Lambda

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Reviewed	☐

Lambda phases regulation

1. Infection: phage attaches to the bacterium — exploit the bacterial pili to inject the nucleic acid into the bacterium (for lambda phage it is the DNA)
2. Early development: decide whether to enter the lytic stage, depending on the conditions
    a. high nutrient level
    b. low MOI (multiplicity of infection) — measured as phage:bacteria ratio — if it is low: means that there are ample number of bacteria to infect — favour replication and infection
    c. therefore, in labs, normally use low [phage] to infect — more likely to enter lytic stage
3. phage DNA replication in bacteria
4. Late development: using the bacterial machinery, heads and tails are made, and DNA is packed into the heads, with tails attached
5. Lysis: cell is broken to release progeny phages

In 2. the phage can instead enter the lysogenic phase

- When nutrients level is low
- When MOI is high — far more phages than the number of bacteria
- In these two scenarios, lysogenic phase is preferable because the bacteria can grow

- When nutrients level is low, metabolism and transcription level is also low, phage DNA has to integrate into bacterial chromosome to prevent from being degraded and recycled
    - When conditions turn favourable for lysis, the phage DNA can be excised from bacterial chromosome to re-enter the lytic phase

▼ Lambda genomes

- dsDNA, all characterised
- when infection: dsDNA is **circularised**; other time — **linear**

