

Huntingtin processing in pathogenesis of Huntington disease¹

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ABSTRACT

Huntington's disease (HD) is caused by an expansion of the polyglutamine tract in the protein named huntingtin. The expansion of polyglutamine tract induces selective degeneration of striatal projection neurons and cortical pyramidal neurons. The bio-hallmark of HD is the formation of intranuclear inclusions and cytoplasmic aggregates in association with other cellular proteins in vulnerable neurons. Accumulation of N-terminal mutant huntingtin in HD brains is prominent. These pathological features are related to protein misfolding and impairments in protein processing and degradation in neurons. This review focused on the role of proteases in huntingtin cleavage and degradation and the contribution of altered processing of mutant huntingtin to HD pathogenesis.

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant neurological disease characterized by involuntary movement accompanied by cognitive impairment and emotional disturbance. The most striking pathologic feature of HD is atrophy, neuronal loss, and astrogliosis in the neostriatum^[1]. Although multiple populations of striatal neurons are affected in HD, however, the spiny projection neurons containing γ -aminobutyric acid and substance P or enkephalin are most vulnerable. Other less severely affected brain regions include cerebral cortex and thalamic nuclei. HD is a fatal neurological disease with no effective treatment available at the present. HD affects 5 out of 100 000 people and symptoms usually occur at the age of late 40s.

The gene mutation that causes HD has been linked to an unstable expansion of CAG repeats in a gene encoding a large protein called huntingtin (huntingtin)^[2]. Both wild-type and mutant huntingtins are widely expressed in the central nervous system and peripheral tissues^[3,4]. The normal function of huntingtin is poorly understood at the present but has been proposed to play roles in neurogenesis, apoptosis, and vesicle trafficking^[5,6]. However, how expansion of polyglutamine repeat in the N-terminal region of huntingtin causes selective degeneration of neurons in the brain remains unknown although several mechanisms have been proposed^[7]. Like other neurological diseases including Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis, and prion disease, HD is also a protein misfolding disease. The bio-hallmark of mutant huntingtin is the formation of intranuclear inclusions and cytoplasmic aggregates in neurons in vulnerable brain areas. Expression of mutant huntingtin in cultured cells also causes the formation of intranuclear inclusions and aggregates in the cytoplasm. The inclusions and aggregates are usually formed by small N-

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terminal huntingtin fragments and are co-localized with other cellular proteins involved in proteolysis, vesicle trafficking and protein degradation. The formation of huntingtin aggregates and intranuclear inclusions has been proposed to play a role in HD pathogenesis.

Polyglutamine expansion causes at least nine different neurodegenerative diseases. The common feature among these neurological diseases is the formation of abnormal aggregates in different populations of neurons. The increase in the length of polyglutamine tract alters biochemical and biophysical properties of proteins. As a result, these proteins are prone to form stable β -sheet structure and assemble into oligomers. Accumulation and aggregation of mutant polyglutamine-containing proteins indicate a failure of normal processing of these proteins in cells^[8,9]. Thus study of the normal and abnormal trafficking and processing of huntingtin by proteases will provide important information for a better understanding of the mechanisms of pathogenesis in HD. Trafficking and processing of full-length and truncated wild-type and mutant huntingtin remain to be characterized. Expressed huntingtin preferentially accumulates in perinuclear regions and are associated with membranous structures where cleavage may occur. Proteasome, autophagy/lysosome, caspases and calpains are found to be involved in cleavage and degradation of huntingtin, thus playing important roles in huntingtin processing. However, proteasome function is compromised in HD brains, while caspase- and calpain-mediated cleavage of huntingtin produces harmful N-terminal huntingtin fragments, which increased in the HD brain. The present review will discuss the relationships between huntingtin cleavage, catabolism and HD pathogenesis.

CLEAVAGE OF HUNTINGTIN BY CASPASES AND CALPAINS

Caspases play pivotal roles in apoptosis in many cell types^[10-12]. Caspases are produced as inactive pro-enzymes and become activated by proteolytic cleavage at internal aspartate residues upon apoptotic stimulation^[13]. Different caspases are activated at different stages during apoptotic process and play different roles in cell death. Some of them, including caspase-8, -9, and -10, are involved in the initiation phase of apoptosis; while others, including caspase-3, -6, and -7 are involved in the execution phase of apoptosis^[14]. When caspase-8 and -9 are activated, they can activate down-

stream caspases such as caspase-3, -6, and -7^[15]. Two signaling pathways involved in activation of initiator caspase have been well documented in response to apoptotic triggers. In death receptors, such as Fas or tumor necrosis factor receptor (TNFR)-induced apoptosis, caspase-8 is recruited by the adaptor molecules (such as FADD) and activated when these receptors bind to Fas ligands or TNF^[16]. Some other apoptotic stimuli cause release of cytochrome c from mitochondria. Cytochrome c binds to Apaf-1 in the cytoplasm and this leads to the activation of caspase-9^[17].

