CHAPTER 19

Replication of Cytoplasmic DNA Viruses and "Large" Bacteriophages

- POXVIRUSES – DNA VIRUSES THAT REPLICATE IN THE CYTOPLASM OF EUKARYOTIC CELLS
- The pox virion is complex and contains virus-coded transcription enzymes
- The poxvirus replication cycle
- Pathogenesis and history of poxvirus infections
- Is smallpox virus a potential biological terror weapon?
- Replication of "Large" DNA-containing Bacteriophages
- Components of large DNA-containing phage virions
- Replication of phage T7
- The practical value of T7
- T4 bacteriophage: the basic model for all DNA viruses
- Replication of phage λ a "simple" model for latency and reactivation
- Biochemistry of the decision between lytic and lysogenic infection in *E. coli*
- A GROUP OF ALGAL VIRUSES SHARES FEATURES OF ITS GENOME STRUCTURE WITH POXVIRUSES AND BACTERIOPHAGES
- QUESTIONS FOR CHAPTER 19

If the classification of DNA viruses into large and small is arbitrary, and in the terms of this text, it certainly is, then the grouping of large DNA viruses that replicate in the cytoplasm of eukaryotic cells with large DNA-containing bacteriophages is even more so. Indeed, this grouping is only defensible in that bacteria have no nuclei, so *any* virus infecting them by necessity will replicate in the cytoplasm. Indeed, there is evidence based on the details of capsid assembly and some very limited genetic sequence homologies that the bacteriophages discussed in this chapter have a distant relationship to the herpesviruses discussed in the last chapter! Still, organizational criteria can be satisfied best by inclusion of these viruses in this chapter, and the inclusion can be operationally defended because these bacteriophages—like poxviruses and unlike herpesviruses and many other DNA viruses utilizing the nucleus as a site for genome replication—encode many transcriptional enzymes required for replication.

WBV19 6/27/03 4:05 PM Page 337

POXVIRUSES – DNA VIRUSES THAT REPLICATE IN THE CYTOPLASM OF EUKARYOTIC CELLS

The poxviruses are a very successful group of double-stranded (ds) DNA–containing viruses that have evolved a highly specialized mode of replication and pathogenesis in animal hosts: the ability to replicate in the cytoplasm of infected cells. Study of smallpox disease and attempts to control it over the past three centuries are responsible for generating much of our basic understanding of virus-induced immunity, virus epidemiology, and viral pathogenesis.

The pox virion is complex and contains virus-coded transcription enzymes

Poxviruses are physically complex, brick-shaped objects with a highly organized subvirion structure, which contains an inner core surrounded by a double membrane derived from the host. A schematic diagram of the structure is shown in Fig. 19.1. They comprise the largest known viruses, with virion dimensions on the order of 250 to 300 nm by 250 nm by 200 nm. This size is just large enough to be resolved with ultraviolet light, and if great care is taken in sample preparation, poxvirus virions can be observed as refractile points with a high-quality optical ultraviolet microscope.

One consequence of the cytoplasmic site of poxvirus replication is that the virus has no access to cellular transcription and DNA replication machinery—at least during the earliest times after infection. It must, then, supply all or most of the nuclear functions that other nuclear-replicating DNA viruses appropriate from the cell; therefore, it is hardly surprising that poxviruses have large genomes. The genomes of orthopox viruses, which include smallpox and vaccinia, contain the replication genes clustered in the center 50% or so of the genome. This is flanked on either side by genes specific to the actual type and strain of poxvirus. The core replication sequences are highly conserved, but the flanking sequences diverge widely.

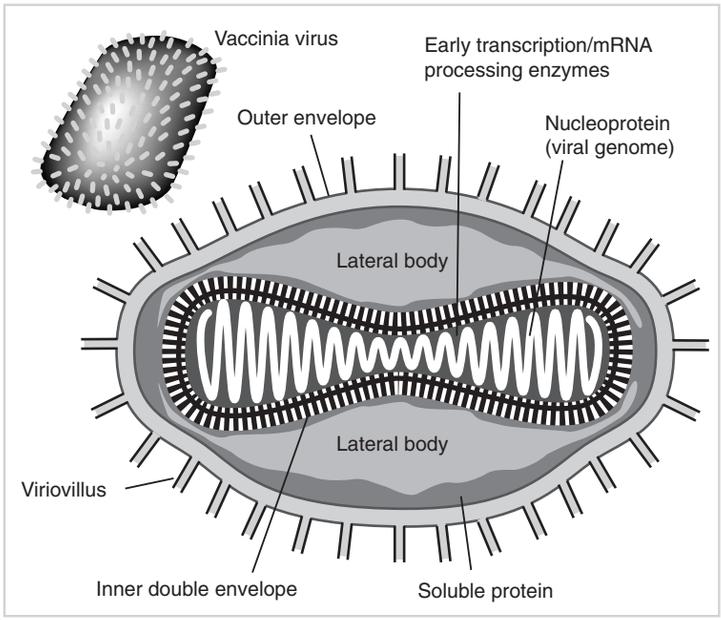

Fig. 19.1 The vaccinia virus virion. The structure of poxviruses is the most complex known among the animal viruses, and rivals that of some bacterial ones. The particles are on the order of 400 nm in their longest dimension. The virion contains numerous enzymes involved in RNA expression from the viral genome concentrated as a nucleoprotein complex in the core. The lateral bodies have no known function.

WBV19 6/27/03 4:05 PM Page 338

The complex virions contain all the enzymes necessary for transcription, polyadenylation, and capping of a specific class of viral mRNAs—those encoding the enzymes required to begin the replication process in the cell. In this, the replication strategy is vaguely reminiscent of some negative-sense RNA viruses. A partial list of enzymes found in the virion is as follows:

1 RNA polymerase
2 Early transcription factors
3 mRNA capping enzymes
4 ATPases
5 DNA helicase, ligase, and topoisomerase
6 Protein kinase(s)

The poxvirus replication cycle

The replication cycle of a typical poxvirus is shown in Fig. 19.2. Most details of this replication cycle were established by studying the poxvirus vaccinia used as the human vaccine against smallpox. Interestingly, the ultimate origins of neither vaccinia nor cowpox virus are known. The reservoir for cowpox virus is thought to be rodents, and the virus is unusual in its extremely broad host range. Vaccinia for vaccine production has been traditionally grown on the skin of horses, and may have originated as a horse virus. The high degree of conservation of core replication genes for all poxviruses means that the molecular details of their replication are very similar, this is not necessarily the case for the ways that the different types of orthopox viruses evade host defenses.

Viral replication can be separated into three specific temporal phases or stages in the infected cells, as was the productive replication cycle of other DNA viruses described in preceding chapters.

Early events

Productive infection involves the virus interacting with specific receptors on the surface of susceptible cells, followed by virus entry into the cytoplasm where *partial uncoating* occurs. This partial uncoating is very important in the virus's life cycle, because following this, the virion-associated transcription enzymes are able to begin expression of early viral mRNA, which is translated by the host's cellular translation machinery.

Partial uncoating can be accomplished in vitro by treating infectious virions in the laboratory with nonionic detergents such as NP-40. These detergents are highly selective in the lipid-associated structures they solubilize, and the treatment of pox virions with such reagents results in *core particles* that will transcribe viral mRNA as long as ribonucleoside triphosphates are supplied. Early enzymes accomplish complete uncoating of the infected cell's core particles, and this signals the end of the early period of infection, which (depending on the exact poxvirus) occurs during the first 4 to 8 hours following infection. While the virus replicates in the cytoplasm, one early protein mobilizes a cellular nuclear protein, Vitf2, into the cytoplasm where it has a major role in expression of the next set of viral transcripts—the delayed early or intermediate class.

The virus also expresses a number of proteins, which are clear homologues to cellular ones. These interfere with host response by blocking MHC-1 presentation, inhibiting a number of arms of the interferon response pathway, and block apoptosis. Further, various pox viruses encode genes, which were derived from cellular growth factor genes. These act to cause localized cellular proliferation—one of the characteristics of the distinct pock or pustule containing infectious virus that erupts on the skin, and (perhaps) providing further cells for continued virus replication.

