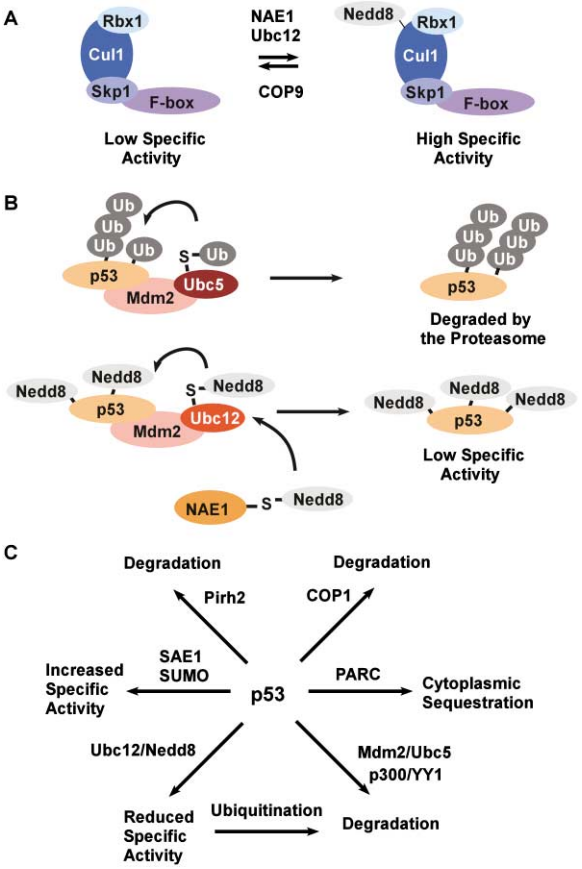

Figure 1. Nedd8 Conjugation Pathways

(A) Nedd8 is covalently attached to members of the cullin family including Cul1 via the activity of NAE1 and Ubc12. COP9 is responsible for removing Nedd8 from cullins.

(B) The C-terminal RING domain of Mdm2 is used to promote neddylation of at least three lysine residues in p53, which interacts with the N terminus of Mdm2. Neddylation appears to reduce the transactivation activity of p53. In contrast, ubiquitination occurs through recruitment of conjugating enzymes such as Ubc5 to the RING domain of Mdm2, leading to degradation of p53.

(C) Multiple pathways impinge on p53 modification by ubiquitin-like proteins.

DNA damage response or could simply be a consequence of increased p53 levels. Unlike ubiquitination, DNA damage-dependent phosphorylation does not appear to block neddylation. This raises the issue of whether Mdm2 is responsible for p53 neddylation after damage. DNA damage-dependent phosphorylation blocks association with Mdm2 with p53 (Yang et al., 2004) and, based on mutagenesis experiments (Xirodimas et al., 2004), would presumably block neddylation as well. An additional question concerns removal of Nedd8. In SCF complexes, Nedd8 is removed by the action of the COP9 complex (Cope and Deshaies, 2003). How and under what circumstances Nedd8 is removed from p53 is unknown, although at long times after DNA damage, the levels of neddylated p53 were reduced while total p53 levels remained high, suggesting the existence of a deneddylation pathway. However, given the fact that neddylated p53 can also be ubiquitinated, it is possible that neddylated p53 may also undergo proteasome-mediated degradation in certain situations, negating a need for regulated deneddylation. Clearly, understanding the dynamic relationship between p53 neddylation, p53 ubiquitination, and p53 turnover will be required to determine how neddylation fits into the p53 degradation pathway.

As is often the case with novel findings, this work raises as many questions as it answers. In particular, it is not clear under what physiological settings p53 neddylation occurs, nor is it evident precisely how neddylation regulates p53 function. Does neddylation play a critical role overall in p53 biology or is it a minor component of p53's diverse regulatory apparatus? In addition, this work raises the question of whether Nedd8 transfer through RING-based E3s is a frequent event. If Mdm2 is a harbinger of things to come, then there could be many more RING-based E3s that are capable of functioning together with Ubc12 to promote neddylation of proteins that are otherwise targets of a RING-based ubiquitin ligase activity. If this is the case, then an intermingling of neddylation and ubiquitination could be the rule and not the exception.

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Huntington's Disease: New Paths to Pathogenesis

Huntington's disease is a progressive autosomal dominant neurodegenerative disorder caused by expan-

sion of a CAG repeat coding for polyglutamine in the huntingtin protein. A recent report (Gauthier et al., 2004, this issue of *Cell*) suggests a new mechanism involving altered interactions with a protein involved in axonal transport, leading to loss of neurotrophic factor transport. This suggests an intriguing convergence to previously described pathways implicating neurotrophin transcription in HD pathogenesis.

