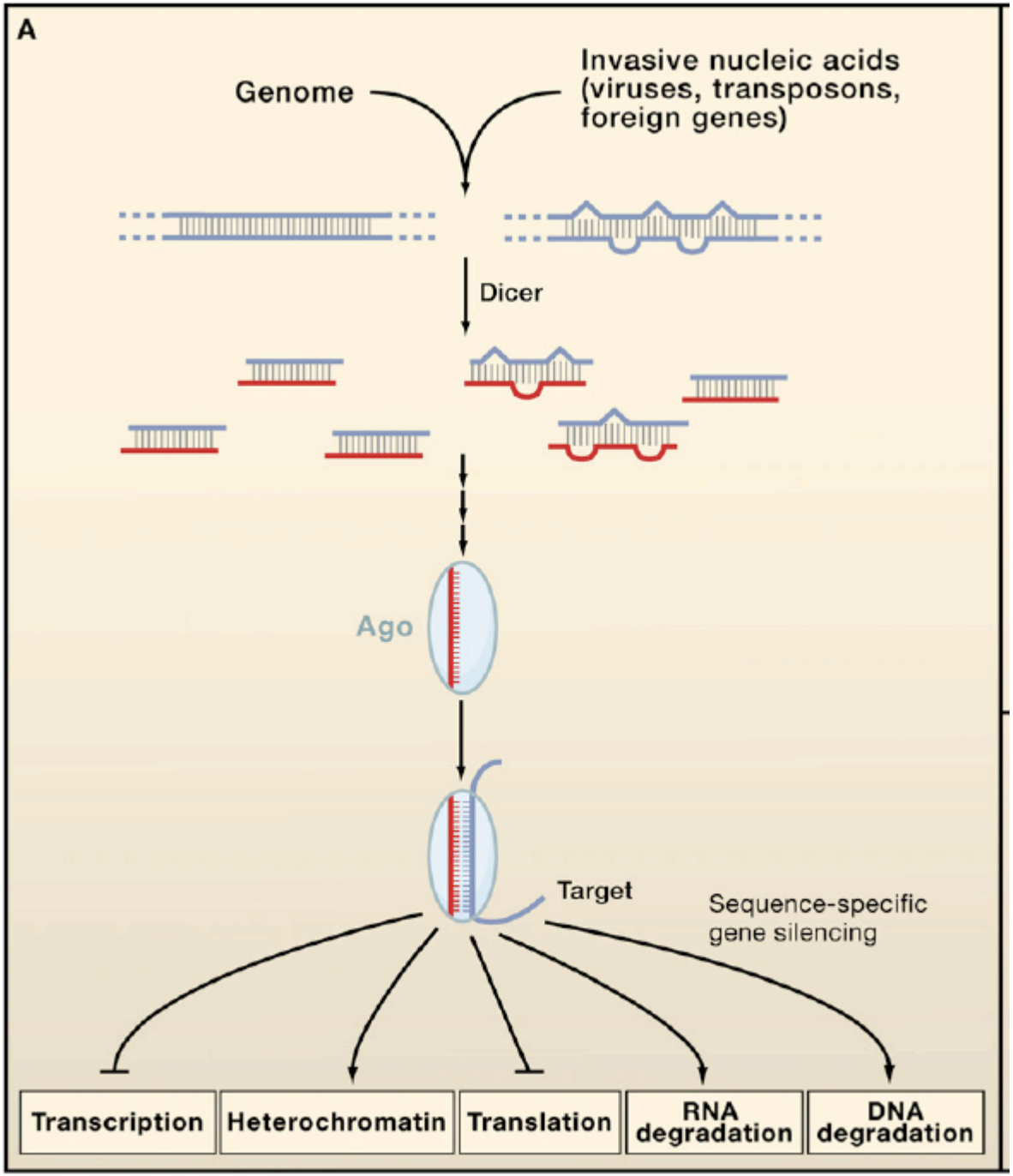
Small RNAs in Regulation of Eukaryotic Gene Expression

1. Silencing by RNA interference (RNAi)
    - Historical context
    - Molecular pathway (common elements w/ miRNA)
    - Outcomes and functions
2. Regulation by micro RNA (miRNA)
    - Historical context
    - Molecular pathway for miRNA production
    - Outcomes and functions

Common Features of Pathways for siRNA and miRNA

Both pathways include:

- dsRNA 'trigger'
- Dicer processing enzyme
- Argonaute (Ago)-containing complex to carry out effector function



Carthew and Sontheimer, Cell (2009) 136, 642-655.

History of RNA Interference

©Courtesy of Dr. Richard A. Jorgensen, The Plant Cell

Fig. 16.28

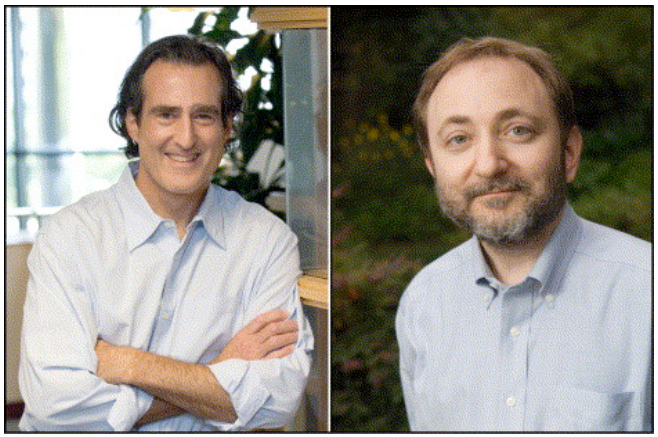
- Instead of enhancing purple color, addition of pigment-producing transgenes eliminated purple color (Jorgensen, 1990)
- Called co-suppression or postranscriptional gene silencing (PTGS)
- Similar phenomenon observed in fungus *N. crassa*, called quelling

RNAi

Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

Andrew Fire*, SiQun Xu*, Mary K. Montgomery*, Steven A. Kostas*†, Samuel E. Driver‡ & Craig C. Mello‡

- Discovered by accident
- An extremely useful tool for researchers



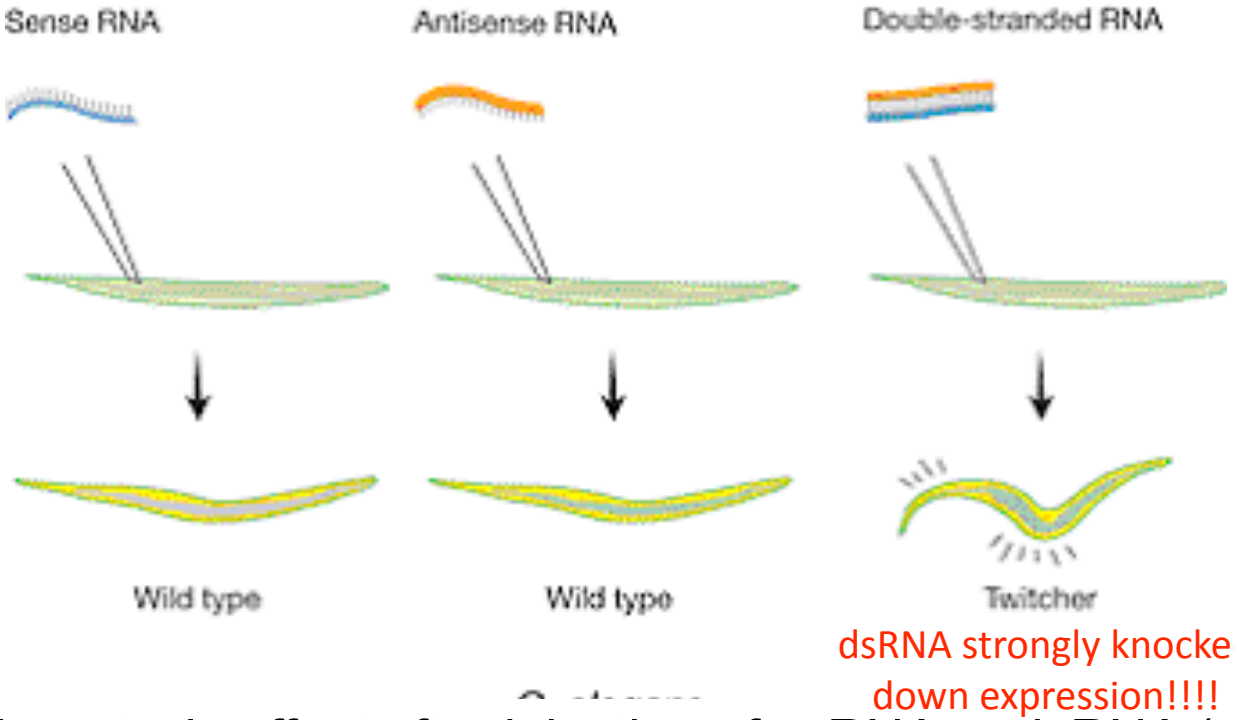
Craig Mello Andrew Fire

Shared the Nobel Prize in Physiology or Medicine for their work on RNA interference in the nematode worm *C. elegans*, which they published in 1998

Accidental Discovery of RNAi

- Goal: silence endogenous mRNAs with antisense RNA
- The *unc-22* gene encodes a myofilament protein.
- Decrease in *unc-22* activity is known to produce severe twitching movements.

