Advances in VIRUS RESEARCH

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VEGETATIVE BACTERIOPHAGE AND THE MATURATION OF THE VIRUS PARTICLES*

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I. Introduction

A. Scope of the Contribution

To deal with all the problems connected with the growth of bacteriophage in a paper like this would be pretentious; hence, we will restrict ourselves mainly to some physical aspects of it. We will concentrate on a definition of vegetative phage and will deal in particular with the contribution made by electron microscopy. For other aspects of the subject we refer the reader to the accompanying paper by Mahler and Fraser* and to the excellent reviews by Sinsheimer (1960) for information concerning the chemistry of the phage DNA and to Stent (1959) for intracellular growth. Phage genetics will be considered here only as far as it is related to the subjects under discussion. Reviews on phage genetics exist (Levinthal, 1959; Bresch, 1959; Brenner, 1959). We should not forget, however, that the final aim in the study of morphogenesis of phage is to understand at the molecular level how the genetic information governs the building and assembly of phage particles.

The direct method of studying phage growth involves kinetic experiments by counting particles, by measuring chemical substances, and similar procedures. However, an indirect approach using radiobiological methods has been developed, as in the Luria-Latarjet type of experiment (see review articles, by Mahler and Fraser and also Stent, 1958). Unfortunately, the connection between these two types of experiments is not yet very clear. We will see in this paper that, while the chemical measurements, the genetic studies, and the investigations involving electron microscopy all give consistent results, it is sometimes very difficult to reconcile them with the Luria-Latarjet type of experiments (see also Kellenberger, 1959). In our opinion, some facts are missing which are necessary to clarify the relation between these experiments and for this reason we will not discuss the Luria-Latarjet experiments in the present paper, hoping that the future will provide us with the missing link.

Ever since the first virus particles were seen by electron microscopy, it was evident that this new technique was very promising. The early

* In this paper, the citation "Mahler and Fraser" refers to the accompanying review.

applications of this technique to phage were rather disappointing, indicating that the preparation techniques had to be improved. The scope of this paper however will not allow us to go into technical problems at all. Despite many improvements, the amount of time that one must spend on purely technical problems is still enormous and this explains why so few phage workers stay permanently interested in the use of the electron microscope.

B. A Summary of the Problems to Be Discussed

After phage adsorbs onto bacteria, the bulk of the phage protein remains outside the cell; all of the DNA is injected along with some minor components of protein-like nature amounting to less than 10% of the total protein content of the phage (Hershey and Chase, 1952). This fact, together with other evidence, makes it quite certain that the deoxyribonucleic acid (DNA) is the carrier of genetic information. After injection, many new synthetic processes are started and changes in the metabolism of the cell are initiated, all of which are concerned with the synthesis of parts of the phage and particularly of its DNA. Hence these early functions are of great interest and are being investigated in many laboratories. We will summarize recent observations concerning these early functions and discuss only one of them in detail. For some phages, probably the very first action by the phage once it is inside the cell is to promote the breakdown of the bacterial nucleus, by the synthesis of some protein. Since an empty phage coat does not produce nuclear breakdown one may conclude that something which is injected is responsible for it. We will discuss whether the responsible agent is produced as a consequence of information contained in the DNA or if another injected substance activates already existing bacterial pathways for its synthesis.

DNA is produced after a certain delay necessary for the formation of its synthesizing apparatus. For T2, Hershey (1953) has shown that its DNA is a true precursor, i.e., DNA found free at an early time of the cycle is later observed integrated into phage particles. On the other hand, it is well known that genetic recombination in phage occurs also in a stage where phage is not yet organized in its final form. We will discuss in some details the arguments which make us believe that phage DNA is able to self-replicate, mutate, and recombine. This leads us to a definition of vegetative phage. We will report, for instance, on experiments which show that recombination is accompanied by a material transfer of parental DNA to the recombinant.

