Functional analysis of the microRNA genes of *C. elegans*: Roles of the *lin-4* and *let-7* families of microRNAs in developmental timing. *Allison L. Abbott[1], Ezequiel A. Alvarez-Saavedra[2], Eric A. Miska[2, 3], Nelson C. Lau[4], David P. Bartel[4], Bob Horvitz[2], Victor Ambros[1].* 1) Dept. Genetics, Dartmouth Medical School, Hanover, NH; 2) HHMI, Dept. Biology, MIT, Cambridge, MA; 3) Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge; 4) Whitehead Institute for Biomedical Research and Dept. Biology, MIT, Cambridge, MA.

lin-4 and *let-7* are microRNAs that act to regulate developmental timing events during larval development in *C. elegans*. The *lin-4* family consists of *lin-4* and *mir-237*, while the *let-7* family consists of *let-7*, *mir-48*, *mir-84* and *mir-241*. By screening a deletion library, we obtained strains with deletions in *mir-237*, *mir-48*, *mir-84* and *mir-241*. Worms with single mutations in *mir-237*, *mir-84* and *mir-241* have no observable mutant phenotype. *mir-48* single mutant and *mir-48; mir-84* double mutant animals display a supernumerary adult molting phenotype. *mir-48; mir-84* worms display a retarded phenotype in the hypodermal syncytium, hyp7. Worms missing both *mir-48* and *mir-241* display a retarded heterochronic phenotype during larval stages: reiteration of the second larval stage developmental program results in the generation of extra seam cells.

Our results indicate that multiple pathways involving microRNAs act to regulate the L2-to-L3 transition. *mir-48*, *mir-84* and *mir-241* appear to act to promote L3-stage events by inhibiting the expression of *hbl-1* post-transcriptionally, likely through putative binding sites in the *hbl-1* 3' UTR. *lin-4* controls early developmental timing events, in part, through the post-transcriptional repression of *lin-28*. Genetic evidence suggests that *mir-237* functions with *lin-4* to control early development, although targets for *mir-237* have not yet been identified. Our data indicate that the *lin-4/lin-28* pathway functions in parallel with *mir-48*, *mir-84* and *mir-241* to control the L2-to-L3 transition.