Transcription in Prokaryotes

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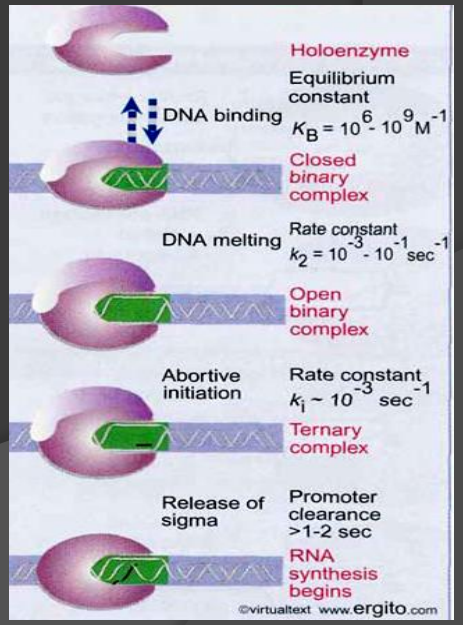
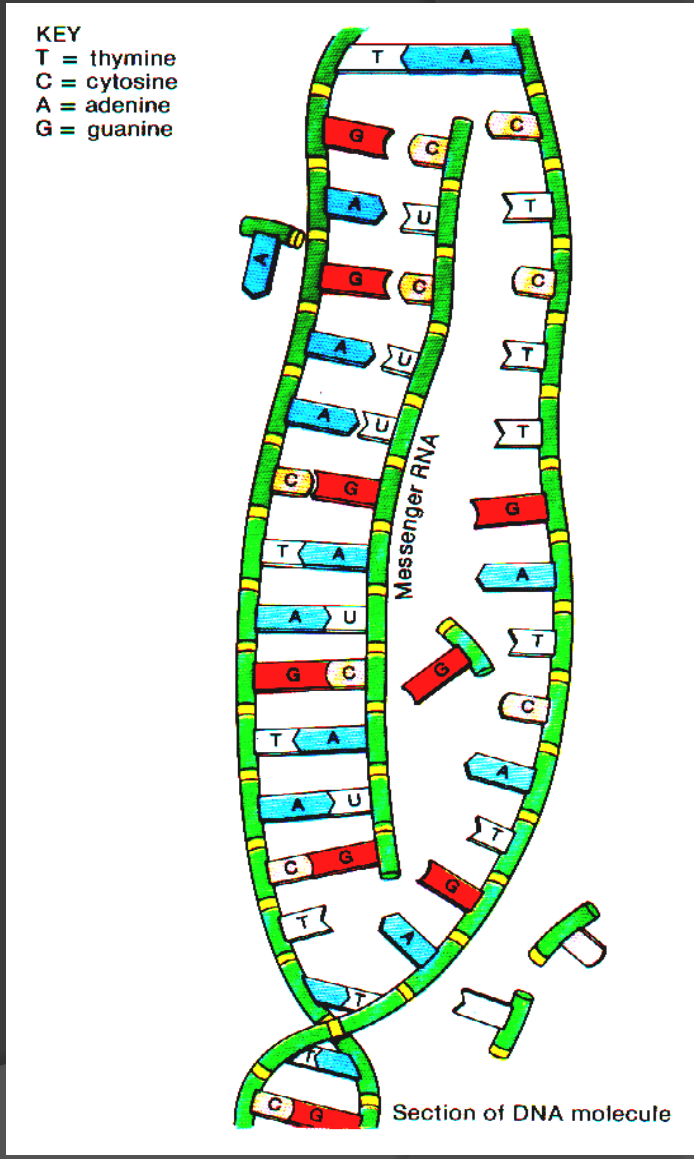


Transcription

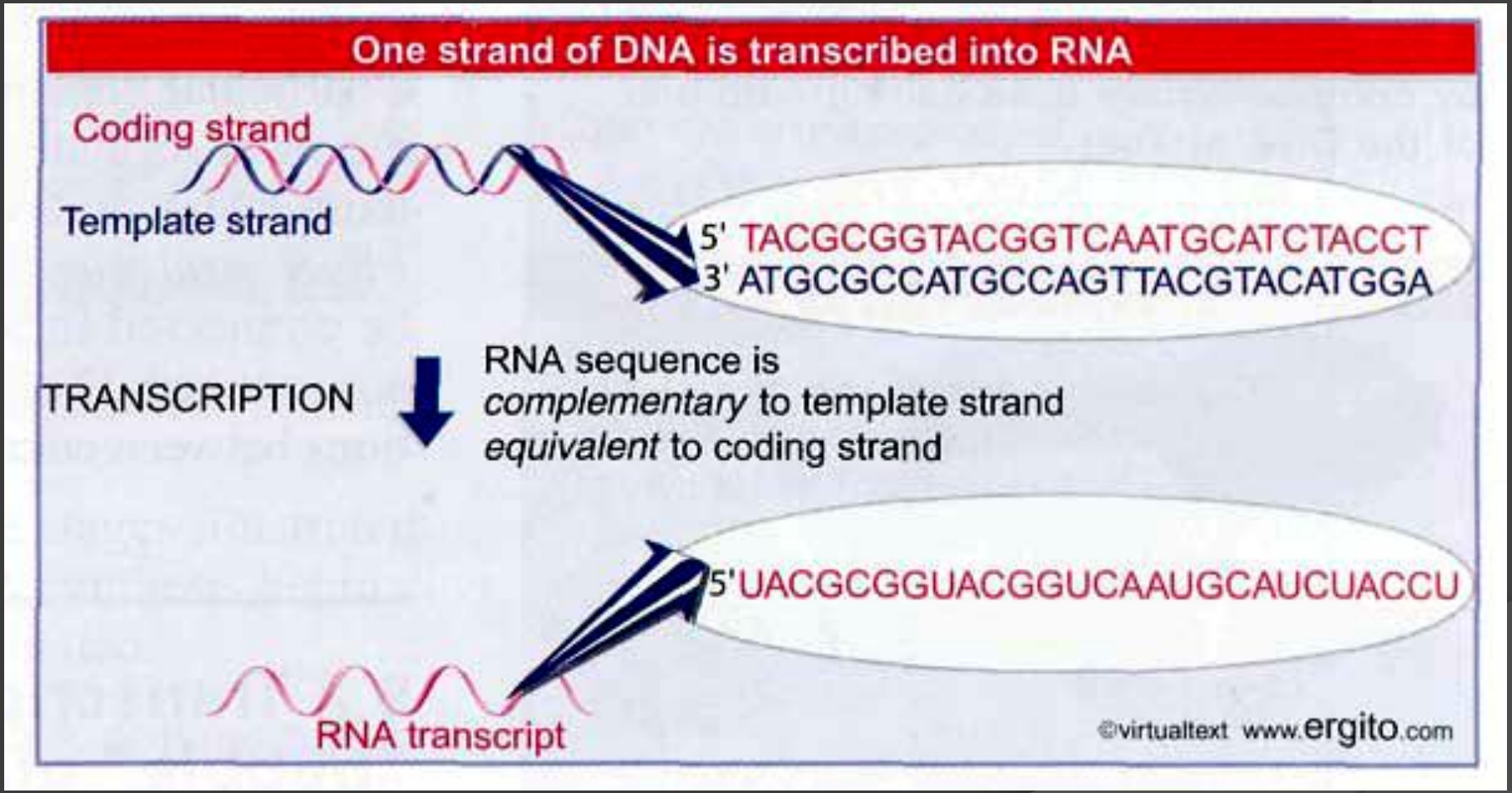
- Transcription is the DNA-directed synthesis of RNA

