Chapter 7

Gene Regulation in Prokaryotes

Outline

1. Principles of Transcriptional Regulation

2. Regulation of Transcription Initiation: Lac Operon

3. The Case of Phage λ: Layers of Regulation

The Nobel Prize in Physiology or Medicine 1965

"for their discoveries concerning genetic control of enzyme and virus synthesis"

François Jacob

1/3 of the prize

France

Institut Pasteur
Paris, France

b. 1920

André Lwoff

1/3 of the prize

France

Institut Pasteur
Paris, France

b. 1902
d. 1994

Jacques Monod

1/3 of the prize

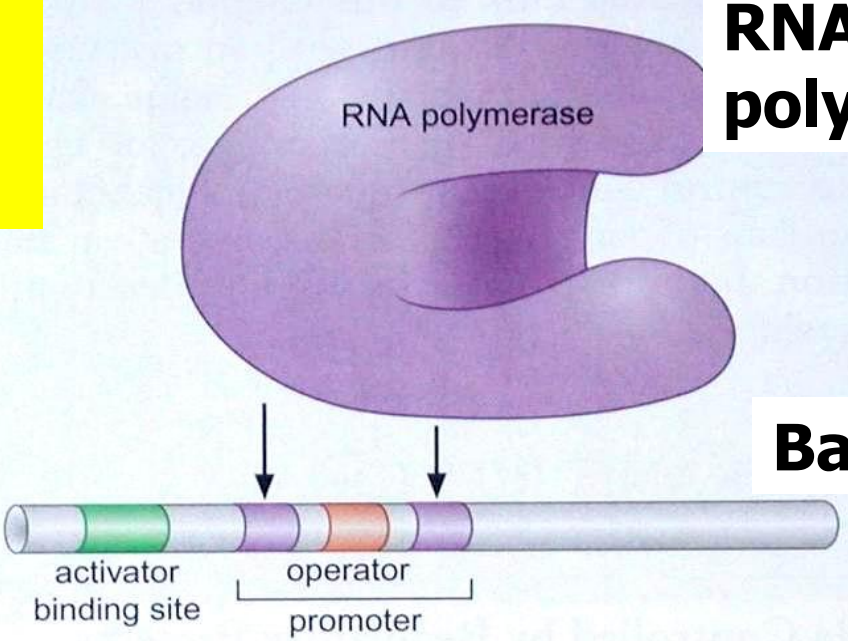
France

Institut Pasteur
Paris, France

b. 1910
d. 1976

Part 1: Principles of Transcription Regulation

Fig 7-1 Activation by recruitment of RNA polymerase



RNA polymerase

Basal level

a. Absence of Regulatory Proteins:

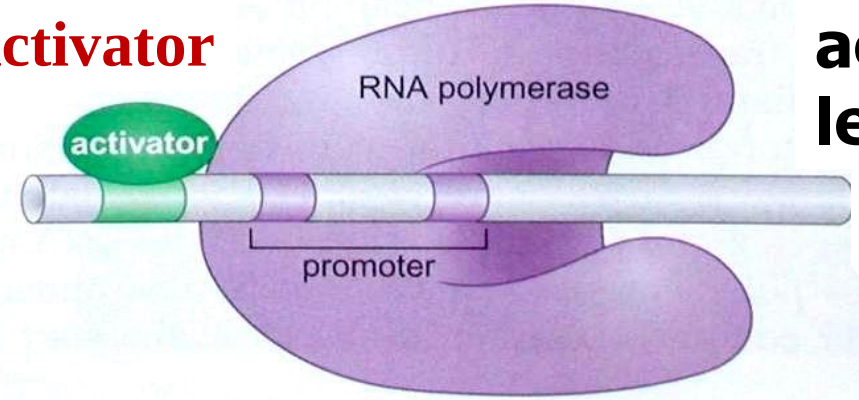
b. Repressor binding to the operator represses expression

Repressor

No transcription

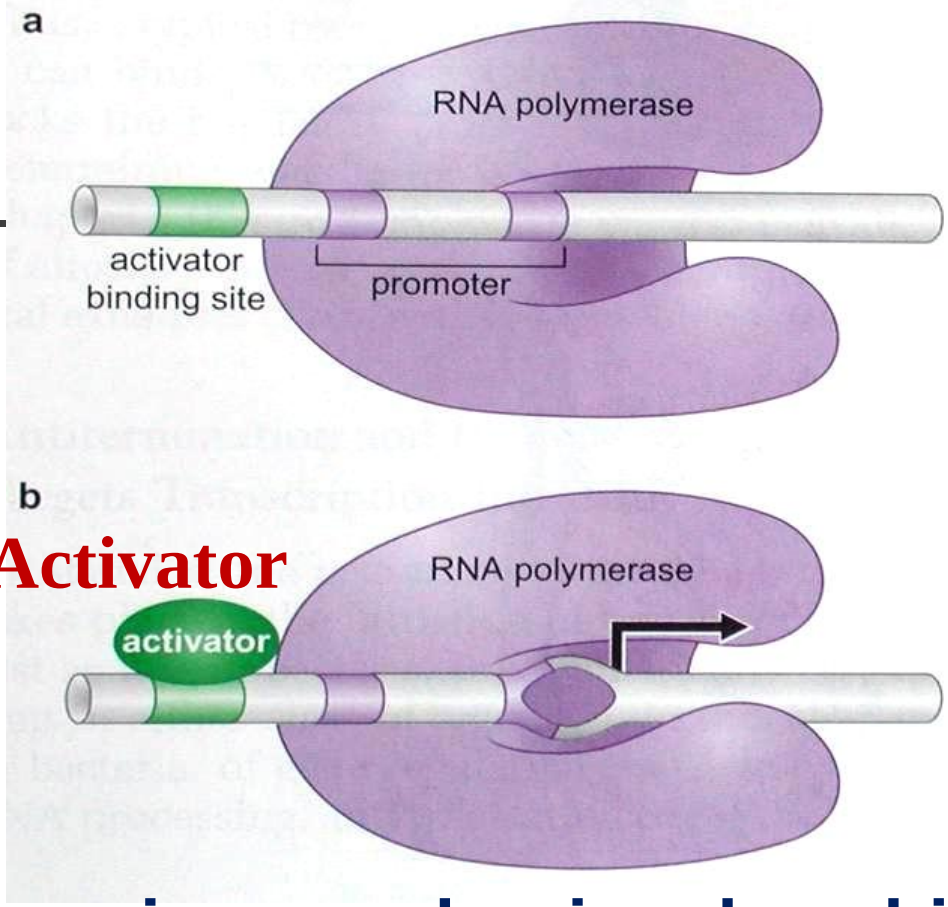
c. Activator binding activates expression

Activator



activated level

Fig 7-2 Allosteric activation of RNA polymerase



No transcription

activated level

Allostery is a mechanism by which activators interact with the stable closed complex and induce a conformational change that causes transition to the open complex.

Action at a Distance and DNA Looping

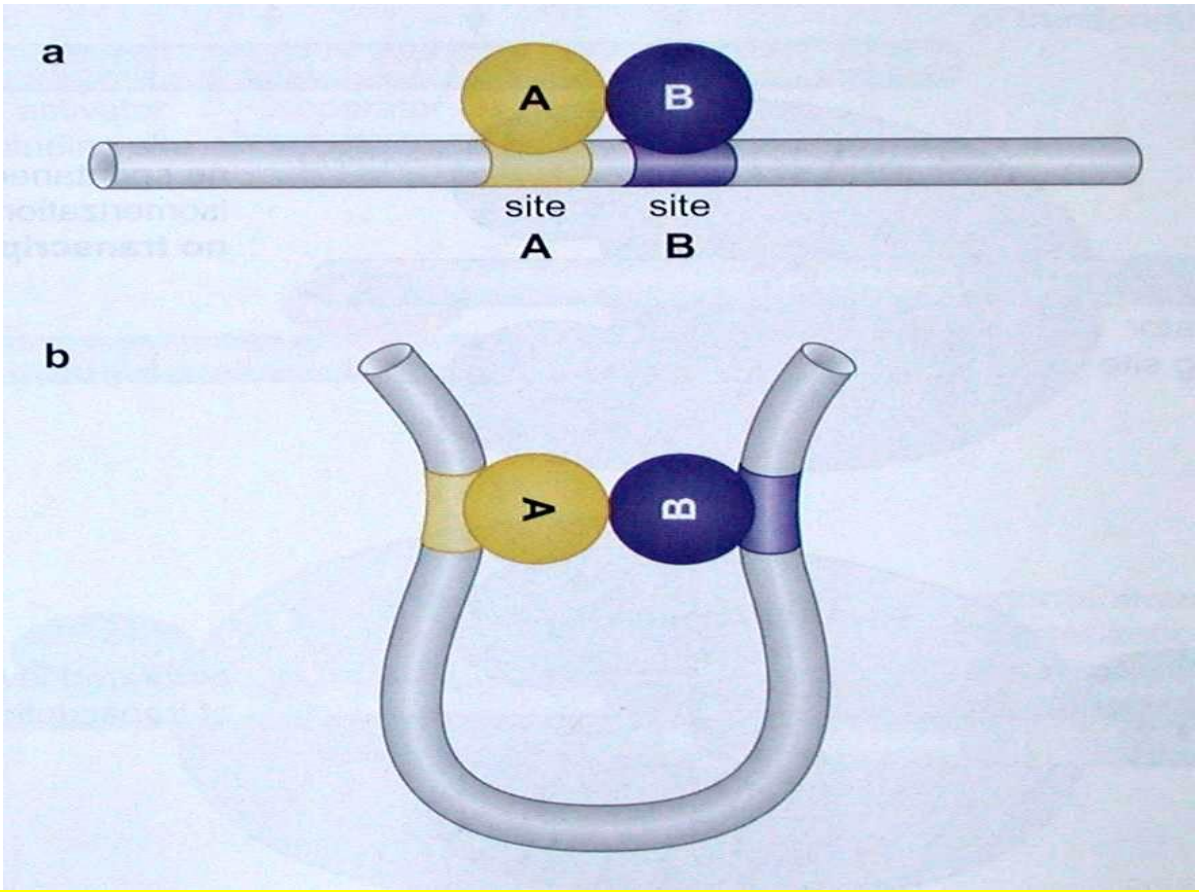
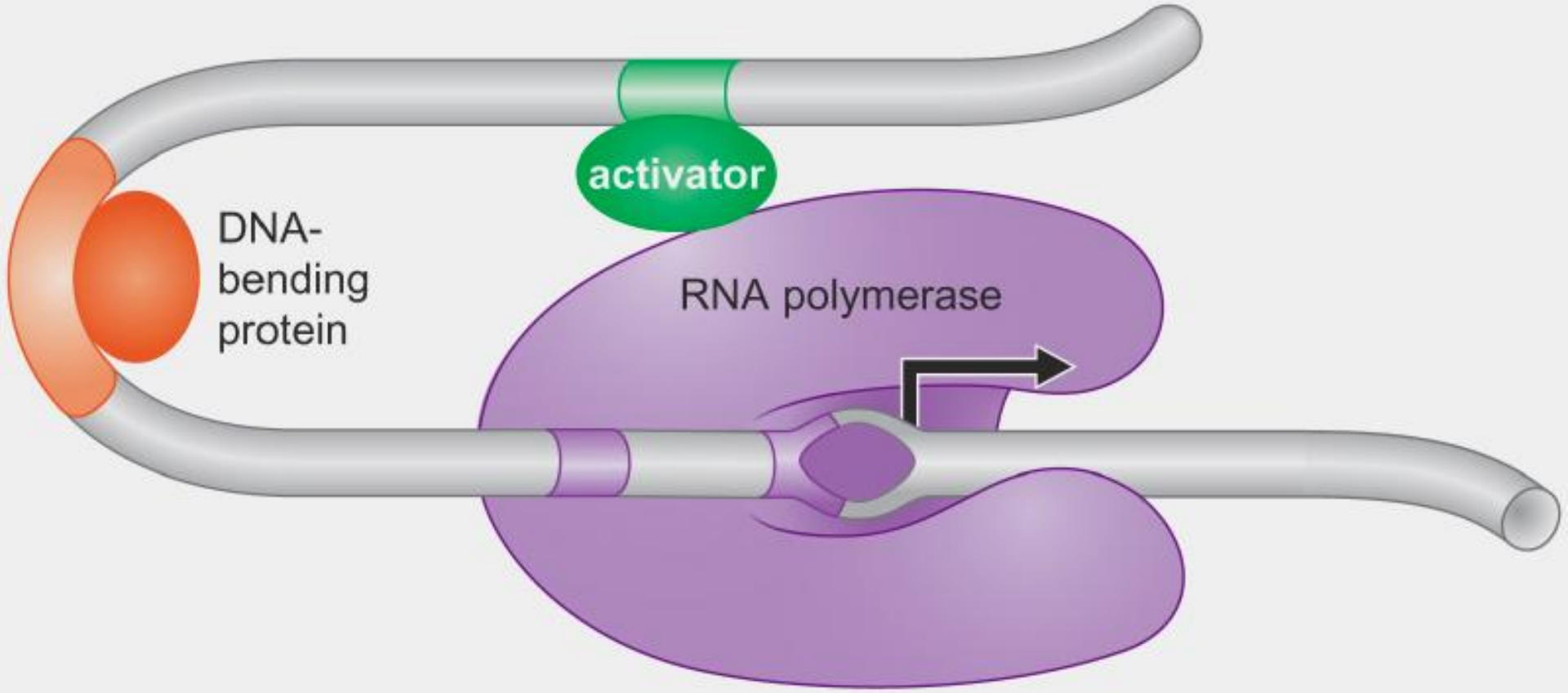


