Small non-coding RNAs

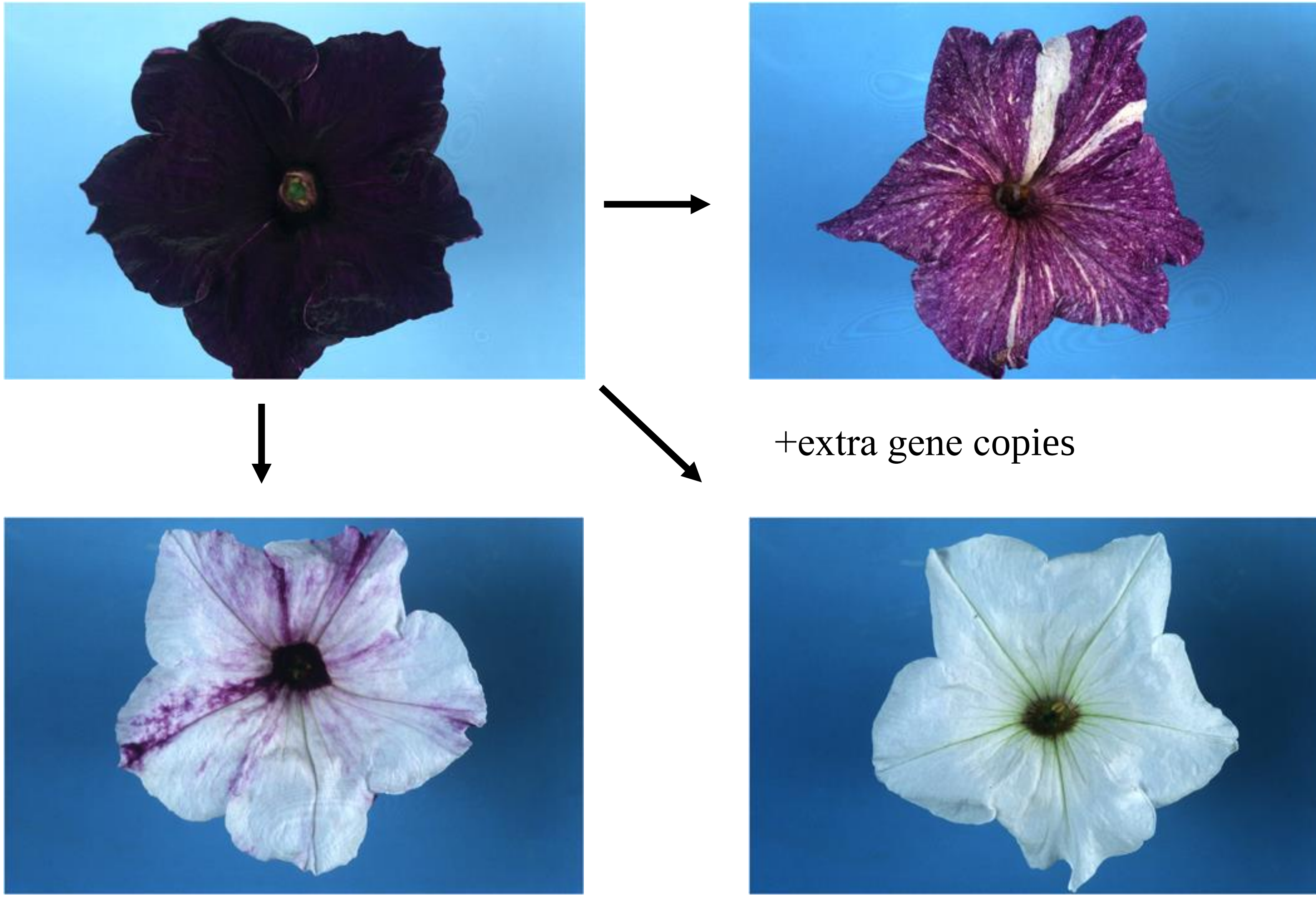
-History on RNAi-discovery

-core-players (Argonaute proteins and small RNAs)

-miRNAs:
- -Expression
- -miRNA families
- -Biogenesis
- -Silencing mechanism

-piRNAs:
- -Targets
- -Mechanism of silencing
- -Biogenesis of piRNAs (Clusters)

A long time ago…



Petunia (1990; Jorgensen)

DNA:

RNA:

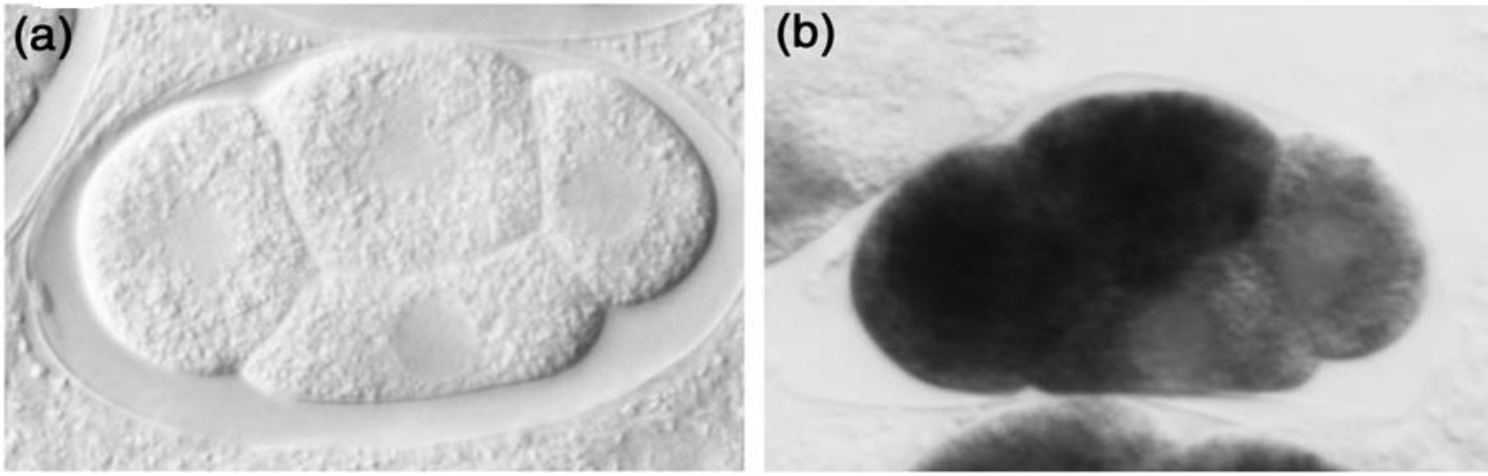
Not plant-specific:

Arabidobsis thaliana

Drosophila melanogaster

Caenorhabditis elegans

Observations in *C. elegans*

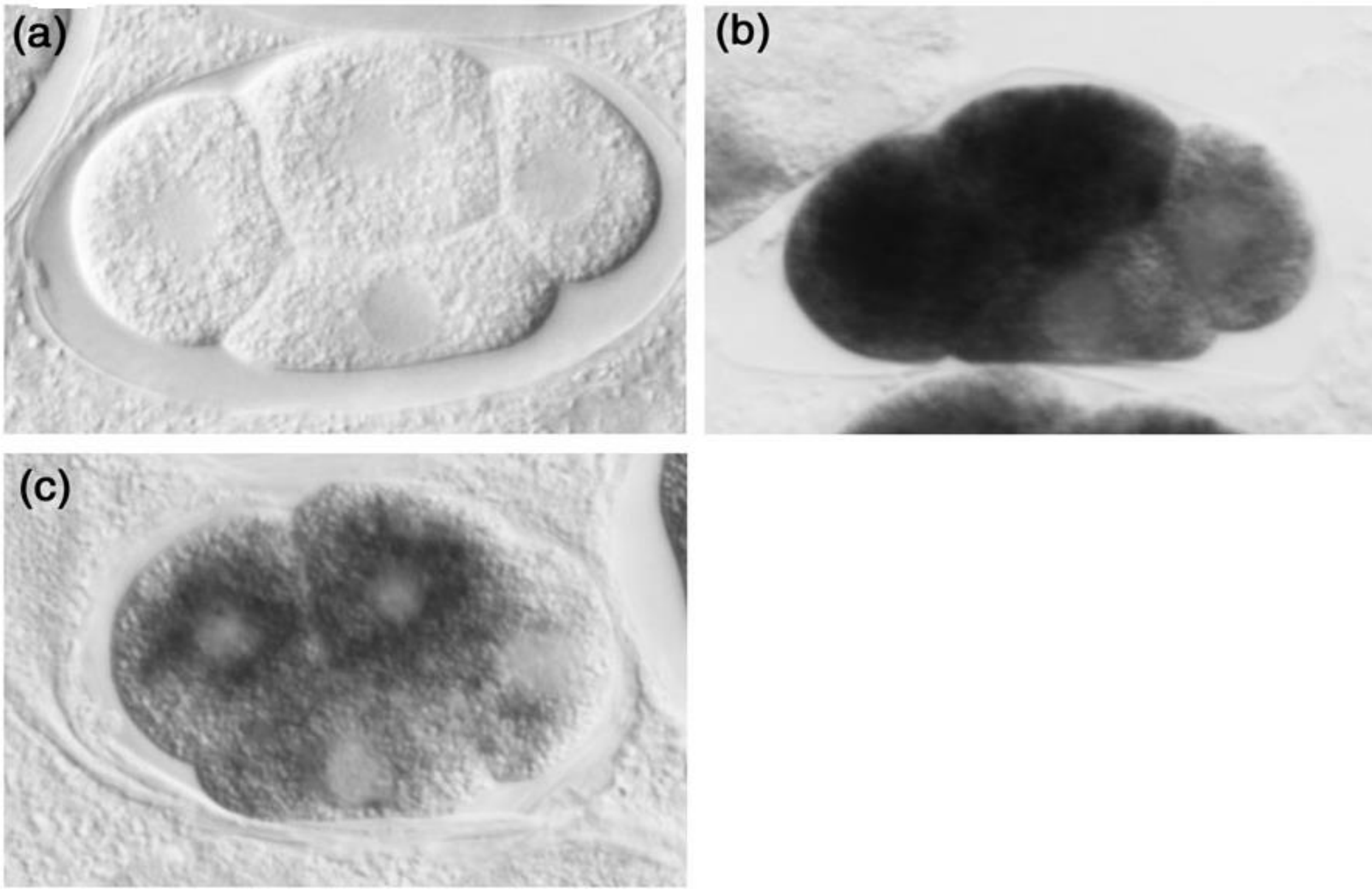


Control probe *mex-3* probe

***mex-3* RNA staining**

Fire et al (1998) Potent and specific genetic interference by double stranded RNA in Caenorhabditis elegans
Nature 391:806-811

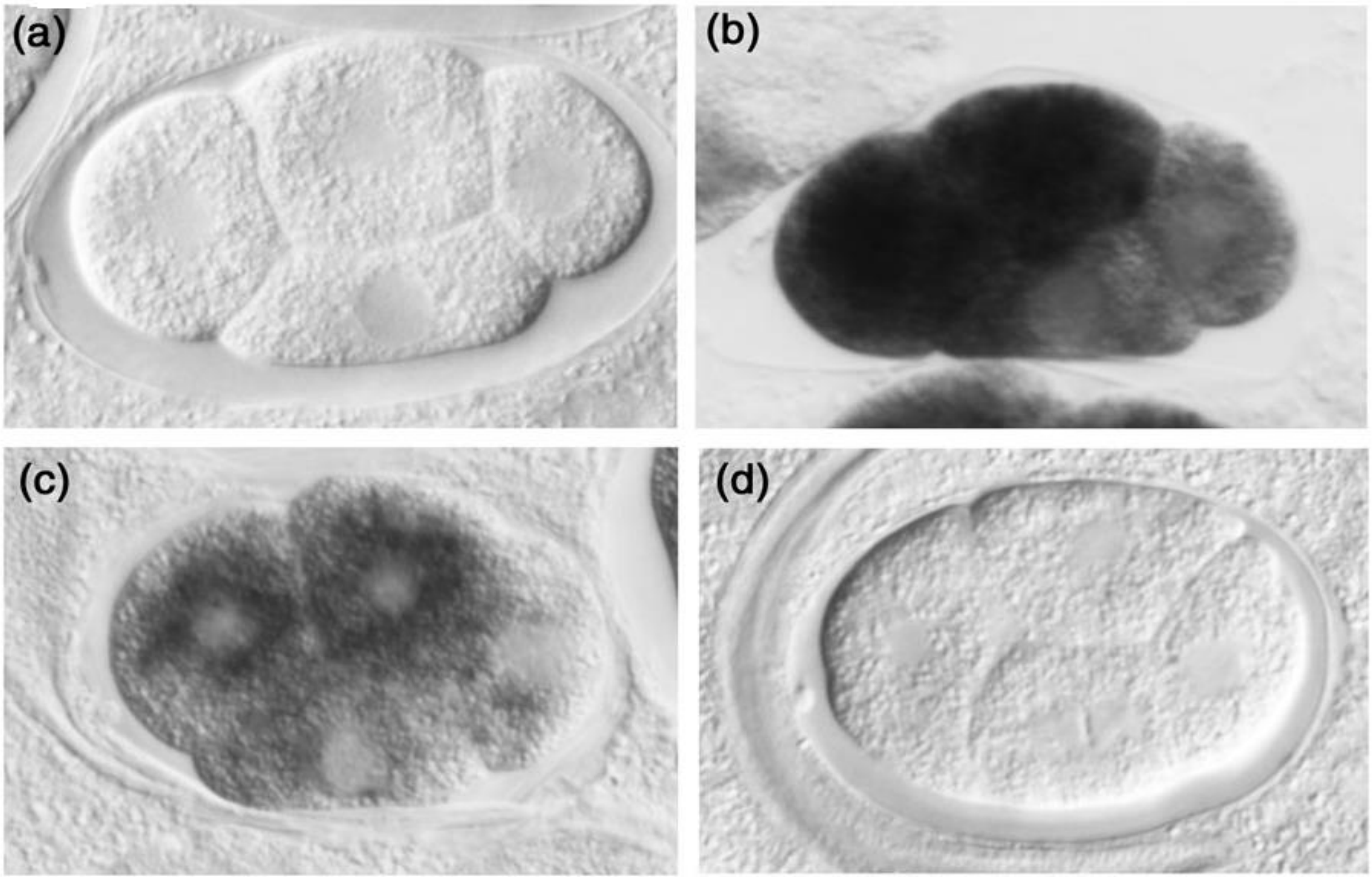
Observations in *C. elegans*



+anti-sense RNA

Fire et al (1998) Potent and specific genetic interference by double stranded RNA in Caenorhabditis elegans Nature 391:806-811

