

HUMAN GENETICS '99: TRINUCLEOTIDE REPEATS

Protein Fate in Neurodegenerative Proteinopathies: Polyglutamine Diseases Join the (Mis)Fold

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Protein misfolding is now recognized to be a central feature of neurodegenerative diseases. Alzheimer disease (AD), Parkinson disease (PD), amyotrophic lateral sclerosis (ALS), prion diseases, and the polyglutamine (polyglu) diseases all represent proteinopathies—diseases in which a particular protein or set of proteins misfolds and aggregates. In the past decade, mutations have been identified in an increasing number of genes underlying specific forms of neurodegeneration—for example, genes encoding amyloid precursor protein (APP) and presenilins 1 and 2 in AD, parkin and alpha-synuclein in PD, Cu/Zn superoxide dismutase in ALS, tau in frontotemporal dementia, prion protein in Creutzfeldt-Jacob and Gerstmann-Straussler diseases, and at least eight different proteins in the polyglu diseases (Hardy and Gwinn-Hardy 1998; Price et al. 1998). Identification of these mutations was a first step in understanding the disease process; next, it will be necessary to explain how specific changes in disease proteins may alter the neuron's physiology and to further question how neuronal responses to the mutant protein influence the disease process. This review examines the fate of mutant protein in neurons and the potential role of posttranslational events in pathogenesis, with particular focus on the polyglu diseases.

Posttranslational events have already been implicated in various neurodegenerative diseases (Hardy and Gwinn-Hardy 1998; Price et al. 1998). For instance, mutations in familial AD—whether in APP or presenilins 1 or 2—influence the processing of amyloid precursor protein, leading to increased production of amyloidogenic forms of A- β peptide (Price et al. 1998). In frontotemporal dementia linked to chromosome 17, mutations in the microtubule-associated protein tau reduce its ability to bind to and promote the assembly of microtubules (Hong et al. 1998). In a recently identified

hereditary form of PD, a mutation in the enzyme ubiquitin carboxy-terminal hydrolase L1 implicates the ubiquitin-proteasome-degradation pathway in pathogenesis (Leroy et al. 1998). Similarly, the amino-terminus of parkin, the disease protein in another form of familial parkinsonism, resembles ubiquitin, suggesting that the molecular mechanism in this disease also involves protein-degradation pathways (Kitada et al. 1998). Several posttranslational events may contribute to pathogenesis in polyglu diseases, including one or more listed in table 1. Some of these modifications of polyglu proteins can be observed *in vitro* or in cell culture, but evidence from human disease tissue is still lacking (e.g., transglutamination).

Polyglu expansion is now recognized to be a major cause of inherited neurodegenerative disease. To date, eight polyglu diseases have been identified (table 2). All eight are caused by expansion of a CAG repeat that encodes an abnormally long glutamine repeat in the otherwise unrelated disease proteins. These progressive, ultimately fatal disorders typically begin in adulthood. The diseases display distinct, yet overlapping, clinical and pathological findings. However, in each disease the phenotype and severity can vary greatly, primarily because of differences in repeat length. Longer repeats cause more severe disease that begins earlier in life, sometimes even in early childhood. With the very longest glutamine repeats, the individual diseases seem to lose some of their specific clinical and pathological features, becoming characterized instead by a more extensive, less selective pattern of neurodegeneration. Very long polyglu expansions may be so neurotoxic that they override their specific disease context, causing a more widespread “glutaminopathy.”

Polyglu Protein Misfolding and Aggregation

The basis of these diseases is a dominant toxic gain of function that occurs at the protein level and that increases with longer glutamine repeats. The threshold for disease (and the dominant toxic property) is a glutamine repeat of ~35–40 residues. As shown in table 1, there are exceptions: the much shorter repeat in spinocere-

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Table 1**Protein Fate in Polyglutamine Diseases: Potential Mediators/Modulators of Pathogenesis**

