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Bacteriophage lambda: early pioneer and still relevant

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Abstract

Molecular genetic research on bacteriophage lambda carried out during its golden age from the mid 1950's to mid 1980's was critically important in the attainment of our current understanding of the sophisticated and complex mechanisms by which the expression of genes is controlled, of DNA virus assembly and of the molecular nature of lysogeny. The development of molecular cloning techniques, ironically instigated largely by phage lambda researchers, allowed many phage workers to switch their efforts to other biological systems. Nonetheless, since that time the ongoing study of lambda and its relatives have continued to give important new insights. In this review we give some relevant early history and describe recent developments in understanding the molecular biology of lambda's life cycle.

Keywords

bacteriophage; lambda; lambdoid phages

Introduction

From the earliest studies on lambda genetics by Jean Weigle (1953), Francois Jacob and Elie Wollman (1953, 1954) and Dale Kaiser (1955 in volume number 1 of VIROLOGY) in the 1950's until it helped usher in the age of genetic engineering in the late 1970's and early 1980's, phage lambda was in its golden age at the center of the molecular genetic universe. During those decades lambda was one of the few experimental "organisms" that was sufficiently experimentally accessible to be viewed as potentially completely understandable. Its genome was small enough not to be overwhelming, yet it was capable of making a decision of whether to lysogenize or grow lytically and was clearly complex enough to be interesting and informative on many fronts. In particular it was unique in that a

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genetic and molecular understanding of the control of gene expression seemed to be within almost immediate reach. Those of us lucky enough to be involved in the study of phage lambda during this time were tremendously excited about future possibilities and threw ourselves into the work with all the energy we had.

Lambda was originally discovered in 1951 by Esther Lederberg (1951) at the University of Wisconsin (Madison), when she serendipitously found it was released from the laboratory *Escherichia coli* strain K-12 after ultraviolet irradiation. Since that time all aspects of lambda's lytic and lysogenic lifestyles have been studied. Lambda became a more important and approachable experimental system when Allan Campbell (1961) isolated a set of suppressible nonsense mutants of lambda (or *amber* mutations, originally called suppressor sensitive or *sus* mutations) that identified genes essential for its lytic growth. Additional *amber* mutations isolated by Sandy Parkinson (1968) and Goldberg and Howe (1969) and prophage mutations isolated by Clarence Fuerst (Mount *et al.*, 1968) that were lethal for lambda lytic growth identified a few more essential genes. Genetic complementation tests of these mutations succeeded in defining all but one of lambda's 29 genes that are essential for plaque formation under laboratory conditions (this includes the frameshifted *G* and *G-T* gene pair and the alternate starts of the *S105* and *S107* genes as well as the *C* and *Nu3* genes as two genes each; see below). We note that two additional genes, *Rz* and *Rz1* should probably also be considered essential genes even though plaques often form in their absence. They are absolutely required for disruption of the outer membrane and lysis unless external physical solution forces that are often present during laboratory growth help to destabilize the outer membrane of infected cells (Berry *et al.*, 2012). Only the small *cro* gene was not found by the above random mutant hunts. It was discovered during the genetic analysis of the control of lysogeny (Eisen *et al.*, 1970), and its absence leads to the lysogenic state and hence no lytic growth. Recombination frequency and deletion mapping of these and various promoter and operator mutations isolated by others during this time allowed the construction of a detailed genetic map. Analysis of the phage DNA molecule led to the most detailed physical map of any organism's genome at the time. Detailed study of the phenotypes of phage mutants defective in known, mapped genes led to an early overall picture of the lambda lifecycle. Indeed, the careers of both authors here started at about the same time with the analysis of lambda virion morphogenesis by utilizing the various *amber* mutants to identify the proteins expressed from the lambda morphogenetic genes and determining the nature of the defects in their absence - SRC in the laboratory of Dale Kaiser at Stanford U. and RWH in the laboratory of Jim Watson at Harvard U. (Happily, rather than generating animosity, our early competition in this arena led to mutual respect and a lifelong friendship). We believe that the clever use of forward genetic selections and screens to isolate informative mutations and combinations of mutations and the use of these molecular genetic experiments to deduce the underlying molecular mechanisms reached its zenith in the study of phage lambda during this period. Those were heady days with lambda running at the front of the scientific pack.

We discuss here early experiments that led to our current understanding of phage lambda and molecular biological processes in general. During this period VIROLOGY published many of these important findings. We also mention recent developments to show how far

the field has come in the past 60 years (we assume a basic knowledge of the phage lambda life cycle; see Hendrix *et al.*, 1983; Hendrix and Casjens, 2006). We include discussion of phages related to lambda, which are commonly referred to as "lambdoid" phages. This ill-defined (and often incorrectly used) term refers phages with very similar lifestyles to lambda and genomes that are mosaically related to lambda. This definition included the notion that a lambdoid phage is capable of recombination with lambda itself to produce a functional hybrid phage, as was first shown with phage 434 by Kaiser and Jacob (1957). Recent phage genome sequences have caused an expansion of this term to mean a phage with the same functional gene order as lambda and that carries patches of nucleotide sequence homology with lambda or another lambdoid phage. Thus, in theory a single recombination event between lambdoid phages could give rise to a fully functional phage that has all the necessary genes (Hendrix, 2002; Casjens, 2005; Hendrix and Casjens, 2006; Grose and Casjens, 2014). In this discussion we use lambdoid to include for example, the three phages HK97, P22 and N15, which typify three of the best-studied lambdoid groups that have significant differences from lambda. Citations are in general not meant to bestow credit for the original discoveries but to allow the reader access to the literature. Other more detailed historical treatments of some of the topics covered below can be found in the books *Bacteriophage lambda* (Hershey, 1971) and *Lambda II* (Hendrix *et al.*, 1983) (and in Gottesman and Weisberg, 2004; Stahl, 2005; Georgopoulos, 2006; Campbell, 2007; Court *et al.*, 2007a).

Historical importance and recent progress

The lambda genome

Phage lambda DNA was one of the earliest model systems for studying the physical nature of DNA and genes, and a substantial amount of early research examined the hydrodynamic properties of the lambda chromosome with the goal of, among other things, determining its absolute molecular weight. At the same time methods (now considered to be primitive) were devised for separating the two halves of the lambda chromosome and for mapping genes identified genetically to these halves and to smaller physical intervals (reviewed by Davidson and Szyblaski, 1971). Soon thereafter electron microscopic analysis of absolute length and of lambda DNA in heteroduplex with various altered lambda DNAs gave rise to a major step forward, the construction of a detailed physical map of lambda DNA - a gene map in base pairs (bps) rather than recombination frequency units (Fiandt *et al.*, 1971; Simon *et al.*, 1971). The lambda virion chromosome was also among the first natural linear DNAs whose end structure was understood in detail; it was found not to have blunt ends, but to have complementary 12 bp protruding 5'-ends called cohesive (or sticky) ends, since the two single-strand ends can pair and thus bind to each other (Hershey *et al.*, 1963; Hershey and Burgi, 1965). Meanwhile, lambda contributed greatly to the ultimate mapping of DNA, the complete nucleotide sequence. It is not generally appreciated that the 12 bp lambda cohesive ends were the subject of the first direct nucleotide sequencing of a biological DNA. Ray Wu devised methodology for using DNA polymerase to fill in the cohesive ends with specifically labeled nucleotides, followed by analysis of the product to determine this exact 12 bp nucleotide sequence (Wu and Kaiser, 1968; Onaga, 2014). This sequencing was not a trivial undertaking, as determining this 0.012 kbp of sequence required several years of

painstaking work. Fourteen years later, Fred Sanger *et al.*, (1982) used lambda as the subject for the first determination of the complete genome sequence of a dsDNA virus - 48,502 bp - using his dideoxynucleotide chain termination method. This, along with anecdotal sequencing of various lambda genes and mutants of them by the laborious and more error-prone chemical sequencing method (Maxam and Gilbert, 1977) in the previous few years, unleashed a rapid avalanche of studies relating the many extraordinary genetic studies on lambda from the previous decades to the genome sequence. This in turn gave rise to an understanding of phage lambda genes and their expression that was unprecedented at the time (reviewed in Hendrix *et al.*, 1983). Figure 1 shows a map of the lambda chromosome; more detailed annotated maps of lambda, HK97, P22 and N15 can be found in the supplementary material of Hendrix and Casjens (2006).

In a sense the complete nucleotide sequence of the lambda genome and publication of the *Lambda II* book (Hendrix *et al.*, 1983) heralded the demise of lambda's golden age and its exalted position in molecular genetic research. The complete sequence gave the (false) impression to researchers outside this field that everything important about lambda was known. This impression, along with the approximately simultaneous development of simple molecular cloning techniques that could be used in virtually any molecular biology laboratory, allowed new and powerful molecular genetic access to essentially any biological system a researcher wished to study. Ironically, lambda played a major part in the development of these techniques (below).

Control of transcription during lytic growth

Lambda was one of the first biological entities whose transcriptional regulation was studied and understood in detail. It was found early on, like other phages under study at the time, to have a temporally controlled pattern of transcription and gene expression, called in this case immediate early, early (sometimes called “delayed early”) and late transcription (reviewed by Echols, 1971; Friedman and Gottesman, 1983). Lambda's major transcription units are shown in figure 1. When Jeffrey Roberts (1969), then a student in the Watson/Gilbert lab at Harvard, used purified RNA polymerase to transcribe lambda DNA *in vitro*, it initiated RNA synthesis at the immediate early promoters P_L and P_R, but the transcripts made were longer than those observed *in vivo*. He then used this system to discover a host protein (that he named Rho) that caused shorter transcripts to be made that were the same length as those observed *in vivo*. Rho was later shown to cause transcription termination at the normal lambda sites *in vivo*, and to be required for a subset of transcription termination events in *E. coli*. Thus lambda was directly responsible for the discovery of this first transcription controlling “factor” (if the sigma subunit is considered to be part of the basic RNA polymerase enzyme). Lytic infection by lambda phage carrying a conditional lethal mutation in its *N* gene drastically lowered protein and transcript synthesis from nearly all lambda genes compared to a wild type infection (*e.g.*, Radding, 1964; Echols, 1971). These findings were rapidly followed by many ingenious molecular genetic studies that converged on the idea that N protein causes termination of the P_L and PR immediate early transcripts to fail. The only immediate early genes, *N* and *cro*, lie upstream of these terminators, in the P_L and P_R transcripts, respectively.. Further work showed that transcription is antiterminated at terminators tL1 and tR1 (and other downstream terminators; figure 1) by N protein to allow

expression of the downstream (delayed) early genes (summerized in Echols, 1971; Franklin, 1974; Friedman and Gottesman, 1983 and references therein). N protein does not, however, simply inactivate Rho. Instead N somehow makes the transcribing RNA polymerase that has passed over a *nut* (N utilization) site in its presence insensitive to downstream termination at Rho-dependent and Rho-independent terminators.

Since its identification as a transcription antiterminator, N protein has been the subject of many studies aimed at elucidating the mechanism by which it accomplishes this feat. Jack Greenblatt first succeeded in accomplishing N action *in vitro* (Greenblatt, 1972), and many subsequent studies have learned much about the detailed roles of the several host proteins that participate in N-mediated antitermination (see ***Interaction between phage lambda and its host*** section below for a more detailed discussion of these factors). N protein, a natively unfolded protein (van Gilst and von Hippel, 1997; Goldenberg and Argyle, 2014), binds to the *boxB* sequence of the *nut* site in the nascent P_L and P_R mRNAs, as well as to the host NusA protein (Mogridge *et al.*, 1998; Mishra *et al.*, 2013) and RNA polymerase. It probably binds RNA polymerase on its β subunit near the RNA exit channel (Cheeran *et al.*, 2005). Exactly how N protein blocks termination is not yet known, but the recent observation that it reduces transcriptional slippage may provide a clue if slippage contributes to termination (Parks *et al.*, 2014).

All characterized lambdoid phages possess an N protein-mediated transcription antitermination mechanism, with the sole exception of phage HK022. In lambda antitermination, as outlined above, the nascent P_L or P_R transcript interacts through its *nut* sites with N protein, RNA polymerase and other host proteins to create a transcription elongation complex that is insensitive to termination signals. HK022 accomplishes the same end without N and without the known host factors. We now know that nascent P_L and P_R transcripts of HK022 interact directly with the β' subunit of the transcribing RNA polymerase through secondary structure formed at the *put* sites in the nascent RNA (which correspond in position to the *nut* sites on the lambda transcripts) (Oberto *et al.*, 1993; Sen *et al.*, 2002; Komissarova *et al.*, 2008). The resulting transcription elongation complex, as with lambda, is insensitive to termination signals. Thus, HK022 early transcript antitermination is apparently mediated by RNA alone, rather than by RNA in conjunction with the N and host transcription factors.

