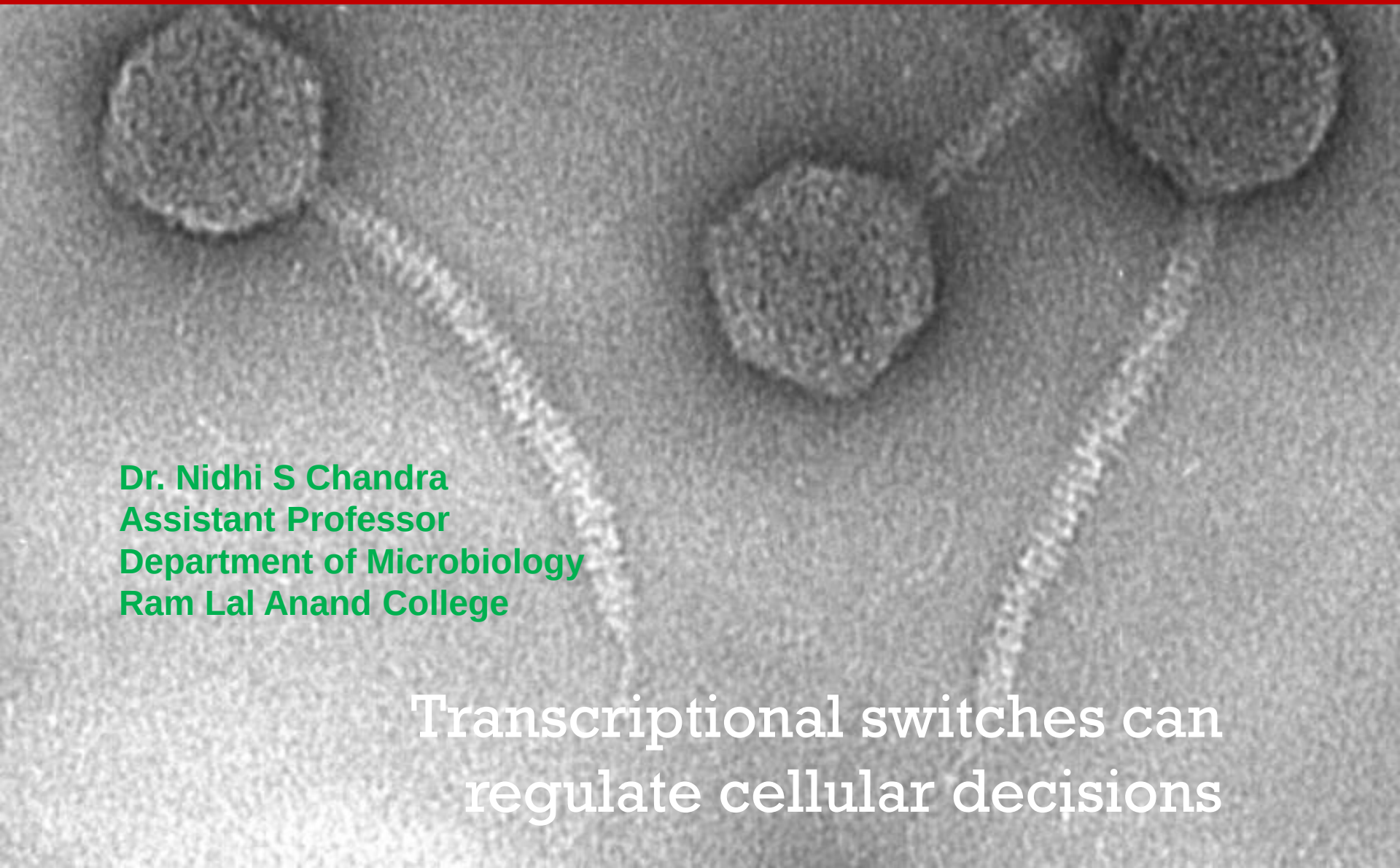
# Bacteriophage lambda (l)



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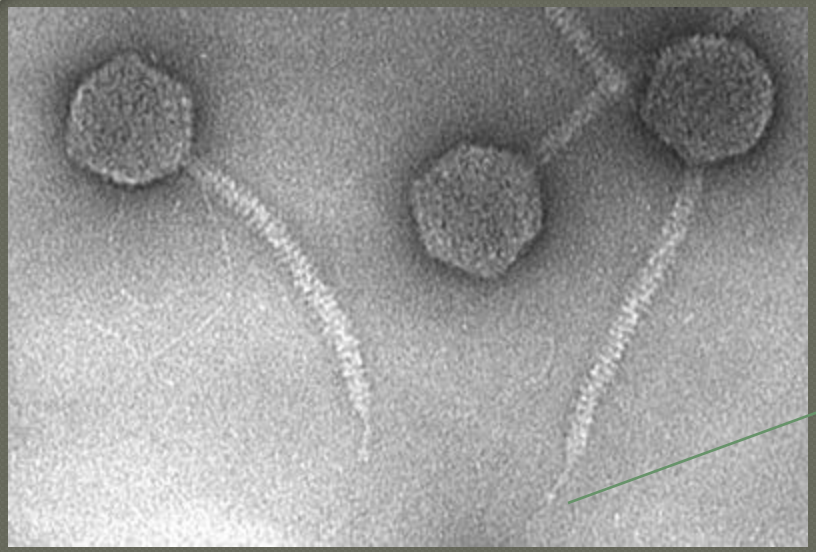
## Transcriptional switches can regulate cellular decisions

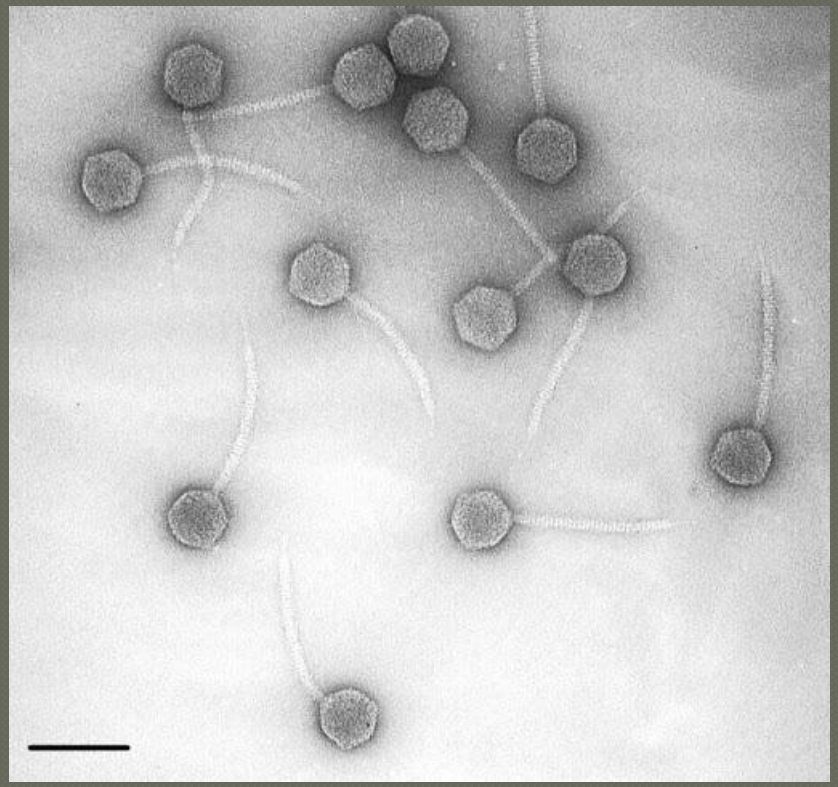
# OUTLINE

- Morphology of **phage λ**
- **Discovery**
- **Lytic vs. Lysogeny**
- **General features of phage genome**



# Electron micrograph

J chain



## Classification

- Virus classification
- Group: Group I (dsDNA)
- Order: Caudovirales
- Family: Siphoviridae
- Genus: λ-like viruses
- Species: λ Phage

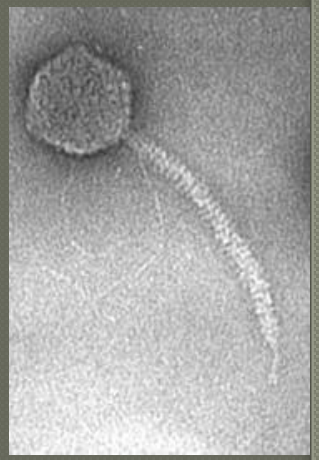
Q. Differentiate between the structure of $T_4$ and lembda phage

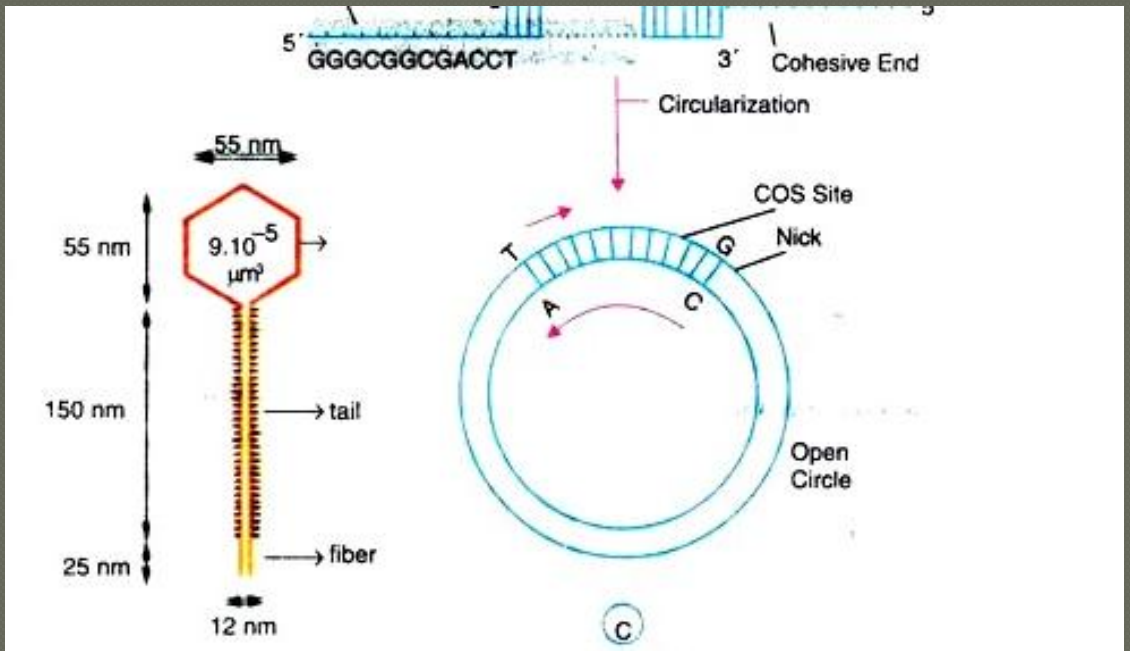
# Classification

- falls under the family **Siphoviridae** of the **Group I** (dsDNA viruses).
- Phage lambda is a virus of *E. coli* K12 which after entering inside host cell normally does not kill it.
- Therefore, it leads its life cycle **in two different ways**, one **as virulent virus** and the second **as non-virulent**. The virulent phase is called lytic cycle and the non-virulent as temperate or lysogenic one

# Morphological structure of phage λ

- The head has 20 faces. A 20- faces 3-D picture is called an icosahedron. The head is made of protein and contains a **48,450 (48.45 kbp) bp long genomic (g) DNA.**
- The phage λ contains double stranded DNA of about 17 μm in length packed in protein head of capsid.
- The head is 55 nm in diameter consisting of 300-600 capsomers (subunits) of 37,500 Daltons.
- The capsomers are arranged in clusters of 5 and 6 subunits i.e. **pentamers and hexamers**.
- The head is joined to a non-contractile **150 nm** long tail. The tail consists of 35 stacked discs. It ends in a fiber.






