JCB Mini-Review

The ubiquitin proteasome system in synaptic and axonal degeneration: a new twist to an old cycle

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The ubiquitin proteasome system (UPS) contributes to the pathophysiology of neurodegenerative diseases, and it is also a major determinant of synaptic protein degradation and activity. Recent studies in rodents and in the fruit fly *Drosophila* have shown that the activity of the UPS is involved in axonal degeneration. Increased knowledge of the UPS in synaptic and axonal reactions may provide novel drug targets for treatments of neuronal injuries and neurodegenerative disorders.

Neurodegenerative diseases are characterized by the selective loss of neurons due to aggregation of different intra- or extracellular proteins (Bence et al., 2001). Apart from the deleterious effects of the protein deposits on the nerve cell soma, the axons and dendrites also degenerate in these diseases. The functional changes observed early in neurodegenerative disorders are reflected by alterations in synaptic dysfunctions and loss of connectivity. Recent evidence indicates that the degeneration of axons and synapses plays an important role both in chronic diseases and after injuries. The molecular mechanisms governing axonal degeneration and synaptic maintenance are not fully understood, but a crucial role has been ascribed to the activity of the UPS.

UPS and neurological disorders

Ubiquitin is a 76 aa–long protein that becomes attached to other proteins through a multi-enzyme system (Weissman, 2001; Adams, 2003). The first step is the activation of ubiquitin by an enzyme, E1, followed by transfer to an ubiquitin conjugating enzyme, E2, and to the ubiquitin ligase, E3 that covalently attaches the ubiquitin moiety to a lysine residue on target proteins (Fig. 1 A). There are several E2 and many E3 enzymes that are specific for different protein substrates that increase the diversity of this system. Reiteration of the cycle produces polyubiquitin chains on target proteins that are then degraded by the UPS to small peptides. At the heart of the UPS is the 26S proteasome that consists of a 20S core particle and two 19S regulatory particles (Fig. 1 A). Apart from ubiquitination, there are deubiquitination enzymes that replenish the cellular pool of ubiquitin and are important for the proper function of the proteasome. Modifications of protein substrates through attachment of a monoubiquitin or polyubiquitin chain are important in many cellular processes ranging form cell cycle control, DNA repair, transcription, cell signaling, and regulation of protein trafficking (Hicke, 2001; Weissman, 2001; Adams, 2003). In the nervous system, ubiquitination plays a role, among others, in neuronal signaling, synapse formation and function, and in different diseases (Hegde and DiAntonio, 2002; Ciechanover and Brundin, 2003).

It is becoming increasingly evident that altered activities of the UPS are crucially involved in the pathophysiology of Parkinson's disease (PD), Huntington's disease (HD), and in spinocerebellar ataxia (Bence et al., 2001; Lindsten et al., 2002; Ciechanover and Brundin, 2003). Mutations in *parkin*, encoding an ubiquitin-E3 ligase result in juvenile recessive PD (Dawson and Dawson, 2003). α-Synuclein, which is mutated in some familiar forms of PD, is highly enriched in presynaptic terminals and Lewy-bodies (Kaplan et al., 2003). Recently, the higher than normal level of wild-type α-synuclein was found in a family with early onset PD, and a contributing mechanism could be an insufficient clearance by the UPS (Eriksen et al., 2003; Singleton et al., 2003). Apart from PD, other neurodegenerative disorders also display accumulation of mutated proteins in inclusion bodies and aggregates in conjunction with ubiquitin (Lindsten et al., 2002; Ciechanover and Brundin, 2003). The presence of protein deposits has been amply demonstrated in neuronal cell bodies, but less is known about their occurrence in axons and synapses. Recent studies on HD and other PolyQ diseases show that the respective mutated proteins can interfere with axonal transport (Gunawardena et al., 2003; Szebenyi et al., 2003). The Huntingtin protein involved in HD also interacts with synaptic vesicles and proteins involved

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Key words: neurodegeneration; synapse function; Wallerian degeneration; polyubiquitination; monoubiquitination

Abbreviations used in this paper: HD, Huntington's disease; PD, Parkinson's disease; PSD, postsynaptic density; UPS, ubiquitin proteasome system.

