- Regulation of gene expression
  - Prokaryotes
  - Eukaryotes
- Epigenetics
- None-coding RNAs and their importance
- Reprogramming, Transdifferentiation and their importance
- Study of genomic function (Genomics, transcriptomics and proteomics)
  - Sequencing
  - Mutation screening methods
  - Hybridization
  - Gene expression analysis methods & applications
  - Molecular markers and applications
  - Cytogenetic (Basic Laboratory Procedures)

- Molecular genetic of stem cells
  - iPS production
- Manipulating gene and cells
  - How to clone a gene and applications
  - Gene transfer to animal cell and applications
    - Stem cell gene therapy

Regulation of Gene expression in Prokaryotes

DNA function

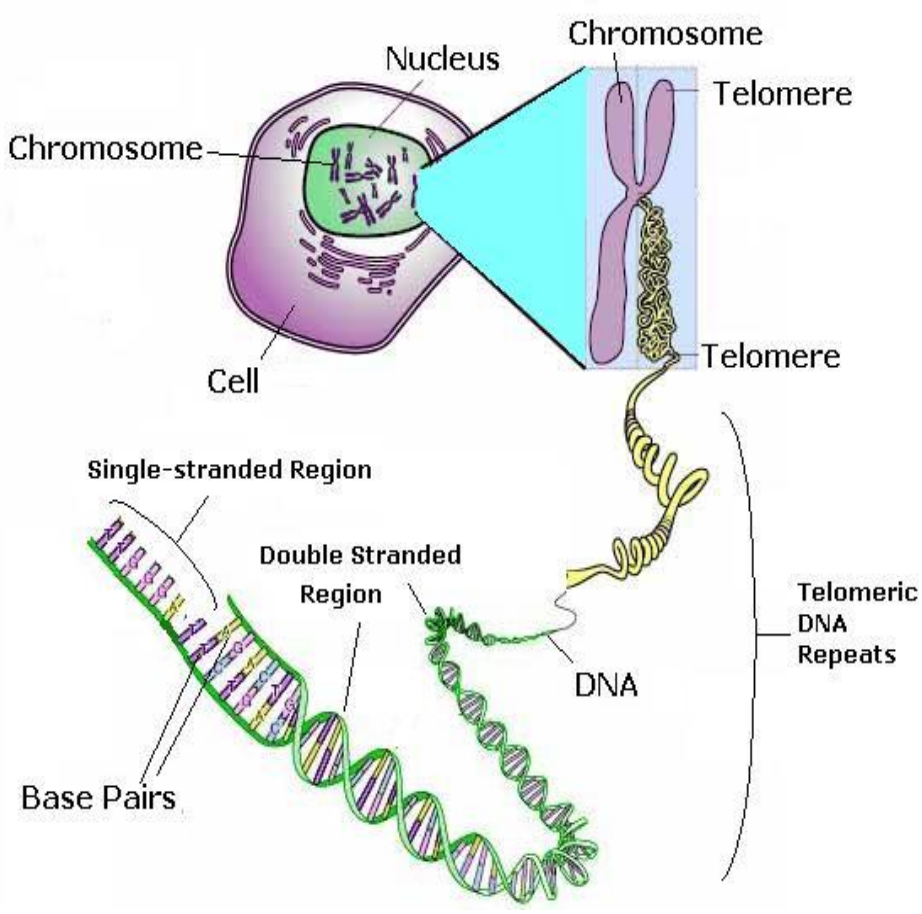
- **Store and transmit** genetic information needed for **all cell functions**

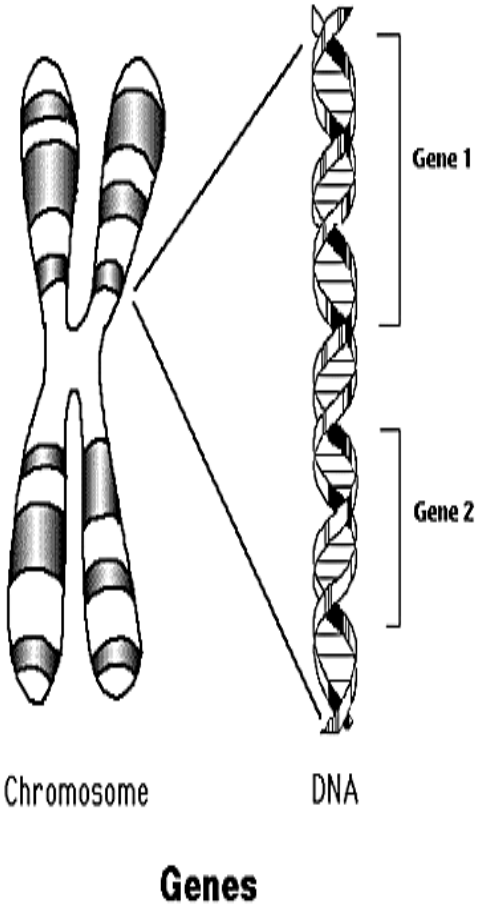
- Put information to work to **determine an organism's characteristics**

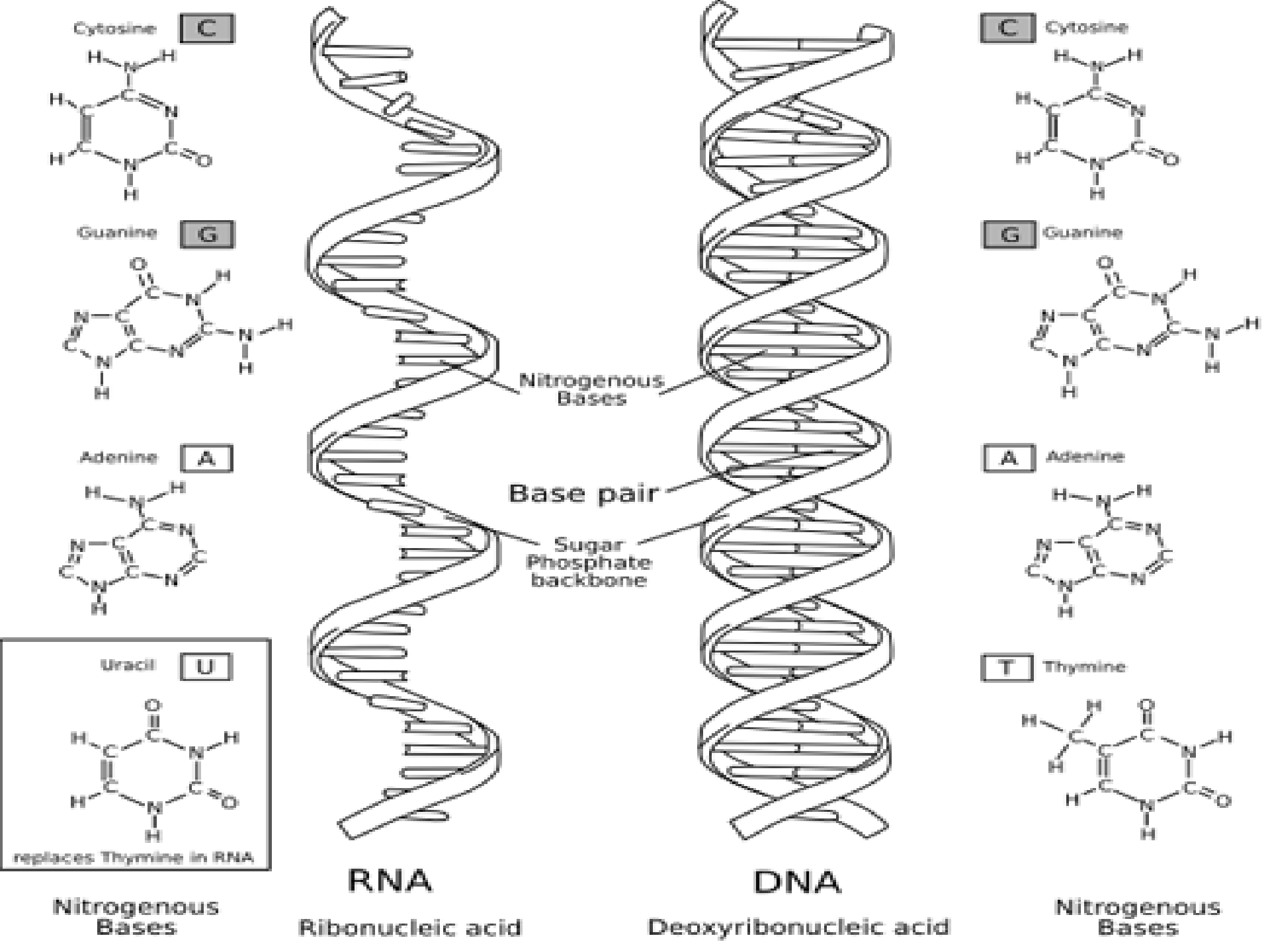
Understanding DNA

- **Our knowledge of DNA put to use:**
  - Inheritance/ Genetic Counseling
  - Cell function/protein synthesis
  - Embryonic development/gene regulation
  - Evolution/phylogenetic relationships
  - Medicine/genetic diseases
  - Genetic engineering/ recombinant DNA

DNA structure






Cytosine
C
Guanine
G
Adenine
A
Uracil
U
replaces Thymine in RNA
Nitrogenous Bases
Base pair
Sugar Phosphate backbone
RNA
Ribonucleic acid
DNA
Deoxyribonucleic acid
C
Cytosine
G
Guanine
A
Adenine
T
Thymine
Nitrogenous Bases

Central dogma

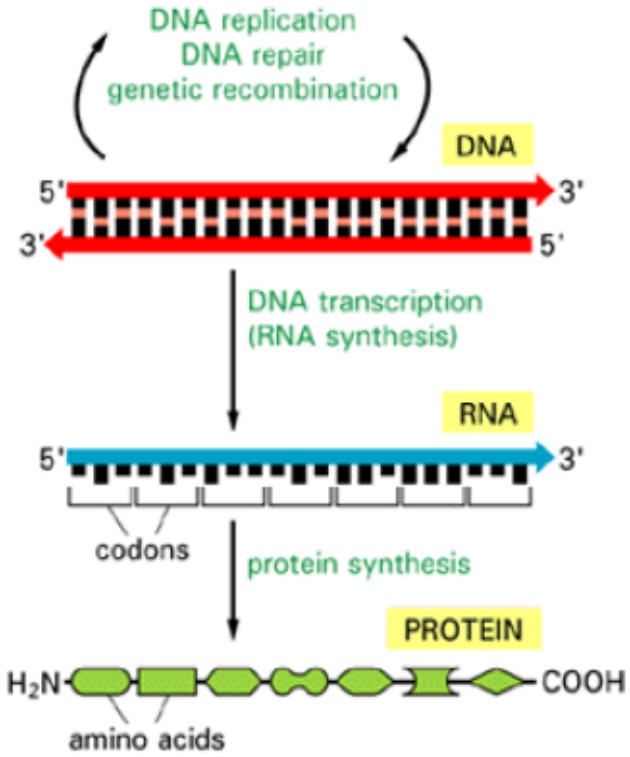


Figure 6-1. The basic genetic processes. The processes shown here are thought to occur in all present-day cells. Very early in the evolution of life, however, much simpler cells probably existed that lacked both DNA and proteins (see Figure 1-11). Note that a sequence of three nucleotides (a codon) in an RNA molecule codes for a specific amino acid in a protein.

Introduction

- In same time all genes in cells are not active.
  - Constitutive genes
  - Regulated genes
- Different amount of protein synthesis form genes

- In terms of ATP consumption, gene expression is expensive (3000 ATP molecules per protein), so this process has to be controlled precisely to prevent wasteful synthesis of unnecessary materials.

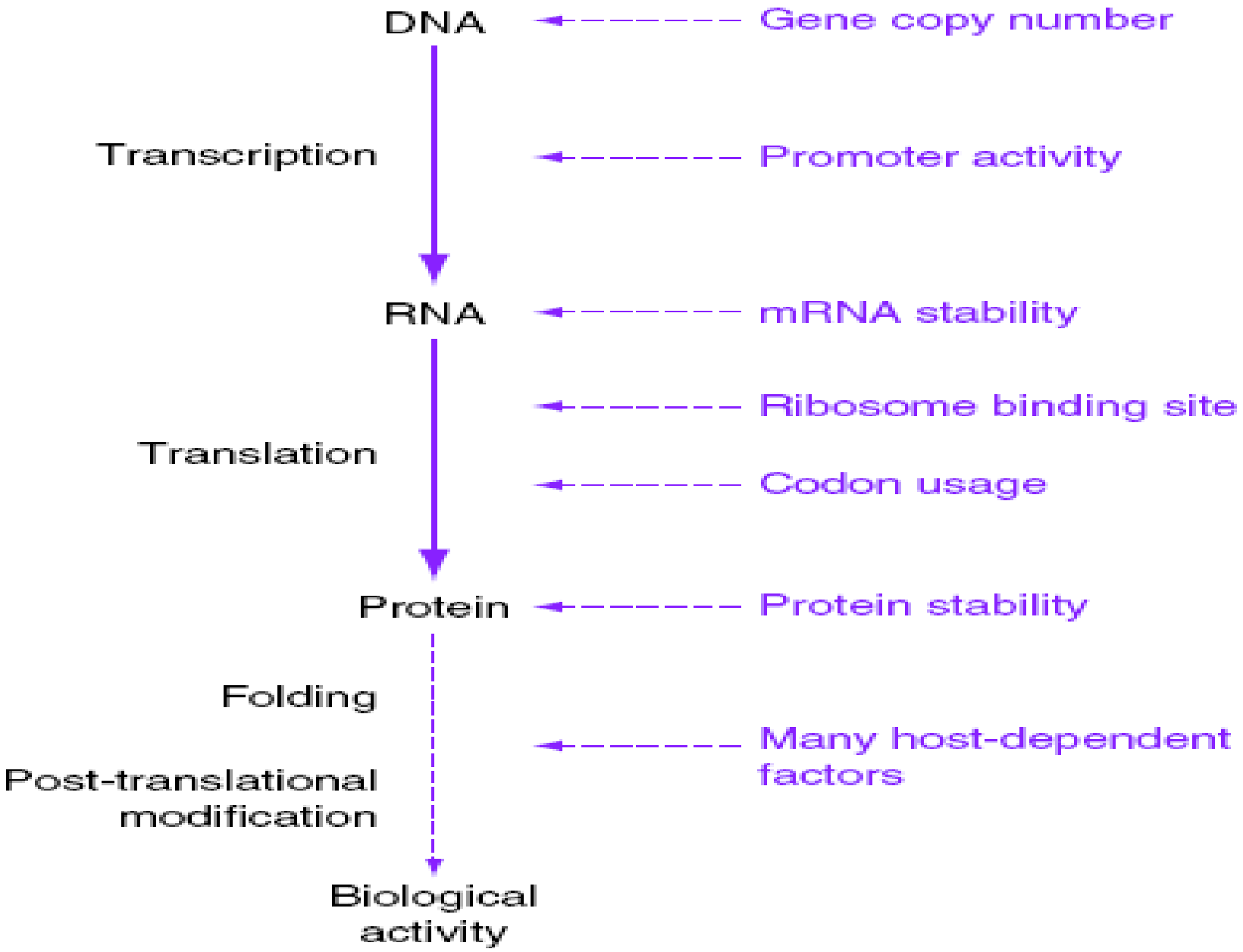
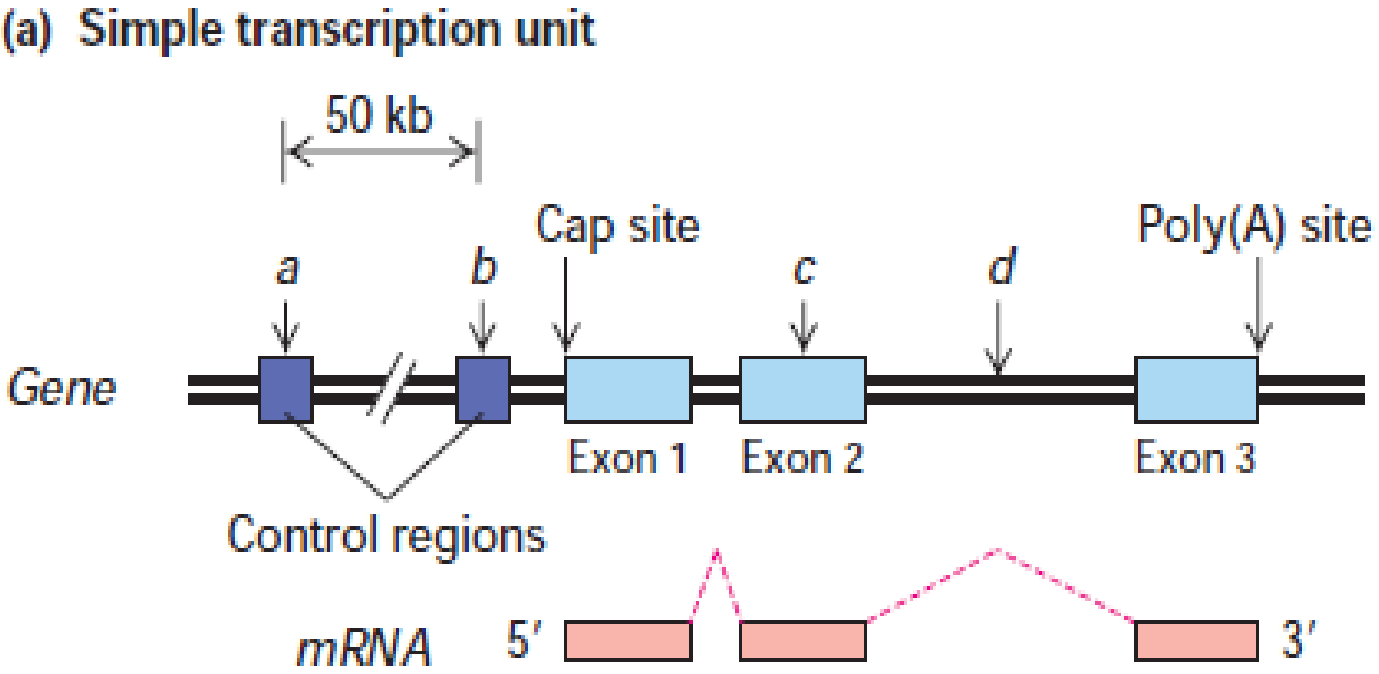


