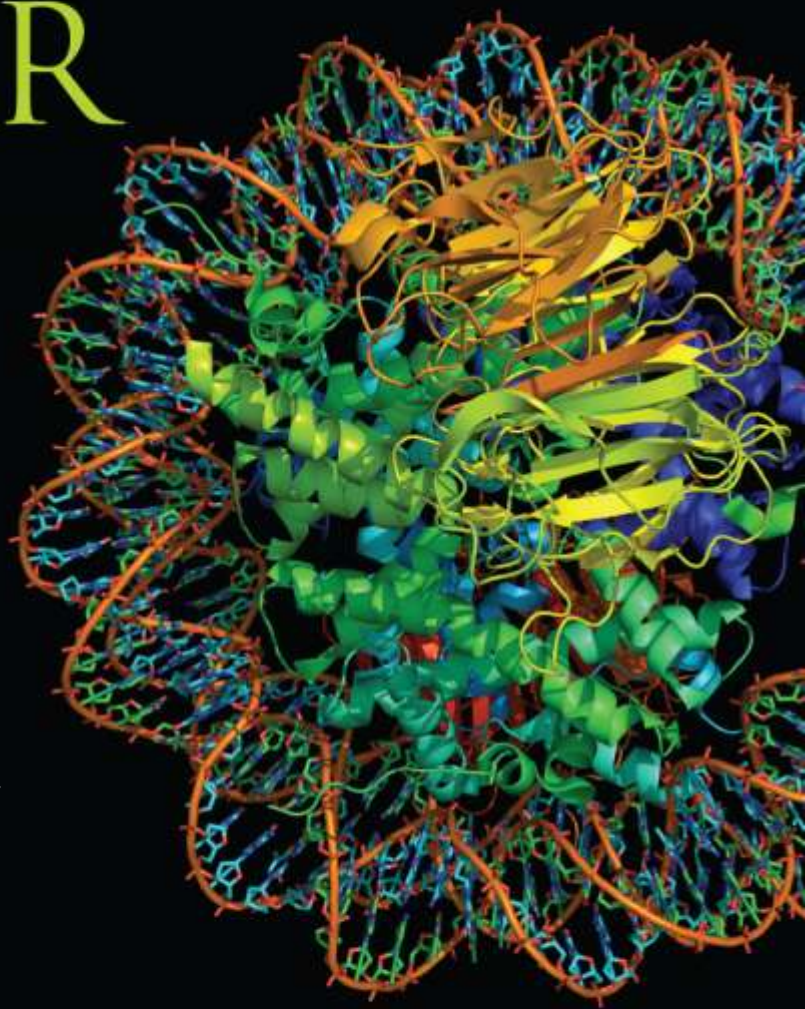
Principles of

MOLECULAR BIOLOGY

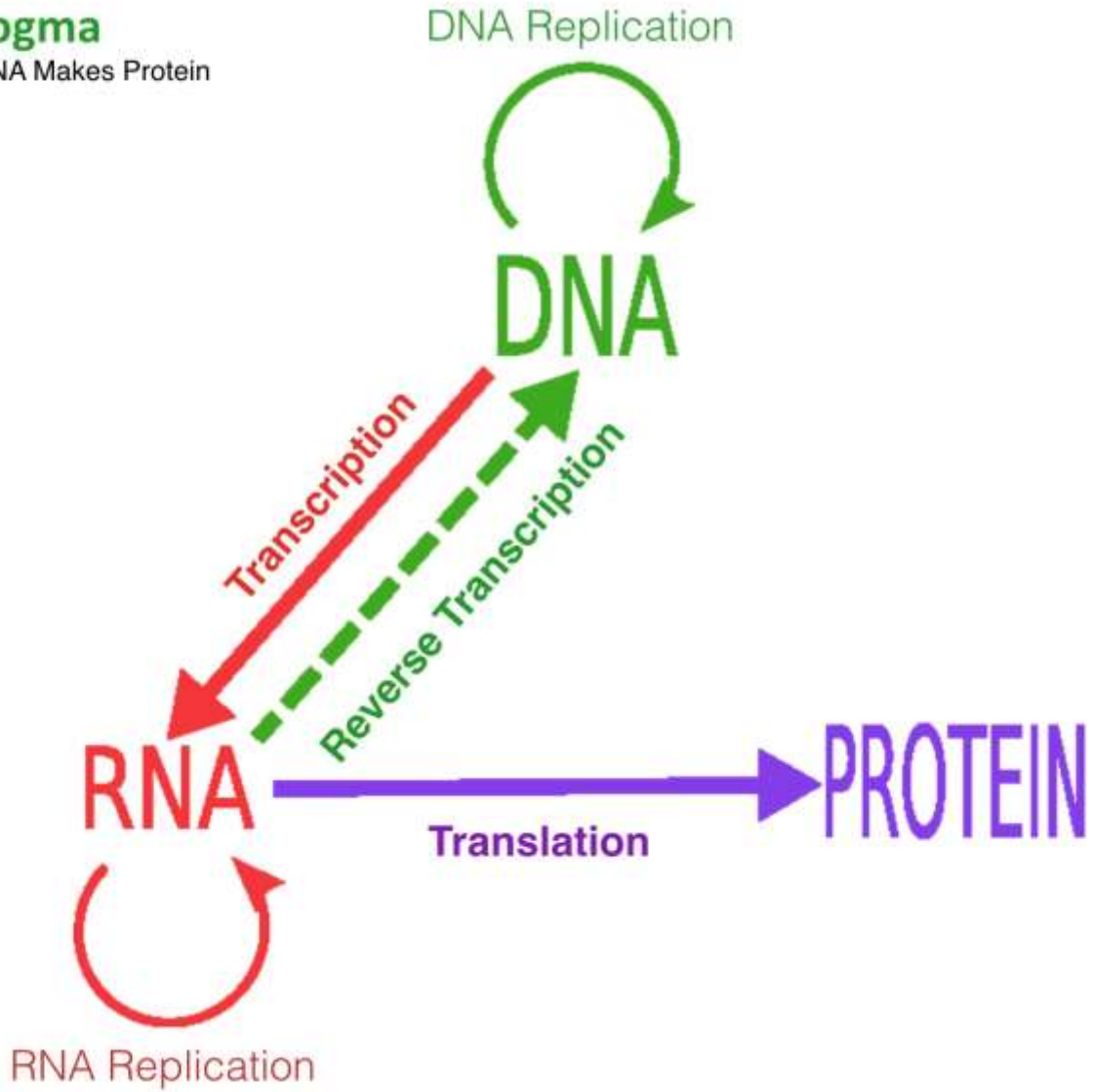
BURTON E. TROPP



Chapter 12
Bacterial Transcription and its Regulation

Central Dogma

- DNA Makes RNA Makes Protein



Transcription

- DNA Makes RNA

- Begins Near a Promoter
- Requires ATP, GTP, CTP, and UTP
- Uses Base-Pairing Rules of DNA
- Requires RNA Polymerase
- Proceeds in 5’ to 3’ Direction
- Only One Strand is Copied

Introduction to the Bacterial RNA Polymerase Catalyzed Reaction

RNA polymerase requires a DNA template and four nucleotide triphosphates to synthesize RNA

- *E. coli* has about 1,000 to 2,000 RNA polymerase molecules/cell
- Catalyzes nucleoside monophosphate group transfer from a NTP to the 3’-end of the growing RNA chain (or the first nucleoside triphosphate) in a 5’→3’ direction
- RNA polymerases can initiate chain growth **without a primer**

