

188. Functional Analysis of the MicroRNA Genes of *C. elegans*

Eric A Miska¹, Ezequiel Alvarez-Saavedra¹, Allison L Abbott², Andrew B Hellman¹, Nelson C Lau³, David P Bartel³, Victor Ambros², Bob Horvitz¹

¹HHMI, Dept. Biology, MIT, Cambridge, MA 02139, USA

²Dept. Genetics, Dartmouth Medical School, Hanover, NH 03755, USA

³Whitehead Institute for Biomedical Research and Dept. Biology, MIT, Cambridge, MA 02142, USA
Whitehead Institute for Biomedical Research and Dept. Biology, MIT, Cambridge, MA 02142, USA

The heterochronic genes *lin-4* and *let-7* encode small (21-22 nt) non-protein coding regulatory RNAs^{1,2}. Strains carrying a mutation in either of these genes are heterochronic, displaying retarded development with some cell lineages having an altered temporal pattern of cell division and differentiation. *lin-4* and *let-7* normally inhibit translation of target genes that when mutated lead to a phenotype opposite that of *lin-4* and *let-7* mutants: precocious development and early expression of certain paths of cell division and differentiation.

Recently, molecular and bioinformatic approaches have identified many genes encoding small RNAs in *C. elegans*, *Drosophila* and mammals³⁻⁵. All of these genes encode 21-25 nt RNAs derived from longer transcripts that contain partially double-stranded RNAs. These small RNAs, termed microRNAs (miRNAs, *mir*s), define a large, new class.

To understand the biology of the *C. elegans* microRNA genes, we decided to combine the generation of loss-of-function mutants with GFP expression studies and target prediction using bioinformatics. To date we have generated deletion strains corresponding to 51 microRNAs. We will present the initial characterization of mutant phenotypes (for information about the *mir-35* and the *let-7* families of microRNAs, see poster by Alvarez-Saavedra *et al.*). One focus will be the issue of redundancy within families of microRNA genes.

In a complementary approach we are generating knockout strains for the *argonaute* family of genes. The *argonaute* genes have been implicated both in RNAi and microRNA function^{6,7}. We will present the initial characterization of the knockout phenotypes of two *argonaute* genes, *prg-1* and *prg-2*. We will present the defects of these mutants in germline development and the relationship of these genes to the microRNAs and to RNAi pathways.

1. Lee, R. C., Feinbaum, R. L. & Ambros, V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. **Cell** 75, 843-54 (1993).

2. Reinhart, B. J. *et al.* The 21-nucleotide *let-7* RNA regulates developmental timing in *Caenorhabditis elegans*. **Nature** 403, 901-6 (2000).

3. Lagos-Quintana, M., Rauhut, R., Lendeckel, W. & Tuschl, T. Identification of novel genes coding for small expressed RNAs. **Science** 294, 853-8 (2001).

4. Lau, N. C., Lim, L. P., Weinstein, E. G. & Bartel, D. P. An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. **Science** 294, 858-62 (2001).

5. Mourelatos, Z. *et al.* miRNPs: a novel class of ribonucleoproteins containing numerous microRNAs. **Genes Dev** 16, 720-8 (2002).

6. Grishok, A. *et al.* Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control *C. elegans* developmental timing. **Cell** 106, 23-34 (2001).

7. Tabara, H. *et al.* The *rde-1* gene, RNA interference, and transposon silencing in *C. elegans*. **Cell** 99, 123-32 (1999).