

ЛК 1

Цели и задачи клеточной биотехнологии.

Генная и клеточная инженерия.

Биологические системы, используемые в клеточной биотехнологии. Особенности транскрипции и трансляции у про- и эукариотических организмов, их регуляция.

- **Биотехнология** – исследование и использование биологических процессов, живых организмов, клеток и клеточных компонентов (в том числе направленное их изменение) для разработки новых технологий (реализации процессов) и получения продуктов для нужд человека.
- **Клеточная биотехнология** - технология, реализуемая в результате манипуляций с отдельными клетками.

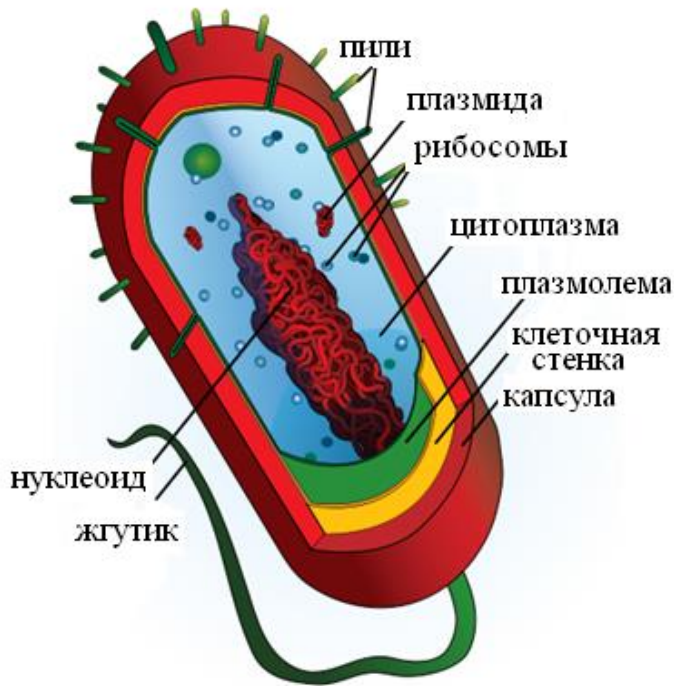
Два уровня клеточной биотехнологии:

- **Клеточная инженерия** – манипуляция с клетками и органеллами. Методы ведения культур клеток для получения новых важных форм и линий растений и животных (устойчивость, продуктивность и качество) размножение ценных генотипов, получение ценных биологических препаратов пищевого, кормового и медицинского назначения.
- **Генная инженерия** – целенаправленное изменение генотипа с целью получения заранее заданных свойств. Получение трансгенных организмов с новыми или усиленными свойствами и признаками.

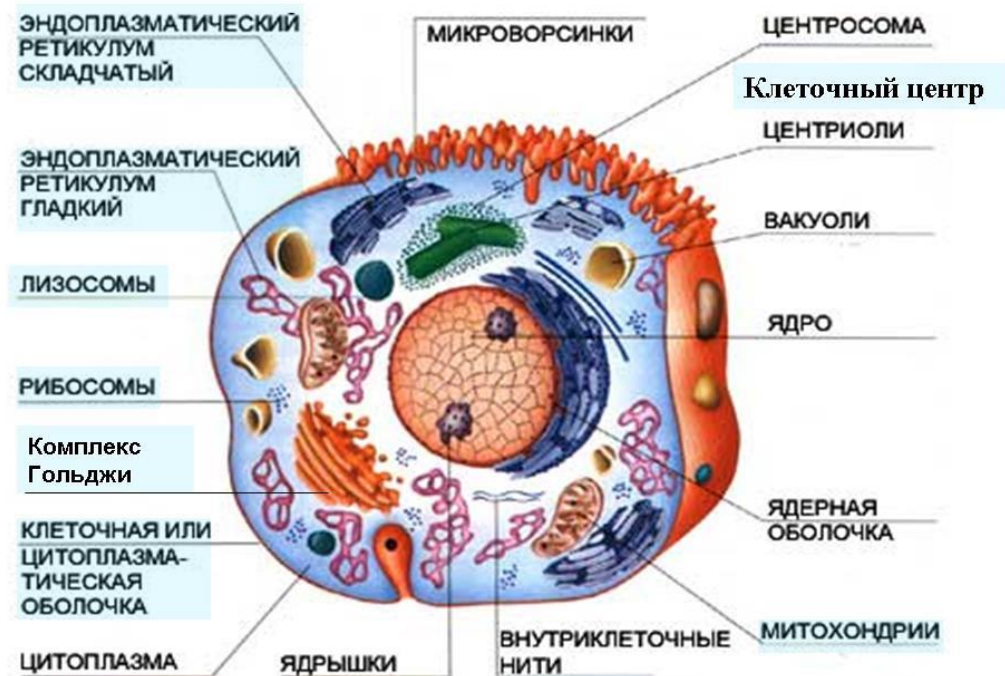
Манипуляции с генетическим материалом клетки - генетическая трансформация.

Перенос чужеродных генов и других материальных носителей наследственности в клетки растений, животных, микроорганизмов.

Строение бактериальной клетки



Строение животной клетки



Транскрипция – экспрессия гена

Траснкрипиция+трансляция=экспрессия белка

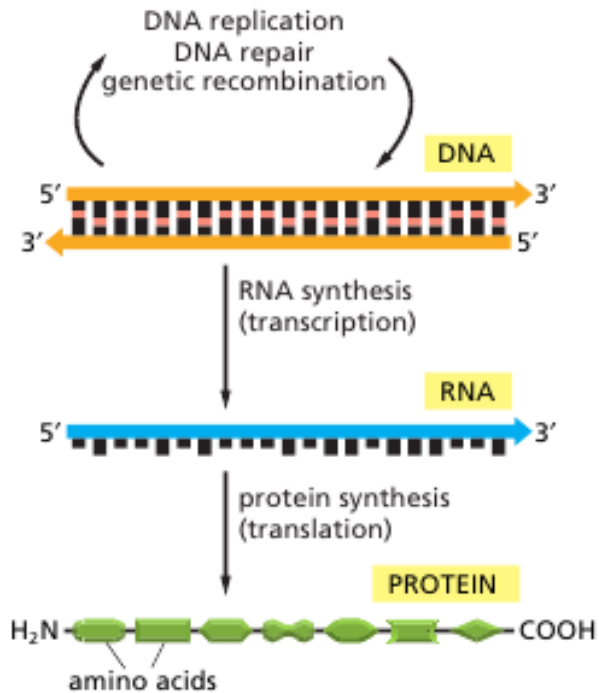
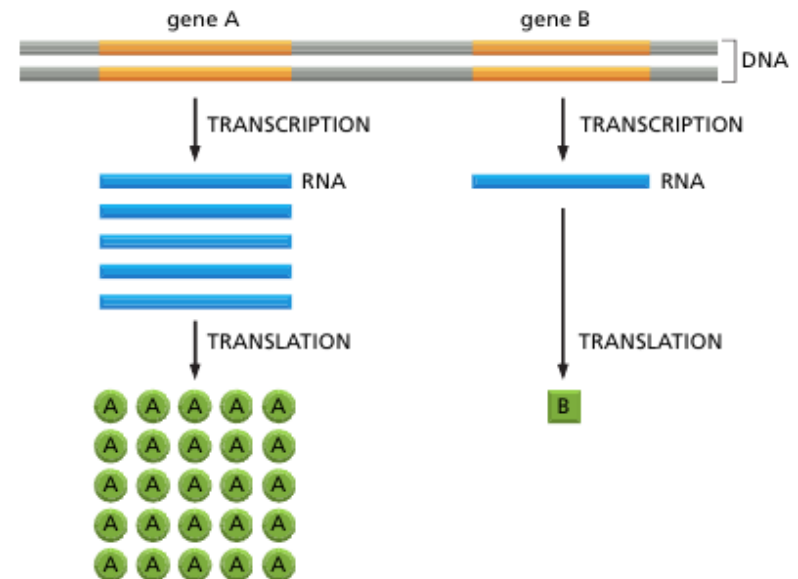


Figure 6–2 The pathway from DNA to protein. The flow of genetic information from DNA to RNA (transcription) and from RNA to protein (translation) occurs in all living cells.



Транскрипция

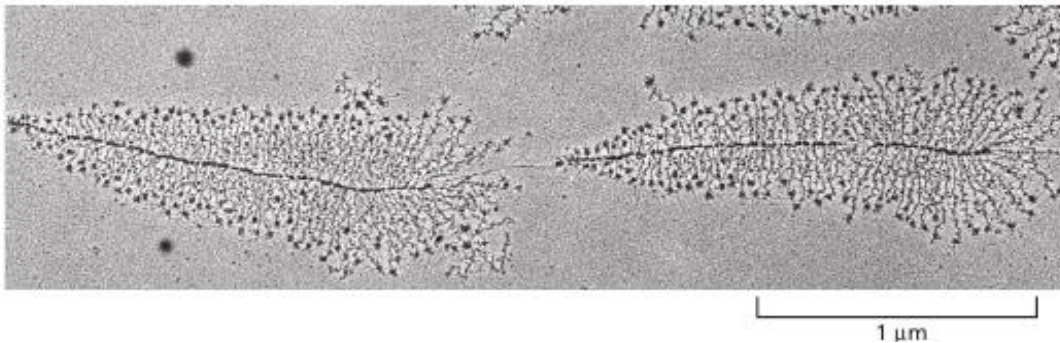
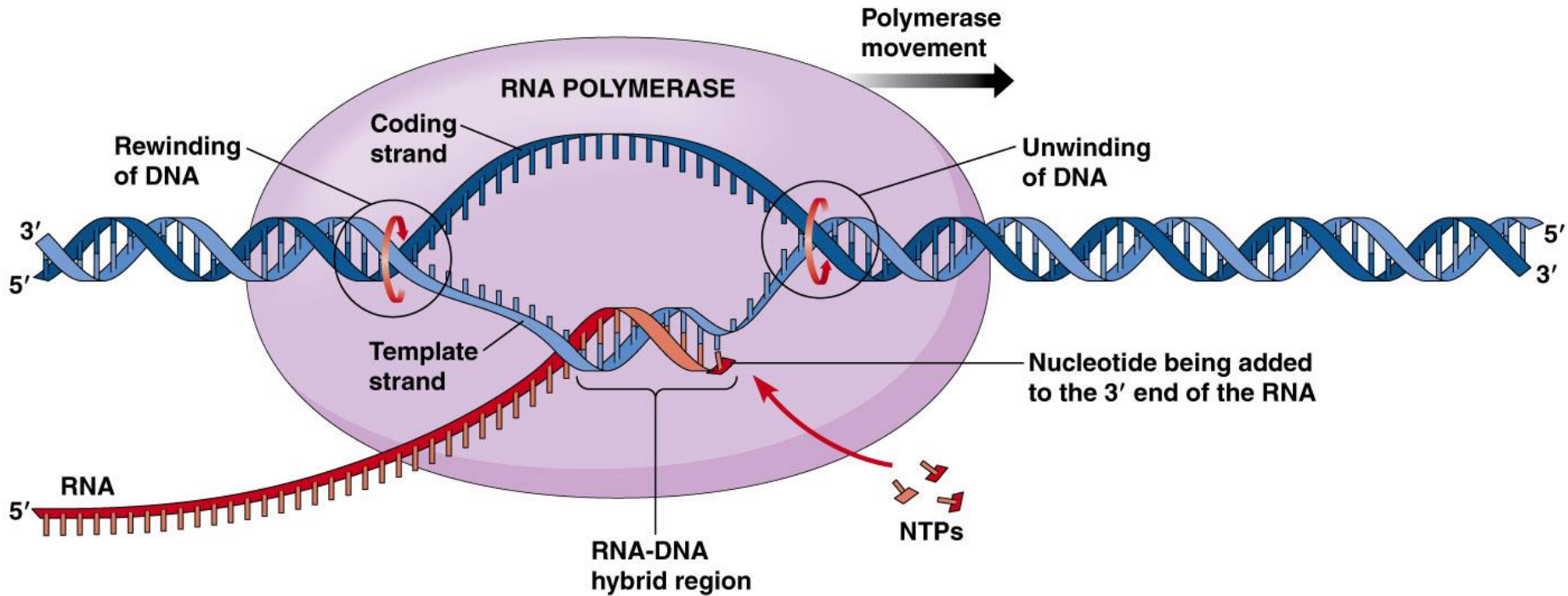