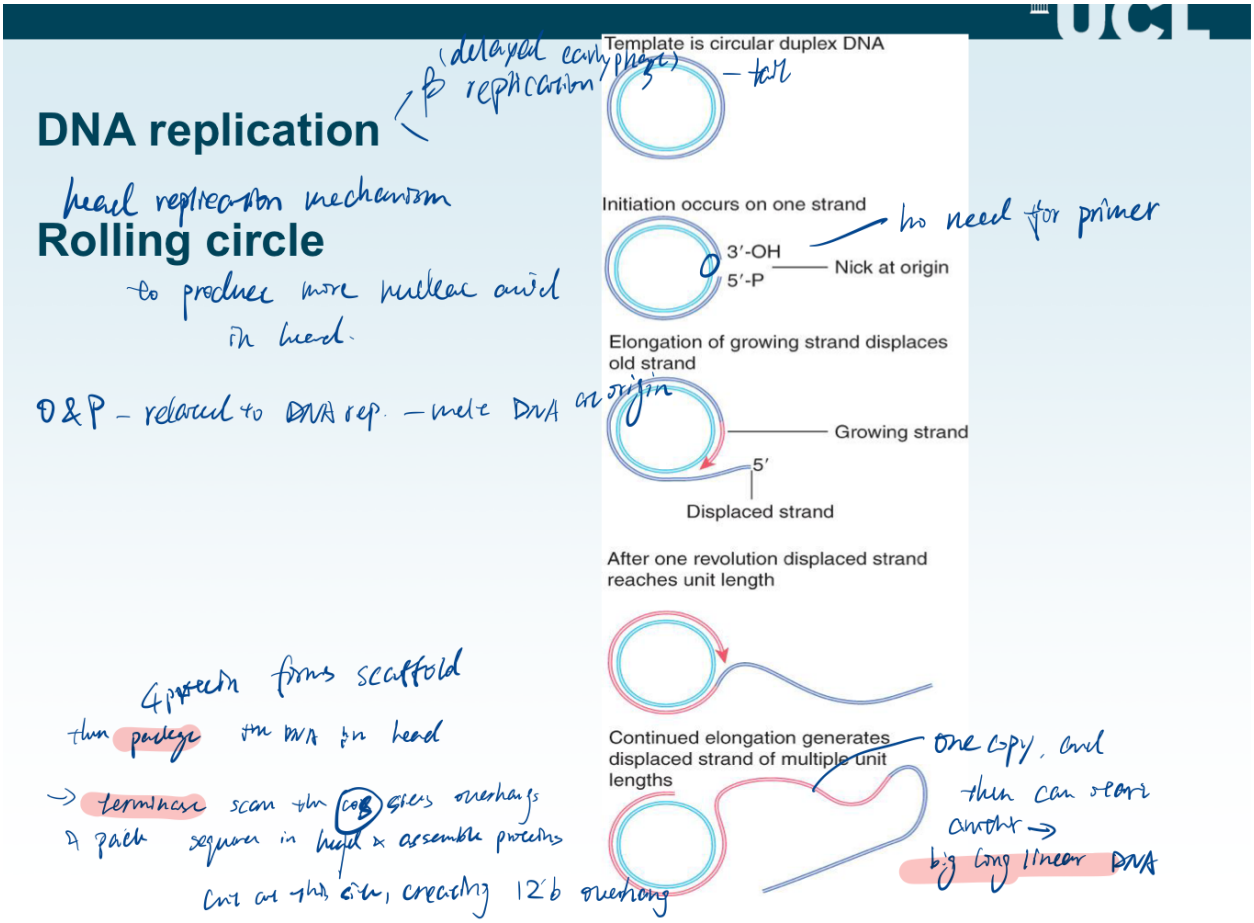
Transcription Anti-termination

- use processive anti-termintion
- anti-terminator proteins conjugate with RNA pol & these bind to the nascent mRNA — RNA pol incapable of termination at many downstream sites
- anti-termination proteins: N and Q (Q is then used to turn on the synthesis of its late proteins, including the head and tail proteins)

1. When lambda DNA first enters the cell, transcription immediately begins from pL and pR
    - these are strong promoters, allow transcription without the presence of activators
    1. rightwards: encodes **Cro — inhibitor of repressor synthesis;** also nutR (N utilisation site)
    2. leftwards: **N protein**: allow the RNA pol to bypass the termination site
        a. the N transcript is leftward from pL
        b. nutL is upstream of the start codon of N protein

2. RNA synthesis terminates at transcription termination sites
    a. if N protein is present, & nutR bind to RNA pol — the **RNA pol can bypass tR1, and tL1**
3. after passing tR1, transcribe delayed early genes: ***CII*, *CIII* regulators are transcribed**, O and P are expressed, to allow replication; another antiterminator **Q is also transcribed**
4. Q by binding on the **qut** site, can load on RNA pol to increase its affinity to binding to the late promoter: **pR′** allows the late genes to be transcribed: head genes, tail genes, **lysis genes**
5. after passing tL1, gam, red are transcribed — lambda recombination & int, xis — integrating DNA and excising

Replication



Decision making between lysogeny and lytic phase

- depends on the outcome of a competition between the product of the cII gene
  - form lysogens
- cro gene — lytic cycle

▼ CII

1. Once the CII protein is made from the pR promoter, it acts as the **transcriptional activators** — activate three promoters:
   - pRE: initiate the expression of CI repressor
   - pI: expression of *int* gene encoding for **integrase**

- pAntiQ: makes an **anti-sense transcript for Q** — hybridise with Q transcript to degrade the level of the transcript — downregulate the synthesis and activity of Q protein — prevent synthesis of lytic proteins

