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I. Introduction

A. General Properties of Episomes

The concept of the episome was introduced by Jacob and Wollman (1958b) as a generic term for those genetic elements able to exist in two
alternative states: (1) an *integrated* state, in which the element is associated with some point on, or small region of, a chromosome, and apparently multiplies synchronously with it, and (2) an *autonomous* state, in which the element multiplies independently of, and frequently faster than, the cell which carries it. They further stipulate that the integrated state must represent an addition to the chromosome rather than a replacement of homologous genetic material.

Most of the episomes of bacteria fall into one of two classes: (1) temperate bacteriophages (or, strictly, the genetic material thereof), and (2) transfer factors, which can pass from cell to cell during conjugation independently of the bulk of the bacterial genome. Some transfer factors play a causative role in the conjugation process itself, such as the fertility factor (F) of *Escherichia coli* K12. We shall consider such independent transfer as indicating that a given factor is an episome even where an alternation between integrated and autonomous states has not been shown.

The episomic nature of temperate bacteriophage was established earlier than that of the transfer factors, and we have a great deal more information about them, which has been the subject of several excellent reviews (Lwoff, 1953; Bertani, 1958; Jacob and Wollman, 1959a). The purpose of the present review will be to ask some of the same questions about episomes in general which have been asked about phage in particular. Before proceeding, we shall give a brief historical account of the known episomes other than phage as well as some instances of possibly similar elements in higher organisms.

**B. Brief Description of Episomes Other than Phage**

1. *The F Agent*

Genetic recombination resulting from cellular conjugation was discovered in *Escherichia coli* K12 in 1946 (Lederberg and Tatum, 1946), but it was several years before some aspects of the mechanism became clear. The initial observation leading to this clarification was that some strains had spontaneously lost their ability to mate with each other, although retaining the ability to mate with the parent strain. The parent strain was thus designated as fertile (F+) and the derived strain as infertile (F−). Subsequent studies showed that mating is an asymmetric process, wherein one partner functions as a donor and the other as a recipient of genetic material. Only F+ strains can function as donors in crosses (Lederberg et al., 1952; Hayes, 1953a,b).

It was also found that the F+ property could be transferred from cell to cell at a rate in excess of the transfer of bacterial genes in general,
and that F+ cells could be "cured" of the F property by exposure to acridine dyes (Hirota, 1960), a treatment known to remove nonchromosomal genetic determinants from yeast (Ephrussi et al., 1949) and other organisms (see Lederberg, 1952). From these facts, the existence of a discrete F factor could be inferred, which was carried by F+ cells but not by F− cells.

F+ strains give rise to variants (Hfr), in which the frequency of transfer of bacterial markers to F− cultures is greatly elevated, and that of the F property itself greatly depressed. It can be verified by microscopy and micromanipulation that when an Hfr and F− cell come together, they form a cytoplasmic connection, and that recombinants appear only among the descendants of the F− exconjugant (Lederberg, 1956, 1957; Anderson and Mazé, 1957). The mechanism of mating has been studied in some detail (Jacob and Wollman, 1956a,b, 1957a, 1958a; Skaar and Garen, 1956; Wollman and Jacob, 1955, 1958a,b). Starting from a given point of origin on a formally circular linkage structure, genetic markers enter the recipient in a temporal order which corresponds to their linkage relationships. The entire transfer requires about 2 hours, and the final character transferred is the fertility property itself. The point of origin and also the direction around the circle are characteristic of the particular Hfr isolate used. Hfr strains can revert with various degrees of ease to F+, demonstrating the presence of F as a genetic element in Hfr strains. Hfr strains are not cured by acridine dyes (Hirota, 1960). The F agent in the Hfr strains is thus in the integrated rather than the autonomous state.

The F agent seems to contain DNA, because incorporation of radioactive phosphorus into F (as judged by subsequent "suicidal" inactivation on storage due to disintegration of phosphorus atoms within the F particle) is inhibited by mitomycin (Driskell and Adelberg, 1961). Naturally occurring variants of F have been reported (Lederberg and Lederberg, 1956; Bernstein, 1958) and laboratory variants mediating high frequency gene transfer (Jacob and Adelberg, 1959) are now common. From phosphorus suicide experiments, the nucleic acid content of F can be estimated as 90–120 million molecular weight (Lavallé and Jacob, 1961). This calculation is made from the relative sensitivities of F and of bacteriophage lambda, assuming a molecular weight of $60 \times 10^6$ for lambda.

Another type of Hfr strain (very high frequency, Vhf) has recently been described (Taylor and Adelberg, 1960). These differ from ordinary Hfr in that the whole genome is transferred with high frequency. With these strains it has been possible to confirm by linkage studies the circular chromosome which Jacob and Wollman had proposed. It is not known
whether the Vhf character is a genetic property of F, of the bacterium, or of the position or mode of attachment of F.

