厦门大学
生命科学学院
SCHOOL OF LIFE SCIENCES XIAMEN UNIVERSITY

MICROBIOLOGY

Lecture 10

Bacterial genome replication and expression

Regulation of bacterial cellular processes

JING YUAN

MICROBIOLOGY

Chapter 10 Molecular Genetic

10.1 Griffith Experiment

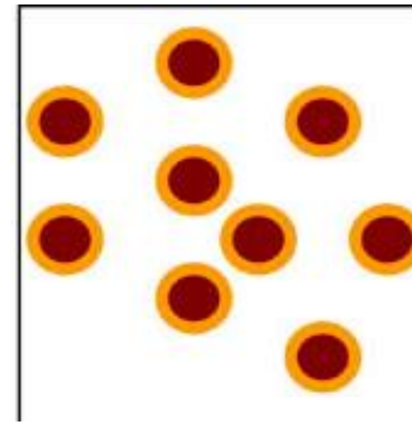
Griffith实验 1928

Griffith Frederick (1879 - 1941) 英国细菌学家和流行病学家

肺炎球菌(Diplococcus pneumoniae)

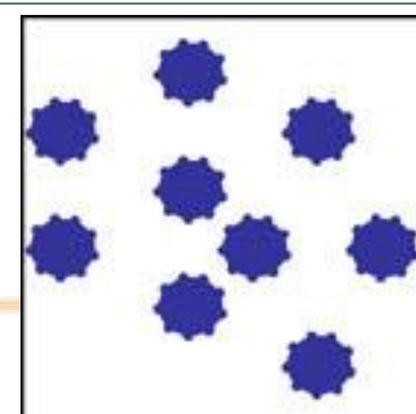
光滑型菌株 (Smooth，简称S型)

- 体外培养时，细胞产生荚膜，细菌菌落光滑；
- 感染人体导致肺炎；
- 感染小鼠导致败血症并且死亡。



粗糙型菌株 (Rough，简称R型)

- 体外培养时，细胞不产生荚膜，菌落粗糙；
- 感染人和小鼠，不发病。



Griffith实验

实验一　S 菌株感染小鼠，致死

实验二　R 菌株感染小鼠，正常

实验三　S 菌株热灭活后感染小鼠，正常

实验结果符合预期

Griffith实验

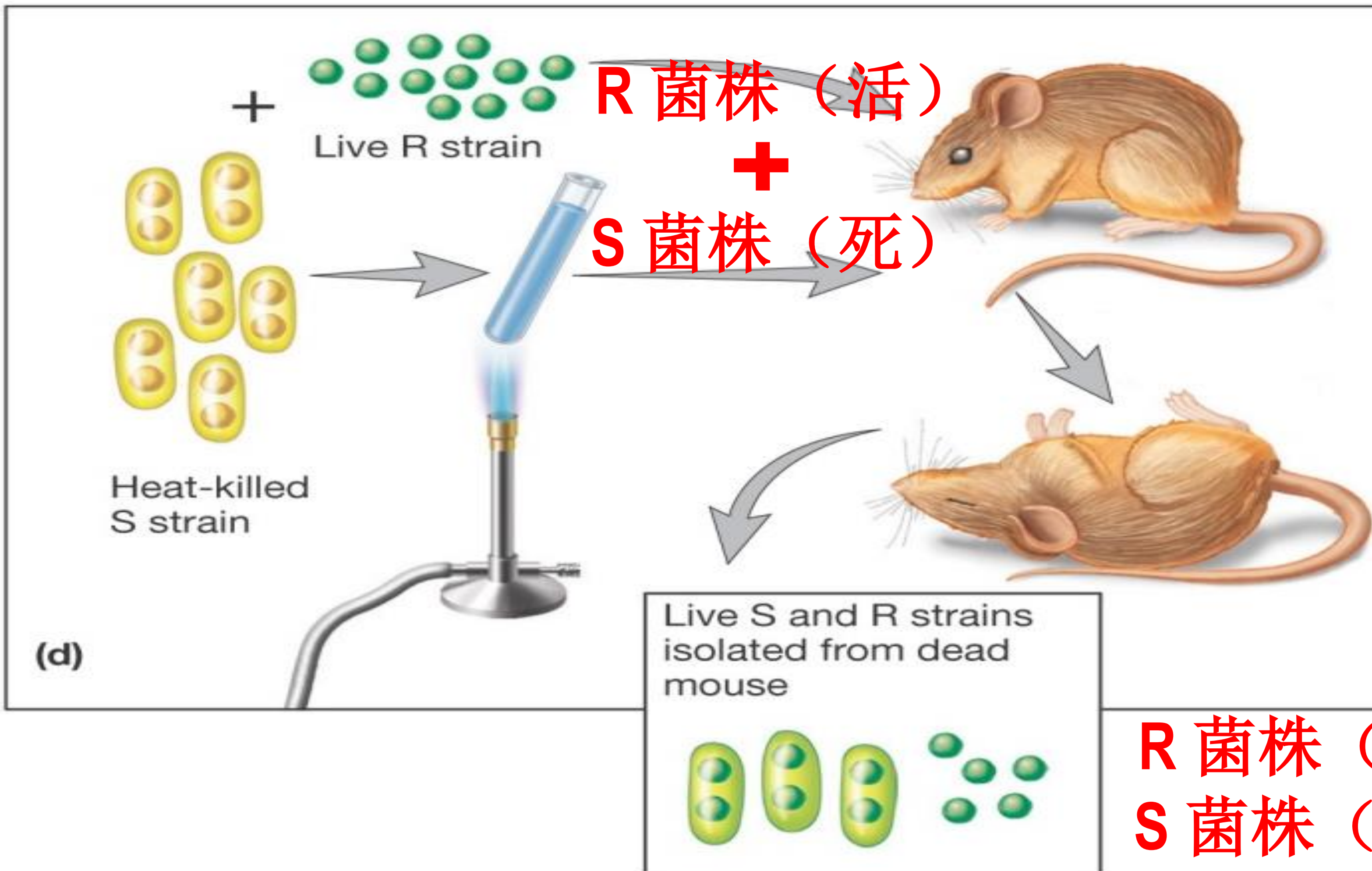
实验一　S 菌株感染小鼠，致死

实验二　R 菌株感染小鼠，正常

实验三　S 菌株热灭活后感染小鼠，正常

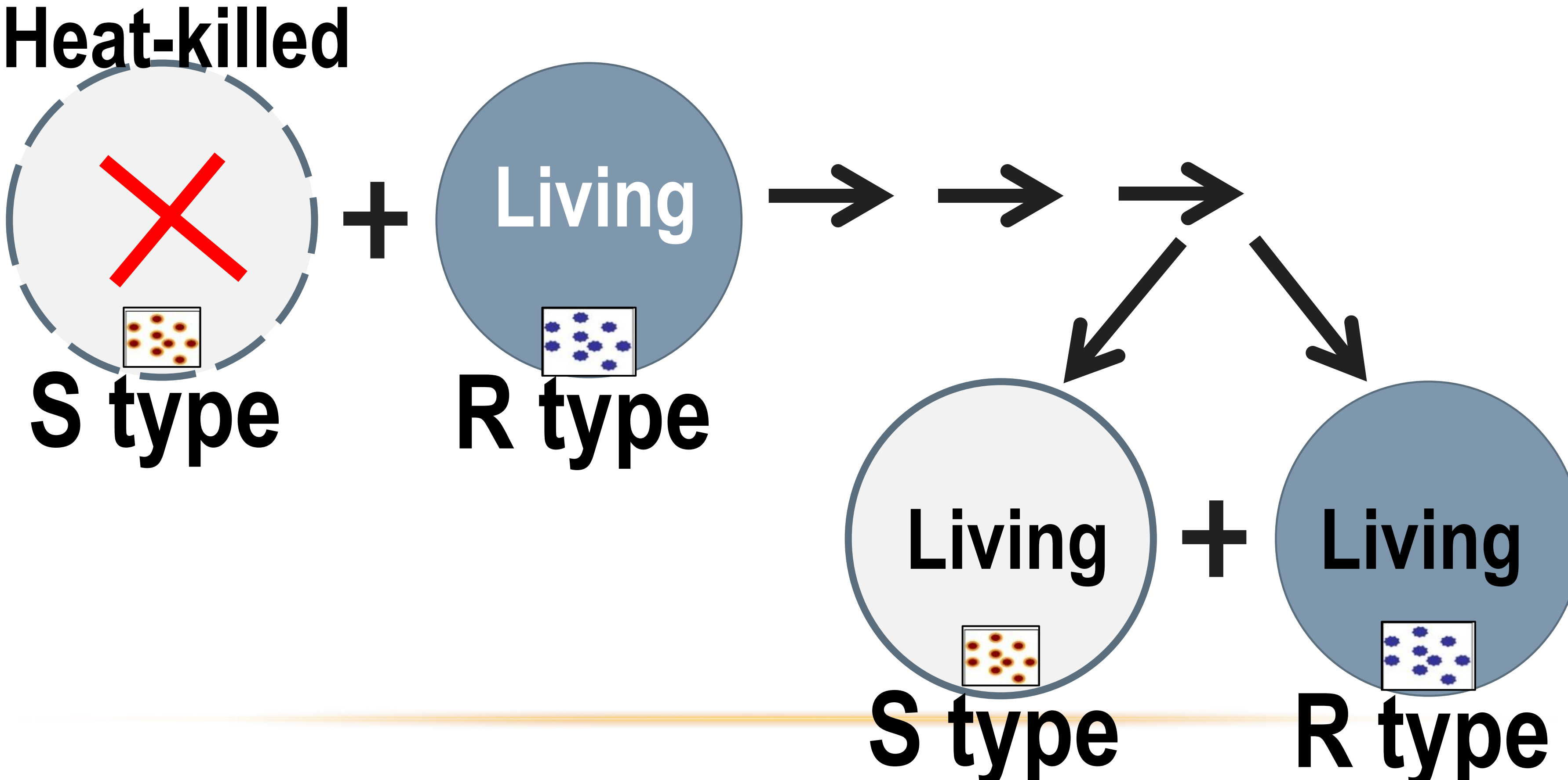
实验四　S 菌株热灭活　+ R 菌株，混合后感染小鼠，什么结果？

实验四的意外发现：细菌转化

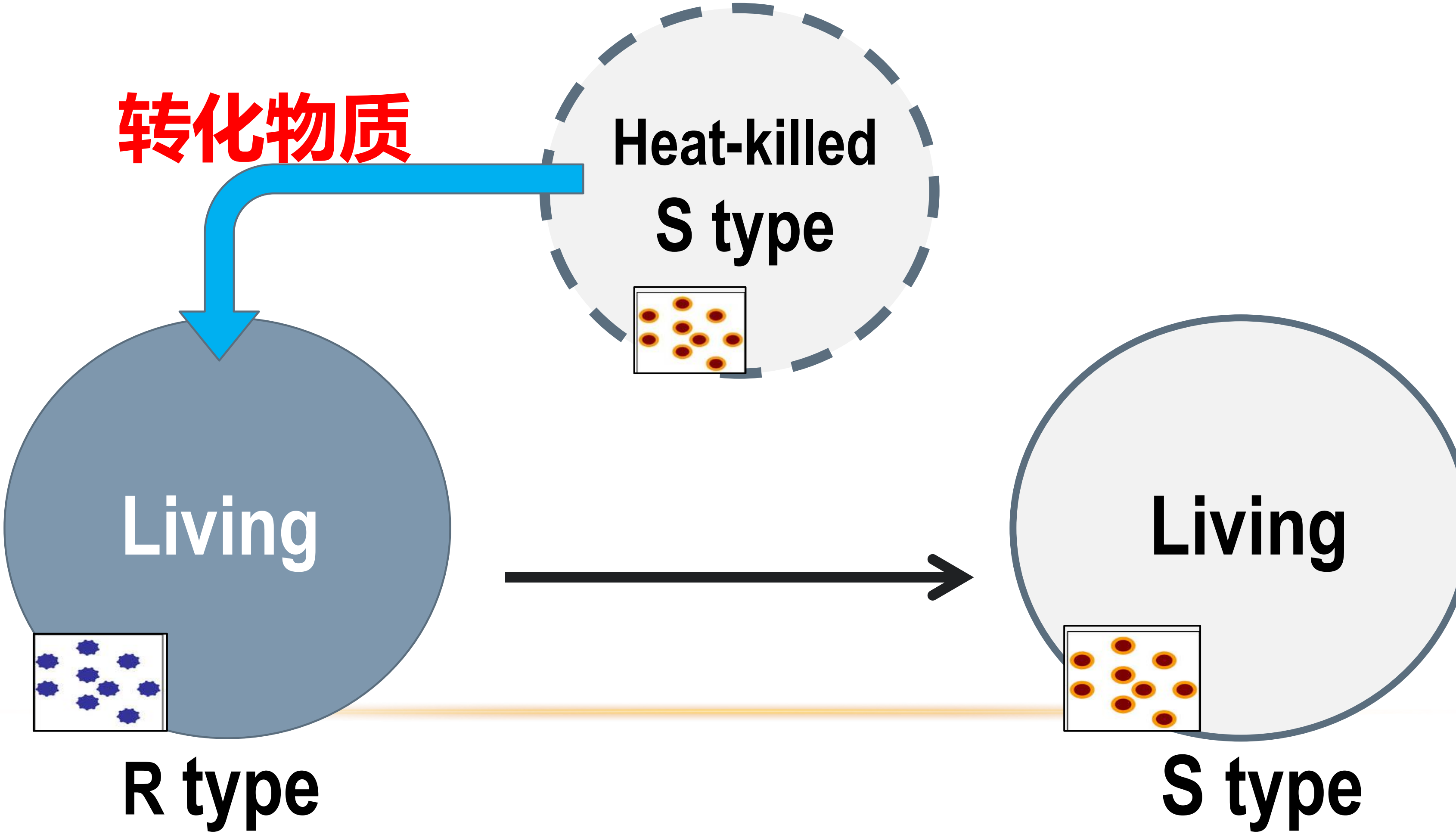


R 菌株（活）
S 菌株（活）

细菌转化（Transformation）



逻辑推导：肺炎球菌细胞中存在“转化物质”



Chapter 10 Molecular Genetic

10.2 Avery Experiment

肺炎球菌中的转化物质具体是什么？

What is the transforming agents in *D. pneumoniae*?

肺炎球菌转化物质的化学本质研究

年代：**1944**

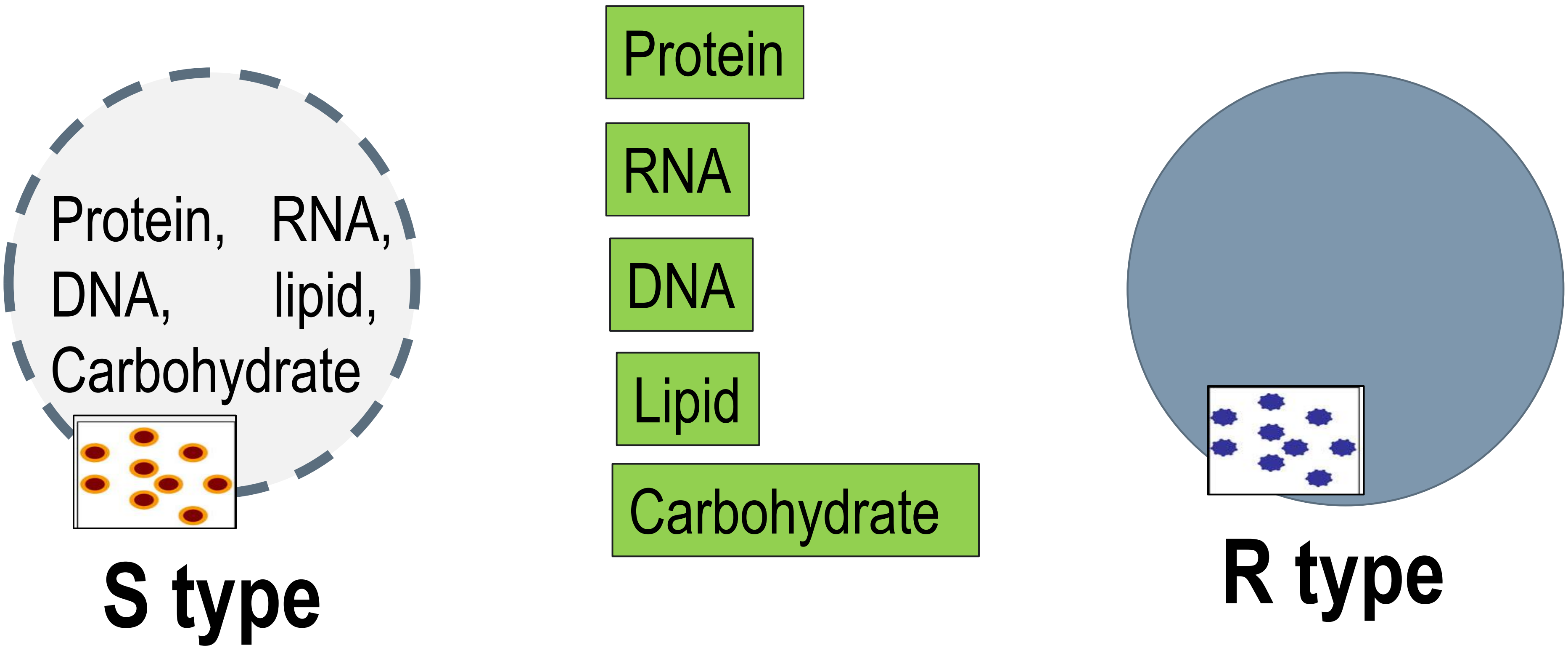
杂志：*J. Experimental Medicine*

作者：Avery, MacLeod, and McCarty

Oswald Avery

美国细菌学家 1877---1955

如何确定“转化物质”是什么?



思路：单组分分离，然后验证其转化能力

Avery实验之功能获得分析

Killed S-type cell	+ R type cell	→	S type colony
Purified protein	+ R type cell	→	R type colony
Purified lipid	+ R type cell	→	R type colony
Purified RNA	+ R type cell	→	R type colony
Purified DNA	+ R type cell	→	S type colony

分离纯化的单组分

Avery实验之功能缺失分析

R type cell + (S cell extract) → S type colony

R type cell + (S cell extract + 蛋白酶) → S type colony

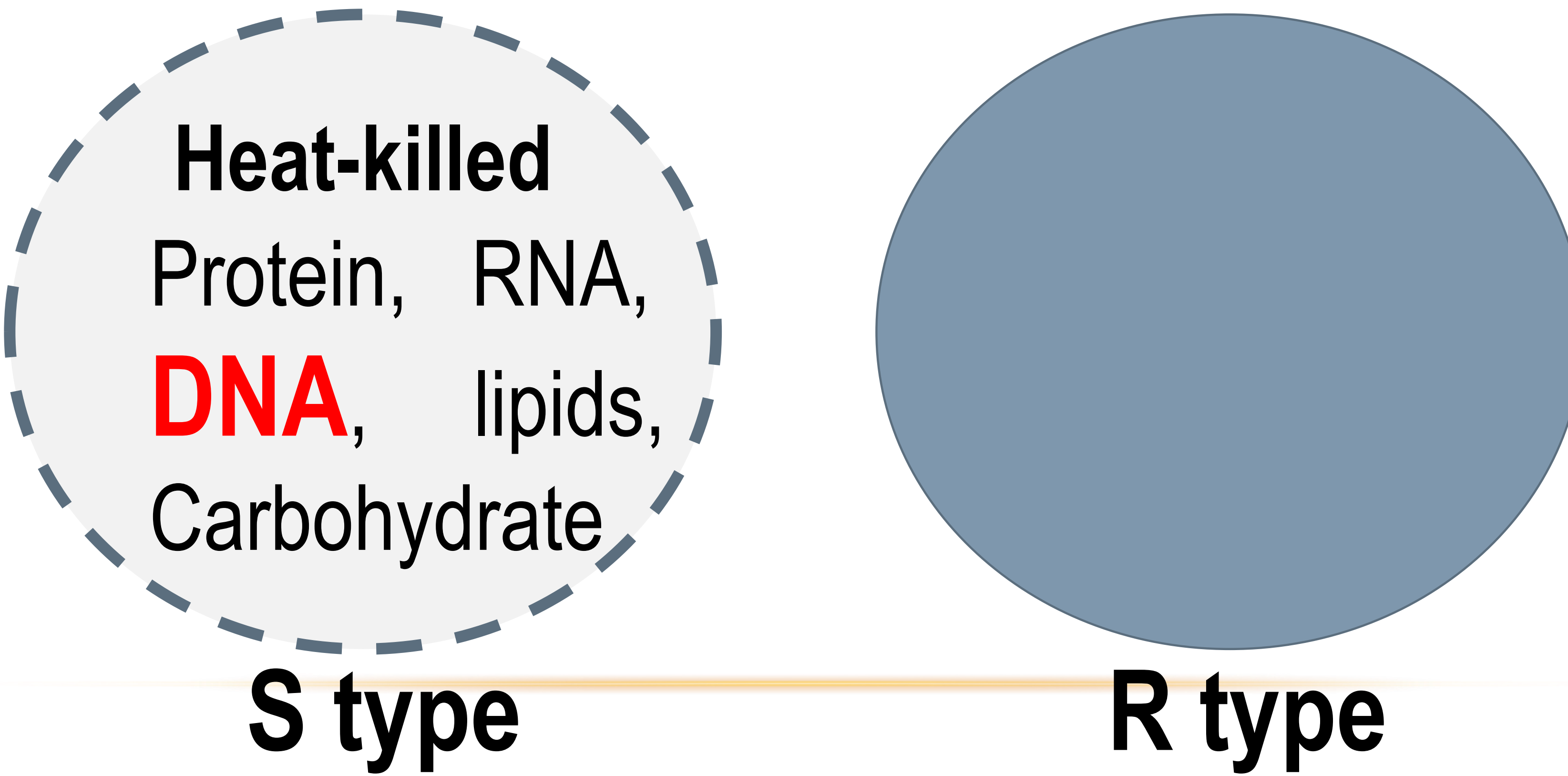
R type cell + (S cell extract + 脂酶) → S type colony

R type cell + (S cell extract + RNA酶) → S type colony

R type cell + (S cell extract + DNA酶) → R type colony

细胞提取物　　降解酶

结论：DNA是肺炎球菌的遗传转化物质



1850s 孟德尔发现看不见的因子

1928 Griffith发现细菌转化

1943 Avery揭示DNA是转化物质

1953 Crick/Waston 揭示DNA的双螺旋结构

MICROBIOLOGY

Chapter 10

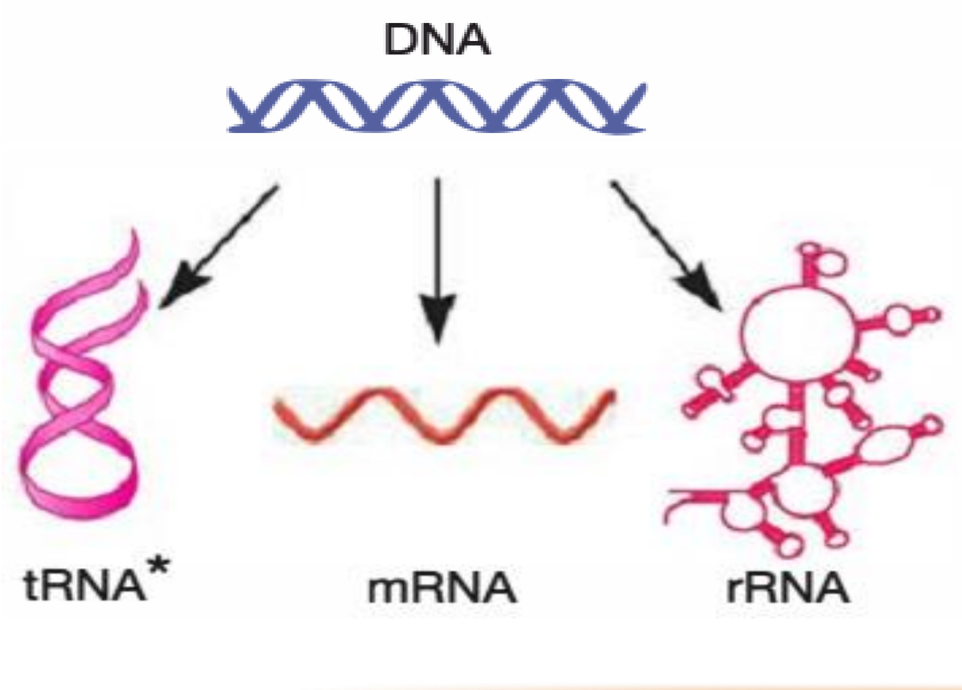
10.3 Bacterial Gene Classification and Structure
细菌基因的分类和结构

Concept of “Gene”

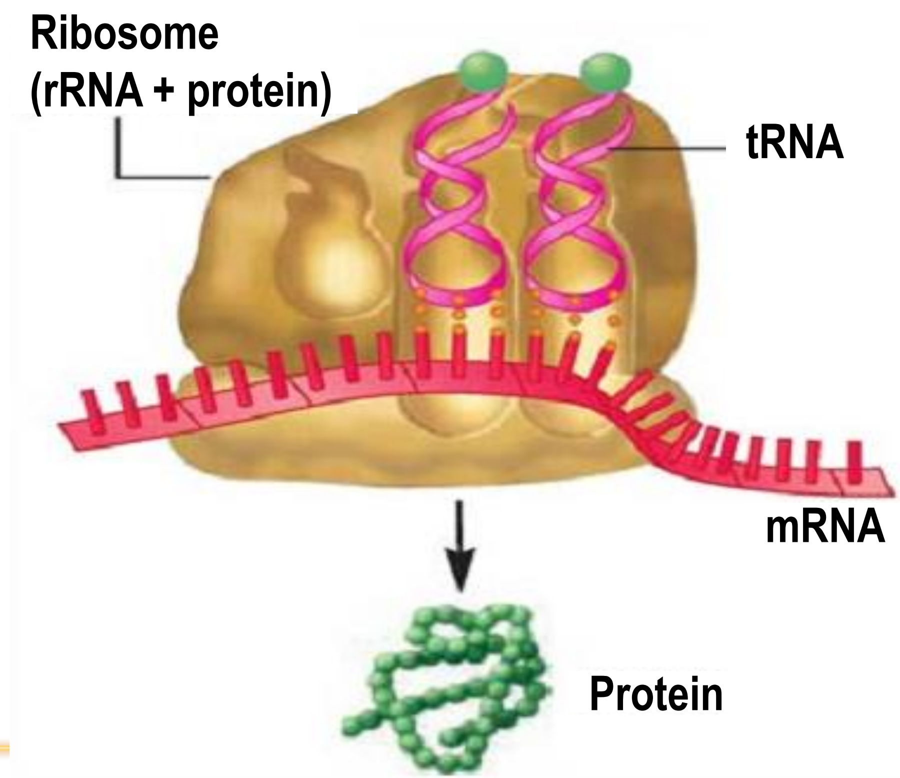
- One gene -------One protein
- One gene -------One polypeptide

- Transfer RNA (tRNA) gene
- Ribosome RNA (rRNA) gene

Transcription



Translation



Concept of “Gene”

- One gene -------One protein
- One gene -------One polypeptide

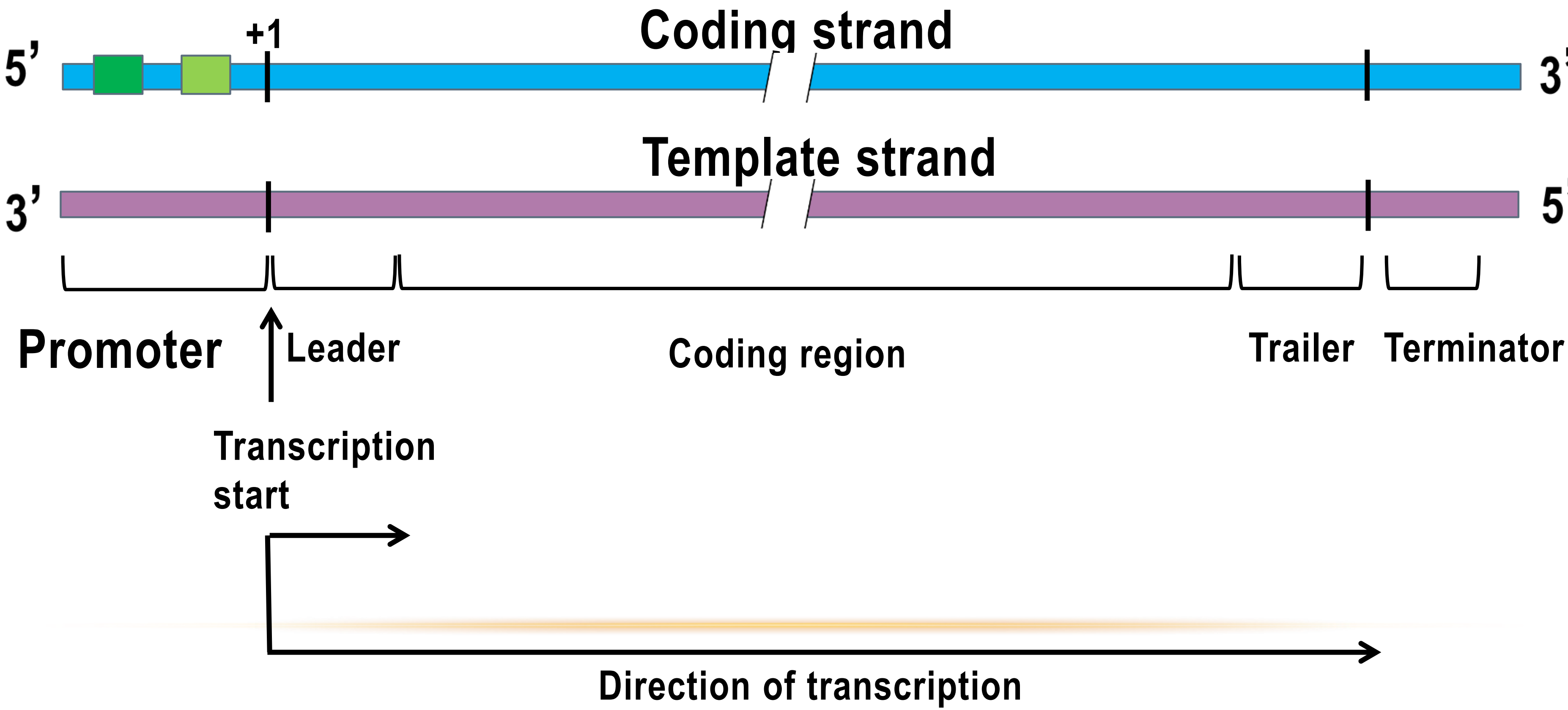
- Transfer RNA (tRNA) gene
- Ribosome RNA (rRNA) gene

