Part IV Regulation of gene expression

An overview of gene expression regulation

1. Types of gene expression

- 根据生物对内外环境刺激的反应，将基因的表达方式分为：

  组成型(constitutive)表达

  适应型(adaptive)或调节型(regulated)表达

2. Types of gene expression regulation

2.1 Positive regulation (正调控)

Activate / promote (激活/促进) gene expression

2.2 Negative regulation (负调控)

Inhibit (抑制) gene expression

3. Levels of gene expression regulation

3.1 Regulation at levels of DNA and chromosome (DNA和染色体水平调控)

DNA修饰、基因重排、基因扩增、染色质结构变化等。（真核）

▲ 3.2 Transcriptional regulation (转录调控)

顺式作用元件 ⟷ 反式作用因子

⇩

正调控或负调控基因的表达

(1) Cis-acting elements (顺式作用元件)

Cis-acting elements are regions of non-coding DNA which regulate transcription of the genes in the same DNA molecule. e.g. promoter, enhancer, silencer, attenuator, insulator…

能调控同一DNA分子上基因转录的非编码DNA序列称为顺式作用元件。e.g.启动子、增强子、沉默子、弱化子、绝缘子……

(2) Trans-acting factors (反式作用因子)

Trans-acting factors are **proteins** that bind to the cis-acting elements to control gene transcription. e.g. transcription factor, repressor, activator…

与顺式作用元件结合，调控基因转录的蛋白质。如转录因子、阻遏蛋白、激活蛋白……

cis-acting – A site that affects the activity only of sequences on its own molecule of DNA.

trans-acting – A product that can function on any of its target DNA. This implies that it is a diffusible (扩散的) protein or RNA.

3.3 Regulation of RNA processing (RNA加工调控)

3.4 Translational regulation (翻译调控)

对mRNA稳定性的调控，某些反义RNA、siRNA、miRNA对翻译水平的调控等。

3.5 Post-translational regulation (翻译后调控)

蛋白质的剪切、修饰与转运等。

转录后调控 (post-transcriptional regulation)

Chapter 9 Regulation of transcription in prokaryotes

1. Operon (操纵子): an overview

In 1961, Francois Jacob and Jacques Monod proposed the operon model of gene regulation in bacteria.

Nobel Prize in Physiology or Medicine (1965)

François Jacob

Jacques Monod, 1910–1976

The operon is a DNA unit of transcriptional regulation, which typically includes: promoter, operator sequence and functionally related structural genes.

操纵子是由启动子、操纵基因和一群功能相关的结构基因所组成的转录调控功能单位。是原核生物基因表达调控的主要方式。

Structural gene

A structural gene codes for any RNA or protein product other than a regulator.

结构基因编码除调节物之外的任何蛋白或RNA。

• Regulator gene

A regulator gene codes for a product (typically protein) that controls the expression of other genes (usually at the level of transcription).

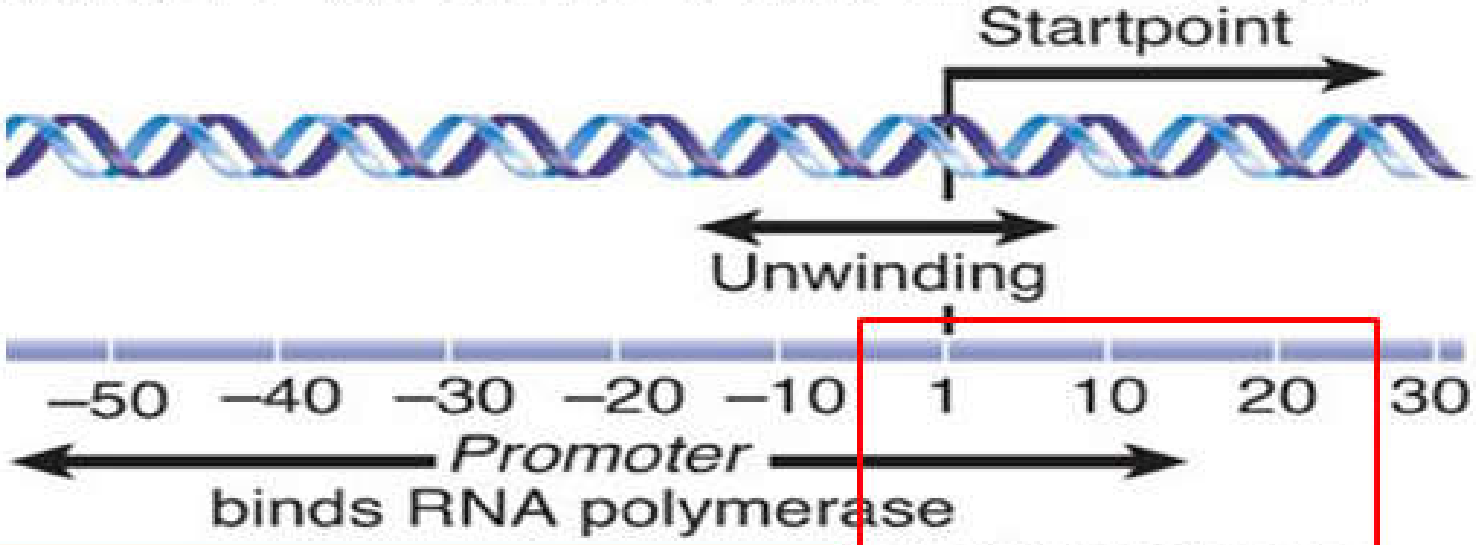
调节基因的产物调控其他基因的表达（通常是转录）。

调节基因的产物通常是变构蛋白，是一个寡聚体，根据调节作用分为阻遏蛋白和激活蛋白。

• Operator

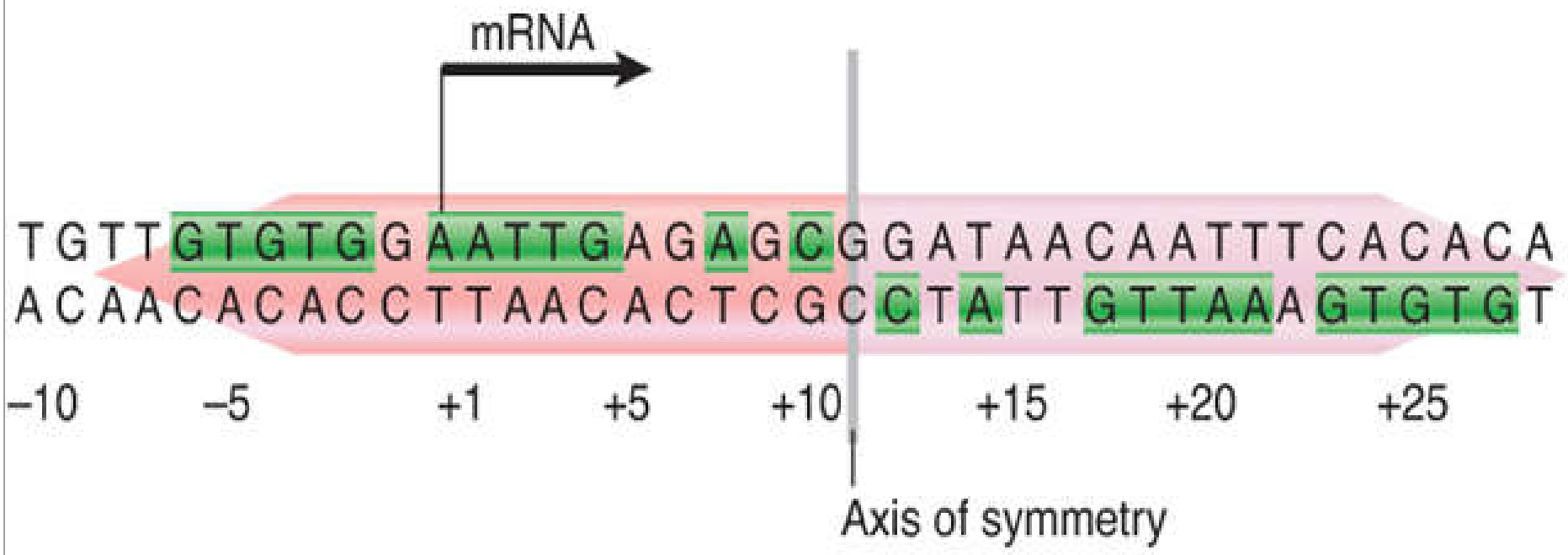
An operator is a segment of DNA to which a regulator gene product binds to regulate structural genes expression.

操纵基因是一段可以通过与调节基因产物结合，调控结构基因表达的DNA序列。



操纵基因与启动子部分重叠

Inverted repeat



2. The lactose operon

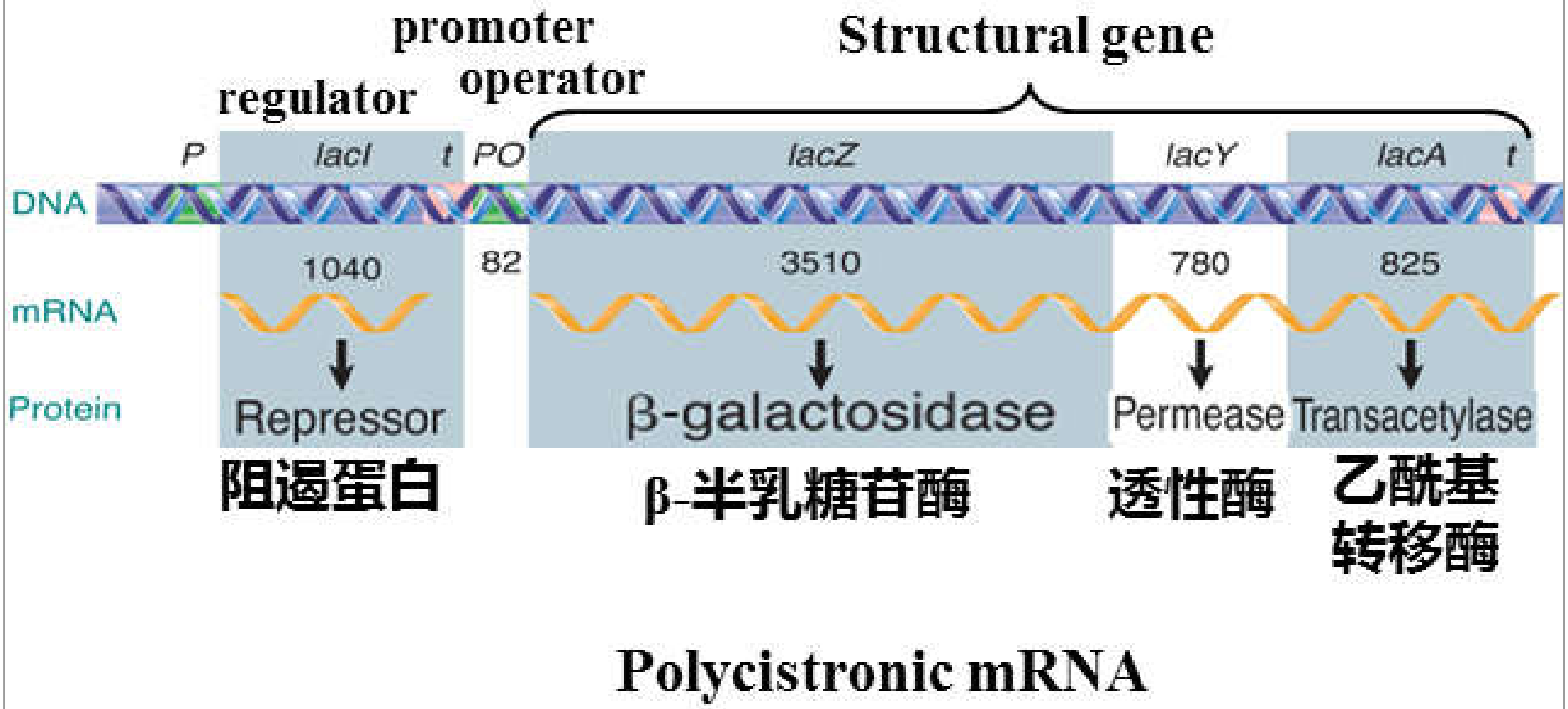
E. coli can use lactose as a source of carbon. The enzymes required for the use of lactose as a carbon source are only synthesized when **lactose is available as the sole carbon source**.