Control Experimental Control



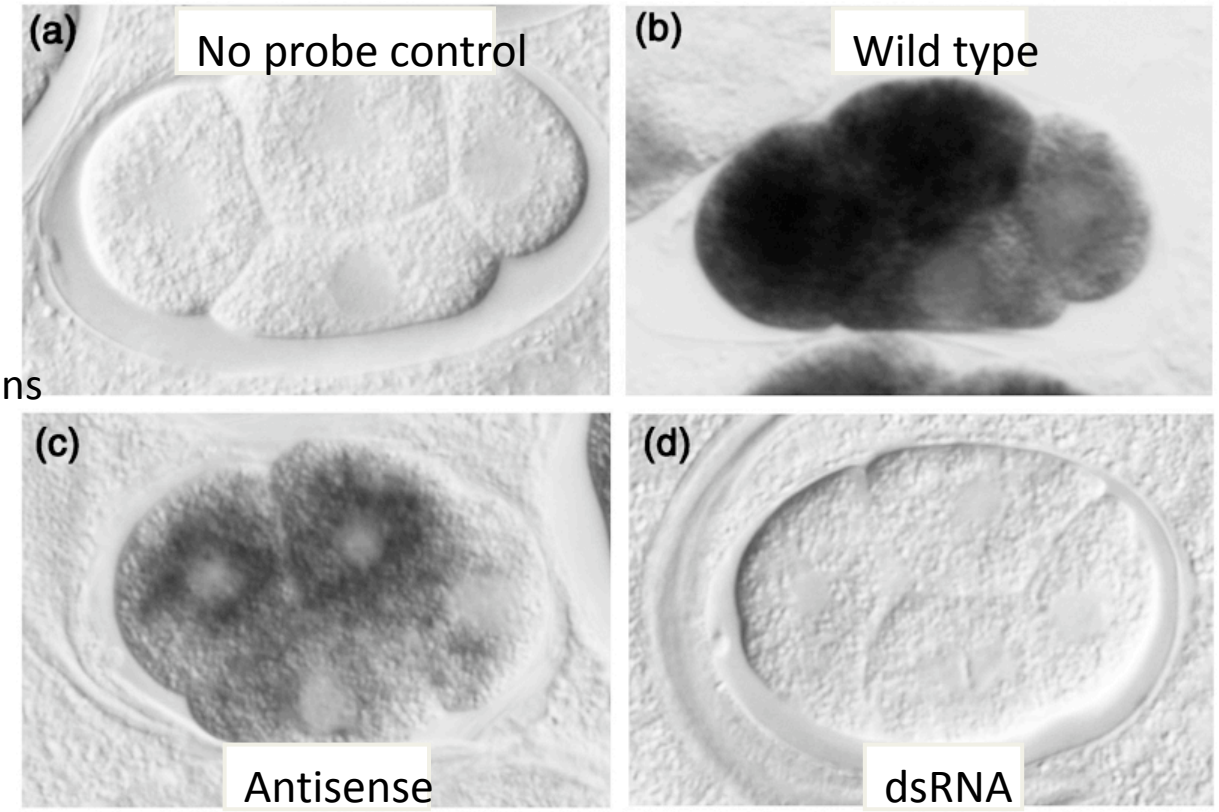
Fire *et al.* *Nature* 1997

dsRNA strongly knocked down expression!!!!

Phenotypic effect after injection of ssRNA or dsRNA (*unc-22)* into the gonad of *C. elegans.*

Injection of dsRNA in *C. elegans* Shown To Cause Destruction of Specific mRNA

- Mello and colleagues, 1998
- Injection in gonads of dsRNA for *mex-3* (abundant RNA) gave much more efficient inhibition in embryos than antisense RNA
- dsRNA had to include exons; introns and promoter didn't work
- Effect was incredibly potent and even spread to other cells within the worm
- Termed 'RNA Interference'
- Incredibly useful as a tool for molecular biology



in situ hybridization four-cell stage embryo

Fire *et al. Nature* 1998

- dsRNA from mature mRNA elicits RNAi
- dsRNA from introns does not
- RNAi results in decreased mRNA levels
- RNAi is heritable (for a few generations)
- RNAi only requires a few molecules of dsRNA per cell
- RNAi is applicable to many different transcripts

dsRNA Is Processed to Small Fragments of Well-defined Length

- Bartel and colleagues, 2000
- Used a system from *Drosophila* embryo lysate
- Added dsRNA corresponding to luciferase gene
- Fragments were the same length regardless of which strand was labeled
- Production of fragments did not require the luciferase mRNA, suggesting that the processing is upstream of effect on mRNA

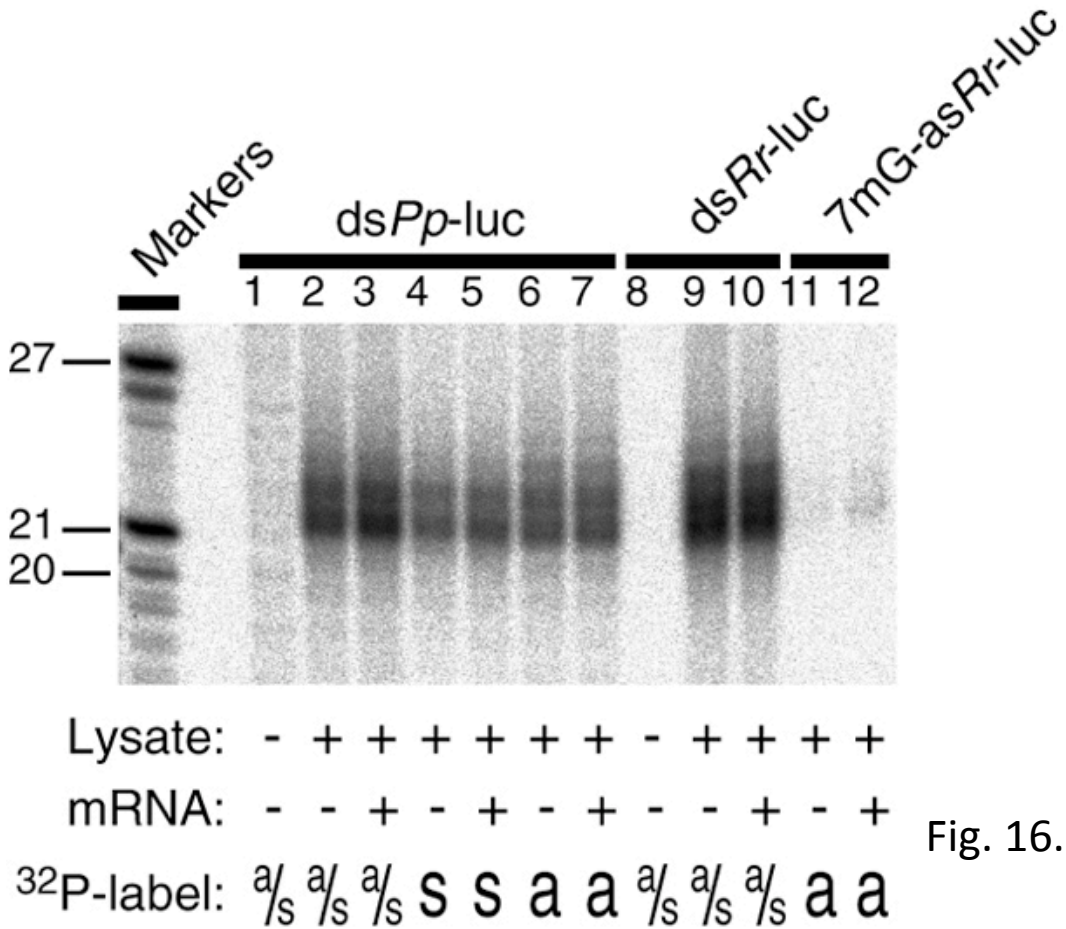
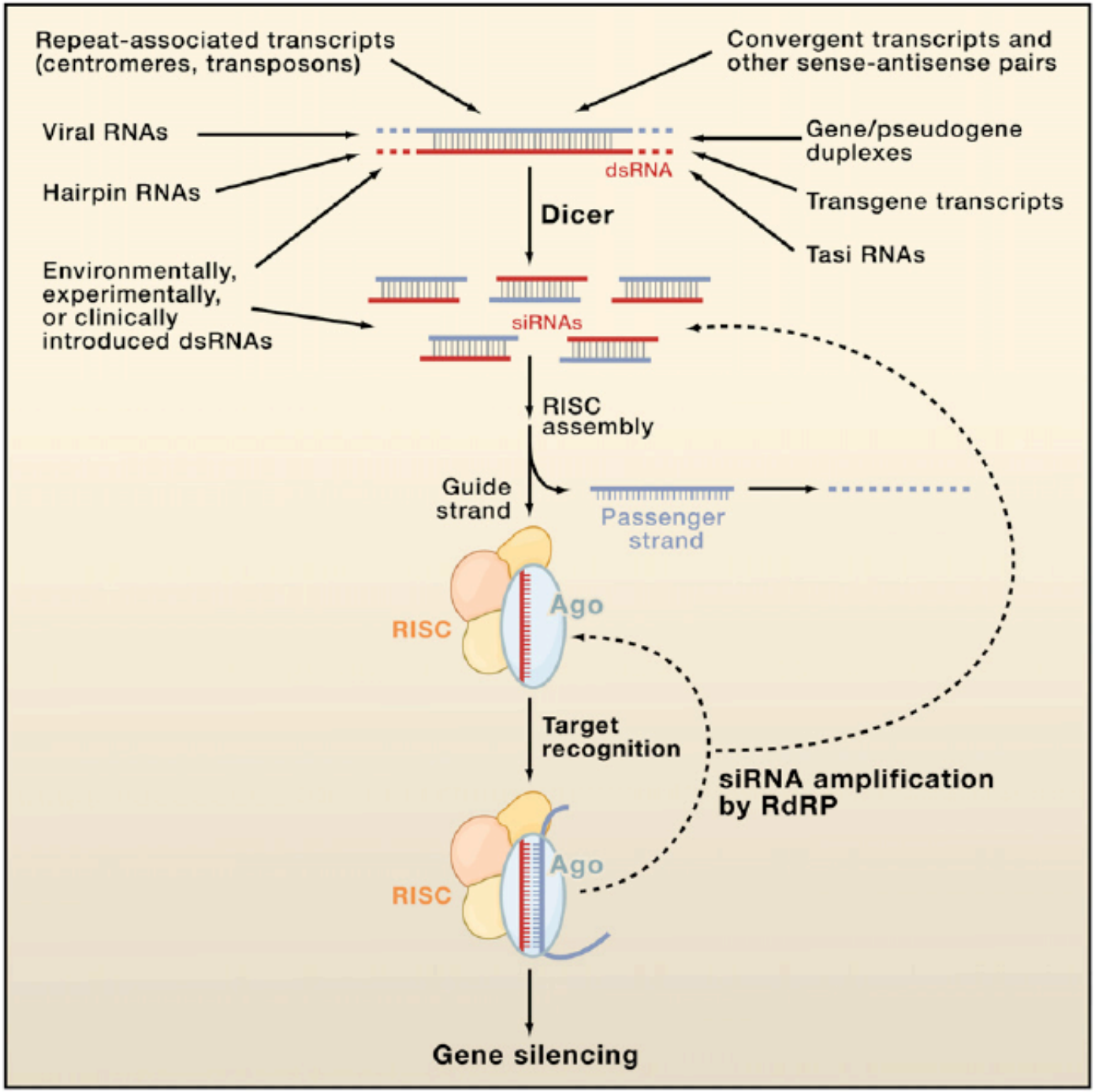