- 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



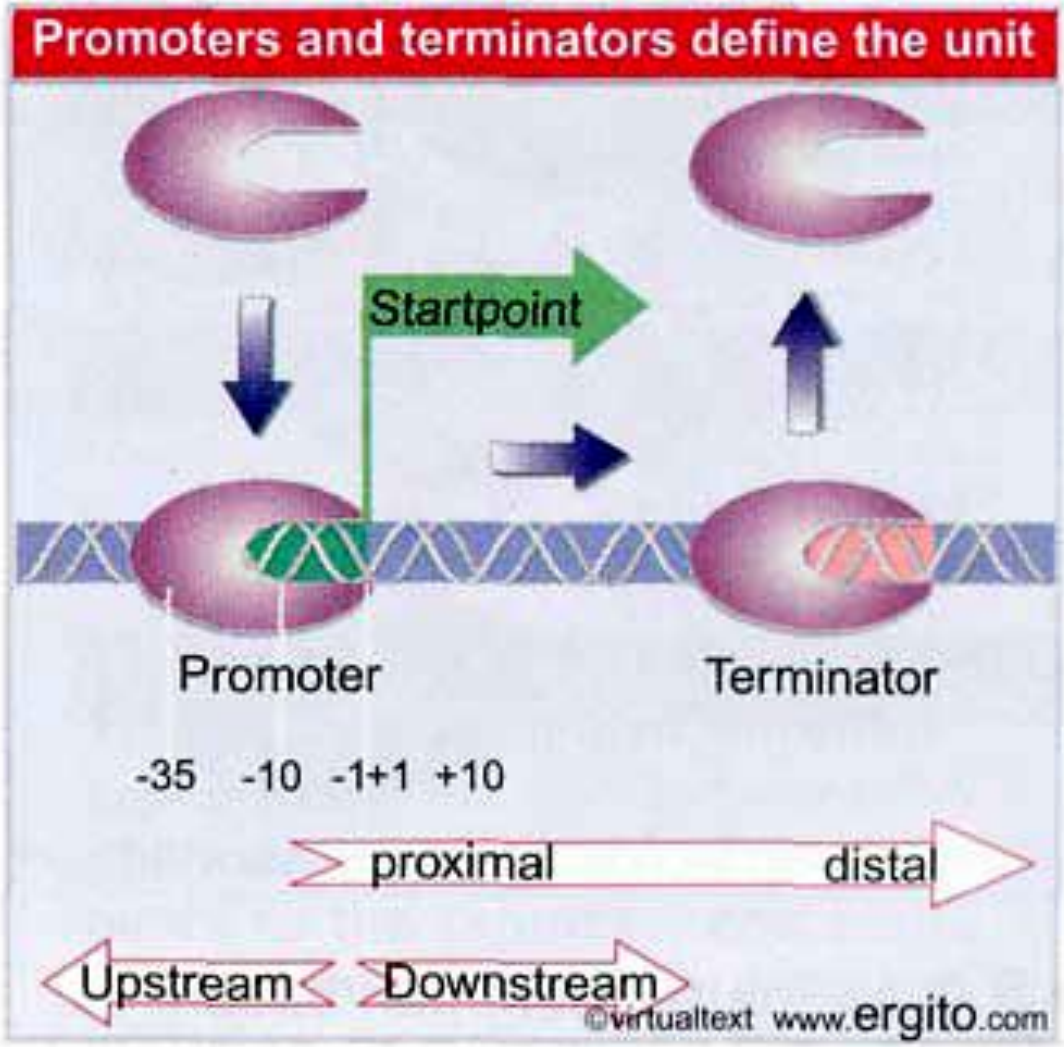
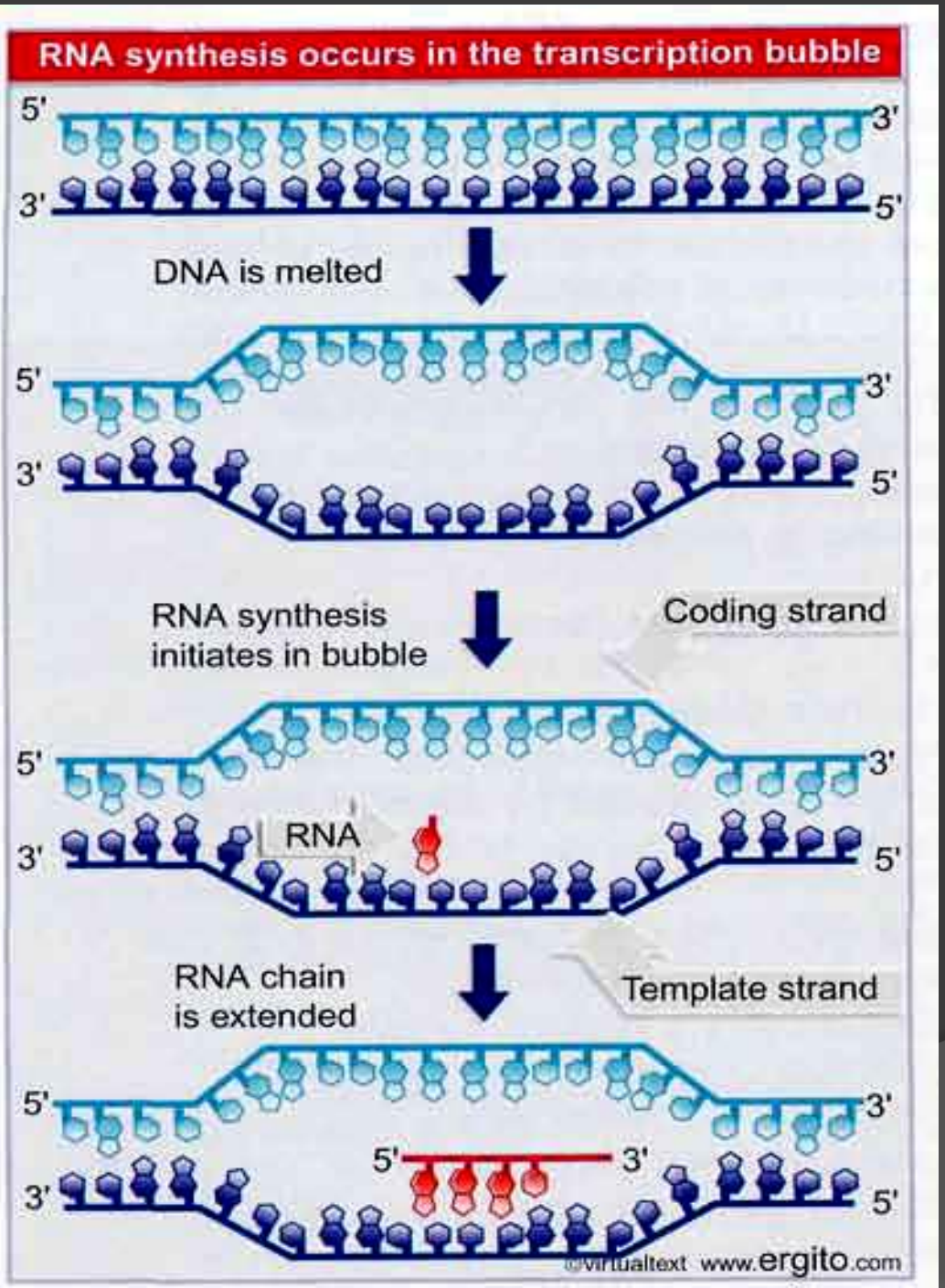


Figure 9.2 A transcription unit is transcribed into a single RNA.