HK022 has not finished its dalliance with non-canonical mechanisms of regulating transcription with its unusual mechanism of accomplishing antitermination. It also has a mechanism to promote inappropriate termination in lambda-like phages during co-infection of the same bacterium. While HK022 does not have an *N* gene, it does have a heterologous gene, called *nun*, at the same position in the gene order as *N* in lambda. The Nun protein interacts with the *nut* sites of lambda as well as with the other host factors and RNA polymerase (Burova *et al.*, 1999; Watnick and Gottesman, 1998; Vitiello *et al.*, 2014). This resembles what happens with N protein, but the resulting transcription elongation complex is biased *toward* termination. For a phage like lambda such inappropriate termination of transcription would be a lethal event. These stories and others about how HK022 deviates in informative ways from the lambda paradigm, have largely come out of the laboratories of Max Gottesman and the late Bob Weisberg (Weisberg *et al.*, 1999). Ongoing work on these

topics provides a good illustration of how phage investigations can still give new information on how RNA polymerase receives and responds to signals.

Following N-mediated antitermination of P_R transcription, gene *Q* is transcribed at the distal end of the early right operon. The Q protein in turn acts as a transcriptional antiterminator that allows read-through of the tR' terminator (figure 1) to allow expression of the late operon (Roberts *et al.*, 1998 and references therein). However, Q protein works by a very different mechanism from that of N protein. Studies with phage lambda and the lambdoid phage 82 have shown that it is a DNA-binding protein that joins the transcription complex at the Q binding element (QBE) between the −35 and −10 parts of the late operon promoter P_R' and stays with the transcription elongation complex as it traverses the late operon. In contrast to N-mediated antitermination, there are no known host proteins that participate in its action. The recent structure of the DNAbinding domain of lambda Q protein sheds considerable light on its contacts with the QBE DNA sequence, the R4 region of sigma factor and the tip of the RNA polymerase β flap (Deighan and Hochschild, 2007; Shankar *et al.*, 2007; Muteeb *et al.*, 2012; Vorobiev *et al.*, 2014).

Control of lysogeny

In 1961 Jacob and Monod put forward their operon hypothesis, deduced entirely from their genetic work on the *lac* operon and phage lambda model systems (Jacob and Monod, 1961). The early observations by Kaiser that CI is required for maintenance of lambda lysogeny and that it is responsible for immunity to superinfecting lambda phages strongly suggested, but did not absolutely prove, that *cI* encoded a protein that *directly* turns off all the lambda lytic genes in the prophage state (Kaiser, 1957; Kaiser and Jacob, 1957). The subsequent isolation of suppressible *amber* nonsense mutants in the *cI* gene proved that this function is mediated by the protein product of the *cI* gene (Jacob *et al.*, 1962; Thomas and Lambert, 1962). By the mid 1960s experiments had demonstrated quite clearly that the lack of lytic gene expression from the lambda prophage was explained by blockage of mRNA synthesis by the *cI* gene product (Jacob and Campbell, 1959; Thomas, 1971; Ptashne, 1971 and references therein). In 1965–66 a competition developed between Mark Ptashne (with the lambda CI repressor) and Wally Gilbert (with the *E. coli* LacI repressor) to be the first to isolate a transcriptional repressor and thus absolutely prove the existence of repressor proteins that regulate gene expression. They worked in laboratories on different floors of the same building at Harvard, and this race for the repressor caught the attention of ABC television, which filmed a documentary, “The Scientist,” giving CI and LacI a brief moment of popular fame (and somewhat amusing and irritating the other scientists in the building who experienced the filming). Gilbert and Müller-Hill (1966) attained their goal first, but Ptashne (1967) was not far behind, and today both repressors remain important model systems for the study of sequence-specific DNA binding by proteins.

While maintenance of lambda lysogeny only requires one phage protein, the process of establishment of the lysogenic state is more complex. Dale Kaiser's original genetic studies identified two additional genes, *cII* and *cIII*, in which defects lower the frequency of establishment by several orders of magnitude (Kaiser, 1957). These two genes lie in the early right and early left operons, respectively. After the discovery of Cro it was first

thought that the lysis/lysogeny decision might simply be explained by a direct competition for operator binding between CI and Cro (a bistable genetic switch), but more recent experiments have shown that it is not this simple. We now know that the CII protein is a transcriptional activator that stimulates strong *cI* gene transcription from the P_{RE} promoter, and that high CII protein levels are critical in ensuring the high CI concentrations required to establish, but not maintain, the lysogenic state (Court *et al.*, 2007a and references therein). The *cIII* gene encodes a protein that controls the stability of the CII protein by inhibiting the action of the hostencoded HflB(FtsH)-HflC-HflK protease complex (Kobiler *et al.*, 2007; Bandyopadhyay *et al.*, 2010). In addition to this host protease control of CII stability, the host physiological state impacts the lysis/lysogeny decision through the following: (1) cAMP and ppGpp levels may control HflBCK levels (Hong *et al.*, 1971; Rao and Raj, 1973; Joung *et al.*, 1994; Slominska *et al.*, 1999). (2) RNase III is under strong growth rate regulation (Wilson *et al.*, 2002), and its action controls *cIII* mRNA stability (Altuvia *et al.*, 1991). (3) Integration host factor (IHF) modulates CII and CIII levels (Mahajna *et al.*, 1986; Krinke and Wulff, 1990; Giladi *et al.*, 1998) and N gene translation (Kameyama *et al.*, 1991; Wilson *et al.*, 2002). (4) RecA is activated by DNA damage to cause CI inactivation (see below). (5) HflD may directly inhibit DNA binding by CII protein (Parua *et al.*, 2010). (6) It has also been suggested that host proteins DnaA and SeqA may affect PL transcription and thus the lysis/lysogeny decision (Wegrzyn *et al.*, 2012). Finally, (7) the OOP RNA made from the lambda Po promoter is antisense to the 3' portion of *cII* gene mRNA and acts to destabilize that message with the help of RNase III, and the OOP promoter is repressed by the host LexA protein (Krinke and Wulff, 1990; Krinke *et al.*, 1991; Lewis *et al.*, 1994). Thus, the current consensus is that CII is the central player in establishing lysogeny, and figure 2 summarizes this complex regulatory circuitry.

When a decision is made to establish lysogeny, lambda ensures that the proteins that favor the lytic growth pathway disappear quickly, thereby minimizing stuttering between the lytic and lysogenic life cycle directions and ensuring that a decision is definitive. The O, N, CII, CIII and Xis proteins are all extremely metabolically unstable and are destroyed within a few minutes of being made. Thus, when their synthesis stops, their DNA replication initiation, delayed early gene expression and lysogeny promoting activities are quickly lost (Kobiler *et al.*, 2004). The host proteases utilized for this purpose are ClpX/ClpP for O (Gonciarz-Swiatek *et al.*, 1999; Thibault and Houry, 2012), Lon for N (Gottesman *et al.*, 1981; Maurizi, 1987; Mikita *et al.*, 2013) and FtsH for CII and CIII (Shotland *et al.*, 2000; Kobiler *et al.*, 2002; Kobiler *et al.*, 2007), and Lon for Xis (Leffers and Gottesman, 1998).

In addition to preventing the lytic gene transcription cascade, the CI protein has two other known functions, turning down expression of the host *pckA* gene and autoregulating its own synthesis. Array analysis of mRNAs showed that the *E. coli pckA* gene (which encodes phosphoenolpuruvate carboxykinase) is the most highly down-regulated host gene in a lambda lysogen (Chen *et al.*, 2005). This gene is required for growth on succinate and some other carbon sources. It is not known why lowering its expression might be beneficial, but the fact that the *pckA* promoter also overlaps lambdoid phage 21, P22, 434 and H-19B repressor binding targets (all different from lambda CI operator specificity), suggests that this regulation is evolutionarily important to the lambdoid phages. Louis Reichardt, a

student in Kaiser's lab in the early 1970's, devised a quantitative assay for CI repressor protein and performed an early set of intricate and elegant experiments that showed that CI activates its own synthesis at low CI levels and represses at high levels (Reichardt and Kaiser, 1971). This was one of the first demonstrations of autoregulation of a gene. Other experiments illuminated the amazing intricacies of CI and Cro binding to the three tandem operators at P_L to regulate the divergent P_{RM} and P_L promoters (Ptashne, 1992 and references therein). More recent study of the lambda prophage repressor has shown that it forms multimers that bind simultaneously to operators O_L and O_R at P_L and P_R, respectively, thus looping the DNA between these two promoters, and that this looping is important in the autoregulation that keeps the CI concentration within narrow limits in lysogens (Lewis *et al.*, 2011; Cui *et al.*, 2013a and 2013b; Hensel *et al.*, 2013; Michalowski and Little, 2013; Priest *et al.*, 2014). Host supercoil density impacts this looping, but its role *in vivo* is not known (Ding *et al.*, 2014).

The CI-CII-CIII circuitry is nearly universally present in the lambdoid phages, but some of these phages have additional controls on lysogeny. The first of these to be discovered was the P22 antirepressor (Ant), which binds to repressor and causes it to release repression (Susskind and Botstein, 1975). Transcription of the *ant* gene in a prophage is kept off by two additional phage-encoded repressors, Mnt and Arc, and a small antisense RNA, Sar, that controls the translation of *ant* mRNA (Prell and Harvey, 1983; Liao *et al.*, 1987; Wu *et al.*, 1987; Schaefer and McClure, 1997). *Salmonella* lambdoid phage gifsy-1 encodes an antirepressor GfoR that is repressed by the host LexA repressor (Lemire *et al.*, 2011). Other phages such as N15 encode a different type of antirepressor control system. Here, transcription of AntA (an antirepressor whose mode of action is not known) is negatively regulated by the N15 prophage repressor and by premature transcriptional termination that is mediated by processing of a portion of the leader of the antirepressor operon mRNA (Ravin *et al.*, 1999; Mardanov and Ravin, 2007). The exact biological role(s) of antirepressors remains unclear; they could be prophage induction modules (see below) but could also impact the lytic/lysogeny decision.

Integration and excision

An aspect of the establishment of lysogeny not discussed above is the physical integration of the phage chromosome into the host chromosome. The phenomenon of lysogeny had been known but poorly understood for several decades before lambda was discovered (Lwoff, 1953). The quiescent phage genome (prophage) in a lysogen appeared to be attached to the host chromosome in some way that could be mapped in bacterial conjugation experiments (Lederberg and Lederberg, 1953; Wollman, 1953), but the nature of such an attachment was not known. Our current understanding of prophage insertion derives from the idea of site-specific recombination between a circular phage lambda genome and the host chromosome put forward by Allan Campbell (1962). Numerous subsequent genetic and physical tests of this idea by Campbell and others soon validated this model (see Gottesman and Weisbeg, 1971). The existence of a lambda encoded function, integrase (Int), that was necessary for prophage insertion was simultaneously demonstrated by the isolation of *int* defective mutants by Zissler (1967; Zissler *et al.* 1971), Gingery and Echols (1967) and Gottesman and Yarmolinsky (1968). Howard Nash and coworkers accomplished *in vitro* integration

(reviewed by Weisberg and Landy, 1983), and many subsequent ingenious genetic and biochemical studies elucidated the complex mechanism of the integration reaction. The host integration host factor (IHF; named for this reaction but now known to have many pleiotropic functions in *E. coli* and Fis protein were found to be involved (Ball and Johnson, 1991). Genetic studies by Ethan Signer, Robert Weisberg and others combined with biochemical studies from the Art Landy lab have resulted in a marvelously detailed picture of the integration protein DNA complex bound to its target (the attachment site, *att*) and its actions that result in *att* site-specific recombination and lambda prophage insertion (Seah *et al.*, 2014; Tong *et al.*, 2014 and references therein).