WBV19 6/27/03 4:05 PM Page 339

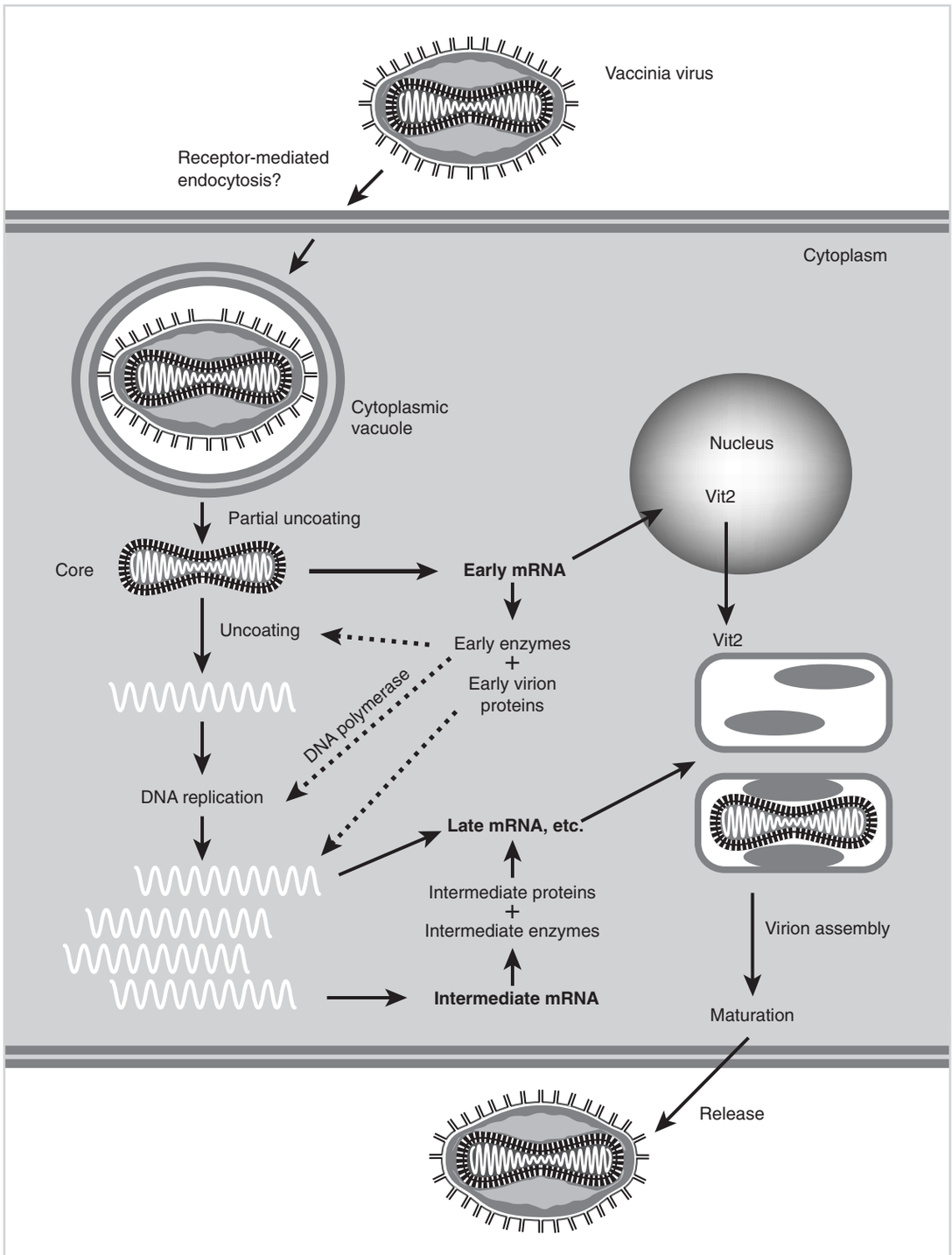

Fig. 19.2 The replication cycle of vaccinia virus. Following viral attachment to cellular receptors, internalization is thought to be by receptor-mediated endocytosis. Virions are partially uncoated in the vesicles and core particles are then released into the cytoplasm where early mRNA synthesis and expression of early viral proteins occur. These proteins function to continue the uncoating of the core and to replicate viral DNA. Late mRNA expression from replicated genomes leads to expression of structural and other proteins involved with virus maturation. Viral gene expression and genome replication cease by approximately 6 hours after infection, but morphogenesis of the complex virion requires a further 14 to 16 hours.

WBV19 6/27/03 4:05 PM Page 340

Intermediate stages of replication

During the intermediate stages of replication, a series of intermediate mRNAs are expressed by virtue of early proteins interacting with existing viral polymerase and Vitf2, and a new polymerase. These intermediate proteins include the enzymes required for viral DNA replication. The poxvirus genome ranges in size from 160 to over 200 kb, depending on the virus under study, but all viral genomes share some basic features. The double-stranded DNA molecules have closed ends, and with relatively long stretches of inverted terminal repeat sequences at the ends. Viral DNA replication apparently initiates at nicks near the closed ends, but the details of priming and exact means of chain elongation are not well characterized. It is clear, however, that poxvirus DNA replication does not require association of a specific origin-binding protein with viral DNA, and *any* circular DNA molecule present in the cytoplasm at the time of infection is replicated to high copy numbers. This is of great practical value, since recombination also takes place in the cytoplasm during DNA replication, and the system can be exploited to generate defined recombinant viruses that have a number of potential medical and research uses. Some promising applications of such viruses are described in Part III.

Late events in the replication cycle

Some intermediate enzymes mediate the transcription of late mRNA, which is translated into late proteins. These late proteins include a large number of virion proteins as well as the proteins required for the morphogenesis of complex pox virions. These proteins also include the virion-associated transcription enzymes required to initiate the next round of viral replication. Morphogenesis is a two-step process; first the inner core particles assemble and are enveloped with a double membrane within the Golgi apparatus. These then are released into the cytoplasm where they mature into intracellular infectious virions, which can spread to neighboring cells through cellular junctions, and which can be released upon cell lysis. Some of these virions are incorporated into exocytotic vesicles, which are released to comprise a second population of mature virions containing an exterior membrane envelope.

Pathogenesis and history of poxvirus infections

Poxvirus infections are relatively unusual among the viruses presented in this text in that they cause a high rate of mortality during the natural course of spread in the host population. This is well illustrated by a consideration of smallpox in humans. This disease was a scourge for thousands of years, and occurs in two forms: variola major, with a mortality rate approaching 20% in immunologically naive populations, and variola minor, with lower mortality rates (2% to 5%). The high mortality rate is associated with the pattern of virus spread and pathogenesis in the host, as described in Chapter 4, but remember that in humans, the virus is spread by inhalation. Primary infection is in the lungs, and only after the virus erupts in the skin as characteristic pox is virus available for further spread. The formation of open sores on the skin leads to a high incidence of superinfection with opportunistic pathogens, as well as relatively nonspecific physiological responses to a disseminated infection of the skin.

The mode of spread of the virus in humans is related to the fact that unlike almost all other animal viruses, pox virions are very resistant to inactivation by desiccation. Infectious virus can be recovered in contaminated clothing, bedding, house wares, and soils for significant periods following the resolution of infection in a particular individual.

Many (if not most) poxviruses induce a rapid proliferation of cells in the neighborhood of the infected cell by means of expression of a virus-encoded protein related to epidermal growth factor. This secreted viral growth factor is expressed in the early stages of infection and serves to

WBV19 6/27/03 4:05 PM Page 341

induce metabolic activity in neighboring cells to provide a more optimal environment for virus replication. The proliferation of cells in the vicinity of an infection center on the surface of the skin is also important in the development of the characteristic pox. The virus also is able to modulate host immunity by virtue of expressed viral proteins that sequester lymphokines important in mediating the antiviral immune response, including tumor necrosis factor (TNF) and interferon-γ.

