Functional screen identifies factors that coordinate with miRNAs to regulate gene expression in *C. elegans*

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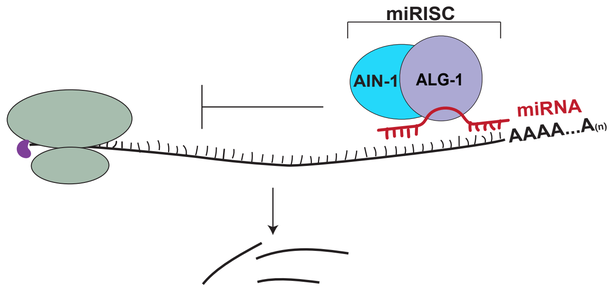
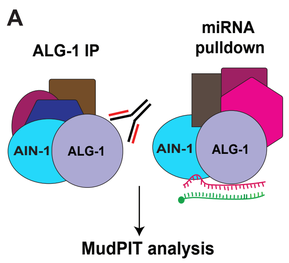
1. miRNA mediated gene regulation

Regulation of gene expression is essential for normal physiology and development. One of the ways cells regulate gene expression is through a class of non-coding RNAs called microRNAs (miRNAs). miRNAs associate with Argonaute proteins to form miRNA Induced Silencing Complexes (miRISCs), which post-transcriptionally repress gene expression by targeting mRNA 3'UTRs through partial sequence complementarity. We have previously identified proteins that co-precipitate with the *C. elegans* miRISC component, Argonaute ALG-1[1]. In addition, we also identified potential physical interactors of specific miRNAs. Functional assays in genetically sensitized backgrounds in a RNAi based screen on 56 factors identified 30 factors to modify phenotypes associated with miRNA mutants. Currently we are characterizing the mechanism through which one such factor, HRP-1 coordinates with miRNA mediated gene regulation.



2. ALG-1 immunoprecipitation and miRNA pulldown identified physical interactors



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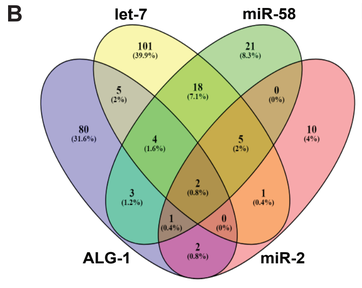
ALG-1 IP

GO term	# genes	P value
Biological Processes		
Embryo development	64	3.72E-17
Translation	18	2.57E-23
RNA splicing	4	5.3E-3
Protein catabolic process	7	1.5E-3
Cellular Components		
Intracellular ribonucleoprotein	22	1.19E-21
P granule	3	8.2E-2
Ribosome	20	4.05E-18
P bodies	4	2.29E-3

let-7 PD

GO term	# genes	P value
Biological Processes		
Embryo development	72	7.33E-15
mRNA splicing, via spliceosome	14	2.86E-13
Translation	18	8.41E-10
mRNA processing	10	4.96E-8
Gene silencing by RNA	4	1.15E-3
Cellular Components		
Intracellular ribonucleoprotein	20	4.77E-17
P granule	14	1.18E-14
Mitochondrion	19	5.46E-8
P bodies	4	4.03E-3



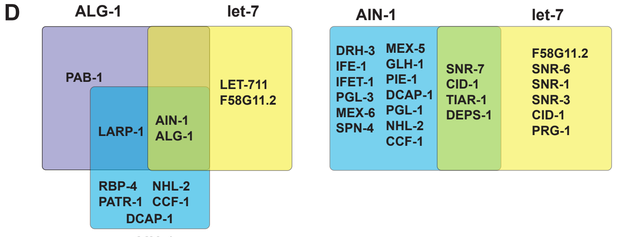


Figure 1: (A) Schematic of ALG-1 IP[1] and miRNA PD followed by MudPIT analysis to identify physical interactors. (B) Venn diagram showing the number of common and unique miRNA and ALG-1 co-factors. (C) Gene Ontology analysis for Biological Processes and Cellular Components for ALG-1 and let-7 co-factors. (D) Subset of ALG-1 and let-7 co-factors overlap with AIN-1[2] co-factors.

