# Bi 8 Lecture 17

# Regulation by RNA Interference

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# Protein is not the only regulatory molecule affecting gene expression:

## RNA itself can be negative regulator

- RNA does not need to be translated to encode regulatory function
- RNA can work through mRNA recognition while acting as a scaffold for complex of proteins

- Key forms of regulatory RNA:
  - **miRNA (natural)**
  - siRNA (artificial)
  - shRNA (artificial)
  - Long ncRNA (natural: “Lnc RNA”)
  - Long dsRNA (pathological)

# Problems that RNA regulation is needed to solve: where transcriptional regulation can't

- Reducing levels of specific RNAs in cells that have stopped dividing (terminally differentiated) & therefore can't dilute them out
- Modulating protein expression from existing RNAs
- Destroying genomes of RNA viruses in cytoplasm before they have a chance to make templates for translation of viral proteins
- Helping to detect "parasitic" repeated DNA sequences integrated into genome in tandem arrays

# Cells naturally make miRNA for their own regulatory fine-tuning of mRNA pools

Processing and action: initial overview (more later)

Translational inhibition and RNA degradation from binding to targets in mRNA 3'-UT

This is highly regulated and normal

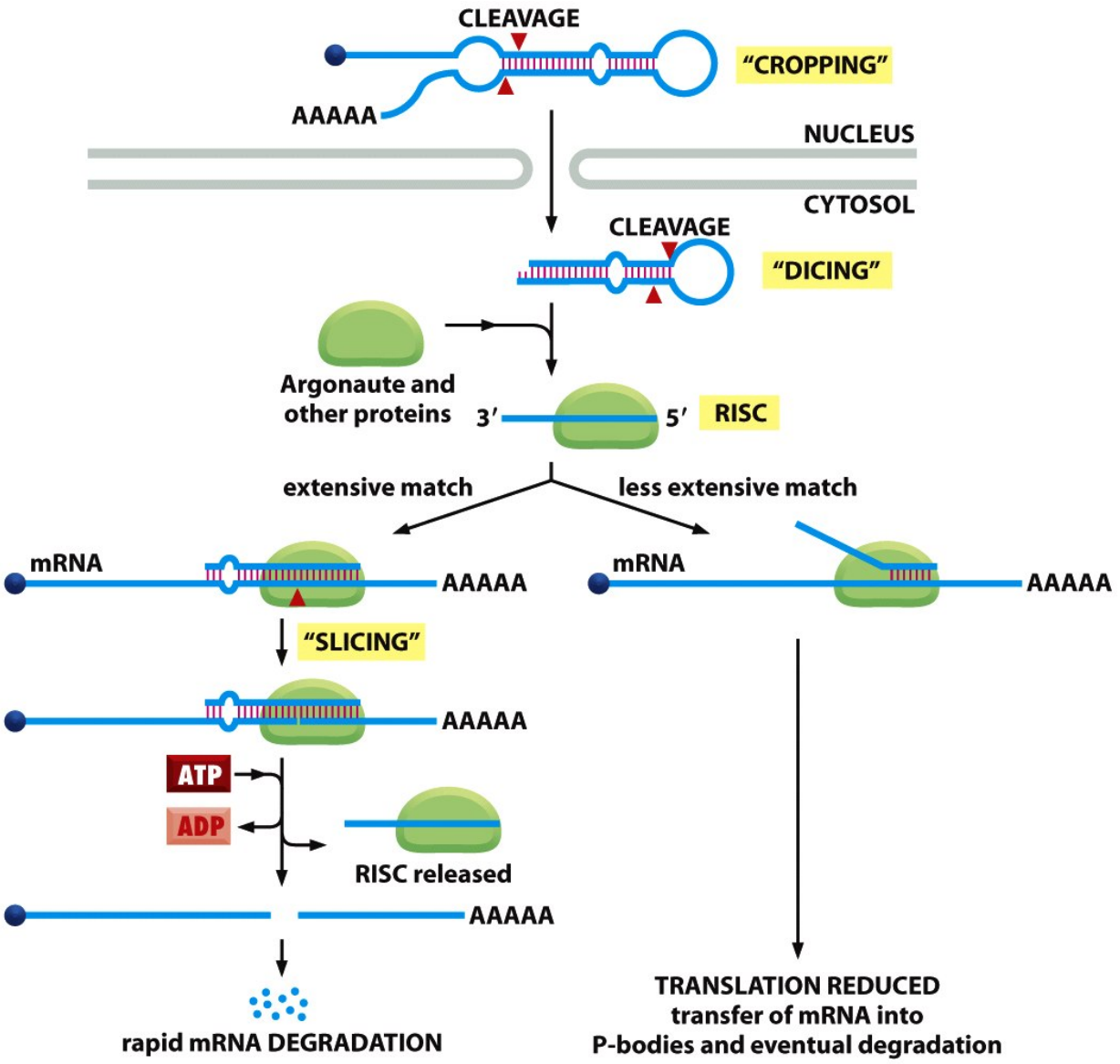



Figure 7-112 *Molecular Biology of the Cell* (© Garland Science 2008)

# miRNA vs. RNAi vs. siRNA

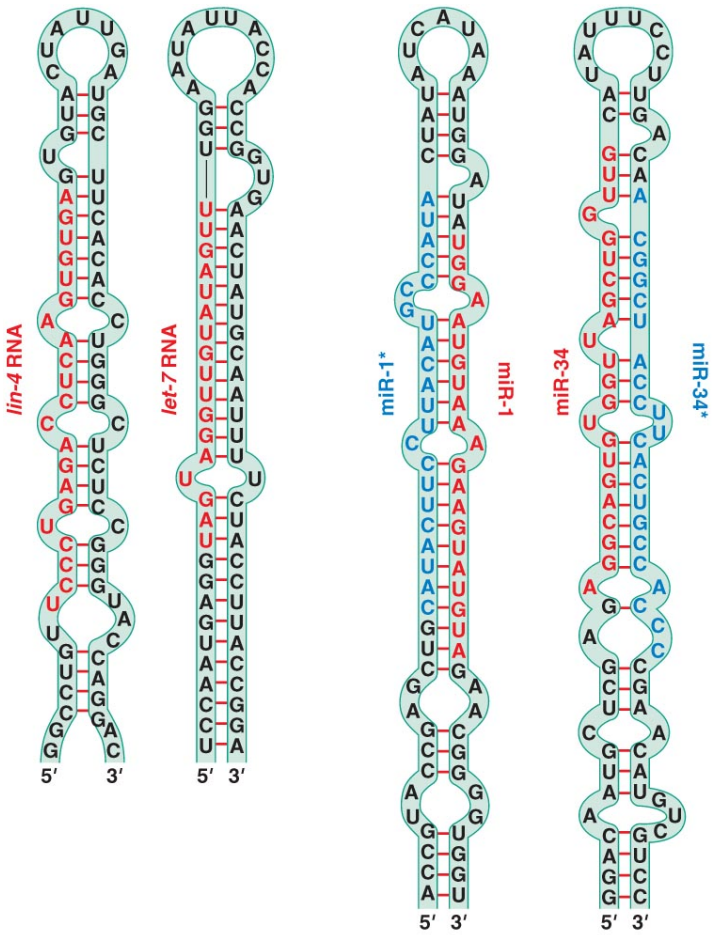
- MicroRNA (miRNA) is a natural molecule
  - Made from special genetic loci (pol II transcripts) that are specially cleaved for action

- RNA interference (RNAi), a regulatory phenomenon, was originally an "experimental artifact"
  - but taps into mechanism designed to neutralize viruses and overexpression of repeat sequence DNA