While many huntingtin protein interactors have been described (Harjes and Wanker, 2003), and a number of mechanisms linking these interactors to HD pathogenesis—including alteration in gene transcription, activation of apoptosis, metabolic poisoning, or blockage of axonal transport—have been proposed, the role of huntingtin interacting proteins in the disease has remained elusive. The group led by Frederick Saudou (Gauthier et al., 2004) now describes functional consequences of the interaction between huntingtin and huntingtin-associated protein 1 (HAP1). HAP1 was the first huntingtin interactor identified, and it in turn interacts with proteins involved in vesicle transport, including the $p150^{Glued}$ subunit of dynactin. The current study provides evidence that huntingtin enhances vesicle transport of brain-derived neurotrophic factor (BDNF) along microtubules. BDNF transport was attenuated by reducing the levels of wild-type huntingtin, or by the introduction of mutant huntingtin. The alteration of the complex of huntingtin, HAP1, and $P150^{Glued}$ correlated with reduced association of motor proteins with microtubules. The loss of BDNF transport appeared to be associated with reduced neurotrophic support and cell toxicity.

Previous cell culture and conditional knockout mouse studies have suggested a different mechanism for the effect of huntingtin on BDNF, involving a neuroprotective role for huntingtin (Trottier and Mandel, 2001; Cattaneo et al., 2001), mediated through enhancement of BDNF gene transcription by normal huntingtin in the nucleus. Additionally, mutant huntingtin may affect BDNF transcription by altering the transcriptional activities of CBP, Sp1, TAF130, and other molecules, presumably via a gain-of-function mechanism (Sugars and Rubinsztein, 2003). The new study complements this previous work by suggesting that, in addition to actions on BDNF transcription, huntingtin may also regulate BDNF transport and activity (Figure 1).

The current study also complements the previous seminal study by Saudou et al. (1998), which demonstrated that N-terminal mutant huntingtin fragments targeted to the nucleus cause neuronal toxicity via a gain-of-function mechanism. Considered together, these two studies raise in a new form many of the overarching questions in the field of polyglutamine disease mechanisms (Ross, 2002; Tobin and Signer, 2000).

One question relates to gain versus loss-of-function effects. As a disease with almost pure genetic dominance, HD has often been presumed to result from a gain of a toxic function. Loss-of-function mechanisms (perhaps as dominant-negatives via polyglutamine interactions) have also been proposed. A pure loss-of-function mechanism appears unlikely. Homozygous huntingtin knockouts are embryonic lethals. Furthermore heterozygous knockouts are normal, but early loss of huntingtin expression to between 0% to 50% of normal has severe neurodevelopmental effects. However, these findings do not exclude a role for a slowly developing late-life loss of huntingtin function in disease pathogenesis. In the present study, mutant huntingtin has an effect similar to inhibiting wild-type huntingtin with RNA interference, suggesting a loss-of-function or dominant-negative mechanism. This loss-of-function mechanism then would complement the previous gain-of-function mechanism originally described by Saudou et al. (1998). Whether this kind of a loss-of-function mechanism is relevant to disease pathogenesis still needs to be demonstrated.

A related issue is the question of protein aggregation. There is a striking correspondence between the polyglutamine length threshold for disease and the polyglutamine length at which huntingtin aggregates, though the relationship among aggregation, inclusions, and disease remains controversial (Ross and Poirier, 2004). Previous studies had suggested that mutant huntingtin could also block axonal transport via purely mechanical effects of aggregates (Feany and La Spada, 2003). In the current study, no aggregates were detected using light microscopy, suggesting effects due to mutant huntingtin monomers or perhaps small oligomeric assemblies.

A third issue is the cellular location of toxic effects. Most (though not all) previous studies have suggested that nuclear localization of mutant huntingtin enhances toxicity, as Saudou et al. initially observed (1998). It is suggested in the discussion of the current study that neuronal dysfunction due to interference with neurotrophin transport (a cytoplasmic effect) might be a precursor to later neuronal cell death.

A fourth issue is the relevance of proteolytic cleavage (DiFiglia, 2002). It has been proposed that huntingtin can undergo proteolytic cleavage, generating a toxic fragment, which may be transported to the nucleus. However, in the current model, full-length mutant huntingtin interferes with BDNF transport, while truncated mutant huntingtin does not.

A fifth issue relates to whether cell death in HD is cell autonomous or might involve cell interactions, or as it is sometimes put, "murder vs. suicide." In cell expression studies, it would appear that mutant huntingtin could be toxic in a cell autonomous fashion. However, it is possible that cell interactions are relevant in vivo. Interestingly, BDNF can be transported to the striatum via cortico-striatal axon projections. Furthermore HAP1 interacts not just with components of retrograde transport motors such as dynactin, but also with components of anterograde transport machinery. Thus, it is conceivable that huntingtin interference with BDNF transport could relate to the depletion of BDNF seen in HD striatum. There may be other effects of aberrant neurotrophic support. Previous studies by DiFiglia's group suggest that HD may involve not just neuronal degeneration but also changes which appear to be attempts at neuronal regeneration.

