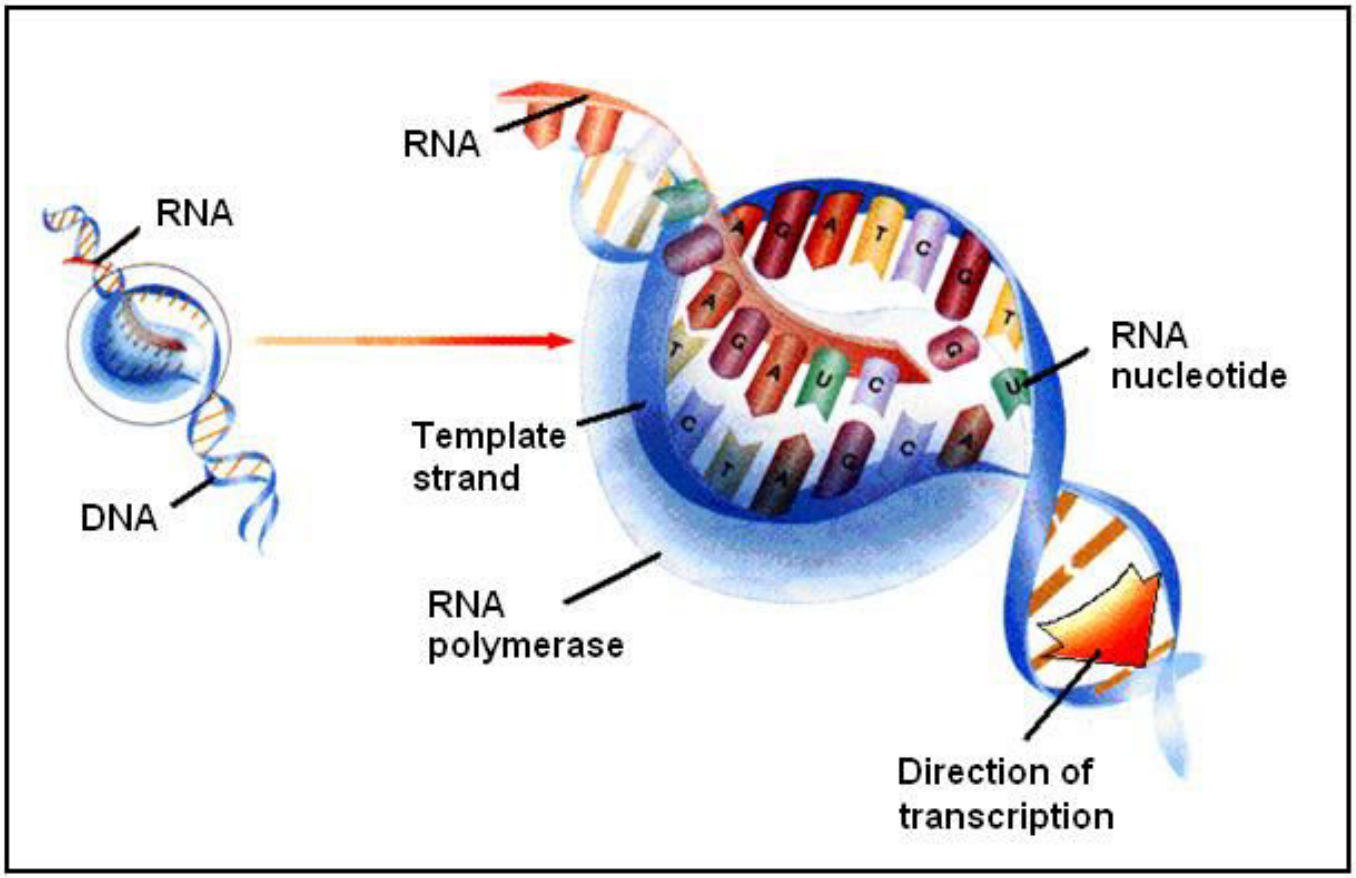
DNA TRANSCRIPTION



Compared structures of DNA and RNA

- DNA-Deoxyribonucleic acid
- Bases-cytosine, guanine, adenine and thymine
- Double stranded
- Function-store genetic information