The Journal of Cell Biology, Volume 165, Number 1, April 12, 2004 27–30
http://www.jcb.org/cgi/doi/10.1083/jcb.200311091

The Journal of Cell Biology

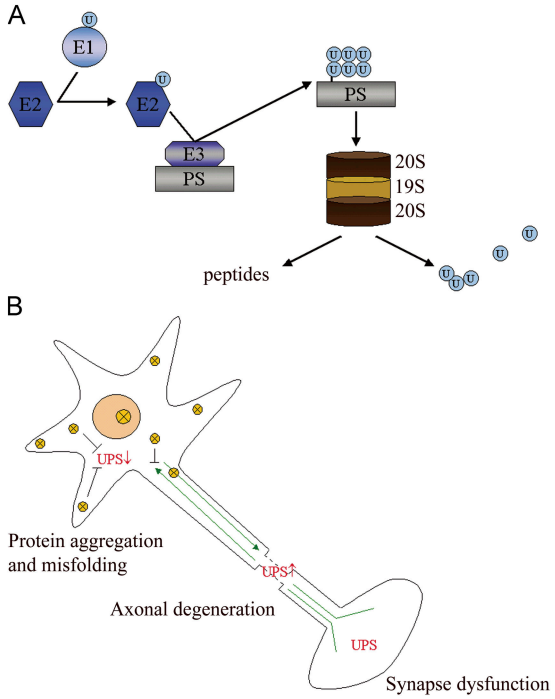

Figure 1. **The activity of the UPS and neuronal compartments.** (A) UPS, ubiquitin proteasome system. U, ubiquitin; E1, ubiquitin activating enzyme; E2, ubiquitin conjugating enzyme; E3, ubiquitin ligase; 20S, catalytic core; 19S, regulator particle; PS. Protein substrate. (B) Neurons consist of three major subcellular compartments functionally linked to each other: the cell body, axon, and nerve terminals. In neurodegenerative diseases, there is an accumulation of mutant or misfolded proteins (circles) due to insufficient clearance or the relative dysfunction of the UPS. The protein aggregates can further disrupt the UPS and affect the axonal transport and the synapses. Molecular insights into axonal reactions show an involvement of the UPS in axonal degeneration. In nerve endings, the UPS is crucial for protein turnover and synapse maintenance and function. The exact role of the UPS in synaptic dysfunction is not known, but disturbances in its activity may seriously affect protein trafficking and neuronal connectivity. Protein components of the UPS, including specific E2 and E3 molecules, and their corresponding protein targets may differ between compartments. This opens up the possibility for specific and local interference with the UPS activity under different conditions and in neurodegenerative diseases.

in neurotransmission (Song et al., 2003). In spinocerebellar ataxia 1, there is an altered trafficking of glutamate receptor subunits and PKCγ in Purkinje cells (Skinner et al., 2001). Given the role of the UPS in disease pathophysiology, it is important to study the key proteins in the axons and in the synapses that may be altered in the different disorders.

UPS and the function of synapses

Synapses undergo large structural changes during maturation and in response to neuronal activity. As shown by Ehlers (2003), the UPS regulates the degradation of molecules, belonging to the postsynaptic densities (PSDs) that contain various receptors and scaffolding proteins. Synaptic activity influences the ubiquitination and turnover of a subset of PSD proteins, important for the control of synapse function and maintenance (Ehlers, 2003). Results with *Aplysia* have shown that protein ubiquitination is important for synaptic plasticity (Hegde and DiAntonio, 2002). Likewise, the activity of the ubiquitin ligase, Ube3A, is crucial for long-term potentiation in mouse hippocampus, and the gene is mutated in Angelman's syndrome, a human disorder causing mental retardation (Miura et al., 2002). Together, these studies show that the UPS and protein modifications via ubiquitination play an important role in regulation of synaptic maintenance and function in different organisms.