Fig 7-3 Interaction between proteins and DNA

Fig 7-4 DNA-bending protein can facilitate interaction between DNA-binding proteins at a distance



[Watch the animation-regulation of the transcription initiation!]

Apply your knowledge!

Summary

- In the absence of regulatory proteins, RNA is often expressed at a low, basal level
- An activator increases the level of transcription
- Activation can occur by recruitment or by allostery
- A repressor decreases the level of transcription
- The site on DNA where a repressor binds is called an operator

Part 2: Regulation of Transcription Initiation : Lac Operon

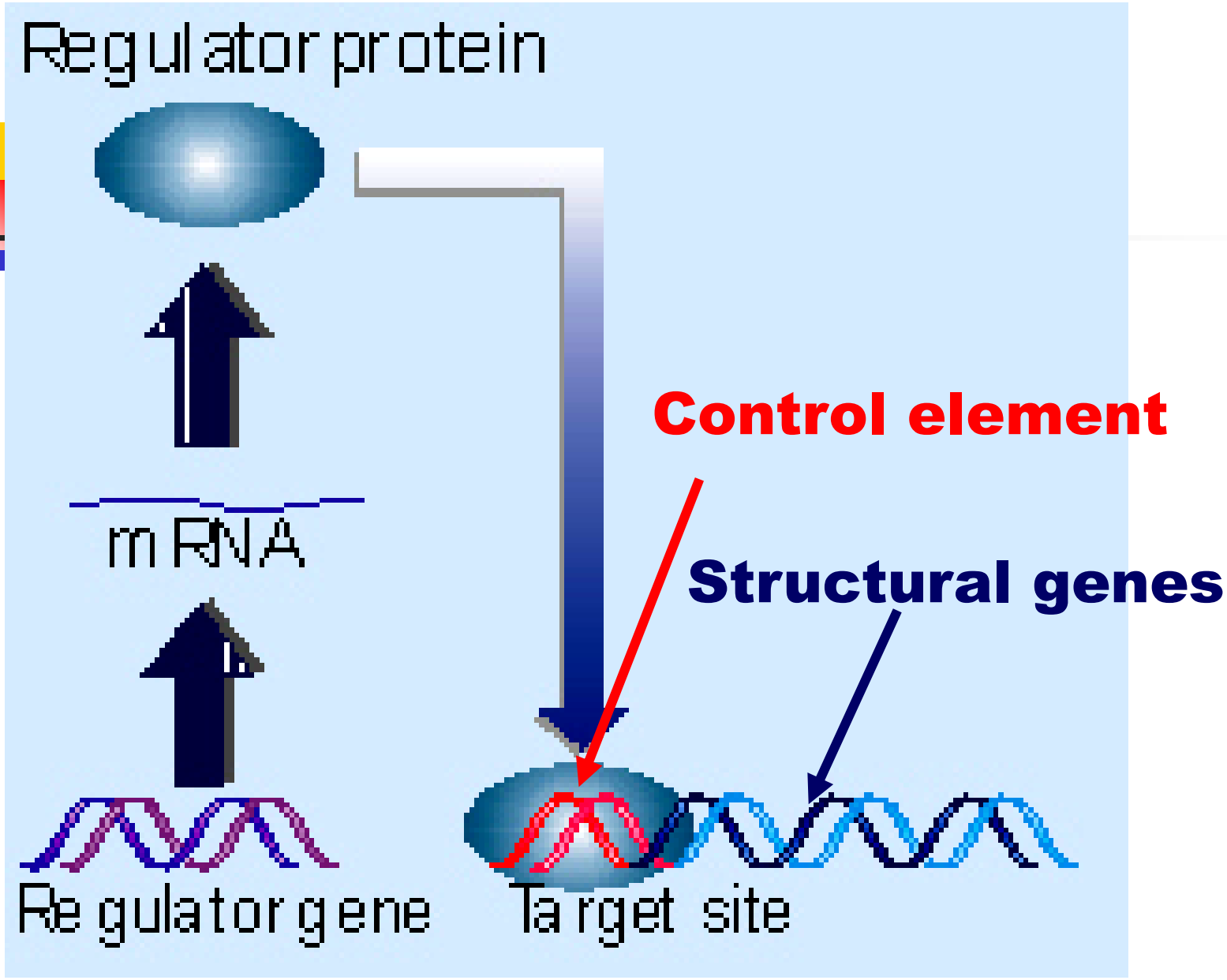


Fig 7-5 Operon

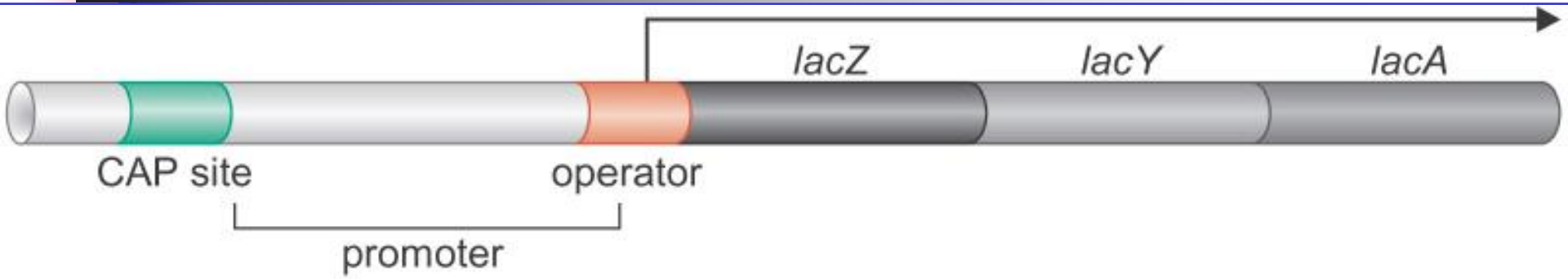
Operon: a unit of prokaryotic gene expression and regulation which typically includes:

1. Structural genes for enzymes in a specific biosynthetic and metabolic pathway whose expression is coordinately controlled.

2. Control elements, such as operator sequence.

3. Regulator gene(s) whose products recognize the control elements. These genes are usually transcribed from a different promoter.

Fig 7-6 Lactose operon



The enzymes encoded by *lacZ, lacY, lacA* are transcribed at a high level only when lactose is available as the sole carbon source.

The *lacZ, lacY, lacA* genes are transcribed into a single *lacZYA* mRNA (polycistronic mRNA) under the control of a single promoter P_{lac}.

lacZ codes for β-galactosidase (半乳糖苷酶) for lactose hydrolysis

lacY encodes a cell membrane protein called lactose permease to transport lactose across the cell wall

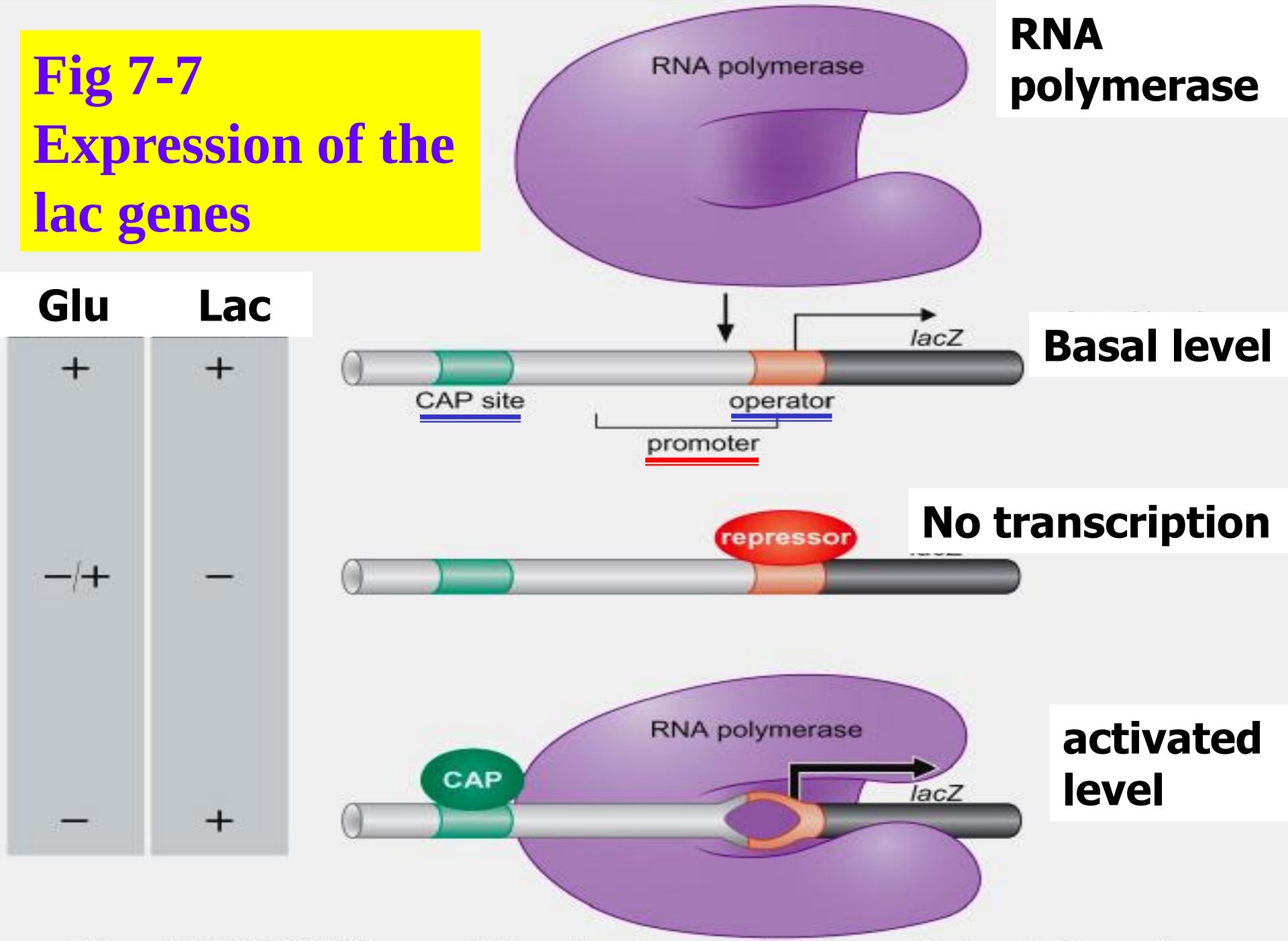
lacA encodes a thiogalactoside transacetylase to get rid of the toxic thiogalacosides

An activator and a repressor together control the *Lac* operon expression

The activator: **CAP** (Catabolite Activator Protein) or **CRP** (cAMP Receptor Protein); responding to the **glucose** level.