We will be concerned frequently with the "organizational state" of vegetative phage, that is, the physical condition of the surroundings of

the individual particles. We will show, for example, that the vegetative T2 phages are assembled into a "DNA-plasm" occupying a delimited portion of space which is neatly separated from the cytoplasm while the DNA-plasm of T5 is dispersed in the cytoplasm. For phage λ, the organizational state seems to be still different. In all these cases, the DNA-plasm is highly hydrated and probably penetrated by the cellular sap. In the first maturation step, the organizational state of the DNA changes drastically: the DNA of every individual phage condenses into a body of low water content and of a shape comparable to the finished phage. This step of condensation is related to changes in what we will call the "chemical state" of the phage DNA. Indeed, the acid groups of the DNA will be occupied by substances which may give functional properties to the DNA. We will postulate that these substances are responsible for DNA being able either to replicate or to condense, or to express its information content. In all cases where we think the future definition of the substances associated with DNA are important we will refer to them as "DNA complexes" or for brevity "DNA_c."

The physical state of vegetative phage concerns the structure of individual particles. Questions of interest are: Is vegetative phage-DNA a single-stranded or a multistranded structure? Is its DNA in one single molecule or is it formed by an association of smaller molecular subunits linked together by units of chemical composition different from DNA? Do such subunits multiply individually in a dissociated form? Most of these problems are not solved definitely; therefore little emphasis will be placed on the physical state of vegetative phage in this paper.

The first steps in the maturation of phage particles will be considered. The condensed DNA represents what we will call a precursor particle of the first type, which is very labile and as yet cannot be observed outside the cell. The precursor particle of the second type has a protein coat which is released at lysis and found as an empty head ("doughnuts"). Later maturation steps, which are barely known, will be considered in an effort to establish finally a tentative scheme for the different phases of the growth of T-even phages.

C. A Summary of the Structure and Composition of Phages T2 and λ

The chemical composition of phage T2 is given in Table I.

The DNA of the T-even phages contains hydroxymethylcytosine instead of cytosine (Wyatt and Cohen, 1952). T2, T4, and T6 also contain glucose bound to the DNA (Sinsheimer, 1956, 1960; Jesaitis, 1956, 1958; Loeb and Cohen, 1959). Small quantities of other substances are associated with the DNA inside the head of T-even phages. For the other phages, information is lacking. Let us consider in more detail the minor

components of phage T2. Nonsedimentable and acid-insoluble protein, representing 3–5% of the total amount of protein of phage T2, was discovered by Hershey (1955) and then thoroughly investigated by Levine and co-workers (Levine *et al.*, 1958; Levine, 1960). These authors studied the antigenic and chemical properties of one such internal protein (which we will call L-protein) contained in the acid-insoluble, nonsedimentable fraction of osmotically disrupted phage. Its antigenicity is very specific for either T2 or T4. In low salt concentrations, it associates with DNA; with increasing concentrations of NaCl it dissociates progressively, being completely dissociated in 0.2 *M* NaCl.

TABLE I
THE CHEMICAL COMPOSITION OF PHAGES T2 AND λ

Component, etc.	T2	λ
Particle weight	5×10^{-16} gm.	2.2×10^{-16} gm.
DNA content	2×10^{-16} gm.[a] = 40%	1.1×10^{-16} gm.[b] = 50%[c]
Proteinlike substances inside the head:		
Acid-soluble		
Spermidine, Putrescine	1.5% of total carbon[d]	Unknown
Polypeptide	1% of total carbon[d]	
Acid-insoluble		
L-protein	5–7% of total protein[a,e]	Unknown

[a] Hershey (1955).
[b] Séchaud (1960).
[c] Weigle *et al.* (1959); Kaiser and Hogness (1961).
[d] Hershey (1957).
[e] Levine *et al.*, (1958); Minagawa (1961).

The acid-soluble substances amount to about 2% of the total protein and in chromatography show three components (Hershey, 1957). Two of them have been identified by Ames *et al.* (1958) and Ames and Dubin (1960) as polyamines (spermidine and putrescine). The third compound seems to be a polypeptide containing mainly aspartic acid, glutamic acid, and lysine (Hershey, 1957).

For the two polyamines it has been suggested by Ames and Dubin (1960) that they act together with Mg mainly as nonspecific cations for the neutralization and stabilization of the DNA. These cations can indeed be found in different relative proportions in viable phage, depending upon the experimental conditions. Spermidine, for example, can easily be replaced by spermine without any harm to the phage. In some other

phages polyamines are absent. Ames and Dubin (1960) think this is due to a different permeability of these phages, allowing a replacement of initially present polyamines by other cations.

The role of the internal protein, as well as of the polypeptide, is still unknown. We will discuss the possibility of an eventual identification of these substances with the "condensation principle" in a later section (V,C).

