48. Characterization of the class A synMuv proteins LIN-56 and LIN-15A

Ewa M. Davison[1], John DeModena[2], Linda S. Huang[2,3], Paul W. Sternberg[2], Bob Horvitz[1]

[1]HHMI, Dept. Biology, MIT, Cambridge, MA 02139 USA

[2]HHMI, Division of Biology, California Institute of Technology, Pasadena, CA 91125 USA

[3]Present address: Dept. Biochemistry and Biophysics, UCSF, San Francisco, CA 94143 USA

The receptor tyrosine kinase/Ras pathway essential for vulval induction in *C. elegans* is negatively regulated by the functionally redundant class A and B synthetic Multivulva (synMuv) pathways. Hermaphrodites mutant in only one of these two pathways appear wild-type for vulval induction. Hermaphrodites mutant in both pathways exhibit the synMuv phenotype: cells that normally adopt a hypodermal fate instead adopt a vulval fate and generate ectopic protrusions of vulval tissue along the ventral side of the animal. Various genetic screens for Multivulva animals have defined four genes in the synMuv class A pathway: *lin-8*, *lin-15A*, *lin-38*, and *lin-56*. Of these genes, only the *lin-15A* locus was cloned previously. *lin-15A* encodes a novel protein with no readily apparent functional or structural motifs. The class B synMuv genes antagonize Ras-mediated vulval development via an Rb/E2F/DP-mediated pathway. This inhibition is thus likely effected by transcriptional repression of genes required for vulval development. The class A synMuv genes function in parallel to this Rb pathway, but the molecular mechanism by which they inhibit vulval development is not known.

We have cloned *lin-56*and found it to encode a novel acidic protein. Although no standard motifs are evident in the LIN-56 protein sequence, it does contain a C_3H Zn^{2+}-finger-like motif of atypical spacing and shares this motif with LIN-15A. Immunohistochemistry using antibodies directed against LIN-56 indicates that this protein is localized to the nuclei of many, if not all, cells throughout development. LIN-15A is also nuclear and broadly expressed. The wild-type LIN-56 expression pattern is maintained in *lin-8* and *lin-38* mutant animals. By contrast, LIN-56 protein, but not *lin-56* mRNA, is greatly reduced in *lin-15A* mutants, indicating a role for LIN-15A in the translation or stability of LIN-56 protein. Overexpression of a *lin-56* cDNA under the control of the heat-shock promoters fails to rescue the *lin-15A* synMuv phenotype despite production of LIN-56 protein. Preliminary experiments suggest that the level of LIN-15A protein may be reduced in a *lin-56*, but not in a *lin-8* or a *lin-38*, mutant background. We favor a model wherein LIN-56 and LIN-15A normally coexist in a functional complex, with absence of one of the subunits resulting in the instability of its binding partner(s).