

ROLE OF THE HOST CELL IN BACTERIOPHAGE T4 DEVELOPMENT

Thesis by

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In Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

1978

(Submitted February 22, 1978)

ACKNOWLEDGMENTS

The experiments reported in this thesis are the results of a collaborative effort. The bulk of Chapter 3 is the work of Helen Revel, aided by Ilga Lielausis and Marie Beall. The following exceptions, which are my work, are noted: the fine-structure mapping of goA-1, goD-1, and goA2820; the isolation and characterization of mutants from selection II, and the characterization of mutants from classes of potential interest from selection III. Chapter 4 is my work, again with the technical help of Ilga Lielausis, with the following exceptions: all phage DNA synthesis experiments and the mapping and dominance studies of goF-1 were done by Helen Revel, and the preparation of thin sections for electron microscopy was the work of Lore Ann McNicol. Others in the lab group and elsewhere in the Division of Biology contributed helpful ideas, discussion, instruction, and advice. Bill Wood provided a well-organized laboratory, financial support, and a constant model of the desired level of achievement. Shirley Brown, Jenny Cortenbach, Lilly Frederick, and Irene von Hartmann supplied vast quantities of media and clean, sterile glassware. Bernita Larsh, Donna Lathrop, and Connie Katz transformed page after page of my scrawls and arrows into a readable manuscript and elegant tables. I thank the rest of the personnel of the Division of Biology for their help; I am sure that they are responsible for the smooth course of my stay here in ways that I still do not fully appreciate. Many friends have supported me in more personal ways; I express my warmest thanks to them. I thank the following sources for financial support: the National Institutes of Health, U.S. Public Health Service, grants GM-00086 and AI-09238, Caltech Special Institute Fellowships, the Arthur McCallum Fund, and the Division of Biology.

ABSTRACT

More than six hundred mutant host-defective bacteria were selected that were able to adsorb and be killed by phage $T4^+$ but were unable to support its growth. The mutants were categorized into seven classes and representatives were screened to determine the nature of the host block. The majority of the mutants clearly blocked in phage assembly interfered with $T4^+$ capsid formation at the very early level of gene 31 function. gp31 acts at or before head core formation, which precedes the assembly of proheads and the formation of the capsid proper. Mutant phage able to overcome the host block carried mutations in either gene 31 or in the gene for the major capsid protein. The host mutations all mapped near purA, at two or possibly three different sites.

A host mutant class that only marginally passed our screens for assembly-related mutants had a pleiotropic effect on $T4^+$ growth: specific, unidentified early phage proteins were made in reduced quantity or not at all; phage DNA synthesis was depressed 50%; specific late, structural phage proteins including some tail-baseplate and phage collar components were reduced or missing, and host cell lysis was delayed and slow. Phage that could overcome this block mapped in functionally undefined regions of the T4 genome, most near gene 39. The host mutation mapped to within 0.5 minutes of nalA.

Possible mechanisms of action for the various host mutations are discussed.

TABLE OF CONTENTS

	<u>Page</u>
Acknowledgments	ii
Abstract	iii
Table of Contents	iv
Chapter 1 Introduction: The Role of the Host Cell in Virus Assembly	1
I. Introduction	1
II. Known roles of the host in virus reproduction; virus self-assembly?	2
III. The host role in animal virus infections	5
IV. The bacterial role in phage assembly	7
A. Educated guesses	7
B. Mutant virus and various standard hosts	9
C. Mutant virus and wild bacteria	9
D. Wild type phage and wild bacteria	10
E. Wild type virus and mutant bacteria	11
Chapter 2 Materials and Methods	17
I. Materials	17
A. Bacteria and phage strains	17
B. Media, chemicals, and enzymes	17
II. Methods	30
A. Maintenance of bacteria	30
B. Phage stocks	30
C. Mutagenesis of bacteria	30
D. Selection of host-defective mutants	31
E. Streak tests and spot tests	31

F.	<u>go</u> phage classification of <u>E. coli</u> host-defective mutants	33
G.	Mutagenesis of T4	33
H.	Standard methods for growth and assay of bacteria and phage	33
I.	Burst size determinations	34
J.	Intracellular phage growth curves	34
K.	Measurement of infected-cell lysis	35
L.	Measurement of phage DNA synthesis	35
M.	Labeling of extracts for gel electrophoresis	35
N.	SDS-polyacrylamide gel electrophoresis	36
O.	Fluorography	37
P.	Electron microscopy	37
Q.	Assay of phage components by <u>in vitro</u> complementation in mixed extracts	38
R.	Tail fiber antigen determinations	39
S.	Temperature shift experiments	39
T.	Phage genetics	40
U.	Bacterial growth curves	40
V.	Assay of bacterial protein synthesis	40
W.	Assay of bacterial DNA synthesis	41
X.	Assay of bacterial cell wall synthesis	41
Y.	Assay of bacterial phospholipid synthesis	41
Z.	<u>E. coli</u> genetics	42
AA.	Abbreviations and terminology	44
Chapter 3	Isolation and Characterization of Host-Defective Mutants of <u>E. coli</u> K-12 Blocked in T4 ⁺ Assembly	46

	I. Introduction	46
	II. Isolation and preliminary characterization of host-defective mutants	50
	A. An overview	50
	B. Clonal selection of host-defective mutants	50
	C. Choice of parental bacterium and phage used as selection agents	52
	D. Selection I	53
	E. Selections II and III: Isolation of host-defective mutants using <u>go</u> phage as selecting agents	73
	III. Characterization of T4 ⁺ assembly defects in host-defective mutant bacteria	77
	A. Introduction	77
	B. The course of T4 ⁺ infection in HD strains	77
	C. Genetics of <u>go</u> phage	84
	D. Genetic analysis of HD strains	110
	E. Characterization of the "other" class	117
	F. Summary	118
	IV. Discussion	122
	A. HD strains block T4 ⁺ head assembly at the level of gp31 function	122
	B. Proposed mechanism of gp31 inactivity in <u>mop</u> mutants and the function of <u>go</u> mutations	126
	C. The host component(s)	131
	D. Attempt to select HD strains blocked in other subassembly pathways	133
Chapter 4	Characterization of Host-Defective Mutants (<u>hdf</u>) that have a Pleiotropic Effect on T4 ⁺ Growth	137
	I. Isolation, classification, and properties of HDF bacteria	137

II.	The defective phenotype of T4 ⁺ -infected HDF cells . . .	141
A.	Growth of T4 ⁺ and <u>goF</u> -1 on HDF strains	141
B.	Lysis of HDF cells following T4 infection	141
C.	Synthesis of T4 DNA in HDF cells	147
D.	Synthesis of T4 proteins in HDF cells	150
E.	Synthesis of T4 structural components in HDF cells	158
F.	Biological activity of T4 structural components made in HDF cells	165
G.	Temperature shift experiments with HDF cells before and after infection	174
III.	T4 mutants that overcome the <u>hdf</u> block to phage growth	181
A.	Introduction	181
B.	<u>go</u> phage initially selected on HDF strains	181
C.	Additional <u>go</u> phage selected on HDF strains	195
D.	Additional properties of <u>goF</u> phage	202
IV.	Characterization of HDF bacteria and mapping of the <u>hdf</u> mutation	205
A.	Temperature sensitivity of HDF cells	205
B.	Location of <u>hdf</u> on the <u>E. coli</u> chromosome	210
V.	Comparison of <u>hd590</u> and K-12 HDF strains	225
VI.	Discussion	227
A.	Introduction	227
B.	The phenotype of HDF strains	228
C.	Model 1: A defective bacterial membrane	228
D.	Model 2: <u>hdf</u> has transcriptional or translational effects	230

	E. Further experiments.	235
Appendix	SDS-Polyacrylamide Gel Electrophoresis of Extracts of T4-infected HDF Cells	237
Literature Cited		245

Chapter 1

INTRODUCTION: THE ROLE OF THE HOST CELL IN VIRUS ASSEMBLY

I. Introduction

The structure and assembly of many kinds of virus particles, most notably that of phage T4, have been extensively investigated through the use of virus mutants. The characterization of structures produced when cells are infected with viruses carrying mutations in genes for virion components has allowed the assignment of many proteins to roles in the formation of particular virion substructures. These assignments have been confirmed by gel electrophoresis and in vitro complementation experiments performed on mutant-infected cell extracts and on virus components purified from such extracts (for a review, see Casjens and King, 1975). An examination of virus assembly (and other aspects of virus infections) oriented towards the identification of the roles played by the host cell in this process has only recently been extensively pursued, perhaps most thoroughly by Georgopoulos and coworkers using phage λ (Georgopoulos, 1971; Georgopoulos and Herskowitz, 1971; Georgopoulos et al., 1972, 1973; Georgopoulos, 1977). The approach has been readily accepted, however, because of the new information it provides concerning macromolecular interactions. We will here review the kinds of investigations that have been done to find out how the host participates in virus growth, and will concentrate on those that deal with the role of the cell in virion assembly.

II. Known roles of the host in virus reproduction; virus self-assembly?

All cells that can host a virus infection supply the virus with precursors and the machinery with which to synthesize macromolecules. "Machinery" means ribosomes and transfer RNAs at the very least, and, depending on the virus, may include other translational factors, polymerases or polymerase subunits, and the variety of enzymes that modify molecules by glucosylation, adenylation, methylation, cleavage, and so on. The virus must interact with the cell's machinery in such a way as to redirect part or all of it towards virus replication. It would seem advantageous for the virus to use the host whenever possible, as long as the interaction does not become so specific as to unduly restrict the virus' host range. Many bacteriophages, for example, use the cell's RNA polymerase for at least part of their transcription, although often the host enzyme is modified by the virus in some way (Losick and Pero, 1976).

Although interaction between host and virus components is inevitable if the virus is to use the cell's transcriptional and translational machinery, it is not a priori necessary in the process of assembling the progeny virions. Whether any viruses can, in fact, assemble completely independently of the host cell is a question that has not been precisely answered yet. Examples of infectious virus particles that have been reconstituted in vitro exist (tobacco mosaic virus (Fraenkel-Conrat and Williams, 1955), several of the three-cistron RNA phages (Hohn and Hohn, 1970), cowpea chlorotic mottle virus (Bancroft, 1970)), but in all cases the precursors for the in vitro reaction have been obtained by dissociation of infectious particles, and the possibility exists that these molecules are not identical to the in vivo precursors. For the three-cistron RNA phages, the determination of partial or complete sequences of the proteins and messenger RNAs

involved has eliminated the possibility of protein precursor molecules different from those which comprise the virion (Eoyang and August, 1974), but a problem still remains with regard to the RNA genome. For reconstitution experiments, the appropriate virion-derived single-stranded RNA is added to the in vitro reaction mixture. It is possible, however, that in vivo the processes of replication and packaging are interdependent: it is known, for example, that large quantities of single-stranded RNA do not exist in vivo in E. coli infected with the small RNA phages (Hohn and Hohn, 1970). Thus, although these simple viruses can self-assemble in vitro, whether they do so in vivo is still unclear. In any case, proper conditions of temperature, ionic strength, and virion component concentrations are necessary for the demonstrated in vitro self-assembly, and at least the latter two of these conditions clearly may be influenced by the host cell.

There are, however, indisputable cases in which the host is essential for virion assembly: all animal viruses with lipid coats acquire these coats by budding through a suitably modified host cell membrane, perhaps with the aid of actin and associated molecules (Casjens and King, 1975; Wang et al., 1976). There are equally clear cases among prokaryotic organisms: in filamentous bacteriophage infections, for example, the virus coat protein accumulates in the cell membrane and replaces a DNA binding protein as the binding protein-coated phage DNA is extruded through the bacterial membrane (Ray, 1977). In other phage-host systems there is circumstantial evidence for a host role in virus assembly; thus for T4, for example, based on electron microscopic results Simon (1969,1972) has suggested that both phage head and tail-baseplate assembly may be initiated in association with the cell membrane.

One way to ask about the host role in virus assembly is to look for altered hosts in which a particular virus strain will not grow. This type of investigation has been pursued in a variety of different systems, which we will now review in detail. Specifically excluded from the following discussion are cases in which extra-genomic elements are known to be responsible for the block to virus growth, such as the exclusion of T7 by F factors and P1 (Morrison and Malamy, 1971; Condit and Steitz, 1975; Condit, 1975; Eskridge et al., 1967), the exclusion of T4rII by λ (Benzer, 1955), and the exclusion of T5 by ColI (Moyer et al., 1972), to name a few. We start with a discussion of relevant research involving animal viruses, which is still in a primitive state, and then consider the much better-characterized interactions between prokaryotic cells, chiefly those of Escherichia coli, and their phages.

III. The host role in animal virus infections

The first cultured animal cells that were reported to adsorb a virus which they could normally grow but that were unable to propagate the virus were derived from HeLa cells in the course of studying poliomyelitis virus (Vogt and Dulbecco, 1958). The cells were obtained by repeated exposure to virus followed by subculturing in the presence of anti-virus serum; the resistant cells had a distinctive fusiform rather than polygonal morphology and were non-virogenic (i.e., not persistently infected). In 1958 the technology to explore the nature of the host defect was not available, so nothing further was done.

In 1960, the first report of cells resistant to a virus at the level of uncoating of the genome appeared (Darnell and Sawyer, 1960), again using HeLa cells and poliovirus. The step at which infection was blocked was determined by the observations that virus adsorption and penetration into the host were normal but nothing further happened, yet the cells were still fully susceptible to infection by naked viral nucleic acid. Several other cell lines have subsequently been isolated that appear to block virus infection at the uncoating step (Butel et al., 1971; Reznikoff et al., 1972; Wilson et al., 1976). Wilson (Wilson et al., 1976; Wilson, 1977) has taken the investigation one step further and isolated virus mutants, in this case of SV40, that are able to grow on the resistant cells and has found that the mutations lie in the major virus capsid protein, supporting the hypothesis that the block may be at the uncoating level.

Taber et al. (1976) have recently started to study virus-host interactions using the genetically (relatively) well-defined Chinese hamster ovary cell as the host. So far they have isolated five different and distinct clones that are resistant to encephalomyocarditis virus. It will be interesting to see whether the use of a genetically better-defined animal cell aids in this type of investigation.

Two other reports are worth mentioning for their different approaches to the problem of virus-host interaction. 1) Robb and Huebner (1973) simultaneously cloned several monkey cell sublines and found that those with the smallest average number of chromosomes were the most resistant to infection by SV40; again, the block seemed to be at the level of virus uncoating. As the subdiploid mean chromosome number of the resistant cells increased with passage time towards a subtetraploid number, the cells became more SV40-sensitive. 2) Most resistant cells are isolated following direct challenge with virus, followed by efforts to rid the culture of virus particles and persistently infected cells. Morgan et al. (1973) exploited a temperature-sensitive mutant of Sindbis virus by infecting cells and allowing one cycle of virus growth at the permissive temperature, and then shifting to nonpermissive conditions. Repetitions of this procedure for two months produced cells that were resistant to Sindbis and seven other viruses. The viruses were adsorbed and taken up normally, but what happened then was unclear; infections with viral nucleic acid only were not tried.

Very few studies have been published that directly address the role of the host cell in the assembly of animal viruses. The only system that seems to have yielded information of this kind is that of poliovirus in HeLa cells, investigated by Perlin and Phillips (Phillips, 1969, 1971; Perlin and Phillips, 1973, 1975). These workers have found that 73S empty capsids, which are virion precursors, can be assembled from 14S subunits in vitro. The reaction is very concentration-dependent unless the rough membrane fraction of infected cells is added; this fraction has two activities in the assembly process, one that "activates" the 14S precursors and is depleted as the incubation proceeds, and one that assembles the 73S capsid and is not consumed.

IV. The bacterial role in phage assembly

A. Educated guesses

One approach to the problem of determining how the host participates in virus development is to make educated guesses about which host genes might be relevant and to ask if cells carrying mutations in these genes can support virus growth. Such an approach has worked in the cases of bacteriophages with small genomes which obviously must use host functions for transcription and genome replication. For example, phage ØX174, which has 9-10 genes, was shown to need the products of E. coli genes dnaB, dnaC, dnaE, dnaG, dnaH, and dnaZ by its failure to grow in cells containing any one of these mutations (Denhardt, 1977). Mosig et al. (1972) have performed similar experiments with host dna gene mutants and small-genomed variants of phage T4. Other workers, knowing that the host RNA polymerase is used by T4 and that rifampicin-resistant E. coli cells are altered in the β subunit of RNA polymerase, have isolated Rif^r E. coli strains and found among them bacteria unable to grow T4⁺ (Snyder, 1972). This approach has also been used to obtain hosts that do not grow phage λ (Sternberg, 1973a,b). A more sophisticated type of investigation (Takahashi et al., 1975; Coppo et al., 1975a,b), based on a model of physical interactions of phage and host gene products, has used phage carrying missense mutations in the virus portion of such hybrid complexes to select for host mutants that can no longer support virus growth due to a mutation in the host portion of the complex. This approach has been used to probe the known interactions of T4-coded proteins with E. coli RNA polymerase. Although the technique is applicable to situations in which the phage-host interactions are undefined, a similar interpretation of direct, physical contact between the relevant gene products can only be made with caution.

Mutations that affect the relative amounts of virus or host components or that change the intracellular conditions (e.g., ion concentrations [Hartman and Roth, 1973; Colb and Shapiro, 1977]) could produce formally similar phenotypes.

With respect to guesses about assembly processes, Cronan and Vagelos (1971) tested an E. coli mutant temperature-sensitive for a lipid biosynthetic enzyme, glycerol-3-phosphate acyltransferase, for its ability to support T4⁺ infection at the nonpermissive temperature. In shift up experiments they found that the production of progeny viruses stopped soon after the shift, an expected phenotype for mutants affecting phage assembly. However, the production of T4 lysozyme also ceased after the shift and further observations showed that the infected cells lysed at the high temperature. Lysis did not occur with uninfected cells or with cells infected by phage carrying a deletion for the lysozyme gene, although in the latter case phage production still stopped following the shift. It was therefore concluded that phospholipid synthesis by the host is necessary to maintain the integrity of the infected cell membrane, but the effect on phage assembly is probably only an indirect one due to cell lysis. Simon et al. (1975), however, have reported the absence of T4 capsid assembly in an E. coli mutant presumably defective in the conversion of palmitoleic acid to cis-vaccenic acid; the block is probably at the level of gene 31 function, early in capsid assembly. Other mutants of E. coli fatty acid, lipid, and membrane biosynthesis have not been investigated. Chao et al. (1974) discovered that endA polA E. coli make an abnormally high proportion (33%) of short-headed T4, an observation which they interpreted to mean that DNA processing was somehow involved in the length determination of T4 heads. Only a small amount of DNA, however, must be necessary for this determination (Hsiao and Black, 1977).

B. Mutant virus and various standard hosts

A second approach is to start with a mutagenized virus stock and several different laboratory hosts that are capable of supporting wild type virus growth, and to ask what kind of virus mutants can be obtained that can grow in one host but not in others. The identity of the virus genes in which the mutations lie then provides a clue to the nature of the virus-host interaction. Georgopoulos et al. (1977) have recently done such an experiment with phage T4 and E. coli strains B and K-12, and have found that certain phage mutations in virion baseplate structural genes 8 and 53 are able to plate on the K-12 strain but not on the B strain. The mutant genes have been identified by complementation tests; this implies that other late phage proteins are synthesized normally by the mutant phage. If the membranes of E. coli K-12 and B are different, this result could be interpreted as support for the hypothesis (Simon, 1969) that tail-baseplates of T4 assemble on the host membrane; alternatively, specific translational factors such as tRNAs could be different in the two bacterial strains, resulting in the synthesis of different forms of the virus proteins that could be inactive. Similar observations of host-specific growth have been made for T4 phage carrying mutations at a particular site in gene 21, a gene whose protein product is involved in capsid formation (Castillo et al., 1977), and gene 60, which has a function in phage DNA synthesis (Yegian et al., 1971).

C. Mutant virus and wild bacteria

The above approach can also be taken using wild bacteria (i.e., non-laboratory strains), and in the case of T4 has been exploited to investigate mutants in nonessential phage genes—nonessential for growth on standard laboratory bacteria (summarized in Mathews, 1977). For example, Wilson (1973) has found

that T4 phage deleted for the tRNA genes which they normally carry do not grow on some wild E. coli strains that wild type phage successfully infect. The exact nature of the host block in these and other wild strains has not, however, been intensively investigated.

D. Wild type phage and wild bacteria

Another way to approach the question of what the host does in virus infections is to start with a wild type phage and isolate wild bacteria which it infects but on which it does not grow. This has been done for phages T3 and T7 (Hausmann, 1973), and the phenotypes of virus growth on the defective hosts were examined. Of 150 E. coli isolates from hospital patients, two were able to grow T3 and T7, and five were able to adsorb the phages satisfactorily but did not propagate them. Two of the latter were found to restrict the entering phage DNA, and two more blocked T7 DNA replication. One host, E8, showed normal incorporation of radioactive thymidine into T7 phage DNA, made normal amounts of early and late phage enzymes, and lysed at approximately the normal time after infection. Examination of the proteins made during infection by gel electrophoresis showed that most were present in normal amounts. One protein found in reduced quantity was gp17, the tail fiber structural protein; this protein was also found to be present in only 10-30% of its normal amount by antigen assay. Further investigation of this strain has not been reported, so whether the host interferes with T7 assembly or with gp17 transcription or translation is not clear.

Hausmann (1968, 1973) also investigated one strain of Shigella sonnei (No. D₂ 371-48) that was unable to grow T7. Phage development in this strain

starts normally, but late in infection the newly-synthesized T7 DNA is degraded. This degradation can be prevented by the addition of chloramphenicol to infected cells 4.5' after infection; under these conditions, phage DNA synthesis continues, implying that phage gene products are responsible for the phenotype. A T7 mutant, ss^- , which grows on this Shigella strain, was isolated by Hausmann, and recently Vetter et al. (1977) have found that the phage carries a mutation in gene 10, the major T7 capsid protein. The system thus appears to be one in which aberrant head formation leads to suicide, but a confirmatory investigation of the status of capsids in $T7^+$ -infected Shigella D₂ 371-48 has not been done.

E. Wild type virus and mutant bacteria

By far the most common way of attacking the problem of host-virus assembly interactions is to start with a laboratory bacterial strain that grows the virus of interest, preferably a well-characterized strain, and to select mutants of it that no longer allow normal phage growth. Obviously, the better the host strain is understood, the better the chances of ultimately understanding the nature of the mutants selected. Host mutants that block virus development at all possible levels are obtained in this way, and either all must be investigated or a screening procedure must be devised to identify those mutants specifically involved in virus assembly. A screen of this kind could be impossible, however, since in many systems the two major virus activities, genome replication and packaging of the nucleic acid, are integrated processes. Thus, for example, the only host mutants that have been found to block the growth of phage ØX174 interfere with the appearance of single-stranded progeny genomes (Denhardt et al., 1967, 1972; Tessman and Peterson, 1976). This may reflect the fact that almost all

virus genes, including structural virion components, are necessary for ØX174 DNA replication (Denhardt, 1977).

In some systems, however, such as cells infected with phage λ or T4, virus DNA synthesis is more independent of the packaging step (Kaiser, 1971; Hsiao and Black, 1977). In these cases, a screening stage following host mutant isolation in which the normalcy of virus DNA synthesis is checked can serve to discriminate possible blocks at the virus assembly level from those involved with genome replication. A screen for assembly mutants that do not act via effects on genome replication is also possible for viruses such as λ and T4 that actively lyse their host, because the synthesis of both lysis enzymes and virus structural proteins tends to occur after the start of genome replication.

The most intensive investigations of mutant bacteria that block phage assembly have been done using phage λ . The only host interactions necessary for λ assembly so far found work at the level of head morphogenesis (Georgopoulos et al., 1972, 1973; Sternberg, 1973a,b; Georgopoulos and Eisen, 1974). E. coli groE mutants adsorb λ^+ normally and the phage is able to inject and replicate its DNA, synthesize all phage proteins, assemble active virus tails, and lyse the host cell (the latter process is delayed and slow). No infectious progeny virus are released, however, and electron microscopic examination of cell lysates shows an absence of λ heads and the presence of petit λ (head precursor) and aberrant structures composed of capsid proteins and lacking DNA (often two headlike structures connected together, and also long tubular forms). Abnormal structures similar to these are found in infections of wild type E. coli with λ carrying a mutation in head structural genes B or C. Several steps in head assembly fail to occur in groE hosts: the fusion of head proteins pC and pE does not occur,

and the cleavage of pB to form h3 does not take place (Georgopoulos et al., 1973; Hendrix and Casjens, 1975). In addition, the petit λ that are formed contain substantial amounts of pC, unlike wild type petit λ , and sediment at 190S rather than 150S. The latter property may be due to changes in the hydrodynamic properties of the groE petit λ rather than to changes in their mass (Hendrix and Casjens, 1975).

λ mutants that can grow on groE hosts carry a mutation in gene E, the major capsid component, or gene B, a minor protein of the capsid (Georgopoulos et al., 1973; Sternberg, 1973b). In this system it thus appears that there is phage-host interaction at the level of virus prohead assembly. In some groE mutants the defective assembly process can be corrected by mutations in λ that reduce the quantity of gene E protein (Sternberg, 1973b; Georgopoulos et al., 1973), thus apparently restoring a balance between the amount of active host groE product and the λ proteins with which it interacts. It is not clear, however, whether all groE mutations are of this kind or whether some cases involve changes in protein structure and compensating alterations in the structure of an interacting gene product, or whether an alteration in the internal environment of the host is involved.

groE mutants also block λ DNA maturation—the cutting of concatameric λ DNA into genome-sized pieces with the generation of sticky ends (Georgopoulos et al., 1973). This process is normally dependent on capsid formation and is blocked in wild type cells infected with λ head gene mutants. Henderson and Weil (1977) have found further evidence for a host function in λ DNA packaging, one which is involved in cutting the DNA at the cos sites. Their mutants, which are altered at a site different from groE (93'), near 59' (cysC), block the packaging of λ DNA

containing large deletions. This effect can be corrected by mutations that either increase the length of the DNA (duplications) or by point mutations that probably map in head gene B, Nu2, or C. Some phage in which amber mutations in gene D, another head component, are suppressed, can also grow on these mutants. How the host participates in the DNA cutting event is not understood. In vitro investigations of the DNA packaging process in phage λ (Becker et al., 1977) have uncovered an early step, the binding of pA (=ter function?) to λ DNA concatemers, which is dependent on the presence of uninfected host cell extracts; whether extracts from groE cells will catalyse this step has not been investigated.

Two of the groE mutants, groEA639 and groEA36, isolated by their inability to grow λ^+ , are also unable to grow phage T5 (Georgopoulos et al., 1973). Zweig and Cummings (1973) have pursued the nature of the block to T5 growth and, as is the case for λ , have found a protein cleavage-related block to virus assembly, this time in a phage tail precursor. Protein cleavage reactions are necessary for both T5 head and tail formation, and although abnormally large quantities of aberrant head forms are found in $T5^+$ infections of groEA639 and groEA36, substantial amounts of active heads containing appropriately cleaved proteins are also made, whereas no active tails are detected.

One λ -related groE mutant was found to block phage T4 growth (Georgopoulos et al., 1972), and for the third time protein cleavages were found to be defective. $T4^+$ infection of the strain groEA44, like λ^+ infection, is blocked at the level of phage capsid assembly. The major T4 capsid protein, gp23, which is found in cleaved form in virions, is not cleaved in groEA44 cells and is found in insoluble aggregates on the cell's inner membrane. This phenotype is like

that found in wild type cells infected with T4 mutants in gene 31; the product of gene 31 is thought to act normally in the solubilization of gp23 or to direct its assembly early in T4 head formation (Laemmli et al., 1970a). Consistent with the idea of a block at this level, T4 mutants that can grow in the groEA44 strain have been found to carry mutations in gene 31.

Since all three phages unable to grow in groE bacteria show defective protein cleavage reactions, one reasonable hypothesis is that the groE⁺ gene product is either itself a protease or is otherwise involved in cleavage reactions. Georgopoulos and Eisen (1974) investigated this hypothesis by looking for host proteins that are not cleaved under conditions where the groE product makes cell growth temperature-sensitive; they were unable to find evidence for cleavage abnormalities. It thus seems more likely that the block is at a step prior to and essential for cleavage reactions.

Takano and Kakefuda (1972), Coppo et al. (1973) and Wood et al. (1973) have isolated E. coli mutants similar to groEA44 which block T4⁺ head morphogenesis. All mutations so far seem to act at the same level, and the host mutations mapped all lie near purA on the E. coli chromosome.

Simon (1969), however, has suggested that T4 baseplates may assemble in association with the host cell's membrane, and Pulitzer and Yanagida (1971) have found a host mutant that causes T4⁺ to produce defective tail fibers. Revel et al. (1976) have reported the existence of E. coli mutants that bypass T4's need for gp57, a product necessary for the assembly of long and short tail fibers. Since phage-host interactions at several places in the T4 assembly process therefore seemed likely, we undertook the project of isolating and characterizing as wide a variety of assembly-related host-defective mutants as possible. More

than six hundred mutants were isolated and sorted into classes; representatives of those classes most likely to block $T4^+$ development at a step of virus assembly were characterized. All mutants that clearly blocked phage assembly interfered at the early stage of capsid assembly in which T4 gp31 participates. A mutant class that marginally passed our screens for hosts that block T4 assembly was found to have a pleiotropic effect on phage growth; the primary inhibitory interaction between phage and host in these strains is probably early in the T4 life cycle, before or during genome replication. A preliminary report of these results has appeared elsewhere (Wood et al., 1973).

Chapter 2

MATERIALS AND METHODS

I. Materials

A. Bacteria and phage strains

All bacterial strains used are listed in Table 2-1. T4D⁺ and amber, ts, and deletion-containing T4 mutants were chiefly from the Caltech collection; single mutants used are listed in Table 2-2, multiple mutants in Table 2-3, and deletion mutants in Table 2-4. Mutants from other sources are listed and acknowledged in these tables. Phage other than T4 are listed in Table 2-5. go phage are mutants of T4 selected as described in Part F of this chapter.

B. Media, chemicals, and enzymes

Gelatin, casamino acids, nutrient broth, Bacto-peptone, Bacto-tryptone, yeast extract, Bacto agar, and Bacto EMB agar base were obtained from Difco. Media for phage and bacterial growth were prepared as follows.

H broth. Nutrient broth, 8.0 g; Bacto-peptone, 5.0 g; NaCl, 5.0 g; and dextrose, 1.0 g were added to H₂O, 1 liter; the pH was adjusted to 7.4 with 3 ml 4% NaOH. For growth of phage P1, CaCl₂ was added to 5 x 10⁻³ M.

LB broth. Bacto-tryptone, 10.0 g; yeast extract, 5.0 g; and NaCl, 5.0 g were added to H₂O, 1 liter; the pH was adjusted to 7.4 with 1.5 ml 4% NaOH. For growth of phage λ, maltose was added to 0.3%.

M9. Na₂HPO₄·7H₂O, 15.1 g; KH₂PO₄, 2.0 g; NaCl, 0.5 g; and NH₄Cl, 1.0 g were added to H₂O, 957 ml. The following solutions were made up separately and added after autoclaving: 0.1 M CaCl₂, 1 ml; 1.0 M MgSO₄, 1.0 ml; 10% dextrose, 40.0 ml.

TABLE 2-1. E. coli strains

Bacterium	Strain	Characteristics	Source or reference ^a	Comments
<u>Indicator bacteria</u>				
CR63	K-12	F ⁺ supD60	Caltech collection	Standard permissive host for T4 am mutants.
CR63(λ)	K-12	F ⁺ supD60	Caltech collection	Nonpermissive for T4 rII mutants.
S/6/5	B		Caltech collection	Standard nonpermissive host for T4 am mutants, usually called S/6.
G(λ)	B		Caltech collection	Used as indicator in serum blocking assays.
B/5	B		Caltech collection	
Bb	B		Caltech collection	
<u>Parents of HD strains</u>				
SKB178	K-12	galE RgII	A. D. Kaiser	Adsorbs T4 well. Usually called B178.
B011'	B	thy sup	L. D. Simon	
<u>Strains used for phage genetics</u>				
CT196			Caltech collection Wilson, 1973	Caltech "hospital strain"

TABLE 2-1 (continued)

Bacterium	Strain	Characteristics	Source or reference ^a	Comments
CT439			Caltech collection Wilson, 1973	Caltech "hospital strain"
WH-1	K-12	F'(F196 supD32) <u>his-45</u> <u>trp-37 phoA4 recA1</u> <u>str144 "valR-116"</u>	CGSC #4612 Hoffman and Wilhelm, 1970	F196 used to make HD strains <u>supD</u> .
C600	K-12	<u>thr-1 leu-6 thi-1</u> <u>supE44 lacY1 stable</u> <u>tonA21 λ⁻</u>	Caltech collection	
<u>Strains used for</u> <u>bacterial genetics</u> <u>Hfr strains^b</u>				
KL96	K-12	Hfr <u>thi-1 rel-1 λ⁻</u>	CGSC #4243 Low, 1973	
KL983	K-12	Hfr <u>xy1-7 lacY1 or Z4</u> <u>mg1P1 λ⁻</u>	CGSC #4240 Low, 1973	
PK191	K-12	Hfr Δ(proB-lac) XIII <u>sup-56 λ⁻ Col V⁻</u> <u>ColVR</u>	CGSC #4316 Low, 1973	
KL16	K-12	Hfr <u>thi-1 rel-1 λ⁻</u>	CGSC #4245 Low, 1973	

TABLE 2-1. (continued)

Bacterium	Strain	Characteristics	Source or reference ^a	Comments
<u>F' strains</u>				
KL17/KL132	K-12	F' (F117) <u>pyrB31</u> <u>thi-1</u> <u>thyA25</u> <u>his-1</u> <u>pro-27</u> <u>leu-6</u> <u>thr-1</u> <u>recA1</u> <u>xy1-7</u> <u>ma1A1</u> <u>ara-13</u> <u>gal-6</u> <u>lacY1</u> <u>tonA2</u> <u>str-9</u> <u>rel-1?</u> <u>λ^R</u> <u>λ⁻</u>	CGSC #4255 Low, 1972	
KL18/KL132	K-12	F' (F118) <u>thi-1</u> <u>pyrB31</u> <u>pro-27</u> <u>thr-1</u> <u>leu-6</u> <u>his-1</u> <u>thyA25</u> <u>recA1</u> <u>xy1-7</u> <u>ma1A1</u> <u>lacY1</u> <u>gal-6</u> <u>ara-13?</u> <u>tonA2</u> <u>str-9</u> <u>λ^R</u> <u>λ⁻</u>	CGSC #4259 Low, 1972	Grows very slowly
KL13/JC1552	K-12	F' (F103) <u>argG6</u> <u>metB1</u> <u>his-1</u> <u>trp-31</u> <u>leu-6</u> <u>mt1-2</u> <u>xy1-7</u> <u>ma1A1</u> <u>gal-6</u> <u>lacY1</u> <u>strA104</u> <u>tonA2</u> <u>tsx-1</u> <u>λ^R</u> <u>λ⁻</u> <u>supE44</u>	CGSC #4249 Low, 1972	
KL13-1/JC1553	K-12	F' (F131) <u>supD43</u> <u>argG6</u> <u>metB1</u> <u>his-1</u> <u>leu-6</u> <u>recA1</u> <u>mt1-2</u> <u>xy1-7</u> <u>ma1A1</u> <u>gal-6</u> <u>lacY1</u> or <u>Z4</u> <u>strA104</u> <u>tonA2</u> <u>λ^R</u> <u>λ⁻</u> <u>supE44</u>	CGSC #4246 Low, 1972	

TABLE 2-1 (continued)

Bacterium	Strain	Characteristics	Source or ^a reference	Comments
<u>Source of λ transducing phage</u>				
AN151 (λ dnaIA1, λ cI857)	K-12	HfrR1 <u>ubiG423</u> <u>metB</u>	K. Kruezer	
AN151 (λ dubG1, λ cI857)	K-12	HfrR1 <u>ubiG423</u> <u>met B</u>	K. Kruezer	
<u>Strains used in P1 transductions</u>				
T832	K-12	<u>argH</u> <u>his</u> <u>pro</u> <u>thi-1</u> <u>purA</u> <u>ampA</u> <u>mal</u> <u>tsx-9</u> <u>StrR</u> λ^R λ^- <u>sup</u> <u>EMBS</u>	T. Takano	
AB352	K-12	Hfr <u>thi-1</u> <u>thr-1</u> <u>leu-6</u> <u>lacZ4</u> <u>purF1</u> <u>strA8</u> λ^- <u>supE44</u>	CGSC #352	
E101	K-12	<u>thr-1</u> <u>leu-6</u> <u>thi-1</u> <u>thyA6</u> <u>deoC1</u> <u>nrdA1</u> <u>lacY1</u> <u>strA67</u> <u>tonA2</u> λ^- <u>supE44</u>	CGSC #5146 Wechsler and Gross, 1971	<u>nrdA1</u> = <u>dnaF101</u>
AT2243-11 ^c -25	K-12	Hfr <u>metB1</u> <u>uhp-1</u> <u>fpk-1</u> <u>pyrE41</u> <u>relA1</u> <u>tonA22</u>	CGSC #4864 Ferenci and Kornberg, 1973	

TABLE 2-1 (continued)

Bacterium	Strain	Characteristics	Source or reference ^a	Comments
SB1803	K-12	<u>thi-1</u> <u>thr-1</u> <u>leu-6</u> <u>metG83</u> <u>hisC3</u> <u>proA2</u> <u>mtl-1</u> <u>xy1-5</u> <u>ara-14</u> <u>galK2</u> <u>lacY1</u> <u>strA25</u> <u>λ⁻</u> <u>supE44</u> <u>f1a?</u> <u>lpcB?</u>	CGSC #4929 Blumenthal, 1972	
EM1101	K-12	<u>dsdA1</u> <u>dsdC1</u> <u>lac</u> <u>str</u>	W. Dempsey McFall, 1964	
HNG2	K-12	F ⁺ <u>polA</u> <u>endA</u> <u>thyA</u> <u>strA</u> <u>na1A</u> <u>glpT</u>	K. Kruezer	
KK325	K-12	HfrR1 <u>ubiG423</u> <u>metB</u> <u>thyA</u> <u>λ⁻</u>	K. Kreuzer	
KK333	K-12	<u>thr</u> <u>leu</u> <u>arg-4</u> <u>strA</u> <u>xy1</u> <u>gal</u> <u>mtl</u> <u>lac</u> <u>glpT</u> <u>na1A</u>	K. Kreuzer	

^aStrains with CGSC numbers were obtained from B. Bachmann, E. coli Genetic Stock Center, Yale University.

^bThe complete set of Hfr's listed by Low (1973) was used, but only those strains which either transferred hdf⁺ in the mapping experiments or whose failure to do so increased the resolution of the position of the hdf locus are included.

TABLE 2-2. Single mutants of T4

Gene	Mutation	Gene	Mutation
3	<u>am</u> NG131	27	<u>am</u> N120
5	<u>am</u> N135	28	<u>am</u> A452
6	<u>am</u> N102 or <u>am</u> B251	29	<u>am</u> B7
7	<u>am</u> B16	30	<u>am</u> H39x5
8	<u>am</u> N132	31	<u>am</u> N54
9	<u>am</u> E17		<u>am</u> NG71
10	<u>am</u> B255		<u>ts</u> A70
11	<u>am</u> N128		<u>ts</u> A56
12	<u>am</u> N69	34	<u>am</u> A455
15	<u>am</u> N133	35	<u>am</u> B252
16	<u>am</u> N66	37	<u>am</u> N52
18	<u>am</u> E18	39	<u>am</u> N116
19	<u>am</u> E1137		<u>am</u> E480
20	<u>am</u> B8		<u>am</u> NG457
22	<u>am</u> E209	41	<u>am</u> N81
	<u>am</u> B270	42	<u>am</u> N122
23	<u>am</u> B17	51	<u>am</u> S29
	<u>am</u> H11	53	<u>am</u> H28
	<u>am</u> B272	54	<u>am</u> H21
	<u>am</u> E506	60	<u>am</u> E300
25	<u>am</u> N67	<u>e</u>	<u>am</u> H26
	<u>am</u> S52		<u>ts</u> C3
26	<u>am</u> S105	<u>r</u> III	<u>r</u> 67

TABLE 2-3. Multiple mutants of T4^a

Strain name	Mutations	Defective genes
X18	<u>amB256</u> : <u>amB251</u> : <u>amB16</u> : <u>amN132</u> : <u>amE17</u> : <u>amB255</u>	5:6:7:8:9:10
X19	<u>amN128</u> : <u>amN69</u> : <u>amE609</u> : <u>amB20</u> : <u>amN133</u> : <u>amN66</u>	11:12:13:14:15:16
X76	<u>amB280</u> : <u>amN52</u>	37:37
X77	<u>amB25</u> : <u>amA455</u> : <u>amN52</u>	34:34:37
X112 = X4E	<u>amB25</u> : <u>amA455</u> : <u>amN52</u> : <u>amB262</u> : <u>amB252</u>	34:34:37:38:35
X113	<u>amS52</u> : <u>amS105</u> : <u>amS29</u> : <u>amS92</u> : <u>amB6</u> : <u>amS7</u> : <u>amN85</u>	25:26:51:27:29:48
X143	<u>amE18</u> : <u>amN120</u>	18:27
X208	<u>r67</u> : <u>amN54</u>	<u>rIII</u> :31
	<u>r67</u> : <u>tsA70</u>	<u>rIII</u> :31
X379	<u>amB17</u> : <u>amM69</u> : <u>rEDdf41</u>	23:63: <u>rII</u>
X381: <u>t</u>	<u>amB256</u> : <u>amB251</u> : <u>amB16</u> : <u>amB5</u>	5:6:7: <u>t</u>
X446	<u>amE1</u> : <u>amE198</u>	36:57
X499	<u>rEDdf41</u> : <u>amN116</u>	<u>rII</u> :39
	<u>rEDdf41</u> : <u>amNG457</u>	<u>rII</u> :39
	<u>amN133</u> : <u>amB5</u>	15: <u>t</u>
	<u>amE1137</u> : <u>amB5</u>	19: <u>t</u>
	<u>amN85</u> : <u>amB5</u>	48: <u>t</u>
	<u>amN132</u> : <u>amB252</u>	8:35
	<u>amN135</u> : <u>amN85</u>	5:48

^aStrains involving go mutations constructed during this investigation are not listed here; see tables in text.

TABLE 2-4. Deletion mutants of T4

Strain name	Defective genes	Comments and reference for extent of deletion
<u>e</u> G19	<u>e</u> :IPIII:(?)	T4B; Wilson <u>et al.</u> , 1972
<u>e</u> G79	<u>e</u> :IPIII:(?)	T4B; Wilson <u>et al.</u> , 1972
<u>e</u> G223	<u>e</u>	T4B; Wilson <u>et al.</u> , 1972
<u>e</u> G298	<u>e</u> :IPII:IPIII:(?)	T4B; Wilson <u>et al.</u> , 1972
psu _b ⁻ Δ64	tRNA:(?)	T4B; Wilson <u>et al.</u> , 1972
psu _b ⁻ Δ33	tRNA:(?)	T4B; Wilson <u>et al.</u> , 1972
<u>r</u> EDdf41	<u>r</u> II:(?)	Kim and Davidson, 1974
<u>r</u> 1589	<u>r</u> II	Benzer, 1961
del(39-56)1: <u>r</u> 1589	(39-56): <u>r</u> II	T4B; ^a Homyk and Weil, 1974
del(39-56)3: <u>r</u> 1589	(39-56): <u>r</u> II	T4B; Homyk and Weil, 1974
del(39-56)4: <u>r</u> 1589	(39-56): <u>r</u> II	T4B; Homyk and Weil, 1974
del(39-56)5: <u>r</u> 1589	(39-56): <u>r</u> II	T4B, Homyk and Weil, 1974
del(39-56)12: <u>r</u> 1589	(39-56): <u>r</u> II	T4D; Homyk and Weil, 1974

^aThese strains were obtained and used prior to the transfer of genetic markers from T4B to T4D described in Homyk and Weil (1974).

TABLE 2-5. Non-T4 phage

Phage	Source, comments, reference
T2L	Caltech collection
T6	Caltech collection
RB69	Caltech collection, A T-even relative. Russell and Huskey, 1974
T3	Caltech collection
T5	Caltech collection
T7	Caltech collection
λ	Caltech collection
λ cI857	K. Kreuzer
λ dnaIA1	K. Kreuzer cI857; deleted genes include J and probably U but not N, O, S, R, Q
λ dubiG1	K. Kreuzer cI857; deleted genes include J and probably U but not N, O, S, R, Q
λ cIb ₂	C. P. Georgopoulos
λ eA30	C. P. Georgopoulos; spontaneously derived from λ cIb ₂
λ eB764	C. P. Georgopoulos; spontaneously derived from λ cIb ₂
f1	H. R. Revel; male-specific
M13	W. Wickner; male-specific
Plkc	H. R. Revel

Supplemented M9. Individual supplements in sterile aqueous solutions were added to M9 in the following quantities: amino acids, purines, and pyrimidines, 20-50 µg/ml; vitamins, 0.1 µg/ml; FeCl_3 , 10^{-5} M; sugars used in place of dextrose, 0.3%.

K medium. Casamino acids at a final concentration of 1.5% were added to M9.

EHA top agar. Bacto-tryptone, 13.0 g; Bacto agar, 6.5 g; NaCl, 8.0 g; Na citrate, 2.0 g; and dextrose, 3.0 g were added to H_2O , 1 liter.

EHA plates. The same recipe as for EHA top agar was used except that 10.0 g agar and 1.3 g dextrose were used per liter.

EMB-galactose plates. Bacto EMB agar base, 27.5 g; and yeast extract, 1.0 g were added to H_2O , 900 ml; after autoclaving, 100 ml sterile 10% D-galactose were added.

LB plates. The same recipe as for LB broth was used, plus 10.0 g Bacto agar per liter.

Tryptone top agar. Bacto-tryptone, 10.0 g; Bacto agar, 6.5 g; and NaCl, 5.0 g were added to H_2O , 1 liter. For growth of phage P1, CaCl_2 was added to 5×10^{-3} M.

Tryptone plates. The same recipe as for tryptone top agar was used, plus 10.0 g agar per liter.

M9 plates. The same recipe as for M9 was used, plus 10.0 g agar per liter.

Slant medium. Bacto-tryptone, 8.0 g; yeast extract, 1.0 g; NaCl, 5.0 g; and Bacto agar, 18.0 g were added to H_2O , 1 liter and the pH was adjusted to 7.4 with 12 drops 4% NaOH.

Stab medium. The same recipe as for slant medium was used, except that 6.5 g agar were used per liter.

Dilution medium (DM). Na_2HPO_4 , 7.0 g; KH_2PO_4 , 3.0 g; NaCl , 4.0 g; and gelatin, 0.5 g were added to H_2O , 1 liter. 1 ml sterile 1.0 M MgSO_4 was added after autoclaving.

Tris-maleate buffer (TM). Tris-HCl, 6.05 g; maleic acid, 5.8 g; $(\text{NH}_4)_2\text{SO}_4$, 1.0 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g; $\text{Ca}(\text{NO}_3)_2$, 5×10^{-3} g; and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 2.5×10^{-4} g were added to H_2O , 1 liter, and the pH was adjusted to 6.0 with NaOH.

Tris-magnesium buffer (TMg). pH 6.8 buffer was 0.05 M Tris-HCl, 0.02 M MgSO_4 ; pH 7.4 buffer was 0.01 M Tris-HCl, 0.02 M MgSO_4 . The pH's were adjusted with NaOH.

Additions to TMg buffer. DNase was added to a final concentration of 10 $\mu\text{g/ml}$ and RNase to 50 $\mu\text{g/ml}$.

Chemicals and enzymes. DN-CL deoxyribonuclease I, 5x recrystallized ribonuclease A, 3x recrystallized egg white lysozyme, PPO (2,5-diphenyl oxazole), nalidixic acid (1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid), crystalline vitamin B_{12} , D-serine, L-homocystine, TWEEN 40 (polyoxyethylene sorbitan monopalmitate), and DL- α -glycerophosphate were from Sigma Chemical Company. 2'-deoxyadenosine was from Sigma or Calbiochem. Streptomycin sulfate, U.S.P., was from Eli Lilly; Liquifluor from New England Nuclear; acrylamide and N,N'-methylenebisacrylamide from Eastman; TEMED (N,N,N,N'-tetramethylethylenediamine) was from Matheson, Coleman and Bell; oleic acid from Mallinkrodt; stearate and palmitate from Serdary Research Laboratories, London, Ontario, Canada; and nitrosoguanidine (N-methyl-N'-nitro-N-nitrosoguanidine) from Aldrich Chemical Company, Inc. All other chemicals were reagent grade.

Radioactive compounds. ^{14}C amino acids were reconstituted protein hydrolysate No. 3122-10 from Schwarz-Mann (individual amino acid specific activities 106-460 mCi/mmole, and concentrations 15-125 $\mu\text{Ci/mCi}$ of mixture) or $[\text{U-}^{14}\text{C}]$ protein hydrolysate (56 mCi/matom carbon) from Amersham. L-[4,5- ^3H] leucine (57 Ci/mmole) and $[2\text{-}^{14}\text{C}]$ thymidine (61 and 54.5 mCi/mmole) were from Amersham. $[2\text{-}^3\text{H(N)}]$ glycerol (200 mCi/mmol) and 2,6-diaminopimelic acid $[1,7\text{-}^{14}\text{C}]$ (105 mCi/mmol) were from New England Nuclear. ^{14}C leucine was 312 mCi/mmole.

