

HOST-CONTROLLED MODIFICATION OF BACTERIOPHAGE¹

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INTRODUCTION

Host-controlled modification of viruses is a general term applied to those cases in which passage through certain host strains imparts one or more new, nonheritable properties to the virus without altering its genetic information content. The terms of host-induced modification, host-controlled variation, or host-induced variation are sometimes used as synonyms to designate the same phenomena. However, we would like to recommend the use of "host-controlled modification," at least for the cases discussed in this paper, since control mechanisms are involved here rather than induction phenomena and since the term "variation" may erroneously suggest some change in the genetic message.

Several examples of host-controlled modification were found independently in the early 1950's by Luria & Human (1) for phage T2 grown on strains of *Escherichia coli* B; by Bertani & Weigle (2, 3) for phages λ grown on either *E. coli* K12 or C and for phage P2 grown on either *E. coli* B or *Shigella dysenteriae*; by Anderson & Felix (4, 5) for Vi-phage II of *Salmonella typhi*; and by Ralston & Krueger (6, 7) for a phage called P1 of *Staphylococcus aureus*. Since these early findings were reviewed by Luria (8), a great number of other bacteriophages have been shown to undergo host-controlled modification which suggests that this phenomenon is very widespread.

Nothing was known concerning the mechanism of host-controlled modification until recently, when two of the above mentioned cases were more

¹ The survey of the literature pertaining to this review was concluded in December 1964.

thoroughly analyzed: that of phage λ and of phage T2. The present article will deal primarily with these two best known systems. Although most of the other examples of host-controlled modification so far studied are superficially similar to one or the other of these two, it is not yet clear whether the underlying mechanisms are the same as those to be described here. It should be emphasized that each new phage-host system needs to be properly analyzed before any conclusions can be drawn regarding the mechanism of the process.

HOST SPECIFICITY OF BACTERIOPHAGE λ DNA

The phage-host system and definitions.—This survey will deal with bacteriophage λ grown on the following hosts: *E. coli* K12, *mal*⁺— λ ^s strains of *E. coli* B (9), *E. coli* C, P1-lysogenic derivatives of K12, B, and C, and restrictionless (*r*⁻) and modificationless (*m*⁻) mutants of K12 and B. Genetic markers of the phage are indicated, if relevant, by the symbol immediately following the λ (example λc for clear plaque-forming λ). The host strain to which the phage has been adapted is given last, separated from the phage symbols by a dot (example $\lambda \cdot K$ for phage grown on K12).

Phage λ variants can be classified with respect to their state of adaptation either by consideration of the history of the phage stock or by determination of the efficiency of plating (eop) on various hosts (10). Table I shows that $\lambda \cdot K$ has an eop of one on K12 and C, i.e., it is accepted, nonrestricted. The same phage $\lambda \cdot K$, however, plates only exceptionally on B or on P1-lysogenic strains: it is said to be restricted. Phage $\lambda \cdot C$ is accepted only by C (or by *r*⁻ mutants or K12 and B to be discussed later), but not by K12, B, or P1-lysogenic strains. Two restriction systems exerted in the same cell result in a restricting action which is to some extent additive, as was pointed out originally by Lederberg (11) for the restricting action of prophage P1 superimposed on that of the host cell itself. This is illustrated in Table I, for example, by the eop of $\lambda \cdot B$ which is 4×10^{-4} on K12 and 10^{-6} to 10^{-7} on K12(P1).

