129. A SCREEN FOR ESSENTIAL CELL-DEATH GENES

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Genes important for programmed cell death in *C. elegans* may remain unidentified because they are also essential for early embryogenesis. We are planning to perform a screen for such essential cell-death genes using the RNAi feeding library developed in the Ahringer laboratory (1). We plan to use two approaches. First, we will place wild-type eggs on bacteria expressing double-stranded RNA and score their phenotype later in development (postembryonic RNAi). This approach ensures that double-stranded RNA will be delivered only postembryonically, allowing animals to develop normally until hatching. Second, we will mate wild-type males with *rde-1* RNAi-deficient homozygotes that have been exposed to double-stranded RNA and score the phenotype of their progeny (2; zygotic RNAi). With this method, RNAi will be effective in cross progeny only after *rde-1* is expressed zygotically. In a pilot experiment, we tested the feasibility of using a *lin-11::gfp* reporter to assay cell death in RNAi experiments. In *ced-3(n717)* animals, *lin-11::gfp* allows the detection of five undead Pn.aap cells, because the deaths of these cells (normally in the L1 larval stage) are prevented (see abstract by Galvin, Reddien, and Horvitz; a sixth undead cell, P1.aap, is not reliably scored). Standard feeding of *ced-3* RNAi yielded an easily observable cell-death defect of, on average, three extra Pn.aap cells per animal. Postembryonic or zygotic RNAi resulted in a much weaker effect of, on average, fewer than one extra Pn.aap cell per animal. We will explore the use of different reporters and the RNAi-hypersensitive strain *rrf-3* (3) to increase the sensitivity of the proposed screen.

In cells that have initiated programmed cell death, CED-4 translocates from mitochondria to the perinuclear region, where it may bind and activate CED-3 (4). However, the localization of CED-3 in living or in dying cells is unknown. To address this issue, we are making polyclonal antibodies to CED-3.

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3 Simmer, J., Ahringer, J., and Plasterk, R.H.A. European *C. elegans* Meeting 2002, Abstract 31

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