3.11 : Structure of bacteriophage lambda (λ). (A), single standard cohesive ends of 12 bases (B), and circularization of complementary DNA to form *cos* site (C).

- There is a hole in capsid through which passes this narrow neck portion expanding into a knob like structure inside.
- The tail possesses a thin **tail fiber (25 nm long)** at its end which recognizes the hosts. Also the tail consists of about 35 stacked discs or annuli. Unlike T-even phage, it is a simple structure devoid of the tail sheath.

# Discovery of bacteriophage λ by E. Lederberg (1951)

DONOR *E. coli*

\+

RECIPIENT *E. coli*

conjugation →

Recipient cell lysis

(In both the cases, lysis was examined under microscope)

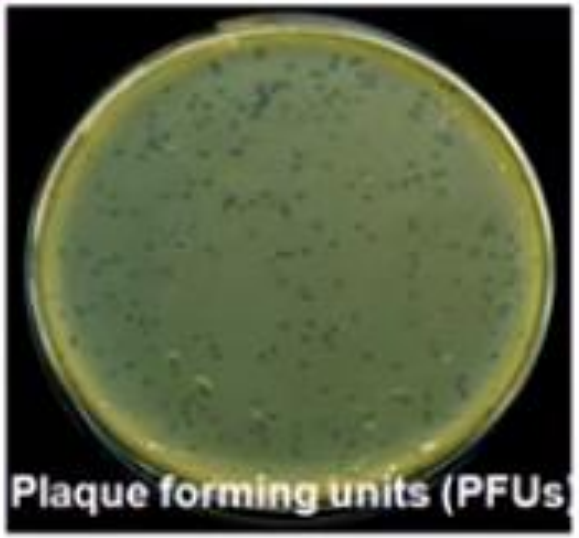
(DONOR) *E. coli*

UV →

Cell lysis

Josua Lederberg

Esther Lederberg



Plaque forming units (PFUs)

- Two strains of lembda phage were isolated independently one at Pasadena, USA and other one at Paris, France.
- Both of these strains could produce only tiny **plaque.**

  **$\lambda_{Paris}$ crossed with $\lambda_{Pasadena}$** $\longrightarrow$ $\lambda_{papa}$

- $\lambda_{papa}$ was taken as wild type $\lambda$ and used for research which could be produce large **plaque**.

# Lytic–Lysogenic

- https://www.youtube.com/watch?v=gU8XeqI7yts

- The process of **a phage infecting a bacterium and producing progeny** is referred to as a lytic infection or **phage life cycle culminate with host cell lysis.**
  - Eg **T4, are only capable of lytic growth**.
- A phage that reproduces only by the lytic cycle is called a **virulent phage**
- While, Some phage are capable of maintaining their chromosome in a stable, silent state within the bacteria. This is called **lysogeny.**

# Lytic–Lysogenic

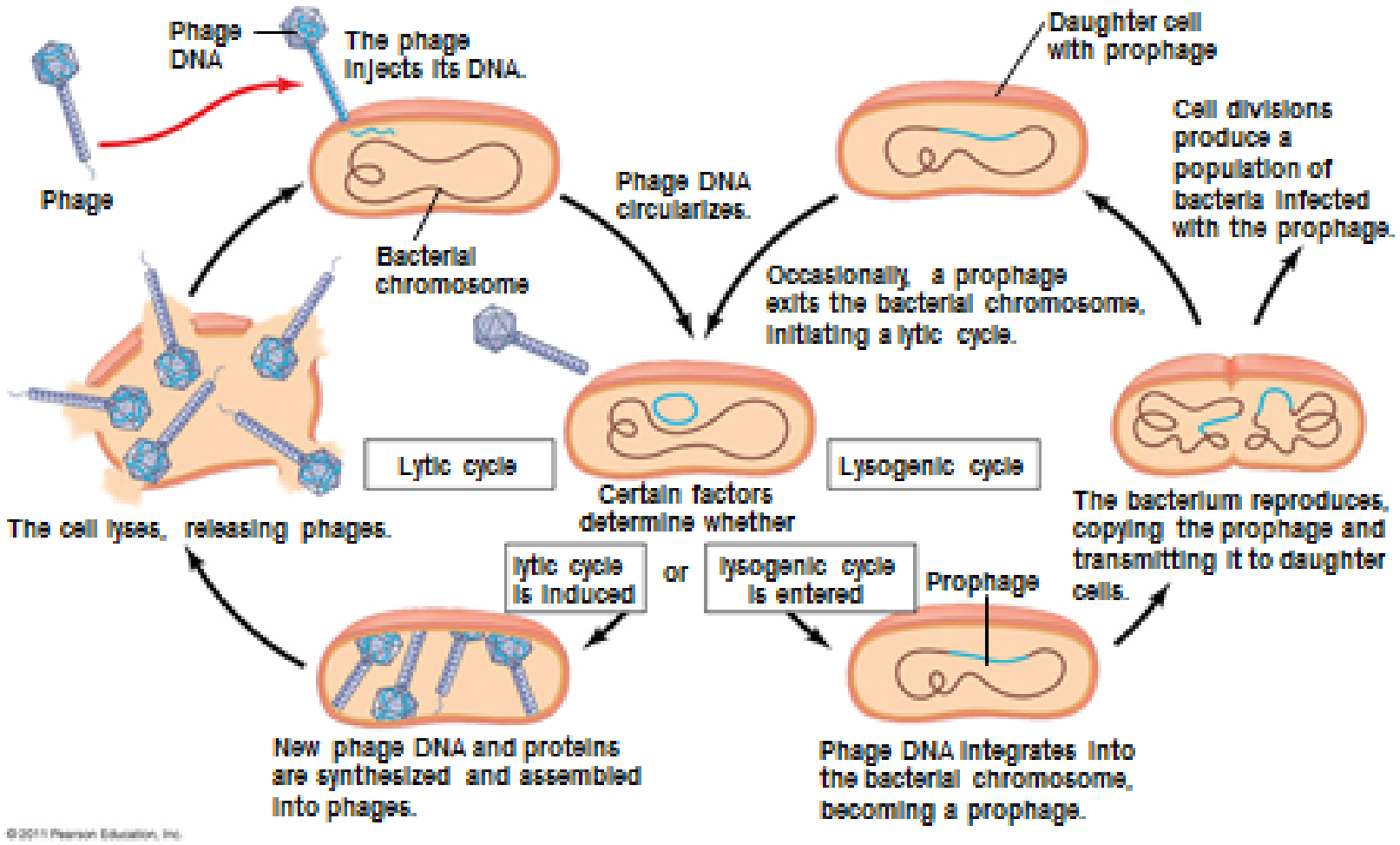
- Phage that are capable of both **lytic and lysogenic pathway** are called **temperate phage**.
  - **Eg. P1 and Lembda**
- **M13 is unusual** because phage continually exit from a bacterium without killing it. For this reason, **M13 is not considered to have a true lysogenic state and not a temperate phage.**

# Lytic–Lysogenic

- The bacterium contains a silent phage chromosome referred as **lysogen.**
- The incorporated phage genome is referred to as **prophage**.
- Lysogens are **immune** to further infection by similar phage because the phage functions are **repressed** in *trans*.

- **Induction of the lysogen leads to excision of the prophage, followed by replication of the phage DNA, and lysis of the host bacterium.**

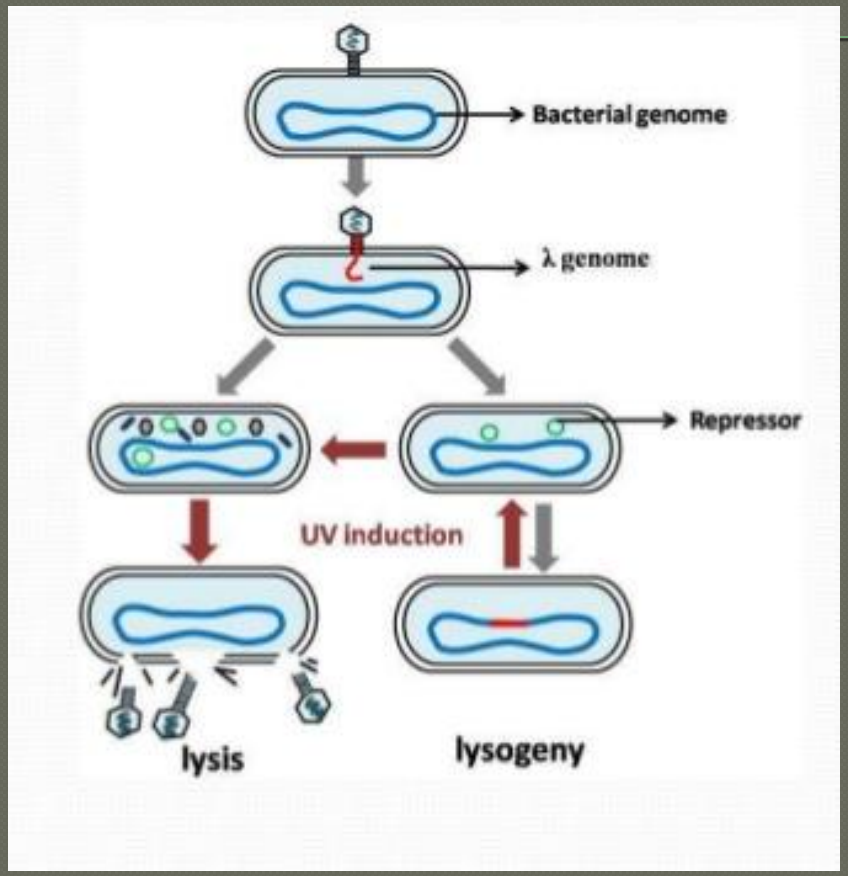
Figure 19.6



# lysis-lysogeny decision of the **temperate lambda phage**

- It has emerged as a novel paradigm for understanding developmental genetic networks.
- ***E.coli* and the lambda** phage establish synergistic relationships. The lambda phage may exist in a dormant lysogenic state, passively replicating with the host chromosome or may fall into the lytic cycle generating progeny phages, killing their hosts.

