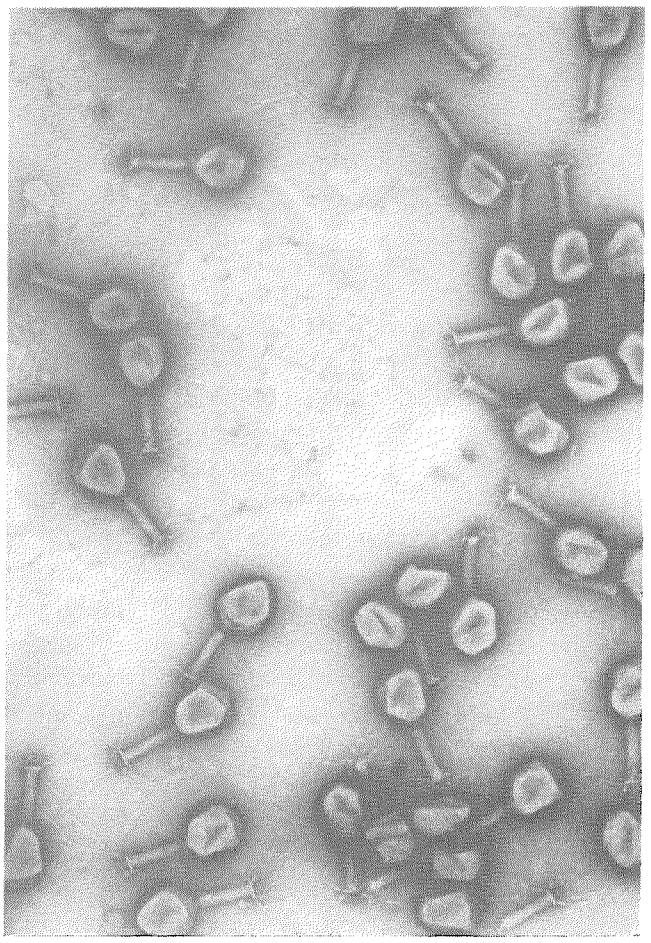
Genetics and Development
at the Threshold of Life—

The Caltech Phage Group

By ROBERT S. EDGAR and WILLIAM B. WOOD

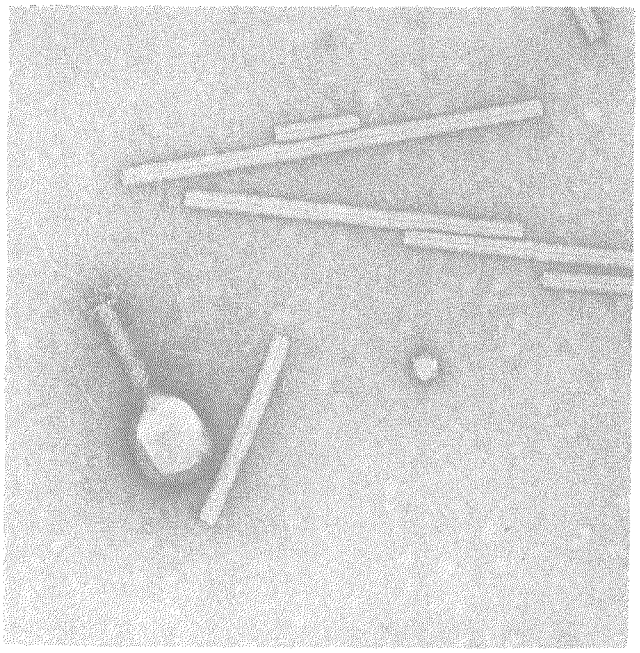


Heads, tails, and tail fibers of T4 bacteriophages can be distinguished in this electron micrograph taken by Ronald Luftig. Magnification is about 150,000 diameters.

