Interaction between bacteriophage λ and its *Escherichia coli* host

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Bacteriophage λ relies to a large extent on processes requiring interactions between viral- and host-encoded proteins for its lytic growth, establishment of lysogeny, and release from the prophage state. Both biochemical and genetic studies of these interactions have yielded new information about important host and λ functions. In particular, mutations in *Escherichia coli* that compromise λ DNA replication, genome packaging, transcription elongation, and site-specific recombination have led to the identification of bacterial genes whose products are chaperones, transcription factors, or DNA-binding proteins.

Introduction

Viruses are the quintessential exploiters, expropriating most classes of the host's macromolecular workforce for their own selfish interest. They accomplish this by multiple and diverse mechanisms that involve processes basic to the expression and replication of the host genes. Consequently, these viral–host interactions provide invaluable means to study virus development, to identify host functions involved in the basic processes listed above, and to isolate mutations in host genes encoding essential functions.

Studies with bacteriophage λ and its *E. coli* host provide excellent examples of the utility of this approach. They have yielded significant information on the physiological processes involved in λ development, and have contributed to the isolation of mutations in previously unidentified *E. coli* genes encoding products that are involved in basic cellular activities. These mutations, which affect interactions between phage and host proteins, can exert significant effects on λ growth without noticeably affecting the host. Thus, selection for mutations that influence λ development avoids some of the problems inherent to searches for mutations in genes whose products are essential for host growth. What is remarkable about this collection of studies is the wide array of host functions that could be identified by genetic selections and screens based on interactions between the virus and its host [1].

Bacteriophage λ, a temperate phage, has two life styles, lytic growth and lysogeny. Lytic growth leads to phage production and requires the regulated expression of most phage genes. Lysogeny results in stable maintenance of the quiescent phage genome by the bacterium, and requires the integration of the phage genome into the host chromosome and repression of most phage genes. This review focuses on recent work on the more prominent activities identified in studies of λ–*E. coli* interactions. Table 1 provides a list of many of these interactions, but because of space limitations, I have had to limit the number of topics discussed below. For a more complete, but dated, review of the subject, see Friedman *et al.* [1].

DNA-binding proteins: site-specific recombination

Integration host factor

Integration of λ into the *E. coli* chromosome (see Fig. 1) occurs by recombination between two unique sites on the phage and bacterial chromosomes (*attP* and *attB*, respectively), and is catalyzed by the phage *int*-encoded product (reviewed in [2–4]). Although *att* sites vary in size and may cover hundreds of nucleotides, recombination occurs within the 15 nucleotide common core that is shared by *att* sites. Fig. 1 represents details on the mechanism of λ site-specific recombination. The Int recombinase is a Type-I topoisomerase; that is, it cuts and rejoins DNA strands one at a time. Although much of the information about the DNA sites and phage proteins involved in integrating and excising the λ genome from the host chromosome was obtained from mutational analysis, two host proteins, DNA gyrase and integration host factor (IHF), were identified through *in vitro* studies of λ site-specific recombination. DNA gyrase, which places negative superhelical turns into DNA, is required for this recombination process because the substrate molecule

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Table 1. λ–*Escherichia coli* interactions.

A. *E. coli* genes influencing λ development

Gene	Map position	Protein	Function in *E. coli*	Function for λ
nusA	min 69	NusA	Trc elong; term	Trc elong.
nusB	min 10	NusB	Trc elong; term	Trc elong.
rpsJ (nusE)	min 72	Ribosomal protein S10	Trn	Trc elong.
nusG	min 90	NusG	Trc elong; term	Trc term.
rho	min 85	Rho	Trc term	Trc term.
dnaB	min 92	DnaB	Rep	Rep.
dnaJ	min 0	DnaJ	Rep-chap	Rep-chap.
dnaK	min 0	DnaK	Rep-chap	Rep-chap.
grpE	min 57	GrpE	Rep-chap	Rep-chap.
groEL(mopA)	min 94	GroEL	Chap	Morph-chap.
groES(mopA)	min 94	GroES	Chap	Morph-chap.
himA	min 38	IHF α-subunit	DNA-binding; trc; rep; trs	Morph; site-specific recomb; trc.
himD(hip)	min 20	IHF β-subunit	See *himA*	See *himA*.
gyrA	min 48	DNA gyrase A-subunit	Gene expr; recomb; rep	Site-specific recomb; gene expr, morph.
gyrB	min 83	DNA gyrase B-subunit	As for *gyrA*	As for *gyrA*.
fis	min 72	Fis	DNA-binding; trs; site-specific recomb	Site-specific recomb (Xis).
hupA	min 90	HU2 subunit	DNA-binding; recomb; rep (?); cell div; trs	Morph.
hupB	min 10	HU1 subunit	As for *hupA*	Morph.
hflA	min 95	HflA	Unknown	Stability of cII maintained.
hflB	min 69	HflB	Unknown	Same as for *hflA*
cya	min 85	Adenyl cyclase	Multiple operon regulation; trc	Controls synthesis of cI.
crp	min 74	cAMP-binding protein	As for *cya*	As for *cya*.
rap	min 26	Peptidyl-tRNA hydrolase	Trn	Mutations cause failed λ growth.
recA	min 58	RecA	Recomb, protease	Repressor inactivation for induction; recomb.

