GENE ACTIVITY

Gene structure

Transcription

Transcript processing

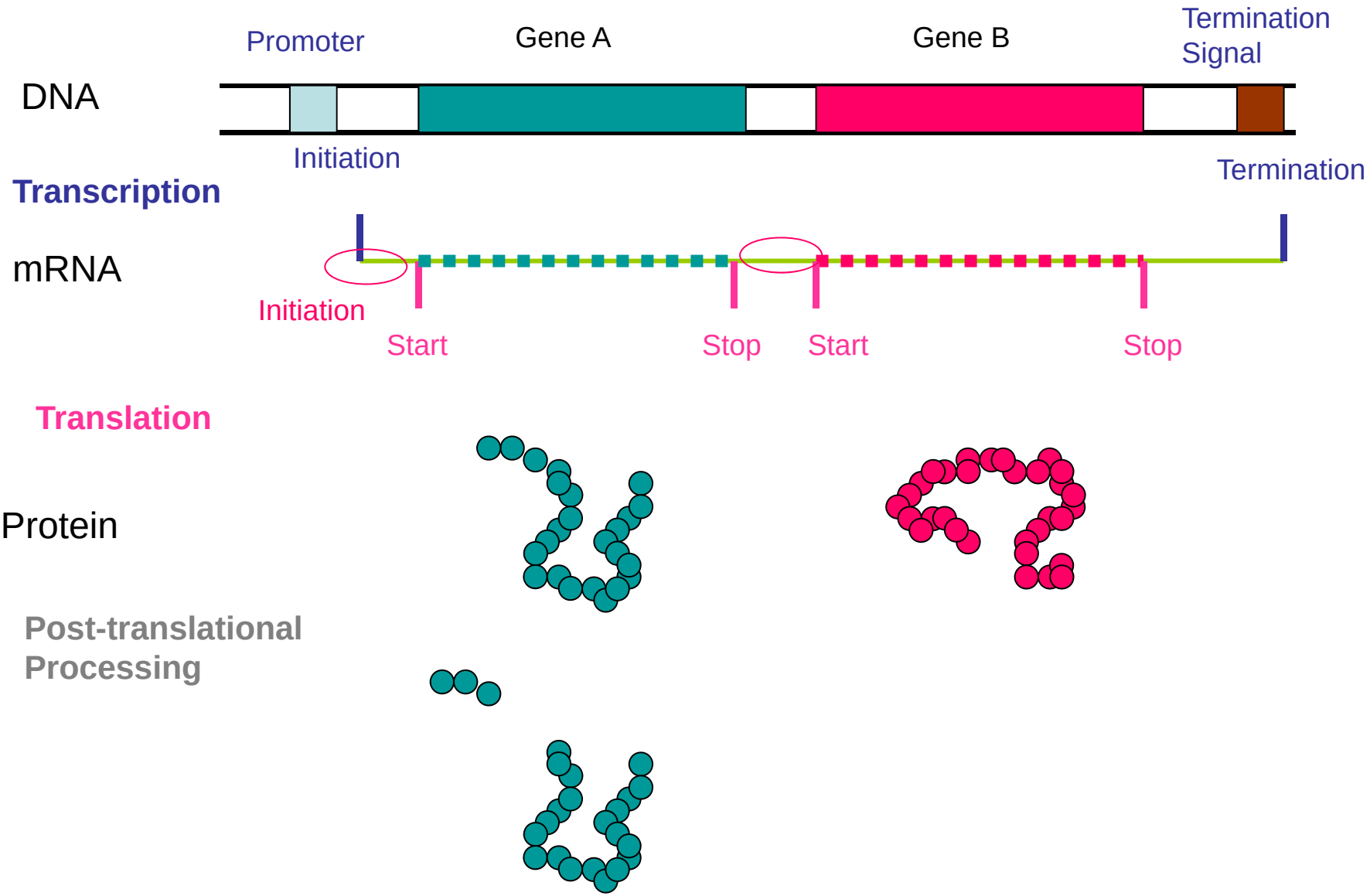
mRNA transport

mRNA stability

Translation

Posttranslational modifications

Genetik und Gentechnik I



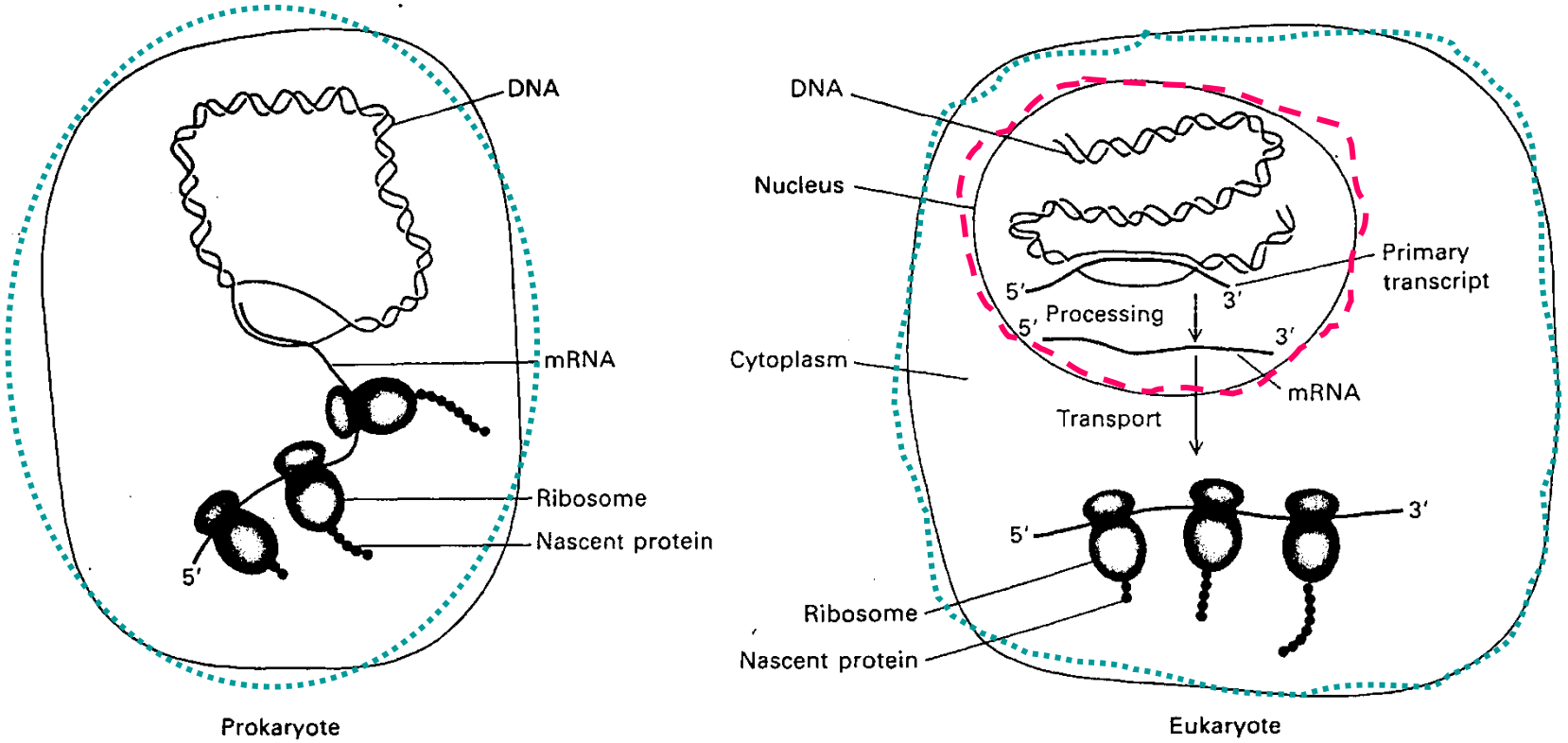


Figure 8-1 The production of functioning mRNA is very different in prokaryotes and eukaryotes. In prokaryotes, the RNA transcript serves directly as the mRNA, and translation begins before transcription is completed; that is, transcription and translation are coupled. In eukaryotes, the primary RNA transcript must be modified in the cell nucleus to form mRNA. Translation takes place only after the completed mRNA is delivered to the cytoplasm.

Figure 5.13 Transcription units can be visualized in bacteria. Photograph kindly provided by Oscar Miller.

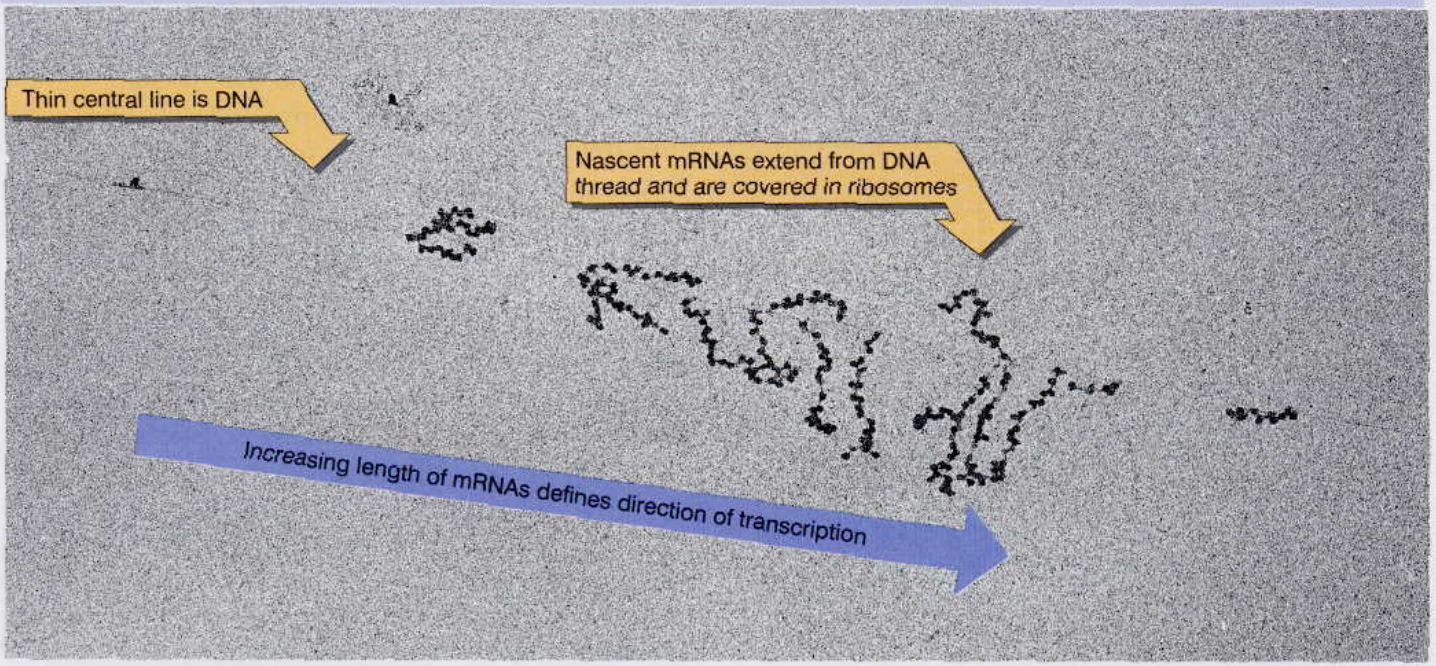
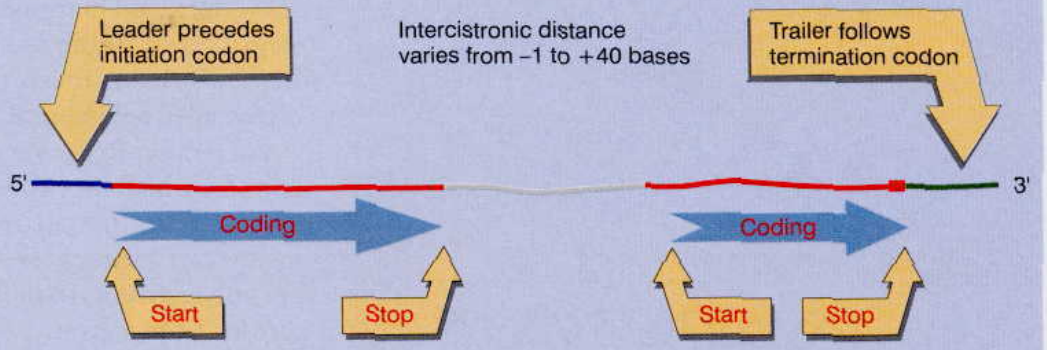


Figure 5.14 Bacterial mRNA includes non-translated as well as translated regions. Each coding region has its own initiation and termination signals. A typical mRNA may have several coding regions.

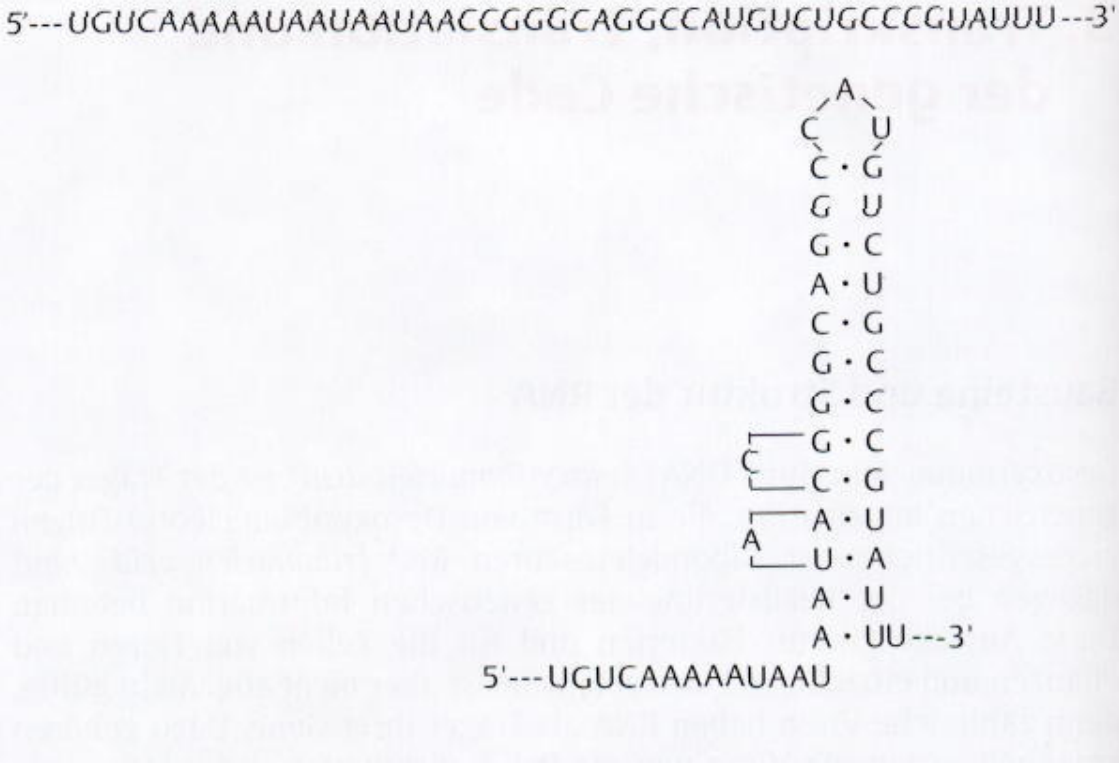


Tab. 3.1 Die drei RNA-Arten.

	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 10 000 Nucleotide)	die Boten-(*messenger*-)RNA überbringt dem Proteinsynthese-Apparat eine Abschrift des Gens

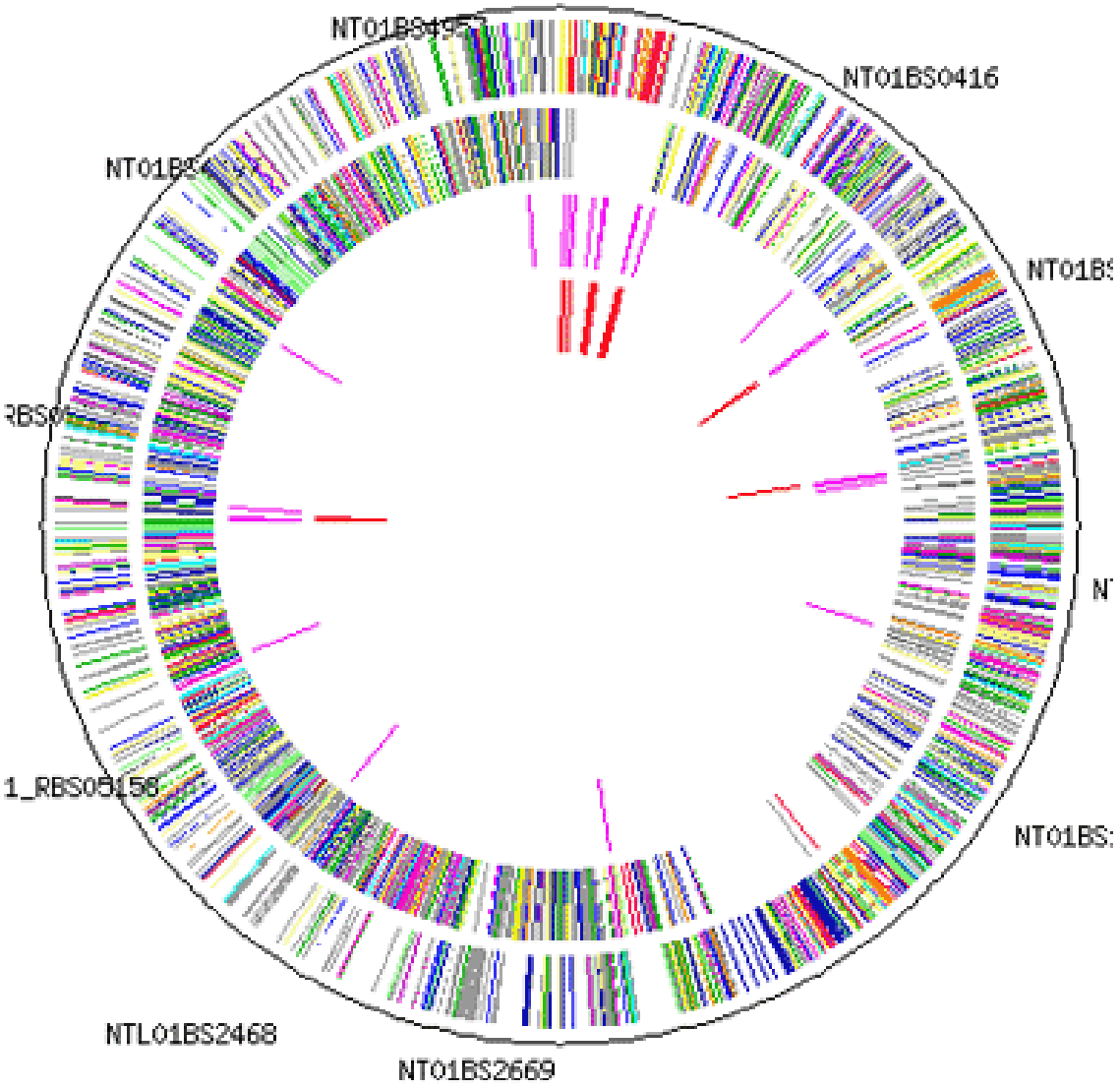
RNA is single stranded but is organized partly as ds RNA by internal base pairing

Abb. 3.2 Schleifen-(Sekundärstruktur-)Bildung in einem Abschnitt einer mRNA von *E. coli*. Die mRNA hat eine Länge von mehreren tausend Nucleotiden. Nur ein kleiner Abschnitt davon ist gezeigt. In diesem Abschnitt befinden sich komplementäre Nucleotidfolgen, *die sich zu einem* doppelsträngigen Abschnitt zusammenlegen können, so daß in dem langen RNA-Molekül an dieser Stelle eine Schleife entsteht. Beachte, daß Cytosin mit Guanin und Uracil mit Adenin paart. Anders gesagt, *Uracil hat in der RNA die Basenpaarungseigenschaften*, die Thymin in der DNA hat. Doppelsträngige RNA hat eine Geometrie, die in manchen Einzelheiten der A-Form einer DNA-Struktur (s. Abb. 2.**14**) entspricht.



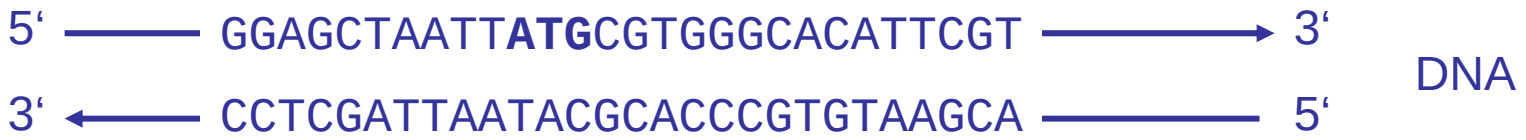
Genome map of Bacillus subtilis

Genes are transcribed from both strands



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

Transcription: Only one strand is transcribed



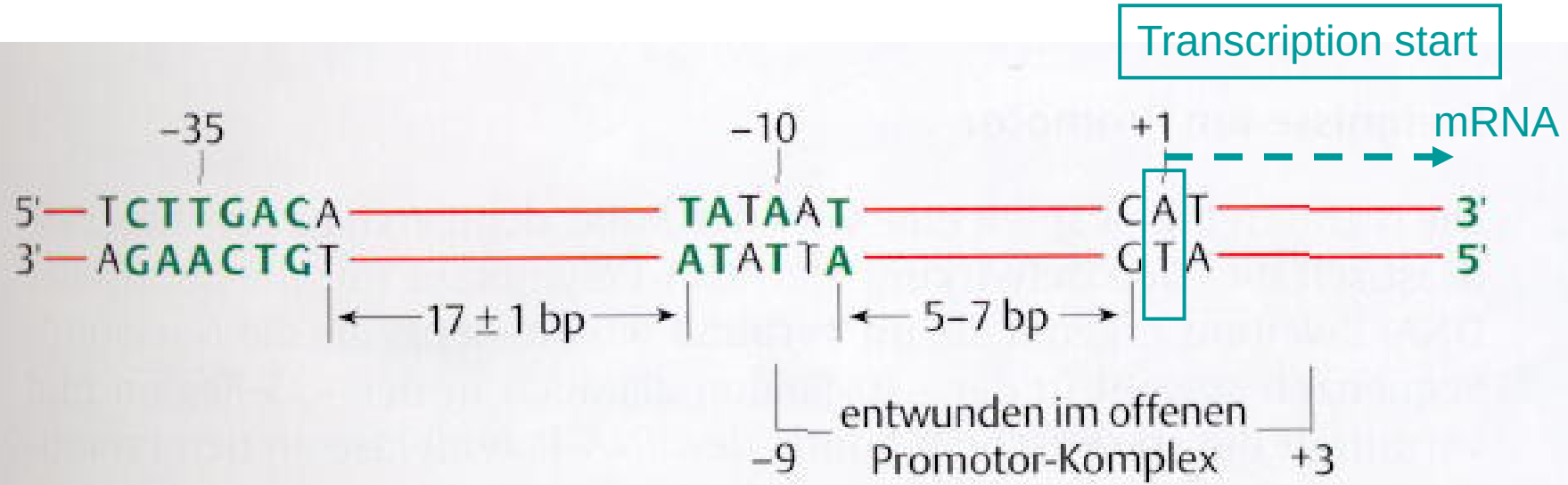


Abb. 3.5 Ein Musterpromotor des *E. coli*-Genoms. Der Abstand zwischen dem Transkriptionsstart und dem ersten Nucleotid der −10-Region beträgt 5–7 Basenpaare (bp); der Abschnitt zwischen der −10-Region und der −35-Region 17 ± 1 bp. *Der untere der beiden DNA-Stränge ist der transkribierte „codogene"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

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

E. coli sigma factors recognize promoters with different consensus sequences.
In addition to 70, E. coli has several sigma factors that are induced by particular environmental conditions

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	?

