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Role of the Host Cell in Bacteriophage T4 Development

I. Characterization of Host Mutants That Block T4 Head Assembly

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To study the role of the host cell in bacteriophage T4 infection, we selected more than 600 mutant host-defective bacteria that adsorbed and were killed by phage $T4^+$ but were unable to support its growth. The mutants were grouped into seven classes by the growth patterns of T4 phages carrying compensating mutations (*go* mutants [*g*rows *o*n]), selected on four prototype host-defective strains. Lysis and DNA synthesis experiments indicated that classes A, AD, D, and B (the majority of the host-defective mutants) block $T4^+$ development at an assembly step, class C mutants affect an early stage in phage development, and class F mutants appear to act at more than one stage. Analysis of $T4^+$ infection in the assembly-defective mutants by in vitro complementation, electron microscopy, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that the host-defective mutations interfere with $T4^+$ capsid formation at the level of phage gene 31 function, before assembly of any recognizable capsid structure. The mutations map near *purA*, but at two or possibly three different sites. The *go* mutant phages able to overcome the host defect carry mutations in either gene 31, as found by others for similar defective hosts, or in the gene for the major capsid protein (gene 23). The gene 23 *go* mutations do not bypass the requirement for gene 31 function. These results suggest that at least three components must interact to initiate T4 head assembly: gp31, gp23, and one or more host factors. The compensatory effects of mutational alterations in these components are highly allele specific, consistent with the view that phage and host components interact directly in protein complexes.

The morphogenesis of bacteriophage T4 is known to proceed in stepwise fashion by the subassembly of heads, tails, and tail fibers, which are subsequently joined in sequence to form active phage particles (40). Although most of the assembly steps can proceed in infected-cell extracts (5), the earliest steps in these subassembly pathways have not yet been demonstrated in vitro (14, 22, 28). Consequently, we wondered whether the host cell might play an obligatory role in initiating some assembly processes. Viruses utilize much of the existing host cell synthetic machinery, specifically redirecting it to make virus particles. Perhaps the host also supplies scaffolding, templates, or other components essential for initiating viral assembly. Electron microscopic evidence suggests that the first steps in the assembly of T4 heads, and perhaps that of baseplates, proceed on the host membrane (29, 30).

We sought to investigate these possibilities by the study of host mutants that specifically block T4 assembly. When this work was initiated, two host mutants that interfere with T4 head assembly had been described. Georgopoulos et al. (9) had reported that *groEA44*, a K-12 mutant selected for its inability to support phage λ growth and found to block λ head assembly, also blocked T4 morphogenesis early in capsid formation, at the step controlled by gene 31. A single mutant derivative of *Escherichia coli* B called *mop* (37) gave a similar phenotype after T4 infection. Furthermore, Pulitzer and Yanagida (20) had reported that a mutant of W3350 was deficient in the assembly of functional tail fibers, and we had inferred from genetic experiments that host involvement in T4 tail fiber assembly occurs at the level of the gene 57-controlled step in polymerization of the tail fiber components (22). These results encouraged us to search for more assembly-defective mutant hosts.

Accordingly, we selected host-defective (HD) bacteria unable to propagate T4 and grouped

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them into classes based on their ability to propagate mutant T4 *go* (*g*rows *o*n) phages selected for growth on various HD strains. We report here genetic and physiological studies on four classes of HD mutants that block T4 assembly and on the T4 *go* phage mutants that overcome these blocks. All of these HD mutant hosts appear to interfere with T4 capsid assembly at the step controlled by gene 31, in agreement with results found for similar mutants by other investigators (3, 9, 36, 37). Genetic analysis shows that the HD mutations define at least two and perhaps three host genes and that compensating phage *go* mutations can occur in either of two phage genes. The patterns of compensation among these mutants are discussed with reference to the detailed model of Takahashi et al. (36) and the possible nature of phage-host interactions in the initiation of T4 capsid assembly.

HD mutants of a fifth class affect T4 assembly but also exhibit additional pleiotropic effects on T4 development. We shall describe the characterization of these mutants in a subsequent paper (B. L. Stitt, H. R. Revel, I. Lielausis, and W. B. Wood, J. Virol., submitted for publication). We also found mutants of a sixth class, which affect an early step in T4 growth; we have not further investigated these mutants.

(A preliminary report of some of the work described here has been published [39]. These studies are included in the doctoral dissertation of B.L.S., submitted in partial fulfillment of the requirements for the Ph.D. degree, California Institute of Technology, Pasadena, 1978.)

MATERIALS AND METHODS

Media. H broth for phage and bacterial growth and EHA top and bottom agars for plating were prepared as described previously (33). LB broth and top and bottom agars (21) were supplemented with 2.5×10^{-3} M $CaCl_2$ for growth of P1 or 0.3% maltose for growth of phage λ. M9, a synthetic, phosphate-buffered medium (13), was used when phage-infected cells were labeled with ^{14}C-amino acids. M9 top and bottom agars contained 6.5 and 10.0 g of agar (Difco Laboratories) per liter, respectively. M9 was supplemented as required with amino acids, purines, or pyrimidines at 20 to 50 μg/ml and vitamins at 0.1 μg/ml for the growth of auxotrophic bacterial strains in P1 transduction and F′ transfer experiments. Buffers used were the dilution medium (DM) described by King (15); Tris-maleate (TM) buffer, pH 6.0 (1); and Tris-magnesium buffer (TMg), pH 6.8 or pH 7.4, which was 0.05 M Tris-hydrochloride–0.02 M $MgSO_4$.

Chemicals and enzymes. Crystalline DNase I and RNase A and 2′-deoxyadenosine were obtained from Sigma Chemical Co. Acrylamide and *N,N′*-methylenebisacrylamide were from Eastman Organic Chemicals. TEMED (*N,N,N′,N′*-tetramethylethylenediamine) was from Matheson, Coleman & Bell. Nitrosoguanidine (*N*-methyl-*N′*-nitro-*N*-nitrosoguanidine) was from Aldrich Chemical Co. Liquifluor was from New England Nuclear Corp. Casamino Acids were from Difco Laboratories. All other chemicals were reagent grade.

