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Studies on the In Vitro Assembly of Bacteriophage φ80 and φ80-Lambda Hybrids

SAMIR S. DEEB

Biology Department, American University at Bierut, Beirut, Lebanon

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Suppressor-sensitive (*sus*) mutants of bacteriophage φ80 defective in late functions were classified, by means of in vitro assembly tests, into two complementation groups: head donors and tail donors. Each group of mutants was subdivided, by means of two-factor crosses, into six cistrons. Deletion mapping revealed clustering of tail and also of head cistrons. The two clusters were located in the left arm of vegetative φ80 (the tail specifying cluster being distal). In vitro cross complementation between φ80 and lambda *sus* mutants revealed that whereas lambda heads could quite efficiently bind φ80 tails to form viable phage, the union of φ80 heads and lambda tails was very much less efficient. Deletion mapping of the φ80 *sus* mutants, using both φ80 and $i^{\phi 80}h^{\lambda}$ deletion lysogens indicated congruent gross gene arrangement in the two related bacteriophages.

The in vitro assemby of viable bacteriophage particles from components such as heads and tails was recently demonstrated for bacteriophages T4 (6) and lambda (13). Studies with T4 showed that several steps in the morphogenetic pathway could take place in vitro including tail fiber assembly, union of tail fibers to fiberless particles, and head to tail interaction. The work in vitro assembly of bacteriophage lambda from heads and tails revealed that genes involved in morphogenesis fell into two groups: tail-specifying and head-specifying genes. The two gene clusters corresponding to tail and head formation were located on the left arm of the vegetative lambda map, the former being distal. These studies have provided a powerful tool for investigating mutationally altered variants of a bacteriophage with respect to the morphogenetic steps affected. In addition, these studies were valuable in that they provided in vitro systems for the study of morphogenetic mechanisms by biochemical methods.

This paper describes complementation in vitro among suppressor-sensitive (*sus*) mutants of bacteriophage φ80 and presents some genetic analysis of these mutants. In addition, cross complementation in vitro between head- and and tail-containing lysates of φ80 and lambda is also described here. The related phages φ80 and lambda differ in immunity specificity, host range, plaque morphology, serological properties, and density. They do, however, recombine in mixed infection, resulting in lambda-φ80 hybrid genomes (7, 11).

The results show that, as in the case of lambda, the φ80 *sus* mutants affecting phage morphogenesis can be classified into two complementing groups: head donors and tail donors. Deletion mapping and two-factor crosses indicate the same pattern of gene clustering and order as that found in lambda. As far as cross complementation between φ80 and lambda is concerned, it is found that whereas the tails of φ80 combine quite efficiently with the heads of lambda to form viable phage, the combination of φ80 heads with lambda tails is very inefficient.

MATERIALS AND METHODS

Media. Penassay Broth (Difco) supplemented with 0.5% NaCl was used for growth of bacterial cultures, unless otherwise indicated. The agar plates used for phage assay consisted of Nutrient Agar (Difco) for the bottom layer and Nutrient Broth (Difco) containing 0.2% agar for the overlay. The K medium-used for preparation of defective lysates was described elsewhere (13).

Bacterial strains. All bacterial strains used are derivatives of *Escherichia coli* K-12. Strain 594, a nonpermissive (*pm*$^-$) host for both φ80 and lambda *sus* mutants, was obtained from J. Weigle. It is a streptomycin-resistant variant of strain W3350. Strain W1485, a permissive host, was kindly provided by N. Franklin.

Phage strains. Bacteriophage φ80 has been described previously (10). The host range variant φ80h is a spontaneous mutant selected for its ability to lyse strain 594 $T1^r$ try^{del} (14). The heat-inducible *sus* mutants of lambda, Allcltl and Lcltl, which are defective in head and in tail formation, respectively, were a gift of J. Weigle (*see* 3, 9). Mutant φ80 *sus*-1

was isolated by irradiation of a suspension of ϕ80 (5×10^{10} particles per ml) with ultraviolet light from a 15-w G.E. germicidal lamp at a distance of 40 cm for a period of 30 sec. The survivals (6%) were plated on W1485, and single plaques were tested for lysing lawns of 594 and W1485. All other ϕ80 *sus* mutants were isolated by the following procedure. W1485 (ϕ80) or W1485 (ϕ80-h) was grown in Penassay Broth to a concentration of about 2×10^8 to 3×10^8/ml. The cells were then treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (150 μg/ml) for a period of 45 min at 37 C. The cells were exposed to an inducing dose of ultraviolet light and aerated at 37 C for 180 min. The phage produced were plated on W1485, and single plaques were tested for lysis of 594 and W1485.