Effect of mutations on promoter function

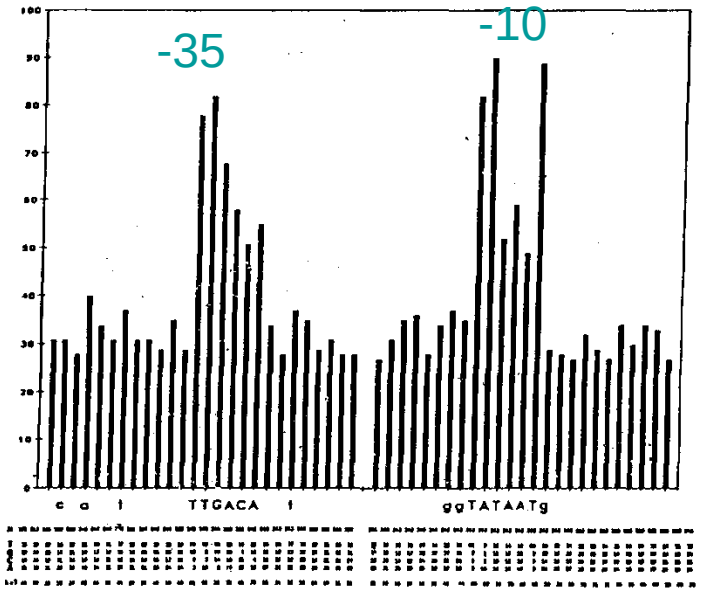


Figure 1. Base distribution of 263 analyzed promoters from Table 1. (a) Frequency histogram of the most highly conserved base on the non-template strand from 12 bp upstream of the -35 hexamer to 11 bp downstream of the -10 hexamer. Highly conserved (upper case) and weakly conserved (lower case) bases, 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.

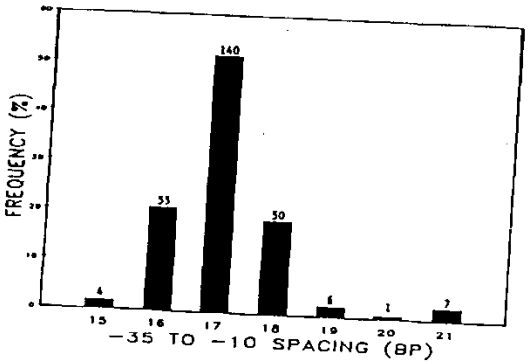


Figure 2. Distribution of promoters with 15-21 bp separating the -35 and -10 hexamers. The number of promoters in each group is indicated on top of the bars.

Spacing between -35 and -10 region

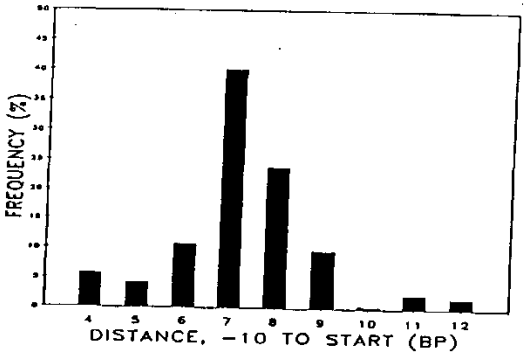
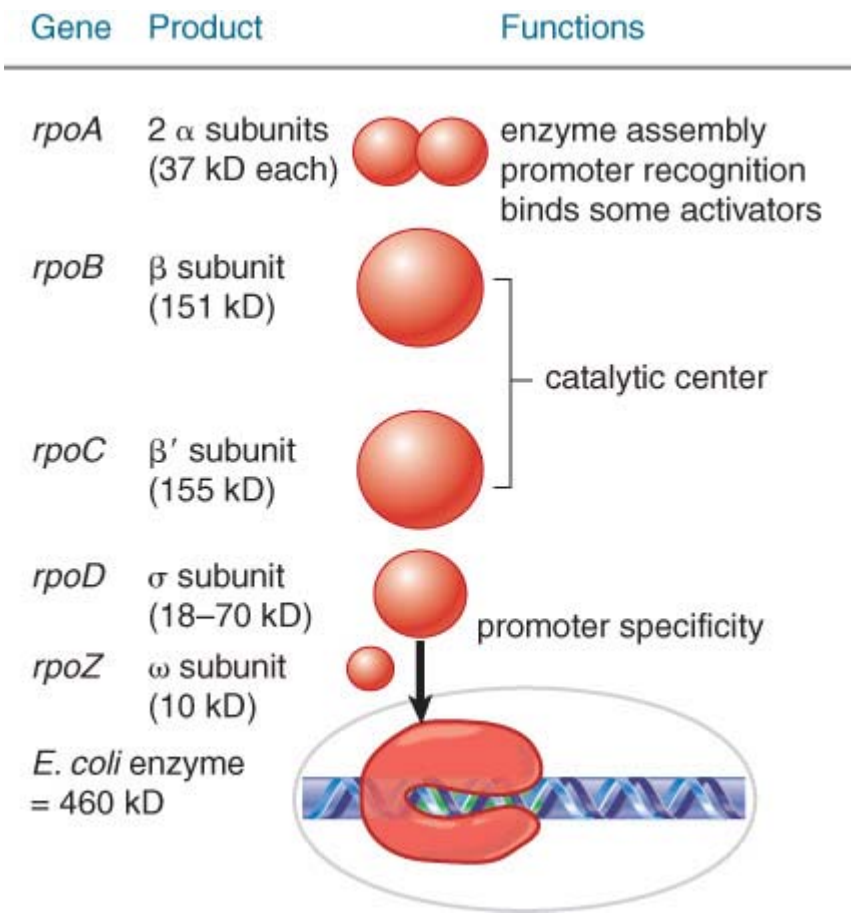
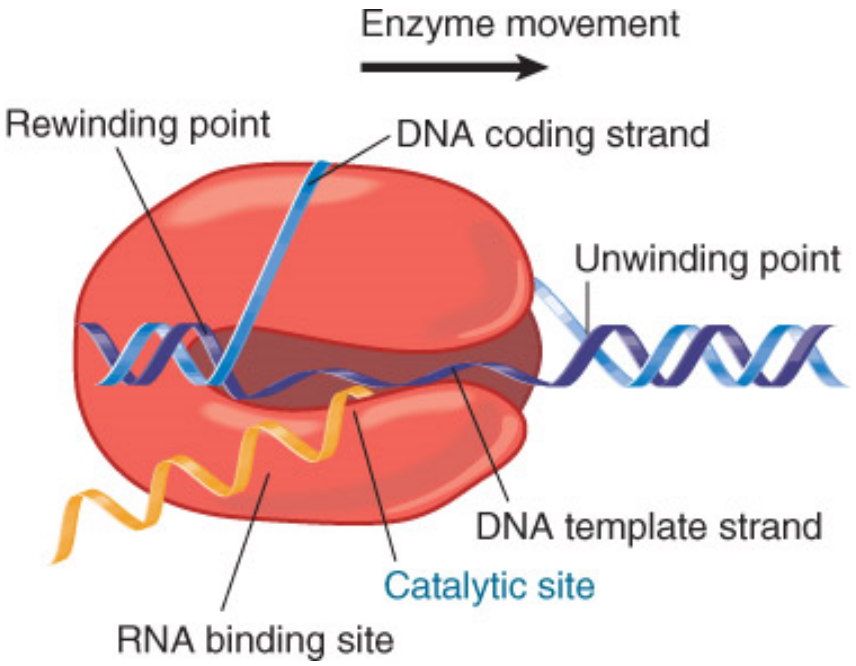


Figure 3. Distribution of promoters with transcription start points initiating 4-12 bases downstream of the -10 hexamer. Only promoters with uniquely defined start points are included in this analysis.

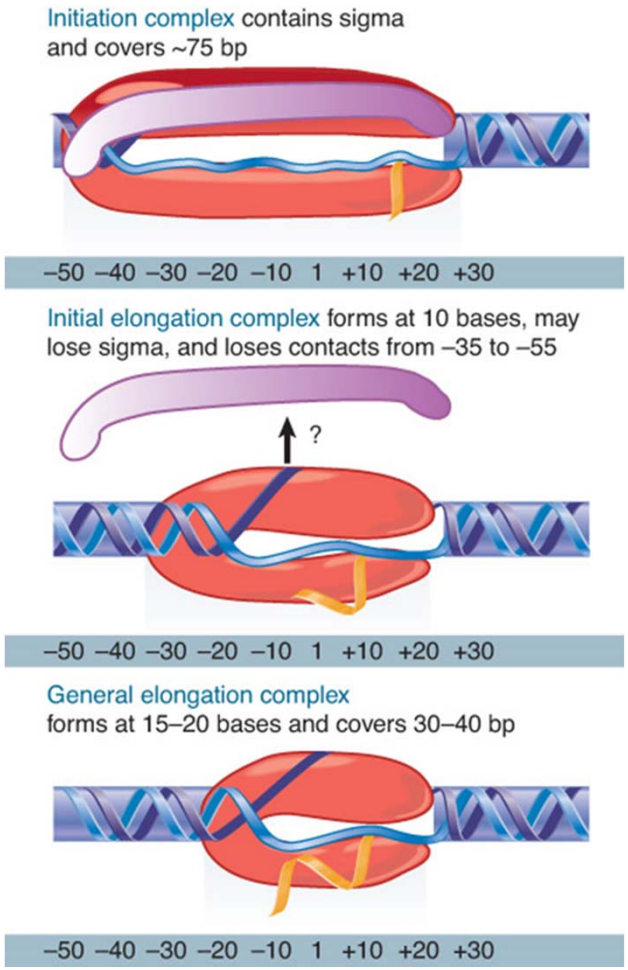
Spacing between -10 region and transcription stra

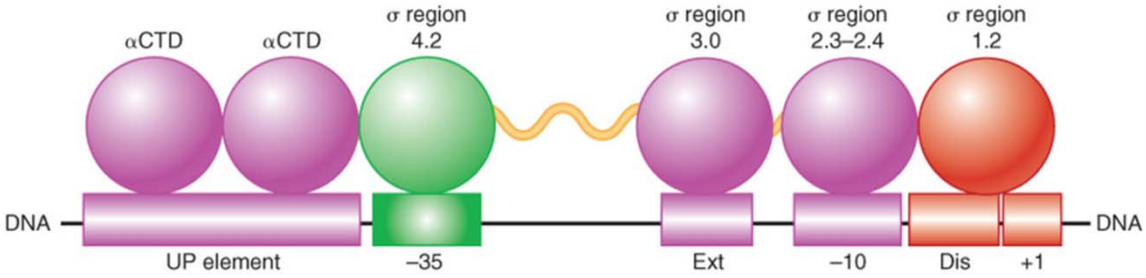


Eubacterial RNA polymerases have five types of subunits.



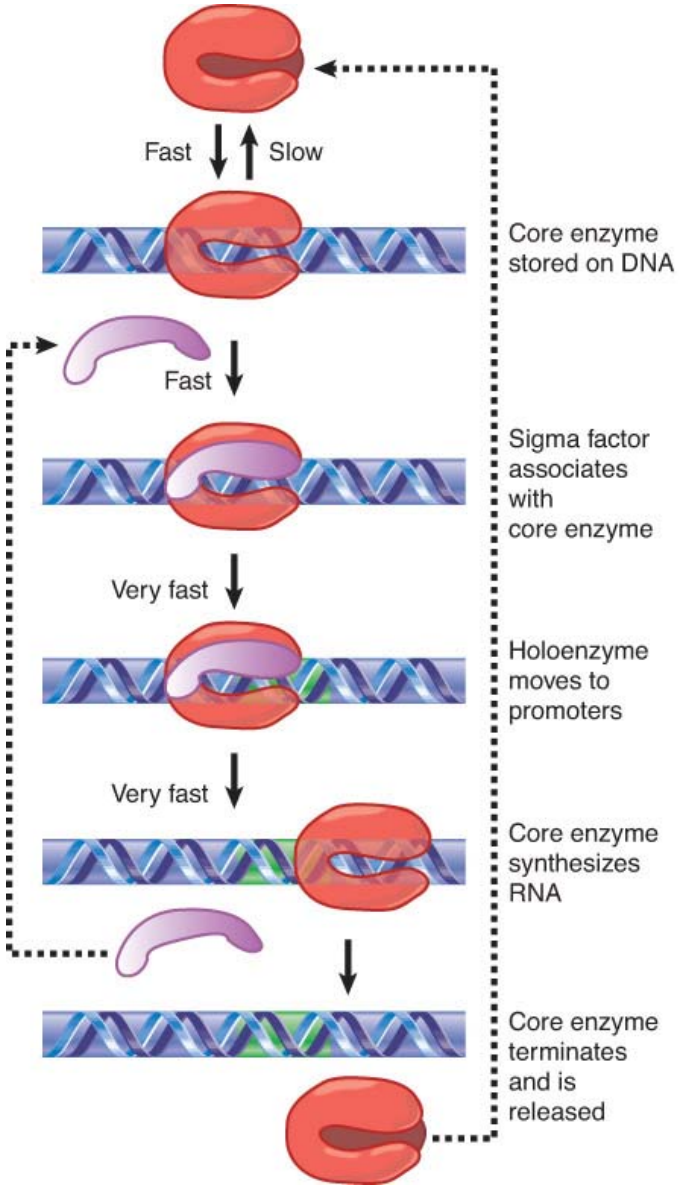
During transcription, the bubble is maintained within bacterial RNA polymerase.





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

RNA polymerase initially contacts the region from –55 to +20.



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

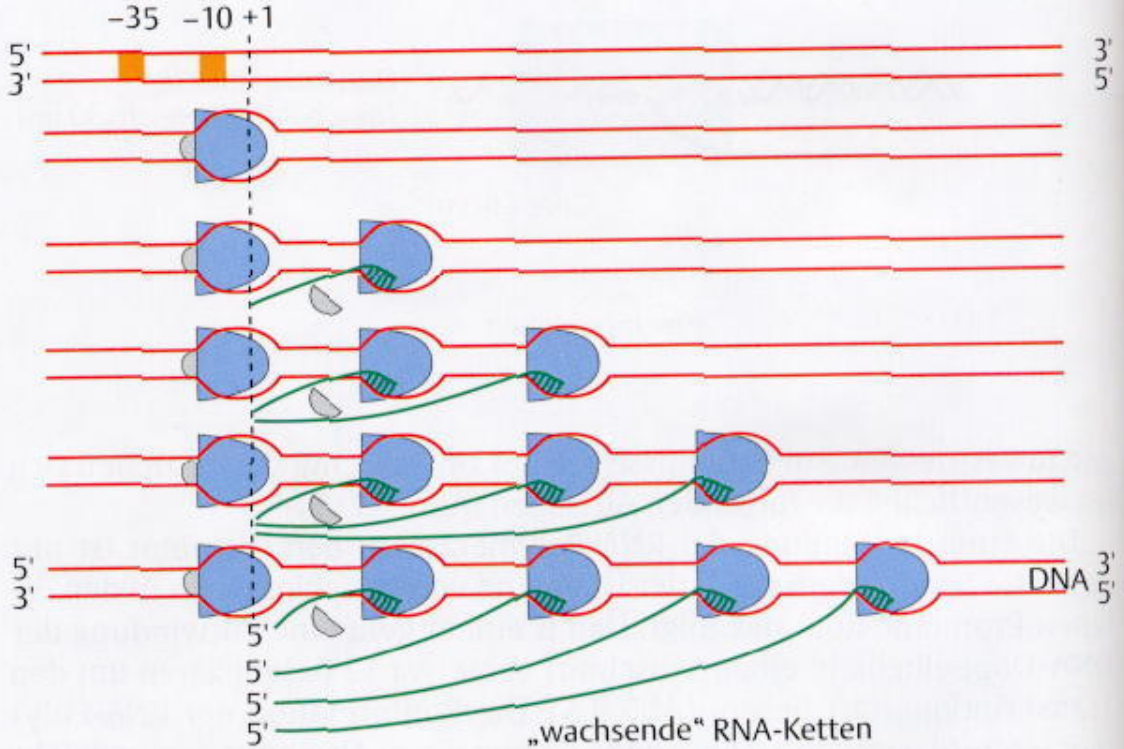


Abb. 3.8 Schema der Transkription. Die Transkription beginnt mit dem „offenen Promotor-Komplex". Das Holoenzym führt die Entwindung eines engen Bereiches um den Startpunkt der Transkription (+1) herbei. Nach wenigen Polymerisationsschritten verläßt der σ-Faktor (Halbkreis) das Core-Enzym, das nun seinen Weg entlang des transkribierten DNA-Stranges fortsetzt. Der freigewordene Promotor wird wieder besetzt. Gleichzeitig sind mehrere RNA-Polymerasen mit der Transkription beschäftigt. Beachte, daß die RNA-Polymerasen auf ihrem Weg eine umschriebene Region entwundener DNA gleichsam wie eine Bugwelle mit sich führen.

Genetik und Gentechnik I

11.11.14

Figure 9.27 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.

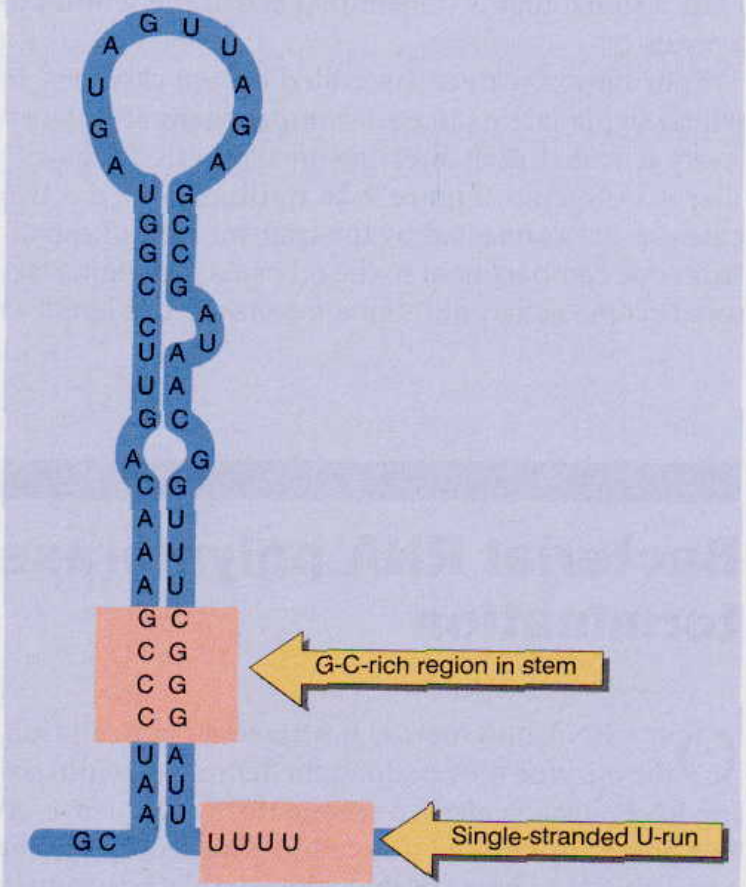
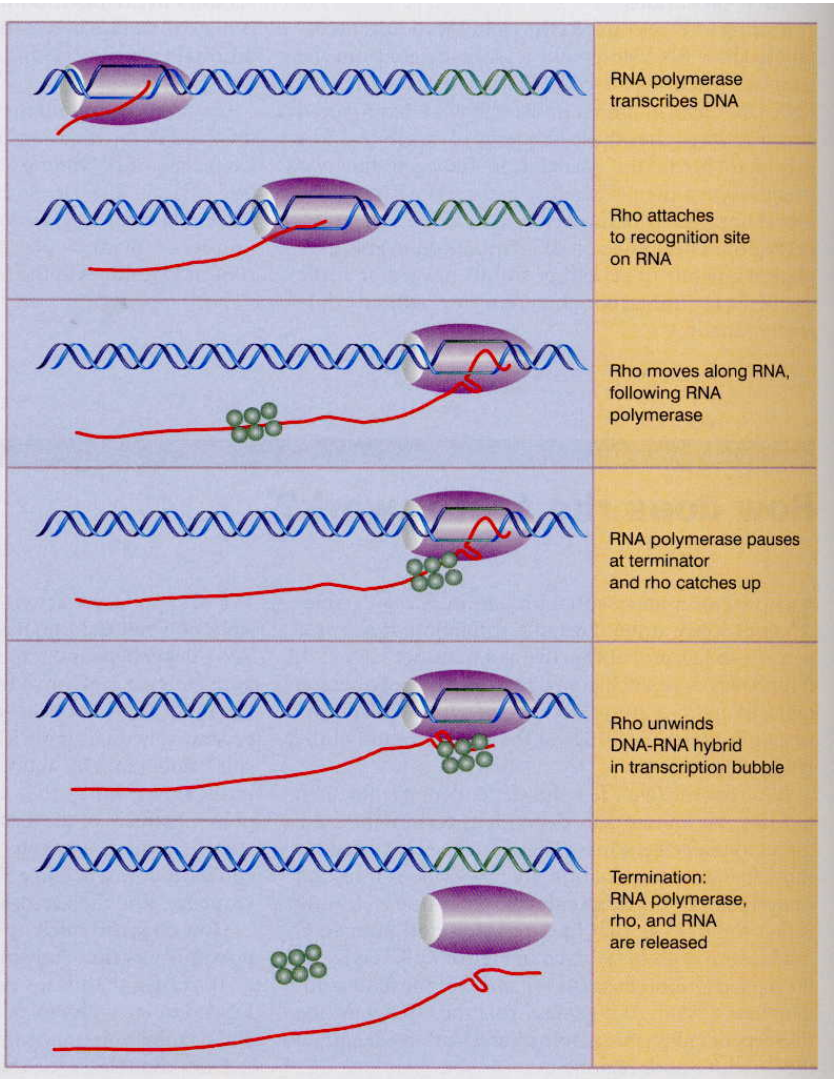


Figure 9.29 Rho factor pursues RNA polymerase along the RNA and can cause termination when it catches the enzyme pausing at a rho-dependent terminator.