- Non-coding RNA genes

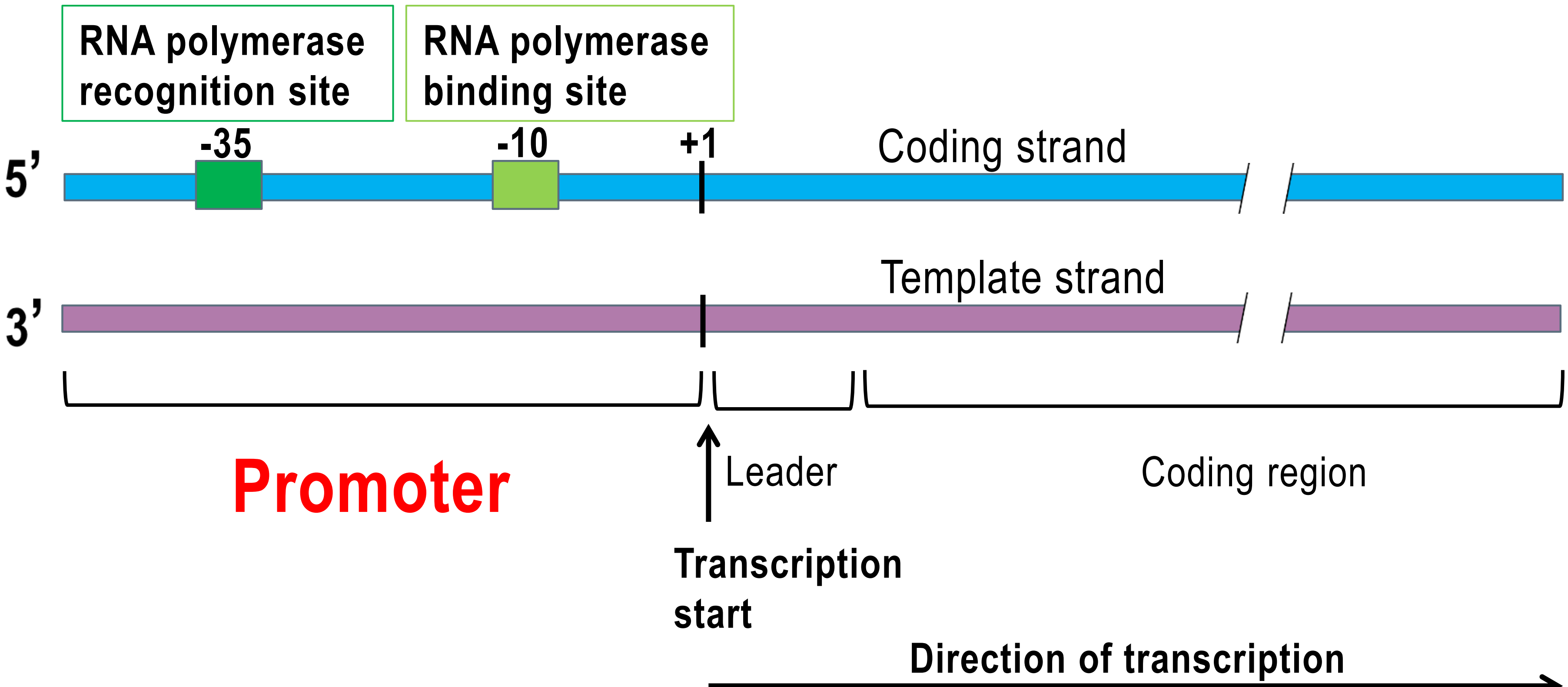
Gene: The basic unit of genetic information

- Protein-coding genes
- tRNA and rRNA genes
- Non-coding RNA genes

A Bacterial Protein-coding Gene Structure



Promoter

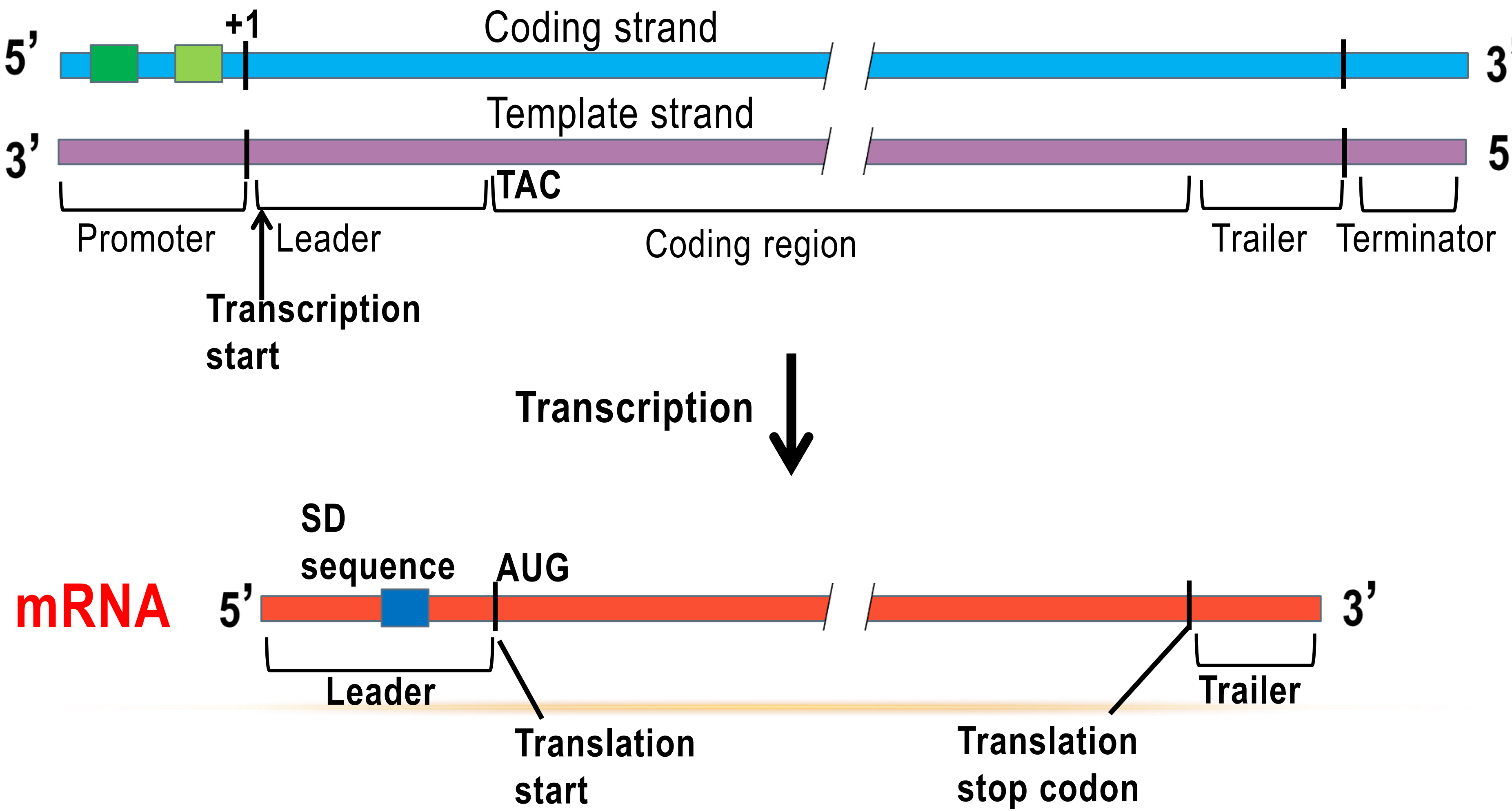


RNA polymerase recognition site
RNA polymerase binding site
-35
-10
+1
Coding strand
5’
Template strand
3’
Promoter
Leader
Coding region
Transcription start
Direction of transcription

Gene promoter

- Promoter is locating upstream of the gene.
- Promoter contains the recognition and binding sites for RNA polymerase.
- Promoter functions to orient the RNA polymerase for transcription.

A Bacterial Gene and Its mRNA Product

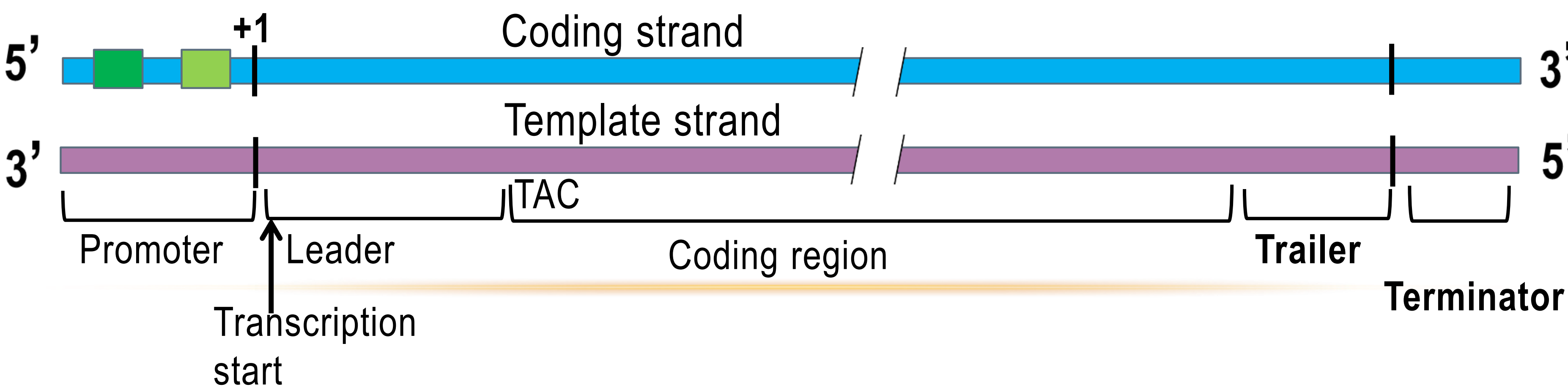


Gene structure

- **Leader sequence** is transcribed into mRNA, but is not translated into amino acids.
- In bacteria, the leader sequence includes a region called the **Shine-Dalgarno sequence**, which is important for initiation of protein translation.

Gene structure

- **Trailer** sequence is transcribed but not translated. It contains sequences that prepare the RNA polymerase for release from the DNA template strand.
- **Terminator** is a sequence that signals the RNA polymerase to stop transcription.



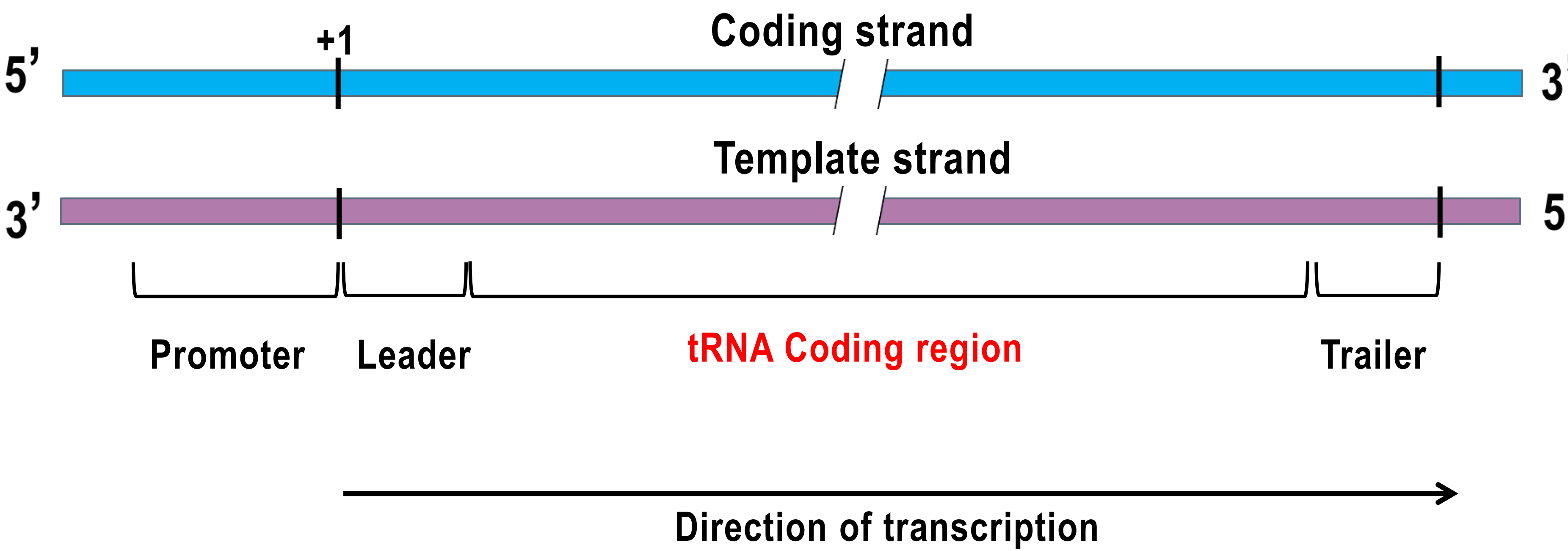
Coding region

- **Linear sequence of nucleotides with a fixed start point and end point**
- **<u>Codons</u> are found in DNA coding strand and code for single amino acids**

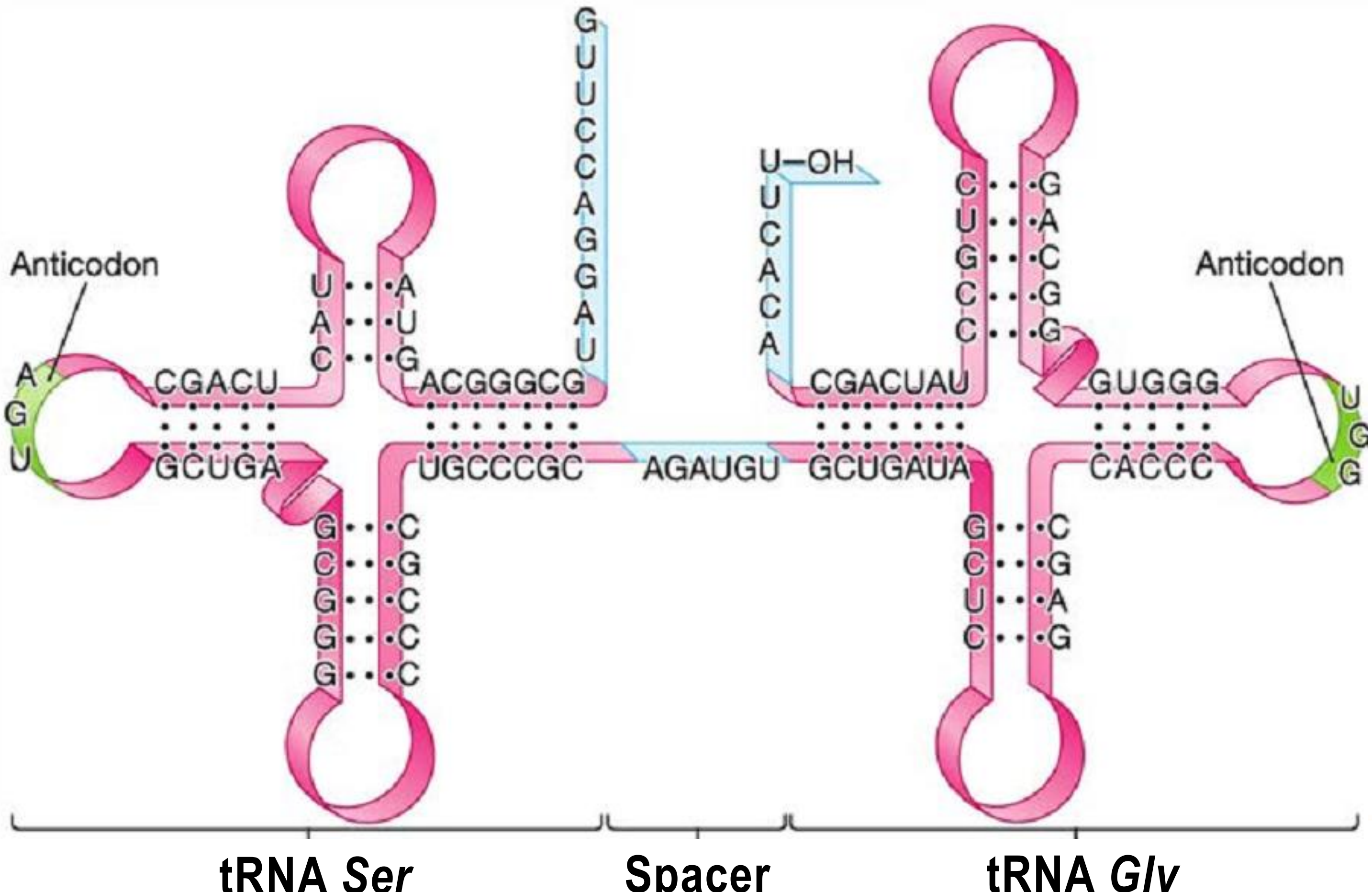
Gene structure in Bacteria

- Bacterial and archaea coding information in gene is normally continuous.
- In eukaryotic, genes that code for proteins (exons) are often interrupted by noncoding regions (introns), which must be removed by splicing.

Bacterial tRNA Gene Structure

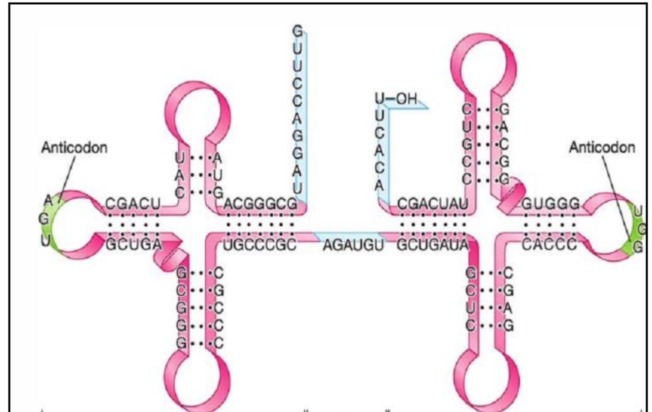


A tRNA precursor from *E.coli*



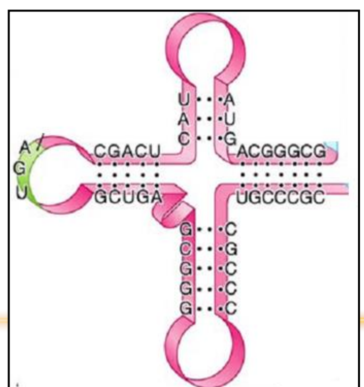
A tRNA precursor from *E.coli*

tRNA precursor



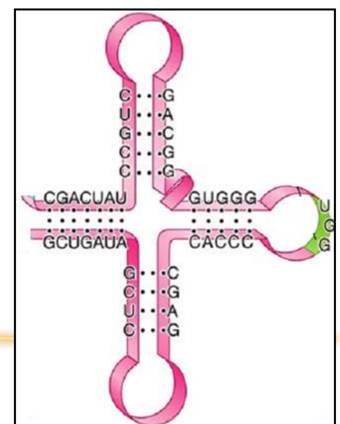
↓ **Ribozyme**

tRNA



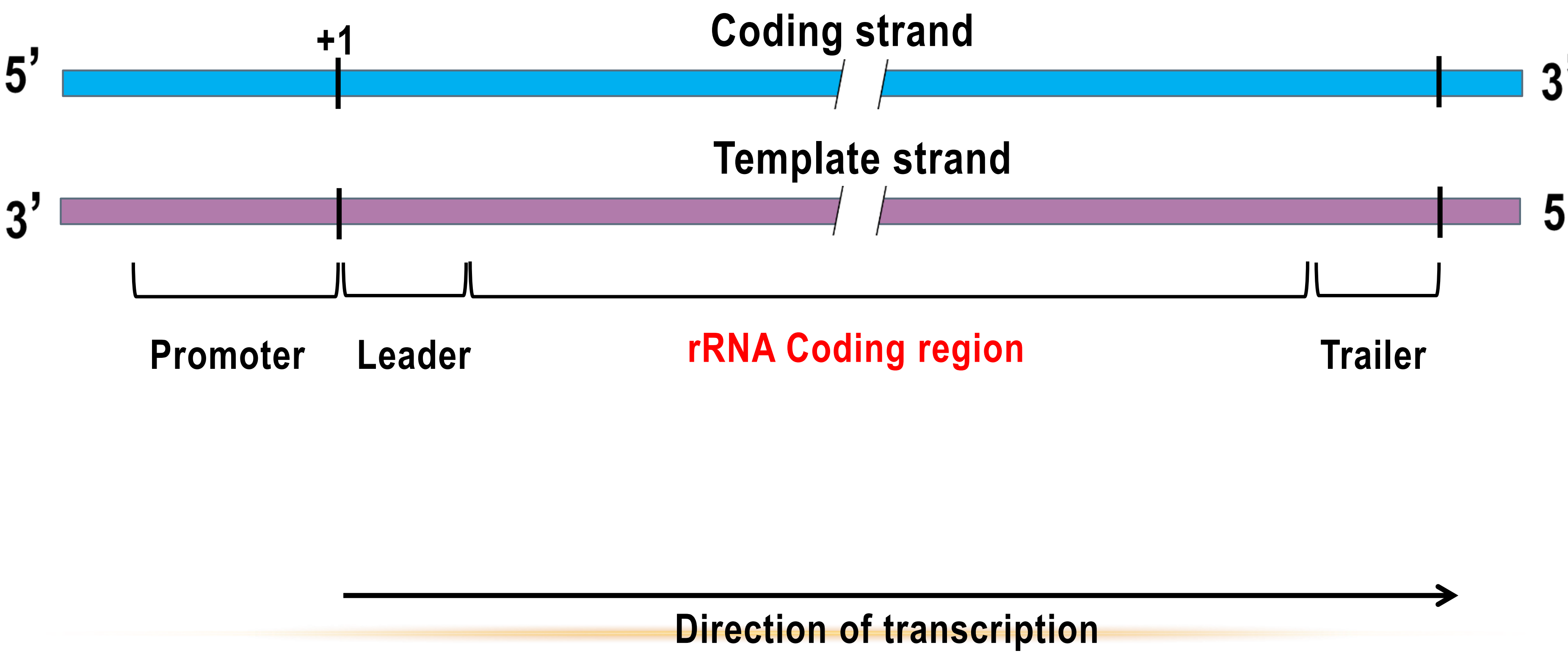
tRNA *Ser*

+

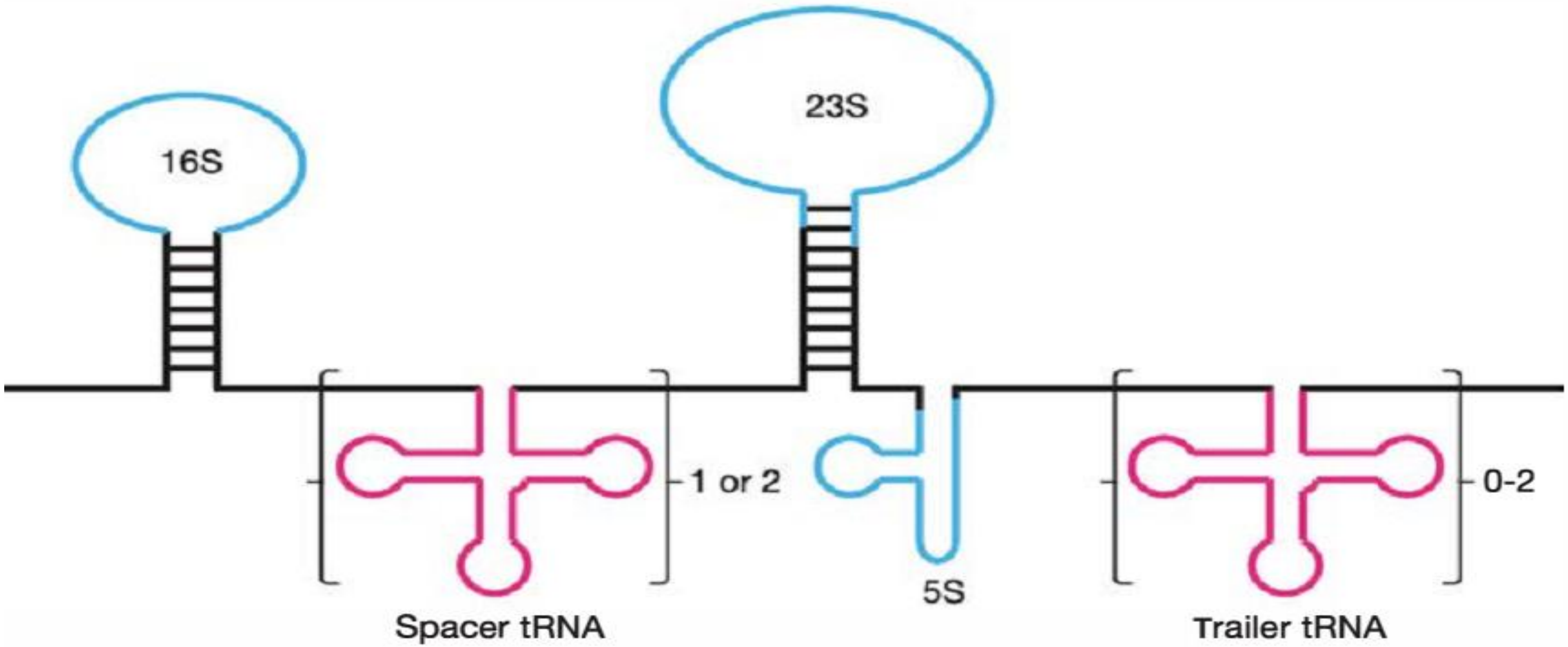


tRNA *Gly*

Bacterial rRNA Gene Structure

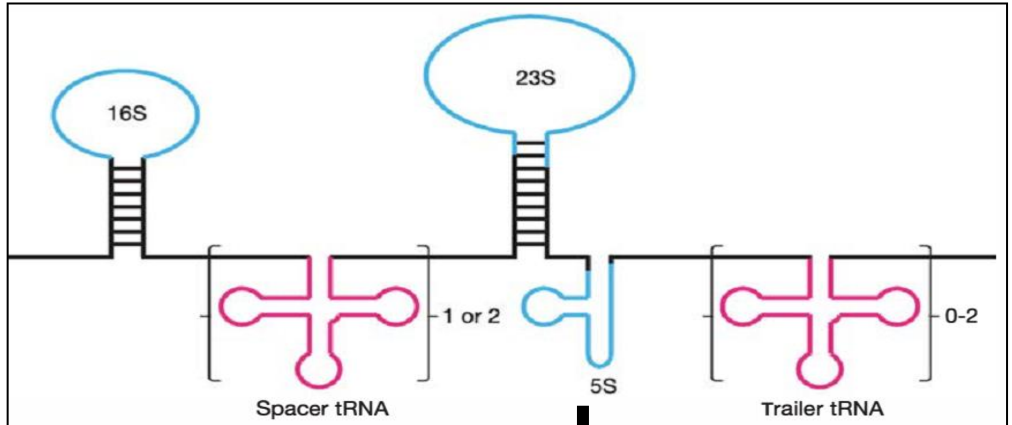


A rRNA precursor from *E.coli*



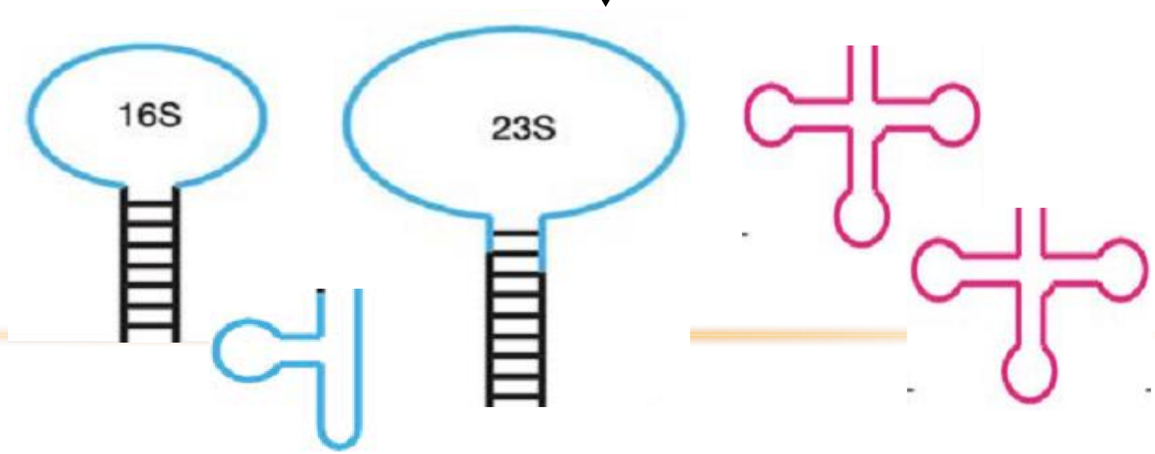
A rRNA precursor from *E.coli*

rRNA precursor



↓ **Ribozyme**

rRNA and tRNA



tRNA and rRNA genes

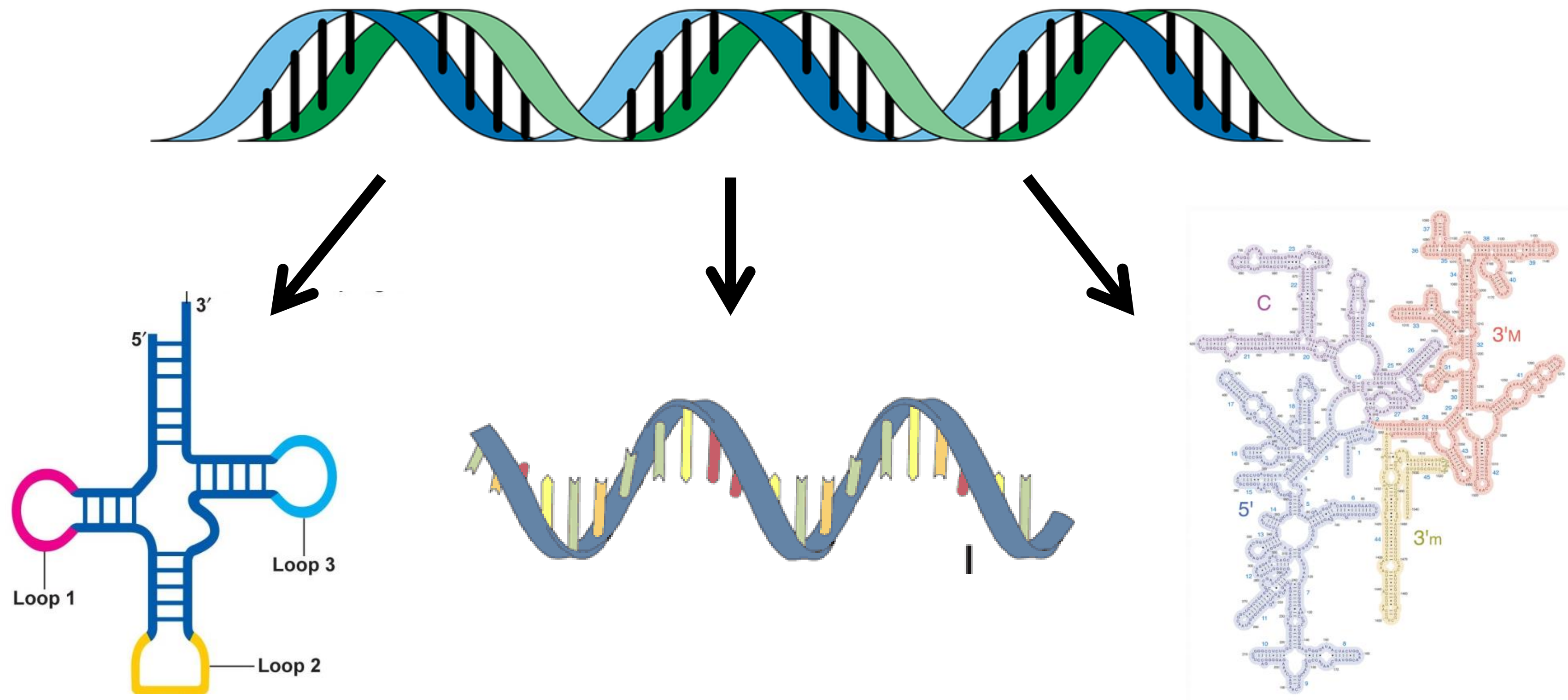
- tRNA genes code for more than one single or one type of tRNA molecule.
- rRNA genes (5S, 16S, 23S) are transcribed as single and large precursor.
- Spacers between the coding regions are removed after transcription, via special ribonucleases called ribozymes.