A large body of evidence suggests that caspases are involved in pathogenesis in HD. This notion is supported by a few lines of evidence:

(a) Several caspase cleavage sites (caspase-3, -2, and -6) have been identified in huntingtin^[18,19];

(b) Recent studies have suggested that CAG-repeat containing proteins including mutant huntingtin can directly activate caspase-8 presumably through protein-protein interactions^[20]. Activation of caspase-8 by CAG-repeat proteins may represent a novel pathway in activation of initiator caspase. Increases in activation of caspase-3 and -9 by mutant huntingtin have also been reported although underlying mechanisms are not fully understood^[21].

(c) Caspase inhibitors attenuate cell death induced by mutant huntingtin in cultured cells^[22,23]. Furthermore, caspase inhibitors Z-VAD-fmk and minocycline can slow down the progression of HD in transgenic mice^[24,25].

Cleavage of huntingtin by caspase-3 was first confirmed by Dr Wellington's group. Using site directed mutagenesis, they identified four caspase-3 cleavage sites near N-terminal region (amino acid residue 513, 552, 530, and 589)^[19]. Cleavage of huntingtin by caspase-3 produces N-terminal fragments containing polyglutamine tract. Intranuclear inclusions and cytoplasmic huntingtin aggregates found in cultured cells expressing mutant huntingtin and brains of HD patients and transgenic mice are formed mainly by N-terminal fragments of huntingtin. Caspase cleavage of huntingtin may promote the formation of intranuclear inclusions due to increases in concentration of N-terminal huntingtin fragments^[26]. Cleavage of huntingtin by caspases has been confirmed in HD brains and is preceded by the neurodegeneration using a specific antibody against caspase-3-cleaved huntingtin fragments^[27]. Cleavage of huntingtin by caspases results in the production and accumulation of small N-terminal huntingtin. N-terminal huntingtin fragments are prone to form in-

tranuclear inclusions and cytoplasmic aggregates and induce apoptosis. Thus N-terminal huntingtin plays a role in the pathogenesis of HD. It has been demonstrated that inhibition of caspase activity reduced the formation of intranuclear inclusions and attenuated cellular toxicity of mutant huntingtin^[27]. It is still not clear whether the formation of intranuclear inclusions is a necessary step in mutant huntingtin-induced neurodegeneration, but cleaved N-terminal fragments from huntingtin seem to be toxic to neurons^[28-30]. Recent studies report that other caspases, including caspase-2 and -6 also cleave huntingtin and play important roles in HD pathogenesis^[31].

Calpains are activated by the rise in intracellular calcium levels and play a role in apoptosis. In 3-nitropropionic acid-induced animal models of HD, activation of calpains were found to contribute to degeneration of striatal neurons^[32]. Calpain mediated cleavage of wild-type and mutant huntingtin was also reported. Exposure of primary neurons to glutamate or 3-nitropropionic acid causes degradation of wild-type huntingtin. This process can be blocked by calcium chelators and calpain inhibitors^[33]. Focal ischemia insult also causes reduction in the levels of full-length wild-type huntingtin, leading to an increase in its cleavage products with a molecular mass about 55 kDa^[34]. Dr Gafni and Ellerby^[35] found that calpain cleavage of huntingtin produced two N-terminal huntingtin fragments, 72 kDa and 47 kDa, which are smaller than or close to those produced by caspase-mediated cleavage. Mutant huntingtin is more easily cleaved by the calpain. These fragments easily enter the nucleus to form intranuclear inclusions^[34-36]. Two putative calpain cleavage sites have been identified between amino acid residues 535-537 and 468-470. Calpain-mediated cleavage of huntingtin mainly occurs in dying cells, suggesting the pathological processing of huntingtin in dying cells. Blockade of calpain-mediated cleavage of huntingtin reduces huntingtin aggregates and toxicity^[37]. Activation of calpain and calpain cleavage products in HD brains has also been observed^[35]. These results suggest calpain cleavage of huntingtin plays a role in HD pathogenesis.