While the pathogenesis of poxvirus results in its being a very successful pathogen in a number of animal hosts, it carries an "Achilles heel." Like some RNA viruses, the poxviruses do not remain associated with the host after primary infection; whether or not the host is killed, the virus is cleared. Hosts that survive have permanent immunity to reinfection. Further, many animal poxviruses are immunologically closely related to human smallpox virus, and the viral proteins expressed by such viruses can induce immunity to smallpox in humans. Indeed, Jenner's original regularization and characterization of vaccination techniques, described in Chapter 8, were based on common knowledge that dairy workers infected with the relatively mild cowpox virus were refractory (immune) to infection with human smallpox.

Is smallpox virus a potential biological terror weapon?

The characteristics of its pathogenesis and spread make smallpox uniquely subject to public health prevention measures, and a careful worldwide program of vaccination, disease reporting, and isolation of infected individuals led to eradication of smallpox from the human population at large in the 1970s. Currently, the only known smallpox viruses exist in public health laboratories in Russia and the United States, and there has been an active debate concerning the desirability of destroying these last vestiges of this terrible scourge.

While this success story is heartening, the political instability in the Middle East as well as degradation of Russian security measures with the collapse of the Soviet Union has made it very evident that declaration of full victory in the war against human suffering caused by poxviruses is premature. With the discontinuation of active vaccination campaigns, susceptibility to infection is now widespread throughout the world. Convincing scenarios have been discussed in which organized and trained terrorists could, for whatever motivation, penetrate within a large open population such as those in the developed world and instigate wide-spread outbreaks of infection. These would not need to lead to a mass epidemic to cause severe political and economic dislocations, since modern health care facilities are ill equipped to deal with the active disease and its containment. Further, mass vaccination would require large stocks of vaccine, and stocks have waned since victory against smallpox was declared.

Currently, the US government, at least, has decided to manufacture large stocks of vaccine with the idea that preparing for the worst-case scenario makes sense in light of the events of September 11, 2001. Still, this requires time and significant resources to do properly, and mistakes and problems in production distribution and use are almost inevitable.

Such considerations point, again, to the fact that no scientific or medical program of prevention is anywhere near as effective against a biological threat of this magnitude as is the absence of motivation to develop the threat in the first place. If the political efforts and international organization needed for the eradication of the stocks of smallpox in the world had been effective and complete, the problem would be greatly lessened, but still would not be solved, because there exists plenty of expertise available for the construction of virulent pathogens such as smallpox from its component genes. Again, the only real defense against potential biological terror weapons is for societies to ensure that there are no strong motivations for an organized effort by an actual or self-considered disenfranchised minority to resort to them. This would not, of course, guard against the threat of an occasional psychopath, but an isolated outbreak of a virulent disease in the face of an informed and empowered populace is no real threat.

WBV19 6/27/03 4:05 PM Page 342

REPLICATION OF "LARGE" DNA-CONTAINING BACTERIOPHAGES

As briefly outlined in Chapter 1, the study of three groups of DNA-containing bacterial viruses infecting *E. coli* (the T-even, T-odd, and λ phages) has occupied a central and seminal place in the development of molecular biology and the functional understanding of gene expression and gene manipulation. Aspects of the replication and structure of these viruses are still studied for their own sake, and the λ phages are in general use as agents for molecular cloning of large DNA segments.

Components of large DNA-containing phage virions

Bacteriophages display a variety of structures, as briefly outlined in Chapter 5. While the size and complexity of specific structural features vary with the size of the viral genome and the nature of the virus, the phages discussed here all share similar structural features. The structure of bacteriophage T7, shown in Fig. 19.3, illustrates some of these features. Notable structural elements include a complex icosahedral head containing the viral genome, a noncontractile tube or sheath for injection of the viral genome into host bacteria, and a **base plate** and fiber structure for attachment of the phage to the host.

Replication of phage T7

The genome

Phage T7 has a linear, dsDNA genome 39,936 base pairs long. Its genetic map is shown in Fig. 19.3. An interesting feature not seen in the other DNA viruses discussed herein is that the viral genes are "clustered" into specific regions that are expressed at specific times. As with the T-even phages discussed in a following section, phage T7's DNA genome is terminally redundant—the 160 base pairs occurring at the beginning of the genome are directly repeated at the other end. This redundancy serves to allow the viral genome to circularize during replication so that no sequences are lost during the replication process (see Chapter 14).

Phage-controlled transcription

As with other DNA viruses, replication of T7 can be divided into temporal phases. Unlike nuclear-replicating DNA viruses that infect vertebrates, the bacteriophage T7 transcription program is mediated by substitution of a phage-encoded RNA polymerase with a specificity different from that of the host cell. Infection is initiated by insertion of the genome into a host bacterial cell.

The first molecular event occurring after insertion of the phage genome is transcription of a set of phage genes called the early genes. These are generally equivalent to the immediate-early or pre-early genes expressed by adenoviruses and herpesviruses. Early transcription is carried out by host RNA polymerase and results in production of a series of five mRNAs that encode phage proteins. Encoded in this set of phage genes is the T7-specific RNA polymerase, the enzyme that will carry out the balance of gene expression (delayed early and late) for the virus.

T7 RNA polymerase differs from host enzyme in the nucleotide sequence of the promoter regions that it recognizes. Phage RNA polymerase only recognizes promoter sequences found upstream of the delayed early and late classes of T7 genes.

Another immediate-early gene, encoding a protein kinase, catalyzes phosphorylation and inactivation of host RNA polymerase, effectively stopping transcription of host mRNAs. Thus, within a few minutes after infection, phage T7 takes over the host cell and converts it into a factory for production of new virus particles.

WBV19 6/27/03 4:05 PM Page 343

The T7 delayed early genes are essentially equivalent to the early genes described for numerous DNA-containing animal viruses. These include genes encoding a T7 DNA polymerase that carries out replication of the viral genome. Late genes include genes encoding structural proteins for the phage capsid, as well as a gene required for cell lysis. The infectious cycle continues with the replication of phage DNA and assembly of progeny virus. This assembly process is generally described in Chapter 6.

The practical value of T7

Phage T7 provides another look at how a virus can completely take over a host cell and convert it into a factory for making progeny particles. In this case, rather than altering an existing host enzyme for transcription, the virus encodes a completely new RNA polymerase that recognizes an entirely different set of promoter sequences. So effective is this system that virtually no host mRNA is transcribed. Because of this specificity, the T7 polymerase and its promoter sequences have become the basis for several expression vectors used in recombinant DNA technology.

T4 bacteriophage: the basic model for all DNA viruses

The study of bacteriophage T4, along with other related T-even phages, occupies a unique place in the annals of molecular biology and molecular genetics. The speed and ease of manipulation and the facility of doing genetics with mixed phage infections, plus the convenience of using *E. coli* as a host, made these viruses a major subject of experimental investigation from the 1930s through the mid-1960s. Many "firsts" were recorded in the study of T-even phages, and even now, their study continues to offer new insights and concepts, especially with regard to understanding large-scale macromolecular structures. Further, gene products encoded by phage T4 provide a valuable resource for biotechnology and genetic manipulation.

