

TWO SCREENS FOR NEW GENES INVOLVED IN PROGRAMMED CELL DEATH

Brendan Galvin, Scott Cameron, and Bob Horvitz

HHMI, Dept. Biology, MIT, Cambridge, MA 02139

Many genes involved in programmed cell death in *C. elegans* have been identified by screening for mutations that allow survival of cells that are normally destined to die. The opposite approach, screening for mutations that result in the deaths of cells, has been comparatively unexplored, in part because of a lack of efficient methods to identify such mutations. A *lin-11::gfp* reporter (see abstract by Cameron and Horvitz) expresses in Pn.aap cells. In wild-type animals, only the six Pn.aap cells of the P3-P8 lineages survive and express GFP, while in mutants defective for cell killing all 12 Pn.aap cells survive and express GFP. A strain carrying this reporter allows one to monitor the survival of cells using a dissecting microscope rather than Nomarski optics, greatly facilitating screening. In this way, I am performing a *ced-4* suppression screen by looking for a reduction in the number GFP-positive cells and thus an increase in cell death in the ventral cords of partial loss-of-function *ced-4* mutants.

To identify substrates of the caspase CED-3, which is required for programmed cell death, I am using a modified two-hybrid screen. An active caspase is composed of two subunits proteolytically derived from an inactive procaspase. The active site of the caspase contains a cysteine, which when mutated prevents the cleavage activity of the enzyme. In this screen the two active subunits of the CED-3 caspase are expressed separately, with the active-site cysteine changed to a serine. This amino acid substitution should permit an interaction with substrates but prevent their cleavage, allowing for the identification of interacting proteins. Other groups have identified substrates of mammalian caspases using this approach (1, 2). Candidates from the screen will be analyzed by testing *in vitro* cleavage by CED-3 and assayed by RNAi to examine their roles in programmed cell death.

1. Van Crielinge, W., van Gorp, M., Decoster, E., Schotte, P., Van de Craen, M., Fiers, W., Vandenabeele, P., and Beyaert, R. *Analytical Biochemistry*. 263, 62-66 (1998).
2. Kamada, S, Kusano, H., Fujita, H., Ohtsu, M., Koya, R. C., Kuzumaki, N., and Tsujimoto, Y. *Proc. Natl. Acad. Sci. USA*. 95, 8532-8537 (1998).