RNA-Ribonucleic acid

Base-Cytosine, guanine, adenine and uracil

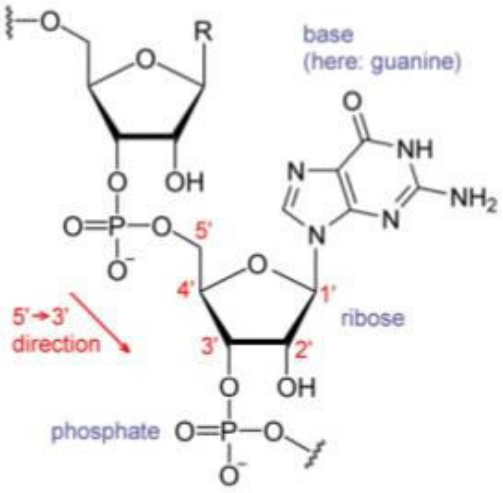
Single stranded

Functions-
rRNA-ribosomal RNA (makes up about 60% of ribosomal structure

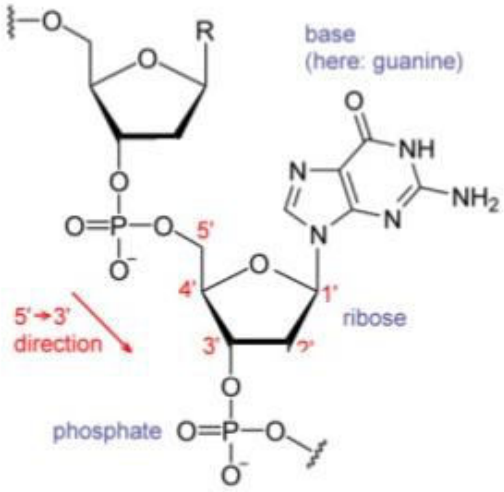
mRNA-messenger RNA (record information from DNA and carry it to ribosomes)

tRNA-transfer RNA (delivers amino acids to proteins at the ribosome to extend the chains)