As Esther Lederberg originally discovered (above), lambda prophages are induced by DNA damaging agents such as UV light or mitomycin C to enter a cycle of lytic phage growth. Release of CI repression causes the lytic gene expression cascade to initiate, and the prophage is excised from the host chromosome to allow circle-to-circle DNA replication (below). Interestingly, the CI repressor contains a cryptic autoprotease activity that is activated by binding to the host RecA recombination protein, but only when RecA is activated by ssDNA (Little, 1984; Kim and Little, 1993; Mustard and Little, 2000). Integration and excision are directional; while Int (with its host factors) is sufficient to integrate lambda DNA, another phage-encoded protein Xis is required for the reverse excision reaction. Control of the relative amounts of Int and Xis are necessary to ensure that integration occurs during establishment of lysogeny and excision occurs during induction of a lysogen. This is accomplished in two ways. First, when lysogeny is being established by high CII levels (above) the Int/Xis ratio needs to be high to ensure that integration occurs. To accomplish this, CII protein activates the promoter P_I, and its transcript encodes Int but not Xis. Second, a process dubbed retroregulation lowers the level of Int expression during lytic infection. Here, a site *sib* in the RNA downstream of *int* and the P_I transcript causes more rapid decay of P_L initiated *int* mRNA early after infection, ensuring that Int is made in large amounts only when a high CII level commits the cell to lysogeny. In addition, the *sib* site is separated from P_L in the integrated prophage, so after induction moderate levels of *both* Int and Xis made from P_L ensure excision (Schindler and Echols, 1981; Gottesman *et al.*, 1982; Guarneros *et al.*, 1982). CII also activates a third promoter, Pa_Q, whose product is a small antisense RNA of the *Q* gene, which presumably helps keep the late operon off while a decision is being whether to lysogenize (Ho and Rosenberg, 1985; Hoopes and McClure, 1985). *In vivo* visualization experiments have recently shown that, after injection, lambda DNA is confined near its point of entry and replication-driven movement of the host DNA allows proper juxtaposition of the host and phage attachment sites. These experiments also demonstrate that even bacterial attachment site location in the chromosome affects the frequency of lysogenization (Tal *et al.*, 2014).

In an interesting variation of lysogeny, lambdoid phages N15, øKO2 and PY54 do not integrate into their host's genome, but exist as linear plasmid prophages (Ravin and Shulga, 1970; Hertwig *et al.*, 2003; Casjens *et al.*, 2004). In these phages the *int* gene is replaced by a *ptl* gene that encodes the enzyme, called protelomerase, that creates the ends of these plasmids. These ends are covalently closed hairpins in which one DNA strand turns around and becomes the other strand with no break in the phosphodiester backbone (Rybchin and

Svarchevsky, 1999). The mechanism of action of protelomerase has been examined, and it recognizes a specific inverted repeat sequence, makes staggered nicks 6 bp apart in the two DNA strands, separates the strands between the nicks, and after strand swapping ligates the ends to form two hairpins (Huang *et al.*, 2004; Aihara *et al.*, 2007). These prophages encode their own plasmid segregation and replication proteins (Ravin, 2003; Ravin *et al.*, 2003) and so do not turn off their early genes as completely as integrated prophages (Ravin *et al.*, 2000).

DNA replication and homologous recombination

Lambda DNA replication initiates at a single origin and proceeds bidirectionally from that point to generate circle-to-circle replication until several tens of circles accumulate in the infected cell. At that point replication switches (by a still poorly understood mechanism) to rolling circle mode to generate the concatemeric DNA molecules that are the substrate for DNA packaging into virions (below) (Skalka *et al.*, 1972; Wake *et al.*, 1972; Narajczyk *et al.*, 2007). The lambda genome carries two essential DNA replication genes, *O* and *P*. Four dimers of O protein bind at four sequence repeats in the origin, which lies inside the *O* gene, to form an "O-some" complex. The other essential replication protein, P, binds to the host DnaB helicase, and this binary complex binds the O-some to form the ori:O:P:DnaB preprimosomal complex (Zylicz *et al.*, 1998 and references therein). Host chaperones DnaK-DnaJ-GrpE (see below) are involved in activating the preprimosomal complex to recruit the rest of the host replication machinery (Learn *et al.*, 1997). Thus, the early study of lambda replication contributed greatly to an understanding of the detailed steps required to accomplish controlled initiation. In addition, the origin is activated by rightward transcription from P_R, but this phenomenon remains poorly understood (Leng and McMacken, 2002; Hayes *et al.*, 2012; Olszewski *et al.*, 2014). In some other lambdoid phages like P22, the P gene is replaced by a gene that encodes an actual DnaB type helicase (Wickner, 1984a and 1984b), and phages APSE-1 and N15 encode a putative DNA polymerase (van der Wilk *et al.*, 1999) or a bifunctional helicase-primase (Ravin *et al.*, 2000; Mardanov and Ravin, 2006), respectively.

Genetic recombination was first demonstrated for lambda by Jacob and Wollman (1954), and mutants of lambda that recombine at reduced frequency were isolated by Franklin (1967), Echols and Gingery (1968) and Signer and Weil (1968). These mutations were found to affect genes *exo* and *bet* that lie in the early P_L transcript. Exo is an exonuclease (Zhang *et al.*, 2011 and refs therein) and Bet is a strand-annealing protein (Matsubara *et al.*, 2013 and refs therein). These two proteins can substitute for RecA in homologous recombination. In addition, a host RecBCD nuclease inhibitor is encoded by the *gam* gene, which is located immediately upstream of *exo* and *bet* (Court *et al.*, 2007b and references therein). Lambda also carries two genes in its *nin* region that affect recombination, *orf/ ninB* and *rap/ninG*. Orf protein is a recombination mediator that binds to host single-strand binding protein and ssDNA (Maxwell *et al.*, 2005; Curtis *et al.*, 2011 and 2014), and Rap protein is a DNA structure-specific endonuclease that cleaves Holliday junction branch points (Sharples *et al.*, 1998; Poteete *et al.*, 2002). Homologous recombination occurs by several different pathways, and these phage-encoded proteins participate with host proteins in various ways (see for example Poteete, 2013). We will not review these in detail here, but

these lambdoid proteins continue to be important in ongoing studies of the systems that catalyze homologous recombination.

Early work on lambda recombination also led directly to our current understanding of host RecBCD action. In about 1969 mutants were isolated by David Henderson in Ken and Noreen Murray's laboratory that increased the growth of lambda that was missing its recombination genes. These were studied further by Frank Stahl, Gerry Smith and coworkers (Stahl, 2005 and references therein; Smith *et al*. 1981a and 1981b). These mutations turned out to have created a unique asymmetric 8 bp nucleotide sequence (now called the Chi site, for crossover hotspot instigator), and their study gave rise to our current understanding that the RecBCD nuclease enters DNA at double-strand breaks and degrades both strands (differentially) from there until it hits a properly oriented Chi site. Passing over a Chi site "civilizes" the RecBCD exonuclease (to use Stahl's words) so that it continues along the DNA stands as a helicase, separating but not degrading them. Finally the host RecA protein is recruited to initiate a recombination event. Wild type lambda has no native Chi site, but its *E. coli* host has one every 5 kbp on average, and they are highly preferentially oriented according to the direction replication passes over them. It is now believed that Chi plays a major role in RecBCD-mediated recombinational repair of collapsed *E. coli* replication forks (Kuzminov, 2001). The lack of Chi sites in most invading foreign DNAs may also allow the RecBCD nuclease to help protect *E. coli* from such "invaders" (Stahl, 2005).

Other lambdoid phages carry different recombination genes in the *exo/bet/gam* chromosomal location. The best studied of these are genes *abc1, abc2, erf* and *arf* of phage P22. The two Abc proteins inhibit RecBCD nuclease, Erf is a strand annealing protein, and Arf is a poorly understood recombination accessory function (Botstein and Matz, 1970; Poteete and Fenton, 1983; Poteete *et al.*, 1988 and 1991). Other lambdoid phages carry a third type of recombination genes, *recE* and *recT*, that encode an exonuclease (ExoVIII) and a strand annealing protein, respectively (Kushner *et al.*, 1974; Hall *et al.*, 1993; Zhang *et al.*, 2009). The latter two genes were originally identified as *E. coli* genes, but were later found to lie in the defective lambdoid prophage Rac (Clark *et al.*, 1993); various fully functional lambdoid phages such as gifsy-1 carry *recE* and *recT* genes (Lemire *et al.*, 2008). In addition, some other lambdoid phage *nin* regions carry a *rusA* gene which encodes a Holliday junction resolvase that is different from lambda Rap (Sharples *et al.*, 1994).

Recently the lambda *exo* and *bet* recombination functions have been put to valuable technological use in accomplishing *very* efficient homologous recombination *in vivo*. Don Court and coworkers devised and optimized systems in which short 30–50 bp homologous tails on a selectable gene (generated by amplification by PCR primers with such tails) are inserted by homologous recombination into any desired site in a bacterial genome according to the sequence of the tails. Then, since the inserted gene is chosen to be counterselectable, homologous recombination can replace the inserted gene with any DNA that has the proper homologies (summarized in Thomason *et al.*, 2014). This now widely used strategy, called "recombineering," is dependent upon high levels of ectopically expressed Exo and Bet and allows rapid, efficient and scarless engineering of bacterial genomes. Very recently, Farzadfard and Lu (2014) have used lambda *bet*-mediated ssDNA recombineering to

develop a genomic memory system that "writes" (recombines) a chosen sequence into a second site in the genome of *E. coli* in response to endogenous expression of ssDNA containing the sequence. The system thus genomically encodes a memory of past expression of the promoter engineered to express the ssDNA.

Interactions between phage lambda and its host

In the early 1970s Costa Georgopoulos, David Friedman and Nat Sternberg independently devised methods of isolating mutations in the *E. coli* host that allowed lambda virions to adsorb and DNA to be injected, but which block phage infection at some later stage. These mutations affect lambda's head assembly, DNA replication and early transcription. They alter specific host proteins that are required for successful transcription and for proper protein folding/unfolding dynamics.

The host mutations that affect head assembly identified the host GroEL-GroES protein folding chaperone (also now known as HSP60-HSP10) (Georgopoulos *et al.*, 1973; Sternberg, 1973). The functions defined by these host mutations were named "*gro*" for to their inability of grow lambda (Georgopoulos and Herskowitz, 1971), and the "E" was added to reflect the fact that mutations in lambda gene *E* (major head protein) overcome this *gro* block (Georgopoulos *et al.*, 1973). However, in actual fact, mutations in either gene *E* or *B* (portal protein) have this property, and although this particular reaction has still not been studied in biophysical detail, subsequent work suggests that it is probably folding of the gene *B* protein that actually requires this host chaperone (Murialdo and Becker, 1978; Kochan and Murialdo, 1983; Georgopoulos, 2006). GroEL was originally known simply as GroE, but it acquired the L when its smaller partner, GroES, was discovered (Tilly *et al.*, 1981). In agreement with the genetic evidence that GroEL-GroES interacts with lambda head proteins, examination of the results of lambda infection of a *groEL* mutant host showed that head production was seriously aberrant as seen in a *B* or *C* minus infection (Georgopoulos *et al.*, 1973). The mechanism of GroEL-GroES protein folding chaperone action has now been studied in detail with other more tractable substrates (Horwich and Fenton, 2009).

The *groE* gene was one of the first bacterial genes to be cloned. Its cloning relied on the confluence of microbial genetics in the form of the *groE* mutants isolated by Costa Georgopoulos, and one of the earliest recombinant DNA techniques in the form of a shotgun (random) library of *E. coli* DNA fragments in a phage lambda vector. One of us (RWH) packed some of Costa's *groE* mutants in a suitcase and went to visit Ron Davis at Stanford. Ron had constructed one of the first libraries of *E. coli* DNA cloned into phage lambda genomic DNA and packaged in vitro into lambda virions (below), and when this phage library was plated on a *groE* mutant bacterial strain it contained plaque-forming phages at a frequency of 10^{-4} relative to wild type *E. coli.* The rare plaque-forming phages carried an 8 kbp insert of *E. coli* DNA. (As an indication of how new the recombinant DNA technology was, the size of the insert was measured not with restriction enzymes and agarose gels but by the much better established technique of measuring the density of phage carrying the insert in an equilibrium CsCl gradient.) The 8 kbp insert was subsequently shown to contain both the *groEL* and *groES* genes and led to the identification of the GroEL protein as a 60

kDa protein with ATPase activity that assembles into a cylindrical 14 subunit structure (two stacked rings of seven subunits) (Hendrix, 1979). Sequencing of this cloned DNA led to the realization, which was very surprising at the time, that GroEL had homologues in the chloroplasts of green plants in the form of “rubisco large subunit binding protein” (Hemmingsen *et al.*, 1988). Rubisco is the photosynthesis enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase, and the plant GroEL homologue has a role in folding its large subunit (Gatenby, 1996). While we were cloning and characterizing GroE, very similar experiments were done by Barbara and Tom Hohn in Basel, Switzerland, using another early lambda library of *E. coli* DNA that was constructed by Ken and Noreen Murray (Georgopoulos and Hohn, 1978; Hohn *et al.*, 1979).