Figure 6–9 Transcription of two genes as observed under the electron microscope. The micrograph shows many molecules of RNA polymerase simultaneously transcribing each of two adjacent genes. Molecules of RNA polymerase are visible as a series of dots along the DNA with the newly synthesized transcripts (fine threads) attached to them. The RNA molecules (ribosomal RNAs) shown in this example

Транскрипция у прокариот

cess of transcription again.

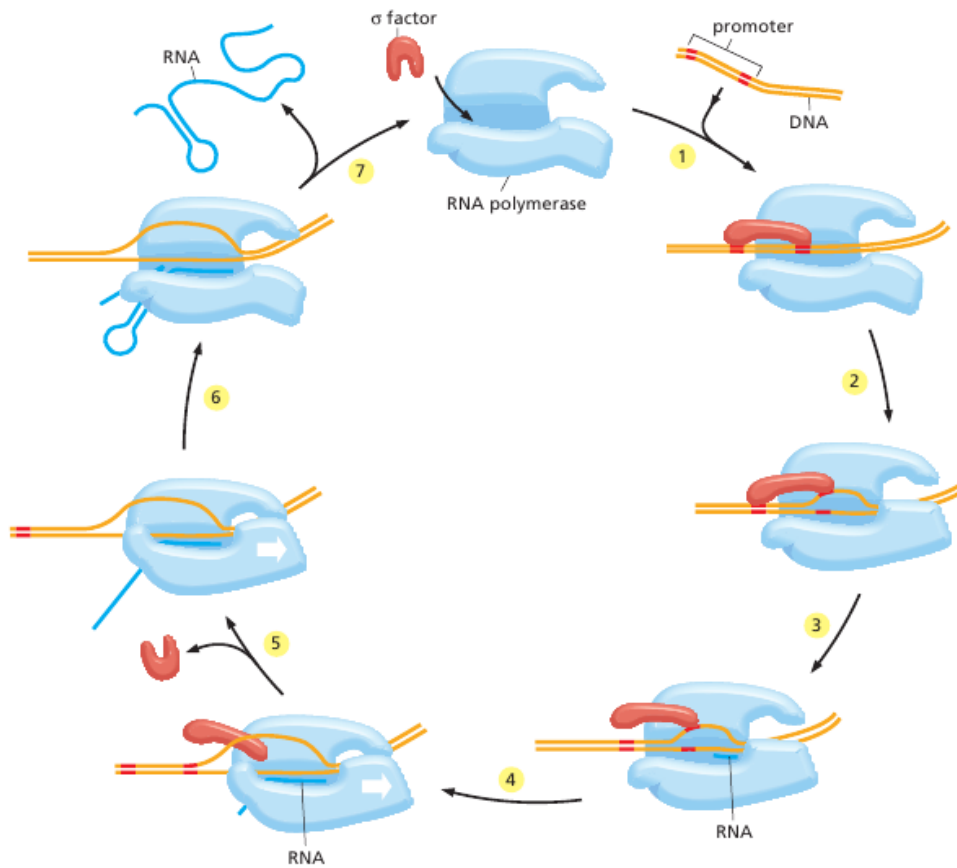


Figure 6–11 The transcription cycle of bacterial RNA polymerase. In step 1, the RNA polymerase holoenzyme (polymerase core enzyme plus σ factor) assembles and then locates a promoter (see Figure 6–12). The polymerase unwinds the DNA at the position at which transcription is to begin (step 2) and begins transcribing (step 3). This initial RNA synthesis (sometimes called “abortive initiation”) is relatively inefficient. However, once RNA polymerase has managed to synthesize about 10 nucleotides of RNA, it breaks its interactions with the promoter DNA and weakens, and eventually breaks, its interaction with σ . The polymerase now shifts to the elongation mode of RNA synthesis (step 4), moving rightward along the DNA in this diagram. During the elongation mode (step 5), transcription is highly processive, with the polymerase leaving the DNA template and releasing the newly transcribed RNA only when it encounters a termination signal (steps 6 and 7). Termination signals are typically encoded in DNA, and many function by forming an RNA structure that destabilizes the polymerase’s hold on the RNA (step 7). In bacteria, all RNA molecules are synthesized by a single type of RNA polymerase and the cycle depicted in the figure therefore applies to the production of mRNAs as well as structural and catalytic RNAs. (Adapted from a figure courtesy of Robert Landick.)

Промоторы прокариот

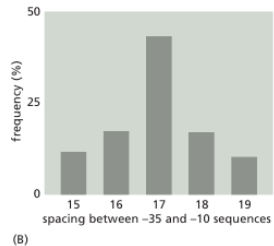
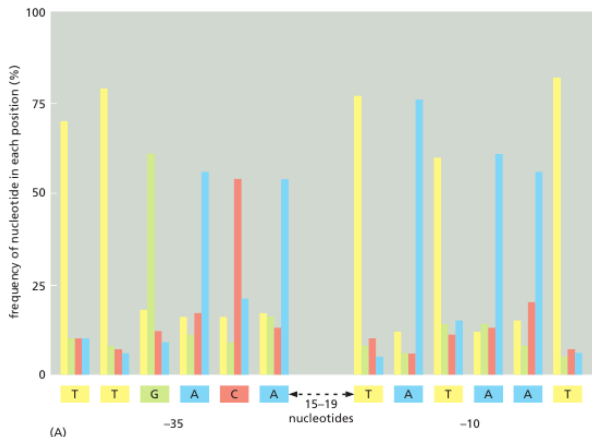


Figure 6-12 Consensus sequence for the major class of *E. coli* promoters. (A) The promoters are characterized by two hexameric DNA sequences, the -35 sequence and the -10 sequence named for their approximate location relative to the start point of transcription (designated +1). For convenience, the nucleotide sequence of a single strand of DNA is shown; in reality the RNA polymerase recognizes the promoter as double-stranded DNA. On the basis of a

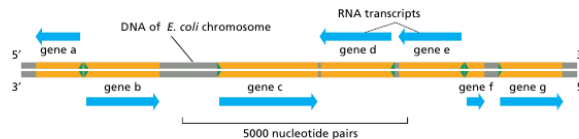
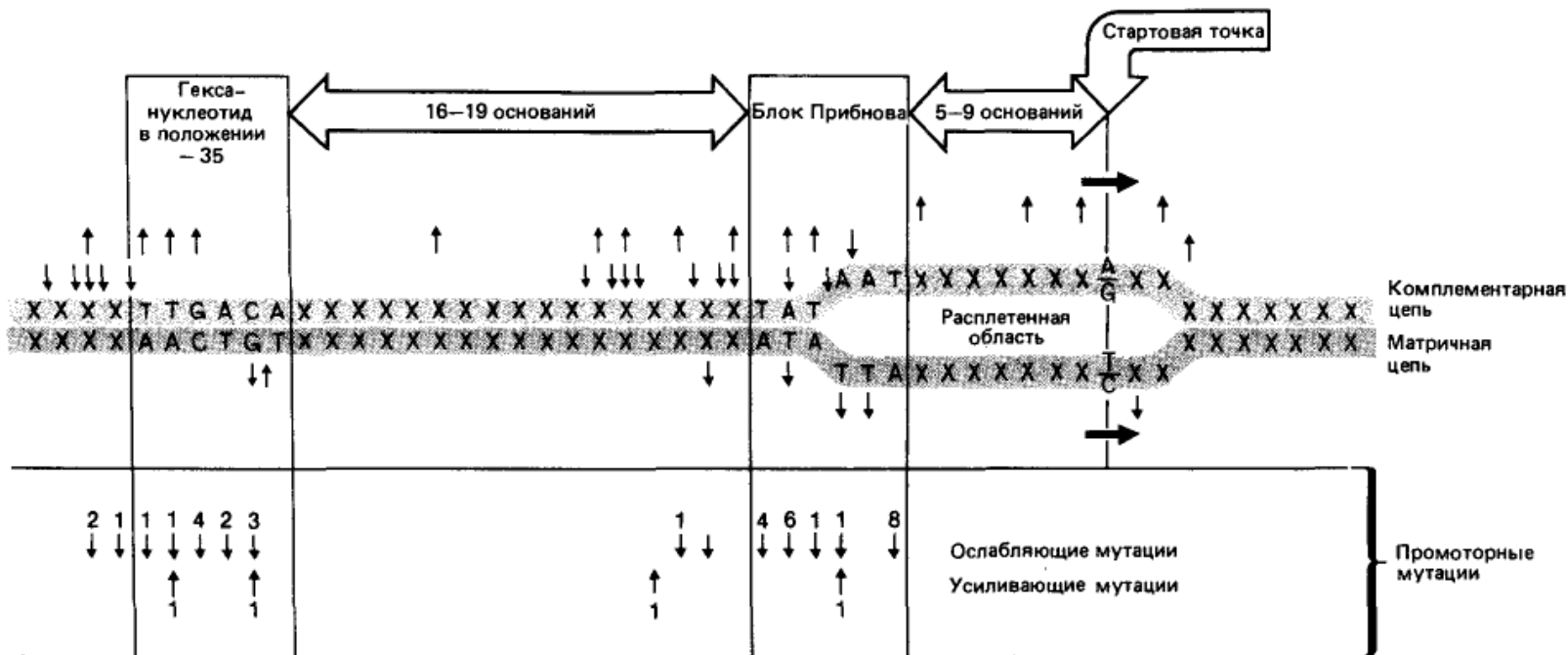


Figure 6-14 Directions of transcription along a short portion of a bacterial chromosome. Some genes are transcribed using one DNA strand as a template, while others are transcribed using the other DNA strand. The direction of transcription is determined by the promoter at the beginning of each gene (green arrowheads). This diagram shows approximately 0.2% (9000 base pairs) of the *E. coli* chromosome. The genes transcribed from left to right use the bottom DNA strand as the template; those transcribed from right to left use the top strand as the template.



