HOST-INDUCED MODIFICATIONS OF VIRUSES

S. E. LURIA
University of Illinois, Urbana, Illinois

Viruses exhibit extensive adaptability to growth in various hosts or tissues. It was widely held in the past that virus adaptability reflected a peculiar plasticity of virus heredity, which allowed it to be directly influenced by its host cells. The alternative interpretation of virus adaptation to new host cells as due to spontaneous mutations, which provide a range of genotypes for the new hosts to select from, always had authoritative proponents (see Findlay, 1939). This viewpoint finally gained wide recognition (see Burnet, 1946), partly as a consequence of the development of phage genetics and of the interpenetration of various branches of virology. It is now recognized that most variation in virus properties is caused by viral mutations, and that virus plasticity results from the variety of genotypes present in the large viral populations.

It was, therefore, an unexpected development when a new type of virus variation was discovered in bacteriophages. This has been called host-induced or host-controlled variation (Luria and Human, 1952; Ralston and Krueger, 1952; Anderson and Felix, 1952; Bertani and Weigle, 1953). Its outstanding characteristics are that it is strictly phenotypic, non-hereditary, and that it is determined by the host cell in which a virus has been produced. In this paper, I shall summarize the features of this phenomenon; I shall compare it with other instances of nonhereditary phage variation; and I shall attempt to assess its possible bearing on certain problems in other areas of virology.

HOST-INDUCED MODIFICATIONS IN BACTERIOPHAGE

Host-induced modifications have been described in coliphages, in salmonella phages, and in staphylococcus phages. The instances that have been recognized affect, by restricting or enlarging it, the host range of bacteriophages. There is no reason to assume that other phage properties cannot be affected by host-induced variation. Indeed, the phage-mediated "transduction" into a bacterial strain of some property of the host strain in which a phage has been formed (Zinder and Lederberg, 1952) is itself a host-controlled variation of phage, although it is recognized by changes of the host cell rather than of the phage itself.

The common features of all host-induced modification of host-range thus far recorded are, (a) a restriction of the ability of the phage to grow in some host as a result of one cycle of growth in one type of cell; and (b) a release of this restriction following one cycle of growth in some other host.

For example, phage P2 grown on Shigella dysenteriae strain Sh can grow in every cell of Sh but only in about one of $10^4$ cells of Escherichia coli strain B. The same phage P2 grown in B (for example, the phage liberated by the one cell in $10^4$ above) can grow in all cells of B and in all cells of Sh. But, if grown in Sh, it is again restricted to one in $10^4$ cells of B. We say that P2 grown on Sh is in the P2 Sh form, whereas P2 grown on B is in the P2 B form (Bertani and Weigle, 1952). The variation P2 Sh $\rightarrow$ P2 B is adaptive, since it permits continued growth on the host B.

The first instances of host-induced variation included only two alternative forms of each phage, but a more general situation may involve several such forms. The situation is conveniently described by a scheme proposed by Weigle and shown in Table 1. The restricted ability of a phage to grow in some host is characterized by a specific "yielder frequency" or "acceptance frequency," that is, by the proportion of the cells of that host that, if infected, can support growth of that phage. The restriction depends only on the strain in which the phage has undergone the last reproductive cycle. The full situation of Table 1 has been demonstrated.

**Table 1. The Scheme of Adaptive Host-Induced Modification**

The figures correspond to the yielder frequencies (= proportion of cells that liberate phage) for the various host-phage combinations.

<table>
<thead>
<tr>
<th>Phage forms</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_A$</td>
<td>1</td>
<td>$10^{-4}$</td>
<td>$10^{-6}$</td>
</tr>
<tr>
<td>$P_B$</td>
<td>$10^{-4}$</td>
<td>1</td>
<td>$10^{-6}$</td>
</tr>
<tr>
<td>$P_C$</td>
<td>1</td>
<td>$10^{-4}$</td>
<td>1</td>
</tr>
<tr>
<td>$P_{BC}=P_C$</td>
<td>1</td>
<td>$10^{-4}$</td>
<td>1</td>
</tr>
<tr>
<td>$P_{CB}=P_B$</td>
<td>1</td>
<td>$10^{-4}$</td>
<td>$10^{-6}$</td>
</tr>
<tr>
<td>$P_{BA}=P_A$</td>
<td>$10^{-4}$</td>
<td>$10^{-6}$</td>
<td></td>
</tr>
<tr>
<td>$P_{CA}=P_A$</td>
<td>$10^{-4}$</td>
<td>$10^{-6}$</td>
<td></td>
</tr>
</tbody>
</table>