Although bacteriophages—viruses which attack bacteria—have been known for over 60 years, the detailed study of their reproductive cycle began only in the early 1940's. At that time Max Delbrück, a former physicist and visiting research fellow at Caltech who had become interested in the mechanism of heredity, recognized in the phage an ideal experimental material for exploring the nature of the gene. In the succeeding years, many of today's leading contributors to the field now known as molecular biology passed through Dr. Delbrück's "Caltech Phage Group" as graduate students and postdoctoral fellows, and research with bacteriophages led directly or indirectly to much of the recent explosive progress in this new science.

Bacteriophages, as Delbrück suspected, proved ideally suited to studies of the molecular basis of heredity. They represent the simplest genetic systems we know—life trimmed to its barest essentials. The bacterial hosts on which they grow are themselves simple (as cellular organisms go), generally harmless, and easy to culture and manipulate in the laboratory. Despite their extreme simplicity, however, bacteriophages transmit and utilize their genetic information by the same basic mechanisms that are common throughout the biological world, with the consequence that phage research has been of value in understanding not only the process of virus multiplication but also problems of heredity and development in higher animals.

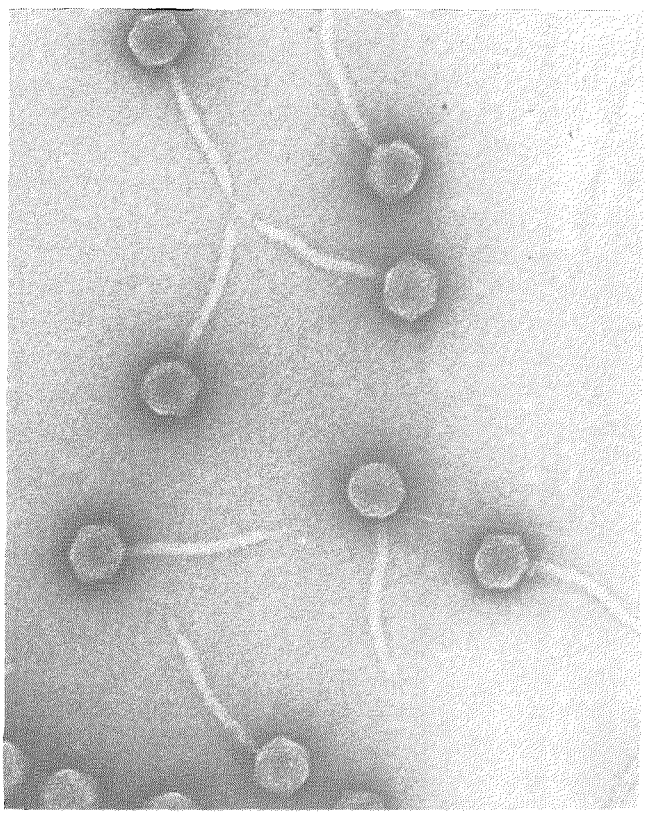
Some forms of life are little more than DNA and a protective coat. But even here countless generations of adaptation and survival have bred a complexity of structure and synthesis.



This micrograph, taken by Fred Eiserling of UCLA, clearly shows the large size and complexity of T4 relative to two of the simplest viruses. The long structures are particles of TMV, a virus that infects tobacco plants, and the small regular polyhedral object is a particle of Phi X 174, which infects E. coli.

Some of the fascination which bacteriophages hold for those who have worked with them can perhaps be understood through a more detailed description of two of these viruses and their life cycles. By accident initially, but increasingly by choice, the most intensely studied phage has been T4, a virulent virus that invades the common colon bacillus, *Escherichia coli* (*E. coli*). T4 is large for a virus. Its architecture is complex, and its effects on the host cell after infection are profound. T4 attaches to the wall of the host cells by means of six slender fibers located at the end of the tail. The syringe-like tail structure then contracts to plunge the end of the central tube of the tail through the wall of the host. This provides a passageway through which the viral DNA, located in the phage head, passes into the interior of the cell.

