*Human Molecular Genetics, 2009, Vol. 18, Review Issue 1* ***R40–R47***
*doi:10.1093/hmg/ddn412*

# Post-translational modifications of expanded polyglutamine proteins: impact on neurotoxicity

**Maria Pennuto[1,*], Isabella Palazzolo[2,3] and Angelo Poletti[3]**

[1]Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA, [2]Neurogenetics Branch, NINDS, NIH, MD 20892, USA and [3]Centro InterUniversitario sulle Malattie Neurodegenerative (Universita' di Firenze, Roma "Tor Vergata" e Milano) and Dipartimento di Endocrinologia, Fisiopatologia e Biologia Applicata, 'Centro di Eccellenza per lo studio delle malattie neurodegenerative', Università degli Studi di Milano, 20133 Milan, Italy

Received October 6, 2008; Revised October 6, 2008; Accepted December 4, 2008

**Polyglutamine diseases are a family of nine neurodegenerative disorders caused by expansion in different genes of a CAG triplet repeat stretch, which encodes an elongated polyglutamine tract. This polyglutamine tract is thought to confer a toxic gain of function to the bearing proteins, which leads to late onset and progressive loss of neurons in specific regions of the central nervous system. The mechanisms underlying specificity for neuronal vulnerability remain enigmatic. One explanation is that the polyglutamine tract is not the only determinant of neurodegeneration and that protein context and post-translational events may also be crucial for pathogenesis. Here, we review how post-translational modifications of the polyglutamine proteins contribute to modulate neurotoxicity.**

## INTRODUCTION

One of the most intriguing aspects of polyglutamine (polyQ) disease is that unique populations of neurons are affected in each disorder, despite the widespread and overlapping distribution of the disease-causing proteins in the central nervous system (Table 1). The molecular bases for neuronal specificity are unknown. The expansion of polyQ confers a multifaceted toxic gain of function to the bearing protein (Fig. 1). The expanded polyQ alters protein folding, thus leading to generation of aggregates (visible by western blotting and filter retardation assay) and inclusions (visible by immunofluorescence techniques) in the nucleus, cytosol and neuropil. PolyQ protein aggregates may also sequester essential cellular constituents (11), with consequent alteration of both cellular protein folding homeostasis (12,13) and gene transcription (14–16), as well as cleavage by cellular proteases, whose effect is to produce small fragments of mutant proteins that may be neurotoxic (17). Recent evidence has suggested that protein context and post-translational modifications influence the neurotoxicity of the polyQ proteins. In this review, we analyze two aspects of the effect of post-translational modifications on polyQ toxicity: their general effect on cell homeostasis and their specific effect on the polyQ proteins.

## PHOSPHORYLATION

Phosphorylation, a highly regulated post-translational modification that regulates protein localization, turnover and function, can be initiated in neurons by various neurotrophin and growth factor signaling and is propagated by kinase cascades. Among the growth factors, insulin-like growth factor 1 (IGF-1) possesses remarkable neuroprotective properties (18). Binding of neurotrophic factors and IGF-1 to their receptors activates two major signaling pathways: the mitogen-activated protein kinase (MAPK) and the phosphatidyl-inositol 3-kinase/Akt pathways. Alterations in neurotrophic or growth factor signaling are associated with several neurodegenerative conditions such as spinal and bulbar muscular atrophy (SBMA) (19,20) and Huntington's disease (HD) (21). Although dysfunction of these signaling pathways is not likely to be the primary cause of neurodegeneration in polyQ disease, it may contribute to disease progression and manifestation.

PolyQ huntingtin (htt), androgen receptor (AR) and ataxin 1 are substrates for Akt. Of remarkable importance is that phosphorylation by Akt has different consequences on these polyQ proteins. Phosphorylation of polyQ htt at serine 421 by Akt results in the decreased formation of nuclear inclusions and reduced toxicity in cultured striatal neurons (22) and in

*To whom correspondence should be addressed. Tel: +1 2155731729; Fax: +1 2155732107; Email: pennutom@mail.med.upenn.edu

© *The Author 2009. Published by Oxford University Press. All rights reserved.*
*For Permissions, please email: journals.permissions@oxfordjournals.org*

**Table 1.** CAG trinucleotide repeat disorders

| Gene | Disease | Wild-type | Mutant | Reference | Neuropathology |
|---|---|---|---|---|---|
| Huntingtin (htt) | HD | 6–34 | 36–121 | (1) | Neostriatum and cortex |
| Androgen receptor (AR) | SBMA | 6–39 | 40–63 | (2) | Spinal cord and brainstem (lower motor neurons) |
| Atrophin 1 | DRPLA | 6–36 | 49–84 | (3,4) | Basal ganglia (globus pallidus, subthalamic nucleus), cerebellum (dentatorubral pathway) |
| Ataxin 1 | SCA 1 | 8–44 | 39–83 | (5) | Cerebellum (Purkinje cells), brainstem |
| Ataxin 2 | SCA 2 | 13–33 | 32–77 | (6) | Cerebellum (Purkinje cells), brainstem |
| Ataxin 3 | MJD/SCA 3 | 12–40 | 54–89 | (7) | Cerebellum (dentate nucleus), brainstem, basal ganglia |
| α1a-subunit of the voltage-dependent calcium channel | SCA 6 | 4–18 | 21–33 | (8) | Cerebellum (dentate and inferior olivary nuclei, Purkinje cells) |
| Ataxin 7 | SCA 7 | 4–35 | 37–306 | (9) | Retina and cerebellum |
| TATA-binding protein | SCA 17 | 29–42 | 47–55 | (10) | Striatum and cerebellum |