Introduction to the Bacterial RNA Polymerase Catalyzed Reaction

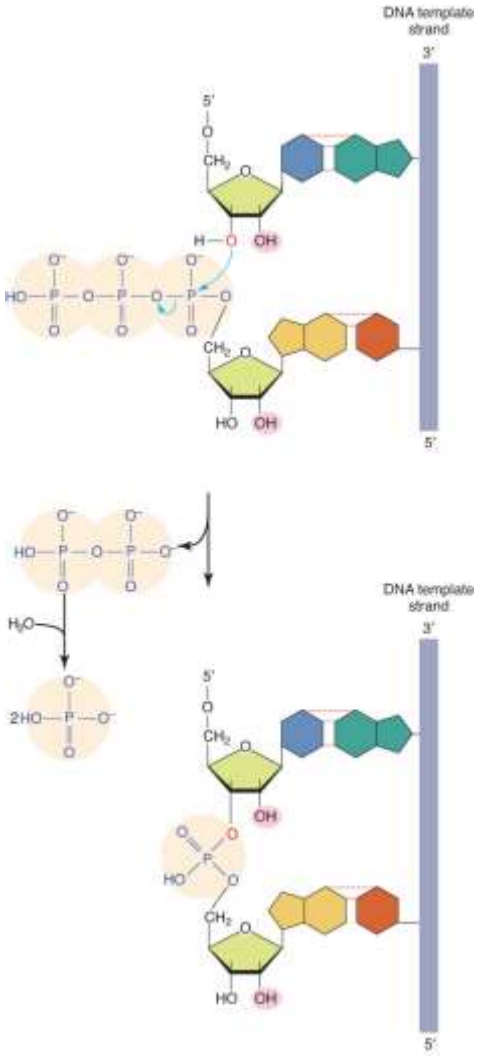


Figure 12.01: RNA polymerase catalyzed phosphodiester bond formation.

(Adapted from Berg, J. M., et al. 2002. Biochemistry (5th ed). W. H. Freeman and Company.)

Introduction to the Bacterial RNA Polymerase Catalyzed Reaction

- Only one of the two DNA strands in a given double-stranded DNA region acts as the **template strand**
- The complementary strand has the same sequence as the RNA (except U replaces T). This strand is referred to as the **coding** or **sense strand**
- The nucleotide at the transcription start site is designated +1
- Sequences that come after +1 (on the 3’ side) are downstream

Introduction to the Bacterial RNA Polymerase Catalyzed Reaction

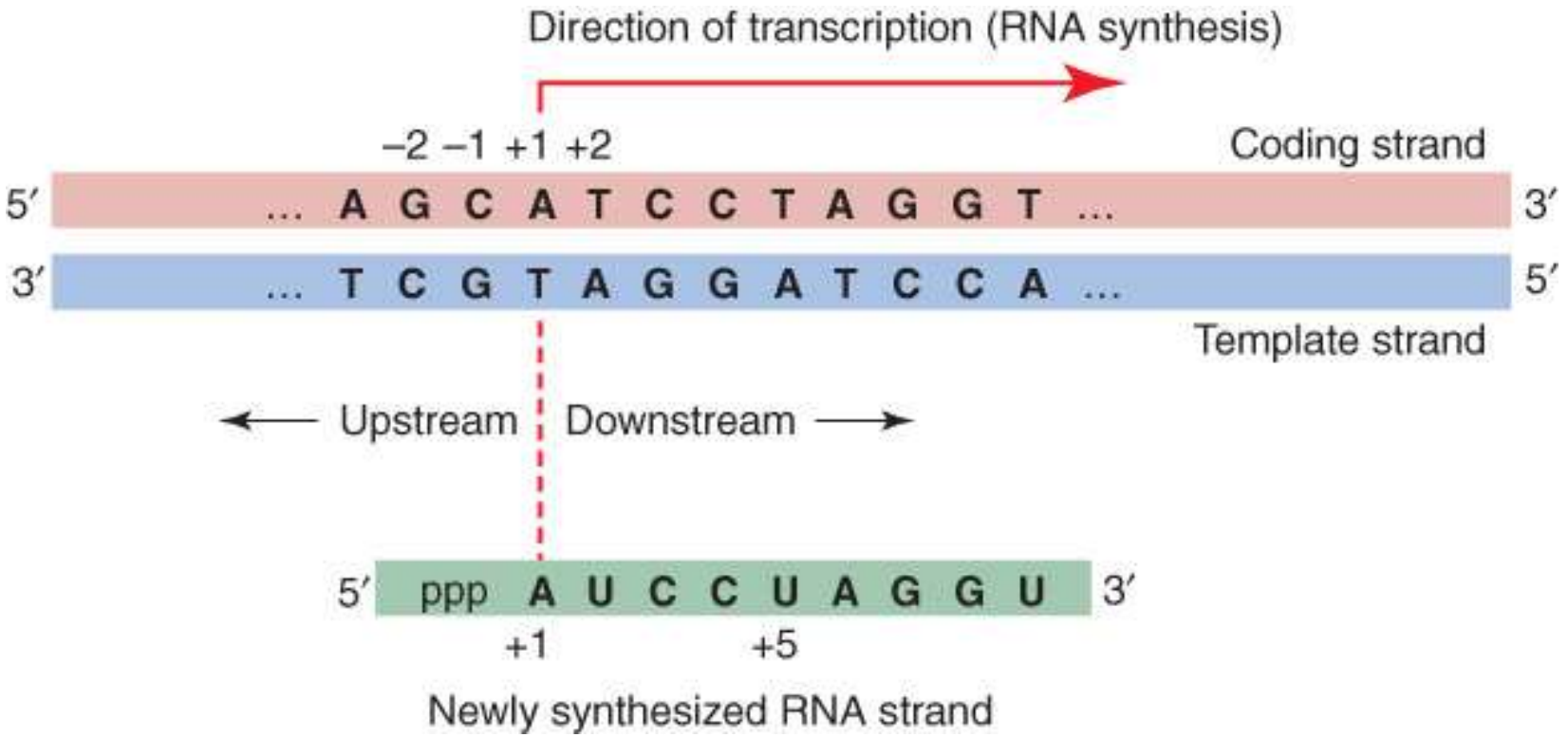
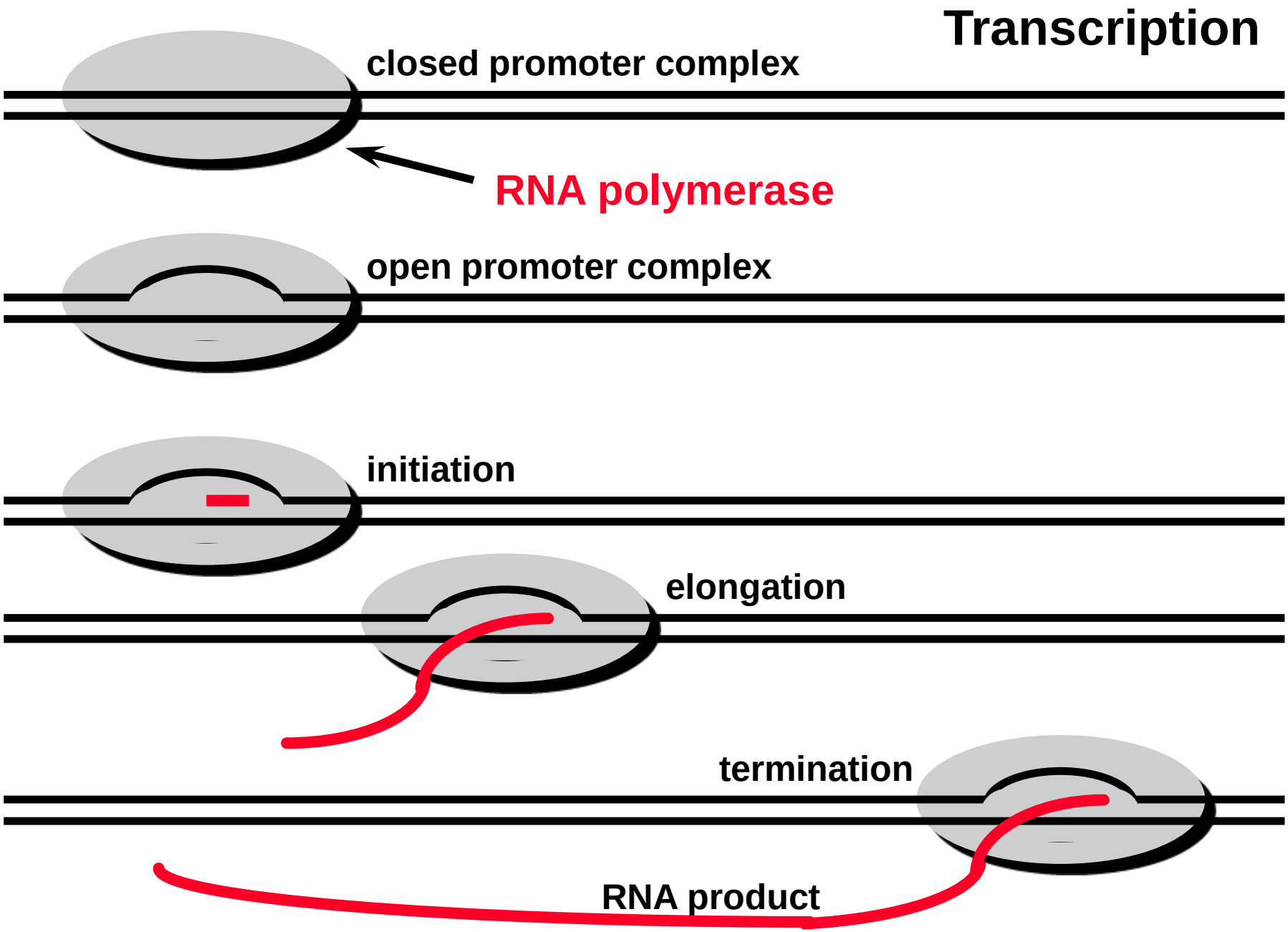