II. Methods

A. Maintenance of bacteria

Bacterial strains were maintained on agar slants at 4°C and periodically transferred by streaking a loop of cells from one slant over the surface of a fresh slant and incubating overnight at 37°C. For some HD strains the transfer interval had to be as short as 2-3 months in order to maintain viability.

Long term (5⁺ years) storage of bacteria was on stabs at room temperature or in glycerol at -20°C. To make stab cultures, a loop of a freshly-grown culture of cells was stabbed into agar, incubated at 37°C overnight, and sealed with paraffin. For glycerol storage, overnight cultures of cells were made ~30% in glycerol and were placed in screw-capped vials at -20°C (Low, 1973).

Strains in use were inoculated from slants into H broth, grown with aeration at 37°C overnight, and stored at 4°C. These cultures were used as the source of inocula for experiments over the next 1-2 weeks, at which time fresh overnights were made. Overnight cultures were not serially transferred. Strains carrying an F-prime factor were grown in the appropriate selective medium to ensure plasmid retention.

B. Phage stocks

T4 stock preparation and plaque assays were as described by Adams (1959) and Stent (1963); stocks were usually started from single plaques. P1 and λ stocks were made on LB or T plates; cells to be infected with λ were grown in LB broth containing 0.3% maltose and those to be infected with P1 in LB broth containing 5×10^{-3} M CaCl_2 . P1 stocks were made as described by Miller (1972).

C. Mutagenesis of bacteria

Nitrosoguanidine mutagenesis under nongrowing conditions was essentially

as described by Adelberg et al. (1965). B178 was grown to 2×10^8 cells/ml, chilled, pelleted, rinsed 3x in 1 volume cold TM buffer and finally resuspended in cold TM at 2×10^9 cells/ml. 1.25 ml of concentrated bacteria (4°C) were mixed with 3.5 ml prewarmed TM (37°C) and 0.25 ml of a fresh aqueous solution of nitrosguanidine (2 mg/ml) and incubated at 37°C for 15'. Cells were then chilled, rinsed 3x with 2 volumes of cold TM and resuspended in 5.0 ml cold H broth. Subcultures were made at this point by inoculating 5.0 ml H broth with 0.1 ml of the mutagenized culture and growing overnight at 37°C .

D. Selection of host-defective mutants

The rationale of the selection procedure and the variations used in selections I, II, and III are discussed in the results (Chapter 3); here we present the empirically determined technical details. Subcultures of mutagenized cells (see Part C, above) were incubated at 37°C overnight and then diluted 1:25 and grown at 37°C to log phase (about 2.5 hours). 0.05 ml samples containing $\sim 10^6$ cells were spread on relatively dry EHA plates. The plates were incubated for two doubling times (60-90') at the desired selection temperature and then sprayed with 10^8 phage in a small volume (1 μl) using an aerosol sprayer and rotating the plate to ensure uniform coverage. After overnight incubation there were 50-100 surviving colonies per plate, indicating a mutant frequency of $\sim 10^{-4}$. Cells from these colonies were purified by streaking over the surface of a fresh EHA plate. No mutants were found when the selection procedure was applied to nonmutagenized cells.

E. Streak tests and spot tests

For rapid testing of phage growth on bacterial strains, streak tests or spot tests were done.

1. Streak tests

A streak test was used to screen presumptive host-defective mutants to distinguish them from resistant bacteria that fail to adsorb T4 or sensitive cells that managed to survive the selection procedure. About 10 μ l (2 loops) of two T4 phage stocks containing 10^8 particles/ml and 10^{11} particles/ml, respectively, were streaked across EHA plates and allowed to dry. Bacterial cultures ($\sim 4 \times 10^8$ cells/ml) were cross streaked separately over each phage streak and the plates were incubated overnight. Cell lysis or its absence in the streak overlap areas differentiates between sensitive, resistant, and host-defective cells: sensitive cells are lysed at both phage concentrations, resistant cells are not lysed at either concentration, and host-defective mutants are lysed at the high phage concentration but not at the low concentration.

Streak tests were also sometimes used to test colonies obtained in bacterial genetics experiments for the ability to grow phages T4, λ , and f1. These tests were like the streak tests described above except that the concentration of phage in the solution applied to the plate was 10^6 - 10^8 pfu/ml.

2. Spot tests

Small drops (~ 5 μ l) of phage solutions at concentrations of 10^8 , 10^6 , and 10^4 particles/ml were spotted on agar plates seeded with 10^8 cells of the bacterial strain to be tested, allowed to dry, and incubated overnight at the appropriate temperature. The test is semiquantitative: a sensitive host will show complete lysis at the two higher phage concentrations and about 40-50 plaques on the spot from 10^4 phage/ml. Tight host-defective strains show only a slightly turbid area at the highest phage concentration, indicative of cell killing. Host strains of intermediate defectiveness give plaques on the spots from phage concentrations of 10^8 phage/ml and/or 10^6 phage/ml.

F. go phage classification of E. coli host-defective mutants

Bacterial mutants that were sensitive to $T4^+$ but unable to support its growth (determined by streak tests, see Part E1, above) were classified according to the results of spot tests on them of a "standard" set of T4 mutants. These mutants were obtained by plating 10^6 - 10^8 $T4^+$ on various host-defective bacteria and picking and purifying the resulting large plaques. The mutants are called go (grows on) followed by a classification letter and a mutant number. goA-1 was selected on HDAD01, goC-1 on HDC013, goD-1 on HDD018, and goF-1 on HDF026. Bacterial mutants that gave similar spot test patterns were classified together.

G. Mutagenesis of T4

3 ml $T4^+$ at 1.4×10^{10} /ml in DM were placed in a petri dish and exposed to ultraviolet radiation for 0.2 minutes at 36 cm from a germicidal lamp previously calibrated to deliver 5 lethal hits/min/phage particle at that distance. Mutants obtained in this manner were backcrossed 3x to wild type T4 at a low ratio of mutant to $T4^+$ phage in order to remove possible extraneous mutations.

H. Standard methods for growth and assay of bacteria and phage

The following procedures were standard: experiments were performed in H broth at 37°C; phage and bacteria were plated on EHA plates with EHA top agar and incubated overnight at 37°C except that phage carrying amber mutations were incubated at 30°C; cells from overnight cultures were inoculated 1:100-1:1000 into H broth and grown with aeration by bubbling at 37°C; infections were at a multiplicity of infection (moi) of five phage per cell (surviving bacteria were generally <1%); superinfections at moi=5 were 8' after infection at 37°C

or 17-20' after infection at 25°C; and infected cultures to be spun down were moved, still under aeration, to ice-water slurries 5-10' prior to centrifugation. Centrifugation was at 2500-4000 x g for 5-10' at 4°C. Phage used in labeling experiments were pelleted by centrifugation at 16,000 x g for 60' and resuspended in DM. Optical density measurements were made in a Zeiss PMQ II spectrophotometer.

I. Burst size determinations

Cells were either grown to 5×10^7 /ml and concentrated to 4×10^8 /ml by centrifugation or grown to $2-4 \times 10^8$ /ml. Phage were added to cells which had just been made 0.004 M in KCN. After five minutes of adsorption, anti-T4 serum was added to a final k of 2 min^{-1} and five minutes later the infected cells were diluted 1:40,000 into prewarmed H broth at 37°C (growth tube). At this point samples were taken to determine the number of infected cells (by plating on permissive bacteria), surviving bacteria (measured as colony formers), and phage which failed to adsorb to cells or be killed by antiserum (samples shaken with a few drops of CHCl_3 and plated under permissive conditions). A few drops of CHCl_3 were added to the growth tube at $t=60'$ (42°C), $t=60-100'$ (37°C), or $t=120'$ (25°C) and the tubes were vortexed. Progeny phage were plated under permissive conditions and the burst size was calculated as progeny phage per ml $\div$ (input bacteria/ml - survivors/ml). For wild-type cells the number of infected bacteria usually equalled input bacteria/ml - survivors/ml.

J. Intracellular phage growth curves

The same procedure was used as for burst size determinations except that samples of the growth tube were taken throughout the latent period, added to a few drops of CHCl_3 , vortexed, and plated on a permissive host.

K. Measurement of infected-cell lysis

Cells were grown to $2-4 \times 10^8$ /ml and infected under standard conditions. Samples were taken at various time intervals and their optical density at 660 nm was measured. In some cases, lysis was observed visually (Epstein et al., 1963).

L. Measurement of phage DNA synthesis

Bacterial cells were grown in K medium or H broth to 1×10^8 /ml and concentrated to 2×10^8 /ml in the same medium. A 10-ml flask containing 2 ml of cells was placed in a shaking water bath at 37°C at $t=-2'$. At $t=0$ phage were added at $\text{moi}=6-8$ and three minutes later deoxyadenosine at a final concentration of $200 \mu\text{g/ml}$ and ^{14}C thymidine at a final concentration of $6 \mu\text{g/ml}$ and a specific activity of $5-10 \mu\text{Ci}/\mu\text{mole}$ were added. At various times 0.1 ml samples were pipetted into 2 ml ice-cold 5% TCA containing $50 \mu\text{g/ml}$ cold thymidine. After 30' or more at 0°C the samples were filtered through Whatman GF/A glass filters, the filters were washed with 4 volumes 5% TCA containing thymidine followed by 2 volumes 95% ethanol, dried, and counted in 5 ml toluene-Liquifluor scintillation fluid.

M. Labeling of extracts for gel electrophoresis

1. Labeling with ^{14}C amino acids

Cells were grown in M9 (supplemented if necessary) to 5×10^7 /ml and concentrated to 4×10^8 /ml; 2.0 ml cultures were used and were always super-infected. ^{14}C amino acids were added to a final concentration of $1-2 \mu\text{Ci/ml}$ to infected cultures at the desired time. After the labeling period, cultures were usually chased for at least one minute by the addition of 0.5 ml of 10% casamino acids. Chilled cells were pelleted and then resuspended in 0.1 ml of

TMg + DNase, pH 6.8, and frozen in a dry ice-ethanol bath. Upon thawing, the preparation was made 2% in SDS and β -mercaptoethanol and was boiled for 1-3 minutes. Incorporated radioactivity was determined by TCA precipitation of a 2 μ l portion, and samples containing equal numbers of counts (and equal volumes when identical labeling conditions were used) were loaded onto gels.

2. Labeling with ^3H leucine

Cells were grown to 8×10^7 /ml in M9 containing purines, pyrimidines, thiamine, pyridoxine, FeCl_3 , and all amino acids, concentrated by centrifugation, and resuspended at 4×10^8 /ml in the same medium minus leucine. ^3H leucine was added at a final concentration of 10 $\mu\text{Ci/ml}$ at 15', 25', and 27' after infection, the cells were chilled at $t=35'$, spun, resuspended in 150 μl TMg pH 6.8 + DNase, and treated as in Part M1, above.

N. SDS-polyacrylamide gel electrophoresis

The gel system of Laemmli (1970) was used in a slab gel apparatus similar to that of Studier (1974). For details of gel preparation, see plate legends. Gels were run at a constant current of 10 milliamps until the bromphenol blue marker dye entered the separating gel, and then at 20 milliamps until the marker dye reached the bottom of the gel. Occasionally the dye was allowed to run off the bottom of the gel to achieve better separation of higher molecular weight proteins. Gels were fixed for several hours in 10% acetic acid:10% methanol and dried on Whatman 3MM chromatography paper (0.33 mm thickness) under a vacuum with external heating and exposed to Kodak No-Screen Medical X-Ray film. Gels with 100,000 TCA-precipitable counts per minute per sample were exposed ~ 3 days. Films were developed in Kodak D-19 developer for 5' and fixed for 10' in Rapid Fix.

O. Fluorography

Fluorography of gels containing ^3H -labeled samples was according to Bonner and Laskey (1974). Gels with 300,000 cpm/sample were exposed to Kodak RP Royal X-Omat Medical X-Ray film at 4°C for 10 days or -70°C for 24-48 hours.

P. Electron microscopy

1. Preparation of grids for negative staining

A thick layer of carbon was deposited on freshly cleaved mica and floated onto a clean water surface; 400-mesh copper grids held in forceps were then brought up under water through the film and were gently blotted dry from below.

2. Preparation of samples and negative staining

Cells were grown to $5 \times 10^7/\text{ml}$, concentrated to $4 \times 10^8/\text{ml}$, infected, superinfected, moved to an ice bath at 33' after infection, spun, resuspended in 1/10 volume TMg pH 7.4 containing DNase and RNase, frozen at -70°C and thawed once to achieve lysis. A drop of a 1:10-1:100 dilution of sample was mixed with a drop of 0.5-1.0% uranyl acetate, placed for 60" on a carbon-coated copper grid, and then flicked off. The grid was rinsed with two drops of water for 30" each and excess liquid was removed by touching the edge of the grid to filter paper. After air drying, grids were examined in a Phillips 201 electron microscope at 60-80 KV. Eastman Fine Grain Release Positive film No. 5302, not perforated, was used for photography.

3. Thin sections

The procedure is essentially that of Simon (1972) except that samples were embedded in epon (Luft, 1961). Cells were infected, superinfected, and moved to an ice bath at 33' after infection. One ml 2.5% glutaraldehyde in 0.06 M

cacodylate buffer, pH 7.2, was immediately added to 4 ml cells and the mixture was aerated for 5', allowed to stand at 4°C for 40', and then centrifuged. The cells were post-fixed according to Simon and Anderson (1967) and Kellenberger et al. (1958) at 4°C overnight in 1% OsO₄ + 0.5% uranyl acetate in veronal acetate buffer, and dehydrated at room temperature in 20' steps through 40%, 60%, 80%, and 3 x 100% acetone. The samples were embedded in epon [46 parts epon 812, 28 parts dodecenyl succinic anhydride, 25 parts nadic methyl anhydride, 2% (2,4,6-tri(dimethylaminomethyl)phenol)] by incubating them at room temperature in a mixture of 1 volume of acetone plus 1 volume epon for 20', adding 1 volume of epon and incubating for a further 20', replacing the epon-acetone mixture with 100% epon and incubating at room temperature overnight, and placing in epon in a gelatin capsule at 70°C for at least 8 hours. Sections were cut with a glass knife on a Reichert Om U2 microtome and collected onto carbon-coated formvar copper grids. Grids were stained with 2% aqueous uranyl acetate for 11 minutes followed by three 5' washes with water. Grids were then blotted dry on filter paper, rinsed once more with water (5'), further stained with lead citrate (Reynolds, 1963) for three minutes, dipped in water, rinsed twice for 5' with water, blotted, rinsed for 1' with water, blotted, and air dried.

Q. Assay of phage components by in vitro complementation
in mixed extracts

1. Phage heads, tail-baseplates, and tail fibers

Cells were grown to 4×10^8 /ml with vigorous aeration, infected at moi=7, superinfected at the same moi, chilled at t=35' and pelleted at 45'. The pellets from a 250-ml culture were resuspended in 0.5-1.0 ml TMg + DNase, pH 7.4, and frozen (-70°C) and thawed once. 20 µl samples were mixed at 4°C

with either 20 μ l TMg or 20 μ l of a different extract, incubated at 30°C for a minimum of 2 hours, and assayed for phage.

2. Whole baseplates and tail-baseplate gene products

Extracts for baseplate and tail-baseplate gene product complementation reactions were 5-10x more concentrated than those used in Part Q1, above, and were prepared as described by Kikuchi and King (1975a) with the following modifications. 250-ml cultures of E. coli strain Bb were infected (and superinfected in cases where no t mutation was present) at moi=7; HDF cell extracts were made at 37°C and chilled at 35' after infection; and 20 μ l DNase and 40-60 μ l TMg buffer, pH 7.4, were vortexed with the cell pellets prior to freezing (rather than during thawing). The reactions were set up and conducted as in Part Q1, above.

R. Tail fiber antigen determinations

The end-point serum blocking assay described by Ward et al. (1970) was used except that twofold dilutions of the sample were made in the serum and 2500 tester phage were added.

S. Temperature shift experiments

Cells were grown to 5×10^7 /ml and concentrated to 4×10^8 /ml. KCN, phage, and antiserum were added as under Part I, above, and five minutes after the addition of antiserum cultures were diluted 200x into H broth prewarmed at the pre-shift temperature (t=0). At the desired times a further 200x dilution was made into H broth prewarmed at the post-shift temperature. CHCl_3 was added to the latter tubes at t=140' and phage were assayed on a permissive host.

T. Phage genetics

1. Phage crosses

Phage crosses, except as noted, were performed as described by Wilson and Kells (1972). Special conditions used to distinguish recombinant progeny are described in the appropriate table legends. Sterile pins, first dipped in a 1:1 mixture of glycerol and food coloring for ease in seeing which plaques had been sampled, were used for stabbing plaques.

2. Construction of go:amber mutants

Doubly mutant phage strains containing a go mutation and an amber mutation were constructed by crossing the appropriate phage strains and selecting progeny that would plate on HDsup bacteria but not on HDsup⁺. Both HDsupD and HDsupE strains were used; HDsupD bacteria were obtained by introducing the F' plasmid F196 carrying supD32 into HDsup⁺ strains, and HDsupE bacteria were obtained by P1kc transduction of supE from E. coli strain C600. In the latter case galE was the selected marker; supE and galE show ~1% cotransduction.

U. Bacterial growth curves

Cells were grown to 4×10^8 /ml and diluted 1:100 into fresh H broth. Samples were removed at ~20' intervals for measurement of optical density at 660 nm and for determination of colony formers by dilution, plating, and incubation overnight.

V. Assay of bacterial protein synthesis

Cells were grown to 8×10^7 /ml in M9 plus all amino acids, thiamine, FeCl₃, and thymine, concentrated to 4×10^8 /ml in the same medium minus leucine,

and diluted 40x into the supplemented medium minus leucine and containing 2 $\mu\text{Ci/ml}$ ^{14}C leucine. 40 μl samples were taken every 15' and added to 2 ml ice cold 10% TCA containing 50 $\mu\text{g/ml}$ leucine. After at least 30', samples were treated as described under Part L, above.

W. Assay of bacterial DNA synthesis

Cells were grown to $4 \times 10^8/\text{ml}$ and diluted 40x into prewarmed H broth containing 100 $\mu\text{g/ml}$ deoxyadenosine and 1 $\mu\text{Ci/ml}$ ^{14}C thymidine. 50 μl samples were added to 2 ml ice cold 10% TCA containing 50 $\mu\text{g/ml}$ thymidine and treated as in Part L, above.

X. Assay of bacterial cell wall synthesis

Cells were grown to $4 \times 10^8/\text{ml}$ and diluted 40x into prewarmed M9 containing 0.05% casamino acids, thiamine, FeCl_3 , and 2 $\mu\text{Ci/ml}$ ^{14}C diamino-pimelic acid. 50 μl samples were taken at the desired times into 2 ml ice cold 10% TCA and treated as in Part L, above.

Y. Assay of bacterial phospholipid synthesis

The procedure is a modification of methods described by Mindich et al. (1976), Goldfine (1969), and Radin (1969). Cells were grown to $8 \times 10^7/\text{ml}$ in M9 plus 0.05% casamino acids, thiamine, FeCl_3 , and 20 $\mu\text{g/ml}$ glycerol, concentrated to $4 \times 10^8/\text{ml}$ and diluted 40x into fresh prewarmed medium without glycerol and containing 25 $\mu\text{Ci/ml}$ ^3H glycerol. 20 μl samples were taken in duplicate every 15', applied to Whatman GF/C glass fiber filters, and plunged into 5 ml ice cold 10% TCA. After 30' the 10% TCA was decanted and replaced by 5% TCA for 15' and then by two 30' rinses of cold water. One set of samples was

then dried and counted; the second was rinsed 3x with 3 ml of 2:1 chloroform:methanol prior to drying and counting. Incorporation of glycerol into phospholipid was determined by subtracting the radioactivity in the chloroform:methanol extracted samples from that in the unextracted samples.

Z. E. coli genetics

1. Mapping HDF strains with Hfr's

The Hfr rapid mapping technique was that described by Low (1973), using ts⁺ as the selected phenotype, except that instead of replica plating, log phase cultures of Hfr's were spotted onto HDF Str^R-seeded plates with a pasteur pipette and allowed to dry. Streptomycin was applied either by spraying or by allowing drops of a 20-200 µg/ml solution to fall from a pasteur pipette on top of the dried Hfr spots. Cells were picked from spots showing bacterial growth after incubation at 42°C or 45°C overnight and tested by spot or streak tests for f1 and T4 phage growth and colony color on EMB-galactose plates (gal⁺ colonies are purple, gal⁻ colonies, pink). Hfr's were f1^S, T4^S, and most were purple; HDF strains were f1^R, T4^{"R"}*, pink, and recombinants were f1^S, T4^S, pink. The Hfr strains used are those mentioned by Low (1973) and were obtained from Dr. B. Bachmann at the E. coli Genetic Stock Center, Yale University. Only those which either transferred hdf⁺ under the conditions used for rapid mapping or whose failure to do so increased the resolution of the position of the hdf locus are listed in Table 2-1.

2. Mapping with F' plasmids

F' plasmid-containing strains are listed in Table 2-1. Cells were grown

* Means sensitive to T4⁺ but unable to support its growth. Such strains look like true T4^R bacteria on spot and streak tests unless high concentrations of phage (10⁸-10¹⁰/ml) are used, at which point cell killing becomes visible.

to 2×10^8 /ml either conventionally with aeration or more anaerobically in para-film-sealed test tubes in order to obtain cells more efficient at plasmid transfer (Curtiss et al., 1969). Matings were carried out at a ratio of 10 donor cells to 1 recipient (aerobically grown to 2×10^8 cells/ml) for 60' without agitation. The culture was then diluted $\times 10^5$ and plated on minimal plates to select against the multiply auxotrophic donors. Single colonies were purified by restreaking on minimal plates and were tested for f1 or M13 and T4 phage growth and colony color on EMB-galactose plates.

3. Transductions of HDF strains with defective λ phage

Phage strains used are listed in Table 2-4. The defective λ transducing phages, λ dnalA1 and λ dubiG1, kindly provided by K. Kreuzer and N. Cozzarelli, carry the ts repressor mutation cI857 and were supplied as double lysogens with a cI857-containing helper phage. Stocks of 10^{10} pfu/ml were made by shifting doubly lysogenic cells grown to 2×10^8 /ml in H broth at 30°C to 42°C for 30' and then incubating at 37°C until lysis. CHCl_3 was then added to kill any surviving bacteria. Because HDF strains had to be tested at 37°C for T4 growth and because at this temperature the cI857 mutation allows λ to induce and kill the host, transductions were performed by coinfecting HDF cells grown in LB broth + 0.3% maltose to 2×10^8 /ml with the induction mixture (λ dnalA1 or λ dubiG1 plus λ cI857 helper phage) at moi=1-3 pfu plus wild-type λ at moi=3-5. Phage adsorption was allowed for 10' at 37°C and then cultures were diluted and plated for single colonies on EHA plates at 37°C. Colonies arising after overnight incubation were restreaked on EHA plates at 37°C and tested for the ability to grow T4^+ .

4. P1 transductions

Bacterial strains used are listed in Table 2-1. Transductions using phage P1_{kc} were according to Miller (1972) or Rothman (1965). In most cases at least one marker other than that selected for was tested to ensure that colonies examined were not revertants or contaminants. f_{pk}⁺ cells were selected by spreading P1-infected f_{pk}⁻ cells on M9 plates containing 0.3% D-fructose as the carbon source (Ferenci and Kornberg, 1973); dsd⁺ cells were selected on M9 plates containing 500 µg/ml D-serine (McFall, 1964); nalA⁻ transductants were selected as described by Hane and Wood (1969); glpT⁺ cells were selected on M9 plates containing 0.9% DL-α-glycerophosphate as the carbon source (Cozzarelli et al., 1968); and ubiG⁺ transductants were selected for by their ability to grow on M9 plates containing 30 mM sodium succinate as the carbon source (Stroobant et al., 1972). Plates for transductions of wild-type alleles into strains auxotrophic for amino acids, purines, or pyrimidines lacked the appropriate compound.

AA. Abbreviations and terminology

am, amber; cpm, counts per minute; cs, cold-sensitive; del, deletion; DNase, deoxyribonuclease; EDTA, ethylenediaminetetraacetic acid; eop, efficiency of plating; kb, kilobase; moi, multiplicity of infection; MW, molecular weight; pfu, plaque forming unit; PPO, 2,5-diphenyl oxazole; RNase, ribonuclease; SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid; ts, temperature-sensitive; UV, ultraviolet light.

For experiments involving phage infection, t=0 is the time when the infection starts and all other times are measured from that instant.

The product of a particular gene is denoted by "gp" ("gene product") followed by the name of the gene, as in gp7, gpwac. A phage carrying a mutation

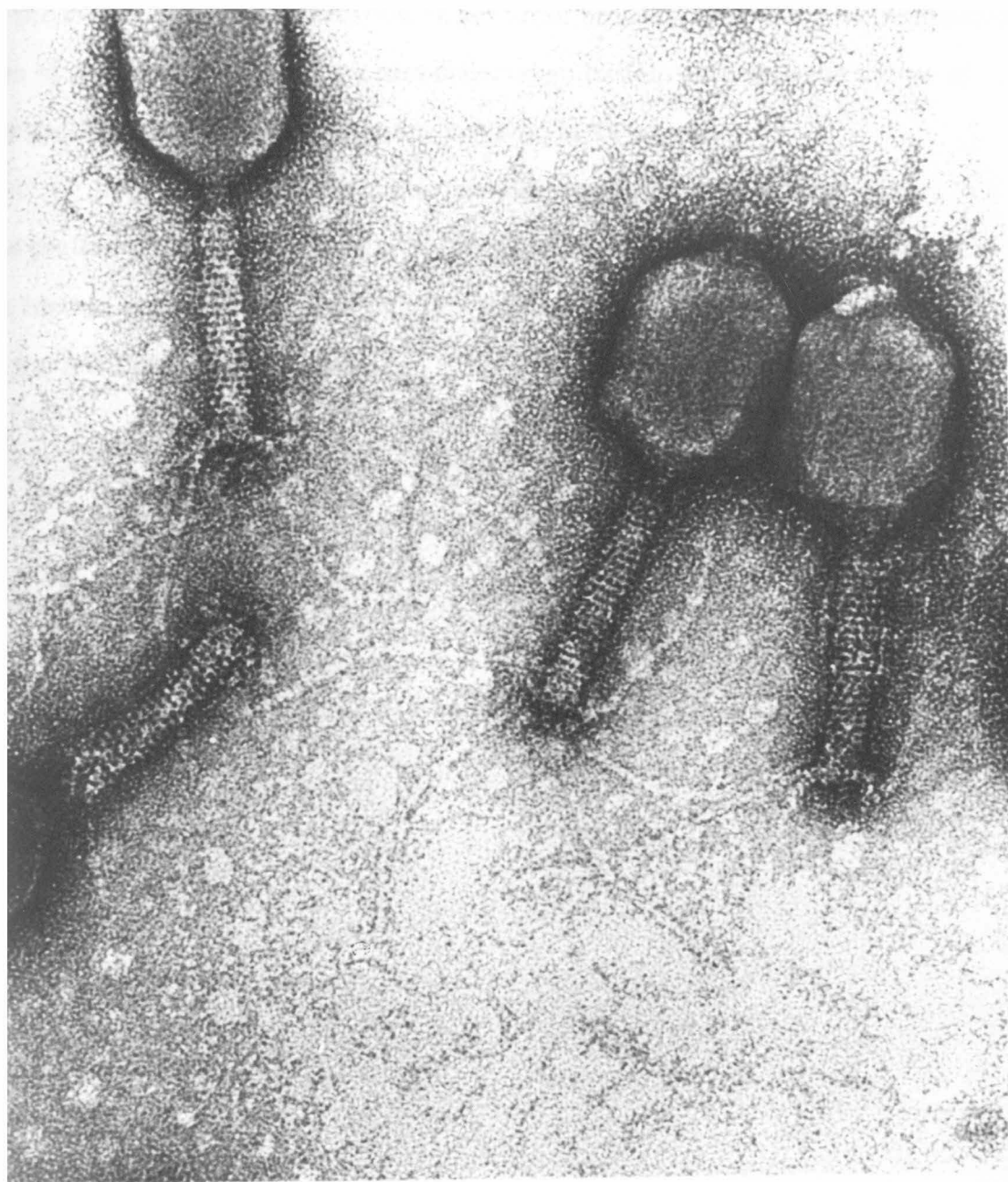
in gene 7 is called a 7⁻ strain. Multiple mutations in the same phage strain are written as, 7:wac. A particular mutation in a gene is noted in parentheses following the name of that gene, as in 7 (amB16) or 7:wac (amB16:amE727J). The gene name and the mutation name may be reversed when the particular mutation used is of primary importance, as in amB16(7⁻). A protein of known molecular weight (for example, 43,000 daltons) but which has not been identified as the product of a particular gene is referred to as "p" followed by the molecular weight, as in p43,000. Host-defective bacterial strains are called HD followed by a classification letter, a subculture number, and an isolate number, as in HDB45. Phage that can grow on HD strains are called go and, except for the standard go phages (see Part F, above), are named after the bacterium on which they were selected, as in goB45. The term go refers to phage strains and to mutations; we leave those presently unidentified genes in which go mutations lie unnamed until such time as their functions are better understood. The host mutation responsible for the HD phenotype is designated by mop (morphogenesis of phage) for cells in which the T4 heads fail to assemble, and hdf (host defective) for mutants mapping near nalA that have a pleiotropic effect on T4⁺ development.

Chapter 3

ISOLATION AND CHARACTERIZATION OF HOST-DEFECTIVE MUTANTS
OF E. COLI K-12 BLOCKED IN T4⁺ ASSEMBLYI. Introduction

The T-even phages (T2, T4, and T6) are large nucleoprotein aggregates consisting of an elongated icosohedral head attached to a contractile tail (Plate 3-1). The distal end of the tail is fixed to a complex baseplate from which extend six adsorption organelles, the tail fibers. The morphogenesis of T4 has been found to proceed in a step-wise fashion by the subassembly of heads, tail-baseplates, and fibers, all of which are subsequently joined in sequence to form active phage particles (Wood et al., 1968). Much has been learned about the specific sequence of gene product interactions in the construction of the phage particles, and it has been found that most of the assembly steps can proceed in vitro. However, the earliest steps in the subassembly of heads, baseplates, and fibers have not been demonstrated in vitro (Kikuchi and King, 1975b; Showe and Kellenberger, 1975). Consequently, we wondered if the host cell might play an obligatory role in initiating assembly. It is known that T4 (and viruses in general) utilizes much of the existing host cell machinery, specifically redirecting it to make virus particles. Perhaps the host supplies scaffolding, templates, or other components essential to initiate assembly processes. Electron microscopic evidence suggests that the first steps in the assembly of T4 heads, and perhaps that of baseplates, proceed on the host membrane (Simon, 1969, 1972). We have sought to investigate this problem by the isolation of host mutants specifically blocked in T4 assembly. By analysis of the defective phenotype of T4⁺ infection of these mutants with the electron microscope, in vitro complementation experiments, and SDS gel

Plate 3-1. Phage T2. Phage at a concentration of $\sim 10^{10}$ pfu/ml were negatively stained and photographed in the electron microscope as described in Materials and Methods, Part P. Magnification, $\sim 330,000\times$.



electrophoresis, and by genetic studies on the nature of phage mutants that can overcome the assembly block, we hoped to identify the altered step. An analysis of the effect of the host mutation on bacterial processes and the genetic identification of the host mutation were also undertaken to help elucidate the nature of the specific phage-host interactions involved in T4 assembly.

In Section II of this chapter we discuss the isolation, classification, and preliminary characterization of 605 host-defective mutants; in Section III we further characterize those mutants that unambiguously blocked T4⁺ growth at the level of virion assembly.

II. Isolation and preliminary characterization of host-defective mutants

A. An overview

After obtaining bacterial mutants that allowed $T4^+$ adsorption and DNA injection but that failed to support the production of progeny phage (Section IIB), a rapid streak test for T4 killing was used to distinguish host-defective from resistant cells. Since we clearly could not investigate all 605 isolates in equal detail, we developed a way to classify the strains (Section IID2) and then further screened representatives of each class for blocks to $T4^+$ growth at the level of phage particle assembly (Section IID6). Those strains that clearly passed the screening tests are characterized in more detail in Section III of this chapter; those that clearly failed were not further investigated. A study of the mutants that gave ambiguous results in the screening tests is presented in Chapter 4. The 605 host-defective mutants were isolated using three slightly different selection criteria. The choice of agents for the second and third selections was based on the results obtained in the first selection. The 279 strains from selection I are discussed together, followed by a section in which the isolates from selections II and III are considered.

B. Clonal selection of host-defective mutants

Our working premise was that host mutants blocked in T4 assembly would adsorb T4 normally and be killed. The phage should be able to carry out all early functions as usual, and also be able to synthesize all virion structural proteins, but fail to assemble them properly. Lysis of the infected cells should occur at the normal time without the release of infectious progeny.

Upon consideration of this expected phenotype, we found that we lacked a way to select specifically for host mutants blocked at the level of T4 assembly. We therefore used a more general procedure that allows any cell clone unable to grow $T4^+$ to survive, and then screened representative mutants for normalcy of phage DNA synthesis and infected-cell lysis.

A selection procedure involving a frontal attack in which all cells of a culture are infected obviously would not work because the desired mutants are still T4-sensitive. Our selection conditions therefore depend on immobilizing bacterial cells by spreading them on an agar surface and allowing microcolony formation prior to challenge with phage. When the outermost cells of colonies composed of wild-type bacteria are infected, the virus propagates and progeny particles infect sensitive cells in the immediate vicinity. After one or more such infection cycles all the cells in these microcolonies have been infected, so that these clones are eliminated. On the other hand, when the microcolony is comprised of host-defective cells, only those outermost cells that come in contact with a selecting phage are killed. These infected cells produce no progeny virus, so the inner cells of such host-defective clones escape infection and continue to multiply, forming a visible colony. In preliminary reconstruction experiments, we explored the effects of varying phage and bacterial concentrations and the optimum time of preincubation prior to phage challenge, and arrived empirically at the procedure described in detail in the Materials and Methods (Part D).*

* groEA44 (Georgopoulos et al., 1972) was the host-defective mutant used in these reconstruction experiments. It and groEB515 are nitrosoguanidine-induced derivatives of B178 that were selected for their inability to grow phage λ (Georgopoulos et al., 1972, 1973) and also fail to support the growth of $T4^+$ and $T4\epsilon$, respectively. $T4\epsilon$ is a go phage selected by plating $T4^+$ on groEA44 (Georgopoulos et al., 1972).

C. Choice of parental bacterium and phage used as selection agents

At least three characteristics were considered desirable in the parent strain from which host-defective mutants were to be selected. The bacterium should be an F^- , K-12 strain to facilitate genetic studies of the mutants, and it should not carry a nonsense suppressor. The latter characteristic would permit genetic crosses and complementation tests of standard T4 amber mutants with potential phage mutants that could overcome the host block, and thus facilitate identification of the defective step. B178 has all these characteristics, and in addition it adsorbs T4 better than do other K-12 strains. B178 is also galE, and in other experiments we found that galE strains give rise to spontaneous T4-resistant derivatives at a lower frequency ($<10^{-8}$) than galE⁺ strains ($\sim 10^{-6}$). Since resistant cells were expected to survive our selection procedure, the galE mutation was desirable. Finally, B178 has an RglI⁻ phenotype (Revel, 1967; Revel and Luria, 1970)* which proved to be useful in eliminating the galU mutant clones that could be selected (Mathews, 1970; Hewlett and Mathews, 1973). galU host mutants have a defective UDPG pyrophosphorylase and fail to supply the glucose required by phage-induced glucosyl transferases to glucosylate T-even DNA. Since T4 phage with nonglucosylated DNA are restricted by the host rgl restriction system (rglA⁺ + rglB⁺) (Revel and Luria, 1970), the selecting phage would get

* In Revel (1967) and Revel and Luria (1970), the genes of K-12 that control the restriction of T-even phages with nonglucosylated DNA were designated r6⁻ and r24⁻. To be consistent with the guidelines for naming bacterial mutant loci (Demerec et al., 1966), the former designations have been changed as follows (Revel, personal communication).

Former notation

r6⁻
r24⁻

Present notation

Genotype	Phenotype
<u>rglA</u>	RglI
<u>rglB</u>	RglII

through only one infection cycle and clones of galU⁻ cells would therefore not be eliminated. Bacterial cells with an RglI⁻ phenotype restrict nonglucosylated T4 phage but not nonglucosylated T6. Since T4 and T6 are closely related phages and had been shown to behave identically in the one host-defective strain studied before the present work was initiated (Georgopoulos et al., 1972), we reasoned that a mixture of T4 and T6 as selecting agents would yield the same type of host-defective mutants as would T4 used alone, and in addition the T6 phage would eliminate the galU mutants. Furthermore, since the two viruses adsorb to different components of the bacterial surface (Jesaitis and Goebel, 1952, 1953), T6 would eliminate cells resistant to T4 that arose as a result of mutagenesis (and vice versa). Reconstruction experiments with strain U95 galURglI⁻ (Shedlovsky and Brenner, 1963) verified this hypothesis. Parenthetically, the 29 surviving clones purified from the O subculture (O for Original subculture) turned out to be resistant to T6 because of an inadvertent contamination of this subculture with T6 phage. Seven of these host-defective strains (or 25%) failed to give rise to go phage mutants. Further investigation revealed that T4⁺ could grow in a single cycle on these strains to produce progeny, but the progeny could only grow on permissive hosts that lack the complete rgl restriction system. Thus 25% of the host-defective mutants in this subculture were galU mutants.

D. Selection I

1. Identification of host-defective mutants

Mutagenized B178 cells from 26 subcultures were spread on agar plates, challenged with a mixture of T4 and T6 phages, and incubated overnight at 37°C or 42°C. Cells from 29 surviving clones of the O subculture and from 10 surviving clones each of subcultures Nos. 1-25 were purified and tested by streak test

at 30, 37, and 42°C to distinguish host-defective cells from resistant or sensitive cells that might have survived. All 279 proved to be host-defective: they adsorbed T4⁺ and were killed but failed to produce progeny phage. These strains are called HDX01 through HDX029 and HDX10 through HDX259, where "X" is a classification letter (see Subpart 2b, below).

2. Classification of HD strains by the plating properties of go mutant phages

a) Theory. Host-defective strains probably block T4⁺ growth by disrupting specific phage-host interactions at various points throughout T4 development, from initial adsorption to final cell lysis. Mutant phages that can overcome or compensate for the host defect should be equally specific. It thus seemed likely that go phage mutants (go is an acronym for "grows on") might be useful for identifying similar host defects, and thereby classifying the host-defective strains.

b) Selection and use of standard go phages. We obtained the first four standard go phages by plating high concentrations of T4⁺ on all HD strains of the O subculture and plaque-purifying phage isolated from resulting large plaques (frequency, 10⁻⁶-10⁻⁷). The purified phages were then analyzed by spot test on all 29 HD strains from subculture O. From their plating patterns we identified four different go phages which we call standard go phages: goA-1 (selected on HDAD01), goC-1 (selected on HDC013), goD-1 (selected on HDD018), and goF-1 (selected on HDF026).

The plating properties of the standard go phages define at least six classes of HD strains among the mutants isolated in selection I. Table 3-1 shows the plating patterns of the go phages on 75 independent HD strains. The majority

Table 3-1. Classification of host-defective mutants by plating properties of T4 go phages. Host-defective mutants were classified by their ability to grow the four standard go phages as determined by spot tests (see Materials and Methods, Part E2) at 37°C and 42°C. The symbols used in the table are as follows: o, no phage growth; +, phage growth; +sm, phage plaques are small (eop $\sim$ 1.0); $\pm$, leaky phage growth (eop $\sim$ 0.1); (+), phage growth better than T4⁺ by a factor 100-1000 (eop $\sim$ 10⁻²-10⁻³; i.e., 5-20 plaques on a spot from a solution of phage at 10⁶ particles/ml).

TABLE 3-1. Classification of host defective mutants by plating properties of T4 go phage

Class	HD strains (Independent mutants ^a)	Growth of phage					
		T4 ⁺	<u>go</u> A-1	<u>go</u> C-1	<u>go</u> D-1	<u>go</u> F-1	T4 ϵ
parent	B178	+	+	+	+	+	+
A	175	0	+	0	0	0	0
AD	01, 11, 21 + 47 others	0	+	0	+ sm	0	+ sm
B	15, 90, 173	0	(+)	0	0	0	0
	45, 84	0	0	0	(+)	0	(+)
C	013, 184	$\pm$	$\pm$	+	$\pm$	$\pm$	$\pm$
D	36, 43, 71	0	0	0	+	0	+
43°	{018, 98, 105} 122, 259}	0	0	0	+	0	0
F	026, 125, 183	0	0	0	0	+	0
<u>gro</u> EA44		0	0	0	+	0	+
<u>gro</u> EB515		+	+	+	0	+	0

^aMutants derived from the same subculture were considered to be independent if they showed different plating patterns. We claim that there are 50 independent mutants of the AD class derived from 26 subcultures on the basis that Class AD mutants isolated at 42°C proved to be T4⁺-sensitive at 37°C, suggesting an independent origin from Class AD mutants isolated as defective for T4⁺ growth at 37°C.

of the host-defective mutants were classified as AD because they allowed both goA-1 and goD-1 to grow. D strains, defective for $T4^+$ growth at either 37°C or 42°C, could support the growth of only goD-1. The A, C and F Classes are represented by 1, 2, and 3 strains, respectively, that can grow only the corresponding go phage mutant. HD strains classified in the B group failed to grow any go phage with an eop of 1.0 but allowed either goA-1 or goD-1 to grow better than $T4^+$.

c) Comparison of goD-1 and $T4\epsilon$. Data for $T4\epsilon$ and bacterial strains groEA44 and groEB515 are included in Table 3-1 for comparison. goD-1 exhibits the same plating pattern as $T4\epsilon$ on the HD strains and on the two groE strains, but differs from $T4\epsilon$ in one respect: goD-1 grows on B178 and D class strains at 42°C whereas $T4\epsilon$ does not. The plating patterns summarized in Table 3-1 suggest that $T4^+$ development in D strains and possibly AD strains may be blocked at the same step as in groEA44, where extensive studies have shown that $T4^+$ infection is interrupted early in T4 head assembly at the level of gene 31 function (Georgopoulos et al., 1972).

3. Growth of other phages on host-defective mutants

We have investigated the ability of other phages to grow on HD strains from selection I. T2 and T6 behaved like $T4^+$. T3, T5, T7 and P1 grew in all strains with one exception: T5 failed to grow on HDB173. λ_{cIb_2} grew on most HD strains. Three exceptions should be noted: 1) strains HDB45, HDB84 cs^+ (see Footnote [a], Table 3-3), HDB173, and HDD43 inhibited the growth of λ_{cIb_2} but allowed the growth of the λ mutant $\lambda_{\epsilon A30}$. 2) the Class C strains grow λ_{cIb_2} but not the λ mutant $\lambda_{\epsilon B764}$. 3) HDF026 and HDF125 grow λ_{cIb_2} but neither $\lambda_{\epsilon A30}$ nor $\lambda_{\epsilon B764}$. The λ mutants $\lambda_{\epsilon A30}$ and $\lambda_{\epsilon B764}$ have been isolated

Table 3-2. Temperature-dependence of host-defectiveness and bacterial growth of selected host-defective strains. Streak tests were performed as described in Materials and Methods, Part E1. After overnight incubation at the indicated temperature, plates were examined for bacterial growth (area of bacterial streak before its intersection with the phage streak) and phage growth (area of overlap between bacterial and phage streaks). The symbols used in the table are as follows:

For growth of bacteria: +, growth; cs (cold-sensitive), poor or no growth at 30°C; ts (temperature-sensitive), poor or no growth at 42°C.

For growth of T4⁺: +, growth; +sm, plaques small but eop ~1; ±, leaky phage growth (eop ~0.1); 0, no growth; -, poor growth of the bacterial host precluded a meaningful test.

TABLE 3-2. Temperature dependence of host-defectiveness and bacterial growth of selected host-defective strains

Bacterial strain	Growth of bacteria				Growth of T4 ⁺			
	25°	30°	37°	42°	25°	30°	37°	42°
B178	+	+	+	+	+	+	+	+
HDA175		+	+	+		0	0	0
HDAD01		+	+	+		0	0	0
HDAD11								
HDAD21								
HDB45		<u>CS</u>	+	+		-	0	±
HDB84		<u>CS</u>	+	+		-	0	0
HDB173		+	+	<u>ts</u>		0	0	-
HDC013		+	+	<u>ts</u>		+	±	-
HDC184		+	+	<u>ts</u>		+	±	-
HDD36		+	+	+		0	0	±
HDD43		+	+	<u>ts</u>		+ sm	0	-
HDD71		<u>CS</u>	+	+		-	0	±
HDD018		+	+	+		+	+ sm	0
HDD259		+	+	+		+	+ sm	0
HDF026	+	+	+	<u>ts</u>	+	+ sm	0	-
HDF125	+	+	+	<u>ts</u>	+	+ sm	-	-
HDF183	+	+	+	+	+	+ sm	0	-
<u>groEA44</u>		+	+	<u>ts</u>		+ sm	0	-
<u>groEB515</u>		+	+	+		+	+	+

on groE strains that block λ head assembly and have been mapped in the λ E and B genes, respectively (Georgopoulos et al., 1973).

4. Temperature dependence of bacterial growth and host defectiveness

The preliminary streak tests (Section IID1) yielded additional information about the host-defective strains which is summarized in Table 3-2 and briefly discussed here.

1) The majority of host-defective strains, that is, Class AD and the one Class A mutant, grew equally well at all temperatures. The AD Class fell into two groups: those isolated at 42°C that were defective for T4⁺ growth only at 42°C (data not shown), and those isolated at 37°C that were defective at all temperatures tested. The single representative of Class A, HDA175, was also defective at all temperatures.

2) Mutants of the B Class were either cs or ts for bacterial growth when isolated. These strains proved to be difficult to maintain because cs⁺ or ts⁺ derivatives appeared to have a growth advantage. (As will be noted in Table 3-3, a cs⁺ derivative of HDB84 is able to grow T4⁺ but inhibits the growth of goA-1. This behavior is unlike that of B178, so HDB84cs⁺ is probably a pseudo-revertant.)

3) All Class C mutants isolated at 37°C in selection I and in subsequent selections were ts for bacterial growth.

4) Mutants in Class D showed growth properties that ranged from normal to ts or cs phenotypes. The ability to support T4⁺ growth appeared in all cases to be temperature-dependent: some strains were defective at low temperature but sensitive at 42°C, whereas others showed the converse behavior.

5. eop and burst sizes of T4⁺ and go phages on HD strains

a) eop. The efficiencies of plating and burst size determinations of

$T4^+$ and go phages are presented in Tables 3-3 and 3-4. The data in Table 3-3 can be summarized briefly as follows: 1) Mutants of Classes A and AD, like groEA44, have tight blocks in the sense that go phage mutants appear at a frequency of less than 10^{-6} . 2) Class D host-defective mutants show two types of go phage mutant plaques: large plaques occur at a frequency of 10^{-6} (goD-1 was isolated from such a plaque), and plaques that are smaller and somewhat turbid occur at a frequency of about 10^{-4} (standard go phages goD-2, goD-3 and goD-4 were isolated from such plaques and will be described in Section III of this chapter). All mutants of Class D characteristically show an eop for goA-1 about 1000-fold lower than that for $T4^+$, suggesting a relationship of negative complementation. 3) Class B mutants appear to be related to either Class A or Class D strains because of the following kinds of behavior: HDB173 grows goA-1 with an eop 10,000x higher than that of $T4^+$ and therefore resembles the Class A mutant. HDB45 resembles the Class D strains because it grows goD-1 better than $T4^+$ and shows the characteristic Class D inhibition of growth of goA-1. 4) HDB84 was originally isolated as a cs strain. It was very unstable and difficult to maintain. The cs⁺ derivative is listed in Table 3-3; it is like groEB515 in the sense that it has regained the ability to grow $T4^+$ but inhibits the growth of goD-1 and also goA-1. 5) HD018 is defective for $T4^+$ growth at 42°C but sensitive at 37°C. At 37°C, however, it inhibits the growth of goA-1 phages. In summary, the plating properties of goA-1 and goD-1 on various host-defective strains or their derivatives in Classes A, AD, B and D strongly suggest that the interruption of $T4^+$ development may occur at the same stage in mutants of these classes. 6) Class C mutants are quite leaky for the growth of $T4^+$ and standard go phage that were selected on host-defective strains of other classes. 7) Class F strains, like Class D strains, give two types of go mutant plaques when $T4^+$ is plated at high concentration.

Table 3-3. Efficiency of plating of T4⁺ and standard go phages on selected host-defective strains. Efficiency of plating was measured as the ratio of the number of plaques produced by a phage strain on a given bacterial host relative to the number produced on the parent strain, B178. The measurement was made at 37°C unless otherwise specified. Underlining is used to emphasize the major differences discussed in the text.

