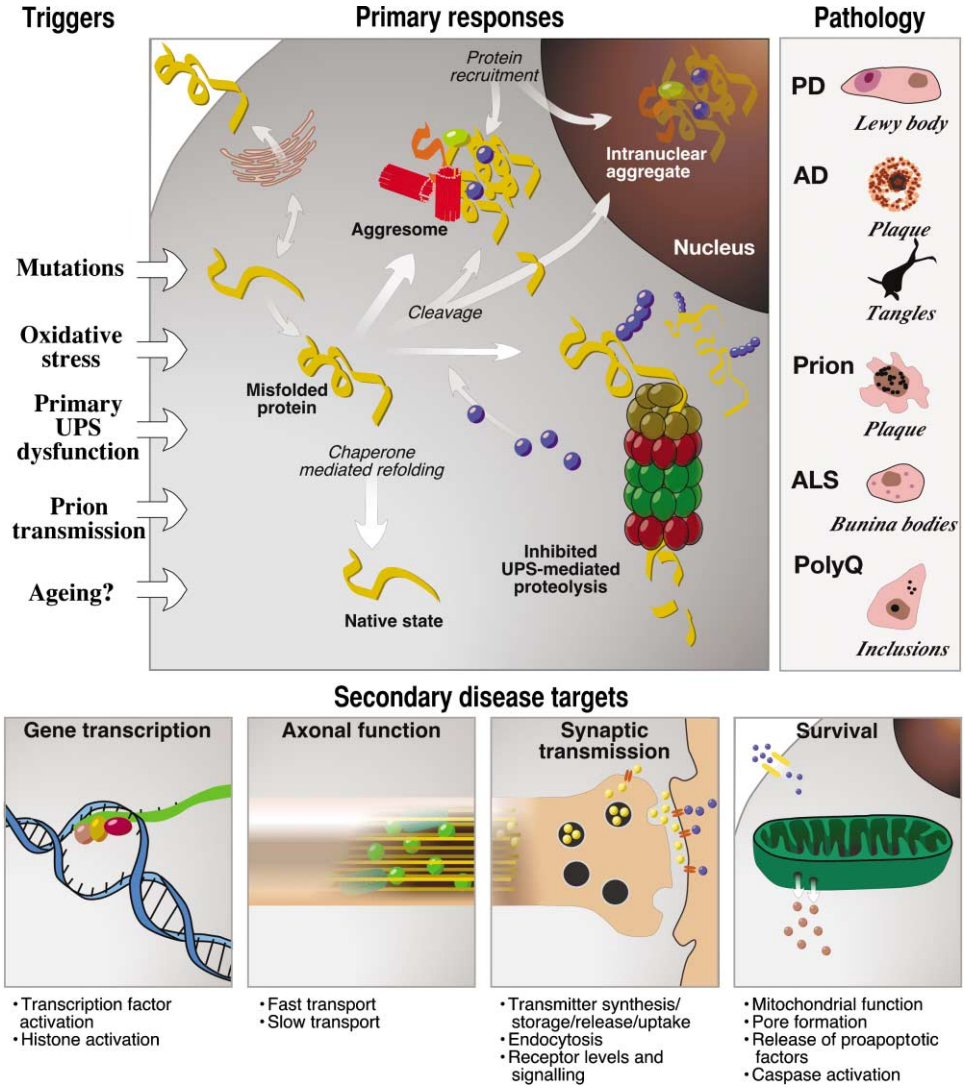

Figure 2. The UPS and Pathogenesis of Neurodegeneration

The figure describes four different aspects related to protein misfolding and neurodegenerative diseases; all are discussed in the text. (1) *Triggers* that can cause the accumulation of misfolded proteins—these include both mutations and epigenetic factors. (2) The *primary responses* to accumulating misfolded proteins—these are related to the reduced capacity of the UPS that is the consequence of protein overload/inhibition by aggregated substrates. The misfolded proteins that accumulate may be refolded by chaperones or accumulate in aggregates in the cytoplasm, nucleus, or extracellular space. The aggregates may sequester additional proteins (3) Types of *neuropathological* extracellular and intracellular protein deposits that can be found in the central nervous system of patients with PD, AD, Prion disease, ALS, and polyglutamine (PolyQ) disorders. Finally, (4) four *secondary disease targets*, i.e., the different cell functions that are affected by the protein conformational disease. In different protein conformation disorders, it has been shown that gene transcription can be markedly disturbed at the levels of histone regulation and individual transcription factors. There is evidence for disrupted axonal transport of different cell constituents. At the presynaptic level, neurotransmitter synthesis can be impaired; vesicular storage is sometimes disrupted; proteins involved in vesicle cycling are altered. At the postsynaptic level, the misfolded proteins can change receptor densities and downstream signaling transduction pathways. Finally, the diseases can cause neuronal death by several mechanisms, including impairment of mitochondrial function and mitochondrial release of cytochrome c with subsequent caspase activation. It has also been suggested that aberrant protein folding can lead to pore formation in cell membranes and loss of ionic homeostasis. For details and references, see the text.

group of putative, yet to be discovered, SCF-like complexes (similar to the RING finger protein Rbx1/Hrt1/Roc1 in the $SCF^{\beta TrCP}$, SCF^{Skp2}, and VBC [von Hippel Lindau, Elongin B and C] ligase complexes). It is the hSel-10 component in this particular complex that is involved in specific substrate recognition. According to this hypothesis, it is possible that other SCF complexes that contain Parkin recognize their different substrates via specific, yet to be identified F-box proteins or other substrate binding subunits (for known/putative Parkin substrates, see below). Most of the point mutations described in Parkin reside in its RING-IBR-(In Between-RINGS)-RING domain and result in its inactivation (Lucking et al., 2000). Like all other known RING finger-containing ligases, Parkin has an autoubiquitinating activity. An interesting finding is that not all mutations found in Parkin in AR-JP patients are inactivating mutations (see, for example, Chung et al., 2001; Corti et al., 2003; Imai

et al., 2001). It is possible that the mutations only reduce the activity of the enzyme (the autocatalytic one and/or the one displayed toward the exogenous substrates). Alternatively, other mechanisms, such as interaction of the E3 with essential partners, are affected by the mutations. Such a partner can be, for example, the cochaperone CHIP (Carboxy terminus of the Hsc70-Interacting Protein) that was found to increase the activity of Parkin, possibly by acting as an E4 (Imai et al., 2002) or the proteasome (see above). In this respect, it should be mentioned that not only recessive loss of Parkin constitutes a risk factor in AR-JP, but haploinsufficiency has been also suggested to cause the disease in some cases (West et al., 2002). Thus, it appears that a decrease in enzyme activity rather than its complete absence can underlie the pathogenesis of AR-JP related to Parkin mutations. It is also possible that the mutated allele has a dominant effect. In this scenario, it codes for a dominant-negative enzyme that binds the substrate and sequesters it but cannot ubiquitinate it, which results in its stabilization. Some of the enigmas regarding Parkin function could have been resolved by the generation of Parkin null mutant mice. However, the phenotype described in the first published report on such knockout mice has complicated the state of affairs rather than simplifying it (Goldberg et al., 2003). The mice display increased extracellular concentrations of dopamine in the striatum and reduced synaptic excitability in the striatal target neurons. The resulting behavioral syndrome includes deficits in motor coordination and somatosensory function. Quite contrary to the expectations, the mice do not exhibit a reduction in dopaminergic neurons in the substantia nigra, and the steady-state levels of CDCrel-1, synphilin-1, and α-synuclein—three putative substrates of Parkin (see below)—appear to be normal. It is hard to reconcile these data with what we currently know regarding Parkin, its substrates, and the mutations found in AR-JP patients. It is possible that Parkin is partially redundant in the mouse, and another E3 can catalyze some but not all of the reactions catalyzed by Parkin. On the other hand, Parkin appears to have a role in synaptic transmission that has not been described previously and that was not possible to observe in patients.