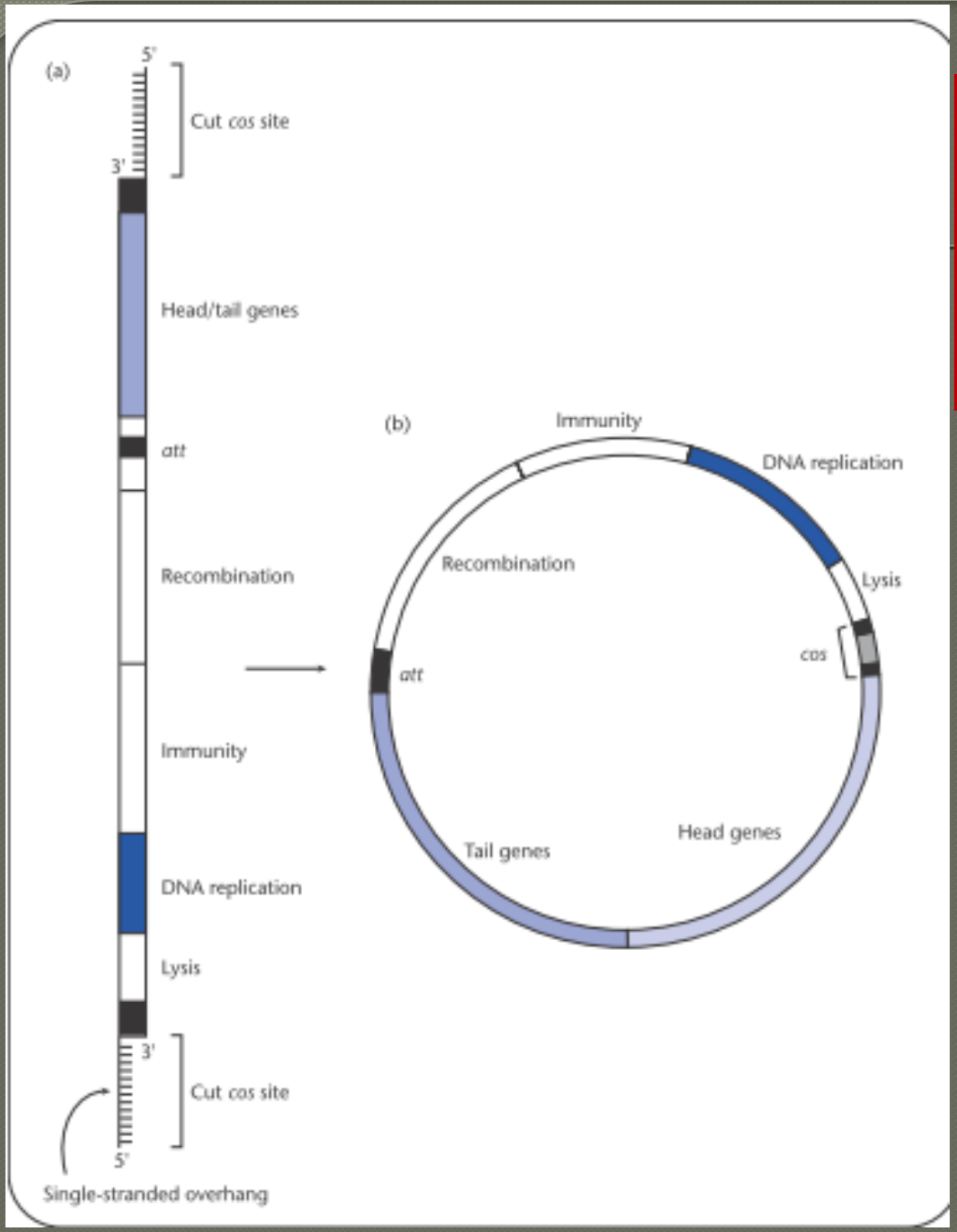
- Therefore, the lambda phage makes a decision to follow either the lytic or the lysogenic pathway.
  - When the lambda phage follows the lytic pathway, it replicates its DNA autonomously, expresses a set of genes, and assembles the virions, resulting in lysis of the host.
  - If the lysogenic state continues over a long time, a stable lysogen is established in the circuit and the phage DNA is integrated to the host genome. **This turns OFF the expression of the lytic genes.**




Inducing signals like UV light that damage the DNA, force the lambda phage to a SOS response and the lysogenic state switches irreversibly to the lytic phase. The lambda phage thus behaves as a biphasic switch.

- **What is the characteristic of phage genome in phage particle?**

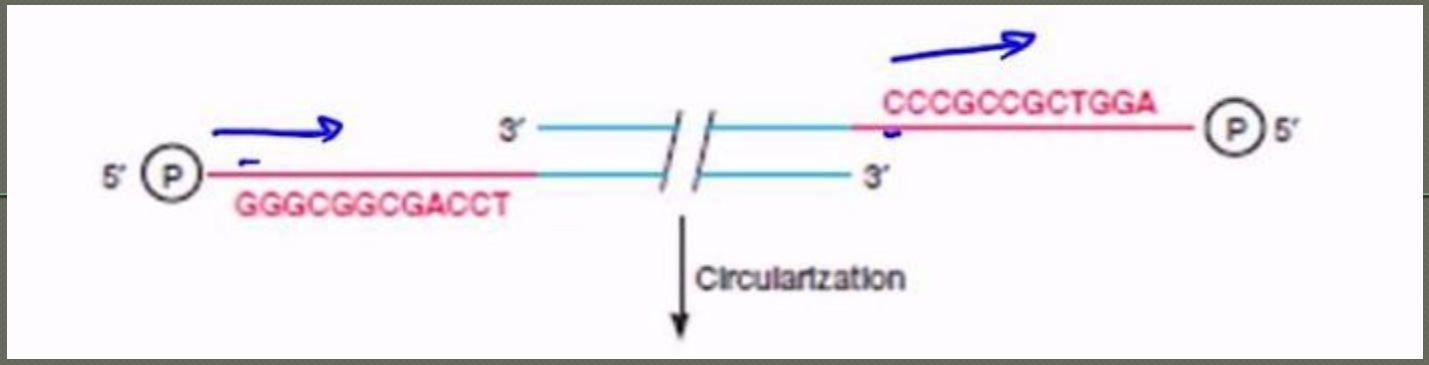
- **What is the characteristic of phage genome in host cell?**




The structure of the λ DNA
(a) in the phage capsid
(b) after circularization in the cytoplasm
**The DNA circularizes via the cos site**

In Phage body:

- λ Phage has a linear genome (ds DNA) enclosed a protein body.
- The linear genome contains **sticky ends/overhang (single strand DNA on each end).**
- The sticky ends are complementary to each other

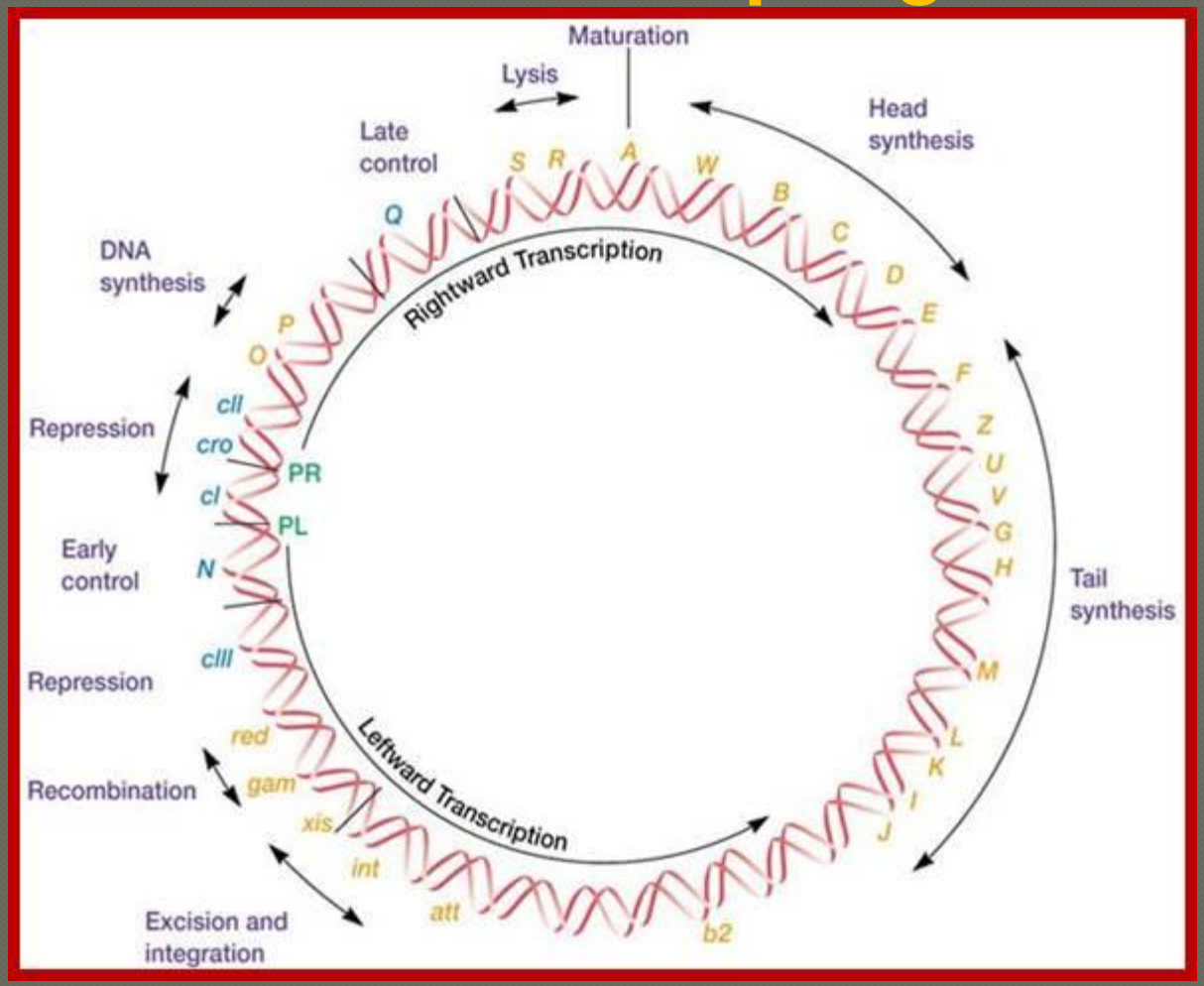



- λ has a specific site on its DNA, termed the **cos site**, which it uses to circularize the DNA.
- The **cos site** is a **22bp sequence** that is cut asymmetrically when the λ DNA is packaged .
- The cut cos site has a **12bp overhang**. There is one cut cos site at the left end of the λ genome and another cut cos site at the right end of the λ genome
- When λ DNA is injected into the cytoplasm, the cut cos sites at either of the linear λ genome anneal
- **A host enzyme, DNA ligase, seals the nicks** at either end of the cos site generating a covalently closed, circular λ genome. **The host encoded enzyme, DNA gyrase, supercoils the λ molecule.**

