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CURRENT PROBLEMS IN RESEARCH

Bacteriophage Genes and Bacterial Functions

The viruses that attack bacteria control properties of their host cells by action of specific genes.

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Knowledge acquired in the last few years has made it a tenable generalization that virus infection at the cellular level consists of the introduction into a cell of a fragment of genetic material composed of nucleic acid. This genetic material is delivered into the host cell by a specialized structure, the virus particle or virion (*1*), and carries the information needed to produce new virus. Highly purified nucleic acid from plant, animal, or bacterial viruses (*2*) can initiate infection and production of new virions if proper conditions are provided for the penetration of the nucleic acid into cells. Accordingly, all effects of viruses on cells are probably traceable either to early events connected with virus entry or to the genetic activity of the viral nucleic acid, expressing itself within the framework of the genetic potentialities of the cell. In considering the activities of the viral genome in the host cell, we must make two important distinctions, one concerning the type of nucleic acid, the other concerning the type of gene functions.

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Activities of the Viral Genome

DNA and RNA viruses. The genetic material of a virus is either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). For DNA viruses there is no difficulty in visualizing a genetic role, in line with current ideas on the nature of chromosomal genes. The problem is different with RNA viruses, because outside the field of viruses no genetic determinant has yet been proved to be composed of RNA (*3*). More correctly, there has been no definite evidence that cellular RNA plays any primary role in the determination of the specificity of cellular components—that is, that it carries genetic information not present also in DNA. Either we are willing to consider the RNA viruses as a special category of genetic elements, possibly remnants of organisms now fully reduced to parasitism, or, more plausibly, we must consider the RNA of viruses as homologous to some type of cellular RNA. Three classes of cellular RNA are now recognized: soluble, amino-acid-carrier RNA; ribosomal RNA; and messenger RNA (*4*). The last two are the main candidates for relationship to viral RNA. At present any evidence of relationship is bound to be indirect because no enzyme system capable of synthesizing RNA in vitro under the direction of an RNA template [as DNA polymerase does for DNA (*4a*)] has yet been reported. Clearly, if viral RNA shared with one category of cellular RNA the ability to act as template for replication, the problem under consideration would be solved.

Ribosomal RNA is found in molecular sizes ranging from 5×10^5 to 3×10^6 molecular weight units in various classes of ribosomes, which are the particles where proteins are synthesized. Nothing is known of the role of ribosomal RNA in the protein-synthesizing function of the ribosomes. Ribosomal RNA has not been shown to carry genetic information, but critical proof that the ribosomal RNA has gene-derived specificity does not exist. Conceivably, this RNA might be relatively independent genetically and might serve as an ancestor of viral RNA.

Messenger RNA, which carries to the ribosomes the gene-dictated instructions for protein synthesis, possesses intrinsic, gene-derived information. Whether messenger RNA may be used as a template for its own replication is uncertain. Its size is variable, ranging up to at least 10^6 molecular weight units, a size compatible with the amounts of nucleic acid in RNA viruses (0.7 to 2.5×10^6 molecular weight units). At least in bacteria, the messenger RNA is characteristically impermanent, being continually repleted by gene action. Viral RNA, on the other hand, is very stable in the host cell.

Several alternatives are open: either (rather improbably) viral RNA is a variety of ribosomal RNA with a greater endowment of specific information than has yet been attributed to the latter; or it is related to some still unrecognized class of cellular RNA, possibly nuclear RNA; or it is messenger RNA, which like the messenger RNA of animal cells, is more stable than that of bacteria. The difference may, in fact, be only an apparent one, due

to differing amounts of RNA-destroying enzymes in different organisms.

It is conceivable that, while being related to one of the classes of cellular RNA, the viral RNA may owe its genetic autonomy to a past history of mutation and selection. It is also possible that, having gained access to a foreign cell line, viral RNA may have succeeded in escaping the control of certain mechanisms that specifically prevented its replication in cells where it was originally made as a gene product.

Structural and regulatory gene functions. If viral genes act like other genetic elements, their possible functions (aside from replication) are of two kinds: structural functions (the specification of the molecular structure of enzymes and other proteins) and control functions (the specification of the level of activity of other genes). Two types of control genes are recognized in bacteria (*5*): regulator genes, which produce repressors that specifically block the expression of certain other genes in the same cell, and operator or switch genes, which control the function of one or more adjacent structural genes along the same chromosome. The operator genes supposedly are the sites of action of the repressors.

We should expect that the effects of viruses on their host cells may reflect both the activity of structural genes, determining new virus-specific proteins, and that of regulator and operator genes. Regulator genes in the virus, acting through cytoplasmic repressors, may act not only on other viral genes but also on genes of the host cells; in turn, the function of viral genes may be controlled by host genes, either indirectly through metabolic interactions or directly through specific regulator genes of the host.

Types of viral control over cellular functions. In the light of these considerations, we expect cellular functions to be altered by a virus in three main ways: through (i) new protein syntheses, controlled by structural genes of the virus; (ii) new specific repressions by regulatory genes of the virus; and (iii) alterations of the host cell genome brought about either by some virus-controlled enzymatic process or by the physical integration of viral genes into the cellular genome.