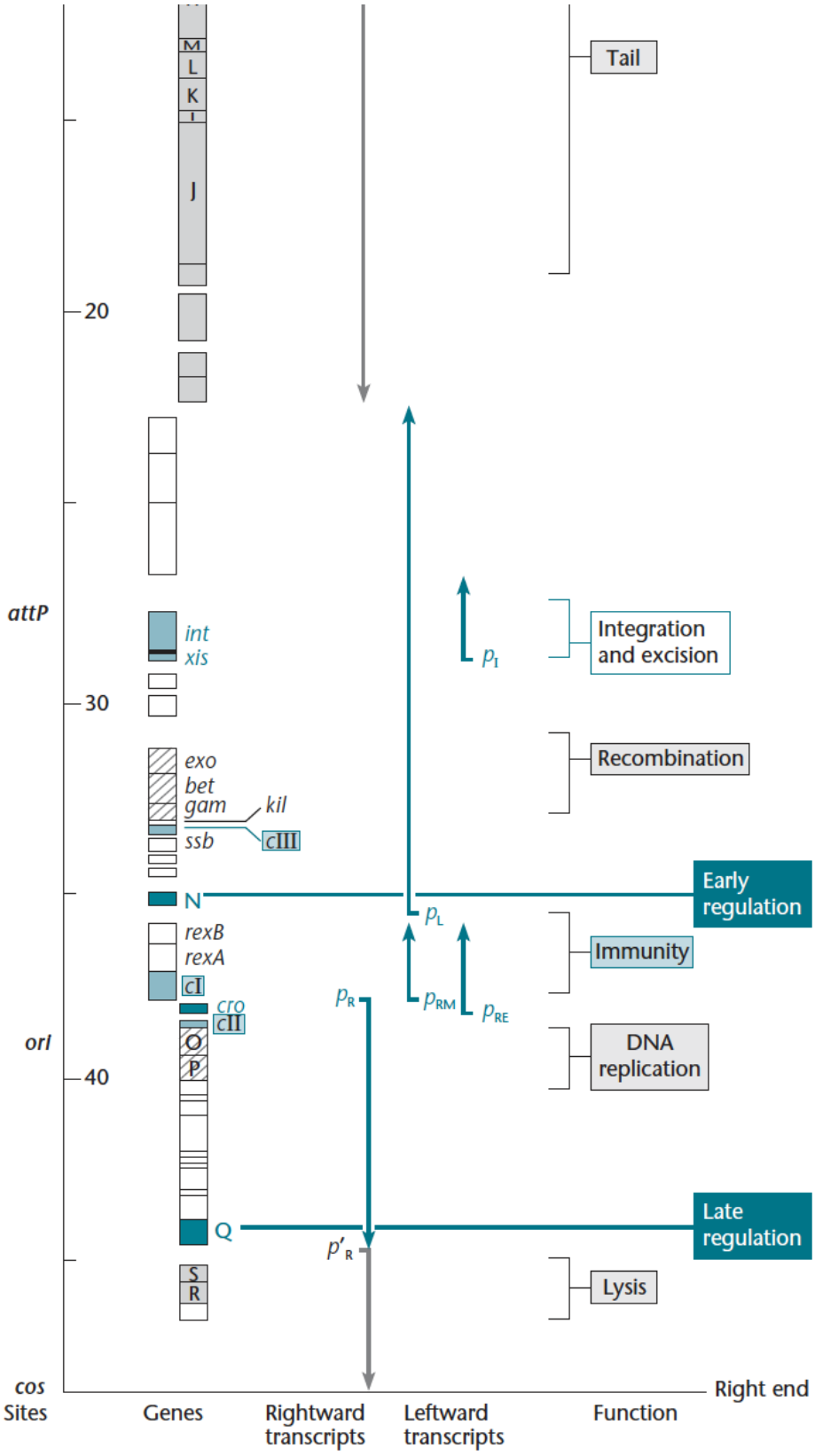
2. Expression of CI repressor:
    a. C-termini dimerise
    b. This increases the affinity N-termini bind to Operator R (oR) and oL
    c. **This prevents the transcription by RNA pol — see below the affinity**
        i. reduced level of protein N and Cro, and also CII
        ii. transcription of CI repressor is actually self-regulated: RNA pol can still bind to an alternative promoter pRM: when CII level is low, CI can still be constantly expressed — CI level is maintained even CII level is low
        iii. immunity
            1. this can cease replication (transcription of integrase allows integration_
            2. free CI and also bind newly entered phage — prevent lytic phase
3. The role of CIII is to prevent CII from degradation

▼ Affinity to the operator region

- The operator has three sites, each composed of two half sites
- The RNA pol binding site for promoter pRM, pR and the three sites of operator have overlapping regions.
- CI repressor has the highest affinity to oR/L1:
    - when the repressor binds to oR1/ oL1, no N protein transcribed, no cro and cII transcribed
    - **cooperative binding**: binding to oL/R1 increases the affinity of repressor to bind to oL/R2

    - instead, RNA pol can bind to region near oR3 — allow RNA pol to recognise and start transcription from pRM — encodes CI — maintain the repressor & lysogeny state
- Cro has the highest affinity ot oR/L3, not in cooperative manner
    - this can still prevent RNA pol from binding to oR/L3, such that it hinders the binding of RNA pol at pRM — one inhibitory mechanism of CI transcription
    - RNA pol can transcribe Cro, N, and replication, lysis proteins — into lytic cycle
    - When Cro binds to the other operator sites it prevents RNA pol from binding to pL and pR to turn off early gene expression (when Cro conc. too high — no longer need those for proliferation etc)

So the critical event is whether cII causes sufficient synthesis of CI repressor to outcompete the action of Cro


M
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Sites
Genes
Rightward transcripts
Leftward transcripts
Function
Right end
p_I
Integration and excision
Recombination
Early regulation
p_L
Immunity
p_R
p_{RM}
p_{RE}
DNA replication
Late regulation
p'_R
Lysis