The host mutants that affect DNA replication identified the DnaK-DnaJ-GrpE protein folding/unfolding chaperone (DnaK is also called HSP70) (Georgopoulos and Herskowitz 1971; Georgopoulos and Welch, 1993 and references therein), which is now known to be required for disassembly/loosening of the protein complex that forms at the origin of lambda (above), thus enabling the replication fork to move away from the origin (Hoffmann *et al.*, 1992; Osipiuk *et al.*, 1993; Wyman *et al.*, 1993). In addition to pointing out that protein folding, assembly and disassembly can be catalyzed by other proteins, it was noticed early that transcription of the genes encoding the GroE and DnaK protein chaperone systems is enhanced at high temperatures. Further examination of this latter phenomenon by many workers has led to our current understanding of the mechanisms of stress control of gene expression (Tilly *et al.*, 1983; Georgopoulos and Welch, 1993). Thus, lambda work was seminal in the nucleation of the idea of catalyzed protein folding and in the discovery and understanding of two important chaperone systems. Before this, all protein folding was thought to be spontaneous.

The host mutants in which lambda fails to express (delayed) early genes affect the ability of the lambda N protein to cause RNA polymerase to bypass terminators and thus transcribe the downstream essential early genes (above). These mutations alter several transcription factors now called NusA, NusB, NusE and NusG (originally for N under supplied but more recently N utilization substance) that are all required for successful N antitermination (Georgopoulos, 1971; Friedman, 1971; Friedman and Baron, 1974; Friedman and Gottesman, 1983; Friedman and Court, 1995). NusA is an RNA-binding protein that interacts with the α subunit of RNA polymerase and modulates transcriptional pausing (Prasch *et al.*, 2009; Yang *et al.*, 2009; Yang and Lewis, 2010; Schweimer *et al.*, 2011; Zhou *et al.*, 2011; Muteeb *et al.*, 2012; Kolb *et al.*, 2014), as well as interacting with lambda N protein (Mishra *et al.*, 2013). The NusB-NusE complex binds the BoxA RNA sequence of the *nut* sites and binds RNA polymerase (Burmann *et al.*, 2010; Stagno *et al.*, 2011). NusG protein homologues are present in all three domains of life, and in *E. coli* NusG is a transcriptional pause-suppressing factor that might also be involved in suppressing aberrant anti-sense transcription in *E. coli* (Herbert *et al.*, 2010; Peters *et al.*, 2012; Yakhnin and Babitzke, 2014). It has two domains, an N-terminal domain that binds RNA polymerase (Belogurov *et al.*, 2009) and a C-terminal domain that binds NusE and Rho (Burmann *et al.*, 2010). NusE, which is now known to be ribosomal small subunit protein S10, binds NusG and as such is thought to be involved in ensuring that a ribosome closely follows the

transcribing RNA polymerase, thus coupling translation to transcription (Burmann *et al.*, 2010; McGary and Nudler, 2013). In addition, NusA interacts with translesion DNA polymerases to affect DNA repair (Cohen *et al.*, 2009), and with NusB it may even be involved in intracellular RNA folding and processing (Bubunenko *et al.*, 2013). Thus, these early lambda-host interaction genetic studies revealed previously unsuspected complexities of transcription and led directly to the discovery and characterization of factors that control RNA polymerase elongation and termination. The study of the mechanisms of action of these factors continues to the present.

Virion assembly

The study of the assembly of phage lambda virions began in the 1960s when Jean Weigle (1966 and 1968) showed that lambda heads and tails assemble independently and spontaneously join *in vitro* to form infectious virions and with the deduction in the Eduard Kellenbeger, Louis Siminovitch and Dale Kaiser laboratories that gene *E* encodes its major capsid protein (MCP—also called coat protein) (Karamata *et al.*, 1962; Kemp *et al.*, 1968; Casjens *et al.*, 1970). Figure 3A shows an electron micrograph of the phage lambda virion (with the side fibers that are not present in laboratory strains, see below).

At that time, the study of viral structural proteins and their assembly was a non-trivial enterprise. For example, one of us (SRC) began his Ph.D. thesis project in 1968 by simply attempting to determine how many different proteins are present in the lambda virion. I had been toiling over various strategies for separating lambda virion proteins solubilized with urea, acetic acid or guanidine hydrochloride for nearly a year without noticeable success, when Bill Studier (who was studying phage T7 at the Brookhaven Laboratory) gave a seminar at Stanford describing the sodium dodecyl-sulfate (SDS)-polyacrylamide gel electrophoresis technique that he and Jake Maizel had developed (Studier and Maizel, 1969; Maizel, 2000). The combined facts that boiling in 0.1% SDS disassembles virtually all protein complexes and that electrophoresis could be performed in SDS solutions instantly made the prospects for my thesis very bright. This, along with what was probably the first published use of a slab gel apparatus that allowed accurate comparison of adjacent lanes, allowed me to get my thesis started with a definitive proof that gene *E* encodes the major head protein of lambda (Casjens *et al.*, 1970). I note here that it was the ability to do the rigorous controls necessary to really prove (rather than surmise or strongly suggest) our conclusions that first attracted me to phage work and has kept me here for nearly 50 years. Uli Laemmli (a phage T4 worker) and Jake Maizel soon modified this technique to give considerably higher resolution (called discontinuous SDS gel electrophoresis). In a curious twist of fate this now universally used and extremely highly cited method was published only in a figure legend in Uli's paper on T4 morphogenetic protein cleavage (Laemmli, 1970; cited 215,930 times at the time of this writing).

With the advent of discontinuous SDS gel electrophoresis and methods to tamp down host protein synthesis relative to lambda protein synthesis in infected cells (Ptashne, 1967; Hendrix, 1971) came the rapid identification of the rest of the morphogenetic gene products as specific bands in such gels (Buchwald *et al.*, 1970; Hendrix, 1971; Georgopoulos *et al.*, 1973). This in turn led to analysis of the structures made by infections with various mutant

phages and the gene products in them. These studies also illuminated the common protein cleavages that accompany lambda and many other virus assembly processes and make them irreversible and/or allow access to final conformations not available to the full-length proteins (Murialdo and Siminovitch, 1972; Georgopoulos *et al.*, 1973; Hendrix and Casjens, 1974 and 1975). This information allowed the deduction of the basic assembly pathway of lambda virions (the order of action and general role of each of the morphogenetic proteins; summarized in Georgopoulos *et al.*, 1983; Hendrix and Casjens, 2006), but it did not elucidate the detailed functions of each of the twenty-one phage-encoded proteins required for virion assembly. Work continues in this direction to this day.

The fact that lambda procapsids (precursor head structures) are assembled first and DNA is subsequently pumped into them by a DNA translocase was initially suggested by analysis of head-like structures made *in vivo* (Hendrix and Casjens, 1975) and was rigorously proven by Kaiser *et al.* (1975). Two proteins, the products of the *Nu1* and *A* genes are required for inserting DNA into the procapsid. The Nu1 protein recognizes the DNA to be packaged (Shinder and Gold, 1988; de Beer *et al.*, 2002). The A protein cleaves lambda DNA at the *cos* site during packaging to create the cohesive ends (Wang and Kaiser, 1973; Feiss *et al.*, 1983; Davidson *et al.*, 1991) and is also the ATP cleavage-driven translocase that moves the DNA into the procapsid (Duffy and Feiss, 2002; Tsay *et al.*, 2010). Neither of these proteins is present in the completed virion. Mike Feiss and coworkers performed extensive and informative genetic analyses of lambda DNA packaging (Sippy *et al.*, 2014; Feiss and Rao, 2012 and references therein), and Carlos Catalano's laboratory has studied its biochemistry (*e.g.*, Andrews and Catalano, 2013; Singh *et al.*, 2013). Most viruses do not depend on the host to help build their virions (except for chaperones, above), but lambda DNA packaging initiation requires the host IHF protein, which binds to a sequence near the Nu1 protein recognition sites and bends the DNA. This bend allows formation of the DNA:Nu1:A nucleoprotein complex that initiates *cos* site cleavage and DNA packaging (Xin *et al.*, 1993; Sanyal *et al.*, 2014). Laser tweezer measurements of the force generated by this molecular motor *in vitro* by Douglas Smith and collaborators have shown it to be among the most powerful nanomotors known (Tsay *et al.*, 2009 and 2010).

Lambda tail assembly work has focused largely on how its shaft is assembled. Isao Katsura and Roger Hendrix found that the length of the gene *H*-encoded tape measure protein determines the length of the shaft. It acts as a measuring device, most likely by serving as a template for shaft assembly (Katsura and Hendrix, 1984; Katsura, 1987). Curiously, the H protein is proteolytically cleaved before tail attachment to heads, but the role of this cleavage and the protease that causes it remain unknown (Hendrix and Casjens, 1974; Tsui and Hendrix, 1983). In addition, Levin *et al.* (1993) found that a programmed translational frameshift is required for expression of the essential *T* reading frame as a C-terminal extension of the gene G protein (called G-T protein). The frameshift ensures the proper ratio (~30:1) of G and G-T proteins. This was one of the first demonstrations of programmed frameshifting in prokaryotes, and it is now known to be an almost universal feature of the assembly of long phage tails, with the curious apparent exception of phage T4 and its allies (Xu *et al.*, 2004a). This phenomenon has only been studied in more detail in lambda, where both G and G-T proteins are required for tail assembly, but neither is present in completed

tails. The G and G-T proteins act as a chaperone in which the G domain binds the tape measure protein, and the T domain of G-T binds the major tail subunit (the gene *V* protein) (Xu *et al.*, 2013 and 2014). An understanding of the detailed dynamics of this assembly process awaits future study.

The different lambdoid phages have quite diverse virion structures (below) and as such have proven to be very fertile ground in studies of virion assembly - in particular studies of phages P22 and HK97 have been very informative. *Salmonella* phage P22 was of interest early because it was the first phage found to carry out generalized transduction (Zinder and Lederberg, 1952). Horst Schmieger isolated P22 mutants with greatly increased frequencies of transduction (Schmieger, 1972; Casjens *et al.*, 1992b), which made P22 the easiest transducing phage to use in the laboratory. As such it contributed significantly to the rise of *Salmonella enterica* as one of the important bacterial model organisms (Neidhardt, 1996). Myron Levine, David Botstein and Jonathan King, studied P22 on its own merits with the (prescient) view that phages related to lambda would benefit from the early lambda studies but would be different enough from lambda to be interesting and informative. Transduction is the result of errors in phage DNA packaging, and the study of P22 has led to a much better understanding of headful DNA packaging (Tye *et al.*, 1974; Jackson *et al.*, 1978; Casjens and Hayden, 1988; Casjens *et al.*, 1992c; Wu *et al.*, 2002; Leavitt *et al.*, 2013). P22 was also the first virus found to require a scaffolding protein for proper assembly of its MCP into a capsid shell. Scaffolding protein is present in large numbers inside of procapsids, but it exits the structure before DNA enters to form complete virions. This was one of the first discoveries of a protein that catalyzes the assembly of other proteins (King and Casjens, 1974; Earnshaw *et al.*, 1976; Weigele *et al.*, 2005; Chen *et al.*, 2011; Padilla-Meier *et al.*, 2012), and scaffolding proteins have since been found to be a general feature of the assembly of large virus particles.