B. λ genes influencing *E. coli* physiology

Gene	Function in *E. coli*
rexA	Exclusion of other phages
rexB	Same as *rexA*
lom	Outer membrane protein; similar to virulence protein identified in other enterobacterial strains
bor	Confers increased resistance to serum killing

(A) The *E. coli* genes encoding proteins that interact with λ proteins. Their map location (given as minutes (min) on the 90 min *E. coli* genetic map) and biological function in *E. coli* as well as their λ function is detailed. Abbreviations include: trc, transcription; elong, elongation; trn, translation; term, termination; rep, replication; chap, chaperone; morph, morphogenesis; IHF, integration host factor; trs, transposition; recomb, recombination; expr, expression; div, division. (?) represents uncertainty. (B) Lists some of the λ proteins expressed from the prophage that influence the phenotype of the host. Appropriate references for these functions can be found in the text or in a previous review on the subject. Functions not referenced in this way include *rap* [111], *lom* [112] and *bor* [113].

with *attP* must be supercoiled for recombination to occur [5,6].

IHF is composed of two small polypetides, IHFα (11 kD) and IHFβ (10.5 kD) encoded by *himA* and *himD* (*hip*), respectively [7]. It belongs to the widely distributed HU family of bacterial DNA-binding proteins [8]. The HU protein of *E. coli* is also a heterodimer composed of two 9.5 kD polypeptides encoded by the *hupA* and *hupB* genes. Although IHF binding is sequence-specific and HU binding is not [9], HU, in some cases, can

substitute for IHF. IHF is not an essential function for *E. coli*, but it does influence a number of phage and bacterial processes [10]. These include the expression of many operons, other recombinational events, replication, and morphogenesis.

IHF appears to influence this wide array of DNA interactions by facilitating contortion of the DNA duplex into structures compatible with the required protein–DNA interactions [4]. Most of the signals required for λ integration are contained in the substrate DNA with *attP*,
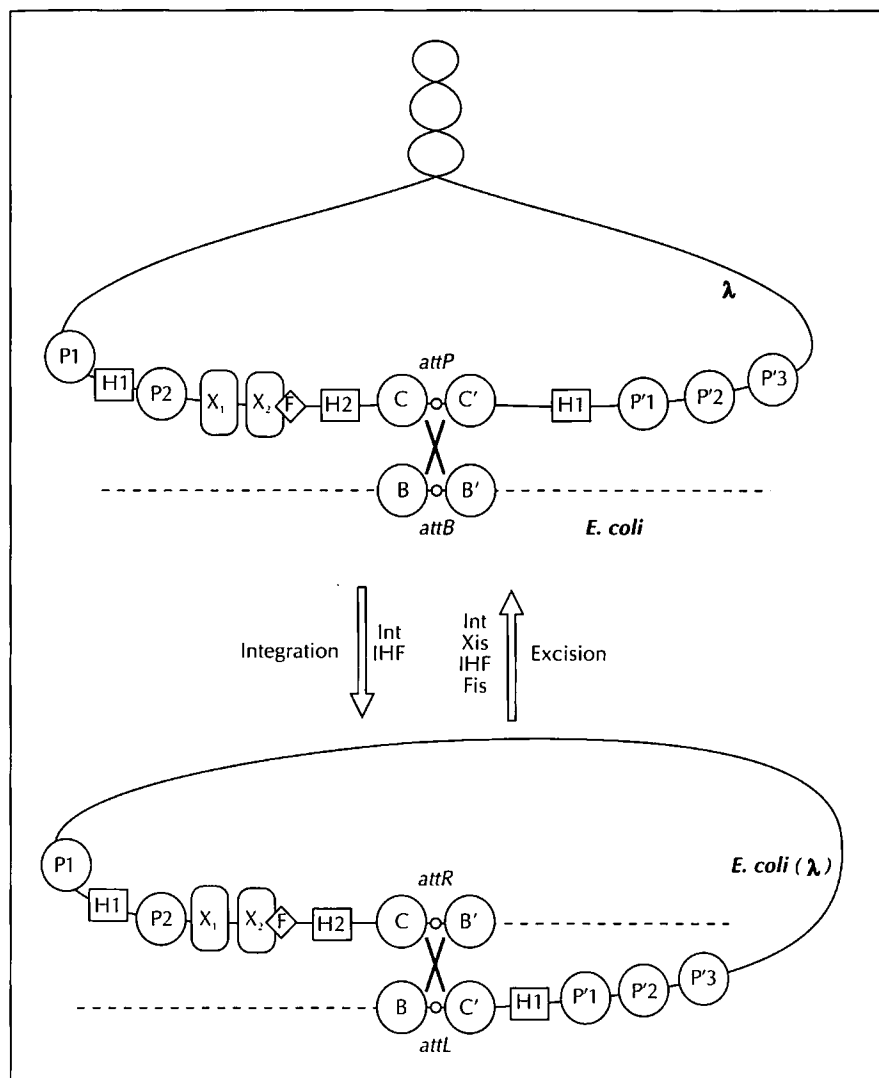
Fig. 1. Structural elements of λ site-specific recombination. The λ DNA is shown as a solid line, while *Escherichia coli* DNA is indicated as a broken line. The four *att* site are shown with the relevant placement of the binding sites for the various factors: C,B, and P are Int-binding sites, H represents an IHF-binding site; X shows an Xis-binding site, and F is a Fis-binding site. The cross-over between the common cores is represented (x) and the small open circles above and below these points represent the 15 bp of homology in which recombination occurs. The central arrows indicate the direction of the integration and excision reaction. Listed alongside the arrows are the λ and *E. coli* functions required for the reaction. The coils shown for λ indicate that DNA with *attP* must be supercoiled. The drawing is not to scale.