This DNA, a single molecule 54 microns in length, or about 80 times the length of the virus, is the viral genetic program. It includes about 150 genes, each carrying the information for the synthesis of a different enzyme or structural protein. Almost immediately after infection, the bacterial DNA is destroyed, and the synthetic machinery of the cell is reprogrammed to turn out the components necessary for phage reproduction under the control of the viral genes. The cell is "under new management." The infected bacterium can be thought of as a new organism, with a new set of genes and a drastically altered purpose in life. Its previous goal,



Phage λ is a simpler phage than T4, having a smaller, more regular head, a less intricate tail, and no tail fibers. This electron micrograph was taken by Fred Eiserling at a magnification of about 200,000 diameters.

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self-duplication, has been completely abandoned in favor of virus production, accompanied by the eventual death of the cell.

Following this coup d'état, viral development proceeds by an orderly sequence of precisely timed events. Among the first proteins to be synthesized are enzymes for catalyzing the production of additional copies of the viral DNA. At 8 minutes after infection DNA replication begins. About 5 minutes later synthesis of the early enzymes ceases, and the machinery begins to turn out a new class of proteins which will eventually comprise the progeny virus particles. Packaging of the new DNA molecules and assembly of the viral structural components proceeds until at 23 minutes the first phage particle is complete. By 35 minutes, more than a hundred finished virus particles have accumulated. At this point the infected cell bursts or lyses, and the newly formed phage enter the world in search of new host cells to invade.

The inevitable consequence of T4 infection is death of the host cell. Many bacterial viruses, termed temperate phages, are less pugnacious. Phage λ, which also infects *E. coli*, is such a temperate virus. Depending on conditions at the time of infection, λ can either carry out a coup d'état of the host similar to that of T4, or it can establish a nondestructive long-term association with the host, by a process called lysogenization. Under conditions that favor lysogenization, the viral DNA, following its entry into the host cell, becomes inserted into the larger DNA molecule of the host bacterium. The host then resumes its growth and reproduction, unaware that hidden away in its own genetic material there is now a subversive plan to convert the cell to the manufacture of virus! The viral DNA, which is not distinguished as foreign by the host's replicating machinery, is faithfully reproduced at each generation and passed on to the daughter cells. When suitable conditions arise, perhaps generations later, the viral DNA takes command, and the bacterial cell is reprogrammed to produce the virus.

Over the years phages T4 and λ remained the principal objects of study of the Delbrück phage group and its descendants. T4 and its close relatives T2 and T6 were the phages which Delbrück and his co-workers originally selected for study. The principal source of inspiration for studies of phage λ at Caltech has been Jean Weigle, who joined the phage group as a research associate in 1948. Like Delbrück, Dr. Weigle was a fugitive from physics, having been at one time professor of physics at the University of Geneva. Weigle and his co-workers have successively exploited λ for studies on the general mechanism of genetic recombination, and in particular for the integration of λ DNA into the DNA of the host, a problem which promises to have some practical significance in view of the recent findings that some tumor viruses exhibit life cycles which closely resemble that of λ.

In the late 1950's Delbrück's interest in phage waned as his interest in phototropism in fungi waxed, but the phage group kept its continuity under the new leadership of Robert Edgar, now professor of biology. During the Delbrück years, the work on T4 consisted largely of always brilliant and often successful attempts to apply formal genetics—the transfer of hereditary traits of the virus to its offspring—to the problems of phage reproduction