RNA synthesis occurs in the transcription bubble
5'
3'
3'
5'
DNA is melted
5'
3'
3'
5'
RNA synthesis
initiates in bubble
Coding strand
5'
3'
RNA
3'
5'
RNA chain
is extended
Template strand
5'
3'
5'
3'
3'
5'
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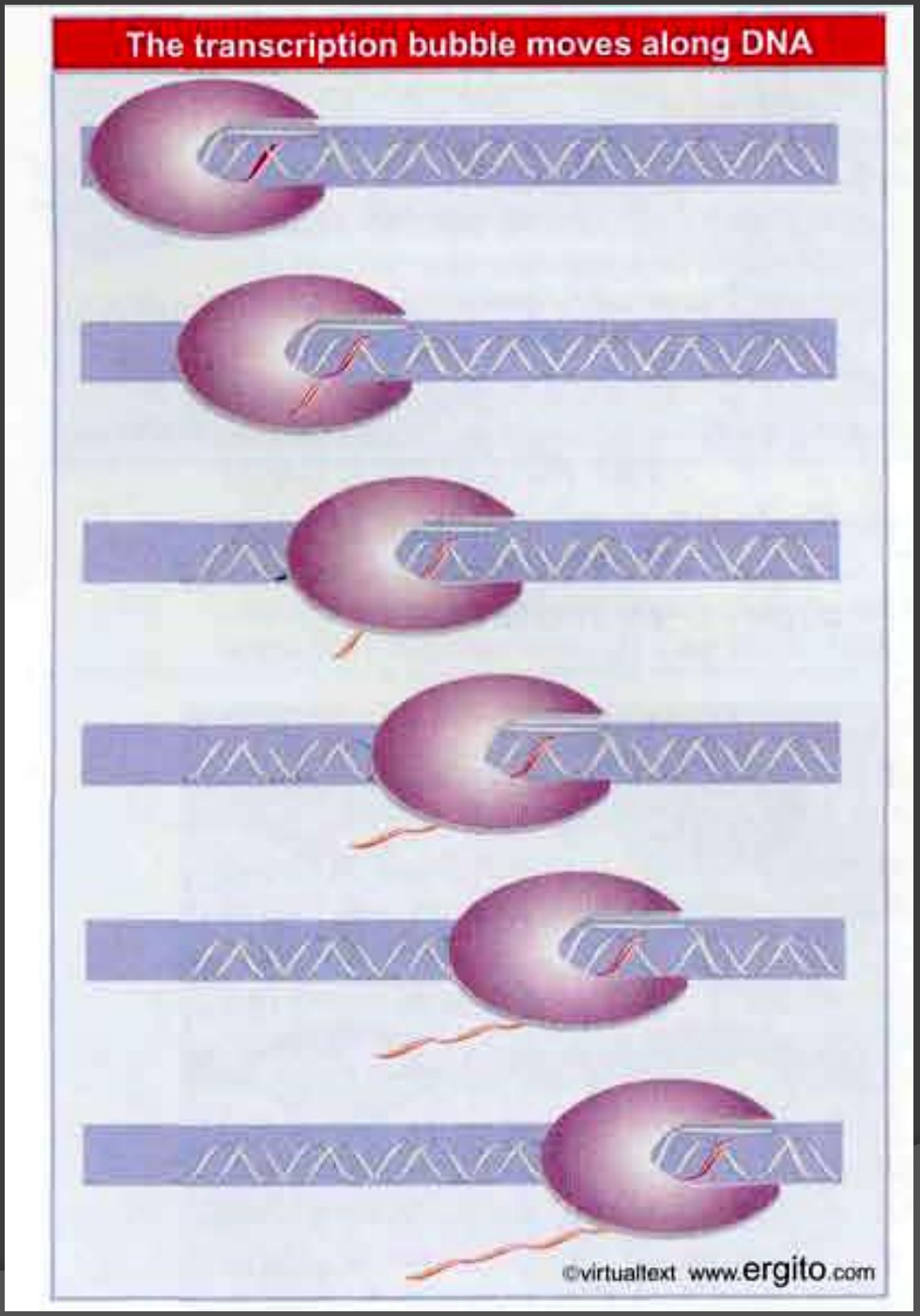
The transcription bubble moves along DNA
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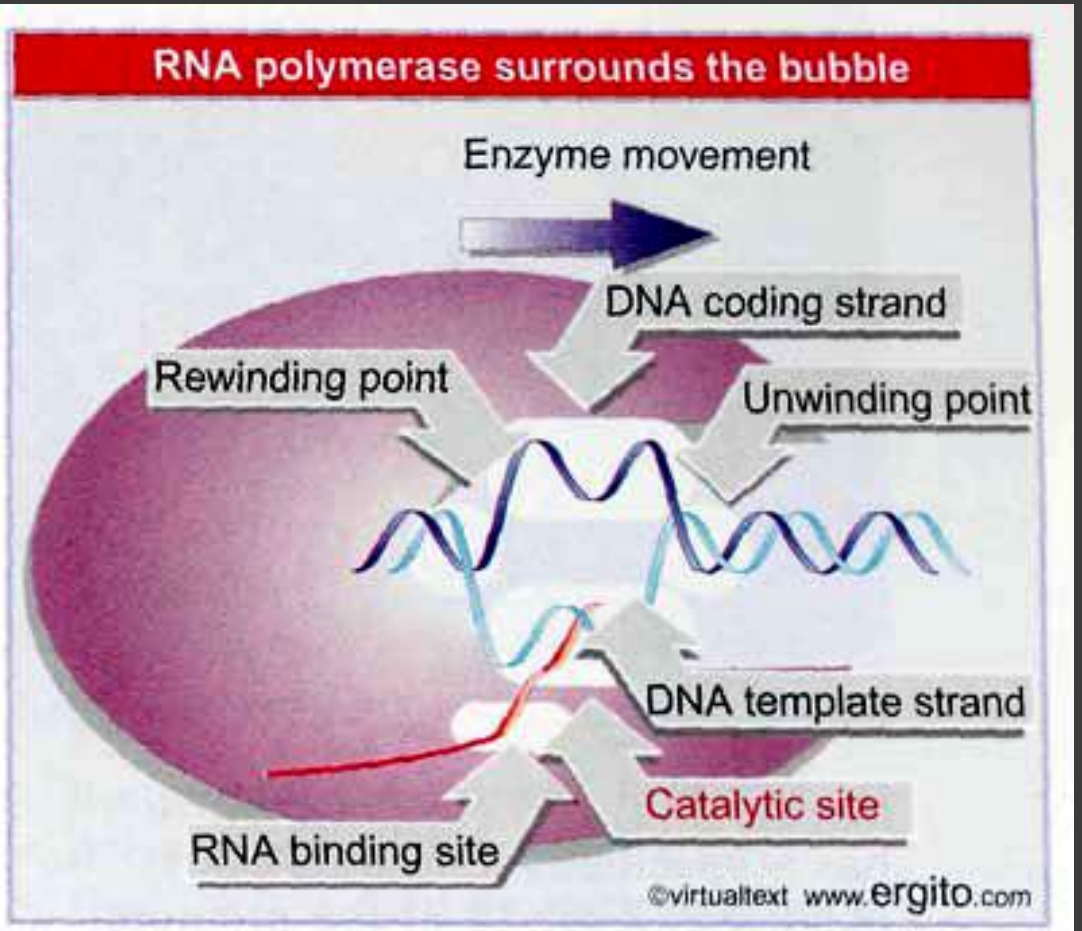


