

# Reversing Neurodegeneration: A Promise Unfolds

# Minireview

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Few human ills have seemed as hopelessly intractable as neurodegenerative diseases. Until recently, there has been little reason for optimism that we might halt the degeneration, let alone cure it. But the work of René Hen and colleagues (Yamamoto et al., 2000), reported in this issue of *Cell*, shows that it is possible to arrest neuronal degeneration, at least in early stages of the disease process. By creating a conditional mouse model of Huntington's disease (HD) that uses the tetracycline-responsive gene system—and thus allows transgene expression to be turned off by oral administration of tetracycline—they discovered that HD-like pathology is dependent on continuous expression of a fragment of huntingtin with an expanded polyglutamine tract.

## *Glutamine Repeat Diseases:*

### *Progressive Neurodegeneration*

Huntington's disease is one of the more familiar members of a group of neurodegenerative disorders known as triplet repeat diseases. HD and the spinocerebellar ataxias (SCAs), among others, result from the expansion of an unstable CAG trinucleotide repeat tract in the relevant disease gene that causes an expanded stretch of glutamines in the resultant proteins (Kaytor and Warren, 1999). The polyglutamine tract itself seems to be the source of toxicity: transgenic mice expressing either intact protein with an expanded polyglutamine tract or a truncated protein containing the polyglutamine expansion develop neurological phenotypes and neuropathology reminiscent of the human diseases (see Lin et al., 1999 and references therein). Transgenic mice expressing protein with a normal polyglutamine tract (or null mice lacking the relevant protein function) do not develop the disease phenotype. Although the chief sites of neuropathology vary from one triplet repeat disease to the next, and the function of most of the proteins remains unknown, this gain-of-function mechanism predominates and all the diseases are progressive. The longer the repeat tract, the more severe the disease and the earlier its onset. In HD, as in most of the other triplet repeat diseases, the mutant proteins form nuclear aggregates that have resisted the most strenuous biochemical efforts to solubilize them. Even truncated proteins with an expanded polyglutamine tract form these aggregates; indeed, truncated proteins seem to be even more toxic than the full-length proteins. It is important to note that symptoms arise from neuronal dysfunction

long before neuronal death becomes significant. Although apoptosis plays a role in some neurological diseases, data from both humans and animal models indicate that neurodegeneration is often a protracted process that culminates in cell death only after a prolonged period of visible disease.

### *The Model of Reversible Neurodegeneration*

Could this process be slowed if expression of a mutant polyglutamine protein were suppressed after the disease process has begun? This is the question that Yamamoto et al. address in their elegantly simple study. They first established a line of mice carrying a transgene with the tetO bidirectional promoter flanked by a *lacZ* reporter gene and exon 1 of huntingtin (*htt*) containing 94 CAG repeats (HD94). They then crossed these mice with transgenics expressing the tetracycline-regulated transactivator (tTA) under the control of the calcium/calmodulin kinase IIa promoter, which have high expression of the transgene in the forebrain (including the striatum, a primary site of HD pathology). Expression of tTA activates expression of both genes flanking the tetO promoter. Thus, in double transgenic mice both transgenes are constitutively expressed. Doxycycline, a derivative of tetracycline that readily crosses the blood/brain barrier, abolishes expression from the tetO promoter by binding to tTA and decreasing its affinity for tetO. Interestingly, in order to obtain the expected yield of double transgenic offspring, pregnant females from the heterozygote crosses had to be treated with doxycycline between gestation day E15 and birth. The explanation for this is somewhat unclear. Full-length mutant huntingtin with an expanded polyglutamine tract rescues the embryonic lethality caused by the absence of wild-type huntingtin (Hodgson et al., 1999). The embryonic lethality seen in the double transgenic mice could reflect the greater toxicity expected of the truncated huntingtin with an expanded polyglutamine tract, or it could indicate ectopic and/or high expression of huntingtin due to the calcium/calmodulin kinase II $\alpha$  promoter.

The double transgenic HD94 mice, in which the bidirectional transgene expression was activated by the removal of doxycycline at birth, revealed high levels of both HD94 and *lacZ* in the striatum, cortex, and hippocampus. A majority of striatal neurons displayed immunoreactivity to a huntingtin antibody, showing diffuse nuclear staining as well as nuclear aggregates. By 8 weeks of age, striatal morphological alterations in the HD94 mice included a reduced size, reactive gliosis, and a decrease in D1 receptors (a feature seen in HD patients). All of the HD94 mice at this age also showed a behavioral abnormality common to mouse models of HD: when suspended by their tails, they clasp their limbs. This behavioral phenotype grew more severe over time. By 20 weeks some of the HD94 mice also developed a mild tremor.