TABLE 3-3. Efficiency of plating of $T4^+$ and standard go phage on selected host-defective strains

Bacterial strain	Phage				
	$T4^+$	<u>goA</u> -1	<u>goC</u> -1	<u>goD</u> -1	<u>goF</u> -1
B178	1.0	1.0	1.0	1.0	1.0
HDA175	$<10^{-6}$	<u>1.0</u>	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$
HDAD01 } HDAD11 } HDAD21 }	$<10^{-6}$	<u>1.0</u>	$<10^{-6}$	<u>1.0</u>	$<10^{-6}$
HDB45	10^{-4}	$<10^{-7}$	10^{-4}	10^{-2}	10^{-4}
HDB84 ^{cs} ^a	<u>1.0</u>	$<10^{-3}$	<u>1.0</u>	10^{-3}	<u>1.0</u>
HDB173	$<10^{-7}$	<u>10^{-3}</u>	$<10^{-7}$	$<10^{-7}$	$<10^{-7}$
HDC013 } HDC184 }	10^{-2}	10^{-2}	<u>1.0</u>	10^{-2}	10^{-2}
HDD36 } HDD43 } HDD71 }	10^{-4} - 10^{-6}	$<10^{-7}$	10^{-4} - 10^{-6}	<u>1.0</u>	<u>10^{-3}</u>
HDD018 } HDD259 }	1.0	<u>10^{-3}</u>	1.0	1.0	1.0
HDD018 } HDD259 } ^{42°}	10^{-4} - 10^{-6}	$<10^{-7}$	10^{-4} - 10^{-6}	<u>1.0</u>	10^{-4} - 10^{-6}
HDF026 } HDF125 }	10^{-5} - 10^{-6}	10^{-5} - 10^{-6}	10^{-5} - 10^{-6}	10^{-5} - 10^{-6}	<u>1.0</u>
HDF183	10^{-2}	10^{-2}	10^{-2}	10^{-2}	<u>1.0</u>
<u>groEA</u> 44	$<10^{-6}$	$<10^{-7}$	$<10^{-6}$	<u>1.0</u>	$<10^{-6}$
<u>groEB</u> 515	1.0	1.0	1.0	<u>10^{-5}</u>	1.0

^aHDB84 was originally classified as a B strain because it failed to grow any standard go phage well but showed an increased eop for goD-1. As originally isolated the strain was cs. HDB84 throws off cs⁺ derivatives that grow much better than HDB84^{cs} so it was difficult to maintain the original strain. The HDB84^{cs}⁺ derivative has the unique property of allowing the growth of $T4^+$ but not goA-1 or goD-1; this made it useful for mapping the goA-1 and goD-1 mutations (Section III).

The standard go phage goF-1 was isolated from a large plaque occurring at a frequency of 10^{-6} . The isolation of other goF mutants is discussed in Chapter 4, Section III.

b) Burst sizes. In the burst size experiments shown in Table 3-4 we found that $T4^{+}$ and go phages adsorbed as well to host-defective strains as to B178 and killed these strains with equal efficiency. $T4^{+}$ infections yielded no progeny phage. goA-1 grew well in Class A and AD strains, as did goD-1 in groEA44 (burst sizes 60-100% of those on B178). goD-1 did not fully compensate for the host defect in D strains (burst sizes 20-50% of those found in B178). Similarly, goF-1 appears to restore phage growth only partially in the Class F strains (burst sizes $\sim 20\%$ of those on B178).

6. Presumptive identification of host-defective mutants blocked in $T4^{+}$ assembly

a) Theory. Since our primary interest was to identify host-defective mutants specifically blocked in T4 assembly and our selection procedure was not specific for blocks to $T4^{+}$ growth at this level, we next screened selected mutants of each class (the mutants listed in Tables 3-3 and 3-4) for the ability to lyse at the normal time after $T4^{+}$ infection. In early studies with amber mutants of T4, Epstein et al. (1963) classified the T4 genes into two major groups: those exhibiting defects in DNA synthesis and those altered in assembly and maturation. Mutants in the former class failed to make DNA and to lyse at the proper time, presumably because late genes including those for lysis were not turned on, whereas the latter class made DNA and lysed normally. We reasoned that mutations in host components involved early or late in the T4 life cycle should cause similar behavior.

Table 3-4. Burst sizes of T4⁺ and standard go phages on selected host-defective strains. Burst sizes were determined as described in Materials and Methods, Part I, with the exception that unadsorbed phage were measured prior to the addition of T4 antiserum: after 5 minutes of phage adsorption in the presence of KCN, a sample was removed from the adsorption tube into H broth containing chloroform and after 30 minutes suitable dilutions were plated on a permissive host. Measurements were at 37°C unless otherwise specified.

TABLE 3-4. Burst sizes of T4⁺ and standard go phage
on selected host-defective strains

Bacterial strain		Phage			
		T4 ⁺	<u>go</u> A-1	<u>go</u> D-1	<u>go</u> F-1
B178	37°	186	185	152	150
	42°	30	41	33	
HDA175		.002	122	.22	n.t.
HDAD01		.09	156	94	n.t.
HDAD11		.01	102	41	n.t.
HDB45		< .1	n.t.	n.t.	n.t.
HDB84 ^{cs} + ^a		.36	.4	1.0	n.t.
HDB173		.002	n.t.	n.t.	n.t.
HDC013		.50	n.t.	n.t.	n.t.
HDC184		.62	n.t.	n.t.	n.t.
HDD36		.3	.002	34	n.t.
HDD43		1.0	.004	89	n.t.
HDD71		1.0	.35	63	n.t.
HDD018		52	2.6	220	n.t.
HDD259		76	.3	360	n.t.
HDD018	42°	< .1	.001	2.0	n.t.
HDD259	42°	.2	.003	23.0	n.t.
HDF026		.5	n.t.	n.t.	30
HDF125		.2	n.t.	n.t.	32
HDF183		.1	n.t.	n.t.	n.t.
<u>gro</u> EA44		.03	n.t.	251	n.t.

^aSee Table 3-3, Footnote (a).

b) Lysis of phage-infected cells. Figure 3-1 shows lysis curves for representatives of the different HD classes. $T4^+$ -infected mutants of Classes A, AD, B and D lysed a few minutes before wild-type cells infected with an amber mutant in a late structural gene (amN52 in gene 37). $T4^+$ -infected HD Class F strains lysed, but later and more slowly than the control. However, rapid lysis ensued upon the addition of chloroform. Class C mutants did not lyse readily even when chloroform was added.

c) DNA synthesis in phage-infected cells. The lysis behavior of $T4^+$ -infected host-defective strains suggested that DNA synthesis should be normal in HD strains of Classes A, AD, B and D but might be aberrant in Classes C and F. Figure 3-2 shows the results of the measurement of DNA synthesis by the continuous incorporation of ^{14}C -thymidine into acid-insoluble material. Thymidine uptake appeared to be at least as efficient in HDD36 as in B178, but was depressed in HDC184 and HDF125. In addition, HDF125 showed a 3 to 6 minute delay in the onset of $T4^+$ DNA synthesis. To establish that the thymidine incorporation was due to phage DNA synthesis, we carried out a control experiment in which B178 was infected with 42^- (amN122) phage that are defective in the synthesis of deoxycytidylate hydroxymethylase, a phage-induced early enzyme required for T4 DNA synthesis. No thymidine incorporation was observed in this control experiment (Fig. 3-2, Panel b). We have not measured DNA synthesis in the remaining HD classes but from other properties of these mutants to be discussed below we infer that DNA synthesis is also normal in these classes.

7. Summary of the preliminary classification and characterization of host-defective mutants

Table 3-5 summarizes the characteristics of the host-defective mutants isolated in selection I. All mutants isolated were host-defective in the sense

Figure 3-1. Measurement of infected-cell lysis. Bacterial cells were grown in 20 ml of H broth to $2-3 \times 10^8$ /ml (O.D. 660 = 0.50-0.60) and infected with phage at an moi of 6-7. 1 ml samples were withdrawn at intervals and the optical density measured at 660 nm. Surviving bacteria measured at 7' were less than 1%. A few drops of CHCl_3 were added to the HDC and HDF cultures at 80' after infection and the optical density was measured once more at 90'. x, B178 + T4 amN52(37⁻); o, HDA175 + T4⁺, HDAD11 + T4⁺, HDD36 + T4⁺, HDB45 + T4⁺; ●, HDC184 + T4⁺; Δ, HDF183 + T4⁺. An amber mutant in a late structural gene was used as a control because T4⁺-infected B178 cells fail to lyse due to lysis inhibition of the culture by the first progeny released.

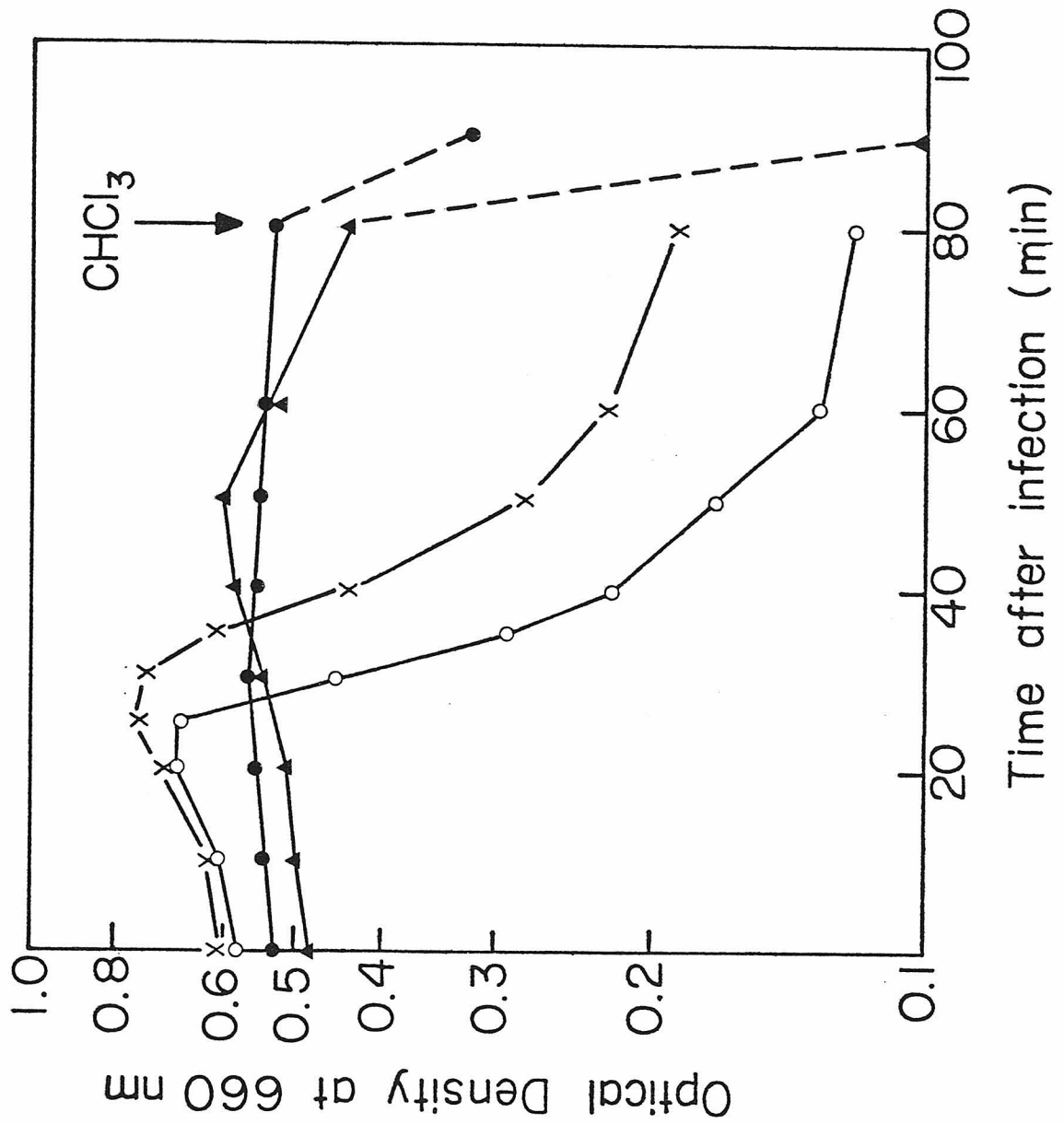


Figure 3-2. Measurement of DNA synthesis in phage-infected cells. The procedure is described in the Materials and Methods, Part L.

a. The experiments were performed in K medium. ●, HDD36 infected with $T4^+$; o, B178 infected with $T4^+$; x, HDC184 infected with $T4^+$.

b. The experiments were performed in H broth. o, B178 infected with $T4^+$; Δ, HDF125 infected with $T4^+$; □, B178 infected with 42^- phage (amN122).

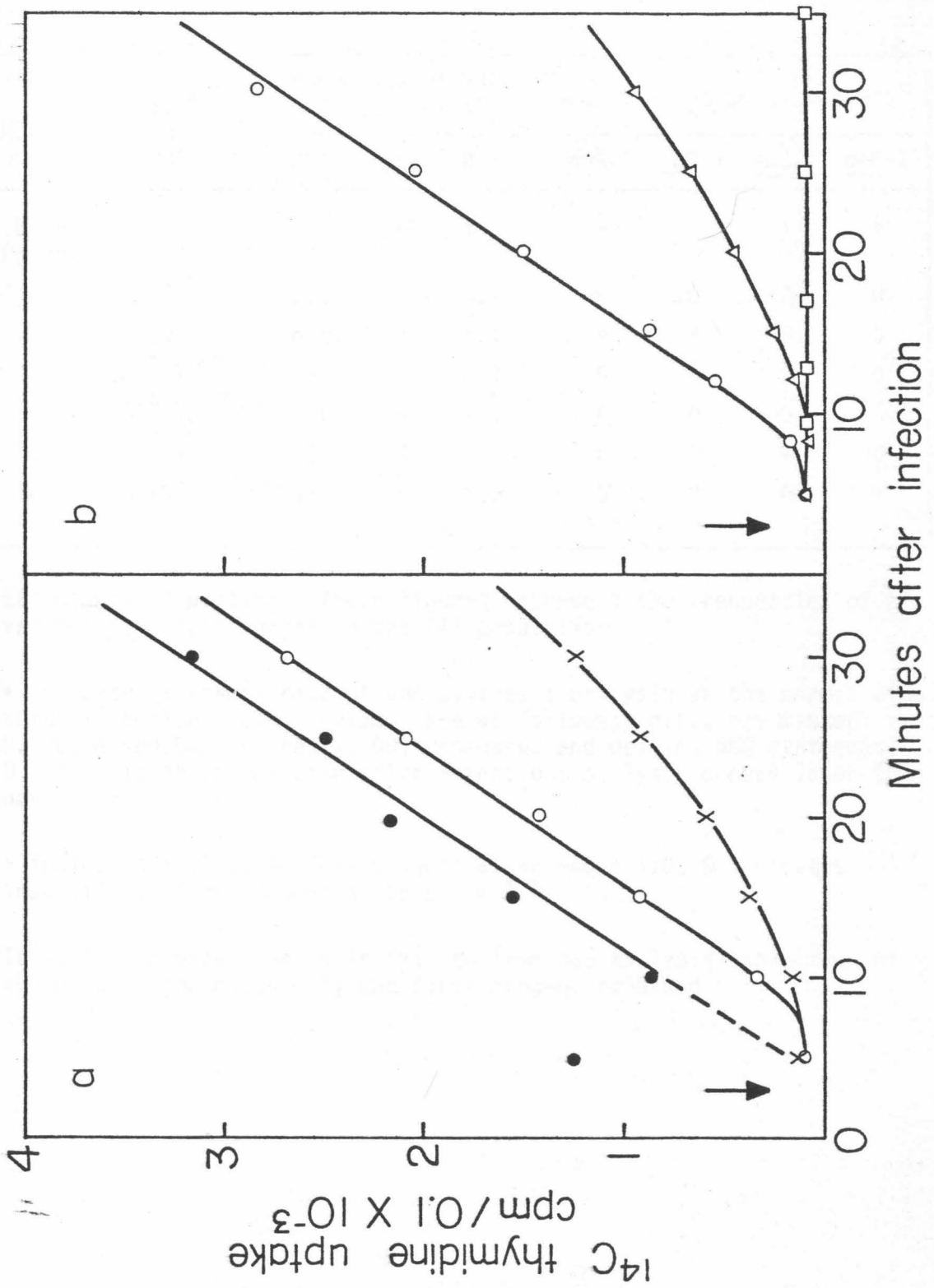


TABLE 3-5. Summary of characteristics of host-defective bacteria

Mutant class	eop ^a of T4 ⁺	Phenotype of T4 ⁺ - infected cells ^b			Growth of <u>go</u> phage mutants ^c			
		DNA	Lysis	B.S.	<u>goA</u> -1	<u>goD</u> -1	<u>goC</u> -1	<u>goF</u> -1
B178 (Parent)	1.0	+	0 ^d	180	+	+	+	+
A	<10 ⁻⁶	n.t.	+	<.1	+	0	0	0
AD	<10 ⁻⁶	n.t.	+	<.1	+	+	0	0
D	10 ⁻⁴ -10 ⁻⁶	+	+	<.1	0	+	0	0
B	10 ⁻⁴ -10 ⁻⁷	n.t.	+	<.1	0	0	0	0
C	10 ⁻² -10 ⁻⁴	D	0	<.5	0	0	+	0
F	10 ⁻⁵	DD	±	<.3	0	0	0	+

^aEfficiency of plating. These figures represent the frequencies of the various go mutant phages in the T4⁺ population.

^b+ indicates a normal rate of DNA synthesis or lysis at the normal time after infection. Other symbols are as follows: n.t., not tested; D, depressed DNA synthesis; DD, depressed and delayed DNA synthesis; 0, no lysis at normal time after infection; ±, lysis occurs later than normal; B.S., burst size.

^c+ indicates ability to form plaques at an eop = 1.0; 0 indicates inability to form plaques at an eop = 1.0.

^dT4⁺-infected wild type cells fail to lyse due to lysis inhibition of the bulk of the culture by the first progeny released.

that they adsorbed $T4^+$ normally and were killed but failed to produce progeny phage. The analysis of go phage plating properties on these strains defined at least six classes of host-defective mutants. From the results of lysis and DNA synthesis experiments we conclude that the host-defective Class A, AD, B and D mutants probably block $T4^+$ growth at an assembly step, Class C mutants are affected at an early stage of phage development, and Class F mutants occupy an ambiguous, intermediate position.

E. Selections II and III: Isolation of host-defective mutants using go phage as selecting agents

1. Introduction

As will be demonstrated in Section III of this chapter, representatives of host-defective strains of Classes A, AD, B and D are blocked at an early step in $T4^+$ head assembly at the level of gene 31 function. HDF strains have pleiotropic effects on $T4^+$ development and assembly and their characterization is discussed in detail in Chapter 4. By our criteria, HDC strains, characterized by failure to lyse, are blocked early in $T4^+$ development and have not been further investigated.

In an effort to identify other classes of host-defective mutants blocked in the assembly of baseplates, tails, or fibers, we have carried out two additional selections. Our idea was to use go phage mutants as selecting agents to eliminate the most frequent classes of HD strains and thereby increase the probability of finding representatives of rarer classes.

2. Selection II

Selection II was carried out with a mixture of goA-1 and T6 at 30°C and 37°C. Only a single subculture was examined. Twenty-six mutants were

purified and tested for support of growth of standard go phage. It can be seen in Table 3-6 that the objective of eliminating the very common AD Class was achieved: 11 mutants allowed growth of goD-1, 7 fell into the F Class, and a single mutant grew goC-1. The seven mutants in the "other" class (304, 305, 306, 307, 310, 321 and 328) failed to grow standard go phage and were further investigated (see Section III of this chapter). Since a D-1 type go phage isolated from HD324 and shown to map in gene 31 (0.87% recombination with amN54(31)) grew on all the "other" strains, we concluded that these strains were most likely to be variations of the head defective phenotype--an hypothesis substantiated by an extensive investigation of HD310 (see Section III).

3. Selection III

A third selection was carried out with a double mutant, goD-1:F-1 (constructed by crossing goD-1 and goF-1) in combination with T6. Ten subcultures of mutagenized B178 were exposed to phage at 30°C, 37°C or 42°C, and 10 surviving colonies from each subculture at each temperature were screened (300 mutants, see Table 3-6).

At 42°C we did not reduce the number of AD strains recovered because goD-1 failed to propagate on AD strains at 42°C (burst size on HDAD01 at 42°C = 0.5).

At 37°C the objective of eliminating the frequent AD, D and F Classes was achieved: 64% of the mutants were placed in the "other" category because they failed to grow the standard go phage better than T4⁺. The 4 Class A mutants, 5 Class B mutants, and 18 independent mutants of the "other" class (categorized as independent according to bacterial and T4⁺ growth properties at 30°C, 37°C and 42°C [patterns similar to those listed in Table 3-2 were found]) were further investigated (27 mutants). Mutants of the "other" class that were leaky (21),

TABLE 3-6. Selection of host-defective bacteria using T4 go mutant phages

Selection	Selecting phage (T)	Sub-cultures	Mutants	Host-defective class, %						
				A	AD	D	B	C	F	Other
I	T4 + T6 (37°, 42°)	26	279	1	87	3	2	1	1	5
II	T4 _{goA-1} + T6 (30°, 37°)	1	26	0	0	42	0	4	27	27
III	T4 _{goD-1} : F-1 + T6 (42°) (37°) (30°)	10	100	1	48	0	0	34	0	17
			100	4	1	0	5	26	0	64
			100	(see text)						

resistant (5) or defective in growth of goD-1 (5) were not examined further. Of the 27 mutants, 3 failed to grow at the time of testing, 3 were apparently unstable and had become sensitive to T4⁺, and 6 failed to lyse at the normal time following T4⁺ infection and were not examined further. The remaining 15 mutants lysed normally after T4⁺ infection and were investigated by in vitro complementation tests. The developmental block in all these mutants was characterized by the production of active T4 tails but inactive heads. Again we concluded that these mutants were variations on the defective head assembly phenotype described in Section III and did not pursue the investigation.

At 30°C selection III yielded no new host-defective classes. The majority of surviving colonies contained cells that were quite leaky with respect to wild-type growth but proved to be defective to varying degrees for goA-1, goD-1, or goD-3, like HDB84cs⁺.

III. Characterization of T4⁺ assembly defects in host-defective mutant bacteria

A. Introduction

Much has been learned about the overall pathway of T4 assembly and the specific sequence of gene product interactions involved in this process by using a combination of electron microscopy, serology, in vitro complementation, sucrose gradient analysis, and SDS gel electrophoresis. For example, in extracts of nonpermissive cells infected with T4 amber mutants defective in late gene functions, various structural intermediates accumulate that can be visualized in the electron microscope (Epstein et al., 1963). The activity of these intermediates can be assessed by an in vitro complementation assay, a test that involves mixing different extracts of T4 amber mutant infected cells and measuring the production of active phage (Edgar and Wood, 1966). Smaller intermediates can be detected by serological techniques (Edgar and Lielausis, 1965) or isolated on sucrose gradients (King, 1968). Specific primary gene products and in some cases their covalently modified forms that are a result of the assembly process can be identified by SDS polyacrylamide gel electrophoresis (Laemmli, 1970; O'Farrell et al., 1973; Vanderslice and Yegian, 1974). We have applied some of these techniques to define the nature of the block to T4⁺ assembly in our host-defective strains. A genetic analysis of go phages has aided in the identification of the particular step that is nonfunctional in the mutant bacteria. The host-defective mutations have been mapped on the E. coli chromosome.

B. The course of T4⁺-infection in HD strains

We have seen in the preliminary classification of host-defective strains (Chapter 3, Section II) that mutants of Classes A, AD, B and D adsorb T4⁺ normally

and are efficiently killed, and that infected cells of these strains lyse at the normal time but few progeny phage are produced. In this section we further investigate the phenotypic characteristics of $T4^+$ infection of representative host-defective strains by in vitro complementation, electron microscopy, and SDS gel electrophoresis.

1. In vitro complementation with extracts of $T4^+$ -infected host-defective cells

We have carried out in vitro complementation tests to ask if any active intermediate structures accumulate in $T4^+$ -infected HD strains. Extracts of $T4^+$ -infected host-defective cells were mixed with either fiberless particles (F^-) or with extracts of nonpermissive cells that had been infected with $T4$ amber mutants and that provided heads and fibers ($T-BP^-$) or tail-baseplates and fibers (H^-). The production of infectious phage particles in these mixtures was assayed. As shown in Table 3-7 (columns 2 and 3), $T4^+$ infection of representatives of all four classes of host-defective strains presumed to affect assembly produced active fibers and tail-baseplates comparable in quantity to those in the H^- control, but no active heads (Table 3-7, column 4). These results show that the assembly of tail-baseplates and fibers is normal and indicate that the interruption of $T4^+$ development is at some step in head morphogenesis.

2. Electron microscopy of extracts of $T4^+$ -infected host-defective cells

The extracts of $T4^+$ -infected host-defective cells that were used for in vitro complementation were also examined by electron microscopy using negative staining techniques. In agreement with the in vitro complementation data, the host-defective extracts contained normal numbers of tails but no heads or head-related structures. A representative field is shown in Plate 4-4D. The

TABLE 3-7. In vitro complementation with extracts of $T4^+$ -infected host-defective strains

Complementing extract	F^-	$T-BP^-$	H^-	HDD36	HDB45	HDAD11	HDA175
HDA175	355	544	4.7	-	-	-	0.4
HDAD11	408	488	4.1	-	-	0.5	
HDB45	242	294	5.0	-	0.6		
HDD36	456	370	5.0	5.0			
H^-	290	487	4.1				
$T-BP^-$	413	0.3					
F^-	0.3						

50 μ l each of two extracts were mixed, incubated for 300 minutes at 30°C, and then assayed for plaque-forming phage. Results are expressed as titer (active phage/ml) $\times 10^{-9}$ in the reaction mixture. Numbers at the bottom of each column represent titers after incubation for 300 minutes of each extract alone. Extracts were prepared as described in Materials and Methods, Part Q. F^- (head and tail + baseplate donor) was a preparation of fiberless particles purified from X4E-infected cells: 5×10^{11} particles/ml were used in the complementation reactions. $T-BP^-$ (head and fiber donor) was an extract of B178 infected with amN120 (27). H^- (tail + baseplate and fiber donor) was an extract of B178 infected with amB17 (23).

controls, the head or tail donor extracts used in the complementation test, showed normal numbers of heads or tails.

3. Synthesis of proteins in phage-infected host-defective strains

The early steps in T4 capsid formation are controlled by 7 genes (20, 21, 22, 23, 24, 31, and 40), the products of which are required for the proteolytic processing of gp22, gp23, gp24, and internal proteins (Laemmli, 1970). In non-permissive infections, amber mutants in head genes except 23 and 31 produce head-related structures containing the uncleaved gene products (Laemmli et al., 1970a,b). The simultaneous absence of gp22 and gpIPIII also prevents polymerization of gp23 into ordered structures (Showe and Black, 1973). Thus the absence of capsid-like structures when lysates of T4⁺-infected HD cells were visualized in the electron microscope suggests three possibilities: 1) a block in the synthesis of gp23, 2) failure of synthesis or function of gp31, or 3) the nonavailability of core proteins. To distinguish among these possibilities, we have examined phage proteins labeled at late times after T4⁺ infection of host-defective strains by SDS acrylamide gel electrophoresis; the results are shown in Plate 3-2A. The growth properties of T4⁺, goA-1, and amN54(31) on B178 (HD⁺), HDA175, and HDB84cs⁺, which were used in this experiment, are shown in Table 3-8. It can be seen that in all infections gp23 (gp23 + gp23*) is synthesized to approximately the same extent, and the core proteins gp22 and gpIPIII are present. In the permissive infections (T4⁺ infection of B178 and HDB84cs⁺ and goA-1 infection of HDA175) gp23 was cleaved to yield the smaller polypeptide gp23*. In the nonpermissive infections (amN54(31) infection of B178, goA-1 infection of HDB84cs⁺, and T4⁺ infection of HDA175) cleavage of the major capsid component has not occurred. Plate 2B shows similar results for HD310 infected with T4⁺ (see Part E2). Identical

plate 3-2. SDS polyacrylamide gels of extracts of phage-infected cells. Cells were infected with phage, labeled with ^{14}C amino acids from 13-24 minutes after infection at 37°C , and prepared for SDS polyacrylamide gel electrophoresis as described in Materials and Methods, Part M1. Samples containing about 40,000 cpm were electrophoresed on 10% polyacrylamide slab gels and processed as described in Materials and Methods, Part N. Phage and bacteria used in the preparation of extracts are listed above each track: + means T4^{+} , 31 refers to phage carrying amN54(31), and 23:63:rII to phage carrying amB17, amM69, and rEDdf41. The growth properties of these phage on these bacteria are shown in Table 3-8. 23, 23*, 22, and IPIII designate, respectively, the product of gene 23, the cleaved product of gene 23, the product of gene 22, and the product of gene IPIII.

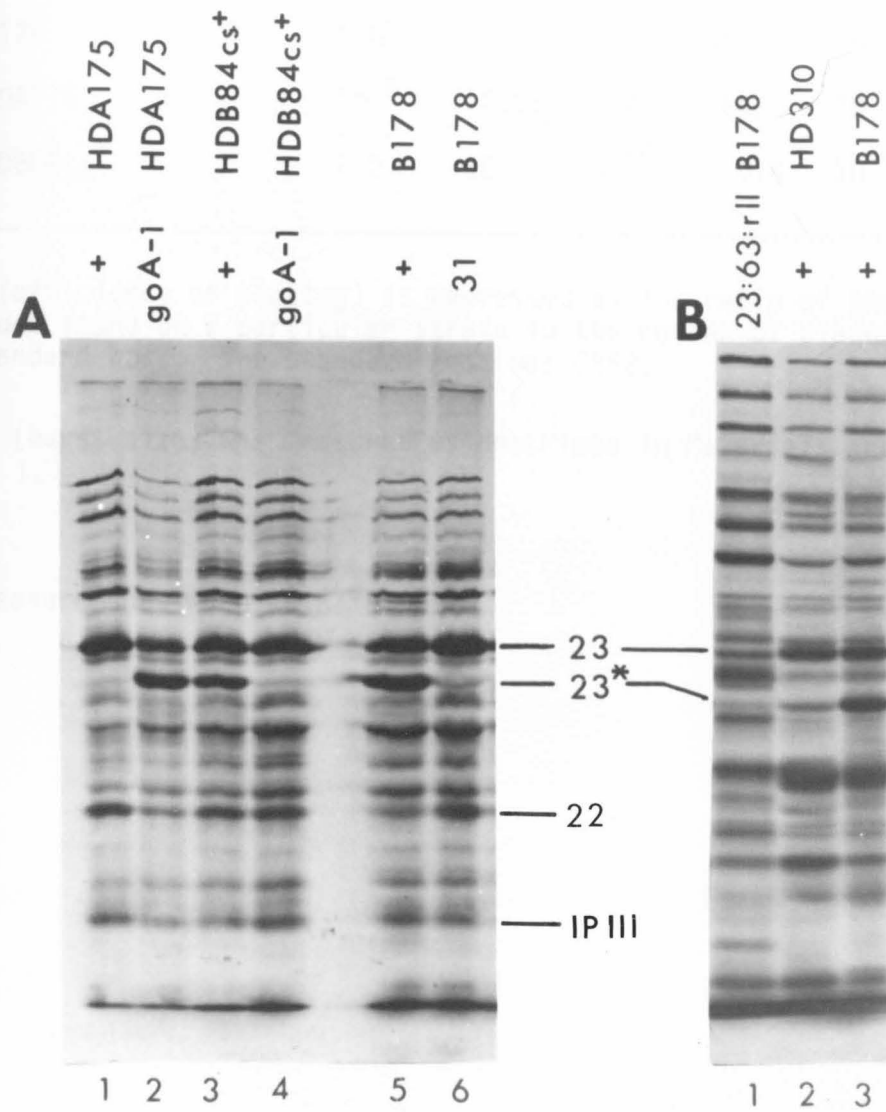


TABLE 3-8.. Phage growth in HDA175 and HDB84 $\underline{cs}^+$

Phage Host	T4 ⁺		<u>goA-1</u>		<u>amN54(31)</u>	
	eop ^a	B.S. ^b	eop	B.S.	eop	B.S.
B178	1.0	137	1.0	153	10 ⁻⁶	0.005
HDA175	10 ⁻⁶	0.02	1.0	84	10 ⁻⁶	0.005
HDB84 $\underline{cs}^+$	1.0	36	10 ⁻⁴	0.4	10 ⁻⁶	0.005

^aeop (efficiency of plating) is expressed as the ratio of the number of plaques found on a particular strain to the number of plaques found on a standard host. The standard host was CR63.

^bB.S. (burst size) was measured as described in Materials and Methods, Part I.

All measurements were at 37°C.

results have been found with extracts of $T4^+$ -infected HDD36 and HDAD11. These results support the conclusion that all the classes of host-defective strains presumed to affect phage morphogenesis block $T4^+$ development at an early stage in head assembly at the level of gene 31 function. The data also suggest that the alteration in $HDB84cs^+$ that prevents growth of goA-1 is probably at the same level.

C. Genetics of go phage

To gain further insight into the nature of the phage-host interactions in $T4$ head assembly, we undertook a genetic analysis of the go phage mutants that can compensate for the host defects. We have determined the genetic locus of the mutations of goA-1 and goD-1. More go phages, including three new prototype standard go phage mutants, have been selected on a number of HD stains and have been characterized genetically.

1. Genetic analysis of goA-1 and goD-1

The standard go phages goA-1 and goD-1 that define the bacterial mutant Classes A, AD, B, and D, are spontaneous mutants that were obtained from large plaques appearing at a frequency of 10^{-6} when high concentrations of $T4^+$ were plated on HDAD01 and HDD018, respectively. The data in Table 3-3 show that at 37°C HDD018 and groEB515 support the growth of $T4^+$ but not goA-1 and goD-1, respectively. Since neither HDD018 nor groEB515 contains a nonsense suppressor, we were able to carry out complementation tests in these bacteria between the go phage and amber mutants to determine the functional block to go phage growth. The results, shown in Table 3-9, indicate that goA-1 and goD-1 failed to complement amN54(31) but were able to complement amber mutations in all other late genes tested. Two- and three-factor genetic crosses shown

TABLE 3-9. Complementation analysis of phages goA-1 and goD-1

<u>amber</u> mutant	Defective gene(s)	Complementation	
		<u>goA</u> -1 ^a	<u>goD</u> -1 ^b
X18	5, 6, 7, 8, 9, 10	+	+
X19	11, 12, 13, 14, 15, 16	+	+
B17	23	+	+
X113	25, 26, 51, 27, 29, 48	+	+
N54	31	-	-
X446	36,57	+	+

^aHost, HDD018, 37°C^bHost, groEB515, 30°C

Tray test complementation was carried out as follows. Phage solutions were prepared at 10^8 /ml. 0.05 ml of each of a pair of phage solutions to be tested was placed into a well of a UV-sterilized clear plastic multiwelled disposable tray. Restrictive plating bacteria, grown in H broth to 10^8 /ml and concentrated to 2×10^9 /ml, were diluted 1/20 into soft top agar at 45°C and 1 ml was added to each well. Control wells contained 0.05 ml of each phage alone. After overnight incubation, tests were scored as negative if there were about the same number of plaques as in control wells (few or none). Positive tests had many more plaques than the controls and usually showed confluent lysis.

in Table 3-10 and summarized in Figure 3-3 have established that the go mutations in goA-1 and goD-1 phage map in gene 31 between tsA70 and amNG71. The crosses confirm the order amNG71, T4ε, tsA70, amN54, r67, previously determined by Georgopoulos et al. (1972), and show that the mutations in goD-1 and goA-1 are closely linked to tsA70 and located distally to it with respect to the r67 marker. goD-1 and T4ε give similar plating patterns on our HD strains and both grow on groEA44 but not on groEB515 (Table 3-1). They differ, however, because T4ε fails to grow at 42°C even on the HD⁺ parental strain, B178. Crosses between goD-1 and T4ε do not give wild-type recombinants, so we have placed these mutations at the same site although the above physiological evidence suggests a different base alteration. goA-1 appears to be further from tsA70 (Table 3-10, Exp. 10, %R = 0.13) than is goD-1 (Table 3-10, Exp. 6, %R = 0.02) and gives wild-type recombinants when crossed with goD-1 (Table 3-10, Exp. 11, %R = 0.6). The percentage recombination is higher than expected but consistent with a different location for goA-1 and goD-1. We have verified that the plaques that we counted as wild-type recombinants were indeed wild type by stab test analysis on an appropriate set of HD strains.

These experiments show that both the goA-1 and goD-1 mutations map in gene 31 and that the different plating patterns of the mutant phages are correlated with different mutational sites.

2. Gene dosage experiments with goD-1

Gene dosage experiments determine the dependence of phage yield on the amount of a given phage gene product by measuring the burst size in cells simultaneously infected with varying amounts of two phage, one carrying a functional allele and the other a nonfunctional allele of the given gene. If the functional gene product is normally produced in limiting amounts, then the burst

Table 3-10. Genetic crosses locate goA-1 and goD-1 within gene 31. Genetic crosses were done in CR63 at 30°C under standard conditions described in Materials and Methods, Part T1. Total progeny was determined by plating on CR63. In Experiments 1-9 wild-type recombinants were determined by plating on groEB515 at 42°C where goD-1, T4 ϵ , tsA70 and amber mutants cannot grow. In Experiment 10, wild-type recombinants were determined by plating on HDD43 at 30°C where goA-1 and tsA70 cannot grow. In Experiments 11, 13, and 14, wild-type recombinants were determined on HDB84cs⁺ where goA-1, goD-1 and goA2820 cannot grow. The genotype of the wild-type recombinants was verified by stab tests onto an appropriate set of HD strains. In Experiment 12, total progeny was determined by plating on CR63. go:am⁺ parental phage and go⁺:am⁺ wild-type recombinants were recovered on S/6 and the go⁺:am⁺ recombinants identified by stabbing onto HDA2820 where they cannot grow.

$$\text{percent recombination (\%R)} = \frac{\text{wild-type progeny}}{\text{total progeny}} \times 200$$

$$\text{The ratio } \frac{\underline{r}}{\underline{r} + \underline{r}^+} = \frac{\text{wild-type progeny with } \underline{r} \text{ phenotype}}{\text{total wild-type progeny}}$$

This ratio permits the ordering of the mutant alleles with respect to the outside marker r67 in gene rIII.

TABLE 3-10. Genetic crosses locate goA-1 and goD-1 within gene 31

Cross #	Phage 1	Phage 2	% R	$\frac{r}{r + r^+}$
1	<u>r67:amN54</u> (31)	T4 ϵ	0.61	0.11
2	"	<u>goD</u> -1	.73	.15
3	"	<u>amNG71</u> (31)	.80	.13
4	"	<u>tsA70</u> (31)	.80	.23
5	<u>r67:tsA70</u> (31)	T4 ϵ	.03	.05
6	"	<u>goD</u> -1	.02	.03
7	"	<u>amN54</u> (31)	.8	.72
8	"	<u>amNG71</u> (31)	.3	.18
9	T4 ϵ	<u>goD</u> -1	<.01	
10	<u>r67:tsA70</u> (31)	<u>goA</u> -1	.13	.16
11	<u>goD</u> -1	<u>goA</u> -1	.6	
12	<u>goA2820</u>	<u>amN54</u>	<.5	
13	<u>goD</u> -1	<u>goA2820</u>	.17	
14	<u>goA</u> -1	<u>goA2820</u>	.16	

Figure 3-3. Genetic location of goA-1 and goD-1. The map of the gene 31 region of the T4 genome showing the positions of amNG71 and amN54 in gene 31 relative to outside markers pseT (to the left) and rIII and gene 30 (to the right) is based on the data of Revel and Lielausis (1978). The top line indicates the relative order of the genes. The second line is the genetic map showing the positions of phage mutations. Each number in the figure is the observed percentage of recombination in a cross between mutants at the ends of the corresponding arrow. Recombination percentages between amNG71 and amN54, amN54 and r67, and between amNG71 and r67 are from the data of Revel and Lielausis (1978) (upper set of arrows). The three-factor crosses in Table 3-10 locate tsA70 between amNG71 and amN54 and position goA-1, goD-1 and T4 ϵ to the left of tsA70. goD-1 and T4 ϵ showed no recombination and are placed at the same site.

gene 31

pseT

rIII

30

NG71
g0A-1
g0D-1
ts470^e

NS4

r67

90

1.50

1.09

1.95

0.80

0.3

0.80

size in gene dosage experiments will be proportional to the input frequency of the functional allele; if the product is normally made in excess, the burst size will not be directly proportional to the input of the functional allele. In our experiments with HD strains as hosts, the go allele is functional, and the wild-type allele is nonfunctional.

The results in Figure 3-4a show that when the parental strain, B178, is mixedly infected with either $T4^+$ or goD-1 and amN54(31), both the wild-type gp31 and the altered gp31 induced by goD-1 are clearly in excess and there is no depression of burst size at equal input ratios. In HDD36 infections, however, the altered gp31 made by goD-1 behaves in a limiting manner in mixed infections with either amN54 (no gene 31 product) or $T4^+$ (wild-type gp31). This result is probably explained by a reduction in efficiency or activity of the goD-1 gp31 in the mutant host rather than a reduction in quantity; the altered protein is only partially able to overcome the host block. An inefficient gp31 would also explain the low burst size of goD-1 in HDD36 (Table 3-4) and the depression of the burst size in mixed infection experiments.

3. Failure of growth of ts mutants in gene 31 on some HD strains at 30°C

A mutant protein whose function is temperature-sensitive usually is the result of a missense mutation that causes an unfavorable conformational change in the molecule at the restrictive temperature. In cases where the wild-type form of the protein normally interacts with other gene products, the ts protein may have aberrant interactions, even at the permissive temperature. We have found this to be the case for two ts mutations in T4 gene 31 when they are grown in certain HD bacteria. As noted previously (Table 3-2) the host-defectiveness of HDD018, HDD43 and HDD259 is temperature-dependent. Table 3-11

Figure 3-4. Gene dosage experiments in B178 and HDD36 at 37°C. The data are presented as percentage phage yield per cell as a function of the go phage input frequency in mixedly infected cells. For B178 infected with T4⁺ and amN54, percent yields are plotted as a function of the input frequency of T4⁺. Infections were carried out under standard cross conditions as described in Materials and Methods, Part T1. Phage were added at a constant moi of about 10-12, varying the proportions of the two phage types. B.S. stands for burst size.

Panel a: ○, B178 infected with goD-1 and T4⁺; B.S. = 237

X, B178 infected with goD-1 and amN54(31); B.S. = 266

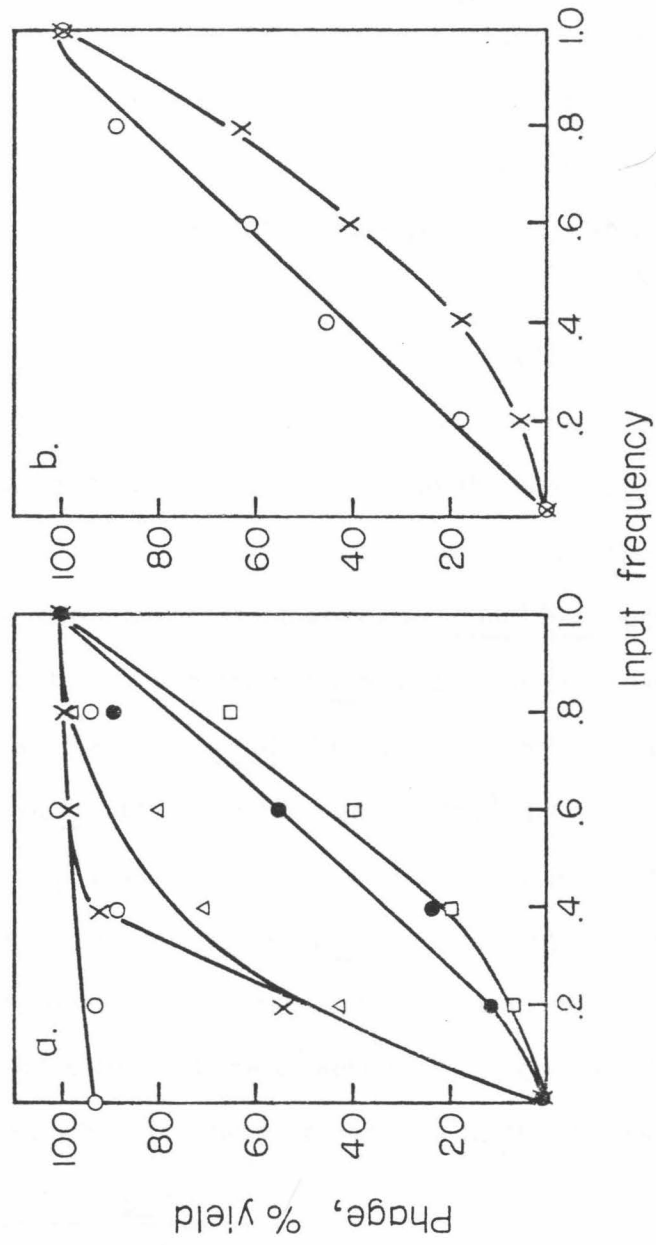
△, B178 infected with T4⁺ and amN54; B.S. = 255

●, HDD36 infected with goD-1 and T4⁺; B.S. = 23

□, HDD36 infected with goD-1 and amN54; B.S. = 39

Panel b: ○, B178 infected with goD-3 and goD-3:amN54; B.S. = 272

X, HDD36 infected with goD-3 and goD-3:amN54; B.S. = 8.0



shows that $T4^+$ grows on these strains at 30°C but not at 42°C. tsA70(31) grows on the HD^+ parent strain B178 at 30°C but on none of these three HD strains at that temperature. tsA56(31) appears to be less fastidious: it can grow on HDD018 and HDD259 as well as on B178, but does not grow on HDD43 at 30°C. We interpret these observations as support for the idea that gp31 interacts in some fashion with a host component. In phage carrying tsA70 or tsA56, gp31 can still interact with that component in wild-type bacteria at 30°C, but are no longer able to do so in certain HD strains.

Coppo et al. (1973) have also observed that tsA70(31) and tsA56(31) failed to grow on tabB212 at 30°C. TabB212 is a host-defective mutant, similar to our HD strains, which blocks T4 development at the level of gp31 function at 42°C but not at 30°C. T4 mutants that compensate for the tabB212 defect at high temperature map in gene 31 and are called com^B (compensates for B). ts mutants in gene 31 that fail to grow on tabB212 at the permissive temperature are called k^B (kills B). Takahashi et al. (1975), in the same laboratory, made an extensive survey of ts mutants in other genes for k^B activity: only one mutant, tsN37 in gene 23, had k^B plating properties on tabB212 at 30°C. The remaining mutants gave an eop of 1.0. In addition, 7 ts phage mutants with k^B properties on tabB212 isolated from a mutagenized stock of T4 mapped in either gene 31 or 23. This survey suggested that the k^B activity may be a specific property of ts mutants in genes whose products interact in a tabB-virus protein complex.

4. Selection of more go mutants

a) Introduction. We selected more go phages on a number of different HD strains to further investigate the idea that go phage plating patterns are a reflection of the particular mutational alteration in the go phage; that is, that

TABLE 3-11. Plating properties of ts mutants in gene 31 on host-defective strains that are sensitive to T4⁺ at 30°C

Bacterial strain	Efficiency of plating			
	T4 ⁺		<u>tsA70</u>	<u>tsA56</u>
	42°	30°	30°	30°
B178	1.0	1.0	1.0	1.0
HDA175	10 ⁻⁶	10 ⁻⁶	10 ⁻⁶	10 ⁻⁶
HDAD11 HDAD21	10 ⁻⁶	10 ⁻⁴	10 ⁻⁶	10 ⁻⁴
HDD018	10 ⁻⁴	1.0	10 ⁻⁶	1.0
HDD36	10 ⁻¹	10 ⁻⁴	10 ⁻⁶	10 ⁻⁴
HDD43	10 ⁻⁴	1.0	10 ⁻⁶	10 ⁻⁴
HDD259	10 ⁻⁴	1.0	10 ⁻⁶	1.0

Efficiencies of plating were estimated from the semi-quantitative spot test described in the Materials and Methods, Part E2.

the compensating mutations are specific. We also wanted to know if mutations allowing phage growth on defective hosts occur only in gene 31 or might also be found in other late genes whose products are required at an early stage in T4 head morphogenesis.

Table 3-12 summarizes the plating properties and indicates the map locations of go phages selected on a number of the host-defective strains.

b) goA mutants. 1) Spontaneous go phage with goA-1 plating properties, selected as large plaques (frequency, 10^{-6}) by plating independent stocks of T4⁺ on several Class A, AD or B host-defective strains, carried a mutation that mapped in the same region of gene 31 as goA-1 (%R with tsA70 = 0.13-0.20). 2) We identified four Class A host-defective mutants in selection III by the fact that they could grow goA-1 but no other standard go phage. A spontaneous go phage mutant selected on HDA2820 (frequency, 10^{-5}) failed to grow on HDA175 or HDAD11. goA2820 mapped in gene 31 but gave wild-type recombinants with both goA-1 and goD-1 (see Table 3-10). The results found for goA-1 and goA2820 are consistent with the suggestion (Part C1) that different plating patterns of the go phage are correlated with different mutational sites. The change in gp31 induced by the goA-1 mutation allows the phage to compensate for defects in both Class A hosts but the alteration induced by the goA2820 mutation corrects only a subset of this group.

c) goD-1 mutants. Spontaneous go phage with goD-1 plating properties, selected as large plaques (frequency, 10^{-6}) by plating independent stocks of T4⁺ on several AD and D strains as well as on groEA44, all carried mutations that mapped close to tsA70, and all those tested (selected on groEA44, HDD259 and HDD324) failed to give wild-type recombinants in crosses with T4c. We conclude

TABLE 3-12. Plating properties of go phage mutants

T4 ⁺ eop	<u>go</u> A-1	<u>go</u> 2820	T4 ϵ	<u>go</u> D-1	<u>go</u> D-2	<u>go</u> D-4	<u>go</u> D-3	<u>go</u> 310
Prototype HD strain on which selected	HDAD01	HDA2820	<u>gro</u> EA44	HDD018	HDD018	HDD36	HDD36	HD310
Frequency of <u>go</u> phage	10 ⁻⁷	10 ⁻⁵	10 ⁻⁷	10 ⁻⁶	10 ⁻⁴	10 ⁻⁴	10 ⁻⁴	UV
B178	+	+	+	+	+	+	+	+
HDA175	+	0	0	0	0	0	0	0
HDA2820	+	+	n.t.	0	n.t.	n.t.	0	+
HDAD11	+	0	+	+	0	0	0	0
HDB45	0	n.t. ^c	0	0	0	0	+	+
HDD36	0	0	+	+	+	+	+	+
HDD018 ^a	0	n.t.	0	+	+	0	+	+
HDD43	0	n.t.	+	+	+	+	+	n.t.
<u>gro</u> EA44	0	0	+	+	0	0	0	0
<u>gro</u> EB515	+	+	0	0	+	+	+	+
Map position	31	(31) ^b	31	31	(23)	(23)	23	(23)
Additional HD strains on which <u>go</u> were selected	HDA175 HDAD11 HDAD31 HDB15 HDB173	HDA3312 HDA3511	—	HDAD11 HDAD21 HDAD31 HDAD41 HDD259 HDD324 <u>gro</u> EA44	HDD43 HDD105 HDD312	HDD71	HDB45 HDD71 HDD105 HDD259 HDD312	—
Map position	31	(31)		31	n.m. ^d	n.m.	n.m.	