Figure 15.1 Gene expression

Regulation at gene level

- Covalently modifications (Epigenetic modifications)
  - DNA methylation → me-A & me-C
- Gene copy number

- Gene structure
  - Regulatory region (-)
  - Structural region (+)



gene copy number

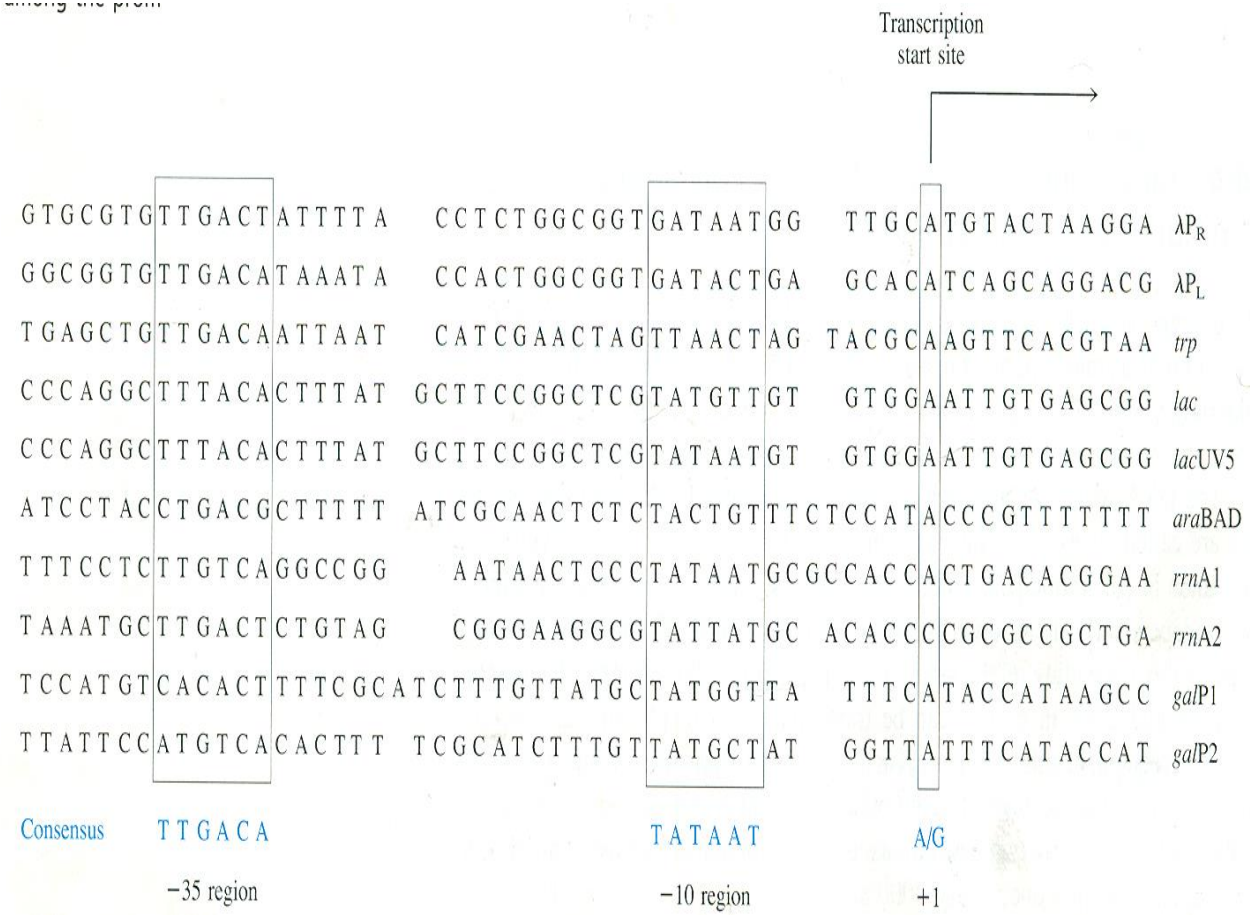
- Most genes on the bacterial chromosome are present as single copies

- Gene copy number is not therefore an important method of control for most of the normal metabolic activities of a bacterial cell.

Gene control at transcription level

- Important stage for control → Initiation
  - Constitutive control → depend on promoter structure
  - Regulatory control → depend on regulatory proteins

Constitutive control



- Based rate
- Mutation
  - Deletion
  - Insertion
  - Substitution

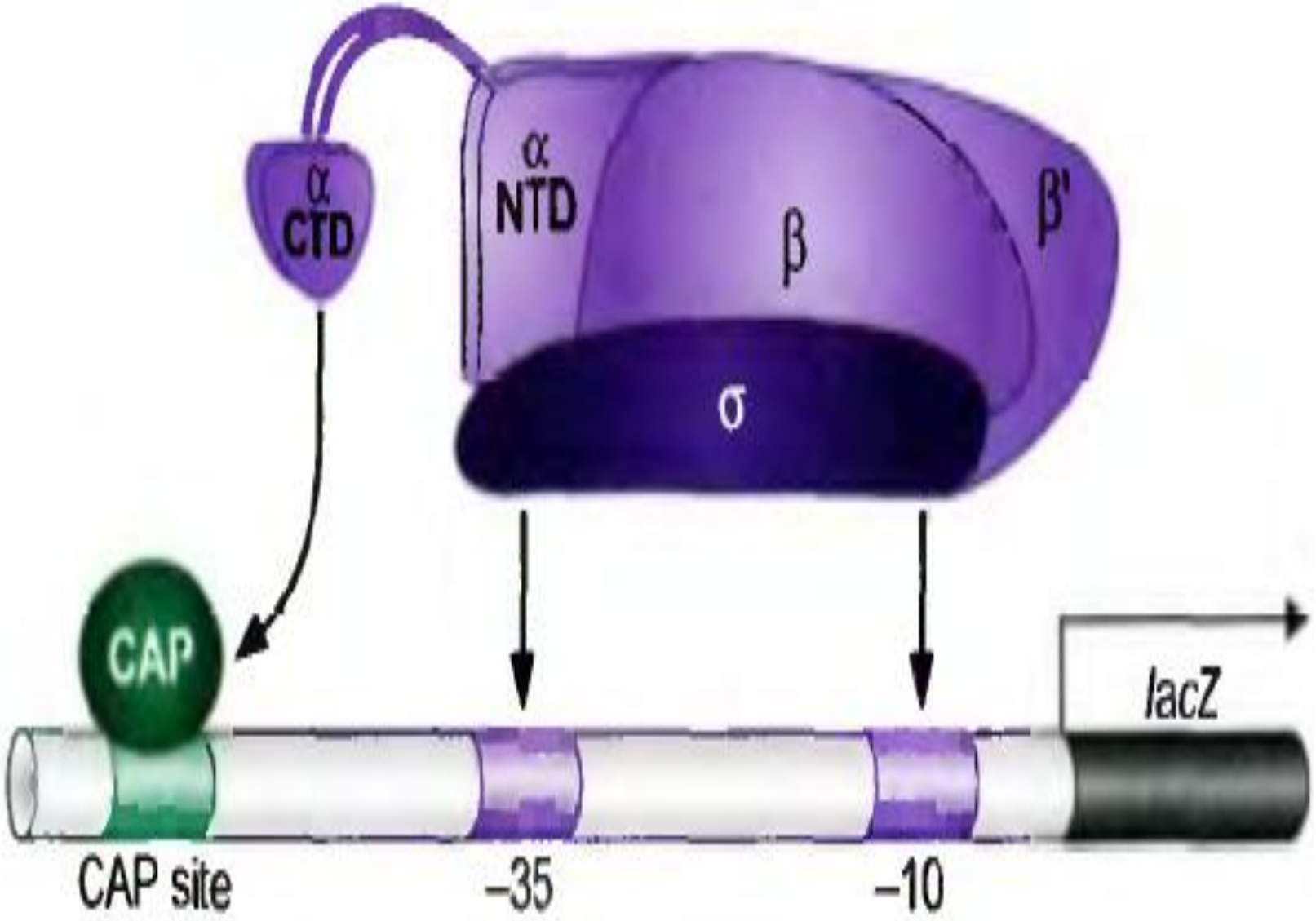
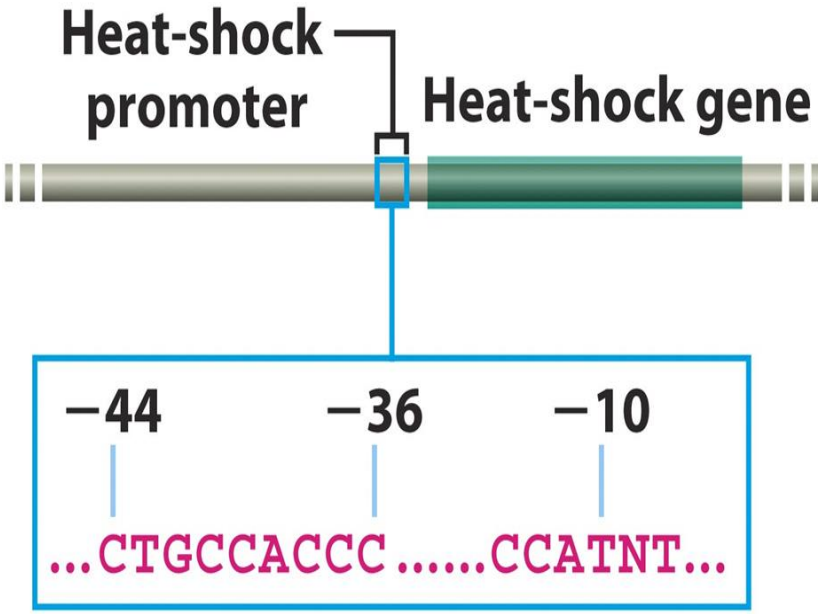

α
CTD
α
NTD
β
β'
σ
CAP
lacZ
CAP site
–35
–10

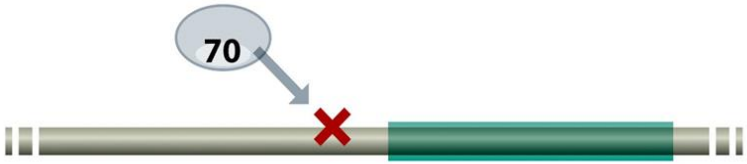
Table 24·3 *E. coli* σ factors

Factor	Gene	Consensus sequence −35	Consensus sequence −10
σ^{70}	Housekeeping	TTGACA	TATAAT
σ^{32}	Heat shock	CTTGAA	CCCCAT-TA*
σ^{60}	Nitrogen metabolism		CTGGCAC-----TTGCA*
σ^{gp55}	T4 late gene	none	TATAAATA