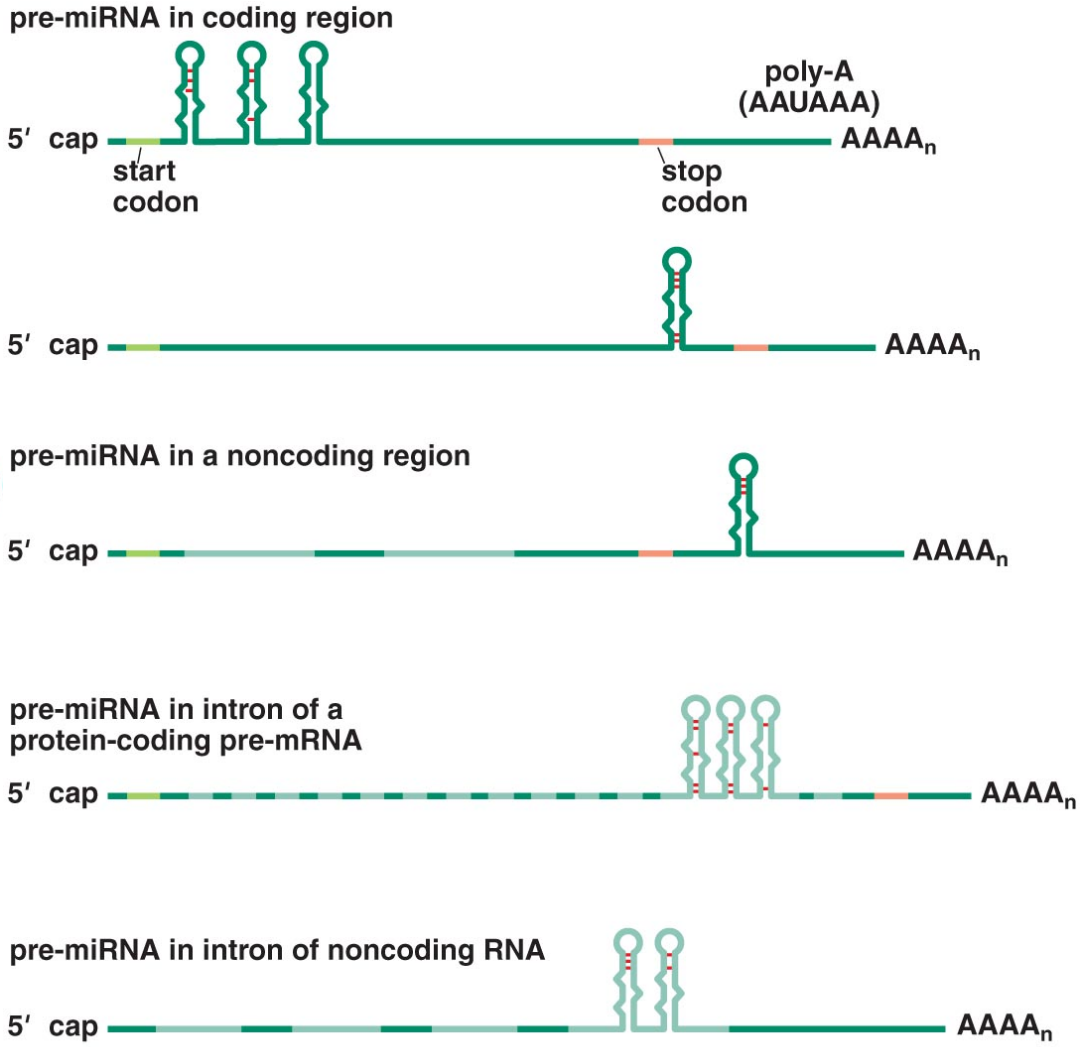
- siRNA is a tool to do RNAi with short synthetic RNA
  - avoids triggering "doomsday option" mammalian cell response to dsRNA

…But they all use major parts of the same machinery

# How pre-miRNAs look and where they are found… they normally do *not* come from loci they regulate





# microRNA processing: clipping out hairpin ("pre-miR") from long RNA primary transcript ("pri-miR") in nucleus

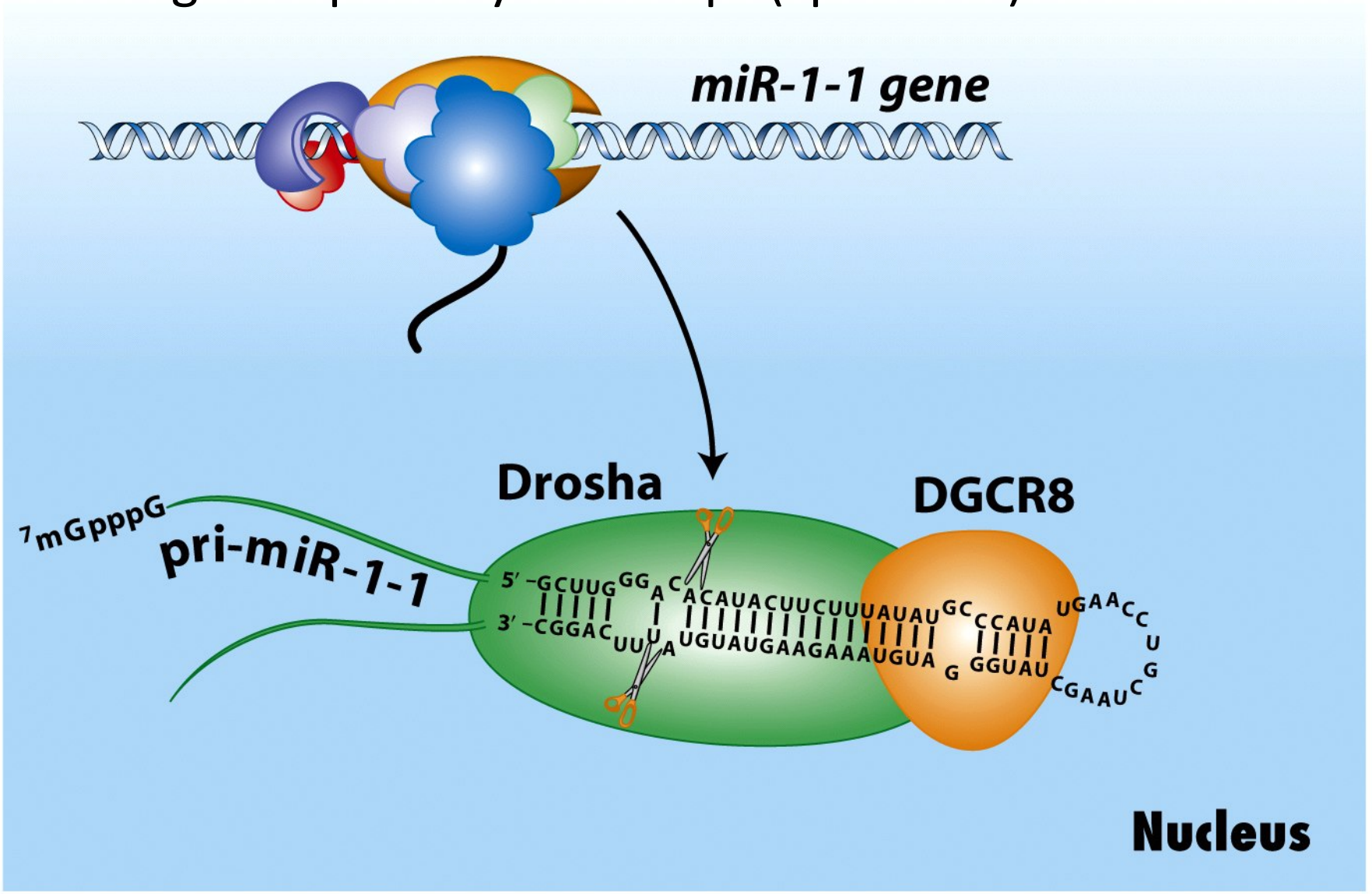



**Figure 8-26 part 1**
***Molecular Cell Biology, Sixth Edition***

pre-miR goes to cytoplasm to finish processing: hairpin converted to short duplex by Dicer clipping off loop