It is further seen from Table I that, in general, phage λ grown on a given host is afterwards fully accepted by this host. It is said to be modified, having undergone host-controlled modification. In considering a system involving only two host strains one encounters either (*a*) symmetry in restriction

TABLE I
EFFICIENCY OF PLATING OF PHAGE λ

Host strains Phage variants	K12	K12(P1)	B	C K12 <i>r</i> ⁻ B <i>r</i> ⁻
$\lambda \cdot K, \lambda \cdot K r^{-}m^{+}$	1	2×10^{-5}	10^{-4}	1
$\lambda \cdot K(P1)$	1	1	10^{-4}	1
$\lambda \cdot B, \lambda \cdot B r^{-}m^{+}$	4×10^{-4}	10^{-6} to 10^{-7}	1	1
$\lambda \cdot C, \lambda \cdot K r^{-}m^{-}, \lambda \cdot B r^{-}m^{-}$	4×10^{-4}	10^{-8} to 10^{-7}	10^{-4}	1

(which does not need to be quantitative), for example, with strains K12 and B ($\lambda \cdot K$ is restricted by B and $\lambda \cdot B$ is restricted by K12) or (b) asymmetry in restriction, for example, with strains K12 and C ($\lambda \cdot C$ is restricted by K12, but $\lambda \cdot K$ is accepted by both K12 and C). Asymmetry is encountered also in the system of K12 and K12 (P1).

The nature of the restriction process.—Bertani & Weigle (2) excluded non-adsorption of phage as a possible reason for restriction. They found no difference in adsorption of $\lambda \cdot K$ and of $\lambda \cdot C$ to K12. They also showed that adsorption of restricted λ neither kills nor even delays the growth of the host cells. The fate of the DNA from restricted phage was investigated with ^{32}P as a DNA label [(12), see also (11)]. After a few minutes of contact of the ^{32}P -labeled $\lambda \cdot K$ phage with the restricting host K12(P1), most of the radioactivity sedimented with the cells, indicating a rapid phage adsorption. But when the adsorption time was prolonged, and particularly when the medium was changed so as to favor a rapid injection of the phage DNA, then less and less ^{32}P label sedimented with the cell fraction. Most of the ^{32}P recovered in the supernatant of a low speed centrifugation was soluble in cold 2 per cent perchloric acid. Hence, this label could not come from nonadsorbed phage but was explained rather as breakdown products from phage DNA which had been injected into the restricting bacteria and shortly thereafter degraded.

Although the site in the cell at which the restricting action takes place has not been identified, various observations suggest that the DNA of a restricted phage is indeed injected before being degraded. First, the amount of acid-soluble ^{32}P recovered, which ranges between 25 per cent and 80 per cent upon assay at 30 min after the addition of phage to bacteria, depends on the method by which it is determined. It is lowest if only the supernatant fraction from low speed centrifugation of the adsorption mixture is treated with perchloric acid. Intermediate values are obtained if the adsorption mixture is treated directly with the acid. The highest proportions of acid-soluble ^{32}P are found if the infected cells are broken open previous to the acid precipitation. Second, genetic markers from injected restricted phage genomes can be rescued by superinfection of the host cell with nonrestricted phage variants [(12), see also (11, 13, 14)]. The frequency of such rescue is higher the earlier the nonrestricted phage is added, suggesting a competition between DNA breakdown and genetic rescue for any marker considered. Dussoix & Arber (12) estimated the average length of the rescued region as about 10 to 20 per cent of the λ genome by measuring joint rescue of more than one marker by a single event. These experiments also indicated that the DNA degradation is either not polar for the whole population of restricted DNA molecules, or else that it occurs very fast once a single DNA molecule is attacked.

DNA breakdown was observed in all cases of restriction of λ that have been tested to the present time, i.e., restrictions governed by K12, by B, by prophage P1, and a resistance transfer factor (12, 15–18). Degradation yields ^{32}P partly in inorganic form, partly in small organic fragments which have not yet been further characterized (12). It is not known whether only

one or several enzymes are involved in the DNA breakdown. One might like to assume that a highly specific "restriction enzyme" only initiates the degradation, for example, by cleavage of the DNA, and that these cleavage products are then subject to the action of less specific nucleases. It was observed that breakdown occurs also in cells infected in the presence of chloramphenicol (16, 19), suggesting that the production of the restriction enzyme does not depend on the infection with λ phage. On the other hand, restricted DNA is degraded independently of whether or not its replication is inhibited (18): immune K12(λ)(P1) cells, for example, do degrade $\lambda \cdot K$ DNA, but not that of nonrestricted $\lambda \cdot K$ (P1) nor, of course, their own bacterial DNA. It should be mentioned finally that hybrid DNA molecules, carrying one DNA strand provided with host specificity and the other lacking it, can give rise to phage progeny as will be described later. It was shown that in this case neither of the two DNA strands is degraded (20).

