Chapter 26.1: RNA metabolism Part II

Transcription

- Common themes of RNA polymerases

- *E. coli* RNA polymerase and sigma factors
  Step-wise control of RNA synthesis

- Eukaryotic RNA polymerases I, II and III
  Many protein factors play a role

DNA-dependent synthesis of RNA

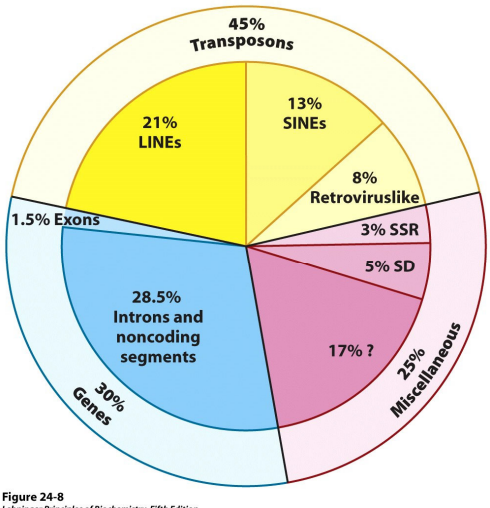

Figure 24-8
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- mRNA: exons + introns

- rRNA, tRNA: translation

- other noncoding RNA:
  regulatory or catalytic
  snRNA, snoRNA,
  miRNA, tmRNA, piRNA,
  etc…

RNA polymerases copy one strand of DNA

Conventions: coding strand, RNA transcript

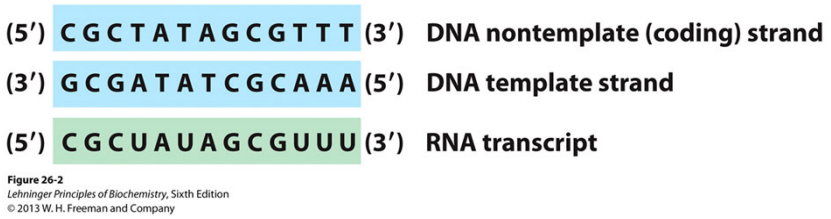

Figure 26-2
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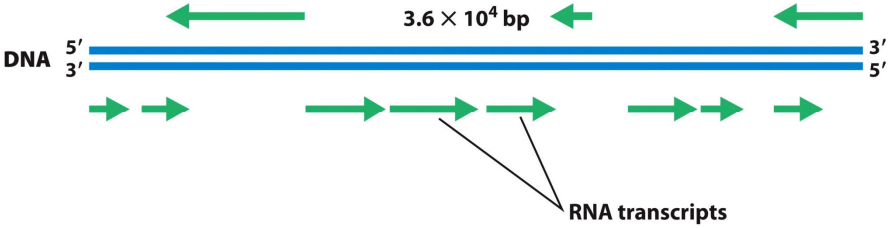
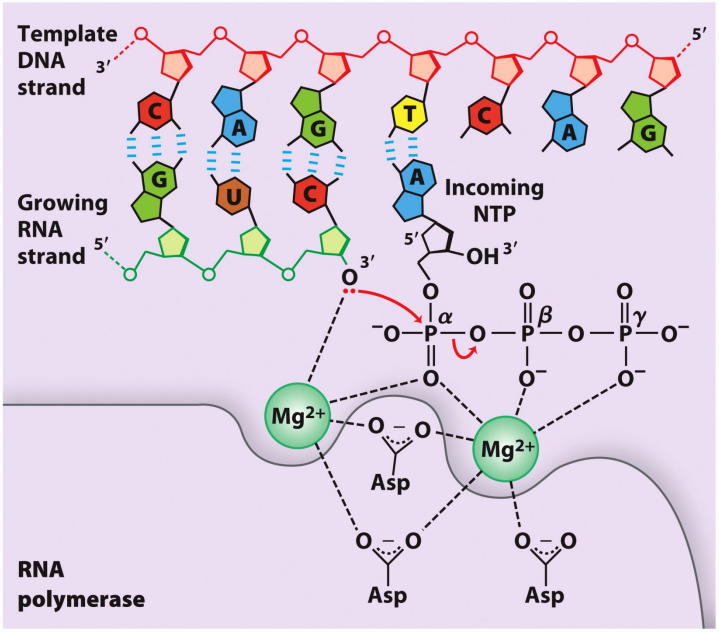
RNA can be transcribed in either direction, often overlapping