Figure 12.02: Rules for numbering nucleotides on the sense strand.

Introduction to the Bacterial RNA Polymerase Catalyzed Reaction

Bacterial RNA polymerases are large multisubunit proteins

- Total of six subunits
- $\alpha^2\beta\beta'\omega\sigma$
- Can recognize ≈ 4,300 genes, signaled by about 1,000 different binding sites


Transcription
closed promoter complex
RNA polymerase
open promoter complex
initiation
elongation
termination
RNA product

Initiation Stage

Bacterial RNA polymerase consists of a core enzyme and sigma factor

Early studies with holoenzyme without **ω** showed that $\alpha^2\beta\beta'\sigma$ can dissociate to form:

- core polymerase ($\alpha^2\beta\beta'$)
- Sigma factor (σ)

- $\alpha^2\beta\beta'$ core polymerase can synthesize RNA using single-stranded DNA or nicked double-stranded DNA as template, but it cannot use intact double-stranded DNA as a template
- Neither ω nor σ is required for phosphodiester bond formation

Initiation Stage

A transcription unit must have an initiation signal called a **promoter** for accurate and efficient transcription to take place

- In contrast to the core polymerase ($\alpha^2\beta\beta'$), the holoenzyme ($\alpha^2\beta\beta'\sigma$) can use intact double-stranded DNA as a template
- Transcription begins when the holoenzyme recognizes a specific transcription initiation sequence called a **promoter**
- Sigma factor is essential for promoter DNA recognition
- The primary *E. coli* σ factor is σ^{70}

