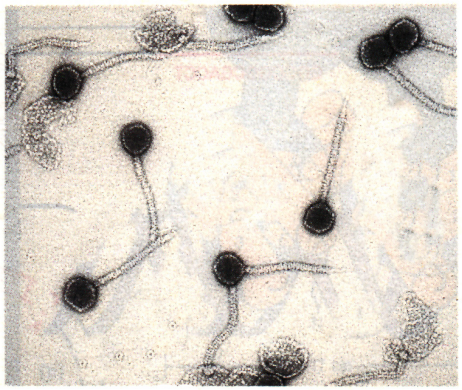
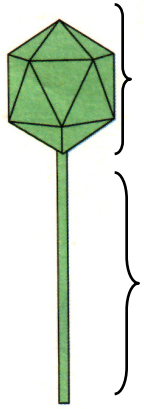
Complex procaryotic gene expression systems: The bacteriophage/batteriofago λ





testa icosaedrica

64 nm

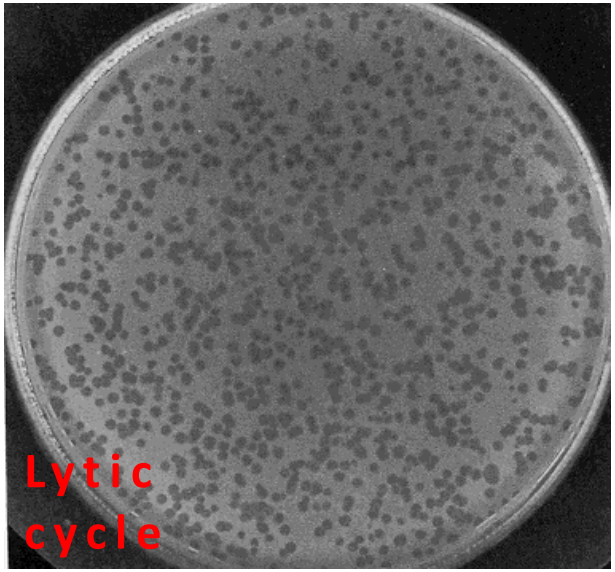
coda non contrattile

150 nm

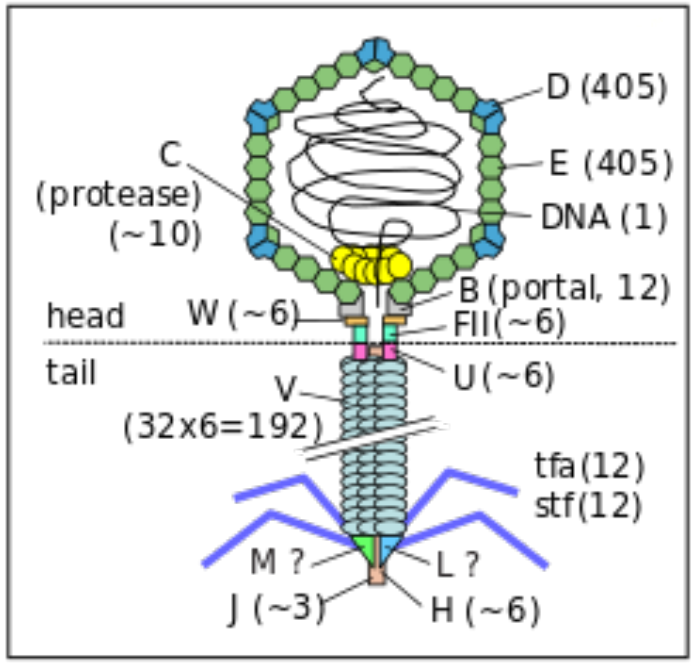
Infects bacteria

Lysogenic cycle

Lytic cycle



Placche di lisi da batteriofago λ su una piastra petri

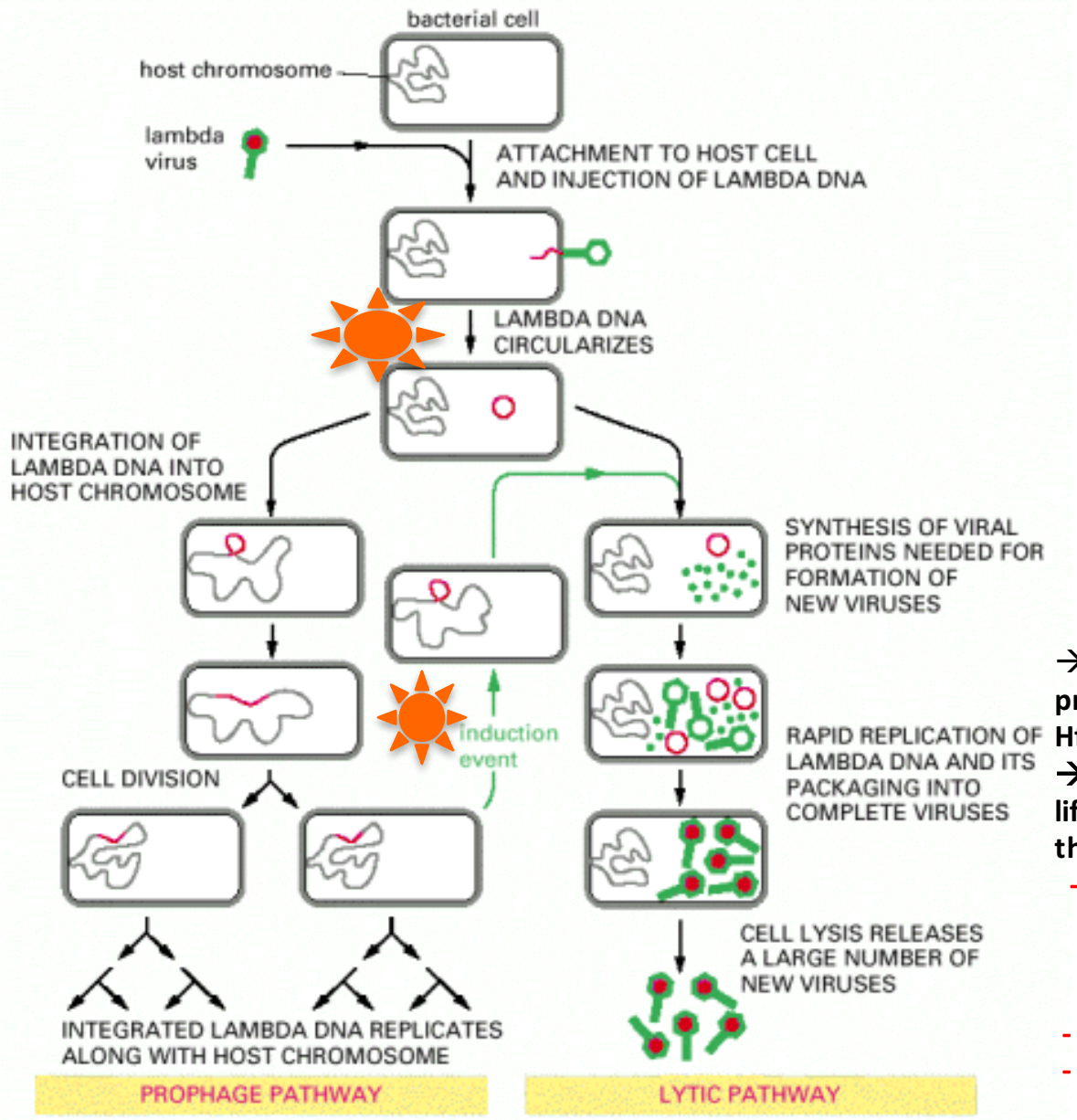


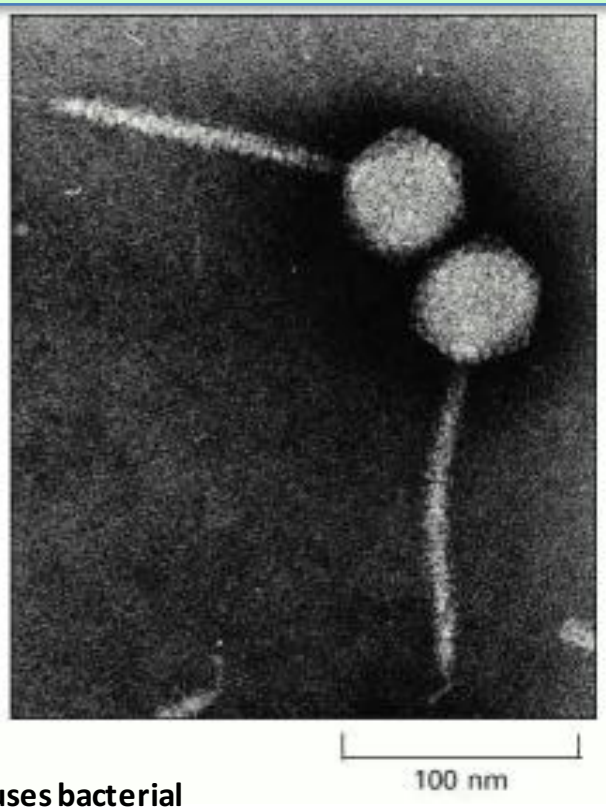
E' un batteriofago che infetta *Escherichia coli*.

E' costituito dal **genoma di DNA lineare a doppio filamento** lungo circa **48500** paia di basi avvolto dentro un **capside** proteico su cui è innestata una **coda**, le cui proteine terminali servono a riconoscere la membrana della cellula infettabile al cui interno inietta il suo DNA, che subito dopo assume la forma **circolare covalentemente chiusa**.

Successivamente, a seconda delle condizioni della cellula infetta può adire al **ciclo litico**, che porta alla produzione di numerose particelle fagiche figlie che si liberano all'esterno provocando la lisi del batterio, oppure può adire al **ciclo lisogeno**, in cui il suo genoma si integra in quello del batterio e rimane quiescente per un numero indefinito di replicazioni batteriche, per poi deintegrarsi in risposta a determinate condizioni ambientali e riprendere il ciclo litico.