A main task of virus research is to interpret viral activities at the cellular level in terms of these various types of genetic function, and to identify those functions that are responsible for the significant changes observed in virus-infected organisms. For example, the problem of the role of viruses in the abnormal cellular differentiation observed in virus-induced tumors should be reducible to questions of the following kind: Is the viral genome directly responsible for the specific properties of the tumor cells? That is, are there viral mutations which specifically affect the properties of the tumor cells (*6*)? And, if so, are the abnormal growth and differentiation of cells due to the presence of virus-determined enzymes or of virus-produced repressors?

Persistent cellular abnormalities might also result from irreversible effects of viruses on cellular constituents—for example, the removal of some self-priming or self-maintaining but inessential component of the cells. In bacteria there are accessory genetic elements, or *episomes*, which can be lost without damage to the cell (*7*). Temperate phages, carried by bacteria as prophages, are themselves dispensable genetic determinants. Even the bacterial cell wall, or some essential cell-wall forming system, can be lost permanently as a result of a transient stimulus such as exposure to penicillin (*8*). A loss of accessory genetic elements may conceivably follow a transient viral infection, so that a cell line may become permanently altered even without becoming a persistent carrier of the virus.

A program of interpretation of viral functions in terms of gene action may clarify some of the most obscure aspects of cellular biology, such as tissue and organ differentiation and the role of various types of genetic and nongenetic cellular constituents in cellular regulation. This program is also a promising approach to the problems of virus origin and of the role of viruses in the evolution of genetic systems. For example, what relation is there between the shells, or capsids (*1*), of the viral particles and those cellular organelles, such as sperm achrosome or mating tubes, whose function it is to facilitate genetic transfer?

In bacteria, the analysis of bacteriophage infection has become an integral part of bacterial genetics. In this article I discuss some situations in which specific properties of phage-infected bacteria can be traced to actions of viral genes, to interactions between viral and host genes, or to alterations of the bacterial genome.

Genetic Controls of Biosynthesis in Phage-Infected Bacteria

The developmental cycle of bacteriophages. The typical overall cycle of phage development may be summarized as follows. Attachment of phage to specific receptors on the bacterial cell wall is followed by penetration of the phage genome into the cell. There follows a period of vegetative multiplication of the phage genome, reflected in an increase in phage nucleic acid to produce a pool of phage genetic elements. The initiation of vegetative multiplication requires the prior synthesis of some essential proteins. With *temperate* bacteriophages, which can establish lysogeny, one of two alternative sequences of events follows: either there occurs an irrevocable reaction that leads to synthesis of virion, proteins, maturation of phage particles, and lysis of the bacterium, or there sets in an immunity reaction, which prevents the step toward lysis, arrests vegetative phage multiplication, and permits the establishment of the phage genome as prophage in the bacterial nucleus (lysogenization). Phage production by lysogenic bacteria is a consequence of the breakdown of immunity.

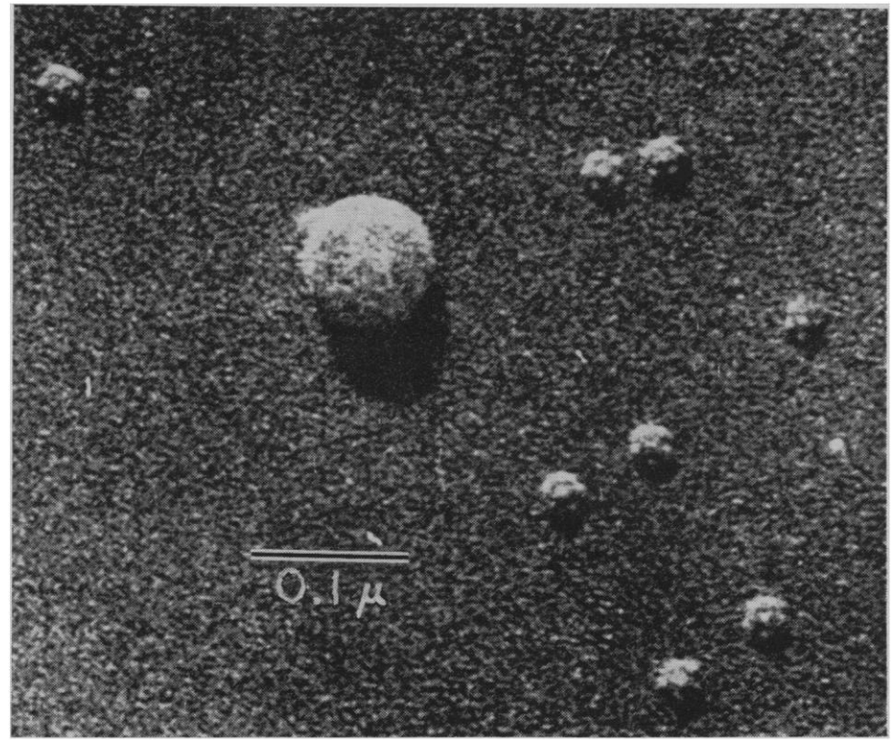
It is not yet known whether this description of the cycle applies to the recently discovered RNA phages (*9*), which have not yet been investigated in detail. With certain phage types some phases of the cycle are missing. Thus, virulent mutants of temperate phages fail to establish lysogeny because of genetic blocks in the immunity reactions. Other phages, which may be called *intemperate*, cannot establish lysogeny because an early step of their action in the bacterial cell leads to an irreversible destruction of the cell nucleus.

