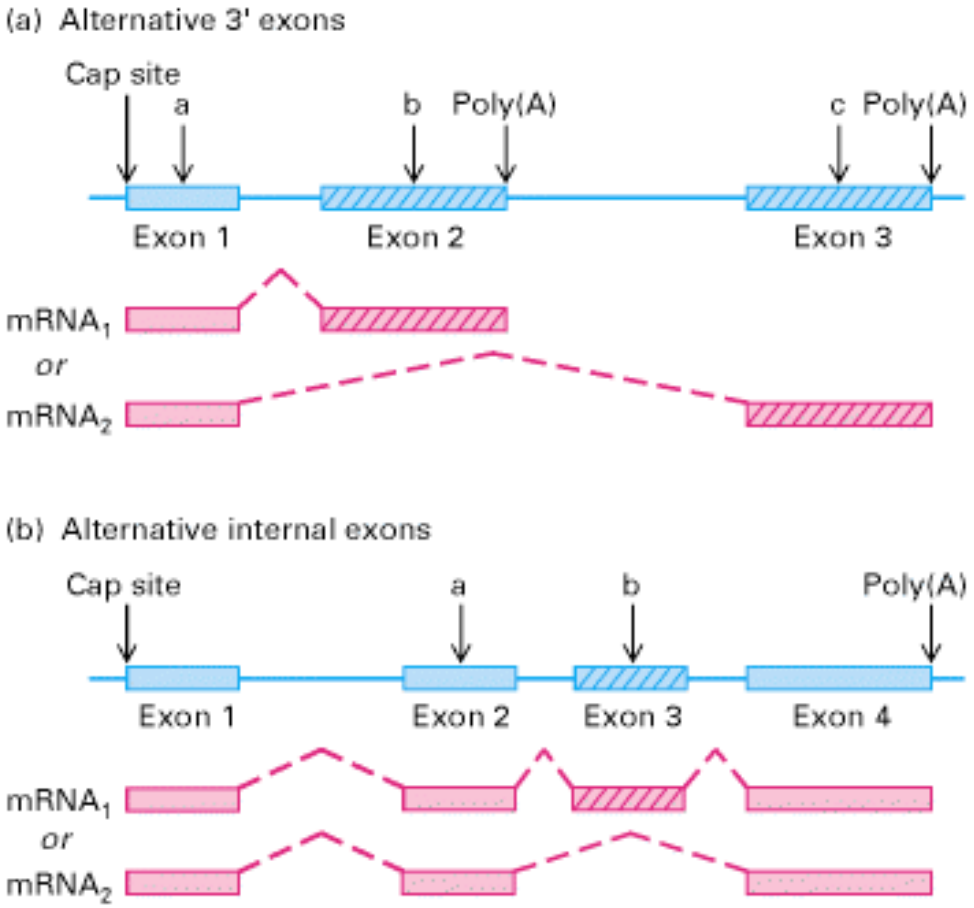
Alternative RNA processing:
Two examples of complex eukaryotic transcription units and the effect of mutations on expression of the encoded proteins.

The RNA transcribed from a complex transcription unit (blue) can be processed in alternative ways to yield two or more functional monocistronic mRNAs. Dashed lines indicate spliced-out introns.

(a) A complex transcription unit whose primary transcript has two poly(A) sites produces two mRNAs with alternative 3′ exons.

(b) A complex transcription unit whose primary transcript undergoes exon skipping during processing produces alternative mRNAs with the same 5′ and 3′ exons. In this example, some cell types would express the mRNA including exon 3, whereas in other cell types, exon 2 is spliced to exon 4, producing an mRNA lacking exon 3 and the protein sequence it encodes.

In (a) and (b), mutations (designated *a*) within exons shared by the alternative mRNAs (solid red) affect the proteins encoded by both alternatively processed mRNAs. In contrast, mutations (designated *b* and *c*) within exons unique to one of the alternatively processed mRNAs (red with diagonal lines) affect only the protein encoded by that mRNA.



Negative and positive control of alternative RNA splicing.

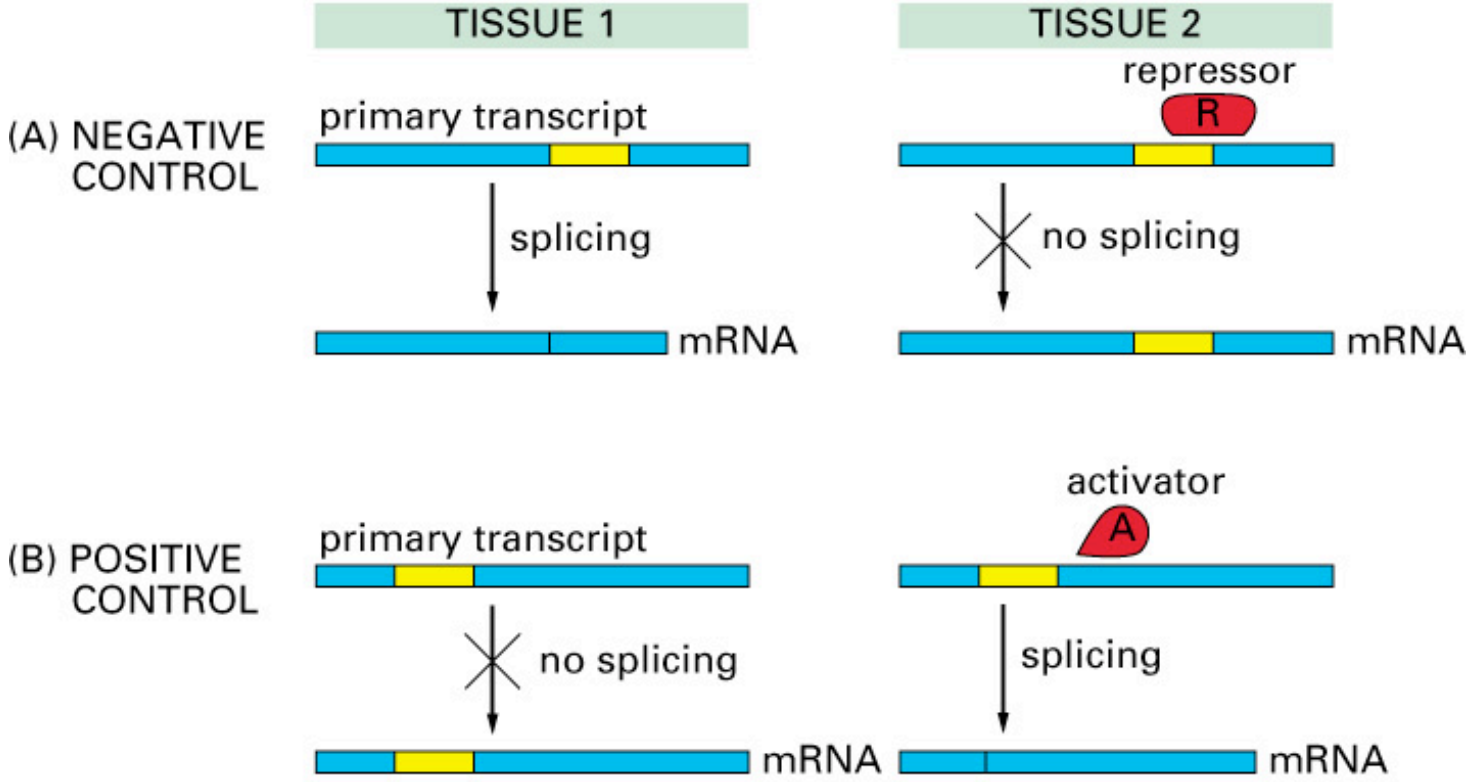
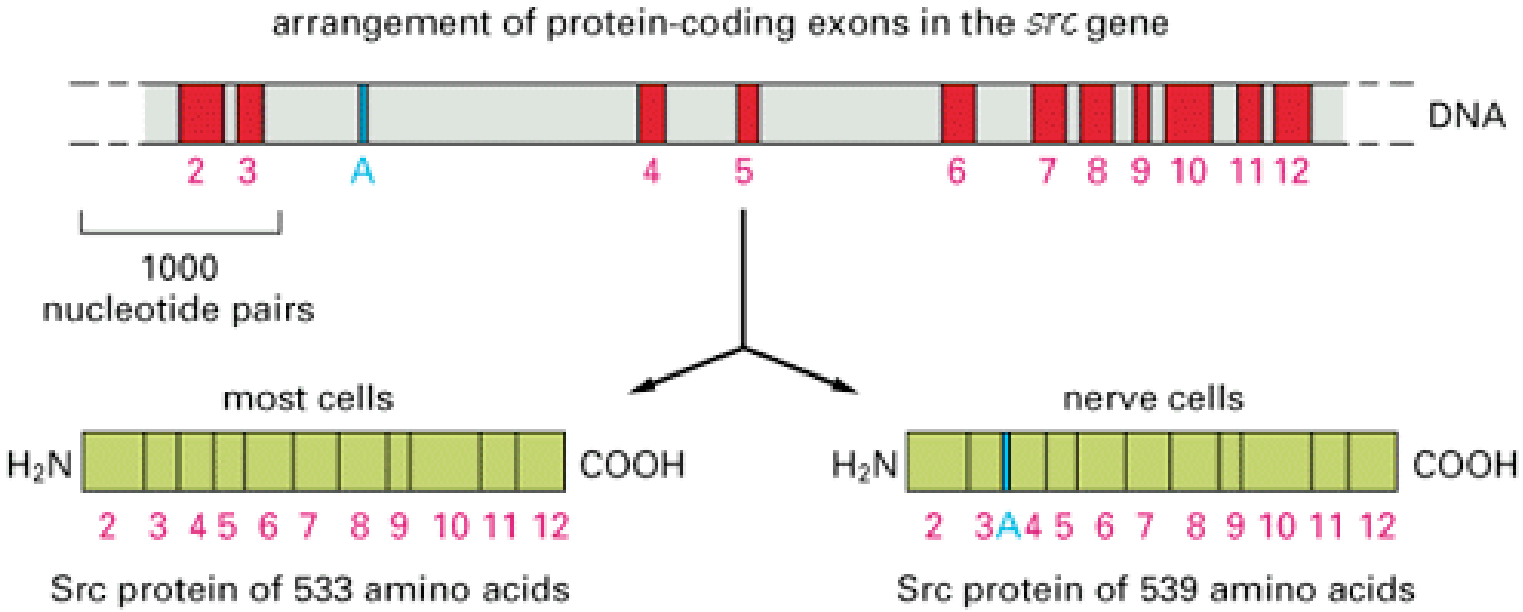