Radioactive compounds. ^{14}C-amino acids were reconstituted protein hydrolysate no. 3122-10 from Schwarz/Mann; [2-^{14}C]thymidine (61 mCi/mmol) was from Amersham Corp.

Bacteria and bacteriophages. *E. coli* K-12 CR63, permissive for amber (*am*) mutants, was the host for growth of phage stocks. *E. coli* B S/6/5, nonpermissive for *am* mutants, was used for selective plating of *am*$^+$ phage. SKB178, an *E. coli* K-12 strain that is F$^-$ *galE* and nonpermissive for *am* mutants, is the parent of the HD strains to be described (9). WH-1 (F′ [F196 *supD32*] *his-45 trp-37 phoA4 recA1 strA144 "val^r-116"* [12; CGSC 4612]) was used to make HD strains *supD*. C600 (*thr-1 leu-6 thi-1 supE44 lacY1 tonA2* λ$^-$) was the donor in P1 transductions of *supE*. F′ strains KLF17/KL132 (F′ [F117] *pyrB31 thi-1 thyA25 his-1 pro-27 leu-6 thr-1 recA1 xyl-7 malA1 ara-13 gal-6 lacY1 tonA2 str-9 rel-1* λ^r λ$^-$ [CGSC 4255]) and KLF18/KL132 (F′ [F118] *pyrB31 thi-1 thyA25 his-1 pro-27 leu-6 thr-1 recA1 xyl-7 malA1 ara-13 gal-6 lacY1 tonA2 str-9 rel-1* λ^r λ$^-$ [CGSC 4259]) (18) were used for mapping mutations in HD strains. K-12 strain T832 (*argH his pro thi-1 purA ampA mal tsx-9* Str^r λ^r λ$^-$) was obtained from T. Takano (37) and used as a recipient in transduction experiments. Our isolate of this strain carries an unidentified *am* suppressor. *groEA44* and *groEB515* were obtained from C. Georgopoulos (9).

T4D$^+$ and T4 (*am*) and temperature-sensitive (*ts*) mutants were from the California Institute of Technology (Caltech) collection (now maintained at the University of Colorado, Boulder). *am* mutants used were *amB*8 (gene 20), *amE*209 (gene 22), *amB*270 (gene 22), *amH*11 (gene 23), *amB*17 (gene 23), *amB*272 (gene 23), *amE*506 (gene 23), *amN*67 (gene 25), *amN*120 (gene 27), *amN*54 (gene 31), *amNG*71 (gene 31), *amN*52 (gene 37), *amN*122 (gene 42), and X4E [*amB*25 (gene 34):*amA*455 (gene 34):*amN*52 (gene 37):*amB*262 (gene 38):*amB*252 (gene 35)]. *ts* mutants used were *tsA*70 (gene 31) and *tsA*56 (gene 31). *r*67 in gene *r*III was included as an outside marker in crosses between gene 31 mutants. T2L, T3, T5, T6, and T7 were from the Caltech collection. T4ϵ (a T4 mutant that grows on *groEA44*) λc1*b*2, and λϵ*A*30 were received from C. Georgopoulos (9). P1 (P1*kc*) was used for transduction experiments; f1 phage is male specific and was used to identify F′-carrying strains.

HD mutant bacteria. SKB178 cells grown in H broth, washed three times, and suspended at 4×10^8/ml in TM buffer were treated with nitrosoguanidine (100 μg/ml) for 15 min at 37°C by the procedure of Adelberg et al. (1). The survival rate was 40%. Nitrosoguanidine was removed by washing three times with cold TM buffer, and 26 subcultures (numbered 0 through 25) were made by inoculating 5 ml of H broth with 0.1 ml of mutagenized cells and growing overnight at 37°C.

To select HD mutants, subcultures were diluted 1:25, grown to log phase at 37°C, and 0.05-ml samples containing about 10^6 cells were spread on relatively

dry EHA plates. The plates were incubated for 60 to 90 min at the desired selection temperature and then sprayed with 10^8 phage in 1 μl, using an aerosol sprayer and rotating the plate to ensure uniform coverage. A mixture of T4 and T6, related phages with different host ranges, was used to reduce the recovery of phage-resistant cells. In SKB178, which is $r6^-$ (permissive for T6 with nonglucosylated DNA [21, 24]), this procedure also prevented the selection of *galU*-defective host mutants which produce nonglucosylated T4 and T6 progeny. Such mutants comprised about 25% of the recovered colonies when selection was with T4 alone. After overnight incubation, there were 50 to 100 surviving colonies per plate. Cells from these colonies were purified by streaking on EHA plates. No mutants were found when the selection procedure was applied to nonmutagenized cells. Three separate selections were carried out (see below).

Mutants from the three selections were called HDX0.1-HDX0.29 and HDX1.0-HDX1.9 through HDX25.0-HDX25.9 (selection I), HDX3.01-HDX3.26 (selection II), and HDX26.01-HDX26.30, HDX27.01-HDX27.30, and so on through HDX35.01-HDX35.30 (selection III). HD is a phenotype designation; "X" is a classification letter (see below). The digits preceding the decimal point indicate the subculture of origin. In the genetic analysis of the HD strains and in the Discussion, we use the genotypic designation *hdh* for the HD strains blocked in T4 head assembly. A particular mutation is designated by the number of the HD strain; e.g., HDA17.5 carries the mutation *hdh-17.5*, the hyphen to be replaced by a letter when the identity of the host genes is better defined.

go mutant phage. The *go* phages were selected by plating 10^7 to 10^8 $T4^+$ particles per plate on each of the first 29 HD strains isolated in selection I (HDX0.1-HDX0.29). Mutant phage were purified by stabbing and replating from large plaques that appeared with frequencies of 10^{-6} to 10^{-7}. An analysis of plating patterns by spot testing with purified phage on the 29 HD host strains resulted in the identification of four different classes of *go* mutant phage, designated *goA*, *goC*, *goD*, and *goF* (see below). The following representatives of each of the four classes were used as the standard *go* phage for typing of HD strains: *goA*1 (selected on HDAD0.1), *goC*1 (selected on HDC0.13), *goD*1 (selected on HDD0.18), and *goF*1 (selected on HDF0.26). Additional *go* mutants were purified similarly from the smaller plaques that appeared at frequencies of 10^{-4} to 10^{-5} when $T4^+$ was plated on some HD hosts (see below). *am:go* double mutants were constructed by appropriate crosses and screened for their ability to grow on HD hosts that carried *supD* or *supE* and for their inability to grow on the corresponding nonsuppressing HD hosts.

