137. A Screen for Genes Synthetically Lethal with lin-35 Rb

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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*mutant animals 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)1 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 allele2. We have compared the phenotypes following RNAi of *lin-35(n745)* animals to the published RNAi phenotypes of the wild-type N2 strain1.Of the 2,445 dsRNAs we have screened, 2,128 did not cause abnormalities in either *lin-35(n745)* or N2 worms. 212 dsRNAs caused the same abnormalities in *lin-35(n745)* and N2 worms. 105 dsRNAs resulted in abnormalities in *lin-35(n745)* worms 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 of them caused severe growth defects in *rrf-3(pk1426)* worms, suggesting that the effects seen in *lin-35(n745)* worms might be related to differential effects of RNAi in *lin-35* worms. Of the remaining 31 genes, 20 have human counterparts, defined as having at least 25% identity over 75% of the length of the gene. These 31 genes may function in Rb-containing or parallel pathways. We found no dsRNAs that were reported to have effects on the growth of N2 worms but did not have any effects on *lin-35(n745)* worms.

Because *lin-35(n745)*animals are less healthy than N2 animals (decreased brood size and rare sterile animals)3, it is possible that some of the abnormal RNAi phenotypes seen in *lin-35*mutants but not in N2 animals are caused by the 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 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 assess the effect of inactivation of *lin-35* in a single tissue in combination with inactivation of any of the genes identified in our primary screen.

1Fraser*et al.* Nature **408**: 325-30, 2000.

2Lu, X., and H. R. Horvitz. Cell **95**: 981-91, 1998.

3Fay *et al*. Genes & Develop **16**: 503-17, 2002.