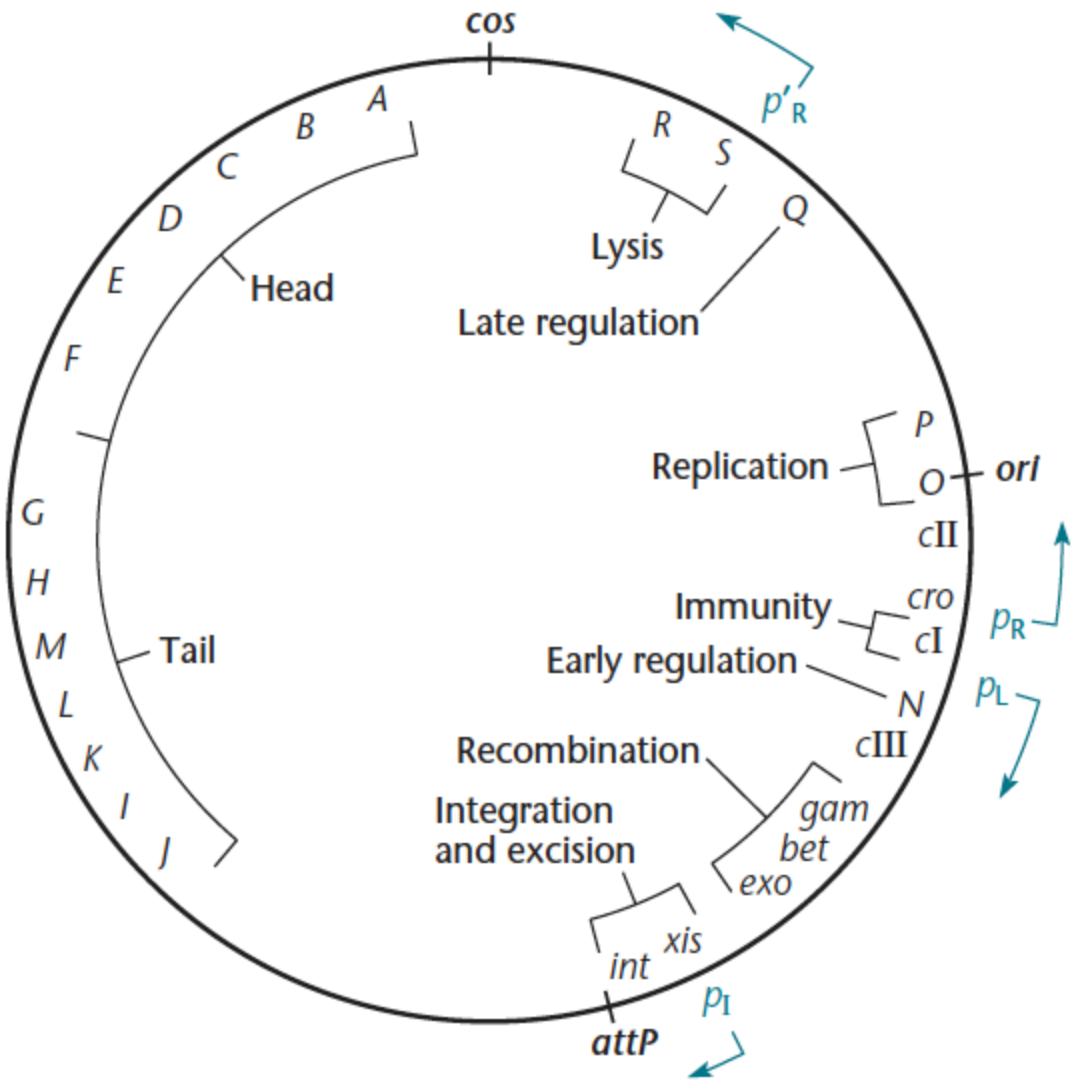