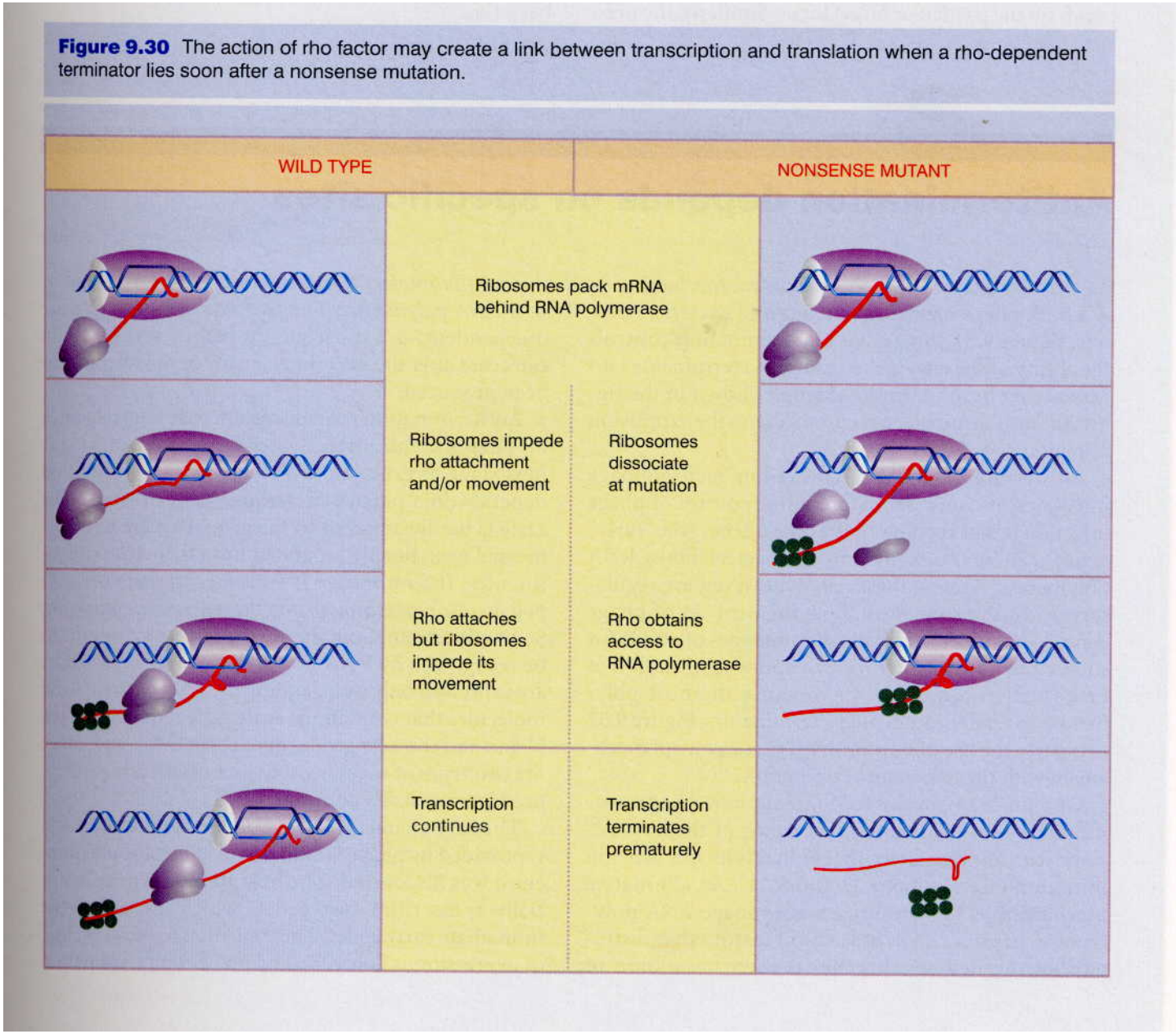
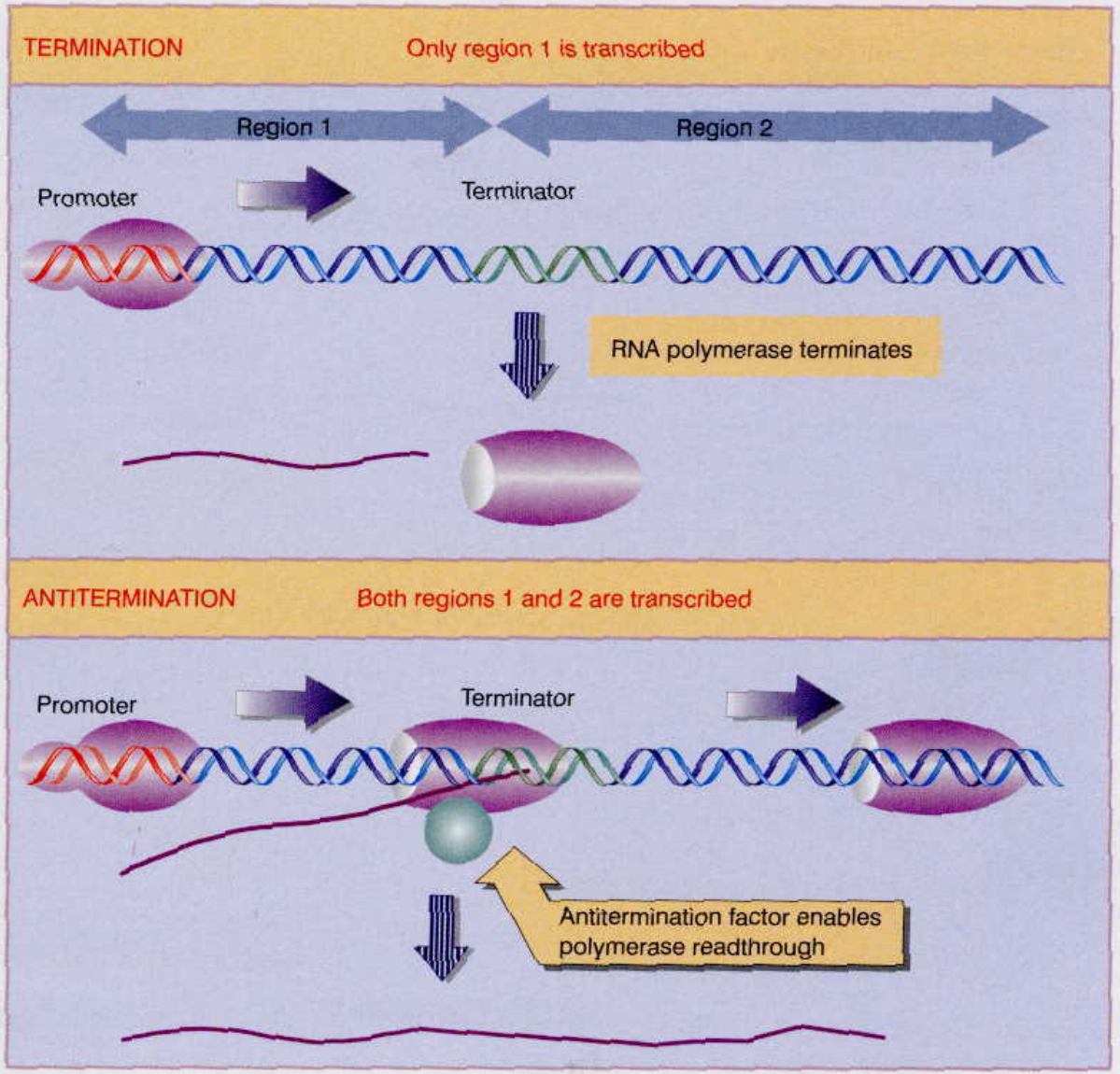


Figure 9.30 The action of rho factor may create a link between transcription and translation when a rho-dependent terminator lies soon after a nonsense mutation.

Figure 9.31
Antitermination can be used to control transcription by determining whether RNA polymerase terminates or reads through a particular terminator into the following region.



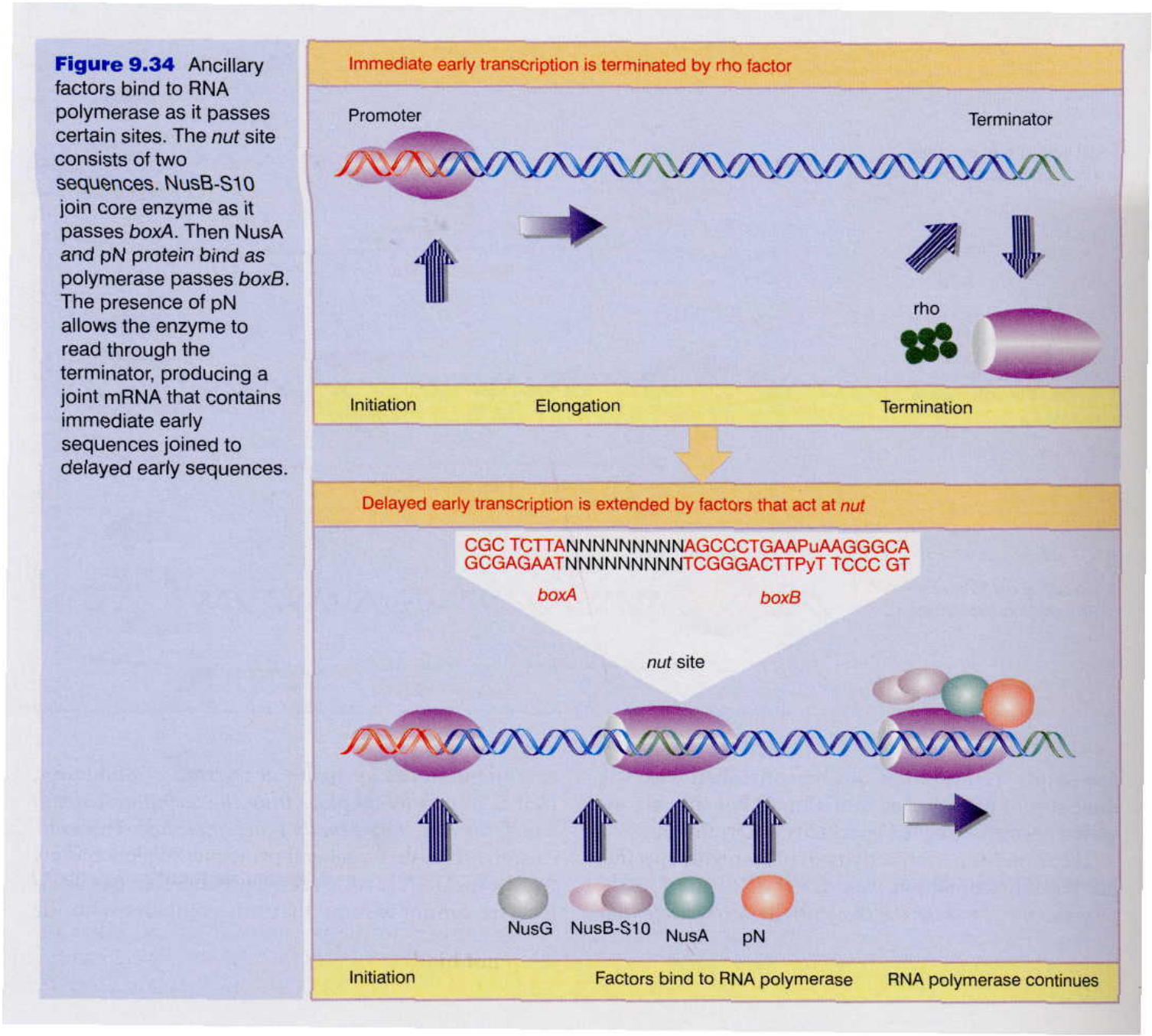
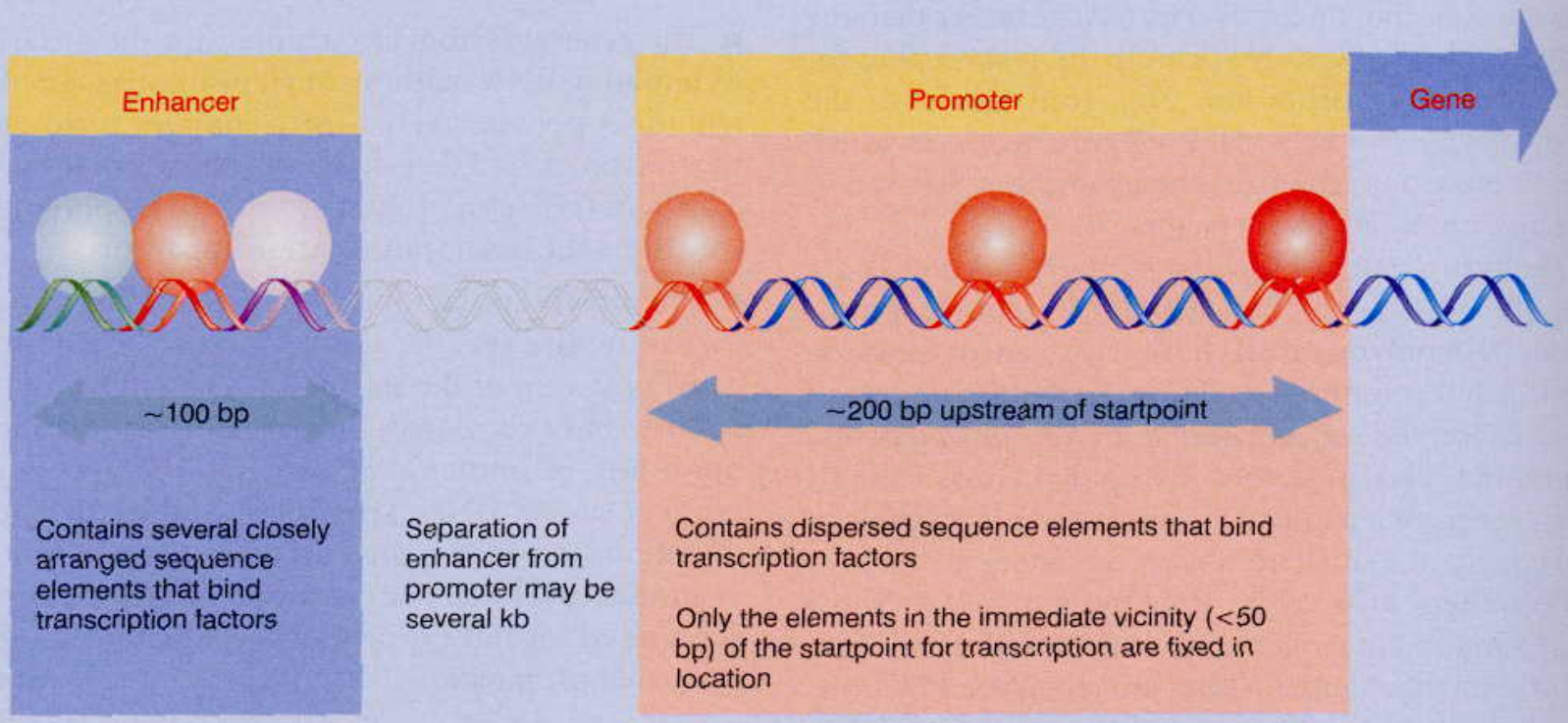


Figure 9.34 Ancillary factors bind to RNA polymerase as it passes certain sites. The *nut* site consists of two sequences. NusB-S10 join core enzyme as it passes *boxA*. Then NusA and pN protein bind as polymerase passes *boxB*. The presence of pN allows the enzyme to read through the terminator, producing a joint mRNA that contains immediate early sequences joined to delayed early sequences.

Figure 20.1 *Overview:* 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 (<10 bp) sequence elements that bind transcription factors, dispersed over >200 bp. An enhancer containing a more closely packed array of elements that also bind transcription factors may be located 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.)



Gene Expression in Eukaryotes -- Introns

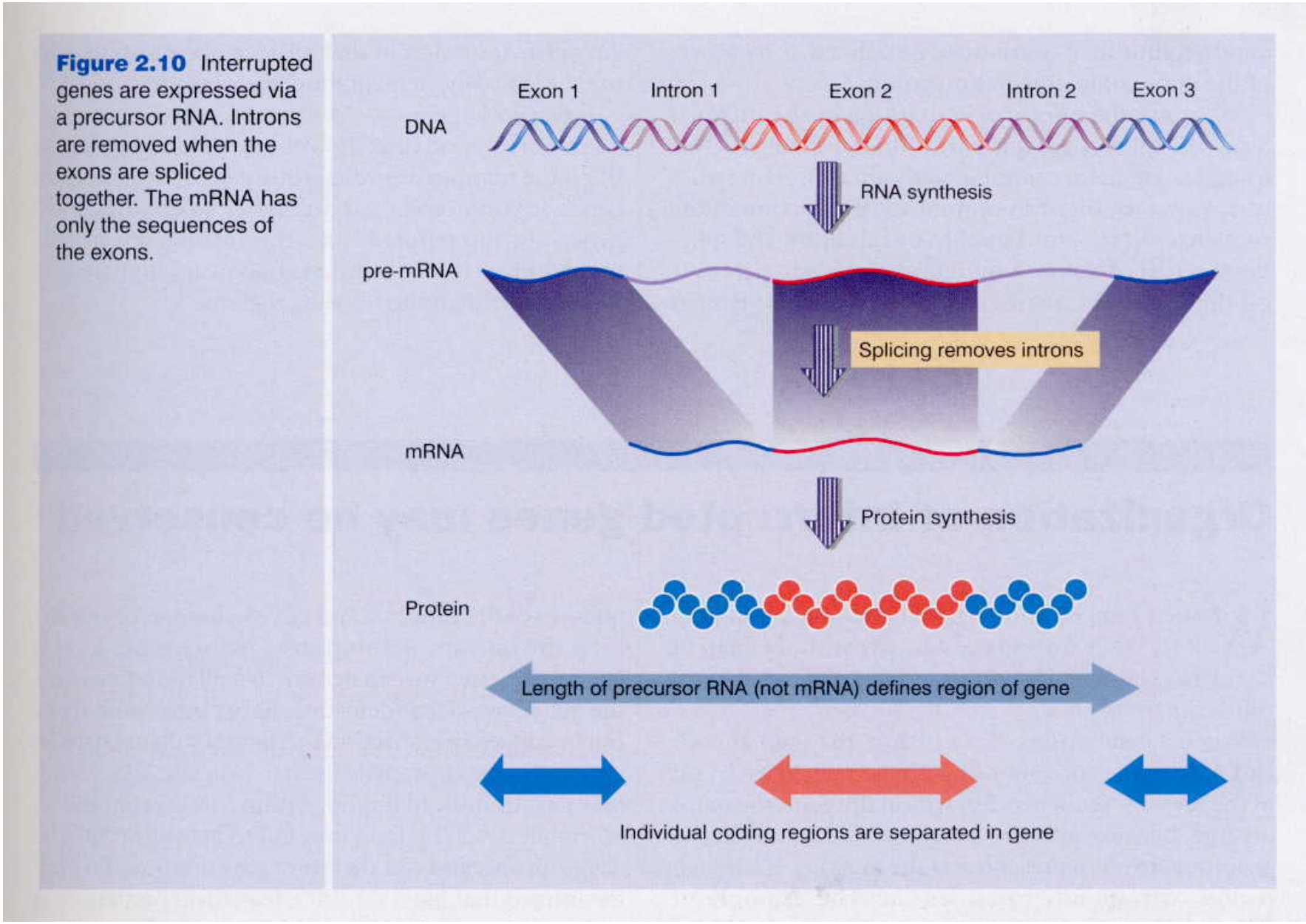


Figure 2.10 Interrupted genes are expressed via a precursor RNA. Introns are removed when the exons are spliced together. The mRNA has only the sequences of the exons.

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.

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

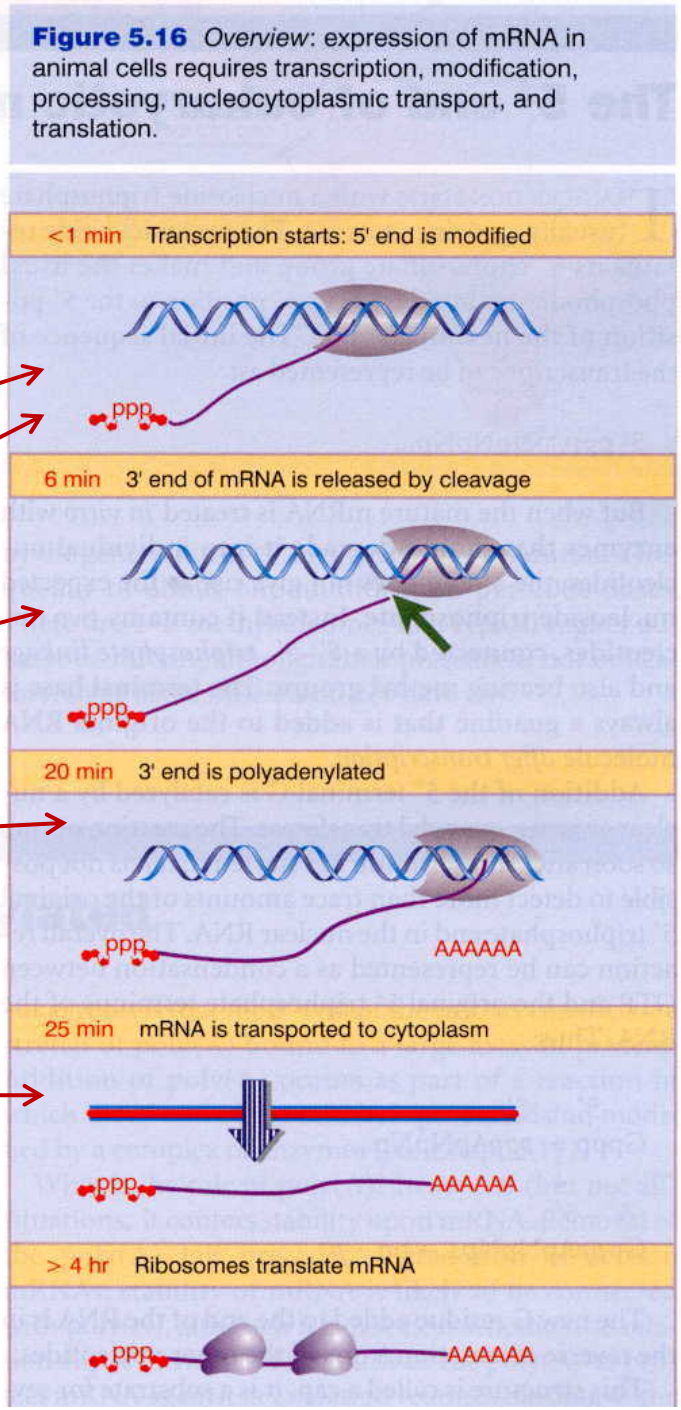


Figure 5.16 *Overview*: expression of mRNA in animal cells requires transcription, modification, processing, nucleocytoplasmic transport, and translation.