A crucially important development in our understanding of Parkin function is the identification of its exogenous native cellular substrates. The overriding hypothesis is that a defect in Parkin will result in accumulation of this protein(s), which is toxic to the dopaminergic neurons. Several substrates have been identified that are ubiquitinated by Parkin, yet it is not clear whether it is the accumulation of one or several of these proteins that underlies the pathogenesis of this familial form of PD. One of these substrates is Cell Division Control related protein (CDCrel-1) (Zhang et al., 2000), an ~44 kDa member of the septin family of proteins that includes GTPases required for cytokinesis. CDCrel-1 has been implicated in the inhibition of exocytosis through its interactions with syntaxin. A large fraction of it is bound to synaptic vesicles. Following transfection to a cell line, wild-type CDCrel-1 inhibits secretion of growth hormone, while the dominant-negative species increases secretion (Beites et al., 1999). It is possible that CDCrel-1 is involved in regulating transmitter release via its role in regulating synaptic vesicle dynamics, and its accumulation in patients with a mutation in Parkin perturbs the process. A second important substrate of Parkin is the Parkin-associated endothelial-like (Pael) receptor, a putative G protein-coupled transmembrane polypeptide (Imai et al., 2001). When overexpressed in cells, the receptor becomes misfolded. It then aggregates, and the insoluble protein elicits cell death via the Unfolded Protein Response (UPR). The UPR is a mechanism that involves a stress response in the ER, including increased biosynthesis of ER chaperones, in response to accumulation of misfolded/denatured/mutated proteins in this organelle (for a recent review on UPR, see Kaufman, 2002). Parkin ubiquitinates and promotes the degradation of the insoluble Pael receptor, acting in this reaction along Ubc6 and Ubc7, two E2s located in the ER. Importantly, the insoluble Pael receptor accumulates in the brains of patients with AR-JP. Overexpression of Parkin can rescue cells from the UPR elicited by a variety of stresses, such as exposure to H_2O_2, DNA alkylating agents, short-wavelength UV light, high osmolarity, and heat shock (Imai et al., 2000). Specifically, it can protect dopaminergic cells in *D. melanogaster* from neurotoxicity induced by overexpressed Pael receptor (Yang et al., 2003). Of note is that overexpression of Parkin also suppresses α-synuclein (αSYN) toxicity, suggesting that Parkin may play a role in regulating this protein as well (see below). Importantly, the insoluble form of Pael accumulates in the brains of AR-JP patients that have a mutated Parkin. Another substrate of Parkin is a novel 22 kDa form of O-glycosylated αSYN (αSp22) (Shimura et al., 2001). It is important to note that while mutations in the nonglycosylated 14 kDa form of αSYN have been linked to the pathogenesis of PD (see below), to date, they have not been linked with Parkin-associated AR-JP. Since αSp22 accumulates in a nonubiquitinated form in the brains of AR-JP patients, it is postulated that this accumulation can be toxic. It should be noted, however, that the finding of accumulation of this new form of modified αSYN in PD patients has to be further corroborated, along with elucidation of the possible role the modification plays in the pathogenetic process. Parkin also ubiquitinates Synphilin-1, a protein of hitherto unknown function that contains a coiled-coiled domain and an ATP/GTP binding motif and that associates with αSYN (Chung et al., 2001). Overexpression of Synphilin-1 with αSYN results in the formation of protein inclusions, yet the function of Synphilin in this process as well as the role of its ubiquitination are still elusive. Since inclusion bodies are lacking in most cases of AR-JP, it is possible that the ubiquitination of Synphilin by wild-type Parkin plays a role in their formation, by targeting ubiquitinated Synphilin to these bodies and removing it from the cytosol where it can be toxic. An interesting finding relates to the role of Parkin in the elimination of proteins containing an expanded polyglutamine stretch. Overexpression of Parkin reduced the accumulation of Ataxin-3-derived, polyglutamine-containing fragment (Tsai et al., 2003). Concomitantly, it reduced the inhibition of the proteasome and the activation of caspase 12 that are induced by accumulation of the polyglutamine-containing fragment. The affinity of the polyglutamine fragment to Parkin was increased by Hsp70, in agreement with the hypothesis that molecular chaperones

may play a role in the recruitment of misfolded proteins to the conjugation machinery (see, for example, Bercovich et al., 1997). A rather surprising, recently discovered substrate of Parkin is cyclin E (Staropoli et al., 2003) that is ubiquitinated by the SCF complex that contains Parkin. Consistent with the notion that cyclin E is targeted by the Parkin ligase complex is the finding that Parkin deficiency leads to accumulation of cyclin E in cultured postmitotic neurons exposed to the glutamatergic excitotoxin kainite and promotes their apoptosis. Overexpression of Parkin is reported to attenuate cyclin E accumulation and rescue the cells from apoptosis. While cyclin E can still be a substrate for Parkin, it is not clear whether its accumulation plays a direct role in cell toxicity. Another recently described substrate of Parkin is the p38 subunit of the aminoacyl-tRNA synthetase complex (Corti et al., 2003). Overexpression of p38 in cells resulted in a formation of aggresome-like inclusions which sequestered Parkin. In the dopamine-producing neuroblastoma cell line SH-SY5Y, Parkin rescued the cells from p38-induced cell death. An interesting point is that the mutated Parkin species K161N, which is associated with AR-JP, could still ubiquitinate p38, suggesting that it is not loss of function that underlies the human disease (see above). Finally, Synaptotagmin XI has also recently been reported to be a substrate of Parkin (Huynh et al., 2003). It is possible that ubiquitination of this substrate affects synaptic vesicle transport and/or transmitter release.

Parkin has been reported also to associate with actin filaments but not with microtubules (Huynh et al., 2000). It has not been demonstrated, however, to modify actin. Yet, it is possible that the ligase regulates actin activity by ubiquitinating an actin-associated protein, which in turn affects the function of actin and thereby influences the movement of synaptic vesicles. Detailed study of *Drosophila parkin* null mutant revealed reduced life span, locomotor disorders (in flight and climbing), and selective male sterility (Greene et al., 2003). Only a subset of dopaminergic neurons was affected. The disorders in locomotion were caused initially by disruption in muscle integrity that later evolved into cell death. Sterility was caused by a late defect in spermatogenesis, occurring at the individualization stage. Importantly, the earliest defect observed in both muscles and spermatids was mitochondrial dysfunction—displayed by swelling and disintegration of matrix and cristae. Thus, while the *parkin* dysfunction syndromes in the fly and human appear to be distinct, they may share a similar basic pathogenetic mechanism—aberration in mitochondrial function.

In summary, recent findings regarding Parkin clearly point toward different direct etiological roles that this enzyme may have in the pathogenesis of AR-JP. As noted, however, it is not clear whether the collective accumulation of all of its substrates—the known and those left to be discovered—is toxic to the dopaminergic cells or if there is one protein in particular that is toxic. It is also unclear why catecholaminergic neurons of the substantia nigra and locus ceruleus are selectively vulnerable to the loss of Parkin in AR-JP patients. It is possible that the high oxidative state associated with cathecolamine biosynthesis does not leave a large enough reserve of defense mechanisms to protect against an additional stress, such as occurs during accumulation of aggregated proteins, the Pael receptor, for example. Importantly, the finding that most AR-JP patients do not display LBs supports a few interesting assumptions. First, these inclusions may not play a role in promoting the pathogenetic process. Second, one can argue that ubiquitination of proteins may play a role in their aggregation and precipitation and result in the formation of inclusions, as LBs are not formed in the absence of ubiquitinated proteins. Further supporting this hypothesis is the finding of Cummings and collaborators (Cummings et al., 1999) that inactivation of the gene coding for the E6-AP (Ube3a) ligase in a transgenic model of SCA-1 resulted in a reduced number of neurons containing intranuclear aggregates (see below). In conclusion, the discovery of Parkin, elucidation of its role as a ubiquitin ligase, identification of certain protein substrates that are targeted by the ligase, and the finding that many of mutations found in Parkin in AR-JP patients inactivate its ubiquitin ligating activity or possible association with its partners are of utmost importance and may provide keys to the enigmatic puzzle of PD pathogenesis (for recent reviews on Parkin and its possible involvement in the pathogenesis of PD, see Dawson and Dawson, 2003; Dev et al., 2003b; McNaught and Jenner, 2001; Mizuno et al., 2001).

