THE GENETICS & BIOLOGY of BACTERIOPHAGE λ

GENE REGULATION / TRANSCRIPTION REGULATION, LYTIC VS. LYSOGENIC INFECTION CYCLES

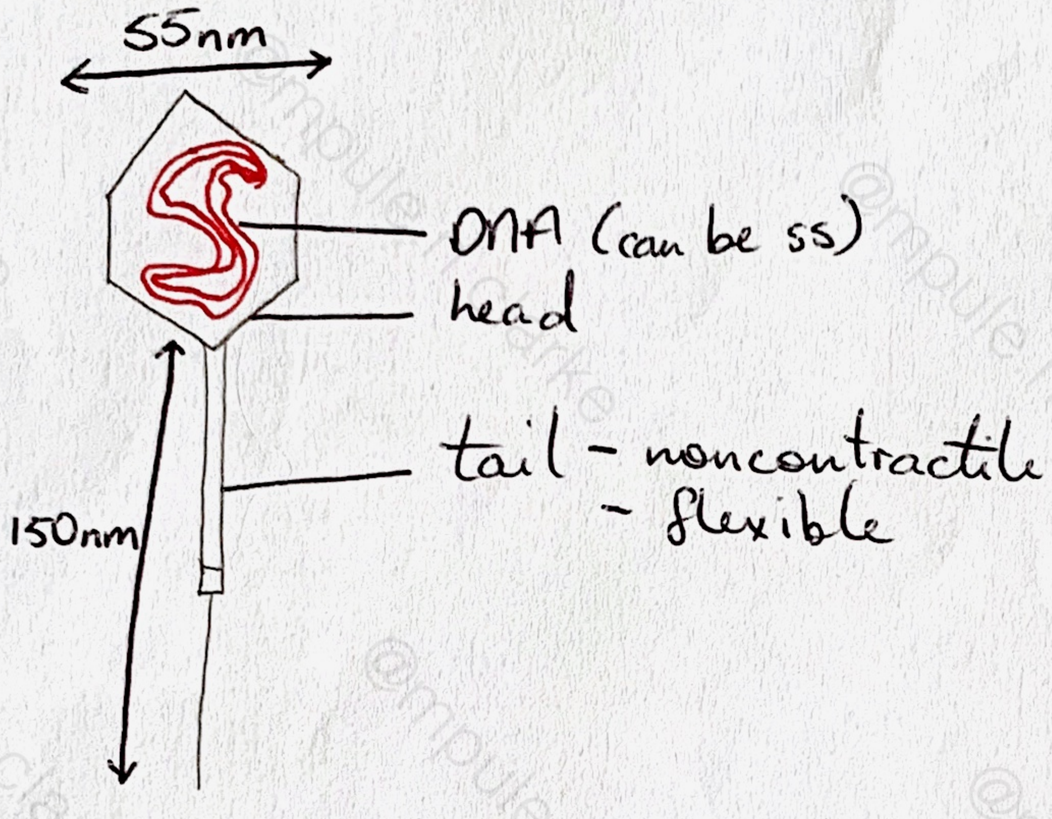
Objectives

- To explain gene organization & expression in bacteriophage λ
- To describe the role of CI & Cro in λ phage transcription regulation.
- To explain the factors determining lytic growth & lysogenic growth.
- To discuss antitermination & retroregulation.

Outline

- Morphology of λ phage
- Gene organization of λ phage
- Gene expression of λ phage
- The immunity region
- Control of transcription by CI & Cro
- Factors determining lysogenic & lytic growth.
- lysogenic growth of λ phage
- Lytic growth of λ phage
- Antitermination & Retroregulation.