Conserved two metal ion mechanism



Differences from DNA polymerases

- RNA polymerases can initiate with free NTP- don't need primers

- Multiple transcripts but limited segment of DNA to be copied

- Only one strand is copied at a time (don't have to worry about semi-discontinuous synthesis)

E. coli RNA polymerase

- Core: 5 subunits ($\alpha_2\beta\beta'\omega$) (plus one σ)

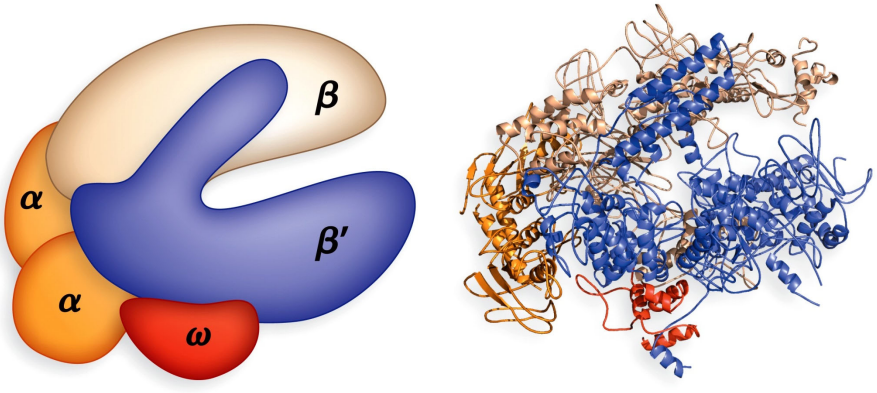


Figure 26-4
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Anatomy of the Transcription bubble in *E. coli*

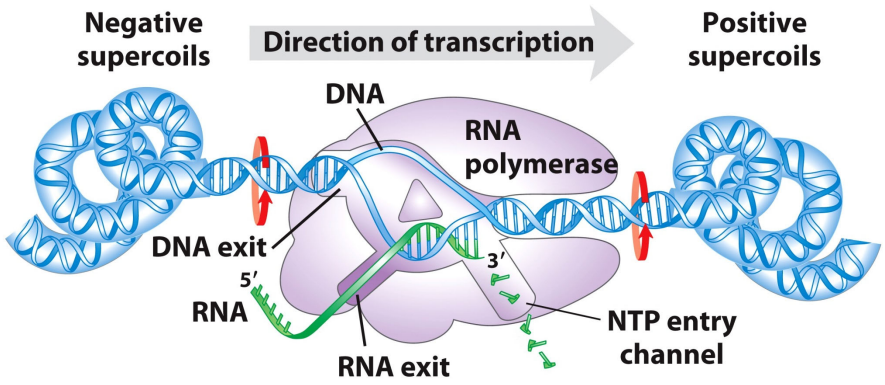

Figure 26-1c
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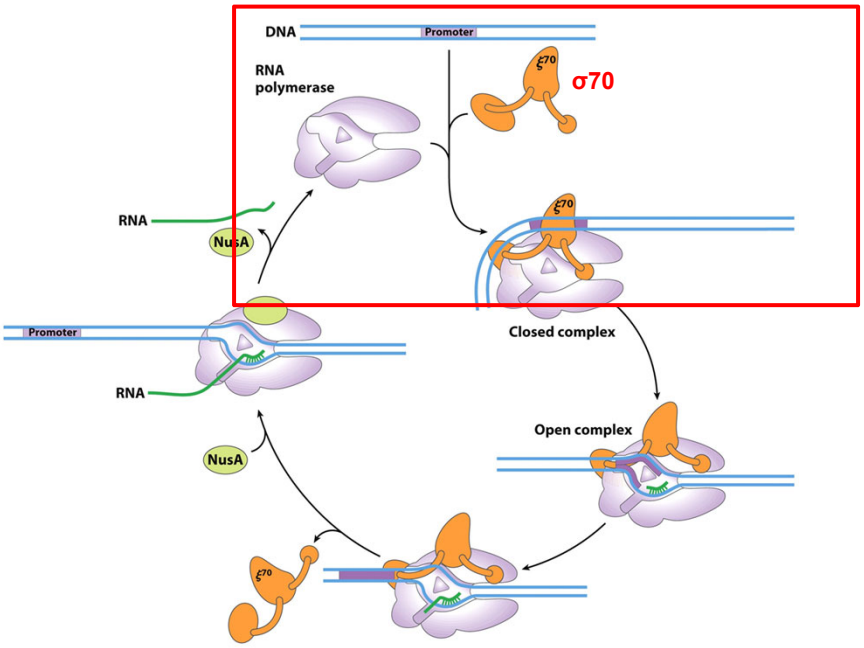
Multiple steps provide points to control



