Supplementary data

Deciphering the biochemistry of necrosis

The availability of many well established models of necrosis in *C. elegans* and *Drosophila,* coupled with the sophisticated genetics and molecular biology at hand in these organisms, allows detailed dissection of the necrotic cell death process. The power of this approach has already been demonstrated in the case of apoptotic cell death, by the many groundbreaking discoveries in *C. elegans* (Metzstein et al., 1998). Here, we provide supplementary/explanatory information that further demonstrates the power of genetic approaches in simple model systems.

Intriguing analogies: Necrosis in *C. elegans* and neurodegenerative diseases in humans

The striking internalized membranous inclusions in dying neurons that express hyperactivated degenerins suggest that intracellular trafficking may contribute to degeneration. Interestingly, in some mammalian degenerative conditions such as neuronal ceroid lipofuscinosis (Batten's disease; the *mnd* mouse; Cooper et al., 1999) and that occurring in the *wobbler* mouse (Pioro and Mitsumoto, 1995), cells develop vacuoles and whorls that look similar to internalized structures in dying *C. elegans* neurons. Furthermore, disrupted trafficking has been implicated in Alzheimer's disease (Sherrington et al., 1995), Huntington's disease (DiFiglia et al., 1995), and amyotrophic lateral sclerosis (ALS; Brown, 1995). Together, these observations suggest that at least some degenerative processes may be similar in nematodes and mammals.

Because many mutant *C. elegans* degenerins inflict necrosis, the neuronally expressed mammalian family members are logical candidates to examine for the ability to mutate into neurodegeneration-causing froms in higher organisms. It is therefore interesting that neuronally expressed homologues engineered to encode amino acid substitutions analogous that found in the protein encoded by *mec-4(d)* induce degeneration when expressed in *Xenopus* oocytes and embryonic hamster kidney cells (Waldmann et al., 1996). Degenerin-induced cell death in these systems is reminiscent of vertebrate excitotoxic cell death, which is triggered by the release of

excess glutamate, an excitatory neurotransmitter, into the synaptic cleft (Choi, 1992). The consequent opening of glutamate-gated cation channels leads to sodium influx and calcium uptake by the cells, which ultimately kills them (Sattler and Tymianski, 2000). Thus, vertebrates and *C. elegans* share a death mechanism that involves hyperactivation of ion channels and consequent excess cation influx, which ultimately leads to neuronal swelling and death.

Modeling neurodegeneration: The versatility of *Drosophila*

Drosophila homologs have been identified for the human genes encoding the β-amyloid precursor protein (APP) and the Cu^{2+}/Zn^{2+} superoxide dismutase, which have been implicated in Alzheimer's disease and in a familial form of ALS, respectively. Interestingly, mutations in the respective fly genes were shown to confer striking neuropathology in *Drosophila* (Mutsuddi and Nambu, 1998).

Similar neuropathology is observed by the expression of either wild-type or mutant forms of human α-Synuclein in *Drosophila*. This replicates all of the key features of the Parkinson's disease pathology: adult-onset loss of dopaminergic neurons, the formation of filamentous intraneuronal inclusions containing α-Synuclein and dysfunction in locomotion (Feany and Bender, 2000). Recent studies have also demonstrated that flies expressing wild-type or mutant human *tau* undergo brain neuron degeneration that reproduces a number of features of the human disorder, including adult onset, progressive necrosis, accumulation of abnormal *tau* and relative anatomic selectivity. Although the *Drosophila* neurodegeneration occurs without the neurofibrillary tangle formation that is seen in human disease and in other experimental models, This may be due to the smaller average size of the neurons, and the lack neurofilaments. The dissociation of tangle formation from Tau neurotoxicity in *Drosophila* suggests that neurofibrillary tangles may not be the primary cause of death. Alternatively, however, the mechanism of toxicity in *Drosophila* may differ from that in mammals (Wittmann et al., 2001).

In *Drosophila*, overproduction of the molecular chaperone Hsp70, which was found to co-localize with polyglutamine protein aggregates, suppresses polyglutamine toxicity and cell death (Bonini, 2001). Elevated Hsp70 expression also ameliorates α-Synuclein toxicity in *Drosophila*

dopaminergic neurons (Auluck et al., 2001). Consistent with these findings, genetic screens have identified suppressors of neurodegeneration directly related to HSP70: Overexpression of DNAJ-1, which is homologous to the human chaperone HSP40/HDJ1, as well as overexpression of dTPR2, a tetratricopeptide repeat protein, suppresses polyglutamine toxicity in *Drosophila*. These J-related proteins may prevent aggregation by stimulating the ATPase activity of HSP70 (thus, enhancing the heat-shock response), and also by directly binding to and trapping abnormally folded proteins, increasing proteasome-mediated degradation (Kazemi-Esfarjani and Benzer, 2000). Consistently, mutations in genes of the ubiquitin-proteolytic pathway behave as enhancers of polyglutamine-induced neurodegeneration in *Drosophila* (Fernandez-Funez et al., 2000).

A second group of polyglutamine-induced, neurodegeneration enhancers in *Drosophila* is defined by transcription co-factors such as Sin3A, Rpd3, Sir2, dCtBP etc. (Fernandez-Funez et al., 2000). Since many transcription factors contain short polyglutamine tracts, it was suggested that polyglutanine-repeat proteins interfere with specific transcriptional regulators through direct glutamine-glutamine interactions, trapping them into aggregates. Indeed, a fragment of mutant huntingtin (Htt; a polyglutanine protein associated with Huntington's disease), directly interacts with CBP and represses transcription in cell culture (Steffan et al., 2000). Furthermore, it was shown that Htt peptide, through its polyglutamine stretch, interacts primarily with the histone acetyltransferase (HAT) domain of CBP as well as other HAT co-activators (p300 and P/CAF), and inhibits their HAT function (Neri, 2001). Thus, pathogenic Htt peptides can lead to reduced levels of acetylation and transcription, both by binding to HAT domains and by sequestering polyglutamine-containing transcription factors. Remarkably, inhibition of deacetylation in flies expressing polyglutanime proteins by pharmacological treatment with histone deacetylase (HDAC) inhibitors, and by genetic means such as reduction of Sin3A activity, quelled necrosis of neurons expressing the toxic proteins. Such findings implicate the state of histone acetylation in the pathogenesis of Huntington's disease and suggest that HDAC inhibitors may hold potential as therapeutic agents (Steffan et al., 2001).

Additional References

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