Analysis of programmed cell death in Drosophila

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Abstract

The *Drosophila reaper, head involution defective* (*hid*), and *grim* genes play key roles in regulating the activation of programmed cell death. In this first part of this study we use the Gal4/UAS targeted gene expression system to demonstrate that unlike *reaper* or *hid*, expression of *grim* alone is sufficient to induce ectopic embryonic CNS midline cell death. We also show that in both the midline and adult eye, *grim*-induced cell death is not blocked by the *Drosophila* anti-apoptosis protein Diap2, which does block both *reaper* and *hid*-induced cell death. *grim* can also function synergistically with *reaper* or *hid* to induce midline cell death. Finally we analyzed the function of a truncated Reaper-C protein which lacks the NH2-terminal 14 amino acids that are conserved between Reaper, Hid, and Grim. Ectopic expression of Reaper-C revealed cell killing activities distinct from full length Reaper, and indicated that the conserved NH2-terminal domain acts in part to modulate Reaper activity. ^ In the second part of this study, we have analyzed the importance of the RHG motifs in Reaper and Grim for their different abilities to activate cell death during development. Analysis of chimeric R/Grim and G/Reaper proteins indicated that the Reaper and Grim RHG motifs are functionally distinct and help to determine specific cell death activation properties. A truncated Grim-C protein lacking the RHG motif retained an ability to induce cell death and unlike Grim, R/Grim, or G/Reaper, it's actions were not efficiently blocked by the cell death inhibitors Diap1, Diap2, P35, or a dominant/negative Dronc caspase. Finally, we identified a second region of sequence similarity in Reaper, Hid, and Grim, that may be important for shared RHG motif-independent activities. ^ In the third part of this study, we describe a genetic modifier screen that was performed in an attempt to identify other genes that act in cell death pathways. Three proteins from the Ubiquitin/Proteasome (Ub/Pro) pathway were identified as enhancers of the eye cell death phenotype induced by a Reaper/Grim chimeric protein. In particular, a gene encoding a novel F-box protein, Morgue, has been identified. ^

Subject Area

Molecular biology|Genetics

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