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Interactions of Bacteriophage and Host Macromolecules in the Growth of Bacteriophage λ

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"The problem of lysogenization is complicated by the fact that the bacterial response does not depend solely on the genetic constitution of the phage and of the bacterium as such, but on the interrelation or interaction of genetic materials."
André Lwoff (167).

INTRODUCTION

Viruses represent the ultimate in parasites; not only do they exploit the host as a source for energy, but also they utilize the host's machinery for DNA, RNA, and protein syntheses. In some cases, viral development may simply involve the use of host products, whereas in other cases, development of the virus requires interactions between host and viral functions. Thus, mechanisms used by viruses to subvert the normal host processes for their own purposes constitute a unique system for studying regulation of gene expression as well as protein-protein interactions.

One of the best-studied host-virus systems is that of bacteriophage λ and its host, *Escherichia coli*. This system is amenable to analysis because both λ and *E. coli* are extremely well characterized. Furthermore, λ development depends on interactions between host and viral functions. Analysis of these interactions has proven to be fruitful in two ways. First, it has led to a substantial increase in our knowledge about the phage and its development. Second, it has uncovered heretofore unknown information about host functions.

There are several different themes that run throughout this review and that we hope make it useful to readers with widely different interests. One of the themes is the experimental strategy that has formed a basis for identifying many of the interactions to be discussed. The rationale is as follows: if a phage protein interacts with a host protein to form the functional product, then it might be possible for the host to become refractory to the phage by a mutation affecting this particular interaction. This rationale for identifying protein-protein interactions is, of course, complementary to the rationale for identifying such interactions by finding mutations in one polypeptide that compensate for a mutation in another, interacting polypeptide (18, 126).

The general strategy for isolation of such host mutants (often called *gro* mutants) is to select for bacteria that survive phage infection or prophage induction. Bacterial mutants can be selected by a suicide method, in which a nutrient agar plate is spread with a low number of phages (10^6 to 10^7 per plate) so that each bacterium placed on the agar can begin to form a colony, but eventually some of the cells in the fledgling colony will become infected by the phage. If these infected cells release a burst of phage, then the sibling cells become infected and killed; hence colony formation is aborted. In contrast, if the infected cells do not liberate phages (even though these cells may themselves be killed), a colony is formed because siblings remain uninfected. Mutants of the *gro* type can also be selected as survivors of prophage induction. For example, lysogens carrying a λ O^- prophage die upon prophage induction due to a killing event that is dependent on the phage N protein. Hence, most survivors carry mutations in the *N* gene or in host genes that affect phage N protein function. These isolation strategies have turned up several different classes of mutations blocking different stages of phage development, transcription, DNA replication, and particle morphogenesis.

The strategy for identifying such phage and host product interactions raises an important question at the outset. Given that such a physical interaction exists, is it possible for the host protein to be so modified that it can maintain its own function while altering its interaction with the phage component? This is obviously an important question for essential bacterial genes. Remarkably, the answer is yes, at least for several diverse *Escherichia coli* genes. One example is alteration of the essential gene *dnaB* in certain *gro* mutants. Because the *gro* mutants produce an altered gene product, they often exhibit an additional phenotype. In particular, many of the *gro* mutants are now temperature sensitive for their own growth. Notable examples include *groP* and *groE* mutants, to be described later.

The types of mutations that lead to the inability of a cell to support phage growth not only can be obtained in the laboratory, but also are naturally occurring. An exquisite example of such natural resistance is seen in *Salmonella typhimurium*, which behaves vis-à-vis phage λ as if it were an *E. coli* strain with a *nusA* mutation. It is thus clear that differences between bacteria with respect to ability to support phage growth can result from not only differences in phage adsorption specificity but also intracellular events. Several of the phage-host interactions that we discuss have been identified by analysis of bacterial mutants that influence lytic development of λ. These variants have mutations in genes such as *nusA*, *groP*, *dnaJ*, *dnaK*, *groEL*, and *groES*. We also discuss influences on the lysogenic mode of development. In particular, we discuss host genes *hflA* and *hflB*, which affect the mode of λ growth, and *him* and *hip* genes, which regulate phage integration. Other phage and host proteins also physically interact to regulate phage behavior. Notable among these are the interactions of the phage *c*I protein with host RNA polymerase to stimulate *c*I transcription and phage *c*I protein cleavage mediated by host RecA protein. These topics are discussed extensively by Hochschild et al. (116) and Roberts and Devoret (208) and are not discussed as fully here. The adsorption of the λ phage particle to the host bacterium and subsequent DNA injection obviously involve interaction between host and viral products. However, the focus of this review is on intracellular interactions and therefore it does not cover these topics. For a recent review of the subject, the reader is referred to the article by Katsura (130).

The studies described here address many questions that are of interest to the related disciplines of biochemistry and molecular biology, parasitology, and bacterial physiology. The following groups of questions address some of the highlights. (i) What types of phage and host products interact to determine the specificity of phage macromolecular syntheses? What are the mechanisms by which gene expression and DNA replication are regulated? (ii) How can hosts become refractory to virus infection? Do such mutants occur naturally? Is it possible to understand relationships among related strains by identifying changes at one or a few key loci? (iii) Do the loci identified by virtue of being important for phage λ also have an important role for the host? We

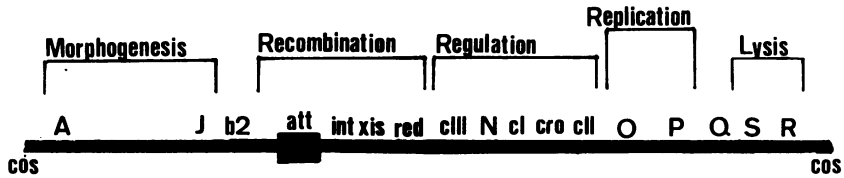

FIG. 1. Genetic map of λ. Identified are representative genes discussed in this review. The clustering of genes by function is indicated above the map. The map is not drawn to scale.

hope that we shall provide answers to some of these questions and provoke the reader into answering the others.

To give the reader a background sufficient to understand the studies that will be reviewed, a brief summary of the phage λ life cycle follows. More detailed discussions of the relevant λ and *E. coli* functions are found in the sections in which they are discussed.

The Life of λ

Lambda has a double-stranded DNA genome consisting of 48,514 base pairs (bp) (220). It is a temperate phage, which means that it can adopt either of two mutually exclusive life styles: lytic or lysogenic. The phage DNA is injected into the bacterium in a linear form and then circularizes by joining the cohesive single-stranded homologous ends (*cos*) followed by sealing by the host DNA ligase (277). In the lytic pathway, phage gene expression leads to the production of phage particles and lysis of the bacterium. In the lysogenic pathway, two separate events occur: the phage genome recombines with the bacterial chromosome to integrate the phage DNA into the host chromosome, and the phage produces a repressor that binds to phage DNA to shut off synthesis of most phage-encoded products. The integrated phage is called a prophage and the bacterium harboring it is a lysogen. The prophage is maintained by the bacterium in the quiescent state until an inducing event such as DNA damage occurs. Upon induction, repression is removed, the prophage is excised from the bacterial chromosome, and phage particles are produced as in a lytic infection. Below we discuss features of both the lytic and lysogenic processes and refer primarily to the appropriate chapters in the recent monograph on λ, *Lambda II* (109; see also references 111, 112, and 262).

Lambda Functions

The λ genome is organized in functional units (Fig. 1). We will base our preliminary discussion of λ on these units. The regulatory region includes both the genes involved in repression and the early regulatory signals. This region contains the early leftward and rightward promoters (p_L and p_R, respectively), which are recognized by host RNA polymerase (Fig. 2). Transcription from p_L and p_R stops at nearby termination sites, to yield mRNA for genes *N* and *cro*. N protein stimulates transcription of the delayed early genes flanking *N* and *cro* by antagonizing transcription termination, resulting in expression of genes including *c*III, *c*II, *O*, *P*, and *Q*. The interaction of N and host transcription machinery is discussed in depth in a following section. Transcription initiation at p_L and p_R is regulated by two repressors, coded by the *c*I and *cro* genes, which bind to operator sites (o_L and o_R) that overlap the corresponding promoters (105).

Downstream of *cro* are the delayed early genes, *c*II, *O*, *P*, and *Q* (53). The *c*II gene encodes a protein needed for initiation of *c*I synthesis. The *O* and *P* gene products are involved in DNA synthesis (67). Within the *O* gene is *ori*, the site at which DNA replication initiates. The *Q* gene codes for a second transcription antitermination function, which positively regulates expression of late genes (53, 90, 112). On the far right end of the genome are functions involved in lysis. The left side contains the genes for particle morphogenesis (head and tail assembly) (84, 130) and includes the functions for packaging phage DNA (45). The genes for the morphogenetic functions are transcribed from the same promoter as the genes for lysis; circularization of the genome results in these two sets of genes being contiguous.

Downstream from gene *N* in the p_L operon are delayed early genes that are not essential for normal phage growth (29). Several of these genes are involved in recombination, notably the *red* genes (whose products catalyze generalized recombination) and the *int* and *xis* genes (whose products catalyze site-specific recombination).

Lambda is one example of a group of related temperate phages, collectively called lambdoid phages, that can recombine with each other. In addition to λ, this class of phages includes 434, 21, 82, Φ80, and *Salmonella* phages such as

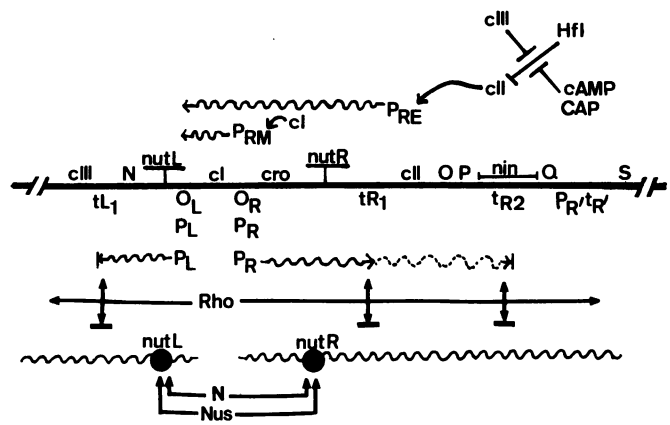

FIG. 2. Action of host factors in regulating expression of λ early and delayed early functions. Wavy lines indicate mRNA transcripts. The heavy line in the center is a genetic map of the early regulatory region (see Fig. 1) showing relevant markers. Shown above the map are the transcription patterns for *c*I (repressor) synthesis. At the top are shown phage and host factors involved in initiation of *c*I transcription. Directly below, features of the maintenance mode of *c*I transcription are shown. Shown below the map are the transcription patterns from the early promoters p_L and p_R, in the presence and absence of the λ *N* gene product. The vertical arrows indicate transcription termination signals overcome by the action of *gp*N. The *nut* sites where gp*N* and Nus proteins act are shown on the RNA based on the experiments discussed in the text.

TABLE 1. Glossary of *E. coli* genes that affect phage lambda

Transcription Termination

nus (N utilization substance): Class of mutants in which wild-type λ early gene expression is blocked and in which λ N-independent mutants can grow

nusA (min 69). Transcription termination factor, antitermination factor (?), [a] interacts with gp*N* and with host RNA polymerase, also called "L factor"

nusB (min 11). Also called *groNB*, transcription termination factor (?), antitermination factor (?), interacts with gp*N* (?)

rpoB (min 88). Beta subunit of RNA polymerase

Various effects on λ: (i) the *ron* mutation blocks growth of λ *mar* mutants; (ii) some alleles increase severity of block imposed by *nus* mutations; (iii) other alleles (also called *lycA*) support growth of N^- phages. A Nus phenotype due to a mutation of *rpoB* is termed NusC

rho (min 84). RNA release factor involved in transcription termination

Various effects on λ: (i) some alleles support growth of lambda N^- phages; (ii) other alleles block λ wild type. A Nus phenotype due to a mutation at *rho* is termed NusD

rpsJ (min 72). Encodes ribosomal protein S10; a Nus phenotype due to a mutation at *rpsJ* is termed NusE

DNA Replication

groP: Class of mutants in which wild-type λ DNA replication is blocked and in which λ mutants with alterations in their *P* genes can grow. Mutations conferring this phenotype occur in the following genes:

dnaB (min 92). Essential for host replication; part of "primosome"; encodes a DNA-dependent ATPase activity which is inhibited by P protein

dnaJ (min 0.3). Essential for host DNA replication and RNA transcription at high temperature (?); heat shock protein; synthesis induced by λ infection; membrane associated

dnaK (min 0.3). Essential for host DNA replication and RNA transcription at high temperature (?); possesses ATPase and autophosphorylating activities; heat shock protein, necessary to turn off heat shock response, homologous to eucaryotic Hsp70; synthesis induced by λ infection

grpD (min 72). Role in host replication unknown

grpE (min 56). Role in host replication unknown

Morphogenesis

groE: Class of mutants in which wild-type λ head morphogenesis is blocked and in which λ mutants with alterations in their *B* or *E* genes can grow

groEL (min 94). Also called *mopA*, codes for large *groE* gene product; a heat shock protein, forms decatetrameric structure, interacts with the GroES protein; possesses weak ATPase activity; synthesis induced by λ infection

groES(min 94). Also called *mopB*, codes for small *groE* gene product; a heat shock protein; interacts with GroEL protein

groEL and *groEs* are both required for λ head morphogenesis (assembly of the preconnector structure); major proteins of *E. coli*; required for host cell division (septation)?; required for host DNA and RNA synthesis at high temperature (?)

mat (min 76). Required for head and tail assembly (?); an allele of the *htpR* (heat shock activator) gene

himA and *hip-himD*. Required for DNA packaging of some lambdoid phages and phage mutants (see below)

Site-Specific Recombination

him (host integration mediator)

hip (host integration protein):

himA (min 28). Codes for alpha subunit of IHF (integration host factor); required for both Int synthesis and activity; required for growth of Mu and some lambdoid phages

himB (min 82). Mutations of *gyrB* (the beta subunit of DNA gyrase); required for Mu growth

himC (unmapped)

himD-hip (min 20). Alternate names for the beta subunit of IHF; required for growth of Mu and some lambdoid phages

Lysis-Lysogeny Decision

Mutations in these genes increase the efficiency of lysogenization:

hflA (min 95). High frequency of lysogeny; codes for at least two polypeptides (HflK and HflC); controls stability of *c*II; inhibited by *c*III (?)

hflB (min 69). Controls stability of *c*II; inhibited by *c*III(?)