This brings up the related issue of anatomic specificity. While polyglutamine expansion diseases share many pathological features, the exact distributions of neurons

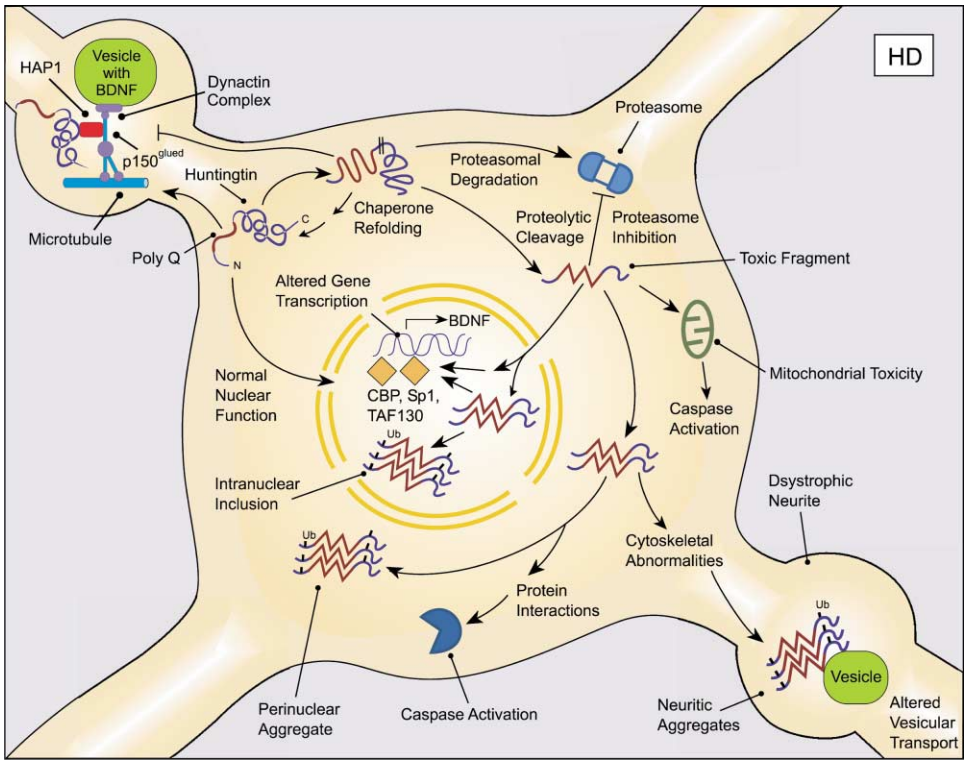

Figure 1. Model for HD Cellular Pathogenesis Involving Both Cytoplasmic and Nuclear Effects on BDNF

Huntingtin is normally predominantly cytoplasmic. One of its normal functions involves regulation of vesicle transport, including transport of BDNF. It may play a role in the regulation of gene transcription in the nucleus, especially for neurotrophic molecules such as BDNF. The mutation causes a conformational change and abnormal folding of the protein, which can be corrected by molecular chaperones. Mutant huntingtin has effects both in the cytoplasm and in the nucleus. In the cytoplasm, full-length mutant huntingtin can interfere with BDNF vesicular transport on microtubules. Mutant huntingtin can also undergo proteolytic cleavage, both in the cytoplasm and in the nucleus (shown here as taking place only in the cytoplasm for simplicity), which may involve several steps. The N terminus with the expanded repeat can assume a β sheet structure. Toxicity in the cytoplasm may involve soluble monomers or oligomers or possibly insoluble aggregates, via inhibition of the proteasome or activation of caspases directly or via mitochondrial effects. Cytoplasmic aggregates accumulate in perinuclear or neuritic regions and are ubiquitinated. Neuritic aggregates can interfere with BDNF transport by mechanical disruption. 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, leading to loss of transcription of neuroprotective molecules such as BDNF.

that degenerate differ among the diseases. The effects described in the current study are likely not to be general events for polyglutamine pathogenesis, but could explain some of the anatomic selectivity to the striatum seen in HD.

A final issue involves the question of whether neuronal dysfunction or death is most relevant to HD pathogenesis. Striatal atrophy begins long before HD can be diagnosed, and striatal cell death correlates with the most debilitating effects of the disease. However, mouse models and clinical evidence suggest the possibility that neuronal dysfunction as well as neuronal death might contribute to symptoms.

The effects seen in the current study suggest the possibility that there might be two broad complementary mechanisms for HD pathogenesis. One set of effects might involve full-length mutant huntingtin, and result in, among other abnormalities, transport defects of BDNF leading to increased neuronal vulnerability. This would sensitize the cell to the toxic effects of truncated mutant huntingtin in the nucleus. The combination of the two would ultimately lead to cell dysfunction and death, striatal atrophy, and progression of disease. Understanding of these new pathways to pathogenesis should lead to new approaches to rational therapeutics.

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