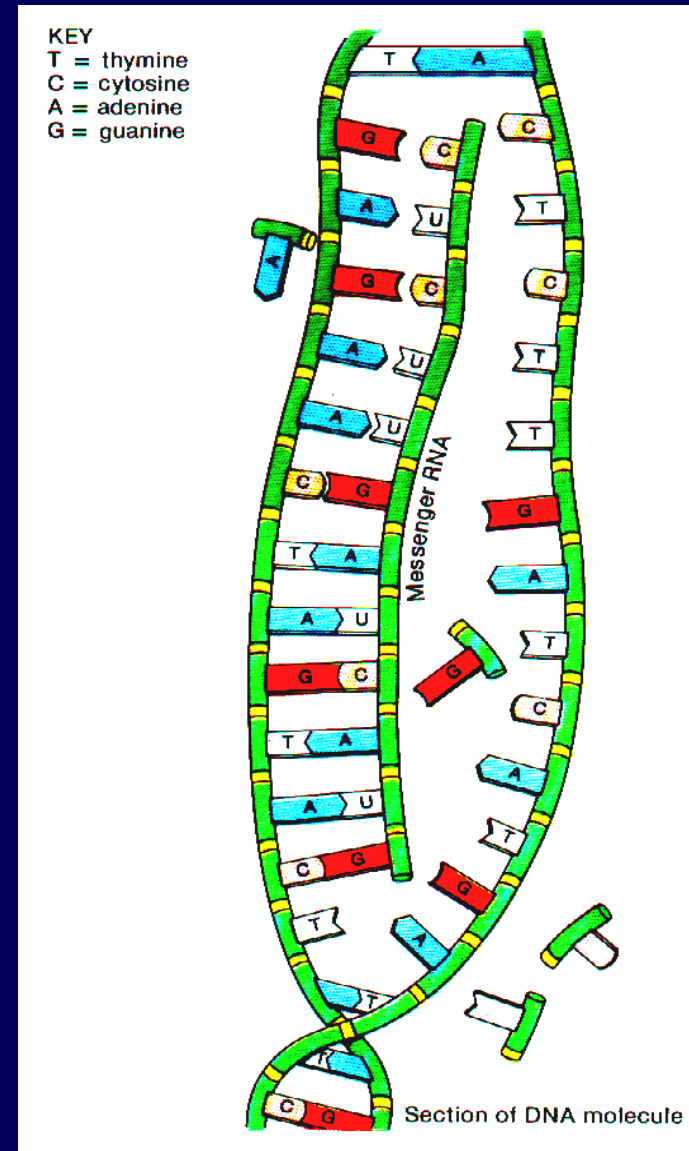


Transcription

Transcription

- Transcription is the DNA-directed synthesis of RNA
- RNA synthesis
 - Is catalyzed by RNA polymerase, which pries the DNA strands apart and hooks together the RNA nucleotides
 - Follows the same base-pairing rules as DNA, except that in RNA, uracil substitutes for thymine