Posttranslational Event	Component, Enzyme, or Pathway
Protein refolding/ disaggregation	Chaperone proteins
Degradation of misfolded polypeptide	Ubiquitin-proteasome pathway
Cleavage of mutant protein	Specific protease(s)
Altered nuclear transport	Passive diffusion or active transport
Crosslinking of mutant protein	Transglutaminase
Altered binding to normal substrates	Disease-specific interacting proteins

bellar ataxia type 6 (SCA6) suggests, perhaps, a different mechanism related to the calcium-channel properties of the disease protein. In SCA3/Machado-Joseph disease (SCA3/MJD), there is a gap between the largest normal repeat (40) and the smallest expanded repeat (55), so that an exact threshold cannot be determined. Evidence increasingly suggests that the novel toxic property is misfolding of the polyglu domain. Unique structural features of polyglu (Perutz et al. 1994) likely cause it to adopt an altered conformation when it is expanded (perhaps a β -sheet hairpin structure). Direct evidence for an altered conformation is the existence of antibodies that specifically bind expanded, but not normal, polyglutamine (e.g., Trottier et al. 1995). A vast amount of indirect evidence also suggests misfolding: numerous studies in transfected cells and transgenic animals have shown that expanded polyglu forms insoluble aggregates, presumably derived from misfolded protein (for example, Ikeda et al. 1996; Paulson et al. 1997; Cooper et al. 1998; Martindale et al. 1998; Merry et al. 1998). In vitro studies of recombinant huntingtin fragments have been particularly informative. Scherzinger et al. (1997) demonstrated that huntingtin polyglu aggregation is a self-driven process that occurs in a concentration- and repeat length-dependent manner and results in the formation of amyloid-like insoluble fibrils. The threshold repeat length for aggregation closely mirrors the threshold for disease, supporting the view that polyglu misfolding is the dominant toxic property. It remains controversial whether the aggregation that results from this misfolding contributes to pathogenesis or is simply a by-product of the disease process.

A major clue that protein misfolding actually occurs in vivo was the discovery of neuronal intranuclear inclusions (NIs) containing the mutant protein (fig. 1). These spherical, ubiquitinated aggregates have now been described in all but two polyglu diseases and in many transgenic models (table 1) (see also Warrick et al. 1998;

Davies et al. 1997); intriguingly, the two exceptions, SCA2 and SCA6, have the shortest glutamine repeats. In addition, aggregates *outside* the nucleus have been noted in HD (dystrophic neurites or neuropil aggregates), and increased perinuclear staining for ataxin-2 in SCA2 may represent aggregated material (Huynh et al. 1999).

The discovery of NIs was exciting because they seemed to represent a unifying pathological structure found primarily in the neurons most susceptible to degeneration. This fact suggested that NI formation might be important both to pathogenesis and to the selective vulnerability seen in each disease. On the basis of recent findings, however, the link between NIs and neuronal susceptibility now seems less compelling. For example, certain striatal neurons are among the most vulnerable neurons in HD but reportedly do not develop abundant NIs (C.-A. Gutekunst and S. Hersch, personal communication). Mouse models of disease also suggest a dissociation between NIs and degeneration: transgenic mice expressing a fragment of mutant huntingtin have massive numbers of NIs, with relatively little neurodegeneration (Davies et al. 1997), whereas mice expressing full-length mutant huntingtin undergo significant striatal degeneration without many NIs (Reddy et al. 1998). An in vitro neuronal model of HD also failed to show a correlation between neuronal death and NI formation, suggesting that NIs may be irrelevant or even beneficial (Saudou et al. 1998). Most importantly, recent results in the study of SCA1 transgenic mice strongly suggest that visible nuclear aggregates are not required for the initiation of pathogenesis (Klement et al. 1998).

These findings notwithstanding, it is still too early to conclude that NIs have no role in pathogenesis. The slow progression of polyglu disease in humans, together with results from transgenic mouse models, suggest a multi-step model of pathogenesis characterized by at least two phases, an early and prolonged period of neuronal dysfunction and a later period of neuronal demise. If NIs do play a role in pathogenesis, it is likely to be late, near the point of neuronal demise. One way that NIs might compromise neuronal function is by sequestering transcription factors or other regulatory nuclear proteins. Many transcription factors contain glutamine-rich or pure polyglu domains, and recent studies of transfected cells, transgenic flies, and human tissue all indicate that NIs can sequester certain other polyglu-containing proteins, such as the basal transcription-factor TATA-binding protein (Perez et al. 1998).