Streak tests. About 10 μl of two T4 phage stocks containing 10^8 and 10^{11} particles per ml, respectively, was streaked across EHA plates and allowed to dry. Bacterial cultures (~4 × 10^8 cells per 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 sensitive, resistant, and HD cells: sensitive cells are lysed at both phage concentrations, resistant cells are not lysed at either concentration, and HD mutants are lysed at the high phage concentration but not at the low concentration.

Spot tests. Small drops (~5 μl) of phage solutions at concentrations of 10^8, 10^6, and 10^4 particles per 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.

Mapping of phage mutations. Phage crosses were performed as described by Wilson and Kells (38). Special conditions used to distinguish recombinant progeny are described in the appropriate figure legends.

Burst size measurements. Burst sizes were determined as described previously (22).

Measurement of infected-cell lysis. Bacterial cells were grown in 20 ml of H broth to 2 × 10^8 to 3 × 10^8/ml (optical density at 660 nm = 0.50 to 0.60) and infected with phage at a multiplicity of infection of 6 to 7. Samples (1 ml each) were withdrawn at intervals, and the optical density was measured at 660 nm. Surviving bacteria measured at 7 min were less than 1%. A few drops of $CHCl_3$ were added to the HDC and HDF cultures at 80 min after infection, and the optical density was measured once more at 90 min. The time of lysis was also determined visually for many phage-infected HD strains.

Labeling phage proteins with ^{14}C-amino acids. Cells were grown in M9 at 37°C to 5 × 10^7/ml and concentrated to 4 × 10^8/ml; 2.0-ml cultures were infected with phage at a multiplicity of infection of 5.0 at $t = 0$ and were superinfected with the same amount of phage at $t = 7$ min. ^{14}C-amino acids were added to a final concentration of 1 to 2 μCi/ml to infected cultures at the desired time. After the labeling period, cultures were usually chased for at least 1 min by the addition of 0.5 ml of 10% Casamino Acids. Chilled cells were pelleted, suspended in 0.1 ml of TMg, pH 6.8, containing 1 μg of DNase, and frozen in a solid CO_2-ethanol bath. Upon thawing, the preparation was made 2% in sodium dodecyl sulfate and β-mercaptoethanol and was boiled for 1 to 3 min. Incorporated radioactivity was determined by trichloroacetic acid precipitation of a 2-μl portion, and samples containing equal numbers of counts (and equal volumes when identical labeling conditions were used) were loaded onto gels.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Samples prepared as described above were electrophoresed in the discontinuous sodium dodecyl sulfate buffer system described by Laemmli (16), as modified by Dickson (4), and adapted for use with slab gels by the method of Studier (35). Gels were run at a constant current of 10 mA until the bromophenol blue marker dye entered the separating gel and then at 20 mA until the marker dye reached the bottom of the gel. Gels were fixed and stained for 1 to 2 h in an aqueous solution of 50% (wt/vol) trichloroacetic acid and 0.2% Coomassie brilliant blue (Schwarz/Mann). Destaining was for 10 to 20 h in 10% methanol–10% acetic acid. The gels were dried onto Whatman 3MM chromatography paper under vacuum and exposed to Kodak No-Screen X-ray film.

In vitro complementation tests. In vitro complementation assays were performed by the procedure of Edgar and Wood (5). For the preparation of defective

extracts, nonpermissive cells were grown to 4×10^8/ml in H broth with vigorous aeration, infected at $t = 0$ with an appropriate *am* mutant at a multiplicity of infection of 7, superinfected at 7 min at the same multiplicity of infection, chilled at $t = 35$ min, and pelleted at 45 min. The pellets from a 250-ml culture were suspended in 0.5 to 1.0 ml of TMg, pH 7.4, containing 10 μg of DNase, frozen at −70°C, and thawed once. Samples (20 μl each) were mixed at 4°C with either 20 μl of TMg or 20 μl of a different extract, incubated at 30°C for a minimum of 2 h, and assayed for phage.

Electron microscopy. Samples of infected-cell extracts prepared as described above were placed on carbon-coated grids and negatively stained with 1% uranyl acetate. Grids were examined in a Philips 201 electron microscope at 60 kV.

P1 transductions. Transductions using phage P1*kc* were according to Miller (19) or Rothman (25).

F' transfer experiments. Cells grown in H broth were mated at a ratio of 10 donor cells to 1 recipient cell for 60 min at 37°C without aeration. The mixture was diluted and plated on M9 agar plates to select against the multiply auxotrophic donors. F-ductants were purified by streaking on M9 plates and tested by the streak test for their ability to support f1 and T4 growth.

RESULTS

Selection and classification of HD bacterial mutants. HD mutants, isolated as colonies unable to propagate T4 or T6, were grouped into at least six classes according to the plating properties of four standard *go* phage mutants that were selected for growth on four prototype HD mutant hosts (Table 1). Lysis measurements, shown by Epstein et al. (6) to differentiate between T4 early and late nonsense mutants, were used to distinguish those bacteria that caused an early arrest of T4 development from those that blocked morphogenesis. Mutants of classes A, AD, D, and B showed normal lysis, suggesting an assembly defect. Lysis did not occur in T4-infected class C mutants and was delayed in class F mutants. T4 DNA synthesis measurements supported these tentative assignments of phenotype: DNA synthesis was normal in the putative assembly mutants of classes A, AD, D, and B but delayed and depressed in mutants of classes C and F, which exhibited defective lysis (data not shown).

When physiological and genetic studies revealed that mutants from all four assembly-defective classes blocked T4 development at the same stage, two further mutant isolations were done, using either *goA*1 (selection II) or *goD*1: *goF*1 (selection III) in combination with T6 as selecting agents to favor the detection of rarer classes of altered bacteria that might affect other morphogenetic pathways. After these selections, the majority AD class was eliminated; other defined classes were enriched, and many mutants (17 to 64%) appeared in the "other" category (data not shown). These mutants, however, did not represent blocks in different assembly pathways. Measurements of lysis, in vitro complementation analysis, and genetic studies revealed the new mutations to be variations of the same defect (see HD3.10, a representative of the "other" class, below).

