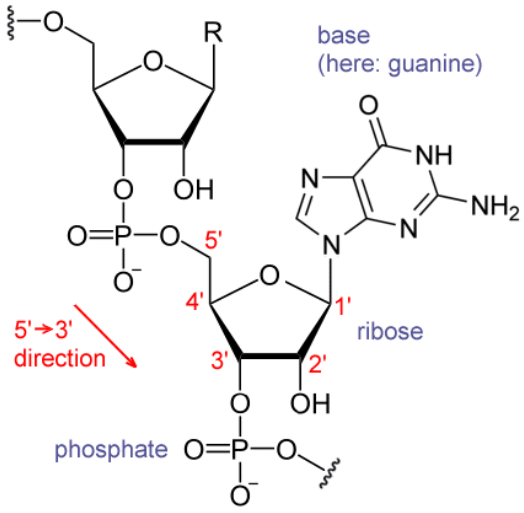
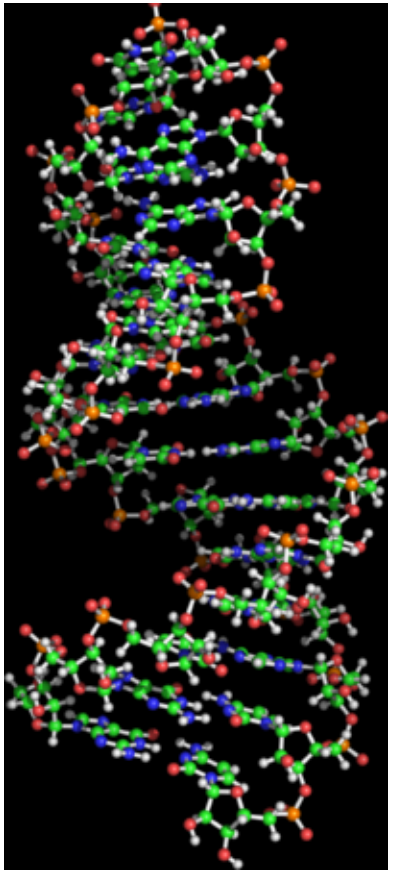
Ribo-gnome: The Big World of Small RNAs