Lactose
(乳糖)

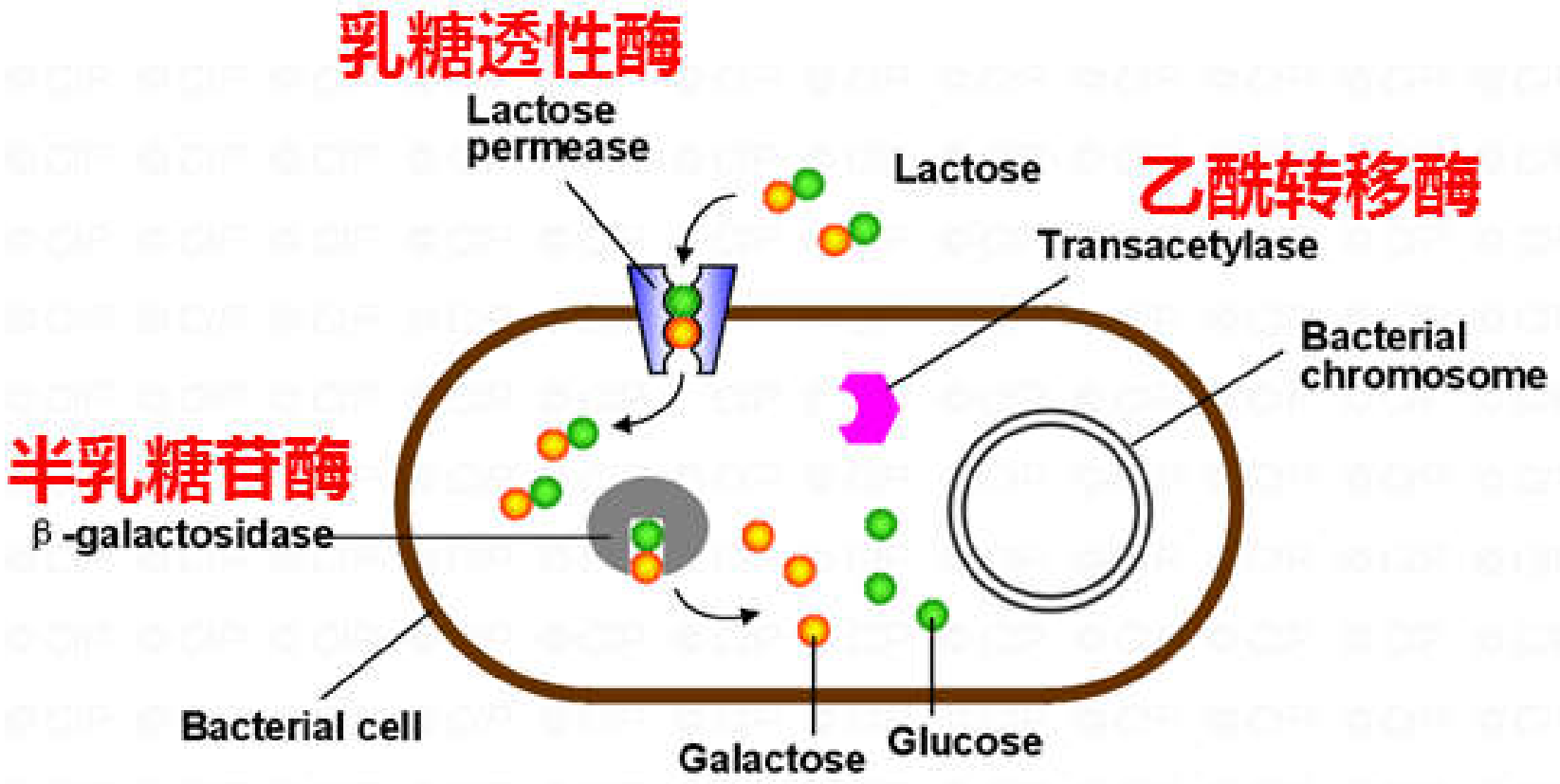
Galactose
(半乳糖)

Glucose
(葡萄糖)

2.1 The structure of the lactose operon



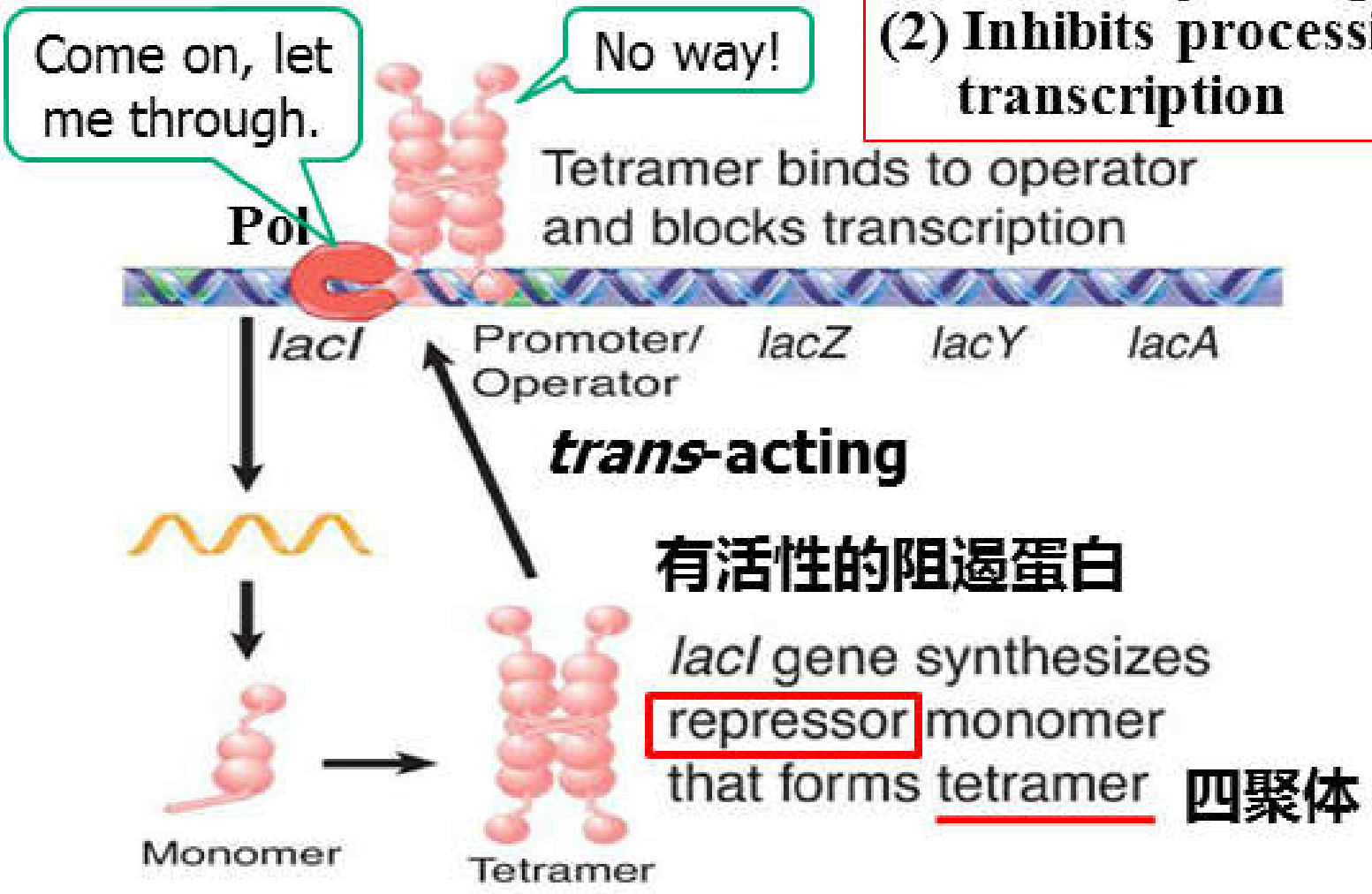
β-galactosidase: for lactose hydrolysis
Permease: transport lactose across the cell wall
Transacetylase: ?



2.2 Negative regulation by repressor protein

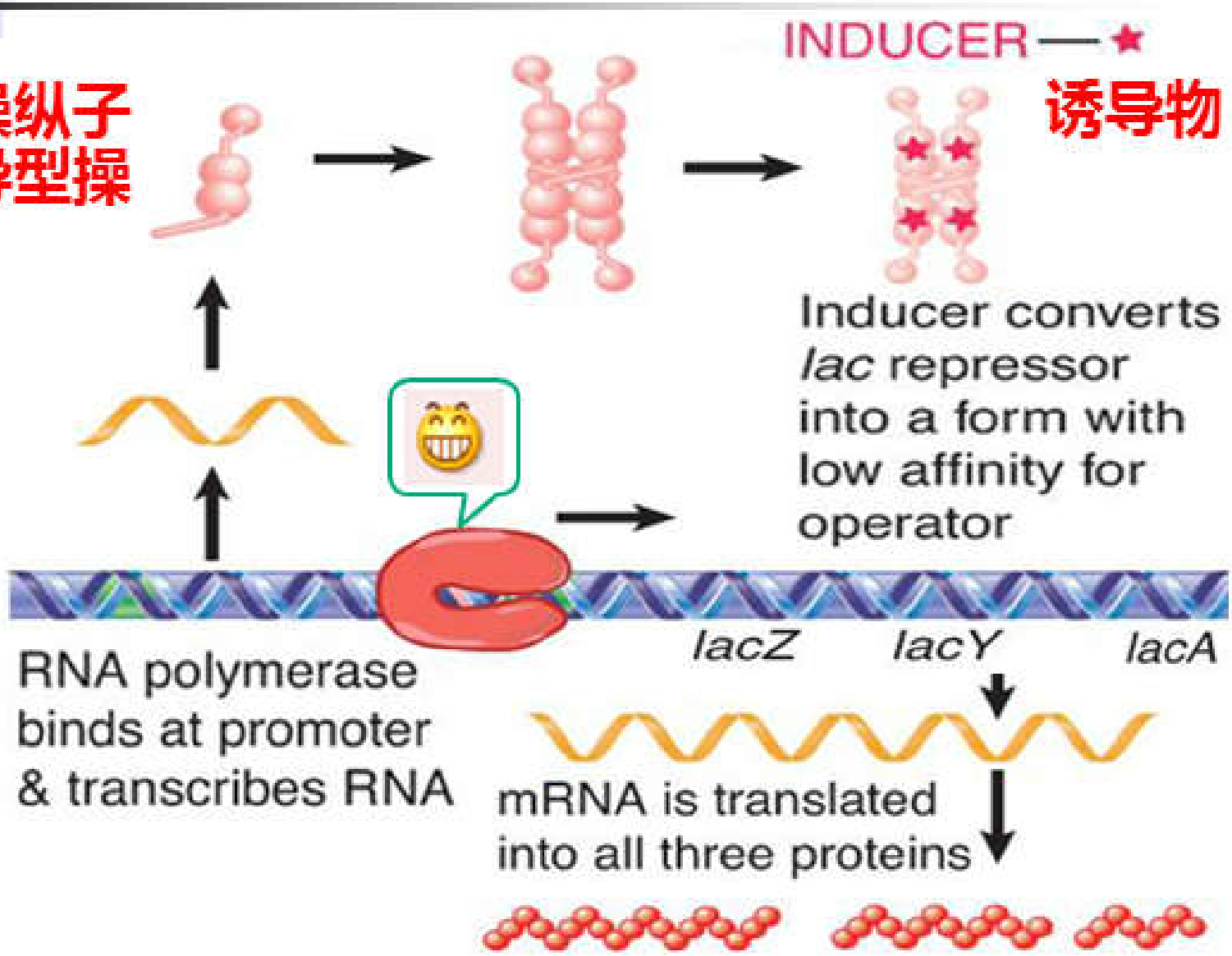
2.2.1 OFF (lactose abscent)

