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Genetic Studies of Coliphage 186

I. Genes Associated with Phage Morphogenesis

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In coliphage 186, 22 essential genes were defined by complementation studies with amber mutants. Eighteen genes were associated with phage morphogenesis: 11 with phage tail formation, and 7 with phage head formation. The remaining four genes are discussed in the accompanying paper (S. M. Hocking and J. B. Egan, J. Virol. **44**:1068–1071, 1982).

The temperate coliphages P2 and 186 both contain linear, nonpermuted, double-stranded DNA of approximately 30 kilobase pairs in size (25), in icosahedral heads of approximately 60 nm in diameter and with tails of approximately 135 nm long attached (4). That the two phages are closely related is clearly shown both by their extensive DNA homology (26) and by their ability to form P2-186 intervarietal hybrids (5). However, whereas P2 is the prototype noninducible, nonexcisable temperate coliphage (4), phage 186 is both inducible and excisable (23). We are ultimately interested in the further description of prophage induction and excision, and we therefore have examined the function of the genes of coliphage 186 with a view to comparing them with those of its noninducible relative, P2.

MATERIALS AND METHODS

Bacteria. All bacterial strains used were derivatives of *Escherichia coli* K-12. Their genotypes and sources are listed in Table 1.

Bacteriophages. Most of the phage strains used were derivatives of 186 *c*I *ts*p, which is the 186p mutant described by Baldwin et al. (2).

The phage 186 amber mutants numbered 1 to 47, 51, and 52 were obtained from 186 *c*I *ts*p by W. H. Woods in this laboratory after irradiation of the strain with UV light and plating on a mixed Su^+/Su^- indicator medium. Small plaques were picked and tested for ability to plate on Su^+ and Su^- strains (W. H. Woods, Ph.D. thesis, University of Adelaide, Australia, 1972). Amber mutants numbered 57 to 67 and temperature-sensitive mutants numbered 59, 68, and 69 were obtained by mutagenesis of 186 *c*I *ts*p with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NNG) and selection on a mixed Su^+/Su^- indicator plate. Amber mutants numbered 48, 49, and 50 (derived from wild-type phage 186) were gifts from A. D. Kaiser, Stanford University, and were previously known as 186 *am*4.1, 186 *am*6.1, and 186 *am*9.1, respectively. The amber mutant numbered 56 (previously known as 186 *am*E) was a gift from C. P. Georgopoulos, University of Utah, and carries an additional mutation in the *c*II gene. It was derived from wild-type phage 186 and can grow on *groE* strains of *E. coli* K-12. Of the amber mutants isolated in this laboratory, four (*am*1, *am*51, *am*52, and *am*62) have an additional clear plaque mutation. *am*40 carries two amber mutations. Several of the 186 amber mutants were poorly suppressed by one or more of the three suppressor hosts used. In particular, mutants *Gam*27 and *Nam*47 failed to grow on the *supE* suppressor host strain C600, and mutants *Mam*60 and *Pam*16 fail to grow on the *supF* suppressor host strain H12R8A.

The phage 186 *c*I *am*53 *vir*1 was isolated as a spontaneous clear-plaque mutant in a stock of the phage 186 *c*I *am*53 (Woods, Ph.D. thesis). It is a virulent phage able to grow on a phage 186 lysogen.

The phage 186 *c*I *ts*p *Pam*16 *vir*2, another virulent mutant, was isolated as a spontaneous clear-plaque mutant in a stock of the phage 186 *c*I *ts*p *Pam*16 (Woods, Ph.D. thesis).

The phage 186 *c*I10, a clear-plaque mutant of phage 186 which fails to complement with 186 *c*I *ts*p, was isolated after irradiation of phage 186 with UV light (V. Huddleston, B.Sc. [Honours] thesis, University of Adelaide, Australia, 1970).

Media and buffers. The media and buffers used included: T broth (2% tryptone [Difco Laboratories] and 0.5% NaCl); L broth (1% tryptone, 0.5% yeast extract, 1% NaCl); LG broth (L broth supplemented with 0.1% glucose); T agar plates (T broth plus 1.2% agar [Difco]); T soft agar for phage assays (T broth plus 0.7% agar); H-1 medium [0.1 M potassium phosphate buffer (pH 7.0), 0.015 M $(NH_4)_2SO_4$, 0.001 M $MgSO_4$, 1.8×10^{-6} M $FeSO_4$]; H-1 plus glucose (H-1 supplemented with 0.2% glucose); and TM (0.01 M Tris [pH 7.1], 0.01 M $MgSO_4$).

Bacterial and phage assays. Bacteria were diluted in H-1, and 0.1-ml samples were spread on T agar plates. Colonies were counted after overnight incubation at 37 or 30°C. Phage were diluted in TM buffer, and 0.1-ml samples were incubated with 0.2 ml of log-phase (4×10^8 colony-forming units [CFU] per ml) indicator bacteria at 37°C for 20 min. T soft agar (3 ml) at 47°C was then added and mixed, and the mixture was

TABLE 1. Bacterial strains

E. coli K-12 derivative	Relevant phenotype	Genotype	Reference or source
594	Su^-	F^- *galK galT str-594*	6
C600	Su^+	F^- *thr leu thi lacY tonA supE*	1
S26R1e	Su^+	HfrC *rel tonA* $T2^r$ *phoA supD*	8
H12R8A	Su^+	HfrC *rel tonA* $T2^r$ *phoA supF*	8
XA7007	Su^- SuA	F^- Δ(*lac-pro*) *mal thi str rho*	D. E. Morse (no. 1013); isolated by Beckwith (3)

poured over a T agar plate. Plaques were counted after overnight incubation at 37°C. The indicator strain used was usually C600 grown to log phase in T broth.

Recombination on the plate. The technique for recombination on the plate was used to determine whether mutations in the same gene occurred at identical or distinct sites within that gene. Stocks of two different amber mutants were assayed separately and together on the Su^- indicator strain 594. An increase in PFU when the two phage stocks were mixed indicated that recombination on the plate had occurred and therefore that the mutations resided at different sites within the genome.

Construction of lysogens. Su^+ lysogens of phage 186 amber mutants were isolated from the center of turbid plaques. Lysogenic bacteria were identified either by their ability to cause lysis when stabbed into an appropriate indicator lawn or by their immunity to superinfection by 186 *c*I10 and their sensitivity to 186 *c*I *am*53 *vir*1. Lysogenic bacteria were purified by three successive single-colony isolation steps; phage production or immunity was determined at each step. Su^- lysogens of phage 186 amber mutants were prepared by spotting 10^7 to 10^8 PFU on indicator lawns and, after overnight incubation, isolating bacteria from the center of the spots. Lysogenic bacteria were identified by their immunity to superinfection by 186 *c*I10 and their sensitivity to 186 *c*I *am*53 *vir*1. They were purified and tested as described above for Su^+ lysogens.

Phage stocks. 186 phage stocks were prepared by heat induction, liquid infection, or plate stocks.

(i) Heat induction. Lysogens of heat-inducible mutants, grown with aeration at 30°C to 3×10^8 CFU/ml, were incubated at 47°C for 10 min and then at 37°C (with aeration) until lysis occurred. Bacterial debris was removed by centrifugation for 10 min at 7,800 × *g* and 4°C, and the supernatants were stored over chloroform at 4°C.

