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Growth and Inheritance in Bacteriophage  
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Period from 1950 to 1957

A. Summary. Our genetic experiments with phage T2, 1945 to 1951, established that inheritance in this phage is regulated by a linear linkage system functionally equivalent to the visible chromosomes of other organisms. A phage particle is now believed to contain only one such "chromosome." During 1948-1952 Doermann, combining our materials and techniques with his, demonstrated that phage particles multiply in a noninfective form, called vegetative phage, inside bacterial cells.

Beginning about 1950, our interest shifted to chemical problems relating to growth and inheritance as exemplified by the transition from resting to vegetative phage and back again during the infectious cycle. We found that only the phage DNA passes from phage particle into bacterium at the time of infection, that such chromosomal DNA can replicate autonomously in the cell, and that its incorporation into new phage particles occurs only as a terminal phase of growth during which protein synthesis is the dominant process. Needless to say, these statements skip over many agonizing questions.

B. Statement of progress. The purely genetic phase of our work, ending in 1951, will not be summarized in detail. Its general nature is indicated by the titles of papers in the bibliography given below. Genetic experiments with phage are being continued in several laboratories in this country and abroad, including that of Streisinger at Carnegie Institution. The main fact pertinent to this report is that a phage particle contains a single linear "chromosome" comparable in function to the visible chromosomes of higher organisms which, however, are ca. 1000-fold greater in mass.

In 1948 to 1950, Hershey, Kamen, Kennedy, and Gest showed that phage particles containing assimilated radio-phosphorus are subject to "suicide" as a consequence of nuclear reactions occurring in their DNA. This formed the starting point of the lines of research outlined below. We did not, however, follow up our suicide experiments, chiefly because of the difficulty of obtaining suitable radioisotopes at the time. The method is being fully exploited at present in several laboratories, notably by Stent at University of California.

By 1950, the pioneer work of Doermann and Luria and (further afield) Iwoff and Bertani was beginning to focus attention on new aspects of the multiplication of phage; namely, qualitative transformations as opposed to simple numerical increase. The new point of view was summarized in the paper by Hershey (1952), written in 1950, which introduced the term "vegetative phage" to distinguish multiplying from resting viral structures. We quote from this paper as follows: "...deoxyribose nucleic acid synthesis changes from a minor to a major activity of the cell after infection...one of the big questions of biology is whether this is a qualitative as well as a quantitative change...Luria has suggested that

the bulk of the nucleic acid may be synthesized during the conversion of vegetative into resting phage, rather than during the period of multiplication proper. (If so) genetic specificity of the phage is independent of its major DNA content. ....perhaps the initial question (about vegetative phage) could be formulated as follows. Does or does not the replication of phage-specific substances occur within a phage-specific membrane?"

At that time chemical experimentation with phage was developing in interesting directions under the leadership of Cohen, Putnam, and Kozloff, but had not yet furnished any clues to the nature of vegetative phage. It was known that phage particles are composed of about equal parts of protein and nucleic acid, that the infected bacterium devotes its major energy to the synthesis of these materials, and that some of the atomic constituents of the infecting phage particle reappear among the offspring.

The first step in the identification of vegetative phage was reported by Hershey and Chase (1952) who showed that viral DNA but not viral protein entered the cell at the time of infection. Consistent with this fact, only the atoms of the parental viral DNA could be found among the offspring particles.

The second step consisted in showing that all the DNA synthesized after infection is viral precursor, that such precursor DNA is synthesized in advance of the phage particles in which it is to appear, and that the DNA in this precursor pool is sampled at random to make phage particles, which are not therefore manufactured on the assembly line principle (Hershey et al., 1953; Hershey 1953). In the early stages of this work we discovered and made use of the unique base 5-hydroxymethyl cytosine in the DNA of T2 but did not identify it. Wyatt and Cohen independently discovered and identified it.

The third step in the identification of vegetative phage consisted in showing, by the use of chloramphenicol and isotopic labeling, that phage particles can be prepared containing DNA synthesized almost entirely in the presence of the antibiotic, and protein synthesized almost entirely during a subsequent period after chloramphenicol is removed (Hershey and Melnick, 1957).

The fourth and final step, proof that phage precursor DNA synthesized in the presence of chloramphenicol includes chromosomal DNA, is still incomplete. The following discovery by Tomizawa in our laboratory indicates one of two independent lines of attack that we are pursuing. If phage precursor DNA formed in the presence of chloramphenicol is irradiated with ultraviolet light while still in the bacteria, and these are afterward transferred out of chloramphenicol so that protein synthesis can begin, phage particles form that are almost all noninfective. The dead particles have properties similar to those produced by irradiating phage particles themselves. Owing to the remarkable nature of these properties, the dead particles are subject to genetic analysis, as shown by Doermann for particles irradiated outside the bacterium.

In this history we have necessarily neglected the work of many people, including much of our own.

Our conclusion, subject to possible upsets by work in progress, is that the phage chromosome is a single DNA molecule that multiplies in infected bacteria independently of all those processes involving protein synthesis by which the typical, finished phage particle is formed.

C. Future plans. (1) To complete the line of thought summarized above. (2) To attempt to identify chromosomal and nonchromosomal DNA among fractions that can be separated by chromatography on columns of basic protein. (3) To study further the genetic significance of the transfer of nucleic acid from parental to offspring phage.

D. Bibliography. (Asterisks denote review papers that do not acknowledge USPHS support).

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