Prokaryotic Control Sequences

-10 Sequence

T	A	T	A	A	T
77%	76%	60%	61%	56%	82%

-35 Sequence

T	T	G	A	C	A
69%	70%	61%	56%	54%	54%

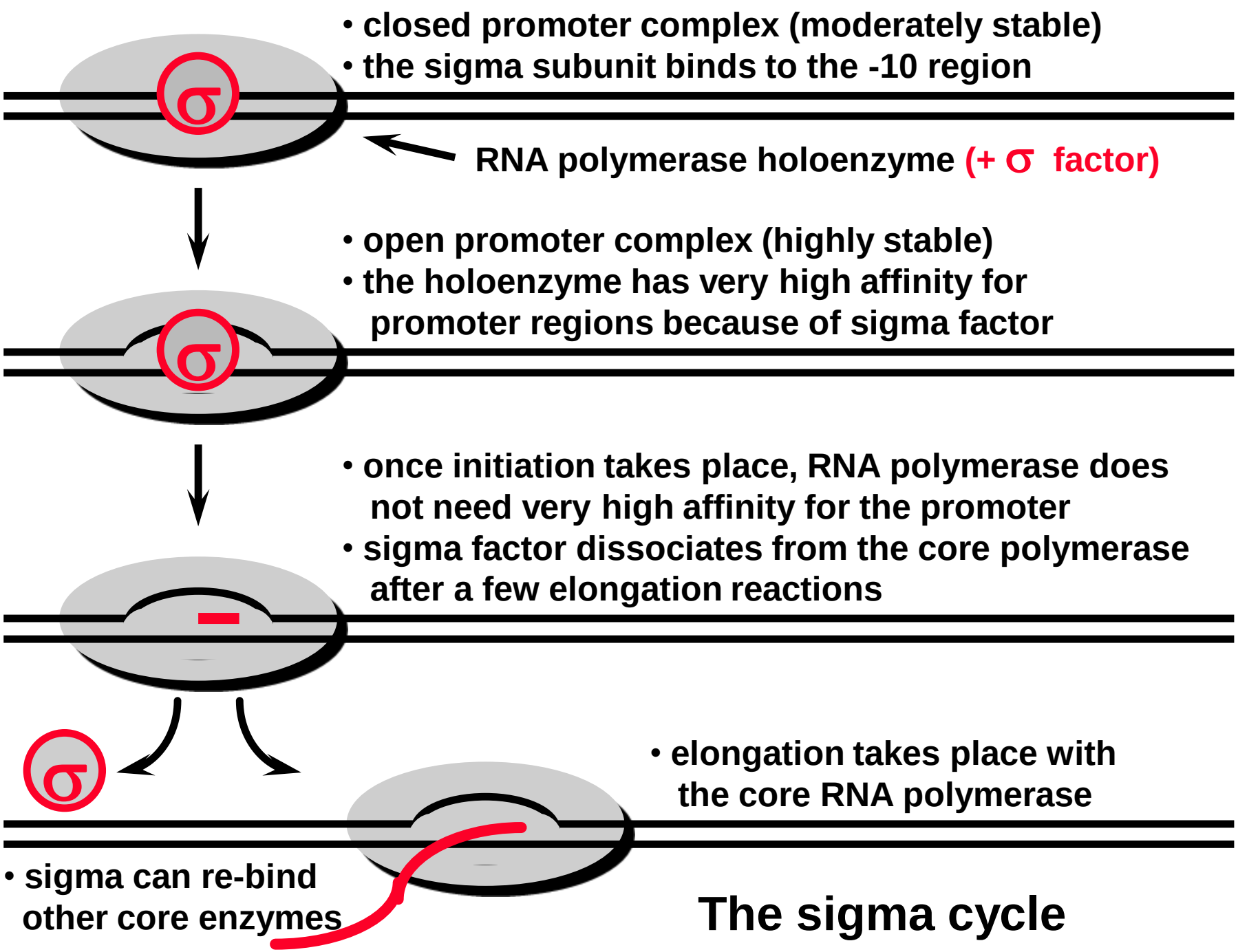
Initiation Stage

- -10 box
  - $T_{77}A_{76}T_{60}A_{61}A_{56}T_{82}$
- -35 box
  - $T_{69}T_{79}G_{61}A_{56}C_{54}A_{54}$
    - Subscript is the frequency of occurrence in 300 *E. coli* σ^{70} promoters
- Idealized sequence is termed a **consensus sequence**
- **Strong promoters** are close to the consensus sequence and have a spacer between the two boxes that is 17±1 bp

Initiation Stage

The σ^{70} RNA polymerase holoenzyme goes through several rounds of abortive initiation before promoter escape

- A significant amount of **abortive initiation** occurs during the early stages of the polymerase reaction
  - RNA synthesis halts and the nascent RNA is released (8-10 nts)
- Escape from abortive initiation is termed **promoter escape**



The sigma cycle

Initiation Stage

RNA polymerase "scrunches" DNA during transcription initiation

- RNA polymerase holoenzyme unwinds adjacent DNA and pulls it into itself during initial transcription (scrunching)
- Polymerase then rewinds the DNA when it leaves the initiation site and begins to move down the DNA (unscrunching)
- Energy stored during scrunching is used to break polymerase initiation/site interactions during promoter escape

Transcription Elongation Complex

The transcription elongation complex consists of core RNA polymerase, template DNA and a growing RNA chain

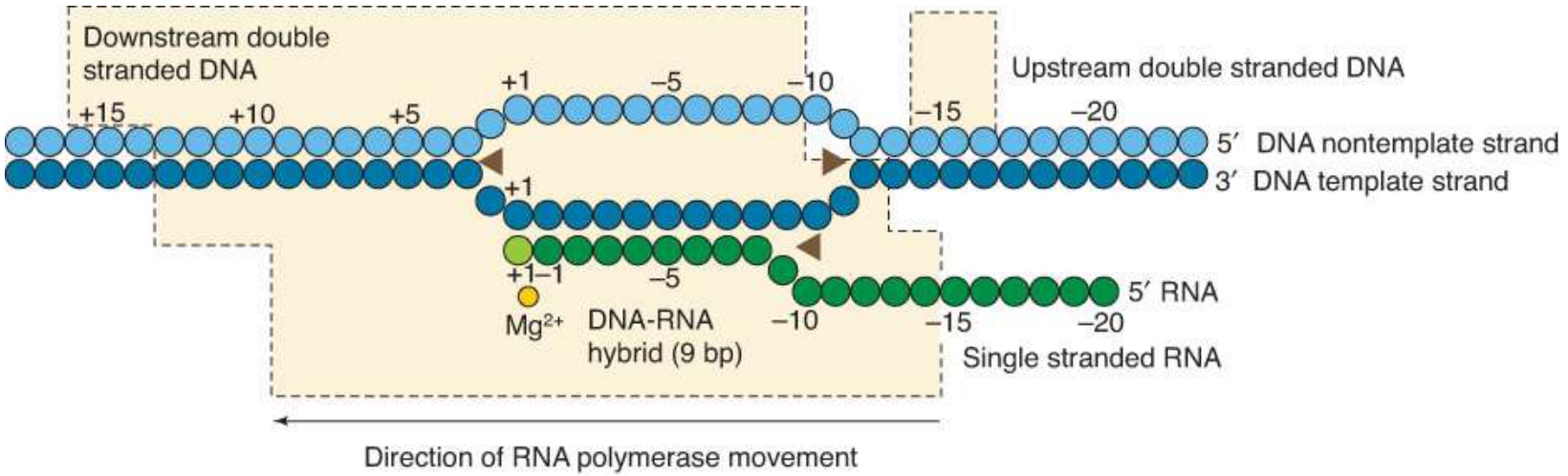


Figure 12.16: A schematic representation of nucleic acids within the transcription elongation complex. Transcription is taking place from right to left.

(Adapted from Darst, S. A. 2001. Curr Opin Struct Biol 11:155–162.)

Transcription Elongation Complex

- RNA chain elongation catalytic cycle

1. NTPs move through the secondary channel to reach the catalytic site (rate limiting step because only a 1 in 4 chance the correct nucleotide will move through)
2. 3’-hydroxyl group at the growing end of the RNA strand makes a nucleophilic attack on the α-phosphoryl group of the incoming NTP to form the phosphodiester bond
3. Polymerase moves one nt downstream at a rate of 30nts/sec
   - Incoming NTPs provide driving force energy

Transcription Elongation Complex

Pauses influence the overall transcription elongation rate

- RNA polymerase does not move along the DNA template at a constant rate
  - Spends more time at **transcriptional pause sites**
    - Helps to synchronize transcription and translation
    - Slows RNA polymerase to allow regulatory protein interaction
    - Leads to **transcription arrest** and **transcription termination**