CAP structure at 5‘ end of mRNA

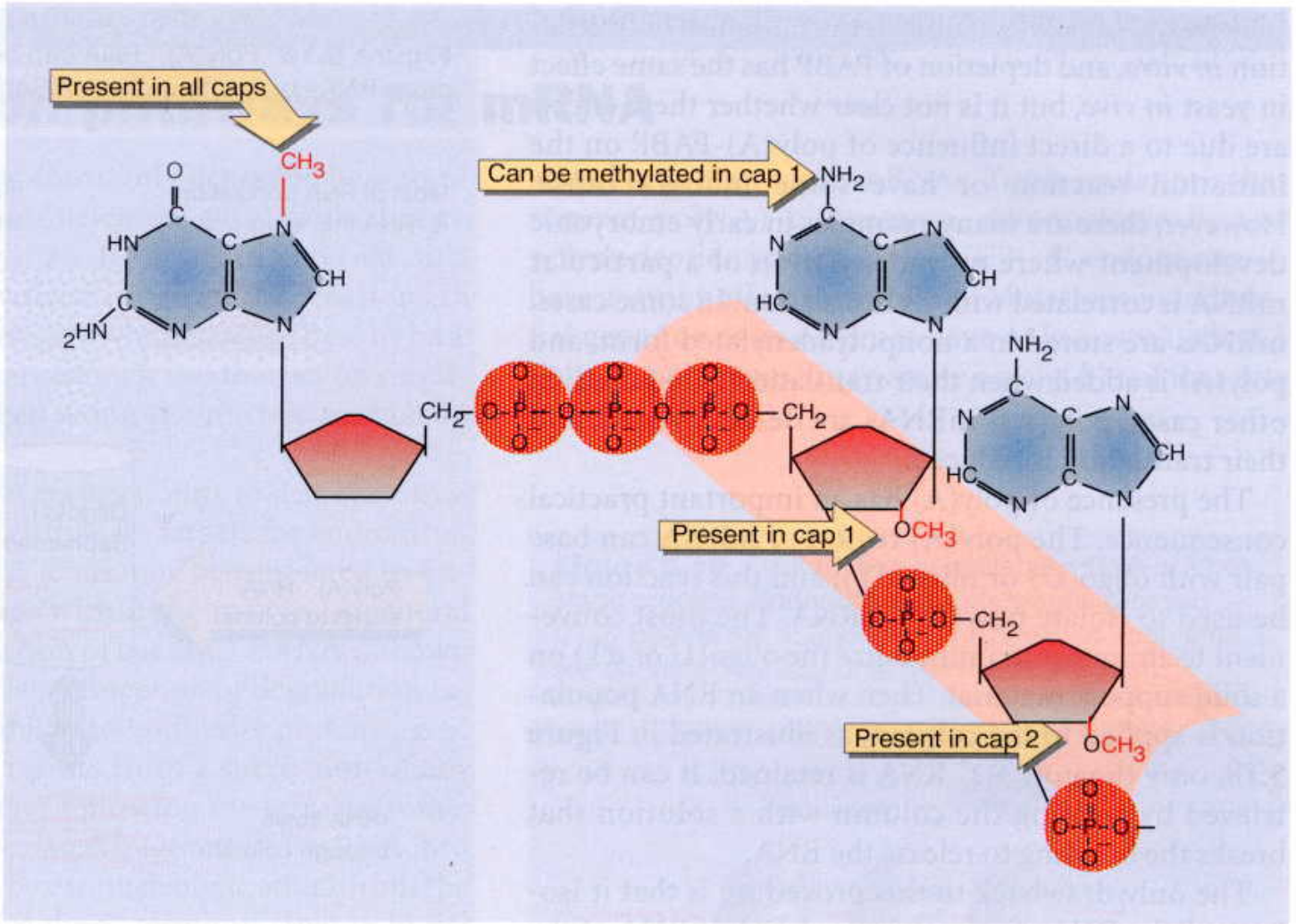


Figure 5.17
The cap blocks the 5’ end of mRNA and may be methylated at several positions.

Figure 2.12 An intron is a sequence present in the gene but absent from the mRNA (here shown in terms of the cDNA sequence). The reading frame is indicated by the alternating open and shaded blocks; note that all three possible reading frames are blocked by termination codons in the intron.

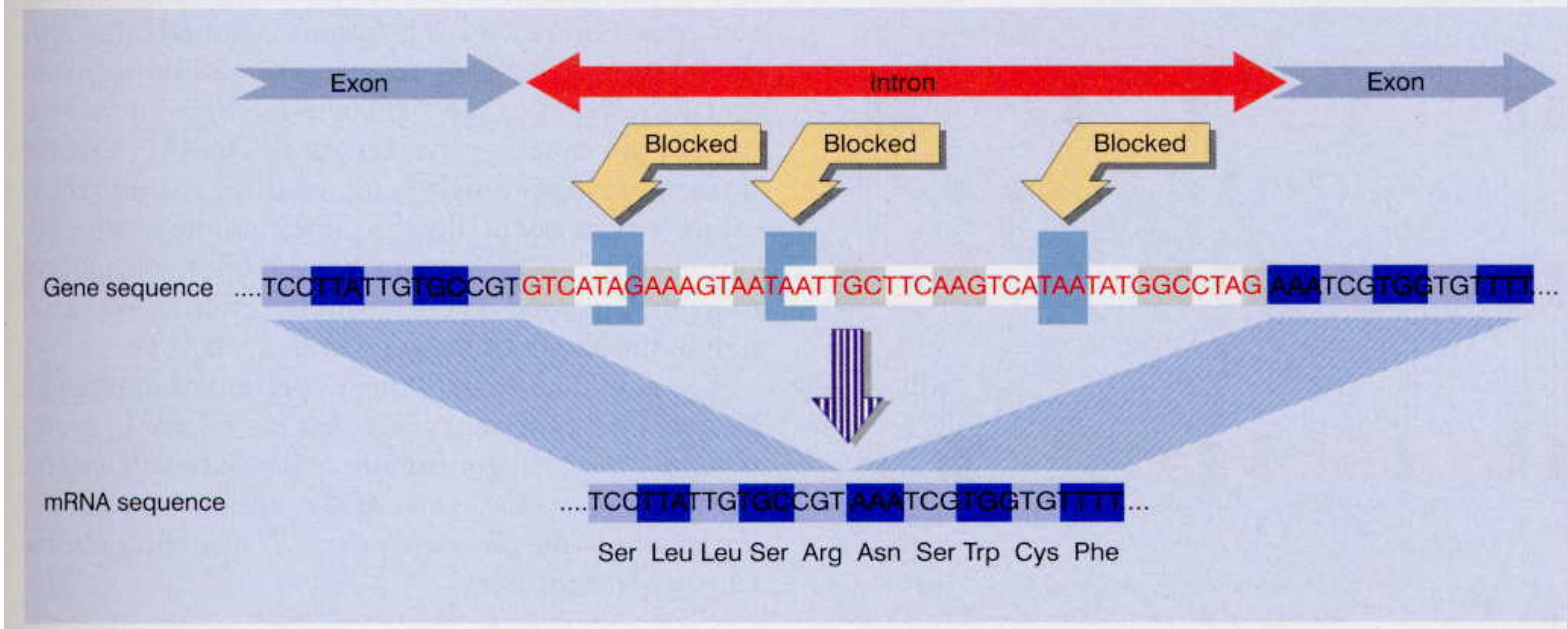
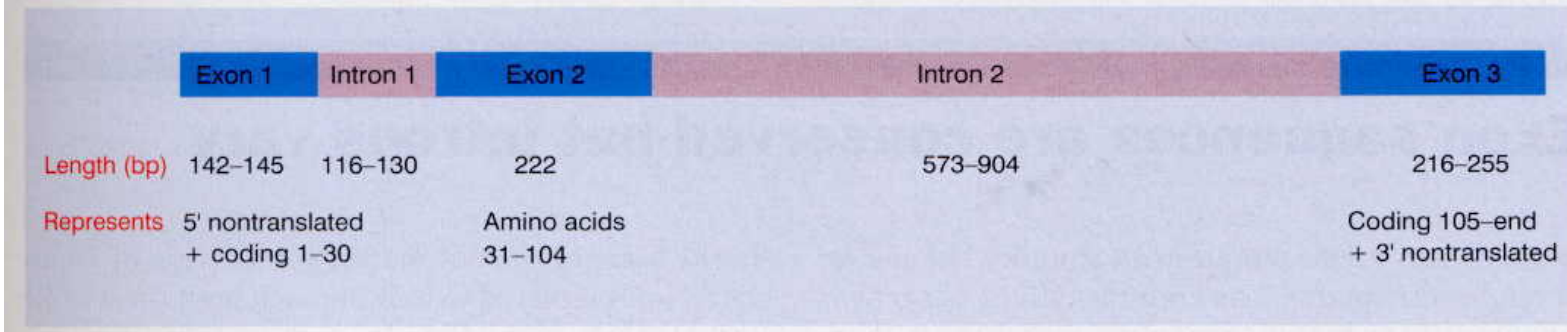


Figure 2.13 All functional globin genes have an interrupted structure with three exons. The lengths indicated in the figure apply to the mammalian -globin genes.



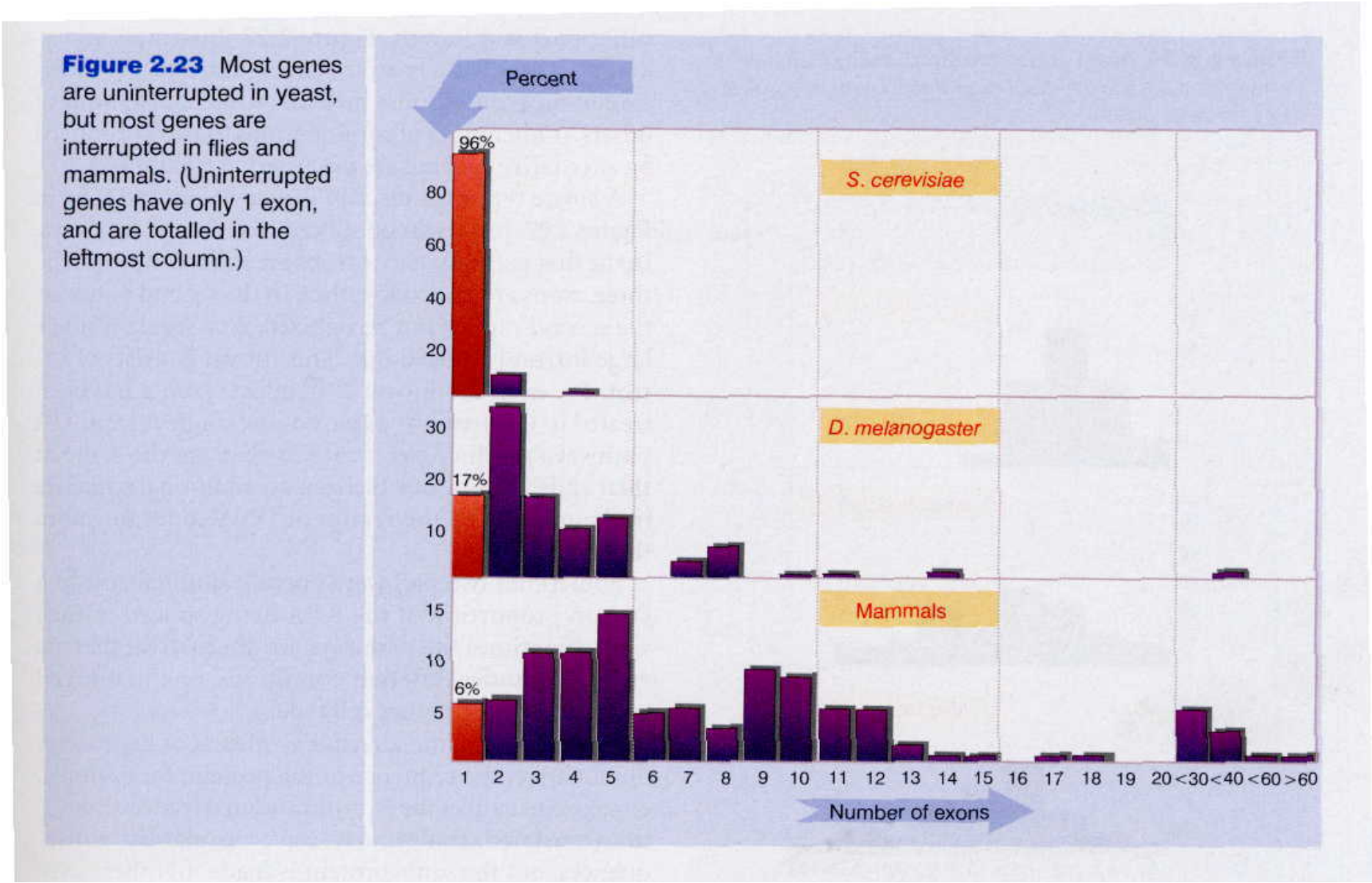
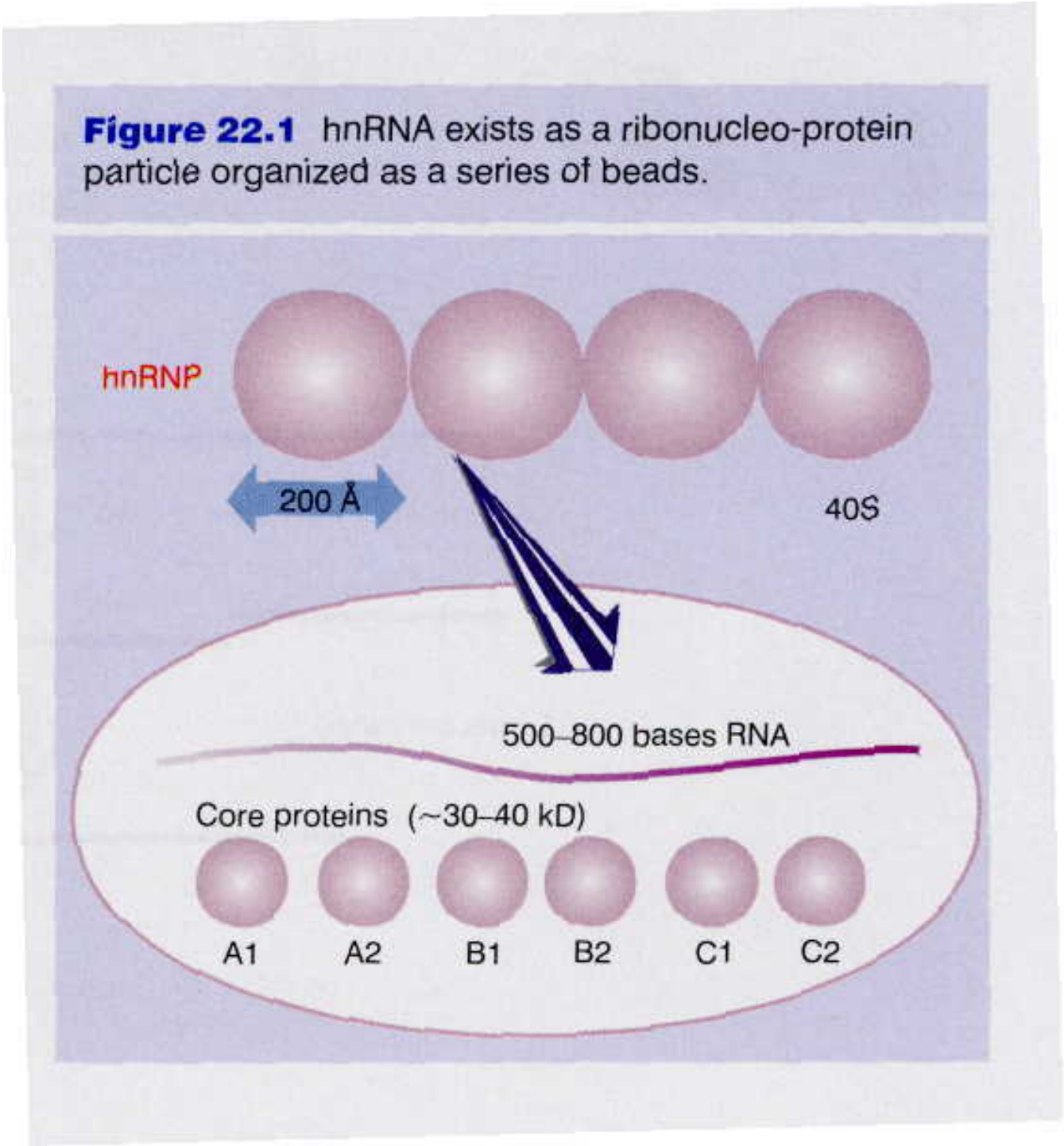


Figure 2.23 Most genes are uninterrupted in yeast, but most genes are interrupted in flies and mammals. (Uninterrupted genes have only 1 exon, and are totalled in the leftmost column.)



hnRNA: hetergeneous nuclear RNA

hnRNP: hetergeneous nuclear Ribonucleoprotein

H.Schwab

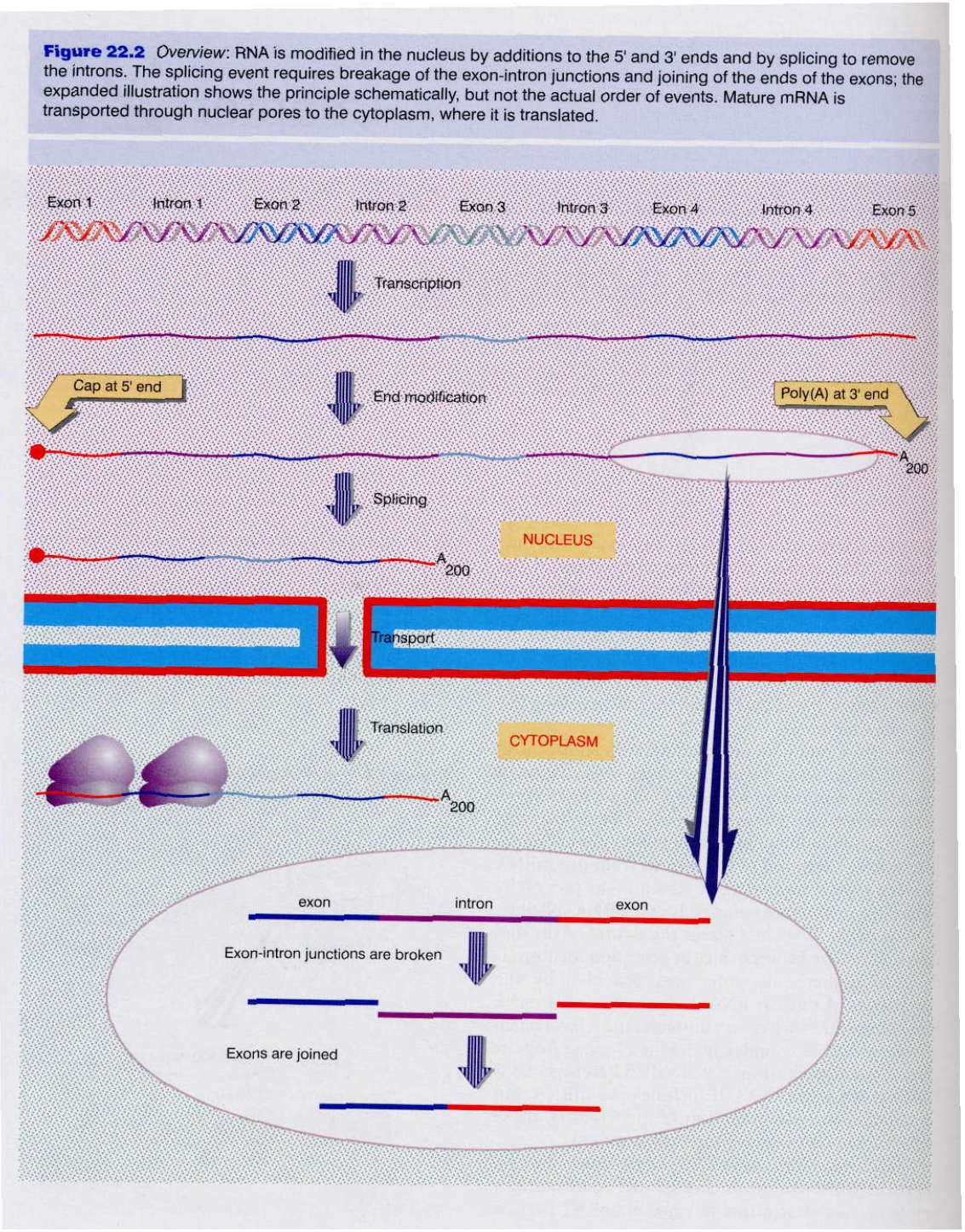


Figure 22.2 *Overview*: RNA is modified in the nucleus by additions to the 5' and 3' ends and by splicing to remove the introns. The splicing event requires breakage of the exon-intron junctions and joining of the ends of the exons; the expanded illustration shows the principle schematically, but not the actual order of events. Mature mRNA is transported through nuclear pores to the cytoplasm, where it is translated.

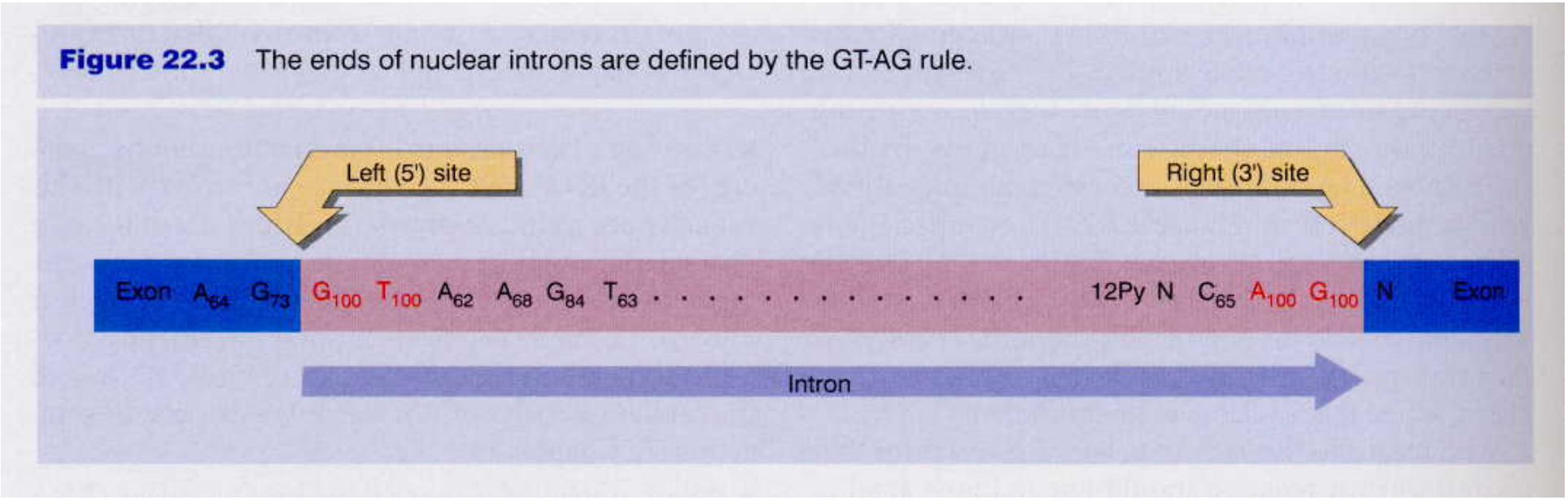


Figure 22.3 The ends of nuclear introns are defined by the GT-AG rule.

Figure 22.6 Splicing occurs in two stages, in which the 5' exon is separated and then is joined to the 3' exon.

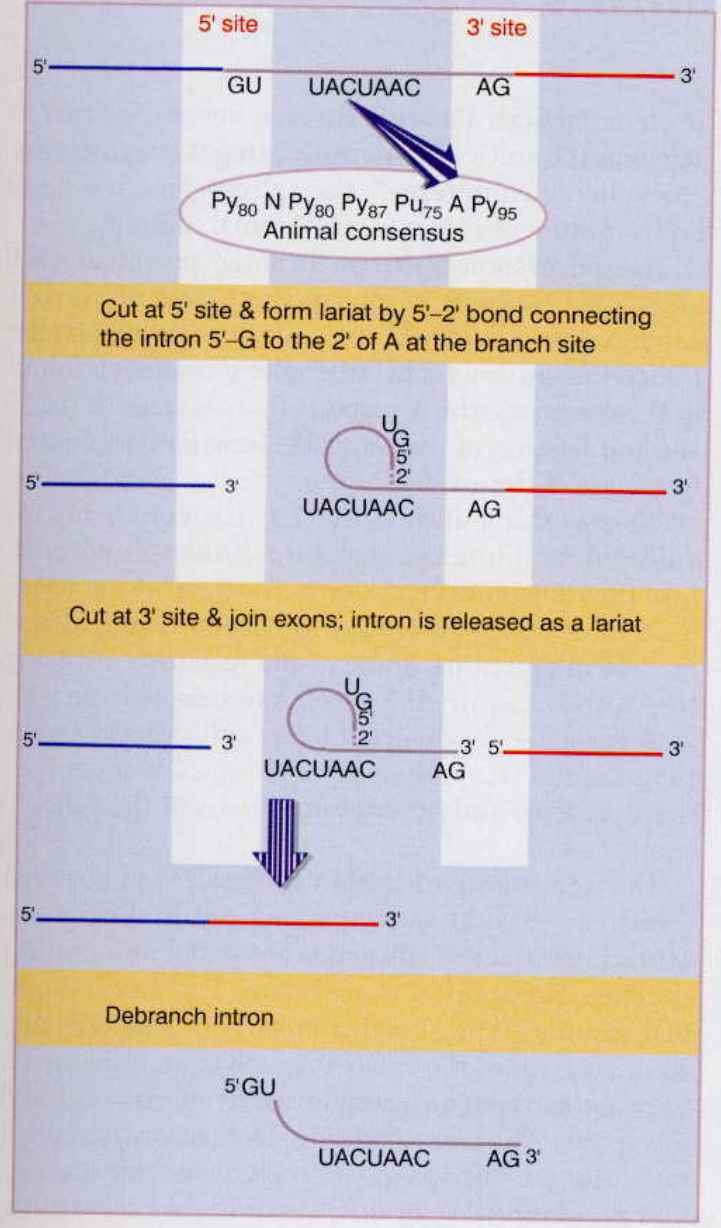


Figure 22.7 Nuclear splicing occurs by two transesterification reactions in which a free OH end attacks a phosphodiester bond.