Mutations in Ubiquitin Carboxy-Terminal Hydrolase-L1 Can Cause Parkinson's Disease

Recent findings in a German family with PD have revealed a mutation in the gene coding for the ubiquitin carboxy-terminal hydrolase UCH-L1 (Leroy et al., 1998). While the mutation did not lead to complete inactivation of the enzyme, the hydrolase was clearly less active compared to its wild-type counterpart. The simple explanation is that the mutation leads to a shortage in free ubiquitin that should have been recycled from conjugates, which results in general impairment of the function of the UPS. This impairment leads, in turn, to accumulation of certain proteins that may be toxic to neurons. Interestingly, inactivation of UCH-L1 in mice does not lead to dopaminergic neuronal death but to Gracile Axonal Dystrophy (GAD) syndrome. This is a recessive autosomal disease that results in sensory ataxia at an early stage, followed by motor ataxia at a later stage (Saigoh et al., 1999). The GAD mice display axonal degeneration in the gracile tract of the spinal cord and medulla oblongata but not cell death in the substantia nigra. The mutation in UCH-L1 that was found linked to PD was identified as I93M. It should be noted that the mutation was not identified via gene linkage studies but rather through a gene association study. Although the causative relationship between the mutation and the disease appears to be strong, it is nevertheless circumstantial at this stage. Since the mutation is not 100% penetrant and since the GAD mice do not display a Parkinsonian syndrome—neither clinically nor histopathologically—it is not clear whether loss of the ubiquitin hydrolytic activity underlies the entire pathology observed in patients with the I93M mutation. Therefore, it was suggested that, in addition to the loss of the ubiquitin hydrolytic activity, additional factor(s) may be involved in the pathogenetic process involved in PD. It has been reported recently that while the monomeric form of UCH-L1 catalyzes deubiquitination, the dimers

display a ubiquitin ligase activity that generates ubiquitin-K63 bonds (Liu et al., 2002). Mono- and diubiquitinated αSYN were polyubiquitinated by the enzyme, suggesting that it acts as an E4 (Koegl et al., 1999). Incidentally, UCH-L1 and αSYN colocalize with synaptic vesicles and can be coimmunoprecipitated (Liu et al., 2002). The ligase activity was diminished significantly in a S18Y mutant of UCH-L1. Interestingly, mutation in this site is a polymorphic variant that protects the carrier population from PD (Satoh and Kuroda, 2001). Thus, it is possible that the activity of the ligase plays also a pathogenetic role in the UCH-L1 I93M hydrolase mutation, as a decreased activity of the hydrolase may result in a net increased activity of the ligase. Supporting this notion is the finding that inactivation of the ligase is protective. It should be noted, however, that the physiological role of the conjugating activity as well as the function of the mono-, di-, and polyubiquitinated αSYNs have remained elusive. In particular, the pathogenetic role of polyubiquitinated Lys63 αSYN is not known. One, although unlikely, possibility is that the ligase activity depletes the free pool of ubiquitin that cannot be replenished by the mutated hydrolase. Alternatively, the Lys63 polyubiquitin chains of αSYN that cannot be trimmed/removed by the mutated hydrolase accumulate and are toxic to the cells. Since many of the ubiquitin recycling enzymes have redundant function, it was important to find whether the activity of UCH-L1 is complemented by another ubiquitin hydrolytic enzyme. An interesting candidate is UCH-L3. Mice homozygous for a targeted deletion of the UCH-L3 gene are indistinguishable from the wt animals (Kurihara et al., 2001). However, mice homozygous for both UCH-L1 and UCH-L3 deletions die early due to dysphagia and display degeneration of the nucleus tractus solitarius and area postrema in addition to the degeneration of the gracile tract that is observed in GAD mice that only have a UCH-L1 deletion. Interestingly, mutant UCH-L3 was found in a modifier screen for ataxin-1-interacting proteins in *Drosophila* as an enhancer of the SCA-1 phenotype (Fernandez-Funez et al., 2000).

Autosomal-Dominant Parkinson's Disease Caused by Mutations in α-Synuclein

A third important player that links aberrations in the UPS to the pathogenesis of PD is αSYN (*PARK1*). αSYN is a small, 140 amino acid residue protein that is thought to regulate/participate in dopamine neurotransmission/release via effects on vesicular storage. Wild-type αSYN is monomeric, but at high concentration, it oligomerizes to β-pleated sheets known as protofibrils. Structural analysis reveals that the protofibrils are made of 10–12 nm fibrils. The protofibrils can further aggregate and precipitate as amyloid fibrils that are present in Lewy bodies, the hallmark of sporadic, late-onset PD. The function of αSYN is also impaired in familial forms of PD where mutant αSYN is found. In the late 1990s, it was reported that two mutations in the N-terminal domain of αSYN, A30P (Kruger et al., 1998) and A53T (Polymeropoulos et al., 1997), were associated with a rare form of autosomal-dominant familial PD. This caused a major shift of attention within the research field to the role of protein misfolding in the pathogenesis of PD (reviewed recently in Dev et al., 2003a). The mutant proteins have a higher tendency to generate protofibrils. In parallel, the wild-type protein was shown to be a major component of LBs and Lewy neurites in sporadic PD, dementia with LBs (DLB), and the LB variant of AD. αSYN fibrils also constitute the central core of oligodendroglial (in contrast to neurons in all other diseases) inclusion filaments in multiple system atrophy (MSA) (Gai et al., 2003). The autosomal-dominant nature of the disease associated with mutant αSYN strongly suggests that a gain as opposed to a loss of function underlies the disease mechanism. Overexpression of wild-type, but in particular mutant αSYN in many cell types but not in all, induces apoptosis or sensitizes the cells to toxic agents, including proteasome inhibitors (see, for example, Lee et al., 2001a). While direct connection between Parkin and αSYN has not been established, overexpression of wild-type Parkin can rescue cultured catecholaminergic primary mesencephalic neurons from the toxic effects of αSYN (Petrucelli et al., 2002), suggesting that the two proteins share a common pathway involved in cell death.

Why is αSYN oligomerization toxic? As noted above, it is still debatable whether LBs that sequester the aggregated proteins are toxic or protective. From recent studies in several neurodegenerative disease models, including PD, SCA, and HD, it appears that it is the soluble, oligomeric forms of the disease-characteristic proteins (αSYN in the case of PD, ataxins in the case of SCAs, and Huntingtin in the case of HD) that are responsible for their toxicity (for αSYN, see, for example, Petrucelli et al., 2002; Xu et al., 2002). The linkage between αSYN and the UPS system is not that clear. AR-JP is characterized by lack of LBs and αSYN aggregates. On the other hand, it is not clear whether polyubiquitination of αSYN is necessary for its degradation. It has been shown that the protein is targeted by the proteasome (Bennett et al., 1999) and that the mutant form is slightly less susceptible, but it is not clear whether aberrations in this process play a role in the pathogenesis of PD. It is possible, although it has not been shown yet, that modification by ubiquitin leads to aggregation and precipitation of the protein and that the unmodified protein is more soluble. The suggestion that the glycosylated form of αSYN is targeted by Parkin (Shimura et al., 2001) may resolve part of the enigma. Accordingly, it is plausible that the accumulation of this, otherwise normally degradable, form of αSYN is toxic. Therefore, patients with AR-JP—who cannot degrade it because of the mutation in the Parkin E3 (see above)—develop neurodegeneration. However, this model does not explain αSYN toxicity in most sporadic PD patients who have a functional and active Parkin and αSYN (as noted, mutated αSYNs and Parkin were found only in rare cases of familial PD). An important finding, however, in that respect is that aggregated and even monomeric αSYN bind to the S6′ proteasome subunit and inhibit proteasomal function (Snyder et al., 2003). Thus, aggregation, which is the primary event, may lead to secondary damage by inhibiting the UPS (Bence et al., 2001). It is also possible that polyubiquitination of αSYN itself is not relevant at all to the pathogenesis of PD. Instead, impairment of αSYN function may disturb ubiquitination of αSYN-associated proteins, such as Synphilin (Chung et al., 2001) and tyrosine hydroxylase (Doskeland and Flatmark, 2002), and thereby perturb neuronal homeostasis.