Synthesis of enzymes after phage infection. The intemperate phages seem to redirect the biosyntheses in the infected bacteria entirely to the task of phage formation. Synthesis of many bacterial enzymes and of ribosomal RNA is arrested. New enzymatic activities appear, concerned mainly with the biosynthesis of phage DNA. This is especially evident with the T-even phages, whose DNA contains 5-hydroxymethyl deoxycytidylic acid [dHMP (*10*)] and its mono- and di-glucosylated derivatives (*11*) instead of deoxycytidylic acid (dCMP). The new enzyme activities that appear after infection

are directed to the synthesis of hydroxymethyl deoxycytidine triphosphate, the precursor of the dHMP of phage DNA (*12, 13*); to the specific glucosylation of dHMP after its incorporation into DNA (*13*); and to the degradation of deoxycytidine triphoshate to dCMP (*13, 14*) and further to deoxyuridylic acid [dUMP (*15*)]. These enzyme activities are not found in the uninfected bacteria.

In addition, other changes occur in certain enzyme activities that were already present in uninfected cells. These changes are such as to increase production of essential precursors for phage DNA. For example, the activity of the methylase that converts dUMP to thymidylic acid (dTMP) increases at least tenfold (*16*). Similar increases in enzyme activities are found in infection with the intemperate phage T5, which does not require any qualitatively new DNA constituent (*13, 17*).

How are these findings to be interpreted? It was suggested some years ago (*18*) that the arrest of synthesis of host-specific enzymes was due to the rapid destruction of the nuclear apparatus of the bacteria, which is observed after infection with intemperate phages and is accompanied by depolymerization of the bacterial DNA to the mononucleotide units. This hypothesis postulated that the continued presence of the structural gene was necessary for the biosynthesis of the corresponding enzyme, a view validated at least for bacteria by studies on enzyme biosynthesis (*5*). A corollary of this hypothesis is that any new enzyme activities, as well an any increase in the level of preexistent activities after infection with intemperate phages, should be due to enzymes synthesized under the structural control of genes in the phage itself. Several indirect lines of evidence support this interpretation: (i) the intemperate phages of the T-even group contain over 10^8 molecular weight units of DNA, enough to provide structural information for 100 average-size proteins; (ii) new antigens, distinct from the proteins of the phage capsid, appear in the infected bacterium during the phase of enzyme increase (*19*); (iii) the increase in some preexistent enzyme activities corresponds to the formation of new enzymes, different in physicochemical properties and in activation requirements from the preexistent bacterial enzymes (*20, 21*); and (iv) the increased enzyme activities are often directed to the utilization of the deoxyribotides liberated in the breakdown of host DNA.



Particles of bacteriophage ΦX-174, one of the smallest viruses known. The large particle in the center is a polystyrene latex ball. [Cecil E. Hall, Elizabeth C. MacLean, and Irwin Tessman, Massachusetts Institute of Technology]

An interesting question arises: Why is the DNA of an intemperate phage protected from the destructive processes that break down the bacterial DNA? The simplest explanation, that breakdown only affects the host chromosome, does not seem valid. In fact, cellular genes become incapacitated even if they are present in extrachromosomal form. For example, infection with an intemperate phage immediately stops the synthesis of the enzyme β-D-galactosidase, whether the galactosidase gene is in the bacterial chromosome or is part of an episome like the fertility factor or the genome of a transducing phage (*22*). We conclude that the DNA of the intemperate phage is protected from breakdown either by some intrinsic quality of its molecular configuration or by some special location in the infected bacteria.

There has been a peculiar difficulty in analyzing the role of phage genes in the control of enzymes formed after phage infection. Obviously, what one needs is a set of phage mutants such that some of the early enzymes are altered or absent. But phage mutants of this kind are hard to obtain; the corresponding mutations would generally be either lethal or undetectable.

A new approach to this problem has been provided by the discovery of classes of phage mutants that can grow under one set of conditions but not under another, because in the latter their developmental cycle is arrested at some specific stage. One such class is that of heat-sensitive mutants (*23*). Another class consists of host-dependent mutants, found both in phage λ [*sus* mutants (*24*)] and in phage T4 [*am* mutants (*25*)]. The mutants can reproduce in some bacterial host strains but fail to do so in other hosts because phage development is arrested at some specific stage. These mutations can occur at many different sites in the phage genome, and the stage of arrest of phage development corresponds to a block in the corresponding gene locus. The special hosts in which the mutant phages can grow have a genetically determined permissiveness with respect to certain altered genes, which can perform their function adequately in these hosts and not in others.

It is among these mutants that one can search for mutations that affect the enzymes needed for phage DNA synthesis. With one of these mutants, T4-*am122*, the enzyme that converts dCMP

to dHMP fails to appear in the infected, nonpermissive bacterial host (*26*). In addition, this mutant has another abnormality: the DNA polymerase activity, which greatly increases after infection with normal phage T4 (*21*), fails completely after infection with T4*am-122*. By extending this approach it should be possible to specify the nature of the control by intemperate phages over the synthesis of enzymes in infected bacteria.

