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Molecular Genetics of Bacteriophage P22†

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INTRODUCTION

The viruses of bacteria are undoubtedly the easiest living organisms to study and to understand at the molecular level. Throughout the past 25 years, the study of these organisms has yielded information and insights concerning the basic nature of life (especially the molecular nature of heredity) and thus nourished the science of molecular biology. Accordingly, molecular biologists continued to lavish attention upon a chosen few bacteriophages, with the result that a handful of these organisms are by far the best understood living things. It should be emphasized that our understanding of even these organisms is still incomplete; nevertheless, it is true that for some bacteriophages the function of virtually every gene is known in at least a general way; the protein products of most genes have been identified; and, especially in the temperate phages, there now exists a reasonably precise understanding of the way in which the expression of phage genes is regulated.

In this article we summarize what is known about the temperate bacteriophage P22, whose normal host is *Salmonella typhimurium*. Phage P22 was involved in the very birth of bacterial genetics, when Zinder and Lederberg (143) discovered generalized transduction of *Salmonella* genes by P22. The phage has been studied ever since in its own right.

† This review includes much of the material discussed by David Botstein in his address to the 78th Annual Meeting of the American Society of Microbiology, Las Vegas, Nev., 14–19, May 1978 in response to his receiving the Eli Lilly Award for 1978.

The earliest studies concentrated on regulation of lysogeny. Levine's work on clear-plaque mutants of P22 showed that establishment of lysogeny is regulated by a group of linked genes, only one of which is required for the maintenance of lysogeny (78, 79) and that these genes have a sequential order of action (81, 109). These studies, together with Kaiser's parallel studies of clear mutants of coliphage λ (69), led to intensive use of P22 and λ in studies of gene regulation at the molecular level.

Other concepts in molecular biology which were first proposed on the basis of research with P22 include:

(i) Regulation: the idea of an "antirepressor" which inactivates phage repressors by binding to them in a noncovalent way (12, 82, 116); the idea that control of the establishment of lysogeny interacts with the cyclic adenosine 5'-monophosphate/catabolite repression system of the host, possibly through a transcription-termination mechanism (56, 100).

(ii) Morphogenesis: the idea of a "scaffolding protein" which ensures correct assembly of capsid subunits by acting as a transient major constituent (one scaffolding protein molecule per two capsid monomers) of a head precursor ("prohead") (14, 74); the idea that such a scaffolding protein, despite its stoichiometric mode of action, can be reutilized after leaving a prohead for the assembly of further proheads, becoming in effect a "morphogenetic enzyme" (19, 73, 74); the idea that deoxyribonucleic acid (DNA) encapsulation can proceed sequentially from a specific site on a long concatemer (127).

(iii) Genetic analysis of biological pathways: the idea that the order of gene function in vivo can be determined from the results of reciprocal temperature shifts with *cs-ts* (cold sensitive-heat sensitive) double mutants (65); the development of a general method for assessing protein interactions by isolating mutations that suppress *cs* or *ts* mutations and confer new conditional-lethal phenotypes (66).

(iv) DNA replication: the idea that circularization of a genome by recombination can be essential to extensive DNA replication and the concomitant evidence that DNA concatemers can arise as a result of replication of the DNA (13, 130, 131).

(v) Prophage integration: the idea that prophage integration is catalyzed by phage-specified site-specific recombination enzymes (111); the idea that excision of prophage involves the same system (108, 111).

(vi) Recombination: direct evidence that bacterial recombination systems are capable of double-strand breakage and joining events (31); the idea that R-factors contain translocatable drug-resistance elements capable of insertion into nonhomologous DNAs (21, 22, 27, 76, 129).

(vii) Virus evolution: the observation that phages of different species of bacteria can recombine in several ways to make viable, normally regulated hybrids (i.e., that phage P22 of *S. typhimurium* is a cousin to phage λ of *Escherichia coli*); the resultant idea that the family of lambdoid temperate phages (of which P22 seems now to be a member) evolve together as a group through the independent evolution and reassortment of segments of the genome specifying particular phage functions (9, 39, 47, 52).

GENETIC ORGANIZATION OF THE P22 GENOME

Gene Order and Gene Function

In 1968, Gough and Levine showed that the genetic map of phage P22, as determined from vegetative crosses, is circular (43). However, when the P22 genome is integrated as a stable prophage into the *Salmonella* chromosome, the genes assume a unique linear order (20, 110). The circular genetic map is shown in Fig. 1; the map in the unique prophage order is shown in Fig. 2. The two maps are related by a unique site on the phage map, called the attachment site (*att*), and a site (*ataA*) between the *proA* and *proC* genes on the genetic map of the host. If one envisions a site-specific recombination event at these two sites between a circular phage DNA and the host chromosome (also circular), then one can generate the prophage orientation. This way of envisioning prophage integration was first proposed for coliphage λ by Campbell (18); all available evidence indicates that this way of looking at the integration process applies to phage P22 as well.

It is convenient to discuss P22 genetics in terms of the prophage map, as shown in Fig. 2. In this figure are listed all the known P22 genes, the function which has been determined for each gene from the phenotypes of mutants, and the characteristics of the proteins specified by each gene, where known. The most striking observation about the genetic organization of the phage is that related functions are clustered. This generalization applies to all the vegetative functions: DNA replication, lysis, and head assembly. This clustering applies even to the subdivisions of the head assembly process: prohead formation (genes 5 and 8); prohead maturation and DNA encapsulation (genes 1, 2 and 3); maturation of full heads (genes 4, 10, and 26); and injection by full heads (genes 7, 20, and 16). On the other hand, the regulatory genes controlling maintenance of lysogeny occur in two widely separated clusters. The "superinfection exclusion" genes

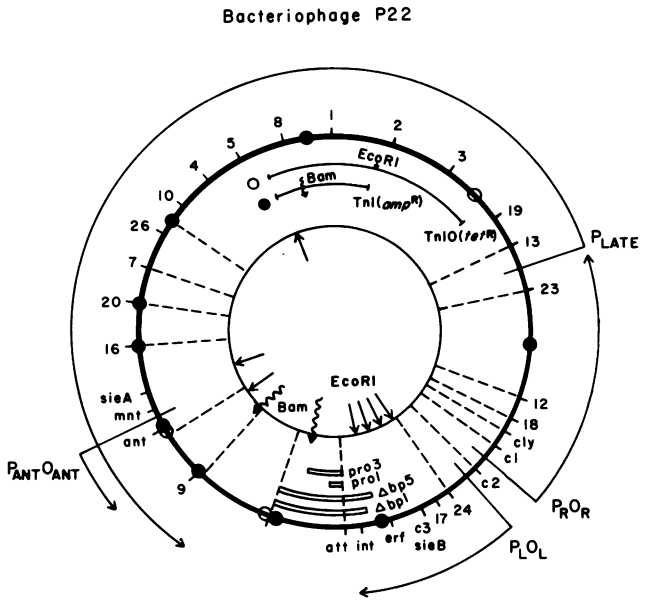


FIG. 1. *Circular genetic and physical map of the genome of P22. The inner circle shows the approximate location of cleavage sites made by the specific endonucleases EcoRI (straight arrows) and BamHI (wavy arrows). The outer circle shows the genetic map of P22 drawn approximately to scale. Dotted lines connecting the two circles show the assignment of genes to restriction fragments, where known (64; Weinstock, Ph.D. thesis). The position of genes within fragments is not drawn to scale. The filled and open circles represent particular insertions of Tn1 (amp^R) and Tn10 (tet^R), respectively (21; Weinstock, Ph.D. thesis; Susskind, unpublished data). For reference, a physical representation of the size and restriction sites of these insertion elements is given at the top between the two circles. The open bars near att show the extents of P22 material deleted in the indicated deletion and substitution variants of P22 (21). Finally, the patterns of transcription and the corresponding promoters are thought to be as shown by arrows drawn outside the genetic map.*

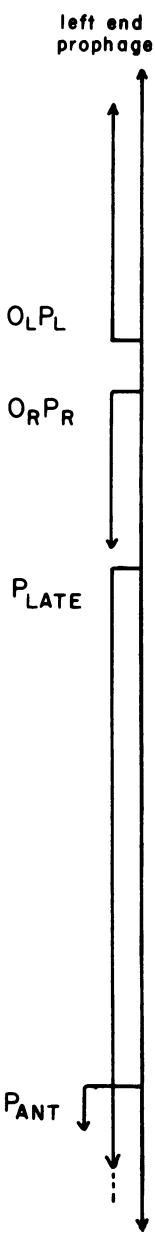
are similarly separated; each of the two known systems is linked to one of the regulatory regions.

The second striking fact about the genetic organization of phage P22 is its similarity to that of coliphage λ (Fig. 3). This similarity is neither the result of coincidence nor the consequence of convergent evolution; a great deal of evidence now exists showing that P22 and λ are related phages containing some homologous DNA sequences (25, 107) which are able to recombine in many places to produce viable hybrids (9, 39, 52). Thus, it is not surprising that P22 and λ share many features of genetic organization and physiology.

If there were an organizing principle for the P22 and λ genomes, it might be the arrangement of units of transcription so that regulatory proteins can exert their effects upon a large number of genes at a small number of sites. It appears that the P22 late genes are all transcribed "rightward" (downward in Fig. 2) from a site just beyond gene 23 (K. K. Lew, Ph.D. thesis, Massachusetts Institute of Technology [MIT], Cambridge, 1975; G. Weinstock, Ph.D. thesis, MIT, Cambridge, 1977). Late gene expression is apparently dependent upon the activity of the regulatory protein specified by gene 23 (14, 83). Gene 23 is similar, if not identical, to the corresponding λ gene *Q* (9; S. Hilliker, Ph.D. thesis, MIT, Cambridge, 1974). The early genes of P22 are organized into two operons which flank the *c2* repressor gene (15, 16). Transcription of each of these operons starts at a promoter/operator complex near the *c2* gene and proceeds in the direction away from the *c2* gene. The transcription of these operons is affected not only by the *c2* repressor, but also by the product of gene 24, which is analogous to the λ gene *N* (51, 52).

Correlation of Genetic and Physical Maps of P22

Two methods of measuring physical distance have been used in correlating genetic and physical distance on the P22 genome: electron microscopy of DNA heteroduplexes (21) and agarose gel electrophoresis of DNA digested with specific restriction endonucleases (64). These methods have been applied to variants of P22 with insertions, deletions, and substitutions whose positions on the genetic map are known and to λ/P22 hybrid phages which contain ge-



GENE	FUNCTION	PROTEINS: Subunit M.W.	PROTEINS: Presence in Mature Particle
int	integration	46,000	No
erf	recombination	23,000	No
c3	establishment of lysogeny	not identified	
sieB	superinfection exclusion	not identified	
17	escape from FelS-I exclusion	14,000	
24	early control	not identified	
c2	maintenance of lysogeny; repressor	25,000	No
cI	establishment of lysogeny	not identified	
18	DNA replication	not identified	
12	DNA replication	54,000**	uncertain**
23	late control	not identified	
13	lysis	not identified	
19	lysis	20,000***	not tested
3	heads; DNA encapsulation	9,500	No
2	maturation of proheads	63,000	No
I	maturation of proheads	94,000	Yes
8	heads; scaffolding protein	42,000	No*
5	heads; major structural protein	55,000	Yes
4		15,000	Yes
10	heads; maturation of full heads	56,000	uncertain**
26	heads; maturation of full heads	23,000	Yes
7		18,000	Yes
20	heads; injection	50,000	Yes
16	heads; injection	67,000	Yes
sieA	superinfection exclusion; injection	not identified	
mnt	maintenance of lysogeny; repressor	not identified	
ant	antirepressor	27,000	No
9	baseplate (tail)	76,000	Yes
a_1	antigen conversion	not identified	

* P8 is in prohead precursor, but not in mature phage
** Cannot be determined because subunit runs too close to P5, the major capsid protein.
*** Determined from enzymatic activity, not from SDS-gel electrophoresis.