The repressor: ***lac*** **repressor** that is encoded by *LacI* gene; responding to the **lactose**.

Fig 7-7 Expression of the lac genes



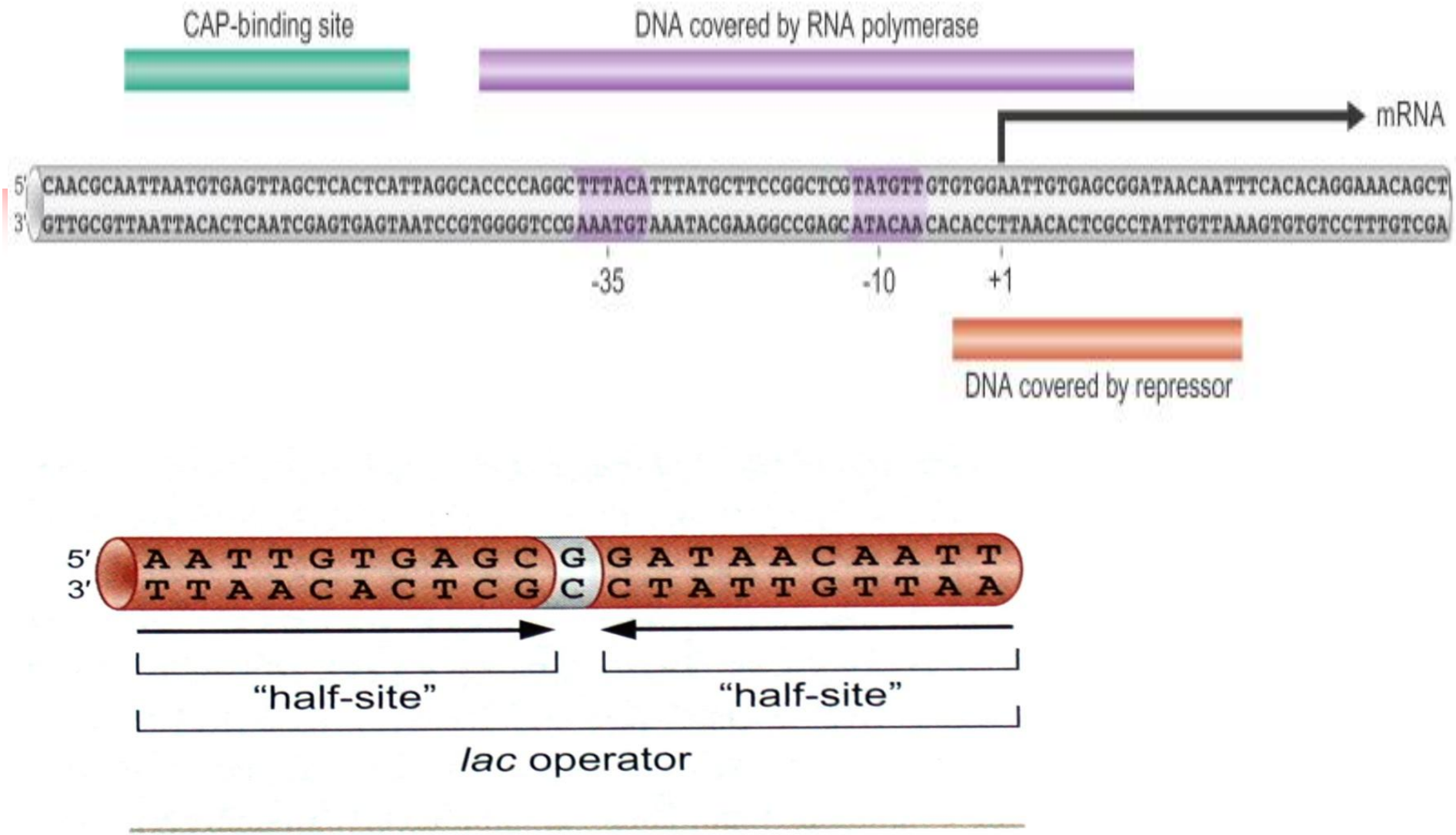
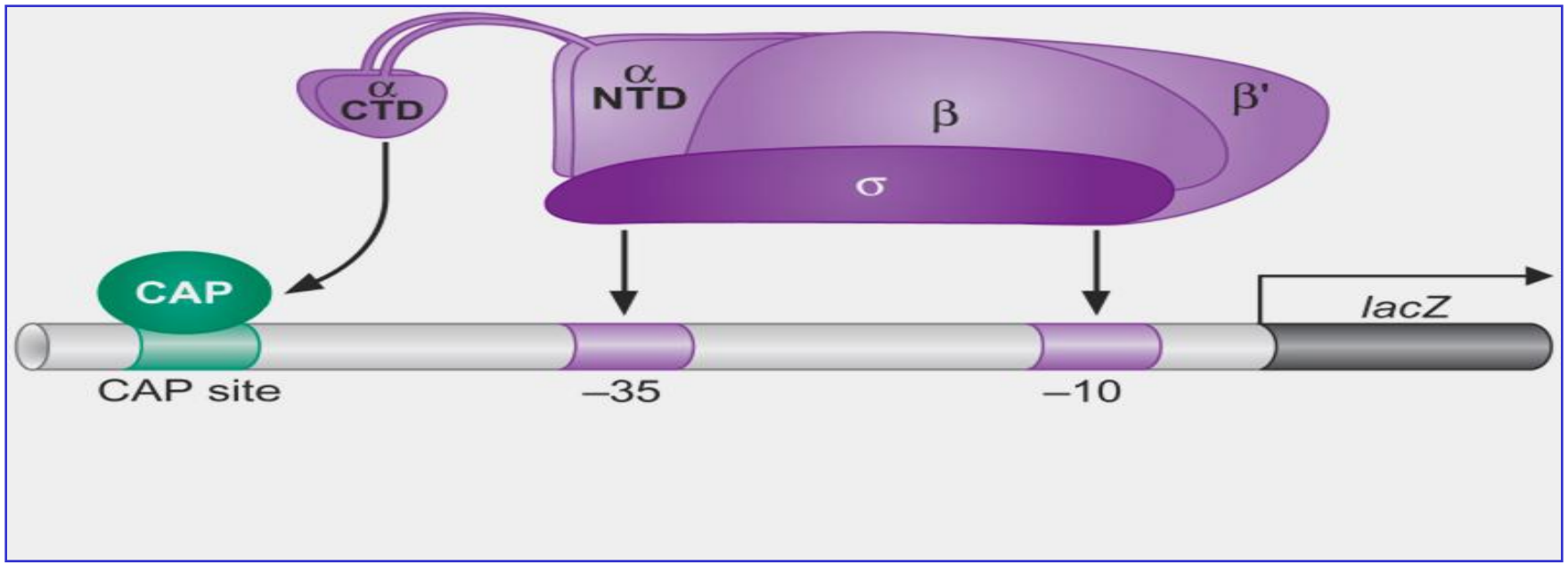


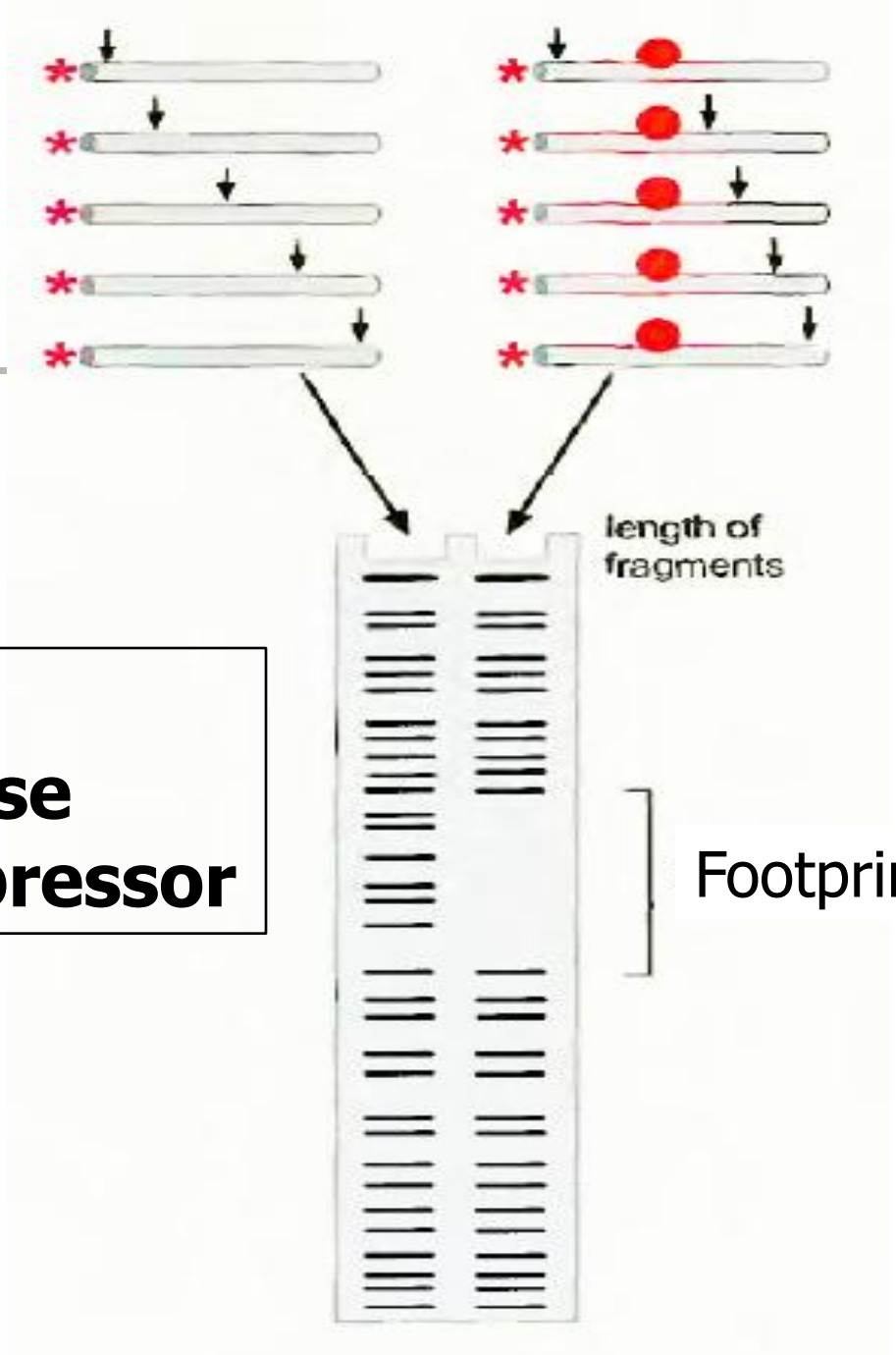
Fig 7-8 The control region of the lac operon