Footnotes for TABLE 3-12. Plating properties of go phage mutants

^a42°C.

^b() indicate that the go mutation has been mapped close to a known marker in the gene indicated but has not been definitively mapped.

^cn.t. = not tested.

^dn.m. = not mapped.

that the mutations in go phages that give the goD-1 plating pattern are tightly clustered or at the same site.

d) Other goD mutants. As noted previously (Section IID5a), plating high concentrations of $T4^+$ on all Class D host-defective strains gave rise to two types of plaques: large plaques occurred at a frequency of 10^{-6} and medium sized turbid plaques at a frequency of 10^{-4} . To investigate the genetic nature of the phage mutants that occurred at the higher frequency, we selected three more prototype standard go phages, goD-2, goD-3, and goD-4, on HDD018, HDD36, and HDD36, respectively. These goD phages could be distinguished by their plating properties. They failed to grow on any Class A or AD strain but gave characteristic plating patterns on several Class B and D strains (Table 3-12, see box). Preliminary mapping experiments shown in Table 3-13 indicated that the mutations in these goD phages were not in gene 31 but were closely linked to amB17 in gene 23. As shown in Table 3-12, goD phages with these different plating patterns could be selected on a variety of host-defective strains of Classes B or D. goD-3 has been mapped within gene 23 (see Part C5, below).

go310 is the only go phage mutant selected from a mutagenized stock of $T4^+$ plated on a host-defective strain. It was necessary to mutagenize the phage because HD310 (from selection II, see Part E2) failed to grow any standard go phage or allow the selection of spontaneous go phage mutants that made plaques of any reasonable size. The go310 phage (backcrossed 3x; see Materials and Methods, Part G) gave a plating pattern similar to goD-3 (except that it also grew on HD310) and has also been mapped close to amB17(23) (%R = 1.0).

5. Genetic analysis of goD-3

To determine whether the go mutations that show linkage to amB17(23) were in gene 23, which codes for the major capsid protein, or in gene 22, which

Table 3-13. Preliminary mapping of goD phage. Crosses were done in CR63 under standard conditions described in Materials and Methods, Part T1. Progeny were plated on CR63 for total yield and on S/6 to recover parental go:am⁺ phage and recombinant go⁺:am⁺ phage. The progeny recovered on S/6 was one-half the total progeny measured on CR63. The go⁺:am⁺ recombinants were identified by stabbing individual plaques onto the appropriate HD strains where the go⁺:am⁺ recombinant would not grow. For the recombination values between 1 and 6%, 1000 plaques were stabbed. For higher recombination values, 200 plaques were stabbed.

$$\text{percent recombination} = \frac{\text{go}^+:\text{am}^+ \text{ (non-platers on HD strain)}}{\text{number of plaques stabbed}} \times 100$$

Stab conditions:

goD-2: stabs were made onto HDD018 at 43°C.

goD-3 and goD-4: stabs were made onto HDD36 at 37°C.

go310: stabs were made onto HD310 at 37°C.

TABLE 3-13. Preliminary mapping of goD phage

<u>amber</u> mutant <u>go</u> mutant	Percent recombination			
	<u>amB8</u> (20)	<u>amB17</u> (23)	<u>amN67</u> (25)	<u>amN54</u> (31)
<u>goD</u> -1	n.t.	n.t.	n.t.	0.61 ^a
<u>goD</u> -2	5	1.2	22	31
<u>goD</u> -3	5.4	1.3	13	21.5
<u>goD</u> -4	5.5	1.6	15.5	20.0
<u>go310</u>	n.t.	1.0	n.t.	n.t.

^a go⁺:am⁺ recombinants were determined directly by plating progeny on groEB515 which plates wild type phage but not goD-1 or amber mutants.

codes for the core or scaffolding protein of T4, we carried out a more definitive mapping analysis with goD-3. We concentrated on this phage because it grew on most D strains. goD-3:amX double mutants were constructed and used for complementation tests and genetic crosses with wild-type phage in the restrictive host HDD36. The results of complementation experiments, shown in Table 3-14, indicate that an altered gene 23 product is necessary for phage growth in HDD36. When goD-3 carries amber mutations in gene 23, no go mutation-modified gp23 is made and complementation fails. When goD-3 carries amber mutations in other late genes, the burst size is similar to that of goD-3 plus wild-type phage in mixed infection. The burst sizes in the mixed infections are reduced about 6-fold relative to the burst size with goD-3 alone. This could reflect a gene dosage effect or active interference of a nonfunctional gp23 with the modified goD-3 gene 23 product (see Part C6).

To map the goD-3 mutation we have backcrossed goD-3:amX double mutants with wild-type phage and scored the go:am⁺ recombinants by plating on HDD36. This allowed many more progeny to be examined than was possible in the stab test analysis employed in the preliminary mapping. The goD-3 mutation was located within gene 23 near amB272 (Tables 3-15 and 3-16; Figure 3-5). We conclude that go mutant phage with alterations in the major capsid protein (gp23) can partially overcome host defects that interrupt T4⁺ head assembly.

6. Can goD-3 bypass gene 31 function?

goD-3 has a mutation in gene 23 that enables it to grow on host-defective strains blocked in T4⁺ head assembly at the level of gene 31 function. We asked if goD-3 could grow because gp31 had become dispensable. Two types of experiments suggest that goD-3 cannot bypass gene 31 function. 1) The data in Table 3-17

TABLE 3-14. Complementation between goD-3:amX and wild type phage

Gene	Phage 1	Phage 2	Burst size
20	<u>goD-3:amB8</u>	++	2.4
22	<u>goD-3:amE209</u>	++	1.6
23	<u>goD-3:amH11</u>	++	0.1
23	<u>goD-3:amB17</u>	++	0.1
25	<u>goD-3:amN67</u>	++	1.7
42	<u>goD-3:amN122</u>	++	2.9
Controls:	<u>goD-3</u>	--	19.0
	++	--	0.1
	<u>goD-3</u>	++	2.7

Complementation tests were carried out under standard cross conditions described in Materials and Methods, Part T1, in HDD36 at 37°C where neither the goD-3:amX double mutant nor the wild type phage grows. At t = 100 minutes, chloroform was added to the growth tube and progeny were assayed on CR63. The burst size was calculated as progeny per infected bacterium.

TABLE 3-15. Genetic crosses locate T4 goD-3 in gene 23

Cross #	Phage 1	Phage 2	Percent recombination
1	<u>goD</u> -3: <u>am</u> B8(20)	++	6.6
2	<u>goD</u> -3: <u>am</u> E209(22)	++	3.5
3	<u>goD</u> -3: <u>am</u> H11(23)	++	1.5
4	<u>goD</u> -3: <u>am</u> B17(23)	++	1.6
5	<u>goD</u> -3: <u>am</u> B272(23)	++	0.04
6	<u>goD</u> -3: <u>am</u> E506(23)	++	0.78
7	<u>goD</u> -3: <u>am</u> N67(25)	++	13.6
8	<u>goD</u> -3: <u>am</u> N54(31)	++	26.9
9	<u>goD</u> -3: <u>am</u> N122(42)	++	32

goD-3:amX double mutants were constructed as described in Materials and Methods, Part T2. The goD-3:amX double mutants were backcrossed to wild type T4⁺ in CR63 under standard conditions described in Materials and Methods, Part T1. Total progeny were determined by plating on CR63. goD-3:am⁺ recombinants were determined by plating on HDD36 at 37°C where wild type and amber phage cannot grow.

$$\text{Percent recombination} = \frac{\text{goD-3:am}^+ \text{ recombinants}}{\text{Total progeny}} \times 200$$

Figure 3-5. Genetic map of the gene 23 region of the T4 genome. The top line gives the relative order and sizes of genes 21-23 (Wood and Revel, 1976). The second line is the genetic map showing the positions of amber mutations.

a. Recombination percentages between mutants in gene 20, 22, and 23 (F. Eiserling, personal communication).

b. Recombination percentages between mutants in gene 23 (Celis et al., 1973).

c. Recombination percentages between mutants in genes 20, 22, 23, and 25 (Table 3-16).

d. Recombination percentages from the crosses between goD-3:amX double mutants with wild-type phage (Table 3-15).

Each number in the figure is the observed percentage of recombination in a cross between mutants at the ends of the corresponding arrow.

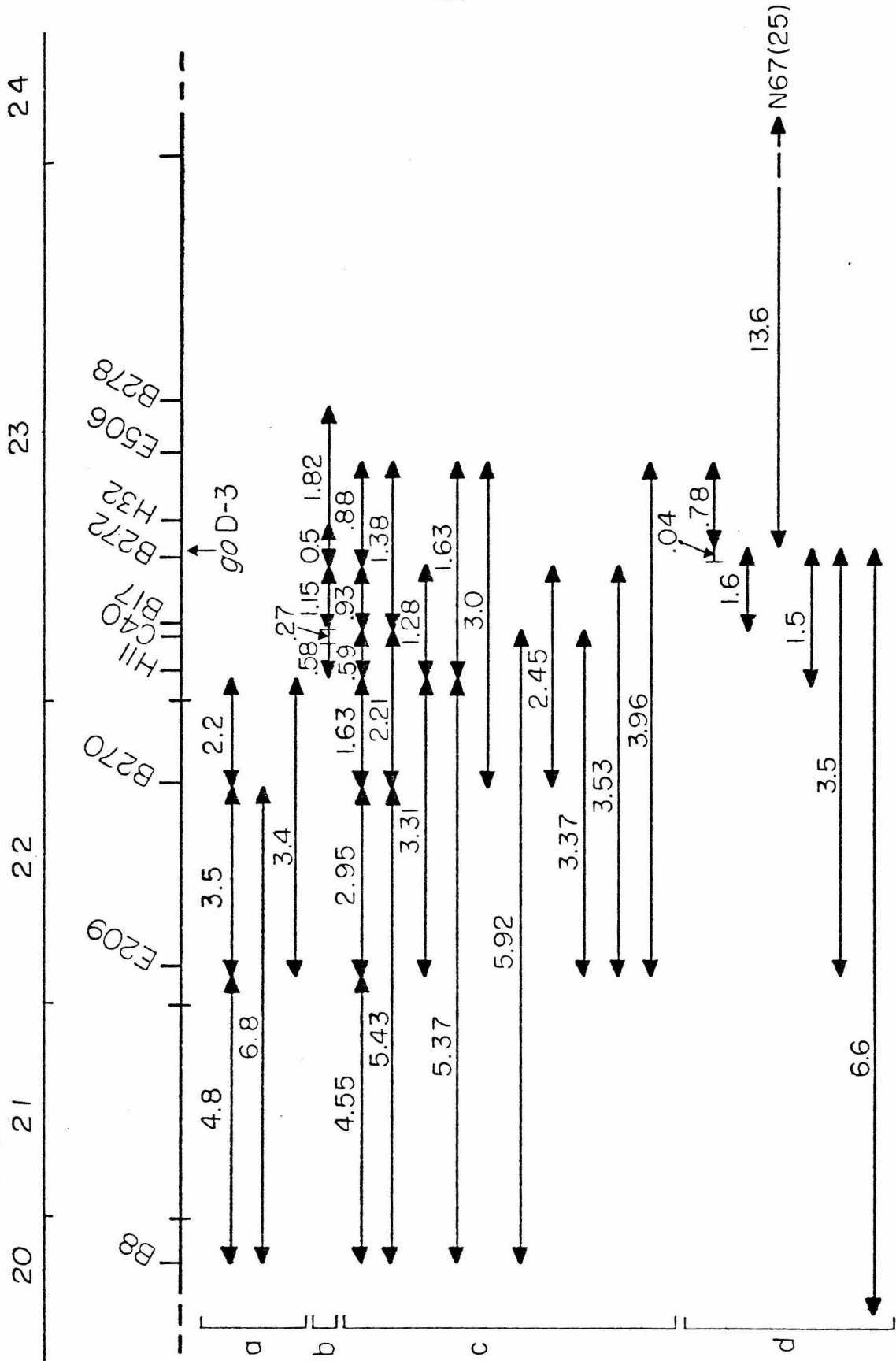


TABLE 3-16. Recombination between amber mutants in genes 20, 22, and 23

<u>amber</u> mutations (gene)	B8(20)	E209(22)	B270(22)	H11(23)	B17(23)	B272(23)	E506(23)
E506(23)	5.62	3.96	3.0	1.63	1.38	0.88	0
B272(23)	5.58	3.53	2.45	1.28	0.93	0	
B17(23)	5.92	3.37	2.21	0.59	0		
H11(23)	5.37	3.31	1.63	0			
B270(22)	5.43	2.95	0				
E209(22)	4.55	0					
B8(20)	0						

Genetic crosses were done in CR63 under standard conditions described in Materials and Methods, Part T1. Total progeny was determined by plating on CR63. am⁺ recombinants were determined by plating on S/6. Values are percents of recombination calculated as:

$$\frac{\text{am}^+ \text{ recombinants}}{\text{Total progeny}} \times 200$$

and are the average of two or more crosses. The data are presented graphically in Figure 3-5.

show that the double mutant goD-3:amN54 could not grow on HDD36 unless the wild-type gene 31 products was present. goD-3:amN54(31) was constructed by crossing goD-3 with phage carrying amN54 and identifying the desired recombinant by screening progeny plaques for those with the ability to grow on HDD36 infected with amB17(23) (to supply gp31 function) but not on HDD36. The goD-3:amN54 double mutant occurred at the same frequency (20%) as wild-type recombinants in the same cross (identified by plating progeny on B178 and determining the number that failed to grow on HDD36 by stab test). In backcrosses of goD-3:amN54 with wild-type phage, we were able to recover both single mutants.

2) Furthermore, in gene dosage experiments we found that wild-type gp31 is limiting in goD-3 infection of both B178 and HDD36 (Fig. 3-4b). When cells were mixedly infected with goD-3 and goD-3:amN54 at varying input ratios, the phage yield proved to be a function of the amount of wild-type gp31 present. The reason that this effect was observed in wild type as well as HDD36 cells is probably because the wild-type gp31 does not work at full efficiency with the altered gp23 produced by the go phage.

Surprisingly, goD-3:amN54 failed to grow on an HDD36^{supE} strain which was subsequently constructed, although goD-3:amB17(23) and several other goD-3amX double mutants grew well (Table 3-17). Investigation revealed that although amN54 mutant phage plated on B178^{supE} with almost normal efficiency, the plaques were small and turbid, in contrast to amB17 mutants that gave wild-type plaques on this host. We conclude that amN54 is poorly suppressed by supE, and when it is combined with goD-3 in the goD-3:amN54 double mutant and asked to grow on HDD36 where wild-type gp31 is already limiting, the further impairment of gene 31 product synthesis prevents phage growth.

TABLE 3-17. Growth of goD-3:amN54 (31)

	CR63	B178	HDD36	HDD36 + <u>am</u> B17	B178 <u>supE</u>	HDD36 <u>supE</u>
<u>goD</u> -3: <u>am</u> N54 (31)	+	0	0	+	+ sm	0
<u>am</u> N54 (31)	+	0	0	0	+ sm	0
<u>goD</u> -3	+	+	+	+	+	+
++	+	+	0	0	+	0
<u>goD</u> -3: <u>am</u> B17 (23)	+	0	0	0	+	+
<u>am</u> B17 (23)	+	0	0	0	+	0

All + and 0 values are derived from eop experiments with the exception of phage growth on HDD36 + amB17(23) (column 5) which was determined by spot test.

The symbols used are +, normal phage growth; 0, no phage growth; sm, small plaques.

D. Genetic analysis of HD strains

1. P1 transduction with purA^+

Since Takano and Kakefuda (1972) reported that the mop mutation in a host-defective derivative of E. coli B that blocked T4^+ head morphogenesis was close to purA, we initiated genetic studies on our HD strains by determining the linkage of the host defect to purA by P1 transduction experiments. P1kc was grown on selected purA⁺ HD strains and used to transduce T832 purA⁻ to purA⁺. PurA⁺ transductants were purified and tested for the ability to support T4^+ growth. The results are summarized in Table 3-18 and Figure 3-6. The frequencies of cotransduction of the host-defective mutations in the HD strains with purA⁺ fell into two, and possibly three, groups. Two strains, HDB45 and HDD71, were clearly different from the rest: there was less than 1% cotransfer of the host-defective mutation with the purA⁺ marker. The mutations that were cotransduced with purA⁺ gave frequencies ranging from 8.5-19%. We have bracketed this region of the E. coli chromosome in Figure 3-6. For convenience of further discussion, we will call these mutations mop (morphogenesis of phage) since one end of the bracket covers the E. coli B mop mutation site as defined by Takano and Kakefuda (1972) and the phenotypes of the original mop mutant and the strains presently under discussion are the same, although we have not shown that the mutations lie in the same gene. The range of cotransduction frequencies found could be viewed as a continuum and the differences attributed to some unknown factors influencing the recovery of purA⁺mop⁻ transductants: for example, in the genetic background of T832, purA⁺mop⁺ transductants might have a selective growth advantage over some of the different purA⁺mop⁻ transductants. Alternatively, one could consider that the mop mutations that cotransduce

Table 3-18. Frequency of P1 cotransduction of the host-defective phenotype of HD strains with purA⁺. P1kc was grown on the host-defective strains and used to transduce T832 purA⁻ as described in the Materials and Methods, Part Z4. purA⁺ transductants were selected on supplemented minimal medium in the absence of purines, purified, and tested by streak test at 37°C for the ability to grow T4⁺ (except HDB84cs⁺ [see footnotes]). Frequencies of purA⁺ transductants ranged from 5-100 x 10⁻⁷ in different experiments.

TABLE 3-18. Frequency of P1 cotransduction of the host-defective phenotype of HD strains with purA⁺

Host-defective strain (Donor)	Host-defective transductants <u>purA</u> ⁺ transductants tested	Cotransduction %
HDD324 ^{b,d}	16/188	8.5
HDA175	19/200	9.5
HDD43	8/75	10.6
HDAD11	22/200	11.0
HDB173	24/200	12.0
HDB84 <u>cs</u> ^{+a}	29/200	14.5
HDD018 ^c	19/100, 32/199	19.0, 16.1
HDD36	19/100, 33/200	19.0, 16.5
HDD71	0/90	<1.0
HDB45	0/199	<0.5
<u>groEA</u> 44	22/200	11.0

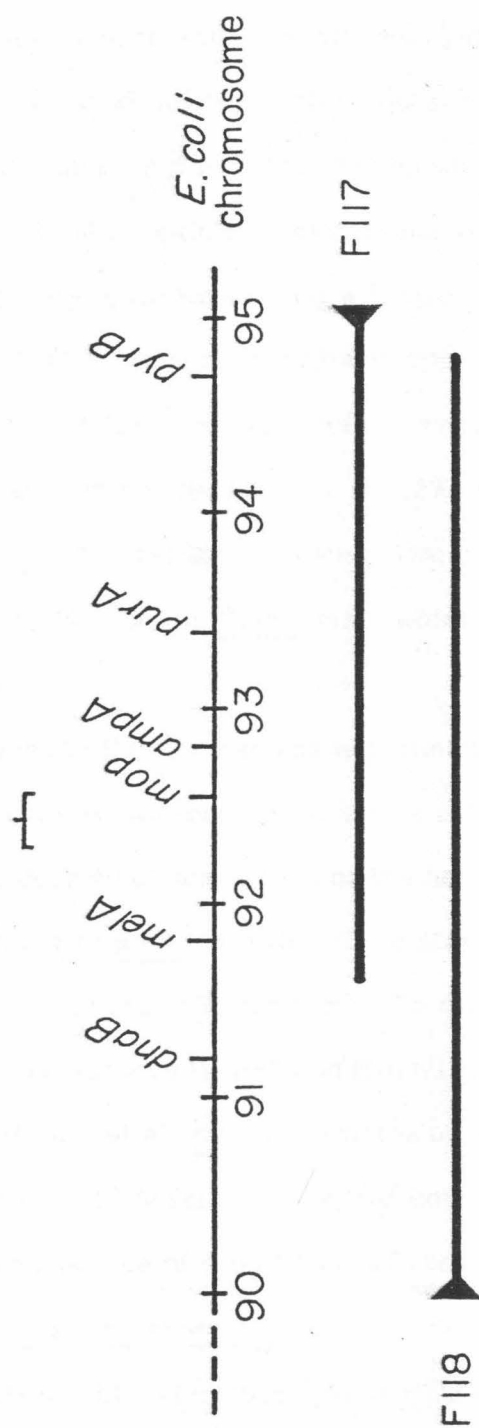
^aHDB84cs⁺ is sensitive to T4⁺ but defective for goA-1; therefore, goA-1 was used to screen the purA⁺ transductants for host-defectiveness.

^bHD strain from selection II.

^cTest for host-defectiveness was at 42°C.

^dTest for host-defectiveness was at 30°C.

Figure 3-6. Location of host-defective mutations (mop) on the E. coli chromosome. The figure shows the 90-95 minute segment of the E. coli chromosome with relevant markers (Bachmann et al., 1976). The heavier lines below the chromosome show the extent of F-prime factors F117 and F118 based on the data of Low (1972) and Takano and Kakefuda (1972) as adjusted to correspond to the most recent map of the E. coli chromosome (Bachmann et al., 1976). The bracket indicates the location of mutations in our host-defective strains as obtained by P1 cotransduction of host-defectiveness with the purA⁺ marker (Table 3-18). Cotransduction frequencies have been converted to minutes on the E. coli chromosome using the equation of Wu (1966) as described by Bachmann et al. (1976).



with purA⁺ fall into two clusters: a group of 5 mop mutations averaging 10% cotransduction, and 2 mop mutations that give on the average 17.5% cotransfer with purA⁺. It is of interest in this respect to compare these values with those published for similar host-defective mutations. Table 3-19 lists the purA⁺ cotransduction frequencies of host-defective mutations variously labeled mop, tabB, and groE. All these mutations have been shown to affect the head morphogenesis of lambda or T4 or both, in a way similar to that described here for HD strains. For simplicity of discussion, we will also call all these mutations mop. Table 3-19 shows that there appears to be an apparent clustering of mop mutations among these strains. A lower cotransduction frequency of 8-12% (average 10%) and a higher cotransduction frequency of 18-25% (average 21.5%) are found in relation to purA. The average distance between these two loci can be calculated to be about 0.25 minutes on the E. coli map, which is the equivalent of about 10,000 base pairs.

Subsequent to the HD mapping experiments, we discovered that T832 carries a strong nonsense suppressor. This means that a possible explanation for the failure to detect cotransduction of the host-defective mutations in strains HDB45 and HDD71 with purA⁺ was that these strains carry nonsense mutations that were suppressed in the T832 recipient. To explore this possibility we transferred a supD suppressor into HDB45 and HDD71 (Materials and Methods, Part T2). The observation that all supD derivatives of these strains remained host-defective indicates that the failure to detect cotransductants with purA⁺ was probably not a consequence of suppression of nonsense mutations.

2. F-prime transfer experiments

To assess whether the mutations of HDB45 and HDD71 were in the same general region of the E. coli chromosome as purA or at some more distant

TABLE 3-19. Frequency of cotransduction of host mutations that block T4⁺ and/or λ head assembly with melA and purA

Head assembly defective host mutant	Phage affected	% cotransduction with <u>melA</u> ⁺ <u>purA</u> ⁺		Reference
<u>mop</u> ^a	T4, λ	22	18	Takano and Kakefuda, 1972
<u>groE-1</u>	λ	32		Sternberg, 1973a
<u>tabB</u> 212	T4		20.3 25.0	Coppo <u>et al.</u> , 1973 Takahashi <u>et al.</u> , 1975
<u>tabB</u> 127	T4		19.2 24.0	Coppo <u>et al.</u> , 1973 Takahashi <u>et al.</u> , 1975
<u>tabB</u> ^{com}	T4 ^{comB}		12.0	Takahashi <u>et al.</u> , 1975
<u>tabB</u>	T4		12.0	" "
<u>tabB</u> ^{k372}	T4 ^{comB}		8.0	" "
<u>groEA</u> 44	T4, λ		9-10	Georgopoulos and Eisen, 1974
<u>groEA</u> 7	λ		9-10	" "
<u>groEA</u> 97	λ		9-10	" "
<u>groEB</u> 764	λ		18.0	" "

^aDerived from E. coli B.

locus, we carried out F-prime transfer experiments using F117 and F118 which cover the 90-95 minute segment of the chromosome (see Fig. 3-6). Transfer of either plasmid rendered HDB45 and HDD71 cells able to support $T4^+$ growth, indicating that the wild-type allele is probably dominant and that the host-defective mutations in these strains must lie between the ends of the F117 factor. Examination of Figure 3-6 suggests that P1 transduction experiments with melA and pyrB markers located at either end of the region covered by F117 should definitively locate the host-defective mutations in these two strains. These experiments have not yet been done.

F117 was transferred to HDD36. HDD36 carrying F117 was capable of supporting $T4^+$ growth, again indicating that the mop⁺ allele is probably dominant to mop⁻. F117 also made HD2820 nonrestrictive for $T4^+$ growth but the mutation responsible for host-defectiveness in this strain has not been tested for P1 co-transduction with purA⁺.

E. Characterization of HD strains from selection II that failed to grow the standard go phage

1. Characterization of the "other" class

Seven bacteria from the second selection were classified initially as "other" because none of the standard go phage grew well on them. On three strains, HD305, HD307, and HD321, both goD-1 and goF-1 grew with slightly better efficiency than $T4^+$ but the difference was not sufficiently great to warrant placing them in the B Class. Only one, HD310, lysed at the normal time when infected by $T4^+$; those of the remaining six that were tested for lysis (strains HD305, HD321, and HD328) gave a more delayed response.

go phage isolated from HD307, HD321, and HD328 were able to grow on Class D bacteria from selection I, and go324, a goD-1 type phage that mapped

at a location near amN54(31) (%R = 0.87%) (see Table 3-12) was able to make small plaques (eop ~1) on all seven "other" strains. We therefore concluded that these HD strains were most likely to be variations on the head-defective theme and not worthy of investigation because of the poor growth of available go phages on them. HD310 was investigated further because of its prompt lysis phenotype.

2. Characterization of HD310

HD310 was cs for growth at 30°C, made nearly 100% long filamentous rods (snakes) when grown in broth at 37°C, and formed colonies with poor efficiency (about 10-15% of the light microscope cell titer). The cells lysed normally following T4⁺ infection and yielded less than 0.1 progeny phage per cell. Phage DNA synthesis was normal, in vitro complementation experiments (Table 3-20) and electron microscopy (Plate 4-4D) showed that active tails and tail fibers but no heads or head-related structures were made. Examination of late phage proteins on gels showed uncleaved gp23 (Plate 3-2B). It proved necessary to UV-mutagenize a T4⁺ phage stock to obtain a go310 phage; the phage subsequently was backcrossed three times to T4⁺ at an input ratio of 1:100. Its eop on HD310 was 0.75 compared to B178 and its burst size was 2-5; on B178 the burst was also low, ~20 phage per cell. go310 grew on all D and B strains tested but not on A or AD strains. The mutation in go310 mapped near amB17(23) (%R = 1).

F. Summary

A summary of the phenotypic characteristics of host-defective strains blocked in T4⁺ head assembly is shown in Table 3-21. A study of the course of T4⁺ infection in HD strains presumed to be blocked in assembly, by a combination of in vitro complementation tests, electron microscopy and SDS polyacrylamide gel

TABLE 3-20. In vitro complementation of an extract of T4⁺-infected HD310

Complementing extract	F ⁻ ^a	T-BP ⁻	H ⁻	HD310
HD310	25	105	5.6	0.3
H ⁻	17	150	5.5	
T-BP ⁻	35	0.6		
F ⁻	0.1			

^aMaximum value = 50.

The experimental procedure is as described for Table 3-7 except F⁻ was only 5×10^{10} particles in the reaction mixtures.

TABLE 3-21. Summary of the phenotypic characteristics of host-defective strains blocked in T4⁺ head assembly

Property investigated	Representatives of mutant classes				
	HDA175	HDAD11	HDB45	HD310	HDD36
T4 ⁺ eop	10 ⁻⁶	10 ⁻⁶	10 ⁻⁴	10 ⁻³ -10 ⁻⁴	10 ⁻⁴ -10 ⁻⁶
Adsorption and killing	normal	normal	normal	normal	normal
DNA synthesis	n.t.	n.t.	n.t.	normal	normal
Lysis	+	+	+	+	+
T4 ⁺ burst size	0.002	0.01	<0.1	<0.1	0.3
<u>In vitro</u> complementation					
active heads	0	0	0	0	0
active tails	+	+	+	+	+
active fibers	+	+	+	+	+
Electron microscopy					
heads	0	0	0	0	0
tails	+	+	+	+	+
Gels - P23 cleavage					
P23	+	+	n.t.	+	+
P23*	0	0	n.t.	0	0
Genetic locus of <u>go</u> phage that grow	31 ? 23	31 ? 23	? 31 23	31 23	31 23
Genetic locus of HD mutation	<u>mop</u>	<u>mop</u>	?	?	<u>mop</u>
F117 covers locus of HD mutation	+	+	+	n.t.	+

electrophoresis, has provided evidence that the block to $T4^+$ development is at an early stage in T4 head assembly at the level of gp31 function. We have shown that go mutant phage that compensate for the host defect map in genes 31 and 23, and that the plating patterns of the go phage are correlated with specific mutation sites. Phage mutants that give negative complementation (k mutants) in temperature-dependent defective hosts at temperatures where $T4^+$ can grow also map in gene 31. Most of the mutations in these HD strains are linked to purA on the E. coli chromosome and have been designated mop (morphogenesis of phage).

IV. Discussion

A. HD strains block $T4^+$ head assembly at the level of gp31 function

The HD strains that we have studied all arrest $T4^+$ development early in head assembly. We will first consider what is known about the initial steps in $T4$ head morphogenesis, summarize the evidence that supports the view that assembly is interrupted at the level of gp31 activity, briefly describe related strains, and discuss current knowledge of the properties of gp31. In Part B we will consider possible mechanisms of gp31 inactivity in the mutant hosts and the function of the go mutations.

1. $T4$ head morphogenesis

The early steps of $T4$ head assembly, leading to the formation of a multi-molecular core-containing tau particle prior to the morphogenetic cleavage reactions necessary for head maturation, occur on the bacterial inner membrane (Simon, 1972). Seven head gene products (gp20, gp21, gp22, gp23, gp24, gp31, and gp40) as well as the dispensable internal proteins participate (Laemmli et al., 1970b; Showe and Black, 1973). gp31 either modifies gp23, the major capsid protein, or directs its orderly assembly so that a structure can be formed (Laemmli et al., 1970a). This process begins at an initiation site and requires the presence of an assembly core (Showe and Black, 1973; Laemmli and Favre, 1973). In normal infection, the initiation is at a site containing gp20, a structural component of the phage head probably located at the head-tail join in virions and at the vertex most proximal to the host membrane in preheads (Müller-Salamin et al., 1977). gp40, not a component of the mature virion, acts catalytically in an unknown way in the formation of this initiation complex. The assembly core consists of gp22, internal proteins, and gp21. When tau particle formation is completed

by the addition of a minor structural component, gp24, gp21 is cleaved to become a proteinase that in turn cleaves head precursor proteins and then self-destructs.

Head precursor modification does not occur in nonpermissive cells infected with amber mutants in any of the prehead genes described above (Laemmli, 1970). However, with the exception of infections with mutants in genes 23 or 31, the uncleaved head proteins are found in aberrant, capsid-like structures such as single and multilayered polyheads and incomplete tau particles (Laemmli et al., 1970b). In 31⁻-infected cells, no head-like structures are seen and gp23 accumulates as lumps on the bacterial membrane (Kellenberger, 1966; Laemmli, 1970). Since lump formation is partially reversible when a temperature-sensitive gp31 is reactivated by temperature shift down, Laemmli (1970) suggested that gp31 acts either to solubilize gp23 or to direct its polymerization into ordered structures. A later observation, that similar lumps of gp23 aggregate on the membrane even in the presence of gp31 if the core components gp22 and the internal proteins are simultaneously missing, indicates that gp31, although necessary, is not sufficient for polymerization of the major capsid protein into structures (Showe and Black, 1973).

2. HD strains block T4⁺ head assembly at the level of gp31 function

Our data indicate that the block to T4 head assembly in HD strains is at the level of gp31 function. Although we have not shown that gp23 accumulates as lumps on the membranes of T4⁺-infected HD cells, the observations that gp23 is made and not cleaved and that no aberrant head-related structures are seen in negatively stained cell lysates in the electron microscope place the block at the level of either gp31 activity or core formation. Genetic evidence supports the former conclusion. We have shown that go phage mutants that compensate

for the host defect map in genes 31 and 23 and not in genes for the core components. In addition, two ts mutants in gene 31 (called k) show a negative interaction with certain mutant hosts: the k mutants fail to grow on temperature-dependent HD strains at 30°C where wild type T4 grows.

3. Other *E. coli* mutants associated with gp31

Several other laboratories have investigated host mutants that appear to be functionally related to our HD strains: they give similar phenotypes after T4⁺ infection, the defect can be compensated for by phage mutants that map in gene 31, and the mutations for the defects are linked to purA on the *E. coli* chromosome. These mutants are mop (Takano and Kakefuda, 1972), groE (Georgopoulos et al., 1972; Georgopoulos et al., 1973; Georgopoulos and Eisen, 1974), and tabB (Coppo et al., 1973; Takahashi et al., 1975). Because of their similarities, the mutations in these strains have all been designated mop on the recent *E. coli* map (Bachmann et al., 1976), and we will use this term in further general discussion. The groE strains, selected for inability to grow λ , arrest λ head morphogenesis. λ phage mutated in gene E, which codes for the major capsid protein, or in gene B, also structurally involved in λ head assembly, overcome the groE defect. A small fraction of groE strains also block phage T4 head assembly; the block is at the gp31-associated step. Conversely, the mop, tabB and HD strains were all selected with T4⁺, and a small fraction of these mutants also block λ head assembly. We think the mutants are basically similar and will eventually be explained by one model.

Two other host mutants that block T4⁺ development are also overcome by phage carrying a mutation in gene 31, but differ from mop mutants with respect to either the genetic locus of the host mutation or the phenotype of T4⁺-infected

cells. 1) hdB3-1 is cold-sensitive for $T4^+$ growth and arrests phage capsid assembly at the level of gp31 function, as evidenced by the absence of head structures and the presence of aggregates of gp23 on the membranes of $T4^+$ -infected cells (Simon et al., 1975). Unlike mop, the defective mutation in hdB3-1, called fatA, maps near proline at about 8 minutes on the E. coli map. The phospholipids of the inner membranes of hdB3-1 cells have less than normal amounts of cis-vaccenic acid and exhibit decreased fluidity. The authors propose that at low temperature the reduced fluidity of the membrane prevents interaction of a membrane structural site with gp31 to initiate head assembly, but the complex formation becomes possible as the temperature is raised. Presumably, a conformational change in gp31 might allow productive interaction with the membrane site at low temperatures. We have found that a cis-vaccenic acid defective mutant (cvc⁻) of E. coli K-12, obtained from J. Cronan (Germann and Cronan, 1972), grows $T4^+$ at all temperatures between 25°C and 42°C. It is not clear whether the block to cis-vaccenic acid production in the cvc⁻ host is at a different level than that in hdB3-1, or whether this unexpected behavior reflects a difference between B and K-12 bacterial strains. 2) A second unusual mutant host in which the block to $T4^+$ growth can be overcome by phage mutant in gene 31 is hd590, a strain that has a pleiotropic effect on $T4^+$ development involving DNA synthesis and tail formation (Simon et al., 1974). In contrast to the situation in $T4^+$ -infected mop cells, both empty heads and polyheads are made in $T4^+$ -infected hd590. We have no explanation for this phenomenon aside from suggesting that since gp31 is made early in infection (see below) and DNA synthesis also occurs at early times, gp31 may have a role in the latter process in addition to that which it plays in head assembly.

4. The function of gp31

What is known about the function of gp31? The product of gene 31 has been recently identified as a 16,000 MW protein whose synthesis starts early in phage-infected cells (Castillo et al., 1977) but whose only known function is at late times during capsid assembly; gp31 is not, however, found as part of the virion. Laemmli et al. (1970a) have shown that gp31 is required continuously throughout the phage assembly period: as mentioned above, in the absence of gp31, the major capsid protein, gp23, accumulates as lumps on the cell membrane. Several investigators (Snustad, 1968; Laemmli et al., 1970a; Coppo et al., 1973) have reported that gp31 behaves in gene dosage experiments like proteins known to function catalytically; this may mean that it acts enzymatically, that it acts stoichiometrically but is made in excess, or that it acts stoichiometrically but is continuously recycled, like the gp8 scaffolding protein of phage P22 (King et al., 1973; King and Casjens, 1974). An apparent exception to the requirements for gp31 in the assembly of head-like structures has been reported recently by Van Driel (1977). Van Driel has isolated gp23 from polyheads in $21^-:22^-$ -infected cell cultures and has reassembled identical polyhead structures in vitro from the purified gp23 in the absence of gp31 and all core proteins. However, the gp23 that he used had already had the opportunity to interact with gp31 in vivo and furthermore was in the form of trimers when used for the in vitro polyhead reconstruction. It is therefore by no means clear that he achieved gp31-independent polymerization of gp23.

B. Proposed mechanism of gp31 inactivity in mop mutants and the function of go mutations

The 31^- phenotype exhibited by $T4^+$ -infected mop mutants could be

due to a failure of gene 31 synthesis or to an impairment of gene 31 function. In the former case, the mop defect would work by inhibiting gp31 production, and go phage that carry mutations in gene 31 would grow because of a restored capacity to make the gene product. In short, the wild type and go mutant phage would carry alternative forms of the gene 31 promotor. This hypothesis seems unlikely because of the internal location of the go mutations between known amber sites in gene 31. Additional evidence against the altered promotor hypothesis is the observation that whether or not a missense mutant in 31 has go (compensatory) or k (killing) activity is apparently host-allele specific. For example, goA-1-type phage were selected on Class A and AD hosts and in these situations are compensatory mutants. The same phages, however, act as k mutants on HDB84 $\underline{cs}^+$ and on HDD018 and HDD259 at low temperature, conditions where T4 $^+$ can grow. An analogous situation exists for goD-1, which has go properties on HD Class D strains but k properties on HDB84 $\underline{cs}^+$ and groEB515. An overproduction of gp31 by go mutants due to an altered promotor is difficult to reconcile with this phenomenon. The recent identification of the gene 31 product on gels should make it possible to confirm this conclusion.

A more likely hypothesis is that the mop mutation interferes directly or indirectly with gp31 function. gp31 might normally directly interact with the mop $^+$ gene product in one of several ways: it might modify a mop $^+$ membrane protein, thus creating an initiation site for gp23 assembly; mop $^+$ might activate gp31 by, for example, promoting its dimerization or covalently altering its structure; or gp31 could combine with the mop $^+$ protein to form an active complex and only this active complex would have true "gene 31 function." All these hypothetical models involve quite specific interactions between gp31 and the mop $^+$ protein. Takano and Kakefuda (1972) have suggested that the mop mutation of E. coli

B disrupts the normal interaction of head precursors with the bacterial membrane or acts as a cofactor for gp31 proteolytic activity. Georgopoulos et al. (1972) postulated that an obligatory interaction between gp31 and a host component is altered in groEA44 and that mutations in gene 31 compensate for the alteration in the groE protein.

The most comprehensive model to explain mop interaction with T4 gene 31 mutants has been presented by Takahashi et al. (1975) as a result of studying the tabB/com/k system. These authors suggest that a modification of host cell machinery to carry out virus functions, involving specific interactions between viral proteins and preexisting host proteins, as occurs in the case of T4 modification of the host RNA polymerase, is a mechanism generally exploited to redirect host synthetic capacities and might be extended to the level of host participation in assembly processes. They propose that viral and host proteins interact to form a specific hybrid complex that is essential for virus growth. Such a hybrid complex would be affected by missense mutations in viral or host genes causing conformational changes in either component that would interfere with the specific complementary (lock and key) interaction of the heterologous subunits. For example, a mutation might change the configuration of the host moiety so that it could no longer interact productively with the phage protein. A mutation in the phage gene coding for the interacting virus component could induce compensatory conformational changes in this protein and allow formation of an active complex with the mutant host protein. Similarly, a mutation affecting the phage subunit might block hybrid complex formation but be compensated for by a mutationally altered configuration of the host component. A third model for possible interactions between viral and host proteins envisions that slight

missense mutations, innocuous to either phage or host growth or to complex formation with the corresponding wild type component of virus or host, might, in combination, negatively complement and prevent virus growth.

Much of our data is consistent with this model. We have shown that both go (compensatory) mutations and k (killing) mutations map in gene 31. In the HD strains, an alteration in the mop protein would block effective interaction with wild type gp31. Specific go mutations would change the configuration of the gene 31 protein to compensate for alterations in the mop protein and restore partial or full gp31 activity. tsA70(31), goA-1(31), and goD-1(31) would act as k mutants on certain HD strains under conditions where wild type T4 can grow because small configurational changes in their gp31's would be incompatible with small conformational changes in the altered host component that are innocuous to T4⁺ growth under these conditions.

Our host mutation mapping data suggest the possibility that several different genes in the host can mutate to produce phenotypically identical blocks to T4⁺ growth (Part C, below). Thus we have proposed that the mop function may be a multimer comprised of two or more different polypeptides. Furthermore, we have found that host mutations at all these sites can be overcome by a single spontaneously arising go phage (goD-1; see Tables 3-3 and 3-18). How can these observations be reconciled with a prediction of the hybrid complex model, that the host component of the complex is a single polypeptide chain (Takahashi et al., 1975)? An explanation compatible with the hybrid complex model would be that mutations in different subunits tend to affect one particular region of the host multimer, the site with which gp31 interacts. An example of heterologous subunit interaction affecting the specificity of interaction of one of the subunits

with a third component is the enzyme aspartate transcarbamylase, a multimolecular protein comprised of two distinct types of subunits, one for regulation and the other for catalysis. Conformational changes in the regulatory subunit due to interaction with effector molecules are transmitted by subunit interaction to the catalytic site (Schachman, 1972). An alternative explanation, that goD-1 might simultaneously be able to overcome mutations in any one of the subunits, is possible, but it lacks the specificity required by the hybrid complex model.

We have made a unique observation: we find that compensatory go mutations (for example, goD-3) also map in gene 23, which codes for the major T4 capsid component, presumably the substrate that interacts with the gpmop-gp31 complex. How can the growth of goD-3 (23) be accounted for? Not by a reduction in the amount of gp23 synthesized, as has been proposed for phage λ mutants in gene E that can grow on certain groE bacteria by restoring a balance between groE function and gene E protein (Georgopoulos et al., 1973; Sternberg, 1973b): the goD-3 mutation lies far from the gene 23 promotor, and phage carrying amber mutations in gene 23 do not grow on HDD36supE strains, although this is a situation in which lowered amounts of gp23 should be produced because of the low efficiency of the supE suppressor. A conformational change at the site of the missense mutation in goD-3 gene 23 could, however, lead to a less functional gene product; if this is the case, it is not manifest in the wild type host where the burst size of goD-3 is normal. A conformational change in the goD-3 gene 23 protein could, on the other hand, lead to productive interaction of the altered gene 23 product with an altered gpmop⁻-gp31 complex that is inactive with wild type gp23. In support of this model, we have shown that goD-3 cannot bypass gene 31 function.

Another major possibility is that the mop mutation indirectly influences gp31 function by altering the internal condition of the cell; for example, by changing the concentration of an ionic species. If ion X has to bind to gp31 in order for the latter to function properly, then various forms of mutant bacterial permeases could cause the concentration of X to be too high, too low, or could admit competing Y ions into the cell in abnormal amounts (Hartman and Roth, 1973; Colb and Shapiro, 1977). In support of such a model, Takano and Kakefuda (1972) have reported a membrane protein alteration in the case of E. coli B mop, and have found that this mutant is insensitive to the tripeptides tri-L-serine and tri-L-tyrosine, unlike its parent strain. We find, however, that this model is less flexible than that of compensating conformational interactions; there are not enough variables to explain all the phage growth patterns we have observed. Takano and Kakefuda may be observing the effects of other mutations induced in their mop strain by nitrosoguanidine mutagenesis; this question could be settled by examination of mop⁺ revertants.

C. The host component(s)

We have shown that the mutations for host-defectiveness in seven HD strains cotransduce with purA, as has been reported for mop (Takano and Kakefuda, 1972), and for several groE (Georgopoulos and Eisen, 1974) and tabB strains (Coppo et al., 1973; Takahashi et al., 1975). For convenience, in this discussion we will call all these mutations mop. The remarkable aspect of our data is that the P1 cotransduction frequencies of mop with purA⁺ range from 8.5% to 19% in two apparent clusters (≈10% and ≈18%). Frequencies for mop, tabB, and groE also show wide ranges and a tendency to cluster at ≈10% and ≈21% cotransduction. As stated in the results, this range could be due to unknown factors that

influence the recovery of purA⁺mop⁺ and purA⁺mop⁻ transductants; the former might have a growth advantage in the genetic background of the recipient strain, T832. Alternatively, the two clusters of transduction frequencies could reflect mutational sites in different genes (the distance between the two clusters represents approximately 0.2 minutes of the E. coli chromosome, or about 8000 base pairs, enough to code for about 300,000 daltons of protein), suggesting the possibility that the mop⁺ function may be comprised of more than one polypeptide. The observation that two HD strains show <1% cotransduction with purA⁺ supports this possibility.

The above hypothesis can be tested by in vivo complementation studies as follows. The plasmid F117 covers the mop mutation in HDD36 and, because of the length of the bacterial segment on this plasmid, also must cover the mutations in all the other HD strains studied here. From an F117/HDD36 merodiploid it should be possible to isolate the homozygous diploid recombinants that occur at a frequency of about 1% in F-prime carrying strains in rec⁺ hosts (Jacob and Wollman, 1961), and transfer the episome with its mop⁻ allele into HD strains that map in both cotransduction frequency clusters. If the multiple polypeptide hypothesis is correct, complementation should occur when the episome is transferred to a mop mutant at a different site but not when transferred to a mop mutant at the same site.

It is of interest in this regard that the isolation of the groE function on two different λ transducing phages has recently been accomplished (Hendrix and Tsui, 1978; Georgopoulos and Hohn, 1978). The groE transducing phages grow on all groE strains, in contrast to $\lambda\epsilon$ mutants that only propagate on certain subsets of groE mutants. Both groups report that a 65,000 MW protein is synthesized

following infection of UV-treated bacterial cells with the transducing phages. Hendrix and Tsui have isolated amber mutants in the groE gene on their transducing phage: the 65,000 MW protein disappears in these mutants in a nonsuppressing host but reappears in a suppressor-carrying host. Georgopoulos and Hohn also searched for amber mutants, but instead found missense mutants that could grow on only certain groE hosts, unlike the parental phage. In these mutants, the 65,000 MW protein was altered to 67,000 MW in one case and to 70,000 MW in another; revertants regained the 65,000 MW species and the ability to grow on all groE hosts. The authors postulate that the active groE product could be a dimer and that what is observed is intragenic complementation between two homologous subunits to give a partially functional groE product, that permits growth of the transducing phage on some groE hosts but not on others. Another possible explanation is that the active groE product is a heterodimer. In this case, the λ transducing phage that carries a missense mutation in one of the groE genes would be able to grow only on a groE strain that could provide the missing polypeptide. The λ groE transducing phages carry a piece of the bacterial genome large enough to code for at least 300,000 daltons of protein (~ 8000 base pairs), so the possible existence of more than one groE gene is not excluded. It is puzzling that only one band has been identified in the gel experiments. In conclusion, these observations with the λ groE transducing phages reinforce the idea suggested by the P1 cotransduction data, that there may be more than one mop gene at different sites near purA.

D. Attempt to select HD strains blocked in other subassembly pathways

Our general selection procedure was very efficient in yielding host-defective mutants. The vast majority of the survivors showed abortive $T4^+$ infection

rather than resistance due to lack of phage receptors, tolerance due to a galU mutation, or full sensitivity to T4 because they fortuitously escaped phage infection during the isolation procedure.