The ϕ80 deletion mutants used for mapping the ϕ80 prophage were isolated as lysogens of W3350. The procedure (7) involved selection for mutants of W3350 (ϕ80) that were resistant to bacteriophage T1 and that have simultaneously become tryptophan dependent. These mutants are a result of deletions of various lengths of the T1^r-tryptophan segment of the bacterial chromosome (14). These deletions often extend into prophage ϕ80, which is located close to the T1^r gene. All the deletion lysogens used in this study are immune to ϕ80 and give no viable phage upon ultraviolet induction.

Preparation of defective lysates and in vitro complementation. The procedure used for the preparation of defective lysates of the various ϕ80 *sus* mutants and for in vitro complementation tests were the same as those described for bacteriophage lambda (13), except that in in vitro complementation tests equal volumes of the undiluted defective lysates were incubated at 37 C. The incubation period was 3 hr, after which the reaction was generally complete.

Deletion mapping of ϕ80 sus mutants. A deletion lysogen together with a ϕ80 *sus* mutant were spotted on a lawn of 594 in top agar, and the whole plate was exposed to 12.5% inducing dose of ultraviolet light. The ϕ80 *sus* mutant and the deletion lysogen were spotted separately as controls. The plates were incubated at 37 C for approximately 12 hr and were scored for lysis of the lawn. In certain cases where spot tests gave ambiguous results, mapping was done by assaying the number of *sus*$^+$ recombinants in lysates of induced and superinfected deletion lysogens. Deletion lysogens were grown in Penassay Broth to a concentration of about 2×10^8/ml, induced with ultraviolet light, and superinfected with a ϕ80 *sus* mutant at a multiplicity of one. The cells were aerated at 37 C for 120 min and treated with chloroform. The lysates were assayed for *sus*$^+$ recombinants on lawns of strain 594.

In vivo complementation tests. The two factor crosses employed to classify ϕ80 *sus* mutants into various cistrons were done by spot testing (1, 2).

RESULTS

In vitro complementation among ϕ80 sus mutants. A few defective lysates of 594 (ϕ80 *sus*) were tested initially in all possible combinations for in vitro complementation. Certain combinations resulted in at least a 100-fold increase in active phage titer, whereas others gave negligible stimulation. The complementation pattern was consistent with the existence of only two complementing groups. Head- and tail-donor mutants were recognized by analyzing the genotypes of the reconstituted phages. A head donor, ϕ80 *sus*-109, and a tail donor, ϕ80 *sus*-9, were selected for the classification of all other *sus* mutants into head and tail donors. As in the case of bacteriophage lambda, the ϕ80 *sus* mutants fell into two classes: head donors and tail donors (Table 1). An exception to this rule, however, was encountered in the case of ϕ80 *sus*-31 which could complement both head-and tail-donor mutants, although much more efficiently in case of the latter. A more detailed

TABLE 1. *In vitro complementation among ϕ80 sus mutants and between ϕ80 and lambda head and tail donors*[a]

ϕ80 *sus* Mutant	Plus ϕ80 tails/ control	Plus ϕ80 heads/ control	Plus λ tails/ control	Plus λ heads/ control
1	1.2	485	1.6	280
2	1.1	588	1.2	650
3	937	1.7	0.7	0.9
5	29	1.9	1.8	2.3
7	1.2	13		
8	0.9	2.7×10^4	6.0	2.0×10^4
9	1.0	138	1.3	5.0×10^5
12	1.2	600		
21	97	1.4		
22	1,014	0.6		
23	240	0.7		
24	83	1.1	6.8	1.2
27	49	1.2	3.8	1.6
28	167	0.7	3.7	0.6
29	83	0.9		
30	1.0	149		
31	347	19		
32	1,733	1.0		
33	39	1.6		
35	1,200	0.9		
100	3.7	133	1.4	4.2×10^4
101	18	2.3	1.0	1.8
102	369	0.7	5.1	1.6
103	1.2	533	1.0	2.3×10^5
104	185	1.3		
105	136	1.0	1.4	1.4
106	1.1	364		
107	93	1.0		
108	342	1.8	4.7	2.7
109	137	1.0	2.0	5.2
sus A 11			1.0	3.9×10^7