(1) Blocks access of RNA Pol to the adjacent promoter
(2) Inhibits processive transcription

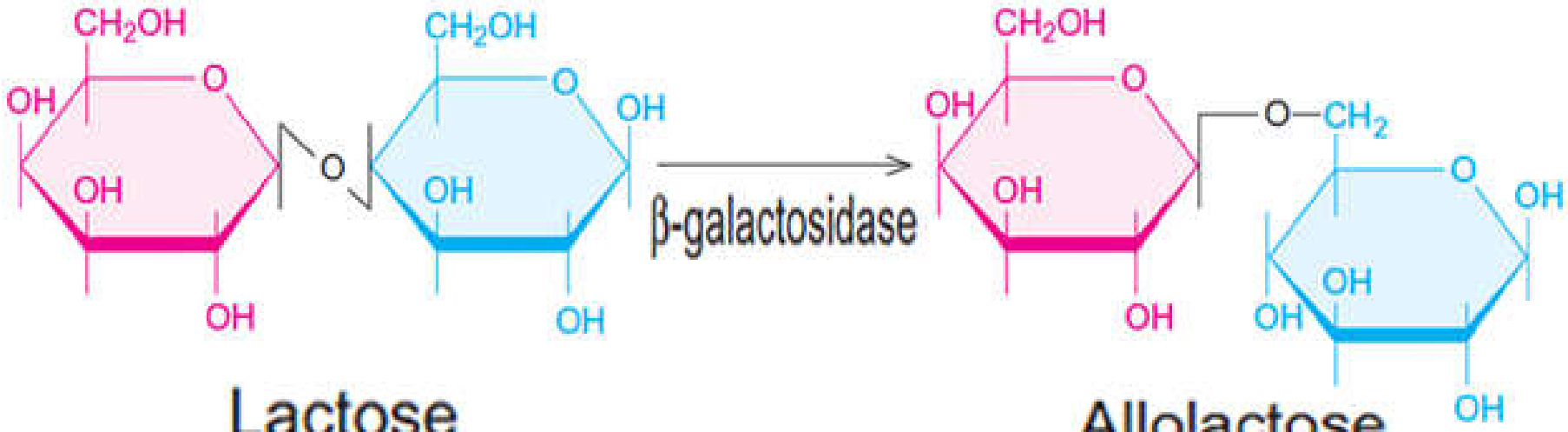


2.2.2 ON (lactose present)

乳糖操纵子是诱导型操纵子。



异/别乳糖才是乳糖操纵子真正的诱导物



Lactose
(β-1,4 linkage)

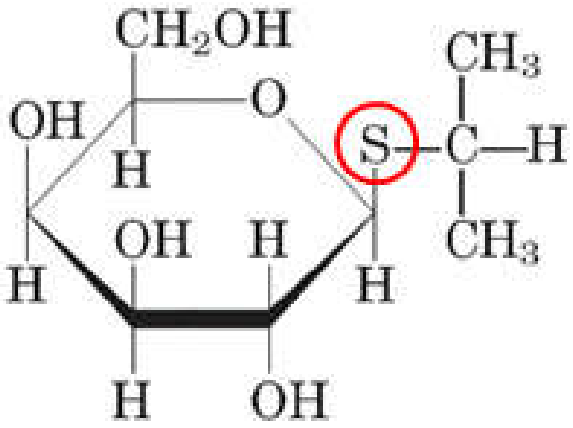
Allolactose
(β-1,6 linkage)

异/别乳糖

Native (天然) inducer

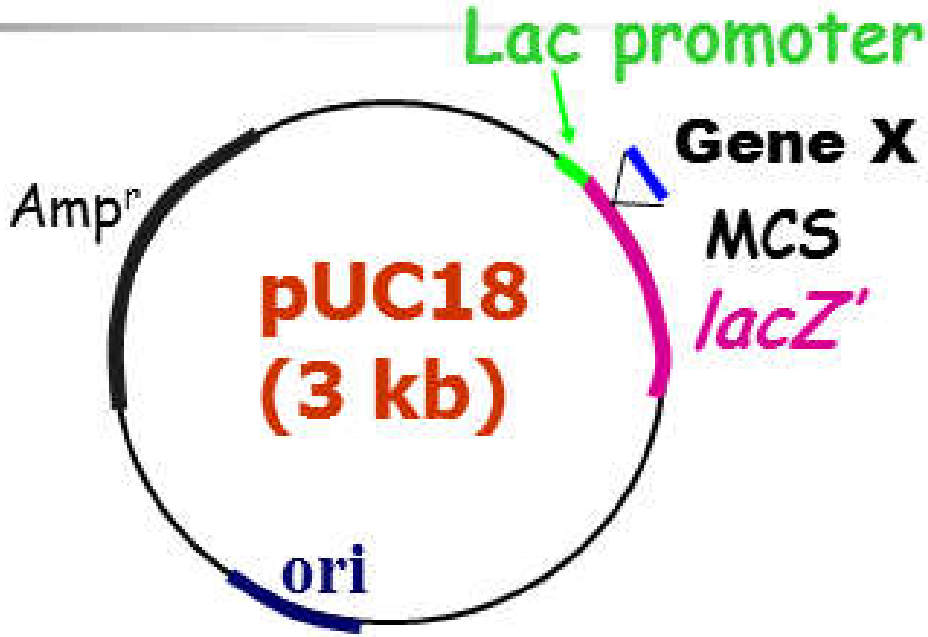
Inducer of *lac* operon

Lack of inducer: the lac repressor block all but a very low level of transcription of *lacZYA* .



Isopropylthiogalactoside
(IPTG)

异丙基硫代
半乳糖苷



No IPTG, little expression of X gene

With IPTG, efficient expression of X gene.

安慰性诱导物（gratuitous repression）：与转录调控中天然诱导物相似的一类人工合成的高效诱导物，但不是该诱导酶的底物。

repressor与operator特异结合

↳ The operon off

inducer (allolactose)与repressor特异结合

↓

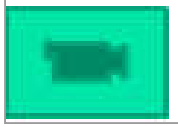
repressor tetramer 变构 → 特异结合力下降

↳ The operon on

作用于O位点上的repressor → 变构 → 脱离O位

作用于游离的repressor

↳ 变构 → 失去结合于O位的能力

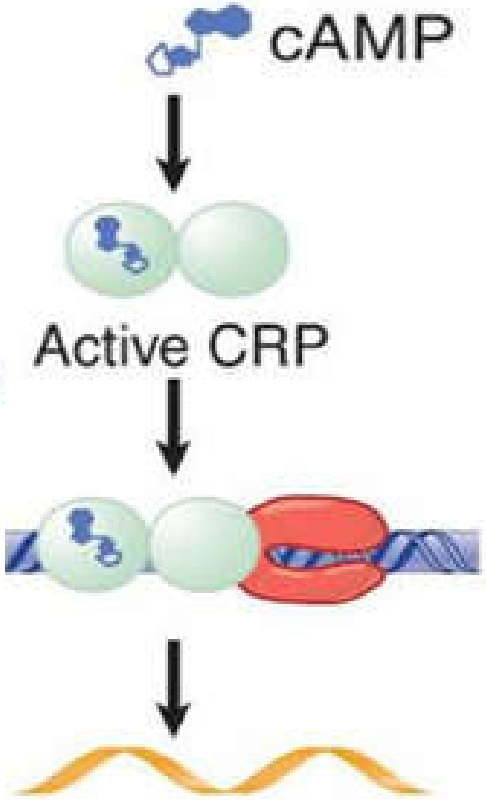


2.3 Positive regulation by cAMP receptor protein (CRP)

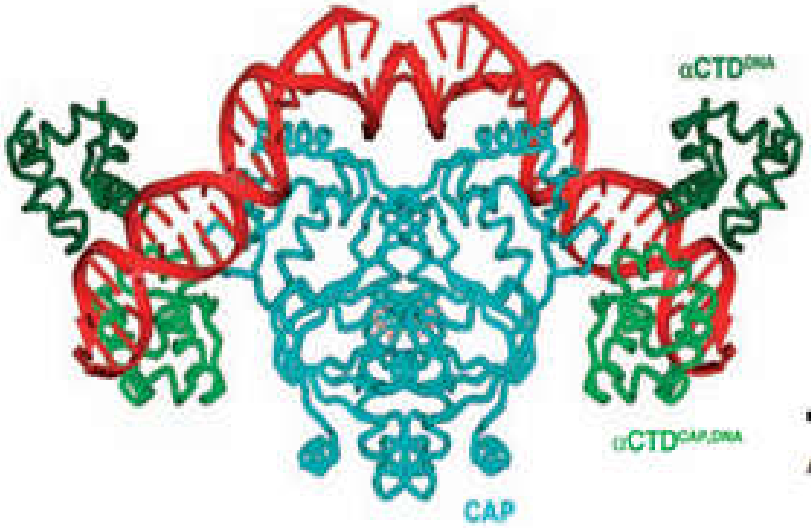
cAMP receptor protein (CRP) or catabolite activator protein (代谢物激活蛋白, CAP)

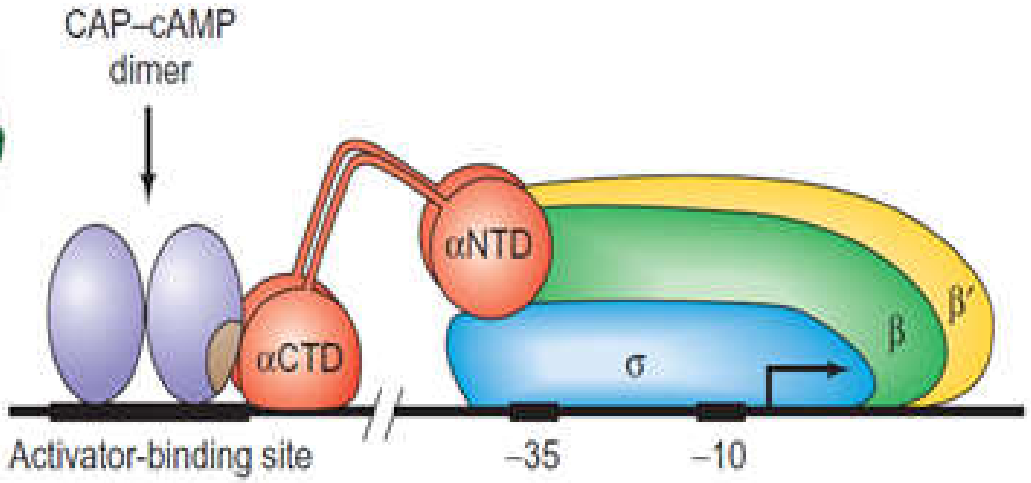
2.3.1 Glucose absent

- The role of cAMP is to change the conformation of CRP to increase its affinity for the activator-binding site (激活因子结合位点).
- The CRP–cAMP complex helps RNA polymerase bind to the promoter.