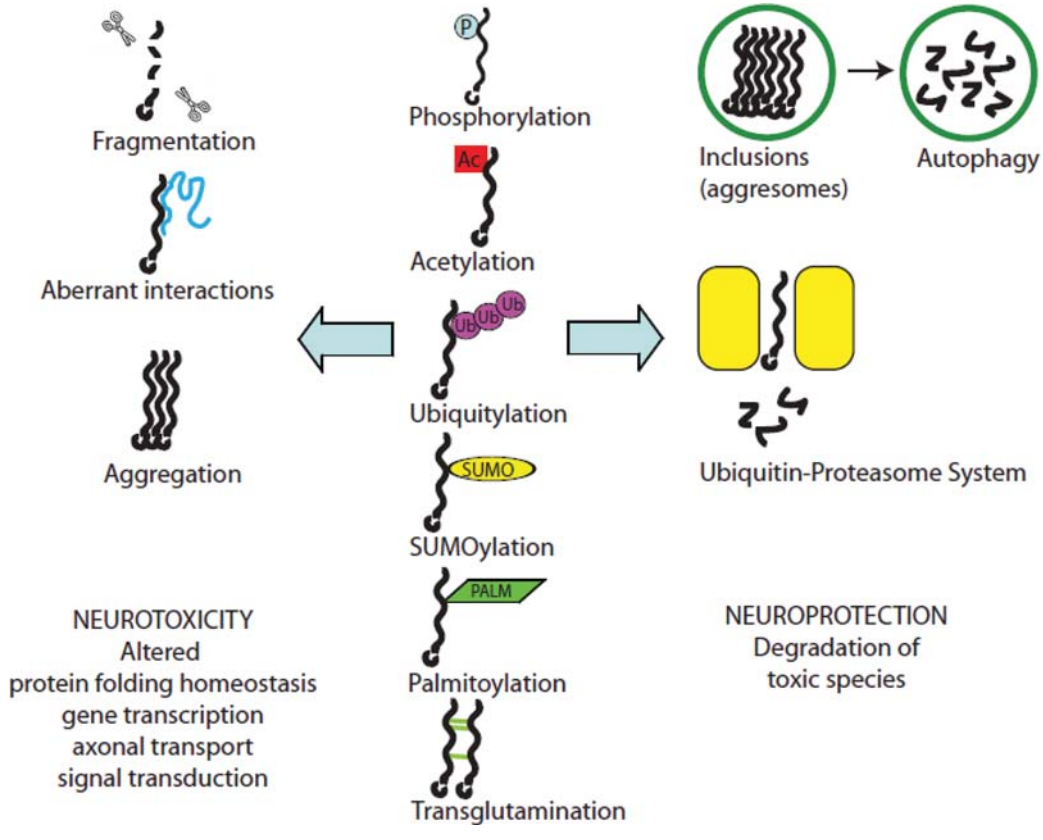


**Figure 1.** Post-translational modification of polyQ proteins. PolyQ proteins undergo numerous post-translational modifications, including phosphorylation, acetylation, ubiquitylation, SUMOylation, palmitoylation, transglutamination and proteolytic cleavage. The effect of these modifications can be neurotoxic, leading to alterations in gene transcription, protein homeostasis, axonal transport and signal transduction, or neuroprotective if it results in protein disposal via autophagic or proteasomal degradation.

a rodent model of HD (23). This phosphorylation is reduced in the cortex and striatum of mouse models of HD (24). Further strengthening the importance of this pathway in HD is the finding that phosphatase calcineurin inhibition increases polyQ htt phosphorylation at serine 421 and reduces striatal neuron death (23). Recently, Saudou and co-workers have shown that htt regulates vesicle axonal transport (25). Noticeably, this function is altered by the expansion of polyglutamine, but restored by phosphorylation at serine 421 (26). Phosphorylation of polyQ AR at serines 215 and 792 by Akt leads to decreased ligand binding and reduced toxicity in cell models of SBMA (27). If phosphorylation of polyQ htt and AR protects against toxicity, phosphorylation of ataxin 1 by Akt has opposite effects. Phosphorylation of ataxin 1 at serine 776 increases interactions with the molecular chaperone 14-3-3 and results in augmented protein stabilization and formation of neuronal inclusions (28). In fly models of spinocerebellar ataxia 1 (SCA 1), activation of the phosphatidyl-inositol 3-kinase/Akt pathway can worsen the eye degeneration associated with the expression of mutant ataxin 1. In mouse models of SCA 1, substitution of serine 776 with alanine, a non-phosphorylatable residue, reduces the accumulation of the protein into nuclear inclusions and decreases the toxic properties of the expanded polyQ protein (29).

PolyQ proteins are known substrates of other kinases. For instance, polyQ ataxin 3 is a substrate of casein kinase 2 (30) and glycogen synthase kinase 3β (31). PolyQ ataxin 3 is phosphorylated by glycogen synthase kinase 3β at serine 256. Substitution of this serine with alanine increases ataxin 3 polymerization, suggesting a role for phosphorylation in ataxin 3 aggregation.

A comprehensive study of htt phosphorylation performed in cell models of HD revealed six serine phosphorylation sites: serines 536, 1181, 1201, 2076, 2653 and 2657 (32). Of these sites, serines 1181 and 1201 have been shown to be phosphorylated by CDK5 (33). CDK5 is also known to phosphorylate htt at serine 434 (34). Interestingly, phosphorylation of htt by CDK5 has various consequences, depending on the serine involved. For example, phosphorylation at serine 434 reduces htt cleavage by caspases and decreases the toxicity of the expanded polyQ protein. Phosphorylation of polyQ htt at serines 1181 and 1201 protects against toxicity, whereas the absence of phosphorylation of normal htt at the same sites confers toxic properties similar to the polyQ protein. Interestingly, CDK5 has recently been shown to suppress polyQ htt toxicity via an additional, htt phosphorylation-independent mechanism (35).

Several residues have been identified as phosphorylation sites in the normal AR, including serines 16, 83, 96, 258, 310, 426, 516 and 651 (NM_000044) (36). Phosphorylation of polyQ AR by MAPK (the MEK 1/2 pathway or 44/42 MAPK) at serine 516 is associated with increased toxicity in cell models of SBMA (37). Interestingly, phosphorylation at serines 426 and 516 has opposite consequences on wild-type and polyQ AR. Loss of phosphorylation converts normal AR into a toxic protein, whereas it reduces the toxicity of polyQ AR (38). AR phosphorylation at serine 651 is induced by stress kinase signaling and modulates nuclear export, at least in prostate cancer cells (39). AR is also phosphorylated by tyrosine kinases such as c-src (40). Phosphorylation of AR at tyrosine 537 regulates ligand-induced transcriptional activation, nuclear translocation and prostate cancer cell proliferation. Cdc42-associated tyrosine kinase Ack1 phosphorylates AR at tyrosines 269 and 365, resulting in enhanced AR transactivation and androgen-independent prostate cancer proliferation (41). Unfortunately, nothing is known about whether phosphorylation of these sites modulates polyQ AR toxicity.

## ACETYLATION

The covalent binding of an acetyl group to lysine residues is a reversible reaction catalyzed by histone acetyltransferase (HAT) enzymes, such as CREB-binding protein (CBP), and its removal is operated by histone deacetylase (HDAC) enzymes. The major targets of acetylation are histones and transcription factors such as AR. Histone and transcription factors acetylation leads to transcriptional activation of specific genes. The disruption of the delicate equilibrium between HAT and HDAC activities results in reduced expression of vital genes in HD (42). CBP is an acetyltransferase, whose activity is altered in polyQ disease. The polyQ proteins/CBP interaction causes loss of CBP function by decreasing its availability. In fact, the interaction of polyQ htt, atrophin 1, ataxin 3 or AR with CBP sequesters CBP into inclusions, thereby decreasing the amount of soluble CBP (43–45) and enhancing its ubiquitylation and degradation through the proteasome (46). Noticeably, the overexpression of CBP protects neurons against the toxicity of expanded polyQ (43,44,47).

Are polyQ proteins direct substrates for acetyltransferases? Normal AR is acetylated at positions 631–634, with an acetylation sequence of KXKK, where K is the lysine and X any amino acid (48). Loss of function mutations at the acetylation sites of normal AR result in increased aggregation to an extent similar to that observed for polyQ AR (49). Although these observations suggest a possible role of acetylation in SBMA, further characterization is required to establish whether acetylation alters the toxic properties of polyQ AR.

## UBIQUITYLATION

The binding of ubiquitin to a protein occurs through an isopeptide bond formed between lysine residues of a target protein and the carboxy-terminal group of ubiquitin. Ubiquitylation is a reversible and multi-step process that involves at least three classes of enzymes acting in concert with ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin-protein ligase (E3) activities. Proteins can be mono- or poly-ubiquitylated. Mono-ubiquitylation of transcription factors such as the AR regulates gene transcription (50). Poly-ubiquitylation is generally a signal for protein degradation, which mainly occurs through the proteasome. The inclusions formed by the polyQ proteins incorporate ubiquitin and ubiquitylated proteins, suggesting that the polyQ proteins are targeted for degradation through the proteasome, but are not efficiently disposed of by the cell (51).