[a] Values represent ratios of infective particles in mixtures of two lysates to those in uncombined lysates. Defective lysates of each of the ϕ80 *sus* mutants were combined with a lysate of ϕ80 *sus*-9 (a tail donor) in one reaction mixture and with a lysate of ϕ80 *sus*-109 (a head donor) in another. Several of the ϕ80 *sus* mutant lysates were also combined with lysates of lambda *sus*-A11 (a tail donor) and lambda *sus*-L (a head donor). Conditions of the reaction are given in Materials and Methods.

description of φ80 *sus*-31 will be given in a later publication.

In vitro complementation between φ80 and lambda sus mutants. Defective lysates of the various φ80 *sus* mutants were tested for in vitro complementation with lysates of lambda *sus*-A11c1t1, a tail donor, and lambda *sus*-Lc1t1, a head donor. In all cases tested, φ80 tails could combine with lambda heads to form viable phage, whereas the combination of φ80 heads and lambda tails resulted in little increase in titer (Table 1). The joining of φ80 tails to lambda heads was as efficient as the combination of the heads and tails of φ80.

The inability of lambda tails to form viable phage with φ80 heads could be because one of these components in the reaction mixture is not free to bind the other, or because the components are free but have little affinity for each other. The following experiment was done to differentiate between the two possibilities. A defective lysate of φ80 (*sus*-109), containing heads, was first mixed with a lysate of 594 (lambda *sus*-A11), containing tails. The mixture was incubated at 37 C for 3 hr and then assayed for viable phage. Samples of the incubation mixture were subsequently incubated for 3 hr at 37 C with lysates of each of 594 (φ80 *sus*-8), a tail donor, and 594 (lambda *sus*-L), a head donor. The results (Table 2) showed that in the incubation mixture of φ80 heads and lambda tails, both components are in the free form since the former can still bind to φ80 tails and the latter to lambda heads, forming infective phage. This would suggest that φ80 heads have low affinity for lambda tails under the conditions of the experiment.

Unlike bacteriophage lambda, φ80 loses its ability to form plaques on a lawn of sensitive bacteria when assay plates are incubated at 43 C. Ability to adsorb to the host, however, is not lost at that temperature. The availability of φ80 tail-lambda head hybrids provided a means of investigating the temperature-sensitive component of φ80. This hybrid was shown to be temperature-sensitive as evidenced by its inability to plate on strain W1485 pm^+ at 43 C. The sensitivity of φ80, therefore, seems to be in the tail component that is not involved in adsorbtion to the host.

In vivo complementation among φ80 sus mutants. Classification of φ80 *sus* mutants into head donors and tail donors was followed by in vivo complementation tests aimed at grouping the mutants into various cistrons. This was done by spot testing all possible combinations of tail-donor pairs on a lawn of the pm^- host 594. The plates were scored for lysis of the host after about 12 hr of incubation at 37 C. The results shown in Table 3 allow the assignment of the 10 tail-donor mutants to 6 cistrons on the phage genome. These two-factor crosses were done at least twice in all cases. Similar tests were performed with the 20 head-donor mutants (Table 3). Here again, the mutants involved in tail formation could be classified into six cistrons.

TABLE 2. *Availability of φ80 heads to bind lambda tails (and vice versa) in in vitro complementation reaction mixtures*

Combination of *sus* mutant lysates	Determination[a]
Lambda *sus* L (heads)	26
Lambda *sus*-A11 (tails)	18
φ80 *sus*-8 (tails)	4.7×10^5
φ80 *sus*-109 (heads)	8.2×10^5
φ80 *sus*-109 + φ80 *sus*-8	2.8×10^8
Lambda *sus*-A11 + lambda *sus*-L	7.9×10^8
Lambda *sus*-A11 + φ80 *sus*-109	2.0×10^5
Lambda *sus*-A11 + φ80 *sus*-109 + lambda *sus*-L[b]	1.7×10^8
Lambda sus-A11 + φ80 sus-109 + φ80 sus-8[b]	2.9×10^8

[a] Expressed as plaque-forming units per milliliter of reaction mixture.