Figure 7–90. Molecular Biology of the Cell, 4th Edition.

(A) Negative control, in which a repressor protein binds to the primary RNA transcript in tissue 2, thereby preventing the splicing machinery from removing an intron sequence.

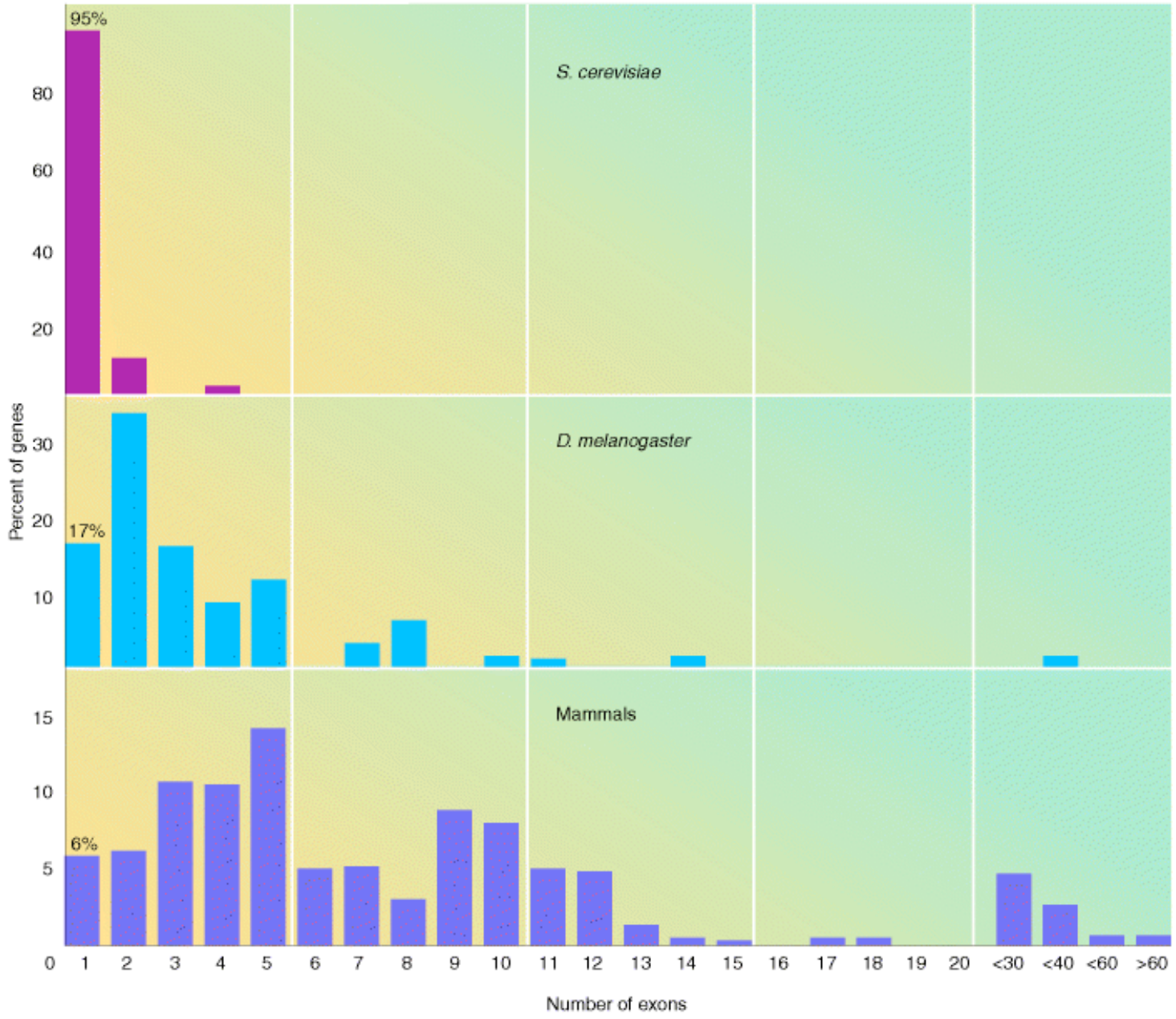
(B) Positive control, in which the splicing machinery is unable to remove a particular intron sequence without assistance from an activator protein.

Regulated alternative RNA splicing produces cell-type-specific forms of a gene product.



