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Uptake of Homologous Single-Stranded Fragments by Superhelical DNA: A Possible Mechanism for Initiation of Genetic Recombination

(marker rescue/S_1 nuclease/D-loops)

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ABSTRACT **Superhelical [^{3}H]DNA (replicative form I, RFI) of bacteriophage ϕX174 slowly but spontaneously took up ^{32}P-labeled homologous single-stranded fragments at 4°. Uptake was accelerated by heating to 75°. RFI did not take up single-stranded fragments derived from DNA of *Escherichia coli* or from separated strands of phage λ. Uptake was inhibited by low concentrations of ethidium bromide. Relaxed circular ϕX174 DNA did not take up homologous fragments. Per molecule of RFI, the complexes contained as much as 90 nucleotide residues of homologous fragment. The ^{32}P-labeled fragments were largely resistant to digestion by exonuclease I, and were not displaced by heating complexes at 60° for 1 min in 16 mM or 100 mM NaCl. Under comparable conditions of temperature and salt all of the fragments were displaced from complexes in which at least one phosphodiester bond was cleaved by pancreatic DNase, but a significant fraction of the fragments was retained in complexes that were relaxed by digestion with S_1 nuclease. These observations are interpreted to mean that S_1 nuclease digested the plus (viral) strand of the recipient RF at the site of uptake in some instances. Transfection of *E. coli* by heterozygous complexes produced recombinant progeny, thereby showing that genetic information can be transferred from the fragment of plus strand to progeny plus strands. We propose that both uptake of a third strand by superhelical DNA and the action of nucleases on the resulting complex may simulate early steps in genetic recombination.**

One of the most obscure aspects of genetic recombination is its initiation. How are long and densely packed molecules of DNA brought into register and how is an exchange started? According to one hypothesis, as illustrated in Fig. 1, the first step in recombination is the enzymic production of a redundant single strand which invades a neighboring homologous double-stranded molecule. As part of a general model for genetic recombination, this hypothesis was used to account for the possible origin of an intermediate in which a single strand is transferred from one double-stranded molecule to another (1). We were prompted to wonder how a homologous single strand might invade double-stranded DNA. Observations made in recent years on superhelical DNA led to the speculation that superhelicity itself might promote the uptake of a homologous single strand: (*1*) In its closed superhelical form, circular DNA is endowed with free energy which is available to do work (2). (*2*) Regions of superhelical DNA behave as if they were transiently single-stranded, an observation that reflects the available free energy (3–7). (*3*) Circular DNA from numerous sources contains regions called D loops in which a third strand is associated with the helix (8, 9). (*4*) Superhelicity is a property not only of relatively small circular genomes, but is also associated with the folded chromosome of *Escherichia coli* (10). To test the hypothesis that superhelicity may promote the uptake of homologous single strands we have investigated the association of small single-stranded fragments with the superhelical form of bacteriophage ϕX174 DNA.

METHODS

DNA. ϕX174 RFI* [^{3}H]DNA was purified from *E. coli* HF 4704 infected with ϕX174*am*3 according to Godson and Vapnek (11) as modified by Godson and Boyer (12). The specific activity of the DNA was 0.5 to 2.2 × 10^4 cpm/nmol of nucleotide. RFI DNA used in these studies was not further purified by equilibrium centrifugation in CsCl gradients containing ethidium bromide since very low concentrations of the latter were found to inhibit the uptake reaction, as will be discussed in *Results*. ^{32}P-Labeled ϕX174*am*3 phages were prepared according to Francke and Ray (13) and further purified by glass bead exclusion chromatography (14). ^{32}P-Labeled ϕX174 wild-type phages were prepared according to Benbow *et al.* (15). DNA with a specific activity between 0.5 and 4 × 10^4 cpm/nmol of nucleotide was isolated from purified phage particles by phenol extraction, followed in some cases by centrifugation in alkaline sucrose gradients. Phage λ [^{32}P]DNA was prepared (16) and the strands were separated and isolated (17) as described previously. Poly(U,G) was removed by alkaline hydrolysis (0.1 N NaOH, 5 hr, 37°) and the ribonucleotides were removed from the DNA by chromatography on a small column of Sepharose 2B in 1 mM Tris·HCl, pH 7.5, 0.1 mM EDTA. The λ DNA appearing at the exclusion volume was concentrated under a stream of dry nitrogen. The DNA had a specific activity of 1.4 × 10^4 cpm/nmol of nucleotide.