Fig. 16.30

Sources of dsRNA

- Some dsRNAs have viral origin, but not all
- Genomic repetitive sequences also are source of siRNA
- Some even regulate other genes (ta-siRNA for trans-acting in plants)
- exo siRNAs (viral etc)
- endo siRNAs –the precursor has a nuclear phase (hairpins, sense-antisense transcripts etc)



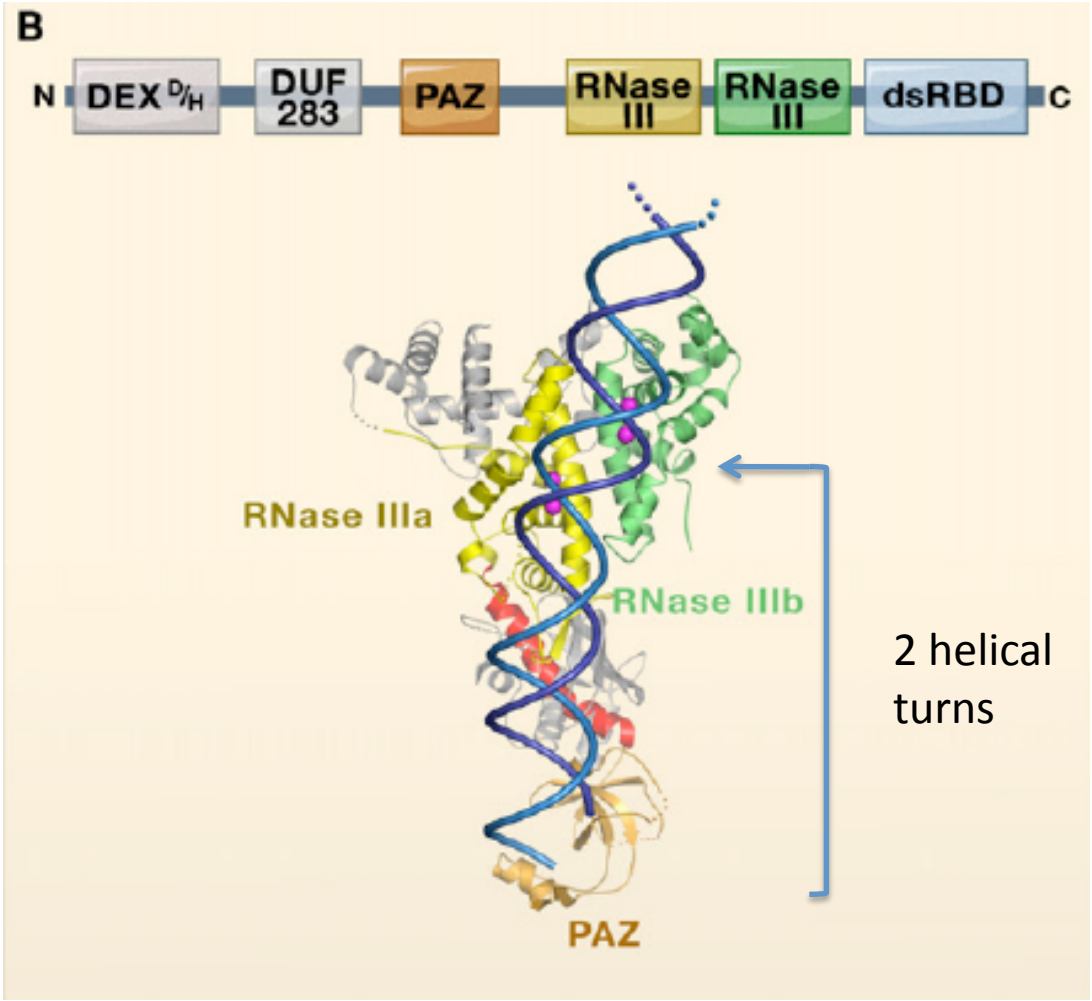
Carthew and Sontheimer, Cell (2009) 136, 642-655.

Signature components of RNA silencing

- Dicer
- Argonaute Proteins (Ago)
- 21-23 nt duplex-derived RNAs

Dicer: Producer of Small (21-23 bp) RNA Fragments

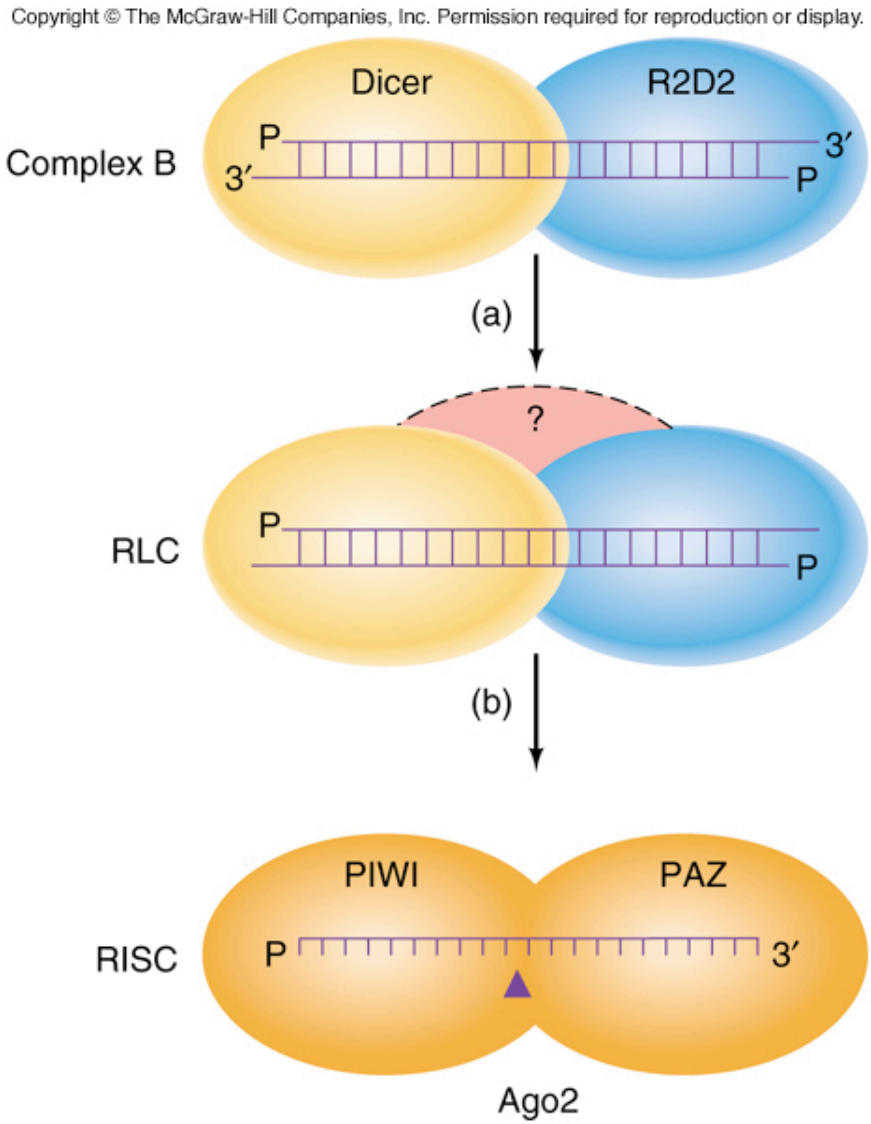
- Structure solved by Doudna and colleagues (2006)
- PAZ domain binds RNA end, RNase III domains cut RNA to produce 2 nt 3'-overhang
- Roles of other domains (not present in structure) remain unclear



Carthew and Sontheimer, Cell (2009) 136, 642-655.