Bacteriophage λ Morphology



Family : Siphoviridae
Host : K12 strain of E. coli

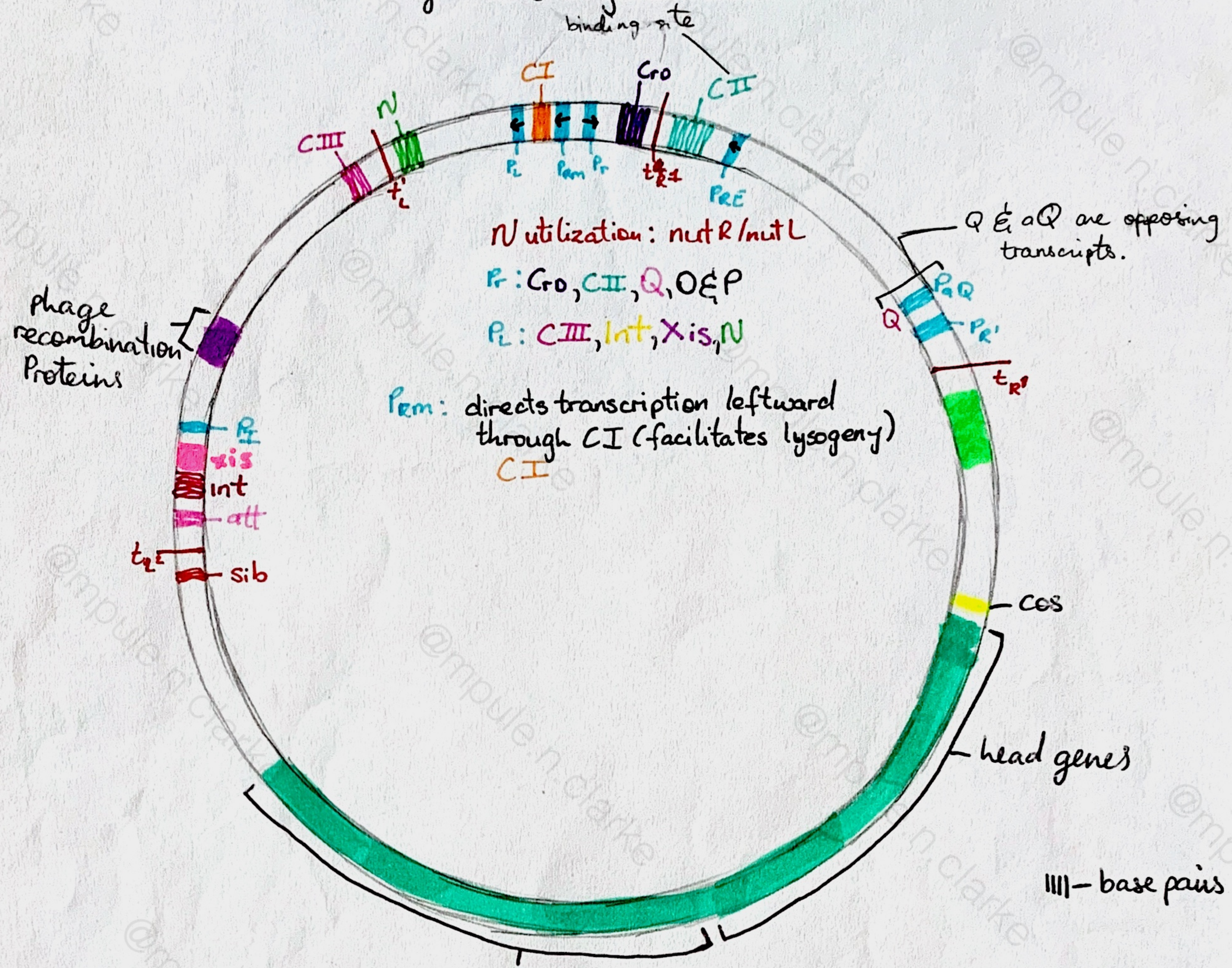
Bacteriophages are viruses that infect bacterial cells. λ phages infect specific E. coli cells. Once the health of the cell is ideal & and the phage quantity does not outnumber the bacterial cell quantity; the multiplicity of infection will be low. The phage will favor lysis if the protease levels outnumber the CIII & CII molecules being produced by the phage DNA in the lysogen.

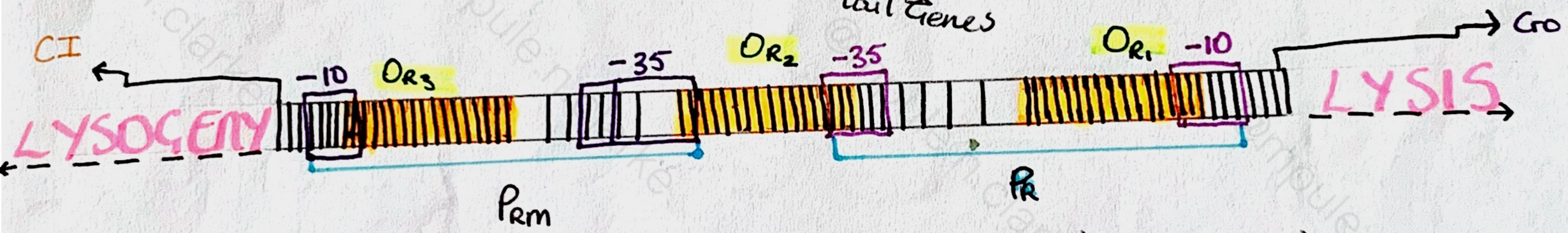
⇒ The phage will enter the lytic pathway as there will be more bacterial cells in the environment for it to infect.