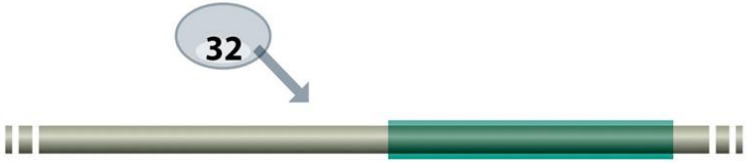
An *E. coli* heat shock gene



Recognition by the σ^{32} subunit

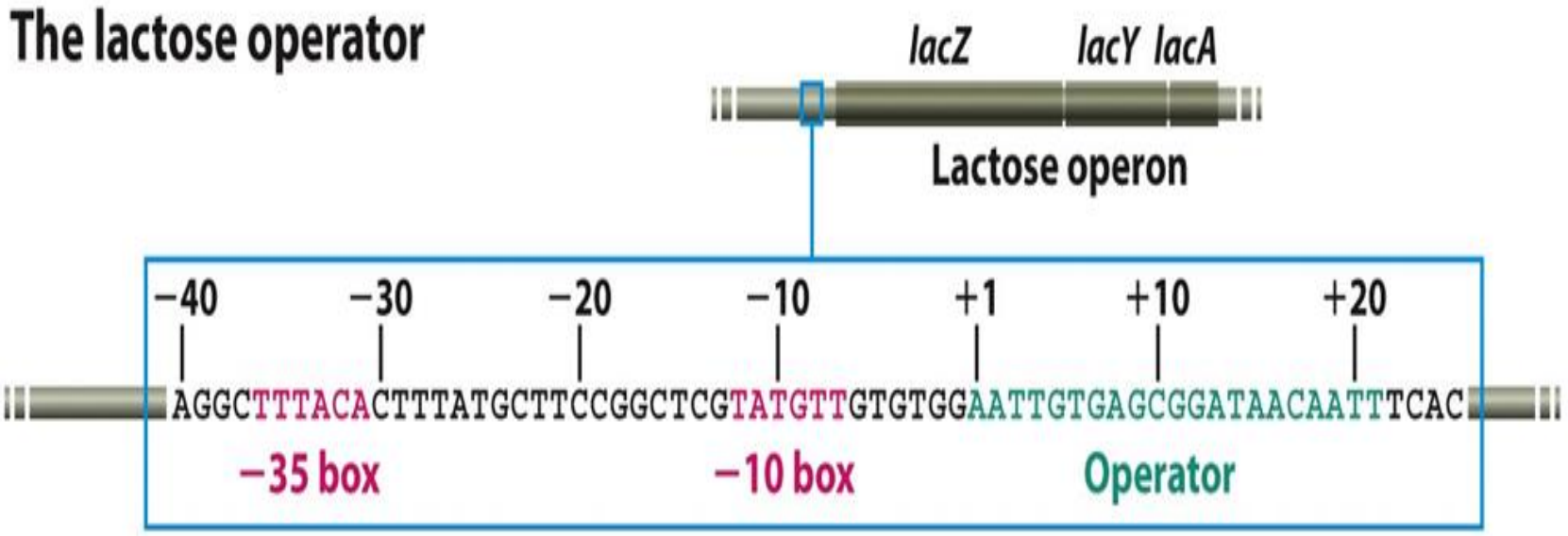


σ^{70} RNA polymerase cannot bind

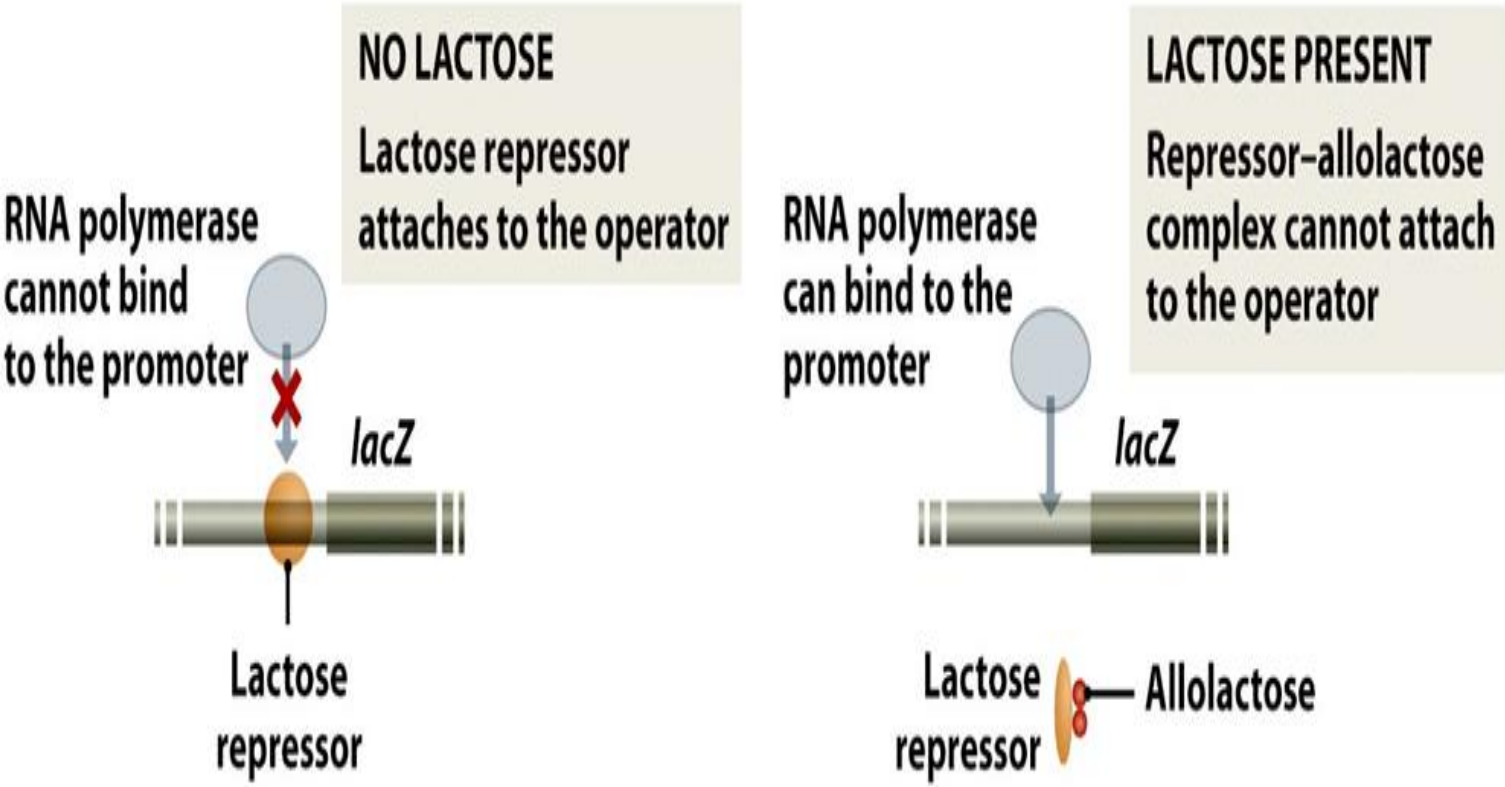


σ^{32} RNA polymerase binds to the heat-shock promoter

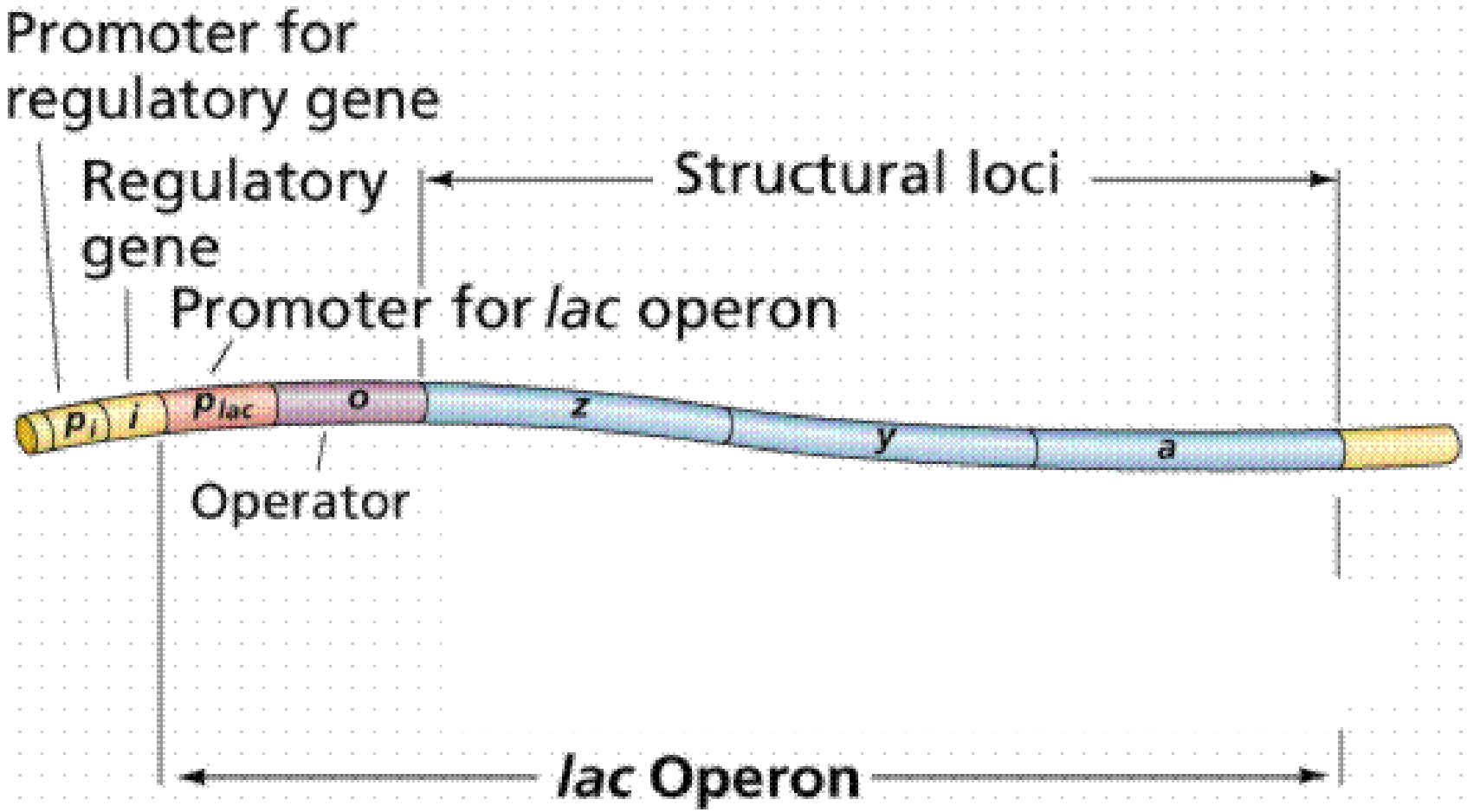
Regulatory control



The original model for lactose regulation



Lac operon


Promoter for regulatory gene
Regulatory gene
Structural loci
Promoter for lac operon
p_I
i
p_{lac}
o
z
y
a
Operator
lac Operon

Lac operon

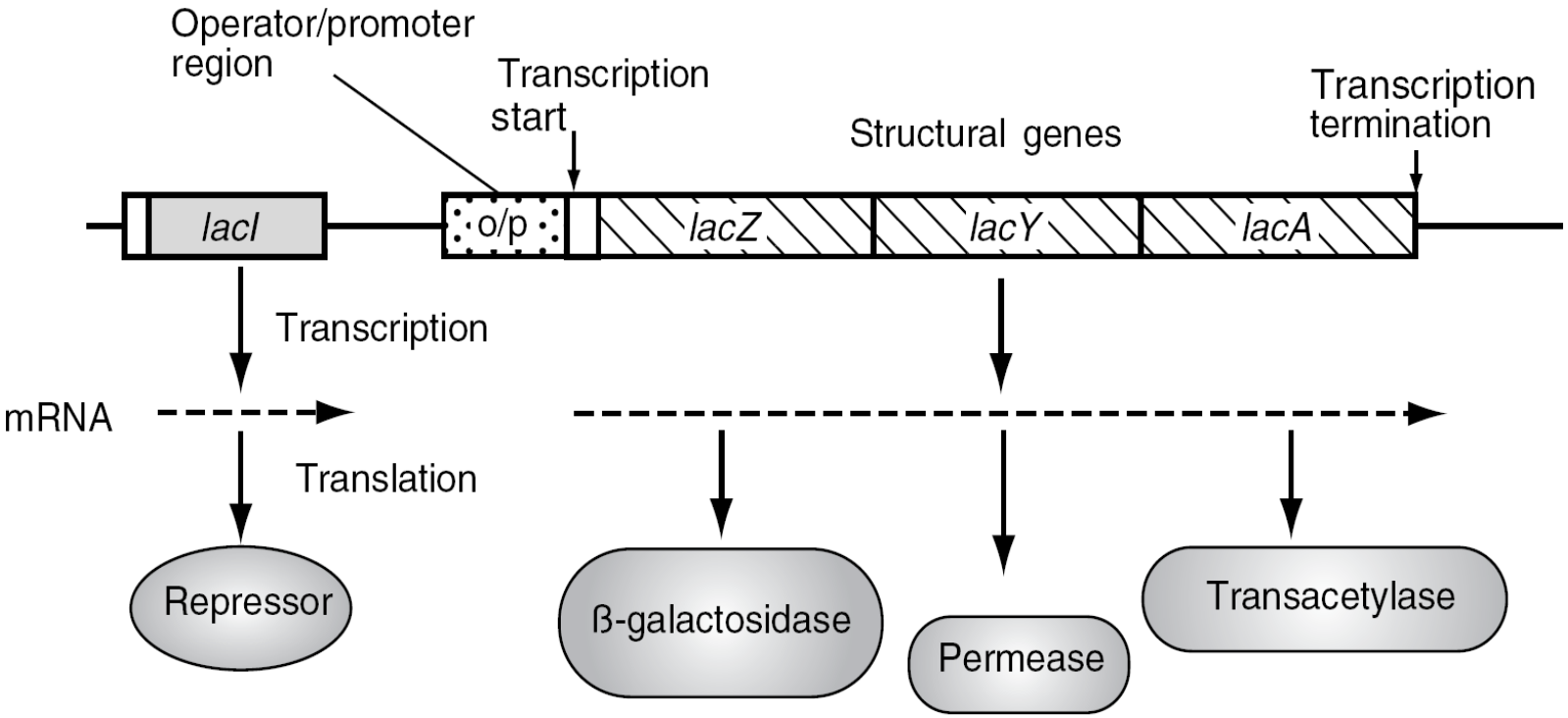
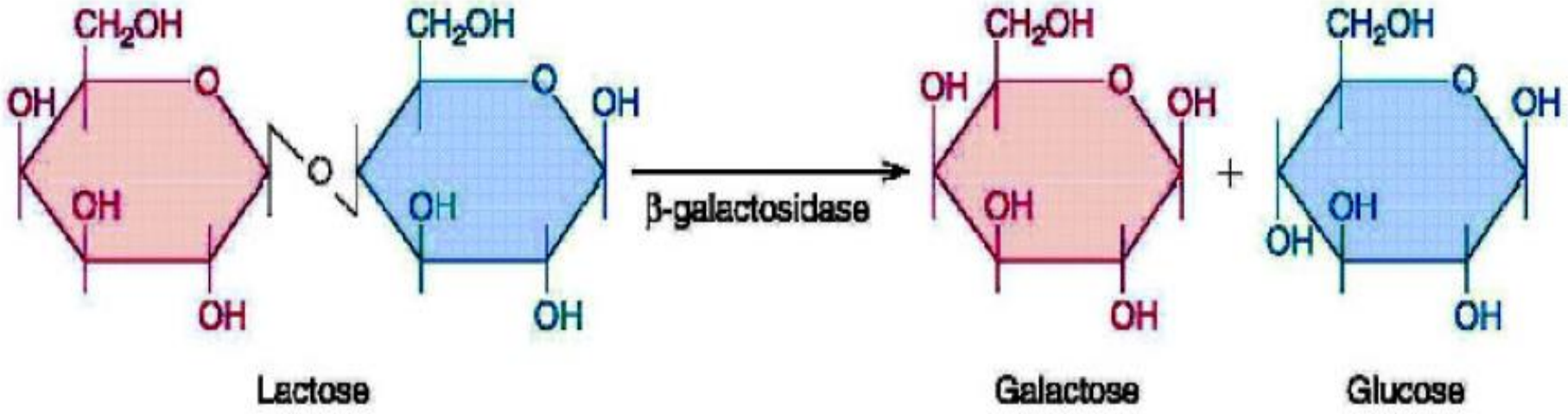
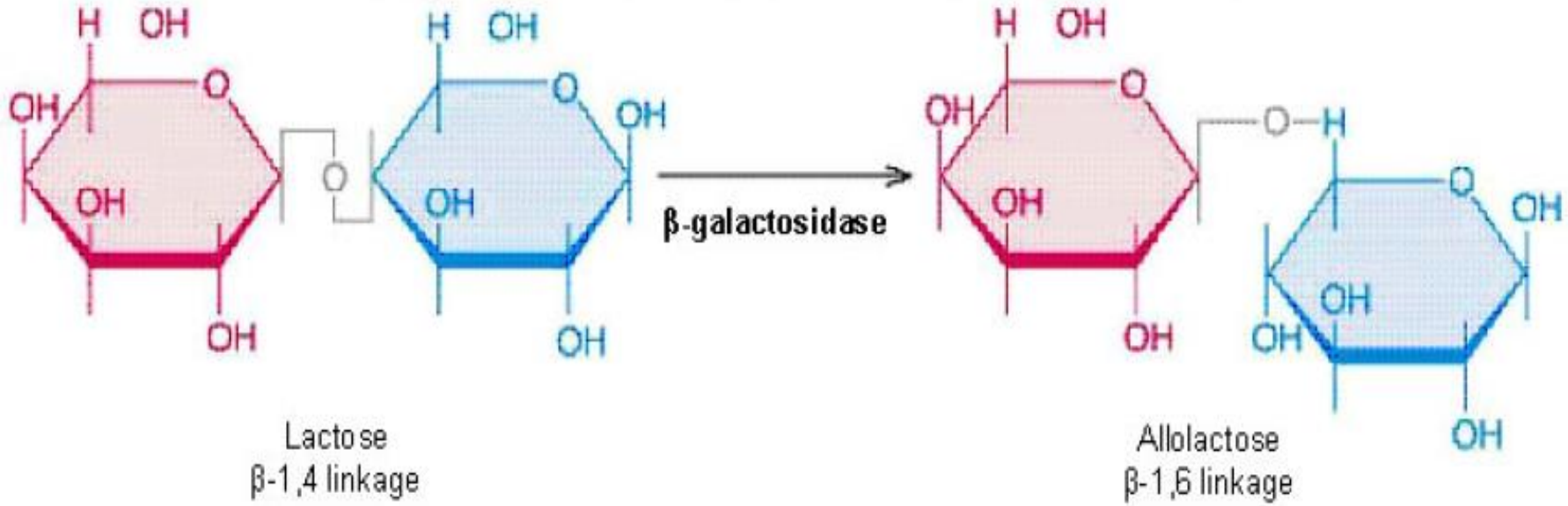
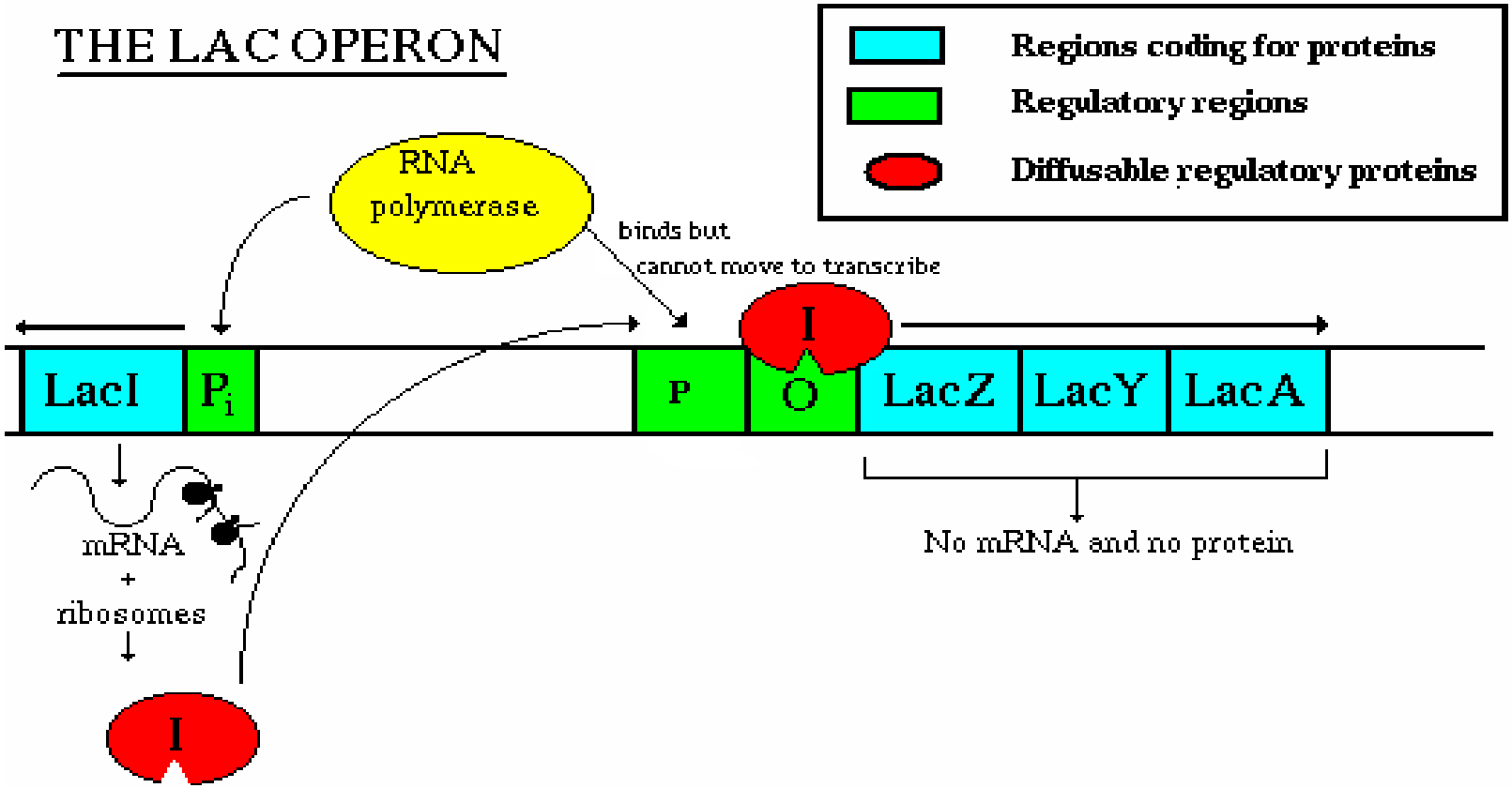