The DNA molecule as the carrier of host specificity.—From what is said above about the breakdown of DNA of restricted phage, one may expect that the difference between restricted and nonrestricted λ variants resides in the phage DNA. Direct evidence for this statement comes from one-cycle growth experiments performed by Arber & Dussoix (10). These authors used K12 and K12(P1) as host strains. As seen from Table I, K12 accepts both $\lambda \cdot K$ and $\lambda \cdot K$ (P1), while K12(P1) accepts only $\lambda \cdot K$ (P1) but restricts $\lambda \cdot K$ (eop = 2×10^{-5}). However, if $\lambda \cdot K$ (P1) is passed through one single cycle of growth on K12, the lysate plates with an eop of about 5×10^{-3} on K12(P1), as if the P1-specific adaptation were not entirely lost in the passage. By labeling the DNA of the parental phage—either with ^{32}P at very high specific activity or with deuterium, the above authors showed that all of those one-cycle progeny phages that were still able to grow on K12(P1) had also inherited parental DNA. The amount of parental DNA in these " $\lambda \cdot K$ (P1)" phage particles corresponded to that of semiconserved, hybrid DNA molecules. If the multiplicity of infection was much higher than one phage per cell, phages with fully conserved parental DNA were recovered in the progeny in addition to those with semiconserved DNA (see 21), and they also were still accepted by K12(P1). But in none of the experiments did phages with entirely newly synthesized DNA give an eop higher than that of $\lambda \cdot K$ on K12(P1). These observations leave no doubt that it is the λ DNA molecule which is provided with host specificity in host-controlled modification governed by prophage P1. One-cycle transfer experiments have also been done on strains C, K12 r^- , and Br^- and with phage variants $\lambda \cdot K$, $\lambda \cdot K$ (P1), and $\lambda \cdot B$. The results permitted the same conclusions regarding the host specificity produced by K12 and B as those outlined above for the P1-directed host specificity (15, 20): host specificity stayed associated with the parental DNA and was recovered only in progeny phage particles which had inherited at least one strand of the parental DNA. Since no genetic or functional changes can be observed in host-controlled modification, it appears that host specificity does not alter the content, the copying, or the reading of the genetic message.

Host specificity is transferable serially together with parental DNA. When progeny phages of P1-host specificity carrying semiconserved DNA molecules, obtained by growth of $\lambda \cdot K(P1)$ for one cycle on K12, are passed through a second cycle of growth on K12, they again yield phages with semiconserved DNA and parental P1-host specificity. The efficiency of transfer in the first and second cycle is constant per DNA strand (15), a result which suggests that both strands of the original DNA molecule are provided with host specificity and have the same probability of reappearing in a semiconserved DNA progeny molecule. Indeed, single K12 cells infected at low multiplicity with $\lambda \cdot K(P1)$ were shown to liberate two $\lambda \cdot K(P1)$ progeny phage particles at a rate corresponding to that of the product of the probabilities of finding only one $\lambda \cdot K(P1)$ phage (22).

The biological activity of λ DNA can be assayed on helper-infected host cells showing a low but reproducible probability of taking up free λ DNA molecules, which then may initiate vegetative phage growth (23, 24). Applied to DNA extracted from different λ variants and to different host strains, this technique reveals the same pattern of restriction as found for λ phage particles (16, 25). The progeny from one cycle of growth is composed, as after infection with phage particles, of phages with semiconserved DNA molecules, which continue to exhibit their parental host specificity in addition to that given by the new host, and of a majority of phages with only newly synthesized DNA and, of course, not provided with the parental host specificity. These facts demonstrate once more the stability of the association between DNA and its host specificity which is not destroyed by the deproteinizing purification.