σ^{70} promoter sequences in *E. coli*

Numbering **convention**: transcription start site = +1

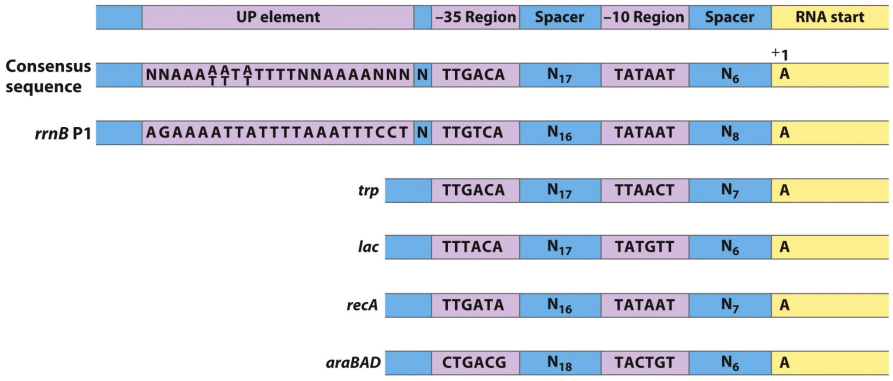


Figure 26-5
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Consensus: most preferred (ideal) sequence, but real promoters vary from this sequence

Sigma subunits recognize a variety of genes

TABLE 26–1 The Seven σ Subunits of *Escherichia coli*

σ subunit	K_d (nM)	Molecules/cell*	Holoenzyme ratio (%)*	Function
σ^{70}	0.26	700	78	Housekeeping
σ^{54}	0.30	110	8	Modulation of cellular nitrogen levels
σ^{38}	4.26	<1	0	Stationary phase genes
σ^{32}	1.24	<10	0	Heat shock genes
σ^{28}	0.74	370	14	Flagella and chemotaxis genes
σ^{24}	2.43	<10	0	Extracytoplasmic functions; some heat shock functions
σ^{18}	1.73	<1	0	Extracytoplasmic functions, including ferric citrate transport

Source: Adapted from Maeda, H., Fujita, N., & Ishihama, A. (2000) *Nucleic Acids Res.* 28, 3500.

Note: σ factors are widely distributed in bacteria; the number varies from a single σ factor in *Mycoplasma genitalium* to 63 distinct σ factors in *Streptomyces coelicolor*.

*Approximate number of each σ subunit per cell and the fraction of RNA polymerase holoenzyme complexed with each σ subunit during exponential growth. The numbers change as growth conditions change. The fraction of RNA polymerase complexed with each σ subunit reflects both the amount of the particular subunit and its affinity for the enzyme.

Table 26-1
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Identification of promoter sequences

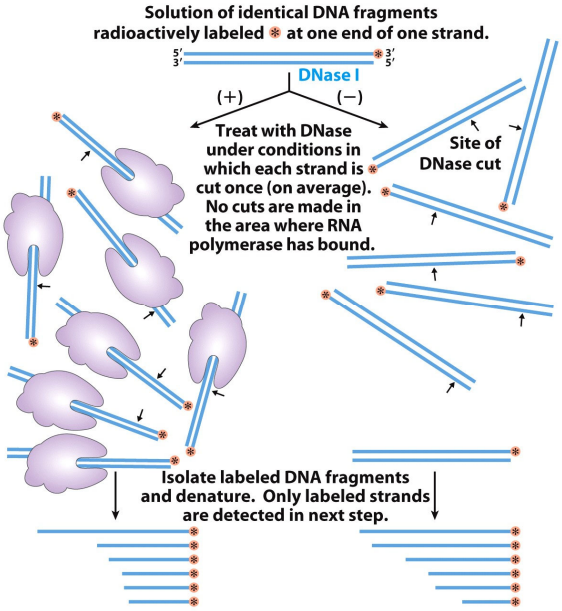
Sequence analysis
- look for most preferred sequences (consensus)

Mutational analysis
- do mutations affect expression?