Figure 3.4 Structure of the *lac* operon

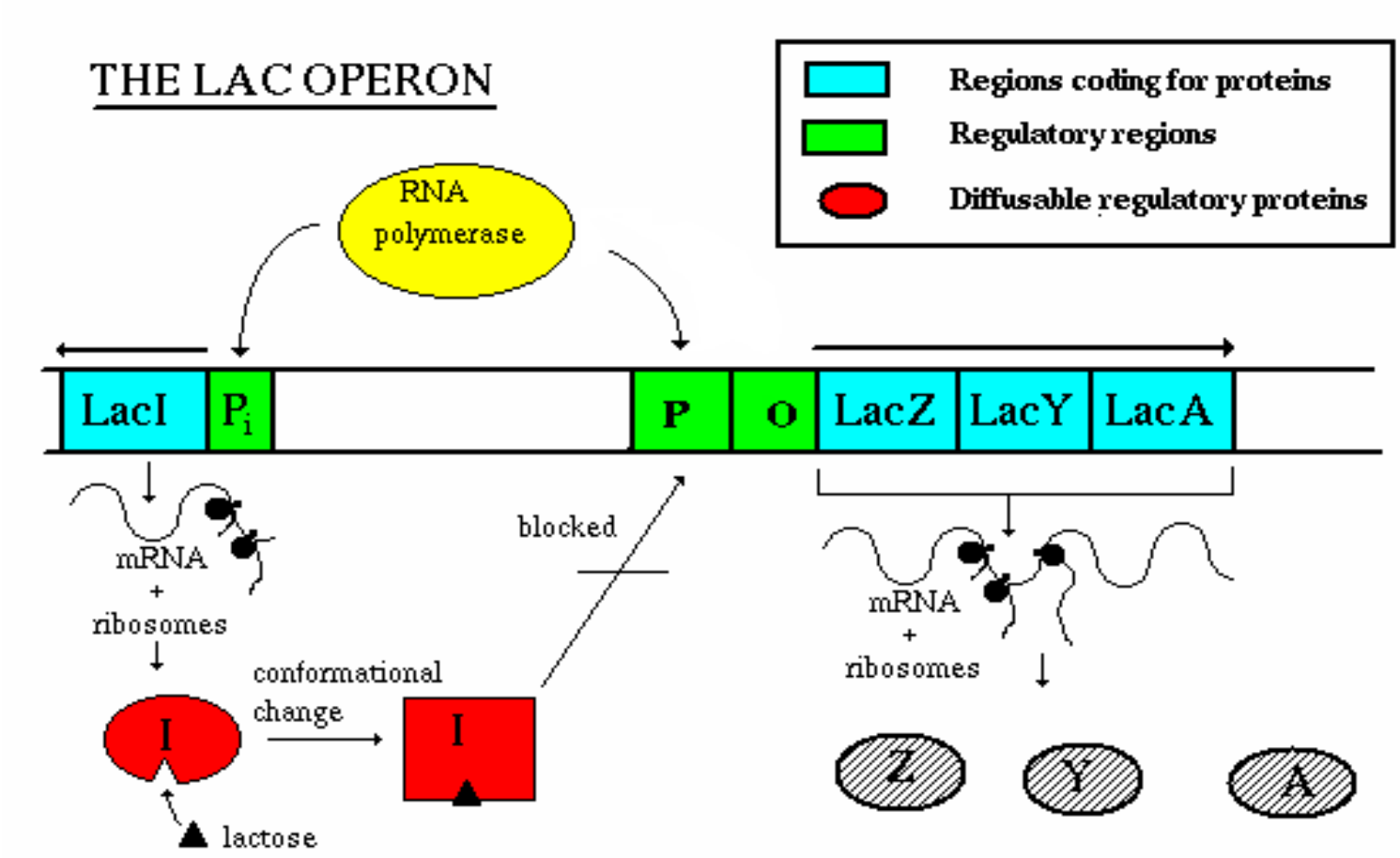

β-galactosidase
Lactose
Galactose
Glucose


β-galactosidase
Lactose
β-1,4 linkage
Allolactose
β-1,6 linkage

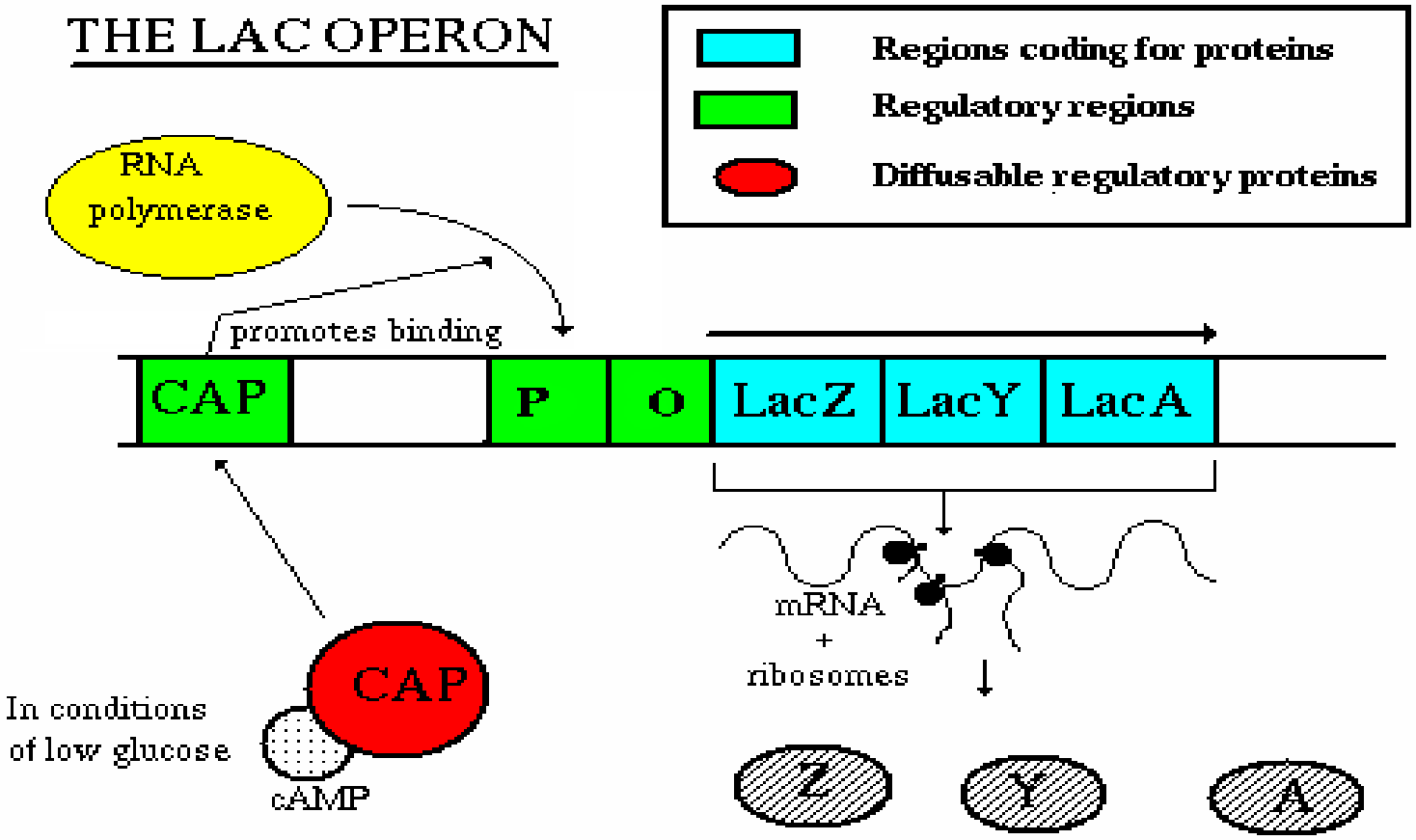
Lac off


THE LAC OPERON
Regions coding for proteins
Regulatory regions
Diffusable regulatory proteins
RNA polymerase
binds but
cannot move to transcribe
I
LacI
Pi
P
O
LacZ
LacY
LacA
mRNA
+
ribosomes
I
No mRNA and no protein

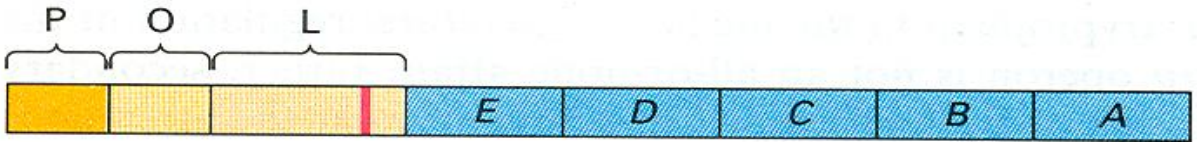
Lac on

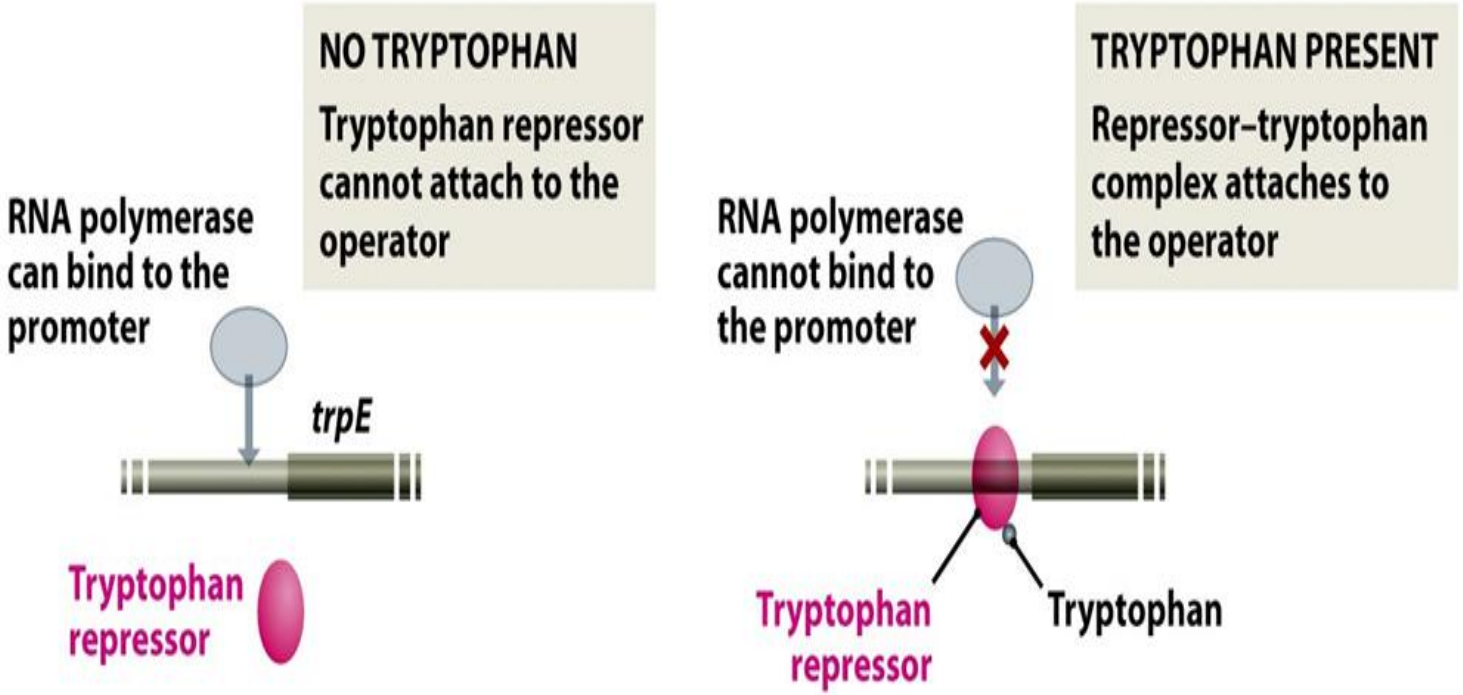

THE LAC OPERON
Regions coding for proteins
Regulatory regions
Diffusable regulatory proteins
RNA polymerase
LacI
Pi
P
O
LacZ
LacY
LacA
mRNA + ribosomes
blocked
conformational change
I
I
lactose
mRNA + ribosomes
Z
Y
A