Here two slightly different tyrosine protein kinases are produced from the *src* gene because exon sequence A is included only in nerve cells. The neural form of the Src protein contains an extra site for phospho-rylation and is also thought to have a higher specific activity. Only the protein-coding exons (*colored*) are shown in this diagram (exon 1, which forms the 5' leader on the mRNA, is not shown). (After J.B. Levy et al., *Mol. Cell Biol.* 7:4142-4145, 1987.)

Distribution of exon numbers in the genes of three organisms.



Major Points

1. **Primary eukaryote RNA transcripts can undergo alternative splicing events giving rise to multiple gene products**

2. **The regulation of alternative splicing is mediated by both negative positive protein factors**

3. **Splice variants and their gene products can account for a significant percentage of the added "complexicity" of higher eukaryotes**

RNAi regulates numerous biological processes

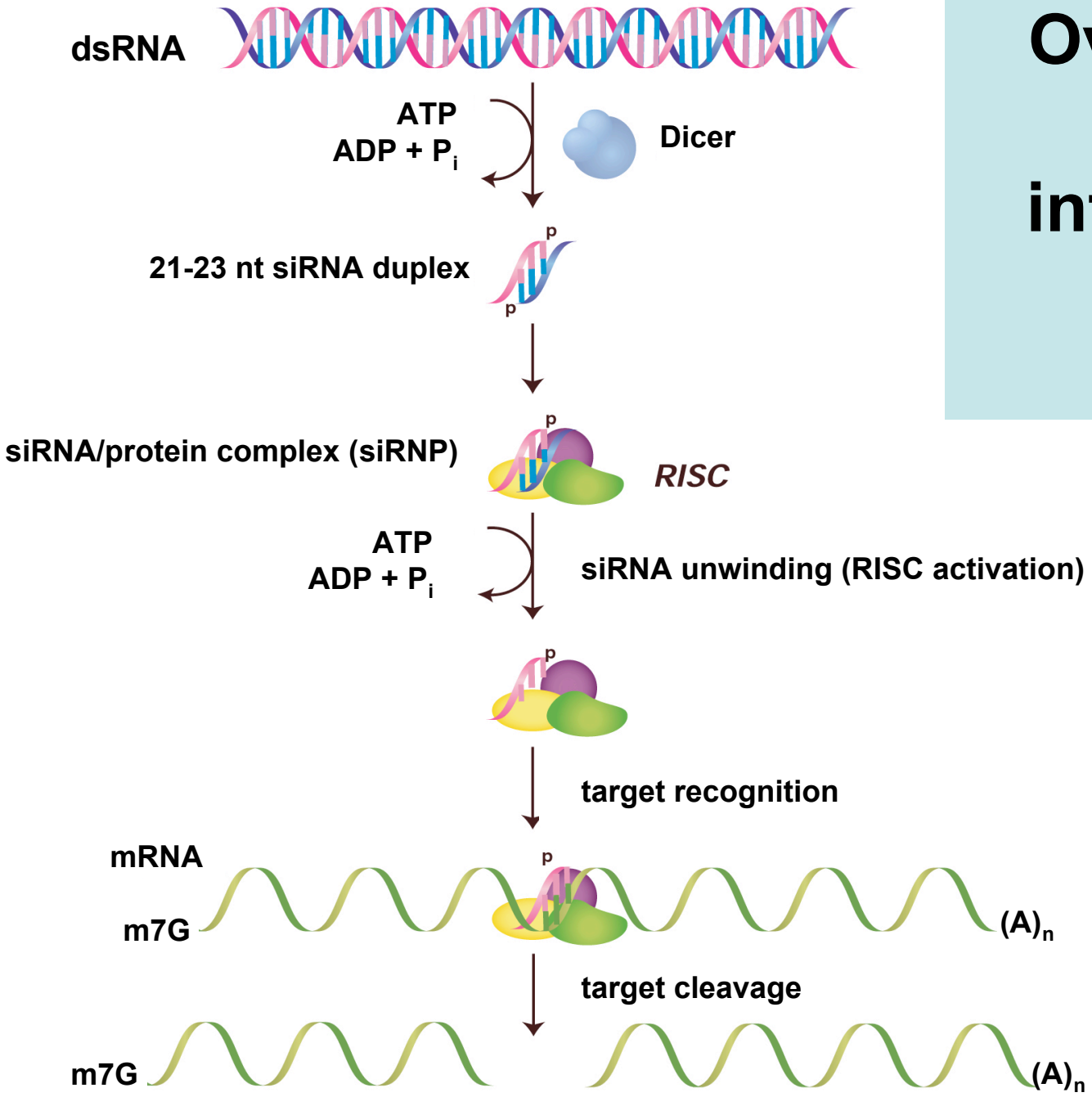
1. RNAi is required for chromosome segregation, differentiation, and limb patterning in mammals.

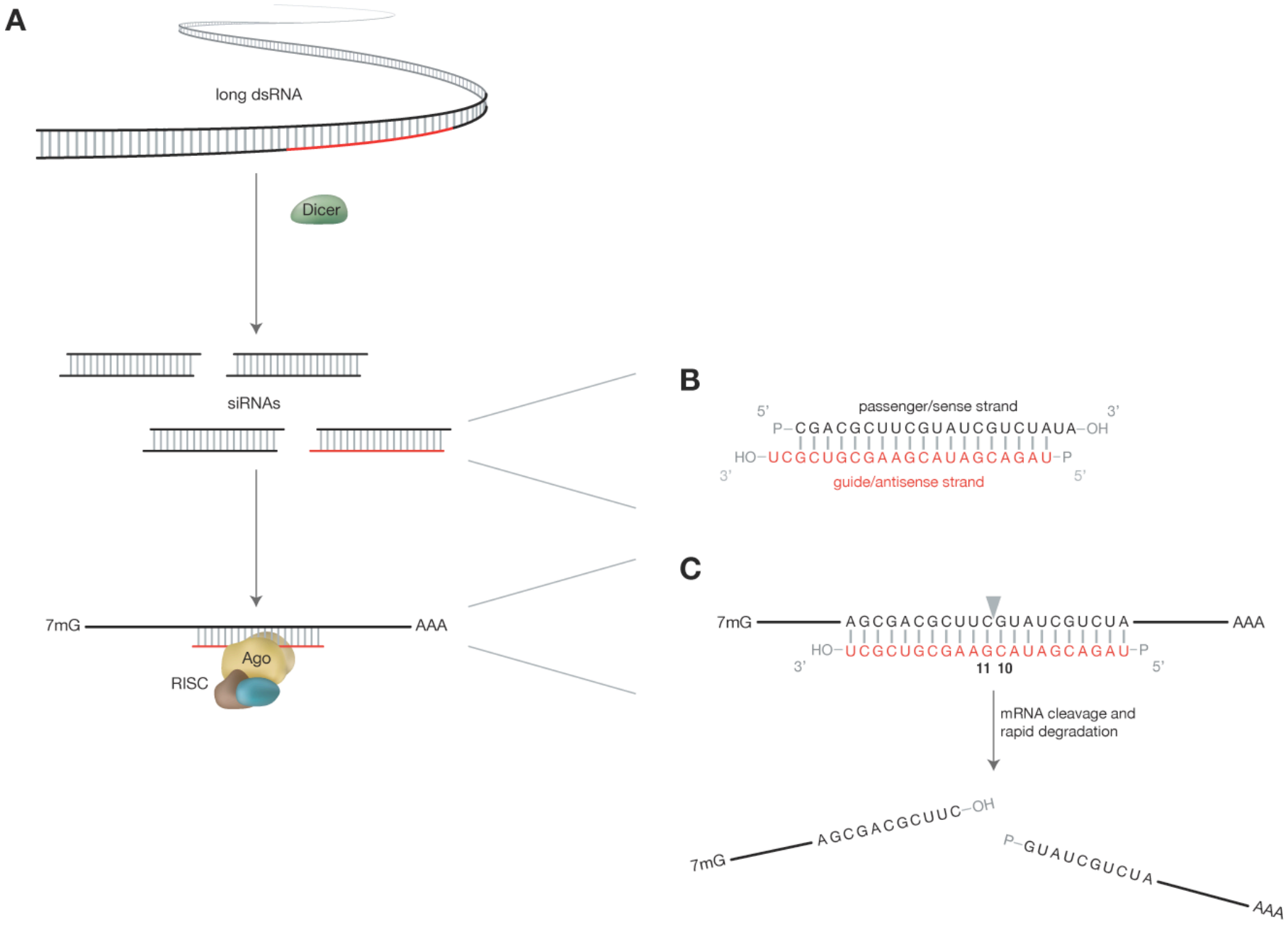
2. Many microRNAs are conserved across phyla.