including three required IHF-binding sites, called H1, H2 and H'. Goodman and Nash [11] have demonstrated that, at least at the H2 site, the only role for IHF is to bend the DNA. These workers 'swapped' the H2 IHF sequence for either the DNA-binding sequence for the cycli-AMP (cAMP)-binding protein, Crp, or for a naturally bending 'A-tract' DNA. In the presence of the usual *in vitro* requirements, both replacements allowed site-specific recombination to occur, providing that the swapped signals were appropriately phased and Crp protein was present when its cognate site was employed. When DNA without a bend was used in the swap, few recombinant molecules were observed.

Results of hydroxyl-radical footprinting experiments demonstrated that IHF contacts DNA primarily in the minor groove of DNA [12]. A structure for IHF, based on X-ray crystallography of the closely related HU protein of *Bacillus stearothermophilus* [13] as well as the DNA protection and interference experiments with IHF, has each subunit contributing a two-stranded β-sheet with extended arms that could contact the DNA in the minor groove. Consistent with this model, mutations that change binding specificity were located in the

flexible arm of IHFα [14••,15••]. However, similar mutations were not found for IHFβ [14••]. G Mengeritsky *et al.* (personal communication) have created a series of mutations changing 'arm' amino acids of IHFβ and have shown that many have little or no effect on IHF activity. Perhaps sequence-specificity resides in IHFα, which initiates the binding, while IHFβ DNA contacts are sequence independent.

Although the arm of an IHF monomer can cover only 5 or 6 bp, Yang and Nash [12] found that at least 25 nucleotides are protected by IHF binding. The obvious explanation that multiple copies of IHF might bind at a single site was ruled out by showing that one site binds one IHF molecule, leading the authors to propose that the DNA duplex may bend around the IHF molecule and contact it at other positions in addition to the arms. Because it bends DNA, IHF can serve to facilitate the wrapping of the *attP* DNA into a higher-order structure that has been named the 'intasome' [16,17]. A more detailed and highly illuminating discussion of the roles of IHF and DNA gyrase in forming the intasome can be found in the review by Nash [4]. Confusing this neat picture of integrative recombination is the *int-h* mutation (*intE174K*)

[18]. The mutant Int protein supports a low level of integrative recombination in the absence of IHF *in vitro* [19] and *in vivo*, suggesting either that a higher-order structure can form, albeit less efficiently, without IHF, or that recombination can occur in the absence of such a structure if Int-h is present. Int-h supports recombination at secondary bacterial *att* sites more effectively than the wild-type protein and this relaxed specificity might be selected against.

Nash and Granston [15••] have identified similarities in the DNA-binding regions of IHFα and the family of eukaryotic TFIID transcription factors. Like IHF, TFIID appears to bind to the minor groove [20•,21•] and recognizes a sequence [22] that is similar to part of the IHF-binding signal [23]. These and other results led Nash and Granston to suggest that "IHF and TFIID might even use similar DNA contacts to recognize their specific targets". However, IHF makes contact with a much larger target site on the DNA. Lef-1, another eukaryotic transcription factor, was postulated to be a DNA-bending protein because, in part, it and its cognate DNA-recognition site could substitute for IHF in a 'bend-swap' similar to that done with Crp [24••]. Lef-1 is a member of the HMG family of DNA-binding proteins that, like IHF, contact DNA primarily in the minor groove [25•].

Factor for inversion stimulation

Excision of the λ prophage, although ostensibly a reversal of integration, is functionally quite different [2,3]. This is understandable given that the two processes must be both efficient and directional in the sense that once committed, λ either integrates or excises. Integrative recombination between the information-loaded *attP* site and the *attB* site results in the formation of two new *att* sites, *attL* and *attR*, which flank the integrated λ prophage and divide the information originally carried by *attP*. Excision of the prophage results from site-specific recombination between *attL* and *attR* and requires the λ Xis and the *E. coli* Fis proteins, in addition to the proteins involved in integration (see Fig. 1).