Assembly of the RNA-Induced Silencing Complex (RISC) Involves Additional Proteins

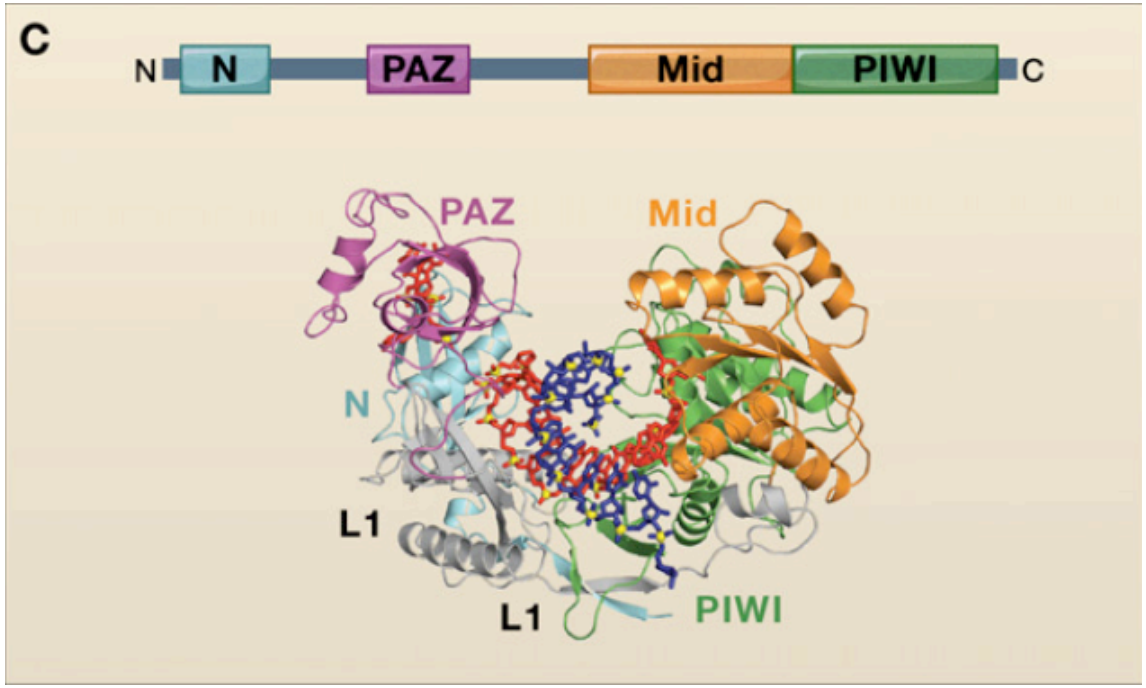
- Processing of dsRNAs into RISC requires accessory proteins: TRBP (R2D2 in *Drosophila*) forms complex with Dicer
- Other unknown proteins bind to form RISC Loading Complex
- Ago2 cleaves the passenger strand, leading to its ejection



Argonaute: Central Component of the RNA-Induced Silencing Complex (RISC)

- One strand of the dsRNA produced by Dicer is retained in the RISC complex in association with Argonaute
- Structure first solved by Leemor-Tor and colleagues (2004), more recent structures by Patel and colleagues include RNAs mimicking guide ssRNA and target mRNA

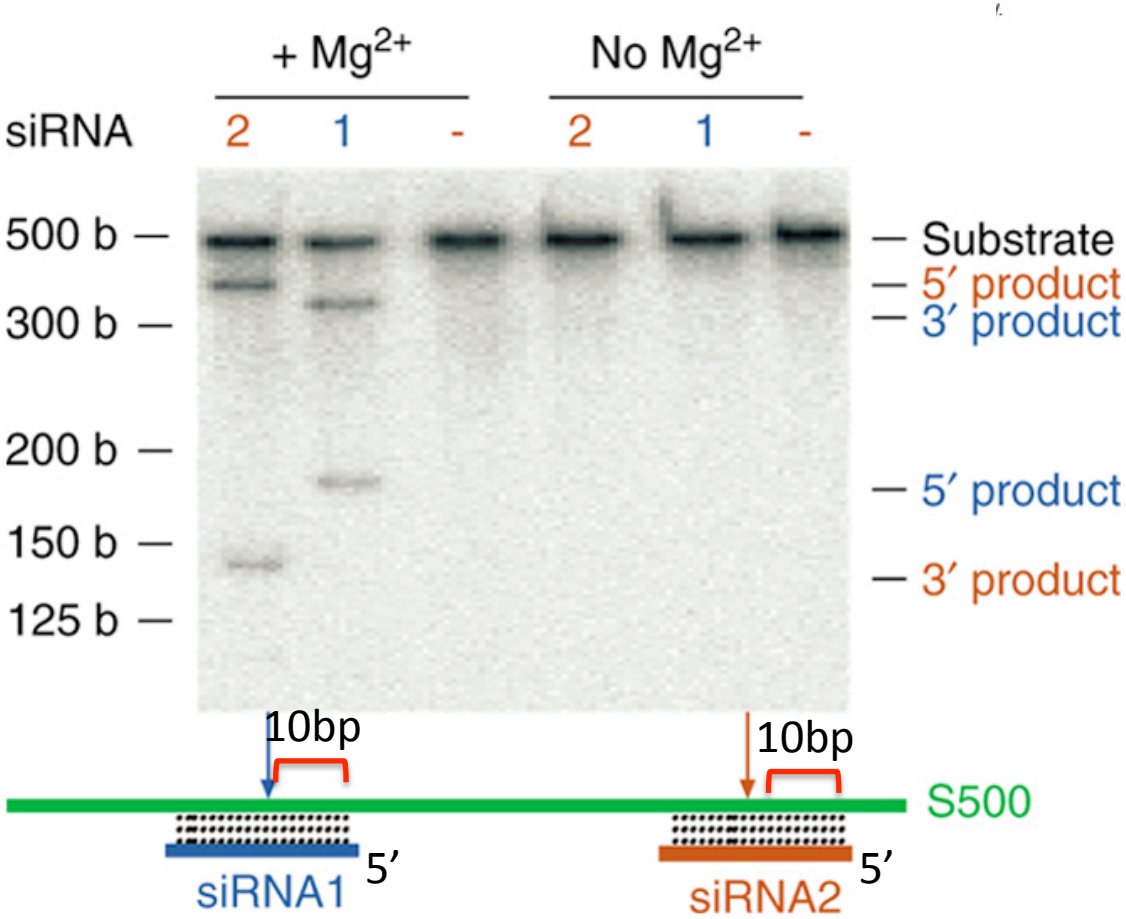
- The PAZ domain has RNA 3' end binding activity
- In structure without mRNA, guide strand nucleotides 2-6 have bases exposed and available for base-pairing
- PIWI domain adopts RNase H fold and in some Ago proteins can cleave the 'passenger strand' : I.e. the mRNA



Carthew and Sontheimer, Cell (2009) 136, 642-655.

In vitro Demonstration of Slicer Activity

- Human Ago2 mixed with 2 siRNAs and a 500-nt RNA target
- Products of expected size were produced, dependent on siRNA, target RNA, and Mg^{2+}
  - The sllicer activity is very precise (10bp from 5' end)
  - in vivo cellular nucleases attack the fragments to complete degradation

