The "Green Pharynx" Phenotype of Transgene Misexpression Shows synMuv Genes Acting in Different Processes Can Act in Different Combinations

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In the course of a screen for mutants defective in the control of a specific cell death using the reporter *pkd-2::gfp*, we found 29 isolates that had strong, inappropriate GFP expression in the pharynx. From several clonal and nonclonal screens, we identified 68 mutants with this "green pharynx" phenotype. This phenotype is not dependent on chromosomal integration, high transgene copy number, or choice of co-injection marker, and can be seen with at least one other GFP reporter. The green pharynx phenotype requires vector sequence in the reporter construct, consistent with previous reports of a cryptic pharyngeal promoter in the Fire vectors. Vector-driven pharyngeal expression is often inhibited by the inclusion of a promoter in the reporter construct; for some promoters, including *pkd-2*, this inhibition depends on a mechanism absent in green pharynx mutants.

We found that mutations in certain synthetic Multivulva (synMuv) genes could produce the green pharynx phenotype. Animals mutant in two of three described classes of synMuv genes, but not animals mutant in one or more members of the same class, display a Multivulva phenotype. Several class B and class C synMuv genes encode homologs of proteins implicated in transcriptional modification and chromatin remodeling. The synMuv genes required to prevent the green pharynx phenotype include one class A and three class B synMuv genes; thus, a class A gene and three class B genes may act together to prevent inappropriate transgene expression, although class A and class B synMuv genes act separately and in parallel to prevent vulval cell fates. Mutations in 27 other synMuv genes did not cause the green pharynx phenotype. 67 of 68 mutations isolated based upon this phenotype appear to be alleles of three of these four synMuv genes; alleles of the fourth synMuv gene could not be recovered in these screens, as this gene shows maternal rescue.

The one green pharynx mutation that was not an allele of a synMuv gene defined the gene *pag-6* (*pag*, pattern of reporter gene expression abnormal). *pag-6(n3599)* causes altered function of a gene encoding a novel protein. *pag-6(n3599)* mutants are not synMuv; instead, *pag-6(n3599)* is synthetically lethal with a subset of class B synMuv mutations, including *lin-35 Rb*. The synMuv genes that are synthetically lethal with *pag-6(n3599)* may share a normal function redundantly required for viability. This function, like the apparent defect of green pharynx mutants, is likely one of transcriptional regulation, especially as this subset of class B synMuv mutants differs from the subset of class B synMuv mutants defective in cell-cycle regulation.