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Polyglutamine Pathogenesis: Emergence of Unifying Mechanisms for Huntington's Disease and Related Disorders

Minireview

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Summary

The mechanisms of neurodegeneration in the CAG repeat polyglutamine diseases, including Spinal and Bulbar Muscular Atrophy (SBMA), Huntington's disease (HD), DentatoRubral and PallidoLuysian Atrophy (DRPLA), and Spino-Cerebellar Ataxia (SCA), have been controversial. Issues have included the role of polyglutamine aggregation and possible amyloid formation, localization in the cell nucleus, and possible proteolytic processing. Proposed mechanisms have included activation of caspases or other triggers of apoptosis, mitochondrial or metabolic toxicity, and interference with gene transcription. Recent studies using transgenic mouse and *Drosophila* models have helped resolve some of these issues and raise hopes for development of therapeutic targets.

Nine neurodegenerative disorders are caused by expanding CAG repeats coding for polyglutamine (Margolis and Ross, 2001). They include Spinal and Bulbar Muscular Atrophy (SBMA), the first to be discovered; Huntington's disease (HD), perhaps the most actively studied; DentatoRubral and PallidoLuysian Atrophy (DRPLA), which is similar to HD; and several forms of Spino-Cerebellar Ataxia (SCA). Each of the disorders is characterized by selective neuronal cell death in specific regions of the brain. While the exact areas affected in each disease differ, there is considerable overlap, including basal ganglia, brainstem nuclei, cerebellum, and spinal motor nuclei (Ross, 1995). Pathology in these regions is characteristic of the polyglutamine disorders, and is distinct from other neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease.

The genes which cause these disorders have no homology with each other except for the polyglutamine stretch itself. While the other portions of the proteins are likely to exert a modulatory effect, giving rise to some of the disease-specific features (Orr, 2001), there are likely to be common pathogenic mechanisms due to expanded polyglutamine itself. In all of the diseases, there is a striking threshold effect of the minimal polyglutamine length to cause disease. The exact length is different in each disease but is generally in the range of 35 to 45 (except for SCA 6, which may have a different mechanism from the others). All of the mutant proteins appear to undergo a conformational change and aggregate in cells, forming characteristic inclusion bodies—though their role in pathogenesis has been controversial. The causative gene with the best-characterized function is the androgen receptor (AR) of SBMA, which is a DNA binding, ligand-activated transcription factor.

The literature on the polyglutamine expansion diseases is itself rapidly expanding. Some of the major controversies in the field are surveyed in the excellent Review of Tobin and Signer (2000). Other Reviews include Merry (2001), Rubinsztein (2002), and Taylor et al. (2002). There have been many conflicting observations and hypotheses. Nevertheless, some of the major issues are becoming resolved, and potential unifying principles are emerging. Two articles in this issue of *Neuron*, using models of SBMA, have provided striking in vivo evidence for some of these principles. One study (Katsuno et al., 2002) reports a new transgenic mouse model generated using the androgen receptor with a long polyglutamine repeat. The other (Takeyama et al., 2002) involves *Drosophila* models constructed using several different AR constructs with expanded repeats. The ability to manipulate the AR within cells, using agonists and antagonists, and then assay transcriptional activation makes SBMA an attractive disease to study. Of the other diseases, HD is probably the best studied. This Minireview will highlight these two diseases in order to examine potential unifying principles (see Figures 1 and 2).

One fundamental issue is whether polyglutamine pathogenesis involves a genetic gain of function or loss of function. A genetic gain of function is suggested by the autosomal dominant inheritance of most of the disorders (except SBMA, which is X linked and possibly dominant at the cellular level) and by most of the results with cell transfection and transgenic and knock-out animal experiments. HD in particular has classic pure genetic dominance, with homozygotes having an almost identical phenotype to heterozygotes. Nevertheless, some recent evidence does point to loss of function as a contributor to pathogenesis. For instance, huntingtin appears to have a neuroprotective function and enhances production of neurotrophic factors, such as brain-derived neurotrophic factor (Cattaneo et al., 2001). The two current studies find that AR agonists greatly potentiate the disease phenotype, providing further support for a gain-of-function model. This suggests that neuronal degeneration in SBMA arises predominantly from a genetic gain of function, while the partial androgen insensitivity seen in some patients would arise from loss of receptor function in the periphery.