(ii) Liquid infection. Log-phase cultures of sensitive bacteria (10 ml; 2×10^7 to 3×10^7 CFU/ml) in LG broth were inoculated with either a single plaque or a phage stock at a multiplicity of infection (MOI) of 0.1 and incubated at 37°C, with aeration, until lysis occurred or for 4 h. Bacterial debris was removed by centrifugation for 10 min. at 7,800 × *g* and 4°C, and the supernatants were stored over chloroform at 4°C.

(iii) Plate stocks. Approximately 10^5 to 10^6 PFU obtained from a single plaque were plated with 8×10^7 CFU of log-phase bacteria and incubated overnight at 30°C. Plates were chilled and flooded with 3 ml of ice-cold T broth, and the supernatants were removed after 16 h at 4°C. Bacterial debris was removed by filtration (pore size, 0.45 μm; Millipore Corp.), and the filtrates were stored over chloroform at 4°C.

NNG mutagenesis. C600 (186 *c*I *ts*p), grown at 30°C in LG broth to 4×10^8 CFU/ml, was incubated with aeration at 40°C for 25 min. NNG (8 or 40 μg/ml) was added, and after 15 min the culture was diluted 10^{-3} into fresh broth and incubated until lysis occurred.

Complementation. (i) Plate complementation. Samples (≈5 μl) of phage 186 amber mutant stocks (3×10^8 to 10^9 PFU/ml) were cross-streaked on plates containing 8×10^7 log-phase Su^- bacteria (strain 594 or XA7007) and incubated overnight at 37°C.

(ii) Liquid complementation. Log-phase strain 594 cells (3×10^8 CFU/ml), grown at 37°C in LG broth, were infected with two different amber mutants at an MOI of approximately 10 for each mutant, incubated without aeration at 37°C for 20 min, diluted 10^{-1} into LG broth containing phage 186 antiserum ($K = 8$ min^{-1}), and incubated without aeration at 37°C for another 5 min. (The MOI values refer to the number of phage added per bacterium. Since 186 adsorbs poorly [only approximately 50% in 20 min], the effective MOIs are about half those given. The antiserum treatment inactivated more than 99.9% of the unadsorbed phage.) The mixtures were then diluted 10^{-3} into prewarmed LG broth and incubated with aeration at 37°C for 90 min. The cultures were chilled, and chloroform was added. Phage yields were determined on Su^+ (strain C600 or S26R1e) and Su^- (strain 594) indicator bacteria. This procedure was repeated for single infections with each phage (at an MOI of 20) and for infection with the phage 186 *c*I *ts*p (MOI of 20). Phage yields in the complementation tests were expressed as percentages of the phage yield of the 186 *c*I *ts*p control.

Preparation of defective lysates. Strain 594 lysogens of 186 *c*I *ts*p amber mutant phages, grown to log phase (3×10^8 to 4×10^8 CFU/ml) at 30°C in H-1 plus glucose medium, were heat induced by incubation at 47°C for 15 min and then by incubation with aeration at 37°C until lysis was complete (2.5 to 3 h) as determined by absorbance measurements on a Zeiss PMQII spectrophotometer. The lysates were centrifuged for 10 min at 7,800 × *g* and 4°C to remove bacterial debris.

Electron microscopy of defective lysates. Samples of defective lysates were mounted on carbon-coated grids, negatively stained with 2% uranyl acetate, and viewed in either a Siemens Elmiskop I or a Siemens 102 electron microscope.

In vitro reconstitution. The procedure used for in vitro reconstitution was that described by Weigle (23). A 0.1-ml amount of each of two defective lysates was added to 0.8 ml of H-1 plus glucose and incubated without aeration at 37°C for 1 h. Viable phages were counted by assaying the mixture on an Su^+ strain. Each lysate was also tested for viable phages by diluting 0.1 ml of it into 0.9 ml of H-1 plus glucose and incubating and assaying the mixture as described above.

TABLE 2. Intercistronic liquid complementation tests

Coinfecting strain	Phage yield (%)[a] after coinfection with:										
	Aam5	*Bam17*	*Eam7*	*Ham50*	*Lam21*	*Mam19*	*Nam47*	*Pam16*	*Qam49*	*Tam8*	*Wam15*
Aam5	0.03	3.8	0.52	0.56	0.29	2.1	1.3	5.8	0.69	0.42	0.34
Bam17		<0.01	63	36	72	48	48	29	34	55	78
Eam7			0.01	26	32	37	15	38	41	30	16
Ham50				<0.01	28	30	13	51	51	31	27
Lam21					<0.01	29	16	36	37	43	19
Mam19						<0.01	34	37	35	24	32
Nam47							<0.01	36	16	17	36
Pam16								0.05	57	46	32
Qam49									<0.01	6.5	32
Tam8										0.01	42
Wam15											<0.01

[a] The figures given are the phage yields for each coinfection expressed as percentages of the phage yield of 186 cI *tsp* which, under the conditions used in the complementation tests, was 42. The phage yields were measured on the *supE* strain C600 in all cases except those involving *Nam47*, for which the *supD* strain S26R1e was used. For the coinfection *Nam47* + *Pam16*, the phage yield was calculated by the addition of the titers obtained on C600 and S26R1e. The number of wild-type recombinants present in each burst was assayed on the Su^- strain 594. In tests not involving *Aam5*, the number of wild-type recombinants formed was <0.01% of the 186 cI *tsp* phage yield, and in tests involving *Aam5*, the number of wild-type recombinants was ≤0.11% of the 186 cI *tsp* phage yield.

RESULTS

Liquid complementation. The results of initial plate complementation tests suggested the presence of at least 11 genes. (In this paper, "gene" has been used instead of "cistron" to refer to the groups of mutations defined by complementation studies.) A representative mutant for each of these 11 genes was tested by liquid complementation, and the results (Table 2) confirmed that their mutations were in different genes. In tests not involving the *Aam5* mutant, the phage yield after mixed infection was 6.5 to 78% of the wild-type yield and at least 500-fold greater than the yield after infection by either mutant alone, and the frequency of wild-type recombinants in the burst was always less than 0.01% of the 186 cI *tsp* phage yield. These mutations defined 10 genes. In those tests involving mutant *Aam5*, the phage yields were lower (0.29 to 5.8% of the wild-type phage yield), and the frequency of wild-type recombinants was higher (0.01 to 0.11% of the wild-type burst yield). Although some complementation appeared to be occurring with *Aam5*, it produced, on average, less than one phage per cell. These results suggested the existence of an 11th gene, whose representative (*Aam5*) complements poorly with mutants carrying mutations in other genes.

The results of other liquid complementation tests (data not shown) suggested the existence of strong polarity effects in coliphage 186. For example, coinfection with *Eam7* and *Fam20* gave a phage yield of 0.4% of the wild-type yield, whereas coinfection with *Eam7* and *Gam25* gave 0.33% of the wild-type yield. These values, representing 1 PFU for every six or seven infected bacteria, were at least 10-fold greater than the values for single infections but approximately 100-fold less than those obtained in most intercistronic tests.

Plate complementation. In most cases, plate complementation was found to be a satisfactory method for defining the genes of phage 186. Complementation was indicated by the presence of a confluent square of lysis at the intersection of the two streaks. No lysis at this intersection indicated that the mutants did not complement each other. Positive plate complementation results on strain 594 indicated the presence of at least 11 genes. However, apart from clearly positive and clearly negative results, a third, intermediate result was often obtained; an increase in phage activity in the region where the two streaks crossed was observed, but the increased activity was in the form of plaques rather than a confluent square of lysis.