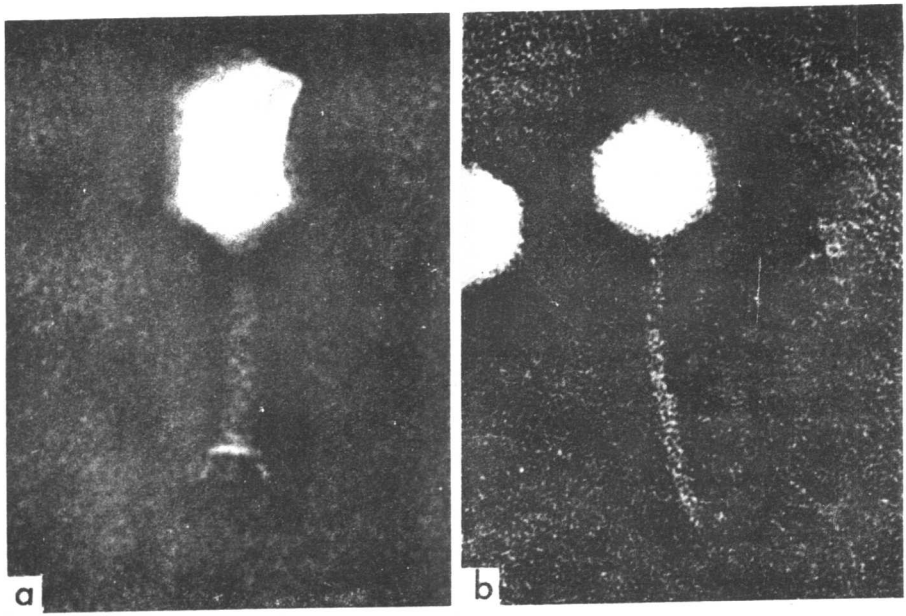

FIG. 1. Phage T2 (a) and phage λ (b), both prepared by the sodium phosphotungstate method. Magnification: ×280,000.

Most of our information concerning the coat comes from structural investigations with the electron microscope. Recent techniques of embedding the particles in sodium phosphotungstate have enabled Brenner *et al.* (1959) to advance greatly the knowledge of the structure of phage T2. Extension of such observations to other phages is now being made (Anderson, 1960). Electron micrographs of T2 and λ are given in Fig. 1, a and b. The dimensions can be seen in the schematic drawing of Fig. 2.

The protein of T2 is organized into a head membrane, presumably composed of subunits of a single type of protein (Levine *et al.*, 1958; Brenner *et al.*, 1959) and a complex tail. An inner hollow core is surrounded by a contractile sheath (Kozloff and Lute, 1959; Brenner *et al.*, 1959) and on its tip is fixed a base plate with six spikes (Brenner, per-

sonal communication; Kellenberger, unpublished results) and fibers, probably six in number (Franklin *et al.*, 1961). From recent experiments on the neutralization by antiserum (Franklin, 1961) it is clear that the adsorption process (see Tolmach, 1957; and Garen and Kozloff, 1959) of T2 is very complicated. It is believed now that the fibers, the spikes, and, perhaps, still other parts of the base plate are involved in the adsorption process. The sheath is not connected to the head (see Fig. 1)

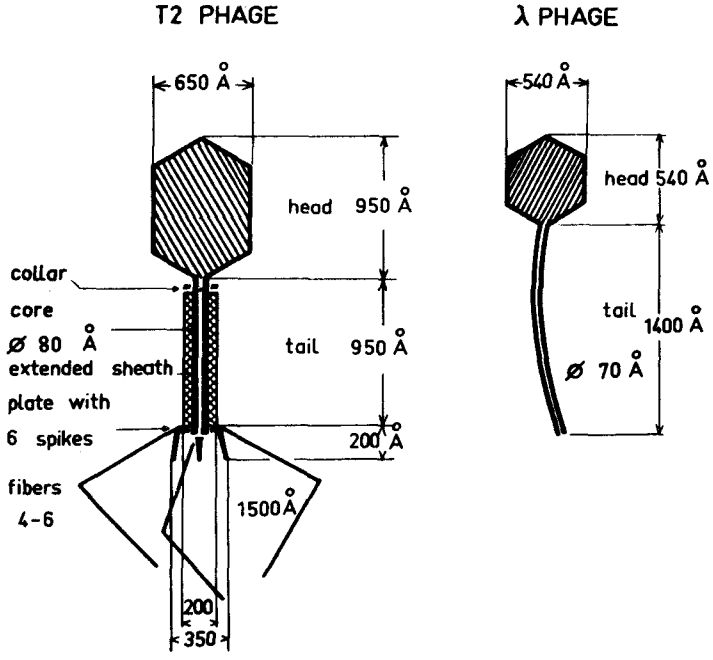

FIG. 2. Dimensions of phages T2 and λ.

and in some cases there may even be an intermediate collar (Anderson, 1960).