Observations in *C. elegans*



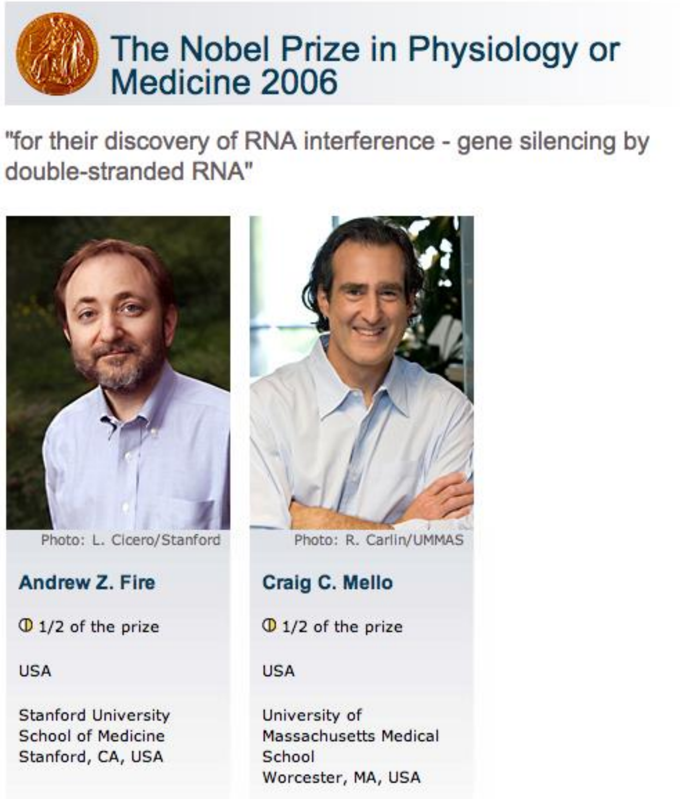
+anti-sense RNA

+double stranded RNA

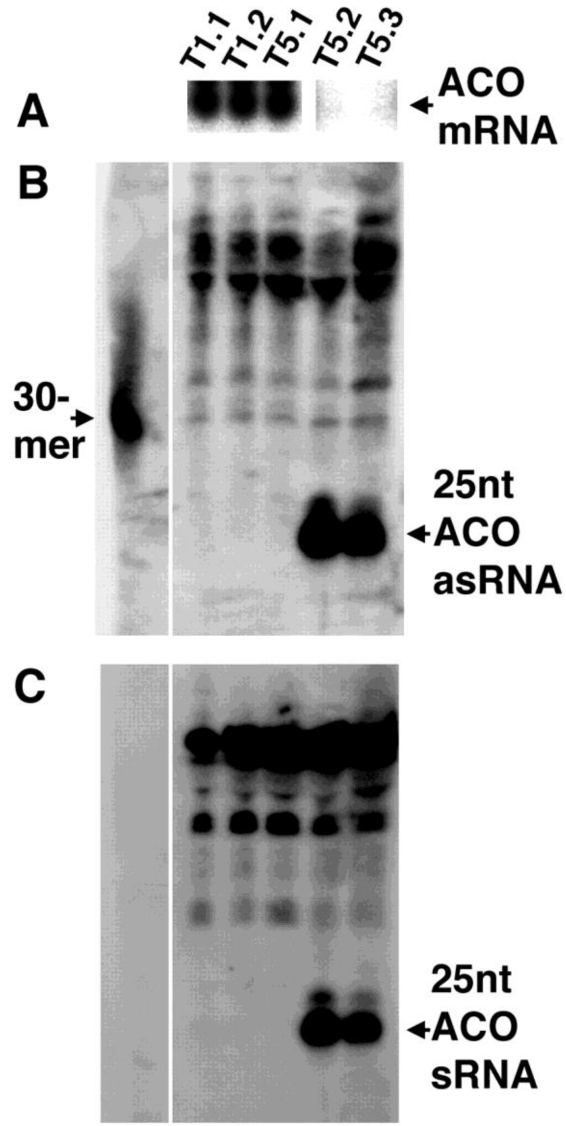
Fire et al (1998) Potent and specific genetic interference by double stranded RNA in Caenorhabditis elegans Nature 391:806-811

RNAi Characteristics

-Very potent response to small amount of dsRNA
-Decreases RNA levels in cytoplasm
-Works only against cDNA sequences
-Can be inherited
-Systemic

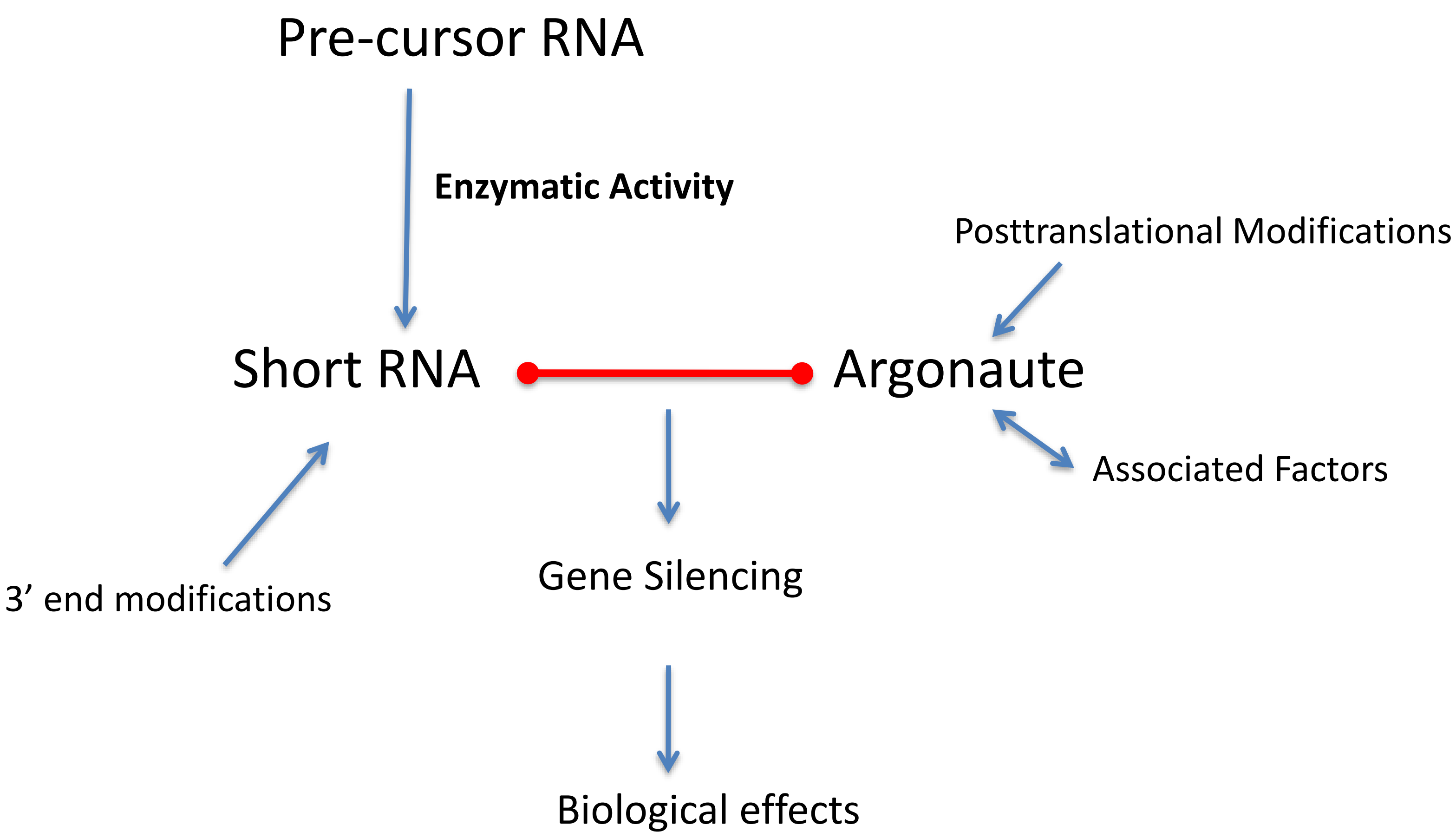


Discovery of small RNAs

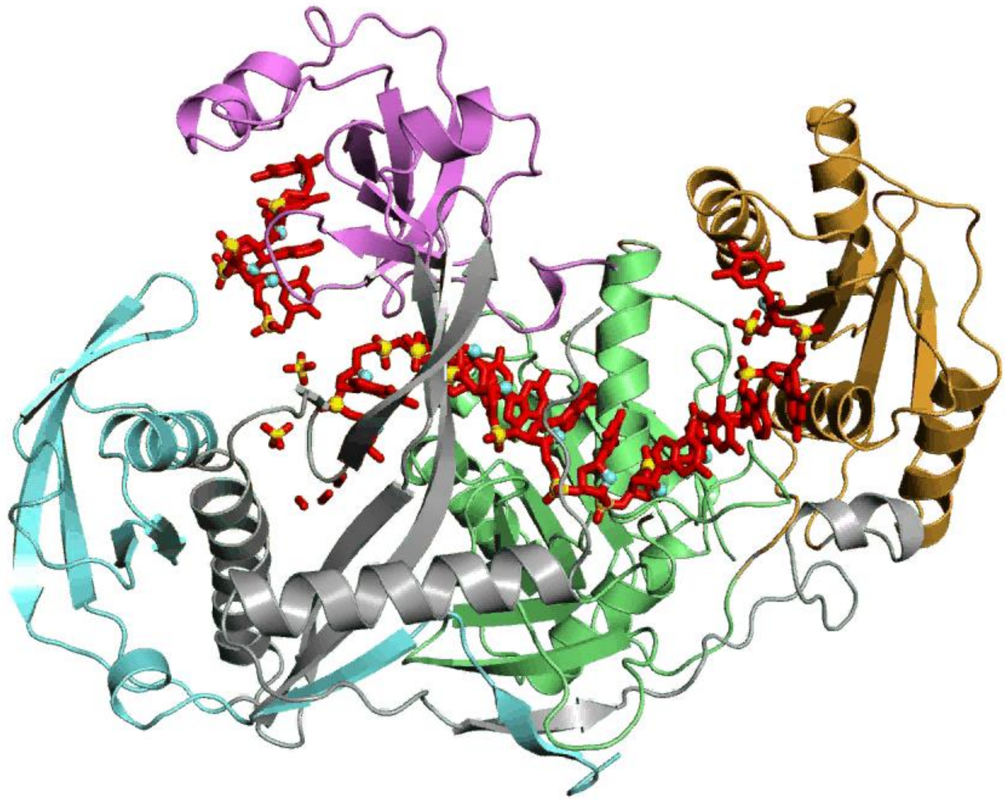


Hamilton & Baulcombe (1999) A species of small anti-sense RNA in posttranscriptional gene silencing in plants. Science 286:950-952

Core Mechanism



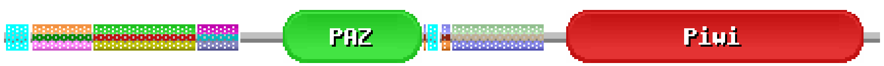
Argonaute:RNA complex

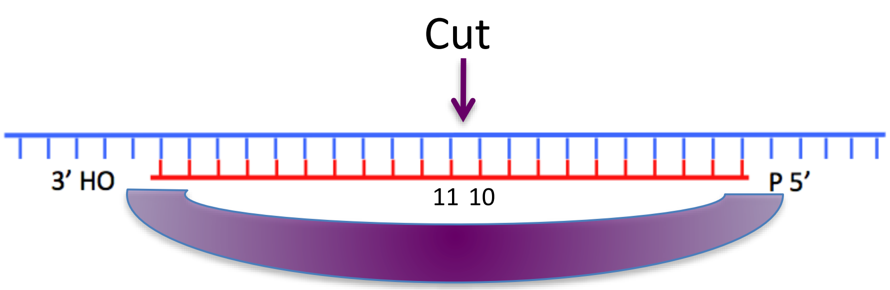


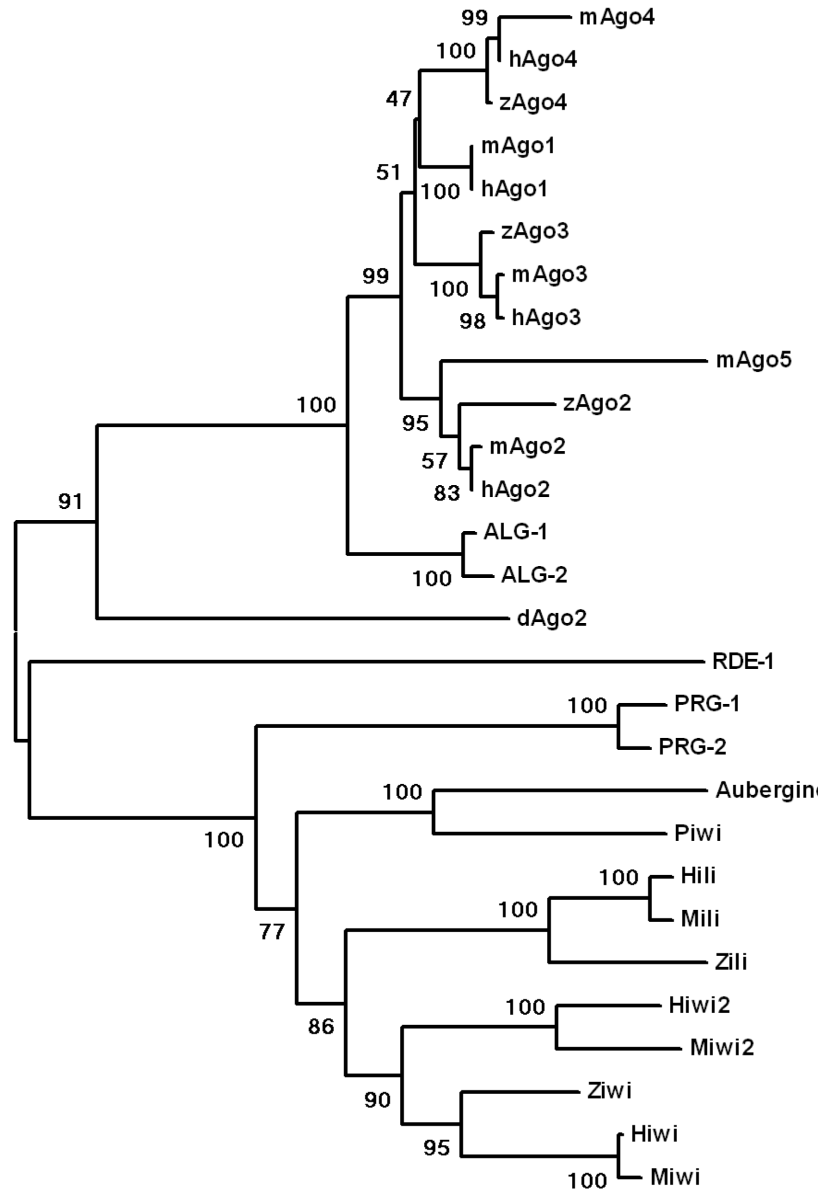
Wang et al. (2007) Structure of the guide-strand-containing argonaute silencing complex. Nature 456:209-213