The DJ-1 Gene Is a New Player in Parkinson's Disease

Recently discovered mutations in the *DJ-1* gene have also been associated with AR-JP (Bonifati et al., 2003). The function of the DJ-1 protein is not known, but it is supposed to be involved in oxygen sensing, acting as an antioxidant, probably protecting/rescuing other proteins from oxidative damage. DJ-1 activity may be regulated by SUMOylation, as it was found that it binds to the SUMO E3 PIASx (Takahashi et al., 2001). Interestingly, one of the DJ-1 mutations that is associated with AR-JP is a point mutation, L166P. Miller and colleagues (Miller et al., 2003) have recently shown that this mutation destabilizes DJ-1 via promotion of its rapid, UPS-mediated degradation. Initial data show that the mutated protein is active, and it is its rapid degradation that leads to loss of function of the protein that may underlie the pathogenesis of AR-JP in this case. Thus, in this case, the UPS may contribute to PD pathogenesis by removing a mutated yet active protein that is recognized more efficiently by the system compared to its wild-type counterpart. This case may be analogous to the loss of function observed during the rapid degradation of mutated ΔF508 Cystic Fibrosis Transmembrane conductance Regulator (CFTR), which is also an active protein, and that the rapid degradation of which contributes to the pathogenesis of cystic fibrosis (CF) in patients carrying the mutation. It should be noted that CFTR degradation is mediated by Endoplasmic Reticulum Associated Degradation (ERAD—a quality control mechanism that eliminates, via the UPS, misfolded/mutated/abnormal membrane or lumenal ER proteins following their retrotranslocation to the cytosol via the Sec61 translocation channel). For a recent review on ERAD, see (Kostova and Wolf, 2003).

The Role of the Ubiquitin Proteasome System in Sporadic Parkinson's Disease

Along with an age-related tendency to accumulate oxidized damaged proteins, failure of the UPS to adequately remove misfolded/abnormal proteins may underlie the degeneration of nigral cells also in sporadic PD (McNaught and Olanow, 2003), which constitutes the most common form of the disease. It has been reported that α but not β subunits of the core catalytic 20S subcomplex of the 26S proteasome are lost (McNaught et al., 2002), and 20S proteasomal enzymatic activities are impaired in the substantia nigra in sporadic PD (McNaught and Jenner, 2001). The 19S/PA700 proteasome activator was also decreased, and the levels of the PA28 regulator were almost undetectable in the substantia nigra compared to other brain areas (McNaught et al., 2003). However, it is not clear why these changes were so striking in nigral tissue samples that contained many more cells than those actually affected by neuronal death in PD. In any event, one should be careful in the interpretation of these data, as it is not clear whether the inhibition of the proteasome indeed results in a reduced capacity of the UPS in vivo and, if so, whether the reduction is large enough to inflict damage (see also below).

So what can one learn from the rare genetic diseases that affect components and substrates of the UPS on the involvement of the system in the vastly more prevalent sporadic cases of PD? A common pathogenetic base to all these diseases has been proposed by several authors. These models suggests that proteolytic stress with an accompanying defect in protein handling is crucial and that the substantia nigra neurons are particularly vulnerable due to their high content of dopamine (summarized in Lotharius and Brundin, 2002; McNaught and Olanow, 2003). The increased vulnerability of dopaminergic neurons may be due to a high basal rate of protein oxidation which is due to the enzymatic and autooxidation machineries involved in dopamine oxidation. This, along with low steady-state levels of the 19S/PA700 and PA28 regulators of the proteasome may render these cells more susceptible to proteolytic stress than other brain cells.

Alzheimer's Disease and Related Tauopathies

In AD, the dominant symptom is dementia, initially characterized by a loss of short-term memory which gradually develops into a loss of most higher faculties. In 1906, Alois Alzheimer was the first to describe neuropathological changes involving protein aggregates and, much ahead of his time and unknowingly, he introduced neuropathologists to the field of aberrant protein folding—before it had even been discovered! Patients with AD display two types of protein deposits: extracellular amyloid plaques and intracellular neurofibrillary tangles (Hardy and Selkoe, 2002). The latter neuropathological change is also observed in a series of other neurological conditions that have been collectively named tauopathies and include some forms of Parkinsonism, Pick's disease, and boxing-induced dementia (Dementia pugilistica) (Lee et al., 2001b).

The plaques in AD are rich in amyloid β peptides (Aβ) that are produced by proteolytic cleavage of the amyloid precursor peptide (APP), a glycolipid located in the outer cell membrane. Three different proteases, called α, β, and γ secretases, can cleave APP at specific sites and generate products that are well characterized. Concomitant cleavage of APP by β and γ secretase at specific sites can result in fragments (Aβ1−40 or Aβ1−42) that can misfold and form extracellular fibrils. These fibrils are around 6–12 nm long and consist of β sheets. It is debated whether the fibrils are toxic through formation of pores (Caughy and Lansbury, 2003) (Figure 2) or whether the extracellular amyloid deposits that they can form are the main culprits. In contrast, the neurofibrillary tangles are intracellular and are rich in tau, a structural protein that is normally associated with microtubuli. In conjunction with the formation of neurofibrillary tangles, the synthesis of the tau protein increases, and it undergoes an abnormal posttranslational modification characterized by hyperphosphorylation. Proteolytic processing/degradation of tau is also believed to be important for the formation of the neurofibrillary tangles, although the molecular pathways involved in this process are not fully understood. Mutations in the *tau* gene can cause the tauopathy Pick's disease (mentioned above). Mutant tau alone does not cause AD, favoring the idea that accumulation of erroneously processed Aβ is a key event in AD pathogenesis. Familial cases of AD with an autosomal-dominant inheritance constitute only 5% of the cases (Forloni et al., 2002) but have been instructive in unraveling possible pathogenetic mecha-

nisms involved in the sporadic cases. They have demonstrated that AD can be caused by mutations in the *APP* gene, either in the vicinity of the secretase cleavage sites, causing abnormal APP processing, or in the sequence coding for Aβ, giving rise to a peptide that is more likely to self-aggregate. Finally, some patients express mutant presenilin proteins 1 and 2 (PS1 and PS2) that can change the processing of APP by altering γ secretase activity, thereby promoting the generation of amyloidogenic Aβ (Hardy and Selkoe, 2002).

Is impairment of the UPS involved in the pathogenesis of AD? It has been shown that Aβ can be degraded by the proteasome in cultured neurons and astrocytes, and treatment with the proteasome inhibitor lactacystin decreased viability of cells exposed to Aβ (Lopez Salon et al., 2003). Postmortem tissue studies have shown an interesting region-specific reduction in proteasome activity in AD patients (Keller et al., 2000). Thus, brain regions thought to be particularly involved in the lost brain functions (e.g., hippocampus and related limbic structures and inferior parietal lobe) displayed reduced activity, although the levels of the α and β subunits of the proteasome were not changed. In contrast, the proteasomal activity was normal in the occipital lobe and cerebellum—regions involved in vision and motor coordination, functions that are relatively spared in AD. A recent study confirmed a brain region-specific reduction in proteasomal activity, with a frontal cortical region being affected whereas the striatum, occipital, and temporal lobes were spared (Keck et al., 2003). The authors argue that paired helical filaments present in neurofibrillary tangles and containing the tau protein are the perpetrators. They present evidence that paired helical filaments obtained from AD brain or generated in vitro can inhibit proteasome function and that in AD brain tissue these filaments coimmunoprecipitate with the proteasome (Keck et al., 2003). Other studies in cultured neuroblastoma cells show that unfolded tau is normally destroyed by the proteasome without prior ubiquitination, and induction of conformational changes in tau by treatment with SDS prevents its degradation (David et al., 2002). Finally, there is also evidence for a reduced activity of E1 and E2 enzymes in cerebral cortex samples from AD patients compared to age-matched controls (Lopez Salon et al., 2000). Taken together, several lines of evidence point to a reduced UPS function in AD and suggest that both Aβ and tau are important players in the game. One should be careful, however, in the interpretation of these data. It is not clear whether the inhibition of the proteasome is large enough to inflict damage (see also above). Also, the observation of proteasome-dependent, yet ubiquitin-independent degradation of tau clearly requires further corroboration.