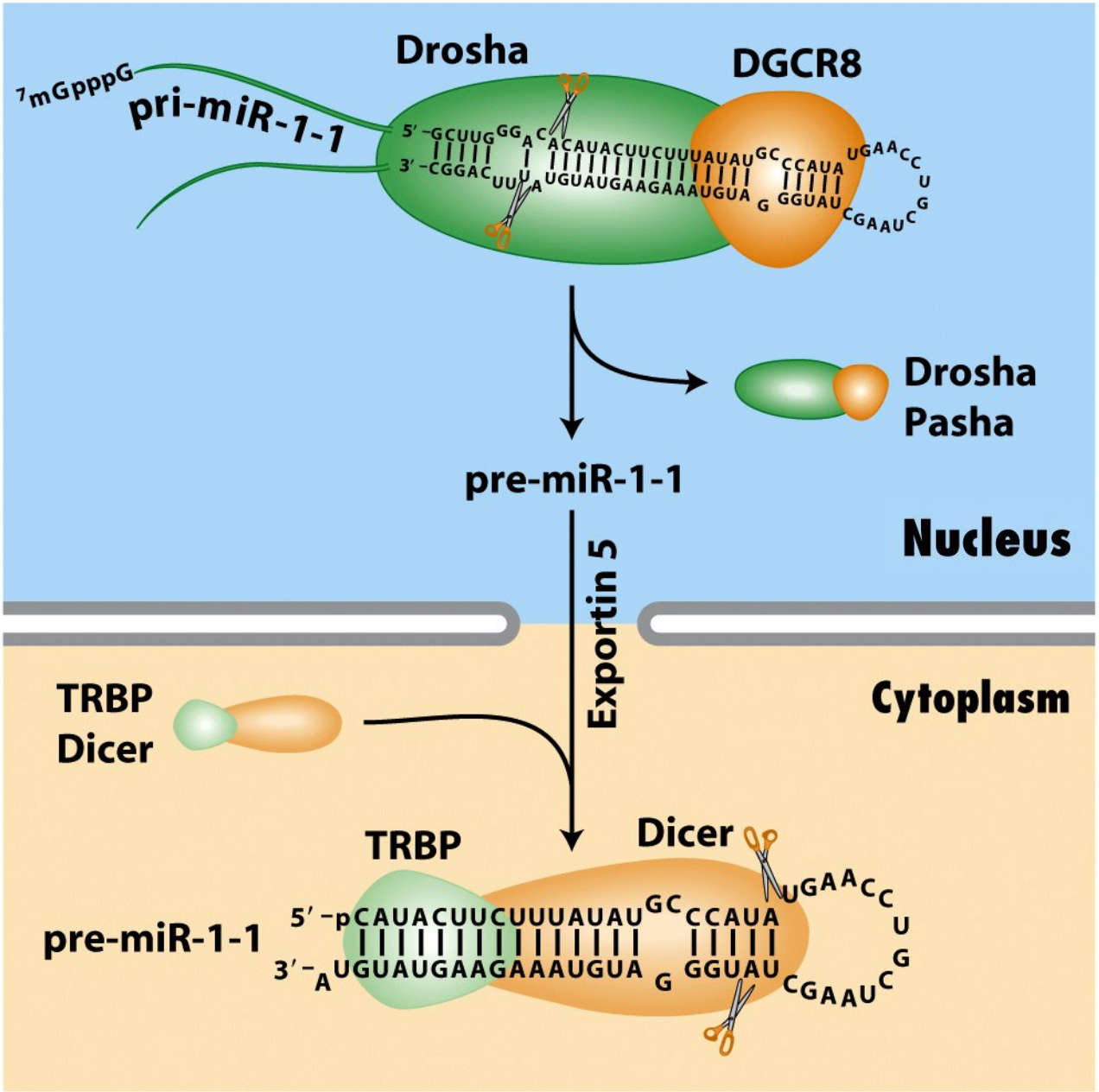



Figure 8-26 part 2
*Molecular Cell Biology, Sixth Edition*

Active form is one strand from duplex loaded onto Argonaute protein in "RISC" complex

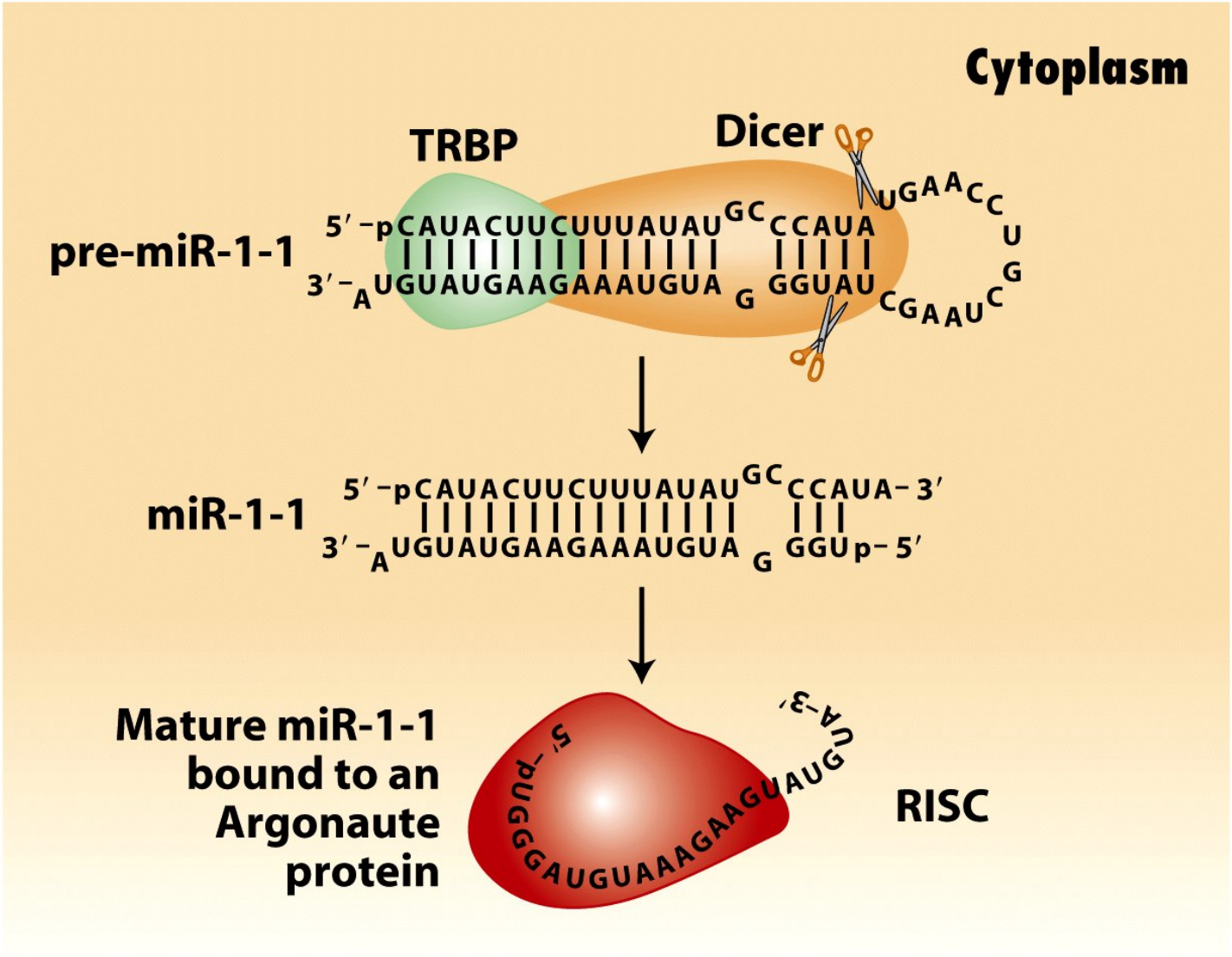



Figure 8-26 part 3
*Molecular Cell Biology, Sixth Edition*


(RNA-Induced Silencing Complex)