Many strains of *Escherichia coli* (Cavalli and Heslot, 1949; Lederberg *et al*., 1952; de Haan, 1954, 1955; Lieb *et al*., 1955; Bertani and Six, 1958; Ørskov and Ørskov, 1961) and the related genera *Salmonella* (Baron *et al*., 1959*b*; Demerec *et al*., 1960; Miyake and Demerec, 1959; Zinder, 1960*a,b*; Ørskov, *et al*., 1961) and *Shigella* (Luria and Burrous, 1957) can act as recipients in crosses with F+ or Hfr strains. In most of these cases, it has been shown that the F agent is actually transferred to and subsequently carried by these strains, although in some instances it no longer functions as a fertility factor in the new environment and is detectable only by the ability of the strains which carry it to transfer the F property back into K12. Mating by conjugation has been described also in the genera *Pseudomonas* (Holloway, 1955, 1956) and *Serratia* (Belser and Bunting, 1956). In *Pseudomonas*, fertility is controlled by an infectious agent similar to F (Holloway and Jennings, 1958).

2. The Colicin Factor(s)

Certain strains of *Escherichia coli* produce proteins (colicins) which are highly toxic for other strains. The large literature on colicins and other bacteriocins will not be reviewed here (see Fredericq, 1957, 1958). We are concerned only with the genetic control of colicin biosynthesis.

In order that the symbols used should be clear, we explain first that colicins attach themselves to specific receptors on the surface of their victim, and that colicins are classified according to their attachment specificity. Thus, colicin V will attack a certain group of bacterial strains and colicin E another. If two different colicins are found with the same attachment specificity, they are called, for example, E1 and E2.

Much of the genetic work on colicinogeny concerns the colicin formed by strain K30 of *E. coli*, which belongs to the colicin “E” family and has been called ER, E(1) or E1 (Fredericq, 1957). It has also on occasions been improperly referred to as “colicin K30.” It was found (Fredericq, 1953; Fredericq and Betz-Bareau, 1953*a,b,c*) that, in crosses between colicinogenic and non-colicinogenic parents, one could not map this colicin factor in relation to other markers.

After our understanding of and control over the mating process had been improved by Jacob and Wollman, more definitive experiments were possible. Using the colicin factor of strain K30, which had been transmitted into strain K12, Alfoldi *et al.* (1957, 1958) showed that the transfer of a col− determinant from the donor into a col+ recipient resulted in the death of the zygote—a curious situation whose cause remains unexplained but which allows genetic localization of the colicinogeny factor by time of
entrance experiments. By making different Hfr strains colicinogenic, they showed that the time of entrance of the col+ determinant into the F− recipient depended on the Hfr strain used. The reciprocal crosses showed that lethal zygosis showed a similar dependence on time of entry. One can thus conclude that the col+ determinant occupies a chromosomal site in these strains. Single cell pedigree analysis revealed that all of the progeny of a single zygote which had received col+ were themselves col+. This is similar to the behavior of F (Lederberg, 1959) and in marked contrast to the results obtained in comparable experiments with ordinary genetic markers (Lederberg, 1957; Anderson and Mazé, 1957). It explains the absence of linkage observed by Fredericq, and necessitates the assumption that, following conjugation, the col+ determinant can multiply faster than the rest of the genome.

Is colicin E1 unique, or are all colicinogeny determinants episomes? Crosses involving colicinogenic parents are complicated by various effects on the fertility of crosses (Fredericq and Betz-Bareau, 1956; Fredericq and Papavassiliou, 1957), which probably reflect differences in the pattern of lethal zygosis (Fredericq, 1956a). However, the rapid transfer of colicinogeny in mixed culture between distantly related strains which has been shown for many different colicins (Fredericq, 1954a, 1956b, 1957; Hamon, 1956) is certainly suggestive. In some cases, including that of colicin E1, this transfer requires that the donor be F+ (Fredericq, 1954a). However, the transfer of colicinogeny was distinct from that of other markers. With strains which produced more than one colicin, frequently only one determinant was transferable.

The transfer of the colicin I factor to non-colicinogenic Salmonella does not require the presence of F (Stocker, 1960; Ozeki et al., 1961). Furthermore, colicinogenic strains can transfer other markers of the bacterium at low frequencies. The colicin I determinant has therefore as much right to be considered a fertility factor as does F itself. It differs from F in having a much higher rate of integration; so that rapid transfer only occurs from cell lines very recently made colicinogenic.

From its sensitivity to phosphorus decay, the nucleic acid content of the colicinogeny factor must be less than a molecular weight of 2.4 million (Lavallé and Jacob, 1961).