So is it the chicken or the egg? That is, does a reduction in the activity of the UPS lead to a greater tendency of Aβ to accumulate and generate amyloid plaques, or do conformational changes in Aβ or tau cause proteasome inhibition? One characteristic of AD is that it shows a higher prevalence with age. The ability to increase ubiquitin conjugation in response to stressors decreases in ageing tissue (Shang et al., 1997). The proteasome activity in the mammalian brain decreases with increasing age (Keller et al., 2002), suggesting that the aged brain is less able to handle the aberrantly folded Aβ. However, Aβ has been demonstrated to reduce proteasome activity in reticulocyte lysates (Gregori et al., 1995), suggesting that increased levels of the peptide could underlie the reduction in UPS function observed in the AD brain.

Perhaps the most compelling evidence for the involvement of the UPS in AD pathogenesis comes from one of the experiments of nature. A transcriptional misreading can cause the deletion of two nucleotides in the mRNA coding for ubiquitin (van Leeuwen et al., 2000). The resulting frameshift mutant form of ubiquitin is called UBB^{+1}, and it has a 19 amino acid residue C-terminal extension. UBB^{+1} was first identified in neurons of patients with AD and Down's syndrome (who also exhibit AD-like brain pathology when middle-aged) (van Leeuwen et al., 1998). UBB^{+1} has also been observed at low levels in brains of elderly individuals with mild cognitive impairment and patients with other neurodegenerative diseases (van Leeuwen et al., 2000) as well as in Mallory bodies in the liver afflicted by disease (French et al., 2001). Due to the absence of the crucial C-terminal RGG, UBB^{+1} cannot bind to target proteins. However, it can be ubiquitinated by wild-type ubiquitin—produced from correctly generated transcripts in the same cell—that can bind to two specific lysine residues in UBB^{+1} (Lindsten et al., 2002). The polyubiquitinated UBB^{+1} cannot be degraded by the proteasome and, by binding to it, probably inhibits its activity toward other substrates (De Vrij et al., 2001; Lam et al., 2000). Furthermore, it cannot be deubiquitinated by the ubiquitin recycling enzymes and, therefore, when present at high levels, causes a "dominant-negative" inhibition of the 26S proteasome both in model cell systems (Hope et al., 2003; Lam et al., 2000; Lindsten et al., 2002) and in cultured cells obtained from a transgenic mouse specifically generated to assay UPS function in vivo (Lindsten et al., 2003). Overexpression of UBB^{+1} by adenoviral transduction in cultured neuroblastoma cells can impair the UPS to the extent that they finally undergo apoptosis (De Vrij et al., 2001). In summary, there is little question that high levels of UBB^{+1} can be detrimental to cell function. It is unlikely that an increased level of UBB^{+1} is a direct cause of AD because it occurs also in other neurodegenerative conditions. However, the recent reports showing that UBB^{+1} can inhibit the proteasome suggest that it may contribute to disease pathogenesis.

In summary, the evidence that the UPS plays a pivotal role in the pathogenesis of AD is compelling. The recent unraveling of the fascinating UBB^{+1} story in AD suggests that we may soon see many more completely novel pathogenetic mechanisms involved in neurodegenerative diseases, and primary failure of the UPS could be a key element in several of them.

Prion Disease

Prion diseases have brought the linkage between abnormal protein conformation in its purest form and neurodegeneration to the center stage. This family of diseases is considered to be caused by a protein component of the proteinaceous infectious (prion) particles, originally proposed to be the causative agent of these diseases by Stanley Pruisner (1982). As discussed later, recent studies suggest that failure of the UPS could trigger prion pathogenesis (Hooper, 2003).

Human prion diseases include Creutzfeld-Jakob disease, fatal familial insomnia, Gerstmann-Sträussler-Scheinker disease, and kuru. They can be sporadic (80%), inherited (15% of cases have one of over 23 known mutations), or transmitted (5%) (McKintosh et al., 2003). This latter feature gives them a unique status among protein conformational neurological disorders and hence the name Transmissible Spongiform Encephalopathies (TSEs). In cattle, prions can cause Bovine Spongiform Encephalopathy (BSE), called in lay terms "mad cow disease." Over the past two decades, this disease has become increasingly common in the European Community due to the inclusion of cow carcasses in cattle feed. The demonstration that it can be transmitted to humans, where it causes a special form of a new variant of Creutzfeld-Jakob disease, has compounded the problem and raised fears of a major outbreak of prion diseases over the next few decades, although mathematical modeling suggests that such an occurrence is unlikely (McKintosh et al., 2003).

The clinical features of prion diseases in humans vary. They have in common a progressive development of severe motor disturbance and dementia leading to death within a few months to a few years after diagnosis, which can be years to decades after the initial infection in transmissible cases. In fatal familial insomnia, the clinical presentation also includes a striking, untreatable inability to sleep. Prion disease neuropathology is characterized by widespread neuronal death, accompanied by spongiform vacuolation and astrogliosis, usually combined with widespread deposits of extracellular amyloid aggregates. These aggregates contain the causative agent, i.e., an insoluble form of the prion protein (PrP), which appears in a β sheet secondary structure and is termed PrP-scrapie (PrP^{sc}). The normal cellular isoform of PrP is called PrP^{c}, and it is a 33–35 kDa protein made of 209 amino acids with a high content of α-helical secondary structure. PrP^{c} is abundant in many cells throughout the body, particularly in the immune and nervous systems, and is typically anchored to the outer cell membrane, where it may act as a copper binding protein with antioxidant properties (Brown, 2002). The conformational change can be transmitted, i.e., if cells are exposed to PrP^{sc}, the normal PrP^{c} can be induced to undergo misfolding. Experiments in knockout mice have demonstrated unequivocally that development of the disease is dependent upon the presence of PrP^{c}, and therefore, transmitted PrP^{sc} cannot exert toxicity in animals that are null mutants for PrP^{c} (Bueler et al., 1993).

There are three different ways by which PrP^{sc} can gain access to the brain. First, by transmission through exposure of the cells to PrP^{sc}, either through ingestion of infected material or by iatrogenic causes (e.g., exposure to infected surgical instruments, hormone preparations, or grafted tissues); second, by inheritance of one of over 30 known mutations in the *prion* gene; and third, by spontaneous mutations in the *prion* gene in a single cell, with subesquent spread of PrP^{sc} to neighboring cells, as has been suggested to occur in the most common sporadic forms of prion disease (McKintosh et al., 2003).

What is the evidence that the UPS may be involved in the pathogenic processes once a mutant prion protein is expressed? The Prion protein normally interacts with the chaperone BiP and undergoes assisted folding (Jin et al., 2000). Alternatively, if misfolded, it is believed to be directed to degradation via ERAD (Yedidia et al., 2001). In a cell culture model, mutant PrP associated with Gerstmann-Sträussler-Scheinker disease can be rapidly degraded (half-life < 10 min), possibly even without prior ubiquitination (Zanusso et al., 1999). Following UPS inhibition with lactacystin, the protein accumulates and forms aggregates in the ER, Golgi apparatus, and nucleus. In the absence of proteasome inhibition, the mutant PrP does not form aggregates, suggesting that impairment of UPS may also be involved in pathogenesis of the human disease (Zanusso et al., 1999).

Recent work has highlighted a novel possible role for failure of the UPS in initiating prion disease, which can explain the cause of some cases of sporadic prion disease. Lindquist and colleagues proposed that inhibition of UPS, which can be caused by ageing or following a pharmacological treatment, can lead to accumulation of PrP^{c} in the cytoplasm where it is spontaneously converted into a PrP^{sc}-like species because it is not rapidly degraded by the UPS (Hooper, 2003; Ma and Lindquist, 2002; Ma et al., 2002). Consequently, the cytosolic PrP^{sc}-like isoform triggers cell death, with subsequent release of PrP^{sc}-like particles and infection of neighboring cells. Specifically, Lindquist and collaborators have shown that experimental inhibition of the UPS by proteasome inhibitors can lead to the appearance of PrP^{sc}-like species in otherwise normal cultured cells (Ma et al., 2002). They suggest that the PrP^{sc} is derived from misfolded PrP^{c} that has been retrotranslocated from the ER to the cytoplasm (Ma and Lindquist, 2002) (Figure 2). Once in the cytoplasm, the PrP^{c} would be efficiently degraded by the UPS via the ERAD pathway, but this does not occur in the presence of the proteasome inhibitors. A small fraction of the accumulated PrP^{c} is converted into the PrP^{sc}-like isoform, initiating the vicious cycle of cell death and transmission to neighboring cells. The generation of PrP^{sc} is suggested to continue even after the proteasome inhibitor is removed from the medium, suggesting that the process is self-perpetuating once it has been triggered (Ma and Lindquist, 2002). The researchers have found additional support for the cytotoxic capacity of PrP^{c} from experiments with transgenic mice expressing the gene coding for a cytoplasmic form PrP^{c}. The mice gradually develop ataxia and cerebellar degeneration and display an insoluble form of prion protein, suggesting that they have generated a PrP^{sc}-like isoform (Ma et al., 2002).