The classification of host-defective mutants by using go phage plating properties appears to be valid in the sense that mutants plating a given phage type all show the same phenotype in subsequent tests. The fact that four different classes of HD strains identified in this way were all blocked in $T4^+$ head assembly at the level of gene 31 function simply means that the resolution of the go phage classification technique is greater than needed. The go phage mutants goA-1 and goD-1 that identified the four classes both map in gene 31, albeit at different sites. As a first approximation in the recognition of strains with similar defects, this classification technique was quite useful in reducing the number of mutants to be analyzed.

Since all the HD strains blocked in assembly that were obtained in the first selection arrested $T4^+$ development early in phage head formation, we attempted to select additional mutants that might be blocked in other subassembly pathways by using the standard go phages as selecting agents to eliminate the most frequent classes of HD strains. We hoped in this way to detect rarer mutants that might affect baseplate, tail or tail fiber assembly. There is suggestive evidence that the host plays a role in these processes: electron microscopic evidence implicates the host cell membrane in the initiation of baseplate formation (Simon, 1969); Pulitzer and Yanagida (1971) have reported that a mutant of W3350 is deficient in the assembly of functional tail fibers; and host involvement in tail fiber assembly has been inferred from genetic experiments to occur at the level of gp57 facilitation of polymerization of the tail fiber components (Revel et al., 1976). In this effort

to isolate other types of mutants, however, we were unsuccessful. Although the technique did screen out the common HD classes that we had found previously, analysis of new host-defective strains from selections II and III revealed them all to be apparent variations of the defective head assembly theme.

A possible explanation for this negative finding is that the frequencies of different classes of host-defective mutants may vary in different bacterial strains. Takano and Kakefuda (1972) reported that out of 93 E. coli B host-defective mutants that restricted T4⁺ growth and were classified by susceptibility to phage mutants that could grow on a particular host, mop was the only strain examined that was permissive for a phage mutant in gene 31. Starting with K-12 strain CT3 as the parent, Takahashi et al. (1975) recovered tabB mutants at a frequency of 37%. These results are in striking contrast with the situation we encountered with B178, where 90% of the bacterial mutants could grow phage mutants with alterations in gene 31. A host-defective mutant previously mentioned that affects functional tail fiber assembly was selected using a different K-12 strain, W3350 (Pulitzer and Yanagida, 1971), again suggesting that the frequencies of host-defective classes may be strain-dependent.

The third selection did, however, reveal some novel T4 head assembly defective mutants. After selection with goD-1:F-1 at 30°C, many of the survivors that were defective for both T4 and T6 under the selection conditions turned out to be sensitive to these phages at higher temperatures. At 37°C these mutants, now sensitive to wild type T4, were specifically defective for goA-1, goD-1, and/or goD-3, go phages with mutations in gene 31 or gene 23 that we had shown could compensate for head assembly defective hosts of Classes A, AD, B and D. In this sense these mutants resemble groEB515, which is specifically defective

for goD-1; HDB84cs⁺, which grows T4⁺ but not goA-1, goD-1, or goA2820; and HDD018, HDD259, and HDD43, which grow T4⁺ at 30°C but not tsA70, a temperature-sensitive mutant in gene 31. Thus selection with particular go phages or temperature-sensitive missense mutants should provide a system to specifically select host mutants that are defective in a particular T4 assembly step. This procedure has been investigated systematically by Takahashi et al. (1975) for the tabB system. The principle has also been applied as a specific selection procedure to identify host mutants that block T4 growth at the level of its interaction with the host RNA polymerase (Coppo et al., 1975a,b). The logical extension of these observations with respect to the selection of host mutants blocked in the subassembly of either baseplates or tail fibers would be to use as selecting agents temperature-sensitive phage mutants in genes whose products are suspected to interact with host components.

Chapter 4

CHARACTERIZATION OF HOST-DEFECTIVE MUTANTS (hdf)THAT HAVE A PLEIOTROPIC EFFECT ON $T4^+$ GROWTHI. Isolation, classification, and properties of HDF bacteria

HDF bacteria were obtained as described in Chapter 3, Section II, by nitrosoguanidine mutagenesis of the K-12 strain B178 followed by selection for cells that were unable to grow $T4^+$. The mutants were classified according to the abilities of spontaneously arising $T4$ mutants (go) to grow on them; HDF strains were able to grow the $T4$ mutant goF-1 but not $T4^+$ or any other standard go phage. All strains classified in this manner as HDF gave similar results when $T4^+$ infections of them were characterized (see Section II, below).

The properties of HDF strains are listed in Table 4-1. Most are ts for growth at 42°C or 45°C and make very poor bacterial lawns at these temperatures on EHA, LB, or T plates. When cells able to grow at high temperatures were selected from these strains, 10-25% of the ts⁺ revertants also had acquired the ability to grow $T4^+$, implying that a single mutation may be responsible for the HDF and ts phenotypes. The original isolate of HDF026 was ts but was inadvertently discarded, and an hdf ts⁺ derivative was retained. Both the latter strain and the strain designated HDF026R were isolated from a slant of HDF026 on which hdf⁺ derivatives had appeared; HDF026R is unusual in that it is cs for $T4^+$ growth.

Several of the HDF strains carry a mutation which renders them unable to incorporate glycerol into phospholipids. As will be discussed in Section IV, this mutation is unrelated to the hdf lesion and probably results from the known tendency of nitrosoguanidine to make multiple, clustered mutations at DNA

TABLE 4-1. Properties of HDF strains

Bacterium	Characteristics	T4 ⁺ eop	T4 ⁺ burst size ^a		Other properties
		37°	25°	37°	
<u>K-12 strains</u>					
SKB178	<u>galE</u> RglI	1	60	120	
HDF125	<u>galE</u> <u>metG</u> ^b <u>hdf</u> RglI Suc ^c	10 ⁻⁶ -10 ⁻⁷	42	0.5	<u>ts</u> at 45°; 4% of the bacteria are snakes in H broth (0% in M9) at 37°
HDF125R	<u>galE</u> <u>metG</u> ^b RglI Suc ^c	1	60	70	Selected to be <u>ts</u> ⁺
HDF026	<u>galE</u> <u>glp</u> ^d <u>hdf</u> RglI T6 ^R	10 ⁻⁵	60	0.5	<u>ts</u> ⁺ derivative of original <u>ts</u> HDF026; 100x higher spontaneous mutation rate than other HDF strains
HDF026R	<u>galE</u> <u>glp</u> ^d RglI T6 ^R	1	20	80	<u>ts</u> ⁺ ; T4 ⁺ grows poorly at 25°
HDF341	<u>galE</u> <u>glp</u> ^d <u>hdf</u> RglI	10 ⁻⁴	17	0.1	<u>ts</u> at 42°; adsorbs T4 poorly after growth in M9
HDF341R	<u>galE</u> <u>glp</u> ^d RglI	1	108	400	selected to be <u>ts</u> ⁺ ; 4% of the bacteria are snakes in H broth at 37°; dies easily on slants
HDF303	<u>galE</u> <u>glp</u> ^d <u>hdf</u> RglI	10 ⁻²	56	1	<u>ts</u> at 42°; <u>hdf</u> allele is leaky; fails to grow phage P1

TABLE 4-1 (continued)

Bacterium	Characteristics	T4 ⁺ eop	T4 ⁺ burst size ^a		Other properties
		37°	25°	37°	
HDF183	<u>galE</u> <u>hdf</u> <u>sup</u> RglI	0.8		0.6	<u>hdf</u> allele is leaky; contains a weak <u>amber</u> suppressor
HDF311	<u>galE</u> <u>hdf</u> RglI				leaky for T4 ⁺ growth at 30° and 37°; permissive at 42°
<u>B strains</u>					
B011 ¹	<u>thy</u> <u>sup</u>	1.0	60	200	carries an undefined <u>amber</u> suppressor
<u>hd590</u>	<u>thy</u> <u>sup</u> <u>hdf</u>	10 ⁻⁷	6	≤0.01	<u>ts</u> at 42°

^aBurst sizes given in the table are the average of at least 2 determinations.

^bIt was determined that HDF125 carries a mutation in metG by 1) the amino acid requirement, 2) the inability of the strain to grow in M9 supplemented with vitamin B₁₂ and homocystine (Blumenthal, 1972), and 3) the map position of the met mutation (it is covered by the F' plasmid F103).

^cHDF125 is unable to grow on plates supplemented with methionine when sodium succinate is the carbon source and therefore may carry a mutation affecting electron transport (Cox et al., 1968). The mutation may be in ubiG.

^dHDF026, HDF303, and HDF341 are all thought to have mutations in glp genes, probably glpT or glpA. HDF026 and HDF303 do not grow when DL-α-glycerophosphate is the carbon source. HDF341 grows on DL-α-glycerophosphate or glycerol as the carbon source, but does not incorporate ³H-glycerol into phospholipids (see Section IV, Part A2).

replication forks (Cerdeña-Olmedo et al., 1968). These mutations are so tightly linked that P1 transduction of the desired mutation, a method often used to "clean up" multiple mutants, also transduces unwanted mutations along with the one of interest. The metG mutation in HDF125 and the mutation responsible for the Suc^- phenotype of the same strain (Table 4-1) may have arisen in this manner as a cluster of mutations that includes hdf.

Because of the use of nitrosoguanidine as the mutagen, we were uncertain about the relevance of observed phenotypes to the hdf lesion; therefore, all experiments were done with several HDF strains. We present results here chiefly for HDF125 and mention other strains for which similar results were obtained. Occasionally a strain other than HDF125 was the strain of choice for a particular experiment; the reasons are discussed in each case.

B011' and hd590 are, respectively, a strain of E. coli B and a nitrosoguanidine-induced HDF-type derivative which have been characterized by L. D. Simon et al. (1974) and kindly supplied by him for comparative purposes. Data for these strains appear throughout the results; a summary comparison of hd590 and our K-12 strains comprises Section V.

II. The defective phenotype of $T4^+$ -infected HDF cells

A. Growth of $T4^+$ and goF-1 on HDF strains

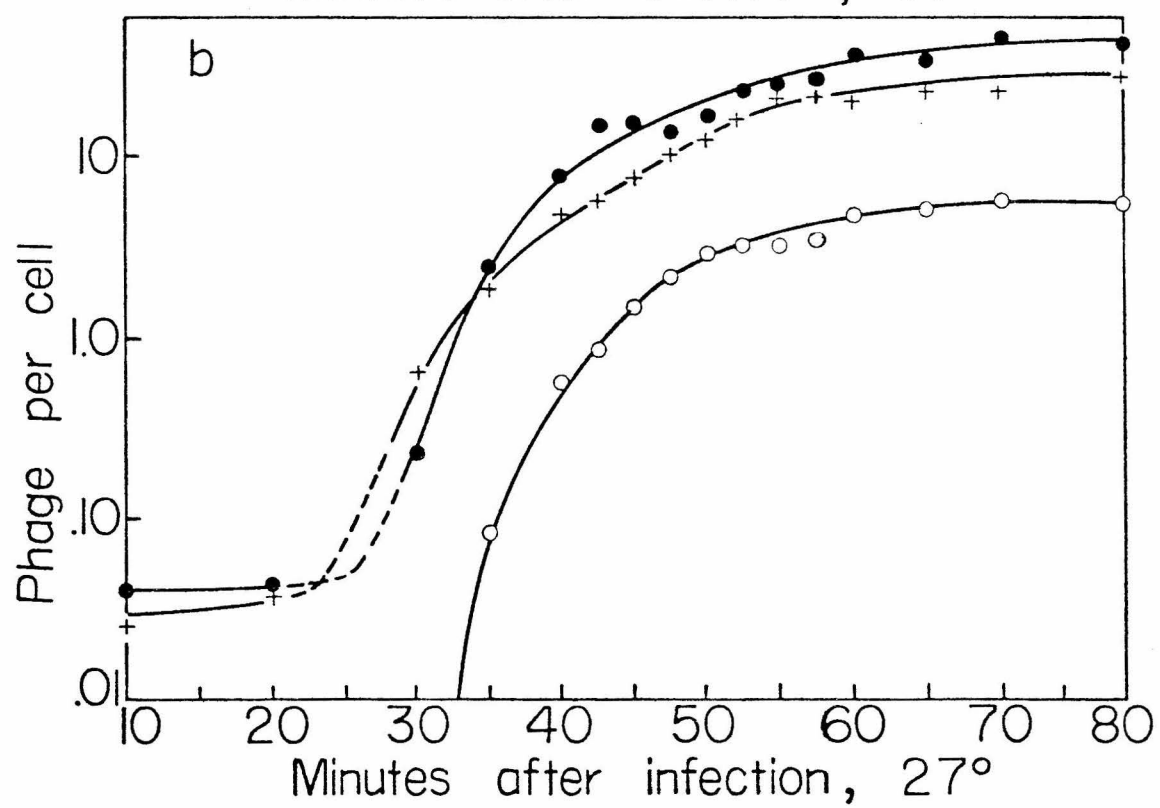
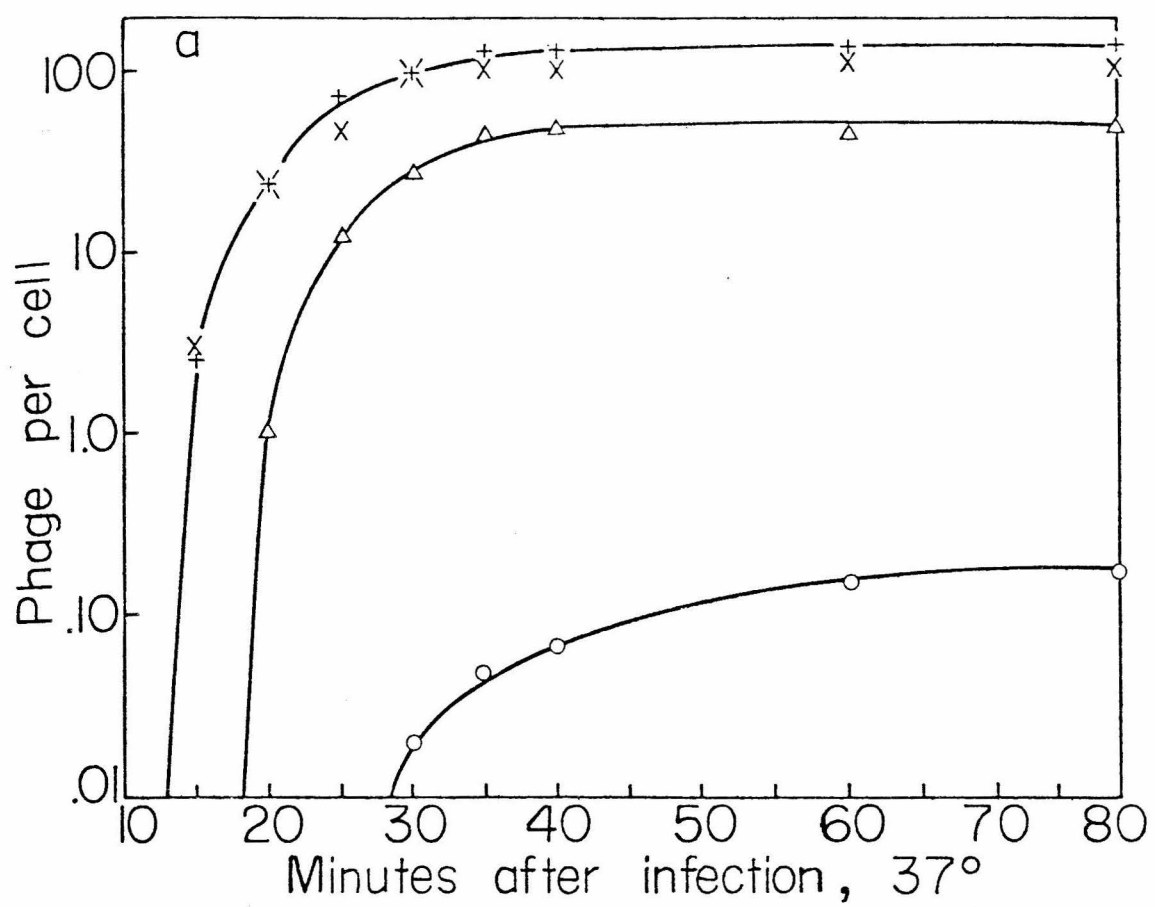
Figure 4-1a shows typical intracellular phage growth curves for $T4^+$ and goF-1 on HDF125 at 37°C. It is clear that phage production is virtually eliminated in $T4^+$ -infected HDF125 and is restored to about half the normal level by the goF-1 mutation in $T4$. Figure 4-1b shows a similar experiment performed at 27°C where the production of wild-type phage is improved 100-fold over that at 37°C. These two experiments illustrate the ts nature of the hdf mutation. Similar results were obtained for HDF026, HDF341, HDF303, and hd590 (see Tables 4-1 and 4-4 for burst sizes). Figure 4-1c shows intracellular phage growth curves at 25° C and 37°C for HDF026 and HDF026R, illustrating the observation that HDF026R is cs for $T4^+$ growth.

B. Lysis of HDF cells following $T4$ infection

In order to lyse its host, $T4$ must disrupt at least two barriers: the cell's inner membrane and the cell wall. The mechanism of inner membrane disruption is not understood; the destruction of the cell wall is accomplished by a phage-coded lysozyme which, however, can act only if inner membrane disruption has already started, thus giving the lysozyme access to the cell wall. In Chapter 3, Section II, we presented curves that illustrate the slow and delayed lysis of $T4^+$ -infected HDF183; Figure 4-2 summarizes the lysis data for HDF bacteria and shows complete curves for several additional strains. Lysis at 37°C is clearly delayed from 5-30' past the normal time. We also tested $T4^+$ -infected HDF cells for the presence of intracellular lysozyme by shaking samples of the cultures with chloroform, which disrupts the membrane, at various times after infection. Both HDF cells and wild-type cells showed prompt lysis in response

Figure 4-1. Intracellular growth of phage on HDF strains.

- a. +, B178 infected by $T4^{+}$; x, B178 infected by goF-1; Δ , HDF125 infected by goF-1; o, HDF125 infected by $T4^{+}$.
- b. +, B178 infected by $T4^{+}$; ●, HDF125R infected by $T4^{+}$; o, HDF125 infected by $T4^{+}$.
- c. ●, HDF026R infected by $T4^{+}$; o, HDF026 infected by $T4^{+}$. The solid lines indicate experiments performed at 37°C and the dotted lines, experiments performed at 25°C.



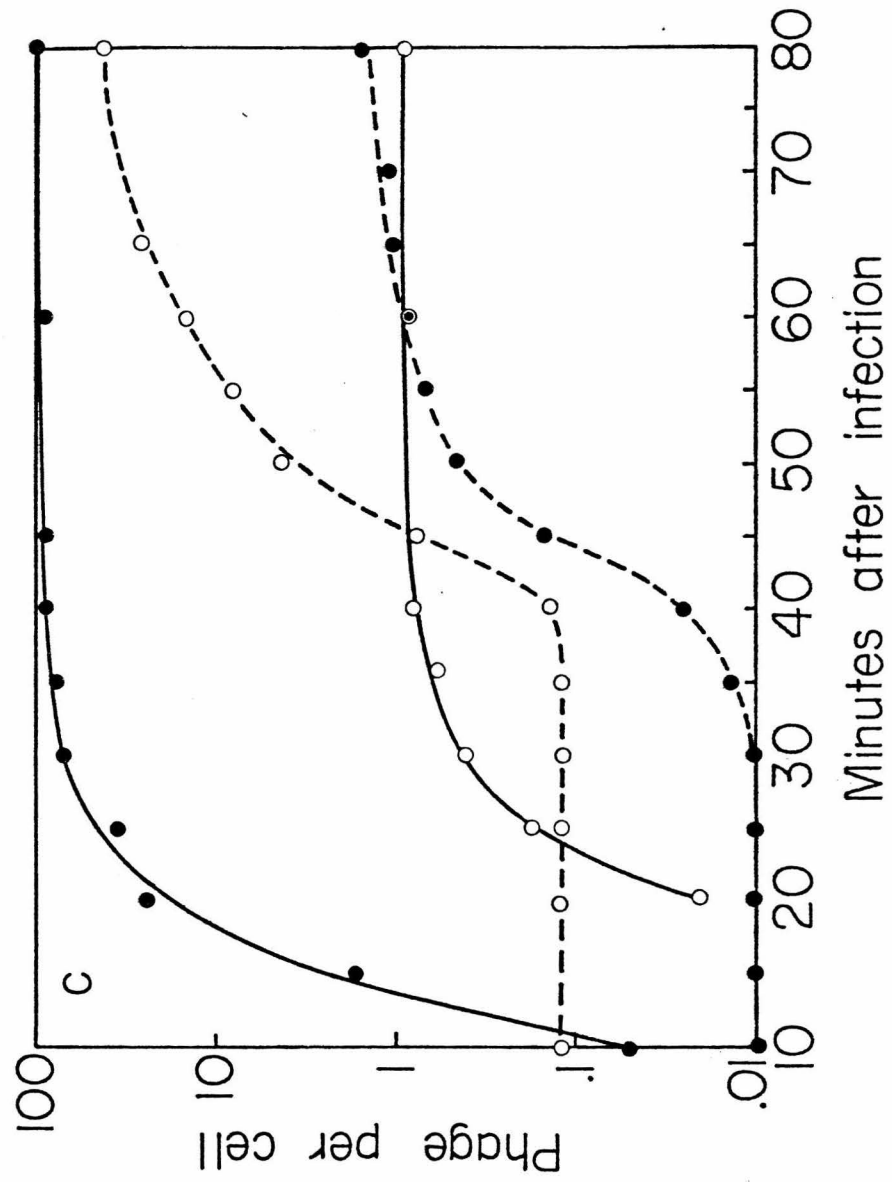
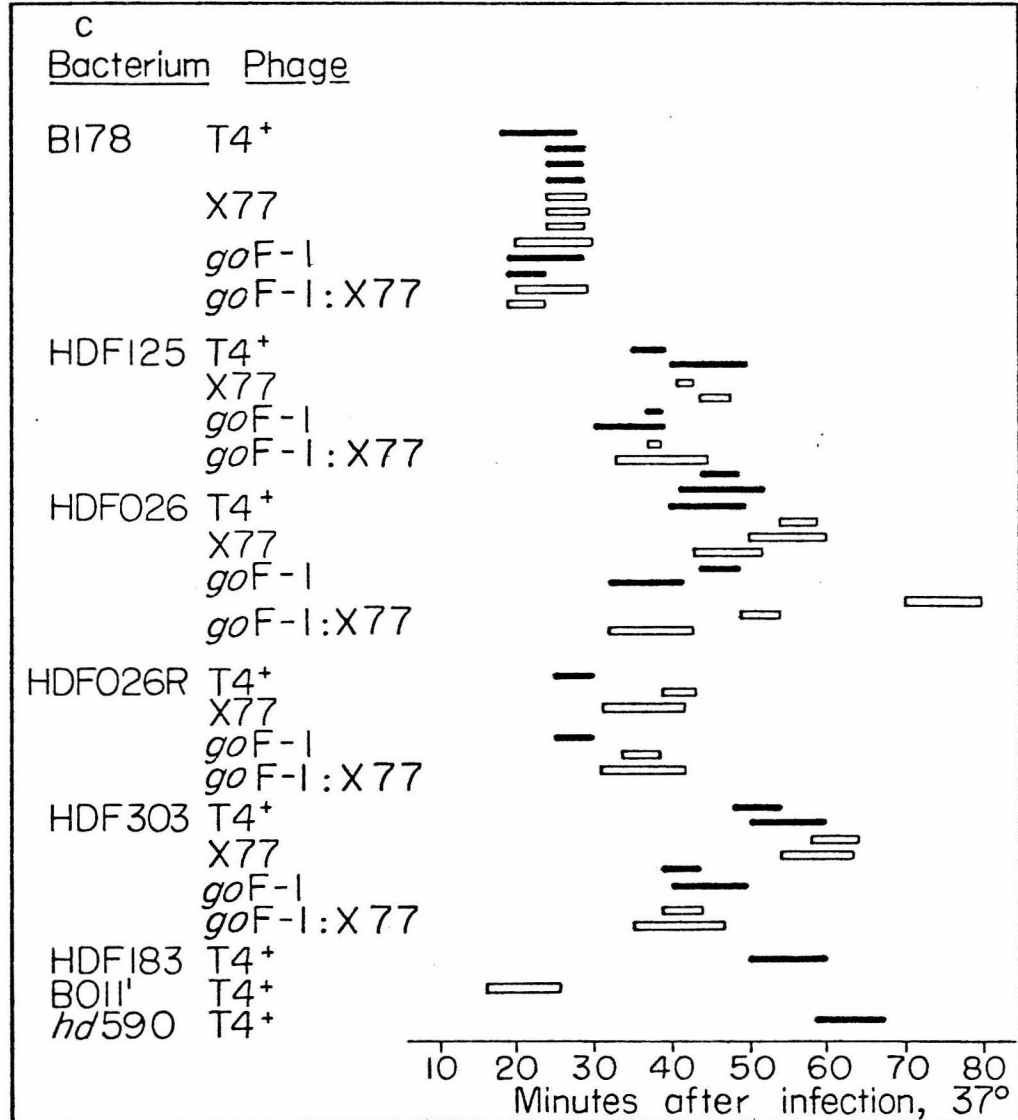
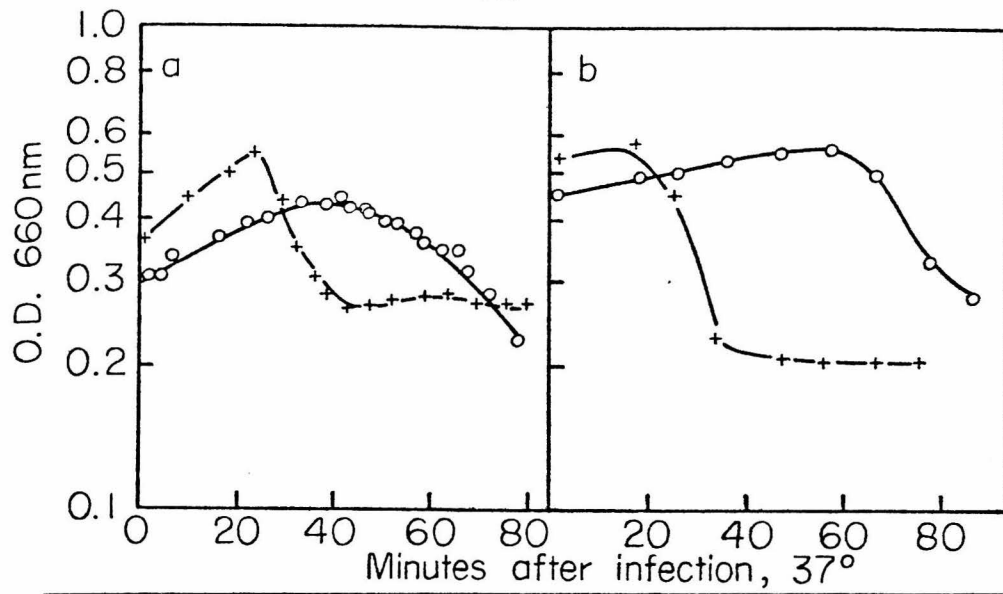


Figure 4-2. Lysis of HDF cells following phage infection. Phage used are $T4^+$, goF-1, X77, and the multiple mutant goF-1:X77.

a. +, B178 infected with X77; o, HDF125 infected with X77.

b. +, B011' infected with $T4^+$; o, hd590 infected with $T4^+$.

c. Summary of lysis data. Each bar indicates for one experiment the time interval within which lysis started, as determined by a drop in O.D. between two successive measurements. Some experiments were performed with phage carrying amber mutations in virion structural proteins to avoid the possibility of lysis inhibition by superinfecting progeny phage. This was not always necessary; a drop in O.D. was often detected at the expected time even in infections of wild-type bacteria with $T4^+$. Whether such an O.D. drop occurs may depend upon the synchrony of infection. Included here are all experiments in which an O.D. drop was seen.



to this treatment starting at 12-15' after infection, a crude indication that lysozyme production in HDF cells is normal (data not shown). This result implies that the delayed lysis shown by $T4^+$ -infected HDF cells may be due to defective breakdown of the inner membrane. However, lysis inhibition, another process that must affect inner membrane disruption, seems normal in HDF cells: an increased burst size results when goF-1 infects HDF cells under superinfection conditions, and either superinfection or the presence in the infecting phage of a mutation in gene t (Josslin, 1970) makes it possible to centrifuge infected HDF cells without lysing them.

When HDF cells are infected by goF-1, a slight shift towards earlier lysis may be seen (Fig. 4-2) but a delay is still clearly evident. This result agrees with the still-subnormal burst sizes found under the same conditions (see Part A, above).

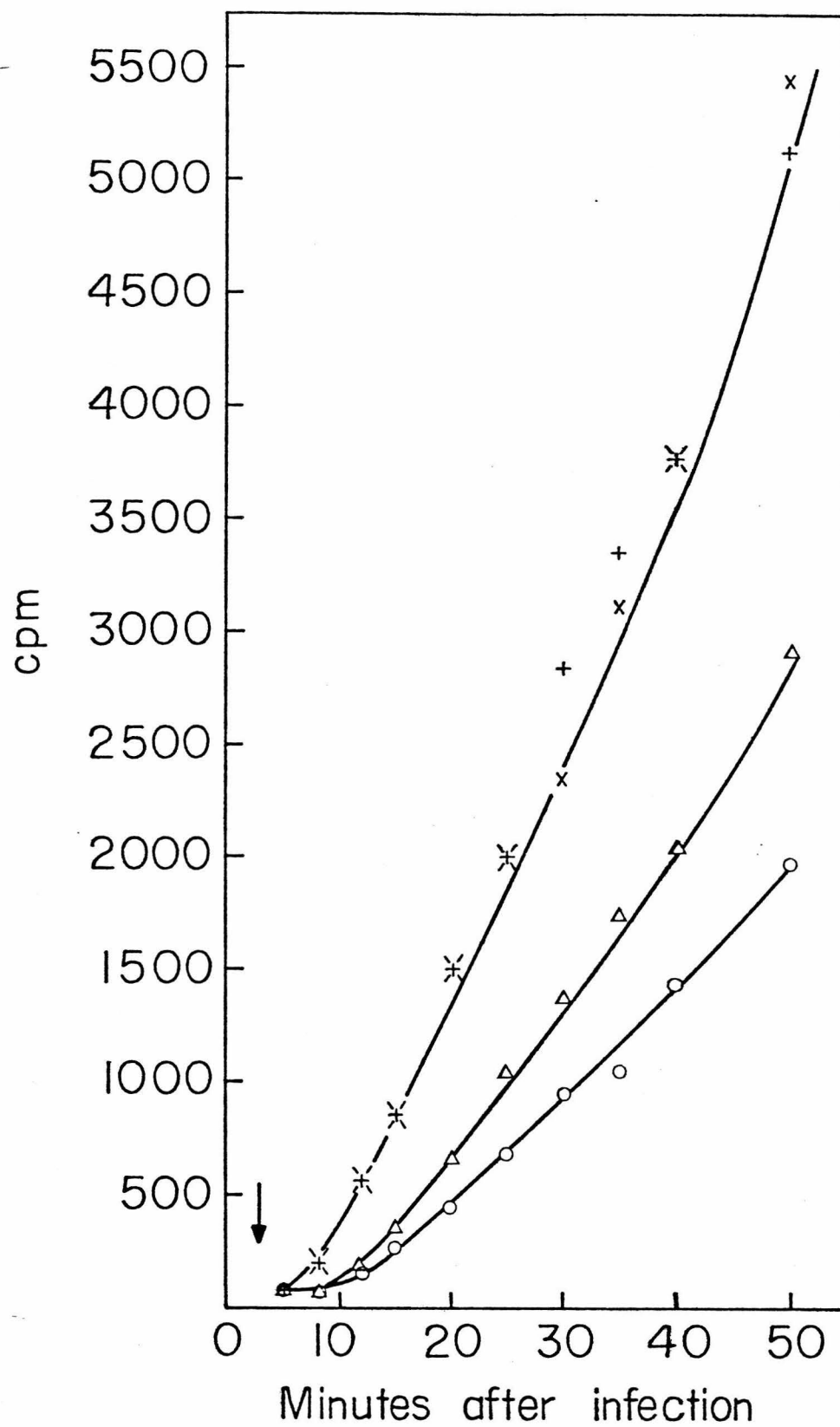
At 25°C, $T4^+$ -infections of HDF strains continue to show delayed lysis, but, as in infections with goF-1 at 37°C, the delay is less severe than that seen in $T4^+$ infections at 37°C (data not shown).

Is the observed delay unique to lysis in HDF cells or does it reflect delays in other processes of the $T4$ infection? In Parts C and D, below, we demonstrate slight delays in both the onset of phage DNA synthesis and in the appearance of certain late phage proteins. Neither of these delays, however, is as severe as the lysis delay.

C. Synthesis of $T4$ DNA in HDF cells

As shown in Figure 4-3, the rate of $T4$ DNA synthesis in HDF125 is about half that in the parental strain, and there is a 3-6' delay in the start of incorporation. Infection with goF-1 improves the incorporation rate but does

Figure 4-3. Cumulative T4 DNA synthesis in HDF cells. The arrow indicates the time of addition of ^{14}C thymidine to infected cells. For experimental details, see Materials and Methods, Part L. +, B178 infected with T4^+ ; x, B178 infected with goF-1; Δ , HDF125 infected with goF-1; o, HDF125 infected with T4^+ .



not fully restore it or correct the initial delay. We therefore conclude that the delay in DNA synthesis is not crucial to the impairment of phage production in these strains, but the depression in rate probably is. In agreement with this conclusion, Simon et al. (1974) found that in $T4^+$ -infected hd590, DNA synthesis is depressed (but not delayed). Results that show both the depression and the slight delay were found for HDF026, HDF303, and HDF183.

D. Synthesis of T4 proteins in HDF cells

T4 protein synthesis was investigated using polyacrylamide gel electrophoresis to analyze samples of $T4^+$ -infected B178 and HDF026 labeled at various times with ^{14}C amino acids. As shown in Plate 4-1, there is no delay evident in the appearance of early proteins in HDF026, and early protein shutoff is more prompt than in B178 (note gprIIA and gp42 + gp45). The times of appearance of late proteins, however, do show some delays; for example, gp34 and gp37 are at least 2' late in Plate 4-1A. gp18 and gp23, on the other hand, appear at the normal time. No delay is seen in Plate 4-1B, although gp34 and gp37 are under-produced. We therefore conclude that a broad inhibition of the synthesis of T4 proteins does not occur in HDF026.

It is clear from the gels in Plate 4-1, however, that a few specific early and late proteins are decreased in quantity or missing in $T4^+$ -infected HDF cells. We have not been able to identify all the bands that are affected or to meaningfully interpret the specific changes that are seen. A more detailed discussion of band identities and appearance is given in the Appendix; we limit the present discussion to the following points.

- 1) Four specific early proteins of molecular weights 58,000, 43,000, 36,000, and 26,000 daltons, none of which has been identified, are reduced or

plate 4-1. Time of appearance of phage proteins in $T4^+$ -infected HDF026. HDF026 was used for most gel experiments because HDF125 behaves anomalously under the conditions used for labeling phage proteins (see text). Samples were labeled for the indicated periods, chased, and prepared for electrophoresis as described in Materials and Methods, Part M1. "+" indicates $T4^+$ infection of B178; "F" indicates $T4^+$ infection of HDF026. "A" and "B" indicate independent samples run on different 10 cm 10% SDS-polyacrylamide gels. The portion of Plate 1A above the main gel is an overexposure of the upper bands, included for better visualization of gp34, gp7, and gp37. At early times in Plate 1A numerous bacterial bands are visible, particularly in the 2-4' labeling period and to a lesser extent in the 4-6' labeling period. Most phage bands were identified by the comparison of proteins made in wild-type infections to those made in infections of nonpermissive cells with phage containing known amber mutations. The identifications of gp7, gp43, gp32, and gp22 were made by comparison with published gels (Vanderslice and Yegian, 1974 and O'Farrell et al., 1973). Molecular weights of unidentified bands were estimated from a plot of the distances which known bands migrated through a 10% gel against the log of their molecular weights. Such plots were found to be linear from approximately 80,000 MW to at least 20,000 MW. 23:63:rII indicates infection of B178 with a phage carrying the mutations amB17, amM69, and rEDdf41. Bands are labeled with the name of the gene whose product they are or by a "p" followed by their estimated molecular weight.

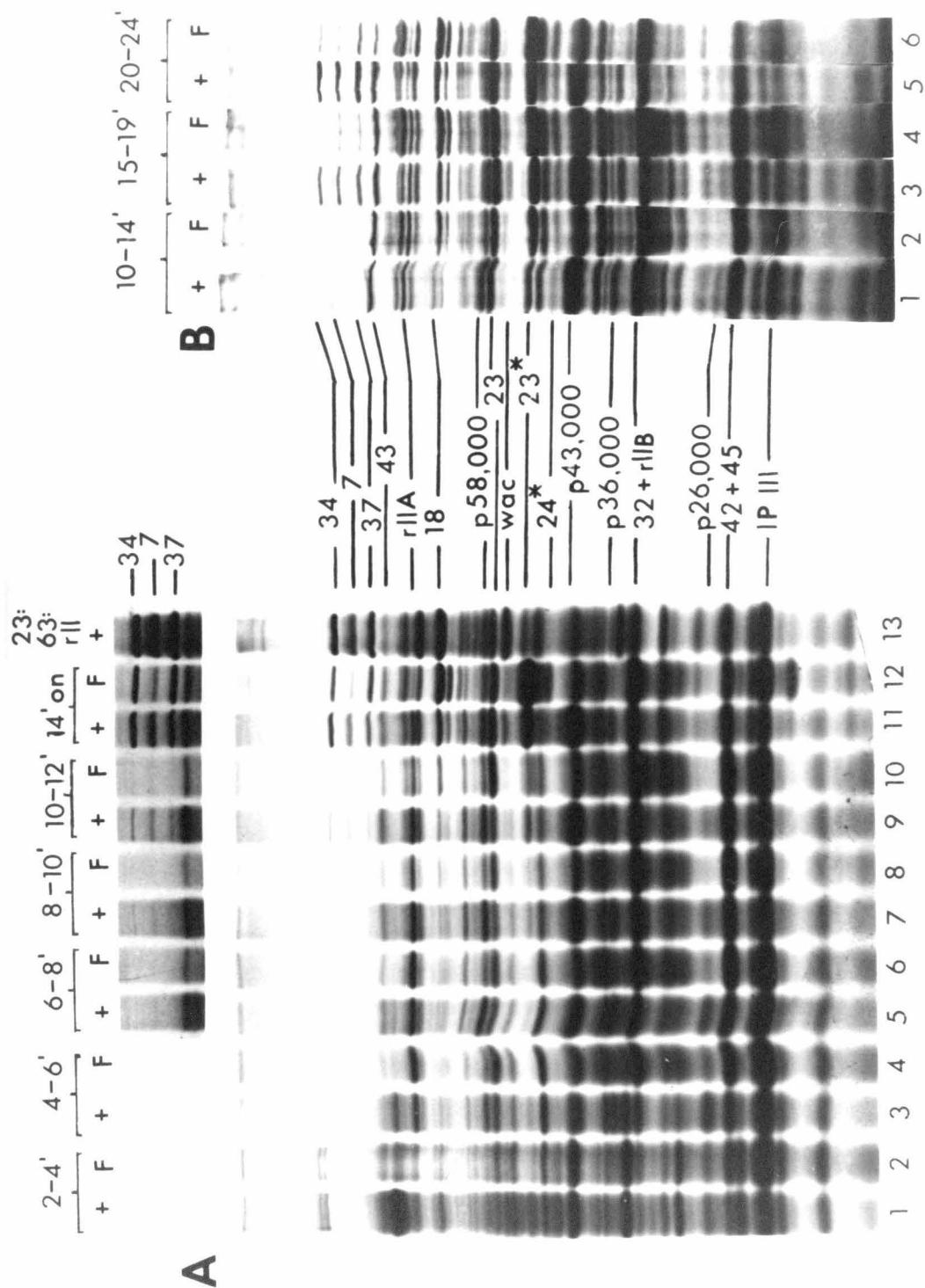
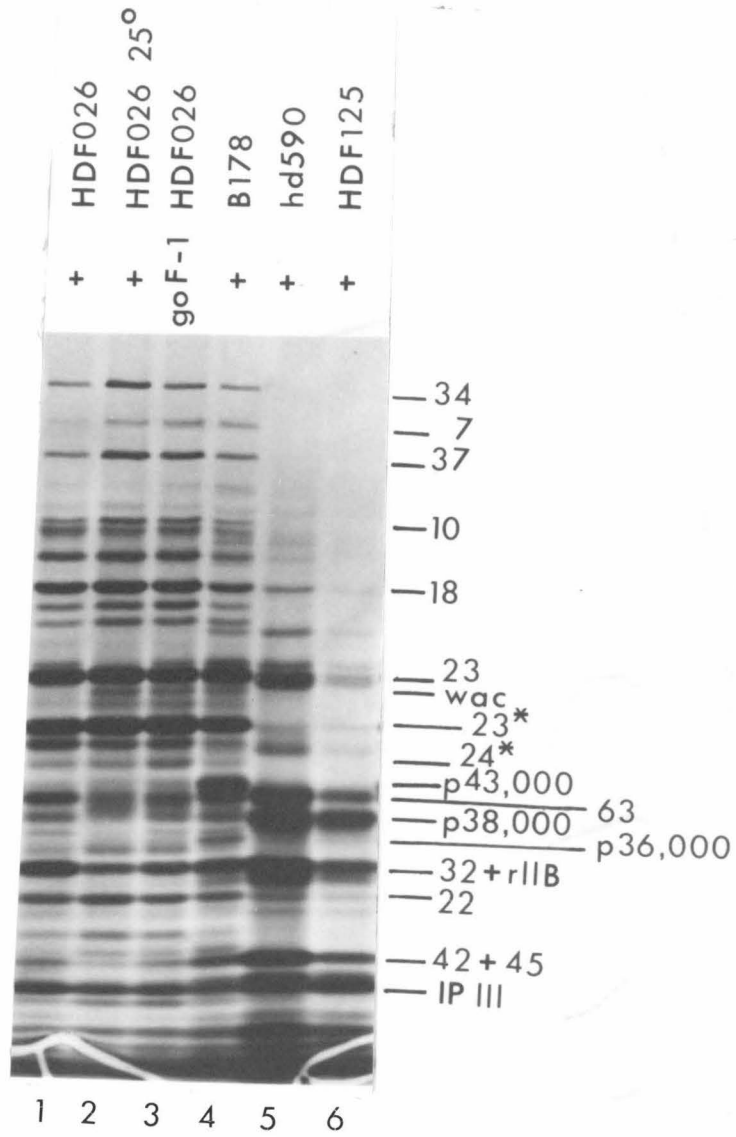


plate 4-2. Gel electrophoresis of T4 proteins synthesized in HDF026, HDF125, and hd590, and in HDF026 under permissive conditions. The phage and host used for sample preparation are indicated immediately above each track; + means $T4^+$. All infections were at 37°C unless otherwise indicated. Cells labeled at 37°C received 1 μ Ci of 14 C amino acids at 20' and 25' after infection (the label was chased at 30') and those labeled at 25°C, 1 μ Ci at 45' and 57' after infection (the label was chased at 70'). The gel is a 12 cm, 30 ml exponential gradient of 7 1/2-20% acrylamide poured by holding the volume of acrylamide in the mixing chamber constant at 5 ml.



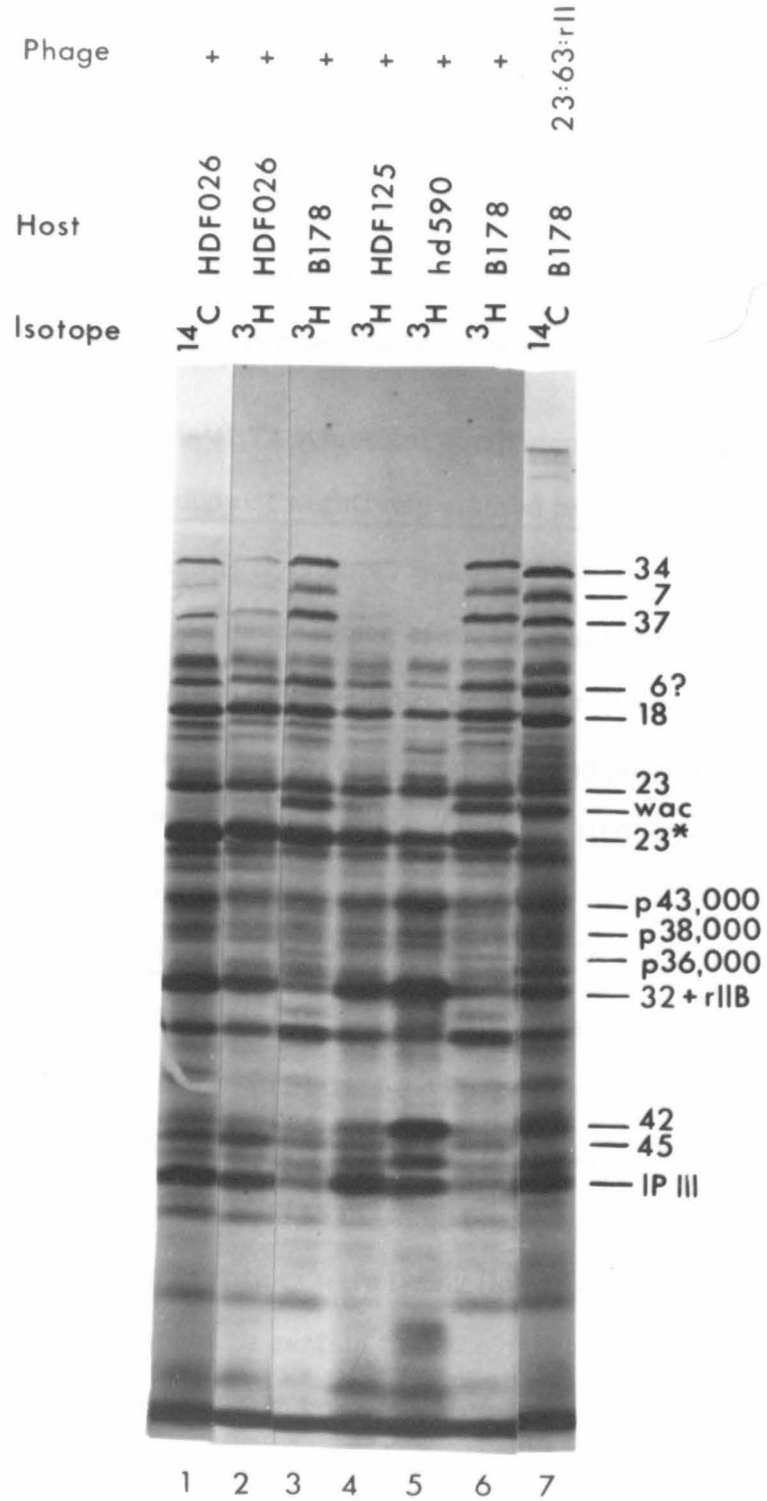
missing in $T4^+$ infections of HDF026 (Plate 4-1). p43,000 and p36,000 are also missing at late times, both under nonpermissive (Plate 4-1) and permissive (Plate 4-2) conditions.

2) There are increases in the amounts of some proteins made in $T4^+$ -infected HDF cells. gp32 + gpIIIB and gpIPIII are overproduced throughout infection (Plates 4-1 and 4-3), and at late times a band just below gp23* is present in greater than normal quantity (Plates 4-1 and 4-2). An overproduction of gp32 might be caused by the defective DNA synthesis found in HDF cells (Krisch et al., 1974).

3) At least four virion structural proteins, gp34, gp7, gp37, and gpwac, and probably a fifth, gp10, are reduced under nonpermissive conditions in HDF026 (Plate 4-1). All return under permissive conditions (Plate 4-2). Similar observations were made for HDF125, HDF303, and hd590.

4) HDF125 and hd590 gave anomalous gel patterns when extracts for gels were made under the usual conditions (in M9 supplemented with methionine or thymine, respectively) (Plate 4-2, tracks 5 and 6). Proteins known to be present from electron microscopy or in vitro complementation experiments such as gp18 and gp23* are scarcely visible. In addition, sometimes only gp23, but not gp23*, is seen, although we find empty heads (and therefore gp23*) at these times (see Part E and Plate 4-5). Several protein bands are overproduced, including gp32 + gpIIIB, gp 42 + gp45, gpIPIII, and a band of approximately 38,000 MW. Since all other experiments investigating the phenotype of $T4^+$ infections of HDF cells were performed in H broth, we decided to prepare new extracts, making the labeling medium as rich as possible. Gels of cells grown and infected in heavily supplemented M9 and labeled with 3H leucine are shown in Plate 4-3; under these

plate 4-3. Gel electrophoresis of proteins synthesized in ^3H leucine-labeled T4^+ -infected HDF025 and hd590. ^3H - and ^{14}C -labeled samples of T4^+ -infected HDF026 are included as a control for label-dependent differences in the gel patterns, and 23:63:rII (amB17:amM69:rEDdf41) is included for band identification purposes. For details of the medium and labeling procedure for ^3H samples, see Materials and Methods, Part M2. All samples were labeled from 15-35' after infection. ^{14}C - and ^3H -labeled samples were made on the same day and run on different halves of the same 13 cm, 10% acrylamide gel. After electrophoresis, the ^3H -containing half was cut away and impregnated with PPO. Both halves were then dried, autoradiographed, and printed. The desired ^{14}C -labeled tracks were cut out of the print, aligned with the tracks in the print of the ^3H -labeled samples, and photographed.



conditions the patterns of phage proteins in $T4^+$ -infected HDF125 and hd590 resemble that seen in HDF026.