Argonaute Proteins

- PAZ and PIWI domain proteins
- Sub families:
  - AGO and PIWI

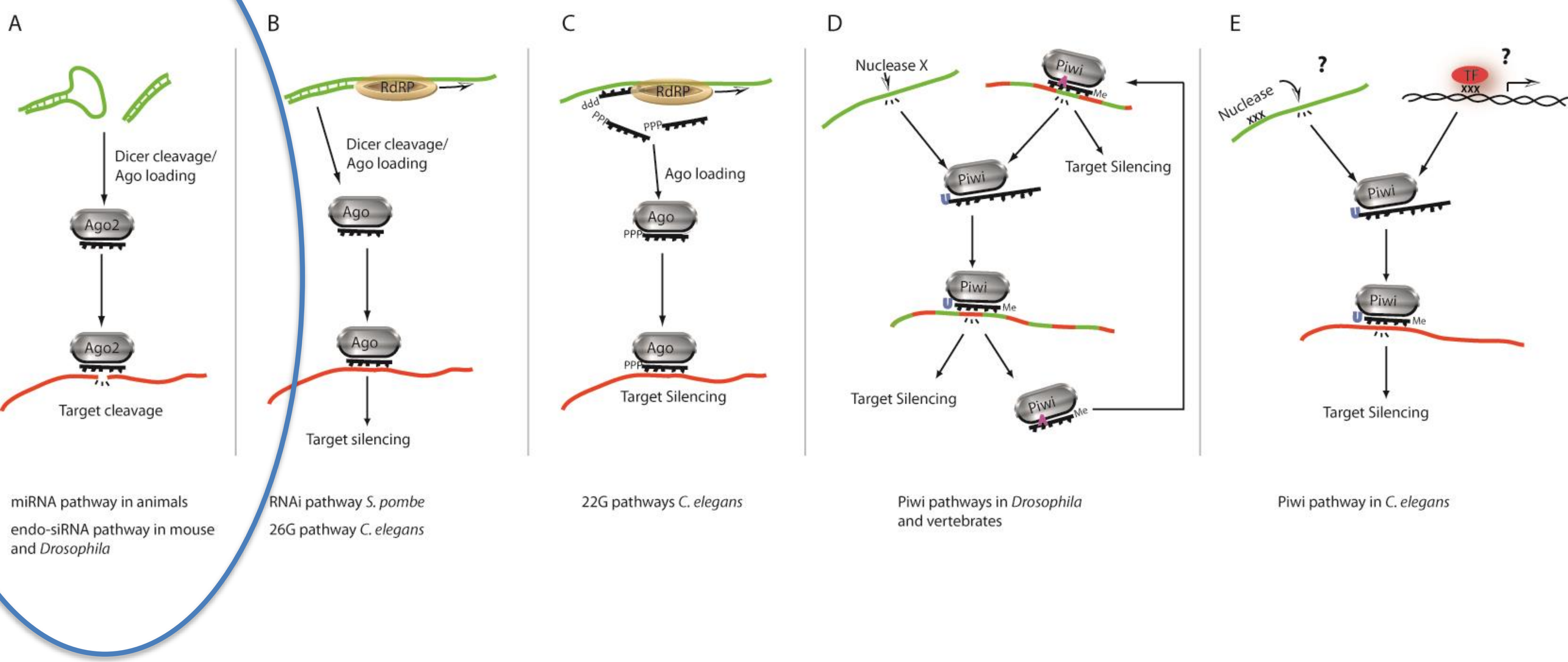






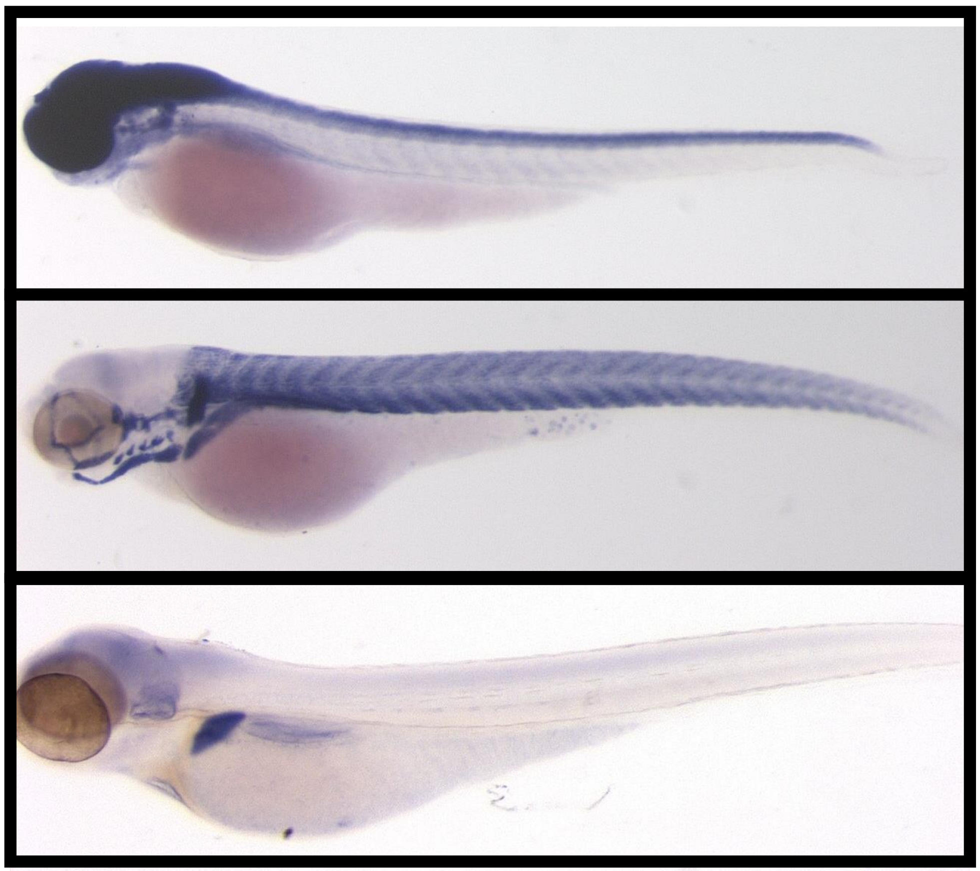
Many Different RNAi-like pathways exist

miRNA pathway



Ketting (2011) The many faces of RNAi Dev Cell 20:148-161

miRNAs are expressed as ‘regular’ genes

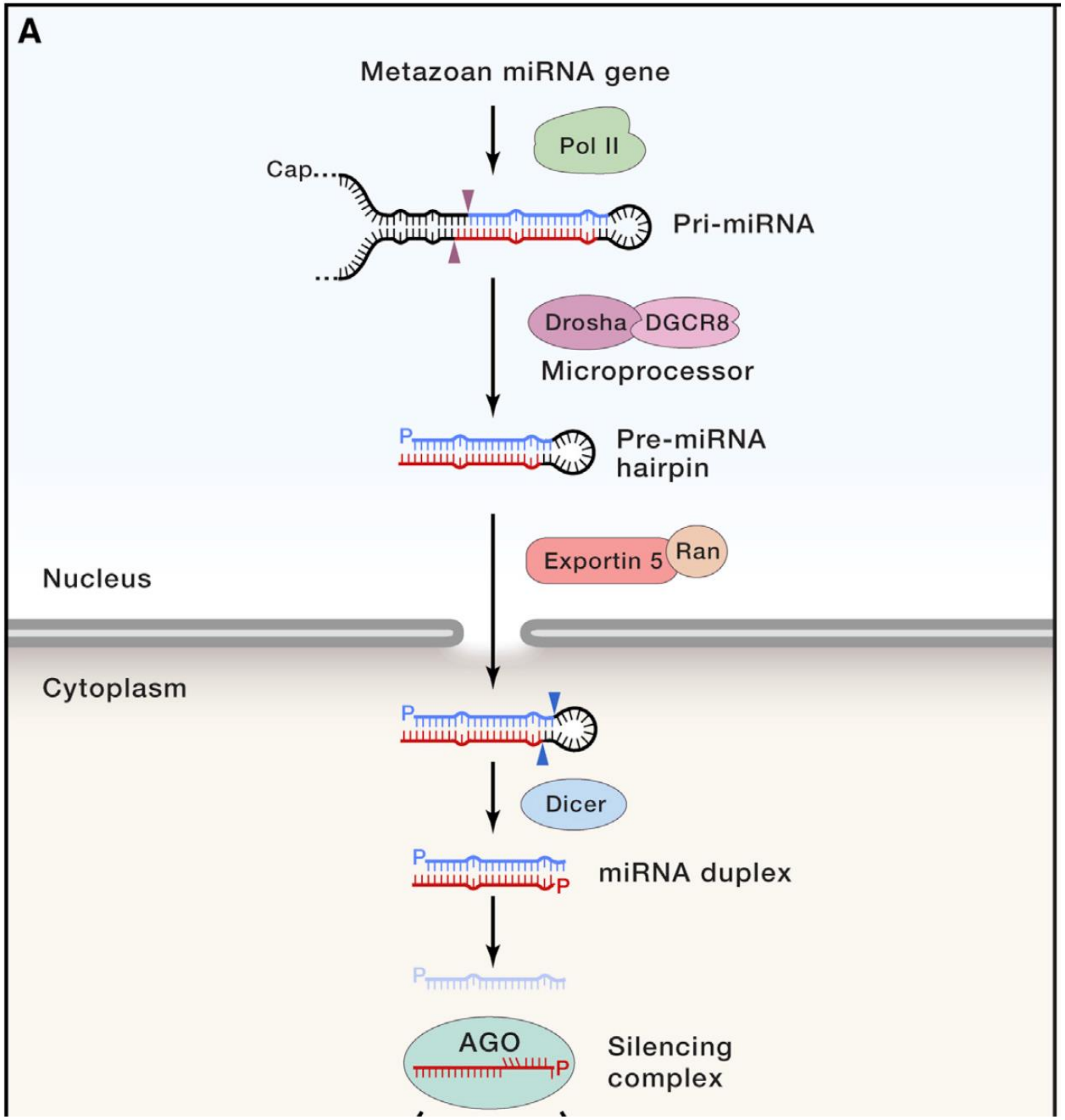


miR-124a — Brain-specific

miR-206 — Muscle-specific

miR-122a — Liver-specific

The miRNA pathway



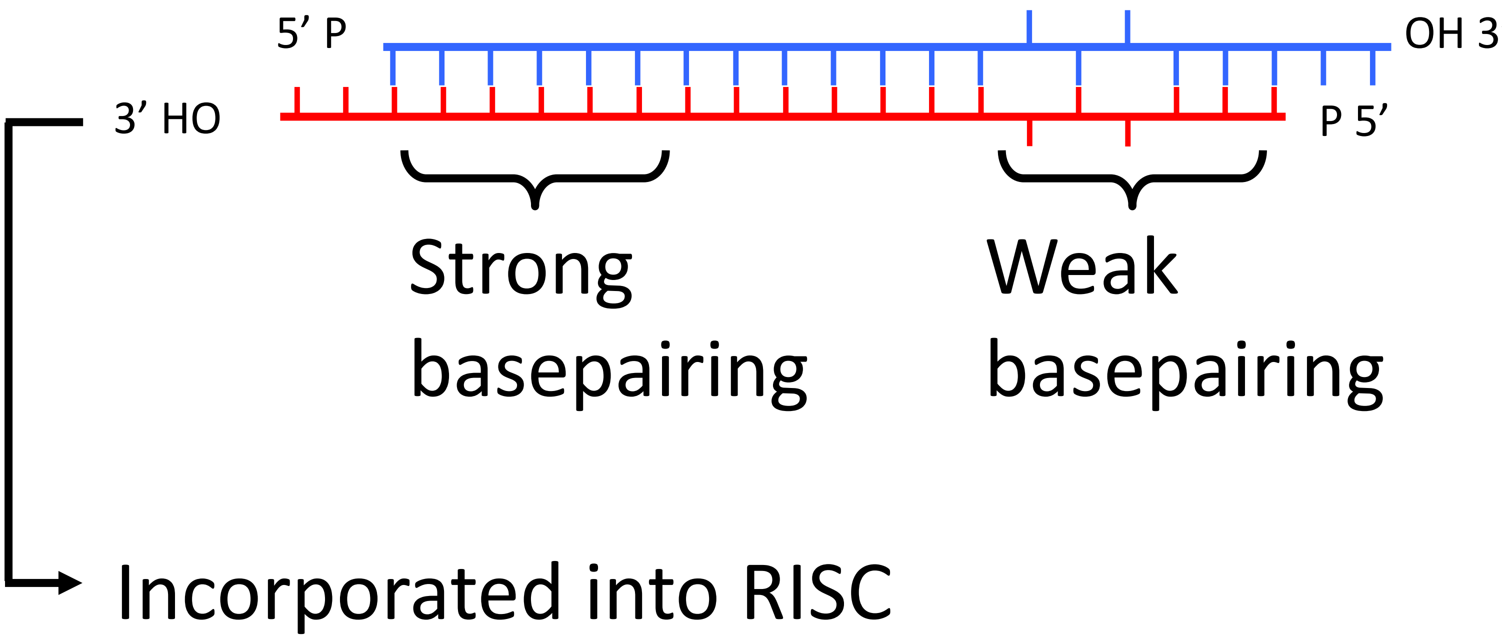
Drosha

Dicer

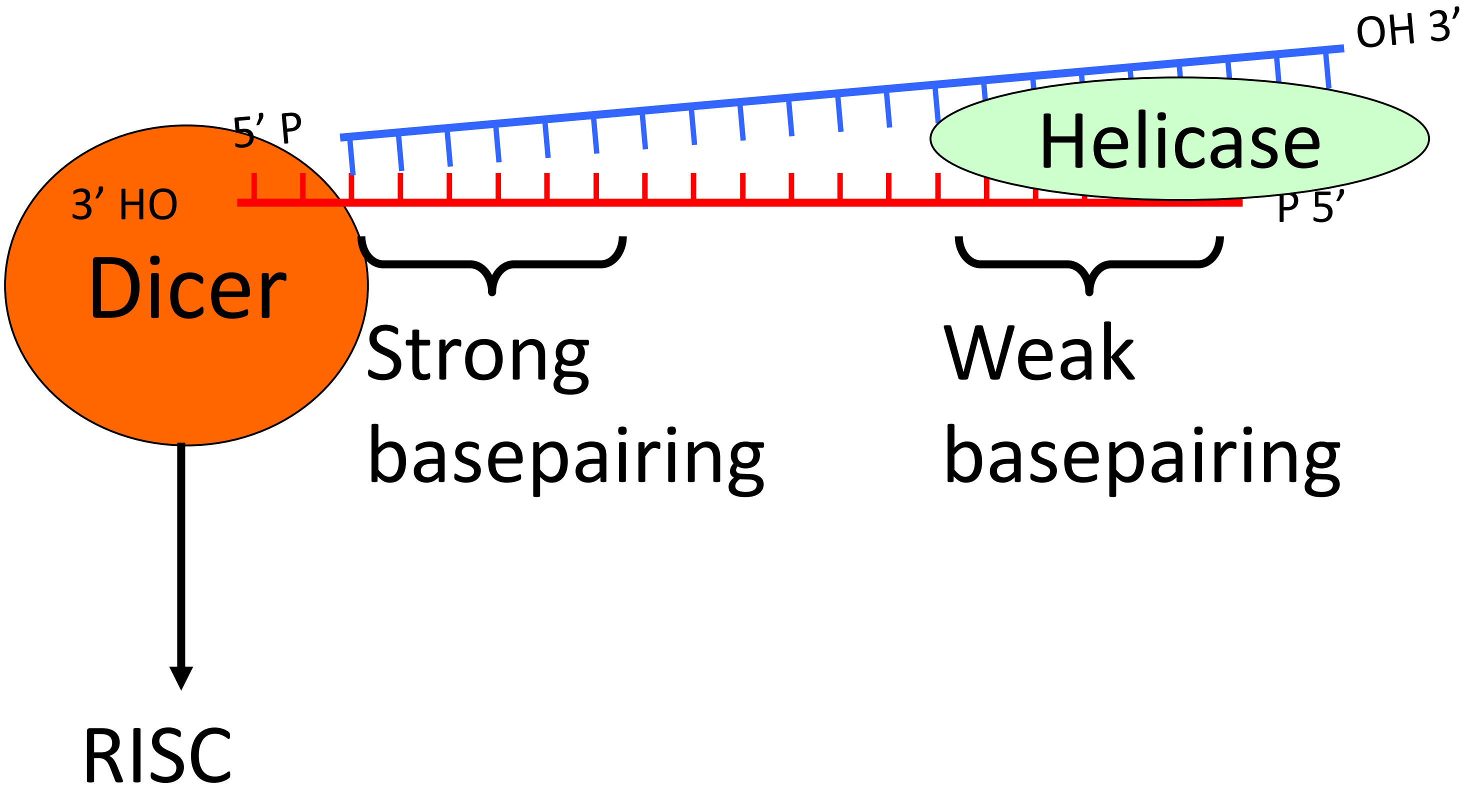
Argonaute

Bartel (2018)
Metazoan MicroRNAs.
Cell 173:20-51

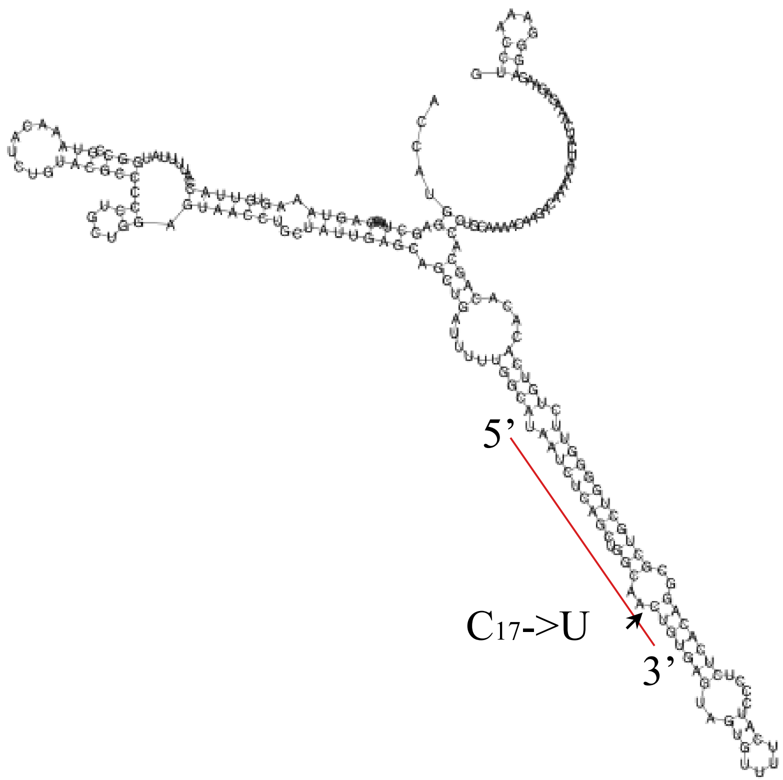
Which strand is selected?