When the *am5* mutant was tested by plate complementation, it gave this intermediate result with all mutants carrying mutations in the 10 other genes defined by liquid complementation. This was in agreement with the very poor complementation observed in liquid medium for tests involving this mutant. Amber mutants 11, 12, 13, 24, 30, 33, and 43 also gave intermediate results when tested with mutants carrying mutations in other genes. When tested among themselves, these eight mutants failed to show any complementation. Their mutations were therefore assigned to the same gene (gene *A*), and poor complementation appeared to be a characteristic of this gene.

Mutant 40 behaved like the strains with muta-

tions in gene *A* in that no squares of confluent lysis were obtained in complementation tests. However, rather than producing intermediate results, mutant 40 generally gave negative results with other mutants. Subsequent results have shown that this mutant is, in fact, a double mutant, one amber mutation being in gene *I* and the other in gene *A*. These mutations are referred to as 40a and 40b, respectively. When the 40a mutant was separated from 40b by recombination, it no longer showed the poor complementation characteristic of gene *A* mutants and complemented all other mutants except nos. 50, 56, and 41.

Intermediate complementation results were also obtained for various other pairs of mutants neither of which carried a mutation in gene *A*. However, in these cases the mutations involved would only show intermediate results with other mutations in one, or at most two, of the 10 non-*A* genes shown in Table 2. For example, the 14 mutants numbered 14, 23, 26, 48, 7, 35, 46, 20, 9, 25, 10, 28, 27, and 29 showed either negative or intermediate results when tested among themselves but showed good complementation with all of the other mutants. Their mutations were initially assigned to a single gene, although it is probable that they belong to more than one gene, the poor complementation being due to polarity effects.

To avoid the problem of polarity and to determine the number of genes represented by the known alleles of phage 186, we used a different bacterial host in complementation tests. The host, XA7007, contains the polarity-suppressing *rho* mutation originally known as SuA (3, 18). When tested by plate complementation on XA7007, the 14 mutation-carrying strains mentioned above could be divided into four groups (by mutations in gene *D*, *E*, *F*, or *G*), the members of each group complementing the members of the other three groups but not those of their own group.

Table 3 summarizes the results obtained from plate and liquid complementation tests. Mutations assigned to a particular gene showed either no phage activity at the intersection of the streaks or, if several plaques did appear, no improvement in activity when retested on strain XA7007. Twenty-two genes were defined by this method, comprising various polarity groups (Table 3). Mutants carrying mutations in different genes within the same polarity group gave intermediate results on strain 594 but showed definite complementation on strain XA7007. Four polarity groups were defined, involving 16 of the 22 genes. Genes *D* to *G*, *H* to *J*, *K* to *M*, and *Q* to *V*, formed the four polarity groups, whereas genes *A*, *B*, *N*, *O*, *P*, and *W* were apparently independent of these polarity groups.

Although the use of strain XA7007 led to the definition of many additional genes in phage 186, there were several problems associated with its use. The appearance of the squares of lysis on this strain was more turbid and indistinct than it was on strain 594; e.g., even mutants in different polarity groups which produced very clear squares of confluent lysis on strain 594 showed a poorer (although still positive) response on strain XA7007. Also, although there was often a clear-cut improvement in complementation on strain XA7007 as compared with strain 594 for mutants in the same polarity group, there were many cases in which only a slight improvement occurred. In some cases no improvement was observed, even though the results with other pairs of mutants suggested that the mutations in question belonged to different genes. Such results could reflect *rho*-independent termination.

These problems seemed to be particularly prevalent in the polarity group containing genes

TABLE 3. List of the genes, their alleles, and the polarity groups of phage 186 as defined by complementation tests

Polarity group	Gene	Amber alleles[a]
	A	5, 11, 12, 13, 24, 30, 33, 43
	B	17, 57
D–G	*D*	14, 23, 26, (48)
	E	7, 35, 46
	F	20
	G	9, 25, (10, 28), 27, 29
H–J	*H*	50, 56
	I	40a
	J	41
K–M	*K*	22, 42, 58
	L	2, 21, (44)
	M	19, 31, 60
	N	47
	O	61, 62
	P	16, 36, 45, 65, 67, (66)
Q–V	*Q*	1, 49
	R	6, (51)
	S	4, (18), 34
	T	8
	U	37, 63, 64
	V	38
	W	15, 39, 52

[a] Three temperature-sensitive alleles have also been assigned by complementation tests. *ts*59 is in gene *K*, and *ts*68 and *ts*69 are in gene *W*. Mutations in brackets appeared to be identical with the ones preceding them, as judged by failure to show recombination on the plate.

TABLE 4. In vitro reconstitution with strains with mutations in genes *D*, *E*, *F*, *G*, *K*, *L*, *M*, *N*, *R*, *S*, *T*, *V*, and *W*

First lysate	Phage titer (PFU/ml)[a] with second lysate:												
	Wam15	*Vam38*	*Tam8*	*Sam4*	*Ram6*	*Nam47*	*Mam19*	*Lam21*	*Kam42*	*Gam25*[b]	*Fam20*	*Eam7*	*Dam14*
Wam15	$<1 \times 10^2$	7×10^5	1×10^2	$<1 \times 10^2$	2×10^2	7×10^8	2×10^9	2×10^9	1×10^9	1×10^9	2×10^9	2×10^9	1×10^9
Vam38		$<1 \times 10^2$	$<1 \times 10^2$	$<1 \times 10^2$	5×10^5	2×10^9	2×10^9	1×10^9	2×10^9	9×10^8	1×10^9	2×10^9	1×10^9
Tam8			$<1 \times 10^2$	$<1 \times 10^2$	2×10^2	8×10^7	1×10^8	1×10^8	1×10^8	1×10^8	2×10^8	9×10^7	1×10^8
Sam4				$<1 \times 10^2$	1×10^2	2×10^9	2×10^9	1×10^9	2×10^9	1×10^9	1×10^9	2×10^9	1×10^9
Ram6					2×10^2	2×10^9	2×10^9	1×10^9	2×10^9	1×10^9	1×10^9	2×10^9	1×10^9
Nam47						$<1 \times 10^2$	6×10^4	5×10^5	6×10^5	4×10^5	9×10^5	6×10^4	7×10^5
Mam19							2×10^2	5×10^2	6×10^2	2×10^2	2×10^2	5×10^2	1×10^2
Lam21								2×10^2	1×10^2	2×10^2	2×10^2	5×10^2	2×10^2
Kam42									$<1 \times 10^2$	$<1 \times 10^2$	$<1 \times 10^2$	3×10^2	$<1 \times 10^2$
Gam25										$<1 \times 10^2$	$<1 \times 10^2$	2×10^2	$<1 \times 10^2$
Fam20											$<1 \times 10^2$	2×10^2	$<1 \times 10^2$
Eam7												2×10^2	2×10^2
Dam14													$<1 \times 10^2$

[a] Titers were obtained after a 60-min incubation of 10^{-1} dilutions of the two defective lysates.
[b] *Gam27* and *Gam28* mutants were also tested, and the results were found to be qualitatively identical to those for *Gam25*.

