*COHESIVE SITES AND HELPER PHAGE FUNCTION OF P2, LAMBDA, AND 186 DNA'S**

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Cohesive sites have now been found on the DNA's of a large number of temperate coliphages.[1–4] In the case of coliphage λ, it has been shown that the cohesive sites are at the ends of the DNA molecule[5–7] and that the sites at the two ends are complementary. The DNA's from phages φ80, 21, 424, and 434 have cohesive sites like those of λ, as shown by their capacity to form mixed dimers with λ, while phage 186 DNA has cohesive sites that are different than λ, although Wu and Kaiser[9] have identified the 5′-terminal nucleotides of the DNA of phages λ, φ80, 21, and 186 as being identical. Previous work by one of the authors (M. M.) has indicated that phage P2 DNA has cohesive ends[4] and that there is a strong tendency to concatenize.

The work reported here shows that P2 DNA has cohesive ends like those of 186 DNA and, as reported by Baldwin[3] *et al.* for 186 DNA, it does not cohere to λ DNA.

There seems to be correlation between the nature of the cohesive end and the capacity to serve as a helper phage for DNA infectivity.[4, 10]

Materials and Methods.—(a) *Bacterial strains: Escherichia coli* K12 strain C 600[11] and strain W 3350 lysogenic for 186p; *E. coli* C 1-a[12] and its derivatives lysogenic for P2 Hy dis (collection number 119) and P2; and C-1055, an auxotroph on which P2 plates very well.

(b) *Bacteriophages:* Phage P2;[13] phage P2 Hy dis vir[14], a heteroimmune phage largely isogenic with P2;[14] and phage 186p,[3] a temperature-inducible mutant.

P2 phage preparations were made by infection of C-1a in LB broth and then concentrated and purified by differential centrifugation. The N^{15}-labeled P2 was made in a similar manner using Davis medium with $(N^{15}H_4)_2SO_4$. Phage 186p was made according to the procedure of Wu and Kaiser[9] and then concentrated and purified by differential centrifugation.

For DNA infectivity assays, phage DNA was extracted according to the procedure of Kaiser and Hogness,[10] as modified by Wu and Kaiser.[9]

(c) *Media:* LB broth and LB agar;[13] Davis medium;[12] Tryptone broth, tryptone agar, P medium;[10] and supplemented P medium.[15] TCM medium: 0.01 *M* Tris, 0.01 *M* $CaCl_2$, 0.01 *M* $MgSO_4$, pH 7.1. Ca-saline: 1% NaCl, 5×10^{-3} *M* $CaCl_2$, 10^{-4} *M* $MgSO_4$.

(d) *DNA infectivity assays:* The 186 DNA, P2 Hy dis vir[14] helper assay procedure was similar to the P2 DNA assay described previously,[4] with the following modifications: Strain C 119 (P2 Hy dis), the recipient of the infection, was grown in P medium instead of in Davis medium. Since phage 186 will not plate on *E. coli* C, we used *E. coli* K12 C 600 as a plating indicator where it is quite easy to distinguish 186 plaques and P2 Hy dis vir[14] plaques.

The P2 DNA, 186 helper phage assay was similar to the λ DNA assay described previously.[4] *E. coli* K12, a derivative of strain S or K12s of Weigle and Delbruck[16] (collection number K49) was used as recipient of infectious DNA and *E. coli* C-1055 was used as plating indicator. Phage 186 does not plate on *E. coli* C-1055.

(e) *Analysis for mixed dimers and trimers:* Similar to procedure described by Baldwin

et al.[3] Solutions of light and heavy phage at a concentration to give a final DNA concentration of several μg/ml were mixed together in a small tube, then an equal volume of 1.2 *M* NaCl, 0.01 potassium phosphate buffer (pH 7) was added. The phages were lysed by adding $^1/_{10}$ vol of a 10% solution of sodium lauryl sulfate, and the mixture was incubated 30 min at 45°C. A volume of 0.1 ml of DNA mixture was added to 0.6 ml of CsCl buffered with 0.01 *M*-Tris chloride (pH 7.0). We used a Kel-F centerpiece (12 mm, 4°) whose filling hole had been drilled out to allow filling with a Pasteur pipette. We also used a synthetic boundary cell in such a way that we could use a wide-mouth pipette to fill the cell.

Results.—Cohesive sites on P2, 186, and λ: Using heavy labeled (N^{15}) P2 phage and light 186, we have found that their cohesive sites are sufficiently alike to allow formation of mixed dimers and trimers as shown in Figure 1. If the 186 and P2 DNA molecules have identical cohesive ends, we would expect to find a distribution of dimers such that for each HH and LL dimer formed, two LH molecules would be formed. Of course, the experimental distribution pattern is complicated by the unequal concentration of input DNA monomers and by the formation of trimers. The fact that both the dimer band LH and trimer band LLH are more intense than the H (HH, HHH) and L (LL, LLL) bands is indicative that the 186 and P2 cohesive ends are nearly identical. In similar experiments with heavy P2 and light λ, we have found only two bands corresponding to the two buoyant densities of the input phage DNA. In an experiment with heavy P2 and light P2, we see multiple bands indicating the formation of dimers and trimers and corroborating previous results obtained on P2 DNA by means of sucrose gradient centrifugation[4] and electron microscopy.[17] The proportion of oligomers to monomers is very dependent on conditions and duration of storage. In the work on P2 DNA in a sucrose gradient using DNA infectivity to assay the fractions, it was shown that practically all the monomers had been converted to higher-molecular-weight forms. We have been able to increase the intensity of the dimer and trimer bands at the expense of the monomer bands by storage in high salt concentrations (7 *M* CsCl) for several days. As a control we ran each of the DNA's separately (normal density) under the same conditions and found only a single band.