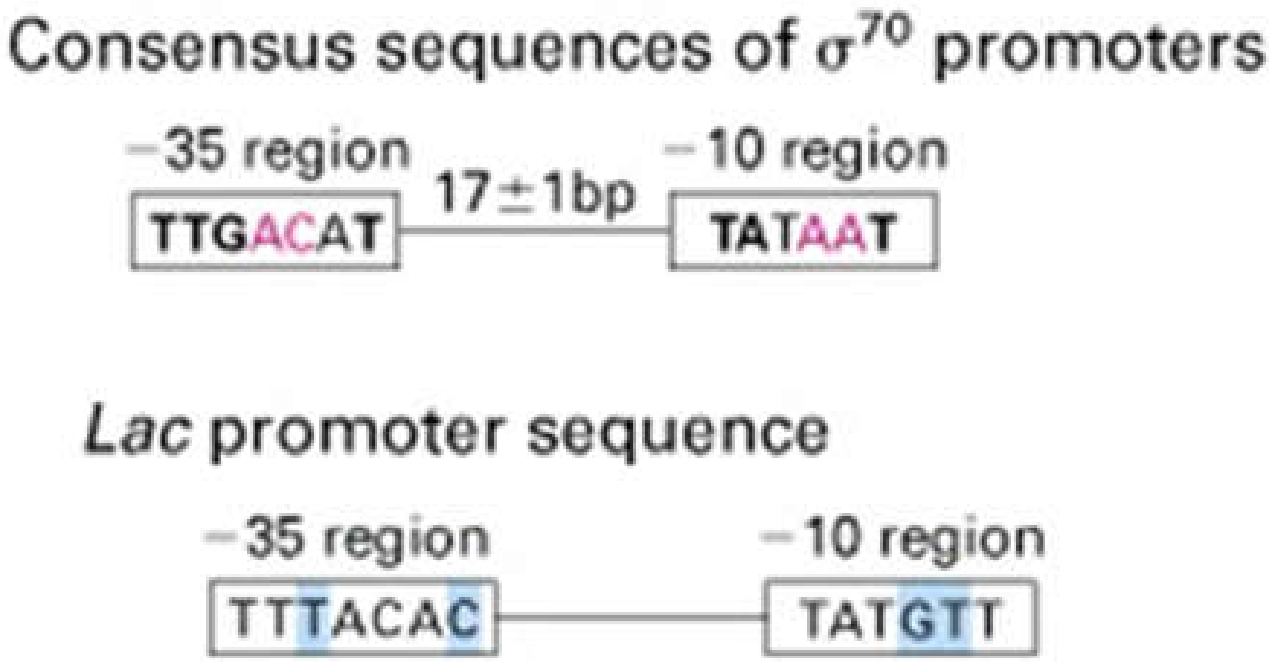


- Binding of CRP-cAMP to its DNA target bends the DNA about 90°, which is believed to enhancing RNA pol binding to the promoter.
- CRP-cAMP interacts with the C-terminal domain of the α subunit (αCTD) of RNA polymerase to activate it.





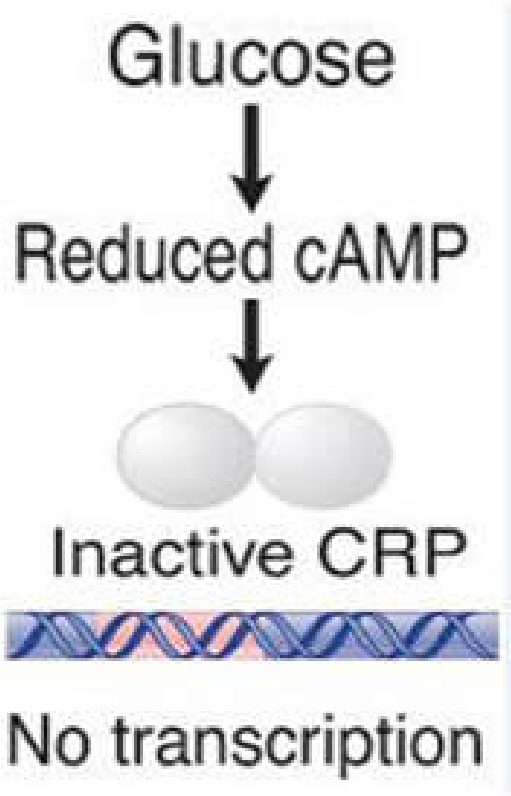
- The P_{lac} promoter is not a strong promoter.
- P_{lac} and related promoters do not have strong –35 sequences and some even have weak –10 consensus sequences.

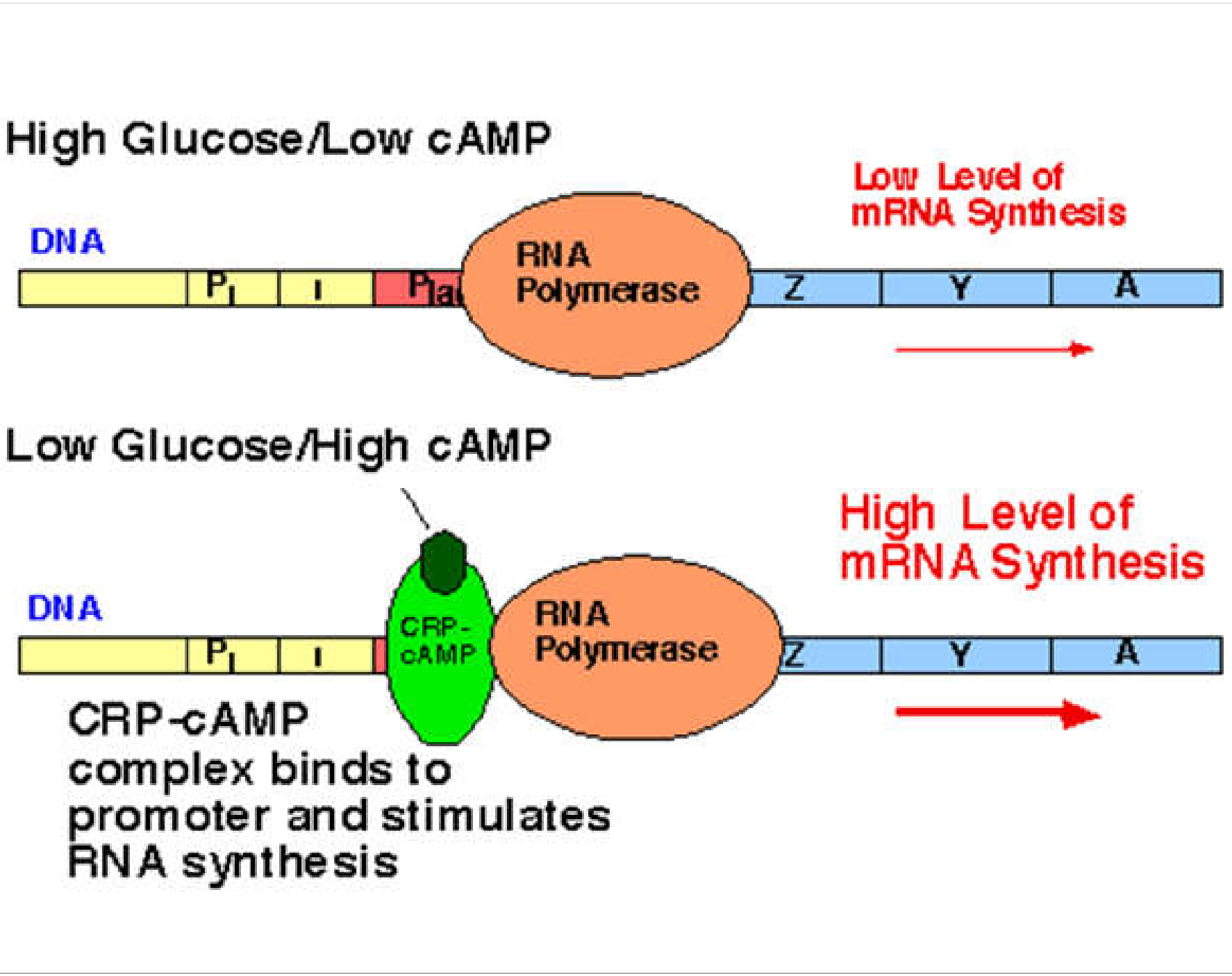


2.3.2 Glucose present

Catabolite repression
(分解代谢产物阻遏)

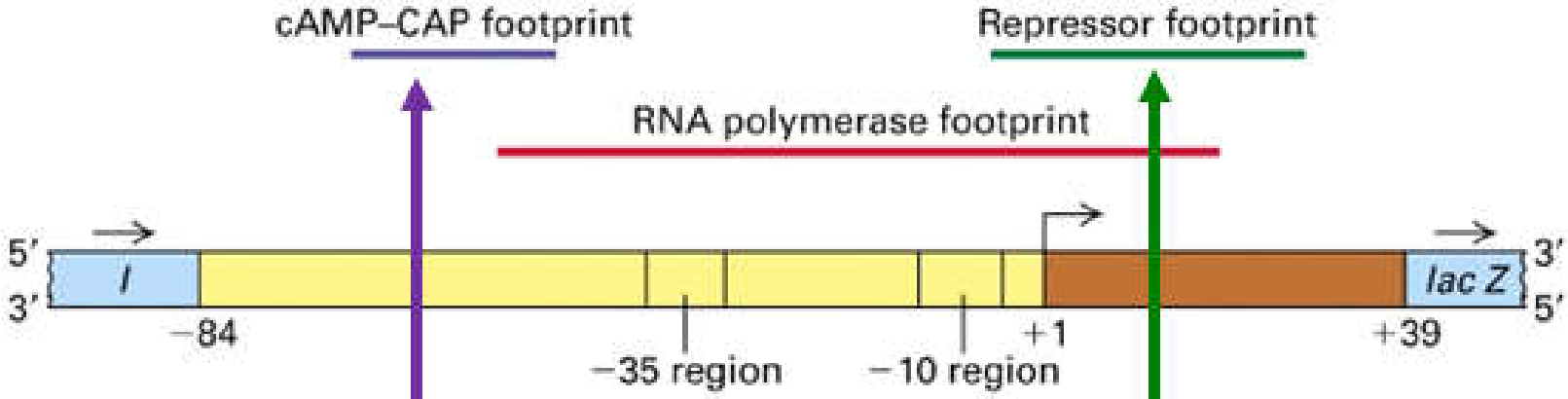
- **Catabolite of glucose can inhibit the activity of adenylate cyclase (腺苷酸环化酶) and activate phosphodiesterase (磷酸二酯酶), thereby reducing the concentration of cAMP.**




High Glucose/Low cAMP
Low Level of mRNA Synthesis
DNA
RNA Polymerase
Z
Y
A
Low Glucose/High cAMP
High Level of mRNA Synthesis
DNA
CRP-cAMP
RNA Polymerase
Z
Y
A
CRP-cAMP complex binds to promoter and stimulates RNA synthesis