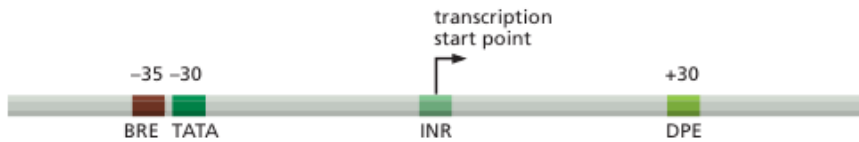
Полимеразы эукариот

Table 6–2 The Three RNA Polymerases in Eucaryotic Cells

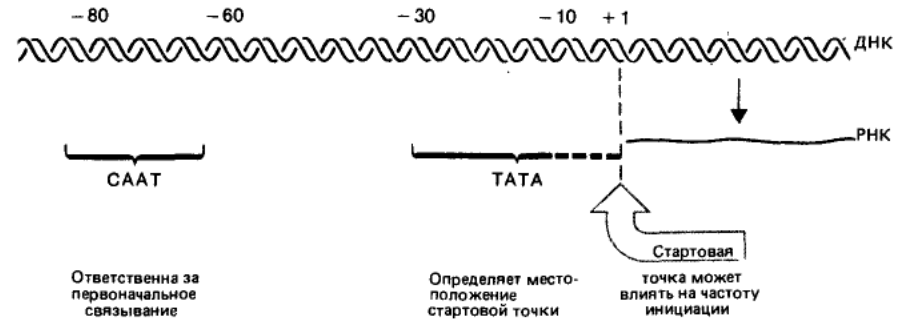
TYPE OF POLYMERASE	GENES TRANSCRIBED
RNA polymerase I	5.8S, 18S, and 28S rRNA genes
RNA polymerase II	all protein-coding genes, plus snoRNA genes, miRNA genes, siRNA genes, and most snRNA genes
RNA polymerase III	tRNA genes, 5S rRNA genes, some snRNA genes and genes for other small RNAs

The rRNAs are named according to their "S" values, which refer to their rate of sedimentation in an ultracentrifuge. The larger the S value, the larger the rRNA.

Точка старта транскрипции у эукариот



element	consensus sequence	general transcription factor
BRE	G/C G/C G/A C G C C	TFIIB
TATA	T A T A A/T A A/T	TBP
INR	C/T C/T A N T/A C/T C/T	TFIID
DPE	A/G G A/T C G T G	TFIID



Консенсусные последовательности промоторов эукариот. Для многих РНК-полимераз II в точке начала транскрипции присутствуют 2-3 из данных 4 последовательностей. *N-любой, **или/или с равной вероятностью

Table 6-3 The General Transcription Factors Needed for Transcription Initiation by Eucaryotic RNA Polymerase II

NAME	NUMBER OF SUBUNITS	ROLES IN TRANSITION INITIATION
TFIID		
TBP subunit	1	recognizes TATA box
TAF subunits	~11	recognizes other DNA sequences near the transcription start point; regulates DNA-binding by TBP
TFIIB	1	recognizes BRE element in promoters; accurately positions RNA polymerase at the start site of transcription
TFIIF	3	stabilizes RNA polymerase interaction with TBP and TFIIB; helps attract TFIIE and TFIIH
TFIIE	2	attracts and regulates TFIIH
TFIIH	9	unwinds DNA at the transcription start point, phosphorylates Ser5 of the RNA polymerase CTD; releases RNA polymerase from the promoter

TFIID is composed of TBP and ~11 additional subunits called TAFs (TBP-associated factors); CTD, C-terminal domain.

Инициация транскрипции РНК-полимеразой II у эукариот

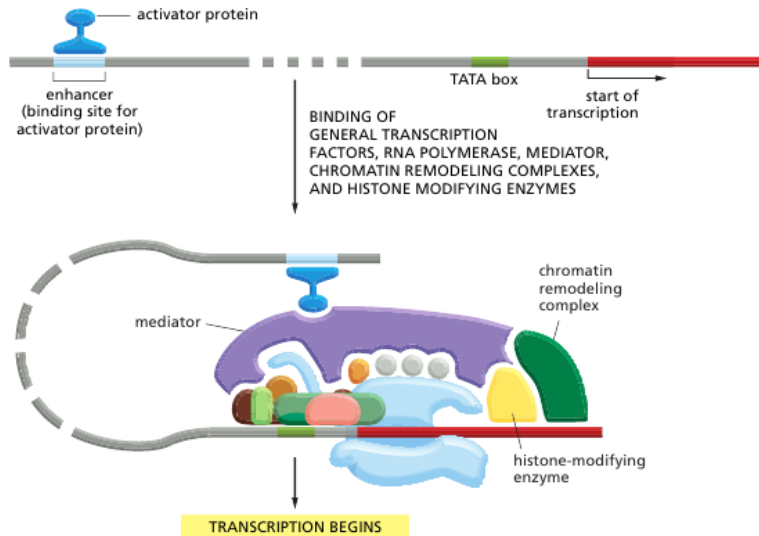
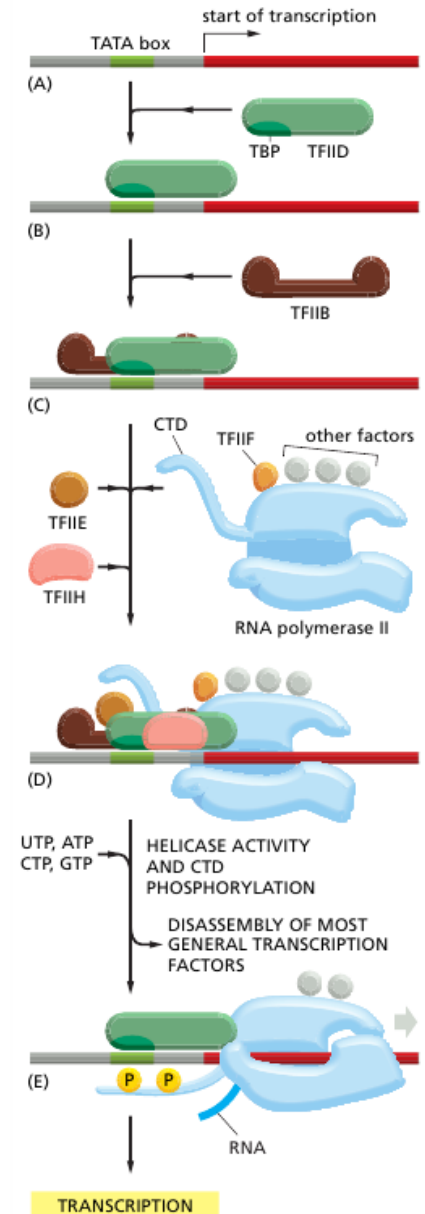
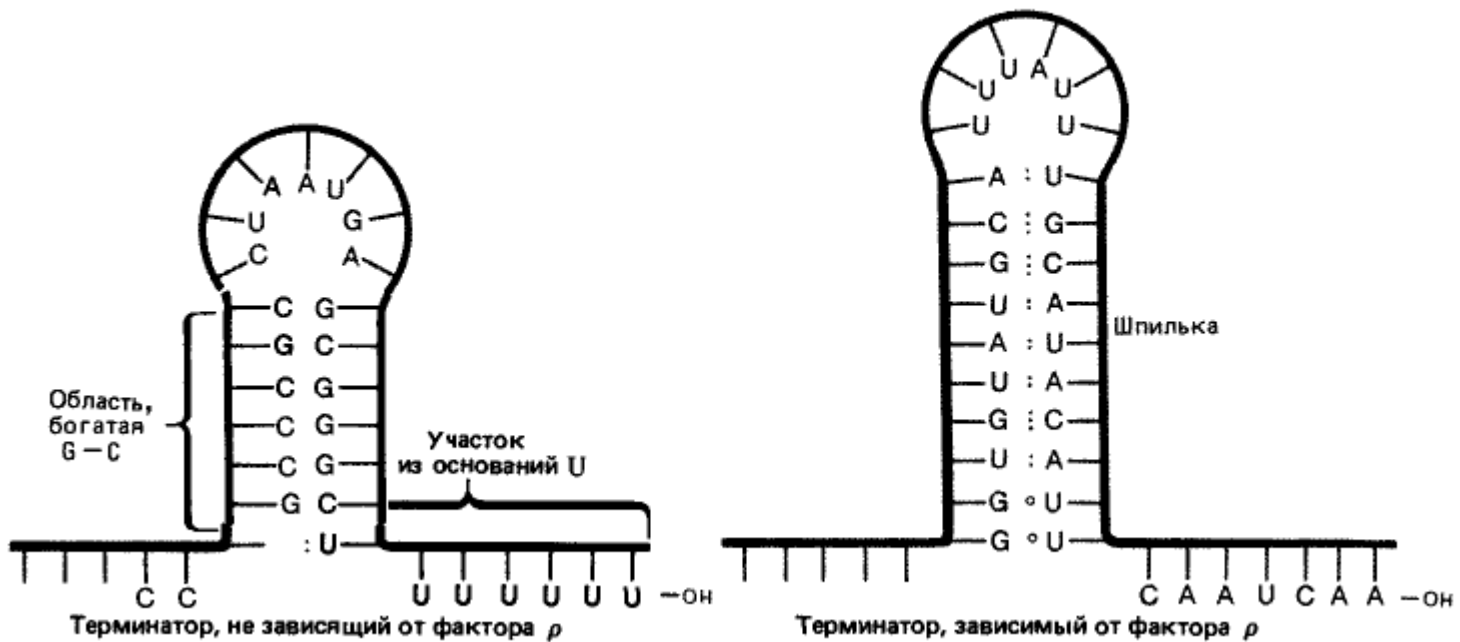