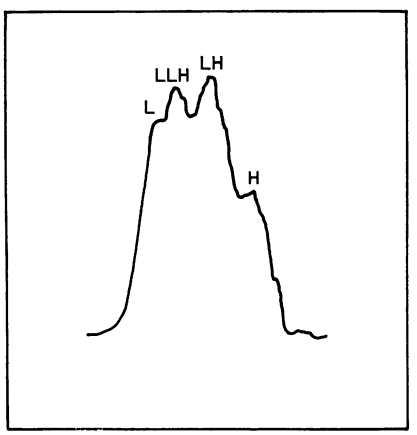

FIG. 1.—Mixed DNA dimers and trimers from phage 186 (*L*) and N^{15}-labeled P2 (*H*).

The dimer band is closer to the heavy than to the light DNA band, as the heavy monomer is larger in size than the light monomer. By accident the amount of light DNA was in excess to the amount of heavy DNA.

(CsCl, mean density approximately 1.7, 44,770 rpm, 25°C.)

Helper phage function: Infection by temperate phage P2 DNA occurs only if the bacteria exposed to P2 DNA are simultaneously infected with a related helper phage. We have found that 186 phage can serve as a helper phage for P2 DNA and that P2 Hy dis vir[14] (usual helper phage for P2 DNA) can help 186 DNA cause infection—with about equal efficiency. Under the conditions used in this experiment, we have obtained an activity of approximately 150 infectious units per microgram of DNA for: (*a*) P2 Hy dis vir[14] phage helping P2 DNA, (*b*) P2 Hy dis vir[14] phage helping 186 DNA, and (*c*) 186 phage helping P2 DNA. The P2 phage can also help 186 DNA cause infection, but with slightly lower efficiency. (See *Note added in proof.*) It has been shown that phage λ cannot help P2 DNA to infect.[4] This was done by using *E. coli* C lysogenic for λ as the recipient of infection. Lambda as a prophage does not exclude P2. Because P2 as prophage excludes λ, we did the reverse experiment in a nonlysogenic recipient (*E. coli* K12 strain C-600) and used the same bacteria as plating indicator where it is easy to distinguish P2 Hy dis vir[14] plaques from λ plaques. The result of this experiment was negative.

Discussion.—We have found that the cohesive sites of 186 and P2 DNA are identical or nearly so and that the specificity of the cohesive sites is different from that of the λ-related phages (λ, 424, 434, φ80, and 21). There seems to be a correlation between specificity of cohesive site and the capacity to serve as helper phage for DNA infectivity. Thus P2-type phage can help 186 DNA to infect and 186 phage can help P2 DNA to infect, but λ phage cannot help either P2 or 186 DNA to infect and P2 or 186 phage cannot help λ DNA. On the other hand, Barrand and Kaiser (private communication) have shown that the λ-type phages can serve as helper for each other's DNA. Now the phages which lysogenize *E. coli* have been classified into two groups:[18, 19] those which are inducible by ultraviolet light and those which are noninducible by ultraviolet light. If we take λ and P2 as respective models of these two classes, we find that they contain two other exclusive properties: (1) recombination—recombination frequency for λ is high; recombination in P2 is low,[20, 21] about 10^{-3} that of λ; and (2) cohesive sites—specificity of sites is different for inducible phages and noninducible phages. With this specificity difference, there seems to be associated a difference in melting temperature of the cohesive ends. Wang[22] has reported a T_m increase of 12°C for the cohesive ends of 186 DNA as compared to the cohesive ends of λ.

Since those phages which recombine genetically with λ have DNA's which will cohere to λ DNA, it has been suggested by Baldwin *et al.*[3] that the cohesive ends may have a role in the recombination of λ. That is, the first step in recombination may be the formation of mixed dimers or double circles. However, the fact that P2 DNA has very cohesive sites and extremely low recombination frequency compared to λ suggests the possibility that the P2-recombinase system is so inefficient that there has been a selection for cohesive ends. This would ensure that the two P2 DNA's remain in close proximity long enough for the inefficient recombinase system to work. Another possibility is that the sites are so cohesive that inside the cell each injected DNA molecule immediately closes and forms a ring by itself, thus preventing recombination.

Note added in proof: Using Davis media to grow the recipient bacteria for the infectious DNA and a mixture consisting of 0.125 *M* $CaCl_2$, 0.2 *M* NaCl, 0.005 *M* sodium citrate, pH 7, and 0.1% glucose to resuspend the helper phage-infected bacteria, we have achieved activities of approximately 10^5 infectious units per microgram of both P2 DNA and 186 DNA.

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