Q, *R*, *S*, *T*, *U*, and *V*. The assignment of alleles to this group (Table 3) represents the most likely arrangement, although the assignment of the three alleles 37, 63, and 64 to a single gene is doubtful. On strain 594, *am*37 and *am*64 showed higher phage activity than would be expected for mutants carrying mutations in the same gene. However, the use of strain XA7007 led to no improvement in complementation for any pair of mutants from the group 37, 63, and 64.

The gene assignments (Table 3) are supported by genetic mapping results previously described (S. M. Hocking and J. B. Egan, Mol. Gen. Genet., in press). Mutations assigned to the same gene by complementation results in strain XA7007 were also found to be located in adjacent positions on the genetic map.

Functional assignments. The functions of genes involved in the formation of the phage particle were determined by examination of the defective lysates produced by the various amber mutants.

(i) Lysis of a nonpermissive host. When Su^- lysogens of 186 *c*I *ts*p amber mutants carrying mutations in genes *D*, *E*, *F*, *G*, *H*, *J*, *K*, *L*, *M*, *N*, *Q*, *R*, *S*, *T*, *U*, *V*, or *W* were heat induced, bacterial cell lysis, as determined by absorbance measurements, commenced 30 to 60 min later, producing lysates containing very few active phage particles (usually less than 10^3 PFU/ml). Although these defective lysates lacked infective phages, they contained many incomplete phage structures. The single known mutation in gene *I* (*am*40a mutant) was not tested by this method.

After heat induction of Su^- lysogens in mutants carrying mutations in the remaining four genes (*A*, *B*, *O*, and *P*), cell lysis was either absent or very delayed. At equivalent times after heat induction, the Su^- lysogens of mutants with mutations in these four genes also differed from those of mutants with mutations in all of the other genes in containing a large number of active phages (10^6 to 10^8 PFU/ml). However, this still represents less than one phage per bacterial cell. Further studies on the function of genes *A*, *B*, *O*, and *P* are described in the accompanying paper (10).

(ii) In vitro reconstitution. Those mutants capable of lysing a nonpermissive host were assumed to carry mutations affecting the phage morphogenetic process. To determine which part of the phage structure was affected by these mutations, the defective lysates were tested for in vitro reconstitution by adding them in paired combinations and determining the increase in phage titer.

The results (Table 4) showed that functionally there were two main groups of mutations. Mutations in genes *D*, *E*, *F*, *G*, *K*, *L*, and *M* appeared

to affect the same phage structure, since the addition of the defective lysates produced by strains with these mutations, in any paired combination, did not lead to any increase in phage titer. Similarly, when tested among themselves, the defective lysates produced by mutants with mutations in genes *R*, *S*, *T*, or *W* did not give rise to any increase in phage titer. However, when the lysates produced by the first set of mutants were added to the lysates produced by the second set, a very large (approximately 10^7-fold) increase in phage titer was observed. This suggested that the products of the two groups of genes, *R*, *S*, *T*, and *W* and *D*, *E*, *F*, *G*, *K*, *L*, and *M*, were concerned with the formation of two different parts of the phage particle.

Complementation studies with the reconstituted phages showed that in all cases the DNA of the reconstituted phages was derived from the lysates produced by mutants with mutations in genes *D*, *E*, *F*, *G*, *K*, *L*, and *M* and never from the lysates produced by strains with mutations in genes *R*, *S*, *T*, or *W*. Furthermore, when the plaque morphology of the two parent phages differed, the reconstituted phages always had the plaque morphology of the parent with mutations in genes *D*, *E*, *F*, *G*, *K*, *L*, or *M* and never that of the parent with mutations in genes *R*, *S*, *T*, or *W*. Both of these observations suggested that genes *R*, *S*, *T*, and *W* were involved in the formation of the phage head, and genes *D*, *E*, *F*, *G*, *K*, *L*, and *M* were involved in the formation of the phage tail.

In reconstitution tests, *Nam*47 and *Vam*38 gave unusual results. The lysate produced by *Nam*47, when added to those produced by strains with mutations in genes *R*, *S*, *T*, or *W*, gave rise to a large (approximately 10^7-fold) increase in phage titer, and, as judged by complementation and plaque morphology, the phages produced invariably contained the DNA of *Nam*47. However, at a low efficiency (10^3-fold over background), some reconstitution was also observed when the lysate produced by *Nam*47 was added to lysates produced by strains with mutations in genes *D* to *M*. Complementation tests indicated equal representation of the *Nam*47 mutation and the other tested mutation in the phages produced. It therefore appeared that *Nam*47 was a tail mutation capable of limited in vitro reconstitution with other tail mutations.

By contrast, *Vam*38 showed normal reconstitution with all other tail mutations including *Nam*47 (approximately a 10^7-fold increase in PFU), but a lower reconstitution with some head mutations (approximately a 10^3-fold increase in PFU). Complementation and plaque morphology studies showed that when *Vam*38 was reconstituted with the tail mutations in genes *D* to *N*, the genotype of the reconstituted phages was that of the mutations in genes *D* to *N* and not that of *Vam*38. When *Vam*38 was reconstituted with the head mutations *Wam*15 or *Ram*6, the reconstituted phages all had the genotype of *Vam*38. It therefore appeared that gene *V* was concerned with head formation, but that in the absence of gene *V* protein some nonfunctional DNA-containing heads could be formed. Completion of these heads then occurred after the addition of lysates produced by *Wam*15 or *Ram*6. Although *Vam*38 reconstituted with head mutations *Wam*15 and *Ram*6, it did not reconstitute with head mutations *Tam*8 and *Sam*4. This could perhaps be due to polarity effects, since *V*, *T*, *S*, and *R* are all in the same polarity group, or to the complexities of the formation of the phage 186 head.

Table 5 shows the results of additional in vitro reconstitution experiments involving mutations in genes *H*, *J*, *O*, *Q*, and *U*. The lysates produced by mutations *Ham*56 and *Jam*41 showed reconstitution with the lysate produced by the head mutation *Wam*15 but not with that produced by the tail mutation *Kam*42. Genes *H* and *J* must therefore be involved in phage 186 tail formation. The plaque morphology of the reconstituted phages in both cases was that of *Jam*41 or *Ham*56, not of *Wam*15, and this supports the above conclusion. The reverse occurred with mutations *Qam*1, *Uam*37, *Uam*64, and *Uam*63.

TABLE 5. In vitro reconstitution with strains with mutations in genes *H*, *J*, *K*, *O*, *Q*, *U*, *V*, and *W*

First defective lysate	Phage titer (PFU/ml) with second defective lysate or control[a]		
	Head donor *Kam*42	Tail donor *Wam*15	Control
*Ham*56	$<1 \times 10^2$	1.5×10^9	$<1 \times 10^2$
*Jam*41	3.5×10^3	1.9×10^9	1.8×10^3
*Kam*42	$<1 \times 10^2$	1.5×10^9	$<1 \times 10^2$
*Oam*61[b]	7.3×10^6	5.6×10^5	5.7×10^5
*Qam*1	9.1×10^7	$<1 \times 10^2$	$<1 \times 10^2$
*Uam*37	7.0×10^7	$<1 \times 10^2$	$<1 \times 10^2$
*Uam*63	1.7×10^9	1×10^2	$<1 \times 10^2$
*Uam*64	1.6×10^9	$<1 \times 10^2$	$<1 \times 10^2$
*Vam*38	1.1×10^9	$<1 \times 10^2$	$<1 \times 10^2$
*Wam*15	1.5×10^9	$<1 \times 10^2$	$<1 \times 10^2$

[a] Titers were obtained after a 60-min incubation of 10^{-1} dilutions of two defective lysates or of a 10^{-1} dilution of the defective lysate alone for the control.