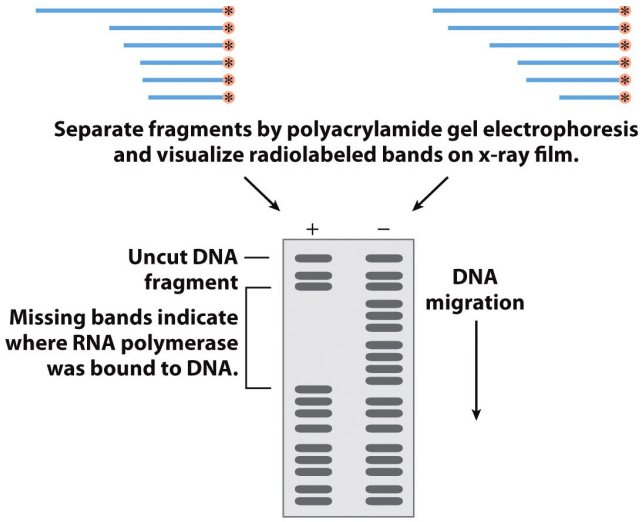
DNA binding and nuclease protection assays
- show that RNAP binds to sequence

Reporter genes: functional in vivo assay
- has to be easy to detect and sensitive
- must be able to quantify (β-Gal, CAT)

Nuclease protection assay (DNA footprinting)



Nuclease protection assay (DNA footprinting)



Box 26-1 Figure 1
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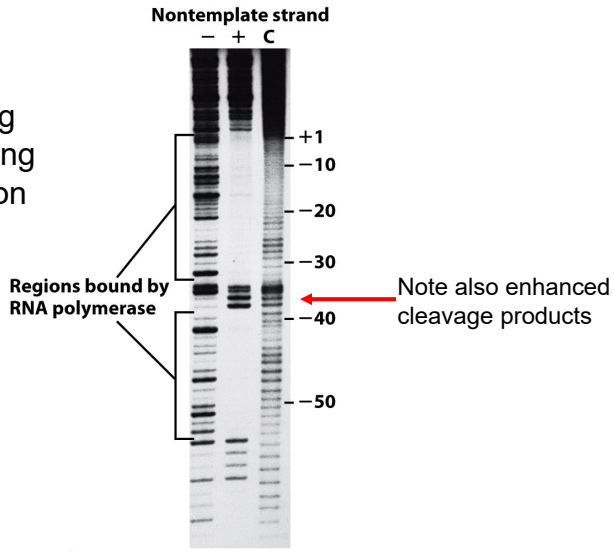
Nuclease protection assay (DNA footprinting)

RNAP is covering 60-80 bp, including -10 and -35 region



Box 26-1 figure 2
Lehninger Principles of Biochemistry, Fifth Edition

Central problem: specificity vs activity

How does RNAP exhibit 10^6-fold specificity for promoter over non-promoter site and then leave?

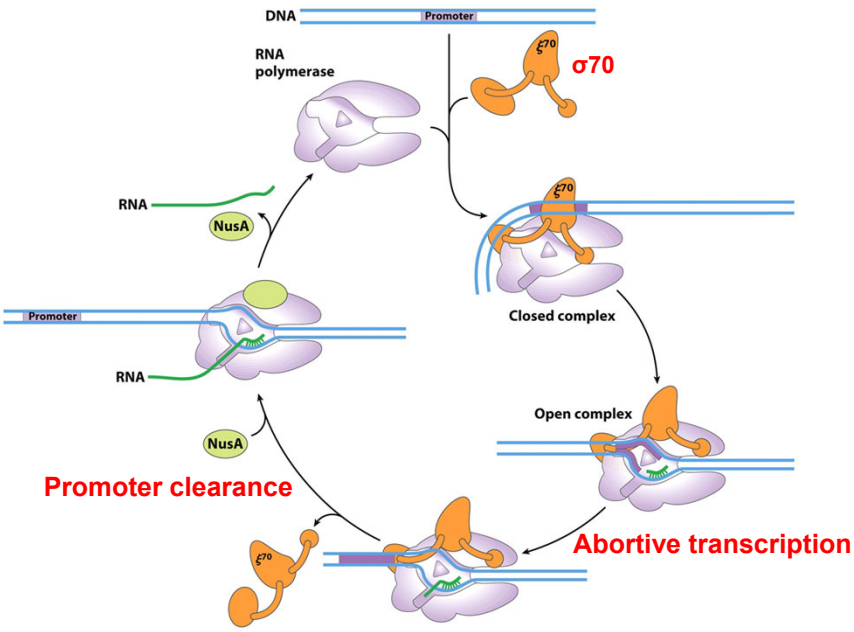
Answer: Steps

- Pol recognizes duplex DNA → closed complex
- Unwinds DNA → open complex (stable)
- Begins transcribing → abortive transcripts
- Transition from initiating pol to elongating pol→ promoter clearance