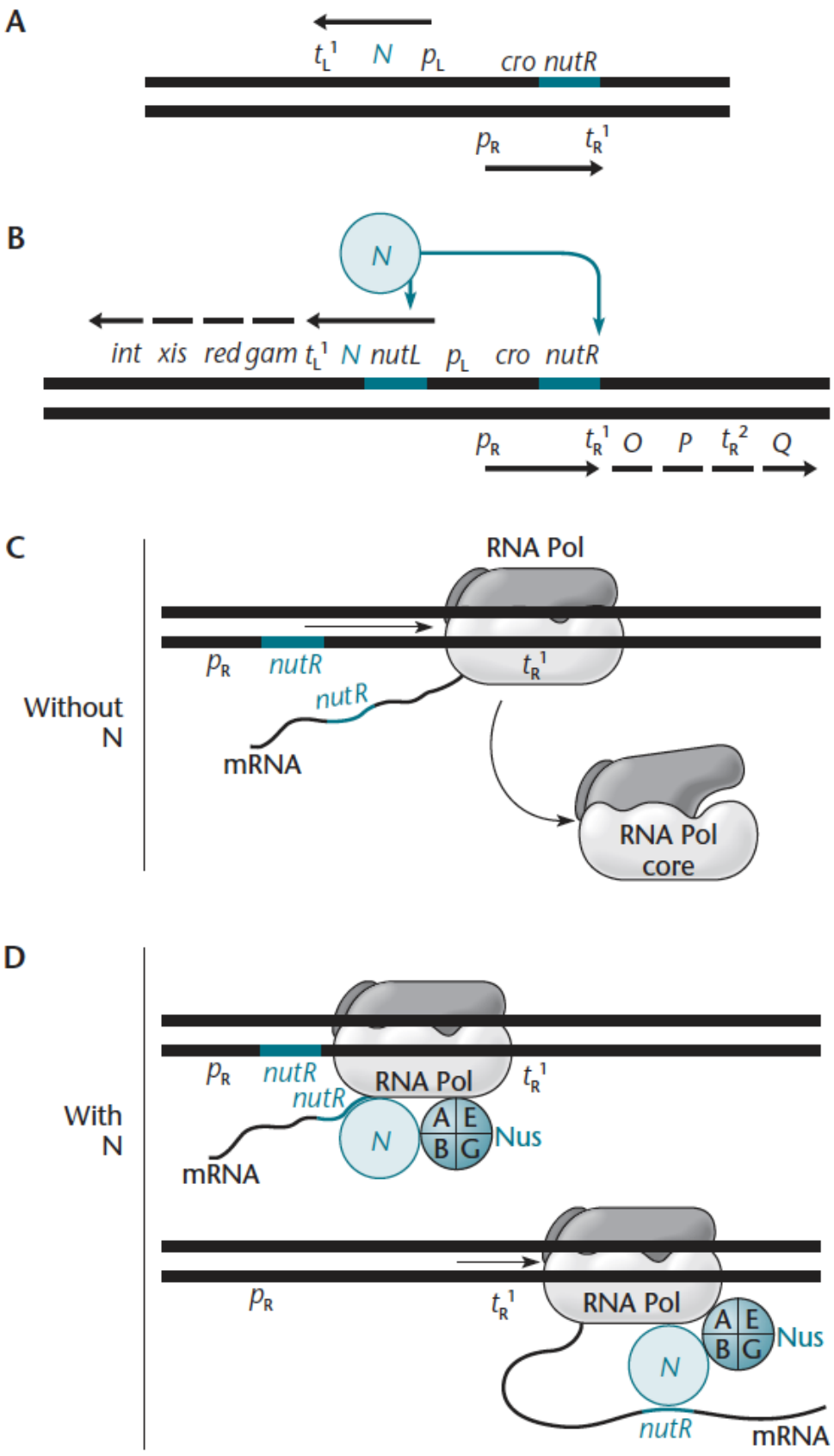