The study of lambdoid phage HK97 began in the Hendrix lab because its tail has a slightly different length from that of lambda, but it was soon noticed that its MCP behaved as a smear near the top of an SDS electrophoresis gel of virion proteins (Popa *et al.*, 1991). Analysis of this phenomenon showed that its head proteins are very different from those of lambda and that its virion MCP is autocatalytically cross-linked into inter-locked rings as "molecular chain mail" (Duda, 1998; Wikoff *et al.*, 2000; Helgstrand *et al.*, 2003; Dierkes *et al.*, 2009). We now know that such a chain mail arrangement is not unique to HK97 and may not be uncommon in phage capsids (Duda, 1998; Gilakjan and Kropinski, 1999). In addition, structural studies in the Alasdair Steven and Jack Johnson laboratories led to a uniquely detailed picture of HK97 procapsid maturation (MCP shell expansion) into virion heads (Duda *et al.*, 2006; Hendrix and Johnson, 2012; Cardone *et al.*, 2014). The study of P22 and epsilon15 (a remote lambda relative that is not quite lambdoid) gave rise to the first asymmetric cryo-electron microscopic (EM) reconstructions of virions that show the tail as well as the MCP at subnanometer resolution (Jiang *et al.*, 2006 and 2008; Lander *et al.*, 2006; Chang *et al*., 2006; Tang *et al.*, 2011). Finally, and perhaps most importantly, the HK97 MCP shell crystallizes and remains today as the only large phage MCP that has been solved to atomic resolution by x-ray crystallography. The fold of the HK97 MCP was novel at the time of its determination (Wikoff *et al.*, 2000) (figure 3B). Subsequent high resolution

cryoEM structures have shown that this protein fold (with minor variations) is present in all tailed phage MCPs examined, including those of lambdoid phages lambda, P22, Sf6 and CUS-3 (Lander *et al.*, 2008; Parent *et al.*, 2012a and 2014a; Singh *et al.*, 2013; Rizzo *et al.*, 2014), as well as herpesvirus MCP (Baker *et al.*, 2005), the MCP of an archaea virus with tailed phage morphology (Pietila *et al.*, 2013), and a cellular nanocompartment (McHugh *et al.*, 2014). In addition, Pell *et al.* (2009) determined the structure of the lambda tail tube subunit (gene *V* protein). This fold is also present in the protein that makes up the tail tubes of both noncontractile and contractile tails of other phages, as well as in the shaft of the bacterial type VI secretion apparatus (reviewed by Davidson *et al.*, 2012). Thus, the lambdoid phages gave us the structures - now called the "HK97 fold" and the "tail tube fold" - of what are surely two of the most abundant proteins on Earth.

Cell lysis

Five lambda genes have been identified that are important in lysis of infected cells, *S105, S107, R, Rz* and *Rz1*. The enzyme responsible for cleaving the cell wall peptidoglycan is encoded by gene *R* (Campbell, 1961; Harris *et al.*, 1967). This endolysin is a true lysozyme in some phages like P22 and a transglycosidase in lambda (Bienkowska-Szewczyk *et al.*, 1981; Weaver *et al.*, 1985; Evrard *et al.*, 1998; Mooers and Matthews, 2006). Ryland Young and colleagues have described the mechanism(s) by which the lambda R protein is released into the periplasm. Release is controlled by the S105 and S107 holin proteins, which are expressed from different start codons of the same reading frame, resulting in one protein, S107 that is two amino acids longer than the other, S105. The shorter one, S105, causes lysis and S107 inhibits lysis; thus, the ratio of the two determines the exact timing of lysis (Wang *et al.*, 2000a). Lambda S105 protein is a small membrane protein that forms large physical holes in the membrane that allow endolysin escape across the cytoplasmic membrane. These holes are irregular, average >300 nm in diameter and involve hundreds of S105 molecules (Savva *et al.*, 2008; To and Young, 2014). Exactly how S105 molecules aggregate to form such large holes after accumulating in the membrane in a non-hole-forming state remains mysterious. More recent study of the lambdoid phage 21 has shown that its mechanism of endolysin release is different from that of lambda. Its endolysin is secreted by the host Sec translocon machinery into the periplasm in a catalytically inactive form, tethered to the inner membrane by an N-terminal transmembrane domain. When the phage 21 "pinholin" forms small holes that depolarize the inner membrane, the endolysin is released from the membrane and is activated to cleave the peptidoglycan (Pang *et al.*, 2009 and 2013; Xu *et al.*, 2004b). In addition to holin disruption of the inner membrane and cell wall breakdown, the outer membrane must also be disrupted for complete cell lysis to occur. The two lambda proteins that perform this still poorly understood process are Rz and Rz1, which are an inner membrane protein and an outer membrane lipoprotein, respectively. Together they form the "spanin" complex (so named because it spans the entire periplasm) (Berry *et al.*, 2008 and 2012; Young, 2014). Spanins are not always essential in laboratory phage infections because buffeting of cells suspended in solution can physically disrupt the outer membrane in their absence; however, their nearly universal presence in tailed phages suggests a critical evolutionary importance. Curiously, in lambdoid and many other phages the *Rz1* gene lies wholly within the *Rz* gene in its +1 reading frame, where it is a spectacular example of complete gene overlap. These three features of cell lysis, a peptidoglycan degrading enzyme,

a holin or pinholin and a spanin, are essentially universal among the tailed phages, and their actions were unraveled through study of the lambdoid phages.

DNA delivery from the virion into target cells

Most mutations that protect bacteria from killing by bacteriophage infection are due to the loss of the primary surface receptors that virions bind to on the cell surface, and studies with lambda identified the LamB protein (an outer membrane porin involved in maltose uptake) as its primary receptor (Thirion and Hofnung, 1972; Randall-Hazelbauer and Schwartz, 1973; Roessner *et al.*, 1983). Other lambdoid phages utilize different surface receptors. For example ø80 binds the FhuA outer membrane protein (Vostrov *et al.*, 1996; Endriss and Braun, 2004), P22 binds (and cleaves) the O-antigen polysaccharide (Wright and Kanegasaki, 1971), and Sf6 utilizes Oantigen and OmpA protein (Parent *et al.*, 2014b). In all phages where it has been studied in detail, fibers or spikes at the distal tip of the tail make the first interaction with the host. In lambda the J protein that resides at the tip of the lambda tail (R. Duda and R. Hendrix, unpublished) and its C-terminal 149 AAs (of 1132 AAs) are sufficient to interact with LamB (Charbit *et al.*, 1994; Wang *et al.*, 2000b; Berkane *et al.*, 2006; Meyer *et al.*, 2012). In addition to receptor alterations, mutations in the host inner membrane protein ManY (mannose phosphotransferase) were found that block injection of lambda DNA. Mutations in the phage *V* or *H* genes overcome this block, and this role of ManY does not depend on its enzymatic activity (Emmons *et al.*, 1975; Scandella and Arber, 1976; Elliott and Arber, 1978; Esquinas-Rychen and Emi, 2001). Phage HK97, which has a tail that is quite similar to that of lambda, requires the host's inner membrane glucose transporter protein PstG and periplasmic chaperone FkpA for successful DNA entry, and these requirements are determined by the tape measure protein (Cumby *et al.*, 2014). The actual role of host proteins in DNA injection remains a mystery.

A reconstruction of the early laboratory history of lambda revealed some unexpected additional information about lambda's tail fibers. When Dale Kaiser began his work on lambda as a graduate student at Caltech he saw that the phage made very small plaques, and he isolated a mutant that made larger, more robust plaques. This large plaque size made his classic experiments defining the *cI*, *cII* and *cIII* genes (above) possible, since plaque turbidity is much more visible in larger plaques. Subsequently, as a postdoc at the Pasteur Institute in Paris, he crossed the large plaque mutant he had brought from Pasadena with the version of lambda in use in Paris at that time to make what came to be called lambda Pasadena/Paris or lambda PaPa. This version of lambda is the one used in virtually all laboratory experiments thereafter, and it soon came to be thought of as lambda wild type. Finally, although lambda PaPa has only one short J protein fiber at its tail tip, and does not have long tail fibers, its genome sequence contains a side tail fiber gene *stf* and a tail fiber addition gene *tfa* downstream of *J*. The STF protein has substantial sequence similarity to the phage T4 gene *37* tail fiber protein but contains a frameshift mutation (Hendrix and Duda, 1992). When it was examined, the original lambda prophage in *E. coli* K-12 (Lederberg, 1951), which has been called Ur-lambda, was found to lack the frameshift mutation in the *stf* gene and to produce virions that have long thin side tail fibers (figure 3A); the two halves of *stf* generated by the frameshift in lambda PaPa were originally called orf401 and orf314 by Sanger *et al.* (1982). These long fibers cause lambda to make smaller

plaques (they probably splay out and slow diffusion in agar), so the *stf* frameshift mutation was present in the plaque picked by Kaiser. The side fibers apparently bind weakly to *E. coli* outer membrane protein OmpC, and, although they are not essential, they speed up adsorption (Hendrix and Duda, 1992).

The mechanism of DNA release from tailed phage virions and passage into cells remains poorly understood. The right end of the DNA in lambda virions extends from the head about one-third of the way into the tail, so it is primed for directional release during injection (Thomas, 1974). The great majority of the DNA is packed very tightly into phage heads with no DNA-binding proteins holding it in place (Casjens and Hendrix, 1974; Earnshaw *et al.*, 1979; Chang *et al.*, 2006; Lander *et al.*, 2006 and 2008). Phosphate charge repulsion, DNA bending, partial dehydration and low entropy state must be overcome to package DNA, and its compression in the phage head creates a kind of DNA pressure inside the capsid. DNA is spontaneously released from lambda virions when they bind purified LamB receptor (Roessner *et al.*, 1983; Roessner and Ihler, 1987); however, Molineux (2006 and 2013) and Panja and Molineux (2010) have argued that cellular turgor pressure acts against phage DNA injection into cells and have reviewed the ongoing discussion of the exact nature of the forces that drive DNA delivery into cells from virions. Walter Gelbart, Alex Evilevitch and co-workers have studied the energetics of lambda DNA condensation and release (*e.g.*, Grayson *et al.*, 2006; Qiu *et al.*, 2011; Liu *et al.*, 2014). Rob Phillips and co-workers have found that lambda DNA release on pure LamB *in vitro* is complete in about 10 sec, while injection into cells appears to average about 5 min (Van Valen *et al.*, 2012). Unless the DNA-bound dye confounds the latter experiments, this suggests that forces inside the cell do affect the rate of DNA entry into cells from lambda virions (Van Valen *et al.*, 2012).

Very little is known about the role of the other lambda tail proteins in DNA injection beyond the fact that the H protein is injected into the host, probably into the inner membrane and ahead of the DNA (Roessner *et al.*, 1983; Roessner and Ihler, 1987), and that there must be an as yet uncharacterized rearrangement of the tail tip that allows release of the DNA and H protein from the virion. Curiously, one of the minor tail tip proteins, the L protein, contains a $(4Fe\text{-}4S)^{+2}$ iron-sulfur cluster (Tam *et al.*, 2013). While the function of the cluster is not known, the ability of the L protein to form the complex with this cluster is essential for both tail assembly and DNA injection (Dai, 2009). While lambda appears to release only a few molecules of one protein from the virion during DNA injection, the short-tailed lambdoid phage P22 releases 6–20 molecules each of three different proteins with its DNA (Botstein *et al.*, 1973; Casjens and King, 1974; Israel, 1977). The roles of these proteins also remain unclear. It is not known how the DNA traverses the periplasm and penetrates the inner membrane DNA. At least in the short tailed phages the injected proteins may form a temporary channel through the periplasm (Perez *et al.*, 2009; reviewed by Casjens and Molineux, 2012; see also Hu *et al.*, 2013). Indeed one of the earliest observations in this area, that bacteria infected with lambda can take up exogenously added lambda DNA only if it has at least one cohesive end is still not understood (Kaiser and Hogness, 1960; Kaiser, 1962).

Non-essential accessory genes

In addition to lambda's 29 essential lytic genes, and *cI*, *cII*, *cIII* and *int*, which are essential for efficient lysogeny, 38 additional open reading frames (ORFs) whose protein products are not absolutely essential to lambda lytic growth or lysogeny in the laboratory were identified in the original genome sequence (as delineated in Hendrix *et al.*, 1983; Hendrix and Casjens, 2006). Many of these have been shown to be functional genes that produce proteins. Recent ribosome profiling analysis of lambda by Jeff Roberts and co-workers (Liu *et al.*, 2013) confirmed that essentially all of these ORFs are translated. This observation, like the determination of the lambda genome sequence before it, provides striking confirmation of the accuracy of the catalog of lambda genes with identifiable functions, which was determined largely by genetic methods, decades before development of many of the sophisticated techniques that contemporary students may assume have always been available. Surprisingly, the ribosome profiling also identified 55 possibly translated ORFs in addition to the 71 previously known genes. These "new" ORFs are all quite small (all but one are ≤76 codons in length), and none completely overlaps a previously identified gene. It is not known if the short putative proteins they might encode (or perhaps the act of their translation?) have functions. The profiling technique used is relatively new, and the authors do not rule out the possibility that some or all of the putative new genes could be the result of artifacts of the method. On the other hand, if ongoing work confirms the reality of even a fraction these new potential genes, lambda will once again have shown that the descriptions found in textbooks fall short, even for a “simple” little virus like lambda.