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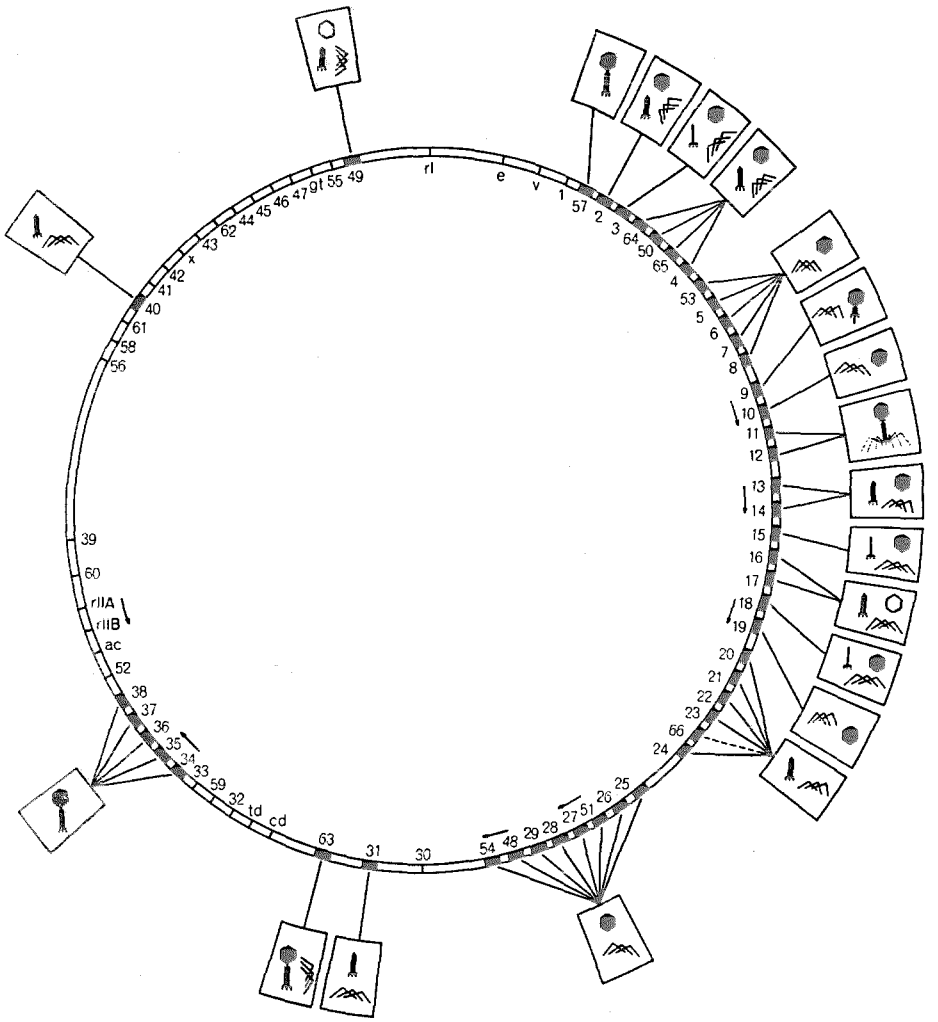
A new direction was introduced in 1960 by the discovery and exploitation of conditionally lethal mutations by Richard Epstein, then a postdoctoral fellow in the group, and Dr. Edgar. Until then, the mutations available in T4 and λ were not of much use for providing insight into the functions of specific viral genes, since they had only slight effects on viral growth. Mutations causing defects in essential genes, being lethal to the virus, were inaccessible to study since mutants carrying them could not be propagated for genetic experiments.

The conditionally lethal mutations provided the first opportunity to study such genes, since they produced lesions whose effects could be controlled by the experimenter. Under one set of conditions, called permissive, these mutations have little effect on the function of the mutant gene, and the virus can be propagated normally. However, under a