FIG. 2. *Genetic and functional map of prophage P22. The conventional left end of the prophage (near proAB) is at the top of the figure and the conventional right end (near proC) is at the bottom. At the left of the figure, operator/promoter sites are shown. At the right are listed genes, their functions, and the subunit molecular weights of proteins they encode. The last column indicates whether the known protein products are found in the mature virion. The pattern of transcription is thought to be as shown by arrows at the left. Data on gene order and gene function are from references 8, 14, 20, 44, 88, and 117. The gene order in the erf-c2 region is from Susskind and Gauger (unpublished data); the order of the c3 and sieB genes is not known. Data on proteins are from 14, 88, and 91; data on antirepressor protein are from Susskind and Botstein (unpublished data).*

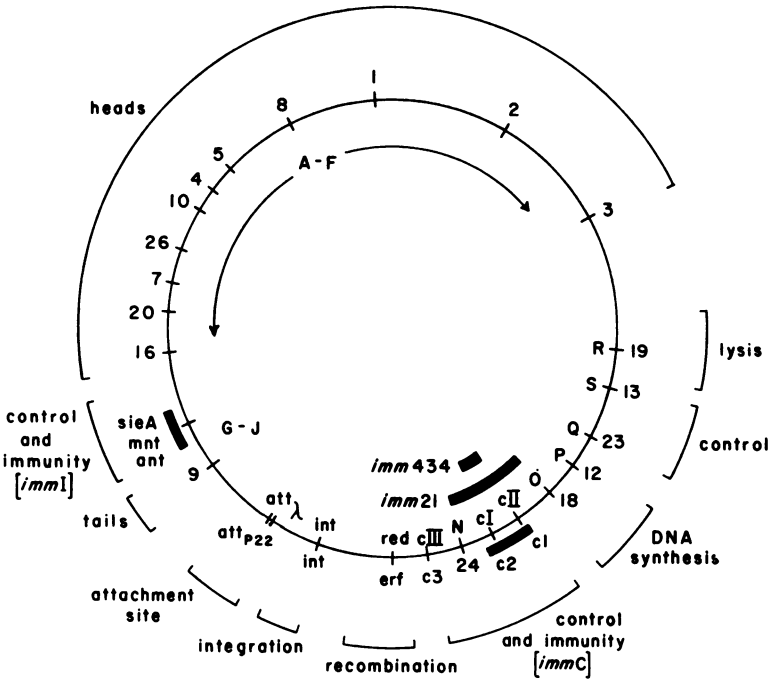


FIG. 3. *Comparison of the maps of phage P22 and coliphage λ. The inside of the circle shows the λ map and the outside shows the P22 map. A solid line connecting markers of the two phages indicates substantial similarity in gene function. The heavy bars inside the λ map indicate the extent of material substituted in the indicated λ variants. The heavy bars outside the P22 map indicate the extents of the immI and immC regions.*

netically defined portions of the P22 genome (9).

Much of the correlation of physical distance with genetic position has involved the use of translocatable drug-resistance elements (10, 21, 75) inserted at genetically defined sites. These insertions can be seen physically in heteroduplex molecules; for example, Chan and Botstein (21) measured the distance between a Tn10 insertion near the attachment site and the attachment site itself by a heteroduplex between P22*pro*1 (which has a deletion/substitution ending at the attachment site) and P22*Tc*-10 (which carries the Tn10 insertion). Since the insertions are very large (Fig. 1), they change substantially the molecular weight of any restriction fragment into which they are inserted. This principle was used by G. Weinstock (Ph.D. thesis) to map physically the *ant* gene; a Tn1 insertion located genetically in the *ant* structural gene changed the mobility of only one *Eco*RI fragment, which must therefore carry some or all of this gene. Finally, the insertions add new cleavage sites to the phage genome; Weinstock (Ph.D. thesis) took advantage of the extra *Bam* site provided by Tn1 to measure the distance from the P22 *Bam* cleavage sites (Fig. 1) to the *Bam* sites in Tn1 insertions located in genes 16 and 20. By a combination of these techniques it has been possible to correlate genetic with physical distance on the P22 map to the degree represented by Fig. 1.

ANATOMY OF THE P22 VIRION

The mature P22 virion is an icosahedral particle ca. 60 nm in diameter with a short baseplate (ca. 20 nm wide) which shows sixfold symmetry (2, 14, 60). A thin spike or fiber (ca. 20 nm long) emanates from the center of the baseplate. Phage heads lacking baseplates can easily be prepared (60); these show a small neck and the spike only (14).

The P22 virion consists of about equal amounts of DNA and protein. The DNA is a single molecule ca. 28×10^6 in molecular weight; its genetic structure and biosynthesis is discussed in detail below. The protein consists of eight (possibly nine) polypeptide chains present in grossly unequal amounts. The major virion protein species is a polypeptide (55,000 daltons) which is the product of gene 5 (14). It is present in about 420 copies per particle and is the major component of the head; it accounts for about 85% of the total protein in the virion. The only known protein of the baseplate (sometimes referred to as the tail) is the product of gene 9 (76,000-dalton polypeptide chain). It is present

in about 18 copies per virion and probably assembles as a trimer. The remaining proteins in the virion are the products of genes 1, 4, 7, 16, 20, and 26; these are present in minor amounts (5 to 20 copies per virion), and their anatomical location is not known for certain. The product of gene 10 might be a ninth polypeptide in the virion; the uncertainty of its presence is due to the fact that its subunit molecular weight is too close to that of the product of gene 5 to permit resolution with the electrophoresis methods which have been used (19, 59).

The remaining products of genes involved in morphogenesis and assembly of the virus have all been identified in infected phage lysates (14, 88). From the phenotypes of mutants in each gene, a role can be assigned in the assembly pathway, as discussed in detail below. The ability to relate genes with their products has clearly been a major factor in the ease with which it was possible to work out the basic features of P22 morphogenesis.

STRUCTURE OF MATURE PHAGE DNA

The DNA found in P22 phage particles is a single linear duplex molecule with a molecular weight of about 28×10^6 and a terminal repetition of ca. 2% (95, 126). In a population of molecules, the DNA sequences are circularly permuted.

The formation of such circularly permuted and terminally repetitious DNA molecules is elegantly explained by a model originally proposed by Streisinger and his collaborators (106, 114, 115) to account for these properties in the DNA of coliphage T4. They proposed that the DNA molecules which serve as the substrate for encapsulation are repeating polymers (concatemers) of the phage genome. If the length of the DNA in mature phage particles is determined by the amount of DNA that can fit into the head, and if the genome length is smaller than the headful, a terminally repeated molecule will be produced when a headful is cut from the concatemer. Thus in the case of P22, a headful is equivalent to the complete wild-type phage genome plus 2%. The Streisinger model also accounts for circular permutation of the phage DNA if it is assumed that headfuls can be cut from the concatemer at different points in the DNA sequence.

The Streisinger model predicts that if the genome size is decreased by a deletion, and the headful size remains constant, the amount of terminal repetition should be increased. This prediction was confirmed for phage T4 by Streisinger et al. (115), who showed by genetic means that crosses of T4 phages with large deletions yield a greater frequency of "terminal redundancy heterozygotes" than crosses of phages carrying short deletions or point mutations. In the case of P22, this prediction was tested physically by examining homoduplexes of DNA molecules from phages with varying genome size with the electron microscope (126). P22*bp*1, a P22 derivative which has a net deletion of about 5%, was found to have a terminal repetition of about 7% (compared to a terminal repetition of 2% for wild-type P22), whereas P22*bp*5, which has a net deletion of about 14%, has a terminal repetition of about 16%. P22Tc-10 has a complete P22 genome plus an insertion of foreign DNA which is about 20% as large as the normal P22 genome; as predicted by the Streisinger model, molecules of this phage have a "negative terminal repetition" of about 18%—that is, they are each missing a portion of the oversized genome corresponding in size to 18% of the wild-type genome. These findings constitute a direct physical proof of the headful packaging model for P22.

The Streisinger model does not specify whether headfuls of DNA are cut randomly from the concatemeric precursor or whether headfuls are cut sequentially once packaging begins on a concatemer. If the DNA is cut randomly, or sequentially starting at random initiation points, the resulting population of DNA molecules is expected to have randomly permuted ends. If, on the other hand, the cutting is always initiated at a specific site and proceeds sequentially on a precursor concatemer that is not too long, the resulting molecules will have limited circular permutation. This is because each sequential headful will have ends differing from the preceding headful by the length of the terminal repetition; if the concatemer is ten headfuls long and the terminal repetition is 2%, the ends of the first and last headfuls will be displaced with respect to each other by only 20% of the genome. The difference between random encapsulation and unique site-sequential encapsulation is illustrated in Fig. 4.

The degree of circular permutation in P22 DNA molecules is in fact restricted; all of the ends are clustered within a region comprising only 20% of the genome. This was demonstrated by Tye et al. (127) by two different electron microscopic techniques. Though other explanations can be made for the clustering of ends on P22, the sequential encapsulation hypothesis makes the unique prediction that the extent of permutation is a direct function of the length of the terminal repetition; i.e., phages with smaller genome sizes and longer terminal repetition should have molecules with ends that are less clustered than those of the wild type. This prediction was confirmed by showing that P22*bp*1,

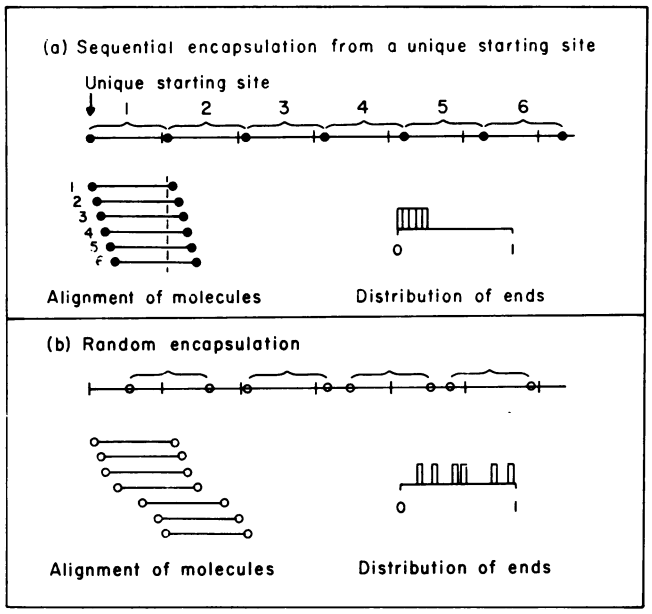


FIG. 4. *Comparison of unique site-sequential encapsulation and random encapsulation. (a) The diagram shows that if the concatemer is only long enough for a small number of headfuls, sequential encapsulation from a unique starting site results in a restricted distribution of ends (i.e., restricted permutation). (b) Random encapsulation results in a random distribution of ends (i.e., random permutation).*

with a terminal repetition of 7%, has ends distributed over 60 to 70% of the map. For P22*bp5*, with 16% terminal repetition, the ends of molecules occur in four discrete regions of the map which are displaced from each other by about 20%; these classes of molecules presumably correspond to the first, second, third, etc., headfuls cut from the concatemer. Tye et al. were able to estimate the number of sequential headfuls taken per concatemer; they found a maximum of 10, although the usual number is probably less.

The electron microscope evidence for limited permutation and sequential packaging of P22 DNA has been fully corroborated by restriction endonuclease maps of the DNA in P22 particles (63, 64). The physical map produced with *Eco*RI endonuclease shows seven cleavage sites in the P22 genome, as illustrated in Fig. 1. The restriction analysis indicates that most of the mature DNA ends are clustered between two particular *Eco*RI sites, confirming limited permutation. This produces a pattern of eight distinguishable fragments, two of which are variable in amount and/or length; these are thought to have one mature end (produced by packaging) and one *Eco*RI end. The other six fragments presumably have two *Eco*RI ends. The details of the restriction patterns of the wild type and various deletion, substitution, and insertion mutants (63) are all consistent with the model derived from the corresponding electron microscope data.

The analysis of mature P22 DNAs, both by electron microscopy (127) and endonuclease digestion (63) indicates not only that packaging of P22 proceeds sequentially from a unique start point, but also that it proceeds in only one direction from that site. The direction of sequential packaging is counter-clockwise relative to the map in Fig. 1 (63). Evidence for unidirectional packaging has also been obtained by characterizing the particles produced by induction of excision-defective P22 lysogens (108, 132).

One fundamental question is the nature and location of the unique start point for encapsulation. The initiation site could be a unique base sequence which signals the encapsulation mechanism to make an endonucleolytic cut and start packaging. Alternatively, the initiation signal could be a physically unique structure such as a free double-stranded end; one possibility is that the end of the tail of a rolling circle (40) is the unique free end at which DNA encapsulation begins. An advantage of this second model is that all encapsulation events would be mechanistically identical. The fact that encapsulation proceeds unidirectionally would simply reflect the structure of such an asymmetric replicative intermediate.

R. K. Chan (Ph.D. thesis, MIT, Cambridge, 1974) obtained genetic evidence that the P22 packaging initiation site is located between genes

12 and 3 on the P22 genetic map. In their *Eco*RI endonuclease analysis of P22 DNA packaging, Jackson et al. (63) concluded that the packaging initiation site is to the right of gene 13. Weaver and Levine (132), using genetic methods, concluded that the packaging initiation site is located near gene 3.