However, the time window and specific proteins regulated by the UPS vary between different studies. In the work on the PSD proteins, a time scale of 24–48 h was used. In this time, the UPS acts on different targets, including ubiquitination of proteins controlling transcription or translation that may indirectly influence protein abundance in the synapse. In their study of the neuromuscular system in *Drosophila*, Speese et al. (2003) noted a relatively short time window for the degradation of synaptic proteins by the UPS. Using drugs to inhibit the activity, in addition to genetic manipulation of the proteasome, DUNC-13 was identified as a selective target for UPS in the presynaptic terminal. Electrophysiological recordings showed that proteasome inhibition also enhanced synaptic efficacy and presynaptic transmitter release. This data demonstrates that the activity of the UPS locally regulates the levels of DUNC-13 and influences presynaptic efficacy in *Drosophila*. The correlative changes observed in synaptic function and regulation of the DUNC-13 by the UPS also suggest that the levels of DUNC-13 may be the crucial mediator for increased neurotransmitter release. However, this functional link has so far not been directly shown neither are other protein excluded as targets for the UPS in this context.

Members of the UNC protein family are found in different species, and are involved in synaptic vesicle priming and regulation of neurotransmitter release. It would be important to study whether the mouse homologue MUNC-1, shown to be important for proper function of glutamatergic synaptic vesicles (Augustin et al., 1999), also undergoes UPS-mediated down-regulation. Mice carrying a gene deletion for Munc-18-1, exhibit loss of neurotransmitter secretion during development, without affecting the initial assembly of the synapse (Verhage et al., 2000). However, after birth there is a widespread neurodegeneration with the loss of synapses. The contribution of the UPS to this phenotype has yet not been studied.

Protein modification by ubiquitin at nerve endings may go beyond effects on protein degradation. Polyubiquitination targets proteins for degradation, but monoubiquitination regulates protein trafficking, involving endosomes, as well as other functions (Hicke, 2001). Receptor tyrosine kinases, such as the EGF receptor are monoubiquitinated upon ligand binding (Haglund et al., 2003; Mosesson et al., 2003). This leads to receptor tyrosine kinase internalization and degradation in lysosomes, preventing recycling to the plasma membrane. Monoubiquitination of receptor proteins can thus exert an important control step in the action of trophic factors. Although not yet studied in brain, this may also occur for neurotransmitter receptors, and other molecules at the synapse. It is crucial to study whether alterations in monoubiquitination per se can affect protein trafficking and synaptic connectivity. Wilson et al. (2002) reported recently that mutation in Usp14, encoding an ubiquitin-spe-

cific protease, causes synaptic dysfunction leading to ataxia in mice.

In recent years, novel proteins have been discovered, such as the ubiquitin-like proteins and those carrying ubiquitin interacting domains that influence the efficacy of the UPS (Weissman, 2001; Adams, 2003). So far little is known about these proteins in the nervous system or locally in the function of the synapse.

Axonal degeneration and the UPS

After transection, the distal segment of the nerve normally undergoes a degeneration process with typical morphological signs, called Wallerian degeneration (Coleman and Perry, 2002; Raff et al., 2002). This type of degeneration occurs also in many neuropathies and neurodegenerative disorders and is distinct from the death of the nerve cell body. Studies of the mouse mutant, *Wld*^{s} that displays a significantly slower Wallerian reaction, demonstrate that axonal degeneration is an active process (Coleman et al., 1998; Mack et al., 2001). In this mouse, gene rearrangements have resulted in the production of an 85-kD chimeric protein consisting of nicotinamide mononucleotide adenylyl transferase and the amino-terminal portion of the ubiquitination factor E4b (Mack et al., 2001). Overexpression of the fusion protein, using the β-actin promoter or viral vectors, can delay axonal degeneration (Coleman and Perry, 2002). The data with the *Wld*^{s} mice suggests an involvement of the UPS in Wallerian degeneration, but there are some caveats. Thus, the cellular targets of the *Wld*^{s} chimeric gene in axonal degeneration are not known. It has also to be shown that the fusion protein with the truncated region of the E4 enzyme can function in polyubiquitination and proteasome-mediated degradation of proteins.

Supporting evidence for the involvement of the UPS in axonal degeneration comes from studies of the Gracile axonal dystrophy (*Gad*) mutant mice. In these mice, there is an inactivation of the ubiquitin carboxy-terminal hydrolase, UCH-L1, causing, among others, degeneration of the gracile tract of the spinal cord (Saigoh et al., 1999). UCH-1 displays dual activities as a deubiquitination enzyme and as an ubiquitin ligase (Liu et al., 2002). The exact roles of UCH-1 and the corresponding protein substrates in the *Gad* mice are so far not known.

