411 Lecture Outline: 18/20 Feb 2009

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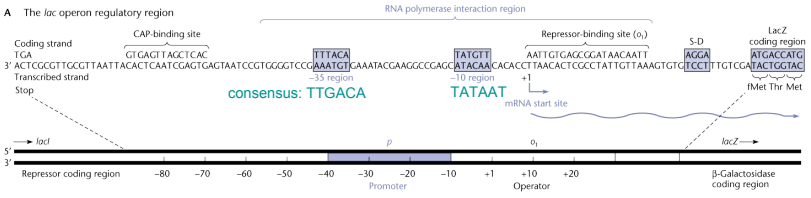
1. Convincing you to care about CRP-cAMP activation
2. Moving on: λ and friends
3. λ lysis-lysogeny choice
4. P1: another temperate phage

Figure 12.6



Differences between *lac* -35/-10 regions and RNA pol σ^{70} consensus contribute to promoter's dependence on CRP-cAMP.

CAP stimulation of initiation

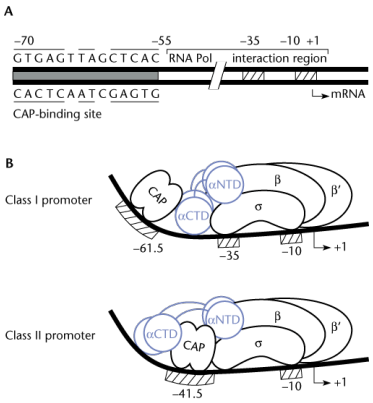


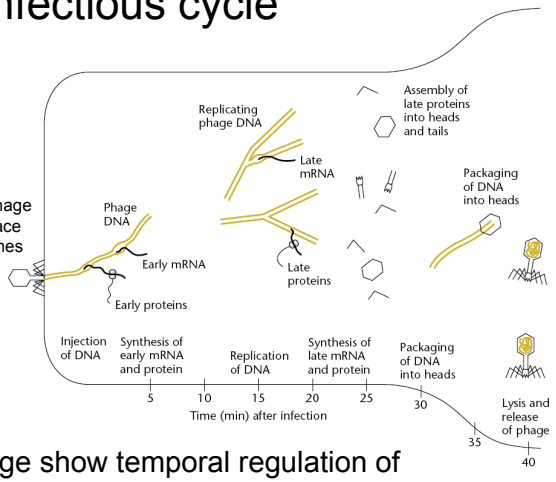
Figure 13.3 (pp 554-556)

lac: increases RNA pol binding/cc formation

gal: increases open complex formation

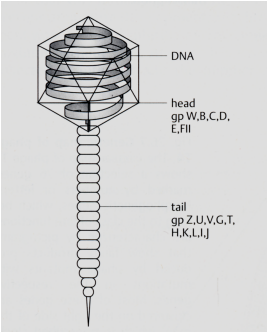
Phage infectious cycle

Figure 7.2



Many phage show temporal regulation of gene expression; usually, transcriptional control is key.

Phage λ



48.5 kB linear dsDNA genome

Receptor: LamB porin in OM

Essential genes for lytic replication with single letter names

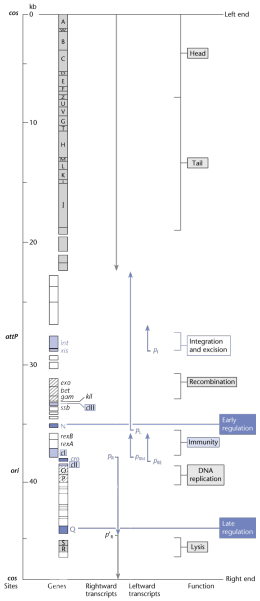
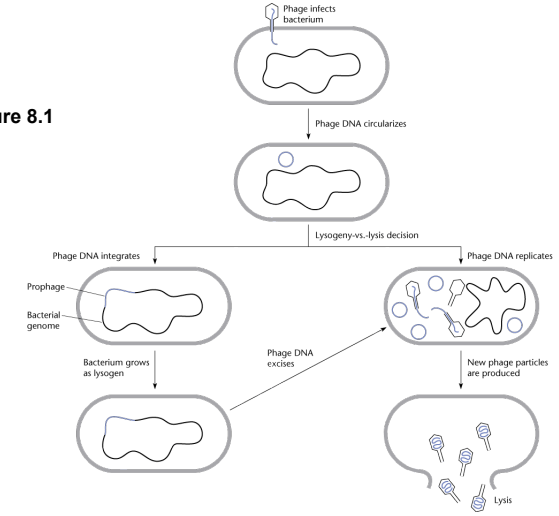