There is much evidence that the sheath contracts, thus causing the core of the phage to penetrate through the cell integuments. Contraction of the sheath can be produced artificially (Kellenberger and Arber, 1955; Kozloff *et al.*, 1957) in two different ways, the base plate either moving with the sheath or staying connected to the tip of the core (Kellenberger and Franklin, unpublished; Brenner, personal communication).

A contractile sheath has also been found recently for phages P1, P2, and P22 (Anderson, 1960), while many other phages like T5 and λ have a much thinner and more flexible tail with no observable sheath; fur-

thermore, there is at most one spike at the tip instead of fibers. Nothing is known about the penetration mechanism for these phages.

In T-even phages an enzyme had been found which is of the lysozyme type (Barrington and Kozloff, 1956; Koch and Weidel, 1956). There is also evidence of the presence of phosphatases (Dukes and Kozloff, 1959). All these enzymes are bound to the coat, since phage ghosts are still active. For more details on the biochemistry of the viral invasion see the review by Garen and Kozloff (1959).

At the present time there is no precise information concerning the arrangement of DNA inside the head. It had been found, however, that when DNA flows out of a broken head one observes the DNA in a series of bundles of decreasing thickness indicating some specific internal arrangement (Rubenstein, 1960; Bertani, unpublished).

Studies on birefringence of intact phage indicate that the DNA fibers are preferentially arranged parallel to the long axis of the head (Bendet *et al.*, 1960).

DNA extracted from phage T2 has been the object of many physicochemical investigations, which lead to the assumption that it is made up of two parts. One fraction, involving a single piece of DNA, represents about 40% of the total amount, while the rest consists of several much smaller pieces. Recent experiments by Hershey and Burgi (1960), however, show that the DNA of phage T2 can be preserved in one single piece or in a very few large pieces. By stirring and pipetting they are broken down to smaller pieces. This has been found to be true also for other DNA's (Davison, 1959). It is not established, however, whether the DNA extracted from phage T2 is an assembly of several molecules of about equal length, as would be indicated by the experiments of Meselson *et al.* (1957) and of Fleischman (1960), or whether it is one single molecule, broken down mechanically. Because of the importance of this problem in understanding the structure of chromosomes new experiments are required.

II. Early Functions in Phage Growth

A. The Mechanism of Invasion of the Cell

Invasion of the cell by phage T2 is a process which may be subdivided into 3 main phases: (1) adsorption, (2) chemical interaction between tail and cell wall and penetration of the inner hollow core of the tail through the cell integuments, and (3) injection of the contents of the head into the cell.

This invasion scheme is based on the following facts: Koch and

Weidel (1956) and Barrington and Kozloff (1956) discovered that material is released from the cell wall upon infection with phage T2. Kellenberger and Arber (1955) found that the phage-tail-sheath is shortened on contact with the cell wall. They also showed that the inner core seems to penetrate through the wall. Brenner and Horne (unpublished) confirmed these observations. Recent studies on thin sections of very heavily infected whole bacteria show that the distance of the head from the cell wall is shorter than the tail length (Kellenberger, unpublished) implying that the tail has penetrated through the wall. Unfortunately the contrast of tail protein in section micrographs is not great enough to show this penetration directly. Brenner *et al.* (1959) have shown clearly that the inner core is hollow and of such dimensions that DNA could just pass through it. Hence the idea of phage acting like a hypodermic syringe is most favored, but not definitively proved.