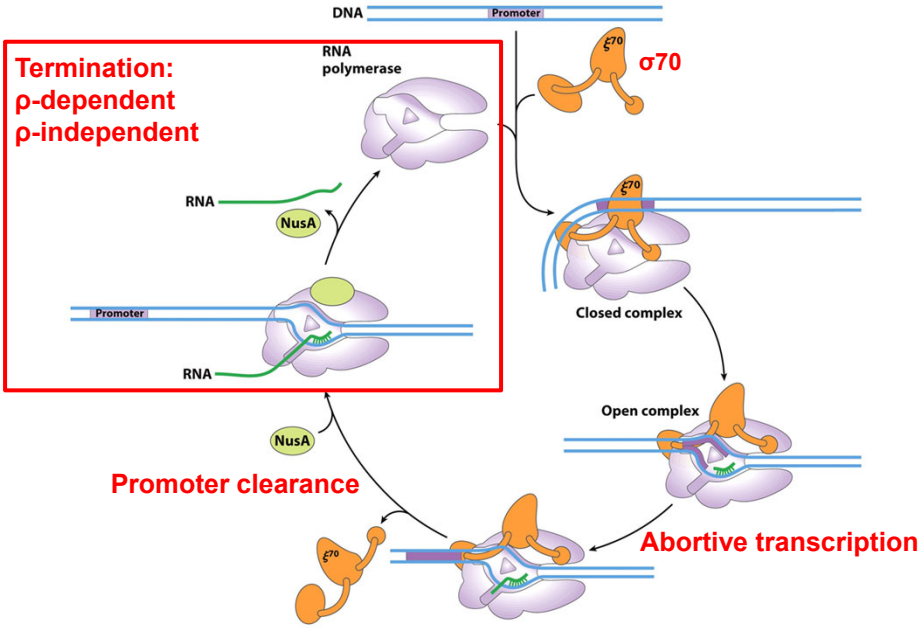
Elongating polymerase

- physical change in structure
- becomes non-specific

Multiple steps provide points to control



Multiple steps provide points to control



ρ-independent termination

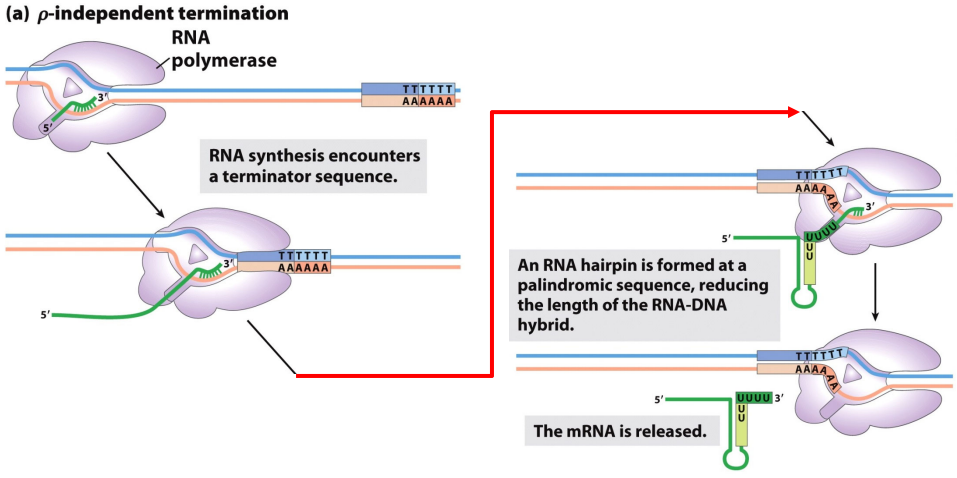


Figure 26-7
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Eukaryotes: three major RNA polymerases

Pol I: rRNA (18S, 5.8S and 28S precursor transcript)

Pol III: tRNA, 5S rRNA, other small RNAs

Pol II: mRNA, snRNA, miRNA

- Similarities to bacterial RNAP, but much more complicated DNA template (chromatin)

- Requires many additional protein factors (transcription factors)

- Still step-wise process: assembly, initiation, elongation, termination

Eukaryotic RNA Pol II promoters

Several types:
- TATA-box, initiator (Inr) (although not most Pol II)
- TATA-less, initiator
- snRNA