Phillip D. Zamore and Benjamin Haley

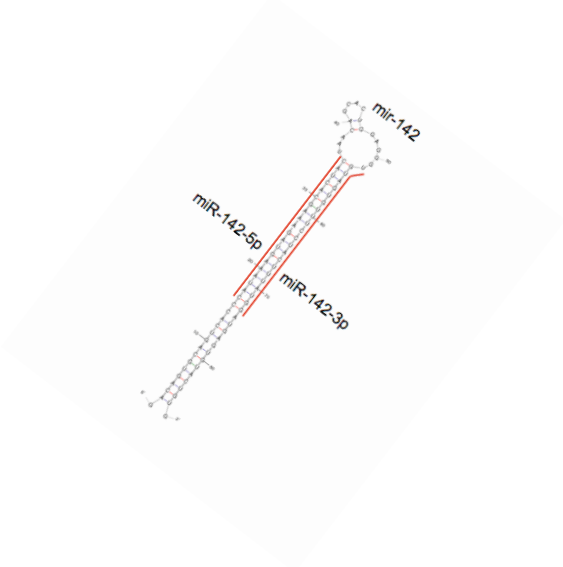




Presentation by: Christopher Jakubowski

Three Classes of small RNAs

- miRNAs



- siRNAs

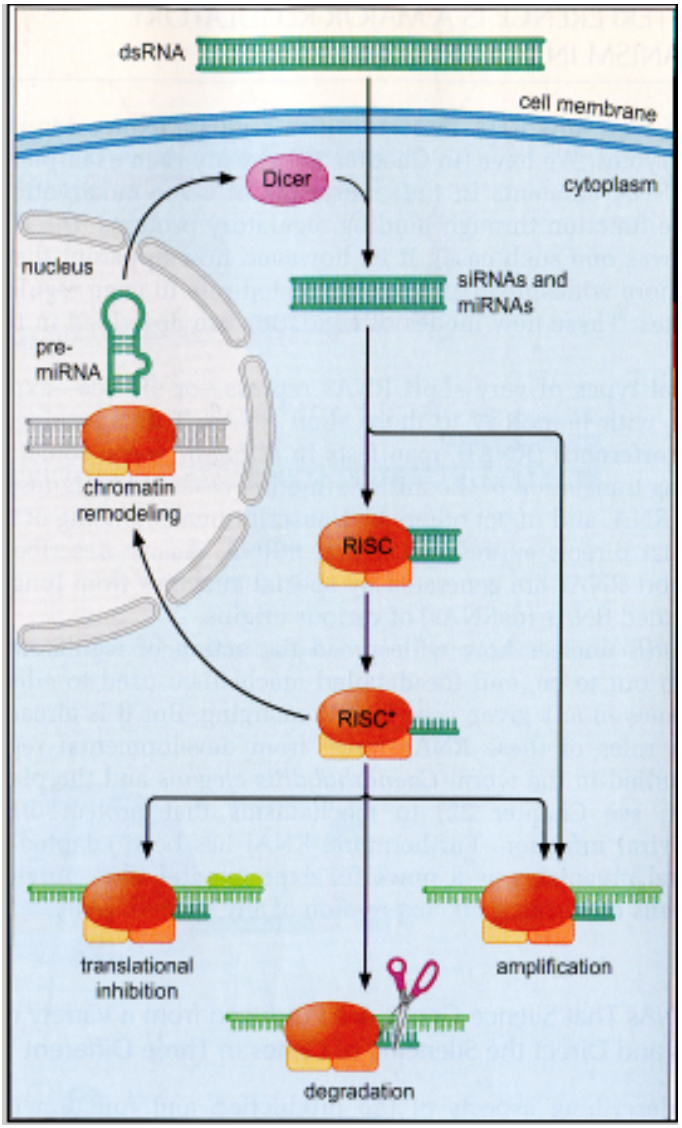
- rasiRNAs

RNA silencing pathway

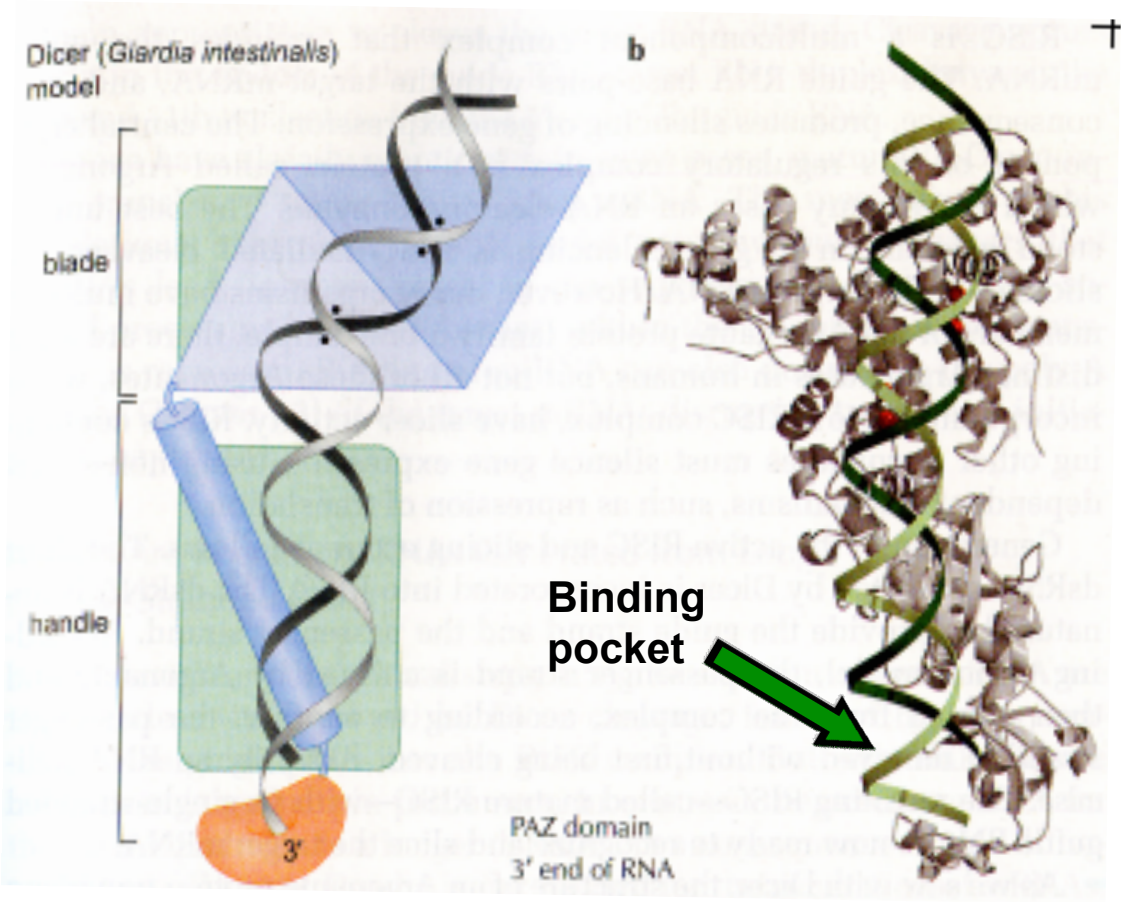
- Double strand molecules must have homology

- Three methods of repression:

1. Digest mRNA
2. Block Translation
3. Chromatin Modification

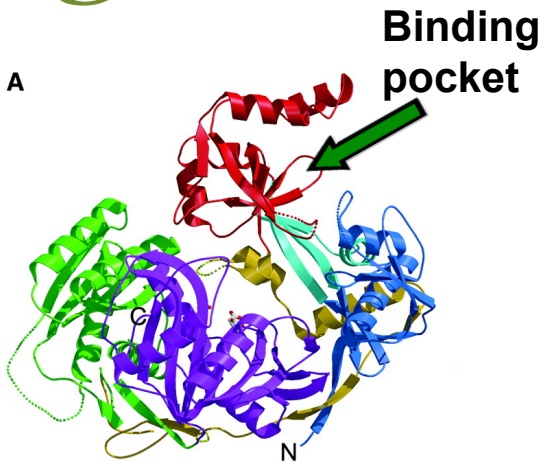


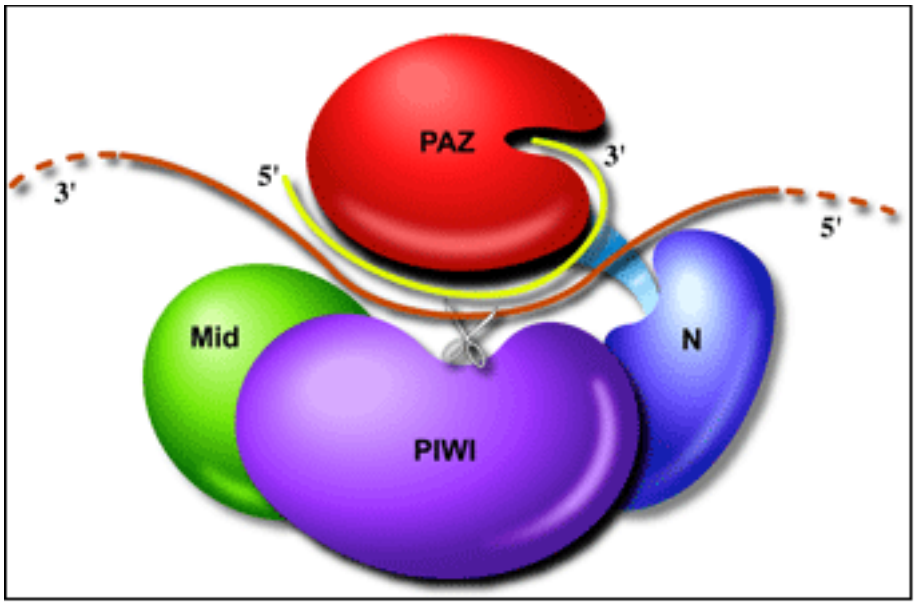
Dicer



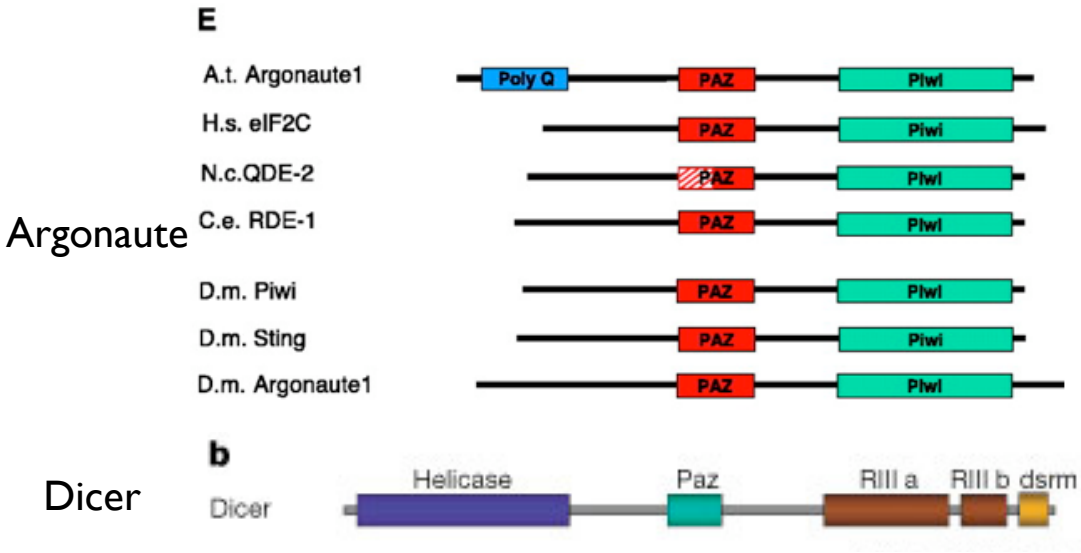
- Selects cleavage sites based on measuring
- Three modules: two RNase III domain and PAZ domain
- Cleaves 22 nucleotides from its end
- Connection between siRNA and miRNA