To ascertain whether disease progression required continuous expression of HD94, doxycycline was administered to the double transgenic mice for 16 weeks, beginning at 18 weeks of age. *LacZ* staining showed

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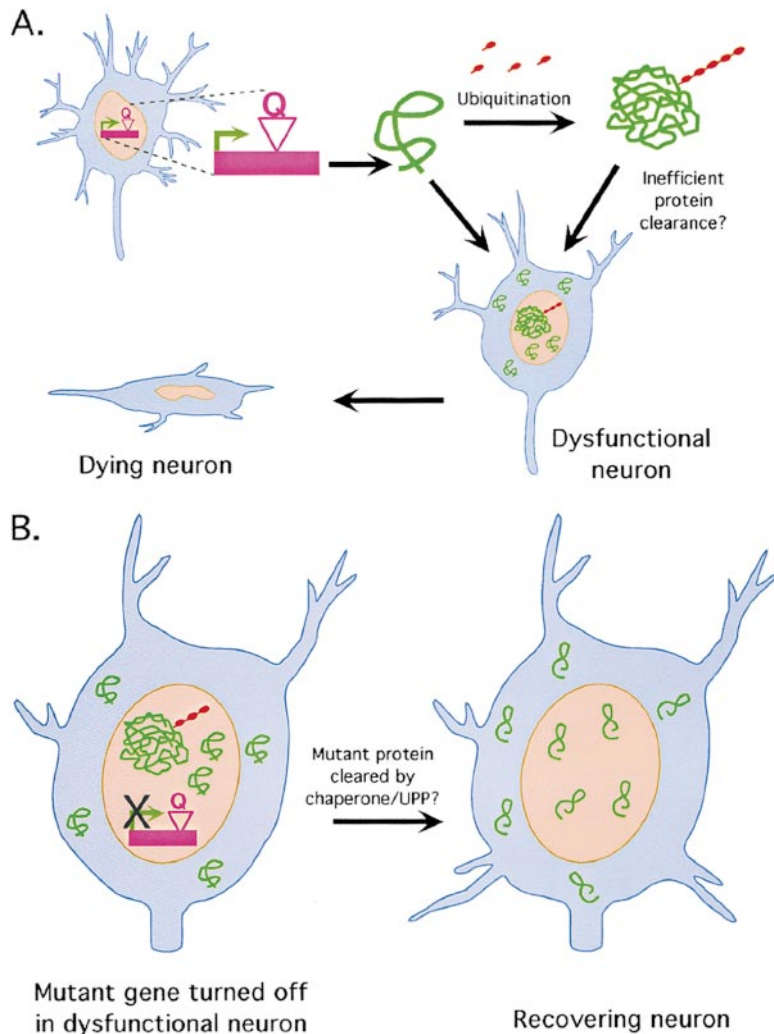


Figure 1. A Model of Polyglutamine-Induced Neurodegeneration and Its Reversal

(A) Expression of a mutant peptide with expanded polyglutamine (Q) tract leads to neuronal degeneration and eventual death. Note that both aggregated and solitary protein induce neuronal dysfunction that precedes death.

(B) Turning off mutant protein expression gives the neuron enough of a reprieve to clear the mutant protein by the chaperone/ubiquitin-proteasome pathway (UPP) and subsequently recover at least some function.

that the doxycycline regimen abolished expression of the transgene. After 16 weeks of treatment the polyglutamine aggregates disappeared in the "gene-off" mice, and all the progressive striatal changes (decrease in size, gliosis, and loss of D1 receptors) were arrested. More promising still, neurological function was partially restored by the doxycycline treatment: the intensity of clasping behavior decreased to a level comparable to that seen in the double transgenics at 8 weeks of age, while the duration of clasping reversed to levels comparable to those of control mice.

#### **Does Impaired Protein Clearance Cause Neurodegeneration?**

Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), prion encephalopathies, and the polyglutamine diseases are all characterized by the accumulation of protein either outside the cell (e.g., amyloid plaques in AD) or within it (e.g., nuclear inclusions in HD and cytoplasmic aggregates in PD and ALS) (Kaytor and Warren, 1999). Although intracellular aggregates are a common feature of polyglutamine disorders, they do not initiate disease. They seem instead to manifest a neuron's efforts to cope with the mutant protein (Klement et al., 1998; Cummings et al., 1999). The

expanded polyglutamine tract could make the protein resistant to degradation by altering its native structure; in vitro studies show that ataxin-1 with an expanded polyglutamine tract is less efficiently degraded than its wild-type counterpart, although both are ubiquitinated equally well (Cummings et al., 1999). The colocalization of various chaperones, ubiquitin, and components of the proteasome with the aggregated proteins further supports this model (Alves-Rodrigues et al., 1998; Lin et al., 1999). Moving beyond the polyglutamine diseases,  $\alpha$ -synuclein with the A53T mutation is associated with familial PD and has a 50% longer half-life than its wild-type counterpart (Bennett et al., 1999). Oligomerization or fibril formation, promoted by the mutations in the respective proteins, could also lead to protein accumulation. Conway et al. (2000) demonstrated that the two disease-causing mutations in  $\alpha$ -synuclein accelerate the oligomerization of the protein. Whether the mutated proteins gradually compromise the function of the proteasome remains to be seen.

