

Transcription regulation (chapters 18, 19)

Regulatory RNAs, RNAi (chapter 20)

LMR05.001 – 19
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Transcriptional regulation

There are various stages at which expression of a gene can be regulated:

- The **most** common is **transcription initiation**
- The mechanisms of transcriptional regulation that operate at steps after initiation, **specifically during elongation and termination.**
- Prokaryotic gene regulation at the **level of translation**
- Different **regulatory RNAs**

Transcriptional regulation

- What controls gene expression?
 - Extracellular, intracellular and intercellular signals
 - Regulatory proteins
- Regulatory DNA-binding proteins
 - Activators
 - Repressors

Transcription initiation regulation in prokaryotes

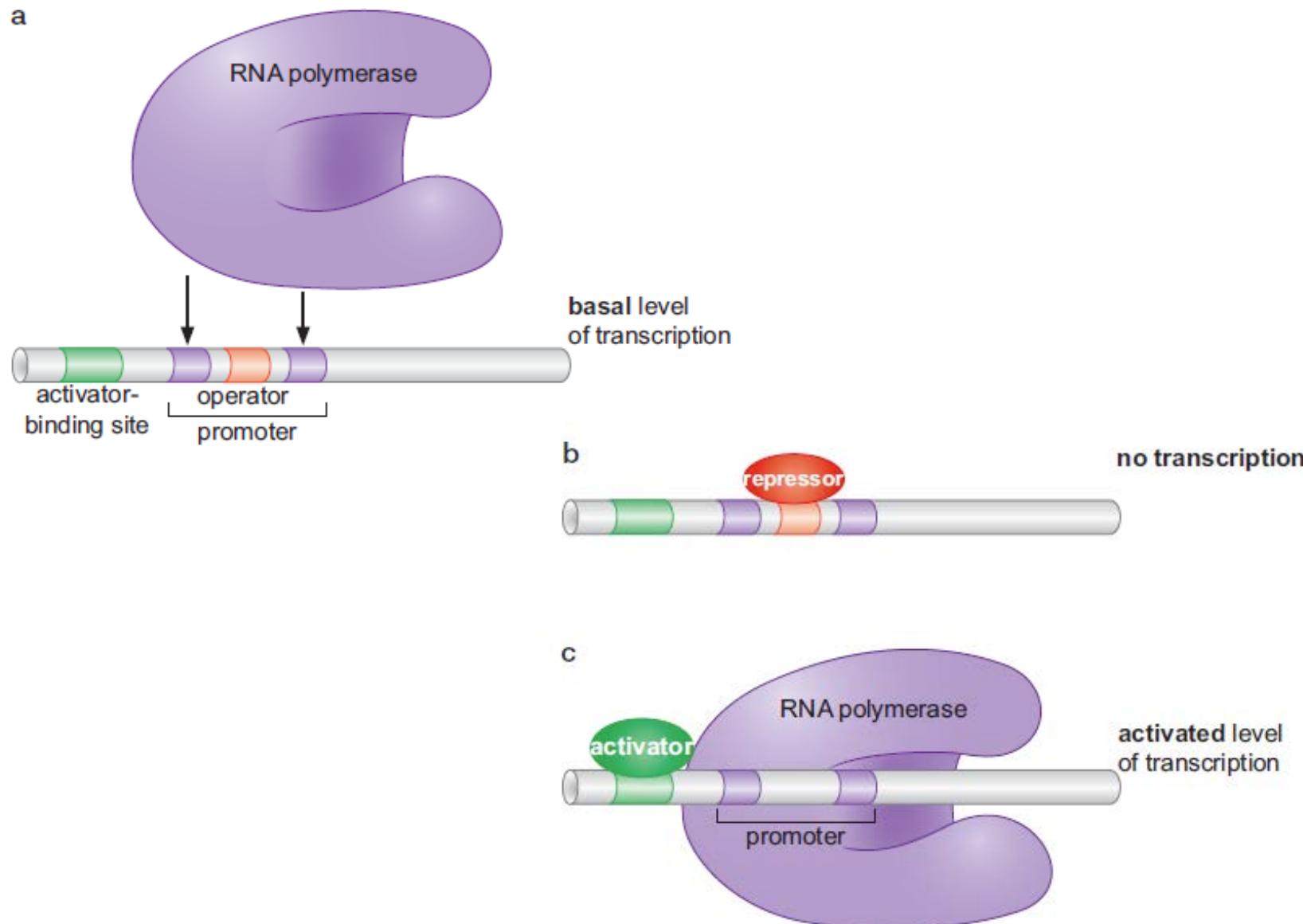


FIGURE 18-1 Activation by recruitment of RNA polymerase. (a) In the absence of both activator and repressor, RNA polymerase occasionally binds the promoter spontaneously and initiates a low level (basal level) of transcription. (b) Binding of the repressor to the operator sequence blocks binding of RNA polymerase and so inhibits transcription. (c) Recruitment of RNA polymerase by the activator gives high levels of transcription. RNA polymerase is shown recruited in the closed complex (see Fig. 13-3). It then spontaneously isomerizes to the open complex and initiates transcription. If both the repressor and activator are present and functional, the action of the repressor typically overcomes that of the activator. (This case is not shown in the figure.)

Are regulatory molecules only proteins?

- Not only proteins, **also RNA!**
- microRNAs – discovered in 1990s
RNA interference (RNAi) – discovered in late 1990s - Craig Mello & Andrew Fire, 1998
- siRNA is also similar to miRNA
miRNAs are derived from shorter stemloop RNA products, typically silence genes by repression of translation, and have broader specificity of action, while **siRNAs typically work by cleaving the mRNA before translation**, and have 100% complementarity, thus very tight target specificity

Regulatory RNAs in eukaryotes

- Between 30-70% of genes are regulated by RNAs
- Short RNA regulators
 - Inhibit translation
 - Guide degradation of mRNA
 - Transcriptionally silence the promoter by recruiting chromatin remodelers
- Long RNA regulators
 - long non-coding RNAs (lncRNAs)

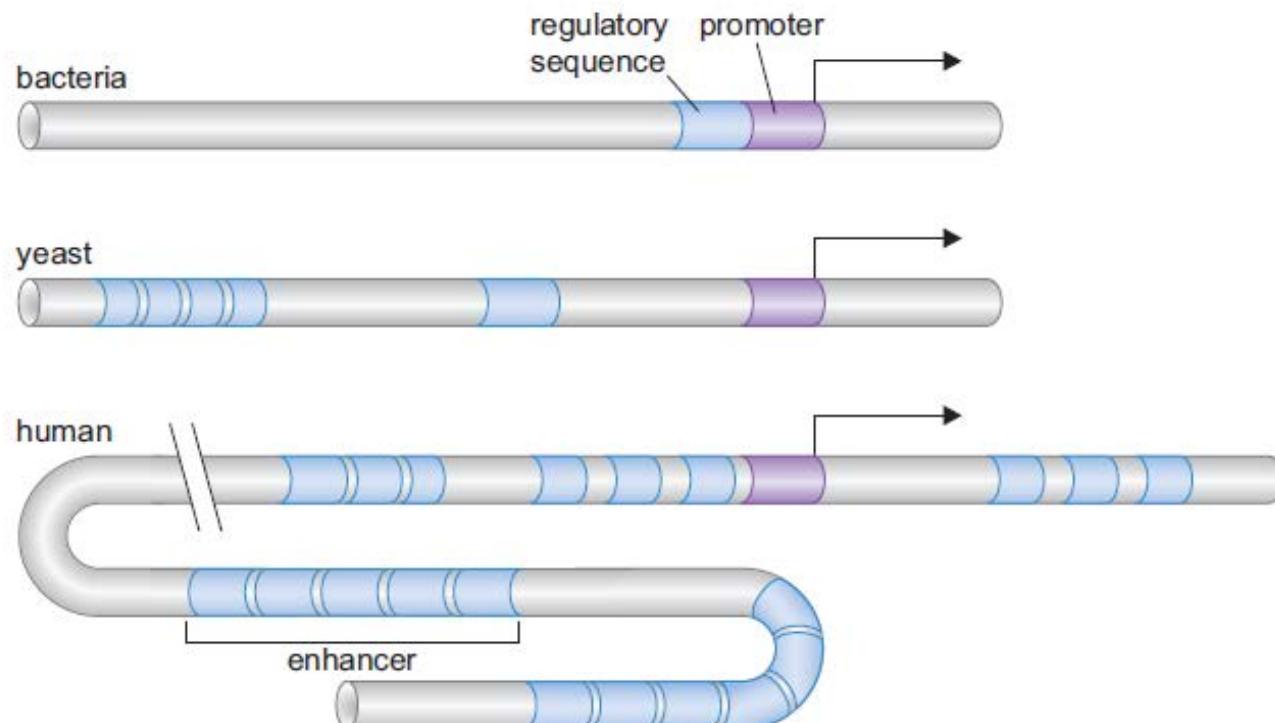
Short regulatory RNAs in eukaryotes

- small interfering RNAs (siRNAs)
 - Produced *in vivo* from dsRNA precursors
- microRNAs (miRNAs)
 - Derived from precursor RNAs encoded by genes expressed in cells where those miRNAs have regulatory roles
- piwi-interaction RNAs (piRNAs)
 - Expressed in germline, distinct from miRNAs

Transcription regulation in eukaryotes

- Two major extra complexities to account for
 - Histones and nucleosomes
 - A problem not faced in bacteria
 - More ways to regulate
 - More regulators and more extensive regulatory sequences
 - Signal integration
 - Enhancers – control expression at a given time and place
 - Insulators – block activation of the promoter
 - Repressors – gene activiti is down-regulated

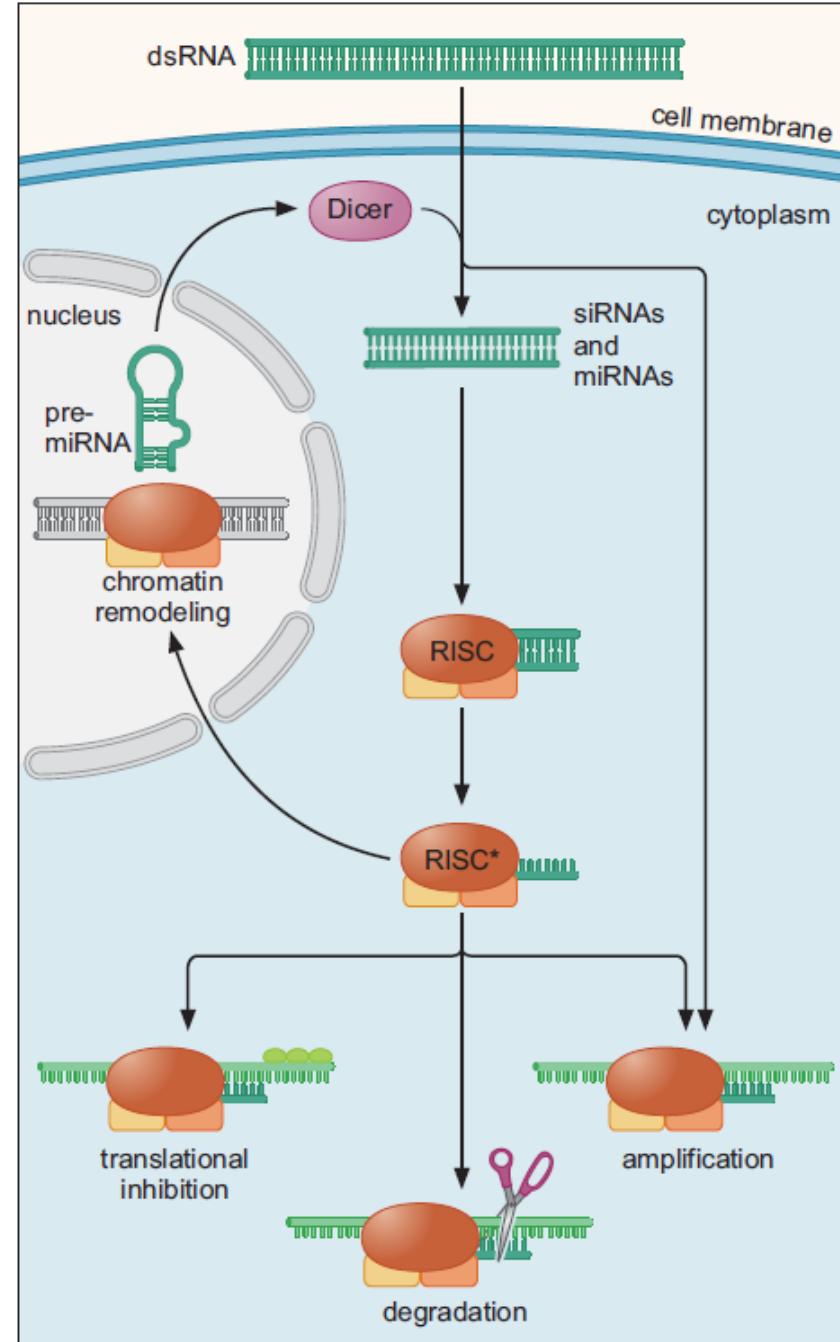
FIGURE 19-1 The regulatory elements of bacterial, yeast, and human genes. Illustrated is the increasing complexity of regulatory sequences from a simple bacterial gene controlled by a repressor to a human gene controlled by multiple activators and repressors. In each case, a promoter is shown at the site where transcription is initiated. Although this is accurate for the bacterial case, in the eukaryotic examples, transcription initiates somewhat downstream from where the transcription machine binds (see Chapter 13). Some groups of regulatory binding sites in the human regulatory sequences represent enhancers, as shown in one case.



How are siRNAs and miRNAs produced?

- miRNA/siRNA
 - 21-23 nt
(19-25 nt)
- piRNA
 - 24-34 nt
- Dicer, RNase III-like enzyme

FIGURE 20-10 Generation of siRNAs and miRNAs, and their mode of action. Processing of dsRNA to make siRNAs and pre-miRNAs to make miRNAs by the enzyme Dicer. Another enzyme involved only in the generation of pre-miRNAs—Drosha—is not shown here but is described later. The siRNAs and miRNAs direct a complex called RISC (RNA-induced silencing complex) to repress genes in three ways. It attacks and digests mRNA that has homology; it interferes with translation of those mRNAs; or it directs chromatin-modifying enzymes to the promoters that direct expression of those mRNAs (Fig. 20-18). By recruiting an RNA-dependent RNA polymerase, siRNAs can generate more double-stranded RNA as fodder for Dicer to make more siRNA. This is the “amplification” step shown on the right and in more detail in Figure 20-11. (Adapted, with permission, from Hannon G.J. 2002. *Nature* 418: 244–251, Fig. 5. © Macmillan.)



Function of RNAi

Gene-censoring mechanisms

To protect plants, animals, fungi against viruses and mobile genetic element

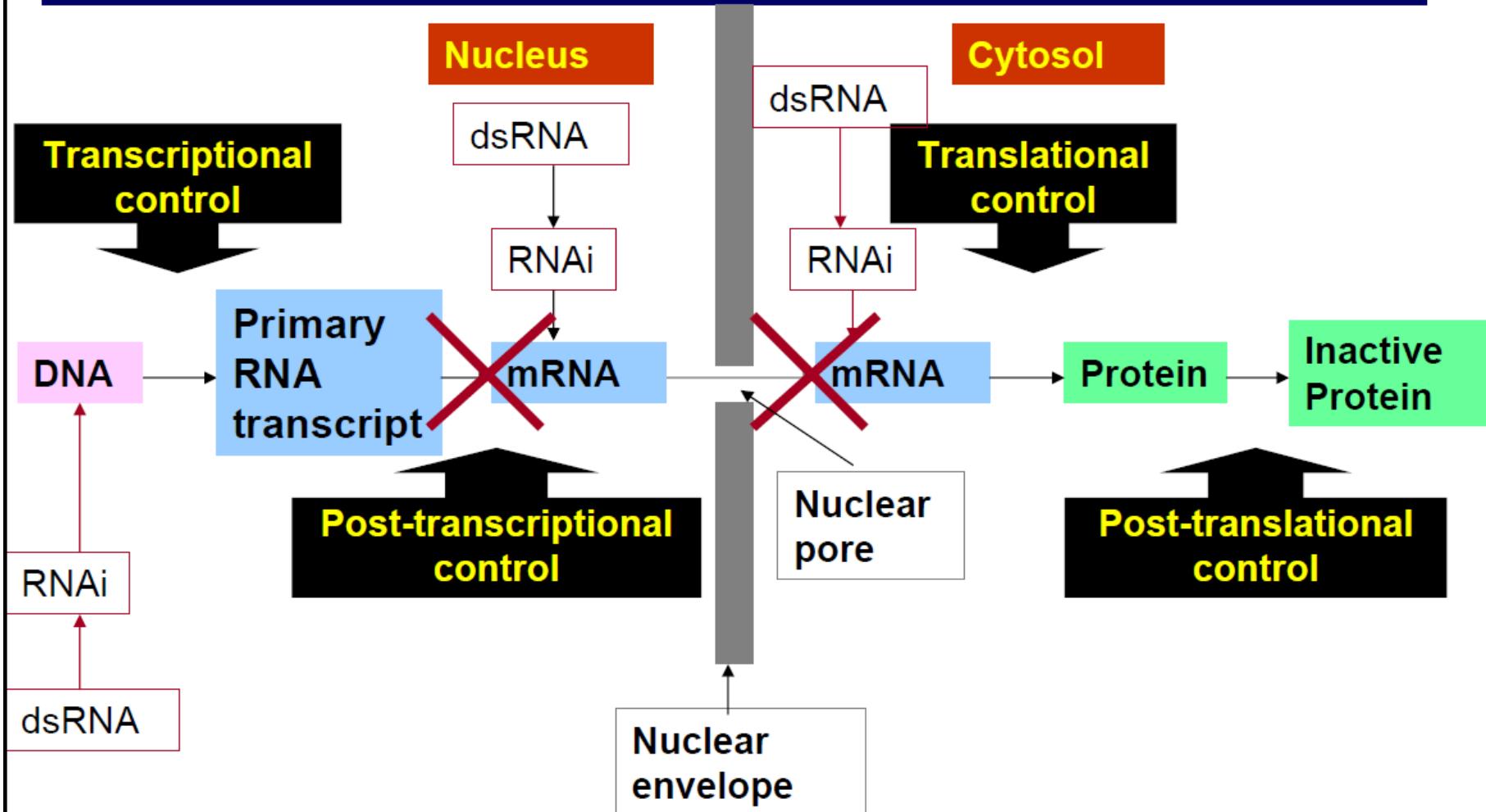
Help silence normal cellular genes:

- during developmental transitions, required for cell differentiation (e.g. into brain, heart, nerve cells, muscle cells, skin, etc.)
- Transposons

Genes are turned 'ON' or 'OFF' during development and as the organ makers

RNAi

Post-transcriptional Gene Silencing (PTGS)





- Fire, A. *et al.* Potent and specific genetic interference by double stranded RNA in *Caenorhabditis elegans*. *Nature* **391**, 806–811 (1998).