Homologies for phage P2: E. coli B = A; Sh. dysenteriae = B
Homologies for phage $\lambda$: E. coli S = A; E. coli C = B
Homologies for phage T1: E. coli B = A; E. coli $\theta$ = B; E. coli F/50 = C.
Homologies for staph phage P1: Staphylococcus #145 = A; Staphylococcus Kc = B.
The figures are yielder frequencies for hypothetical hosts infected with a phage or with its host-range mutants.

TABLE 2. THE SCHEME OF HOST-RANGE MUTATION

<table>
<thead>
<tr>
<th>Phage forms</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>P A (= P_ha)</td>
<td>1</td>
<td>10^{-6}</td>
<td>10^{-7}</td>
</tr>
<tr>
<td>P R (= P_hc)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P B, A (= P_hc)</td>
<td>1</td>
<td>10^{-6}</td>
<td>1</td>
</tr>
<tr>
<td>P B, C (= P_hc)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P C, A (= P_hc)</td>
<td>1</td>
<td>10^{-6}</td>
<td>1</td>
</tr>
<tr>
<td>P C, B (= P_hc)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

The ability to grow on a given strain, once acquired, is permanently maintained (barring rare reverse mutations).

The relations in Table 1 differentiate the host-induced modifications from host-range mutations in phage, which for purposes of comparison are illustrated in the scheme of Table 2. Here, the characteristic feature is the persistence of the "adaptation" after return to the original host.

The instance of host-induced modification that was recognized first (Luria and Human, 1952), and that led to the recognition and interpretation of most other instances, differs from the prototype of Table 1. It is illustrated in Table 3. Here, one growth cycle in a host strain E. coli B/48, differing

TABLE 3. THE SCHEME OF UNADAPTIVE HOST-INDUCED MODIFICATION

<table>
<thead>
<tr>
<th>Phage forms</th>
<th>Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>P A (= T2)</td>
<td>(E. coli B)</td>
</tr>
<tr>
<td>P B (= T2)</td>
<td>(E. coli B/48)</td>
</tr>
<tr>
<td>P C (±*T2)</td>
<td>(S. dysenterae Sh)</td>
</tr>
<tr>
<td>P C, A (±*T2)</td>
<td>(E. coli B)</td>
</tr>
<tr>
<td>P C, B (±*T2)</td>
<td>(E. coli B/48)</td>
</tr>
<tr>
<td>P C, C (±*T2)</td>
<td>(E. coli B)</td>
</tr>
</tbody>
</table>

by a single spontaneous mutation from another host, E. coli B, modifies phage T2 (or T6) to the form T*2 (or T*6), characterized by a restriction of growth ability to a small proportion of the cells of the modifying host and of its relatives (E. coli B and all its derivatives). Growth of both T2 and T*2 is unrestricted in the unrelated host S. dysenteriae Sh, which liberates phage in the T2 form. The modification induced by B on the T*2 form is adaptive, but the modification induced by B/48 on T2 is unadaptive, since it restricts the growth ability on B/48 itself.

In summary, the known instances of host-controlled modification of phage involve, on the one hand, a restriction by one or more hosts of the growth ability of the phage on some host and, on the other hand, a release of the restriction by some other hosts. The latter hosts in turn may or may not impose other alternative restrictions. The modifications imposed by successive hosts are not additive but mutually exclusive. Each host modifies the phage in a characteristic way, independent of the previous host history of the phage (see Table 1). The phage modification imposed by a given host is the same, whether the phage is liberated by a lytic cycle or from lysogenic cells (Bertani and Weigle, 1953). As far as we know, a given modification is similar in all genetic mutants of a phage (for example, in P2 and its virulent mutants; in T2, T2h, T2r . . . ). The modifications generally affect the totality of the phage produced in the modifying host cells.

