126. FUNCTIONAL STUDIES OF THE CLASS B SYNMUVS, GENES REQUIRED FOR NEGATIVE REGULATION OF VULVAL INDUCTION, AND CHARACTERIZATION OF THE CLASS B SYNMUV GENE *lin-61*

Melissa Harrison, Xiaowei Lu, Bob Horvitz
HHMI, Dept. Biology, MIT, Cambridge, MA 02139, USA

The receptor tyrosine kinase/Ras pathway necessary for vulval induction in *C. elegans* is negatively regulated by two redundant pathways defined by the synthetic Multivulva (synMuv) class A and class B genes. Mutations in members of either class alone do not result in a Multivulva phenotype, but animals containing loss-of-function mutations in both a class A and a class B gene are Muv. The identified synMuv class A genes encode novel proteins. Many of the class B synMuv genes, including *lin-35* Rb, *dpl-1* DP, *efl-1* E2F, *lin-53* RbAp48, *hda-1* HDAC, and *let-418* Mi-2, have homologs that are known to be involved in chromatin-modification.

We have molecularly characterized a new class B gene, *lin-61*. LIN-61 is predicted to contain three MBT repeats, which are poorly defined sequences of approximately 100 base pairs found in a number of nuclear proteins, including the *Drosophila* Polycomb group protein Sex Comb on Midleg. We have identified the molecular lesions in all ten *lin-61* alleles. *n3809,* a nonsense mutation at amino acid 159, is predicted to delete two-thirds of the protein and is a presumptive null. Animals homozygous for *lin-61(n3809)* are viable. We are using antibodies and translational GFP constructs to characterize the expression pattern of LIN-61. Additionally, we are trying to identify proteins that interact with LIN-61 by performing a yeast two-hybrid screen.

Some of the proteins acting in the synMuv B pathway have been shown to physically interact *in vitro* (1,2) or in yeast two-hybrid assays (3), indicating that many synMuv proteins may function together in transcriptional regulatory complexes. We are attempting to use co-immunoprecipitation experiments to further explore *in vivo* physical interactions among the synMuv proteins. These studies may allow us to assign functionality to some of the novel class B genes, to analyze the effects of various synMuv mutations on physical interactions, to identify potential sub-complexes among the synMuv proteins, and possibly to determine whether class A and class B proteins play redundant roles in the formation of chromatin-modifying complexes.

1. Ceol and Horvitz. (2001) Mol. Cell **7**: 461.
2. Lu and Horvitz. (1998) Cell **95**: 981.
3. Walhout et al. (2000) Science **287**:116.