3. The Resistance Transfer Factor

Strains of pathogenic Shigella resistant to streptomycin, tetracycline, chloramphenicol, and sulfonamide have been shown to carry on agent which imparts all these resistances simultaneously (Harada et al., 1960; Mitsuhashi et al., 1960a,b). This agent resembles a fertility factor in causing its own transfer from cell to cell by conjugation, but differs in that it
does not render any other genes of the bacterium transferable. If the resistance transfer factor (RTF) is introduced into an F+ or Hfr strain of *Escherichia coli*, the transfer of bacterial genes by the fertility factor is eliminated or strongly reduced. An F+ cell is thus converted into an F− phenocopy, and spontaneous loss of RTF is accompanied by recovery of the F+ character (Watanabe and Fukasawa, 1960h; 1961a,b,c,i).

Our accounts of this work will be based on the available American literature. References to the Japanese literature (Akiba *et al.*, 1960; Ochiai *et al.*, 1959; Watanabe and Fukasawa, 1960a–g; 1961d–h) are included for the linguistically competent reader.

RTF is transferable from *Shigella* into members of the genera *Escherichia* and *Salmonella*. It is susceptible to curing by acridine dyes. Both the ease of transfer and the curing indicate that it is ordinarily in the autonomous state. The results of Hfr(RTF) × F− crosses suggest that, in a fraction of the population, RTF is associated with the host chromosome at or close to the B1 locus (Watanabe and Fukasawa, 1962). A reversible equilibrium between integrated and autonomous states is thus indicated.

The individual determinants of resistance can become stably, irreversibly integrated, probably by replacing their homologs in the bacterial chromosome. This process has no known connection with the integration of RTF.

4. The Sporulation Factor

It has been suggested (Jacob *et al.*, 1960b) that the factor responsible for bacterial sporulation might be an episome. This should provide an interesting opportunity to study the role of an episome in a simple developmental process.

5. The Mycelial Factor in Aspergillus

Roper (1958) studied three strains of *Aspergillus* in which distinguishable obvious morphological variations had been induced by acriflavine. The first (M1) was examined in greatest detail. Heterokaryons were made between genetically marked wild type and M1 individuals. Conidial isolates from these were mostly of the two parental types. Some individuals had the genotype of the originally wild type nucleus, but had become M1. The reciprocal class was never found.

This indicates that M1 is a genetic element which is generally associated with the nucleus but which can, rather infrequently, infect another nucleus. It can be considered a transfer factor which operates between nuclei in a synkaryon rather than between isolated bacterial cells. One point which should be stressed is that M1 arose as an induced mutant of a
normal cell type, and we are therefore not free to assume that the wild type lacks any homolog to it.

6. **Controlling Elements in Maize**

McClintock (1956) has investigated extensively a variety of controlling elements in maize, and has emphasized the difference between these elements and the normal genes of that organism. Genetically, controlling elements are unique in their ability to undergo transposition from one part of the genome to another. Functionally, controlling elements may (1) cause chromosome breakage in neighboring regions, (2) modify or suppress the activity of neighboring genes, or (3) activate other controlling elements far removed from them in the genetic complement. It is their transposability which justifies our inclusion of these elements as episomes. Their functional aspects make them more similar to bacterial regulators and operators, for which transposability has not been found (cf. McClintock 1961).

**C. GENERAL REMARKS**

We see that not all of the episomes mentioned here have been shown to satisfy Jacob and Wollman's definition, nor is there good reason to suppose that they will. They all have in common the property of being associated with the normal genetic complement of a cell but also of being able on occasions to assort independently of it. The ability to *replicate* in alternative states of course implies more than this, and remains to be proven (or disproven) in several cases. At present it seems more important to look for similarities than for differences, and the definition may well be modified with increasing knowledge.

**II. The Autonomous State**

**A. VEGETATIVE REPLICATION OF VIRULENT AND TEMPERATE BACTERIOPHAGES**

A large amount of chemical and genetic work has converged to form a picture of intracellular bacteriophage growth which is satisfying at a certain level (Levinthal, 1959). Most of the evidence comes from the virulent coliphages T2, T4, and T6. The application to other phage-bacterium systems seems a reasonable extrapolation. Following injection of the phage DNA into the bacterial cell, certain new enzymes not found in the non-infected cell begin to appear which are necessary for the synthesis of phage rather than bacterial DNA (Kozloff, 1960). Almost all such early enzymes characterized so far function in the diversion of the synthetic pathways toward the production of DNA containing hydroxymethyl-
cytosine rather than cytosine. Since this base occurs only in these particular virulent phages, such steps seem irrelevant to the vegetative growth of temperate phages. However, some early protein synthesis is prerequisite to the vegetative multiplication of the temperate coliphage lambda (Thomas, 1959).

In T2-infected cells, this early protein synthesis is necessary for phage DNA synthesis, which can then proceed independently of additional protein synthesis (Hershey and Melechen, 1957). The phage DNA thus produced is genetic material and not merely a precursor thereof (Tomizawa, 1958). In the average infected cell the amount of free phage DNA rises to a maximum of about 40 phage equivalents per cell and remains roughly constant until lysis. This constancy does not result from a cessation of synthesis but rather from equality of the rates of synthesis of new phage DNA and its rate of removal from the vegetative state by maturation.