MICROBIOLOGY

Chapter 10

10.4 Transcription in Bacteria
细菌基因的转录

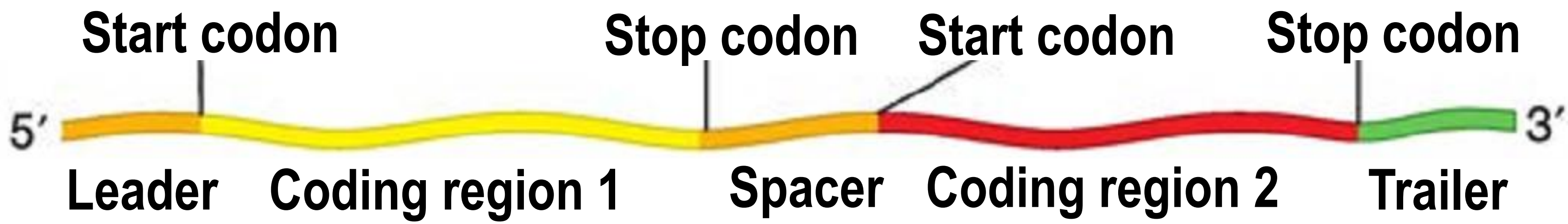
Transcription



tRNA | **mRNA** | **rRNA**

transfer RNA | **messange RNA** | **Ribosome RNA**

Polycistronic mRNA



Monocistronic mRNA

Cell

Volume 73, Issue 3, 7 May 1993, Pages 521–532

Article

线虫

Operons in C. elegans: Polycistronic mRNA precursors are processed by trans-splicing of SL2 to downstream coding regions

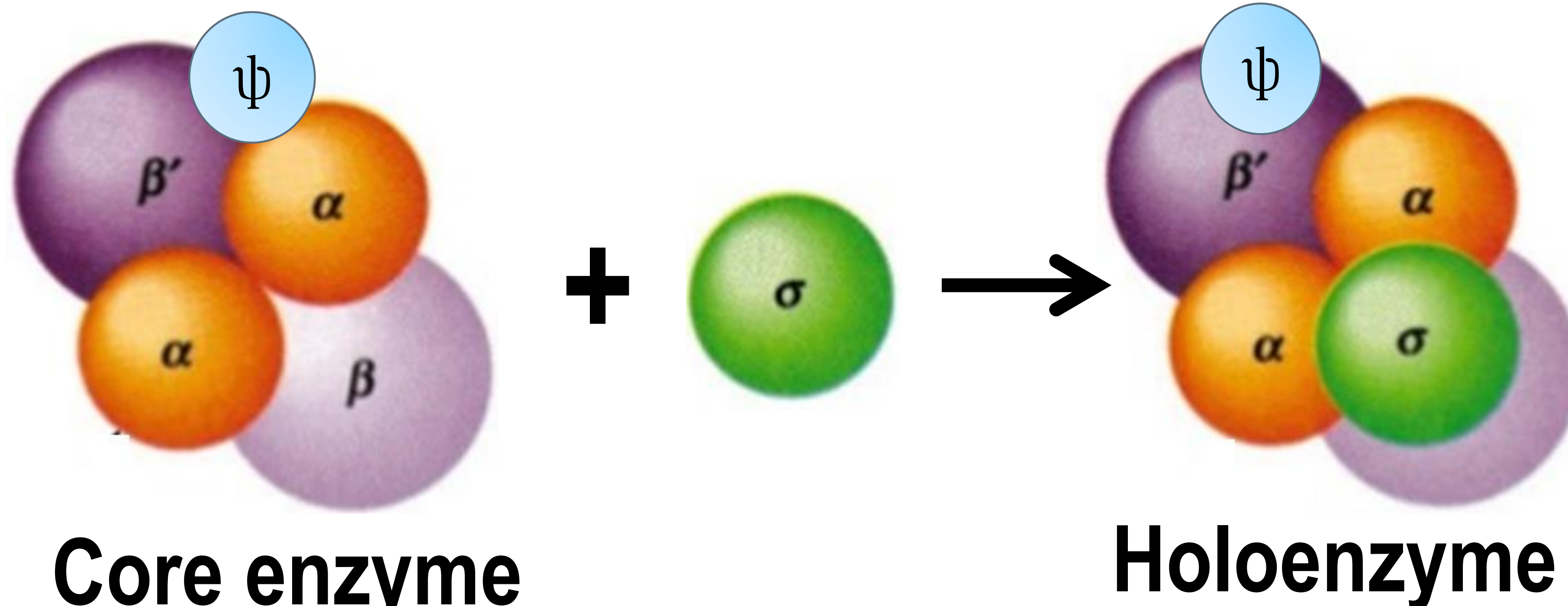
John Spieth, Glenn Brooke, Scott Kuersten, Kristi Lea, Thomas Blumenthal

Department of Biology Indiana University Bloomington, Indiana 47405 USA

Received 25 January 1993, Revised 1 March 1993, Available online 19 April 2004

Show less

Bacterial DNA-Dependent RNA Polymerase

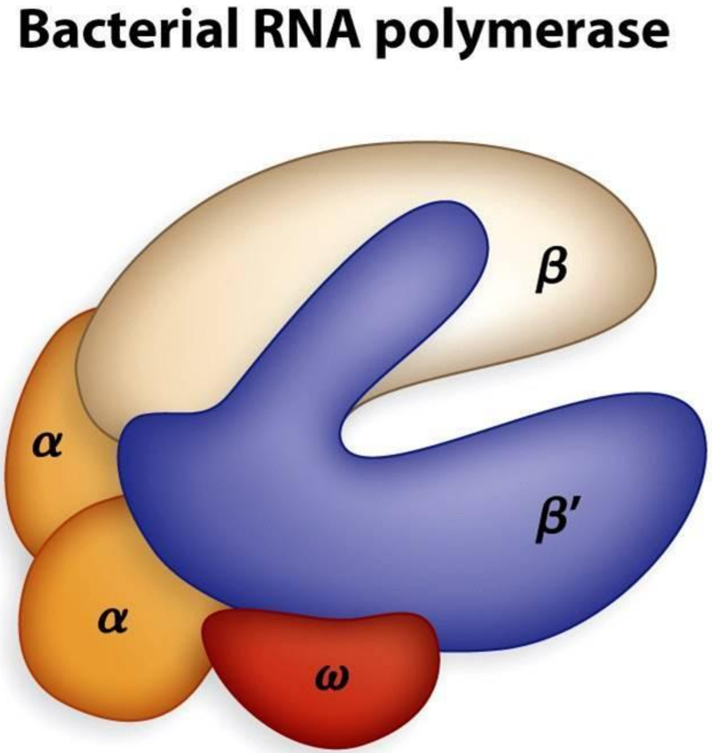


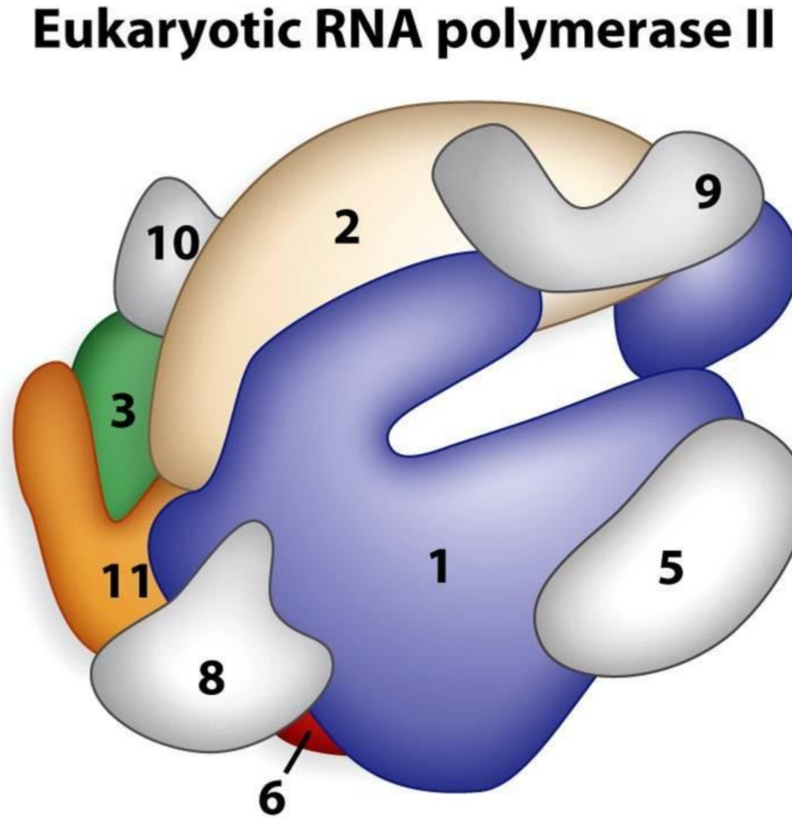
Th RNA polymerase
core enzyme is composed of fie polypeptides (two a
subunits,

Bacterial DNA-Dependent RNA Polymerase

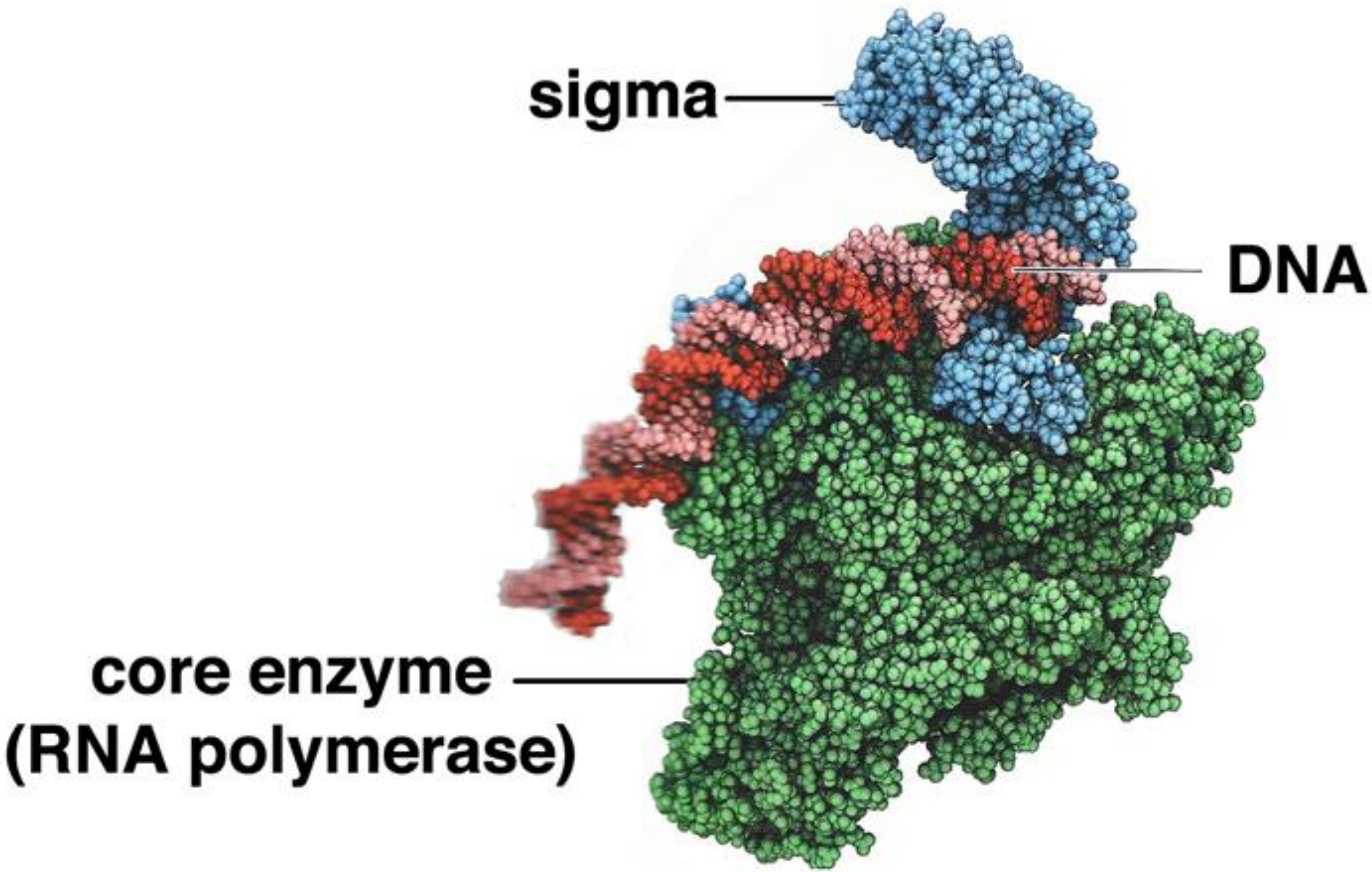
- RNA polymerase core enzyme is composed of five polypeptides and catalyzes RNA synthesis.
- Sigma factor has no catalytic activity but helps the core enzyme recognize the promoter.
- Holoenzyme is required for correct initiation of transcription.

Bacterial and Eukaryotic RNA Polymerase

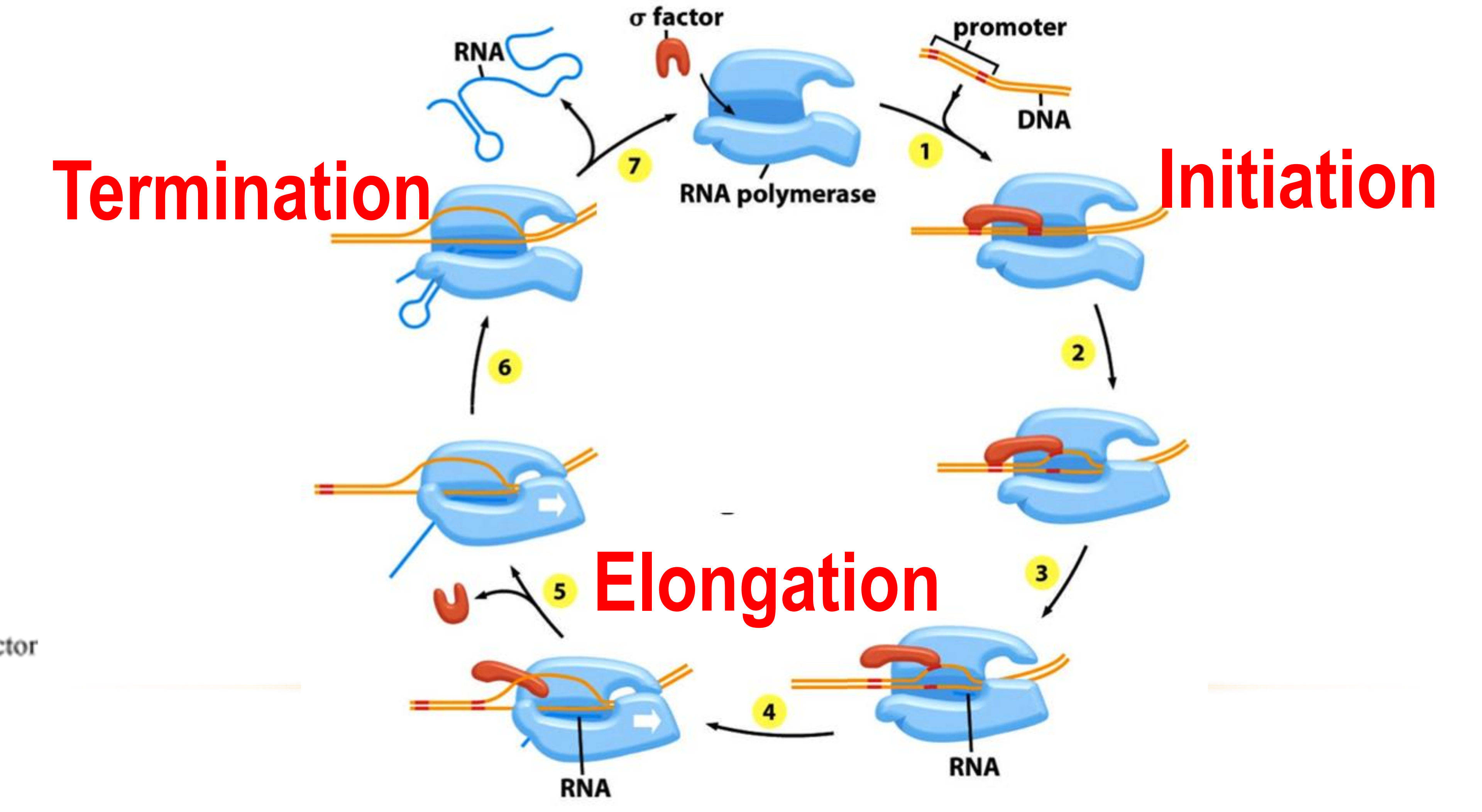




Bacterial RNA Polymerase and binding DNA



The Bacterial Transcription Cycle



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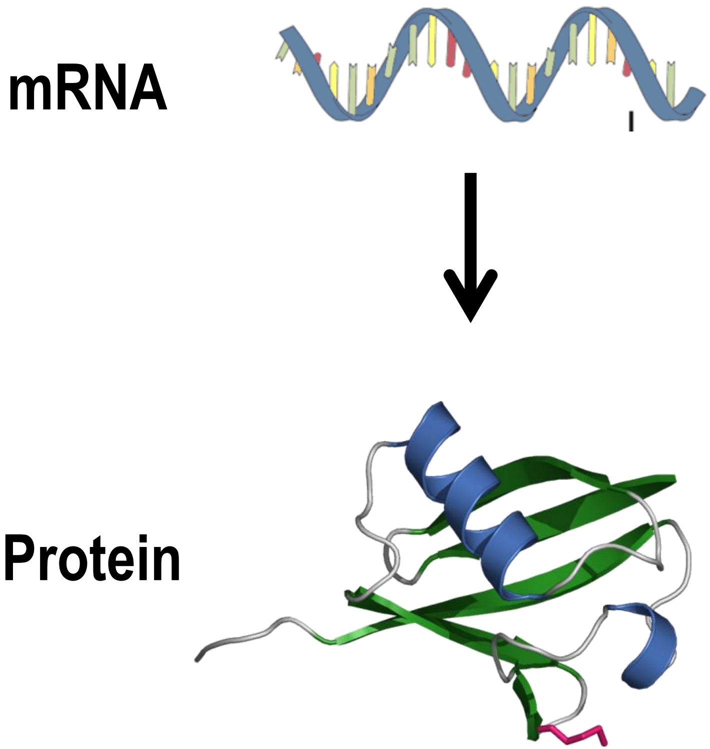
MICROBIOLOGY

Chapter 10

10.5 Translation in Bactria
细菌的翻译



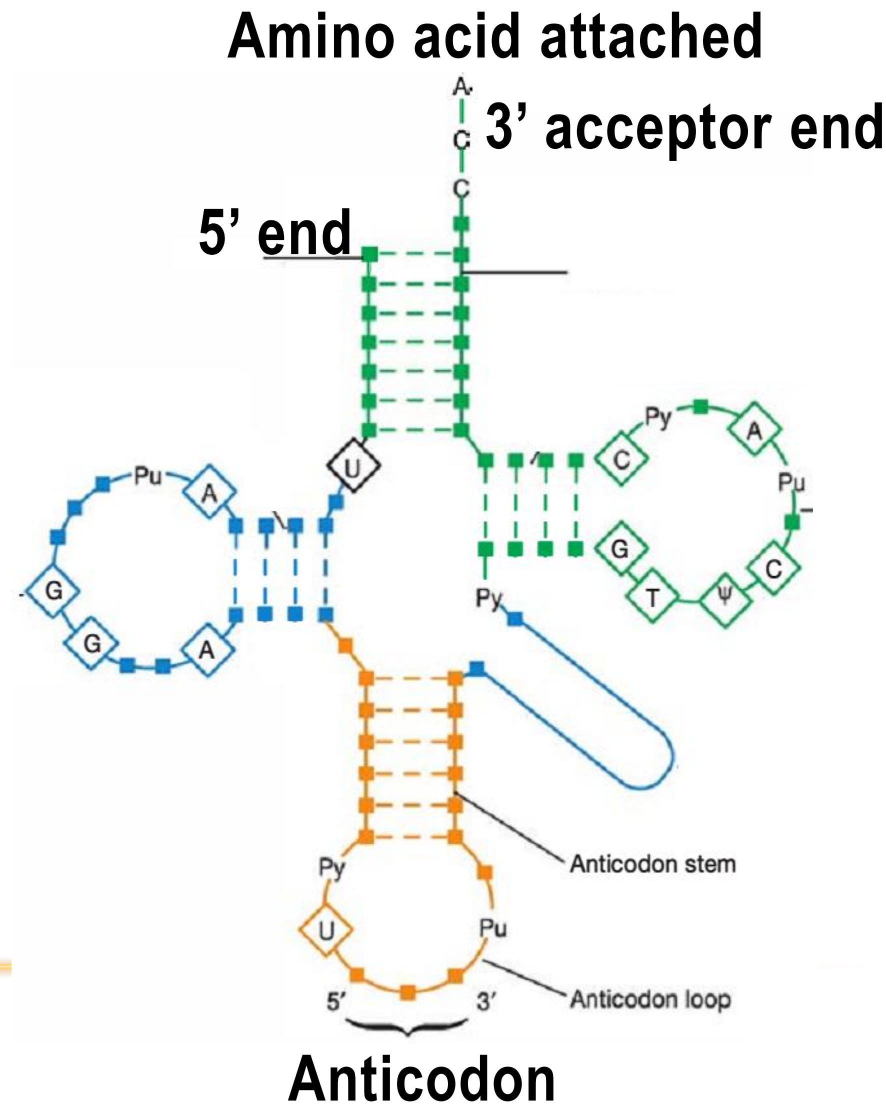
Translation

- mRNA
- tRNA
- Ribosome

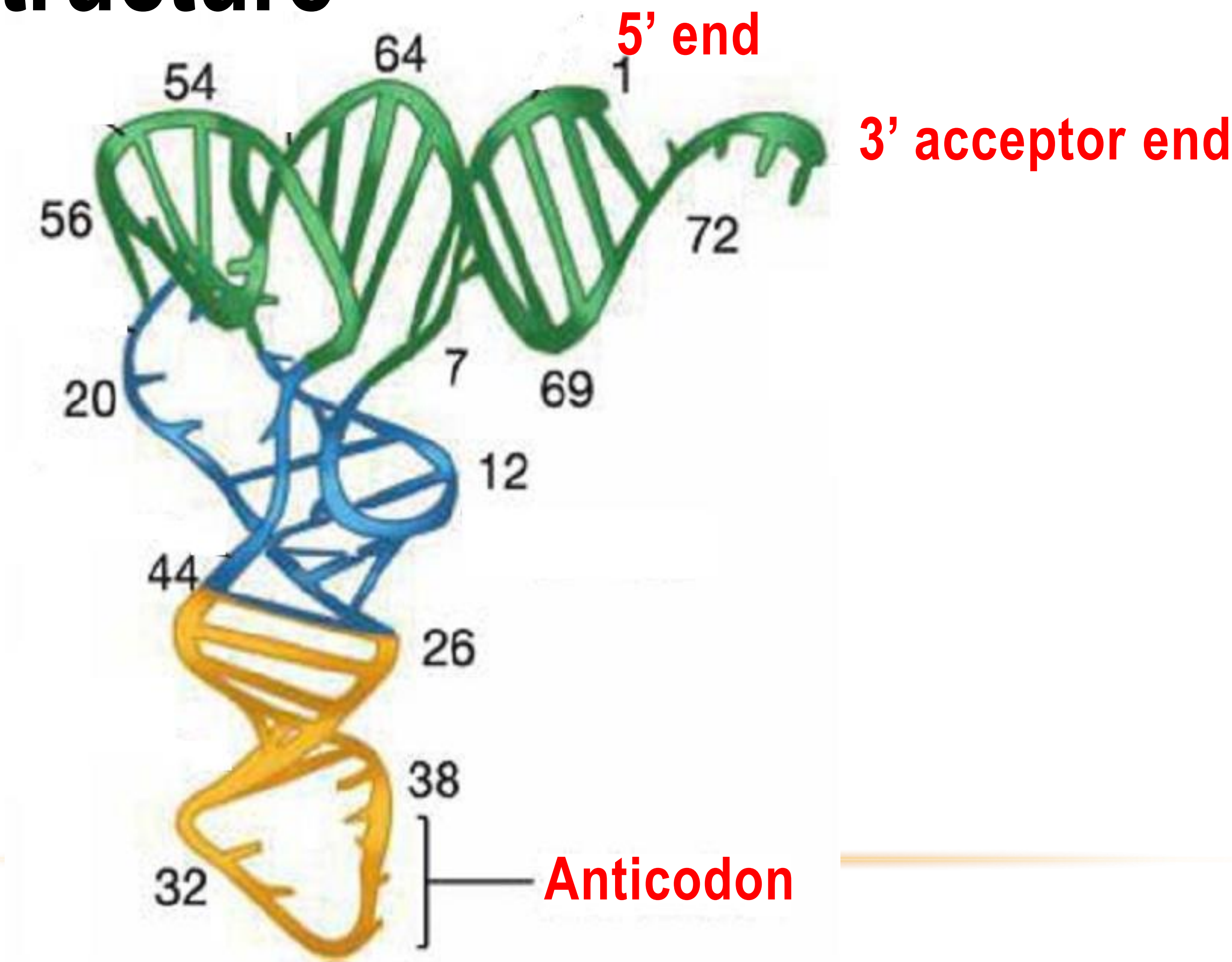
tRNA structure

- Cloverleaf conformation;

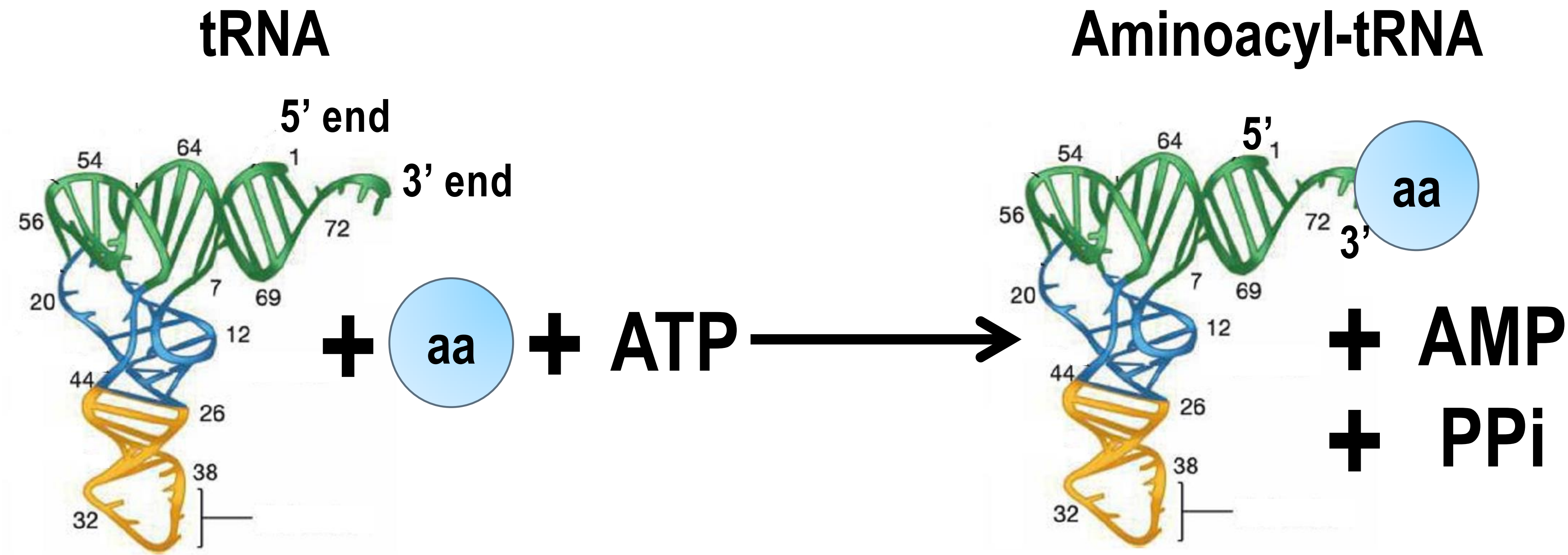
- In E. coli, the 3' end of all tRNAs has the same 5’-C-C-A-3’ sequence;
- Anticodon is complementary to the mRNA codon.