Importance of Nuclear Localization

Although the role of NIs remains uncertain, nuclear expression of polyglu proteins is clearly implicated in pathogenesis. In SCA1 transgenic mice, neuronal degen-

Table 2**Polyglutamine Diseases**

Disease ^a	Protein	Repeat Length in Disease	Evidence for Aggregation
HD	Huntingtin	38–180	NI, dystrophic neurites
DRPLA	Atrophin	49–88	NI
SBMA	Androgen receptor	38–65	NI
SCA1	Ataxin-1	39–83	NI
SCA2	Ataxin-2	34–59	Dense cytoplasmic staining (?)
SCA3/MJD	Ataxin-3	55–84	NI
SCA6	Calcium channel	21–30	?
SCA7	Ataxin-7	34->200	NI

NOTE.—References for aggregation in disease tissue are the following: DiFiglia et al. (1997); Paulson et al. (1997); Becher et al. (1998); Holmberg et al. (1998); Li et al. (1998); Huynh et al. (1999).

^a DRPLA = dentatorubral-pallidolysian atrophy; SBMA = spinobulbar muscular atrophy.

eration does not occur when mutant ataxin-1 is targeted to the cytoplasm instead of to its normal nuclear location (Klement et al. 1998). Also, in transfected neurons, mutant huntingtin induces apoptosis only when the protein is localized to the nucleus (Saudou et al. 1998). Several studies further suggest that, compared with the cytoplasm, the nuclear environment favors aggregation (Klement et al. 1998; Perez et al. 1998), perhaps because the nucleus is less efficient than the cytoplasm at degrading, refolding, or disaggregating misfolded protein. Alternatively, the intricate structure of the nucleus might concentrate mutant proteins in subdomains that foster aggregation. Whatever the mechanism, one wonders whether there is a connection between the aggregation-promoting property of the nucleus and its being the site of toxicity. Ways that mutant protein, either as misfolded monomers or as aggregates, might alter vital nuclear functions are easy to imagine. The existence of glutamine-rich transcription factors suggests that mutant protein might bind such factors inappropriately, altering transcription of genes that are critical for neuronal function; other nuclear functions, such as mRNA splicing or the export of proteins and RNA to the cytoplasm, could be disrupted in a similar manner. Altered mRNA processing is a particularly intriguing hypothesis, because it has been suggested to underlie degeneration in the motor-neuron diseases, ALS and spinal muscular atrophy (Lin et al. 1998; Pellizzoni et al. 1998) and because at least two polyglutamine disease proteins (ataxin-2 and atrophin) contain motifs found in RNA-splicing proteins.

The importance of nuclear localization raises its own set of questions. First, in diseases such as SCA2, in which the protein is apparently cytoplasmic and NIs have not been observed, will the nucleus still be a primary site of pathogenesis? Second, do NIs preferentially form within particular subnuclear domains, such as promyelocytic leukemia protein (PML) nuclear bodies (Hodges et al.

1998), and, if so, does this disrupt nuclear function in predictable ways? Third, how does a large cytoplasmic protein like huntingtin accumulate in the nucleus, and why does this happen only in the disease state? A relatively small polyglutamine protein like ataxin-3 may be able to enter the nucleus by passive diffusion, but transport of full-length huntingtin and other large polyglutamine proteins would require karyopherin-mediated transit through the nuclear pore. Data from cellular and transgenic models are most consistent with a fragment of huntingtin entering the nucleus (Cooper et al. 1998; Martindale et al. 1998). Whether this fragment is preferentially generated from mutant huntingtin remains to be seen.

Proteases and Chaperones in Polyglutamine Disease

In AD, the role of proteolytic processing in pathogenesis is widely accepted. In polyglutamine diseases, such a role for proteolysis is also much anticipated but has not yet been established. Several converging lines of evidence make the proteolysis model compelling. First, immunohistochemical studies of at least three diseases indicate that polyglutamine-containing fragments, not the full-length protein, are the constituents of NIs (ataxin-1 inclusions in SCA1 may be an exception). Second, proteolytic fragments of disease protein have been reported in the HD brain (DiFiglia et al. 1997) and in cell-based models of several diseases; in some of these cellular models, proteolytic fragments are preferentially seen with expanded polyglutamine protein (Goldberg et al. 1996; Merry et al. 1998). Third, studies of recombinant protein and transfected cells have shown that many disease proteins are substrates for caspases (Goldberg et al. 1996; Wellington et al. 1998). Finally, polyglutamine-containing fragments that have been freed from their surrounding protein context are particularly potent pathogenic molecules that are

