LYSOGENY, PROPHAGE INDUCTION, AND LYSOGENIC CONVERSION

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3

Temperate phages can carry genes that affect the phenotype and behavior of their bacterial host. These genes can be considered extra genetic material in that they are not necessary for viral lytic growth or for the lysogenic lifestyle. In this chapter, these extra genes will be termed "foreign genes" for ease of reference. Foreign genes include genes for various toxins that have pathogenic effects. The expression of toxin genes has been documented to occur in two different phases of the viral life cycle. The primary goal of this chapter is to describe circumstances under which foreign genes can be expressed. We will first consider the life cycle of temperate phages. With this background, we will then describe how foreign genes can be expressed in the lysogenic state. We will then turn to a more detailed description of a particular temperate phage, λ, with an emphasis on the regulatory mechanisms that are best understood for this phage. This description will facilitate an understanding of how foreign genes can be expressed during the process of prophage induction, and a particular example will be described.

Temperate phages have two lifestyles, as illustrated for phage λ (Fig. 1). Like most other bacteriophages, they are able to grow lytically in their host, replicating their DNA and producing new progeny that are released into the environment. In addition, temperate phages are able to establish and maintain a stable relationship with their host. In this relationship, termed the lysogenic state, the expression of lytic genes is prevented by the action of a viral gene product termed a repressor. Although the lytic genes are present, their expression is prevented. In most cases, the viral DNA is integrated into that of the host and is replicated along with the host DNA. This arrangement offers the virus an alternative mechanism for making more copies of its genome.

Although the lysogenic state is extremely stable (29), it can switch to the lytic state. For different viruses, this switching occurs by different mechanisms. In the best-studied case, that of bacteriophage λ, switching occurs by the action of a host regulatory system termed the SOS regulatory circuit (26, 28). In turn, the SOS

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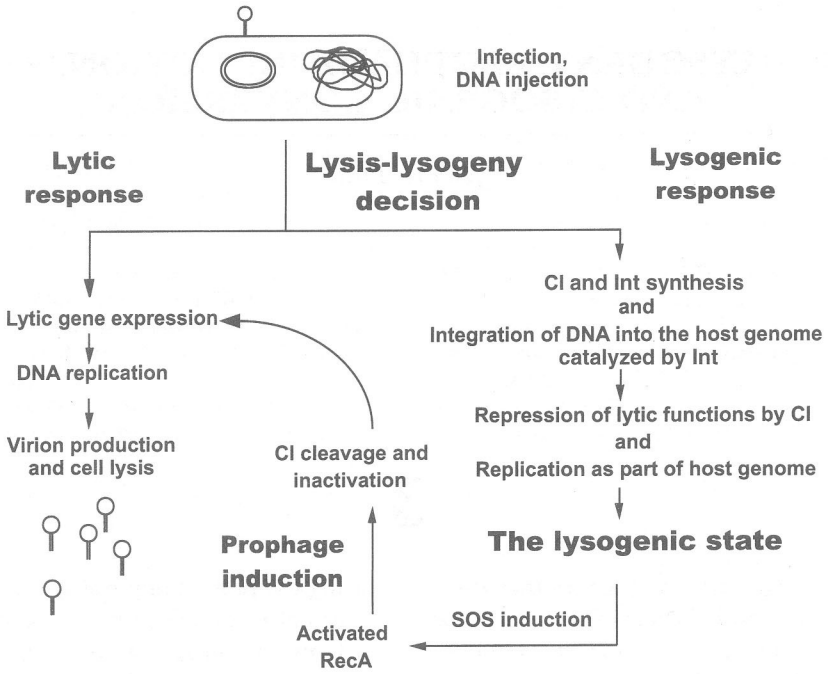

FIGURE 1 Life cycle of phage λ. An infected cell is depicted at the top, in which injected phage DNA has rapidly circularized. Ten to 15 min after infection, a decision is made between two alternative fates. See the text for more details. During the lysogenic response, the circular λ DNA is inserted into the host chromosome by a site-specific recombination event between the phage *att* site (Fig. 2A) and a cognate site on the host genome.

response is triggered by external events. This mechanism is used by many phages related to λ. A related mechanism occurs in phage 186 (23). Other temperate phages can switch to the lytic state by mechanisms that are poorly understood (e.g., see Chapter 9). In almost all cases, it is not known whether external events can affect the rates of switching, and these cases will not be considered further.