In summary, specific early and late proteins are decreased in quantity or missing $T4^+$ -infected HDF bacteria. The late proteins return under conditions of infection permissive for phage growth; we have not obtained clear evidence concerning the early bands under permissive conditions.

E. Synthesis of T4 structural components in HDF cells

1. Electron microscopy of negatively stained preparations

We examined negatively stained lysates of $T4^+$ -infected HDF cells in the electron microscope for the presence of major phage structures. While in control extracts heads, tails, and baseplates were seen (Plate 4-4), $T4^+$ -infected HDF cells showed only empty heads, rare polyheads and polysheaths, and no tail structures (Plate 4-5). The same result was found for HDF strains 125, 026, 303, 183, 311, 341, and hd590.

2. Electron microscopy of thin sections

Thin sectioned preparations of the same infected cells that were examined by negative staining are shown in Plate 4-6. Phage heads full of DNA were seen in $T4^+$ infections and empty capsids in 16^- phage infections of B178, as expected. All cells of strain HDF125 in which phage structures were visible seemed to be ripped open as if they were lysing (lower edge of Plate 4-6A) and for this reason we cannot be certain that the phage heads inside have retained whatever DNA they may have had; however, both full and empty-appearing capsids were found in these cells, indicating that at least some DNA is packaged by $T4^+$

plate 4-4. Phage structures found in negatively stained lysates of T4-infected cells. A and C, B178 infected with 16^- phage; B, B178 infected with $18^-:27^-$ phage; D, host-defective strain HD310 infected with $T4^+$. The following abbreviations are used: eh, empty head; fh, full head; t, tail-baseplate; bp, baseplate; ps, polysheath. The scale of these micrographs is indicated by the following information. The length of the tail sheath is 94 nm, the length of the head of a completed virus particle, 110 nm, and the diameter of the baseplate from vertex to vertex, 40 nm (Eiserling and Dickson, 1972). Approximate magnifications for A and B are X120,000, for C, X180,000, and for D, X100,000.

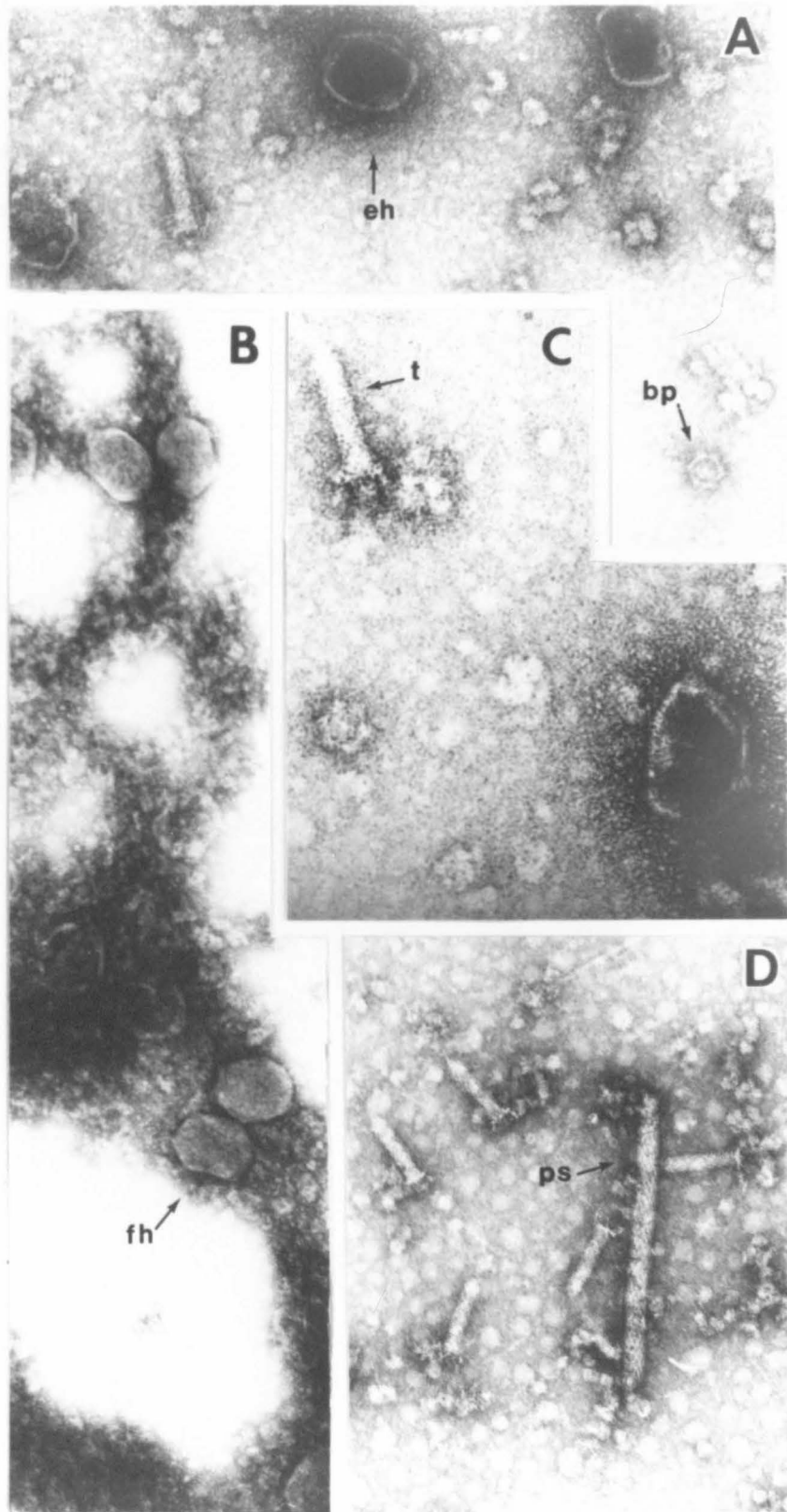


Plate 4-5. Phage structures found in negatively stained lysates of T4⁺-infected HDF cells. A, HDF183; B, HDF125; C, HDF026; D, HDF303. The following abbreviations are used: eh, empty head; ps, polysheath; ph, polyhead; f, flagellum. Approximate magnifications are: A, X70,000; B and C, X110,000; D, X150,000.

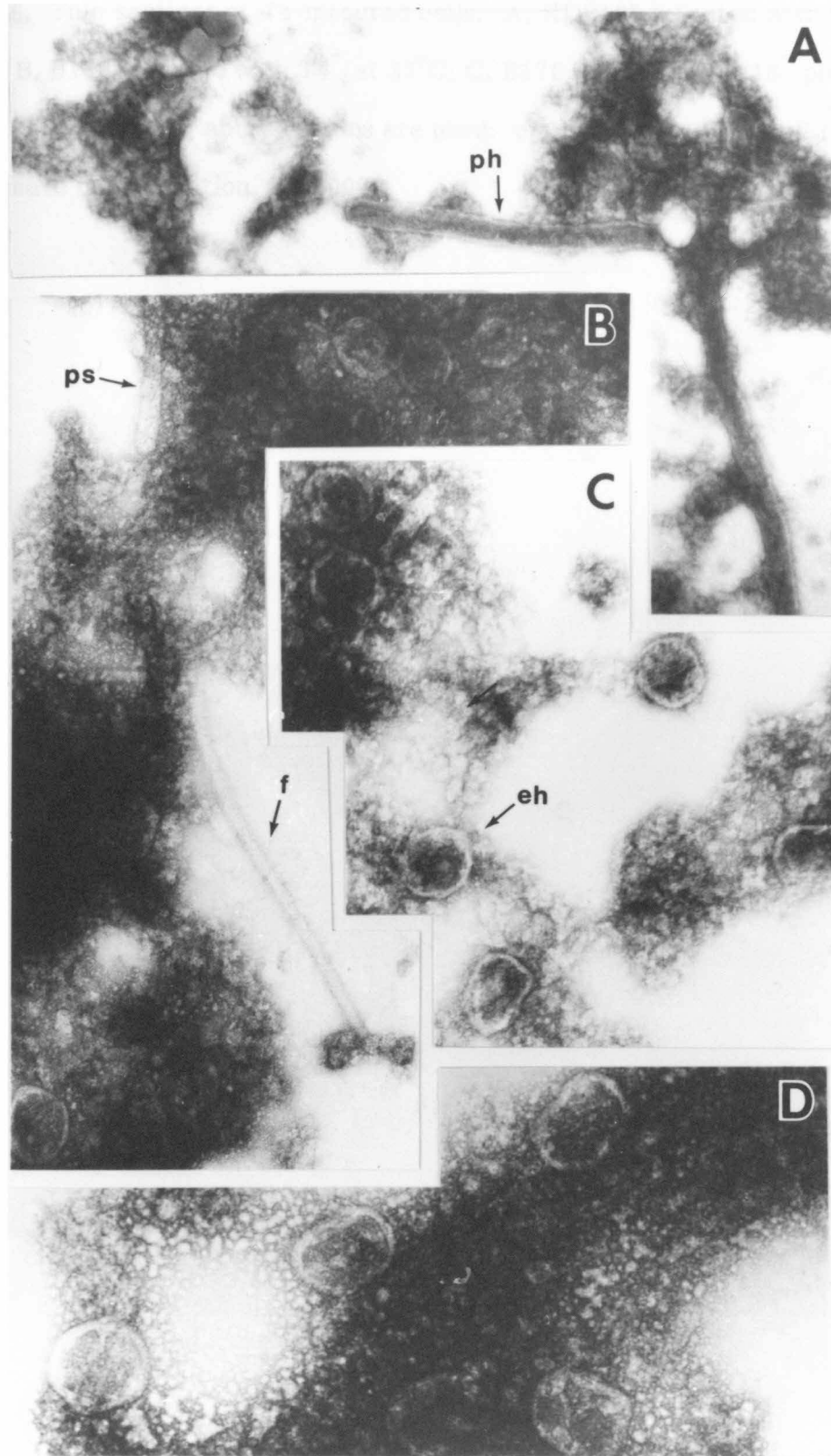
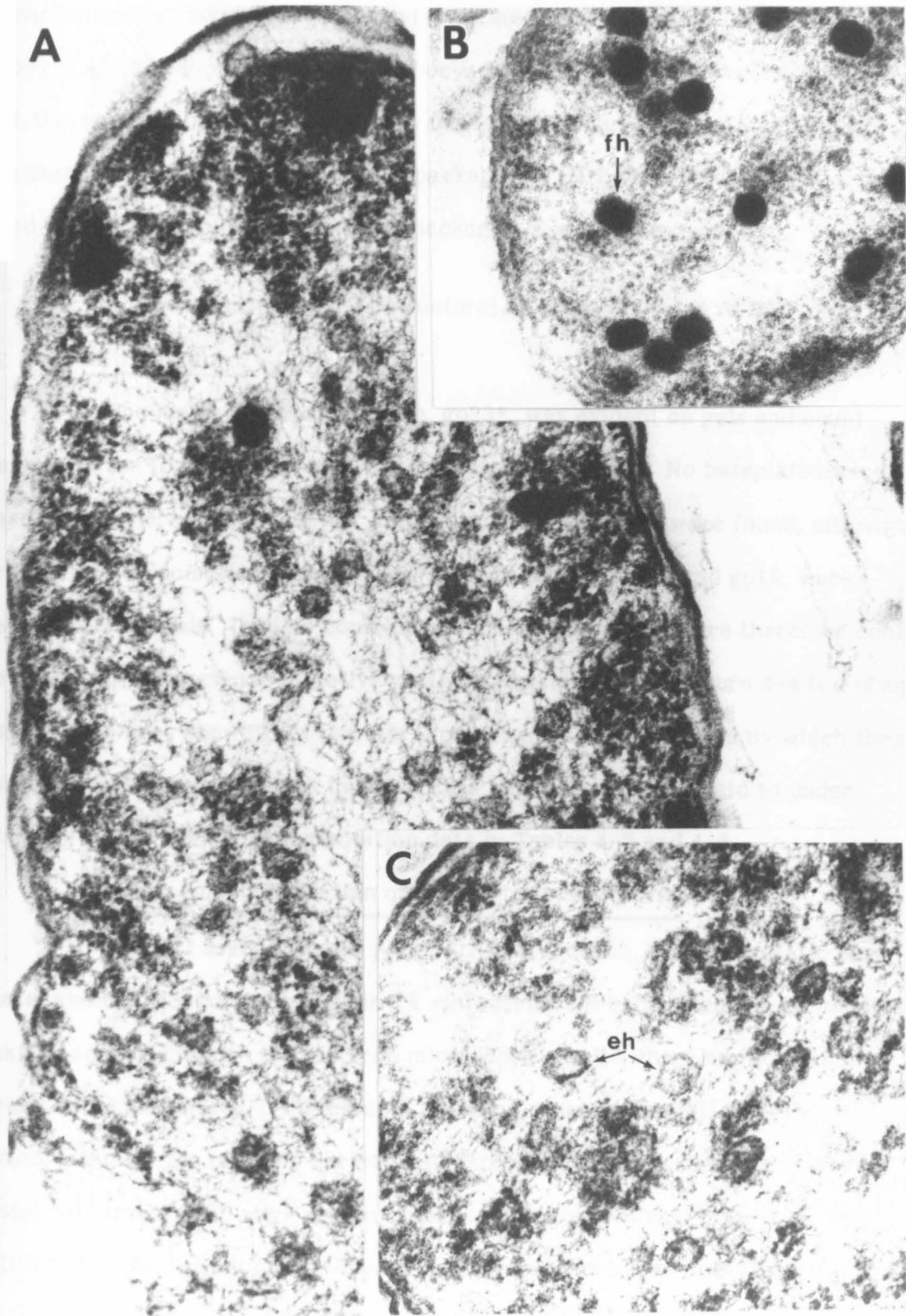


plate 4-6. Thin sections of T4-infected cells. A, HDF125 infected with T4⁺ at 37°C; B, B178 infected with T4⁺ at 37°C; C, B178 infected with 16⁻ phage at 37°C. The following abbreviations are used: eh, empty head; fh, full head. Approximate magnification, X70,000.



in HDF125. A further indication of packaging was found in HDF125 cells infected under permissive conditions: negatively stained preparations of T4⁺ grown on HDF125 at 25°C showed more empty heads than full heads or completed phage, but thin sections of the same cultures showed only full heads (data not shown). We therefore conclude that the DNA packaged in HDF cells is less stably associated with the phage capsid than that packaged in wild-type cells.

F. Biological activity of T4 structural components made in HDF cells

1. Introduction

The major T4 capsid protein, gp23*, was evident on gels and could be seen in the form of capsids in the electron microscope. No baseplates were seen in the microscope, however, and only rare polysheaths were found, although proteins which comprise these structures, such as gp7, gp10, and gp18, were readily seen on gels. In vitro complementation experiments were therefore done to ascertain the presence of active proteins and structures. Figure 4-4 is a phage assembly scheme showing T4 structural proteins and the pathways by which they assemble into phage particles in vitro. It is included here as an aid to understanding the in vitro complementation data in Tables 4-2 and 4-3.

2. In vitro complementation of major phage structures

We first asked if major phage structures (head, tail - baseplate, baseplate, and tail fibers) were made in T4⁺-infected HDF cells. Table 4-2 indicates that, in agreement with the electron microscope results, there are no active heads, tail - baseplates, or baseplates. In some cases active tail fibers were found, although the quantity varied from experiment to experiment and often distal half fibers (BC') were present in excess of proximal half fibers (A). For HDF125 this finding disagrees with the gel electrophoresis results (Part D, above)

Figure 4-4. Pathway of T4 assembly. Structures are shown roughly as they appear in the electron microscope, except for the intermediates in baseplate assembly at the left which have not been seen and are shown schematically. The left hand portion of the baseplate assembly pathway is not in scale with the rest of the figure. Proteins known to be structural components of the phage are denoted "gp" followed by the name of the gene; nonstructural proteins or proteins of unknown status are written with a "g" preceding the name of the gene. Brackets around a structure indicate instability. Proteins and structures interact only in the order shown, with the exceptions of gp9 and gp12, whose placement in the scheme is arbitrary. Baseplates are assembled from one plug and six wedges. Adapted from Kikuchi and King (1975c) and Wood et al. (1968).

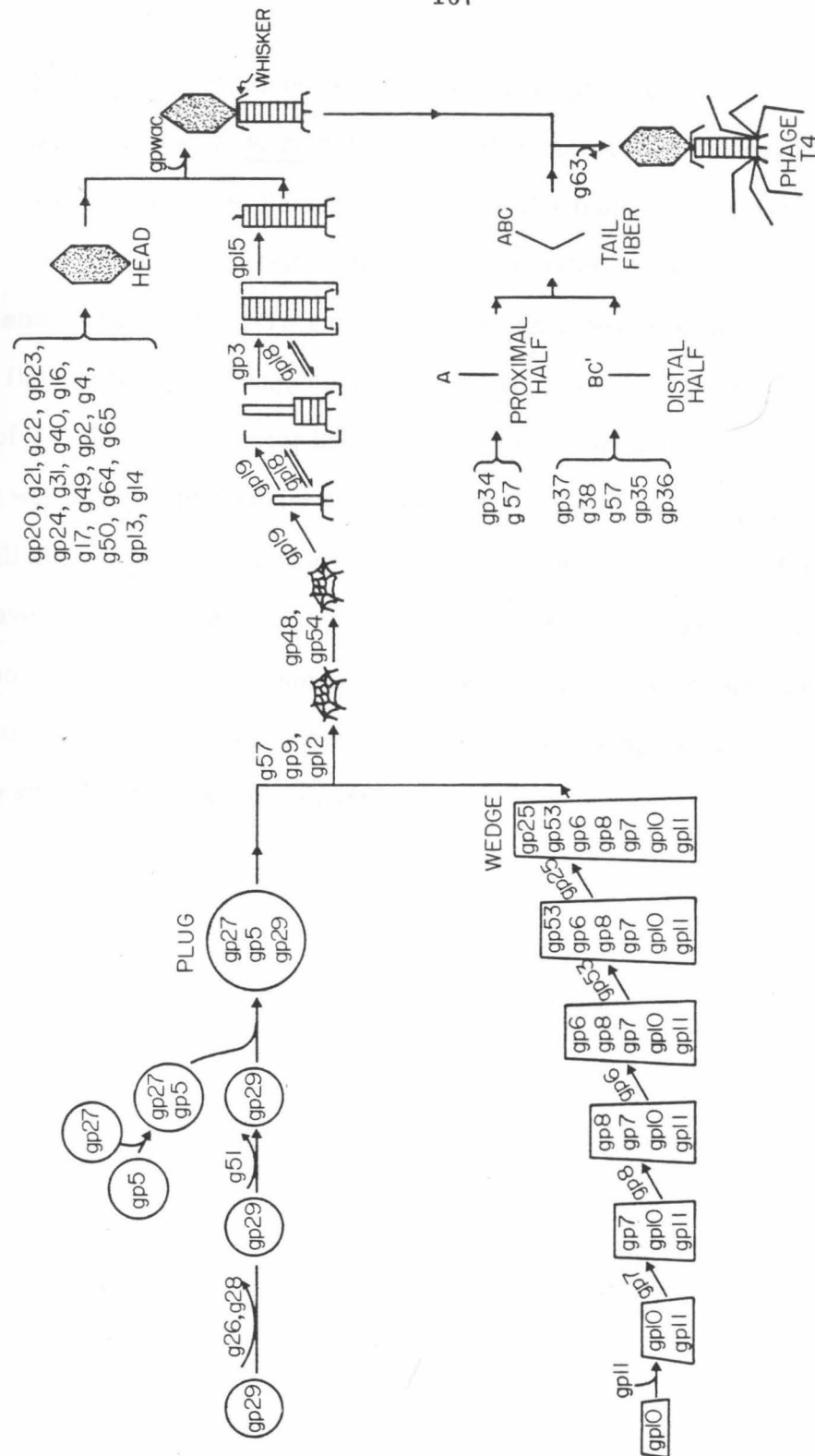


Table 4-2. In vitro complementation of major phage structures and measurement of tail fiber antigens. In vitro complementation and antigen assays are described in Materials and Methods, Parts Q and R. Results from representative single experiments are shown. Phage strains used are listed in Tables 2-2 and 2-3. "bp" stands for baseplate. To achieve low backgrounds in serum blocking assays of tail fiber antigens, a phage containing the rII deletion rEDdf41 was used in place of $T4^+$ and the indicator bacteria were $G(\lambda)$. Growth of this phage on HDF strains was very slightly better than the growth of $T4^+$, but phage production was still always ≤ 1 progeny phage per cell. A "phage equivalent" of antigen is the average amount carried by rEDdf41 phage that have been purified by centrifugation to equilibrium in cesium chloride gradients and which are used in the determination of a standard curve for the serum blocking assay; they are assumed to have six tail fibers per virus particle.

TABLE 4-2. In vitro complementation of major phage structures and measurement of tail fiber antigens

		In vitro complementation: Major phage structures pfu x 10 ⁻⁹							Tail fiber antigens/ phage equivalents/ cell			
Missing structure and mutant phage gene(s)	Bacterial host	Uncomplemented	Head ⁻ 23	Tail+bp ⁻ 18:27	Basepl. ⁻ 5:6:7:t	Tail fiber ⁻			A		BC'	
						Whole 34:34:37	A 34	BC' 37:37	t=0	t=40	t=0	t=40
Head ⁻ 23	B178	1.3		60		480			129			273
Tail+bp ⁻ 18:27	B178	0.4	60			200			170			264
Tail ⁻ 15:t	Bb	8.0			2300							
19:t	Bb	3.6			470							
Baseplate ⁻ 5:6:7:t	Bb	0.5										
Whole fiber ⁻ 34:34:37	B178	3.2							6	7	27	19
Proximal fiber ⁻ (A) 34	B178	0.4						1600	3	4	31	248
Distal fiber ⁻ (BC')	B178	1.2						1600	17	176	16	11
T4 ⁺	HDF12-5	7.9	9.3	11.6	11	62	155	950	4	68	33	321
T4 ⁺	hd590	0.2	0.9	2.2		4	1.6	64	<3	6	22	24

where gp34 and gp37 were only rarely and faintly seen. Table 4-2 also shows the results of serum blocking experiments in which tail fiber antigens were quantitated. For HDF125, these results again disagree with the gel results. One possible explanation for this discrepancy lies in the different media used for the two experiments: H broth for in vitro complementation and antigen measurements, and supplemented M9 for the gels. How the media might cause the different results is not understood. HDF026 and HDF303 also gave varying results when tail fiber antigens and activity were assayed, the former averaging 10% of the antigen found in wild-type cells, the latter 30-50%. Neither gave positive results in head or tail complementation.

3. In vitro complementation of tail and baseplate gene products

The results of in vitro complementation experiments for all tail and baseplate proteins in HDF extracts are shown in Table 4-3. The four tail proteins (possibly excepting gp3) are active in $T4^+$ -infected HDF cells, although their levels of activity are sometimes low. All baseplate proteins, however, with four exceptions, are inactive in extracts of $T4^+$ -infected HDF cells. The exceptions are gp51 and gp11, which showed no activity in control experiments and about which no conclusions can be drawn, and gp9 and gp12, both of which showed high levels of activity. The latter two proteins have no fixed place in the baseplate assembly pathway and can even add to otherwise completed phage particles (Edgar and Lielausis, 1968). Similar results were found for HDF026.

4. Hypotheses to explain baseplate complementation results

One explanation for these results is that all baseplate proteins are synthesized in $T4^+$ -infected HDF cells but the mutation in the host prevents them from assembling properly. The observation that gp9 and gp12 are normally active

Table 4-3. In vitro complementation of tail and baseplate proteins. The results shown are from representative single experiments. Results obtained for a given gene product on separate days are shown on separate lines; results from replicate reactions run simultaneously are shown on the same line and are separated by commas. Numbers in parentheses in columns 3-6 identify the mutant gene; mutants used are specified in Tables 2-2 and 2-3. Columns 3 and 4 show the results of control experiments in which the defective extracts described in column 1 were asked to supply an active baseplate gene product (usually gp7) and an active tail gene product (usually gp15), respectively. Baseplate gene products are unstable and in vitro complementation reactions involving them are very concentration-dependent. It is quite possible, therefore, for an extract to work well in, for example, tail connector complementation reactions but poorly or not at all in baseplate gene product complementations. We therefore did the two controls described; only those experiments in which the control baseplate gene product complementation is positive are considered valid.

TABLE 4-3. In vitro complementation of tail and baseplate proteins

Phage structure and mutant phage gene(s)	pfu x 10 ⁻⁹				
	Uncom-plemented	Baseplate mutant (gene)	Tail mutant 15:t	T4 ⁺ or 19 ⁻ phages on HDF125	T4 ⁺ on hd590
			6.8	(T4 ⁺) 1.2 (19) 0.4	1.5
<u>Tail sheath</u>					
18	1.1	(7) 100	450	12	69
	23.	(10) 570		370	
	0.1	(7) 220	380	30	
<u>Tail tube</u>					
19	0.4	(7) 41	380	2.0	17
	3.8	(5) 670	3300	370	
<u>Tail connector</u>					
3	32.	(7) 1000	840	91	90
15:t	6.8			200, 520	150
<u>Baseplate plug</u>					
29	2.1	(7) 86	930	3.0	2.6
26	1.8	(7) 20	310	2.0	
	2.7	(7) 20	580	3.9	2.6
28	2.6	(7) 194	1300	3.0	1.2
51	2.6	(8) 10	210	3.0	
5	0.4	(7) 130	650	2.2	1.7
		(10) 44			
		(27) 40			
		(28) 62			
27	1.0	(7) 60	710	2.7	1.3
<u>Baseplate wedge</u>					
10	0.1	(7) 67	380	1.7	0.8
11	75.	(7) 65	150	42	62
7	1.0		360, 230, 340, 900, 1100	2.1	3.0
8	3.5	(7) 670	2200		29
	1.3	(7) 52	1600	8.0	
	7.0	(10) 316	(19) 120	20	
6	8.0	(7) 280	1100	10	9.3
53	0.6	(7) 46	430	1.6	1.8
25	5.4	(7) 28	300	2.6	4.0
<u>Baseplate completion</u>					
48:t	2.7	(8) 74	620	7.7	
54	0.2	(7) 31	400	0.8	0.8
9	5.6	(7) 150	1000	310	100
12	3.7	(7) 290	820	80	

in HDF cell extracts supports this explanation. Since proteins such as gp10 and gp7 are seen on gels but are inactive in vitro, the remainder of the negative baseplate complementation results must then, according to this hypothesis, be explained either by the formation of aberrant complexes of baseplate proteins or by the existence of an inhibitor to baseplate assembly in HDF cells. We have no evidence concerning the formation of aberrant complexes of baseplate proteins. Kikuchi and King (1975a,b,c), however, have shown that if baseplate assembly is blocked in wild type cells by an amber mutation in a baseplate gene, all proteins downstream of the affected protein in the assembly pathway accumulate in unassembled form. This observation makes the possibility of aberrant complex formation in HDF cells seem less likely. To test for the existence of an inhibitor to baseplate assembly, T4⁺-infected HDF cell extracts were added to active in vitro baseplate complementation reaction mixtures. Some inhibitory results were obtained, but these observations were not reproducible. Furthermore, control experiments showed that extracts of wild-type cells (infected with the appropriate multiple baseplate mutant so they would not supply any reaction components) could also inhibit in vitro baseplate complementations. We were thus unable to conclude that an inhibitor of baseplate assembly exists in HDF cells.

A second hypothesis is that in HDF cells most baseplate proteins are either not made or are synthesized in subnormal quantities. Since in vitro baseplate complementation reactions are so concentration-dependent, a decrease in the amount of one component could result in the failure of the in vitro assembly reactions. To test this hypothesis, we needed a protein that is active in extracts

of $T4^+$ -infected HDF cells and whose in vitro complementation behavior is sufficiently concentration-independent to allow us to estimate its relative amount when it is present in less than wild-type concentrations. gp15 is such a protein, as the dilution experiment in Figure 4-5 shows. In a comparison of wild-type and HDF125 extracts as sources of gp15 for in vitro complementation reactions (Fig. 4-5), we estimate that there is 5-8x less gp15 activity in the HDF extract. HDF026 gave almost identical results (data not shown), and hd590 shows even less gp15 activity. If certain baseplate proteins in HDF cells are similarly depressed in activity or quantity, then that depression could be responsible for the observed lack of in vitro complementation activity. This hypothesis is supported by the gels of $T4^+$ proteins made at late times after infection: Plates 4-1 through 4-3 clearly show that gp7 and to a lesser extent gp10 are present in reduced quantities in HDF026 at 37°C compared to B178 or to HDF026 under permissive infection conditions.

G. Temperature shift experiments with HDF cells before and after infection

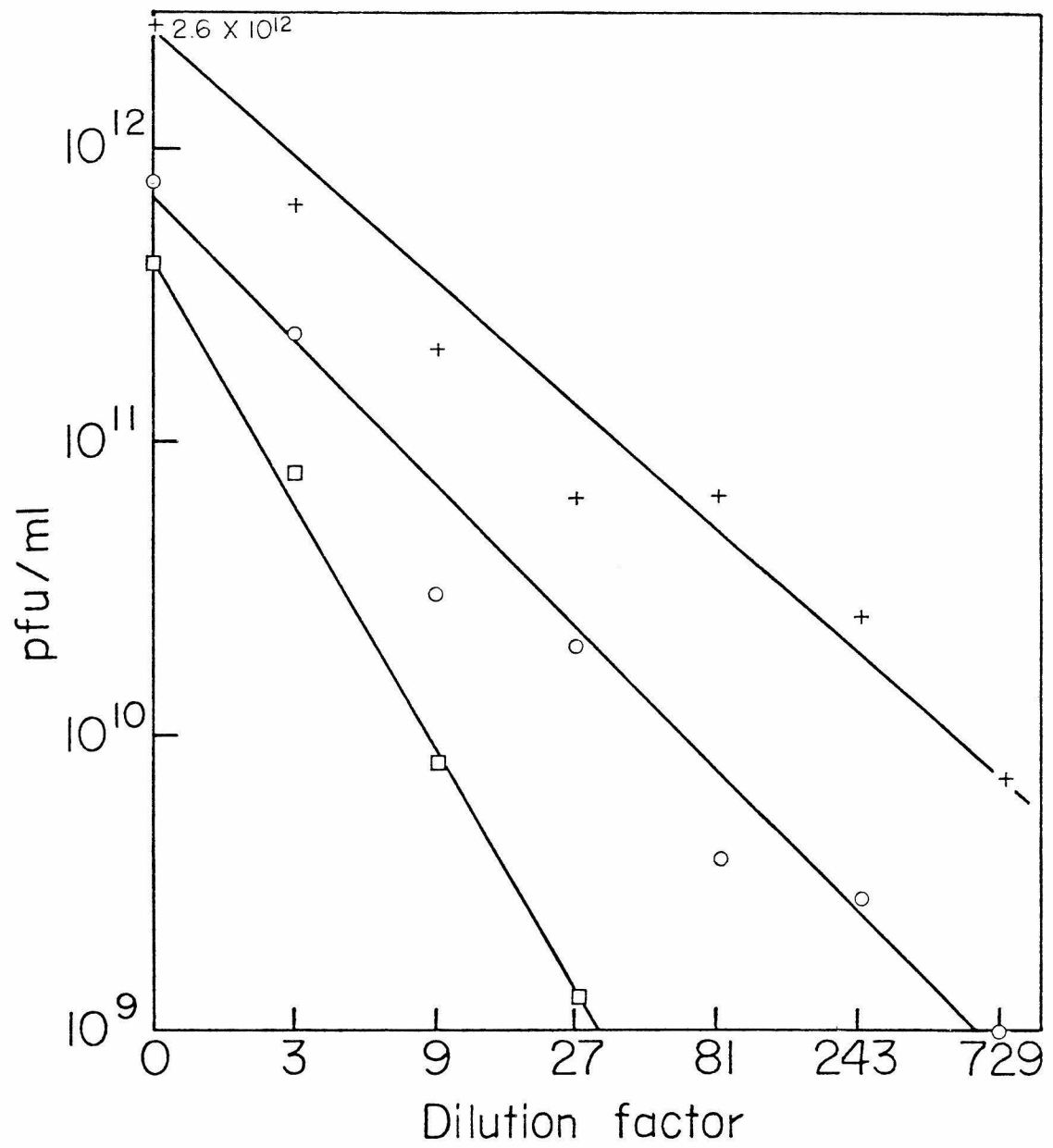
The ts nature of the hdf mutation made possible an investigation of the effects of temperature shifts on $T4^+$ -infected cells.

1. Temperature shifts before infection

Uninfected cells grown at one temperature were mixed with KCN $\sim 3'$ before being added to an equal volume of phage prewarmed at the desired temperature, thus blocking bacterial metabolism at the temperature of cell growth prior to phage infection. We found (data not shown) that the burst size of $T4^+$ on HDF125, HDF303, and hd590 at 25°C or 37° C is unaffected by the temperature at which the cells are grown (only 25°C and 37° C were tested). This result indicates that the temperature-sensitive component in HDF cells is capable of rapid,

Figure 4-5. Dependence of in vitro complementation on the dilution of gp15.

In vitro complementation was as described for baseplate gene products except that serial threefold dilutions of the gp15-containing extracts were used. Reaction components were as follows: +, B178 infected with 19⁻ phage and Bb infected with 15⁻ phage; o, HDF125 infected with 19⁻ phage and Bb infected with 15⁻ phage; □, hd590 infected with T4⁺ and Bb infected with 15⁻ phage.



metabolism-independent conversion from a state in which it blocks T4 growth to one in which phage production is possible, and is suggestive of a temperature-dependent conformational change in the host component.

2. Temperature shifts after infection

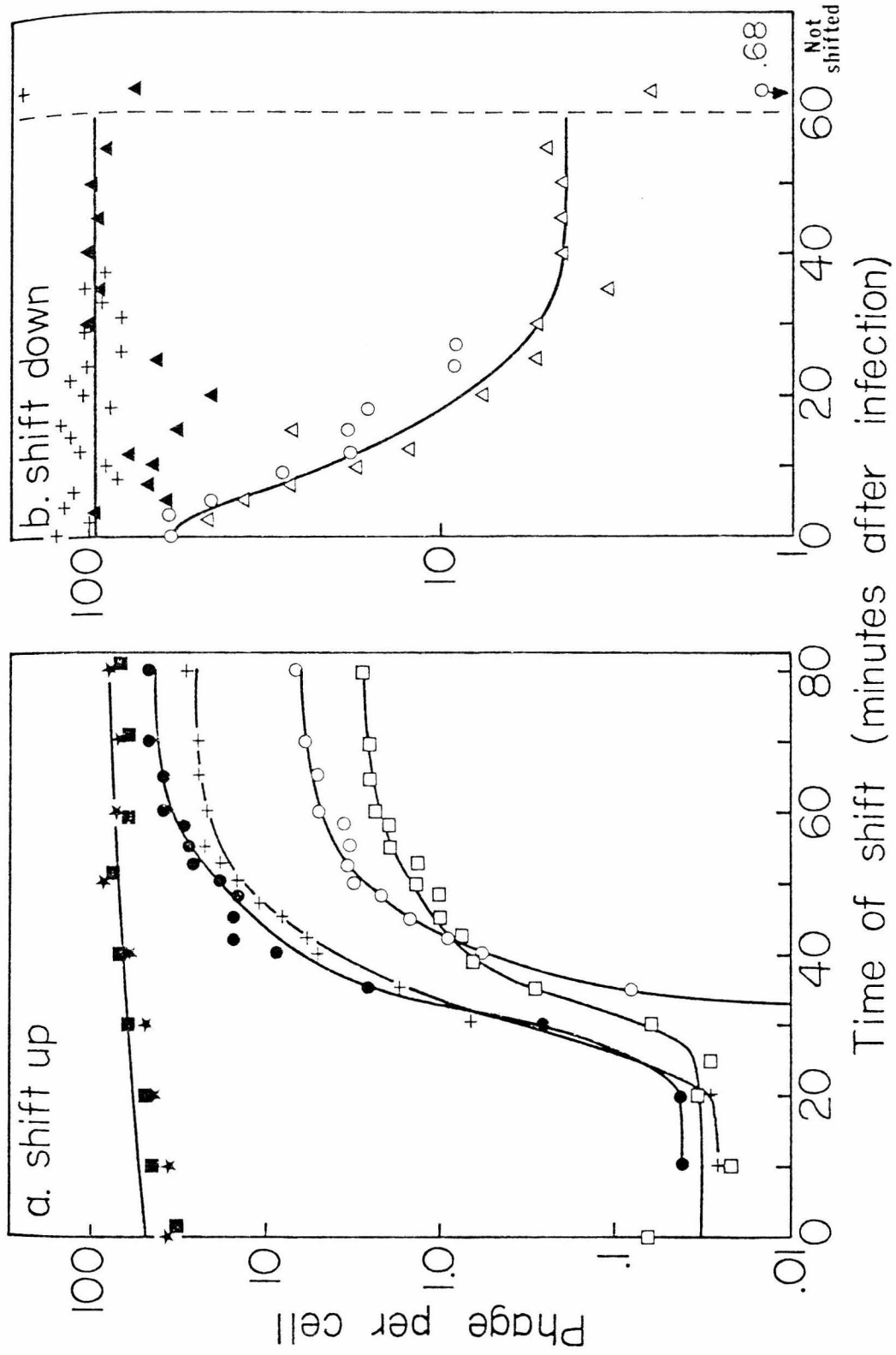
Temperature shift experiments performed after phage infection allowed us to determine the time during the infection cycle when the host function is needed. Figure 4-6a shows the results of a shift-up experiment and simultaneous intracellular phage growth curve at the permissive temperature. There is little difference between the two curves for HDF125; samples shifted up at early times produce no phage while those shifted during the rise stop making phage as soon as they are shifted. This behavior indicates that the host function is inactivated virtually immediately after shift-up and is necessary either throughout the phage life cycle or only at late times for phage assembly.

In shift-down experiments (Fig. 4-6b), the longer the infected HDF cells are held at 37°C before shift down, the smaller the number of phage they produce. That this decrease is not merely related to the amount of time available at the permissive temperature prior to lysis is shown by the curve for superinfected HDF125 cells, which is nearly identical to that for the nonsuperinfected culture. The decrease in burst size is evident as early as five minutes after infection at 37°C, so the host function is clearly needed at early times, perhaps even before phage DNA synthesis starts (at 3-4' in the wild-type host (Miller, 1975) and 3-6' later in HDF strains, see Fig. 4-3). When considered together, the two temperature shift experiments done with infected cells indicate that the host component in question is necessary throughout the phage infection cycle. Similar results were obtained for HDF026, HDF341, HDF303, and hd590,

Figure 4-6. Temperature shift experiments performed on $T4^+$ -infected cells.

a. Infected cells were shifted from 27°C to 37°C at the indicated times by 200x dilution into prewarmed medium. Chloroform was added to all growth tubes at 140' after infection and phage were assayed. Intracellular phage growth curves performed at 27°C are included for comparison. ■, shift-up experiment with $T4^+$ -infected HDF125R; ★, shift-up experiment with $T4^+$ -infected B178; ●, intracellular growth curve at 27°C of $T4^+$ in HDF125R; +, intracellular growth curve at 27°C of $T4^+$ in B178; o, intracellular growth curve at 27°C of $T4^+$ in HDF125; □, shift-up experiment with $T4^+$ -infected HDF125.

b. Infected cells were shifted from 37°C to 25°C at the indicated times and treated as in (a). +, B178 infected with $T4^+$; ▲, HDF125R infected and super-infected with $T4^+$; o, HDF125 infected with $T4^+$; Δ, HDF125 infected and super-infected with $T4^+$.



although inactivation of the host component in shift-up experiments was not equally immediate in all cases.

III. T4 mutants that overcome the hdf block to phage growth

A. Introduction

In the host-defective bacteria described in Chapter 3, Section III, which are unable to grow $T4^+$ because the phage capsid fails to assemble, the bacterial mutation can be overcome by phage carrying a mutation in a gene known to be involved in head assembly (gene 23 or gene 31). By mapping mutants of $T4^+$ that can grow on HDF strains we hoped to similarly elucidate the nature of the hdf block to phage growth. We were unsuccessful.

goF phage were isolated twice, once in the early stages of this work as a means of classifying the newly selected host-defective bacteria, and again in an effort to get a more complete picture of the kinds of mutants that can overcome the HDF block. The plating properties and relevant mapping data for these go phage are summarized in Table 4-4. We will describe the phage in the order in which they were isolated.

B. go phage initially selected on HDF strains

1. goF-1

goF-1 is the T4 mutant used to identify Class F host-defective bacteria, so by definition it plates on all HDF strains. This phage grows normally on wild-type bacteria but is only partly able to overcome the block to phage development in HDF cells (see Figs. 4-1 through 4-3 for growth curves, lysis, and DNA synthesis data and Table 4-4a for other properties). The mutation in goF-1 has been mapped to a previously blank region of the T4 chromosome near gene 39 (Tables 4-5 and 4-6 and Fig. 4-7). The goF-1 mutation probably does not lie in gene 39 because of the properties of a T4 deletion mutant, del(39-56) 12:r1589, which

Table 4-4. goF phage: plating properties, burst sizes, and map locations. The numbers given are the burst sizes found at 37°C in H broth at moi=5 and are the average of at least two determinations. (+) indicates that approximately the same number of plaques were found on the strain in question as on wild-type cells when a drop of phage at a concentration of $\sim 10^4$ /ml was spotted on a seeded plate; (-) indicates that no plaques were found. Selection conditions for scoring recombinants in phage crosses are detailed in the legends to Tables 4-6 and 4-8. Section (a) of the table shows data for T4⁺ and the first three goF phage obtained; section (b) shows data for phage unlike goF-1 that were found in a later, more extensive selection.

TABLE 4-4a. goF phage:
Plating properties, burst sizes, and map locations

	Phage			
	T4 ⁺	<u>goF</u> -1	<u>goF</u> 303.2	<u>go</u> 590-1
Plating properties and burst size				
Bacterium:				
B178	120 (+)	120 (+)	90 (+)	70 (+)
HDF125	0.2 (-)	40 (+)	0.3 (-)	0.01 (-)
HDF026	0.5 (-)	15 (+)	1.0 (-)	0.02 (-)
HDF303	1.0 (-)	15 (+)	5.0 (+)	0.3 (-)
B011'	200 (+)	90 (+)	(+)	150 (+)
<u>hd</u> 590	0.01 (-)	20 (+)	(-)	5.0 (+)
Percent recombination of <u>goF</u> phage with T4 mutants				
T4 mutant:				
39 (<u>am</u> N116)		3.5		
de1(39-56)4: <u>r</u> 1589		3.7		
<u>e</u> (<u>am</u> H26)			1	
31 (<u>am</u> N54)				<1

TABLE 4-4b. goF phage:
Plating properties, burst sizes, and map locations

	Phage					
	<u>goF303b2</u>	<u>goF026c2</u>	<u>goF026a1</u>	<u>goF125b1</u>	<u>go590a3</u> <u>go590a5</u>	<u>go590b9</u> <u>go590b10</u>
Plating properties and burst size						
Bacterium:						
B178	100 (+)	100 (+)	100 (+)	(+)	(+)	(+)
HDF125	0.8 (+)	90 (+)	20 (+)	small (+)	(-)	small (+)
HDF026	2.0 (+)	80 (+)	30 (+)	small (+)	(-)	tiny (+)
HDF303	90 (+)	200 (+)	1 (+) small, turbid	(+)	(-)	(+)
B011'	25 (+)	150 (+)	(+)	(+)	(+)	(+)
<u>hd590</u>	0.05 (-)	6 (+)	(-)	(-)	(+)	tiny (+)
Percent recombination of <u>goF</u> phage with T4 mutants						
T4 mutant:						
39 (<u>amN116</u>)	20	29	25	3.0		7-8
de1 (39-56)4:r1589	39	25	31	3.5		3-7.5
<u>e</u> (<u>amH26</u>)	1	32	20			
31 (<u>amN54</u>)	32	16	23		0-1.0	23-38

TABLE 4-5. Plating properties of phage used to map goF-1

Phage	Bacterium: Temperature:	CR63 37°	CR63(λ) 37°	S/6 25°	HDF125 or HDF026 37°	CT196 30°
<u>r1589</u>		+	-	+	-	+
<u>rEDdf41</u>		+	-	+	-	+
<u>amE300</u>		+	+	-	-	-
<u>amE480</u>		+	+	-	-	-
<u>amN116</u>		+	+	-	-	-
<u>amNG457</u>		+	+	-	-	-
<u>del(39-56)12:r1589</u>		+	-	+ sm	-	-
<u>del(39-56)5:r1589</u>		+	-	+ sm	-	-
<u>del(39-56)3:r1589</u>		+	-	+ sm	-	-
<u>del(39-56)2:r1589</u>		+	-	+ sm	-	+
<u>del(39-56)4:r1589</u>		+	-	+ sm	-	-
<u>del(39-56)1:r1589</u>		+	-	+ sm	-	+
<u>goF-1</u>		+	+	+	+	+
<u>goF-1:r1589</u>		+	-	+	+	+
<u>goF-1:del(39-56)3:r1589</u>		+	-	+ sm	+	-
<u>goF-1:del(39-56)4:r1589</u>		+	-	+ sm	+	-
<u>del(39-56)12:r⁺</u>		+	+	+ sm	-	-
<u>T4⁺</u>		+	+	+ med	-	+

The table lists the parental and recombinant phages that were scored in the genetic crosses presented in Table 4-6 and shows their ability to form plaques on the five indicator bacteria used. + indicates phage growth and - indicates no growth. "sm" and "med" stand for "small" and "medium" and describe the observed plaque morphology.

Table 4-6. Summary of crosses locating goF-1 on the T4 genome. Genetic crosses were carried out in CR63 at 30°C as described in Materials and Methods, Part T1. In all crosses total progeny was measured by plating an appropriate dilution on CR63. The plating properties of phage under various conditions are given in Table 4-5.

Scoring of recombinants and determination of the frequency of recombination were achieved in the following ways.

1) For crosses between amber mutants (4-6, 8-12, 18, and 19), the wild-type progeny were scored by plating samples of the growth tube on S/6 at 25°C. The percent recombination (%R) was calculated as the number of wild-type (wt) recombinants that were scored x 200 divided by the total number of progeny (calculated from the number of plaques found when appropriate dilutions of the growth tube were plated under permissive conditions), or $\%R = \text{wt} \times 200 / \text{total}$ (calculated).

2) For crosses between amber:rEDdf41 double mutants and wild-type phage (crosses 2 and 3), the rII:am⁺ recombinants were determined by plating progeny on S/6 at 25°C and stabbing individual plaques onto lawns of CR63 (λ) to distinguish rII:am⁺ from rII⁺:am⁺. The number of progeny plating on S/6 was equal to one half the total progeny, as expected. $\%R = \text{rII:am}^+ \times 100 / \text{plaques}$ picked from S/6.

3) For crosses between amber mutants and deletions in the (39-56) region that also carry r1589(rII) (crosses 13-16, 20-24, and 27-31), the am⁺:del⁺ recombinants were measured as large plaques on S/6 at 25°C. $\%R = \text{am}^+:\text{del}^+ \times 200 / \text{total}$ (calculated). For crosses 21-24 and 28-31 in which the amber parental phage also carries the rEDdf41 mutation in rII this is a true measure of recombination. In crosses 13-16, 20 and 27, the frequency of recombination determined

Table 4-6 legend (continued)

in this manner may be underestimated by about 10% due to double recombination events yielding $\underline{rII}^+:\underline{am}^+:\underline{del}^+$ wild-type progeny that do not give a large plaque on S/6 at 25°C.

4) For crosses between goF-1 and amber mutants (7, 17, 25, 26, and 32), the $\underline{goF}^+:\underline{am}^+$ recombinants were determined by plating progeny on S/6 at 25°C and stabbing individual plaques onto lawns of HDF125 to distinguish $\underline{goF}^+:\underline{am}^+$ from $\underline{goF}:\underline{am}^+$. The number of progeny plating on S/6 was equal to one half the total progeny, as expected. $\%R = \underline{goF}^+:\underline{am}^+ \times 100/\text{plaques picked from S/6}$.

5) For crosses between goF-1 and the deletions in the (39-56) region (crosses 33-36), progeny were plated on CR63 and individual plaques were stabbed onto lawns of CT196 and HDF125 to identify both recombinant types. $\%R = \text{total recombinants} \times 100/\text{plaques picked from CR63}$.

6) For a cross between goF-1 and $\underline{del}(39-56)12:\underline{r1589}$ (cross 1), it was possible to measure the interval between $\underline{del}(39-56)12$ and $\underline{r1589}$. Total progeny was determined by plating on CR63 and individual plaques were stabbed onto lawns of CR63(λ) and of HDF125 to identify the $\underline{rII}^+:\underline{del}$ and $\underline{rII}:\underline{goF}$ recombinants. $\%R = \text{total recombinants} \times 100/\text{plaques picked from CR63}$.