The provision of the DNA with host specificity.—The fact that phage liberated by bacteria of strain K12 is accepted with the same high chance by K12 itself and by strains not requiring the K12-host specificity, indicates that the provision with host specificity is an efficient process. Still, one finds experimental conditions, to be described later, in which phage DNA is either not modified or is incompletely modified, at the moment of its wrapping in phage coats. These observations suggest that modification is a process that is independent of DNA replication. The following experimental results confirm this expectation (20). Density-labeled $\lambda \cdot K$ is grown for one cycle on strain Br^-m^+ (not requiring specificity for B host to be present on infecting DNA but providing the DNA with it) after infection at high multiplicity. The progeny lysate is centrifuged to equilibrium in a CsCl density gradient and the fractions of phages with conserved, semiconserved, and new DNA molecules assayed on K12 and B indicator. It turns out that the B host specificity has been given to the DNA carried by the progeny phages independently of whether it went through replication or not. In addition, if phages with hybrid DNA molecules are purified and then passed through a second cycle of growth on the same host, it can be revealed by density analysis of the second-cycle lysate that the new strand of the semiconserved DNA from phages in the first-cycle lysate has not acquired the host specificity of the infecting parental phage (15, 20).

An experiment equivalent to the one just described had been performed before r^-m^+ strains were available (10). It was based on the finding that K12 can be successfully coinfecting with P1 and λ and that such complexes produce essentially only λ progeny of which an important fraction carries the P1 directed host specificity [see also (26)]. The use of density-labeled parental phage allowed the demonstration that the proportion of modified $\lambda \cdot K(P1)$ was constant for phages with conserved, semiconserved, and new DNA. This experiment also revealed another interesting fact: that the modification functions carried out by P1 are established quite soon after infection with P1, and even under conditions where P1 is "excluded" by growing λ . On the other hand, the restriction functions cannot be revealed even after delayed superinfection with $\lambda \cdot K$. Hence, this experiment provides a physiological distinction between the two functions of modification and restriction.

The distribution of the host specificity determinants.—It is known that during vegetative growth of phage λ , part of the parental DNA molecules are fragmented by break and reunion recombination (21). It is striking, however, that in the above mentioned one-cycle growth lysates, phages with parental host specificity and densities intermediate between that of semiconserved and new DNA did not show up in measurable proportions. The absence of such phages might be expected if (a) the host specificity of the DNA is not located at one single spot on the λ DNA molecule and if (b) each or at least most of the specific sites for host specificity on the λ genome must be adapted in order to insure a successful infection. The first of these assumptions can be tested by genetic experiments. A bacterial host strain is infected simultaneously with two genetically distinct phage parents, both unrestricted but of different host specificity, for example, K12 infected with $\lambda \cdot K$ and $\lambda \cdot K(P1)$. The one-cycle progeny lysate is then assayed on K12(P1), which reveals only phages with parental host specificity, and the plaques obtained are scored for genetic recombinants having acquired markers from the $\lambda \cdot K$ parent. Unfortunately, only a few experiments of this type have been performed. For the P1-directed host specificity it appears that recombinants for the *mi*-marker from a nonadapted phage are accepted by P1-lysogenic cells, but not recombinants having acquired a nonadapted *c*-region or a nonadapted *h*-region (10, 27). These results indicate that the P1-directed host specificity is not attached at a single site on the λ genome, but they do not permit an estimation of the number of such specific locations. More experiments are also needed before similar conclusions can be drawn for the host specificities produced by K12 and by B.

Another attempt to estimate the number of sites concerned with host specificity on the λ genome was made by Paigen & Weinfeld (28) on the basis of the increased acceptance rate of multiply infected restricting cells. However, their conclusions seem to be questionable in the light of their later investigations on the mechanism of cooperative infection (29).