EXPRESSION OF FOREIGN GENES (LYSOGENIC CONVERSION)

Foreign genes are most commonly expressed from a prophage in the lysogenic state. This pattern of expression is often termed lysogenic conversion, since it can change or convert the phenotype of the bacterial host. Its existence raises several questions, including the following. Why are foreign genes maintained? How are these genes regulated? How can the phage accommodate other genes in its genome? What are the sources of these genes?

It is likely that foreign genes are maintained because they can confer a selective advantage to the host or to the phage. The expression of foreign genes during the lysogenic state can affect the phenotype of the host. This can provide selective pressure to maintain these genes in the virus and to maintain the relationship between the virus and its host. In addition, some foreign genes can confer a selective advantage in the relationship of the bacteria with a metazoan host, either promoting an association with the host or resulting in pathogenic effects. Many examples are described elsewhere in this book.

How are foreign genes regulated? In a lysogenic cell, nearly all of the viral genes are not expressed. In the case of λ, this is because the promoters for the lytic genes are turned off, as detailed below; in other words, the viral cir-

cuitry is designed to keep these genes off. The same is doubtless true for other phages, although the details of regulation will likely vary in many cases. However, in order for foreign genes to offer a selective advantage, they would need to be expressed during some phase of the viral life cycle. We focus for now on lysogenic conversion.

In principle, foreign genes may be regulated either under the control of the viral regulatory circuitry or by other means. The former is apparently not common, but one well-known example exists. In lambdoid phages, there is one class of genes, aside from the CI repressor, that is also expressed under the control of the viral circuitry during the lysogenic state. Many, though not all, lambdoid phages carry other genes just downstream of *cI* in an operon with this gene (see Fig. 3C), and these are expressed from the P_{RM} promoter along with CI. In most cases, these genes have not been analyzed in any detail. In λ, these genes, *rexA* and *rexB*, may confer a selective advantage to lysogens (13); for instance, they prevent the growth of T4*r*II mutants (a property that is important in the history of molecular biology but of questionable significance in a natural setting). Three lines of evidence provide weak support for the idea that genes in this location are foreign genes. First, some lambdoid phages (such as HK022) do not carry genes in a homologous position (35), suggesting that genes found at this location in phages such as λ were inserted as foreign genes and persist due to selective pressure. Second, phage HK97, a phage with the same CI repressor as that of λ, carries two genes that are different from the *rex* genes in the corresponding positions (18). Third, the *rex* genes are not essential for either the lysogenic state or the lytic state of λ, at least under laboratory conditions.

It is apparently more common that the regulation of foreign genes is not subject to viral regulatory controls. In most cases, mechanisms for the regulation of foreign genes have not been studied, but it is likely that most such genes are expressed from autonomously acting regulatory regions during the lysogenic state. These regulatory regions would include a promoter that can be expressed and, probably, a transcription terminator to limit the extent of expression from the prophage, thereby avoiding disruptions of the viral regulatory circuitry. Such genes may be expressed constitutively (that is, all the time), in which case they might alter the host phenotype constitutively. Alternatively, foreign genes may be regulated by a wide variety of mechanisms, simply by coopting a regulatory system of the host, in which case their expression would depend on the environmental conditions impinging on the bacterial host. They might also be expressed only during certain phases of the host growth cycle, such as stationary phase. Examples of many of these patterns are known, and some of them play important roles in pathogenesis, as discussed below.

Importantly, for lysogenic conversion to occur, it is not necessary that the prophage remains functional as a virus that is capable of prophage induction or lytic growth. Genome sequencing of many bacterial species has revealed a multitude of defective or "cryptic" prophages in which these capabilities have been lost. In principle, these defective prophages can retain foreign genes in an active form (for example, see Chapter 7). In addition, for lysogenic conversion to occur, it likely does not matter how lysogeny is maintained, nor if the prophage is integrated (as in the case of λ) or replicates as a plasmid (as in the case of P1).