The existence of a vegetative pool can also be inferred from the occurrence of genetic recombinants among the earliest mature phage particles produced, and from the kinetics of accumulation of recombinants among mature phage. These constitute the principal genetic evidence for a pool. The consistency with the chemical evidence, together with the possibility of artificially divorcing vegetative multiplication from maturation by the addition of chloramphenicol, leave little doubt as to the correctness of the general picture.

In those systems where initial infection does not strongly exclude later superinfection, one can estimate the number of vegetative phage in an infected cell as a function of time by studying the contribution of genetically marked superinfecting phage to the final yield and assuming that the superinfecting phage participates on an equal basis with the vegetative phage present at the moment of superinfection. The method can give self-consistent results (Thomas, 1959).

B. The Carrier State of Phage

Following infection with a virulent phage of the T2 type, a cell does not undergo any further divisions but simply enlarges somewhat during virus growth and ultimately lyses. With temperate phages, a fraction of the infected cells also lyse without dividing. The remainder survive the infection and produce colonies of lysogenic cells in which the phage genome is integrated as prophage. There are some reports which suggest that both temperate phages and some types of weakly virulent mutants thereof can also continue indefinitely in a non-integrated "carrier" (or pseudolysogenic) state, in which the cells multiply and occasionally
lyse and liberate phage (Zinder, 1958; Luria et al., 1958). In those cases most carefully studied (Li et al., 1961), the indefinite maintenance of the carrier state is destroyed by the presence of antiphage serum, which implies that it is an artifact of intraclonal reinfection. There seems little doubt, however, that, during a limited number of generations following infection, a transient state exists in which the cell continues to grow and phage genomes multiply within the cells in a manner not exactly comparable either to the vegetative or to the prophage state.

C. Episomes Other than Phages

The autonomous state of the fertility factor resembles superficially the carrier state of phage, and the highly contagious nature of the F+ property suggests the possibility that its maintenance likewise requires frequent reinfections between cells. There is little direct evidence on this point, but the high frequency of occurrence of F− individuals among motilized isolates from F+ cultures (Skaar et al., 1957) is suggestive. The autonomous state of the colicinogenic factor is only seen transiently (Stocker, 1960; Ozeki et al., 1961). Whether this reflects an incapacity for indefinite autonomous replication or merely a high but constant probability of integration is not certain. The number of F particles per cell is probably about three, as judged by the enzyme levels of cells made partially diploid by F-mediated transduction (Jacob and Monod, 1961; Jacob and Wollman, 1961).

III. The Integrated State

A. Chromosomal Localization of Phage and Other Episomes

A lysogenic culture is defined as one in which the capacity to form phage is perpetuated intracellularly and transmitted from one generation to the next. Since the phage has a specific set of genetic information, each cell of a lysogenic culture must contain one or more copies of this information, and to these copies the name prophage is given. It seemed of fundamental importance to know whether the prophage was present in a few copies, distributed regularly between the daughter cells at each division, or in many copies, distributed at random at division. Studies on superinfection of Shigella lysogenic for phage P2 (Bertani, 1953a) and of E. coli lysogenic for lambda (Jacob and Wollman, 1953) indicated that the former hypothesis was correct, and this was soon confirmed by the results of bacterial crosses of Escherichia coli (E. Lederberg and J. Lederberg, 1953; Appleyard, 1954a), which showed that, in a lysogenic cell, the genome of a temperate phage behaves as though it were located at a
specific point on the bacterial chromosome. Crosses between lysogenic parents carrying genetically different lambda prophages revealed (1) that the attachment sites of the two prophages were allelic and (2) that there was linkage between the prophage and other genes of the bacterium. This observation has been generalized to other systems, although the facts do not completely justify such a generalization.

On the basis of those lysogenic systems for which crosses have been made, three types can be distinguished. (1) The lambda type, in which the phage has a unique point of attachment to the bacterial chromosome. Many different phages, some of them related to lambda, have been shown each to have its own specific attachment site (Jacob and Wollman, 1957a). (2) The P2 type, in which the phage preferentially occupies a particular site, but where it is possible to form, under appropriate conditions, quite stable lysogens in which the preferred site is vacant and one of the several possible secondary sites is instead occupied (Bertani and Six, 1958). (3) The P1 type, for which the segregation in crosses does not permit the assignment of any chromosomal location to the prophage (Jacob and Wollman, 1957b). A separate transfer mechanism for P1 is possible (Boice and Luria, 1961).

The difference between lambda and P2 may be technical rather than fundamental. These two phages differ in the behavior of homopolylysogens, which are bacterial lines perpetuating simultaneously more than one related prophage. In P2 double lysogens the two prophages are located at different places on the bacterial chromosome, whereas with lambda they occupy the same site (or extremely closely linked sites). Double lysogens of lambda are also less stable than those of P2. It seems possible that P2 can also form double lysogens of the lambda type, but that they are even more unstable and are never recovered, thereby rendering easier the isolation of double lysogens in which distinct locations are occupied.