Each of the major lambda transcription units contains accessory genes whose functions are known. The late region includes the *lom* and *bor* genes that are expressed from the prophage and appear to help the host bind to eukaryotic cells and confer blood serum resistance on the host, respectively (Barondess and Beckwith, 1995; Vaca Pacheco *et al.*, 1997). *Ea47*, *Ea31* and *Ea59* (the latter encodes an ATP-dependent endonuclease; Benchimol *et al.*, 1982) are nonessential genes that lie between the tail region and *int* whose biological roles are not known. The early left operon contains a number of useful but nonessential genes. For example, the *exo*, *bet* and *gam* proteins catalyze homologous recombination (see ***DNA replication and homologous recombination*** above), the *kil* gene product disrupts host division septum formation (Greer, 1975; Haeusser *et al.*, 2014), and the less well studied *bin* and *ral* functions block host replication initiation (Sergueev *et al.*, 2001) and modulate host restriction-modification systems (Loenen and Murray, 1986), respectively. Nonessential genes *rexA* and *rexB* are co-transcribed with the *cI* repressor gene and are discussed below with lysogenic conversion genes. Near the 3'-end of lambda's early right operon lie ten contiguous nonessential *nin* genes, so named because they are absent in large deletions that remove tR2 and make transcription of the essential gene *Q* (and thus the late genes) high enough to allow the phage to be N independent (Court and Sato, 1969). The *nin* genes include the *ren*, *orf (ninB)*, *rap (ninG)* and *orf221*, genes that encode protection from *rexAB* exclusion (Toothman and Herskowitz, 1980b), a homologous recombination function (Tarkowski *et al.*, 2002; Curtis *et al.*, 2014), a Holliday junction resolvase active in homologous recombination (Barik, 1993; Sharples *et al.*, 2004) and a serine/threonine/tyrosine protein phosphatase (Voegtli *et al.*, 2000) whose role is unknown, respectively.

Lysogenic conversion

Accessory genes that are expressed from the prophage and alter the host in some way are called lysogenic conversion genes, *e.g.* the lambda *bor* and *lom* genes (above). Many temperate phages carry genes, called superinfection exclusion genes, that protect the prophage-carrying host from attack by other phages, and lambda is no exception. Its *rexAB* (above) and *sieB* genes block infection by certain other phages (*e. g.*, Parma et al., 1992; Engelberg-Kulka *et al.*, 1998; Ranade and Poteete, 1993). In both these cases the detailed mechanism of exclusion remains unclear, but it occurs after DNA from the superinfecting phage has entered the cell and in both cases a second protein, *ren* and *esc*, respectively, protect lambda from self-exclusion (Toothman and Herskowitz, 1980a; Ranade and Poteete, 1993). Other lambdoid phages encode exclusion proteins that operate through other mechanisms. Several examples are as follows: The P22 *sieA* gene blocks injection of DNA by some phages (Susskind *et al.*, 1974), phage 933W encodes a tyrosine kinase that excludes HK97 (Friedman *et al.*, 2011)., and the phage ø80 and N15 *cor* genes block adsorption of superinfecting phages to the outer membrane FhuA protein (Vostrov *et al.*, 1996; Uc-Mass *et al.*, 2004).

Many lambdoid phages encode proteins that modify the bacterial O-antigen surface polysaccharide. These modifications change the spectrum of phages that can adsorb to the O-antigens of such lysogens and also alter the antigenicity and thus the pathogenicity of the host bacterium (Allison and Verma, 2000; Broadbent *et al.*, 2010; Kintz et al., 2015). O-antigen modifying genes are present in two common forms in lambdoid phages. Some phages, for example *Shigella* phages Sf6 and Sf101, encode O-acetyl transferases that are expressed from the prophage and acetylate sugar moieties in the O-antigen repeat (Verma *et al.*, 1991; Allison and Verma, 2000; Jakhetia *et al.*, 2014). Other lambdoid phages, for example P22, SfX and epsilon34, carry a three gene *gtrABC* operon; the first two genes in such a cluster, *gtrA* and *gtrB*, are rather highly conserved and are thought to encode an undecaprenol phosphate-glucose flippase that moves the sugar part of its substrate from the cytoplasmic to periplasmic side of the inner membrane (Liu *et al.*, 1996) and a bactoprenol transferase that transfers the glucose from UDP-glucose to form undecaprenol phosphate-glucose (Guan *et al.*, 1999), respectively. The third gene, *gtrC*, is much more variable and different *gtrC*'s encode different glycosyltransferases that add various sugars as side chains to the O-antigen backbone (Markine-Goriaynoff *et al.*, 2004; Thanweer *et al.*, 2008; Villafane *et al.*, 2008; Davies *et al.*, 2013). Interestingly, the phage P22 prophage *gtr* operon is subject to on/off phase variation that is epigenetically controlled by the host Dam methylase and OxyR regulatory protein (Broadbent *et al.*, 2010).

A variation on lysogenic conversion in the lambdoid phages is the presence of genes that affect the host during lytic infection. It has been known for over half a century that prophages often encode virulence factors that are important in bacterial diseases, *e.g.*, diphtheria toxin (Freeman, 1951; Uchida *et al.*, 1971; Pappenheimer and Murphy, 1983), but it was assumed that these are expressed from uninduced prophages. A surprising more recent observation is that the Shiga-like toxins do not follow this paradigm. The Shiga-like toxins are important in the human disease *E. coli* hemorrhagic colitis, and the *stxA* and *stxB* genes that encode the toxin are carried mostly by lambdoid phage 933W and its close

relatives (Plunkett *et al.*, 1999; Smith *et al.*, 2012), where they lie within the late operon between the promoter and lysis genes (Neely and Friedman, 1998). David Friedman, Matthew Waldor and colleagues have studied two of these phages, 933W and H-19B, and they find that high level expression of *stxAB* is largely dependent on prophage induction and replication of the prophage to increase the DNA copy number. In addition, phage mediated cell lysis is required to release the toxin from the cell (Wagner *et al.*, 2002; Tyler *et al.*, 2004). Thus, it appears that "spontaneous" induction, lysis and death of a small fraction of these bacteria produces the toxin that is presumably advantageous to the bacterium during disease. This seems to be an example of sib selection and/or altruism in which lysis of a few members of a bacterial clone give an advantage to their clonal siblings (Livny and Friedman, 2004).

Molecular cloning of DNA

In 1968 Peter Lobban, a Ph.D. student in Dale Kaiser's laboratory in the Biochemistry Department of Stanford University was the first person to realize that the tools to clone DNA existed and that it would be a useful thing to do. He presented this proposal at a Kaiser lab group meeting, defended the idea in his Ph.D. preliminary exam, and proceeded to do his thesis work on cloning methodology (Lobban, 1972; Lobban and Kaiser, 1973). This idea, which in part sprang from the fact that Kaiser's laboratory was very near those of Arthur Kornberg and Robert Lehman where DNA polymerase and DNA ligase were being characterized at that time (*e.g.*, Olivera and Lehman, 1967; Englund *et al.*, 1968), was so powerful that others devised more rapidly attainable cloning goals and were able to publish before Lobban (Jackson *et al.*, 1972; Cohen *et al.*, 1973). Nonetheless, the idea of molecular cloning originated with him (Kornberg, 1991; Berg, 1993; Casjens, 1994; Yi, 2008; for more extensive historical treatments of molecular cloning and genetic engineering see Lear, 1978; Judson, 1979; and McElheny, 2014).

In addition, other important parts of cloning technology were also developed with phage lambda. Werner Arber's discovery and characterization of bacterial restriction-modification systems was done using phage lambda (Arber, 1971 and references therein). Ron Davis at Stanford developed phage lambda into a powerful cloning vector by showing that some restriction enzymes leave sticky ends and then utilizing these enzymes for DNA cloning (Mertz and Davis, 1972; Cameron *et al.*, 1975), Kaiser and Masuda (1973) devised a procedure for packaging exogenously added lambda DNA into lambda virions, and lambda-based cosmid vectors were developed for cloning relatively large DNA sections up to about 40 kbp (Hohn and Murray, 1977; Collins and Hohn, 1978).

Thus, lambda contributed greatly to the development of DNA cloning technology, but these advances contributed to the demise of the golden age of lambda, since molecular cloning became the first step in being able to understand cellular (both prokaryotic and eukaryotic) DNA function at a molecular level. Because lambda was no longer uniquely experimentally accessible, and because the basic aspects of its lifecycle were by then fairly well understood, the 1980s and 1990s saw fewer and fewer laboratories dedicated solely to the study of phage lambda. Nonetheless, a handful of laboratories persisted in delving deeper into the details of the life cycles of lambda and its relatives. In addition, the well-understood lambda system

has often been used for proof-of-principle demonstrations for new technologies. Thus, even though they are the sole objects of study in relatively few laboratories at present, the lambdoid phages continue to give new insights into the amazing details of gene expression, gene function and virus life cycles to this day.

Bacteriophage diversity and the evolution of modular viral genomes

The early decision to study a small number of chosen bacteriophages (including the most highly studied phages lambda, T4 and T7) for highly focused hypothesisdriven research certainly sped up the attainment of our current overall knowledge of gene expression mechanisms and numerous viral functions immensely. However, at the same time it restricted our view of the diversity of life on Earth and in particular the diversity and natural abundance of bacteriophages. Even in the early days of the study of phages several lambda relatives (*e.g*., phages 21, 434, P22 and ø80) had been isolated and were known to form viable hybrids with lambda (Kaiser and Jacob, 1957). Because of their different repressor-operator specificities, these other phages were of seminal use in developing an understanding of the control of early gene expression and the reasons why phage lambda cannot infect a lambda lysogen (*e.g.*, Bode and Kaiser (1965) showed that the lambda *cII* and *cIII* genes can't complement from a heteroimmune repressed hybrid prophage). Analysis of heteroduplex DNA pairs with lambda showed early on that these phages have mosaically related genomes in which patches of highly related sequence are interspersed with patches of less related or unrelated sequences (Fiandt *et al.*, 1971; Simon *et al.*, 1971; Botstein, 1980; Campbell and Botstein, 1983; Casjens *et al.*, 1992a; Campbell, 1994). Phage genome sequences have greatly increased our understanding of this genome mosaicism in recent years (Hendrix *et al.*, 2000; Juhala *et al.*, 2000; Hendrix, 2002; Casjens, 2005; Casjens and Thuman-Commike, 2011; Grose and Casjens, 2014). Figure 4 shows some of the better-characterized variations among lambdoid phages.

Many relatives of phage lambda have now been isolated, and at present over 80 such phage genomes have been completely sequenced (compiled in Grose and Casjens, 2014), along with hundreds of related prophages in bacterial genome sequences (see, for example, Casjens and Thuman-Commike (2011) for the 45 such P22-like prophages known at that time). All these phages infect members of the *Enterobacteriaceae* bacterial family, but their diversity is nonetheless huge. These 80 phages fall into seventeen different "types" or "clusters" that all have lambda-like gene (functional module) orders and apparently similar gene expression cascades (Grose and Casjens, 2014). (We note that there are a very small number of notable exceptions to this synteny; some phage N15 and ASPE-1 early genes have an order that is different from that of lambda; van der Wilk *et al.*, 1999; Ravin *et al.*, 2000.) The seventeen lambdoid phage clusters were defined in terms of overall sequence similarity, in which a dot matrix plot comparison of members of different clusters at relatively low stringency shows recognizable nucleic acid similarity over more than 50% of the genome within but not between clusters (Grose and Casjens, 2014). A very preliminary survey of prophages present in the hundreds of reported enterobacterial genome sequences has revealed at least four additional lambdoid phage clusters (S. Casjens, unpublished). Clearly, the lambda life-style has been extremely successful and has diverged to fill many presumed niches.