How can viruses accommodate extra genetic material? There are two parts to the answer. First, although viral genomes are quite compact, all temperate phages for which detailed analyses have been performed appear to have room for foreign genes. This can be evaluated to some extent by sequence analysis, but a detailed analysis of gene function is needed to identify which viral genes are essential. The first, and most detailed, analysis of this type was done with phage λ. λ has ~30 genes that are essential for lytic growth under laboratory conditions, and three more are required to establish and/or maintain the lysogenic state (for extensive reviews of all aspects of λ, see reference 16). However, it also has large regions that are not essential for either state, and these regions contain nonessential genes. These genes are

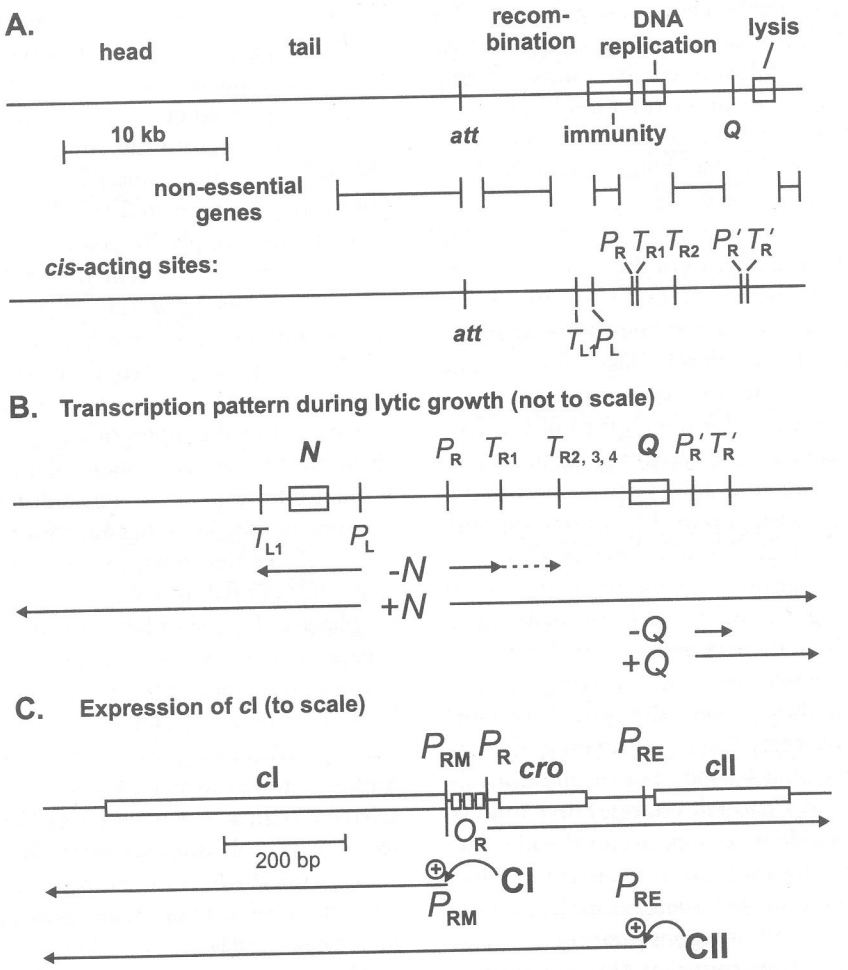

FIGURE 2 λ genome organization and transcription patterns. (A) Map of the λ genome. The map is drawn to scale. Genes with related functions are grouped together into modules (see the text for more details). The immunity region, discussed at length in the text, includes the regulatory sites O_L, P_L, O_R, and P_R and the regulatory genes *cI* and *cro*. The locations of nonessential genes are shown (see the text), as are those of *cis*-acting sites that are important for the lytic program of gene expression. The GenBank accession number for the λ genome is NC_001416. (B) Lytic gene expression. The map is not drawn to scale. Transcripts are indicated by lines with arrowheads depicting their 3′ ends, except that those indicated by "+N" or "+Q" are antiterminated and would continue beyond the ends of the map. The T_R1 terminator is inefficient; transcripts reading through it terminate at T_R2 in the absence of N function. Several other terminators lie to the left of T_L1 and are not shown. (C) Expression of *cI* from two different promoters, P_{RE} and P_{RM} (see the text). The map is drawn to scale.