tRNA structure



Amino Acid Activation



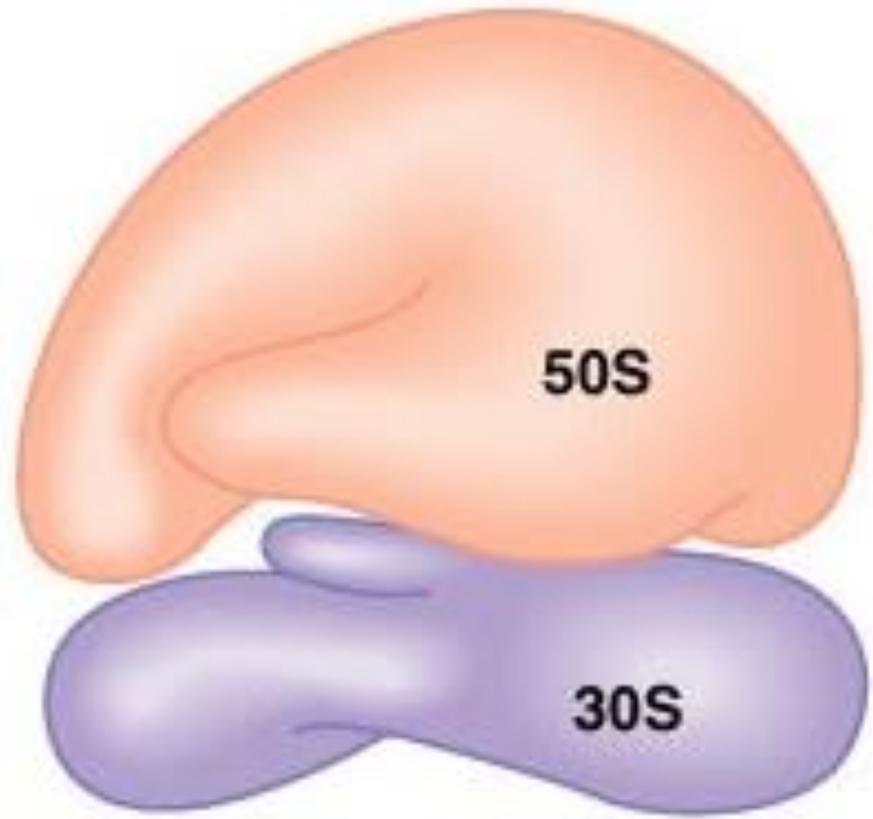
Aminoacyl-tRNA Syntheses

氨酰tRNA合成酶

Ribosome: Translation machine

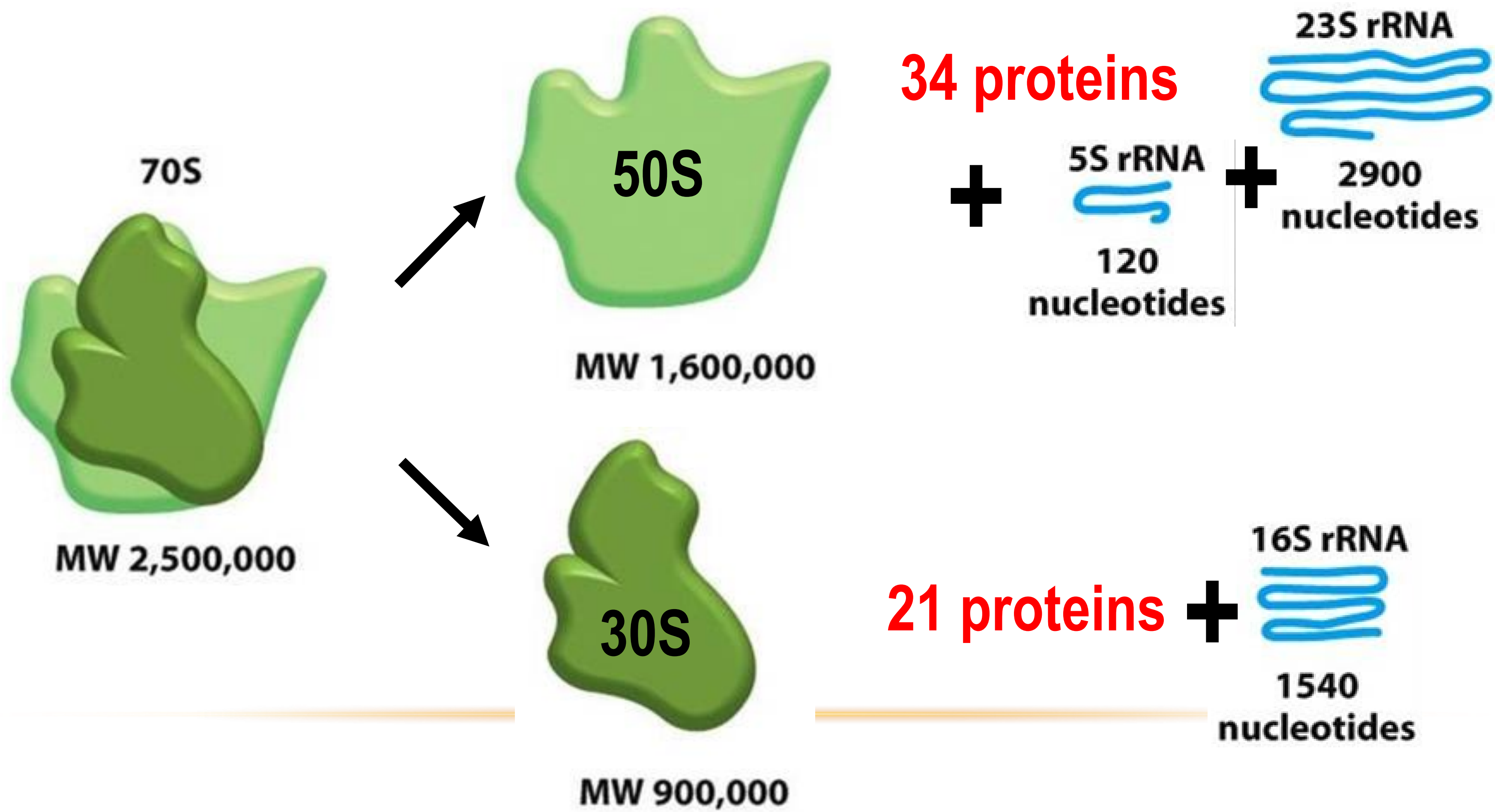
50S subunit (Large)

30S subunit (Small)

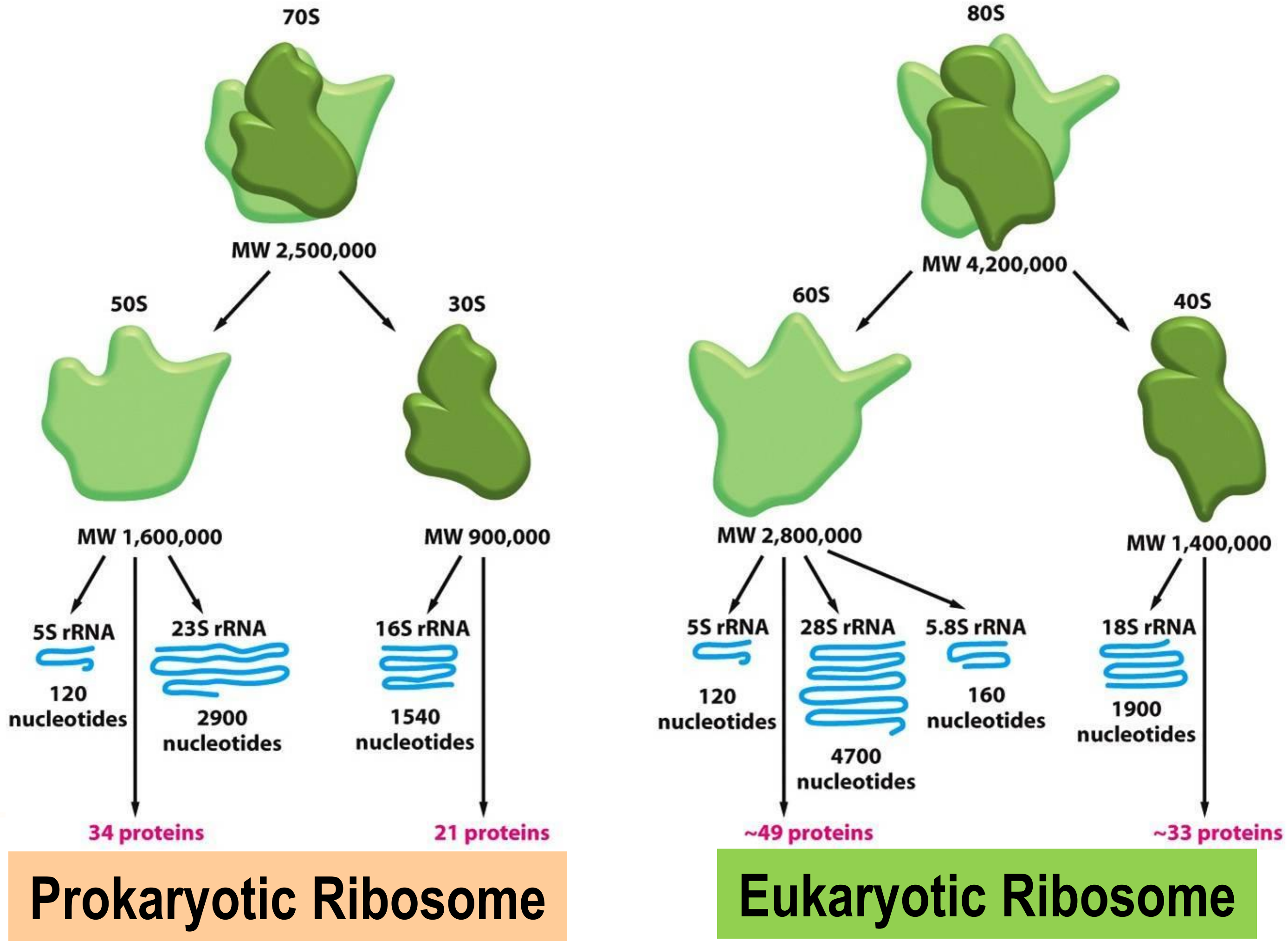


Protein/ribosome RNA complex

Prokaryotic Ribosome



Ribosome in Evolution



Translation

- mRNA
- Aminoacyl-tRNA
- Ribosome

Initiation

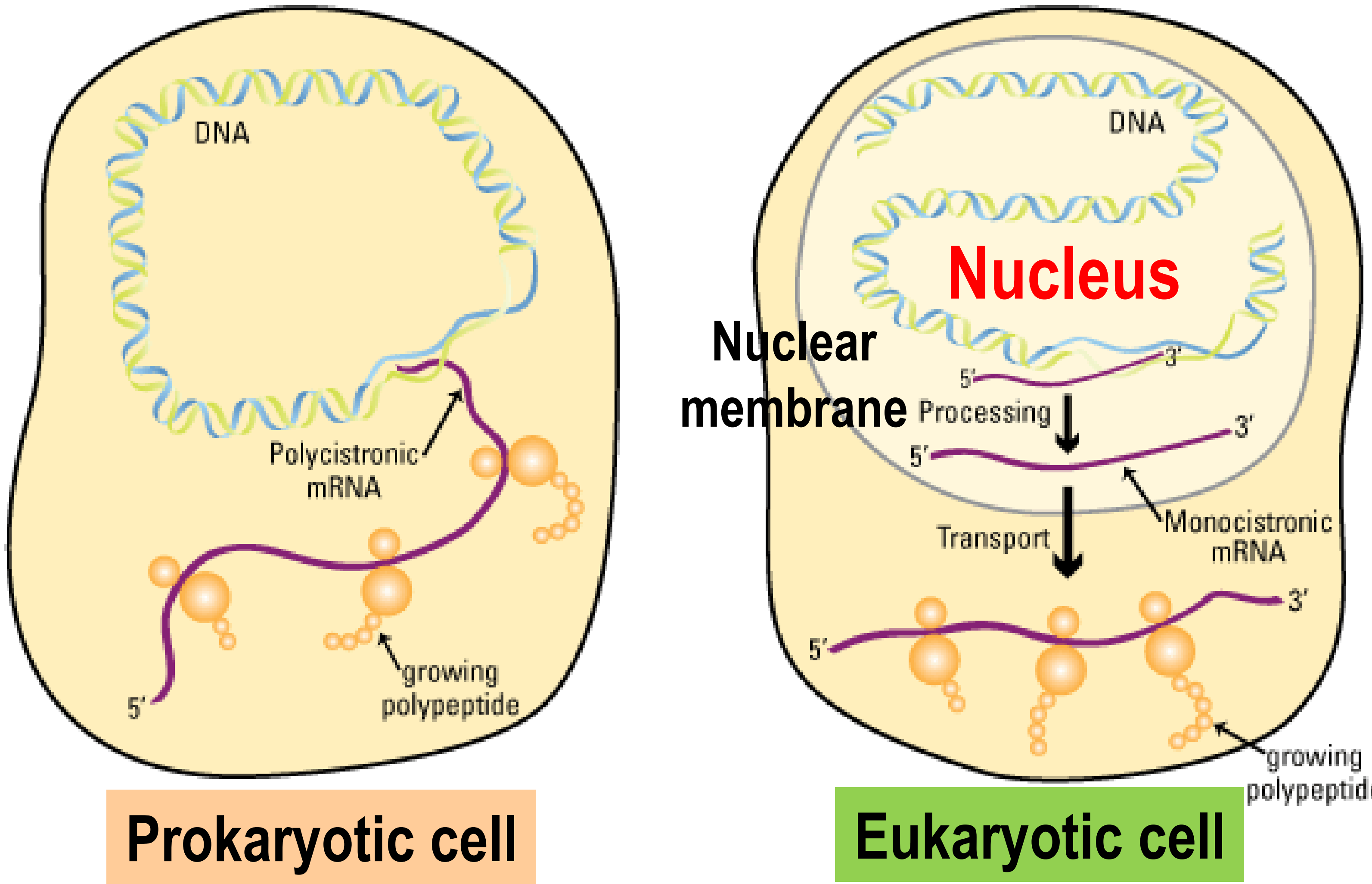
↓

Elongation

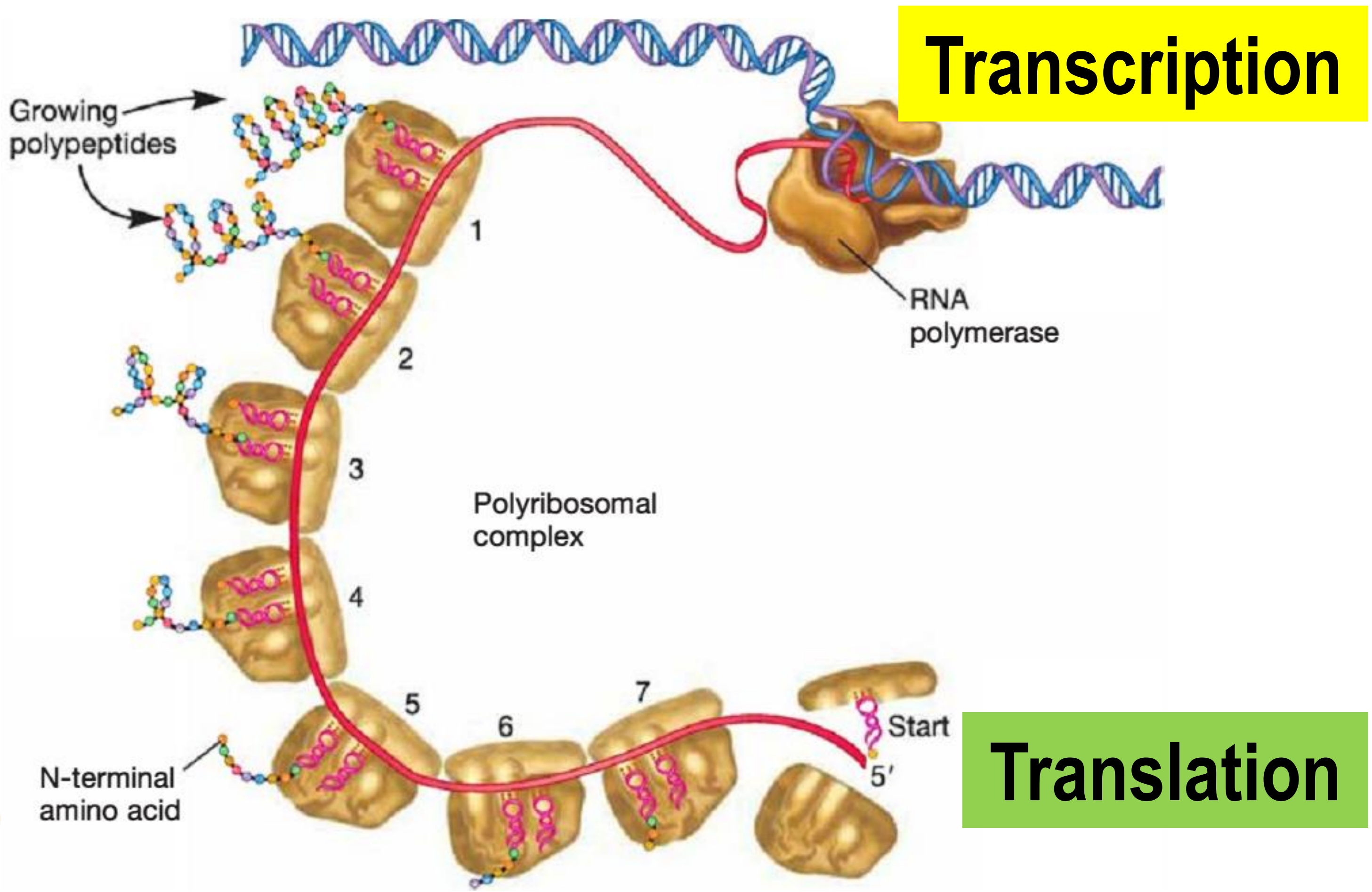
↓

Termination

No Nucleus Structure in Bacteria



Coupled Transcription and Translation in Bacteria



Rapid Protein Synthesis in Bacterial

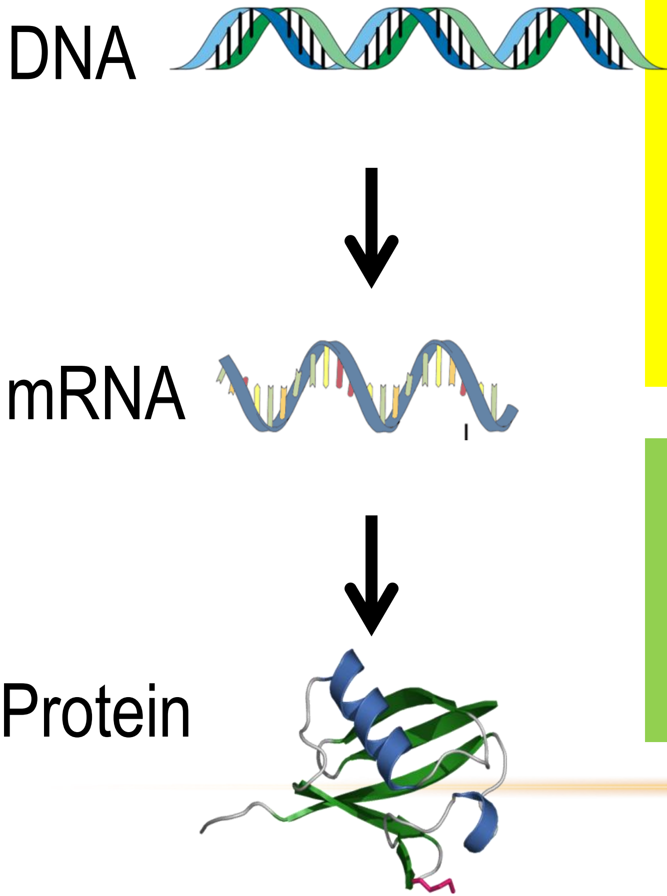
- In E. coli, synthesis occurs at a rate of at least 900 amino acids added per minute;
- Translation in Eukaryotic is slower, about 100 amino acids residues per minute.

MICROBIOLOGY

Chapter 11

11.1 Regulation levels of Gene Expression
基因表达的不同水平调控

Regulation of gene expression



- Transcription initiation
- Transcription elongation
- Transcription termination

- Translation initiation
- Translation elongation

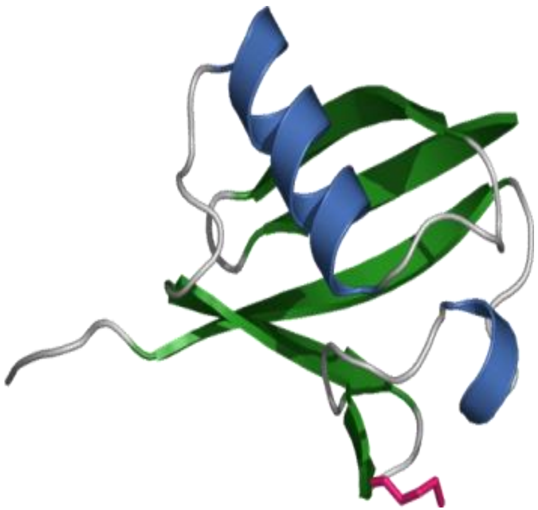
Transcription Regulation

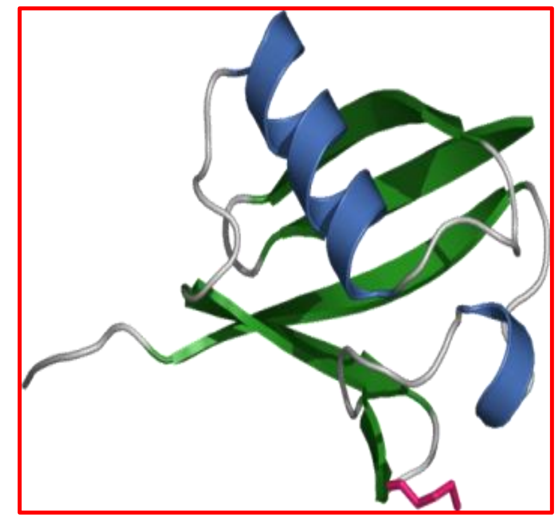
- Genetic regulatory proteins can bind to the DNA and control whether or not transcription.
- Attenuation: Transcription can terminate very early after it has begun due to the formation of a transcriptional terminator.
- Binding of a metabolite to a Riboswitch in mRNA can cause premature termination of transcription.

Translation Regulation

- Translational repressor proteins can bind to the mRNA and prevent translation from starting.
- Antisense RNA can bind to mRNA and control whether or not translation begins.
- Binding of a metabolite to a riboswitch in mRNA can block translation.

Posttranslational Regulation





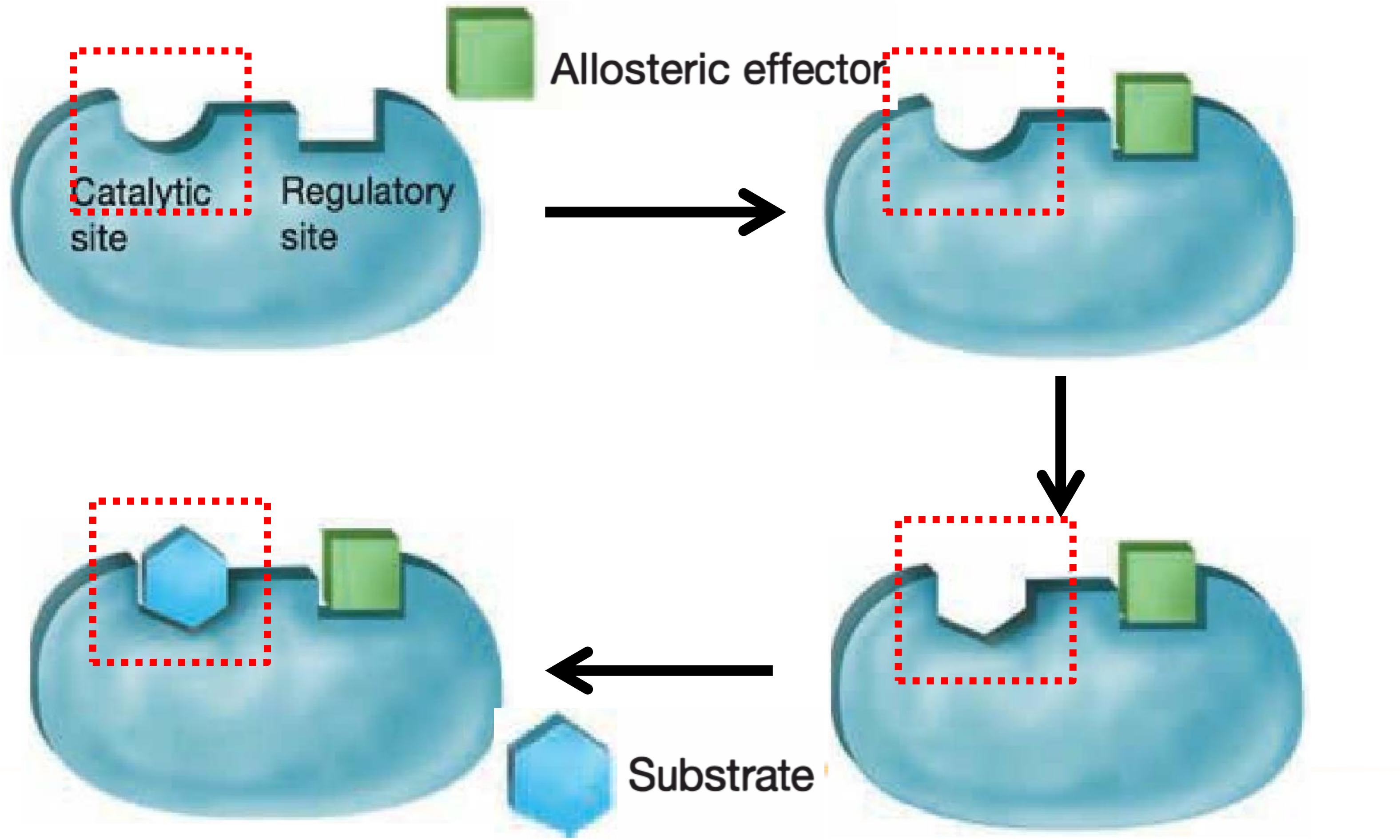
Nonfunctional protein/enzyme

Functional protein/enzyme

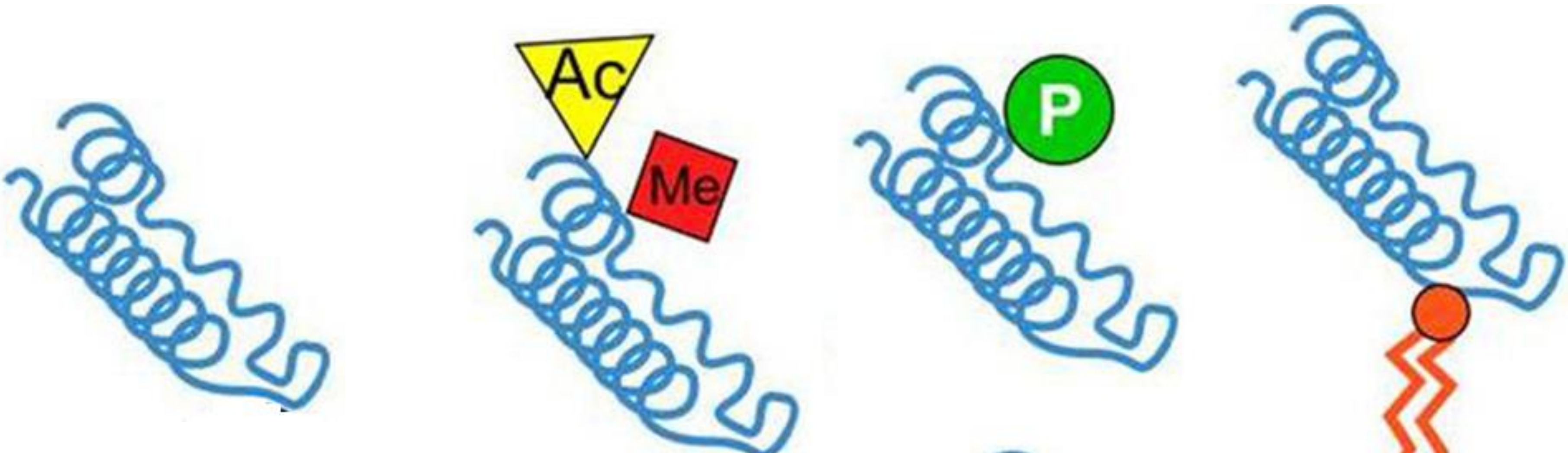
Posttranslational Regulation

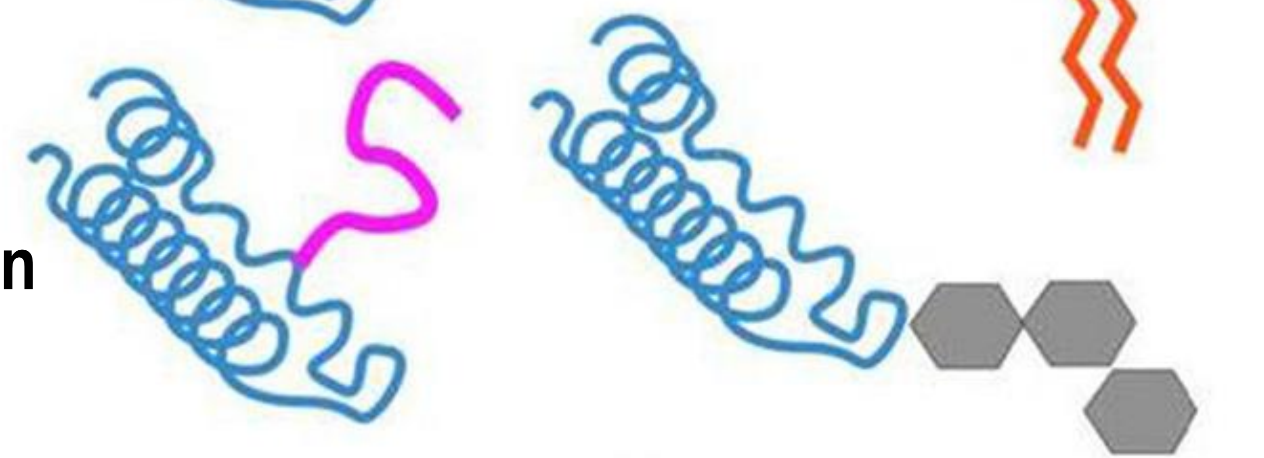
- Allosteric Regulation: small molecules can bind (noncovalently) to a protein and affect its function.
- Covalent Modification: the structure and function of a protein can be altered by covalent changes to the protein.