GENERAL CHARACTERISTICS OF THE HOST-INDUCED MODIFICATIONS IN HOST RANGE

Host-induced modifications, as opposed to host-range mutations, are characterized, not only by their ready reversibility, but also by the determination of the few successful particles of a restricted phage form that succeed in overcoming the restriction. The observations can be listed as follows:

1. The success of the few particles that manage to grow is not due to a difference in adsorbability. All alternative forms of a phage are equally well adsorbed by any given host, whether they grow in it or not.

2. The ability to overcome the growth restriction results from the attachment of particles of the restricted phage form to some exceptional cell of the host. The evidence for this statement is as follows:

(a) The proportion of cells in which a restricted phage succeeds in growing (as for P2 Sh on B) can be altered by a variety of environmental factors acting on the host before infection.

(b) In mixed infection of bacteria with two mutants of a restricted phage (for example, P2 Sh and P2 vir Sh on B, or T*2 and T*2r on B) the frequency of mixed yielders may be, say 30 per cent when the total frequency of yielding complexes is only one per cent. If the yielders were bacteria infected with exceptional phage particles, the fre-
quency of mixed yielders should reflect the coincidence of two exceptional particles of different mutants infecting the same cell (less than $10^{-3}$ in the experiment quoted above).

(c) Phage mutant particles, when they first appear in nonmutant populations, are present in characteristic clones of identical sibs, each clone deriving from one mutation (Luria, 1951). Instead, when single bursts of a restricted phage are tested for the number of particles that succeed in overcoming the restriction, the rare yielders are distributed at random (at least as long as the bacteria are in large excess, as in platings) (Bertani and Weigle, 1953). This is explained if we consider that a yielder is an exceptional bacterium infected with a nonexceptional phage particle.

The facts listed above do not exclude completely that the particles of a restricted phage may be heterogeneous in their ability to grow in exceptional bacteria. The fact that in single infection experiments the number of yielder bacteria is a linear function of the phage inoculum is explained by the linear increase of exceptional cells that become infected. In multiple infection, some complications appear, which have not yet been adequately investigated.

In summary, the modification of a phage by growth in a host towards which it was restricted is due to the accidental acceptance of some particle of the restricted phage by some exceptional, "active" cell of the host. On the basis of experiments with phage T1 grown on various hosts, Fredericq (1950a, b) has questioned the hypothesis of a spontaneous origin of host-range mutants. Prominent among Fredericq's findings was the random, nonclonal distribution of the T1 particles with extended host range in platings on various hosts (strains E. coli B and E. coli 9 respectively). Apart from some inadequacies of methodology used in this work (small samples from each of six large cultures), most of the observations are easily interpreted in terms of host-induced modifications. The critical test of reversibility of the modifications of T1 upon return to other hosts, lacking in the original work, was done for T1 B and T1 9 by N. Collins Bruce (personal communication), who showed complete transitions between the two forms in single growth cycles on E. coli B and E. coli 9 respectively.

The Nature of the Restricted Growth Ability of Modified Phage

The stage of arrested development. This stage varies from phage to phage. It always follows adsorption; adsorption is equal for restricted and unrestricted forms of the same phage. In some instances, for example with P2 Sh on B, there is no killing of the host; after adsorbing the restricted phage, the host is not slowed down at all in its development. We have been unable to observe any gross nuclear changes in B cells that had adsorbed several particles of P2 Sh.

With other systems, there is complete suppression of cell division (T*2 on B or on B/44). The infected cells may elongate before dying. Desoxyribonucleic acid synthesis is stopped. The nuclear changes are not those characteristic of the normal infection with the corresponding unrestricted phage forms. No infectious phage can be revealed in the infected bacteria by artificial lysis.

It is possible that the differences between the T2 and the P2 situations are related to other differences between the infection of E. coli B with phages of the T group (suppression of enzyme syntheses, rapid nuclear disintegration) and the infection of bacteria with phages that do not produce these changes. The difference is not simply between temperate and virulent phages (as defined with relation to lysogenicity) since a highly virulent mutant of P2 Sh fails to kill the B cells in which it does not grow.

Zinder (personal communication) observed that phage PLT-22, the agent of "transduction" in Salmonella, is modified, by growth on S. gallinarum, to a form restricted in growth ability on S. typhimurium (yielder frequency $10^{-8}$). The restricted form can still transduce genetic properties of gallinarum to typhimurium with about normal frequency. This observation suggests that the interaction between restricted phage and host goes far enough to permit introduction and acceptance of the accompanying host-genetic material.