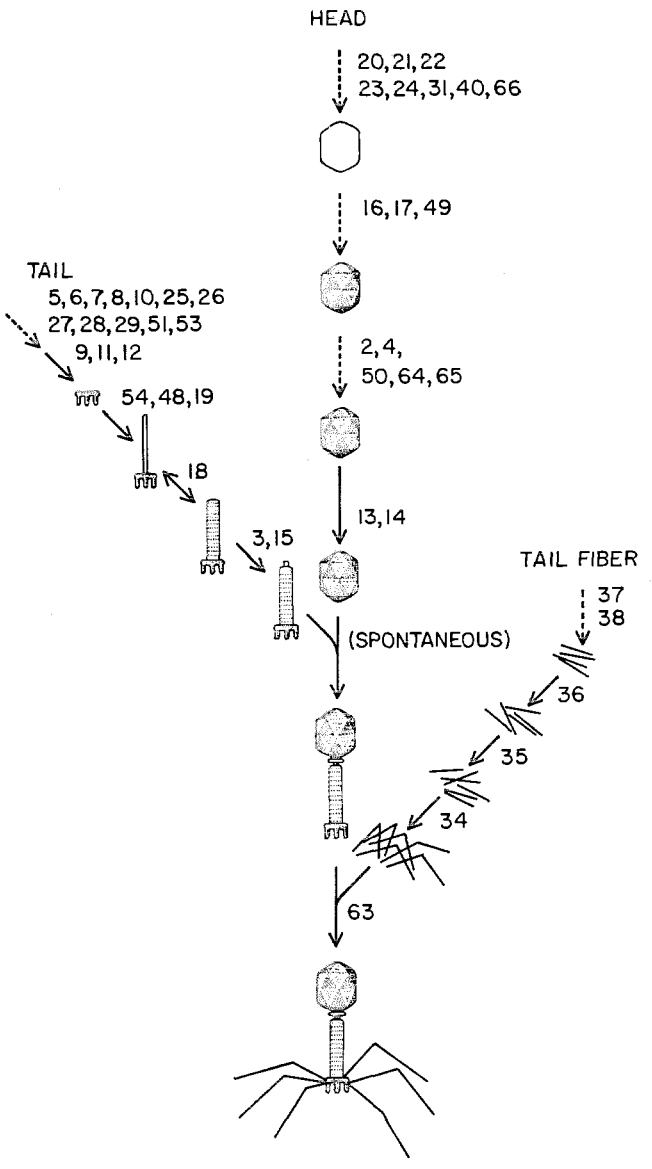
A Genetic Map of T4 Virus

In a genetic map of T4, genes identified by conditionally lethal mutations are numbered for identification. The genes represented by narrow black lines control replication of the viral DNA and other events occurring early after infection. The genes represented by heavier black lines control the synthesis of phage components and their assembly into virus. The aspect of the assembly process controlled by the various genes can be inferred from the symbols in the accompanying boxes, which represent the major phage components found in cells infected with a mutant defective in the corresponding gene.

second set of conditions, termed restrictive, the mutation blocks gene function completely. If the mutant gene is an essential one, viral development under restrictive conditions can proceed only to the point where that gene's function is required for continuation of the program; there the process must stop.

By examining such abortively infected cells to determine where development is blocked, or which phage components are missing, it is possible to infer a good deal about the normal function of the mutant gene. For instance, cells infected under restrictive conditions with a mutant defective in a gene essential for the synthesis of viral DNA exhibit the early stages of normal viral development—the destruction of the host cell DNA and the appearance of new virus-specific enzymes. But no new viral DNA is synthesized, and the latter half of the program, for synthesis of phage parts, never gets under way. Cells infected with a mutant defective in a gene controlling the formation of the head of the virus, on the other hand, go through a normal growth cycle and lyse but produce only virus tails rather than active virus.

During the last eight years, much of the work of the phage group has centered around the study of the conditionally lethal mutations in attempts to identify and determine the function of as many genes as possible in T4. So far about 80, or approximately half of the estimated total number, have been mutationally identified. Through studies at Caltech and other laboratories, the detailed functions of many of these genes are now known, as indicated on the genetic map above. A good many genes were found to be clearly involved with the synthesis of the various enzymes required for main-



The process of T4 assembly as currently understood can be represented as an assembly line with three major branches. Solid arrows are used here to indicate steps which can be carried out in the test tube. The numbers indicate the gene control of the various steps.

tenance of the host cell and reproduction of the viral DNA. However, more than half of the genes identified appeared to control the synthesis of viral components and the assembly of the phage particle.