Fig 7-9 Activation of the lac promoter by CAP



α CTD: C-terminal domain of the α subunit of RNAP

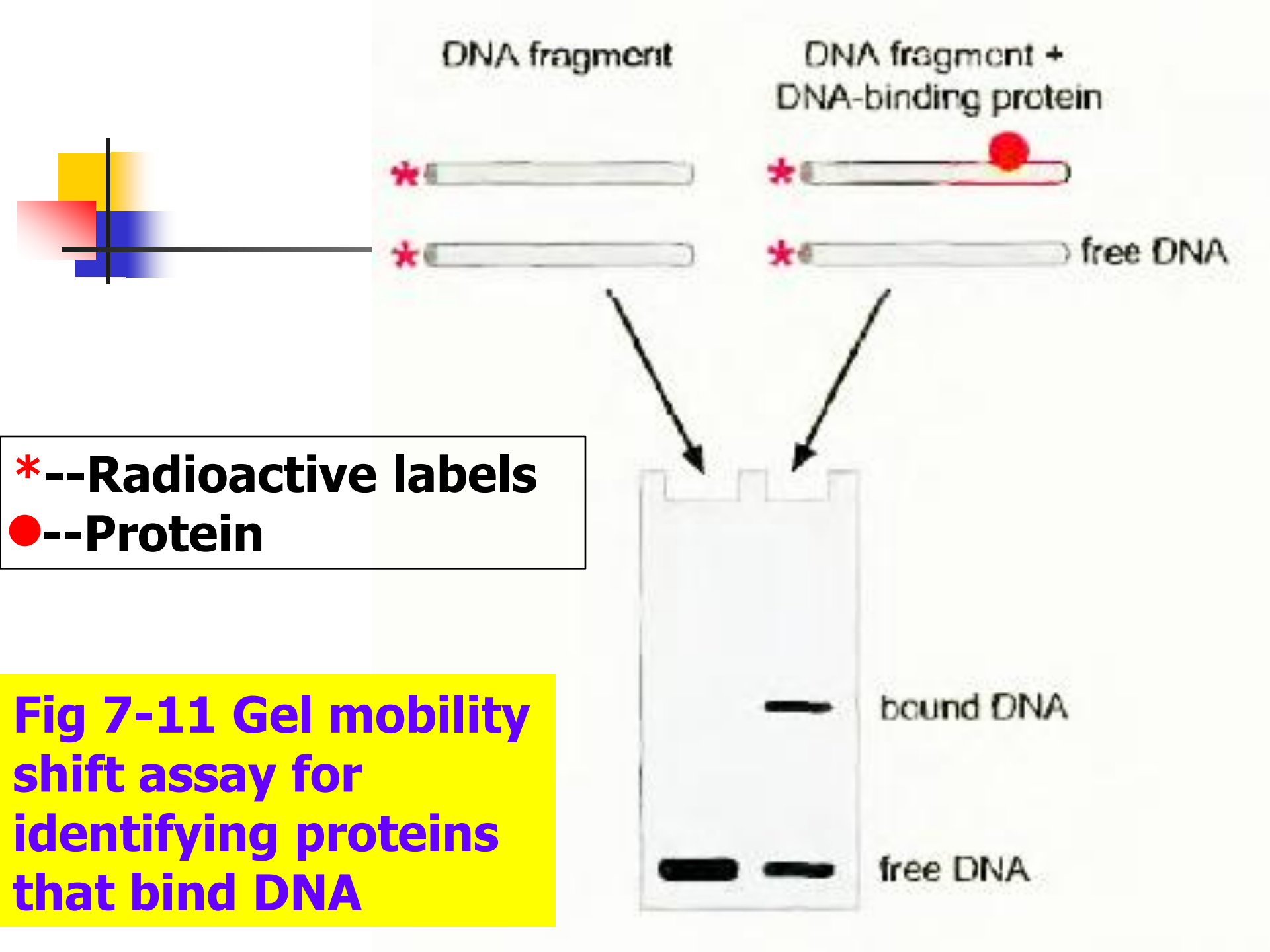
Fig 7-10 Footprinting method for detecting protein-binding site in DNA



*--Radioactive labels

↓ --cutting sites by DNase

●--Protein, e.g., *Lac* repressor



*--Radioactive labels
●--Protein

Fig 7-11 Gel mobility shift assay for identifying proteins that bind DNA

CAP and *Lac* repressor bind DNA using a common structural motif: helix-turn-helix motif

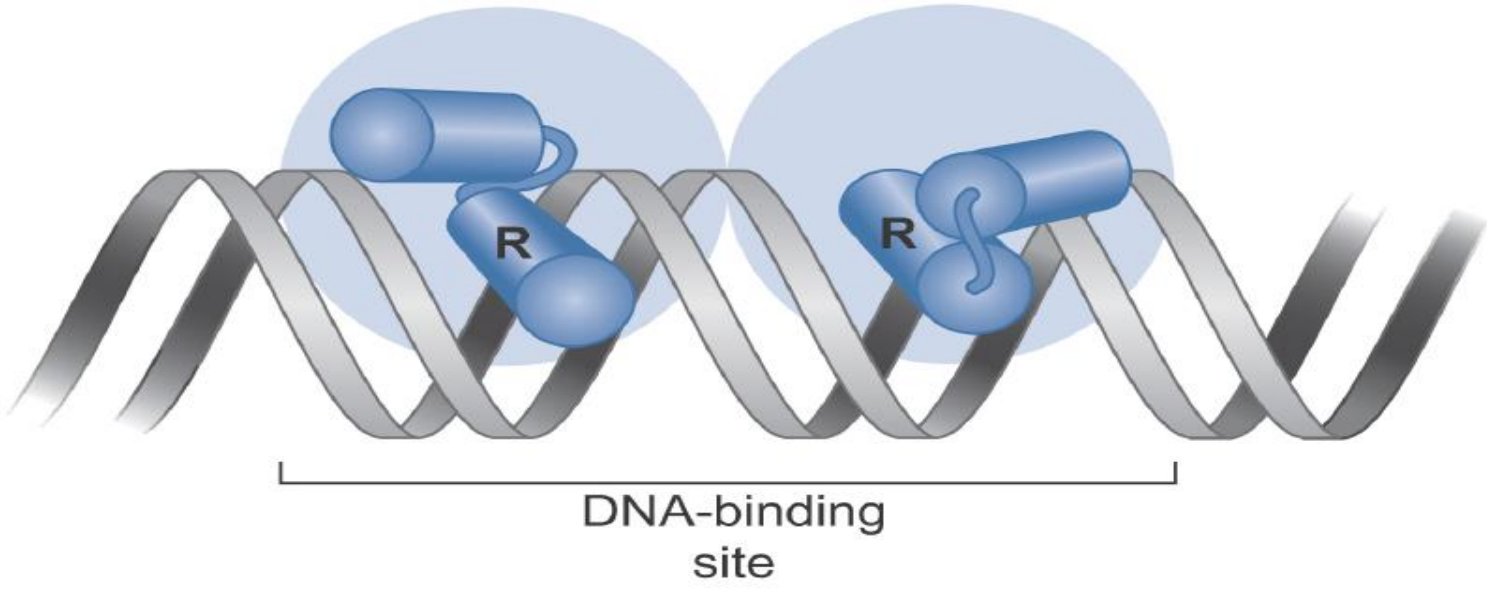


Fig 7-12 Binding of a protein with a helix-turn-helix domain to DNA

***Lac* operon contains three operators: the primary operator and two other operators located 400 bp downstream and 90 bp upstream, respectively.**