In the case of prophage, one can infer a chromosomal location of the phage genome itself rather than of some other gene affecting prophage maintenance because each parent of a bacterial cross can be marked with a genetically different prophage. It has not been possible to do the analogous experiment with other episomes. One cannot therefore exclude totally the possibility that the $F^+ \rightarrow Hfr$ change, for example, might involve, rather than the fixation of $F$ itself at a particular site, a mutation at that site which affects the behavior of $F$ during conjugation. Richter's (1957, 1961) finding of $F^-$ recombinants which become Hfr upon infection with $F$ fits this hypothesis. It can be explained alternatively by assuming that in these strains a part of $F$ has adhered to the chromosome. Wollman and Jacob (1958a) have presented other evidence which can also be interpreted in this manner.
B. Mode of Attachment of the Prophage to the Bacterial Chromosome

The manner of attachment of the prophage to the bacterial chromosome has been much discussed in the past. At one time, the problem was how a small linear structure (the phage genome) could act as part of a large linear structure (the bacterial chromosome). The most appealing solution was that the small structure was inserted into the continuity of the large one so that one linear genome would result. In recent years, many data have appeared which seem to contradict such a co-linear structure, and popular taste has tended to favor some sort of branched model, in which the prophage is not really attached to the bacterial chromosome at all but rather portions of the two are permanently synapsed together. These two extreme cases are illustrated in Fig. 1 as Model 1 and Model 2, respectively. Bertani (1958) has categorized more completely the various possible modes of juxtaposition.

To evaluate much of the recent evidence on this subject, one must understand first that it is primarily evidence against the insertion hypothesis rather than for any particular kind of branched structure. One is thus really setting a model which makes quite specific predictions against all other possibilities, and whenever one prediction fails, the specific model is discarded. This seems a somewhat unfair procedure to the reviewer, and we will react to it by discussing primarily insertion hypotheses. To explain some of the facts in this way requires additional ad hoc assumptions, but insertion, with complications, is not inherently less desirable than an undefined model of branching or sticking together.

Besides such data which bear on the answer, there is also information...
which changes the question. Our large linear structure (the bacterial chromosome) behaves, on formal genetic analysis, not as a line but as a circle (Jacob and Wollman, 1957a). This really does not matter very much, but what if the small structure is also a circle? Detailed linkage studies lead to the conclusion that the genome of one phage (T4) is indeed circular Streisinger, Edgar, and Harrar, quoted by Stahl (1961). If circularity is a property of phages in general, the equivalent of the insertion hypothesis is to make one circle out of two, and we will discuss later the simplest model for accomplishing this.

The most obvious approach to distinguishing the various models is to examine the results of crosses between two lysogenic parents in which both the prophages and the bacterial chromosomes are well marked. The most complete study of this type thus far published is that of Calef and Licciardello (1960) on phage lambda. The assortment of prophage markers among bacteria recombinant for a pair of bacterial genes on either side of the prophage indicates a linear order with the prophage genes lying between those of the bacterium. However, the order of prophage markers on this map is different from that of the vegetative lambda phage. Whereas crosses of vegetative phage give the order “h-cl-mi,” the order in the lysogenic cell is “try-h-mi-cl-gal.”

This surprising result is supported by some other data suggesting a singularity in the region between h and cl. For example, Whitfield and Appleyard (1958) found with doubly lysogenic strains marked at these two loci that one recombinant type was liberated in excess of either parental type. The type preferred depended on the order of lysogenization and on the parental couplings, not on any selective advantages of the markers employed. This seems quite possible if the phage genome splits into two or more pieces at the time of lysogenization and is reassembled later. The results of Calef and Licciardello also allow one to contemplate mechanisms for the transduction of the galactose genes by phage lambda which otherwise would be unthinkable. This point will be amplified in a later section.

If the phage genome is circular rather than linear, the lambda chromosome need not be split into parts, but rather could be cut at a specific point on the circle when it lysogenizes. It is actually very simple (on paper) to insert a circular phage chromosome into a linear bacterial chromosome by reciprocal crossing-over (Fig. 2).

Figure 3 shows the genetic constitutions predicted for the chromosome of doubly lysogenic bacteria and for those carrying the defective, galactose-transducing lambda (Section VIII,A) on the model described here. If induction is imagined to entail a reversal of the process shown in Fig. 2, it is easily seen that one can make many different complete loops from
a double lysogen, which would explain the result of Whitfield and Appleyard. The instability of double lysogeny in lambda (Appleyard, 1954b; Arber, 1960), the unstable lysogeny of transducing lambda (Campbell, 1957), and the apparent correlation between loss of transducing lambda and recombination in the gal region (Arber, 1958) could all be explained as consequences of the presence of a longer region of duplication.