Fis was identified because of its role in enhancing the action of a recombinase that catalyzes a DNA inversion [26]. It is a homodimeric protein that binds to a rather degenerate consensus sequence [27], presumably through a region with a helix-turn-helix motif [28–30]. Fis, which is required for effective λ excision [31•], binds to the F site found in the *attR* arm that overlaps X_2, one of the two adjacent Xis-binding sites [32,33]. Int binding at the P2 site of *attR* is facilitated by Xis binding cooperatively either with another molecule of Xis at the X_1 and X_2 sites or Fis at the X_1 and F sites [34]. Presumably, Fis is required for excisive recombination *in vivo* at least in part because the concentration of Xis is not sufficient to facilitate Int binding to P2. Employing an *in vivo* integrative recombination assay, Ball and Johnson [35••] found that Fis binding at the F site stimulates integrative recombination in the absence of Xis, but inhibits it in the presence of Xis. Indeed, because Fis levels vary significantly depending on the growth phase (levels

are high during exponential growth) [32], it has been postulated that Fis levels could influence the direction of the site-specific recombination reaction; in stationary phase where there are low levels of Fis, integration would be favored. In contrast, Ball and Johnson [35••] report that production of λ from a lysogen growing in late exponential phase (where Fis levels are low) is not significantly affected by high levels of Fis expressed from a plasmid. However, phage production may not be a sufficiently sensitive assay to assess the effects of excision on the ultimate fate of the lysogen and the prophage.

For an informative discussion of excisive recombination see Kim and Landy [36••]. They present evidence consistent with a model of excisive recombination in which the IHF, Xis and Fis proteins wrap the two *att* sites into a structure that is aligned for recombination. This would allow Int molecules bound on the arm of one *att* site to bind to the core on the other *att* site.

DNA-binding proteins: transcriptional control

Transcription from the pL promoter (see Fig. 2), which is regulated by repressor and directs synthesis of the important N regulatory protein (see below), is enhanced both by IHF and supercoiling [37,38•]. There are two IHF-binding sites 86 and 180 bp upstream of pL, and IHF enhancement of transcription requires proper phasing between the promoter and the IHF sites [38•]. This suggests an interaction between IHF and RNA polymerase (pol) at pL and, as pL and the IHF sites do not overlap, this interaction probably requires some localized deformation of the DNA. A second promoter (the function of which has yet to be defined) that is repressed both by IHF and repressor has been identified 42 bp upstream of pL [38•].

Initial transcription of the cI repressor gene of λ from the pRE promoter requires the product of the cII gene [39]. Expression of cII is regulated, in part, by IHF [40]. However, this regulation appears to be post-transcriptional [41]. The cII-encoded protein also activates two other λ promoters, p_{int} [42] and p_{aq} [28], by binding to a unique sequence located in each of their -35 regions. Transcription from these promoters helps direct λ toward lysogeny. Thus, by regulating cII expression, IHF indirectly influences expression from a number of λ promoters.

DNA-binding proteins: genome packaging

In light of its multifarious roles supporting lysogeny, it is tempting to conclude that IHF serves as a physiological sensor of the physiological state of the cell, at higher concentrations directing the phage toward lysogeny. Nevertheless, this model fails to explain why IHF participates in an event occurring late in the lytic pathway — the terminase reaction, which cleaves the concatameric λ DNA
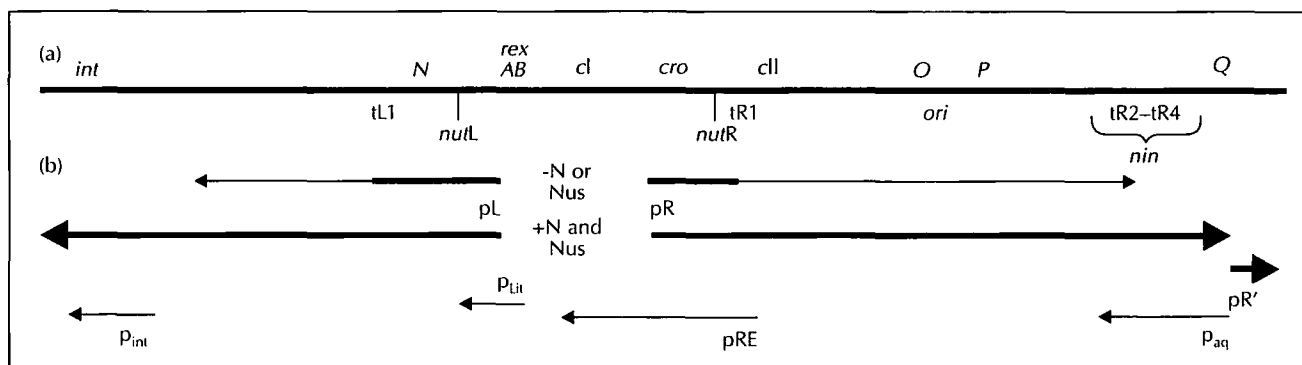
Fig. 2. The early regulatory region of λ. (a) The λ genetic map, which is not drawn to scale. Listed above the line are relevant genes and below the line are the relevant signals: the termination signals tL1 and tR1, and the *nin* region of multiple terminators (tR2–tR4); the *nut* sites (see text for details); and the origin of replication (*ori*). The positions of the relevant promoters (p) are indicated below the map (see text for details). (b) The direction of transcription is indicated by the arrows. The thickness of the arrows indicates the relative level of transcription. The nature of transcripts from pL and pR with (+) and without (−) N modification, in the presence of the group of host factors, Nus, is also indicated.

into the unit-length genomes required for packaging (reviewed in [43–45]).