Transcription

One strand of DNA is transcribed into RNA

Coding strand

Template strand

5' TACGCGGTACGGTCAATGCATCTACCT
3' ATGCGCCATGCCAGTTACGTACATGGA

TRANSCRIPTION



RNA sequence is
complementary to template strand
equivalent to coding strand

5' UACGCGGUACGGUCA AUGCAUCUACCU

RNA transcript

Promoters and terminators define the unit

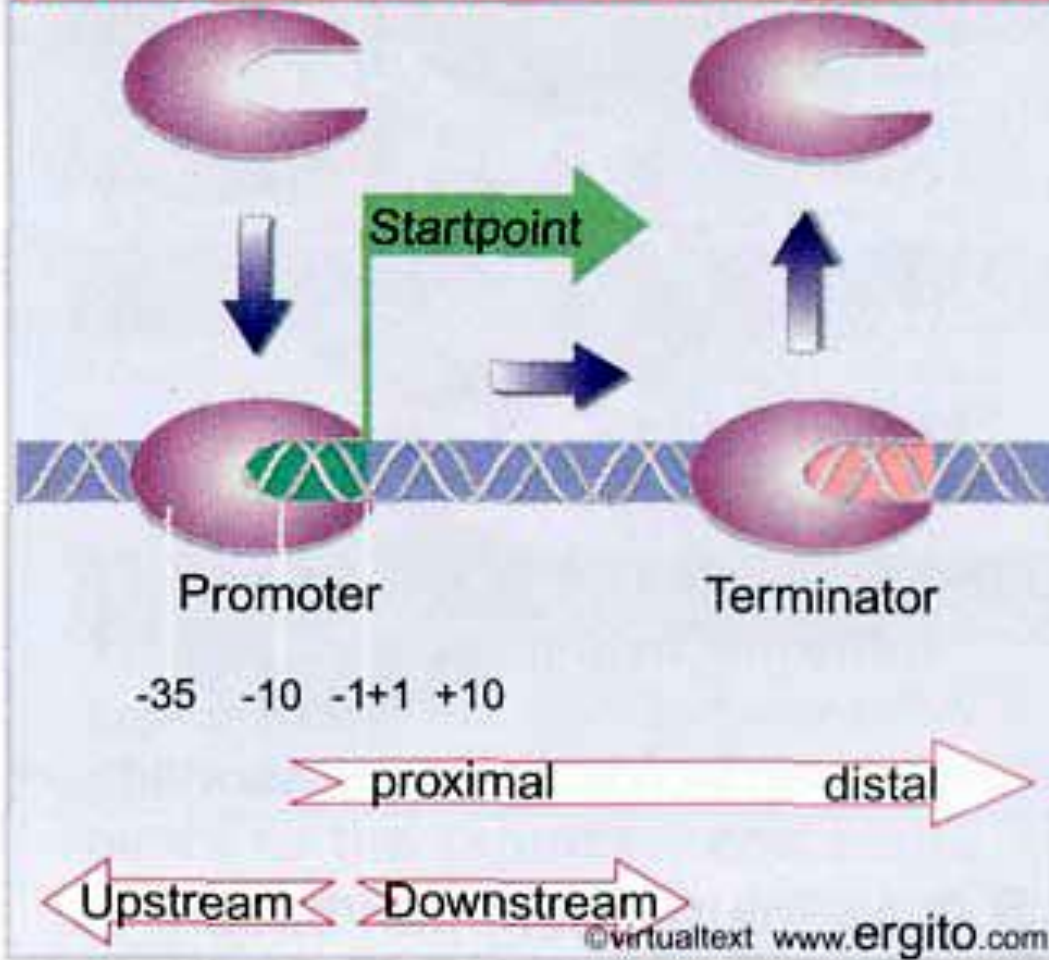
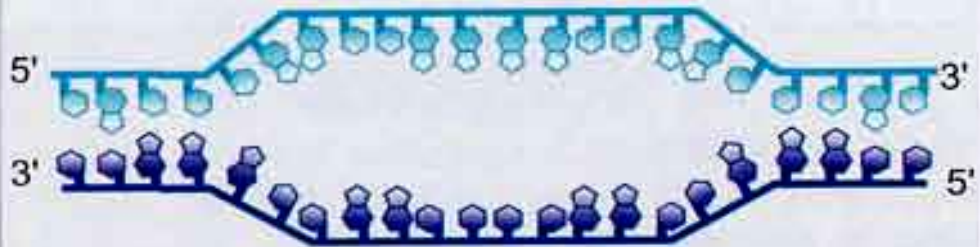


Figure 9.2 A transcription unit is transcribed into a single RNA.

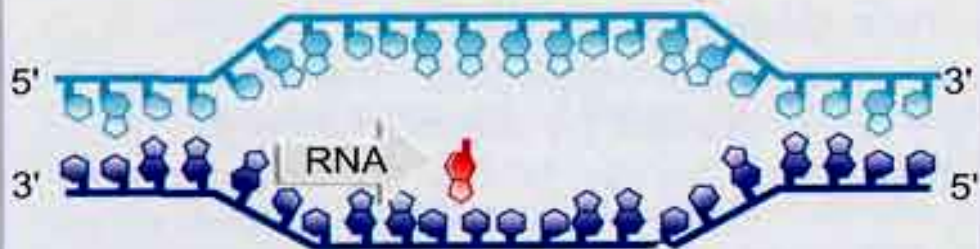
RNA synthesis occurs in the transcription bubble