Recent studies in rats using transection of cultured neurons or crush lesions of the optic nerve in vivo showed that inhibition of the UPS by drugs targeting the proteasome can retard the onset of Wallerian degeneration (Zhai et al., 2003). Similar results were obtained by viral expression of an ubiquitin protease that can reverse ubiquitination of protein substrates. Using specific antibodies for axonal components, the fragmentation of microtubuli was identified as an early sign of the axonal degeneration that was also sensitive to proteasome inhibition (Zhai et al., 2003). These results lend credence to the view that the local activity of UPS, acting on selective protein targets, is important during axonal degeneration.

Further evidence comes from studies in *Drosophila*, showing that the degeneration of axons occurring during pruning requires the UPS (Watts et al., 2003). Axon pruning is an important process for the refinement of the neuronal connections in both vertebrates and invertebrates. Studying development of particular projecting neurons of the *Drosophila* mushroom bodies it was shown that overexpression of an ubiquitin protease or a mutant form of the *Drosophila* E1 ubiquitin-activating enzyme, inhibited pruning (Watts et al., 2003). Likewise, mutations in two of the subunits in the 19S regulatory particle (Fig. 1 A) also impaired pruning. This genetic evidence strongly suggests that local degeneration of protein via the UPS activity is a necessary requirement for axon pruning during development of the mushroom bodies. The relevance of these findings for Wallerian degeneration and axonal reactions in neurodegenerative diseases is not straightforward. However, there are similarities between these processes both with regard to molecular mechanisms and the cellular reactions involved. First, studying different fly mutants, it was shown that axon pruning is independent of the activity of *grim*, *hid*, and *rpr* that regulate cell death in *Drosophila*. This is in keeping with results obtained in rodents in the *Wld*^{s} mice (Finn et al., 2000; Raff et al., 2002), and shows that the programs governing axonal degeneration and death of the nerve cell body via apoptosis are inherently different. Second, local UPS activity seems instrumental for both pruning and Wallerian degeneration to occur. Third, looking at the cellular changes, the disruption of the microtubuli was identified as an early step in both pruning and Wallerian degeneration. Thus, it is tempting to suggest that the two processes may be governed by similar mechanisms involving local regulation of axonal proteins via the UPS.

The results on inhibition of the UPS in axons, also suggest therapeutic targets to preserve axons after injury and in degeneration. The caveat is that a general inhibition of proteasome activity may be harmful to the cell. To arrive at a specific inhibition of the UPS, the exact nature of the different molecules involved in ubiquitination and degradation of protein targets in the axons needs to be studied in more detail. The first attempt toward this was made by Watts et al. (2003) in *Drosophila*. However, of the over a dozen of E2 and E3 enzymes studied, none was found important for axon pruning. This does not come as a surprise in view of the fact that in *Drosophila*, as in mammalian cells the diversity of these enzymes and their corresponding protein substrates is large. However, the use of modern large-scale techniques for proteome analysis may provide new insights into these important questions.

Concluding remarks

Synaptic dysfunction and decreased connectivity herald many of the neurodegenerative diseases. The UPS play an important role in synaptic function and may contribute to the disease-induced changes. The activity of the UPS is involved in local axonal degeneration after nerve injury. The mechanisms controlling axonal degeneration are different from those regulating death of the nerve cell body. One could then envision a two-stage approach in neurodegeneration with treatments of the axon and the soma separately. However, with regard to such therapies and the effects of reducing UPS activity, there is a *Scylla and Charybis* situation for these two compartments. Inhibition of local UPS activity is beneficial for retarding axonal degeneration. However, a drawback is that there is already a relative inhibition of the

UPS in the neurological disorders, caused by protein aggregates and toxic products in the cell (Fig. 1 B). A further inhibition of the UPS may thus aggravate the underlying disease. Although one could foresee a local delivery of UPS inhibitors, this may prove cumbersome. Many of the compounds used to date are also rather toxic and show unspecific effects. To circumvent these problems, we need to know more about the occurrence and the nature of molecules, including specific E2 and E3 enzymes and the corresponding protein substrates in the neuron. Such information may allow designing novel drug targets to influence the UPS separately in the axon and the nerve cell body in different diseases. Given the current interest in the UPS, increased knowledge on these matters is likely to occur rapidly. The door to new discoveries in this field is now more than ajar.