Photo: L. Cicero

Andrew Z. Fire



Photo: J. Mottern

Craig C. Mello

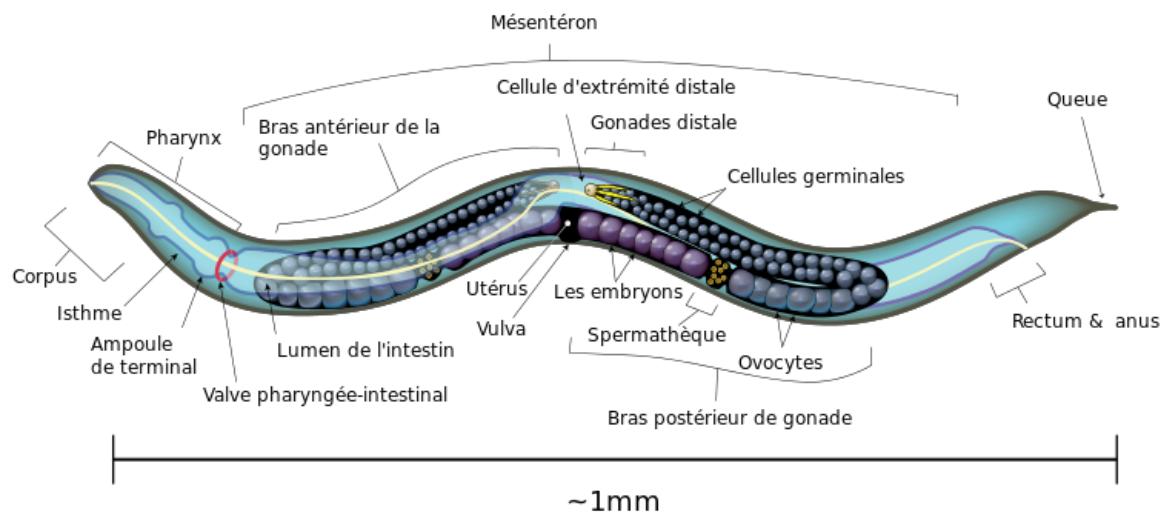
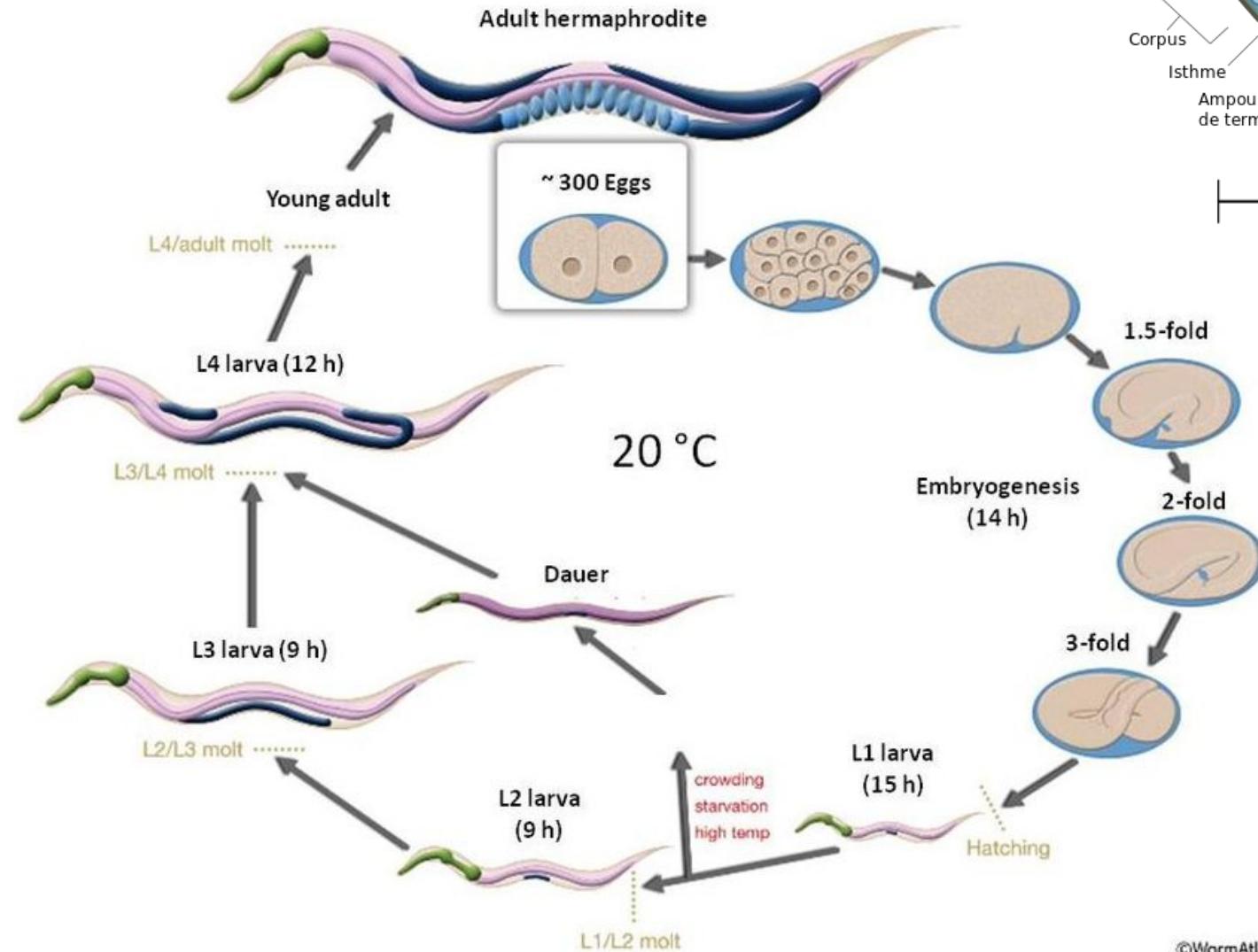
C. elegans – roundworm is a free-living, transparent nematode, about 1 mm in length, that lives in temperate soil environments.



<https://upload.wikimedia.org/wikipedia/commons/b/be/CrawlingCelegans.gif>

This Nobel Prize-winning landmark paper provides the first description of the phenomenon of RNAi.

Caenorhabditis elegans



1 male : 100 hermaphrodites
hermaphrodite has 959 somatic cells
and the male has 1033 cells

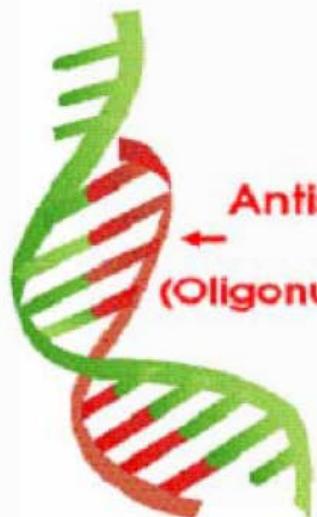
In the nematode *C. elegans*, more than 12 genes have been identified that function in the **apoptotic killing** and elimination of **131 of the 1090** cells that are generated during hermaphrodite development.

Guo and Kemphus used an antisense RNA to specifically inactivate a gene called *par-1* (controls symmetry).

↓
Antisense RNA (if present in large amounts) pairs up with mRNA to produce an RNA-RNA helix

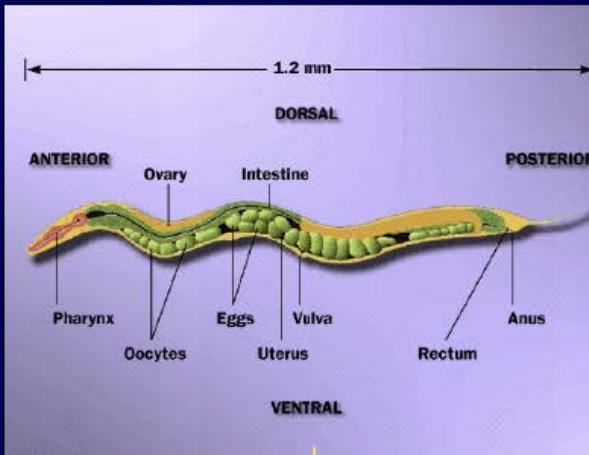
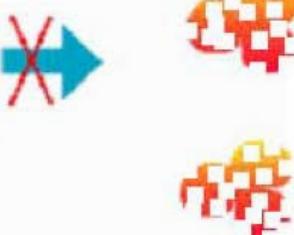
↓
Translation inhibition. (No gene expression). No muscle protein

mRNA



No Proteins

No Translation



↓
Twitching due to defect in muscles

Results of Experiments of Fire et al (1998)

Pure ss antisense RNA of *Unc-22* gene



C.elegans

Pure ss sense RNA of *Unc-22* gene



C.elegans

ds RNA corresponding to *Unc-22* gene (antisense/sense mixture)



C.elegans

Marginal loss of muscle function

Severe decrease in *Unc-22* activity (silencing)

Severe uncontrolled twitching and muscle defect in the worms

(Even a few molecules per cell were enough to produce severe twitching)

Other observations from the experiments on *C.elegans*

- RNAi phenomenon is very specific (only occurs if dsRNA sequence exists within the target gene).
- dsRNA must contain exonic sequences to produce the RNAi effect.
- dsRNA corresponding to various introns and promoter sequence do not produce RNAi.
- dsRNA-mediated interference could cross cellular boundaries (injection in body cavity, produced an effect throughout the organism).
- A specific amplification mechanism exists (as few as 2 molecules per cell can effectively induce silencing).
- Effect of RNAi is long lived and can be transmitted to future generations.
- RNAi is a reversible process.

Action in cell

Small interfering RNA (siRNA), sometimes known as short interfering RNA or silencing RNA, is a class of double-stranded RNA at first non-coding RNA molecules,

- **RNAi (RNA interference)** is RNA-depending gene silencing process
- **RNA interference (RNAi)** is a biological process in which **RNA molecules inhibit gene expression or translation**, by neutralizing targeted mRNA molecules.
- Historically, RNAi was known by other names, including *co-suppression*, *post-transcriptional gene silencing* (PTGS), and *quelling*.
- Important is **RISC (RNA induced silencing complex)**.
- **Small dsRNA molecules initiate the RNAi** in cytoplasm where they bind to the RISC catalytical component — with **argonaut**

Cis or trans?

- siRNA – *cis*
 - Typically generated by transcripts of the regions on which they act
- miRNA – *trans*
 - Encoded by a gene but act on other genes



siRNA

Gavrillo K, Saltzman WM. Therapeutic siRNA: principles, challenges, and strategies. *Yale J Biol med.* 2012 Jun;85(2):187-200.

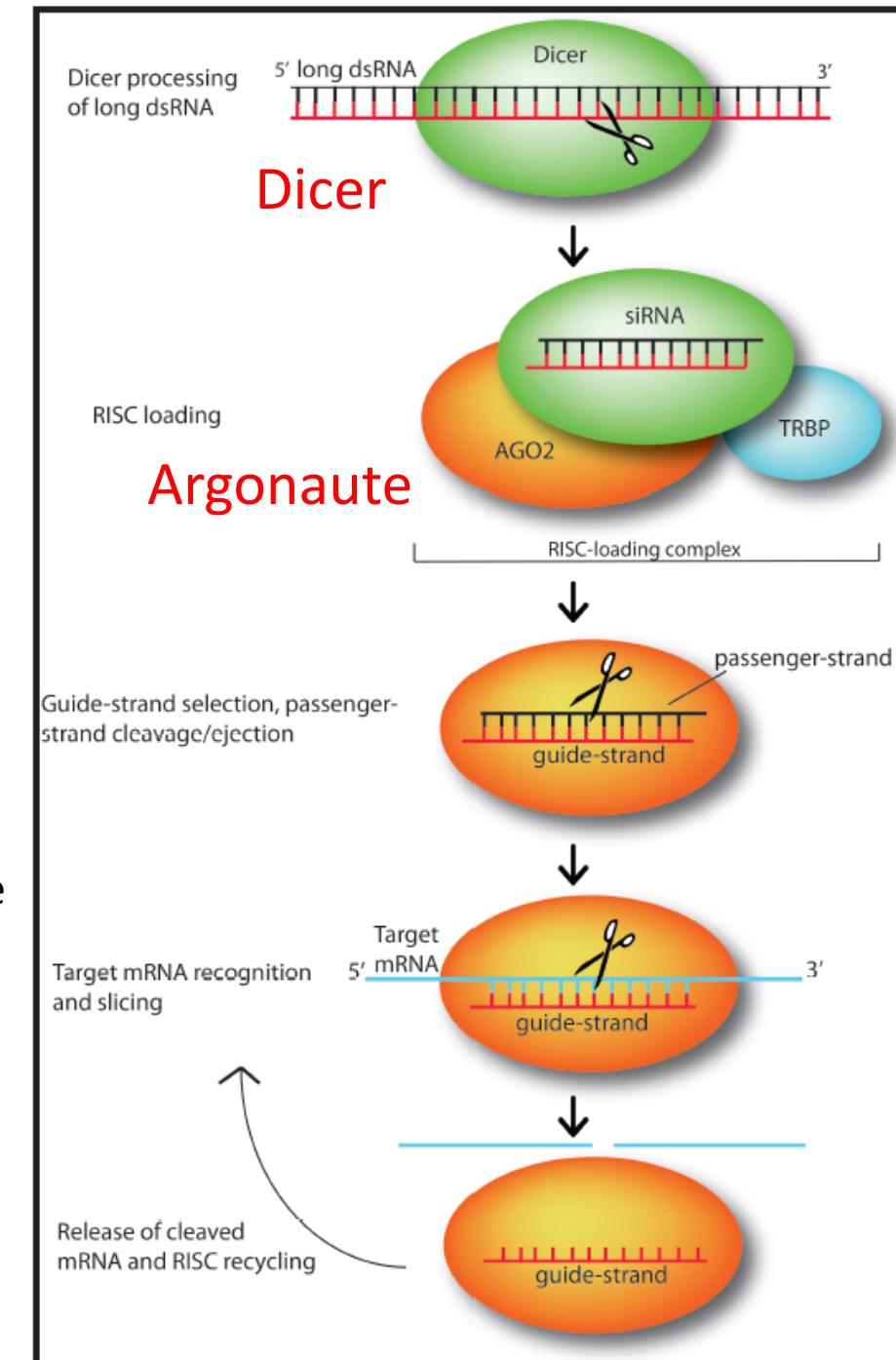
siRNA – short interference or small interfering RNA

They mediate silencing of target genes by guiding sequence
Dependent silicing of their target mRNAs

These non-coding, silencing RNAs begin as long **double-stranded RNA (dsRNA) molecules**, which are processed by **endonuclease Dicer** into short, active ~21-25 nt constructs. Once generated, a **siRNA duplex** is loaded by Dicer, with the help of **RNA-binding protein TRBP**, onto **Argonaute (AGO2)**, the heart of the RNA-induced silencing complex (which here is represented just by AGO2). upon loading,

AGO2 selects the siRNA guide strand, then cleaves and ejects the passenger strand.

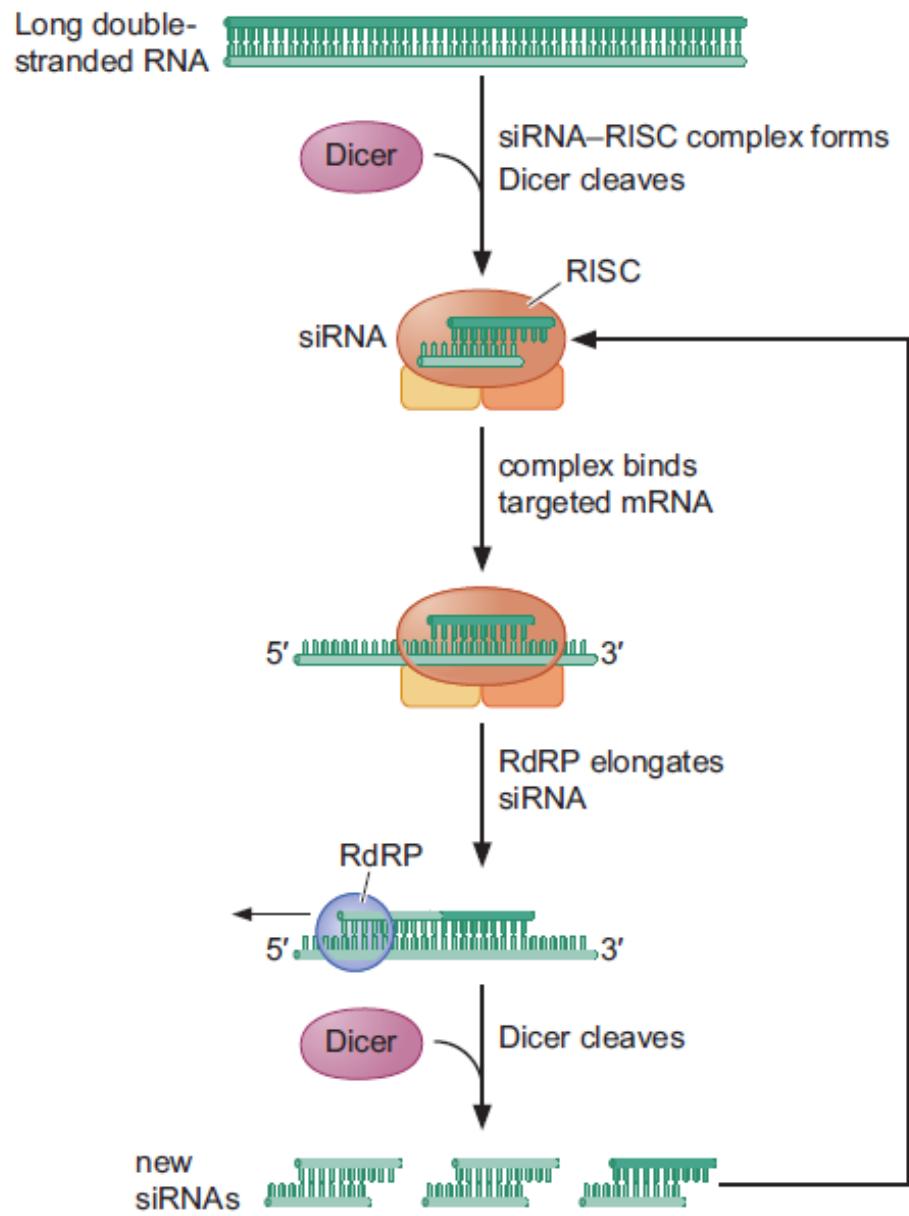
transactivation response RNA binding protein (TRBP)



Amplification of siRNA

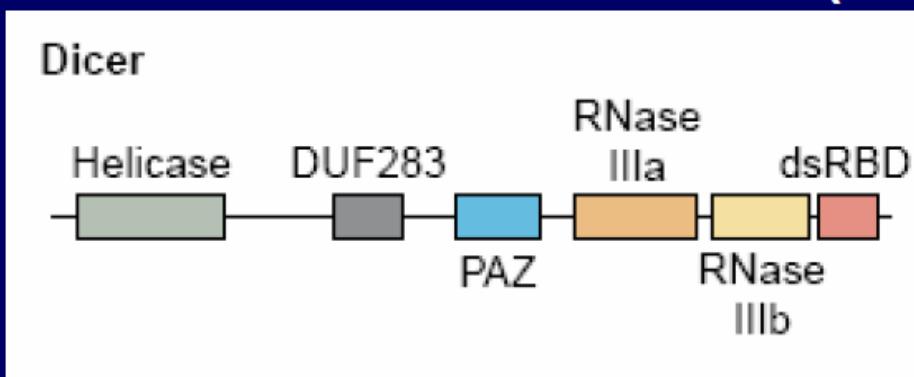
- RNAi silencing is highly efficient
 - RdRP – RNA-dependent RNA polymerase
 - *S. pombe*, not found in mammals

FIGURE 20-11 Amplification of the siRNA signal by RdRP. As shown on the right in Figure 20-10, the siRNA signal can be amplified, generating more dsRNA for Dicer to process into more siRNAs. This is achieved because the siRNA–RISC complex can recruit an enzyme, RNA-dependent RNA polymerase to the targeted RNA, and the siRNA acts as a primer for that enzyme to transform the target into dsRNA, which can itself then be acted on by Dicer. RdRPs are found in plants, worms, and the yeast *Schizosaccharomyces pombe* (*Saccharomyces cerevisiae* does not have the RNAi machinery at all), and we will see the importance of this amplification step in the case of centromere silencing in *S. pombe* (Fig. 20-18).