Figure 9.5 During transcription, the bubble is maintained within bacterial RNA polymerase, which unwinds and rewinds DNA, maintains the conditions of the partner and template DNA strands, and synthesizes RNA.

Stages of Transcription (4)

1. Promoter recognition
2. Initiation
3. Elongation
4. Termination

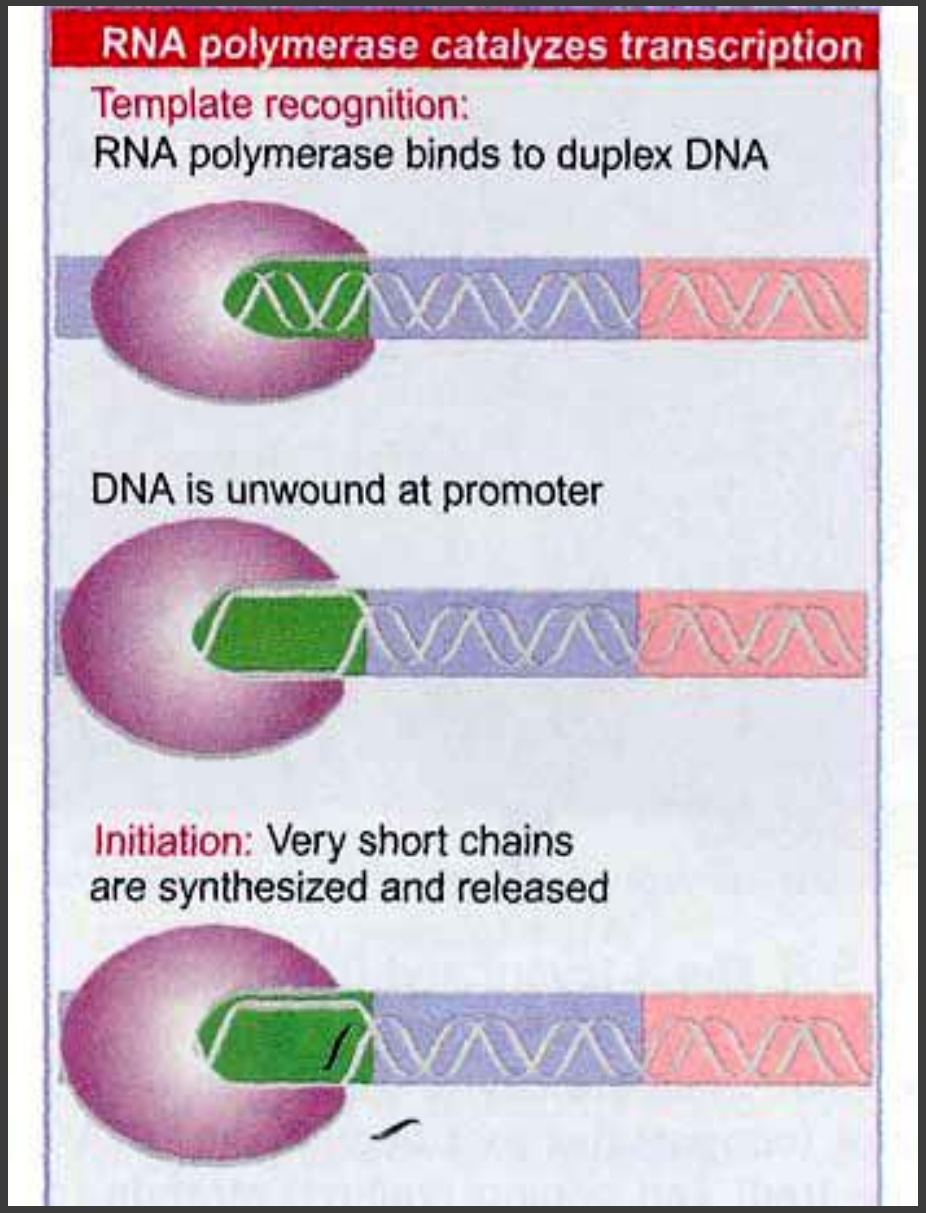


Figure 9.6 Transcription has four stages, which involve different types of interaction between RNA polymerase and DNA. The enzyme binds to the promoter and melts DNA, remains stationary during initiation, moves along the template during elongation, and dissociates at termination.

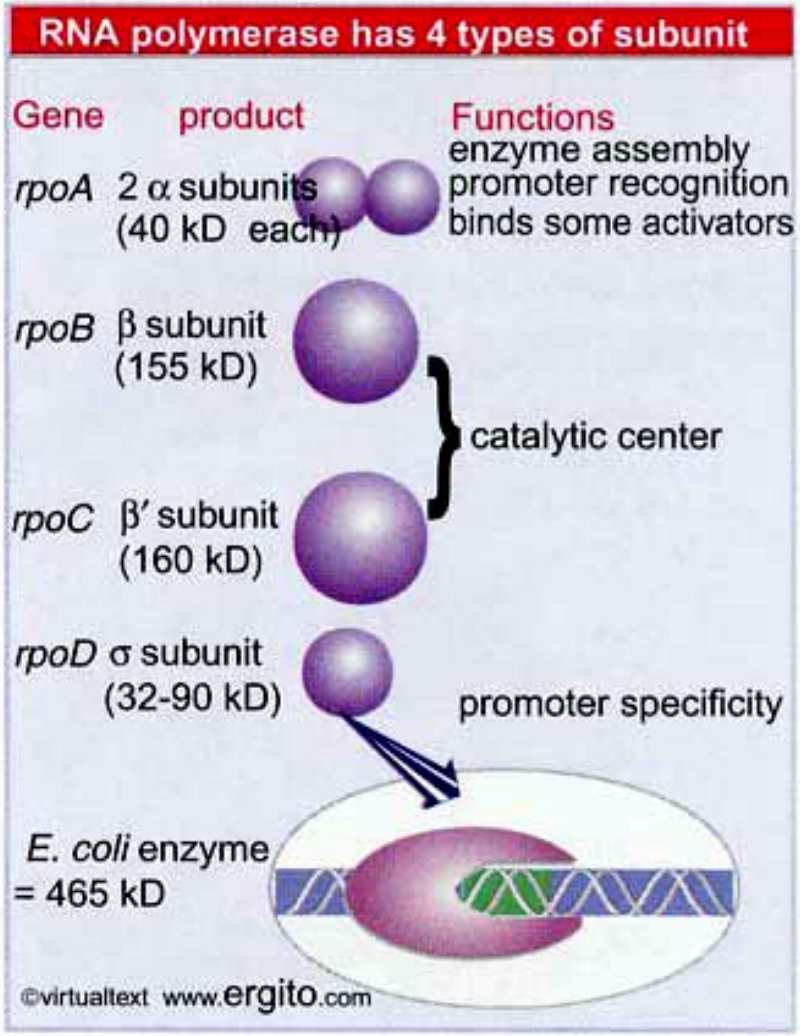


Figure 9.16 Eubacterial RNA polymerases have four types of subunit; α, β, and β have rather constant sizes in different bacterial species, but σ varies more widely.