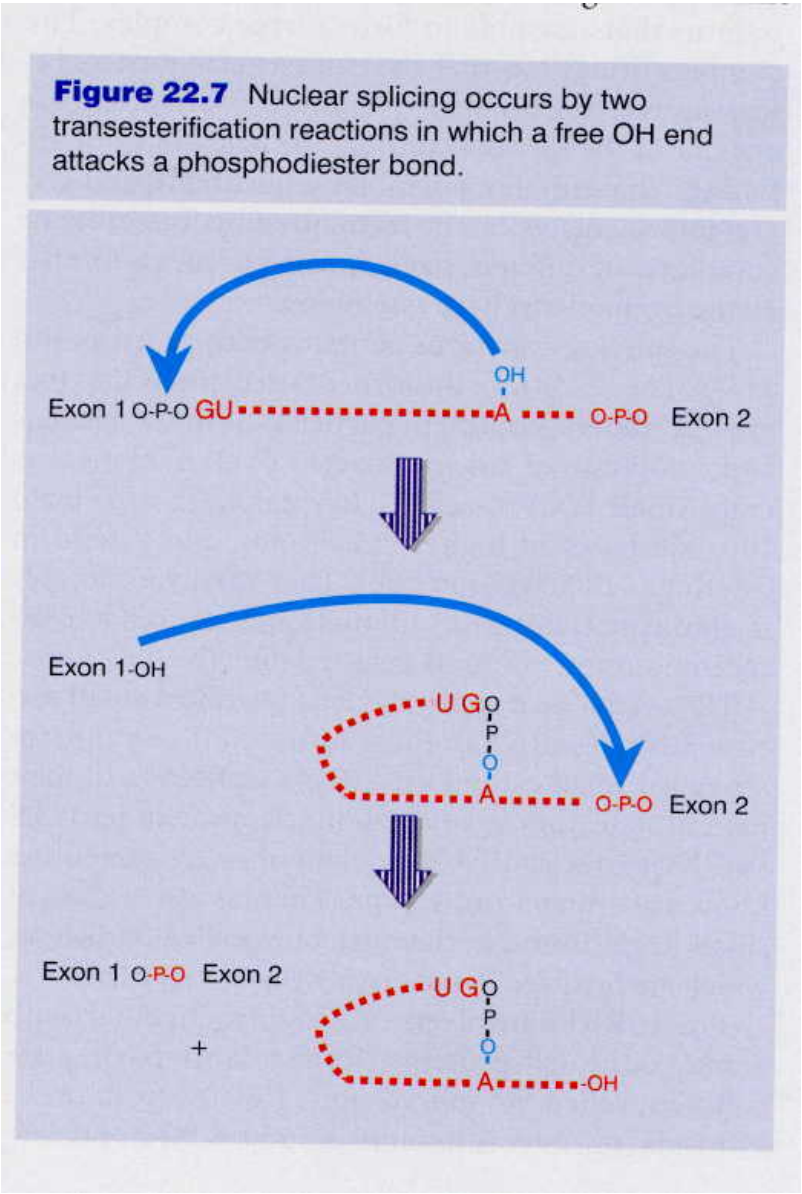
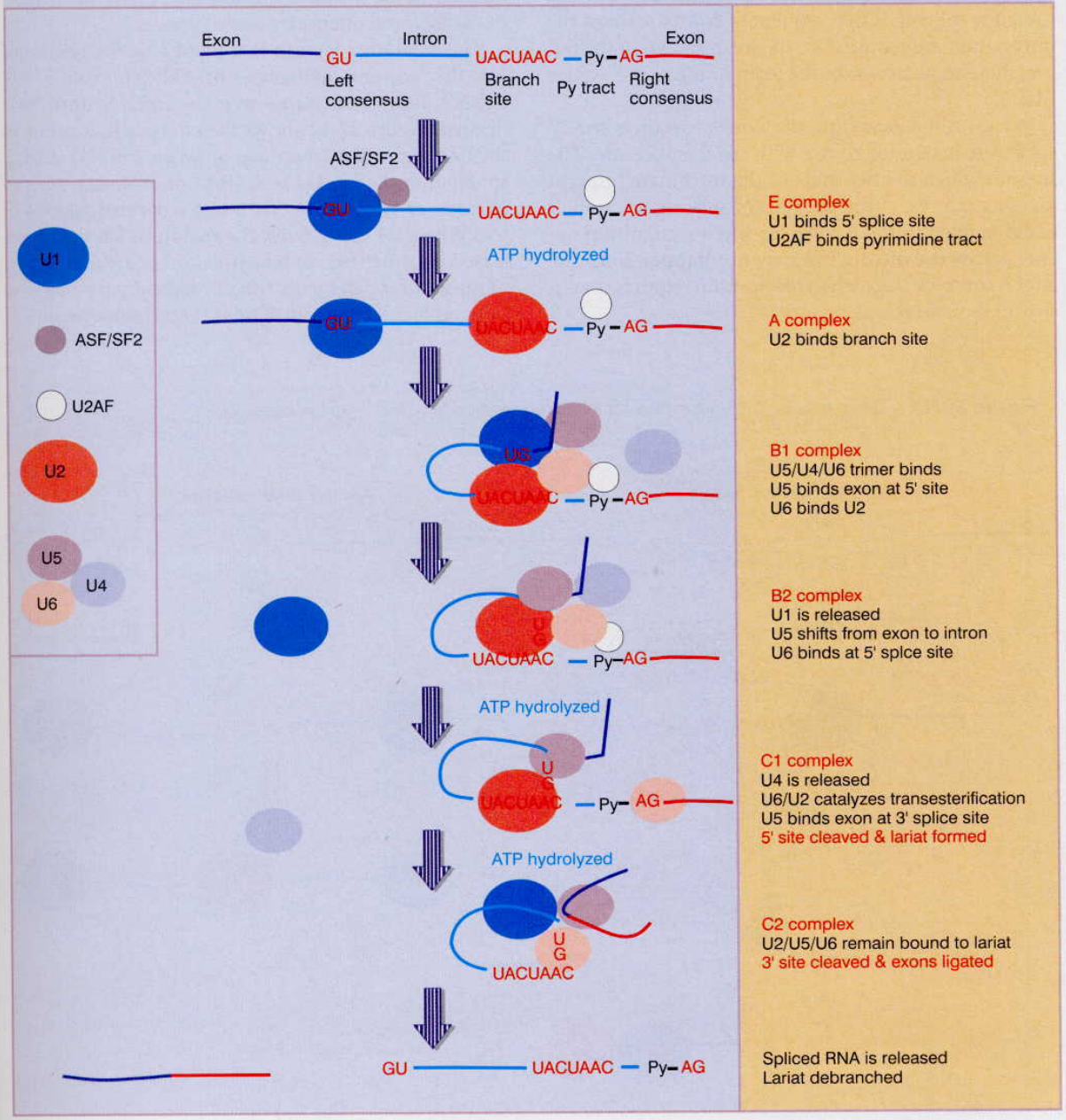


Figure 22.16 Mitochondrial group II introns are released by splicing in the form of stable lariats. Photograph kindly provided by Leslie Grivell and Annika Arnberg.

Figure 22.10 The splicing reaction proceeds through discrete stages in which spliceosome formation involves the interaction of components that recognize the consensus sequences.



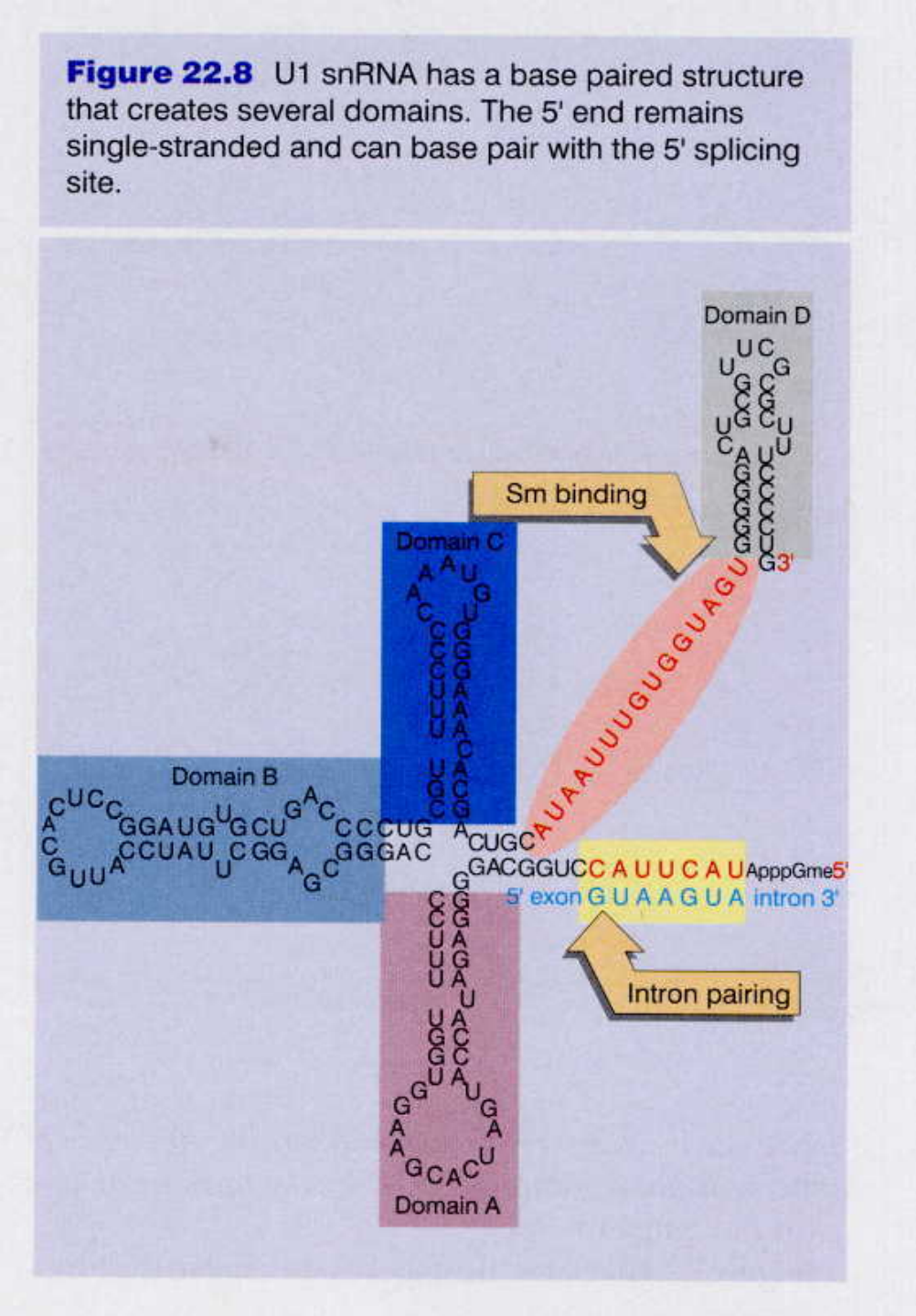


Figure 22.8 U1 snRNA has a base paired structure that creates several domains. The 5' end remains single-stranded and can base pair with the 5' splicing site.

snRNA: small nuclear RNA

snRNP: small nuclear Ribonucleoparticle „snurps“

Figure 22.18 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.

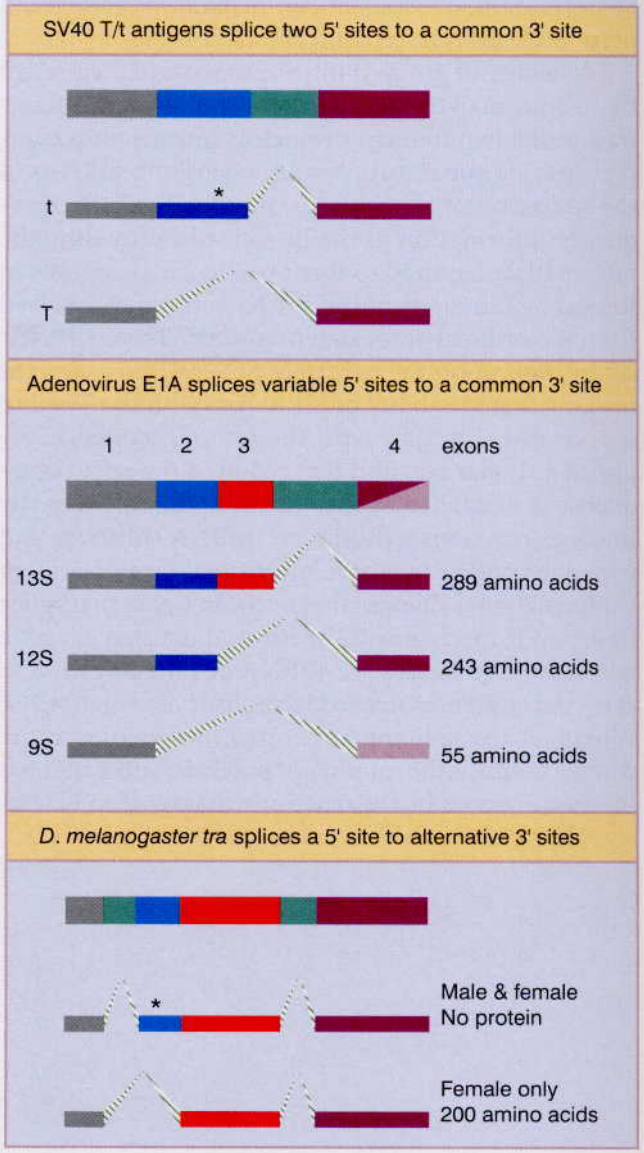
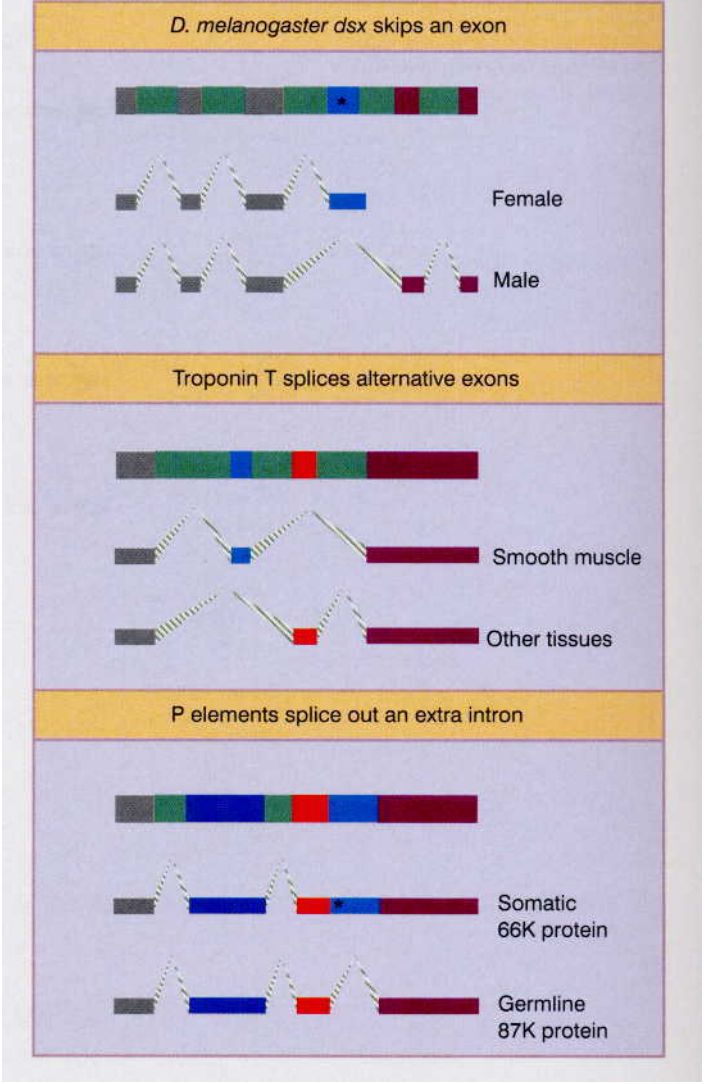


Figure 22.20 Alternative splicing events that involve both sites may cause exons to be added or substituted.



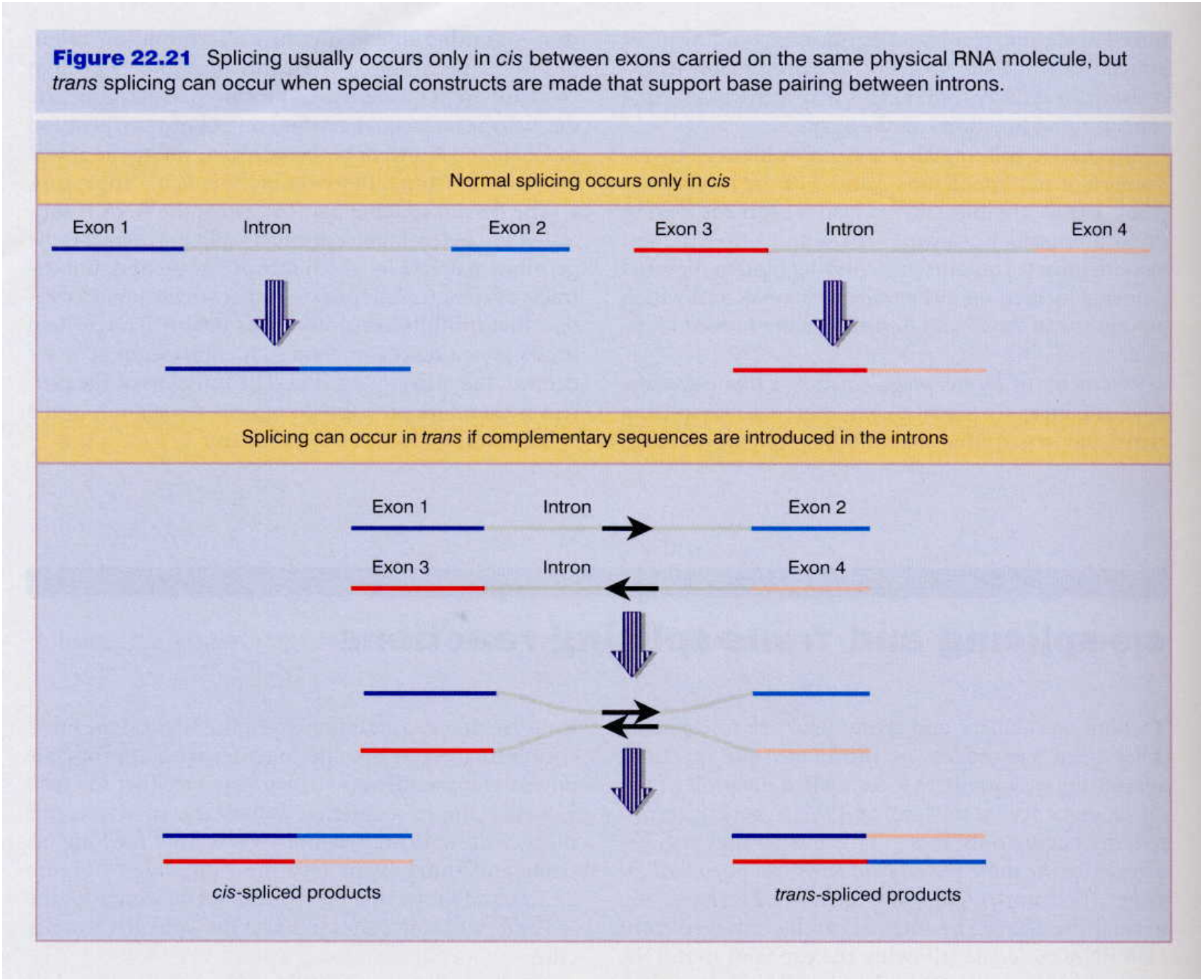


Figure 22.21 Splicing usually occurs only in *cis* between exons carried on the same physical RNA molecule, but *trans* splicing can occur when special constructs are made that support base pairing between introns.