Domain structure of Dicer

- Dicer—multi domains protein; ~200kDa protein
 - ATP dependent RNA helicase for unwinding dsRNA
 - PAZ (Piwi, Argonaute and Zwille) for protein-protein interactions/RNA binding
 - Two RNase III domains for digesting one strand of RNA
 - dsRNA binding domain (dsRBD).
 - A domain of unknown function (DUF283)

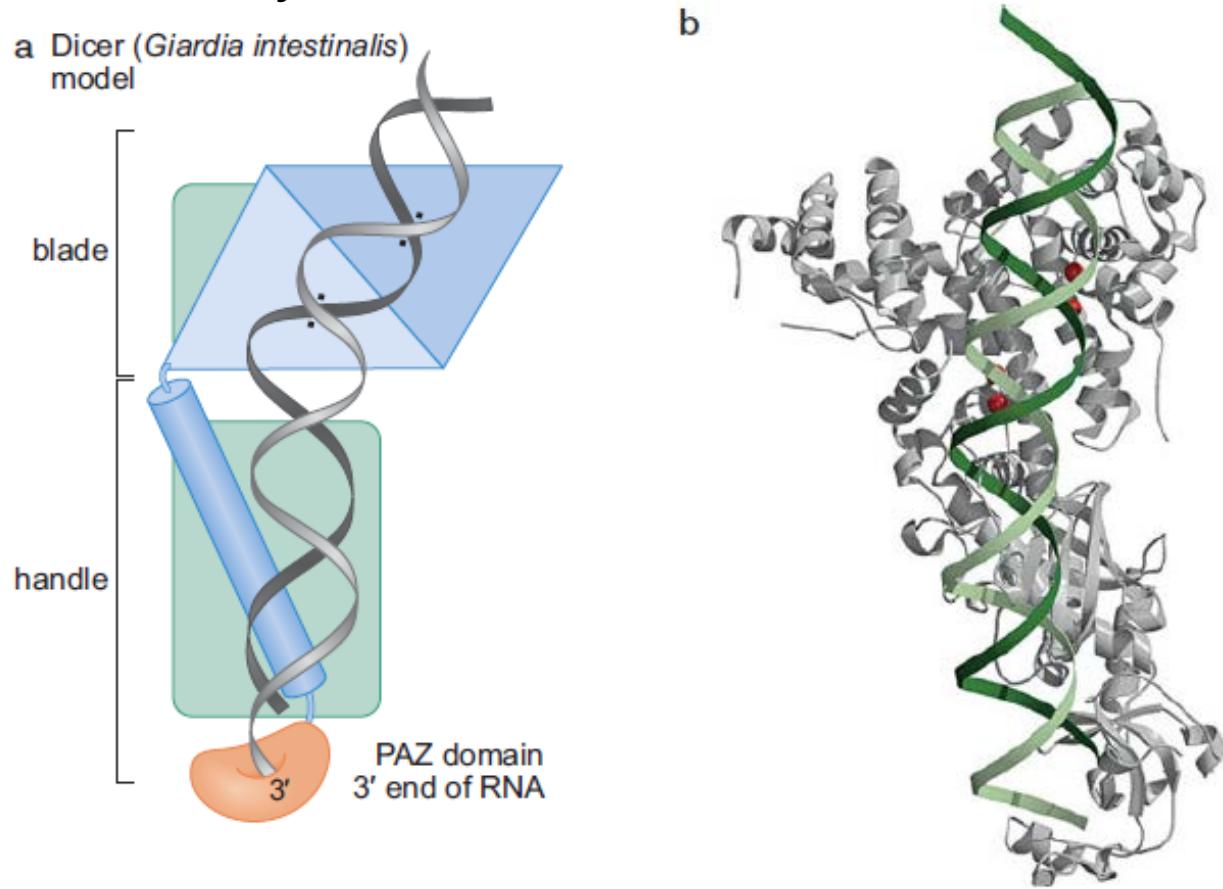


Dicer

evolutionarily conserved PAZ (Piwi/Argonaute/Zwille) domain
The structure consists of a left-handed, six-stranded β -barrel capped at one end by two α -helices and wrapped on one side by a distinctive appendage, which comprises a long β -hairpin and a short α -helix. Using structural and biochemical analyses, we demonstrate that the PAZ domain binds a 5-nucleotide RNA with 1:1 stoichiometry.

- Cleaves RNA ~22 nt from the end
 - PAZ defines length
- Sequence unspecific

FIGURE 20-16 Dicer structure and organization. (a) The scheme shows Dicer organization. (b) Dicer structure modeled with dsRNA reveals how length is measured. The protein is shown in gray, with nuclease active sites indicated by the red spheres (and as black dots in part a). The RNA is in green. The structure shown contains only the RNase III and PAZ domains. The Dicer protein also contains ATPase and other domains. (b, MacRae I.J. et al. 2006. *Science* 311: 195–198. PDB Code: 2FFL; note that the RNA was modeled into the structure and was not part of the crystal structure.) Image prepared with MolScript, BobScript, and Raster3D.



Argonaute

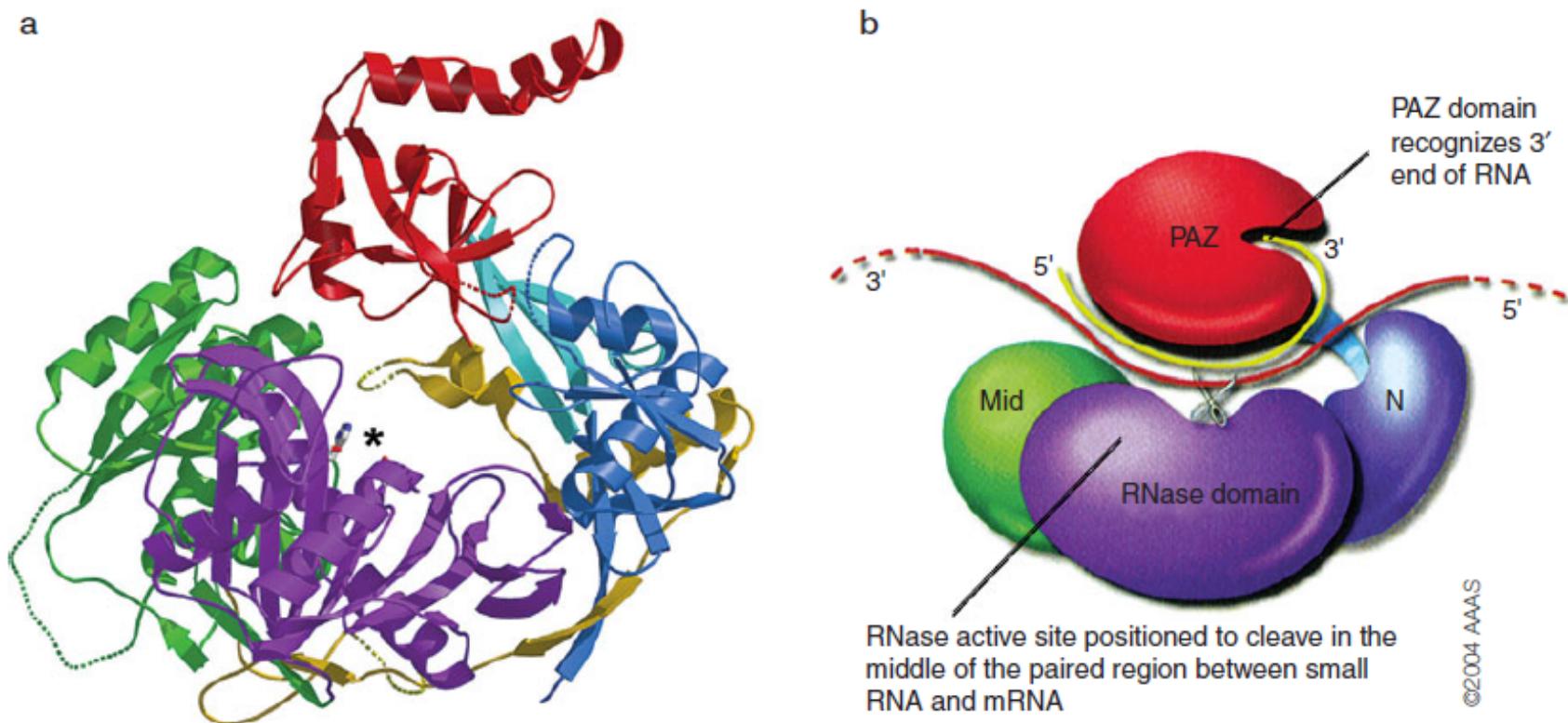


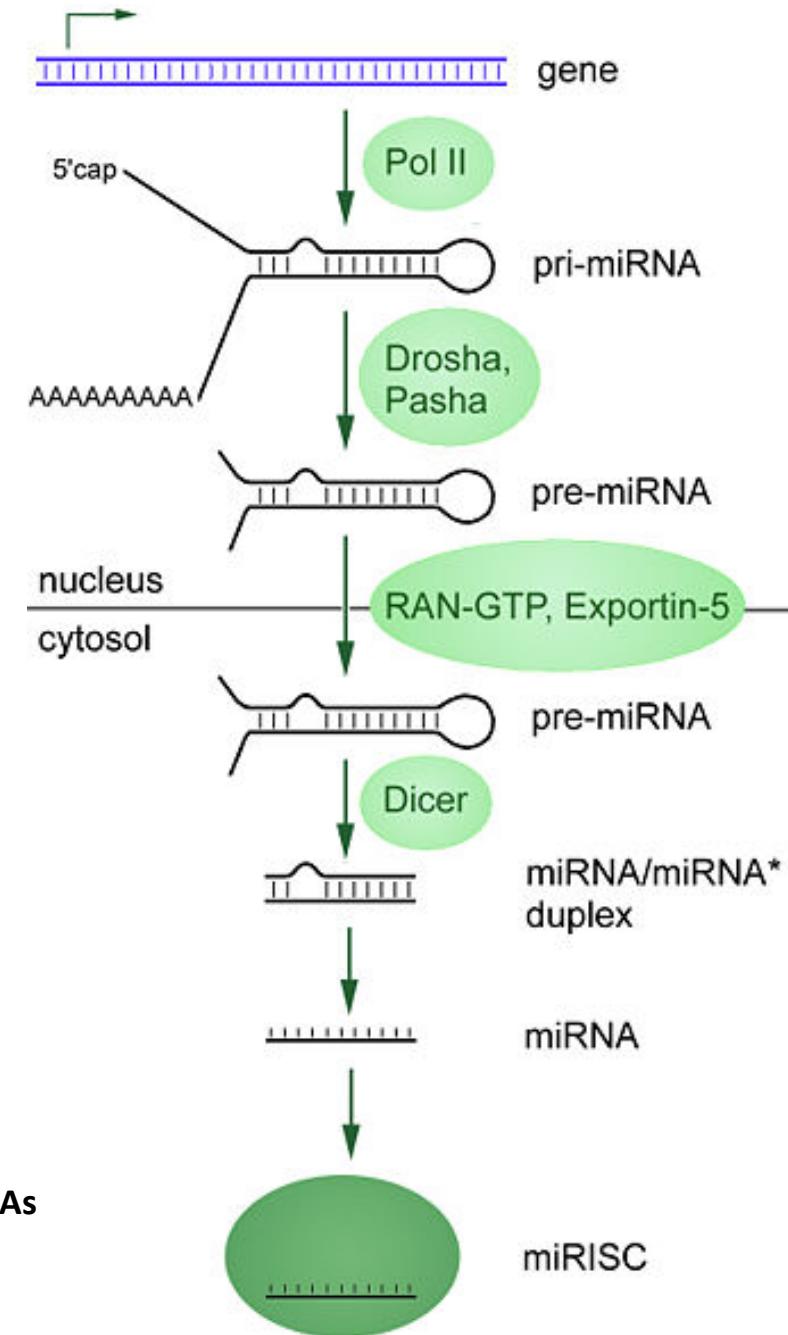
FIGURE 20-17 Argonaute structure, showing RNA-binding regions and an RNase H-like nuclease domain. (a) Crystal structure of Argonaute. The domains are colored as in part b, with the blue domain being the amino-terminal part of the protein, and the green domain in the middle. (b) Cartoon of the Argonaute domains. The arrow shows the RNase active site positioned to cleave in the middle of the paired region between small RNA and miRNA. (a,b, Adapted, with permission, from Song J.J. et al. 2004. *Science* 305: 1434–1437, Fig. 4C. PDB Code: 1u04. © AAAS.) Images prepared with MolScript, BobScript, and Raster3D.

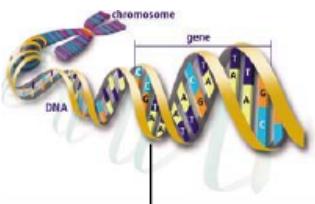
miRNA

- A microRNA (abbreviated miRNA) is a small non-coding RNA molecule (containing about 22 nucleotides) found in plants, animals and some viruses, that functions in RNA silencing and post-transcriptional regulation of gene expression.
- miRNAs function via base-pairing with complementary sequences within mRNA molecules.
- As a result, these mRNA molecules are silenced, by one or more of the following processes:
 - (1) Cleavage of the mRNA strand into two pieces,
 - (2) Destabilization of the mRNA through shortening of its poly(A) tail,
 - (3) Less efficient translation of the mRNA into proteins by ribosomes.

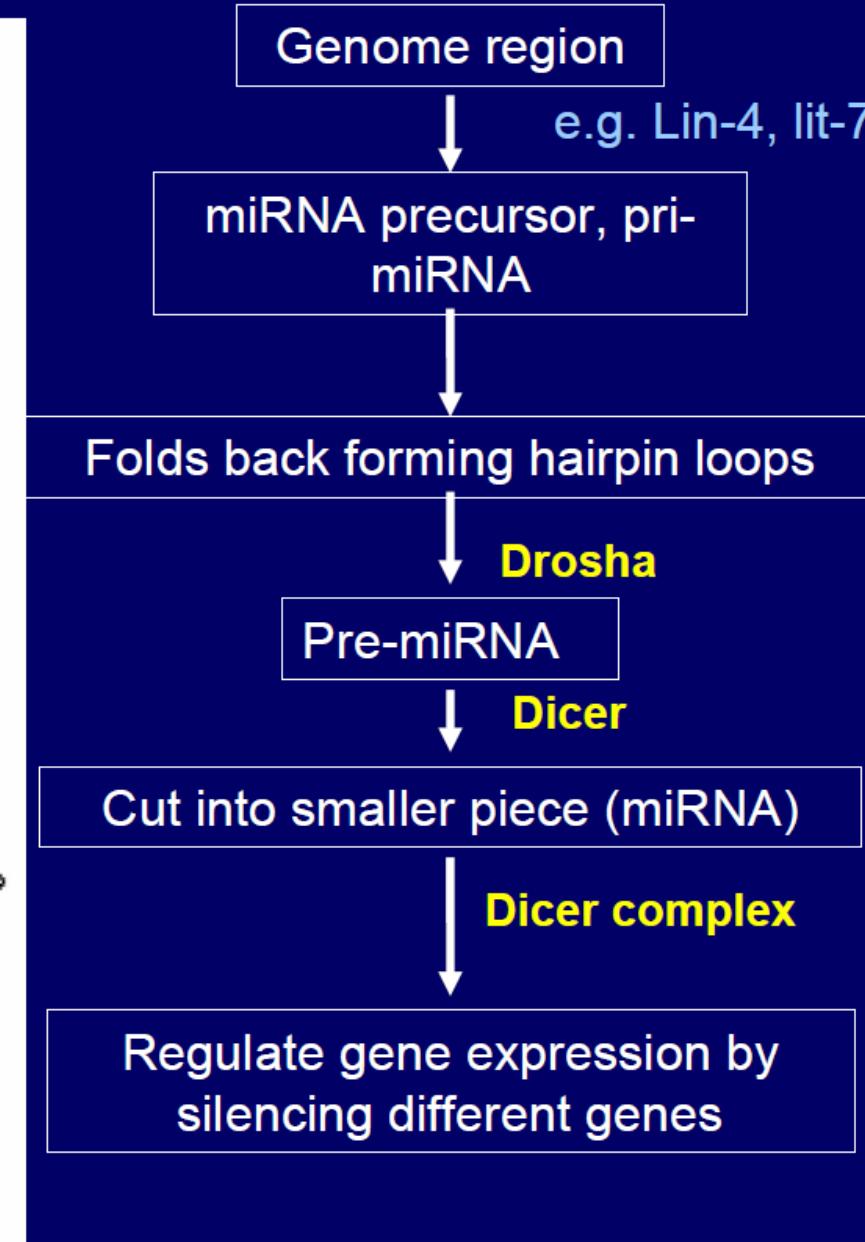
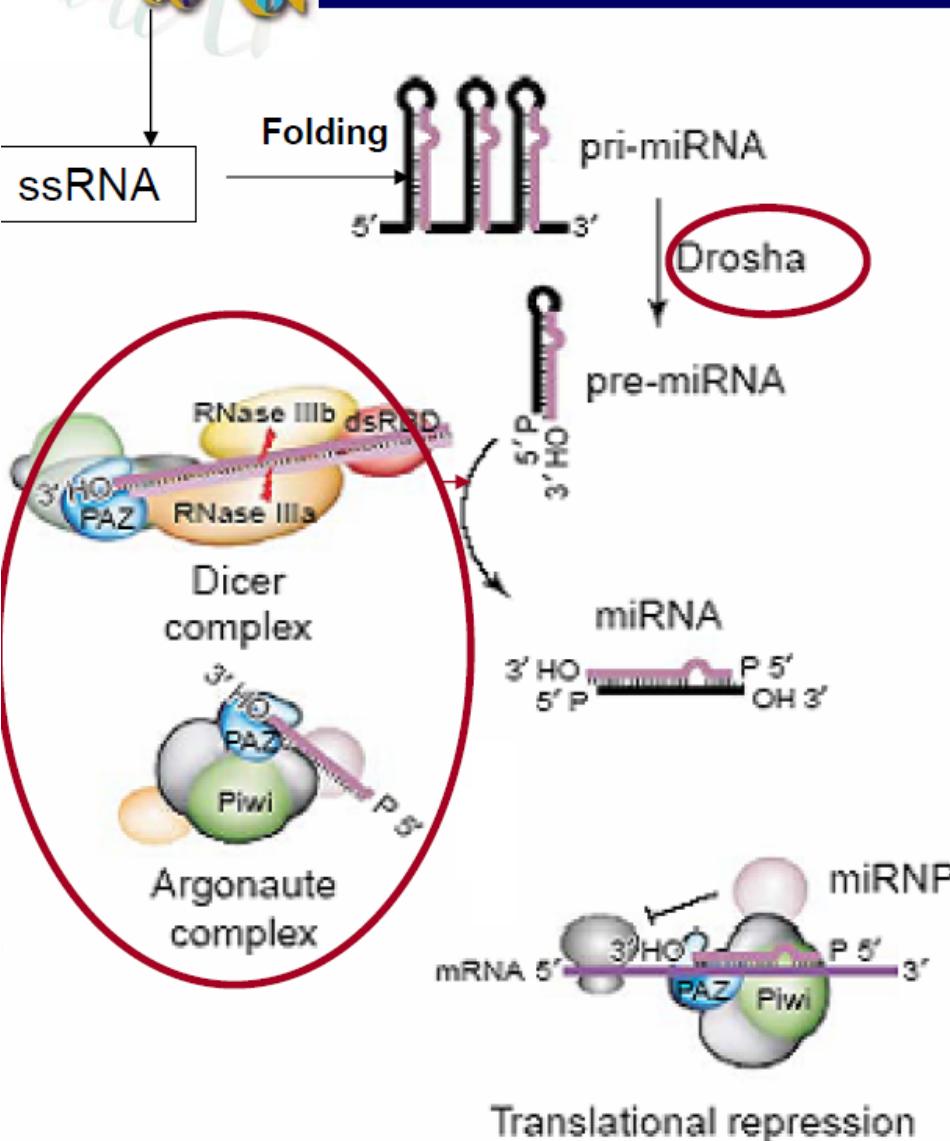
miRNA

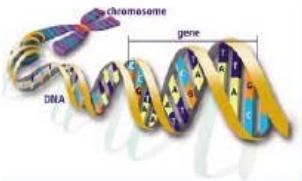
Esquela-Kerscher A, Slack FJ (2006). "Oncomirs – microRNAs with a role in cancer". *Nature Reviews Cancer* 6: 259–69.