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**Fig. 2.** Possible mechanism of lysogenization by reciprocal crossing-over between a circular phage chromosome and a linear bacterial chromosome. Arrows indicate possible rare points of breaking and joining in the formation of transducing lambda. (See Section VIII.) The genes ABCD are hypothetical and indicate a small region of homology between host and phage. X and Y are unspecified bacterial genes.

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**Fig. 3.** Genetic constitution of doubly lysogenic bacterium and of defective heterogenote, assuming the mechanism of Fig. 2. The origin of the transducing lambda is described in the text (Section VIII).

On the other hand, this model would predict that it should be easier to lysogenize an already lysogenic strain than a non-lysogenic one, since the former will present a larger region of homology to an entering phage. This prediction is not fulfilled. Lysogeny creates a strong steric hindrance against lysogenization by another phage at the same site (Six, 1961a).

This illustrates well what we pointed out to begin with—that ad hoc hypotheses are required to fit most insertion models to the facts. We would assume that the pairing between phage and bacterial chromosomes may have other requirements besides the homology of the regions which pair. This is almost required by the model anyway, because the specificity of the attachment is determined by the immunity region (Kaiser and
Evidence indicating that the prophage is not inserted into the bacterial chromosome has been presented by Jacob and Wollman (1959b). The most important concerns the non-inducible prophage 18, which is located close to two methionine markers (M1 and M2). Two Hfr strains by which this region was injected early but in opposite directions were isolated, and it was shown that the prophage appears to enter the bacterium after the marker M1 in both cases. They therefore suggest that the prophage may be synapsed parallel with the bacterial chromosome, overlapping the M1 gene, inasmuch as "prophage entrance" would require the entrance of the entire prophage genome. They also observed that the distance between M1 and M2 is not altered by lysogenization of both parents in a cross.

These facts should not be disregarded. They do not, in the reviewer's opinion, necessitate the abandonment of the insertion hypothesis, although it requires some ingenuity to circumvent them. In this review we will discuss mostly insertion hypotheses, not because we strongly favor them, but because we believe alternative ideas have received rather more than their share of attention at the hands of others.

Any model for prophage attachment must ultimately be extended to explain the behavior of homopolylysogenic strains of the lambda type. Strong asymmetries in the patterns of phage liberation and of segregation are seen with such strains, and there are reproducible differences between strains in the type of asymmetry shown (Arber, 1960a). Our picture in Fig. 2 is therefore at best a naïve first approximation to the true situation. It seems permissible at present because no simple explanation of the facts has been provided by alternative hypotheses.

IV. Immunity

A. Superinfection Immunity and Repression

In order that a stable lysogenic culture should exist, it is necessary that the cells of this culture should be unable to support the lytic growth of the phage which other cells have liberated. This conclusion has been verified by experimental superinfection experiments, which have shown, in addition (1) that the lysogenic cells are neither killed or lysed by superinfection with phage of the carried type but that (2) the superinfecting phage are still able to attach to the lysogenic host, and, from genetic data, must also be able to inject their DNA into it. The lysogenic cell is thus immune to superinfection.

Another *sine qua non* for lysogeny is that those genes of the prophage
which determine functions involved in growth or maturation of phage or of lysis of the cell—i.e., all those functions which can be characterized as viral—must not express their potentialities in the lysogenic cell. There is much negative evidence that this is indeed the case. The analogy between this repression of viral functions in lysogenic cells and the repression of enzyme synthesis by internal repressors has been brilliantly developed by Jacob (Jacob, 1960; Jacob and Campbell, 1959; Jacob and Monod, 1961).

We have therefore two operational phenomena—the superinfection immunity and the repression of prophage genes—which are similar in that in both cases a phage genome in a cell is prevented from functioning in the same manner that it would upon entering a non-lysogenic cell. One may hypothesize that the two are identical, and that the immunity to superinfection is due to the same repressor(s) which are necessary for the stability of the lysogenic state. A complete proof of this hypothesis will not be possible until the key steps have been defined biochemically, but the available evidence is encouraging.

B. CYTOPLASMIC NATURE

In the first place, both effects are mediated physiologically through the cytoplasm rather than sterically by the attachment of the prophage to the bacterial chromosome. This is indicated by the following facts in the two cases:

1. Immunity

As mentioned earlier (Section III,A), the prophage of P2 can occupy various alternative sites on the E. coli C chromosome. However, cells carrying P2 in any position are immune (Bertani and Six, 1958; Bertani, 1956). Moreover, the related phage P2 $Hy$ Dis, which has a different immunity specificity, does not create immunity against P2, although it can occupy the same sites and interferes with lysogenization by P2 (Cohen, 1959; Six, 1961a).

Equivalent evidence comes from studies of cells recently infected with P22 (Luria et al., 1958; Zinder, 1958) and from merozygotes (Jacob, 1960) and stable partial diploids (Jacob and Monod, 1961) in which one chromosome or one nucleus of a cell has become lysogenized, but its non-lysogenized homolog can segregate from it. This segregation of sensitive progeny from an immune individual implies that immunity is a dominant character. Evidence for its cytoplasmic nature comes also from the persistence of immunity for some time after the genetic segregation has occurred (Luria et al., 1958).