Transcription Elongation Complex

RNA polymerase can detect and remove incorrectly incorporated nucleotides

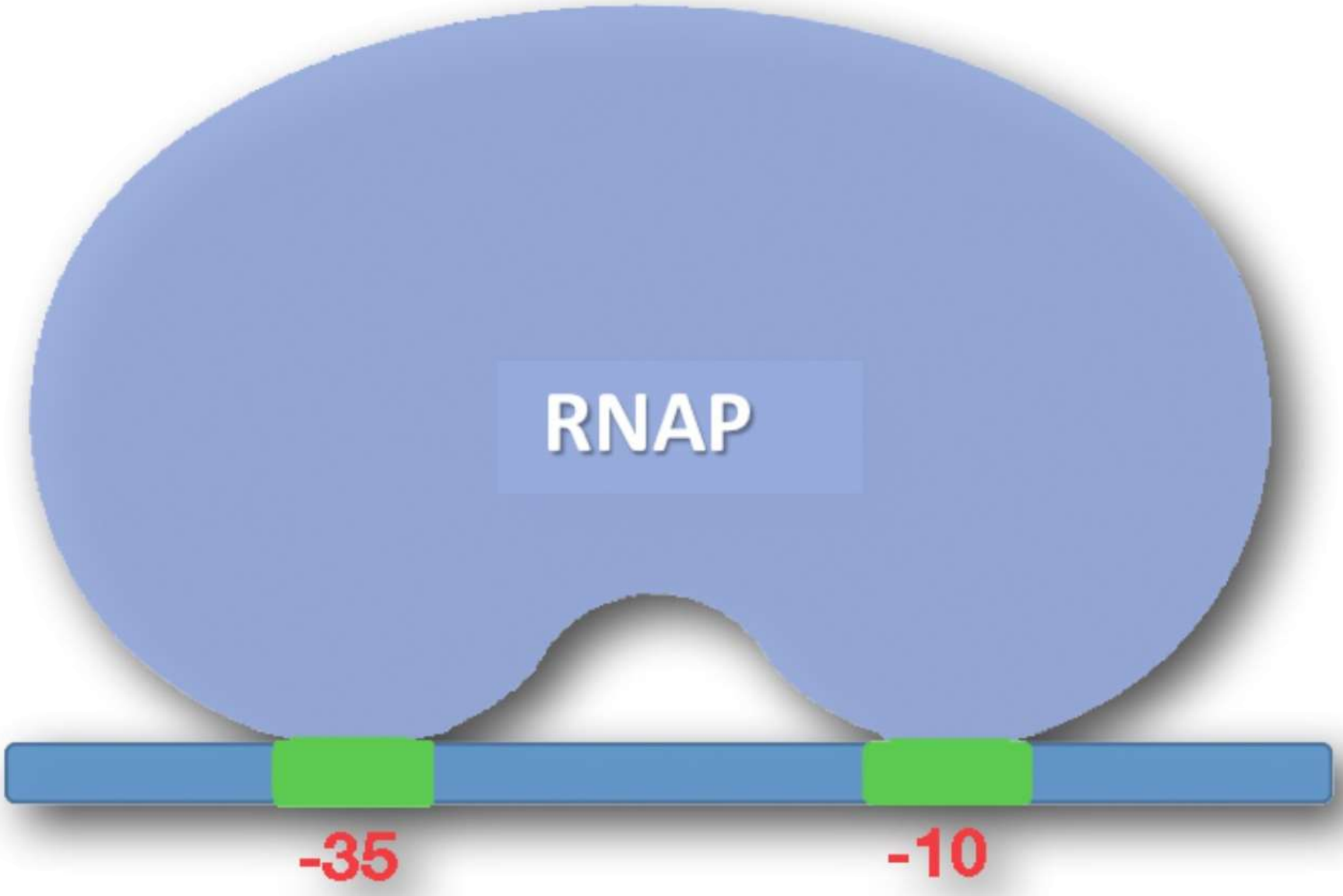
- Pausing is an important step in proofreading
- When a nucleotide mismatch is present in the DNA-RNA hybrid region, RNA polymerase pauses then backtracks
- The mismatch can be removed by RNA polymerase or by transcription elongation factors GreA (2-3 nt removal) or GreB (up to 18 nts removed)

Transcription Termination

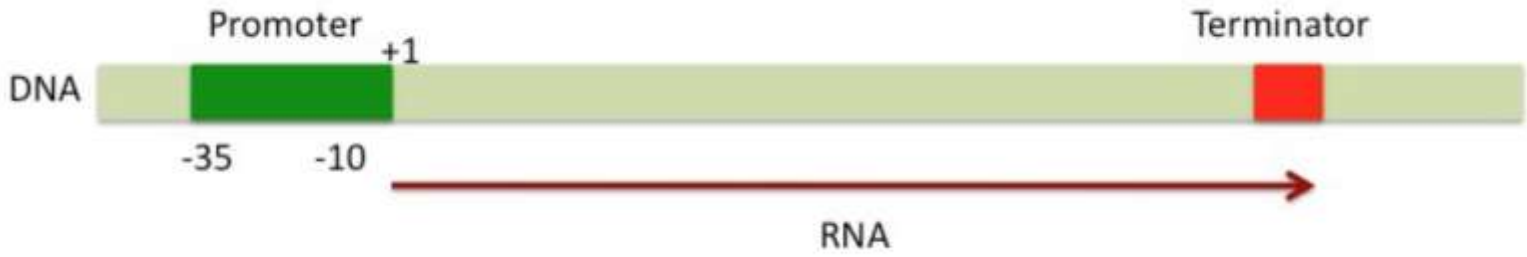
Bacterial transcription machinery releases RNA strands at intrinsic and Rho-dependent terminators

- RNA polymerase is a highly processive macromolecular machine
- Two transcription termination pathways contribute about equally in E. coli
  - **Intrinsic termination**
    - Utilizes a secondary structure that is formed in the 3’ end of the nascent RNA strand
  - **Rho-dependent termination**
    - Requires the **Rho factor**

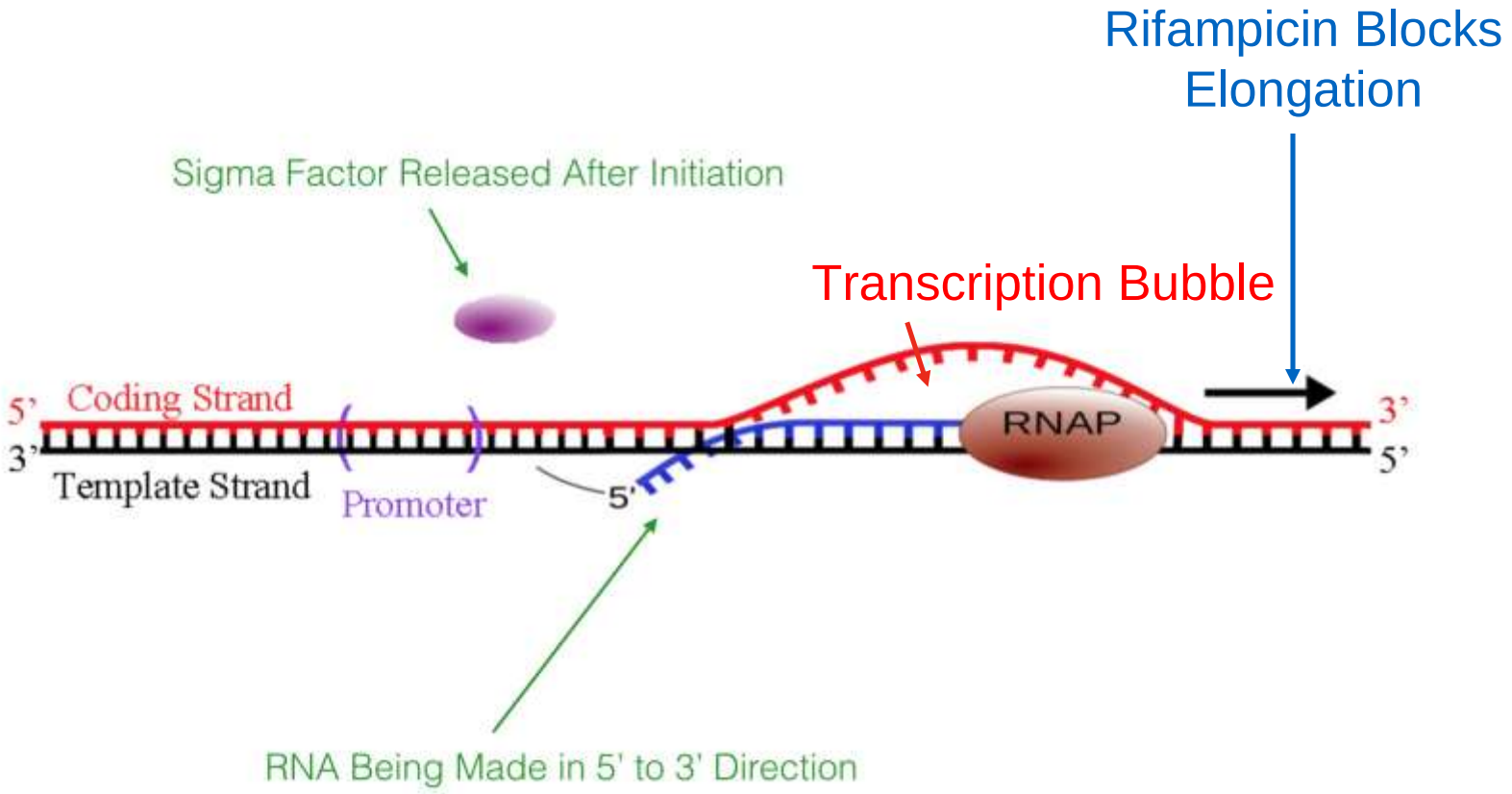
Prokaryotic RNA Polymerase (RNAP)