A contentious question has been whether pathogenesis is primarily activated in the cytoplasm or in the cell nucleus. Studies using manipulation of localization signals in polyglutamine disease proteins, including huntingtin, have concluded that a primary site of cellular toxicity is the nucleus (Saudou et al., 1998; Peters et al., 1999). However, other groups have reported a cytoplasmic site of toxicity, including two recent papers. One paper reports direct mitochondrial toxicity of mutant polyglutamine-containing proteins (Panov et al., 2002), which, if specific, could represent an interesting gain-of-function mechanism, and a striking coalescence of current genetic methodology with previous hypotheses regarding

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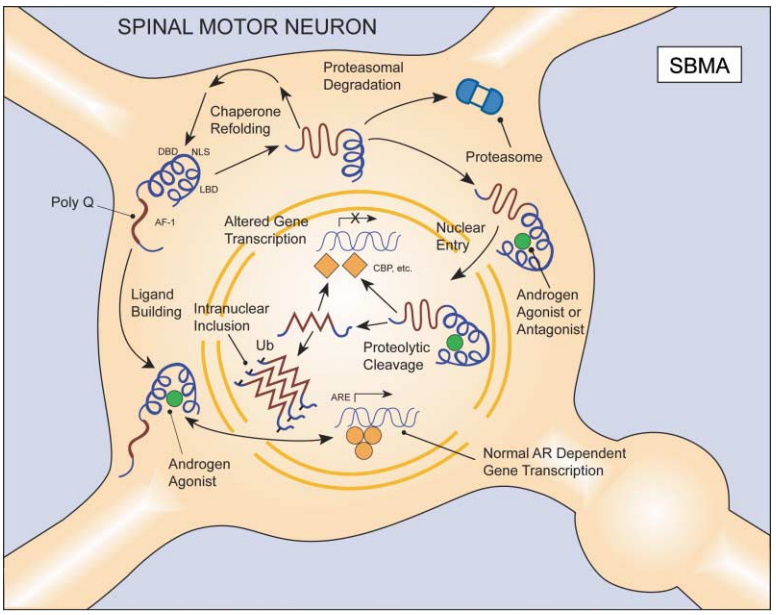

Figure 1. Model for SBMA Cellular Pathogenesis

The Androgen Receptor (AR) is cytoplasmic (and bound to heat shock proteins, not shown) in its basal state. It consists of several domains, including an N-terminal transcriptional regulatory region (AF-1), a zinc finger containing DNA binding region (DBD), a hinge region with the nuclear localization signal (NLS), and a C-terminal ligand binding domain (LBD). Upon ligand binding, there is a conformational change which results in homodimerization and dissociation from heat shock proteins (not shown) and exposure of the NLS, resulting in nuclear translocation. In the nucleus, agonist binding results in association with androgen response elements (ARE), leading to the recruitment of coactivators and activation of gene transcription. By contrast, antagonist binding results in nuclear translocation without targeting to the androgen response elements or recruitment of coactivators, and thus, no gene transcription. The pathogenesis of SBMA may result from targeting of the mutant receptor to the nucleus. The expanded polyglutamine stretch causes altered conformation of the protein. Upon binding of either agonist or antagonist, the receptor translocates to the nucleus. After proteolytic cleavage (either in the nucleus or the cytoplasm), the polyglutamine stretch assumes an altered conformation, leading to aggregation and the formation of intranuclear inclusions. The mutation may confer a gain of a novel toxic property on the AR, such as abnormal interactions with CBP, leading to loss of neuronal survival signaling.

excitotoxicity or metabolic toxicity. The other recent paper (Gervais et al., 2002) proposes a caspase 8-mediated triggering of apoptotic machinery after a chain of protein interactions—though this would appear to act via a loss-of-function mechanism.