Thus, the evidence for the genetic location of the origin of sequential DNA packaging indicates that it is near gene 3; this would mean that it is not near the only known origin of P22 DNA replication, which is linked to genes 18 and 12 (52). The P22 packaging origin appears similar in location to the unique packaging site of coliphage λ, which packages DNA with specific ends (Fig. 3; 63). However, this finding does not enable us to decide among the possible mechanisms of packaging. An interesting compromise possibility was raised by Tye (125), who proposed that the product of gene 3 (P3) might bind and cut specifically at the unique site for the first encapsulation event and stay with the DNA through the encapsulation process. When a head is finished, P3 would remain bound to the new end of the DNA concatemer to initiate another headful. All encapsulation events would be similar in being initiated by P3 bound to a DNA end. In addition to providing a mechanistic similarity between first and subsequent packaging events, this model has the virtue of easily accounting for the properties of the high-transducing (HT) mutants which are alleles of gene 3 (90; see below).

P22 DNA REPLICATION

Evidence for Concatemers

As demanded by the Streisinger model, concatemeric phage DNA molecules do exist in P22-infected cells; furthermore, these molecules appear to be precursors of the DNA in progeny phage particles (mature DNA). Botstein (7) showed that at early times after infection with ^{32}P-labeled P22, some of the parental phage DNA is extracted in a form (intermediate I) which sediments in neutral sucrose gradients at a rate exceeding 1,000S. Replication apparently takes place in this fast-sedimenting form, since it contains most of the radioactivity incorporated in a short pulse of [^{3}H]thymidine administered at any time after the onset of phage DNA synthesis. The extremely high sedimentation coefficient of intermediate I suggests that it is formed by attachment of phage DNA to some other cell constituent, possibly the cell membrane or wall. If a short pulse is followed by incubation in the presence of excess unlabeled thymidine (chase), the pulse-labeled DNA disappears from the intermediate I position in neutral sucrose gradients and appears, eventually, in a form indistinguishable in sedimentation coefficient from mature phage DNA.

Sedimentation analysis of newly synthesized DNA (native and alkali denatured) indicates that much of it consists of strands at least two to five times the length of the P22 genome. In other words, newly replicated DNA (in intermediate I) has all the properties expected of P22 DNA concatemers.

Maturation of Concatemers

Implicit in the Streisinger model is the idea that cutting the phage DNA to mature size should occur as part of the encapsulation process, since the volume of the phage head is assumed to be the extrinsic factor that determines the length of the DNA. In the case of P22, several observations suggest that removing phage DNA from intermediate I and cutting concatemers to mature size occur as an intimate part of the encapsulation process (11, 14, 88). P22 DNA is normally released from intermediate I and is cut to mature size during the late stage of phage development, when assembly of progeny virions is taking place. Mutants in the late genes 1, 2, 3, 5, and 8 synthesize concatemeric DNA normally, but do not cut it or remove it from the replication complex. These mutants also have in common their production of either no normal head-related structures (genes 5 and 8) or an early precursor form (the prohead; genes 1, 2, and 3). Mutants in all the other known late genes both cut the DNA and encapsulate it. Thus, DNA cutting does not occur in the absence of a proper head structure; when it occurs, it is always accompanied by encapsulation (14, 88).

Replicative Origin of Concatemers

In principle, concatemers could be formed during phage infection by any of several mechanisms, all of which involve recombination events within the terminal repetition. Streisinger et al. (115) pointed out that recombination at the ends of two identical daughters of the infecting linear molecule would produce a dimer. Alternatively, recombination of a molecule with itself would produce a circular monomer. This could produce concatemers via replication by a rolling circle mechanism (40) or via recombination between daughter circles.

In the case of P22, recombination is essential for phage growth, since mutants defective in the phage-specified recombination system (*erf*$^{-}$ mutants, for *e*ssential *r*ecombination *f*unction) cannot grow after infection of recombination-deficient (*rec*$^{-}$) hosts (13, 139). Circularization of

the infecting DNA appears to be the step for which recombination is required, since *erf*⁻ phage grow normally in *rec*⁻ cells after induction of a lysogen (13); in this case, the DNA presumably is circularized by the prophage excision mechanism. In *rec*⁻ hosts infected with *erf*⁻ phage, DNA synthesis is initially normal in timing and in extent, but does not continue beyond about one round of replication, suggesting that circularization is topologically essential for late replication (13). Weaver and Levine (130) carried out temperature-shift experiments with *erf-ts* mutant phage to show that the essential recombination step occurs early after infection, at approximately the time that DNA synthesis stops under totally recombination-deficient (*erf*⁻*rec*⁻) conditions (13). The exact nature of the essential recombination event and the required structure it produces remain somewhat obscure. Closed circular monomers may be the required intermediate, since they first appear after infection at about the same time that the essential event occurs and fail to appear under *erf*⁻*rec*⁻ conditions (131). However, covalent circular molecules never account for more than about 1% of the parental or pulse-labeled DNA present in productive infections (96, 131). Thus, as pointed out by Weaver and Levine (131), the required intermediate could be a nicked circular structure which for technical reasons could not be detected by standard methods.

Summary Model of P22 DNA Metabolism

A simple model for the replicative origin of concatemers is the rolling circle model of Gilbert and Dressler (40). Current information on P22 is clearly consistent with this kind of mechanism, although the existence of the critical intermediate (the rolling circle itself) has not been demonstrated. A consistent scheme summarizing P22 DNA metabolism (13) is shown in Fig. 5.

Upon infection of the host, DNA replication initiates at a specific, unique origin of replication and proceeds to the end of the molecule. Phage DNA synthesis shows an absolute requirement for the products of genes 18 and 12 (8, 11, 80); in the absence of either of these functions, the infecting DNA can enter intermediate I (11), but density transfer experiments show that it is not replicated at all (8). In Fig. 5, replication proceeds unidirectionally to the left; however, Weaver and Levine (132) have obtained evidence that early P22 replication is bidirectional.

For DNA replication to proceed further, recombination must occur (crossing of the dotted line in Fig. 5). In the case of an unreplicated

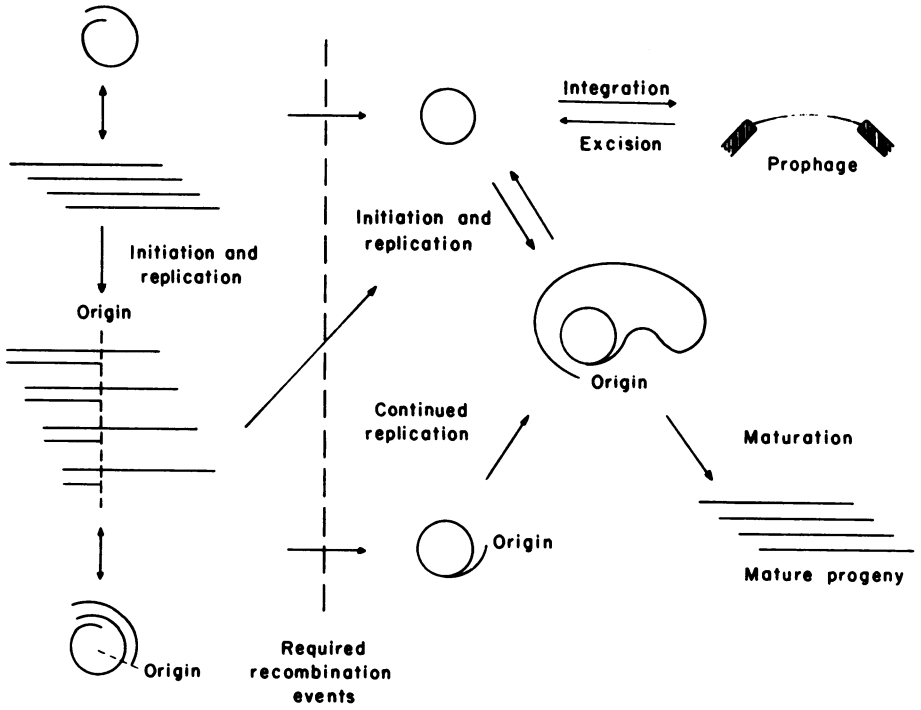


FIG. 5. *Model for the topological transformations in phage DNA during the life cycle of phage P22. Single-headed arrows indicate a change in structure; double-headed arrows show topological equivalence and imply no change. Arrows which cross the dotted line indicate changes that require recombination events.*

molecule, a single recombination event within the terminal repetition can circularize it. In the case of partially replicated molecules (shown with the homology lined up), recombination events between replicated and unreplicated portions of the molecules produce rolling circle forms, which replicate to produce concatemers. As described above, the concatemers are cut to mature length during the sequential headful packaging process, producing a set of permuted and repetitious progeny molecules.

During infections leading to lysogeny, replicated or unreplicated molecules must be circularized to undergo integration as envisioned by the Campbell model (18). This circularization requires recombination events (crossing of the dotted line). P22 $18^{-}ts$ and $12^{-}ts$ mutants fail to lysogenize at 37°C, suggesting a requirement for DNA replication in lysogeny (80). $18^{-}am$ and $12^{-}am$ mutants also fail to lysogenize su^{-} hosts at 37°C, but they lysogenize nearly normally at 26°C (51). Thus, the requirement for phage DNA replication for lysogeny is temperature sensitive. Since the P22 wild-type *int* function is itself somewhat temperature sensitive (51, 111), the temperature-sensitive requirement for DNA replication may mean that the "normal" temperature sensitivity of integration is exaggerated when fewer copies of phage genomes are available as substrate for the integration system (51).

Finally, after induction of lysogens, the prophage excision mechanism produces a circular form. The rolling circle form can therefore be produced without generalized recombination events.

GENERALIZED TRANSDUCTION

Origin of Transducing Particles

The ability of P22 to mediate generalized transduction (141) can be considered to be a simple consequence of the packaging mechanism of the phage. Generalized transducing particles probably result when the host chromosome, rather than the phage concatemer, serves as a substrate for the sequential headful packaging mechanism. In agreement with this view, P22 generalized transducing particles have been shown to contain DNA molecules of the same molecular weight as mature P22 DNA. Furthermore, the DNA in generalized transducing particles consists primarily of host DNA synthesized before phage infection (30, 102).

Schmieger (102) reported that generalized transducing particles contain a small amount of DNA synthesized after infection which is covalently attached to the host DNA synthesized before infection. If the newly synthesized DNA consists of phage sequences, as Schmieger proposed, the mechanism of formation of generalized transducing particles obviously would have to be a good deal more complicated than the mechanism discussed above. Evidence against the joining of host and phage sequences is that normal numbers of generalized transducing particles are formed by erf^{-} phage in rec^{-} hosts—that is, in the absence of any known general recombination system (30).

Several observations suggest that encapsulation of the host chromosome to form transducing particles (like encapsulation of the phage concatemer to form infectious phage) involves sequential packaging from preferred starting points. Chelala and Margolin (23) found that a deletion in the chromosome of a transductional donor can alter the cotransduction frequencies for a pair of markers located wholly to one side of the deletion. This is true even if both the donor and the recipient carry the deletion, so that homology in the region of the deletion is preserved. In one case, the effect is observed even though the deletion is known to be too far away to be included in the same headful of DNA as the selected markers.

These results can easily be explained if it is assumed that transducing DNA fragments are formed by sequential encapsulation from a small number of preferred starting points in the host chromosome. If the headful cutting mechanism were reasonably precise, each start point for encapsulation would produce a set of transducing fragments with fixed genetic end points. The cotransduction frequency for a pair of markers would then be determined by the number and efficiency of those start points that generate transducing fragments carrying both markers, relative to the number and efficiency of those start points that generate fragments carrying the markers separately. A deletion near a pair of markers could alter the position of the markers with respect to start-point sequences, eliminate one or more such sequences, or create a new start-point sequence; any of these changes could significantly alter the frequency with which the markers are both included on the same transducing fragment, thereby altering their cotransduction frequency.

As Chelala and Margolin (23) point out, the above model can also explain the fact that in P22 transductions some markers are carried on genetically homogeneous transducing fragments (85), while others are carried on transducing fragments of heterogeneous genetic composition (33, 72, 86, 101). By the sequential encapsulation hypothesis, the former would be markers carried on transducing fragments formed primarily from only one start point for encapsulation or from two or more start points that have the same

sequential register. The latter would be markers that are encapsulated from two or more start points of approximately equal efficiency that are not in register with each other.