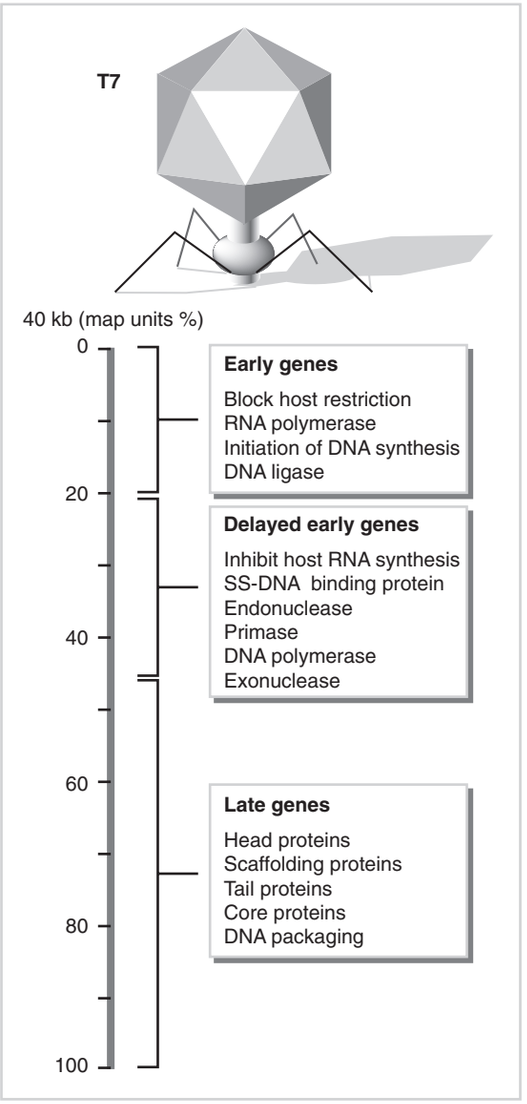

Fig. 19.3 The structure and genetic map of T7 bacteriophage. The 40 kb gene map shows that genes are clustered according to function, with those involved in the earliest stages of infection shown to the left. Transcription begins as the DNA is injected into the host, so the left portion of the genome must be injected first. The early genes include an RNA polymerase that transcribes later genes from the viral genome.

The T4 genome

The genome of T4 has some unique structural features. A complete genetic map and model of phage structure (quite similar to T7, but more complex) are shown in Fig. 19.4. The map is really no more complex than that of HSV whose genome is about 90% the size of T4 (150 versus 168 kb). One obvious difference is that nearly 50% of T4's genetic complexity is devoted to encoding proteins of the complex capsid; this value is nearer 30% for HSV. Also, for obvious reasons, T4 does not need to encode a large number of genes important for dealing with host immune defenses.

Although the viral genome is a linear, dsDNA molecule, the genetic map is circular. This results from the DNA being terminally redundant—a situation similar to that seen for phage T7. As with other linear DNA virus genomes, terminal redundancy allows the genome to become circular by recombination.

The T4 genome is circularly permuted. Circular permutation means that the starting point in the linear genome (one end of the molecule) differs for various members of a particular virus

WBV19 6/27/03 4:05 PM Page 344

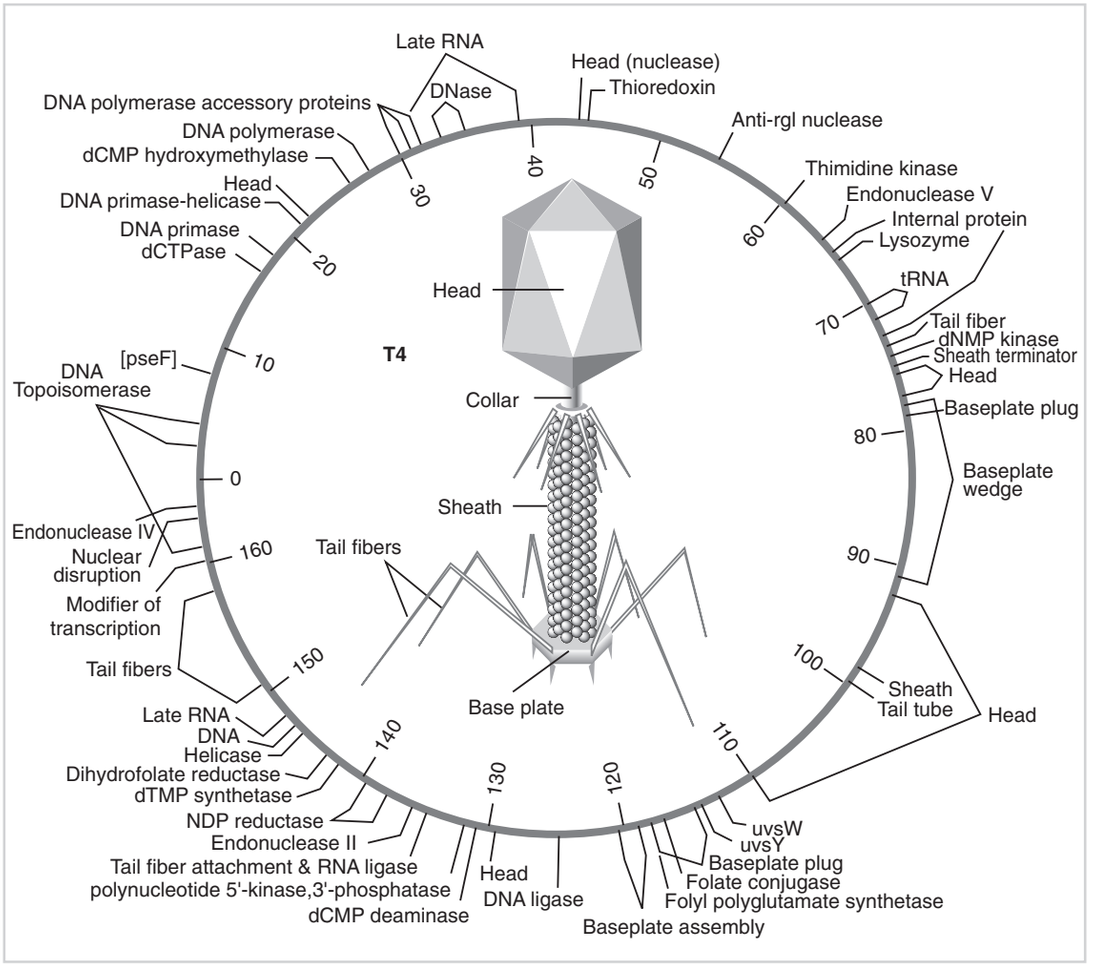

Fig. 19.4 The genetic map and structure of bacteriophage T4. By convention, this map is divided into kilobases instead of map units. Since the viral DNA ends are redundant, the starting point shown is entirely arbitrary. Considerably larger than T7, the phage particles have similar head shapes, but T4 has a contractile sheath and a base plate important in attachment (see Chapter 6). The viral genetic map is as complex, but not more so, than that of HSV shown in Fig. 18.2. Note that unlike HSV but similar to T7, many genetic elements are functionally clustered.

population. Essentially, all possible starting points in the linear sequence are represented, as shown diagrammatically in Fig. 19.5. Circular permutation is a consequence of the viral genome being replicated by a complex rolling circle mechanism (much like that of HSV discussed in Chapter 18). Each phage head encapsidates one full genome length of DNA *plus a bit more*. The generation of such circularly permuted genomes also means that there is no unique packaging signal for T4 DNA. A possible mechanism for this packaging of a "genome-plus" piece of DNA is shown in Fig. 19.5.

