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Mutations in selected synMuv genes cause the green pharynx phenotype of transcriptional derepression. **Hillel Schwartz**, Dawn Wendell, Bob Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA 02139 USA.

In screens for mutants defective in the control of CEM neuron 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 73 such "green pharynx" mutants. The green pharynx phenotype requires vector sequence in the *pkd-2::gfp* reporter, consistent with the presence of a cryptic pharyngeal promoter in the Fire vectors (Susan Mango, personal communication). Vector-driven pharyngeal expression is often inhibited by the inclusion of a promoter in the reporter; for some promoters, including *pkd-2*, this inhibition depends on a mechanism absent in green pharynx mutants.

Mutations in certain synthetic multivulva (synMuv) genes produce the green pharynx phenotype. Animals mutant in two classes of synMuv genes (A and B), but not animals mutant in one or more genes of the same class, display a multivulva phenotype. Several class B synMuv genes encode homologs of proteins that regulate transcription and modify chromatin. Of the 32 synMuv genes we tested, four were required to prevent the green pharynx phenotype: the class A gene *lin-8* and the class B genes *hpl-2*, *lin-13*, and *lin-61*. Of the 73 green pharynx mutations isolated in our screens, 57 were alleles of *lin-8*, *lin-13*, or *lin-61*; maternal rescue explains the absence of *hpl-2* alleles.

14 of the remaining 16 mutations are alleles of *lnes-1*. LNES-1 and the closely related protein LIN-8 are each required to prevent the green pharynx phenotype. One mutation is a late nonsense allele of *gei-4*; RNAi of *gei-4* can cause a class B synMuv phenotype and lethality (Poulin *et al.*, EMBO J 24: 2613-23, 2005). The remaining mutation, *n3599*, causes altered function of the MSP domain protein PAG-6. *pag-6(n3599)* is synthetically lethal in selected class B synMuv mutants, including *lin-35 Rb*. The synMuv mutants synthetically lethal with *pag-6(n3599)* might lack a function that acts redundantly with *pag-6* to promote viability. This function, like that missing in green pharynx mutants, is likely one of transcriptional regulation.

Our results suggest that, although the class A and class B synMuv genes act separately and in parallel to prevent vulval cell fates, synMuv genes can act in different combinations in different biological contexts. We propose that the class A synMuv gene *lin-8* and the class B synMuv genes *gei-4*, *hpl-2*, *lin-13*, and *lin-61* act together, with *lnes-1* and possibly with *pag-6*, to prevent expression driven by weak or cryptic promoters, ensuring proper transcriptional regulation of endogenous genes.

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Distinct populations of primary and secondary effectors during RNAi in *C. elegans*. **Julia Pak**, Andrew Fire. Dept Pathology, Stanford Univ, Stanford, CA.

RNA interference (RNAi) is a phylogenetically widespread gene silencing process triggered by double-stranded RNA (dsRNA). In plants and *C. elegans*, two distinct populations of small RNAs have been proposed to participate in RNAi : "Primary siRNAs" (derived from Dicer nuclease-mediated cleavage of the original trigger) and "Secondary siRNAs" (additional small RNAs whose synthesis requires an RNA-directed RNA polymerase [RdRP]). Analyzing small RNAs associated with ongoing RNAi in *C. elegans*, we found secondary siRNAs to comprise the vast majority. The bulk of secondary siRNAs exhibited structure and sequence indicative of a biosynthetic mode where each molecule derives from an independent *de novo* initiation by RdRP. Analysis of endogenous small RNAs indicated that a fraction derive from a biosynthetic mechanism that is similar to that of secondary siRNAs formed during RNAi, suggesting that small antisense transcripts derived from cellular mRNAs by RdRP activity may have key roles in cellular regulation. We are currently further characterizing the roles of primary and secondary siRNAs and the genetic requirements for their biogenesis.

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ANTAGONISTIC FUNCTIONS OF SET-2/SET1 and HPL/HP1 PROTEINS in C. ELEGANS DEVELOPMENT. Thomas Simonet, Remi Dulermo, Fabien Ramos, Samia Sheribet, **Francesca Palladino**. Dept. of Molecular and Cellular Biology, ENS , Lyon, France.

Cellular identity during metazoan development is maintained by epigenetic modifications of chromatin structure. The balance between repressive and activating chromatin is maintained by the dynamic activity of specific protein which mediate histone variant incorporation, histone modifications, nucleosome remodeling and DNA methylation. HP1 family proteins function in both silencing and activation of gene expression by directly modifying chromatin structure depending on the chromosomal context. In mammalian cells, recruitment of HP1 to specific promoters by co-repressor proteins including TIF1 and Rb has been shown to be associated with gene repression. The *C. elegans* HP1 proteins HPL-1 and HPL-2 are required for several aspects of post-embryonic development by regulating the expression of specific genes. To better understand how HP1 proteins may influence gene expression in a developmental context, we carried out a candidate RNAi screen to identify suppressors of *hpl-1* and *hpl-2* phenotypes. We identified *C. elegans* SET-2, the homologue of yeast and mammalian SET1, as an antagonist of HPL-1 and HPL-2 activity in growth and somatic gonad development. Yeast Set1 and its mammalian counterparts SET1/MLL are H3 lysine 4 (H3K4) histone methyltransferases associated with gene activation as part of large multisubunit complexes. We show that the nematode counterparts of SET1/MLL complex subunits, including the WDR5 orthologue WDR-1, also antagonize HPL function in post-embryonic development. However, not all *hpl* phenotypes are equally suppressed. Analysis of the mutant phenotypes associated with *wdr-1* and *set-2* deletion alleles are consistent with SET1/MLL complex subunits having both shared and unique functions in development. Furthermore, as observed in other species, we find that SET1/MLL complex homologues differentially affect global H3K4 methylation. Altogether, our results suggest that in *C. elegans*, HP1 and a SET1/MLL related complex may play antagonistic roles in the epigenetic regulation of specific developmental programs.