Argonaute





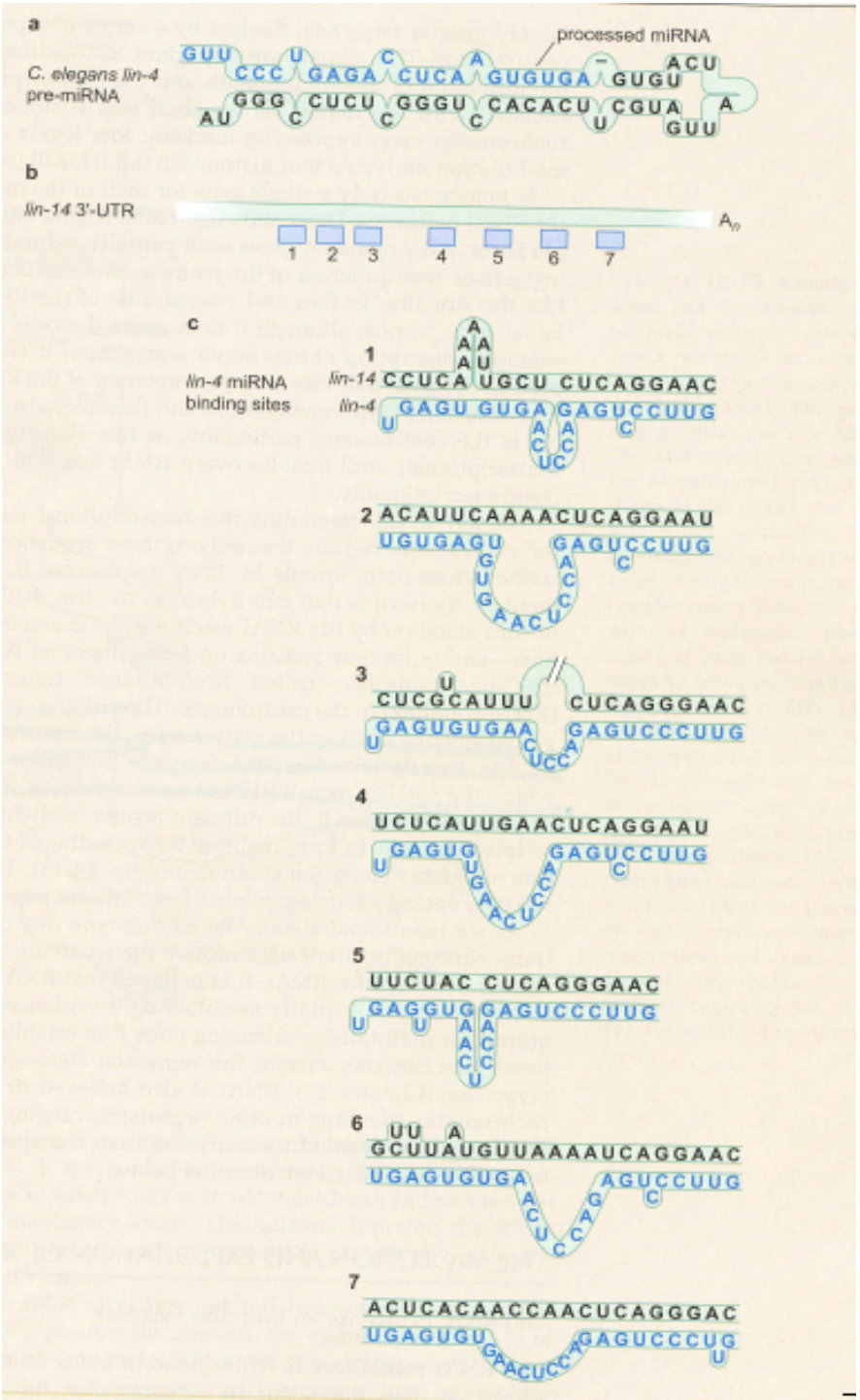
Along with Dicer both have PAZ domain and RNase Domain