The SBMA studies address the issue of nuclear versus cytoplasmic toxicity. In both the *Drosophila* and mouse models, androgen agonists coordinately induce nuclear translocation and toxicity. Furthermore, in the *Drosophila* paper (Takeyama et al., 2002), an ingenious twist is added to this paradigm. Some AR antagonists, unlike agonists, induce nuclear translocation of the AR but do not activate AR-dependent gene transcription. Takeyama et al. found that androgen antagonists also strongly enhanced toxicity. Furthermore, flies expressing only the N-terminal portion of the AR with an expanded repeat and the AR nuclear localization signal (but no DNA binding region or ligand binding region) also had a severe neurodegenerative phenotype, with nuclear localization of AR. These results strongly sug-

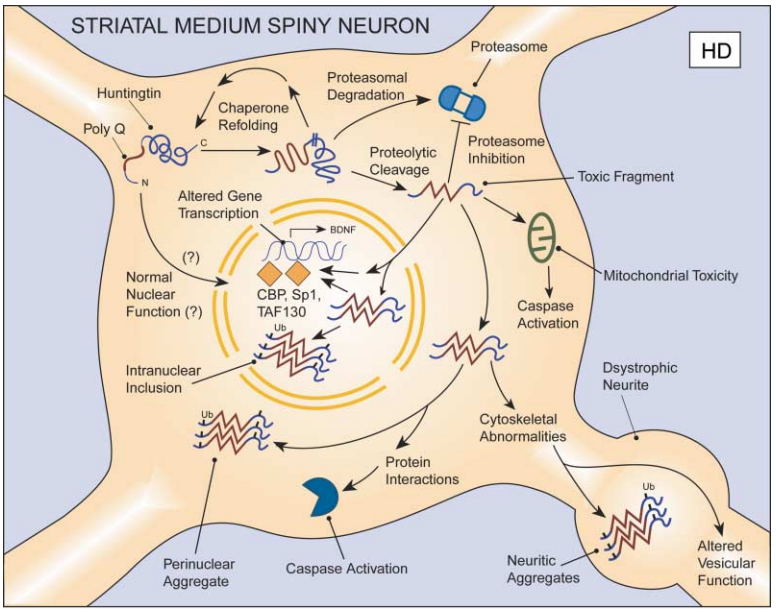

Figure 2. Model for HD Cellular Pathogenesis

Huntingtin is normally predominantly cytoplasmic. Its normal function is not well understood but likely involves cytoskeletal function or vesicle recycling (not shown). It may cycle to the nucleus and have a normal role in the regulation of gene transcription, but this is uncertain. The mutation causes a conformational change and likely leads to partial unfolding or abnormal folding of the protein, which can be corrected by molecular chaperones. Proteolytic cleavage of mutant huntingtin takes place. For simplicity, this is shown as taking place in the cytoplasm, though it may also take place in the nucleus, and may involve several steps. The N terminus with the expanded repeat can assume a β pleated sheet structure. Toxicity in the cytoplasm may be caused by mutant full-length protein or by cleaved protein, and the toxic species may be soluble monomers or oligomers or, possibly, insoluble aggregates. Toxicity may involve inhibition of the proteasome or activation of caspases directly or via mitochondrial effects. Cytoplasmic aggregates accumulate in perinuclear or neuritic regions and are ubiquitinated. The mutant protein translocates to the nucleus, where it forms intranuclear inclusions, though they are not primarily responsible for toxicity. Nuclear toxicity is believed to be caused by interference with gene transcription.

gest that the presence of the expanded polyglutamine in the nucleus is sufficient to induce toxicity. They also suggest that the "gain" may be a novel function and not one related to the AR's usual action to activate gene transcription.