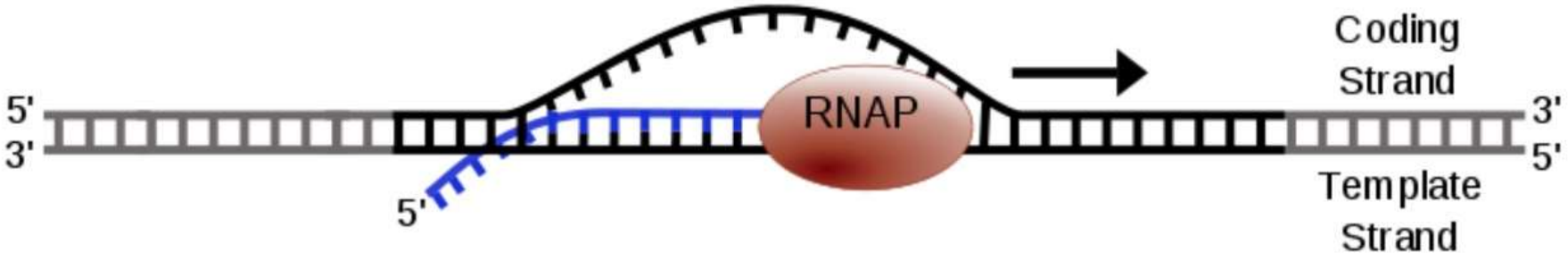
Structure



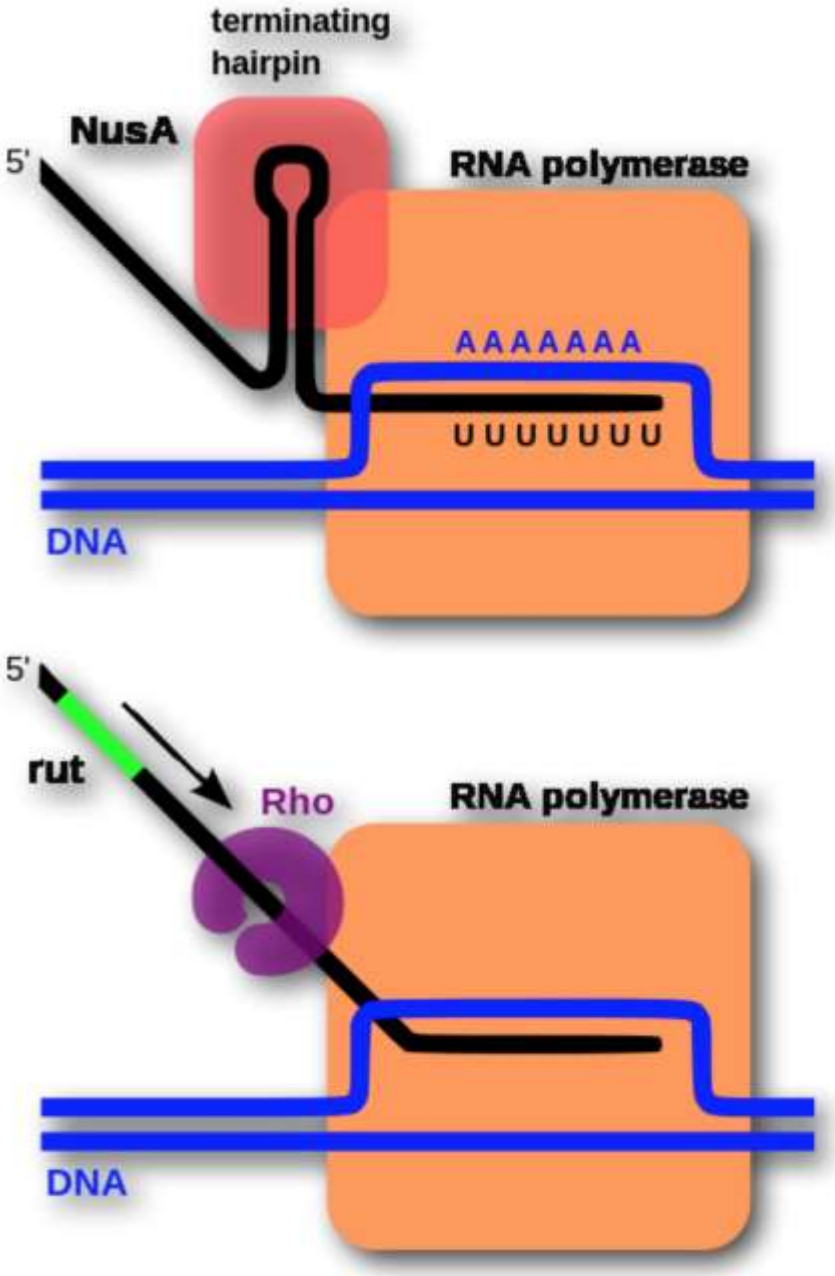
Transcription Initiation and Elongation



Transcription Elongation

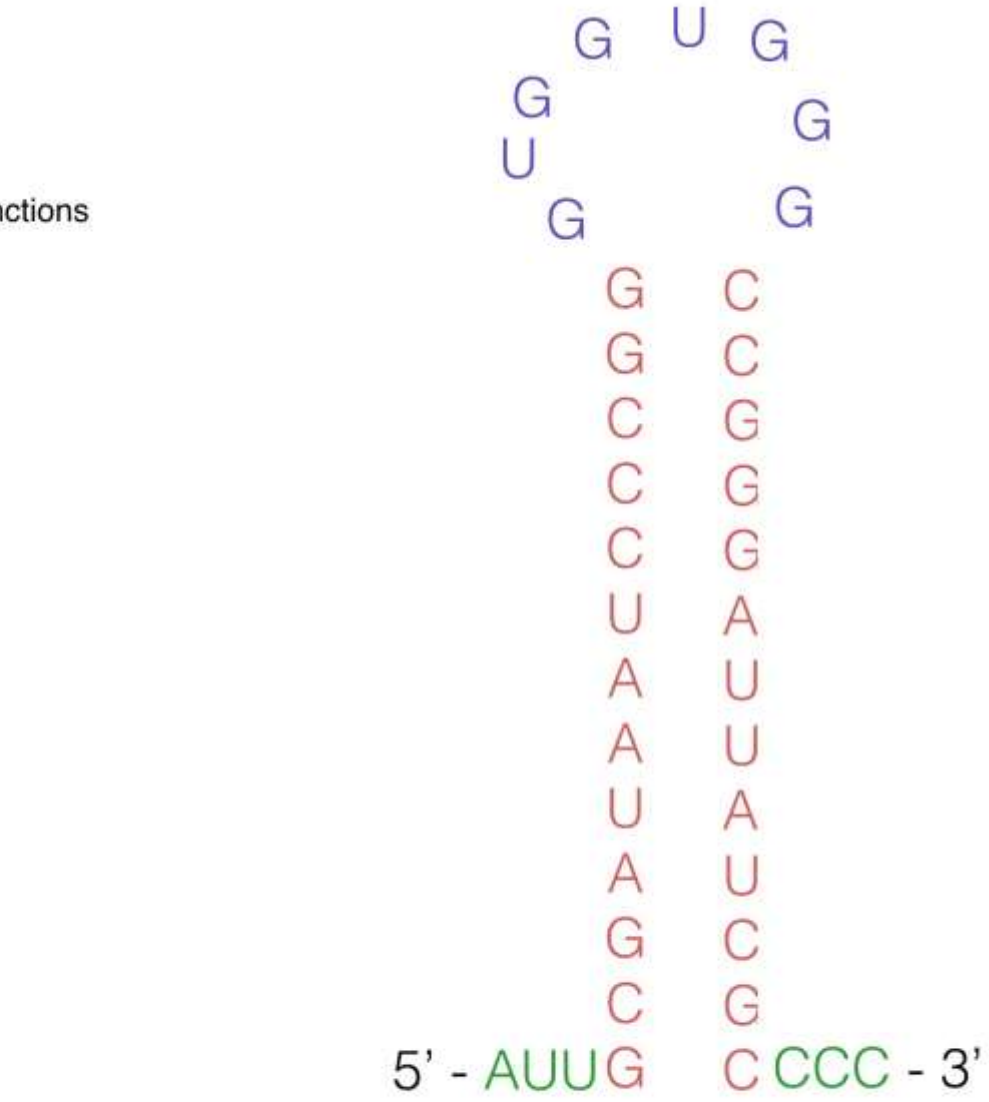


Transcription Terminatio



RNA

- Structures and Functions



Stem-Loop Structure

5' - AUUGCGAUAAUCCCGGGUGGUGGGCCGGGAUUAUCGCCCC - 3'

Termination

- Rho Factor Termination - Rho Protein "Climbs" RNA Being Synthesized and Removes RNA Polymerase from DNA
- Factor Independent - Sequence in RNA Causes RNA Polymerase to Fall Off of DNA

Transcription Termination

Rho factor loads onto the nascent RNA at the **Rho utilization (*rut*) site**

- Rho factor moves up the RNA strand in pursuit of RNA polymerase and allows polymerase to release

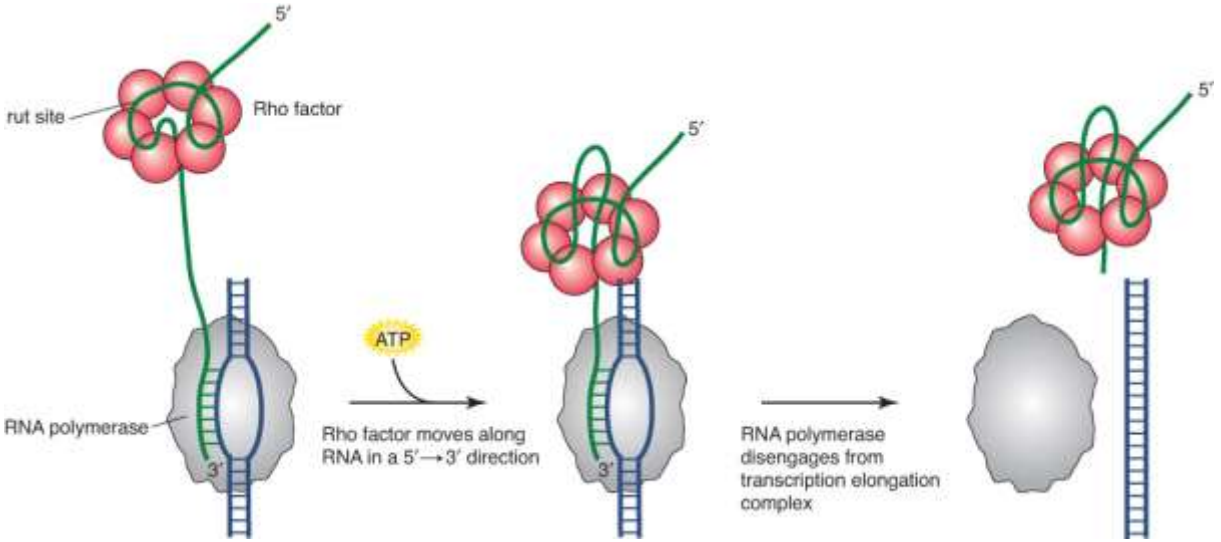


Figure 12.20: Rho factor transcription termination.

(Modified from Curr. Biol., vol. 13, D. L. Kaplan and M. O'Donnell, Rho Factor: Transcription Termination in Four Steps, pp. R714–R716, copyright 2003, with permission from Elsevier [http://www.sciencedirect.com/science/journal/09609822].)

Messenger RNA