DEGRADATION OF HUNTINGTIN BY PROTEASOME

Proteasome is an important protease complex involved in degradation of misfolded proteins and proteins involved in signaling pathways^[38,39]. Proteasome

is comprised of multiple protein subunits. About 40 peptides with molecular weight ranging from 20-110 kDa make up two different proteasomes (20S and 26S proteasomes) with similar protease activity. The 20S proteasome (700-kDa) is a bell-shaped or soccer ball-like protein with catalytic activity. A PA700 and a PA28 modulatory subunits are attached at the both ends. An additional 19S modulatory subunit is present in 26S proteasome. Proteasomal degradation of proteins requires ubiquitination of proteins. Ubiquitin is a protein that consists of 76 amino acids. Polyubiquitin is added to the residue of lysine through enzymatic reactions. Ubiquitinated proteins then are degraded to amino acids by proteasome. In addition to degradation of proteins, proteasome also plays a role in intracellular signaling, such as activation of NF- κ B, caspase-3, and regulation of cell death and proliferation.

Several studies reported degradation of huntingtin by proteasome. Dr Hayden's group^[40] found that huntingtin interacted with a human ubiquitin-conjugating enzyme (hE2-25K). This enzyme mediates ubiquitination of target proteins. Ubiquitination of proteins leads to degradation by 26S proteasome. The Huntingtin is ubiquitinated in a polyglutamine length independent manner by E2-25K^[40]. Expression of truncated huntingtin forms SDS-resistant aggregates in the perinuclear regions at or close to centrosome, a cellular structure that has been proposed as a place for degradation of misfolded proteins by proteasome. The formation of huntingtin aggregates is significantly increased in the presence of proteasome inhibitor lactacystin^[41]. These studies support a role of proteasome in the degradation of huntingtin. Proteasome degradation of huntingtin plays a role in modulating the intracellular levels of huntingtin fragments. This mechanism helps to prevent an accumulation of N-terminal huntingtin to toxic levels. In cells expressing exon 1 huntingtin with expanded polyglutamine, subunits of 26S proteasome including 20S, 19S, and 11S were found to be sequestered into huntingtin aggregates. Some molecular chaperones were also found co-localized with huntingtin aggregates^[41]. These findings suggest that proteasome function could be compromised by mutant huntingtin. Recent studies revealed more direct evidence showing that aggregated proteins including mutant huntingtin inhibited proteasome functions. In transgenic HD mice, the activity of proteasome decreases with age and this is accompanied by an increase in accumulation of N-terminal huntingtin fragments. The relationship be-

tween decreases in proteasome activity and accumulation of N-terminal fragments has been established by inhibiting proteasome function as the later significantly increases the levels of huntingtin fragments and huntingtin aggregates in young animals^[42]. These results could explain why mutant huntingtin, but not wild-type huntingtin accumulates in the brain. Furthermore, expression of certain molecular chaperones such as HSP70, HSP40 inhibits the formation of huntingtin aggregation and increased clearance of mutant huntingtin, thus reducing the toxicity of mutant huntingtin^[43].

DEGRADATION OF HUNTINGTIN BY AUTOPHAGY/LYSOSOME

Autophagy, a process involved in bulk cellular degradation, is first found in yeast and plant cells in response to nutrition restriction. It is formed by the expansion and fusion of a double membrane structure originating from ER or other undefined organelles. The double membrane structure elongates and closes to form a vacuole called autophagosomes. Autophagosomes then fuse with lysosomes and become a single membrane bounded autophagic vacuoles^[44]. The formation of autophagy involves membrane expansion and fusion, targeting of cytoplasmic proteins to vacuoles and thus require complex protein-protein interactions^[45]. Many regulatory proteins, such as APG, AUT, and CTV have been identified in *Saccharomyces cerevisiae*^[46]. Autophagy is also involved in apoptosis as an alternative mechanism for active cell death^[47]. Autophagy may also be involved in neurodegenerative diseases. Recent studies reported increased autophagy in Alzheimer disease, Parkinson disease, and prion disease^[48-50]. Previous studies from the laboratories of Dr DiFIGLIA and others have found increases in lysosomal/endosomal staining and multivesicular bodies in HD brains^[4]. *In vitro* expression of full length and large N-terminal huntingtin fragments induces the formation of huntingtin-positive autophagic vacuoles^[51]. These studies suggest an autophagic mechanism is activated by huntingtin. Also, increased autophagy has been found in striatal cells derived from HD transgenic mice in response to oxidative stress and may play a role in degeneration of striatal neurons^[52].