Formation of miRNA





miRNA Genes

- **Unusual genes. Arranged in tandem repeats.**
- **First genes identified were *lin-4* and *let-7* in *C.elegans***
- **First make a multiply bulged, partially duplexed precursors (such as stRNA) which are cleaved to miRNA.**
- **Final product is an RNA, not a protein.**
- **To-date, hundreds of different miRNA genes discovered in worms, flies, plants and humans.**
- **In different organisms they make up from 0.5-1.0% of the total genome.**
- **Humans are estimated to have 200-250 miRNA genes (~ 1% of total human genes). Over 175 confirmed by biochemical analysis.**

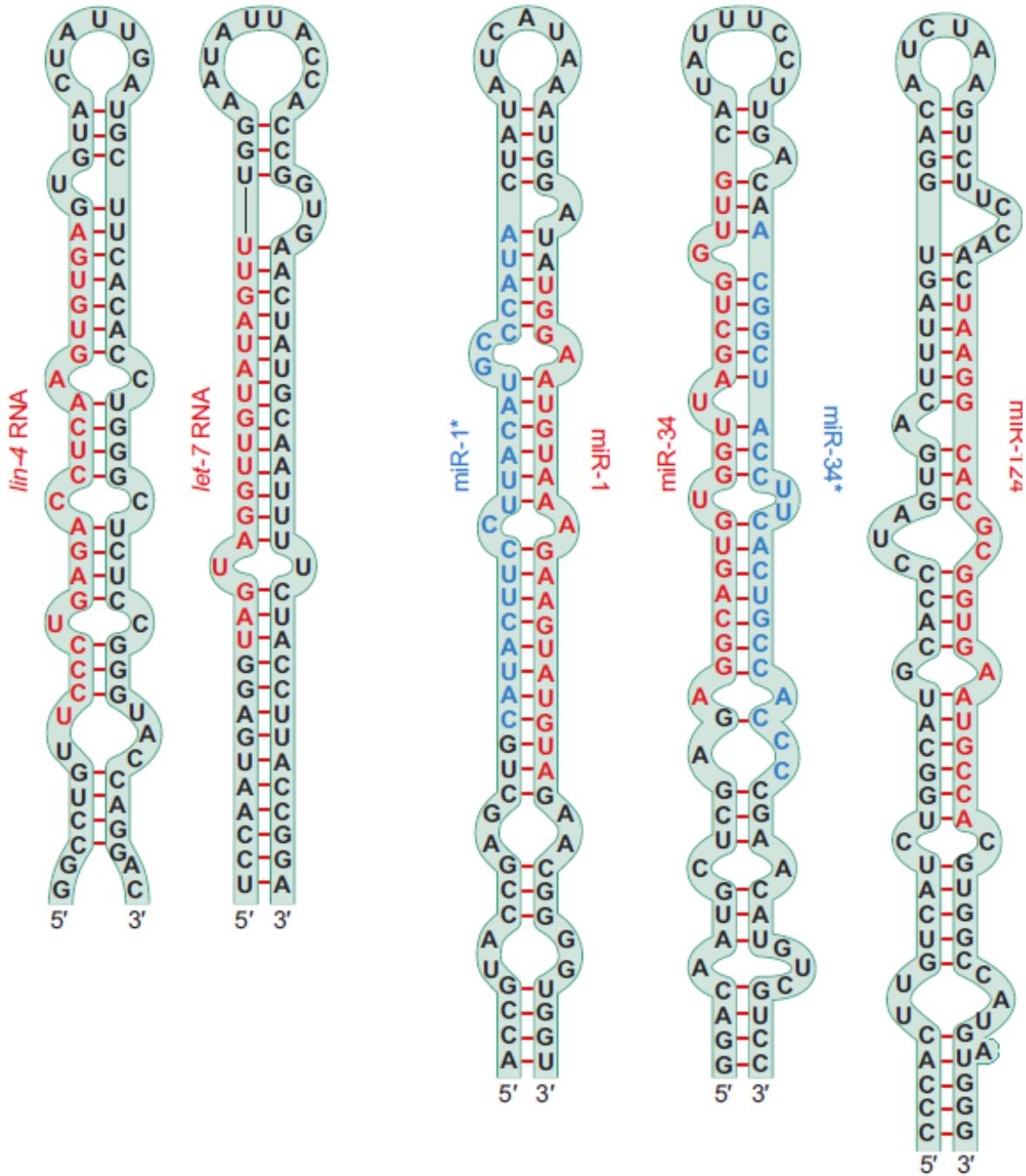
The human genome may encode over 1900 miRNAs, although more recent analysis suggests that the number is closer to 2,300.

stRNA – small temporal RNA
It binds to the DNA and inhibits the producing mRNA (post-transcriptional control)

miRNA in detail

- Characteristic hairpin structure of pri-miRNA
 - Generated by first cleavage
 - Second cleavage generates miRNA

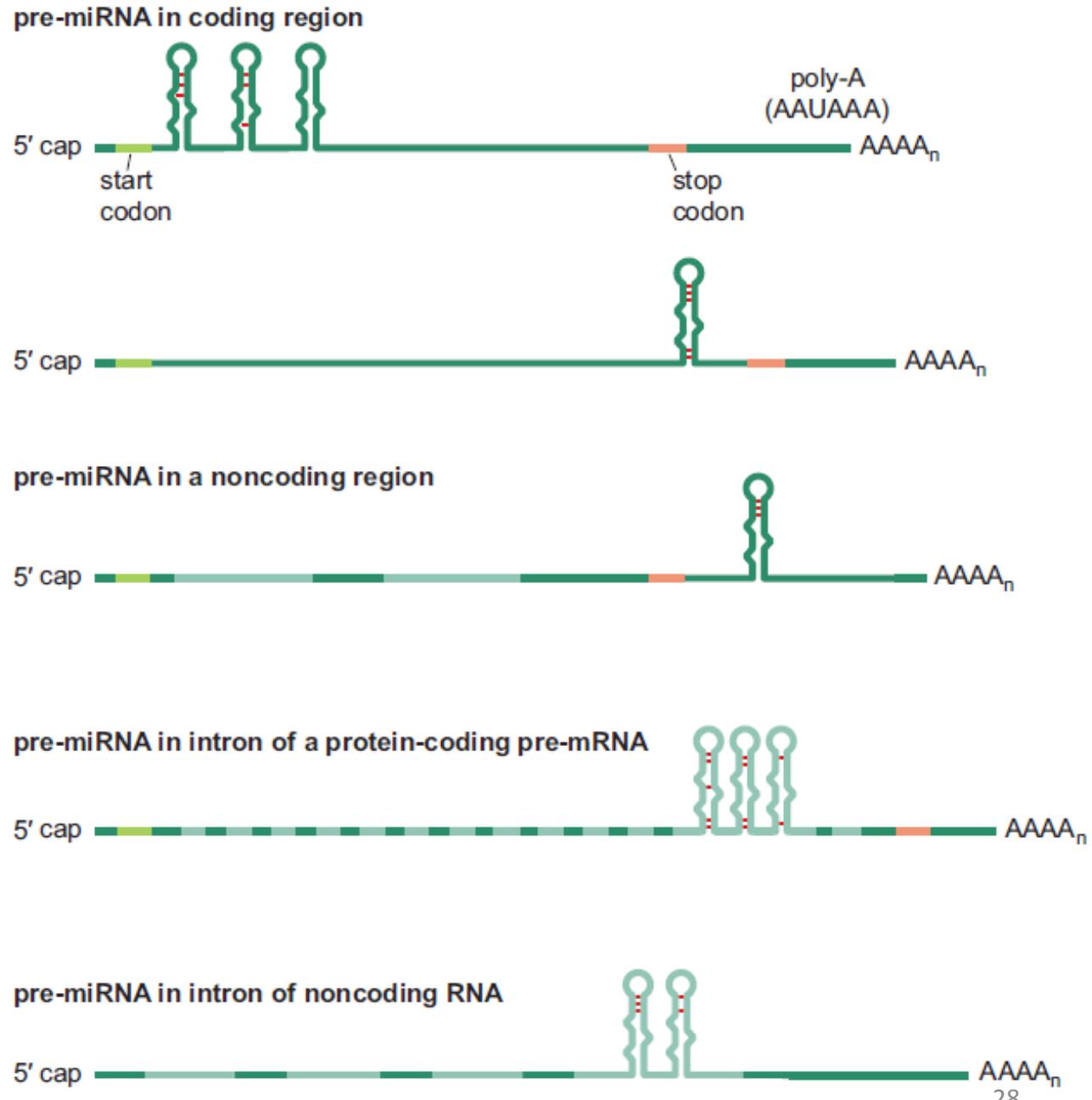
FIGURE 20-12 Structure of some pre-miRNAs before processing to generate the mature miRNAs. The sequences in red are miRNAs. In some cases, both “arms” of a stem-loop can generate a functional miRNA. In such cases, the second miRNA is shown in blue—for example, miR-1 (red) and miR-1* (blue), as well as with miR-34 (red) and miR34* (blue). The miRNAs shown are all from the worm. *lin-4* and *let-7* were identified genetically; those called miR were found by bioinformatics. (Modified, with permission, from Lim L.P. et al. 2003. *Genes Dev.* 17: 991, Fig. 6. © Cold Spring Harbor Laboratory Press.)



miRNA in detail

- Characteristic structure
 - pre-miRNAs can be encoded by any part of a transcript:
 - coding regions,
 - within leader regions,
 - or within introns
- Produced by two nucleases
 - Dicer
 - Drosha

FIGURE 20-13 miRNAs are coded in both introns and exons in RNA. Intronic sequences are shown in light green. Start and stop codons are indicated by lime green and pink, respectively.



miRNA production

- Produced by two nucleases that recognize the structure, rather than sequence
 - Dicer (cytoplasm)
 - Drosha (nucleus)

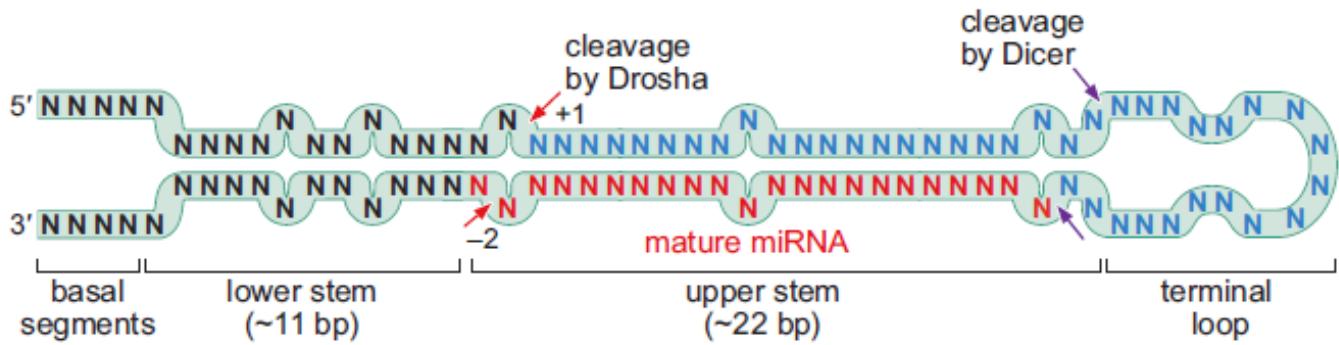
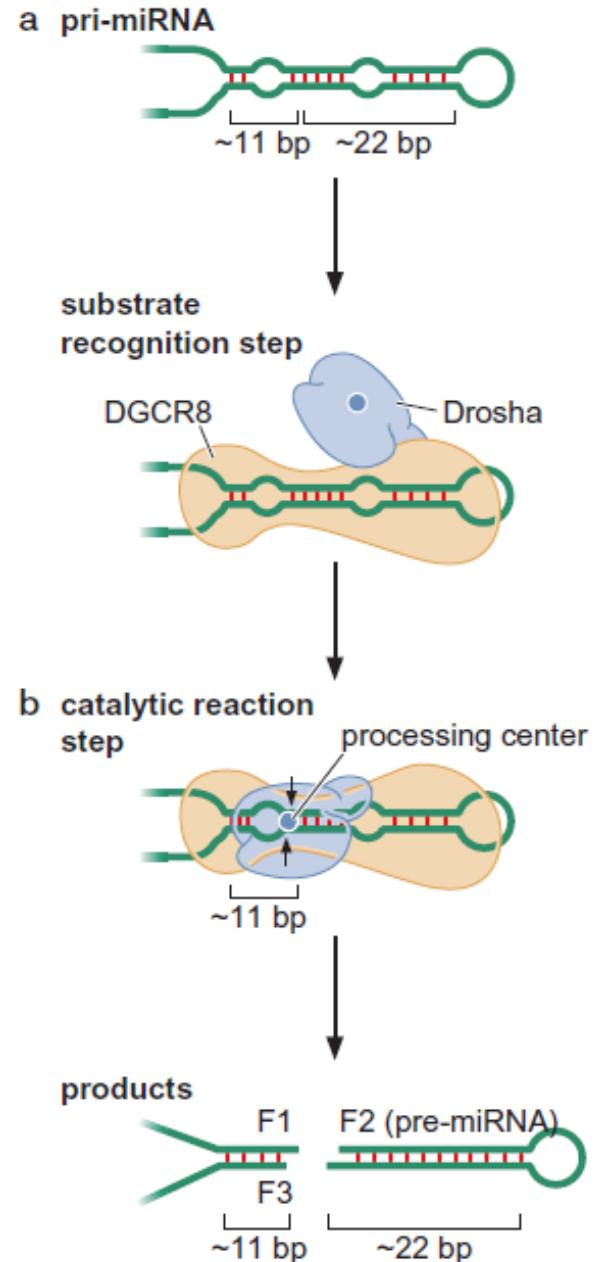


FIGURE 20-14 Overview of the structure of pri-RNA showing Dicer and Drosha cleavage sites. The region in red becomes the mature miRNA. Note that the basal segments must be single-stranded for proper recognition by the Drosha complex.

FIGURE 20-15 Recognition and cleavage of pri-miRNA by the Microprocessor complex. Three fragments are generated by cleavage, labeled F1, F2 (the pre-miRNA), and F3.



miRNA functions

In plants and animals miRNA genes play a very important **role in development**. (By clearing certain messages from cells during development, RNAi help cells to mature into correct type and proper structure)

General role in gene regulation of other genes

Control stability and translation of mRNA of other genes

To specify temporal progression of cell fate

May amplify and spread throughout the source

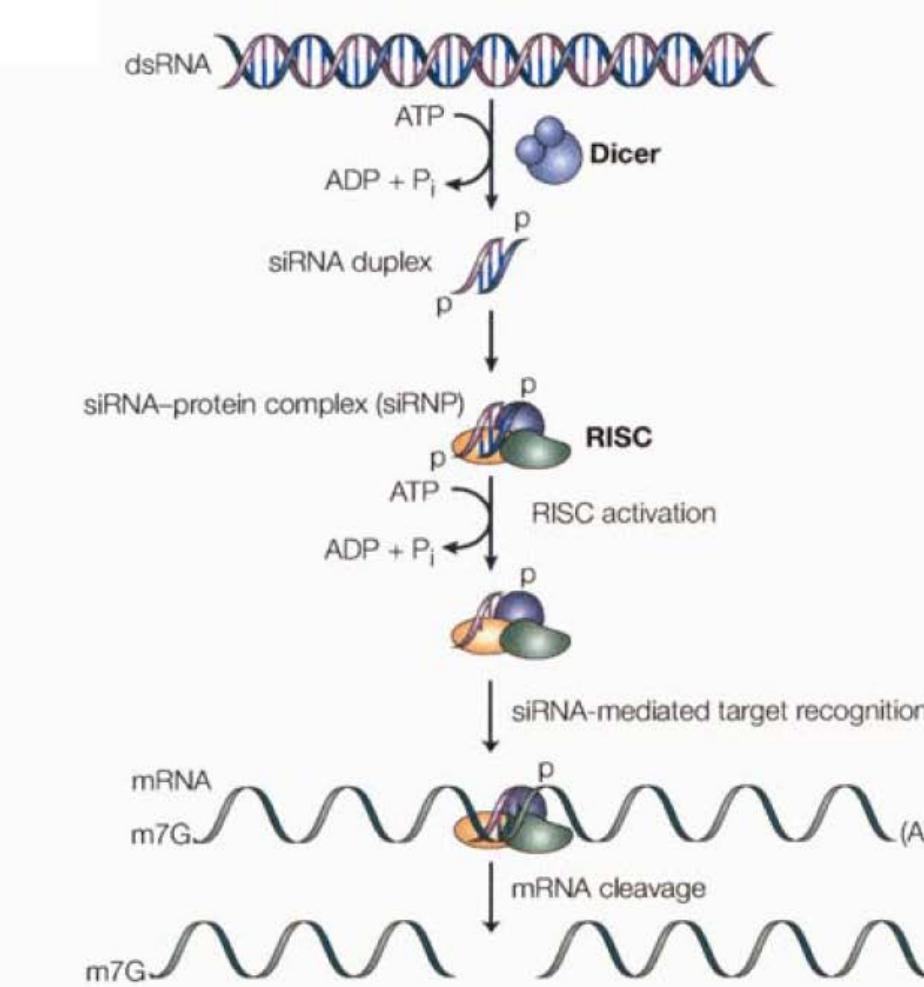
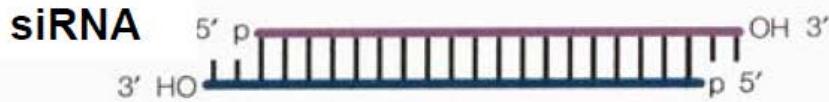
Developmental Control