Growth and plating properties of assembly-defective HD mutants. The ability to propagate T4 phages and the growth properties of the assembly-defective mutants described in this paper are shown in Table 2. Although most HD strains grew normally, some class D and all class B mutants showed temperature-dependent growth patterns. Phage propagation in these two classes also appeared to be influenced by temperature: some strains were defective at high temperatures but supported phage growth at 30°C (HDD0.18, HDD25.9, and HDD4.3), whereas others showed the converse behavior (HDD3.6, HDD7.1, and HDB4.5).

Several observations indicated that the four

TABLE 1. *Classification of HD mutants*

HD class	Growth of phage[a]					Lysis[b]	Frequency (%)[c]
	T4⁺	*goA*1	*goD*1	*goC*1	*goF*1		
HD⁺	+	+	+	+	+	+	
A	0	+	0	0	0	+	1
D	0	0	+	0	0	+	3
AD	0	+	+	0	0	+	87
B	0	(+)	(+)	0	0	+	2
C	0	0	0	+	0	0	1
F	0	0	0	0	+	D	1
Other	0	0	0	0	0	+ or D	5

[a] The four standard *go* phage mutants were isolated as large-plaque formers on the appropriate HD host strains as described in the text. Phage growth was determined by spot tests. Symbols: 0, no phage growth; +, phage growth; (+), phage growth is better than T4⁺ by a factor of about 1,000.

[b] Lysis measurements are described in the text. Symbols: +, lysis; 0, no lysis; D, delayed lysis.

[c] A total of 279 mutants derived from 26 subcultures were classified to yield 75 independent HD strains.

TABLE 2. *Properties of HD strains that block T4 assembly*

Bacteria		Growth of phage					
		EOP[b]			Burst size[c]		
Strain[a]	Growth	T4+	*goA*1	*goD*1	T4+	*goA*1	*goD*1
HD+		1.0	1.0	1.0	186	185	152
HDA17.5		$<10^{-6}$	1.0	$<10^{-6}$	0.002	122	0.2
HDAD1.1		$<10^{-6}$	1.0	1.0	0.01	102	41
HDD3.6		10^{-4}-10^{-6}	$<10^{-6}$	1.0	0.3	0.002	34
HDD4.3	*ts*	10^{-4}-10^{-6}	$<10^{-6}$	1.0	1.0	0.004	89
HDD7.1	*cs*	10^{-4}-10^{-6}	$<10^{-6}$	1.0	1.0	0.35	63
HDD0.18[d]		10^{-4}-10^{-6}	$<10^{-6}$	1.0	<0.1	0.001	20
HDD25.9[d]		10^{-4}-10^{-6}	$<10^{-6}$	1.0	0.2	0.003	23
HDB4.5	*cs*	10^{-4}	$<10^{-6}$	10^{-2}	<0.1	NT[e]	NT
HDB8.4	*cs*+	1.0	$<10^{-3}$	10^{-3}	36	0.4	1.0
HDB17.3	*ts*	$<10^{-6}$	10^{-3}	$<10^{-6}$	0.002	NT	NT
HD3.10	*cs*	10^{-4}	$<10^{-6}$	10^{-4}	0.1	NT	NT
groEA44	*ts*	$<10^{-6}$	$<10^{-6}$	1.0	0.03	NT	251
groEB515		1.0	1.0	10^{-5}	107	NT	0.3

[a] The course of T4 infection was characterized in detail in the underlined strains.
[b] EOP, Efficiency of plating; measurements were at 37°C unless otherwise specified.
[c] Phage adsorption and killing were normal.
[d] Efficiency of plating and burst size measurements at 42°C.
[e] NT, Not tested.

assembly-defective classes of HD mutants are related. (i) Both *goA* and *goD* phages grew on HDAD hosts. However, *goA* phages showed a lower efficiency of plating than did T4+ on HDD hosts. This property was most obvious at permissive temperatures in host strains with temperature-dependent defectiveness; for example, at 37°C on HDD0.18 and HDD25.9, T4+ grew well, but *goA*1 showed an efficiency of plating of 10^{-3} (data not shown). (ii) Both *goA*1 and *goD*1 showed efficiencies of plating about 10^3 times higher than that of T4+ on HDB hosts. However, a cold-sensitive (*cs*+) derivative of HDB8.4 that had regained the ability to propagate T4+ still inhibited the growth of both *go* mutants. (iii) After selection with *goD*1:*goF*1 in combination with T6 at 30°C, many of the survivors that were HD for both T4 and T6 under the selection conditions propagated T4+ at 37°C but were specifically defective for *goA*1, *goD*1, and *goD*3. These observations suggest that the four HD mutant classes impose a common block to T4 development. Additional evidence for this suggestion is presented below.

The ability of other phages to grow on the assembly-defective HD mutants was investigated. T2 and T6 behaved like T4. T3, T5, T7, and P1 grew on all strains with one exception: T5 failed to grow on HDB17.3. HD strains HDB4.5, HDB8.4*cs*+, HDB17.3, and HDD4.3 inhibited the growth of λ*cIb*$_2$, but allowed the growth of its derivative λϵ*A*30, a phage that compensates for many *groE* host mutations (8).

Course of T4 infection in assembly-defective HD mutants. The course of T4 infection was studied in a mutant host of each class. First, in vitro complementation tests were carried out to determine whether active structural intermediates in assembly accumulate in T4+-infected HD strains. Extracts of T4+-infected HD cells were mixed with either particles lacking tail fibers or with an extract (prepared by using the appropriate mutant phage [see above]) that provided heads and tail fibers (tail defective) or tails and tail fibers (head defective). The production of infectious phage particles in these mixtures was assayed. As shown in Table 3, T4+ infection of representatives of the four classes of assembly-defective HD mutants produced active tail fibers and tails comparable in quantity to those in the head-defective control, but yielded no active heads. These results show that in the HD hosts, assembly of tail baseplates and fibers is normal, whereas assembly of heads is defective.