# Argonaute protein (Slicer) embracing a paired miRNA-mRNA complex

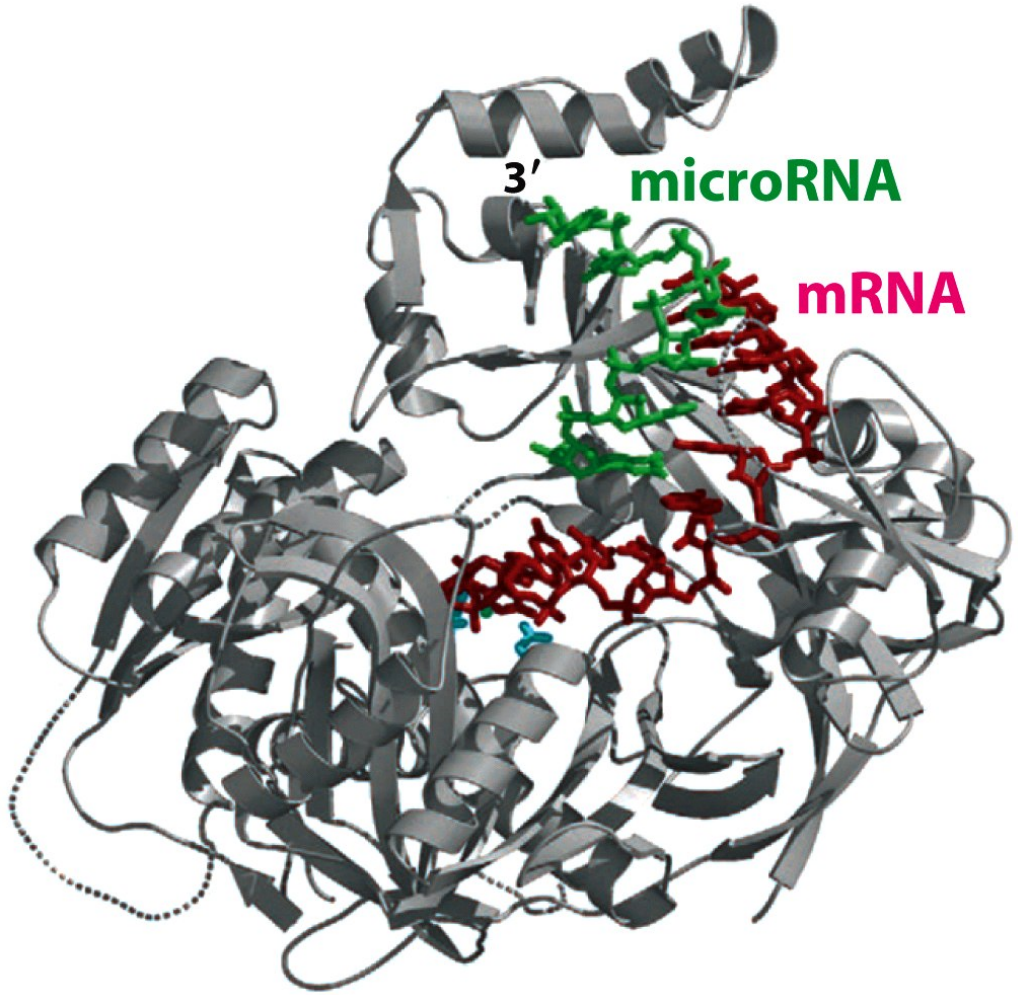
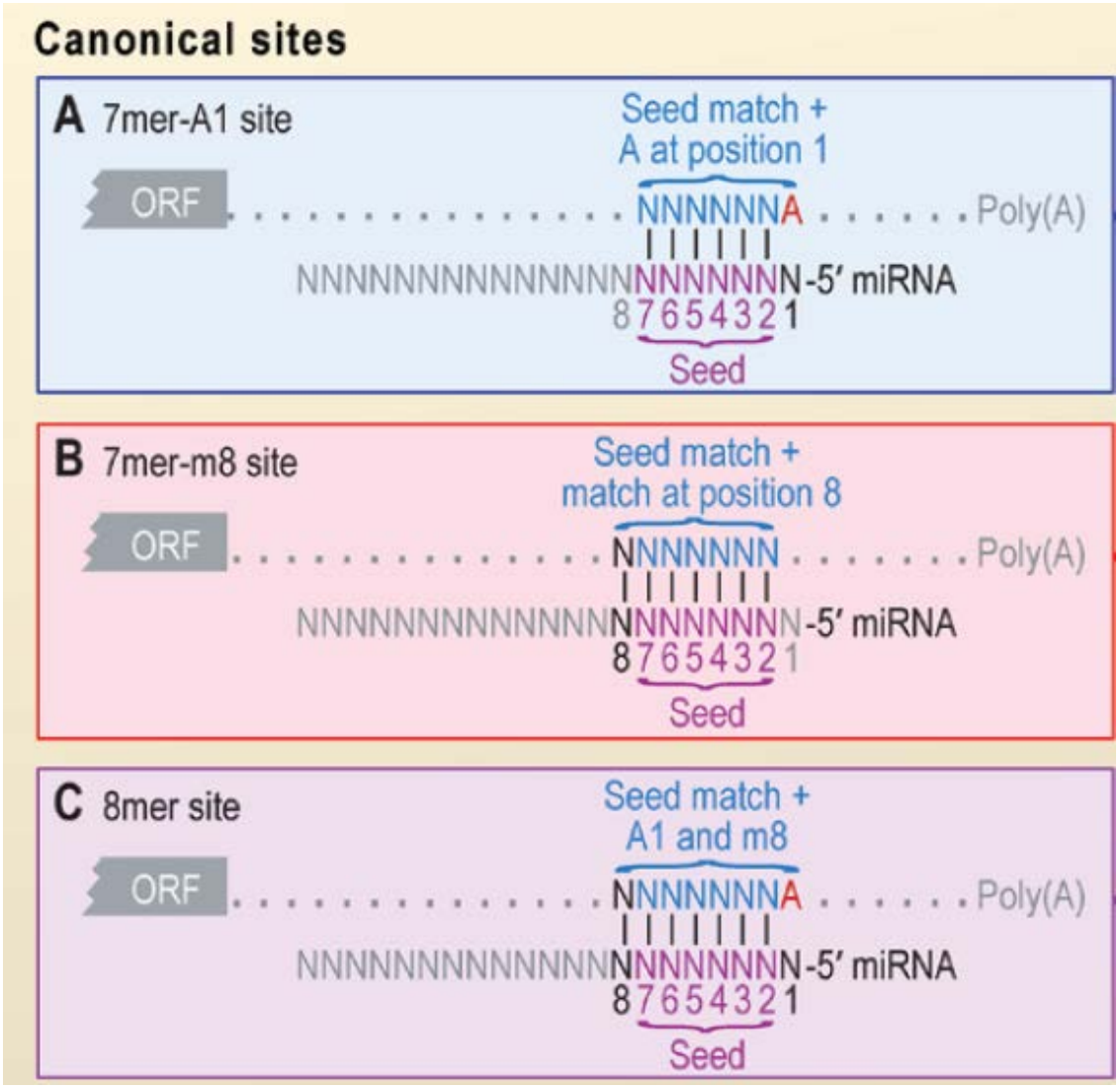



Figure 7-113 *Molecular Biology of the Cell* (© Garland Science 2008)

# 7-8 bases at 5’ end of mature miRNA bind to target sites in mRNA

This is the “seed”



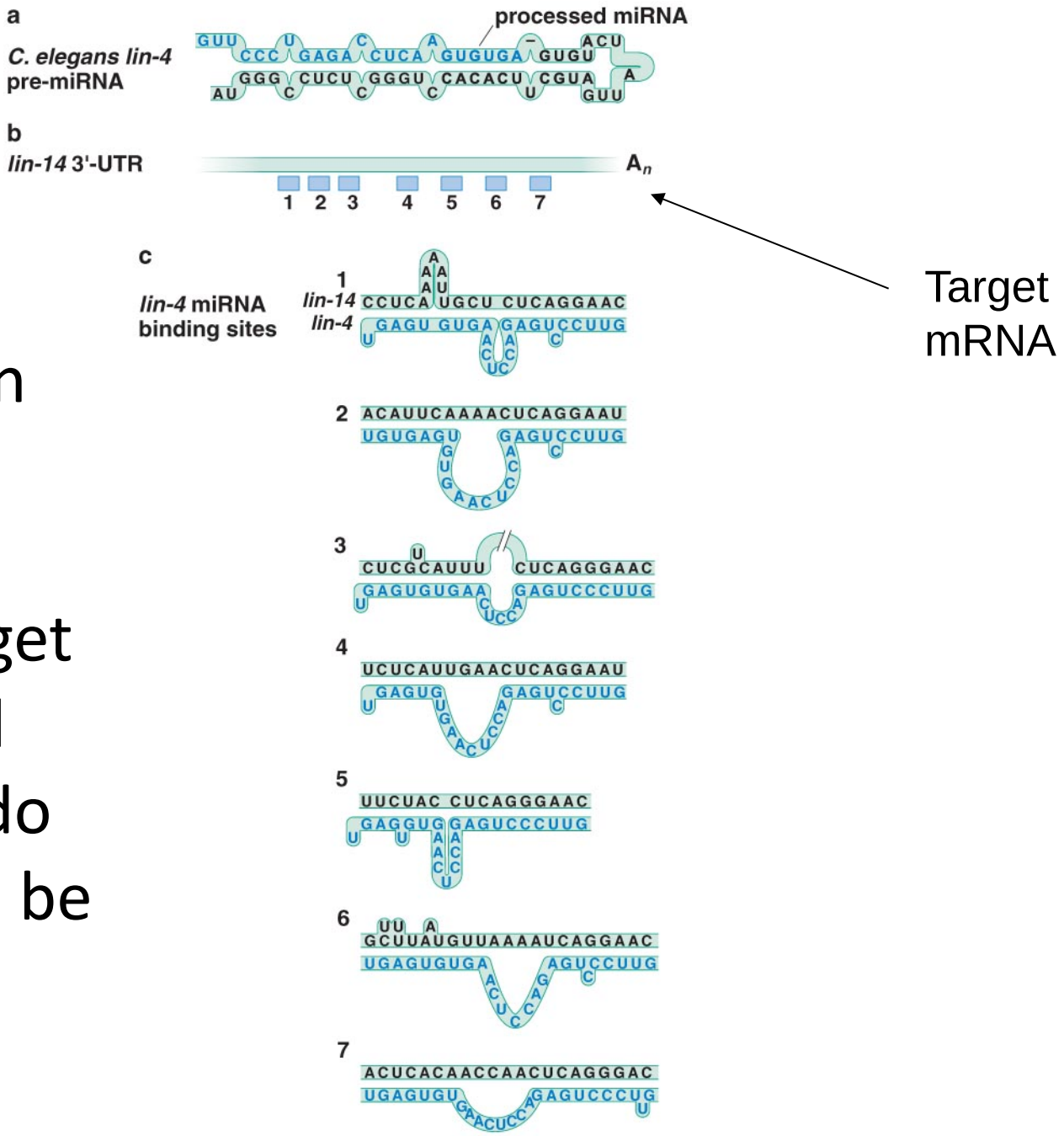

*Bartel, Cell, 2009*

# Targeting of miRNAs: pleiotropic & redundant

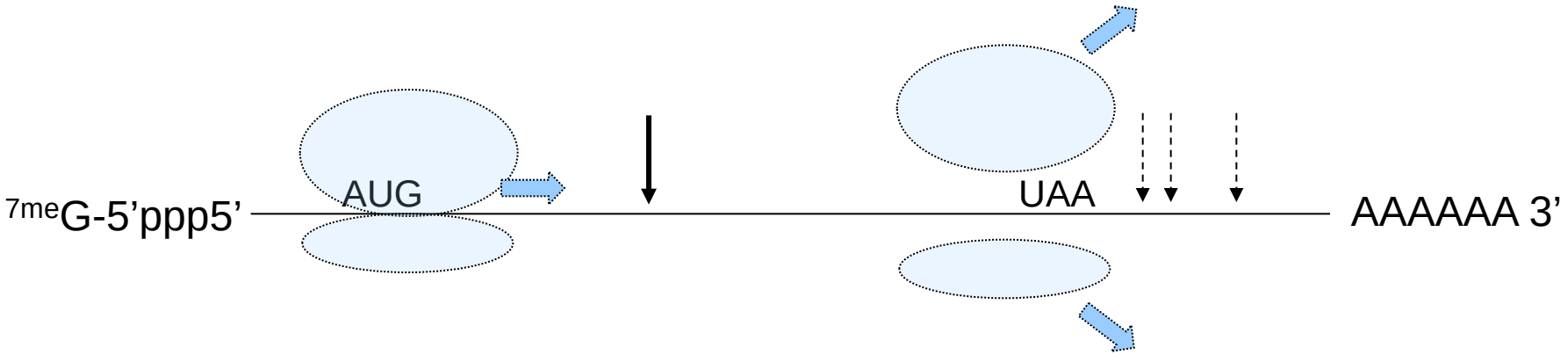
(many targets/effector) (many effectors/target)