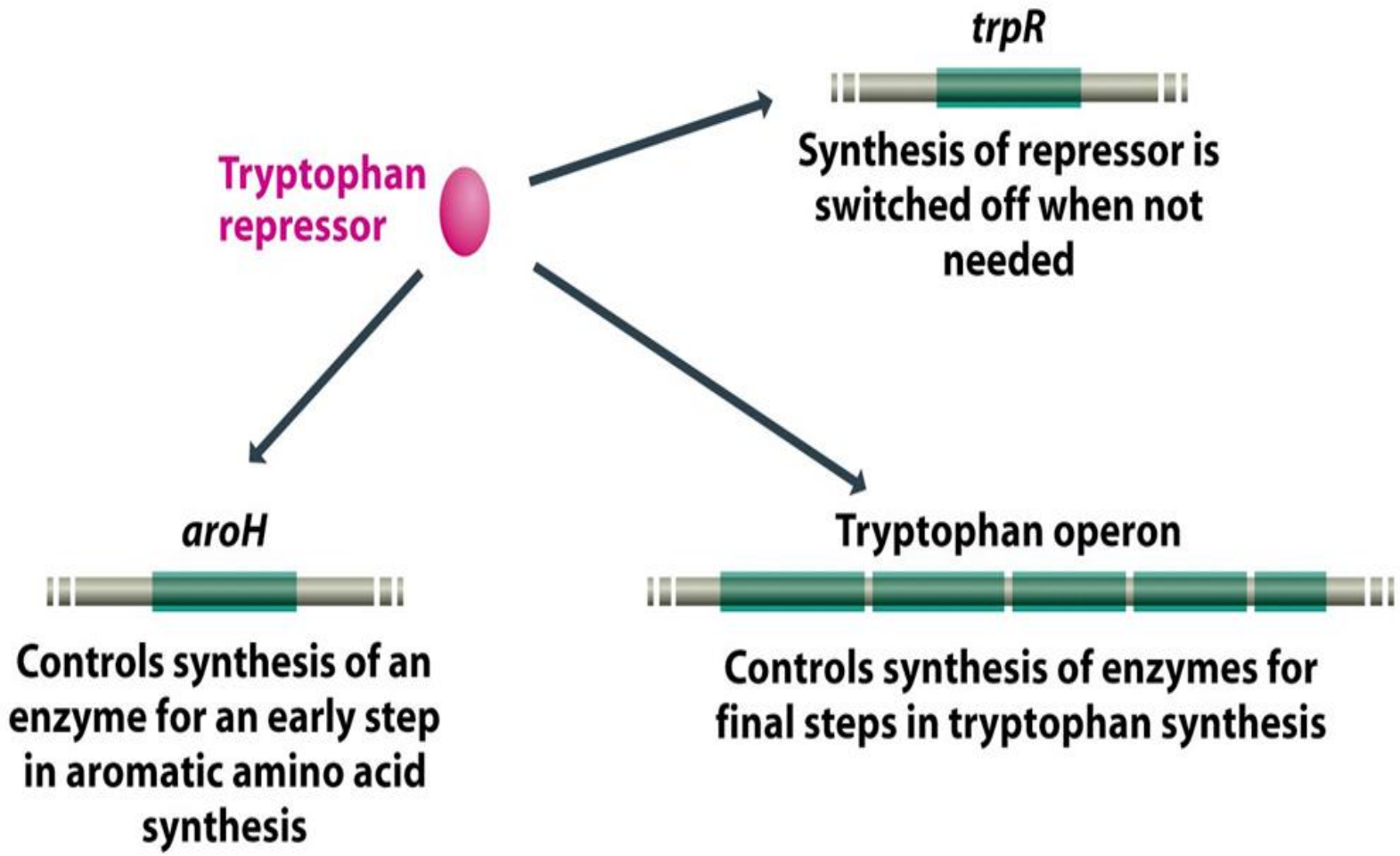
Multi-control operon



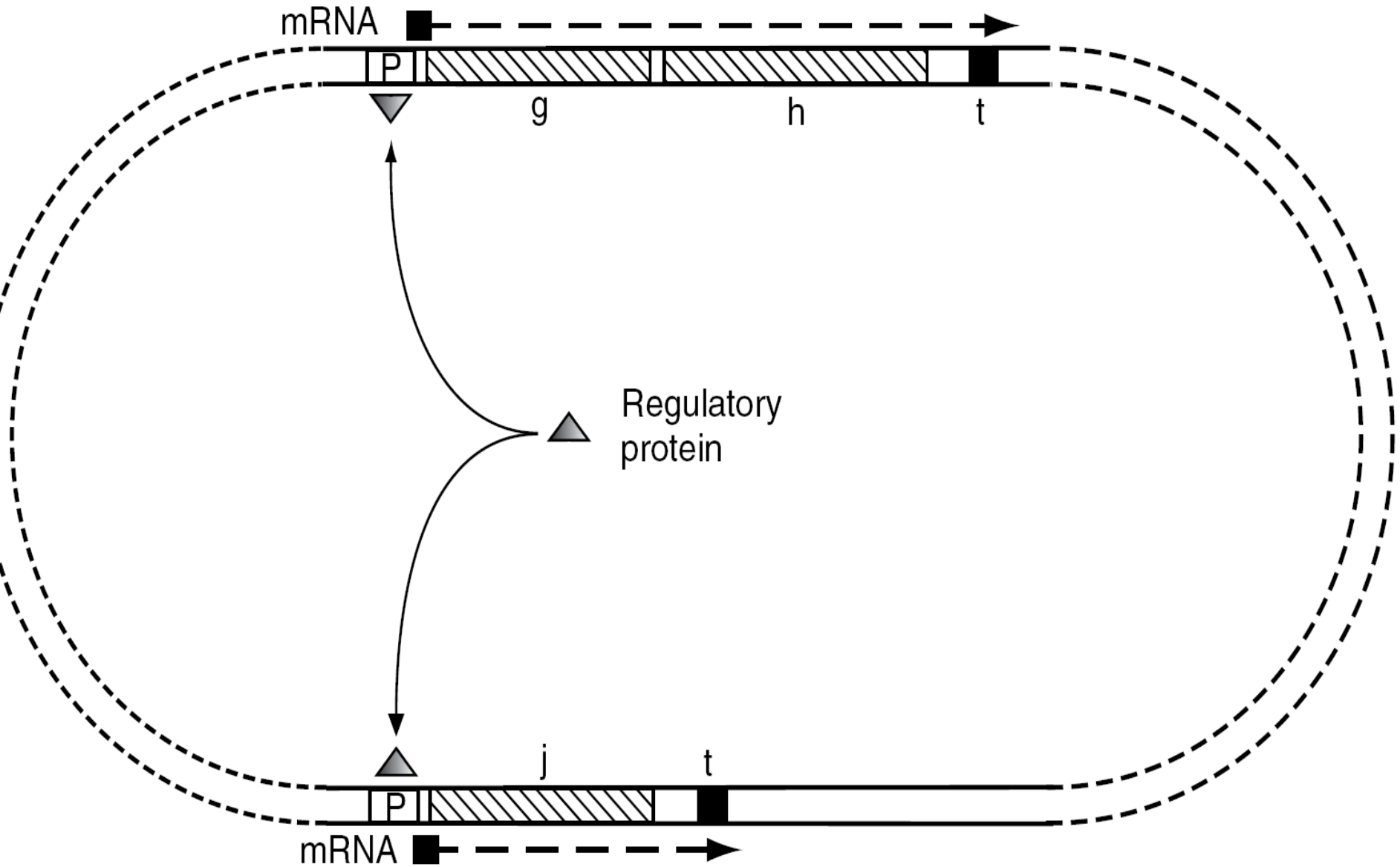
Trp operon


P
O
L
E
D
C
B
A

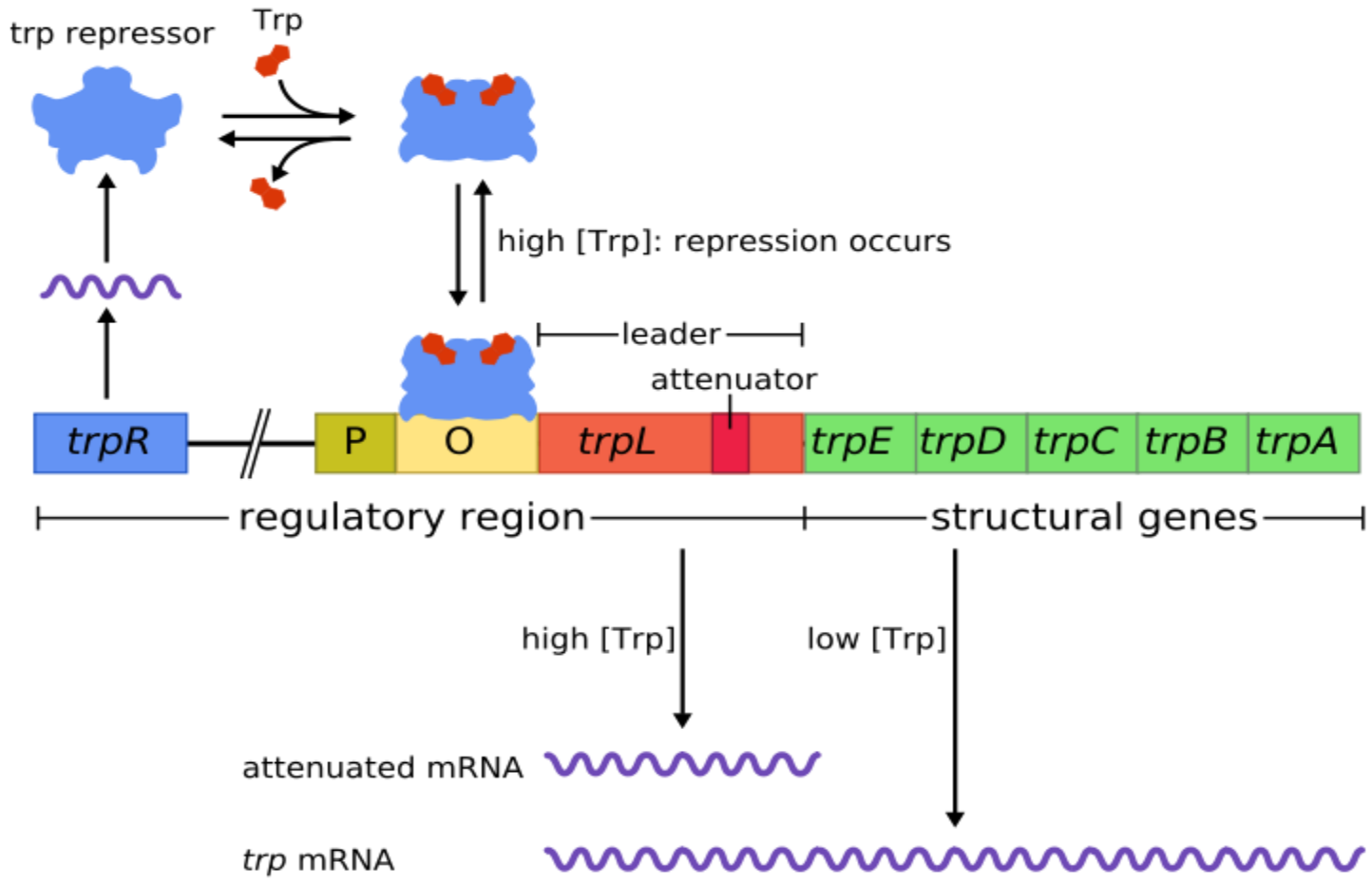

NO TRYPTOPHAN
Tryptophan repressor cannot attach to the operator
RNA polymerase can bind to the promoter
trpE
Tryptophan repressor
TRYPTOPHAN PRESENT
Repressor–tryptophan complex attaches to the operator
RNA polymerase cannot bind to the promoter
Tryptophan repressor
Tryptophan


trpR
Synthesis of repressor is switched off when not needed
Tryptophan repressor
aroH
Controls synthesis of an enzyme for an early step in aromatic amino acid synthesis
Tryptophan operon
Controls synthesis of enzymes for final steps in tryptophan synthesis

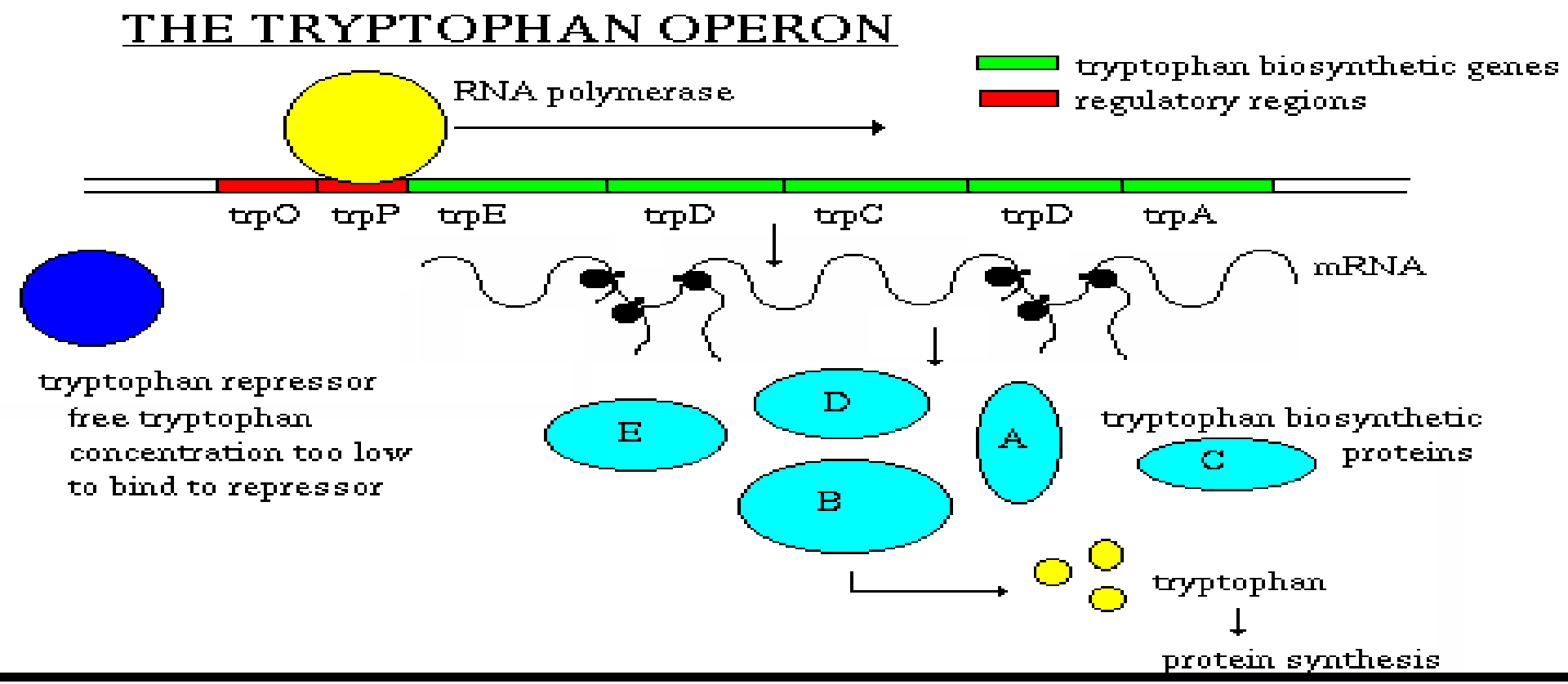
(c) Regulon. Regulatory protein interacts with several operators, controlling genes on different parts of the genome

