GENE ACTIVITY

Gene structure

Transcription

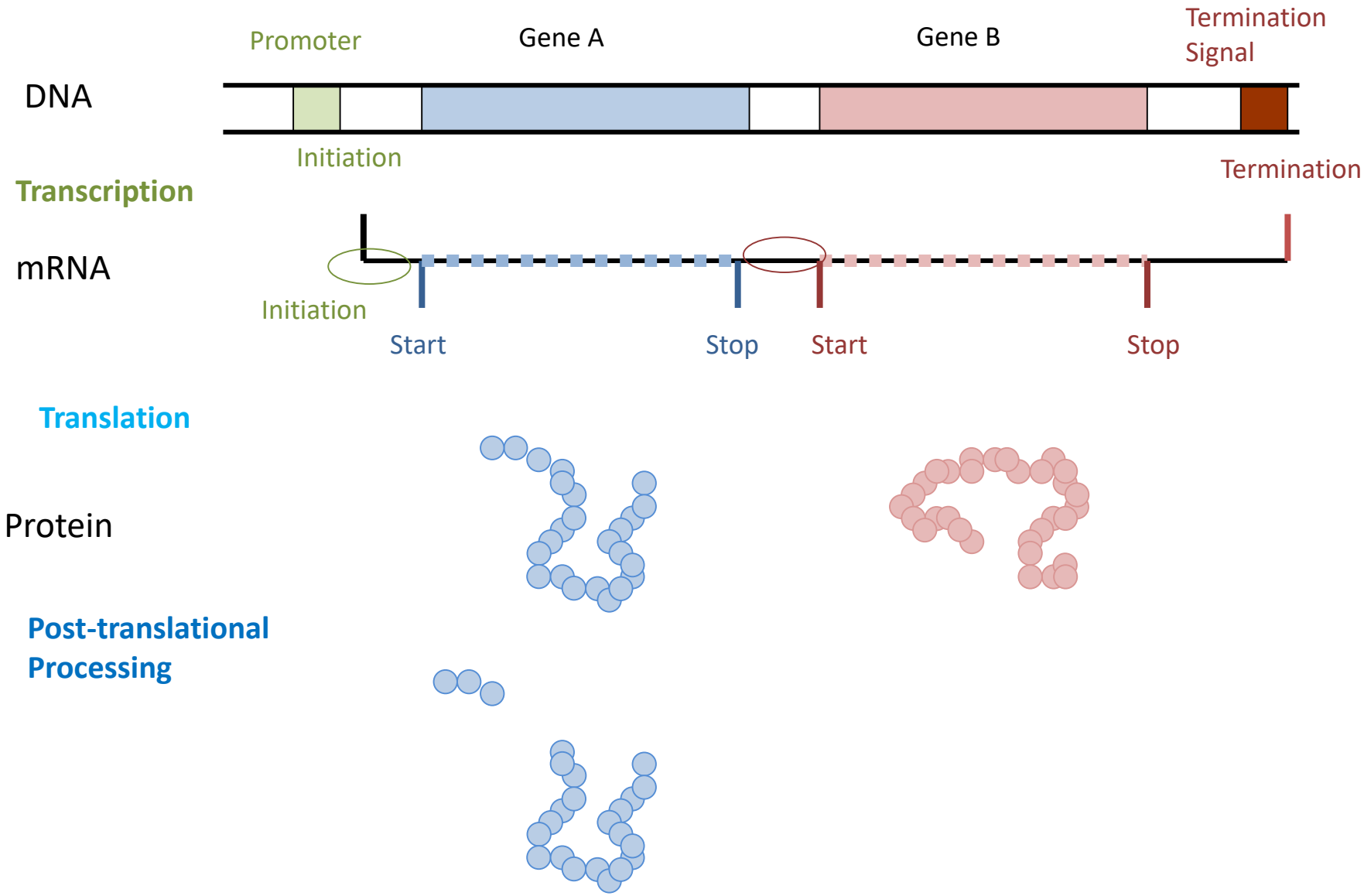
Transcript processing

mRNA transport

mRNA stability

Translation

Posttranslational modifications


Promoter
Gene A
Gene B
Termination Signal
DNA
Initiation
Termination
Transcription
mRNA
Initiation
Start
Stop
Start
Stop
Translation
Protein
Post-translational Processing

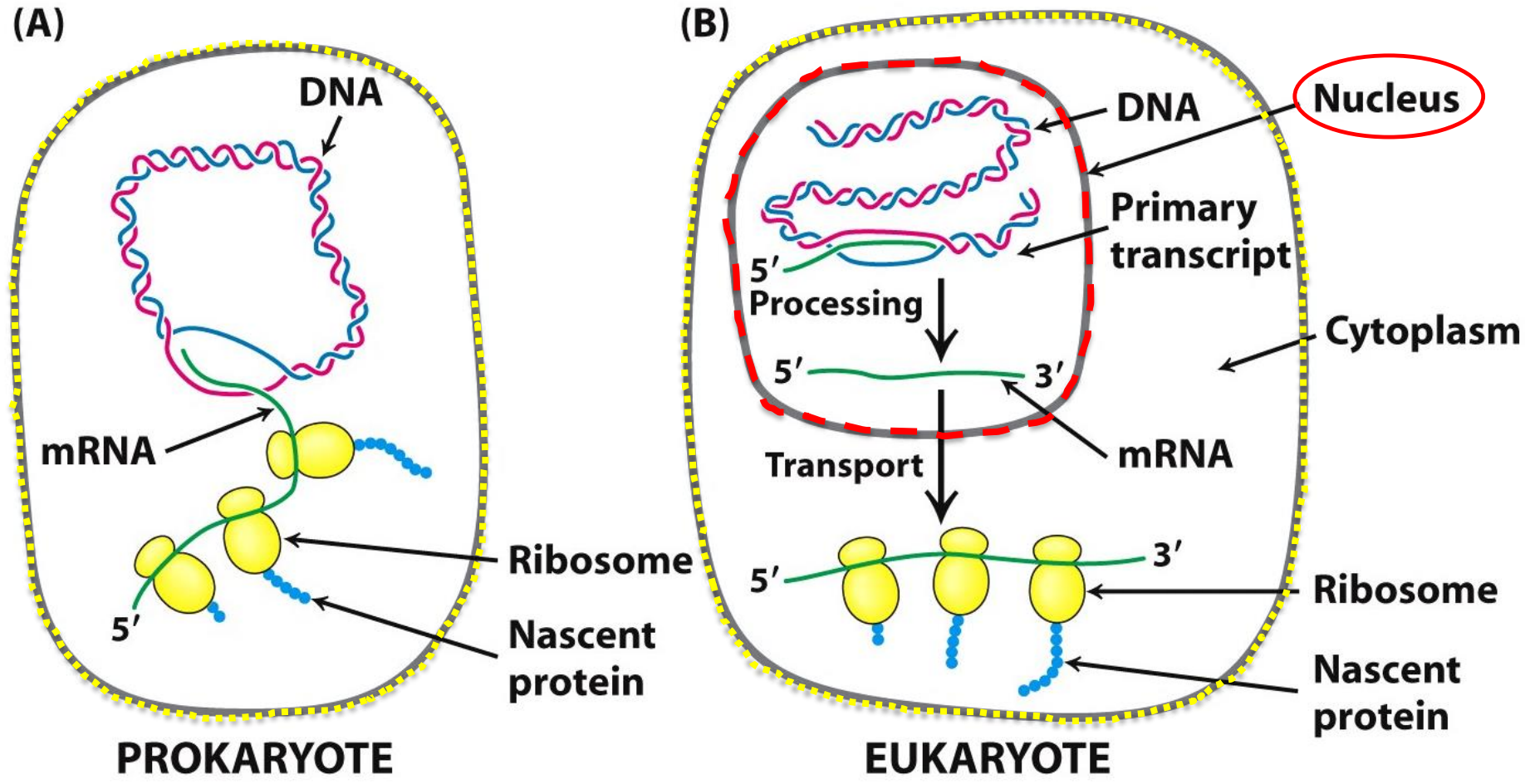


Figure 29.21
***Biochemistry*, Seventh Edition**

The production of functioning mRNA is very different in prokaryotes and eukaryotes. In prokaryotes, the RNA transcript serves directly as the mRNA and translation begins before transcription is completed; that is, transcription and translation are coupled. In eukaryotes, the primary RNA transcript must be modified in the cell nucleus to form mRNA. Translation takes place only after the completed mRNA is delivered to the cytoplasm.

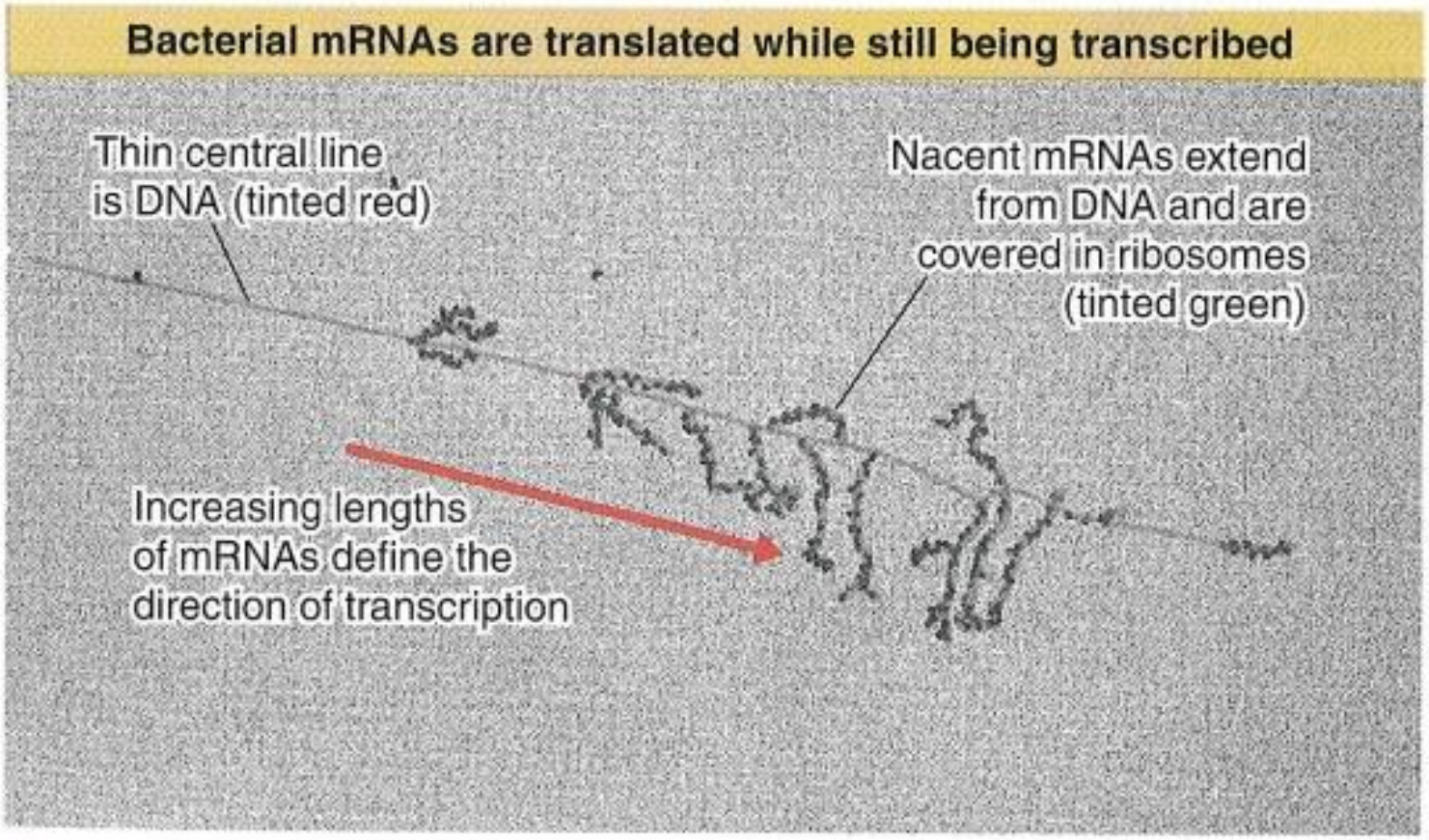


Figure 7.14 Transcription units can be visualized in bacteria. Photograph kindly provided by Oscar L. Miller, Department of Biology, University of Virginia.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

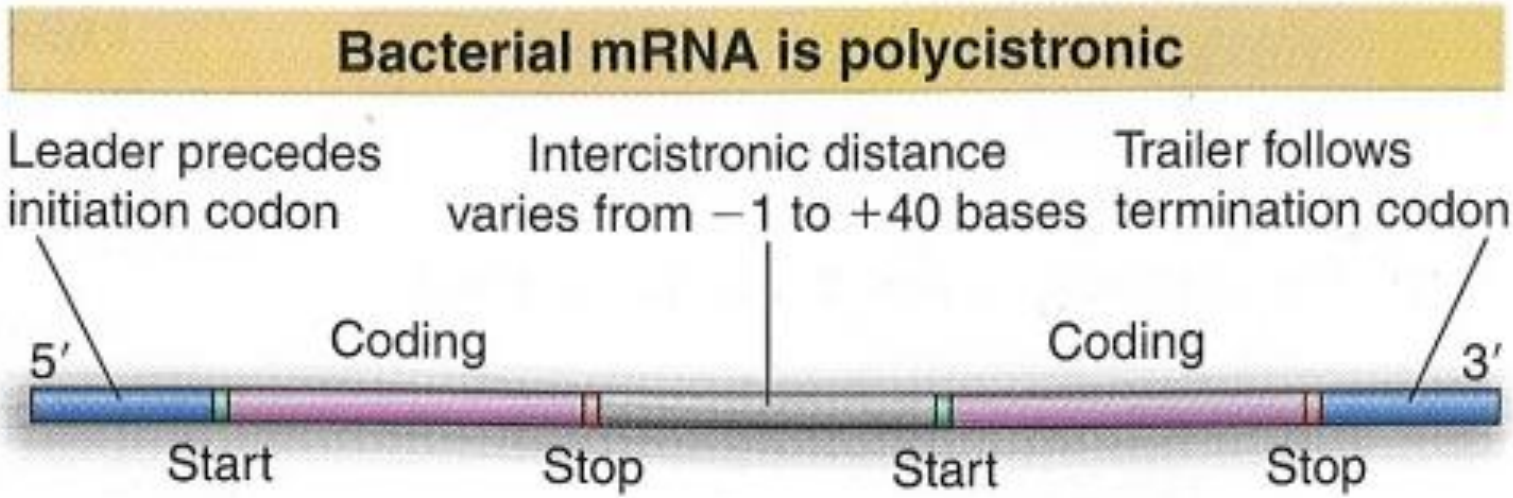


Figure 7.15 Bacterial mRNA includes untranslated as well as translated regions. Each coding region has its own initiation and termination signals. A typical bacterial mRNA has several coding regions.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

Tab. 3.1 Die drei RNA-Arten.

	Größe (ungefähre Angaben)	Funktion
transfer-RNA (tRNA)	80–90 Nucleotide	Übertragung von Aminosäuren zum Proteinsynthese-Apparat der Zelle
ribosomale RNA (rRNA)	4 Arten (bei Eukaryoten) mit je ca. 120, 150, 1700, 3500 Nucleotiden	Struktur und Funktionselemente der Ribosomen
messenger-RNA (mRNA)	sehr verschieden (einige 100 bis über 10000 Nucleotide)	die Boten-(*messenger*-)RNA überbringt dem Proteinsynthese-Apparat eine Abschrift des Gens

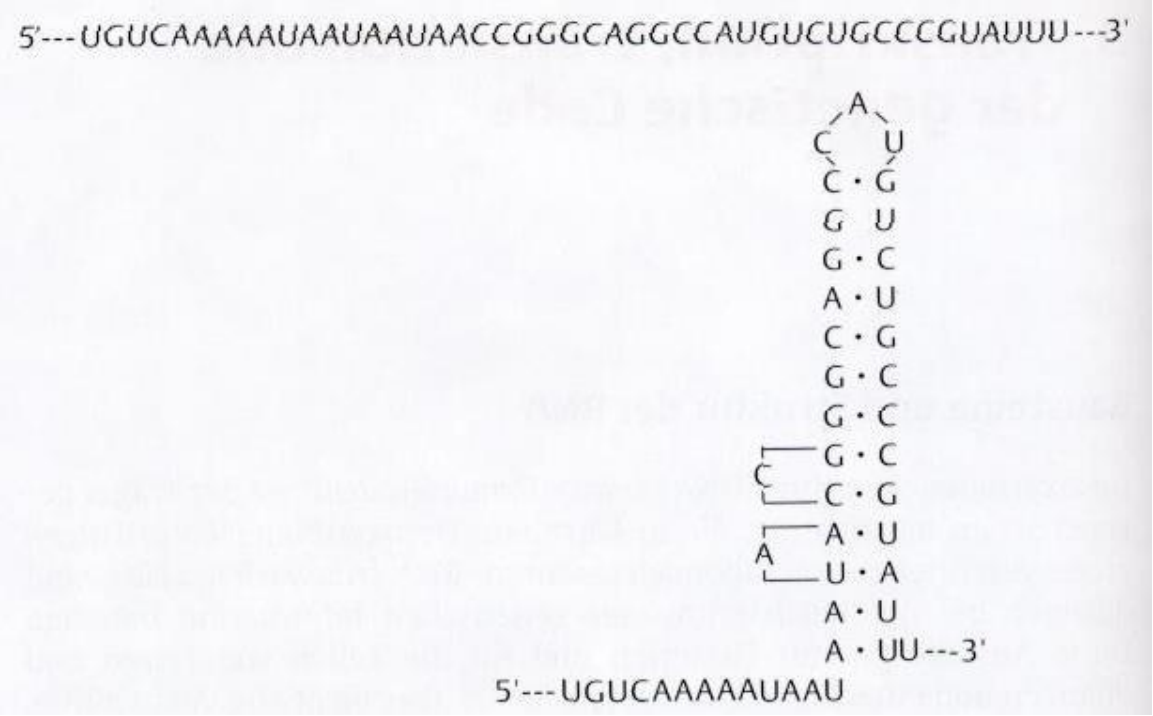
The 3 RNA types

	Size (approximate)	Function
transfer-RNA (tRNA)	80-90 nucleotides	Transfer of amino acids to the protein synthesis apparatus of the cell
Ribosomal RNA (rRNA)	4 types (in eukaryotes) with each ca. 120, 150, 1700 and 3500 nucleotides	Structural and functional elements of the ribosomes
messenger-RNA (mRNA)	Very different (from several 100 to more than 10000 nucleotides)	The mRNA delivers a gene copy to the protein synthesis apparatus of the cell

Taken from: R. Knippers, Molekulare Genetik, 9th Ed., Thieme

Types of RNA	Characteristics and key functions
messenger RNA (mRNA)	• varies in length, depending on the gene that has been copied • acts as the intermediary between DNA and the ribosomes • translated into protein by ribosomes • RNA version of the gene encoded by DNA
transfer RNA (tRNA)	• functions as the delivery system of amino acids to ribosomes as they synthesize proteins • very short, only 70–90 base pairs long
ribosomal RNA (rRNA)	• binds with proteins to form the ribosomes • varies in length

RNA is single stranded but is organized partly as ds RNA by internal base pairing

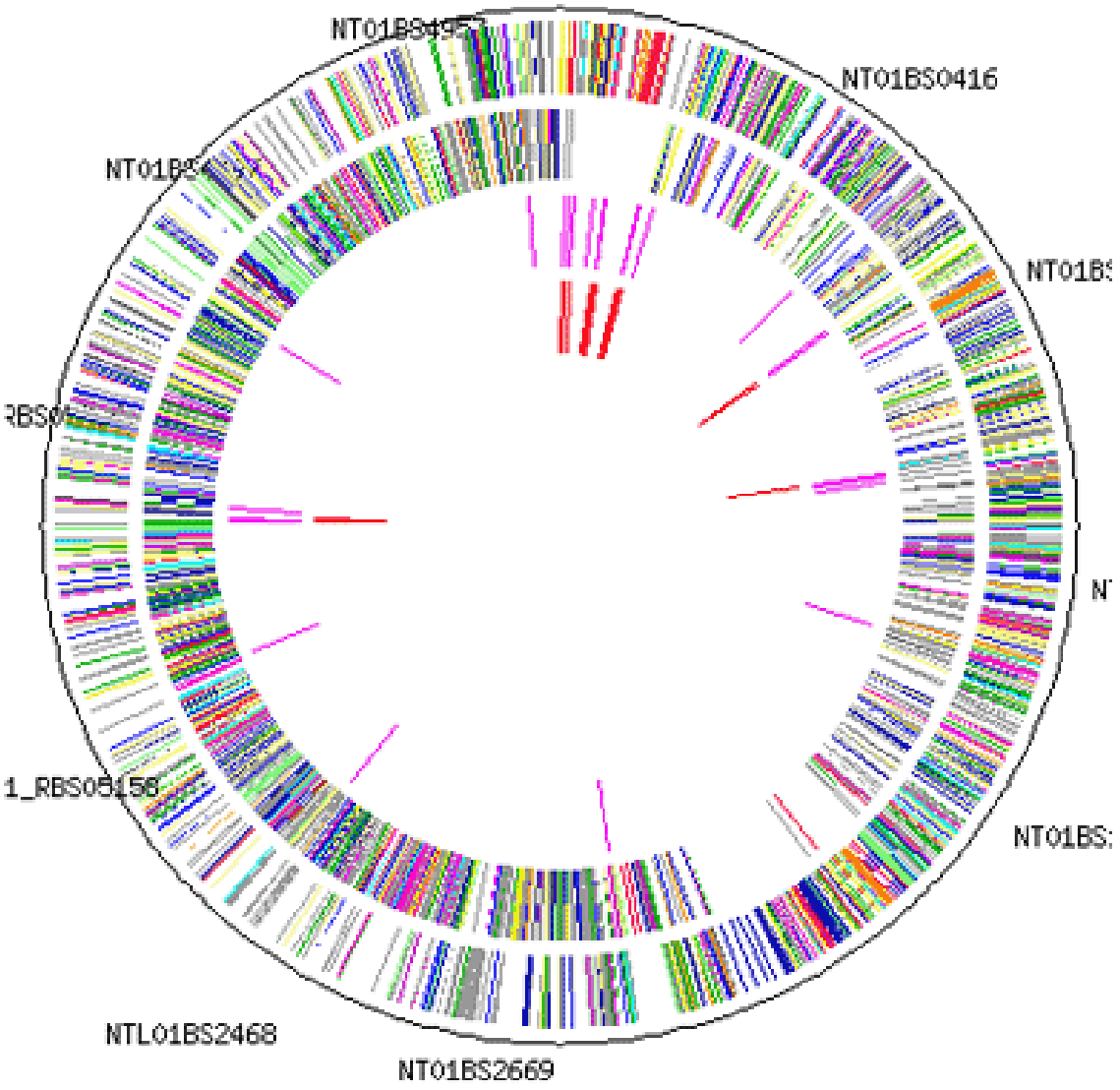


Loop (secondary structure) formation in the *E.coli* mRNA.
The mRNA has a length of several thousand nucleotides, only a short part is shown here. This part contains complementary nucleotide sequences that can combine to a double stranded region so that a loop is created at this site. Cytosine pairs with Guanine and Uracil with Adenine.