The "double-life" of the bacteriophage λ



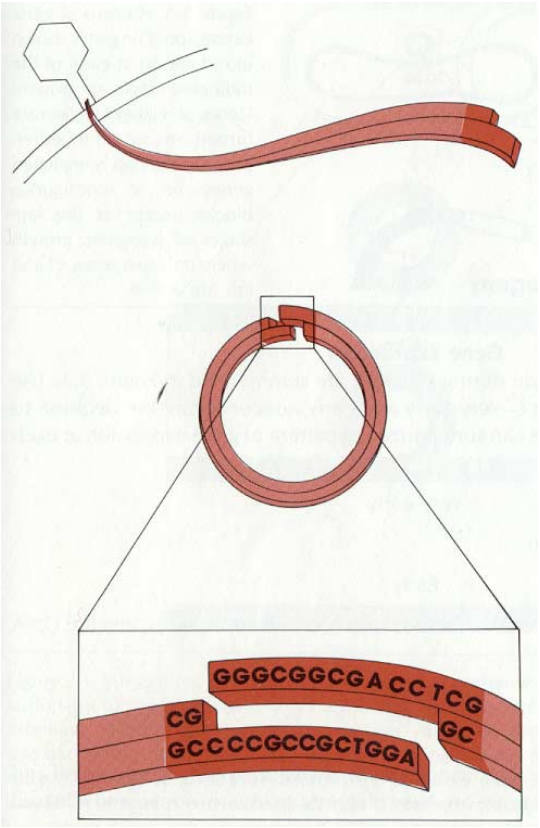


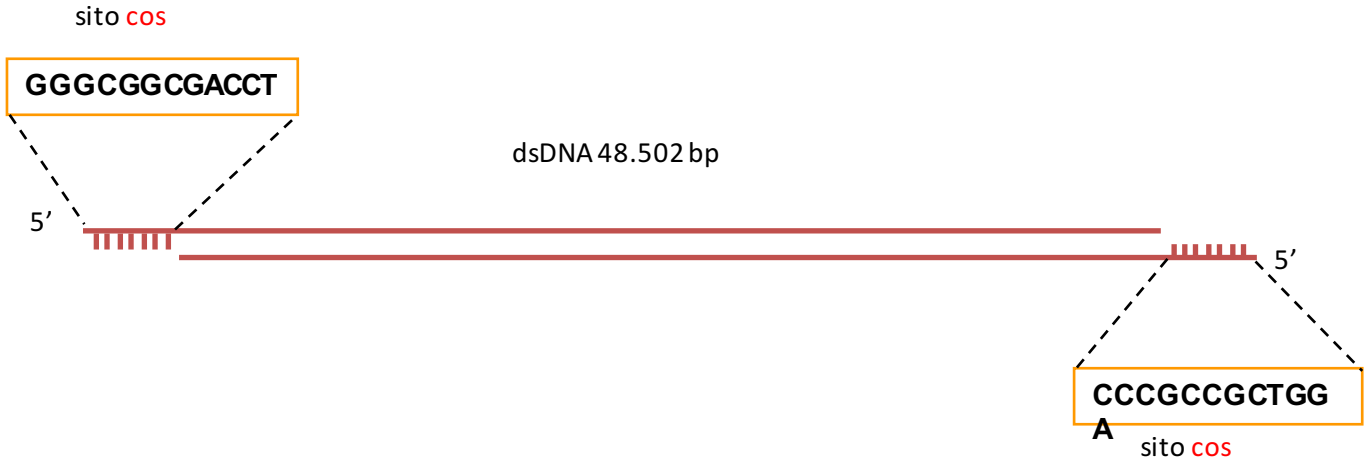
→Lambda uses bacterial proteins for gene regulation (RNA polymerase, Hfr, etc)

→ Switch from lysogenic to lytic lifecycle is tightly regulated on the gene expression level

- depends on phage/bacteria ratio in culture conditions
  - -b:p=1: lytic life cycle (enough bacteria to infect)
  - -b:p<1: lysogenic life cycle
- DNA damage in host
- growth conditions for E.coli

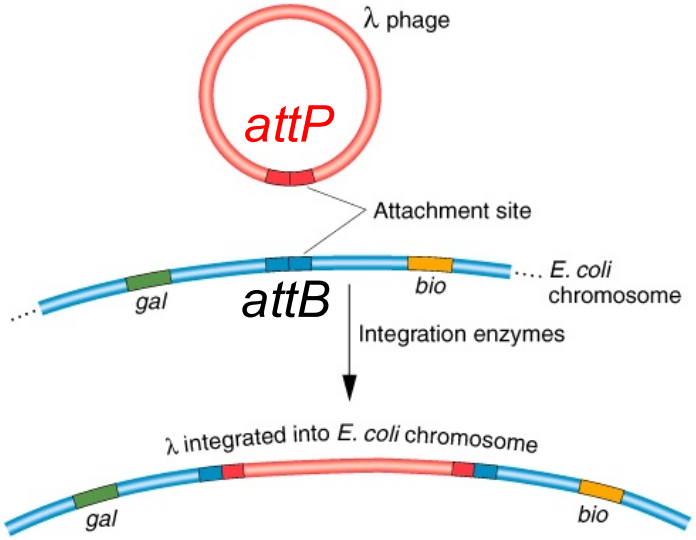
Linear Lambda DNA circularizes after entry into bacteria





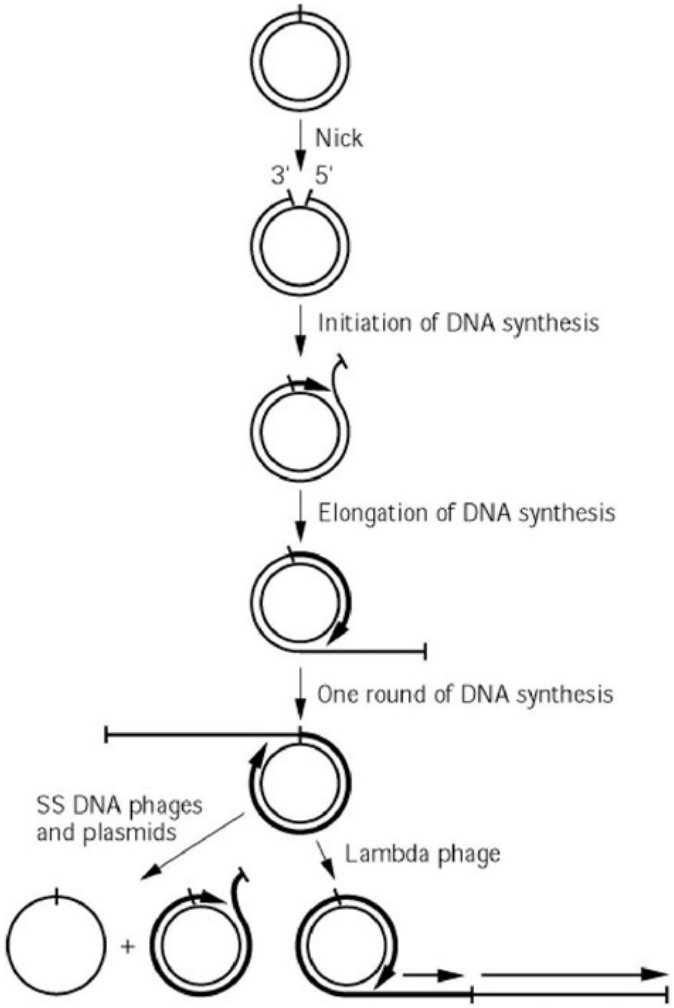
In seguito all'infezione di un batterio sensibile, il fago lamda inietta il suo DNA lineare che circolarizza all'interno del batterio grazie alle sequenze complementari dell'estremità (siti cos).

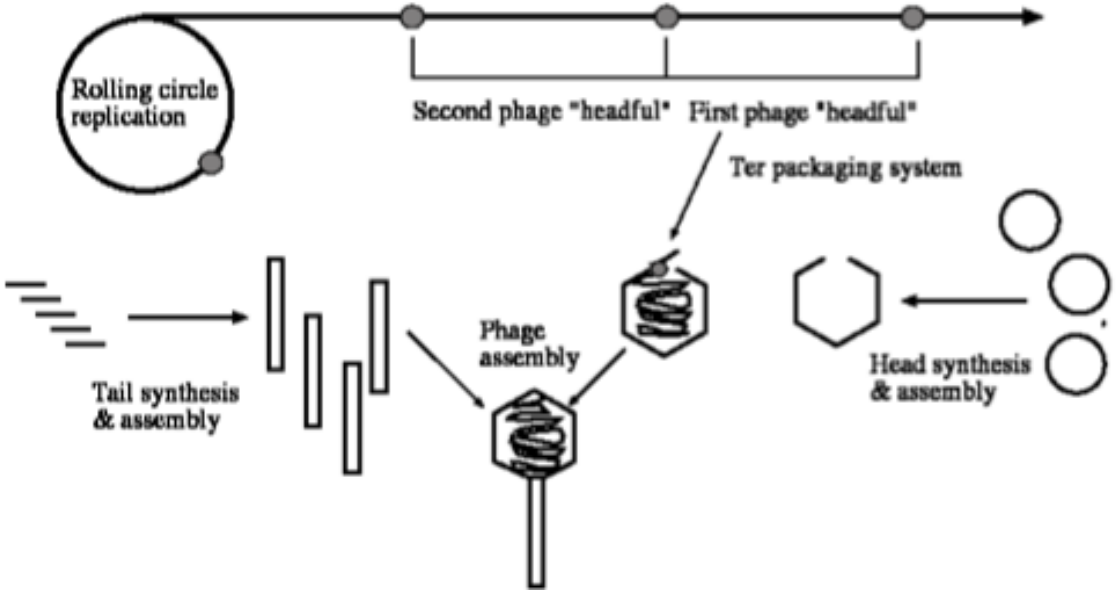
Circulated lambda DNA can integrate Into the bacterial genome using attachment sites
=lysogenic cycle
If circular phage DNA remains circular = start of lytic cycle



Rolling cycle" type of DNA replication (="Induction") precedes the induction of the LYTIC CYCLE (host lysed)

1. Circular DNA after infection
2. Insertion into host genome (activation of lysogenic life cycle)
3. Shift from lysogenic to lytic life cycle upon stimulus
4. Exit of cicular Phage DNA from host genome --> rapid replication via "rolling cycle replication"