Ubiquitylation plays an important role in polyQ disease. Ubiquitylation reduces polyQ toxicity by enhancing protein degradation. For instance, the overexpression of the ubiquitin ligase E4B increases ataxin 3 ubiquitylation, reduces the accumulation of insoluble species and suppresses neurodegeneration in a fly model of SCA 3 (52). Overexpression of the ubiquitin ligase C-terminus of Hsp70-interacting protein (CHIP) reduces the aggregation and toxicities of polyQ ataxin 3 and htt (53), AR (54) and ataxin 1 (55). Overexpression of the E3 enzyme parkin enhances ataxin 3 ubiquitylation and degradation and reduces toxicity (56). Normal ataxin 3 is a poly-ubiquitin-binding protein whose overexpression reduces the accumulation of various polyQ proteins and suppresses neurodegeneration *in vivo* (57). Importantly, the ubiquitin-associated activity of ataxin 3 is required to protect against neurodegeneration.

In contrast, ubiquitylation can also enhance toxicity. Inhibition of the E2 enzyme Hip 2 reduces the accumulation of insoluble fragments of polyQ htt and its toxicity (58). Recently, CHIP was shown to enhance the ubiquitylation of polyQ ataxin 1, leading to a reduction in solubility and increased accumulation in insoluble aggregates (59).

## SUMOYLATION

The covalent and reversible attachment of the SUMO (small ubiquitin-related modifier) proteins to the side chain of target protein lysine residues is referred to as SUMOylation. One sequence motif for SUMOylation is $\Psi KX[^{D}/_{E}]$, where $\Psi$ is a hydrophobic residue, $K$ is the acceptor lysine, $X$ is

any amino acid and *D*/*E* is an aspartate or glutamate. SUMO co-localizes with neuronal inclusions in autopsy brain and cell models of dentatorubral pallidoluysian atrophy (DRPLA) (60) and HD (61). Interestingly, the amount of SUMOylated proteins is increased in autopsy brain tissues of DRPLA, SCA 1, MJD and HD patients and in mouse models of SCA 1, suggesting that SUMO modification contributes to neurodegeneration in polyQ disease (62).

An even more intriguing aspect of the genetic interaction between SUMOylation and polyQ disease emerges from the finding that polyQ proteins, including htt (61), AR (63) and ataxin 1 (64), are direct target for SUMOylation. Although it is unknown whether other polyQ proteins are SUMOylated, a neural network analysis of protein sequences indicates the presence of putative SUMOylation sites (http://bioinformatics.lcd-ustc.org/sumosp/index.php). SUMO modification of the polyQ proteins is important for their toxicity. Loss of SUMOylation in polyQ htt reduces neurodegeneration (61), and SUMO modification of atrophin 1 enhances cell death (60). SUMO modification may influence neurodegeneration with different mechanisms. Enhanced SUMOylation increases the stabilization of htt, possibly due to competition with ubiquitylation at the same lysine residues, and reduces aggregation of polyQ htt (61). Conversely, SUMO modification increases the aggregation of polyQ atrophin 1 (60). SUMO modification enhances the repressive effect that the expanded polyQ has on htt-mediated transcription (61), and a loss of SUMOylation enhances AR-mediated transcription (63). SUMOylation can modify the subnuclear localization of target proteins and may be important for nuclear localization of polyQ htt (61) and ataxin 1 (64).

## PALMITOYLATION

Palmitoylation is the reversible covalent attachment of a saturated palmitic fatty acid chain to a cysteine residue of proteins. Interestingly, both htt (65) and AR (66) are palmitoylated. Palmitoylation has been implicated in different cellular processes, including protein trafficking, protein–protein interactions and protein stability. For instance, palmitoylation of AR is required for its localization to the plasma membrane (66). Palmitoylation seems to be particularly important in neurons, where it regulates protein trafficking along neurites and synapse formation (67). In most polyQ diseases, defects of the antero-retrograde fast axonal transport have been reported as one possible cause of polyQ protein-triggered neurodegeneration. However, whether palmitoylation can impact the toxicity of expanded polyQ AR is not known. A role for palmitoylation in polyQ disease has been proposed for HD (65). Hayden and co-workers have shown that htt is palmitoylated by the huntingtin-interacting protein (HIP) 14. Expansion of the polyQ tract reduces the interaction between htt and HIP14 and decreases the amount of palmitoylated polyQ htt. Importantly, loss of htt palmitoylation exacerbates toxicity, as it results in increased protein aggregation and cell death.

## TRANSGLUTAMINATION

Transglutaminases are a family of calcium-activated enzymes that catalyze the acyl transfer reaction between glutamine residues of one protein and lysine residues of another, leading to the generation of isopeptide bonds that cross-link proteins. In mammalian cells, at least nine members of the transglutaminase family have been identified to date. Transglutaminase type 2 is highly expressed in neurons. PolyQ ataxin 1 (68), AR (69) and htt (70) are substrates for transglutaminases. In the early 1990s, expansion of the polyQ tract was postulated to be associated with increased transglutaminase activity (71). With time, compelling evidence emerged, showing that alteration of transglutaminase activity may contribute to polyQ disease. Type 2 transglutaminase is upregulated in spiny neurons in the striatum of HD patients (72,73) and transgenic mice (74,75). The evidence that transglutaminase activity is important in polyQ disease came from the observation that ablation of the gene encoding type 2 transglutaminase in HD mice alleviates disease manifestations (76). Interestingly, the overexpression of type 2 transglutaminase in cultured striatal neurons expressing either wild-type or polyQ htt had opposite effect on cell survival, suggesting that the sensitivity of striatal neurons to transglutaminase activity may depend on cellular context (77).

## PROTEOLYTIC CLEAVAGE

In the 1990s, the idea emerged that proteolysis of polyQ proteins may generate the shorter, diffusible fragments that are thought to be responsible for aggregation. This process, referred to as the 'toxic fragment hypothesis', is strongly supported by evidence, showing that, in most polyQ diseases, aggregates are mainly formed by small polyQ-containing fragments rather than the full-length polyQ proteins. Moreover, most polyQ proteins generate fragments that differ from those released by the corresponding wild-type proteins, suggesting that these post-translational events occur in a polyQ-dependent manner. Htt (17,78,79), AR (17), atrophin 1 (17,80) and ataxin 3 (81,82) are cleaved by specific caspases, with htt also being a substrate for calpain (79,83). In the striatum and cortex of HD patient brains, intranuclear inclusions contain the amino-terminal-truncated fragments of polyQ htt (51), and mouse models generated with an N-terminal htt fragment have a more severe phenotype than mice expressing full-length htt.