- One miRNA can have “seed matches” in 3’-untranslated regions of ***hundreds*** of mRNAs
- miRNA families of many members with similar target specificities: encoded in different loci
- Complex control, as with transcription factors…but with differences
  - Primarily “OR” logic for inhibition
  - rather than “AND” logic for stimulation

Target can contain multiple miRNA target sites and duplexes do not need to be precise



# Targets for miRNA can have complementary sites in different places on the mRNA




- Evolutionary conservation of miRNA target sites in same genes across species imply that miRNA control is ***valuable***
- Target sequences in 3' untranslated region upstream of poly(A): often multiple targets, "seed" only
- Targets in body of open reading frame (often seen in plants) are perfect matches: maybe ribosome can displace weaker match

# Different results for mRNA target depend at least partly on extent of hybridization to small regulatory RNA

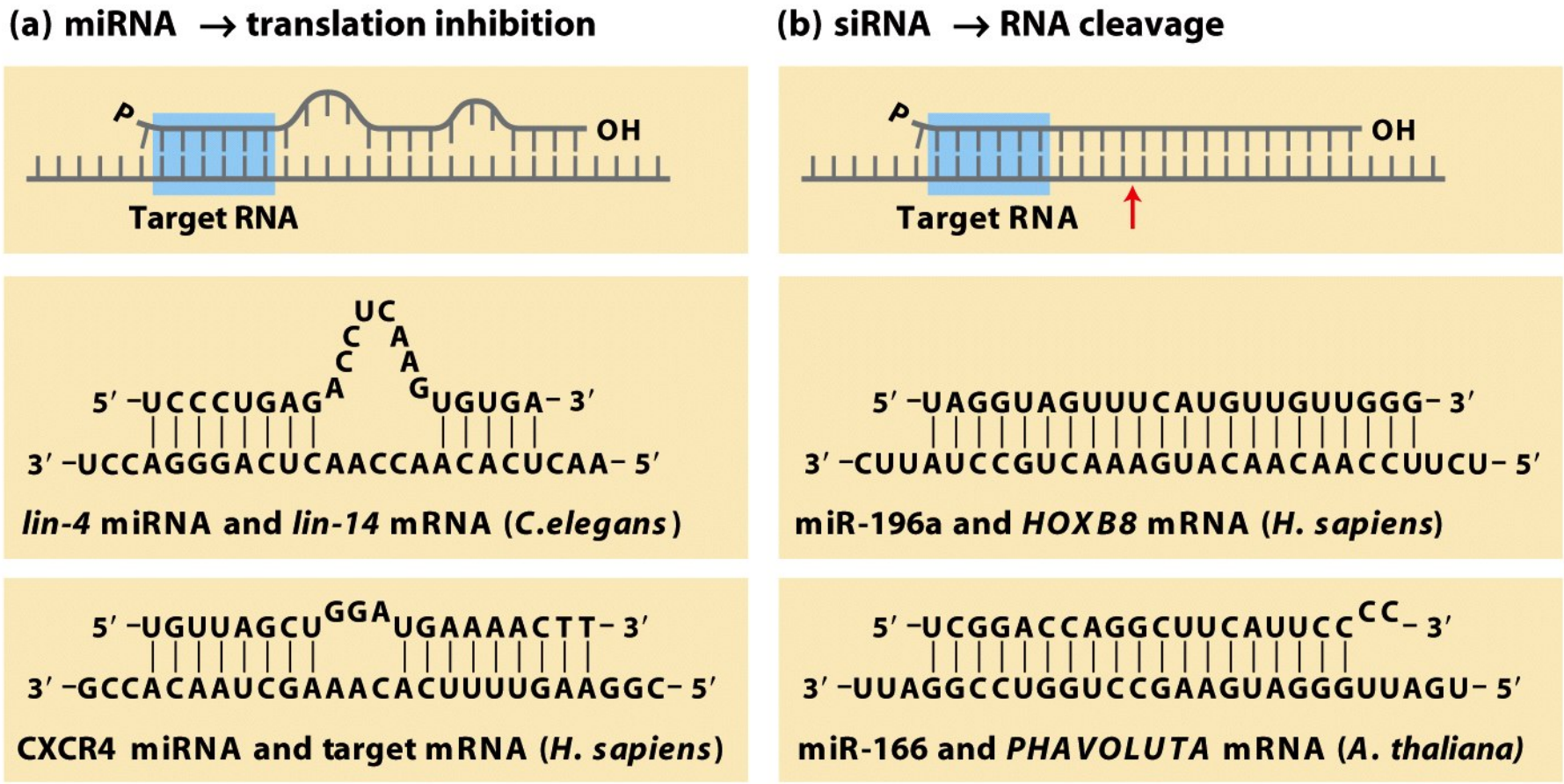
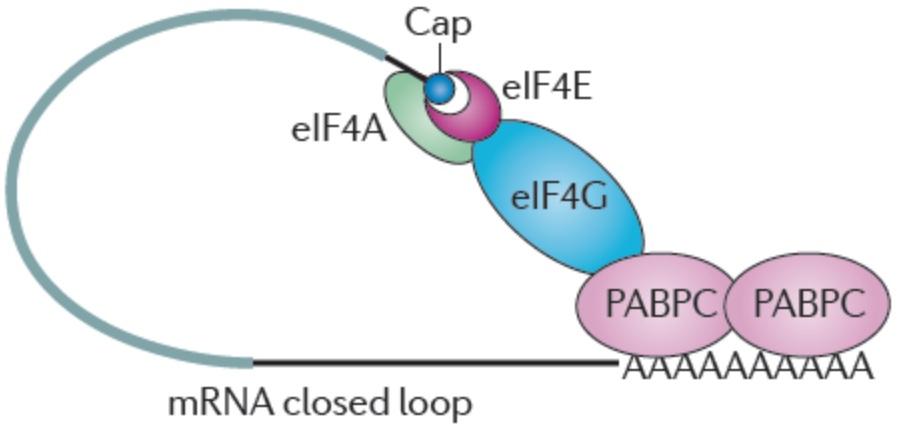