Taken from: R. Knippers, Molekulare Genetik, 9th Ed., Thieme

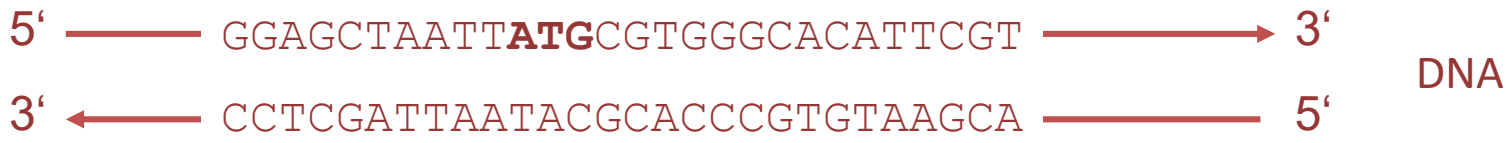
Genome map of *Bacillus subtilis*

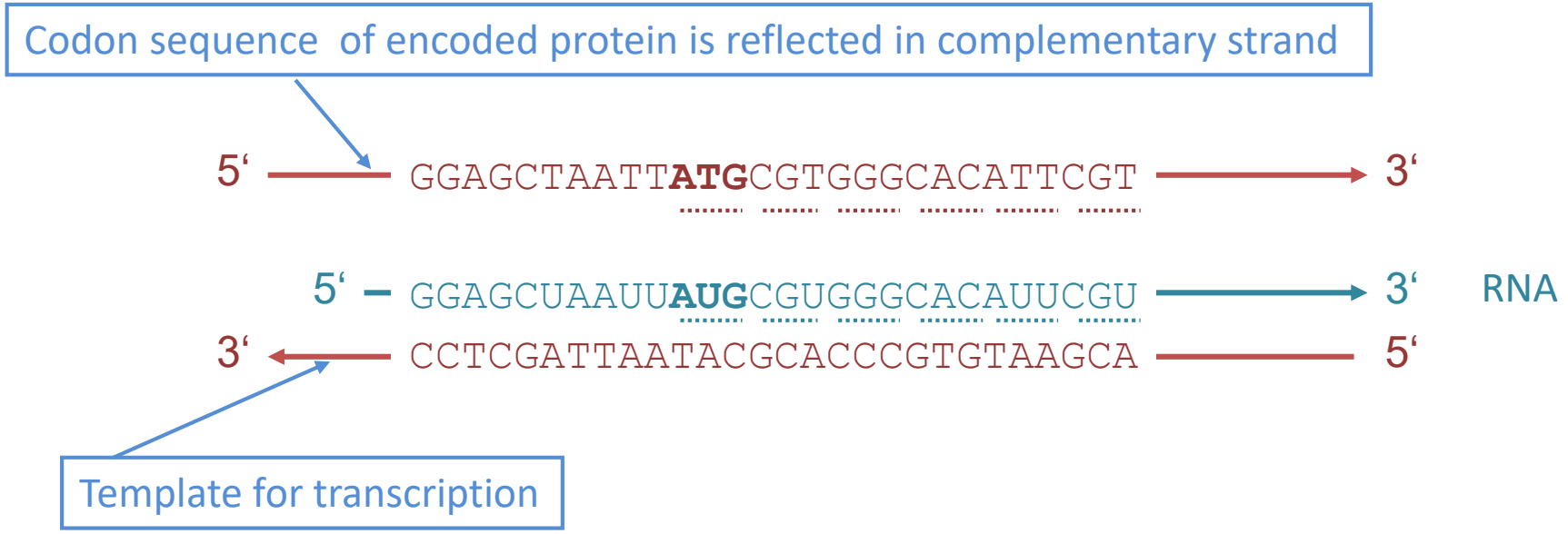
Genes are transcribed from both strands



www.expeditions.udel.edu/extreme08/genomics/genomemap.php

Transcription: Only one strand is transcribed





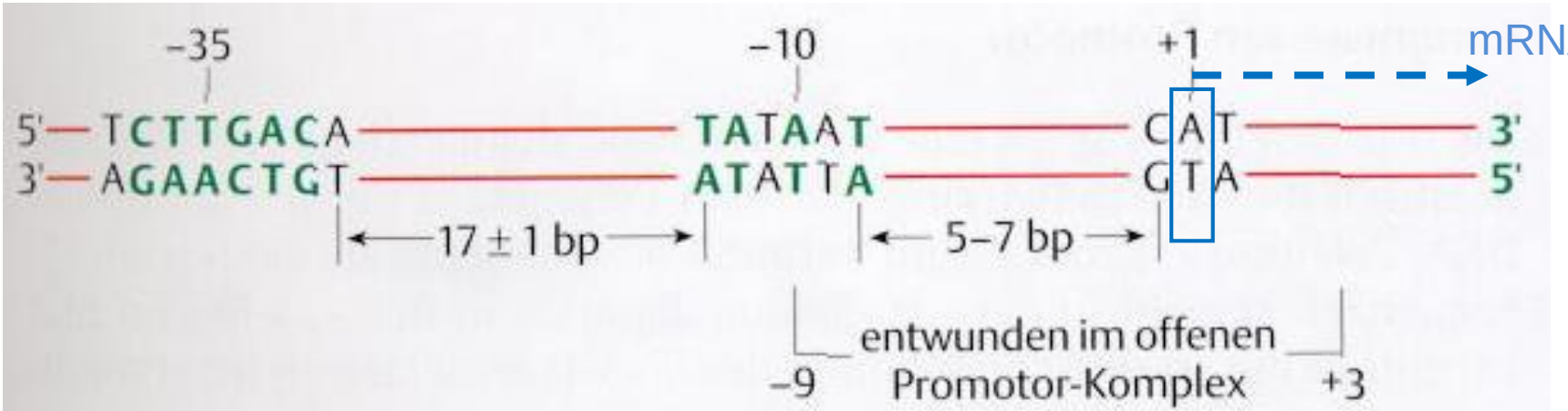


Abb. 3.5 Ein Musterpromotor des *E. coli*-Genoms. Der Abstand zwischen dem Transkriptionsstart und dem ersten Nucleotid der –10-Region beträgt 5–7 Basenpaare (bp); der Abschnitt zwischen der –10-Region und der –35-Region 17 ± 1 bp. Der untere der beiden DNA-Stränge ist der transkribierte „codogene" oder Sinnstrang, der obere der nichttranskribierte Gegensinn-Strang [nach 13].

Taken from: R. Knippers, Molekulare Genetik, 9th Ed., Thieme

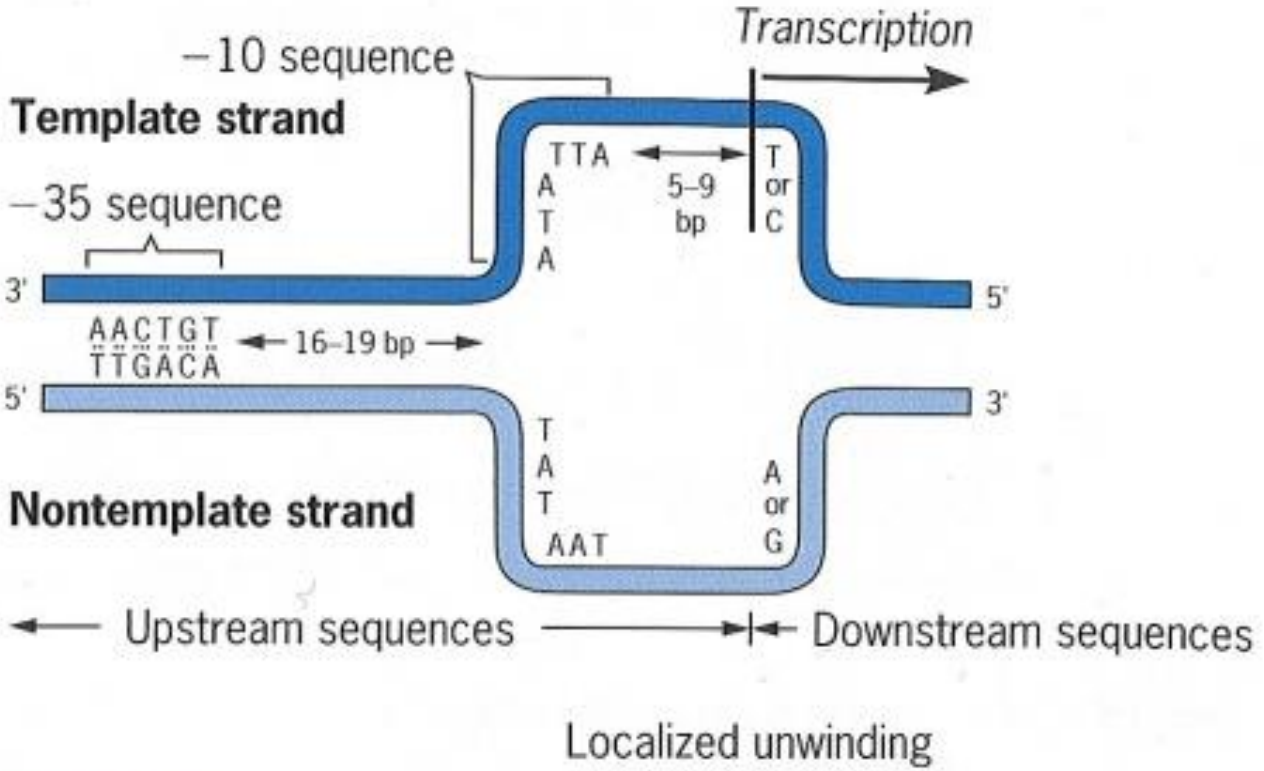


Figure 11.9 ▶ Structure of a typical promoter in *E. coli*. RNA polymerase binds to the –35 sequence of the promoter and initiates unwinding of the DNA strands at the AT-rich –10 sequence. Transcription begins within the transcription bubble at a site five to nine base pairs beyond the –10 sequence.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

Gene	Factor	Use
rpoD	σ^{70}	most required functions
rpoS	σ^{S}	stationary phase/some stress responses
rpoH	σ^{32}	heat shock
rpoE	σ^{E}	periplasmic/extracellular proteins
rpoN	σ^{54}	nitrogen assimilation
rpoF	σ^{F}	flagellar synthesis/chemotaxis
fecI	σ^{fecI}	iron metabolism/transport

Fig. 19.37: In addition to σ70, *E. coli* has several sigma factors that are induced by particular environmental conditions.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

Subunit/gene	Size (# aa)	Approx. # of promoters	Promoter sequence recognized
Sigma 70 (*rpoD*)	613	1000	TTGACA-16 to 18-bp-TATAAT
Sigma 54 (*rpoN*)	477	5	CTGGNA-6 bp-TTGCA
Sigma S (*rpoS*)	330	100	TTGACA-16 to 18-bp-TATAAT
Sigma 32 (*rpoH*)	284	30	CCCTTGAA-13 to 15-bp-CCCGATNT
Sigma F(*rpoF*)	239	40	CTAAA-15 bp-GCCGATAA
Sigma E (*rpoE*)	202	20	GAA-16 bp-YCTGA
Sigma FecI (*fecI*)	173	1–2	?

Fig. 19.15: *E. coli* sigma factors recognize promoters with different consensus sequences.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

Effect of mutations on promoter function

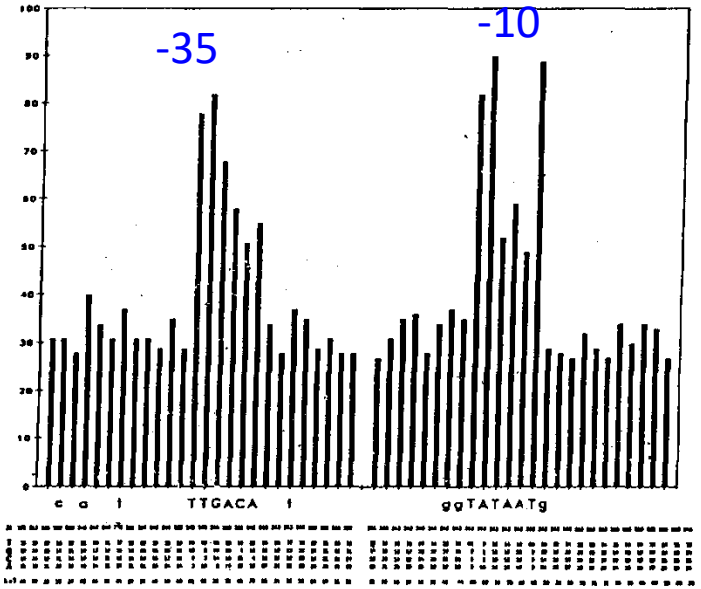


Figure 1. Base distribution of 263 analyzed promoters from Table 1. (a) Frequency histogram of the most highly conserved base on the non-template strand from 12 bp upstream of the -35 hexamer to 11 bp downstream of the -10 hexamer. Highly conserved (upper case) and weakly conserved (lower case) bases, as defined in the text, are shown below the histogram. (b) Frequency of bases (T,C,G,A and T+A) in aligned promoters as a percentage of total number of bases (N) at each position.

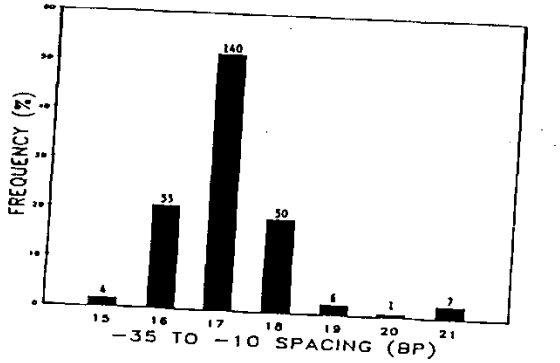


Figure 2. Distribution of promoters with 15-21 bp separating the -35 and -10 hexamers. The number of promoters in each group is indicated on top of the bars.

Spacing between -35 and -10 region

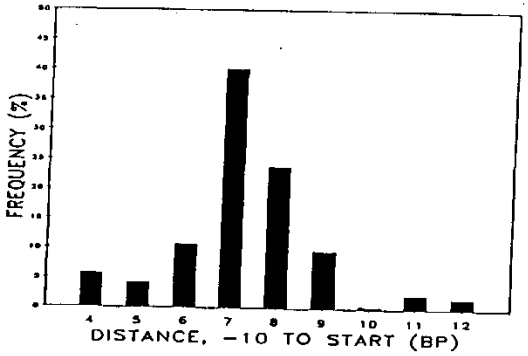


Figure 3. Distribution of promoters with transcription start points initiating 4-12 bases downstream of the -10 hexamer. Only promoters with uniquely defined start points are included in this analysis.

Spacing between -10 region and transcription start

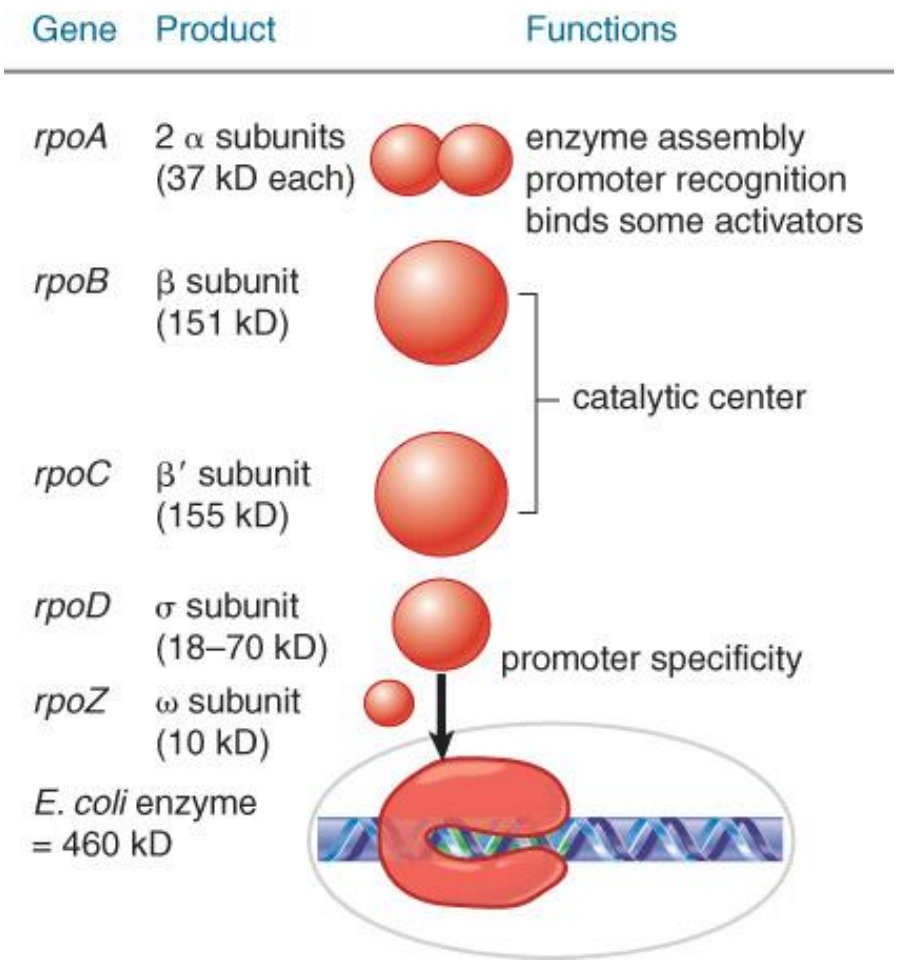


Fig. 19.7: Eubacterial RNA polymerases have five types of subunits.

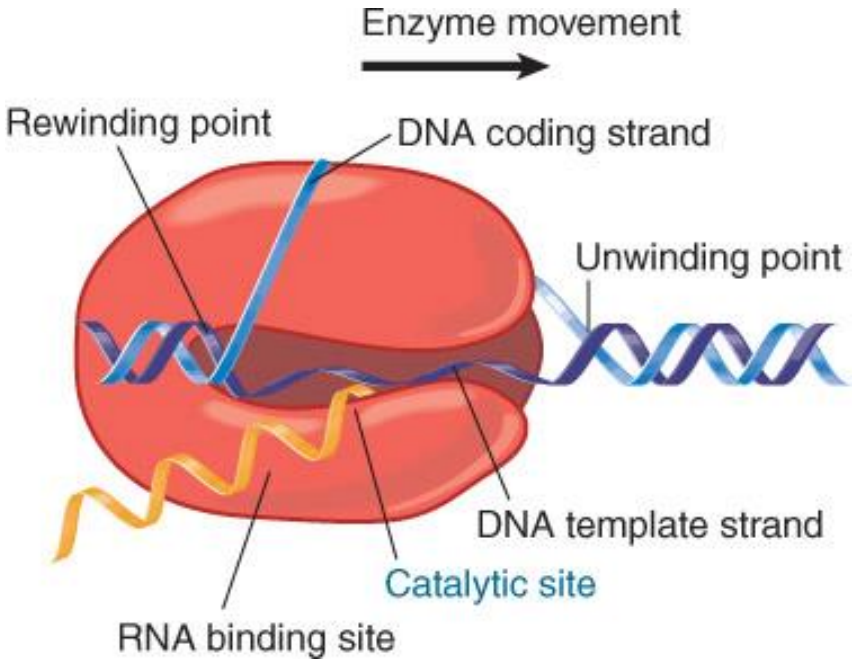
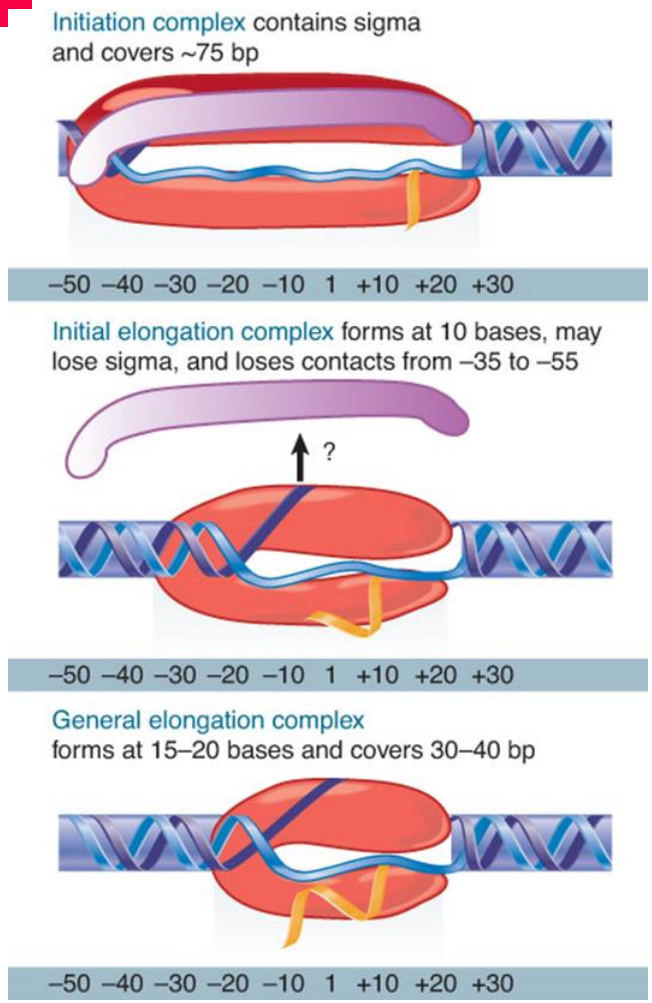


Fig. 19.5: During transcription, the bubble is maintained within bacterial RNA polymerase, which unwinds and rewinds DNA and synthesizes RNA.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning



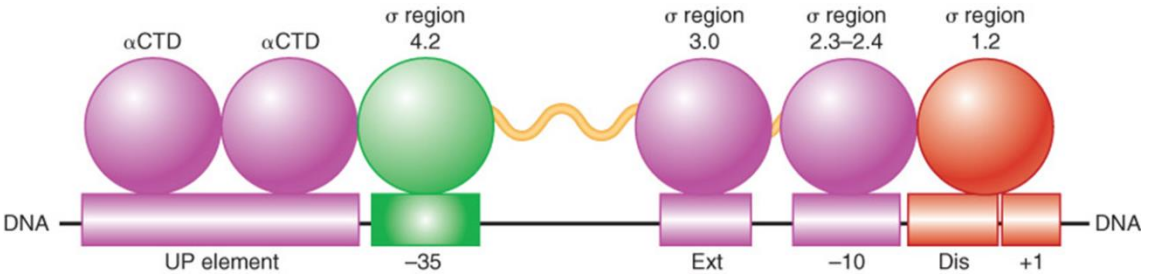


Fig. 19.14: DNA elements and RNA polymerase modules that contribute to promoter recognition by sigma factor.