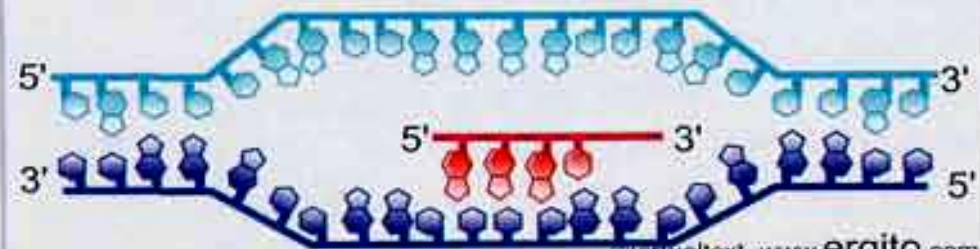
DNA is melted



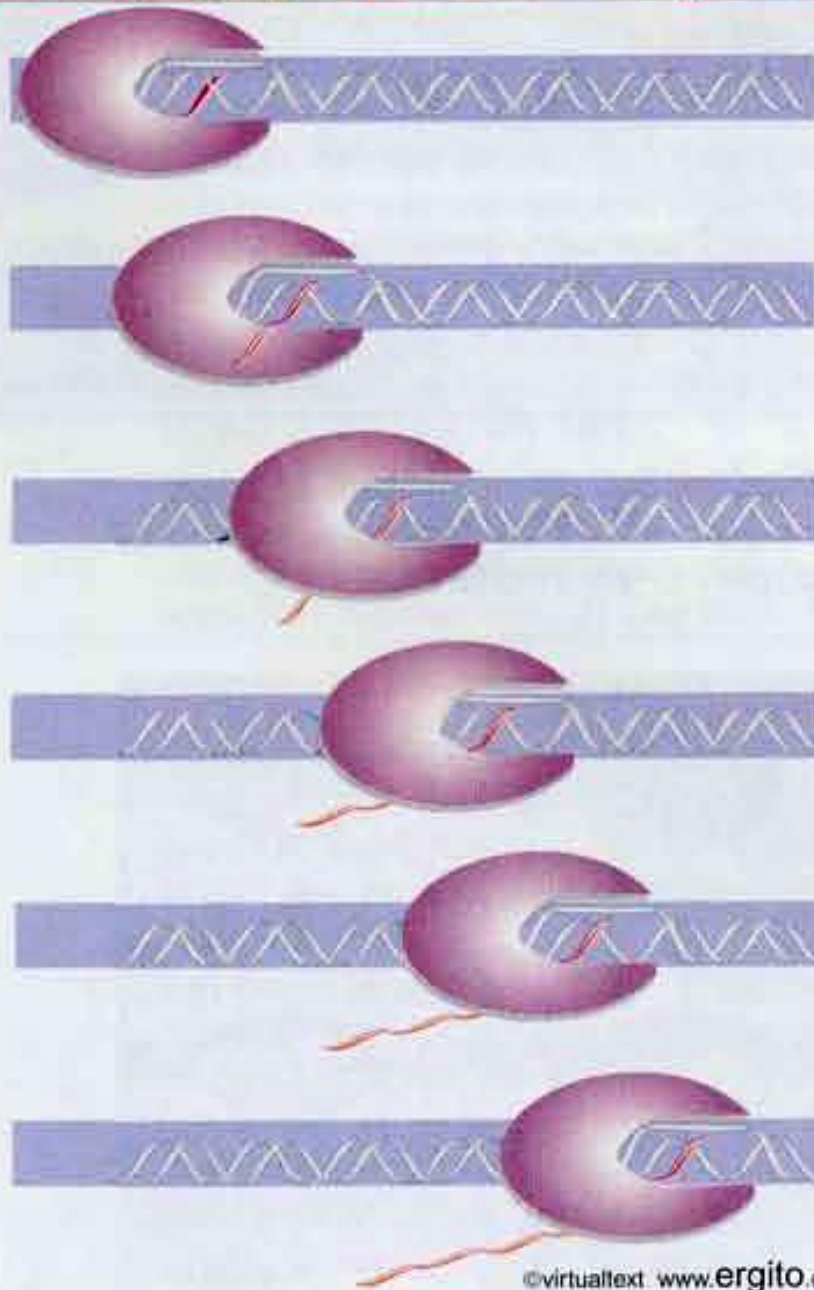
RNA synthesis initiates in bubble



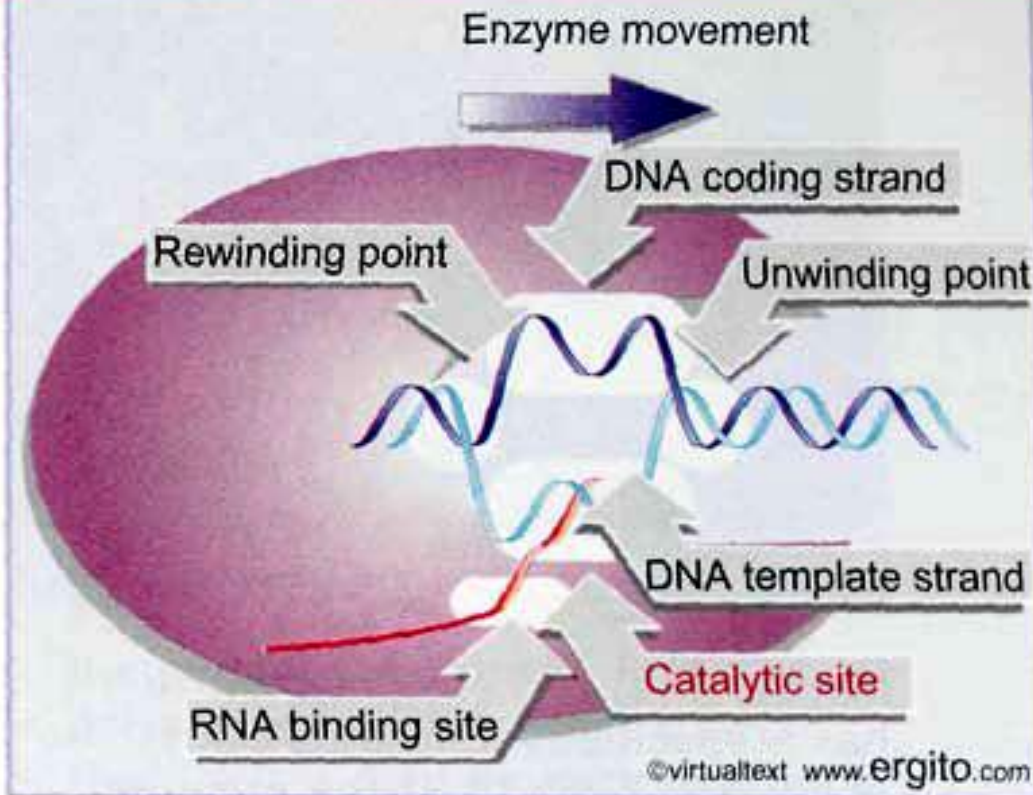
RNA chain is extended



The transcription bubble moves along DNA



RNA polymerase surrounds the bubble

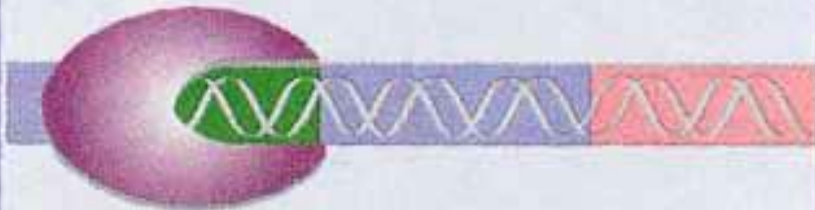


- : **Figure 9.5** During transcription, the
- : bubble is maintained within bacterial RNA
- : polymerase, which unwinds and rewinds