3. More than 1/3 of all human genes are potential microRNA targets.

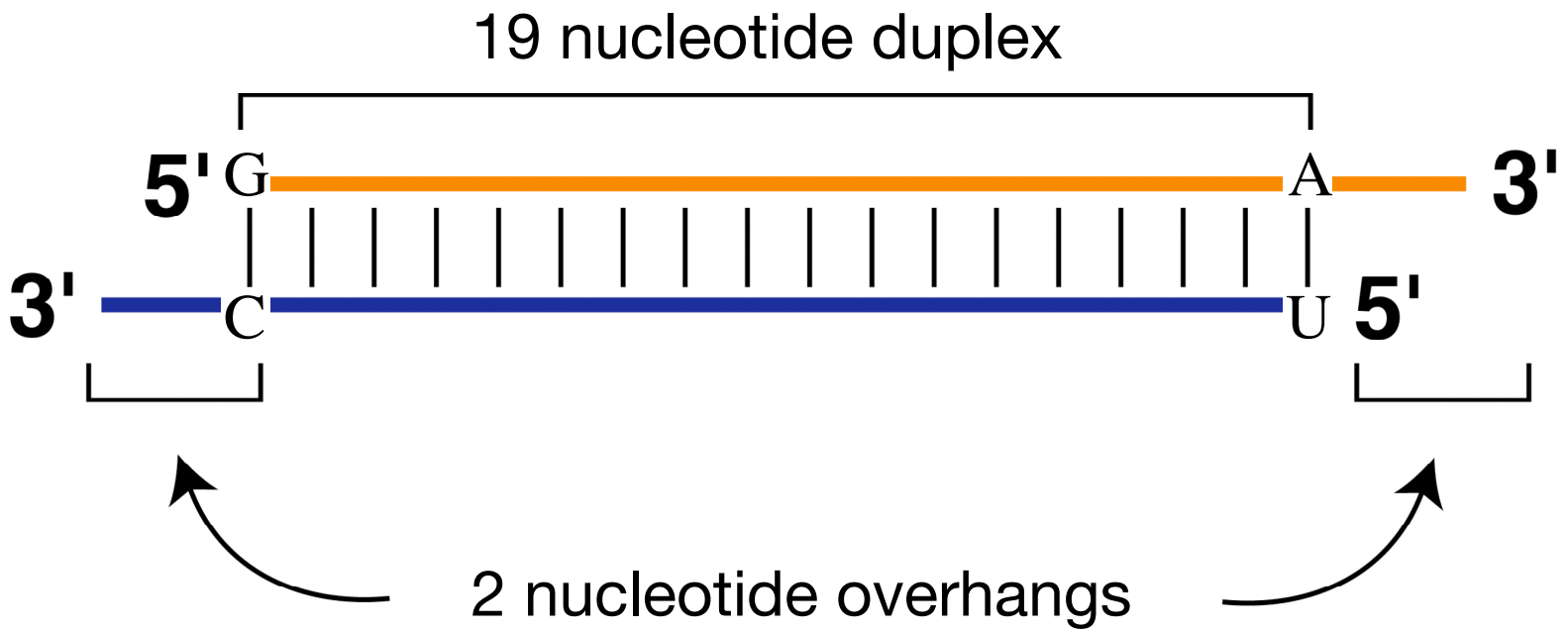
4. microRNAs have been linked to several diseases including diabetes and cancer.