[b] In a 10^{-1} dilution of another lysate produced by *Oam*61 there were 4.8×10^5 PFU/ml. After incubation with the lysates produced by the head mutant *Wam*15 and the tail mutant *Jam*41, there were 6.5×10^5 and 6.8×10^6 PFU/ml, respectively. In the latter case, of the 68 plaques scored, 5 had the plaque morphology of *Oam*61 and 63 had the plaque morphology of *Jam*41.

For these mutations reconstitution occurred with the tail but not with the head mutation lysate. Genes *Q* and *U* must therefore be involved in the formation of the phage 186 head particle.

The results for *Oam*61 were quite different. This mutant showed very late lysis and a large number of mature, active phages in the lysate. No reconstitution was obtained when the *Oam*61 lysate was added to the *Wam*15 lysate, but 10-fold increases in phage titer were obtained when it was added to either a *Kam*42 or a *Jam*41 lysate. The plaque morphologies of *Oam*61 and *Jam*41 were easily distinguishable, and 90% of the reconstituted phages in this test had the plaque morphology of *Jam*41, indicating that the increase in phage titer was due to genuine reconstitution. (The 10% of plaques with the morphology of *Oam*61 were to be expected, since 1 in 10 of the phages present after reconstitution should have been derived as a complete phage from the *Oam*61 lysate.) It would appear that there was an excess of phage tails in the *Oam*61 lysate, perhaps suggesting a defect in head formation. However, the level of reconstitution obtained was very much lower than the normal level, and this fact, coupled with the late-lysis phenotype of the *Oam*61 mutant, suggested that the product of gene *O* was involved in some function other than morphogenesis.

(iii) Electron microscopy. To confirm the functional assignments obtained from in vitro reconstitution experiments, we observed the defective lysates directly in the electron microscope. The phage particles seen and the behavior of each mutant in reconstitution experiments are shown in Table 6.

Genes *D*, *E*, *F*, *G*, *K*, and *L* were clearly involved in formation of the phage tail, since head particles but no tail particles were visible in the defective lysates produced by strains with mutations in these genes. In addition to the DNA-containing heads visible in these lysates, there were also empty heads present. Tail mutation lysates showed both full and empty heads, and both types of head were present in all tail mutation lysates examined (Fig. 1b). In addition to phage heads, the lysate produced by *Gam*27 did show one particle which appeared to be a tail core without a sheath (Fig. 1e). Strains with mutations in all of the genes listed above donated heads in reconstitution experiments, as was expected for mutations in tail genes. The defective lysate produced by *Mam*19 contained heads but no normal tails. However, some tail-like protein (possibly a sheath protein) was observed (Fig. 1d). *Mam*19 behaved as a head donor in reconstitution experiments and was clearly a tail mutation.

The lysates produced by strains with mutations in *R*, *S*, and *W* contained normal tails, but the heads observed were obviously defective (Fig. 1c). No full heads were observed in any of the lysates. The results of reconstitution experiments also suggested that the heads in these lysates were not active. It was therefore concluded that genes *R*, *S*, and *W* are involved in the formation of the phage head, although the presence of head-related structures in the lysates showed that these genes did not code for the major capsid protein.

Only tail particles were visible in the lysates produced by strains with mutations in *T* and *U*, and these genes must therefore be involved in head formation. Since no head-related structures were observed in these lysates, it was possible that one of these genes coded for the major capsid protein. The absence of head-related structures in the other lysate could have been due to polarity effects, since genes *T* and *U*

TABLE 6. Phage 186 genes involved in phage head and tail formation

Gene	Allele	Particles observed in defective lysates[a]	Active phage particles[b]	Morphogenetic function
D	*am*14	Heads	Heads	Tail
E	*am*7	Heads	Heads	Tail
F	*am*20	Heads	Heads	Tail
G	*am*25[c]	Heads	Heads	Tail
H	*am*56	Heads, tails	Heads	Tail
J	*am*41	Heads, tails	Heads	Tail
K	*am*42	Heads	Heads	Tail
L	*am*21	Heads	Heads	Tail
M	*am*19	Heads, tail protein	Heads	Tail
N	*am*47	Heads, defective tails	Heads (many), tails (few)	Tail
Q	*am*1	Heads, tails	Tails	Head
R	*am*6	Tails, defective heads	Tails	Head
S	*am*4	Tails, defective heads	Tails	Head
T	*am*8	Tails	Tails	Head
U	*am*37[d]	Tails	Tails	Head
V	*am*38	Defective tails, defective heads	Tails (many), heads? (few)	Head?
W	*am*15	Tails, defective heads	Tails	Head

[a] Heads, Both full and empty heads present; defective heads, only empty heads present; defective tails, tails showed variation in length. Figure references: tail protein, Fig. 1d; defective tails, Fig. 1g and 2a and b; defective heads, Fig. 1c and 2a.

[b] Determined by in vitro reconstitution.

[c] *G* alleles *am*27 and *am*28 gave identical results.

[d] *U* alleles *am*63 and *am*64 gave identical results.