The extracts of T4+-infected HD cells used for in vitro complementation also were examined by electron microscopy using negative-staining techniques. In agreement with the in vitro complementation data, the HD extracts contained normal numbers of tails but no heads or head-related structures. As controls, the head-defective and tail-defective extracts used in the complementation tests showed normal numbers of tails and heads, respectively (data not shown).

The phage protein gene products (gp) labeled during the latter half of the latent period after T4+ infection of HD hosts were analyzed by sodium dodecyl sulfate-polyacrylamide gel elec-

TABLE 3. *In vitro complementation with extracts of T4⁺-infected HD strains*[a]

Complementing prepn	None	Tail fiber defective	Tail defective	Head defective
T4⁺ + HDA17.5	0.4	355	544	4.7
T4⁺ + HDAD1.1	0.5	408	488	4.1
T4⁺ + HDB4.5	0.6	242	294	5.0
T4⁺ + HDD3.6	5.0	456	370	5.0
T4⁺ + HD3.10	0.3	25[b]	105	5.6
Head defective	4.1	290	487	
Tail defective	0.3	413		
Tail fiber defective	0.3			

[a] Two extracts (50 μl of each) were mixed, incubated for 300 min at 30°C and then assayed for plaque-forming phage. Results are expressed as the titer (active phage per milliliter) $\times 10^{-9}$ in the reaction mixture. Extracts of phage-infected cells were prepared as described in the text. The tail fiber-defective preparation (head and tail donor) was particles lacking tail fibers purified from X4E-infected cells; 5×10^{11} particles per ml were used in the complementation reactions. The tail-defective preparation (head and tail fiber donor) was an extract of SKB178 infected with *amN*120 (gene 27). The head-defective preparation (tail and tail fiber donor) was an extract of SKB178 infected with *amB*17 (gene 23).

[b] The maximum possible value in this experiment was 50 because only 5×10^{10} particles were added to the reaction mixture.

trophoresis (Fig. 1). In all infections, the major capsid protein (gp23 plus its cleavage product gp23*) was produced at approximately the same level. In the permissive infections (T4⁺ infection of SKB178 and HDB8.4*cs*⁺ and *goA*1 infection of HDA17.5), gp23 was cleaved to gp23*. In the nonpermissive infections [*amN*54 (gene 31) infection of SKB178, *goA*1 infection of HDB8.4*cs*⁺, and T4⁺ infection of HDA17.5 and HD3.10], all of the gp23 remained uncleaved. Similar results also were found in T4⁺-infected HDD3.6 and HDAD1.1. These findings support the conclusion that all of the HD strains presumed to affect phage morphogenesis block T4⁺ development at an early stage in head assembly at the level of gene 31 function (17). The data also suggest that the mutation in HDB8.4*cs*⁺ which prevents the growth of *goA*1 probably acts at the same level.

Genetic analysis of *go* mutants. The *goA*1 and *goD*1 mutations map in gene 31 at an internal site near *tsA*70 and give wild-type recombinants in crosses of *goA*1 by *goD*1 (Fig. 2). Complementation tests with gene 31 *am* mutants confirmed the assignment to gene 31 (data not shown). Further crosses showed that *goD*1 and a number of spontaneous, independently isolated *go* phage mutants with identical plating patterns, selected on class A, AD, or B mutant hosts, failed to recombine with T4ϵ. This *go* mutant, selected previously on *groEA44* (9), has *goD*1 growth characteristics at 37°C but differs by its inability to grow at 42°C. Similarly, a number of independently isolated mutants with *goA*1 plating properties showed the same frequency of recombination with *tsA*70 as did the prototype phage *goA*1. Thus, *goA*1-like and *goD*1-like mutations appear to recur at sites identical or closely linked to those of the original mutations.

However, not all *go* mutants selected on HDA strains were similar to *goA*1. Four such mutants, isolated in selection III, failed to plate on HDAD0.1 or HDA17.5. One of these novel mutations mapped in gene 31 but gave wild-type recombinants with both *goA*1 and *goD*1 (percent recombination = 0.16 and 0.17, respectively). These results are consistent with the view that the different plating characteristics of *go* mu-

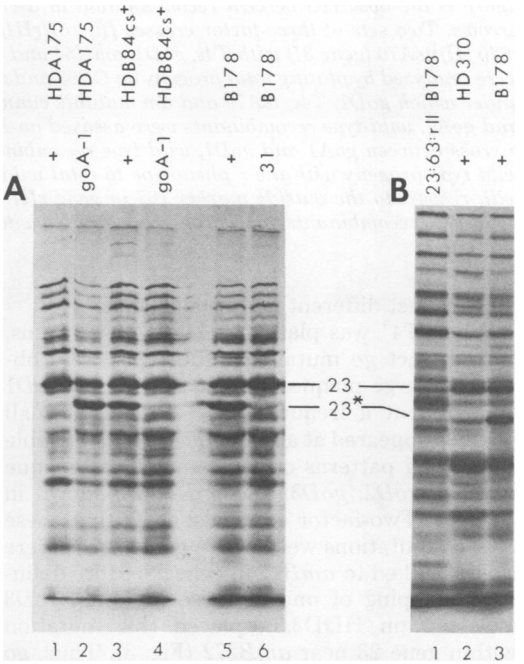

FIG. 1. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of extracts of phage-infected cells. Cells were infected with phage, labeled with* ^{14}C*-amino acids from 13 to 24 min after infection at 37°C, and prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described in the text. Samples containing about 40,000 cpm were electrophoresed on 10% polyacrylamide slab gels and processed as described in the text. Phage and bacteria used in the preparation of extracts are listed above each track: "+" means T4⁺; "31" refers to phage carrying amN54 (gene 31), and "23:63:rII" refers to phage carrying amB17, amM69, and rEDdf41; "B178" refers to SKB178. 23 and 23* designate, respectively, the product of gene 23 and the cleaved product of gene 23. (A) and (B) are gels from two different experiments.*