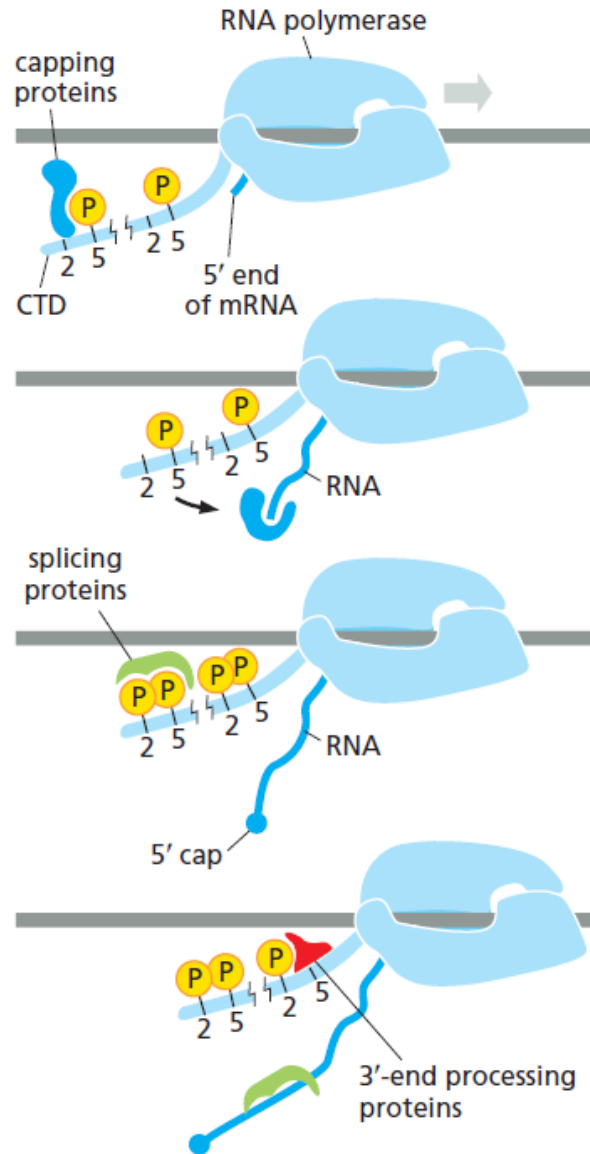
Figure 6–19 Transcription initiation by RNA polymerase II in a eucaryotic cell. Transcription initiation *in vivo* requires the presence of transcriptional activator proteins. As described in Chapter 7, these proteins bind to specific short sequences in DNA. Although only one is shown here, a typical eucaryotic gene has many activator proteins, which together determine its rate and pattern of transcription. Sometimes acting from a distance of several thousand nucleotide pairs (indicated by the dashed DNA molecule), these gene regulatory proteins help RNA polymerase, the general transcription factors, and the mediator all to assemble at the promoter. In addition, activators attract ATP-dependent chromatin remodeling complexes and histone acetylases. As discussed in Chapter 4, the “default” state of chromatin is probably the 30-nm filament (see Figure 4–22), and this is likely to be a form of DNA upon which transcription is initiated. For simplicity, it is not shown in the figure.



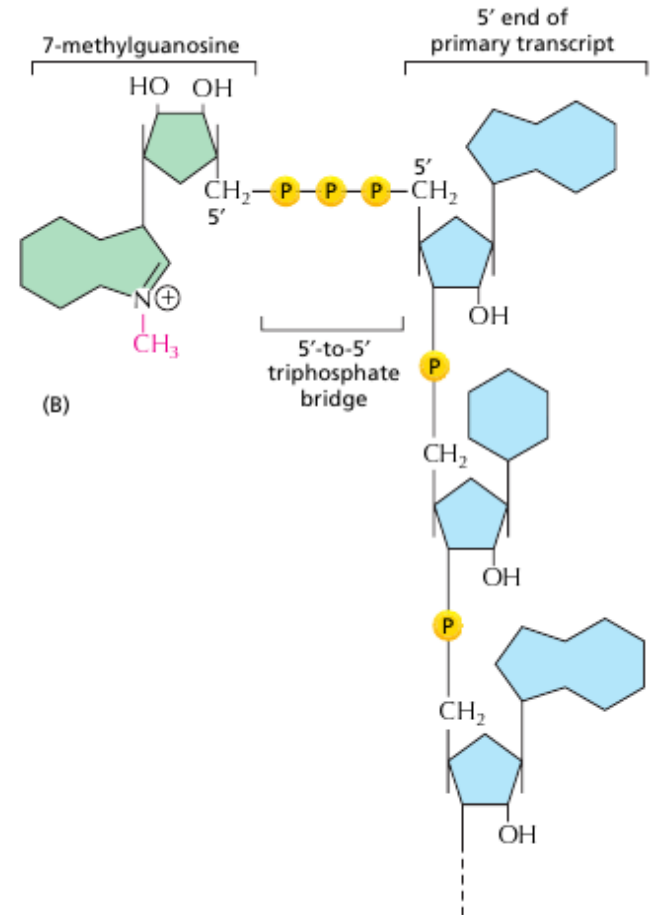
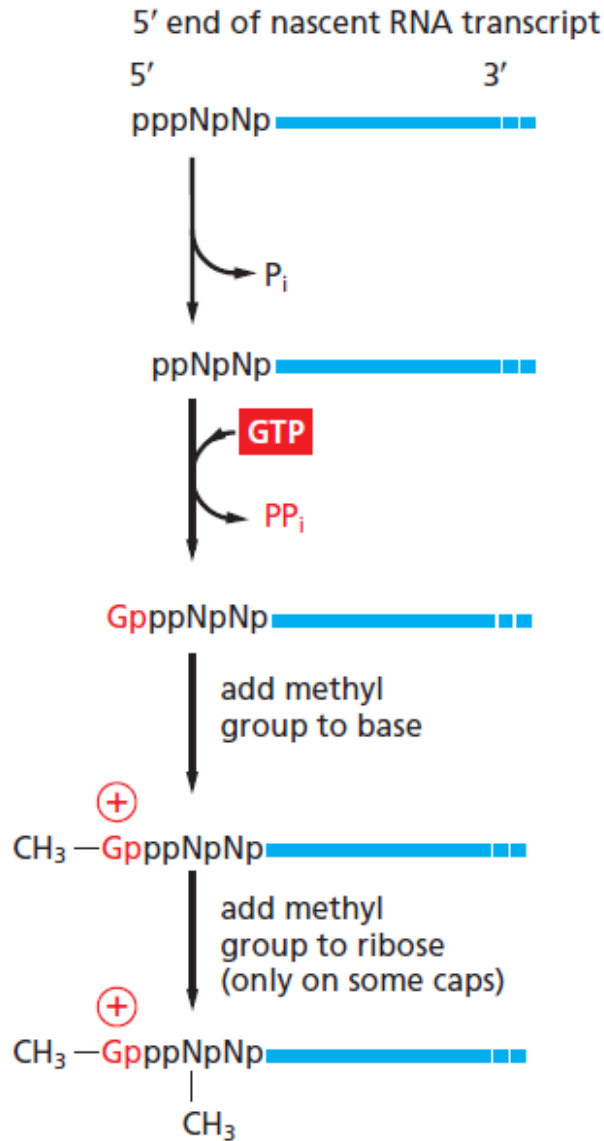
Структура терминаторов прокариот



Посттрансляционные модификации РНК эукариот



«Кэпирование» РНК



Сплайсинг мРНК эукариот

Экзоны: 150-200 bp

Инторыны: 40-1000 bp

Левая граница



Правая граница



Экзон ... A G G T A A G T ... Интрон ... 6Pu N C A G ... Экзон



Alternative Splicing

mRNA



Protein A



Protein B

Процессинг 3'-конца мРНК. ПолиА

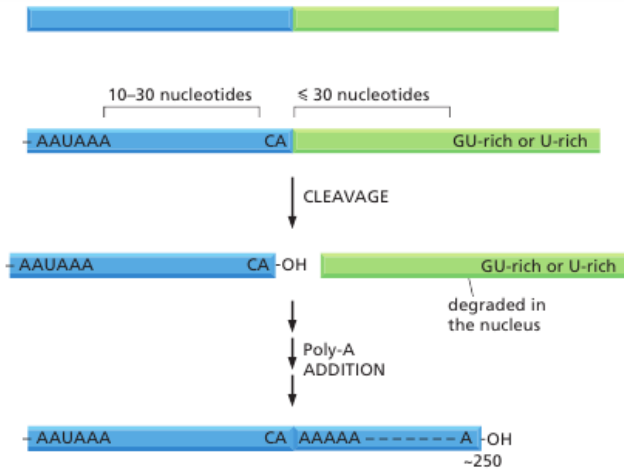
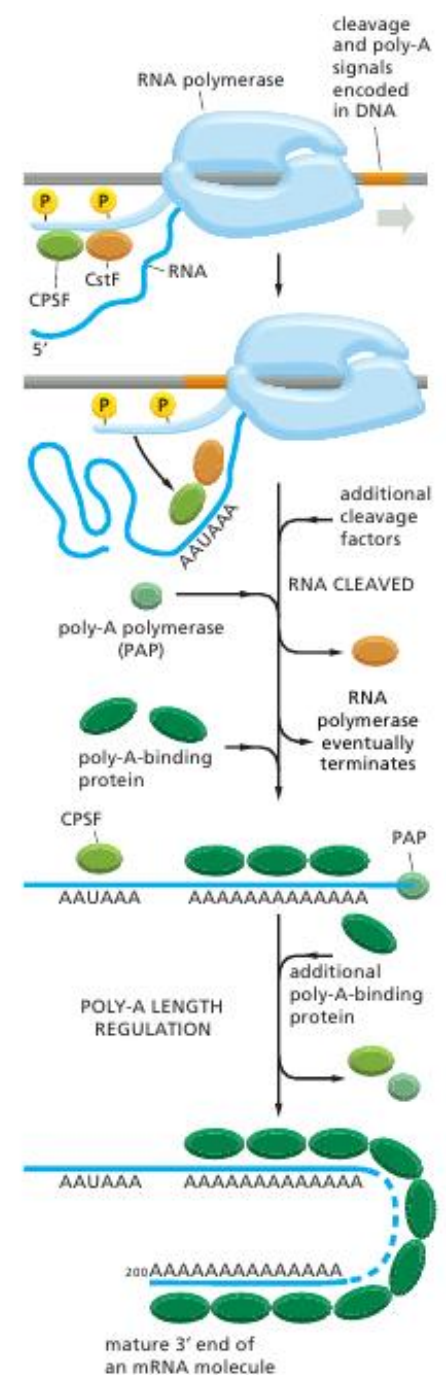


Figure 6-37 Consensus nucleotide sequences that direct cleavage and polyadenylation to form the 3' end of a eucaryotic mRNA. These sequences are encoded in the genome; specific proteins recognize them after they are transcribed into RNA. The hexamer AAUAAA is bound by CPSF, the GU-rich element beyond the cleavage site is bound by CstF (see Figure 6-38), and the CA sequence is bound by a third factor required for the cleavage step. Like other consensus nucleotide sequences discussed in this chapter (see Figure 6-12), the sequences shown in the figure represent a variety of individual cleavage and polyadenylation signals.



