Developmental Cell
Supplemental Data
Li et al.

Regulatory mutations of *mir-48*, a *C. elegans let-7* family microRNA, cause developmental timing defects. Ming Li, Matthew W. Jones-Rhoades, Nelson C. Lau, David P. Bartel, Ann E. Rougvie

Supplementary Experimental Procedures

Nematode strains

veIs51 let-7(mn112) unc-3(e151) mutants were generated by crossing *veIs51* males, which contain an integrated array of pR58 plus *sur-5::gfp*, to SP231 *mnDp1*(X:V)/+; *let-7(mn112) unc-3(e151)* hermaphrodites (Meneely and Herman, 1979). GFP+ cross progeny were picked and *veIs51* + +/+ *let-7 unc-3* animals identified. Following self-fertilize, GFP+ Unc animals were picked to identify *veIs51 let-7 unc-3*/+ *let-7 unc-3* recombinants, and homozygous animals were then identified from among their progeny.

The extrachromosomal transgenic lines described in Supplemental Table S1 were generated by co-injecting the indicated test DNA with *rol-6(su1006)* (pRF4; 100 ng/µl; Mello et al., 1991) into animals bearing *wIs78,* an integrated array containing *scm::gfp* plus *ajm-1::gfp* which mark seam cell nuclei and cell borders, respectively (a gift from M. Maduro and J. Rothman; see Abrahante et al., 2003 for details). PCR fragments were injected at 1 ng/µl and pR56 at 5 ng/µl.

Molecular biology

The sequence of each oligonucleotide used for PCR, site directed mutagenesis, or as radiolabeled probes for northern analysis, is present in Supplemental Table S2.

PCR products. Fragments A, B, and C were amplified from wild-type or *lin-58(ve33)* genomic DNA using the following primer pairs: A, 25.36 plus 22.53; B, 20.113 plus 22.53; C, 25.36 plus 20.114.

Genomic clones. For pR58, the SmaI-MluI fragment of pR56 was replaced with the corresponding fragment from ve33C to introduce the *ve33* lesion. pLM45 contains the 1.05 kb BamHI-SphI fragment upstream of *mir-241* cloned into the corresponding sites of pR58. The SmaI-EcoRV fragment was deleted from pLM45 to give pLM47. The 23 bp *mir-48* coding region was deleted from pLM45 to create pLM48 using the primer set LM31 plus LM32. pR67 is the StuI fragment from pR58 cloned into pBluescript SK(-) (Stratagene).

GFP clones. The 1.0 kb SmaI-SpeI fragment upstream of miR-48 conding sequence was inserted into pPD95.67 (SmaI-XbaI) to generate *mir-48::gfp* (pR40). *mir-48(ve33)::gfp* (pLM36) was derived from pR40 by replacing the SmaI-MluI fragment with the corresponding fragment from ve33C to introduce the *ve33* lesion. In *mir-48(AT)::gfp* (pLM41), the inverted repeat sequence 5'-GGGCGCCGCCC-3' in pR40 was replaced with 5'-ATTTATTATAA-3' by site-directed mutagenesis using the QuikChange XL kit (Stratagene) and the primer set LM17 plus LM18. *mir-241::gfp* (pR59) contains a 1.27 kb fragment upstream of miR-241 coding sequence cloned into the SmaI site of pPD95.67. pLM42 is the 0.73 kb fragment upstream of miR-241 coding sequence amplified with primers LM26 and LM27 and cloned into pCRII (Invitrogen). *mir-241(Δ)::gfp* (pLM43) contains the 0.7 kb BamHI-ScaI fragment of pLM42 cloned into pPD95.67.

Supplemental References

Abrahante, J. E., Daul, A. L., Li, M., Volk, M. L., Tennessen, J. M., Miller, E. A., and Rougvie, A. E. (2003). The *Caenorhabditis elegans hunchback*-like gene *lin-57/hbl-1* controls developmental time and is regulated by microRNAs. Dev Cell *4*, 625-637.

Mello, C. C., Kramer, J. M., Stinchcomb, D., and Ambros, V. (1991). Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. EMBO J *10*, 3959-3970.

Meneely, P. M., and Herman, R. K. (1979). Lethals, steriles and deficiencies in a region of the X chromosome of Caenorhabditis elegans. Genetics *92*, 99-115.