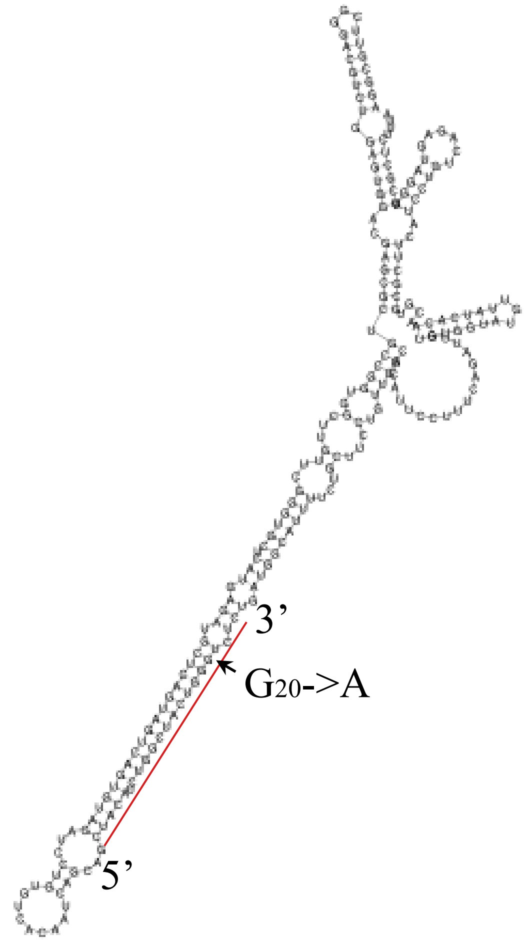
Which strand is selected?



miRNA point mutants in zebrafish

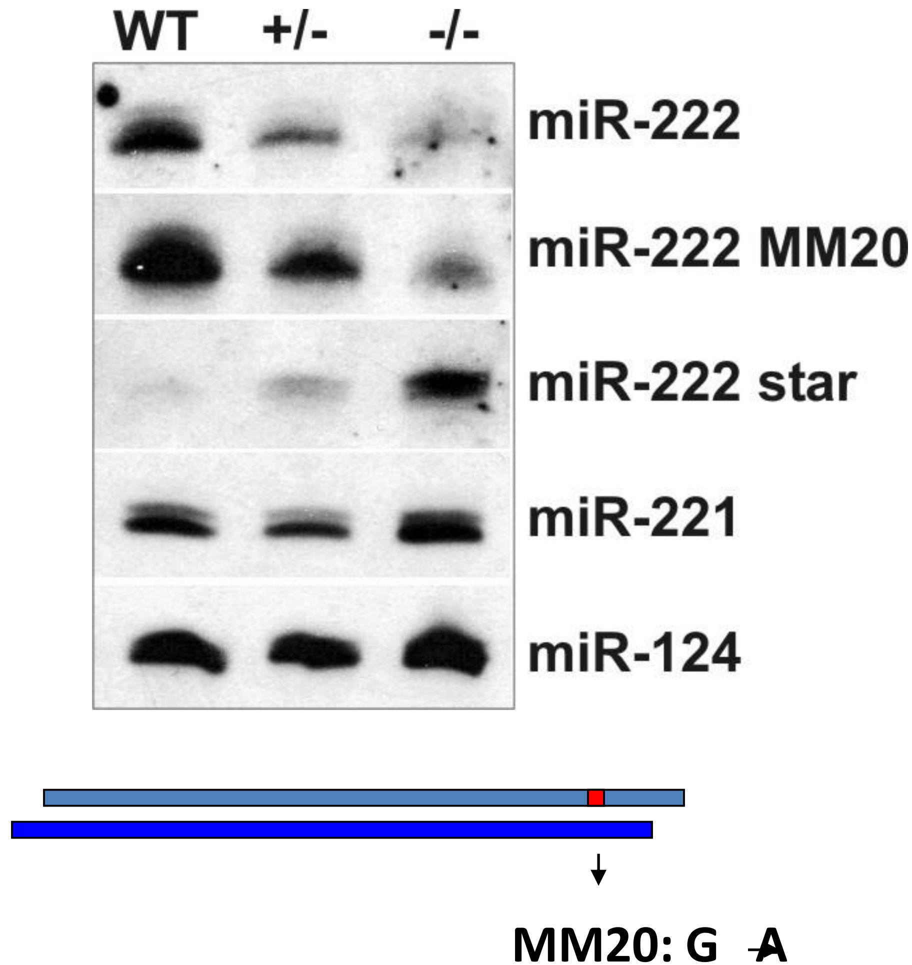


dre-miR-216

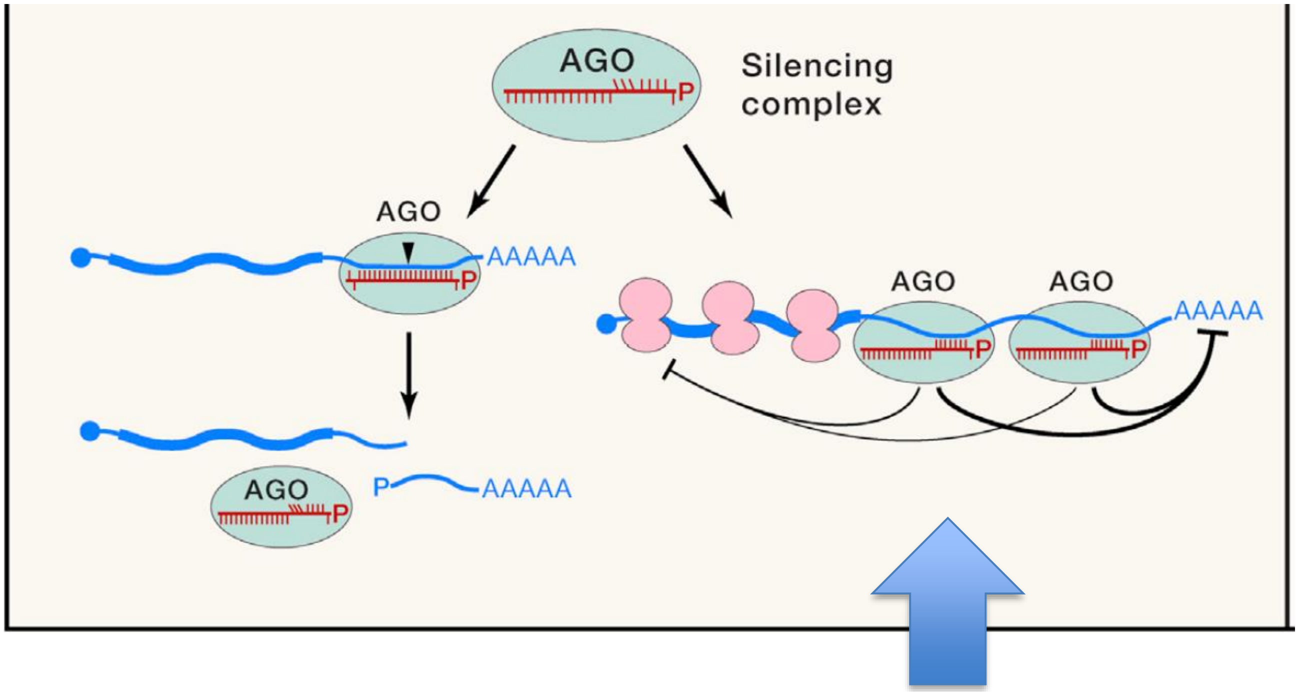


dre-miR-222

miR-222 expression in mutants



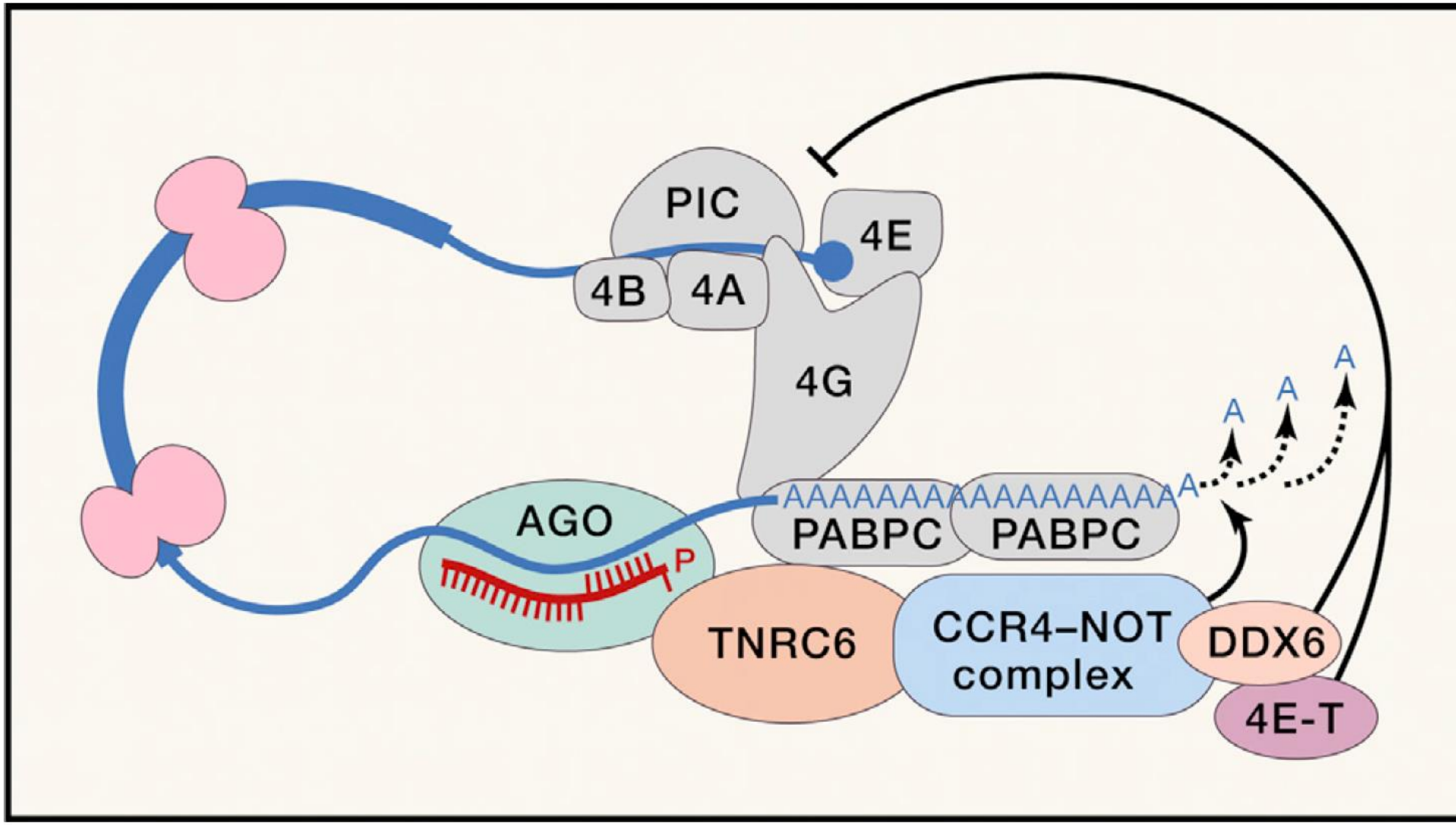
miRNA induced silencing



Dominant mechanism in animals

Bartel (2018)
Metazoan MicroRNAs.
Cell 173:20-51

miRNA induced silencing



Bartel (2018)
Metazoan MicroRNAs.
Cell 173:20-51

miRNA Families

In *C. elegans*: *let-7, mir-48, mir-241*

```
let-7      UGAGGUAGUAGGUUGUAUAGUU
miR-48     UGAGGUAGGCUCAGUAGAUGCGA
miR-241    UGAGGUAGGUGCGAGAAAUGA
           ********            *
```

let-7 family

In human: 13 *let-7* members (a-k, mir-98, mir-202)

Often organized in genomic clusters: One transcript containing multiple miRNA precursors

miRNA Take-home messages

miRNAs are bound by Argonaute proteins

miRNAs can be expressed tissue specifically

miRNAs are made from dsRNA precursors, through two main enzymes: Drosha and Dicer

miRNAs in animals mostly do not cleave their target transcripts

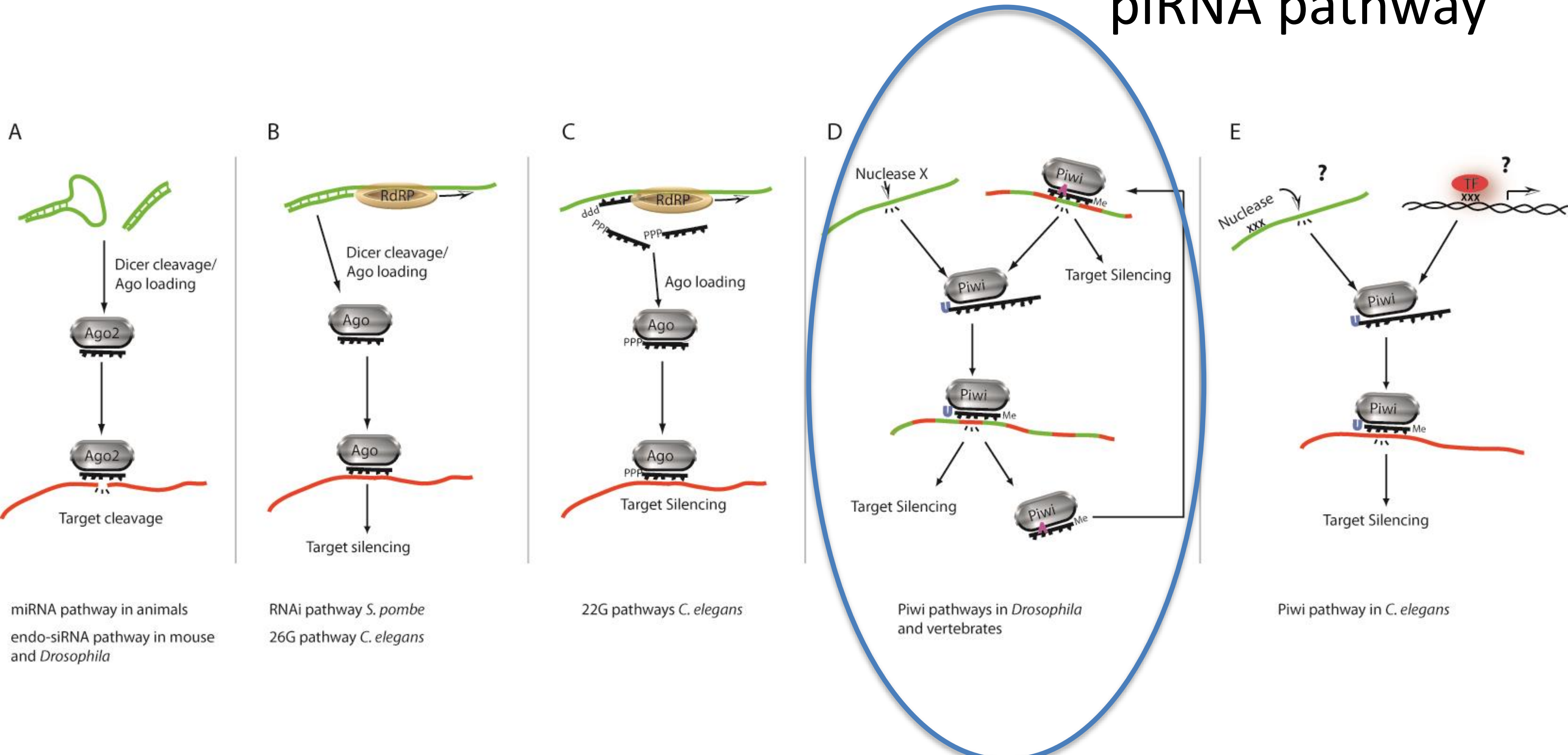
miRNAs in animals mostly inhibit translation and/or induce de-adenylation