In addition to the distinctive genome structure of these viruses, the base composition of the DNA differs from that of the host cell. T-even phage DNA contains the unusual base 5-hydroxymethyl cytosine (5′-OHMeC) in place of cytosine. The precursor triphosphate, 5′-OHMeCTP, is synthesized in the cell by phage-specific enzymes. In fact, T4 hydroxymethylase, identified by Seymour Cohen in 1957, was the first viral-encoded enzyme ever to be described. T-even phage DNA is further modified in that a portion of the 5′-OHMeC base has one or two glucose residues

WBV19 6/27/03 4:05 PM Page 345

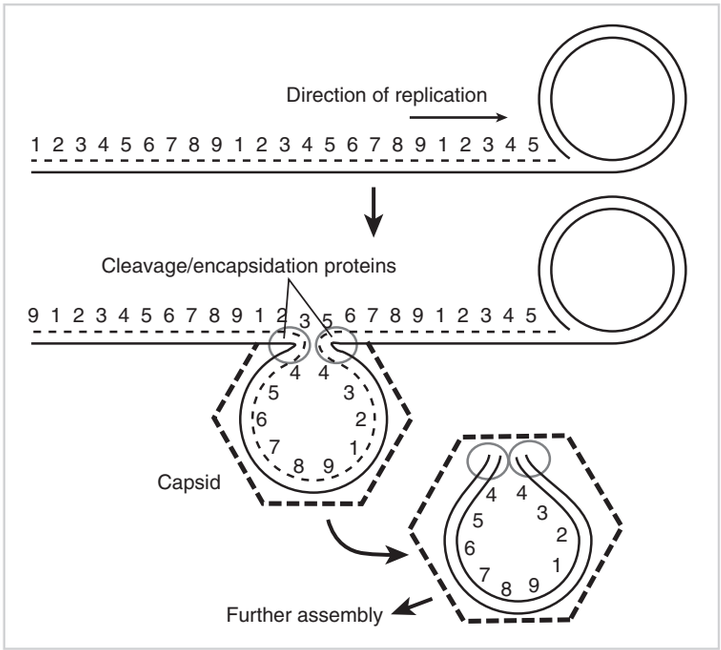

Fig. 19.5 Rolling circle replication and packaging of phage T4 DNA. The process has similarities to that of HSV shown in Fig. 18.5, but there is no specific packaging signal in the viral genome. Packaging begins at random sites and once the phage head is filled with an amount of DNA that is equivalent to 110% or so of the full genome, the ends are cleaved and packaging is completed. This results in the encapsidated DNA ends being redundant. This redundancy leads to this linear DNA molecule producing a circular genetic map, as shown in Fig. 19.4.

covalently linked to the hydroxymethyl residue. This glycosylation has a role in the virus abrogation of host restriction defenses.

Regulated gene expression during T4 replication

As is usual in DNA virus replication, the transcription of T-even phage genes is temporally controlled during infection; thus, expression of T4 genes varies with time after infection and can be divided into four stages: immediate-early, delayed early, quasi-late, and late (or strict-late). This temporally regulated pattern is shown in Fig. 19.6. The transcriptional switching in T4 infection involves use of the bacterial host's DNA-dependent RNA polymerase; this mechanism is distinct from the encoding of a novel RNA transcription enzyme like T7. During T4 infection, the specificity of RNA polymerase for particular promoters is altered by expression of phage-specific sigma (σ) factors, and by modification of the core enzyme by phage-encoded enzymes. The role of such factors in bacterial RNA polymerase specificity is outlined in Chapter 13.

T4 gene expression begins with transcription of the immediate-early genes. This takes place utilizing the host's RNA polymerase and σ factors. The transition to delayed early gene expression involves recognition by host enzyme of certain phage promoter sequences and modification of host enzyme, possibly by the phage-catalyzed covalent addition of ADP-ribosyl groups to each of the RNA polymerase alpha (α) subunits. *This change occurs in two steps: The first is catalyzed by a phage gene that enters the cell along with viral DNA (gp alt), and the second is catalyzed by a phage gene that is itself the product of immediate-early expression (gp mod).* ADP-ribosylation may not be required for the transitions, since double mutants in both gp alt and gp mod are able to carry out delayed early gene expression in a normal manner.

The two other phases of viral gene expression—quasi-late and strict-late—require replicating DNA structures and involve sequential replacement of host σ factors by phage proteins.

WBV19 6/27/03 4:05 PM Page 346

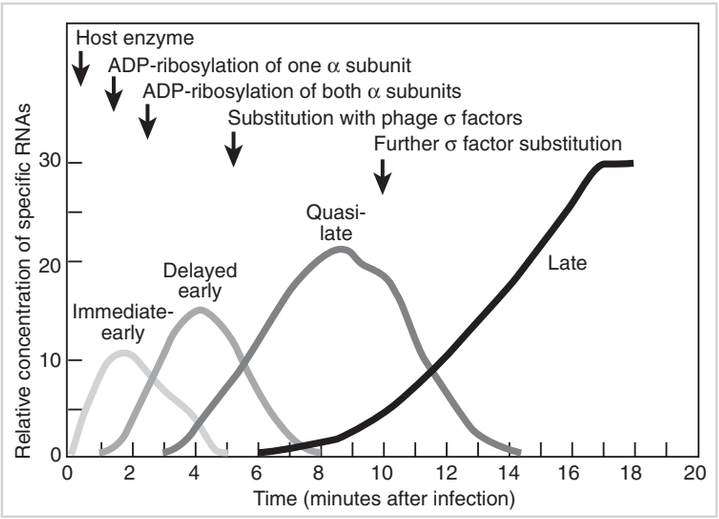

Fig. 19.6 Time of appearance of various functions encoded by T4 bacteriophage. Each class of transcripts is transcribed by modified *E. coli* RNA polymerase. The modifications are sequential: First one and then the second alpha (α) subunit of the RNA polymerase is modified by covalent linkage of an ADP molecule; then various phage-encoded proteins displace first the host sigma (σ) factor and then one another to generate enzymes of altered specificity.

Capsid maturation and release

The patterns of T4 maturation are known in exquisite detail. A broad outline is shown in Fig. 19.7. In essence, the process of head assembly and DNA encapsidation is very similar to that of the other large DNA viruses described herein. After the filling of the head, the other components of the complex virion, which have preassembled to form subassemblies, come together to form the complete particle. As complex as it is, all these steps are simple biochemical reactions driven by mass action, and can be mimicked in a test tube. Mature phage is released from the infected cell by expression of a late lysozyme that disrupts the bacterial cell wall, releasing virus.

Replication of phage λ: a "simple" model for latency and reactivation

Many eukaryotic viruses, especially those with DNA genomes, have evolved complex mechanisms for remaining associated with the individual that they have infected long after the disease caused by the initial infection is resolved. A good example of this is found with the latent phase of infection by herpesviruses; another is the limited transformation of cells induced by papillomavirus infections of epithelial cells.

The mechanism for continued association between virus and host varies with the virus and host in question, but nowhere is it more fully described than in the replication cycle of bacteriophage λ. In phage λ, one encounters a virus with two very different outcomes of infection with very different consequences for the host bacterial cell — either lysis or lysogeny.

Productive or lytic infection entails expression of the phage genes required for replication of phage DNA, synthesis of phage structural proteins, assembly of viral particles, and lysis of the host cell. Although different in some details from the process outlined for T7 and T4 phages, the process is essentially the same.

Lysogeny, on the other hand, is characterized by the virus suppressing its own vegetative DNA replication. Instead, the viral genome becomes integrated into the bacterial chromosome where the virus exists in a lysogenic or latent state until a set of metabolic stimuli reinitiates productive infection. When integrated, the phage λ genome is called a **prophage**. It is clear that there are phenomenological parallels with herpesvirus and papillomavirus latency as well as with retroviruses discussed in the next chapter, but the details are unique to bacteriophage λ.