Synthesis of the virion proteins. The genetic determination of the phage-virion proteins unquestionably rests with structural genes of the phage. The phage loci concerned with the determination of several of these proteins (including phage head proteins, tail fibers, and cofactor combining protein), as well as with the control of the lysozyme-like lytic enzymes produced in phage infection, have been mapped by phage crosses (*27*). Even the morphology of the phage tail may be altered by what appear to be mutations (*28*), although other possibilities, such as recombination with defective proviruses, are not excluded. An internal protein found in some phages is specific for each phage type and is synthesized very early after infection (*29*). In addition, certain steps in the process of phage maturation, such as the "condensing" step that initiates the folding of phage DNA into compact structures, require the synthesis of new proteins (*30*), apparently under phage control.

Sequential expression of phage genes. A remarkable feature of the phage-determined protein syntheses is their characteristic sequentialism. For example, virion proteins and phage lysozyme are synthesized only in the second half of the period of phage development. Even more remarkably, the new enzymes initiated by an intemperate phage such as T2 stop increasing at about the time the new phage proteins appear. This arrest is due to the inception of some later step in phage development. If the infecting phage has been treated with ultraviolet light so that no DNA synthesis occurs, or if it is an *am* mutant blocked in DNA synthesis, the production of phage-initiated enzymes does not stop, but continues for a much longer time (*26, 31*). Apparently, some of the late phage functions have a repressive effect on the genes that initiate production of the enzymes.

The situation is certainly simpler in the case of phages with only 1 to 2 $\times$ 10^6 molecular weight units of nucleic acid, sufficient to specify only very few proteins. With such phages, all cellular alterations should be traceable, directly or indirectly, to the action of a few phage genes. The same is true for infection with the small plant and animal viruses.

Genetic Functions in Lysogeny

Lysogenization and immunity. Temperate phages do not destroy the genome of their host bacteria; hence, they can establish a persistent relation with the bacteria. This compatibility may reflect a molecular compatibility of the DNA; the temperate phages that have been analyzed all have a DNA base composition similar to that of the host DNA. There may actually be some degree of genetic homology between temperate phage and host cell.

The added features of the lysogenic relation reveal genetic functions of phage that are not easily observed with intemperate phages. One group of functions controls the ability to establish lysogeny. The initial decision as to lytic or lysogenic response to infection depends on the function of several phage genes; mutations in any one of these genes can reduce or suppress the ability to lysogenize (*32*). These genes function through cytoplasmic products; in mixed infection, mutants altered in different gene loci can complement each other and make lysogenization possible. The critical step in decision is probably the production of a repressor, which prevents an irreversible, chloramphenicol-sensitive step toward maturation and lysis (*33*).

The lysogenic bacteria are immune to superinfection with homologous phages, in the sense that the superinfecting phage (as well as the prophage itself) is prevented from starting the path to vegetative multiplication and maturation. As a rule, the superinfecting phage genome does not multiply and is passed on to one daughter cell at each division. Its presence is revealed, through suitable genetic markers, by its participation in phage production whenever the cell that carries it happens to produce phage (*34*). The mechanism that prevents vegetative multiplication in lysogenic bacteria is supposed to be an immunity repressor (*35*), which blocks a function needed to initiate replication and maturation. Phage mutations affecting the production of the immunity substance (by decreasing or increasing it) occur in gene loci located in the same region as the genes that control the early decision (*36*). These gene loci also control the specificity of the immunity substance, which is different in otherwise closely related phages and can be altered by mutation (*37*). Other mutations in the same genetic region make the phage genome insensitive to the immunity repressor, hence incapable of becoming prophage (*35*).

The regulated expression of phage genes is well illustrated in lysogeny; the functions of all the genes concerned with vegetative replication, virion biosynthesis, and lytic processes are repressed in lysogenic bacteria by the action of the immunity repressor (*35*).

We do not know whether the immunity repressor and the decision repressor are one and the same substance. The former inhibits a function needed for vegetative multiplication; the latter, a function required to initiate maturation. Possibly the same repressor blocks the function of several genes through a common operator. Mutations in other phage genes alter the stability of the prophage condition; they may act either through the immunity repressor and operator or by some other mechanisms —for example, by preventing adequate pairing between prophage and attachment site.

The lysogenic condition makes it possible to analyze the genetic determination of various steps of phage maturation, which, if blocked in intemperate phages, would generally act as lethals (*38*). A mutant prophage blocked at some stage in the production of mature phage particles is a defective prophage. It can persist and produce immunity, but, if a shift to lytic development occurs (for example, by induction with ultraviolet light), the development is arrested at the blocked step. If the blocked function is supplied in the same cell by a normal related phage, the defective phage genome can enter mature phage particles. These defective phages can then infect bacteria and may even establish lysogeny, if the product of the altered phage gene is not needed for the decision and immunity functions.