Mutations in these genes decrease the efficiency of lysogenization:

cya (min 85). Codes for adenyl cyclase, which catalyzes cAMP synthesis

crp (min 74). Codes for cAMP binding protein (CAP); activator for family of genes coding for catabolic enzymes

himA Necessary for synthesis (transcription and/or translation?) of *c*II

hip-himD Necessary for synthesis (transcription and/or translation?) of *c*II

Prophage Induction

recA (min 58): Bifunctional protein required for recombination and for induction of proteolysis; involved in autoproteolytic activity of certain repressors (e.g., λ *c*I protein and host LexA protein); activity stimulated by the binding of single-stranded DNA and nucleotide

[a] (?) means that the point has not been established definitively.

phage P22. Although these phages share a nearly common gene order, many of their analogous gene products are specific. For instance, these phages all encode antitermination functions analogous to that of the λ N protein, coded by genes located in an analogous position on their maps. Except for the cases of λ and 434, however, these *N* gene products are specific for their own genomes. N. Franklin (personal communication) has determined the DNA sequence for the *N* genes of phages 21 and P22 as well as λ (50). Although the sequences show that these proteins are all small (around 100

amino acids), there is little homology among the three proteins. Similarly, although all of these phages have two genes encoding replication functions, located in positions analogous to the *O* and *P* replication genes of λ, the gene products are specific for the corresponding genomes.

Nomenclature

In this review, bacterial genes and their products are designated by using the standard convention: genes are described in lower case, as in *nus*; the corresponding product is capitalized, as in Nus. Lambda genes have been named primarily by letters, e.g., *A*, and its gene product, gp*A*. As an aid to the reader a glossary of key terms is provided in Table 1 and a map of the bacterial genes is shown in Fig. 3.

Our discussion focuses on phage-host interactions involved in transcription antitermination, DNA replication, prophage integration, the lysis-lysogeny decision, morphogenesis, and prophage induction.

TRANSCRIPTION ANTITERMINATION

Background

Gene expression of λ, like that of most other viruses, is temporally regulated. For λ, this regulation is accomplished primarily by a system of transcription termination-antitermination (53). Two λ gene products promote antitermination at the appropriate times to allow transcription into previously unexpressed regions of the genome. The product of the *N* gene, gp*N*, acts to permit transcription initiating at the early promoters, p_R and p_L, to overcome termination barriers. Similarly, the product of the *Q* gene, *gpQ*, permits transcription from the late promoter, $p_{R'}$, to overcome termination barriers. Our understanding of this type of gene regulation began with the studies of J. Roberts on the *E. coli* Rho transcription termination protein (206). Using Rho in an in vitro transcription system, Roberts identified the immediate-early transcripts of λ and inferred that gp*N* acts as an antiterminator (206).

After the discovery of this type of gene regulation in λ, transcription termination (in this case called "attenuation") was also found to play an important role in regulation of biosynthetic operons of bacteria (276). In the case of attenuation, the efficiency of transcription termination at a termination site located between the promoter and the first structural gene, of a biosynthetic operon such as *trp*, is governed by the efficiency with which the leader transcript is translated. It has also been proposed that the polar effect on downstream gene expression exerted by nonsense mutations and some insertion elements is due to the premature termination of translation which uncouples transcription and translation, leading to the uncovering of a termination signal (3, 34). In both cases a plausible model (3) has the trailing ribosomes interfering with the secondary structure of the newly synthesized mRNA, which ultimately perturbs the signal for transcription termination and chain release (198).

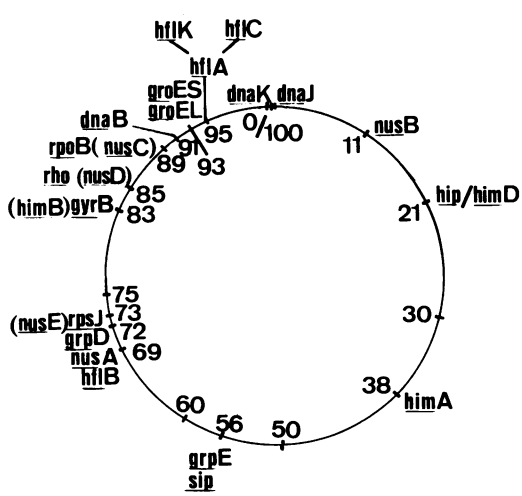

FIG. 3. Position on the *E. coli* genetic map of the various host genes whose products are utilized by λ. Location is based on the standard *E. coli* map of Bachmann (8).

The *N* gene encodes a 12,000-M_r protein and is located immediately downstream of the p_L promoter (50, 94). It is one of two gene products expressed immediately after infection by λ or upon induction of a λ prophage. The studies outlined below show that gp*N* acts as an antiterminator by a remarkable and unprecedented mechanism in which the properties of the transcription machinery become modified. When RNA polymerase, initiating at a promoter such as p_L or p_R, traverses a special N-recognition site (*nut*), gp*N* causes this enzyme to ignore most subsequent transcription termination sites. Thus the recognition sites for gp*N* (and the sites that determine the specificity of gp*N* action) are different from the sites of gp*N* action (4, 49, 63, 243). The recognition sites, *nut*, consist of regions of hyphenated dyad symmetry downstream from the early promoters p_L and p_R (see Fig. 2). The *nut*L site, downstream of p_L, was identified by isolation of *cis*-acting mutations that lead to a defect in ability of N to work on transcription initiating at p_L (219). The *nut*L mutations result in changes in the *nut* region of hyphenated dyad symmetry. A site downstream of p_R between *cro* and *c*II was identified as a possible *nut*R since it was identical to the *nut*L hyphenated dyad symmetry in 16 of 17 bp (212). Functional studies consistent with this assumption showed that a cloned fragment of DNA that includes this putative *nut*R sequence could produce termination-resistant transcription if it was placed between a non-λ promoter and a termination signal (36). In summary, RNA polymerase initiates transcription at the early λ promoters and travels to the *nut* sites where it is somehow modified by gp*N*, which results in subsequent downstream transcription being resistant to many termination signals.

Mutants that do not require N protein for growth have been invaluable in studies of the mechanism of gp*N* action. Such mutants, *nin*, N independent (30), or *byp* (23, 121), provide a means of defining the class of bacterial mutants that block phage growth by blocking gp*N* action. In particular, λ *nin* phages can grow on such mutants, whereas λ^+ phages cannot. As expected from our discussion of the mode of gp*N* action, λ *nin* mutants have changes that eliminate termination signals. Since all essential functions except for gp*N* are encoded in genes downstream from p_R, it is only necessary for terminators in this region to be altered to free the phage from N dependence. In point of fact, N independence occurs when one set of termination signals, the t_{R2} region, is eliminated as is the case with the λ *nin* deletions/substitutions (30) (see Fig. 2). Although there is another terminator upstream of t_{R2}, called t_{R1}, this terminator is weak and apparently there is sufficient transcription through this terminator in the absence of gp*N* to allow N-independent growth if t_{R2} is eliminated (27).

Host Factors

Genetic studies have demonstrated that, in addition to gp*N*, a number of *E. coli* products are required for efficient modification of transcription at the *nut* sites. Mutations in five loci of *E. coli* have been identified that reduce the bacterium's ability to support the gp*N* modification reaction (62). This is most commonly observed as a failure to support growth of λ under nonpermissive conditions, while supporting the growth of λ *nin* under the same conditions. Taking cognizance of this phenotype, the following nomenclature has been adopted. The previously unidentified loci are named *nus* for N-utilization substance, whereas mutations in known loci also will be identified by including in their names the phenotypic character, Nus. The five loci and their map positions (Fig. 3) are *nusA* (min 69), *nusB* (also called *groNB*; min 11), *rpoB* (NusC; min 88), *rho* (NusD; min 84), and *rpsJ* (NusE; min 72).

Although they map in different loci, many of the *nus* mutations cause very similar phenotypes. (i) The mutants fail to support gp*N* action rather than its synthesis. (ii) The mutations are recessive, indicating that they result in the loss of an activity necessary for gp*N* action. (iii) The mutants exhibit a temperature-sensitive block in λ growth. When hosts carrying the *nusA1*, *nusE71*, or *nusB5* mutations are used as plating lawns, λ forms plaques at 32°C but not at 42°C. This apparent temperature sensitivity might mean that either all of these Nus mutants have mutations that result in the synthesis of temperature-sensitive proteins or, more likely, some aspect of the termination or antitermination reaction requires more gp*N* activity at high temperature. These explanations are not mutually exclusive. Favoring the latter possibility are observations showing that Rho-dependent termination is more efficient at higher temperatures (33).

Although the *nus* mutants share a common phenotype with respect to λ growth, the roles of the Nus products in the host bacterium, as we will now discuss, are not all the same.

nusA. The *nusA* gene was first recognized by the identification and characterization of the *nusA1* mutation (52, 54, 56). Wild-type λ behaves as a λ*N*$^-$ mutant upon infection of a *nusA* mutant (52, 56). It was subsequently shown that *nusA* encodes a 69,000-M_r protein that binds to the N protein (93) and binds directly, with equimolar stoichiometry, to *E. coli* core RNA polymerase (92). Shimamoto and co-workers (229), using an in vitro rapid kinetic method for studying transcription, showed that NusA interacts with polymerase after σ is released. The uptake of NusA is therefore not a displacement of σ. Greenblatt (personal communication) has used photoaffinity cross-linking to demonstrate that NusA binds tightly to core polymerase but weakly to holoenzyme. He postulates that σ traps RNA polymerase in a conformation with a lower affinity for NusA. The complete NusA protein is not necessary for biological activity since an N-terminal 45,000-M_r NusA fragment complements NusA$^-$ defects (both for λ growth and for bacterial viability [see below]) (Y. Nakamura and S. Mizusawa, personal communication; R. Haber, S. Adhya, L. S. Baron, D. Kopecko, and D. I. Friedman, unpublished data).

In vitro experiments reveal a role for NusA in recognizing transcription termination signals. In particular, NusA protein (termed L protein at the time) stimulated β-galactosidase synthesis in a coupled transcription-translation system by allowing transcription to continue through the *lacZ* gene (95, 154). In a variety of transcriptional systems, NusA protein increases pausing and termination (44, 96, 137, 157, 223, 232). Recent studies by Lau et al. (158) demonstrate that NusA mediates pausing without termination in the t_{R1} region of λ. In vivo studies showing a reduction of both attenuation and polarity in the *trp* operon due to *nusA1* are consistent with NusA being a termination function (261). Although the isolation of conditional lethal mutations in the *nusA* gene (187; B. Bigelow, D. Carver, and D. Friedman, unpublished data) indicates that NusA mediates an essential function for the bacterium, it is not known if the essential activity lost under nonpermissive conditions is transcription termination.

The *nusA* gene from the closely related genus *Salmonella* can be substituted for that of *E. coli*. Hybrid bacteria have been constructed that carry mostly *E. coli* genetic information but have the *nusA* gene and surrounding genes from *S. typhimurium* (10, 54). Although this NusA protein performs the essential roles required of NusA for bacterial viability (see below), it cannot support the action of the λ gp*N*. However, it can support the action of the N analogs of lambdoid phages 21 and P22 (54).

Studies on λ have led to identification of a presumptive NusA recognition sequence (57, 195). This sequence, called *boxA*, was first identified as a sequence present in all of the lambdoid phages for which sequence information was available. It was always found promoter proximal to the putative *nut* region of dyad symmetry (see Fig. 2). For λ *nut*L and *nut*R, the sequence 5′CGCTCTTA3′ is found 8 and 9 bp, respectively, from the region of dyad symmetry. Examination of the nucleotide sequences from 21 and P22, which have their own unique N products and putative *nut* sites, leads to a canonical *boxA* sequence of 5′C/TGCTCTT(T)A3′.

Two mutations in the λ *boxA* sequence of the *nutR* region support the contention that *boxA* is required for N action and is important for NusA action. The first mutant, *boxA5*, contains a *boxA* region synthesized in vitro. It differs from wild type in a single position (a G-to-T change at the second position) and results in the sequence 5′CTCTCTTA3′. In contrast to wild-type *boxA*, *boxA5* does not allow N action (E. R. Olson, C. C. Tomich, and D. I. Friedman, J. Mol. Biol., in press). Evidence for a role for *boxA* in NusA action came from studies of a second mutation in *boxA*, *boxA1*. Ordinarily λ cannot use the NusA protein of *Salmonella*, whereas P22 and 21 can (54). Correlating with this functional difference is a structural difference: the *boxA* sequences of both P22 and 21 have TTT at their 3′ ends, whereas λ has TT. The *boxA1* mutation is an A:T to T:A transversion that results in the *boxA* sequence of the *nut*R region having a TTT sequence at its 3′ terminus. This change (along with certain other mutations in *N* that are not important for this discussion) allows λ to use the *Salmonella* NusA product. This result suggests that NusA requires a specific *boxA* sequence for N to function.

Another set of mutations in this region led to the conclusion that the *nut* mRNA per se plays a significant role in the N-modification reaction. Mutations originally called *nut*R (e.g., *nut*R62) were isolated that interfered with the N-modification reaction at *nut*R (195). Although these mutations mapped in the *nut*R region, DNA sequence analysis showed them all to be single-base-pair deletions located slightly upstream of the *nut* site in the *cro* gene. The mutations have been renamed *cro*Δ (e.g., *nut*R62 is now called *cro*Δ62) to reflect their structure and true location (see Fig. 2). The deletions are in a run of A's resulting in a change in the reading frame of *cro* so that translation of *cro* extends four bases into the *nut*R region. When translation terminates upstream of the *cro*Δ62 mutation, there is no longer a Nut$^-$

phenotype (Olson et al., in press). This argues that it is the extension of translation past the normal *cro* termination signal that is responsible for the Nut$^-$ phenotype of *cro*Δ62. That a ribosome extending into the *nut* region can interfere with the N-modification reaction suggests, first, that a sequence adjacent to *cro* is important in the modification reaction and, second, that at least part of the interaction among Nus proteins, gp*N*, and nucleic acid must occur at the level of the RNA. Specifically, it has been further suggested that the shift of the terminating ribosome four bases into the *nut*R region occludes a sequence in the RNA required to bind one or more of the proteins involved in N modification. That *boxA* is only seven bases downstream from the site of translation termination has led to the suggestion that the shifted ribosome interferes with a NusA-*boxA* (RNA) interaction (Olson et al., in press).