If the moi is high or the protease levels are ~~high~~ low the phage DNA will favor lysogeny to preserve ~~the~~ its genetic material until conditions are ideal for it to reproduce & lyse from the bacterial cell.

low moi = lysis if protease levels are ~~low~~ high
low moi = lysogeny if protease levels are low
high protease levels = lysis
low protease levels = lysogeny

λ Phage Gene Organization





O_{R3} is completely in the Promoter for regulatory maintenance (P_{RM})

O_{R3} is overlapped with P_{RM} by 2 bp ; ovelapped with P_R by 3 bp

⇒ obstruction of O_{R3} blocks tranciption activity at P_{RM}

⇒ obstruction of O_{R1} blocks transciption activity at P_R

⇒ obstruction of O_{R2} blocks transciption activity at P_R but ~~allows~~ activates transciption at P_{RM} as it would be the only available promoter.

Operator regions obstruct sigma binding regions (-35 & -10)

CI attacks/has a prefrence for O_{R1} first so it will be obstructing the transciption of Cro. at P_R.

Cro attacks ~~or~~ O_{R3} first so it will be obstructing the transciption of CI at PRM.

20/11

Lysogeny:

1. infection ⇒ cell is now a lysogen
2. phage DNA circularizes at cos site.
3. Host cell holoenzymes attach to the strong promoters ~~& begin~~
   ⇒ P_L & P_R are strong promoters
4. PL transcribes the sequence for N protein which allows the core enzyme to ~~go~~ continue past the terminator sequences. ⇒ N is always present.
5. The presence of CII & CIII are what determine wether lysogeny ~~&~~ or lysis occurs. ⇒ P_R expresses ~~for~~ CII; if CII is favored then lysogeny occurs
6. Binding of CII blocks core enzyme from moving past nutR;
7. Restriction of RNAP at nutR results in P_{RM} directing RNAP through CI Towards ~~P~~ P_L
8. Cro favors O_{R3} ⇒ P_{RM} ~~is overlapped by O_{R3}~~ spans O_{R3} so cro binding blocks lysogeny; why Cro facilitates lysis. Whereas CI favors O_{R1}, O_{R1} obstruction will block lysis as RNAP will only be able to bind to P_L & P_{RM} ⇒ O_{R1} is a central part of P_R so CI will facilitate lysogeny
9. RNAP proceeds through P_{RM} & P_L towards t_L'; N allows RNAP to pass through terminator sequence. CIII is transcribed ~~for~~ to stabilize the CII at the CII binding site. by P_L CIII inhibits proteases.
10. CII binds upstream of P_{RE}; P_{RE} is a promoter for CI; Absecance of Q ~~does~~ prevents RNAP from moving forward
11. Int & Xis are transcribed; RNAP is facilitated by N protein through P_I for Int & Xis to be transcribed.

9.1 ⇒ CII activates P_{aQ}; P_{aQ} transcribes for a termination sequence
⇒ low CII = low CI = no more lysogeny.

Regulatory Proteins:

Where & how they interact with phage DNA in A bacterial cell
(How regulatory proteins interact with viral DNA in the lysogen.)

NB
Understanding the functionality of molecular systems relies on understanding how these systems work in relation to the chemical & mechanical properties of the molecules involved.

recall: How things work
• proteins • DNA • RNA
• Enzymes • Transcription

CI / The repressor protein.