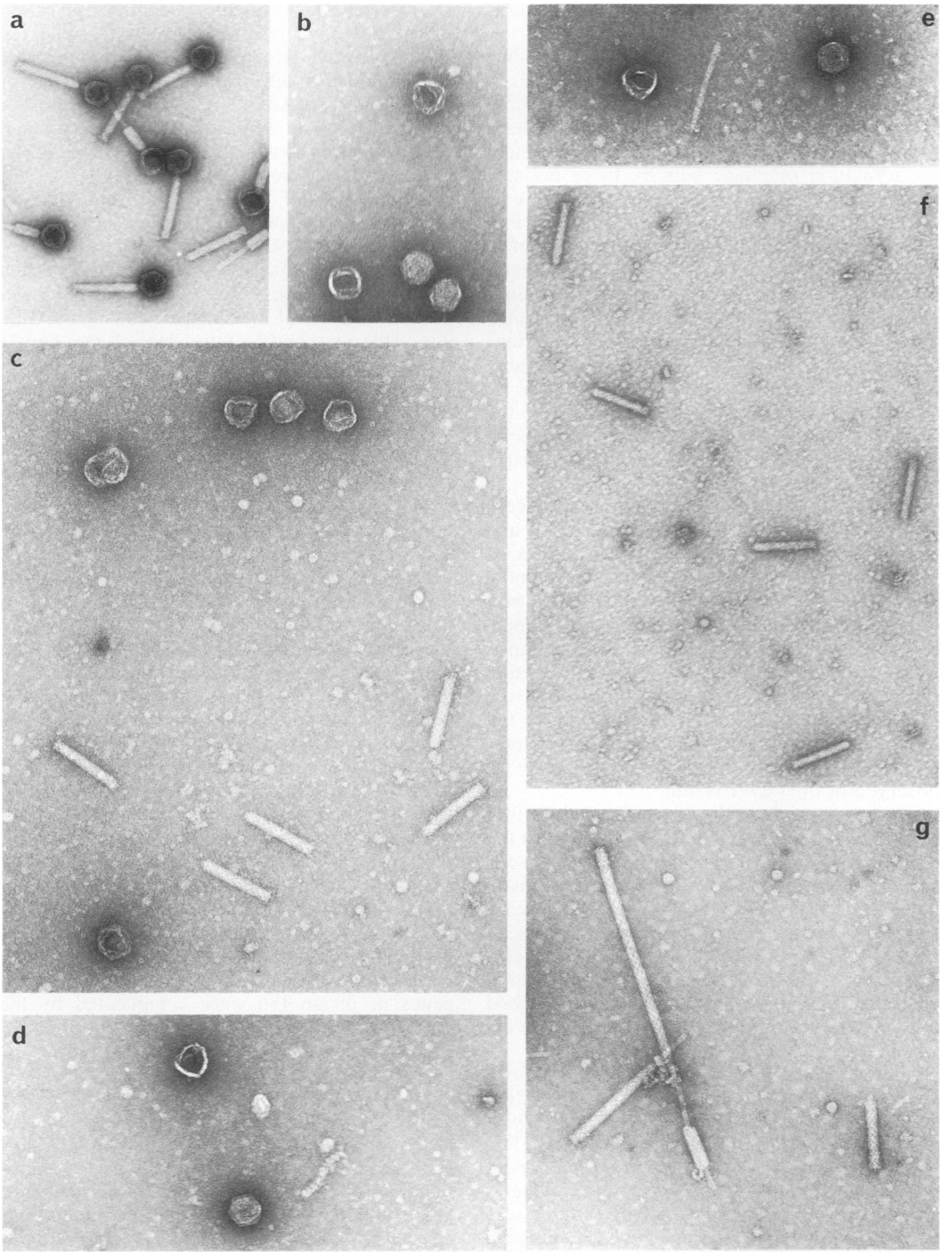

FIG. 1. Electron micrographs of 186 *c*I *ts*p (a) and defective lysates produced by 186 *c*I *ts*p mutants *Gam*28 (b), *Sam*4 (c), *Mam*19 (d), *Gam*27 (e), *Ham*56 (f), and *Nam*47 (g). Magnification, about 74,000×. Two different microscopes were used to produce the micrographs shown in Fig. 1 and 2, and small differences in apparent particle size resulted. No internal standard was present, so an exact size for the particles could not be determined.

belong to the same polarity group. The results of reconstitution also suggested that genes *T* and *U* were involved in head formation.

Both phage heads and phage tails were visible in the lysates produced by *Ham*56 and *Jam*41. In vitro reconstitution results showed that these two mutants were able to donate active phage heads but not active phage tails, suggesting that the formation of phage tails by these mutants was defective. However, the tails appeared to be normal as seen in the electron microscope (Fig. 1f), and it therefore appeared that genes *H* and *J* were not involved in the formation of the major tail structures, the core and the sheath. A minor structure, such as a collar or the tail fibers, may instead have been involved. The number of tails appearing in the *Ham*56 lysate was considerably greater than the number of heads, since no quantitative studies were done on any of the lysates it is not known whether this was significant. It may be that an excess of tails is normal for a phage 186 infection.

The lysate produced by *Qam*1 showed very few phage structures, but both phage heads and phage tails were observed. The heads appeared to be normal despite the fact that the results of in vitro reconstitution showed that the lysate produced by this mutant was unable to donate active heads. A head function has been assigned to gene *Q* on the basis of the reconstitution results. The level of reconstitution with the *Qam*1 and *Kam*42 lysates was lower than the normal level of reconstitution, and this is consistent with the low numbers of phage particles observed in the *Qam*1 lysate.

The lysate produced by *Nam*47 contained full, apparently active phage heads together with the usual empty heads, but the tail structures seen varied greatly in length and seemed to be unstable, the core often protruding from the sheath (Fig. 1g). Some apparently normal tails were also observed. The results of electron microscopic observation and in vitro reconstitution were therefore in agreement, and both suggested that some step in the assembly of the phage tail was defective in this mutant.

The electron microscope results with *Vam*38 were hard to reconcile with the reconstitution results. Reconstitution suggested that *Vam*38 was an efficient tail donor, yet under the electron microscope very few tails of normal length were observed (Fig. 2a). Most varied in length, some being exceedingly long (Fig. 2b). It was not known whether these long tails were capable of attaching to heads, and if so whether the resultant phage were infectious. Defective heads were seen in these lysates; this was in agreement with reconstitution results. The function of gene *V* was therefore rather obscure, both heads and tails appearing to be abnormal.

With *Lam*21 the results of both in vitro reconstitution and electron microscopic observation of the defective lysate suggested that the tail particles were defective. Confirmation of this was provided by electron microscopic observation of a stock of *Lam*21 grown in the suppressor host strain C600. *Lam*21 produced very small plaques on this host, and the tails of many of the phage produced were visibly defective (Fig. 2c). When a stock of *Lam*21 grown in C600 was centrifuged to equilibrium in a CsCl density gradient (ρ = 1.425 g/cm^3), two bands were produced. The upper (less dense) band consisted of whole 186 phages (Fig. 2c), whereas the lower band consisted of 186 phage heads only (Fig. 2d). This instability of the 186 *c*I *ts*p *Lam*21 phage can be used to purify 186 phage heads. The tail particles, if still intact, did not band under the conditions used.

DISCUSSION

In coliphage 186, 22 genes were defined by complementation tests. These were assigned letters of the alphabet after consideration of the mapping data (Hocking and Egan, in press), with the alphabetical order being the order of genes from right to left.

Mutations in gene *A* are characterized by their very poor complementation with mutations in any other gene. Lindahl (15) reported similar behavior for phage P2 gene *A* mutants and showed that this was a result of the *cis* action of the *A* protein and the dependence of all other known essential genes on its expression. These two properties are also therefore expected for the phage 186 gene *A* product. Since *Aam* mutants of phage 186 lysogenize Su$^-$ strains, it is assumed that the gene *A* product is not required for the expression of the nonessential genes *c*I, *c*II, and *int*, whose products are required for lysogen formation (unpublished data).

The complementation results for other mutant genes of phage 186 were complicated by the presence of polarity. Polarity describes the phenomenon in which a nonsense mutation in one gene can reduce the expression of other genes located distal to it within the same transcription unit. In phage λ, polarity effects which cause a reduction in intergene complementation have been described by Parkinson (20). These polarity effects were relatively weak in that the phage yields obtained in coinfections with mutations affected by polarity were still 100 to 300 times greater than the yields obtained for mutations in the same gene. By contrast, much stronger effects have been observed for phage P2, with phage yields in some cases for mutations in different genes being no greater than those for mutations in the same gene (16, 22).