The idea that packaging of the host chromosome is initiated at specific sites of varying preferability could also explain the observation that certain bacterial markers are transduced by wild-type P22 at much higher efficiency than others (30, 103). The specificity of the encapsulation process is also demonstrated by the fact that P22 encapsulates far more phage DNA than host DNA, although there is at least as much host DNA as phage DNA in the infected cell (30, 102); presumably this is because P22 DNA has more or better initiation sites than host DNA.

The relationship of the phage DNA encapsulation mechanism to generalized transduction is most clearly demonstrated by the properties of the HT mutants of P22 isolated by Schmieger (103). These mutants, some of which appear to be alleles of gene 3 (90), encapsulate a larger proportion of host DNA than wild-type P22 encapsulates (as much as 50% as opposed to 1 to 5%) (30, 103). HT mutants display altered specificity for host sequences; different bacterial markers are transduced at widely varying efficiencies by wild-type P22, but are transduced at a constant high frequency by HT phage (103). The HT mutants also exhibit alterations in cotransduction frequencies for linked markers (104).

The altered specificity of packaging by HT mutants extends to packaging of phage DNA as well. Tye (125) demonstrated that phage DNA produced by one HT mutant, unlike wild-type DNA, is randomly permuted. This observation suggests that the same DNA encapsulation mechanism is responsible for the formation of both generalized transducing particles and infectious phage particles, since the HT mutation alters the specificity of both processes.

Mechanism of Integration of Transducing DNA

On the basis of genetic evidence, it has long been thought that most DNA from generalized transducing particles, after entry into a new (recipient) host, expresses the information it carries but does not replicate or recombine with the host chromosome (85, 113); the transducing DNA persists indefinitely, forming "abortive" transductants. Only a small fraction (<10%) of the transducing particles mediate stable or "complete" transduction, in which transducing DNA undergoes recombination with the host DNA. Ebel-Tsipis et al. (31) investigated the physical fate of transducing DNA in recipient bacteria and found that most of this DNA retains its original size, does not replicate, and is not integrated. Presumably, these molecules account for the phenomenon of abortive transduction.

Ebel-Tsipis et al. also found that a small fraction (2 to 5%) of the total DNA in generalized transducing particles becomes integrated into the host chromosome as substantial segments of conserved duplex DNA. Incorporation of these segments (2×10^6 to 10×10^6 daltons) appears to be responsible for the recombinant bacteria called "complete" transductants. This finding shows that the integration of DNA in generalized transduction (i.e., by the host recombination functions) involves double-strand breakage and joining of DNA.

SPECIALIZED TRANSDUCTION

Specialized transduction is the process by which bacterial genes are carried from one host to another by becoming incorporated physically into the genome of a temperate phage. Recipient bacteria acquire these bacterial genes when they are lysogenized by the specialized transducing phage. Specialized transduction therefore usually results in gene addition; the transductant carries the transducing material as long as the prophage is present (either in the integrated state or as an extrachromosomal element), but loses the transducing material if the prophage is lost. If the bacterial genes carried on the specialized transducing phage are homologous to genes in the recipient chromosome, recombination can result in gene substitution, producing transductants that are stable regardless of whether the transducing phage remains. However, the formation of such substitution transductants via recombination is usually rare compared to the formation of addition transductants via lysogenization of the specialized transducing phage.

Specialized transducing phages can be formed by at least two different mechanisms: (i) aberrant excision of an integrated prophage, resulting in incorporation into the phage genome of bacterial genes adjacent to the prophage attachment site on the bacterial chromosome; and (ii) direct insertion into the phage genome of translocatable elements. The first of these mechanisms was proposed by Campbell (18) to account for the formation of certain specialized transducing derivatives of coliphage λ, but appears to apply also in the case of some P22 specialized transducing phages (21, 58, 67, 68, 71, 112, 137). For example, P22 *pro*-1 and *pro*-3 are derivatives that carry the *proA* and *proB* genes which nor-

mally are located on the *S. typhimurium* chromosome adjacent to the P22 prophage attachment site (67). Genetic and physical evidence strongly indicates that these transducing phages were formed by an aberrant prophage excision event as envisioned in the Campbell model (21, 68).

The second mechanism listed above was proposed by Chan and Botstein (21) and Kleckner et al. (76) to explain the origin of P22Tc-10, a specialized transducing phage carrying genes for tetracycline resistance from an R-factor (22, 129). This mechanism also seems to apply to derivatives of other phages which have acquired R-factor drug resistance determinants (17).

Although formed by different mechanisms, P22 *pro*-1 and Tc-10 share certain properties which can be explained by taking into account the headful packaging mechanism of the phage (21, 22, 68). Because of the addition of bacterial DNA, both Tc-10 and *pro*-1 have genomes that are too large to fit inside the phage head. When a lysogen of Tc-10 or *pro*-1 is induced, a normal number of particles is produced, since both phages have all of the phage genes required for vegetative growth. Circularization of the genome, which is required for extensive DNA replication (see above), is presumably accomplished by the prophage excision mechanism (as in *rec*$^-$ lysogens of P22 *erf*$^-$). When the Tc-10 or *pro*-1 concatemers are packaged, the DNA is cut to the usual size, so that the resulting molecules in the progeny particles are each missing part of the oversize genome and do not have terminal repetition. Without terminal repetition, these DNA molecules are unable to grow or to lysogenize on single infection. However, because they are circularly permuted, different DNA molecules in a population are missing different regions of the genome. In a double infection, the DNA molecules from two particles will usually be able to form a circle by recombining with each other. In this way the oversize genome is reconstituted, and can either lysogenize or produce progeny particles (22).

An interesting corollary of these findings is the idea that P22 can mediate high-frequency specialized transduction of very large segments of DNA. Even if the specialized transducing phage genome were almost as large as two headfuls, the permuted molecules in particles produced on induction of a lysogen could still occasionally transduce recipients by double infection. This situation can be contrasted with that of phages such as λ, which package DNA by a site-specific mechanism that produces molecules with unique ends. Such phages have a much lower limit on the size of specialized transducing phage genomes; genomes larger than about 109% of wild-type λ are not efficiently packaged (34, 133).

LYSOGENY

Since P22 is a temperate phage, there is an alternative to the lytic cycle of growth after infection of a host cell. This alternative, called lysogeny, results in the survival of the infected cell. The survivor usually contains in its DNA the genome of P22 in the prophage state; all P22 genes are present, but those involved in growth of the virus (and the concomitant destruction of the host cell characteristic of lytic growth) are not expressed. The P22 prophage can remain in this dormant state for an unlimited number of host cell generations; only certain conditions (e.g., irradiation with ultraviolet light, interference with host DNA replication) will cause the prophage to become active and enter into the lytic cycle once again. The means by which this dormant prophage state is maintained is the subject of the next section. In the section which follows that, we will summarize what is known about how the lysogenic state is established by P22.

Repression and Immunity

The primary mechanism of maintenance of P22 lysogeny is repression of gene activity. In principle, a repression mechanism works by the elaboration of one or more repressors which act to prevent the expression of genes involved in phage reproduction and concomitant killing of the host cell. In a cell in which repression has been established, a superinfecting phage homologous to the prophage also cannot grow, since it too will be sensitive to repression. This property of lysogens is called immunity; it is defined as prevention of growth of superinfecting homologous phage specifically by the same repression system which prevents lytic growth of the prophage.

The repression/immunity phenomenon lends itself to genetic analysis. Several classes of mutants can be found which shed light on the way in which repression works. Mutants in the gene(s) encoding the repressor protein(s) should be unable to maintain the lysogenic state. In particular, temperature-sensitive alleles in repressor genes should allow maintenance of the lysogenic state at a permissive temperature, but repression should fail if mutant lysogens are shifted to a nonpermissive temperature. Another expected mutant class should be insensitive to repression; such mutants can be recognized by their ability to express some or all lytic functions after superinfection of an immune lysogen. Mutants which can express all lytic functions will grow in the immune lysogen; such mutants are

called virulent mutants. The exact phenotypes of virulent mutants, their dominance relationships, and their location on the genome have been very informative about the nature of the P22 repression system (Table 1).

Using such a genetic approach, the immunity/repression system of phage P22 has been elucidated in some detail (12, 15, 16, 20, 42, 45, 78, 81, 82, 116, 123, 142). P22 prophages maintain the lysogenic state by directing the synthesis of two repressors, both of which are continuously required for the maintenance of repression in the cell. Each of the two repressors contributes to immunity, and mutants insensitive to either repressor are able to grow in immune lysogens. (A technical complication in the study of P22 repression is that P22 prophages express, in addition to the repressors, several other relatively nonspecific barriers to superinfection by related temperate phages. These systems, called superinfection exclusion systems to distinguish them from immunity, act on phages whether or not they are sensitive to the repression system elaborated by the prophage. Mutant P22 phages have been isolated (called *sie*$^-$ for *s*uper*i*nfection *e*xclusion) which are defective in the superinfection exclusion systems (92, 118, 128). Such *sie*$^-$ mutants have been used in all the experiments involving immunity and repression.)

In having two repressors, P22 differs from coliphage λ, which maintains its immunity/repression system with a single repressor. The molecular basis of repression and immunity in the case of λ is quite well understood. λ repressor binds DNA specifically at two operator sites; binding prevents essentially all transcription from the λ prophage, except for the transcription of the repressor gene itself (see 32 and 48 for reviews).

The current model (12, 82) for the maintenance of P22 immunity and repression by the two repressors is presented in Fig. 6. The *immC* region contains the gene for the *c*2 repressor, which acts at two sites (O_L and O_R) to prevent expression of the early genes. In this way, P22 is like λ. This view of the *immC* region is supported by the finding that λ and P22 can recombine to form hybrids which have substituted the P22 *immC* region for the λ immunity region; these λ *immP22* hybrids are normally regulated temperate lambdoid phages (9, 39, 52). A protein which binds specifically to P22 DNA and which appears to be the *c*2 gene product has been detected in several laboratories (45, 105; M. Ballivet and H. Eisen, Université de Genève, Geneva, Switzerland, unpublished data; A. R. Poteete and M. Ptashne, Harvard University, Cambridge, Mass., unpublished data).

The P22 *immI* region contains the gene for the *mnt* repressor. It is not known how the *mnt* repressor exerts its effect. However, it seems to involve a site on the P22 DNA marked by the *Vy* mutations, which are partially or completely insensitive to the *mnt* gene product. The *immI* region also contains the gene for an antirepressor (*ant*) which is expressed only when the *mnt* repressor is absent or inactive. P22 antirepressor is a protein which inactivates phage repressors, including the P22 *c*2 repressor and the *c*I repressor of coliphage λ (116).

The model thus envisions different reasons for the requirement for each of the two P22 repres-

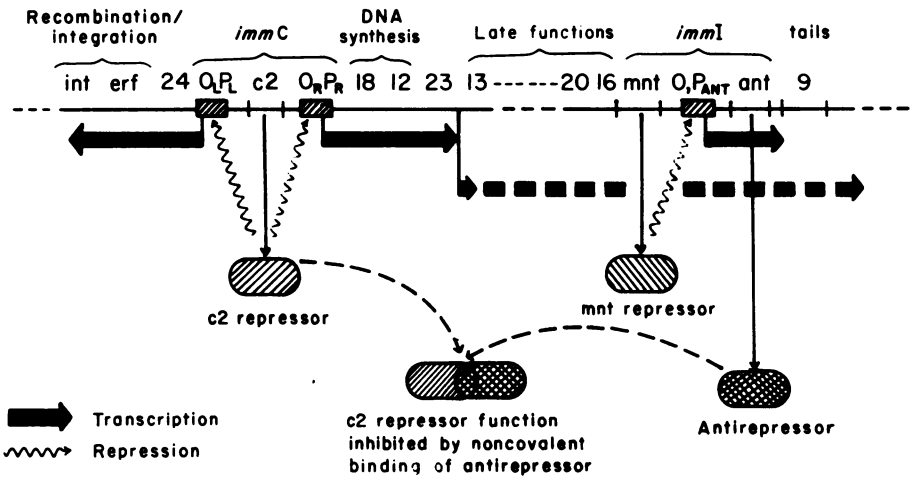


FIG. 6. *Antirepressor model for the bipartite repression and immunity system of phage P22. The prophage genetic map of P22 is shown. The probable pattern of transcription is shown by thick arrows; discontinuous arrows indicate transcription dependent on gene 23 function. Wavy arrows indicate negative control by repressors acting at operator/promoter sites, shown in hatched boxes.*

sors in maintenance of repression and immunity. When the *c*2 repressor is not active, the lytic functions are expressed directly. When the *mnt* repressor is not active, antirepressor is produced, which results in inactivation of the *c*2 repressor and consequent expression of the lytic functions. This distinction between the functions of the two repressors is reflected in the properties of mutants in the various genes involved. The phenotypes of all these mutations are summarized in Table 1; they constitute the genetic evidence substantiating in detail the bipartite immunity model (12, 82).