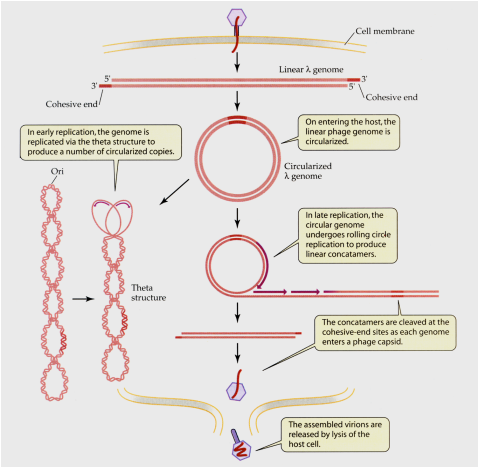
Figure 8.2

The temperate phage λ: lysis or lysogeny

Figure 8.1



λ Lytic replication



- Upon entering cell, linear λ DNA circularizes
- Early replication is θ form
- Late replication for production of progeny genomes is rolling circle, producing concatmers
- Sequence-specific cleavage of DNA concatamers at *cos* prior to packaging in progeny capsids

λ Lysis-lysogeny up close

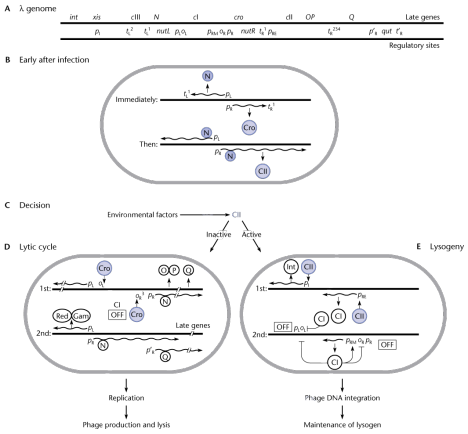


Figure 8.13

N: making it all possible

Figure 8.4

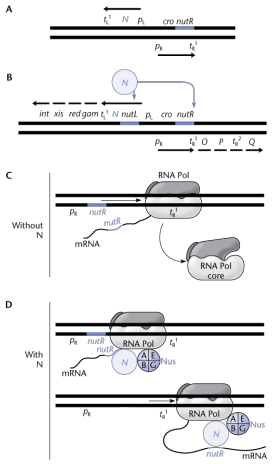


Figure 8.8

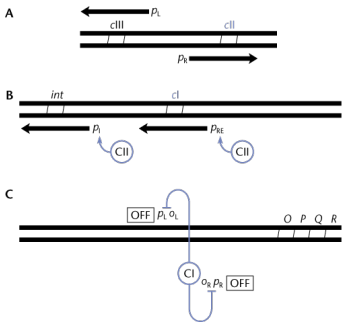


Table 8.3

TABLE 8.3 Steps leading to lytic growth and lysogeny

Steps leading to lytic growth	Steps leading to lysogeny
1. Transcription from p_L and p_R	1. Same as for lytic growth
2. N and Cro are made	2. Same as for lytic growth
3. N allows CII expression	3. Same as for lytic growth
4. **CII degraded**	4. **CII stable**
5. Low CII concentration means that little CI is made	5a. High CII concentration activates p_I, and so Int is made and λ DNA integrates
	5b. High CII concentration activates p_{RE}, and so CI is made
6. Cro binds at O_R3 and O_L3, blocking binding by any low level of CI that is made	6. CI outcompetes Cro, and so CI binding at o_R and o_L both represses p_L and p_R and positively autoregulates at p_{RM}, maintaining lysogeny
7. Meanwhile, N allows O and P replication gene transcription	
8. A second antiterminator, Q, allows late-gene transcription, and so λ phage particles are made	

