Supplementary Material

Drosophila Bruce Can Potently Suppress Rpr- and Grim-Dependent but Not Hid-Dependent Cell Death

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Supplementary Experimental Procedures

Construction of Transgenic Flies

GMR-Grim-lys$^-$ was made by PCR amplifying Grim using a reverse primer that incorporated nucleotides specifying arginine (CGG) instead of the single endogenous lysine (AAG.) A version of Rpr with all five lysines mutated to arginine was made using site-directed mutagenesis and was a generous gift from Sally Kornbluth. Both Grim-lys$^-$ and Rpr-lys$^-$ were introduced into GMR. A GMR-RNAi construct was generated as described in [S1], by PCR-amplifying the GMR promoter/enhancer sequence and reintroducing it into GMR, downstream of the existing promoter, in the opposite orientation. A 732-bp fragment of dBruce encompassing nucleotides (as determined from the dBruce start codon) 13,354–14,086 was introduced into the GMR-RNAi construct between the GMR promoters, so that both sense and antisense would be transcribed. GMR-Strica flies were generated by introducing the Strica coding region into GMR. Transformants were generated using standard techniques.

Characterization of the dBruce Gene and Generation of dBruce Mutants

The exon-intron structure of dBruce was determined as follows: first-strand cDNA was made from *Drosophila* embryo mRNA (Clontech) using three different primers spaced 5 kb apart along the length of the predicted dBruce sequence. Fragments (1 kb) were PCR amplified from this cDNA using primers that spanned dBruce, based on the BDGP predicted sequence. These fragments were sequenced and assembled into a contiguous dBruce coding sequence. To generate dBruce deletions E12 and E16, we first used recombination to remove a background lethal from the EP(3)3731 chromosome. The transposon was mobilized as described in [S2], and balanced excision lines were generated. Deletions were characterized by carrying out PCR on genomic DNA from the excision lines using sequential sets of primers spanning dBruce and sequencing products that bridged the deletions.

Antibody Generation and Immunostaining

Antibodies were raised in rabbits using a GST-fusion protein corresponding to amino acids 361–693 of dBruce. Antisera were applied to an affinity column containing protein used as the immunogen, and bound antibodies were eluted using 100 mM glycine (pH 2.5). For immunolabeling, dissected larval eye discs were fixed in PBS + 4% formaldehyde for 20 min and then permeabilized in PBS + 0.3% Trition X-100, 0.3% deoxycholate, 5% BSA for 1 hr. Following an overnight incubation with purified dBruce antibody (1:100), eye discs were washed for 1 hr in PBTB (PBS + 0.1% Trition X-100, 5% BSA) and were incubated for 1 hr in Alexa Fluor 488 goat anti-rabbit IgG (1:500) (Molecular Probes). Discs were then washed for an hour, mounted in Vectashield mounting medium (Vector Labs), and viewed on a Leica TCS-NT confocal microscope.

Supplementary References

S1. Giordano, E., Rendina, R., Peluso, I., and Furia, M. (2002). RNAi triggered by symmetrically transcribed transgenes in *Drosophila melanogaster*. Genetics *160*, 637–648.

S2. Hay, B.A., Wassarman, D.A., and Rubin, G.M. (1995). *Drosophila* homologs of baculovirus inhibitor of apoptosis proteins function to block cell death. Cell *83*, 1253–1262.

S3. Hawkins, C.J., Yoo, S.J., Peterson, E.P., Wang, S.L., Vernooy, S.Y., and Hay, B.A. (2000). The *Drosophila* caspase DRONC cleaves following glutamate or aspartate and is regulated by DIAP1, Hid, and Grim. J. Biol. Chem. *275*, 27084–27093.

S4. Colussi, P.A., Quinn, L.M., Huang, D.C., Coombe, M., Read, S.H., Richardson, H., and Kumar, S. (2000). Debcl, a proapoptotic Bcl-2 homologue, is a component of the *Drosophila melanogaster* cell death machinery. J. Cell Biol. *148*, 703–714.

S5. Wing, J.P., Zhou, L., Schwartz, L.M., and Nambu, J.R. (1998). Distinct cell killing properties of the *Drosophila reaper*, *head involution defective*, and *grim* genes. Cell Death Differ. *5*, 930–939.

S6. Wing, J.P., Schwartz, L.M., and Nambu, J.R. (2001). The RHG motifs of *Drosophila* Reaper and Grim are important for their distinct cell death-inducing abilities. Mech. Dev. *102*, 193–203.

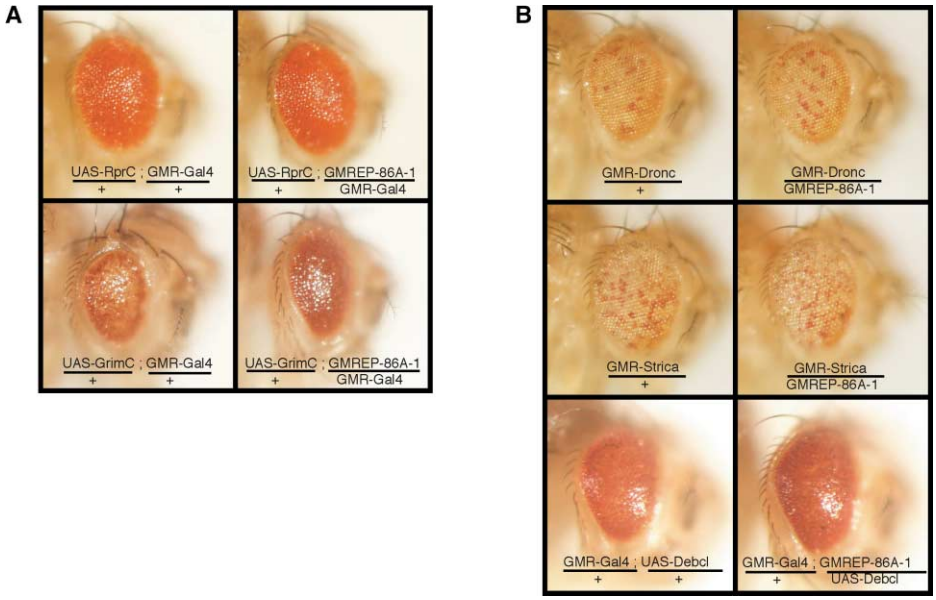

Figure S1. Interactions of dBruce with Apoptosis Regulators

(A) GMREP-86A-1-driven dBruce does not suppress death induced by eye-specific expression of versions of Rpr (RprC) and Grim (GrimC) that lack their N-terminal DIAP1 interaction domains.

(B) GMREP-86A-1-driven dBruce does not suppress cell death induced by eye-specific expression of the long prodomain caspase Dronc, the long prodomain caspase Strica, or the proapoptotic bcl-2 family member Debcl/Drob-1/dBorg-1/Dbok (referred to in the legend as Debcl). GMR-Dronc flies were previously described [S3]. GMR-Gal4/CyO; UAS-Debcl/TM6B flies were a generous gift from Sharad Kumar [S4]. Flies carrying UAS-RprC on the X chromosome or UAS-GrimC on the second chromosome were a generous gift from John Nambu [S5, S6].