- : DNA, maintains the conditions of the
- : partner and template DNA strands, and
- : synthesizes RNA.

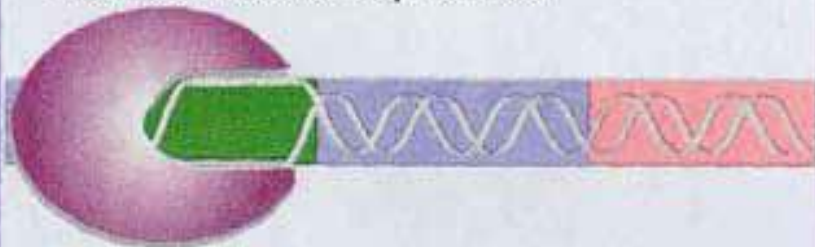
RNA polymerase catalyzes transcription

Template recognition:

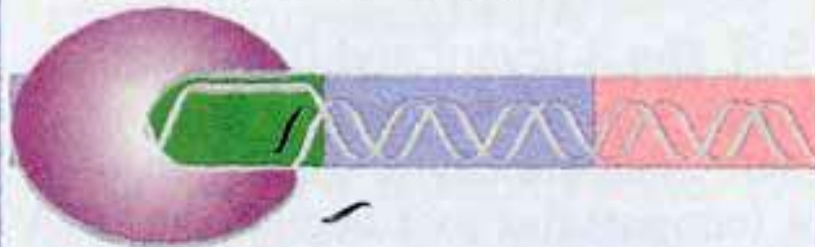
RNA polymerase binds to duplex DNA



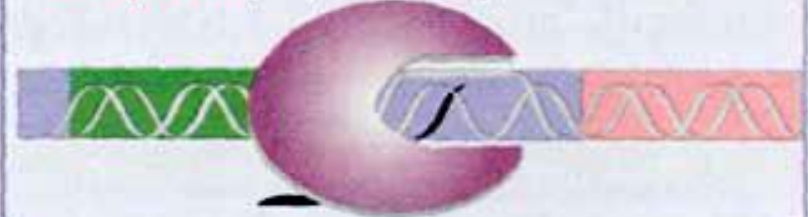
DNA is unwound at promoter



Initiation: Very short chains are synthesized and released



Elongation: polymerase synthesizes RNA



Termination:

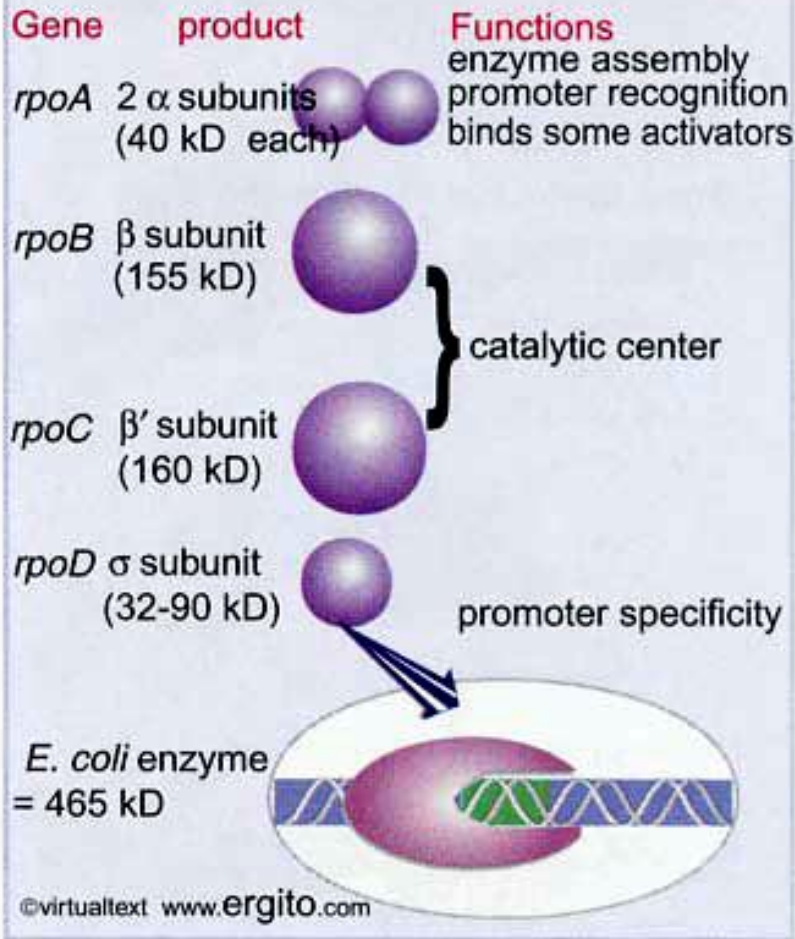
RNA polymerase and RNA are released



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Figure 9.6 Transcription has four stages, which involve different types of interaction between RNA polymerase and DNA. The enzyme binds to the promoter and melts DNA, remains stationary during initiation, moves along the template during elongation, and dissociates at termination.

RNA polymerase has 4 types of subunit



- In Bacteria single type of RNA polymerase synthesise all type of RNA (rRNA, mRNA, tRNA)
- The complete enzyme or holoenzyme in *E. coli* has a molecular weight of ~465 kD.
- Holoenzyme have five types of subunits ($\alpha_2\beta\beta'\sigma$).

Figure 9.16 Eubacterial RNA polymerases have four types of subunit; α , β , and β' have rather constant sizes in different bacterial species, but σ varies more widely.