In vitro experiments examining the effect of NusA on transcription initiating at the λp_R promoter offer further proof that the *boxA* sequence plays a role in NusA action. Addition of NusA protein inhibits termination at the first of three sites of transcription termination identified by these experiments in the t_{R1} region (157). Using linker scanning mutations, Lau and Roberts (L. F. Lau and J. W. Roberts, J. Biol. Chem., in press) demonstrated that elimination of the *boxA* sequence eliminates this antitermination activity of NusA.

Other in vitro transcription experiments argue against the role of a specific sequence in NusA activity. Schmidt and Chamberlin (223) showed that NusA protein inhibits RNA chain elongation with a variety of templates. Since NusA inhibition is observed when the template is polydeoxyadenylate-polydeoxythymidylate, it is obvious that in this system there can be no *boxA* sequence. Moreover, these authors present evidence that the interaction of NusA with elongating RNA polymerase is not processive, but rather shows a pattern of association and dissociation. Greenblatt (personal communication) observed that NusA protein imposes pausing at the λt_{R2} termination region if added any time before polymerase reaches that site. Thus, the *boxA* sequence in *nutR* cannot be responsible for this activity of NusA.

The *nusA* gene and surrounding genetic material have been cloned (119, 156, 199; L. Baron and D. Kopecko, personal communication; S. Adhya, R. Haber, and J. Levin, personal communication) and the DNA sequence has been determined (124). Analysis of this sequence suggests that the *nusA* gene is in an operon that includes at least four genes, with the following order: a minor Met-tRNA, an open reading frame coding for a 15,000-M_r protein, *nusA*, and an open reading frame coding for a third protein. The end of the operon has not been determined.

In vitro studies show that transcription of this operon is repressed by cyclic AMP (cAMP) and NusA (S. Ishii and F. Imamoto, personal communication). One possible consequence, if this type of regulation is operative in vivo, is that NusA-directed transcription termination might be reduced when catabolite repression is removed. NusA might also negatively regulate its own synthesis. Indeed, the presence of a high-copy-number plasmid with a cloned expressed *nusA* gene reduces expression of the chromosomal *nusA* operon with a Mu d(*lac* Ap^r) insertion in *nusA* (D. Friedman and L. Eades, unpublished data).

We terminate this discussion by noting the widespread occurrence of *boxA*-like sequences and speculating about the significance of that fact.

Lambda has a second antitermination function, gp*Q*, that acts in a manner similar to gp*N* (32, 48, 113, 207). The *Q* gene lies downstream of t_{R2} (see Fig. 2) and is responsible for the turn-on of late gene expression. gp*Q* acts at a site adjacent to the *Q* gene to antiterminate transcription initiating at the p_R promoter. In vitro studies show that gp*Q*-mediated antitermination is enhanced 10-fold by NusA (90). Examination of the nucleic acid sequence in the region where gp*Q* is thought to modify RNA polymerase reveals a sequence that resembles the canonical *boxA* sequence (195); in this case the sequence is 5'CGCTCGTT3'. Functional studies show that deletion of this sequence reduces gp*Q* antitermination (J. Roberts, personal communication).

Sequences similar to *boxA* are found in *E. coli* operons where NusA is known to mediate in vitro transcription termination. These included *trp* terminators, t-5'CGCAGTTA3' and t'-5'CGCGCTTA3' (270). Upstream of a NusA-stimulated termination site within the leader region of the *rrnB* operon is the sequence 5'TGCTCTTTA3' (20, 137). Moreover, transcription initiating at the *rrnB* promoters becomes termination resistant, and some of the mutations interfering with this antitermination activity have been shown to alter the *boxA* sequence (M. Cashel, personal communication). Thus, the 5' three bases, C/TGC, and a 3' run of two or three T's with a spacer of two bases make up the consensus *boxA* sequence, 5'C/TGCNNTT(T)3'. In many cases an A follows the terminal run of T's.

Examination of sequences at 3' ends of mRNAs from a variety of species reveals that in many cases *boxA*-like sequences are located at these ends. We offer three examples. (i) Birchmeier et al. (17) have derived a consensus sequence at the authentic 3' end of a large number of histone mRNAs from a variety of species. This consensus includes the sequence 5'CGGC(T/C)CTTT3', located 12 bp from the 3' terminus. (ii) The mRNA for maize sucrose synthetase has the sequence 5'TGCTCGTT 10 bp from the end of the mRNA (226). (iii) Approximately 9 bp upstream of the site for termination of the yeast *CYC1* gene transcript is the sequence 5'CGTTATTTA3' (280). Deletions that include this sequence also read through the usual site of termination. We speculate that *boxA* might be a sequence conserved through evolution that has maintained its function as a signal for ending the message. This signal might be involved either in true transcription termination or in signaling 3' message processing.

***nusB*.** The most common class of *nus* mutations maps at min 11 of the *E. coli* chromosome, a locus called *nusB* (also referred to as *groNB*) (55, 83, 134). The *nusB* gene encodes a 14,500-M_r protein (237, 242). The isolation of cold-sensitive (*cs*) mutations in *nusB* shows that the NusB protein is essential for bacterial growth (83).

There is evidence independent of studies with λ indicating that NusB is involved in transcription termination. Bacteria carrying the *nusB5* mutation show partial relief of nonsense polarity (261), a phenomenon thought to be caused by premature transcription termination (3). In vitro studies indicate that termination of at least one λ transcript depends on NusB (A. Das, personal communication). However, other in vitro studies are contradictory, suggesting that NusB encodes an antitermination function (J. Greenblatt; F. Imamoto; J. Swindle; M. Gottesman and M. Cashel, personal communications).

The NusB protein has been purified from a host carrying the cloned *nusB* gene on a "runaway" plasmid or under p_L control (J. Swindle and C. Georgopoulos, unpublished data; F. Imamoto, personal communication). Its N-terminal sequence, as well as the overall amino acid composition,

corresponds to those predicted by the DNA sequence (123a, 241a). Analysis of the DNA sequence shows that NusB is a protein of 139 amino acids with an M_r of 15,689, consistent with the size obtained from analysis of the protein discussed above.

***rpoB*-NusC.** A number of mutations exhibiting a variety of effects on gp*N* action have been mapped to the *rpoB* gene, which encodes the β subunit of RNA polymerase. One mutation in *rpoB*, *ron*, specifically blocks growth of λ derivatives with certain mutations in the *N* gene ["*mar*" mutations (87)]. The *nusC60* mutant has at least two mutations mapping either in or very close to *rpoB* that confer a very strong Nus phenotype: λ fails to grow in this host at either low or high temperatures (61, 62). Sternberg (236) has isolated *rpoB* mutations that delay production of λ.

Other *rpoB* mutations, *snu* (11), are known that have no observable effects on λ growth unless coupled to certain *nus* mutations (e. g., *nusA1*, *nusB5*, *nusE71*). The resulting double mutants are far more restrictive for λ growth than the *nus* mutants by themselves (11; A. Schauer, E. Olson, and D. Friedman, unpublished data).

A third class of mutations in *rpoB*, represented by the *lycA* mutation, have been isolated. LycA$^-$ hosts support growth of λ derivatives that express little or no gp*N* activity (159). Although a definitive basis for this phenotype is unknown, Greenblatt (91) suggests that it might result from an apparent defect in the ability of the LycA RNA polymerase to recognize pause signals.

***rho*-NusD.** Two types of mutations in the *rho* gene influence gp*N* activity. The first results in a partial Nus$^-$ phenotype and the second permits partial relief of termination at Rho-dependent terminators.

The HDF026 mutant of *E. coli*, originally isolated because it failed to support growth of phage T4, also exhibits a partial Nus phenotype (231). The responsible mutation maps in the *rho* gene. Bacteria with the HDF026 mutation are hyperdegradative for abnormal proteins. The Nus phenotype of HDF026 is only apparent when growth of a λ variant requiring higher than normal levels of gp*N*, e.g., λ32, is tested. Wild-type λ grows at both 32 and 42°C, but λ32 fails to grow in HDF026 hosts at both temperatures. Degradation of the *N* gene product is not the cause of the Nus phenotype because the half-life of gp*N* in an HDF026 host is identical to that observed in a wild-type host (89). Experiments by Das and co-workers (35) indicate that the HDF026 mutation specifically interferes with gp*N* action at a Rho-dependent terminator, t_{L1}, but not at an adjacent Rho-independent terminator, t_{L2}. Thus, gp*N* can act in the HDF026 host, but the mutant Rho protein must be resistant to gp*N* action.

Many *rho* mutants allow N-independent expression of phage genes, the Sun phenotype (suppressor of N [21]). In these hosts, significant transcription initiating from the early λ promoters overcomes termination barriers in the absence of gp*N* (33, 123, 204). Since the early λ terminators are Rho dependent, it was not surprising that such hosts would allow N-independent transcription through termination signals.

***rpsJ*-NusE.** The *nusE71* mutation maps in the *rpsJ* gene (min 72) encoding ribosomal protein S10 (61). Two-dimensional gel electrophoresis of ribosomal proteins revealed that the *nusE71* mutation causes a shift in the electrophoretic mobility of ribosomal protein S10. That a change in a ribosomal protein results in a Nus phenotype suggests a role for the ribosome in gp*N* action. However, a role for some free ribosomal proteins or the 30S subunit is not ruled out. If the complete ribosome is involved in the action of gp*N* at *nut*, then the question arises as to how the ribosome gets to the appropriate site. One possibility is that ribosomes are delivered to the *nut* region after termination of translation of the adjacent *cro* gene. Two lines of evidence argue against such a model of ribosome presentation. First, there is no translation of the message upstream of *nut*L (50) and it would be expected that *nut*R and *nut*L function similarly. Second, stopping translation in *cro* does not influence the action of gp*N* in rendering subsequent transcription termination resistant (Olson et al., in press; F. Warren and A. Das, Proc. Natl. Acad. Sci. U.S.A., in press; C. Debouck and M. Rosenberg, personal communication). If the ribosome plays a role in gp*N* action, it must enter the complex by some mechanism other than being presented by the termination of translation of an upstream gene.

A genetic approach has been used to address the question of the role of S10 in the action of gp*N*. Second-site mutations that suppress the *nusE71* defect, yielding a Nus$^+$ phenotype in the presence of the *nusE71* mutation, have been selected. One of these mutations has been mapped to the region of the S10 operon (A. Schauer and D. Friedman, unpublished data). Moreover, a high-copy-number plasmid with genes cloned from the S10 operon (coding for ribosomal proteins S17, L29, and L16) reverses the suppression, making the bacterium Nus$^-$. It has not been determined which of these ribosomal proteins is responsible for the complementation. The suppressor does not influence the phenotype of a *nusA1* mutant and only slightly suppresses a *nusB5* mutant. That a change in one ribosomal protein specifically suppresses the Nus$^-$ character imposed by a change in another, S10, suggests that the two proteins interact in supporting gp*N* action. One obvious way in which ribosomal proteins can physically interact is on the ribosome. Although in no way can this be taken as a definitive proof, the identification of this *nusE71* suppressor lends support to the argument that there is a role for the ribosome or one of its subunits in the action of gp*N*.

In vitro coupled transcription-translation systems have been used to directly assess the role of NusE in gp*N* action by looking for functions that complement a *nusE* extract for gp*N* antitermination activity. As discussed below, Das and Wolska (A. Das and K. Wolska, Cell, in press) have presented evidence showing that factors both tightly associated with the ribosome and in the S100 ribosome-free supernatant can complement *nus* mutant extracts, including *nusE*. R. Horwitz and J. Greenblatt (personal communication) have purified a complementing activity from the postribosomal supernatant of *nusE*$^+$ extracts. This activity has been purified about 200-fold. Curiously, it has a molecular weight of 25,000, whereas ribosomal protein S10 has a molecular weight of approximately 12,500 (267). The nature of the complementing protein is not known. However, purified S10 will complement a *nusE* defect (A. Das and B. Ghosh, personal communication).

Combination of *nus* mutations. Multiple mutants carrying combinations of *nus* mutations have been constructed. The *nusA1 nusB5*, *nusA1 nusE71*, and *nusE71 nusB5* double mutants are all more restrictive for λ growth than any of the mutants with the corresponding single *nus* mutations. The *nusA1 nusB5 nusE71* triple mutant has been constructed, demonstrating that the presence of all three mutations does not interfere with cell viability (62). However, the triple mutant is more restrictive for λ growth than any of the double mutants. One of the first Nus type of mutants isolated, *groN785* (75, 76), has recently been shown to contain mutations in three genes contributing to the Nus$^-$ phenotype: *nusA*, *rpoB*, and *rpsJ*-NusE (C. Georgopoulos, unpublished data).

Interaction Between gpN and Nus Products

Biochemical and genetic studies suggest that there are interactions between some Nus proteins and gpN. As noted earlier, Greenblatt and Li (93, 94) showed that NusA protein binds to gpN. These same studies showed that a second protein, of 25,000 M_r, also binds to gpN but has not yet been shown to be the product of a known gene. Consistent with these biochemical studies are genetic studies showing that mutations in *N*, *punA* (60–62; Franklin, personal communication), can be isolated that enable λ to grow in the *nusA1* mutant host under nonpermissive conditions. Although the *punA* mutations do not permit λ to grow in NusC$^-$ or NusB$^-$ hosts, they allow growth in the *nusE71* host (61; A. Schauer, personal communication). *punA* mutations do not free λ from the need for NusA activity since the *punA* phage does not grow in a hybrid *E. coli* with the *nusA* gene of *Salmonella*. Additional mutations in *N*, *N**, can be isolated that permit λ to utilize the NusA from *Salmonella* (57). Genetic studies (60) and DNA sequence analysis (Franklin, personal communication; Schauer and Friedman, unpublished data) place the *punA* mutations within the *N* structural gene rather than in the regulatory region preceding the *N* gene. It is thus unlikely that these mutations influence the amount of gpN synthesized. Rather, they might result in a more stable N protein or an N protein with increased affinity for either a Nus protein or the *nut* region. All *punA* mutations have been found to change any of 3 amino acids in a run of 10 amino acids and all increase the net charge of the N protein (Franklin, personal communication). This has led Franklin (personal communication) to suggest that *punA* changes may allow effective N action with the *nusA1* gene product by compensating for a decrease in acidity of the NusA1 protein. Thus, the more highly charged *punA* products could effectively interact with the less acidic *nusA1* protein. In considering this specific model, it should be kept in mind that the *punA* mutations also suppress the effect of the *nusE71* mutation, calling into question the idea of such a NusA-specific interaction.