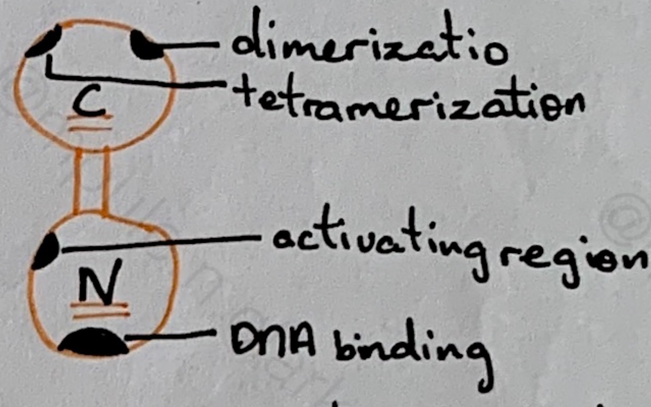
what is a repressor?
- A regulatory protein that binds to specific sites on DNA & blocks transcription. they are involved in negative control of gene expression.

The CI proteins are helix-turn-helix (α-helices) binding protiens (as is the σ factor of RNAP). They bind at the operator sequences on the phage DNA; each monomer occupies half of the binding site (O_R) so they exist as dimers.

CI proteins have an ~~preferential~~ observed preferential binding order: $O_{R1} > O_{R2} > O_{R3}$

=> as CI accummulates eventually O_{R3} will be occupied & P_{RM} will be blocked (CI production will stop - lysogeny is nearing completion).

=> CI gene ~~negati~~ contiols its production by feedback of accumulated CI (all the other phage DNA will also be concentrated)



N-terminal is the operator binding site with the HTH motif

C-terminal domain is responsible for dimerization

DNA binding site amino acid residues make interactions with ~~the~~ specific bases at the site.

During Induction recA protein or papain cleaves the dimer & splits the terminal domains.

Cooperativity allows the repressor to bind to O_{R1} & O_{R2} at lower Conc.

CI conc. is established by P_{RE}.

CI conc. is maintained by P_{RM}.

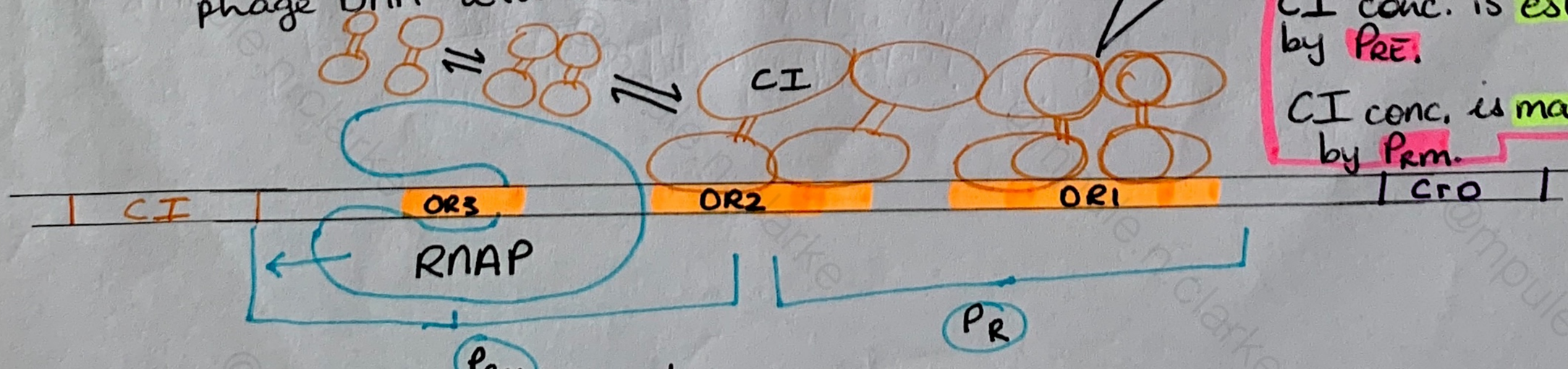


fig. Binding of CI to Operators ; σ ~~fa~~ can only bind to P_{RM} CI is transcribed.

CII protein.

CII mRNA is transcribed by P_R during Early expression (After phage ~~has DNA~~ DNA has been replicated)

CII supports lysogeny by binding upstream of P_{RE} & stimulating the production of CI from the promoter for repressor establishment (A weak promoter). P_{RE} has a very poor -35 sequence; CII binding site overlaps the -35 sequence of P_{RE}, this helps polymerase bind to the promoter when CII binds.

CI is expressed from P_{RE} until there are sufficient amounts of it available to bind to OR1 & turn off the transcription of Cro mRNA.

