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Hunting for the function of Huntingtin

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Huntington's disease (HD) is a devastating neurodegenerative disorder, afflicting three to ten individuals per 100,000 in Western Europe and North America (Gil and Rego, 2008). In 1993, the gene responsible for HD (*HTT*) was cloned, representing a major breakthrough in the field. *HTT* encodes a large protein that was named Huntingtin (Htt). The N-terminal portion of Htt contains a stretch of glutamines (the polyQ tract) and HD patients harbor pathogenic polyQ expansions (Andrew et al., 1993; Bates et al., 1998). Although the polyQ length can vary, all healthy individuals have fewer than 37 glutamines and those with greater than 40 are certain to develop HD (Rubinsztein et al., 1996). Moreover, the length of the polyQ expansion correlates inversely with the age of disease onset (Andrew et al., 1993). Experiments in cell culture and animal models have clearly established that an expanded polyQ tract is toxic (Cattaneo et al., 2001). However, little is known about the normal cellular function of Htt and how this might be disrupted in HD. In vitro studies identified a wide range of Htt-interacting proteins and suggest that wild-type Htt may be involved in such diverse biological processes as protein trafficking, vesicle transport, anchoring to the cytoskeleton, clathrin-mediated endocytosis, postsynaptic signaling, transcriptional regulation and anti-apoptotic functions (Gil and Rego, 2008; Imarisio et al., 2008).

As a model organism, the fruit fly *Drosophila melanogaster* has many experimental advantages, including a short life span; large and rapid reproductive capacity; a vast array of available genetic information from its fully sequenced genome; and an extensive collection of powerful genetic tools. Importantly, Drosophila shares many essential features with higher-order organisms. Approximately 75% of human disease genes have at least one Drosophila homolog. Compared with other simple systems, Drosophila is particularly suitable for studying neurodegenerative disease because of its complex nervous system and behaviors (Bilen and Bonini, 2005). Jackson et al. first showed that the human Htt-Q75 and -Q120 transgenes induce degeneration of fly photoreceptor neurons (Jackson et al., 1998). Li et al. later identified a Drosophila homolog of *HTT* (*htt,* hereafter referred to as *dhtt*) (Li et al., 1999). However, the normal function of Drosophila Htt (dHtt) remains elusive.

A new study by Zhang et al. in the current issue of *DMM* explores the function of dHtt in a unique model of HD (Zhang et al., 2009). First, the authors used reverse transcription-PCR and in situ hybridization to demonstrate that *dhtt* is expressed ubiquitously during all stages of fly development (see fig. 1 in Zhang et al.). Also, with an anti-dHtt antibody, they confirmed that, like its mammalian homolog, dHtt is a cytoplasmic protein.

To explore the normal function of dHtt, they created a *dhtt* knockout (*dhtt-ko*) fly using flipase–FRT-mediated recombination (see fig. 2 in Zhang et al.). Previous mouse models that are null for the murine Htt homolog, Hdh, have been difficult to study since they die very early in embryogenesis (Duyao et al., 1995; Nasir et al., 1995; Zeitlin et al., 1995). Unlike Hdh homozygous knockout mice, the *dhtt-ko* flies survived into adulthood. Further, in contrast to a previous report that used RNAi (Gunawardena et al., 2003), knockout flies developed at a similar rate to their wild-type counterparts, with embryonic and larval CNS, muscles, eyes and other imaginal discs appearing morphologically normal (see fig. 3 in Zhang et al.). The authors also observed normal axonal pathfinding, muscle innervation, overall synapse structure, axonal transport and typical crawling behavior during larval stages (see figs 3, 4 in Zhang et al.). Immunostaining revealed normal pre- and post-synaptic structures, and proper synaptic component delivery (including synapsin, FasII and Dlg). However, the *dhtt-ko* flies were not completely normal; they displayed phenotypes in mushroom bodies (MBs), particularly a decrease in FasII staining, as well as reduced numbers of varicosities and branches in the axonal termini of giant fiber neurons (see fig. 7 in Zhang et al.). The mature *dhtt-ko* flies also demonstrated late-onset defects in mobility and viability, and phototransduction deficits during temperature-induced stress (see figs 5, 6 in Zhang et al.).

To test the hypothesis that HD pathogenesis is caused by both a toxic gain of function, resulting from the polyQ expansion, and reduced protection from the normal function of the wild-type Htt protein, Zhang et al. used the Gal4-UAS system to express a polyQ-expanded Htt transgene (HD-Q93) in all fly neurons. Interestingly, they found that *dhtt* knockout exacerbated HD-Q93 toxicity (see figs 8, 9 in Zhang et al.), suggesting that loss of the normal dHtt function is a component of the toxicity from polyQ expansions in HD.

It is surprising that *dhtt-ko* flies display no developmental defects, in contrast to the early embryonic lethality observed in Hdh knockout mice. It remains possible that a redundant pathway might compensate for the loss of dHtt. There may also be subtle defects in *dhtt-ko* flies that escaped detection under normal conditions that might intensify with stress (e.g. aging, environmental insults, oxidative stress, injury). It would be interesting to further investigate whether the mild abnormalities in MBs and axonal termini are responsible for the reduced adult mobility and viability. Since intranuclear inclusions are a pathological hallmark of HD, determining whether aggregates are formed in HD-Q93; *dhtt-ko* flies will be important. Although Zhang et al. argue that dHtt may have distinct functions from human Htt, no solid conclusion can be made until we know whether human Htt can rescue the phenotypes of *dhtt-ko* and HD-Q93; *dhtt-ko* flies.

This fly HD model offers a number of exciting possibilities for delineating the role of Htt in normal biology and disease. In the future, researchers could measure several

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additional parameters to more clearly define the role of dHtt in Drosophila. Genetic modifier screens will probably identify genes and pathways that interact with *dhtt*, to expand our understanding of dHtt function and HD pathogenesis. Also, because the HD-Q93; *dhtt-ko* model mimics both gain- and loss-of-function aspects of the disease, this may be a useful system for identifying and testing candidate compounds that could ameliorate HD pathogenesis [e.g. histone deacetylase inhibitors (Steffan et al., 2001)]. Thanks to the simplicity and elegance of the fly, we may soon step closer to understanding the normal and pathogenic functions of Huntingtin in humans.

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