Caspase and calpain cleavage of polyQ htt occur in a discrete region of the protein. The caspases that cleave htt include caspase 2, 3, 6 and 7 and act on cleavage sites located between amino acids 513 and 586 (17,84,85). Calpains 1 and 2 cleave htt at sites located mainly between amino acids 469 and 536 (86,87). Other smaller putative amino-terminal fragments of htt have been reported in post-mortem brain material of HD patients (51). Noticeably, wild-type htt overexpression results in caspase 3 inhibition, as htt physically interacts with active caspase 3 (88). PolyQ htt has lower affinity for, and a lower inhibitory effect on, active caspase 3 than wild-type htt, suggesting a contribution of the loss of this function in HD. The relationship between caspase proteolysis and HD pathogenesis *in vivo* was largely unknown. Only recently, Graham *et al*. (89) have shown that mice expressing an htt mutated at the caspase 6 cleavage site do not develop behavioral deficits and the pathological signs of HD.

PolyQ atrophin 1 is also cleaved by caspases at aspartate 109, and cleavage site mutations reduce nuclear localization, aggregate formation and cytotoxicity (63,90).

In the brain of SCA 3 transgenic mice, neuronal loss is associated with the appearance of a toxic 28 kDa fragment of ataxin 3 containing the polyQ (91). Ataxin 3 cleavage is blocked by caspase inhibitors, with caspase 1 being one possible cleavage protease, and ataxin 3 fragmentation also correlates with increased ataxin 3 aggregation (81).

Truncated ataxin 7 fragments are present in the nuclear aggregates of transgenic mice and in SCA 7 patient specimens (92). Ataxin 7 is cleaved by caspase 7 at two putative cleavage sites, at positions 266 and 344. Mutation of these sites in polyQ ataxin 7 attenuates cell death, aggregate formation and transcriptional dysfunction (93).

A fragmented form of polyQ AR is present in SBMA nuclear inclusions (94). PolyQ AR seems to be specifically cleaved by caspase 3 at aspartate 146, and this cleavage is increased during apoptosis (95). Mutation of this site inhibits perinuclear aggregate formation (96). Using biochemistry and atomic force microscopy approaches to immunopurify soluble oligomers after ultracentrifugation, Li *et al*. (97) recently showed that a 50 kDa terminal AR fragment containing the polyQ tract is present in AR oligomers and that these species appear several weeks prior to the onset of disease symptoms in animal models of SBMA.

## INTERPLAY BETWEEN POST-TRANSLATIONAL MODIFICATIONS

Post-translational modifications can be reciprocally linked to one other. For instance, phosphorylation affects the proteolytic cleavage of polyQ proteins. AR phosphorylation by MAPK at serine 516 increases the caspase 3-mediated cleavage of polyQ AR (37). Htt phosphorylation by CDK5 at serine 434 reduces caspase-mediated cleavage at aspartate 513, whereas a serine-to-alanine mutation prevents this CDK5-mediated effect (34). Phosphorylation of htt at serine 536, a residue located in the proteolytic susceptibility domain, prevents the cleavage of htt by calpain and reduces the toxicity of the polyQ protein (32).

Phosphorylation of the polyQ proteins can influence SUMOylation and ubiquitylation. Expansion of the polyQ tract reduces SUMOylation of ataxin 1, which is rescued by a loss of phosphorylation of ataxin 1 at serine 776 (64). CHIP-mediated ubiquitylation and aggregation of ataxin 1 are prevented when serine 776 is mutated to alanine (59). Phosphorylation of AR by Akt regulates AR ubiquitylation and degradation (98). SUMOylation and ubiquitylation can compete for the same lysine residues, as suggested for htt (61).

## CONCLUDING REMARKS

The research analyzed in this review brings us back to the question: why are specific populations of neurons vulnerable in each polyQ disease? Or in other words, is polyQ the only determinant for neurodegeneration, or is protein contest responsible for cell-specific death? Although the first polyQ disease was discovered in 1991 (2), the identification of an effective therapy for polyQ disease remains a major challenge. Dissecting the role of post-translational modifications in polyQ disease can shed light into the mechanisms of polyQ neurodegeneration and could ultimately help in developing novel therapeutic strategies to counteract disease progression and manifestations.

## ACKNOWLEDGEMENTS

We apologize to those authors whose work has not been cited due to limitation of space. We thank Katie Edmondson (University of Pennsylvania, PA, USA) for revision of manuscript.

*Conflict of Interest statement*. The authors declare that no conflict of interest exists.

## FUNDING

This work was supported by NINDS intramural funding and Telethon—Italy (GFP04005), Kennedy's Disease Association and Muscular Dystrophy Association Development grants to M.P. and Telethon—Italy grants (GGP06063 and GGP07063), Fondazlone Cariplo (Italy) and Ministero del Lavoro, della Salute e delle Politiche Sociali (Italy) to A.P.

## REFERENCES

1. The Huntington's Disease Collaborative Research Group. (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell*, **72**, 971–983.
2. La Spada, A.R., Wilson, E.M., Lubahn, D.B., Harding, A.E. and Fischbeck, K.H. (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature*, **352**, 77–79.
3. Koide, R., Ikeuchi, T., Onodera, O., Tanaka, H., Igarashi, S., Endo, K., Takahashi, H., Kondo, R., Ishikawa, A., Hayashi, T. *et al.* (1994) Unstable expansion of CAG repeat in hereditary dentatorubral-pallidoluysian atrophy (DRPLA). *Nat. Genet.*, **6**, 9–13.
4. Nagafuchi, S., Yanagisawa, H., Sato, K., Shirayama, T., Ohsaki, E., Bundo, M., Takeda, T., Tadokoro, K., Kondo, I., Murayama, N. *et al.* (1994) Dentatorubral and pallidoluysian atrophy expansion of an unstable CAG trinucleotide on chromosome 12p. *Nat. Genet.*, **6**, 14–18.
5. Orr, H.T., Chung, M.Y., Banfi, S., Kwiatkowski, T.J. Jr, Servadio, A., Beaudet, A.L., McCall, A.E., Duvick, L.A., Ranum, L.P. and Zoghbi, H.Y. (1993) Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type 1. *Nat. Genet.*, **4**, 221–226.
6. Imbert, G., Saudou, F., Yvert, G., Devys, D., Trottier, Y., Garnier, J.M., Weber, C., Mandel, J.L., Cancel, G., Abbas, N. *et al.* (1996) Cloning of the gene for spinocerebellar ataxia 2 reveals a locus with high sensitivity to expanded CAG/glutamine repeats. *Nat. Genet.*, **14**, 285–291.
7. Kawaguchi, Y., Okamoto, T., Taniwaki, M., Aizawa, M., Inoue, M., Katayama, S., Kawakami, H., Nakamura, S., Nishimura, M., Akiguchi, I. *et al.* (1994) CAG expansions in a novel gene for Machado–Joseph disease at chromosome 14q32.1. *Nat. Genet.*, **8**, 221–228.
8. Zhuchenko, O., Bailey, J., Bonnen, P., Ashizawa, T., Stockton, D.W., Amos, C., Dobyns, W.B., Subramony, S.H., Zoghbi, H.Y. and Lee, C.C. (1997) Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the alpha 1A-voltage-dependent calcium channel. *Nat. Genet.*, **15**, 62–69.
9. David, G., Abbas, N., Stevanin, G., Durr, A., Yvert, G., Cancel, G., Weber, C., Imbert, G., Saudou, F., Antoniou, E. *et al.* (1997) Cloning of the SCA7 gene reveals a highly unstable CAG repeat expansion. *Nat. Genet.*, **17**, 65–70.
10. Nakamura, K., Jeong, S.Y., Uchihara, T., Anno, M., Nagashima, K., Nagashima, T., Ikeda, S., Tsuji, S. and Kanazawa, I. (2001) SCA17, a novel autosomal dominant cerebellar ataxia caused by an expanded