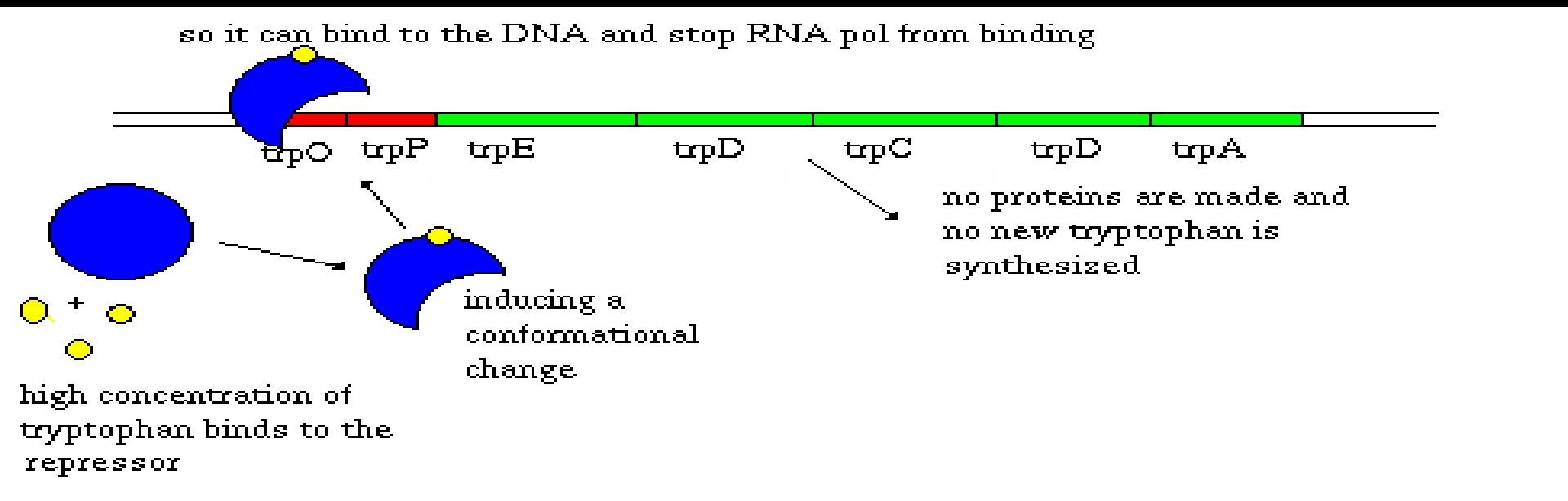


Trp operon

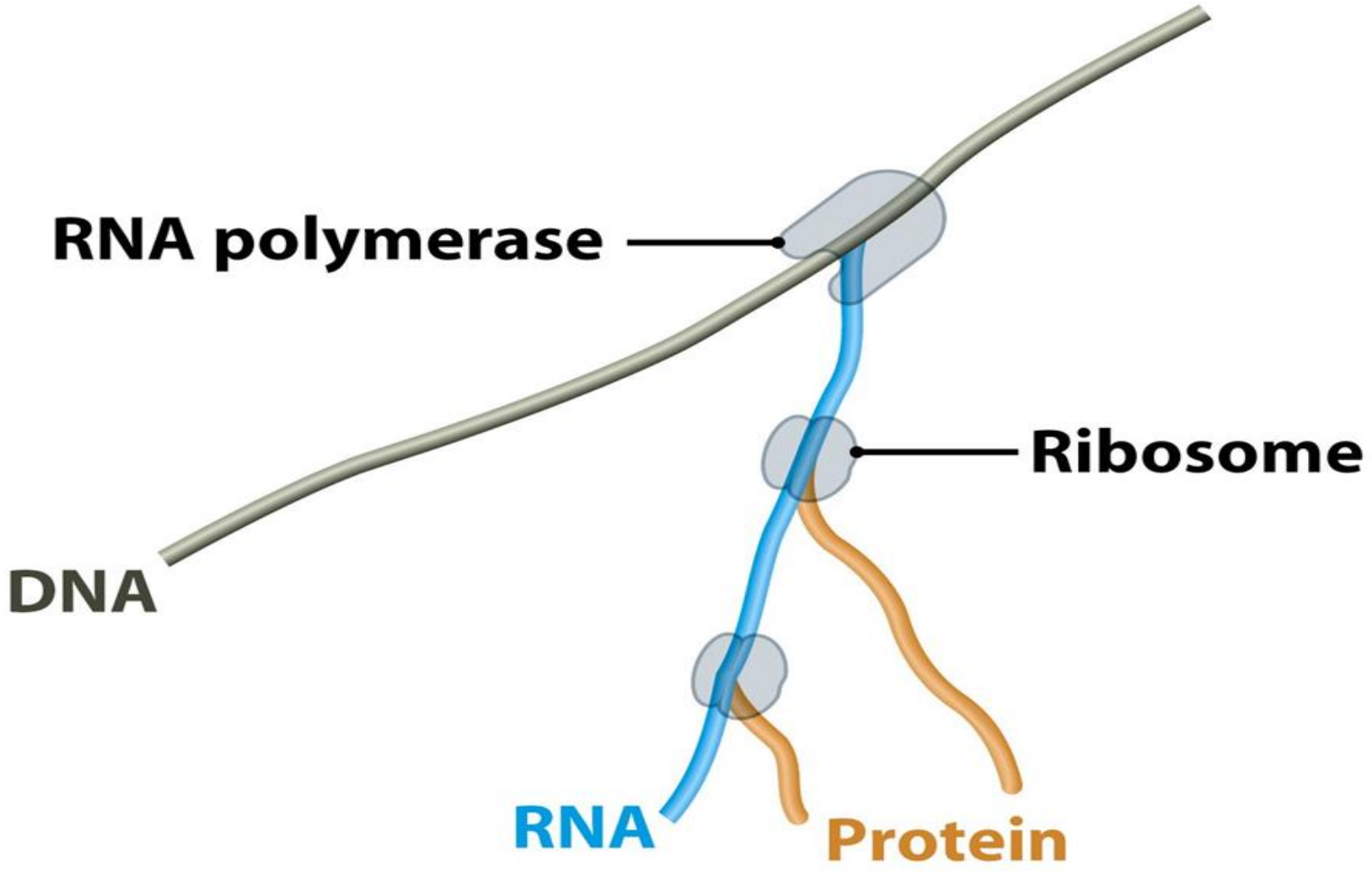

trp repressor
Trp
high [Trp]: repression occurs
leader
attenuator
trpR
P
O
trpL
trpE
trpD
trpC
trpB
trpA
regulatory region
structural genes
high [Trp]
low [Trp]
attenuated mRNA
trp mRNA

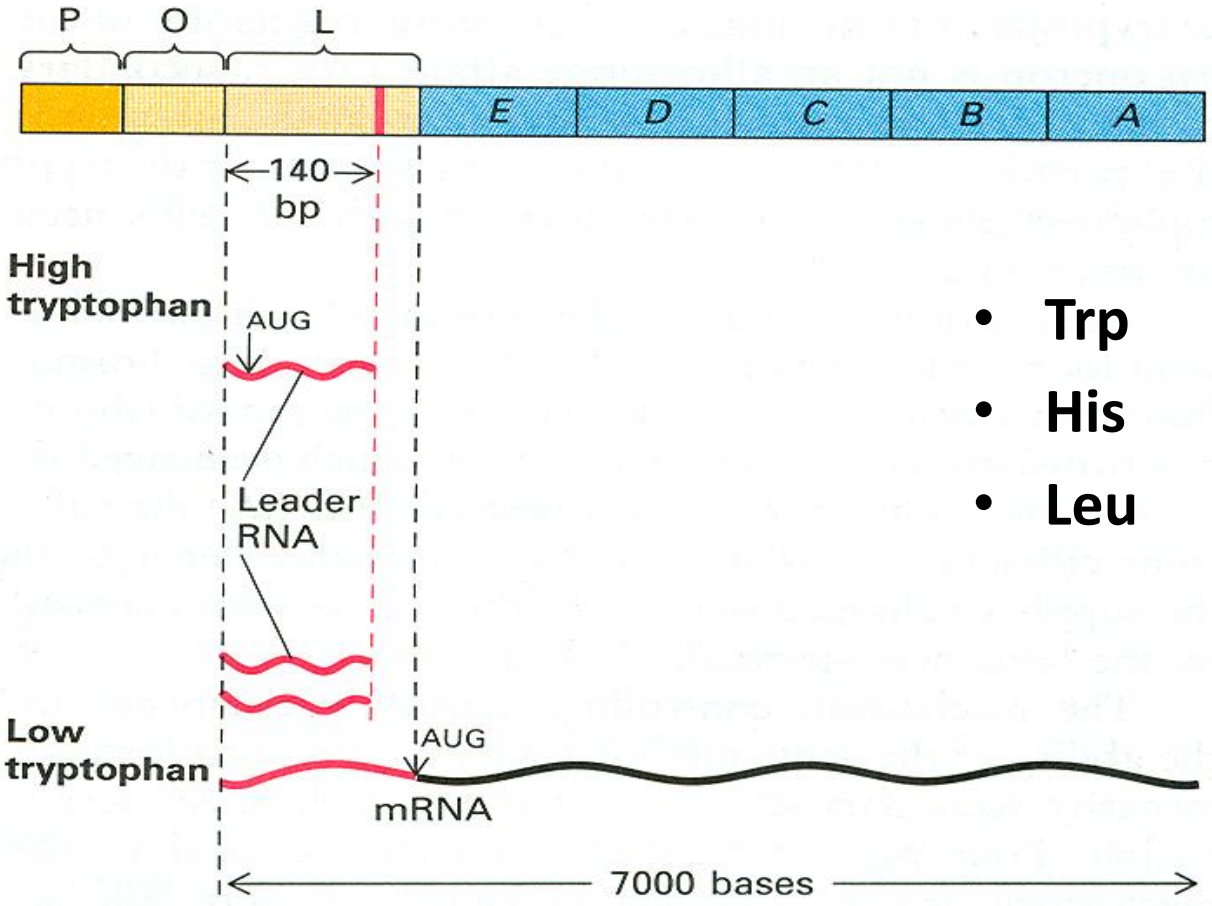
THE TRYPTOPHAN OPERON



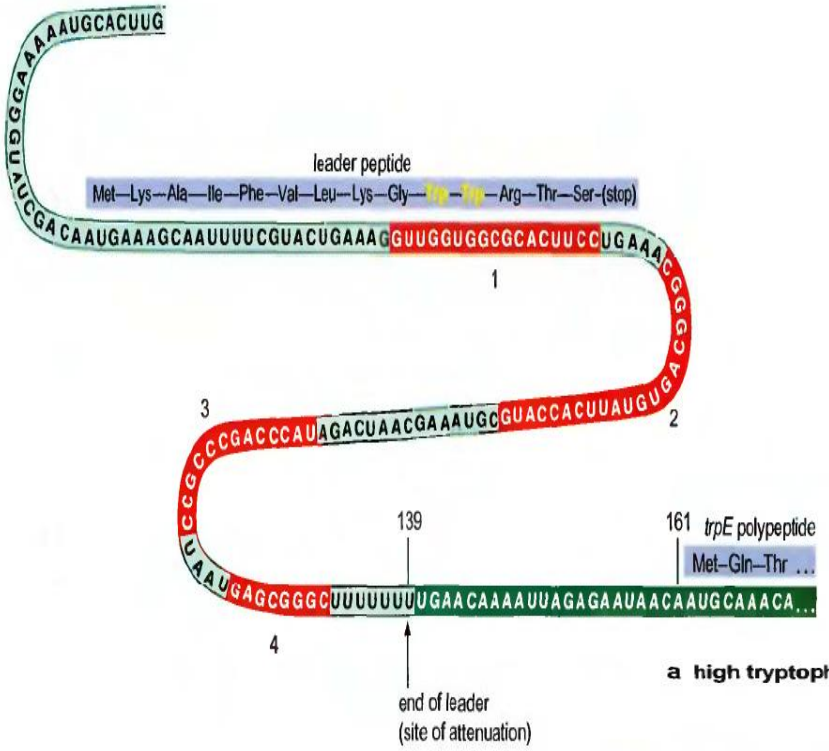


Attenuation

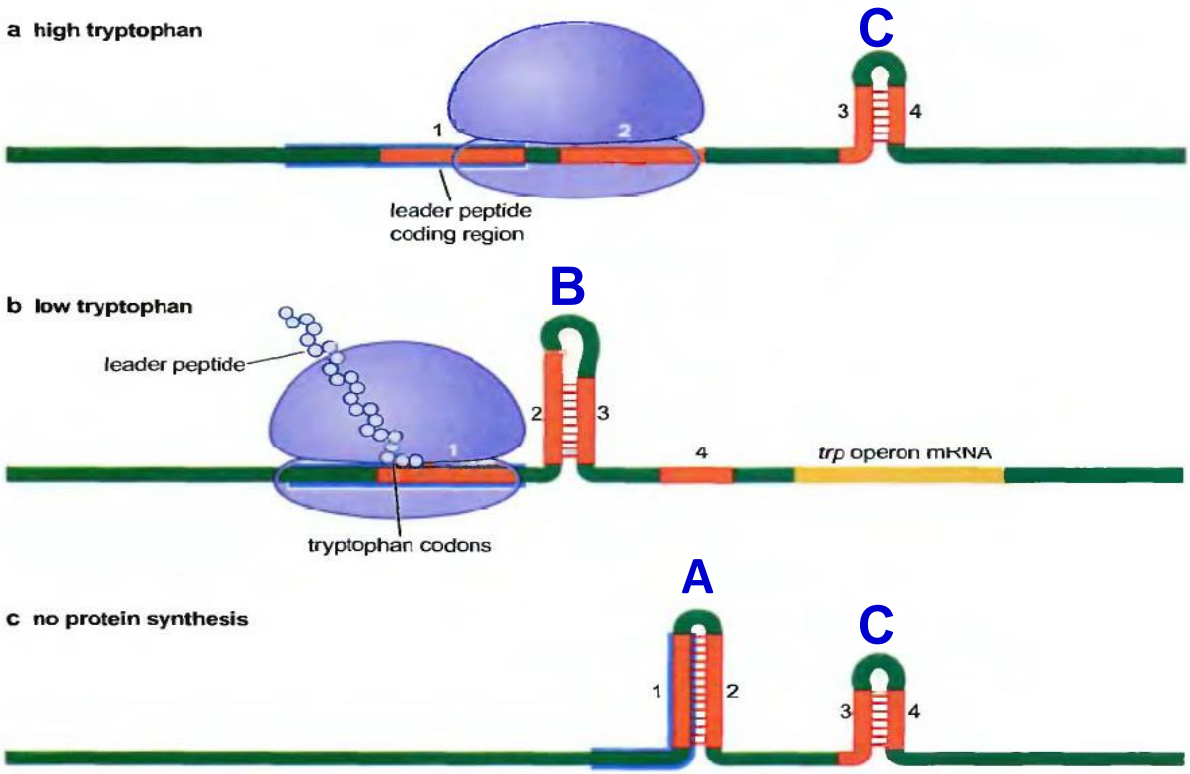



P
O
L
E
D
C
B
A
140
bp
High
tryptophan
AUG
Leader
RNA
Low
tryptophan
AUG
mRNA
7000 bases

