The Significance of Bacteriophage in Bacterial Classification. A Review

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A given race of phage grows in a relatively limited range of bacteria. A coli phage, for instance, will not lyse a staphylococcus or a corynebacterium. Within these limits, however, some phages have a much wider host-range than others: some attack only one or a few bacterial strains; some a whole species; and some can lyse members of several species which on other grounds are considered to be not too distantly related. For instance, some pasteurilla phages also attack strains of *Salmonella* and *Shigella* (Lazarus & Gunnison, 1947). The phage-sensitivity of a strain as a basis for bacterial classification can be interpreted in two ways, just as there are two levels at which bacterial classification can itself be regarded. That is to say, either as just another phenotypic character which the two strains may have in common; or at the level of the genetic material, the nucleic acid, so that, if two bacterial strains interact with the same phage at the genetic level, each of the strains is manifesting some degree of genetic compatibility with the phage, and thus with each other.

Receptors

To consider the first of these, one might argue that the phenotype indirectly portrays the genetic make-up of the strain, but we know, in fact, that it does this to a lesser extent than at first appears. Thus, although different kinds of organisms may produce enzymes with the same function, the actual enzyme proteins will probably differ structurally in different species (Markert & Møller, 1959; Signer, Torriani & Levinthal, 1961; Levinthal, Signer & Fetherolf, 1962). Nevertheless, the first step in phage-bacterial interaction being attachment of the phage particle to the surface of the cell, it is immediately apparent that the first necessary condition for sensitivity of a bacterium is that the receptor for the phage should be present, and accessible. The receptor may, with some phages, be part of the O somatic antigen, or, instead, part of the surface of a rough strain (Burnet, 1930). It may be a superficial envelope antigen, such as the Vi antigen (Craigie & Brandon, 1936), or it may even be provided by filamentous appendages such as fimbriae (Crawford & Gesteland, 1964), or flagella (Sertic & Boulgakov, 1936). Resistance of a bacterial culture arising from lack of attachment of the phage may sometimes be for only a trivial reason, for any manipulation or event, not necessarily genetic in origin, which does away with the receptor or prevents the phage particles having free access to it makes the bacterium resistant. There are innumerable examples of such a situation: a phage adsorbing to flagella will not attack bacteria growing at a temperature too high for flagella to be formed (Meynell, 1961); and phages for *Bacillus anthracis* will not attack bacteria

grown under conditions in which the capsule is produced, for the capsule obscures the receptor on the cell surface (Meynell, 1963). The example perhaps most often quoted is that of the strain of *Escherichia coli* which produces abundant Vi antigen at 20°, and at this temperature is sensitive to a Vi phage but resistant to another phage which uses the O somatic antigen as its receptor. When the bacteria are grown at 41·5°, however, they do not produce Vi antigen, and are now resistant to the Vi phage and sensitive to the O phage (Nicolle, Jude & Diverneau, 1953). A bacterium without an accessible phage receptor is thus in fact resistant to the phage, although it may be potentially sensitive, as has actually been demonstrated by infection of bacterial protoplasts by phage DNA (Spizizen, 1957) and the introduction of prophage by conjugation (Luria & Burrous, 1957). On the other hand, attachment or lack of attachment is not the only factor concerned in phage-sensitivity: certain staphylococcal phages adsorb to *Bacillus subtilis*, but produce no effect on the bacteria (Rakieten & Rakieten, 1937).

Killing without multiplication

In phage typing, the phages are used at 'routine test dilution' (Anderson & Williams, 1956) to distinguish the different bacterial strains, for it is the activity of the *bulk* of the phage population that is being tested, and its ability to *multiply*. If clearing occurs only at higher concentrations of phage, this can indicate either that the effect is due to host-range mutants of the typing phage or even to contaminant phage particles present in small numbers in the typing preparation (Rountree, 1956), or that, although the phage cannot multiply, it can nevertheless kill the bacteria, producing clearing like that caused by a colicine. Examples of this sort of killing include the lysis of Vi + *Salmonella typhi* by unadapted preparations of Vi phage II (Anderson & Fraser, 1955), and also the killing of *Pseudomonas* and *Serratia* species by coliphage P1 (Amati, 1962). The fact even that killing occurs must indicate some interaction of the phage with the bacteria, and a group of bacterial strains which are all killed by the same phage must, at least, share the receptors to adsorb it. But without knowing more about what the process of killing by phage consists of, we cannot put its significance in assessing bacterial relationships any higher than this.