The model proposed by Lindquist and collaborators is important as it identifies malfunction of UPS as a potentially important player in prion pathogenesis. However, this concept is still controversial. For example, Harris and collaborators found no evidence for retrograde translocation of PrP^{c} from the ER to the cytoplasm. They suggest that the increased production of PrP^{c} in the presence of proteasome inhibitors may reflect an increase of gene transcription by the inhibitor via an effect on its promoter (Drisaldi et al., 2003).

Amyotrophic Lateral Sclerosis

There are different forms of amyotrophic lateral sclerosis (ALS), all primarily display widespread death of brain-

stem and spinal motoneurons, corticospinal degeneration, paralysis of skeletal muscle, and eventual neuronal cell death. Around ~5%–10% of all cases of ALS are hereditary, and in 20% of these patients (1%–2% of all patients), 100 different autosomal-dominant mutations—mostly single amino acid substitutions—have been identified in the gene coding for Copper-Zinc superoxide dismutase (SOD1) (Valentine and Hart, 2003). There is very little evidence to suggest that alterations in SOD are a common feature of sporadic ALS (Alexander et al., 2002). The normal function of SOD1 is to scavenge oxygen radicals and prevent oxidative stress. Mutations in the *SOD1* gene do not induce cell loss by affecting cellular antioxidant defenses. Rather, mutant SOD1 may act through one of two different mechanisms (Valentine and Hart, 2003). The first is that the mutant protein promotes oxidative stress through a gain-of-function mechanism where improper handling of copper may lead to the enzyme catalyzing aberrant pro-oxidant reactions (Cleveland and Liu, 2000). Alternatively, the enzyme itself may misfold and generate protein aggregates, thereby implicating a role for the UPS in disease pathogenesis (Cleveland and Liu, 2000; Julien, 2001; Valentine and Hart, 2003). The ubiquitinated SOD1-containing aggregates appear as cytosolic, hyaline inclusions called Bunina bodies (Figure 2), reminiscent of LBs, in neurons of patients and transgenic rodents carrying disease-related mutations (Bruijn et al., 1998; Katsuno et al., 2003; Tu et al., 1996). They also contain components of the proteasome and neurofilaments (Alves-Rodrigues et al., 1998; Johnston et al., 2000).

What have laboratory experiments told us about the interplay between misfolded SOD1 and the UPS? In transgenic animals, the aggregates form relatively late in the disease process (Morrison et al., 1998). Mutant SOD1 proteins are less soluble than the wild-type protein (Johnston et al., 2000; Shinder et al., 2001) and sediment more readily in vitro (Okado-Matsumoto et al., 2000). The mutant SOD1 proteins, unlike the wild-type form, are degraded by the UPS (Hoffman et al., 1996; Johnston et al., 2000). In transgenic cells, mutant SOD1 generates nonnative oligomers, and the subsequent inhibition of the UPS increases the tendency for the protein to form aggresomes (Johnston et al., 2000). Overexpression of the chaperone HSP70 has been found to reduce aggregate formation and death in nonneuronal cultured cells expressing mutant SOD1 (Bruening et al., 1999). Similarly, overexpression of the putative SOD1 E3 ligase dorfin can inhibit cell death induced by the mutant protein (Niwa et al., 2002), presumably by promoting its removal via the UPS. Interestingly, dorfin expression is increased in the spinal cord of ALS patients (Ishigaki et al., 2002), which may suggest that the expression of the E3 ligase is increased in an attempt to enhance clearance of the mutant SOD1. The E3 enzyme is also enriched in aggregates observed in PD and ALS (Hishikawa et al., 2003). Spinal cords from sporadic ALS cases also express increased levels of the gene for ubiquitin-like protein 5. While the function of this protein is not known, the high expression nevertheless serves as an additional indicator that the UPS is affected by the neurodegenerative process (Ishigaki et al., 2002).

Recent studies employing cultured cells have provided additional insight into the effects of mutant SOD1 on cell function. Urushitani and coworkers (Urushitani et al., 2002) showed that mutant SOD1 is degraded by the UPS in cultured cells and that oxidative damage increases the degree of ubiquitination of mutant but not of wild-type SOD1. They found that the proteasome catalytic activity was decreased in neuroblastoma cells following 1 week of expression of a mutant *SOD1* gene. Accordingly, they proposed that, in familial ALS, oxidative damage to mutant SOD1 leads to misfolding and polyubiquitination of the protein and eventually inhibits the UPS. Unlike in some other neurodegenerative diseases, where the misfolded proteins are thought to recruit subunits of the proteasome into the aggregates, there is no evidence for this occurring in the cellular model of familial ALS (Urushitani et al., 2002). In an attempt to understand the preferential death of spinal motoneurons in ALS, the investigators examined the effect of proteasome inhibitors on primary mixed cultures derived from embryonic spinal cord. They observed a selective death of the motoneurons in the culture and proposed that these neurons are particularly sensitive to perturbations of the UPS function (Urushitani et al., 2002). Thus, as a disease model they suggested that continued breakdown of mutant SOD1 eventually overloads the UPS in spinal neurons. This leads to accumulation and aggregation of mutant SOD1 and also to impairment of the defense against oxidative stress, which is partially dependent upon proper UPS function. Consequently, due to the increased stress, mutant SOD1 will misfold more rapidly. This, in turn, will further exacerbate substrate overload on the UPS of the already sensitive spinal motoneurons, as part of a self-perpetuating vicious cycle (Urushitani et al., 2002). This hypothesis, linking misfolding of SOD1 and impairment of the UPS to cell death, is conceptually attractive. However, the support for the idea that aggregates of mutant SOD1 are toxic is not unanimous. In another recent study, it was reported that expression of mutant SOD1 in PC12 cells increases formation of aggregates and that aggregate formation is further enhanced by oxidative stress and experimental inhibition of UPS (Lee et al., 2002). However, contrary to the hypothesis that the aggregates per se are toxic—e.g., by causing functional UPS inhibition—the mutant SOD1 aggregates did not kill cells (Lee et al., 2002). Two recent studies have demonstrated that cells expressing mutant SOD1 may be particularly vulnerable to additional inhibition of the proteasome. Thus, exposure to lactacystin resulted in increased cell death in human cell lines expressing mutant SOD1 (Aquilano et al., 2003; Hyun et al., 2003). In one model, the treatment led also to increased nitric oxide production and protein nitration, and inhibition of nitric oxide synthase both reduced aggregate formation and cell death (Hyun et al., 2003). In the other model, the expression of mutant SOD1 alone was sufficient to induce oxidative stress, giving rise to increased proteasome activity, possibly due to the need to remove oxidatively damaged proteins (Hyun et al., 2003). These studies imply that mutant SOD1 can induce oxidative stress through different mechanisms in manners that are coupled to changes in the UPS system.

In conclusion, the role of protein aggregates in familial ALS remains unclear. There is no doubt that aggregates of SOD1 do exist in a small subset of patients. However,

as is also the case in other neurodegenerative disorders, some discussed in this review, the interplay between protein aggregates, UPS function, and disease pathogenesis remains an area for exciting and important future research.