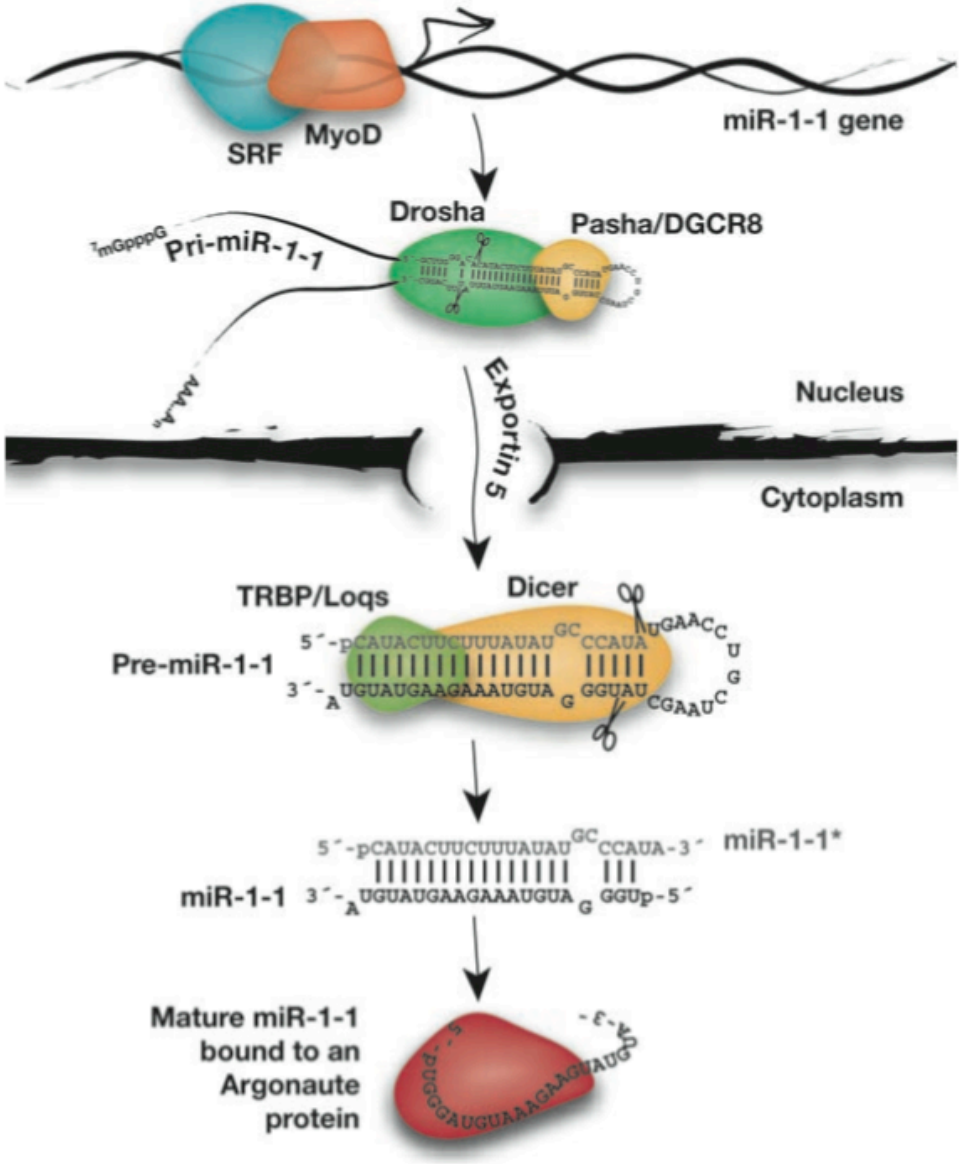
Discovery of miRNA

- While siRNAs are found in an assortment of eukaryotes, miRNAs have only been found in plants, animals, and their viruses

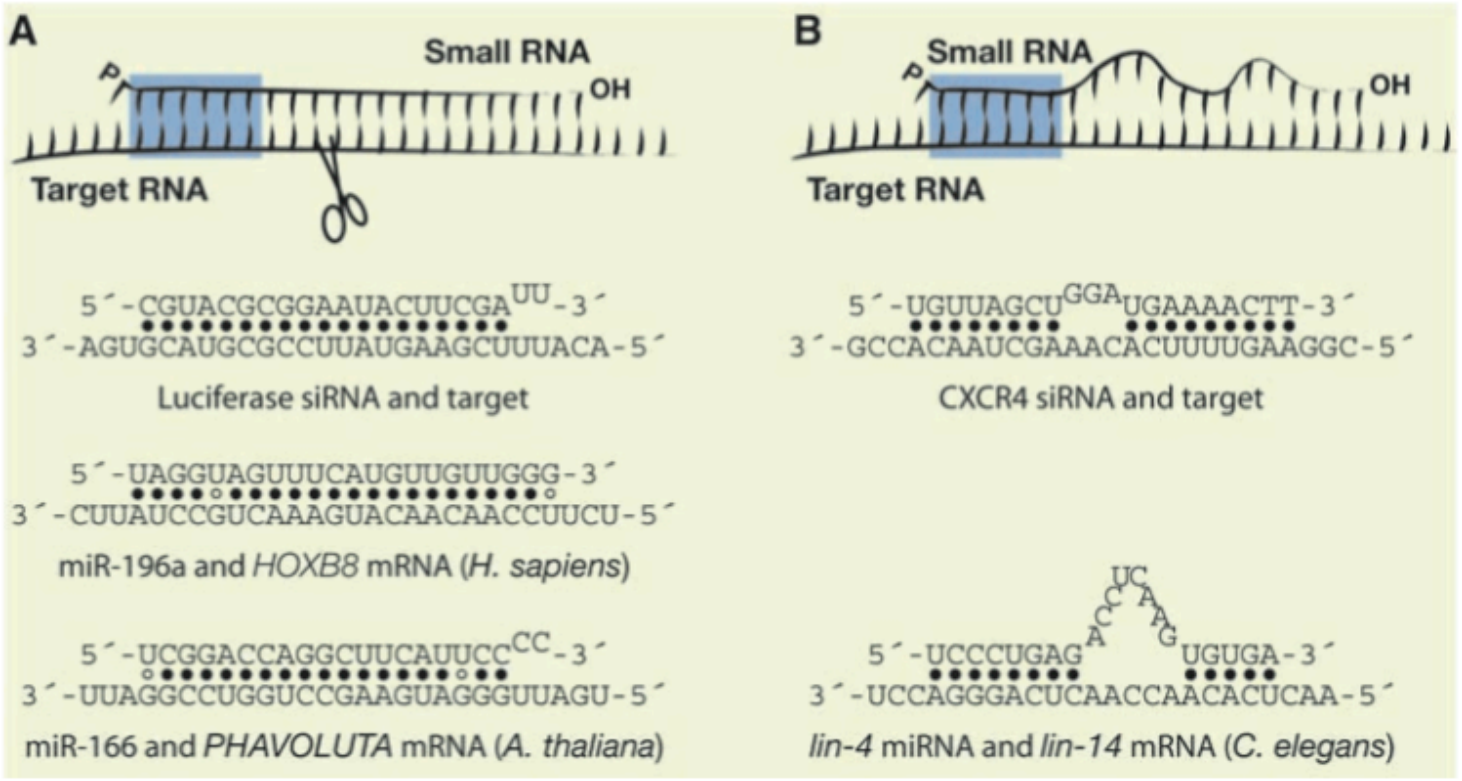
- Ambros and co-workers found two RNA transcripts from *lin-4* locus and *lin-4* could base pair sites within *lin-14*



miRNA miR-1


SRF
MyoD
miR-1-1 gene
Drosha
Pasha/DGCR8
Pri-miR-1-1
Exportin 5
Nucleus
Cytoplasm
TRBP/Loqs
Dicer
Pre-miR-1-1
miR-1-1
miR-1-1*
Mature miR-1-1
bound to an
Argonaute
protein

Small RNAs act in two distinct ways



- "Seed" sequence highlighted in blue nucleates binding
- Figure (A) presents extensive RNA binding. Argonaute proteins are then directed to cut a single phosphodiester bond leading to destruction
- Figure (B) presents partial base pairing. With attached Argonaute protein translation is prevented