Only part of the miRNA (mostly the 5’ part) is involved in target recognition

Many many gene transcripts are targets of miRNAs

Many Different RNAi-like pathways exist

piRNA pathway



miRNA pathway in animals

endo-siRNA pathway in mouse and *Drosophila*

RNAi pathway *S. pombe*

26G pathway *C. elegans*

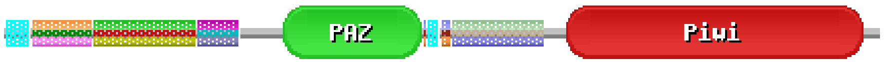
22G pathways *C. elegans*

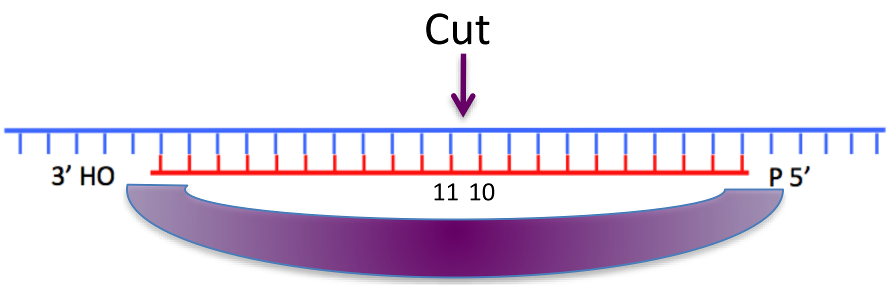
Piwi pathways in *Drosophila* and vertebrates

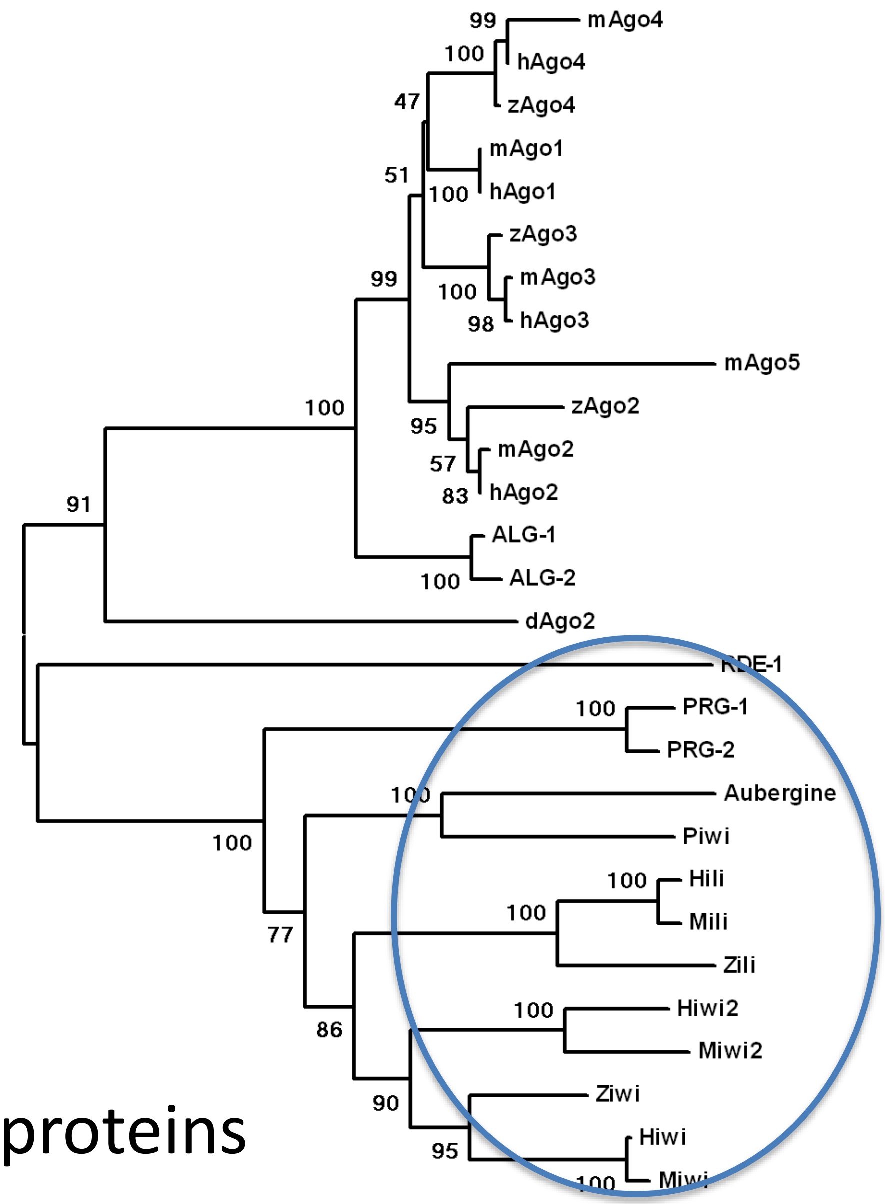
Piwi pathway in *C. elegans*

Argonaute Proteins

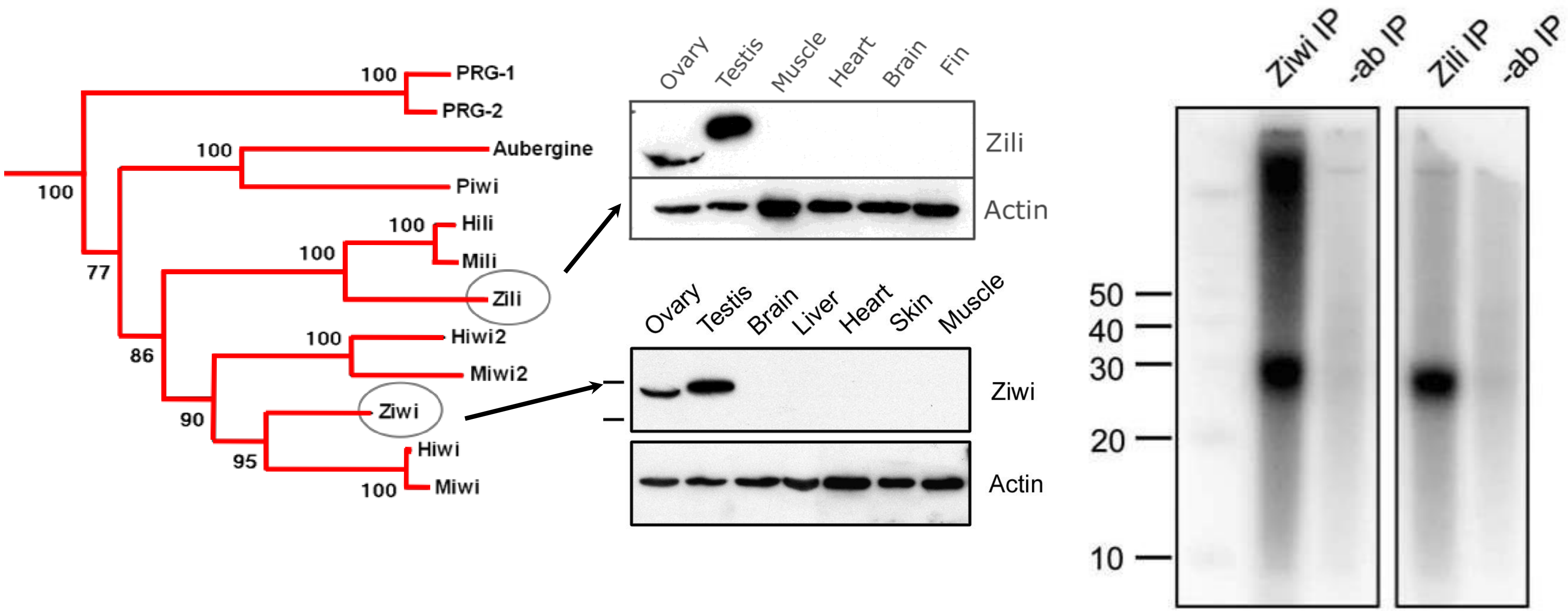
- PAZ and PIWI domain proteins
- Sub families:
  - AGO and PIWI





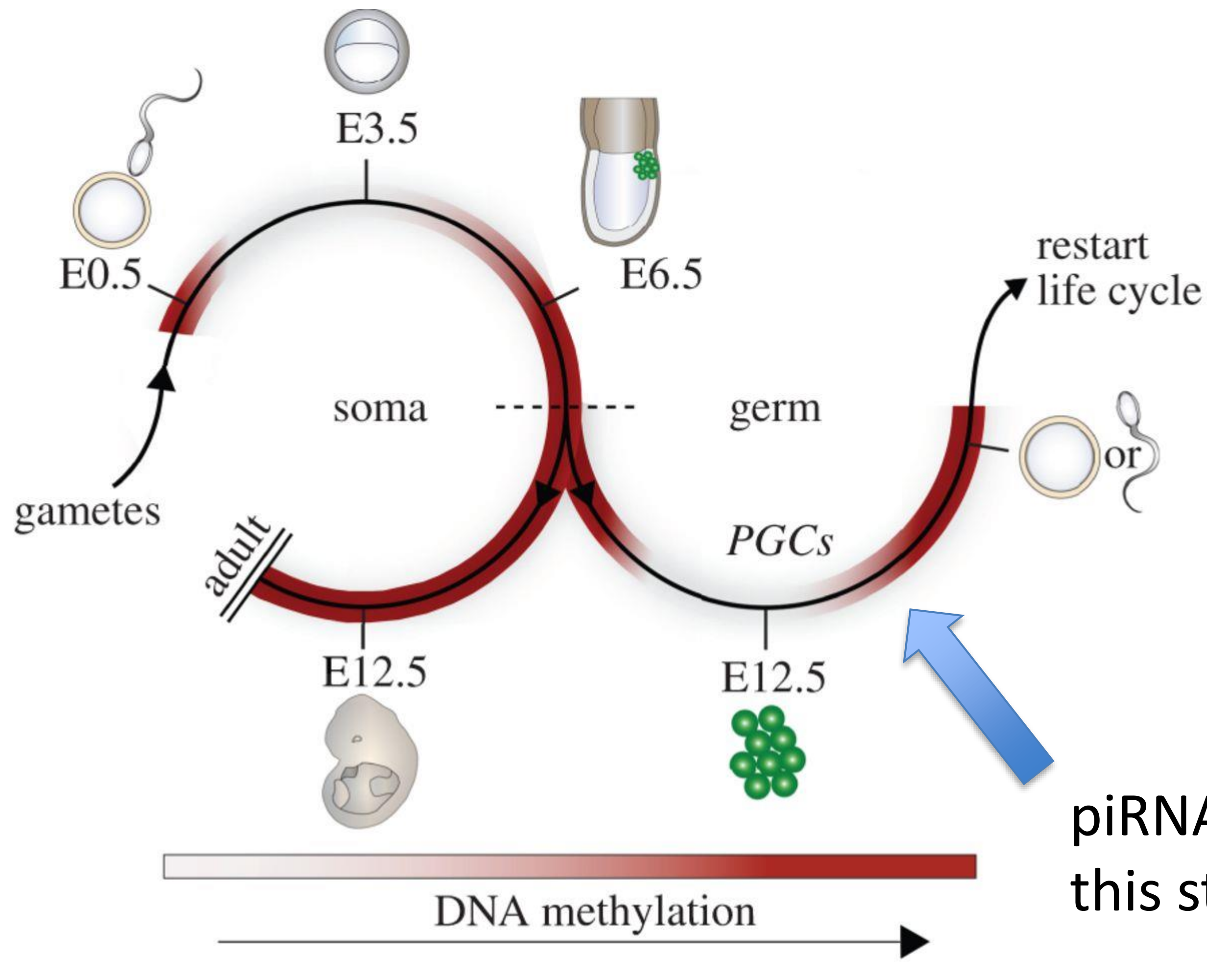


Piwi Proteins are usually germ cell specific



Houwing et al Cell 2007
Houwing et al EMBO J 2008

Why specifically in germ cells?