Gene silencing by small RNAs

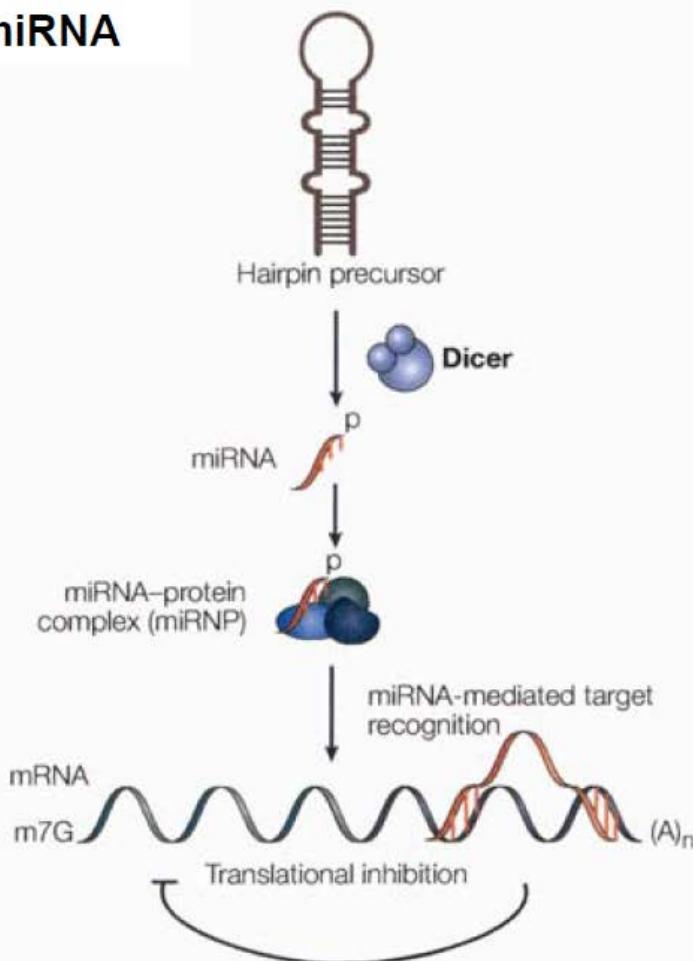
- ~22 nt is long enough for unique sequence recognition
- Argonaute, the central component of RISC
 - RNA-cleaving enzyme
 - Not all Argonautes are nucleolytically active
- Mechanism of translational repression by miRNAs not fully clear
 - mRNA degradation -> less translation
 - Translation inhibition -> mRNA degradation

small RNAs direct chromatin modifications

- Telomere regions are silenced in yeast
 - Histone modifications
- Centromeres are silenced in *S. pombe*
 - Histone modifications
 - Directed by RNAi



Inhibition of protein synthesis by cutting mRNA



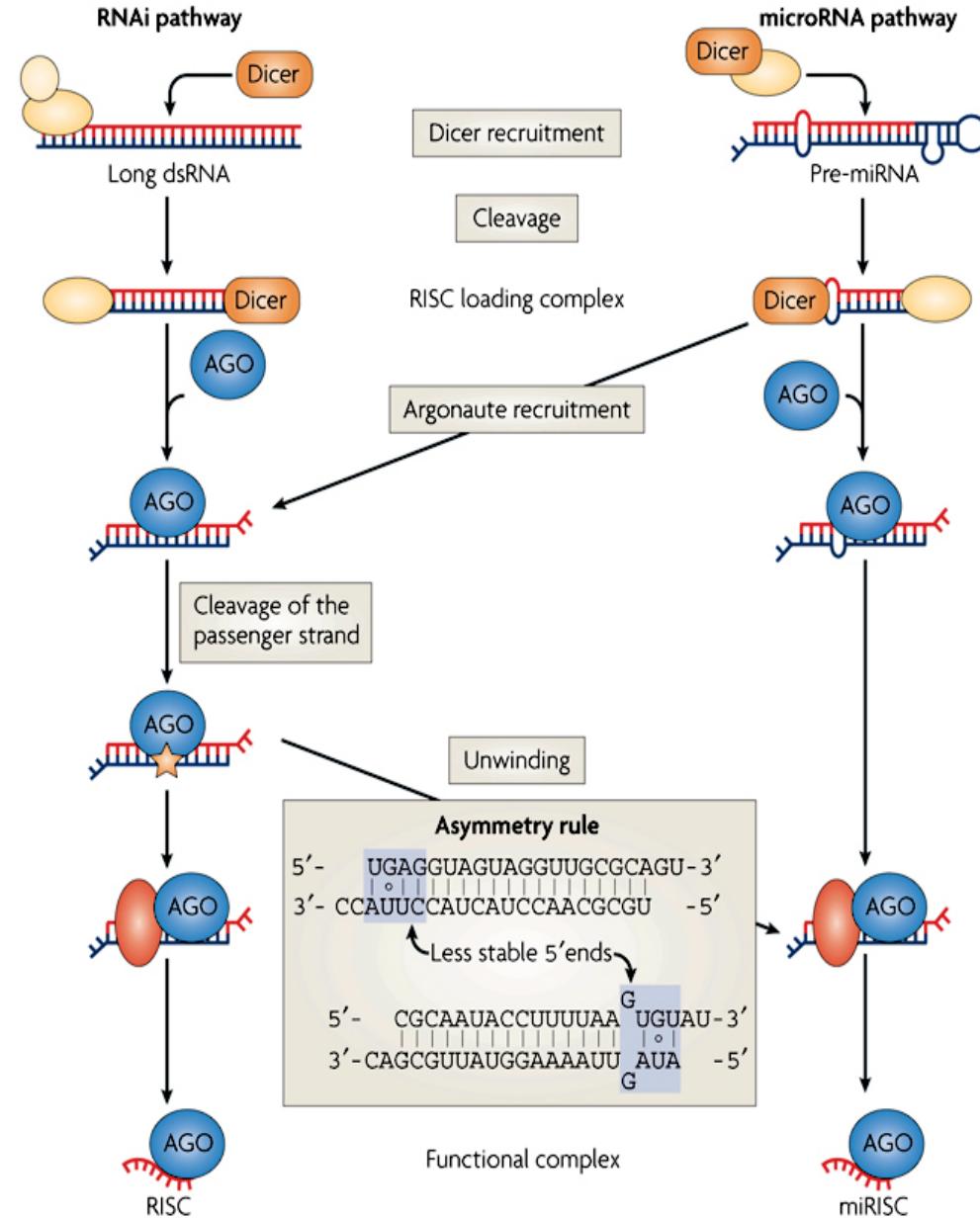
Inhibition of protein synthesis by binding to the mRNA

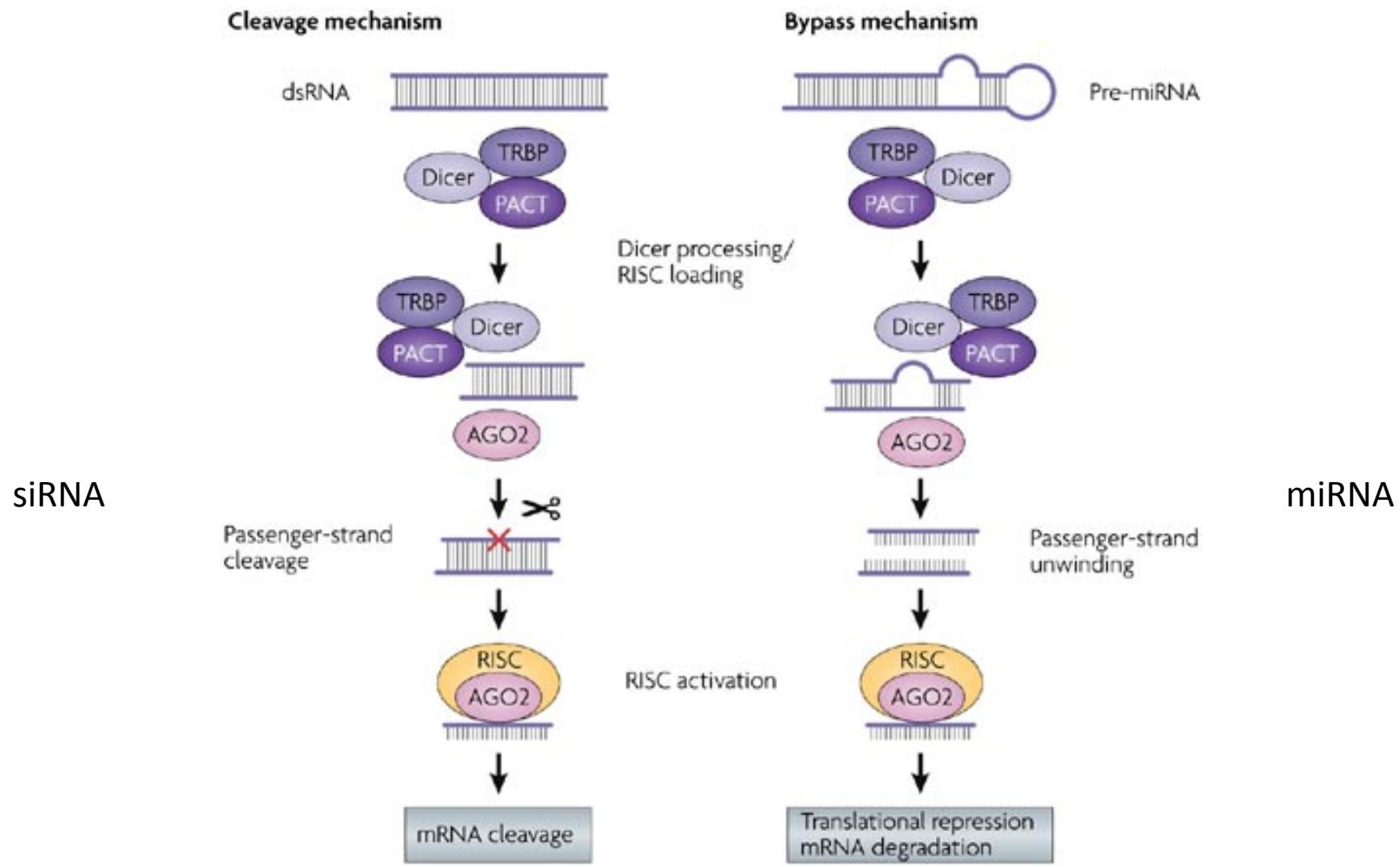


miRNA vs siRNA

- siRNA need to have the binding to the mRNA with the full complementarity and the cleavage site is on this and only on this one point on the mRNA chain
- miRNA don't need the full complementarity

Hutvagner G, Simar MJ. Argonaute proteins: key players in RNA silencing. *Nat Rev Mol Cell Biol.* 2008;9(1):22-32.



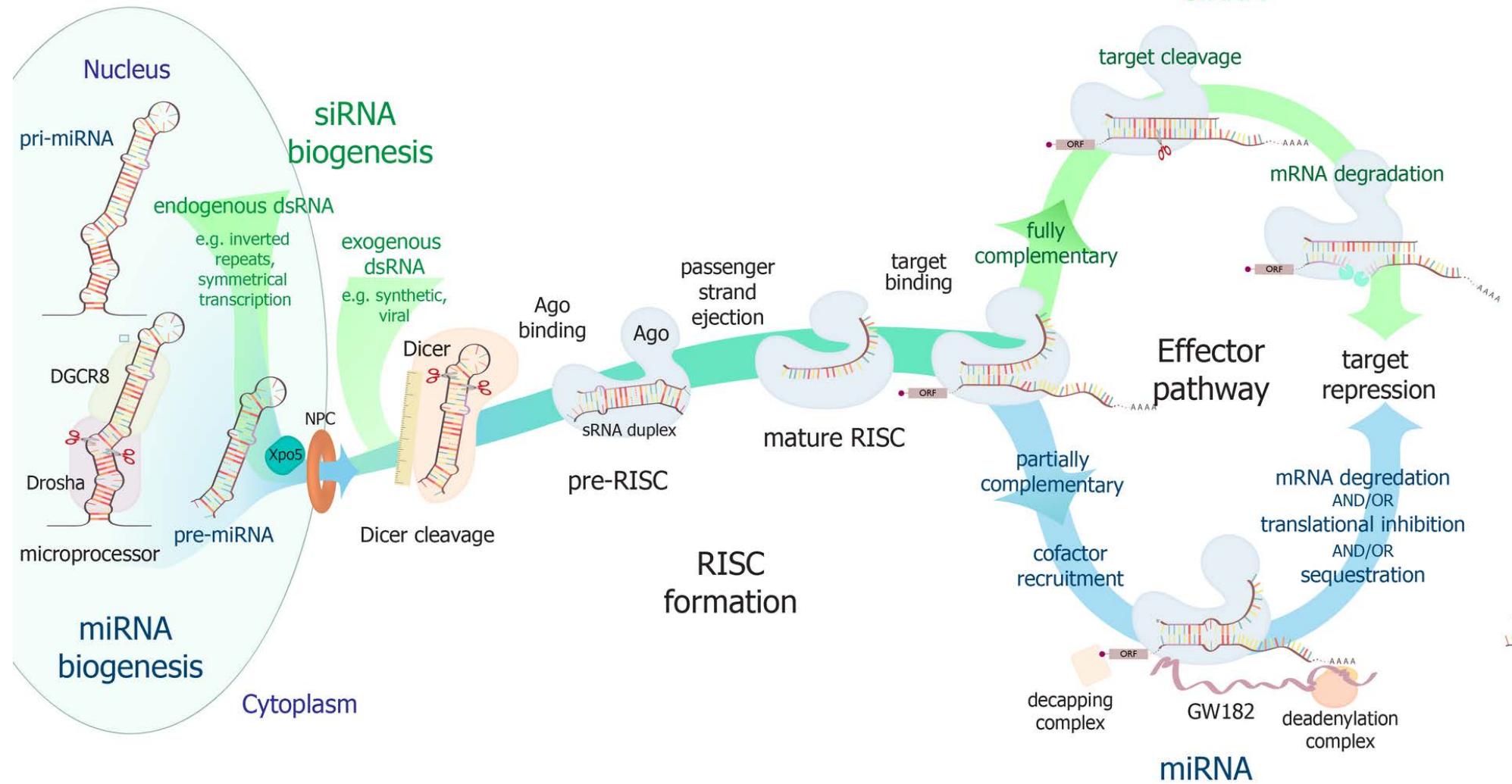


Nature Reviews | Genetics

Kim and Rossi *Nature Reviews Genetics* 8, 173–184 (March 2007) | doi:10.1038/nrg2006

TAR RNA-binding protein (TRBP)
protein kinase RNA activator (PACT)

nature
REVIEWS GENETICS

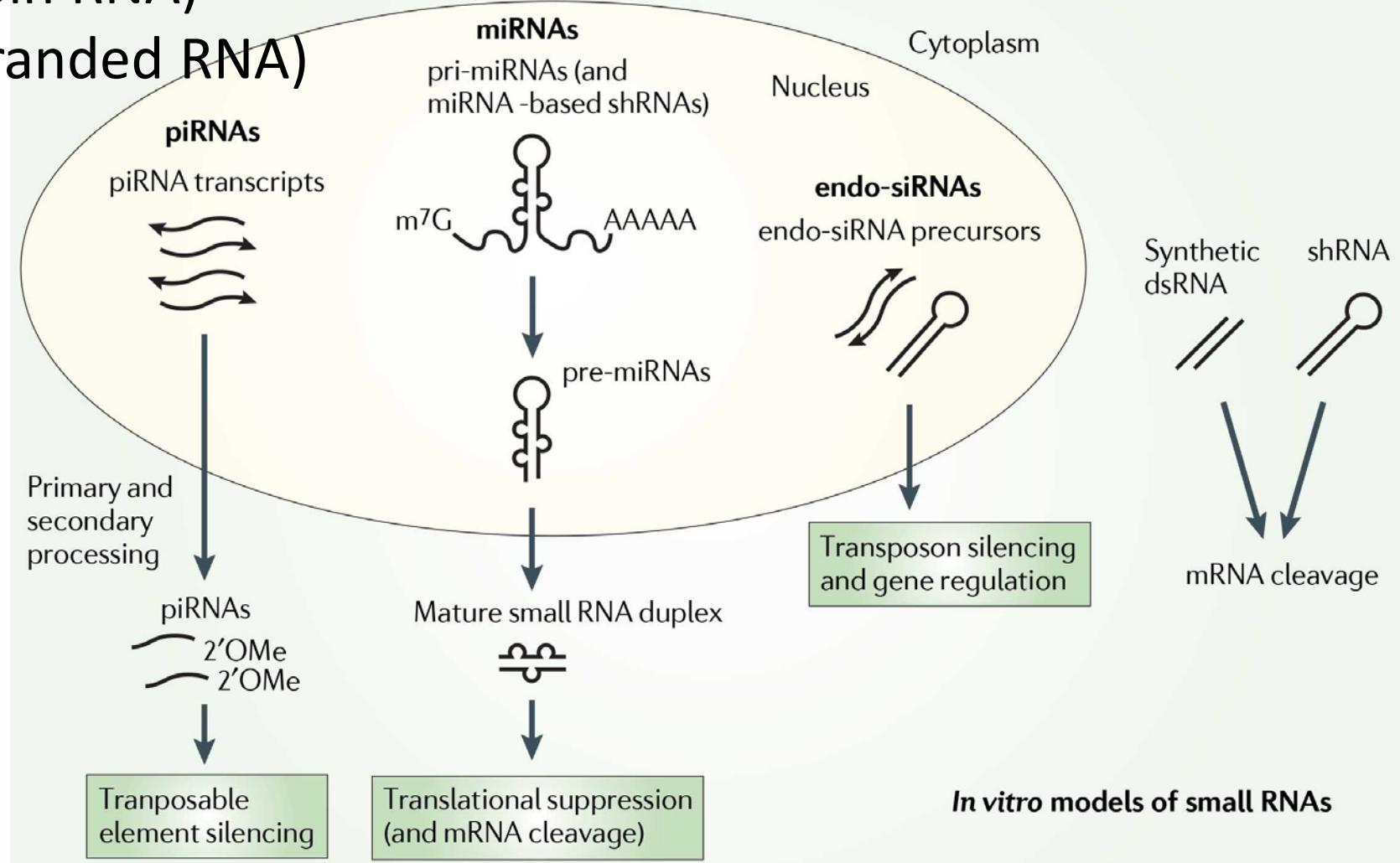


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small RNA biogenesis: primary miRNAs (pri-miRNAs) are transcribed in the nucleus and fold back onto themselves as hairpins that are then trimmed in the nucleus by a microprocessor complex to form a ~60-70nt hairpin pre-RNA. This pre-miRNA is transported through the nuclear pore complex (NPC) into the cytoplasm, where Dicer further trims it to a ~20nt miRNA duplex (pre-siRNAs also enter the pathway at this step). This duplex is then loaded into Ago to form the “pre-RISC(RNA induced silencing complex)” and the passenger strand is released to form active RISC.

piRNA – piwi protein binding RNA; **pri-miRNA** - primare transcript of miRNA;
pre-miRNA; **endo-siRNA** – endogenous small interfering RNA (esiRNA);
rasiRNA (*repeat associated small interfering RNA*);
shRNA (short hairpin RNA)
ds RNA (double-stranded RNA)

Some others
small RNAs
in the cells:



RNAi pathways for gene silencing in Eukaryotes

The siRNA pathway

siRNA formed from dsRNA

Cleaves mRNA

Degraded by nucleases

Gene silenced

The miRNA pathway

miRNA formed from dsRNA

Binds mRNA

Prevents translation

Gene silenced

Chromatin based pathway

Small RNAs from dsRNA

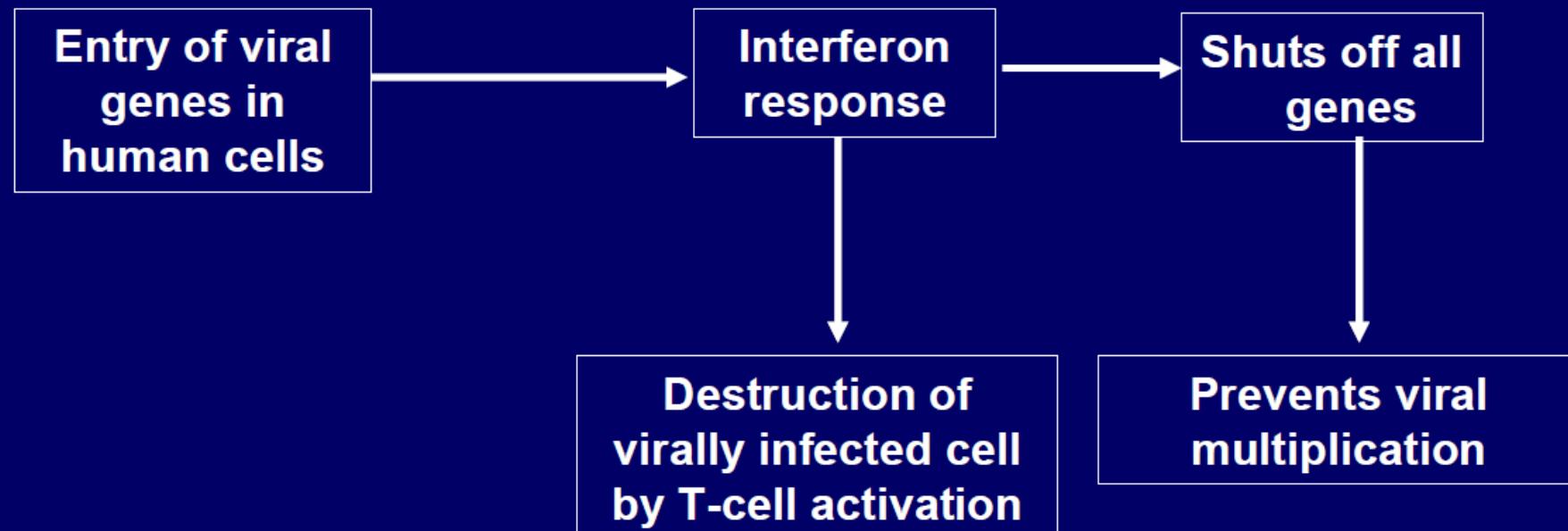
Bind chromatin

Prevents transcription

Gene silenced

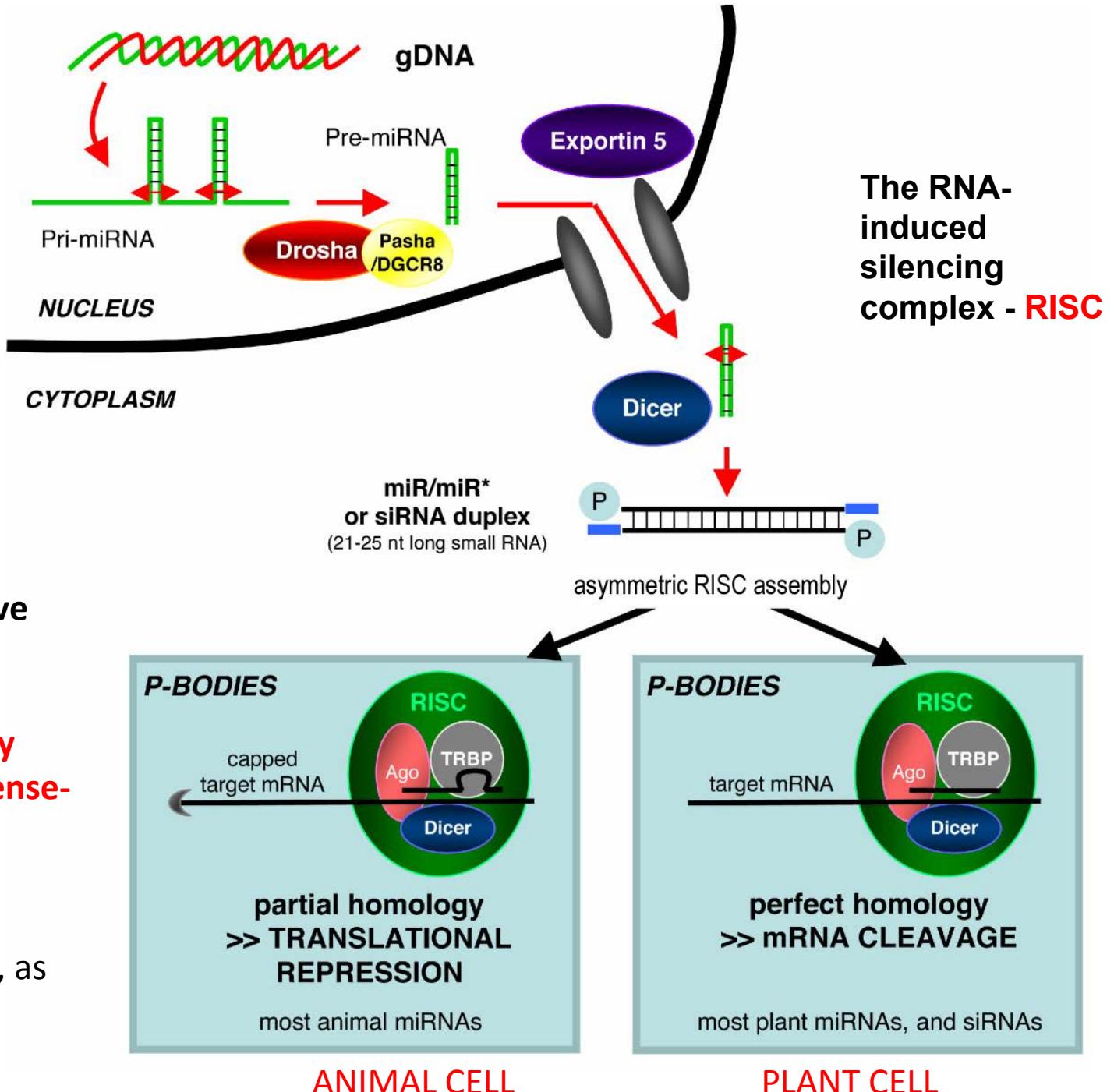
RNAi in vertebrates Cells

- Transfection of long dsRNA (>30 nt) in vertebrates cells caused a non-specific suppression of gene expression.
- A response similar to **interferon response**.



RISC
Ago
TRBP
Dicer
Drosha
Pasha
P-body (processing body)

Illustration of the major differences between **plant and animal gene silencing**. Natively expressed microRNA or exogenous small interfering RNA is processed by dicer and integrated into the RISC complex, which mediates gene silencing.



- The following activities have been demonstrated to occur in or to be associated with P-bodies:
 - decapping and degradation of unwanted mRNAs
 - storing mRNA until needed for translation
 - aiding in translational repression by miRNAs (related to siRNAs)
- In neurons, P-bodies move by motor proteins in response to stimulation. This is likely tied to local translation in dendrites.

P-bodies were first described in the scientific literature by Bashkirov et al.

In 1997, in which they describe "small granules... discrete, prominent foci" as the cytoplasmic location of the mouse exoribonuclease mXrn1p.

In 2002, researchers demonstrated that multiple proteins involved with mRNA degradation localize to the foci.

Regulation by RNAs in bacteria

- Small RNAs (sRNA)
 - Plasmid replication regulation
 - Gene expression regulation
 - 6S RNA
- 6S RNA binds to σ^{70} and down-regulates transcription
 - Accumulates in stationary phase
 - Helps to shift expression to σ^S promoters



sRNAs (bacterial regulatory RNAs)

- Act in *trans*
 - Control translation of target genes
 - 80-110 nt in length (larger than their eukaryotic counterparts which range from 21-30 nt)
 - Encoded by small genes, not produced by processing dsRNA as in eukaryotes
 - More than 100 sRNAs in *E. coli*
 - work by base pairing with complementary sequences within target mRNAs
 - direct destruction of the mRNA,
 - inhibit its translation
 - stimulate translation

sRNAs – mode of action

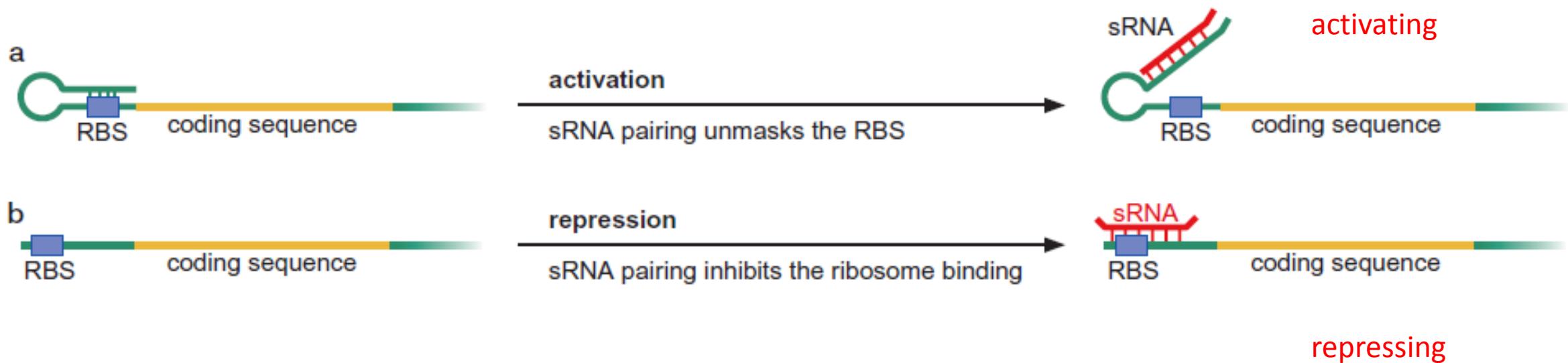


FIGURE 20-1 Activation and repression of translation by sRNAs. When the ribosome-binding site (RBS) is occluded by base pairing with another RNA molecule (as in part b) or another region of the same RNA molecule (as in part a), translation is inhibited. (Adapted, with permission, from Gottesman S. et al. 2006. *Cold Spring Harbor Symp. Quant. Biol.* 71: 1–11, Fig. 1. © Cold Spring Harbor Laboratory Press.)



sRNAs (bacterial regulatory RNAs)

- RybB RNA
 - Binds several target mRNAs and triggers their destruction by RNase E
 - Mostly iron storage proteins
- SigmaS
 - *rpoS* mRNA translation is stimulated by two sRNAs
 - Negatively regulated by OxyS sRNA
- Antisense RNAs
 - encoded by the strand opposite the coding strand of a gene, act through homologous base pairing to inhibit expression of the mRNA
 - act in *cis* because they act only on the gene from which they are made (in contrast to the *trans*-acting sRNAs described above).

Trans or *cis*?

- *Trans-acting RNA* regulatory elements
 - Control expression of the genes not linked to their own gene
- *Cis-acting RNA* regulatory elements
 - Control expression of the genes within whose mRNAs they reside
- *Riboswitches – cis-acting*
 - Control metabolic operons
 - Control attenuation in biosynthetic operons

Riboswitches

- Control gene expression in response to changes in the concentrations of small molecules (many hundreds or thousands found)

- Usually found within the 5'-untranslated regions (5'-UTRs)
- Can regulate expression at transcription or translation level
- Work through changes in RNA secondary structure

- Two components
 - Aptamer
 - Expression platform

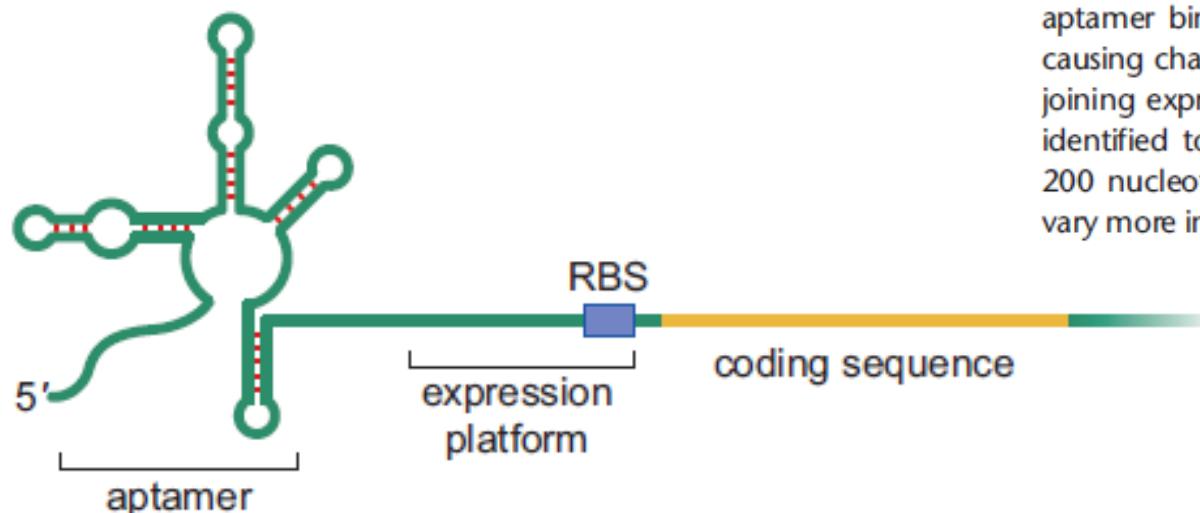


FIGURE 20-2 Organization of ribo-switch RNAs. As described in the text, the aptamer binds the controlling metabolite, causing changes in the structure of the adjoining expression platform. The aptamers identified to date vary in size from 70 to 200 nucleotides; the expression platforms vary more in both size and character.

Each riboswitch is made up of two components: the aptamer and the expression platform (Fig. 20-2).

The aptamer binds the small-molecule ligand and, in response, undergoes a conformational change, which, in turn, causes a **change in the secondary structure of the adjoining expression platform.**

These conformational changes alter expression of the associated gene by either **terminating transcription or inhibiting the initiation of translation.**

How do riboswitches work?

- SAM-sensing riboswitch in *Bacillus subtilis*
- SAM—the ligand for this **riboswitch**—binds to the aptamer and stabilizes the secondary structure that includes this transcriptional terminator

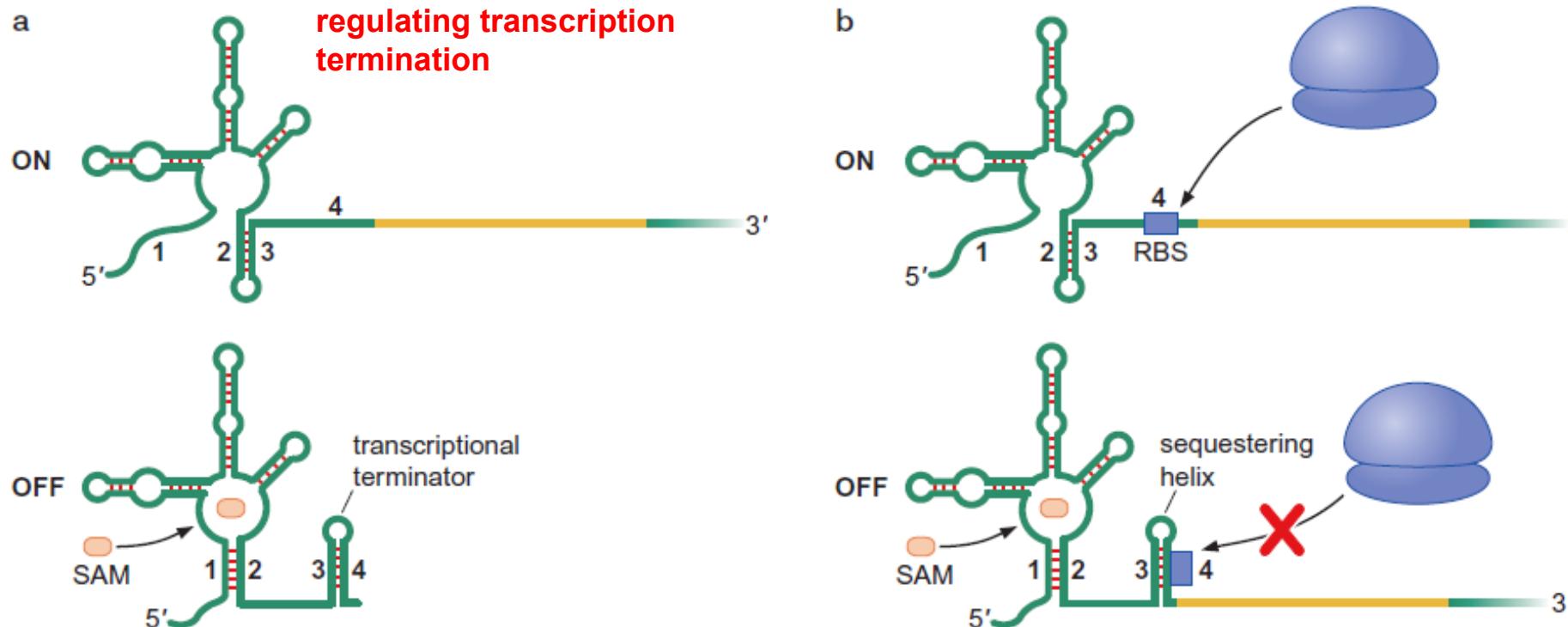
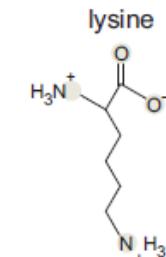
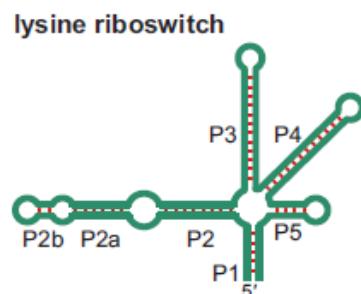
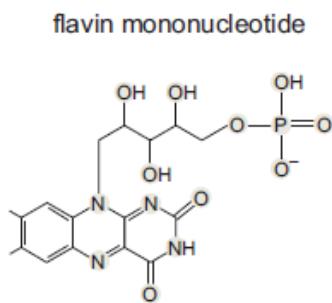
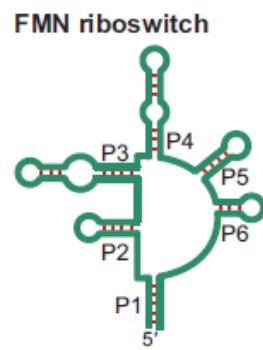
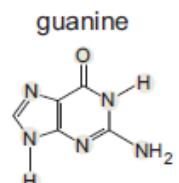
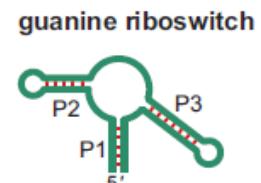
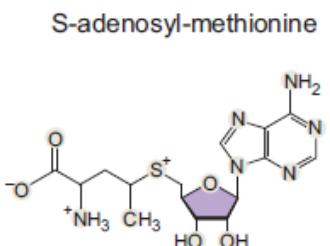
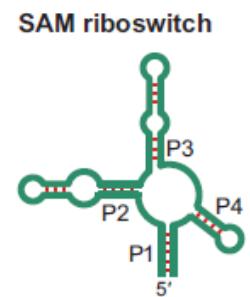
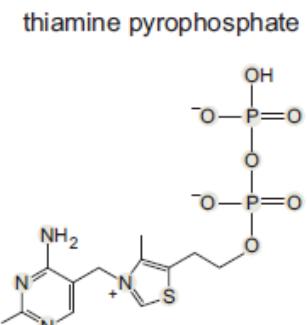
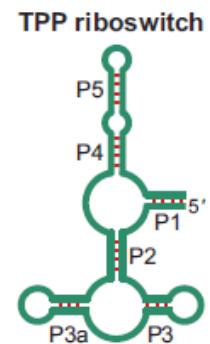


FIGURE 20-3 Riboswitches regulate transcription termination or translation initiation. Two examples of a SAM-sensing riboswitch, in one case (a) regulating transcription termination, in the other (b) translation initiation. Numbers 1–4 indicate different sequence elements within the RNA upstream of the coding region (yellow). In the absence of SAM, regions 2 and 3 form a stem-loop; in the presence of SAM, regions 1 and 2 form a stem-loop, and regions 3 and 4 do likewise. The consequence of that change in secondary structure controls transcription or translation as shown. (a) A stem-loop of regions 3 and 4 produces a transcriptional terminator, which triggers RNA polymerase to terminate transcription immediately after transcribing those regions and before entering the downstream coding region. The stem-loop in this case is followed by a stretch of Us in the mRNA, another feature of the transcriptional terminator (Chapter 13, Fig. 13-13). (b) The stem-loop formed by regions 3 and 4 inhibits translation initiation by sequestering the ribosome-binding site, as shown.

