Analysis of microRNA interactions with mRNA target sites in the *C. elegan* nervous system. *Dominic M Didiano*[1], *Eric Miska*[2,3], *Ezequiel Alvarez-Saavedra*[2], *Bob Horvitz*[2], *Oliver Hobert*[1]. 1) Biochemistry, Columbia University, New York, NY; 2) HHMI, Dept. Biology, MIT, Cambridge, MA; 3) Gurdon Institute, University of Cambridge, Cambridge, CB2 1QN, United Kingdom.

Two microRNA-regulated transcription factors, *cog-1* and *die-1*, are required for the establishment and maintenance of left/right asymmetry in the ASE cell type. The microRNA *lsy-6* has been shown to regulate the translation of *cog-1* in ASEL through deletion analysis and 3' UTR sensor constructs (1). We are systematically mutating the *lsy-6* target site in the *cog-1* 3'UTR in the context of sensor constructs in order to determine the specific requirements for functional microRNA/target interaction. This analysis will provide valuable insight into microRNA/target interaction, which will provide prediction specialists with a verified data set. The status of this analysis will be presented. Furthermore, we hypothesize that a battery of microRNAs, including *mir-273, mir-55, mir-56* and *mir-265* are acting in a combinatorial manner to regulate *die-1* via several evolutionarily conserved target sites in its 3' UTR. *die-1* encodes a Zn finger transcription factor essential for the generation of ASE asymmetry (2). GFP-reporter analysis shows a strong bias for *die-1* expression in ASEL vs. ASER. *die-1* is primarily responsible for inhibiting the expression of *cog-1* in ASEL via activation of the microRNA *lsy-6*. Through the use of a *ceh-36::gfp-die-1* 3' UTR sensor construct it was determined that *die-1* is down regulated in ASER through its 3' UTR (2). This sensor construct analysis indicates that two phylogenetically conserved sites in the *die-1* 3' UTR are required for ASER down-regulation; both sites display complementarity to *mir-273*, *mir-55* and *mir-56* and an as yet untested site displays similarity to *mir-265*. All of these microRNAs are expressed predominantly in the ASE cell type, with some showing a bias toward ASER expression. Additionally, ectopic expression of *mir-55*, *mir-56*, and *mir-273* is each sufficient to disrupt ASE asymmetry; while *mir-265* has yet to be tested. We are determining which miRNAs act on which site/s in the 3' UTR of *die-1* to regulate its expression. This analysis will involve a systematic scanning deletion analysis of the *die-1* 3' UTR, in conjunction with an analysis of microRNA deletion alleles that we retrieved from a deletion library. This analysis will provide a paradigm for combinatorial regulation of target genes by microRNAs. References: (1) Johnston RJ, Hobert O. Nature. 2003 Dec 18;426(6968):845-9. (2) Chang S, Johnston RJ Jr, Frokjaer-Jensen C, Lockery S, Hobert O. Nature. 2004 Aug 12;430(7001):785-9.