Fig. 19.13: RNA polymerase initially contacts the region from –55 to +20. When sigma dissociates, the core enzyme contracts to -30; when the enzyme moves a few base pairs, it becomes more compactly organized into the general elongation complex.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

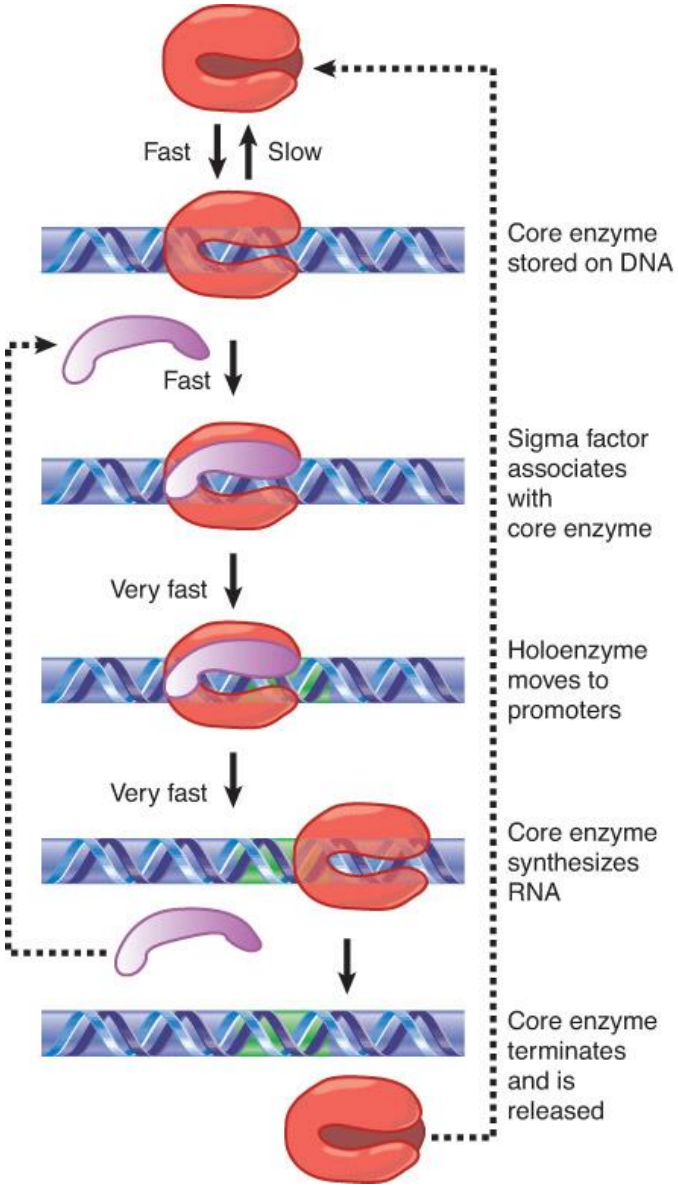
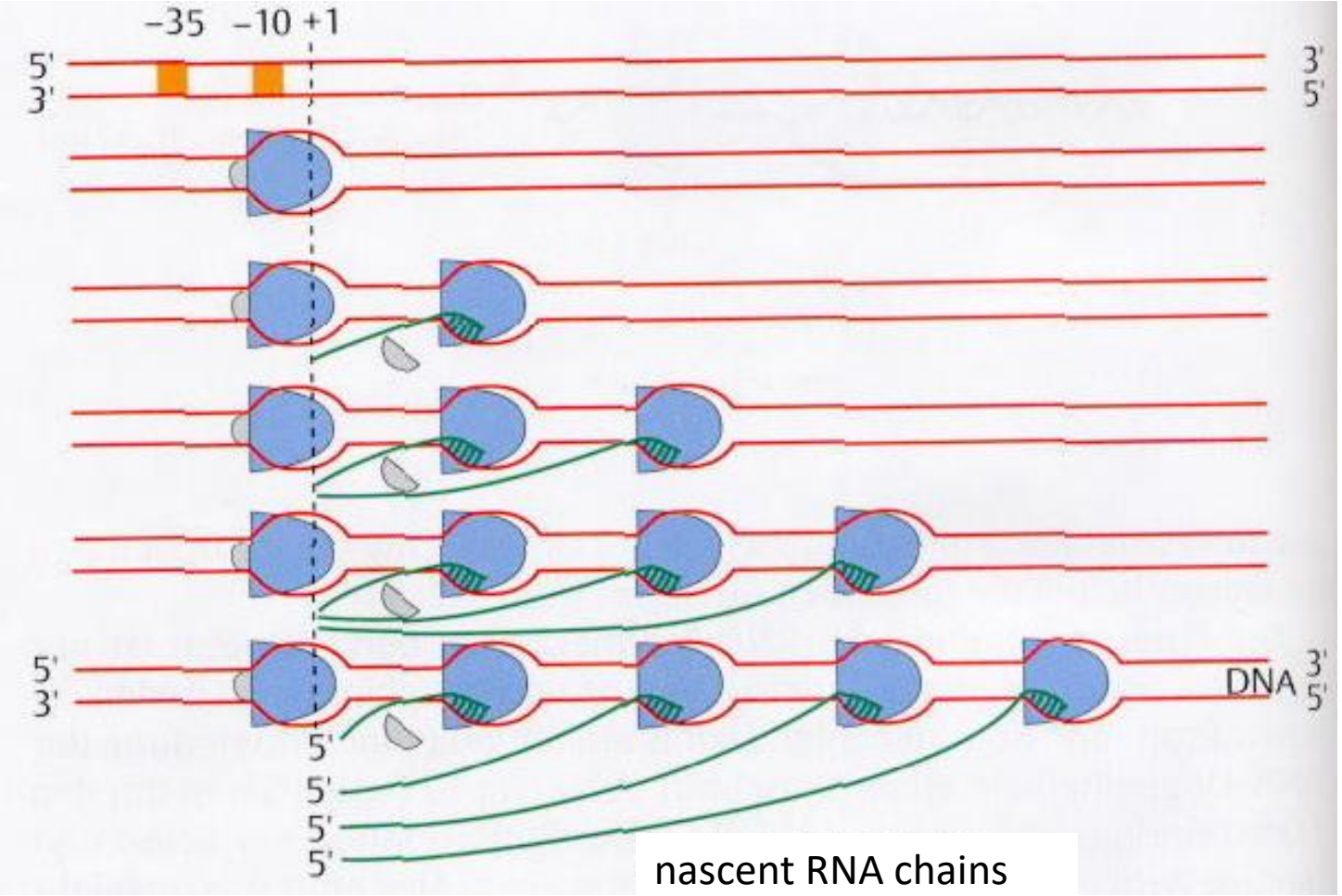


Fig. 19.24: Sigma factor and core enzyme recycle at different points in transcription.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning



Scheme of transcription. Transcription starts with an „open" promoter complex. The holoenzyme causes the unwinding of the region close to the transcription start point (+1). After a few polymerisation steps, the sigma factor leaves the core enzyme which continues its way along the transcribed DNA strand. The released promoter is re-occupied. In parallel, several RNA polymerases are occupied with transcription

Taken from: R. Knippers, Molekulare Genetik, 9th Ed., Thieme

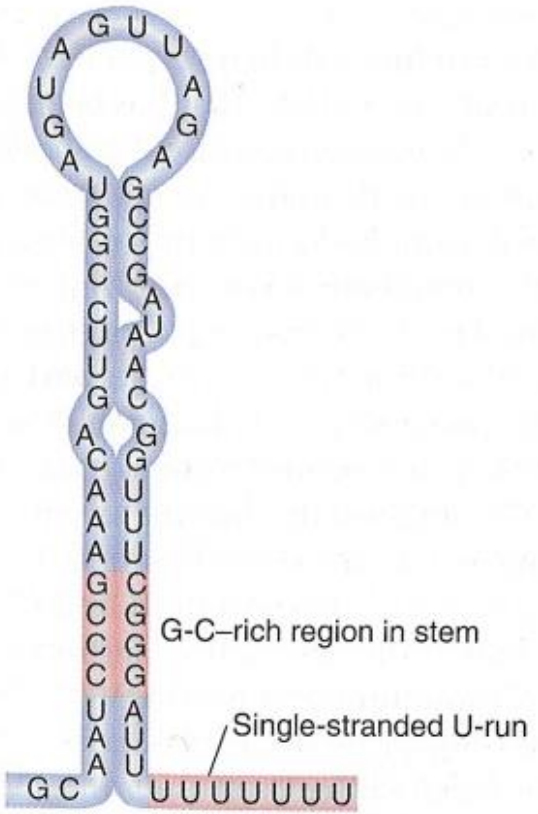


FIGURE 19.29 Intrinsic terminators include palindromic regions that form hairpins varying in length from 7 to 20 bp. The stem-loop structure includes a G-C–rich region and is followed by a run of U residues.

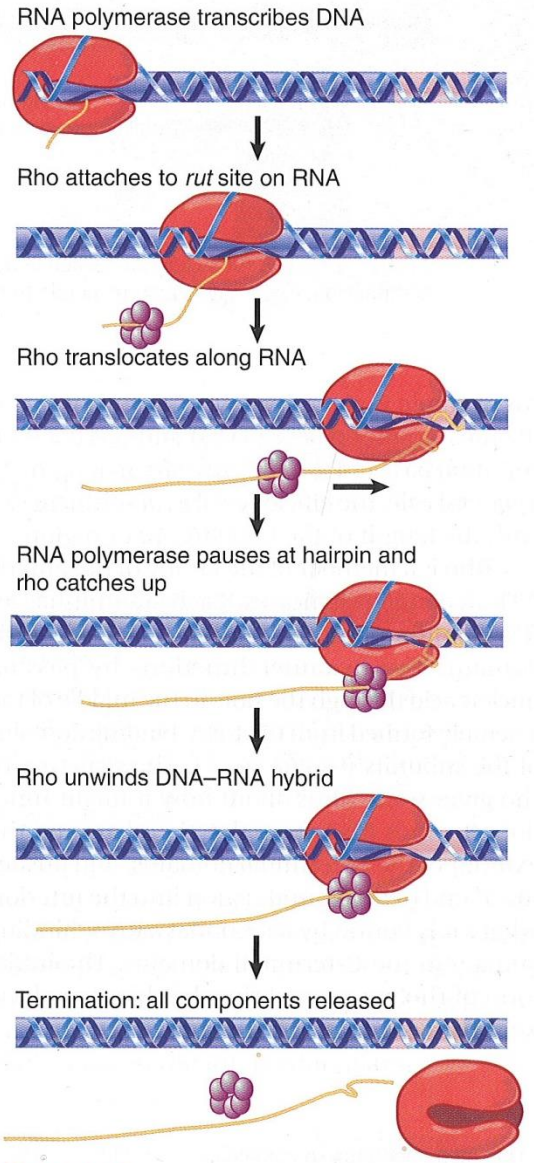


FIGURE 19.30 Rho factor binds to RNA at a *rut* site and translocates along RNA until it reaches the RNA–DNA hybrid in RNA polymerase, where it releases the RNA from the DNA.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

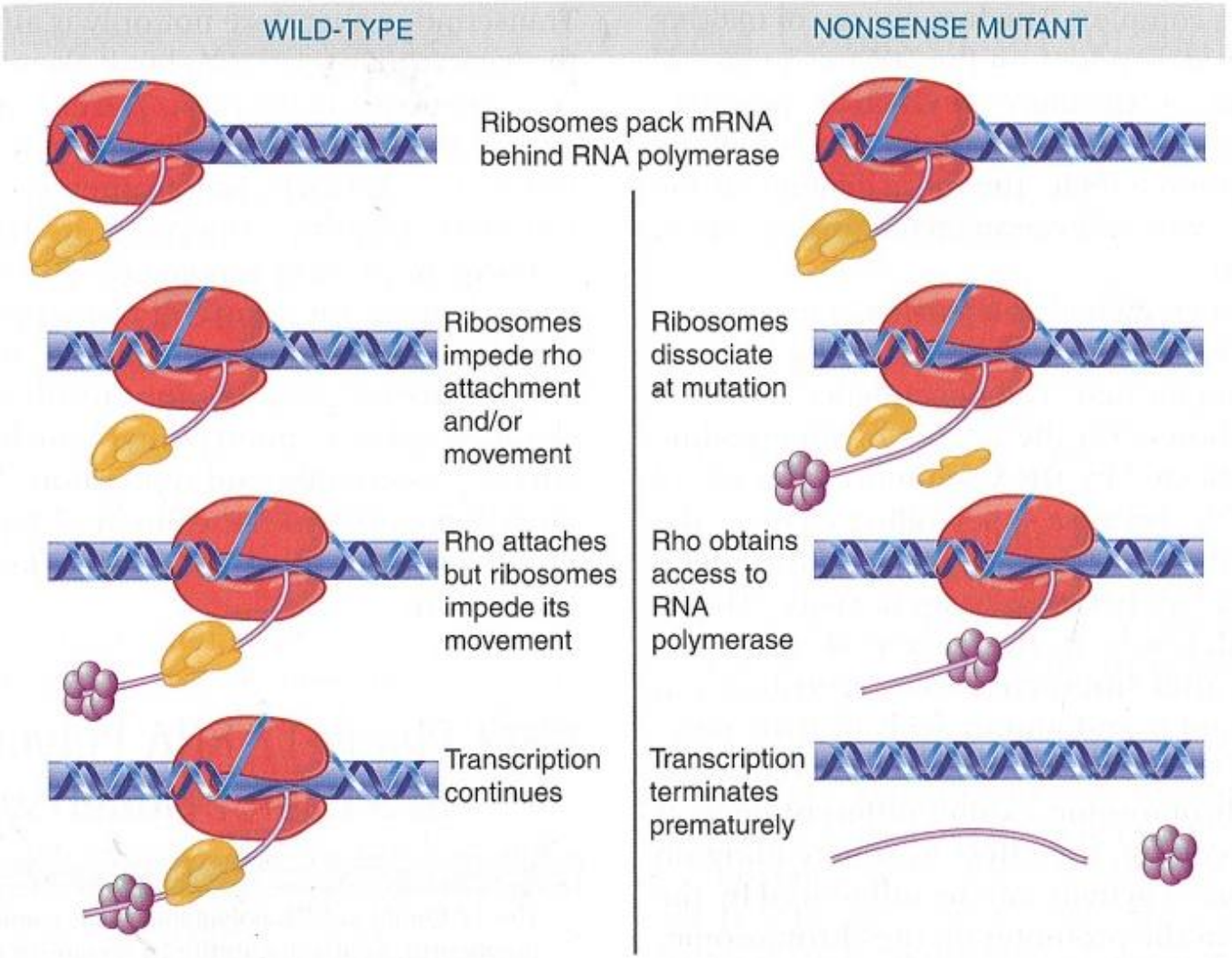


FIGURE 19.33 The action of rho factor may create a link between transcription and translation when a rho-dependent terminator lies soon after a nonsense mutation.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

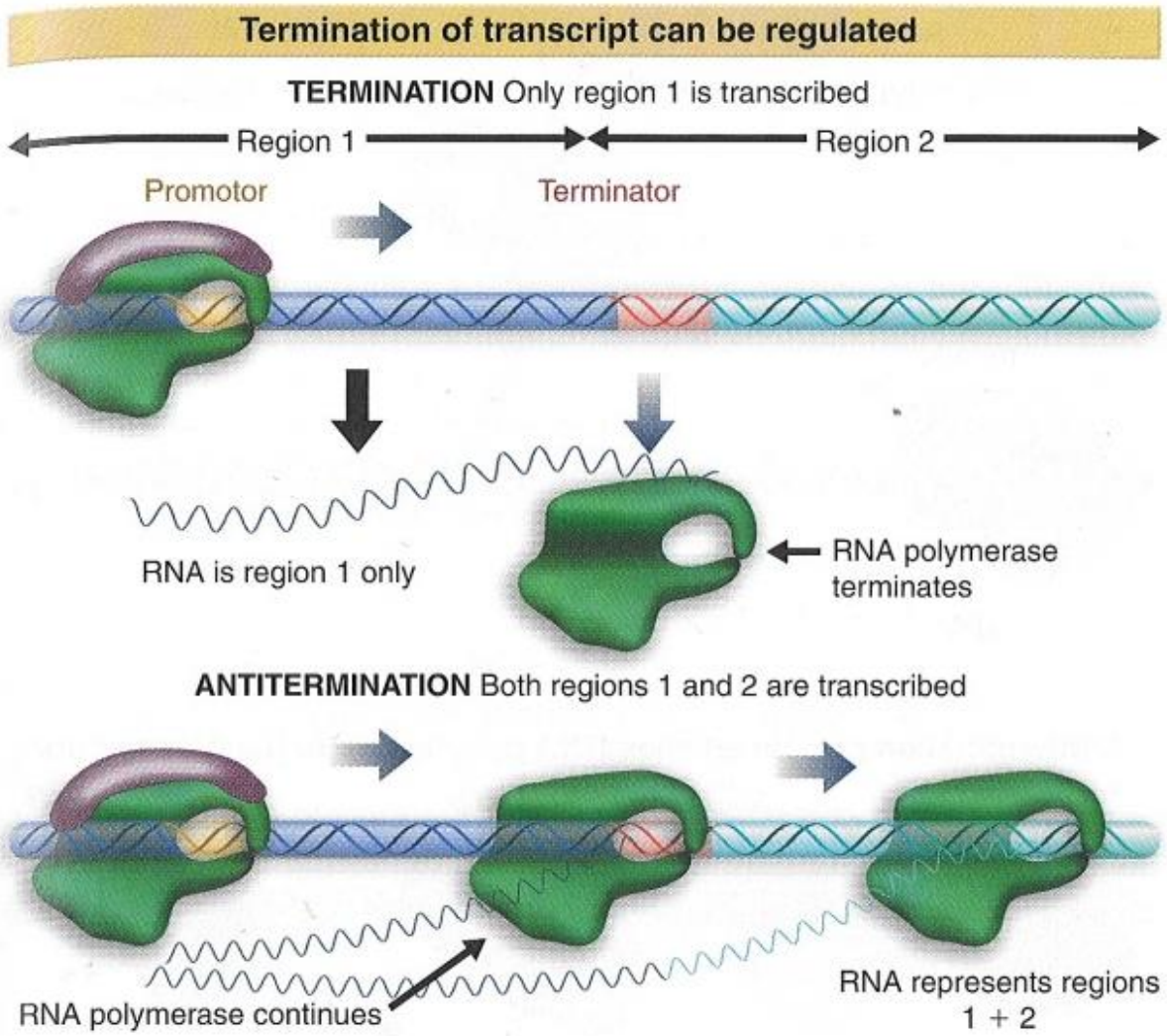


Figure 11.36 Antitermination can control transcription by determining whether RNA polymerase terminates or reads through a particular terminator into the following region.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

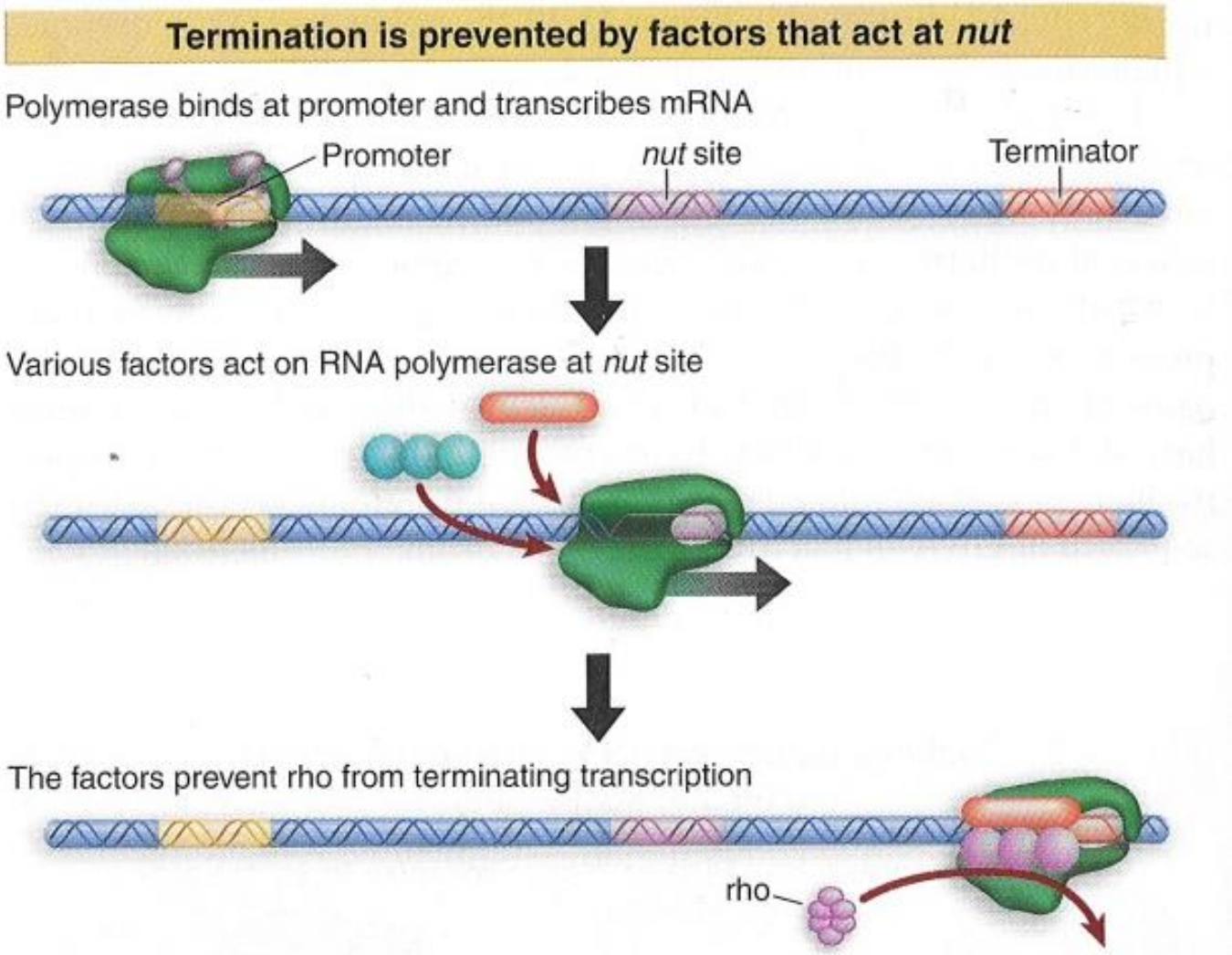


Figure 11.39 Ancillary factors bind to RNA polymerase as it passes the *nut* site. They prevent rho from causing termination when the polymerase reaches the terminator.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

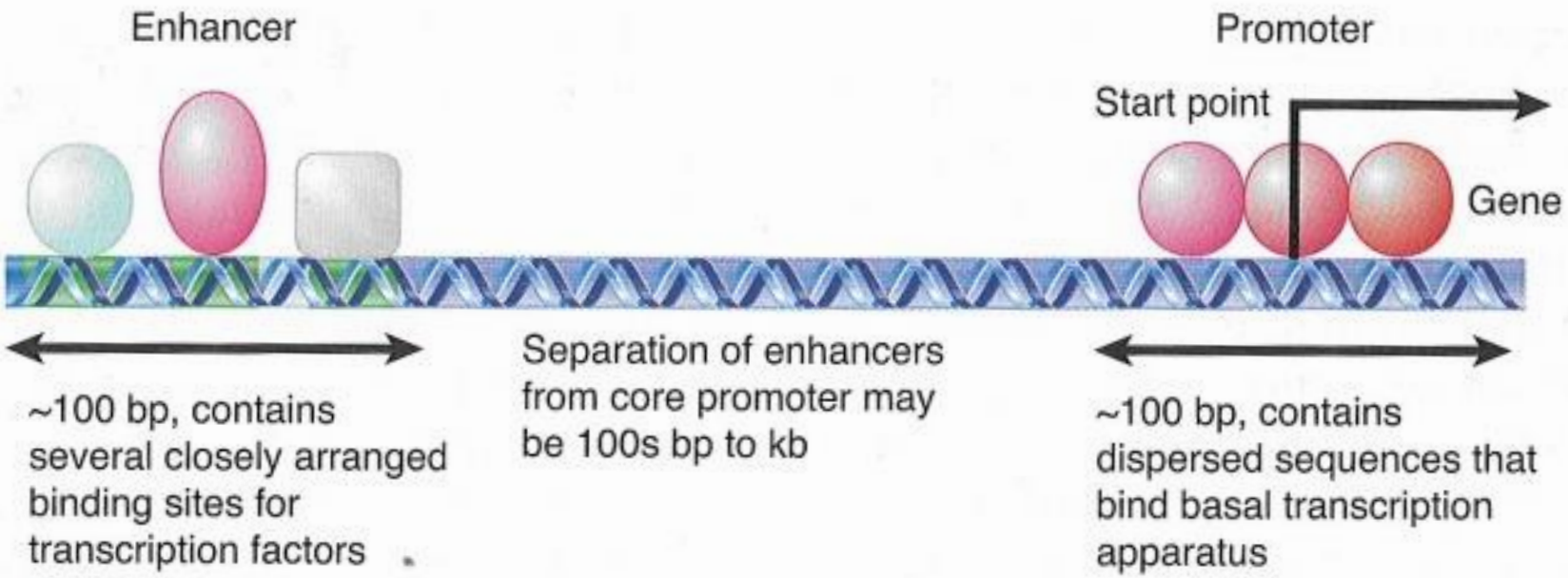


FIGURE 20.1 A typical gene transcribed by RNA polymerase II has a promoter that extends upstream from the site where transcription is initiated. The promoter contains several short-sequence (~10 bp) elements that bind transcription factors, dispersed over ~100 bp. An enhancer containing a more closely packed array of elements that also bind transcription factors may be located several hundred bp to several kb distant. (DNA may be coiled or otherwise rearranged so that transcription factors at the promoter and at the enhancer interact to form a large protein complex.)