Overview of RNA interference

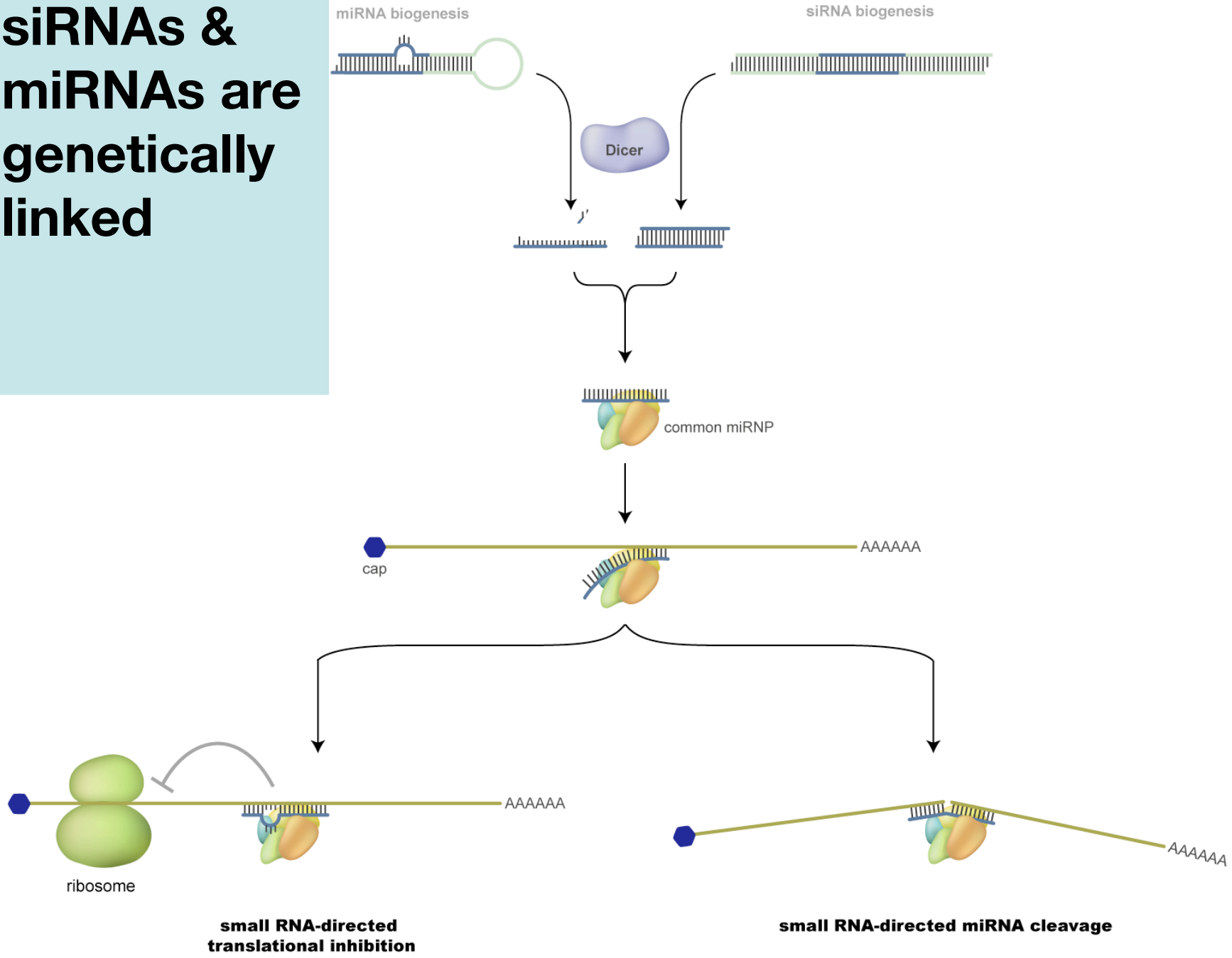



A
long dsRNA
Dicer
siRNAs
7mG
AAA
Ago
RISC
B
5'
passenger/sense strand
3'
P–CGACGCUUCGUAUCGUCUAUA–OH
HO–UCGCUGCGAAGCAUAGCAGAU–P
3'
5'
guide/antisense strand
C
7mG
AGCGACGCUUCGUAUCGUCUA
AAA
HO–UCGCUGCGAAGCAUAGCAGAU–P
3'
5'
11 10
mRNA cleavage and
rapid degradation
7mG
AGCGACGCUUC–OH
P–GUAUCGUCUA
AAA

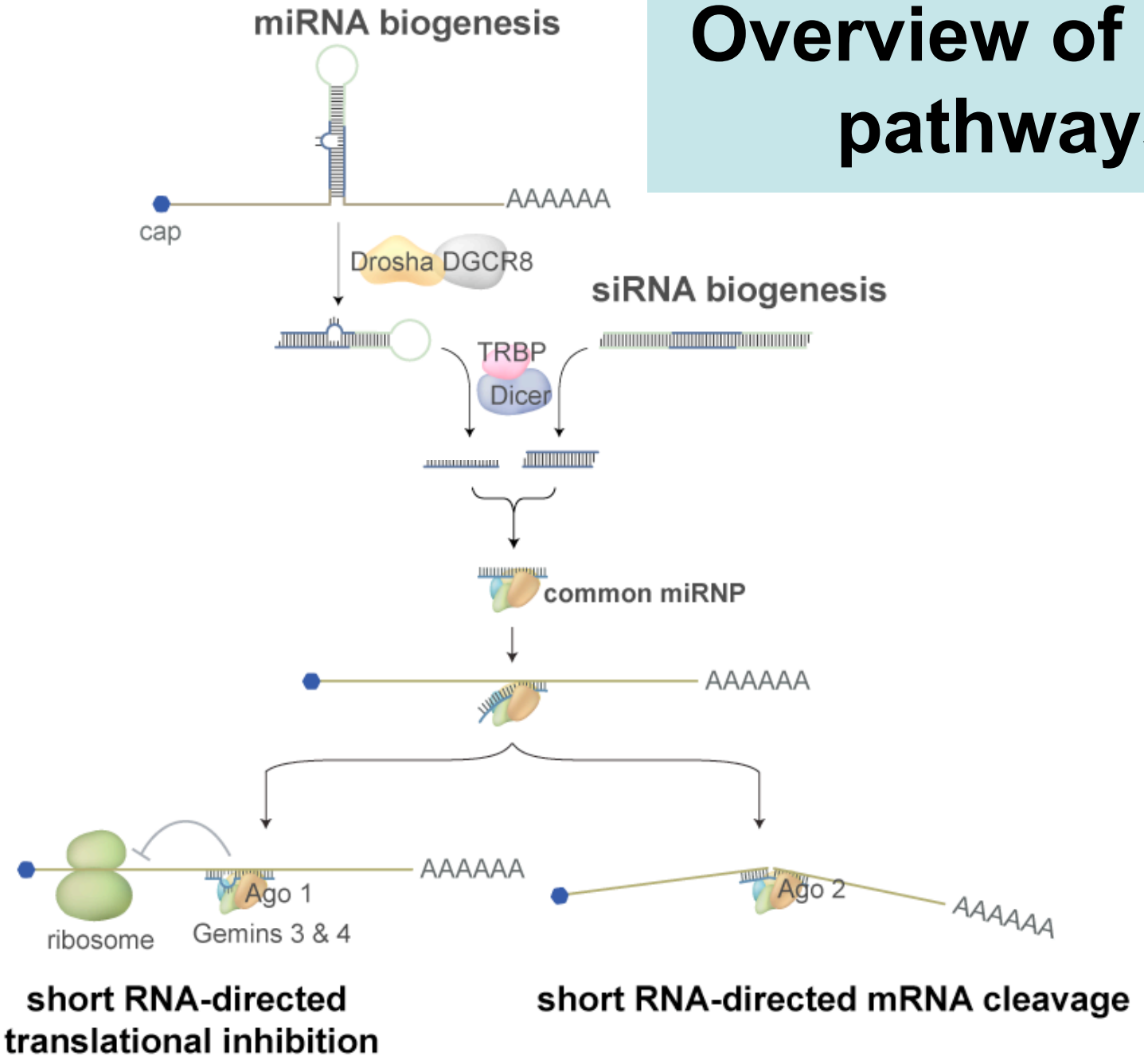
siRNAs have the molecular hallmarks of an RNaseIII-like cleavage