The DNA sequence of two independently isolated *punA* mutations reveals that, although they affect different base pairs, they both cause a change from a serine to an arginine in amino acid 50 of gpN (Franklin, personal communication). Another mutation resulting in a change from a lysine to an arginine at amino acid 45 is necessary for λ gpN to be active with NusA of *Salmonella* (Schauer and Friedman, unpublished data).

The isolation of *nusB* mutations that suppress the effects of the *nusA1* mutation has led to the suggestion that gpN and NusB interact (260). The unique feature of these mutations is their specificity: the *nusB101* allele allows gpN_λ but not gpN_{21} to be active in the presence of the *nusA1* mutation. Another mutation, *nusB102*, acts in the opposite manner, allowing gpN_{21} but not gpN_λ to be active in the presence of the *nusA1* mutation. Such specificity is most easily explained by a gpN-NusB interaction.

Das and Wolska (in press) have studied the role of Nus factors in gpN action, using an in vitro-coupled transcription-translation system in which *galK* expression depends on antitermination by gpN. By using S30 extracts from *nusA*, *nusB*, and *nusE* mutants, it was shown that extracts from each *nus* mutant, in the presence of gpN, complemented extracts made from the other two mutants for *galK* expression. This confirms that all three Nus functions are required for the action of gpN. Moreover, the complementing activities are found both associated with ribosomes and in the S100 ribosome-free fraction. Das and co-workers (in press and personal communication) have shown that purified NusA, NusB, and NusE (S10) proteins will complement extracts from *nusA* and *nusB* mutants, respectively. These experiments prove the direct interaction of these proteins in gpN action.

Host Factors and Models of N Action

Although the studies discussed here do not lead to a definitive model of N action and interaction with Nus products, they do permit an assessment of the extant models. Two basic models have been proposed: (i) a negative model, in which gpN antagonizes the action of Nus factors; and (ii) a positive model, in which gpN action requires a Nus product(s). Formally, these models are distinguishable in the following way: in a host totally defective in Nus activity, the first model predicts that both wild-type and λ N^- phage should grow equally well, whereas the second model predicts that under these conditions neither phage should grow.

Nakamura and Uchida (187) have proposed that gpN inhibits the action of Nus factors. They found that reducing the levels of functional NusA leads to a reduction in the amount of functional gpN required for λ growth, suggesting that the proteins act in opposition. The bacterial mutants, however, were selected for their ability to support the growth of λ *Nam* mutants (the N^- mutants used in the experiment) so their existence cannot be construed to support the model.

Experiments with hybrid *E. coli* with the *nusA* gene of *Salmonella*, which address the formal means of distinguishing the two models proposed above, favor the alternative model. Remembering that NusA-*E. coli* supports gpN_λ action and that NusA-*Salmonella* does not support this N activity, we examine the results of diploid experiments. If NusA-*Salmonella* cannot be opposed by gpN_λ action, then the *Salmonella nusA* should be dominant in diploid strains. This is not the case: in diploids with *nusA* genes from *E. coli* and *S. typhimurium*, gpN_λ is active as an antitermination function, whereas in hosts with only the *nusA-Salmonella* gene, gpN_λ cannot antiterminate (54). This dominance pattern argues that the NusA of *E. coli* is the active gene product. Therefore, in the diploid, the NusA of *E. coli* supports antitermination rather than the NusA of *Salmonella* acting to terminate transcription. A further argument against an antagonism between Nus and gpN derives from comparing the growth characteristics of λ and P22. As described above, the *boxA*1 mutation, which makes the λ *boxA* sequence identical to the P22 *boxA*, allows λ to grow in *E. coli* strains with the *Salmonella nusA* gene. Since this sequence is thought to be a NusA recognition site, it can be argued that functional interaction between NusA and gpN is required for gpN action. If gpN served only to oppose NusA-mediated termination, the lack of a functional NusA binding site would make gpN unnecessary.

Therefore, we favor the second type of model. We summarize the essential elements of this model. gpN, along with Nus factors, alters the RNA polymerase at the *nut* site to render the transcription machinery termination resistant. This might occur by the addition of one or more proteins onto the RNA polymerase. A detailed model of how all of these factors interact has been proposed by Greenblatt (91).

Identification of the *nusE71* mutation in the *rpsJ* gene led to the suggestion that the N-modification process might involve a tight coupling between RNA polymerase and a ribosome. Subsequent genetic and biochemical experiments discussed above lend further support to this idea. How the

ribosome might become associated with RNA polymerase at the *nut* site is not apparent. As argued before, it is unlikely that the ribosome is presented by translation from an upstream gene. Perhaps the ribosome is captured in an unusual way, unrelated to the normal translation process. For instance, there are translation initiation codons about 20 bp downstream of each *nut* stem-loop structure in a 7-bp conserved sequence called *boxC* by Friedman and Gottesman (53). Regardless of whether the ribosome itself is involved, we feel that there is strong evidence favoring the idea that there is concerted action of a number of factors at *nut* to alter the polymerase, creating a termination-resistant complex.

We leave the subject of N-Nus interactions by looking at the question of whether gp*N* can influence *E. coli* gene expression. Nakamura and Yura (188) have reported increased levels of the sigma subunit of RNA polymerase in the presence of gp*N*. The *rpoD* gene (encoding sigma) is in an operon with a gene order of *rpsU dnaG rpoD* (22, 166). A transcription terminator site has been identified downstream of *rpsU*. Analysis of the sequence of *rpsU* revealed a nut_{λ} stem-loop within the gene (166). Seven bases upstream is the sequence 5'CGTCGCTTC3', a reasonable approximation of a *boxA* sequence, and 20 bases downstream is a reasonable *boxC* sequence. Study of N-controlled sigma synthesis in a *nusA1* host will indicate whether this *boxA* sequence plays any biological role.

DNA REPLICATION

Background

For successful λ DNA replication to take place in vivo, the following four requirements must be met. (i) Initiation of DNA synthesis requires a functional, *cis*-acting site, termed *ori*, which is located in a region approximately 80% from the left end of the λ chromosome (39). The *ori* sequence is different in the various lambdoid phages analyzed so far, but some of its features have been conserved. These are tandemly arranged 19-bp repeats separated by a few bases, with four repeats in phages λ and φ80 and five repeats in phage 82. Mutations causing inability to replicate, called ori^-, map in this region. For more details, see the review on λ DNA replication by Furth and Wickner (67). (ii) Active forms of the phage *O* (34,000 M_r) and *P* (26,000 M_r) gene products must be present. The O protein has been shown to bind to the tandem 19-bp repeats in the *ori* region, which are located within the *O* coding sequence (254). Studies with hybrid *O* genes have shown that the specificity of interaction with *ori* resides in the amino-terminal portion of the gp*O* (68), whereas the carboxyl-terminal portion of the protein is responsible for the specificity of interaction with gp*P* (66). The isolation of suppressors of an O^{ts} mutation that had alterations in the *P* gene suggested that gp*O* and gp*P* interact in vivo (253). Recent studies offer evidence that purified gp*O* and gp*P* interact in vitro since they cosediment in metrizamide-sucrose gradients (281b). (iii) Transcription originating at p_R (38) or even downstream from the *ori* region (65) is required to activate phage DNA replication. This transcriptional activation is necessary even when excess gp*O* and gp*P* are supplied in *trans*. (iv) Functional forms of the majority of the *E. coli* proteins necessary for host DNA replication must be present. These include at least the *dnaB* and *dnaG* (primase) gene products, which are essential for the formation of the RNA primer for DNA synthesis, and the *dnaE*, *dnaX*, *dnaQ*, and *dnaZ* gene products, which code for some of the subunits of DNA polymerase III holoenzyme. Other gene products are also involved (see below; 67, 147, 148).

The process of λ DNA replication is marked by distinct stages. A few minutes after infection, λ DNA replication is initiated at *ori*, provided that the above four requirements have been met. Replication has been shown to proceed bidirectionally resulting in the generation of characteristic theta forms (224). The early mode of λ DNA replication results primarily in the formation of unit-length, covalently closed circles. Recently three different laboratories have reported successful in vitro DNA replication of a plasmid, λ *dv*, that contains λ *ori* as the only origin of replication. The reaction requires concentrated *E. coli* extracts from uninfected cells supplemented with purified O and P proteins (6, 255, 268). The O protein is extremely unstable in vivo, whereas the P protein is stable (162), suggesting that the rate of λ DNA replication may be governed by the functional intracellular level of gp*O*. Later in infection, large concatemeric DNA molecules accumulate, even under recombination-deficient conditions, suggesting that replication shifts to a rolling-circle mechanism (although some rolling-circle-type replication may also occur early in infection [16]). Evidence has been presented that both the O and P proteins are essential for the late mode of DNA replication in addition to their roles in early replication (140). The concatemers are the natural substrates for encapsidation into mature proheads (45).

Host Factors

The identification of *E. coli* proteins involved in λ replication has been facilitated by the isolation of bacterial mutants that fail to support the phage-directed replication. Although two different selection strategies were used (80, 217, 241), in both cases similar types of mutants were isolated.

Mutations in three bacterial loci (see Fig. 3) whose products are essential for λ replication were isolated by the same selection procedure (75). These mutants were called *groP* because the block to λ replication could be overcome by phage mutations (called π) that map in the *P* gene (see below) (80).

dnaB. The majority of the original *groP* mutants, about 30 isolates, carried mutations in the *dnaB* gene as judged by the following criteria. First, they mapped at min 92 on the *E. coli* genetic map (80). Second, the defects were complemented by λ $dnaB^+$ transducing phages (86). Third, some previously isolated *dnaB* mutants exhibited GroP phenotypes (80).

Studies with *groP* and two classes of λ mutants, πA and πB, led to a division of *groP* mutations into two phenotypic classes, GroPA and GroPB (80). The πB mutations permit the phage to plate on all $groP^-$ hosts, whereas the πA mutations permit the phage to plate only on $groPA^-$ hosts. Since the π mutations allow λ to bypass the GroP block, it was proposed that the DnaB and P proteins interact (80). That the classification of *groP* mutations into two classes indeed has biological significance and does not represent a peculiarity of λ physiology was demonstrated by studies with φX174. These studies showed that extracts from $groPB^-$ cells do not support φX174 DNA replication, whereas extracts from *groPA* mutants do (103). This observation probably correlates with the presence of only low amounts of DnaB protein in *groPB* as opposed to normal amounts in *groPA* mutant extracts (103). In the presence of ATP or ADP, however, the labile mutant *groPB* gene products can be stabilized (104).

The DnaB protein has been shown to possess a DNA-dependent ATPase activity and to exist as a hexamer of ~300,000 M_r. This protein is part of a mobile multienzyme

complex, the primosome, that helps to initiate the RNA primer synthesis necessary for lagging-strand DNA replication (147, 148). The λ P protein may confer phage specificity for initiation of replication by the primosome in the same manner that the *E. coli* DnaC protein confers specificity for initiation of bacterial replication. In vitro studies show that both the DnaC and P proteins interact with the DnaB protein and inhibit its ATPase activity (139, 253a, 264). Thus, because it binds both proteins λ O and *E. coli* DnaB, the λ P protein directs the *E. coli* DNA replication machinery to the λ *ori* site. Moreover, by competing with DnaC protein for binding to DnaB protein, the λ P protein assures preferential replication of λ DNA over host DNA sequences.

***dnaJ* and *dnaK*.** Two mutations, *groP*C756 (77, 82) and *groP*C259 (241, 278), confer a GroP phenotype and do not map at the *dnaB* locus; they map at 0.3 min (see Fig. 3). Saito and Uchida (217, 218) demonstrated that both mutations affected *E. coli* DNA metabolism. Further experiments showed that the mutations define separate complementation groups (278). The *groP*C756 mutation identified the *dnaK* gene (217), and the *groP*C259 mutation identified the *dnaJ* gene (218). These two genes constitute an operon with the gene order promoter-*dnaK-dnaJ* (218).

The DnaK and DnaJ proteins belong to a set of *E. coli* proteins whose rate of synthesis is transiently increased after a shift to higher temperatures and are called the heat shock proteins (85; M. Zylicz and C. Georgopoulos, unpublished data). The 15 identified heat shock proteins compose a regulon that is controlled by *E. coli* through the action of the *htpR* gene product (192). In addition to DnaK and DnaJ, this regulon includes two other proteins (GroEL and GroES) that are necessary for λ growth (for head assembly, see below). Drahos and Hendrix (40) have found that the synthesis of the DnaK and GroEL proteins, both of which are required for phage growth, is elevated after λ infection. Deletion analysis suggests that the products of either or both the λ *ssb* and *ral* genes (which are located just to the left of *N*) are involved in the turn-on of these heat shock proteins, although the experiments by Kochan and Murialdo (145) suggest that the products of other genes may be involved.

The DnaK protein appears to modulate the heat shock response, since bacteria with the *dnaK756* mutation are unable to turn off the heat shock response of *E. coli* at nonpermissive temperature (250). This regulatory role for the DnaK protein is supported by the observation that bacteria that overproduce DnaK protein at low temperature show a diminished heat shock response when shifted to high temperature (250).