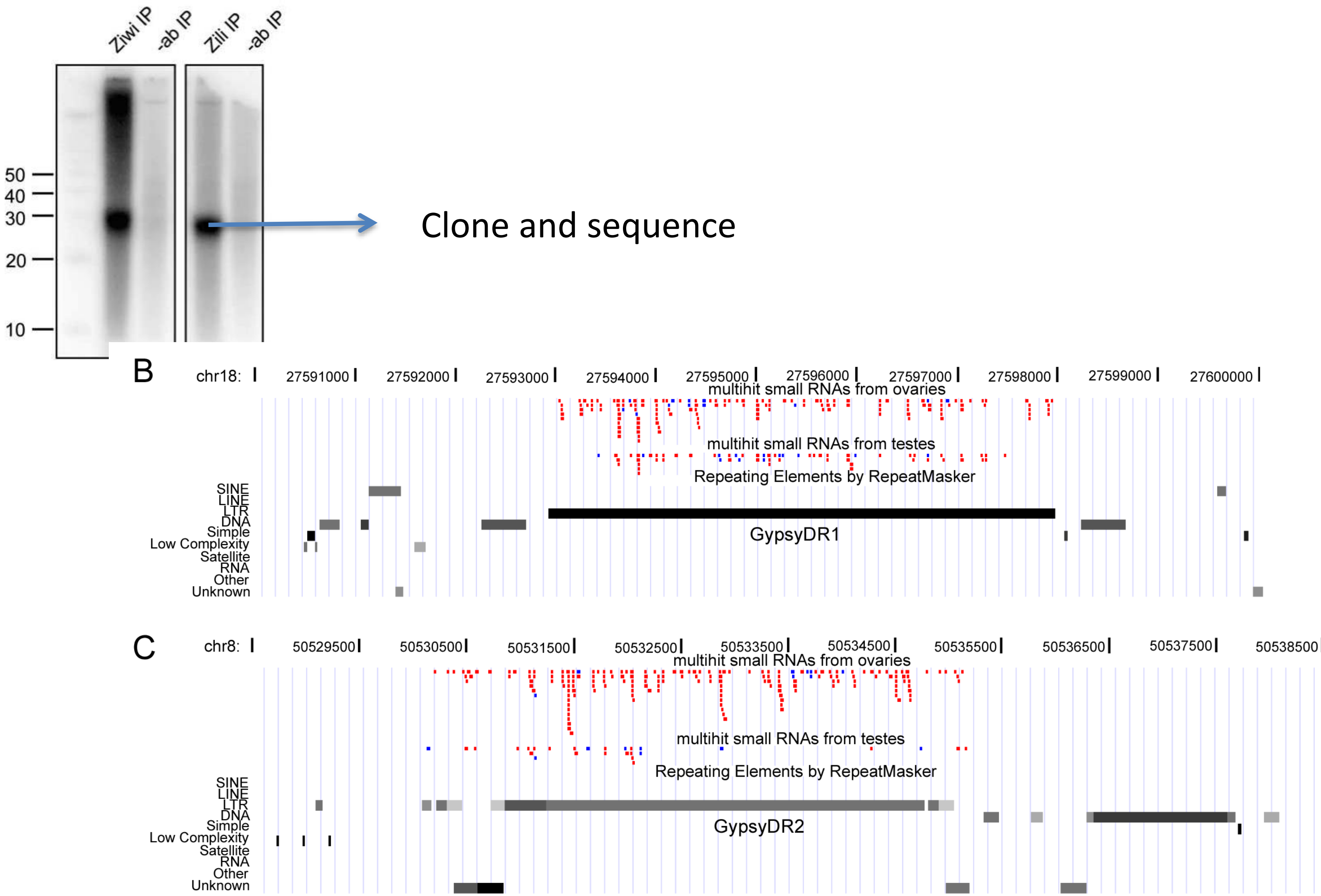


piRNAs help
this step

Jamie A. Hackett, and M. Azim Surani Phil. Trans. R. Soc. B
2013;368:20110328

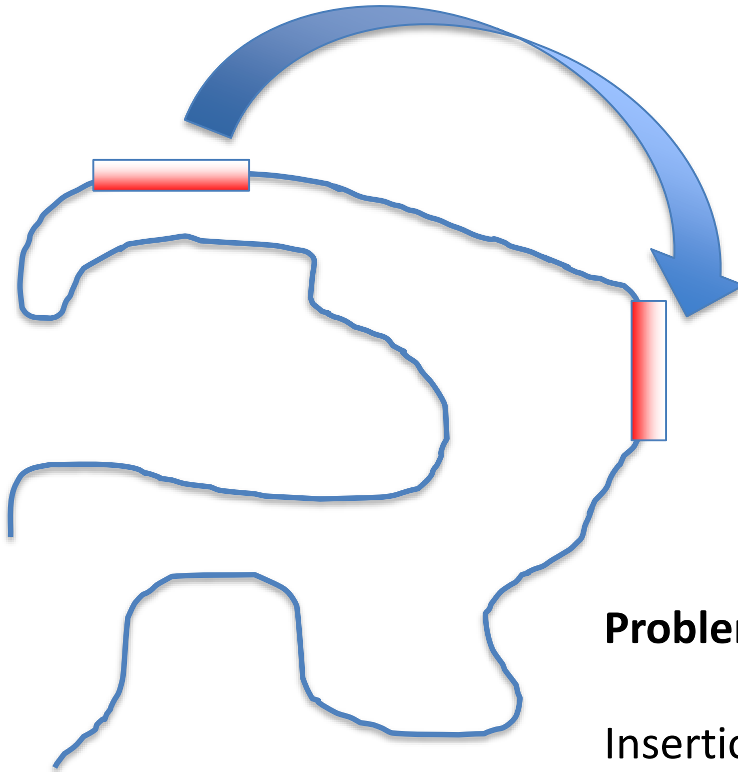
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

Piwi Proteins target transposons



Houwing et al. 2007 Cell

Transposable Elements = Jumping genes



Problems associated with transposition:

Insertional mutagenesis

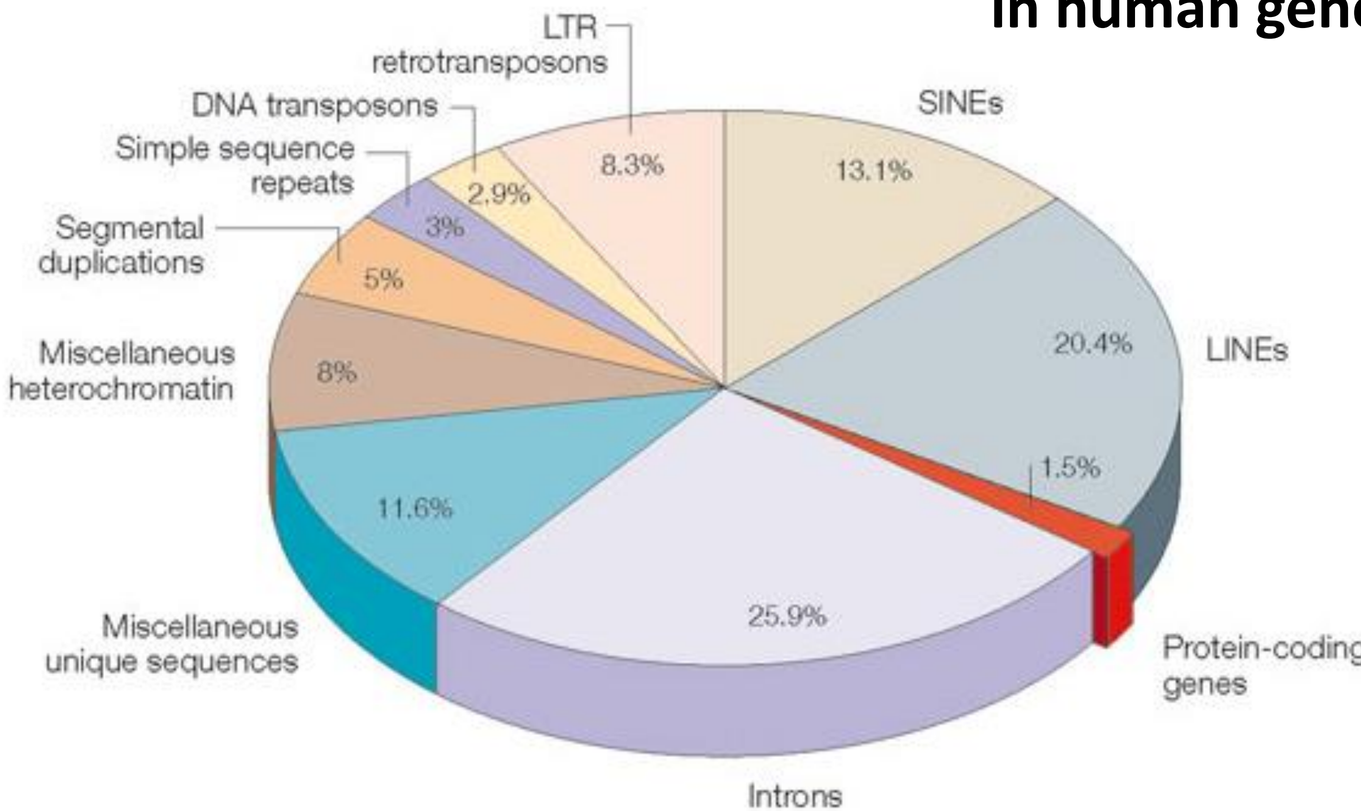
Non-allelic homology

Generation of pseudogenes

Increase in copy number over time

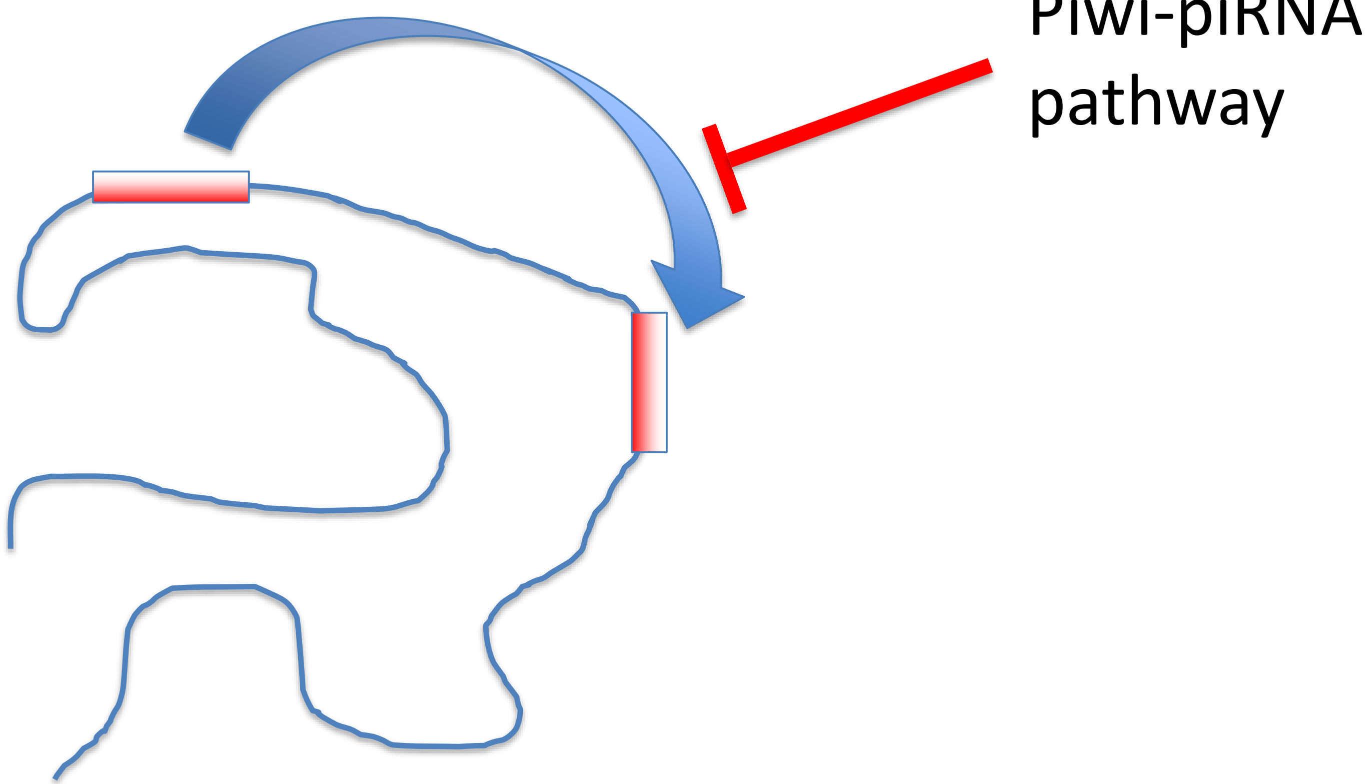
TEs are major constituents of our genomes