# Why λ phage genome circularized in the host cytoplasm?

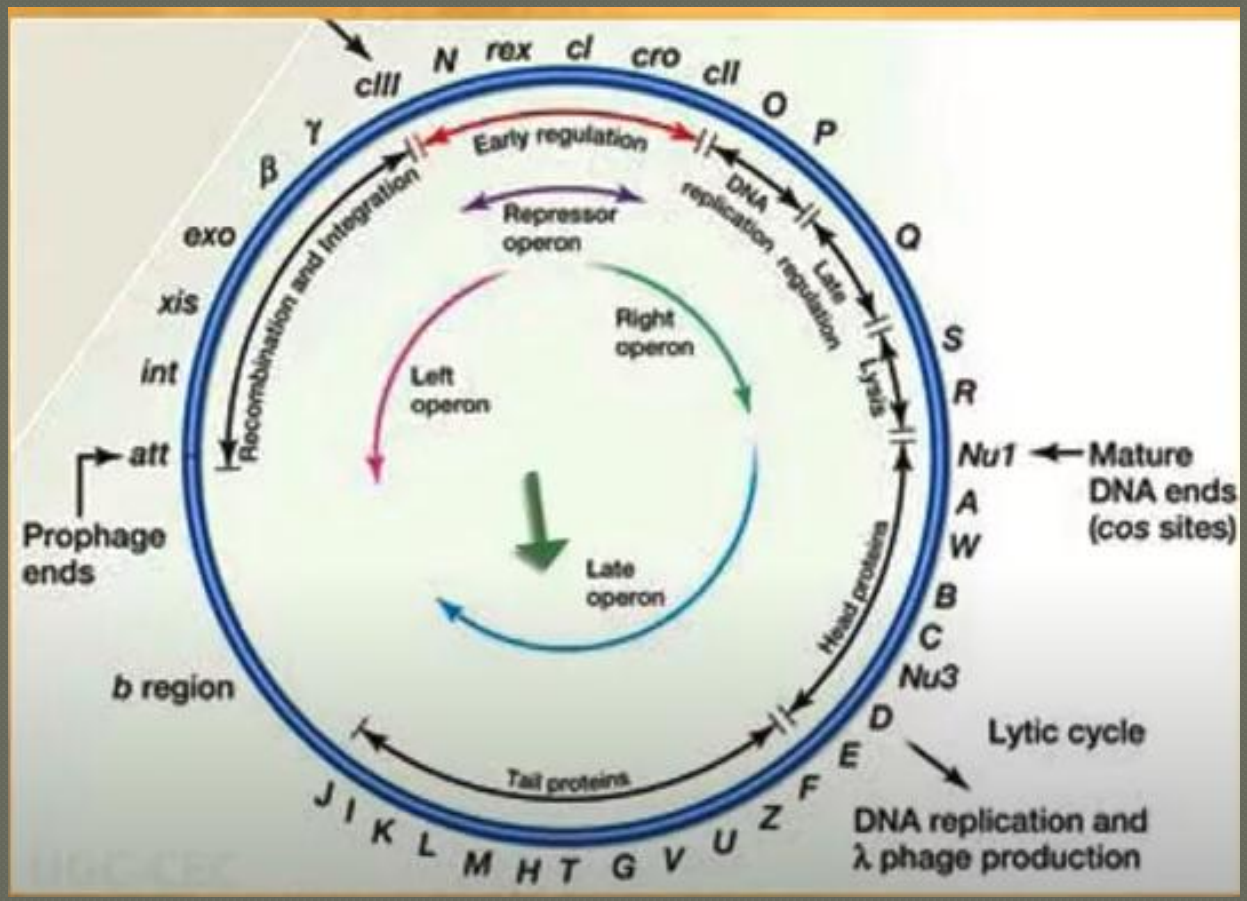
- λ contains a linear double-stranded DNA molecule in its capsid.
- In the bacterial cytoplasm, **dsDNA molecules** are subject to **degradation by exonucleases** that need a free end to digest the DNA.
- To overcome this problem newly injected λ DNA (linear) is circularizes

# Genome of λ phage

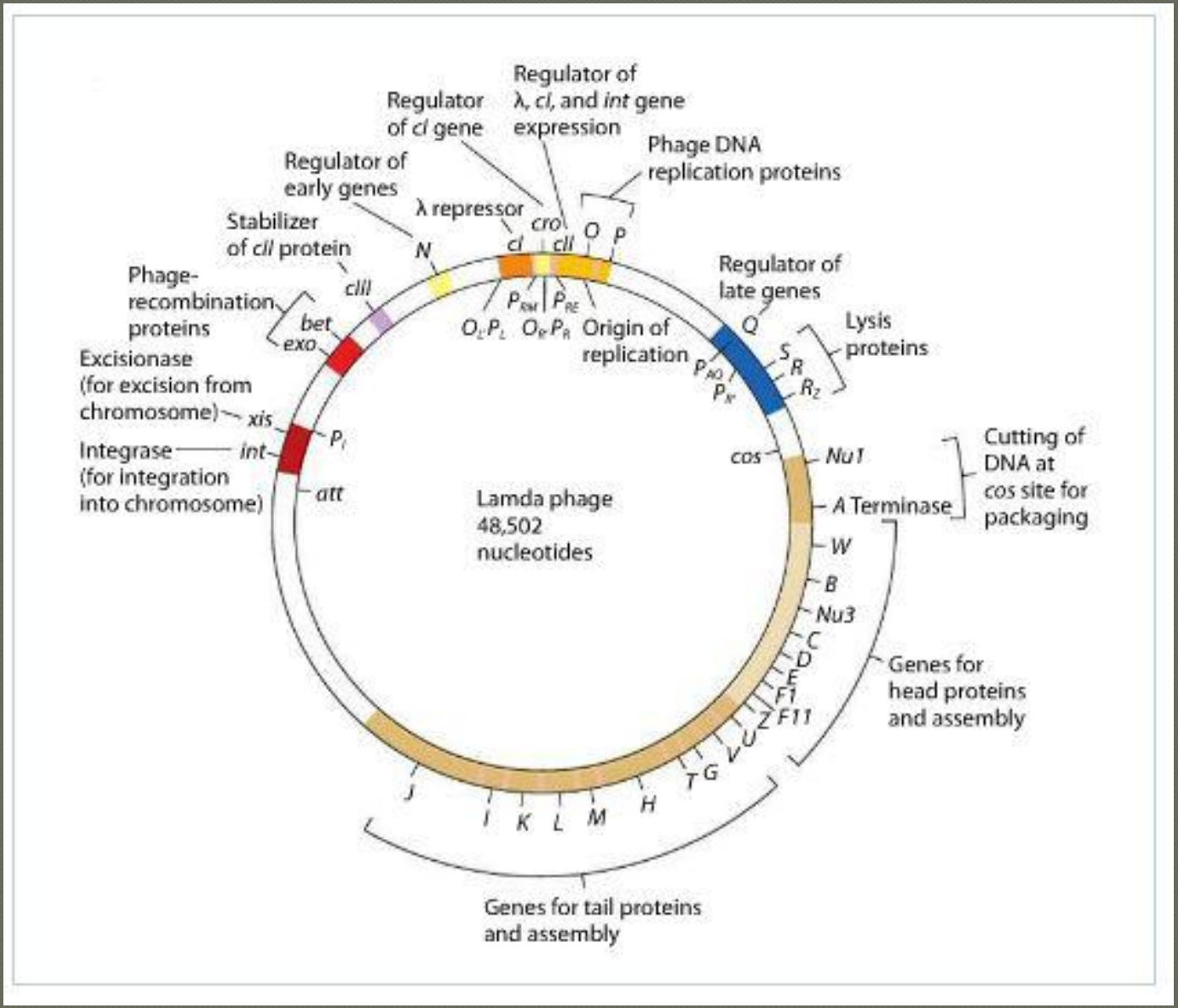



**Frederick Sanger determined the complete nucleotide sequence of the DNA molecule in 1979. over 40 genes have been mapped in the λ genome that are clustered according to their function.**

**Francois Jacob and Jacques Lucien Monod led to propose a famous operon modal for gene regulation in prokaryotes in 1961**



# Genome of λ phage



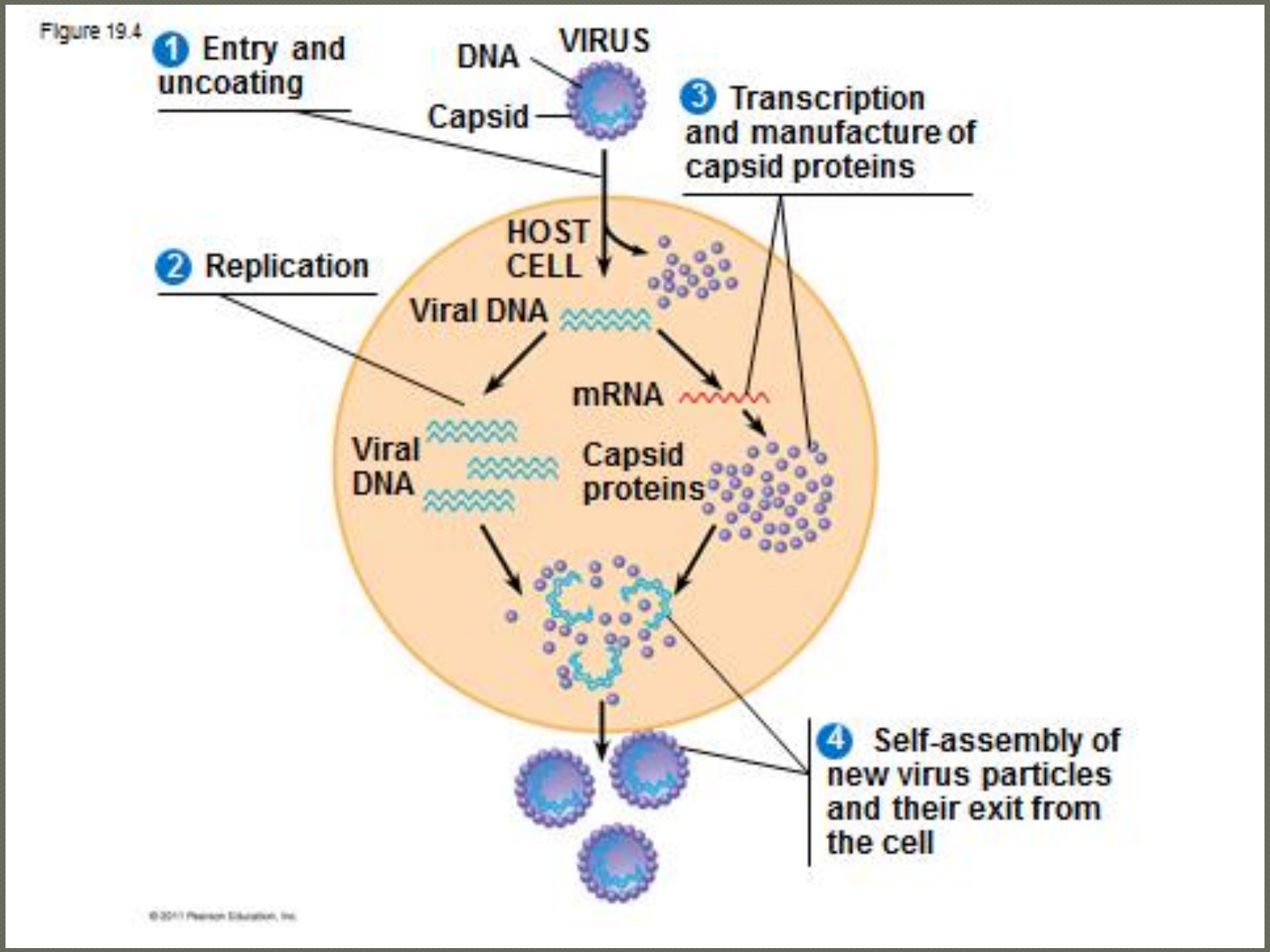
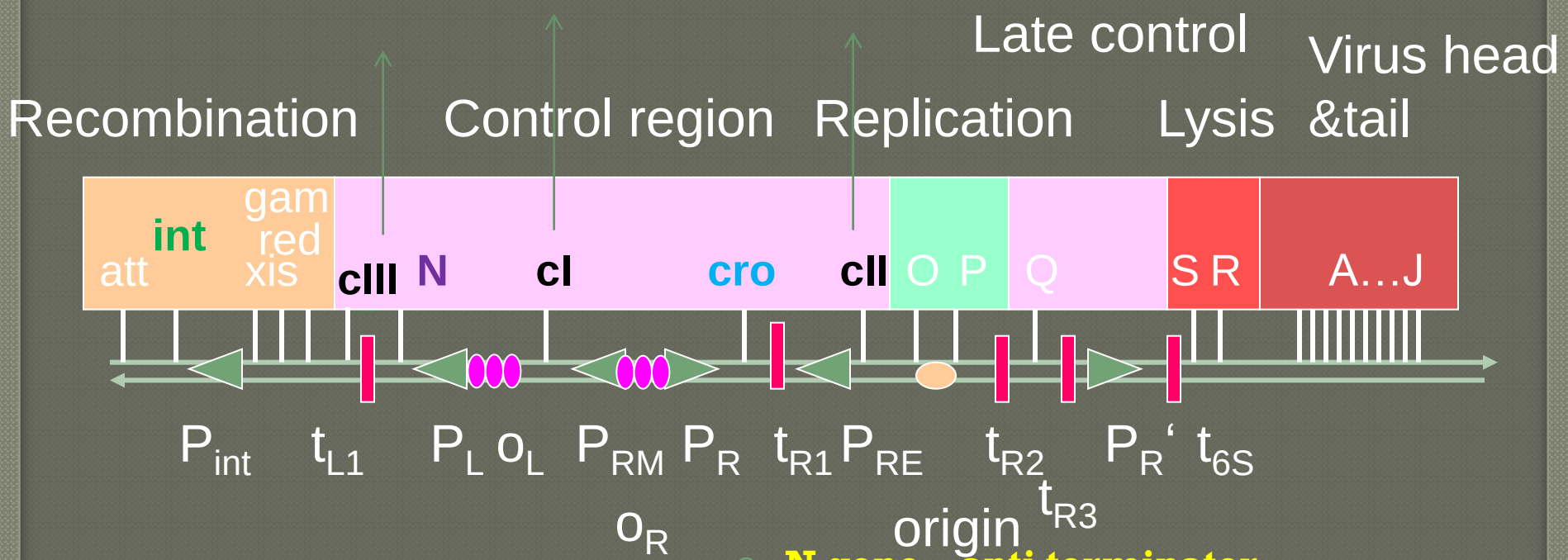


