Comprehensive identification of *C. elegans* miRNA pathway genes

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Little is known about the actual mechanism by which miRNAs down-regulate target gene expression. An exploration of what protein co-factors are necessary for a miRNA to down regulate a target gene should reveal more fully the molecular mechanisms by which miRNAs are processed, trafficked, and regulate their target genes. We used a weak allele of the *C. elegans* miRNA gene *let-7* as a sensitized genetic background for a whole-genome RNAi screen to detect miRNA pathway genes, and 213 candidate miRNA pathway genes were identified (1).

Genes in one large class of candidate miRNA pathway genes encode proteins with known or predicted roles in RNA binding or processing. Included in this class is *dcr-1*, with its well-established role in miRNA processing. Also included in this category is *imb-4*, which encodes the worm ortholog of exportin-1, an importin-ß-like protein involved in nuclear trafficking. Several of the genes identified encode proteins predicted to act in mRNA processing. A translation initiation factor itself, eif-3.D, was also identified, as was a DEAD-box RNA helicase, F01F1.7. W04D2.6, which encodes a conserved protein with an RNP-1 RNA binding domain and a PWI domain was also detected. PWI domains bind both single-stranded and double-stranded nucleic acids, making this protein an intriguing candidate for a miRNA pathway component.

A number of the candidate genes encode proteins that play a structural or regulatory role in the cytoskeleton. Y19D2B.1 and *tba-2* both encode α-tubulins and *mec-7* encodes a β-tubulin. *spd-1* encodes a microtubule interacting protein and *dyce-1*, which encodes an intermediate chain of the motor protein dynein, and *dnc-1*, which encodes the dynein interactor dynactin, also interact with microtubules. Two genes encoding members of the ADP-ribosylation factor family, *arf-1.2* and *arf-3*, were also identified. Members of this protein family play roles in controlling microtubule dynamics as well as intracellular trafficking. *unc-59*, which encodes a septin, a filament-forming protein with known roles in cytokinesis, was also identified. Evidence for a connection between small RNAs and the cytoskeleton does exist. The discovery that RNAs interact with and play a regulatory role in mitotic spindle formation {Blower, 2005 #284} provides a precedent for this potential miRNA/cytoskeleton regulatory connection. Although we have no direct evidence, it is tantalizing to imagine localized zones of miRNA regulation guided by association with the cytoskeleton.

Several transcription factors were also identified, for example the Polycomb Group gene *sop-2*. The identification of the *sop-2* inactivation as an enhancer of *let-7(mg279)* raises the possibility of a similar transcriptional silencing mechanism involving miRNAs. Several known or predicted transcription factors are also *let-7* enhancing gene inactivations, including *ceh-18, elt-1*, H20J04.3, Y53G8AR.9 and F13H6.1. Many miRNAs are expressed at higher levels in differentiated cells than tumor cells, suggesting that as cells differentiate during development, the expression of miRNAs is
under significant transcriptional control. The transcription factors identified in our screen could control the expression of general miRNA pathway components, including other genes identified in this screen. In addition, *dpy-21*, which encodes a component of the dosage compensation complex was identified. It is also possible that miRNAs associate with these chromatin factors in the nucleus, in analogy to the siRNA regulation of heterochromatin formation in fungi.

To determine if any of the targeted genes are required for *let-7* biogenesis, Northern blots with *let-7* probes were performed after inactivation of each of the candidate miRNA pathway genes. Besides *dcr-1*, no other dsRNA treatment induced a dramatic increase in precursor or decrease in mature *let-7*, suggesting that these miRNA pathway genes act at a later step.

*lin-4*, the first miRNA identified downregulates *lin-14* mRNA translation during larval development. For many of the *let-7*-enhancing gene inactivations, the down-regulation of LIN-14 at late stages was not as pronounced as in wild-type. Taken together with the *let-7* Northern blot data, these LIN-14 results suggest that a major subset of the genes identified in our screen function between the point of miRNA biogenesis and the point of target mRNA down-regulation. Given how little is known about how miRNAs actually induce down-regulation of target mRNA translation and/or stability, the genes identified in this screen are promising leads towards discerning the molecular mechanisms by which miRNAs downregulate their targets. In this set are several genes from the RNA binding/processing class, including the RNA binding W04D2.6, the nuclear cap-binding F37E3.1 and the predicted U5 snRNP interactor F32B6.3. It may be that these factors facilitate the interaction of the target mRNA with its corresponding miRNA or detect that interaction to in-turn recruit the mRNA::miRNA complex to P bodies and down-regulate translation.

DCR-1 is an example of a protein that functions in both the miRNA and RNAi pathways. Kim et al. performed a whole-genome RNAi screen designed to identify genes required for RNAi (2). They identified only three genes (*dcr-1*, *pop-1* and *kin-10*) from their list of 90 candidates that when inactivated caused significant enhancement of *let-7*(mg279), suggestive of a role in the miRNA pathway as well. Consistent with this, only two of our 44 confirmed *let-7* pathway genes (*dcr-1* and *pop-1*) appear on the RNAi candidate list. It appears that, by these assays, the RNAi and miRNA pathways are largely molecularly distinct.