polyglutamine in TATA-binding protein. *Hum. Mol. Genet.*, **10**, 1441–1448.
11. Donaldson, K.M., Li, W., Ching, K.A., Batalov, S., Tsai, C.C. and Joazeiro, C.A. (2003) Ubiquitin-mediated sequestration of normal cellular proteins into polyglutamine aggregates. *Proc. Natl. Acad. Sci. USA*, **100**, 8892–8897.
12. Gidalevitz, T., Ben-Zvi, A., Ho, K.H., Brignull, H.R. and Morimoto, R.I. (2006) Progressive disruption of cellular protein folding in models of polyglutamine diseases. *Science*, **311**, 1471–1474.
13. Satyal, S.H., Schmidt, E., Kitagawa, K., Sondheimer, N., Lindquist, S., Kramer, J.M. and Morimoto, R.I. (2000) Polyglutamine aggregates alter protein folding homeostasis in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA*, **97**, 5750–5755.
14. Hodges, A., Strand, A.D., Aragaki, A.K., Kuhn, A., Sengstag, T., Hughes, G., Elliston, L.A., Hartog, C., Goldstein, D.R., Thu, D. *et al.* (2006) Regional and cellular gene expression changes in human Huntington's disease brain. *Hum. Mol. Genet.*, **15**, 965–977.
15. Lieberman, A.P., Harmison, G., Strand, A.D., Olson, J.M. and Fischbeck, K.H. (2002) Altered transcriptional regulation in cells expressing the expanded polyglutamine androgen receptor. *Hum. Mol. Genet.*, **11**, 1967–1976.
16. Lin, X., Antalffy, B., Kang, D., Orr, H.T. and Zoghbi, H.Y. (2000) Polyglutamine expansion down-regulates specific neuronal genes before pathologic changes in SCA1. *Nat. Neurosci.*, **3**, 157–163.
17. Wellington, C.L., Ellerby, L.M., Hackam, A.S., Margolis, R.L., Trifiro, M.A., Singaraja, R., McCutcheon, K., Salvesen, G.S., Propp, S.S., Bromm, M. *et al.* (1998) Caspase cleavage of gene products associated with triplet expansion disorders generates truncated fragments containing the polyglutamine tract. *J. Biol. Chem.*, **273**, 9158–9167.
18. Trejo, J.L., Carro, E., Garcia-Galloway, E. and Torres-Aleman, I. (2004) Role of insulin-like growth factor I signaling in neurodegenerative diseases. *J. Mol. Med.*, **82**, 156–162.
19. Sopher, B.L., Thomas, P.S. Jr, LaFevre-Bernt, M.A., Holm, I.E., Wilke, S.A., Ware, C.B., Jin, L.W., Libby, R.T., Ellerby, L.M. and La Spada, A.R. (2004) Androgen receptor YAC transgenic mice recapitulate SBMA motor neuronopathy and implicate VEGF164 in the motor neuron degeneration. *Neuron*, **41**, 687–699.
20. Yu, Z., Dadgar, N., Albertelli, M., Gruis, K., Jordan, C., Robins, D.M. and Lieberman, A.P. (2006) Androgen-dependent pathology demonstrates myopathic contribution to the Kennedy disease phenotype in a mouse knock-in model. *J. Clin. Invest.*, **116**, 2663–2672.
21. Colin, E., Regulier, E., Perrin, V., Durr, A., Brice, A., Aebischer, P., Deglon, N., Humbert, S. and Saudou, F. (2005) Akt is altered in an animal model of Huntington's disease and in patients. *Eur. J. Neurosci.*, **21**, 1478–1488.
22. Humbert, S., Bryson, E.A., Cordelieres, F.P., Connors, N.C., Datta, S.R., Finkbeiner, S., Greenberg, M.E. and Saudou, F. (2002) The IGF-1/Akt pathway is neuroprotective in Huntington's disease and involves Huntingtin phosphorylation by Akt. *Dev. Cell*, **2**, 831–837.
23. Pardo, R., Colin, E., Regulier, E., Aebischer, P., Deglon, N., Humbert, S. and Saudou, F. (2006) Inhibition of calcineurin by FK506 protects against polyglutamine-huntingtin toxicity through an increase of huntingtin phosphorylation at S421. *J. Neurosci.*, **26**, 1635–1645.
24. Warby, S.C., Chan, E.Y., Metzler, M., Gan, L., Singaraja, R.R., Crocker, S.F., Robertson, H.A. and Hayden, M.R. (2005) Huntingtin phosphorylation on serine 421 is significantly reduced in the striatum and by polyglutamine expansion *in vivo*. *Hum. Mol. Genet.*, **14**, 1569–1577.
25. Colin, E., Zala, D., Liot, G., Rangone, H., Borrell-Pages, M., Li, X.J., Saudou, F. and Humbert, S. (2008) Huntingtin phosphorylation acts as a molecular switch for anterograde/retrograde transport in neurons. *EMBO J.*, **27**, 2124–2134.
26. Zala, D., Colin, E., Rangone, H., Liot, G., Humbert, S. and Saudou, F. (2008) Phosphorylation of mutant huntingtin at S421 restores anterograde and retrograde transport in neurons. *Hum. Mol. Genet.*, **17**, 3837–3846.
27. Palazzolo, I., Burnett, B.G., Young, J.E., Brenne, P.L., La Spada, A.R., Fischbeck, K.H., Howell, B.W. and Pennuto, M. (2007) Akt blocks ligand binding and protects against expanded polyglutamine androgen receptor toxicity. *Hum. Mol. Genet.*, **16**, 1593–1603.
28. Chen, H.K., Fernandez-Funez, P., Acevedo, S.F., Lam, Y.C., Kaytor, M.D., Fernandez, M.H., Aitken, A., Skoulakis, E.M., Orr, H.T., Botas, J. *et al.* (2003) Interaction of Akt-phosphorylated ataxin-1 with 14-3-3 mediates neurodegeneration in spinocerebellar ataxia type 1. *Cell*, **113**, 457–468.
