

## The Role of *mir-237* in Stage-Specific Regulation of LIN-14 and LIN-28 Levels in *C. elegans*

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Heterochronic genes control developmental timing in *Caenorhabditis elegans*. These genes control the progression through larval stages (L1 to L4) to the terminal adult stage. One of the heterochronic genes in *C. elegans*, *lin-4* encodes the first miRNA discovered. My project involves the *lin-4* family member, *mir-237*, with the purpose of determining whether it has an effect on the expression of *lin-14* and *lin-28*. Changes in the levels of two heterochronic regulators, LIN-14 and LIN-28, control the L1 to L2 and the L2 to L3 transitions. The protein levels of these two genes are downregulated by the activity of the *lin-4* microRNA. In addition, there is a feedback loop between *lin-14* and *lin-28* that acts in a *lin-4* independent manner and is an important component of the developmental pathway that regulates progression from the L1 to L3 stages (Moss *et al.*, 2007).

We hypothesize that *mir-237* functions in the *lin-4* independent feedback loop between *lin-14* and *lin-28*. In order to test this hypothesis, we analyzed LIN-14 and LIN-28 levels in protein samples from staged L1 and L3 larvae using Western blot analysis in wild type, and *mir-237* mutant strains in the *lin-4 (0); lin-14 (ts)* background. Normal development in the *lin-4 (0); lin-14 (ts)* background is dependent upon the *lin-4* independent feedback loop. We optimized Western blotting conditions for LIN-14 and LIN-28 detection and used empirically determined optimal concentrations of antibodies to perform Western blot analysis of strains: N2, RF190, VT1175, VT1176 and VT1390. L1 and L3 larvae were synchronized to ensure that protein samples we obtained were representative of each stage. Results to date have been inconclusive likely due to bacterial contamination in our protein preps and possible degradation of protein samples. Additional work will be directed towards isolating L1 and L3 stage protein samples for Western blots.