How does Pol II recognize a promoter?
- Transcription factors (not Pol subunit like σ) bind to DNA

TBP (TATA binding protein) plays a central role
- even at promoters that don't have a TATA box

TBP induces structural alteration in DNA

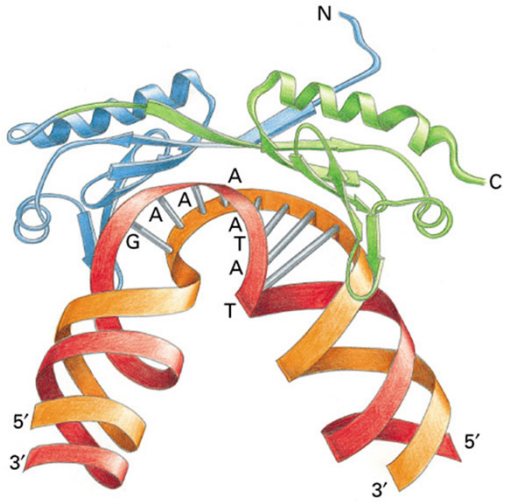
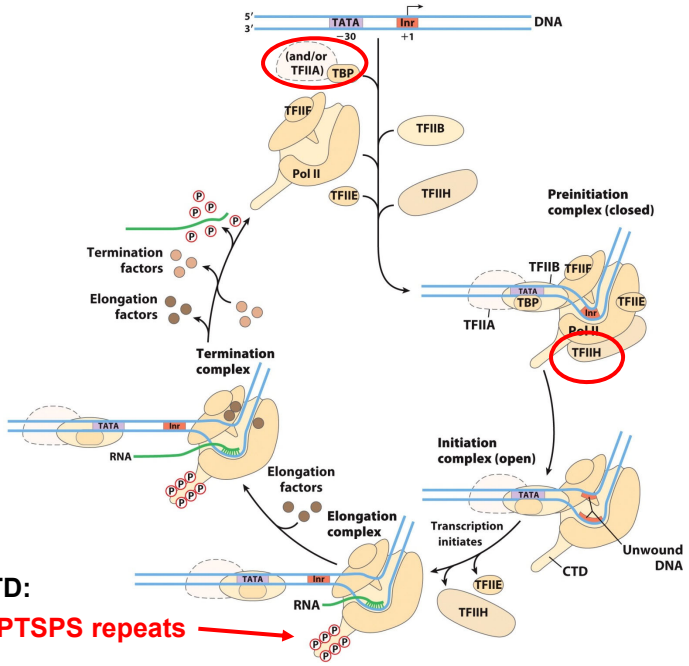


Figure 6–18. Molecular Biology of the Cell, 4th Edition.

Transcription by RNA Pol II in eukaryotes



Pol II CTD:

YSPTSPS repeats

Transcription by RNA Pol II in eukaryotes

TABLE 26–2 Proteins Required for Initiation of Transcription at the RNA Polymerase II (Pol II) Promoters of Eukaryotes

Transcription protein	Number of subunits	Subunit(s) M_r	Function(s)
Initiation			
Pol II	12	10,000–220,000	Catalyzes RNA synthesis
TBP (TATA-binding protein)	1	38,000	Specifically recognizes the TATA box
TFIIA	3	12,000, 19,000, 35,000	Stabilizes binding of TFIIB and TBP to the promoter
TFIIB	1	35,000	Binds to TBP; recruits Pol II–TFIIF complex
TFIIE	2	34,000, 57,000	Recruits TFIIH; has ATPase and helicase activities
TFIIF	2	30,000, 74,000	Binds tightly to Pol II; binds to TFIIB and prevents binding of Pol II to nonspecific DNA sequences
TFIIH	12	35,000–89,000	Unwinds DNA at promoter (helicase activity); phosphorylates Pol II (within the CTD); recruits nucleotide-excision repair proteins
Elongation*			
ELL†	1	80,000	
pTEFb	2	43,000, 124,000	Phosphorylates Pol II (within the CTD)
SII (TFIIS)	1	38,000	
Elongin (SIII)	3	15,000, 18,000, 110,000	

*The function of all elongation factors is to suppress the pausing or arrest of transcription by the Pol II–TFIIF complex.
†Name derived from *e*leven-nineteen *l*ysine-rich *l*eukemia. The gene for ELL is the site of chromosomal recombination events frequently associated with acute myeloid leukemia.

Table 26-2
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