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

Gene Expression in Eukaryotes -- Introns

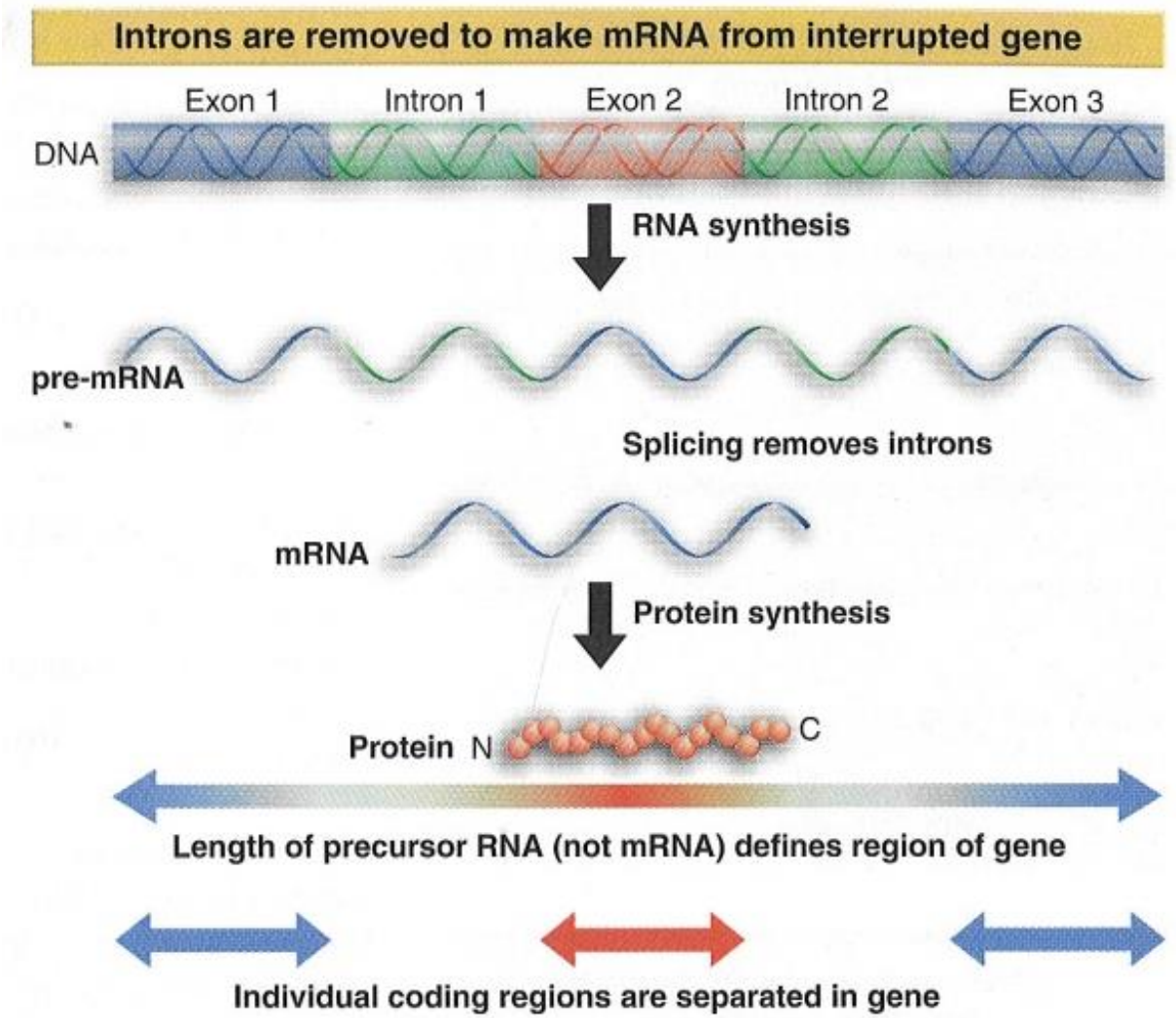


Figure 3.1 Interrupted genes are expressed via a precursor RNA. Introns are removed when the exons are spliced together. The mRNA has only the sequences of the exons.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

Table 9–1 The Size of Some Human Genes in Thousands of Nucleotides

	kb Gene Size	kb mRNA Size	Number of Introns
β-Globin	1.5	0.6	2
Insulin	1.7	0.4	2
Protein kinase C	11	1.4	7
Albumin	25	2.1	14
Catalase	34	1.6	12
LDL receptor	45	5.5	17
Factor VIII	186	9	25
Thyroglobulin	300	8.7	36
Dystrophin*	more than 2000	17	more than 50

The size specified here for a gene includes both its transcribed portion and nearby regulatory DNA sequences. (Compiled from data supplied by Victor McKusick.)

*An altered form of this gene causes Duchenne muscular dystrophy.

Taken from: B. Alberts, Molecular Biology of the Cell, Garland Science

mRNA Synthesis in Eukaryote is a complex Process

Transcription Initiation

Transcription Elongation

5’ Transcript Processing (CAP)

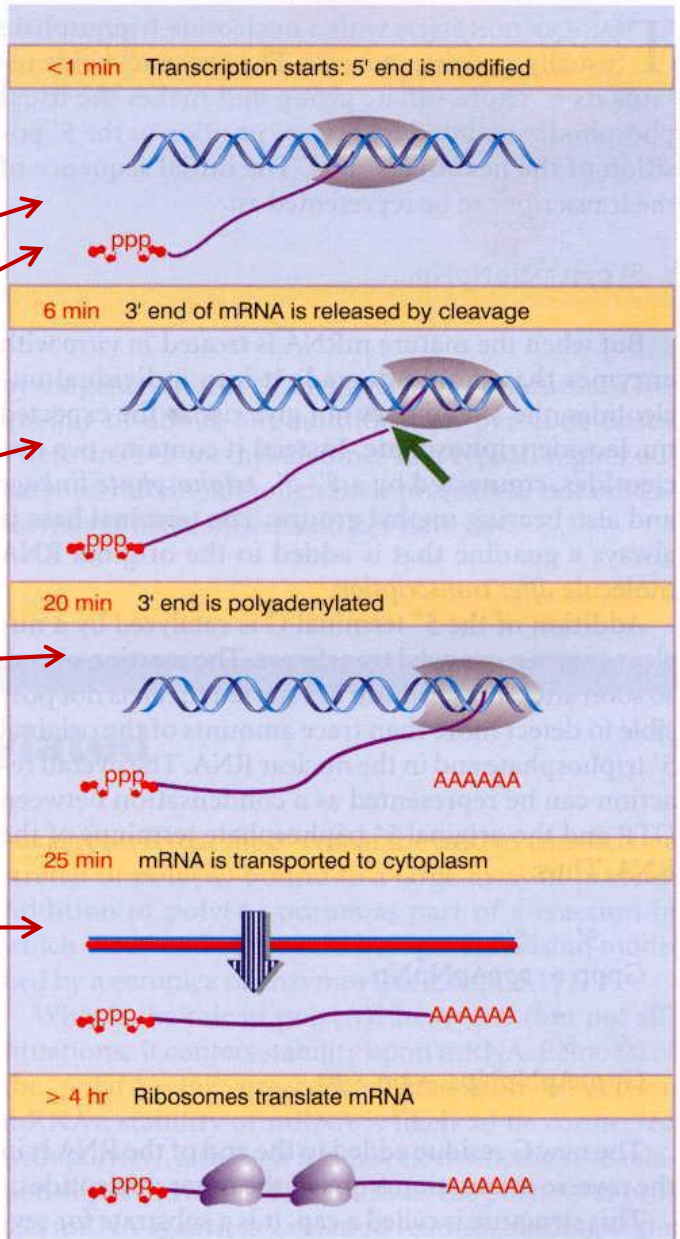
Transcription Termination

3’ Transcript Processing

Intron Splicing

Transport into Cytoplasm

Figure 5.16 *Overview*: expression of mRNA in animal cells requires transcription, modification, processing, nucleocytoplasmic transport, and translation.



mRNA Synthesis in Eukaryote is a complex Process

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Transport into Cytoplasm

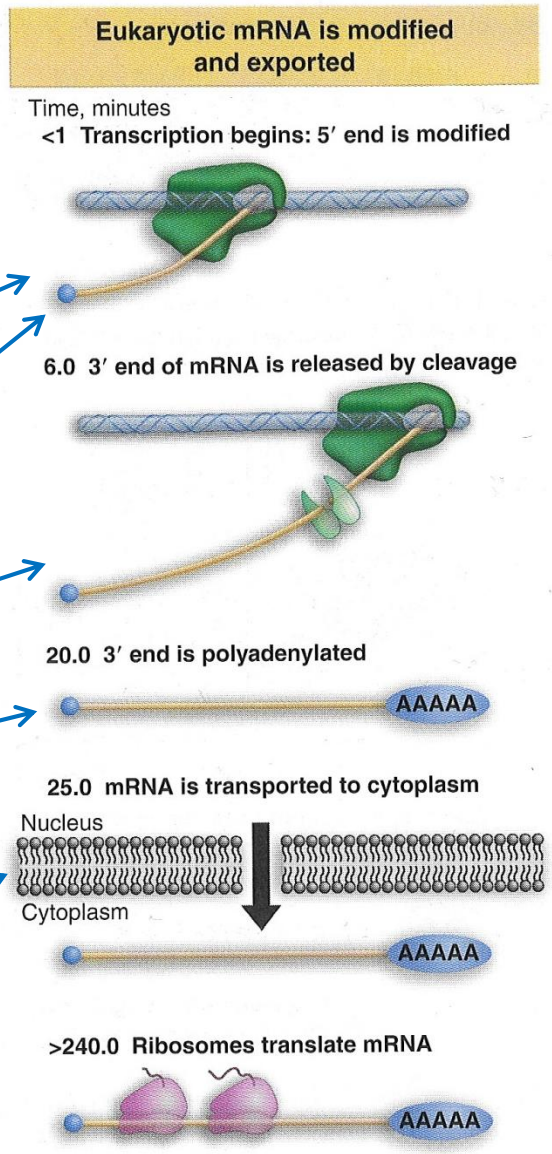


Figure 7.17 Overview: expression of mRNA in animal cells requires transcription, modification, processing, nucleocytoplasmic transport, and translation.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

CAP structure at 5‘ end of mRNA

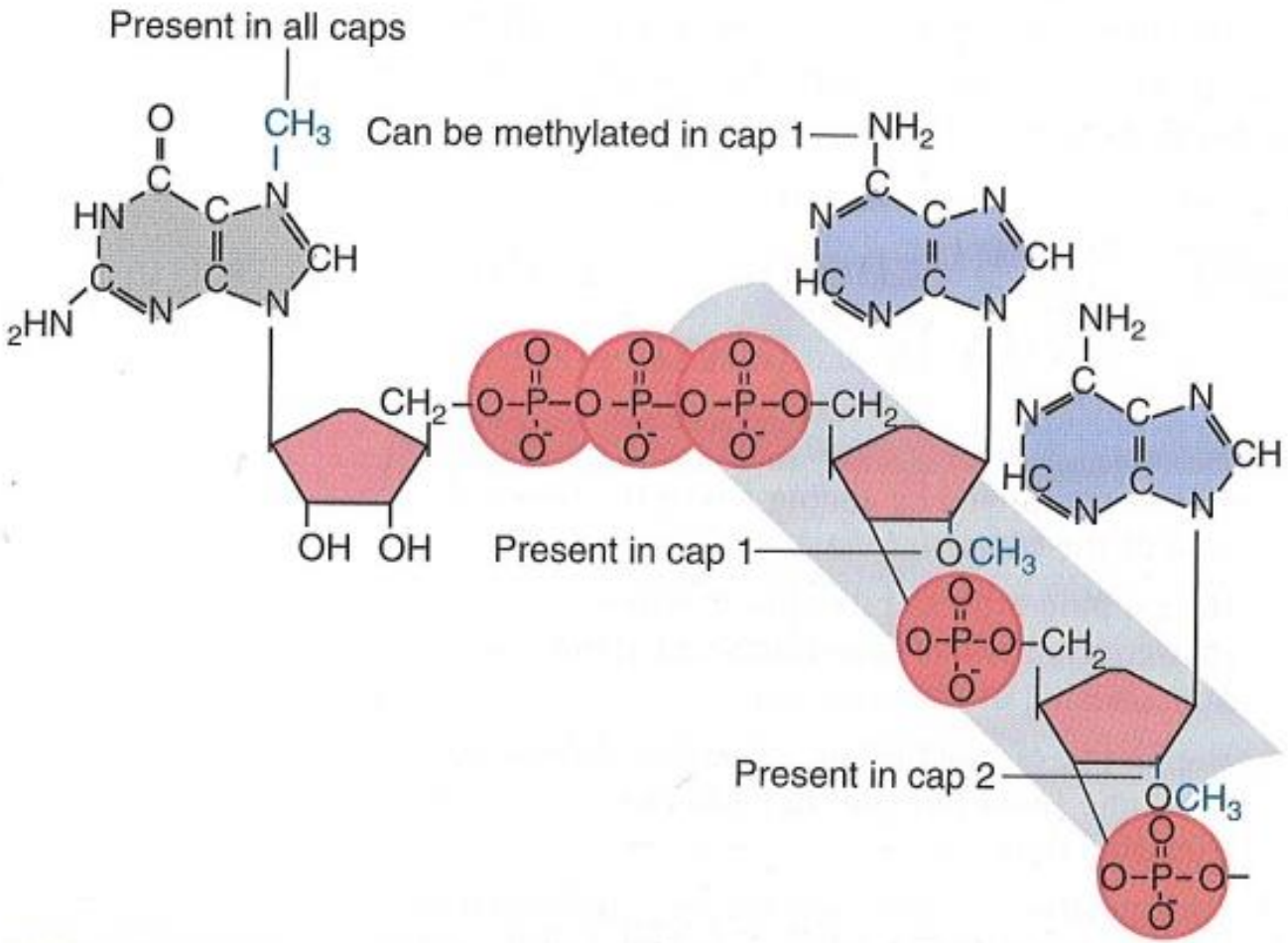


FIGURE 21.2 The cap blocks the 5′ end of mRNA and can be methylated at several positions.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI“; Jones&Bartlett Learning

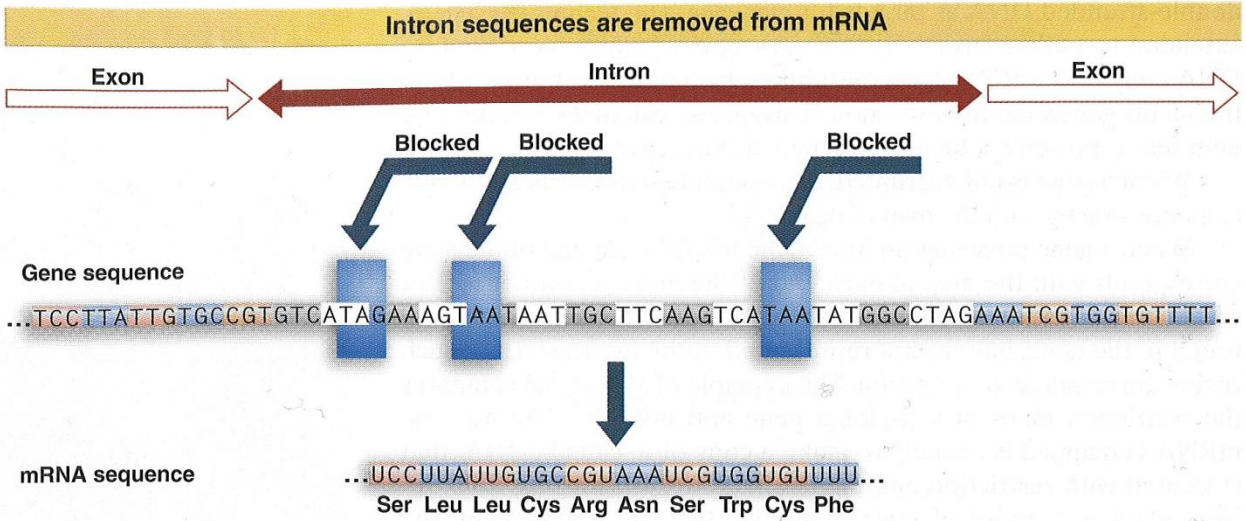


Figure 3.6 An intron is a sequence present in the gene but absent from the mRNA (here shown in terms of the cDNA sequence). All three possible reading frames are blocked by termination codons in the intron.

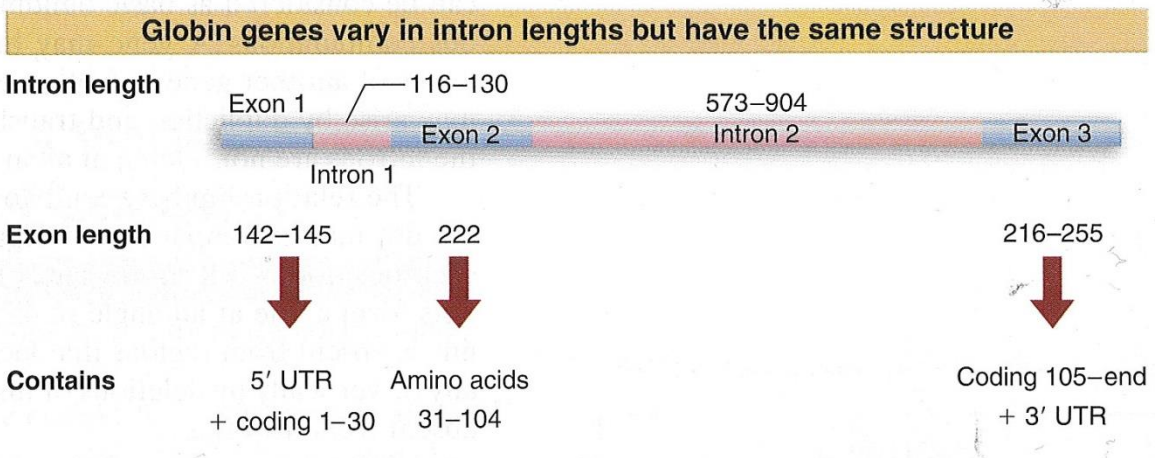


Figure 3.8 All functional globin genes have an interrupted structure with three exons. The lengths indicated in the figure are those of the mammalian β-globin genes.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

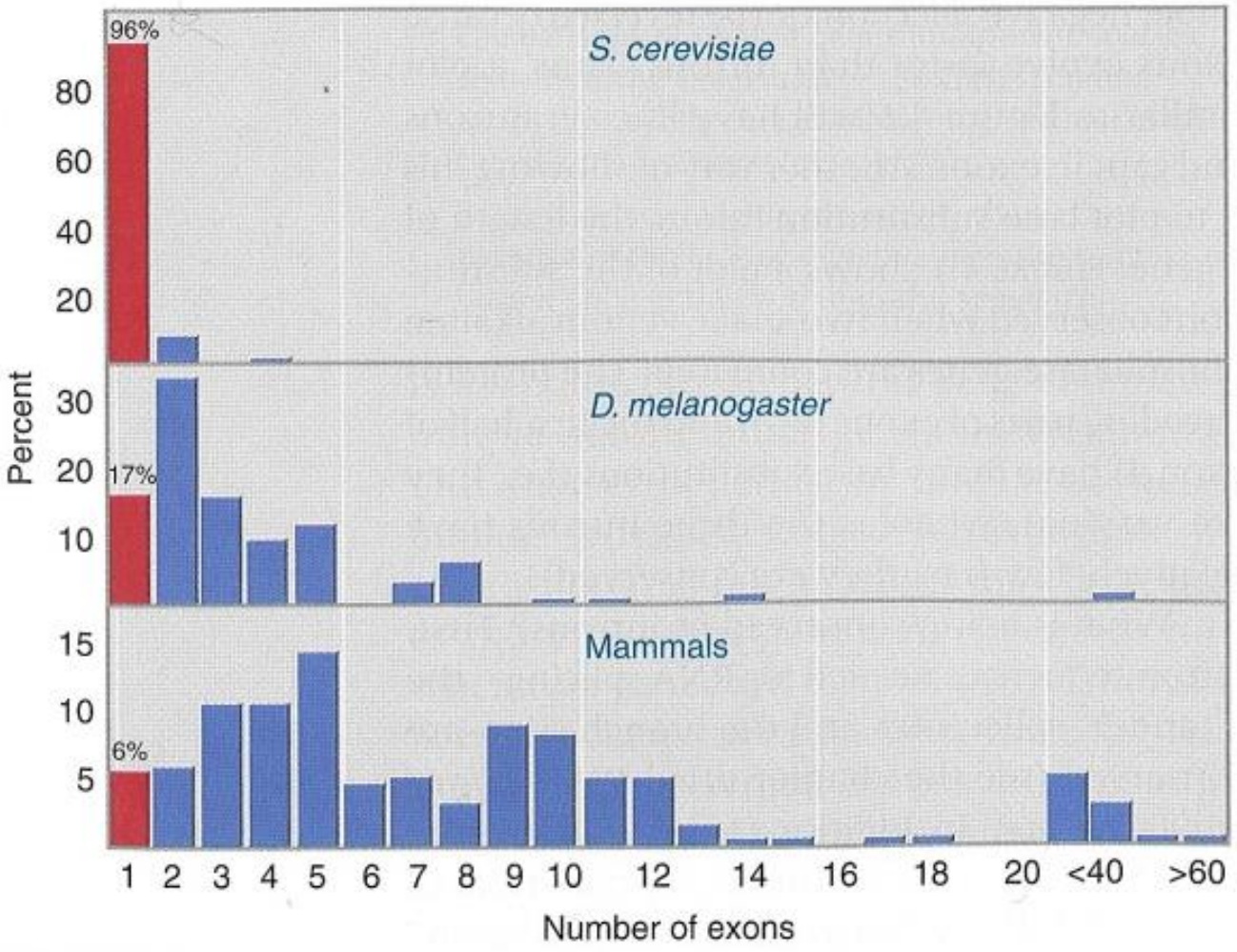
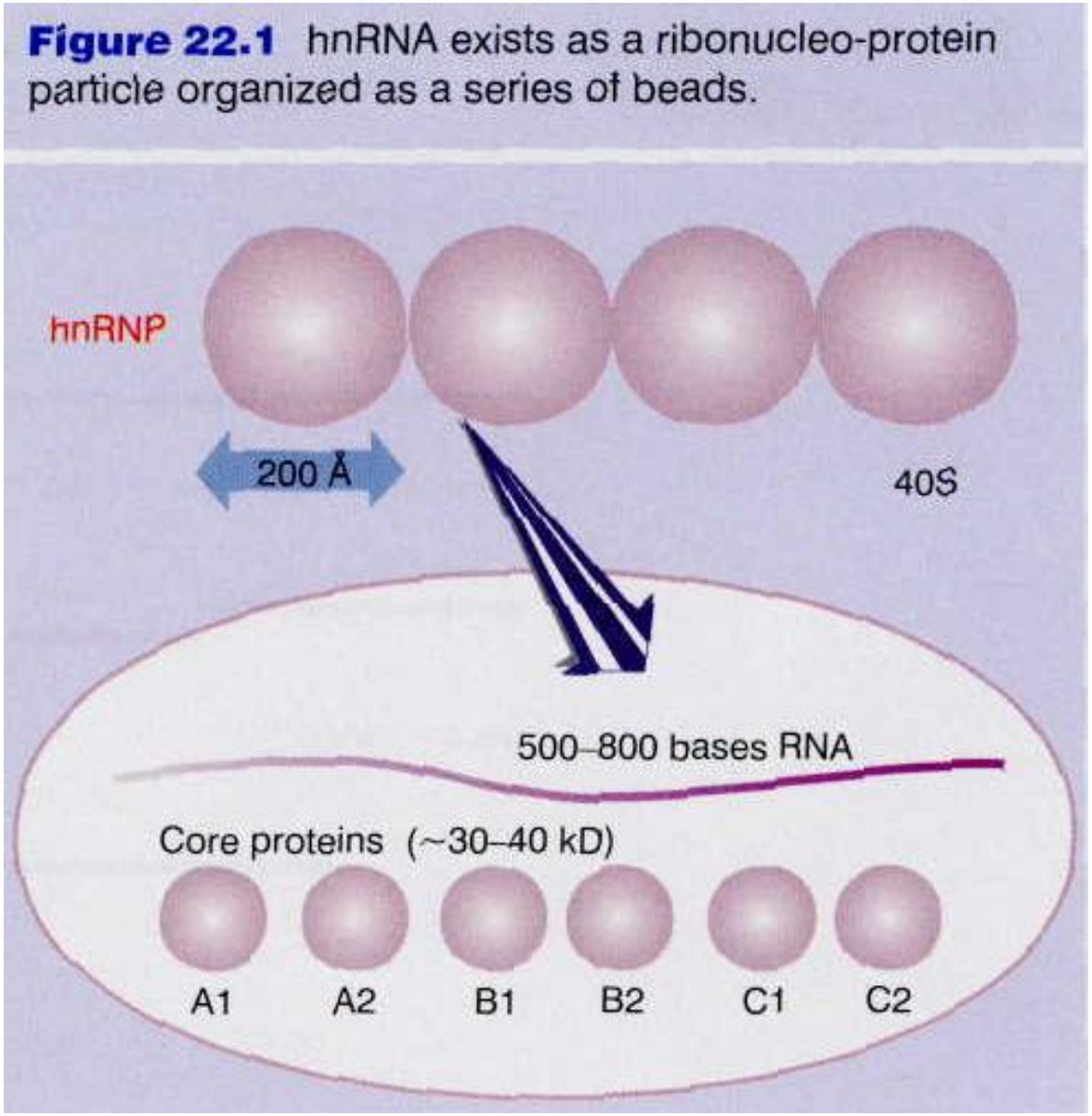


FIGURE 4.8 Most genes are uninterrupted in yeast, but most genes are interrupted in flies and mammals. (Uninterrupted genes have only one exon and are totaled in the leftmost column in red.)