In 1964 William Wood, a biochemist with an interest in genetics, joined the faculty of the biology division. Shortly thereafter, he and Edgar decided to collaborate in an attempt to attack the problem of virus assembly more directly. Using disrupted preparations of cells which had been infected with appropriate mutants as sources of unassembled phage parts, they were able to find conditions under which these components could be put together in the test tube to form complete infectious virus. Edgar, Wood, and graduate students Jon King and Jeffrey Flatgaard characterized the incomplete components produced by various mutants to yield a progressively more detailed picture of the process of phage assembly (left).

Construction of the virus seems to take place by a stepwise assembly line process, in which each step is under the control of a different gene. The heads, tails, and tail fibers are assembled independently of one another, so that the assembly line is branched. These subcomponents are then put together, also in a fixed sequence; that is, the fibers are attached to the tails only after head and tail are united.

Virus assembly in the test tube is not restricted to T4. Weigle, also using mutants, has shown that isolated heads and tails of phage λ, when mixed together, can unite to form normal active virus. In an intriguing series of experiments, he has shown that the free tails of the virus can attach to bacterial host cells. If heads are now added, they attach themselves to the tails, inject their DNA through them, and the bacteria become infected.

The encapsulation of viral DNA within a protective coat, a process common to the assembly of all viruses, is now under investigation.

For Wood and his collaborators, Delbrück's early consideration of T4 as a purely genetic system has now shifted to an emphasis on the phage particle as a complex supramolecular structure which is built up under the control of the viral genetic program. Molecular biology has provided a clear understanding of how genes direct the synthesis of single protein molecules; however, little is known about the assembly of higher order structures such as cellular organelles or viruses like T4 which are made up of many different kinds of protein molecules, or about how genes control this assembly.

In the hope of better understanding such processes, Wood and his co-workers are continuing the study of T4 assembly in the test tube. The earlier work in collaboration with Edgar, Flatgaard, and King established a sequence of gene-controlled