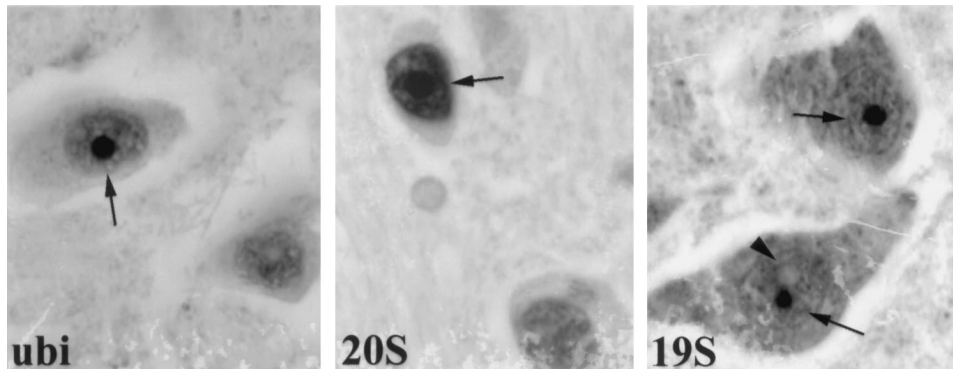


Figure 1 NIs in SCA3/MJD contain the proteasome. Pontine neurons from disease brain contain NIs (*arrows*) that immunohistochemically stain for ubiquitin (*left*), the 20S proteasome core (*middle*), and the 19S cap of the proteasome complex (*right*) (immunostaining for the disease protein, ataxin-3, is not shown). The arrowhead (*right*) indicates a nucleolus. Sections were not counterstained.

more prone than full-length protein to misfold, aggregate and cause cell death in cell-based and transgenic models (Ikeda et al. 1996; Paulson et al. 1997; Martindale et al. 1998; Merry et al. 1998). Although this last point does not constitute evidence for proteolysis, it suggests how proteolysis might accelerate the disease process. However, well-characterized, disease-specific proteolytic fragments are still lacking. Moreover, evidence in SCA1 suggests that pathogenesis and aggregation may occur via the full-length protein in this disease. Definitive answers will most likely come from transgenic models, in which potentially relevant proteolytic fragments should be easier to characterize.

Cells have a complex array of chaperone proteins that assist in the folding, refolding, and elimination of proteins under both normal and stress conditions such as heat shock. If the central problem in polyglN disease is protein misfolding, then the cell might be expected to mount a chaperone stress response that assists in the refolding, elimination, and/or disaggregation of expanded polyglN protein. Studies of at least two diseases, SCA1 and SCA3/MJD, suggest that this may indeed be the case. In disease tissue and in transfected cells, specific cellular chaperones are redistributed into NIs and polyglN aggregates, most notably heat-shock protein (Hsp) 40 family members (Bailey et al. 1998; Cummings et al. 1998; Paulson et al. 1998). In certain transfected cell lines, polyglN aggregates also cause an upregulation of Hsp70 that colocalizes to the aggregates; however, neurons from the SCA3/MJD brain only rarely show induced Hsp70. Hence, if this response occurs in human disease, it must take place near the point of neuronal death (Paulson et al. 1998; author's unpublished data). The simplest interpretation of chaperonin redistribution is that it is an appropriate cellular response to handle misfolded and aggregated polypeptides. It is possible,

however, that this redistribution into aggregates could titrate out chaperonins that otherwise would serve normal chaperoning functions in the neuron. Importantly, overexpression of the Hsp40 protein, human DnaJ homolog HDJ-2/HSDJ, can reduce the aggregation of polyglN proteins (Cummings et al. 1998; Paulson et al. 1998). This ability to reduce aggregation by overexpressing chaperonins now allows researchers to test directly whether modulating aggregation has a beneficial or deleterious effect. A fly model of polyglN disease (Warrick et al. 1998) that recapitulates many cellular features of polyglN disease, including NI formation and late-onset degeneration, will be particularly useful for such experiments.