The DnaK protein exhibits remarkable conservation throughout evolution, from *E. coli* to humans. The *E. coli* DnaK protein is 48% identical at the amino acid sequence level with the *Drosophila* heat shock protein, Hsp70 (9). Immunological cross-reactivity has been found between *Drosophila* Hsp70 and the analogous protein in other organisms, such as humans and yeasts (132). Studies with amino acid analogs have implicated Hsp70 as a negative regulator of the heat shock response in *Drosophila* (37), as is the case with DnaK in *E. coli* (250). Whether the DnaK and Hsp70 proteins share other functions remains to be determined. A further interesting similarity between procaryotic and eucaryotic Hsp70 proteins has been the recent discovery that the proteins are phosphorylated at threonine residues in both *Dictyostelium discoideum* (165) and *E. coli* (282). In the case of DnaK protein, it appears to be due to an autophosphorylation reaction (282).

The complex phenotype exhibited by *dnaK756* bacteria suggests a prominent role for the DnaK protein in various *E. coli* regulatory circuits. Through its ATPase activity and capacity to phosphorylate itself (282) and potentially other *E. coli* proteins, and DnaK protein could control the levels of various intracellular activities. It may be relevant that temperature-sensitive alleles of *dnaK* result in altered ATP pools at the nonpermissive temperature (258).

The DnaK (75,000 M_r) and DnaJ (38,000 M_r) proteins have recently been purified to homogeneity (281a, 282). The DnaJ protein has been localized in the membrane fraction of *E. coli*, whereas DnaK is found in the cytoplasm and the membrane. Both proteins have been shown to be active in an in vitro λ*dv* replication system. In this system (268), crude extracts from either the *dnaK756* or *dnaJ259* mutant do not support the replication of λ*dv* but can be complemented by the addition of the corresponding purified protein (281a; unpublished data). Recently it has been shown in a purified system that the O and P proteins, in conjunction with *E. coli* DnaB, DnaJ, DnaK, Ssb, and DnaG proteins and DNA polymerase III holoenzyme, convert M13 single-stranded DNA to a double-stranded form (J. LeBowitz, M. Zylicz, C. Georgopoulos, and R. McMacken, unpublished data). The DnaJ and DnaK proteins appear to act at a very early step in DNA replication, preceding the formation of the primer RNA molecule.

***grpD* and *grpE*.** Saito and Uchida (217) described the isolation of two additional *E. coli* mutants that fail to support λ DNA replication. Mapping of the responsible mutations identified two loci, *grpD* and *grpE*, each represented by only a single mutation. Since mutations in λ that allow phage growth in either of these bacterial strains map in the *P* gene, it was concluded that the *grpD* and *grpE* gene products influence the action of the P protein. The *grpE* locus has been mapped at min 56 and is closely linked to *tyrA* (216). A transducing phage, λ *grpE*$^+$, was isolated from an *E. coli* library as a plaque former on the *grpE280* mutant strain. Deletion analysis of this transducing phage indicated that the *grpE* gene codes for a 24,000-M_r weakly acidic protein (216). Interestingly, the genes coding for the *rplP* and *rplS* ribosomal proteins are also carried on the λ *grpE* transducing phage (216). The product of another gene, *sip*, involved in growth of lambdoid phage λ *imm*P22 is also closely linked to but easily separable from *grpE* (M. Strauch, M. Baumann, L. S. Baron, and D. I. Friedman, unpublished data).

The *grpD* locus maps at 72 min and is cotransducible with *aroE* (217). The *grpD* gene product has not yet been identified. Also, it is not known whether the *grpD* and *grpE* gene products are necessary for *E. coli* viability since the only known mutations do not affect bacterial growth (216, 217).

Phage Mutants

It is possible (at frequencies of 10^{-8} to 10^{-5}) to isolate λ mutants able to overcome the blocks exerted by the various bacterial mutants affecting λ DNA replication (80, 217, 241). As already discussed, the mutations in these phages, called π, were mapped to the *P* gene by marker rescue experiments with defective deleted prophages (80). Some allele-specific mutants were isolated, able to grow only on the host on which they were isolated (77, 241; Georgopoulos, unpublished data). This allele specificity can be simply explained as the result of alteration of a protein-protein interaction between the P protein and the host proteins. A more surprising mutant, λπB1, has a mutant *P* gene that permits the phage to propagate on all *groP*$^-$ hosts. The apparent lack of allele specificity could be due to an alteration in the P protein making it independent of all host factors. The λπB1

phage, however, does not grow on *dnaB*, *dnaJ*, or *dnaK* bacteria at 42°C (Georgopoulos, unpublished data), suggesting that this is not the case. Instead, we favor the explanation that there is a multienzyme replication complex in infected cells, consisting of at least the DnaB, DnaK, DnaJ, GrpD, and GrpE host proteins and the phage O and P proteins. A single alteration in the *P* gene may stabilize all mutant protein complexes. It could also be that the host mutations create a situation in which too high levels of active P protein destroy the complex, and the lowering of activity resulting from the λπB1 mutation restores its stability (see below). Recently, Reiser et al. (202) have determined the base sequence change in 14 π mutants. They found that (i) all π mutations are due to amino acid substitutions toward the carboxyl end of the P protein, (ii) deletions of the carboxyl end of the P protein that retain P-protein function simultaneously acquire the πA phenotype, (iii) four independently isolated πB mutants had exactly the same base substitution in the *P* gene (this πB mutation allowed growth on *dnaK756* and *dnaJ259* bacteria; it is not known whether there is any relationship between this πB mutation and πB1), and (iv) none of the π mutations tested affected the intracellular levels or chemical half-life of the P protein. The P protein interacts in vitro with both the DnaB (139, 253a, 264) and the DnaK (282) proteins, lending support to the idea of a multiprotein complex. The DnaK protein has been shown to interact in vitro with the O protein as well (282). This suggests that it should be possible to find mutations in *O* that compensate for the *dnaK* block.

A host mutant, *groPA15*, which was renamed *dnaB15* when the mutation was subsequently shown to map in the *dnaB* locus (80, 86), exhibited an interesting and unique phenotype. Originally isolated in a *supE* background, *dnaB15* bacteria blocked growth of λ^+ but allowed growth of all λ *Pam* mutants tested. This phenotype was not observed with other bacterial mutants upon introduction of the *supE* allele (Georgopoulos, unpublished data). An explanation for this result is that in the *dnaBA15* host high levels of the P protein are poisonous to phage DNA replication. The *supE* mutation suppresses polypeptide chain termination by inserting glutamine at the amber codon with a low efficiency (69). Hence, all *Pam* mutations would result in lower levels of P protein, which could permit a bypass of the block. The alternate explanation, namely, that the insertion of glutamine at each suppressed amber codon per se is responsible for the observed phenotype, appears less likely. The importance of the relative intracellular levels of the DnaB and P proteins in determining the GroP phenotype was also suggested by the work of Ogawa (194). She was able to demonstrate that *E. coli* strain *dnaB266*(Am) *supE* exhibited a $GroP^+$ phenotype when the suppression level of the amber polypeptide was high and a $GroP^-$ phenotype when the level of suppression was low. Similarly, increasing the intracellular levels of the DnaB534 mutant protein, using a multiple-copy plasmid, resulted in the suppression of its GroP phenotype (102). It appears that both of the models suggested originally (80) to explain the occurrence of the GroP phenotype, namely, that it is due to either (i) a failure of direct protein-protein interaction between the λ P protein and the host DNA replication proteins or (ii) a change in the relative intracellular levels of the λ P protein and the host DNA replication proteins, are still possible and not mutually exclusive.

Summary

The isolation of *E. coli* mutants that fail to support λ replication led to the identification of three groups of genes necessary for λ replication: (i) those already known to be involved in bacterial replication, namely, *dnaB*; (ii) those not previously identified, but encoding products necessary for bacterial replication, namely, *dnaJ* and *dnaK*; and (iii) those encoding functions apparently used by λ, but not as yet shown to be necessary for bacterial replication, namely, *grpD* and *grpE*.

SITE-SPECIFIC RECOMBINATION

Background

When λ establishes lysogeny, the phage genome is integrated into the *E. coli* chromosome. The reversal of this reaction is excision of the phage genome from the bacterial chromosome after induction. These recombination reactions are referred to as site specific because they occur at unique sites on both the host and phage genomes (263). Genetic and biochemical studies have led to a rather complete picture of this recombination, details of which are shown in Fig. 4.

In the integration reaction, recombination occurs at the λ *attP* site on the phage and the *attB* site on the bacterial chromosome. The *attP* site is internal to the ends, *cos*, of the linear λ genome. Therefore, the prophage genome has a permuted gene order in comparison to the vegetative phage genome. The prophage is bounded by hybrid *att* sites, *attL* and *attR*. Prophage excision occurs by recombination between these *att* sites, regenerating *attB* and a circular phage genome with *attP*. Functional studies demonstrate that each *att* site responds differently in the site-specific recombination reaction. The products of two phage genes play crucial roles in this reaction. Immediately adjacent to *att* is the *int* gene (43). The *int* gene product is a 40,000-M_r protein (136)

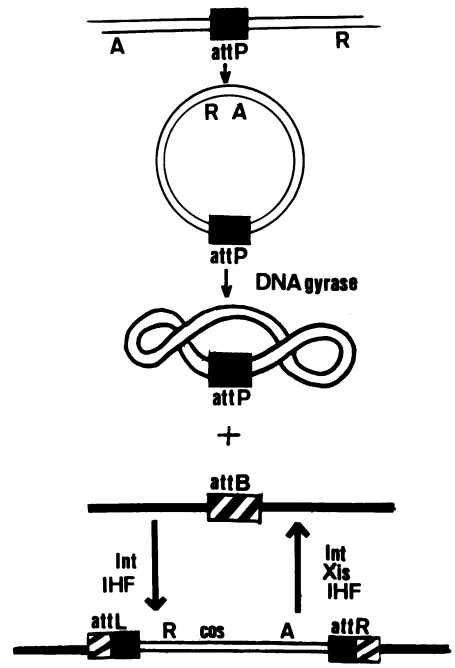

FIG. 4. λ site-specific recombination. The linear phage genome circularizes upon infection. The supercoiled phage DNA integrates by recombination at unique sites found on both the phage (*attP*) and bacterial (*attB*) genomes. The integrated prophage generates a new set of *att* sites, *attL* and *attR*. Excision involves a reversal of the integration reaction. The factors participating in these recombination reactions are listed.

that is involved in both integration and excision. Next to the *int* gene is the *xis* gene whose product, an 8,630-M_r protein (2), is needed for excision but not integration.

Host Factors

In vitro studies on integrative recombination by Kikuchi and Nash (135) first demonstrated that a host factor (IHF) is required for integrative recombination. Mutants of *E. coli* were isolated that are not able to support site-specific recombination (172–174, 265). The mutations responsible for the defect map at four loci on the *E. coli* chromosome (see Fig. 3): *himA* (host integration mediator) at min 38, *himB* (*gyrB* mutations) at min 82, *himC* (unmapped), and a locus known by either of two names, *himD* or *hip* (host integration protein), at min 20. (We shall refer to the latter locus as *hip-himD*.) In addition to their effects on site-specific recombination, the *him* mutants, except *himC*, fail to support growth of bacteriophage Mu (173, 174, 279).

***himA* and *hip-himD*.** The in vitro studies by Nash and colleagues have provided definitive information on the elements necessary for integrative recombination (190). In addition to Int protein, the 20,000-M_r IHF protein is required (190). Analysis of IHF under denaturing conditions revealed two polypeptides, α of 10,500 M_r (177) and β of 9,500 M_r (175).

The *himA* gene has been shown to encode the IHF alpha protein (177). The *hip-himD* gene encodes the IHF beta subunit (175). Although there are Int protein binding sites in both the *attP* and *attB* regions (213, 214), IHF binding sites are found only in the *attP* region (N. Craig and H. Nash, personal communication). Three 30-bp sequences are protected from nuclease digestion by bound IHF. The consensus sequence 5′-AANNNPuTTGAT-3′ is found in all of the protected fragments.

Study of bacteria with *him-hip* mutations revealed additional roles for IHF. Miller (171) showed that, in addition to being necessary for Int action, IHF is necessary for Int synthesis. The lack of Int synthesis results, in turn, from an absence of synthesis of the λ *c*II protein in *himA* or *hip-himD* mutants (122, 196). Transcription of the *int* gene can initiate at either of two promoters, p_I or p_L (43). Transcription from p_I requires *c*II protein. Mutations in *c*II were first identified among a collection of clear-plaque mutants isolated by Kaiser (129). The *c*II gene product was initially identified because of its role in the transcription of the *c*I (repressor) gene (see "Control of Lysis-Lysogeny Decision by *c*II and host factors"). In this case *c*II acts at the −35 region of the p_E promoter to promote initiation of *c*I transcription (271). Thus, in a *himA* host, failure to synthesize *c*II gene product interferes with the essential elements of lysogeny, repressor synthesis and integration (175). An IHF binding site has been identified near *c*II (Craig and Nash, personal communication).

The isolation of deletions in both the *himA* and *hip-himD* genes (H. Miller, personal communication) demonstrates that neither subunit of IHF is essential for bacterial viability. Some bacterial gene expression, however, is under IHF control. The expression of cloned flagellar antigen genes of *Salmonella* (M. Simon and H. Miller, personal communication) and the *ilvB* gene (51, 58) as well as at least one of the genes involved in xylose utilization (58) is under control by the *himA* and *hip-himD* gene products. It is not known in any of these cases whether this control is exerted at the level of transcription or translation or both.

Study of the regulation of *himA* expression also suggests that there is a role for Him product in some cellular processes (175). The *himA* gene is under SOS control, and it has been suggested that HimA may play a subtle role in DNA repair (see "Prophage Induction"). Interestingly, *himA* expression is autogenously regulated (175): in a *himA* mutant, there is a substantial increase in *himA* expression. Since a similar increase is also seen in a *hip-himD* mutant, it seems likely that the complete IHF molecule is involved in this regulation. Miller et al. (175) suggest that *hip-himD* expression is also autogenously regulated in a manner similar to *himA*.

***gyrB*-HimB.** Mutations producing a Him phenotype have been isolated in the *gyrB* gene (59, 174). The *gyrB* gene encodes the B subunit of DNA gyrase, an enzyme that places negative superhelical turns in DNA (71). Mutations conferring resistance to the antibiotics coumermycin A and novobiocin map in *gyrB* (73, 215). The *gyrA* gene encodes the second subunit, A, and mutations conferring resistance to the antibiotics nalidixic acid and oxolinic acid (74, 238) have been mapped in this gene.