Degradation of truncated huntingtin by an autophagic mechanism was reported by Dr Ravikumar^[53]. They found that inhibiting autophagy with 3-methyladenine increased accumulation of mutant huntingtin and huntingtin aggregates, while stimulating autophagy with

rapamycin reduced both huntingtin accumulation and huntingtin aggregates. Our recent studies found that autophagy was involved in activation of cathepsins and caspase-3 induced by overexpression of huntingtin^[54]. Autophagy may stimulate huntingtin cleavage and degradation through activation of caspase-3 and cathepsin D. The autophagic mechanism may also contribute to the formation of huntingtin bodies, a unique structure participating different molecular forms of huntingtin in the shell and the core^[55]. Thus an autophagic mechanism may play different roles in HD pathogenesis at early and later stages of HD. At early stages of HD, stimulation of autophagy could enhance clearance of huntingtin fragments thus preventing accumulation of N-terminal huntingtin fragments. At later stages of HD, overstimulation of autophagy could be detrimental to cells since autophagy (if macroautophagy) could damage cellular organelles. The linkage of autophagy and HD pathogenesis could be related to increased cleavage of huntingtin and alterations in mitochondria localization and function, activation of caspase-3 and possible programmed cells death.

ALTERED PROCESSING OF MUTANT HUNTINGTIN AND HUNTINGTON DISEASE

Protein misfolding may be a common molecular mechanism in neural injury and death in several neurodegenerative diseases. Protein misfolding and abnormal deposition intracellularly or extracellularly causes neuronal dysfunction and death. In HD, loss of normal functions of huntingtin contributes to HD pathogenesis^[56]. Accumulation of N-terminal mutant huntingtin fragment in vulnerable neurons plays an important role in neurodegeneration. Accumulation of mutant huntingtin fragments could result from altered processing and/or insufficient degradation of huntingtin. Expansion of polyglutamine tract in huntingtin activates caspases and calpains and increases caspase and calpain mediated cleavage of huntingtin. Cleavage of huntingtin by caspases and calpains produces N-terminal fragment containing polyglutamine tract. It has also been found that expansion of the length of polyglutamine repeat in huntingtin inhibited proteasome function and rendered resistance to degradation by lysosomal protease cathepsin D. Combined effects of these would increase levels of N-terminal huntingtin fragments. N-terminal fragments, particularly with expanded polyglutamine tract are prone to form dimmers and oligomers. The later assemble into a structure named protofibril. Protofibrils are interme-

diate products of stable fibril proteins, which are main components of insoluble aggregates and inclusions in the nucleus in HD. Protofibrils actively interact with cytoplasmic proteins and are toxic to cells^[57]. N-terminal huntingtin fragments can get into the nucleus and interfere with transcriptional regulation. They are also toxic to cells and can induce cell death by apoptosis. Thus cleavage of mutant huntingtin by caspases and calpains and compromised proteolysis play important roles in HD pathogenesis. This notion is now supported by a few lines of evidence including alleviation of HD pathology and symptoms of HD by inhibitors of caspases and calpains, by molecular chaperones, and increased proteolysis^[24, 58, 59].

SUMMARY

In HD, N-terminal fragments of mutant huntingtin accumulate in neurons and then form detergent insoluble aggregates and inclusions. As revealed in cellular models, these could result from enhanced cleavage by caspases and calpains and/or a failure of proteasome function. Increased cleavage of huntingtin by caspases and calpains produces more N-terminal fragments. These fragments are prone to form stable oligomers and protein fibrils if proteasome is unable to remove them efficiently. Autophagy may be activated when huntingtin accumulates to high levels. Autophagy, on the one hand, helps in degrading huntingtin to reduce accumulation of mutant huntingtin. On the other hand, based on our observation, autophagic activity also promotes caspase-mediated cleavage of huntingtin. Moreover, overactivity of autophagy may cause damage to cellular organelles. Thus, autophagy degradation of mutant huntingtin may eventually result in cell dysfunction. Preserving and boosting the capacity of cellular proteolytic function may be one of the useful strategies in delaying onset and retarding progression of HD.