The genetic determinants for restriction and modification.—Coding for the functions involved in host-controlled modification is either on the bacterial

chromosome or on additional genetic elements as, for example, the prophage P1 (11), or a resistance transfer factor (17, 30). As far as is known, production of one host specificity does not exclude the production of another host specificity in cells containing genetic information for both; similarly, two different restrictions are additive at least to some extent (see Table I).

It was discussed that the two functions of modification and of restriction governed by the genome of phage P1 are physiologically distinct. Glover et al. (31) demonstrated also their genetic separation by the isolation of r^- mutants from phage P1 which no longer restricted a nonadapted phage λ . Some of the mutants still carried out modification (r^-m^+), whereas others were also defective in this function (r^-m^-).

More recently, Wood (32) has isolated a considerable number of r^- mutants from K12 and from B. Cultures of these strains were found to contain spontaneous r^- mutants at the rather high proportion of about 1 in 10^5 cells. Treatment with the mutagen ethyl-methane sulfonate increased the frequency of r^- mutants, and for both strains K12 and B, about half proved to be r^-m^+ . The majority of the remainder was r^-m^- and a small fraction exhibited intermediate rates of modification. Most of the r^- mutants lacked entirely the restriction and they accepted $\lambda \cdot K$, $\lambda \cdot B$, and $\lambda \cdot C$ equally well with an eop of approximately 1 (Table I). A small number of mutants were obtained which gave intermediate restriction. The high frequency of fully restriction-deficient r^- mutants observed implies that in K12 as well as in B, restriction is lost by a one-step mutation, i.e., each of these strains requires only one type of host specificity. This fact facilitates the analysis of the genetic determinants of restriction.

Mapping experiments in several laboratories using conjugation and phage-mediated transduction have shown that the r character is linked to the *thr* locus, on the side opposite to the *leu* markers (32, 33, 34). Furthermore, the r characters of K12 seem to be allelic with those of B, since no recombinants have been obtained which give B- and K12-type restriction simultaneously.

The consideration of the m^- mutants is handicapped not only by the lack of selective techniques to isolate such mutants, but also by the theoretical expectation (see following) that m^- mutations occurring in r^+ cells should be lethal. Indeed, no r^+m^- mutants have yet been detected. As shown above, r^-m^- mutants (which are also linked to *thr*) occur about as frequently as r^-m^+ mutants, so that they could hardly be explained as two-step mutants. An explanation for the high frequency of this simultaneous disappearance of functions might provide a clue to the relationship between the factors responsible for restriction and modification. One appealing hypothesis would be the following: Modification acts on the DNA molecule, providing it with host specificity. Restriction occurs only if this host specificity is absent. Hence, both restriction and modification must recognize the absence of host specificity at specific locations on the DNA. The high degree of specificity is perhaps best explained if these locations are recognized as particular base

trolled modification and are susceptible to restriction if passed through the strains given above as hosts for λ . None of these cases have yet been studied as thoroughly as that of λ , but the similarity of the observed phenomena suggests that the same host specificity determinants are provided to the DNA of these phages as in the case of phage λ . It would nevertheless be desirable to have more extensive experimental data before a conclusion on the general nature of the phenomena is put forward. Such studies might reveal phage genomes carrying none or only a very limited number of sites for host specificity, as has been suggested recently for the K specificity on phage ϕ 80 (40). The irregularities in host-controlled modification with T3 (41) may perhaps be connected with the production, in T3-infected cells, of an enzyme which cleaves S-adenosylmethionine (42). The T-even phages do not seem to be submitted to the same control as λ (see later discussion). No report on host-controlled modification of phages with single-stranded DNA or with RNA has yet been made.

Host specificity of the bacterial DNA.—It might be predicted as a further generalization that the hypothetical enzymes of modification and restriction would act also on bacterial DNA. The DNA of strain K12 would thus carry the K12 host specificity, DNA from K12(P1) could carry both the K12 and P1 host specificities, and so on. Good evidence supporting this prediction comes from bacterial conjugation and phage-mediated transduction experiments.