Two pieces of evidence suggested that mutations in a *gyr* gene should influence site-specific recombination. First, supercoiled DNA is required in the in vitro reaction (1, 178). Analysis of the role of gyrase shows that the DNA with *attP* must be supercoiled, whereas that with *attB* may be linear (179). When both *att* sites are on the same molecule, the DNA must be supercoiled (178). Second, in vivo experiments show that both integrative (135) and excisive (1) recombination are inhibited when bacteria are treated with coumermycin.

The isolation of *gyrB* mutations that cause the bacteria to be temperature sensitive (Ts) for site-specific recombination [called *gyrB-him*(Ts)] confirms that gyrase plays an important role in site-specific recombination (59). The gyrase activity from these mutants has been demonstrated to be significantly reduced in vitro at high temperature. Genetic studies locate the mutation either within or extremely close to *gyrB*. As expected for hosts with gyrase mutations (247, 269), bacteria with *gyrB-him*(Ts) mutations (59) lose plasmids, both pBR322 and F′*gal*, at high frequency. Interestingly, these studies also showed that the P1 prophage plasmid is stably maintained in *gyrB-him*(Ts) mutants at high temperature. This result is not surprising in light of the knowledge that P1 has a partitioning system for ensuring coordinate replication of the prophage with the replication of the bacterial chromosome (7). Another aspect of the Him phenotype, failure to support growth of bacteriophage Mu, is also temperature sensitive in the *gyrB-him*(Ts) mutants.

Both the *himA* (Miller, personal communication) and *hip-himD* (E. Flamm and R. Weisberg, personal communication) genes have been cloned and sequenced. The amino-terminal residues of the IHF α and β proteins have been determined (H. Nash, personal communication) and correspond respectively to the *himA* and *hip-himD* nucleotide sequences (R. Weisberg, personal communication). Comparison of these sequences reveals a striking similarity: nearly one-third of the amino acid sequences are identical (H. Miller and R. Weisberg, personal communication).

The combination of both the *himA* and the *gyrB-him*(Ts) mutations has a far more profound effect on DNA supercoiling than do the single *gyrB-him*(Ts) mutations (58, 72). Upon shift to 42°C, there is virtually no supercoiling of phage DNA in the double mutant at 15 min postinfection. Under the same conditions, there is substantial supercoiling in the *gyrB-him*(Ts) hosts and normal supercoiling in the *himA* mutant. This physical difference is reflected in the physiology of the double-mutant bacteria. The following differences from the single *himA* or *gyrB-him*(Ts) mutants have been noted when assaying the double mutant at 42°C (58): λ fails to grow, the

bacteria become isoleucine auxotrophs, the bacteria are unable to use xylose as a carbon source, and maintenance of pBR322 is reduced another order of magnitude when compared with the *gyrB-him*(Ts) parents. Similar results were observed in the case of double mutants constructed with *hip-himD* and *gyrB-him*(Ts) mutations.

The apparent synergistic effect of *himA* and *gyrB-him*(Ts) mutations suggests that there is some type of interaction, direct or indirect, between the gene products. There is evidence that the level of gyrase activity is controlled by the *himA* gene product or, more likely, IHF. The amount of the *gyrA* gene product is reduced fivefold in a *himA* mutant host (72). This suggests that in the double mutant a relatively inactive *gyrB* product coupled with a low level of the *gyrA* product results in low gyrase activity.

It should be noted that, although the studies with all of these mutants demonstrate lowered site-specific recombination, more than one possible mechanism could explain the result. Thus lowered gyrase activity could influence site-specific recombination by one or more of the following means: (i) failing to properly supercoil the substrate molecule; (ii) reducing the replication of the λ genome, which supplies Int and Xis, and thereby reducing gene dosage; or (iii) reducing the supercoiling necessary for normal levels of transcription, resulting in a reduction in the amount of Int and Xis synthesized.

Regulation of *int* Expression

A fascinating aspect of the regulation of *int* gene expression involves differential degradation of mRNA from the 3′ end. This differential processing depends on where the transcription ends and, indirectly, on where it begins. The effect of downstream sequences on the expression of an upstream gene has been termed "retroregulation." An in-depth discussion of this subject can be found in the article by Echols and Guarneros (43).

The basis of retroregulation for the *int* gene is the fact that transcription of *int* can initiate from either the p_I or the p_L promoter. Transcription initiated at p_L is N modified and hence passes through a termination barrier downstream of *int*. In contrast, transcription initiated at p_I (which is stimulated by cII) terminates at this site. In the absence of cII, transcription of *int* occurs only from p_L. Strikingly, it was found that p_L-determined *int* expression occurred if there was a deletion of the genome immediately promoter-distal to the *int* gene (99). Point mutations in this region, *sib*, also increased *int* expression from p_L (100).

A number of lines of research have led to the following model for retroregulation of the *int* gene (43). The 3′ end of the *int* message, which terminates early, is not a good substrate for RNase III. The longer p_L message is a good substrate for RNase III action. The *sib* point mutations and the deletion mutations that show a Sib phenotype eliminate the sequences necessary to form the secondary structure that yields the appropriate substrate for RNase III action in the larger p_L message. Therefore, the Sib^- message is not degraded by the RNase III and the *int* gene is expressed. In the presence of an intact *sib* region, the p_L *int* message is cleaved by RNase III and degraded by other RNases, resulting in low levels of *int* expression.

In summary, retroregulation of *int*, the ability of downstream sequences to influence expression of an upstream gene, results from the degradation of *int* mRNA from the 3′ end. The differential degradation of the alternative *int* transcripts ultimately depends on the presence of both termination-sensitive and termination-resistant transcription of the *int* gene.

Phage Mutants

Two mutations in *int*, *int*-h3 (176) and *xin* (C. Gritzmacher, L. Enquist, and R. Weisberg, personal communication), reduce the levels of IHF required for integrative and excisive recombination. These mutations are not allele specific, being able to suppress the effects of a variety of *himA* and *hip-himD* mutations. Moreover, the *int*-h3 protein supports low levels of integrative recombination without host factor (B. J. Lange-Gustafson and B. A. Nash, J. Biol. Chem., in press). These experiments suggest that the mutant proteins catalyze site-specific recombination more efficiently than does wild-type protein.

CONTROL OF THE LYSIS-LYSOGENY DECISION BY cII AND HOST FACTORS

Background

Establishment of lysogeny by λ requires synthesis of repressor (cI gene product) and integration of the phage DNA into the host chromosome. The cII protein is critical for both of these processes (42, 131, 201) and is thought to be the key determinant in the phage's decision to choose a lytic or lysogenic program of growth.

There are two modes of synthesis of λ repressor, the product of the cI gene (42, 201). Each mode of synthesis has a characteristic activator protein. After infection, cI transcription initiates at p_E (the promoter for repressor establishment). This transcription requires the products of the phage cII and cIII genes. The cI synthesized from this promoter activates cI gene expression from a second promoter, p_M (the promoter for repressor maintenance). In the latter case it is argued that the cI protein bound to the right operator makes physical contact with host RNA polymerase to stimulate transcription initiation. Mutants of the cI gene (*pc* mutants) have been obtained that maintain ability to bind the operator but are unable to activate cI transcription. These *pc* mutants appear to define the sites in the cI protein that interact with RNA polymerase (106, 116). Second-site suppressors of *pc* mutations mapping in *rpoB* have been isolated (N. Irwin and M. Ptashne, personal communication). No mutations have yet been identified that result in polymerase being refractory to stimulation by cI protein.

The cII protein promotes lysogeny in two ways. First, it is a positive regulator that activates transcription of the *int* and cI genes. The *int* gene product is required for integration of the phage DNA (discussed in the preceding section). Second, cII protein causes a delay of lytic growth. Most evident is the delay of late gene products (28, 170), although inhibition of DNA replication can also be observed (151, 222, 233).

The cII gene codes for a polypeptide of 97 amino acids (225), which undergoes a distinctive processing at its amino terminus: the first two amino acids of the chain (f-Met and Val) are removed (115). Removal of this second amino acid may have an important role in influencing the stability of the cII protein. The cII polypeptide chains form a tetramer (115) which is probably the active form that is required for the transcription of cI and *int* (230). The binding site for this protein is well defined (115). Genetic and nucleotide analyses of mutations that abolish p_E promoter function show that these mutations fall into two clusters, *cy*L and *cy*R, which correspond to the −10 and −35 regions of the p_E promoter, respectively (222, 271–273). The *cy*R region contains the cII binding site, which consists of direct repeats of the sequence

TTGC with six intervening nucleotides, yielding the sequence TTGCN$_6$TTGC (115). Mutations in either TTGC reduce binding of *c*II to p_E, whereas mutations in the N$_6$ region do not. This TTGCN$_6$TTGC sequence is also found in the promoter for the *int* gene, p_I. DNase protection and methylation experiments indicate that *c*II binds to a region of 24 bp in the *cy*R region (115). For details on the biochemistry and mode of action of the *c*II protein, see Wulff and Rosenberg (271) and Shih and Gussin (227, 228).

Two main environmental factors influence the lysis-lysogeny decision: nutritional conditions (particularly starvation of the cells before infection) and the multiplicity of infection (MOI). We shall present arguments indicating that the *c*II product is critical in monitoring these environmental factors.

Starvation of exponentially growing cells in $MgSO_4$ for one generation before infection leads to a 50- to 100-fold increase in lysogenization. This dramatic, but unexplained, effect appears to be independent of phage replication and MOI (149). One possibility is that starvation, which is known to lead to increased proteolysis, causes the appearance of a proteolytic activity that degrades an inhibitor of lysogenization. Another possible explanation is that starvation conditions cause an increase in the levels of cAMP (169), which then influence phage growth.

It is known that the catabolite gene activation system (cAMP and its binding protein, CAP) can affect the phage lysis-lysogeny decision, as indicated by the observation that lambdoid phages lysogenize mutants lacking adenyl cyclase (*cya*) or the cAMP binding protein (*crp*) at a frequency of 10 to 50% that for wild-type hosts (97, 120). A simple explanation for the cAMP effect postulates that the cyclic nucleotide stimulates transcription from p_E and p_I. This is unlikely since addition of cAMP to *cya* mutants at the time of infection has no effect on lysogenization (128). Although in vitro studies cannot be taken as definitive evidence against an in vivo role for cAMP-CAP, *c*II protein binds to p_E and stimulates transcription from this promoter in the absence of cAMP-CAP in an in vitro transcription system (230).

A high MOI, between 2.5 and 10, favors lysogeny, whereas a low MOI favors lysis (149, 152). Several lines of evidence suggest that the MOI dependence of lysogenization efficiency is monitored by the *c*II and *c*III gene products. First, *c*II and *c*III appear to be limiting at low MOI in replication-proficient phages (149, 150). Second, mutations have been identified that map in or affect the *c*II and *c*III genes and greatly enhance lysogenization in singly infected cells. In particular, mutants (*can*) selected for *c*III-independent *c*I synthesis show efficient lysogenization at low MOI (127). These mutants exhibit increased *c*II activity, due to a more stable *c*II product (see below). Similarly, mutations within *c*III, *c*IIIs, that presumably lead to increased *c*III activity also allow increased lysogeny at low MOI (141, 142, 143). As discussed below, *c*III is thought to act by inhibiting host proteins (the Hfl proteins). The link between this inhibitory action and MOI monitoring capacity can be made by proposing that the level of *c*III determines the extent of inhibition of the host proteins. The lability of *c*III protein might mean that several gene copies of λ are necessary to produce sufficient *c*III product for this inhibition.

Host Factors

In addition to the factors discussed above, the genotype of the host influences the lysis-lysogeny decision as shown by the existence of host mutations that alter the course of infection (112, 160). The most important factors so far identified are *hfl*, *himA*, *hip-himD*, and the catabolite gene activation system (*crp* and *cya*). Recent studies of the *hfl* genes have contributed greatly to the view that *c*II and Hfl proteins play a central role in the lysis-lysogeny decision. In particular, the Hfl proteins control the stability of the *c*II protein and hence set the activity of the crucial determinant of this decision. Arguments described below indicate that the phage *c*III protein and the host catabolite gene activation system probably act via Hfl. Him and Hip also affect the activity of *c*II, by positively regulating *c*II synthesis. A detailed discussion of the action of HimA and HimD-Hip is featured in the discussion on site-specific recombination (see above).

hfl. Belfort and Wulff (13) isolated mutants in which λ is preferentially channelled towards lysogeny. In these *hfl* (high frequency of lysogeny) mutants, infection by λ$^+$ leads to nearly 100% lysogeny. A λ *c*III$^-$ mutant, which lysogenizes a wild-type host inefficiently, is able to lysogenize an *hfl*$^-$ host with an efficiency as high as λ$^+$ (13). Thus, *c*III is not needed for efficient lysogeny in an *hfl* host. Because the *hfl* mutations are recessive to *hfl*$^+$, Gautsch and Wulff (70) proposed that the wild-type Hfl protein antagonizes the establishment of lysogeny and that the role of *c*III in promoting lysogeny is to negate the effect of Hfl.

Six of the seven original *hfl* mutants contained mutations tightly linked to the *purA* locus, located at 95 min on the *E. coli* genetic map (70) with P1 cotransduction frequencies ranging from 77 to 100% (14, 70). These mutations define the *hflA* locus. The exceptional strain has recently been shown to contain a single recessive mutation closely linked to *argG* at 69 min; the locus affected by this mutation (*hfl*29) has been designated *hflB* (F. Banuett, unpublished data). This mapping places the mutation in the vicinity of the *nusA* gene. Studies with isogenic *hflB*$^+$ and *hflB*$^-$ strains confirm that the phenotype of *hflB* mutants is like that of *hflA* mutants: λ$^+$, λ *c*III$^-$, and λ *c*17 form plaques with reduced efficiency. The *hflA hflB* double mutant has a more extreme phenotype than either single mutant, as assayed by efficiency of plating of test phages (F. Banuett and I. Herskowitz, unpublished data).