**Figure 8-25**
*Molecular Cell Biology, Sixth Edition*

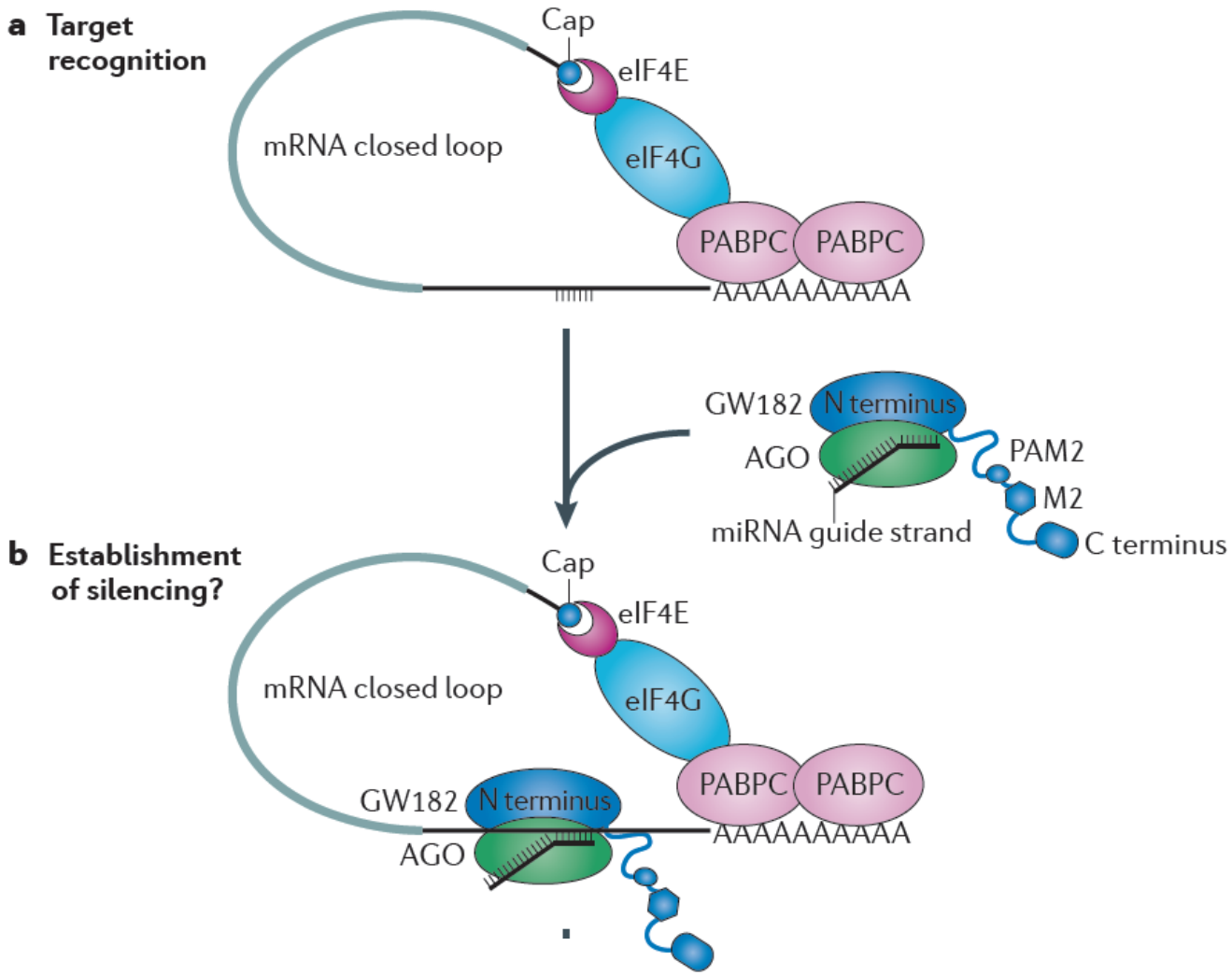

Perfect duplexes are THE RULE if small RNA in RISC complex was processed from a viral replication intermediate

# How to think about roles of miRNAs in translational arrest vs. mRNA decay: back to 5' & 3' end QC mechanism for translation



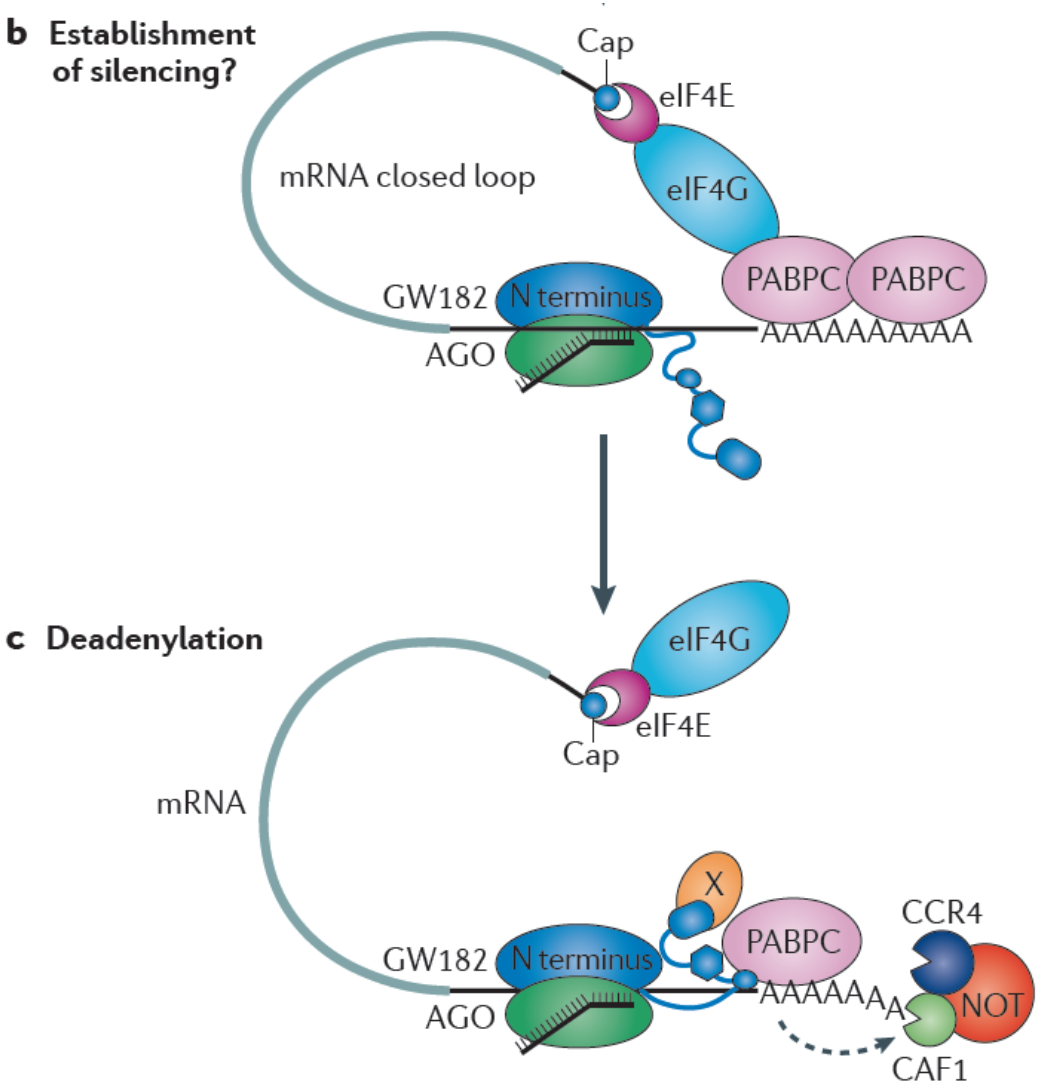

*(from Huntzinger & Izaurralde, 2011, Nat. Rev. Genet.)*

# Translational inhibition starts with “seed” hybridization – miRNA to mRNA – in cytoplasm




*(from Huntzinger & Izaurralde, 2011, Nat. Rev. Genet.)*

# One way to stop translation is to cause removal of poly(A) from mRNA

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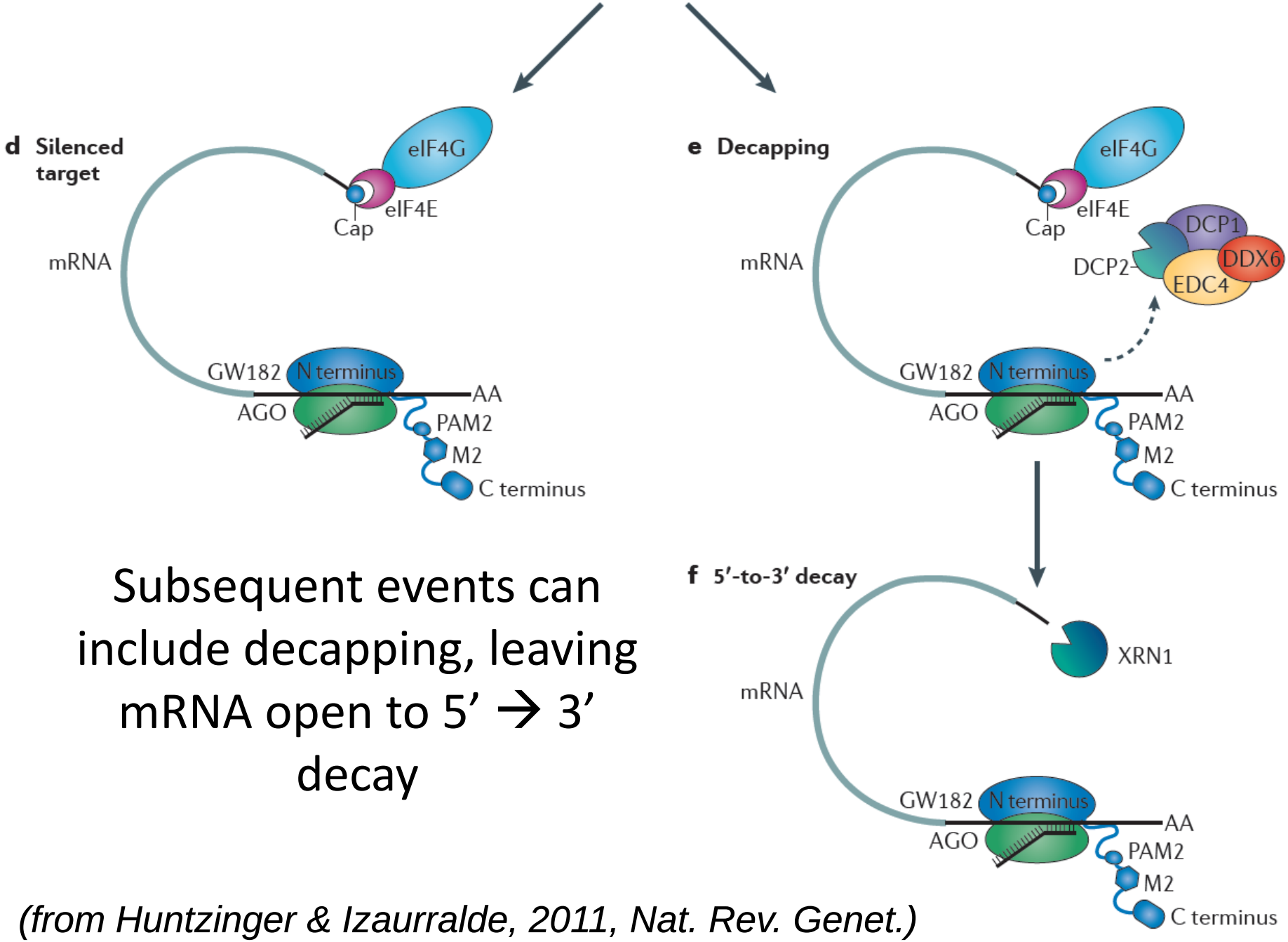