We will refer to the specific cytoplasmic principle involved in immunity as the "immunity substance" (Bertani, 1958).
2. Repression

Bacterial mating is essentially a transfer of part or all of the genetic material of the male parent into the female cell. If the male parent is lysogenic, mating entails the sudden introduction of an established prophage into a non-immune cytoplasm. The dramatic result is that it is induced to multiply vegetatively (Jacob and Wollman, 1954a, 1956b). The implication is that it is ordinarily restrained from entering the autonomous state not by virtue of its physical union with the chromosome but rather by the repressors present in the cytoplasm, which must themselves be synthesized under the direction of the prophage.

C. Genetic Determination of Immunity

In the second place, there is a considerable formal similarity between the genes controlling immunity and the regulator and operator genes which determine the rate of synthesis of an enzyme such as β-galactosidase. For a detailed comparison, the reader is referred to the review of Jacob and Monod (1961). We will concentrate here primarily on the genetic control of immunity.

1. Determination of the Specificity of Immunity

The genetic control of the immunity specificity has been investigated for several phages. Phages independently isolated from nature which are closely enough related to recombine genetically nevertheless frequently differ in their immunity specificity. For example, the two phages lambda and 434 each can form lysogens which are immune to superinfection by the carried phage, but infection of K(λ) by 434 or of K(434) by lambda results in lysis and phage production (Jacob and Wollman, 1956b). If the immunity specificities of two phages are the same, the two are homo-immune; if different, hetero-immune.

Crosses between hetero-immune phages allow one to localize the determinants of immunity on the genetic map. If one crosses a series of lambda mutants with 434, one finds that some wild type lambda is produced in all cases except for those mutants lying within a single genetic locus (cr) of the phage. Kaiser and Jacob (1957) backcrossed phage 434 several times with phage lambda and produced a strain which we can call λimm^434, in which the immunity determinant of 434 is imbedded in a lambda genome. We will refer to the region containing the cr locus within which lambda and λimm^434 differ as the immunity region.

A hetero-immune phage of the P2 type was obtained from infection of E. coli B with phage P2. E. coli B apparently carries a defective prophage related to P2, and the active hetero-immune phage obtained by
recombination with the superinfecting P2 is called $P2 \text{ Hy Dis}$ (Cohen, 1959; Six, 1961a). The rarity of recombination in lytic infection by P2 has discouraged mapping of the immunity determinant. Crosses of Salmonella phage P22 with a hetero-immune relative have shown that the immunity determinant is close to and perhaps allelic with the marker $v_1$ (Zinder, 1958).

2. Determination of the Ability to Generate or to Respond to the Specific Immunity

The immunity region thus determines the specificity of immunity, and this immunity has two components. The phage must be able to send a specific message (e.g., a repressor) and also to respond specifically to the same message by failing to grow in an immune cytoplasm. It is not clear why the genes determining these two functions should occupy the same region of the chromosome, but phage present no unique problem in this respect. Bacterial regulator and operator genes can likewise be closely linked (Jacob et al., 1960a). For phage, the implication is that mutations which abolish or alter the ability either to generate or to respond to immunity should be found in the immunity region. Furthermore, mutations altering the specificity of immunity should rarely if ever be found, because such mutations would require a simultaneous change in both the regulator and the operator to new forms which again match each other.

Present data support the idea that alterations of immunity specificity do not occur by mutation. We feel justified in making this statement even in the presence of some outstanding exceptions, because of the difficulty in distinguishing between true mutation of a phage and recombination between it and an unknown prophage or defective prophage. That the phage P2 $\text{Hy Dis}$ was originally classified as an immunity mutant is a good point to bear in mind. More recently, a mutant of lambda has been described (Kellenberger et al., 1961a) which renders the phage not only hetero-immune but also less dense than wild type lambda. On primary isolation, it carried also a small plaque mutation which was separable from the density-immunity alteration by crossing-over. This mutant is therefore complex and could well have a recombinational rather than a mutational origin.

a. Bacteriophage Lambda. The mutational pattern of bacteriophage lambda fits well with a regulator-operator picture, and therefore this phage will be discussed first. Several interesting types of mutants are found:

i. Mutants unable to generate immunity. Such mutants are the most commonly observed result of mutation in the $c_I$ region. The $c_I$ mutants
are still responsive to the immunity generated by wild type lambda and therefore will not grow in lysogenic cells. They are unable to lysogenize. They are called "clear plaque" mutants because whereas there is no lysogenization within the plaques of these mutants, which therefore look clear like the plaques of a virulent phage, the wild type plaques are turbid due to lysogenization of bacterial cells within the plaque.