WBV19 6/27/03 4:05 PM Page 347

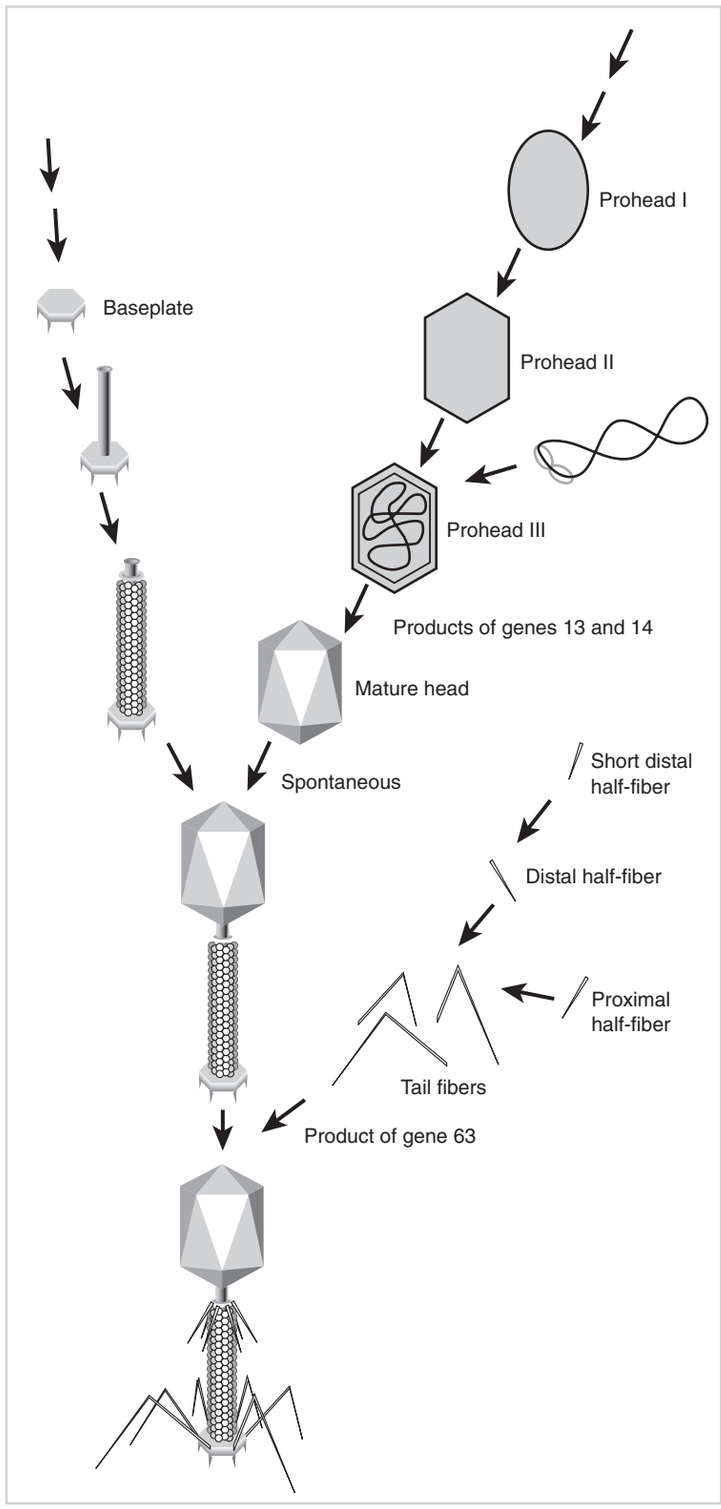

Fig. 19.7 The assembly of T4 bacteriophage. Note that assembly of the phage head is similar to the process seen with HSV. Other components of the virion are assembled as "subassemblies" brought together sequentially to form the full phage. (This figure is drawn from work reviewed and presented by W.B. Wood. Bacteriophage T4 assembly and the morphogenesis of subcellular structure. *Harvey Lectures* 1979;73:203–23.)

WBV19 6/27/03 4:05 PM Page 348

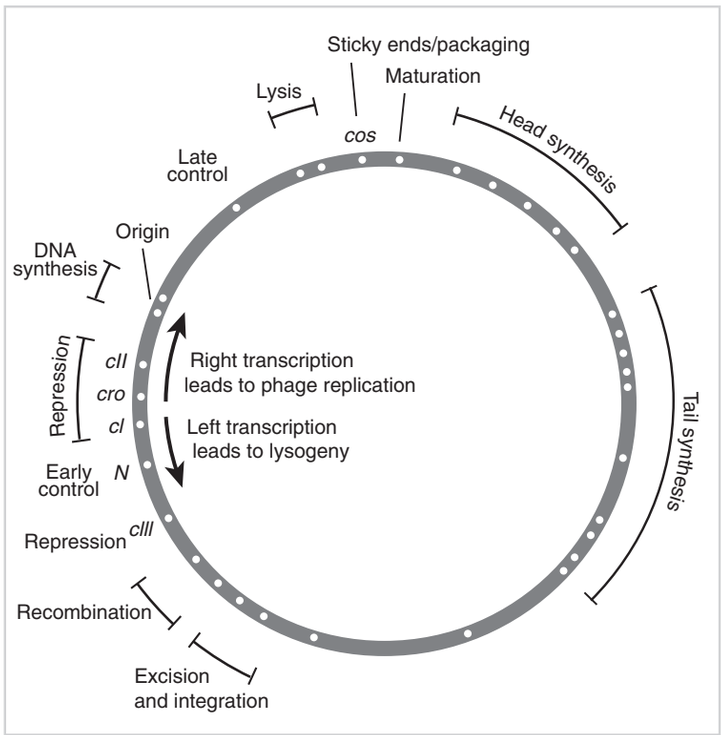

Fig. 19.8 The bacteriophage λ genetic map. Specific clustered functions are indicated. The primary decision of whether to replicate or integrate involves the single question of whether leftward or rightward transcription occurs first. This process is entirely stochastic (random). If transcription takes place to the left, cI repressor is expressed and blocks lytic replication. At the same time, integrase and recombination functions lead to the phage DNA being integrated into the host bacteria's genome.

Infection of a bacterial cell by phage λ leads to a competition between the mutually exclusive processes leading to lytic or lysogenic phases of infection, and the outcome is just a matter of which process happens first. Both outcomes result from the action of proteins encoded by a small subset of phage genes expressed immediately upon infection. Their interaction is complex; indeed, the biochemical "decision" between lysogeny and lytic replication is among the most complex biochemical control pathways known. The original phenomenon of latency was discovered in the 1920s, and only after 60 years or so of development of ever-more sophisticated biochemical, physical, and genetic analyses have the details been fully worked out. It is not too much to say that the successful melding of biochemistry, molecular biology, classic genetics, and molecular genetics was needed to fully decipher the complex and elegant pathways involved in the biochemical decision made by phage λ each time it initiates an infection in *E. coli*. This melding stands both as a triumph of modern biology and as a model to achieve understanding of all biological processes.

The phage λ genome

The phage λ genome whose map is shown in Fig. 19.8 is a linear dsDNA molecule 48.5 kbp in size. Unlike either T7 or the T-even phages, there is no terminal redundancy. Despite this, the genetic map is represented as a circle because λ DNA can become circular following infection. The genome has a complementary stretch of single-stranded bases at each end. These "sticky ends" can anneal in the phage head to form a noncovalently bonded circle, which is converted to a covalently closed molecule by ligation shortly after infection.

Phage λ gene expression immediately after infection

Upon injection of viral DNA, unmodified *E. coli* RNA polymerase can recognize two λ promoters, and can transcribe two mRNAs from λ DNA, as shown in Fig. 19.9. This is the immediate-early