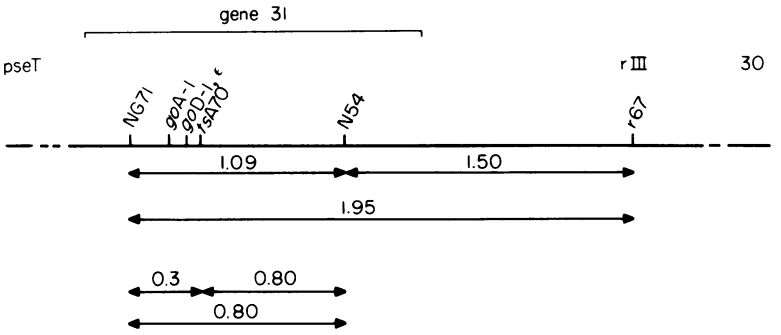

FIG. 2. *Genetic location of goA1 and goD1. The map of the gene 31 region of the T4 genome is from the data of Revel and Lielausis (23) and shows the positions of amNG71 and amN54 in gene 31 relative to outside markers pseT (to the left) and rIII and gene 30 (to the right), as well as recombination percentages between amNG71 and amN54, amN54 and r67, and amNG71 and r67. 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 percent recombination in a cross between mutants at the ends of the corresponding arrows. Two sets of three-factor crosses [(i) r67(rIII):amN54 with T4ε, goD1, amNG71, and tsA70; and (ii) r67(rIII):tsA70 (gene 31) with T4ε, goD1, amN54 and amNG71] and a two-factor cross between T4ε and goD1 were analyzed by plating total progeny on CR63 and wild-type recombinants on groEB515 at 42°C, conditions under which goD1, T4ε, tsA70 and am mutants cannot grow. In a three-factor cross between r67(rIII):tsA70 and goA1, wild-type recombinants were assayed on HDD4.3 at 30°C, where goA1 and tsA70 fail to plate. In a cross between goA1 and goD1, wild-type recombinants were assayed on HDB8.4cs$^+$ at 37°C. The ratio of wild-type progeny with the r phenotype to total wild-type progeny permitted ordering of the mutant alleles with respect to the outside marker r67 in gene rIII. Recombination percentages are calculated as follows: (wild-type recombinants/total progeny) × 200. No wild-type recombinants were found in the cross between T4ε and goD1.*

tants reflect different mutational sites.

When T4$^+$ was plated on HDD host strains, two distinct *go* mutant plaque types were observed. Large plaques similar to those of *goD*1 appeared at a frequency of ~10^{-6}, and small plaques appeared at a frequency of ~10^{-4} (Table 2). Plating patterns of three such small-plaque isolates, *goD*2, *goD*3, and *goD*4, are shown in Table 4. Two-factor crosses showed that these new *go* mutations were not in gene 31 but were closely linked to *amB*17 in gene 23. More definitive mapping of one of these mutants, *goD*3 (selected on HDD3.6), placed this mutation within gene 23 near *amB*272 (Fig. 3). Thus, *go* phage mutants with alterations affecting the gene for the major capsid protein also can overcome the assembly block in some HDD host strains. Two experiments showed that *goD*3 does not simply bypass the requirement for gp31 function: (i) *goD*3:*amN*54 (gene 31) double mutants grew on HDD3.6 only when gp31 was supplied by a complementing phage, and (ii) in mixed infection of HDD3.6 with *goD*3:*amN*54 and *goD*3 in varying ratios, the phage yield was a function of the level of wild-type gp31 present (data not shown).

Inability of T4 *go* mutants to grow on some HD strains. As noted in Table 2, some *go* phage mutants selected on specific HD strains fail to grow on certain other temperature-dependent HD strains at low temperatures at which wild-type T4 can grow. Similarly, we found that *tsA*70 (gene 31) did not plate on HDD0.18, HDD25.9, and HDD4.3 at 30°C. The growth of *tsA*56 (gene 31) was inhibited only on the last host.

TABLE 4. *Plating properties of various goD phage mutants*

Bacterial strain	Growth of phage[a]				
	T4$^+$	*goD*1	*goD*2	*goD*3[b]	*goD*4
HD$^+$	+	+	+	+	+
groEA44	0	+	0	0	0
HDD0.18[c]	0	+	+	+	0
HDD3.6	0	+	0	+	+
HDD4.3	0	+	+	+	+
HDB4.5	0	0	0	+	0

[a] Phage growth was determined by a spot test at 37°C as described in the text. Symbols: +, phage growth; 0, no phage growth.

[b] The burst size of *goD*3 on SKB178 (HD$^+$) was the same as that of T4$^+$ (~150). On HDD3.6, the burst size of *goD*3 was reduced to ~30.

[c] Spot test at 42°C.

Genetic analysis of *hdh* strains. P1 transduction studies showed that most of the host mutations cotransduced with *purA* with frequencies ranging from 8.5 to 17.3% for different mutations (Table 5 and Fig. 4). Two strains, HDB4.5 and HDD7.1, were clearly different from the rest: there was less than 1% cotransfer of the HD mutation with the *purA*$^+$ marker.