In lambda, there are two other cistrons closely linked to \( c_I \) (one on either side), mutations in which also decrease the ability to lysogenize. These cistrons differ from \( c_I \) in several ways: (A) Whereas most \( c_I \) mutants show no detectable lysogenization at all, \( c_{II} \) and \( c_{III} \) mutants exhibit a low but measurable frequency of lysogenization. The lysogens, once formed, are completely stable. (B) Mixed infection with two mutants from different cistrons results in a high frequency of lysogenization. The survivors from such cooperation experiments may be either singly or doubly lysogenic. However, one never finds individuals singly lysogenic for \( c_I \). (C) Kinetic studies show that the \( c_I \) function in lysogenization occurs later than the \( c_{II} \) or \( c_{III} \) functions (Kaiser, 1957).

These data clearly indicate that, of the three cistrons containing clear mutants, all must cooperate in the establishment of the lysogenic condition, but that only the \( c_I \) cistron continues to operate in the maintenance of this condition. The \( c_{II} \) and \( c_{III} \) cistrons perform functions necessary for lysogenization, but not for lysogeny. Their mechanism of action is of interest in its own right, but only the \( c_I \) mutants are directly relevant to the problem of immunity; and of these three closely linked cistrons, only \( c_I \) lies within the immunity region.

ii. Mutants unable to respond to immunity. These are the so-called "inducing virulent" mutations (Jacob and Wollman, 1954b). The mutation to this state is complex and requires several changes from wild type, one of which is in the immunity region. Inducing virulent mutants are characterized by their ability to grow on lysogenic cells. Whether they have also lost the ability to generate immunity is difficult to test experimentally and has not been decided.

iii. Mutants in which the immunity, but not its specificity, has been altered. A mutant of lambda has been isolated (\( \lambda ind \)), lysogens of which are not inducible by ultraviolet. This mutation is located within the immunity region. In double lysogens, non-inducibility is dominant. If a cell of the type K(\( \lambda ind^+ \)) which has been induced is superinfected soon afterwards with \( \lambda ind \), lysis is prevented and the survival of cells increases. Most of these survivors are still K(\( \lambda ind^+ \)). It thus seems that the \( ind \) gene produces something which can reverse induction without the \( ind \) phage's becoming integrated as prophage. The effect is specific for the lambda immunity type. Induced cells of K(\( \lambda ind^+imm^+ \)) or double lysogens K(\( \lambda ind^+imm^\lambda \))
(\(\lambda_{\text{ind}}^{+}\text{imm}\)) are not affected by superinfection with \(\lambda_{\text{ind}}\) (Jacob and Campbell, 1959).

This mutant provides additional evidence for the existence of a cytoplasmic immunity substance. One cannot say at present whether the \(\text{ind}\) mutation alters the substance itself or affects its rate of production. It probably alters the same function which is abolished by the \(c\) mutations, because a phage which carries both mutations is unable to prevent lysis of \(K(\lambda_{\text{ind}}^{+})\) (Jacob and Campbell, 1959).

b. Bacteriophage P2. In P2, where the immunity determinants have not been mapped, there occur, in addition to types 1 and 2 (which are called by P2 workers "weak virulent" and "strong virulent," respectively), some intermediate types which show limited growth on lysogenic strains, or which form plaques on singly lysogenic but not on doubly lysogenic strains (Bertani, 1953b, 1958). These are readily explained as involving quantitative alterations in the pattern of response to immunity.

c. Bacteriophage P22. The findings with Salmonella phage P22 are more difficult to fit into the general scheme constructed for phage lambda. The two phages present certain striking analogies. In both cases, there is a cluster of closely linked cistrons of clear plaque mutants (Levine, 1957; Kaiser, 1957). In both cases, only one of these cistrons is necessary for the maintenance of lysogeny. The others function only in its establishment. In both cases, other mutants are found which form turbid plaques but from which stably lysogenic lines can never be extracted (Lieb, 1953; Zinder, 1958). The function altered in these mutants is apparently not required in order that the infected cell should survive the infection, but is necessary for the establishment or perpetuation of stable lysogeny. These mutants are not closely linked with the \(c\) mutants.¹

In both cases, crosses with related hetero-immune phages allow genetic localization of a determinant of immunity specificity. There is only one important difference between the two systems, but it is one which cannot be ignored. Whereas the immunity determinant of lambda is allelic with the \(c\) cistron, that of the Salmonella phage is close to (perhaps allelic with) the mutant \(v_{1}\)—a mutant of the type which forms turbid plaques but does not lysogenize, and which is unlinked to the clear mutants of P22. This of course does not fit too easily with the idea that the \(c\) phenotype is the result of the absence of a repressor which determines the immunity specificity. One explanation would be that immunity does not involve one repressor but several, and that hetero-immunity may involve

¹ Another mutation of this type has recently been described for phage \(\lambda\) by Kellenberger et al. (1961b). It is of especial interest here because its genetic location would make it a possible candidate for the region of homology we have postulated between phage and host (genes ABCD of Fig. 2).