Assembly and Function of the Lambdoid Bacteriophage Tail: Observations from $\phi 80$ -Lambda Host-Range Phenotypic Mixing

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The host-range proteins of lambda and $\phi 80$, while differing sharply in host-receptor specificity and in sensitivity to phage-killing antiserum, can be assembled into functional phage tail structures of mixed composition.

Structural elements of the bacteriophages lambda and $\phi 80$ can interact at several levels of morphogenetic organization to form phage particles of mixed composition. Genomes of $\phi 80$ have been found encapsulated in the protein package of lambda (5), and lambda *dgal* genomes, deleted of head and tail genes, can be packaged by a co-infecting $\phi 80$ (6). Heads from tail mutants of lambda can become infectious particles by joining to tails from $\phi 80$, although heads from $\phi 80$ are not measurably activated by lambda tails (3, 5; S. Casjens, Fed. Proc., p. 444, 1972). I have observed that $\phi 80$ can, under proper mixed-infection conditions, complement mutants in the A, W, B, C, D, E, and F_{II} head genes of lambda (unpublished results). This indicates that functional phage heads can be assembled from mixed $\phi 80$ and lambda proteins.

In this series of complementation experiments, I also observed that the lambda-genotype progeny of $\phi 80$ -lambda mixed infections were phenotypically mixed with respect to host range. They would not plate efficiently on *Escherichia coli* strain C600, which is $\phi 80$ resistant. They would plate on strain W 1485, which is sensitive to both phages, and would plate on C600 after preadsorption to W 1485.

In addition to the strongly specific head-tail mixing between lambda and $\phi 80$, there is the possibility that tails also might be assembled from mixed proteins. Appleyard et al. (1) reported phenotypic mixing between host-range variants of lambda. We will be concerned here with an experiment designed to test for the mixing of $\phi 80$ and lambda host-range proteins within the host-range-determining substructure of the phage tail. This is the part of the phage

particle responsible for its binding to the host cell and its sensitivity to phage-killing antisera, and it is at least principally composed of the product of the J gene in lambda. Of all lambda mutants tested, only J mutants are defective in the production of serum blocking antigen (2).

RESULTS AND DISCUSSION

A rabbit antiserum prepared against highly purified lambda phage particles was determined to be quite specific for lambda over $\phi 80$ as measured by phage inactivation. This is shown by lines 1 and 2 of Fig. 1; line 1 represents the sensitivity of phage with lambda host range, line 2 that of $\phi 80$. These curves are typical of all such comparisons with this serum. The differential sensitivity is not absolute; some cross-reaction is a consistent feature. The cross-reaction is, however, sufficiently small that sensitivity to this serum can clearly be used as an indicator of the presence of lambda host-range protein.

A mixed infection was performed with wild-type $\phi 80$ and lambda *att⁸⁰imm⁸⁰* (4), in parallel with single infections by each phage. On fresh tryptone plates with mixed lawns of C600 and W 1485, these two phages are easily distinguished by plaque morphology. The $\phi 80$ plaques are large (2-4 mm diameter), with ragged edges, and turbid, owing to failure to infect C600. The lambda *imm⁸⁰* plaques are small (1-1.5 mm) and neat and lyse through both hosts. The plaque types are sufficiently distinct that artificial control mixtures of the two phages could be differentially titered with an accuracy consistently better than $\pm 10\%$ probable error. The mixed lawn has the additional advantage of causing no selection at the level of host range. Single-infection lambda *imm⁸⁰* progeny plate with identical efficiencies on C600, W 1485, and

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FIG. 1. Antilambda serum sensitivities of phenotypically mixed phage particles. Three identical cultures of W 1485 were infected with $\phi 80$, lambda att⁸⁰imm⁸⁰, and both, at a multiplicity of five of each phage per cell. The progeny were assayed for sensitivity to a high-titer antiserum prepared against highly purified lambda phage particles. Phage were incubated at 37 C with a 1:2,000 dilution of serum. Samples were taken at the indicated times by 100-fold dilution and plated on a mixed lawn of C600 and W 1485 (lines 1 through 4) or on C600 alone (line 5). Line 1 is lambda imm⁸⁰ single-infection progeny; line 2, $\phi 80$ single-infection progeny; line 3, total mixed-infection lambda-genotype progeny; line 4, total $\phi 80$ -genotype progeny from the mixed infection; line 5, the lambda-phenotype fraction of the mixed-infection lambda-genotype progeny.

the mixed lawn. Single-infection $\phi 80$ progeny plate with the same efficiency on W 1485 and the mixed lawn. Survival kinetics of the single-infection progeny are likewise independent of the indicator.