WBV19 6/27/03 4:05 PM Page 349

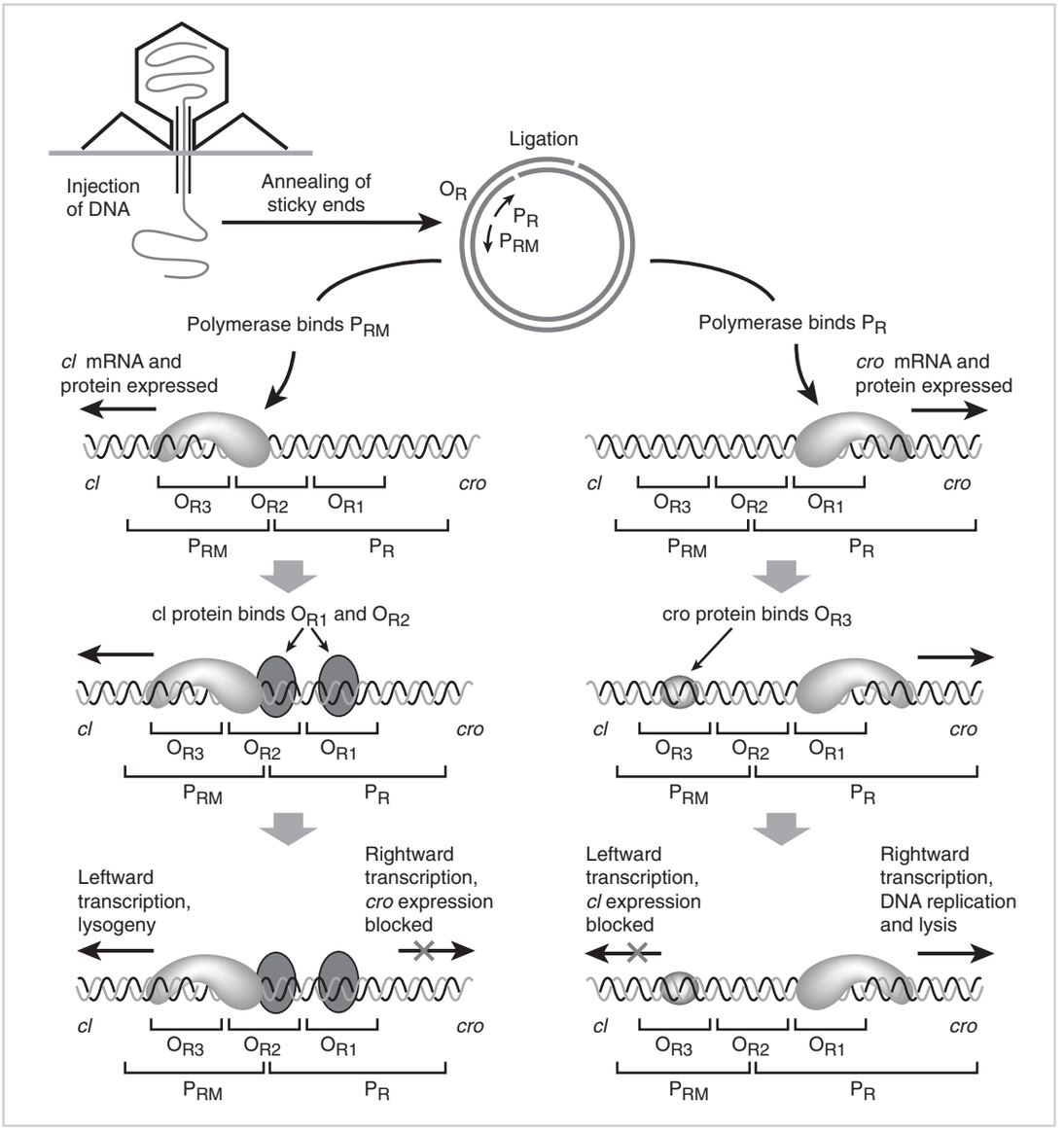

Fig. 19.9 The earliest events in the infection of a bacteria by phage λ. Details of the biochemical decision concerning lysogeny or vegetative replication are outlined here and described in the text. (This figure is based, in part, on work described by M. Ptashne. In *A Genetic Switch: Gene Control and Phage* λ. Palo Alto: Blackwell Science and Cell Press, 1986. Especially chapters 2 and 3.)

phase of gene expression. The rightward promoter, termed P_R, transcribes mRNA encoding the cro protein, which is so named because of its control function. The leftward promoter, P_L1, controls expression of mRNA for the N protein, which acts to modulate the utilization of transcription termination signals (see Chapter 13).

Notice that the earliest stage of transcription from these two promoters terminates at two sites (T_R1 and T_L1), both of which have λ-dependent termination signals. Many of the early regulation events involve the action of competing phage genes on these termination signals to suppress their activity. If one set is suppressed, transcription proceeds through the signal and a further set of transcripts is expressed; if the other set is effectively suppressed, then a *second* set of extended transcripts encoding competing functions is expressed.

WBV19 6/27/03 4:05 PM Page 350

The action of cro: lytic growth The λ cro protein, under appropriate metabolic conditions of growth, can repress transcription of the mRNA encoding another phage regulatory protein called the λ CI repressor by binding to control sites just upstream of these two promoters. The λ repressor is involved in establishing lysogeny and its repression serves as a "green light" for lytic growth, especially when sufficient N protein has been made. The lytic genes include DNA replication and capsid proteins. The result of this rather Byzantine process is production of progeny phage and lysis of the cell.

Modulating the activity of the N protein: priming the cell for lysogeny Expression of high levels of the λ phage N protein leads to expression of a set of delayed early genes. It does this by interacting with a host protein called N-utilizing substance A (nusA). This interaction allows RNA polymerase to read through the two immediate-early termination sites, producing longer transcripts in both directions. The transcripts expressed from rightward transcription encode two further phage proteins, cII and cIII, which together enhance the expression of cI, the λ repressor.

Action of cI, cII, and cIII: establishment of lysogeny Extension of leftward transcription as well as additional rightward transcription will eventually lead to onset of lytic-phase gene expression. Essentially simultaneous with the earliest events on this path, however, phage λ cII and cIII proteins can act in concert to stimulate transcription of mRNA for cI, the λ repressor. Both cII and cIII are encoded in the mRNA that begins at P_R and continues through T_R1. This "read through" expression requires N protein action, and the cII protein stimulates transcription of cI mRNA beginning at P_{RE} (promoter for repressor establishment), while the cIII protein functions to stabilize cII against degradation by inhibiting host cell protease.

The λ repressor (cI) serves two roles: (i) It represses transcription of cro mRNA from P_R and of N mRNA from P_L1; and (ii) It stimulates transcription of its own mRNA. Thus, it blocks expression of genes required to initiate the lytic cycle. Notice that by blocking cro and N synthesis, cI also blocks synthesis of cII and cIII. Therefore, *all subsequent transcription of cI mRNA during the lysogenic* phase takes place from P_{RM} (promoter for repressor maintenance). Recall that promoter switching in response to repression by viral products is seen in the replication of adenovirus, discussed in Chapter 17.

Integration of λ DNA: generation of the prophage Lysogeny is established with λ integrase action, which catalyzes phage genome recombination into a specific site in the *E. coli* chromosome. The phage genome is then found as a linear sequence within host DNA and the host cell is now called a λ *lysogen*. The name comes from the fact that while phage replication is generally repressed, occasionally lytic growth can by triggered; thus, the bacteria harboring the prophage can give rise to lysis. The only phage gene expressed in a λ lysogen is cI, and in most lysogenic cells, phage DNA remains stably integrated, replicating along with the cellular chromosome.

Biochemistry of the decision between lytic and lysogenic infection in *E. coli*

Competition for binding by cro and cI at the operator O_R

Both cro and cI bind to the operator O_R as dimers. The three binding sites in O_R are called O_R1, O_R2, and O_R3, and are shown in Fig. 19.9. The affinity of these sites differs for each protein dimer; cro repressor binds in the order O_R1, O_R2, and O_R3. Binding to O_R3 effectively blocks transcription of cI from P_{RM}. Conversely, cI binds in the reverse order of O_R3, O_R2, and O_R1. The cI protein's binding to O_R1 blocks transcription of cro mRNA from P_R. The additional binding of cI to

WBV19 6/27/03 4:05 PM Page 351

O_R2 acts to stimulate transcription of cI mRNA from P_{RM}. Thus, as noted earlier, cI has the dual ability to repress cro transcription as well as to stimulate transcription of its own mRNA.

This competitive binding and competing repression of the divergent promoters P_R and P_{RM} result in a control system that is quite sensitive to relative concentrations of cro and cI. During the early stages of infection, the rates of synthesis and degradation of these two proteins will dictate which pathway — cro dominated (lytic) or cI dominated (lysogenic) — will be followed. The variety of metabolic factors controlling these rates are described below.

Factors affecting the lytic/lysogenic "decision"

The relative transcription rate of mRNAs for the two critical phage proteins cro and cI is altered by a variety of metabolic conditions that a lysogenic cell may encounter. The strategy of viral gene expression is such that lysogeny will occur in a healthy, rapidly growing cell. When, however, the cell encounters changes that threaten survival of a particular cell such as DNA damage or starvation, λ phage "jumps ship" by inducing lytic replication — the resulting phage particles may survive until a more fruitful time for bacterial growth and lysogeny.