The importance of the ubiquitin-proteasome pathway (UPP) in neuronal degeneration has been recently highlighted (Kaytor and Warren, 1999; MacDonald, 1999). Several neurodegenerative disorders are caused by mu-

tations in genes that play a role in the UPP. For example, a form of early-onset PD is caused by a missense mutation in the ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) gene (Leroy et al., 1998). An in-frame intragenic deletion in the mouse *Uchl1* causes gracile axonal dystrophy (*gad*), an autosomal recessive disorder characterized by sensory and motor ataxia, axonal degeneration, and ubiquitin-positive aggregates in the neurons of the gracile nucleus (Saigoh et al., 1999). *Uchl1* hydrolyzes bonds between degraded end products and ubiquitin to generate free monomeric ubiquitin; the deletion removes 42 amino acids that contain a catalytic residue, thus compromising the free ubiquitin pool necessary for an intact UPP. The SCA1 phenotype was recently found to be accelerated by loss of function of an E3 ligase (*Ube3a*) (Cummings et al., 1999). Finally, mutations in Parkin, a member of the RING finger family of proteins, cause an autosomal recessive juvenile form of PD (Kitada et al., 1998). Several RING fingers interact with ubiquitin-conjugating enzymes and mediate ubiquitination (Lorick et al., 1999); the RING finger protein c-Cbl acts as an E3 ubiquitin ligase that recognizes target substrates and promotes their ligation to ubiquitin (Joazeiro et al., 1999). Although this connection remains speculative, it reinforces the notion that mutations affecting the UPP cause or worsen neurodegeneration.

It thus appears that the pathogenesis of a large number of neurodegenerative diseases involves altered "protein clearance." Neuronal dysfunction could be caused by the mutant protein itself or its targets (as may be the case with UCHL1 and Parkin mutations), aberrant protein-protein interactions, alterations of gene expression, or activation of an apoptotic pathway. The findings of Yamamoto et al. bring hope that if we can decrease the load of mutant proteins, some of the neuronal damage may be reversible.

#### **Therapeutic Potential**

The disappearance of aggregates in the HD mice of Yamamoto et al. suggests that mutant protein(s) can be cleared even after the cells manifest dysfunction (Figure 1). (It also suggests that neurons have abilities of which biologists know little.) Of course, how long a neuron can withstand mutant protein before suffering irreversible damage needs to be determined. More importantly, it remains to be seen whether neurons can recover from the toxicity of full-length mutant proteins. Nevertheless, the discovery that turning off expression of even a truncated mutant protein can halt neuronal degeneration, well after behavioral and cellular dysfunction are apparent, has far-reaching implications.

How might this be accomplished in humans? Turning off expression of the mutant genes or directly enhancing the clearance of the mutant proteins are two obvious answers. Before the first strategy can be successful, we must determine whether the relevant proteins are essential in adulthood. If they are, an antisense strategy might allow partial reduction or targeting of only the mutant allele. Such a strategy requires that a specific antisense approach or transcriptional manipulation be designed for each of the disease genes—and that such transcriptional manipulation would not interfere with the transcription of any other endogenous sequences.

The second strategy, enhancing mutant protein clearance, has the advantage that it might apply to more

than one disease, but it remains to be shown that this approach can be designed to avoid clearance of endogenous critical proteins. Factors that might enhance protein clearance include components of the UPP and the chaperone pathway. Enhancing proteasomal activity, just like interfering with transcription, might have undesirable ramifications for some key neuronal proteins. If specific proteasomal subunits play a role in substrate-specific recognition, one is again faced with the need to have a specific therapeutic approach for each of these diseases. Should future research identify a unique structural property of proteins that undergo oligomerization and accumulation in these diseases, then perhaps a strategy that enhances the activity of a protease that selectively degrades such proteins would become an option. The discovery that overexpression of HSP40 or HSP70 modulates protein aggregation and/or cell death in cell culture models of SCA1, SCA3, and ALS (Cummings et al., 1998; Bruening et al., 1999; Chai et al., 1999) and in *Drosophila* transgenic models of polyglutamine toxicity (Warrick et al., 1999) is exciting and suggests a possible universal approach to this problem. Obviously, the safety of chaperone overexpression needs to be determined, and the delivery of such chaperones to the central nervous system is an open challenge.

In sum, this is truly an exciting time for investigating treatment avenues for late-onset neurodegenerative diseases. The cloning of human disease genes and the study of mouse, fly, worm, and yeast models this past decade provided tremendous insight into the pathogenesis of neurodegenerative diseases. The discovery that neurons are capable of at least some recovery has provided an exciting glimpse into the future use of a new generation of mouse models.

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