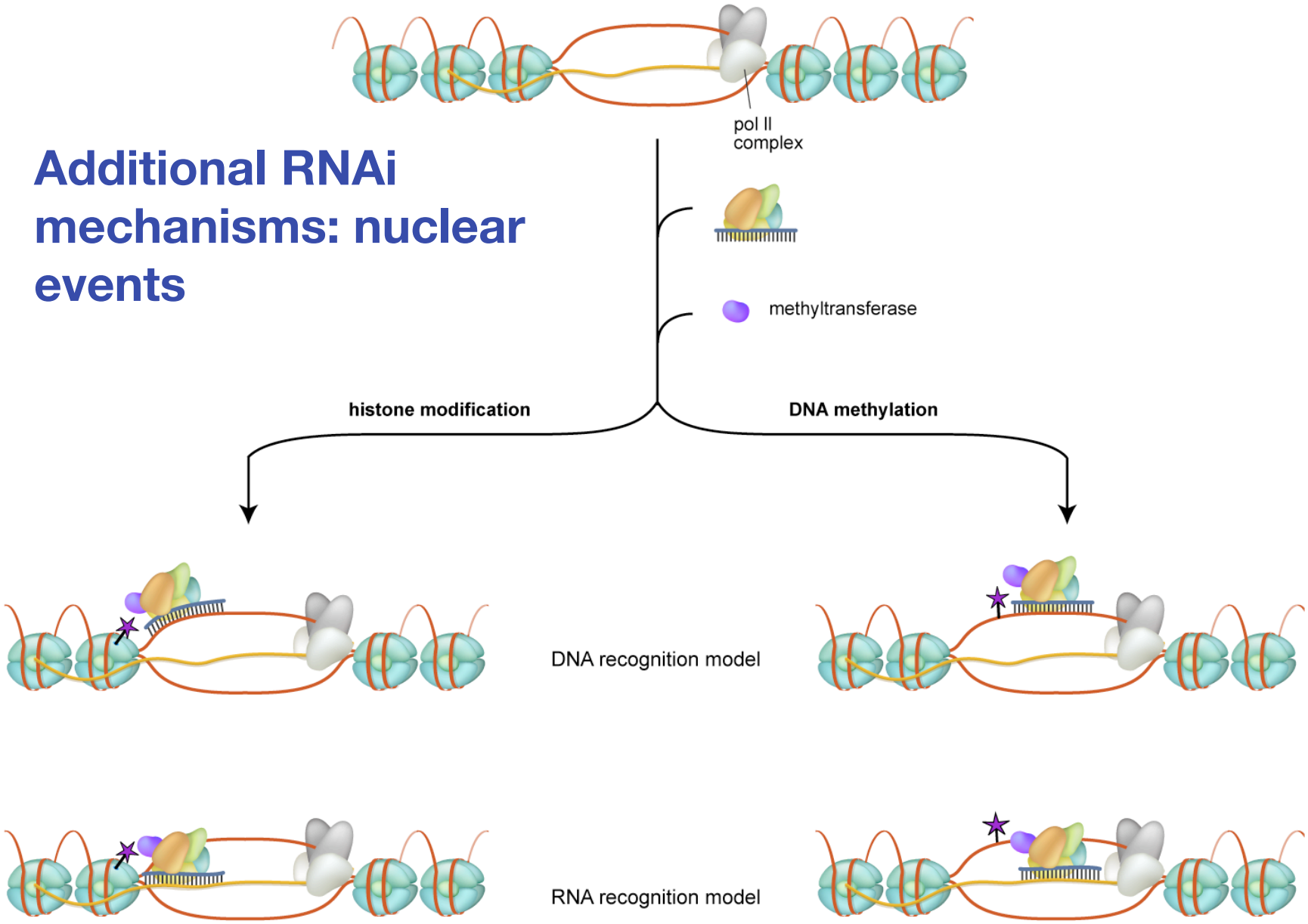
siRNAs & miRNAs are genetically linked



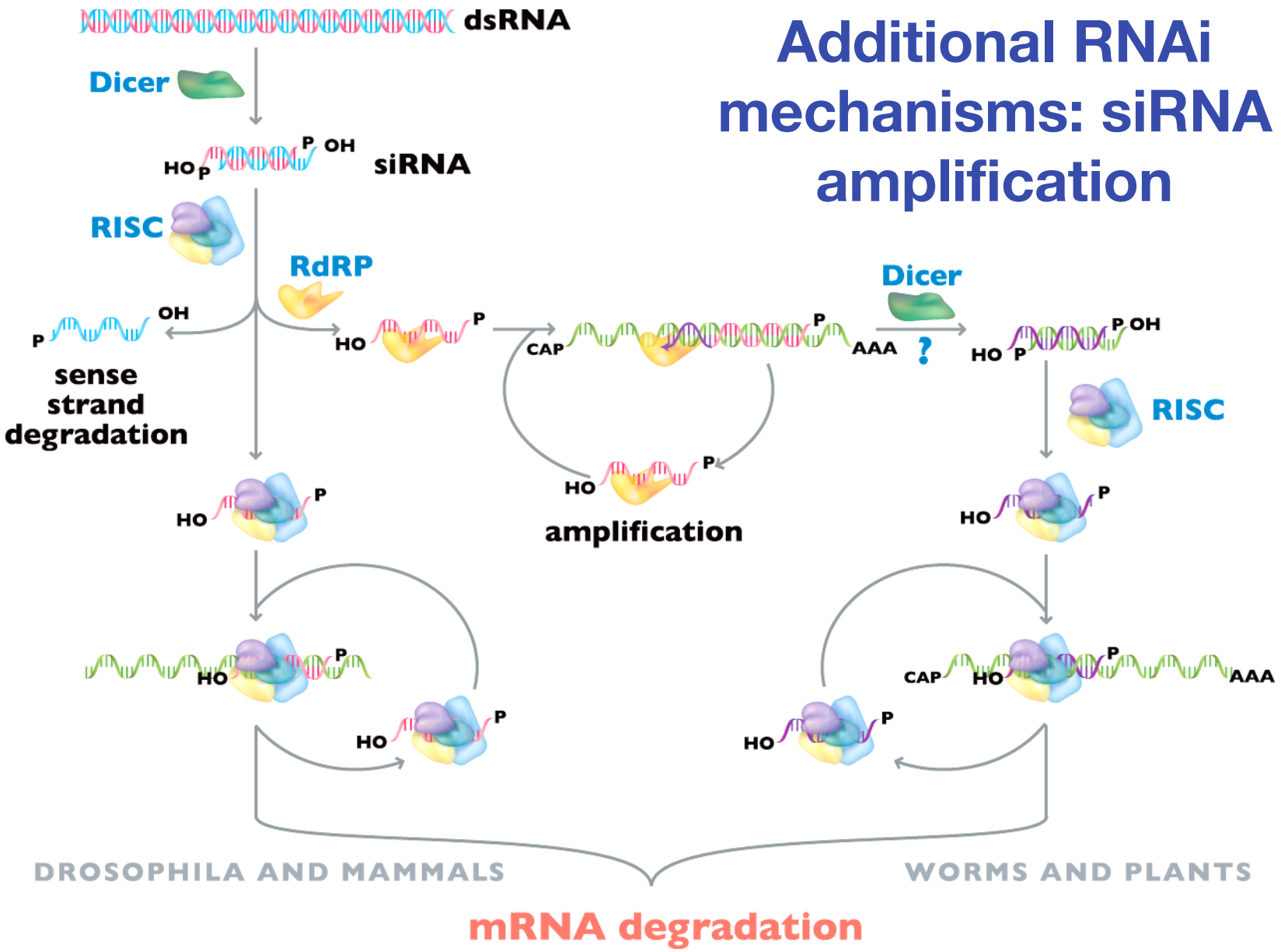
Overview of RNAi pathways



Additional RNAi mechanisms: nuclear events



Additional RNAi mechanisms: siRNA amplification



Major Points

1. **Double stranded RNA in eukaryotic cells undergo a set of processing events that generate "anti-sense" small interfering RNAs that regulate gene expression**

2. **siRNA can target specific genes by interfering with either mRNA integrity or protein synthesis**

3. **There are multiple classes of small regulatory RNA's including siRNAs and miRNAs**