Transduction of *gal* characters with phage λdg is submitted to the same restrictions as is the infection with active phage λ , even under conditions (ultraviolet irradiation of λdg) in which the transduction occurs by stable integration rather than by lysogenization (43). Transduction with phage P1 cannot be compared as easily, since the restriction of nonadapted phage P1 is much weaker than that of λ .

More interesting is the finding that in bacterial crosses the formation of genetic recombinants is very much inhibited with female strains, which— if taken as hosts for phage λ —restrict phage grown on the male strain involved (17, 33, 44, 45, 46). Perhaps the most convincing example is given by the comparison of crosses between K12 and its P1-lysogenic derivatives, where the cross Hfr K12 \times F⁻K12(P1) is 100- to 1000-fold less fertile than the 3 other combinations, Hfr K12 \times F⁻K12, Hfr K12(P1) \times F⁻K12, and Hfr K12(P1) \times F⁻K12(P1). In this case, the donor and recipient chromosomes are the same and nonhomology is thus ruled out as a reason for the low frequency of recombinant formation. Furthermore, zygotic induction of prophage λ , carried by the donor strain K12, is reduced in F⁻K12(P1) by the same factor of 100 to 1000. Poor recombinant formation in Hfr K12 \times F⁻B crosses could be partly explained by incomplete homology between the bacterial genomes, as has been suggested by transduction for the *gal* region (43). However, absence of the required host specificity probably plays the preponderant role, since recombinants are obtained at the same high frequencies with Br⁻, K12r⁻, and K12 females crossed to Hfr K12 (35).

It has not yet been possible to determine whether restricted bacterial

sequences. The enzymes of restriction and of modification may thus have that part in common which recognizes the specific sites, so that mutation arising in this part could cause the loss of both functions in question. The same explanation may apply independently of whether only one gene product exerts the functions of sequence recognition, modification, and restriction or if different gene products are assembled as subunits to the specific enzymes.

Among the mutants showing intermediate modification activity, Wood (35) found some which are temperature-sensitive, giving good modification at low temperature but incomplete or no modification at high temperature. Bacterial strains giving intermediate activity for both restriction and modification have also been found among streptomycin-resistant mutants (11, 12). In this latter case, the mutation affects also many other cell functions (36) and seems thus rather nonspecific with respect to the present discussion. Such cells are perhaps better regarded as being in a particular physiological state, to be discussed in the next paragraph.

Influence of "physiological state."—It was pointed out in the early papers of Bertani & Weigle (2) and Lederberg (11) that the rare phage producing complexes in a culture infected with restricted phage were due to special cells rather than to special phages. Such cells might be explained as r^- mutants, but in general the number of r^- mutants found in a culture is fewer than that of accepting cells. On the other hand, particular physiological states may cause weakness in the restriction (and perhaps also the modification) functions. It is indeed possible to increase the frequency of the productive response by certain treatments (2, 8) applied to a culture, as, for example, ultraviolet irradiation, exhaustion of the growth medium or the use of a synthetic minimal medium. Acceptance is also much increased by a short heating of the restricting recipient cells at temperatures of about 50° C (37, 38). Furthermore, multiplicities of infection higher than one lead to the already mentioned effect of cooperative infection (28, 29). Finally, experiments with amino acid auxotroph mutants have shown a specific decrease of restriction in the absence of methionine (35).

On a theoretical basis, one may expect that cells with high modification activity and transiently low restriction activity can be infected successfully, if one assumes that modification and restriction are competitive and acting both on the same sites on the DNA molecules. But the actual situation may even be more complex; for example, by the formation of intermediate states of the DNA which are resistant to restriction although not yet modified (29). It would be of particular interest in this regard to know something about the topological distribution within the host cell of the two enzymes in question. In any event, these considerations make it clear that eop 's measured on restricting hosts (Table I) are not fixed values and may undergo considerable variations unless the assay conditions are kept rigorously constant. Restriction should thus be judged qualitatively rather than quantitatively.