Riboswitches



B₁₂ riboswitch

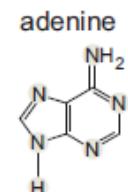
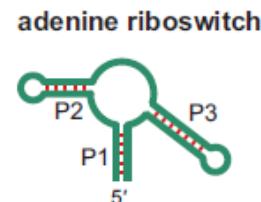
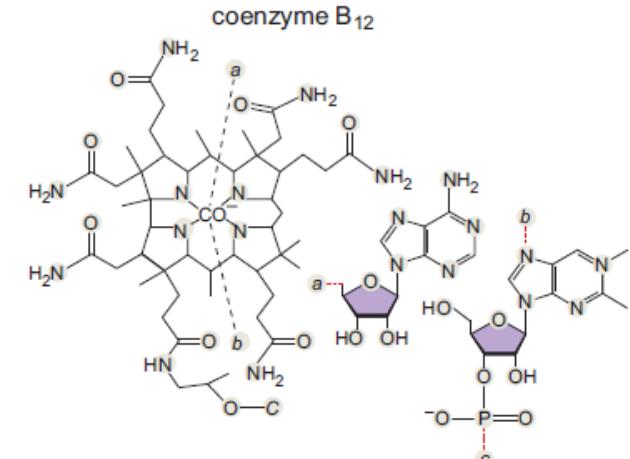
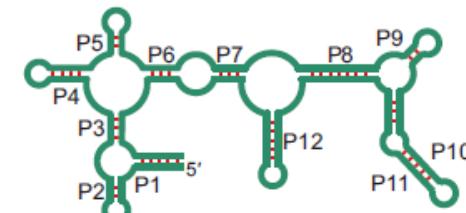
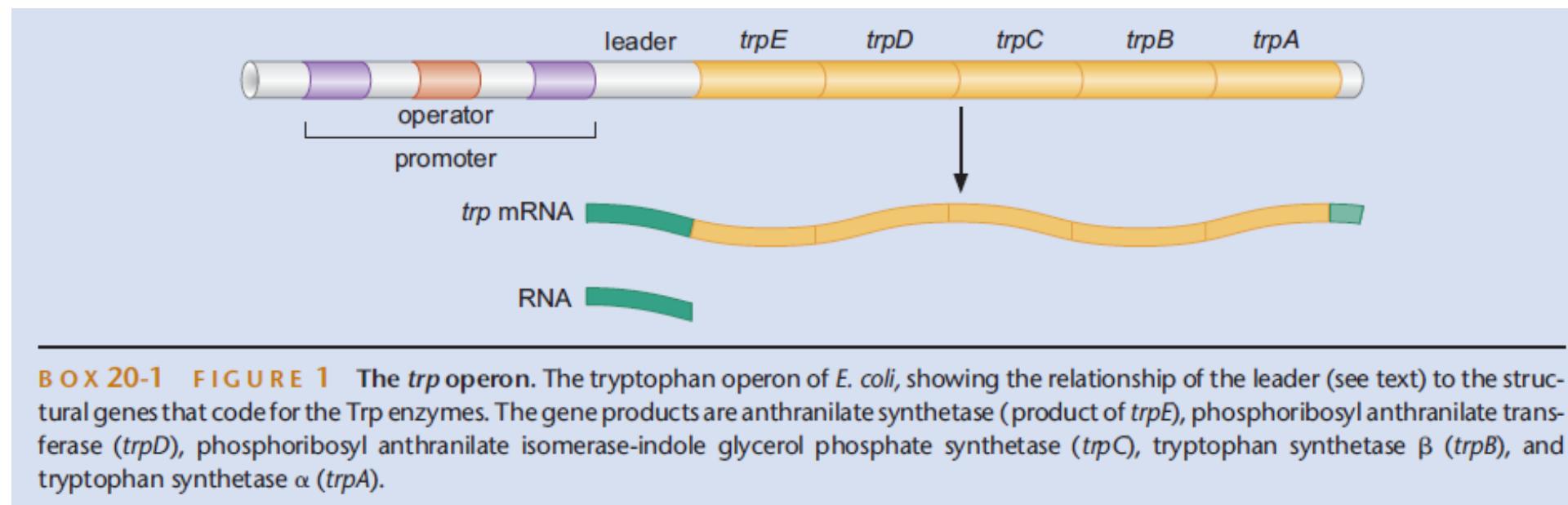


FIGURE 20-5 Riboswitches respond to a range of metabolites. The secondary structure of seven riboswitches and the metabolites they sense are shown here. (Adapted, with permission, from Mandal M. et al. 2003. *Cell* 113: 577–586, Fig. 7A. © Elsevier.)

Attenuation – *trp* example

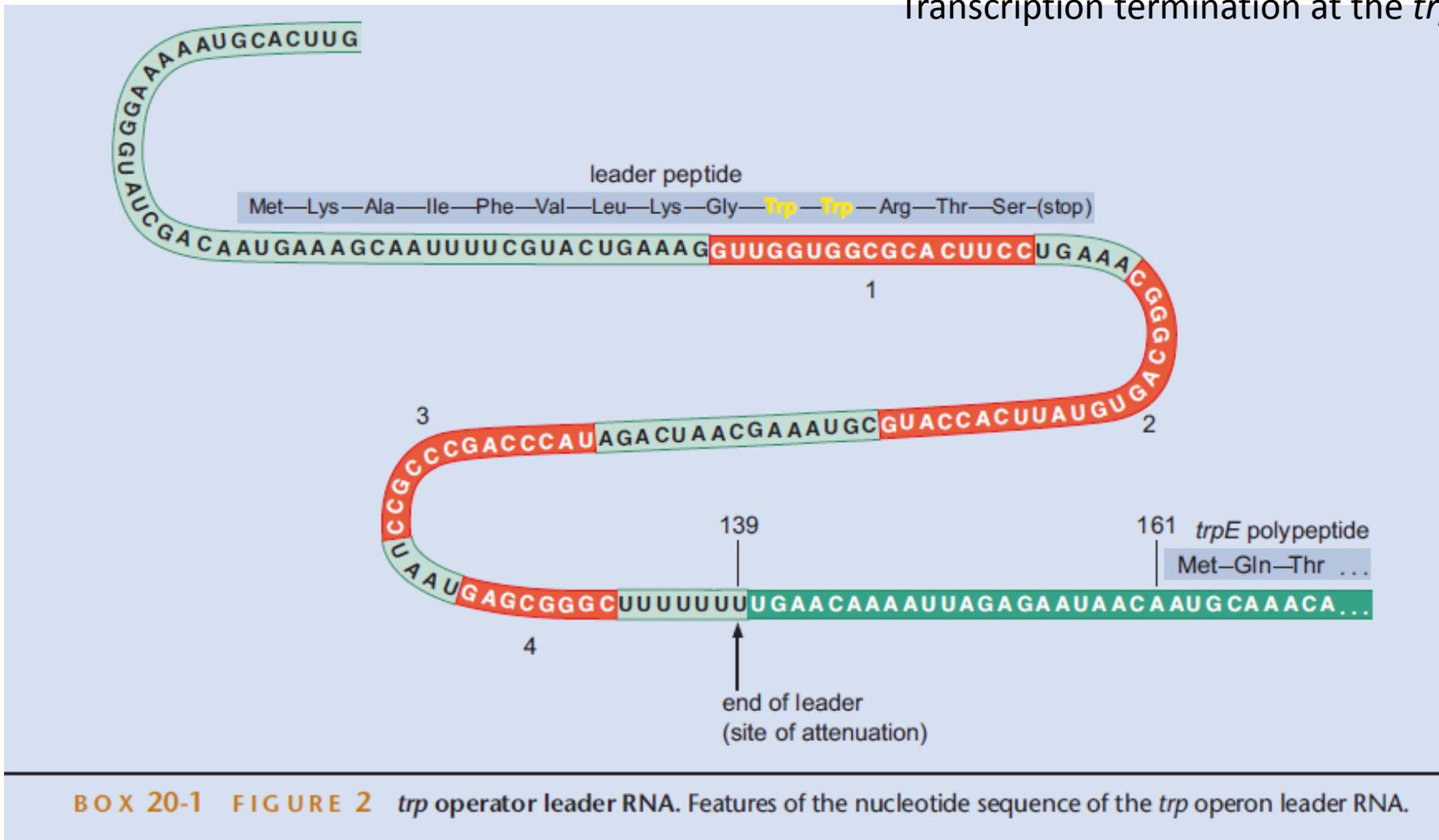
Attenuation - transcription is terminated before polymerase has a chance to transcribe the downstream protein-coding segment of the gene

- Premature termination of transcription, due to translation and transcription being coupled in bacteria



161 nt long leader sequence + transcript. terminaator = characteristic hairpin loop in the RNA = stopping transcription

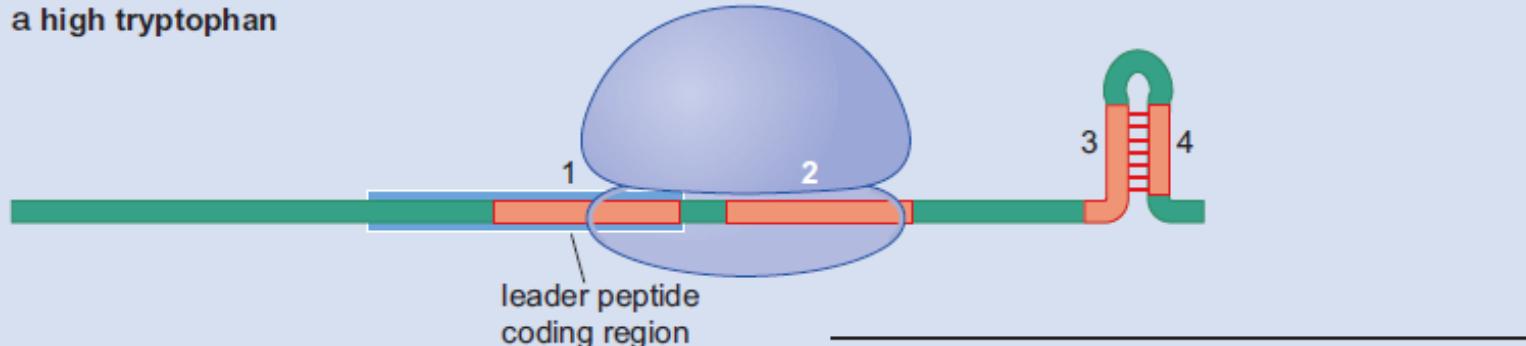
Attenuation – *trp* example



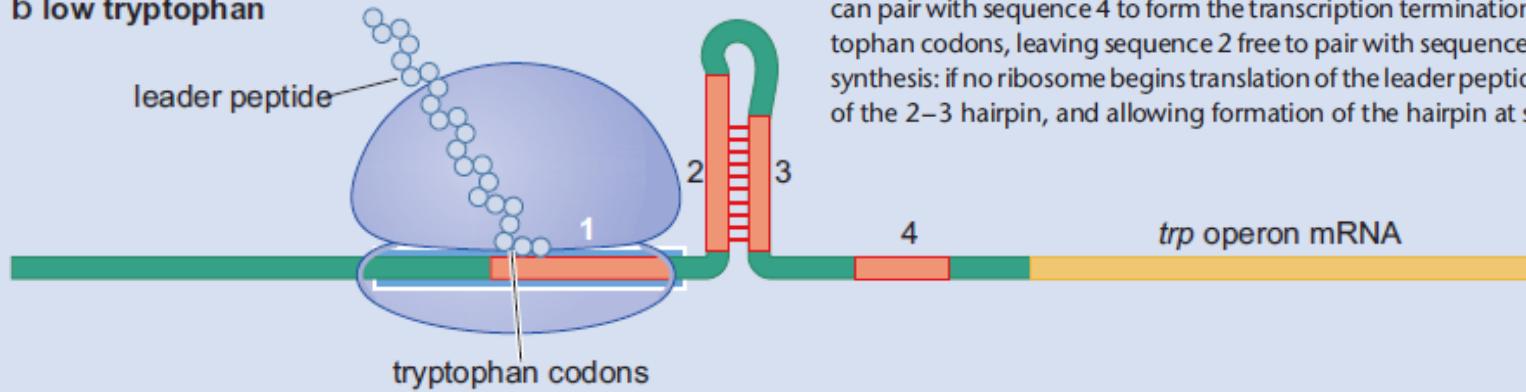
Hairpins: 1+2; 2+3 or 3+4; ORF of leader RNA sequence + RBS

Attenuation – *trp* example

a high tryptophan



b low tryptophan



c no protein synthesis



BOX 20-1 FIGURE 3 Transcription termination at the *trp* attenuator. Transcription termination at the *trp* operon attenuator is controlled by the availability of tryptophan. The blue box shows the leader peptide-coding region. (a) Conditions of high tryptophan: sequence 3 can pair with sequence 4 to form the transcription termination hairpin. (b) Conditions of low tryptophan: the ribosome stalls at adjacent tryptophan codons, leaving sequence 2 free to pair with sequence 3, thereby preventing formation of the 3–4 termination hairpin. (c) No protein synthesis: if no ribosome begins translation of the leader peptide AUG, the hairpin forms by pairing of sequences 1 and 2, preventing formation of the 2–3 hairpin, and allowing formation of the hairpin at sequences 3–4. The Trp enzymes are not expressed.

Regulatory RNAs – system of defence

- CRISPRs – Clustered Regularly Interspaced Short Palindromic Repeats
 - Repeated sequences – ~30 bp long, highly conserved within a cluster
 - Spacer sequences – similar length, highly divergent
- CRISPRs found in half of all bacterial genomes
 - Usually one cluster
 - Up to 400 per genome

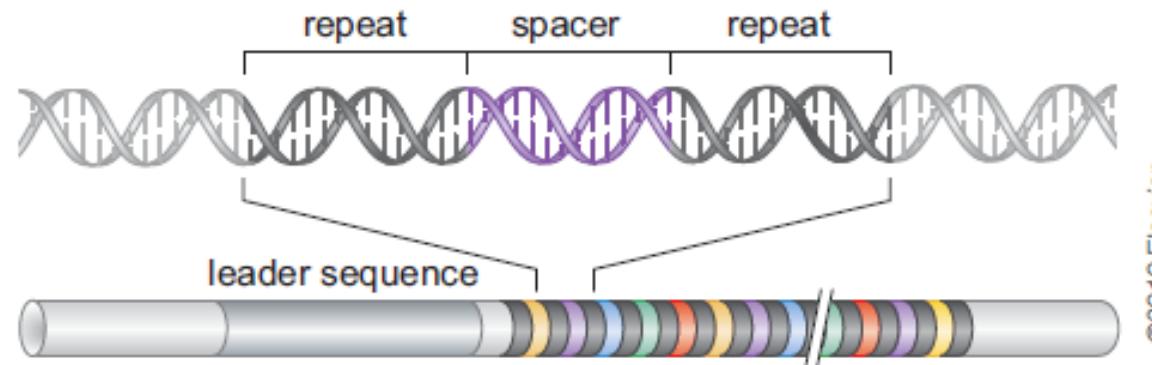


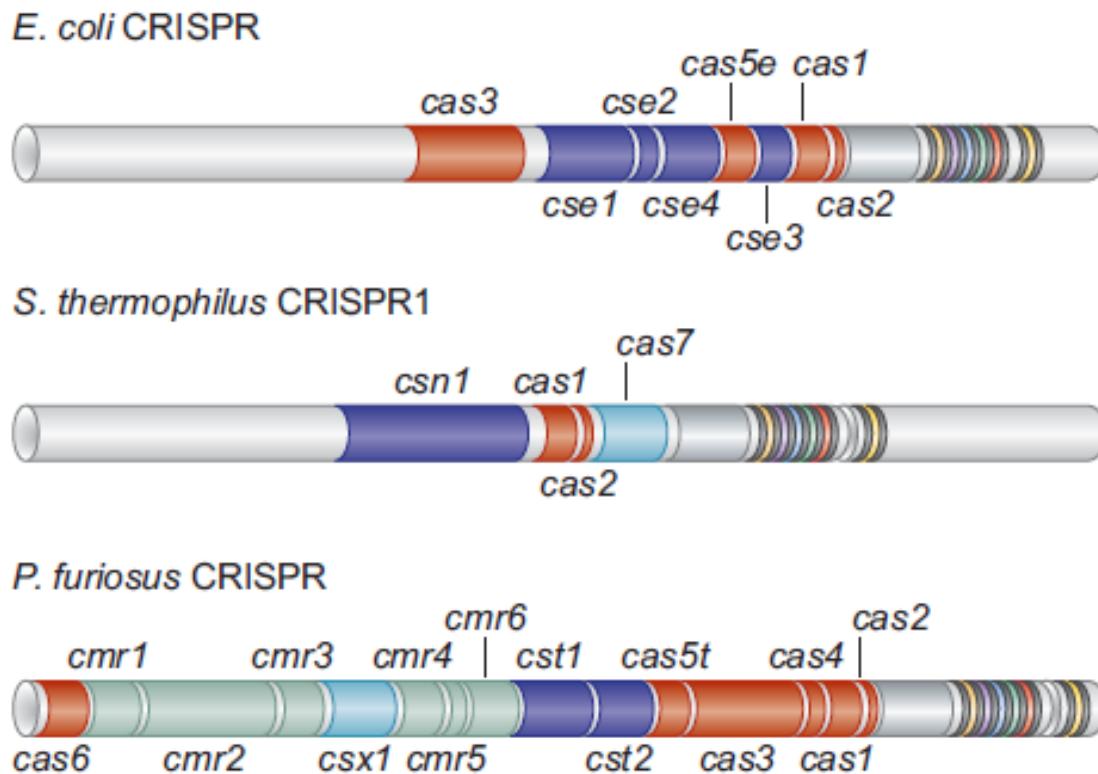
FIGURE 20-6 The organization of the CRISPR locus. The conserved repeat sequences and variable spacer sequences are shown at the top. Underneath is an array of such sequences (the number varies enormously); the proximal leader sequence is also shown. (Adapted, with permission, from Karginov F.V. and Hannon G.J. 2010. *Mol. Cell* 37: 7–19, Fig. 1A,B, p. 8. © Elsevier.)

CRISPR – what do they do?