Genetic similarity. Nature of phage

Before we look for any relationship between a particular phage and bacterium at the genetic level, we must first ask if it has any meaning at all to talk of a fundamental, natural relationship between phage and its host. We must, that is, consider the essence of the relationship between phage, as a biological entity, and bacterium; or, put another way, what is the fundamental nature of the phage? During the lytic cycle, the phage is a parasite, multiplying autonomously. The phage genome controls its own replication, and directs the synthesis of its protein—protein foreign to the bacterial cell. In the lysogenic state, the phage is an integral part of the bacterial heredity; the phage genome is replicated under the same control as the bacterial genome itself (Nagata, 1963), it segregates with it, and is evidently physically associated with it, in some cases at least. The more we learn about the structure and behaviour of phage, the more probable it becomes that the phage originated as a genetic element of the bacterium, and evolved by developing the means for cell-to-

cell transfer. This was first proposed because of the abundance of lysogenic strains and the enormous variety of different phages specifically attacking different races of bacteria (Nicolle, Grabar & Gilbert, 1946; Felix, 1953). Certain phenomena also suggest that genetic exchange can actually occur between phage and host bacterium. For instance, with several different phages, it has been observed that when ultra-violet-irradiated phage is grown in bacteria which have also been irradiated, new genotypes appear which are different from the phage mutants obtained in other circumstances (Jacob, 1954; Tessman & Ozaki, 1960), and which are best explained as the result of genetic exchanges between phage and host genomes occurring with increased frequency as a result of the ultraviolet irradiation (Roman & Jacob, 1957). Some of these new genotypes, formally similar to mutants, are, in fact, apparently mutated at several distinct loci simultaneously, and different kinds are obtained when the phage is grown in different bacterial hosts (Fraser, 1957).

Lysogeny, which involves integration of the phage genome with the bacterial genome implies some degree of genetic homology between them. A genetic map based on recombination frequencies can be made for phage, just as for bacteria, and the phage chromosome, like that of the bacterium, appears to be a circular structure (Jacob & Wollman, 1961; Cairns, 1963; Foss & Stahl, 1963; Epstein *et al.* 1963; Chandler, Hayashi, Hayashi & Spiegelman, 1964). A short region of the genome of a temperate phage is concerned with the ability to lysogenize (Kaiser, 1957; Levine, 1957), and integration of the prophage has been visualized as first involving pairing of this region with a homologous region of the bacterial chromosome. In one mechanism which has been proposed (Campbell, 1962), the pairing of the homologous regions of the two circular structures is followed by a cross-over between them which leads to insertion of the phage genome, opened into a linear structure, into the bacterial genome between the ends of the break where the cross-over took place.

Some phages, such as coliphage λ , have a fixed location for their prophage, while the prophages of other phages may attach at one or other of a few, or even sometimes of many different genetic sites (Lederberg & Lederberg, 1953; Jacob & Wollman, 1957; Bertani, 1958). This immediately presents an analogy with the bacterial fertility factor, F, which in the ordinary way can be integrated anywhere on the bacterial chromosome, but whose site becomes fixed after picking up bacterial genes, like those concerned with lactose fermentation, for then it is determined by the homology of those associated genes with their alleles on the bacterial chromosome (Adelberg & Burns, 1960). The analogy between phage and the bacterial fertility factor can be pressed further, for the λ prophage can acquire bacterial genes located next to it. When the prophage is induced, the resulting phage particles which emerge contain an incomplete phage genome and less than the full complement of phage DNA and carry the bacterial markers into their next host (Arber, 1958; Campbell, 1959; Weigle, Meselson & Paigen, 1959). And again, the bacterial markers on entering the new host may occasionally be integrated into the bacterial genome, or else may remain associated with the carrier genetic fragment, the defective prophage, just as they may remain associated with F, so that there appears to be no fundamental difference between these two genetic elements.

