A Screen for Genes Synthetically Lethal with *lin-35* Rb

Mike Hurwitz[1,2] and Bob Horvitz[1]
[1]HHMI, Dept. Biology, MIT, Cambridge, MA 02139 USA
[2]MGH Cancer Center, Boston, MA 02114

The class B synMuv gene *lin-35* encodes a *C. elegans* protein similar to Rb, a tumor suppressor inactivated in many human solid tumors. *lin-35* mutants may provide a model for mammalian cells harboring mutant Rb genes. Since *lin-35* mutations are not lethal, we can screen for genes with functions required for viability in *lin-35* mutants but not in wild-type animals to identify potential targets for cancer therapy. Pharmacologic inactivation of such target proteins could cause the specific deaths of Rb-deficient cells.

We have screened the LGI RNAi library described by Fraser *et al.* (2000) for genes that are essential specifically in *lin-35(n745*) animals. *n745* causes an early stop codon in *lin-35* and is probably a null allele. We have compared the phenotypes following RNAi of *lin-35(n745)* animals to the published RNAi phenotypes of the wild-type N2 strain. Of the 2445 dsRNAs we have screened, 2128 did not cause abnormalities in either *lin-35(n745)* or N2 worms. 212 dsRNAs caused similar abnormalities in *lin-35(n745)* and N2 worms. 105 dsRNAs resulted in abnormalities in *lin-35(n745)* but not in N2 worms. These 105 dsRNAs were tested in the RNAi-hypersensitive strain *rrf-3(pk1426)* to assess whether a more complete knockdown of gene function by the dsRNAs might generate more severe defects. 74 caused severe growth defects in *rrf-3(pk1426)* worms, suggesting that the effects seen in *lin-35(n745)* worms might be related to increased sensitivity to RNAi of *lin-35* worms.

To determine whether the effects of the remaining 31 dsRNAs with apparent synthetic lethality on *lin-35(n745)* animals are strain-specific and possibly allele-specific, we tested these dsRNAs on four other presumptive *lin-35* null mutants (*n2242, n2239, n2996, n373*). In these strains, only three of the remaining dsRNAs caused lethality. These three genes may act in molecular pathways parallel to Rb for an essential cellular function. Currently, we are obtaining deletion alleles of *lin-35* and these other genes.

It is possible that the abnormal RNAi phenotypes seen in *lin-35* mutants but not in N2 animals are caused by non-specific additive effects of two harmful mutations. For example, RNAi of some genes may affect one cell type and the *lin-35* mutation another cell type, so that together these two distinct defects result in severely affected animals. Since our goal is to identify pathways that are redundant within single cells, it is crucial to identify RNAs that cause cell-autonomous synthetic lethality. To find such genes, we are developing an assay to determine the effect of inactivation of *lin-35* in combination with inactivation of any of the genes identified in our screen in a single tissue.

Poster
Session topic: Cell Cycle regulation, cytokinesis, mitosis
Second session topic: Cell fate specification – post-embryonic
Keyword: Cell cycle
2866 Characters