- CRISPR spacer regions identical to regions of known phages or plasmids
- Targeted weapons of mass destruction?
- Resistance against phages if spacer sequences identical to phage gDNA
 - The more spacers, the more efficient resistance
 - Mutations in those regions abolish resistance

CRISPR – not only DNA

- A set of conserved proteins associated with the CRISPR sequences

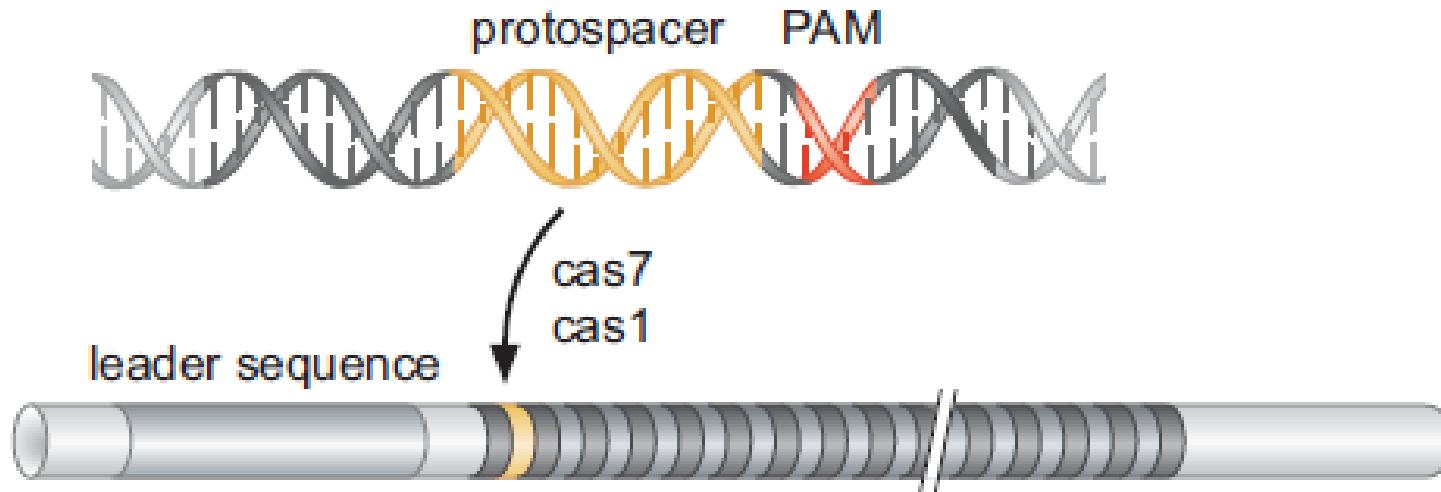


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FIGURE 20-7 The organization of *cas* genes at three CRISPR loci. The varying numbers, orientations, and types of *cas* gene are shown at three well-studied CRISPR loci. The core *cas* genes are shown in red. The repeat and spacer sequences shown in Figure 20-6 are here at the right-hand end. (Adapted, with permission, from Karginov F.V. and Hannon G.J. 2010. *Mol. Cell* 37: 7–19, Fig. 1C, p. 8. © Elsevier.)

CRISPR spacer sequences

- New spacer sequences are acquired from infecting viruses
 - Added to the proximal end, near the leader sequence
 - PAM (proto-spacer adjacent motif) required



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FIGURE 20-8 The mechanism of spacer sequence acquisition. Each new spacer sequence is inserted next to the leader sequence, with the consequence that the array is a temporal record of acquisitions past. The sequence destined to become a spacer is, within the phage genome, known as a “proto-spacer” and lies adjacent to a PAM sequence as described in the text. (Adapted, with permission, from Karginov F.V. and Hannon G.J. 2010. *Mol. Cell* 37: 7–19, Fig. 2B, p. 10. © Elsevier.)

CRISPR – how does it work?

crRNA - crispr RNA

- CRISPR is transcribed as a single long RNA (pre-crRNA)
- Processed into shorter crRNAs
 - 8 nucleotides of the 5' repeat + one spacer + most of the next repeat sequence
- Act either on foreign DNA or RNA

CRISPR

- *Escherichia coli*
- targets DNA
- *Pyrococcus furiosus*
targets RNA

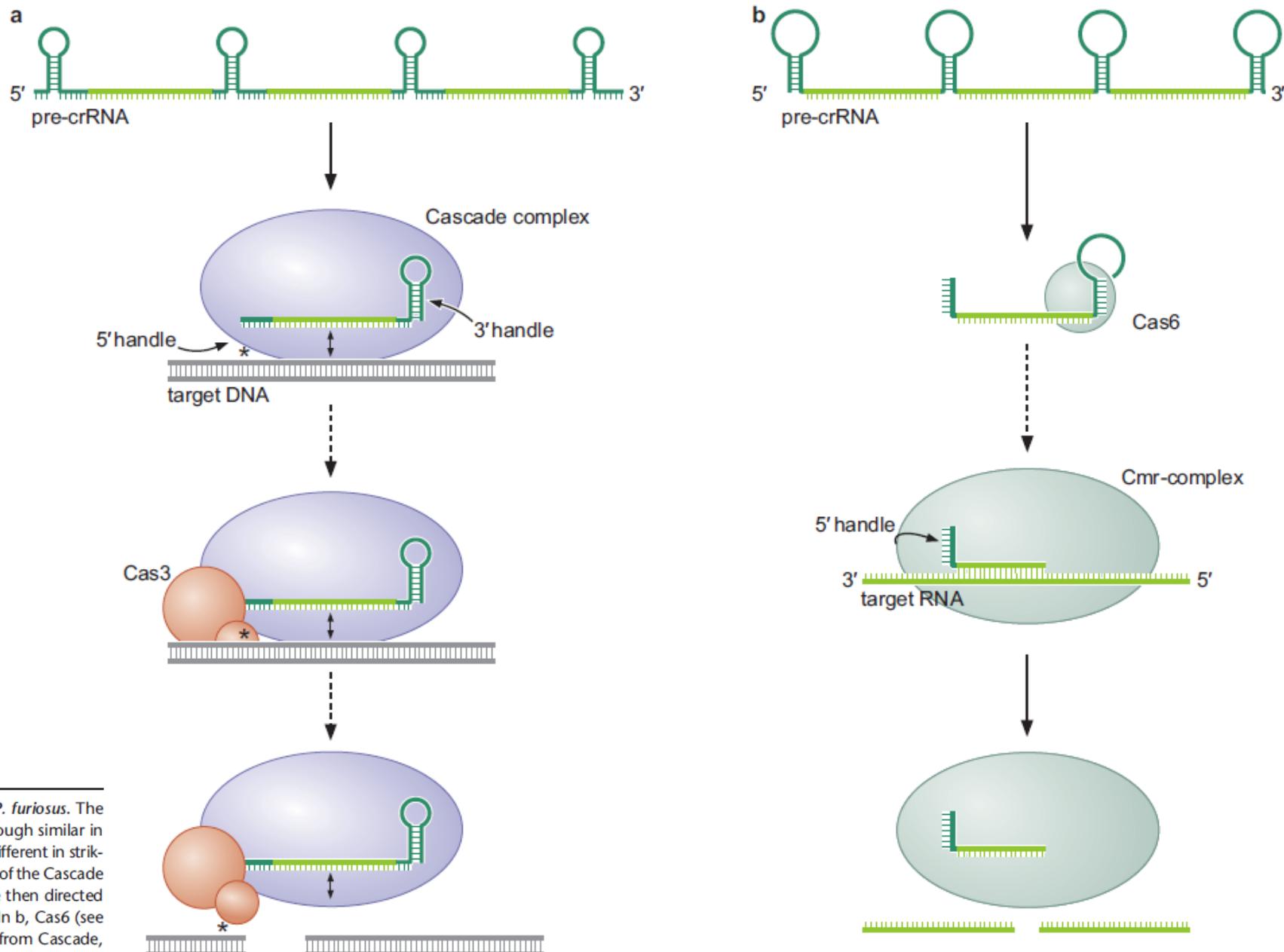


FIGURE 20-9 The antiviral operation of the CRISPR loci from *E. coli* and *P. furiosus*. The *E. coli* (a) system targets incoming DNA, whereas *P. furiosus* (b) targets RNA. Although similar in many ways, the processing mechanism and final operation of the two systems are different in striking ways as outlined in the text. In a, the pre-crRNA is processed by the CasE subunit of the Cascade complex (CasE is encoded by the *cse3* gene in Fig. 20-7). crRNA and Cascade are then directed to and cleave the target DNA with the help of Cas3 in ways not fully understood. In b, Cas6 (see Fig. 20-7) processes the pre-crRNA, and this, in complex with a complex distinct from Cascade, targets viral RNA in a mechanism strikingly analogous to the RNAi system in eukaryotes. (Adapted from Jore M.M. et al. 2012. *Cold Spring Harb. Perspect. Biol.* 4: a003657. © Cold Spring Harbor Laboratory Press.)

RISC does the heavy lifting

- RISC complex (RNA-induced silencing complex)
 - Contains also proteins (Argonaute family members), in addition to the small RNA
- guide RNA – denatured small RNA that gives the RISC specificity
- passenger RNA – usually discarded
 - If guide RNA:target RNA basepairing is highly complementary (siRNAs), mRNA gets degraded (Slicer)
 - If guide RNA:target RNA basepairing is less complementary (miRNAs), then often translation is inhibited

Why RNAi (RNA interference)?

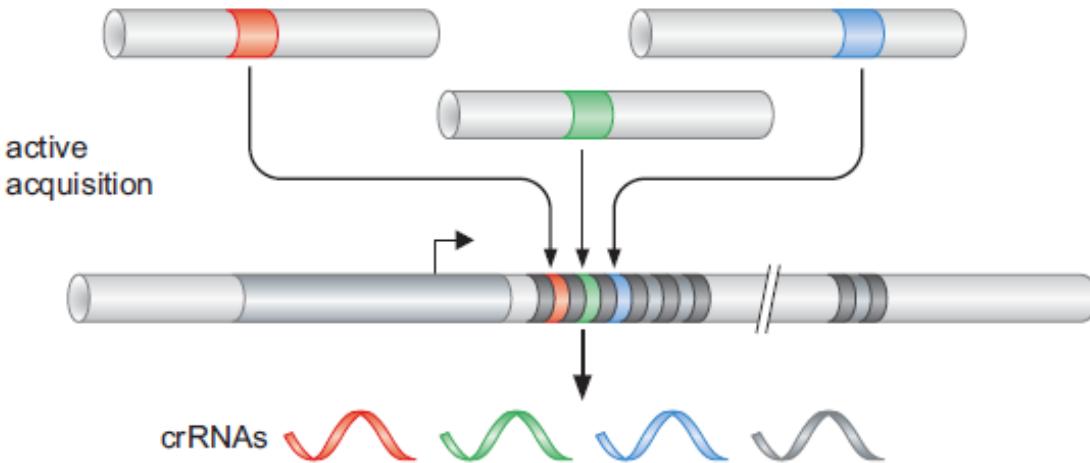
- Some organisms have RNAi machinery but no miRNAs (*S. pombe*)
- miRNAs evolved to take advantage of existing RNAi machinery
- Defence mechanism?

Why RNAi?

- piRNAs
 - Arise from long, single-stranded transcripts of piRNA clusters (“transposon graveyards”)
 - Target nucleic acid parasites – transposons
 - Expressed in germline, why?
- In plants, when one leaf is infected by a virus, silencing factor spreads through the whole plant
 - Does not protect originally infected leaf but stops the infection from spreading
 - Viruses fight back – carry genes to counteract RNAi

CRISPR vs piRNA

a CRISPR system



b piRNA system

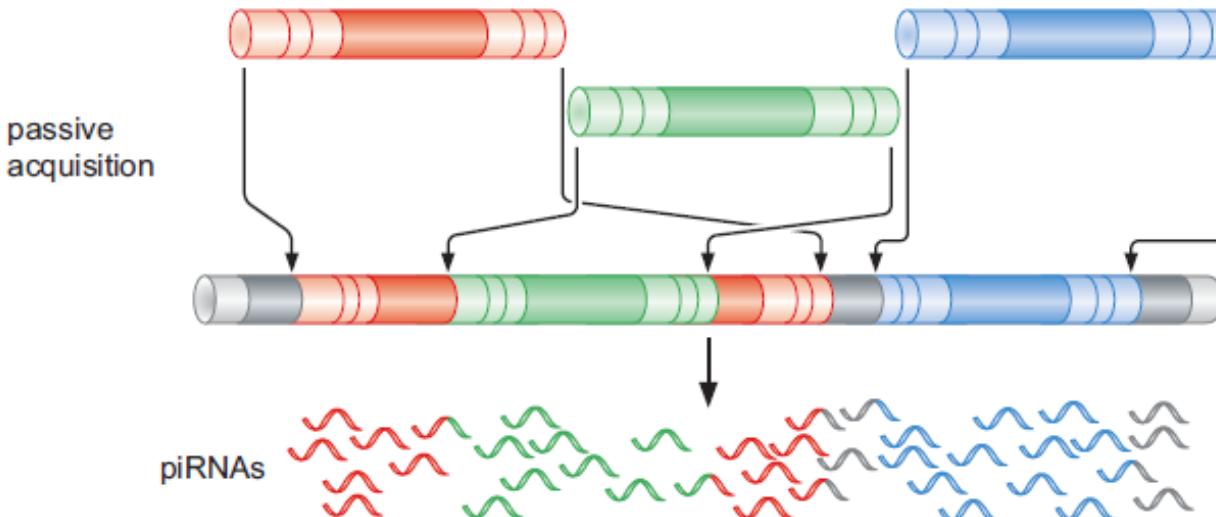


FIGURE 20-19 Comparison of the bacterial CRISPR (a) and animal piRNA (b) defense systems. Although many features are analogous, the molecular components are not conserved. In addition, as discussed in the text, whereas CRISPR actively acquires new spacer sequences from infecting phage (see Fig. 20-8), the transposon sequences that prime the piRNA system arrive passively within the piRNA clusters. (Adapted from Karginov F.V. and Hannon G.J. 2010. *Mol. Cell* 37: 7–19, Fig. 5, p. 16. © Elsevier.)



Long regulatory RNAs in eukaryotes

- X inactivation
 - Dosage compensation
 - Random choice or non-random choice
 - More tightly compacted
 - Methylation
 - Acetylathion

Calico or tortoiseshell cat



FIGURE 20-21 Visualizing X-inactivation: the calico cat. The patches of orange and black fur provide an indirect visualization of X-chromosome inactivation, as described in the text. (Courtesy VG.)

The fur colour can be chosen by activating or non-activating the specialized region of X-chromosome

A consequence of inactivation being random in each cell is that females are mosaics—some of their cells express the paternal and others the maternal X chromosome.

A more familiar example is the calico (or tortoiseshell) cat (Fig. 20-21).

In cats, one gene on the X chromosome influences whether fur is orange or black—one allele of that gene gives rise to orange fur, and another allele gives black.

In cats heterozygous for this gene, the different patches of black and orange fur reveal regions made up of cells in which one or the other X chromosome was inactivated - methylated.

This observation also explains why all calico cats are female.

The white fur colour comes from effects of an autosomal gene.

How does a cell choose which X chromosome to inactivate?

The answer is still proving elusive, but another RNA regulator might be key. This other RNA is also encoded by the **Xic** locus but on the opposite strand and overlapping the *Xist* gene. It is called **Tsix** (*Xist* spelled backward) and acts as a negative regulator of *Xist* (Fig. 20-23). Indeed, if *Tsix* is mutated on a given **X chromosome**, it is that chromosome that will be chosen for inactivation. Thus, a **balance between the production and stability of the *Xist* and *Tsix* RNAs** may tilt the outcome one way or the other in each cell. – **That kind of dosage compensation is necessary in all animals.**

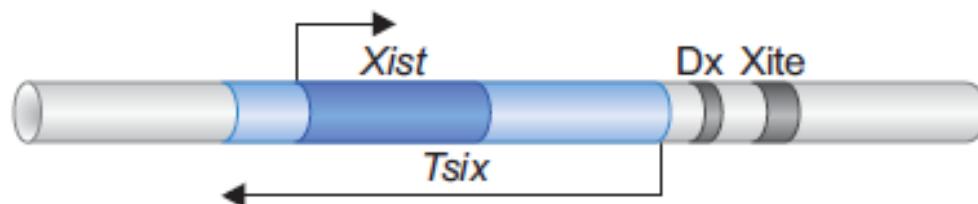
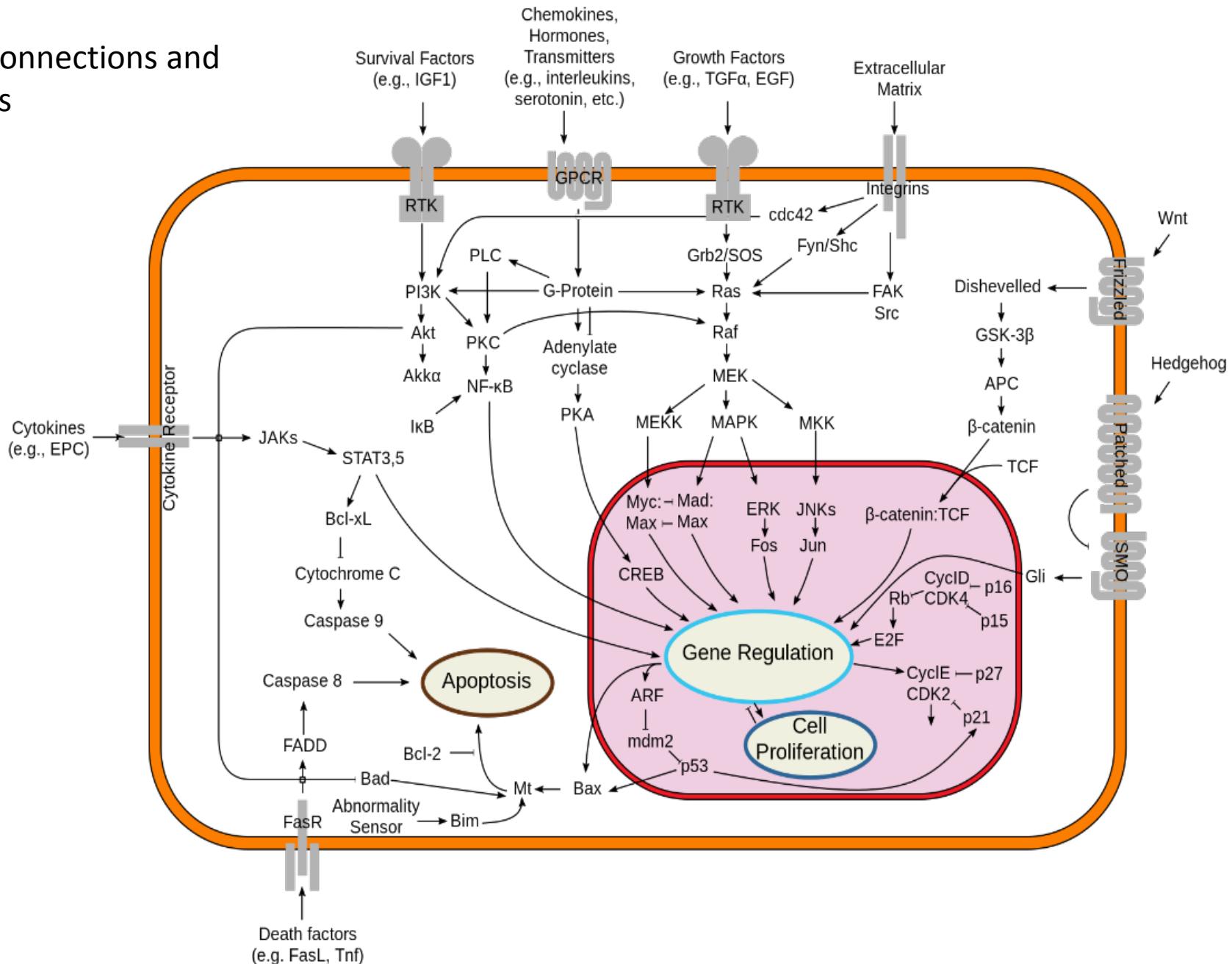


FIGURE 20-23 *Tsix* antagonizes expression and action of *Xist*. *Tsix* (shown in light blue shading) is expressed as an antisense RNA of *Xist* (in dark blue shading) and is longer than *Xist*. The degree of overlap is indicated in the dark blue region. Xite and DxPas34 (Dx) are regulatory elements that control expression of the genes. At the start of inactivation, both *Xist* and *Tsix* are expressed from both X chromosomes, but after awhile, the chromosome that will become inactivated increases expression of *Xist*, whereas expression from that chromosome destined to remain active decreases *Xist* expression. How this change in *Xist* levels is regulated by *Tsix* is still not clear, but if *Tsix* is deleted from either chromosome, it is always that copy that becomes inactivated.

Some examples of connections and regulatory processes in the cell



The Eagle, Cambridge

Discovery of DNA

**On this spot, on February 28, 1953, Francis Crick
and James Watson made the first public
announcement of the discovery of DNA with the
words "We have discovered the secret of life".
Throughout their early partnership Watson & Crick
dined in this room on six days every week**