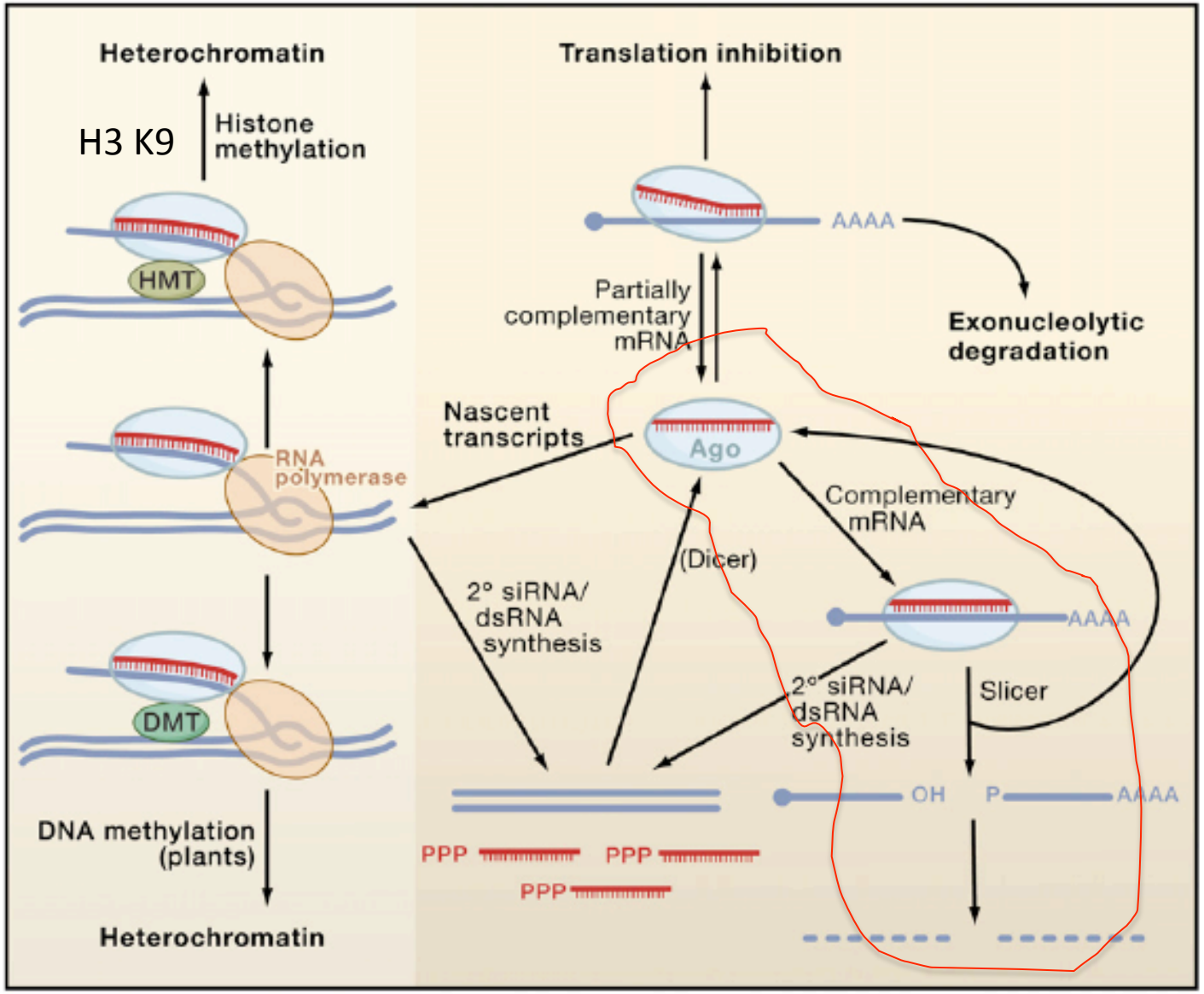


Thermodynamic asymmetry determine the preferential strand

- One strand of the dsRNA produced by Dicer is retained in the RISC complex in association with Argonaute
- strand selection is dictated by the relative thermodynamic stabilities of the two duplex end
- the strand with the 5’ terminus less stable is favored
- but frequently both strands are included in RISC
- in mammals the mechanism is unclear
- example -mir34a and mir34a*

Mechanisms of siRNA Silencing

- canonical RNAi , si RISC recognizes a perfect complementary RNA leading to Ago-mediated cleavage

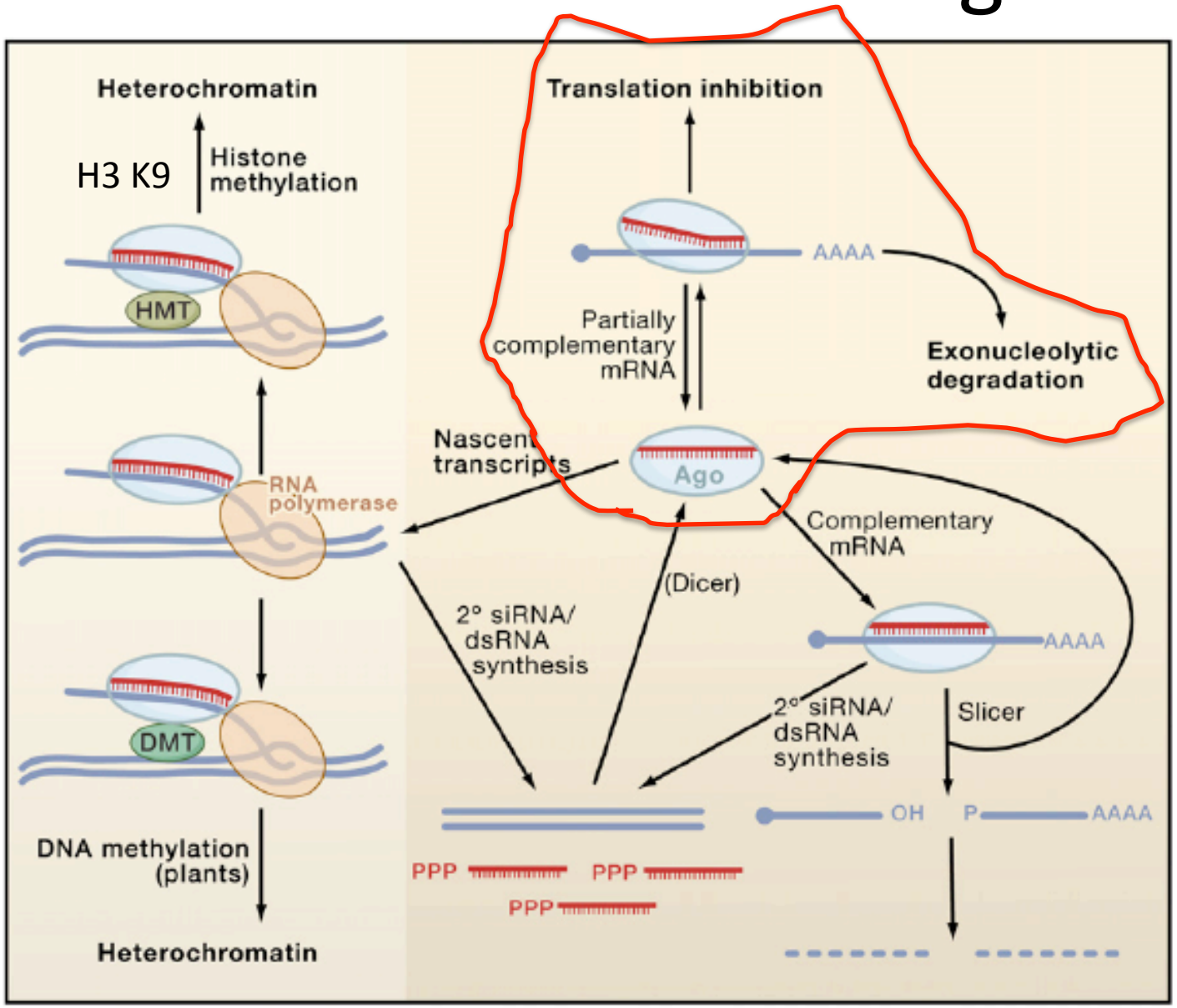


Carthew and Sontheimer, Cell (2009) 136, 642-655.

Mechanisms of siRNA Silencing

- Imperfect base-pairing between guide strand and target can give non-degradative silencing (miRNA pathway) with translation inhibition and/or exonucleolytic degration

(next lesson)



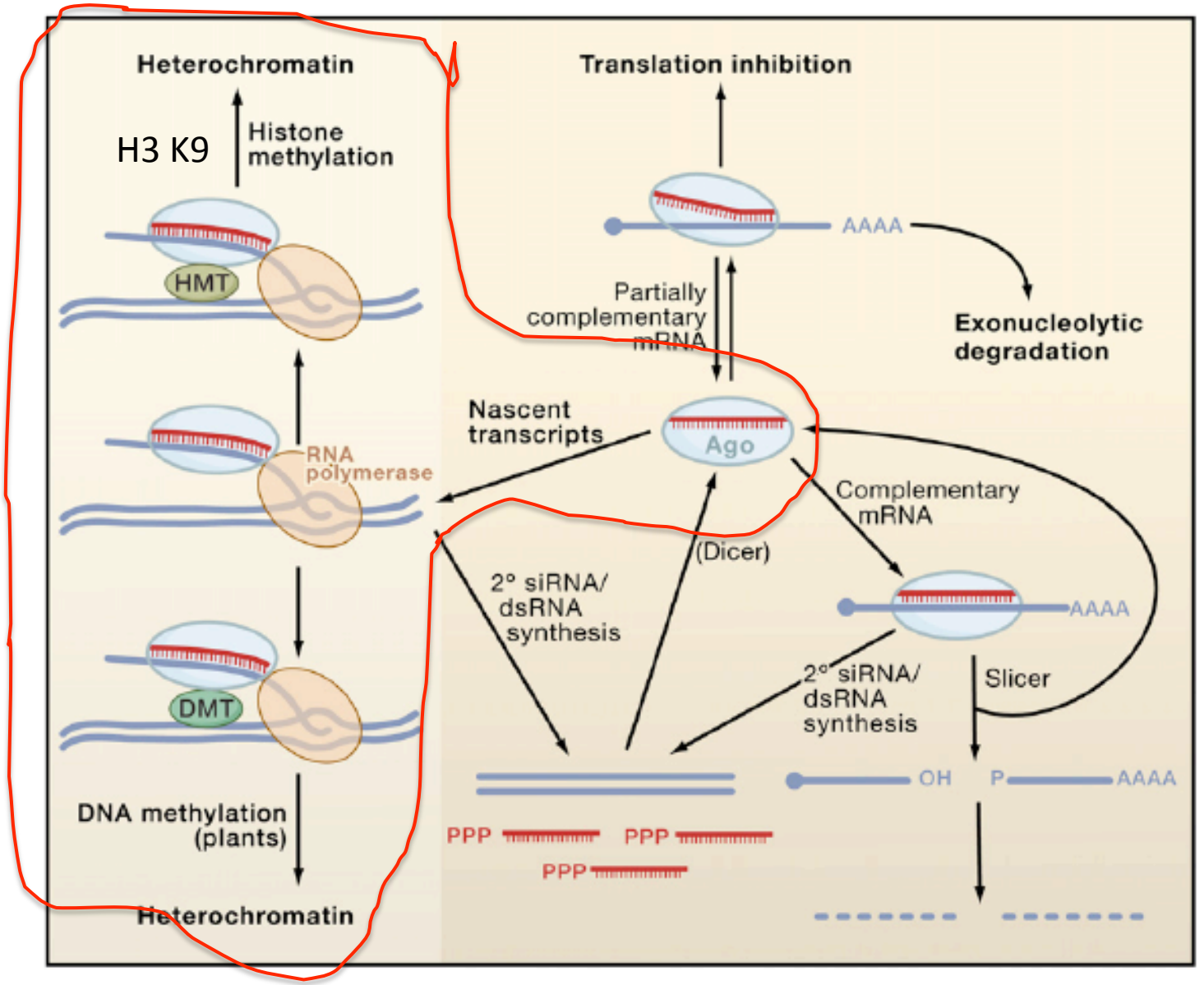
Carthew and Sontheimer, Cell (2009) 136, 642-655.