Because of technical reasons, we have as yet been unable to prepare any P32-labeled, growth-restricted phage suitable for testing whether the phage DNA is inoculated into hosts in which the phage fails to grow.

The nature of the exceptional cells that allow a restricted phage to grow. In various situations, the exceptional yielder cells may be as many as one in 40 (Ralston and Krueger, 1952) or as few as one in $10^8$ (N. Collins Bruce, unpublished). If even fewer, they might not be detected at all and the variation would probably remain undetected.

The conditions that modify the frequency of exceptional cells vary from system to system:

1. Age of cells. Old cells of E. coli B or of its mutants (from aerated cultures in buffered nutrient with exhausted food supply) accept T*2 or T*6 with a frequency of 1 to $4 \times 10^{-2}$ instead of $10^{-4}$ to $10^{-8}$. Rejuvenation (that is, reduction of yielder frequency, or deactivation of "active" cells) occurs rapidly if the old cells are aerated in fresh nutrient broth or in solutions of glucose, lactate, or other oxidizable substrates. The temperature coefficient for this deactivation is high. These observations suggest the removal, by oxidation, of some metabolite that is accumulated in the old cells and is operative in allowing the restricted phage to grow. The age of E. coli B cells has little effect on their acceptance ability for phage P2 Sh.
2. Medium and growth conditions. When E. coli B is grown in a casein hydrolysate medium with at least one per cent glucose and not more than one per cent K₂HPO₄, growth stops as the pH of the medium reaches 4.9-5.0. The cells from the acid cultures are viable. After washing in buffer, these "acid cells" accept T⁴₂, T⁶₆, or P₂ Sh with frequencies of 10 to 50 per cent. (Similar growth conditions barely alter the acceptance frequency of cells of E. coli S for λC.) The requirements for acceptance of T*₂ are less strict than for P₂ Sh. T*₂ is well accepted by acid cells from media of a variety of compositions, synthetic or variously supplemented, whereas the acceptance frequency for P₂ Sh is much higher with acid cells from complete media. The nutritional factors involved have not yet been worked out. The low pH is not itself responsible for the increase in acceptance frequency, since young cells growing (slowly) in media at pH 5.0 are not active. Filtrates of old, low-pH cultures do not activate inactive cells.

The active cells from the acid cultures are deactivated slowly by aeration in fresh media with various carbon sources; the physiology of this activation and deactivation needs further study. We cannot tell at present whether the activity of the "acid cells" depends on storage of phage-needed intermediates or on removal of phage-growth inhibiting mechanisms.

3. Ultraviolet irradiation. The activation by ultraviolet irradiation, discovered first with E. coli S cells as acceptors of λC (Bertani and Weigle, 1953), occurs also with E. coli B and its mutants as acceptors for T*₂, but not for P₂ Sh. In the ultraviolet activation of B for T*₂ acceptance, the remarkable feature is the continued increase in activation at very high doses of ultraviolet (see Figure 1). The activation is almost identical for B and for its radiation-resistant mutant B/r, in spite of the great difference in ultraviolet sensitivity of their colony-forming abilities. Activation by ultraviolet is partly eliminated by exposure to "photoreactivating" light.

Ultraviolet-activated cells of E. coli B are deactivated by aeration in fresh media at a rate similar to the rate of deactivation of acid cells, and much slower than the rate of deactivation of old, starved cells. Yet, ultraviolet and growth to high acidity cannot act by the same mechanism, since ultraviolet activates B only as acceptor for T*₂ whereas acid growth activates B as acceptor for both T*₂ and P₂ Sh.

In summary, a variety of agencies can activate or deactivate cells, that is, change their accepting ability for restricted phages. With a given host, activating agents are specific for a given phage. This emphasizes the differences in the developmental sequences of various phages in the same host and in the stages at which these sequences are blocked with different restricted phages.

**Figure 1.** The number of irradiated cells of E. coli B/r or of E. coli B that yield phage when infected with phage T*₂, as a function of the dose of ultraviolet light. The values in the figures correspond to mixtures containing 1 × 10⁸ bacteria and 7 × 10⁶ phage per ml, with over 90 per cent of the phage adsorbed. The broken lines are the survival curves of uninfected bacteria.