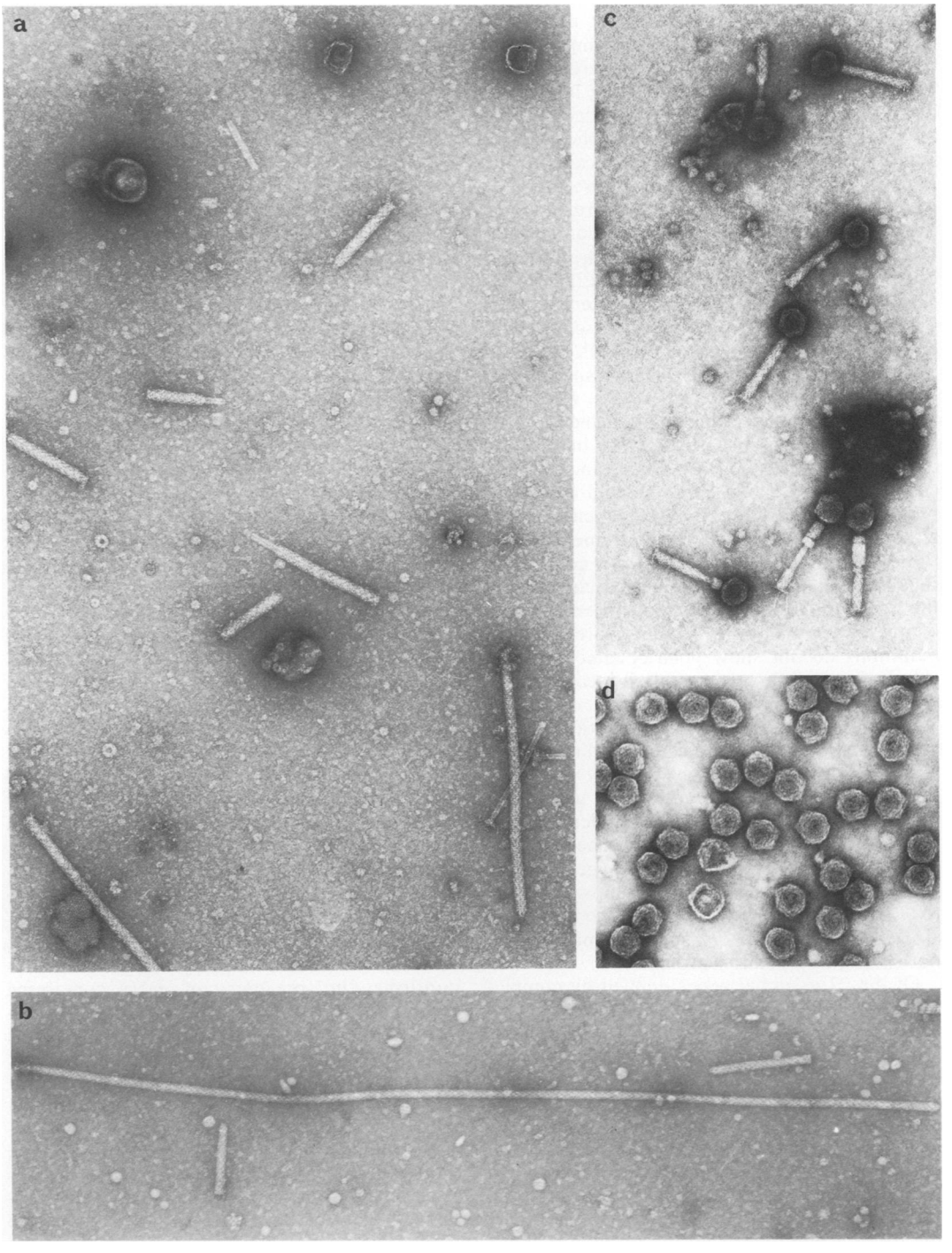

FIG. 2. Electron micrographs of defective lysates produced by mutant 186 *c*I *ts*p *Vam*38 (a and b) and of a CsCl-banded stock of mutant 186 *c*I *ts*p *Lam*21: (c) upper band; (d) lower band. Magnification, about 74,000×.

The polarity effects operating in phage 186 infections are of the strong P2 type rather than the weak λ type. Without the use of the polarity-suppressing host, strain XA7007, the number of genes represented by the known amber mutations would have been greatly underestimated. The use of the polarity-suppressing host overcame this problem and allowed the definition of many additional genes. However, the level of suppression by strain XA7007 appeared to be highly variable, although the increase in phage yield could not be quantitated as all results with this strain were obtained from plate complementation tests. Quantitative results from liquid complementation experiments might help to resolve some of the problems encountered. However, Sunshine et al. (22) reported some liquid complementation results for phage P2 obtained by using a strain with the polarity-suppressing phenotype SuA, and their results were also extremely variable. They reported increases in phage activity of up to 5,000-fold, but the increase was usually less than this, and in some cases no increase could be obtained even with mutations known to be in different genes. Thus, although the SuA mutation is extremely useful, the results obtained with it are not completely reliable. Doubtful results could be checked by the isolation of temperature-sensitive revertants of the amber mutations. Temperature-sensitive mutations do not show polarity effects and so should fail to complement only if located in the same gene.

Such temperature-sensitive mutations could also be employed to indicate the direction of transcription for the various polarity groups as described for P2 (16). Our present use of the polarity-suppressing strain in complementation tests increased the number of genes defined from 11 to 22. Sixteen of these genes were involved in one of four polarity groups: groups *D* to *G*, *H* to *J*, *K* to *M*, and *Q* to *V*. The remaining six genes, *A*, *B*, *N*, *O*, *P*, and *W*, apparently do not belong to a polarity group.

Of the 22 essential genes defined by complementation, only four (*A*, *B*, *O*, and *P*) affected the ability of the infected cell to lyse, and their functions are better discussed in the accompanying paper (10). A fifth gene (*I*) has not been studied. Mutations in the remaining 17 essential genes all caused host cell lysis. In vitro reconstitution results with lysates produced by these mutations allowed their division into two groups. The genes of one group controlled the formation of the phage head, and the genes of the other group controlled the formation of the phage tail. Phage 186 head and tail particles combined in vitro at a very high efficiency. A combination of undiluted head and tail mutant lysates should contain approximately 10^{10} PFU of reconstituted phages per ml (Table 4). This compares with a level of less than 10^8 PFU/ml obtained for phage λ by Weigle (23) under conditions almost identical to those used for phage 186. In vitro reconstitution has also been reported for phage P2 (17), but a comparison with 186 cannot be made since the titers of reconstituted phages were not given and the results were complicated by the presence of a large number of active phage in the lysates (10^7 to 10^8 PFU/ml).

The results (Table 4) also provided evidence of some intratail reconstitution (between the product of the tail gene *N* and those of the other tail genes).

The results of electron microscopy of the defective lysates provided some additional information about the functions of the phage 186 genes. Lysates produced by strains with mutations in the head genes *R*, *S*, and *W* all contained recognizable, although empty, head structures. Thus, these genes do not code for the major structural protein of the head. The lysates of strains with mutations in genes *T* and *U* did not show head-related structures, but this needs to be confirmed by quantitative studies on more lysates. If confirmed, then it is possible that one of these genes codes for the major head protein, and the product of the other gene might be required for the assembly of the major capsid protein. Alternatively, the absence of head-related structures in the second lysate might be due to polarity effects. Defective lysates produced in a polarity-suppressing host could help to distinguish between these possibilities.

Apparently normal, DNA-containing head particles were observed in the lysate produced by the *Qam*1 mutant, although the results of in vitro reconstitution showed that these particles must be defective. DNA-containing but defective head particles have also been found to be intermediates in the assembly of the head particles of T4 (12), λ (7), and P2 (21). If examination of other lysates produced by the *Qam*1 mutant confirms the presence of DNA-containing heads, then it could be concluded that the product of gene *Q* is required for one of the final steps in the assembly of the phage 186 head particle. A gene with a phenotype like that of *Vam*38 (i.e., defective heads and abnormally long tails) has not been found for either λ or P2.