When the progeny of a mixed infection are assayed for their sensitivity to the lambda-specific antiserum, it is clear that the total lambda-genotype progeny (line 3, Fig. 1) have a reduced sensitivity to the serum, and the $\phi 80$ -genotype progeny have acquired greater than normal sensitivity (line 4, Fig. 1).

The isolated behavior of the normal-host-range fraction of the lambda progeny is repre-

sented by line 5 of Fig. 1. These are the progeny which produced plaques directly on lawns of C600. Again, this requires both the phenotypic function and the genotype of lambda host range. This population is clearly more sensitive than the mixed-infection lambda progeny as a whole, and less sensitive than the single-infection progeny.

All points in Fig. 1 represent an average of at least four titers. The average standard error of the mean is $\pm 9.4\%$. The slopes of curves 1, 2, 4, and 5 were fitted by least squares, with standard errors of estimated slope equalling 3.43, 1.65, 0.18, and 0.15%, respectively. No straight line will fit within the probable error envelopes of the points of curve 3. This is consistent with the heterogeneity in antiserum sensitivity of this population that is demonstrated by the distinctly different sensitivity of the normal-phenotype subpopulation represented by curve 5.

These data directly demonstrate that host-range phenotypic mixing between the two phages is occurring in both directions. They also strongly suggest, in the case of the $\phi 80$ -genotype progeny, that the phenotypic mixing is occurring by way of mixing host-range protein types within the tail structure. Over 30% of the viable $\phi 80$ -genotype mixed-infection progeny have become sensitive to the lambda antiserum, which stands in sharp contrast to the absence of detectable activation of $\phi 80$ heads by whole lambda tails in the results of Inokuchi and Ozeki (5) and Deeb (3). The possibility remains that their in vitro assays represent a situation differing from that in mixedly infected cells; for example, the head protein(s) responsible for head-tail joining specificity (S. Casjens, Fed. Proc., p. 444, 1972) might also be capable of mixing. The lambda-genotype population as a whole behaves as though the association of genotype with host-range phenotype were approximately random. On the other hand, association of $\phi 80$ genomes with lambda host range seems relatively restricted. This would require that at least partial head-tail joining specificity be retained, and that there be, in addition, some restriction against the assembly of tail structures combining lambda host range with $\phi 80$ head-joining specificity. The intermediate sensitivity of the normal-host-range subpopulation of the lambda-genotype progeny, represented by curve 5, would be expected if host-range phenotypic mixing occurred within the host-range substructure, but not if mixing occurred exclusively between heads and whole tails. Among the lambda-genotype progeny, host-range phenotypic mixing cannot be occurring exclusively at the level of head-tail joining. Nor

can the host-range-determining substructure of the phage particle be composed of a single molecule of host-range protein. If either of these two situations obtained, the normal-phenotype fraction of the population would be expected to have an exactly normal, and not an intermediate, sensitivity. These data tell us that $\phi 80$ and lambda host-range proteins can be combined in a mixed tail structure, and that functional binding of the phage tail to the bacterial receptor can be achieved with less than a full set of normal host-range protein molecules.

While it remains possible to imagine that the reduced sensitivity of the lambda-host-range lambda-genotype progeny might result from conformational change in the J protein caused by mixing among tail shaft subunits bound to the J protein, it seems very difficult to explain by such a mechanism the reciprocal appearance of intermediate sensitivity among the $\phi 80$ -genotype progeny.

An additional observation which may be drawn from these data is that the host-range proteins of lambdoid phages must have at least two molecular functions: one through which they associate with the other proteins of the tail structure, and one responsible for serum sensitivity and the recognition of host receptor sites. The cell-binding function, further, must be capable of mutation and selection independent

from the function which integrates it with the rest of the tail structure. This suggests that these different regions of the final three-dimensional structure of the protein would not be in intimate structural contact.

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