Here, too, it has been postulated that IHF functions in the formation of a higher-order DNA–protein structure. Genetic and physical studies suggest that this structure contains, in addition to IHF, the products of the λ *nu1* and *A* genes as well as 200 bp of DNA surrounding and including the cleavage site. The DNA surrounding the cleavage site contains binding sites for four Nu1 and three IHF molecules. Becker and Murialdo [46] postulate a DNA structure wrapped around two molecules each of A protein and IHF, and four molecules of Nu1 protein. This would orient A proteins in the appropriate positions to cleave the DNA at unique sites and generate the 12 bp 5' overhangs that form the single-stranded cohesive ends of the linear DNA molecule of the mature phage.

The requirement for IHF in the λ terminase reaction is not absolute because HU protein partly compensates for the missing IHF. Mendelson and colleagues [47] found that while the burst of λ from either an IHF⁻ or an HU⁻ host is reduced fivefold, in the double IHF⁻:HU⁻ host it is reduced 100-fold. This defect was ascribed to a failure in terminase activity, because a mutation in *Nu1* [48,49] allows growth in an IHF⁻:HU⁻ double mutant. HU binding in the *cos* region apparently does not have sequence specificity because, unlike IHF, it failed to provide specific DNase-I protection. HU and IHF are also interchangeable for both *in vitro* replication [50] and transposition [51] systems. This is not surprising considering the structural similarity between IHF and HU; however, it is surprising considering that IHF, but not HU, has sequence specificity.

RNA-binding proteins: transcription elongation factors

In large measure, λ development is regulated by systems of transcription termination and antitermination. For further discussion and more complete reference lists, see the reviews [52–54] on this subject. Early transcription initiating at the pL and pR promoters (see Fig. 2) partially terminates at two terminators, tL1 and tR1, which are activated by the *E. coli* Rho termination protein. Approximately 50% of the transcription from pR proceeds past tR1 through the *cII–O–P–ren* genes to terminate in a region composed of both Rho-dependent and Rho-independent terminators called *nin* [55•]. Early transcription from pL results in expression of the product of the *N* gene. In the presence of N and a group of host factors, Nus, transcription from pL and pR overrides downstream termination signals. In the case of pR, transcription extends through the *nin* region of multiple terminators into the *Q* gene. Q, whose *in vitro* action is stimulated by NusA, is a second antitermination function that maximally stimulates late gene expression (reviewed in [52]). Other Nus proteins do not appear to be necessary for Q action [56].

The Nus factors were identified by analysis of mutations in *E. coli* that result in failure of the mutant host to support N-mediated antitermination [57] and were subsequently shown to be required for the *in vitro* antitermination reaction [58,59]. The NusA and NusB proteins are essential bacterial functions (NusB, only at lower temperatures) [60–62], that at least in part serve as host transcription factors [63]. The NusE protein is ribosomal protein S10 [64]. *In vitro* studies indicate that S10 is bound to NusB in the N transcription complex [65•].

The 23 000 M_r NusG *E. coli* protein participates in the *in vitro* N-antitermination reaction [66••,67•], but by one measure, does not appear to be necessary for *in vivo* N-mediated antitermination. Sullivan and Gottesman [68••], using a plasmid that fails to replicate at high temperature as the sole source of NusG, demonstrated that bacteria grown at high temperature, and thus substantially depleted of NusG, still supported N-mediated read-through of the Rho-independent terminator, tI. Although Rho-independent termination appeared normal, NusG-depleted bacteria exhibited a severe reduction in Rho-dependent termination. A missense mutation in *nusG* allows hosts with mutations in *nusA* or *nusE* to

support N-mediated antitermination [69••]. Because this mutation does not result in a bypass of Nus proteins, it was suggested that NusG is a participant in the N reaction. It is possible that different concentrations of NusG are required for termination and antitermination, and that the depleted cell has a residual level of NusG sufficient to support antitermination or that another factor substitutes for NusG. Alternatively, Sullivan *et al.* [69••] have suggested that NusG may stabilize antitermination complexes and thus may not be essential for their *in vivo* assay. Not surprisingly, mutations in *rpoB* [70–72] encoding the β-subunit of RNA pol, enhance or suppress the inhibitory action of *nus* mutations.

The loading of N and Nus factors onto RNA pol is signalled by *nut* sites located downstream of pL and pR [58,59,66••,73]. The *nut* signals, recognized at the level of RNA [74–76,77•], have two components, *boxA* and *boxB*. The 9 bp *boxA* signal, conserved with only slight variations amongst lambdoid phage *nut* sites [78], is also found in the leader-region antiterminator of the *E. coli rrn* operons [79], and is probably a signal for a host Nus factor (D Court, personal communication) [59,78,80]. The hairpin structure comprising *boxB* has been implicated as the recognition site for N [81,82]. Although this sequence is not conserved amongst lambdoid phages, the structure is conserved. Whalen and Das [83], employing an abbreviated *in vitro* N-antitermination system, showed that N and NusA can be loaded onto RNA pol even if the pol has traversed 176 bp 3′ to the *nut* site. These authors propose that *boxB* anchors N and brings it to the pol. The pol modified only by N and NusA will override a terminator, but only if it is close to the *nut* site [84]. Presumably addition of the other Nus factors produces a more processive antitermination complex.