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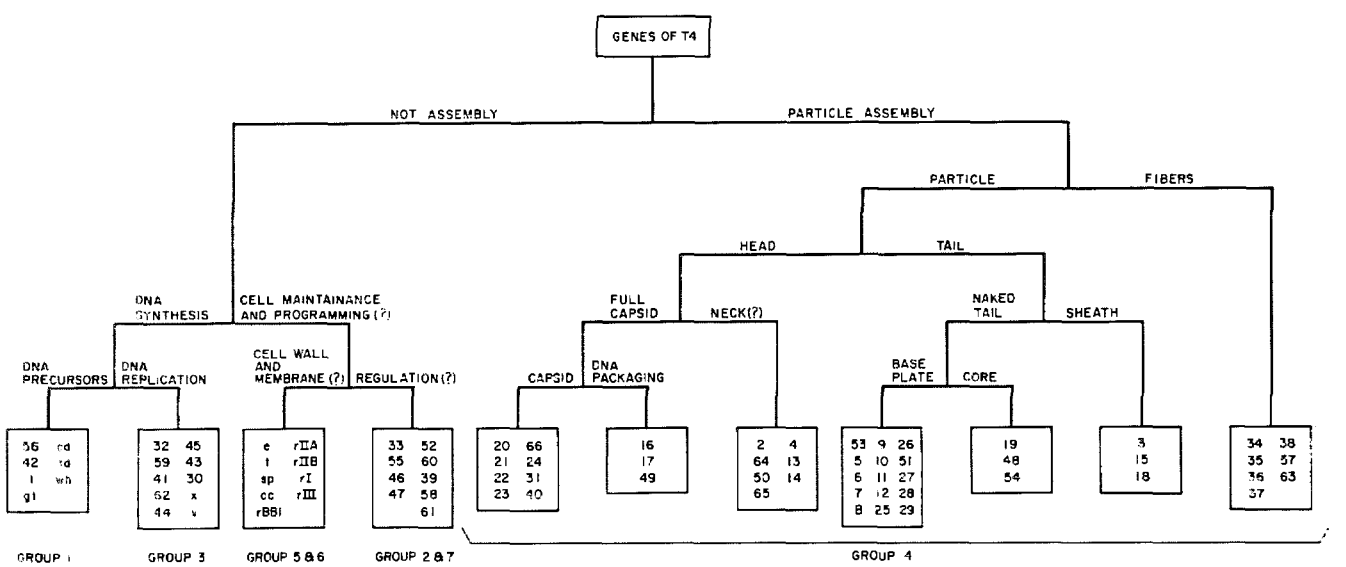
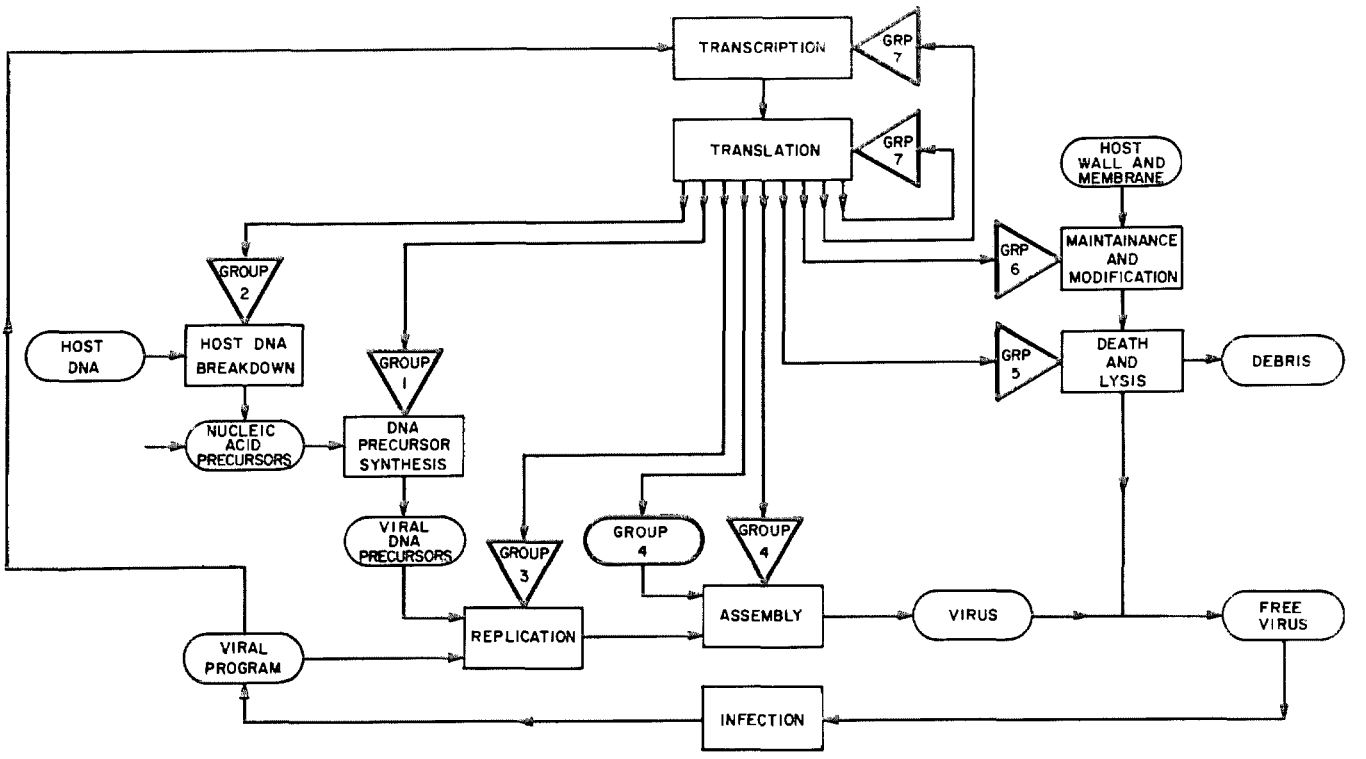
steps but provided little detailed information about their nature at the molecular level. The investigators demonstrated, however, that at least 15 steps could be carried out in the test tube and hence were at least in principle subject to detailed biochemical analysis. Characterization of selected steps is now under way. Graduate students Sam Ward and John Wilson have focused their attention on the sub-assembly pathway concerned with the tail fibers, and they have begun purification of the interacting components for detailed analysis. From work of Wood and research assistant Harriet Lyle on the final step in assembly, it now appears that a phage-induced enzyme catalyzes the attachment of the finished tail fibers to the otherwise completed particle. This is the first direct indication that assembly of complex multi-protein structures may involve enzyme-mediated reactions as well as simple interactions between the protein components themselves.