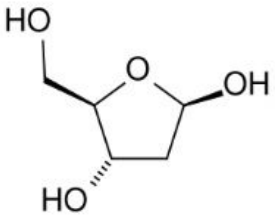
RNA nucleotide



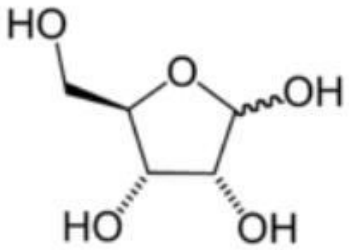
DNA nucleotide



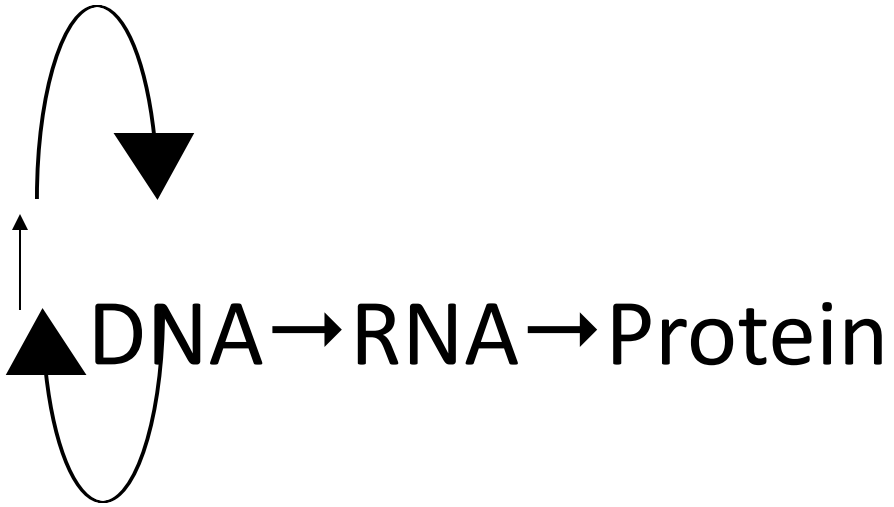
Deoxyribose



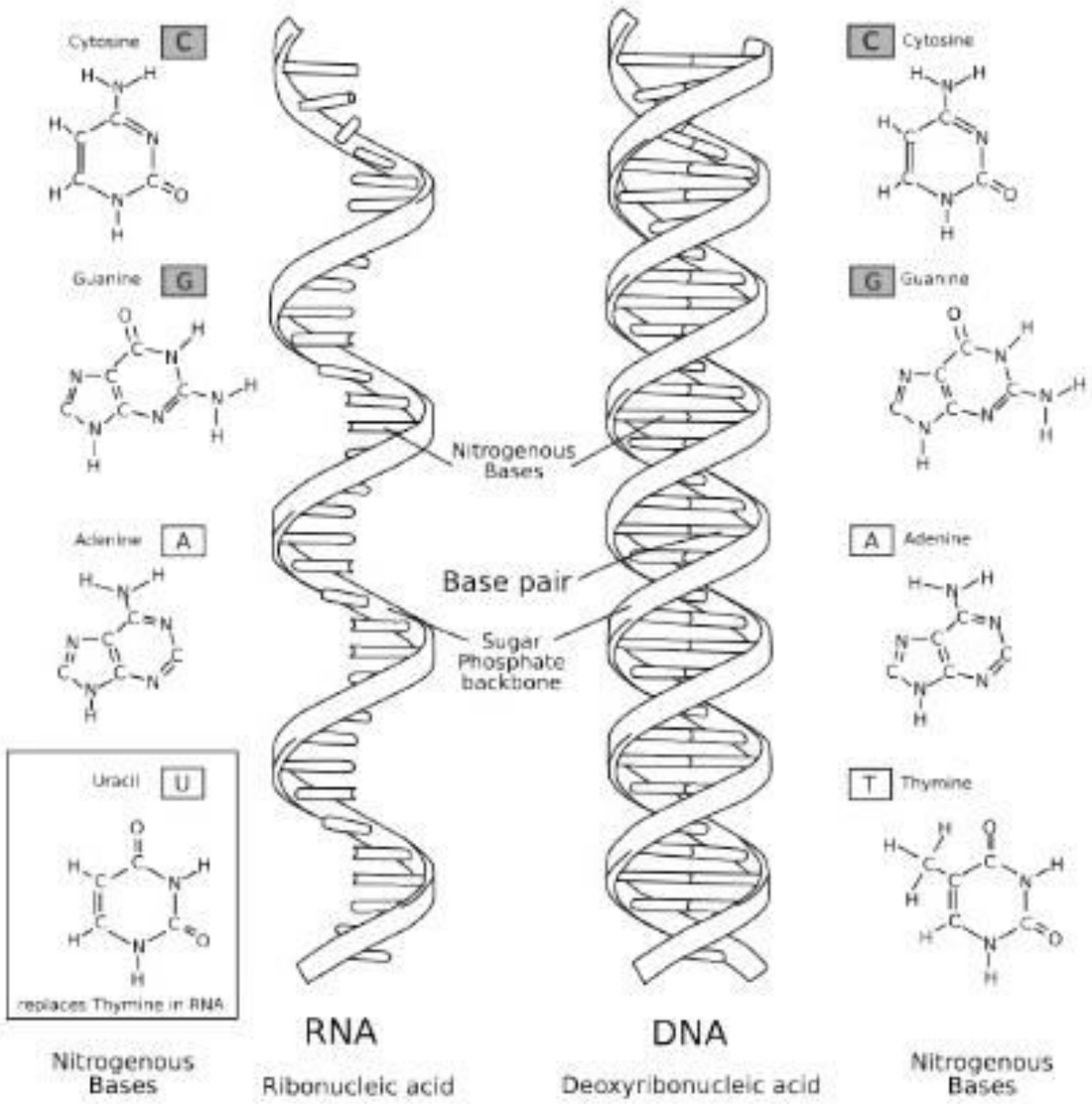
Ribose



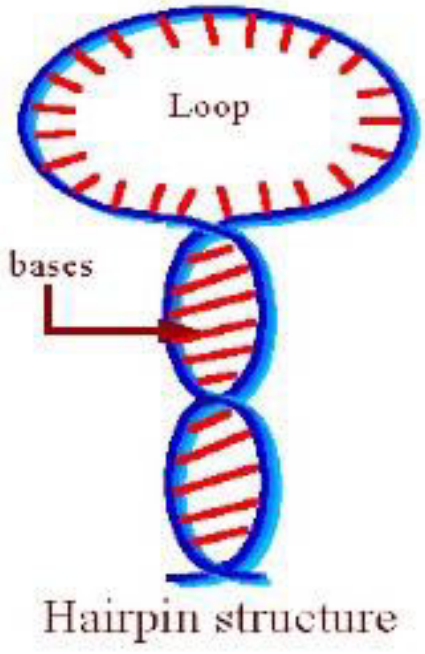
Flow of genetic information

- Information must be transcribed from DNA in order function further.
- 

Compared structure of DNA & RNA



Transcription



RNA single strand with hairpin loop

Transcription- The synthesis of mRNA from a DNA template

Occurs in the 5' ->3' direction

Involves RNA polymerase

mRNA, tRNA and rRNA must all be transcribed for protein synthesis to take place

the sequence of mRNA nucleotides determine the primary sequence of the polypeptides.