HOST LYSIS AND INFECTION OF OTHER BACTERIA

Genomic structure of the Lambda phage

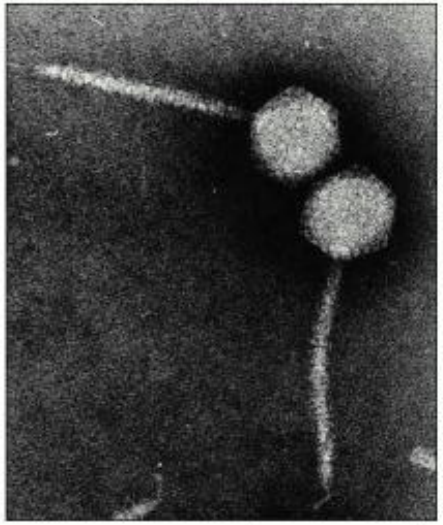
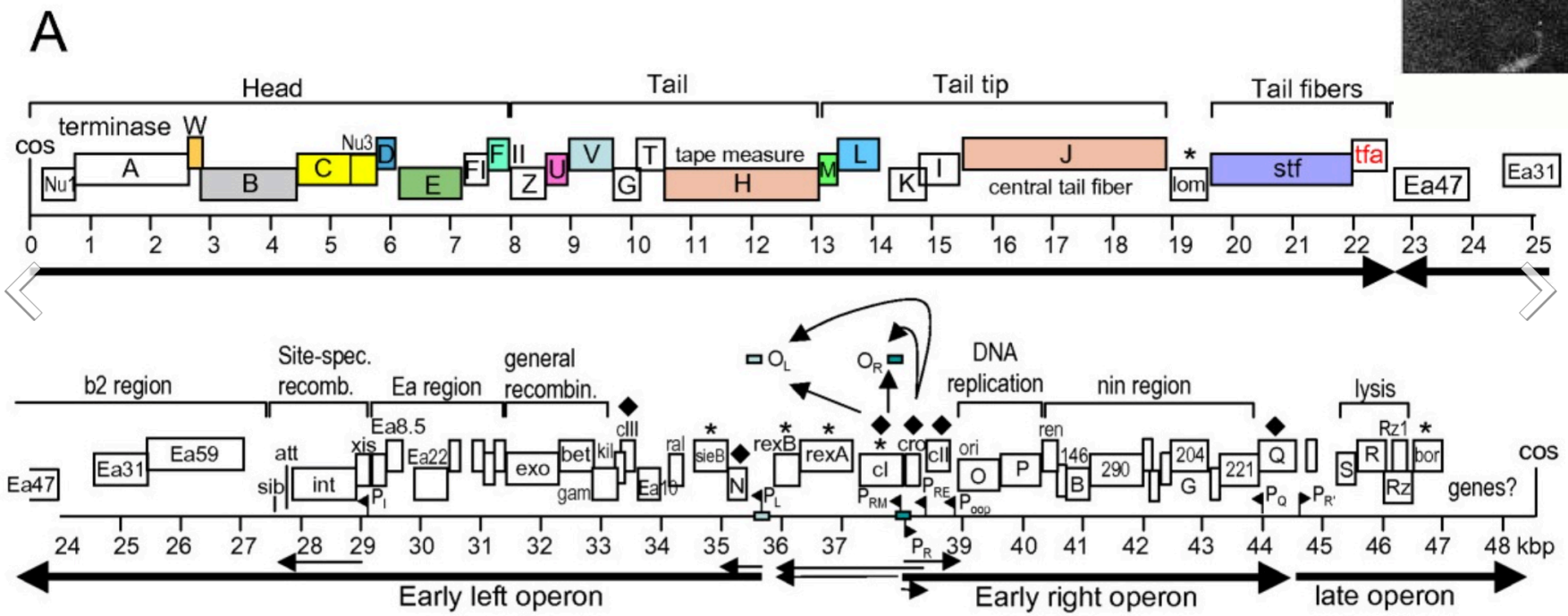


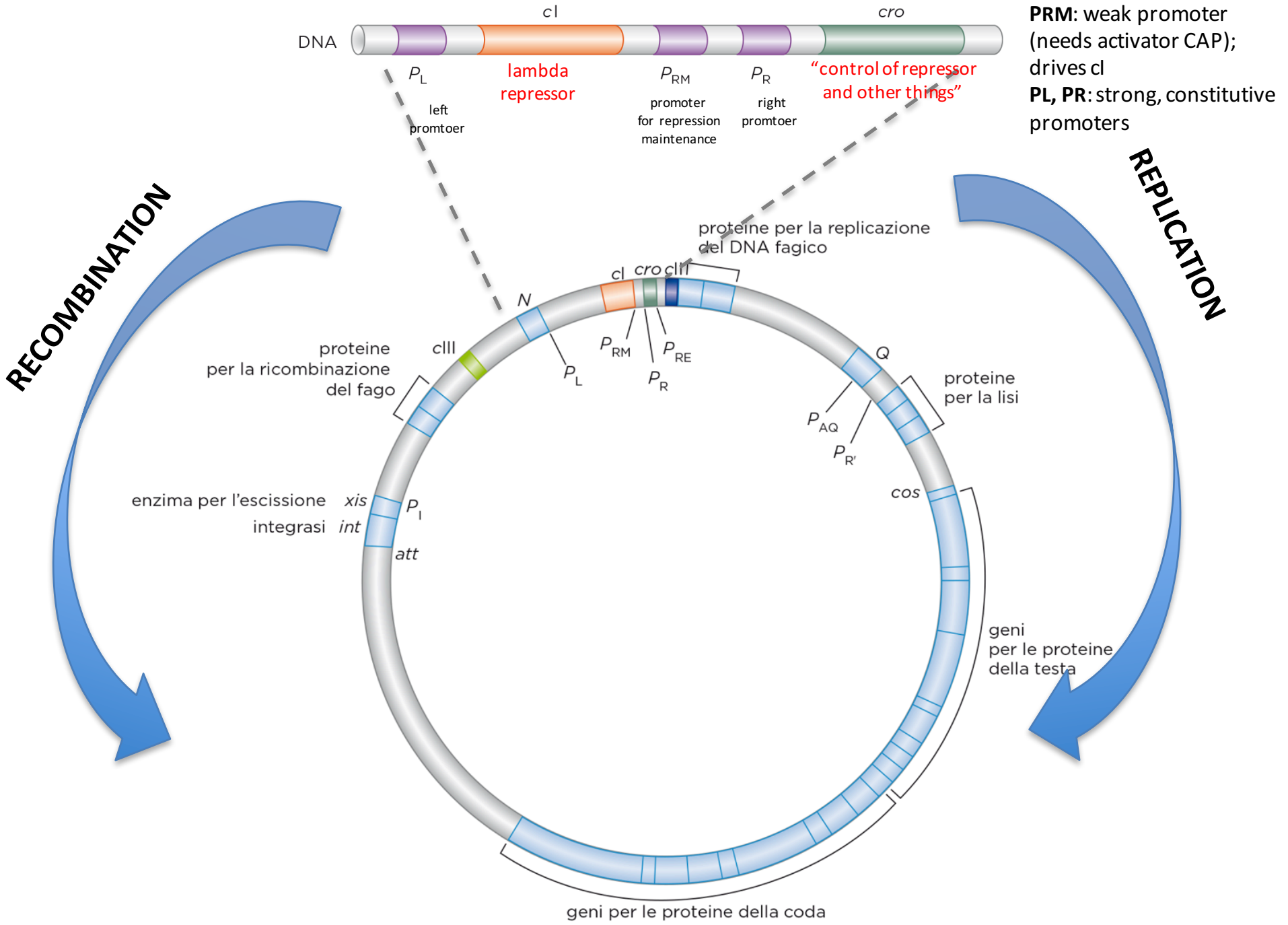
Figure 1.



→ 50 genes, predominantly encoding for proteins for capsid formation, replication, recombination and lysis

→ 7 promoter elements that control the decision between lytic and lysogenic cycle (P_L, P_{RM}, P_R, P_{RE}, P_{AQ}, $P_{R'}$, P_I)

Genomic structure of the Lambda phage

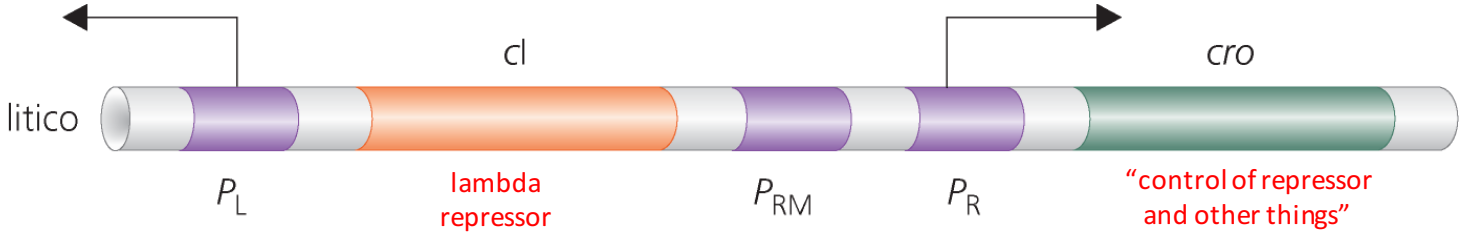


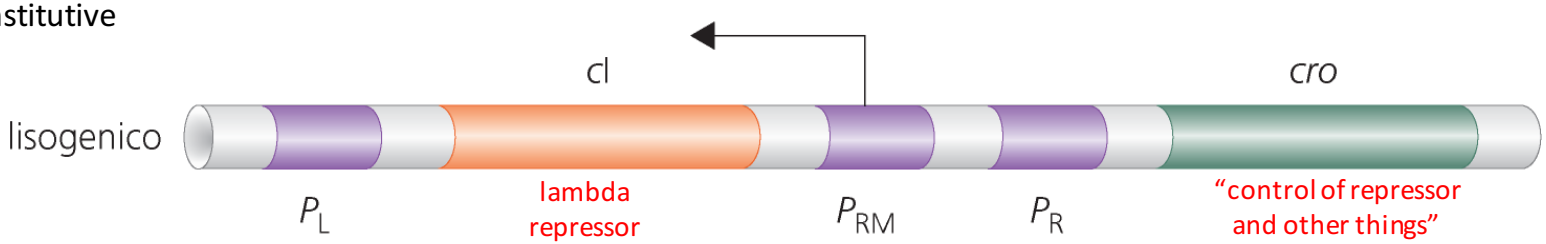
PRM: weak promoter (needs activator CAP); drives cI

