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Homology Between the Deoxyribonucleic Acid of Fertility Factor P and *Vibrio cholerae* Chromosomal Deoxyribonucleic Acid

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The deoxyribonucleic acid (DNA) of the *Vibrio cholerae* fertility factor P was isolated by the dye-buoyant density method and hybridized to *V. cholerae* chromosomal DNA. The DNA of this fertility plasmid had between 35 to 40% homology with the *V. cholerae* chromosomal DNA. Little or no homology was detected between the P factor DNA and the DNA of the *Escherichia coli* sex factor F.

In a previous publication, Datta et al. (4) described the isolation and characterization of deoxyribonucleic acid (DNA) of the fertility factor P, from *Vibrio cholerae* strains. This DNA exists as supercoiled, circular molecules which are 41 nm in length and have a molecular weight of 80×10^6. The average base composition of this P factor DNA was determined by buoyant density centrifugation to be 42% guanine plus cytosine (G+C) as compared to 48% G+C for the *V. cholerae* chromosomal DNA. The average G+C composition values, however, are not very informative in terms of the genetic relatedness of plasmid DNA and the DNA of its host. The *Escherichia coli* fertility factor F, for example, which happens to have the same average G+C composition as its host (50% G+C), is actually a composite element comprising a region of 44% G+C and a region of 50% G+C (6, 9). This F element has a 40% homology with the *E. coli* chromosomal DNA in hybridization studies (7). In similar studies reported here we have found a similar amount of homology between the DNA of the P factor DNA and the DNA of the *V. cholerae* chromosome.

MATERIALS AND METHODS

Isolation of DNA. The ^{3}H-labeled, supercoiled, circular P factor DNA was isolated from *V. cholerae* strain V58 P$^+$ by the dye-buoyant density procedures of Bazaral and Helinski (1), as modified by Datta et al. (4). After fractionation of the CsCl gradients, the samples containing the supercoiled, circular P factor DNA were pooled and dialyzed with 0.14 M phosphate buffer, pH 6.8. To reduce the size of the molecules, the P factor DNA was then sonically treated with a Bronson sonifier at a setting of 4 for 5 min while the sample was cooled in an ice bath. This sonic treatment produced molecules with an average double-stranded molecular weight of 200,000 as determined by sedimentation velocity experiments.

Large quantities of unlabeled *V. cholerae* DNA were isolated from strains V58 P$^+$ and V58 P$^-$ by the procedures described by Brenner et al. (3). The only known difference between these two strains is that V58 P$^+$ contains the P factor. The same procedures were used to isolate DNA from *Serratia marcescens* and *E. coli*. These preparations were sonically treated to reduce the DNA size to a molecular weight of about 200,000. The DNA was denatured by heating to 100 C for 5 min and then quick cooled to prevent reannealing.

Binding of reannealed DNA to hydroxyapatite. Double-stranded DNA in 0.14 M phosphate buffer (pH 6.8) at 60 C, binds to hydroxyapatite, whereas single-stranded DNA is not bound (3). In these experiments, P factor DNA isolated by the dye-buoyant density method was tested for its ability to hybridize with single-stranded DNA isolated either from the V58 P$^+$ or V58 P$^-$ strains. The single-stranded DNA which has reannealed to form double-stranded DNA will then be bound to the hydroxyapatite. The hybridization mixture contained about 10,000 counts/min of ^{3}H-labeled P factor DNA in a concentration of less than 0.05 μg/ml. This labeled DNA was mixed with a large excess (about 2 mg/ml) of unlabeled DNA from either strain V58 P$^+$ or V58 P$^-$. The samples were denatured and incubated at 60 C for 2 h. Several different incubation times were tested and the time of incubation was chosen so that there would be maximal reassociation between the unlabeled chromosomal DNA and the labeled P factor DNA, whereas the reassociation of labeled P factor DNA fragments with one another would be minimal. After incubation, the reaction mixture was passed through a hydroxyapatite column maintained at 60 C, to which the reannealed DNA was bound. The single-stranded DNA was eluted with six 15-ml portions of 0.14 M phosphate buffer (pH 6.8) containing 0.4%

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sodium dodecyl sulfate. This was followed by thermal elution with the same buffer at increasing temperatures until 90 C was reached. At 90 C, the remaining DNA was washed from the column with 15 ml of 0.4 M phosphate buffer. Incubations were also done at 50 and 70 C to observe the effect of temperature on reannealing. In these cases, single-stranded DNA was eluted from hydroxyapatite with buffer held at the same temperature used in the incubation. Each of the eluted samples was treated with 10% trichloroacetic acid to precipitate the DNA. The precipitated DNA was collected on cellulose nitrate filters and then counted in a Packard liquid scintillation counter. When thermal elutions were performed, the T_m was designated as the temperature at which 50% of the bound DNA was eluted from the hydroxyapatite.