Supplemental Table S1. *mir-48* Arrays Cause Seam Cell Phenotypes			
Array[a]	Construct	Seam cell fusion at L3 molt[b]	Seam cell number[c]
none	-	0	16.0±0.0
veEx229	wtA	3	16.0±0.3
veEx361	wtA	0	15.9±0.3
veEx363	wtA	0	16.0±0.2
veEx364	pR56	0	15.9±0.3
veEx230	ve33A	100	12.3±1.2
veEx231	ve33A	86	12.7±1.6
veEx236	ve33B	0	16.0±0.3
veEx241	ve33B	9	15.4±0.9
veEx265	ve33C	67	14.2±1.4
veEx325	ve33C	90	13.3±1.4

Note: For all values, $n \geq 20$.

[a] The background strain contains *wIs78* to facilitate identification of seam cells (see Supplemental Methods).

[b] Percentage of animals with at least one pair of seam cells fused at the L3 molt.

[c] Seam cell number was scored in adults. Seam cell numbers were wild-type at hatching. Skipping of the S2 division at the end of the L1 molt lowers seam cell number (see Figure 3).

Supplemental Table S2. Oligonucleotide sequences	
Name	Oligonucleotide Sequence (5' to 3')
25.36	TGCAAAACCATCTAGAGTATTGGTG
22.53	AGAAGCTGGCGCCGAGTAGTAT
20.113	CTTACCTGTGGGGGAATCAA
20.114	TCACTTGGATTAGGGGTTCG
LM17	ATTTATTATAACTCTCTAGTTCCTTCTGA
LM18	TTATAATAAATACAAATCGGACTAACGGC
LM26	ACTAGTACTTTGACACCCCCGCGG
LM27	TCTTCGACCGCGGCTCATCATAATCC
LM31	TTGAAACTCCCGGGAACTAATGAACGGTATCTCACATC
LM32	GATGTGAGATACCGTTAATAGTTCCCGGGAGTTTCAA
miR-48	TCGCATCTACTGAGCCT
miR-241	CATTTCTCGCACCTACC
Lin-4	TCACACTTGAGGTCTCAGGGA
Let-7	ACTATACAACCTACTACCTCA
U6	TTGCGTGTCATCCTTGCGCAGG

Note: Oligonucleotides were used in PCR or clone construction except for the last five, which were radiolabeled and used as probe in the northern analyses (Figure 1B, C).

Supplemental Table S3. Quantitation of miRNA accumulation during larval development.

Quantitation of Figure 1B					Quantitation of Figure 1C				
Strain	Hours	miR-48	miR-241	*let-7* RNA	Strain	Hours	miR-48	miR-241	*lin-4* RNA
N2	0	<0.2	<7.2	<1.8	N2	6	<0.1	<5.3	<2.3
N2	6	<0.3	<9.9	<2.5	N2	9	<0.2	<4.8	<2.1
N2	9	<0.4	<14	<3.6	N2	12	0.9	<5.2	30
N2	12	7.7	16	<2.8	N2	18	8.7	11	113
N2	18	13	24	<2.9	N2	24	28	40	99
N2	24	33	54	4.6	N2	29	51	65	102
N2	28	66	70	12	N2	31	50	76	100
N2	32	100	100	100	N2	33	100	100	100
ve33	0	0.4	<4.6	<1.2	ve33	6	0.6	<2.0	<0.9
ve33	6	0.8	<7.9	<2.0	ve33	9	0.8	<3.1	<2.1
ve33	9	2.4	<9.7	<2.5	ve33	12	2.4	<3.7	13
ve33	12	9.0	11	<2.3	ve33	18	15	9.4	89
ve33	18	42	25	<3.1	ve33	24	36	14	70
ve33	24	58	51	10	ve33	29	48	63	93
ve33	29	65	69	17	ve33	32	46	79	95
ve33	32	119	101	150	ve33	34	36	73	87

For each probing, signals representing the mature miRNA were quantitated, normalized to the signal of U6, and then normalized to the level of the N2 (wild-type) signal at 33 hours. Upper limits for undetectable bands were estimated based on quantitation values of weekly visible bands on the same blot and normalization to the U6 loading control.