Ribo-gnome: The Big World of Small RNAs
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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
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

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

RdRP can act here

RITS = RNA induced transcriptional silencing complex
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*

- Deletion of 16 CTD heptad repeats

Large Subunit of Pol II

- *ura4*<sup>+</sup> and *ade6*<sup>+</sup> are centromeric markers


Transcription but no repression!
Template-independent RNA polymerases

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

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

Citations