Polyglutamine Diseases: A Family of Mutations, Each Giving Rise to Specific Lesions and Clinical Features

The causative pathological event in the polyglutamine diseases of the nervous system is a mutation involving a CAG triplet repeat expansion (Gusella and MacDonald, 2000). There are a total of nine diseases, and all display an increased number of glutamine residues in the mutant protein; however, they differ in their clinical presentation and neuropathological profile (Ross, 2002; Taylor et al., 2002; Zoghbi and Orr, 2000). Thus, the patients display different combinations of motor, psychiatric, cognitive, and sensory symptoms, dependent on which protein exhibits the polyglutamine expansion. Many of these patients display onset of symptoms in midlife and a relatively slow progression over several years, demonstrating that the disease-associated protein has to be expressed for a prolonged period before the nervous system fails. Although each of these diseases has received a great deal of attention from the research community, the most studied CAG triplet repeat disease is probably HD. The disease is caused by a mutation in the gene coding for Huntingtin—a protein of hitherto unknown function, although it has been recently implicated in the control of gene transcription (Zuccato et al., 2003). In addition, the family of SCAs (SCA 1, 2, 3, 6, 7, and 17), DentatoRubro and PallidoLusyian Atrophy (DRPLA), and SBMA are all characterized by a polyQ expansion in a specific, disease-related protein. In three cases, the functions of the mutant proteins are known. In SBMA, the expanded polyglutamine is located in the androgen receptor (Ross, 2002); in SCA-6, the mutant protein is part of a voltage-gated calcium channel (Zoghbi and Orr, 2000); and in SCA-17, it is a TATA box binding protein (Nakamura et al., 2001). With the exception of SBMA, which is X-linked, the neurodegenerative polyglutamine diseases are autosomal-dominant. Therefore, there is strong argument for a toxic gain of function of the mutation in most cases. However, a contribution to the pathogenesis by loss of function of Huntingtin encoded from the wild-type allele has not been excluded in HD (Cattaneo et al., 2001), since the mutant protein not only aggregates itself but can also promote aggregation of the wild-type protein (Busch et al., 2003). What all the polyglutamine diseases have in common is that the mutant protein misfolds, and, as discussed below, it often forms protein inclusions. Therefore, it is important to understand why these mutant proteins misfold, how the conformational changes mediate toxicity, and in the context of the present review, how the function of UPS is affected.

Despite the mutation appearing in different proteins for each disease, the threshold for development of the pathology is approximately the same, i.e., the existence of a stretch of 35–45 glutamine residues in the mutant protein. The number of CAG repeats is inversely correlated with the age of disease onset (Gusella and MacDonald, 2000), suggesting that the rate at which the mutant proteins misfold is related to the length of the polyglutamine tract. A recent study in HD patients has demonstrated that, in the rare instances that patients are homozygous for the mutation, the rate of disease progression is faster than in the more commonly occurring heterozygous cases (Squitieri et al., 2003). This suggests that protein load may be another important factor in the pathogenesis of polyglutamine disease.

Protein Aggregate Formation and Composition

The polyglutamine disorders exhibit cytoplasmic or intranuclear protein inclusions, partly composed of amyloid-like fibrils consisting of β strands of the mutant protein (Scherzinger et al., 1997; Ross, 1997). The localization of the protein aggregates, intranuclear or cytoplasmic, varies between diseases and cells within the same disease (Zoghbi and Orr, 2000). Intranuclear aggregates have been described for all of the polyglutamine diseases except SCA-2. The events leading to the misfolding of expanded polyglutamine-containing proteins are not fully understood but seem to be partly the result of stochastic processes that require an early nucleation event (Perutz and Windle, 2001) and that are enhanced by the action of transglutaminase (Kahlem et al., 1996). In a second step, the transition from oligomers of misfolded protein to larger intracellular aggregates appears to involve the microtubule organization center (MTOC or centrosomes) which transports the misfolded protein in an orderly fashion to a perinuclear localization (Hoffner et al., 2002; Waelter et al., 2001), akin to the concept of the aggresome as site for accumulation of mutant CFTR in CF (Johnston et al., 1998). Several studies indicate that multiple proteins related to or directly involved in the UPS are recruited into aggregates of different polyglutamine proteins (Figure 2). They include molecular chaperones, the 14-3-3 protein, ubiquitin, enzymes involved in ubiquitin conjugation, and both regulatory and catalytic subunits of the proteasome (Chai et al., 1999; Cummings et al., 1998; Schmidt et al., 2002; Stenoien et al., 1999; Stenoien et al., 2002; Verhoef et al., 2002; Waelter et al., 2001). In addition, other proteins, e.g., αSYN (Waelter et al., 2001) and the transcriptional cofactor CREB binding protein (CBP) (Jiang et al., 2003), colocalize with the protein inclusions. It is possible that intranuclear protein inclusions represent a means for the cell to effectively sequester toxic misfolded proteins, akin to the concept previously mentioned regarding LBs in PD, and thereby shield organelles from damage. In this sense, the aggregates in polyglutamine diseases would be neuroprotective. Tentative support for this idea was obtained in a cell model of HD, where expression of a dominant-negative variant of the E2 Cdc34p decreased the formation of intranuclear inclusions but increased the likelihood of cell death (Saudou et al., 1998). In a similar vein, crossing SCA1 mice expressing mutant ataxin-1 into a mouse that lacks E6-AP (Ube3a) expression resulted in a reduced number of neurons exhibiting intranuclear aggregates (Cummings et al., 1999). Interestingly, the expression of the inactive E3 increased the number of the dying Purkinje cells. It is possible that its wild-type counterpart is somehow involved in the conjugation of the mutant proteins (although it has not been shown directly), and the lack of ubiquitination leads to increased concentration of the

soluble proteins, which is toxic. Ubiquitination in this case may accelerate precipitation and formation of inclusions which may be protective (see above for αSYN in PD).

The Interactions between the UPS and Mutant Polyglutamine Proteins

It has been reported that wild-type as well as mutated forms of different disease-related polyglutamine proteins interact directly with elements of the UPS. Thus, Huntingtin was found to be ubiquitinated and also to interact with E2-25 kDa (Kalchman et al., 1996). Similarly, Ataxin-7 was found to interact with the S4 subunit of the proteasome (Matilla et al., 2001). The biological significance of these interactions is not clear, as substrates of the UPS typically bind to E3s, and the proteasome interacts, prior to proteolysis, only with polyubiquitinated proteins or with proteins that shuttle polyubiquitinated substrates (except for one established case, that of ODC; see above). It is possible that these interactions, while they do not serve to target the substrates for ubiquitination/degradation, interfere with the function of the UPS once the mutated proteins are involved. One case, that of Ataxin-3, deserves special attention (Doss-Pepe et al., 2003). The protein is found in inclusions along with the proteasome. It interacts with the human homolog of Rad23, a cytosolic protein that binds polyubiquitinated proteins and is involved, most probably, in shuttling them to the proteasome. In addition, it shares regions of homology with the proteasome subunit S5a that can recognize polyubiquitinated proteins. It appears therefore that Ataxin-3 can be a proteasome-associated factor that may assist in shuttling/binding of polyubiquitinated proteins and targeting them to the proteasome.

What is the evidence that the UPS is functionally inhibited or overwhelmed in cells expressing expanded polyglutamine proteins? At the outset, it is important to stress that most studies in this area have been conducted in cultured cells where a truncated form of the mutant protein has been overexpressed. Both the level of expression and the length of the protein can have significant impact on how it affects the UPS, and therefore some caution is warranted when extending the findings to the human diseases. An elegant demonstration that an expanded polyglutamine protein can cause functional UPS inhibition was provided by Bence and colleagues (Bence et al., 2001). In a cell culture model, they overexpressed mutant Huntingtin and observed proteasomal inhibition accompanied by cell cycle arrest. Another study has also demonstrated that UPS inhibition is only revealed under conditions of increased stress in cells expressing mutant Huntingtin (Ding et al., 2002). Striatal neurons from R6/2 transgenic HD mice display increased autophagy when they are subjected to oxidative stress (Petersen et al., 2001). Neuronal autophagy has been found to occur following chronic, low-grade proteasomal inhibition in cultured neuroblastoma cells (Ding et al., 2003) and may reflect activation of the lysosomal system as cells try to protect themselves during stress (Larsen and Sulzer, 2002).