Сайты сплайсинга в интронах

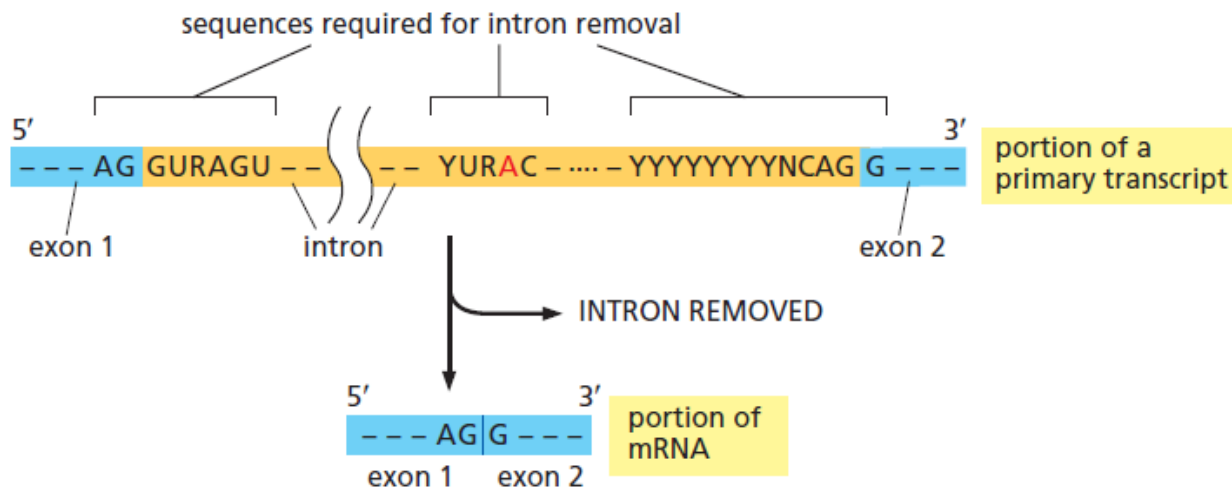
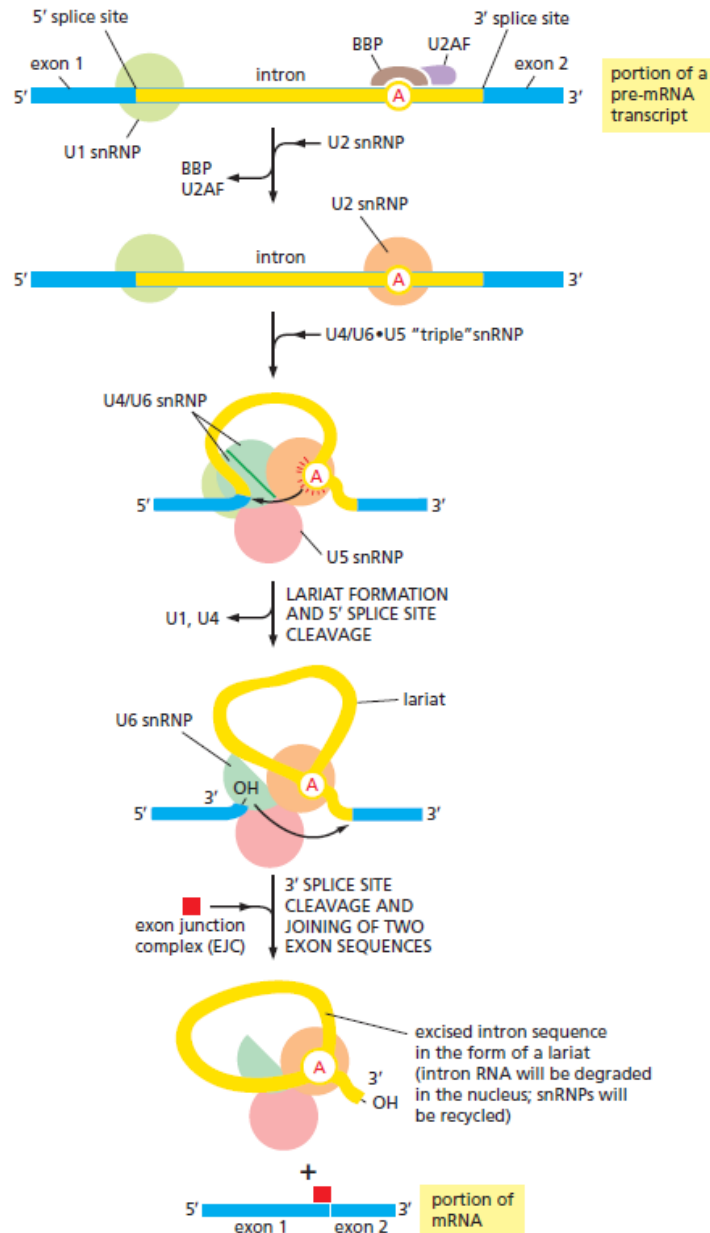


Figure 6–27 The consensus nucleotide sequences in an RNA molecule that signal the beginning and the end of most introns in humans. The three blocks of nucleotide sequences shown are required to remove an intron sequence. Here A, G, U, and C are the standard RNA nucleotides; R stands for purines (A or G); and Y stands for pyrimidines (C or U). The A highlighted in red forms the branch point of the lariat produced by splicing (see Figure 6–25). Only the GU at the start of the intron and the AG at its end are invariant nucleotides in the splicing consensus sequences. Several different nucleotides can occupy the remaining positions, although the indicated nucleotides are preferred. The distances along the RNA between the three splicing consensus sequences are highly variable; however, the distance between the branch point and 3' splice junction is typically much shorter than that between the 5' splice junction and the branch point.

Консенсусные последовательности молекулы РНК, маркирующие начало и конец интронов.

R – пурин (puRines) Y – пиримидин (pYrimidines) A – точка ветвления для формирования петли

Сплайсинг пре-мРНК



portion of a pre-mRNA transcript

The U1 snRNP forms base pairs with the 5' splice junction (see Figure 6–29) and the BBP (branch-point binding protein) and U2AF (U2 auxiliary factor) recognize the branch-point site.

The U2 snRNP displaces BBP and U2AF and forms base pairs with the branch-point site consensus sequence.

The U4/U6•U5 "triple" snRNP enters the reaction. In this triple snRNP, the U4 and U6 snRNAs are held firmly together by base-pair interactions. Subsequent rearrangements break apart the U4/U6 base pairs, allowing U6 to displace U1 at the 5' splice junction (see Figure 6–29). This creates the active site that catalyzes the first phosphoryl-transferase reaction.

Additional RNA–RNA rearrangements create the active site for the second phosphoryl-transferase reaction, which then completes the splice (see Figure 6–25A).

Figure 6–28 The pre-mRNA splicing mechanism. RNA splicing is catalyzed by an assembly of snRNPs (shown as *colored circles*) plus other proteins (most of which are not shown), which together constitute the spliceosome. The spliceosome recognizes the splicing signals on a pre-mRNA molecule, brings the two ends of the intron together, and provides the enzymatic activity for the two reaction steps required (see Figure 6–25A and *Movie 6.5*). As indicated, a set of proteins called the exon junction complex (EJC) remains on the spliced mRNA molecule; its subsequent role will be discussed shortly.

Схема сплайсинга пре-мРНК эукариот

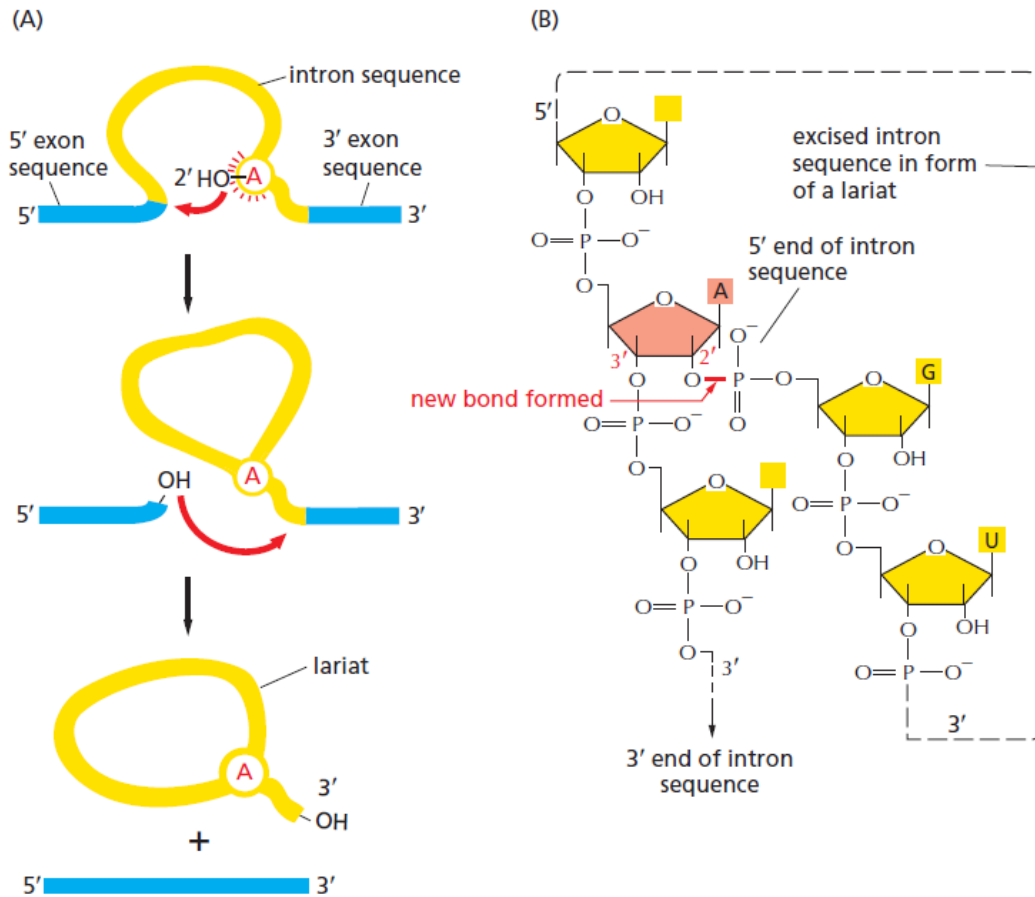
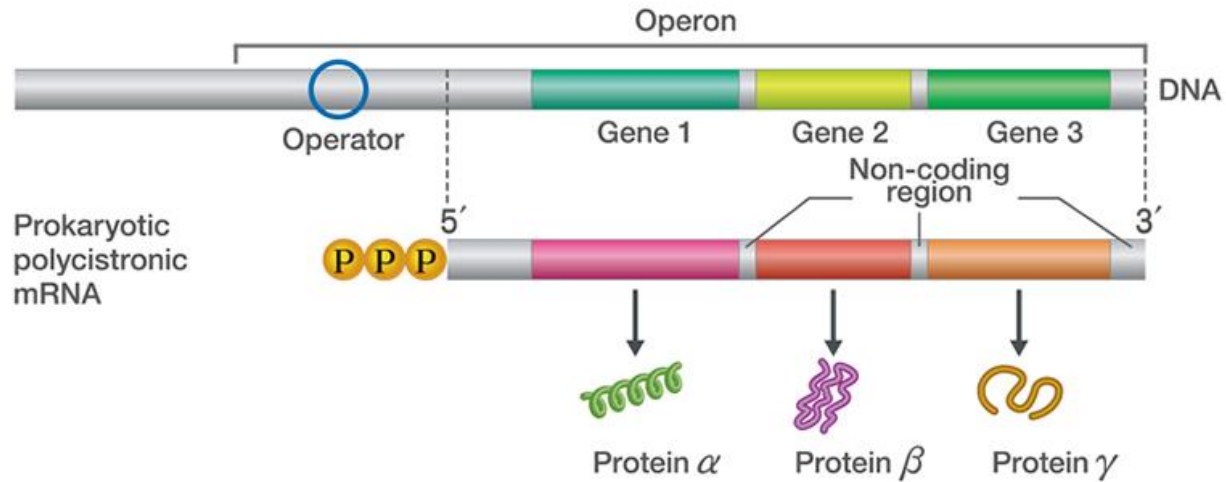