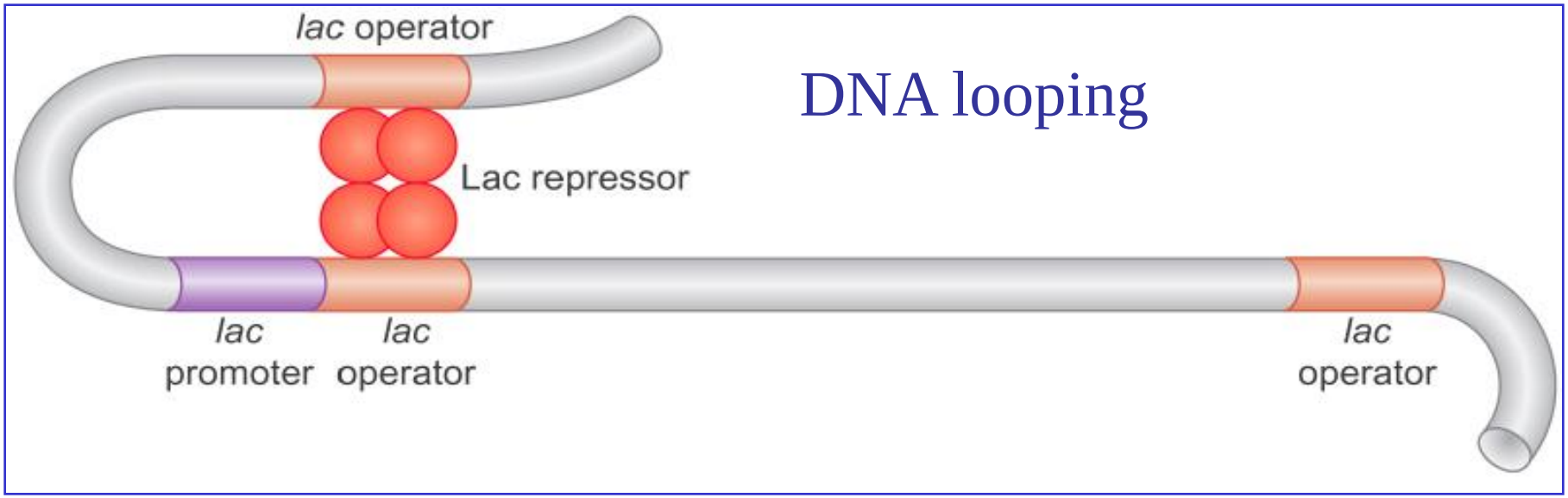


Fig 7-13 Lac repressor binds as a tetramer to two operators

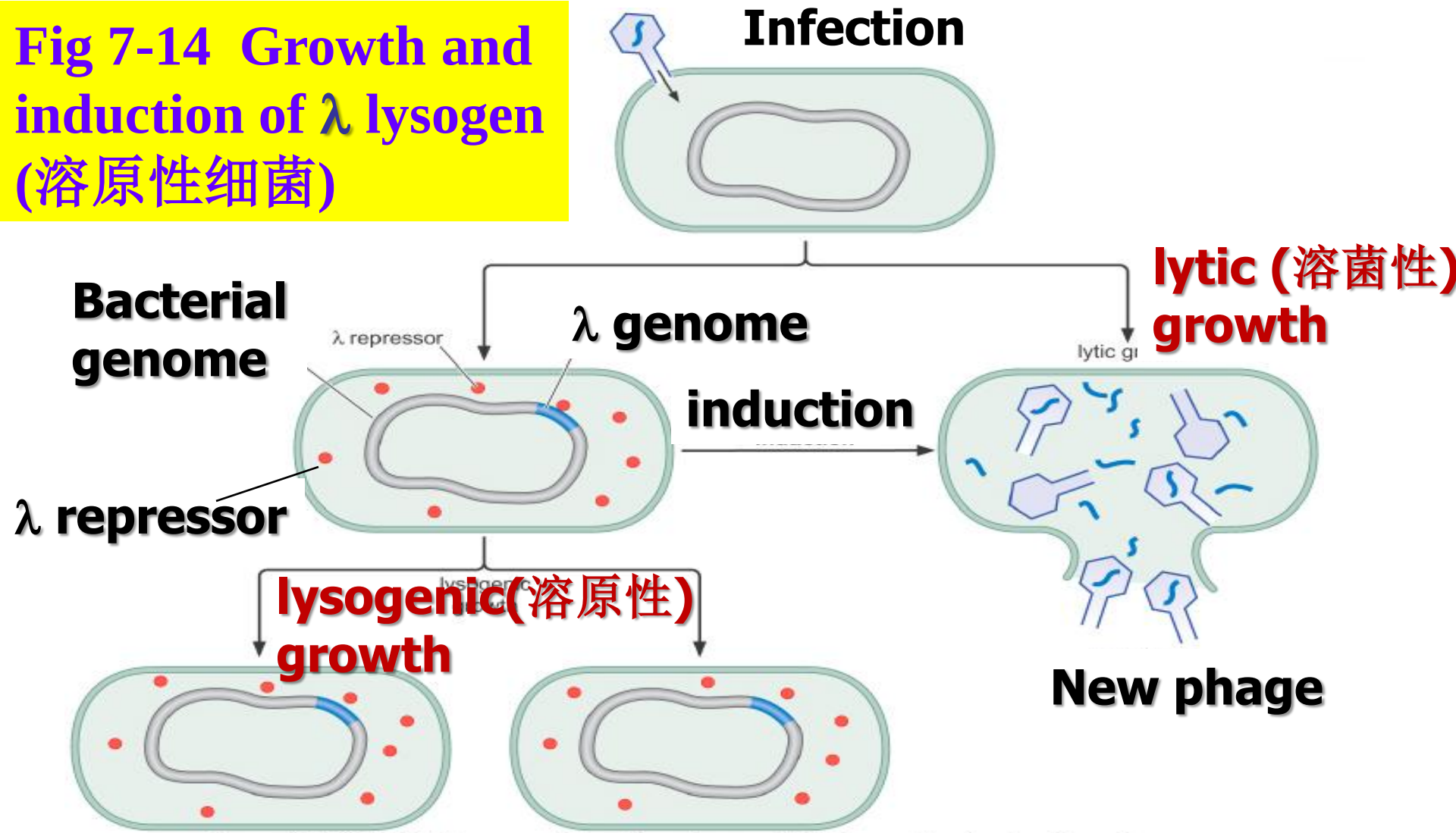
Combinatorial Control : CAP controls other genes as well.

- **A regulator (CAP) works together with different repressors at different genes, this is an example of Combinatorial Control.**
- **In fact, CAP acts at more than 100 genes in *E.coli*, working with an array of partners.**

Part 3: The Case of Bacteriophage λ: Layers of Regulation

Bacteriophage λ is a virus that infects *E. coli*. Upon infection, the phage can propagate in either of two ways : lytically or lysogenically.

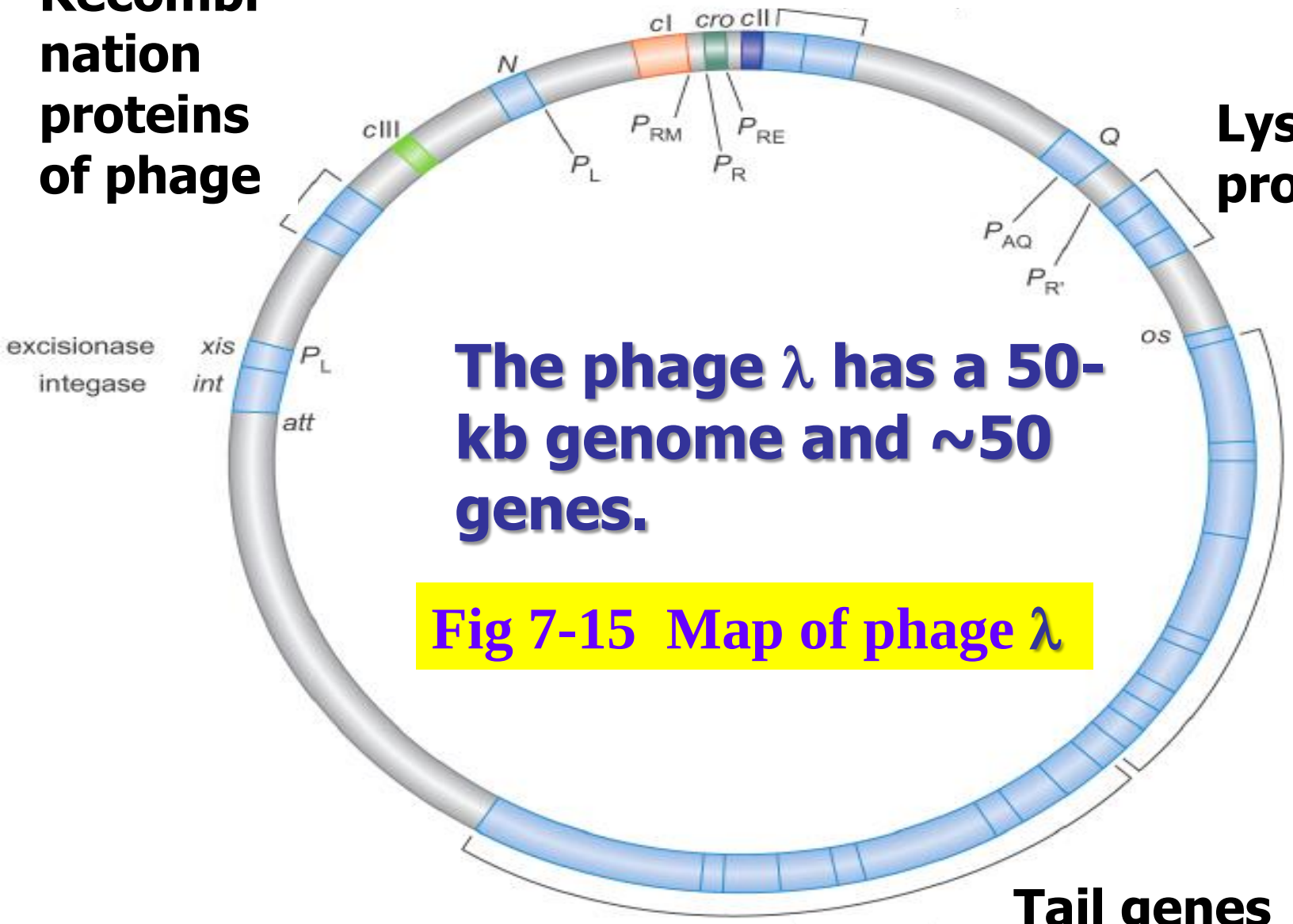
Fig 7-14 Growth and induction of λ lysogen (溶原性细菌)



Phage DNA replication proteins

Recombi-nation proteins of phage

Lysis proteins



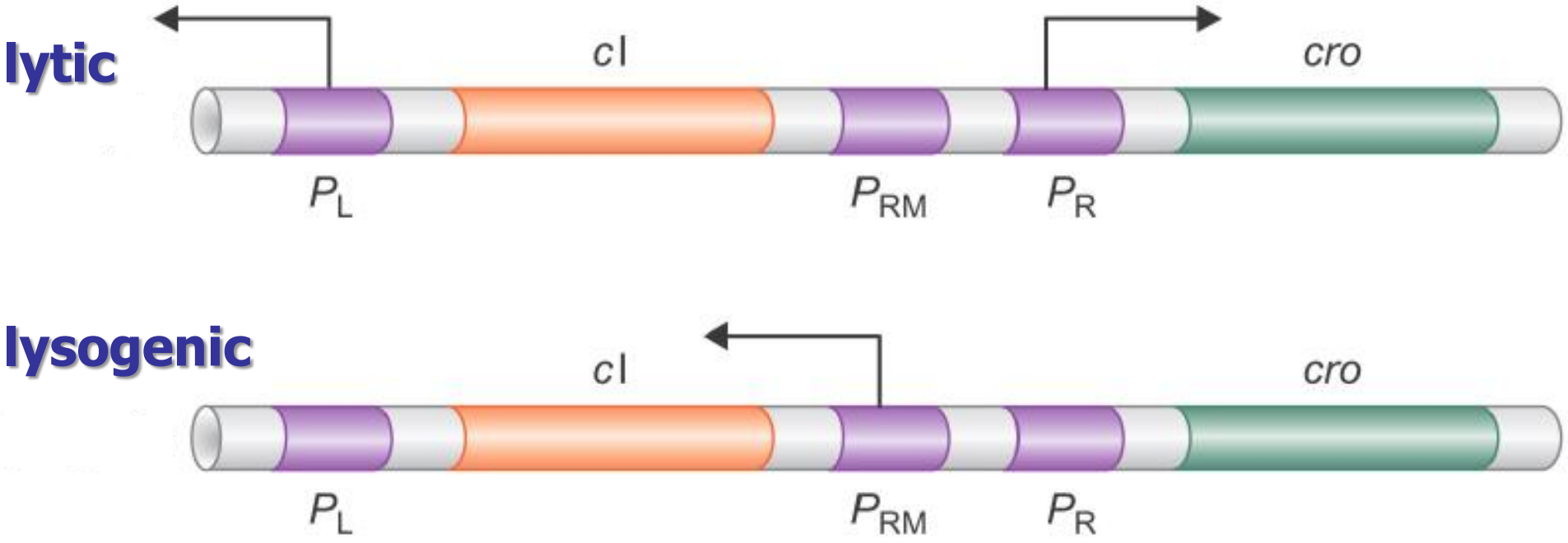
The phage λ has a 50-kb genome and ~50 genes.

Fig 7-15 Map of phage λ

Head genes

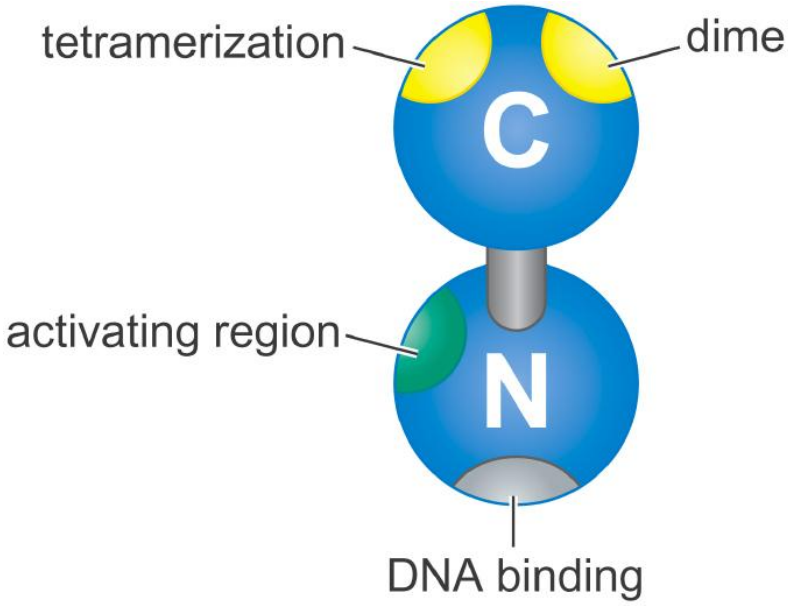
Tail genes

Fig. 7-16: Transcription in the λ control regions in lytic and lysogenic growth



P_R and P_L : rightward and leftward promoter, respectively
P_{RM}: Promoter for repressor maintenance

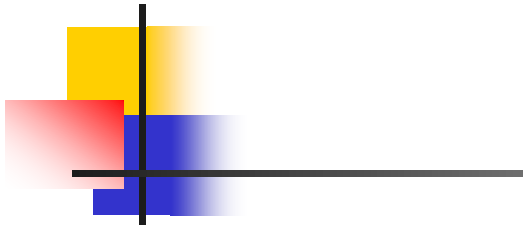
The cI gene encodes λ repressor, which can both activate and repress transcription