What might be the molecular mechanisms of polyglutamine toxicity in the nucleus? A logical candidate would be interference with gene transcription. Several recent studies have suggested abnormal interactions with nuclear coactivator or corepressor molecules, including p53, CBP, Sp1, and TAF130 (Steffan et al., 2000; McCampbell et al., 2000; Nucifora et al., 2001; Dunah et al., 2002). The details of the molecular interactions in the different studies differ. In some of them (Steffan et al., 2000; Dunah et al., 2002), interactions take place between the normal polyglutamine protein and the candidate transcription factors and are altered by the expansion mutation. For instance, in the Dunah et al. (2002) study, both normal and mutant huntingtin interact with Sp1 and TAF130 and inhibit transcription, but the interaction of mutant huntingtin is stronger. Data from post-mortem HD brain material support the involvement of these molecules, beginning very early in the disease course. However, in this model, higher concentrations of normal huntingtin might be expected to have similar effects on Sp1 and TAF130 as mutant huntingtin, but, in many previous cell and mouse studies, expression of normal huntingtin generally does not cause significant toxicity.

In a different kind of mechanism, originally proposed by Perutz and supported by data from the labs of Housman, Paulson, and others, mutant polyglutamine assumes an altered conformation and binds to short polyglutamine stretches normally present in many transcription factors and transcriptional coactivators, such as Cyclic Adenosine Monophosphate Response Element Binding Protein (CBP). This polyglutamine interaction is postulated to alter CBP's structure. Cell transfection studies have shown that this effect can remove CBP from its normal site of survival-promoting action within cells, causing toxicity (McCampbell et al., 2000; Nucifora et al., 2001). This is only seen with the mutant protein, consistent with the idea of a gain of a novel function. However, at present, in vivo support for this mechanism is sparse.

Other interactions could also take place in the nucleus. Another idea is that the abnormal polyglutamine proteins are targeted for the proteasome but cannot be digested and thus inhibit proteasome activity, potentially resulting in toxicity. Cell transfection data support such a mechanism for mutant huntingtin (Bence et al., 2001). The assay used in this study could show loss of cytoplasmic proteasome activity, but proteasomes are present in both the cytoplasm and the nucleus. Also in its favor, this is another mechanism involving a gain of a novel function.

Another controversial issue involves the possible relation between polyglutamine toxicity and the formation of polyglutamine aggregates and inclusions. Expanded polyglutamine aggregates both in vitro and in vivo and forms characteristic inclusion bodies. The aggregates have many of the characteristics of amyloid and are composed of fibers with β sheet structure, like the amyloid of Alzheimer's disease. The threshold for polyglutamine aggregation in vivo and in vitro is strikingly similar to the threshold for disease in human patients, as shown by Wanker's laboratory. However, the neurons with aggregates are not necessarily the neurons most likely to die, and polyglutamine aggregation and cell toxicity can be experimentally dissociated both in vitro and in vivo.

Thus, the relationship between aggregation and toxicity is currently unknown (See Figure 1 of Tobin and Signer, 2000). The recent study of Dunah et al. (2002) proposed that interference with gene transcription and cell toxicity is caused by soluble monomeric polyglutamine species. By contrast, the studies in which mutant polyglutamine is proposed to interact with short polyglutamine stretches present in CBP suggest an interaction in some way related to polyglutamine aggregation, and CBP has been detected in polyglutamine containing inclusions. However, it is also possible that abnormal polyglutamine interactions could involve monomeric protein, causing an altered conformation of monomeric CBP, leading it to be degraded by the proteasome or other cellular processes. One interesting twist on the polyglutamine interaction hypothesis is that the expanded polyglutamine in mutant huntingtin could interact with the normal polyglutamine in the huntingtin expressed via the other allele, potentially leading to partial inactivation of normal huntingtin. This would be a dominant-negative interaction, which could mediate a loss of huntingtin function.