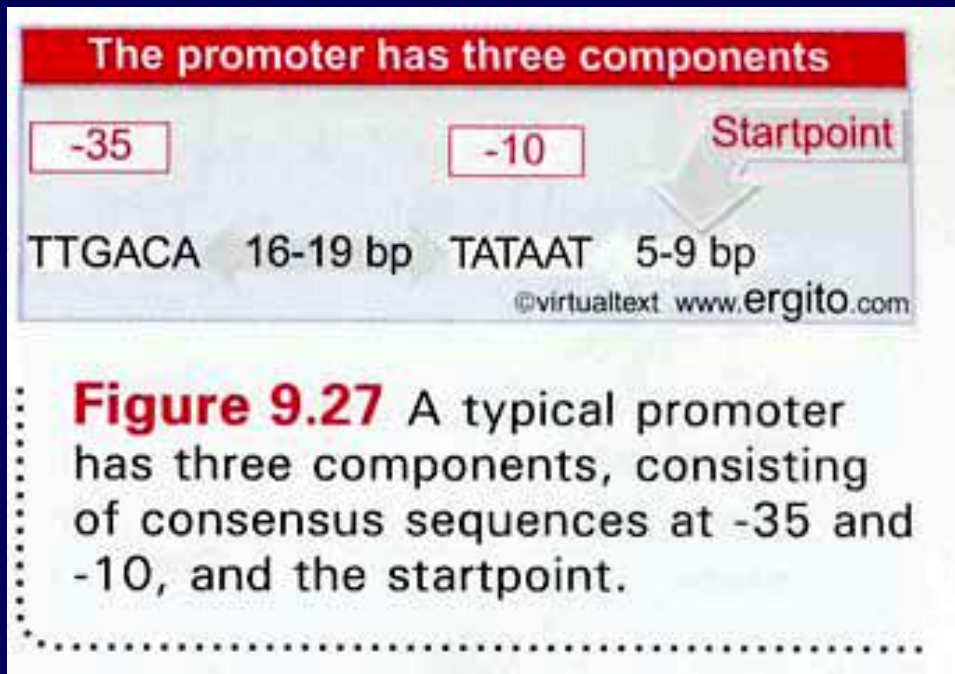
RNA Polymerase

- The holoenzyme ($\alpha_2\beta\beta'\sigma$) can be separated into two components,
- the core enzyme ($\alpha_2\beta\beta'$) and the sigma factor (the a polypeptide).
- *Only the holoenzyme can initiate transcription. Sigma factor ensures that bacterial RNA polymerase binds in a stable manner to DNA only at promoters. The sigma "factor" is usually released when the RNA chain reaches 8-9 bases, leaving the core enzyme to undertake elongation.*
- *Core enzyme has the ability to synthesize RNA on a DNA template, but cannot initiate transcription at the proper sites.*

Subunits of RNA Polymerase

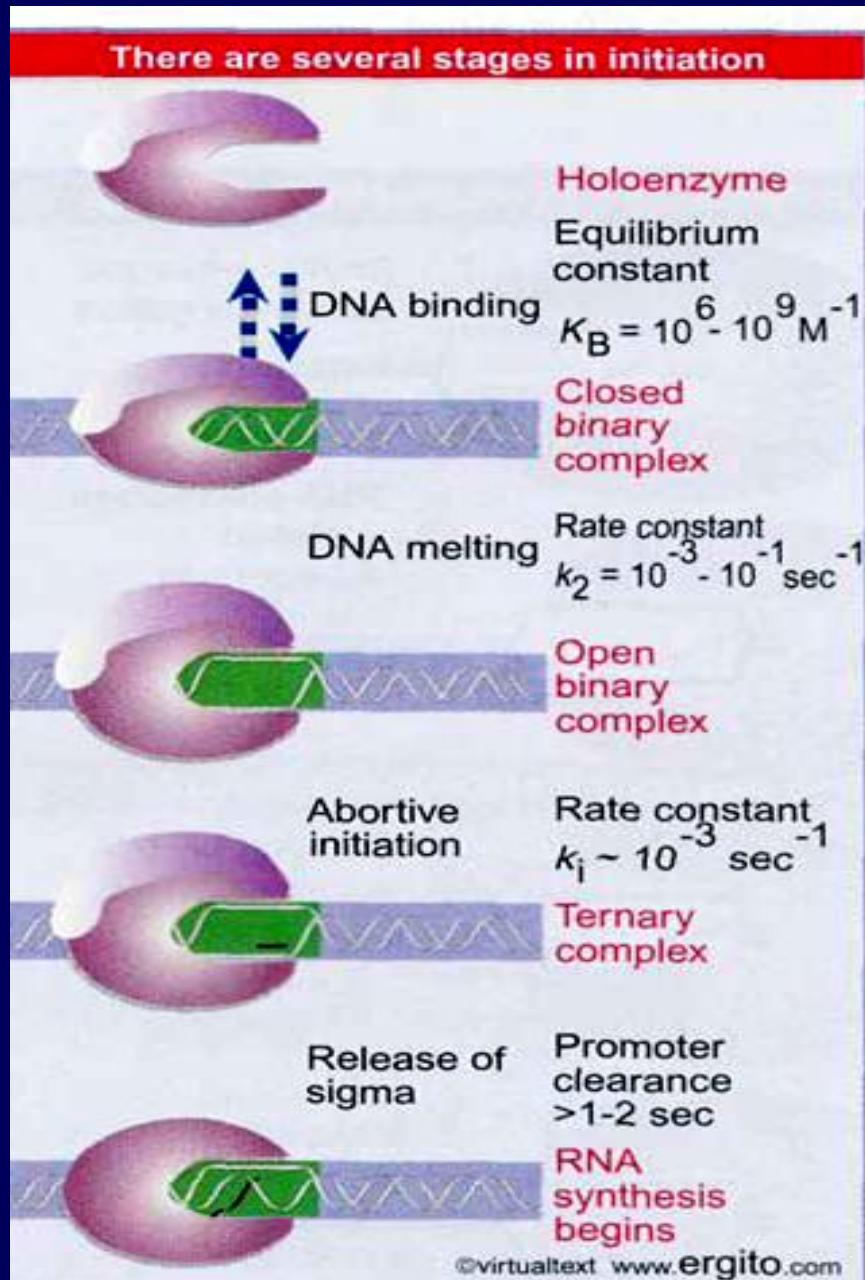
- The α subunit is required for assembly of the core enzyme.
- The β and β' subunits together make up the catalytic center.
- *Sigma factor ensures that bacterial RNA polymerase binds in a stable manner to DNA only at promoters.*