All these lambdoid phages are transitively related by patches of similar sequence that can potentially undergo homologous recombination. The "unrelated" patches that lie in parallel chromosomal locations can be either homologous sequence that has diverged to the point that it is no longer significantly related or entirely unrelated sequence form a different source that performs the same function. An example of the former is the lambdoid prophage repressor. The repressors all perform the same role, shutting off the lytic genes in the prophage, yet they are extremely variable and often barely recognizable or even not recognizable as being homologous. The lambdoid repressors that have been examined have similarities in their DNA-binding domain folds, and so are almost certainly ancient homologues (Pabo *et al.*, 1990; Watkins *et al.*, 2008). We are not aware of a recent analysis, but in 1992 eleven different repressor operator-binding specificities were known (Casjens *et al.*, 1992a), and the currently known sequence diversity of these proteins suggests a very much larger repertoire. Another example of very divergent homologues is the procapsid assembly gene cluster (including the MCP and portal protein) of which there are fourteen *very different* types among the lambdoid phages (Grose and Casjens, 2014). Examples of apparently nonhomologous proteins that perform analogous functions in various lambdoid phages are the three largely unrelated tail types - short, long contractile and long noncontractile (*e.g.*, P22, SfV and lambda, respectively), the integrases of integrating phages like lambda and P22 and the protelomerases of phages N15, PY54 and øKO2 (above), and the multiple types of homologous recombination modules (above). We have previously discussed the differences and similarities among the four best-studied lambdoid phages, lambda, HK97, P22 and N15 in more depth, and the reader is referred to Hendrix and Casjens (2006) for more examples of such mosaic relationships.

We believe that extant lambdoid phage diversity has been attained in several ways. These phages are likely extremely old, and natural divergence has had sufficient time to make even some of their homologous protein sequences no longer significantly related. New mosaic boundaries/novel sequence joints can form in two ways. First, highly divergent lambdoid phages could recombine through illegitimate recombination events to generate a new boundary. Second, illegitimate recombination could incorporate novel sequences into a phage by insertion between or replacement of resident genes. These foreign inserts between pre-existing lambdoid phage genes have been called "morons" since there is more DNA when they are present (Hendrix *et al.*, 2000; Juhala *et al.*, 2000), and the known morons usually carry their own promoters for independent expression. The sources of such inserts and replacements may be divergent lambdoid phages, other phages or genes with no phage relationship, since their sources are currently unknown. Thus, new mosaic boundaries likely form very rarely, but the probability of such a fortuitous event between two phages giving rise to a functional progeny phage is greatly increased if the two participating phages have the same gene order (as all lambdoid phages have). Given the enormous numbers of phages on Earth (Whitman *et al.*, 1998; Wommack and Colwell, 2000; Hendrix, 2002), we imagine that this type of novel joint formation may well be random, and that all possible single-recombination novel joints between divergent lambdoid phage genomes might be formed. However, only the *extremely small* fraction of such recombinants that has a functional, advantageous phage genome would be found as the phages present today. Once a new functional mosaic boundary is formed in a lambdoid phage genome, homologous

recombination between similar sequences in other mosaically related lambdoid phages could rapidly cause reassortment to form new combinations of such boundaries (and sequence patches). Interestingly, although both of these processes, formation and reassortment of mosaic patches, will tend to homogenize the genetic makeup of the participating phages, neither process is fast enough to mask the presence of clear clustering of different types within the lambdoid phage group (Grose and Casjens, 2014).

The future

Lambdoid phage research was seminal in the attainment of our current understanding on such varied fronts as gene and operon structure and function, the mechanisms of control of gene expression by regulatory proteins and small RNAs, protein chaperones, prophage integration, protein and RNA turnover, virion assembly and modular virus genome evolution. Although from outside lambdoid phages may be seen as "fully understood," we believe that there is still much that these phages can teach us. In addition to their roles as model systems, the amazing abundance of tailed phages on our planet means that they are extremely important in all ecological processes that involve bacteria such as global carbon and nitrogen cycling (Suttle, 2007; Ankrah *et al.*, 2014). Phages are involved through both their ability to kill bacteria and so affect their population size as well as modification of bacteria through lysogenic conversion and transduction. Thus, it is important to understand phages in their own right.

Recent work has also found the lambdoid phages to have technological applications and human health importance. Lambda and P22 have been developed into powerful and versatile protein display agents, where both the head decoration proteins lambda D (Sternberg and Hoess, 1995; Mikawa *et al.*, 1996; Nicastro *et al.*, 2013; Chang *et al.*, 2014) and P22 Dec (Parent *et al.*, 2012b), as well as the lambda major tail V protein (Maruyama *et al.*, 1994; Zanghi *et al.*, 2007) and P22 MCP head protein (Kang *et al.*, 2008; Servid *et al.*, 2013) have been used successfully to display foreign peptides and proteins. Lambda virions have also been used as DNA or protein vaccine delivery vehicles in mammals (Jepson and March, 2004; Lankes *et al.*, 2007; Clark *et al.*, 2011; Mattiacio *et al.*, 2011; Saeedi *et al.*, 2014). Trevor Douglas and Peter Prevelige are developing P22 into a flexible multi-use nanoparticle platform, where foreign molecular cargo can be placed on the surface or within the interior of the particle (O'Neil *et al.*, 2011 and 2102; Lucon *et al.*, 2012; Uchida *et al.*, 2012) to make, for example, magnetic resonance contrast enhancing agents (Qazi *et al.*, 2013 and 2014) or enzymecontaining nanoreactors (Patterson *et al.*, 2012 and 2014). Finally, the stability of cross-linked HK97 capsids may give them stability advantages, and HK97 nanoparticles are under development (Huang *et al.*, 2011). Human health relevance comes from the fact that these phages carry bacterial disease virulence factor genes such as the *lom* and *bor* genes of lambda and the Shiga-like toxin *stx* genes (above).

It is now nearly 65 years since Esther Lederberg discovered and named bacteriophage lambda, but we still haven’t reached the mythical goal of a complete understanding of its molecular life cycle. If nothing else, work on these phages should have taught us that there are many levels of intricacy and sophistication in all aspects of phage life cycles, and each time a level is "understood" it opens doors to yet deeper levels of understanding. Each time

we think we have learned something new and important about lambda, we have really learned something new and important about biology more generally, and the lambdoid phages haven't yet run out of interesting biology to illuminate. Despite the changing fashions in research, the vicissitudes of NIH grant funding, and the changing cast of characters studying them, these phages have been a remarkably consistent and productive source of new biological insight that remains an important experimental model system in the vanguard of biological science. This seems to us unlikely to change any time soon.

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Highlights

- Phage lambda research was seminal to our current understanding of molecular biology.
- We describe some of the events that occurred during early lambda research.
- We also describe recent findings in phage lambda research.

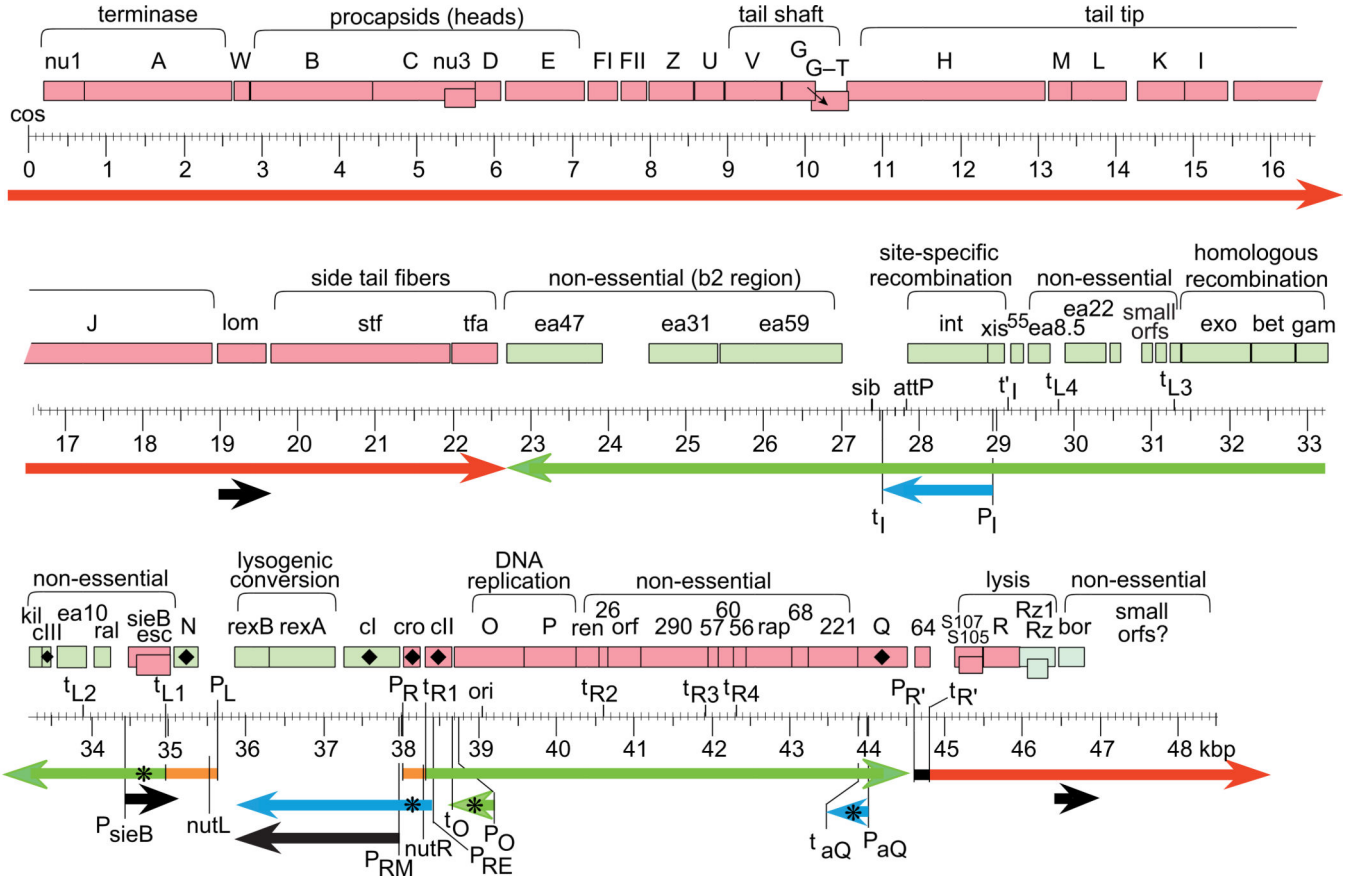

Figure 1. Map of the bacteriophage lambda chromosome

The linear virion chromosome is shown with a scale in kbp below. Rectangles indicate known genes with names and functional regions shown above; red genes are transcribed rightward and green leftward; vertically offset gene rectangles are expressed from reading frames that overlap (*CNu3, S107–S105* and *sieB-esc* in the same reading frame, *rz-rz1* in different frames) or by programmed frameshifting (*G-T*, small arrow in figure). Diamonds (♦) mark regulatory genes. Important DNA sites (*e.g.*, P, promoters; t, terminators) are indicated below the genes. Thick horizontal arrows below indicate mRNAs: black, transcripts made in a lysogen; orange, immediate-early transcripts; green, early transcripts; red, late transcripts; blue, transcripts made in response to high CII levels. Asterisks (✳) mark RNAs with regulatory activity (the detailed role of P_{RE}-initiated *cro* antisense message remains unclear (Spiegelman *et al*., 1972). The chromosome is circularized in the cell during infection, so the P_R'-initiated late transcript is continuous from the lower right to the upper left in the figure.

