


CHAPTER THREE

CONTROL CIRCUITS—SETTING THE SWITCH

Many viruses grow in only one way. Soon after infecting the host cell the viral genes work vigorously—new proteins are synthesized that extensively replicate the viral chromosomes, package them in new viral particles, and lyse the cell.

As we have noted, λ multiplies in such a lytic fashion, but it also has a more subtle alternative. In a lysogenic bacterium the phage genes required for lytic growth are turned off and the phage chromosome is replicated passively by proteins encoded and synthesized by the bacterium. In the parlance of Chapter One we say that the master control element—the switch—has been set so that a single phage protein, the repressor, dominates. Recall that the repressor turns off the other phage genes as it turns on its own gene, cl. This otherwise stable situation is perturbed by inducing agents, such as ultraviolet light, that flip the switch by destroying repressor, and lytic growth begins.

This chapter is concerned primarily with two questions concerning gene regulation in λ.

- How does λ "decide" whether to grow lytically or to lysogenize a newly infected bacterium? Presumably it is a useful trick to multiply surreptitiously, as part of a lysogen, if the conditions for vigorous lytic growth are not optimal.

- How does λ regulate its genes as growth proceeds down either of its two separate pathways?

When the phage grows lytically following infection it must, in an orderly fashion, replicate and package its DNA and then lyse the cell, all the while preventing synthesis of repressor. When a lysogen is induced, a further task is faced by the prophage about to begin lytic growth: an enzyme must be synthesized that releases—excises—the prophage from the host chromosome.

In contrast, when the phage begins the lysogenization process it must synthesize the enzymes that integrate its chromosome into that of the host and begin synthesis of repressor, while preventing expression of the various lytic genes. In other words, the switch of Chapter One must be set in the lysogenic position.

The lysis-lysogeny "decision" is an instructive example of how the environment can influence the choice of developmental pathways. As we shall see, the first few steps of gene regulation that occur upon λ infection are identical whether the phage is ultimately to lyse the cell or to lysogenize it. At the critical step the state of the host
is "sensed" by a phage regulatory protein, and subsequent events are appropriately funneled down one of the two pathways.

We shall see that whether λ is growing lytically or is establishing lysogeny, its pattern of gene regulation is organized as a cascade: one regulatory protein typically turns on (or off) a block of genes; that block of genes typically includes another regulatory gene whose product in turn activates (or represses) a second block of genes, and so on.

We begin with a brief overview of the activities of an infecting λ chromosome as it travels down the lytic or the lysogenic path. We then examine the patterns of gene expression in more detail, and return to the question of how the lysis or lysogeny "decision" is made.

A BRIEF OVERVIEW OF λ GROWTH

The Genetic Map

A simplified map of the λ chromosome is shown in Figure 3.1. Only six genes are named individually in this representation: the regulatory genes cl, cII, cIII, N, cro, and Q. The remaining genes are indicated in groups according to the functions of the proteins they encode.

The region named "recombination" includes the two genes required for DNA replication. The region labelled "lysis" includes three genes whose products lyse the bacterium. The "recombination" region contains some 10 genes, including two whose products integrate the phage chromosome into the host chromosome during lysogenization and excise it during induction. The approximately 10 "head" genes encode proteins that construct the phage head and some 12 more the phage tail. We will identify some of these individual genes as we discuss λ growth.

Circularization

The genetic map is shown as a circle because the λ chromosome, a rod in the phage particle, circularizes immediately upon being injected into the bacterium, as shown in Figure 3.2. The ends of the λ chromosome—called cohesive ends—are joined by a bacterial enzyme, producing a pair of continuous intertwined circular DNA strands. The joining brings together the lysis and the tail genes. At the end of lytic growth, when the new phage chromosomes are packaged into new phage heads, the ends of the chromosomes are separated.

Figure 3.1. The λ chromosome. In general, genes of related function are grouped together. The genes within each of these groups are, as a rule, regulated coordinate-ly. On this map six control genes are named individual-ly, as are two sites, att (attachment site) and cos (cohesive ends).

Figure 3.2. Circularization of the λ chromosome. The "sticky ends" of the λ chromosome are 12 bases of single-stranded DNA that emerge, one from each strand, at the ends of the molecule. They pair spontaneously, and bacterial enzymes link the strands together to produce a continuous circular double-stranded DNA molecule.
If the phage is lysogenizing, only two genes are on—cl and int. The int gene product, located in the recombination region, integrates the phage chromosome into the host chromosome. Finally, in the lysogen, only the repressor gene, cl, is active.

Integration

Figure 3.4 shows how a single recombination event integrates the \( \lambda \) chromosome into the much larger host chromosome, a process that occurs only if the phage is lysogenizing the host. The two DNA molecules are each broken once and their ends rejoined to form the single continuous structure in the lysogen. The site of the crossover on the phage DNA is within a region called attP (for phage attachment site) and the

**Gene Expression**

The patterns of gene expression during \( \lambda \) growth are summarized in Figure 3.3. The first two stages of development—very early and early—occur before the decision to lyse or lysogenize is made. We can summarize the pattern of gene expression at each stage as follows:

- **Very early**
  - Only genes \( N \) and \( cro \) are on.