Studies by Belfort and Wulff (15) provide a link between *hfl* and the catabolite gene activation system in regulating the lysis-lysogeny decision. As already noted, in the absence of either *c*III or cAMP-CAP, lysogeny is reduced but still occurs at significant levels (10 to 50% of wild-type frequencies). Belfort and Wulff found that lysogenization was almost totally abolished when both *c*III and the catabolite gene activation system were inactivated: at an MOI of 10, lysogenization by λ *c*III$^-$ of a *crp*$^-$ *cya*$^-$ host was only approximately 0.1% that in a wild-type host. By comparison, lysogenization by wild-type λ in the *crp*$^-$ *cya*$^-$ host was only modestly affected by the absence of the catabolite gene activation system (~42% of the efficiency observed in its presence). Thus, although lysogeny occurs in the absence of either *c*III or the catabolite gene activation system, the presence of one or the other is critical. *c*III and cAMP-CAP can be viewed as being functionally redundant.

The link between Hfl and the catabolite gene activation system comes from the remarkable finding that neither *c*III nor the catabolite gene activation system is required in the absence of Hfl: the extreme lysogenization defect of λ *c*III$^-$ in a *crp*$^-$ *cya*$^-$ host is completely reversed by an *hflA* mutation (15). The *hflB*29 mutation also eliminates the requirement for *c*III and the catabolite gene activation system (Banuett and Herskowitz, unpublished data). Thus, mutations in either *hflA* or *hflB* act as suppressors of *c*III mutations and of *cya* and *crp* mutations. These results have

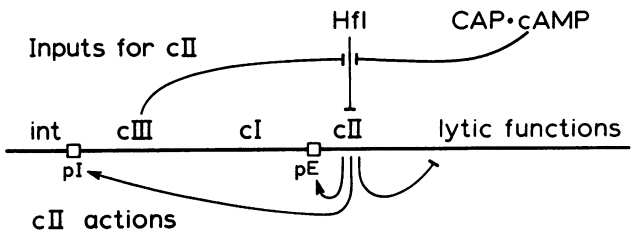

FIG. 5. Central role of cII in the lysis-lysogeny decision. Arrowheads indicate stimulatory interactions; bars indicate inhibitory interactions. (Modified from reference 112.)

been interpreted in the following light (Fig. 5): as noted above, Hfl is viewed as an antagonist of lysogenization and both cIII and cAMP-CAP are viewed as negative regulators of Hfl. It is possible that cAMP-CAP stimulates synthesis of a cIII-like inhibitor of Hfl or that cAMP-CAP acts as a repressor of Hfl synthesis (112, 127). Ample evidence exists for the role of cAMP-CAP as a negative regulator of gene expression (5, 180, 185).

Although Wulff and colleagues proposed that Hfl was an antagonist of lysogenization, its target was not identified. Two pieces of evidence led to the deduction that its target is cII. (i) Mutations in cII (*can*1) and in *cro* (*can*10, *cro*27) bypass the requirement for cIII; hence, it was proposed that cIII stimulates the activity of cII (127). (ii) cIII stimulates lysogeny by antagonizing Hfl (15). Thus Hfl was proposed to inhibit cII activity (127). The basis for this inhibition, as described below, is degradation.

Physiological experiments by Reichardt (200) led to the suggestion that cII activity is unstable. Identification of the cII protein on sodium dodecyl sulfate-polyacrylamide gels made it possible to examine the effects of a variety of conditions on cII synthesis and stability. As originally demonstrated by Epp (C. Epp, Ph.D. thesis, University of Toronto, Toronto, Ont., Canada, 1978) and subsequently by Gottesman et al. (89) and Hoyt el al. (122), the cII polypeptide chain is unstable, and its stability is affected by *hfl*, cIII, and cII (*can*1) mutations.

Hoyt et al. (122) have developed a quantitative system to study cII protein stability. They have constructed a plasmid in which cII and the *galK* (galactokinase) gene form an operon read from the λ promoter p_L. Operon expression is triggered by thermal induction (in the presence of a temperature-sensitive λ repressor). Stability of cII is monitored by pulse-chase experiments in which the ratio of cII to galactokinase is determined as a function of length of chase. Galactokinase, a stable protein, thus acts as an internal control for operon expression and for technical variables. Using this system, it was found that the half-life of cII is increased from 5 min in an *hfl*$^+$ host to 10 min in an *hflA*$^-$ host. Because cII is not completely stable in this *hflA*$^-$ host (which has an insertion mutation in the *hflA* gene), it appears that there are additional proteolytic activities that act on cII.

Stability of cII is increased in strains carrying the *hflB*29 mutation to a level comparable to that in *hflA* mutants, with a half-life of 10 min (A. Hoyt and F. Banuett, unpublished data). In the *hflA hflB*29 mutant, cII half-life is similar to that in the single mutant. Because the *hflB*29 mutation is uncharacterized, it is not known whether the remaining sensitivity of cII results from partial activity of HflB or whether there are additional proteolytic activities that can degrade cII protein. It is unclear whether *hflA* and *hflB* code for components of the same or separate proteolytic systems(s) that can act on cII.

hflA has been cloned in pBR322 (Banuett, unpublished data). Maxicell analysis of *hflA*$^+$ and *hfl*::Tn*1000* plasmids showed that *hflA* codes for two polypeptides of 46,000 and 37,000 M_r. The Tn*1000* (γδ) insertions comprise two nonoverlapping clusters: in one, the 46,000-M_r polypeptide is completely absent and the 37,000-M_r polypeptide is greatly reduced; in the other, the 37,000-M_r polypeptide is completely absent and the 46,000-M_r polypeptide is greatly reduced. These two polypeptides thus appear to be encoded by two adjacent genes, *hflK* and *hflC*, coding for the 46,000- and 37,000-M_r polypeptides, respectively.

Because these genes are apparently transcribed in the same direction (Hoyt and Banuett, unpublished data), they may be cotranscribed in the order, promoter-*hflK-hflC*. The apparent polarity observed for insertions into both *hflK* and *hflC* can be explained by proposing that these two polypeptide chains interact and that the individual species are sensitive to degradation. Mutants obtained recently by Tn*5* mutagenesis of the chromosome contain insertions in *hflK* and *hflC* (K. Thomas, personal communication). Interestingly, some mutations that confer an Hfl$^-$ phenotype are located upstream of *hflK*. This result suggests that the *hfl* genes so far described are perhaps part of a larger gene cluster.

***himA* and *hip-himD*.** The host *himA* and *hip-himD* gene products are remarkable in that they exert control on the establishment of lysogeny in two different ways. As described in the previous section, HimA and Hip-HimD are necessary for the integration reaction, as they comprise the integration host factor, IHF. HimA also affects the rate of synthesis of repressor and integrase (171). Pulse-labeling of infected bacteria at various times after infection shows that in *himA*$^-$ cells little or no repressor or integrase is made as compared with *himA*$^+$ cells. λ mutants that synthesize repressor or integrase constitutively in a cII-independent manner are able to synthesize these products in *himA*$^-$ cells, suggesting that HimA is necessary for synthesis of repressor and integrase and not for post-translational modification of these proteins (171, 172).

HimA and Hip-HimD differ from Hfl and cIII in that they do not affect stability of cII (122). Rather, they are essential for its synthesis (122, 196). For example, the ratio of cII to galactokinase produced by the cII-*galK* plasmid described above is reduced four- and twofold in *himA* and *himD-hip* mutants, respectively, in comparison with wild-type hosts (122). Because these genes are cotranscribed, cII production must be affected at some step after transcription. Perhaps HimA and Hip-HimD facilitate translation initiation (Miller, personal communication) or affect RNA processing. Additional influences by HimA and Hip-HimD on the lysis-lysogeny decision might arise as a result of binding of the integration host factor to the t_{R1}-p_E region (N. Craig and H. Nash, personal communication).

Summary

In summary, the level of activity of the phage cII protein is proposed to be the crucial determinant in the decision for lysogenic or lytic growth, and this level is determined by the interaction of cII with the host HflA and HflB proteins. The Hfl proteins are themselves proteases or control proteolytic activities that degrade cII. cIII and cAMP-CAP are viewed as inhibitors of this proteolysis and thereby are important regulators of the lysis-lysogeny decision. In particular, as discussed above, cIII is thought to be important in monitoring multiplicity of infection, and the catabolite gene activa-

tion system (CAP and cAMP) is thought to be important in monitoring the nutritional status of the host cell.

MORPHOGENESIS

Background

Assembly of the λ head and encapsidation of the DNA is a complicated process that involves at least 10 phage-encoded functions. We briefly summarize the salient features of this process and refer the reader to recent reviews for more detailed summaries of the process (45, 84, 183). Figure 6 summarizes the steps involved in assembly and encapsidation. Proteins gp*B* and gp*C* probably form a complex with the scaffolding protein gp*Nu3*. It is thought that this complex facilitates the correct assembly of the major capsid protein, gp*E*, to give rise to an immature prohead structure consisting of ~420 molecules of gp*E*, ~12 molecules of gp*B*, ~12 molecules of gp*C*, and ~200 molecules of gp*Nu3*. At some stage during or after the completion of this assembly process, all of the gp*C* molecules somehow undergo a fusion/cleavage reaction with an equal number of gp*E* molecules to give rise to the hybrid X1 and X2 subunits (108) found in the mature head. Approximately three-quarters of the gp*B* molecules undergo a cleavage reaction to become p*B**, and the scaffolding protein gp*Nu3* exits after or during its cleavage to p*Nu3** (84). The temporal order of these modification steps is not known at this time. However, there is evidence of an early intermediate, called the preconnector, needed for the correct assembly of the major capsid protein, gp*E*, into the prohead structure (144, 145). The preconnector is formed from 12 subunits of gp*B* (144, 146). The mature prohead structure (also known as petit λ) is ready to encapsidate λ DNA.

After DNA cutting at the unique terminus, *cos*, by the gp*Nu1*-gp*A* terminase complex and encapsidation of a genome length of DNA, an unstable head structure forms, which is stabilized by the addition of the gp*D*, gp*W*, and gp*FII* proteins to produce a stable head structure (45). The full head interacts spontaneously with a finished tail to give rise to an infectious λ particle.

Host Factors

groE. One class of *E. coli* mutants unable to support productive λ growth blocks phage development at the level of morphogenesis (79, 236). In these mutants there is no obvious defect in λ transcription, DNA replication, integration, or tail assembly. Examination of extracts from λ infections by electron microscopy and protein analysis revealed a failure in the formation of normal heads or head precursors. Moreover, these extracts were unable to donate active heads for in vitro complementation assays. Because mutations in the λ *E* gene (which codes for the major λ head protein) permit the phage to overcome the block imposed by this class of *E. coli* mutants (see below), the mutations are called *groE*. All of the *groE* mutations map near *purA* at 94 min on the *E. coli* genetic map (see Fig. 3) (78). Fine-structure mapping has placed the mutations between the *cadA* and *ampC* genes (101, 244). Studies with λ *groE* transducing phages have established that the *groE* locus is composed of two closely linked genes. These genes, named *groEL* and *groES* by their discoverers (251), have also been named *mopA* and *mopB* (8).

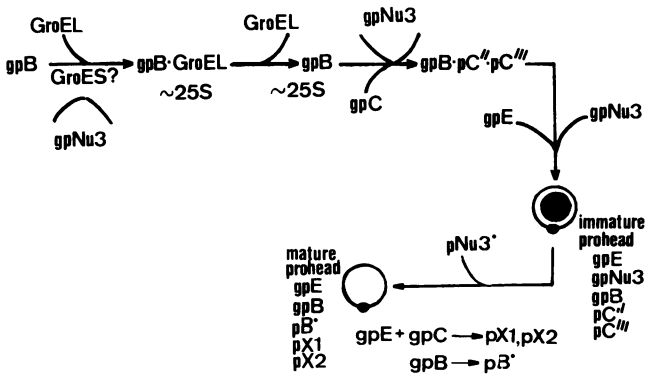

FIG. 6. Steps involved in formation and maturation of λ proheads. Indicated are both phage and host functions contributing to the reaction. See text for detailed discussion.

The large *groE* gene product, GroEL, was identified as a 65,000-M_r polypeptide by analyzing proteins formed after infection of UV-irradiated bacteria with a λ *groE* transducing phage in the presence of [^{35}S]methionine (81, 110). Confirmation of this assignment came from strains with mutant *groE* transducing phage (81, 110, 249). The purified *groEL* protein has a weak ATPase activity and forms decatetramers with sevenfold symmetry (107, 118).

The small *groE* gene product, GroES, was identified by using methods similar to those used in identifying GroEL. Deletion mutants of λ *groE* were selected by treatment with EDTA (197). These mutants defined the existence of two *groE* genes and were used to identify the second *groE* gene product as a protein of 15,000 M_r. This designation was confirmed by the isolation of a λ *groE* point mutant that failed to grow in *groES* mutant bacteria and produced a 15,000-M_r protein with an isolectric point different from the analogous protein synthesized by the *groE*$^+$ transducing phage (249). The base sequence of the *groES* gene has recently been determined (C. Woolford and R. Hendrix, personal communication). The predicted *groES* protein consists of 97 amino acids, whose N-terminal sequence and overall amino composition agree with those of the purified protein (G. N. Chandrasekhar, personal communication).

The role of the *groE* gene products in head assembly was determined by examining the structures formed during infection of *groE* mutants. These studies showed that mutations in both the *groEL* and *groES* genes block λ head assembly at an early stage (79, 249). Only the effects of the *groEL* mutations, however, have been studied in detail. The only step in head production that clearly requires the *groE* gene products is the assembly of the preconnector (144). The formation of this ring-shaped structure composed of gp*B* molecules requires, in addition to the *groE* products, the phage *Nu3* gene product (144). Consistent with a role for GroEL in preconnector formation is the observation that the GroEL protein appears to sediment with gp*B*, the major protein of the preconnector (181). Although this appears to indicate an association between gp*B* and GroEL, it may merely reflect the fact that both the native GroEL and the preconnector have large sedimentation coefficients, the former being a decatetramer of 65,000-M_r subunits (107, 118) and the latter a dodecamer of 56,000-M_r subunits (144, 146). Cosedimentation was also found for both GroEL missense proteins and an amber B-protein fragment, suggesting that the observed association is not a fortuitous event reflecting similar sizes of the two proteins (181).