Analysis of these mutations was primarily responsible for the historical development of ideas about the nature of P22 immunity and repression. In 1957, Levine described the isolation of clear-plaque mutants, defined the tightly-linked *c*1, *c*2, and *c*3 genes by complementation, and showed that *c*2 function is required for maintenance of lysogeny (78, 79). In 1958, Zinder described a mutation (now called *mnt*1) which is not closely linked to Levine's *c* genes but which also affects maintenance of lysogeny (142). Campbell in 1962 was probably the first to suggest that these observations might mean that P22 repression involves more than one repressor (18). In 1964, Levine and Smith used a temperature-sensitive clear-plaque mutation to show more directly that *c*2 product is continuously required to maintain lysogeny (81); later, Gough used a temperature-sensitive *mnt* mutation to make a similar argument about the *mnt* gene product (42). Virulent mutants in the *immC* region of P22 were first isolated in Levine's laboratory; *mnt*-linked virulent mutants and antirepressor mutants were isolated simultaneously in the Levine and the Botstein laboratories (12, 15, 16, 82). Also important in the development of the bipartite immunity model was the discovery by Bezdek and Amati that two independent determinants of immunity specificity segregate in crosses between P22 and a heteroimmune phage (5). Further evidence for a bipartite immunity system was provided by Chan and Botstein, who isolated non-overlapping prophage deletions which have lost superinfection immunity (20). Finally, it should be reemphasized that the characterization of mutations affecting P22 immunity was possible only after 1968, when Rao isolated P22 mutants which, as prophage, do not exclude superinfecting phage (92).

Antirepressor

The strongest prediction of the bipartite im-

TABLE 1. *Phenotypes of regulatory mutants of phage P22*

Mutation	Mutant phenotype	Map location	Function	Reference
c2-ts	Failure to establish or to maintain lysogeny at 40°C; 30°C lysogens are induced at 40°C	*immC*	*c*2 repressor	78, 81
Vx	Partially virulent, constitutive to left of *c*2	*immC*	Operator/promoter (O_LP_L), site of repression by *c*2 repressor to the left	16
K5	Partially virulent, constitutive to right of *c*2	*immC*	Operator/promoter (O_RP_R), site of repression by *c*2 repressor to the right	16
VxK5 = *virB*	Virulent, grows on lysogens without inducing prophage	*immC*		15, 16
mnt-ts (recessive)	Failure to maintain lysogeny (40°C); 30°C lysogens are induced at 40°C	*immI*	*mnt* repressor	42
Vy (cis dominant)	Virulent, inducing (sometimes weak), constitutive for the *ant* gene	*immI*	Operator/promoter (O_{ANT}-P_{ANT}), site of repression by *mnt* repressor	12, 15, 82
Vyc2 = *virA* (dominant)	Virulent (enhanced), inducing (i.e., prophage also grows after superinfection of lysogen)	*immI*		12, 15, 82
ant (recessive)	Fails to grow on *immI*-deletion lysogens; suppresses *mnt* and *Vy* mutations; fails to make antirepressor	*immI*	Antirepressor, inactivates *c*2 repressor	12, 82

munity model for P22 is the existence of an antirepressor protein, the product of the *ant* gene. Susskind and Botstein (116) established an assay for this protein based on its ability to inactivate the coliphage λ repressor in vivo and in crude extracts. They examined the properties of the antirepressor-repressor interaction and concluded that the antirepressor exerts its inactivating effect upon the λ repressor by binding to it in a noncovalent way, thereby preventing binding of the repressor to operator DNA. The antirepressor protein has subsequently been purified to homogeneity and shown to consist of subunits with a molecular weight of 30,000 (Susskind and Botstein, unpublished data).

Several properties of the antirepressor seem worthy of comment. First, the P22 antirepressor acts on a variety of different temperate phage repressors, including those of the *Salmonella* phages L and P22 and the coliphages λ and 21. Second, the antirepressor does not act upon the repressor with the same specificity or by the same mechanism as induction by irradiation with ultraviolet light or other interference with DNA metabolism. The latter kind of induction results ultimately in proteolytic cleavage of the repressor (99). The difference in specificity is further shown by the fact that induction with antirepressor works in *rec*A hosts (where ultraviolet induction does not occur) and by the fact that the antirepressor works on mutant repressors insensitive to ultraviolet induction (12, 116). Third, it must be stressed that the antirepressor function is not essential to any of the normal steps in the P22 life cycle; lysogeny and the lytic pathway are normal in *ant*$^-$ mutants (12, 82) and even in phages carrying deletions of the entire *immI* region (Weinstock, Ph.D. thesis). The only visible effects of antirepressor mutations occur upon superinfection of lysogens. This fact strongly distinguishes mutations in the antirepressor gene of P22 from mutations in the *cro* gene of phage λ which are viable only under special circumstances (i.e., in the presence of moderate amounts of active λ repressor). The P22 antirepressor also differs from the λ *cro* product in that the latter inhibits repressor synthesis and the former acts directly on the repressor protein (see 32 and 48 for reviews).

Establishment of Lysogeny

After a sensitive cell is infected with P22, the cell enters one of two pathways. One pathway leads to phage reproduction and lysis; the other leads to repression and ultimately the formation of stable immune lysogens. The "decision" between these two fates is affected by many factors, both environmental and genetic. Levine showed that the most prominent environmental factor is multiplicity of infection; high multiplicity strongly predisposes toward lysogenization, whereas single infections virtually always follow the lytic pathway (78). The genetic factors include the activities of several phage genes and several host genes. It should be emphasized that a simple blockade (by mutation) of either the lytic or lysogenic pathway does not necessarily predispose the infected cell toward the alternative fate. Mutations in the lytic pathway (e.g., in head assembly or lysis functions) result not in increased frequencies of lysogenization but rather in abortive lytic cycles; likewise, defects in the genes required for prophage integration result in abortive lysogeny and not in lysis. Analysis of phage and host mutations which alter the normal regulation of the decision between lysis and lysogeny has provided evidence that the decision between the alternative fates is made early after infection, primarily through the regulation of *c*2-repressor synthesis. When lysogeny is preferred *c*2-repressor synthesis begins early at very high levels; when the lytic pathway is preferred, one finds little or no synthesis of active repressor early after infection.

***c* genes.** The first requirement for establishment of lysogeny is that the infected cells survive the infection. There are at least two early phage functions whose expression is lethal to the cell; these functions are directly controlled by *c*2 repressor (51; Hilliker, Ph.D. thesis). Thus, the early synthesis of adequate amounts of *c*2 repressor is essential to cell survival and thus establishment of lysogeny.

Mutations in three different complementation groups result in inadequate synthesis of repressor early after infection and consequent poor cell survival; they are easily recognized because they form clear plaques (having no surviving cells in the center of the plaque) (78). One of these complementation groups (*c*2) represents the structural gene for the *c*2 repressor. Levine showed that mutants defective in the other groups (*c*1 and *c*3) have an intact *c*2 gene and can express this gene to levels adequate for the maintenance of lysogeny, since stable lysogens which carry *c*1 or *c*3 mutant prophages can be formed by mixed infection with *c*2 mutants (78). Thus, the *c*1 and *c*3 genes are not essential for *c*2 gene expression per se, but only for stimulation of this expression to the early high levels apparently required for cell survival immediately after infection. Failure of *c*1 and *c*3 mutants to synthesize high levels of *c*2 repressor early after infection has been verified by direct biochemical assay (45, 105).

Thus, the *c*1 and *c*3 genes affect the decision between lysis and lysogeny by strongly stimulating *c*2-repressor synthesis after infection. Even-

tually, *c*2-repressor synthesis continues in the absence of the products of genes *c*1 and *c*3, since the map positions of these genes indicate that they are eventually repressed in lysogens (15, 16, 79). It should be noted that *mnt* mutants do not form clear plaques (42, 142). Cell survival is high after *mnt*⁻ infection, even though stable lysogens are not formed (142). Thus *c*2 repressor, but not *mnt* repressor, is involved in establishment as well as maintenance of lysogeny.

The mechanism of *c*1 and *c*3 stimulation of *c*2-repressor synthesis is probably similar to that proposed by Reichardt and Kaiser (94) to explain the same situation in coliphage λ. They proposed that there are two promoters capable of initiating transcription of the repressor gene: one (P_{RE}, for *p*romoter for *r*epressor *e*stablishment) is stimulated by *c*1 and *c*3, resulting in high levels of repressor soon after infection; the other (P_{RM}, for *p*romoter for *r*epressor *m*aintenance) is independent of the *c*1 and *c*3 gene products and results in low levels adequate for maintenance, but not establishment, of lysogeny.

There is one unusual P22 clear-plaque mutation which strengthens the analogy between P22 and λ repressor establishment. This mutation, *c*27, appears to be defective in *c*2-repressor synthesis during establishment, but not maintenance, of lysogeny. However, it complements all *c*1 and *c*3 mutants (45, 79, 123). It is exactly analogous in phenotype and map position to the λ *cy* mutants (94); both the λ *cy* and P22 *c*27 mutations map beyond O_RP_R and presumably lie outside the repressor structural gene (16, 79). Like the λ *cy* mutations, P22 *c*27 is a candidate for a mutation in the site of *c*1 and/or *c*3 gene action and/or the P_{RE} promoter (45, 123). Arguments are presented below which suggest that *c*27 is not a mutation in P_{RE} itself.

Smith and Levine (109) showed that the patterns of DNA synthesis after infection are strongly affected by mutations in the *c* genes of P22. In particular, they showed that the *c*1 and *c*3 functions are responsible for a transient "repression" of phage DNA synthesis after infection (26). This delay in DNA synthesis is not due to synthesis of *c*2 repressor, since it occurs even in *c*2⁻ mutants. Botstein (Ph.D. thesis, University of Michigan, Ann Arbor, 1967) showed that the delay is also absent in wild-type P22 infections if the multiplicity of infection is low. The *c*1/*c*3-dependent delay in DNA synthesis seems therefore to reflect the process of decision between lysis and lysogeny. The mechanism of this DNA delay is not understood. It has been suggested that the delay may result because high levels of transcription leftward from P_{RE} prevent the rightward transcription from P_R known to be necessary for DNA synthesis (16, 45).

***cly* mutations.** Hong et al. (56) observed that wild-type P22 forms clear plaques on certain mutants of its normal host, *S. typhimurium*. Many different kinds of bacterial mutations cause P22 to make clear plaques, and there is as yet no obvious connection linking all of them. Nevertheless, Hong et al. were able to find mutants of P22 which have regained the ability to form turbid plaques on such mutant hosts. The phage mutants (called *cly* for *c*ontrol of *ly*sogeny) frequently fail to make any plaque at all on wild-type hosts, apparently because they invariably choose the lysogenic pathway over the lytic pathway after infection. The *cly* mutations map between the *c*1 gene and gene 18 (DNA replication) on the P22 genetic map. In mixed infections with wild-type P22, the *cly* mutants are generally dominant (i.e., the mixed infections produce little or no progeny) (100). Biochemical assays of *c*2-repressor activity show that three different *cly* mutants overproduce *c*2 repressor; one of them makes as much as 20 times the normal amount of *c*2 repressor (124; Botstein, unpublished data). This overproduction could nicely account for the dominance of *cly* mutations and suggests that *cly* mutations are not simple loss of a functional protein.

Using an in vitro transcription system, Roberts et al. (100) showed that the *cly* mutations result in an alteration in one of the five primary transcripts made from P22 DNA (see below). M. Rosenberg and S. Hilliker (National Institutes of Health, unpublished data) have evidence that the *cly*3 mutation results in a base change within this RNA.

Transcription-termination model for the regulation of repressor synthesis during the establishment of lysogeny. In Fig. 7, the primary transcripts made from P22 DNA in vitro are shown. The one marked "*b* transcript" is the one altered by *cly* mutations. Clearly, if one imagined that genes *c*1 and *c*3 function directly or indirectly to continue the *b* transcript all the way through the *c*2 gene, then one could nicely account for *c*1/*c*3 stimulation of *c*2-repressor synthesis early after infection (100).

By this model, P_{RE} is not the site marked by *c*27 (as suggested by Gough and Tokuno, 45, 123), but is instead located further to the right at the beginning of the *b* transcript. The *c*27 mutation would then be a mutation which creates, enhances, or renders *c*1/*c*3-insensitive a secondary premature termination site. The *cly* mutations would, by this hypothesis, either decrease the frequency of termination of the *b* transcript or greatly increase the efficiency of the P_{RE} promoter.

