MicroRNA: Basic Understanding

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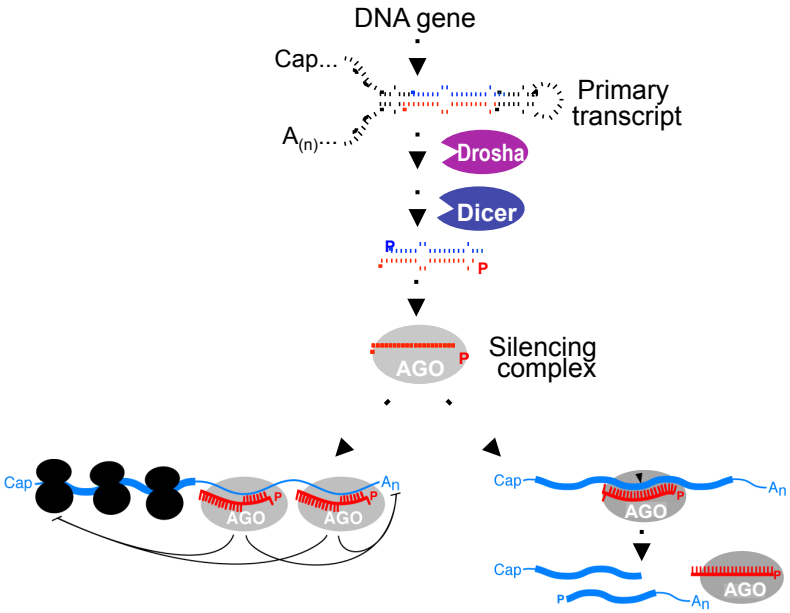
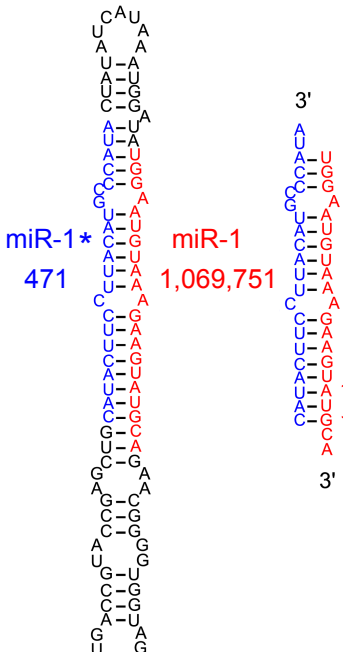
MicroRNAs (miRNAs)



Sequencing of small RNAs from *C. elegans*

330 reads (Lau et al. 2001)
4,078 reads (Lim et al. 2003)
394,926 reads (Ruby et al. 2006)
22,986,137 reads (Ruby, et al. 2011)



MicroRNA genes

M. musculus:
506 miRNA genes
Tuschl lab
plus Chiang et al., 2010

Mammals

D. melanogaster:
148 miRNA genes
Tuschl lab
plus Ruby et al., 2007

Insects

Cnidaria

Nematostella:
40 miRNA genes
Grimson et al., 2008

C. elegans:
159 miRNA genes
Ruby et al., 2006
Jan et al., 2011

Nematodes

Sponge

Amphimedon:
8 miRNA genes
Grimson et al., 2008

Fungi

Arabidopsis:
151 miRNA genes
Rajagopalan et al., 2006
plus Carrington lab

Flowering plants

Physcomitrella:
205 miRNA genes
Axtell et al., 2007

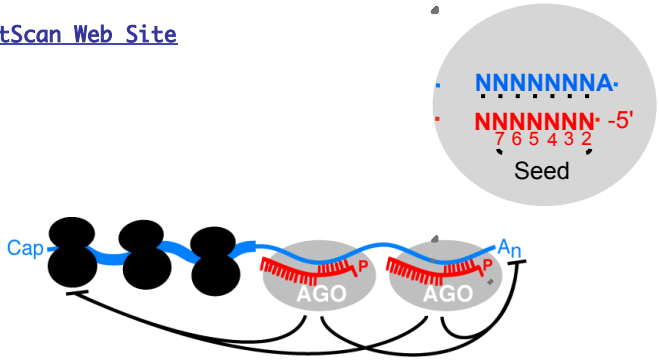
Moss

Abundance of miRNA targets

Each highly conserved miRNA has many preferentially conserved targets (Lewis et al. 2005; Cohen lab, 2005; Rajewsky lab, 2005)