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

hnRNA: heterogeneous nuclear RNA

hnRNP: heterogeneous nuclear Ribonucleoprotein



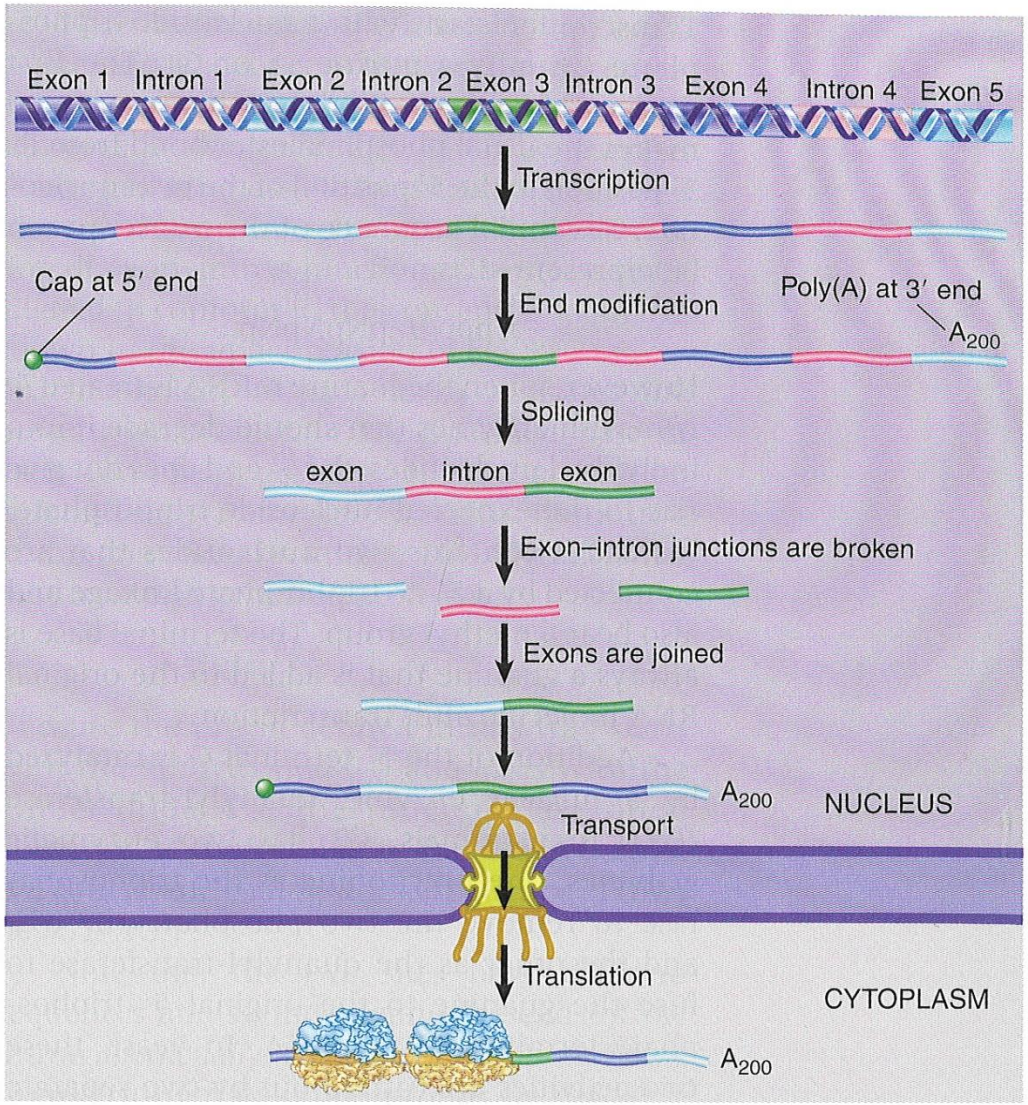


FIGURE 21.1 RNA is modified in the nucleus by additions to the 5′ and 3′ ends and by splicing to remove the introns. The splicing event requires breakage of the exon–intron junctions and joining of the ends of the exons. Mature mRNA is transported through nuclear pores to the cytoplasm, where it is translated.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

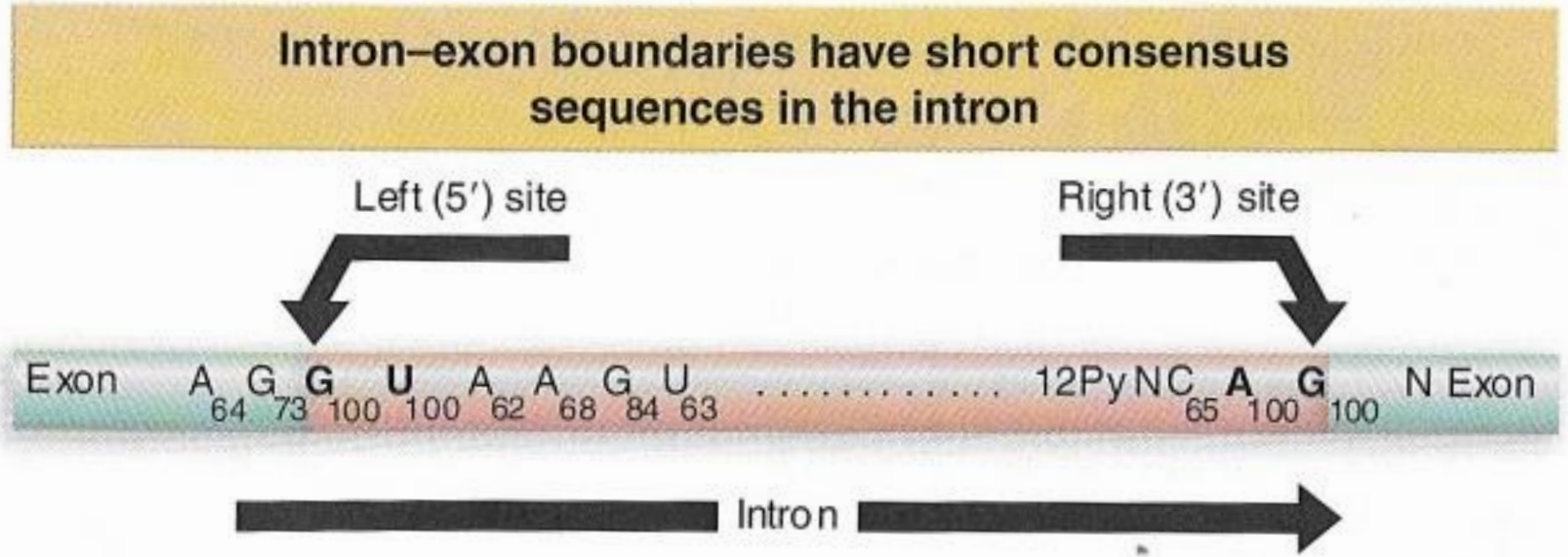


Figure 26.2 The ends of nuclear introns are defined by the GU–AG rule.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

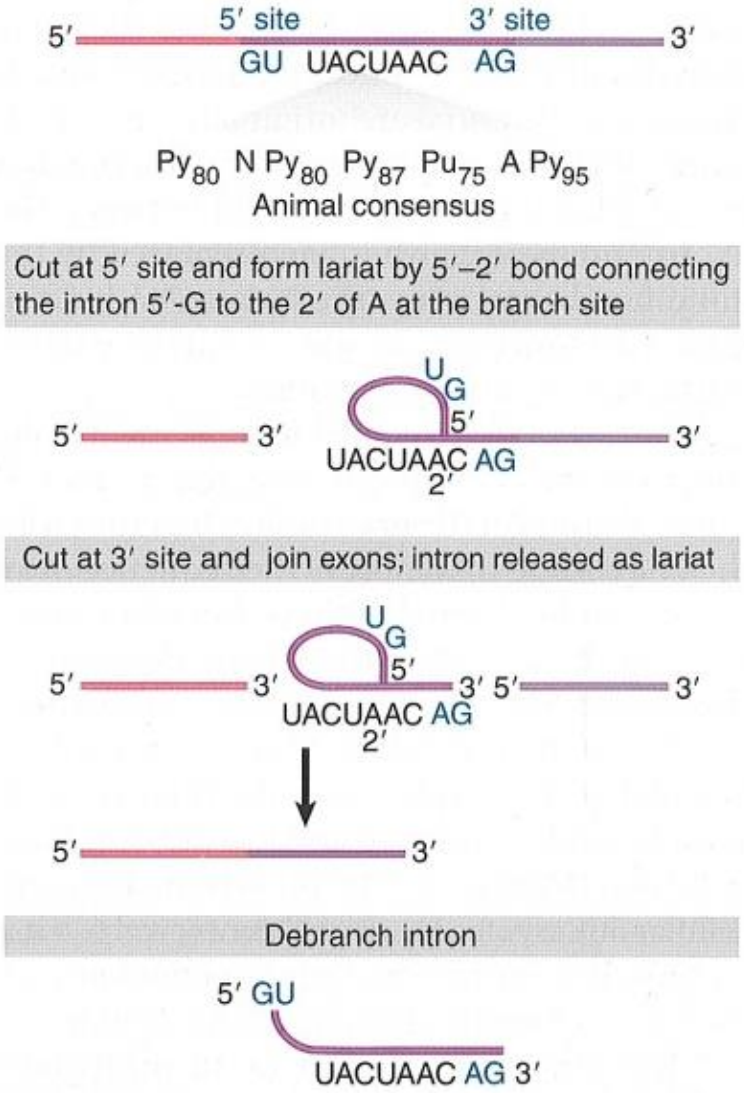


FIGURE 21.5 Splicing occurs in two stages. First the 5′ exon is cleaved off, and then it is joined to the 3′ exon.

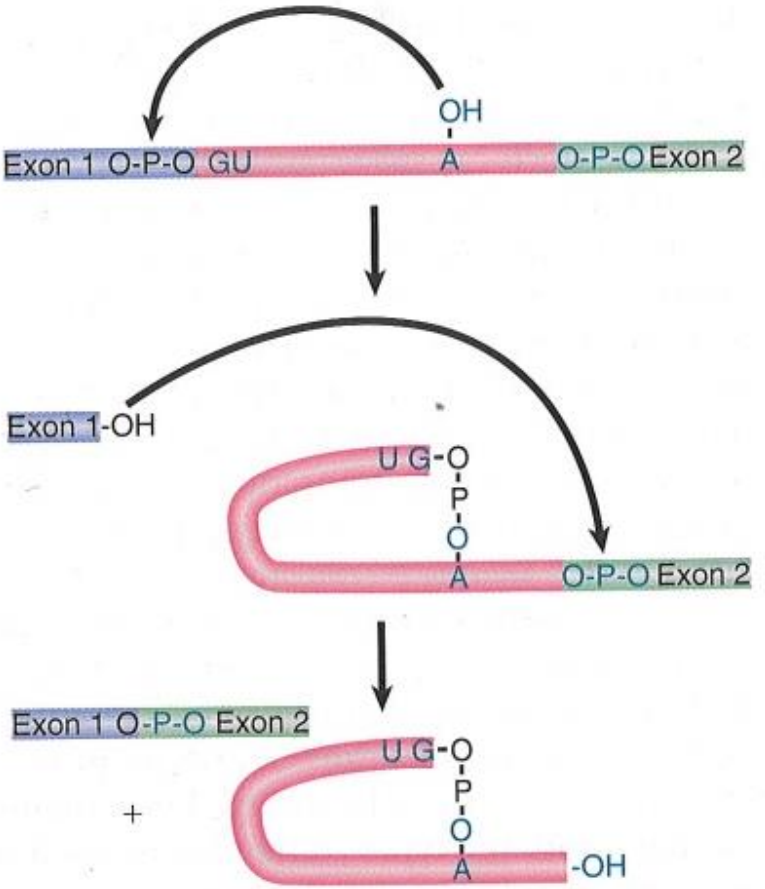


FIGURE 21.6 Nuclear splicing occurs by two transesterification reactions, in which an –OH group attacks a phosphodiester bond.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

Group II introns form lariats

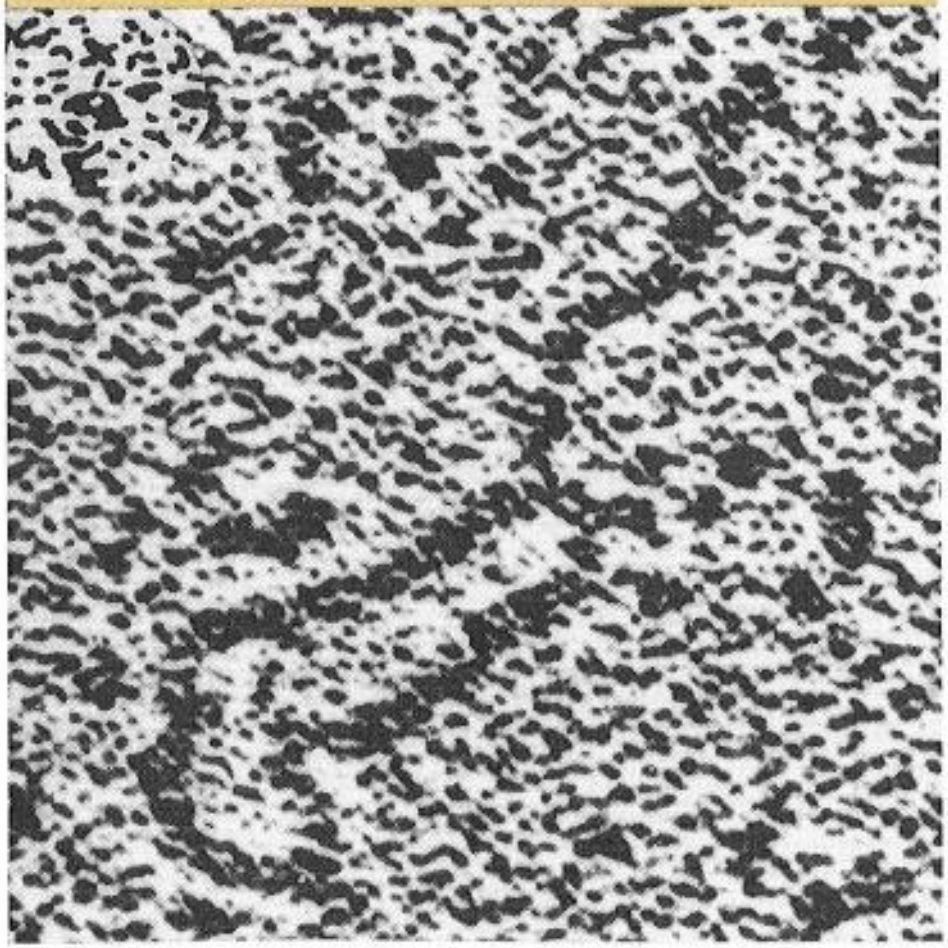
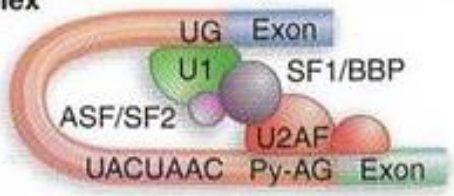


Figure 26.18 Splicing releases a mitochondrial group II intron in the form of a stable lariat. Photograph kindly provided by Leslie Grivell and Annika Arnberg.

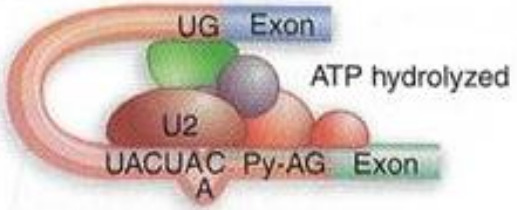
Taken from: B. Lewin, Essential Genes, Pearson Ed. International

A spliceosome forms through several discrete complexes

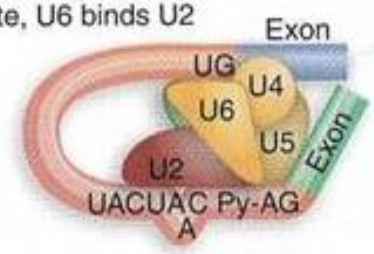
E complex



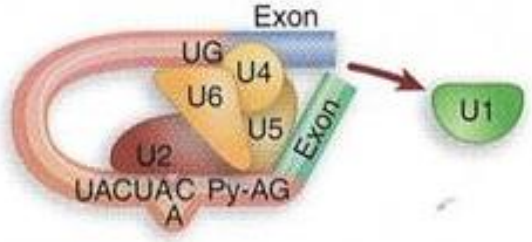
A complex–U2 binds branch site



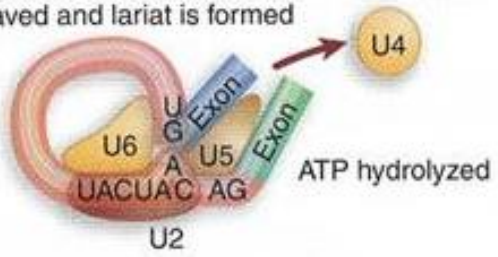
B1 complex–U5/U4/U6 trimer binds, U5 binds exon at 5′ site, U6 binds U2



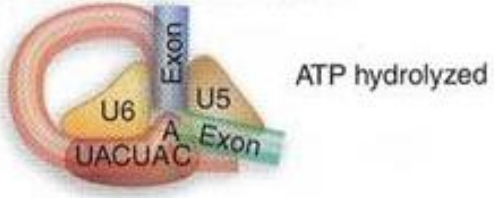
B2 complex–U1 is released, U5 shifts from exon to intron, U6 binds at 5′ splice site



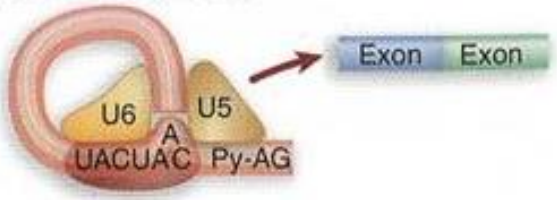
C1 complex–U4 is released, U6/U2 catalyzes transesterification, U5 binds exon at 3′ splice site, 5′ site cleaved and lariat is formed



C2 complex–U2/U5/U6 remain bound to lariat, 3′ site cleaved and exons ligated



Spliced RNA is released



Lariat debranched

Figure 26.12 The splicing reaction proceeds through discrete stages in which spliceosome formation involves the interaction of components that recognize the consensus sequences.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

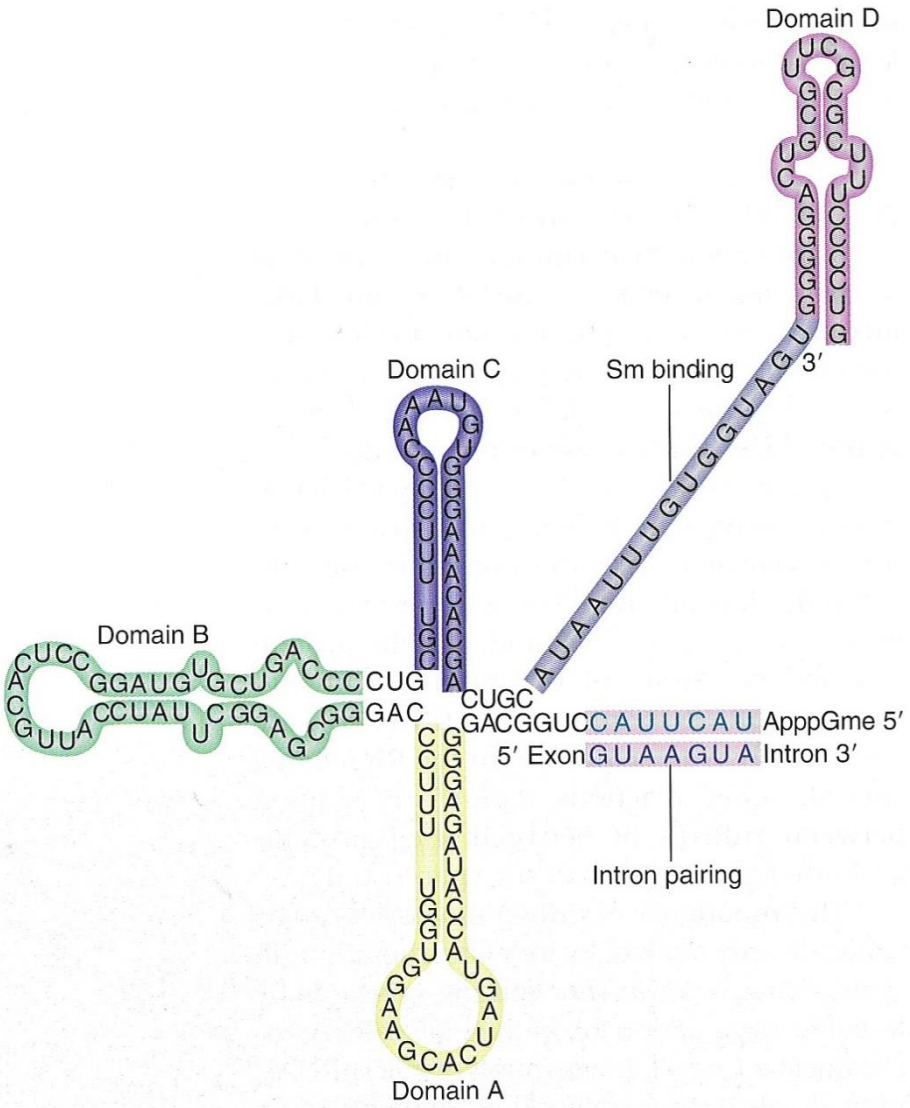


FIGURE 21.8 U1 snRNA has a base-paired structure that creates several domains. The 5′ end remains single stranded and can base pair with the 5′ splice site.