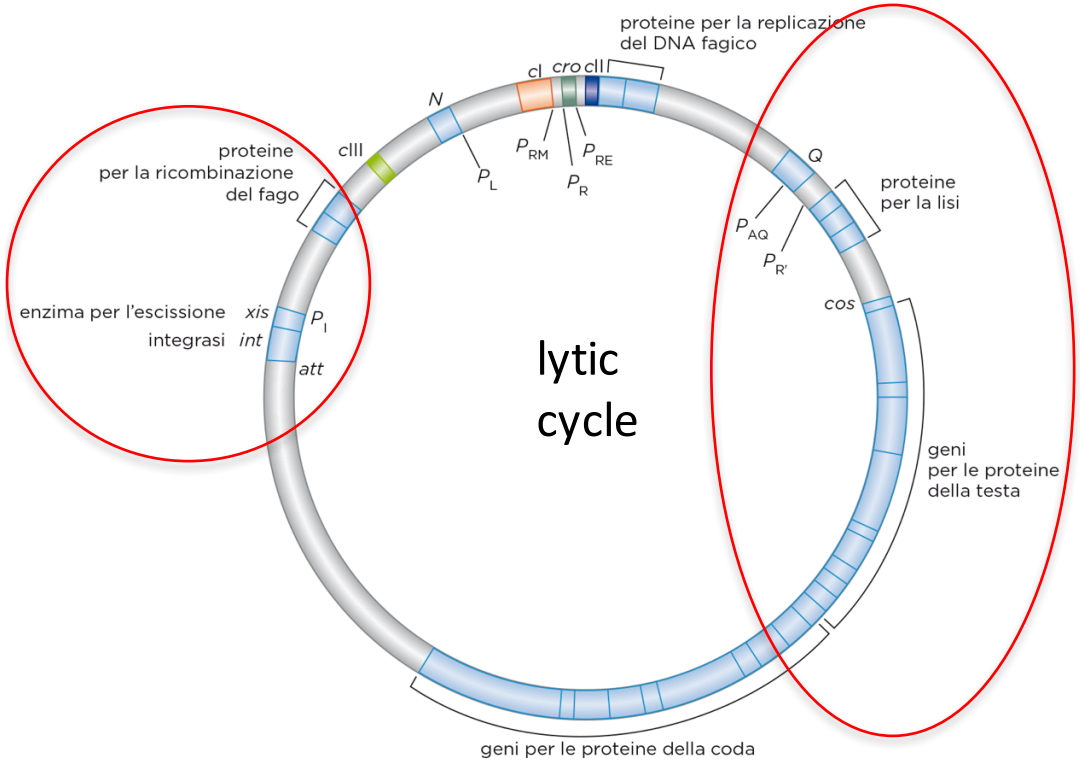
PL, PR: strong, constitutive promoters

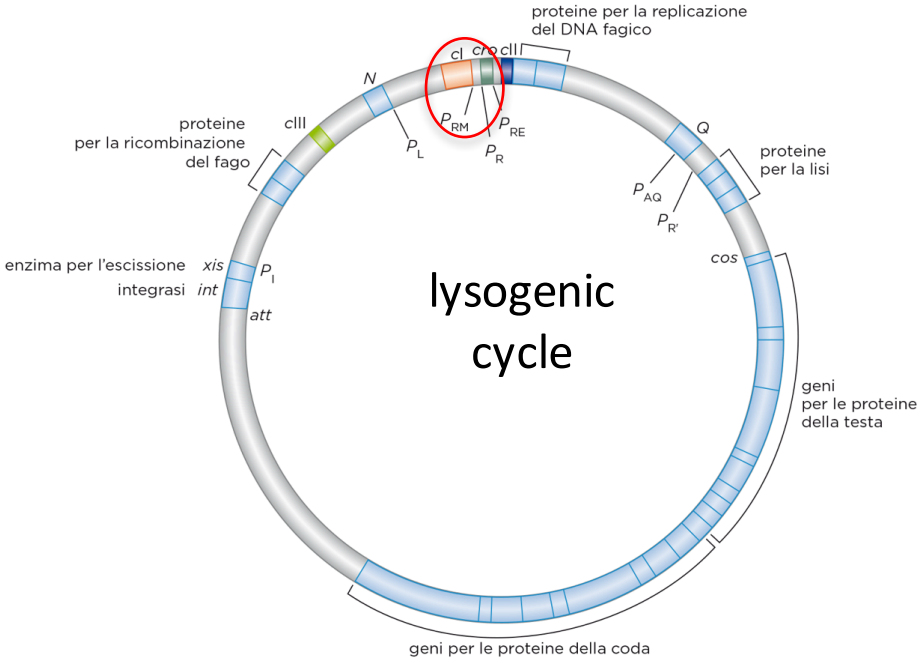
Introduction into the key promoters of the Lambda phage

PRM: weak promoter (needs activator CAP); drives cI
PL, PR: strong, constitutive promoters

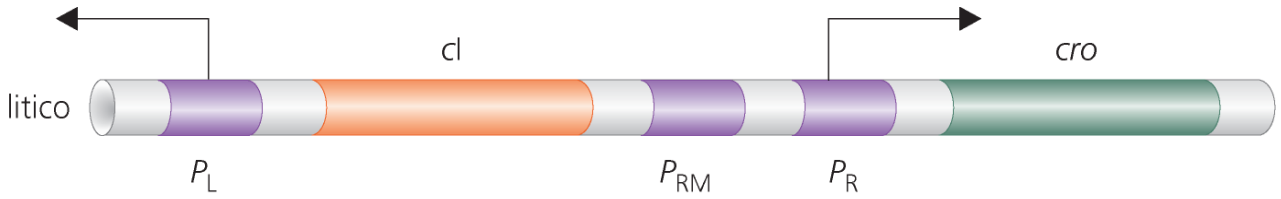






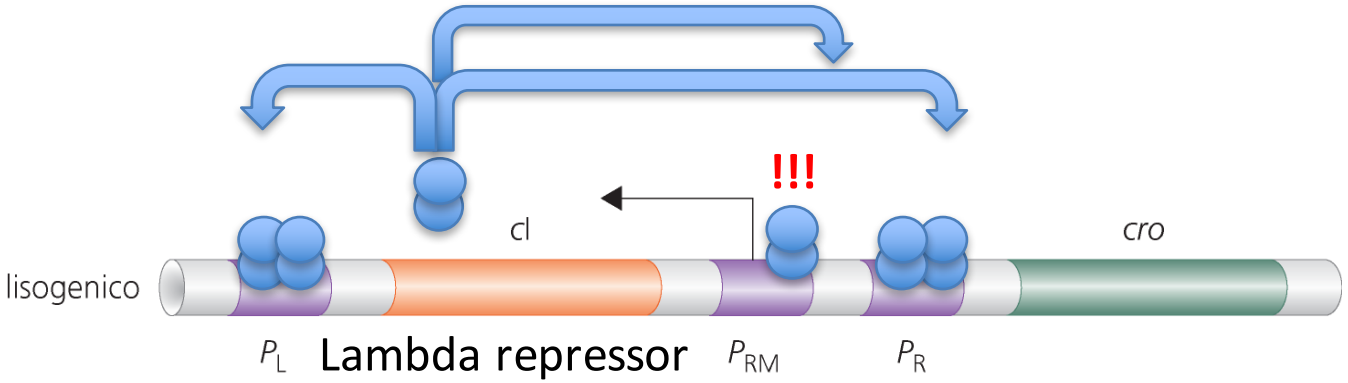


Introduction into the key promoters of the Lambda phage



P_L and P_R promotors are active: expression of genes that are essential for the lytic life cycle

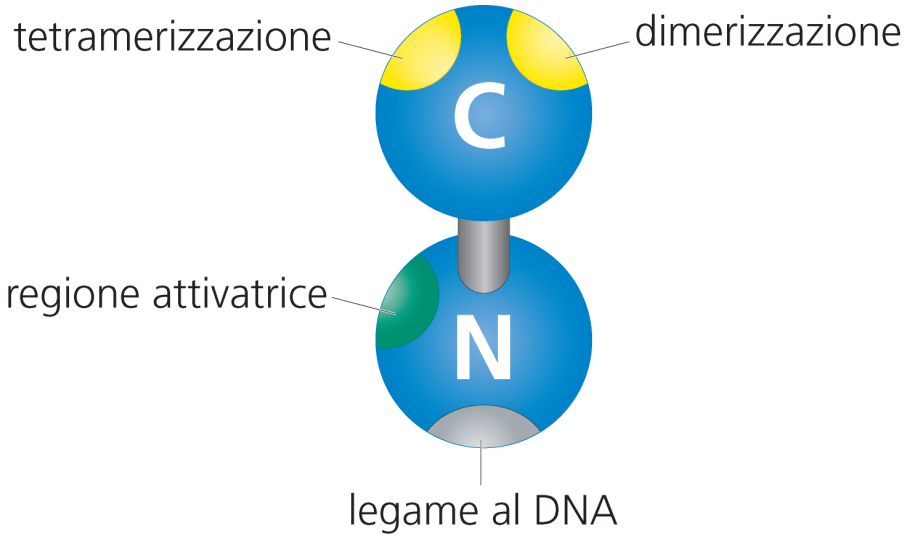
Cro (control of repressor and other things) suppresses the expression of cI (Lambda repressor → P_L and P_R remain active)



Lamda repressor is expressed and represses the P_L and P_R promotors and represses the expression of cro

Introduction into the key promoters of the Lambda phage: The lambda repressor and cro (control of repressor and other things)

LAMBDA REPRESSOR



Encoded by cI:
E' costituito da due regioni unite da una regione flessibile.
Si lega come **dimero**.
→può funzionare da repressore, legandosi a regioni che si sovrappongono al promotore (excludes RNA Pol)
→ funziona anche da attivatore; come CAP. Recruits RNA Pol.