[b] The first two lysates were combined in equal volumes (0.2 ml each) and incubated at 37 C for 3 hr. The third lysate (0.2 ml) was then added, and the incubation was continued for an additional 3 hr before assaying for infective particles.

TABLE 3. *Assignment of the φ80 sus mutants to various cistrons by in vitro complementation*[a]

Cistron	*sus* Mutants
Involved in head formation	
1	100
2	30, 103, 106
3	9
4	1, 2
5	8
6	12
Involved in tail formation	
7	27, 29
8	3, 28, 101, 102, 105, 107
9	21, 27
10	5, 7, 35, 109
11	23, 31, 32, 104
12	22, 33, 108

[a] One drop of a mixture of all possible combinations of two *sus* mutant lysates (containing 2×10^8 to 3×10^8 infective particles of each mutant per ml) was spotted on a lawn of the pm^- host 594. The plates were incubated for 12 to 13 hr at 37 C and the scored for lysis of the lawn. The mutants were spotted separately as controls.

Ordering of ϕ80 sus mutants on prophages ϕ80 and $i^{\phi 80}$ h^{λ}. The ordering of the various head- and tail-donor ϕ80 *sus* mutants on prophage ϕ80 was done by using the deletion mapping technique described in Methods and Materials. The presence of sus^{+} alleles in the various deletion lysogens was detected by the spot-test procedure (3). Unambiguous results were obtained in the majority of these tests. Ambiguous cases were further examined by superinfecting an ultraviolet-induced culture of W3350 (ϕ80-del) $T1^{r}$ with the *sus* mutant in question (multiplicity of infection 1). The lysates were assayed for the presence of sus^{+} phage by plating on 594. The results allowed the arrangement of the various cistrons involved in head and tail formation in a linear order on the prophage genome (Fig. 1). Clustering of the genes responsible for head formation and of those for tail formation is evident. The sequence of genes in the prophage relative to bacterial genes would then be as follows. The bacterial gene UDPG (uridine diphosphoglucose synthesis) is followed by the segment of the prophage genome specifying early phage functions, the head gene cluster, the tail gene cluster, and, finally, the T1 receptor gene and the tryptophan operon.

In view of the previously reported relatedness of bacteriophages ϕ80 and lambda (7, 11), attempts were made to investigate similarities in the relative arrangement of genes involved in head and tail formation. This is made possible by the fact that the various ϕ80 functions involved in head and tail formation are rescuable by bacteriophage lambda in mixed infections. The arrangement of ϕ80 *sus* mutants on the lambda prophage was investigated by using deletion lysogens of the hybrid phage $i^{\phi 80}h^{\lambda}$. These deletion lysogens were isolated and characterized by Franklin et al. (7). The deletion mapping procedure employed with the ϕ80 deletion lysogens was also used here. The results (Fig. 1) show that the order of late function genes on prophage lambda is grossly similar to that on prophage ϕ80. The relative sequence of cistrons 1, 2, 3, and 4 of head formation and of 10 and 11 of tail formation could not be determined with the available phage deletions; therefore, differences in the sequence of these cistrons between the two phages cannot be excluded.

DISCUSSION

Thirty ϕ80 *sus* mutants have been isolated and utilized to study the various late-function cistrons