Figure 19.4
1 Entry and uncoating
DNA
VIRUS
Capsid
3 Transcription and manufacture of capsid proteins
HOST CELL
2 Replication
Viral DNA
mRNA
Viral DNA
Capsid proteins
4 Self-assembly of new virus particles and their exit from the cell

# The λ Life Cycle

- **λ adsorption**
- Phage identify a host bacterium by binding or adsorbing to a specific structure on the surface of the cell. λ binds to an outer membrane protein called **LamB** via a protein that resides at the tip of the λ tail called the **J protein**.
- **LamB** normally functions in the binding and uptake of the **sugars maltose** and **maltodextrin.**

# Genes responsible for phage expression




promoter

operator

terminator

- **N gene – anti terminator**
- **Cro gene – lytic switch ON**
- **C I gene – lysogenic switch ON**
- **C II gene – activation of C I gene**
- **C III gene – protects C II gene**
- **Q gene – anti terminator**
- **int gene – integrase enzyme**

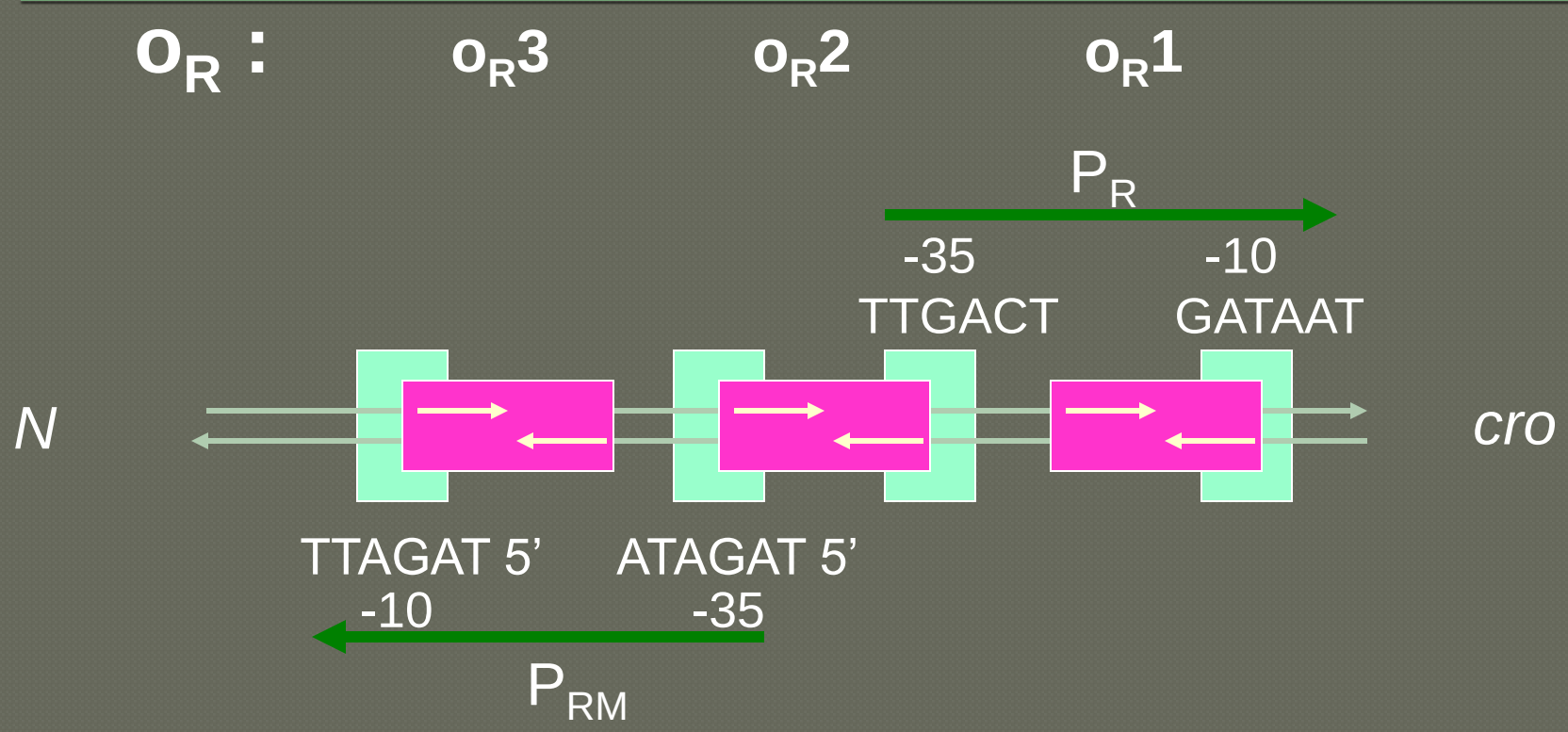
- **Two genes serve as the molecular switch.**
  - **Lambda repressor protein (CI):** activates the lysogenic pathway.
  - **Cro protein:** activates the lytic pathway.

**This system is called the lambda repressor switch**

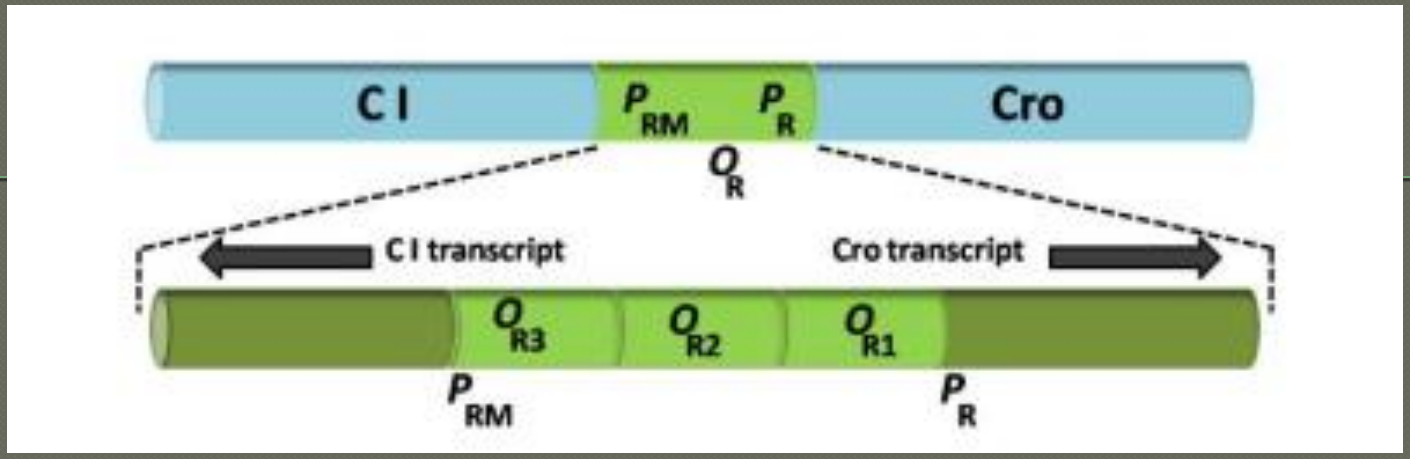
# In order to understand how switching happens between the lysis and lysogeny states in the lambda phage

- we focus on **two regulatory genes *CI* and *cro*** and **a regulatory region OR called the right operator** as shown in Fig.
  - During the **lysogeny phase *CI* is switched ON** and ***cro* is OFF.**

# λ operators overlap promoters



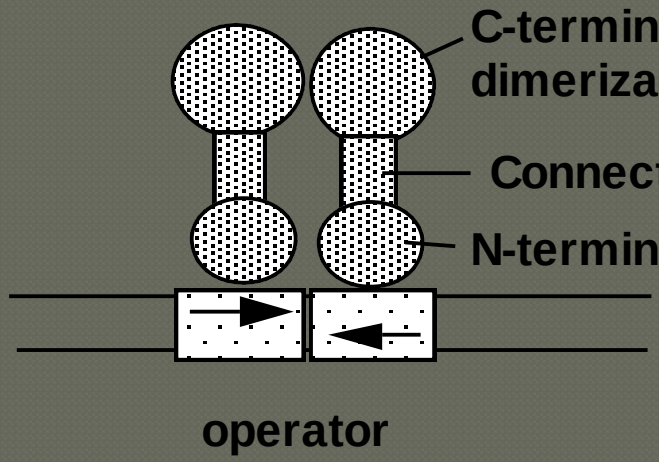

The operator $O_R$ is constituted of three binding sites $O_R1$, $O_R2$ and $O_R3$ which overlap two promoters $P_{RM}$ and $P_R$ which oppose each other.