Conversion of bacterial properties by phage. The precise controls of the functions of those phage genes that play specific roles in phage development makes even more remarkable the behavior of certain classes of phage genes, which appear to function irrespective of the stage of phage infection. These

are the converting genes typified by the antigen-controlling genes of certain *Salmonella* phages. For example, infection with phage PLT22 initiates production of somatic antigen 1 on the bacterial surface (*39*); likewise, phage ϵ^{15} controls antigen 15 and ϵ^{34} controls antigen 34 (*40*). Each *Salmonella* type has a characteristic surface polysaccharide, which carries the somatic antigenic determinants. Antigen 1 results from the addition of a terminal glucose monomer to a side chain of the polysaccharide (*41*). Antigen 34 also corresponds to the addition of glucose to a side chain (*42*). The determination of antigen 15 is not an addition but a changed linkage between monosaccharides (*43*).

In phage ϵ^{15}, the converting property is determined by a mutable genetic determinant: nonconverting phage mutants, as well as mutants with altered forms of antigen 15, have been isolated (*44*). Presumably, the antigen-determining phage genes control the production of enzymes that catalyze specific steps in the biosynthesis of the complex polysaccharides.

Two features of this situation are especially interesting.

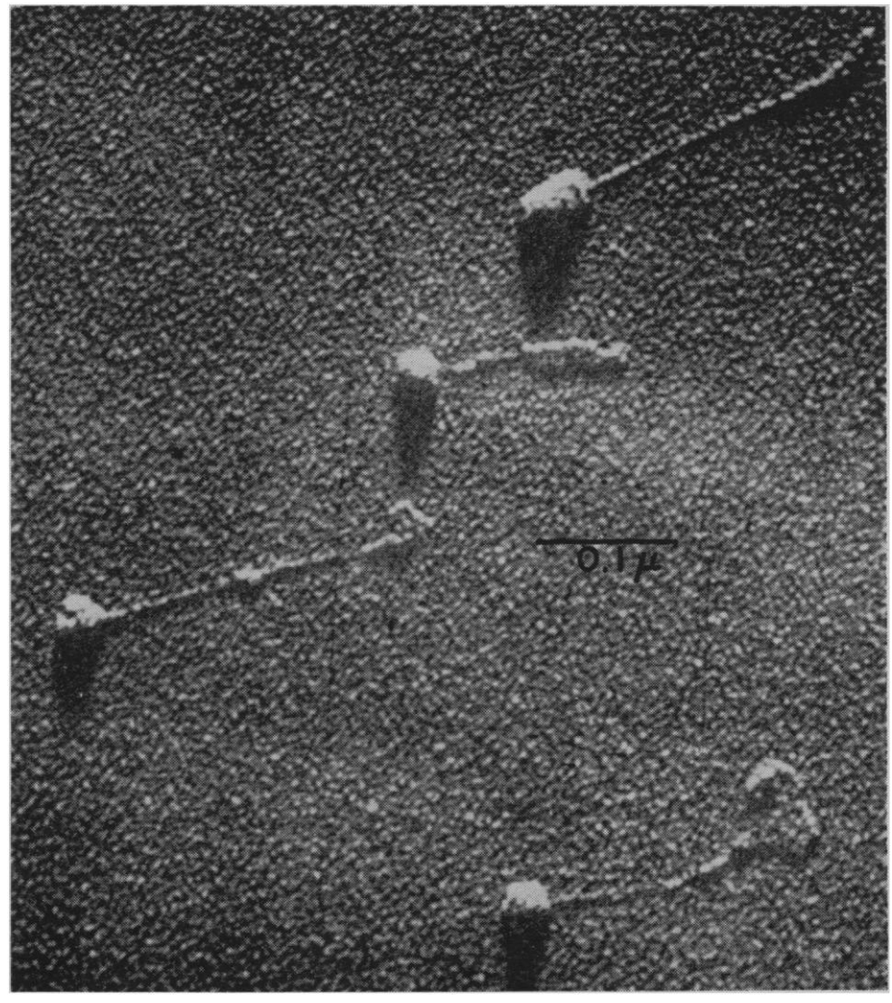
First, the production of the bacterial antigens is apparently not essential to either virus or host. Nonconverting mutants undergo a normal phage cycle, including both lysogenic and lytic phases; thus, both the bacterial antigen and the phage-controlled enzyme are dispensable. Also, antigen 34, determined by phage ϵ^{34}, is formed only in bacteria that carry also phage ϵ^{15} and, therefore, have antigen 15 (*45*). The reason for this is that the portion of polysaccharide determined by ϵ^{15} is needed as substrate for the ϵ^{34} action (*43*). Yet, the developmental cycle of phage ϵ^{34} is quite normal, irrespective of the presence or absence of ϵ^{15}. In general, the converting genes control functions that seem to be unimportant to the phage.

Second, when the gene is present and the substrate is available, an antigen-converting gene operates all the time. The new antigen is formed almost immediately after infection; in the lytic cycle it continues to be made until lysis occurs, and in the lysogenic cycle, as long as the prophage is present. Loss of the prophage stops production of antigen, but prophage mutations to defectiveness do not affect antigen production. Clearly, the antigen-converting genes are not subject to the regulatory repressions that control the activity of other phage genes at specific stages of phage development.