- 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 ($\alpha_2\beta\beta'\sigma$).

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

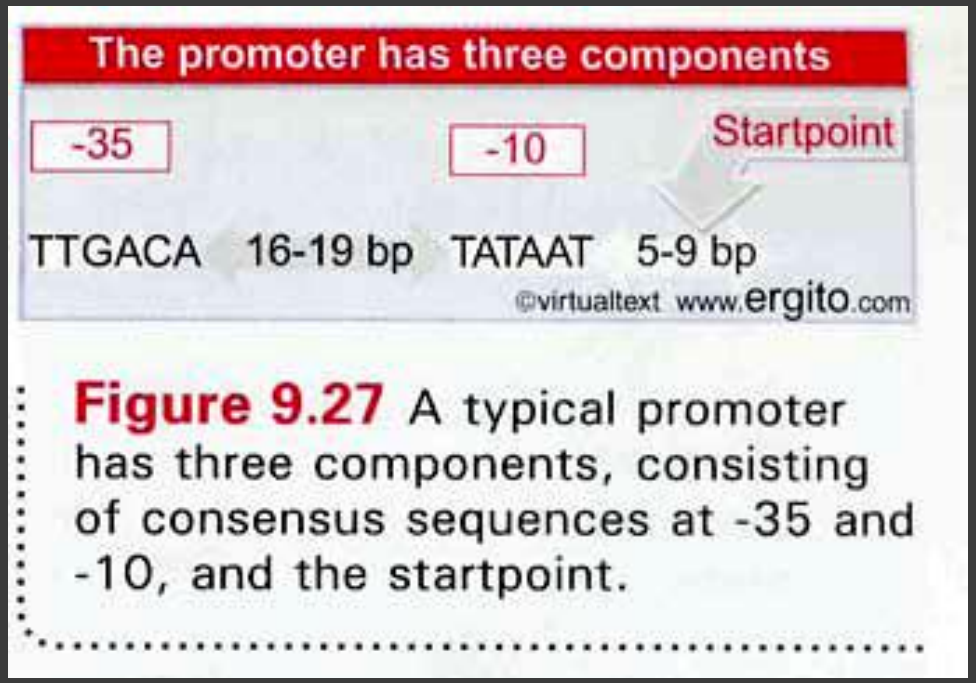
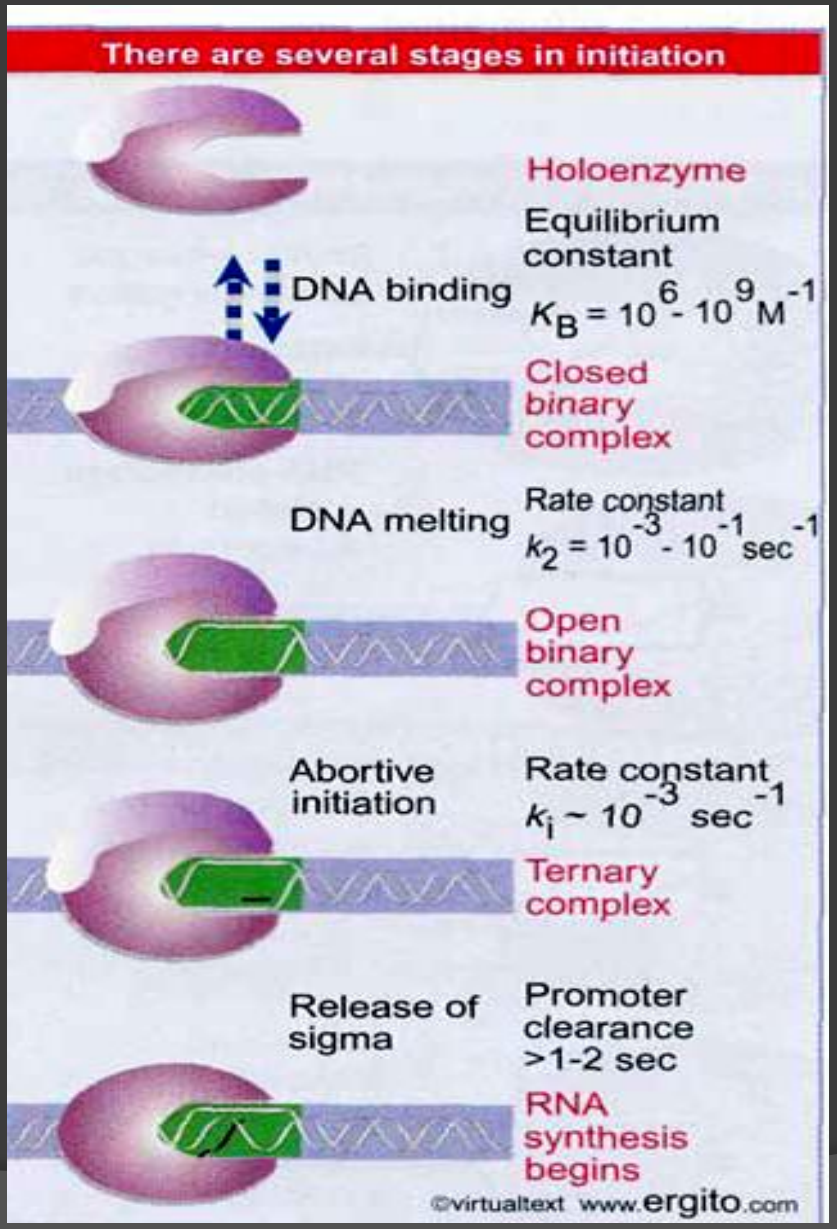


Figure 9.27 A typical promoter has three components, consisting of consensus sequences at -35 and -10, and the startpoint.

- 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.