Host specificity of DNA from phages other than λ .—Many phages other than λ as, for example, T1 (11, 14, 39) or P2 (2, 11) also undergo host-con-

Finally, methionine is readded in order to allow the phage maturation, and the first mature intracellular phage particles are harvested. The DNA of such phage proves to be lacking in or only incompletely provided with, host specificity. If K12 serves as a host, for example, lysates can be obtained which give several hundredfold more plaques on C or K12 r^- indicator than on the host strain K12 itself. The same holds true for host specificities produced by B and phage P1. No effect at all is found upon starvation of auxotrophic hosts for two other amino acids, proline and arginine; other auxotrophs have not yet been tested. It remains unclear, however, how methionine is involved in the provision of host specificity. Since DNA produced in the absence of either proline or arginine is fully modified, it seems unlikely that the deficiency of host specificity which results from methionine deprivation is a consequence of the nonproduction of a specific protein, such as the enzyme responsible for the modification. It would seem more probable that methionine is directly involved in the production of host specificity; for example, in the alkylation of specific sites on the DNA. Although this hypothesis would satisfy all of the criteria put forward so far on the basis of experimental observations, it has not yet been possible to obtain direct evidence for it. For example, it is known that methylation of the bacterial DNA is strain-specific (52), and there is now experimental evidence that DNA of phage λ reflects the methylation specificity of the host strain on which it has been grown (53). However, when the methylation of DNA from K12 is compared with that of K12 r^-m^- mutants which do not produce the K12 host specificity, no measurable differences are found (53). The same holds true in comparisons of B with Br^-m^- . Thus, if host specificity is determined by methylation of the DNA it can only involve a minor fraction of the total methylated bases observed. In this case one might also expect that the DNA methylating enzymes that have been described by Gold & Hurwitz (52) are not necessarily active in host-controlled modification. Experiments are in progress to determine whether or not the aminopropyl moiety of the methionine molecule is transferred specifically to λ DNA (53). Methionine itself does not appear to associate with DNA; after growth of λ in K12(P1) in the presence of S^{35} -labeled methionine, less than one methionine molecule could be detected per purified λ genome (53).

HOST-CONTROLLED MODIFICATION OF T-EVEN PHAGES

The DNA of the phages T2, T4, and T6 differs from that of the bacterial host strains and most other phages with respect to two features: it contains the base 5-hydroxymethylcytosine instead of cytosine and it is glucosylated. The glucosylation reaction requires uridine diphosphoglucose (UDPG) which has to be furnished by the host cell. Bacterial mutants which lack the capacity to synthesize UDPG can still produce T-even phages, but their DNA is then only poorly glucosylated or not at all (54-57). Such phages show the same properties as the T* phages obtained by Luria & Human (1) upon host-controlled modification in strain B/4₆. Hattman & Fukasawa (55) have in-

DNA is degraded upon its transfer into the female, as one might expect by analogy to the degradation of restricted phage DNA. Indirect evidence for such degradation is provided by the observation that in the rare recombinants formed upon transfer of chromosomal DNA to a restricting female, the normal frequency of joint integration of linked markers is sharply reduced, suggesting a fragmentation of the incoming DNA (33, 35, 46).

Restriction acts also against the acceptance of the fertility factor F, of its F' derivatives *Fgal* and *Flac* and of the resistance transfer factors RTF (17, 31). Some of the transfers of a restricted episome exert a still unexplained killing action on the female cells.

Merozygotes formed in conjugation between strains with different host specificities of the DNA sometimes carry the genetic information for restriction and modification from both acceptor and donor strains. It appears that here, too, as upon infection with P1, the modification function is expressed quite fast, as concluded from the proportion of doubly modified λ phage liberated after zygotic induction in merozygotes formed with λ -lysogenic Hfr donors (35, 47). It has not yet been possible to obtain stable heterozygotes for the *r* and *m* characters.