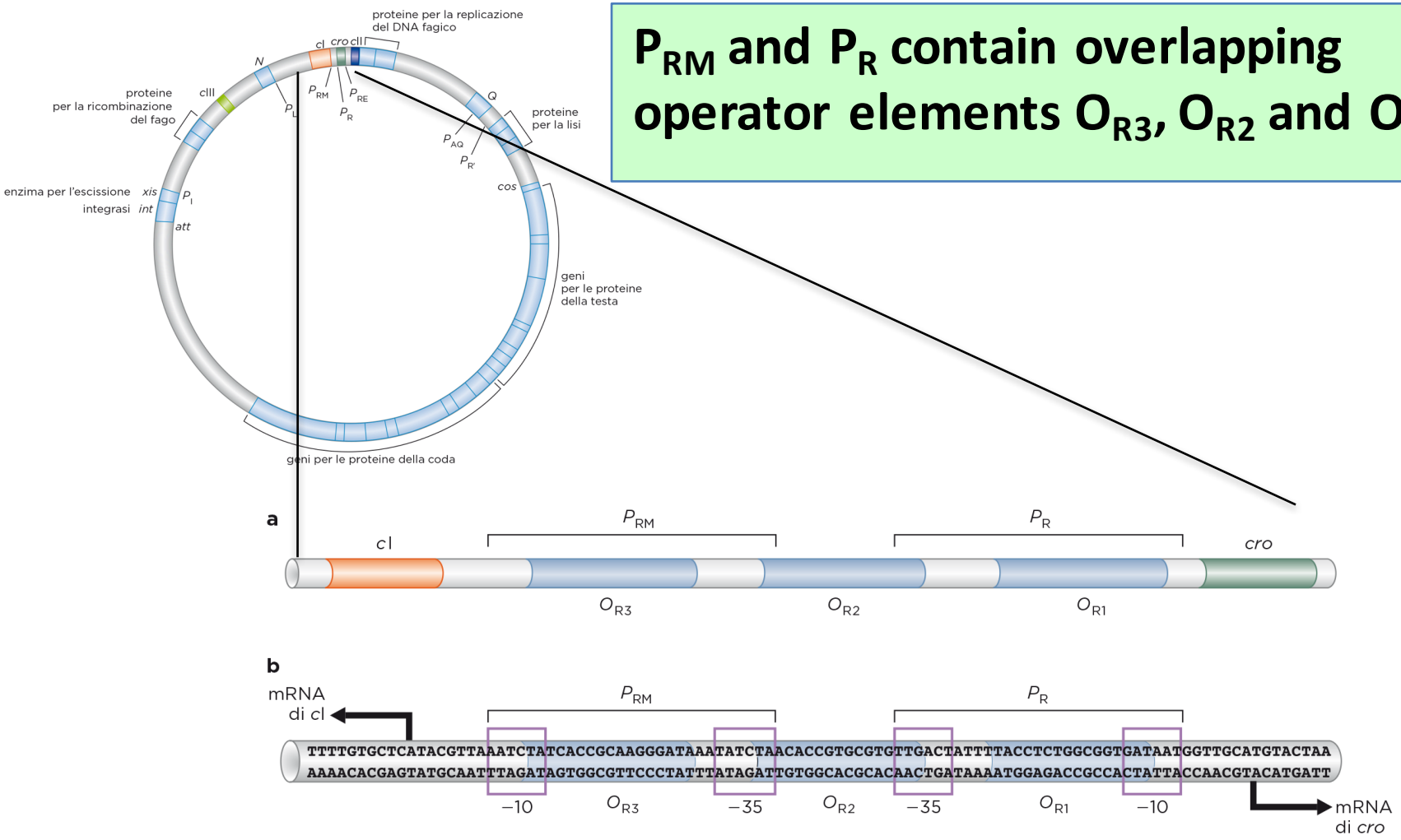
cro
(control of repressor and other things)

-single domain
-acts as dimer

Cro (*control of repressor and other things*) funziona solo come repressore.

Cro and the repressor have several binding sites that can also overlap
→balancing the occupation of promoters by Cro/repressor controls
Decision between from lysogenic and lytic cycle.

P_{RM} and P_R contain overlapping operator elements O_{R3}, O_{R2} and O_{R1}

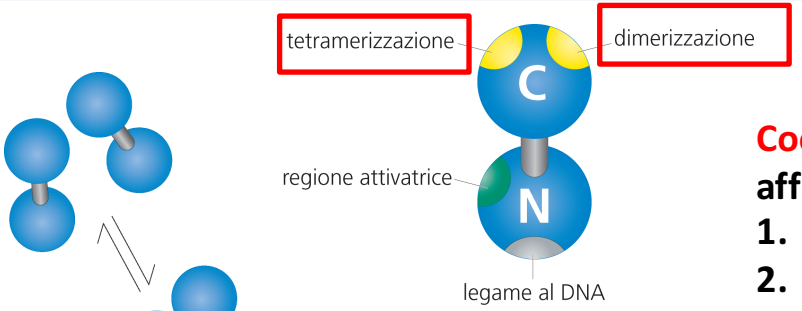


O_{R3}, O_{R2} and O_{R1} are bound by the lambda repressor AND cro

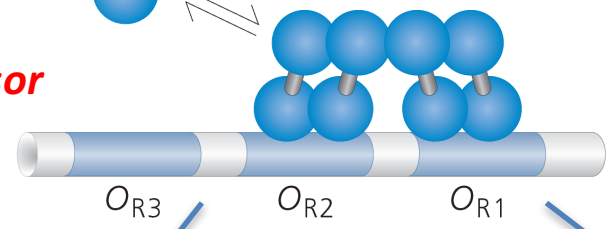
Affinity: lambda repressor dimer: O_{R1}>O_{R2},O_{R3} (10>1,1)

cro dimer: O_{R3}>O_{R2}, O_{R1} (10>1,1)

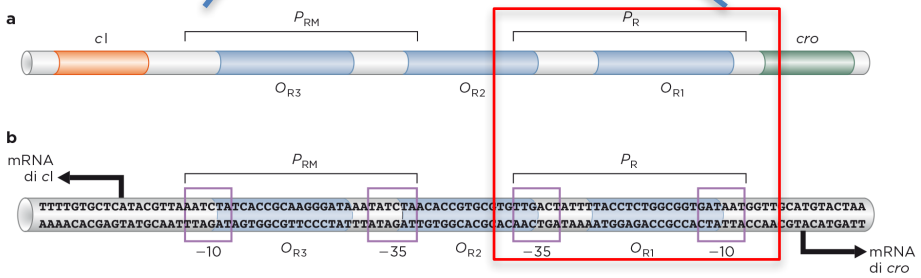
The lambda repressor shuts down the expression of lytic genes



Lambda repressor



Repressor tetramers



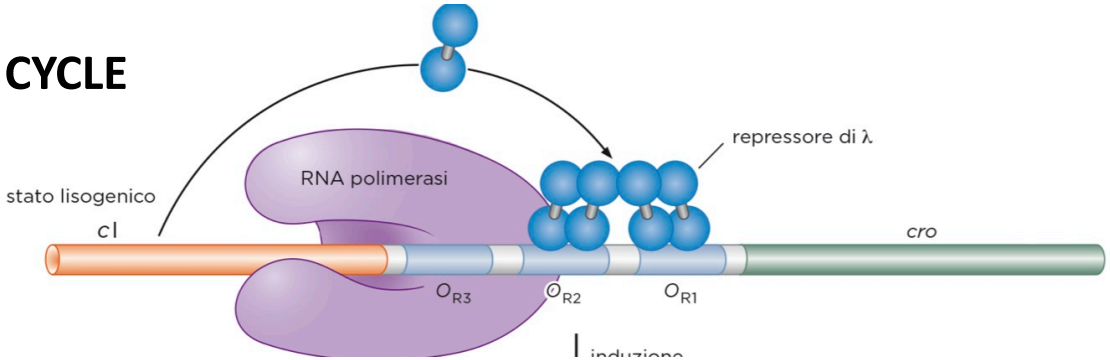
Cooperative binding of the lambda repressor dimer:
affinity: O_{R1}>O_{R2},O_{R3} (10>1,1)

1. Repressor dimer binds with high affinity to OR1
2. tetramerization domain allows the formation off tetramers, thus enhancing the efficiency of binding to OR2

RESULT: repression of P_R; no cro expression; P_{RM} used to transcribe more repressor – cI protein
Recruits RNA pol
-(positive autoregulation/autoregualzione positiva)

Cooperative binding:
- Increasing of binding affinity by additional interaction
- Represents a sensitive tool for gene regulation; changing cooperative binding features can result in immediate changes in gene expression

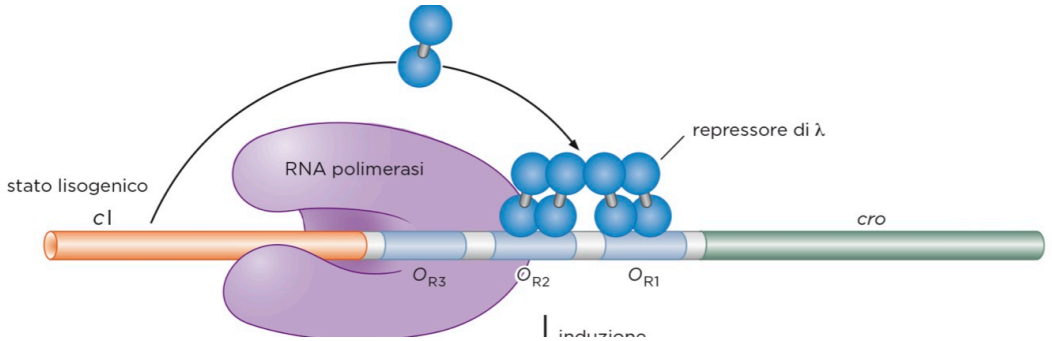
LYSOGENIC CYCLE



1. lambda repressor bound to $O_{R1,2}$ represses P_R ($+P_L$)→ no lytic cycle
2. Lambda repressor bound to $O_{R\backslash,2}$ directs the usage of P_{RM} by RNA Pol
→More repressor production
= POSITIVE AUTOREGULATION

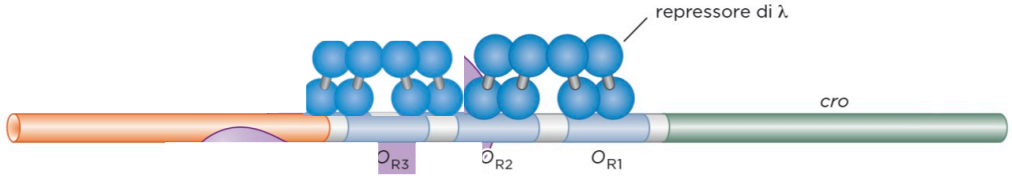
DUAL FUNCTION of the Lambda repressor: Activator and Repressor: Controlled by positive and negative autoregulation