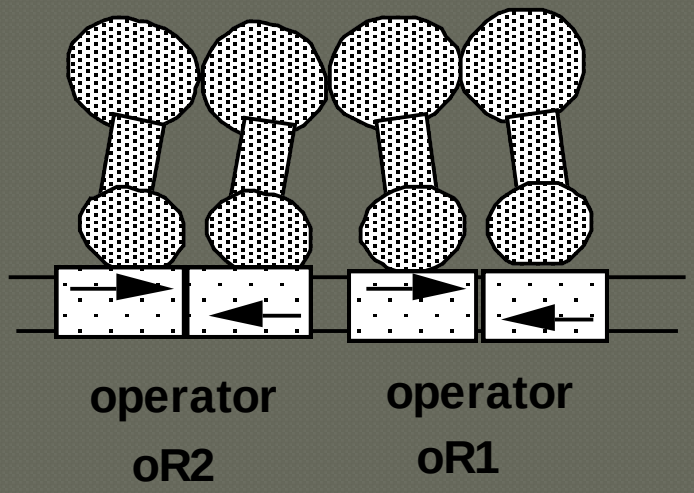



- The promoter $P_R$ drives the transcription of lytic genes (*cro*)
- $P_{RM}$, the transcription of the *CI* gene.
- During the lysogenic state, the lambda repressor at $O_R$ is bound at $O_R1$ and $O_R2$, sites adjacent to each other At these sites, the repressor represses the right ward transcription from the promoter $P_R$. the expression of cro and other lytic genes is therefore **turned OFF.**

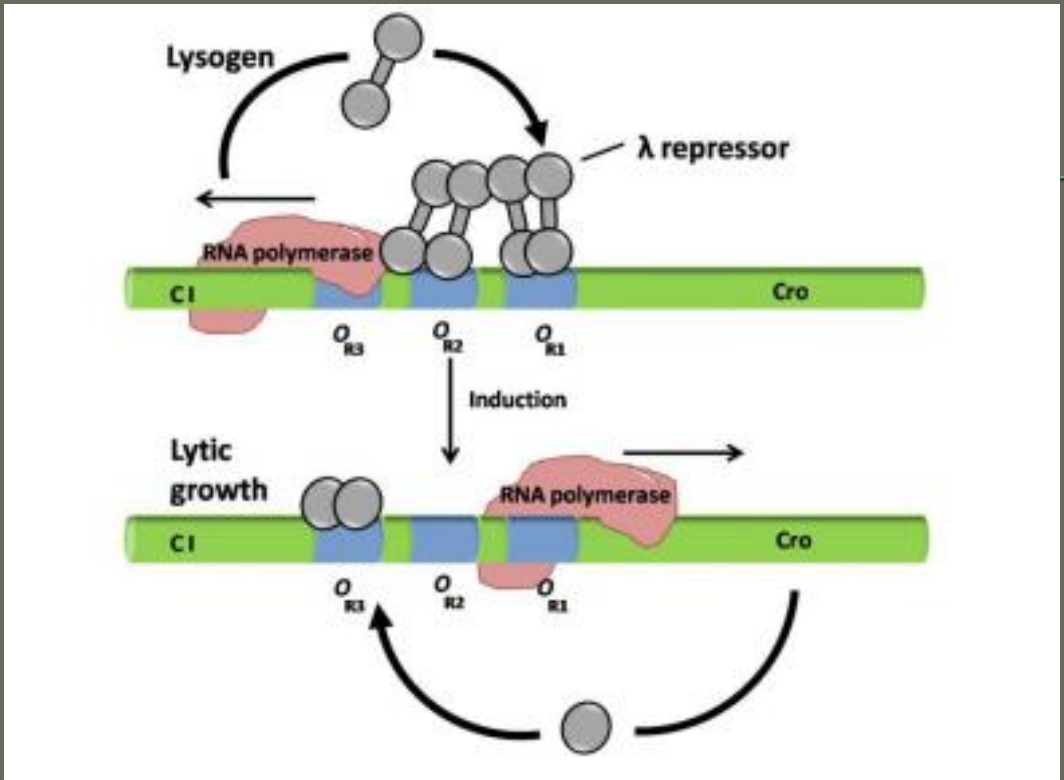
# Repressor structure

λ repressor is a dimer; monomer has 236 amino acids.




λ repressor can bind cooperatively to operator sub-sites.




**Role of λ repressor and CRO**

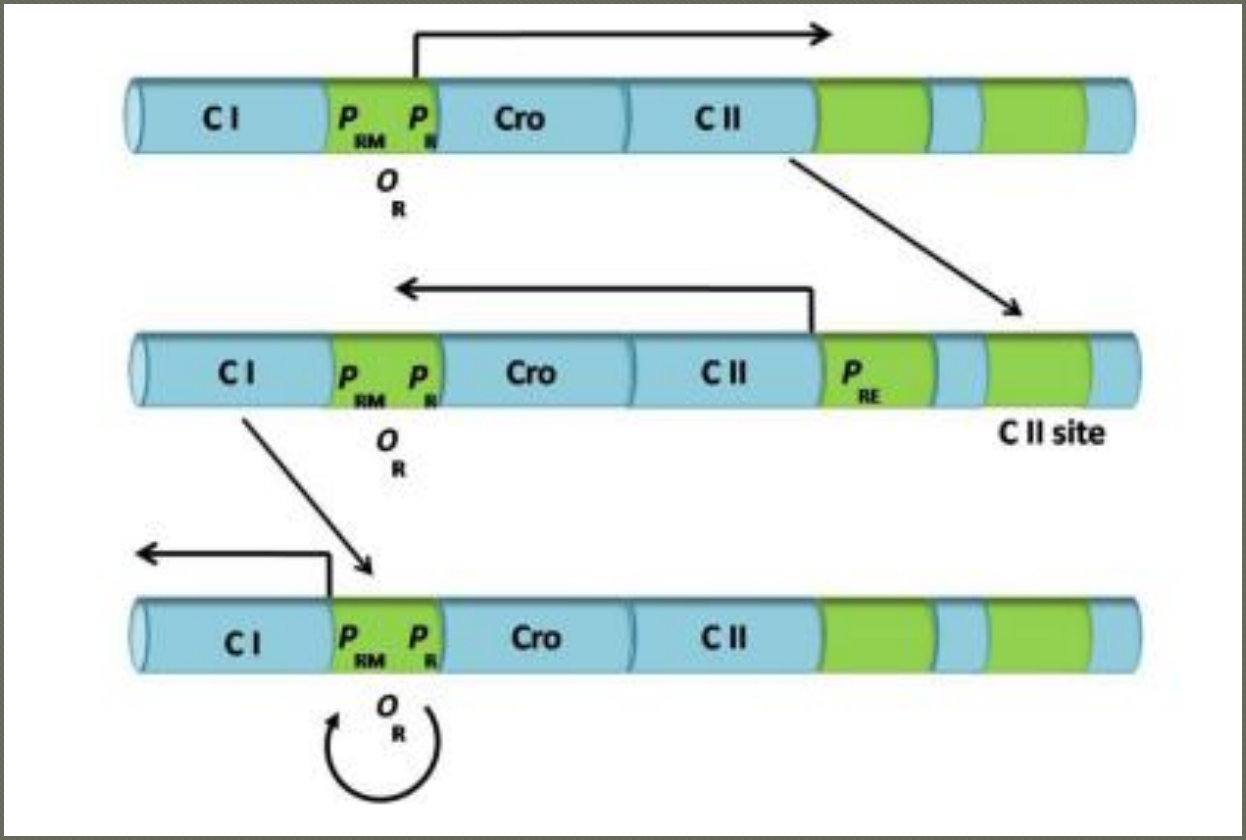
Note: $P_R$ is a stronger promoter than $P_{RM}$.
As transcription begins, the **CRO protein** is made which binds first to $O_R3$ abolishing the synthesis of the repressor.

- At the same time, it also transcribes its own gene from the promoter $P_{RM}$
- When induced, the **repressor leaves** the **operator** and transcription from $P_R$ is initiated spontaneously.

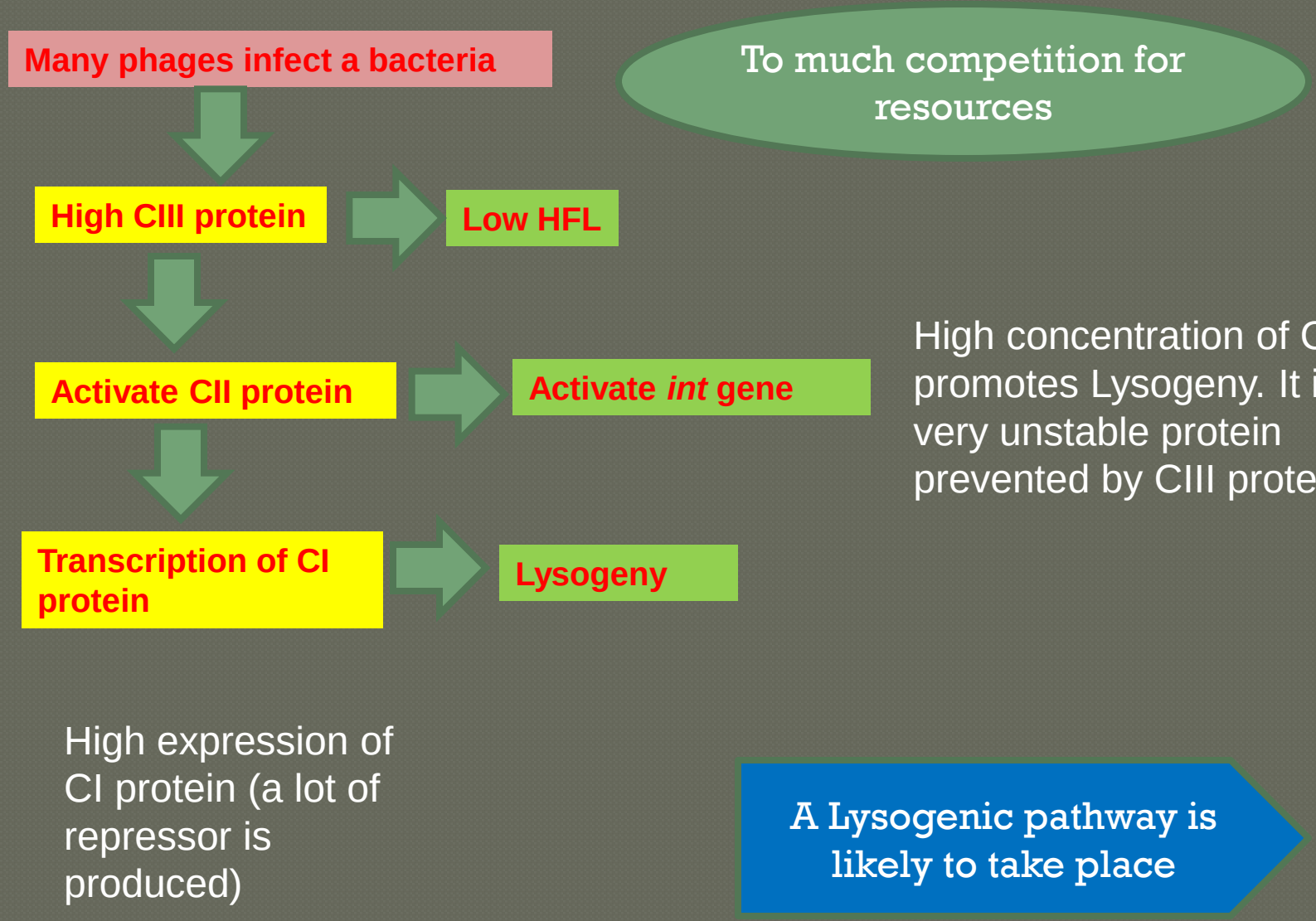
# Lysogeneic state

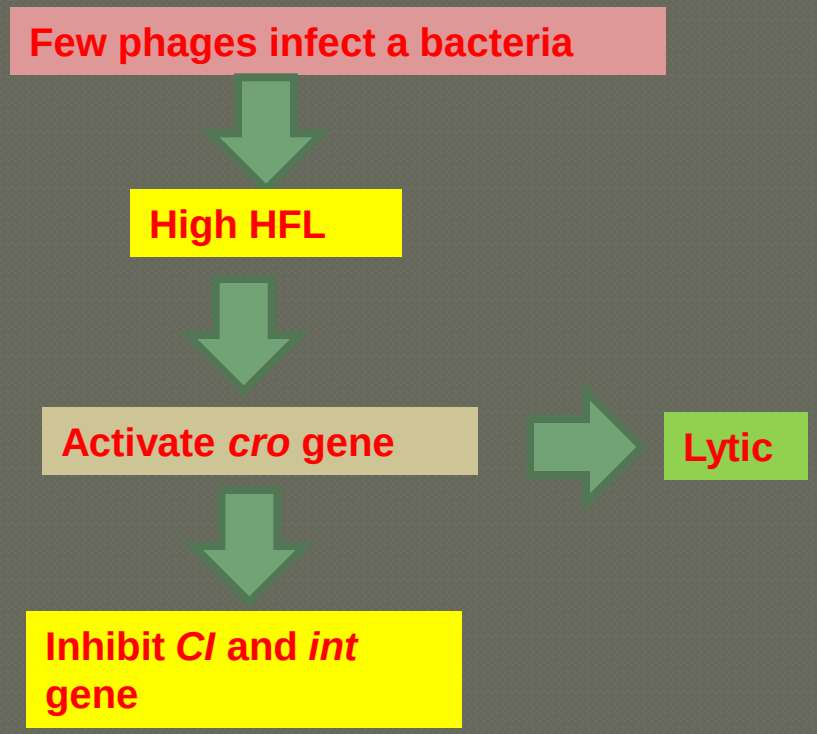
- We know that the **lambda repressor** is required to regulate the transcription of its own gene.