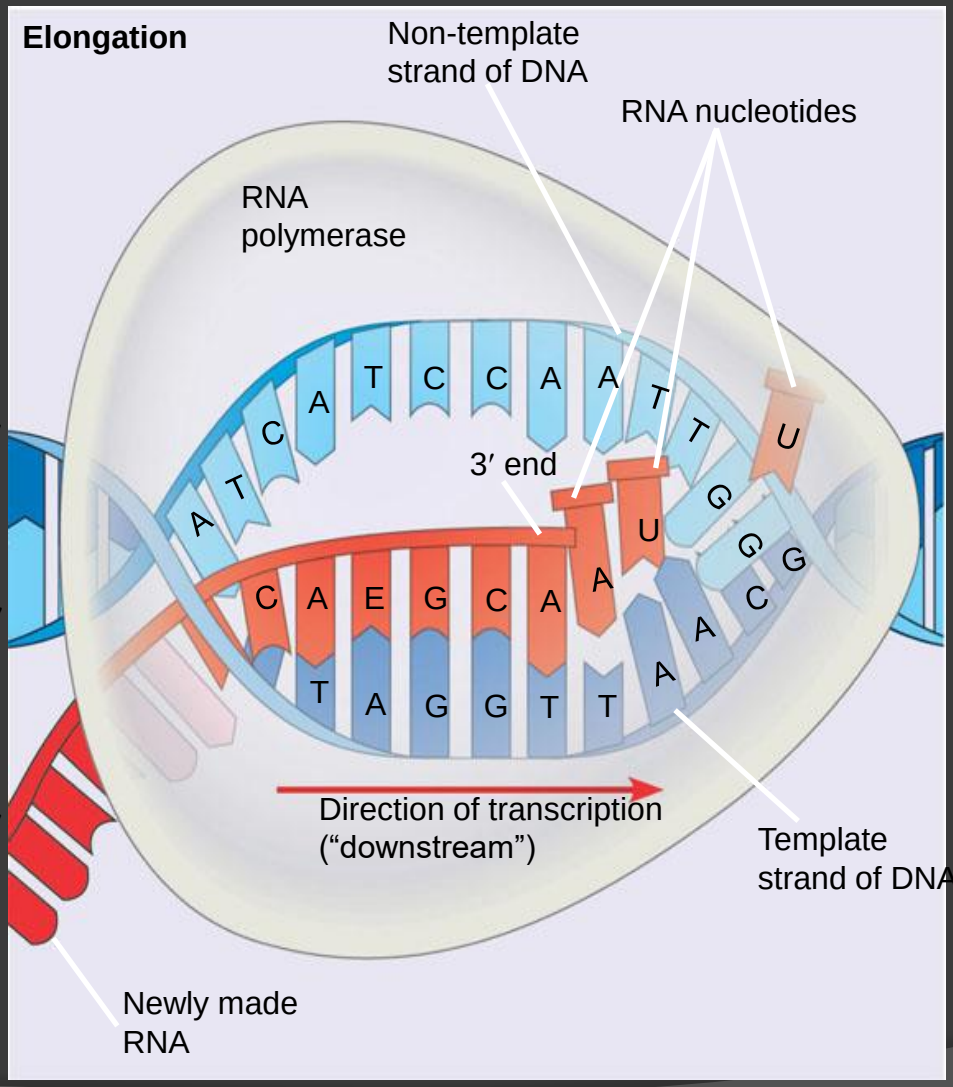
Initiation of Transcription



When RNA polymerase undergoes abortive transcription in the +nce 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