Bacterial gene regulation has several features that eukaryotic systems cannot have

- Transcription and translation are occurring in the same compartment
  - Translation of the 5’ end of the mRNA can occur before the 3’ end is transcribed
  - No chance to alter or process the mRNA
- Bacterial mRNA molecules often contain two or more **cistrons** (a sequence with an open reading frame and translation start and stop signals)
  - **Polycistronic** mRNA

Messenger RNA

- Each cistron in a polycistronic mRNA codes a specific polypeptide chain
  - A way to regulate synthesis of several related proteins

- Bacterial mRNA usually has a short lifetime compared with other kinds of bacterial RNA
  - The half-life of a typical bacterial mRNA is only a few minutes

Messenger RNA

The molecular mechanism of mRNA regulation can be divided into two major categories:

- **Negative regulation**
  - A repressor turns off transcription
- **Positive regulation**
  - An activator turns on transcription

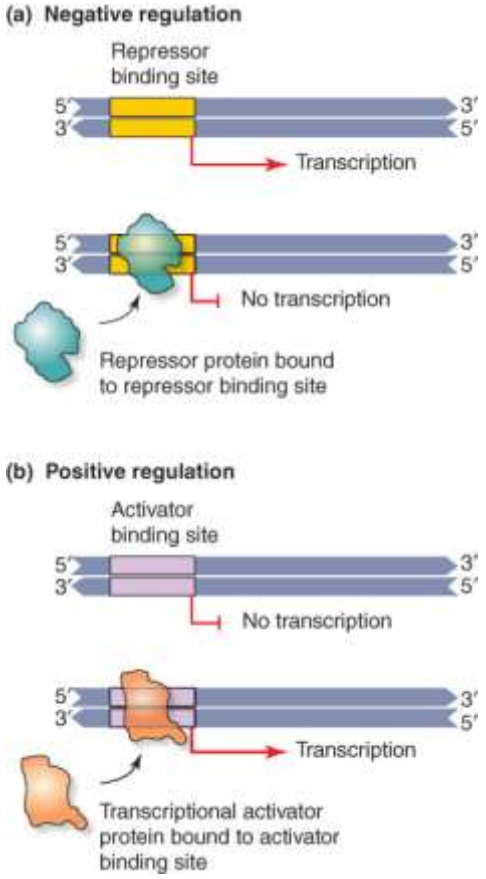


Figure 12.21: The distinction between negative and positive regulation.