Theories regarding translation inhibition

- Animal miRNAs usually act in this mode because of partial complementarity

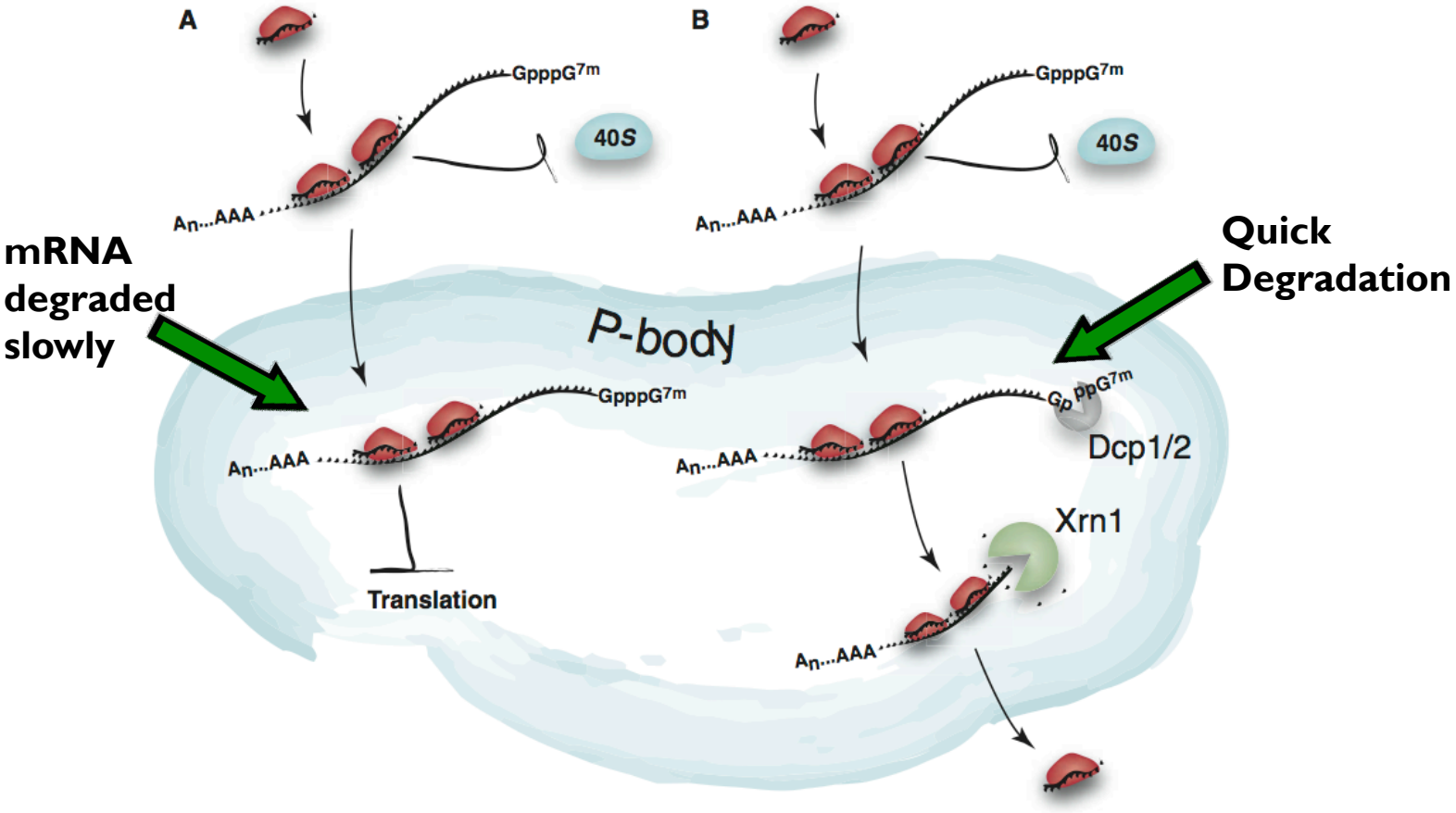
- What happens?

1. Direct degradation of nascent polypeptide
2. “Freeze” ribosomes in place

- Theories called into question
  - Lim and co-workers using microarrays found miRNAs can alter stability of hundreds of mRNAs
  - Studied changes in steady-state mRNA unlikely to be due to cleavage

- **How do miRNAs make mRNA less stable then?**

Sequestration in P-body model

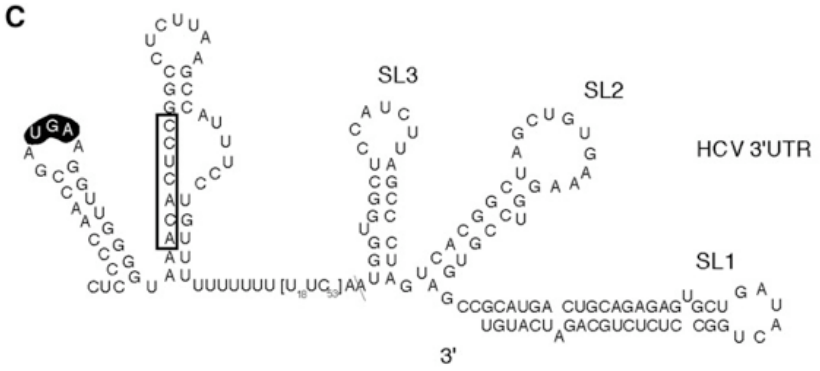


- Cytoplasmic site of mRNA decapping and degradation
- Argonaute concentrates here only when bound to miRNAs or siRNAs
- Mutant Argonautes remain in the cytosol
- Argonaute associates with decapping enzymes

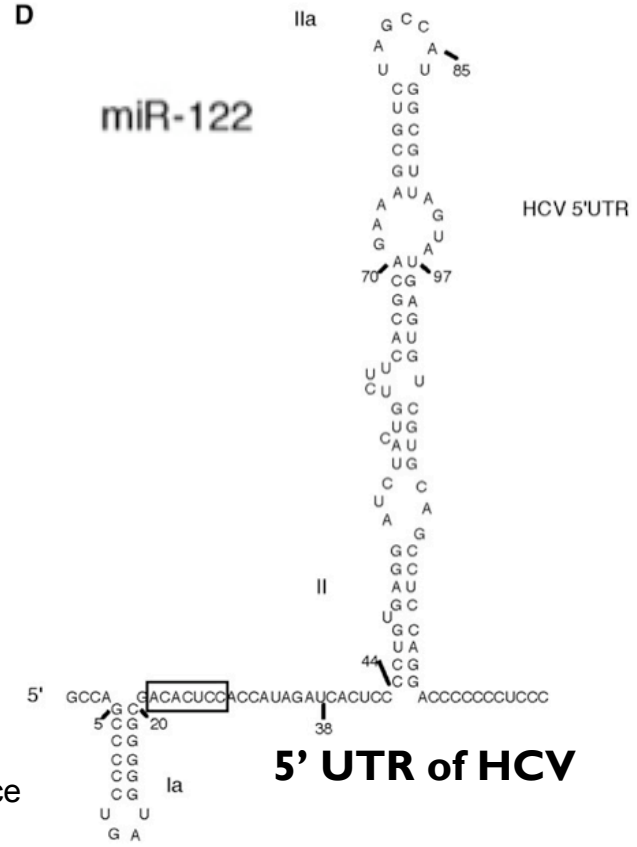
Model of miRNA gene activation

- Specific liver cells, Huh7, produce miR-122
- Hepatitis C virus (HCV) can only replicate in Huh7 cells
- **Connection between presence of miR-122 in permissive Huh7 cells?**