Single-Stranded Fragments of DNA. Single-stranded fragments were prepared by digestion of [^{32}P]DNA by pancreatic DNase. Mixtures contained 0.1 M Tris·HCl, pH 7.5, 5 mM $MgCl_2$, about 0.25 mM DNA nucleotide, and 1–10 ng of pancreatic DNase/nmol of DNA nucleotide. Reactions were carried out for 15 min at 37° and terminated by the addition of EDTA to 10 mM and heating the sample at 100° for 2 min. Fragments generated had average chain lengths of 25–80

* The closed circular superhelical form of ϕX174 DNA is called RFI (replicative form I) or form I. Relaxed double-stranded circular DNA derived from form I is called form II DNA, and the linear double-stranded derivative is called form III DNA. Concentrations of DNA or fragments of DNA are expressed in relation to moles of nucleotide unless otherwise specified.

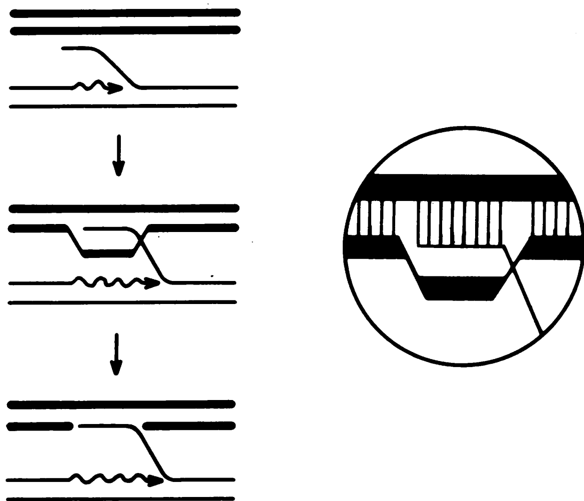

FIG. 1. Hypothesis concerning the initiation and propagation of transfer of a single strand between recombining molecules of DNA (1). A strand, displaced from a donor molecule by the action of a polymerase, is taken up by a homologous recipient. Specific endonucleolytic cleavage of one strand of the recipient in the region of uptake makes extensive strand transfer possible by simultaneous exonucleolytic digestion of the cleaved recipient strand and displacement of a donor strand. The inset illustrates the hypothetical process of uptake, which might involve relatively few base pairs prior to digestion of the unpaired strand of the recipient.

nucleotides, as estimated from the proportion of ^{32}P that was sensitive to the action of bacterial alkaline phosphatase.

Formation of Complexes of RFI and Homologous Single-Stranded Fragments. Reaction mixtures contained 50 mM Tris·HCl, pH 7.5, 5 mM EDTA, 2.5 mM $MgCl_2$, 0.63 M NaCl, 0.07–0.13 mM RFI [^{3}H]DNA nucleotide, and 0.13–0.49 mM single-stranded [^{32}P]DNA fragment nucleotide. Unless specified otherwise, mixtures were heated to 75° for 5 min and cooled to 30° at the rate of 0.6°/min in a water bath controlled by a mechanically driven thermostat. Small samples (about 0.2 ml) were laid on top of 5 ml of a linear gradient of 5–20% sucrose containing 1.0 M NaCl, 10 mM Tris·HCl, pH 7.5, 1 mM EDTA, and centrifuged 180 min at 50,000 rpm in the Spinco SW 50.1 rotor. Larger samples (about 0.6 ml) were laid on 12 ml gradients and centrifuged for 8 hr at 41,000 rpm or 18 hr at 30,000 rpm in the Spinco SW 41 rotor. The conditions for reisolation of complexes are stated in the descriptions of individual experiments. The uptake of fragment nucleotide per mole of RF nucleotide was estimated from the peak samples. To express the results as residues of nucleotide taken up per molecule of RFI, we adopted the round number of 10^4 nucleotides per molecule of RF.