Remarkably, the λ *nut* sites can also serve as transcription-termination signals. The lambdoid phage HK022 prophage expresses Nun, which acts specifically at λ *nut* sites to terminate transcription from the pL and pR promoters [85]. The *nun* gene is located at a position on the HK022 genome analogous to that occupied by *N* genes of other lambdoid phages, and it shares some significant amino-acid homologies with the products of those genes. Nevertheless, the *nun* gene product is not required by HK022 for phage growth [86]. In fact, even though HK022 has an early transcription antitermination system similar to the various N systems, there is apparently no requirement for a phage-encoded product (J Oberto *et al.*, personal communication). The only identified role for Nun is termination of λ transcription. Even more astounding, Nun-mediated termination uses λ *nut* sites and the same host Nus proteins that λ N requires for antitermination [87••]. However, mutational analysis reveals that whereas Nun and N require the same *boxA* sequences, there are differences for *boxB* [88•]. Moreover, mutations in *nus* genes have been isolated that reduce Nun action without affecting N action [87••] and some *nus*, as well as *nutR* mutations convert Nun to a suppressor of termination at the adjacent tR1 terminator [80]. However, this apparent reversal of roles, unlike

N antitermination, does not extend to downstream terminators

These studies suggest that although Nun and N both recognize *boxB* and act with the same complex of host Nus proteins (in part, acting through *boxA*), they promote opposite reactions. Nus proteins themselves, therefore, may not determine transcription elongation, but rather modify pol to accept signals from other effector molecules.

Protein–protein binding: chaperone proteins

One of the most rewarding lines of research with λ has been the characterization of interactions between phage functions and *E. coli* stress proteins, many of which were identified through their roles in λ replication or packaging.

Replication

The following discussion of λ replication is based primarily on the group of excellent reviews [89–91]. Most of the proteins involved in λ replication are supplied by the *E. coli* host [92], but two λ proteins, products of the adjacent *O* and *P* genes (see Fig. 2), are required. The origin of replication, consisting of four adjacent 19 bp direct repeats, is located within the *O* gene. Host proteins involved in the initiation of λ replication include DnaJ, DnaK, and GrpE — functions first identified through the analysis of *E. coli* mutants defective for λ replication. All three are members of the 'heat-shock' family of proteins, identified in *E. coli* as proteins that are induced following a shift to a higher temperature (reviewed in [93]).

The early events in initiation align the host DnaB protein at *ori* (Fig. 3). DnaB is a helicase that forms the structure recognized by the host primase, a role DnaB also plays in host replication. The initial step is thought to be the binding of O protein to *ori*, resulting in an opening of the DNA duplex. DnaB is then delivered to the *ori*-bound O protein by P protein. P binds tightly enough with DnaB to allow it to compete favorably with DnaC, the protein that fulfills the analogous role in host replication. However, DnaB is inactive when complexed with P. The DnaK, DnaJ, and GrpE heat-shock proteins (hsps), in an ATP-dependent reaction, release DnaB from the tight complex formed with O and P at *ori*. Thus freed, DnaB can unwind the duplex to prepare the way for primase. Central to this reaction is the ATPase activity of DnaK.