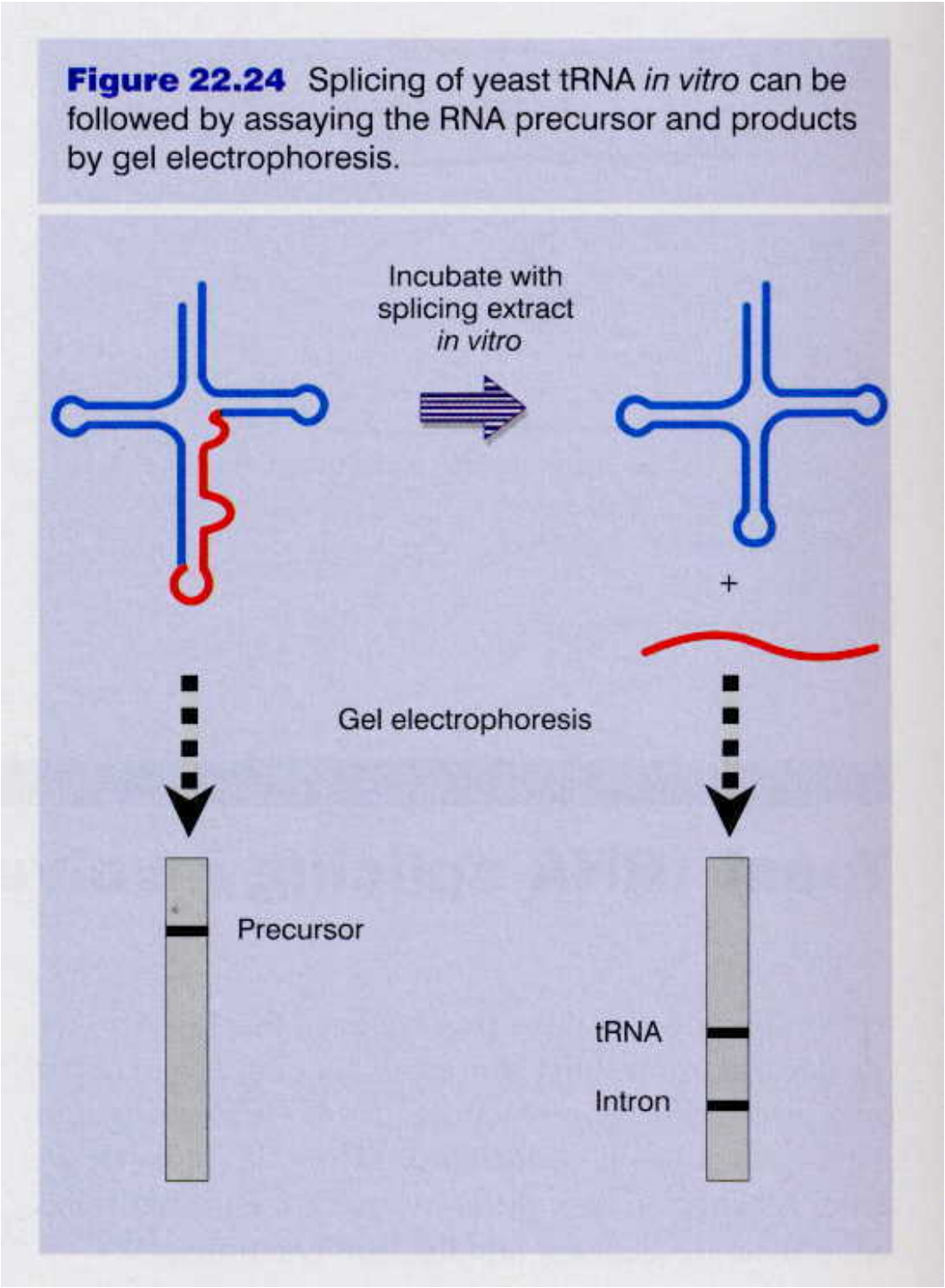
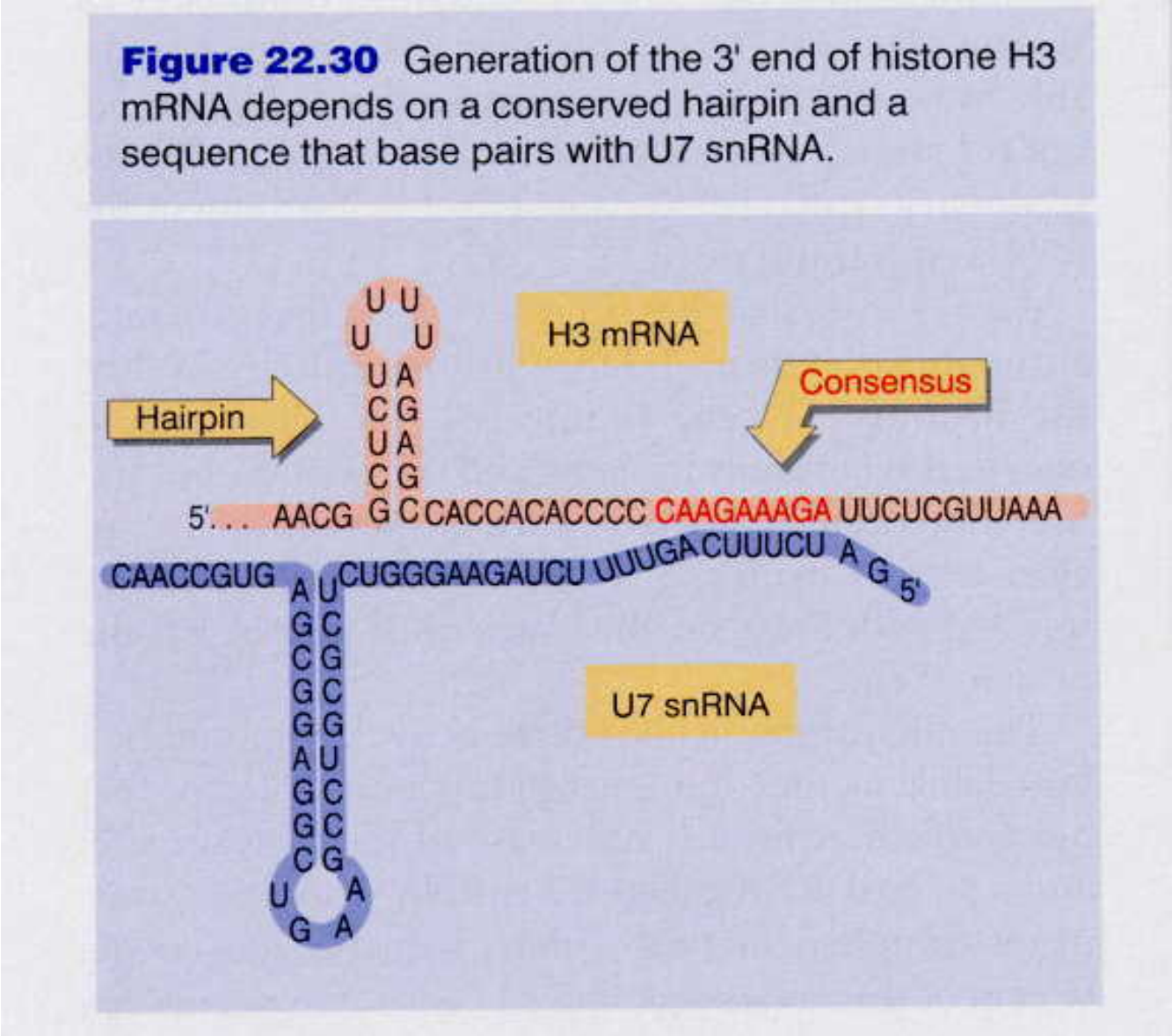
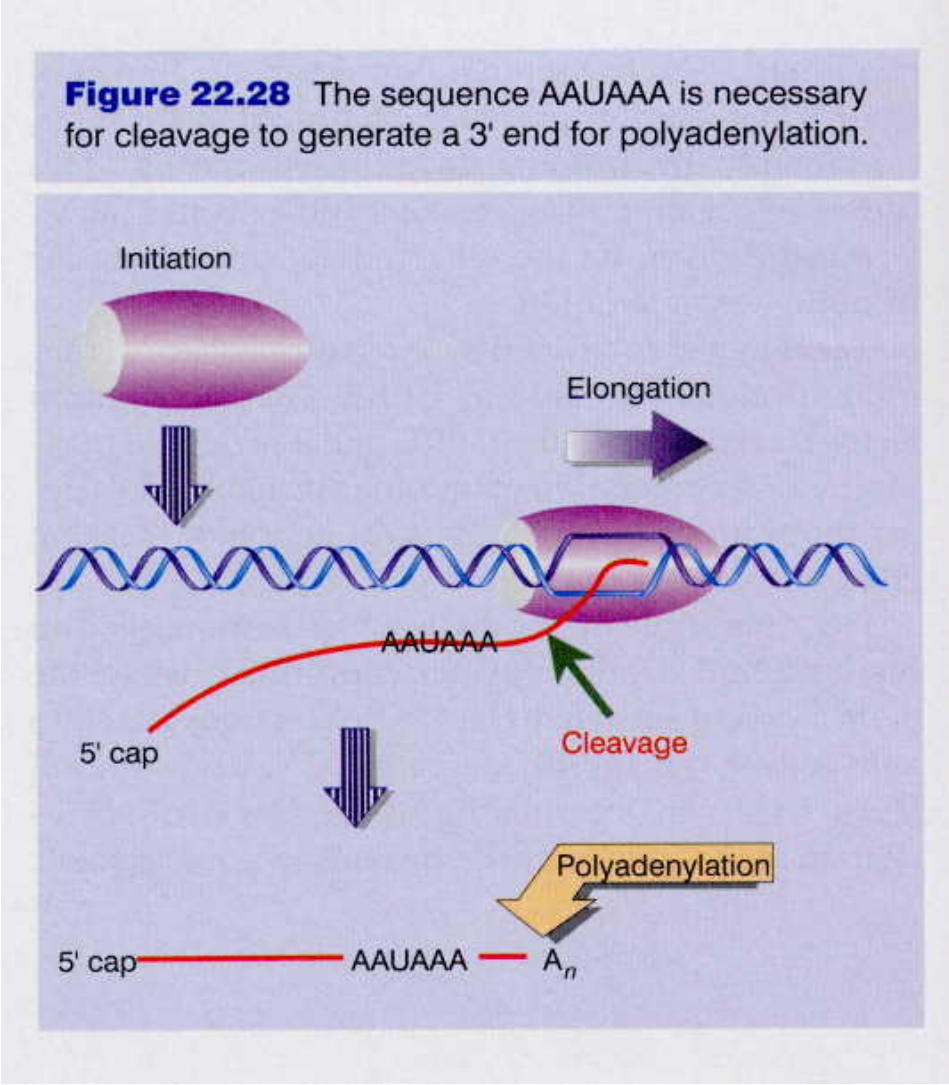


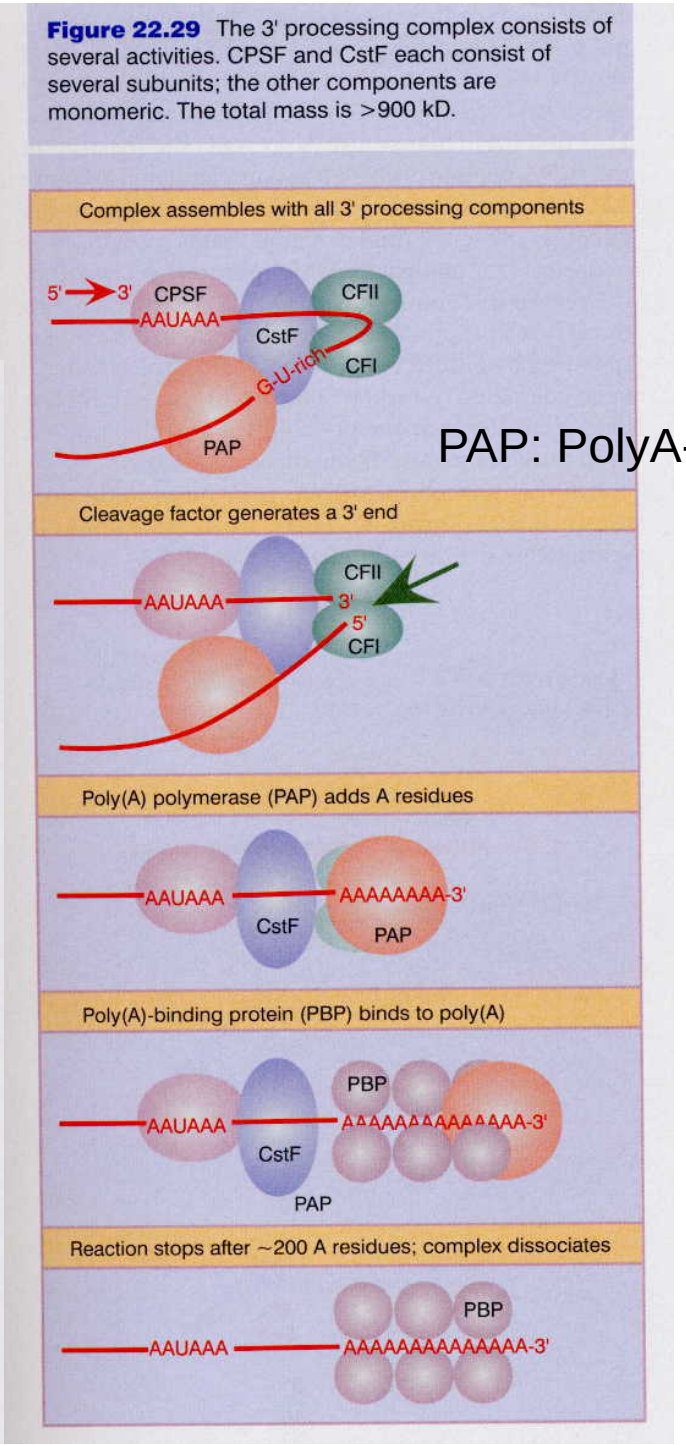
Figure 22.24 Splicing of yeast tRNA *in vitro* can be followed by assaying the RNA precursor and products by gel electrophoresis.

3‘-end processing



3‘-end processing





PAP: PolyA-Polymerase

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

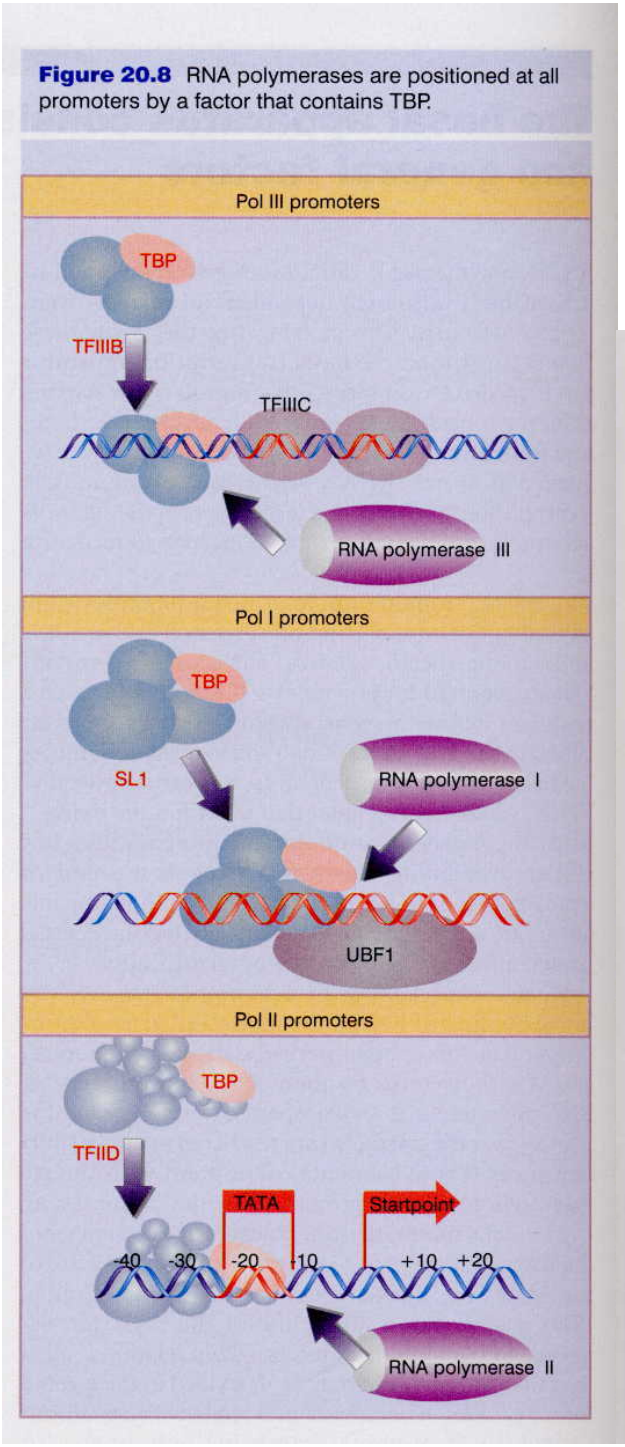


Figure 20.8 RNA polymerases are positioned at all promoters by a factor that contains TBP.

Figure 20.9 A view in cross-section shows that TBP surrounds DNA from the side of the narrow 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 Burley.

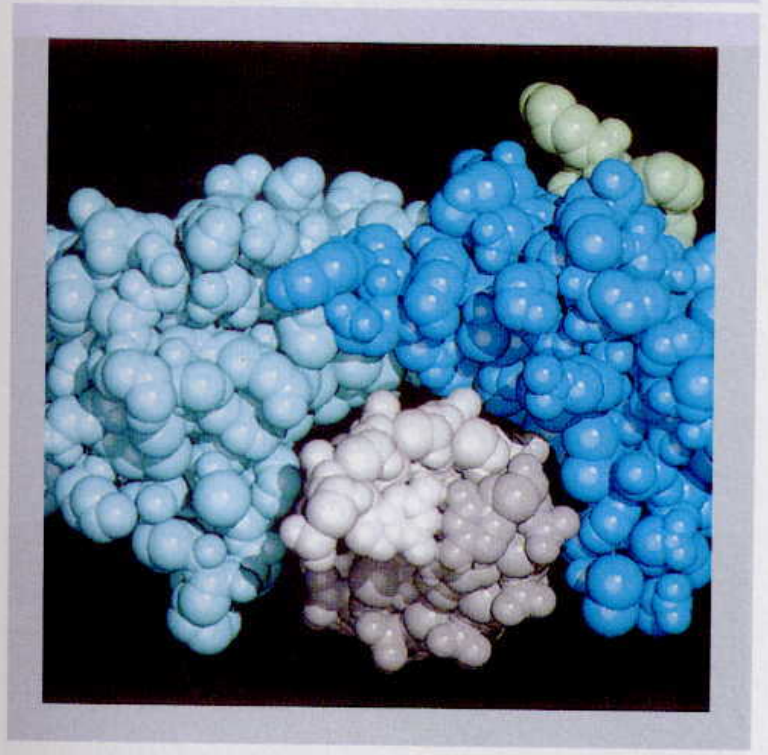
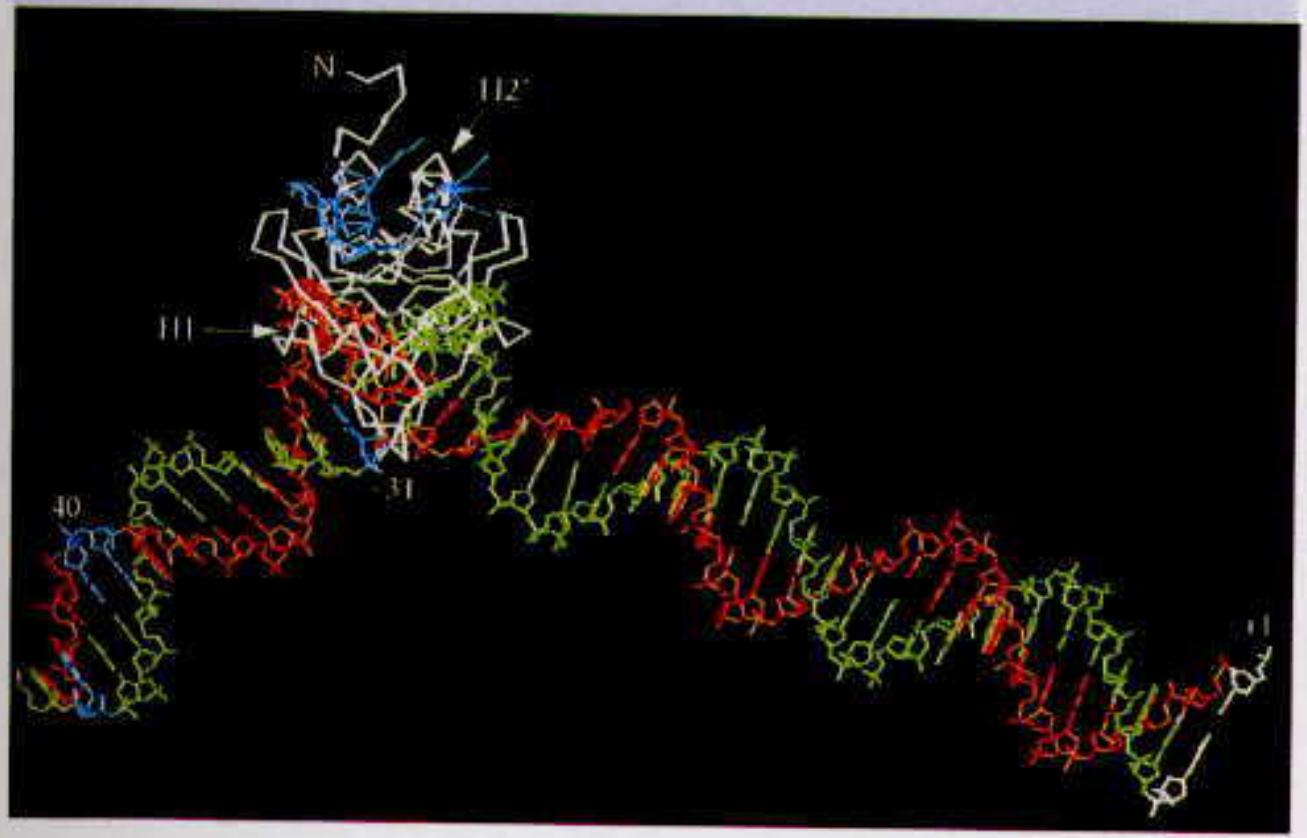
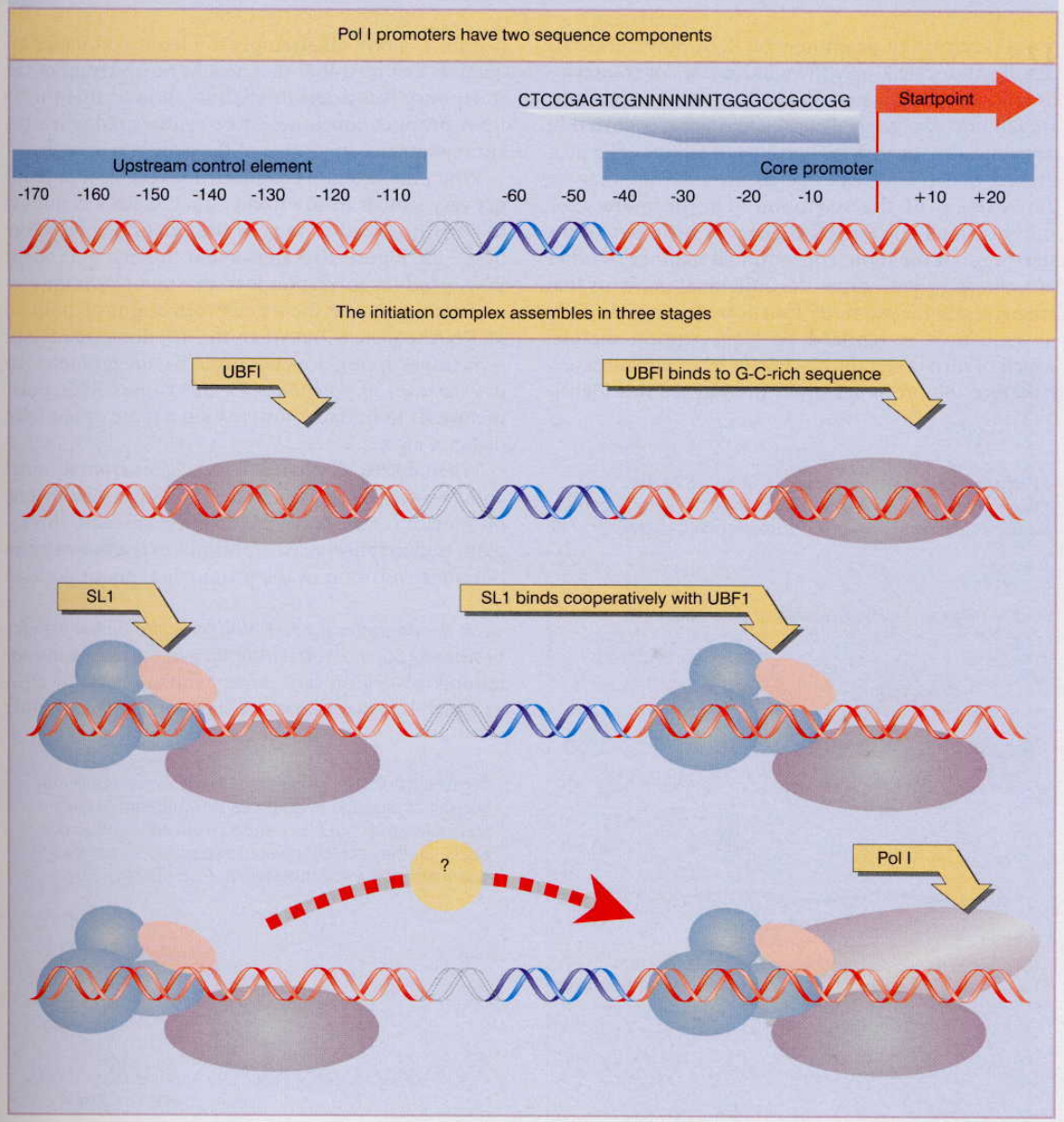


Figure 20.10 The cocrystal structure of TBP with DNA from -40 to the startpoint shows a bend at the TATA box that widens the narrow groove where TBP binds. Photograph kindly provided by Stephen Burley.