Similarities at the genetic level between one bacterium and another have been assessed by the readiness with which genetic interchange occurs between them as well as by physico-chemical tests to compare the base compositions and base

sequences of their nucleic acids (Marmur, Falkow & Mandel, 1963). The overall composition of a sample of DNA, usually denoted by its content of guanine-cytosine pairs, determines its density and also the temperature to which it has to be heated to denature it by separating the two strands of the helix (Sueoka, Marmur & Doty, 1959; Marmur & Doty, 1959). The absolute sequence in which the nucleotides are joined in a DNA strand has been investigated for a few micro-organisms by the 'nearest-neighbour' method; that is, the relative frequencies with which each nucleotide is situated next to itself or to each of the others (Josse, Kaiser & Kornberg, 1961). Finally, the base sequences in nucleic acids from different sources have been compared, using RNA or single strands of DNA. Reforming of the DNA duplex structure or fixing of RNA to DNA needs matching of complementary regions in the individual strands, and so the readiness with which hybrid combinations are formed gives an estimate of the extent to which the nucleic acids are alike in base sequence (Schildkraut, Marmur & Doty, 1961). We are all aware of the agreement between genetic experiments and the results of these physico-chemical tests on the molecular structure of the nucleic acid. We know that a genetic fragment donated by one bacterium to another may or may not become integrated into the recipient's chromosome, and that, broadly, the less similar the DNAs of the two bacterial strains, the less likely is integration to occur. For instance, integration seldom occurs between *Escherichia coli* and *Salmonella* species, which, although they have similar overall base compositions and the same gross ordering of genes on the chromosome, show only localized regions of base sequence homology in physico-chemical experiments (Falkow, Rownd & Baron, 1962).

I can find the base compositions of only a very few phages reported in the literature (Lanni, 1960; Erikson & Szybalski, 1964), but it is held that lysogenization occurs only when the DNA of the phage has the same overall composition as that of the bacterium. Integration requires a base sequence homology sufficient to lead to pairing, but one might imagine that in the integration of prophage, only a very localized homology might be needed, and thus that overall similarity in base composition between bacterial and phage DNA might not necessarily be observed. However, we know that the DNA from any single bacterial source is relatively homogeneous in composition for, in contrast to the enormous differences between different microbial DNAs, no gross local differences in guanine-cytosine content along one single kind of DNA appear when it is broken down into smaller pieces (Marmur & Doty, 1959; Sueoka *et al.* 1959). It has been calculated that there can be few, if any, sizeable DNA fragments in common between organisms with overall base compositions differing by 10 %, or perhaps even less, and it would therefore not be surprising if a bacterium were lysogenized only by a phage whose DNA was similar to its own. Incidentally, phage λ and *Escherichia coli*, which have both been examined for their base sequence, have also shown the same nearest-neighbour frequencies (Josse *et al.* 1961); and a comparison of their base sequences by combination of nucleic acid strands has led to the conclusion that about 8 % of the λ genome is complementary to *E. coli* DNA (Green, 1963).

Significance of lytic multiplication

Foreign bacterial genes in a fragment of genome too dissimilar to be integrated in the recipient can often function nevertheless, and also be replicated if they are

associated with a structure, such as the F factor, itself able to replicate autonomously. Examples of this are the substituted F factor from *Escherichia coli* when it is in *Salmonella typhi*, or even in *Proteus*, *Vibrio*, *Serratia* or *Pasteurella pestis* (Mäkelä, Lederberg & Lederberg, 1962; Falkow, Wohlhieter, Citarella & Baron, 1963; Marmur *et al.* 1961; Martin & Jacob, 1962). The cell can interpret the foreign code and make the enzyme protein characteristic of the donor cell which contributed the genetic fragment (Signer *et al.* 1961; Levinthal *et al.* 1962). By analogy with such a situation, one might expect to find temperate phages (that is, phages inherently capable of lysogenizing) which could multiply autonomously, that is, by the lytic cycle, in many more different kinds of bacteria than they could lysogenize. I can find no instance of exactly this in the literature, but we know, of course, that phages which never lysogenize, like the T even-T5 phages, can multiply in bacteria with a base composition very different from their own (Lanni, 1960; Erikson & Szybalski, 1964). We can also conclude, since one and the same phage can attack bacteria as different as *Escherichia coli* and *Pasteurella pestis*, that there must be many more cases besides the T even-T5 phages where similarity in nucleic acid composition is not needed for the phage to multiply. Thus multiplication does not necessarily imply close genetic relationship of the phage with its host.