POSITIVE AUTOREGUALTION



- Cooperative binding of repressor tetrameres to $O_{P1,2}$ causes repression of P_R.
- P_{RM} active to produce more lambda repressor

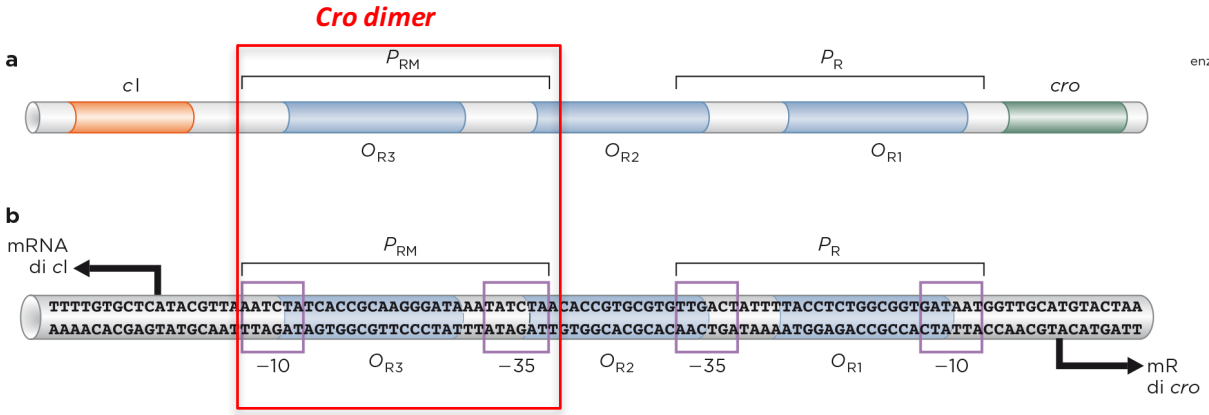
NEGATIVE AUTOREGUALTION

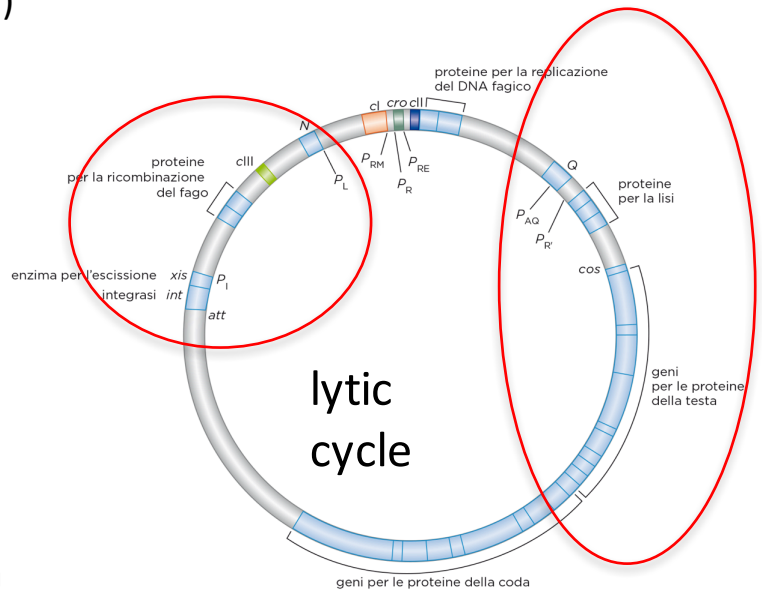


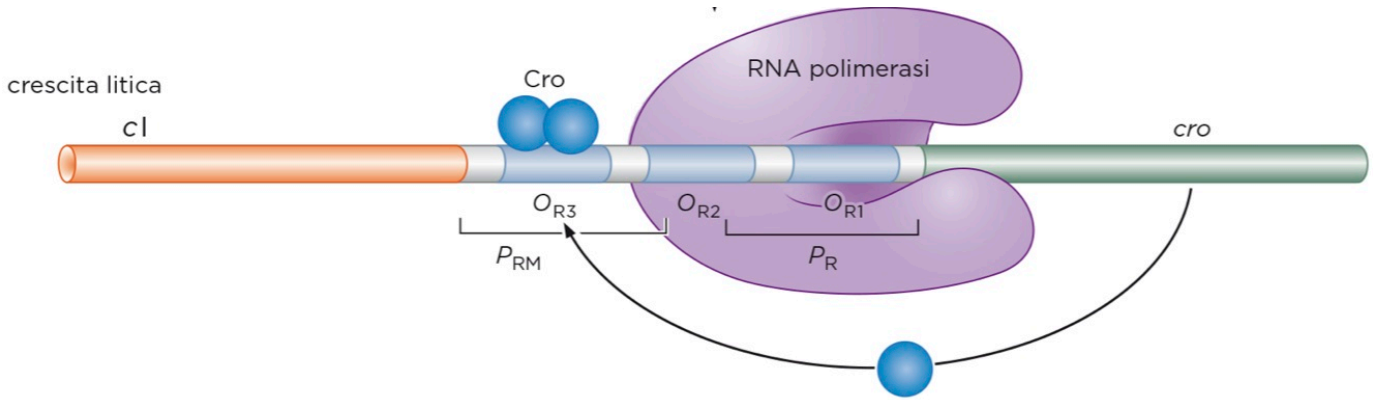
-Production of lambda repressor increases until concentration is high enough to bind O_{R3} (the low affinity binding site for the repressor
-this leads to the REPRESSION of P_{RM}
-When lambda repressor levels decrease repression of P_{RM} is reversed

Cro represses the lamda repressor: activation of lytic cycles

Cro dimer binds OR3 with high affinity (access of RNA Pol to pRM is blocked)
→repression of cI (lambda repressor gene)
→**RNA polymerase uses P_R (and also P_L) to transcribe lytic genes**



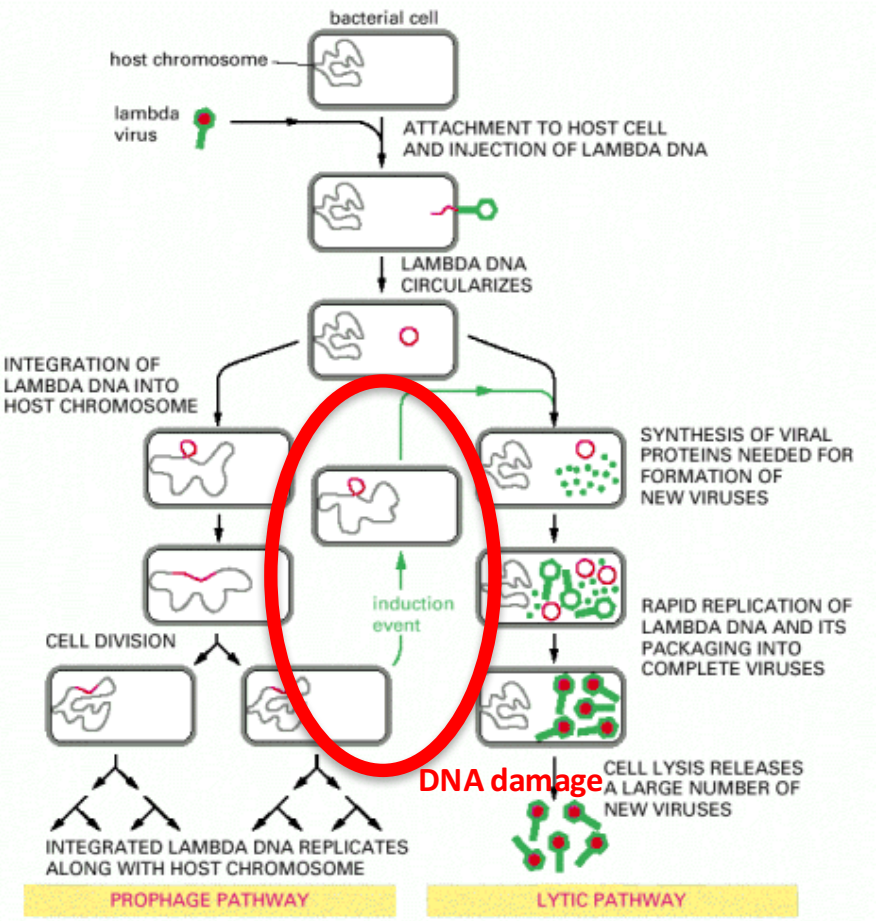




LYTIC CYCLE

INDUCTION: transition from lysogenic to lytic cycle

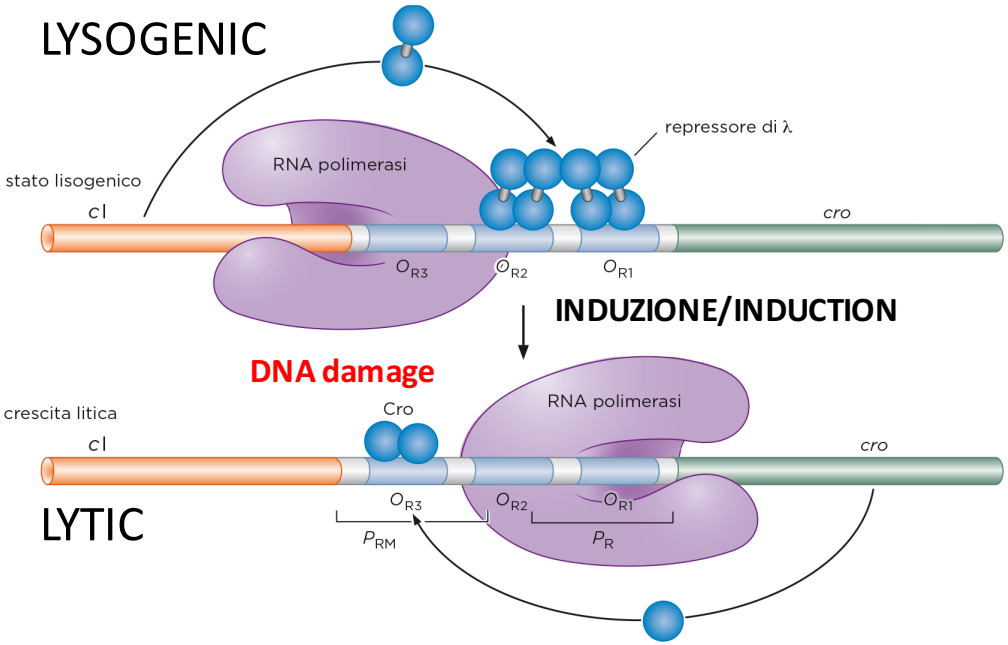
PHYSIOLOGIC CONDITION



Lysogenic:
Low bacteria/phage ratio
(lack of bacteria to infect)