Additional evidence supports this role for the GroE proteins. Normally, late in infection, gp*B* is found in association with cleaved forms of gp*C*. This intermediate is not formed

in *groEL*$^-$ bacteria. Studies of in vitro prohead assembly, using the system of Murialdo and Becker (182, 184), showed that complementation extracts must be prepared from *groE*$^+$ bacteria (46). Moreover, other experiments suggest that the mutations in *groEL*140 and *groEL*764 do not affect assembly stages after preconnector formation (146). It was shown that purified preconnectors from infection of a *groE*$^+$ host will complement appropriate extracts from infections of *groE* bacteria. However, studies with another lambdoid phage, HK97, suggest that the *groEL* protein could play a role in assembly of the major head protein. This phage has been shown to cross-link its major head protein, a gp*E*-like protein, into a very high-molecular-weight macromolecule (M. Popa and R. Hendrix, personal communication). This cross-linked product was not found in an infection of a *groEL* mutant, even though headlike structures were formed.

Headlike structures are also formed by λ during the abortive infection of *groE* mutants. Electron microscopic examination of such infections revealed a variety of abnormal polyhead structures (79). Proheads composed of gp*B*, gp*E*, gp*C*, and gp*Nu3* can be purified after infection of either *groEL*$^-$ or *groES*$^-$ bacteria (41, 155, 249). These are probably dead-end structures, since none of the protein cleavages found in normal head assembly have occurred (117).

Pleiotropic effects of *groE* mutations. Studies with *groE* mutants revealed an effect on growth of nonlambdoid phages T4 and T5. T5 infection of some *groEL* and *groES* mutants was abortive due to a block in an early stage of T5 tail assembly, a stage before recognizable structures were assembled or the proteolytic cleavage in the process was performed (281). In the case of T4, only *groEL* mutations were found to block head assembly, but the block occurs at an early stage of head assembly (26, 79, 203, 245, 246; K. Tilly and C. Georgopoulos, unpublished data).

groE mutations confer a temperature-sensitive growth defect on the host (78). This may not be surprising in light of the fact that GroEL protein represents nearly 2% of the total cellular protein at 37°C (110a). When placed at 42°C, the *groE* mutants form filaments without septa and show an altered protein synthesis pattern (78). Recently, it has been reported that mutations in the *groEL* or *groES* gene interfere with RNA and DNA synthesis at 42°C (257).

Regulation of *groE* expression. Recent evidence indicates that the *groE* genes are among those that are induced to be expressed at high levels in *E. coli* by heat shock (191, 252, 274, 275) and suggests that their products may be essential for survival at high temperature. Although the exact mode of action of GroEL and GroES proteins is not known, it has been established that they functionally interact in vivo and in vitro. The in vivo evidence stems from the isolation of intergenic suppressors of the *groES*619 mutation that map in the *groEL* gene (248). The in vitro evidence consists of (i) inhibition of the ATPase activity associated with the GroEL protein by purified GroES protein (Chandrasekhar, personal communication) and (ii) cosedimentation of the two purified proteins in glycerol gradients in the presence of ATP (Chandrasekhar, personal communication). The functional interaction between the two proteins helps explain why mutations in either gene exhibit an identical phenotype with respect to λ head assembly and bacterial growth.

Other host mutants. Other host proteins appear to be required for the assembly of lambdoid phages. *E. coli mat* mutants seem to affect the growth of λ at a late stage, since heads and tails are not assembled (C. Waghorne and C. Fuerst, personal communication). The proteins composing these structures are made in normal amounts but the structure of at least the *J* gene product is aberrant, since there is no detectable serum-blocking activity in lysates (64). Recent evidence suggests that the *mat* and *hin* (275) or *htpR* (191) genes are identical or very closely linked, since λ *mat*$^+$ transducing phages share many restriction fragments with plasmids carrying the *htpR* gene (Waghorne and Fuerst, personal communication). The functions of the *htpR* gene product in morphogenesis may be manifold, since HtpR is required for induction of the heat shock proteins and this induction can be triggered by λ infection (40, 145; Tilly and Georgopoulos, unpublished data). Because the heat shock proteins include the *groE* gene products (see above), the effects of *mat* mutations on λ morphogenesis may be indirect.

A final case in which host proteins are involved in phage assembly is in the case of DNA packaging. M. Feiss and coworkers (personal communication) have demonstrated that in both *himA* and *hip-himD* mutants, lambdoid phage 21, unlike λ, fails to produce progeny phage. As discussed in the section on site-specific recombination (see above), these two genes encode the two subunits of IHF, the host protein required for λ site-specific recombination. Mutants of 21 (*her*), isolated for their ability to overcome the block, have mutations in the 21 analog of the λ *Nu1* gene, which encodes one of the terminase subunits (88, 239, 240). Thus the growth defect results from a failure in the mechanism making the cleavage at the *cos* sites that creates the unit-copy genomes ultimately packaged into phage. These findings also show that, in addition to phage-encoded functions, IHF is required for this cleavage reaction in phage 21.

In the case of λ, cleavage at *cos* occurs in the absence of active IHF since λ makes progeny phage in both *himA*$^-$ and *hip-himD*$^-$ hosts. However, IHF, at a minimum, plays an auxiliary role in the *cos*-cutting reaction for λ. In vitro studies show that a host factor is required, but either IHF or another *E. coli* product is sufficient to support cleavage at *cos* provided that the appropriate phage functions are also present (88). Although this alternative function(s) has not been identified, a sequence on the λ genome has been identified as a possible candidate for the recognition site of the alternative function (see below).

Phage Mutants

Phage λ mutants able to overcome the block exerted by *groE* mutations were isolated and their compensatory mutations were mapped (79, 236). Some amber and other point mutations in the λ *E* gene, encoding the major head protein, allowed the phage to form plaques on either *groEL*$^-$ or *groES*$^-$ bacteria. The growth in these cases was most likely the result of lowered levels of functional gp*E*, permitting small amounts of active GroE proteins to start and complete a few phage, rather than initiating many and completing none (analogous to the effect described by Floor [47]). λ *B* gene mutants were also specifically isolated by their ability to grow on *groEL*$^-$ hosts. The allele specificity of these mutations suggests that they alter an intermolecular interaction, which adds credence to the specificity of the sedimentation results of Murialdo (181).

A mutant of λ has been isolated that fails to grow in hosts with *himA* deletions or in double mutants with point mutations in both *himA* and *hip-himD* (12). The responsible mutation, *cos*154, was located very near to the left end of λ. DNA sequence analysis revealed a single-base change in a region of hyphenated dyad symmetry 154 nucleotides from the left end. Functional studies show that in the absence of active IHF, λ genomes with this mutation cannot be cleaved

at *cos*. Bear and colleagues (2) have suggested two explanations for the phenotype of *cos*154: the mutation defines the recognition site for either the phage terminase or a host function necessary for *cos* cutting in the absence of IHF.

Genetic material found on the right end of lambdoid phage φ80 results in a requirement for IHF in lytic growth at low temperatures (M. A. Mozola and D. I. Friedman, Virology, in press). Although all lambda derivatives tested grow in *himA* and *hip-himD* mutant hosts at low temperatures, λ-φ80 hybrids that have the rightmost 10% of the λ genome replaced by φ80 genetic material (QSR-80), as well as φ80 itself, show this temperature-dependent growth defect. The gene(s) responsible for this phenotype, *rha*, has been mapped to the QSR-80 region. λ *rha* is partially dominant. Although a possible protein candidate for the *rha* gene product has been identified by two-dimensional electrophoretic analysis (M. A. Mozola, D. L. Carver, and D. I. Friedman, Virology, in press), the precise nature of the defect is not known.

Summary

Although it is clear that host proteins are important for lambdoid phage assembly processes, their mechanisms of action remain obscure. It is likely that even more interactions in morphogenesis remain to be discovered, awaiting the design of new selections or serendipity.

PROPHAGE INDUCTION

Background

The prophage is essentially an inert piece of genetic material kept dormant by virtue of the prophage repressor. The host bacterium plays a crucial role in the induction of the prophage. The central event in induction is the removal of repression, which is accomplished by destruction of repressor (208). Early studies by Lwoff and co-workers (168) identified the triggering event for induction of lysogenic *Bacillus megaterium* to be related to factors that interfere with normal DNA metabolism. The primary example was UV irradiation. Later studies revealed that a similar mechanism operates for λ lysogens.

Host Factors

The first evidence that a host function was involved in the induction of prophages was the observation that UV irradiation failed to induce λ lysogens of a *recA* host (19, 114). The *recA* gene was originally identified as required for generalized recombination (25). Another type of *recA* mutation, "*tif*," has the unusual property of being constitutive for prophage induction at 42°C (24, 138), suggesting that wild-type RecA protein is activated by the inducing treatment.

Roberts and co-workers (31, 209, 210) discovered that induction involves the cleavage of repressor and showed that this activity requires the product of the *recA* gene. Both activities, proteolytic and recombinogenic, are activated by single-stranded DNA. (The *tif* mutation results in a RecA protein that at high temperature has a reduced requirement for single-stranded DNA to stimulate its role in protein cleavage.)

The work of many investigators (164, 210, 211, 266) indicates that RecA plays a prominent role in the response of *E. coli* to DNA-damaging stimuli, the "SOS response." Single-stranded DNA formed as a result of DNA damage is a substrate for several recombination and repair activities of the RecA protein. It also activates the proteolytic activity associated with RecA, which induces expression of a set of genes (*din* [damage inducible]) involved in the SOS response (133). The activation of host genes occurs by cleavage of the *E. coli lexA* gene product by RecA. LexA is a repressor that controls a large number of genes involved in the SOS response (164). This role for *recA* protein has been extensively reviewed (164, 259). Although cleavage of both LexA and λ repressor requires activated RecA in vivo, the sensitivities of the two proteins are very different (164). Low levels of DNA damage induce SOS efficiently, but are not sufficient to induce λ. Only at higher levels of damage is λ induction observed. This suggests that the λ repressor has evolved so as to become sensitive to RecA-stimulated cleavage only when host survival is clearly imperiled.

Recent studies suggest that repressor cleavage may be an autocatalytic reaction facilitated by RecA protein (163). It was shown that both purified LexA and λ repressor cleave spontaneously when placed at higher pH. These cleavages occur at the same sites as those observed when RecA protein facilitates the reactions. Nonetheless, it should be emphasized that RecA protein is required in vivo for the cleavage of repressor.

There is also an interesting connection between the responses of bacteria to DNA damage and to heat shock. Krueger and Walker (153) have shown that at least some of the heat shock proteins are expressed at higher levels after treatment with UV light and nalidixic acid. This induction requires the *htpR*$^+$ gene product but is independent of *recA* and *lexA* and does not have the normal kinetics of the heat shock response.

Phage Mutants

Mutations in the *c*I gene have been identified that result in the prophage being uninducible by DNA-damaging agents or in *tif* mutants (125, 161). These Ind$^-$ mutations map in the *c*I gene, and in the case of one Ind$^-$ mutation the resulting repressor is not cleaved under standard conditions (211). This repressor protein shows a single change at amino acid 117 (221).

Summary

To summarize the events in induction, agents interfering with normal DNA metabolism result in regions of single-stranded DNA. The single-stranded DNA alters RecA protein so that it is now able to facilitate repressor cleavage. Cleavage of LexA protein and of λ *c*I protein allows expression of the gene sets necessary for DNA repair and λ lytic growth.

CONCLUDING REMARKS

The studies reviewed in this article identify a wide variety of phage activities that require interaction with host functions. In some cases, the reasons for the interactions are obvious. For instance, the induction of the λ prophage results from cleavage of repressor with the aid of the RecA protein. The activation of the proteolytic activity of RecA protein is the major event in the induction of the host SOS response, a set of events set into motion when the host DNA has been damaged. This signals the possibility of the imminent demise of the cell and therefore the demise of the prophage. To save itself, the prophage must be able to heed the SOS signal and escape the dying host by growing lytically.

The role of host-phage interactions in other processes may be less evident but is equally important. In a general sense, the reliance on interactions of this type allows a more

flexible response of the phage to its bacterial environment. Most of the processes examined in this review require a large number of host factors acting in a concerted manner. Each of these factors may differ from one host to another. The problem for a phage, particularly a temperate phage, is to be able to utilize the host's machinery effectively. One way for the phage to exploit these host mechanisms is to replace or alter one of the factors in the host complex. By affecting only one host function, the phage might then be able to fit itself into a process requiring a large number of host factors. For instance, in the case of the *N* gene product, merely by adapting to the host *nusA* product, lambdoid phages might be able to utilize a foreign transcription termination-antitermination system.

Regardless of why the phage has evolved such responses, it is obvious that the nature of these λ-*E. coli* interactions has facilitated the analysis of many important physiological processes. Without the lambdoid phages certainly many of the *nus*, *hfl*, *groP*, *groE* or *him* genes would not have been uncovered as easily. Moreover, the identification of mutations in these host factors has permitted the exploitation of back-and-forth genetics. Using phage mutants to select bacterial mutations (and vice versa) permits, in addition, the selection of unique types of phage mutations that allow the phage to use the mutant bacterial products. These mutants have added new tools for studying phage-determined processes. For instance, the isolation of *hip-him* mutants has allowed the isolation of the *int*-h mutation, which results in a more active Int protein. Analysis of this mutant protein should add to the understanding of Int action.

One further significant observation is that some of the *E. coli* gene products required by λ are part of the heat shock regulon. Since infection with λ results in increased synthesis of at least some heat shock proteins, it is conceivable that the phage has exploited the regulation of these genes as a unit, to ensure that it can turn on required host genes in a concerted manner. By acting on expression of the *E. coli* *htpR* gene, the central gene for the heat shock response, λ could influence the expression of at least four genes whose products are required for phage growth. Thus, in addition to subverting individual host functions by protein-protein interactions, λ may also have evolved a means for controlling the expression of groups of host proteins. This mode of operation may not be limited to lambdoid phages; studies with adenovirus indicate that it also influences expression of a host heat shock protein (193).

The detailed knowledge gained by studying λ-*E. coli* interactions should serve as a useful prototype in the study of host influences on the growth of other viruses.

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