Figure 8.3 Genetic map of λ cyclized by pairing at the *cos* sites, shown at the top. The positions and directions of transcription from some promoters are shown in blue.
doi:10.1128/9781555817169.ch8.f8.3


A
t_L^1 N p_L cro nutR
p_R t_R^1
B
N
int xis red gam t_L^1 N nutL p_L cro nutR
p_R t_R^1 O P t_R^2 Q
C
Without N
RNA Pol
p_R nutR t_R^1
nutR
mRNA
RNA Pol core
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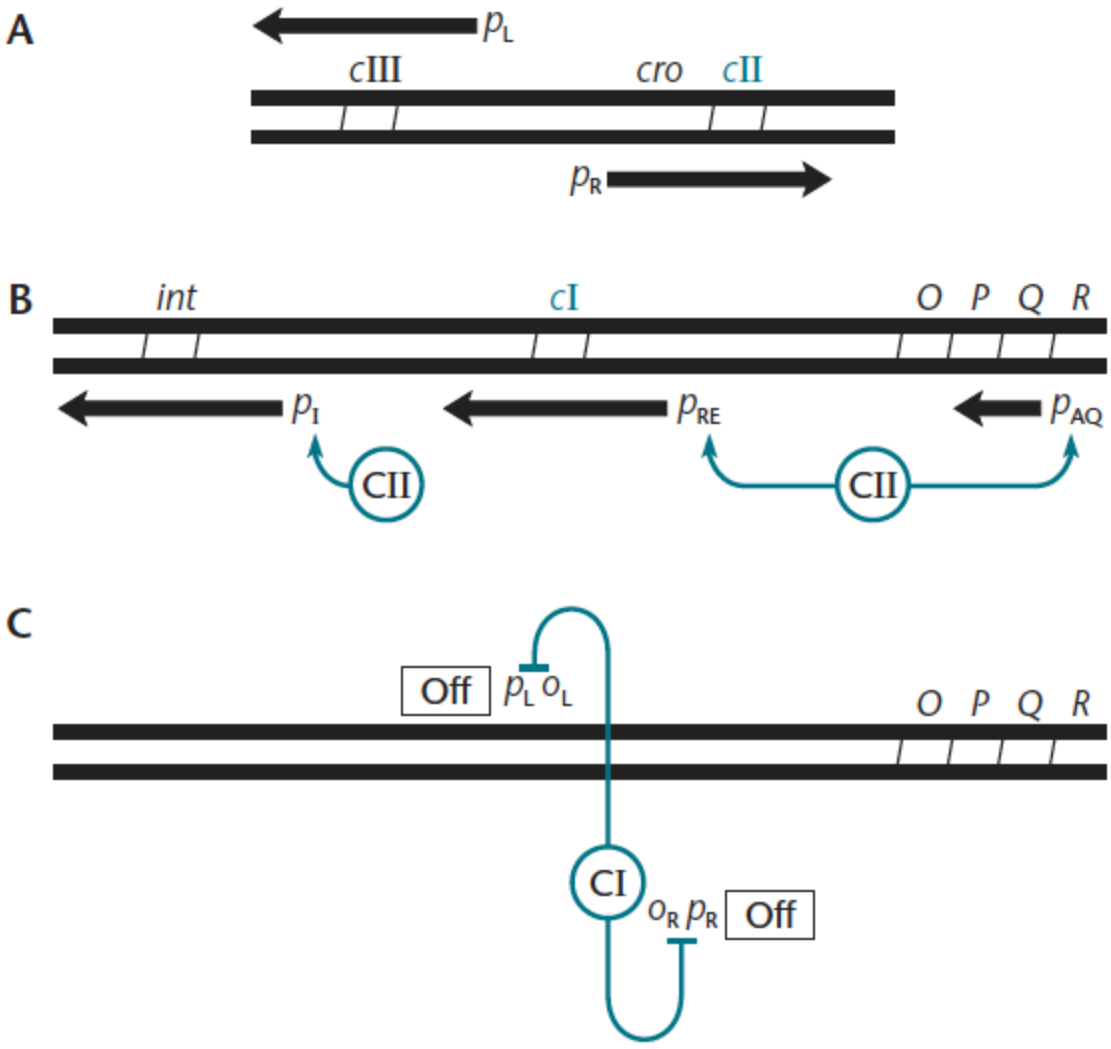

Figure 8.9 Formation of lysogens after λ infection. **(A)** The *cI* and *cIII* genes are transcribed from promoters p_R and p_L, respectively. **(B)** CII activates transcription from promoters p_{RE} and p_I, leading to the synthesis of CI repressor and the integrase Int, respectively. It also activates transcription from p_{AQ}, probably inhibiting synthesis of Q. **(C)** The repressor shuts off transcription from p_L and p_R by binding to o_R and o_L. Finally, the Int protein integrates the λ DNA into the chromosome (Figure 8.10).
doi:10.1128/9781555817169.ch8.f8.9

