129. Analysis of synMuv Protein Complexes *in vivo* and Characterization of the Class B synMuv Gene *lin-61*

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Vulval induction in *C. elegans* is negatively regulated by at least three redundant functions provided by the synthetic Multivulva (synMuv) class A, B, and C genes. Loss-of-function mutations in members of any single class do not result in a Multivulva (Muv) phenotype, but animals with mutations in any two synMuv classes are Muv. The molecularly characterized synMuv class A genes encode novel proteins. Many of the class B and class C synMuv genes, including *lin-35* Rb, *dpl-1* DP, *efl-1* E2F, *lin-53* RbAp48, *hda-1* HDAC, *let-418* Mi-2, *trr-1* TRRAP, *mys-1* HAT, and *epc*-1 E(Pc), have homologs in other species that are involved in chromatin remodeling and transcriptional modulation.

Some of the proteins that act in the synMuv B pathway have been shown to physically interact *in vitro* (1,2), in yeast two-hybrid assays (3), or *in vivo* based on co-immunoprecipitation experiments (4). These results along with homology to proteins in other species suggest that many synMuv proteins may function together in transcriptional regulatory complexes. We are using co-immunoprecipitation experiments to further explore *in vivo* physical interactions among the synMuv proteins. Such studies may allow us to better understand the functions of novel synMuv proteins, to analyze the effects of various synMuv mutations on physical interactions, and to identify potential sub-complexes among the synMuv proteins. We have focused initially on the gene products of the class B synMuv genes *lin-37* and *lin-61* as well as the class A synMuv gene *lin-56,*since null mutants in these genes are viable and can serve as negative controls for the immunoprecipitation experiments. LIN-37 is a small hydrophobic protein with no known homologs outside of nematodes. LIN-61 contains four MBT (malignant brain tumor) repeats, which are loosely defined sequences of approximately 100 amino acids found in a number of nuclear proteins including the *Drosophila* Polycomb group protein Sex Comb on Midleg. LIN-56 is a novel acidic protein with no canonical motifs. We have surveyed the interactions of these proteins with a number of class A and B synMuv proteins by immunoprecipitation followed by western blots and shown that a subset of class B proteins co-immunoprecipitate from embryo extract. We are developing reagents to perform immunoprecipitation studies of other synMuv proteins. We also hope to identify new proteins involved in vulval development on the basis of their interaction with the synMuv proteins by co-immunoprecipitations followed by mass spectrometry.

We are also characterizing the class B synMuv gene *lin-61* in detail as mutations in this gene cause a number of pleiotropic defects that differ from those of most other class B synMuv mutants. *lin-61* loss-of-function alleles cause GFP transgene misexpression (see abstract by Schwartz, Wendell, and Horvitz), and Pothof et al. have shown that RNAi of *lin-61* results in an increased mutation rate (5). We have made polyclonal antibodies that recognize LIN-61 and have shown that it is a ubiquitously expressed nuclear protein that is localized to condensed chromosomes in the germline. We have also shown that this localization remains unchanged in a large number of synMuv mutant backgrounds.

(1) Ceol and Horvitz. Mol. Cell **7**: 461-473, 2001.
(2) Lu and Horvitz. Cell **95**: 981-991, 1998.
(3) Walhout et al. Science **287**: 116-122, 2000.
(4) Unihavaithaya et al. Cell **111**: 991-1002, 2002.
(5) Pothof et al. Genes Dev. **17**:443-448, 2003.