**The Causes of the Host-Induced Modifications**

Except for restrictions of growth ability, no other differences, serological or physiological, have been detected between host-modified forms of the same phage. These alternative forms may be produced either by bacteria that differ by unknown, probably multiple properties (Sh. dysenteriae Sh and E. coli B; E. coli C and E. coli S); or by bacteria that differ by one spontaneous mutation (E. coli B/A₄, and E. coli B). The latter example suggests that one-step genetic differences may also be involved in other situations. E. coli B/A₄, has the phage-resistance pattern B/3,4,7. It can be isolated from E. coli B by the selective action of either T₄, or T₃, or T₇. It does not adsorb these phages and apparently does not carry them lysogenically.

There is another mutant of E. coli B, called B/A∞₄ (Luria and Human, 1952) which in young cultures behaves like B/A₄, transforming T₂ into T*₂. In old, starved cultures most cells behave like B and liberate T₂ instead of T*₂. Here, not only the acceptance frequency, but also the modifying ability of a host for a phage depend on the physiological conditions of the host cells.

An important lead is provided by observations on the Vi-phages of Salmonella typhosa. The original Vi- phage II of Craigie and Yen (1938), plated on each of 30 types of Vi-positive S. typhosa, gave a few plaques, from which more or less specifically "adapted" Vi-phages were isolated. These differ from the nonadapted Vi-phage II because they can grow unrestrictedly on one or more of the Vi-positive host strains. The different host strains are then...
HOST INDUCED MODIFICATIONS OF VIRUSES

recognized or “typed” by their pattern of sensitivity to the adapted phages (see complete scheme of VI-types in Felix and Anderson, 1951). The adapted phages were supposed to be host-range mutants (see Craigie, 1946). Recently, however, many of them were shown to be due to host-induced modifications of Vi-phage II (Anderson and Felix, 1952, 1953a). Reversion to the A (= unadapted) form occurs in single passage on host type A. (This was tested by a single plaque isolation; the occurrence of the modification in a single growth cycle has not yet been established).

These facts are summarized in Table 4, which cor-

Table 4. The Relation of Some Variants of Vi-Phage II with Vi-Strains of Salmonella typhosa

Data from Anderson and Felix (1953a). The — sign indicates a plaque count at least 1000 times lower than on host strain A.

<table>
<thead>
<tr>
<th>Phage forms</th>
<th>Host strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phage II A</td>
<td>C E1 T D5 D6* D1* D4</td>
</tr>
<tr>
<td>Phage II C</td>
<td>1 1 — — — —</td>
</tr>
<tr>
<td>Phage II C, A</td>
<td>1 — 1 — —</td>
</tr>
<tr>
<td>Phage II E1</td>
<td>1 — — — 1</td>
</tr>
<tr>
<td>Phage II E1, A</td>
<td>1 — 1 —</td>
</tr>
<tr>
<td>Phage II T</td>
<td>1 — — 1</td>
</tr>
<tr>
<td>Phage II T, A</td>
<td>1 — —</td>
</tr>
<tr>
<td>Phage II D5</td>
<td>1 — — 1 —</td>
</tr>
<tr>
<td>Phage II D5, A</td>
<td>1 — — 1</td>
</tr>
<tr>
<td>Phage II D6</td>
<td>1 — — 1</td>
</tr>
<tr>
<td>Phage II D6, A</td>
<td>1 — —</td>
</tr>
<tr>
<td>Phage II D1</td>
<td>1 — — — 1</td>
</tr>
<tr>
<td>Phage II D1, A</td>
<td>1 — — —</td>
</tr>
<tr>
<td>Phage II D4</td>
<td>1 — — —</td>
</tr>
<tr>
<td>Phage II D4, A</td>
<td>1 — — —</td>
</tr>
</tbody>
</table>

*Strain T is lysogenic for phage t and identical to A(t) in Vi-type; likewise, D6 is like A(d6); D1 is like A(d1). From: Anderson and Felix (1953b).

Phage forms II C, II E1, II T are presumably host-induced modifications (see Table 1).

Phage forms II D5, II D6, II D1 are presumably host-range mutants (see Table 2). They might be designated:

II D5; II D6 D1; II D4, respectively.