45% Transposons in human genome

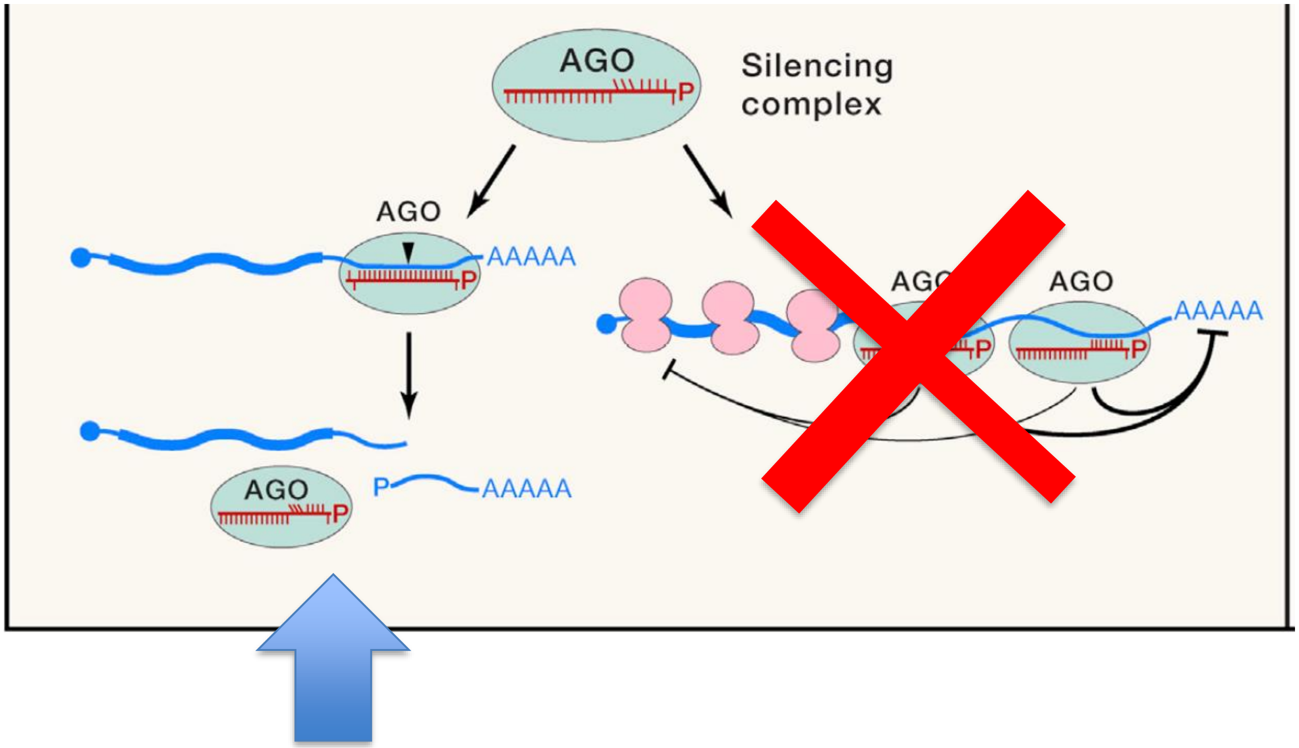


Ryan Grogory, Nat Rev Genet 2005 6; 699-708

Piwi proteins silence transposons

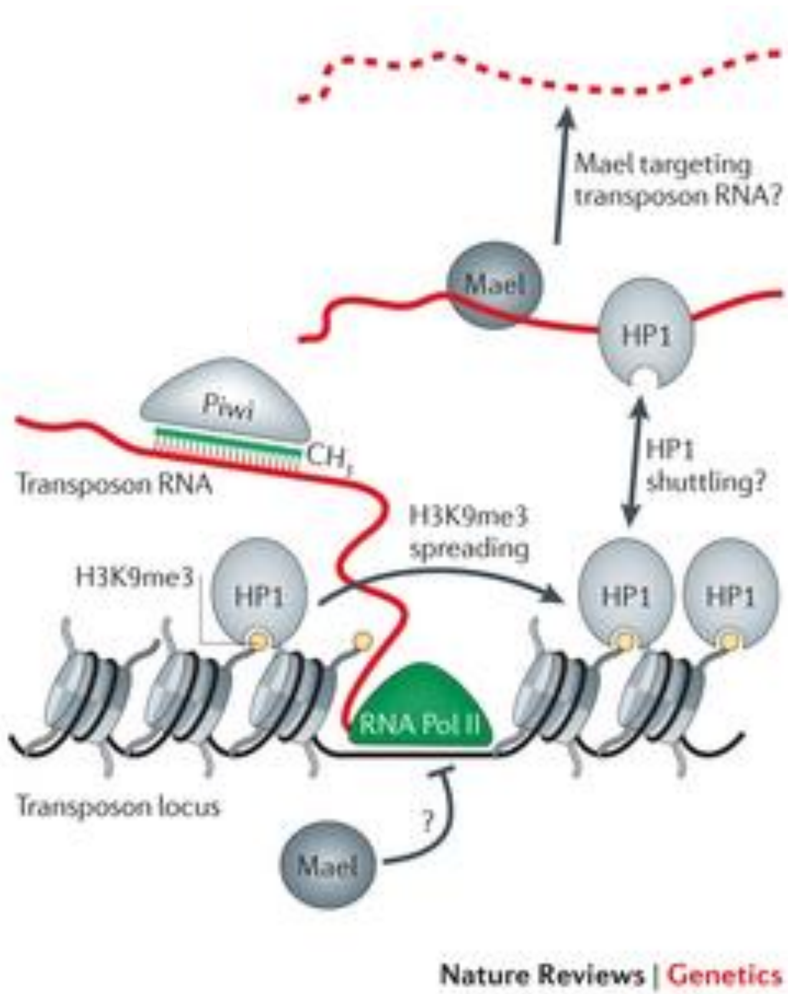


piRNA induced silencing - I



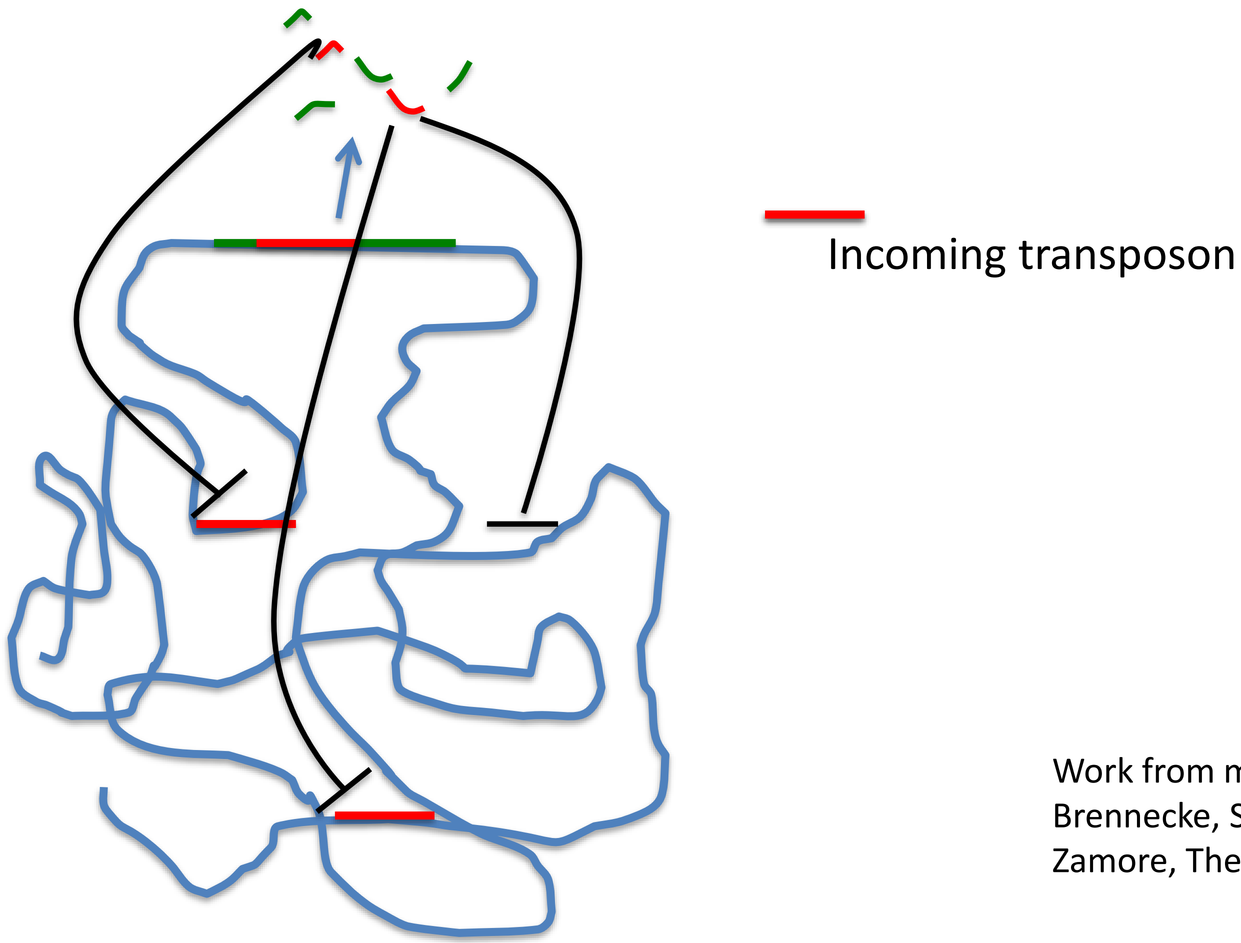
Piwi proteins cleave their target transcripts

piRNA induced silencing - II



Piwi-piRNAs can induce heterochromatin

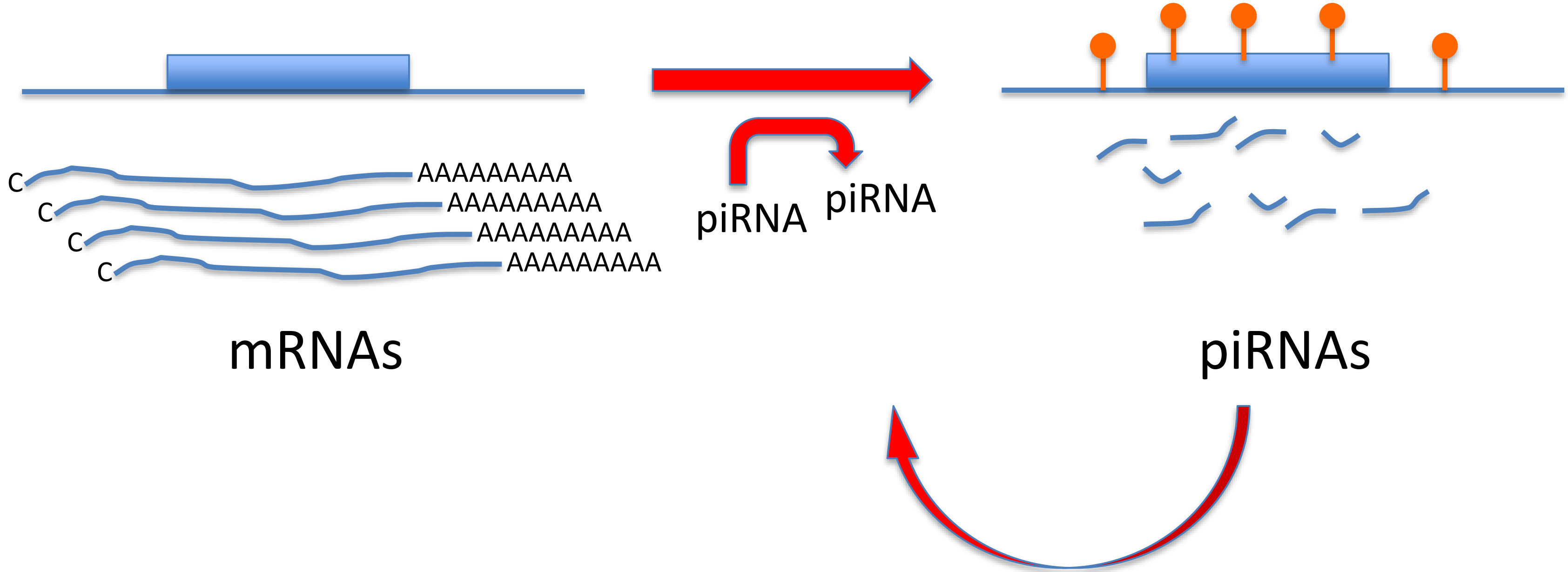
How to identify a transposon?



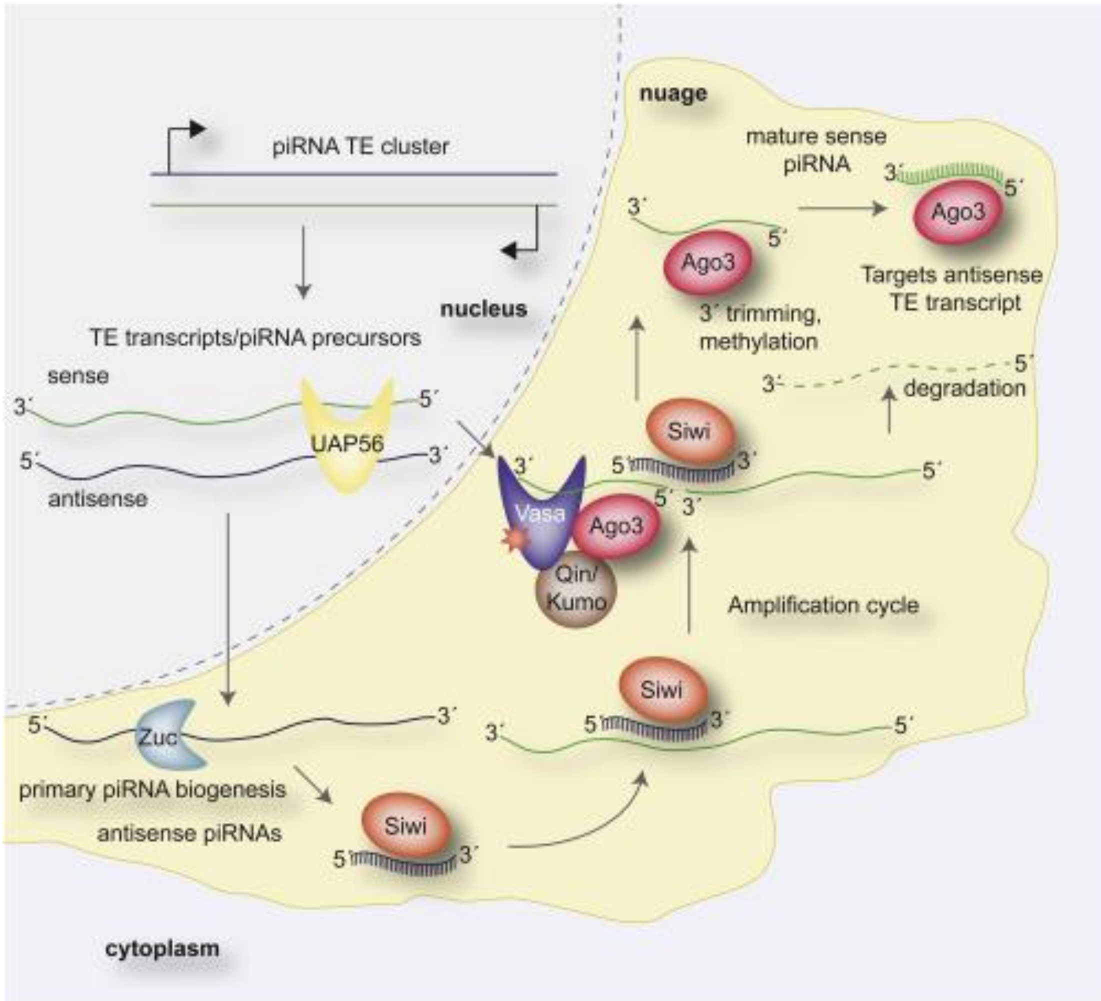
Work from many labs:
Brennecke, Siomi, Hannon,
Zamore, Theurkauf

Specialized chromatin at piRNA clusters that helps piRNA biogenesis!

piRNAs act as initiators of an epigenetic switch



How are ssRNAs turned into piRNAs?



Claycomb (2014) Dev Cell 23:632-634

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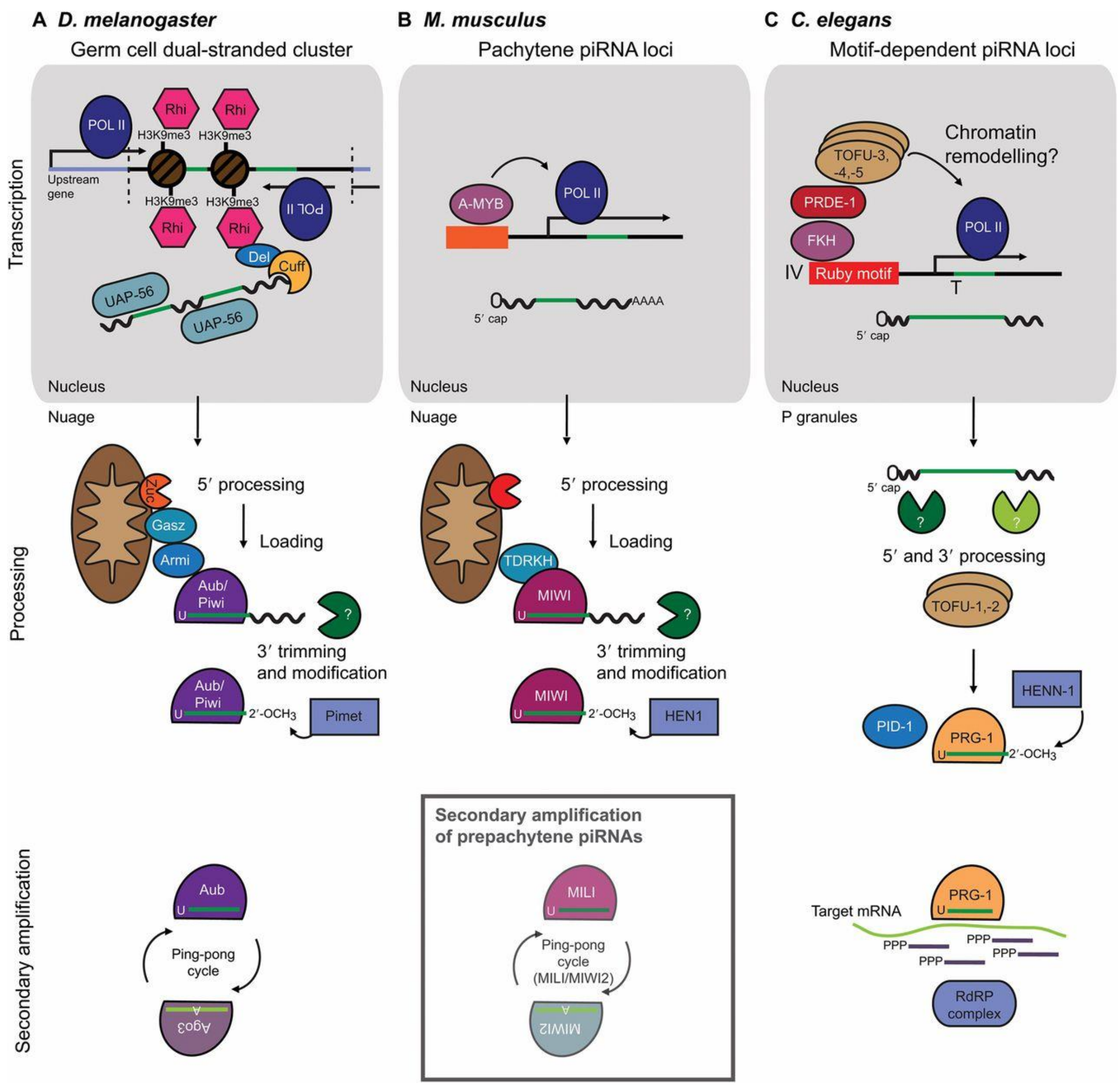