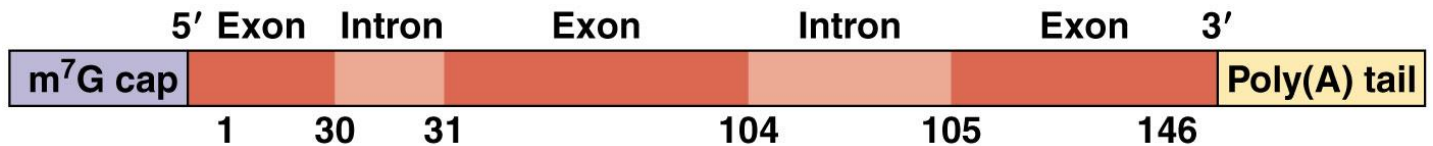
Figure 6-25 The pre-mRNA splicing reaction. (A) In the first step, a specific adenine nucleotide in the intron sequence (indicated in red) attacks the 5' splice site and cuts the sugar-phosphate backbone of the RNA at this point. The cut 5' end of the intron becomes covalently linked to the adenine nucleotide, as shown in detail in (B), thereby creating a loop in the RNA molecule. The released free 3'-OH end of the exon sequence then reacts with the start of the next exon sequence, joining the two exons together and releasing the intron sequence in the shape of a *lariat*. The two exon sequences thereby become joined into a continuous coding sequence. The released intron sequence is eventually broken down into single nucleotides, which are recycled.

Структура мРНК

Полицестронная мРНК
бактерий



Primary transcript (pre-mRNA)



мРНК эукариот



Introns excised and
exons spliced together



Регуляция экспрессии у прокариот

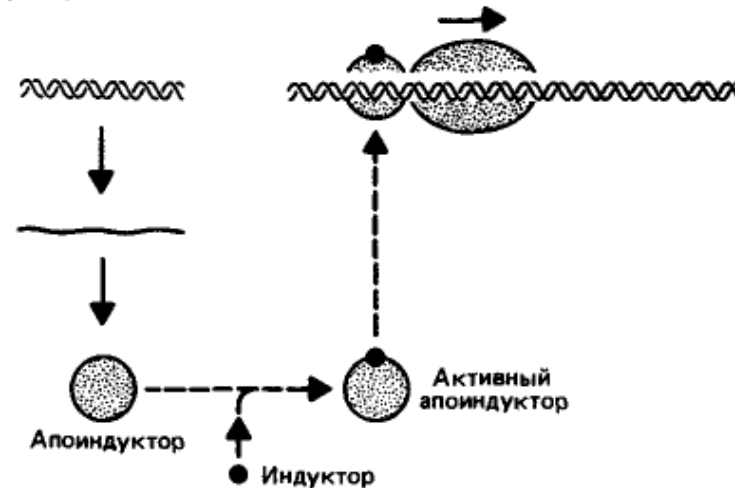
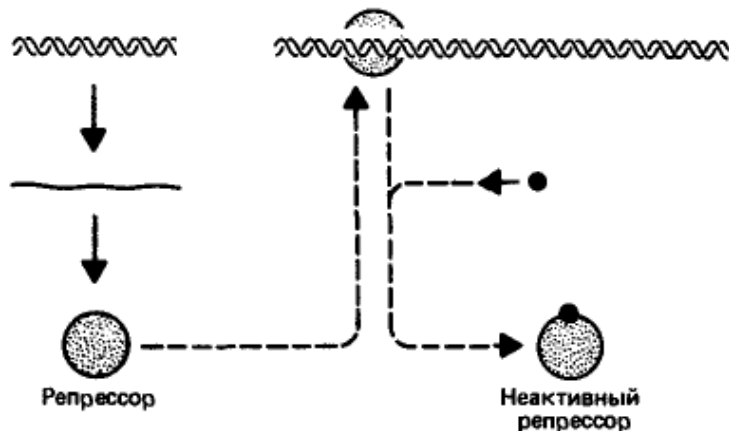
Отрицательная регуляция

Положительная регуляция

Индукция

Делеции в гене репрессора являются рецессивно-конститутивными
Пример: лактозный оперон

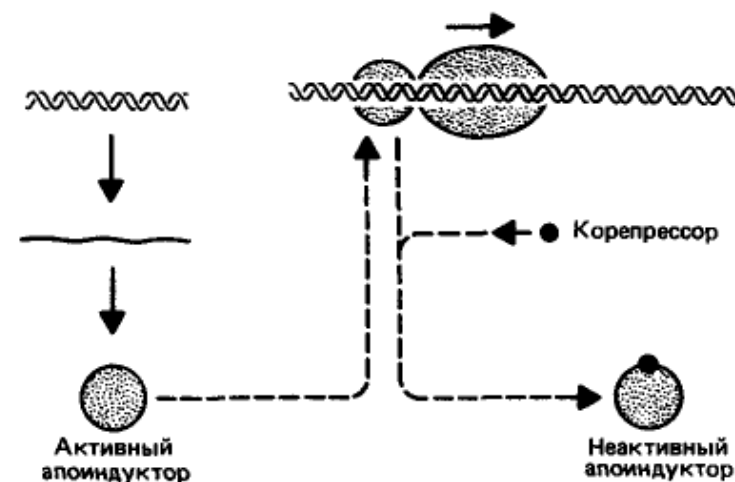
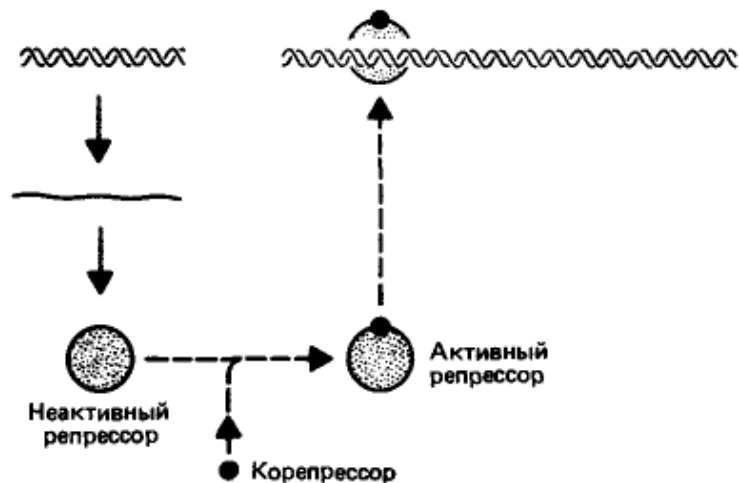
Устранение апоиндуктора ведет к рецессивной
неиндуцибельности оперона
Пример: катаболитная репрессия



Репрессия

Делеции в гене репрессора являются рецессивно-
конститутивными (дерепрессированными)
Пример: триптофановый оперон

Устранение апоиндуктора ведет к рецессивной
суперрепрессии (неиндуцируемости)




Генетический код


	U	C	A	G	
U	UUU → Phe F	UCU → Ser S	UAU → Tyr Y	UGU → Cys C	U
	UUC → Phe F	UCC → Ser S	UAC → Tyr Y	UGC → Cys C	C
	UUA → Leu L	UCA → Ser S	UAA → Stop	UGA → Stop	A
	UUG → Leu L	UCG → Ser S	UAG → Stop	UGG → Trp W	G
C	CUU → Leu L	CCU → Pro P	CAU → His H	CGU → Arg R	U
	CUC → Leu L	CCC → Pro P	CAC → His H	CGC → Arg R	C
	CUA → Leu L	CCA → Pro P	CAA → Gln Q	CGA → Arg R	A
	CUG → Leu L	CCG → Pro P	CAG → Gln Q	CGG → Arg R	G
A	AUU → Ile I	ACU → Thr T	AAU → Asn N	AGU → Ser S	U
	AUC → Ile I	ACC → Thr T	AAC → Asn N	AGC → Ser S	C
	AUA → Ile I	ACA → Thr T	AAA → Lys K	AGA → Arg R	A
	AUG → Met M	ACG → Thr T	AAG → Lys K	AGG → Arg R	G
G	GUU → Val V	GCU → Ala A	GAU → Asp D	GGU → Gly G	U
	GUC → Val V	GCC → Ala A	GAC → Asp D	GGC → Gly G	C
	GUA → Val V	GCA → Ala A	GAA → Glu E	GGA → Gly G	A
	GUG → Val V	GCG → Ala A	GAG → Glu E	GGG → Gly G	G