Phage form II D4 is apparently a host-induced modification of the host range mutant II D1. It might be designated: II D1 D4.

responds to a portion of the classic Vi-typing scheme homologized with the scheme of our Table 1. We introduce the symbol II to indicate the Vi-phage II, and to distinguish between phages and bacteria. Some adapted phage forms are presumably host-range mutants (e.g., II D5); others are host-induced modifications (e.g., II T); others are combinations of both (e.g., II D4 is a host range mutant II D1 modified by host D4, that is, II D1 D4). Thus far, the situation in host-induced modifications in phage II is analogous to the case of Table 1. Several mutually exclusive restrictions and releases are impressed by several host strains on phage II, and a common host is attacked by all phage forms and restricts them against growth in all other hosts.

The additional important feature is that the differences among some of the Vi-strains of S. typhosa, which determine their susceptibility to adapted Vi-phages and their modifying ability for these phages, are due to latent phages carried by individual Vi-strains and completely unrelated to the Vi-phages (Craigie, 1946; Anderson and Felix, 1953b).

Loss or gain of lysogenicity for one of these latent phages can transform one Vi-type bacterium into another type. In its pattern of sensitivity to the adapted Vi-phages, a transformed strain may either correspond to one of the other known strains or may exhibit a new pattern ("untypable strains").

In at least one instance, that of the transformation of host strain A into host strain T by lysogenization with the phage t, the new lysogenic strain A (t) is indistinguishable from T and has presumably acquired the ability to impress onto the Vi-phage II A the phenotypic modification to the form II T (see Table 4). Instances of such transformations may become more numerous as the system is further explored.

The point of importance is that a latent prophage may presumably impress upon the host, not only the inability to accept an unrelated phage (as in many other known examples), but also the ability to discriminate among modified forms of an unrelated phage and, even more important, the ability to impress a specific modification upon that phage.

It would be premature to generalize as to the role of prophages in other instances of host-induced variation in phage. The difference between E. coli B and B/4, for example, is not due to lysogenization. It might, however, correspond to a mutation in an undetected prophage. Indeed, the phenomena of transduction and the presumably close relations between the prophages and the genetic apparatus of their host cells make it difficult and possibly meaningless to distinguish between genotype-controlled and prophage-controlled properties of a bacterium. The influence of the prophages carried by various Vi-strains of S. typhosa on the reaction of these strains to the phages of the Vi-group II might be due either to phage genes or to host genes transduced with the latent phages. The study of successive and multiple transformations induced in Vi-strains by lysogenization may provide some of the answers.

The Mechanism of Phage Modifications by the Host Cell

We have tentatively concluded that host-induced modifications determine the ability or inability of a phage to perform some specific critical step of...
interaction with one or more hosts. When different modifications can be impressed on a phage by different hosts, these modifications are mutually exclusive rather than additive. These facts suggest that the same phage structure, needed for the restricted step, is altered in two or more alternative ways in different hosts. Several phages have been shown to consist of genetic and nongenetic material, the latter including at least the protein skin of the phage and possibly also some of its nucleic acid. Since the host-induced modifications are nonhereditary, we may incline to attribute them to changes in the nongenetic portions of the phage. This may be unjustified, however. As pointed out by Bertani (personal communication), the genetic portion of a phage might be so modified (although not intrinsically mutated) by its intimate relation with the genome of a host as to be unable to establish successful connections with the genome of a different one. The fact that some modifications are adaptive (for example, the change P2 Sh → P2 B, which extends the growth ability on B) and some unadaptive (for example, T2 → T′2 on B/4, ) might reflect different forms of nuclear interactions. It seems desirable to investigate thoroughly the stages at which the development of restricted phages is arrested in a variety of cases, in order to understand the role of various phage structures in phage development and to localize the modifying ability of the host on any one of the phage structures.

The Relation of Host-Induced Variation to Other Forms of Non-Hereditary Changes in Bacteriophage

Apart from transitory changes in the particles that survive certain treatments, such as with ultraviolet light or antisera, two types of nonhereditary modifications have been described in phages, besides host-induced variation.

Phenocopies of Heat-Stable Mutants of Phage T5. Lysates of T5 and of its relatives contain heat-resistant particles, which upon growth give rise to regular T5 phage; these phenotypically heat-stable particles are phenocopies of stable mutants of the same phages (Adams and Lark, 1950). In phage T5, the mutants appear with a frequency of about 10^{-7}, the phenocopies with a frequency of about 10^{-3}.