Sequence of miR-122 with the seed sequences surrounded by a box



3' UTR of HCV

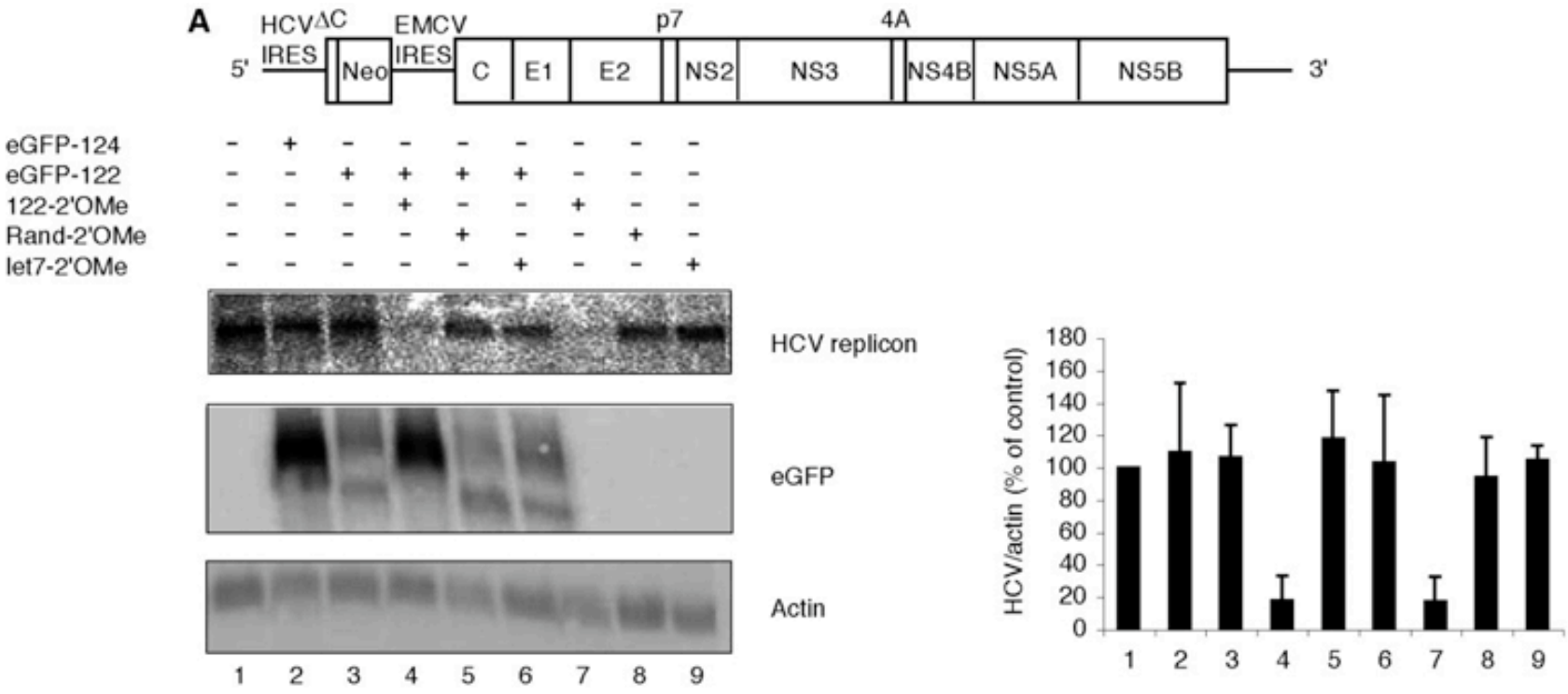


5' UTR of HCV

Jopling, Catherine L. "Modulation of Hepatitis C Virus RNA Abundance by a Liver-Specific MicroRNA." Science 309 (2005): 309.

Testing the predicted models

- Abundance of autonomously replicating, HCV RNA replicon was tested during miR-122 inactivation

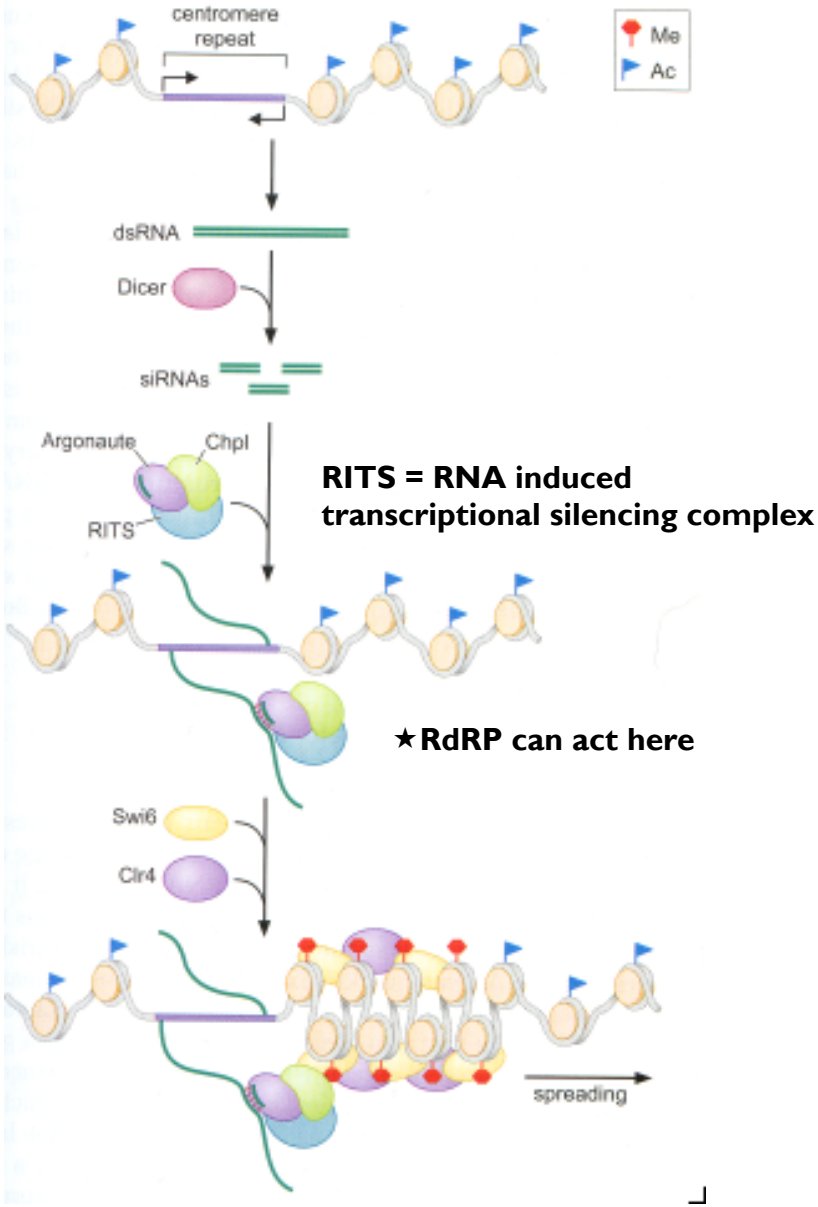


Implications in transcriptional silencing

- Associated with heterochromatin formation
- Marked by H3K9 methylation or hypermethylation
- Topics:
  - *S. pombe*
  - RNA Polymerase IV
  - RNA Polymerase II

si-RNA directed heterochromatin assembly

- In outer regions of centromere in *S. pombe*
- Needed for chromosome segregation
- Repetitive sequences compose out regions (similar to mammals)
- Argonaute can also slice transcripts and RdRP can make further substrates increasing efficiency