H.Schwab

Figure 20.4 Transcription units for RNA polymerase I have a core promoter separated by ~70 bp from the upstream control element. UBF1 binds to both regions, after which SL1 can bind. RNA polymerase I then binds to the core promoter. The nature of the interaction between the factors bound at the upstream control element and those at the core promoter is not known.



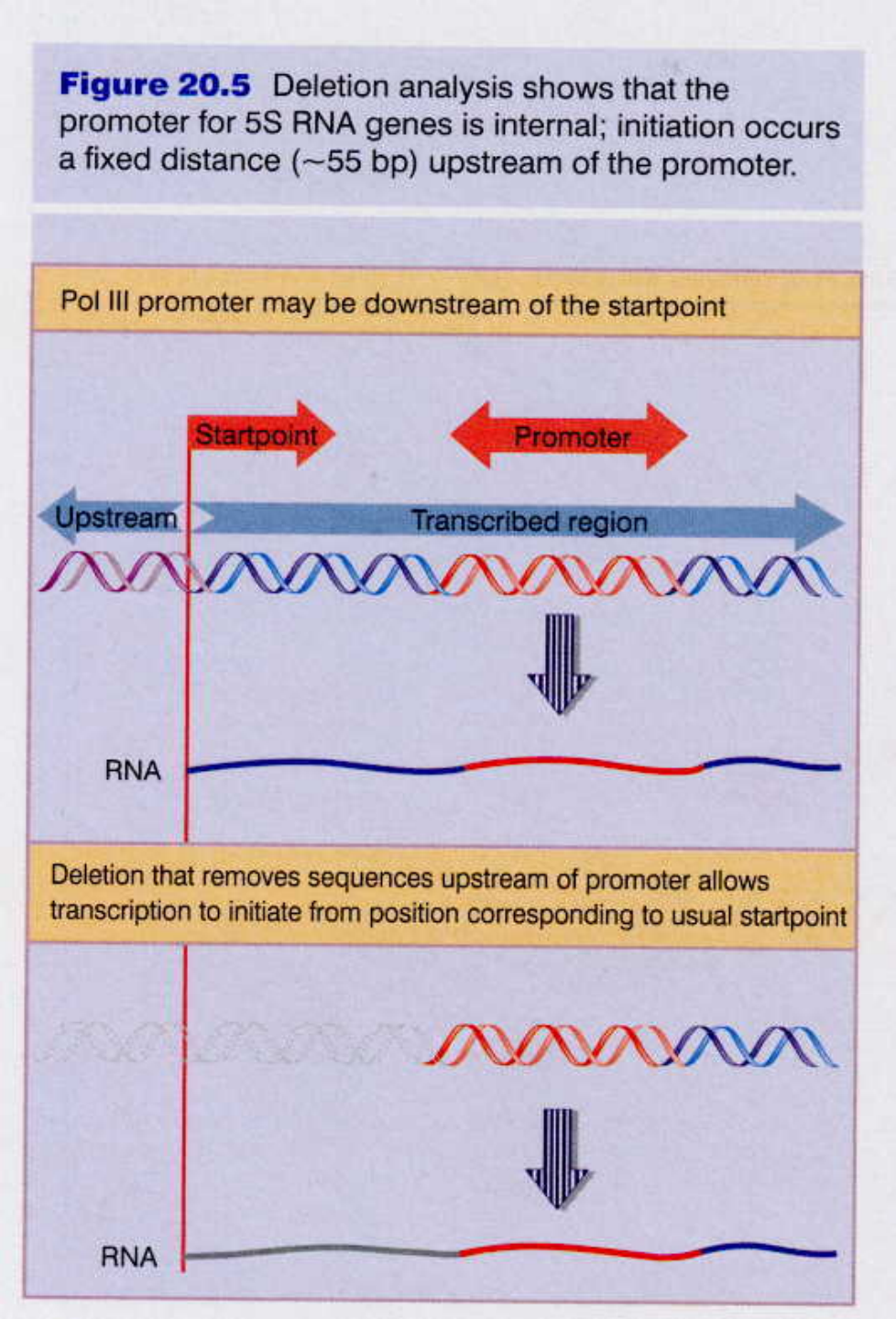


Figure 20.5 Deletion analysis shows that the promoter for 5S RNA genes is internal; initiation occurs a fixed distance (~55 bp) upstream of the promoter.

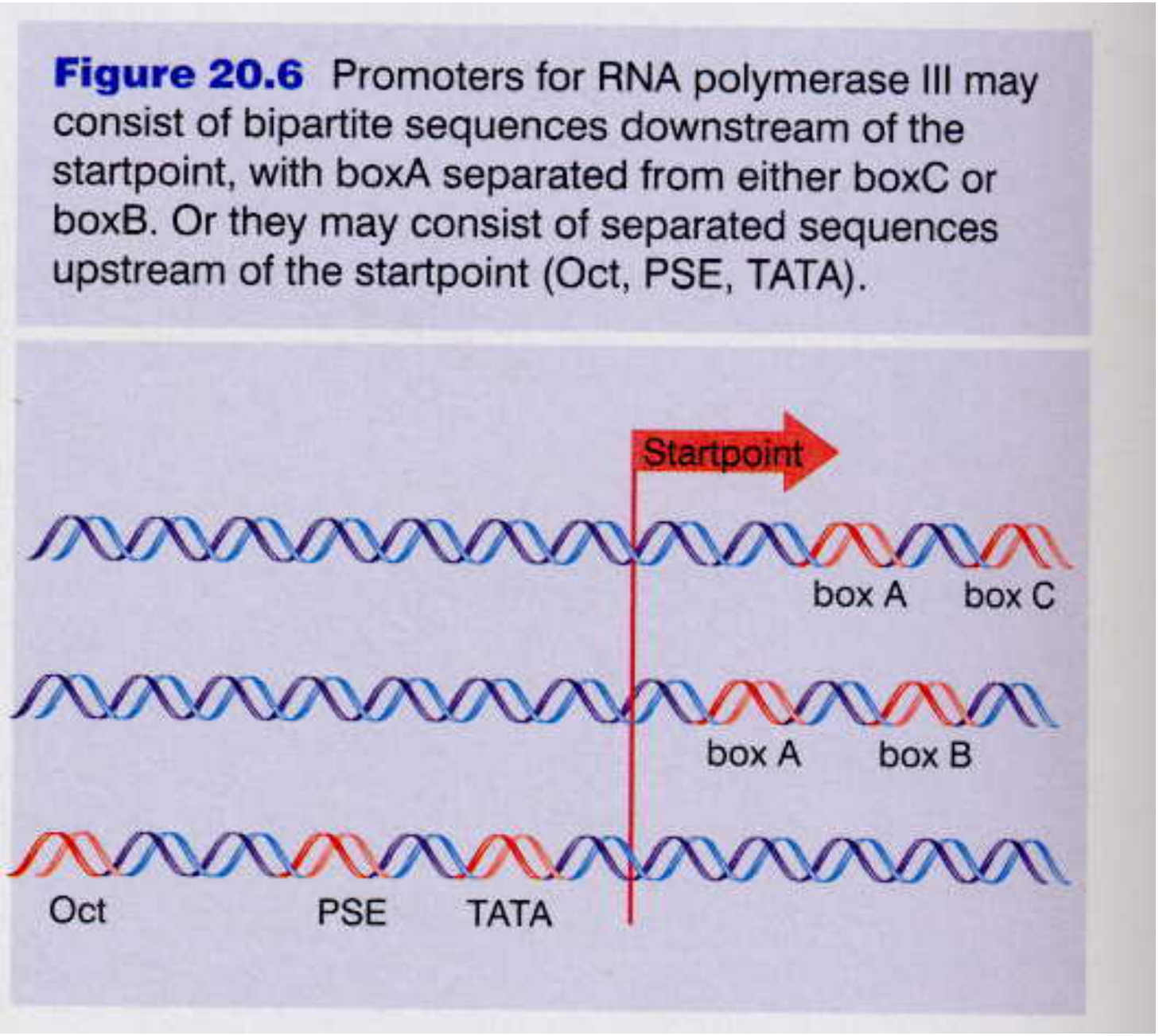


Figure 20.6 Promoters for RNA polymerase III may consist of bipartite sequences downstream of the startpoint, with boxA separated from either boxC or boxB. Or they may consist of separated sequences upstream of the startpoint (Oct, PSE, TATA).

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.

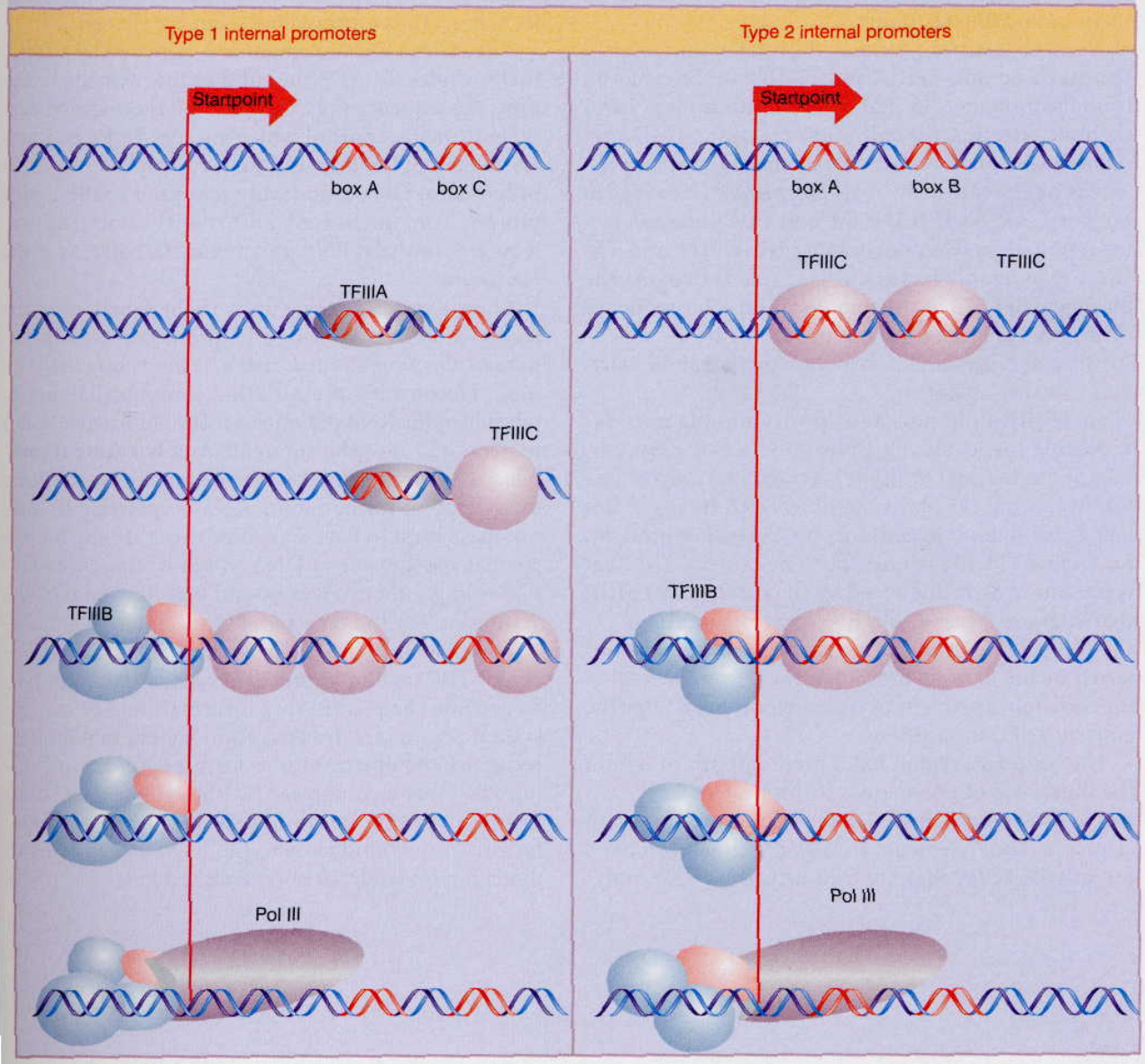
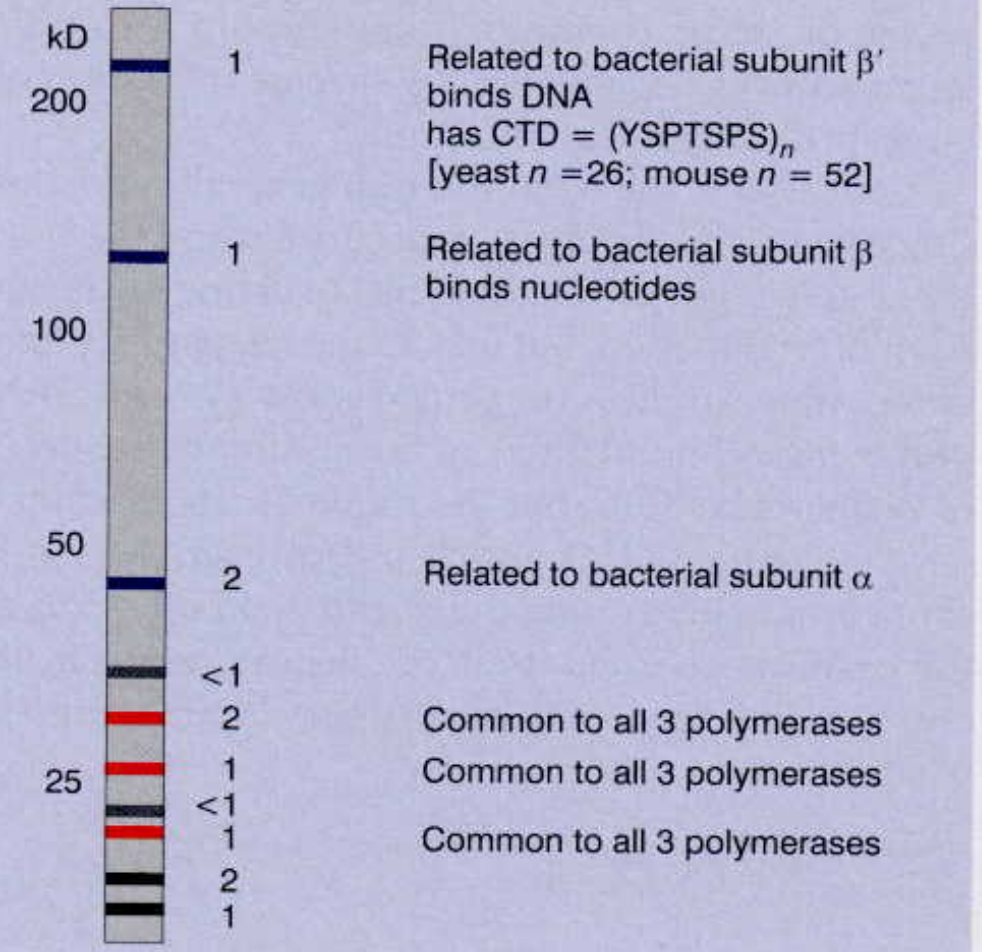


Figure 20.2 Eukaryotic RNA polymerase II has >10 subunits.



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.

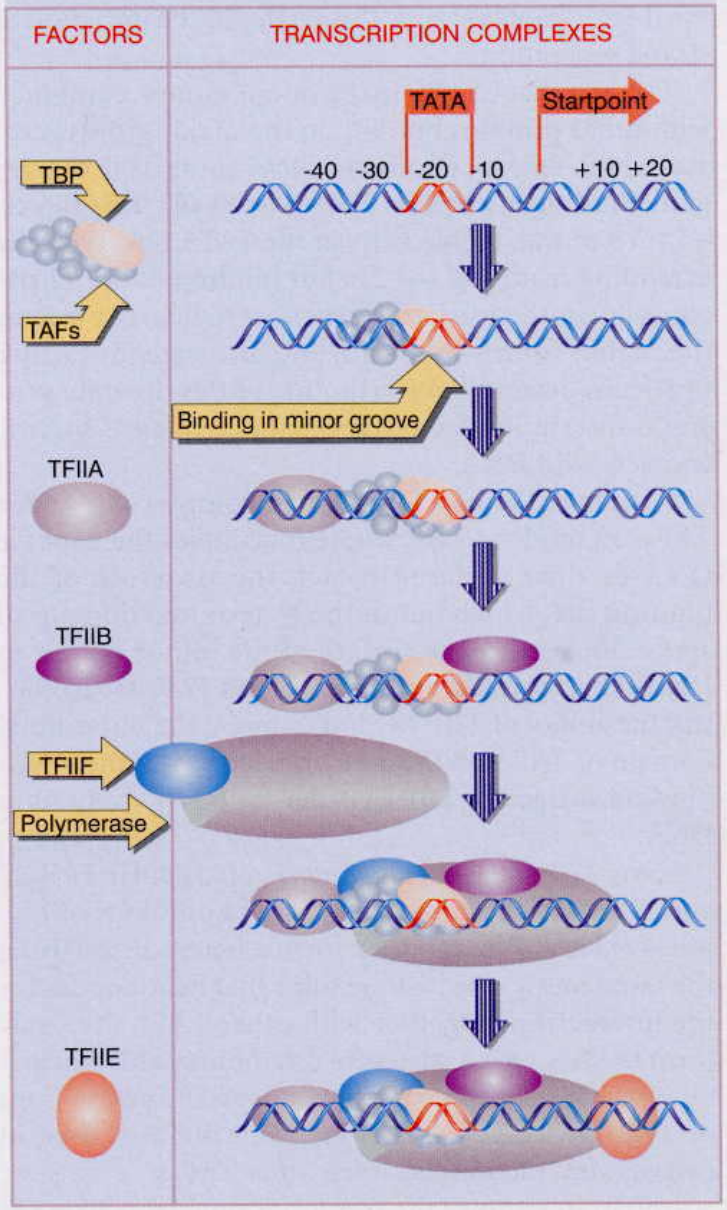
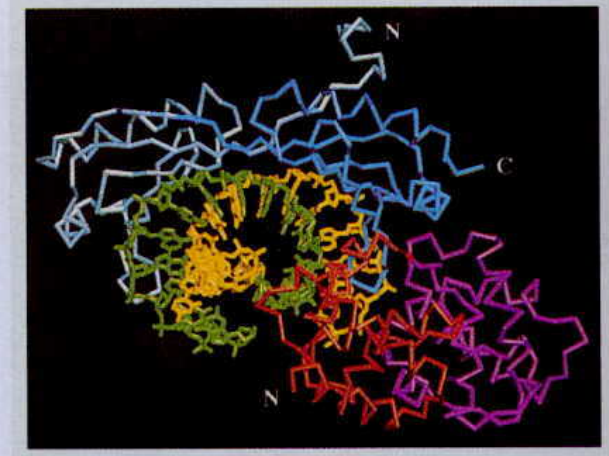
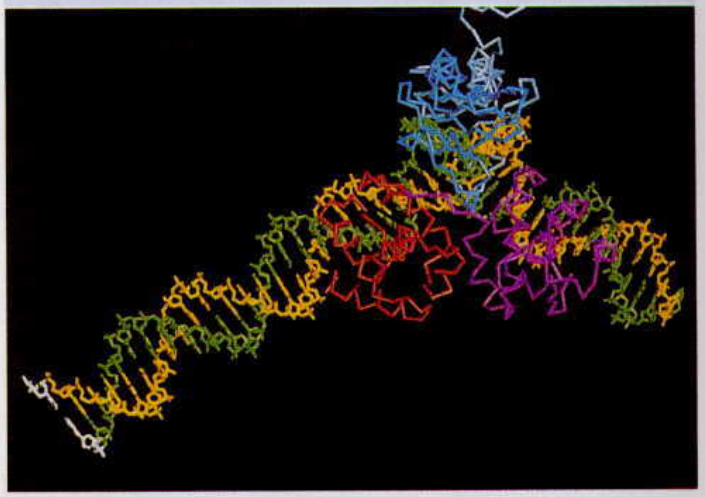


Figure 20.12 Two views of the ternary complex of TFIIB-TBP-DNA show that TFIIB binds along the bent face of DNA. The two strands of DNA are green and yellow, TBP is blue, and TFIIB is red and purple. Photograph kindly provided by Stephen Burley.





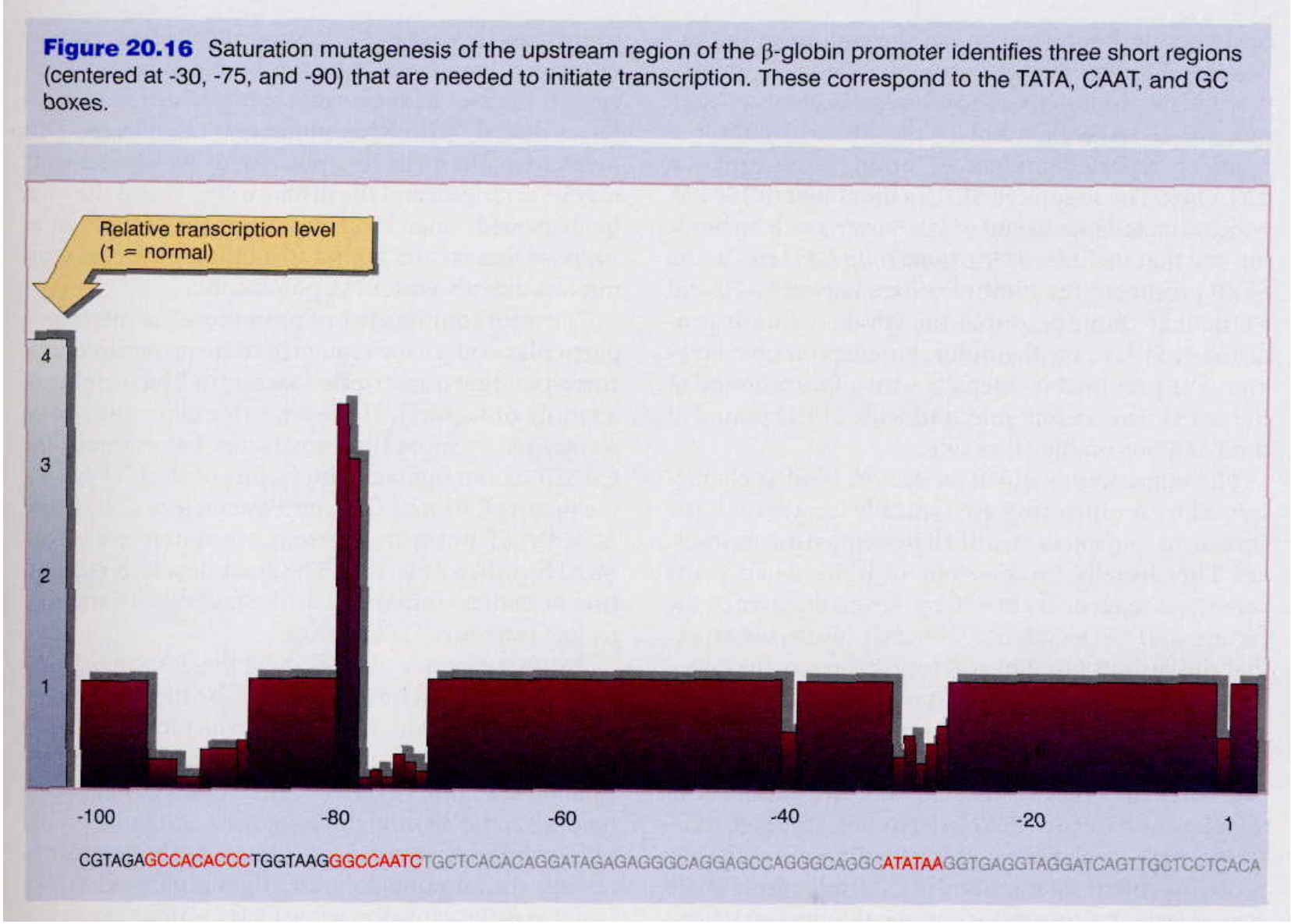


Figure 20.16 Saturation mutagenesis of the upstream region of the β-globin promoter identifies three short regions (centered at -30, -75, and -90) that are needed to initiate transcription. These correspond to the TATA, CAAT, and GC boxes.