Allosteric Regulation



Covalent Modification





- Glycosylation
- Lipidation
- Phosphorylation
- Methylation
- Acetylation

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MICROBIOLOGY

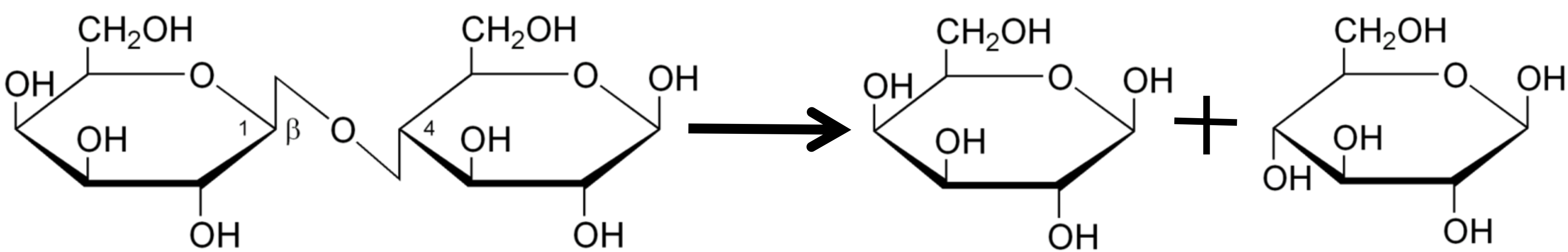
Chapter 11

11.2 Regulation of Transcription Initiation I
转录起始的调控 一

Regulation of gene expression

- Constitutive genes: express continuously
- Regulated genes: express only needed

Only Lactose available and catabolism needed, galactosidase expresses in Bacteria



Lactose 乳糖 → D-galatose 半乳糖 + D-glucose 葡萄糖 → ATP

β-galactosidase

半乳糖苷酶

Observing in bacteria cell：

Galactosidase molecules

If Lactose lack — **< 3**

If Lactose exist — **about 3,000**

Regulatory Decision in Bacteria

- When No Lactose in the environment

  No expression of Lactose catabolism enzymes

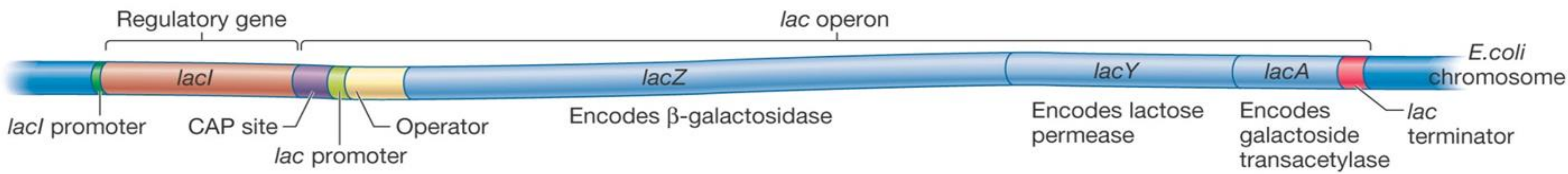
- When Lactose present in the environment

  Expression of Lactose catabolism enzymes

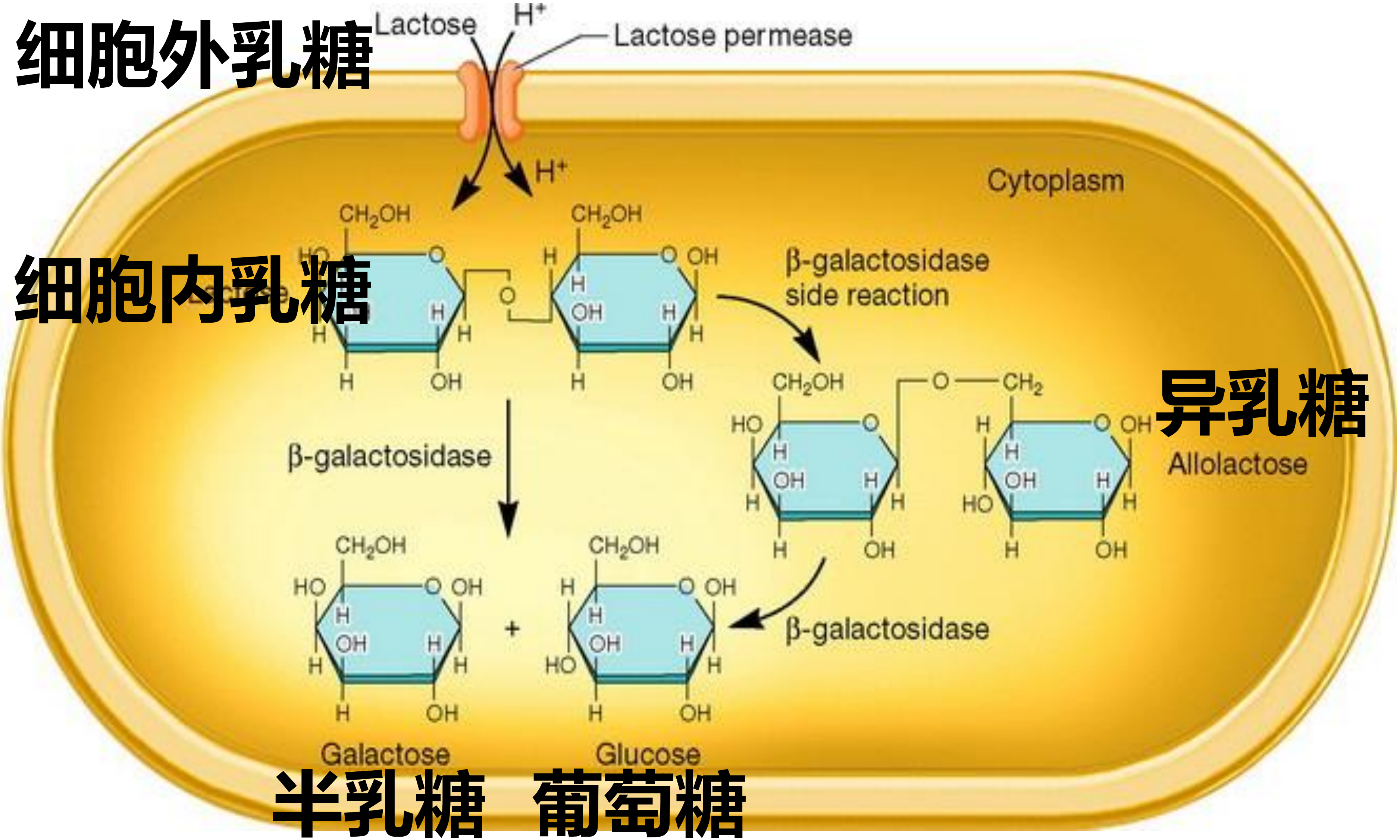
Operon 操纵子

- In bacteria, an operon is a functioning unit of genomic DNA containing a cluster of structural genes under the control of a regulatory DNA including promoter and operator.
- Its expression is regulated as a single unit.
- Lactose Operon（乳糖操纵子）

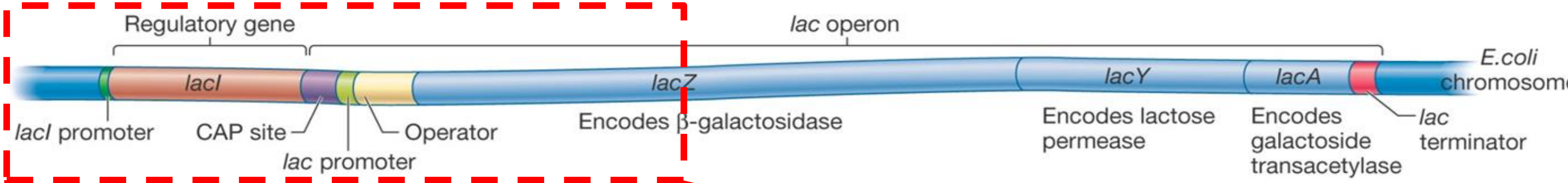
Lactose Operon

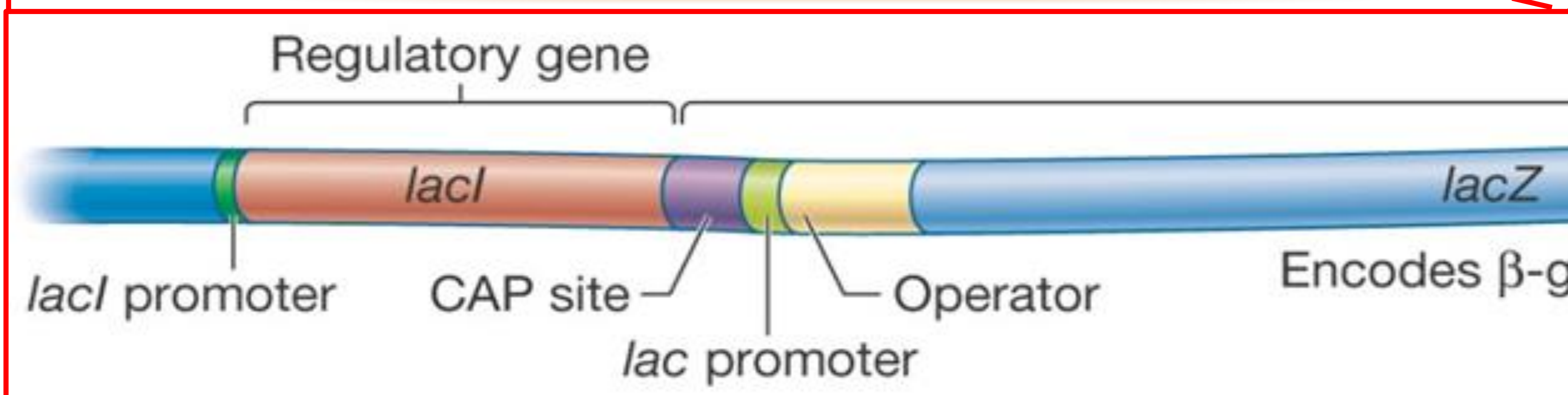


1. Regulator- gene that codes for Repressor(阻遏蛋白)
2. Regulatory DNA- composed of promoter and operator
3. Structural gene cluster- made of three genes each coding for an enzyme needed to catabolize lactose
   - β-galactosidase – hydrolyze lactose
   - Permease – take lactose across cell membrane
   - β-galactosidase transacetylase –function unknown

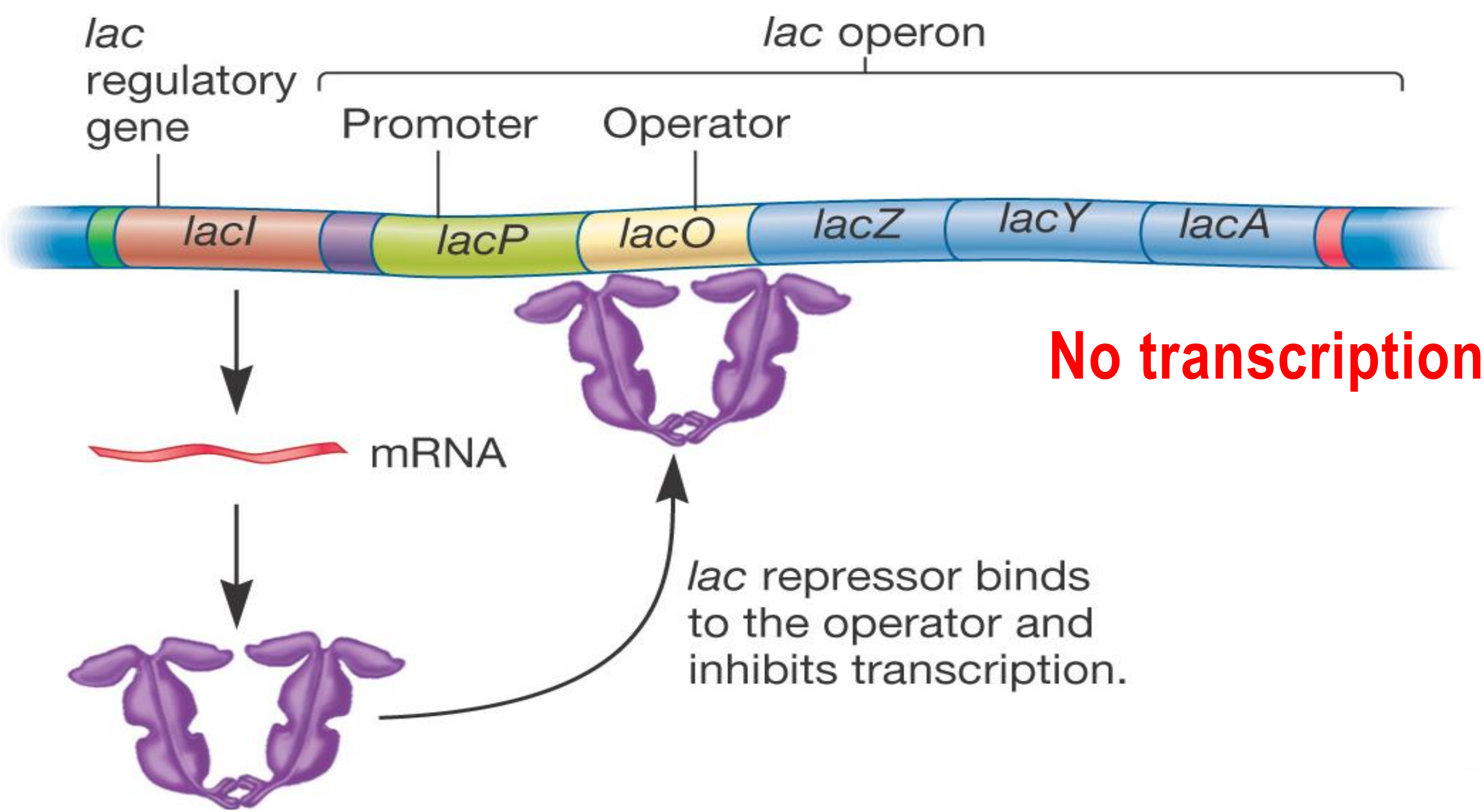

细胞外乳糖
Lactose
H+
Lactose permease
H+
Cytoplasm
细胞内乳糖
CH2OH
O
OH
H
HO
β-galactosidase side reaction
异乳糖
Allolactose
β-galactosidase
β-galactosidase
Galactose
Glucose
半乳糖
葡萄糖

Lactose Operon Structure



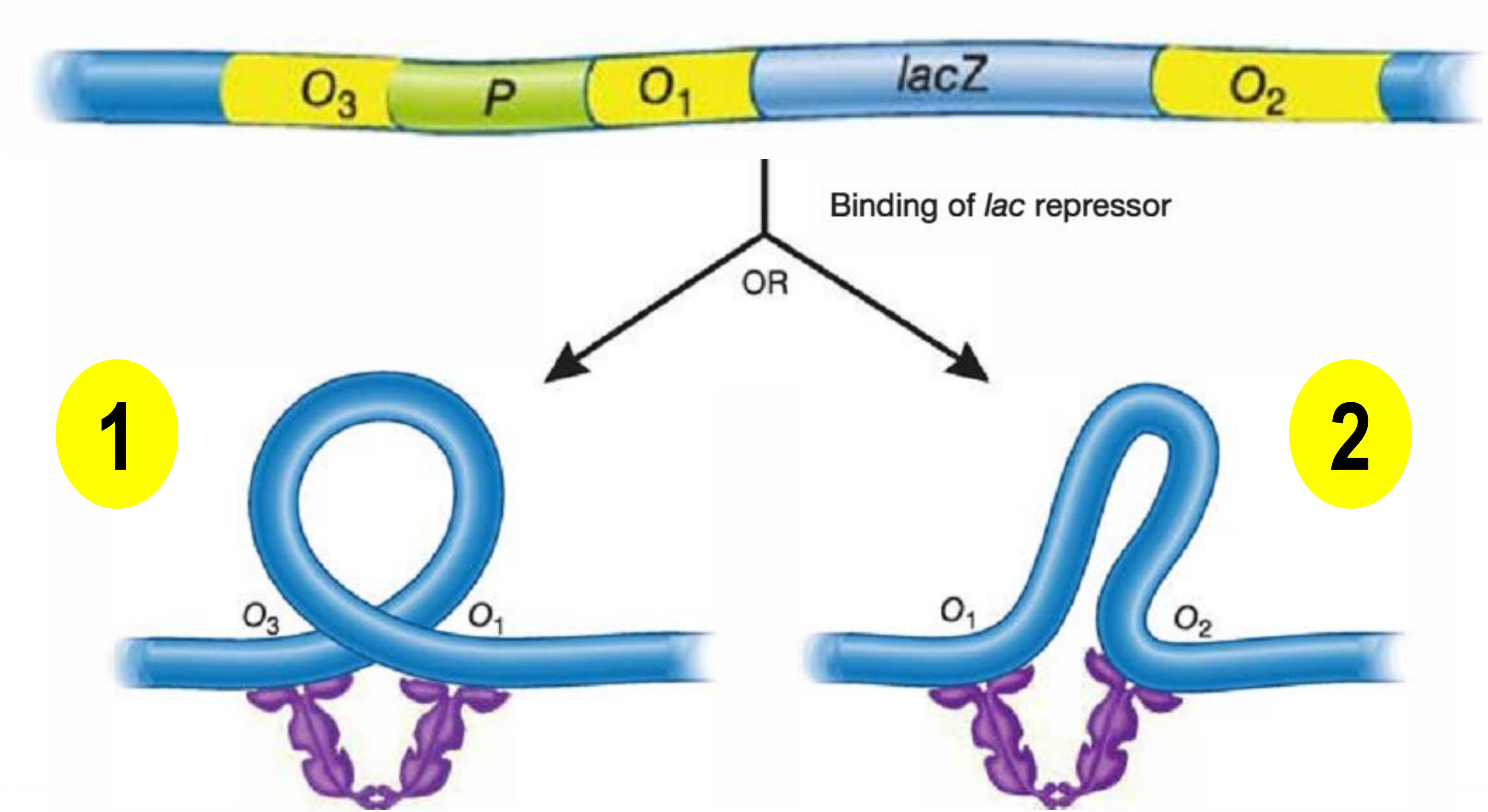


When No Lactose in the environment



Lac repressor (active)

DNA loop forming caused by repressor binding



Lac repressor (active)

Regulatory Decision in Bacteria

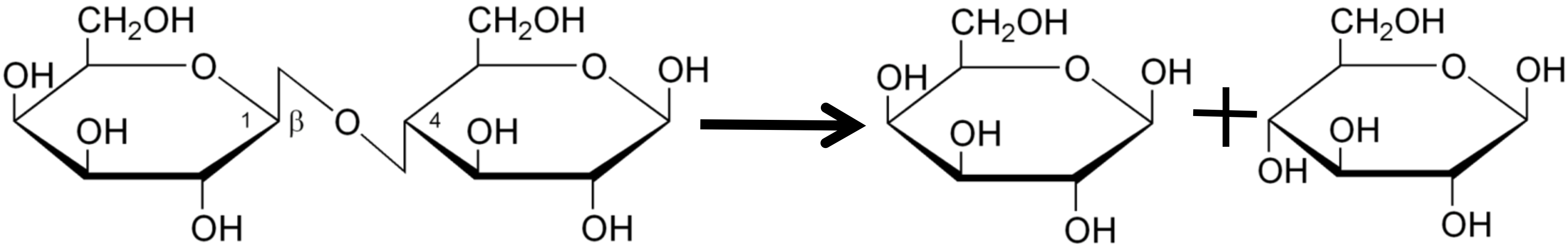
- When No Lactose in the environment

  No transcription of Lactose Operon

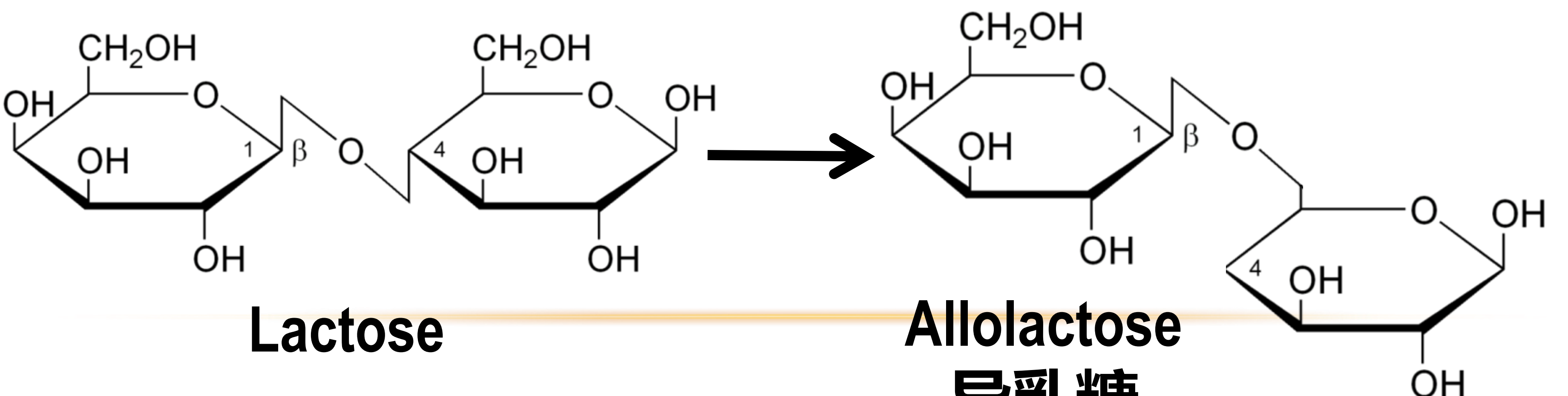
- When Lactose present in the environment