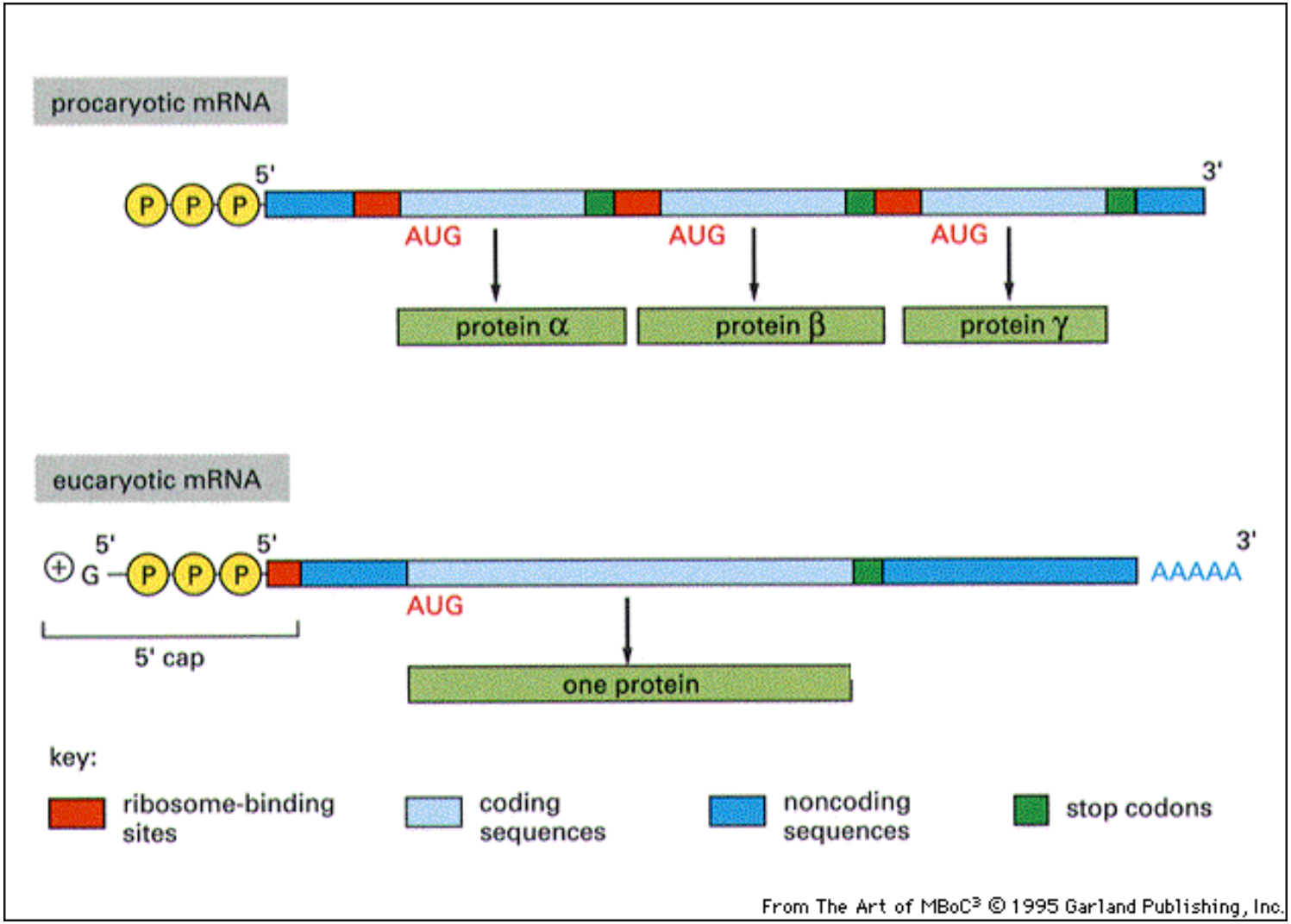
4. **siRNAs can also regulate gene expression by other mechanisms involving chromatin or DNA modifications**

Protein Translation

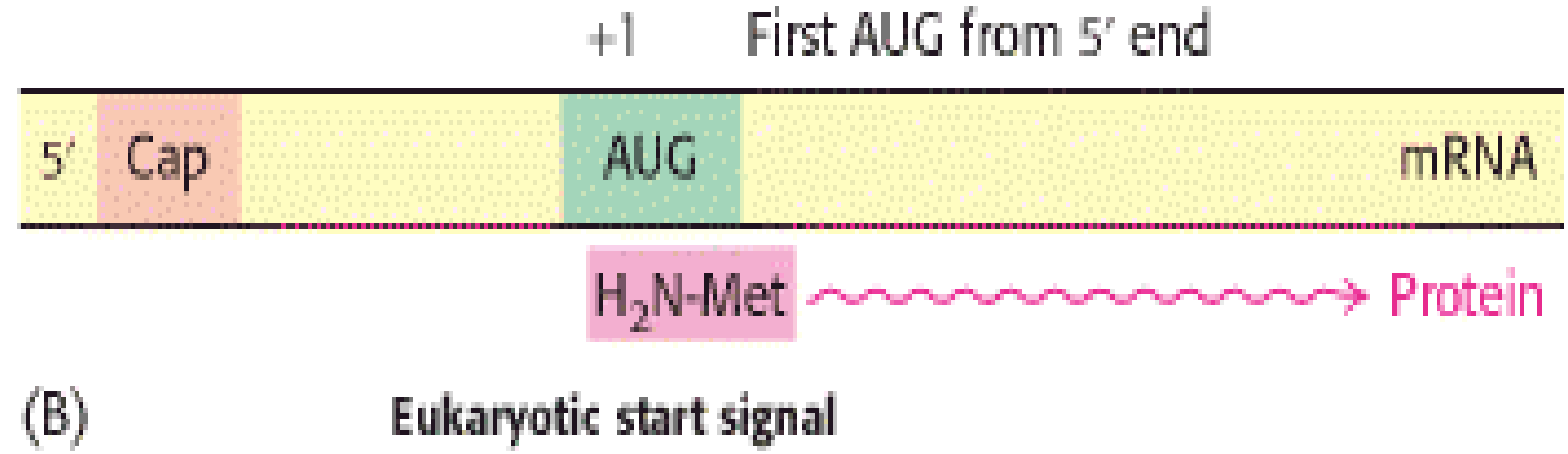
The genetic code

Protein synthesis Machinery

Ribosomes, tRNA's



A comparison of the structures of procaryotic and eucaryotic messenger RNA molecules. Although both mRNAs are synthesized with a triphosphate group at the 5' end, the eucaryotic RNA molecule immediately acquires a 5' cap, which is part of the structure recognized by the small ribosomal subunit. Protein synthesis therefore begins at a start codon near the 5' end of the mRNA. In procaryotes, by contrast, the 5' end has no special significance, and there can be multiple ribosome-binding sites (called *Shine-Delgarno sequences*) in the interior of an mRNA chain, each resulting in the synthesis of a different protein.