Promoter recognition by σ factor



- Promoter recognition depends on consensus sequences
- The promoter consensus sequences consist of a purine at the startpoint, the hexamer TATAAT centered at -10, and another hexamer centered at -35.

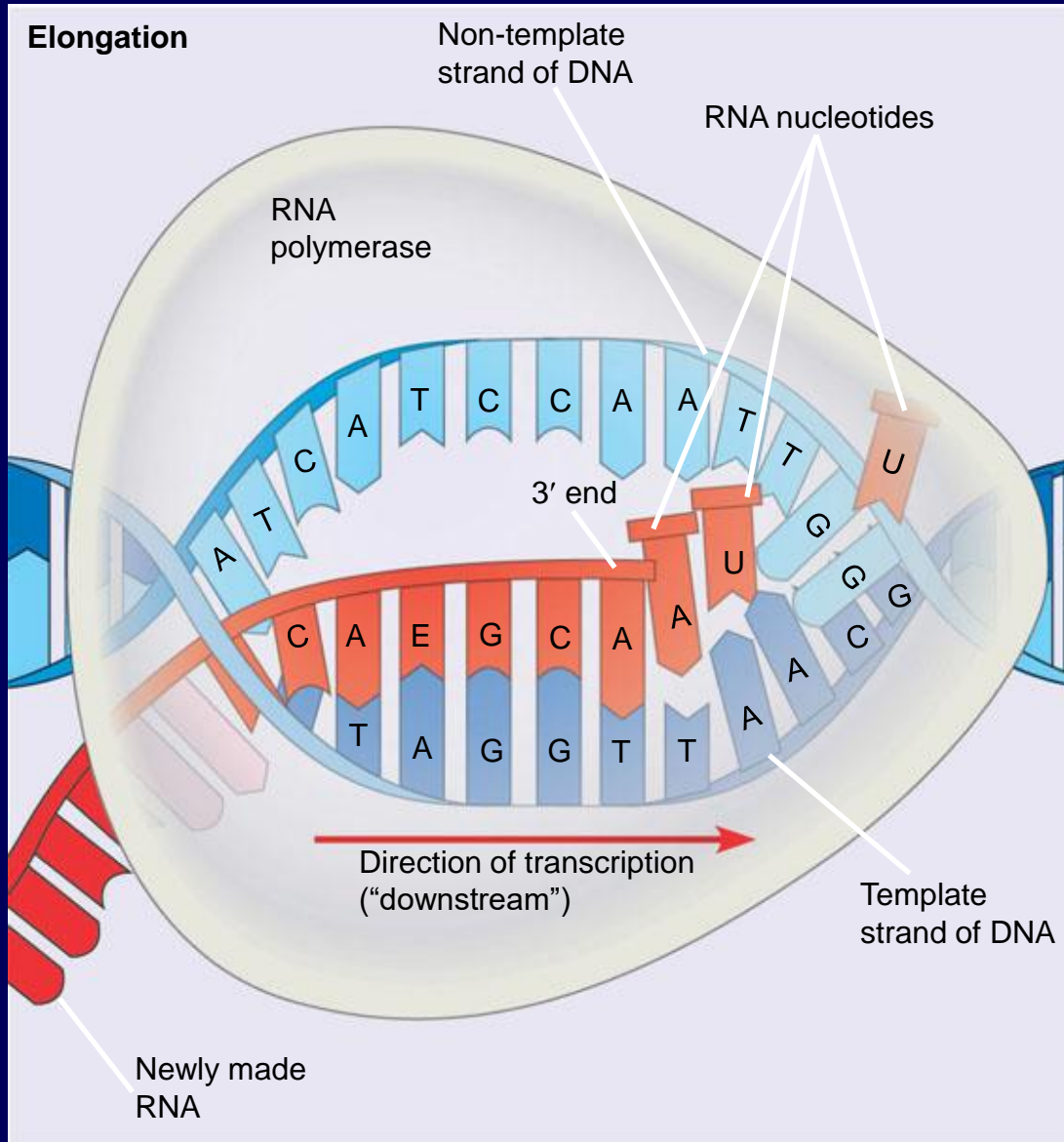
Initiation of Transcription



When RNA polymerase undergoes abortive transcription in the presence of ATP, UTP, and GTP, a complex is formed that has a much lower capacity for abortive recycling and a much higher rate of synthesis of the full-length RNA transcript.

A study in 2010 did find evidence supporting that these truncated transcripts inhibit termination of RNA synthesis by a RNA hairpin-dependent intrinsic terminator

Synthesis of an RNA Transcript - Elongation



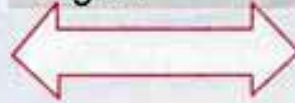
- RNA polymerase synthesizes a single strand of RNA against the DNA template strand (anti-sense strand), adding nucleotides to the 3' end of the RNA chain
- As RNA polymerase moves along the DNA it continues to untwist the double helix, exposing about 10 to 20 DNA bases at a time for pairing with RNA nucleotides

Bacterial RNA polymerase terminates at discrete sites

- Termination may require both recognition of the terminator sequence in DNA and the formation of a hairpin structure in the RNA product.