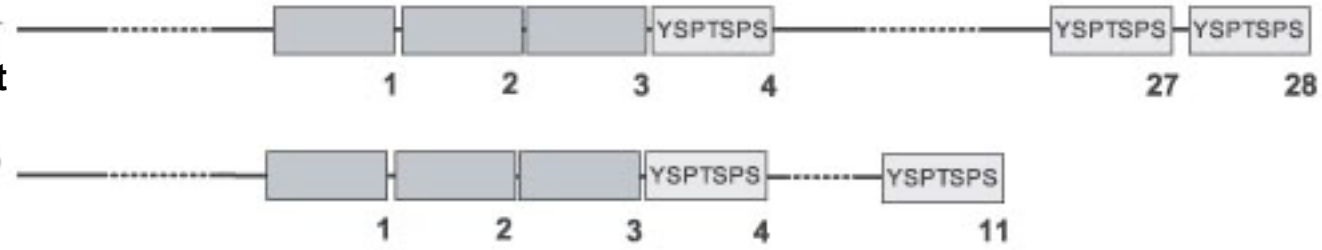
Supplying transcripts for siRNA production

- Previous model requires transcription across silenced regions
- In plants, RNA polymerase IV transcribes silent heterochromatin
- RNA-dependent RNA polymerase (RdRP) can then make substrate for dicer
- Pol II can create targets for small RNAs as well as trigger for small RNA production
- CTD of Pol II might interact with silencing machinery, then Argonaute proteins with loaded siRNA are recruited