**How this gene is switched ON to establish lysogeny during the viral infection?**




The repressor is transcribed initially from the promoter $P_{RE}$ (Promoter for repressor establishment) as shown in Fig. This transcription is activated by **CII**, a product of another phage gene. Thus, a new repressor CI is made and it activates its own transcription from $P_{RM}$. This **switches OFF** the other phage genes including CII. Thus we see the establishment of lysogeny in lambda phage, even in the absence of the inducer signal.


Many phages infect a bacteria
To much competition for resources
High CIII protein
Low HFL
Activate CII protein
Activate *int* gene
High concentration of CII promotes Lysogeny. It is very unstable protein prevented by CIII proteion
Transcription of CI protein
Lysogeny
High expression of CI protein (a lot of repressor is produced)
A Lysogenic pathway is likely to take place




High HFL degrade the *CII* and the concentration of *CII* become Low and no enough repressor will produce

High expression of *cro* gene (a lot of CRO protein is produced)

A Lytic pathway is likely to take place

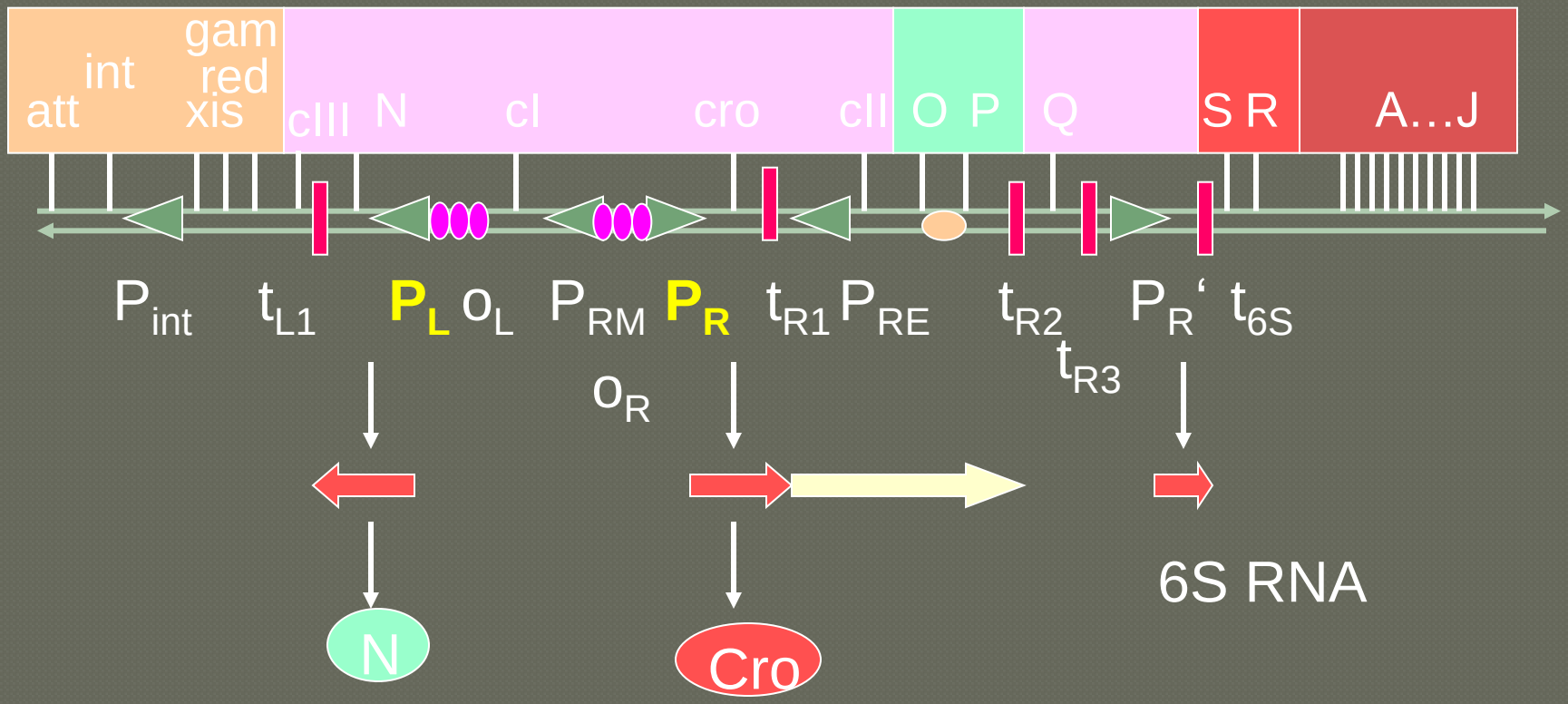
# Gene Regulation

- There are three classes of genes in the phage genome that regulate whether the lytic or lysogenic cycle will occur
  - Immediate early genes
  - Delayed early genes
  - Late genes

# Expression of Immediate early gene

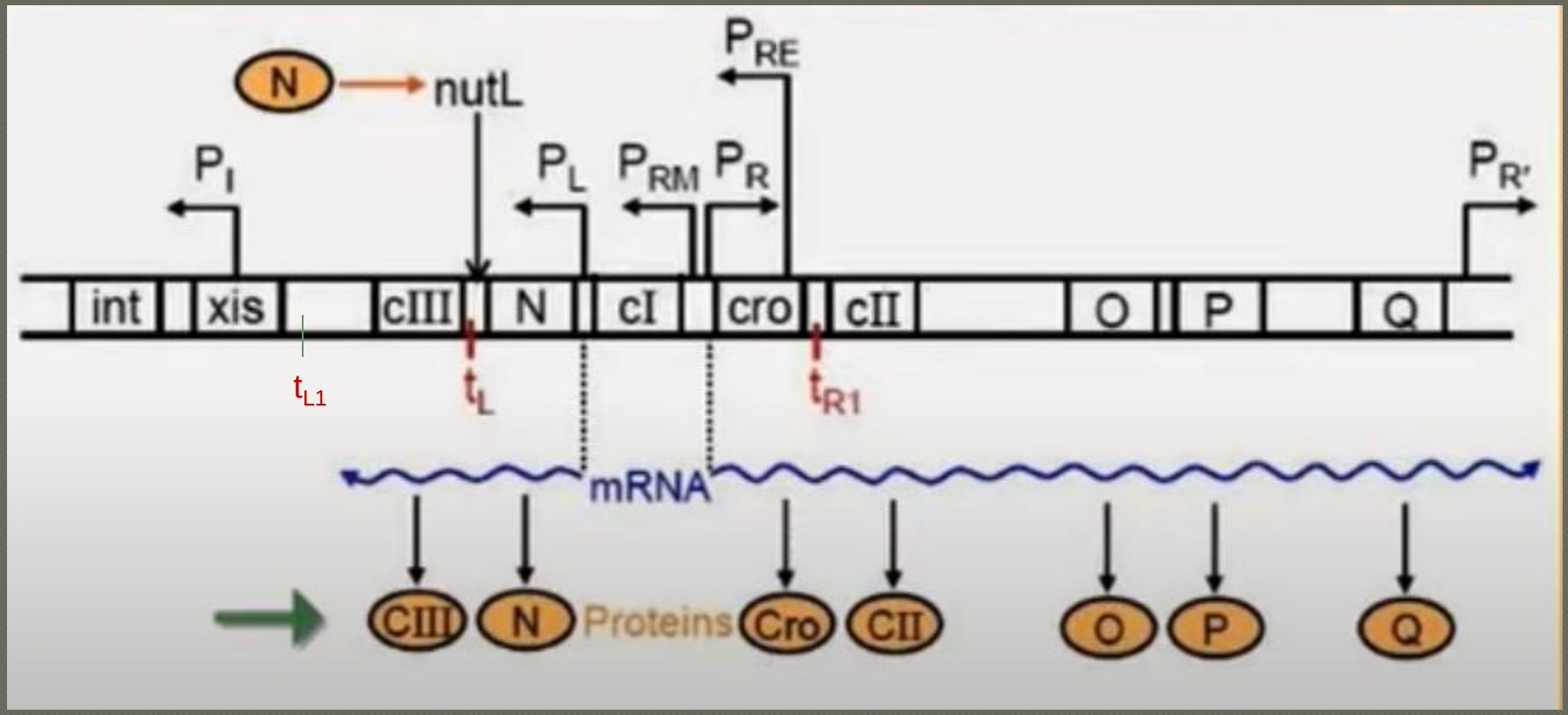
*E. coli* RNA polymerase initiates at strong promoters $P_R$ and $P_L$