tRNA -carries the amino acids to mRNA

-tRNA folds in on itself

rRNA -major components of ribosomes

Steps in DNA Transcription

- Recognition and binding
- Initiation
- Elongation
- Termination and release

RNA polymerase

Polymerization catalyzed by RNA polymerase

- Can initiate synthesis
- Uses rNTPs
- Requires a template
- Unwinds and rewinds DNA

5 subunits, 449 kd (~1/2 size of DNA pol III) Core enzyme

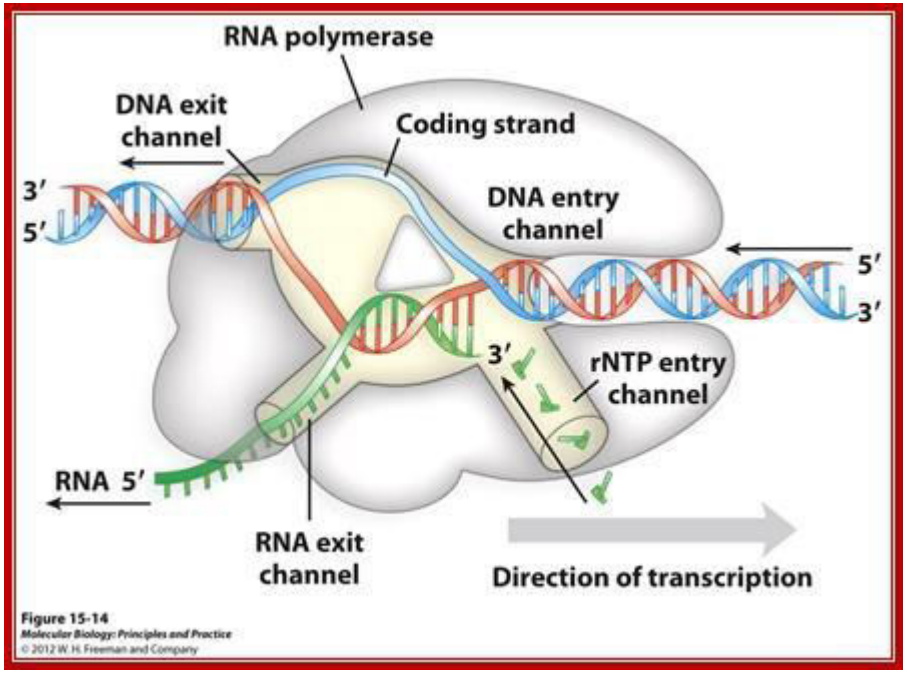
- 2 α subunits---hold enzyme together
- β--- links nucleotides together
- β'---binds templates