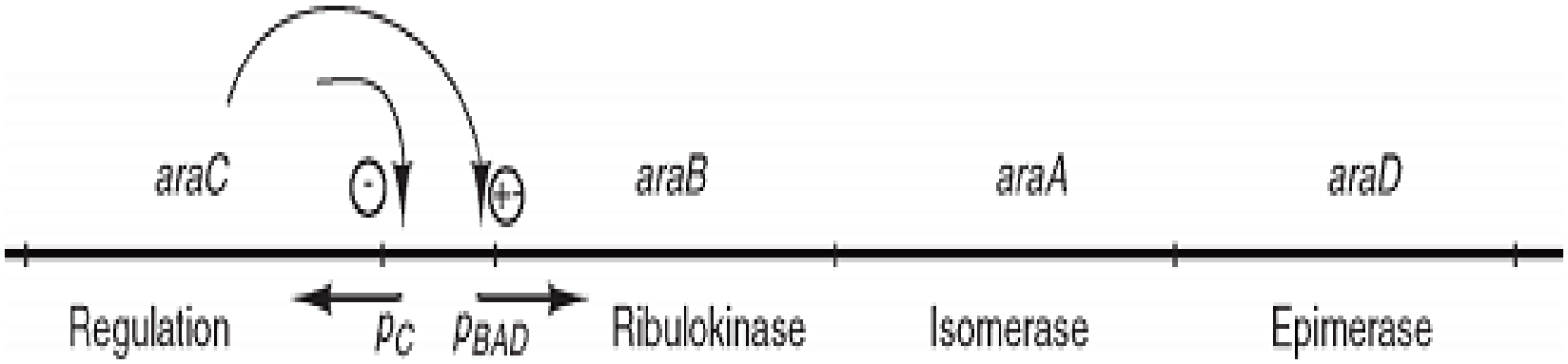
- **Trp**
- **His**
- **Leu**



- Leader RNA & peptide
- 1-2 → loop-A
- 2-3 → loop-B (Anti-termination loop)
- 3-4 → loop-C (Termination loop)

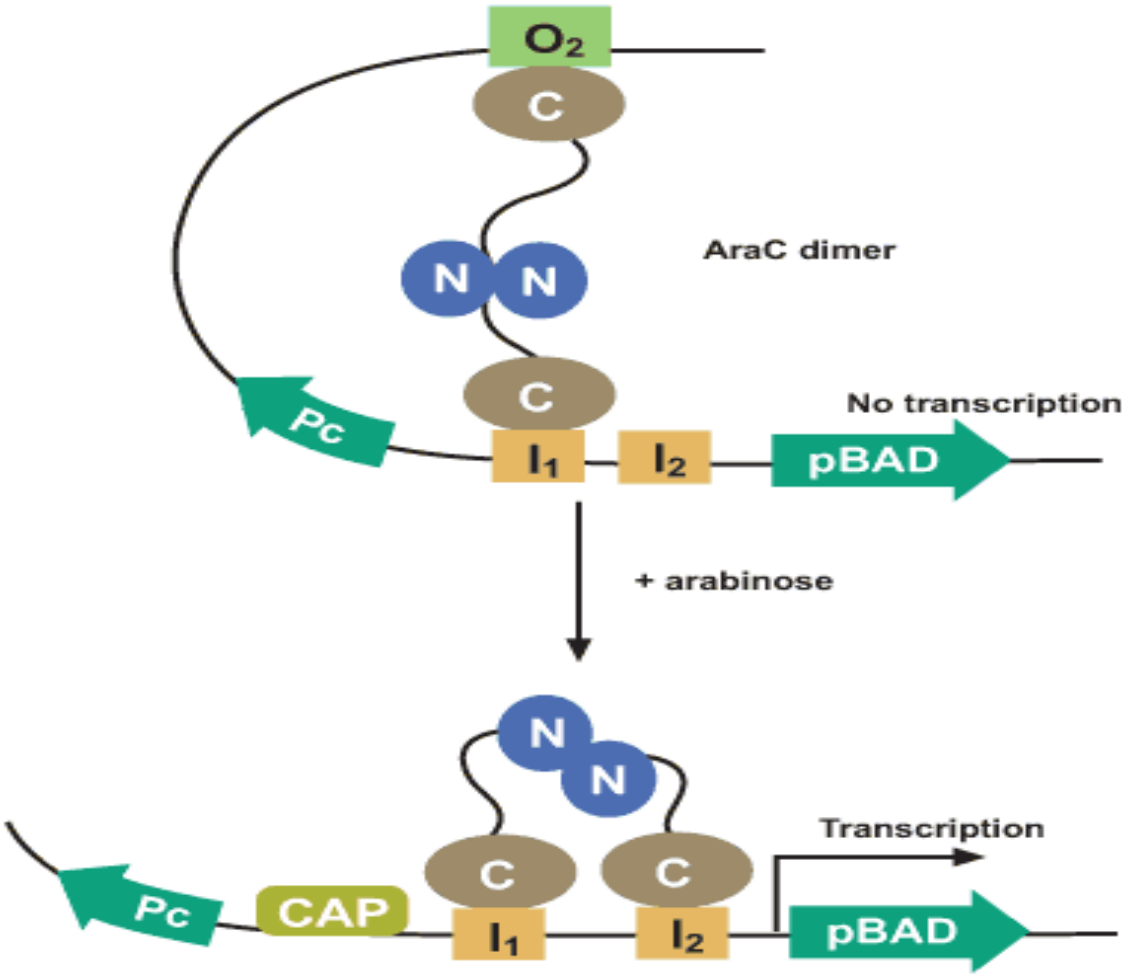


Arabinose operon



- L-arabinose → D-xylose-5-phosphate

Arabinose operon



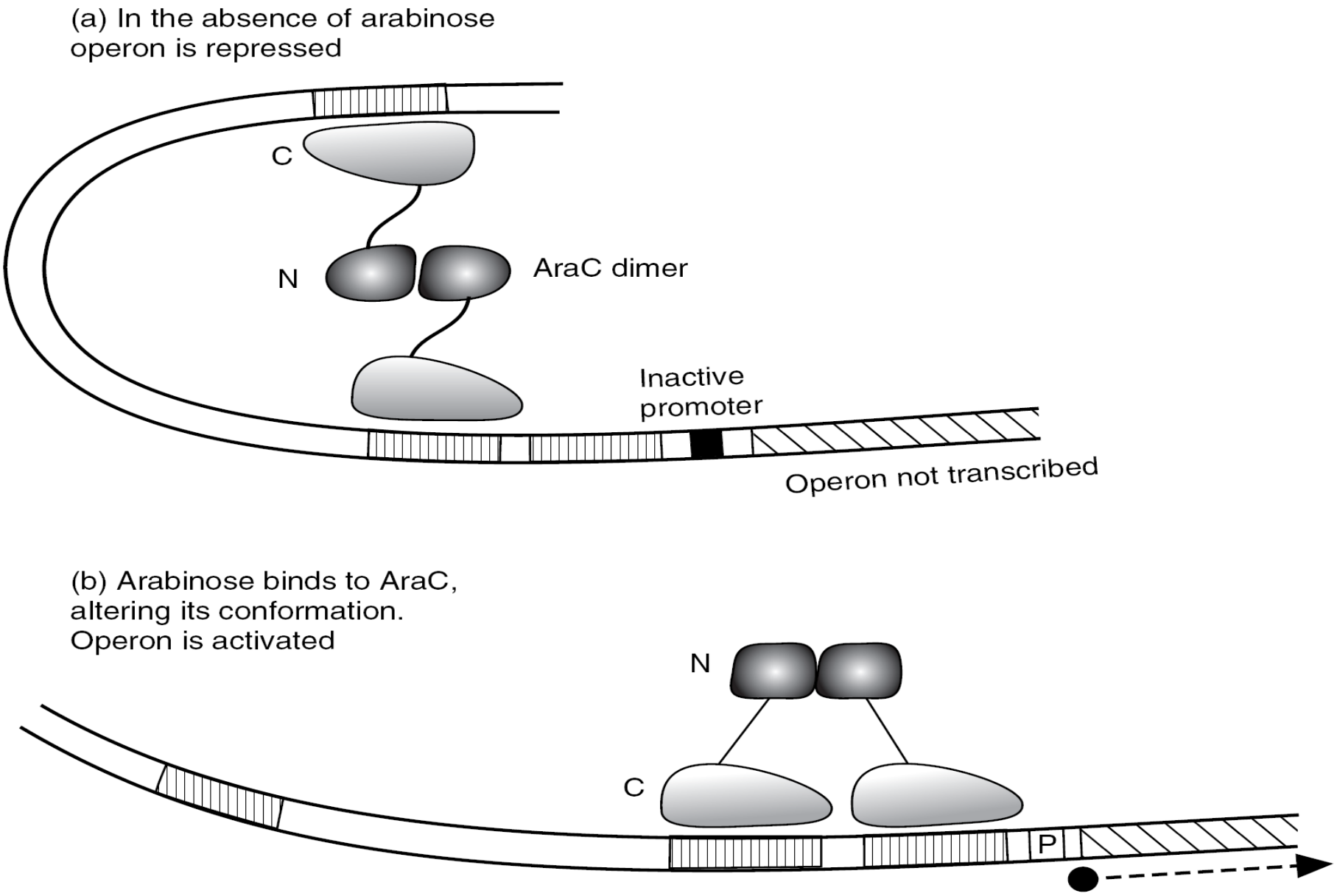
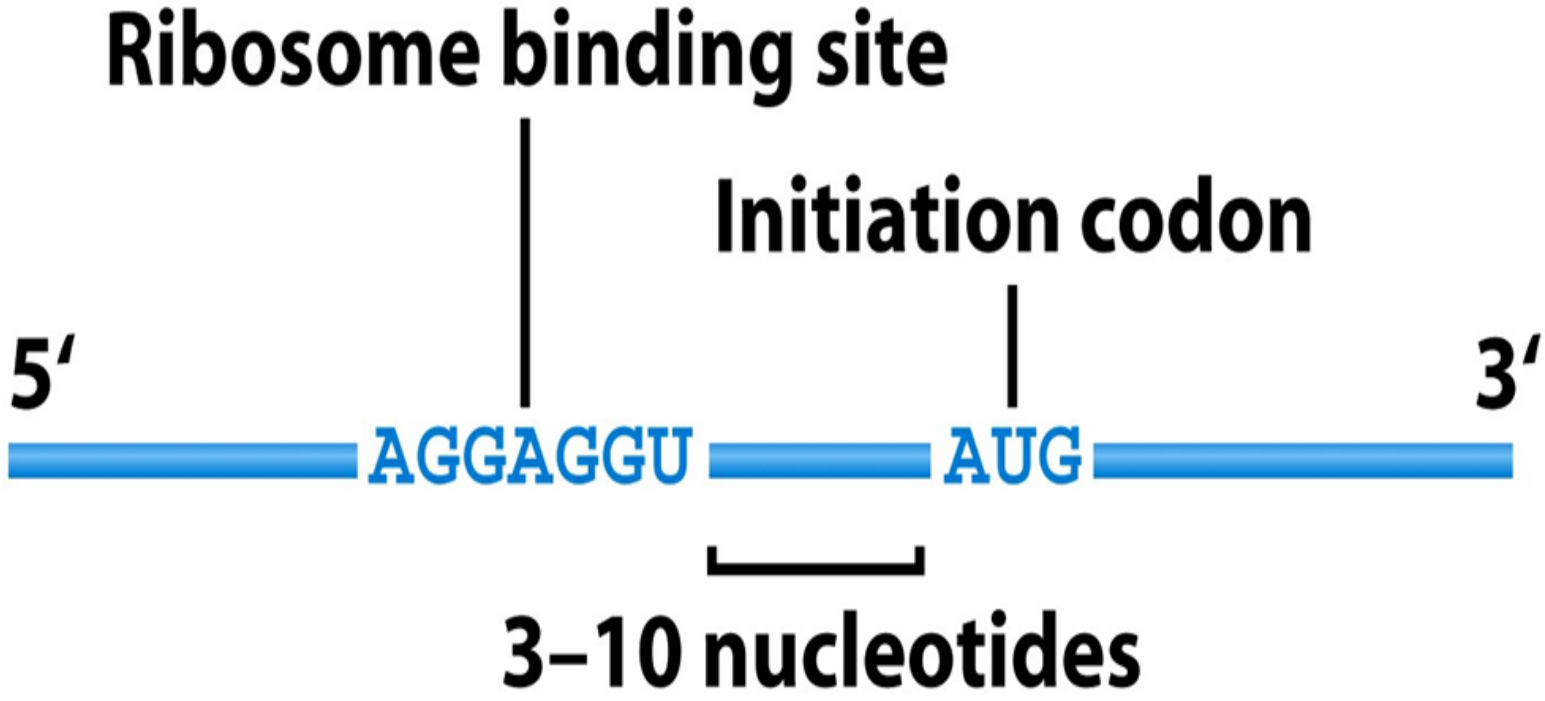
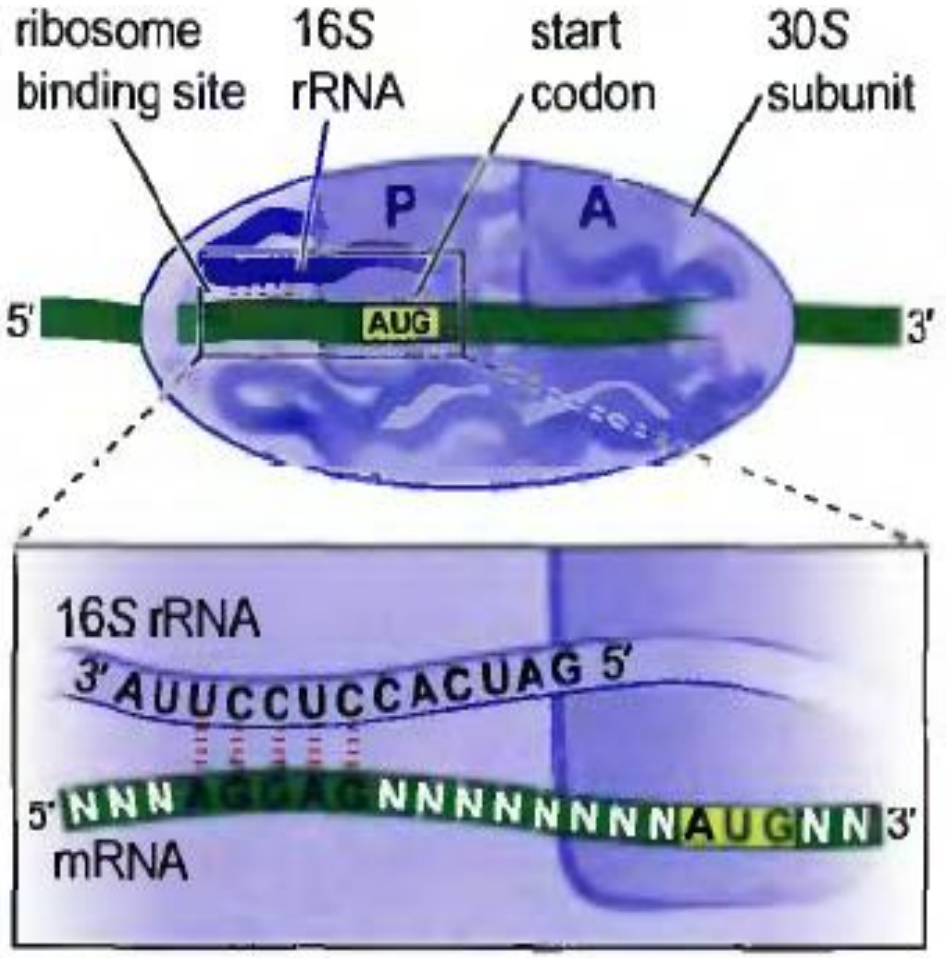