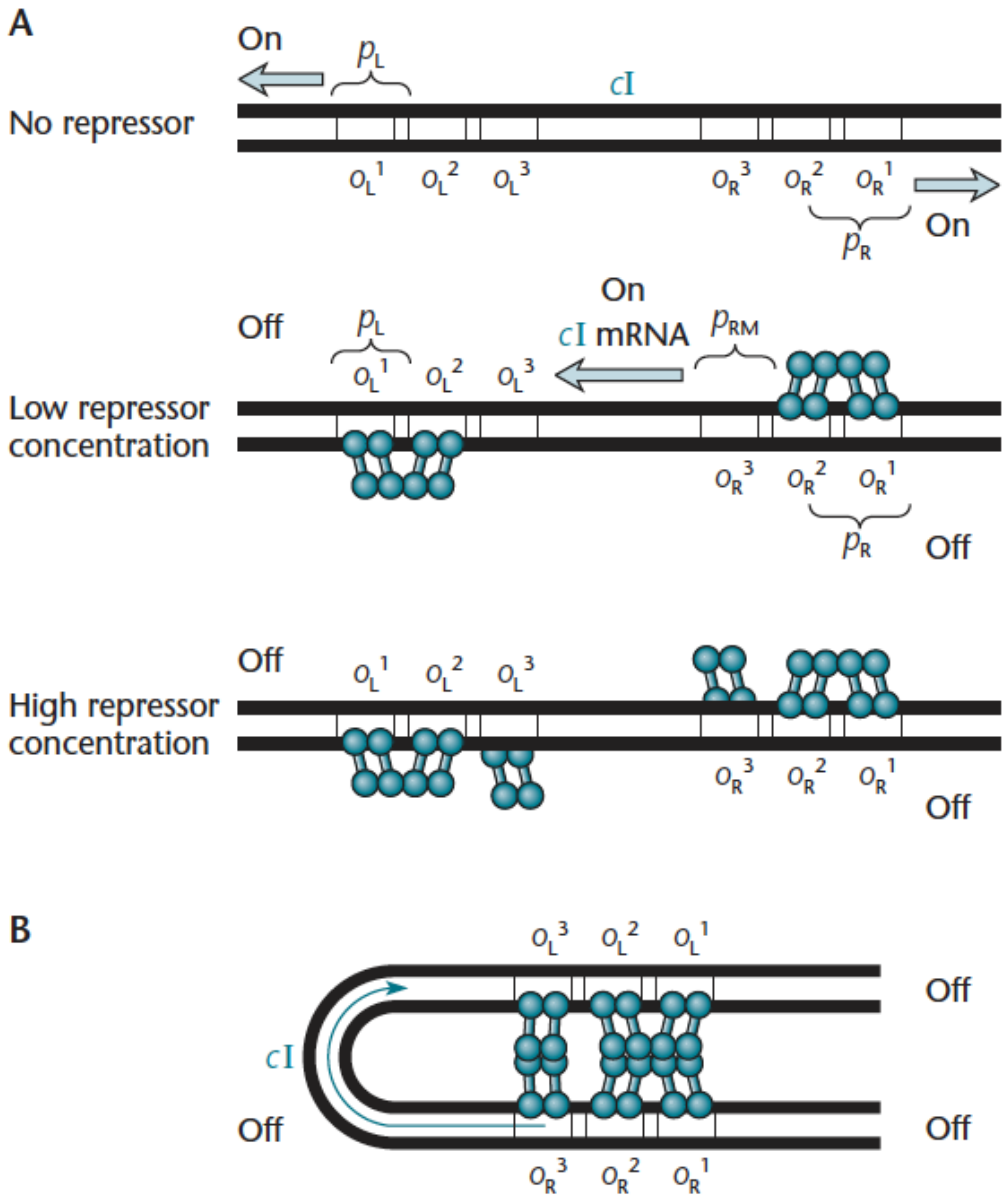

Figure 8.11 Regulation of repressor synthesis in the lysogenic state. The dumbbell shape represents the two domains of the repressor. **(A)** The dimeric repressor, shown as two dumbbells, binds cooperatively to o_R^1 and o_R^2 (and o_L^1 and o_L^2), repressing transcription from p_R (and p_L) and activating transcription from p_{RM}. At higher repressor concentrations, it also binds to o_R^3 and o_L^3, repressing transcription from p_{RM}. **(B)** Still higher concentrations cause the formation of tetramers that bend the DNA, further repressing transcription from p_{RM}. The relative affinities of the repressor for the sites is as follows: $o_R^1 > o_R^2 > o_R^3$ and $o_L^1 > o_L^2 > o_L^3$.

doi:10.1128/9781555817169.ch8.f8.11

Integration

- recombination requires: attP site on the phage and attB site on the bacteria
    - formation of holliday junction
- Integration requires: integrase & integration host factor (IHF)
- Excision requires: integrase & *Xis*
    - The equilibrium depends on the ratio of expressed Int and Xis
        - if Int: Xis high — integration
        - if Int: Xis low — exision
        - equal amount: same rate — equilibrium