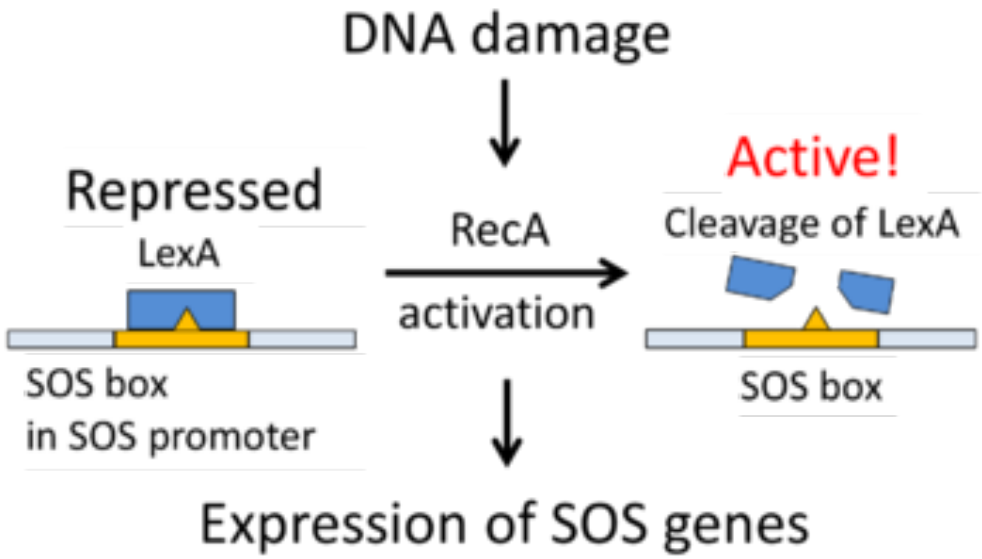
Lytic:
High bacteria/phage ratio
(a lot of bacteria to infect)

GENE EXPRESSION



INDUCTION: transition from lysogenic to lytic cycle for example: DNA damage

Lambda phage uses host SOS DNA damage response to activate the lytic response by degrading the Lambda repressor



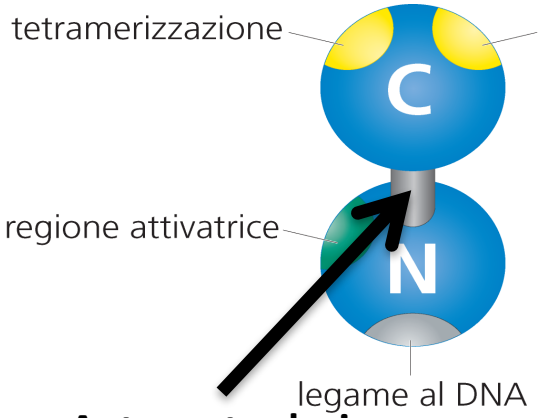
When DNA is significantly damaged (e.g. by exposure to UV radiation or chemicals), several DNA damage-related proteins are synthesized quickly. This reaction to DNA damage is known as SOS response.

RecA of *Escherichia coli* is a 38 kilodalton protein essential for repair and maintenance of DNA. RecA ha multiple activities, all related to DNA repair. In the bacterial SOS response, it has a co-protease function the autocatalytic cleavage of the LexA repressor and the λ repressor. lexA gene is expressed constitutively and prevents expression of damage-related proteins by binding to a SOS box as a repressor. RecA is activated by binding to single-stranded DNA, and the activated RecA then turns on LexA protease activity. Self-cleavage of LexA derepresses the expression of damage-related proteins enabling a response to be mounted.

We decided to employ the promoter of the recA gene of *D. radiodurans* (**BBa J22106**) to detect DNA damage. While RecA is an inducer of SOS genes, it itself is an SOS gene that is auto-induced upon DNA damage. Expression of genes downstream of this promoter is induced by DNA damage.

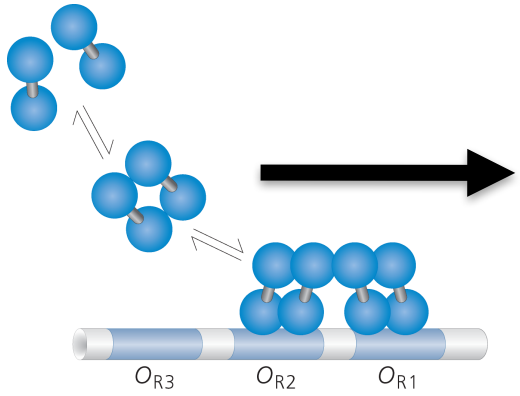
INDUCTION: transition from lysogenic to lytic cycle

DNA damage → bacterial SOS response → bacterial RecA stimulates autoproteolysis of LexA but also autoproteolysis of the lambda repressor
→ induction of lytic cycle

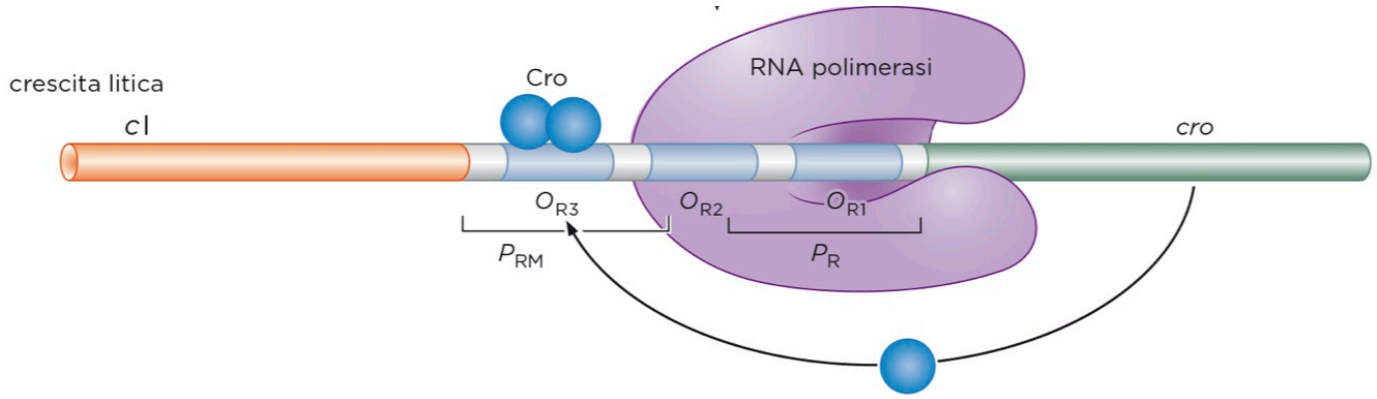


Autoproteolysis (removal of C-terminal domain)

No di/tetramerisation NO COOPERATIVE BINDING OF O_{R2}



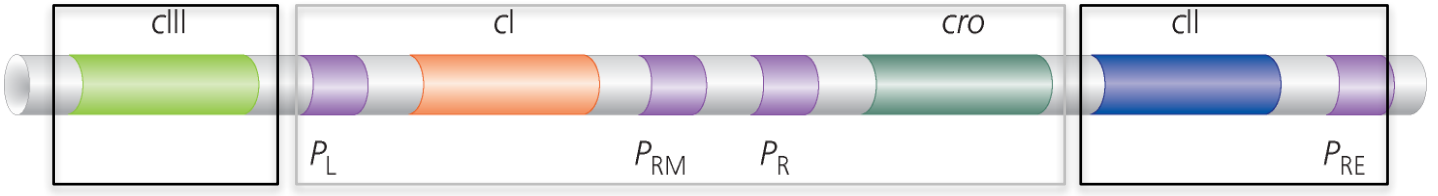
P_{RM} active
→ cro expression
→ repression of P_{RM} (lambda repressor)
→ P_R and P_L active
→ expression of lytic genes



LYTIC CYCLE

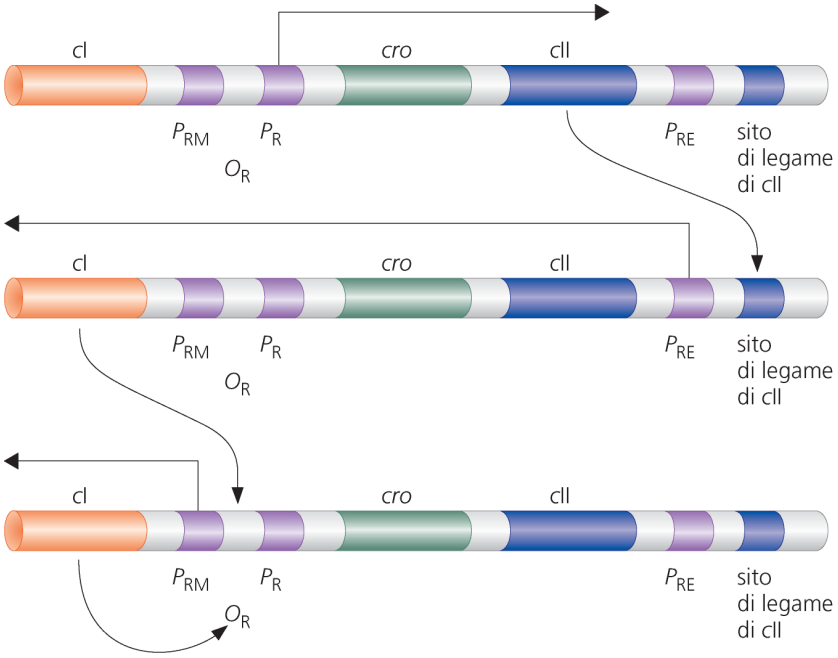
DECISION ON KIND OF LIFE CYCLE UPON INFECTION:
LYTIC or LYSOGENIC CYCLE??