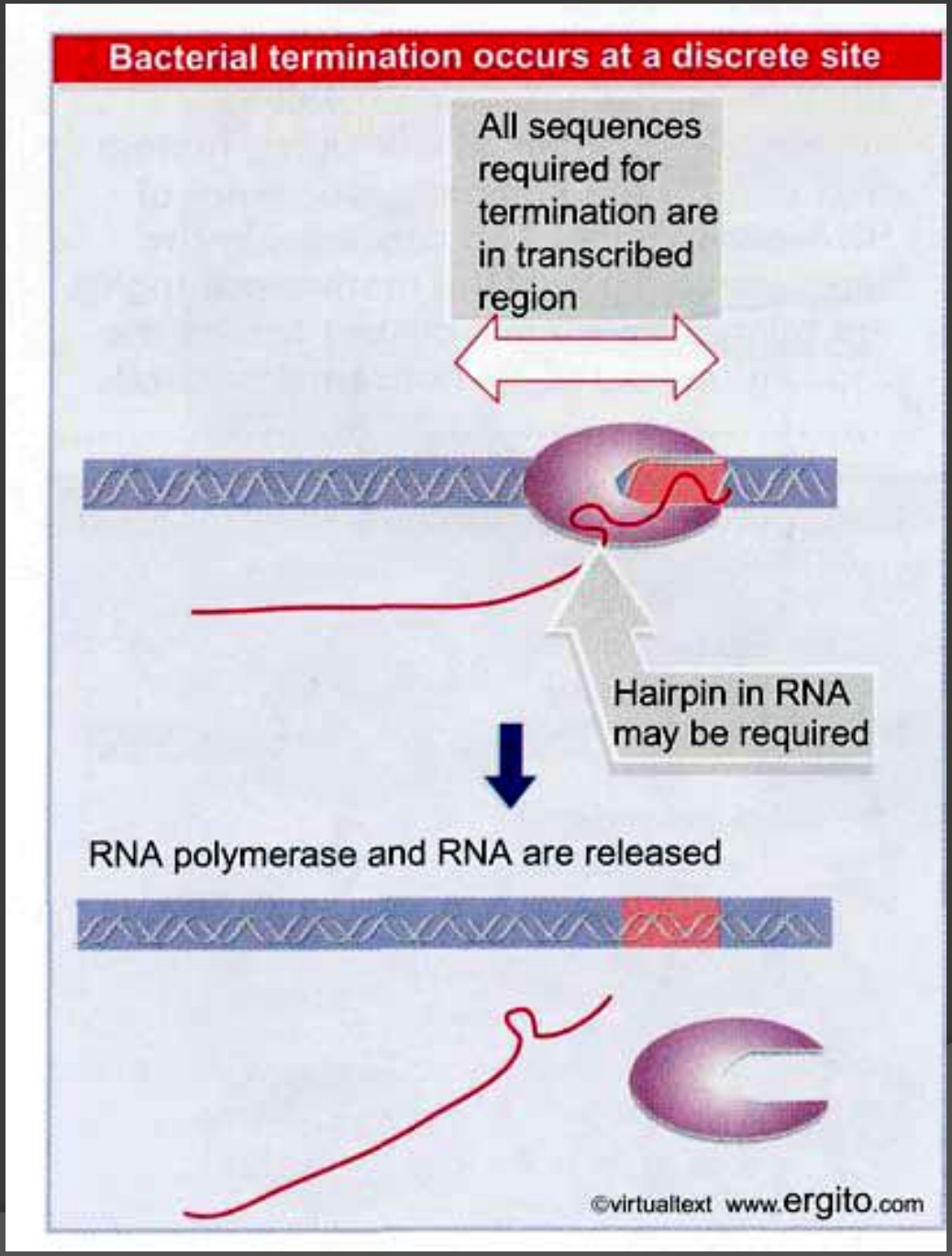


- RNA polymerase synthesizes a single strand of RNA against the DNA template strand (anti-sense strand), adding nucleotides to the 3' end of the RNA chain
- As RNA polymerase moves along the DNA it continues to untwist the double helix, exposing about 10 to 20 DNA bases at a time for pairing with RNA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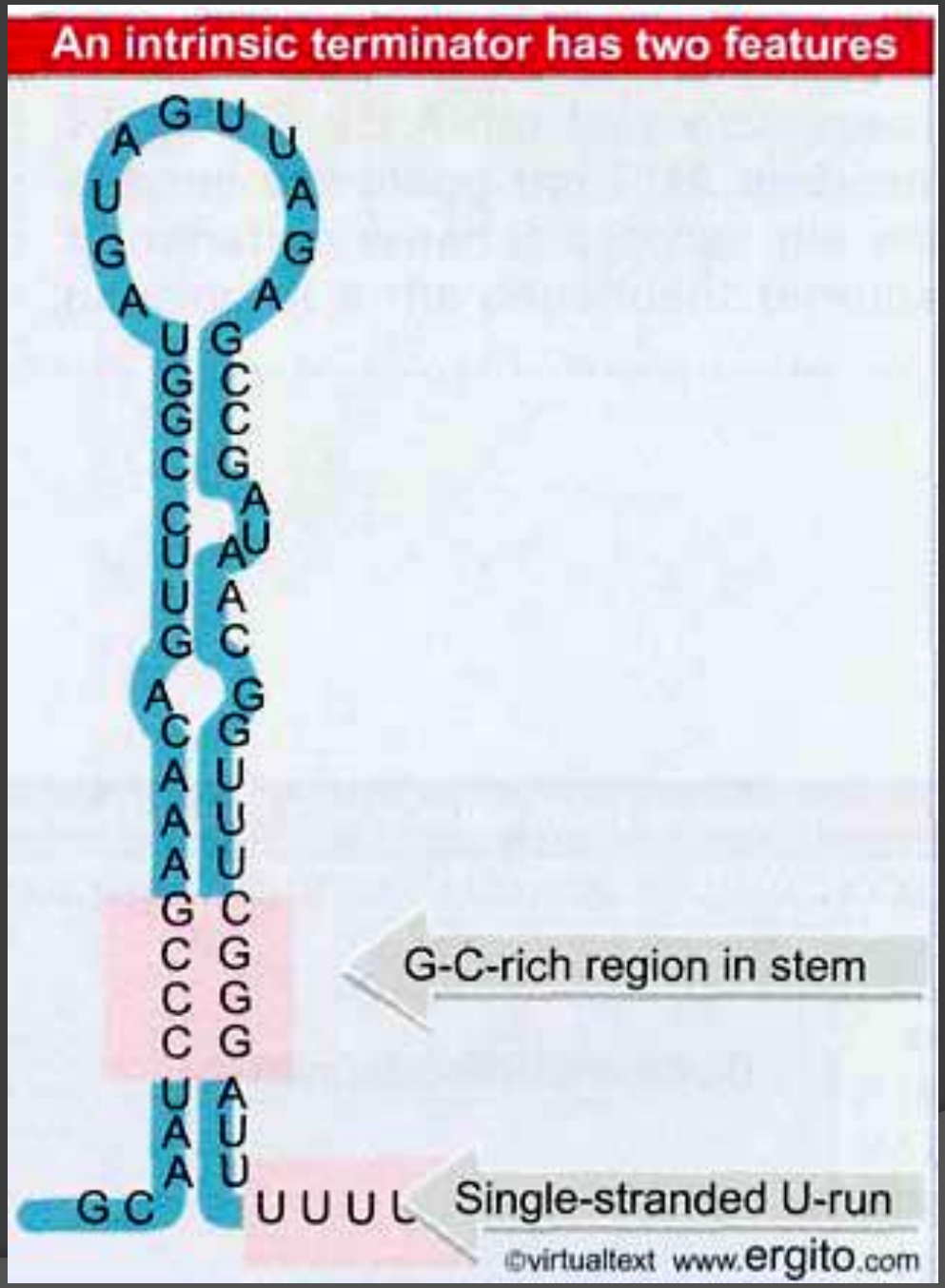
There are two types of terminators in *E. coli*

- **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


An intrinsic terminator has two features
G-C-rich region in stem
Single-stranded U-run
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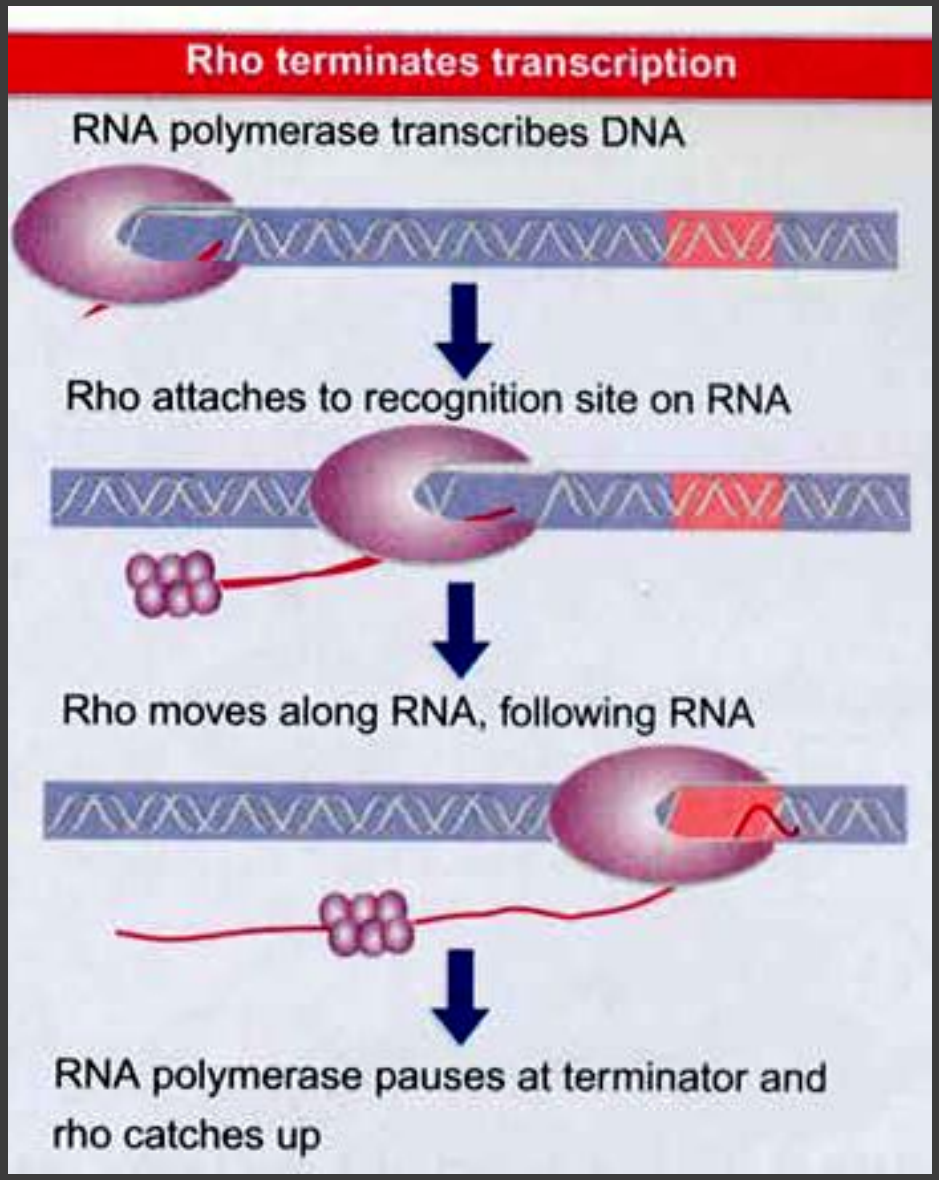