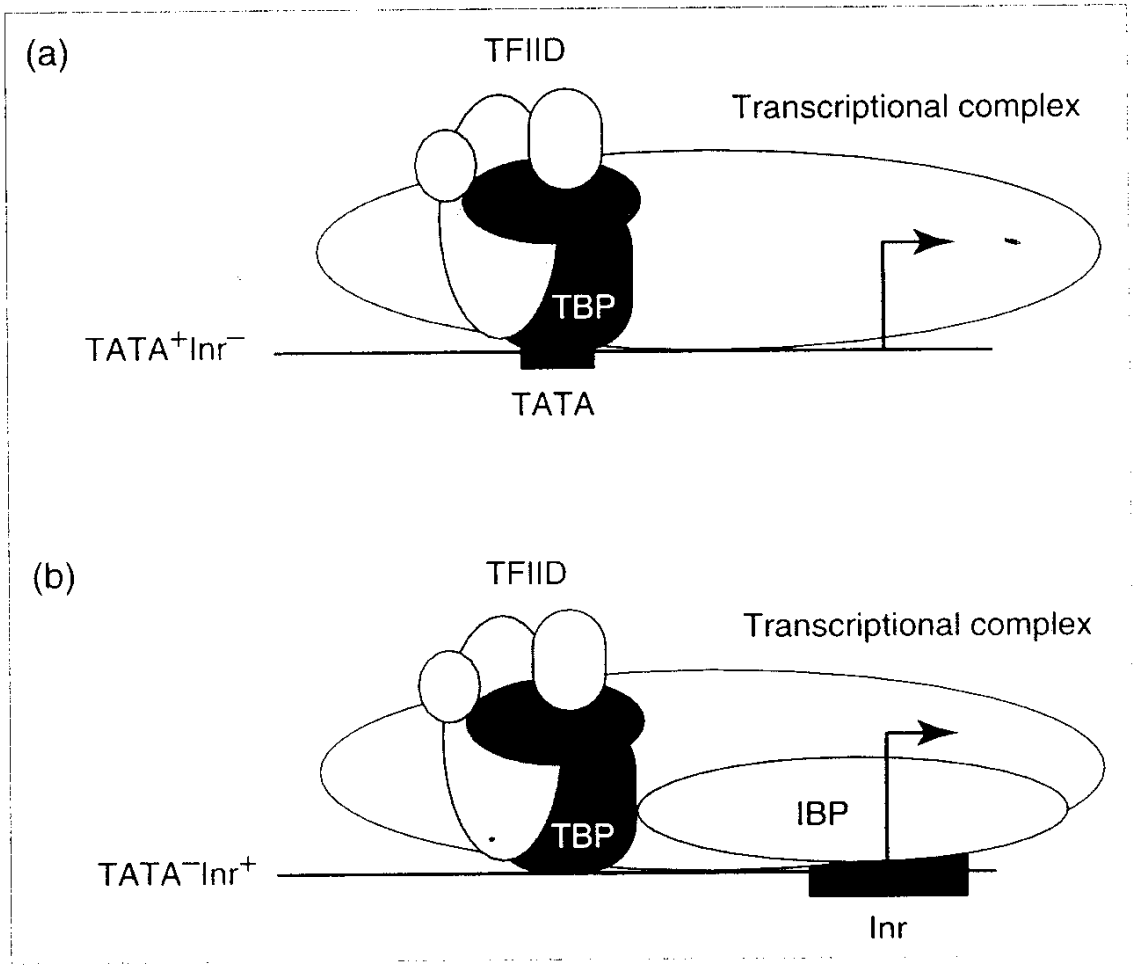


FIGURE 2. Formation of the transcription complex at (a) the TATA box or (b) an initiator (Inr) element. In $TATA^+$ Inr^- promoters, the initial recognition step is the binding of TFIID to the TATA box via its DNA-binding subunit, the TATA-binding protein (TBP). Following this event, other general transcription 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.

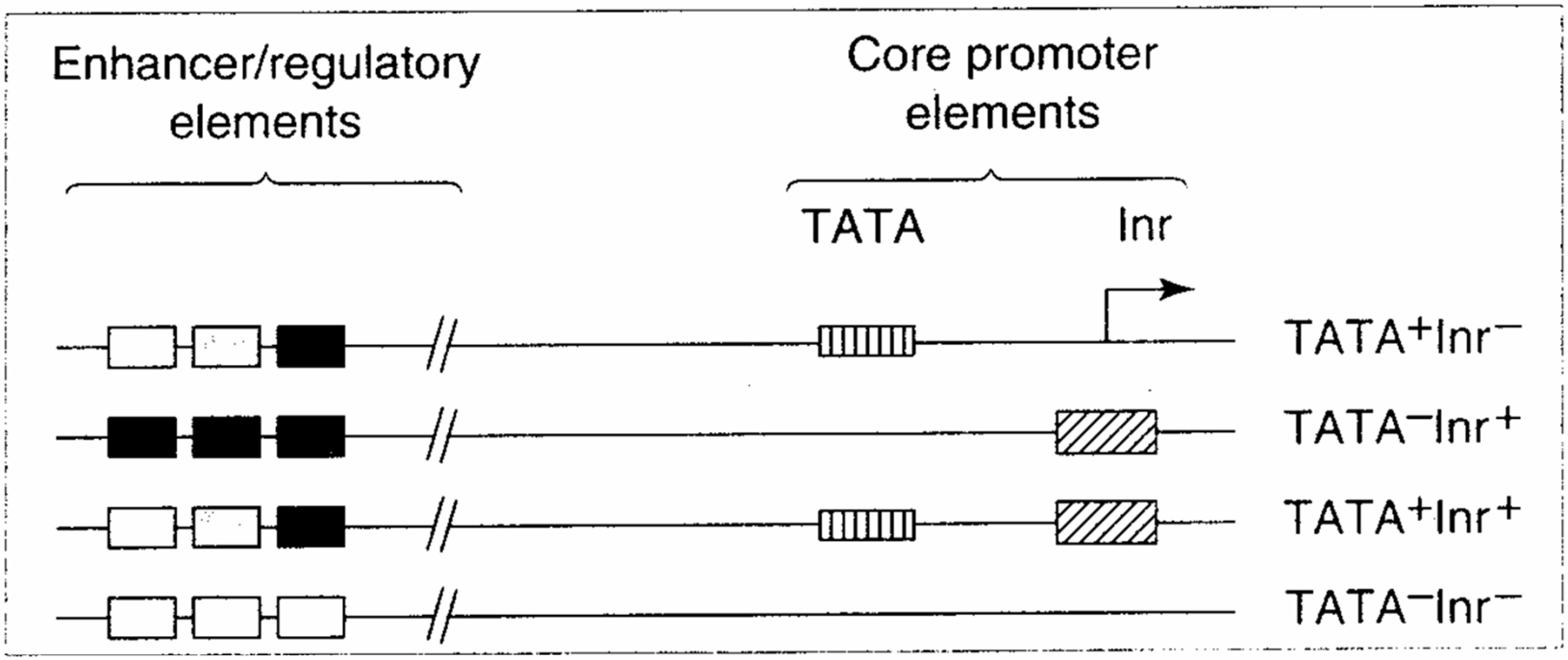


FIGURE 1. Architecture of different classes of eukaryotic RNA polymerase II (Pol II) promoters. The core promoter region may contain either a TATA box ($TATA^+ Inr^-$) or an initiator (Inr) element ($TATA^- Inr^+$). Some promoters might contain both core elements ($TATA^+Inr^+$) and others none ($TATA^- Inr^-$). 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 vivo*.

Regulated Expression in Eukaryotes

Complex Initiation System

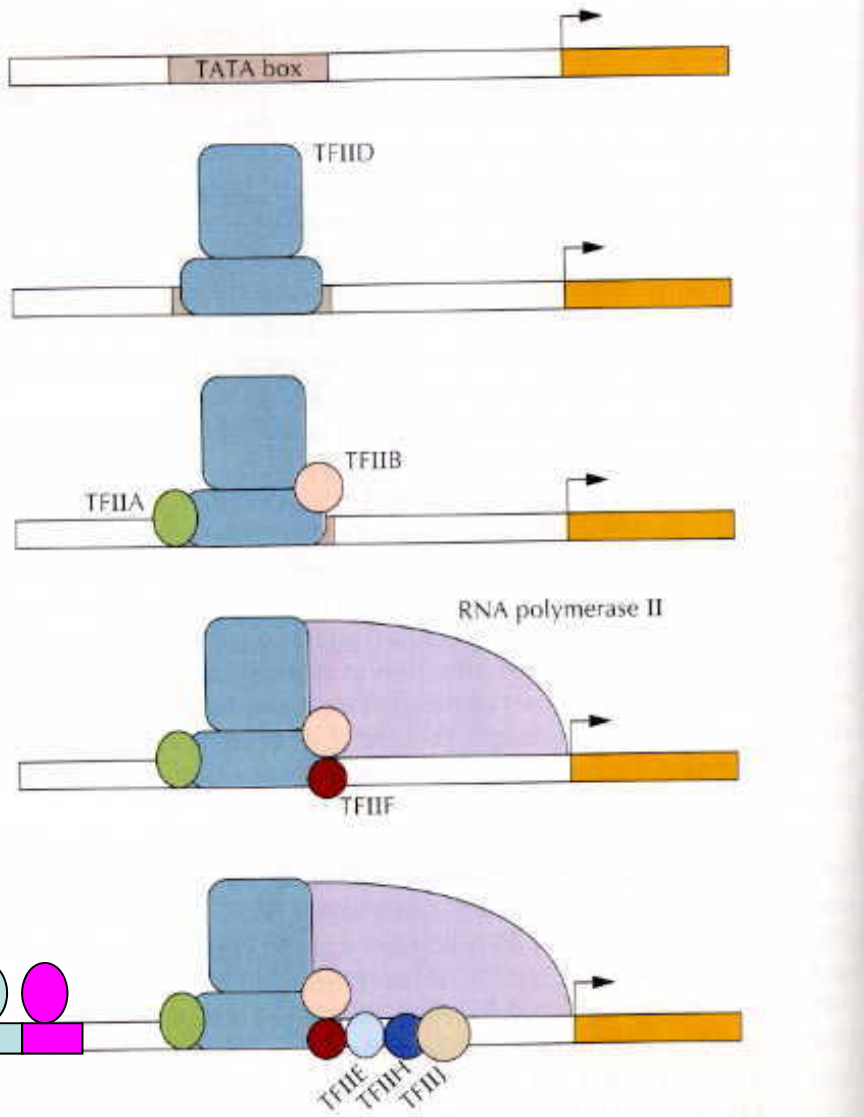


Figure 3.24 Formation of an RNA polymerase II transcription initiation complex at a TATA box. Transcription factor TFIID binds to a TATA box, and, in sequence, other transcription factors and RNA polymerase II bind to form a protein aggregate that is responsible for initiating transcription. The right-angled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

Enhancer

Activators

Repressing Systems

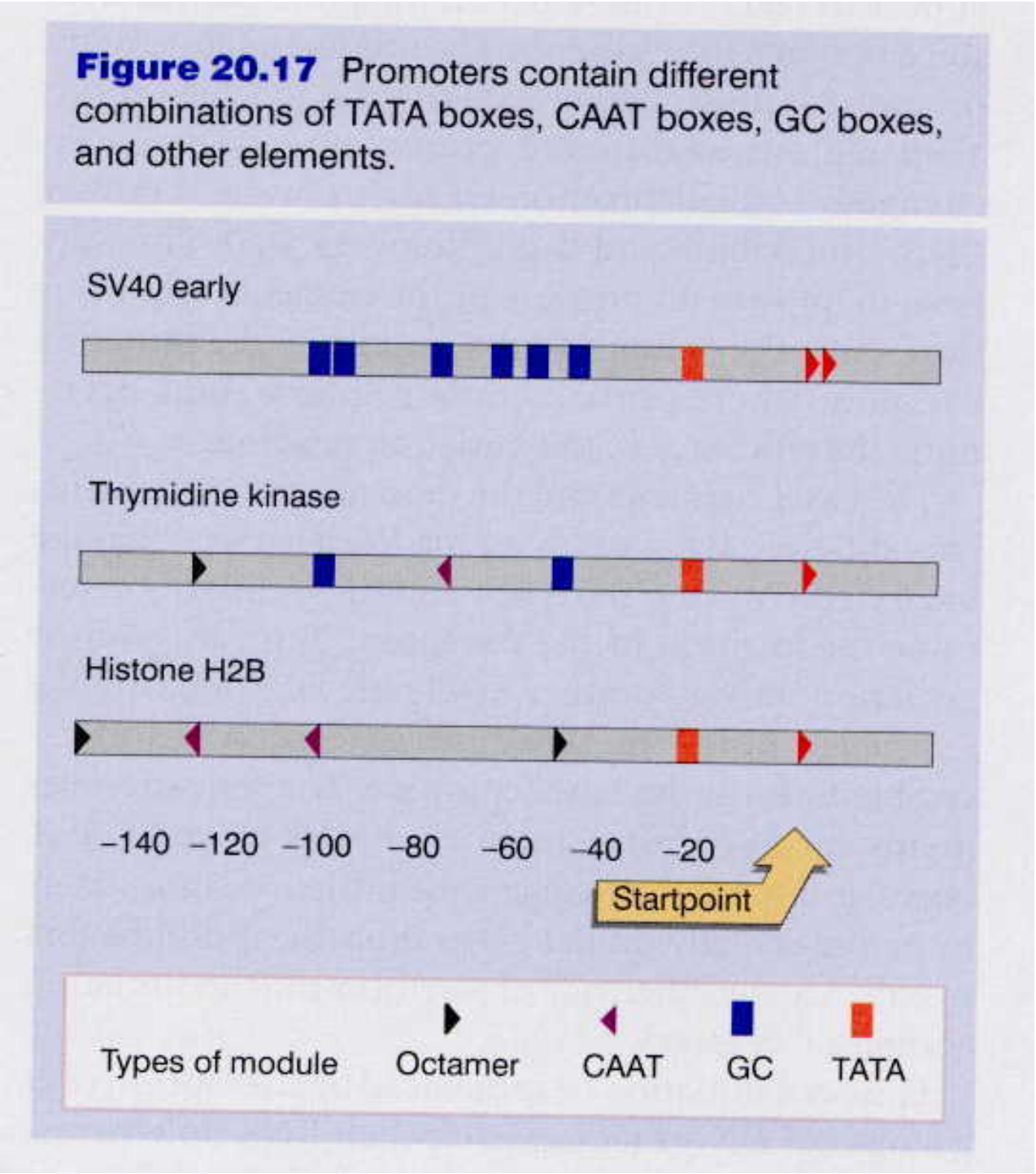


Figure 20.17 Promoters contain different combinations of TATA boxes, CAAT boxes, GC boxes, and other elements.

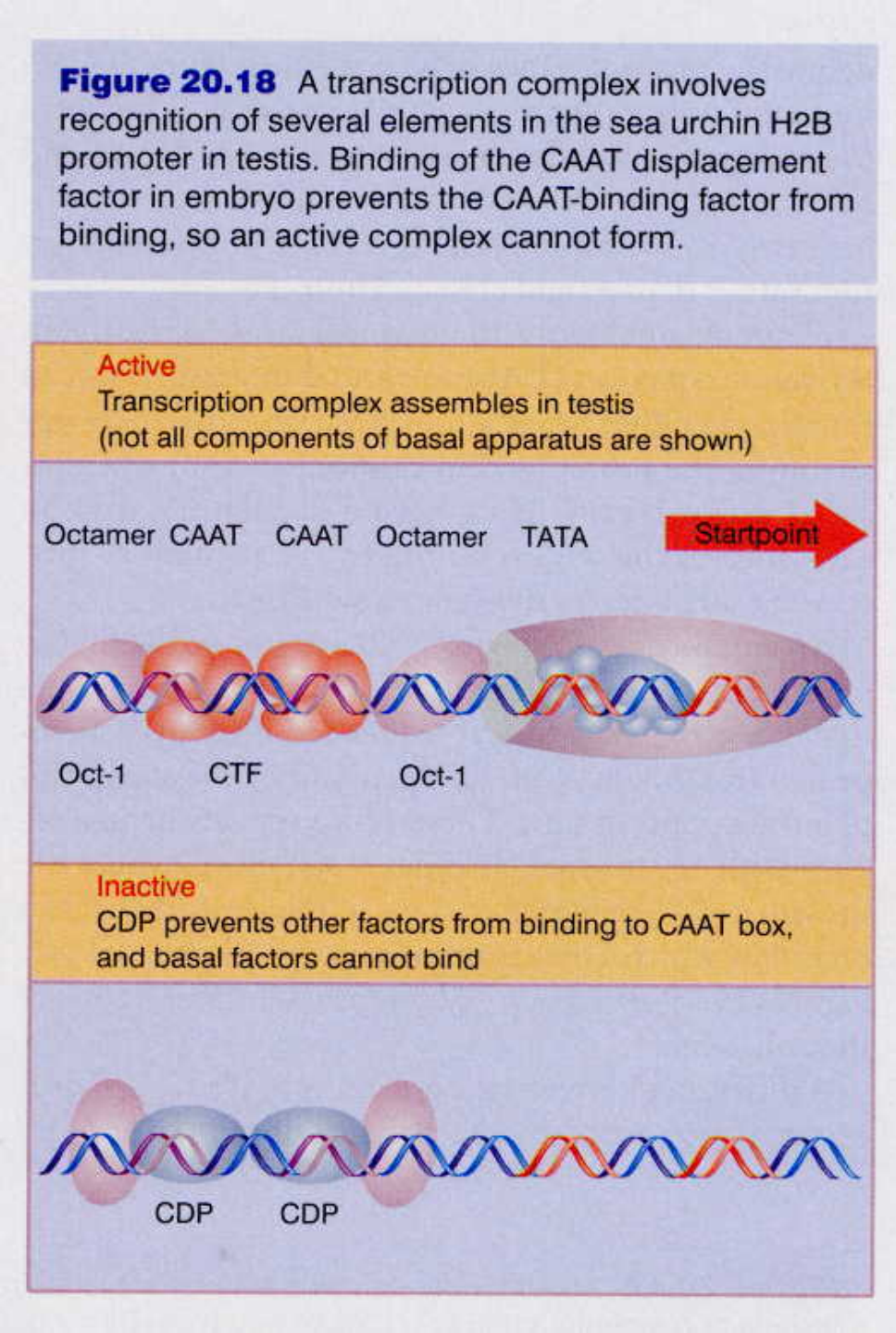


Figure 20.18 A transcription complex involves recognition of several elements in the sea urchin H2B promoter in testis. Binding of the CAAT displacement factor in embryo prevents the CAAT-binding factor from binding, so an active complex cannot form.

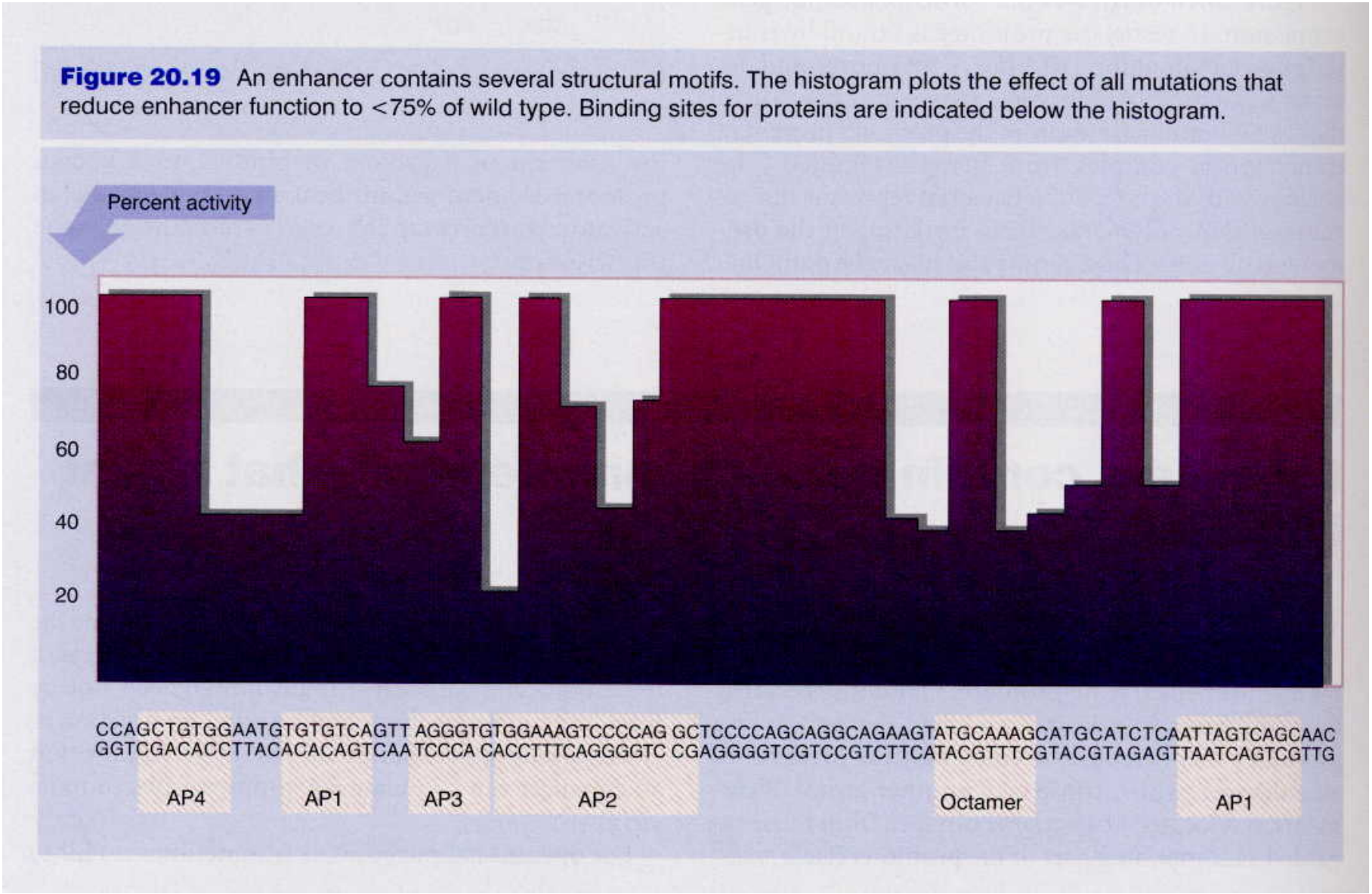


Figure 20.19 An enhancer contains several structural motifs. The histogram plots the effect of all mutations that reduce enhancer function to <75% of wild type. Binding sites for proteins are indicated below the histogram.