σ---recognition

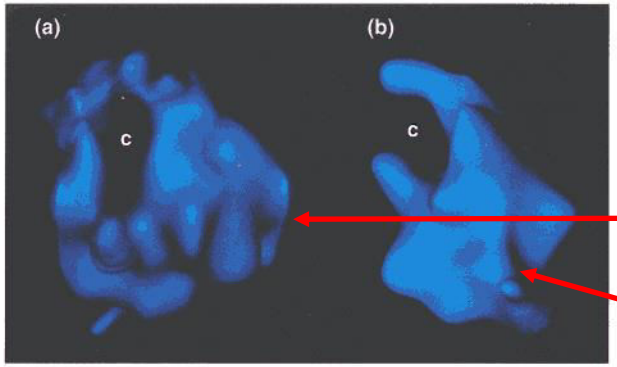
Holoenzyme= Core + sigma

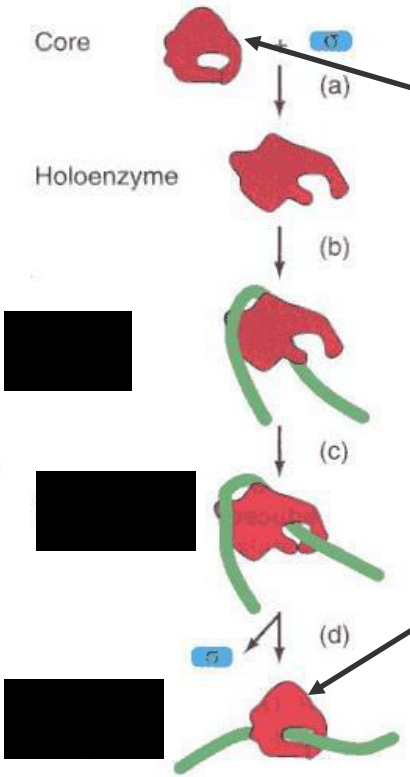
Function of RNA polymerase

- Starts at a promoter sequence, ends at termination signal
- Proceeds in 5' to 3' direction
- Forms a temporary DNA:RNA hybrid
- Has complete processivity



RNA Polymerase



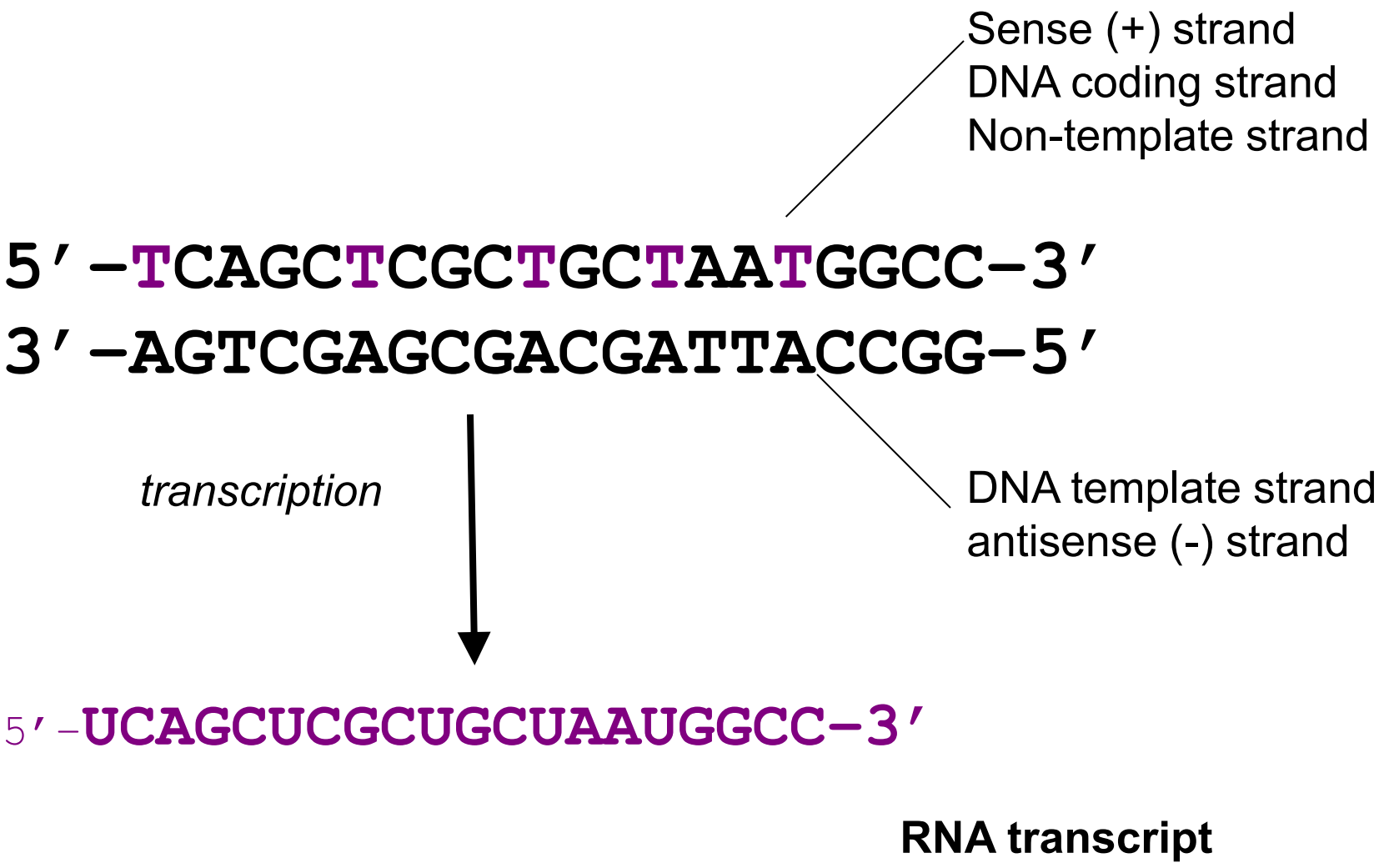


- X-ray studies reveal a "hand"
- Core enzyme closed
- Holoenzyme open
- Suggested mechanism
- NOTE: when sigma unattached, hand is closed
- RNA polymerase stays on DNA until termination.