DnaK is related to the eukaryotic hsp70 family of chaperone proteins (50% homologous at the amino-acid level) [94]. It releases P from the λ replication complex, but does so efficiently only in the presence of DnaJ and GrpE. Liberek *et al.* [95] demonstrated that DnaK ATPase activity is effectively stimulated only in the presence of both DnaJ and GrpE. Moreover, each of these latter two proteins were shown to have unique roles: DnaJ accelerates hydrolysis of ATP; while GrpE stimulates release of ATP or ADP bound to DnaK. As DnaJ and GrpE catalyze these reactions independently of each
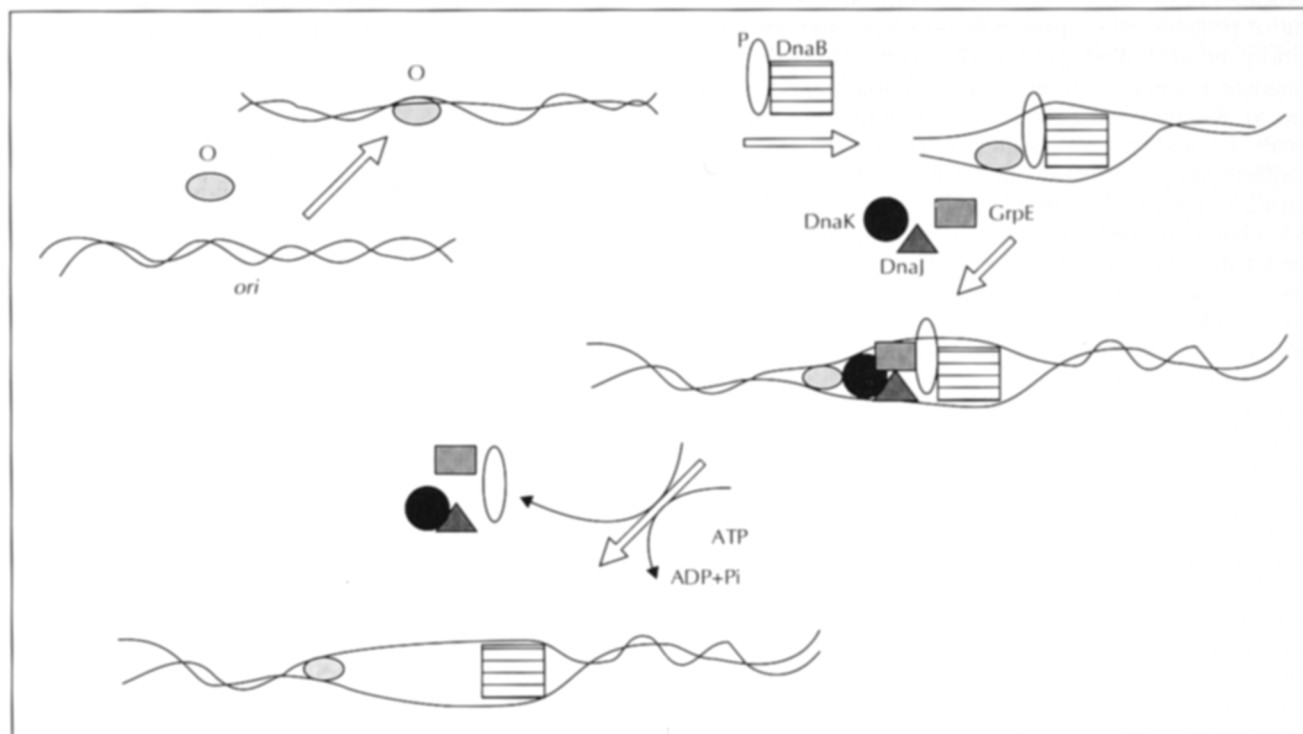
Fig. 3. Schematic representation of early events at the λ origin of replication (*ori*). O protein binds to *ori*, opening the DNA helix. P protein then delivers the host DnaB helicase to the complex. In an ATP-independent reaction the host heat-shock proteins DnaK, DnaJ and GrpE release DnaB. Thus released, and in an ATP-dependent reaction, DnaB unwinds the duplex in preparation for primase and subsequently replication. See text for further details.

other, it was suggested that an intracellular complex of the three proteins need not form.

The *dnaK756* mutation, isolated because it prevented λ replication [96], has proven to be a remarkable tool for characterizing DnaK activity. It has 50-fold higher ATPase activity than the wild-type DnaK protein. Liberek *et al.* [95] offer the interesting hypothesis that DnaK756, which does not function in the *in vitro* λ replication assay, forms a complex with P, but the complex prematurely dissociates because of the high intrinsic ATPase activity of the DnaK756 protein.

These studies suggested that DnaK and associated proteins might normally serve to disassemble inappropriate protein aggregates, as proposed for other related hsp70 proteins. DnaK, in the presence of ATP, not only protects *E. coli* RNA pol from heat inactivation, but disaggregates and reactivates RNA pol when it is heat inactivated [97].

Morphogenesis

Assembly of the λ capsid and the associated packaging of DNA is a complicated process involving a multitude of proteins, making even a summary beyond the scope of this article. Instead, the reader is referred to a number of superb reviews [45,98–100]. Here, discussion will be limited to the products of the *groEL* and *groES* genes, which were identified by mutations that block λ morphogenesis.

The GroEL protein is homologous to proteins in the hsp60 family of heat-shock proteins and is essential for

bacterial growth (reviewed in [98]). The active protein is made of 14 identical 57 259 M_r subunits arranged into a structure with a sevenfold axis of symmetry. The GroES protein is composed of seven identical 10 368 M_r subunits also arranged into structure with a sevenfold axis of symmetry. Like DnaK, GroEL has a weak ATPase activity that is modulated by another protein, in this case GroES. Martin *et al.* [101•], using the intrinsic flourescence properties of tryptophan in bound proteins, directly studied the structure of proteins bound by the GroE structure. This was possible because neither GroE protein contains tryptophan. These studies indicated that GroEL 'stabilizes unfolded substrate proteins in a conformation devoid of ordered structure (globular)' and releases them in the presence of ATP. GroES accelerates the conversion to the fully folded native form. One, or at most two, denatured protein molecules are bound by one GroEL 14-mer, which interacts with one GroES 7-mer.

This GroEL (presumably with GroES) structure functions early in the assembly of the of λ head. These proteins are required for the assembly of the phage-encoded B protein into the 12-fold symmetrical head–tail connector structure. A relationship between the 12 subunits of this structure and the 14 subunits of GroEL during assembly need not necessarily be postulated. Georgopoulos *et al.* [91] suggest that the GroE proteins facilitate formation of B dimers, which then spontaneously associate into the head–tail appendage. Accordingly, GroE proteins would serve to facilitate proper folding of B into a form that can dimerize.