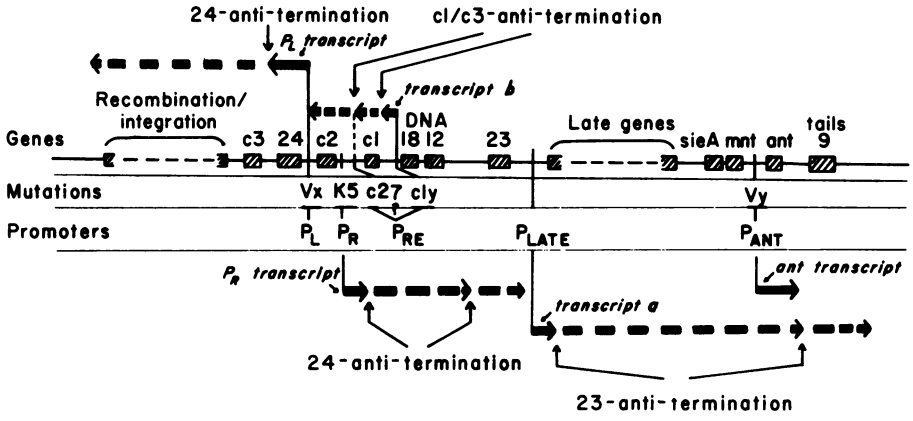


FIG. 7. *Model for transcription of phage P22. The line labeled "genes" shows the prophage map of P22, including the functions of some of the gene clusters. Below the map are shown regulatory site mutations and promoter sites, which are defined both by the regulatory mutations and by in vitro transcription experiments (see text). Leftward transcription is shown above the map. The P_L promoter produces a short transcript in vitro which is thought to be extended (antiterminated) in vivo by the action of gene 24 product, thereby allowing expression of the recombination/integration gene cluster. Roberts et al. (100) proposed that the in vitro transcript b is the product of the P_{RE} promoter and is extended (antiterminated) in vivo by the action of the c1 and c3 gene products. Rightward transcription is shown at the bottom of the figure. The P_R promoter produces a short transcript in vitro which is thought to be extended (antiterminated) in vivo by the action of gene 24 product, thereby allowing expression of genes c1 through 23. The in vitro transcript a is thought to be the product of the P_{LATE} promoter and to be extended (antiterminated) in vivo by the action of gene 23 product, allowing expression of all late genes, including gene 9. A fifth in vitro transcript is thought to be from the P_{ANT} promoter, the early gene 23-independent promoter for ant which is under negative control by mnt-repressor (12, 82). This transcript is not extended into gene 9 in vivo (Lew, Ph.D. thesis; Weinstock, Ph.D. thesis), suggesting that a termination site between ant and 9 is overcome by gene 23 product when transcription initiates at P_{LATE} but not when transcription initiates at P_{ANT}.*

This model can also explain the regulation of repressor synthesis during the establishment of lysogeny by λ. In the case of λ, the corresponding minor leftward transcript differs in size and nucleotide sequence from the P22 *b* transcript and has been called the λ *oop* RNA (6). It maps between the *cII* gene (analogous to the P22 *c1* gene) and the *O* gene (DNA replication)—that is, in a position analogous to that of the *b* transcript of P22. Not only do the map positions of these RNAs correspond, but they also have in common a run of pyrimidines at their 3′ ends. A similar sequence has been found at the 3′ end of the "leader sequence" in the *trp* operon of *E. coli*, which is the site at which transcription termination is regulated by a mechanism sensitive to the level of tryptophan in the cell (4, 77). For λ, this model would place P_{RE} at the beginning of the *oop* RNA. The *cy* mutations would, like P22 *c27*, represent secondary transcription termination sites. The *cII* and *cIII* functions of λ (like the *c1* and *c3* functions of P22) would then be positive control elements preventing termination and thereby ensuring transcription of the repressor gene.

This view of the biological significance of the λ *oop* and P22 *b* transcripts demands that these RNAs be essential parts of the immunity regions of λ and P22, respectively. In crosses between P22 and λ, it was shown that the small RNAs (*b* and *oop*) cosegregate with immunity specificity, rather than with the genes for DNA replication (52, 100). The λ *oop* RNA had previously been thought to be involved in DNA replication (46); the transcription termination model, while not necessarily excluding absolutely a role for the minor leftward RNAs in DNA replication, would suggest otherwise.

It is also possible that both the region from which the *oop* or *b* transcripts derive and the region marked by *cy* or *c27* can act as promoters for repressor synthesis during establishment of lysogeny (57, 84; I. Herskowitz, University of Oregon, Eugene, unpublished data).

Host genes. In addition to the host mutants mentioned above which cause P22 to prefer the lytic pathway after infection (and thus form clear plaques), host mutants have been found which enhance the frequency of the lysogenic response. One of these (*pox*) was isolated as a polymyxin-sensitive mutant and displays greatly enhanced lysogenization by P22, even at low

multiplicity of infection; only *c* mutants of P22 plate on this strain (122).

Although some of these host mutations have suggestive properties (e.g., the hosts on which P22 forms clear plaques include mutants defective in catabolite repression genes and mutants with known alterations in RNA polymerase [56]), no clear conclusion about the nature of host involvement in the decision between lysis and lysogeny after P22 infection has yet emerged.

Integration and Excision of Prophage

After repression and immunity have been established, the final step in the formation of a stable lysogen is integration of the phage genome into the host chromosome. This occurs primarily at a particular site (*ataA*, between *proA* and *proC* on the *Salmonella* genetic map), although secondary sites at which integration can occur are known (93, 110). Smith and Levine (111) showed (for the first time in any temperate phage) that integration is under the control of a specific phage gene, now called *int*. In every way that has been examined, this process is identical to integration of λ prophage into the chromosome of *E. coli* as first understood by Campbell (18) (see 134 for review). Mutations of P22 affecting excision but not integration (*xis* mutants, for *excise*) are also known, although little characterization of these has been done (H. O. Smith, Johns Hopkins University, Baltimore, Md., unpublished data).

One interesting problem in integration of P22 prophage by the Campbell model is the origin of the circular phage DNA substrate. Circularization of P22 DNA after infection is accomplished by recombination within the terminal repetition (13, 130, 131); in the absence of general recombination, integration of prophage does not occur.

Smith (108) showed that integration-deficient mutants of P22 are always also defective in prophage excision. In the absence of *int* function, induced integrated prophages replicate in situ; sequential packaging then proceeds from the start-point of encapsulation into the adjacent portion of the bacterial chromosome, producing a normal burst of defective particles which are highly enriched for transducing particles for nearby host genes (e.g., *proC* and *purE*) (108, 132).

Superinfection Exclusion and Lysogenic Conversion

Like many other temperate *Salmonella* phages (3, 138), P22 prophage expresses functions which act to prevent superinfection of the lysogenic cell. These functions differ in several important respects from the phage immunity/repression system. First, the superinfection exclusion systems do not play a role in maintaining the lysogenic state and are not specific for homoimmune phages. Second, the three known exclusion systems work by three different mechanisms, each of which is different from the immunity/repression mechanism. As mentioned above, these exclusion systems had to be understood and dealt with in order to analyze genetically the immunity/repression system.

The first mechanism that *Salmonella* prophages elaborate to prevent superinfection is chemical modification of the cell surface component (the O antigen), which is the site of adsorption of the phage. In the case of some phages (e.g., ϵ^{15} and ϵ^{34}; 3, 138), this modification completely abolishes the adsorption site. In the case of P22, the alteration of the adsorption site is less drastic; P22 lysogens still adsorb P22 virions, but less efficiently (92, 118). Mutants of P22 which lysogenize normally but which do not, as prophages, alter the cell surface have been isolated (140). These mutations (called *a1*, since they alter the antigenic properties of the lysogenic cell) map at the righthand end of the P22 prophage map (to the right of gene 9) (Fig. 2) (44).

The second mechanism that P22 elaborates to prevent superinfection of P22 lysogens (*sieA*, for superinfection exclusion) works by preventing entry of the DNA of superinfecting phages into the cell (117). Mutants of P22 which lack this property have been isolated (92, 118, 128), and the mutations have been mapped to a locus immediately to the left of the *mnt* repressor gene (117). *SieA*$^+$ lysogens exclude DNA from P22 virions regardless of its genetic content; even the DNA in generalized transducing particles (i.e., host DNA) and heteroimmune phage DNA are excluded (29, 117). The *sieA* exclusion system can be saturated by high multiplicities of infection (29, 117); presumably the cell surface of *sieA*$^+$ lysogens has a limited number of sites or molecules which are specified directly or indirectly by the *sieA* gene and which are responsible for preventing DNA uptake.

The bipartite immunity system of P22 allows virulent phage mutants (i.e., *virA* mutants, which produce antirepressor constitutively) to arise in a single step. In the absence of a mechanism to exclude superinfecting phage, such virulent mutants (which arise at a frequency of about 10^{-7}) would grow, accumulate, and destroy lysogenic cultures. In fact, this phenomenon is observed in cultures of P22 *sieA*$^-$ lysogens (117). It is therefore easy to understand why the antirepressor mechanism of bipartite immunity is associated with superinfection exclusion systems, and, further, why the *sieA* gene is so

closely linked to the *mnt* and *ant* genes.

The third system of superinfection exclusion (*sieB*) is less well understood (118, 119). It acts upon certain heteroimmune *Salmonella* phages, including phage L. Although it does not act on wild-type P22, it does exclude some P22/L hybrids which have both the *immC* and *immI* regions of P22; sensitivity to *sieB* exclusion is thus separable from the immunity/repression determinants. Superinfecting phages sensitive to the *sieB* system adsorb, inject, and express their early functions normally in *sieB*$^+$ lysogens. However, they are incapable of completing a normal lytic cycle of growth. Late functions are incompletely expressed because a synchronous failure in all macromolecular synthesis occurs approximately midway through the developmental cycle. P22 mutants missing the *sieB* system have been isolated; the *sieB* gene maps to the left of *immC* (Fig. 2).

The ability of wild-type P22 to escape *sieB* exclusion is due to a gene (called *esc*, for *esc*ape) which also maps to the left of *immC* (119). Expression of this gene prevents the premature arrest of macromolecular synthesis and allows even *sieB*-sensitive phages to grow in mixed infection with *sieB*-insensitive phages. The *sieB* system thus formally resembles other phage exclusion systems (e.g., exclusion by the λ *rex* system) in that target superinfecting phages can save themselves by expressing certain genes (e.g., T4 *r*II).

P22 elaborates another function which serves to save it from superinfection exclusion by heterologous prophages. The endogenous prophage Fels-1, which is present in most *S. typhimurium* strains, excludes P22 mutants defective in gene 17 (Botstein, unpublished data). Amber mutants in gene 17 grow in *su*$^+$ Fels-1 lysogens but not in *su*$^-$ Fels-1 lysogens, and grow normally in *su*$^-$ nonlysogens; wild-type P22 grows normally in Fels-1 lysogens. Gene 17, like the *esc* gene, maps to the left of the *immC* (M. M. Susskind and A. Gauger, unpublished data). It is tempting to speculate that Fels-1 elaborates a *sieB*-like function which is antagonized by the product of gene 17 of P22. It is known, however, that 17$^-$ mutants are still insensitive to exclusion by the P22 *sieB* system (Susskind, unpublished data), indicating that *esc* and 17 are different genes.

PRIMARY TRANSCRIPTS FROM THE P22 GENOME

In a series of in vitro transcription experiments with P22 DNA using purified *E. coli* RNA polymerase with and without purified *E. coli rho* (transcription termination) factor, Roberts et al. (100; Roberts, unpublished data) has defined five primary transcripts (Fig. 7). By hybridization experiments using DNA from P22 prophage deletion lysogens and separated strands of DNA from various P22/λ hybrids, the five transcripts could be assigned to particular regions of the P22 genome. Each of the five could be associated with promoters whose existence had previously been deduced from genetic and physiological studies of the phage. Two of them correspond to the transcription controlled directly by the *c*2 repressor from the promoters P_L and P_R which lie in *immC* immediately to the left and to the right, respectively, of the *c*2 gene itself. A third transcript could be mapped to the *immI* region and presumably represents transcription of the *ant* gene from the promoter called P_{ANT}, which is regulated by the *mnt* repressor. All three of these promoters are thus negatively regulated in vivo; each of them is associated with mutations (presumably in corresponding operators) in which the site of regulation is altered, making synthesis from the promoter constitutive (12, 15, 16, 82).