Weick and Miska (2014) Development 141:3458-3471

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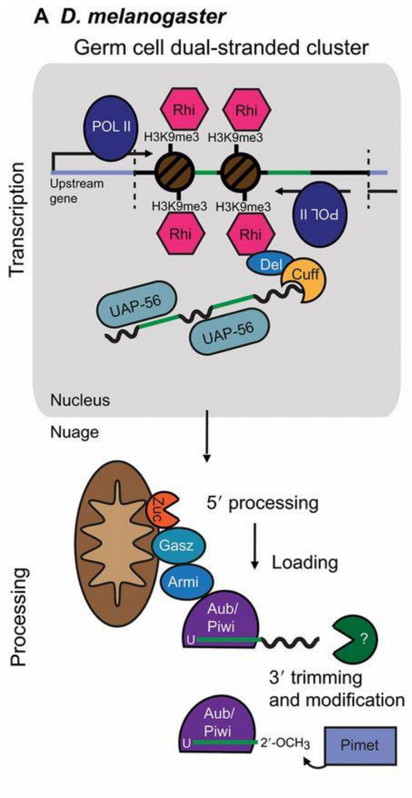

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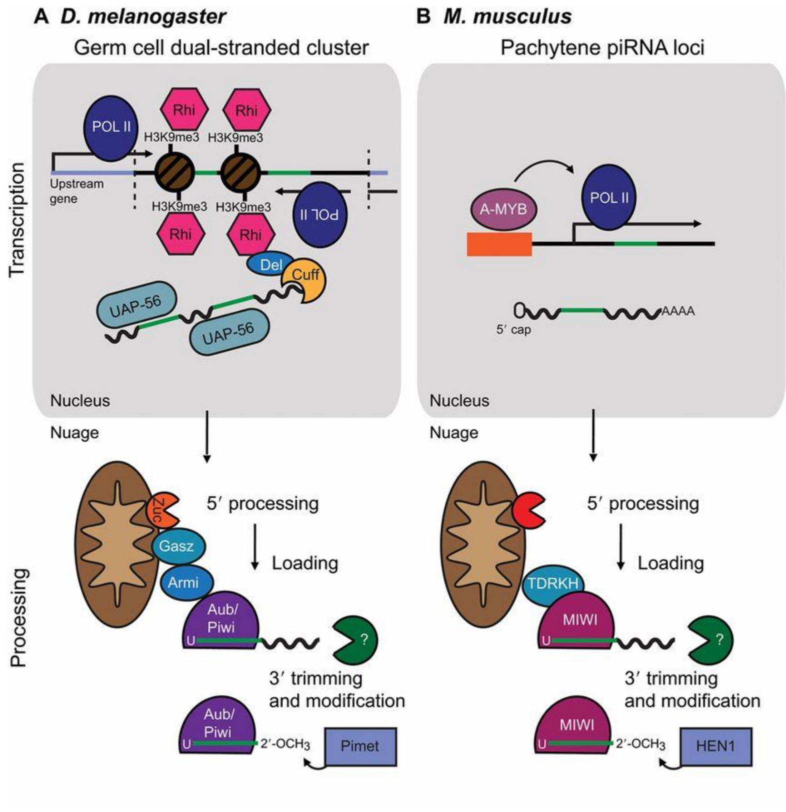

Weick and Miska (2014) Development 141:3458-3471

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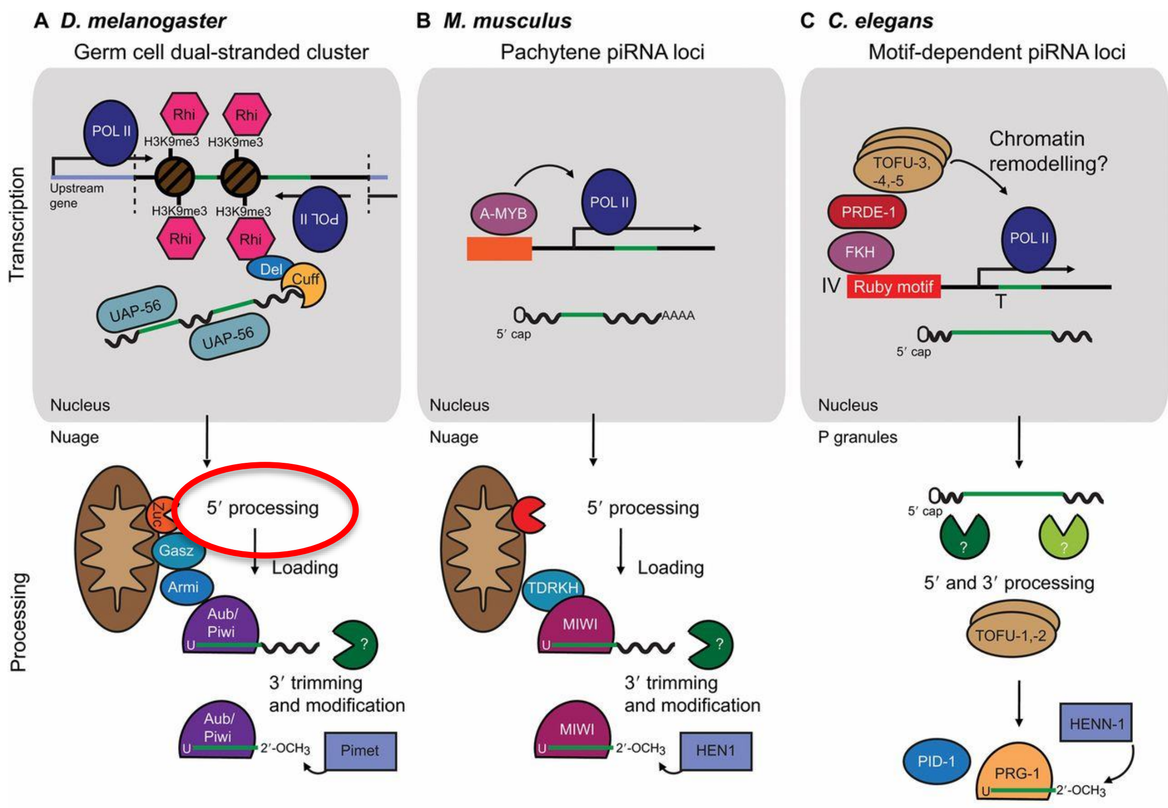
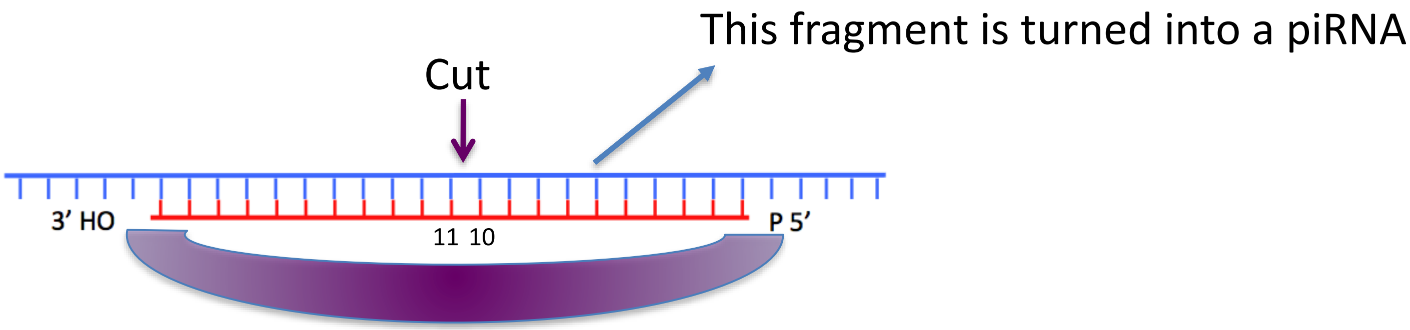


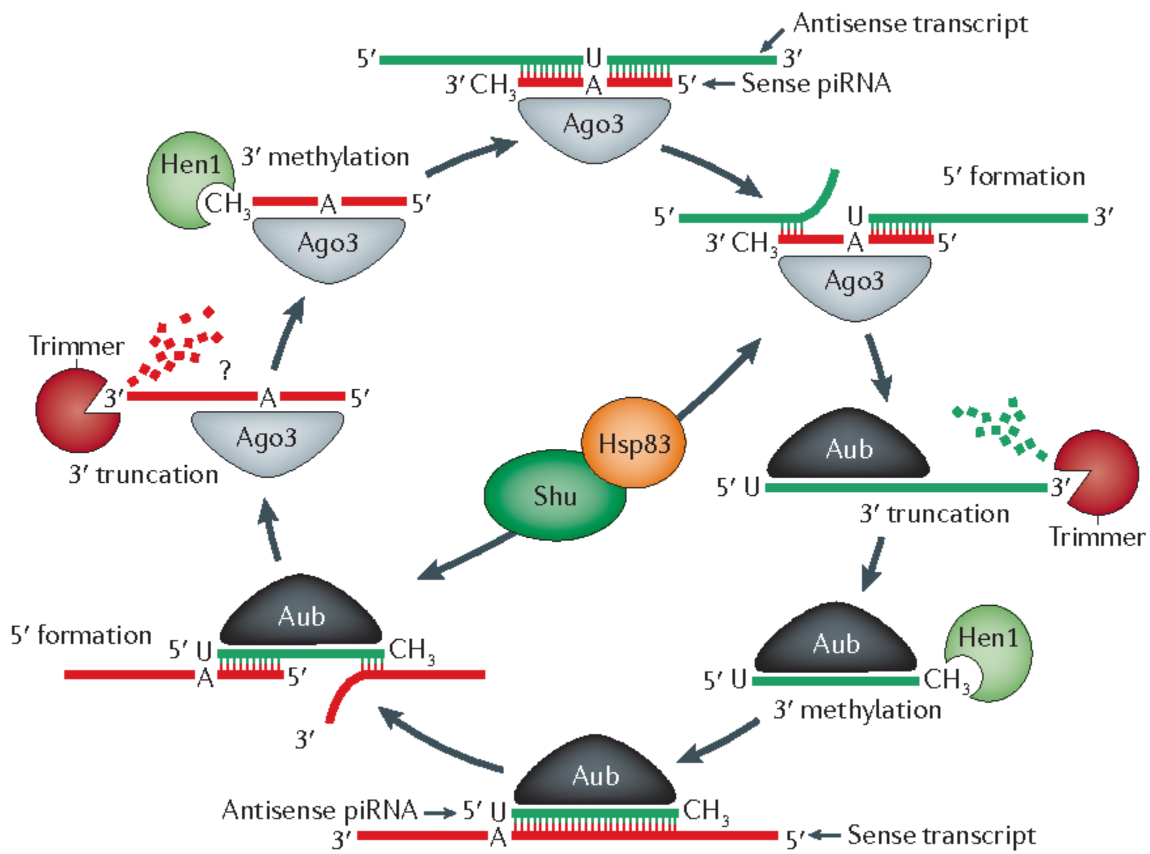
Weick and Miska (2014) Development 141:3458-3471

5’ processing by endonucleases

Piwi itself:



5’ processing by endonucleases



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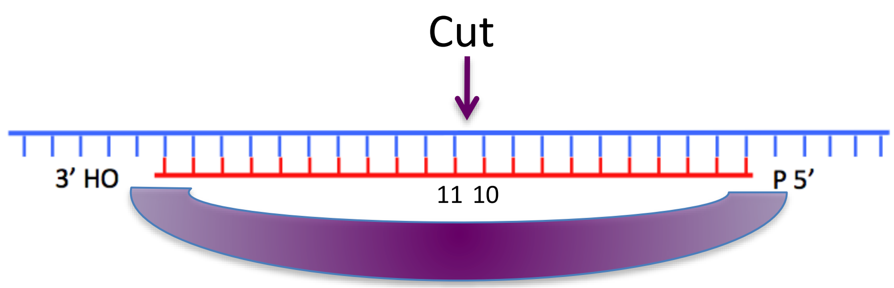
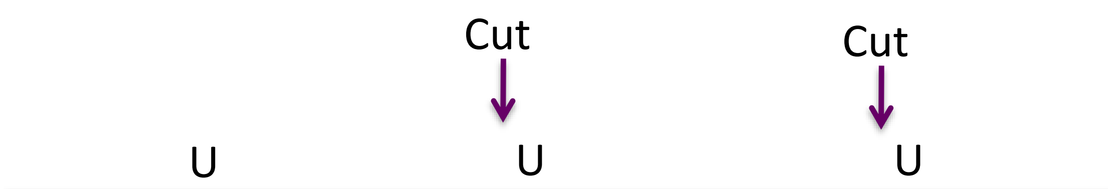


Zucchini:



5’ processing by endonucleases

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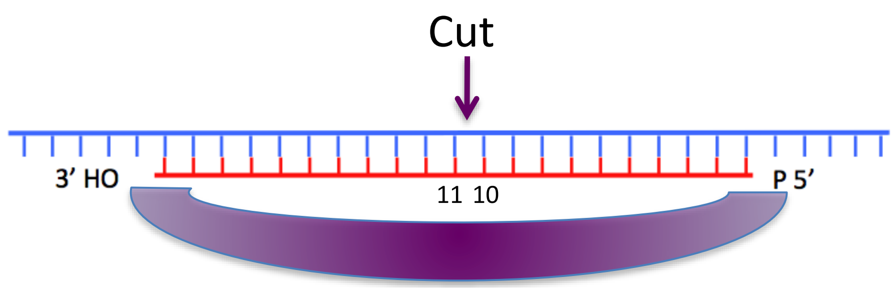

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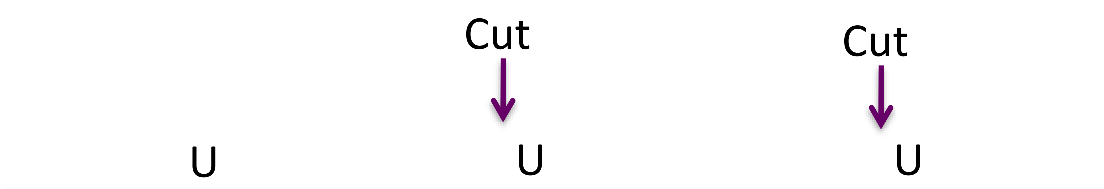
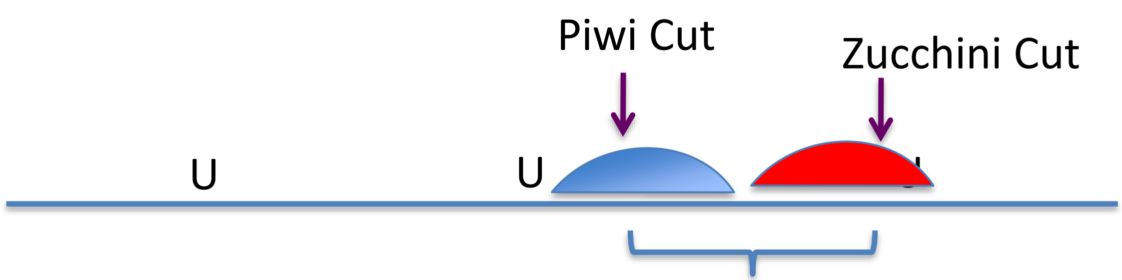


Combination:



piRNA Take-home messages

piRNAs are bound by specialized Argonaute proteins: Piwi proteins

piRNAs and Piwi proteins are mostly germ cell specific (in arthropods also somatic)

piRNAs and Piwi proteins repress activity of transposable elements
(in mosquitoes also viruses)

piRNAs cleave their target transcripts

Nuclear Piwi-piRNA complexes induce heterochromatin

Affects of piRNAs on chromatin are driven though recognition of nascent transcripts

piRNA-driven chromatin drives piRNA biogenesis from ssRNA

Biogenesis occurs in dense, peri-nuclear aggregates (phase-separated structures)

Biogenesis involves many different nucleases and can differ between species