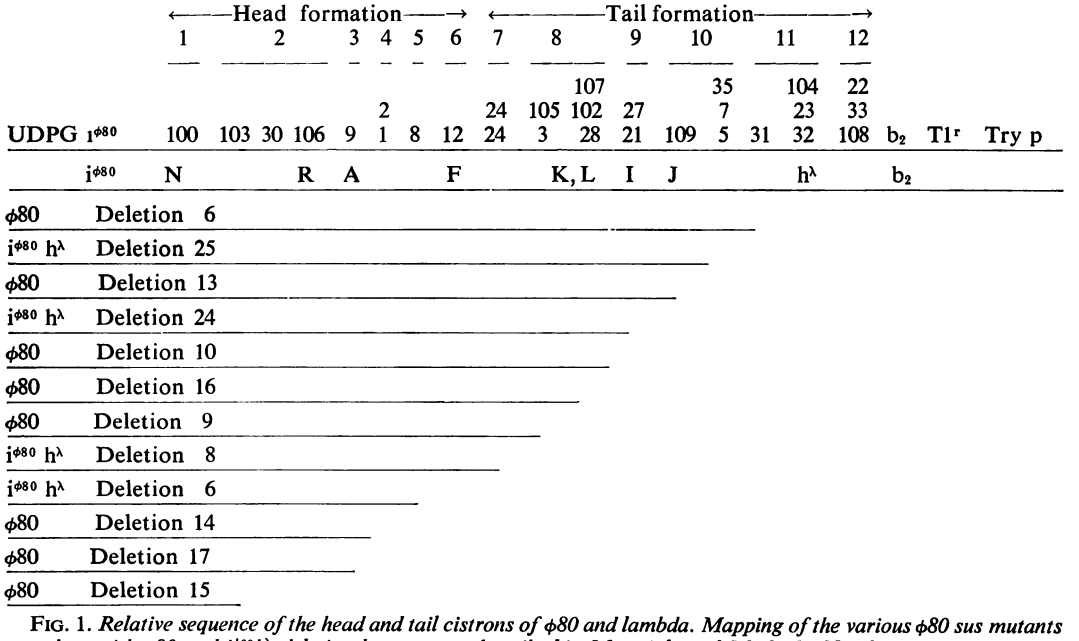

FIG. 1. *Relative sequence of the head and tail cistrons of ϕ80 and lambda. Mapping of the various ϕ80 sus mutants was done with ϕ80 and $i^{\phi 80}h^{\lambda}$ deletion lysogens as described in Materials and Methods. Numbers at top represent the various ϕ80 cistrons followed by the ϕ80 prophage and the mutants assigned to these cistrons. Below the ϕ80 prophage map is the $i^{\phi 80}h^{\lambda}$ prophage map showing the distribution of the lambda head and tail cistrons. Lines below the prophage chromosomes represent prophage segments present in defective lysogens used in mapping the ϕ80 sus mutants.*

involved in phage morphogenesis. All of the mutants isolated were found to have retained the ability to cause lysis of the *pm*$^-$ host, with with very little viable phage production. This type of mutant is analogous to that of lambda mutants defective in late functions. Mutations involving early functions usually result in loss of ability to cause lysis (5, 8). As shown in this study, all of the ϕ80 mutants isolated were of the type affecting late functions, although no selection for this type was exercised in their isolation. Late-function cistrons of lambda have been shown to specify the synthesis of the structural coat proteins and of the proteins necessary for their assembly (13). All of the ϕ80 *sus* mutants isolated could be classified by means of in vitro complementation tests into head donors and tail donors. There are at least 12 late-function cistrons in ϕ80 divided equally between head and tail formation. Cistrons 1 to 6, specifying head formation, form a cluster that is distal to the cluster of cistrons 7 to 12, specifying tail formation, on the late function (left) arm of vegetative ϕ80. This arrangement is the same as that of the head and tail clusters of lambda (13), in which six cistrons have been ascribed to head formation and seven to tail formation. Furthermore, deletion mapping of the ϕ80 *sus* mutants using both ϕ80 and $i^{\phi 80}h^{\lambda}$ deletion lysogens revealed a closer relationship between the two phages insofar as the sequence of individual cistrons is concerned. Although the available deletions did not allow exhaustive comparison, the gross arrangement of the cistrons in the two phages seems to be the same. The uncertainty lies in the relative sequence of cistrons 1, 2, 3, and 4 of head formation and 11 and 12 of tail formation. The arrangement of the immunity gene and of the "b_2" region relative to the head and tail clusters has also been suggested to be similar in the two phages (7, 12).

In addition to gene-sequence similarities, the two phages were also shown to exhibit some structural relationship. The tails of ϕ80 could combine efficiently with lambda heads to form infective particles. The combination of ϕ80 heads and lambda tails occurred with a much lower efficiency, however, suggesting that there is a difference in the structures directly involved in coupling heads to tail. Another difference seems to be in a tail component, which is heat sensitive in ϕ80 but not in lambda.

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