Important factors:
cII and cIII



cII: activator of transcription: activates transcription of cI (lambda repressor) from P_{RE} by recruiting RNA Pol. Note: cI expression driven by P_{RM} and P_{RE} promoters. essential to reach high

cIII: is required to reach repressor levels that allow dimerization/tetramerization



ESTABLISHMENT OF LYSOGENIC CYCLE

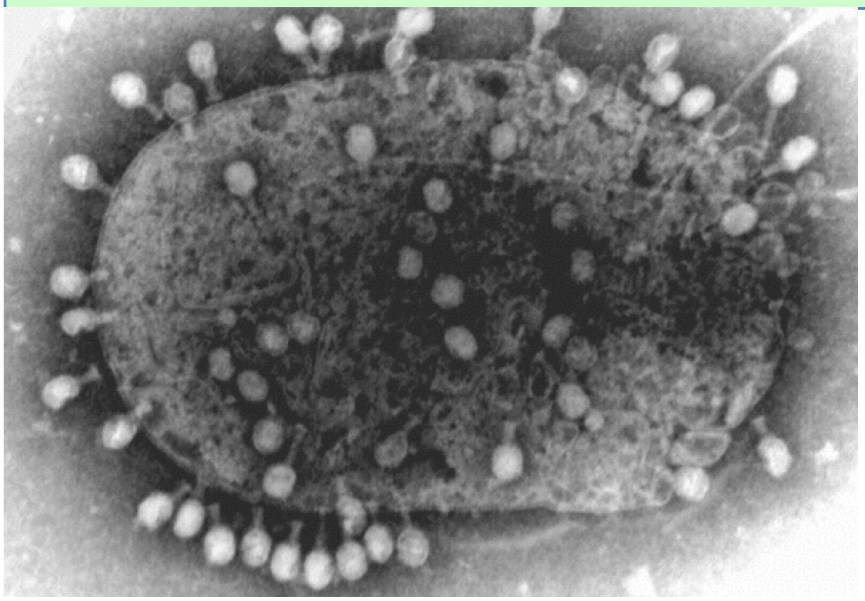
1. Production of cII → lambda repressor production

   =ESTABLISHMENT OF REPRESSOR FUNCTION

2. Negative/Positive autoregulation of lambda repressor levels

   =MAINTENANCE OF REPRESSOR FUNCTION

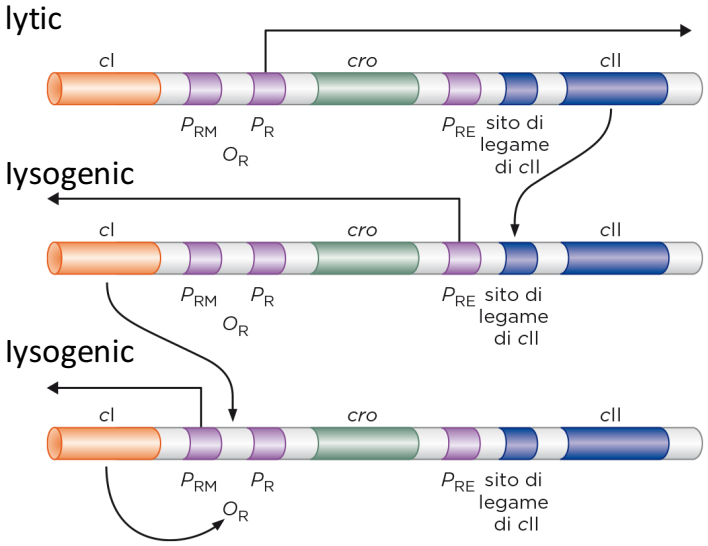
DECISION ON KIND OF CYCLE UPON INFECTION:
LYTIC or LYSOGENIC CYCLE??



Early phase of infection:

Active promtoter:
P_R → drives cro and cII (and as a consequence cI)

cro
Lytic ←→lysogen
cII



Mulitplicity of infection (moi)
(molteplicità d'infezione):

1 or less than 1 phage per bacterium

cro
Lytic ←→lysogen
cII

2 or more than 2 phages per bacterium

cro
Lytic ←→lysogen
cII

DECISION ON KIND OF CYCLE UPON INFECTION:
LYTIC or LYSOGENIC CYCLE??

Early phase of infection:

HOST GROWTH CONDITIONS ARE EXPRESSED BY THE PRESENCE OF THE FtsH Protease → degrades CII

good
(rapid growth,
many bacteria)

FtsH Protease high (hfl gene)

cro

Lytic ←→lysogenic

cII

FtsH

~~cII~~

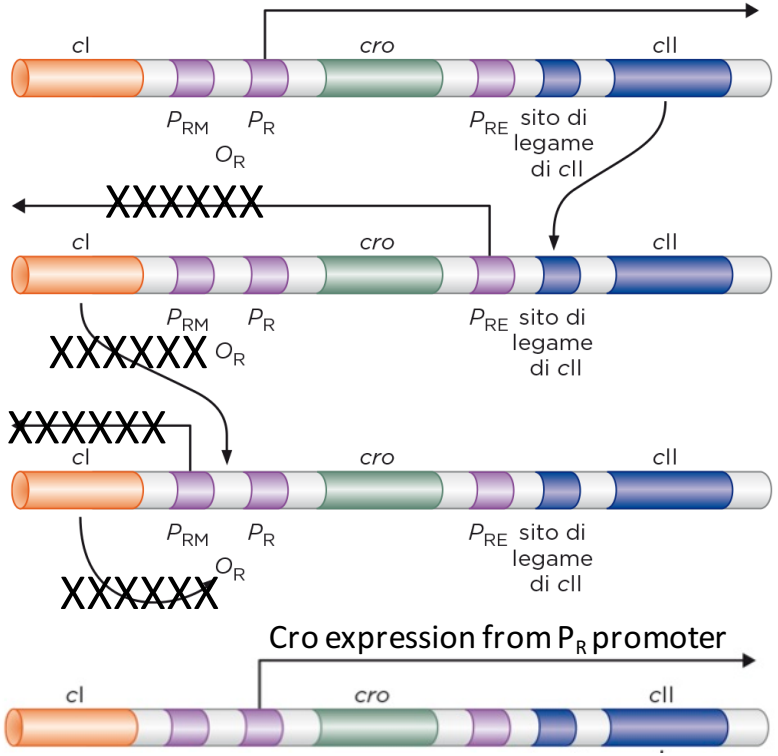
bad
(Slow growth,
low number of bacteria)

FtsH Protease low (hfl gene)

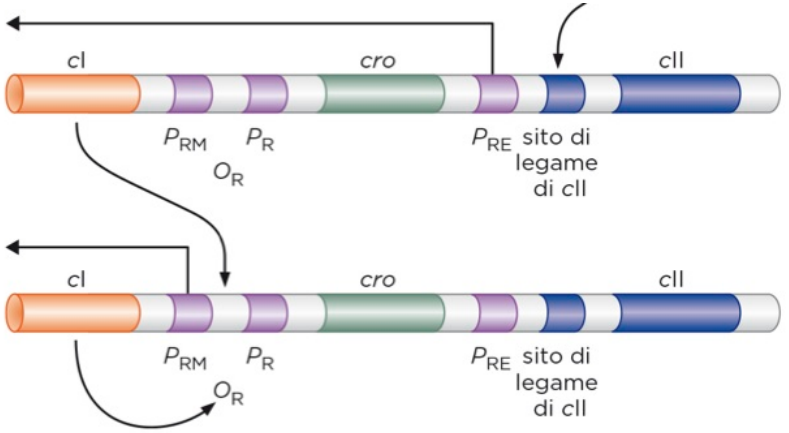
cro

Lytic ←→**lysogenic**

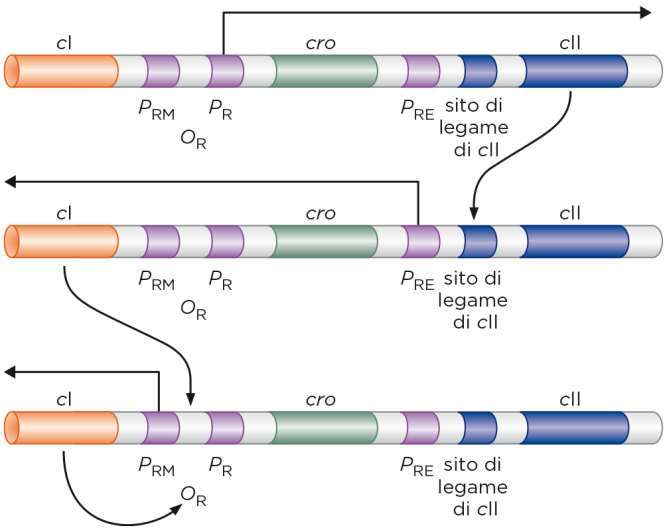
cII



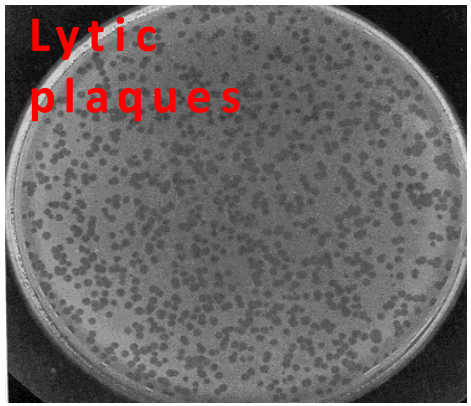
High cII



EXPERIMENTAL IDENTIFICATION OF cI, cII and cIII



Lambda that carries mutations in cI, cII and cIII cannot produce the lambda Repressor
→cannot enter into the lysogenic cycle



Lambda is characterized by **immunity**:
Does not kill all bacteria after infection: some E.coli become immune and continue to grow slowly inside a plaque and carry a lysogenic phage

plaque

"clear"
limpido

"opache"
torbide

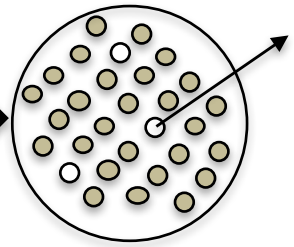
Typcial lambda plaque

culture of bacteria
+
lambda phage

Induction
of
Mutations
(DNA damaging
agents)

purify
Lambda phage
Particles
(mutant)

plate
on
confluent
E.Coli
dish



100% lytic mutant phage!!!
Isolate
→Purify DNA
→Identify cI or cII or cIII mutation by sequencing

Named after phenotype
Of plaque: clear I,
clear II, clear III.

purify
Lambda phage
Particles
(wild-type)

plate
on
confluent
E.Coli
dish