29. Emamian, E.S., Kaytor, M.D., Duvick, L.A., Zu, T., Tousey, S.K., Zoghbi, H.Y., Clark, H.B. and Orr, H.T. (2003) Serine 776 of ataxin-1 is critical for polyglutamine-induced disease in SCA1 transgenic mice. *Neuron*, **38**, 375–387.
30. Tao, R.S., Fei, E.K., Ying, Z., Wang, H.F. and Wang, G.H. (2008) Casein kinase 2 interacts with and phosphorylates ataxin-3. *Neurosci. Bull.*, **24**, 271–277.
31. Fei, E., Jia, N., Zhang, T., Ma, X., Wang, H., Liu, C., Zhang, W., Ding, L., Nukina, N. and Wang, G. (2007) Phosphorylation of ataxin-3 by glycogen synthase kinase 3beta at serine 256 regulates the aggregation of ataxin-3. *Biochem. Biophys. Res. Commun.*, **357**, 487–492.
32. Schilling, B., Gafni, J., Torcassi, C., Cong, X., Row, R.H., LaFevre-Bernt, M.A., Cusack, M.P., Ratovitski, T., Hirschhorn, R., Ross, C.A. *et al.* (2006) Huntingtin phosphorylation sites mapped by mass spectrometry. Modulation of cleavage and toxicity. *J. Biol. Chem.*, **281**, 23686–23697.
33. Anne, S.L., Saudou, F. and Humbert, S. (2007) Phosphorylation of huntingtin by cyclin-dependent kinase 5 is induced by DNA damage and regulates wild-type and mutant huntingtin toxicity in neurons. *J. Neurosci.*, **27**, 7318–7328.
34. Luo, S., Vacher, C., Davies, J.E. and Rubinsztein, D.C. (2005) Cdk5 phosphorylation of huntingtin reduces its cleavage by caspases: implications for mutant huntingtin toxicity. *J. Cell Biol.*, **169**, 647–656.
35. Kaminosono, S., Saito, T., Oyama, F., Ohshima, T., Asada, A., Nagai, Y., Nukina, N. and Hisanaga, S. (2008) Suppression of mutant Huntingtin aggregate formation by Cdk5/p35 through the effect on microtubule stability. *J. Neurosci.*, **28**, 8747–8755.
36. Gioeli, D., Ficarro, S.B., Kwiek, J.J., Aaronson, D., Hancock, M., Catling, A.D., White, F.M., Christian, R.E., Settlage, R.E., Shabanowitz, J. *et al.* (2002) Androgen receptor phosphorylation. Regulation and identification of the phosphorylation sites. *J. Biol. Chem.*, **277**, 29304–29314.
37. LaFevre-Bernt, M.A. and Ellerby, L.M. (2003) Kennedy's disease. Phosphorylation of the polyglutamine-expanded form of androgen receptor regulates its cleavage by caspase-3 and enhances cell death. *J. Biol. Chem.*, **278**, 34918–34924.
38. Funderburk, S.F., Shatkina, L., Mink, S., Weis, Q., Weg-Remers, S. and Cato, A.C. (2008) Specific N-terminal mutations in the human androgen receptor induce cytotoxicity. *Neurobiol. Aging*., Epub ahead of print.
39. Gioeli, D., Black, B.E., Gordon, V., Spencer, A., Kesler, C.T., Eblen, S.T., Paschal, B.M. and Weber, M.J. (2006) Stress kinase signaling regulates androgen receptor phosphorylation, transcription, and localization. *Mol. Endocrinol.*, **20**, 503–515.
40. Guo, Z., Dai, B., Jiang, T., Xu, K., Xie, Y., Kim, O., Nesheiwat, I., Kong, X., Melamed, J., Handratta, V.D. *et al.* (2006) Regulation of androgen receptor activity by tyrosine phosphorylation. *Cancer Cell*, **10**, 309–319.
41. Mahajan, N.P., Liu, Y., Majumder, S., Warren, M.R., Parker, C.E., Mohler, J.L., Earp, H.S. and Whang, Y.E. (2007) Activated Cdc42-associated kinase Ack1 promotes prostate cancer progression via androgen receptor tyrosine phosphorylation. *Proc. Natl. Acad. Sci. USA*, **104**, 8438–8443.
42. Sadri-Vakili, G., Bouzou, B., Benn, C.L., Kim, M.O., Chawla, P., Overland, R.P., Glajch, K.E., Xia, E., Qiu, Z., Hersch, S.M. *et al.* (2007) Histones associated with downregulated genes are hypo-acetylated in Huntington's disease models. *Hum. Mol. Genet.*, **16**, 1293–1306.
43. McCampbell, A., Taylor, J.P., Taye, A.A., Robitschek, J., Li, M., Walcott, J., Merry, D., Chai, Y., Paulson, H., Sobue, G. *et al.* (2000) CREB-binding protein sequestration by expanded polyglutamine. *Hum. Mol. Genet.*, **9**, 2197–2202.
44. Nucifora, F.C. Jr, Sasaki, M., Peters, M.F., Huang, H., Cooper, J.K., Yamada, M., Takahashi, H., Tsuji, S., Troncoso, J., Dawson, V.L. *et al.* (2001) Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. *Science*, **291**, 2423–2428.
45. Steffan, J.S., Kazantsev, A., Spasic-Boskovic, O., Greenwald, M., Zhu, Y.Z., Gohler, H., Wanker, E.E., Bates, G.P., Housman, D.E. and Thompson, L.M. (2000) The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. *Proc. Natl. Acad. Sci. USA*, **97**, 6763–6768.
46. Jiang, H., Nucifora, F.C. Jr, Ross, C.A. and DeFranco, D.B. (2003) Cell death triggered by polyglutamine-expanded huntingtin in a neuronal cell line is associated with degradation of CREB-binding protein. *Hum. Mol. Genet.*, **12**, 1–12.
47. Taylor, J.P., Taye, A.A., Campbell, C., Kazemi-Esfarjani, P., Fischbeck, K.H. and Min, K.T. (2003) Aberrant histone acetylation, altered transcription, and retinal degeneration in a *Drosophila* model of