小结：

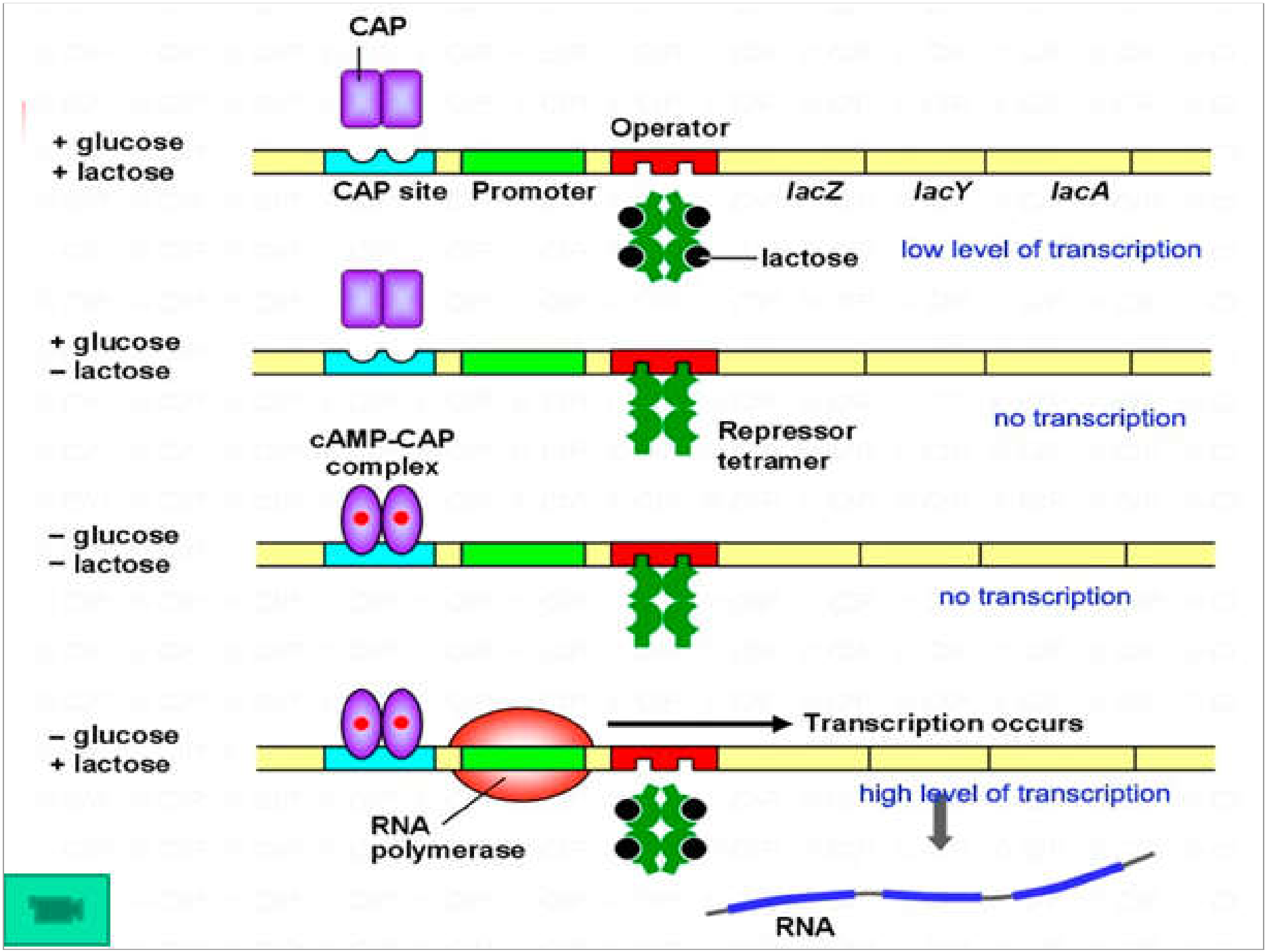
The *lac* operon transcription-control region.



Positive control: CAP protein

Negative control: repressor

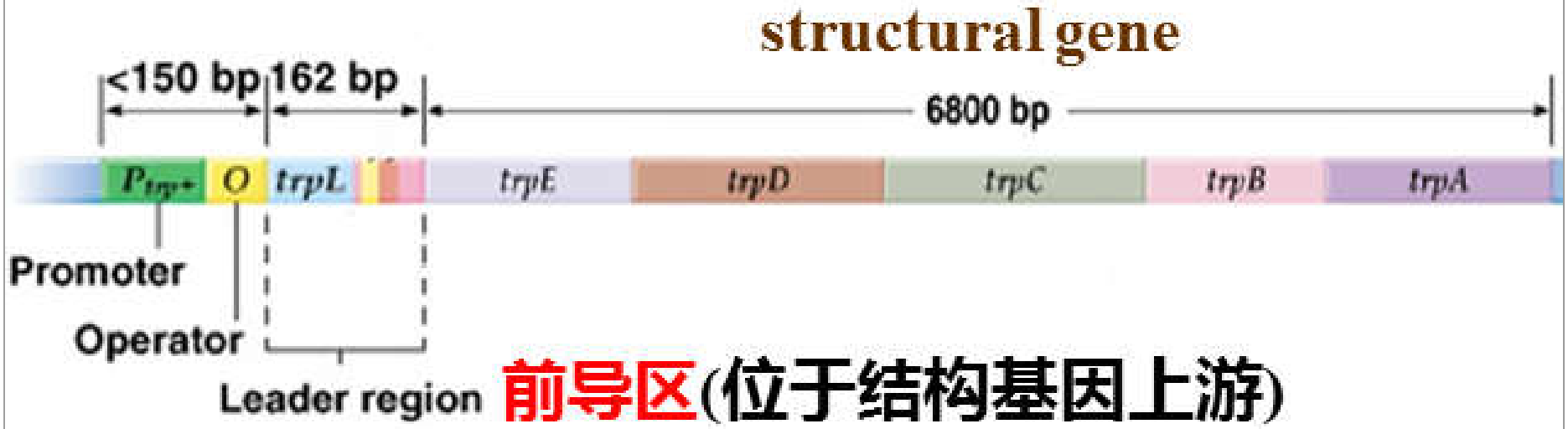
- **阻遏蛋白负调控与CRP正调控两种机制协调合作**
- ***lac*操纵子强的诱导作用既需要乳糖存在又需缺乏葡萄糖。**


CAP
+ glucose
+ lactose
Operator
CAP site
Promoter
lacZ
lacY
lacA
lactose
low level of transcription
+ glucose
– lactose
Repressor
tetramer
no transcription
cAMP-CAP
complex
– glucose
– lactose
no transcription
– glucose
+ lactose
Transcription occurs
high level of transcription
RNA
polymerase
RNA

3. The tryptophan (Trp) Operon

3.1 The structure of the *Trp* operon

合成色氨酸有关的酶（5个）

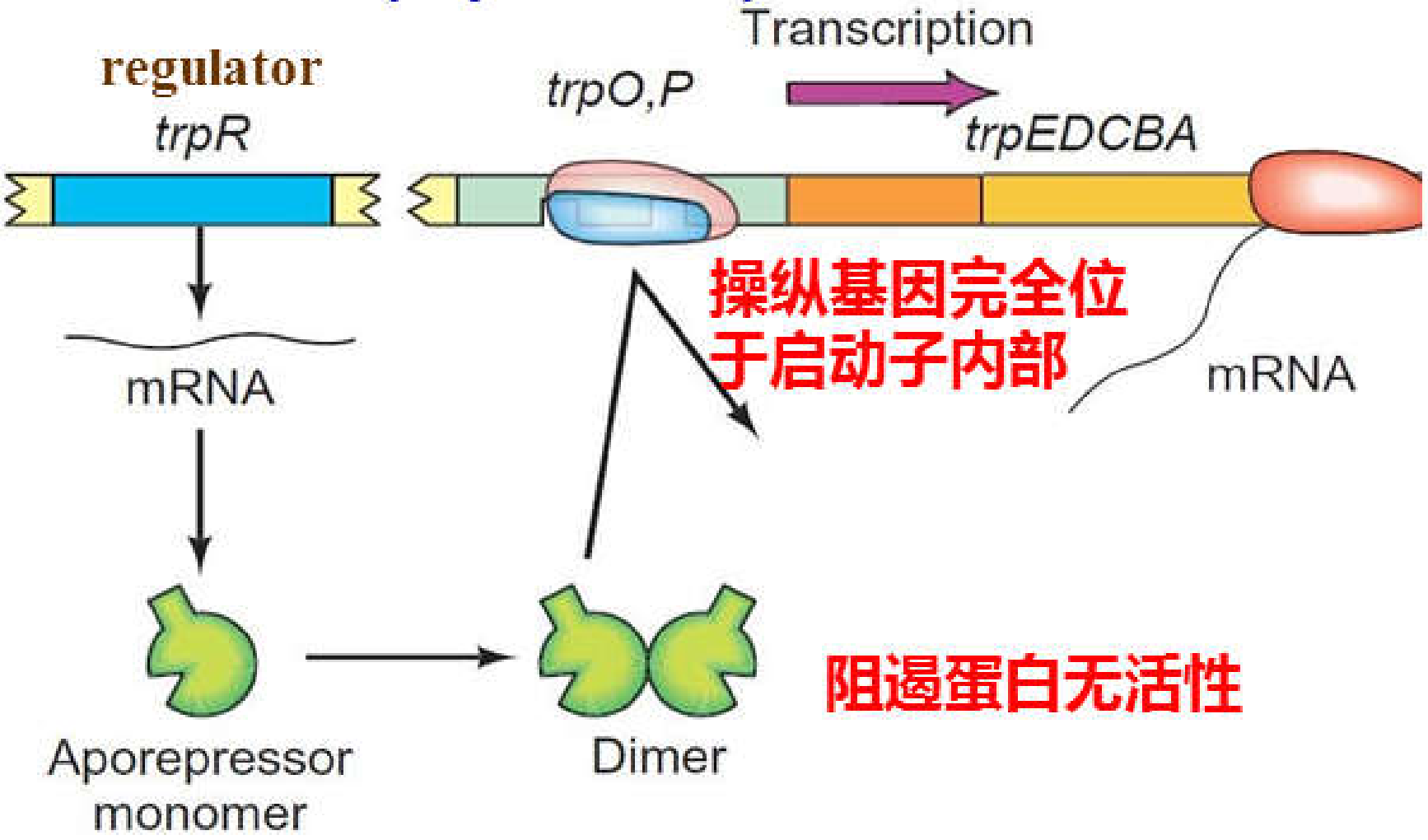


The tryptophan (*Trp*) operon of *E. coli* is one of the most extensively studied operons in amino acids synthesis.