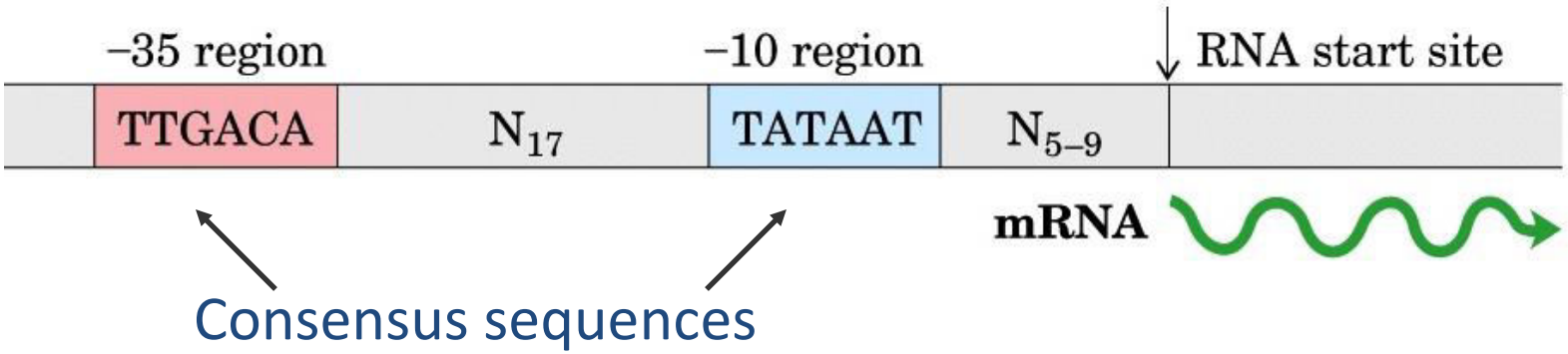
Recognition

- Template strand
- Coding strand
- Promoters
  - Binding sites for RNA pol on template strand
  - ~40 bp of specific sequences with a specific order and distance between them.
- Core promoter elements for E. coli
  - -10 box (Pribnow box)
  - -35 box
- Numbers refer to distance from transcription start site

Template and Coding Strands



Typical Prokaryote Promoter



- Pribnow box located at –10 (6-7bp)
- -35 sequence ~(6bp)