When considering the issue of aggregation, it may be too simplistic to limit the models for the postulated toxic species to either soluble monomers or insoluble fibrilar aggregates. Protein aggregation and fiber formation leading to amyloid is a complex biochemical process and is likely to have a number of intermediate stages, including oligomeric species, and small nascent fibrils, termed "protofibrils." Such intermediates have been reported for huntingtin (Poirier et al., 2002). The AR transgenic mouse study (Katsuno et al., 2002) demonstrated that nuclear label for the AR which appears diffuse at the light microscope level consists of small "microaggregates" at the electron microscope level. A mechanism involving aggregation intermediates would be consistent with recent studies of Parkinson's and Alzheimer's diseases and thus has the attractive feature that it could be a general mechanism for protein misfolding neurodegenerative diseases (reviewed in Taylor et al., 2002). It is possible that several different species—monomer, soluble intermediate, and insoluble aggregate—could all have differing deleterious effects on cell function.

One final question, which remains highly contentious, is the possible role of proteolytic cleavage in polyglutamine pathogenesis. A consistent observation has been that antibodies to the N terminus of huntingtin, but not antibodies to epitopes in the middle of the protein or at the C terminus, recognize the intranuclear inclusions in HD. A number of studies have suggested a role for proteolytic cleavage of polyglutamine proteins (Ellerby et al., 1999; Li et al., 2000; Toneff et al., 2002). The fact that many of the polyglutamine proteins, including the androgen receptor (Ellerby et al., 1999), can be cleaved by caspase enzymes has suggested that caspase cleavage might be involved. However, other studies have equally strongly argued that full-length proteins

are the most relevant species (Dyer and McMurray, 2001; Wheeler et al., 2000).

The two recent studies of the AR provide striking correlative evidence for the existence of a proteolytic fragment of the AR. In the mouse study, truncated fragments of the AR were observed, though the relation to toxicity was not assessed. In the *Drosophila* study, truncated fragments containing the polyglutamine expansion were also observed. An additional model generated using a truncated fragment with the expanded repeat showed enhanced toxicity.

Recent data have provided additional evidence for the cleavage of huntingtin and considerably clarified its nature (Lunkes et al., 2002). In both a stable inducible cell model of HD and in material from HD post-mortem brains, there was evidence for several cleavage events, generating fragment sizes of mutant huntingtin smaller than would be predicted by caspase cleavage. In the cell model, it could be shown that this cleavage was not mediated by the proteasome, as proteasome inhibition actually increased the amount of the fragment. Cell transfection and inhibitor studies indicated that the most relevant fragment was generated by aspartic endopeptidases, though their nature was not identified. These studies are still correlative, and the relation of this cleavage to toxicity has not yet been reported. If proteolytic cleavage could be established as a central pathogenic event, it might be an excellent therapeutic target, since pharmacologic inhibition of proteolytic enzymes may be feasible.

The past few years have seen rapid progress in the study of polyglutamine pathogenesis, though there are still many uncertainties. Some unifying themes do appear to be emerging. There are qualifications, but often in genetics (and biology in general) the exceptions to any simplifying rules may help illuminate the complexities of the system. The two papers on the AR provide strong evidence for the gain-of-function hypothesis and the idea that nuclear events are central to pathogenesis, though with possible contributions from loss-of-function and cytoplasmic toxicity. Roles for gene transcription, intermediates in an aggregation pathway as toxic species, and proteolytic cleavage are intriguing but not yet proved. While the field has not yet resolved all of the controversies, progress toward identifying key toxic mechanisms is being made. Of course, there is likely to be more than one single pathway to toxicity, and the different diseases are each likely to have their own additional twists. Answering some of these questions may help elucidate pathogenic mechanisms relevant to other neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, and will provide targets for therapeutic intervention.

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