Figure 3.18 Repression and activation of the arabinose operon

Regulation of gene expression at translation level

- Ribosome binding
    - Production of secondary structures →e.g. pseudoknots
- Codon usage


Ribosome binding site
Initiation codon
5′
3′
AGGAGGU
AUG
3–10 nucleotides

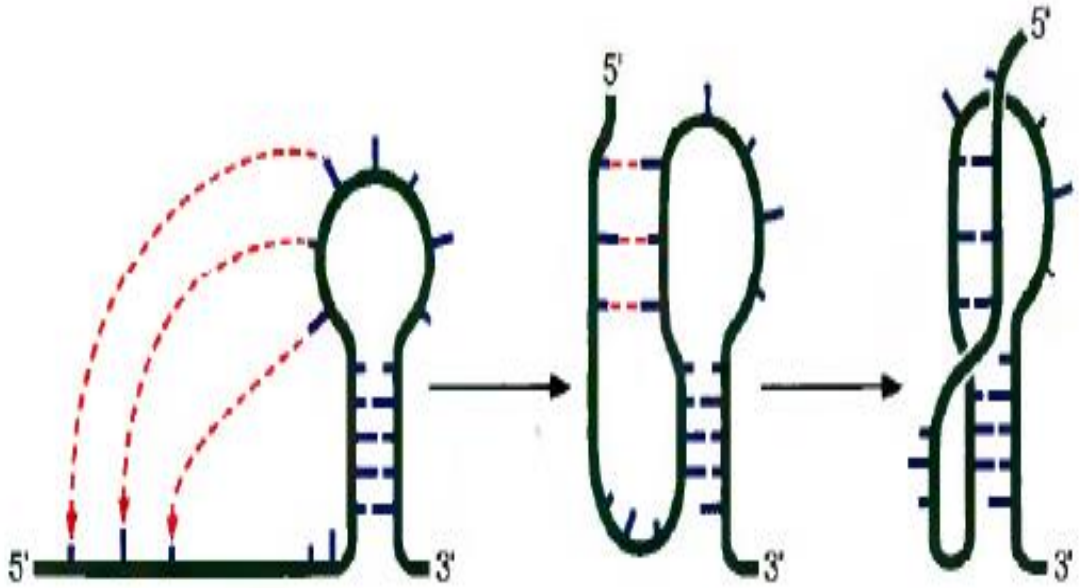

ribosome binding site
16S rRNA
start codon
30S subunit
P
A
5'
AUG
3'
16S rRNA
3' AUUCCUCCACUAG 5'
5' NNN AGGAG NNNNNNNNNAUGNN 3'
mRNA

- ***Ribosome binding***

  - Sequence of RBS and its distance from the start codon can vary, so it is to be expected that there will be weak and strong ribosome binding sites

  - But the distance separating the ribosome binding site from the initiation codon can have a powerful effect on gene expression.

FIGURE 6-32 Pseudoknot. The pseudoknot structure is formed by base pairing between nonconfiguous complementary sequences



Codon usage

- **Codon usage bias** refers to differences in the frequency of occurrence of synonymous codons in coding DNA.

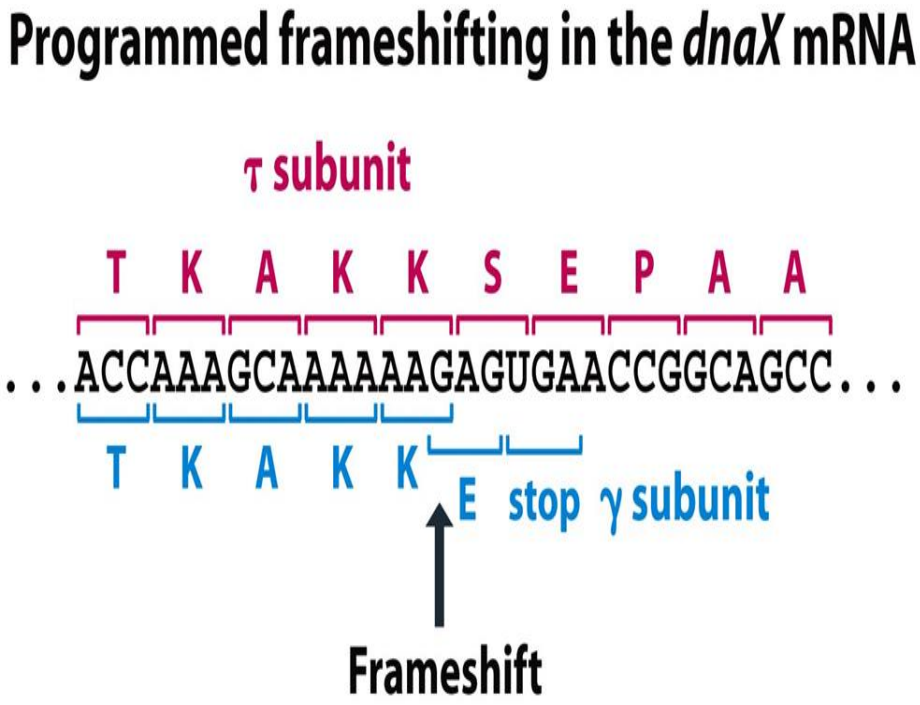
- **Optimal codons** in fast-growing microorganisms, like *Escherichia coli* or *Saccharomyces cerevisiae* (baker's yeast), reflect the composition of their respective genomic tRNA pool.

- It is thought that optimal codons help to achieve faster translation rates and high accuracy.

Unusual events during translation

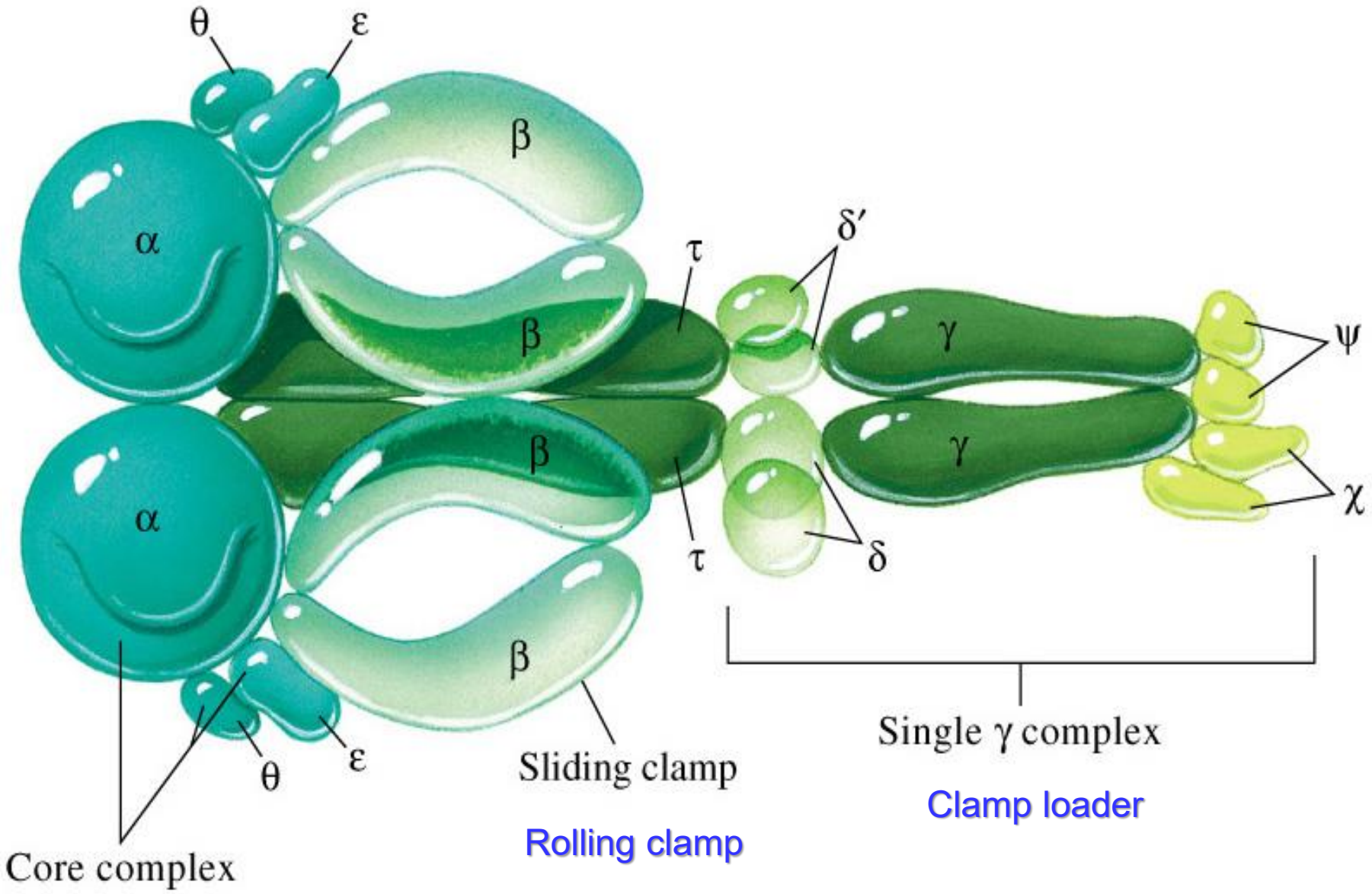
Frameshifting

- Frameshifting Mutation →
  - Deletion / Insertion
    - Change aa sequence
    - Premature stop codon → Produce truncated protein
- Chemical agents
  - Intercalating agents
    - Proflavin
    - Ethidium bromide
    - S-aminoaucridin
    - PI

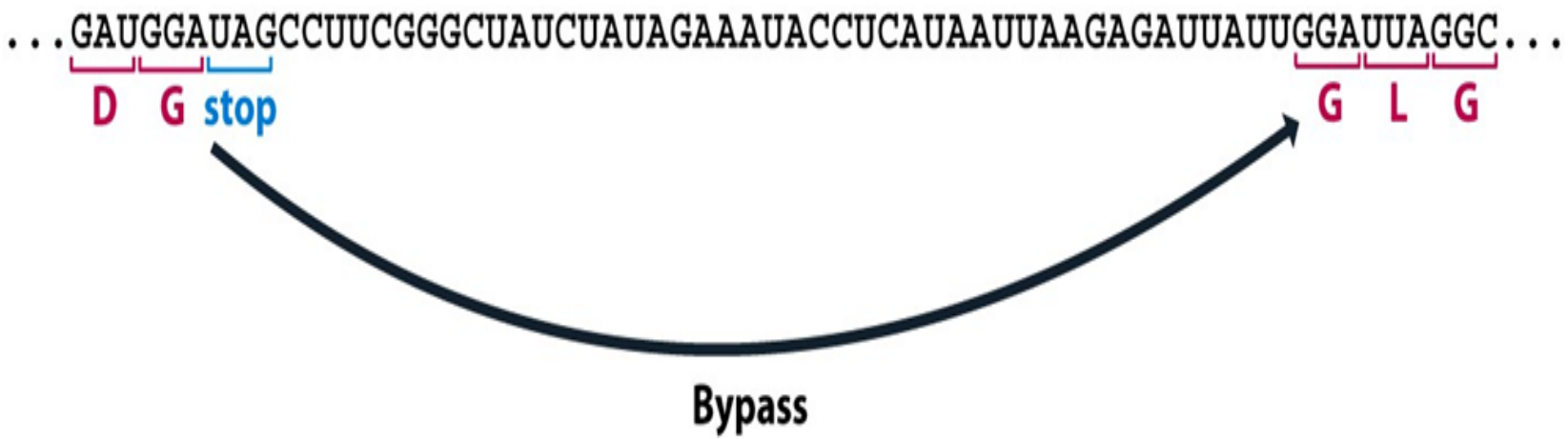


- All organisms
- Example → E.coli DNA pol III
- Causative agents
  - Hairpin
  - Similar sequence for ribosome interaction

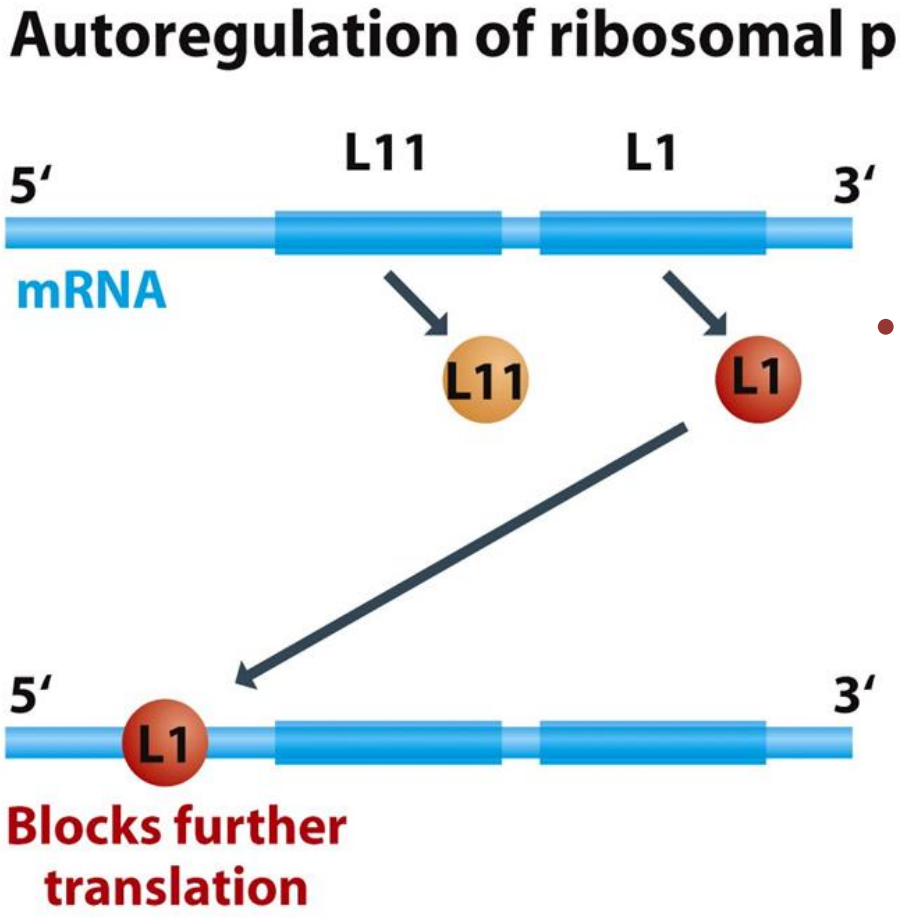
DNA Polymerase III



Translational bypassing in the T4 gene *60* mRNA



Post-translational regulation



- **Feedback inhibition**