CII can however be degraded by proteases that will be present in healthy cells: high frequency lysogeny protease A (HflA) & HflB. ~~If the phage pos DNA outnumbers~~

CIII proteins inhibit the proteases & protecting the CII on the phage DNA. CI levels are allowed to accumulate & CI finally binds to OR1; inhibiting P_R & the transcription of Cro. Lysogeny begins; once the multiplicity of infection (moi) remains low the ratio of proteases to CII remains low.

CII is essential for the maintenance of Repressor expression. ⇒ essential for Lysogeny.

CII activates P_L, N mRNA will be transcribed & RNAP will proceed through t'_L to transcribe CIII

CII protein cont'd

CII activates PaQ.

PaQ codes for short self terminating sequences that function in antisense control of the Q antiterminator

=> Q is not transcribed after early gene expression so lysis is delayed & early Q are degraded

~~Q antiterminator~~

Antiterminators

N protein

- Causes expression/transcription from P_R & P_L to continue past the first termination sequences

=> Nut site is ahead of the termination sequences, N binds to RNAP at these sites; altering the conformation of the molecules which allows it to move through the terminator sequences.

=> Alterations to RNAP by N allows RNAP to be recognized by the weak P_I promoter. P_I allows for the transcription of integrase and exisase by the Int & Xis genes.

Integrase couples with Excisase for the phage to be integated into the host cell DNA

=> Early expressed Int is unstable & degraded by proteases. Only Int Expressed by P_I is stable

=> sib site prevents Int & Xis to be transcribed from any promoter but P_I

=> Int recognizes the attachment sites attP & AttB

Induction

λ remains a prophage until the cell is damaged.

RecA accumulates in the cells when it is damage;

⇒ RecA complexes with ssDNA (damaged DNA) & activates protease activities of other proteins in the host. This complex autocleaves LexA.

⇒ LexA is similar to CI structurally so it is cleaved & the promoters for phage transcription is no longer repressed.

⇒ Protease levels will be high so ~~lysog~~ lysis will be favored. Induction is stimulated by the presence of RecA.

⇒ Cro binds to O_{R3} so repressor maintenance is obstructed. Cro also binds to O_L.

⇒ CII is not present so P_I will not be activated; Int & Xis are transcribed through P_L (which goes through the sib site).

⇒ Xis is produced to excises the prophage from the lysogen DNA ~~& Int is no longer needed after the DNA's recircularizes at all.~~

⇒ Int mRNA is destroyed when RNAP transcribes the sib site sequence after N restructured the RNAP to run through the termination sequence following Int & Xis to the sib site.

⇒ hairpin like mRNA is formed & degraded from the Xis ~~gene pa~~ mRNA. RNAse III cleaves the hairpin. ⇒ retioregulation

Antiterminators

antiterminators act on sequences between the promoter & terminator to allow transcription to proceed through the terminator & beyond.

Q protein

Q antiterminator associates with the QBE in P_R'.

⇒ presence of Q allows for RNAP to transcribe through P_R', the promoter for head & tail genes.

⇒ presencen of CII prevents Q activity because of antisense regulation.

- Immediate early proteins are responsible for: Phage Replication.
- Early: production of transcription factors / Phage transcript
- Late: structural production of virus.

⇒ delayed early gene (often not to decide cycle)

Bacteriophage λ lysogeny

Proteins: CI, CII, Int, Xis, N, CIII

Host cell: Enteric bacteria e.g E. coli

The phage injects phage ~~bacteria~~ DNA into the cytoplasm of the host cell. The cell is now referred to as a lysogen. Two pathway of infection can occur based on the state of the bacterial host.

If the cell is healthy & lots of proteases are present lysis will occur.
If the cell is not very stable or conditions don't allow for the expression of cro then lysogeny will occur.

Recall: RNAP holoenzyme with σ mechanism for prokaryotic cells.

1. The bacterial DNA circularizes by forming bonds with its ends. The linear dsDNA strand has cohesive end (cos site) that are staggered & Complementary to each other.

2. RNAP holoenzyme forms transient bonds with the sigma factors at the strong promoters (P_R & P_L)
   - P_R transcripts code for N, Int, Xis, ~~#~~ CIII
   - P_R transcripts code for CII,

Regulatory Proteins

Why does the occurence of cooperative binding ~~to~~ increasing the effective affinity of the repressor for the operator at low concentrations have serious consequences?