L. Korhonen received a grant from Swedish Brain Foundation. D. Lindholm was supported by Uppsala University and Swedish Cancer Foundation.

Submitted: 18 November 2003
Accepted: 26 February 2004

References

Adams, J. 2003. The proteasome: structure, function, and role in the cell. *Cancer Treat. Rev.* 29(Suppl. 1):3–9.

Augustin, I., C. Rosenmund, T.C. Sudhof, and N. Brose. 1999. Munc13-1 is essential for fusion competence of glutamatergic synaptic vesicles. *Nature.* 400: 457–461.

Bence, N.F., R.M. Sampat, and R.R. Kopito. 2001. Impairment of the ubiquitin-proteasome system by protein aggregation. *Science.* 292:1552–1555.

Coleman, M.P., and V.H. Perry. 2002. Axon pathology in neurological disease: a neglected therapeutic target. *Trends Neurosci.* 25:532–537.

Coleman, M.P., L. Conforti, E.A. Buckmaster, A. Tarlton, R.M. Ewing, M.C. Brown, M.F. Lyon, and V.H. Perry. 1998. An 85-kb tandem triplication in the slow Wallerian degeneration (*Wlds*) mouse. *Proc. Natl. Acad. Sci. USA.* 95:9985–9990.

Ciechanover, A., and P. Brundin. 2003. The ubiquitin proteasome system in neurodegenerative diseases. Sometimes the chicken, sometimes the egg. *Neuron.* 40:427–446.

Dawson, T.M., and V. Dawson. 2003. Molecular pathways of neurodegeneration in Parkinson's disease. *Science.* 302:819–822.

Ehlers, M.D. 2003. Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system. *Nat. Neurosci.* 6:231–242.

Eriksen, J.L., T.M. Dawson, D.W. Dickson, and L. Petrucelli. 2003. Caught in the act: α-synuclein is the culprit in Parkinson's disease. *Neuron.* 40:453–456.

Finn, J.T., M. Weil, F. Archer, R. Siman, A. Srinivasan, and M.C. Raff. 2000. Evidence that Wallerian degeneration and localized axon degeneration induced by local neurotrophin deprivation do not involve caspases. *J. Neurosci.* 20: 1333–1341.

Gunawardena, S., L.S. Her, R.G. Brusch, R.A. Laymon, I.R. Niesman, B. Gordesky-Gold, L. Sintasath, N.M. Bonini, and L.S. Goldstein. 2003. Disruption of axonal transport by loss of huntingtin or expression of pathogenic polyQ proteins in *Drosophila*. *Neuron.* 40:25–40.

Haglund, K., S. Sigismund, S. Polo, I. Szymkiewicz, P.P. Di Fiore, and I. Dikic. 2003. Multiple monoubiquitination of RTKs is sufficient for their endocytosis and degradation. *Nat. Cell Biol.* 5:461–466.

Hegde, A.N., and A. DiAntonio. 2002. Ubiquitin and the synapse. *Nat. Rev. Neurosci.* 3:854–861.

Hicke, L. 2001. Protein regulation by monoubiquitin. *Nat. Rev. Mol. Cell Biol.* 2:195–201.

Kaplan, B., V. Ratner, and E. Haas. 2003. Alpha-synuclein: its biological function and role in neurodegenerative diseases. *J. Mol. Neurosci.* 20:83–92.

Lindsten, K., F.M. de Vrij, L.G. Verhoef, D.F. Fischer, F. W. van Leeuwen, E.M. Hol, M.G. Masucci, and N.P. Dantuma. 2002. Mutant ubiquitin found in neurodegenerative disorders is a ubiquitin fusion degradation substrate that blocks proteasomal degradation. *J. Cell Biol.* 157:417–427.

Liu, Y., L. Fallon, H.A. Lashuel, Z. Liu, and P.T. Lansbury, Jr. 2002. The UCH-L1 gene encodes two opposing enzymatic activities that affect α-synuclein degradation and Parkinson's disease susceptibility. *Cell.* 111:209–218.