Also under investigation in the Wood group is a process common to the assembly of all viruses, the encapsulation of viral DNA within a protein coat. In T4, this step has so far not been accessible to study in the test tube. By radioactive labeling of cells infected with appropriate mutants, however, Ronald Luftig, a postdoctoral fellow working with Wood, has been able to show that in T4 development the protein membrane of the head is first made as an empty bag, and then subsequently somehow filled with the viral DNA. An elucidation of how this unlikely contortional feat is performed, which Luftig hopes to achieve, would be of importance in understanding the general process of viral development, and might conceivably also bear on the problem of how proteins and DNA interact to form the chromosomes of higher animals.

Edgar's interest has recently returned to the general nature of the T4 genetic program. Although highly complex as viruses go, T4 is just simple enough to foster the hope that continued research could lead to identification of all of the phage genes and a complete understanding of the program of viral development. Already, the known genes can be classified into a few groups on the basis of their functional roles (below). If each of these groups is



Using a genetic map of T4, all of the known phage genes can be classified according to their function in the infected cell.



If each of the classified groups of known T4 genes is thought of as a different kind of functional element, they can be incorporated into a tentative "circuit diagram" of T4 development. Transcription and translation refer to the principal stages in gene expression at the molecular level. In the first, the segment of DNA comprising a gene is transcribed to produce a "messenger" RNA molecule, carrying the same information. This information is then translated by the protein synthesizing machinery to produce the enzyme or structural protein controlled by the original gene. The remainder of the diagram indicates how these proteins participate in the various processes of intracellular phage development. Triangular boxes designate enzymes or catalytic proteins, while ovals represent structural components of the finished virus. Each group is numbered according to the classification of the corresponding genes.

taken to represent a different kind of controlling element, a tentative "circuit diagram" of phage development can be written (above). While on the one hand it helps to provide an overall picture of the nature of the genetic program, it also points up gaps in current knowledge of precise gene functions.

There is clearly another gap as well. The 80 genes of T4 so far identified account for only about one-half of the information stored in the DNA of the virus. What of the unknown half? Recent attempts to isolate conditionally lethal mutations in new genes have met with little success, although Richard Josslin, a graduate student, has discovered one new gene that appears to control the lysis of the infected cell. It appears likely that most if not all of the genes which are essential for viral growth, at least under laboratory conditions, have in fact been identified and that the functions of the remaining genes are nonessential. If so, mutations affecting them will be hard to detect.

For phage λ, one approach to this problem has been developed by graduate student John Parkinson, who has developed a simple method for isolating mutants of λ that have less DNA than the normal virus. Among these deletion mutants are strains which have lost up to 30 percent of their DNA and yet can still multiply in the host cell quite normally. This shows that at least 30 percent of the DNA of this virus is not concerned with functions required for reproduction. Parkinson has found, however, that most of these mutants are defective in the process of lysogenization. Since these mutants cannot integrate their DNA into the host DNA molecule, their only recourse is to multiply and kill the host cell.

As living creatures go, phages T4 and λ may be simple, but nevertheless the accumulated biological wisdom of two billion years of evolution is encoded in their DNA molecules. This is challenge enough to keep at least two genetic cryptographers intrigued for some time to come. □