As found for phage λ (11, 13, 19) and P2 (14), mutations in most of the phage 186 tail genes resulted in no tail-related structures being produced. Quantitative studies would be required to determine whether the apparent tail core found in the *Gam*27 lysate and the possible sheath protein found in the *Mam*19 lysate are characteristic of these mutations. Exceptions to the absence of tail structures in tail mutant lysates

were the lysates produced by strains with mutations in *N*, *H*, and *J*. The tails in the gene *N* mutant lysate were obviously defective, most being too long, but the tails in the gene *H* and *J* mutant lysates appeared to be normal. Gene *N* may have a function similar to that of genes *U* of λ (19) and *R* of P2 (14); mutations in either cause abnormally long tails in defective lysates, although such structures could simply reflect an imbalance of morphogenetic components caused by polarity. Genes *H* and *J* may perhaps be involved in the synthesis of a part of the tail structure which is normally not visible or only occasionally resolved (e.g., the tail fibers of phage 186, which are rarely seen). *Ham*56 was originally isolated by Georgopoulos as a mutant capable of growth on a *groE* strain. If it is true that the suppressed *am*56 phenotype is directly related to the mutant's ability to grow on *groE*, then it is noteworthy that *groE* is a host mutant that blocks the utilization of the λ gene *E* product in coliphage λ head assembly (9), whereas the phage 186 gene *H* product is concerned with tail maturation. The *groE* mutation appears therefore to interfere with proper tail assembly in phage 186, as was found also with phage T5 (27). Although it is not known whether an excess of phage tails is normal for a phage 186 infection, such an excess could explain the excess of phage tails seen in the *Ham*56 lysate and apparently present (judging from reconstitution results) in the *Oam*61 lysate. For phage λ it was observed that in lysates produced by wildtype phage the number of free tails was 10-fold greater than the number of free heads (11).

Although the *Iam*40a mutant has not been studied, the *I* gene is tentatively considered to encode a tail function by virtue of its map location in the middle of the tail gene cluster, with five tail genes on either side (Hocking and Egan, in press).

In summary, 22 essential genes were defined, 7 concerned with phage head formation and 11 with phage tail formation. The functions of the remaining four genes are discussed in the accompanying paper (10), and the genome organization is reported in Hocking and Egan (in press).

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LITERATURE CITED

1. **Appleyard, R. K.** 1954. Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from *Escherichia coli* K12. Genetics **39**:440–452.
2. **Baldwin, R. L., P. Barrand, A. Fritsch, D. A. Goldthwait, and F. Jacob.** 1966. Cohesive sites on the deoxyribonucleic acids from several temperate coliphages. J. Mol. Biol. **17**:343–357.
3. **Beckwith, J.** 1963. Restoration of operon activity by suppressors. Biochim. Biophys. Acta **76**:162–164.
4. **Bertani, L. E., and G. Bertani.** 1971. Genetics of P2 and related phages. Adv. Genet. **16**:199–237.
5. **Bradley, C., P. L. Ong, and J. B. Egan.** 1975. Isolation of phage P2-186 intervarietal hybrids and 186 insertion mutants. Mol. Gen. Genet. **140**:123–135.
6. **Campbell, A.** 1965. The steric effect in lysogenization by bacteriophage lambda. I. Lysogenization of a partially diploid strain of *Escherichia coli* K12. Virology **27**:329–339.
7. **Casjens, S., T. Hohn, and A. D. Kaiser.** 1972. Head assembly steps controlled by genes *F* and *W* in bacteriophage λ. J. Mol. Biol. **64**:551–563.
8. **Garen, A., S. Garen, and R. C. Wilhelm.** 1965. Suppressor genes for nonsense mutations. I. The *Su-1*, *Su-2* and *Su-3* genes of *Escherichia coli*. J. Mol. Biol. **14**:167–178.
9. **Georgopoulos, C. P., and B. Hohn.** 1978. Identification of a host protein necessary for bacteriophage morphogenesis (the *groE* gene product). Proc. Natl. Acad. Sci. U.S.A. **75**:131–135.
10. **Hocking, S. M., and J. B. Egan.** 1982. Genetic studies of coliphage 186. II. Genes associated with phage replication and host cell lysis. J. Virol. **44**:1068–1071.
11. **Kemp, C. L., A. F. Howatson, and L. Siminovitch.** 1968. Electron microscope studies of mutants of lambda bacteriophage. I. General description and quantitation of viral products. Virology **36**:490–502.
12. **King, J.** 1968. Assembly of the tail of bacteriophage T4. J. Mol. Biol. **32**:231–262.
13. **Kühl, P. W., and I. Katsura.** 1975. Morphogenesis of the tail of bacteriophage λ. I. *In vitro* intratail complementation. Virology **63**:221–237.
14. **Lengyel, J. A., R. N. Goldstein, M. Marsh, and R. Calendar.** 1974. Structure of the bacteriophage P2 tail. Virology **62**:161–174.
15. **Lindahl, G.** 1970. Bacteriophage P2: replication of the chromosome requires a protein which acts only on the genome that coded for it. Virology **42**:522–533.
16. **Lindahl, G.** 1971. On the control of transcription in bacteriophage P2. Virology **46**:620–633.
17. **Lindahl, G.** 1974. Characterization of conditional lethal mutants of bacteriophage P2. Mol. Gen. Genet. **128**:249–260.
18. **Morse, D. E., and P. Primakoff.** 1970. Relief of polarity in *E. coli* by "*suA*". Nature (London) **226**:28–31.
19. **Mount, D. W. A., A. W. Harris, C. R. Fuerst, and L. Siminovitch.** 1968. Mutations in bacteriophage lambda affecting particle morphogenesis. Virology **35**:134–149.
20. **Parkinson, J. S.** 1968. Genetics of the left arm of the chromosome of bacteriophage lambda. Genetics **59**:311–325.
21. **Pruss, G. J., and R. Calendar.** 1978. Maturation of bacteriophage P2 DNA. Virology **86**:454–467.
22. **Sunshine, M. G., M. Thorn, W. Gibbs, R. Calendar, and B. Kelly.** 1971. P2 phage amber mutants: characterization by use of a polarity suppressor. Virology **46**:691–702.
23. **Weigle, J.** 1966. Assembly of phage lambda *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **55**:1462–1466.
24. **Woods, W. H., and J. B. Egan.** 1974. Prophage induction of noninducible coliphage 186. J. Virol. **14**:1349–1356.
25. **Younghusband, H. B., J. B. Egan, and R. B. Inman.** 1975. Characterization of the DNA from bacteriophage P2-186 hybrids and physical mapping of the 186 chromosome. Mol. Gen. Genet. **140**:101–110.
26. **Younghusband, H. B., and R. B. Inman.** 1974. Base sequence homologies between bacteriophage P2 and 186 DNAs. Virology **62**:530–538.
27. **Zweig, M., and D. J. Cummings.** 1973. Cleavage of head and tail proteins during bacteriophage Ts assembly: selective host involvement in the cleavage of a tail protein. J. Mol. Biol. **80**:505–518.