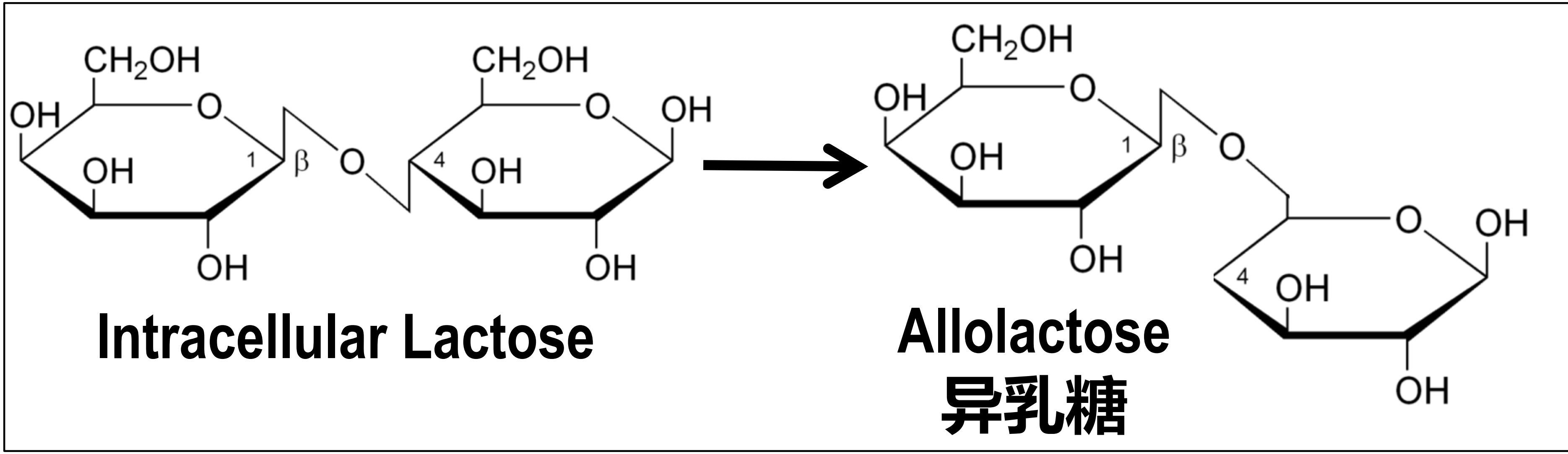
  Transcription of Lactose Operon

β-galactosidase 半乳糖苷酶



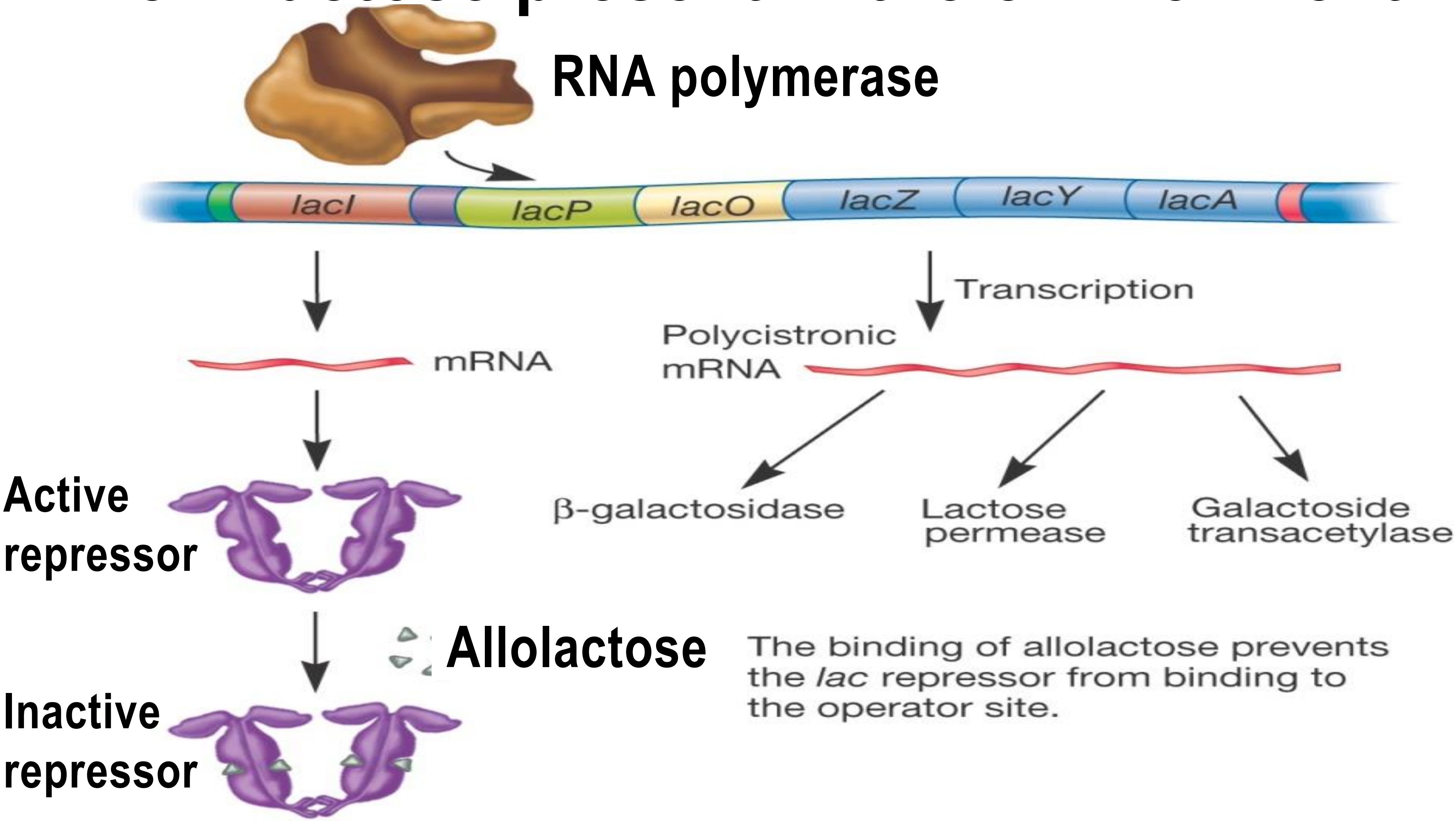
Lactose → D-galactose + D-glucose



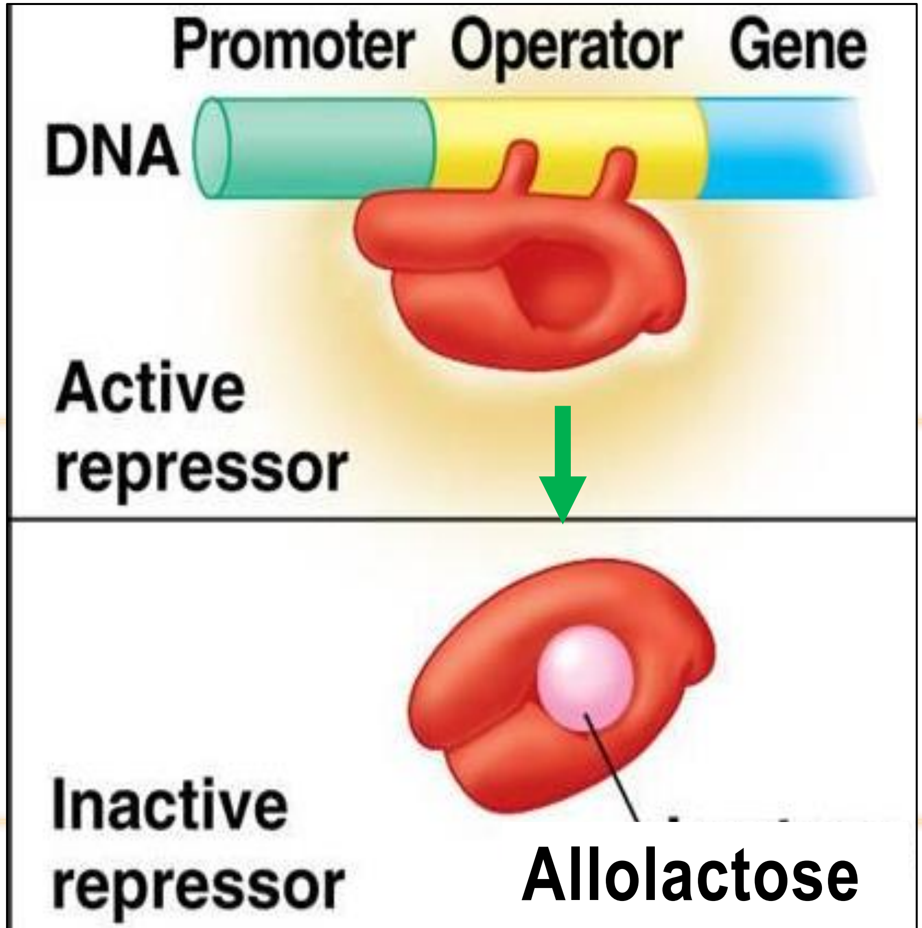


Allolactose acts as the inducer of lac operon by inactivate repressor protein

When Lactose present in the environment



lac operon


Promoter
Operator
Gene
DNA
Active
repressor
Inactive
repressor
Allolactose

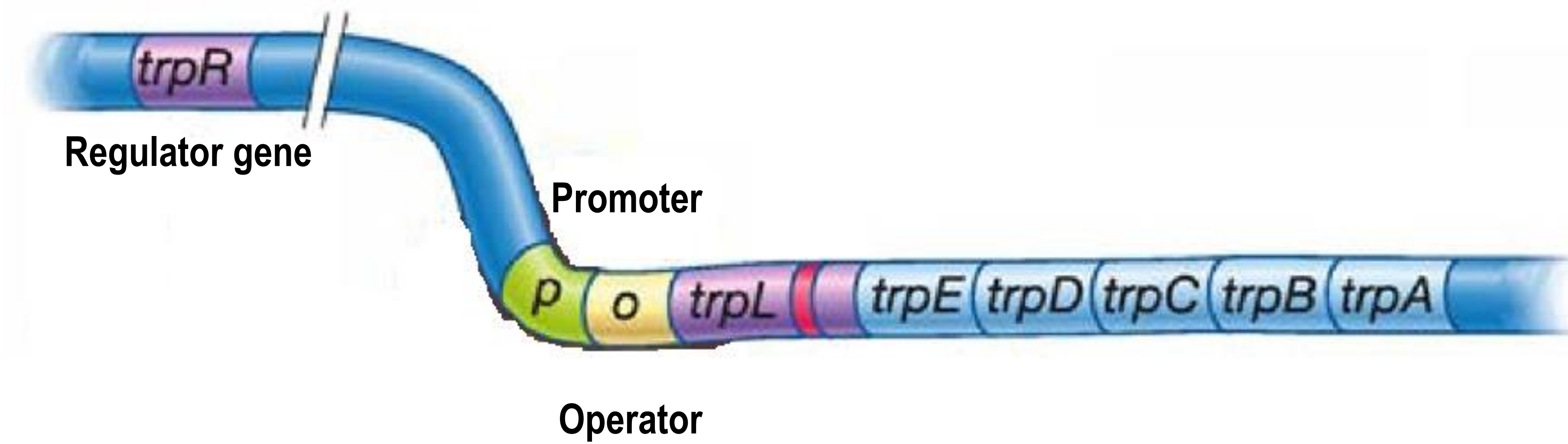
- **Lactose Operon**

Gene encoding enzyme for catabolism

- **Tryptophan Operon 色氨酸**

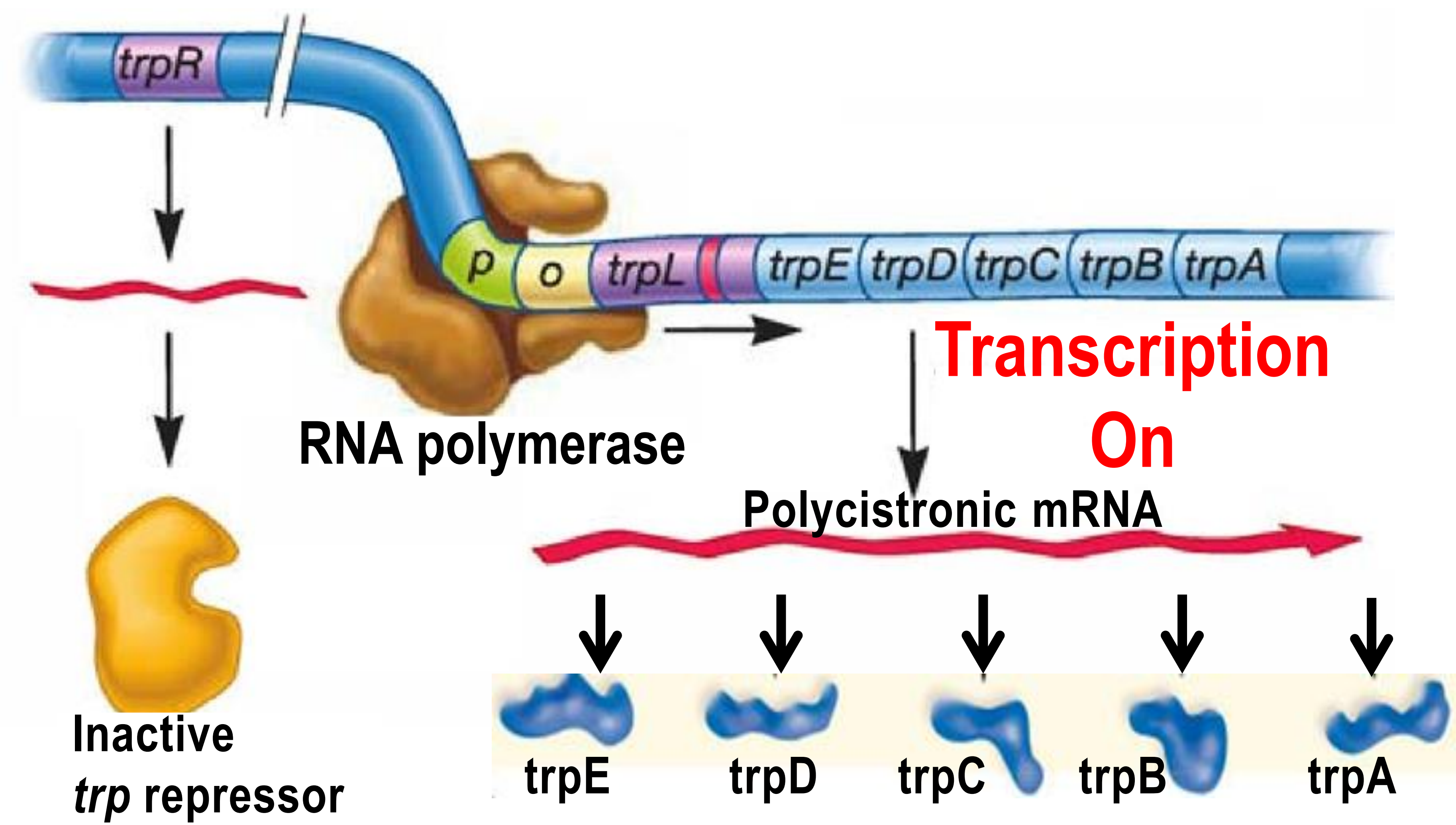
Gene encoding enzyme for synthesizing

Tryptophan Operon

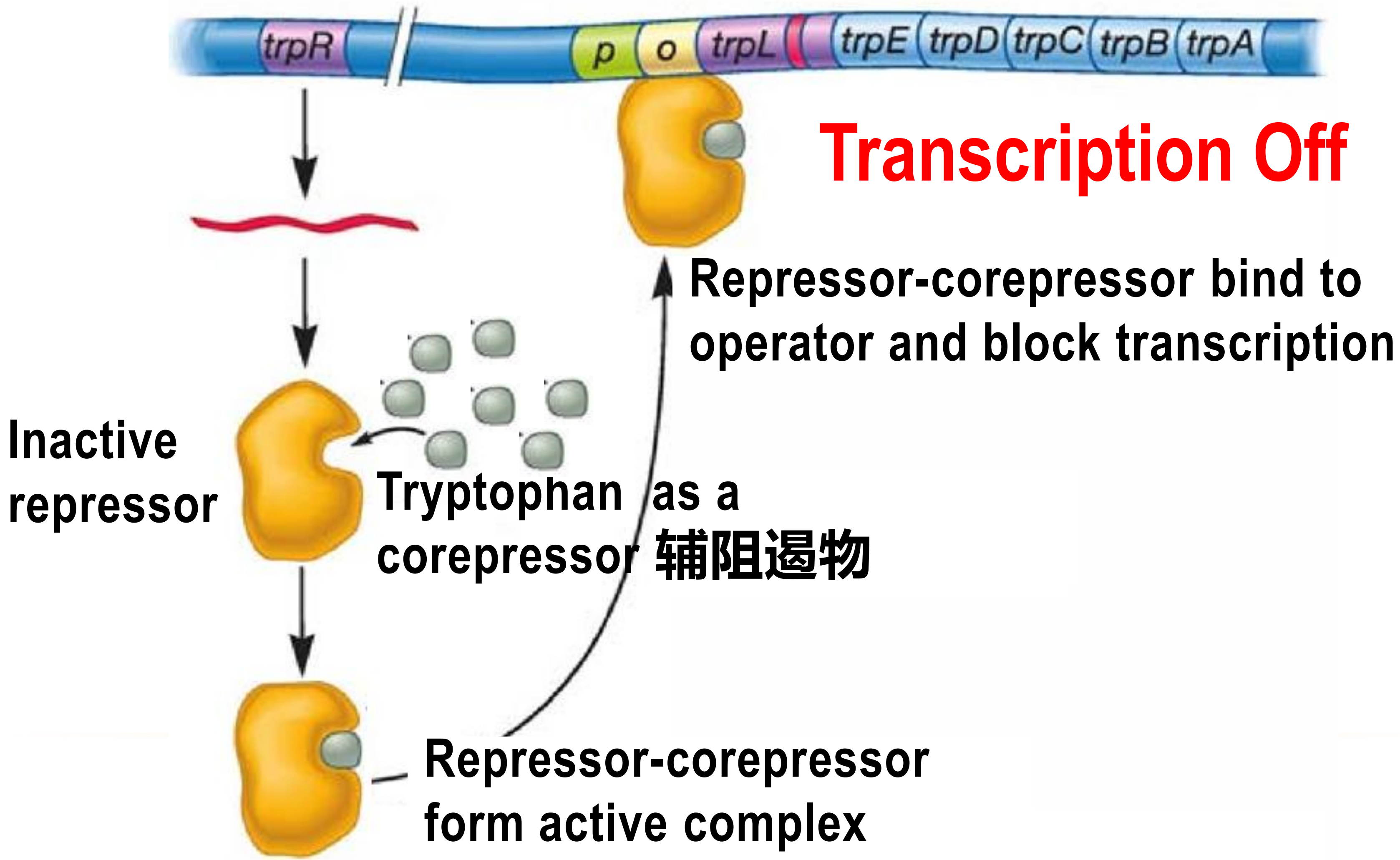


1. <u>Regulator</u>- gene encoding Repressor（阻遏蛋白）
2. <u>Regulatory DNA</u>- composed of promoter and operator
3. <u>Structural gene cluster</u>- made of five genes each coding for an enzyme needed to synthesize tryptophan

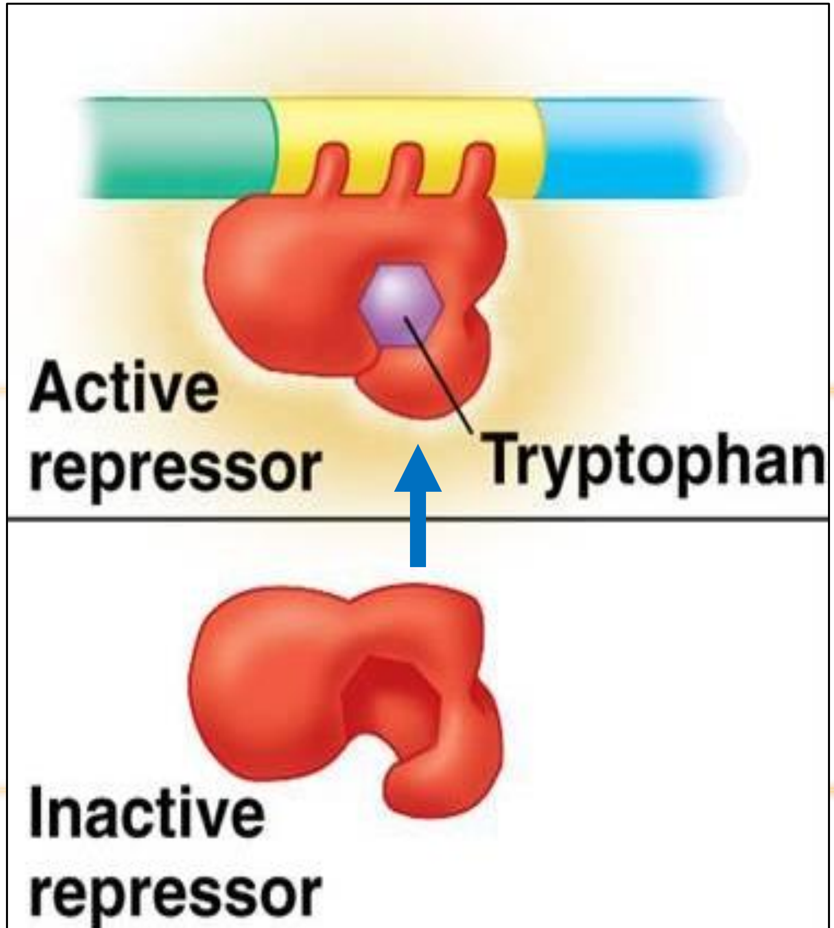
When No/Low Trp in the bacterial cell



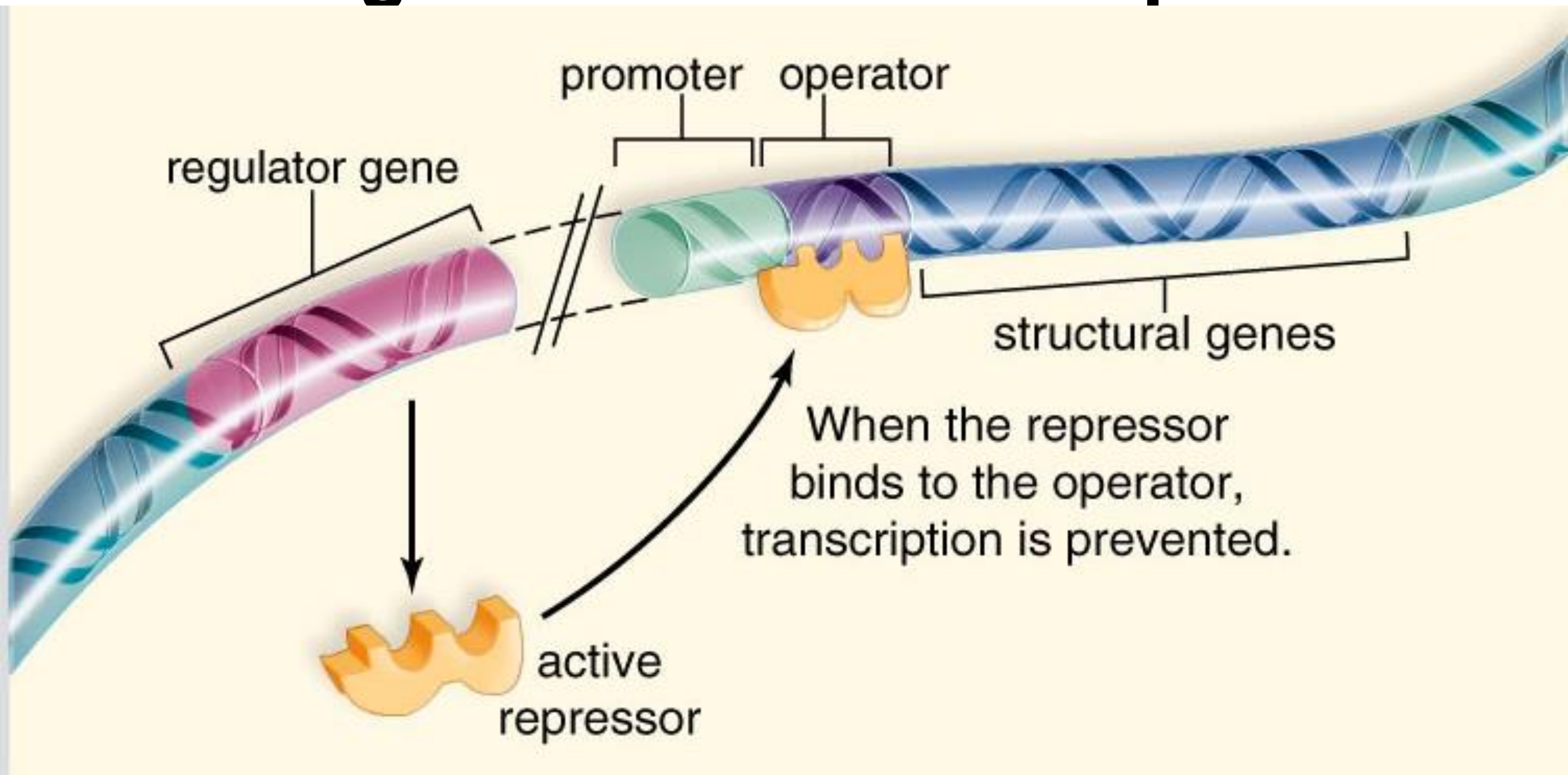
When High/Excess Trp in the cell



trp operon

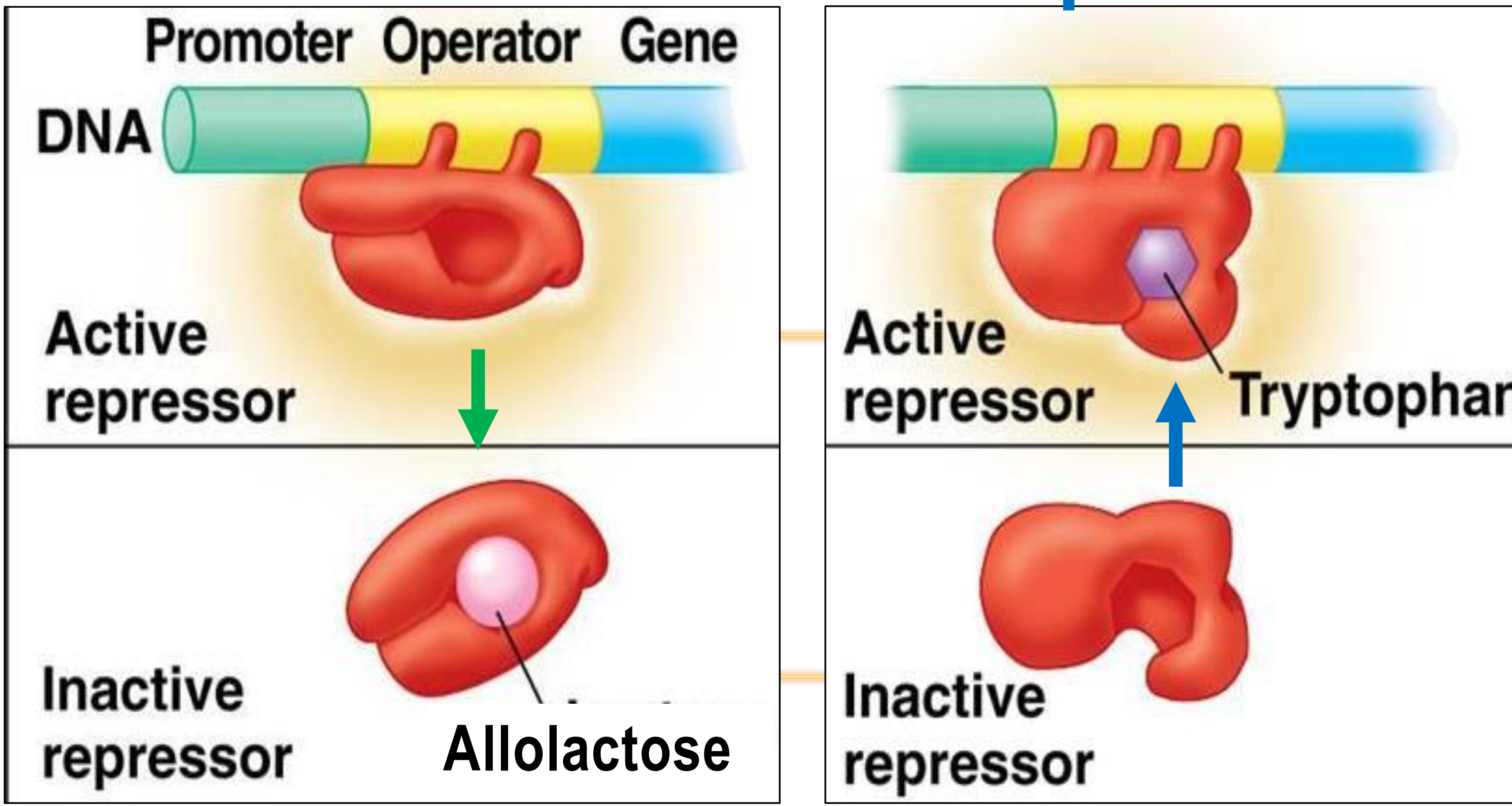


Repressor-mediated Negative Regulation of Transcription



lac operon | *trp* operon

Inducible | Repressible



MICROBIOLOGY

Chapter 11

11.3 Regulation of transcription initiation II
转录起始的调控 二

Negative regulation

Inducible: Lactose operon

Repressible: Tryptophan operon

Positive regulation

?

Negative regulation

Inducible: Lactose operon

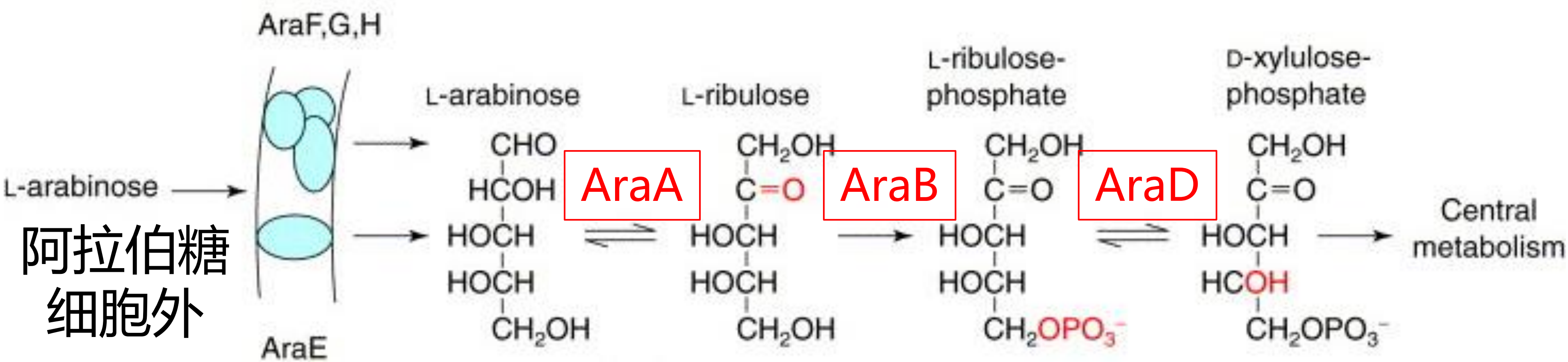
Repressible: Tryptophan operon

Positive regulation

Arabinose operon 阿拉伯糖

The *ara* operon encodes enzymes needed for catabolism of arabinose.

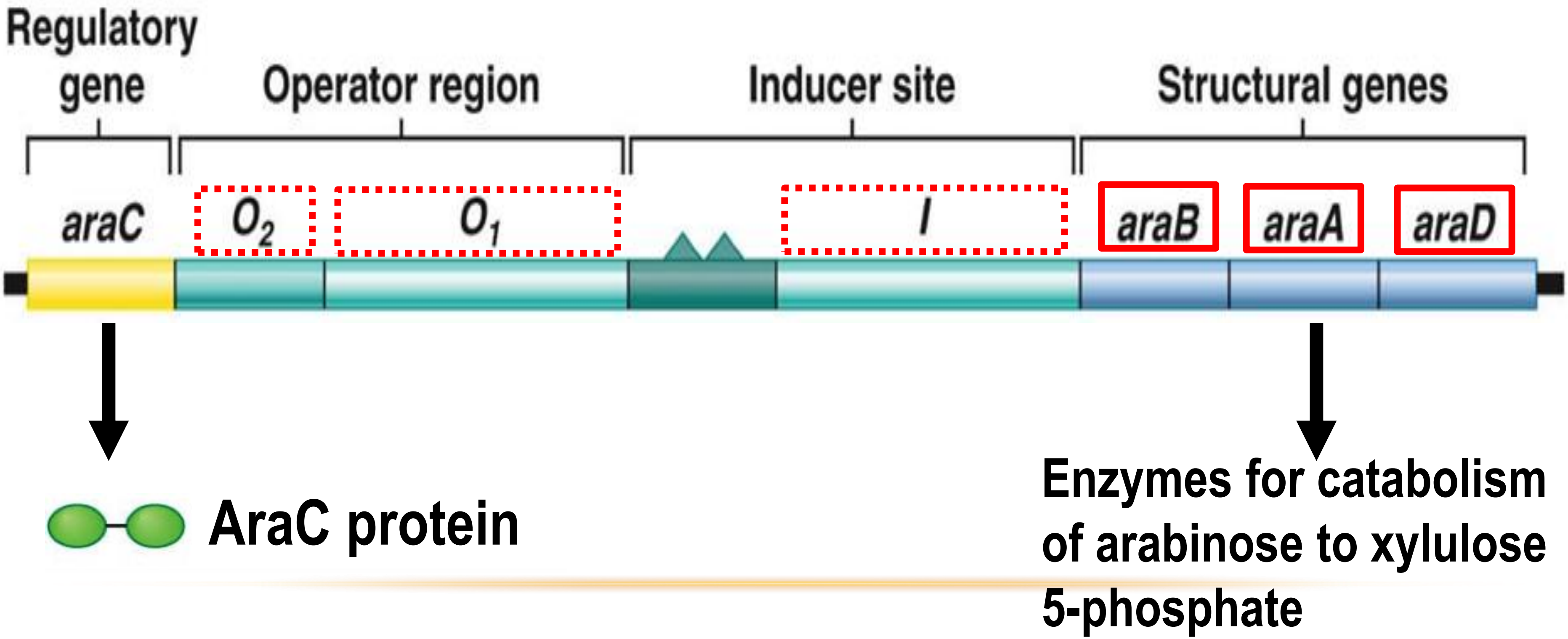
Arabinose Metabolism in Bacteria



阿拉伯糖→核酮糖→磷酸核酮糖→磷酸木酮糖

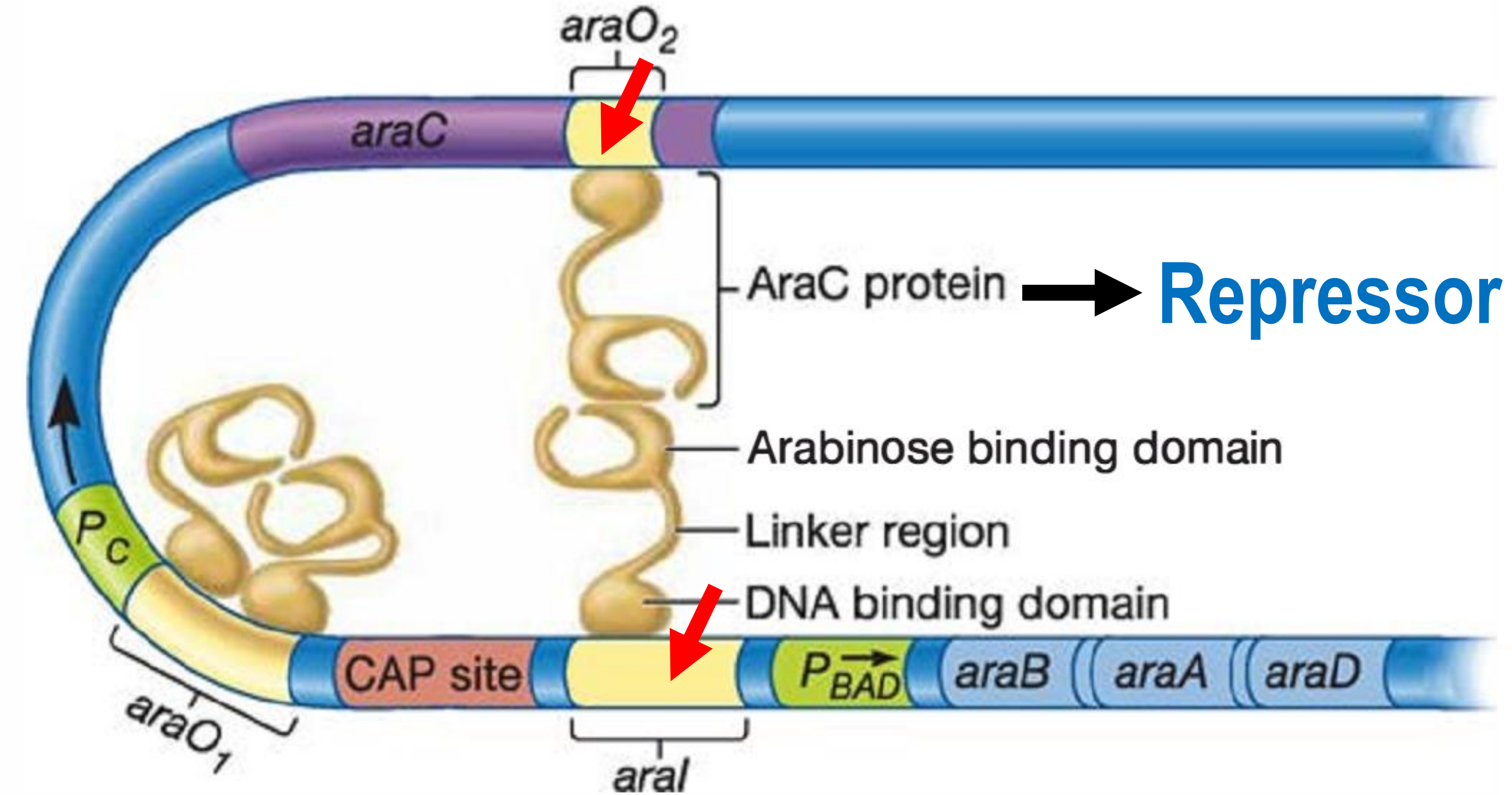
Robert Schleif, 2000, Trends in Genetics