Stability of the cI protein itself can determine the balance between the two competing repressors. The cI protein can be proteolytically cleaved and inactivated by the host's recA protease, which is induced by DNA damage. In the so-called SOS repair system, the recA protease destroys cellular repressor proteins, resulting in expression of a series of bacterial enzymes involved in repair of DNA damage. This cellular response also results in the destruction of cI and induction of the lytic phage pathway in a λ lysogen. Indeed, exposure to ultraviolet light has long been a favored method for the induction of lytic infection in lysogenic strains of *E. coli*.

Action of the cellular protease HflA is sensitive to the cell's nutritional state as moderated by the catabolite repression system. At low glucose levels in the cell (i.e., when metabolic activity is slow), the cell generates high levels of 3′,5′-cyclic AMP (cAMP) as an intracellular signal of metabolic stress. Indeed, similar signals are used in eukaryotic cells in response to a number of extracellular stimuli. At high cAMP concentrations, activity of the HflA protease increases. The result is an increased inactivation of cII and a concomitant decrease in cI synthesis during the early phase of infection.

A GROUP OF ALGAL VIRUSES SHARES FEATURES OF ITS GENOME STRUCTURE WITH POXVIRUSES AND BACTERIOPHAGES

As mentioned, there is a strong resemblance between aspects of the capsid structure and assembly of herpesviruses and T-even bacteriophages. Other viruses of the animal, bacterial, and plant kingdoms also share certain details of structure and replication. Viruses of vascular plants are subject to a strict limitation of genome size because of their problems of penetrating the cellulose cell wall of such plants (see Chapter 17). This limitation does not apply to viruses of "lower" plants such as algae, however. Recently *Paramecium bursaria Clorella-1 virus* (PBCV-1), a virus that infects a type of algae that often lives symbiotically with a species of paramecium, was extensively characterized and shown to have some suggestive similarities to both bacterial DNA and poxviruses.

PBCV-1 has the appearance of an iridovirus (see Chapter 5). It has a genome of 330 kbp, which makes it among the largest viruses known. The genome is linear DNA with closed ends like the genomes of poxviruses. The virus does not infect its algal host like an animal virus; rather, the virion attaches to the cell's surface and the DNA is injected into the cytoplasm (a process analogous to a number of bacteriophages, including those described in this chapter). Also, similar to bac-

WBV19 6/27/03 4:05 PM Page 352

terial viruses, the PBCV-1 genome is extensively methylated. This methylation is in response to the presence of restriction enzymes of limited specificity that are encoded by its host. The ability of algae to encode such enzymes is a rarity among eukaryotes and is indicative of the degree of coevolution between virus and host.

This virus is found at concentrations as high as 4×10^4 PFU/ml in freshwater throughout the United States, China, and probably elsewhere in the world. The virus can only infect free host cells, and since it cannot infect its algal host when that is existing symbiotically with the paramecium, it is a good guess that existence of the virus is a strong selective pressure toward the symbiotic relationship. This virus represents a good example of the extensive coevolutionary interaction between viruses and their hosts throughout many ecosystems, and the importance of viruses in forming those ecosystems.

QUESTIONS FOR CHAPTER 19

1 *E. coli* λ K12 is a λ lysogen, meaning that the λ bacteriophage genome has been stably incorporated into the genome of the host cell.

a In this cell, which viral protein, if any, will you find being expressed?

b When this cell is irradiated with ultraviolet light, the resulting damage to the cellular DNA induces the SOS response. This system results in the proteolytic destruction of several repressor proteins, including λ cI. What is the effect of this treatment on expression of the λ genome?

2 You plan to carry out an experiment in which you infect *E. coli* cells with bacteriophage T4. You have a 10 ml culture of cells containing 3×10^8 cells/ml. You have a stock of phage T4 containing 10^{10} PFU/ml. To start the infection, you add 0.3 ml of this virus stock to the 10 ml culture of cells.

a What is the multiplicity of infection?

b If phage T4 normally produces about 200 virus particles per infected cell, what will be the total yield of virus from this infection *at the end of one cycle of virus growth*?

c You repeat the experiment with four identical *E. coli* cultures. To three cultures you add nalidixic acid, an inhibitor of DNA synthesis, at the times indicated in the table below. The fourth culture receives no inhibitor and is a control. Predict the results of this experiment by completing the following table. (The entire life cycle of bacteriophage T4 takes 20 minutes.) Use a "+" to indicate normal function or activity and a "–" to indicate inhibition.

Time of addition of nalidixic acid	Phage DNA synthesis	Immediate-early gene expression	Yield of progeny per cell
Control (no inhibitor)			
0 minutes			
5 minutes			
18 minutes			

WBV19 6/27/03 4:05 PM Page 353

3 Which of the following treatments will inhibit the *complete expression* of immediate-early genes of the bacteriophage T4 during infection of an *E. coli* host cell? Use "+" to indicate expression and "–" to indicate inhibition of expression.

Treatment	Immediate-early mRNA expression
Rifampicin (an inhibitor of host RNA polymerase)	
Nalidixic acid (an inhibitor of DNA replication)	
A mutation that inactivates the phage DNA polymerase	
A mutation that inactivates the phage lytic enzyme	
Chloramphenicol (an inhibitor of protein synthesis)	

4 The following table lists a series of mutants of bacteriophage T4. Predict the properties of infection of a host cell by each of these mutants with respect to expression of each class of genes shown in the table. Indicate "+" if the class *is* expressed and "–" if the class *is not* expressed for each mutant.

Phage mutant	Immediate-early	Delayed early	Quasi-late	Late
Control (wild type)	+	+	+	+
A phage mutant that cannot catalyze ADP-ribosylation				
A nonfunctional mutant of the phage DNA polymerase				
A nonfunctional mutant of the phage protein gp55				
A nonfunctional mutant of the phage receptor-binding proteins on the tail fibers (infected under permissive conditions and then changed to nonpermissive conditions)				

WBV19 6/27/03 4:05 PM Page 354

5 In an experiment with bacteriophage T7, you have isolated mutants that are defective in specific phage genes. In each case, a nonfunctional version of the phage protein is produced. Indicate by "+" or "–" which of the classes of T7 genes are transcribed in the case of cells infected with each of the indicated phage mutants.

T7 phage	Early (Class I)	Delayed early (Class II)	Late (Class III)
Control (wild type)	+	+	+
T7 RNA polymerase mutant			
T7 DNA polymerase mutant			

6 Bacteriophage T7 has a linear, dsDNA genome. Gene expression of this phage after infection of its *E. coli* host cell occurs in three different phases. Genes that are expressed early are class I, genes that are expressed in a delayed early fashion are class II, and genes that are expressed late are class III.

a For each of the T7 functions listed in the following table, identify into which class of genes they are most likely to be classified: class I, class II, or class III.

T7 function	Class
Head capsid protein	
RNA polymerase	
DNA polymerase	

b Suppose that a cell has been infected with bacteriophage T7 and is in the middle of delayed early (class II) gene expression. At this point you add the drug rifampicin, which is an inhibitor of the host RNA polymerase. What will be the effect of this treatment on the expression of bacteriophage T7 genes in this cell?

WBV19 6/27/03 4:05 PM Page 355

7 Bacteriophage λ infects *E. coli* and can follow one of two pathways: lytic or lysogenic. Several viral genetic locations are involved in these pathways. Assume that you have the phage mutants shown in the table below. Predict which of the pathways, if any, each mutant can follow. Indicate your prediction with a "Yes" or a "No." In each case, assume that the phage enters the cell under conditions where the mutant phenotype will be expressed.

Mutant	Lytic?	Lysogenic?
Wild type	Yes	
N-minus		
CII-minus		
Deletion of the P_{RM} region		
CIII-minus		
Mutated O_R3 that fails to bind cro		
Deletion of the cro gene		