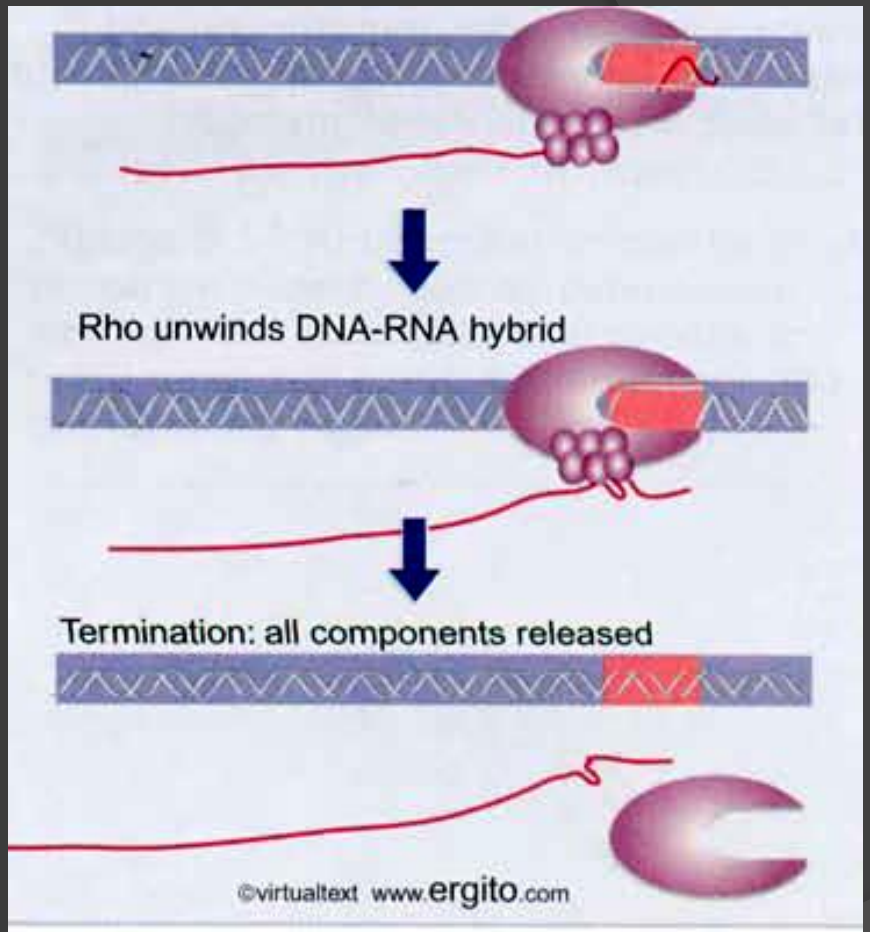
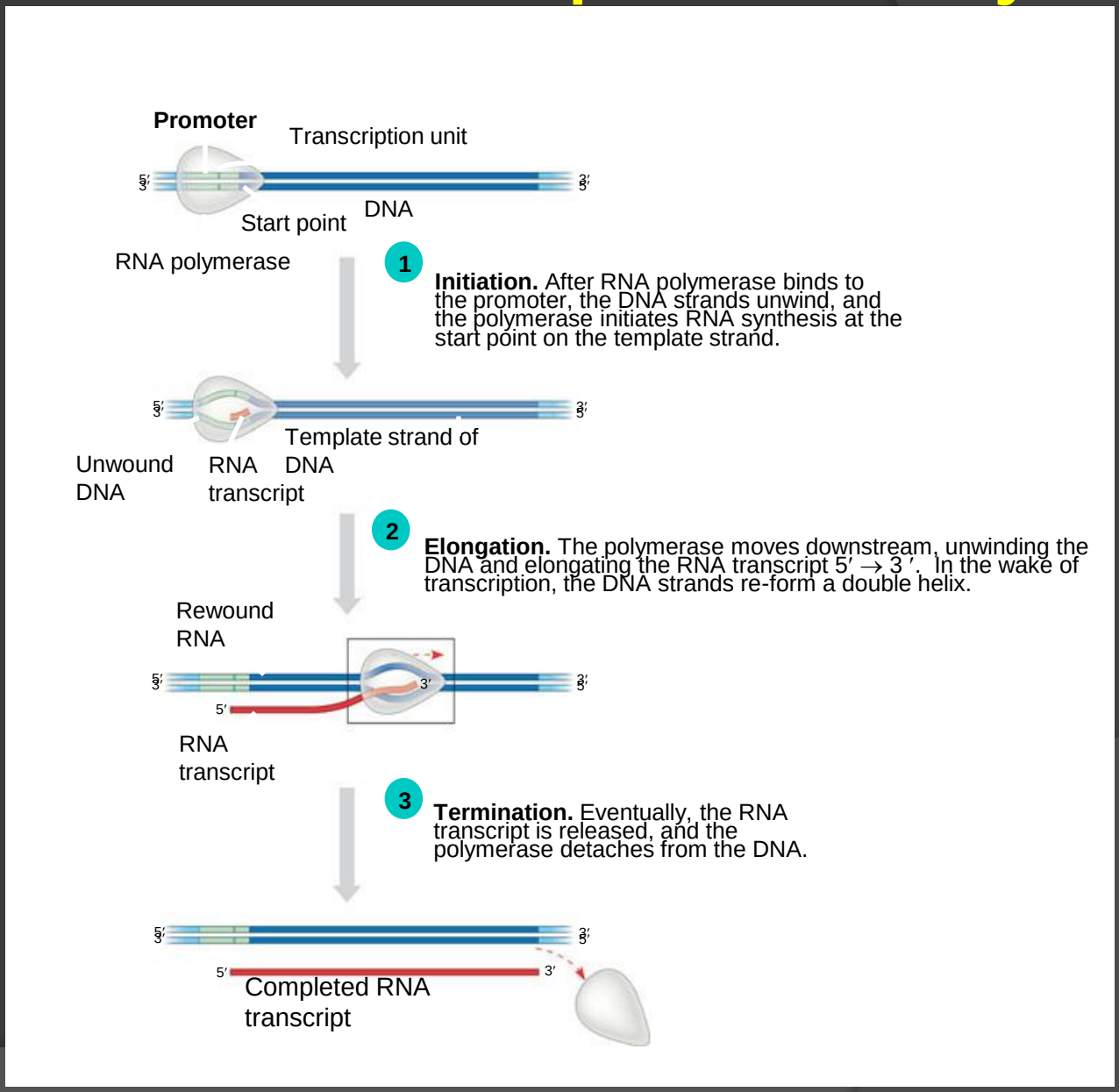


Figure 9.49 A rho factor pursues RNA polymerase along the RNA and can cause termination when it catches the enzyme pausing at a rho-dependent terminator.

Synthesis of an RNA Transcript: Summary

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



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