- **Early**
  - The list of active genes is extended to include the recombination genes, and the DNA replication genes.

- **Late**
  - Here the pathway splits.

  * If the phage chromosome is growing lytically, the various early genes are off and the heads, tails, and lysis genes are on. New phage particles are formed and released when the cell lyses.

  * If the phage is lysogenizing, only two genes are on—cl and int. The int gene product, located in the recombination region, integrates the phage chromosome into the host chromosome. Finally, in the lysogen, only the repressor gene, cl, is active.
bacterial site is called attB. Upon induction of a lysogen this process is reversed and the λ chromosome circularizes as it is excised from the host chromosome.

The integration and excision reactions, shown in the figures as involving only DNA molecules, are driven in the cell by phage proteins that work in conjunction with proteins of the host. The phage protein Int drives the integration reaction, and the combined efforts of Int and Xis promote the excision reaction.

**CONTROL OF TRANSCRIPTION**

We now describe the activities of lambda regulatory proteins at the various stages of λ growth pictured in Figure 3.3. For each stage a figure shows the active regulatory protein(s) and the relevant mRNAs synthesized. The first two stages are identical whether the phage is to lyse or lysogenize.

**Very Early**

The host RNA polymerase binds to two promoters on the phage chromosome, P, and P, and begins transcription. Figure 3.5 shows that these transcribing RNA polymerase molecules stop just at the end of N and cro, respectively. The N and cro mRNAs are translated into their respective proteins.

![Figure 3.5. Very early events. Very early after infection the E. coli RNA polymerase transcribes genes N and cro from different strands of the DNA.](image)

**Early**

The N protein is a positive regulator. Figure 3.6 shows that it turns on genes to the left of N including cIII and the "recombination" genes, and genes to the right of cro including cII and the DNA replication genes O and P and Q. It does not turn on the head, tail, and R genes at the efficiency needed for lytic growth.

The action of N as a positive regulator is entirely different from that of repressor as described in Chapter 1. N works by enabling RNA polymerase to transcribe through regions of DNA that would otherwise cause the mRNA to terminate, and therefore

![Figure 3.6. Early events. N protein turns on the early genes to the left of N and to the right of cro. The pyramid representing N protein is shown hovering near the beginning of the leftward mRNA, but further downstream in the case of the rightward mRNA. This is explained in the text.](image)

**Figure 3.7. The action of N. If no N protein is present polymerase ignores the Nut site and falls off the DNA, releasing the mRNA, when it reaches the stop signal. But in the presence of N polymerase becomes a juggernaut as it passes over Nut and ignores the stop signal.**
N is called an anti-terminator. In the presence of N, the N and cro mRNAs are extended, effectively turning on the flanking genes.

How N works is not known in detail. We do know that it recognizes a specific sequence called Nut (for N utilization); as polymerase passes over this sequence it is evidently modified by N so that it ignores certain (but not all) further termination signals. There is one Nut site between P1 and the beginning of N, and another just to the right of cro. Figure 3.7 emphasizes that the site recognized by N (Nut) is distinct from the site where anti-termination actually occurs.

At this point the pathway bifurcates. During lytic growth Q and Cro proteins are active, while for lysogenic CII, CIII, and finally CI proteins are active. We will describe the activities of these two sets of regulatory proteins separately and then return to the question of how the decision is made to express one or the other set.

**Late Lytic**

Figure 3.8 shows how the Q protein turns on the late genes—those for lysis and for production of heads and tails. Q works like N except that Q anti-terminates specifically a small RNA begun at a promoter called P2, located just to the right of Q. The short (terminated) mRNA is synthesized by the bacterial RNA polymerase. When anti-terminated by Q the mRNA extends around the circular phage chromosome through the head and tail genes.

In the meantime Cro first binds to O3 as described in Chapter One to prevent further synthesis of repressor. It then binds to a second operator, O2, to turn off transcription initiated at P1, and to the remaining sites in O2 to repress P2. Thus, following a burst of synthesis, these early leftward and rightward transcripts are repressed by Cro. At the late stage, sufficient Q has accumulated to activate production of the protein coats that wrap up the newly synthesized DNA molecules. The cell is lysed, and a new crop of phage produced.

**Late Lysogenic**

Figure 3.9 shows that the cl gene product turns on cl and int. The CI protein works like λ repressor in its role as a gene activator—it encourages RNA polymerase to bind and begin transcription at two promoters that would otherwise remain silent: P4 and P5.

We now can resolve a question that arose in Chapter One. We saw that repressor turns on its own gene, thereby maintaining the production of repressor in a lysogen. How then does repressor synthesis begin in the absence of repressor? The answer is that cl can be transcribed from either of two promoters, one of which is activated by repressor, the other by CI protein.