TABLE 4-6. Summary of crosses locating goF-1 on the T4 genome

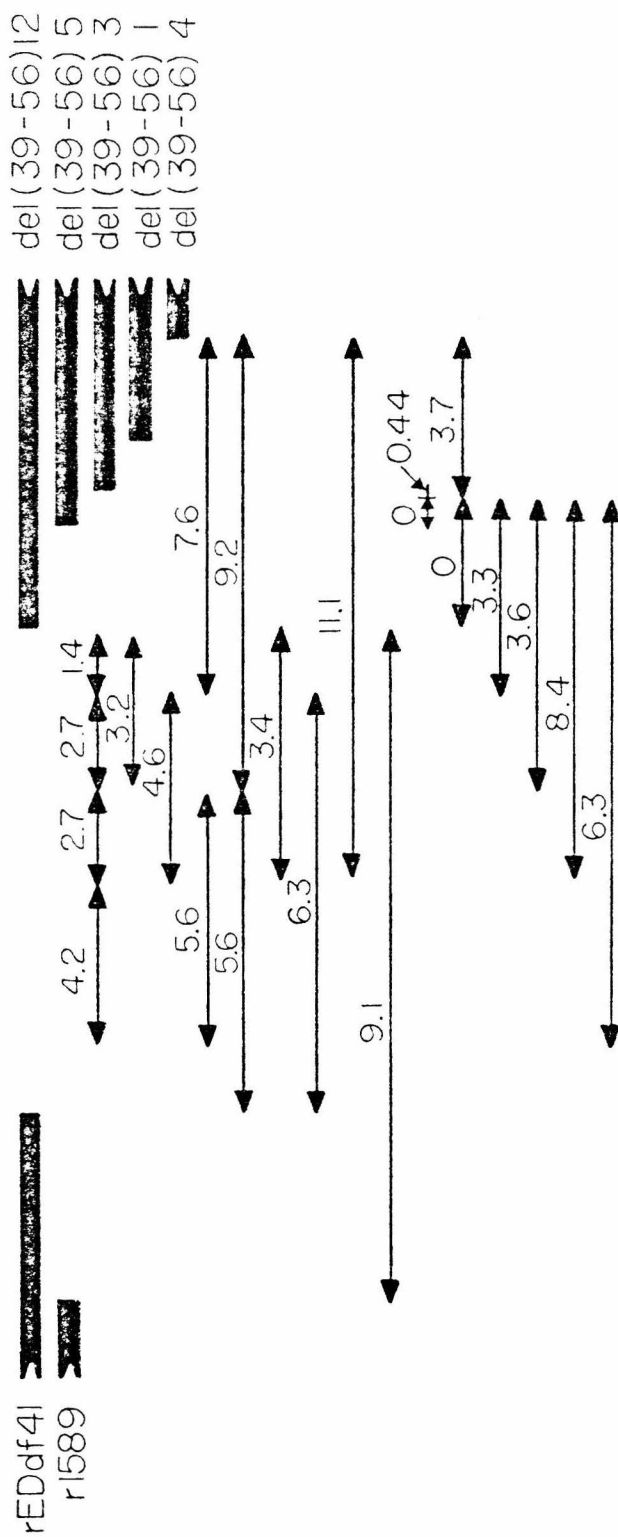
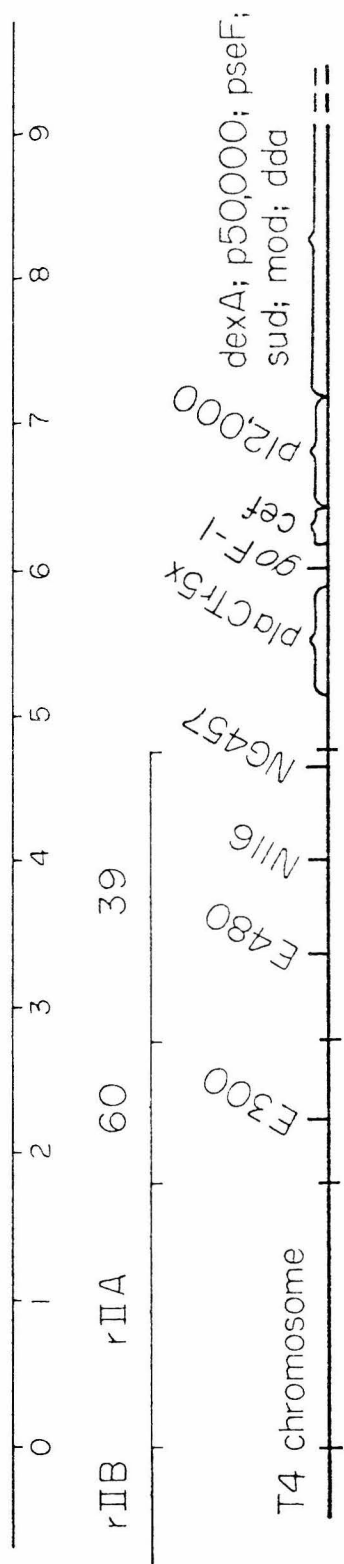
Cross #	Phage used in cross		Interval measured	No. of recombinants/ total progeny scored ^a	Percent recombination
	parent 1	parent 2			
1	<u>goF-1</u>	<u>del(39-56)12:r1589</u>	<u>r1589-del 12</u>	57/622	9.1
2	<u>rEDf41:amN116</u>	<u>goF-1</u>	<u>rEDf41 - N116</u>	48/849	5.6
3	<u>rEDf41:amNG457</u>	<u>goF-1</u>	<u>rEDf41 - NG457</u>	110/1759	6.3
4	<u>amE300</u>	<u>amE480</u>	<u>E300 - E480</u>	448/2.1 x 10 ⁴	4.2
5	<u>amE300</u>	<u>amN116</u>	<u>E300 - N116</u>	338/1.1 x 10 ⁴	6.1
6	<u>amE300</u>	<u>rEDf41:amN116</u>	<u>E300 - N116</u>	482/1.9 x 10 ⁴	5.1
7	<u>amE300</u>	<u>goF-1</u>	<u>E300 - goF</u>	26/415	6.3
8	<u>amE480</u>	<u>rEDf41:amN116</u>	<u>E480 - N116</u>	235/1.4 x 10 ⁴	3.3
9	<u>amE480</u>	<u>amN116</u>	<u>E480 - N116</u>	350/2.9 x 10 ⁴	2.4
10	<u>amE480</u>	<u>amN116</u>	<u>E480 - N116</u>	258/2.3 x 10 ⁴	2.3
11	<u>amE480</u>	<u>rEDf41:amNG457</u>	<u>E480 - NG457</u>	255/1.4 x 10 ⁴	3.6
12	<u>amE480</u>	<u>amNG457</u>	<u>E480 - NG457</u>	429/1.5 x 10 ⁴	5.6
13	<u>amE480</u>	<u>del(39-56)12:r1589</u>	<u>E480 - del 12</u>	430/2.6 x 10 ⁴	3.4
14	<u>amE480</u>	<u>del(39-56)5:r1589</u>	<u>E480 - del 5</u>	392/1.3 x 10 ⁴	6.0
15	<u>amE480</u>	<u>del(39-56)3:r1589</u>	<u>E480 - del 3</u>	397/1.5 x 10 ⁴	5.3
16	<u>amE480</u>	<u>del(39-56)4:r1589</u>	<u>E480 - del 4</u>	657/1.2 x 10 ⁴	11.1
17	<u>amE480</u>	<u>goF-1</u>	<u>E480 - goF</u>	22/260	8.4
18	<u>rEDf41:amN116</u>	<u>amNG457</u>	<u>N116 - NG457</u>	291/1.8 x 10 ⁴	3.3
19	<u>rEDf41:amNG457</u>	<u>amN116</u>	<u>N116 - NG457</u>	373/3.3 x 10 ⁴	2.3

TABLE 4-6. (continued)

Cross #	Phage used in cross		Interval measured	No. of recombinants/ total progeny scored ^a	Percent recombination
	parent 1	parent 2			
20	amN116	del(39-56)12:r1589	N116 - del 12	249/1.9 x 10 ⁴	2.6
21	rEDf41:amN116	del(39-56)12:r1589	N116 - del 12	460/2.5 x 10 ⁴	3.7
22	rEDf41:amN116	del(39-56)5:r1589	N116 - del 5	311/1.0 x 10 ⁴	6.0
23	rEDf41:amN116	del(39-56)3:r1589	N116 - del 3	244/1.1 x 10 ⁴	4.4
24	rEDf41:amN116	del(39-56)4:r1589	N116 - del 4	525/1.1 x 10 ⁴	9.2
25	amN116	goF-1	N116 - goF	16/500	3.2
26	rEDf41:amN116	goF-1	N116 - goF	31/773	4.0
27	amNG457	del(39-56)12:r1589	NG457 - del 12	194/2.6 x 10 ⁴	1.5
28	rEDf41:amNG457	del(39-56)12:r1589	NG457 - del 12	198/2.9 x 10 ⁴	1.4
29	rEDf41:amNG457	del(39-56)5:r1589	NG457 - del 5	338/1.5 x 10 ⁴	4.5
30	rEDf41:amNG457	del(39-56)3:r1589	NG457 - del 3	226/1.5 x 10 ⁴	3.1
31	rEDf41:amNG457	del(39-56)4:r1589	NG457 - del 4	627/1.6 x 10 ⁴	7.6
32	rEDf41:amNG457	goF-1	NG457 - goF	58/1759	3.3
33	r1589:goF-1	del(39-56)12:r1589	goF - del 12	0/1140	<0.1
34	r1589:goF-1	del(39-56)5:r1589	goF - del 5	0/1140	<0.1
35	r1589:goF-1	del(39-56)3:r1589	goF - del 3	5/1140	0.44
36	r1589:goF-1	del(39-56)4:r1589	goF - del 4	42/1140	3.7

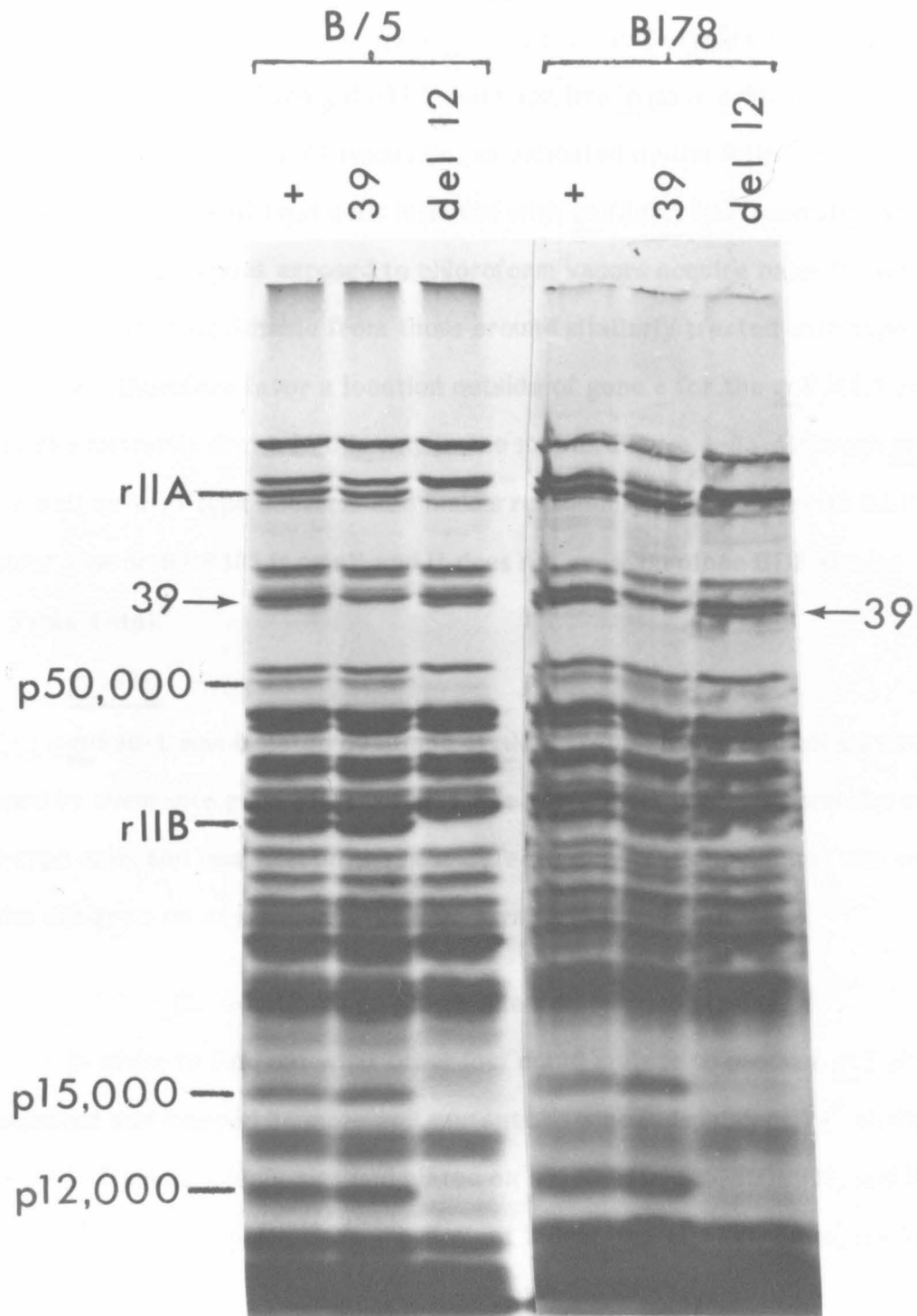
^a See legend for further explanation.

Figure 4-7. Location of goF-1 on the T4 genome. The figure shows the position of mutations (short vertical lines above the line representing the T4 chromosome) and deletions (heavy bars) and the percentages of recombination based on the data presented in Table 4-5 (numbers written over arrows indicating the distance over which recombination was measured). A notch at the end of a heavy bar indicates that the deletion continues in that direction. The ends of genes are indicated by vertical lines drawn all the way through the line representing the T4 chromosome. The positions of genes are indicated by labeled brackets. Amber mutations are indicated by number only, without the prefix am. The scale (top line) is measured in kilobase pairs (kb) from an arbitrary zero point at the boundary between rIIA and rIIB (Wood and Revel, 1976). The size of the rIIA region, and hence the right end point of rEDdf41 (Edgar et al., 1962) is based on the data of Bujard et al. (1970) and Kim and Davidson (1974). By measuring the distance from del(39-56)1 (previously designated D_J) to rII deletions defining the right ends of rIIA (4600 base pairs) and rIIB (6400 base pairs), Bujard et al. estimated the length of the rIIA region to be 1800 ± 70 base pairs. The right hand end of r1589 has been placed at 0.5 kb on the basis of data of Homyk and Weil (1974) who measured the distance between del(39-56)1 and r1589 to be 5900 base pairs. The left end points of the del(39-56) mutants are defined by the data of Homyk and Weil. The size of gene 60 has been estimated from extensive intracistronic mapping (Mufti and Bernstein, 1974). The size of gene 39 has been estimated from the polypeptide molecular weight of 64,000 daltons (O'Farrell et al., 1973). The mutations in gene 39 (amE480, amN116 and amNG457) have been located by extensive intracistronic mapping (Mufti and Bernstein, 1974). Their relative positions with respect to rEDdf41 as an outside marker have been confirmed by three factor crosses (data not shown).



fails to recombine with goF-1 and therefore deletes part or all of the gene in which the goF mutation lies. This deletion-containing phage apparently carries an intact gene 39; the mutant makes plaques under conditions where a gene 39 amber mutant does not (S/6 at 25°C; see Table 4-5), and it makes a product identifiable on gels as gp39 (Plate 4-7). goF-1 lies closest to the genes for plaCTr5x and cef. The former gene is necessary for T4 to plate on the bacterium CTr5x (Homyk and Weil, 1974); goF-1 phage are able to plate on this strain and therefore may contain a wild-type plaCTr5x gene product. cef is a gene defined by an amber mutation (cef76) that prevents plaque formation on a nonsuppressing E. coli mutant, AR8, that was selected for its inability to grow the T4 deletion mutant del(39-56) 12:r1589 (Rodriguez-Prieto, 1976). The cef locus is deleted in the T4 del(39-56)3:r1589 mutant but the goF-1 mutation is not, and goF-1 plates on AR8 at 41°C whereas cef76 does not (Rodriguez-Prieto, personal communication). (The ability of cef76 to plate on HDF mutants has not been tested.) This information cannot confidently be interpreted to mean that the goF-1 mutation lies in a different gene from both plaCTr5x and cef76 because plaCTr5x and cef are defined by mutations that eliminate gene products (deletions in the former case and an amber mutation in the latter) while, as we argue below (Part D), the goF-1 mutation is more likely to result in the alteration of a gene product. It would not be surprising for phage missing a gene product to behave differently from those in which that product is present in an abnormal form. Whether or not the goF-1 mutation affects the gene for plaCTr5x or cef awaits better mapping or characterization of the relevant gene products.

Plate 4-7. Production of gp39 in del(39-56)12:r1589-infected cells. Phage and host strains used are indicated above each track of the gel. Bands are identified as described in Plate 4-1. Bacteria grown in M9 at 37°C to 1×10^8 cells/ml were placed in the bottom of a 150 mm petri dish and gently agitated while being exposed to ultraviolet light. The petri dish was placed 36 cm from a germicidal lamp (previously calibrated with phage T4 to give 5 lethal hits per minute per phage at this distance) and the cells were irradiated for 6' to reduce host transcription and translation. The cells were then incubated in a 30°C shaking water bath for 10' prior to infection. ^{14}C amino acid labeled extracts for gel electrophoresis were prepared essentially as described in Materials and Methods, Part M1 except for the following: cells were infected at 30°C at moi=6 and labeled from 4' to 10', and at 10' 1.5 ml portions of ice cold 3% casamino acids were added to the 2 ml cultures and the cultures were immediately centrifuged. The acrylamide concentration in the separating gel is 10%. gp39, defined by the band missing (arrow) in the amN116 (39⁻) infection, is present in cells infected by either T4⁺ or del(39-56)12:r1589. Other bands missing in del(39-56)12:r1589 are also indicated.



2. goF303.2

goF303.2 is one of four phage originally selected for the ability to grow on HDF303. The other three isolates, like goF-1, map near gene 39, but the goF303.2 mutation lies in or near gene e, the gene for T4 lysozyme (Tables 4-7 and 4-8 and Fig. 4-8). If the goF303.2 mutation lies in gene e, then it does not affect the lysis function of T4 lysozyme, as indicated by the following two pieces of evidence. First, wild-type cells infected with goF303.2 lyse normally, and second, goF303.2 plaques exposed to chloroform vapors acquire halos (Streisinger *et al.*, 1961) indistinguishable from those around similarly treated wild-type plaques. We therefore favor a location outside of gene e for the goF303.2 mutation and have arbitrarily drawn it this way in the map in Figure 4-8. Although goF303.2 grows well on wild-type bacteria and makes reasonably good plaques on HDF303, its burst size on HDF303 is small and it does not grow on other HDF strains (see Table 4-4a).

3. go590-1

go590-1 was isolated by Simon *et al.* (1974) on hd590 and conclusively mapped by them into gene 31. Like all other goF phage, it grows normally on wild-type cells and less well on the host-defective mutant on which it was selected. It does not grow on any of our K-12 HDF strains (Table 4-4a).

C. Additional go phage selected on HDF strains

In order to find out what other loci could mutate to produce goF phage, we selected and mapped 24 more goF mutants. Three independent $T4^+$ stocks were made from single plaques and plated on hd590, HDF125, HDF026, and HDF303 at 10^6 - 10^8 phage per plate. After overnight incubation at 37°C, large, medium,

TABLE 4-7. Plating properties of phage used to map goF303.2

Phage	Bacterium: Temperature:	HDF303 37°	S/6 37°	S/6 + lyso. 30° or 37°	S/6 30°	CT439 30°	CT439 37°	CR63 + lyso. 30°	S/6 42°
<u>eG298</u>		-	-	+	-				
<u>eG19</u>		-	-	+	-				
<u>eG223</u>		-	-	+	-				
<u>eG79</u>		-	-	+	-				
<u>psu_b⁻Δ64</u>		-	+	+	+	-	-		
<u>psu_b⁻Δ33</u>		-	+	+	+	-	-		
<u>amH26 (e)</u>		-	-	+	-	-	-	+	-
<u>tsC3 (e)</u>		-	-	+	+	+	-	+	-
<u>goF303.2</u>		+	+	+	+	+	-	+	+
<u>T4⁺</u>		-	+	+	+	^a +	-	+	+

^a_{eop} = 0.4.

The table lists the phage that were scored in the crosses presented in Table 4-8 and shows their ability to form plaques under various plating conditions. "+lyso." indicates that 500 µg egg white lysozyme were added to 2 ml top agar before it was poured. "+" indicates high efficiency phage growth and "-" indicates no growth.

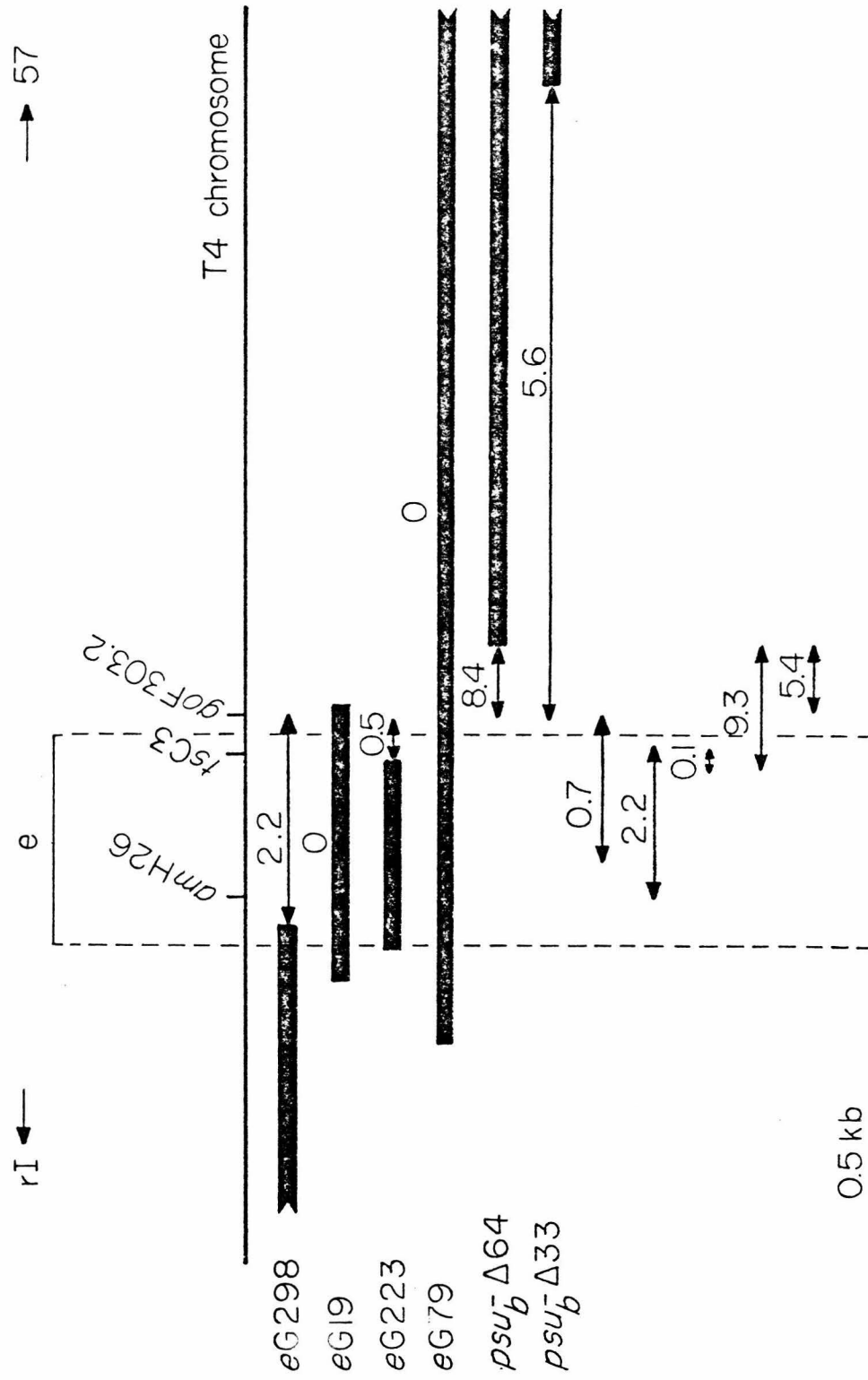
Table 4-8. Summary of crosses locating goF303.2 on the T4 genome. Crosses were performed in S/6 at 30°C (except No. 8) as described in Materials and Methods, Part T1. eG deletions and psu_b strains were crossed to T4D⁺ prior to use to remove an rIV ("spackle") mutation from the former and a gene 15 (amN133) mutation from the latter. eG deletions without rIV were recognized by their inability to plate on S/6 in the absence of added lysozyme, and the psu_b deletions without amN133 by their ability to plate on S/6 but not CT439. Plating properties of phage under various conditions are given in Table 4-7. The following conditions were used to score recombinants. 1) For crosses 1-4 and 7, progeny were plated (a) on S/6 + lysozyme (500 µg/2 ml top agar/plate) for total progeny and (b) on S/6 without lysozyme as a source of plaques for stabbing. Half as many plaques were found under plating condition (b) as under (a), as expected. Plaques were stabbed onto HDF303 and S/6. %R = wt x 100/plaques picked from S/6. 2) For crosses 5 and 6, progeny were plated on S/6 and stabbed onto HDF303, CT439 at 30°C, and S/6. Both recombinant classes were scored and %R = total recombinants x 100/plaques picked from S/6. The two recombinant classes were found in approximately equal numbers. 3) Cross 8 was performed in CR63 at 30°C and progeny were plated on CR63 + lysozyme at 30°C and on S/6 at 42°C. %R = wt x 200/total progeny (calculated). 4) For cross 9, progeny were plated on S/6 + lysozyme at 30°C (total) and S/6 at 37°C (wt recombinants). %R = wt x 200/total (calculated). 5) For crosses 10 and 11 the total progeny was plated on S/6 + lysozyme. Wt recombinants were scored by stabbing from S/6 (no lysozyme) onto CT439 at 30°C and S/6. %R = wt x 100/plaques picked from S/6.

TABLE 4-8. Summary of crosses locating goF303.2 on the 14 genome

Cross #	Phage used in cross		No. of recombinants/total progeny scored ^a	Percent recombination
	parent 1	parent 2		
1	<u>eG298</u>	<u>goF303.2</u>	11/500	2.2
2	<u>eG19</u>	<u>goF303.2</u>	0/673; 0/1206; 0/564	0
3	<u>eG223</u>	<u>goF303.2</u>	8/772; 4/1695; 5/1324	0.5
4	<u>eG79</u>	<u>goF303.2</u>	0/500	0
5	<u>psu_b⁻Δ64</u>	<u>goF303.2</u>	26/347; 28/300	8.4
6	<u>psu_b⁻Δ33</u>	<u>goF303.2</u>	20/360	5.6
7	<u>amH26(e)</u>	<u>goF303.2</u>	3/600; 7/800	0.7
8	<u>amH26(e)</u>	<u>tsC3(e)</u>	488/4.3 x 10 ⁴	2.9
9	<u>tsC3(e)</u>	<u>eG223</u>	110/9.5 x 10 ⁴	0.1
10	<u>eG223</u>	<u>psu_b⁻Δ64</u>	37/400	9.3
11	<u>eG19</u>	<u>psu_b⁻Δ64</u>	37/682	5.4

^aDifferent ratios are the results from different crosses. See legend for further explanation.

Figure 4-8. Location of goF303.2 on the T4 genome. The figure shows the position of mutations (short vertical lines above the line representing the T4 chromosome) and deletions (heavy bars) and the percentages of recombination as determined in the crosses listed in Table 4-7 (numbers written over arrows indicating the distance over which recombination was measured). A notch at the end of a heavy bar indicates that the deletion continues in that direction. The ends of gene e are indicated by vertical dotted lines; the size of gene e is 492 base pairs (calculated from the number of amino acids in the sequenced gene product (Inouye et al., 1970). The extents and positions of the deletions were determined by Wilson et al. (1972). The line at the lower left indicates the scale of the figure.



and sometimes small plaques were found at frequencies that varied depending on the strain (see Table 4-1 for eop's). Only phage from large plaques (frequency, 10^{-7} - 10^{-8}) were purified and further studied. Two plaques were picked on each host for each phage stock for a total of 24 goF phage. Twenty of the twenty-four phage resemble goF-1 in plating properties and map position; four were anomalous and will now be further described (see also Table 4-4b).

A second goF303 mutation, goF303b2, was found which maps near lysozyme. Unlike goF303.2, it plates on other K-12 HDF strains although its burst size on them is only slightly larger than that of goF303.2. It fails to plate on the B strain hd590.

Two of the six plaques picked on HDF026 do not map near gene 39. Preliminary data tentatively place goF026c2 in the region between genes 33 and 31, and goF026a1 between genes 55 and e.

The fourth unusual phage, goF125b1, maps close to gene 39 but may carry a mutation at a different site than goF-1 since it makes small plaques on HDF strains 125 and 026 and fails to grow on hd590.

All six phage initially picked from hd590 lawns (which gave almost exclusively large plaques--very few smaller ones) mapped near gene 39, unlike go590-1. We therefore picked phage from more plaques in order to see if there were any with mutations in gene 31. As a rapid test for presumptive gene 31 mutants, phage from the picked plaques were spotted on several K-12 HDF strains. Those phage which failed to grow on these bacteria (like go590-1) were mapped. Two large plaques out of 35 tested were found not to grow on the K-12 strains and mapped close to amN54 (gene 31). Two small plaques (the only small plaques found on this strain) were also picked; both gave small plaques on all tight K-12

strains tested (larger plaques on the leaky HDF303) and mapped near gene 39. They may represent a third mutational site in this region.

D. Additional properties of goF phage

1. Growth on wild bacteria

All goF phage are able to grow on the wild-type parental bacterium and are also able to grow on all Caltech "hospital" strains (Wilson, 1973) on which $T4^+$ can grow.

2. goF mutations alter, rather than inactivate, gene products

del(39-56)12:r1589, a mutant of T4 which deletes the goF locus near gene 39, and other deletion-containing strains that cover the goF locus near gene e, are unable to grow on HDF hosts but do grow on wild-type hosts (the latter in the presence of exogenous lysozyme). Although these mutants undoubtedly delete more than one gene, we interpret their plating properties to mean that goF phage are the result of an alteration in and not an elimination of some T4 gene product. This interpretation is strengthened by the fact that the multiple mutants goF-1:del(39-56)3:r1589 and goF-1:del(39-56)4:r1589, which contain only slightly smaller deletions than del(39-56)12:r1589 (Homyk and Weil, 1974), are able to grow on HDF strains.

3. Dominance relationship of goF^+ and goF-1

To ask about the dominance relationship of goF^+ and goF-1, we performed mixed infections of HDF125 with wild-type (goF^+) phage and phage carrying the goF-1 mutation. The results are shown in Table 4-9, lines 1-6. Since the burst sizes found are intermediate between those for HDF125 infected with goF^+ alone and goF-1 alone, we should conclude that the two alleles show incomplete dominance. However, the fact that del(39-56)12:r1589, which lacks the goF

TABLE 4-9. Dominance relationship of goF⁺ and goF⁻1

Experiment	Phage	Host	Burst size	% of progeny which are	
				<u>goF</u> ⁺	<u>goF</u> ⁻ 1
1	++ (<u>goF</u> ⁺)	B178	148	100	0
2		HDF125	0.07	100	0
3	<u>goF</u> ⁻ 1	B178	197	0	100
4		HDF125	12.3	0	100
5	++ and <u>goF</u> ⁻ 1	B178	140	47	53
6		HDF125	0.8	25	75
				% of progeny which are del(39-56)12 <u>goF</u> ⁻ 1	
7	del(39-56)12: <u>r</u> 1589	B178	65	100	0
8		HDF125	0.02	100	0
9	<u>goF</u> ⁻ 1: <u>r</u> 1589	B178	145	0	100
10		HDF125	19.6	0	100
11	del(39-56)12: <u>r</u> 1589 and <u>goF</u> ⁻ 1: <u>r</u> 1589	B178	92.5	47	53
12		HDF125	1.9	20	80

The procedure used for these experiments was the same as that for phage crosses except that the moi of each parental phage was 5. goF⁻1:r1589 was used in lines 9-12 instead of goF⁻1 because del(39-56)12 also contains the r1589 deletion and we wanted to avoid possible effects of this rII deletion on the results.

locus, behaves just like $T4^+$ in mixed infections with goF-1:r1589 (Table 4-9, lines 7-12) shows that rather than exhibiting incomplete dominance, the goF⁺ gene product is irrelevant. The burst sizes of all del(39-56):r1589 strains are lower than that of r1589 on wild-type bacteria (Homyk and Weil, 1974; Rodriguez-Prieto, 1976). This observation plus the results of the mixed infections with del(39-56)12:r1589 and goF026:r1589 imply that the wild-type goF⁺ gene product may serve merely as a dispensable enhancer of T4 growth in wild-type cells, becoming essential only in HDF cells in an altered form that partly compensates for the defective host gene product. The skewed progeny obtained in mixedly infected HDF125 cells (Table 4-9) may indicate that the goF gene product is cis-acting.

IV. Characterization of HDF bacteria and mapping of the hdf mutation

A. Temperature sensitivity of HDF cells

1. Bacterial growth

Although most HDF strains make very poor lawns at 42°C or 45°C, they do grow at the nonpermissive temperature in liquid but at a slower rate than wild type. Figure 4-9 shows curves of optical density and colony-forming ability for cells of wild type and two HDF strains in liquid culture before and after a shift up in temperature. All strains divide every 30' at 37°C, but at high temperatures the HDF cells divide more slowly than B178 (see Fig. 4-9 for division times). No morphological change is detectable in the light microscope at the nonpermissive temperature, although for HDF341 a comparison of the curves for O.D. and colony-forming ability indicates that the cells may become smaller. A similar comparison for HDF303 (data not shown) indicates that the cells die at 42°C. In Figure 4-9a although HDF125 grows more slowly than the wild type at 45°C, it is clear that the growth of B178 is also impaired at this temperature. We therefore decided to use HDF341 in place of HDF125 in further experiments because it shows ts behavior at 42°C (Fig. 4-9b) where B178 grows normally.

2. Incorporation of macromolecular precursors

Since we were able to get simultaneous reversion of hdf to hdf⁺ by selecting for ts⁺, it may be that these two properties are caused by the same mutation. By discovering the reason for the ts behavior of these strains we therefore expected to learn about the nature of the block to T4⁺ growth. The incorporation of several macromolecular precursors before and after a temperature shift from 37°C to 42°C was compared in HDF and wild type cells (Fig. 4-10). No

Figure 4-9. Bacterial growth before and after a temperature shift. Samples of broth-grown cultures were taken at the indicated times for measurement of optical density (scale at left) and colony forming ability (scale at right). The temperature shift was accomplished by moving cultures of less than 50 ml contained in 250 ml flasks between shaking water baths set at the desired temperatures. Arrows indicate the times at which the cultures were shifted. Doubling times in minutes are written above the curves to which they apply. The solid curves indicate colony forming ability; the dashed curves, optical density.

- a. Shift was from 37°C to 45°C. +, B178; o, HDF125.
- b. Shift was from 37°C to 42°C. +, B178; Δ, HDF341.

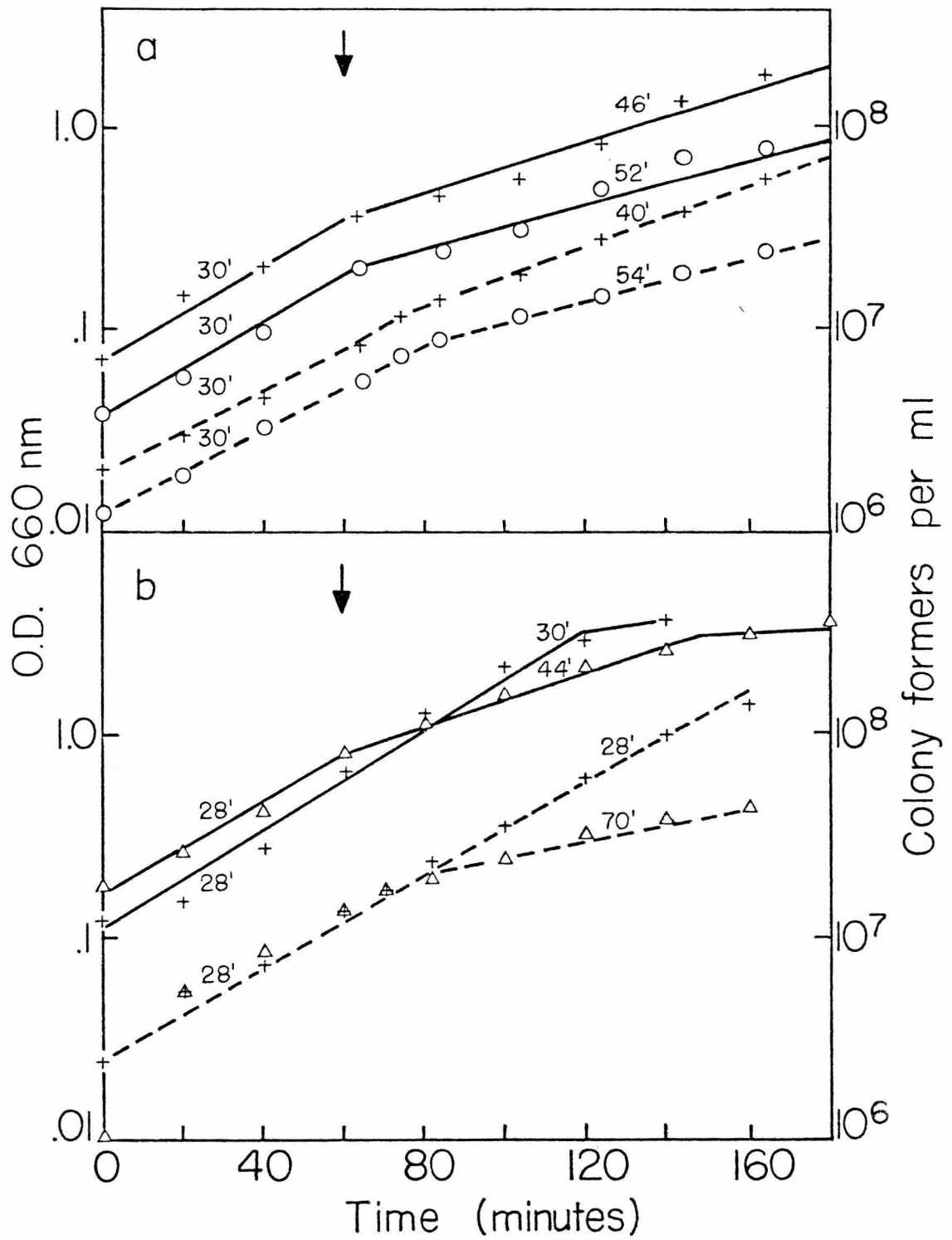
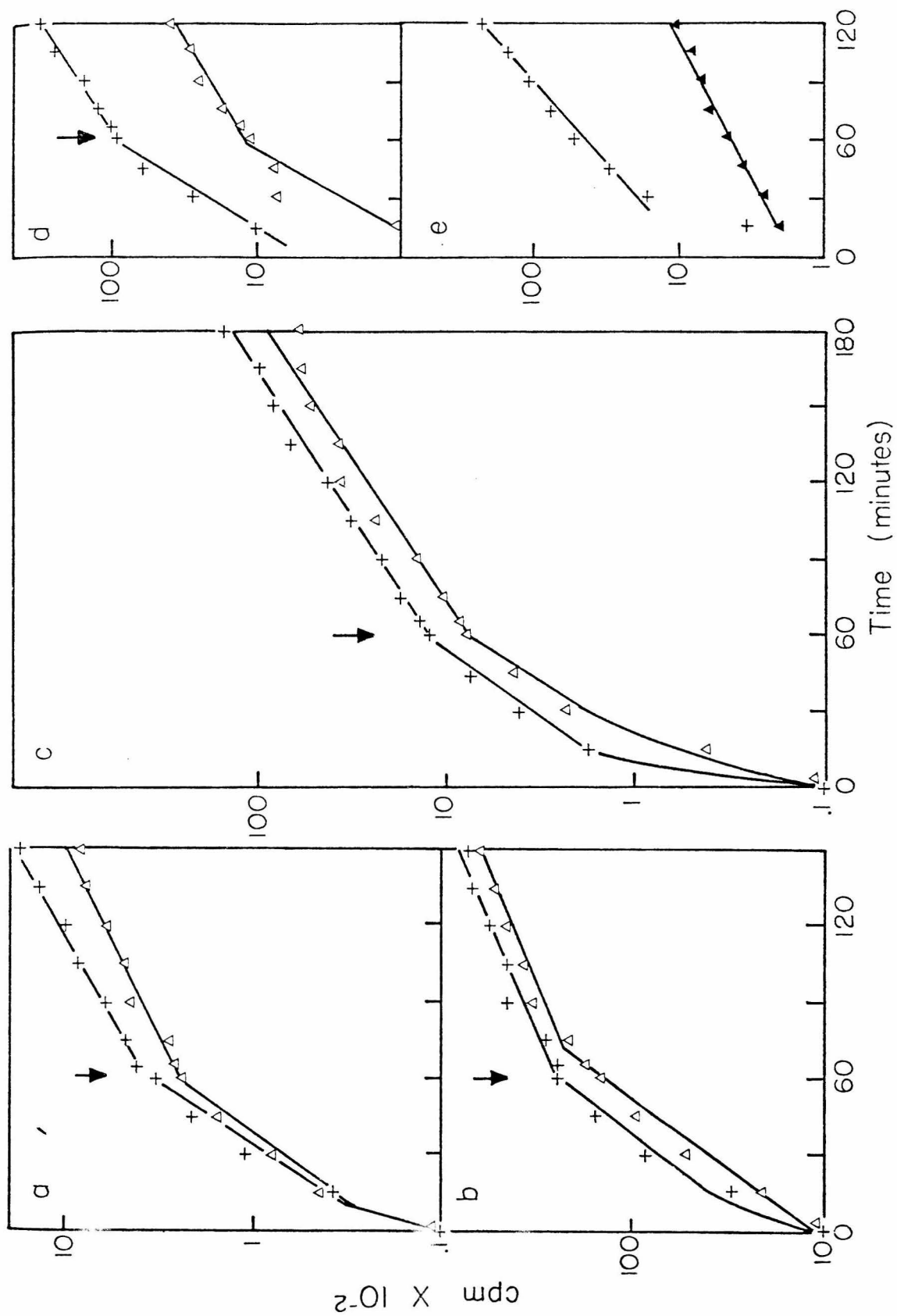


Figure 4-10. Incorporation of macromolecular precursors into HDF341 before and after a temperature shift. For experimental details, see Materials and Methods, Parts V-Y. The arrow in Panels a-d indicates the time of the temperature shift, which was carried out by moving a 10 ml flask containing 1-2 ml cells from a shaking water bath set at 37°C to one set at 42°C. The experiment in Panel e was performed entirely at 37°C. The symbols used are as follows: +, B178; Δ , HDF341; $\blacktriangle$, HDF341(λ dnalA1).

- a. ^{14}C thymidine incorporation
- b. ^{14}C leucine incorporation
- c. ^{14}C diaminopimelic acid incorporation
- d. ^3H glycerol incorporation
- e. ^3H glycerol incorporation



differences were found in the incorporation of ^{14}C thymidine into DNA (this result was found for HDF125 and hd590 also), ^{14}C leucine into protein, or ^{14}C diaminopimelic acid into cell wall. Because of the rapid turnover of mRNA in E. coli, we interpret the unimpaired ^{14}C leucine incorporation into protein to be evidence for the absence of major problems in message transcription at high temperatures in HDF341. A 10-fold difference in the abilities of wild type and HDF strains 341, 303, and 026 to incorporate ^3H glycerol into phospholipids was seen, but HDF125 and hd590 failed to show this behavior and a derivative of HDF341 made ts⁺hdf⁺ by the transducing phage λ dnalA1 continued to incorporate glycerol at the lower level (Fig. 4-10e). We therefore conclude that the poor glycerol incorporation is due to a nitrosoguanidine-induced mutation unrelated to hdf, probably in glpT. As will be shown in the following section, the glpT gene is very close to the map position of hdf. As a further check on the lipids in HDF cells, we tried to correct the temperature-sensitivity of these strains by the addition of the fatty acids palmitate, stearate, or oleate to either liquid medium or top agar (final concentration, 0.01-0.1%, solubilized by 0.01-0.1% TWEEN 40). The cells retained their ts growth characteristics under these conditions. HDF strains are also insensitive to 10 mg/ml SDS in the growth medium, a crude indication that the cell surface is normal. In summary, the cause of the temperature sensitivity of HDF strains remains unknown.

B. Location of hdf on the E. coli chromosome

1. Introduction

Because nitrosoguanidine mutagenesis, which results in multiple mutants, was used to obtain the host-defective bacteria, we decided to characterize more

than one Class F strain in order to avoid being misled by the effects of simultaneously-induced non-hdf mutations. This increased the amount of work that had to be done, but proved worthwhile when, for example, we could show that the inability of several HDF strains to incorporate ^3H glycerol into phospholipids was irrelevant to the hdf phenotype (Part A, above). We also found that particular strains were better-suited for certain experiments; for example, HDF341 was the strain of choice for the investigation of the temperature sensitivity of HDF bacterial growth and HDF026 the best mutant to use for gel electrophoresis work. However, if we are to be justified in discussing the effects of the hdf lesion on T4^+ development in terms of a single gene and its product, we must show that the hdf mutations in strains HDF125, HDF341, HDF026, and HDF303 are allelic.

We find all strains to be qualitatively similar with respect to T4^+ growth; Table 4-10 summarizes the experimental results obtained for each strain. It cannot, of course, be concluded from such phenotypic data that all strains are mutated in the same gene. In Chapter 3, for example, bacteria in which T4^+ heads were unable to assemble and which had identical phenotypes were found to carry mutations at several different loci.

Nor is the ability of a single phage mutant to overcome the block in all strains a criterion for their identity or allelism; again, this point was clearly made in Chapter 3. Proof of identity or allelism can come most directly from sequence data of relevant DNA, RNA, or protein molecules or from genetics; we have pursued the genetic approach and will now present our data concerning the map location of hdf.

TABLE 4-10. Summary of characteristics of T4⁺-infected and uninfected HDF strains

Bacterial strain	Characteristics of T4 ⁺ infection							Characteristics of uninfected cell	
	Phage made	Cell lysis normal	T4 DNA synthesis	Specific early and late T4 proteins decreased	EM shows empty heads, no tails or baseplates	In vitro complementation yields no heads, tails, bp ^a , or bp protein activity	Host product required throughout infection	Bacterial growth <u>ts</u>	hdf mutation maps near 48'
B178	+	+	+	No	-	-		No	
HDF125	-	delayed	±	Yes	+	+	+	Yes	+
HDF026	-	delayed	±	Yes	+	+	+	Yes ^b	+? ^c
HDF341	-	delayed			+			Yes	+
HDF303	-	delayed	±	Yes	+		+	Yes	+
HDF183	-	delayed	±						
HDF311	-				+			No	
B011'	+	+	+	No	-			No	
hd590	-	delayed	±	Yes	+	+	+	Yes	

^abp means baseplate.^bAs discussed in Section I, the original isolate of HDF026 was ts.^cSee Part B2c.