Subsequent events can include decapping, leaving mRNA open to 5' → 3' decay

*(from Huntzinger & Izaurralde, 2011, Nat. Rev. Genet.)*

# Other mechanisms are possible…
# but all these pathways can eventually lead to destruction of mRNA too

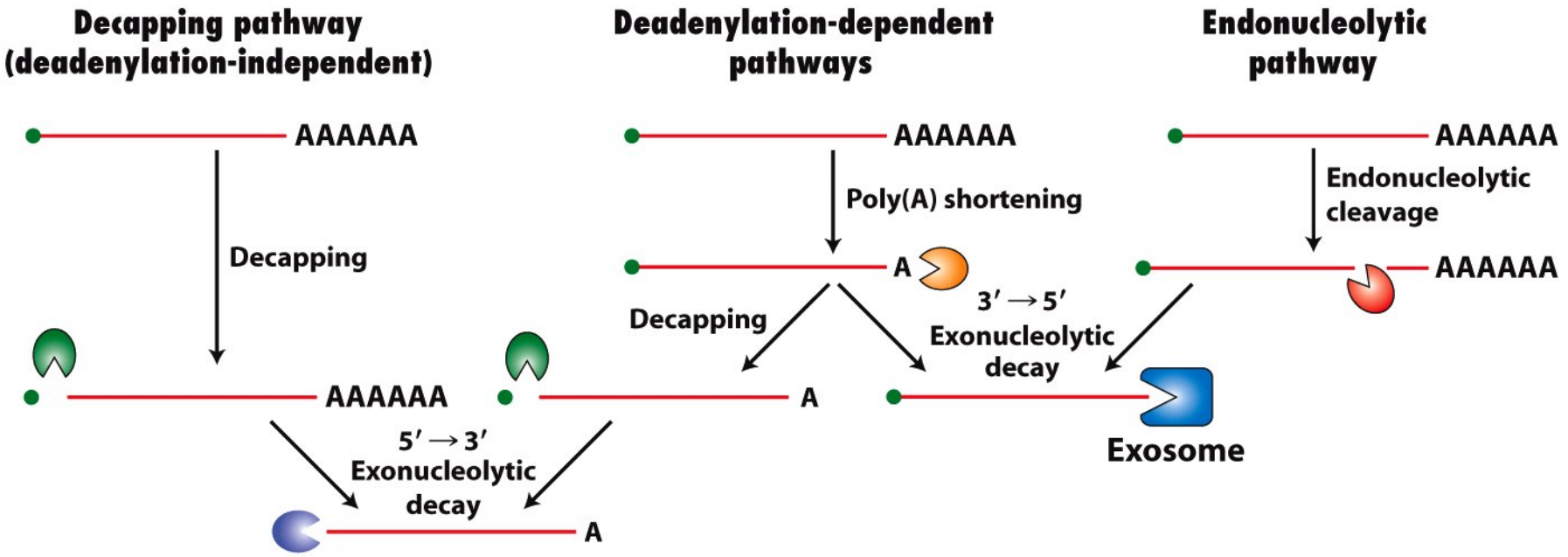
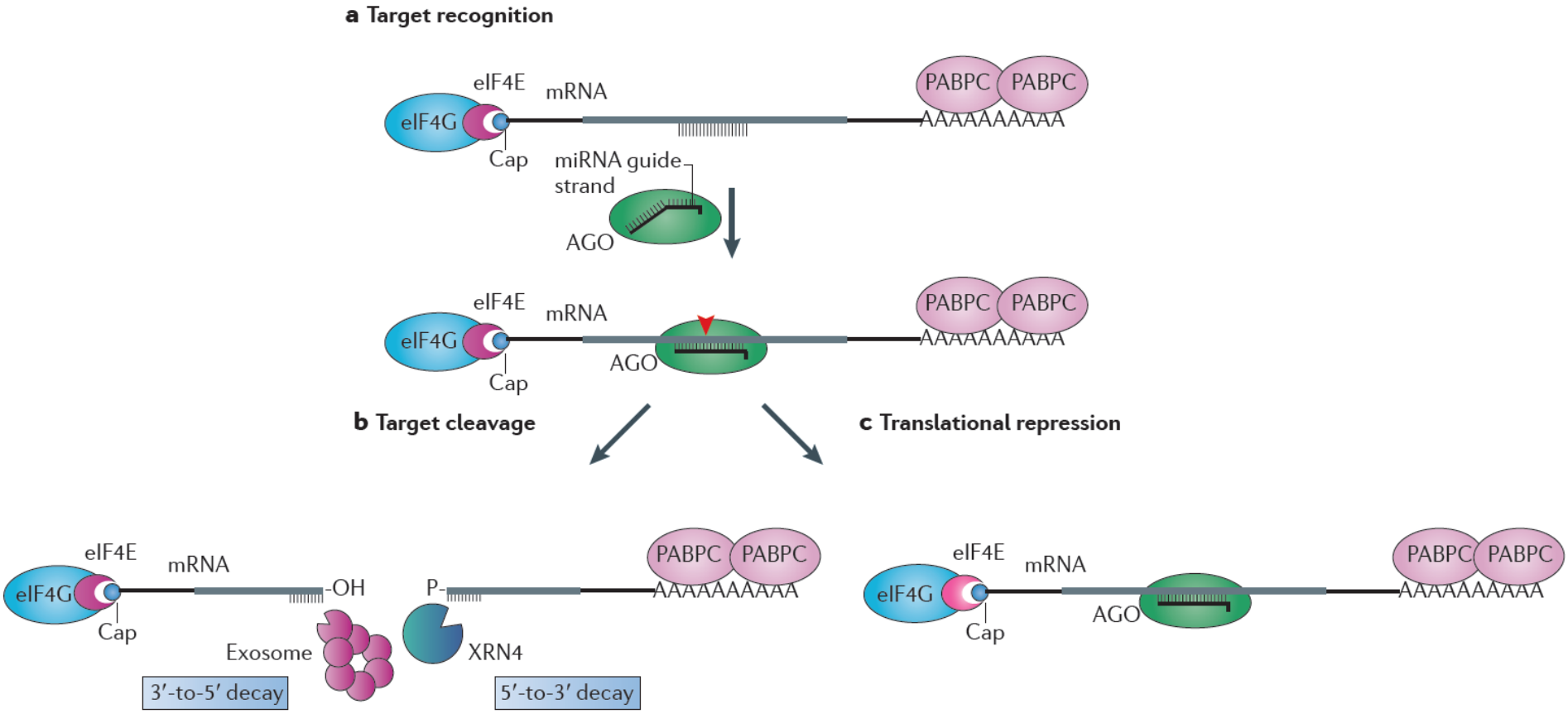