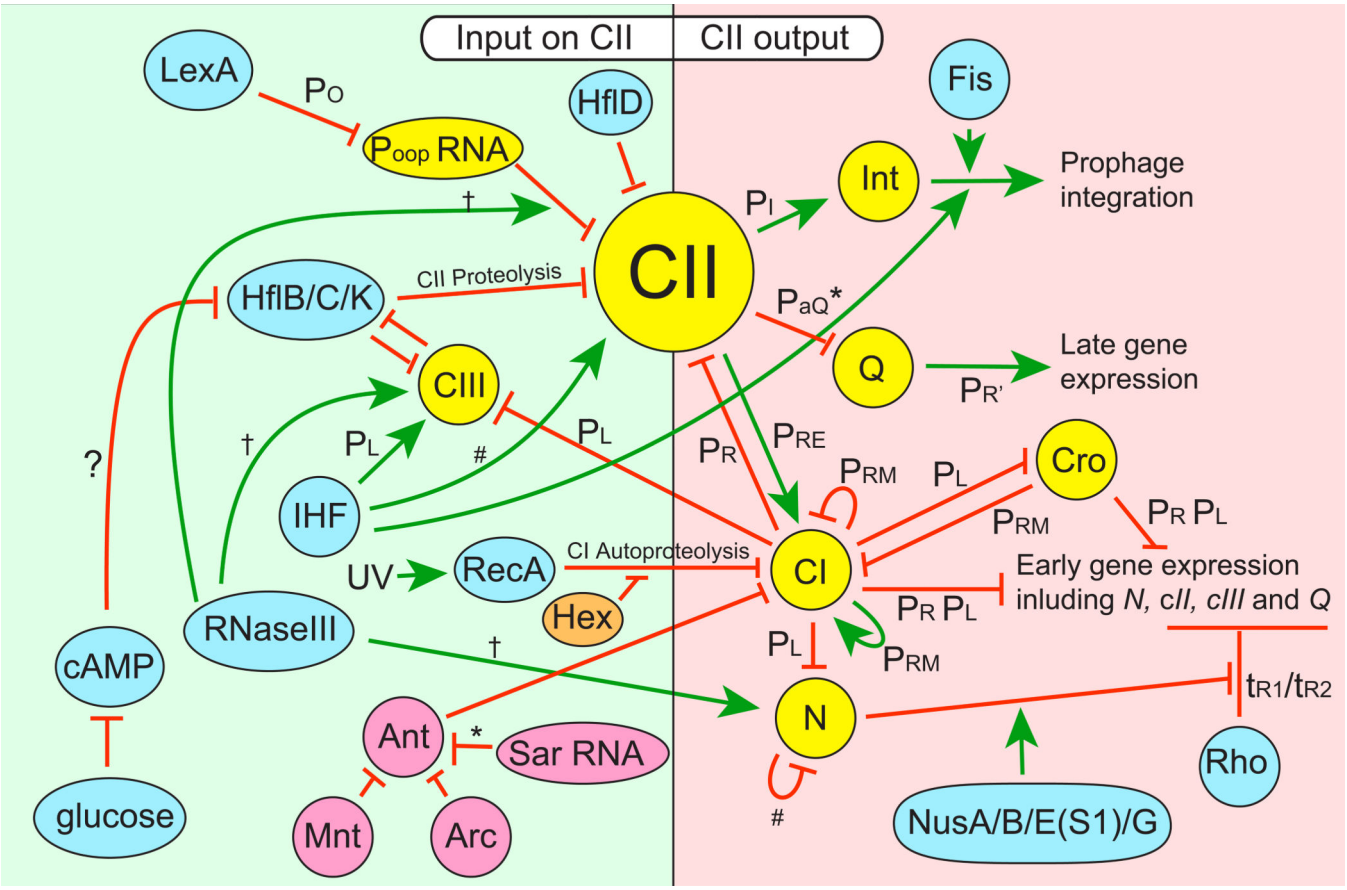

Figure 2. Lambda regulatory circuits

The figure shows phage lambda-encoded functions (yellow ovals) and host encoded functions (blue ovals) that impact the lysis-lysogeny decision and control the lytic gene expression cascade. Phage P22 (Liao *et al.*, 1987;Wu *et al.*, 1987) and phage 434 (Shkilnyj *et al.*, 2013) genes are pink and orange ovals respectively. Green arrows denote positive (activation) functions and red lines denote inhibitory (repression) functions. Asterisks (*) indicate antisense RNA interactions, hashes (#) indicate translational repression, and daggers (†) indicate mRNA stability controls; other regulatory events are at the level of transcription except IHF and FIS physical participation in the integrase complex that catalyzes the integration of prophage DNA.

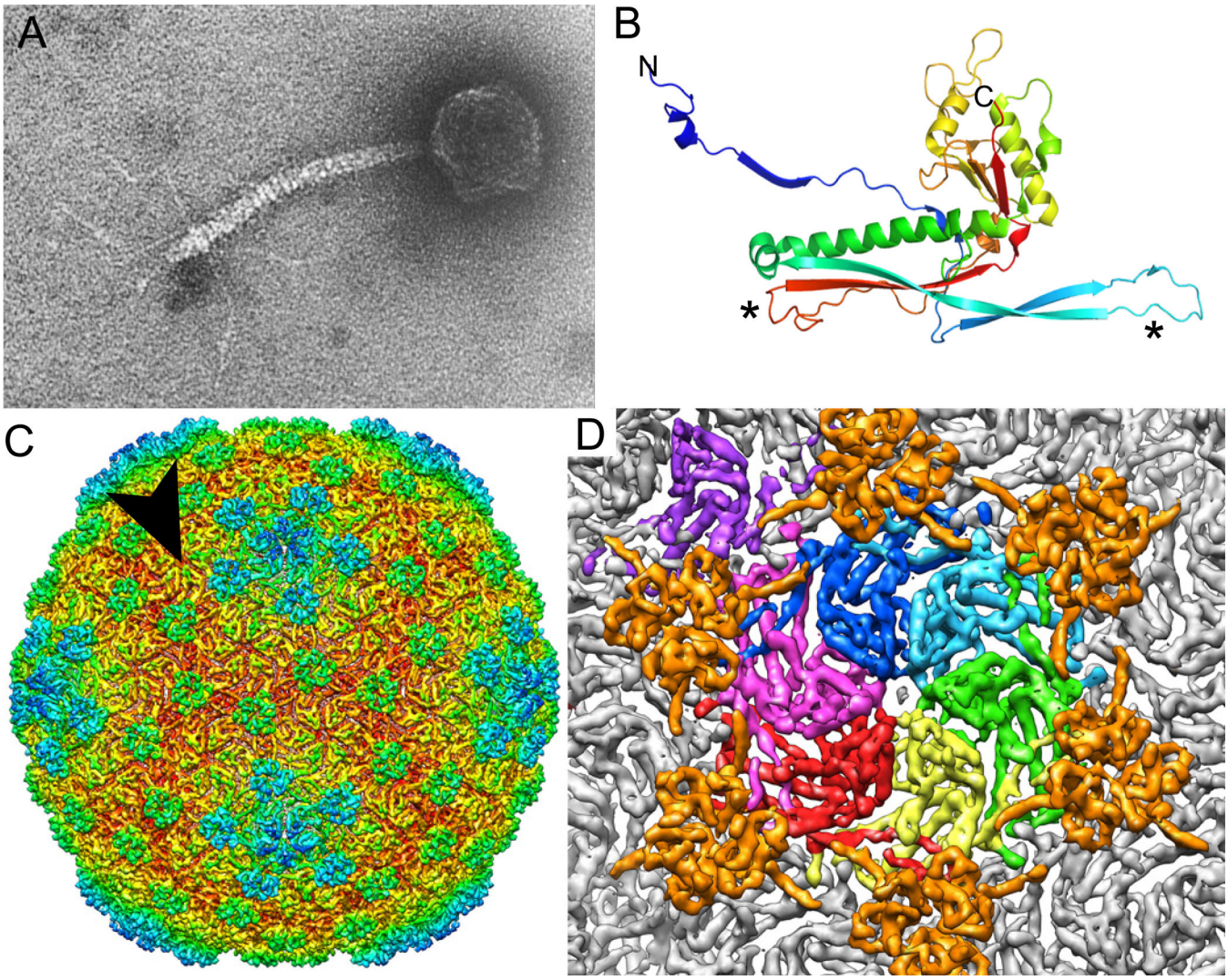

Figure 3. The phage lambda virion

A. Negatively stained electron micrograph of the Ur-lambda virion. Micrograph is modified from Hendrix and Duda (1992) and reproduced with permission of publisher.

B. Ribbon diagram of the phage HK97 virion MCP subunit. The N- and C-termini of the protein are indicated, and the asterisks mark the locations of K169 and N356 whose side chains are crosslinked to adjacent subunits in the virion (from Helgestrand *et al.*, 2003; protein database code 1OHG with rainbow color depiction by MacPyMOL copyright 2009–2010 Schrodinger, LLC).

C. Subnanometer-resolution 3-dimensional cyroelectron microscopic reconstruction of the phage lambda head. Coloration ranges from red to blue with increasing radius, and the black arrowhead indicates the center of a coat protein hexamer as shown in panel D.

D. Close up view centered on a major capsid protein hexamer of the head shown in panel C. Six gene D protein trimers are indicated in orange and seven major coat protein (gene *E* protein) subunits are shown in other colors. These colored subunits form one shell asymmetric unit. Panels C and D are modified from Lander *et al.* (2008) and reproduced with permission of publisher.

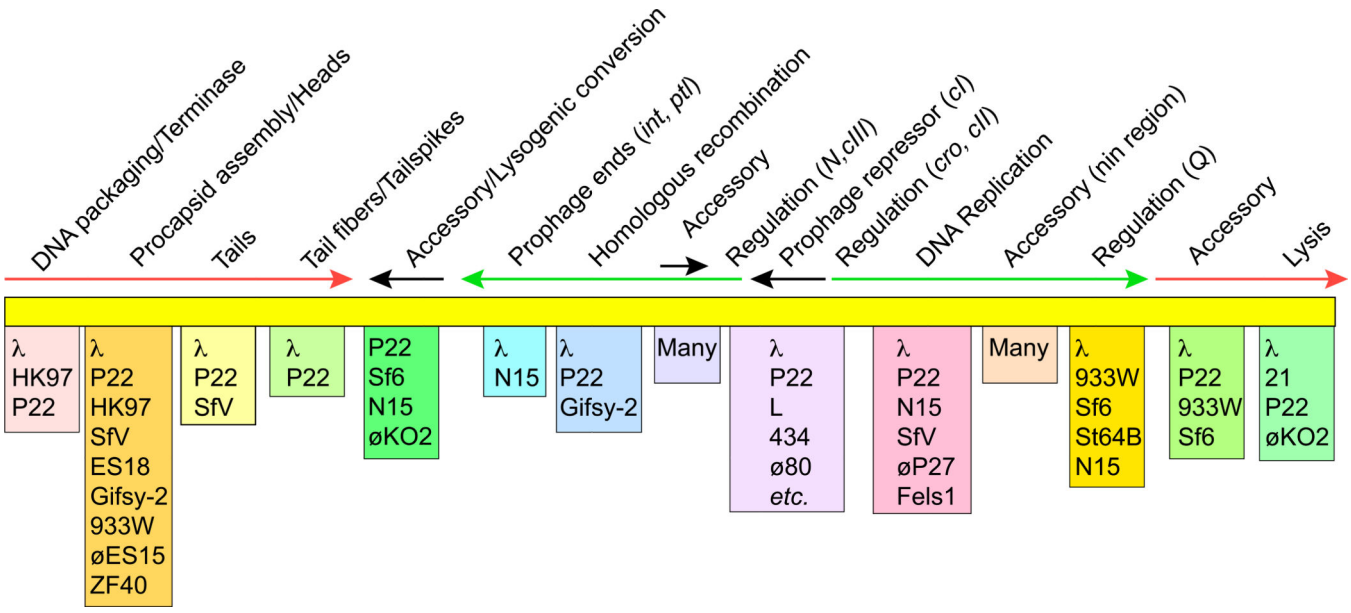

Figure 4. The lambdoid phage diversity menu

A schematic map of the lambda virion chromosome and its major transcripts are shown above (colored as in figure 1). The "menus" of various functional sections are labeled above and listed below the map in colored rectangles. In each section's menu a few lambdoid phages that have very different genes in this region and are best understood in that particular regard are indicated; however, the extant diversity of most sections is larger than shown and remains poorly defined in many cases. In some cases the different phages indicated within one menu section have clearly nonhomologous genes that encode parallel functions (*e.g.*, SfV long contractile, lambda long non-contractile and P22 short tails; lambda Exo, P22 Erf and gifsy-2 RecE type homologous recombination systems), and in some cases the genes are extremely divergent homologues (*e.g.*, portal and MCPs in the procapsid assembly section and probably all the different prophage repressors with different operator binding specificities). Each of the functional sections shown is populated by at least one gene in most lambdoid phages, although there are a few exceptions (*e.g.*, Fels-1 has no *nin* region genes and HK022 has no *N* gene). In addition, any given lambdoid phage may have additional scattered accessory genes (*e.g.*, in lambda the *lom* and *rexAB* genes between the tail and tail fiber genes and immediately downstream of *cI*, respectively, and in P22 the *rha* and *ant* genes between the lysis and DNA packaging genes and between tail and tailspike genes in P22, respectively).