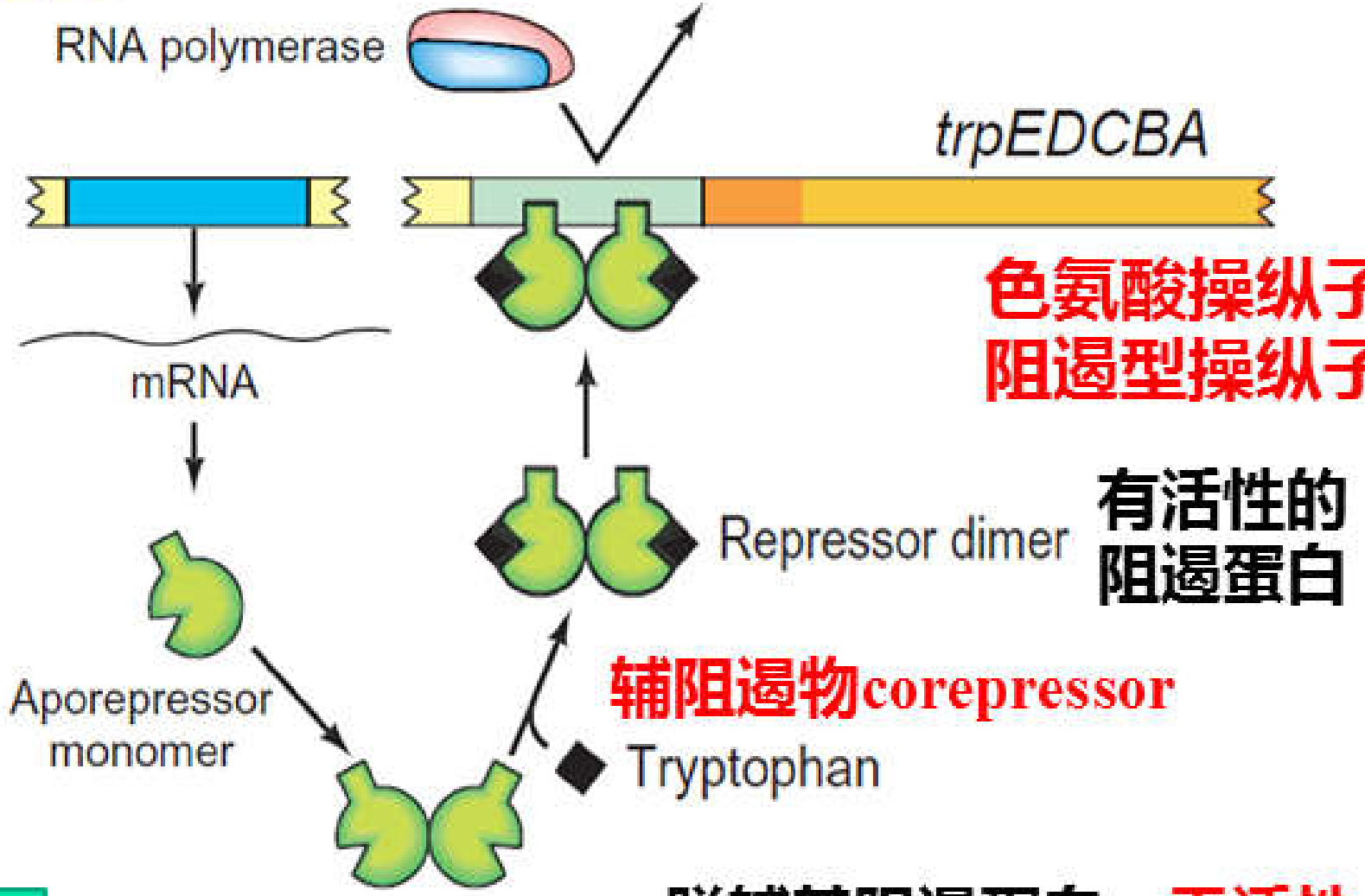
合成代谢操纵子

3.2 Negative regulation by repressor protein

3.2.1 ON (Trp absent)



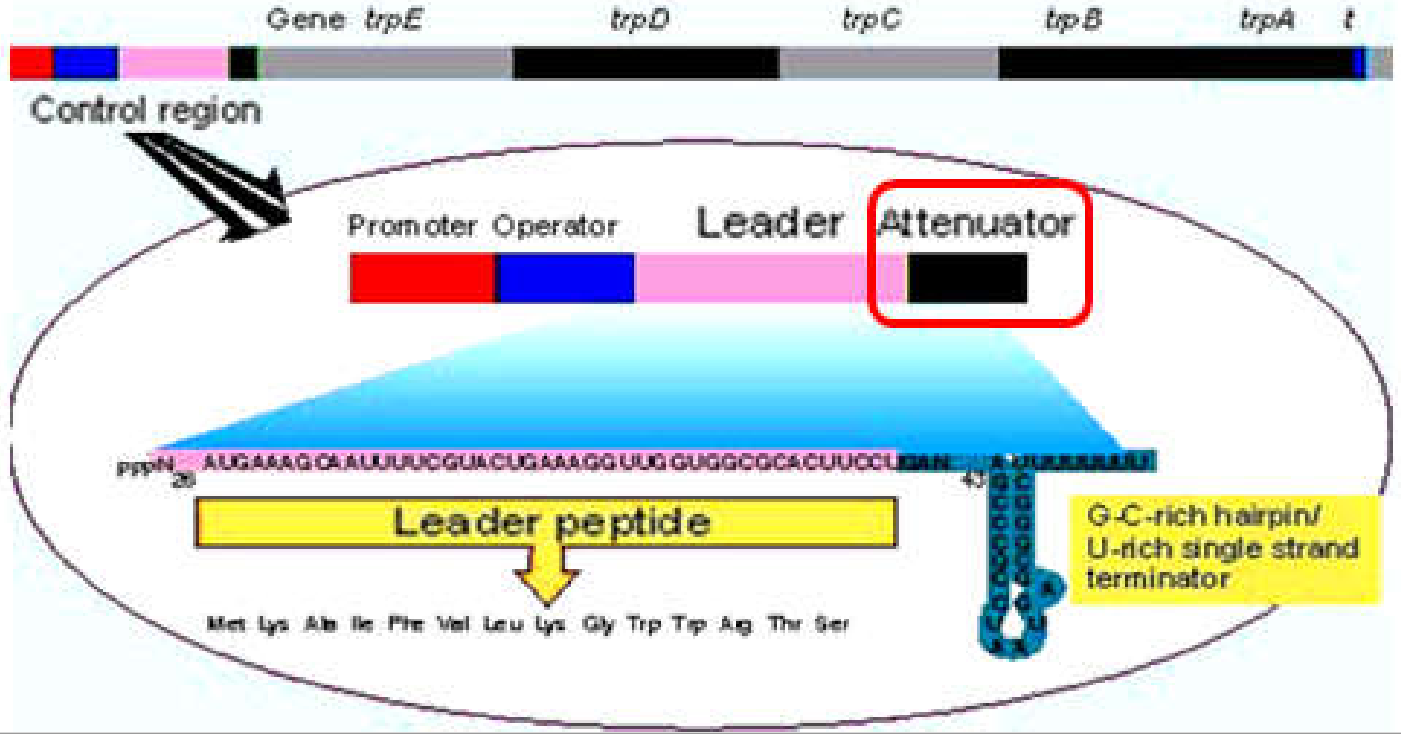
3.2.2 OFF (Trp present)



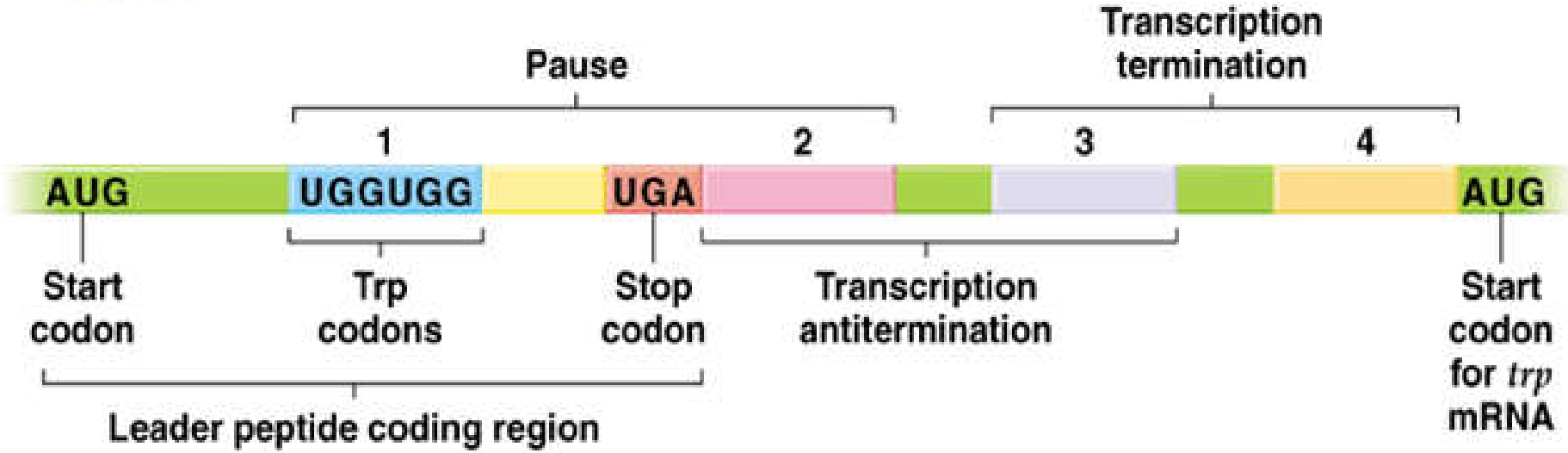
3.3 Negative regulation of the *Trp* operon by attenuator

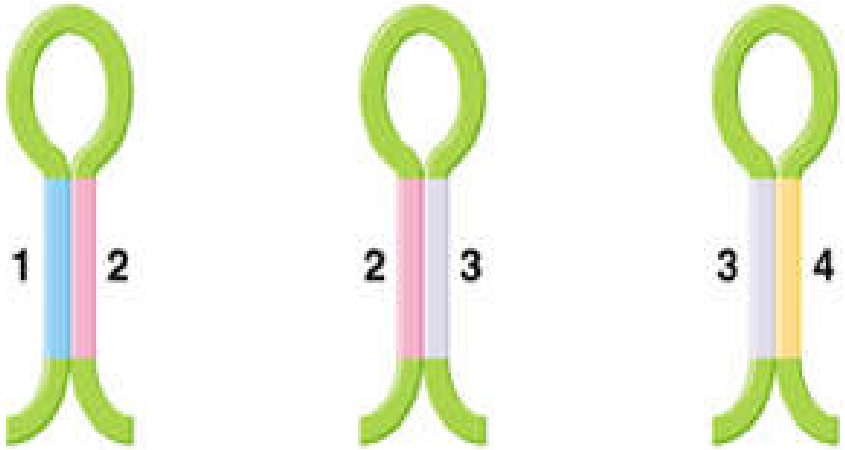
3.3.1 Attenuator(弱化子/衰减子)

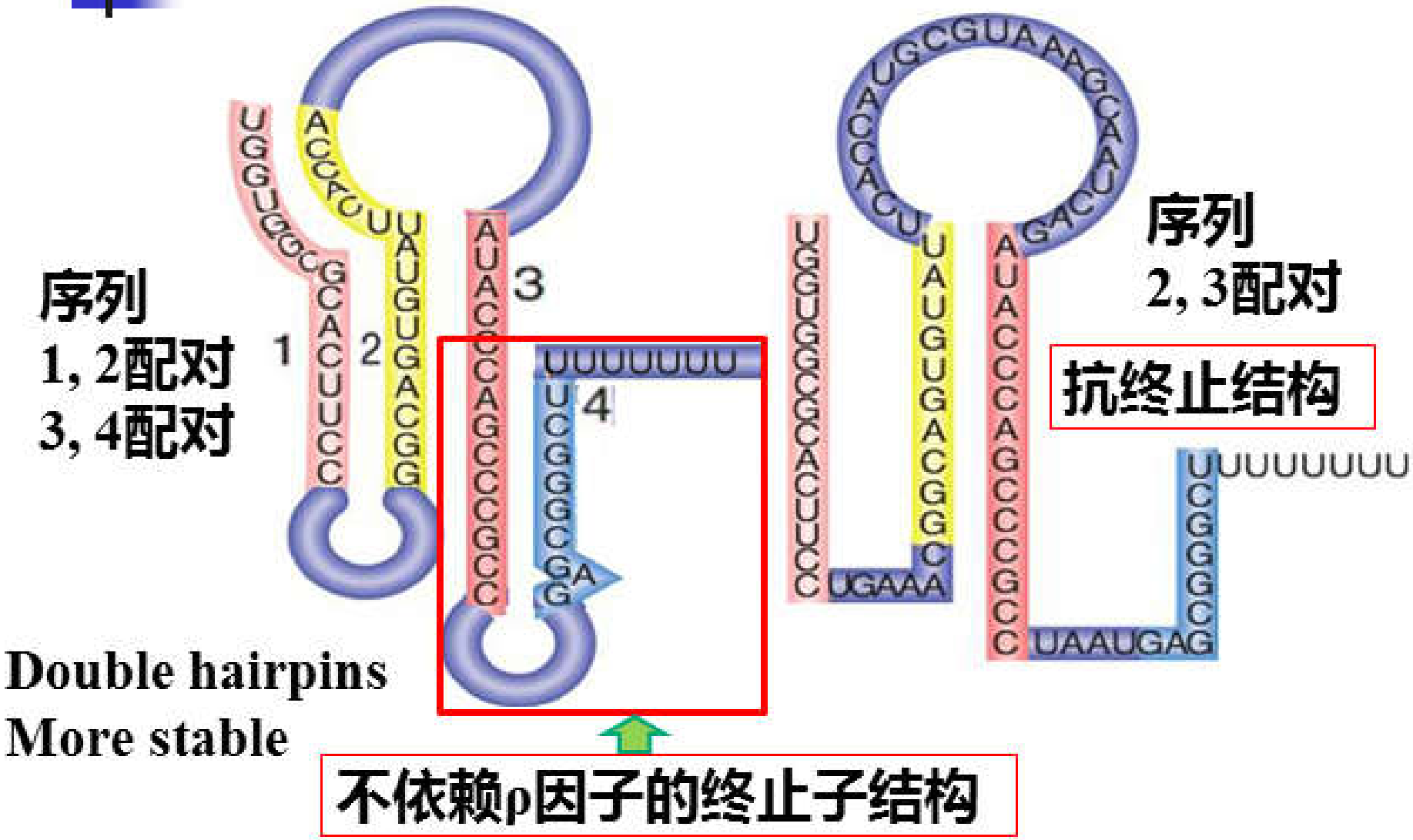
A DNA sequence located at the end of the leader sequence can significantly weaken or even terminate transcription. 位于结构基因上游前导区末端，可以明显弱化甚至终止转录的一段DNA序列。



3.3.2 Structure of the leader RNA






序列
1, 2配对
3, 4配对
1
2
3
4
UUUUUUUU
Double hairpins
More stable
不依赖ρ因子的终止子结构
序列
2, 3配对
抗终止结构
UUUUUUUU

3.3.3 Leader peptide (前导肽)

- The leader RNA contains an efficient ribosome binding site (RBS) and encodes a 14-amino-acid leader peptide.
- Codons 10 and 11 of this peptide encode Trp. Trp is a rare amino acid (1%).
- The availability of trp will affect the translation/ribosome position, which in turn to regulate transcription termination.