polyglutamine disease are rescued by CREB-binding protein. *Genes Dev.*, **17**, 1463–1468.
48. Fu, M., Wang, C., Reutens, A.T., Wang, J., Angeletti, R.H., Siconolfi-Baez, L., Ogryzko, V., Avantaggiati, M.L. and Pestell, R.G. (2000) p300 and p300/cAMP-response element-binding protein-associated factor acetylate the androgen receptor at sites governing hormone-dependent transactivation. *J. Biol. Chem.*, **275**, 20853–20860.
49. Thomas, M., Dadgar, N., Aphale, A., Harrell, J.M., Kunkel, R., Pratt, W.B. and Lieberman, A.P. (2004) Androgen receptor acetylation site mutations cause trafficking defects, misfolding, and aggregation similar to expanded glutamine tracts. *J. Biol. Chem.*, **279**, 8389–8395.
50. Burgdorf, S., Leister, P. and Scheidtmann, K.H. (2004) TSG101 interacts with apoptosis-antagonizing transcription factor and enhances androgen receptor-mediated transcription by promoting its monoubiquitination. *J. Biol. Chem.*, **279**, 17524–17534.
51. DiFiglia, M., Sapp, E., Chase, K.O., Davies, S.W., Bates, G.P., Vonsattel, J.P. and Aronin, N. (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science*, **277**, 1990–1993.
52. Matsumoto, M., Yada, M., Hatakeyama, S., Ishimoto, H., Tanimura, T., Tsuji, S., Kakizuka, A., Kitagawa, M. and Nakayama, K.I. (2004) Molecular clearance of ataxin-3 is regulated by a mammalian E4. *EMBO J.*, **23**, 659–669.
53. Jana, N.R., Dikshit, P., Goswami, A., Kotliarova, S., Murata, S., Tanaka, K. and Nukina, N. (2005) Co-chaperone CHIP associates with expanded polyglutamine protein and promotes their degradation by proteasomes. *J. Biol. Chem.*, **280**, 11635–11640.
54. Adachi, H., Waza, M., Tokui, K., Katsuno, M., Minamiyama, M., Tanaka, F., Doyu, M. and Sobue, G. (2007) CHIP overexpression reduces mutant androgen receptor protein and ameliorates phenotypes of the spinal and bulbar muscular atrophy transgenic mouse model. *J. Neurosci.*, **27**, 5115–5126.
55. Al-Ramahi, I., Lam, Y.C., Chen, H.K., de Gouyon, B., Zhang, M., Perez, A.M., Branco, J., de Haro, M., Patterson, C., Zoghbi, H.Y. *et al.* (2006) CHIP protects from the neurotoxicity of expanded and wild-type ataxin-1 and promotes their ubiquitination and degradation. *J. Biol. Chem.*, **281**, 26714–26724.
56. Tsai, Y.C., Fishman, P.S., Thakor, N.V. and Oyler, G.A. (2003) Parkin facilitates the elimination of expanded polyglutamine proteins and leads to preservation of proteasome function. *J. Biol. Chem.*, **278**, 22044–22055.
57. Warrick, J.M., Morabito, L.M., Bilen, J., Gordesky-Gold, B., Faust, L.Z., Paulson, H.L. and Bonini, N.M. (2005) Ataxin-3 suppresses polyglutamine neurodegeneration in *Drosophila* by a ubiquitin-associated mechanism. *Mol. Cell*, **18**, 37–48.
58. de Pril, R., Fischer, D.F., Roos, R.A. and van Leeuwen, F.W. (2007) Ubiquitin-conjugating enzyme E2–25K increases aggregate formation and cell death in polyglutamine diseases. *Mol. Cell. Neurosci.*, **34**, 10–19.
59. Choi, J.Y., Ryu, J.H., Kim, H.S., Park, S.G., Bae, K.H., Kang, S., Myung, P.K., Cho, S., Park, B.C. and Lee do, H. (2007) Co-chaperone CHIP promotes aggregation of ataxin-1. *Mol. Cell. Neurosci.*, **34**, 69–79.
60. Terashima, T., Kawai, H., Fujitani, M., Maeda, K. and Yasuda, H. (2002) SUMO-1 co-localized with mutant atrophin-1 with expanded polyglutamines accelerates intranuclear aggregation and cell death. *Neuroreport*, **13**, 2359–2364.
61. Steffan, J.S., Agrawal, N., Pallos, J., Rockabrand, E., Trotman, L.C., Slepko, N., Illes, K., Lukacsovich, T., Zhu, Y.Z., Cattaneo, E. *et al.* (2004) SUMO modification of Huntingtin and Huntington's disease pathology. *Science*, **304**, 100–104.
62. Ueda, H., Goto, J., Hashida, H., Lin, X., Oyanagi, K., Kawano, H., Zoghbi, H.Y., Kanazawa, I. and Okazawa, H. (2002) Enhanced SUMOylation in polyglutamine diseases. *Biochem. Biophys. Res. Commun.*, **293**, 307–313.
63. Poukka, H., Karvonen, U., Janne, O.A. and Palvimo, J.J. (2000) Covalent modification of the androgen receptor by small ubiquitin-like modifier 1 (SUMO-1). *Proc. Natl. Acad. Sci. USA*, **97**, 14145–14150.
64. Riley, B.E., Zoghbi, H.Y. and Orr, H.T. (2005) SUMOylation of the polyglutamine repeat protein, ataxin-1, is dependent on a functional nuclear localization signal. *J. Biol. Chem.*, **280**, 21942–21948.
65. Yanai, A., Huang, K., Kang, R., Singaraja, R.R., Arstikaitis, P., Gan, L., Orban, P.C., Mullard, A., Cowan, C.M., Raymond, L.A. *et al.* (2006) Palmitoylation of huntingtin by HIP14 is essential for its trafficking and function. *Nat. Neurosci.*, **9**, 824–831.
66. Pedram, A., Razandi, M., Sainson, R.C., Kim, J.K., Hughes, C.C. and Levin, E.R. (2007) A conserved mechanism for steroid receptor translocation to the plasma membrane. *J. Biol. Chem.*, **282**, 22278–22288.
67. Huang, K., Yanai, A., Kang, R., Arstikaitis, P., Singaraja, R.R., Metzler, M., Mullard, A., Haigh, B., Gauthier-Campbell, C., Gutekunst, C.A. *et al.* (2004) Huntingtin-interacting protein HIP14 is a palmitoyl transferase involved in palmitoylation and trafficking of multiple neuronal proteins. *Neuron*, **44**, 977–986.
68. D'souza, D.R., Wei, J., Shao, Q., Hebert, M.D., Subramony, S.H. and Vig, P.J. (2006) Tissue transglutaminase crosslinks ataxin-1: possible role in SCA1 pathogenesis. *Neurosci. Lett.*, **409**, 5–9.
69. Mandrusiak, L.M., Beitel, L.K., Wang, X., Scanlon, T.C., Chevalier-Larsen, E., Merry, D.E. and Trifiro, M.A. (2003) Transglutaminase potentiates ligand-dependent proteasome dysfunction induced by polyglutamine-expanded androgen receptor. *Hum. Mol. Genet.*, **12**, 1497–1506.
70. Kahlem, P., Green, H. and Djian, P. (1998) Transglutaminase action imitates Huntington's disease: selective polymerization of Huntingtin containing expanded polyglutamine. *Mol. Cell*, **1**, 595–601.
71. Green, H. (1993) Human genetic diseases due to codon reiteration: relationship to an evolutionary mechanism. *Cell*, **74**, 955–956.
72. Lesort, M., Chun, W., Johnson, G.V. and Ferrante, R.J. (1999) Tissue transglutaminase is increased in Huntington's disease brain. *J. Neurochem.*, **73**, 2018–2027.
73. Karpuj, M.V., Garren, H., Slunt, H., Price, D.L., Gusella, J., Becher, M.W. and Steinman, L. (1999) Transglutaminase aggregates huntingtin into nonamyloidogenic polymers, and its enzymatic activity increases in Huntington's disease brain nuclei. *Proc. Natl. Acad. Sci. USA*, **96**, 7388–7393.
74. Dedeoglu, A., Kubilus, J.K., Jeitner, T.M., Matson, S.A., Bogdanov, M., Kowall, N.W., Matson, W.R., Cooper, A.J., Ratan, R.R., Beal, M.F. *et al.* (2002) Therapeutic effects of cystamine in a murine model of Huntington's disease. *J. Neurosci.*, **22**, 8942–8950.
75. Van Raamsdonk, J.M., Pearson, J., Bailey, C.D., Rogers, D.A., Johnson, G.V., Hayden, M.R. and Leavitt, B.R. (2005) Cystamine treatment is neuroprotective in the YAC128 mouse model of Huntington disease. *J. Neurochem.*, **95**, 210–220.
76. Mastroberardino, P.G., Iannicola, C., Nardacci, R., Bernassola, F., De Laurenzi, V., Melino, G., Moreno, S., Pavone, F., Oliverio, S., Fesus, L. *et al.* (2002) 'Tissue' transglutaminase ablation reduces neuronal death and prolongs survival in a mouse model of Huntington's disease. *Cell Death Differ.*, **9**, 873–880.
77. Ruan, Q., Quintanilla, R.A. and Johnson, G.V. (2007) Type 2 transglutaminase differentially modulates striatal cell death in the presence of wild type or mutant huntingtin. *J. Neurochem.*, **102**, 25–36.
78. Goldberg, Y.P., Nicholson, D.W., Rasper, D.M., Kalchman, M.A., Koide, H.B., Graham, R.K., Bromm, M., Kazemi-Esfarjani, P., Thornberry, N.A., Vaillancourt, J.P. *et al.* (1996) Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract. *Nat. Genet.*, **13**, 442–449.
79. Kim, Y.J., Yi, Y., Sapp, E., Wang, Y., Cuiffo, B., Kegel, K.B., Qin, Z.H., Aronin, N. and DiFiglia, M. (2001) Caspase 3-cleaved N-terminal fragments of wild-type and mutant huntingtin are present in normal and Huntington's disease brains, associate with membranes, and undergo calpain-dependent proteolysis. *Proc. Natl. Acad. Sci. USA*, **98**, 12784–12789.
80. Miyashita, T., Okamura-Oho, Y., Mito, Y., Nagafuchi, S. and Yamada, M. (1997) Dentatorubral pallidoluysian atrophy (DRPLA) protein is cleaved by caspase-3 during apoptosis. *J. Biol. Chem.*, **272**, 29238–29242.
81. Berke, S.J., Schmied, F.A., Brunt, E.R., Ellerby, L.M. and Paulson, H.L. (2004) Caspase-mediated proteolysis of the polyglutamine disease protein ataxin-3. *J. Neurochem.*, **89**, 908–918.
82. Haacke, A., Broadley, S.A., Boteva, R., Tzvetkov, N., Hartl, F.U. and Breuer, P. (2006) Proteolytic cleavage of polyglutamine-expanded ataxin-3 is critical for aggregation and sequestration of non-expanded ataxin-3. *Hum. Mol. Genet.*, **15**, 555–568.
83. Lunkes, A., Lindenberg, K.S., Ben-Haiem, L., Weber, C., Devys, D., Landwehrmeyer, G.B., Mandel, J.L. and Trottier, Y. (2002) Proteases acting on mutant huntingtin generate cleaved products that differentially build up cytoplasmic and nuclear inclusions. *Mol. Cell*, **10**, 259–269.
84. Hermel, E., Gafni, J., Propp, S.S., Leavitt, B.R., Wellington, C.L., Young, J.E., Hackam, A.S., Logvinova, A.V., Peel, A.L., Chen, S.F. *et al.* (2004) Specific caspase interactions and amplification are involved in selective