- >400 per gene family (Friedman et al., 2009)
- More than half of all human mRNAs (Friedman et al., 2009)
- Even more non-conserved targets (Farh et al., 2005; Schier lab, 2006)

TargetScan Web Site



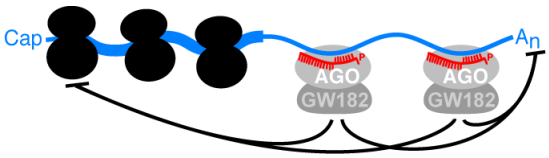
Molecular consequences of miRNA-directed repression

1993–2005 miRNAs repress mRNA translation with essentially no change in mRNA

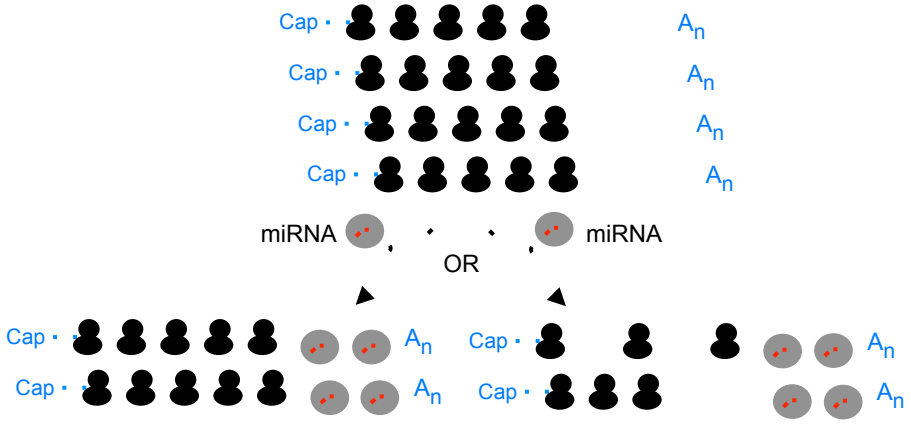
2005 mRNA microarrays: small but detectable decrease for many mRNAs (Lim et al., 2005); occurs through deadenylation (Giraldez et al., 2006; Wu et al., 2006; Behm-Ansmant et al., 2006; Eulalio et al., 2009)

The original *C. elegans* targets also decrease (Bagga et al., 2005)

2008 Proteomics: most of the repression explained by mRNA destabilization/ deadenylation (Beak et al., 2008; our interpretation of Selbach et al., 2008)



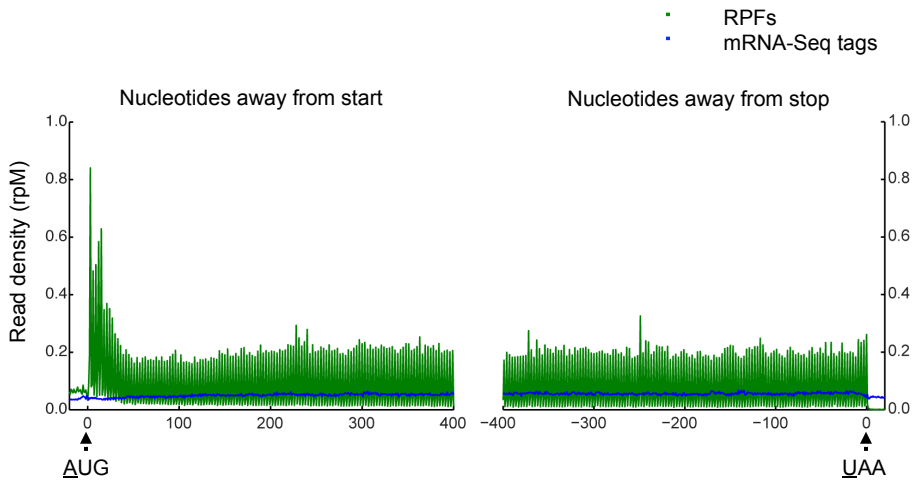
How much repression can destabilization explain?