2. Determination of the map position of hdf

The hdf mutation has been localized to a 20 kilobase region of the E. coli chromosome by the strategy summarized in Table 4-11 and discussed below. Bacterial strains used in mapping hdf are listed in Table 2-1; the extent of bacterial DNA in relevant genetic elements is shown in Figure 4-11.

a) Chromosome transfer. A rapid mapping technique using a set of predominantly wild-type Hfr's (Low, 1973) was initially employed to locate hdf to an 8' segment of the E. coli chromosome. The success of the technique depends on the identity or close linkage of the temperature-sensitive mutation in HDF cells with the hdf mutation. In summary, streptomycin-resistant derivatives of HDF ts strains were briefly mated on plates with 15 streptomycin-sensitive Hfr's, including representatives of both directions of chromosome transfer, whose origins of transfer were fairly evenly distributed around the E. coli chromosome. The plates were then exposed to streptomycin (see Materials and Methods, Part Z1) and incubated at 42°C or 45°C. Only spontaneous revertants and Str^r cells that have become ts⁺ by recombination with Hfr-transferred DNA can grow under these selection conditions. Because of the brief time (30') allowed for mating, only Hfr's whose origins of transfer are close to the necessary ts⁺ marker are able to transfer it before selection conditions are imposed. Very satisfactory results were obtained (see Table 4-11 and Fig. 4-11): strong patches of growth arose after incubation on spots where Hfr's KL983 (at 42°C only) or PK191 had been placed, but not on the KL96 spot. ts⁺ cells from the patches of growth were tested and found to be hdf⁺, thereby locating hdf between his (44') and supN (51.5'), the origins of transfer of KL96 and KL983. The Hfr strain KL16, whose origin of transfer is between lysA (61') and serA (62') and which

TABLE 4-11. Experiments to determine the map location of hdf

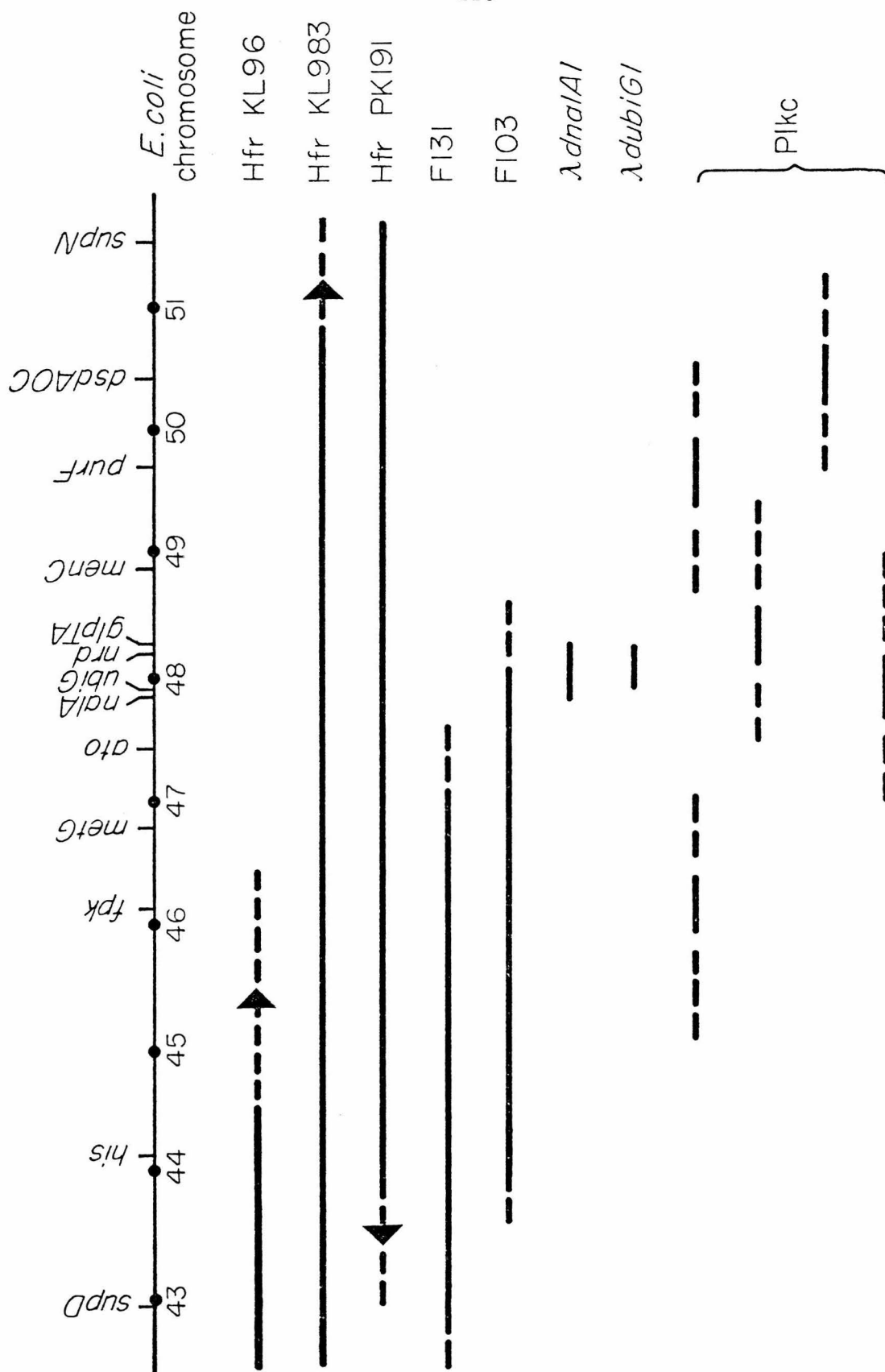
Donor strain and relevant markers	Recipient strain and relevant markers	Selection	Results	Other HDF strains yielding similar results
KL96 Hfr	HDF125 <u>hdf</u> <u>ts</u> <u>gal</u> <u>Str</u> ^r	For <u>ts</u> ⁺ <u>Str</u> ^r	None found.	HDF341, HDF303
KL983 Hfr	HDF125 <u>hdf</u> <u>ts</u> <u>gal</u> <u>Str</u>	For <u>ts</u> ⁺ <u>Str</u> ^r	None found. KL983 unable to grow at 45°C	HDF341, HDF303 <u>ts</u> ⁺ <u>Str</u> ^r are <u>hdf</u> ⁺ <u>gal</u> ⁻ <u>q</u>
PK191 Hfr	HDF125 <u>hdf</u> <u>ts</u> <u>gal</u> <u>Str</u> ^r	For <u>ts</u> ⁺ <u>Str</u> ^r	<u>ts</u> ⁺ <u>Str</u> ^r are <u>hdf</u> ⁺ <u>gal</u>	HDF341, HDF303
KLF3-1/JC1553 (F131) <u>arg</u> <u>met</u> <u>his</u> <u>leu</u>	HDF125 <u>hdf</u> <u>ts</u> <u>met</u> <u>gal</u>	1) Test colonies for <u>f1</u> ^{Sa} 2) <u>met</u> ⁺ ^b	5 <u>f1</u> ^S /45 colonies; all 5 <u>hdf</u> <u>met</u> ⁺ 12/16 <u>met</u> ⁺ are <u>f1</u> ^S ; 16/16 <u>met</u> ⁺ are <u>hdf</u>	
KLF3/JC1552 (F103) <u>arg</u> <u>met</u> <u>his</u> <u>trp</u> <u>leu</u>	HDF125 <u>hdf</u> <u>ts</u> <u>met</u> <u>gal</u>	Test colonies for <u>f1</u> ^S	3 <u>f1</u> ^S /30 colonies; all 3 <u>hdf</u> ⁺ <u>ts</u> ⁺ <u>met</u> ⁺	HDF303
<u>λ</u> dna1A1	HDF125 <u>hdf</u> <u>ts</u> <u>met</u> <u>gal</u>	Test colonies for <u>T4</u> ⁺ growth (<u>hdf</u> ⁺)	12/250 were <u>hdf</u> ⁺ <u>ts</u> ⁺ <u>met</u> <u>gal</u>	HDF341, HDF026
<u>λ</u> dubiG1	HDF125 <u>hdf</u> <u>ts</u> <u>met</u> <u>gal</u>	Test colonies for <u>T4</u> ⁺ growth (<u>hdf</u> ⁺)	5/250 were <u>hdf</u> ⁺ <u>ts</u> ⁺ <u>met</u> <u>gal</u>	HDF341

^af1^S means sensitivity to the male-specific phage f1.

^bIt was confirmed that F131 carries met⁺ by mating KLF3-1/JC1553 with SB1803 metG his and selecting for met⁺, his⁺, and met⁺ his⁺. Colonies were obtained in all cases and all were f1^S met⁺ his⁺.

For procedural details, see Materials and Methods, Part Z. For the extent of bacterial DNA transferred by the various genetic elements, see Figure 4-11.

Figure 4-11. Genetic elements used in mapping hdf. One-minute intervals along the E. coli chromosome are indicated by dots on the line representing the chromosome and by accompanying numbers below the line. The positions of mutations are indicated by short vertical lines rising from the chromosome; these are labeled with the name of the mutant gene (Bachmann et al., 1976; Guest, 1977). Solid portions of the lines representing the various genetic elements indicate that the corresponding areas of the E. coli chromosome above are known to be present on that element. Dotted lines indicate uncertainty about the extent of the bacterial DNA. The directions of chromosome transfer in Hfr strains are indicated by the arrows.



transfers the chromosome in the same direction as KL96 and KL983, also gave strong growth patches, as might be expected.

b) F' transfer. Several F' plasmids were next used to confirm and to define more narrowly the region within which the hdf mutation lies. The amount of bacterial DNA on these plasmids has been determined genetically, which at present means that the ends of the bacterial segments are ill-defined. We have found that both plasmids F131 and F103 carry his⁺ and metG⁺, but only F103 carries hdf⁺ (Table 4-11 and Fig. 4-11). The hdf mutation must therefore be between metG and the undefined end of F103 beyond metG, towards 50'. These results also indicate that hdf is probably recessive to hdf⁺.

c) λ transduction. The mutation was further localized using two defective λ transducing phage, λdnalA1 and λdubiG1. The bacterial DNA in these phage starts at an undefined point within glpT (the same point in both phage) and for the larger phage, λdnalA1, runs at least through nalA⁺, a distance of approximately one-half minute or 20 kilobases (see Fig. 4-11). λdubiG1, which does not transduce the nalA⁺ gene, does carry ubiG⁺, a marker which cotransduces 95-98% of the time with nalA in P1-mediated transduction experiments (Stroobant et al., 1972). Both phage were found to render HDF125 hdf⁺ts⁺ (Table 4-11), thus localizing hdf to a region at 48' slightly less than twenty kilobases in size.

d) P1 transductions. P1-mediated transduction of the genes fpk⁺ (46'), dsd⁺ (50.5') and purF⁺ (49.5') confirmed the λ transduction data: P1kc grown on HDF125 or HDF125 Nal^r and used to transduce fpk⁻, dsd⁻, and purF⁻ strains to fpk⁺, dsd⁺, and purF⁺, respectively, yielded no colonies that were simultaneously hdf (Table 4-12). Further experiments with P1 and markers in the region of interest were unfruitful. The possibly helpful markers are ato,

TABLE 4-12. P1 transduction experiments to determine the map location of hdf

Donor strain and relevant markers	Recipient strain and relevant markers	Selection	Results
1 HDF125 <u>hdf</u>	AT2243-11 ^C -25 <u>fpk</u>	<u>fpk</u> ⁺	0 <u>hdf</u> /180 <u>fpk</u> ⁺
2 HDF125 <u>hdf</u>	EM1101 <u>dsd</u>	<u>dsd</u> ⁺	0 <u>hdf</u> /220 <u>dsd</u> ⁺
3 HDF125 <u>hdf</u>	AB352 <u>purF</u>	<u>purF</u> ⁺	0 <u>hdf</u> /40 <u>purF</u> ⁺
4 HDF125 <u>hdf</u> <u>NaI</u> ^r	AB352 <u>purF</u>	<u>purF</u> ⁺	0 <u>hdf</u> /193 <u>purF</u> ⁺
5 HDF125 <u>hdf</u> <u>NaI</u> ^r	AB352	<u>NaI</u> ^r	None found.
6 B178 <u>NaI</u> ^r	HDF125 <u>hdf</u>	<u>NaI</u> ^r	0 <u>hdf</u> ⁺ /171 <u>NaI</u> ^r
7 HDF125 <u>hdf</u> <u>NaI</u> ^r	KK325 <u>ubiG</u>	<u>ubiG</u> ⁺	None found. HDF125 is <u>Suc</u> ⁻ ; does not grow under selection conditions.
8 HDF341 <u>hdf</u> <u>NaI</u> ^r	KK325 <u>ubiG</u>	<u>ubiG</u> ⁺	0 <u>hdf</u> <u>NaI</u> ^r /96 <u>ubiG</u> ⁺
9 HDF026 <u>hdf</u>	KK325 <u>ubiG</u>	<u>ubiG</u> ⁺	0 <u>hdf</u> /96 <u>ubiG</u> ⁺
10 HDF125 <u>hdf</u> <u>NaI</u> ^r	HNG2 <u>glpI</u> <u>NaI</u> ^r	<u>glpI</u> ⁺	0 <u>hdf</u> /13 <u>glpI</u> ⁺
11 HDF341 <u>hdf</u>	HNG2 <u>glpI</u> <u>NaI</u> ^r	<u>glpI</u> ⁺	0 <u>hdf</u> /69 <u>glpI</u> ⁺ ; 33 <u>hdf</u> ⁺ <u>NaI</u> ^r /69 <u>glpI</u> ⁺ ;
			36 <u>hdf</u> ⁺ <u>NaI</u> ^r /69 <u>glpI</u> ⁺ ;
12 HDF125 <u>hdf</u>	HNG2 <u>glpI</u> <u>NaI</u> ^r	<u>glpI</u> ⁺	0 <u>hdf</u> /48 <u>glpI</u> ⁺ ; 41 <u>hdf</u> ⁺ <u>NaI</u> ^r /48 <u>glpI</u> ⁺

For procedural details, see Materials and Methods, Part Z4. For the location of bacterial markers, see Figure 4-11.

nalA, ubiG, nrd, glpT and A (Bachmann et al., 1976), and menC (Guest, 1977), and the ts phenotype that accompanies the hdf mutation. Mutations in the ato operon allow E. coli to grow when butyrate is the carbon source, but the use of such mutants is limited because a second, constitutive mutation for C-3 degradative enzymes must be simultaneously present for growth on butyrate to occur (Pauli and Overath, 1972); HDF strains do not have this constitutive mutation. We could not use nrd as a marker because the only well-characterized mutant carries a ts allele, and HDF cells are also ts. glpA and menC were avoided because they require anaerobic selection conditions. We found that selection for ts⁺ colonies after transduction of HDF cells with P1 grown on a wild type strain was not successful because of a high background of bacterial growth and the frequency of spontaneous ts⁺ colonies ($\sim 10^{-6}$). We were thus left with nalA, ubiG, and glpT to work with in P1 transduction experiments.

We initially concentrated on nalA because the ease with which nalA⁻ derivatives of bacterial strains can be obtained (by selecting colonies that grow in the presence of 25-50 $\mu\text{g/ml}$ nalidixic acid [Hane and Wood, 1969]) opened the possibility of making the required strains and then transducing both into and out of HDF bacteria. Most transductions in which Nal^r was the selected phenotype, however, failed for unknown reasons: no colonies above the number of spontaneous resistants found on control plates were seen. A few experiments with particular P1 stocks appeared to work, as judged by the number of colonies on plates containing P1-infected cells (100-200) compared to the controls (2-20). In one such apparently successful experiment, P1 grown in B178Nal^r were used to infect HDF125 at moi's from 0.5 to 5 and 2-4 hours were allowed for expression of the recessive nalA allele before selection conditions were imposed. All Nal^r

colonies, however, were still hdf (Table 4-12, Expt. 6), a totally unexpected result since nalA and glpT, which define the limits of the λ transducing phage and between which the hdf mutation must therefore lie, are reported to show 50% cotransduction (Kistler and Lin, 1971), and our own control experiments gave 48-85% cotransduction. Transductions in the opposite direction were equally unsuccessful. Although P1 stocks made in HDF125Nal^r showed normal transduction frequencies of purF⁺ into strain AB352purF⁻ ($\sim 10^{-6}$ /P1 pfu), in the same experiment we were unable to find any transductants when Nal^r was the selected marker. Similar results were obtained when other K-12 strains (B178, E101, KK333) were used as recipients.

When nalA was not the selected marker and the P1 transducing phage were grown in Nal^s cells, more normal results were found with respect to the frequency of cotransduction between glpT and nalA (Table 4-12, Expts. 11 and 12). This behavior is in contrast to that seen in Experiment 8, where P1 was grown in HDF341Nal^r and used to transduce a ubiG strain to ubiG⁺; subsequent testing showed that as well as an absence of transduction of hdf, there was also no cotransduction of Nal^r with ubiG⁺, although these markers have been reported to show 95-98% cotransduction (Stroobant *et al.*, 1972).

Transduction experiments in which ubiG⁺ and glpT⁺ were the selected markers and P1 was grown in HDF strains (Table 4-12, Expts. 7-12) were more successful in terms of transductant recovery than experiments in which Nal^r was the selected phenotype, but in no case was the hdf marker transduced. These observations cast doubt on the validity of the results obtained with the markers fpk, dsd, and purF. Because the results of three other kinds of genetic experiments place hdf in this region of the E. coli chromosome, however, we conclude that the P1 results indicating no linkage are aberrant. One might hypothesize that

all transductant colonies are also P1 lysogens and that P1 carries a gene that influences the expression of hdf to produce the observed results, but to explain all the anomalies it would have to suppress hdf in some cases and not in others, an unlikely situation (Table 4-12; compare Expt. 6 with the rest). These puzzling results made us wonder if hdf could be a strain-specific marker, expressed only in B178 and its derivatives. If this were the case, however, Experiment 6 in Table 4-12 should have worked. It therefore is more likely that the observed effect is specific for hdf. These results must be due to strong selective pressure exerted at the level of P1 transductant particle formation, the level of recombination of the transduced DNA with the recipient's chromosome, or the level of gene expression.

3. Additional unusual results obtained in mapping experiments

A variety of additional unusual results were obtained in mapping experiments which we will now present and discuss. We organize these results according to the HDF strain involved.

a) HDF125Nal^r. A spontaneous derivative of HDF125 selected for growth on 35 µg/ml nalidixic acid and therefore probably containing a mutation in nalA was used in several experiments. It is the strain of choice to use in transductions with λdnalA1, because colonies may then be checked for sensitivity to nalidixic acid as a test for successful transduction (sensitivity to nalidixic acid is dominant to resistance). However, we were unable to find any Nal^S or hdf⁺ colonies (0/514) after infecting cells with λdnalA1, unlike the situation with HDF125 Nal^S (Table 4-11). This result is not due to the inability of λ to lysogenize HDF125 Nal^r, because in separate experiments we were able to obtain lysogens of this strain with wild-type λ.

We were also unable to introduce the plasmid F103 (metG⁺) into HDF125metG Nal^r as determined either by checking potential recipients for sensitivity to the male-specific phage f1 or by selecting for metG⁺ after mating, although F103 was successfully introduced into HDF125 metG Nal^s (Table 4-11).

b) HDF026. Mapping data for HDF026 is desirable because this strain gave us the best gel electrophoresis results. Unfortunately, it could not be used in Hfr experiments because it is ts⁺ and the selection for recombinants in that experiment depends on the ability of Hfr's to supply a ts⁺ allele to ts⁻ HDF strains.

P1 transduction experiments using this strain as a recipient were impractical because HDF026 has a spontaneous mutation rate which is 100-fold higher than that in other strains and is comparable to P1 transduction frequencies ($\sim 10^{-6}$). Transductions in the opposite direction gave the anomalous results described above. Experiments with the defective λ transducing phage were only slightly encouraging: we found one hdf⁺ colony out of 370 tested after transduction with λ dnalA1, and 0/590 with λ dubiG1.

The plasmid F103 could apparently be introduced into HDF026, but with low frequency (2 f1^s colonies out of 173 tested) and with the result that f1^s strains were still hdf⁻. There are several possible explanations for this result: the complete F103 plasmid may not have been transferred; HDF026 could contain a dominant hdf allele; the hdf mutation could map at some other locus; or the plasmid DNA could have recombined with the chromosomal DNA resulting in the loss of some markers. Because of the results reported below for HDF341, we tend to believe the last hypothesis. We would then also have to invoke recombination to explain the results of mating a Nal^r derivative of HDF026 with

the semi-anaerobically grown donor of F103: 13 out of 35 colonies subsequently tested were $f1^S$ and gal^- but were still hdf^- Nal^R .

c) HDF341. HDF341 was anomalous in that we found no $f1^S$ colonies (0/90) after mating it with a strain containing F103 unless the donor was grown semi-anaerobically, and then although both $f1^S$ (1/22) and $T4^S$ (3/22) strains were found, no colony was found which was simultaneously sensitive to both phage. The same experiment performed with a Nal^R derivative of HDF341 yielded 6 $f1^S$ colonies out of 24 tested, all of which were still Nal^R hdf^- gal^- . Because we have data from Hfr and lambda transduction experiments for HDF341 which locate the hdf mutation in the immediate vicinity of 48' (Table 4-11), we think that these results are due to recombination of the F103 plasmid with the bacterial chromosome in the recA⁺ HDF strain.

One finding common to HDF strains 125, 341, and 026 was that irregular behavior occurred when Nal^R derivatives were used in genetic experiments. We are not sure of the significance of this behavior, but it implies that there may be some interaction between the hdf mutation and that responsible for the Nal^R phenotype.

4. Summary

In summary, the hdf mutation in two strains, HDF125 and HDF341, lies between nalA and glpT and the mutation in HDF026 may be in the same region (based on a single λ dnalA1 transductant). The mutation in HDF303 probably also falls in this region, although it has been mapped only to the resolution obtainable with the F' plasmids F131 and F103.

A number of experiments to circumvent the P1 transduction problem could be tried. More λ transducing phage that carry smaller amounts of bacterial

DNA could be isolated and tested for hdf, or recombinant DNA technology could be used to construct such phage. A way to test whether all HDF strains carry allelic mutations would be to isolate F' factors that carry hdf and do complementation tests.

At the beginning of this section we pointed out that in order to legitimately pool phenotypic data from different strains, we must show that the strains involved carry identical or allelic mutations. We have been unable to obtain mapping data of sufficient resolution to conclude that the hdf mutations in our strains are allelic. For the sake of discussion, however, we will assume that allelism is the case.

V. Comparison of hd590 and K-12 HDF strains

hd590 was isolated and characterized by Simon et al. (1974). These investigators found that in $T4^+$ -infected hd590 cells, phage DNA synthesis was depressed, specific late phage proteins were missing from gel electrophoresis patterns, and the only T4 virion structures seen in negatively stained lysates were empty heads, polyheads, and some polysheath.

The results of $T4^+$ infections of hd590 which Simon et al. observed are almost identical to those found for HDF strains, although hd590 shows a more extreme phenotype. We found that hd590 was also like our K-12 strains in the following ways: it was ts for cell growth at 42°C , was late in lysing after $T4^+$ infection, and was partially permissive for $T4^+$ growth at 25°C . The latter property was not revealed by spot tests, but burst size determinations in broth at 25°C yielded 5-20 phage per cell.

In our hands, hd590 was able to grow in M9 + thymine but produced an abnormal pattern of ^{14}C amino acid labeled phage proteins under these conditions (see Plate 4-2). When cells were grown in a richer medium, infected, and labeled with ^3H leucine, we found certain late phage proteins to be missing, as did Simon et al., but the pattern we saw differs from that published by them (see Section II, Part D, and Appendix).

We were able to repeat Simon's observation that 2×10^{-4} M EDTA improves the burst size of $T4^+$ in hd590 to ~ 0.1 phage per cell. We also found that it less dramatically increased the burst of $T4^+$ on the K-12 HDF strains by 2- to 5-fold, to 1-2 phage per cell.

The majority of go phage selected on hd590 lawns were like goF-1, but we also found phage like go590-1 that carry mutations in gene 31. All such

phage were able to grow only on hd590 and on wild-type cells, not on K-12 HDF strains. The ability of phage with a mutation in gene 31 to grow on hd590 is puzzling, since gene 31 is only known to act in the early stages of T4 head assembly. These stages are probably normal in hd590 because T4 capsids are produced. The implication is that gp31 interacts with some E. coli element that is needed for head assembly and also for other T4 purposes, and in hd590 only the latter are affected. In some way this interaction must be unique to hd590 or to B strains of E. coli, since go590 phage carrying mutations in gene 31 were unable to grow on K-12 HDF bacteria. The changed gp31 does not hamper the growth of go590-1 in wild-type cells. go590-1 was also tested for growth on the T4 head assembly defective strains HDAD11, HDA175, HDD36, and groEA44, with negative results.

In summary, we conclude that hd590 and the HDF strains block T4⁺ growth at the same level(s), but differences between B and K-12 strains of E. coli allow T4 to overcome the block in one type of host by routes that are ineffective in the other (phage carrying certain mutations in T4 gene 31 can plate on hd590 but not on the K-12 strains, and vice versa for certain go mutations that map near gene e).

It would be of interest to find out if the mutation in hd590 maps in the same gene as hdf maps in the K-12 HDF strains. If the function of gp31 could then be elucidated, that information would contribute to understanding the nature of the defect in these host mutants which pleiotropically affect T4⁺ development.

VI. Discussion

A. Introduction

Our orientation in the investigation of HDF mutants was towards the possibility of host involvement in T4 virion assembly. Class F host-defective bacteria passed, albeit marginally, our two screens for mutants likely to affect phage assembly: normalcy of lysis following T4⁺ infection and normalcy of phage DNA synthesis. T4⁺-infected HDF cells did lyse, although later and more slowly than normally, and while phage DNA synthesis was depressed by about 50%, a similar depression was seen in T4⁺ infections of groEA44, an E. coli mutant which blocks T4⁺ head assembly (Georgopoulos et al., 1972). We therefore did not immediately eliminate HDF strains as candidates for mutants in host-coded T4 assembly functions. We were further encouraged by data obtained from gels, in vitro complementation reactions, and electron microscopy: although virion structural proteins were found on gels, tail and baseplate structures were not seen in the electron microscope or found in in vitro complementation experiments. More gels and extensive in vitro complementation work led us to the conclusion that several tail proteins (gp7, gp10, gp15) were present but in lower than normal amounts, and that a lack of sufficient concentrations of gene products could explain the failure of the in vitro complementation experiments and perhaps also the failure of in vivo T4 tail assembly. The finding that specific early T4 proteins were also greatly decreased led us to question the likelihood that the major defect in T4⁺-infected HDF cells was in phage assembly and to reevaluate the significance of the depressed phage DNA synthesis. We will now briefly summarize the phenotype of HDF mutants, discuss several models which attempt to account for the available data, and suggest directions for further work.

B. The phenotype of HDF strains

The hdf phenotype is a complex one. Mutant cells are able to adsorb $T4^+$ normally and are killed by the phage, but in the subsequent infection some early proteins are underproduced and others are overproduced, phage DNA synthesis is depressed, phage capsids are either not filled with DNA or if filled are abnormally unstable, the amounts of specific late phage proteins are decreased, and lysis of the infected cell is slow and delayed although phage lysozyme is present. Whatever host component is responsible for this complex phenotype is ts in both uninfected and infected cells, and is needed throughout T4 infection.

C. Model 1: A defective bacterial membrane

1. Direct host membrane participation

One kind of bacterial mutation that could account for such a variety of effects is an alteration in a bacterial membrane component. Such an alteration could conceivably affect all phage processes which are membrane-associated, a list of which closely resembles the effects of the hdf mutation on $T4^+$ infection: there is evidence that the E. coli membrane participates in phage DNA synthesis, capsid formation and DNA packaging, lysis, and perhaps tail assembly (for a review, see Siegel and Schaechter, 1973). In this model the host component would clearly be needed throughout phage infection and could also be responsible for the observed temperature-sensitive bacterial growth. The goF mutations, which can partially overcome the hdf block to T4 growth, are then thought of as changes in T4-coded components that normally affect the host membrane. Changes in both the protein and lipid composition of the membrane have been found in phage-infected cells: at least 13 T4 proteins, most of unknown identity and function, are selectively enriched in membrane preparations of $T4^+$ -infected

wild-type E. coli B (Huang, 1975); and the lipid metabolism of infected cells is different from that of uninfected bacteria, although it is not known if any T4 genes are responsible for these changes (Buller and Astrachan, 1968; Furrow and Pizer, 1968; Peterson and Buller, 1969; Merlie, 1974). It is conceivable that mutations in T4 genes whose products interact with the cell membrane could correct the effects of the hdf lesion. The depression in amount of specific early T4 proteins, however, is not explained by this model.

2. Indirect host membrane participation

We have previously argued (Section IIF4) that in T4⁺-infected HDF strains the problem in tail-baseplate assembly is likely to be due to insufficient quantities of structural proteins rather than a failure in the assembly process as proposed in the model above. We can explain these shortages as the result of a cell membrane defect by changing the model and proposing that the major disruptive effect of the altered membrane is on phage DNA synthesis. In T4 infections it is known that late transcription (primarily of virion structural proteins) is obligatorily coupled to DNA replication; the latter process may provide a "competent" state of the DNA in which it has nicks or single-stranded gaps (Rabussay and Geiduschek, 1977). Defective late transcription in HDF cells could then be caused by an aberrant, nick-free state of the poorly-replicating phage DNA, which in turn might be the result of the improper interaction of that DNA with the mutant bacterial membrane. This aberrant state could also interfere with head-filling reactions (cf. Kemper and Brown, 1976). Defective cell lysis is explained if the necessary lysis proteins are late ones. Another example of an aberrant state might be a disruption of the physical organization of the DNA within the replicating pool (Curtis and Alberts, 1976).

The depressed amounts of certain early proteins during the phage infection, however, is more difficult to explain by this model. It is not known whether membrane attachment is necessary for some T4 early transcription; no phage mutants have been found in which attachment is defective (Miller, 1975). Phage gene products are, however, necessary for the attachment process (R. C. Miller, 1972), so at least some early T4 transcription must occur on free DNA. Experiments with HDF strains that determine the time of attachment of the T4 chromosome to the host membrane (reported to be 3' after infection at 37°C in E. coli B [R. C. Miller, 1972]) relative to the earliest time at which missing proteins can be detected (4-6' after infection in nonultraviolet irradiated cells, Plate 4-1A) would answer the question of whether the messages for the decreased early proteins are transcribed from attached or free DNA and therefore whether the hypothesis of a defective membrane component in hdf cells is a viable one.

D. Model 2: hdf has transcriptional or translational effects

The second model postulates that the altered host component in hdf cells is directly involved in T4 early transcription or translation, and that either the T4 gene products whose expressions are consequently blocked are needed for phage DNA synthesis, or the same transcriptional or translational host component also participates in T4 DNA replication. In either case, a depression in phage DNA synthesis results, and an argument like that in Part C2, above, is then made: the DNA that is synthesized has an abnormal structure and therefore is a poor substrate for packaging and for the transcription of some late genes, including those for tail proteins and substances necessary for lysis. Alternatively, the depressed synthesis of some late T4 proteins could be explained by the participation of gphdf in transcription or translation late in infection

as well as early. (gphdf cannot function solely in DNA replication because its effects on early phage proteins are found at least as early as 4-6' after infection in HDF cells (Plate 4-1A), whereas DNA synthesis does not start until 8' or later [see Fig. 4-3].)

1. Is E. coli RNA polymerase involved?

The most obvious candidate for a host component involved in transcription is E. coli RNA polymerase, which is used in various phage-modified forms throughout T4 infection. The hdf mutation does not map near any of the genes that code for the three polymerase subunits (Bachmann et al., 1976) or for sigma factor (Nakamura et al., 1977; Harris et al., 1977), but it could lie in an as yet unidentified gene whose product modulates the polymerase or which affects one of the T4 gene products that are known to alter, modify, or add to the host enzyme (Losick and Pero, 1976). For this model to be correct, different classes of early T4 promoters distinguishable by the polymerase must exist in order to explain the selective effect of the hdf mutation on the production of T4 early proteins. Such classes have been found (Rabussay and Geiduschek, 1977).

The host mutation could also be at the translational level, affecting ribosomal recognition of certain messages; again, there would have to be some feature of the messages that allows such a discrimination.

In either of these models, the goF mutations are postulated to lie in T4 transcriptional or translational factors which in wild-type infections play enhancing but nonessential roles, and which in altered form can compensate for the cellular hdf mutation.

2. Is hdf an alteration of the nalA gene?

A more intriguing candidate for the hdf host component is the nalA gene product, mutations in which can make cells resistant to nalidixic acid, a

DNA synthesis inhibitor. It will be recalled that the hdf mutation lies very close to nalA on the E. coli genome and could fall within this gene (see Section IV of this chapter).

a) Involvement of gpnaIA in transcription. Although originally described as affecting DNA synthesis only (Goss et al., 1965), nalidixic acid has recently been found to have effects on transcription as well. Puga and Tessman (1973) found that transcription of phage S13 DNA in E. coli C was nonspecifically inhibited to 20% of its normal value by 32 $\mu\text{g/ml}$ of nalidixic acid, although under these conditions bacterial transcription was unaffected. The size distribution of the transcripts produced was normal, and the inhibition was not found when the host cell was nalidixic acid resistant.

Shuman and Schwartz (1975) reported specific inhibition in vivo of the induction of several bacterial proteins in the presence of 50 (but not 10) $\mu\text{g/ml}$ nalidixic acid. The induction of the λ receptor was inhibited by 80%, maltodextrin phosphorylase synthesis was reduced 70%, and β -galactosidase synthesis was reduced 40%; however, no effect was seen on L-tryptophanase, homoserine dehydrogenase, or glucose-6-phosphate dehydrogenase. The effect on β -galactosidase was seen regardless of whether cAMP was present during induction.

Staudenbauer (1976) has shown that ^3H -UMP incorporation is depressed 50% in plasmolysed E. coli cells at nalidixic acid concentrations of 50-100 $\mu\text{g/ml}$, 10-20x higher concentrations than those required for a comparable inhibition of DNA synthesis. This depression is not seen when nalidixic acid resistant cells are used. Further experiments in which nalidixic acid, rifampicin, and ^3H -UTP were simultaneously added to plasmolysed, NaI^{S} cells did not show lower amounts of label incorporation than experiments in which the nalidixic acid was omitted,

implying that the drug does not interfere with RNA chain elongation and therefore must act at the chain initiation step. These data agree with the observation of Puga and Tessman (1973) that S13 transcripts are of normal size in nalidixic acid-treated cells.

b) The occurrence of *gpnaIA* in *E. coli*. The *naIA* gene product (MW 110,000) occurs in at least two protein complexes in *E. coli*. The first complex is the recently discovered DNA gyrase (Gellert *et al.*, 1976a,b). This enzyme is able to introduce negative supercoils into closed circular DNA, and the subunit that contains the necessary DNA nicking-closing activity for the supercoiling function has been found to be *gpnaIA* (Sugino *et al.*, 1977; Gellert *et al.*, 1977). The second complex is a dimer of *gpnaIA*, denoted PnaI (MW 220,000), which has DNA nicking-closing activity (Sugino *et al.*, 1977). The exact roles of DNA gyrase and PnaI in *E. coli* are not understood. Since both are sensitive to nalidixic acid *in vitro* and resistant to it when prepared from *Nal^r* cells, either or both could be involved in *E. coli* DNA synthesis.

c) Proposed role for *gpnaIA* in T4-infected cells. How might the *naIA* gene product function in T4-infected cells? If the *hdf* mutation does indeed map in *naIA*, then our model for what happens in T4⁺-infected HDF cells is as follows. The transcription of specific early phage gene products is depressed due to the altered *naIA* product, and either the lack of these phage products inhibits T4 DNA synthesis, or the *naIA* product also plays a direct role in T4 genome replication. Since the processes of transcription and replication have many features in common (proteins bind to the DNA, locally denature it and use it as a template, and RN^h synthesis is involved in both), this hypothesis is not unreasonable. The resulting poorly synthesized DNA may be an ineffective

substrate for late gene transcription, as described in earlier models, or gpnaIA could directly interfere with the transcription of late genes. The nicking-closing activity of PnaI or the supercoiling ability of DNA gyrase could also conceivably be useful in the packaging of T4 DNA, a process which we have found is defective in HDF cells. Numerous combinations of these possibilities result in models in which specific T4 proteins are missing or made in reduced quantities, phage DNA synthesis is depressed, phage capsids are not correctly filled with DNA, and cell lysis is slow and delayed. The requirement for the host product throughout infection can also be met. T4 goF mutants would lie in a gene whose product normally interacts with gpnaIA and which, in altered form, can partially compensate for naIA mutations of the hdf type. According to this model, since gpnaIA is involved in DNA replication, the temperature-sensitivity of the uninfected HDF host cell would most probably be a consequence of aberrant DNA synthesis. We have not seen any effect of high temperature on the cumulative incorporation of ^{14}C thymidine in HDF cells (Section IVA2), but more sensitive pulse-label experiments or an examination of the structure of HDF DNA at 42°C might be revealing.

If gpnaIA plays a role in T4 infections, one might expect to see an effect of nalidixic acid on infected wild-type cells. This result has been found: the burst size of T4 r48 (rI⁻) in E. coli B is decreased by one-third in the presence of 10 µg/ml nalidixic acid, and by two-thirds in the presence of 50 µg/ml. If the cells are preincubated with nalidixic acid prior to infection, the resulting decrease in phage production is even more severe (Baird et al., 1972). hdf mutations would therefore transform a host protein that has an unnecessary but enhancing role into one that has lethal effects.

It is worth mentioning that Baird et al. (1972) also found that nalidixic acid severely inhibits phage production in cells infected with T5, T7, ϕ R, or λ and with T5 and T7 they showed that this was because phage DNA synthesis was inhibited. These results are of interest because so far DNA gyrase and PnaI have been shown to affect only circular DNAs such as are found in phage λ infections; T7, however, does not replicate through a circular intermediate (Ritchie, 1975) (nor does T4 [Doermann, 1973]).

d) λ dubiG1 may carry part of nalA. The observations that λ dubiG1 carries hdf⁺ but not nalA⁺ (Section IVB2c) cast doubt on the above hypothesis concerning the nature of the hdf mutation, but an explanation is available. The report that λ dubiG1 does not carry nalA⁺ (Sugino et al., 1978) is based on genetic complementation experiments and on a comparison of the proteins transcribed and translated from the two defective transducing phage: λ dnalA1 produces a 110,000 MW protein, the correct size to be the nalA gene product, which is not made by λ dubiG1. All these results necessarily mean, however, is that part of the nalA gene or its controlling region must be absent from the λ dubiG1 bacterial DNA; they do not mean that the entire gene is absent. hdf mutations could occur in whatever parts of nalA remain on λ dubiG1 (the genetic data in Section IVB2c must then be the result of recombination events rather than of integration of λ dubiG1 into the chromosome in HDF strains). The isolation of more defective lambda transducing phage that have less bacterial DNA than the present two could help to settle this question.

E. Further experiments

A variety of experiments could help elucidate the nature of the interaction between phage and host in HDF cells. As mentioned above, the accurate

mapping of the host mutation could clearly direct research either towards or away from the investigation of the role of gpnaIA in T4 infections. It might be worthwhile to reisolate HDF mutants for this purpose, using a mutagen other than nitrosoguanidine. Purification and characterization of gpnaIA from HDF strains might also answer the question of whether hdf and naIA are the same gene. Analysis of the membrane-associated phage proteins in T4⁺-infected and goF-infected HDF cells might help clarify the status of Model 1, as would an investigation of the state of replicating T4⁺ DNA in HDF strains. The identification of the early phage proteins present in decreased amounts in T4⁺-infected HDF cells could also be valuable, and further gel work is needed to determine whether any are restored to more normal levels under permissive infection conditions. The locations on the T4 genome of the two unmapped goF026 mutations (Section IIIC) and the discovery of the functions of the genes in which goF mutations lie should also be illuminating.

Appendix

SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS OF EXTRACTS
OF T4⁺-INFECTED HDF CELLS

In HDF026, four early bands of approximate molecular weights 58,000, 43,000, 36,000, and 26,000 daltons are missing or greatly decreased. Two of these proteins, p43,000 and p36,000, are also visibly decreased at late times in HDF026, HDF125, hd590 (Plate A-1), and HDF303 (not shown) infected with T4⁺. These two proteins are still missing at late times in cells infected under conditions permissive for phage production (Plate A-1, tracks 7 and 8). It is possible that p43,000 and p36,000 are present at early times under permissive conditions but are also shut off early (see under "Bacterial revertants to hdf⁺," below); we do not have gels at early times under permissive conditions in which these bands are clearly identifiable.

p58,000

On 10 cm 10% acrylamide gels on which proteins are electrophoresed for ~1 hour after the marker dye has reached the bottom of the gel, the 58,000 MW band in wild-type infections is resolved into two bands, the lower one of which can be identified as gp41 by comparison with gels of infections with T4 amN81(41⁻) (Plate A-2). On the same long gel we have so far been unable to resolve the 58,000 MW region in infections of HDF026, so the decreased protein could be either gp41 or the unidentified band immediately above it.

p43,000

The 43,000 MW band runs very close to the position of gp63 but it is not gp63. This is clear from Plate A-1 and from in vitro reactions (performed according to Wood and Henninger, 1969) in which HDF extracts were able to supply gp63 to increase the rate of tail fiber attachment to fiberless T4 particles

Plate A-1. Gel electrophoresis of T4-infected HDF cells and T4 baseplate-defective mutant infections. Labeling and electrophoresis conditions are as for Plate 4-2, except that early samples (tracks 1 and 2) were labeled at 37°C from 3-8' after infection. Tracks containing proteins labeled in infections of B178 with T4 amber mutants are identified by the names of the mutant genes written above each track; the specific mutants used are listed in Tables 2-2 and 2-3.

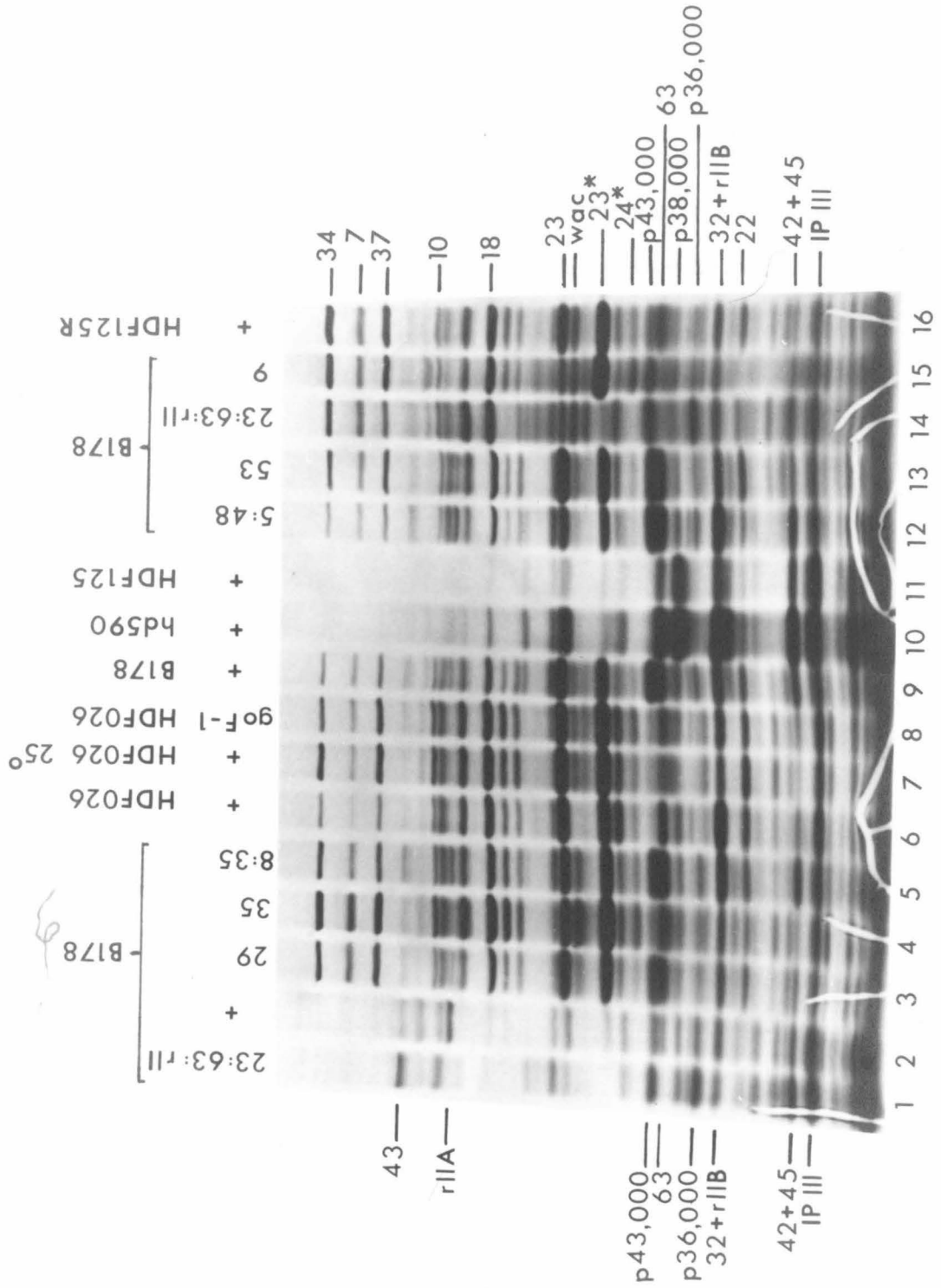
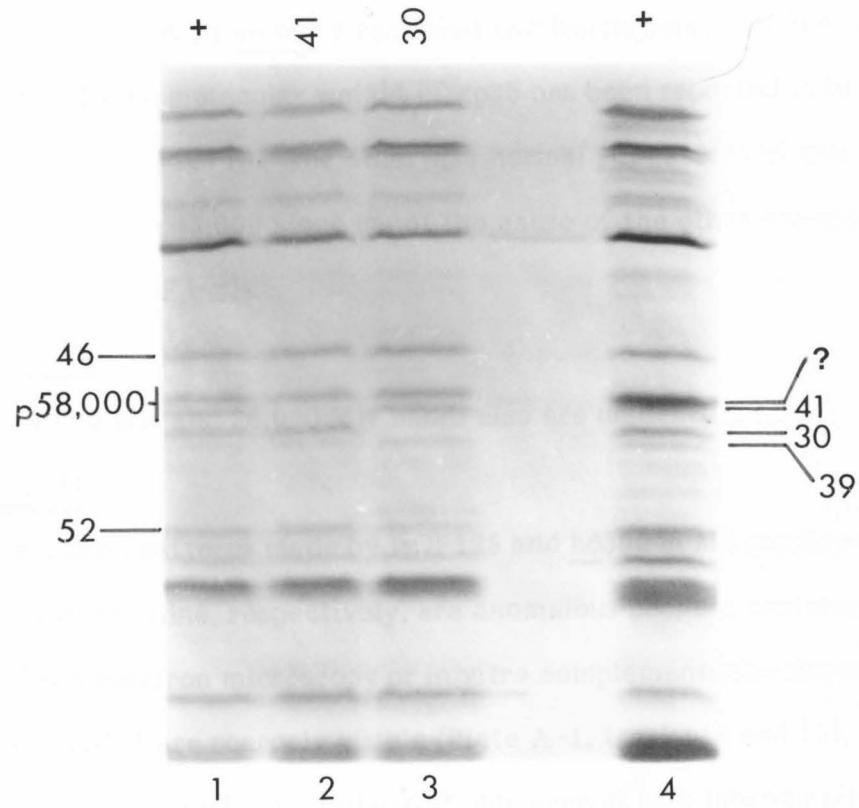


Plate A-2. Identification of gp41 and an additional protein in the 58,000 MW band. B178 was infected at 37°C with wild-type phage (+) or with amber mutants (Table 2-2) in the genes named above each track. Cultures were labeled as described in the Materials and Methods, Part M1, at 4' after infection and were chased at 8' after infection. Electrophoresis was continued for ~1 hour after the marker dye reached the end of the 10 cm, 10% acrylamide gel.



(data not shown). Several published gels have indicated that gp β gt migrates as a 43,000 MW band (Huang and Buchanan, 1974; Sauerbier *et al.*, 1976), but we have not been able to confirm these findings. In Plate A-1, track 4, it may be seen that a sample of 35⁻ (amB252)-infected wild-type cells is also missing the 43,000 MW protein. This deficiency is probably due to the presence of an additional mutation in the T4 amber strain used and has no relationship to the mutation in gene 35 (the molecular weight of gp35 has been reported to be 39,000 [Vanderslice and Yegian, 1974]). The otherwise normal gel pattern of this mutant implies that the lack of p43,000 alone is not the cause of the other changes seen in T4⁺-infected HDF cells.

p36,000 and p26,000

The 36,000 MW and 26,000 MW bands also are unidentified..

HDF125 and hd590

The gel band patterns made by HDF125 and hd590 in M9 supplemented with methionine or thymine, respectively, are anomalous because proteins known to be present from electron microscopy or in vitro complementation experiments such as gp18 and gp23* are scarcely visible (Plate A-1, tracks 10 and 11). In addition, sometimes only gp23, but not gp23*, are seen at late labeling times, although we find empty heads (and therefore gp23*) at these times (Plate 4-5). Several proteins are overproduced, including gp32 + gprIIB, gp42 + gp45, gpIPIII, and a band of approximately 38,000 MW.

Since all other experiments investigating the phenotype of T4⁺ infections of HDF cells were performed in H broth, we decided to prepare new extracts, making the labeling medium as rich as possible. Cells in M9 supplemented with 19 amino acids (except leucine), purines, pyrimidines, vitamins, and iron, were

labeled with ^3H leucine and yielded the gels shown in Plate 4-3. Under these conditions of growth and labeling, gp18, gp23, and gp23* are readily evident and the 38,000 MW protein is not as grossly overproduced. A comparison of ^3H leucine and ^{14}C amino acid labeled T4^+ -infected HDF026 made on the same day and electrophoresed on the same gel (Plate 4-3, tracks 1 and 2) does show a few differences between the patterns as a result of the different labels: gp34, gp37, and the bottom part of the 43,000 MW band (gp63?) seem less heavily labeled with ^3H than with ^{14}C . We are, however, able to conclude from the ^3H labeled tracks that HDF125 and hd590, like HDF026, show reduced quantities of gp34, gp7, gp37, gpwac, and p36,000, and increased gp32 + gprIIB and gpIPIII. They also seem deficient in the band above gp18, which includes gp6. In addition, on this gel hd590 overproduces gp42 and the band above gpIPIII (gp1 or gp11?). Replicate experiments have not been done under these labeling conditions to determine the reproducibility of these observations.

The results shown here for hd590 are similar to those of Simon et al. (1974) in that gp34, gp7, and perhaps gp6 are greatly reduced, although we have not positively identified gp6. We have no clear explanation for the other changes which we see but which Simon et al. did not, although they did use a different labeling medium (minimal medium + 1% tryptone), labeled with ^{35}S methionine in addition to ^{14}C amino acids, and labeled only at late times.

Bacterial revertants to hdf^+

In infections of HDF125R (Plate A-1, track 16) and HDF026R (not shown), all four late virion structural proteins are restored. p43,000 and p36,000 appear to be present at early times but are clearly decreased in amount by 15' after infection (not shown), unlike the situation in B178 where these bands are still

labeled as late as 20' after infection (Plate A-1). The 58,000 and 26,000 MW bands have not been identified on gels of the revertants.

Baseplate proteins

In Plate A-1 we also show the proteins made in cells infected with phage carrying mutations in baseplate genes whose products have previously been identified in gels of infected cell extracts (Vanderslice and Yegian, 1974). We are unable to identify with certainty missing bands of the appropriate molecular weights in these extracts, but the results allow us to conclude that the changes seen in HDF026 gels in the 35,000-43,000 MW region are not due to gp8 (39,000), gp5 (37,000), or gp48 (37,000). This conclusion is reinforced by the gels of T4⁺-infected HDF026 labeled at early times (Plate 4-1) which show that the missing bands in this region are also synthesized at early time and are therefore not baseplate structural proteins.

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