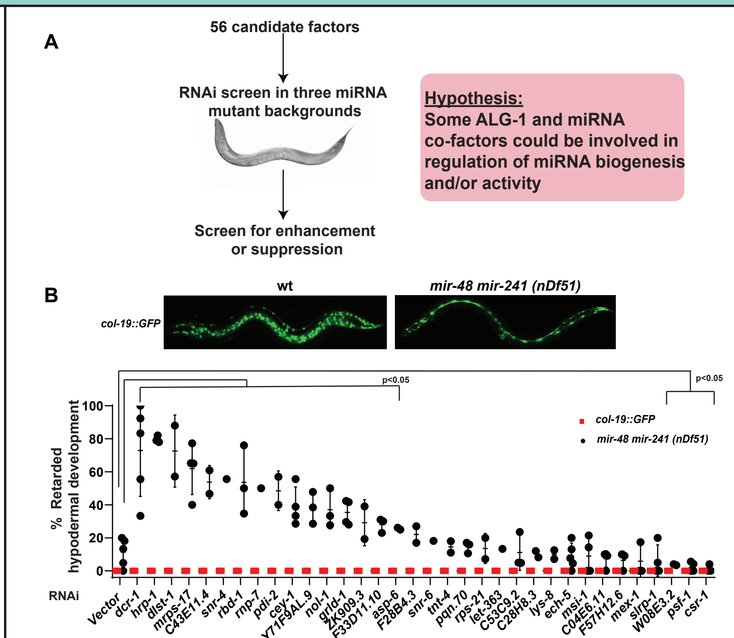
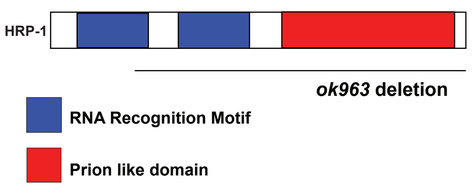


Figure 2: (A) Experimental approach to identify factors that coordinate with miRNA mediated gene regulation. (B) An example of one of the functional assays carried out on a subset of co-factors. Assay in let-7 family miRNA (*mir-48 mir-241 (nDf51)*) sensitized background identified factors that positively and negatively coordinate with miRNA activity.

3. Characterization of mechanism by which HRP-1 coordinates with miRNA mediated gene regulation

- HRP-1 is a homolog of HnRNP A1.
- One of the strongest enhancers of miRNA mutant phenotypes identified through RNAi screen
- Predicted to have RNA binding activity
- HnRNP A1 binds the pri-let-7a-1 and inhibits its processing by Drosha[4] and promotes pri-mir-18a processing[5].



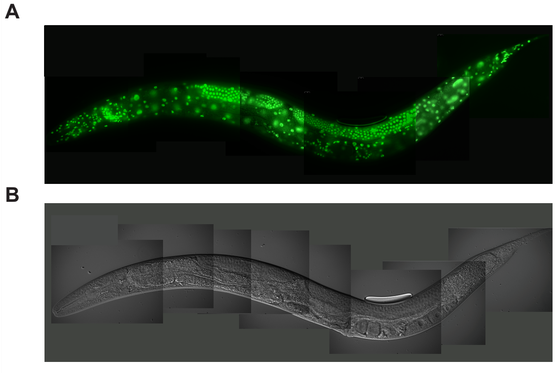


Figure 2: Endogenously tagged *hrp-1::gfp* transgene is expressed ubiquitously in *C. elegans*. HRP-1::GFP expression in somatic and germline nuclei in young adult worm (Fluorescent (A) and DIC (B)).