Mechanisms of siRNA Silencing

- siRNA pathway can act at the level of DNA also (Transcriptional Gene Silencing) pombe, mammals?

Histone Methyl Transferase

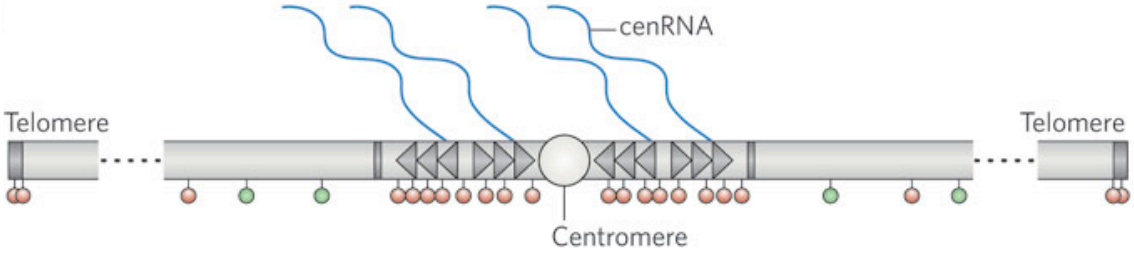
DNA methyl Tansferase



Carthew and Sontheimer, Cell (2009) 136, 642-655.

Gene Silencing by Formation of Heterochromatin

- Pathway best understood in *S. pombe*

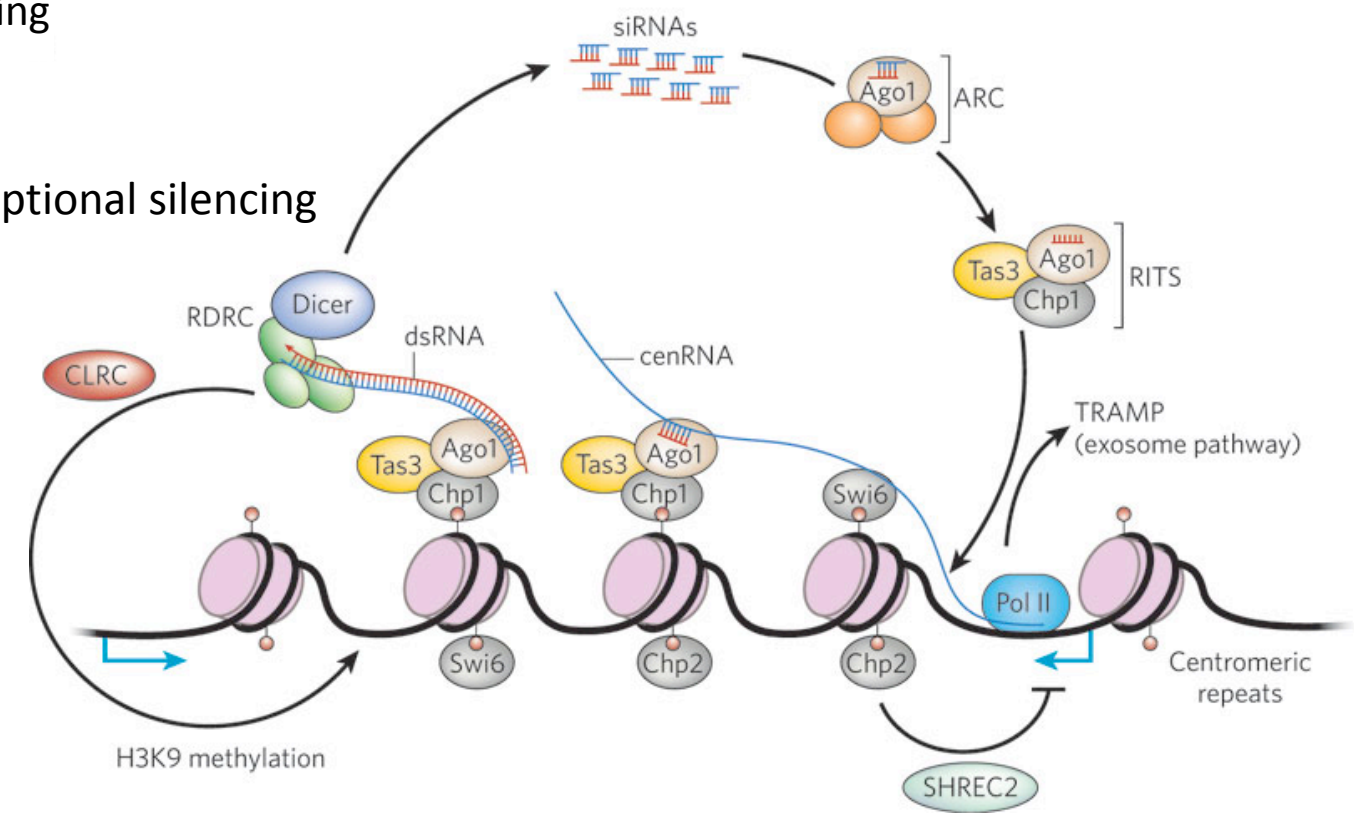


- Silencing involves formation of heterochromatin and resulting transcriptional repression

the RNA-induced transcriptional silencing complex (RITS)

Clr4 methyltransferase complex (CLRC)

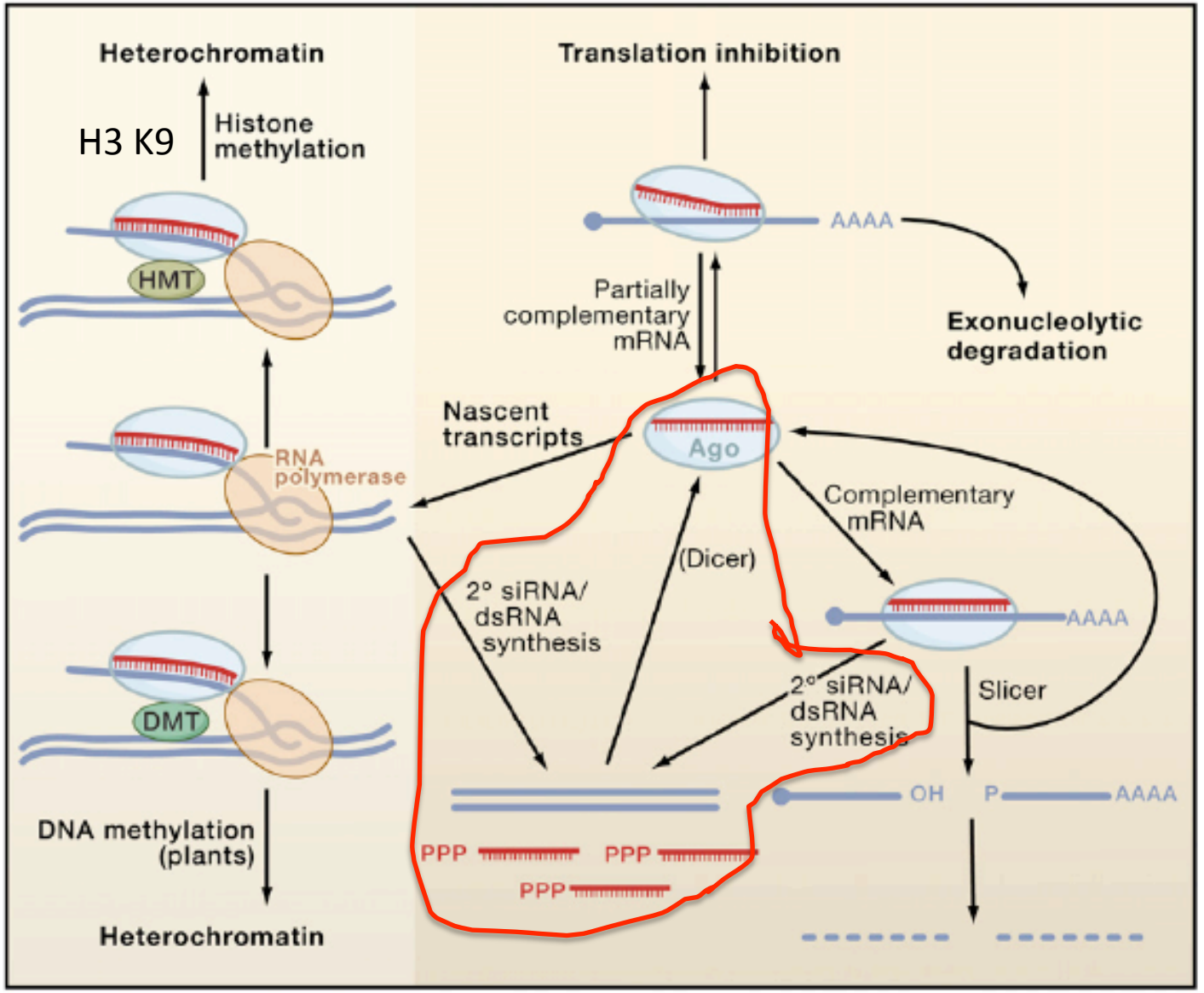
RNA-directed RNA polymerase complex (RDRC)