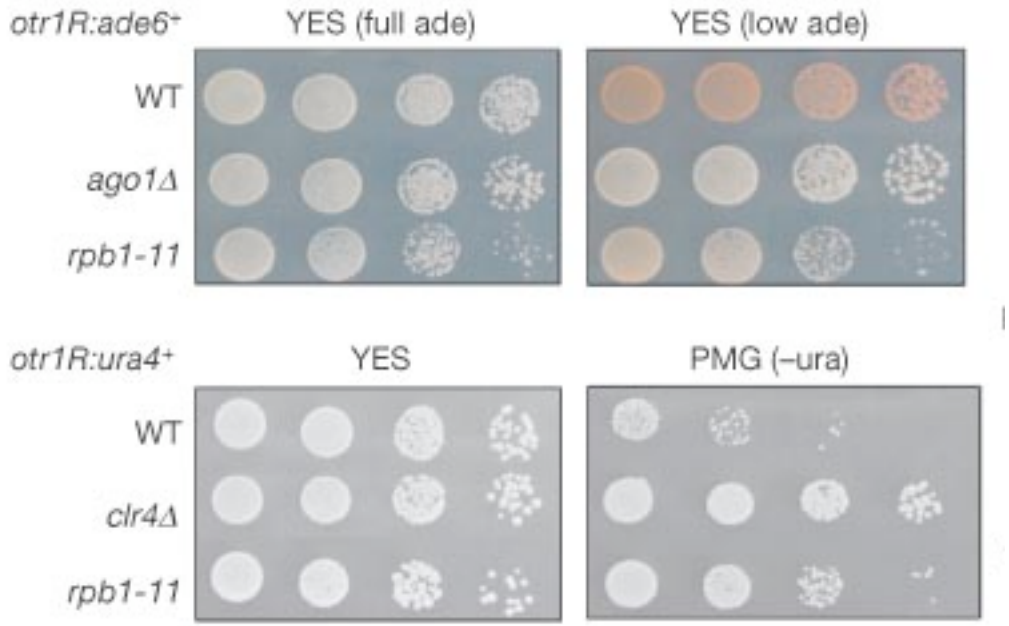
Evidence for CTD interaction

Experiments in *S. cerevisiae*

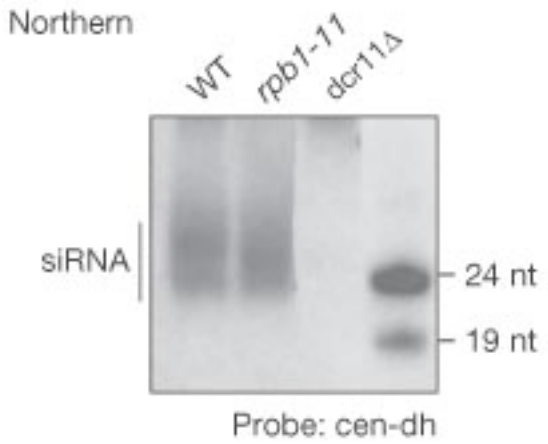
Large Subunit of Pol II



- **Deletion of 16 CTD heptad repeats**



- **ura4+ and ade6+ are centromeric markers**

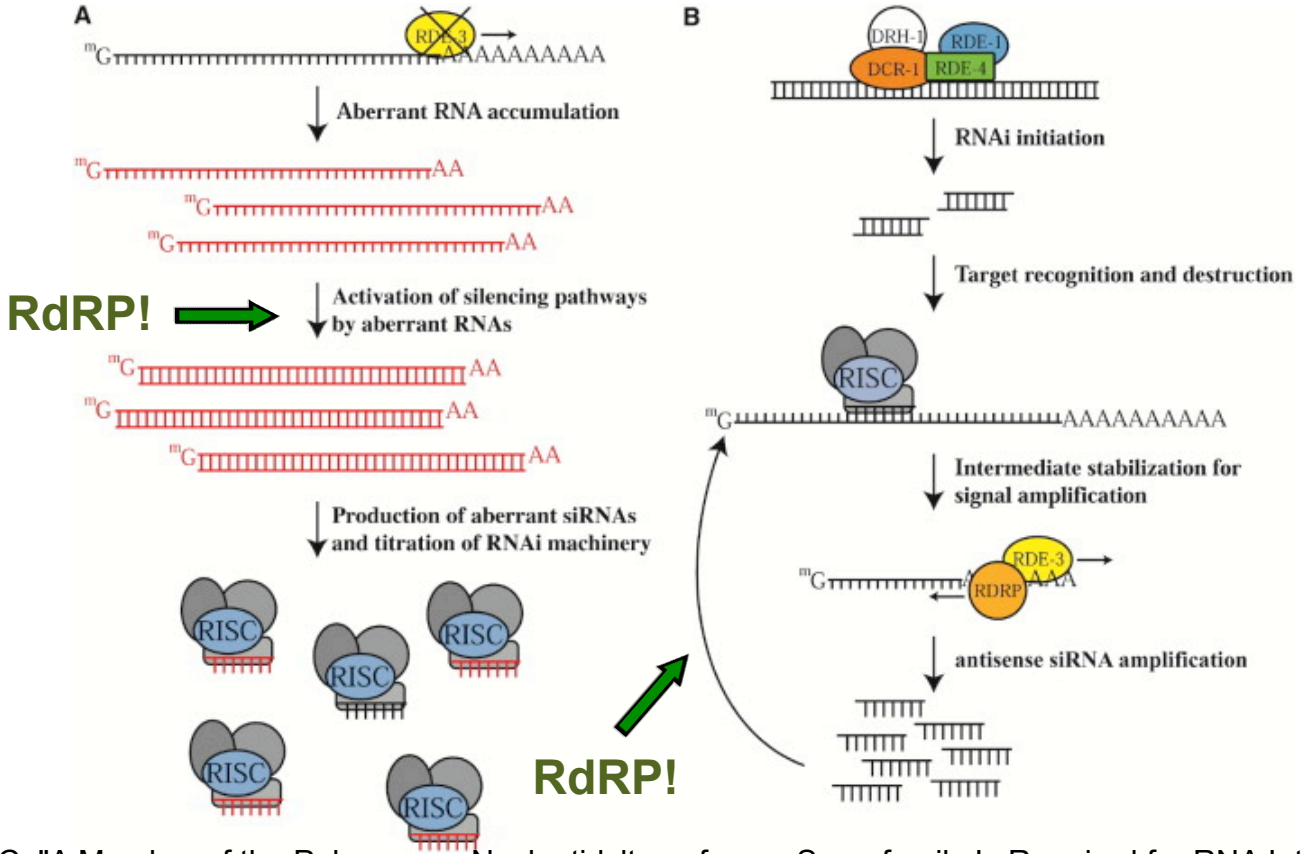


Transcription but no repression!

V. Schramke et al., Nature 435, 1275 (2005)

Template-independent RNA polymerases

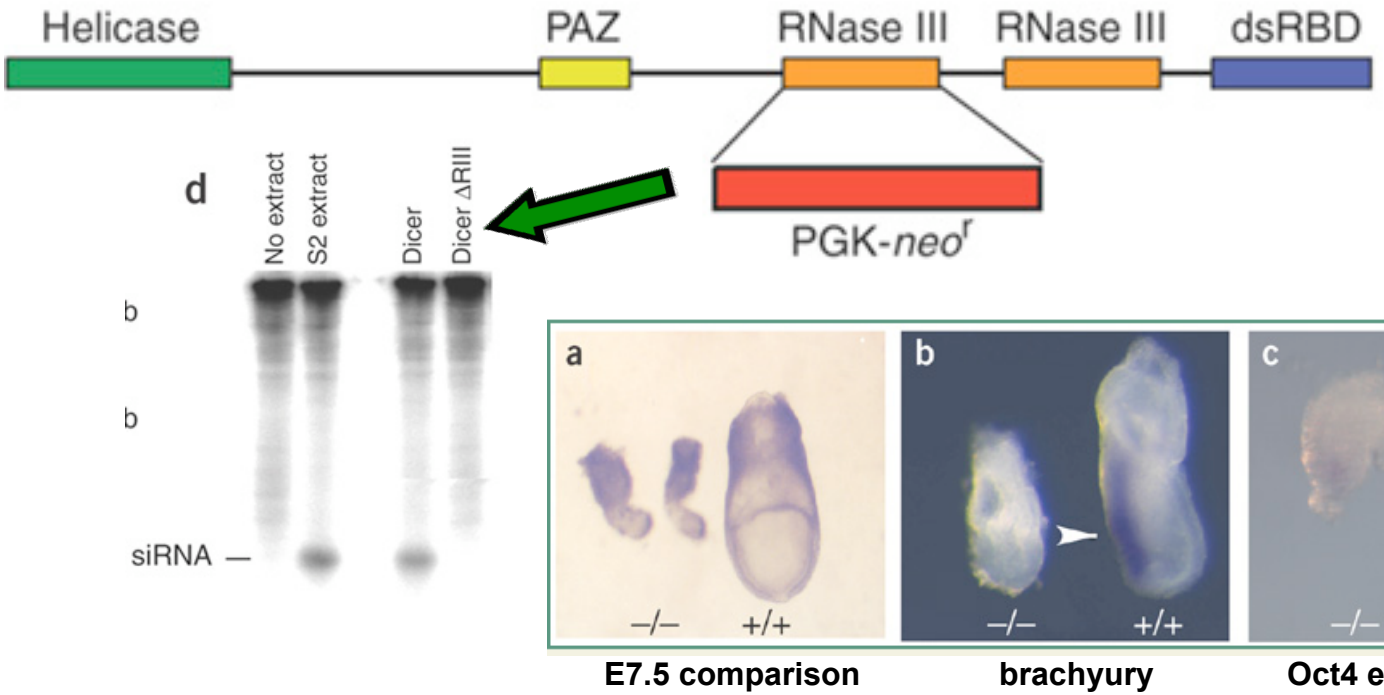
- Required for RNA silencing in worms and yeast
- Polymerase β nucleotidyltransferase superfamily (includes polyA polymerases)



Chen, C. C. "A Member of the Polymerase Nucleotidyltransferase Superfamily Is Required for RNA Interferance in C. elegans." Current Biology 15 (2005): 378.

Small RNAs needed for stem cell life cycle

- Embryonic stems cells lacking Dicer, Argonaute proteins, or dsRNA-binding partners die rapidly
- Defects due to either loss of miRNA or silent heterochromatin



E7.5 comparison **brachyury** **Oct4 expression**

Bernstein, E. "Dicer is essential for mouse development." Nature Genetics 35 (2003): 215.

Citations

Bernstein, E. "Dicer is essential for mouse development." Nature Genetics 35 (2003): 215.

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Jopling, Catherine L. "Modulation of Hepatitis C Virus RNA Abundance by a Liver-Specific MicroRNA." Science 309 (2005): 309.

Onodera, Yasuyuki, and Jeremy R. Haag. "Plant Nuclear RNA Polymerase IV Mediates siRNA and DNA Methylation-Dependent Heterochromatin Formation." Cell 120 (2005): 613.

V. Schramke et al., Nature 435, 1275 (2005)

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