 translation start codon

 translation stop codon

 hydrophobic amino acids

 hydrophilic non-charged amino acids

 negatively charged amino acids

 positively charged amino acids

 cysteine

Рекогниция – подготовка аминокислот

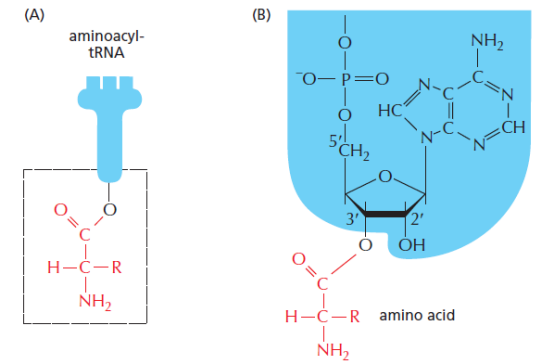
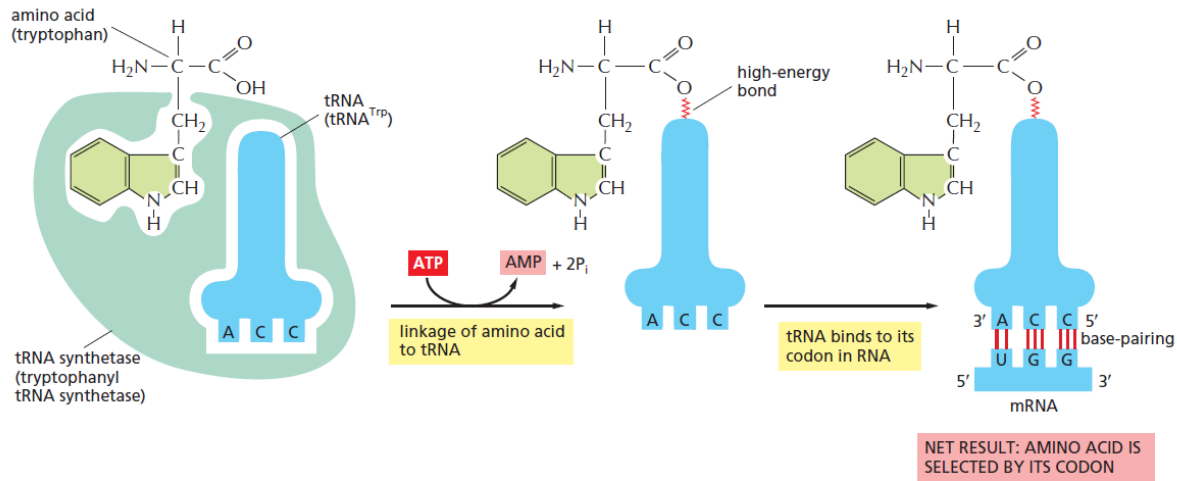
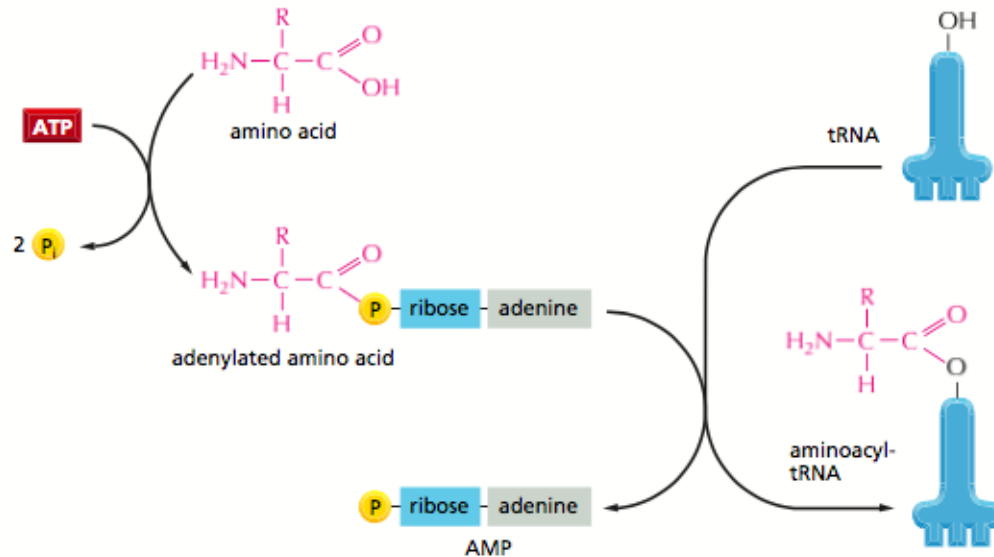
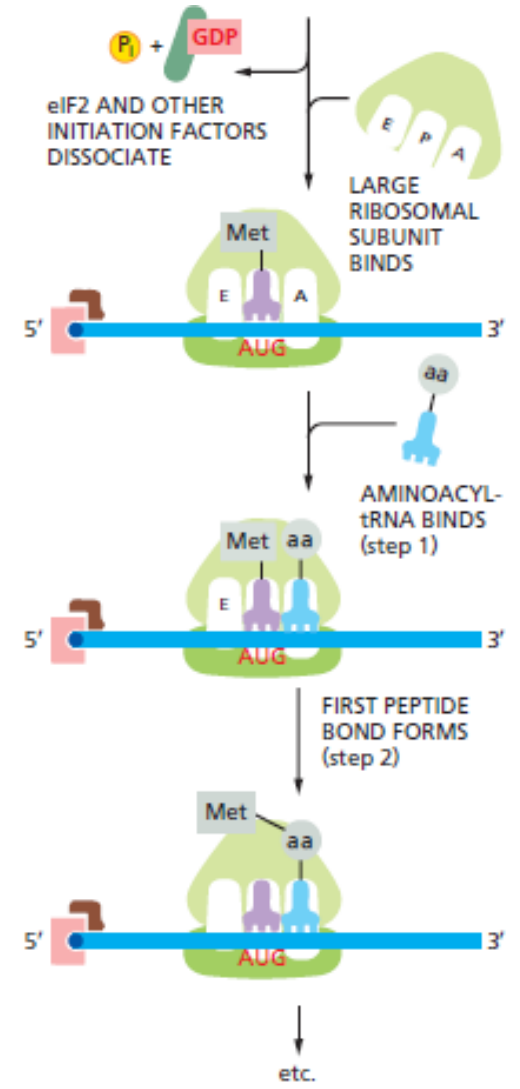
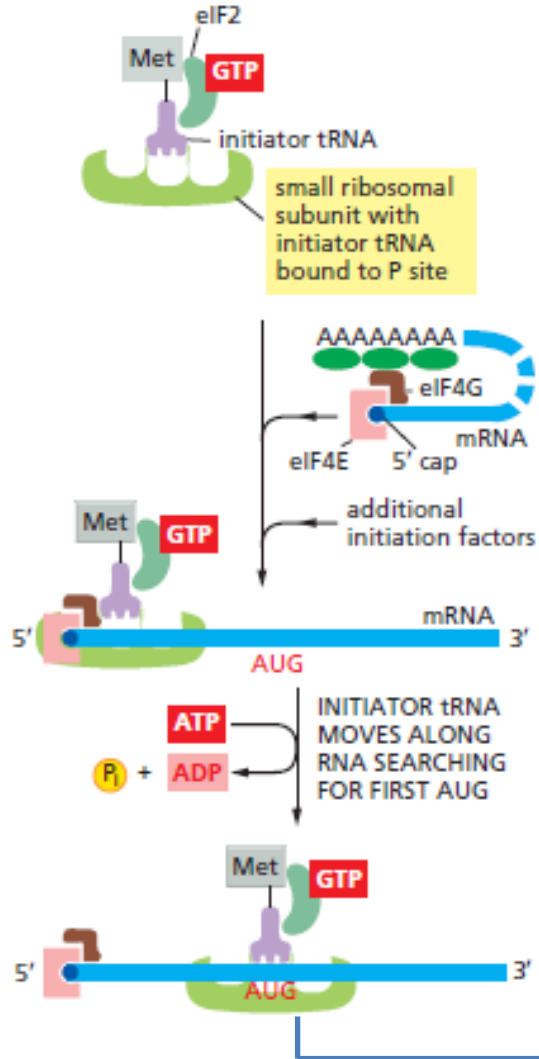
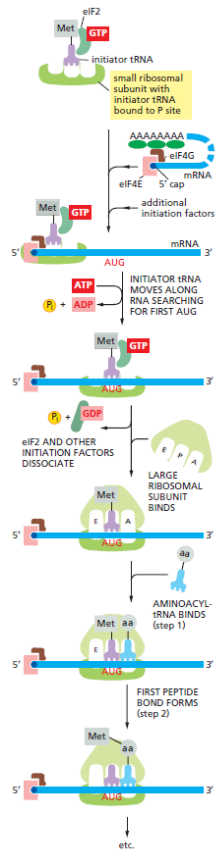


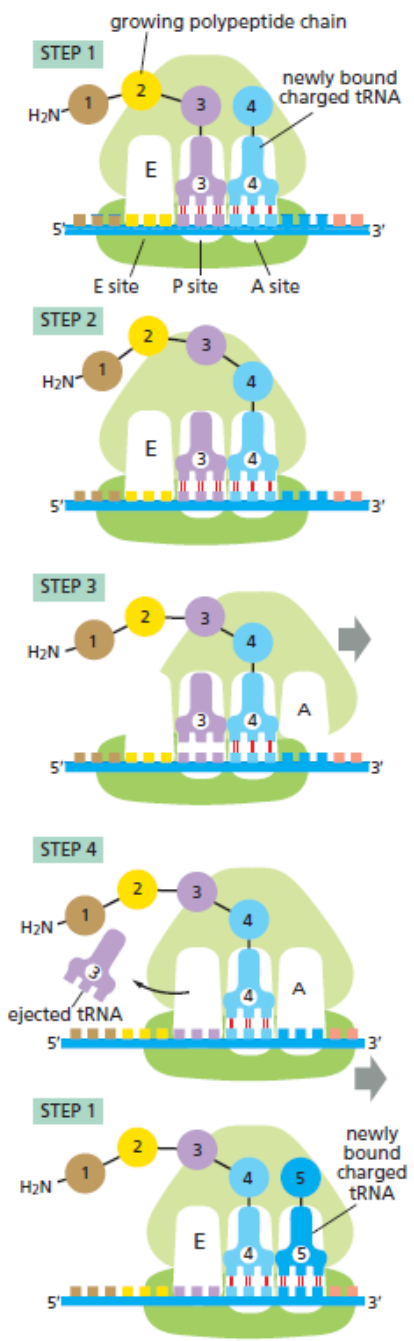
Figure 6–56 The genetic code is translated by means of two adaptors that act one after another. The first adaptor is the aminoacyl-tRNA synthetase, which couples a particular amino acid to its corresponding tRNA; the second adaptor is the tRNA molecule itself, whose *anticodon* forms base pairs with the appropriate *codon* on the mRNA. An error in either step would cause the wrong amino acid to be incorporated into a protein chain (*Movie 6.6*). In the sequence of events shown, the amino acid tryptophan (Trp) is selected by the codon UGG on the mRNA.