- Once RNA polymerase has started transcription, the enzyme moves along the template, synthesizing RNA, until it meets a terminator sequence. At this point, the enzyme stops adding nucleotides to the growing RNA chain, releases the completed product, and dissociates from the DNA template.

Bacterial termination occurs at a discrete site

All sequences required for termination are in transcribed region



Hairpin in RNA may be required



RNA polymerase and RNA are released



There are two types of terminators in *E. coli*

- **Intrinsic terminators**

Intrinsic terminators consist of a G-C-rich hairpin in the RNA product followed by a U-rich region in which termination occurs and do not require any additional factor.

- **Rho-dependent terminators**

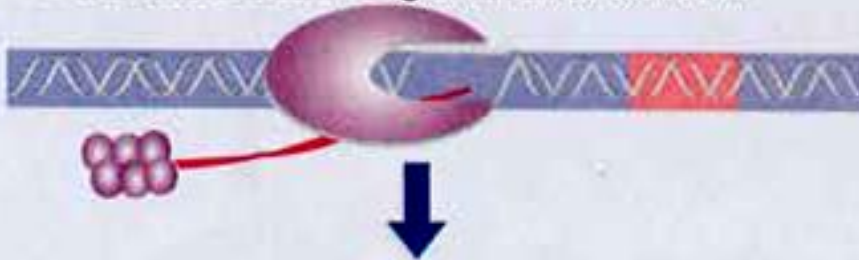
Rho-dependent terminators are defined by the need for addition of rho factor for transcription termination

Rho terminates transcription

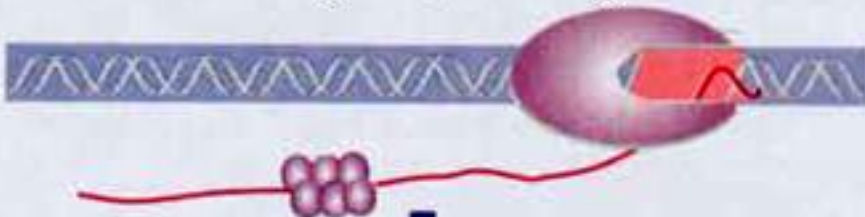
RNA polymerase transcribes DNA



Rho attaches to recognition site on RNA



Rho moves along RNA, following RNA



RNA polymerase pauses at terminator and rho catches up



Rho unwinds DNA-RNA hybrid



Termination: all components released



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Figure 9.49 A rho factor pursues RNA polymerase along the RNA and can cause termination when it catches the enzyme pausing at a rho-dependent terminator.

Synthesis of an RNA Transcript: Summary

- The stages of transcription are
 - Initiation
 - Elongation
 - Termination