The other two transcripts found in vitro are very short (50 to 80 nucleotides) and map in positions consistent with the idea that they are short "leader" sequences which, when their termination is prevented by phage gene products, result in transcription of the late genes (from P_{LATE}) or of the *c*2 gene (from P_{RE}). The latter transcript (called transcript *b* in Fig. 7) is altered in *cly* mutants; as described above, these mutants result in gross overproduction of the *c*2 repressor and abnormally high frequencies of lysogenization.

REGULATION OF THE LYTIC CYCLE OF INFECTION

Like most bacteriophages, P22 regulates gene expression during lytic development. Even after the decision between lysis and lysogeny has been made, not all genes are expressed at once. The first genes to be expressed (the "early genes") are those lying immediately to the left and to the right of the *c*2-repressor gene; these are presumably made from the promoters P_R and P_L in Fig. 7. These genes encode functions involved in DNA replication, recombination, integration, and regulation of gene expression. The regulatory genes are genes 24 and 23. Gene 24 is required for full expression even of the early genes (51, 83, 87); gene 23 is required only for expression of the late genes (14, 83, 87; J. Margolskee and D. Botstein, unpublished data), which include all the genes specifying the proteins required for phage morphogenesis, DNA maturation, and lysis. As is the case with many other phages, defects in early genes (particularly those involved in DNA replication) often result in partial or complete failure to express the late genes (14).

Early Regulation: Gene 24

Hilliker and Botstein (51) isolated mutants in gene 24 of P22 and found that 24^- mutants had properties strikingly similar to those of N^- mutants of coliphage λ. P22 24^- mutants are pleiotropic and fail to express efficiently virtually all the phage-coded proteins (83). They make very little phage RNA after nonpermissive infection (87; E. N. Jackson, University of Michigan, Ann Arbor, unpublished data). The analogy with the λ *N* gene is greatly supported by the finding that gene 24 of P22 can substitute for the *N* gene of λ in P22/λ hybrids. In fact, Hilliker and Botstein (52) and Friedman and Ponce-Campos (37) showed that the P22 24 gene product can act at heterologous target sites (such as those of λ), even though the λ *N* gene product seems to be specific for its own targets.

This kind of evidence suggests that gene 24 works in the same way that the λ *N* gene has been shown to work (98) by preventing termination of transcription at genetically defined sites. Thus in the absence of gene 24 function, the transcripts from P_L and P_R terminate before the early genes are transcribed, but in its presence, the early genes are transcribed efficiently. In this way gene 24 exerts positive control over the two early operons adjacent to the *c*2-repressor gene. Additional evidence that the gene 24 product is mechanistically similar to the λ *N* gene product has been obtained by Hilliker et al. (53), who showed that transcriptional polarity caused by polar insertions can be suppressed by gene 24 product when transcription starts from P_L or P_R, promoters which can be acted upon by the gene 24 product.

The positions of the transcription termination sites in the early P22 operons has not been determined directly. Hilliker and Botstein (52) obtained indirect evidence for a site between the P_RO_R region and the *c*1 gene and for another between gene 24 and *c*3. The other sites shown in Fig. 7 are given by analogy with λ (32, 48).

In summary, gene 24 acts positively to regulate expression of the early genes by preventing termination of transcription at specific sites between the promoters P_L and P_R and the early genes themselves. It should be noted that some of the early genes (*c*1, *c*3, and the integration and recombination genes) which are under control of gene 24 are required for lysogeny. Gene 24 can be shown to be required for efficient lysogeny as well as for lytic development (51).

Late Regulation: Gene 23

Mutants in gene 23 were first described by Botstein et al. (14). None of the late proteins is expressed to a high level in 23^- infections, whereas the early proteins are expressed normally (14, 83). Similarly, 23^- mutants make reduced amounts of RNA late in nonpermissive infections (87; Jackson, unpublished data).

Like gene 24, gene 23 can substitute for its analog (gene *Q*) in coliphage λ (9). Furthermore, the *Q* gene and gene 23 appear to be functionally identical, in that a Q^- λ phage can be complemented by a 23^+ hybrid phage and a 23^- hybrid phage can be complemented by a Q^+ λ phage (9; Hilliker, Ph.D. thesis). Even more striking, recombinants with normal late gene expression can be found in crosses between 23^- mutants and Q^- mutants (Hilliker, Ph.D. thesis). Thus, gene 23 of P22 and gene *Q* of λ seem to be functionally identical and at least partially homologous.

Roberts (97) has proposed a model for the mechanism of action of the λ *Q* gene. He proposed that the *Q* gene product acts by preventing termination of transcription of the small 6S λ RNA, which he showed is made from a promoter at or very near the position predicted for the late promoter by Herskowitz and Signer (49). This mechanism would apply nicely to the case of P22, since it is clear that two termination sites must be dealt with in P22. One termination site is at the end of the small P22 transcript analogous to the λ 6S transcript (transcript *a* in Fig. 7) and the other is at the end of the *ant* gene. This second termination site is inferred from the size of the putative *ant* transcript made in vitro (J. W. Roberts, Cornell University, Ithaca, N.Y., unpublished data) and is consistent with the observation that gene 9 is not expressed (even from P_{ANT}) in the absence of gene 23 function, even though antirepressor is made under the same conditions (Lew, Ph.D. thesis; Weinstock, Ph.D. thesis; Susskind, unpublished data). Polar insertion mutations located in gene 20 or in the *ant* gene are polar to the same degree upon expression of gene 9 (measured biochemically) (Weinstock, Ph.D. thesis). Since the insertions in gene 20 can exert their polarity only upon transcription from P_{LATE}, whereas the insertions in the *ant* gene should affect transcription both from P_{LATE} and P_{ANT}, this result means that little, if any, transcription of gene 9 begins at P_{ANT}, even in the presence of gene 23 function.

Thus, Roberts' transcription termination model for the regulation of the late genes fits the P22 case nicely if one assumes that only transcripts beginning at P_{LATE} under the influence of the gene 23 product are capable of proceeding through the termination sites at the end of tran script *a* and at the end of the *ant* gene. The model then accounts for the observation that gene 9 transcription is absolutely dependent upon the function of gene 23; transcripts from P_{ANT} are not continued into gene 9, since gene

23 product will not affect transcription from P_{ANT}. The assumption that the gene 23 product distinguishes among transcripts made from different promoters has a precedent: the same is thought to be true for the product of gene *N* of λ and gene 24 of P22 (1, 36, 37).

Negative Regulation of Antirepressor Synthesis During Infection

As discussed above, P22 has a gene (*ant*) that codes for an antirepressor protein which can inactivate the P22 *c2* repressor. During lysogeny, the prophage *mnt* gene product is continuously required to repress the *ant* gene of the prophage and thereby prevent antirepressor-mediated prophage induction (12, 82).

Recently, it has become apparent that P22 specifies another negative regulator, the product of the *arc* gene (for *a*ntire*p*ressor *c*ontrol), that decreases the rate of antirepressor synthesis during lytic infection (Susskind, unpublished data). *Amber* mutants in the *arc* gene have been isolated; under nonpermissive conditions, these phages vastly overproduce antirepressor protein and fail to produce progeny phage. An analysis of revertants which have regained the ability to grow under nonpermissive conditions indicates that the conditional-lethal phenotype and the antirepressor-overproduction phenotype are both due to the *arc*$^-$*am* mutation; furthermore, there is evidence that the primary phenotype of the *arc*$^-$ mutants is overproduction of antirepressor and that the lethal phenotype is a secondary result of this effect (Susskind, unpublished data). However, the exact nature of the block to phage development suffered by *arc*$^-$ phage under nonpermissive conditions is not yet well-defined.

The *arc* gene maps between *mnt* and *ant* on the P22 genetic map. *Mnt* and *arc* appear to be different genes, since *arc*$^-$ mutants show no defect in maintenance of lysogeny or superinfection immunity, whereas *mnt*$^-$ mutants (including an *amber* mutant) do not vastly overproduce antirepressor during lytic infection (Susskind, unpublished data).

It therefore appears that negative regulation of the P22 *ant* gene is essential for lytic infection as well as for lysogeny; the *arc* gene product is of paramount importance in performing this function during infection, whereas the *mnt* gene product is of paramount importance during lysogeny.

MORPHOGENESIS OF THE P22 VIRION

The pathway of P22 morphogenesis has been elucidated in some detail by Botstein et al. (14), King et al. (74), and Poteete and King (88). This pathway (Fig. 8) was worked out by identifying the P22 genes involved in morphogenesis, identifying the protein products of these genes, and characterizing the DNA and protein structures

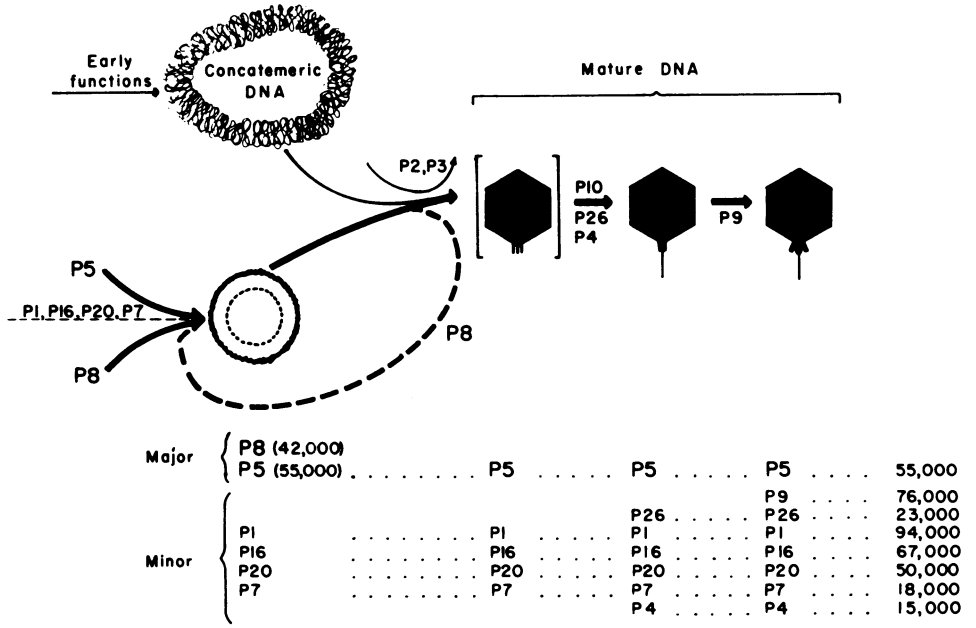


FIG. 8. *P22 morphogenetic pathway. The pathway shown is described in the text. A P before a gene number refers to the polypeptide chain of that gene. The polypeptide chains found in each structure are listed below it; their molecular weights are listed at the right. Brackets around a structure indicate that it is unstable and loses DNA during isolation.*

which accumulate in cells infected with mutants blocked in assembly at various stages as well as in cells infected with wild-type P22.

The earliest known structural intermediate in P22 assembly is the prohead, a spherical structure which contains no DNA and is composed of two major proteins (the products of genes 5 and 8—"P5" and "P8") and several minor proteins (P1, P7, P16, and P20). P5, the major protein of mature phage (420 copies per virion), and P8, a "scaffolding protein" which is not found in mature phage, are present in the prohead in a molar ratio of about 2:1 (73). Together these two proteins make up most of the mass of the prohead, and both are apparently required to determine its correct shape and size. Mutants defective in gene 1 produce recognizable proheads, but are unable to carry out the next step in morphogenesis, DNA encapsulation. With mutants defective in genes 7, 16, or 20, proheads form and all subsequent steps in morphogenesis proceed normally. However, the resulting normal-looking particles, which are missing the product of the defective gene (P7, P16, or P20), are unable to inject their DNA after adsorption to the next host cell.

Proheads containing P1, in the presence of P2 and P3, encapsulate and cut headfuls of DNA from the phage DNA concatemer. P2 and P3 are required for encapsulation of DNA, but are not themselves stably incorporated into proheads or phage. At about the same time that DNA enters the prohead, the scaffolding protein P8 exits. P8 molecules are not cleaved during this process; in fact, released P8 molecules can complex with new P5 molecules to form new proheads in a repeating cycle of prohead assembly. Thus, P8 is reused and can be thought of as a "morphogenetic enzyme" (19, 73).

Newly filled heads are stabilized by the action of P4, P10, and P26 to form complete phage heads. With mutants defective in gene 4, 10, or 26, DNA is encapsulated but then often is released, forming empty heads that are distinguishable from proheads by morphology and sedimentation properties. P26 and P4 are found in mature phage; it is not known whether P10 is present in phage particles, since P10 and P5 (the most abundant protein in the phage) are not resolved by standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The last step in phage morphogenesis is the addition of P9, which assembles on the head to form the base plate or tail, which is required for adsorption of phage to host cells (60).