Adams (1953) reported that in the yield of individual bacteria infected with phage T5 the phenocopies are not distributed at random but are grouped clonally. When present, they constitute a minority portion of the yield. This suggested that the phenocopies might be formed in response to "local conditions" or to a modified "template or pattern" (not a phage-genetic one) in some of the bacteria. The further suggestion was made that the local conditions may have to do with some biochemical irregularity within individual host cells.

This suggestion would relate the production of the heat-stable phenocopies to host-induced modifications. There is a basic difference, however, between the production of heat-stable phenocopies and the established cases of host-induced variation. In these, the modifying influence of a host cell is apparently uniform on all the phage particles that cell liberates.

As shown in Table 5, the heat-stable phenocopies of T5 in individual T5 bursts are grouped clonally

**Table 5. The Distribution of Phenotypically Heat-Resistant Phage Particles of T5**

Data from Adams (1953) analyzed according to Luria (1951).

<table>
<thead>
<tr>
<th>Resistant particles</th>
<th>Found</th>
<th>Expected according to the duplication hypothesis (from the number with 1 or more)</th>
</tr>
</thead>
<tbody>
<tr>
<td>per sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 or more</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>2 ″ ″</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>3 ″ ″</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>4 ″ ″</td>
<td>8</td>
<td>7.5</td>
</tr>
<tr>
<td>5 ″ ″</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>6 ″ ″</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>10 ″ ″</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>20 ″ ″</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

and the frequency distribution of clone sizes is very close to the one expected for groups of identical sibs arising by reduplication of randomly mutated individuals (Luria, 1951). Thus, we are led to an alternative hypothesis. We assume that the phenotypic change in heat stability arises, like a mutation, spontaneously and randomly in individual phage particles during reproduction, and is transmitted from parent to daughter particles within the cell of origin, but is not transmitted to the progeny of the heat-stable particles when they later reproduce in other bacteria. The occurrence in the same phage of similar but permanent mutations (as much rarer events) strengthens this conclusion. We suggest that the phenocopies are due to a genetic change in a portion of phage material that, although "self-reducing" in the bacterium of origin, is not utilized as a model for reproduction in later cycles of multiplication in other bacteria. The identification for this "transitorily genetic" structure in phages of the T5 group awaits further evidence.

Phenotypic mixing in mixed infection. This consists of the production, in cells of E. coli B infected with phages T2 and T4, of particles that, like T4, can attack bacteria B/2 but that give rise to a pure yield of T2 (defined by heritable ability to grow on B/4 and inability to adsorb on B/2) (Novick and Szilard, 1951). Similar particles with mixed phenotype are probably formed also in mixed infection with T2 and T6 (Delbrück and Bailey, 1946). Hershey (unpublished) found
HOST INDUCED MODIFICATIONS OF VIRUSES

phenotypic mixing between T2h and T2h+. Streisinger (unpublished) found that in mixed infection with backcross strains of T2 and T4, especially selected to minimize mutual exclusion, all progeny particles are genetically either T2 or T4 in host range. Both groups include particles of three phenotypes: (a) adsorbed by B/4 only; (b) adsorbed by B/2 only; (c) adsorbed by B/2 and by B/4.

All features of phenotypic mixing can be accounted for by modifications of the protein skin of the phage. The restricted step, when present, is adsorption: whenever adsorption occurs, growth follows. Genotypic T2 with T4 phenotype is neutralized equally well by anti-T2 and by anti-T4 sera, which act on the protein skin (Delbrück, unpub.).

The mechanism of phenotypic mixing is unknown. The host-range specificity of the phage skin in particles from mixed infection may be determined by a complex between phage nucleus and some accessory genetic material of the other phage type that fails to appear in the progeny. Alternatively, the host range specificity of the phage skins might be influenced by many phage genomes through interactions in the host cell at the level of the synthesis of the "adsorption sites" of the phage.