As a repressor, it binds to sites that overlap the promoter and excludes RNA polymerase

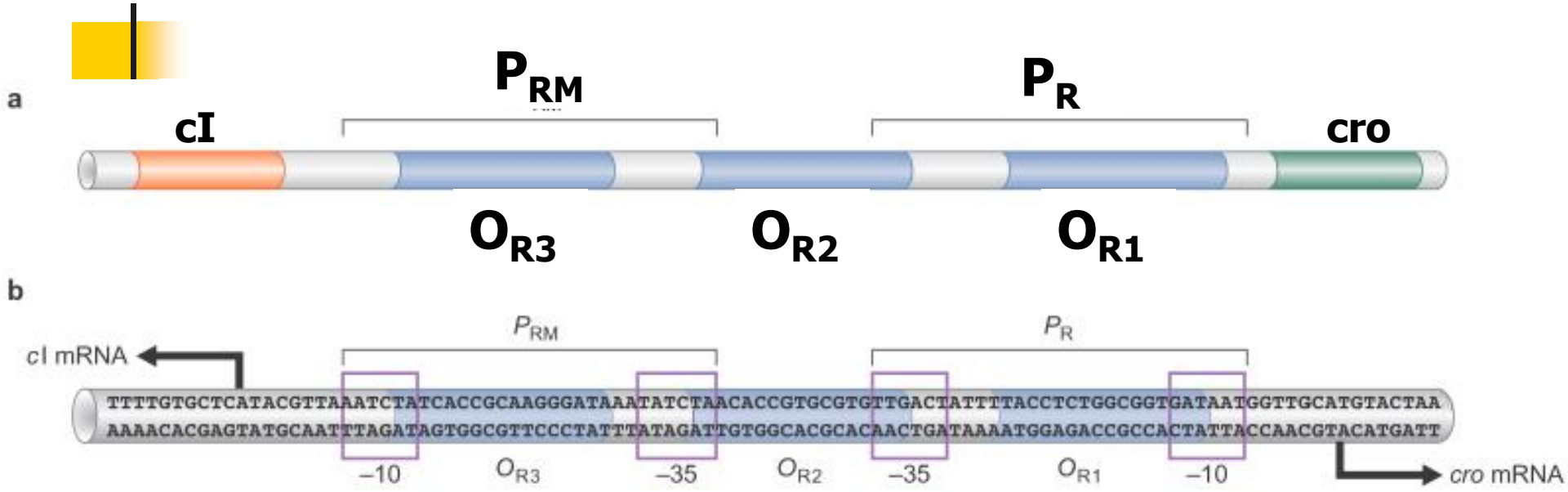
As an activator, it works like CAP by recruitment.

Fig. 7-17 λ repressor



Cro (for control of repressor and other things) only represses transcription. It is a single domain protein that binds as a dimer to 17-bp DNA sequences.

Fig. 18 Relative positions of promoter and operator sites in O_R

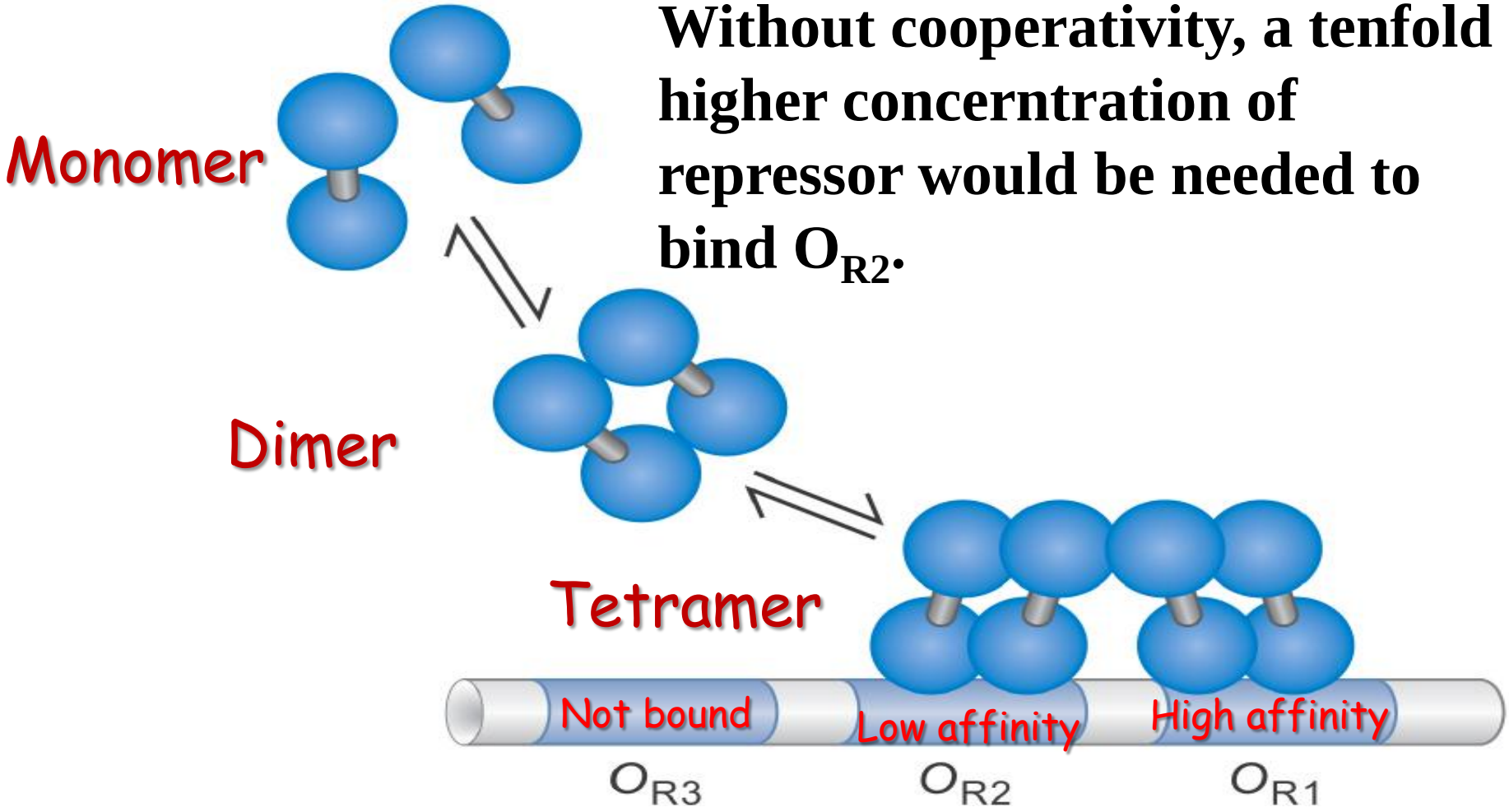


There are 6 operators in the right (3) and left (3) control regions of bacteriophage λ.

λ repressor and Cro can each bind to any one of six operators, but with dramatically different affinity.

λ repressor binds O_{R1} most easily while Cro binds O_{R3} with the highest affinity.

Fig. 7-19 Layer (1): Cooperative binding of λ repressor to DNA



Without cooperativity, a tenfold higher concerntration of repressor would be needed to bind O_{R2}.