Arabinose Operon



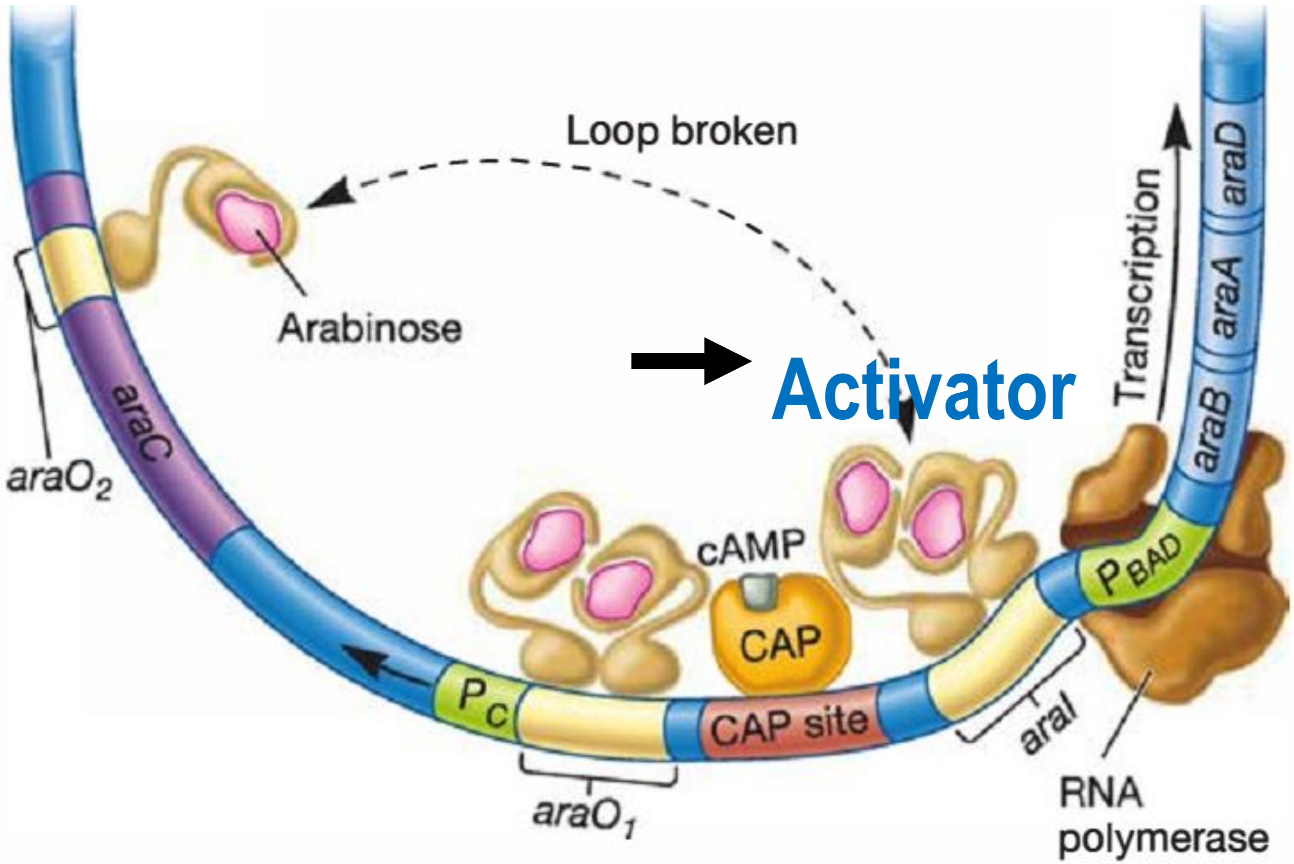
Operon inhibited in the absence of Arabinose

Negative control-monomers of AraC protein bind to **O2** and **I** looping out the intervening sequence(210bp) & blocking access to the promoter by RNA polymerase



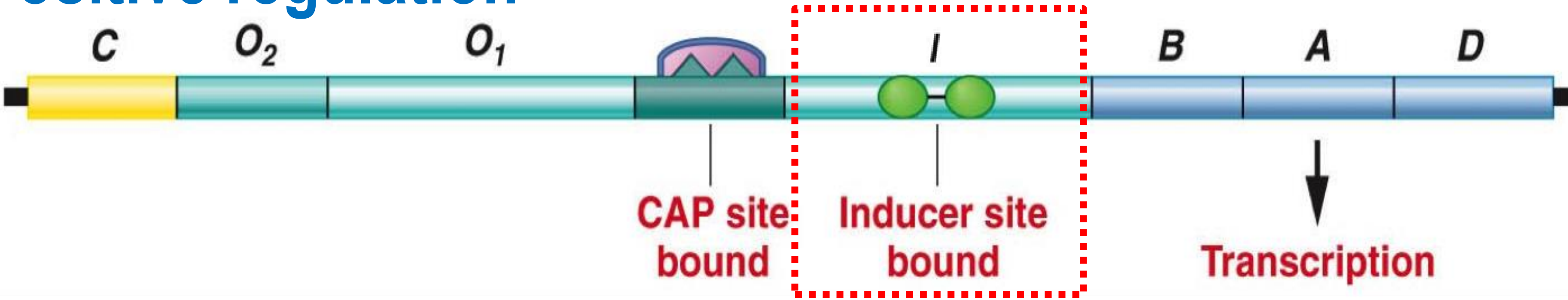
Operon activated in the presence of Arabinose

Positive control-Arabinose binds AraC to change conformation, forms AraC dimer and activate operon transcription



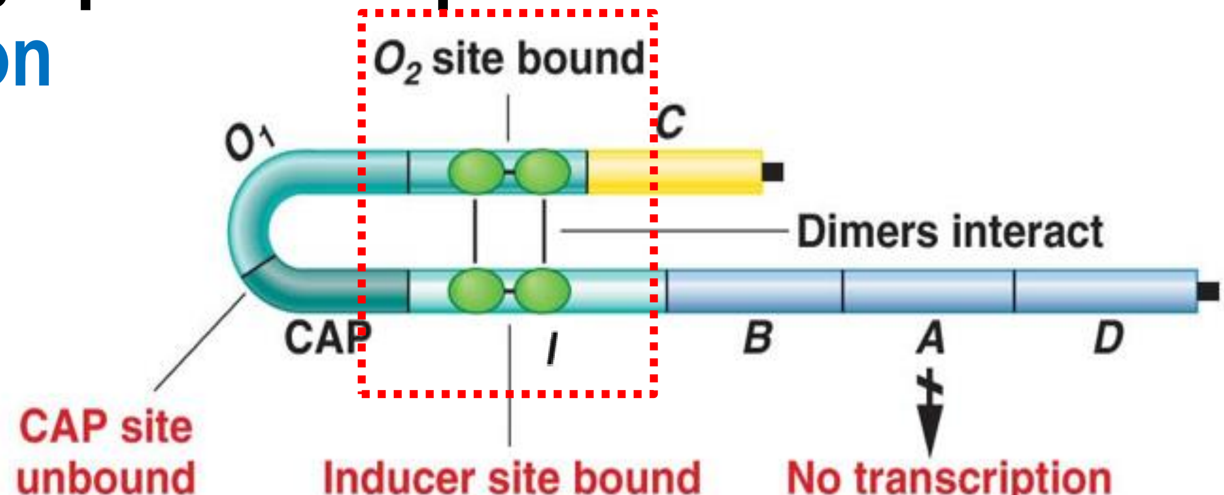
Arabinose present; operon is induced.

Positive regulation

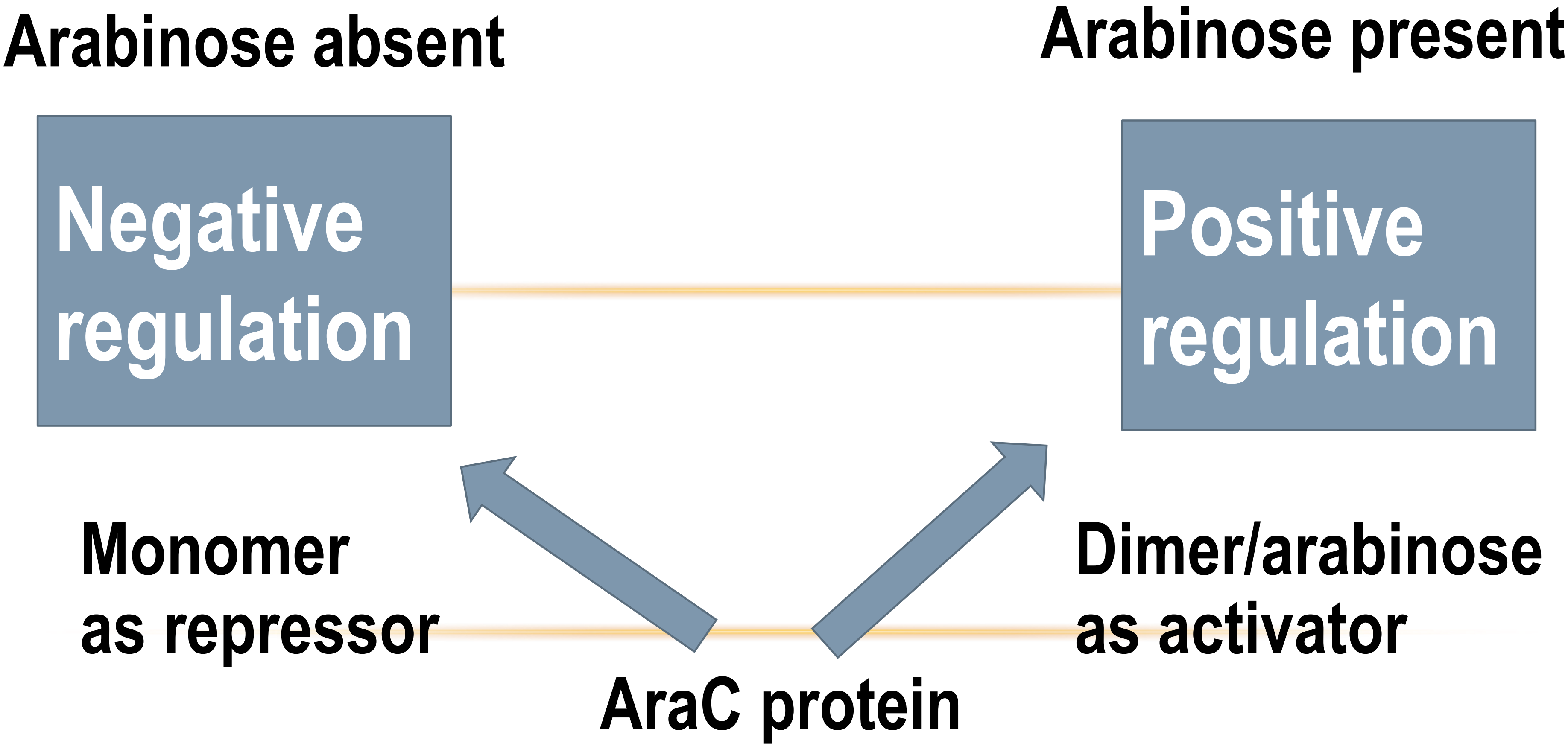


Arabinose absent; operon is repressed.

Negative regulation



Arabinose Operon



负调控
Negative regulation

Inducible: Lactose operon

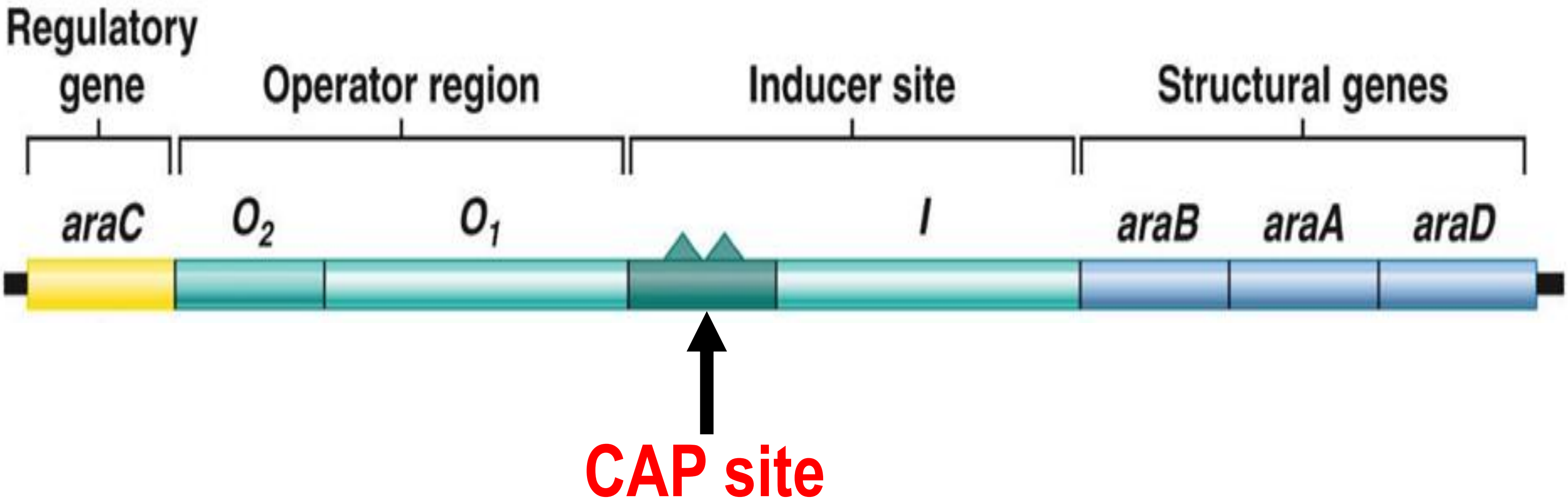
Repressible: Tryptophan operon

正调控
Positive regulation

Arabinose operon

CAP site in Catabolism operons

CAP site for positive regulation in bacteria Operon



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MICROBIOLOGY

Chapter 11

11.6 Regulating Complex Cellular Processes
复杂细胞过程的调控

Carbon source for *E.coli*

Polysaccharide

↓

Monosaccharide

↓

Glycolysis

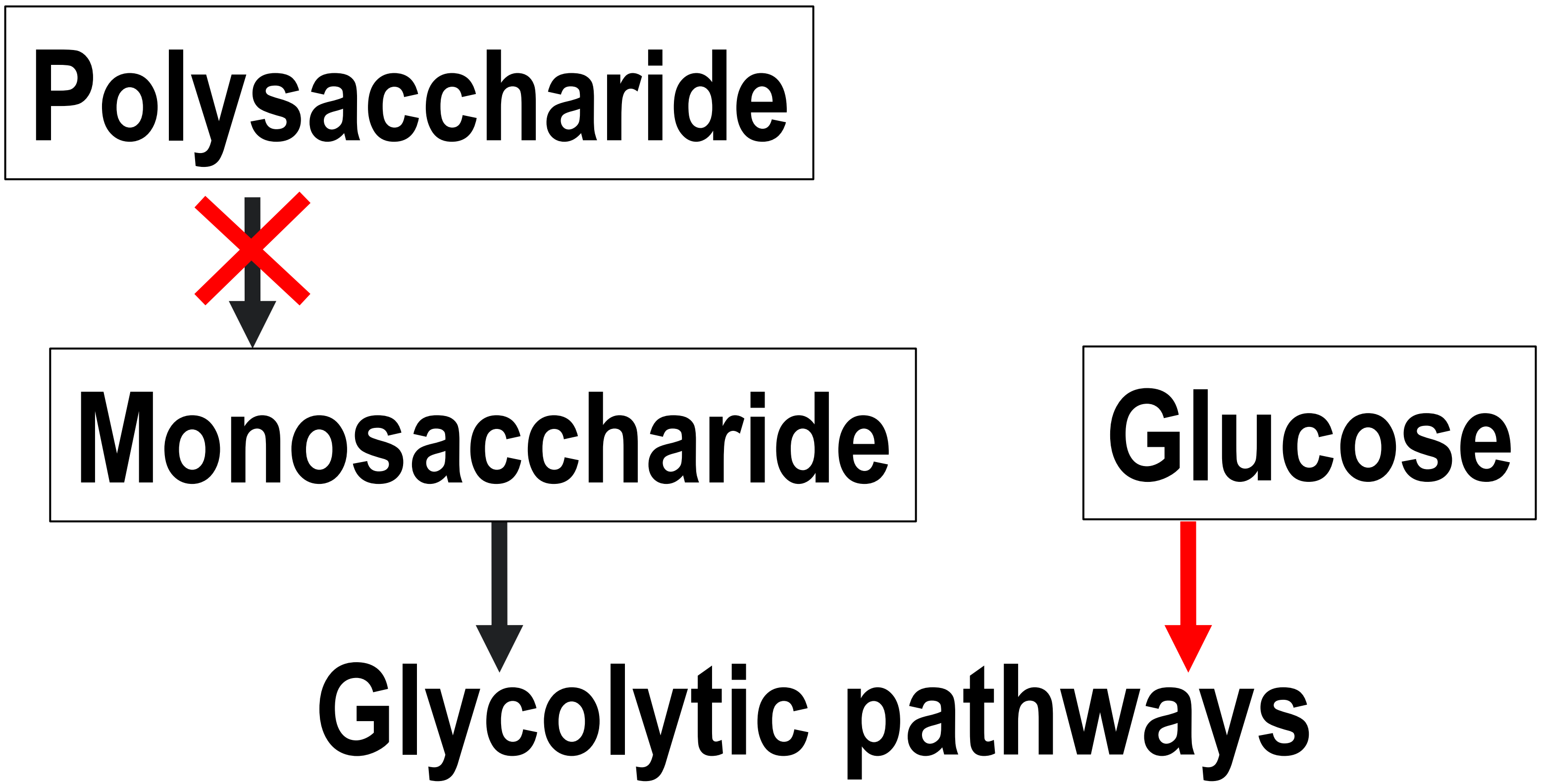
Carbon Catabolism in *E.coli*

- Constitutive genes: enzyme for glucose catabolism
- Regulated genes: enzyme for catabolism of other carbon source
  - Arabinose(*ara*)
  - Lactose(*lac*)
  - Maltose(*mal*)
  - Galactose(*gal*)

Carbon Catabolism in *E.coli*

If Glucose present, Use Glucose first

Catabolite Repression



If Glucose present, Use Glucose first

Catabolite Repression

- The operons that encode enzymes required for the catabolism of carbon sources that must first be modified before entering glycolysis.

- The expression of these catabolite operons is coordinately repressed when glucose is plentiful.

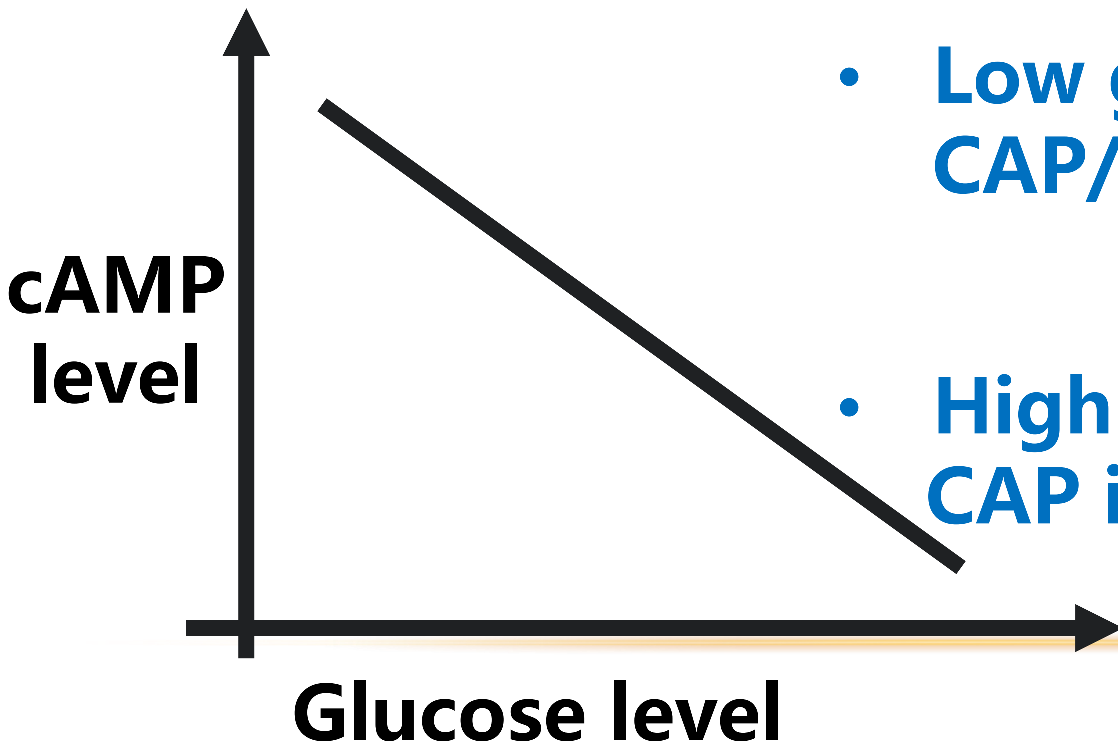
Catabolite Activator Protein (CAP)

- Also named cAMP receptor protein (CRP)

- CAP is active when the cAMP is bound and binding

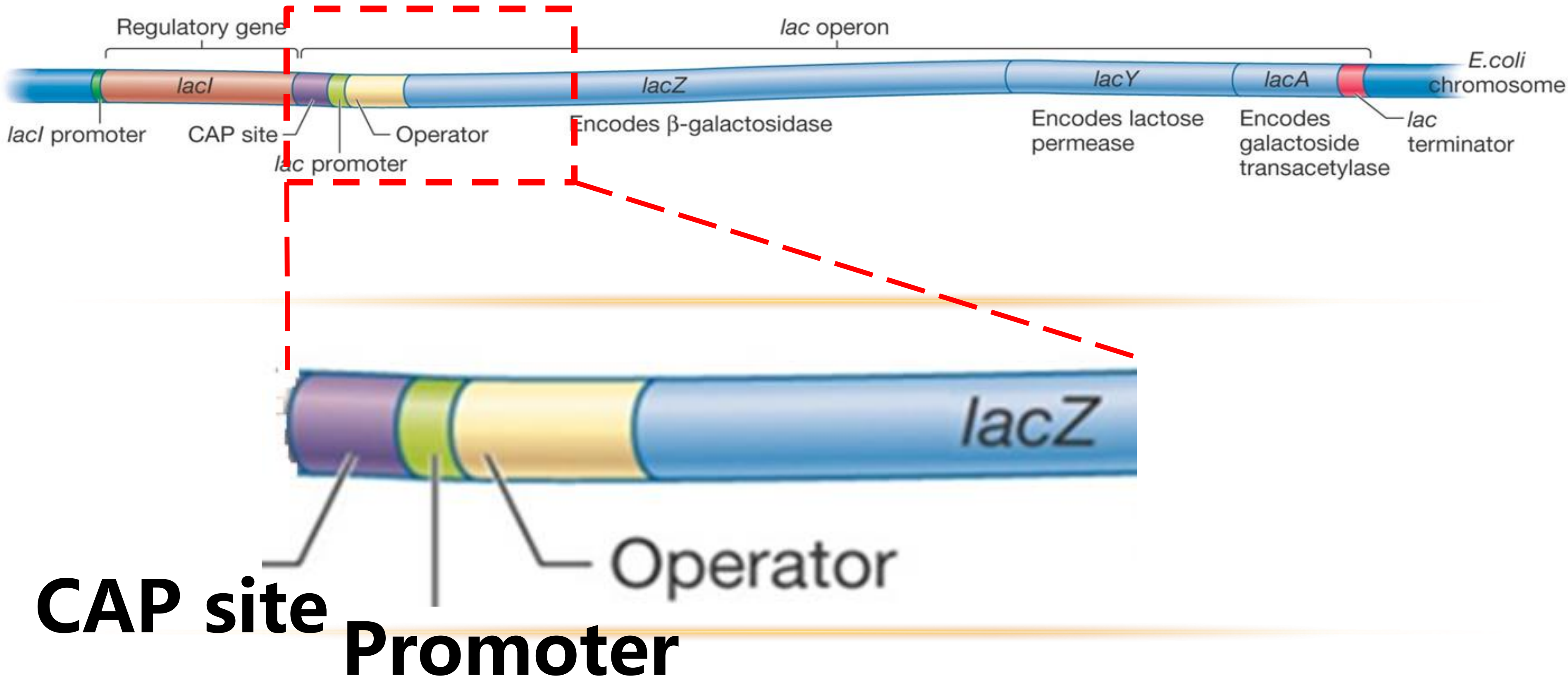
- CAP is inactive when it is free of cAMP.

The level of cAMP varies inversely with that of glucose



- Low glucose, High cAMP, CAP/cAMP activator
- High glucose, Low Camp, CAP inactive

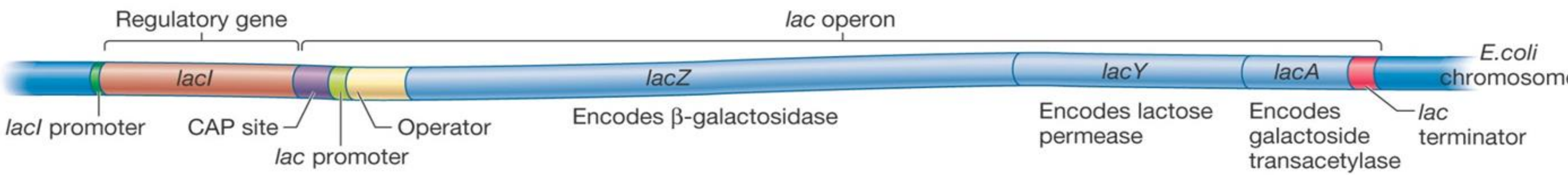
CAP recognition site in all catabolite operons



CAP Site for CAP/cAMP activator binding

High Glucose: Low cAMP **Low Glucose: High cAMP**

Dual regulation in Lactose Operon



- **Regulatory protein specific to each operon**
- **CAP for positive regulation in all catabolite operons**

Regulation of *Lac* Operon in different nutrient environment

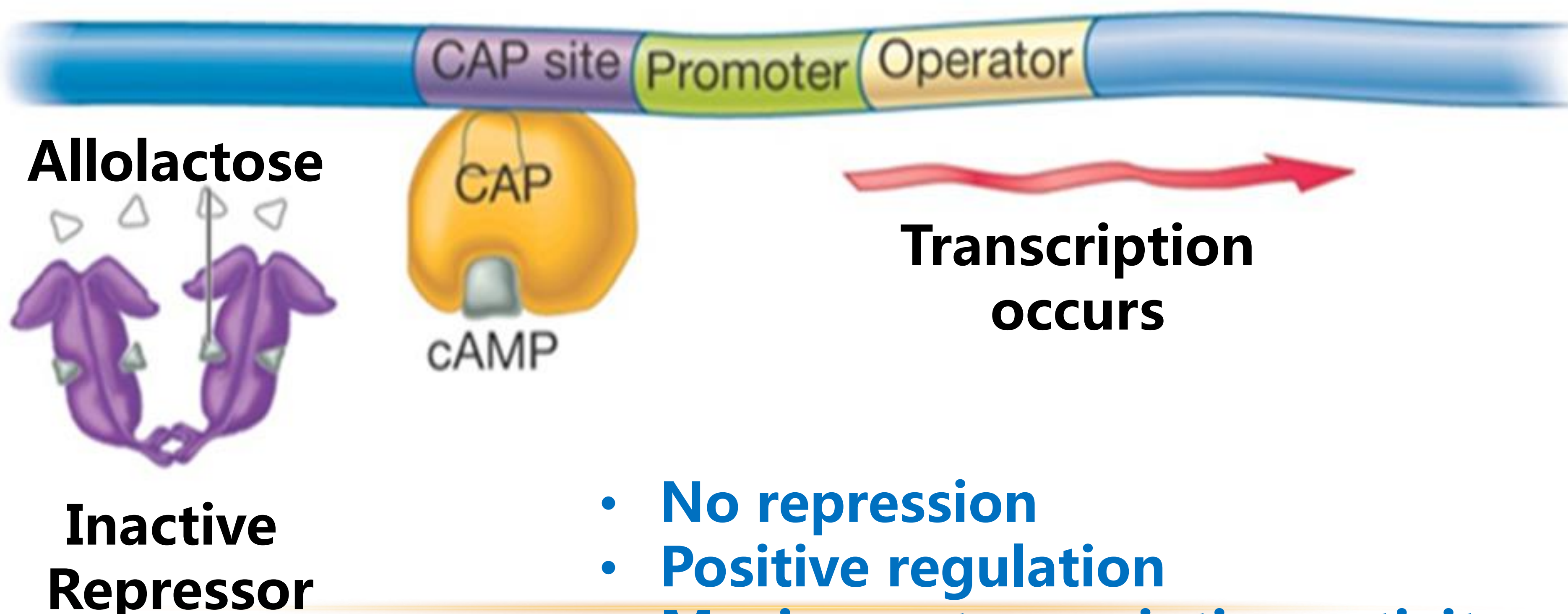
Lactose乳糖 → Galactose半乳糖 + **Glucose 葡萄糖**

(β-D-galactosidase 半乳糖苷酶)

Glucose → ATP

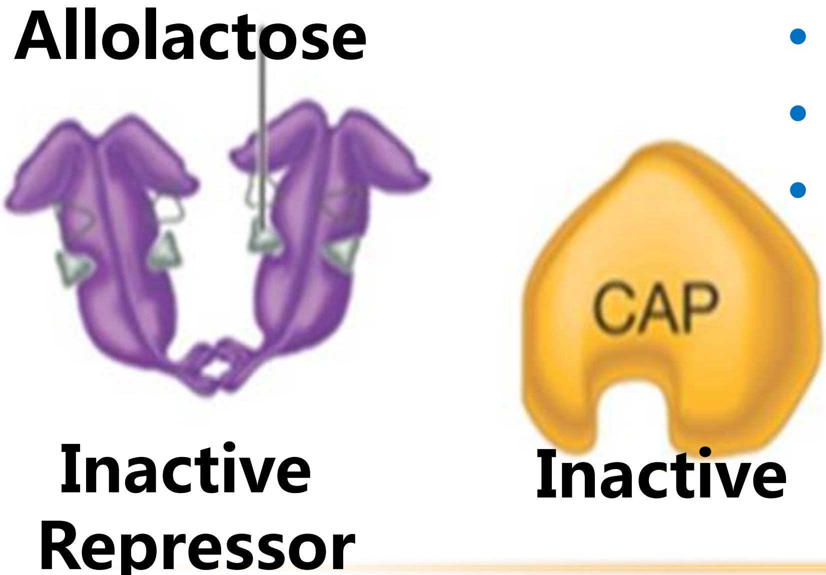
1. **Only Lactose; No Glucose**
2. **Lactose and Glucose**
3. **Neither Lactose Nor Glucose**
4. **No Lactose; Only Glucose**