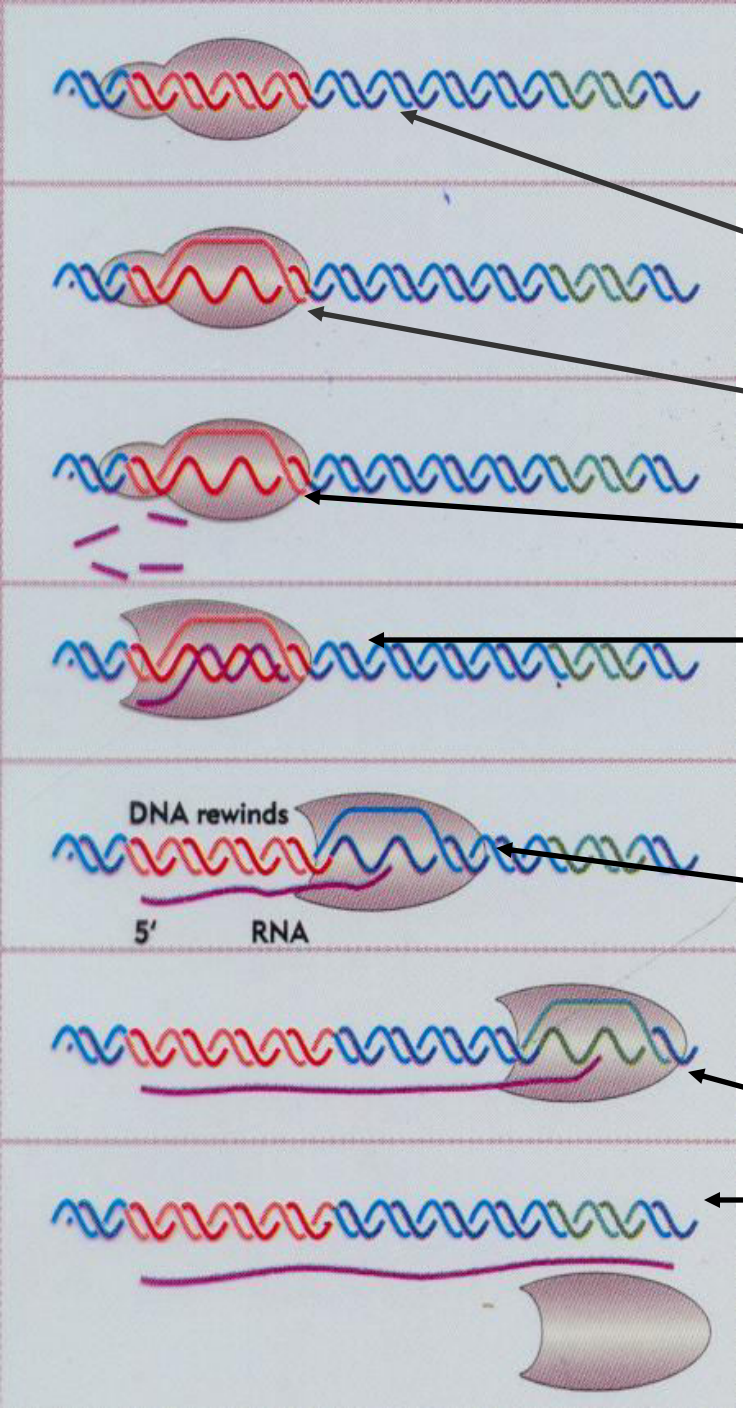
- Consensus sequences: Strongest promoters match consensus

  - Up mutation: mutation that makes promoter more like consensus
  - Down Mutation: virtually any mutation that alters a match with the consensus

In Addition to Core Promoter Elements

- UP (upstream promoter) elements
  - Ex. E. coli rRNA genes
- Gene activator proteins
  - Facilitate recognition of weak promoter

