A New Class A SynMuv Mutation Is a lin-3(gf) Allele that Causes Increased lin-3 mRNA Levels

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C. elegans vulval cell fates require an RTK/Ras signaling pathway and are antagonized by the synthetic multivulva (synMuv) genes. synMuv genes are grouped into two redundant classes: A and B. Animals with one or more mutations in one class are non-Muv, whereas animals with mutations in both classes are Muv. Many class B proteins are homologous to proteins that repress transcription. The class A synMuv genes might also regulate transcription, as four of the five class A synMuv proteins contain zinc-finger or zinc-finger-like motifs, and the two class A synMuv proteins tested are localized to the nucleus.

In a screen for new class A synMuv alleles we isolated the mutation *n4441*, which causes a dominant class A synMuv phenotype. This observation could be explained if *n4441* were a mutation in a synMuv target gene such that class A synMuv-mediated repression was abolished. We determined that *n4441* is an allele of *lin-3*, which encodes the EGF ligand that controls vulval cell fates. Loss-of-function mutations in *lin-3* cause a vulvaless phenotype, whereas overexpression of *lin-3* causes a Muv phenotype. Cui *et al.* (Dev. Cell 10, 667, 2006) recently showed that the two classes of synMuv genes redundantly repress *lin-3* expression. We found that *lin-3(n4441)*, like other class A synMuv mutations, synthetically causes an increase in *lin-3* mRNA levels when combined with a class B synMuv mutation.

lin-3 has multiple transcription start sites several kb apart. Because the *n4441* mutation is close to the start of the *lin-3a* transcript, we tested whether the synMuv genes specifically regulate *lin-3a*. In all double mutants between a class B mutation and either *lin-3(n4441)* or any other class A mutation, the *lin-3a* transcript is upregulated more than total *lin-3* relative to wild-type levels, suggesting that the synMuv genes specifically prevent overexpression of the *lin-3a* transcript.

Because a mutation in *lin-3* can entirely recapitulate the class A synMuv phenotype, *lin-3* is likely to be the major functionally relevant target of the class A synMuv genes in vulval development. We propose that one or more class A synMuv proteins bind to the *lin-3* promoter at the site of the *n4441* mutation and repress transcription of *lin-3a*. Currently, we are determining the exact DNA sequences in the *lin-3* promoter required for class A synMuv-mediated repression and testing whether the class A synMuv proteins directly bind to the *lin-3* promoter.

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