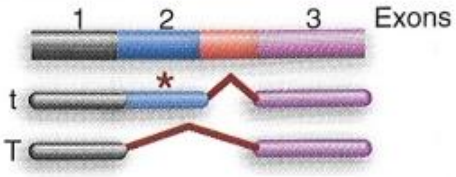
snRNA: small nuclear RNA

snRNP: small nuclear Ribonucleoparticle „snurps“

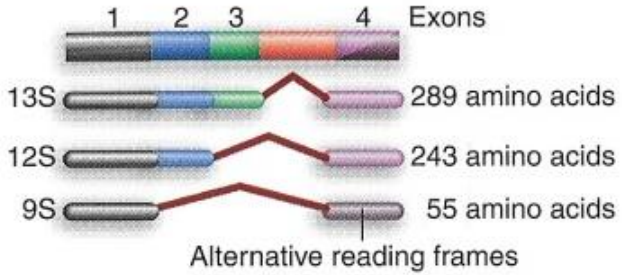
Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI“; Jones&Bartlett Learning

Alternative splicing generates multiple RNAs

SV40 T/t antigens splice two 5′ sites to a common 3′ site



Adenovirus E1A splices variable 5′ sites to a common 3′ site



D. melanogaster tra splices 5′ site to alternative 3′ sites

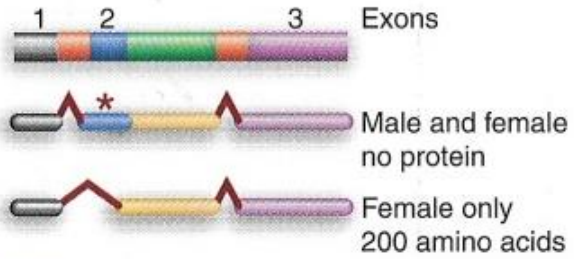
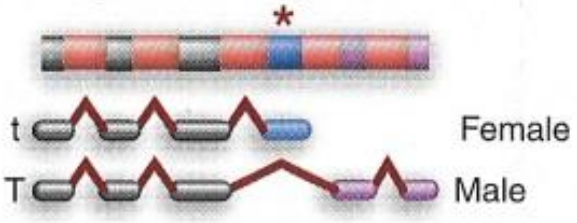


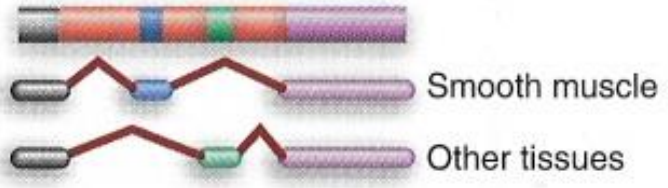
Figure 26.20 Alternative forms of splicing may generate a variety of protein products from an individual gene. Changing the splice sites may introduce termination codons (shown by asterisks) or change reading frames.

Alternative splicing may substitute exons

D. melanogaster dsx skips an exon



α-tropomyosin splices alternative exons



P elements splice out an extra intron

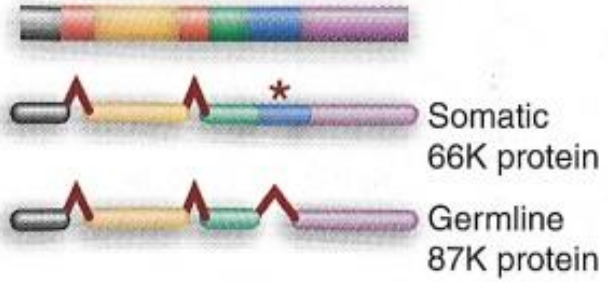


Figure 26.21 Alternative splicing events may cause exons to be added or substituted.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

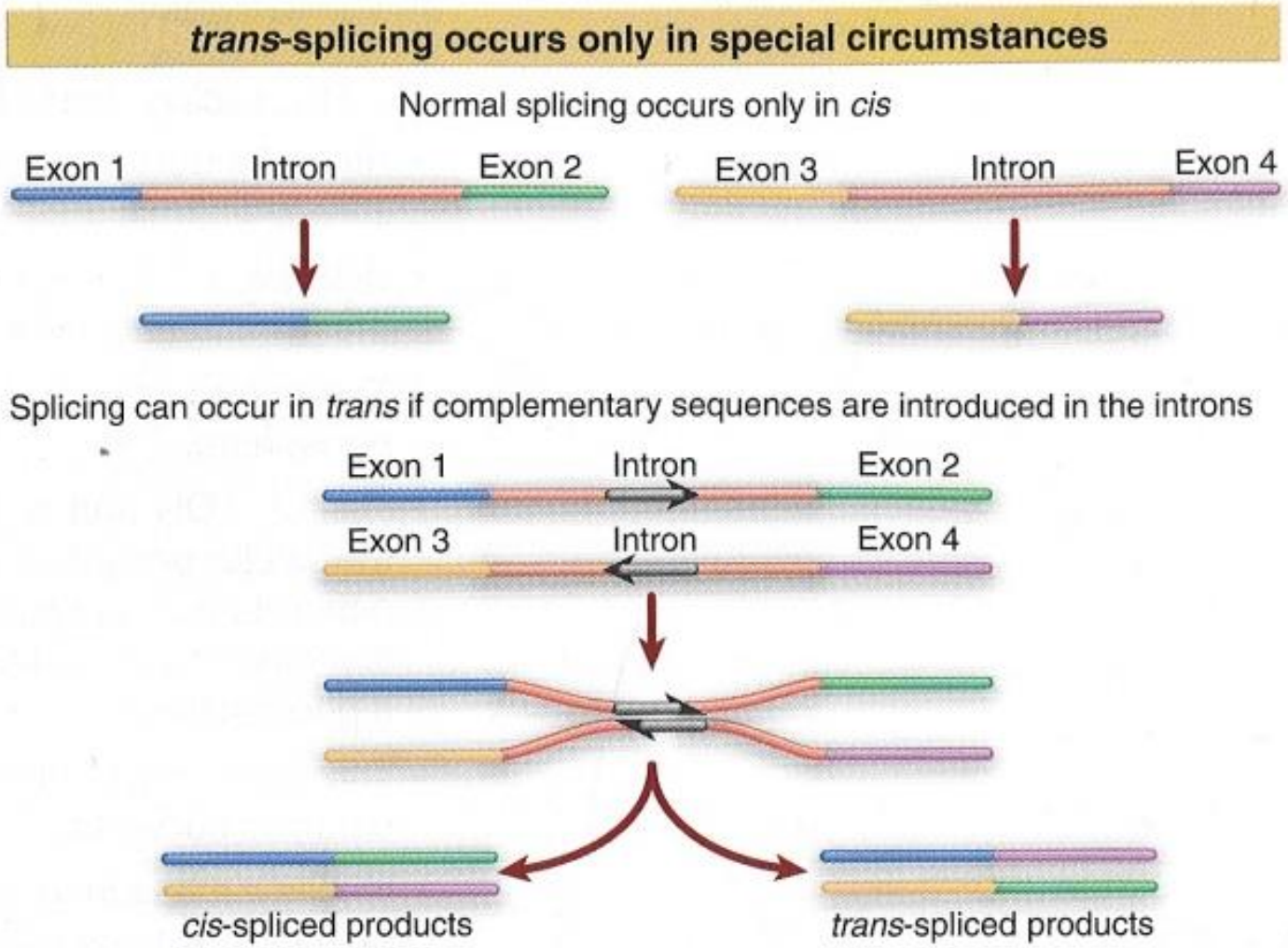
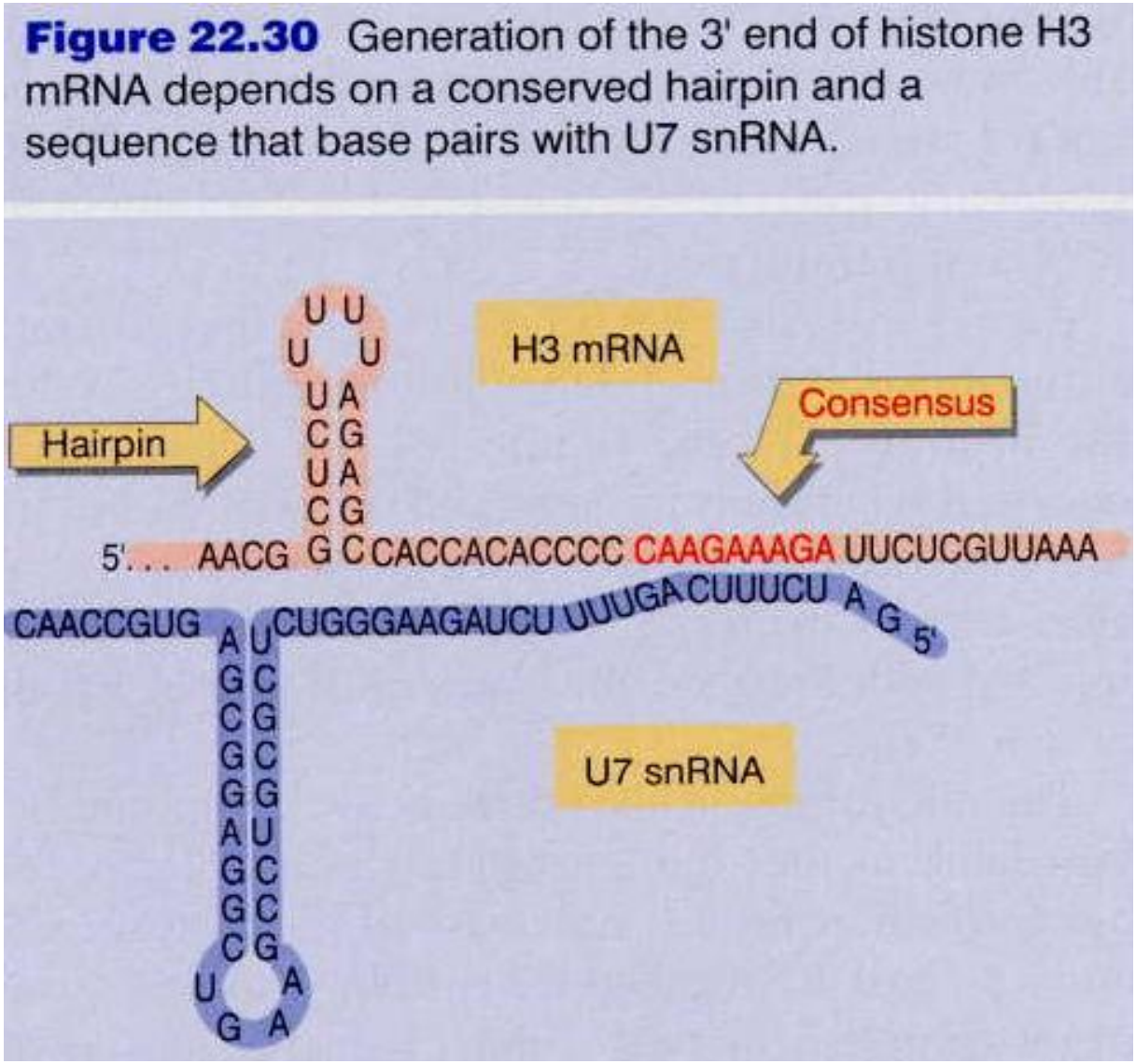


Figure 26.22 Splicing usually occurs only in *cis* between exons carried on the same physical RNA molecule, but *trans* splicing can occur when special constructs are made that support base pairing between introns.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

3‘-end processing



3‘-end processing

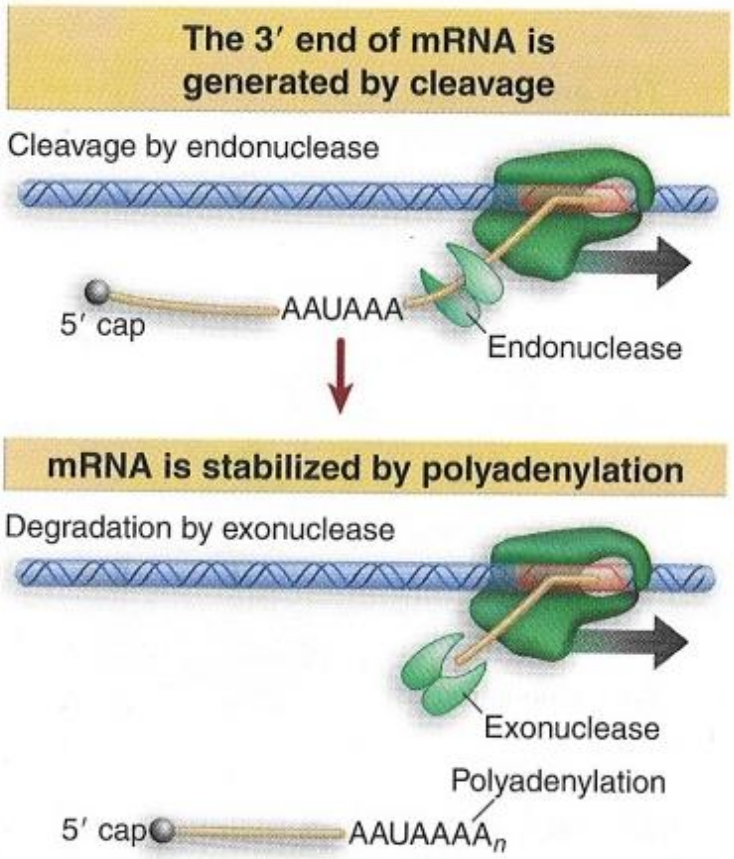


Figure 26.28 The sequence AAUAAA is necessary for cleavage to generate a 3′ end for polyadenylation.

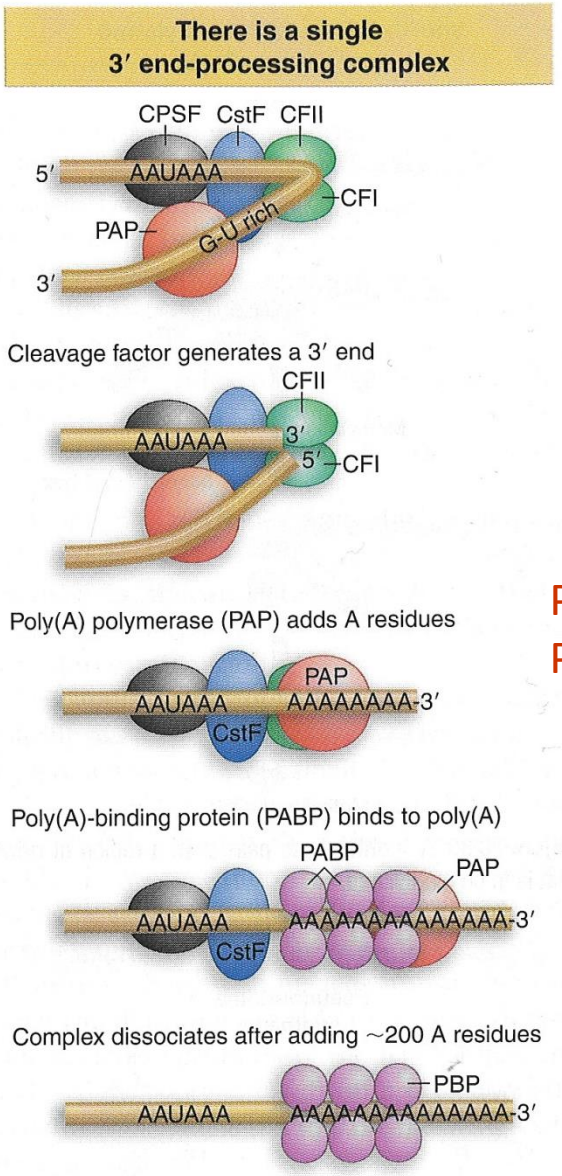


Figure 26.29 The 3′ processing complex consists of several activities. CPSF and CstF each consist of several subunits; the other components are monomeric. The total mass is >900 kD.

PAP: PolyA-Polymerase

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

Transcription in Eukayotes

Pol III:
Transfer RNA
5S rRNA
Small nuclear RNA U6
Repeated DNA sequ.
(e.g. Alu)

Pol I:
Ribosomal RNAs

Pol II:
All coding genes
Small nuclear RNAs

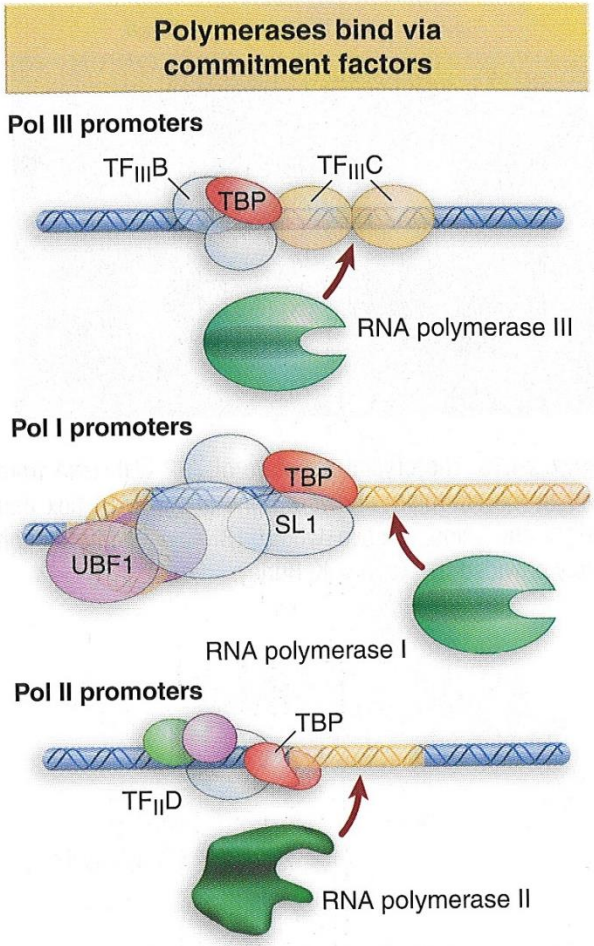


Figure 24.8 RNA polymerases are positioned at all promoters by a factor that contains TBP.

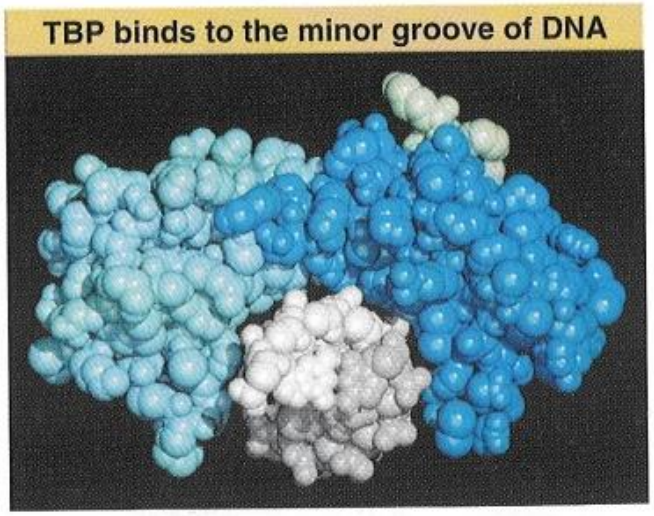


Figure 24.9 A view in cross section shows that TBP surrounds DNA from the side of the minor groove. TBP consists of two related (40% identical) conserved domains, which are shown in light and dark blue. The N-terminal region varies extensively and is shown in green. The two strands of the DNA double helix are in light and dark grey. Photograph kindly provided by Stephen K. Burley.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

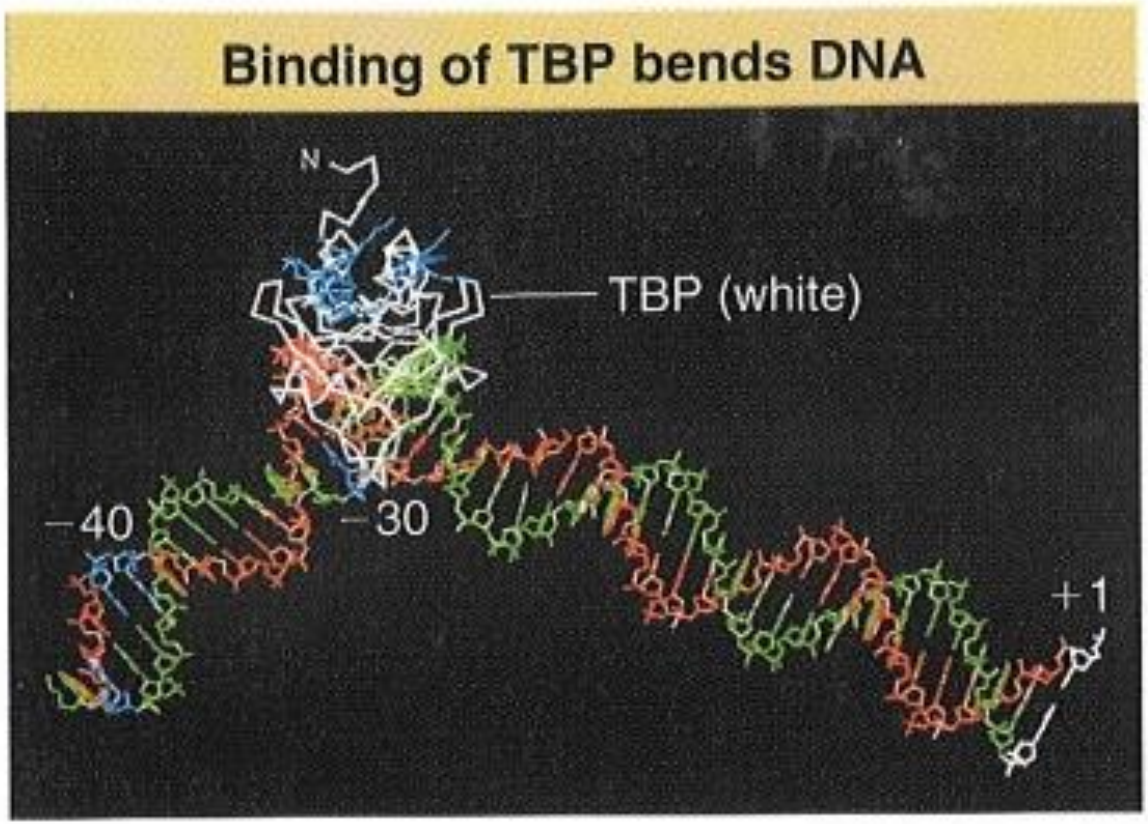


Figure 24.10 The crystal structure of TBP with DNA from −40 to the startpoint shows a bend at the TATA box that widens the minor groove where TBP binds. Photograph kindly provided by Stephen K. Burley.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

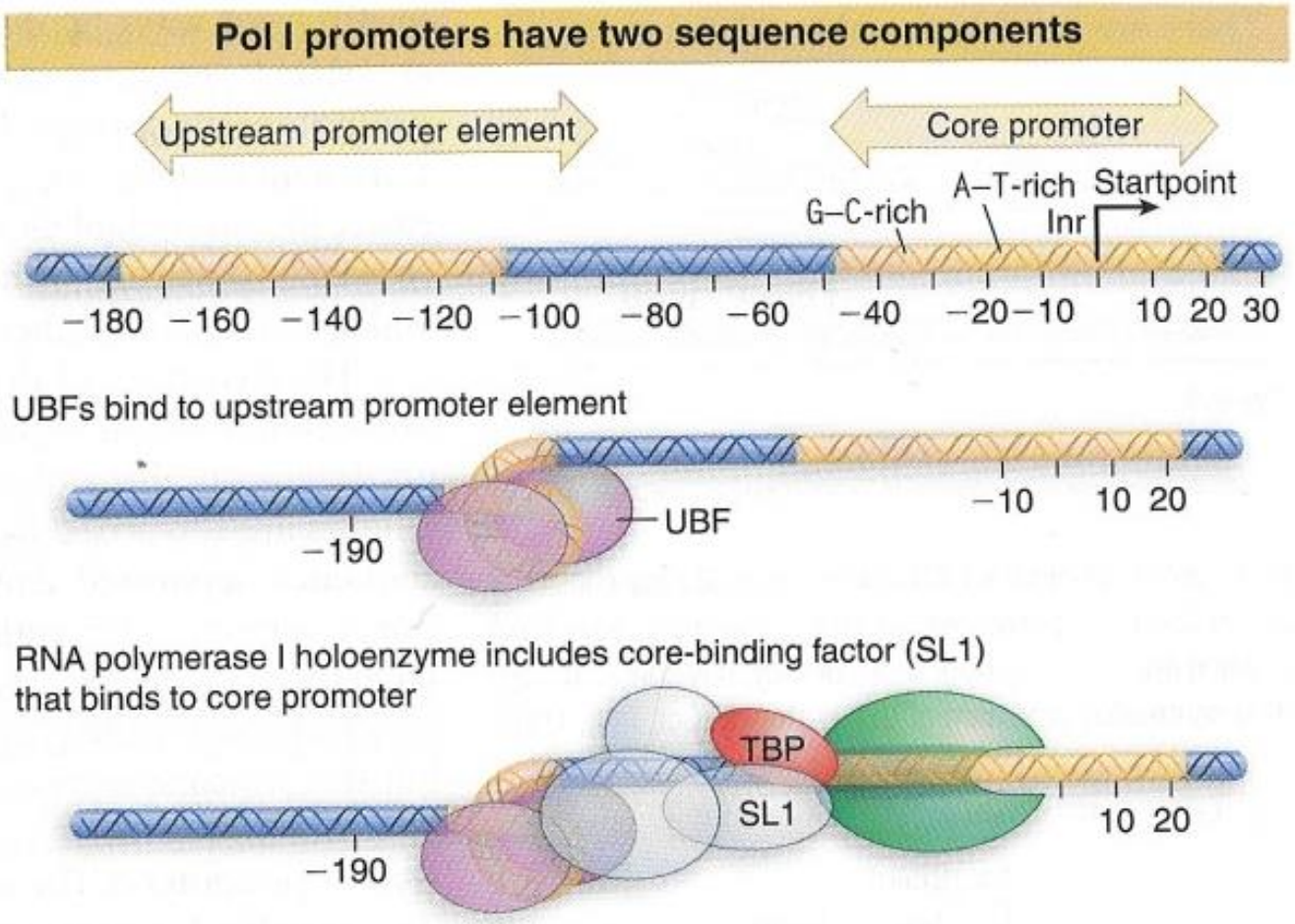


Figure 24.3 Transcription units for RNA polymerase I have a core promoter separated by ~70 bp from the upstream promoter element. UBF binding to the UPE increases the ability of core-binding factor (SL1) to bind to the core promoter.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

Figure 20.5 Deletion analysis shows that the promoter for 5S RNA genes is internal; initiation occurs a fixed distance (~55 bp) upstream of the promoter.

