N3194, IDENTIFIED IN A SCREEN FOR SUPPRESSORS OF *CED-9(N1950)*, MAY BE REQUIRED FOR CELL VIABILITY

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To identify genes that interact with the cell-death protector gene *ced-9*, we screened for suppressors of the Ced phenotype of *ced-9*(*n1950*gf) animals by looking for the reappearance of corpses in *ced-1*(*e1735*); *ced-9*(*n1950*) embryos. We identified one candidate, *n3194*, with a large number of refractile bodies in embryos. *n3194* maps to LG*III*, complements *ced-9*(*lf*), and causes a recessive maternal-effect lethal phenotype. Embryos arrest at approximately the two-fold stage of development. Two additional alleles, *n3264* and *zu223*,were isolated by others in unrelated screens. Neither the accumulation of refractile bodies nor the lethality of *n3194* is suppressed by loss-of-function mutations in *ced-3* or *ced-4*, suggesting that this gene acts downstream of or parallel to *ced-3*. We examined the morphology of the refractile bodies in *n3194* mutants using electron microscopy and determined that the bodies more closely resemble degenerative deaths than they do caspase-dependent apoptotic deaths.

n3194 disrupts the gene R13A5.1, which encodes a predicted protein highly similar to the products of *Drosophila*, human and mouse ESTs of unknown function and with weak similarity to K^+ and Ca^{2+} channels. *n3194* and *zu223* are nonsense mutations that likely represent molecular null alleles, and *n3264* is a missense mutation that alters a conserved glycine. An R13A5.1::GFP fusion capable of rescuing *n3194* is localized to the plasma membrane of all cells in early embryos. Later in development the GFP marker becomes restricted to the excretory canal cell and several neurons in the head. We have isolated a full-length R13A5.1 cDNA, which we will use for rescue experiments. In addition, we will test the effect of R13A5.1 overexpression on cell death.

R13A5.1 appears to be required for cell viability during embryogenesis. Its localization in the excretory canal and its similarity to ion channels suggest a possible role in maintaining ionic homeostasis. Loss of R13A5.1 function may activate a degenerative cell-death pathway or may aberrantly activate some but not all of the apoptotic pathway, leading to abnormal corpses. As redistribution of key ions such as Ca^{2+} may play an important role in regulating apoptosis, R13A5.1 may represent a link between ionic balance and programmed cell death.