neuronal vulnerability in Huntington's disease. *Cell Death Differ.*, **11**, 424–438.
85. Wellington, C.L., Singaraja, R., Ellerby, L., Savill, J., Roy, S., Leavitt, B., Cattaneo, E., Hackam, A., Sharp, A., Thornberry, N. *et al.* (2000) Inhibiting caspase cleavage of huntingtin reduces toxicity and aggregate formation in neuronal and nonneuronal cells. *J. Biol. Chem.*, **275**, 19831–19838.
86. Gafni, J. and Ellerby, L.M. (2002) Calpain activation in Huntington's disease. *J. Neurosci.*, **22**, 4842–4849.
87. Gafni, J., Hermel, E., Young, J.E., Wellington, C.L., Hayden, M.R. and Ellerby, L.M. (2004) Inhibition of calpain cleavage of huntingtin reduces toxicity: accumulation of calpain/caspase fragments in the nucleus. *J. Biol. Chem.*, **279**, 20211–20220.
88. Zhang, Y., Leavitt, B.R., van Raamsdonk, J.M., Dragatsis, I., Goldowitz, D., MacDonald, M.E., Hayden, M.R. and Friedlander, R.M. (2006) Huntingtin inhibits caspase-3 activation. *EMBO J.*, **25**, 5896–5906.
89. Graham, R.K., Deng, Y., Slow, E.J., Haigh, B., Bissada, N., Lu, G., Pearson, J., Shehadeh, J., Bertram, L., Murphy, Z. *et al.* (2006) Cleavage at the caspase-6 site is required for neuronal dysfunction and degeneration due to mutant huntingtin. *Cell*, **125**, 1179–1191.
90. Ellerby, L.M., Andrusiak, R.L., Wellington, C.L., Hackam, A.S., Propp, S.S., Wood, J.D., Sharp, A.H., Margolis, R.L., Ross, C.A., Salvesen, G.S. *et al.* (1999) Cleavage of atrophin-1 at caspase site aspartic acid 109 modulates cytotoxicity. *J. Biol. Chem.*, **274**, 8730–8736.
91. Colomer Gould, V.F., Goti, D., Pearce, D., Gonzalez, G.A., Gao, H., Bermudez de Leon, M., Jenkins, N.A., Copeland, N.G., Ross, C.A. and Brown, D.R. (2007) A mutant ataxin-3 fragment results from processing at a site N-terminal to amino acid 190 in brain of Machado–Joseph disease-like transgenic mice. *Neurobiol. Dis.*, **27**, 362–369.
92. Garden, G.A., Libby, R.T., Fu, Y.H., Kinoshita, Y., Huang, J., Possin, D.E., Smith, A.C., Martinez, R.A., Fine, G.C., Grote, S.K. *et al.* (2002) Polyglutamine-expanded ataxin-7 promotes non-cell-autonomous purkinje cell degeneration and displays proteolytic cleavage in ataxic transgenic mice. *J. Neurosci.*, **22**, 4897–4905.
93. Young, J.E., Gouw, L., Propp, S., Sopher, B.L., Taylor, J., Lin, A., Hermel, E., Logvinova, A., Chen, S.F., Chen, S. *et al.* (2007) Proteolytic cleavage of ataxin-7 by caspase-7 modulates cellular toxicity and transcriptional dysregulation. *J. Biol. Chem.*, **282**, 30150–30160.
94. Li, M., Miwa, S., Kobayashi, Y., Merry, D.E., Yamamoto, M., Tanaka, F., Doyu, M., Hashizume, Y., Fischbeck, K.H. and Sobue, G. (1998) Nuclear inclusions of the androgen receptor protein in spinal and bulbar muscular atrophy. *Ann. Neurol.*, **44**, 249–254.
95. Kobayashi, Y., Miwa, S., Merry, D.E., Kume, A., Mei, L., Doyu, M. and Sobue, G. (1998) Caspase-3 cleaves the expanded androgen receptor protein of spinal and bulbar muscular atrophy in a polyglutamine repeat length-dependent manner. *Biochem. Biophys. Res. Commun.*, **252**, 145–150.
96. Ellerby, L.M., Hackam, A.S., Propp, S.S., Ellerby, H.M., Rabizadeh, S., Cashman, N.R., Trifiro, M.A., Pinsky, L., Wellington, C.L., Salvesen, G.S. *et al.* (1999) Kennedy's disease: caspase cleavage of the androgen receptor is a crucial event in cytotoxicity. *J. Neurochem.*, **72**, 185–195.
97. Li, M., Chevalier-Larsen, E.S., Merry, D.E. and Diamond, M.I. (2007) Soluble androgen receptor oligomers underlie pathology in a mouse model of spinobulbar muscular atrophy. *J. Biol. Chem.*, **282**, 3157–3164.
98. Lin, H.K., Wang, L., Hu, Y.C., Altuwaijri, S. and Chang, C. (2002) Phosphorylation-dependent ubiquitylation and degradation of androgen receptor by Akt require Mdm2 E3 ligase. *EMBO J.*, **21**, 4037–4048.