REFERENCES

- Ferrante RJ, Kowall NW, Beal MF, Richardson EP Jr, Bird ED, Martin JB. Selective sparing of a class of striatal neurons in Huntington's disease. *Science* 1985; 230: 561-3.
- Gusella JF, MacDonald ME. Huntington's disease. *Semin Cell Biol* 1995; 6: 21-8.
- Bhide PG, Day M, Sapp E, Schwarz C, Sheth A, Kim J, *et al*. Expression of normal and mutant huntingtin in the developing brain. *J Neurosci* 1996; 16: 5523-35.
- Sapp E, Schwarz C, Chase K, Bhide PG, Young AB, Penney J, *et al*. Huntingtin localization in brains of normal and Huntington's disease patients. *Ann Neurol* 1997; 42: 604-12.
- DiFiglia M, Sapp E, Chase K, Schwarz C, Meloni A, Young C, *et al*. Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain neurons. *Neuron* 1995; 14: 1075-81.
- White JK, Auerbach W, Duyao MP, Vonsattel JP, Gusella JF, Joyner AL, *et al*. Huntingtin is required for neurogenesis and is not impaired by the Huntington's disease CAG expansion. *Nat Genet* 1997; 17: 404-10.
- Aronin N, Kim M, Laforet G, DiFiglia M. Are there multiple pathways in the pathogenesis of Huntington's disease. *Phil Trans R Soc Lond* 1999; 354: 995-1003.
- Waelter S, Boeddrich A, Lurz R, Scherzinger E, Lueder G, Lehrach H, *et al*. Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation. *Mol Biol Cell* 2001; 12: 1393-407.
- Chen S, Bertheliev V, Hamilton JB, O'Nuallain B, Wetzel R. Amyloid-like features of polyglutamine aggregates and their assembly kinetics. *Biochemistry* 2002; 41: 7391-9.
- Alnemri ES. Mammalian cell death proteases: a family of highly conserved aspartate specific cysteine proteases. *J Cell Biochem* 1997; 64: 33-42.
- Green D, Kroemer G. The central executioners of apoptosis: caspases or mitochondria? *Trends Cell Biol* 1998; 8: 267-71.
- Thornberry NA. The family of cysteine proteases. *Br Med Bull* 1996; 53: 478-90.
- Salvesen GS, Dixit VM. Caspases: intracellular signaling by proteolysis. *Cell* 1997; 91: 442-6.
- Thornberry NA, Lazebnik Y. Caspases: enemies within. *Science* 1998; 281: 312-6.
- Zhuang S, Lynch MC, Kochevar IE. Caspase-8 mediates caspase-3 activation and cytochrome c release during singlet oxygen-induced apoptosis of HL-60 cells. *Exp Cell Res* 1999; 250: 203-12.
- Juo P, Kuo CJ, Yuan J, Blenis J. Essential requirement for caspase-8/FLICE in the initiation of the Fas-induced apoptotic cascade. *Curr Biol* 1998; 8: 1001-8.
- Slee EA, Harte MT, Kluck RM, Wolf BB, Casiano CA, Newmeyer DD, *et al*. Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. *J Cell Biol* 1999; 144: 281-92.
- Goldberg YP, Nicholson DW, Rasper DM, Kalchman MA, Koide HB, Graham RK, *et al*. Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract. *Nat Genet* 1996; 13: 442-9.
- Wellington CL, Ellerby LM, Hacham AS, Margolis RL, Trifiro MA, Singaraja R, *et al*. Caspase cleavage of gene products associated with triplet expansion disorders truncated fragments containing the polyglutamine tract. *J Biol Chem* 1998; 273: 9158-67.
- Sanchez I, Xu CJ, Juo P, Kakizaka A, Blenis J, Yuan J. Caspase-8 is required for cell death induced by expanded polyglutamine repeats. *Neuron* 1999; 22: 623-33.