E. coli can regulate gene expression in many ways



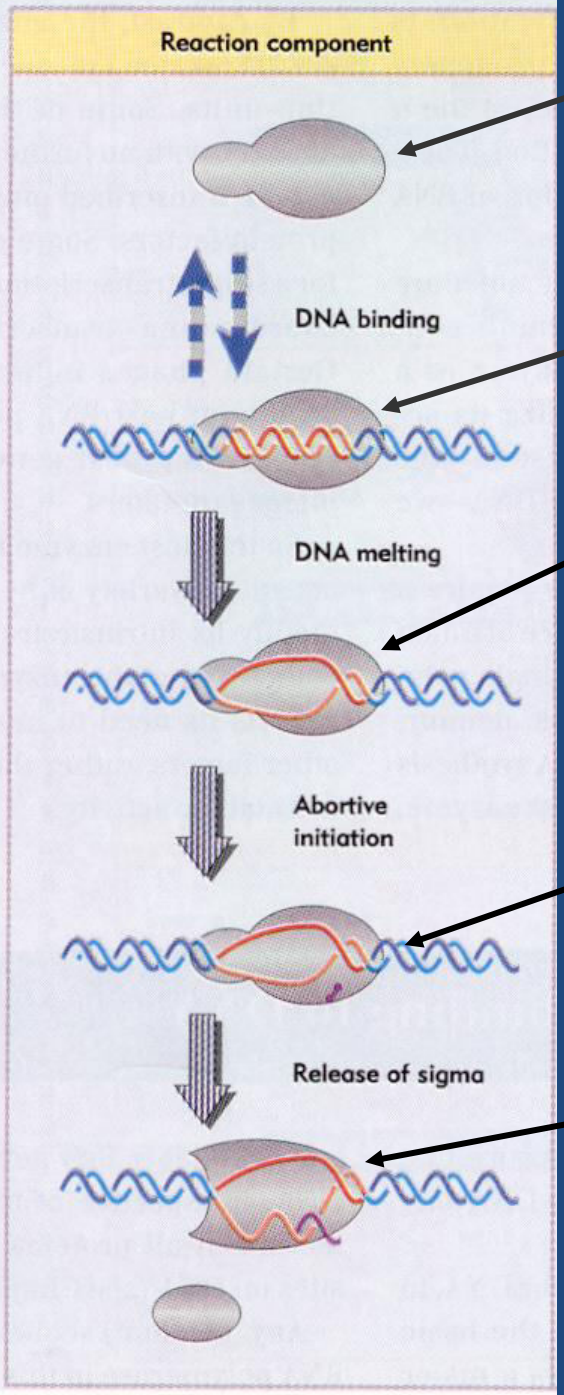
Stages of Transcription

- Template recognition
  - RNA pol binds to DNA
  - DNA unwound
- Initiation
- Elongation
  - RNA pol moves and synthesizes RNA
  - Unwound region moves
- Termination
  - RNA pol reaches end
  - RNA pol and RNA released
  - DNA duplex reforms

Transcription Initiation

- Steps
  - Formation of closed promoter (binary) complex
  - Formation of open promoter complex
  - Ternary complex (RNA, DNA, and enzyme), abortive initiation
  - Promoter clearance (elongation ternary complex)
    - First rnt becomes unpaired
    - Polymerase loses sigma
    - NusA binds - Binding of rho factor to RNA polymerase mediated by **nusA protein**
  - Ribonucleotides added to 3' end

RNA polymerase passes through several steps prior to elongation. A closed binary complex is converted to an open form and then into a ternary complex.



- Holoenzyme
  - Core + σ
- Closed (Promoter) Binary Complex
- Open binary complex
- Ternary complex
- Promoter clearance

Sigma (σ) Factor

- Essential for recognition of promoter
- Stimulates transcription
- Combines with holoenzyme
  - "open hand" conformation
  - Positions enzyme over promoter
- Does NOT stimulate elongation
- Falls off after 4-9 nt incorporated
- "Hand" closes

Much less conserved than other RNA pol subunits

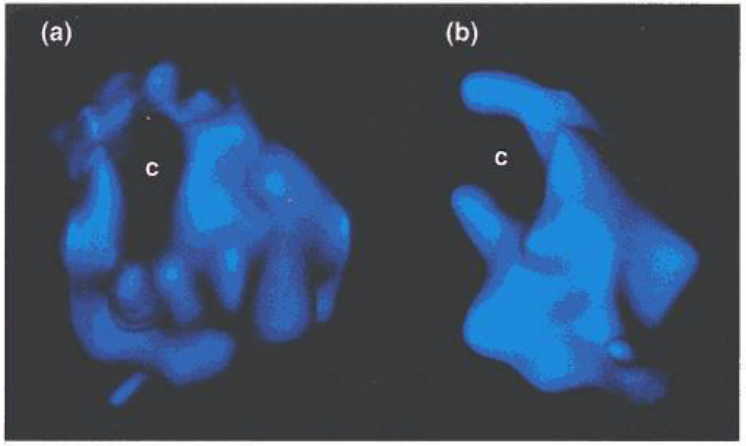


Figure 6.9 Overall shapes of *E. coli* RNA polymerase core (a) and holoenzyme (b) deduced from electron microscopy studies on two-dimensional crystals of the enzymes. The channel through the enzyme (denoted C) is closed in the core and open in the holoenzyme.

Sigma Variability in E. coli

- Sigma70 (-35)TTGACA (-10)TATAAT
  - Primary sigma factor, or housekeeping sigma factor.
- Sigma54 (-35)CTGGCAC (-10)TTGCA
  - alternative sigma factor involved in transcribing nitrogen-regulated genes (among others).
- Sigma32 (-35)TNNCNCNCTTGAA (-10)CCCATNT
  - heat shock factor involved in activation of genes after heat shock.
- POINT: gives E. coli flexibility in responding to different conditions

Promoter Clearance and Elongation

- Occurs after 4- 10 nt are added
- First rnt becomes unpaired from antisense (template) strand. ∴ DNA strands re-anneal
- Polymerase loses sigma, sigma recycled
  - Result "Closed hand" surrounds DNA
- NusA binds to core polymerase
- As each nt added to 3', another is melted from 5', allowing DNA to re-anneal.
- RNA pol/NusA complex stays on until termination. Rate=20-50nt/second.

Termination

- Occurs at specific sites on template strand called Terminators

- Two types of termination
  - Intrinsic terminators
  - Rho (ρ) dependent terminators

- Sequences required for termination are in transcript

- Variation in efficiencies.