Ribosome Profiling

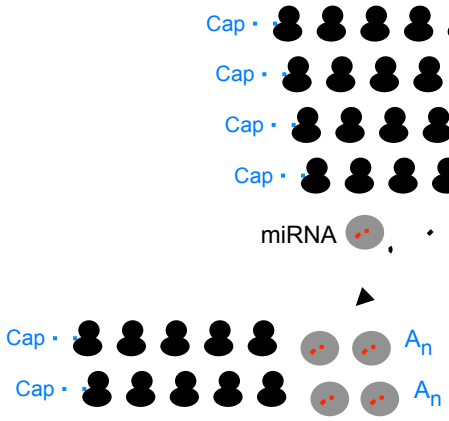
Nick Ingolia & Jonathan Weissman
(*Science* **324**:218 2009)

Ribosome locations along a composite mRNA



Guo et al., 2010

Molecular consequences of miRNA targeting



- Ribosome changes mostly reflect mRNA changes.
- Each interaction leads to modest repression.
- No evidence for targets repressed at only the protein level.
- Detectable change in ribosome occupancy. (must occur before complete deadenylation)

- Some cell-to-cell differences: 70–90% of the repression explained by mRNA deadenylation/destabilization.

Baek, Villén, Shin, et al., 2008; Guo, et al., 2010,& unpublished; also Brown/Herschlag labs

Classical view of miRNA function:

Yes, but they mostly decrease mRNA levels

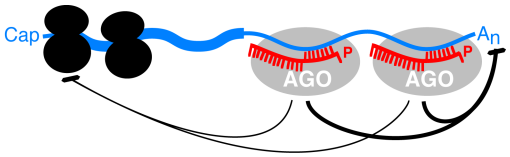
Binary switch: repress translation of a few key mRNA targets to initiate a developmental transition.

Not in animals

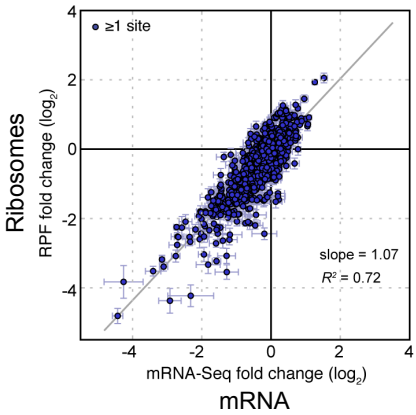
Yes, usually other functions

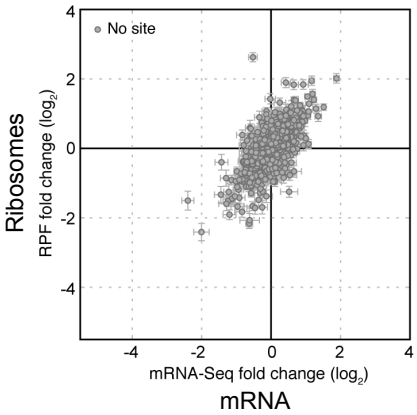
A modern view of miRNA function:

Rheostat: dampen expression of many mRNA targets to optimize expression, reinforce cell identity, and sharpen transitions.



mRNA and ribosome changes after introducing miR-1





Guo, et al., 2010

Response of proteins from messages with sites

Of those with at least one 7mer site, ≥18% respond to miRNA loss.
Of those with at least one 8mer site, ≥33% respond to miRNA loss.

- sites falling in ORFs, fewer respond
- sites that are conserved, more respond
- sites in favorable predicted context, more respond (Grimson et al., 2007)
- miRNAs with stronger seed pairing, more effective (Garcia et al., 2011)
- miRNAs with fewer sites, more effective (Garcia et al., 2011)

TargetScan Web Site

Baek, Villén, Shin, Camargo, Gygi, Bartel, 2008

Thanks to:

miRNA targeting

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miRNA genomics

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Ribosome profiling

Huili Guo
Nick Ingolia & Jonathan Weissman (UCSF)