Another major intracellular pathway for the recognition and handling of abnormal protein is the ubiquitin-proteasome pathway, which is also implicated in polyglN disease. The proteasome complex is responsible for the ubiquitin-dependent degradation of most cytosolic proteins, including misfolded or damaged proteins. Proteasomes are abundant in neurons, where precisely controlled protein degradation is probably required to maintain neuronal function. Several recent studies have shown redistribution of the proteasome complex into NIs (Bailey et al. 1998; Cummings et al. 1998; Paulson et al. 1998). As shown in figure 1, the two major components of the proteasome complex, the 20S proteolytic core and the 19S regulatory cap, both redistribute into NIs in SCA3/MJD. It is unclear whether proteasomal redistribution in polyglN disease benefits or harms neurons. A working model is that the proteasome represents a first-line cellular defense that recognizes and eliminates misfolded polyglN protein before it aggregates. This model of proteasome action is supported by studies showing that proteasome inhibitors promote aggregation of moderately expanded polyglN repeats, possibly

by increasing the intracellular concentration of misfolded monomers (Chai et al., in press). If proteasome redistribution into NIs effectively depletes the neuron of functioning proteasomes, the result would be deleterious to the cell: fewer active proteasomes to degrade abnormal protein, which would lead to a further increase in misfolded polyglu protein. It will be important to determine, by means of reconstitution experiments, how well the proteasome degrades expanded polyglu protein. To be degraded by the proteasome, a protein must first be unfolded, to enter the central proteolytic chamber of the 20S core. Expanded polyglu may adopt an energetically stable structure that resists unfolding and thus blocks entrance to the chamber or that causes incomplete degradation of the polypeptide.

Disease Progression and Selective Vulnerability: Relationship to Misfolding

Two fundamental characteristics of neurodegenerative proteinopathies are the delay in onset and the selective vulnerability. Polyglu diseases are no exception to this general rule. Signs of disease typically do not begin until adulthood, despite expression of the mutant protein throughout life. This delay suggests either an accumulation of a toxic product (possibly protein aggregates) or cumulative damage to one or more critical cellular processes, such as mitochondrial oxidative phosphorylation (Knight et al. 1999 [in this issue]), axonal transport, subnuclear organization, or proteasome function. In either case, the toxic product or cumulative damage likely reaches a threshold level beyond which the neuron can no longer fully compensate.

Despite widespread expression of the various disease proteins, certain populations of neurons are particularly susceptible to degeneration, and the basis of this vulnerability remains a crucial question. Selective vulnerability is not absolute but, rather, represents a continuum of susceptibility in different neuronal populations. For instance, in the most severe early-onset cases due to the longest repeats, degeneration is rather widespread, and, even in typical adult-onset cases, the pathological changes in advanced disease extend beyond the neuronal populations that are known to be most susceptible.

Factors contributing to selective vulnerability can be grouped broadly into two categories: those that increase the level of misfolded protein or directly promote misfolding, and those that act downstream of misfolding. The expression level of the disease protein clearly falls into the first category. Although the various disease proteins are widely expressed in the brain, absolute expression levels in different neurons certainly will vary, which will contribute to the differences in intracellular concentration of misfolded monomer. Some of the post-translational modifications discussed here also would

fall under the first category. In susceptible neurons, for example, misfolding and aggregation could be promoted by specific proteolytic events that release a polyglu fragment or by aberrant targeting of polyglu protein to the nucleus. In addition, specific interacting proteins are likely to contribute to selective vulnerability through both categories. For instance, certain interacting proteins may bind to disease proteins in a way that promotes misfolding and aggregation, as was recently demonstrated for the huntingtin interacting protein, SH3-containing Grb2-like protein (SH3GL3) (Sittler et al. 1998). Other specific interacting proteins are likely to influence events downstream of misfolding, through mechanisms that are linked to the specific functions of the disease proteins. For example, in each disease, a subset of specific interacting proteins may bind more or less avidly to the mutant protein, thereby altering physiological or biochemical properties of one or both proteins. The susceptibility of a neuron to the downstream effects of the mutant protein would depend, in part, on the particular interacting proteins it expresses. The ataxin-1 interacting protein LANP (cerebellar leucine-rich acidic nuclear protein) is just one of a number of recently identified interacting proteins that may contribute in this manner to selective vulnerability (Matilla et al. 1997).

The recent profusion of mammalian and invertebrate transgenic models of polyglu disease will aid considerably in defining cellular pathways and proteins that mediate or modulate pathogenesis. In particular, genetic screens in *Drosophila* models should prove especially useful in identifying modifiers of misfolding and degeneration (Jackson 1998; Warrick et al. 1998). As modifiers are identified, we can expect that some will tie into one or more of the cellular pathways described here, thus leading the way toward novel therapies for these fatal diseases.

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