Why call the genes *cI*, *cII*, *cI*II

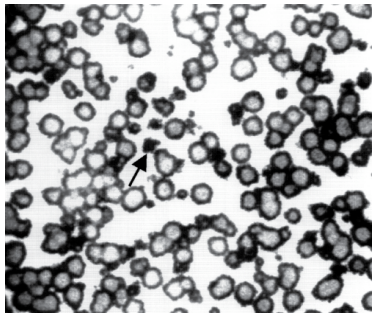


Figure 8.7

Inducing lysogens

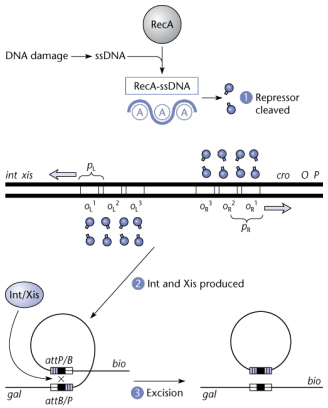


Figure 8.11

λ lysogens

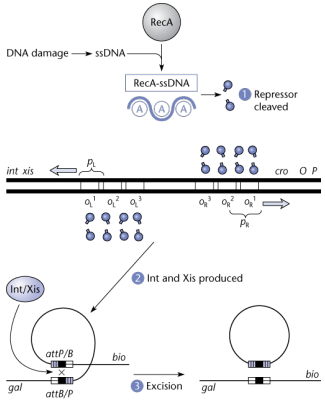


Figure 8.11

- λ regulatory circuit is relatively simple: cI repressor only needs to bind to two operator regions to shut down lytic gene expression.
- cI activates its own transcription (from promoter near o_R2; Fig 8.10)
- Induction of lysogen (DNA damage/SOS response) leads to cI cleavage and loss of repression.

λ: resistance vs immunity

- Resistance to phage: the phage cannot infect.
- Immunity is the resistance of a lysogen to re-infection (super-infection) by a similar phage.

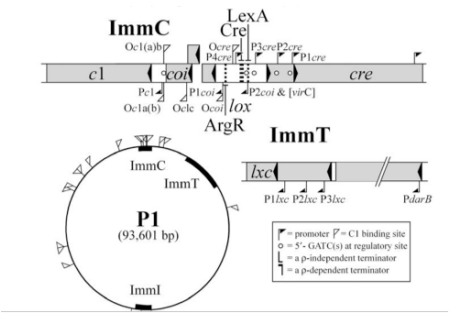
  This can be observed: ability of lysogens to grown in a plaque of phage (turbid plaques). λ lysogens contain about 100 copies cI repressor/cell.

Phage P1

- Infects broad range of bacteria (using LPS core in OM of Gram neg. bacteria), but only replicates efficiently in *E. coli* (and close cousins)
- Linear 92.6 kb dsDNA genome: like many large phages, several accessory genes (*e.g.*, tRNA genes).
- DNA circularizes upon entry to cell.
- Temperate phage
- Approximately 120 genes, organized into 45 operons; only 4 operons involved in lysis-lysogeny choice.
- Lysogeny by P1 depends on its specific repressor protein, C1.

P1: genome & repressor C1

- 17 operons controlled by C1 repressor and transcribed by σ^{70} holoenzyme.
- *c1* gene located in one of the immunity regions of phage genome.
- Site-specific recombination system of *cre-lox*, which we'll talk about later in quarter



P1 lysogens

- Different from λ in that P1 prophage replicates separately from bacterial chromosome as a plasmid.
- P1 lysogens are stable (loss = 10^{-5})
- As the P1 genome is separate from the host cell chromosome, the P1 prophage must express additional functions than λ for maintenance of lysogeny.

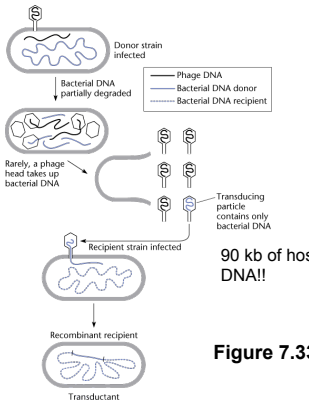
these include?

P1 and molecular genetics

Ability of P1 to move bacterial DNA around as "generalized transducing phage" has been important for molecular genetics.

This property of P1 due to the mechanism of preparing dsDNA for packaging into capsids.

Lytic growth of P1 produces majority of normal phage…



90 kb of host DNA!!

Figure 7.33