The most complex step in phage morphogenesis is the DNA encapsulation step, in which DNA enters the prohead and P8 leaves. Physical studies (28) reveal that proheads probably consist of a thick shell or solid ball of P8 (outer radius, 21.5 nm) surrounded by a thin shell of P5 (radius, 25.6 nm). In mature phage, the outer shell of P5 is larger (radius, 28.5 nm), indicating that the P5 shell expands during encapsulation, apparently without gross rearrangements in the particle surface. We do not know whether these three events—exit of P8, packaging of DNA, and expansion of the P5 shell—occur simultaneously or sequentially in time or whether one process provides a driving force for another. It is known that treatment of proheads with sodium dodecyl sulfate will remove P8 with concomitant expansion of the remaining P5 shell (19).

Three other phage proteins—P1, P2, and P3—perform essential functions in encapsulation. Several observations suggest that P3 is the nuclease responsible for cutting headfuls of DNA from concatemers during encapsulation. Botstein et al. (14) found much less intracellular degradation of phage DNA with 3^- mutants than with other encapsulation-defective mutants. Furthermore, the frequency of generalized transduction (i.e., encapsulation of host instead of phage DNA) is altered by mutations (*amber*, temperature-sensitive, and HT mutations) in gene 3 (90; J. Jarvik, Ph.D. thesis, MIT, Cambridge, 1975). The roles of P1 and P2 are not yet well understood.

Some information about the relationships between proteins implicated in DNA encapsulation has been obtained by the reciprocal temperature-shift method of Jarvik and Botstein (65). In this method, cold-sensitive (*cs*) and heat-sensitive (*ts*) mutations at different sites are combined to make *cs-ts* double mutants. The results of temperature shifts from one nonpermissive temperature to the other can be interpreted to indicate the order of function of the wild-type forms of the mutant proteins. Using this method, it was found that P2 probably functions later than P1 or P3 in DNA encapsulation, a result in accord with the results obtained using the in vitro DNA encapsulation system described below.

Jarvik and Botstein also developed another method of assessing relationships between morphogenetic proteins in vivo (66). Cold-sensitive revertants of heat-sensitive mutants (and vice versa) were isolated and characterized. Many of these suppressor mutants occur in genes which code for proteins known to interact with the protein altered by the original mutation. This kind of experiment indicates that P1 functionally interacts with P5 (the major coat protein), an idea consistent with the observation that P1 is a necessary component of proheads active in vitro, and cannot be added in vitro to proheads made in its absence (A. R. Poteete, Ph.D. thesis,

MIT, Cambridge, 1977).

The most promising approach for analyzing P22 morphogenesis further is to study the encapsulation reaction in vitro. DNA encapsulation (and all subsequent steps in morphogenesis) have been accomplished for P22 by using methods developed by Kaiser and Masuda (70) for coliphage λ. Viable P22 phage can be produced in vitro by using highly purified active proheads (i.e., proheads containing P1), phage DNA concatemers, soluble factors (including P2 and P3), and adenosine 5'-triphosphate as substrates (Poteete, Ph.D. thesis). Two properties of the current in vitro system deserve further comment. First, the system will encapsulate host DNA and thereby produce particles active in generalized transduction. Second, the phage DNA in infected cell lysates is greatly preferred over host DNA as substrate for encapsulation. These and other properties seem to verify that the in vitro system faithfully reflects the process which goes on in vivo.

Although encapsulation of DNA can now be accomplished in a cell-free system, we are as yet no nearer to understanding the basic problem of how the DNA is compressed into the small space within the preformed capsid. Further, attempts to form the prohead from its constituent proteins in vitro have not yet succeeded.

FUNCTIONS OF THE P22 VIRION: ADSORPTION AND INJECTION

It has clearly been established that the phage protein responsible for adsorption of P22 to host cells is the product of gene 9 (14, 60). Particles which lack P9 ("heads") do not adsorb to cells; morphologically they are missing the baseplate structure. Heads purified in CsCl equilibrium gradients can acquire the ability to adsorb normally and infect productively by incubation in vitro with P9 (60), even if the P9 has been purified to homogeneity (P. Berget and A. R. Poteete, MIT, Cambridge, unpublished data). Particles activated in this way acquire the baseplate and are morphologically indistinguishable from normal P22 virions.

The receptor site for adsorption of P22 to host cells is the O antigen, a repeating polysaccharide polymer in the lipopolysaccharide layer of the cell surface (138). P22 adsorption is exquisitely specific for the particular structure found in *S. typhimurium* and certain other related *Salmonella* species. When phage adsorb to cells, the receptor polymer is hydrolyzed; heads lacking P9 cannot catalyze this hydrolysis, but purified P9 can (60, 62; Berget and Poteete, unpublished data). Recently, Berget and Poteete (unpublished data) have obtained evidence that hydrolysis is not essential for phage to adsorb to cells, but is essential for productive infection to ensue.

Thus P9 is a multifunctional protein: it assembles onto heads to form the morphological baseplate; by itself or as part of a phage it adsorbs to cells and hydrolyzes the receptor polysaccharide. In addition, P9 is the major target of neutralizing antibody made against whole P22 phage (60; R. Shea, Ph.D. thesis, MIT, Cambridge, 1977). It thus obeys the general rule that the organ of adsorption is the major target of neutralizing antibody.

Three proteins (in addition to P9) are known to be required for successful infection. These proteins (P7, P16, and P20), like P9, are not required for head assembly, but are present in normal virions and in the earliest proheads (unlike P9) (14, 74, 88). Particles lacking any one of these proteins adsorb normally but fail to inject their DNA into the host (54, 55, 88; M. Osburne, C. H. Waddell, and D. Botstein, unpublished data; Shea, Ph.D. thesis). The DNA is ejected (at least partially) from the defective virions, by the criterion that the DNA of particles adsorbed to cells is subject to hydrolysis by host nucleases. Hoffman and Levine (54, 55) showed that particles lacking P16 can be rescued by coadsorption of normal P22 virions; this helping effect does not occur with particles lacking P20 or P7 (Osburne, Waddell, and Botstein, unpublished data; Shea, Ph.D. thesis). Israel (59) has obtained evidence suggesting that P7, P16, and P20 (as well as P26) are ejected from virions after adsorption.

Thus, one can envision the infection process as having several stages: adsorption to and hydrolysis of the receptor (functions of P9), ejection of the DNA into the periplasm, with concurrent ejection of P16, P20, P7 and P26, and subsequent uptake of the DNA by the cells. The latter functions may be mediated by any or all of the four last-mentioned proteins; P9 might also play a role. It is interesting to recall that particles excluded by an *sieA*$^+$ prophage also adsorb and eject their DNA, but do not succeed in getting their DNA into the lysogenic cell (117). The *sieA* gene is adjacent to the 7-20-16 gene cluster on the P22 genetic map.

EVOLUTIONARY RATIONALE FOR THE MODULAR CONSTRUCTION OF TEMPERATE BACTERIOPHAGES LIKE P22

From all of the foregoing, it seems evident that phage P22 greatly resembles the lambdoid group of coliphages in some respects (overall genetic organization, early and late lytic regulation, the primary repressor (*immC*) system, in-

tegration of prophage), while differing markedly in others (morphogenesis, DNA metabolism, antirepressor, adsorption, and injection). The resemblance is fortified by the observation that many P22 genes between the attachment site and the lysis genes can be substituted (sometimes singly, sometimes in clusters) for their λ analogs, producing perfectly viable, normally regulated lambdoid bacteriophages (9, 39, 52). For some genes, the functional specificity of the P22 gene is virtually indistinguishable from the specificity of a corresponding gene from one or another of the wild-type lambdoid coliphages. One example of this is the *c2* repressor gene of P22, which is homologous functionally and in nucleotide sequence to the *cI* repressor gene of coliphage 21 (9); another example is gene 23 of P22, which is functionally identical and at least partially homologous to gene *Q* of λ (9; Hilliker, Ph.D. thesis). In other cases, the P22 gene is partially or completely divergent in mechanism and in nucleotide sequence from its lambdoid counterparts, although it still functions perfectly well in the lambdoid hybrid. Examples of this include gene 24 (which seems to be mechanistically similar to gene *N* of λ, but is totally nonhomologous at the DNA level and shows markedly different specificity (9, 37, 52); gene 19 of P22, which specifies a true lysozyme (91), whereas the corresponding gene *R* of λ specifies an endopeptidase (121); and the recombination genes of P22, which are largely nonhomologous to those of λ and have different consequences from those of λ after superinfection of cells lysogenic for coliphage P2 (52; Hilliker, Ph.D. thesis). In the latter two cases, P22 genes, while different in phenotype from the corresponding genes of λ itself, show the same characteristics as the corresponding genes of the lambdoid phage 80 (24).

Thus, it would appear that P22, despite the fact that it grows on a different species of bacterium, is a member of the λ family. P22 has not only retained the same genetic organization and some of the very same genes found in its lambdoid cousins, but has also retained the ability to recombine with its cousins at many points (usually between known genes) to produce healthy hybrids. It seems difficult to avoid the implication that retention of compatibility and interchangeability of regions of function (even when the functions themselves have diverged in mechanism and nucleotide sequence) is continually selected in evolution. This selection seems to have persisted even though the cousins have separated into divergent hosts; neither phage can directly infect the other's host. Thus the P22 lysozyme gene, although different in every way from the λ endopeptidase gene, can function in its place and has retained enough homology flanking it so that replacement at the correct position can actually still occur (9). In some ways this idea is not expected, since one might, a priori, imagine that regions of function (i.e., structural genes) would have greater constraints against divergence than the flanking regions. The flanking regions (or linkers) serve to ensure that genes can be replaced by their functional analogs even when nonhomologous.

Given these considerations, it seems more reasonable to think of evolution of this family of bacteriophages in terms of the joint evolution of a set of largely interchangeable modular elements than to think in the more classical terms of evolution and speciation by linear progression from common ancestors. Thus neither λ nor P22 is per se the product of evolution; instead, the individual functional segment is the unit and the entire set of segments is the product of evolution. Thus λ, P22, phage 80, and phage 21 are each a single sampling of the pool of modules selected by the evolutionary process.

It should not be assumed that all the linkers between segments are retained in every pair of cousins one might try to cross; it is enough that any pair retain some few homologous linkers. In this way, each of the segments can ultimately replace any of its analogs. It should not be assumed that new combinations of segments arise only in crosses between intact phages; new combinations have been found as a result of recombination between an intact phage and a defective prophage present in the host (35, 41, 50, 144). It should also not be assumed that all combinations of functional segments are necessarily equally fit; the main pressure for the maintenance of the system is that fit combinations can arise frequently enough to fill all the available niches. Finally, it should not be assumed that all functional segments are either completely homologous or completely heterologous; there are well-known examples of intermediate cases (*exo*, 89, 120; *cI*, 135, 136; *O*, 38).

This view of the evolution of P22 and its cousins has the advantage of providing a rationale for the existence of antirepressor and its attendant regulation. The role of antirepressor, according to this scheme, is to facilitate the reassortment of the functional modular segments to fit changing environmental conditions. Antirepressor, by inducing any prophages present in a new bacterial host, allows prophage replication and thereby stimulates recombination between the superinfecting phage and the prophage. Furthermore, derepression of prophage functions might sometimes be essential if

one of P22's own functions cannot work in the new host. The relative non-specificity of antirepressor for particular phage repressors is nicely consistent with this role.

This view of phage evolution requires both a common regulatory organization and clustering of functions, especially where specificity for sites or neighboring genes is important. The common genetic organization of P22 and λ is itself instructive; repressor works at neighboring sites; gene 24 (*N*) works at nearby promoters; gene 23 (*Q*) works at a nearby site. Regulation of most other genes (e.g., genes for head morphogenesis or recombination) is accomplished through regulation of transcription from promoters far from the genes themselves, thus avoiding the necessity for specific regulatory sites distant from the regulatory genes. Much of the genome is positively controlled by antitermination of transcription. Although the genes for the antiterminator proteins are far from some of the termination sites at which they act, the termination sites are in fact relatively nonspecific; the sites which are specifically recognized by the antiterminator proteins are at or near the promoters (1, 36, 37), which, as noted above, are linked to the regulatory genes themselves. Thus, control by antitermination of transcription accomplishes specific regulation at a distance without requiring distant specific regulatory sites. This facilitates replacement of segments while maintaining proper regulation and can be thought of as a rationale for the distinct preference of these bacteriophages for transcriptional control of very large operons.

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