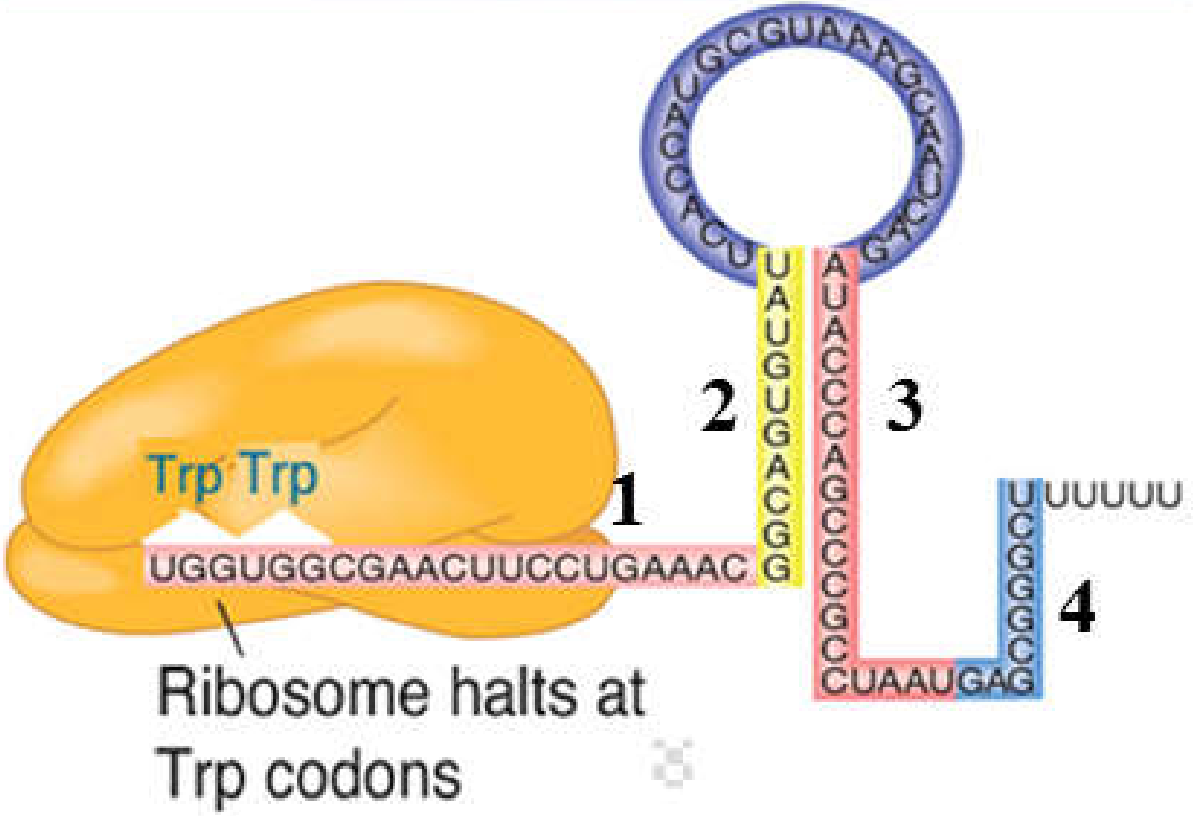
Met Lys Ala Ile Phe Val Leu Lys Gly Trp Trp Arg Thr Ser Stop

pppA---AUGAAAGCAAUUUUCGUACUGAAAGGUUGGUGGCGCACUUCCUGA

3.3.4 Attenuation (弱化/衰减作用)

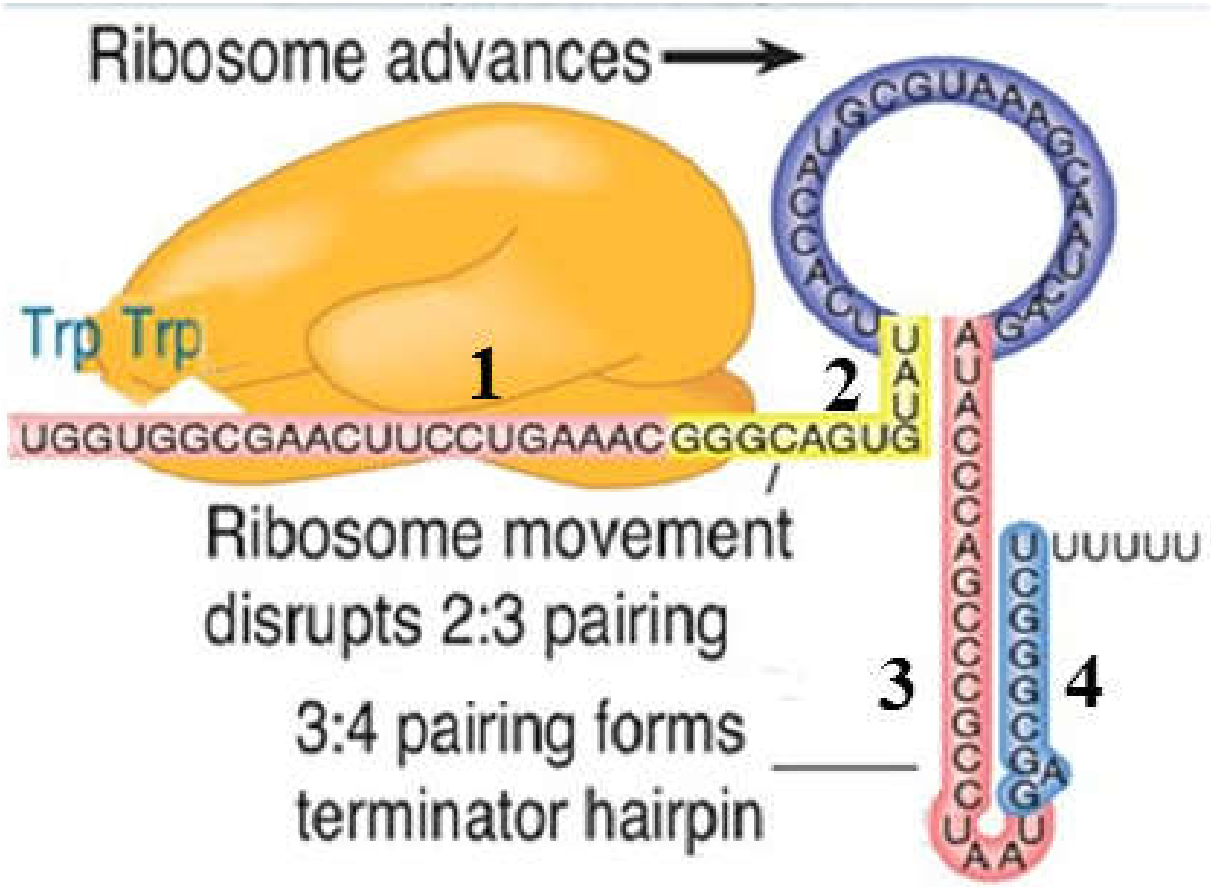
(1) Trp absent

Ribosome pause at Trp codons → 2:3 hairpin (anti-terminator) forms → transcription



(2) High Trp

Ribosome movement → disrupts 2:3 pairing → 3:4 hairpin forms → termination



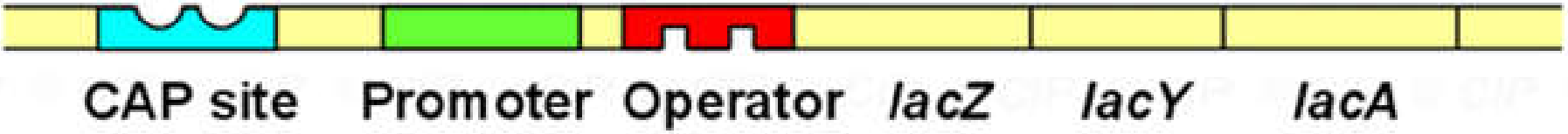
3.3.5 Importance of attenuation

- **A typical negative feed-back regulation**
- **Give rise to a 10-fold repression of the *trp* operon. Faster and more subtle (细微的) regulation of *trp* metabolism in bacteria.**
- **Attenuation occurs in at least six operons that encode enzymes concerned with amino acid biosynthesis.**
- **The *his* operon has no repressor-operator regulation, and attenuation forms the only mechanism of feedback control.**

3.3.6 Conditions for attenuation

- A coupling of transcription and translation, where the latter affects the former.
  转录与翻译偶联
  (Attenuation would not work in eukaryotes.)
- Transcription and translation occurring at about the same rate.
  转录与翻译速度大致相同。

乳糖操纵子和色氨酸操纵子的调控比较



Regulation of the *lac* Operon:

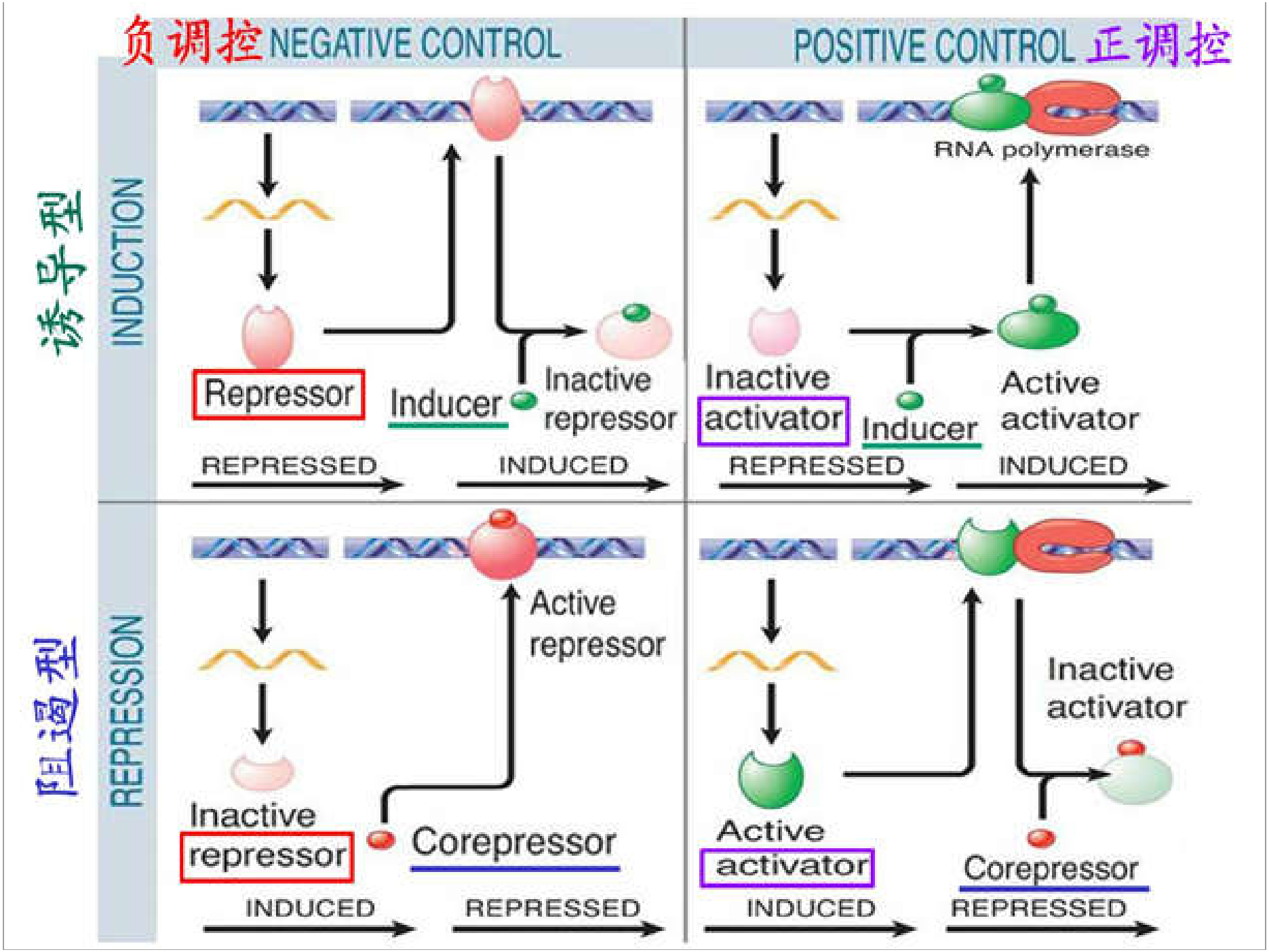
- lac repressor 负调控
- CRP 正调控

诱导型操纵子
（分解代谢）

Regulation of the *Trp* operon:

- Trp repressor 负调控
- Attenuator 负调控

阻遏型操纵子
（合成代谢）


负调控 NEGATIVE CONTROL
POSITIVE CONTROL 正调控
诱导型
阻遏型
INDUCTION
REPRESSION
RNA polymerase
Repressor
Inducer
Inactive repressor
Inactive activator
Inducer
Active activator
REPRESSED
INDUCED
REPRESSED
INDUCED
Active repressor
Inactive repressor
Corepressor
Active activator
Inactive activator
Corepressor
INDUCED
REPRESSED
INDUCED
REPRESSED

4. Transcriptional regulation by alternative σ factors

4.1 σ factor is a bifunctional protein

- **Recognize specific promoter** sequence (-35) in DNA
- **Bind to core RNA Pol** for transcription initiation

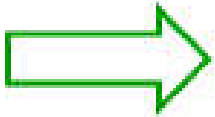
σ^{70} factors is the most common σ factor in *E. coli* under the normal growth condition.

Many bacteria produce alternative sets of σ factors to meet the regulation requirements of transcription under normal and extreme growth condition.

- *E. coli*: Heat shock
- Sporulation in *bacillus subtilis*
- bacteriophage σ factors

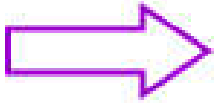
4.2 Heatshock (热休克)

From 37°C to 42°C



Transiently expression of the 17 heat shock proteins

Increase in temperature is more extremely (50°C)



Heat shock proteins are the only proteins made in *E. coli* to maintain its viability

- **HSPs are expressed through transcription by RNA polymerase using an alternative σ factor σ^{32} coded by *rpoH* gene. σ^{32} has its own specific promoter consensus sequences.**

Responsive Promoter	-35	-10
Standard σ^{70}	----TTGACA	----16-18--------TATAAT------
Heat shock σ^{32}	----TTGAA	-----13-15---CCCCATT---------

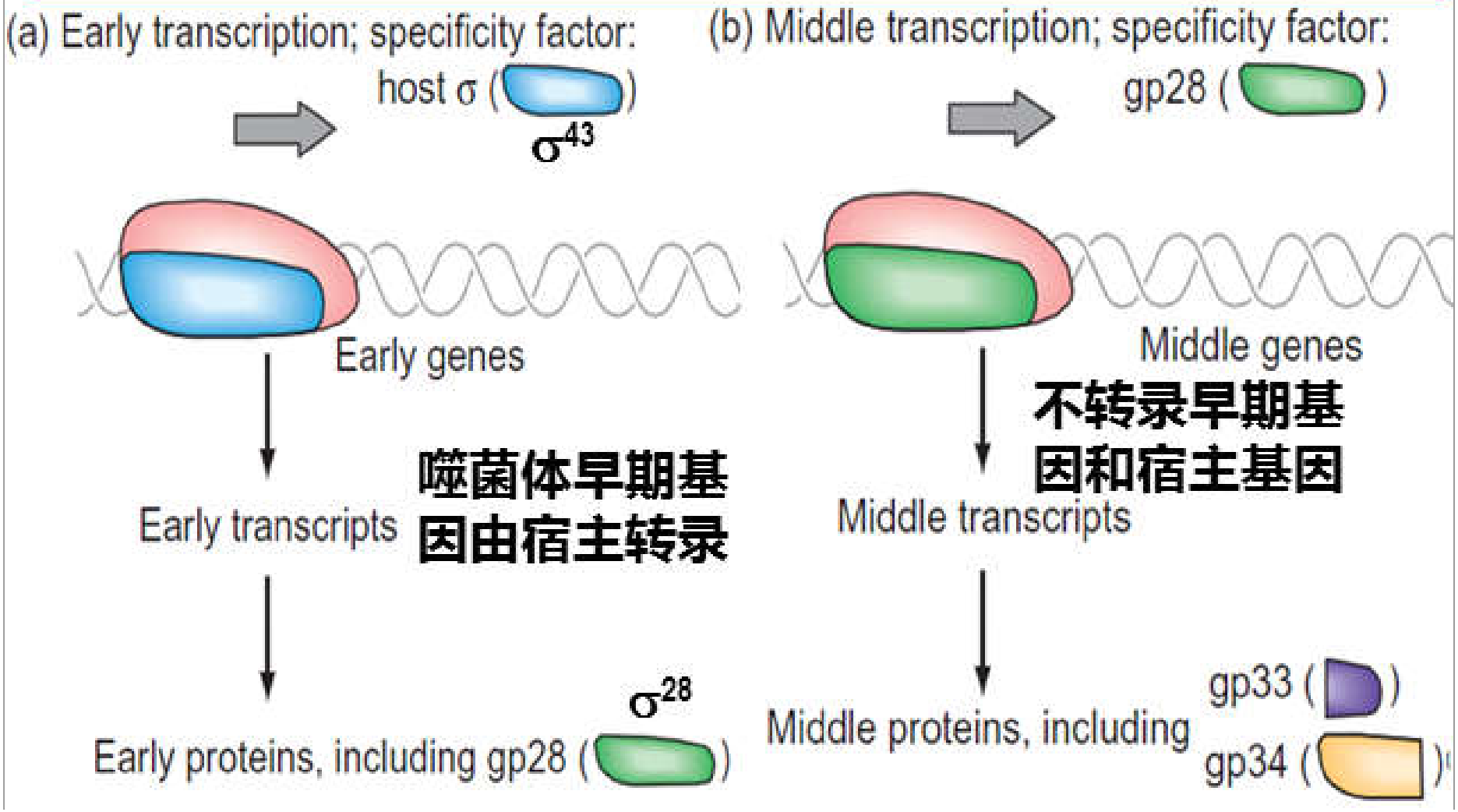
4.3 Sporulation (孢子形成)

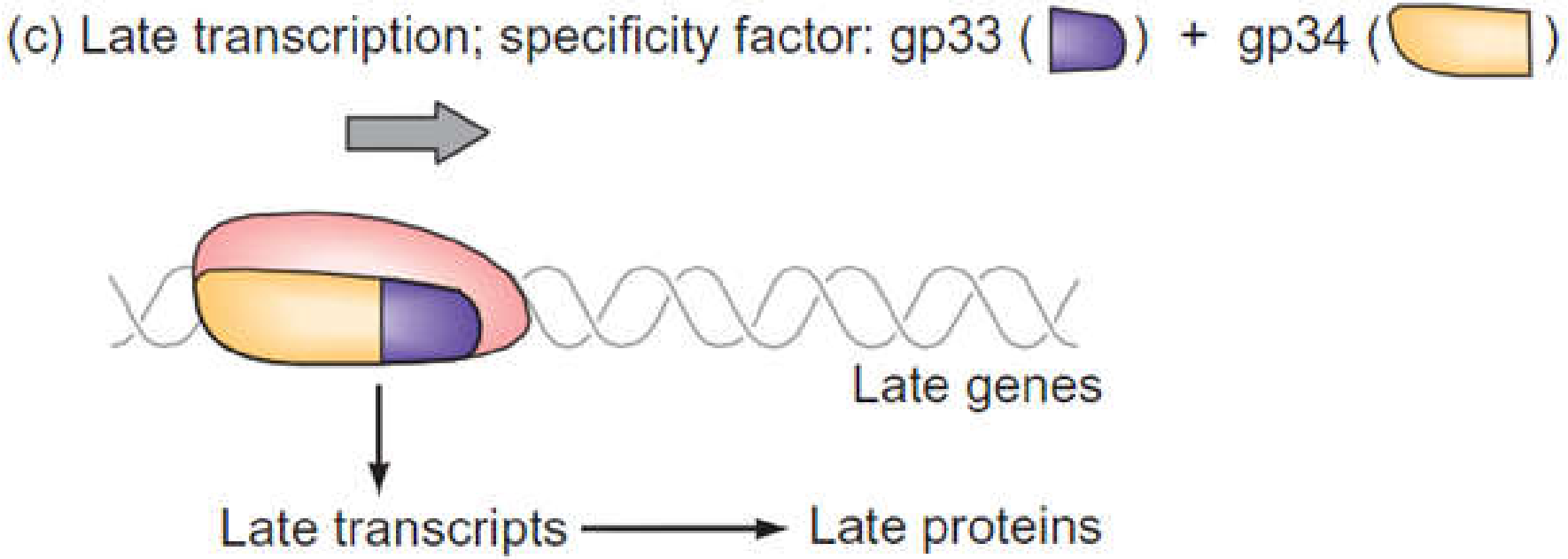
- Under non-optimal environmental conditions *Bacillus subtilis* (枯草芽孢杆菌) cells form spores (孢子).
- When *B. subtilis* sporulates, a whole new set of sporulation-specific genes is turned on, and many, but not all, vegetative (营养生长的) genes are turned off.

- Sporulation is accomplished by several new σ factors that displace the vegetative σ factor from the core RNA Pol.
- σ^F, σ^E, σ^H, σ^C, σ^K
- The sporulation-specific σ factors recognize quite different sequences.

4.4 Bacteriophage σ factor switching

Phage SPO1 in *B. subtilis* (枯草芽孢杆菌); Phage T4 in *E. coli*





Bacteriophage SPO1 expresses a 'cascade' (级联的) of σ factors in sequence to allow its own genes to be transcribed at specific stages during virus infection.

Summary

1. Definition, types and regulatory levels of gene expression
2. Concepts of cis-acting elements, tran-acting factors, operon, operator and regulator gene
3. Regulatory mechanisms of the *lac* operon and *trp* operon