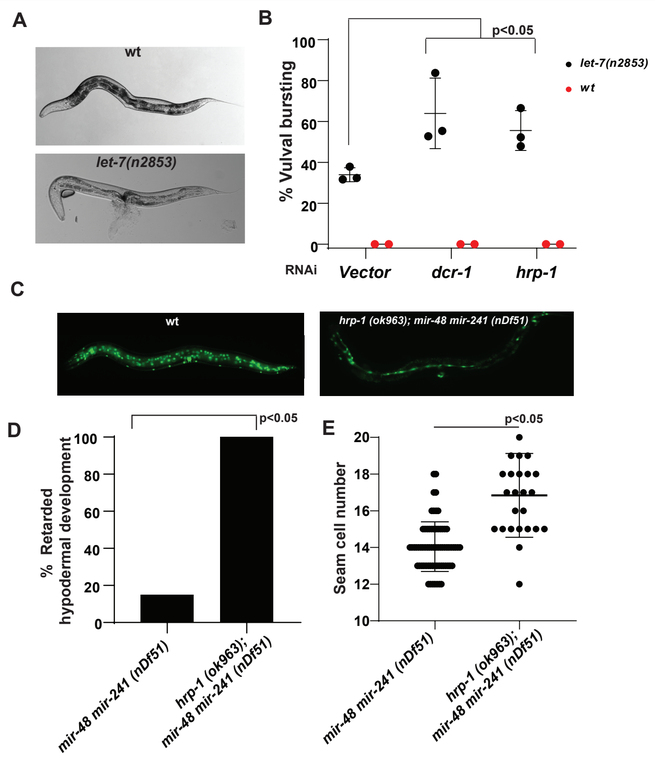
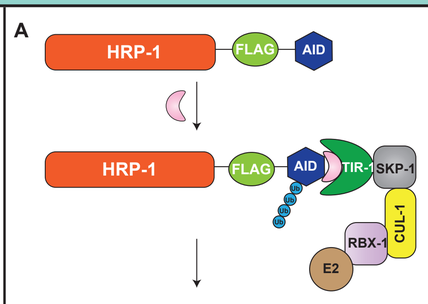


Figure 3: *hrp-1* functionally interacts with multiple miRNAs. (A) *let-7(n2853)* mutants show vulval bursting phenotype. (B) Loss of *hrp-1* enhanced this phenotype in *let-7* mutant background. (C) Young adult worms expressing *col-19::GFP* in seam cells and hypodermal cells (left). *mir-48 mir-241(nDf51)* mutants show delayed development with hypodermal cells lacking the *col-19::GFP* expression (right). Loss of *hrp-1* strongly enhanced the retarded development phenotype in hypodermal (D) and seam cells (E).



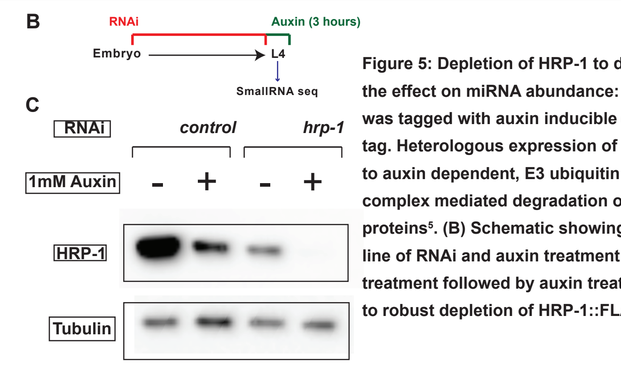


Figure 5: Depletion of HRP-1 to determine the effect on miRNA abundance: (A) *hrp-1* was tagged with auxin inducible degron tag. Heterologous expression of TIR1 leads to auxin dependent, E3 ubiquitin ligase complex mediated degradation of tagged proteins[5]. (B) Schematic showing the time-line of RNAi and auxin treatment. (C) RNAi treatment followed by auxin treatment led to robust depletion of HRP-1::FLAG::AID.

4. Conclusion

RNAi based screen on 56 ALG-1 and miRNA co-factors identified 30 potential modulators of miRNA activity.
HRP-1 was confirmed to genetically interact with multiple miRNAs.

5. Future work

Identify and characterise potential roles of HRP-1 in modulating miRNA processing and/or activity by performing smallRNA seq and CLIP experiments.

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