Significance of transduction

Similarly, a phage can incorporate in its protein coat foreign DNA different in composition from its own. A *Bacillus subtilis* phage which differed from its host in DNA composition was nevertheless found to be able to transduce a variety of bacterial characters. The transduced markers, in the form of DNA with the characteristic bacterial composition, were, however, a physically separate element from the phage DNA (Okubo, Stodolsky, Bott & Strauss, 1963). This finding brings out the difference between specialized and generalized transduction as indicators of similarity at the genetic level. Specialized transduction (such as occurs with phage λ), where the markers are situated next to the location of the prophage and are physically associated with a defective phage genome, probably signifies a degree of homology and fundamental relationship between phage and host. But the same cannot be said about generalized transduction, where any different bacterial character is picked up by the phage in the course of lytic multiplication, and is evidently free of any residual phage genome when it arrives in the new host.

Application of phage sensitivity

It is by now apparent that many factors are involved in phage-bacterium interactions and in the sensitivity of a bacterium to a phage. One question here is how do they apply directly to the application of phage sensitivity in bacterial classification and phage-typing? First, the bacterium may be resistant because it does not adsorb the phage. Between members of larger taxonomic groups the specificity lies largely in adsorption, but one would infer that if adsorption were to take place, one would then see the patterns of behaviour resulting from genetic incompatibility. The main use to which phage-typing is put is to distinguish between very closely similar bacterial strains which cannot be distinguished in other ways, and which are thus likely to be closely related. In the two most widely used typing schemes, those for *Salmonella typhi* and *Staphylococcus aureus* (Anderson & Williams, 1956), all the

strains likely to come under test adsorb all the typing phages, and failure of lysis is due to causes other than non-adsorption. One of these causes is pre-existent lysogenicity of the bacteria with a phage like the typing phage in any scheme using a diversity of different phages for typing (Rountree, 1956). In this situation, of course, it would paradoxically be an absence of reaction with the typing phage that would be significant of a relationship. The presence of the phage genome in the cell is known to be able to affect it in a number of different ways, and two of these may influence its phage-sensitivity. First, the prophage directly renders the cell immune to lytic multiplication of similar phage, and, secondly, an alteration in the cell surface may occur resulting in antigenic change associated with loss of ability to adsorb one phage and perhaps gain of ability to adsorb another (Uetake, Luria & Burrous, 1958).

Finally, I must mention what in this context is perhaps the most subtle relationship of all between phage and host, that is, the phenomenon of host-induced modification (Bertani & Weigle, 1953). This is very much in evidence in the well-known typing scheme for *Salmonella typhi*, in which, as we know, a single phage is used which is altered by growth in different strains, so that it becomes 'adapted' to the strain in which it was last grown. In some cases, the adaptation consists of a mutation or hereditary alteration in the phage, but in others the change is a modification imposed by the last host on the crop of phage to issue directly from it (Anderson & Fraser, 1956). When host-induced modification occurs, a phage which has reproduced in one host is modified in a way that affects its ability to grow in another. Thus, a bacterial strain under test can react differently, not only with different phages, but actually with the same phage grown in itself or in a different host. The modification lies in the DNA of the phage and is such that, while this DNA is not attacked by the DNase of the bacterial strain in which it was made, it is extensively broken down on entering the new strain (Arber & Dussoix, 1962; Dussoix & Arber, 1962; Arber, Hattman & Dussoix, 1963). In this situation, the second strain will equally reject bacterial DNA of the first in the form of its chromosomal genetic markers (Arber, 1962), as well as extrachromosomal DNA structures like the F factor (Glover, Schell, Symonds & Stacey, 1963). Enzymes concerned with DNA, for instance methylating enzymes, are known to distinguish their homologous from heterologous nucleic acid (Gold, Hurwitz & Anders, 1963), so that we can well think of this phenomenon as another example of the recognition of 'self' in nature. But it is interesting to find that it applies to foreign DNA like phage, so that even when the DNA has a foreign code, the bacterium puts the same superficial imprint on its structure as it puts on its own.

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