located in several regions (Fig. 2A) (the organization of the λ genome is detailed below). These include the *b2* region, lying between the tail genes and the *att* site; a region lying between the DNA replication genes and *Q*, containing about 10 genes in the case of λ; and an interval lying to the right of the lysis genes on the linear map. In addition, the region to the right of *att* contains numerous nonessential genes. In all of these regions, genes that are not essential for growth

could in principle be replaced with foreign genes and their own regulatory elements without greatly compromising the ability of the virus to carry out lytic growth, lysogeny, or prophage induction.

A second way to incorporate extra genes is by addition rather than substitution. This is possible because of the mechanisms by which viral DNA is packaged into mature virions. Two primary mechanisms have been analyzed, termed *cos* site and *pac* site mechanisms. Viruses of the *cos* site type, such as λ, can vary the amount of DNA that is carried in the mature virion (4). For instance, λ virions can accommodate about 4 kb more DNA than is present in wild-type λ, allowing the addition of other genetic material. This ability arises from the mechanism by which the DNA is packaged into the viral head. At the ends of the mature virion lie two cohesive sites. These sites stick together after infection and are ligated, causing the viral genome to circularize. The resulting site is termed a *cos* site. When the DNA is replicated, a rolling-circle mechanism generates long linear DNA molecules, termed concatemers, in which many genomes are arranged in a head-to-tail fashion. When this DNA is packaged, the *cos* sites serve as markers for the ends of the new viral DNA. An intricate mechanism ensures that all of the DNA between successive *cos* sites is packaged into a virion, provided that it is less than the maximum allowed in the viral head.

Many other temperate phages are of the *pac* site type, among which the best studied is P22 (47). In such cases, the amount of DNA contained in the viral head is always about the same. The packaging mechanism is not well understood, but it appears again to operate on long linear concatemers. In this case, however, the first molecule to be packaged from a concatemer has a different type of site, termed a *pac* site, from which packaging initiates. The packaging machinery packages a bit more than a full genome equivalent, typically about 105% of a genome equivalent. Packaging of the next molecule starts from the end of the previous one; therefore, it starts a bit beyond the *pac* site. Each molecule contains a few kilobases more than a genome equivalent and carries a terminal redundancy, in which the same genetic material lies at both ends. When these molecules are injected into the next host, homologous recombination between the sites of terminal redundancy generates a circular structure. Accordingly, these *pac* site viruses can also accommodate some extra DNA, provided that it is not so large as to prevent terminal redundancy.

In addition, a few temperate phages are filamentous phages. In these cases, the size of the virion is dictated by the length of the DNA, so there are no severe constraints on DNA size. Among temperate phages, the best analyzed is the cholera toxin phage CTXϕ (discussed in Chapter 9). The packaging mechanism is best understood for nontemperate phages such as M13.

It is much more difficult to know the origins of foreign genes. Again, there are two issues. What is their source, and how do they become inserted into the genomes of temperate phages? Answers to these questions are at best tentative, in most cases. Generally, the source is not known, although a few cases exist in which plausible guesses can be made. Mechanisms by which these genes move are likewise obscure. A more detailed discussion is included in Chapter 4.

GENE REGULATORY CIRCUITRY OF λ

This section focuses primarily on phage λ, which is by far the best-studied member of a large class of related temperate phages termed lambdoid phages. These phages have served as paradigms for complex gene regulatory circuitry (16, 39) and are still the best-understood systems at the mechanistic level. In the case of λ, three different aspects of gene regulation are discussed in detail below. First, a decision is made, soon after infection, whether to follow the lytic or the lysogenic pathway. This decision appears to depend in part on the physiology of the host in ways that are not well understood (12). Second, this decision results in the establishment of one of two stable regulatory states and the exclusion of the other. The chosen state is