Moazed, *Nature* (2009) 457, 413-420

Mechanisms of siRNA Silencing

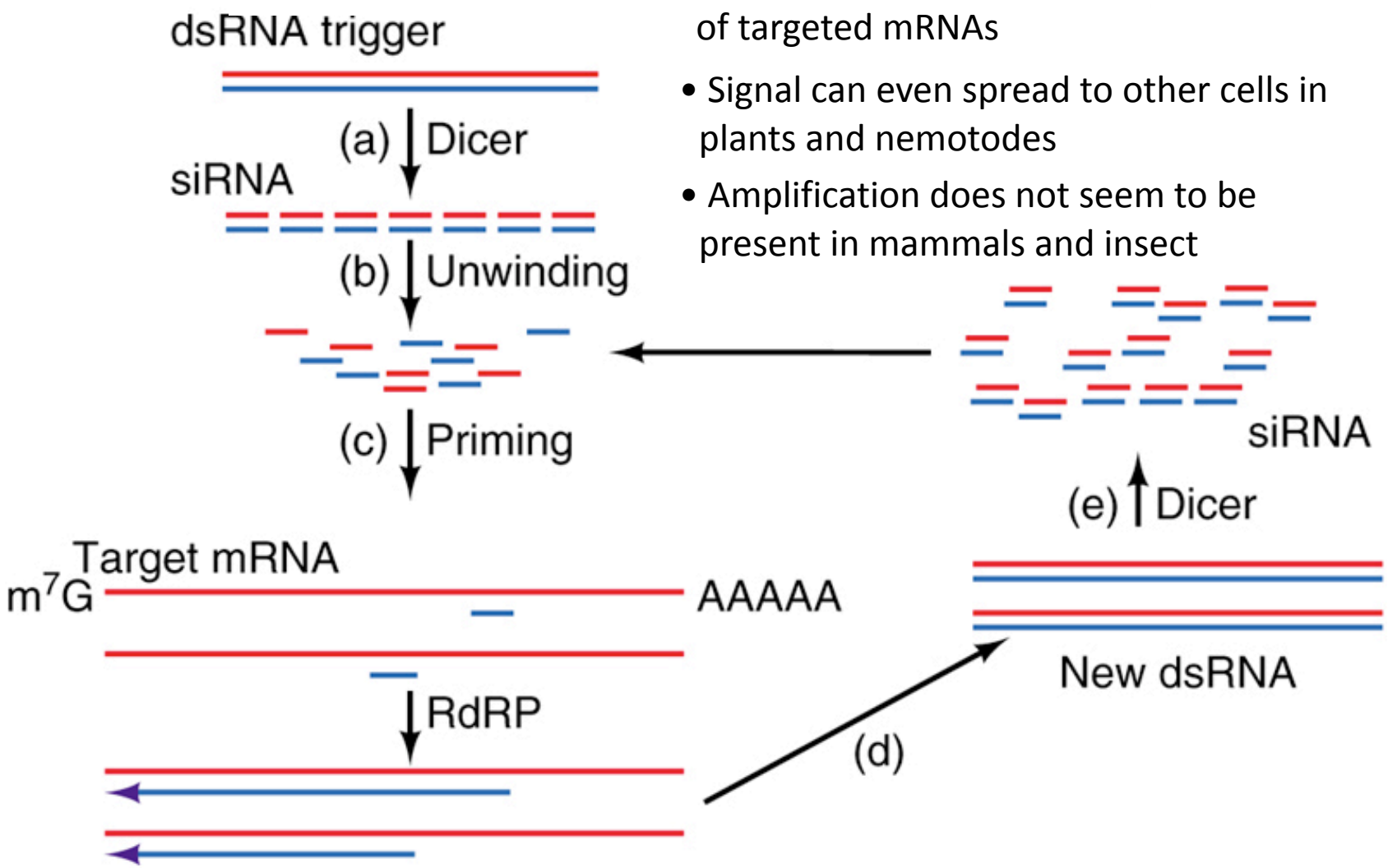
- 2nd siRNA/dsRNA synthesis and amplification not present in mammals and insect



Carthew and Sontheimer, Cell (2009) 136, 642-655.

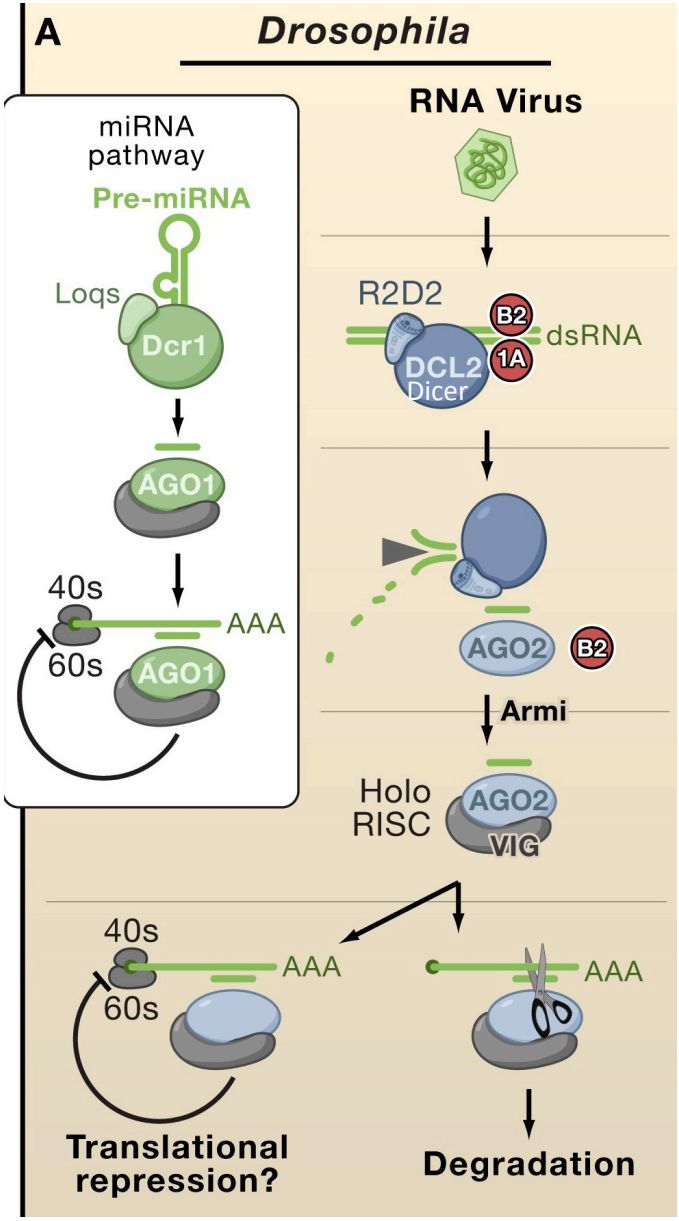
In Some Organisms, siRNA Signal Is Amplified and Spread

- New siRNAs appear against other regions of targeted mRNAs
- Signal can even spread to other cells in plants and nemotodes
- Amplification does not seem to be present in mammals and insect



RNAi in Defense Against Viruses

- Cell co-opts the viral RNA and uses it against itself
- System is easily adaptable to any new virus or foreign invader