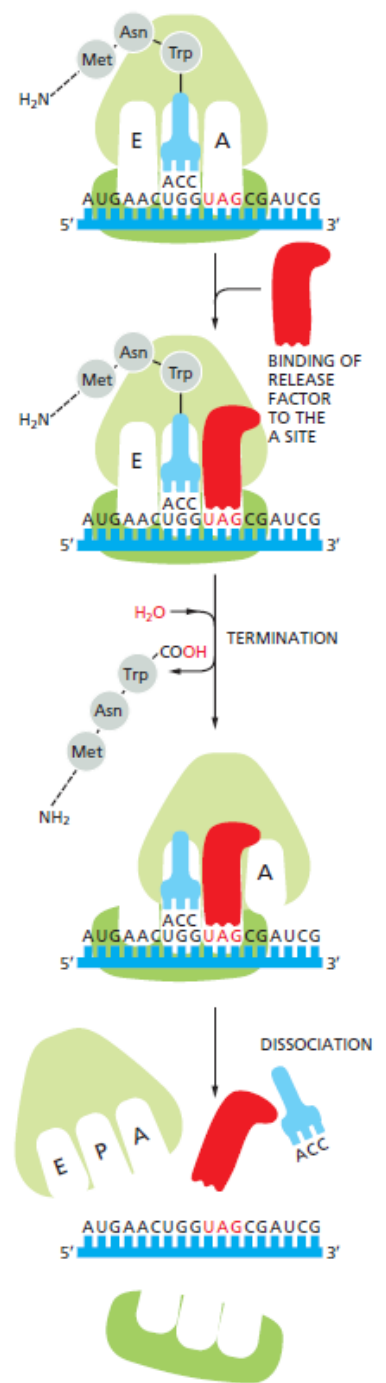
Инициация трансляции



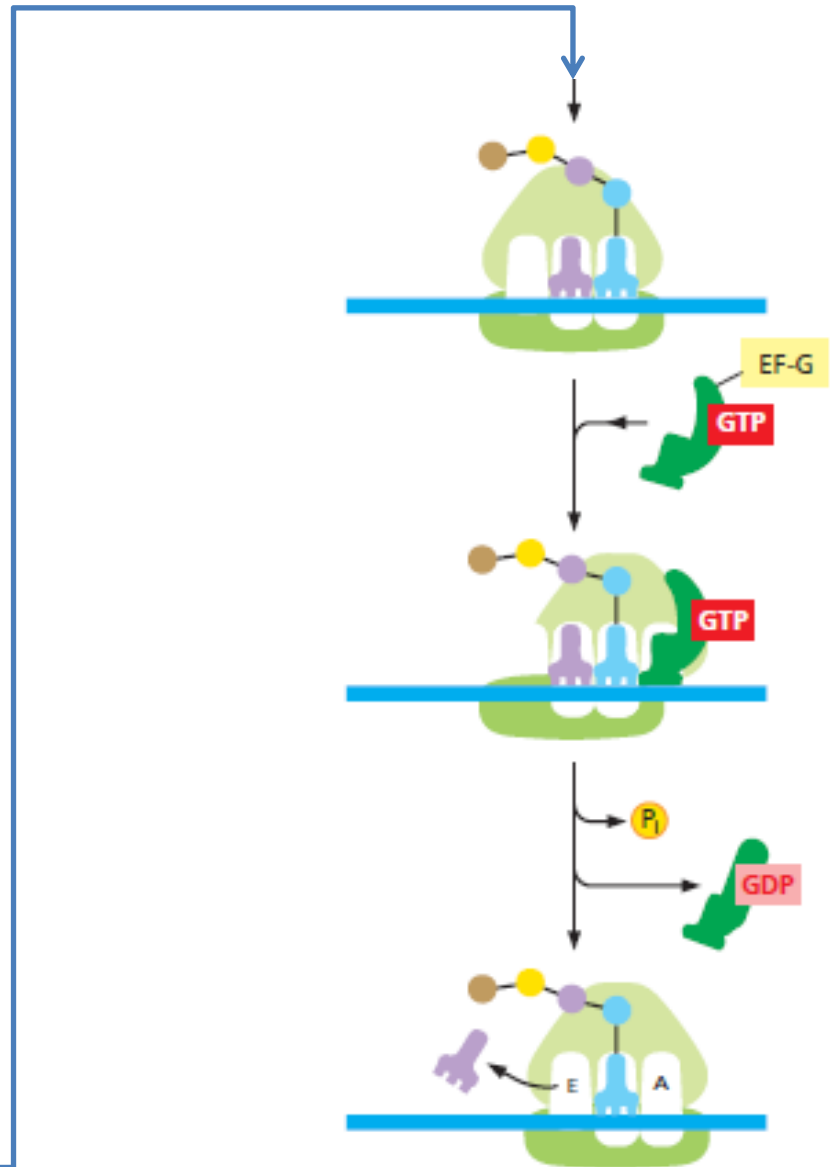
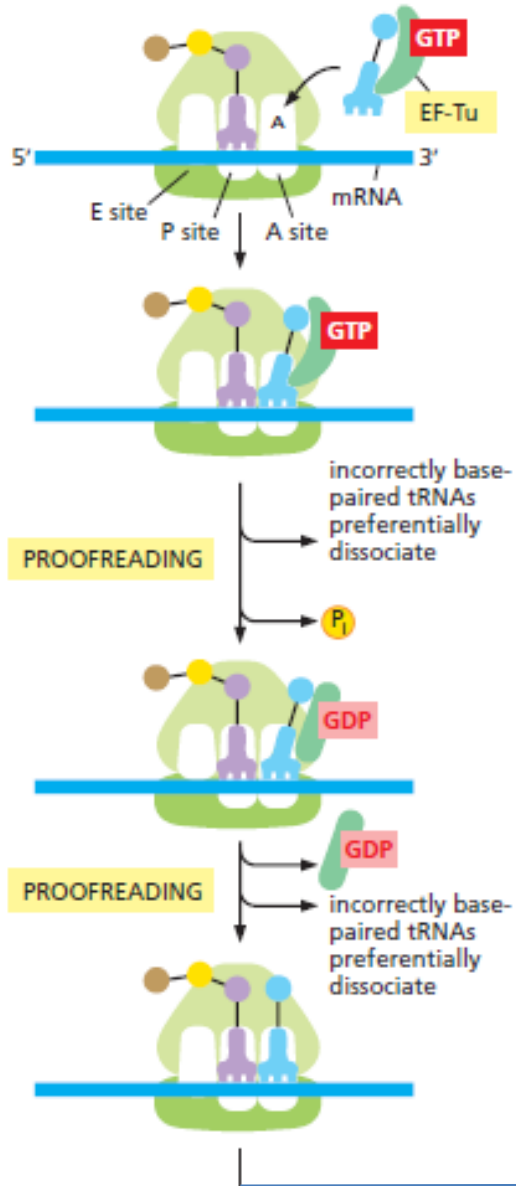
Элонгация трансляции



Терминация трансляции

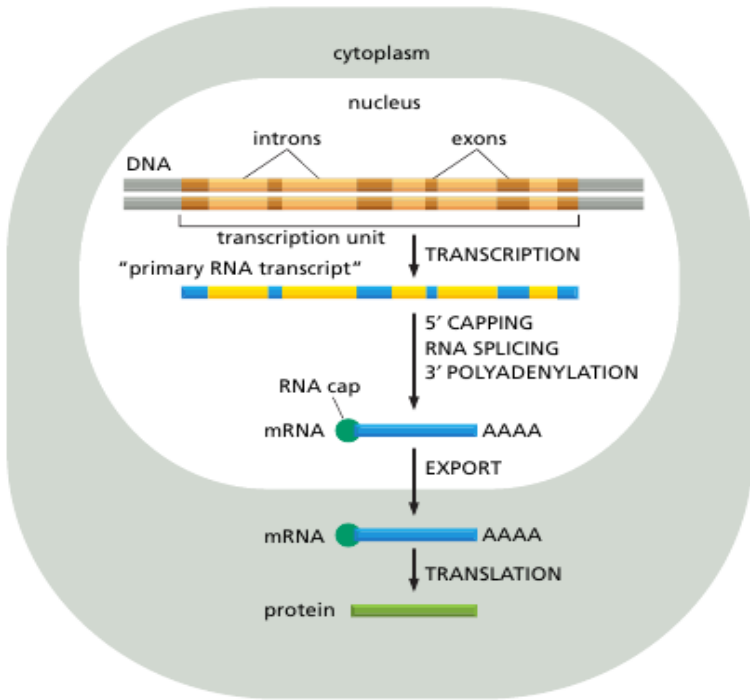


Повышение точности трансляции

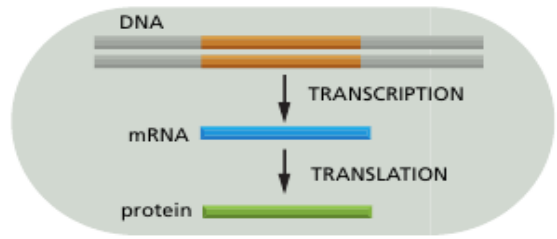


От гена к белку: прокариоты vs эукариоты

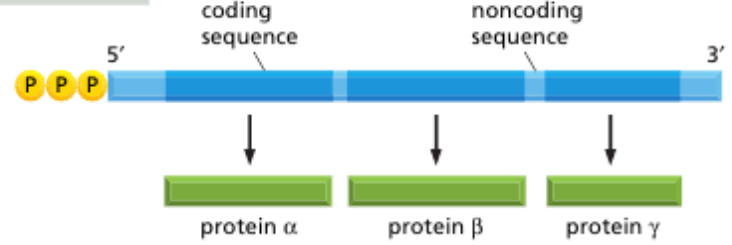
(A) EUCARYOTES



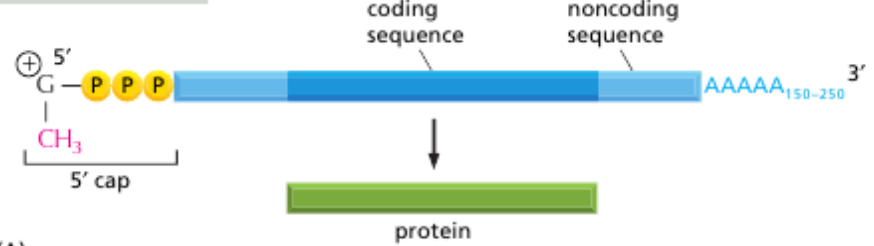
(B) PROCARYOTES



prokaryotic mRNA



eucaryotic mRNA



(A)

Особенности трансляции у про- и эукариот

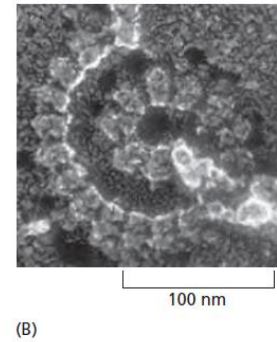
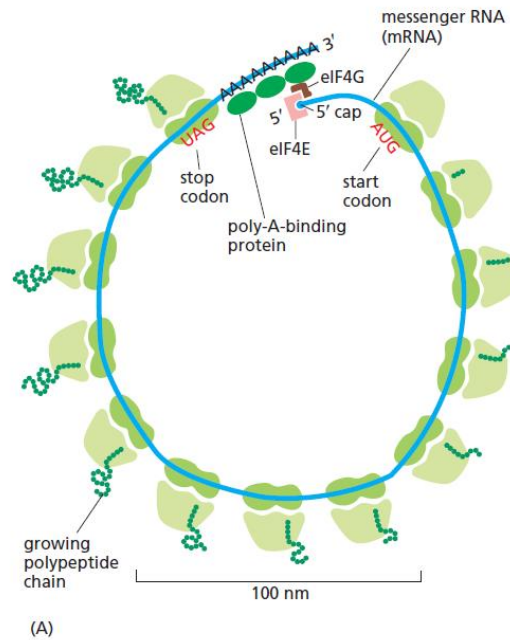


Figure 6-73 A polyribosome. (A) Schematic drawing showing how a series of ribosomes can simultaneously translate the same eukaryotic mRNA molecule. (B) Electron micrograph of a polyribosome from a eukaryotic cell (Movie 6.10). (B, courtesy of John Heuser.)