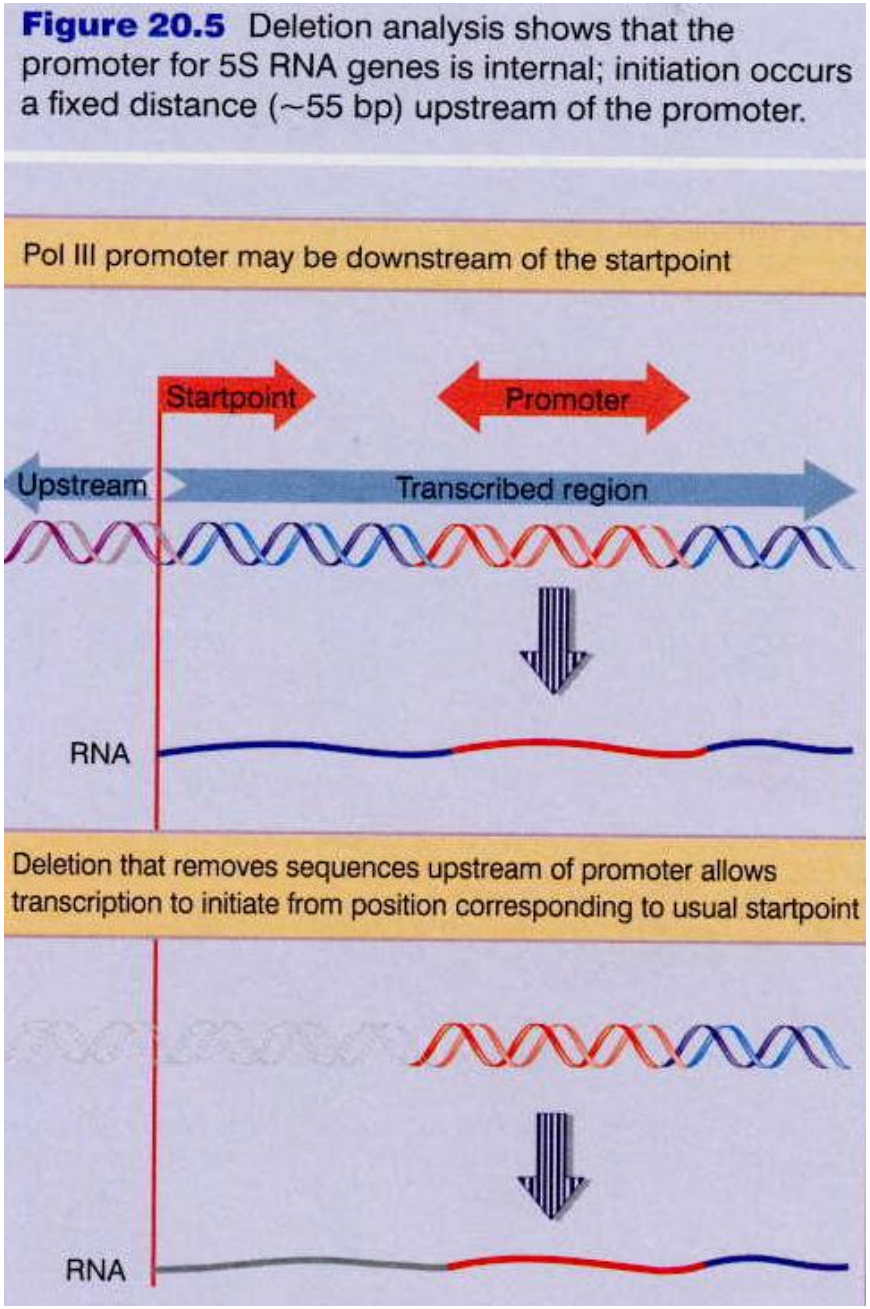


Figure 20.6 Promoters for RNA polymerase III may consist of bipartite sequences downstream of the startpoint, with boxA separated from either boxC or boxB. Or they may consist of separated sequences upstream of the startpoint (Oct, PSE, TATA).

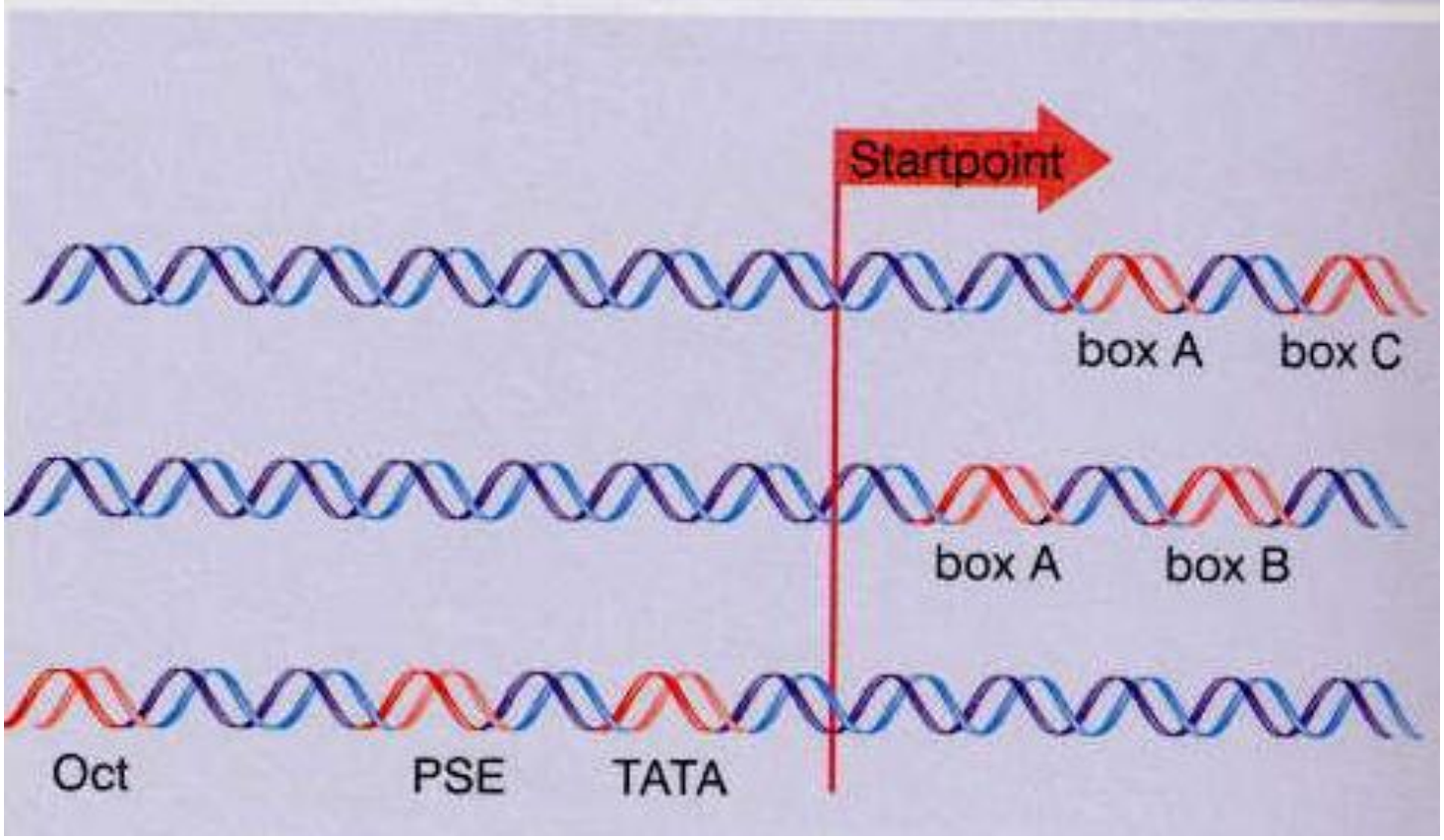
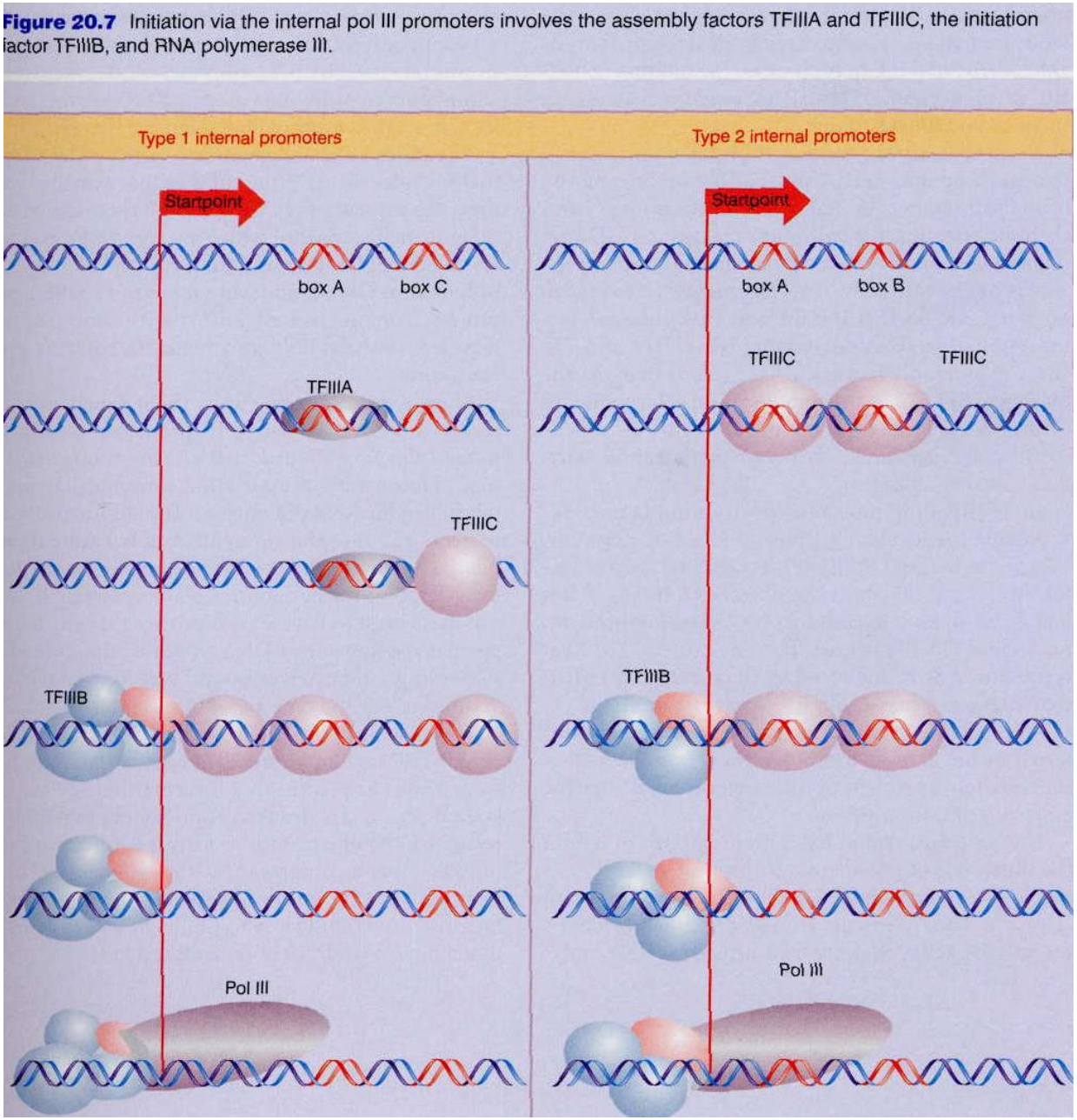


Figure 20.7 Initiation via the internal pol III promoters involves the assembly factors TFIIIA and TFIIIC, the initiation factor TFIIIB, and RNA polymerase III.



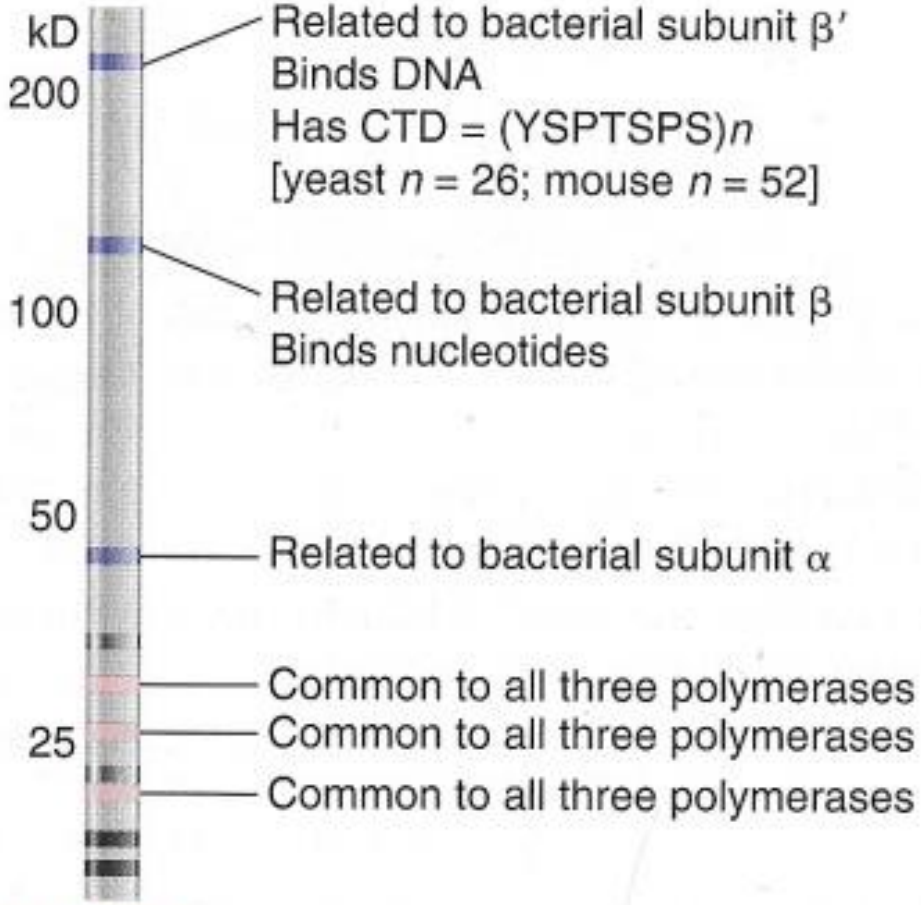


FIGURE 20.2 Some subunits are common to all classes of eukaryotic RNA polymerases and some are related to bacterial RNA polymerase. This drawing is a simulation of purified yeast RNA polymerase II run on an SDS gel to separate the subunits by size.

Pol II Promoters

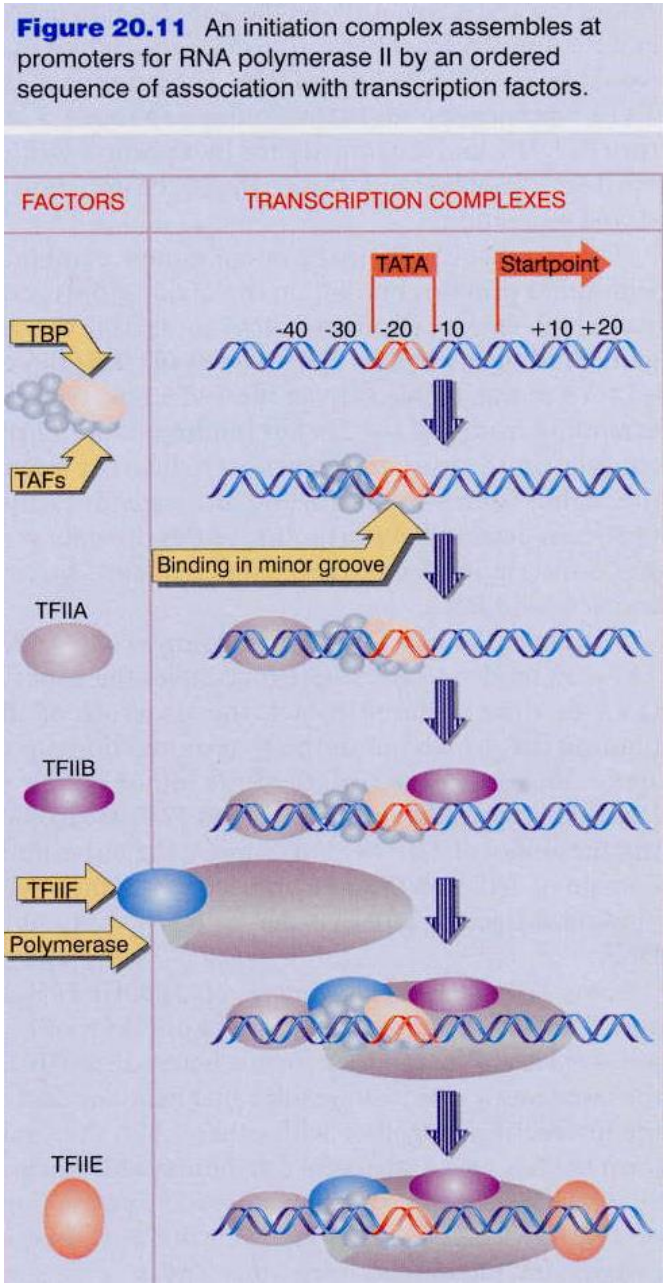


Figure 20.11 An initiation complex assembles at promoters for RNA polymerase II by an ordered sequence of association with transcription factors.

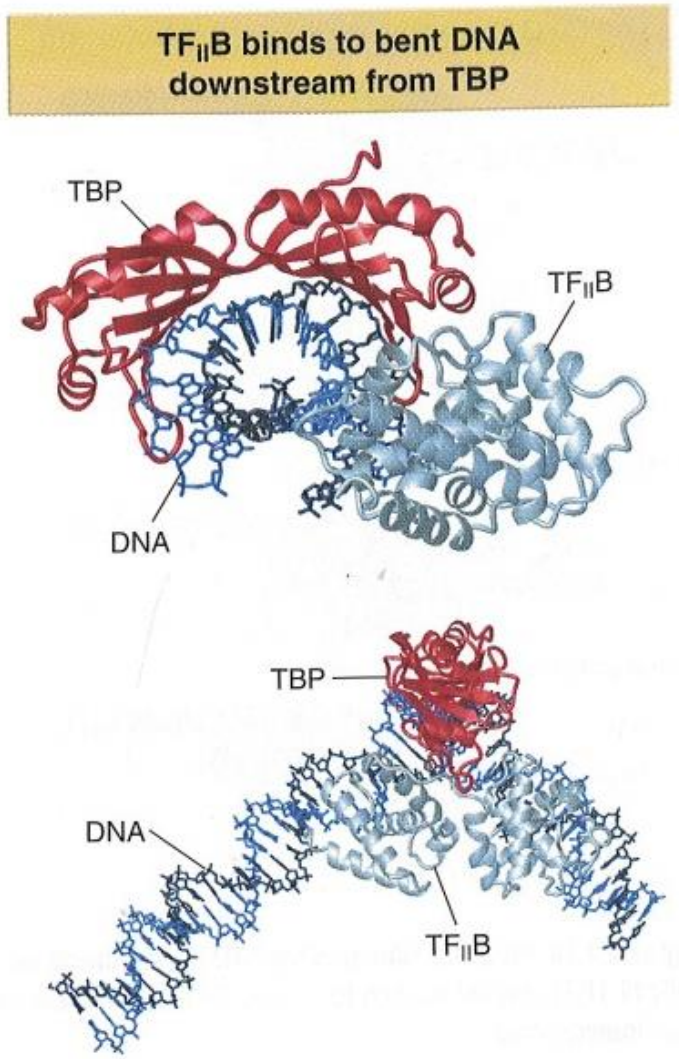


Figure 24.12 Two views of the ternary complex of TF$_{II}$B-TBP-DNA show that TF$_{II}$B binds along the bent face of DNA. Photograph kindly provided by Stephen K. Burley.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

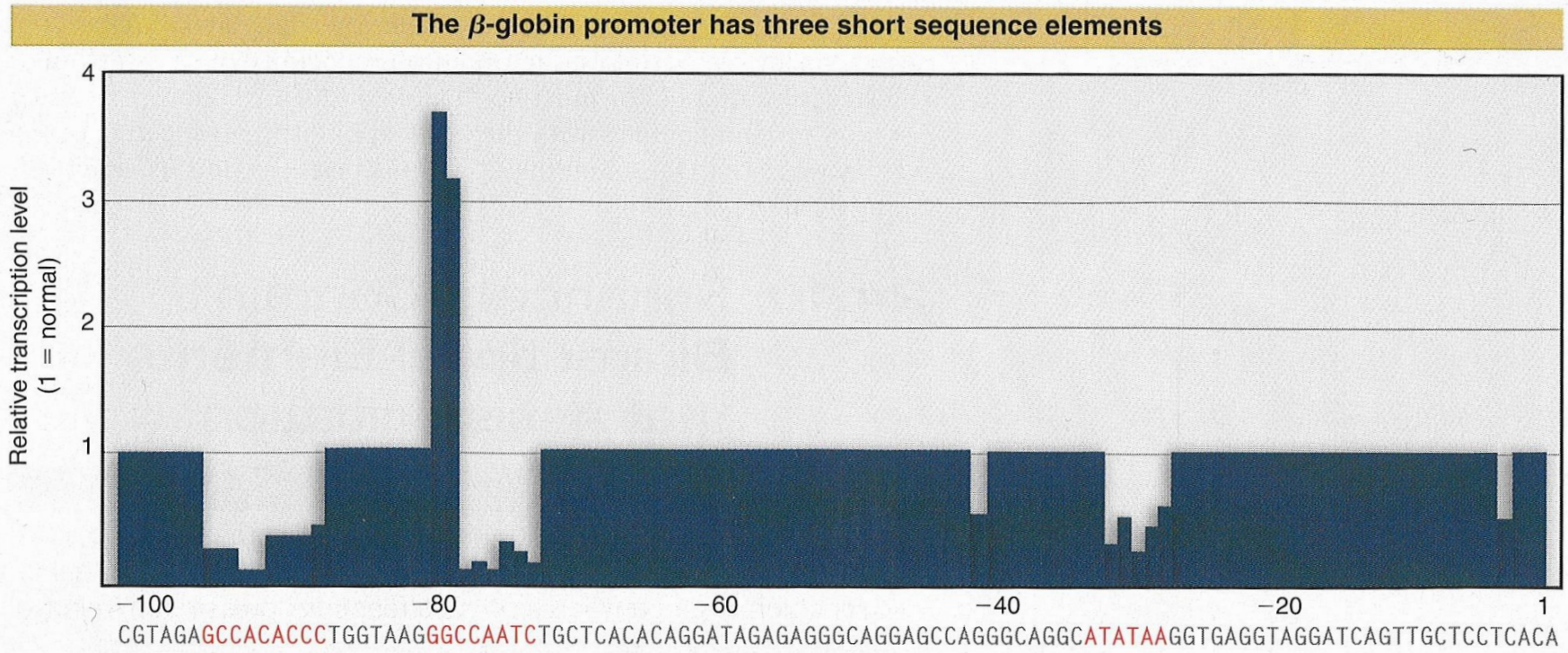


Figure 24.17 Saturation mutagenesis of the upstream region of the β-globin promoter identifies three short regions (centered at −30, −75, and −90) that are needed to initiate transcription. These correspond to the TATA, CAAT, and GC boxes.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