Lactose Operon

The *E. coli* genes *lacZ*, *lacY* and *lacA* code for β-galactosidase, lactose permease and β-galactosidase transacetylase, respectively

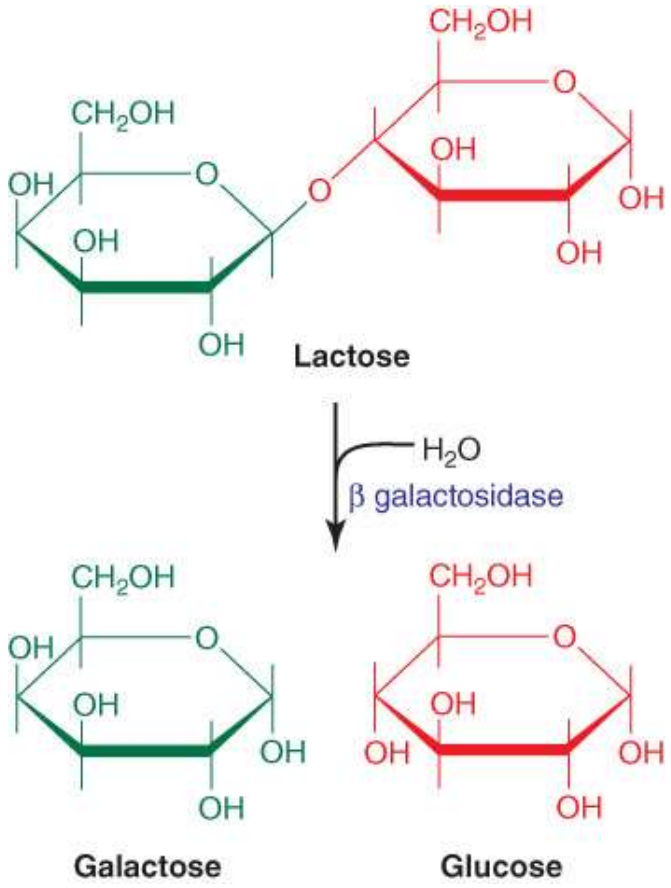


Figure 12.22: b-Galactosidase catalyzed lactose hydrolysis.

Lactose Operon

In lactose free media the *lac* enzymes are scarce (1-2 molecules/cell)

- When lactose is added the concentration of all of the proteins increases simultaneously (10^5 molecules/cell)
- Said to be **inducible** enzymes
- Others are **repressible** or **constitutive**

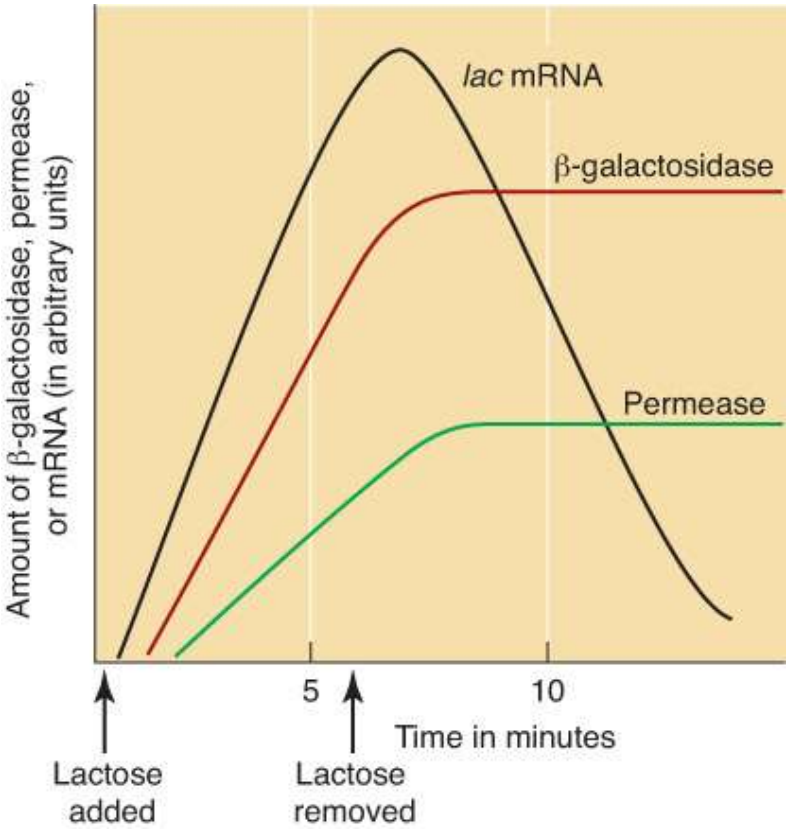


Figure 12.25: The on-off nature of the lac system.

Lactose Operon

The **operon model** explains the regulation of the lactose system

1. The products of *lacZ*, *lacY* and *lacA* are encoded in a single polycistronic mRNA
2. The promoter is adjacent to the *lacO* region
3. The operator is a DNA sequence that binds the repressor
4. When repressor is bound to operator transcription cannot take place
5. Inducer (lactose) binds to the repressor releasing it from the operator **- derepression**

Lactose Operon

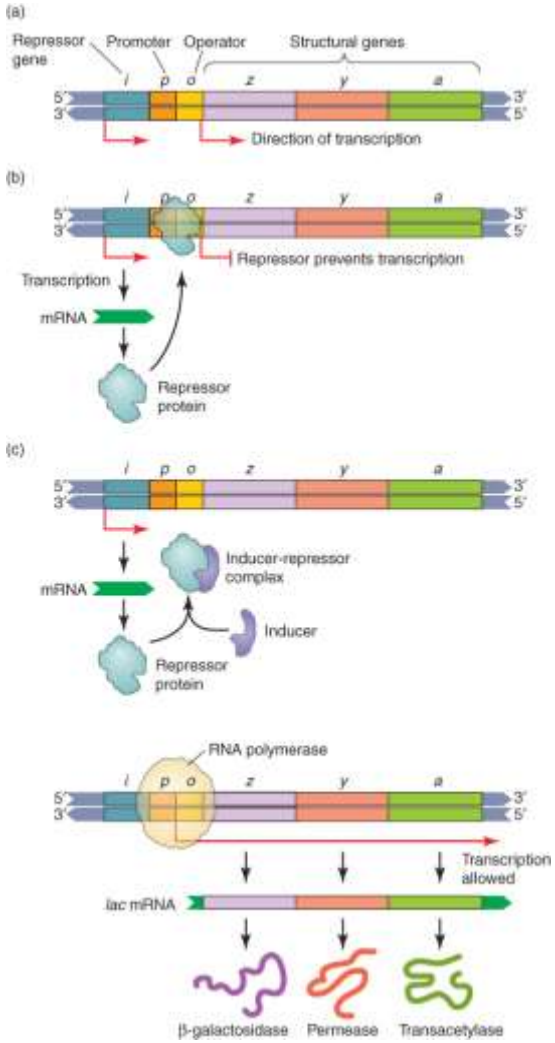


Figure 12.27: Lac operon model.

Lactose Operon

Two related problems became apparent

1. Inducers must enter the cell, but lactose transport requires permease and permease requires induction
2. Lactose does not bind to the repressor!

- Both problems are solved in the same way
  - In the uninduced state a small amount of lac mRNA is still produced (1 mRNA/cell)
    - Basal synthesis keeps a few molecules of permease and β-galactosidase around
  - β-galactosidase converts lactose to allolactose which is a potent inducer