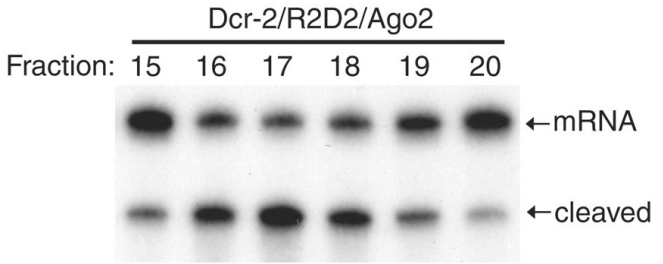
Ding and Voinnet, Cell (2007) 130, 413-422

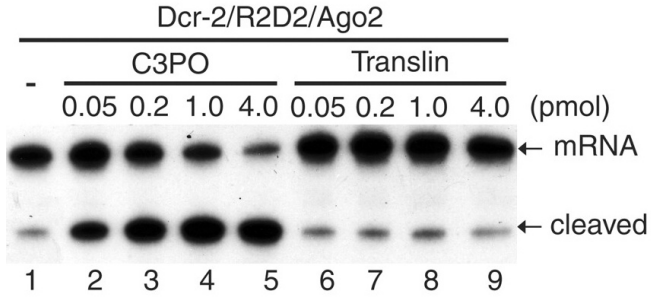
miRNA readings

- Carthew and Sontheimer, Origin and mechanism of miRNAs and siRNAs. Cell (2009) 136, 642-655.
- Jacek Krol, Inga Loedige and Witold Filipowicz The widespread regulation of microRNA biogenesis, function and decay. Nature Reviews Genetics 11 2010, 597
- V. Narry Kim, Jinju Han and Mikiko C. Siomi. Biogenesis of small RNAs in animals. Nature Reviews Mol Cell Biol 10 2009, 126

Removal of Passenger Strand Is Accelerated by C3PO

- Biochemical fractionation of *Drosophila* extract identified protein complex, C3PO, that enhances mRNA cleavage by Ago 2 programmed with dsRNA
- Complex of two proteins, Translin and Trax, both of which are required for activity
- Trax has RNase activity, which is required for promotion of RISC complex
- Suggests that RNase activity is used to remove passenger strand to activate RISC
- Same activity may also remove cleaved mRNA in subsequent functional cycles



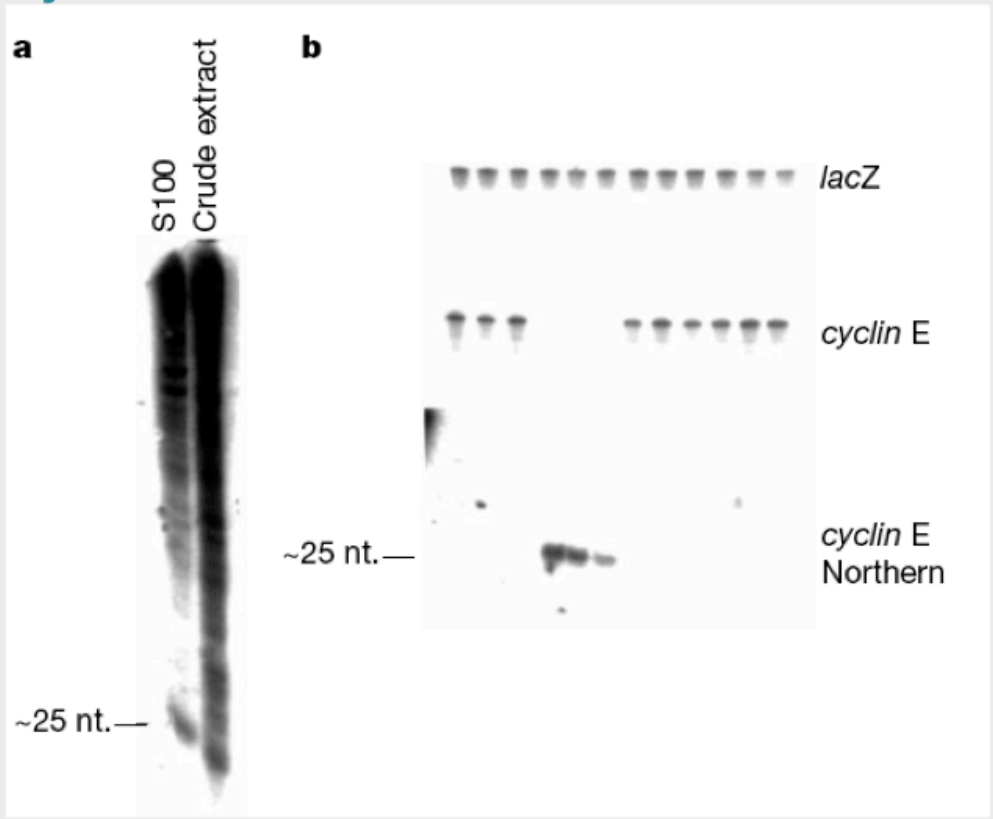


Liu *et al*, *Science* (2009) 325, 750-753

- Ago subfamily
  - miRNA
  - siRNA
- PIWI subfamily
  - transposon silencing

Initial purification of RNA induced Silencing Complex (RISC)

In Vitro Cleavage assay



Probes

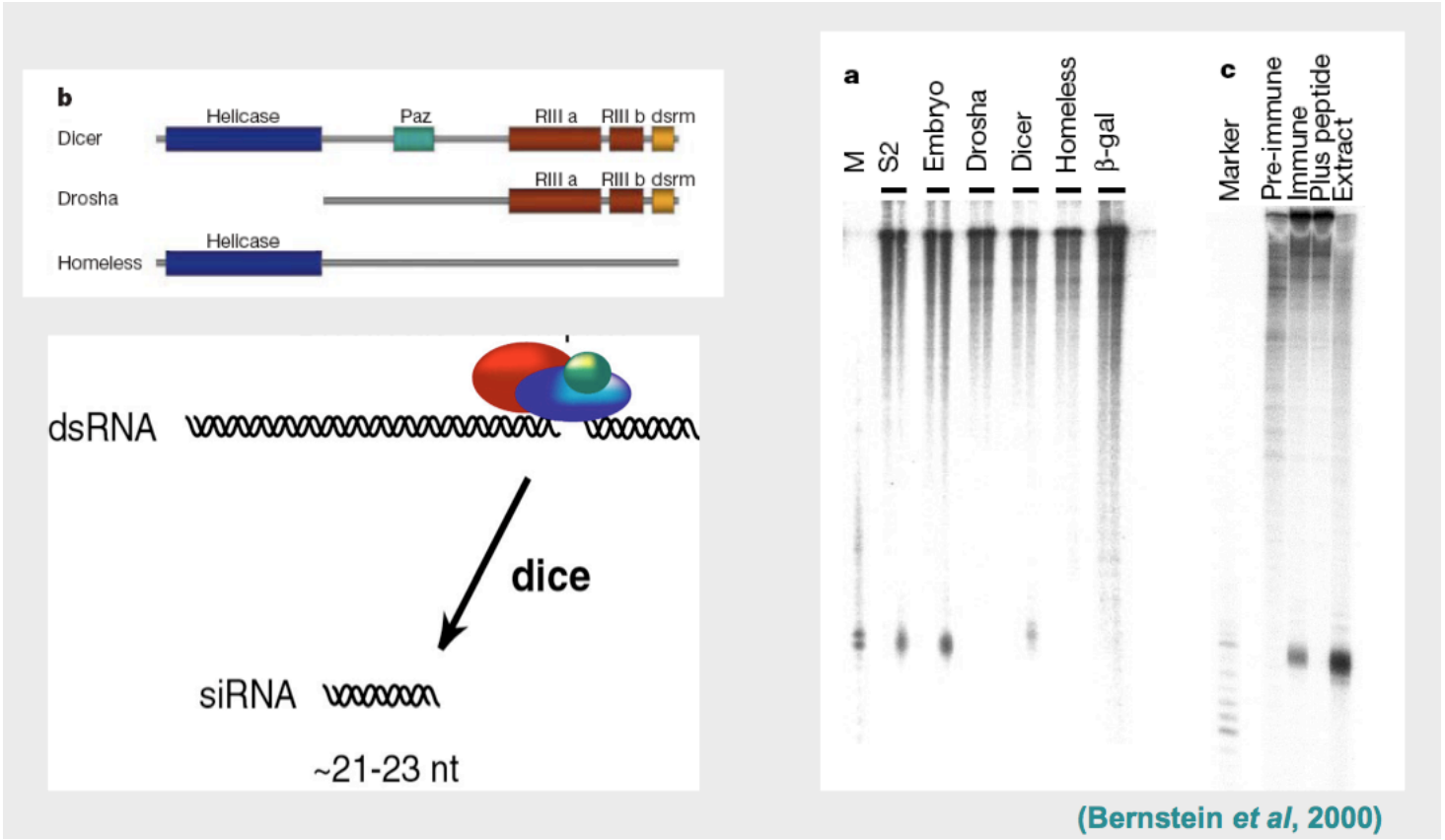
antisense

Sense

Small RNAs antisense to the mRNA identified

(Hammond *et al*, 2000)

Identification of Dicer, the enzyme that generates 21bp sRNAs



(Bernstein *et al*, 2000)

Genetic screening in *C elegans* indentified rde-1 as required for RNA interference

- non obvious developmental defects
- PAZ and PIWI domains
- Argonaute family of proteins
  - C elegans 20 homologues
  - Drosophila
  - Plants
  - Mammals