Why should the UPS be functionally inhibited in the presence of proteins with an expanded polyglutamine stretch? One simple explanation is that the mutant proteins, unmodifed or following ubiquitination, block the proteasome due to the presence of the expanded polyglutamine stretch and do not allow the entrance of the normal substrates of the proteasome into the complex (Goellner and Rechsteiner, 2003). In this context, it is notable that transglutaminases catalyze the formation of protease-resistant glutamyl-lysine crosslinks in polyglutamine proteins, making these proteins even less sensitive to the activity of the proteasome. Formation of such bonds in proteins have been shown to increase proteasomal inhibition, possibly by blocking the pore, thus enhancing toxicity in cell culture models of DRPLA (Igarashi et al., 1998) and SBMA (Mandrusiak et al., 2003). Administration of cystamine, a transglutaminase inhibitor, can reduce aggregate formation and death in cells expressing atrophin-1 (DRPLA model) (Igarashi et al., 1998) and in cells expressing mutant androgen receptor (SBMA model) (Mandrusiak et al., 2003). Similar effects have been observed using a synthetic fusion protein containing GFP and a CAG triplet repeat expansion (de Cristofaro et al., 1999). There are even reports claiming that cystamine can reduce transglutaminase activity in the brain, retard the development of a neurological phenotype, and prolong life span in the R6/2 transgenic mouse model of HD (Dedeoglu et al., 2002; Karpuj et al., 2002), suggesting that transglutaminase may play an important role in preventing the UPS from clearing polyglutamine expansion-containing proteins. Crossing R6/1 HD mice onto the background of transglutaminase knockout mouse also results in an improved neurological phenotype and survival, but there is a concomitant increase in the formation of intranuclear protein aggregates (Mastroberardino et al., 2002).

An alternative explanation is that the UPS is overwhelmed, with rates of formation/delivery of misfolded proteins exceeding the capacity of the UPS to tag, target, and proteolytically degrade them. It has been speculated that there may be a relative shortage of ubiquitin or related enzymes under these conditions. On the other hand, it can be argued that the presence of ubiquitin in the inclusion bodies indicates that the system does not falter at the level of ubiquitination. However, the presence of ubiquitin in the aggregates does not guarantee a functional ubiquitination process, since the polyubiquitin chains may not be sufficiently long, for example, to target the misfolded protein molecules to the proteasome. Also, some of the ubiquitin in the inclusions may be in its free form, and for ubiquitination to occur, the inclusions must contain all three conjugating enzymes and ATP. In favor of the concept of UPS overload are reports that demonstrate that protein inclusions from isolated neurons and brains of transgenic HD mice can recede within 1 week if the inducible transgene is turned off (Martin-Aparicio et al., 2001). Their disappearance is UPS dependent, since the proteasome inhibitor lactacystin prevents the process. In the transgenic mouse, turning off the transgene is also accompanied by a recovery of the behavioral disease phenotype (Yamamoto et al., 2000). Additional support for the UPS overload hypothesis comes from studies in R6/2 HD mice. At 9 weeks of age, these mice normally display widespread Huntingtin aggregates throughout the brain. If oligomerization of mutant Huntingtin is inhibited by administration of Congo red starting at this age, no aggregates can be detected in the brain 5 weeks later (Sanchez et al., 2003).

In a parallel experiment using a cell culture model, Congo red was found to increase proteasomal activity in cells expressing a polyglutamine protein but not in cells expressing a control protein (Sanchez et al., 2003). Somewhat contrary to these observations, Dantuma and coworkers have found that only soluble polyglutamine proteins are efficiently cleared by the proteasome from cultured HeLa cells, and once they are captured in aggregates, they become virtually resistant to proteolysis (Verhoef et al., 2002). Possibly, the type of cell model has some bearing on the results, and the issue of the activity of the UPS in cells expressing expanded polyglutamine proteins will require further investigation.

Could the functional status of the UPS differ between individuals due to genetic or epigenetic influences unrelated to the polyglutamine disease, and can this difference play a role in governing the age of disease onset? The CAG repeat length in the mutant gene accounts for ~70% of the variance of age of onset for HD (Becher et al., 1997), and individual differences in UPS activity could influence the time it takes for mutant proteins to accumulate in the patient's brain. Interestingly, Li and collaborators demonstrated recently that transgenic HD mice do not display reduced proteasomal activity in the brain but rather show a decline in enzyme activity with age (Zhou et al., 2003)—similar in both wild-type and transgenic mice of the same genetic background. Thus, the rate of age-related decline in UPS activity could define the efficacy with which the brain handles the mutant proteins and thereby influence the age of onset of symptoms in an individual patient.

There are several possible consequences of the UPS being inhibited in neurons as a result of expression of expanded polyglutamine proteins. With our expanding knowledge of the multiple roles of the UPS in a broad array of cellular functions, it will not be surprising to find that impairment of the UPS function can be devastating. However, it can be difficult to dissect out the effects of UPS inhibition from the direct effects of the aggregated proteins per se and from the toxic effects of oligomers of mutant polyglutamine proteins. One immediate consequence of inhibition of the UPS in cells expressing a mutant polyglutamine protein is obviously impairment in the clearing of misfolded proteins. Consequently, the formation of aggregates may increase when the UPS fails. Lunkes and colleagues demonstrated that truncated Huntingtin, which has undergone proteolytic cleavage in the cytoplasm, accumulates more rapidly if the proteasome is pharmacologically inhibited (Lunkes et al., 2002). This indicates that the truncated fragments are normally processed via the UPS, and therefore the disposal of fragments of mutant proteins will be impaired. A study in cultured mouse neuroblastoma cells showed that N-terminal mutant Huntingtin inhibited the 20S proteasome catalytic activity, in turn causing impaired proteasomal degradation of p53, subsequent loss of mitochondrial membrane potential, release of cytochrome c, caspase activation, and apoptosis (Jana et al., 2001) (Figure 2). The effects of proteasomal inhibition may not always be as drastic but could still have profound long-term effects on neuronal function and viability. Thus, as mentioned earlier, several transcription factors are either activated (NF-κB, for example) or downregulated (Myc, for example) by the UPS. Changes in UPS function may well contribute to the alterations in gene transcription in polyglutamine disease (Sugars and Rubinsztein, 2003). In conclusion, although the primary cause of the pathology in polyglutamine diseases is clearly defined by the mutation, the actual disease manifestations may also reflect malfunction of the UPS. Therefore, a better understanding of how the UPS interacts with proteins expressing expanded polyglutamine tracts can lead to the development of therapeutic approaches.

Concluding Remarks

As described in this review, rare cases of slowly progressing neurodegenerative diseases, notably PD and AD, exhibit aberrations in the UPS that probably play a primary and direct role in the disease pathogenesis. However, in many others, it appears that inhibition of the UPS by the aggregated disease proteins may lead to a secondary neuronal damage. In a third large group of neurodegenerative diseases, the linkage between UPS impairment and disease pathogenesis is even more remote and indirect, if it exists at all. This current state of knowledge makes it difficult to design mechanism-based therapeutic modalities based on the mode of action of the UPS. In general, it appears that activation of the UPS may have a beneficial effect in many of these disorders, yet, it appears that such an activation is hard to achieve. It is possible that once the mediators of general activation of the system, such as occurs in sepsis, muscle atrophy, and cancer-induced cachexia, are identified, their selective employment in neurodegenerative disorders should be examined. Curiously, the only drug in the market that targets the UPS and that is used to treat multiple myeloma is a potent proteasome inhibitor. From what we currently know, inhibition of the proteasome will, most probably, aggravate the pathology and consequently the symptoms of many of the known neurodegenerative disorders. The effect of "chemical/pharmacological chaperones" to dissolve the aggregated proteins cannot be predicted. While certain findings indicate that the soluble proteins are neurotoxic, alleviation of the inhibition of the UPS may have beneficial effects. Thus, such agents may have different effects in different diseases and therefore should be examined in a systematic manner. Interestingly, such agents are being examined in the treatment of cystic fibrosis, where the mutated yet active ΔF508 CFTR is rapidly degraded via the ERAD pathway. The undegraded protein, however, is accumulated in aggresomes. The hope is that some of the dissolved protein will evade ERAD and be expressed at the cell surface. It appears that a better mechanistic understanding of the role that the UPS plays in neurodegeneration is required before specific and mechanism-based, rather than general, modalities can be developed.

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