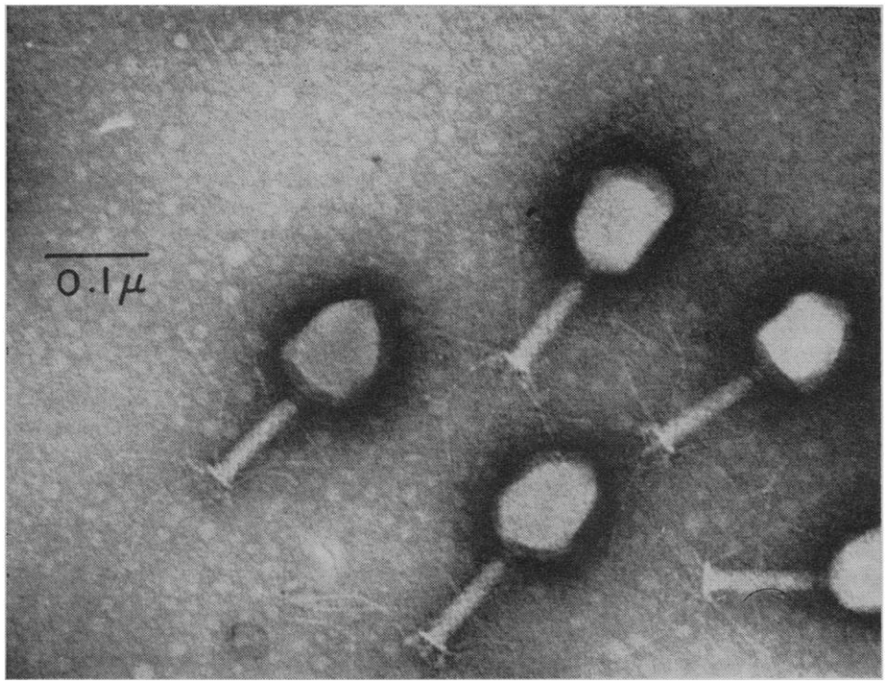
The existence of converting genes can be explained in two ways: either the phage consists of a fragment of bacterial chromosome which, by regressive or progressive evolution, has become a virus, or the phage has acquired bacterial genes by recombination with the host. These two hypotheses are not mutually exclusive; a phage may be able to receive from a bacterial host some genetic elements that already have homologous counterparts in the phage genome.

Origin of phages from elements of the cellular genome would require acquisition of the ability to produce a transfer apparatus, including protein coat, lytic enzymes, and so on. It seems unlikely that such complex devices have arisen repeatedly and independently in many bacterial forms. A more likely hypothesis is that mechanisms for genetic transfer arose early in bacterial evolution and that phages represent one of the forms into which such mechanisms have evolved. In this respect, comparison of phages with other groups of genetic components of bacteria is suggestive. At least two groups of episomes (*7*), the fertility factor and some of the colicinogenic factors, make the carrier bacteria capable of mating with other bacterial cells and of acting as donors of genetic materials (*46*). Presumably, these episomes control the production of specific conjugation apparatus. It is interesting to note that the fertility factor in *Escherichia coli* confers to the carrier cell a new surface antigen, apparently part of a surface polysaccharide (*47*).

The episomes (including fertility factors, colicinogenic factors, and temperate phages) can shift back and forth from chromosomal to cytoplasmic



Particles of bacteriophage ΦX-174 heated for 30 minutes at 75°C at *p*H 8.5. Note the DNA fibers emerging from the disrupted virus particles. [Elizabeth C. MacLean and Cecil E. Hall, Massachusetts Institute of Technology]



Particles of bacteriophage T4, one of the largest bacteriophages. Note the polyhedral head, full of DNA, and the tail with collar, tail plate, spikes, and tail fibers. [Eduard Kellenberger, University of Geneva, Geneva, Switzerland]

states. In doing so, some of them incorporate fragments of the bacterial chromosome (*7*). A chromosomal gene, once incorporated into an episome, becomes transmissible to other cells as part of the episome. Such a gene can then persist with the episome, but it may also become integrated into the chromosome of the carrier cell by genetic recombination. When the transferring episome is a phage, the genetic transfer is called phage-mediated transduction. The mechanism of transduction is illuminating with regard to the general problem of viral origin and viral function.

Transduction by Bacteriophage and the Function of Transduced Genes

The nature of the transducing elements. A temperate phage capable of transduction, after reproducing in a donor bacterium with certain genetic markers, can transfer these markers to some of the cells of a related recipient bacterium (*48*). Joint transfer of two or more markers occurs only for closely linked determinants. What is transferred by a transducing phage particle is a single fragment of genetic material, either from the bacterial chromosome or from an episome (such as the fertility factor). Three questions concern us here: the relation between the transduced genes and the phage genome; the function of the transduced genes; and their status in the recipient cells.

The relation between host genes and phage genes in the transducing particles varies from case to case. Sometimes, the transduced bacteria appear to have received no phage genes at all. Phage functions are missing altogether, and the transduced bacteria are neither lysogenic nor immune (*49*). The transduced host markers have simply become integrated into the recipient cell chromosome. At the opposite extreme are instances in which all transduced bacteria are fully lysogenic, as though they had received both an active phage genome and a bacterial fragment (*50*). In no case, however, has it been proved beyond question that host genes and normal phage genome can be transferred together by the same phage particle.

The most informative situations are those in which the transducing particles contain genetic elements in which a part of the phage genome is replaced by bacterial genes. These composite elements are recognizable if they retain the ability to persist as prophages in the transduced bacteria; the transduced genetic markers remain part of the prophage.