Competition-experiments. Single-stranded DNA binds to nitrocellulose membrane filters (10, 11). Such filters, after treatment with bovine serum albumin, will then only allow added single-stranded DNA which is homologous to bind to the DNA on the filters (5). Thus, it is possible to determine homology by reannealing experiments between single-stranded DNA bound to the nitrocellulose filters and single-stranded DNA in solution (5). When the single-stranded DNA in solution is labeled, the amount of reannealing after an incubation period can be measured by washing the filter and counting the labeled double-stranded DNA attached to it. In addition to its simplicity, this method makes it possible to test whether DNA isolated from different bacterial strains will interfere with reannealing of the labeled DNA to the DNA on the filter. In such competition experiments, the DNA from *V. cholerae* strain V58 P^+ was bound to nitrocellulose filters according to the procedure described by Denhardt (5). These filters were then incubated in a small volume (0.2 ml) of buffer (0.14 M phosphate, pH 6.8) with denatured, ^3H-labeled P DNA containing less than 0.01 μg of DNA per ml with about 3,000 counts/min. Reannealing was performed at 60 C for 12 h. Various amounts of DNA isolated from different bacterial strains were added to determine whether competition with the reannealing of the labeled P factor DNA to the DNA on the filter could occur. If the DNA added as a competitor is homologous to the labeled P factor DNA, it will reanneal with it, thus inhibiting the homologous reaction of the P factor DNA with the filter-bound DNA.

RESULTS AND DISCUSSION

Homology determined by binding of reannealed DNA to hydroxyapatite columns. Supercoiled, circular DNA associated with the fertility factor P was labeled with [^3H]thymidine and isolated from the *V. cholerae* chromosomal DNA as described previously (4). The P factor DNA was then incubated at various temperatures with DNA from different bacterial strains. The extent of reassociation was determined by adsorption of reannealed DNA to hydroxyapatite. When the labeled P factor DNA was incubated with an excess of DNA extracted from strain V58 P^+, reannealing occurred between the labeled P factor DNA and the P factor DNA present in the extracted DNA from the V58P^+ strain. When labeled P factor DNA was incubated with an excess of DNA extracted from the V58 P^- strain, significant hybridization still occurred due to reassociation of labeled P factor DNA to the *V. cholerae* chromosomal DNA.

The results of these reassociation experiments are shown in Table 1. The amount of the labeled P factor DNA bound to the hydroxyapatite column after incubation is reported as the percentage of DNA reannealed. The values in parentheses are the relative percentages of DNA reannealed based on 100% for reassociation of P factor DNA with DNA from strain V58 P^+. Thus, the amount of reassociation between the isolated P factor DNA and the *V. cholerae* chromosomal DNA is between 42 and 50%, depending on the temperature at which the hybridization reactions were performed. These values slightly overestimate the amount of homology between the P factor DNA and the *V. cholerae* chromosomal DNA because they have not been corrected for the binding observed in controls. Binding of P factor DNA to hydroxyapatite due to self-annealing was 6% or less under the conditions and various incubation temperatures used. Reannealing of the isolated P factor DNA with a nonrelated DNA such as *S. marcescens* DNA indicates that the amount of such reannealing is very small and cannot be distinguished from self annealing. Circular DNA molecules with a molecular weight of 40 million are present in a number of *V. cholerae* strains including both the V58 P^+ and the V58 P^- strains used in this study. The function of these smaller circular DNA molecules is at present unknown (4), but they are isolated with the P factor DNA and comprise about 10 to 20% of the DNA in the P factor preparations. This small amount of circular material which is not P factor DNA will probably anneal with similar DNA in the chromosomal preparations, causing an overestimate in the amount of homology between P factor DNA and the *V. cholerae* chromosomal DNA. When the appropriate corrections are made for the presence of this small circular DNA component and self-annealing, the value estimated for the homology between the P factor DNA and the *V. cholerae* chromosome is between 35 and 40%.