**N gene – anti terminator**
**Cro gene – lytic switch ON**

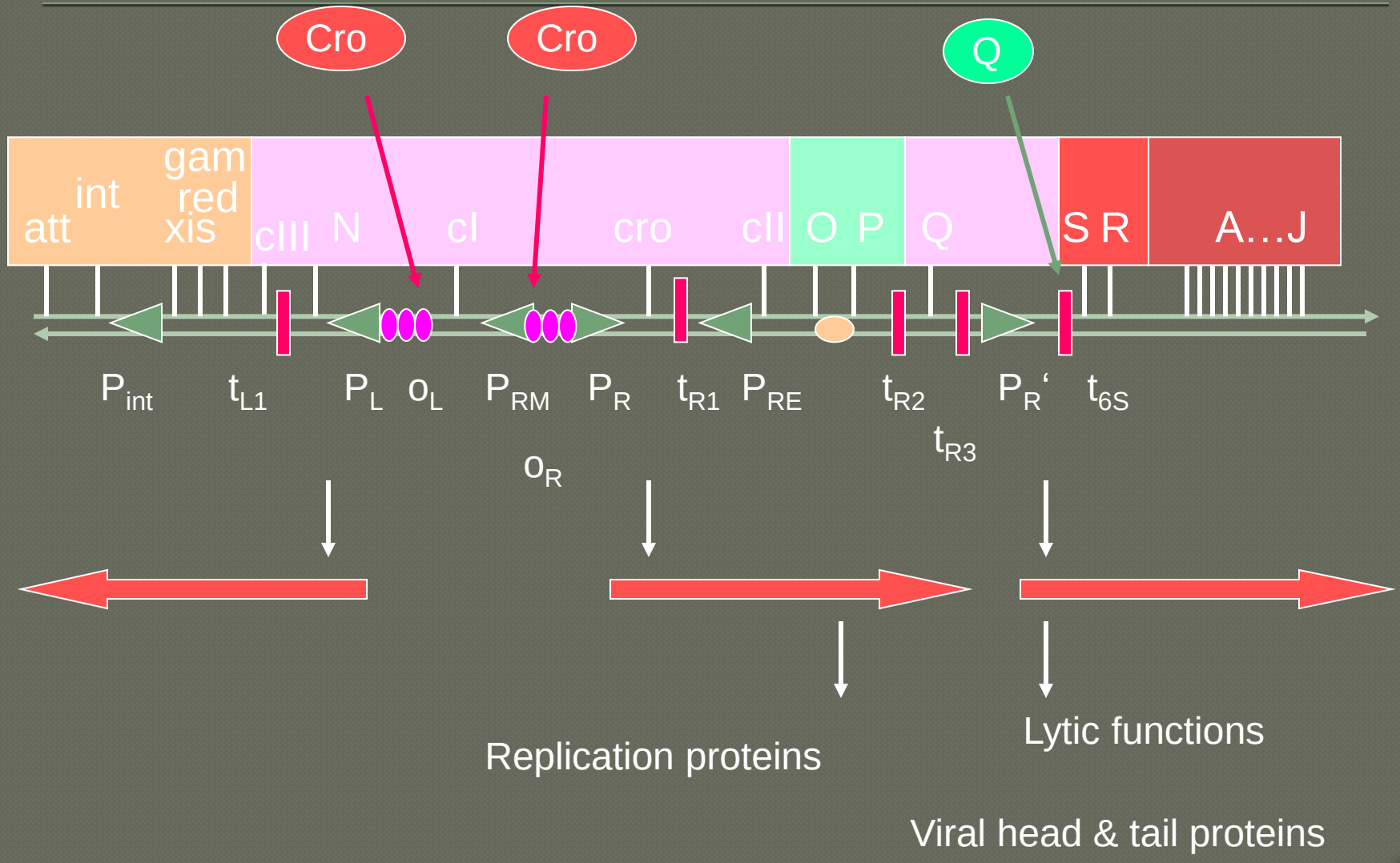


# Expression of delayed early gene

Antiterminator protein (**N protein**) bind to termination site

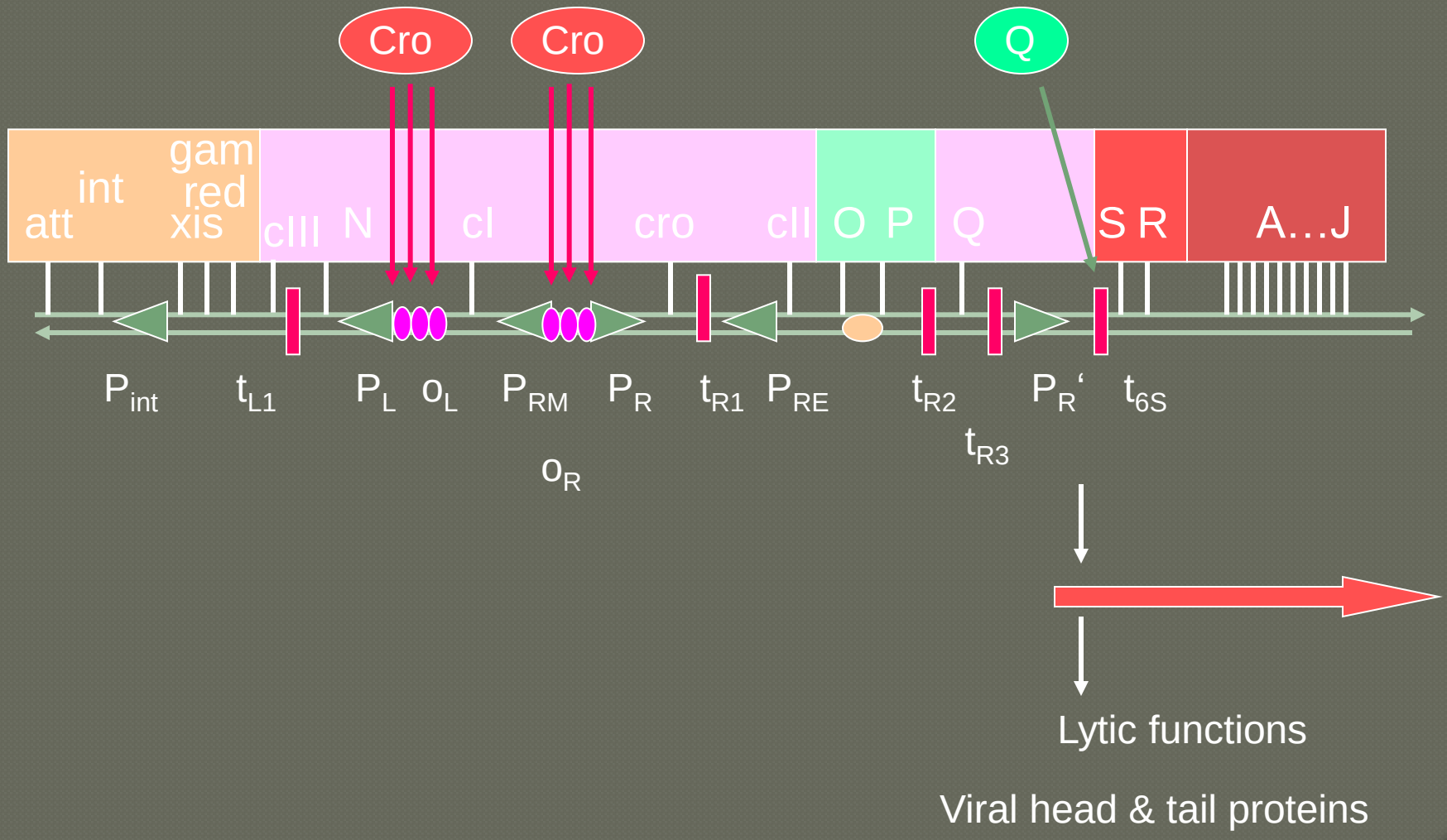


# Lytic cascade: Cro turns off *cI*, Q protein action leads to late gene expression

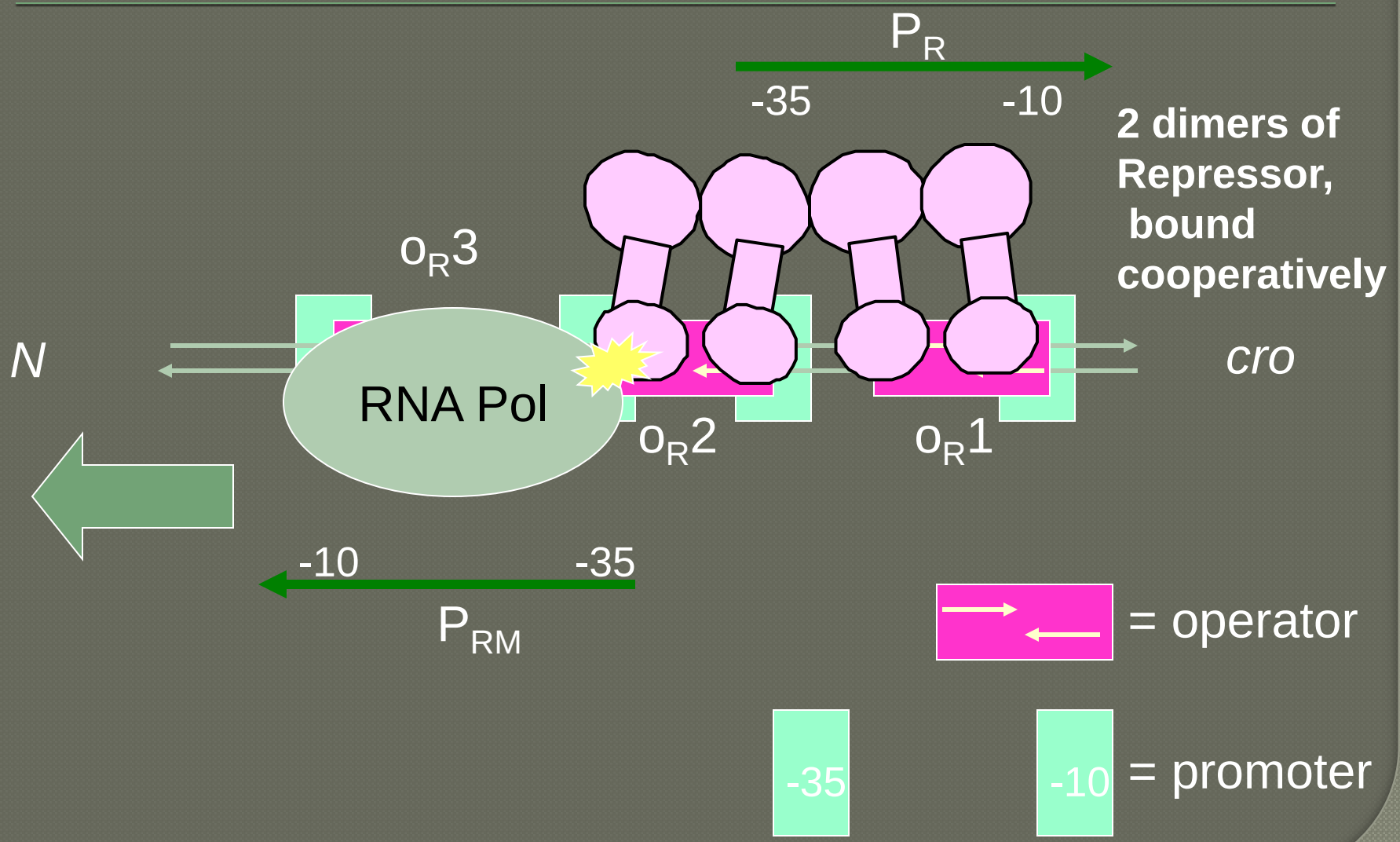


# Late stage of lytic cascade

High concentrations of Cro turn off $P_R$ and $P_L$ .
Abundant expression from $P_R$'.



# Binding of repressor blocks transcription from $p_R$ but activates $p_{RM}$



- The life styles of the phage lambda present a classic case of complex genetic control circuits. It is interesting to understand a small set of regulatory proteins yielding a complex set of temporally controlled macro molecular interactions in a simple organism like the lambda phage.
- Systems biology approaches will help understand the function of specific modules of these regulatory domains and will help understand the kinetic behavior and quantitative picture of the genetic circuit of the lambda phage.

# Events leading to lysis

lysis or lysogeny (cI or Cro?) ?

Both lysis and lysogeny:

- $P_R$, $P_L$, $P_{R'}$ active : synthesize N, Cro
- antitermination by N : synthesize cIII, cII, Q

Lysis:

- Low [Cro] : binds $O_R3$, shuts off $P_{RM}$ (cI)
- High [Cro] : shuts off $P_R$ and $P_L$
- antitermination by Q + activation of $P_{R'}$ by Cro

# Events leading to lysogeny

lysis or lysogeny (cI or Cro) ?

Lysis and lysogeny :

- $P_R$, $P_L$, $P_{R'}$ active : synthesize N, Cro
- antitermination by N : synthesize cIII, cII, Q

Lysogeny:

- cII stimulate expression from $P_{RE}$ (cI repressor) and $P_{INT}$ (integrase)
- cIII stabilizes cII
- cI repressor shuts off $P_R$, $P_L$, $P_{R'}$ (no lytic functions), stimulates $P_{RM}$

# Factors favoring lysogeny cause increased concentrations of repressor vs. Cro

- **High multiplicity of infection**
  - More templates produce more of the CII protein, which stimulates $P_{RE}$.
  - Phage sense that it is too crowded.
- **Poor nutrient conditions for host**
  - Low [glucose] leads to increase in [cAMP].
  - Increased [cAMP] will **repress** the host gene *hflA*.
  - Less HflA (a protease) leads to less degradation of the CII protein.

# Problem ?

provide a quantitative approach to the competition between Cro and repressor for the λ operators.