Fig. 7-20 Cooperative binding reaction

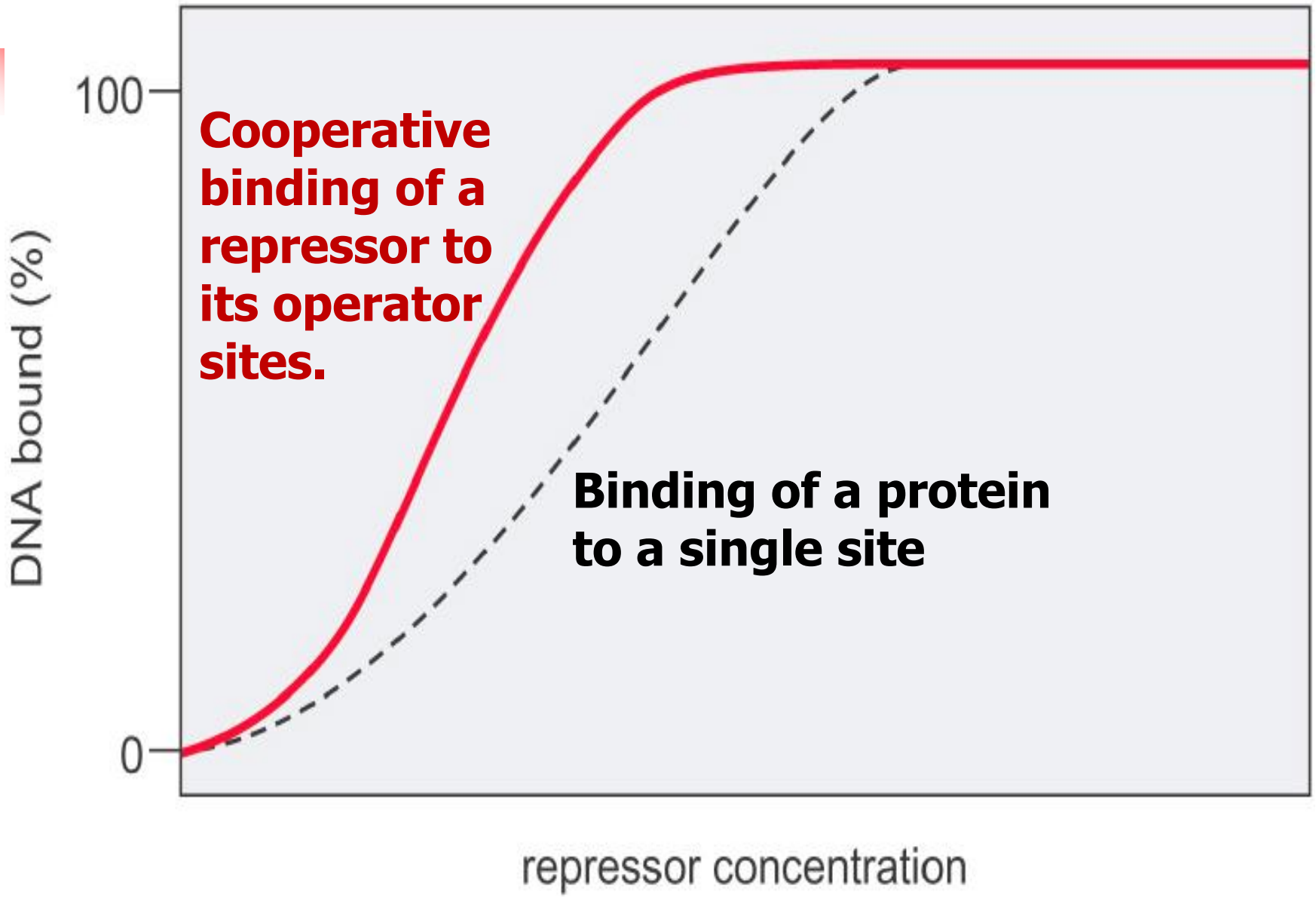
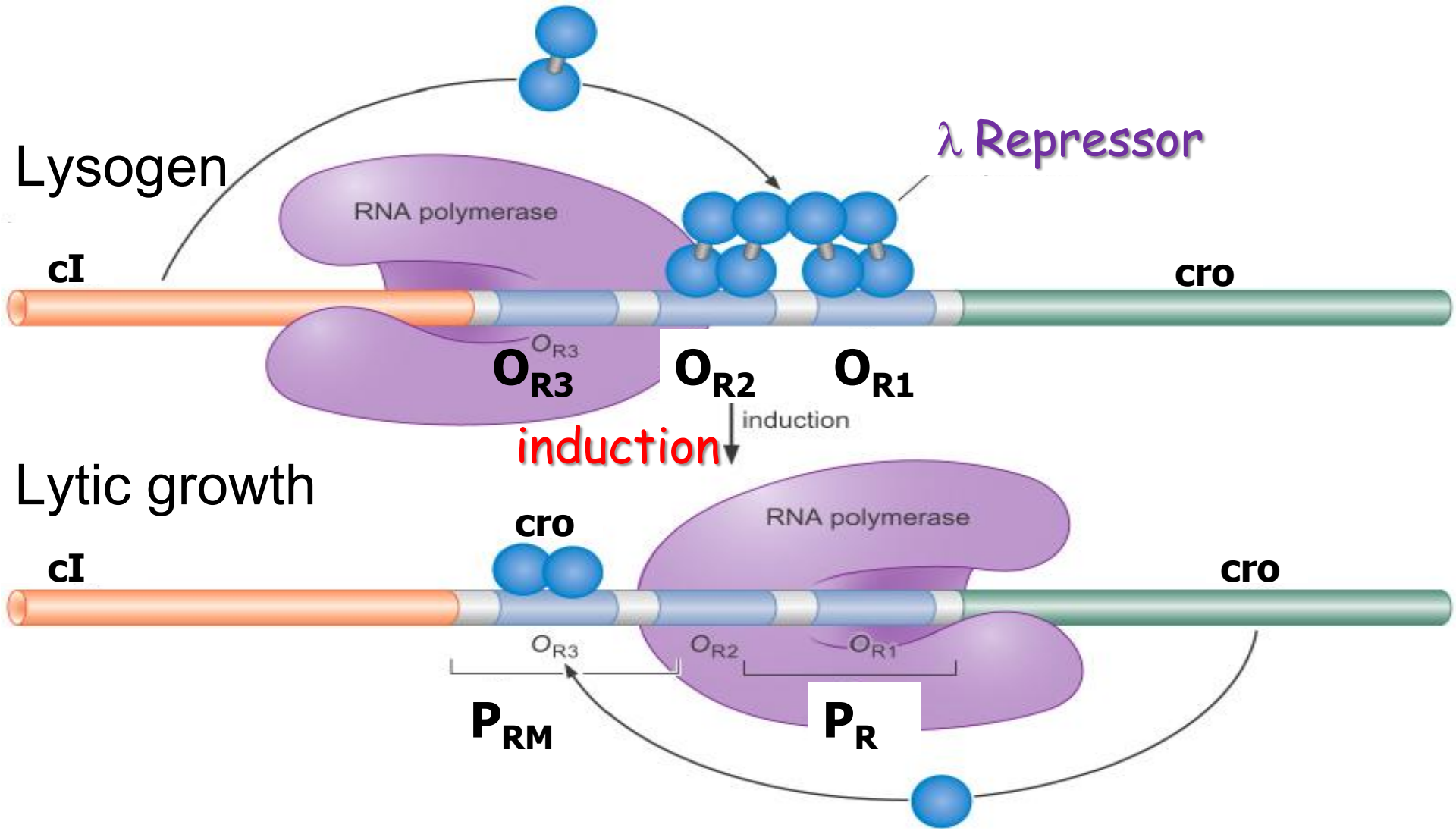
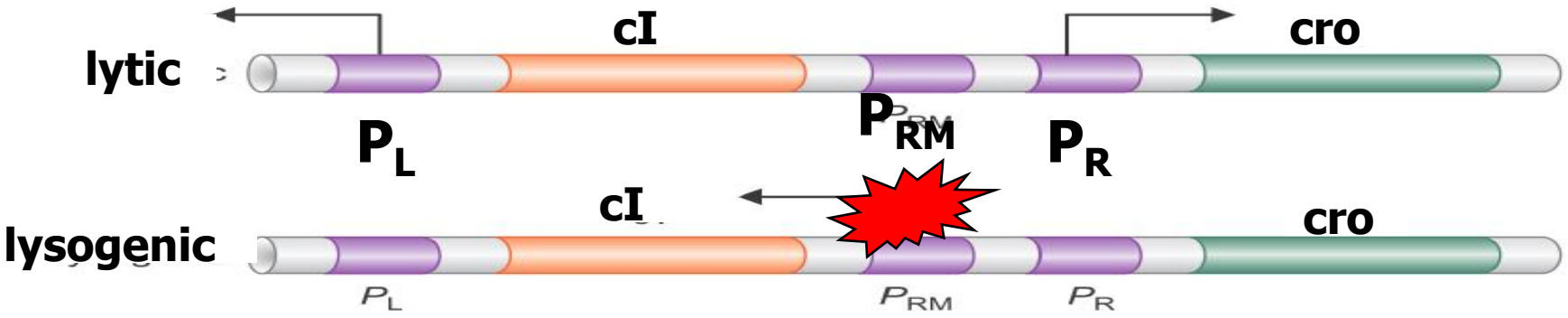


Fig. 7-21 Layer (2): λ Repressor and Cro bind in different patterns to control lytic and lysogenic growth



1. DNA damage activates RecA in *E. coli*
2. RecA stimulates λ repressor to undergo autocleavage, resulting in the removal the C-terminal domain and the immediate loss of dimerization and binding cooperativity.
3. Repressor dissociates from O_{R1}-O_{R2} & O_{R1}-O_{R2}. Loss of repression triggers transcription from P_R and P_L, leading to lytic growth.

Fig. 7-22 Layer (3): Lytic induction requires proteolytic cleavage of λ repressor

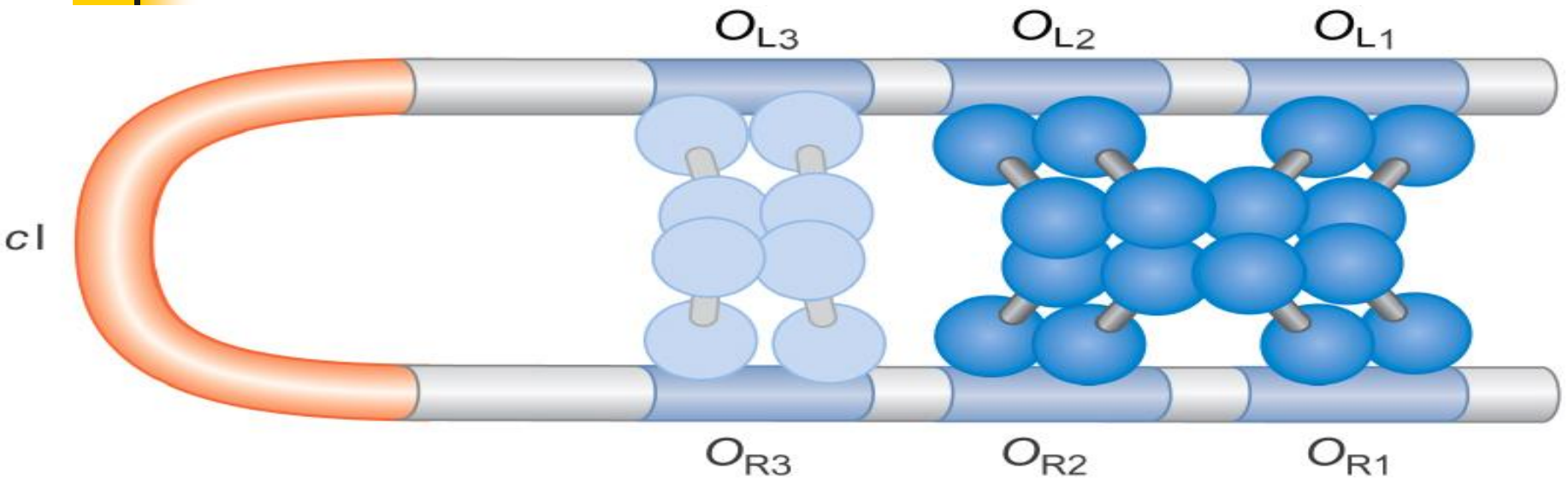


For induction to work efficiently, the level of λ repressor in a lysogen must be tightly regulated.

- **Layer (4):** Keep it not too **low** by positive autoregulation: λ repressor binding at O_{R2} activates its own transcription from P_{RM}.

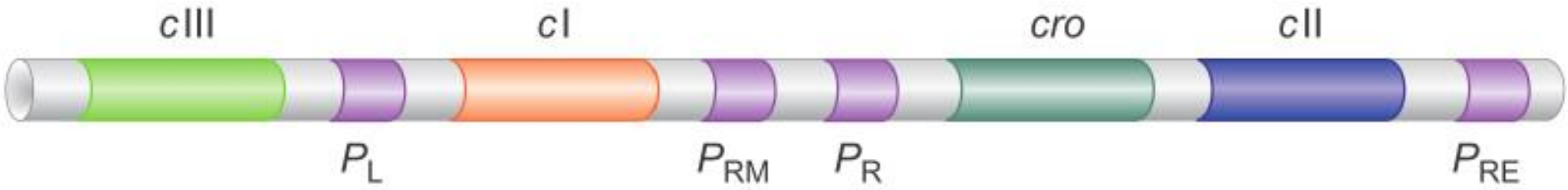
- **Layer (5):** Keep it not too **high** by negative autoregulation: when the repressor level goes too high, it will bind to O_{R3} as well, which will prevent transcription from P_{RM}.

Fig. 7-23 Interaction of λ repressors at O_R and O_L



Negative autoregulation of repressor requires long-distance interactions and a large DNA loop: cooperative binding at O_{R3} and O_{L3} to prevent the synthesis of new λ repressor.