Although both DnaK and GroEL aid in the proper folding of proteins, they apparently do so in different ways. Landry et al [102•] presented evidence from nuclear magnetic resonance studies indicating that peptide binding to the two proteins is quite different: binding to DnaK is mediated by hydrogen bonds, hydrophobic contacts, and side-chain salt bridges; while binding to GroEL is mediated primarily by the hydrophobicity of side chains. Indeed, Langer et al. [103•] postulate that the DnaK–DnaJ–GrpE and GroEL–GroES groups can cooperate to form a pathway for folding certain proteins. They studied the refolding of the Rhodanese protein from bovine mitochondria, a protein that when diluted in the denatured state is quite recalcitrant to refolding. Effective refolding requires successive interactions with DnaK, DnaJ, GrpE, GroEL and GroES in the presence of ATP. Reaction first with DnaK and DnaJ yields a partially folded protein that contains an "as yet undetermined amount of secondary structure but lacks ordered tertiary structure". In the presence of GrpE, this partially folded protein is transferred to the GroEL complex, where in the presence of GroES, it becomes properly folded. The isolation of conditional lethal mutations in these genes suggests that this pathway is required for folding proteins essential for *E. coli* viability.

A membrane-binding protein

Rex exclusion

"A riddle wrapped in a mystery inside an enigma", as Winston Churchill described the Russia of 1939, aptly applies to one of the classical puzzles of molecular biology: the mechanism of exclusion of T4 *r*II mutants (and other phages) by the *rex* gene product expressed from λ prophages. For that now elderly generation that learned its molecular biology in the 1950's and 60's, there was no more perplexing question. Many of the seminal findings of molecular genetics relied on Seymour Benzer's discovery of the ability of a λ lysogen to exclude all *r*II mutants of T4, while supporting growth of wild-type T4. These findings unlocked many secrets of recombination, the basis of nonsense suppression, the nature of the genetic code, the definition of the cistron, and the molecular basis of the action of many mutagens [104,105]. Later, it was shown that the basis of the exclusion depended on the expression by the λ prophage of the *rex* gene, which is located adjacent to the *cI* repressor gene [106]. Although scores of graduate students and postdoctorates spent their youthful energies attempting to unravel the mystery of exclusion, the problem, like the legendary sword Excalibur, resisted the pull of many. Recently, however, Parma and coAuthurs [107••] have come closer to prying it loose.

Three earlier observations are crucial in understanding the arguments of Parma et al. First, physiologically, *rex*-mediated exclusion is characterized by loss of membrane potential, failures of macromolecular synthesis and hydrolysis of ATP, and cell death (reviewed in [104]). Second, there are not one, but two *rex* genes (see Fig. 2), *rexA* and *rexB* [108]. Third, events mimicking this exclusion can occur in the absence of T4 infection if there is expression of *rexB* and over-expression of *rexA* [109].

Using a series of *rexB–phoA* fusions, RexB was shown to be a membrane protein with multiple cytoplasmic and membrane domains. A model was proposed based on the following observed characteristics of the exclusion process and property of the protein: first, depolarization of the cytoplasmic membrane; second, altered RexA/RexB ratio; and third, the gap junction-like structure of RexB. It was proposed that RexB forms membrane depolarizing ion channels and that these channels are activated by RexA. The critical factor is a high ratio of RexA to RexB. If and how T4rII mutants change the RexA : RexB ratio remains unknown.

Interestingly, when over-expressed, the Rex proteins block growth of wild-type T4 and all other phages tested with the exception of λ [109]. It is conceivable that, like restriction, colicin production, and perhaps the termination activity of the HK022 Nun protein, Rex exclusion is also a mechanism for a selfish parasite, in this case λ, to exclude the growth of other similar parasites. As the infected λ lysogen is killed by the exclusion process, a sacrifice of the individual lysogen for the good of the whole is postulated (i.e. the sacrificed bacterium is a dead-end for the infecting DNA) [107••].

How does λ escape its own exclusion? Parma et al. [107••] look to the ancient λ literature for an answer. Hayes and Szybalski [110] identified a transcript, *lit*, that covered the *rexB* gene and apparently initiates at an unidentified promoter in *rexA*, p_{Lit} (Fig. 2). Parma and colleagues propose that the lytically growing λ overcomes Rex exclusion by activating transcription from p_{Lit} and decreasing the ratio of RexA to RexB. Obviously, the cognoscenti await the construction of a λ derivative missing p_{Lit} before assessing the validity of this model.

Concluding remarks

The expropriation of host functions can be considered advantageous to the virus in three ways. First, the expropriated machinery is obviously functional in that host. Second, to the extent that host products are used, much can be accomplished with a small amount of viral genetic material. Third, limited essential functions can be competitively removed from the host. For temperate phage, such as λ, host products might take part in the decision between lysis and lysogeny by indicating the physiological state of the host. Depending on the concentration of such products, the phage would be directed toward one or the other developmental pathways. Although tinkering with one host factor has failed to confirm this idea [35••], it is conceivable that a number of host factors, some of them as yet unidentified, with overlapping activities could contribute to this decision.

For the investigator, study of these interactions has not only yielded a wealth of information about λ, but identi-

fied previously unrecognized functions and nucleic-acid signals important to the host.

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I must apologize to my many colleagues and friends in the field whose work I have failed to cite. Suffice it to say that there are numerous additions to the stories I have told, and also others that are as, if not more, interesting.

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structure. The strategy assumes that only when the binding sites for
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