Two instances have been explored in detail: the association of the *gal* (galactose utilization) loci with phage λ [λ*dg* phage (*51*)] and that of the *lac* (lactose utilization) loci with phage P1 [P1*dl* phage (*52*)]. In both cases the resulting phage elements are defective; the transduced bacteria carry the defective elements but produce transducing phage only if they are superinfected by a normal phage, which supplies the missing phage functions. In the λ*dg* phage, the defect is due to the replacement of a variable portion of the phage genome with the *gal* region of the bacterial chromosome (*53*). As a result of this genetic loss the λ*dg* element is unable to initiate vegetative multiplication, but it retains many phage genes, including those that control maturation, lysozyme formation, and immunity. The λ*dg* element can become prophage, but this is less firmly bound than a normal λ. Thus, the defective λ*dg* element is difficult to recognize as phage-related; an observer unfamiliar with its past history might consider it a nonviral episome. Some of the other episomes of bacteria may in fact have originated from phages in a similar manner.

The transduction of the *lac* genes by phage P1*dl* resembles in many respects the λ*dg* situation. Some of its features are interesting because they suggest a unified interpretation of all phage-mediated transductions (*52*).

Phage P1 can perform general transduction, transferring any genetic marker among many strains of the coli-dysentery bacilli. Thus, the P1*dl* phage, which carries the *lac*$^+$ genes, can give the *lac*$^+$ property to a variety of *lac*$^-$ strains, more or less distantly related to one another. The P1*dl* phage gives unstable lysogeny, but the *lac*$^+$ genes can become integrated into the bacterial chromosome. If the recipient strain is closely related to the donor (as with two derivatives of the same *E. coli* strain), integration is the rule; substrains carrying P1*dl* prophage are very rare among the *lac*$^+$ transductants. When, however, donor and recipient are less closely related (as in transduction from an *E. coli* strain *lac*$^+$ donor to a *Shigella dysenteriae* strain *lac*$^-$ recipient), integration is rare and the *lac*$^+$ transductants are almost all carriers of a prophage P1*dl*. In these cases the existence of the combined phage element P1*dl* as the agent of transduction is detected only because the donor and recipient strains are sufficiently unrelated to prevent integration by genetic recombination. It seems probable that transductions of other host genes by

phage P1 may be mediated by combined genetic elements which are similar to P1*dl*.

All the P1*dl* elements studied carry the full *lac* region from the host cell, but their complement of functional phage genes varies; they are variously defective in phage properties, as though they missed different portions of the phage genome. Some are almost fully competent phages that can make almost normal phage particles. Others, more defective, persist and mature only if a normal P1 prophage is also present. Still other transducing elements give no indication of possessing any phage genes at all, being unable even to produce immunity; these elements can persist and multiply in the transduced clones but are readily lost. A transducing element of the last type may be a chromosomal fragment, without any phage genes; more probably, it may still include some phage genes, which make it capable of multiplying in the transduced cells. In general, one may suppose that certain classes of "episomal" genes are required for an extrachromosomal DNA fragment to multiply autonomously. For example, replication may be restricted to elements of DNA that can themselves initiate the production of a special enzyme, possibly a DNA polymerase (*54*), and the episomal genes may control the production of such an enzyme.

Function of transduced genes. The behavior of bacterial genes introduced by a transducing phage into a recipient bacterium can be illustrated by transduction of the lac^+ genes into a lac^- cell by phage P1*dl* (*22, 55*).

The *lac* region contains at least four gene loci (*5*): locus *y* controls formation of β-D-galactoside permease and transacetylase; locus *z* controls formation of the enzyme β-D-galactosidase; locus *i* produces a specific repressor, whose action is countered by specific inducers; and locus *o* is an operator gene (possibly part of the *z* locus) that controls the function and the repressor sensitivity of the *z*-*y* region. All these genes are present in the transducing P1*dl* elements. The course of galactosidase formation after transduction of the z^+ gene into a z^- recipient resembles the formation of enzyme after entry of the *lac* genes into a lac^- cell by mating (*56*). If no repressor gene is present or if an inducer is provided, enzyme synthesis after a brief initial period of acceleration proceeds at a linear rate, while the bacteria grow exponentially. If a transducing phage with the i^+ and z^+ genes is introduced into an i^- z^- recipient in the absence of inducer, enzyme formation starts, but it soon ceases, due to the production of repressor by the i^+ gene introduced with the phage (*55, 57*). Thus, the bacterial genes that are part of the defective phage element P1*dl* begin to function almost immediately in the recipient cell.

Two findings are worth underscoring.

1) The bacterial synthesis of enzyme continues at a linear rate for several hours after transduction. This is probably due to the fact that the z^+ gene is present in nonreplicating phage elements: transduction is abortive (*58*). Integration of the *lac* genes into the host chromosome, or establishment of the P1*dl* element as prophage, occurs only in a minority of the recipient bacteria and may be delayed for many generations.

There is an interesting difference between genes introduced into a bacterium by a transducing phage and genes introduced as bits of chromosomal DNA in bacterial transformation experiments (*59*). In transformation, the integration between transferred genes and their homologues in the recipient chromosome can occur very promptly (*60*), and only those genes that have accomplished the integrating contact begin to function (*61*). In transduction, instead, genes introduced with the transducing phage can function without being integrated, possibly because of the influence of episomal genes contributed by the phage.