Formally, phenotypic mixing does not resemble host-induced modifications; the two types of alteration apparently modify different phage functions and probably also different phage structures. Phenotypic mixing reflects interactions, at the phage phenotype level, among genetic materials of several phages. Host-induced modification reflects interactions, at the phage phenotype level, between phage and host genotype. The tie-up between the two phenomena, if any, may reside in some general pattern of interactions at the genic level in phage-infected cells.

HOST-INDUCED MODIFICATIONS IN VIRUSES OTHER THAN PHAGE

The essential characteristic of host-induced modification is the complete transformation of one form of virus into another upon a single cycle of intracellular growth in a modifying host. Experiments on animal and plant viruses seldom permit observation of virus after single cycles of growth; a search of the literature reveals no certain example of reversible changes attributable to host-induced modification. The numerous instances of host adaptation or tissue adaptation in animal viruses correspond probably to a selection of host-range mutants. The variation generally appears, gradually or suddenly, after several passages in a new host and persists after return to the previous host. Reversion, when observed, has never been shown to occur by a single-cycle mass transformation. Variation detected after a single animal passage can be due to selection of mutants, since one passage corresponds to many repeated intracellular growth cycles, especially if only a small fraction of the virus inoculated can multiply.

Yet, the existence of host-induced modification in phage suggests that variation in other viruses should be reexamined experimentally in the light of this new phenomenon. In doing so, the following properties of host-induced modification, as observed in phage, should be remembered:

1. It affects the totality of the virus exposed.
2. It may either extend or restrict the host range of a virus.
3. The restriction in host range may concern either the same type of host cell which induces the modification or another type.
4. The modifying ability of the host cell can be affected by its growth stage (which, more generally, corresponds to its developmental history).
5. The ability of a host to accept (hence, to reveal or unmask) a restricted virus may itself depend on the developmental stage of the host cells.
6. Host-induced modification of the adaptive type (see Table 1) can simulate selection of adapted mutants; host-induced modifications of the non-adaptive type (see Table 3) can simulate the production of noninfectious or masked virus.

A single-cycle change in an animal virus is the formation of hemagglutinating, noninfectious particles, following injection of nonneurotropic influenza virus into the mouse brain (Schlesinger, 1950) and possibly in other host tissues as well. This change is certainly a host-induced modification; formally, it can be homologized with the change T2 → T*2 induced by B/4, assuming that only hosts B and B/4 are known (Table 3). That is, the "noninfectious" virus might be virus restricted in growth ability and might appear to be noninfectious only because no unrestricting host is available. In the absence of any evidence, however, that the modified influenza virus from brain can grow in some other host, it seems more reasonable to consider it as incomplete, unfinished virus rather than as infectious virus with a restricted host range.

Some cases of virus masking (Shope, 1950) may also seem to be formally analogous to nonadaptive host-induced modifications. Yet, in the classical cases of masking, for example, with rabbit papillomavirus in domestic rabbit, virus particles seem to be few or absent; masking may reflect partly the small amount of virus present (Friedewald and Kidd, 1944), partly the presence of modified, noninfectious virus antigens.

Rabbit papillomavirus has occasionally been maintained by repeated passages in the domestic rabbit (Shope, 1955). One such domestic rabbit-adapted strain lost its adaptation in a single passage in cottontail hare (Selbie et al., 1948). The conditions did exist for selection in the cottontail of a better growing variant, but the occurrence of a pair of host-controlled forms cannot be excluded.
Another field where host-induced modifications may play a role is exemplified by the determination of the tissue affinities of amphibian tumors derived from the virus carcinoma of the Vermont leopard frog (Rose and Rose, 1952). Passage of tumor cells from frogs to salamanders to frogs of different races, and from one organ to another, induces in the tumors (and presumably in their viral agent) a remarkable series of changes. The extreme specificity of some of these changes is illustrated by the significant coincidence of exactly bilateral periosteal tumors. It is conceivable that by growth in given host cells the tumor-causing agent may become, either specifically restricted to developmentally characteristic cells, or specifically prone to attack such cells. Unfortunately, methods for precise quantitative work with these tumor agents still have to be developed.

In summary, host-induced modification as observed in phage has not been demonstrated with animal or plant viruses. This does not constitute evidence against its occurrence since observations suitable for its detection have not been made. In view of the great importance that this type of variation, if present would have in virus ecology and in the epidemiology and pathology of virus diseases, efforts to determine its range of existence appear desirable.

REFERENCES


