63. Characterization of the Synthetic Multivulva Suppressor Gene *isw-1*, a Homolog of the *Drosophila* Chromatin-Remodeling ATPase ISWI

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The synthetic Multivulva (synMuv) genes are grouped into at least three functionally redundant classes, A, B, and C, that negatively regulate the induction of vulval cell fates. Animals with a mutation in one or more genes within the same class are non-Muv. Animals with mutations in genes within any two classes are Muv. Among some of the identified class B gene products are counterparts of a transcriptional repression complex, and among the identified class C gene products are homologs of a putative transcriptional activation complex.

To identify genes that interact genetically with the synMuv genes, we performed two screens for synMuv suppressors. From these screens, 166 suppressors of the synMuv phenotype of *lin-15AB(n765ts)* animals and 43 suppressors of the synMuv phenotype of *lin-53(n833); lin-15A(n767)* animals were isolated. We cloned one *lin-53(n833); lin-15A(n767)* synMuv suppressor. It encodes a homolog of the chromatin-remodeling ATPase ISWI. We named this gene *isw-1*. RNAi or a presumptive null allele of *isw-1* can suppress the Muv phenotype of most, if not all, synMuv mutant combinations. By contrast, loss of *isw-1* function did not suppress the Muv phenotype of a null mutant of *lin-1*, a RTK/Ras pathway effector. The loss-of-function Vulvaless phenotype of mutants in the RTK/Ras pathway is epistatic to the synMuv phenotype. We conclude that *isw-1* is likely to act downstream of or in parallel to the synMuv genes and upstream of or in parallel to the RTK/Ras pathway.

Immunohistochemistry using antisera specific to ISW-1 suggests that ISW-1 is in the nuclei of most if not all cells throughout development. Levels of ISW-1 are not significantly changed in synMuv mutants as compared to the wild type based on quantitative western blotting. We are currently trying to identify in which cells and during which developmental times *isw-1* is required to allow the synMuv phenotype.

ISWI has been biochemically purified from *Drosophila* embryo extracts as a component of multiple complexes, including the Nucleosome Remodeling Factor (NURF), the Chromatin Accessibility Complex (CHRAC), and the ATP-utilizing Chromatin assembly and remodeling Factor (ACF). RNAi of *C. elegans* homologs of NURF, CHRAC, and ACF complex members in a *lin-15AB(n765ts)* background was used to test for suppression of the synMuv phenotype. A NURF301 homolog and the NURF38 homolog *dhp-2* were identified as synMuv suppressor genes likely to act with *isw-1* to regulate vulval development. Additionally, a deletion allele of the NURF301 homolog can suppress the synMuv phenotype of *lin-15AB(n765ts)* animals. Thus, ISW-1 may be acting as a component of a *C. elegans* NURF-like complex to antagonize the synMuv phenotype. We are mapping and cloning additional mutationally-defined suppressors of the synMuv phenotype in an effort to identify other factors that may act with a putative *C. elegans* NURF-like complex or in parallel processes to antagonize the synMuv phenotype.