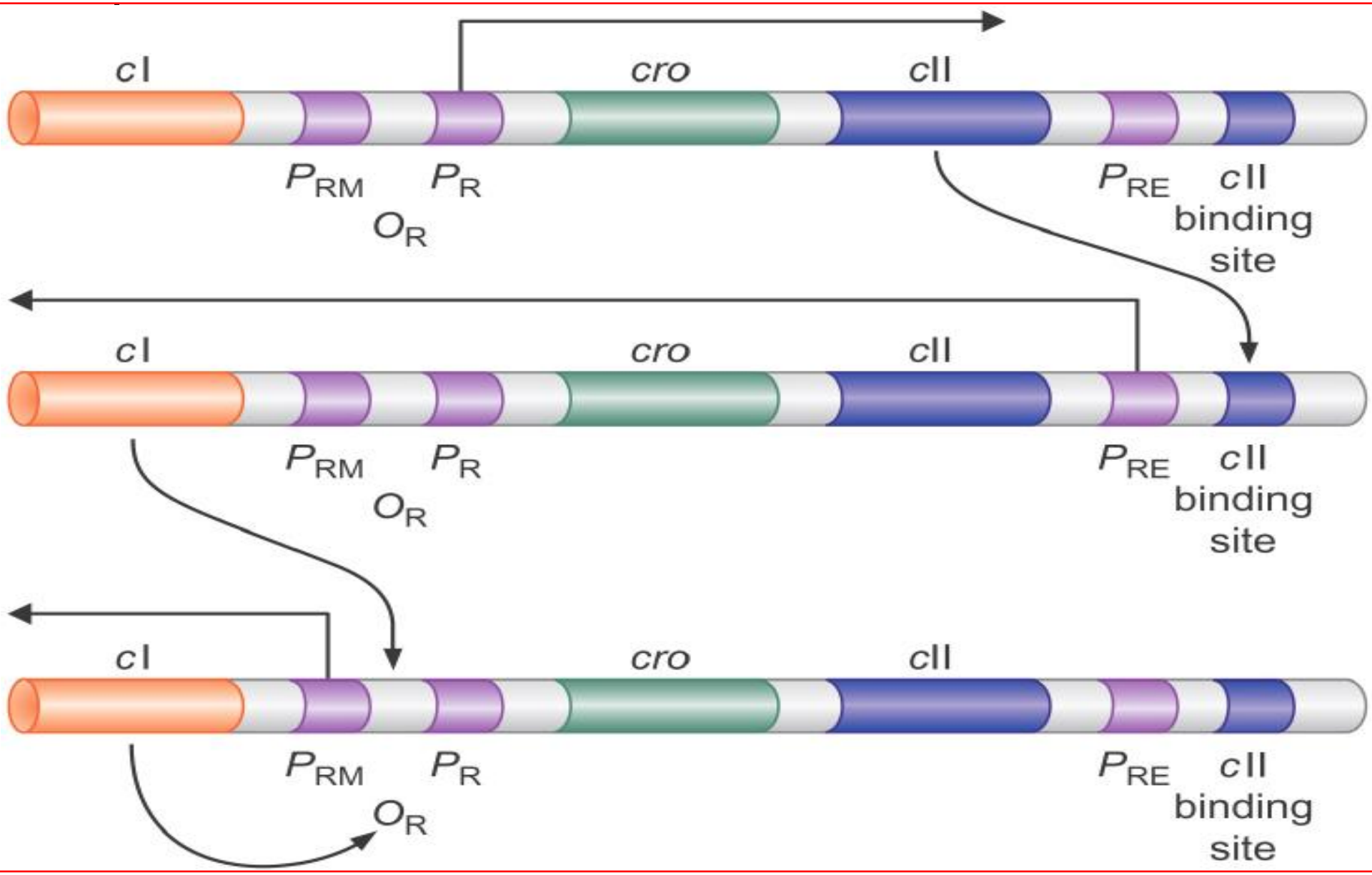
Fig. 7-24 Layer (6): Another activator, λ cII, controls the decision between lytic and lysogenic growth upon infection of a new host.



cII is transcribed from P_R and cIII is transcribed from P_L.

cII protein is a transcriptional activator that binds to P_{RE} and stimulates the transcription of cI gene (λ repressor).

Fig. 7-25 Establishment of lysogeny



Establishment of lysogeny

1. P_R and P_L are constitutive promoters that promote transcription once the phage enter the cells.
2. P_R directs the synthesis of both Cro and cII proteins. Cro favors lytic development while cII favors lysogenic growth by activating the synthesis of λ repressor.
3. The efficiency with which cII directs transcription of cI gene (λ repressor) is critical in deciding the lysogeny.

Growth conditions of *E. coli* control the stability of cII protein and thus the lytic/lysogenic choice.

--cII is degraded by a specific protease FtsH.

--Layer (7): If growth conditions is good, cII is unstable, repressor is not made, and the phage tend to grow lytically.

--Layer (8): When conditions are poor for bacterial growth , cII becomes stable, repressor accumulates, and lysogens form.

--cII are stabilized by cIII, which serves as an alternative substrate for FtsH.

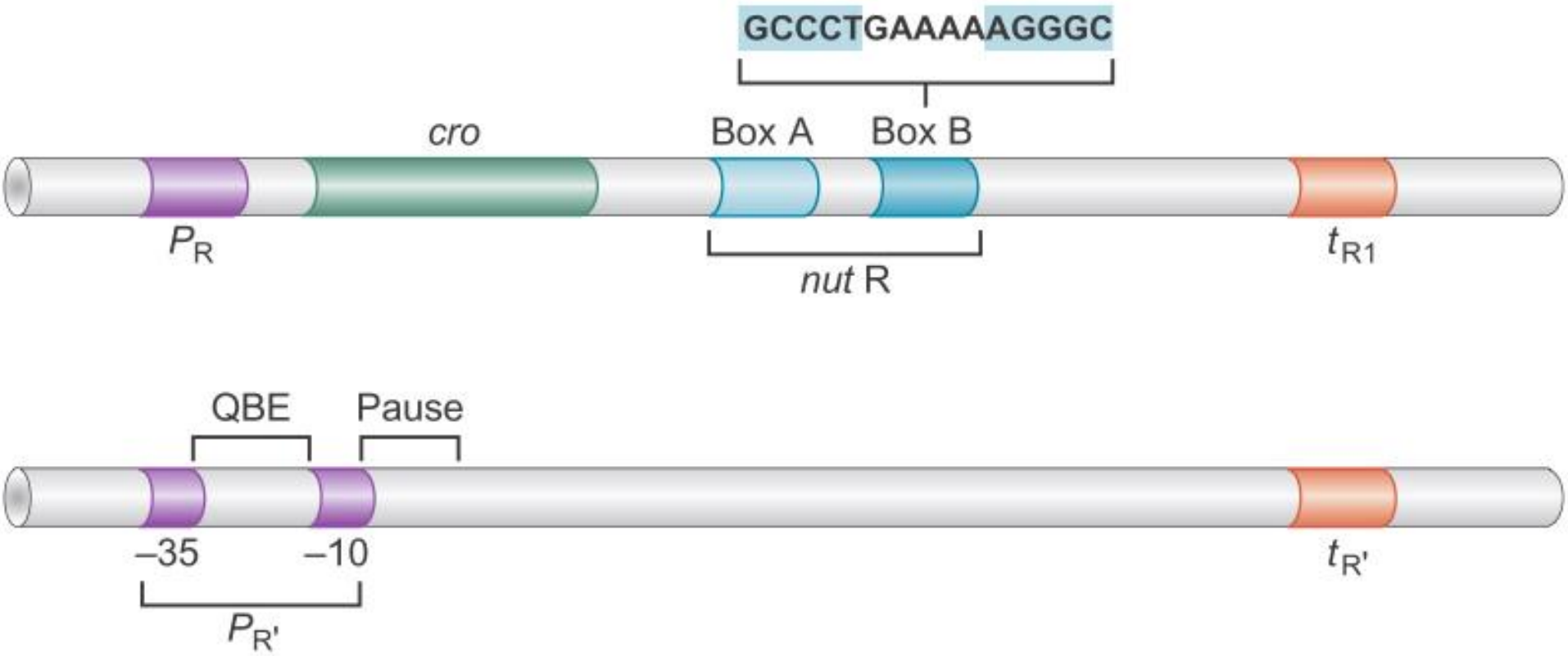
Layer (9): Transcriptional Antitermination in λ development

--Two λ phage regulatory proteins N and Q are called antiterminators.

--The transcripts controlled by λ N and Q proteins terminate a few hundred to a thousand nucleotides downstream of the promoter in the absence of N and Q proteins.

--λ N and Q proteins prevent the termination at some termination sites by regulating RNA polymerase and promote the transcription of the early and late genes for the lytic growth of the phage.

Fig. 7-26 Binding sites for proteins N and Q



N protein binds to the RNA transcribed from DNA containing a nut sequence, while Q protein binds to the QBE DNA site

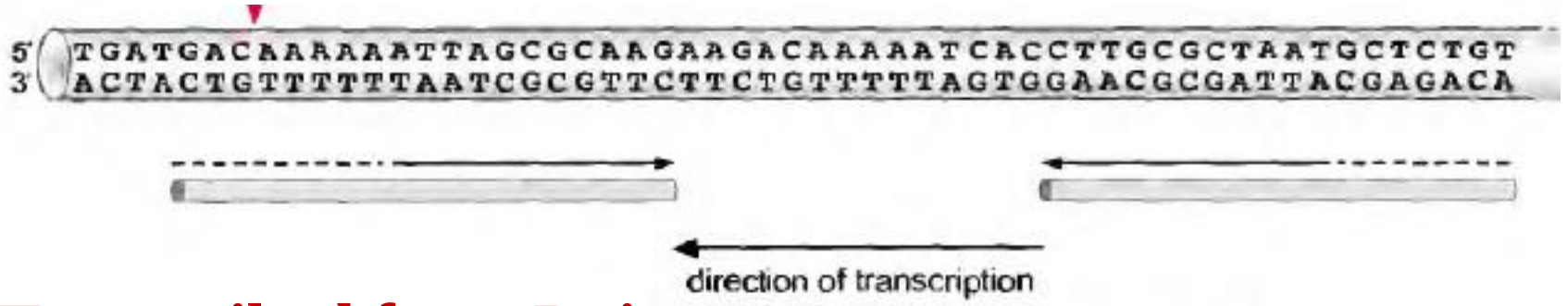
Layer (10): Retroregulation : An interplay of controls on RNA synthesis and stability determines *int* gene expression

The *Int* protein is the enzyme that integrates the phage genome into that of the host cell during formation of lysogen.

The cII protein activates the promoter P_I that directs expression of the *int* gene as well as the promoter P_{RE} responsible for repressor synthesis.

The *int* gene is transcribed from both P_L and P_I, but the *int* mRNA initiated at P_L is degraded by cellular nucleases.

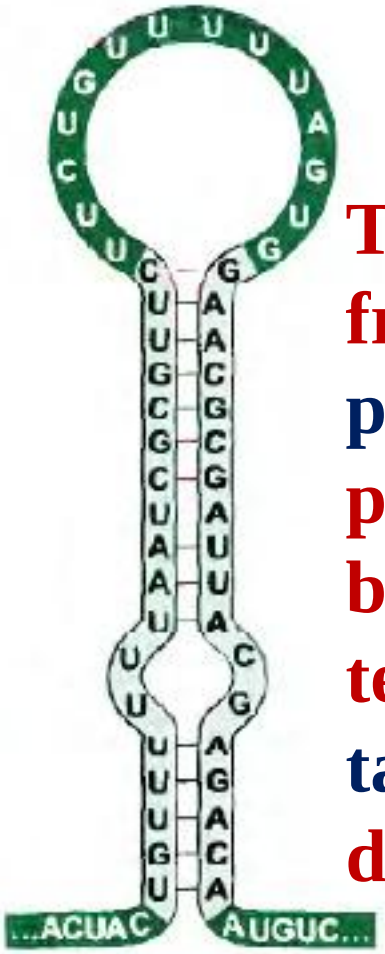
Site of termination in absence of N protein



Int **gene**

Transcribed from P_I, in the absence of N protein, resistant to degradation

Fig. 7-27



Transcribed from P_L in the presence of N protein, going beyond the terminator and targeted for degradation

Summary for 10-layer regulations in phage λ

(1) Cooperative binding of λ repressor to DNA at O_{R2}.
(2) λ Repressor and Cro bind in different patterns to control lytic and lysogenic growth.
(3) Upon DNA damage, proteolytic cleavage of λ repressor.
(4) Positive autoregulation: λ repressor binding at O_{R2}
(5) Negative autoregulation: cooperative binding of repressor at O_{R3} and O_{L3} .
(6) cII protein stimulates the transcription of cI gene (encoding λ repressor) in a new host.
(7) cII degradation by a specific protease FtsH *under good growth conditions*.
(8) cII stabilization by cIII, another substrate for FtsH.
(9) λ N and Q proteins prevent the transcriptional termination of the degradable *Int* mRNA.
(10) Retroregulation : targeted degradation of *Int* mRNA for lytic growth.

Summary of Chapter 7

1. **Principles of gene regulation.** Activator, repressor, operator, recruitment, allostery.
2. **Regulation of transcription initiation in bacteria: the *Lac* operon.**

   The activator: CAP

   The repressor: *Lac* repressor
3. **The case of phage λ—10 layers of regulation**

   λ repressor and Cro and their binding; proteolytic cleavage of λ repressor (lytic induction); autoregulation; control of the decision to lytic or lysogenic growth by λ cII; antiterminators; retroregulation of *Int* gene.