- 21 Zeron MM, Fernandes HB, Krebs C, Shehadeh J, Wellington CL, Leavitt BR, *et al*. Potentiation of NMDA receptor-mediated excitotoxicity linked with intrinsic apoptotic pathway in YAC transgenic mouse model of Huntington's disease. *Mol Cell Neurosci* 2004; 24: 469-79.
- 22 Kim M, Lee HS, LaForet G, McIntyre C, Martin EL, Chang P, *et al*. Mutant huntingtin expression in clonal striatal cells: dissociation of inclusion formation and neuronal survival by caspase inhibition. *J Neurosci* 1999; 19: 964-73.
- 23 Wang GH, Mitsui K, Kotliarova S, Yamashita A, Nagao Y, Tokuhira S, *et al*. Caspase activation during apoptotic cell death induced by expanded polyglutamine in N2a cells. *NeuroReport* 1999; 10: 2435-8.
- 24 Ona VO, Li M, Vonsattel JP, Andrews LJ, Khan SQ, Chung WM, *et al*. Inhibition of caspase-1 slows disease progression in a mouse model of Huntington's disease. *Nature* 1999; 399: 263-7.
- 25 Chen M, Ona VO, Li M, Ferrante RJ, Fink KB, Zhu S, *et al*. Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. *Nat Med* 2000; 6: 797-801.
- 26 Sieradzan KA, Mehan AO, Jones L, Wanker EE, Nukina N, Mann DM. Huntington's disease intranuclear inclusions contain truncated, ubiquitinated huntingtin protein. *Exp Neurol* 1999; 156: 92-9.
- 27 Wellington CL, Singaraja R, Ellerby L, Savill J, Roy S, Leavitt B, *et al*. Inhibiting caspase cleavage of huntingtin reduces toxicity and aggregate formation in neuronal and nonneuronal cells. *J Biol Chem* 2000; 275: 19831-8.
- 28 Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, *et al*. Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell* 1997; 90: 537-48.
- 29 DiFiglia M, Sapp E, Chase O, Davies SW, Bates GP, Vonsattel JP, *et al*. Aggregation of Huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science* 1997; 277: 1990-3.
- 30 Scherzinger E, Lurz R, Turmaine M, Maggiani L, Hollenbach B, Hasenbank R, *et al*. Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates *in vitro* and *in vivo*. *Cell* 1997; 90: 549-58.
- 31 Hermel E, Gafni J, Propp SS, Leavitt BR, Wellington CL, Young JE, *et al*. Specific caspase interactions and amplification are involved in selective neuronal vulnerability in Huntington's disease. *Cell Death Differ* 2004; 4 : 1-15.
- 32 Bizat N, Hermel JM, Boyer F, Jacquard C, Creminon C, Ouary S, *et al*. Calpain is a major cell death effector in selective striatal degeneration induced *in vivo* by 3-nitropropionate: implications for Huntington's disease. *J Neurosci* 2003; 23: 5020-30.
- 33 Goffredo D, Rigamonti D, Tartari M, Micheli AD, Verderio C, Matteoli M, *et al*. Calcium-dependent cleavage of endogenous wild-type huntingtin in primary cortical neurons. *J Biol Chem* 2002; 277: 39594-8.
- 34 Kim M, Roh JK, Yoon BW, Kang L, Kim YJ, Aronin N, *et al*. Huntingtin is degraded to small fragments by calpain after ischemic injury. *Exp Neurol* 2003; 183: 109-15.
- 35 Gafni J, Ellerby LM. Calpain activation in Huntington's disease. *J Neurosci* 2002; 22: 4842-9.
- 36 Sieradzan KA, Mehan AO, Jones L, Wanker EE, Nukina N, Mann DMA. Huntington's disease intranuclear inclusions contain truncated, ubiquitinated huntingtin protein. *Exp Neurol* 1999; 156: 92-9.
- 37 Gafni JG, Hermel E, Young JE, Wellington CL, Hayden MR, Ellerby LM. Inhibition of calpain cleavage of huntingtin reduces toxicity, accumulation of calpain/caspase fragments in the nucleus. *J Biol Chem* 2004; 279: 20211-20.
- 38 Voges D, Zwickl P, Baumeister W. The 26S proteasome: a molecular machine designed for controlled proteolysis. *Annu Rev Biochem* 1999; 68: 1015-68.
- 39 Goldberg AL. Protein degradation and protection against misfolded or damaged proteins. *Nature* 2003; 426: 885-99.
- 40 Kalchman MA, Graham RK, Xia G, Koide HB, Hodgson JG, Graham KC, *et al*. Huntingtin is ubiquitinated and interacts with a specific ubiquitin-conjugating enzyme. *J Biol Chem* 1996; 271: 19385-94.
- 41 Waelter S, Boeddrich A, Lurz R, Scherzinger E, Lueder G, Lehrach H, *et al*. Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation. *Mol Biol Cell* 2001; 12: 1393-407.
- 42 Zhou H, Cao F, Wang Z, Yu ZX, Nguyen HP, Evans J, *et al*. Huntingtin forms toxic NH2-terminal fragment complexes that are promoted by the age-dependent decrease in proteasome activity. *J Cell Biol* 2003; 163: 109-18.
- 43 Muchowski PJ, Schaffar G, Sittler A, Wanker EE, Hayer-Hart MK, Hartl FU. Hsp70 and Hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. *Proc Natl Acad Sci USA* 2000; 97: 7841-6.
- 44 Glaumann H, Ericsson JL, Marzella L. Mechanisms of intralysosomal degradation with special reference to autophagocytosis and heterophagocytosis of cell organelles. *Intl Rev Cytol* 1981; 73: 149-82.
- 45 Kim J, Klionsky DJ. Autophagy, cytoplasm-to-vacuole targeting pathway, and pexophagy in yeast and mammalian cells. *Annu Rev Biochem* 2000; 69: 303-42.
- 46 Noda T, Suzuki K, Ohsumi Y. Yeast autophagosomes: *de novo* formation of a membrane structure. *Trends Cell Biol* 2002; 12: 231-5.
- 47 Xue L, Fletcher GC, Tolkovsky AM. Autophagy is activated by apoptotic signalling in sympathetic neurons: an alternative mechanism of death execution. *Mol Cell Neurosci* 1999; 14: 180-98.
- 48 Jeffrey M, Scott JR, Williams A, Fraser H. Ultrastructural features of spongiform encephalopathy transmitted to mice from three species of bovidae. *Acta Neuropathol (Berl)* 1992; 84: 559-69.
- 49 Hirai K, Aliev G, Nunomura A, Fujioka H, Russell RL, Atwood CS, *et al*. Mitochondrial abnormalities in Alzheimer's disease. *J Neurosci* 2001; 21: 3017-23.
- 50 Stefanis L, Larsen KE, Rideout HJ, Sulzer D, Greene LA. Expression of A53T mutant but not wild-type a-Synuclein in PC12 cells induces alterations of the ubiquitin-dependent degradation system, loss of dopamine release, and autoph-