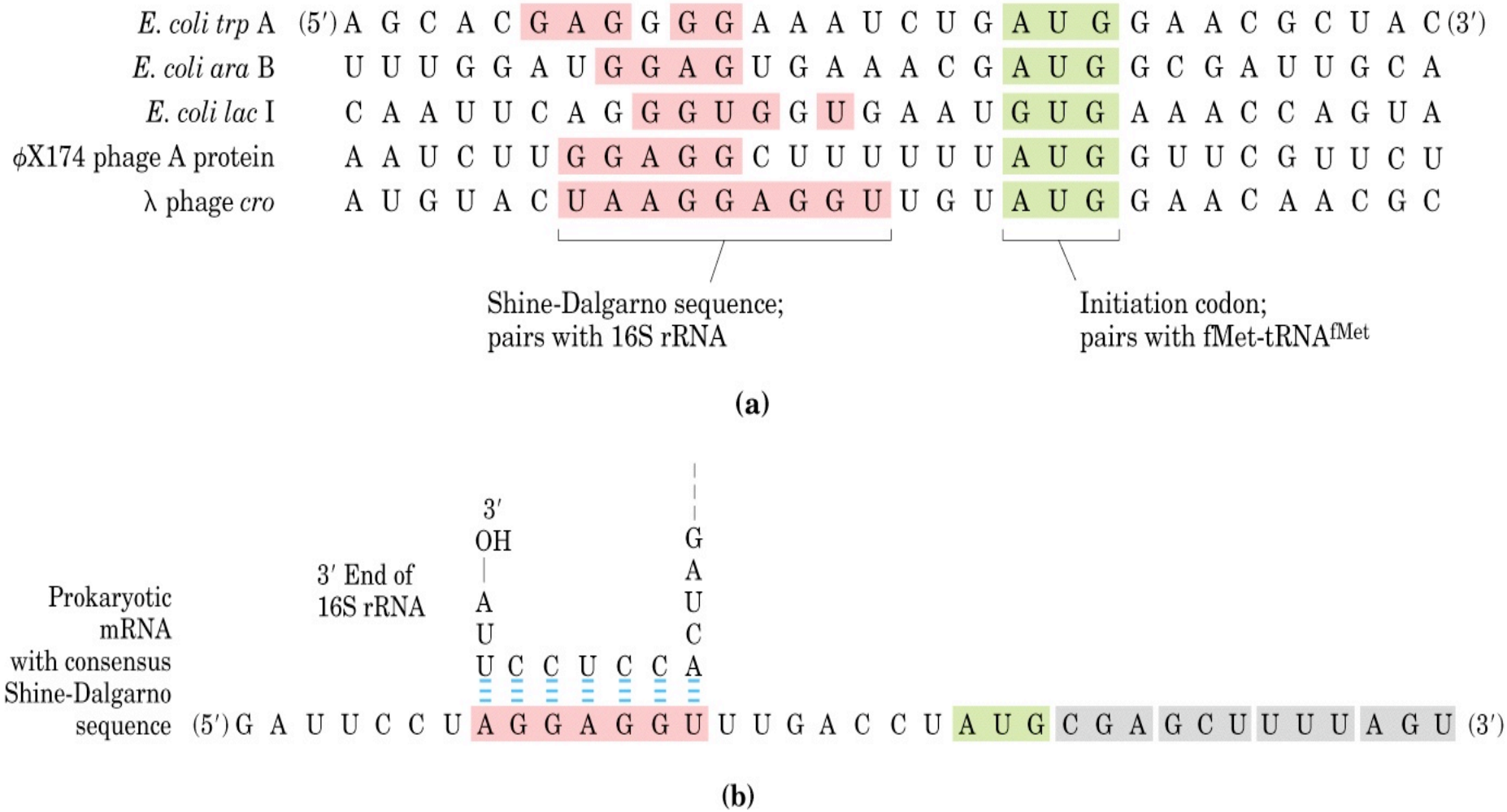


Start signals for the initiation of protein synthesis in (A) prokaryotes and (B) eukaryotes. In eukaryotic mRNAs the 5' end, called a cap, contains modified bases.

First position (5' end)	Second position				Third position (3' end)
	U	**C**	**A**	**G**	
U	UUU Phe UUC	UCU UCC UCA UCG Ser	UAU Tyr UAC	UGU Cys UGC	**U** **C**
	UUA Leu UUG		UAA Stop UAG Stop	UGA Stop UGG Trp	**A** **G**
C	CUU CUC CUA CUG Leu	CCU CCC CCA CCG Pro	CAU His CAC CAA CAG Gln	CGU CGC CGA CGG Arg	**U** **C** **A** **G**
A	AUU AUC Ile AUA	ACU ACC ACA ACG Thr	AAU Asn AAC AAA AAG Lys	AGU Ser AGC AGA AGG Arg	**U** **C** **A** **G**
	AUG Met[b]				
G	GUU GUC GUA GUG Val	GCU GCC GCA GCG Ala	GAU Asp GAC GAA GAG Glu	GGU GGC GGA GGG Gly	**U** **C** **A** **G**

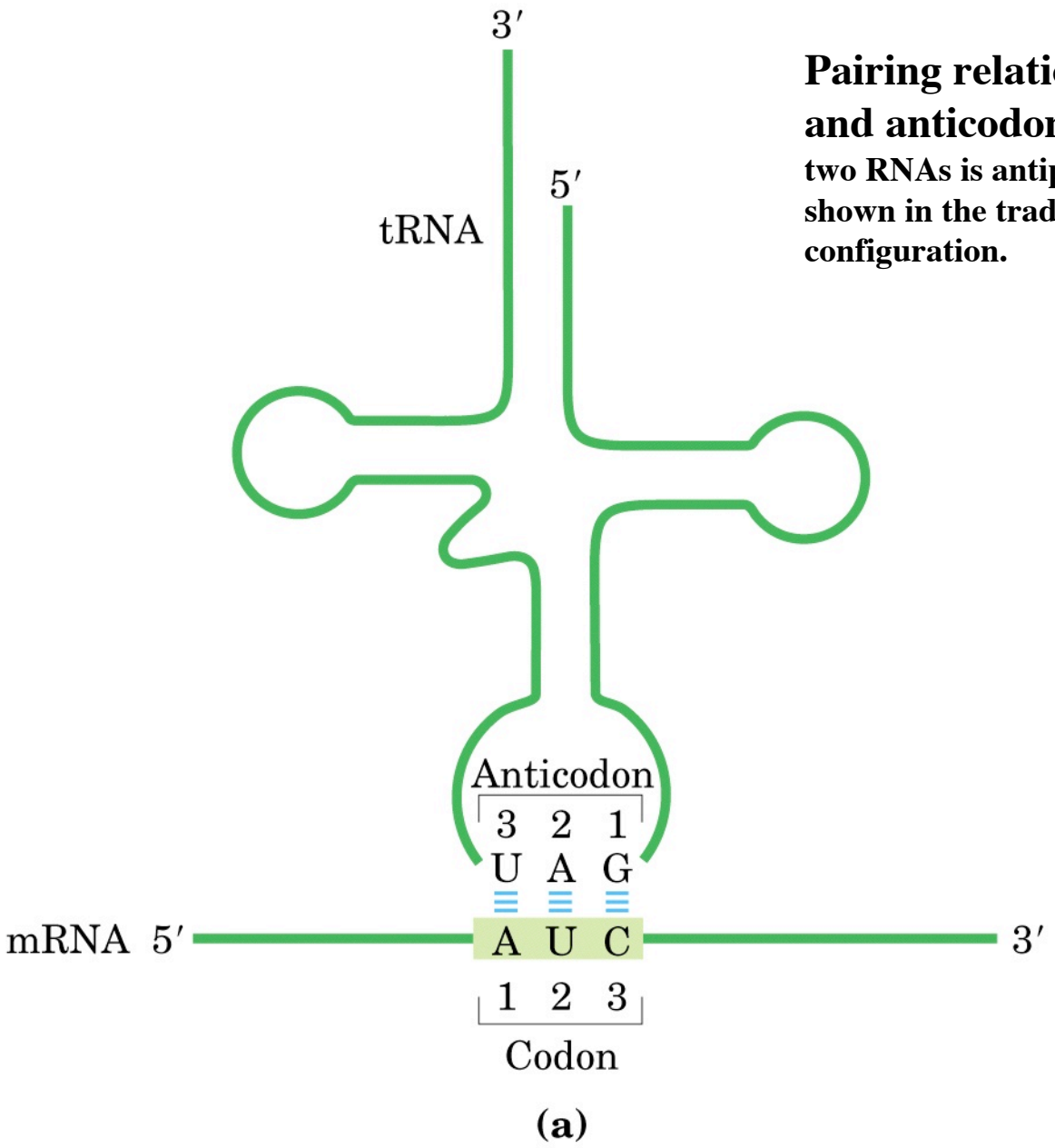
[a]Nonpolar amino acid residues are tan, basic residues are blue, acidic residues are red, and polar uncharged residues are purple.

[b]AUG forms part of the initiation signal as well as coding for internal Met residues.



Sequences on the mRNA that serve as signals for initiation of protein synthesis in bacteria. (a) Alignment of the initiating AUG (shaded in green) at its correct location on the 30S ribosomal subunit depends in part on upstrteam Shine-Dalgarno sequences (shaded in red). Portions of the mRNA transcripts of five prokaryotic genes are shown. **(b)** The Shine-Delgarno sequences pair with a sequence near the 3' end of the 16S rRNA.

a.a.



Pairing relationships of codon and anticodon. **Alignment of the two RNAs is antiparallel. The tRNA is shown in the traditional cloverleaf configuration.**