Thermal elution of the reannealed DNA showed that there was a difference in its stability depending on whether the labeled P factor DNA was reassociated with DNA from the V58

TABLE 1. *Extent of homology between isolated P factor DNA and V. cholerae chromosomal DNA*

Hybridization reaction[a]	Incubation temperature					
	50 C		60 C		70 C	
	DNA reannealed (%)	T_m (C)	DNA reannealed (%)	T_m (C)	DNA reannealed (%)	T_m (C)
P	6		5		4	
P/*S. marcescens*	5		6		4	
P/*V. cholerae* V58 P$^+$	76 (100)[b]	81	78 (100)	81	69 (100)	81
P/*V. cholerae* V58 P$^-$	32 (42)	78	38 (48)	76	35 (50)	77

[a] Isolated P factor DNA was labeled with tritium and used at an input of 2,000 counts/min per ml as described in Materials and Methods.

[b] Figures in parentheses are normalized to the binding observed in the P/V. *cholerae* V58 P$^+$ reaction, which was arbitrarily designated as 100%. These figures were not corrected for the background.

P$^+$ or the V58 P$^-$ *V. cholerae* strain (Table 1). In the hybridization reaction between P factor DNA and the DNA from strain V58 P$^-$, the labeled P factor DNA strands can only anneal with the *V. cholerae* chromosomal DNA. The T_m value for elution of this reassociated DNA was lower than the T_m for elution of the reannealed DNA formed in the P/*V. cholera* V58 P$^+$ reaction. The differences in the T_m values are between 3 and 5 C, indicating that the double-stranded molecules formed between the labeled P factor DNA and the *V. cholerae* chromosomal DNA do not have perfect base pair matching. The difference in these T_m values implies that duplexes formed between P factor DNA and the *V. cholerae* chromosomal DNA contain 3 to 5% unpaired bases (8).

Homology determined by competition experiments with nitrocellulose filters. Competition experiments were performed using the Denhardt procedure (5) and ^{3}H-labeled P factor DNA was hybridized to *V. cholera* DNA from strain V58$^+$ bound to nitrocellulose filters. Various DNAs were added to this reaction mixture to see whether they would compete and interfere with the binding of the labeled P factor DNA. If these DNAs are homologous to the DNA on the filter, then they will reanneal and prevent binding of the labeled P factor DNA.

The results of these competition studies are shown in Fig. 1. As expected, DNA from *V. cholerae* V58 P$^+$ is a good competitor and prevents binding of P factor DNA even when added in low concentrations. *S. marcescens* DNA, which has a G+C composition of 58%, was chosen as a strain which should have little or no homology with P factor DNA. It does not interfere with the binding of P factor DNA to DNA from *V. cholerae* V58 P$^+$, even at 2,000 μg/ml, the highest concentration of *Serratia* DNA used, a ratio of 40,000 to 1.

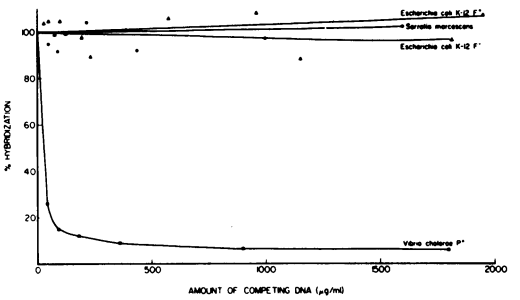

FIG. 1. *Competition experiments. Filters containing DNA from V. cholerae strain V58 P$^+$ were prepared as described in the Materials and Methods section and incubated at 60 C for 12 h with less than 0.01 μg of labeled P factor DNA per ml. Competition was assessed by adding various concentrations of DNA isolated from different bacterial strains to this reaction mixture.*

Little or no interference with binding was observed when *E. coli* F$^-$ or *E. coli* F$^+$ DNA was added. This indicates that little or no homology exists between the F factor of *E. coli* and the P factor of *V. cholerae*. Although not shown in Fig. 1, DNA from *V. cholerae* V58 P$^-$ also competed with the binding of P factor DNA to the filters. The resultant competition curve is similar in shape to that observed for the V58 P$^+$ DNA when appropriate corrections are made for the fact that DNA from V58 P$^-$ contains less homologous DNA, due to the fact that P is not present. Thus, the actual amount of competing DNA in the P$^-$ strain was much less than that in the P$^+$ strain and could not directly be compared on the same figure. However, between 40 and 50% competition was observed with the V58 P$^-$ DNA, confirming the results obtained from the hydroxyapatite experiments.

The amount of homology between the P factor DNA and the *V. cholerae* chromosomal

DNA is about the same as that observed between the *E. coli* sex factor F and the *E. coli* chromosomal DNA (7). This homology may account for the ability of the fertility factor P to transfer some of the *V. cholerae* chromosomal genes as reported by Baskaran (2). However, no high frequency donors have yet been found in the *V. cholerae* mating system similar to those of the *E. coli* mating system which result from integration of the F factor into the *E. coli* chromosome.

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