Mack, T.G., M. Reiner, B. Beirowski, W. Mi, M. Emanuelli, D. Wagner, D. Thomson, T. Gillingwater, F. Court, L. Conforti, et al. 2001. Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/Nmnat chimeric gene. *Nat. Neurosci.* 4:1199–1206.

Mosesson, Y., K. Shtiegman, M. Katz, Y. Zwang, G. Vereb, J. Szollosi, and Y. Yarden. 2003. Endocytosis of receptor tyrosine kinases is driven by monoubiquitylation, not polyubiquitylation. *J. Biol. Chem.* 278:21323–21326.

Miura, K., T. Kishino, E. Li, H. Webber, P. Dikkes, G.L. Holmes, and J. Wagstaff. 2002. Neurobehavioral and electroencephalographic abnormalities in Ube3a maternal-deficient mice. *Neurobiol. Dis.* 9:149–159.

Raff, M.C., A.V. Whitmore, and J.T. Finn. 2002. Axonal self-destruction and neurodegeneration. *Science.* 296:868–871.

Saigoh, K., Y.L. Wang, J.G. Suh, T. Yamanishi, Y. Sakai, H. Kiyosawa, T. Harada, N. Ichihara, S. Wakana, T. Kikuchi, and K. Wada. 1999. Intragenic deletion in the gene encoding ubiquitin carboxy-terminal hydrolase in gad mice. *Nat. Genet.* 23:47–51.

Singleton, A.B., M. Farrer, J. Johnson, A. Singleton, S. Hague, J. Kachergus, M. Hulihan, T. Peuralinna, A. Dutra, R. Nussbaum, et al. 2003. α-Synuclein locus triplication causes Parkinson's disease. *Science*. 302:841.

Skinner, P.J., C.A. Vierra-Green, H.B. Clark, H.Y. Zoghbi, and H.T. Orr. 2001. Altered trafficking of membrane proteins in purkinje cells of SCA1 transgenic mice. *Am. J. Pathol.* 159:905–913.

Song, C., Y. Zhang, C.G. Parsons, and Y.F. Liu. 2003. Expression of polyglutamine-expanded huntingtin induces tyrosine phosphorylation of N-methyl-D-aspartate receptors. *J. Biol. Chem.* 278:33364–33369.

Speese, S.D., N. Trotta, C.K. Rodesch, B. Aravamudan, and K. Broadie. 2003. The ubiquitin proteasome system acutely regulates presynaptic protein turnover and synaptic efficacy. *Curr. Biol.* 13:899–910.

Szebenyi, G., G.A. Morfini, A. Babcock, M. Gould, K. Selkoe, D.L. Stenoien, M. Young, P.W. Faber, M.E. MacDonald, M.J. McPhaul, and S.T. Brady. 2003. Neuropathogenic forms of huntingtin and androgen receptor inhibit fast axonal transport. *Neuron.* 40:41–52.

Verhage, M., A.S. Maia, J.J. Plomp, A.B. Brussaard, J.H. Heeroma, H. Vermeer, R.F. Toonen, R.E. Hammer, T. K. van den Berg, M. Missler, et al. 2000. Synaptic assembly of the brain in the absence of neurotransmitter secretion. *Science.* 287:864–869.

Watts, R.J., E.D. Hoopfer, and L. Luo. 2003. Axon pruning during *Drosophila* metamorphosis. Evidence for local degeneration and requirement of the ubiquitin-proteasome system. *Neuron.* 38:871–885.

Weissman, A.M. 2001. Themes and variations on ubiquitylation. *Nat. Rev. Mol. Cell Biol.* 2:169–178.

Wilson, S.M., B. Bhattacharyya, R.A. Rachel, V. Coppola, L. Tessarollo, D.B. Householder, C.F. Fletcher, R.J. Miller, N.G. Copeland, and N.A. Jenkins. 2002. Synaptic defects in ataxia mice result from a mutation in Usp14, encoding a ubiquitin-specific protease. *Nat. Genet.* 32:420–425.

Zhai, Q., J. Wang, A. Kim, Q. Liu, R. Watts, E. Hoopfer, T. Mitchison, L. Luo, and Z. He. 2003. Involvement of the ubiquitin-proteasome system in the early stages of Wallerian degeneration. *Neuron.* 39:217–225.