Figure 8-29
*Molecular Cell Biology, Sixth Edition*

# Outcome when miRNA is perfectly paired across whole length is more likely to be mRNA destruction



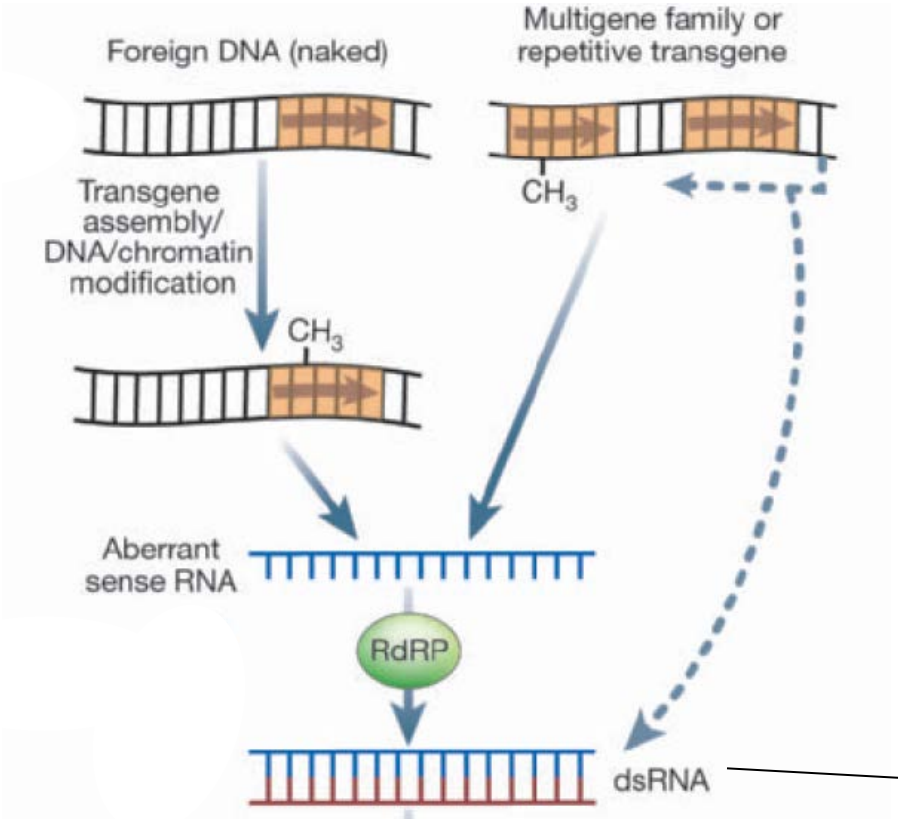

Common in “nonpathological” examples from plants but also in responses to viruses or RNA from parasitic DNA repeats

# dsRNA: problem and solution

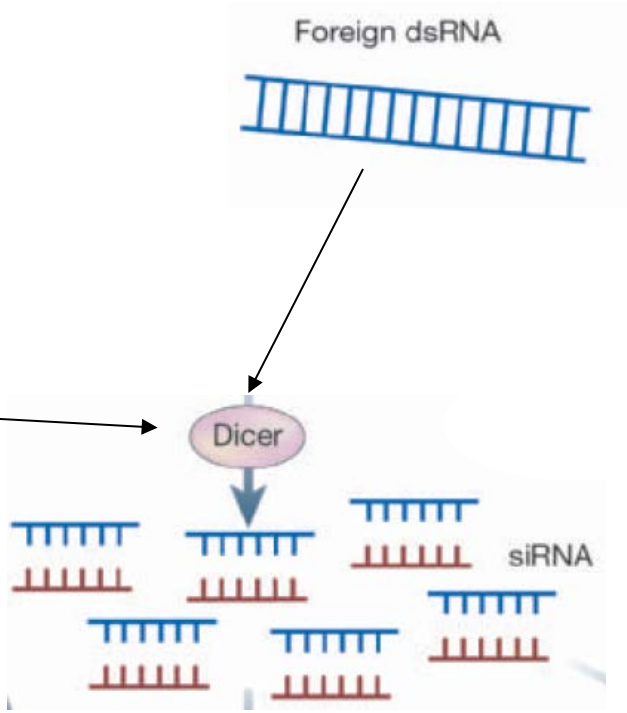
- Normal cells have dsDNA and ssRNA
- Viruses can be a source of dsRNA
- Aberrant transcription across genes in antisense as well as sense orientation can be source of dsRNA
- Multicellular organisms have evolved to detect *long* dsRNA and trigger violent responses: gross translation arrest, suicide

- But RNA complementarity can be useful for cell's regulation
- By processing *desirable* dsRNA to small pieces, can keep it large enough to be specific but small enough to avoid threat

# Pathological intermediates make near-perfect duplexes which can be processed to siRNA




Or… from viral replication intermediate

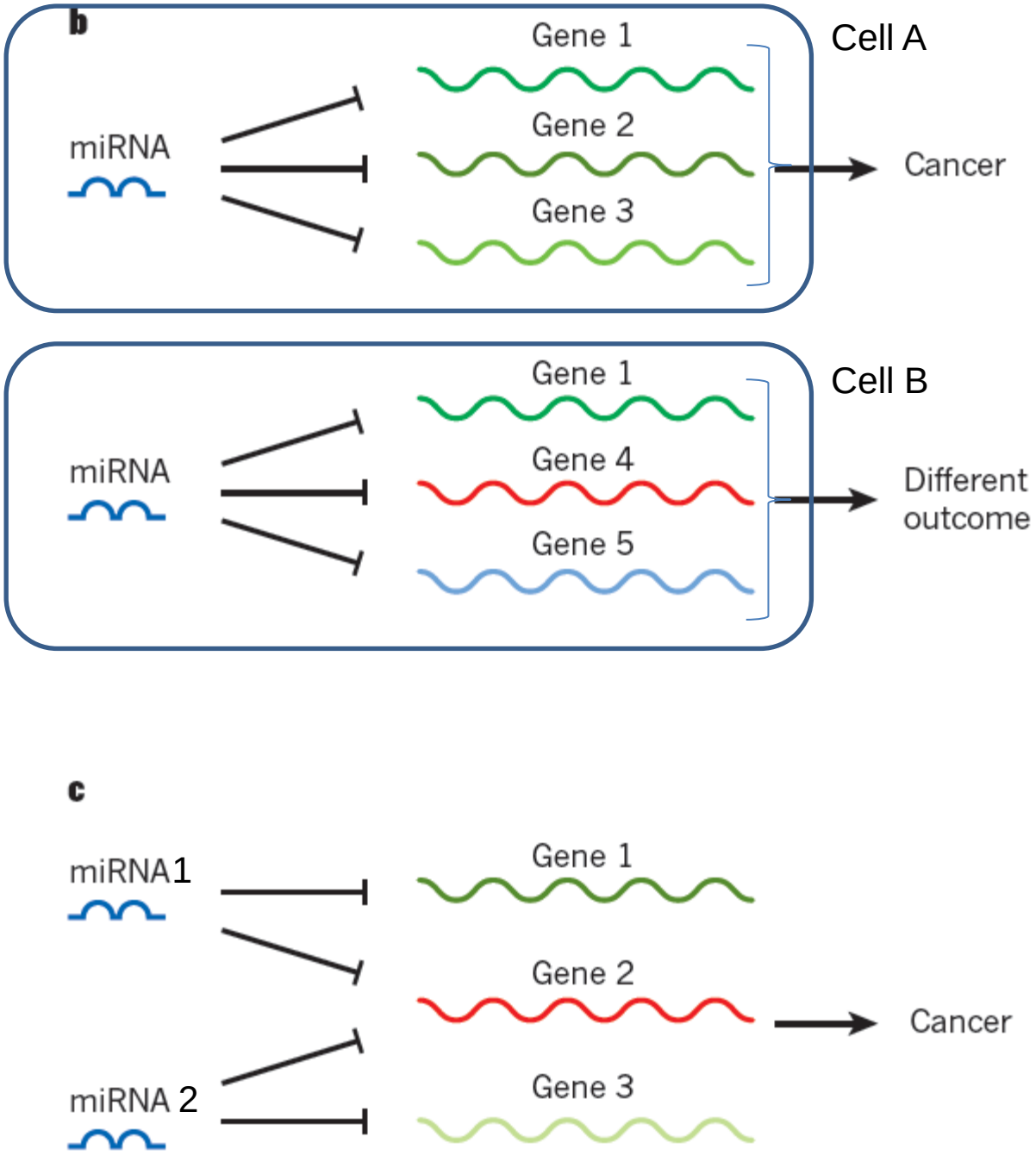



…and these can target their own template strands!

*Mello, Conte 2004 Nature "Insight"*

Processing of an individual mRNA transcript can affect whether or not it can be a target of a particular type of microRNA

(must be transcribed)
(must be processed to include target sequence)
(can be different for different RNAs from same transcription unit in same cell)




Effect of a microRNA on cell biology can depend on the target mRNAs and other microRNAs the cell is expressing

(Lujambio & Lowe, Nature 2012)

# microRNAs vs. transcription factors

- Both kinds of regulators have multiple targets
  - Single TFs participate in regulating multiple genes (activating and/or repressing)
  - Single microRNAs participate in reducing expression of proteins from multiple mRNAs
- Both are expressed in controlled ways
  - Both TF coding mRNA and microRNAs are made by RNA pol II
  - Both are expressed under control of cell-type and physiologically regulated transcription factors
- But:
  - Transcription factors can ***turn on*** genes that are not expressed in the cell before
  - microRNAs can only regulate mRNAs that are already being expressed in the cell