- agic cell death. *J Neurosci* 2001; 21: 9549-60.
- 51 Kegel KB, Kim M, Sapp E, McIntyre C, Castaño JG, Aronin N, *et al*. Huntingtin expression stimulates endosomal-lysosomal activity, endosome tubulation, and autophagy. *J Neurosci* 2000; 20: 7268-78.
- 52 Petersen A, Larsen KE, Behr GG, Romero N, Przedborski S, Brundin P, *et al*. Expanded CAG repeats in exon 1 of the Huntington's disease gene stimulate dopamine-mediated striatal neuron autophagy and degeneration. *Hum Mol Genet* 2001; 10: 1243-54.
- 53 Ravikumar B, Duden R, Rubinsztein DC. Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. *Hum Mol Genet* 2002; 11: 1107-17.
- 54 Qin ZH, Wang Y, Kegel KB, Kazantsev A, Apostol BL, Thompson LM, *et al*. Autophagy regulates the processing of amino terminal huntingtin fragments. *Hum Mol Genet* 2003; 12: 3231-44.
- 55 Qin ZH, Wang Y, Sapp E, Cuiffo B, Erich Wanker E, Hayden MR, *et al*. Huntingtin bodies sequester vesicle-associated proteins by a polyproline-dependent interaction. *J Neurosci* 2004; 24: 269-81.
- 56 Gauthier LR, Charrin BC, Borrell-Page M, Dompierre JP, Rangone H, Cordelie'res F, *et al*. Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. *Cell* 2004; 118: 127-38.
- 57 Poirier MA, Li H, Macosko J, Cai S, Amzel M, Ross CA. Huntingtin spheroids and protofibrils as precursors in polyglutamine fibrilization. *J Biol Chem* 2002; 277: 41032-7.
- 58 Gafni J, Hermel E, Young JE, Wellington CL, Hayden MR, Ellerby LM. Inhibition of calpain cleavage of huntingtin reduces toxicity; accumulation of calpain/caspase fragments in the nucleus. *J Biol Chem* 2004; 279: 20211-20.
- 59 Ravikumar B, Vacher C, Berger Z, Davies JE, Luo S, Oroz LG, *et al*. Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. *Nat Genet* 2004; 36: 585-95.