1. Only Lactose present; No Glucose



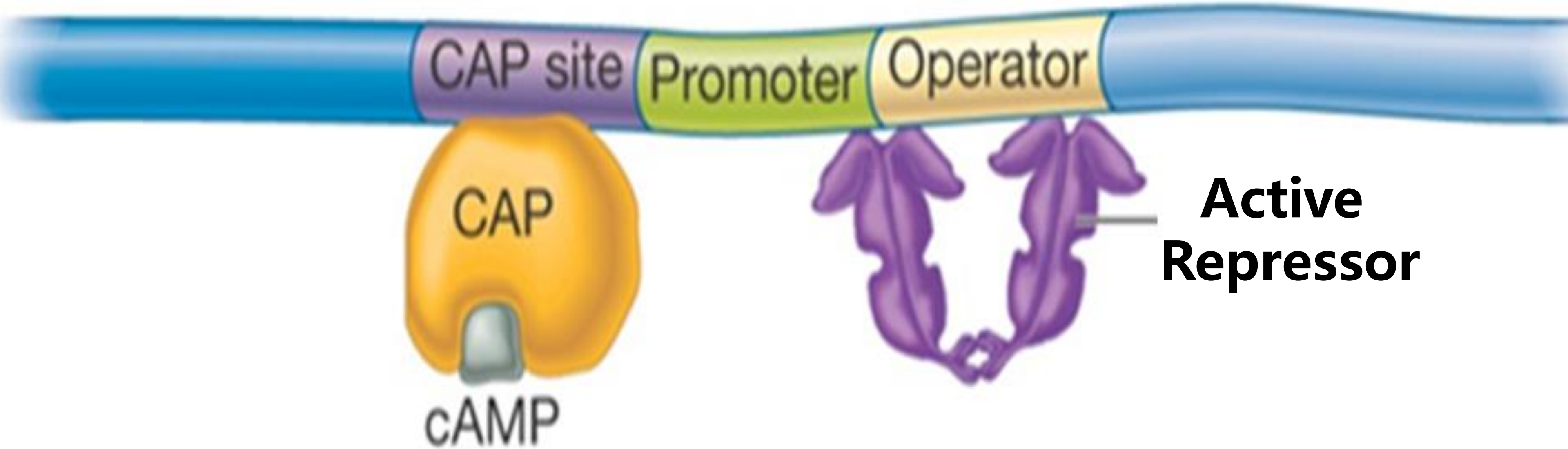
- No repression
- Positive regulation
- Maximum transcription activity

2. Both Lactose and Glucose Present



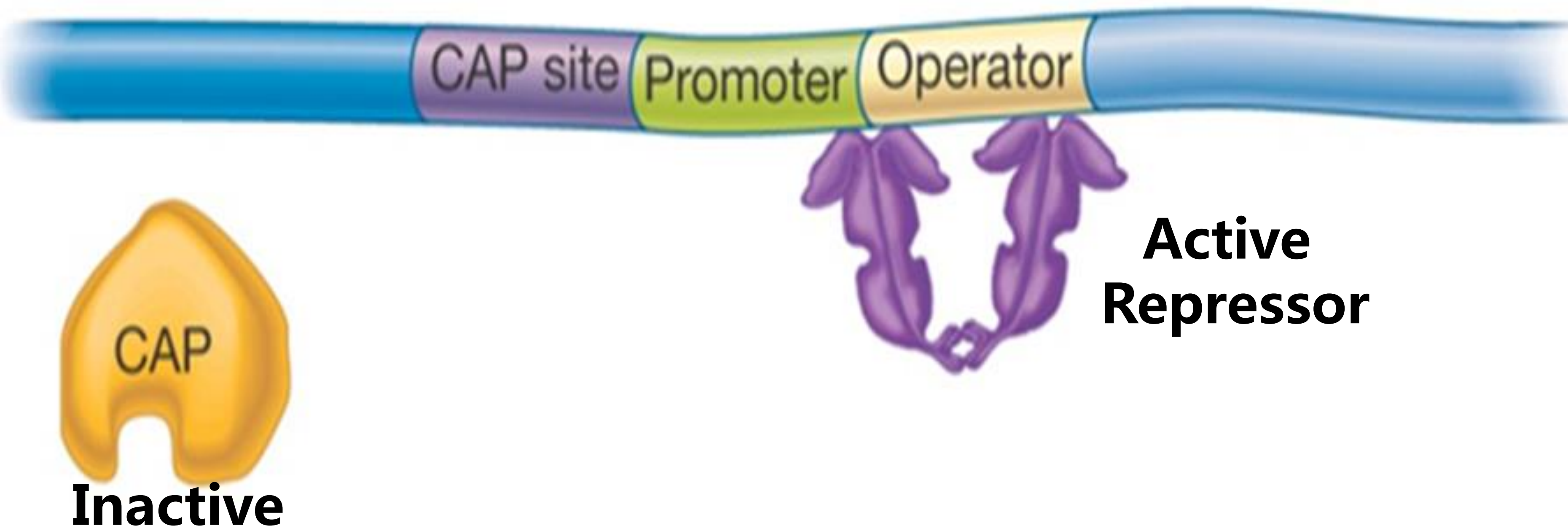
- No repression
- No positive regulation
- Low Transcription activity

3. Neither Lactose Nor Glucose Present



- **Repression**
- **Positive regulation**
- **Transcription blocked**

4. No Lactose; Only Glucose Present



- Repression
- No positive regulation
- Minimum activity of transcription

Negative regulation

- Repression by repressor
- Attenuation by attenuator
- Riboswitch

Positive regulation

- Activator in *Ara* operon
- Active CAP/cAMP as activator in all catabolite operons

Inducible Vs Repressible Operon

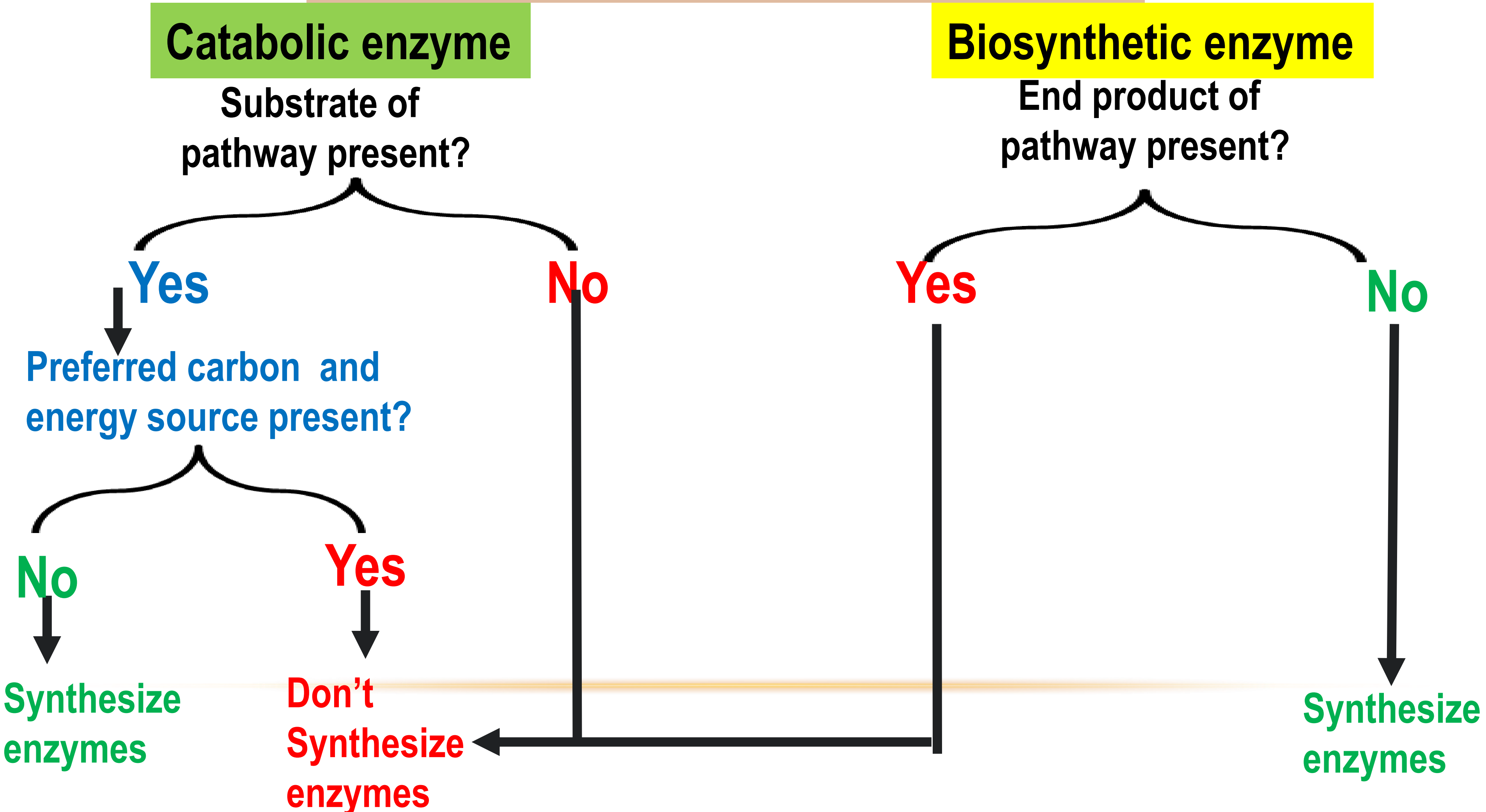
- Inducible operon is turned ON by substrate.

  Lac operon - enzymes needed to catabolize the nutrient/lactose are produced when needed.

- Repressible operon is turned OFF by the product synthesized;

  Trp operon – enzymes used to synthesize Try stop being produced when they are not needed

Regulatory "Decision"



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Chapter 11

11.7 Riboswitch and antisense RNA
核糖开关和反义RNA

Regulation Of Gene Expression

- Transcription
  - Regulation Of Transcription initiation
    - Operon 操纵子
  - Regulation Of Transcription elongation
    - Riboswith 核糖开关
- Translation
    - Riboswith； antisense RNA

Riboswitch 核糖开关

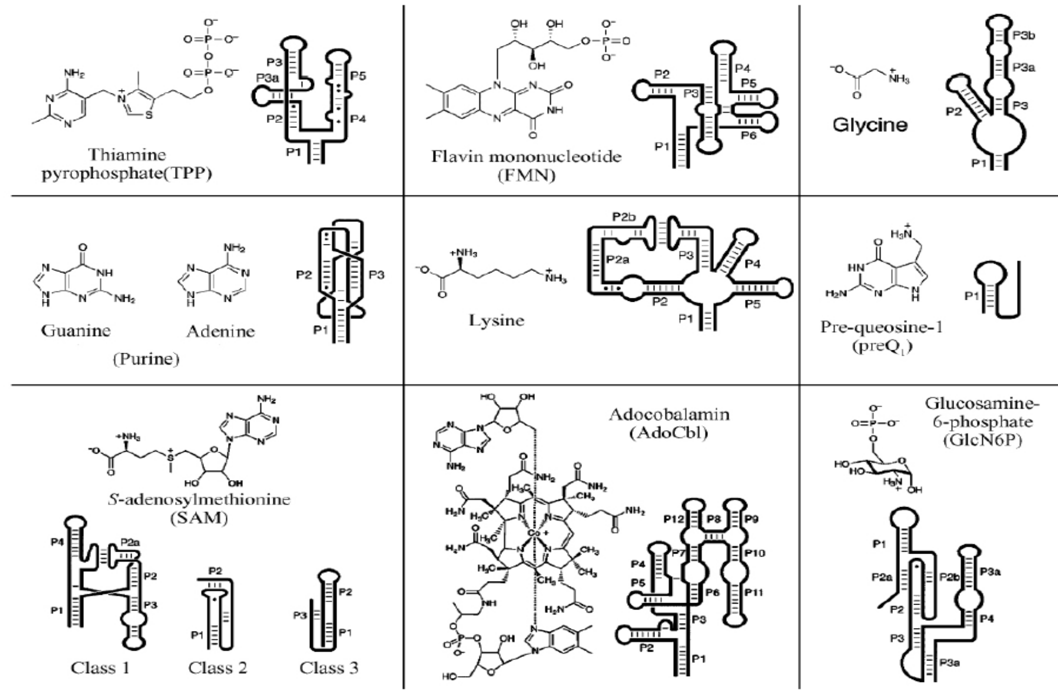
- Regulatory sequences in the leader of an mRNA both sense and respond to environmental conditions by either prematurely terminating transcription or blocking translation.

- Folding pattern altered in response to mRNA binding of an effector molecule.

- Folding of mRNA leader sequence determines whether transcription will continue or be terminated

Riboswitches Bind Specific Metabolites

- amino acids,
- vitamins,
- glucosamine-6-phosphate,
- S-adenosylmethionine,
- thiamine pyrophosphate, ions
- flavin mononucleotide (FMN)

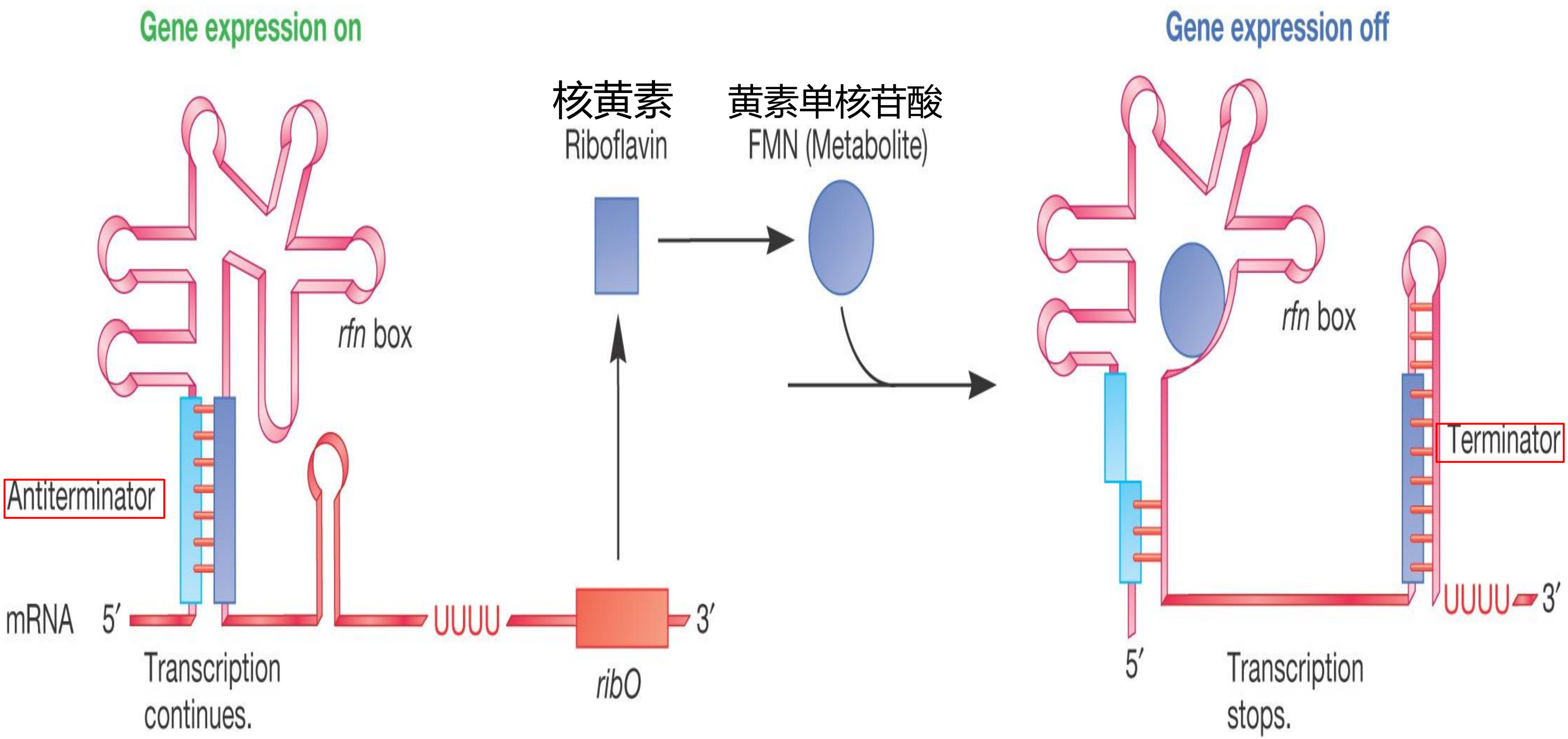


Riboswitches in bacteria

Table 13.1 Regulation of Gene Expression by Riboswitches

System	*Microbe(s)*	*Target genes encode:*	*Effector and Regulatory Response*
T box	Many gram-positive bacteria	Amino acid biosynthetic enzymes	Uncharged tRNA; anticodon base pairs to 5′ end of mRNA, preventing formation of transcriptional terminator
Vitamin B_{12} element	*E. coli*	Cobalamine biosynthetic enzymes	Adenosylcobalamine (AdoCbl) binds to *btuB* mRNA and blocks translation
THI box	*Rhizobium etli* *E. coli* *B. subtilis*	Thiamine (Vitamin B_1) biosynthetic and transport proteins	Thiamine pyrophosphate (TPP) causes either premature transcriptional termination (*R. etli, B. subtilis*) or blocks ribosome binding (*E. coli*)
RFN-element	*B. subtilis*	Riboflavin biosynthetic enzymes 核黄素合成	Flavin mononucleotide (FMN) cases premature transcriptional termination
S box	Low G + C gram-positive bacteria	Methionine biosynthetic enzymes	S-adenosylmethionine (SAM) causes premature transcriptional termination

The riboflavin (*rib*) biosynthetic operon in *bacillus subtilis* 枯草芽孢杆菌



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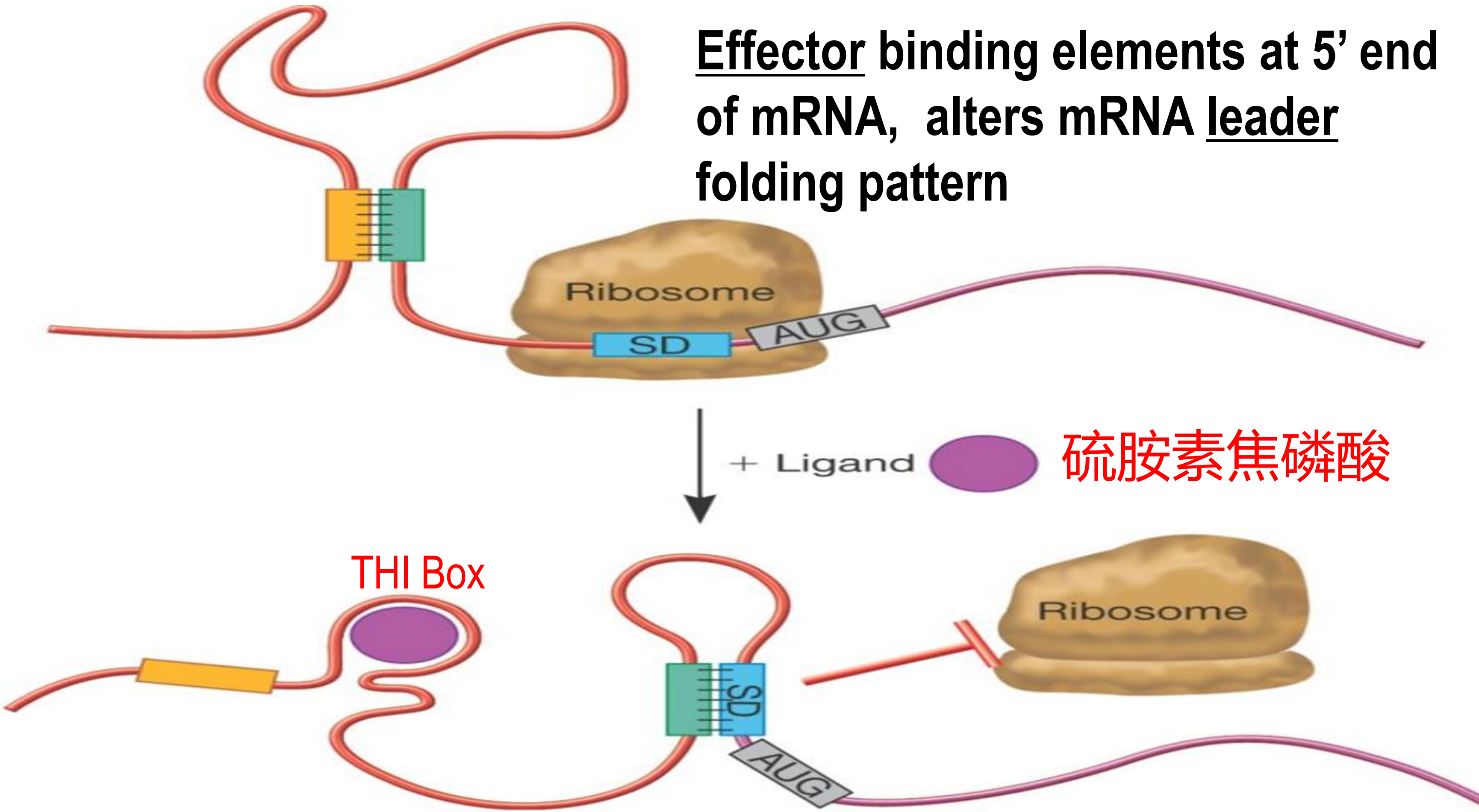
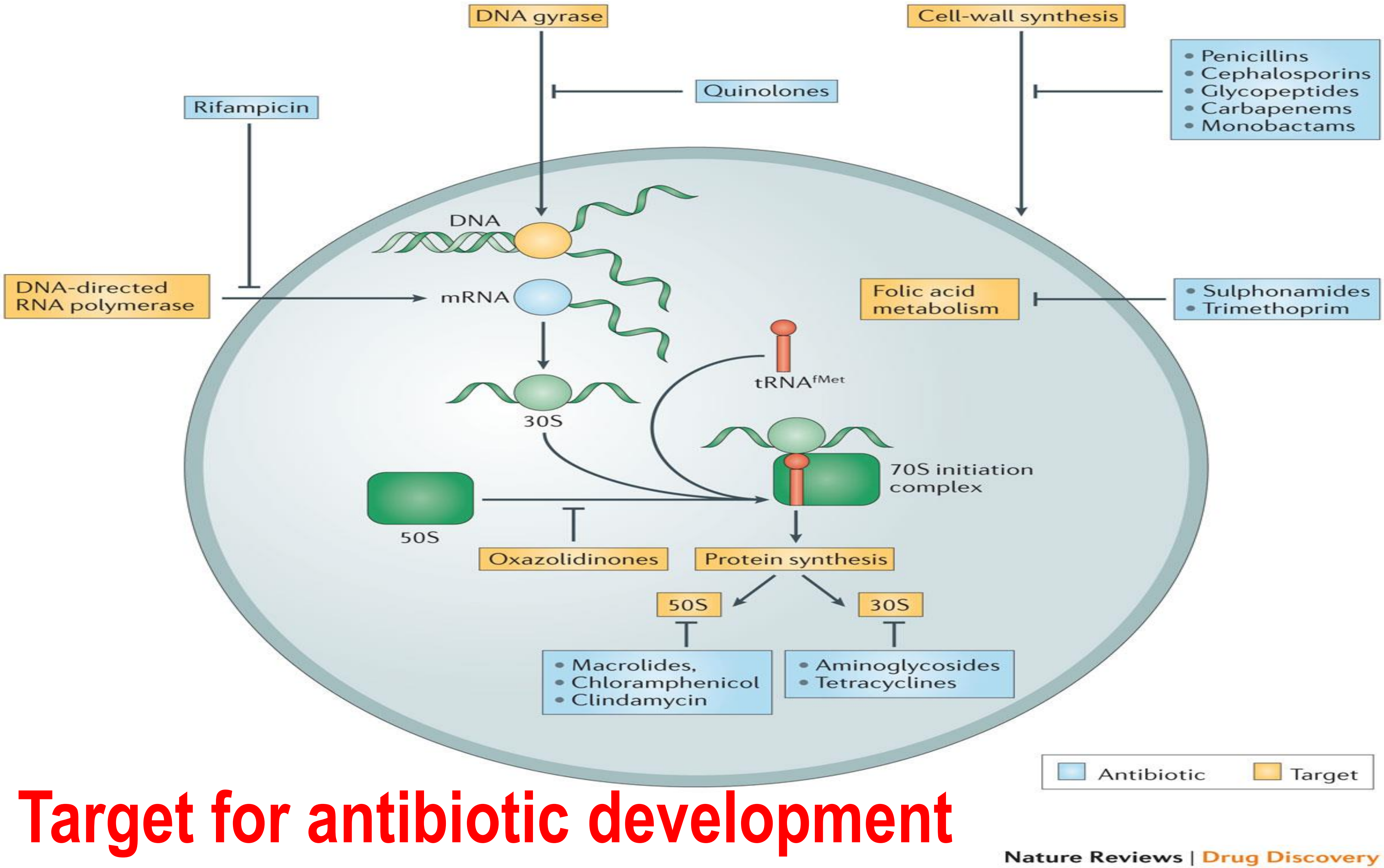
Blocking translation Initiation by a Riboswitch

Effector binding elements at 5' end of mRNA, alters mRNA leader folding pattern





Target for antibiotic development

Riboswitches as novel antibiotic target

2015 Oct 29

ARTICLE

doi:10.1038/nature15542

Selective small-molecule inhibition of an RNA structural element

John A. Howe[1]*, Hao Wang[1]*, Thierry O. Fischmann[1]*, Carl J. Balibar[1], Li Xiao[1], Andrew M. Galgoci[1], Juliana C. Malinverni[1], Todd Mayhood[1], Artjohn Villafania[1], Ali Nahvi[2], Nicholas Murgolo[1], Christopher M. Barbieri[1], Paul A. Mann[1], Donna Carr[1], Ellen Xia[1], Paul Zuck[3], Dan Riley[3], Ronald E.Painter[1], Scott S. Walker[1], Brad Sherborne[1], Reynalda de Jesus[1], Weidong Pan[1], Michael A. Plotkin[1], Jin Wu[1], Diane Rindgen[1], John Cummings[1], Charles G. Garlisi[1], Rumin Zhang[1], Payal R. Sheth[1], Charles J. Gill[1], Haifeng Tang[1] & Terry Roemer[1]

Riboswitches are non-coding RNA structures located in messenger RNAs that bind endogenous ligands, such as a specific metabolite or ion, to regulate gene expression. As such, riboswitches serve as a novel, yet largely unexploited, class of emerging drug targets. Demonstrating this potential, however, has proven difficult and is restricted to structurally similar antimetabolites and semi-synthetic analogues of their cognate ligand, thus greatly restricting the chemical space and selectivity sought for such inhibitors. Here we report the discovery and characterization of ribocil, a highly selective chemical modulator of bacterial riboflavin riboswitches, which was identified in a phenotypic screen and acts as a structurally distinct synthetic mimic of the natural ligand, flavin mononucleotide, to repress riboswitch-mediated *ribB* gene expression and inhibit bacterial cell growth. Our findings indicate that non-coding RNA structural elements may be more broadly targeted by synthetic small molecules than previously expected.

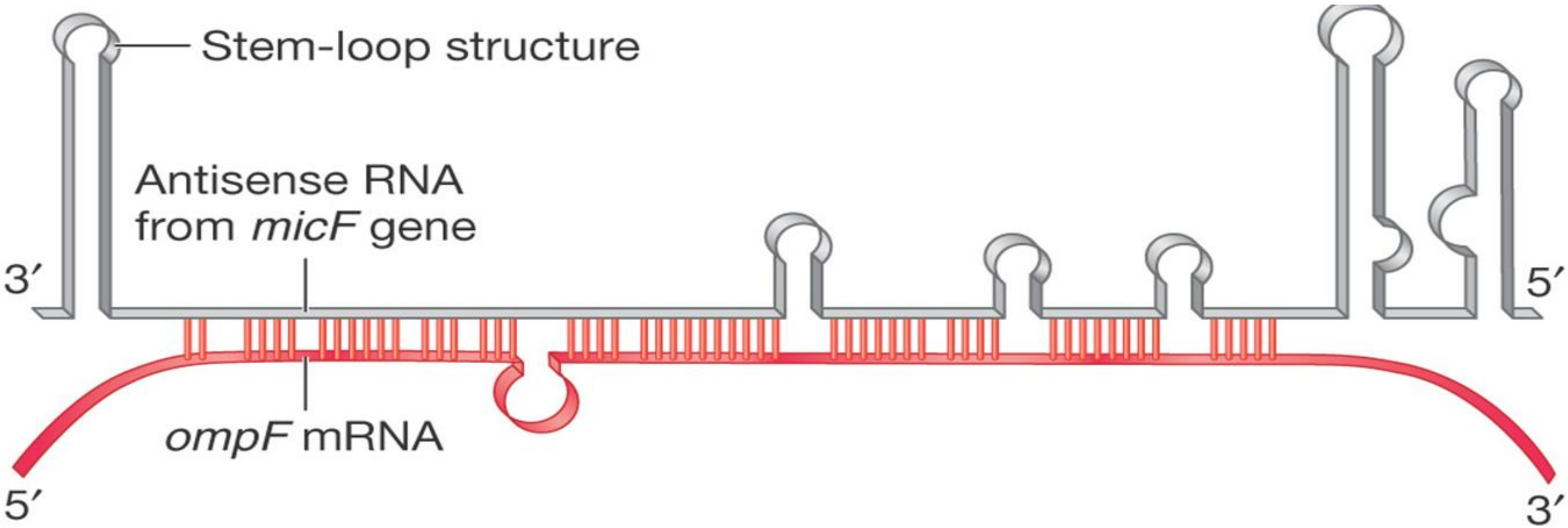
Regulation of translation by small RNA molecules

Key

- small (sRNAs) or noncoding (ncRNAs) RNAs
  - Do not function as mRNA, tRNA, or rRNA
  - May inhibit or enhance translation
  - Antisense RNAs are complementary to mRNA and function as translation blocker by base pairing

Key

Translation repression by "antisense RNA"



Antisense RNA medicines