Recall that in a lysogen, repressor stimulates transcription from Pmax (promoter for repressor maintenance). But in a cell lacking repressor, the CI protein causes polymerase to transcribe the ci gene from a different promoter—P1 (promoter for repressor establishment). Beginning at P1, polymerase transcribes leftward to the end of the cl gene at cl. When this mRNA is translated it produces repressor (but not Cro, because cro is transcribed “backwards” here).

Beginning at another promoter, P4, CII causes polymerase to transcribe leftward the
int gene, the product of which integrates the phage chromosome into the host chromosome.

Repressor, translated from the mRNA initiated at $P_{int}$, binds to $O_3$ and $O_4$. Repressor bound to these two sites turns on transcription of its own gene from $P_{int}$ as it turns off all the other phage genes. In a lysogenic the late genes are off because there is no $Q$ protein, and the $Q$ gene is off because the $N$ gene is off.

CII is believed to promote lysogeny in two additional ways not shown in the figures. First, CII inhibits expression of the late genes by stimulating a promoter called $P_{int}$. That promoter, located within gene $Q$, directs backwards (leftwards) transcription of the $Q$ gene. The 'anti-sense' $Q$ RNA hybridizes with and prevents translation of the $Q$ messenger RNA. Second, the transcript initiated at $P_{tet}$, which includes 'anti-sense' $cro$ sequences, hybridizes with $cro$ messenger RNA and prevents its translation. Thus CII antagonizes $Cro$ and $Q$ production as it stimulates production of Int and repressor.

Lambda's regulatory cascade is controlled by the action of regulatory proteins acting at only a few sites on the chromosome. This is made possible by the fact that genes encoding related functions are grouped together and are transcribed in the same direction.

THE DECISION

Having described the two developmental pathways available to an infecting $\lambda$ phage, we now must ask: what determines which pathway is taken? What factors drive the system toward lysis or lysogeny?

We do not have a complete understanding of these matters but we can construct a plausible scenario. Briefly put, the "decision" is effected by a single protein—CII.

Figure 3.10. The lysogeny-lysis decision. Host proteases regulate the level of activity of CII protein. Although CII protein is not shown here, the host factors may exert their effects by working on CII, which protects CII. It is likely that other host proteins regulate translation of the CII mRNA as well.

Figure 3.10 summarizes the situation. If CII is highly active the infecting phage lysogenizes; otherwise it grows lytically. Once the decision is made, all other steps of $\lambda$ growth are determined by one or the other of $\lambda$'s developmental programs.

The activity of CII is determined by environmental factors. As suggested in Figure 3.10, this protein is unstable—bacterial proteases can attack and destroy it. Environmental conditions influence the activities of these proteases. Growth in rich medium, for example, activates the proteases, whereas starvation has the opposite effect and, consequently, $\lambda$ more frequently lysogenizes starved cells. This makes sense because starving cells are deficient in components necessary for efficient lytic $\lambda$ growth.

Lambda's CII protein also helps establish lysogeny—it's role is to protect CII from degradation. The protective effect of CII is not foolproof, and under some environmental conditions CII is largely inactivated even in the presence of CII. But if CII is absent, CII is virtually always inactivated and the phage can grow only lytically.

Next, we compare the outcome of infection of cells in which the activity of CII-degrading proteases is high and in one case, and low in the other.

- In those cells in which CII is rapidly degraded—protease levels are high—no repressor is synthesized. $Q$ and $Cro$ proteins are synthesized, transcription proceeds as in Figure 3.8, and lytic growth ensues. We shall see that the phage also has a mechanism to diminish the amount of Int protein made from the mRNA that initiates at $P_{int}$.

- In those cells in which CII is highly active—low protease levels—transcription of $cl$ and Int from the promoters $P_{tet}$ and $P_{int}$, respectively, proceeds at a high rate as illustrated in Figure 3.9. Int protein integrates the phage chromosome, and repressor binds to $O_3$ and $O_4$ to turn off all the phage genes except $cl$. Lysogeny is established.

In the latter case, were $Cro$ to bind to $O_3$, before repressor bound $O_3$ then $O_4$, the phage would have difficulty establishing lysogeny. We imagine that this never occurs if CII protein is highly active. The rate of repressor synthesis directed by CII is sufficiently high so that, given the relative affinities of repressor and $Cro$ for the relevant sites, repression is established and the $cro$ gene is shut off before enough $Cro$ is made to shut off $P_{tet}$.

CONTROL OF INTEGRATION AND EXCISION

The integration reaction requires a single phage protein—Int—but the reverse reaction, excision, requires both Int and Xis. The genes encoding these two proteins lie adjacent on the map, and two different kinds of controls ensure that they are expressed as needed. One involves the action of the lysogeny-promoting protein CII; the other involves a form of control we have not yet discussed, namely, modulation of mRNA function.

To set the stage for understanding the logic behind these control mechanisms, let us examine three scenarios: the first is an infecting phage chromosome destined to