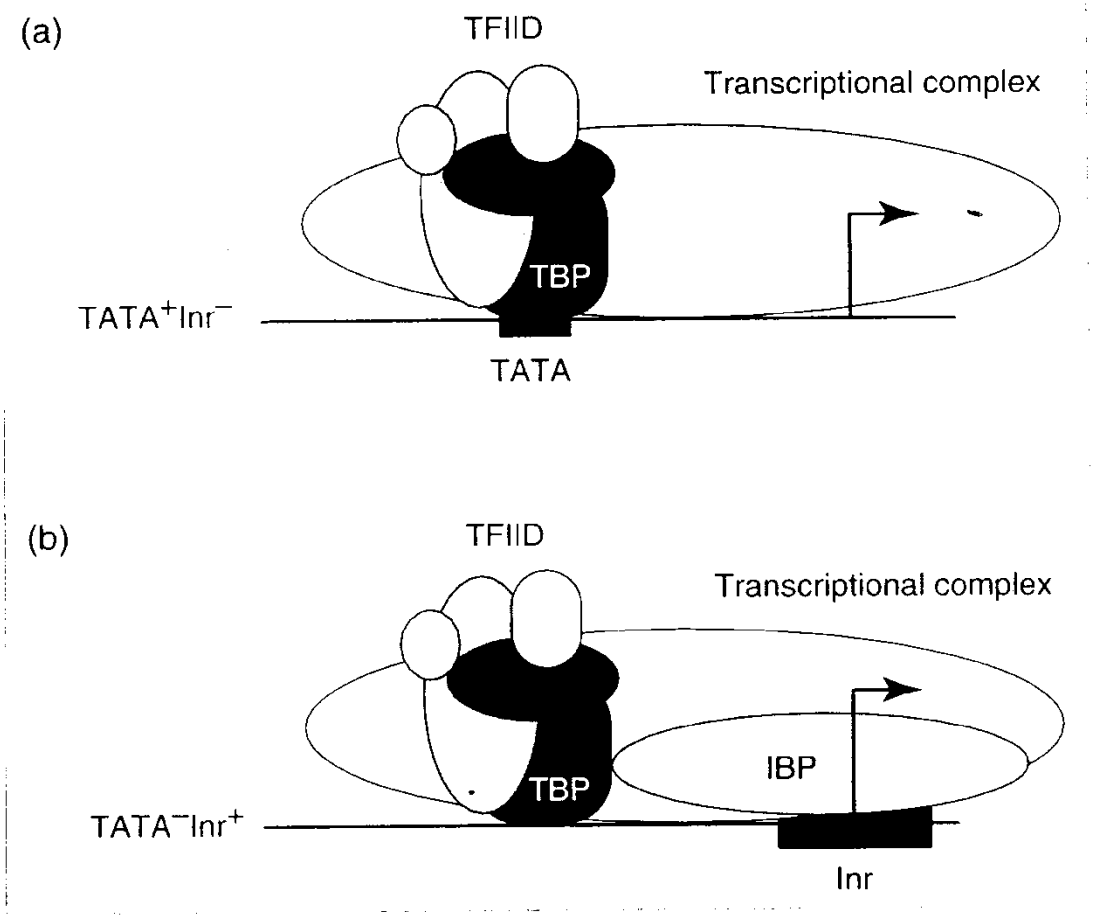


FIGURE 2. Formation of the transcription complex at (a) the TATA box or (b) an initiator (Inr) element. In $TATA^+$ Inr^- promoters, the initial recognition step is the binding of TFIID to the TATA box via its DNA-binding subunit, the TATA-binding protein (TBP). Following this event, other general transcription factors (GTFs) might enter into the complex, either in a stepwise fashion or as a holoenzyme complex, giving rise to a transcriptionally competent complex. In $TATA^-$ Inr^+ promoters, the initial recognition step is the interaction of an Inr-binding protein (IBP). An IBP could be a distinct factor, a GTF, or a subunit of the TFIID complex (TAF, TBP associated factor). TFIID is next recruited to the promoter, potentially via protein–protein interactions, and finally other components enter the transcription complex.

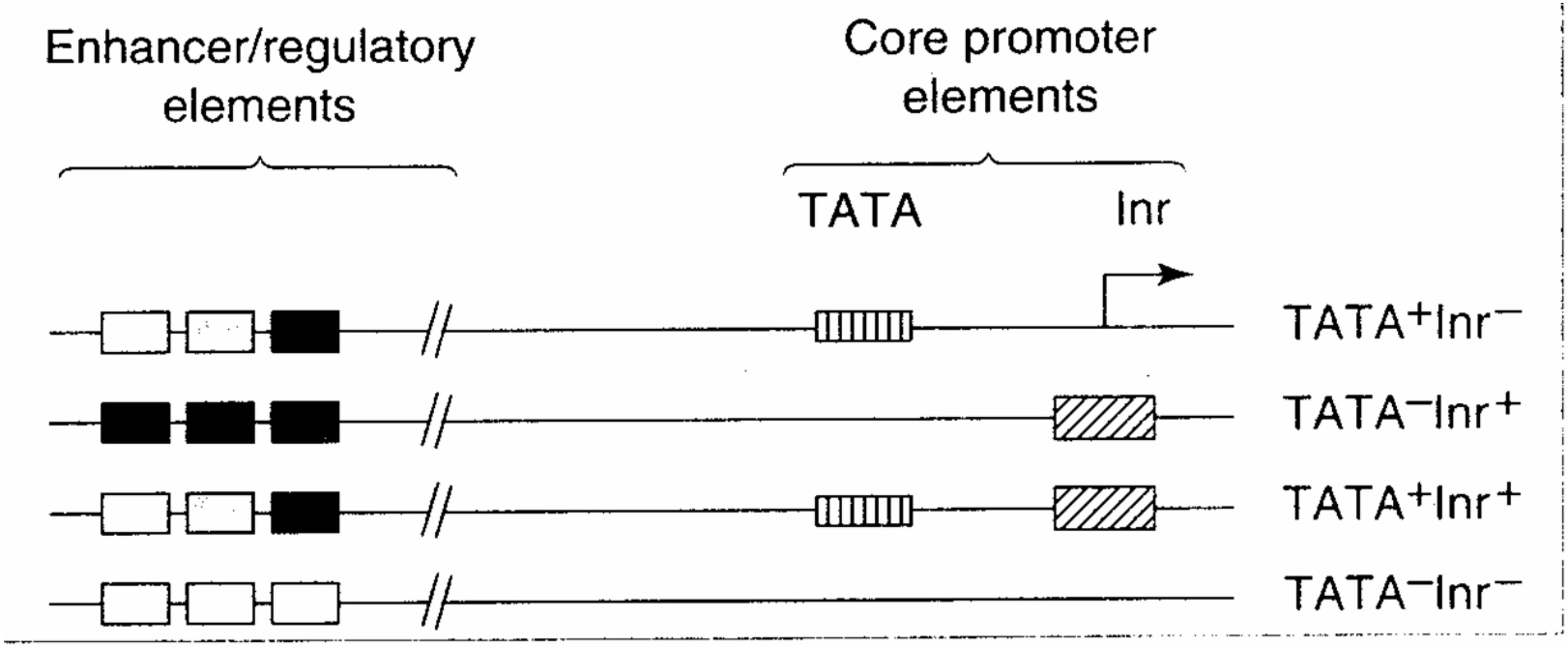
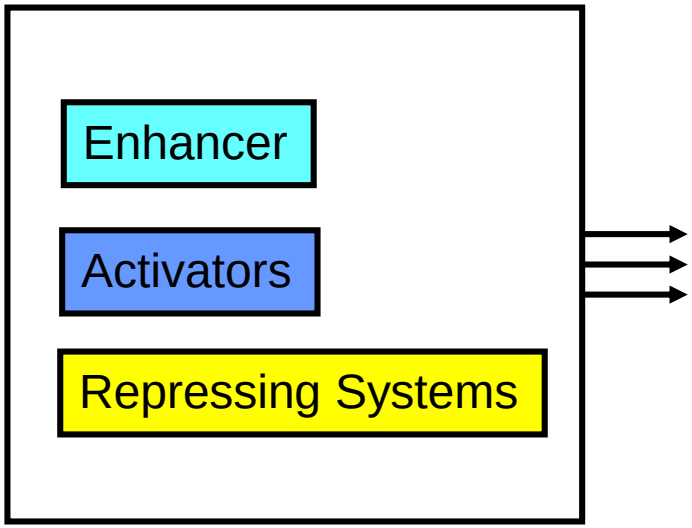


FIGURE 1. Architecture of different classes of eukaryotic RNA polymerase II (Pol II) promoters. The core promoter region may contain either a TATA box ($TATA^+ Inr^-$) or an initiator (Inr) element ($TATA^- Inr^+$). Some promoters might contain both core elements ($TATA^+Inr^+$) and others none ($TATA^- Inr^-$). The transcription start site (+1) is indicated by the arrow. Each promoter may have co-evolved with its associated enhancer region, thereby maintaining specificity of gene expression, especially *in vitro*.

Regulated Expression in Eukaryotes

Complex Initiation System



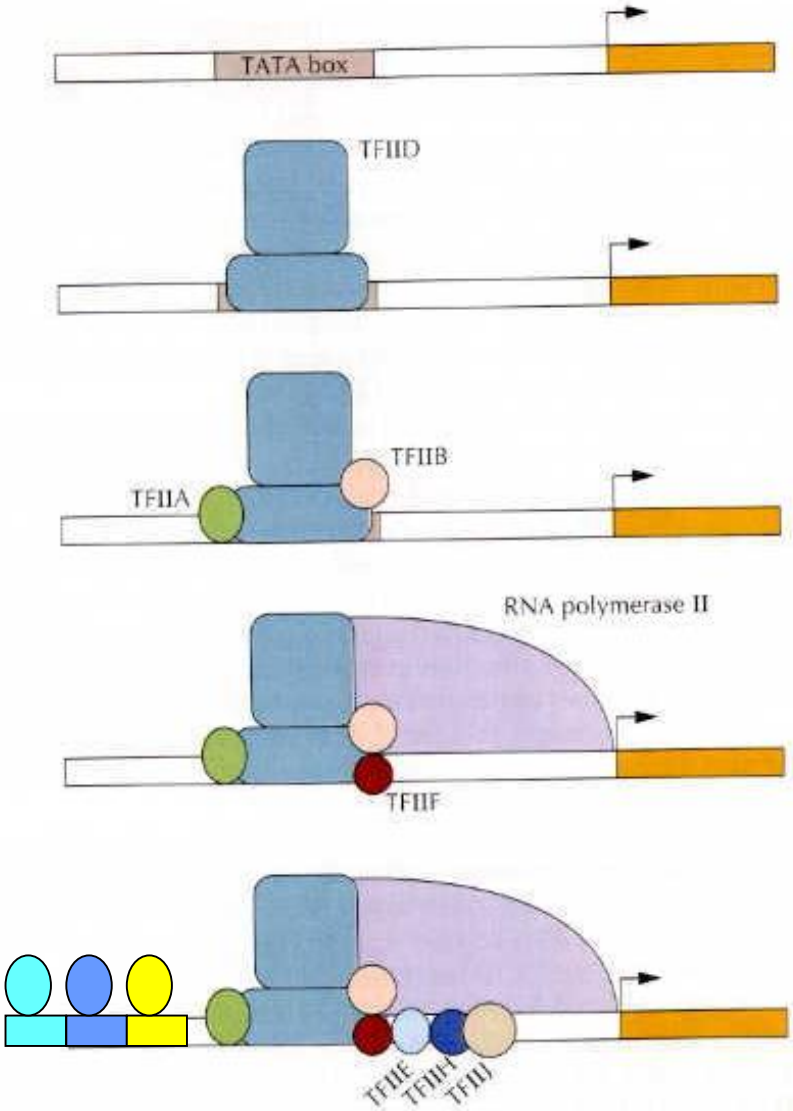


Figure 3.24 Formation of an RNA polymerase II transcription initiation complex at a TATA box. Transcription factor TFIID binds to a TATA box, and, in sequence, other transcription factors and RNA polymerase II bind to form a protein aggregate that is responsible for initiating transcription. The right-angled arrow designates the site of initiation and direction of transcription.

Figure 20.17 Promoters contain different combinations of TATA boxes, CAAT boxes, GC boxes, and other elements.

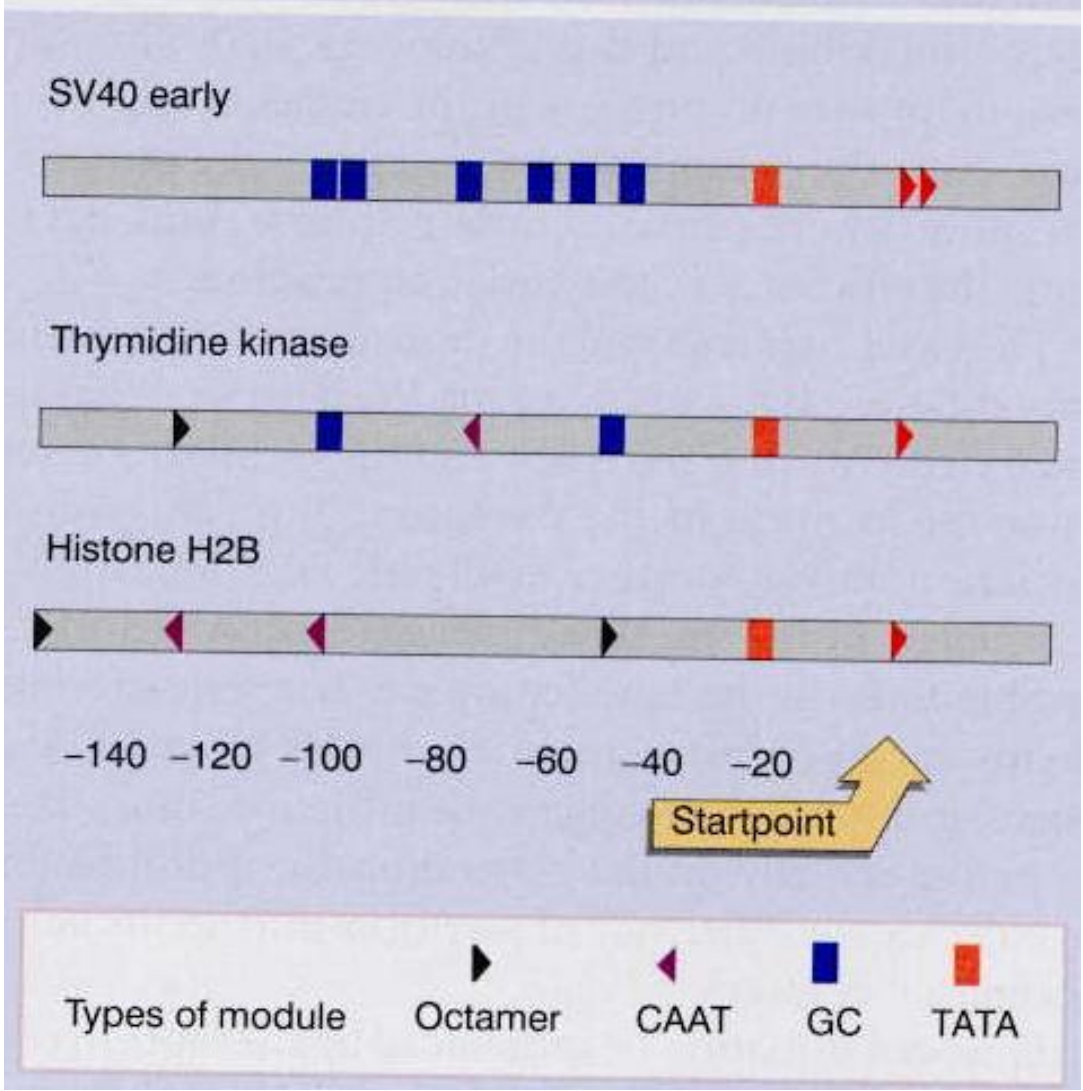
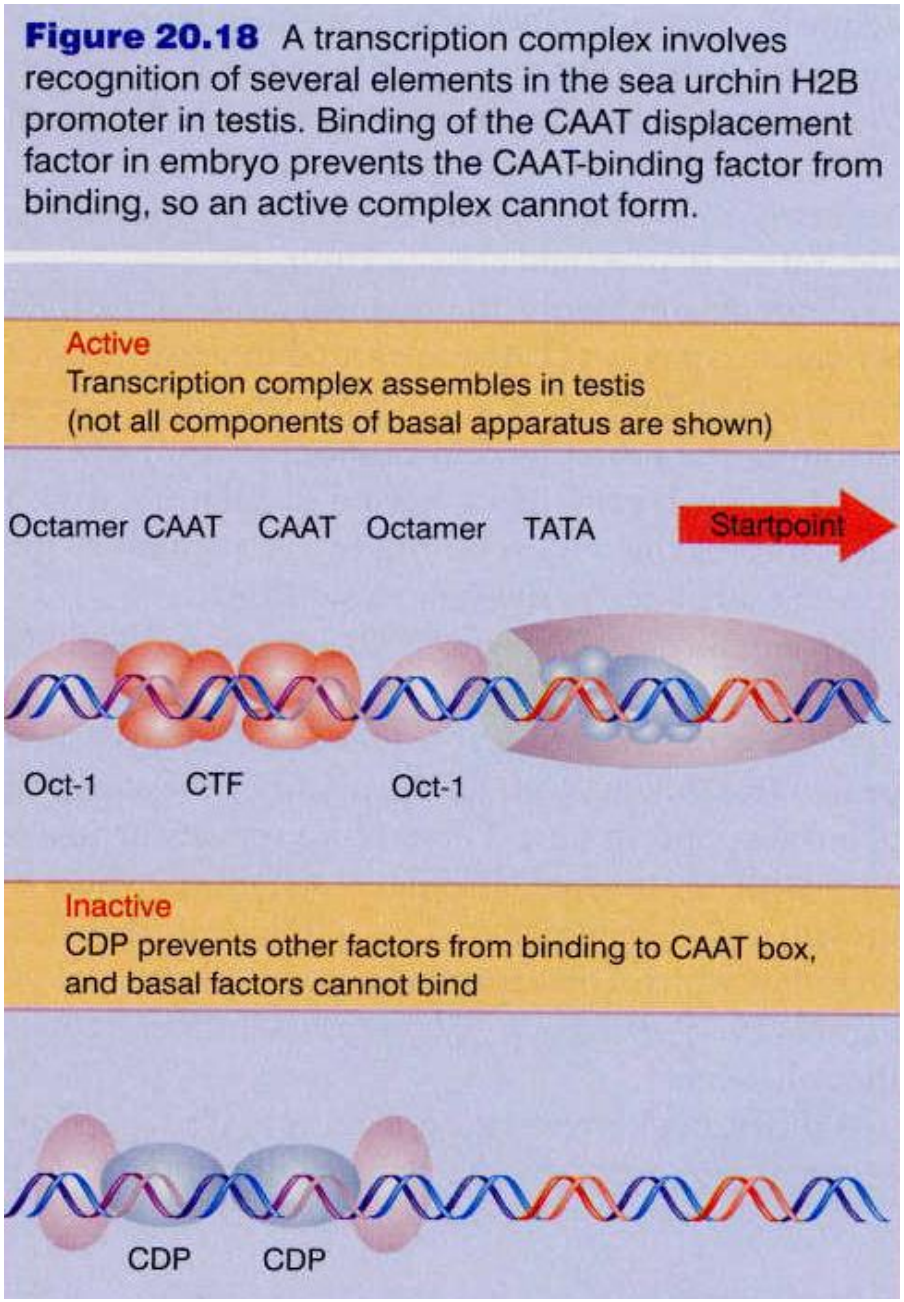


Figure 20.18 A transcription complex involves recognition of several elements in the sea urchin H2B promoter in testis. Binding of the CAAT displacement factor in embryo prevents the CAAT-binding factor from binding, so an active complex cannot form.



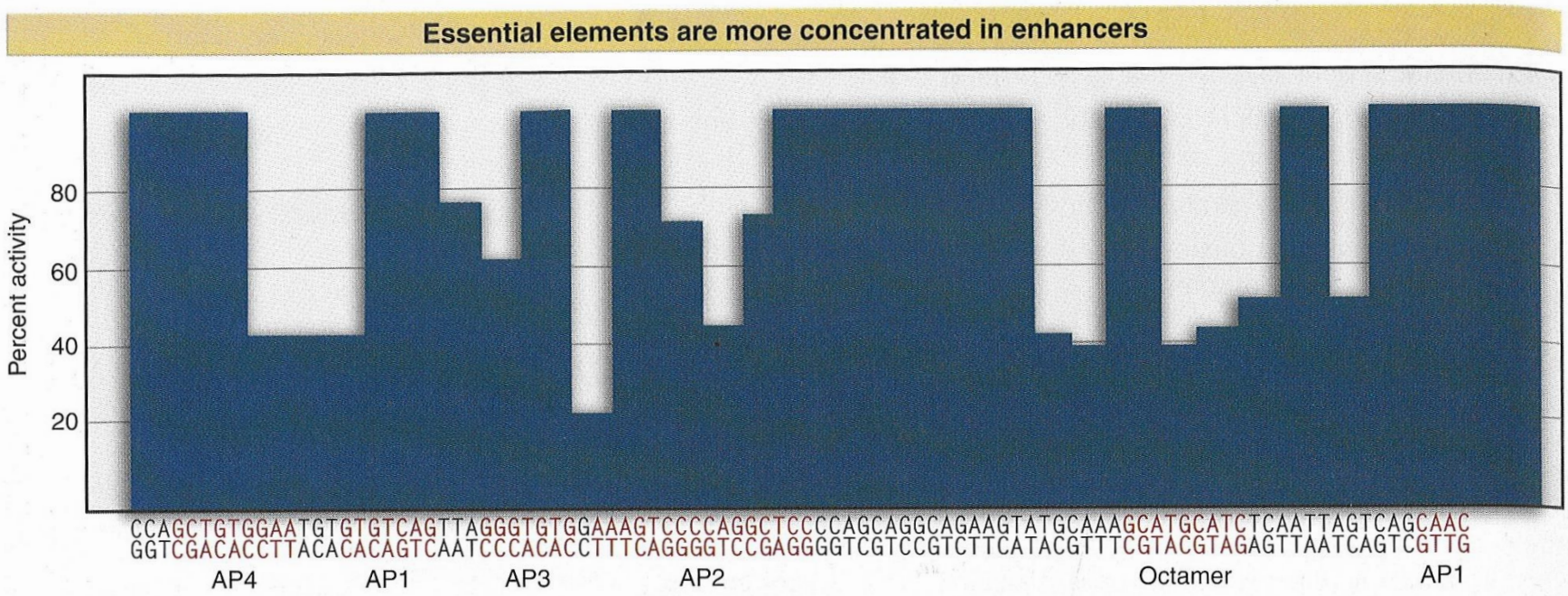


Figure 24.20 An enhancer contains several structural motifs. The histogram plots the effect of all mutations that reduce enhancer function to <75% of wild type. Binding sites for proteins are indicated below the histogram.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

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