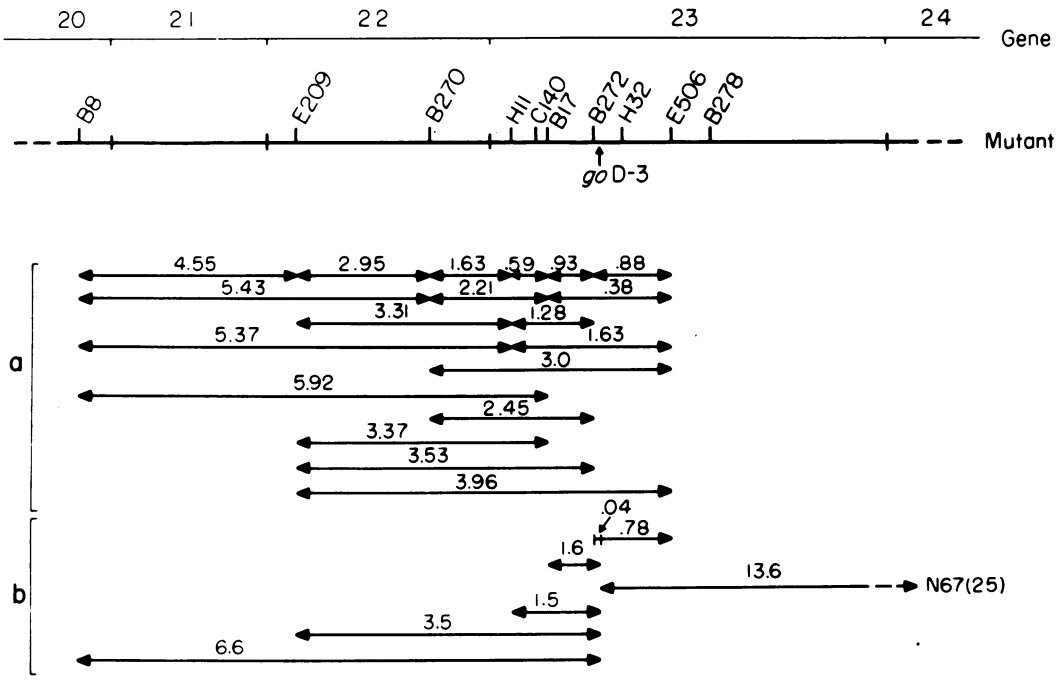

FIG. 3. *Genetic map of the gene 23 region of the T4 genome. The top line gives the relative order and sizes of genes 21 to 23 (41). The second line is the genetic map showing the position of am mutations. (a) Recombination percentages between mutations in genes 20, 22, 23, and 25. Genetic crosses were done in CR63 under standard conditions described in the text. Total progeny was determined by plating on CR63. am$^+$ recombinants were assayed on S/6/5. Recombination percentages are calculated as follows: (am$^+$ recombinants/total progeny) × 200 and are averages obtained in two or more crosses. (b) Recombination percentages from the crosses between goD3:amX double mutants with wild-type phage. goD3:amX double mutants were constructed as described in the text. The goD3:amX double mutants were backcrossed to wild-type T4$^+$ in CR63 under standard conditions. Total progeny were determined by plating on CR63. goD3:am$^+$ recombinants were assayed on HDD3.6 at 37°C, where wild-type and am phage cannot grow. Recombination percentages are calculated as follows: (goD3:am$^+$ recombinants/total progeny) × 200.*

Transfer of either of the F′ factors, F′117 or F′118, into these strains or into HDD3.6 rendered the hosts able to support T4$^+$ growth. This result indicates that the wild-type alleles are probably dominant and that the HD mutations in all three of these strains must lie between the ends of the F′117 factor. P1 transduction experiments with *melA* and *pyrB* (Fig. 4) markers to locate these mutations more precisely have not been done.

DISCUSSION

Bacterial *hdh* mutations block an early step in head assembly. Our combined physiological and genetic data show that the HD mutations described here block T4 morphogenesis at the level of gene 31 action. After infection of these strains by T4$^+$, neither heads nor head-related structures are made, and head proteins are not cleaved as in normal infection. This phenotype is distinct from that of an assembly core defect (27), but is the same as observed after infection of wild-type (nonsuppressing) hosts by gene 31 *am* mutants (17). It is also the same as the phenotype reported for T4$^+$ infection of the previously described mutant host strains *mop* (37), *groEA44* (9), *tabB* (3, 36), and *hdB3* (31). As was reported for these strains, we find that mutations in T4 gene 31 can compensate for the host defect, and that certain gene 31 *ts* mutants (*k* mutants [3, 36]) interact negatively with certain temperature-dependent HD mutant hosts, in that the *k* mutant phage fail to propagate under conditions where T4$^+$ phage can do so. Therefore, the HD mutants, like those described previously, appear to interfere with the normal function of T4 gp31 in head assembly.

However, we have shown, in addition, that a mutation in gene 23, *goD3*, also can compensate for the host defect in certain HD strains such as HDD3.6. Gene 23 codes for the major T4 capsid protein, which presumably must interact with gp31 in order to assemble correctly (17). In possibly analogous mutants of phage λ, mutations

in gene *E* (major capsid protein) restore phage growth on certain HD *groE* bacterial mutants apparently by lowering the amount of gpE produced, thereby restoring a required balance between the levels of this protein and the host *groE* function (8, 34). However, it appears unlikely that the *goD*3 mutation in T4 acts by reducing the amount of functional gp23 produced. The *goD*3 mutation lies far from the gene 23 promoter (Fig. 3), and *goD*3 phage give a normal burst size on wild-type host strains (Table 4). Furthermore, several gene 23 *am* mutants were found not to grow in a HDD3.6 *supE* host (data not shown), in which gp23 should be underproduced because of the low efficiency of the *supE* suppressor. We have also presented evidence that the *goD*3 mutation does not simply bypass the normal requirement for gp31 function. Therefore, the most likely interpretation is that the HDD3.6 host defect can be overcome by a specific change in either gp23 or gp31 of T4, implying interaction between these proteins and one or more host functions in the first step of T4 head assembly. This view is supported by the findings that *k* mutations, as well as compensating mutations, can occur in gene 23 and that the corresponding *k* mutants can be used to select specifically for *tabB*-defective host mutants (36).

TABLE 5. *Frequency of P1 cotransduction of the HD phenotype of HD strains with purA*$^{+}$[a]

HD strain (donor)	HD transductants/ *purA*$^{+}$ transductants tested	Cotransduction (%)
HDD3.24[b]	16/188	8.5
HDA17.5	19/200	9.5
HDD4.3	8/75	10.6
HDAD1.1	22/200	11.0
HDB17.3	24/200	12.0
HDB8.4*cs*$^{+}$[c]	29/200	14.5
HDD0.18[d]	51/299	17.1
HDD3.6	52/300	17.3
HDD7.1	0/90	<1.0
HDB4.5	0/199	<0.5
groEA44	22/200	11.0

[a] P1*kc* was grown on the HD strains and used to transduce T832 *purA* as described in the text. *purA*$^{+}$ transductants were selected on supplemented minimal medium in the absence of purines, purified, and tested by a streak test at 37°C for the ability to grow T4^{+} (except HDB8.4*cs*$^{+}$ [see footnote *c*]). Frequencies of *purA*$^{+}$ transductants ranged from 5×10^{-7} to 100×10^{-7} in different experiments.

[b] The test for host defectiveness was at 30°C.