It may be interesting to consider these facts of restriction in bacterial conjugation in the light of current ideas on how the DNA is donated to the female (48, 49). According to these views, the donor chromosome is replicated near the site of contact between male and female, and one of the new (= semi-conserved) copies is passed immediately into the recipient cell, so that one might expect that the new strand is not yet provided entirely with male host specificity. This seems to be confirmed by recent experiments of Ptashne (50). Here again, as for semiconserved phage DNA, it appears that DNA is accepted upon penetration into a new host cell as long as one of the two DNA strands is provided with the required host specificity. This insensitivity of hybrid DNA to restriction may conceivably also explain how the cell protects its own DNA between the time of synthesis and the completion of modification.

Search for the biochemical nature of host specificity.—The biochemical basis of host specificity is not yet understood. Investigation of the nature of the determinants should be guided by our present knowledge of their properties and the conditions under which they are produced. These determinants are imparted to the DNA at more than one specific site on the λ genome and without changing measurably its buoyant density, in a process independent of DNA replication. Host specificity maintains a firm association with DNA through the process of replication or upon purification and subsequent submission to various physical, chemical, or enzymic treatments (16, 25). Another hint comes from the observation that methionine seems to be specifically required for the production of host specificity (51). These conclusions are based on experiments that consist in initiating vegetative phage growth in methionine-requiring auxotrophs which are then deprived of methionine while phage DNA is being replicated, at least to some extent.

of enzymes of different specificity should then be useful in attempts to determine base sequences of DNA molecules.

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deed shown that B/4₀ is deficient in the enzyme UDPG pyrophosphorylase, so that host-controlled modification of T-even phages is now explained by the incomplete glucosylation of the phage DNA. As expected from these results, glucosyl-transferase-deficient mutants of T2 behave similarly to T* (58).

The T* phages are able to grow only on a limited number of bacterial strains, for example, on *Shigella dysenteriae* strain Sh (1). Most of the other commonly used host strains such as *E. coli* B and K12 are restricting in that they degrade the DNA of the infecting T* phage (59). However, certain early phage functions can be accomplished by T* even in restricting host cells (59). Rates of restriction and the efficiency of expression of early functions show variations with respect to the host strain and to the T* involved (59, 60). This might be related to the differences in the glucosylation patterns of phages T2, T4, and T6 (61) and to the mechanism of recognition of glucosylation by the infected host.

Glucosylation is not required for DNA replication, or for its initiation (59). On the contrary, recent evidence suggests that glucosylation occurs subsequent to replication (54), as predicted on the basis of the specificity of the purified glucosyl-transferases (62). Glucosylation provides the T-even DNA with a natural density label which has been exploited in one-cycle growth experiments (54). Unfortunately, however, initially glucosylated strands of DNA injected into UDPG deficient strains are soon fragmented by the high incidence of recombination events so that such experiments are less informative than those done with phage λ .

CONCLUSIONS

The study of host-controlled modification of bacteriophage λ has revealed unexpected properties of phage DNA molecules and of the bacterial DNA as well. In addition, host specificity has proved to be useful as a biological label of the DNA. It is to be hoped that the chemical nature of the factors providing host specificity will soon be elucidated.

Genetic studies discussed above indicate that bacterial chromosomal and episomal elements are modified and restricted by the same factors which act upon bacteriophage λ DNA. This finding allows a reasonable guess concerning the value of host-controlled modification to the cell. It appears to provide bacteria with a mechanism whereby the expression or integration of foreign genetic elements can be prevented without hindering the potentially beneficial genetic exchange between cells of the same strain.

Looking toward future developments in this field, it is to be hoped that the enzymes involved in production and control of host specificity will be isolated and characterized. Such studies, paralleled with investigations of the genes controlling restriction and modification and of their expression, should eventually permit an explanation of the high degree of strain specificity, for example by a mechanism of recognition of certain base sequences. If this last idea should be correct one may further speculate that a restriction enzyme might provide a tool for the sequence-specific cleavage of DNA. Application

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