Very little is known about the mechanism responsible for the ejection of DNA out of the head. Most experiments show clearly that contraction of the tail-sheath is not necessarily linked to it. In our experiments, and with our strains (Kellenberger and Arber, 1955; and Bolle, unpublished) no DNA is released when phages are absorbed on empty cells even when contraction occurs perfectly. Brenner and Horne (personal communication) have found that two heat-resistant mutants of T2 show sheath contraction upon heating; the one together with release of DNA, the other without it. Nothing is known as yet about the energy requirement of injection and about possible polarity in the DNA transferred. Some of the problems related to a partial injection will be discussed in later sections. Nothing is known either about the reasons why cells reject DNA of superinfecting T-even phages 5–10 minutes after the first infection (French *et al.*, 1951; Graham, 1953). All minor components described in Section I,C are injected together with DNA (Hershey, 1957; Levine *et al.*, 1958).

B. Introductory Remarks on the Early Synthesis Related to Phage Growth

From a number of recent investigations it has become clear that the onset of the synthesis of substances that will later be built into phage particles is preceded by a very important period during which profound changes in the synthetic abilities of the cell take place. Although no net synthesis of RNA is observed (Cohen, 1948b), it has been found that a small amount of a new RNA is produced (Volkin and Astrachan, 1956, 1957; Astrachan and Volkin, 1958; Volkin *et al.*, 1958; Watanabe and Kiho, 1957). Cohen (1948a) found that after phage infection proteins are synthesized. Watanabe (1957a,b) showed that, in bacteria where protein

synthesis is greatly depressed by ultraviolet (UV) irradiation, phage infection induces a large increase in over-all protein synthesis, showing that the phage is responsible for new synthesis. These early proteins have recently been investigated in detail and the results are summarized

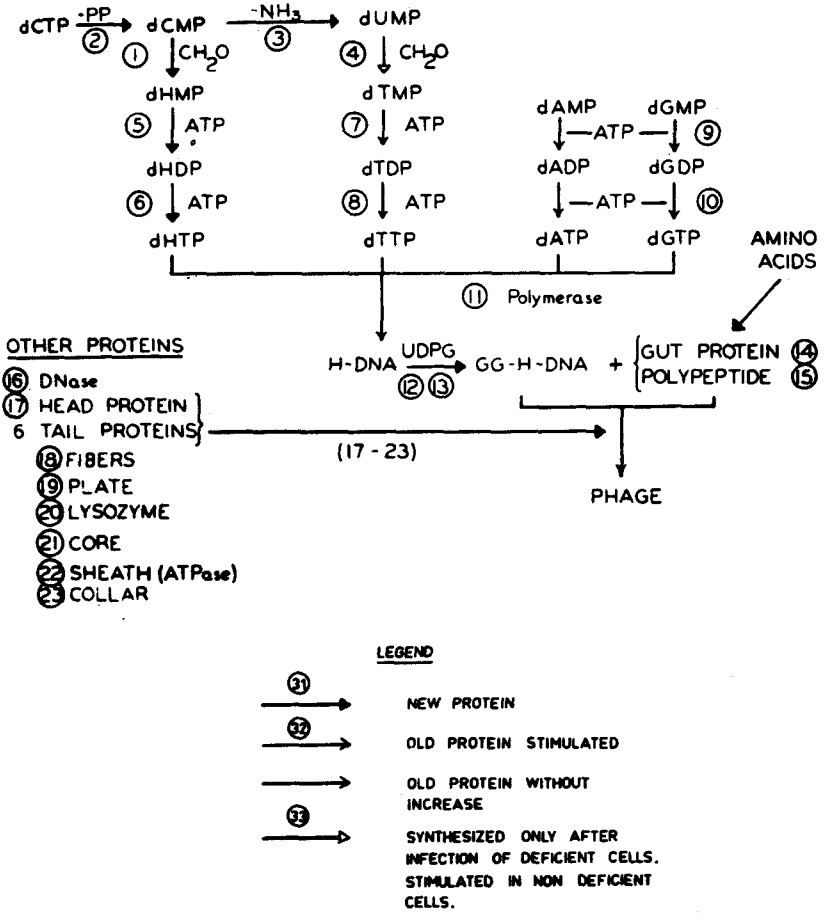

FIG. 3. New and stimulated reactions in T6 infection. (Courtesy of S. S. Cohen.)

in Fig. 3 through the courtesy of S. S. Cohen. The "early" proteins numbered 1 to 13 and 16 are not found as parts of the mature phage but are necessary for the synthesis of DNA (Kornberg *et al.*, 1959) and other structural components of mature phage. The biochemistry of these early phases of replication is discussed in detail by Mahler and Fraser.