[c] HDB8.4*cs*$^{+}$ is sensitive to T4^{+} but defective for *goA*1; therefore, *goA*1 was used to screen the *purA*$^{+}$ transductants for host defectiveness.

[d] The test for host defectiveness was at 42°C.

***hdh* mutations define at least two host functions.** Most of the *hdh* mutations tested can be cotransduced with the *purA* marker, as has been reported for *mop* (37), *groE* (7), and *tabB* (36). In our experiments, the cotransduction frequencies of various *hdh* mutants range from 8.5 to 17.3%, but show two apparent clusters (HDD3.24, HDA17.5, HDD4.3, HDAD1.1, HDB17.3, and *groEA44* and HDB8.4, HDD0.18, and HDD3.6) with means of about 10 and 16%, respectively. Data in the literature for *mop*, *groE*, and *tabB* mutants show similar ranges and tendencies to cluster around 10 and 21%. The finding of two apparent frequencies could be due to an effect of some *hdh* mutations on the recovery of *purA*$^{+}$*hdh* transductants to give a lower apparent cotransduction frequency. Alternatively, the two frequencies could represent two different sites at which *hdh* mutations occur. From the difference in transduction frequency, the two sites would be about 10^4 nucleotide pairs apart and, therefore, probably in different genes. This hypothesis could be tested by genetic complementation analysis. Of interest in this connection is a recent report that a λ transducing phage carrying 8,000 base pairs of bacterial DNA, including a mutant *groE* gene cannot grow on some *groE* hosts but can grow on others,

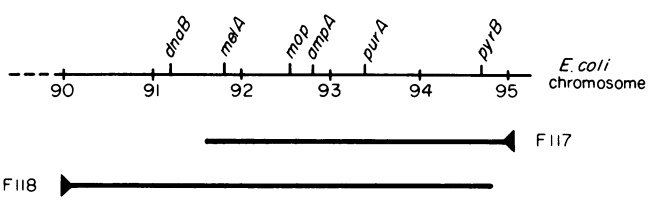

FIG. 4. *Location of hdh mutations on the E. coli chromosome. The upper line shows the 90–95-min segment of the E. coli chromosome with relevant markers (2). The heavier lines below the chromosome show the extents of F' factors F'117 and F'118 based on the data of Low (18) and Takano and Kakefuda (37), as adjusted to correspond to the most recent map of the E. coli chromosome (2). The bracket above the chromosome indicates the location of most of the mutations in our HD strains as obtained by P1 cotransduction of host defectiveness with the purA*$^{+}$ *marker (Table 5). Cotransduction frequencies have been converted to minutes on the E. coli chromosome by the equation of Wu (42) as described in reference 2.*

suggesting that two complementing *groE* genes may be present (10).

In addition to the *purA*-linked mutations, we have found two *hdh* mutations that are not cotransduced with *purA*, although F′ transfer experiments show that they are in the same region of the bacterial chromosome. The defects in strains HDB4.5 and HDD7.1 can be overcome by the phage mutations *goD*3 and *goD*1, respectively. Therefore, *hdh* mutations may occur in three or more host genes.

To complicate the picture still further, two other HD bacterial mutants that block T4 head assembly and can be overcome by mutations in T4 gene 31 have been reported. These mutants differ from our characterized *hdh* mutants and from *mop*, *groE*, and *tabB* mutants in that one of them maps near *pro* on the *E. coli* chromosome (31) and the other shows a dissimilar T4-defective phenotype (32). We have selected *go* mutants on the latter strain, *hd*590, kindly furnished to us by L. D. Simon. Most of the corresponding mutations do not map near gene 23 or gene 31. A few map in gene 31, but these mutants fail to grow on any of our HDA, HDAD, HDB, or HDD strains (Stitt, unpublished observations). A comprehensive model for the interaction of gp31 with host functions must account for these HD mutants as well as the other classes described here.

go and hdh mutations probably define specific phage-host protein interactions. Two kinds of models can be proposed to explain the interactive systems of host and T4 phage mutations described here and by others (3, 9, 31, 36, 37). The interaction could be indirect; for example, host mutations might alter membrane transport properties so as to cause a change in the intracellular ionic environment that could prevent the gp31-mediated step in T4 head assembly. Alternatively, the interaction could be direct; *hdh* mutations might block head assembly by altering a host protein so as to prevent a required specific association of gp31 and gp23 with a host protein complex. In either model, compensating mutations in phage gene 23 or 31 could overcome the block.

From the evidence available so far, we cannot rule out either model. Indirect interaction seems to be supported by our findings that: (i) a single phage mutation in gene 31 can overcome several host defects that may represent mutations in three different host genes, and (ii) certain host defects can be overcome by mutations in either of two phage genes. Either model would be consistent with the findings of both compensatory (*go*) and killing (*k*) mutations in both genes 23 and 31 of T4: in some cases, a phage mutation can compensate directly or indirectly for a detrimental host mutation, whereas in other cases, a phage mutation and a host mutation, both innocuous alone, can interact either directly or indirectly to give a lethal phenotype.

However, one aspect of the data supports the type of direct-interaction model proposed by Takahashi (36), involving a specific active complex of phage and host proteins required for phage head assembly. Host mutants that appear related on the basis of similar cotransduction frequencies with $purA^+$ show different patterns of compensation by different classes of *go* mutant phage. Conversely, different *go* mutations or *k* mutations in a particular gene show different plating patterns on the HD strains. In other words, the properties of *hdh*, *go*, and *k* mutations appear to be allele specific rather than class specific or gene specific. This feature would be less likely if the HD mutations cause alterations in a physiological parameter for which the *go* mutations can compensate. Allele specificity would be more likely if these mutations are causing compensating conformational changes in an interacting complex of proteins. From the observations reported here, the functional complex might consist of at least three host proteins and the viral proteins gp31 and gp23. The apparent ability of compensating mutations to occur in any phage-host pair of these components would seem puzzling. However, a possibly analogous observation has been made with the multimeric regulatory enzyme aspartate transcarbamylase: mutational changes in the regulatory subunit affect the specificity of the catalytic subunit active site, solely by interaction between the heterologous subunits (26). The recent identification of a host protein defined by a *groE* mutation (10, 11) should lead to more direct tests for the existence of a functional phage-host protein complex in head assembly.

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