2) The phage-transduced genes are subject to a dual type of regulation. On the one hand, they are subject to their specific repressors; when no such repressors are present, the transduced genes are immediately expressed. On the other hand, the transduced genes that are part of a phage element are subject to peculiar regulatory effects. For example, when P1*dl* phage carrying the z^+ gene infects lysogenic z^- bacteria that are immune to phage P1 there is much less production of galactosidase than with P1-sensitive recipients (*57*). Thus, the function of the *lac* genes can be affected directly or indirectly, by the specific phage-immunity repressor.

More complex aspects of the relationship between immunity and repression are also observed. When λ*dg* phage start multiplying vegetatively (as a result of ultraviolet treatment of lysogenic bacteria), the gal^+ genes in the phage element begin to function and to produce enzymes in the absence of inducers —that is, they are released from control by their specific repressors (*62, 63*). In the P1*dl* case, a similar constitutive synthesis of enzyme is observed in irradiated bacteria (*64*) when the phage carries the i^+ and z^+ genes. In bacteria lysogenic for phage λ, whose prophage is attached to a chromosomal site near the *gal* loci, even the chromosomal set of *gal* genes begins to function constitutively after ultraviolet induction of λ (*62*). These findings indicate that regulation of gene function can be altered by the presence of episomes. It is conceivable that some instances of abnormal differentiation arise from alterations of gene-repression mechanisms due to changes in state of episomes, including latent viruses.

Role of the Host Genome in the Course of Phage Infection

I have already mentioned the existence of phage mutations that affect the ability of a phage to grow in one host but not in another. In the permissive host, apparently, the functions of the altered phage genes are at least partially restored. There are other cases in which the functions and sometimes even the structure of these genes are altered by the genetic environment of certain host cells.

Lysogenization in chloramphenicol-resistant mutants. One example concerns the phage genes that control the decision toward lysogenic or lytic development. In general, the relative frequencies of these alternative outcomes are similar for a given phage in different hosts. But in some chloramphenicol-resistant mutants of the bacterium *Salmonella typhimurium*, the temperate phage P22 grows well but fails to establish lysogeny (*65*). As we have seen above, the decision to lysogenize requires repression of a critical step toward lysis. In the normal bacteria, chloramphenicol favors lysogenization by blocking the performance of the critical step (*33*); in the chloramphenicol-resistant bacteria the critical step has apparently become irrepressible because of some alteration in protein synthesis. This observation illustrates how a cellular mutation can shift the outcome of viral infection. Similar effects, produced by developmental rather than mutational changes in cells of multicellular organisms, may explain the dif-

ferent outcomes of infection of different tissues with the same virus.

Host-induced variation. Another example of host control over phage function is host-induced modification. Sometimes a phage, because of having grown in one host strain, becomes restricted, that is, unable to grow in another host (*66*). This modification affects most of the phage particles. The few particles that occasionally succeed in multiplying in the second host give rise to a progeny of unrestricted phage, which again becomes restricted en masse upon growth on the first host strain. The host-imposed restriction is apparently due to the production of localized "bad spots" in the phage genome, recognizable by genetic analysis (*67*). Whether the bad spots correspond to breaks, changes, or functional blocks of the phage DNA is not known. They may be due to recombinational events involving phage and host genome, or to functional events, such as an irreversible attachment of inhibitors to specific sites in the phage genome.

Another group of host-directed changes is that of mutations that arise as a result of growth in specific hosts. These host-induced mutations are easily observed with temperate phages after ultraviolet irradiation of both phage and bacteria (*68*). For the same phage the frequency and pattern of host-induced mutations differ in different hosts. Host-induced mutations, as well as the formation of combined transducing genetic elements, suggest homology between parts of the phage and of the bacterial genome, but may also be caused by recombination between an infective phage and some unrecognized prophage, or by mutagenic effects of certain host-cell products on extraneous DNA.

Conclusions

This brief survey of bacteriophage infection has provided support for an obvious but important generalization: that the study of virus infection at the cellular level is a branch of cellular genetics. The interactions between viral and cellular functions show that infection with a virus is not just a disrupting intrusion; it is an addition to the cellular endowment of genetic specificity. The outcome of this addition depends on the nature of the instructions carried by the viral genome, on the ability of the cellular machinery to carry out these instructions, and on the availability of control mechanisms to regulate the functions of the added element.

Inevitably, the most easily recognized viruses are those that produce destructive events in their hosts. Even these destructive effects of virus infections are exerted at the genetic rather than at the metabolic level, contrary to the situation in infectious diseases caused by bacteria and other pathogens. This viewpoint need not foster pessimism in the search for practical approaches to the control of virus diseases, such as antiviral chemotherapy; it simply means that the solutions will probably be quite different from those applicable to other types of infectious diseases.

It may well be that in order to cure virus diseases we may first have to learn to affect selectively the intimate processes of gene function and regulation. Progress in this area of biology is proceeding at a tremendous rate. Virologists find it rewarding to know that virus research is a major contributor to current developments in molecular biology (*69*).

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