Enzymes. S_1 nuclease was prepared by a modification of the method of Ando (18, †). Exonuclease I was purified according to Lehman and Nussbaum (19). Pancreatic DNase was purchased from Worthington. One unit of enzyme activity is the amount of enzyme that renders 10 nmol of DNA nucleotide acid-soluble in 30 min at 37°, under the standard conditions of the assay of each enzyme.

† R. Wiegand, G. Godson, and C. Radding, manuscript in preparation.

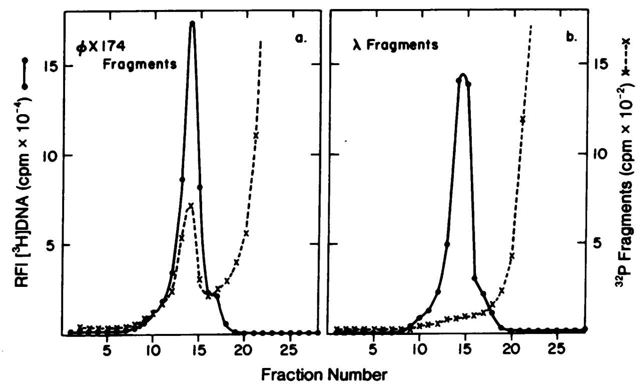

FIG. 2. Influence of homology on the uptake of fragments. (a), φX174 [^{32}P]DNA fragments (3.5 × 10^4 cpm/nmol of nucleotide) and (b) fragments of ^{32}P-labeled λ *r* strand. (1.4 × 10^4 cpm/nmol of nucleotide) with average chain lengths of 27 were each incubated with φX174 RFI [^{3}H]DNA (2.3 × 10^4 cpm/nmol of nucleotide) and centrifuged as described in *Methods*.

Strains. E. coli C Su^+ (suppressor) and Su^-, HF4714 and HF4704, respectively, φX174*am3* and φX174 *wild-type* (20) were obtained from G. N. Godson. The C600 strain of *E. coli* K12, which is Su^+, served as the recipient for transfection by φX174 DNA.

Transfection. Transfection of spheroplasts of strain C600 was carried out according to Wackernagel (21). After the DNA was added the spheroplasts were incubated at 37° for 2 hr. Phage titers were determined after the addition of chloroform and lysis of the spheroplasts by three rounds of freezing and thawing.

RESULTS

Uptake of Homologous Single-Stranded Fragments by Preparations of Superhelical DNA. When homologous single-stranded fragments in the range of 25–80 nucleotides in length were incubated with preparations of superhelical φX174 DNA, stable complexes were formed, as assayed by sedimentation through gradients of neutral sucrose (Fig. 2a). No association of fragments with superhelical φX174 DNA was detected when fragments were made from *r* strands of phage λ DNA (Fig. 2b). Similarly, single-stranded fragments derived from *E. coli* DNA were not taken up by superhelical φX174 RFI (data not shown). Complexes of homologous single-stranded fragments and superhelical DNA were formed slowly at low temperatures. Uptake was accelerated by heating to temperatures that are appropriate for annealing homologous single strands. The addition of salt also promoted uptake (Table 1). Once formed, complexes were stable for at least a month when stored in 0.1 M NaCl at 4°. Complexes also survived reisolation from gradients of neutral sucrose and precipitation by ethanol (Figs. 3 and 5). In an alkaline sucrose gradient, complexes dissociated, yielding a peak of rapidly sedimenting form I DNA and a peak of fragments (Fig. 5c).

Lack of Uptake by Relaxed DNA. Under controlled conditions, the S_1 endonuclease of *Aspergillus oryzae* will convert superhelical φX174 DNA to a relaxed circular molecule (form II) by cutting one strand more or less at random (7, †). A preparation of φX174 double-stranded DNA consisting largely of form I (Fig. 3a) was treated with S_1 nuclease such that about half of the molecules were converted to form II

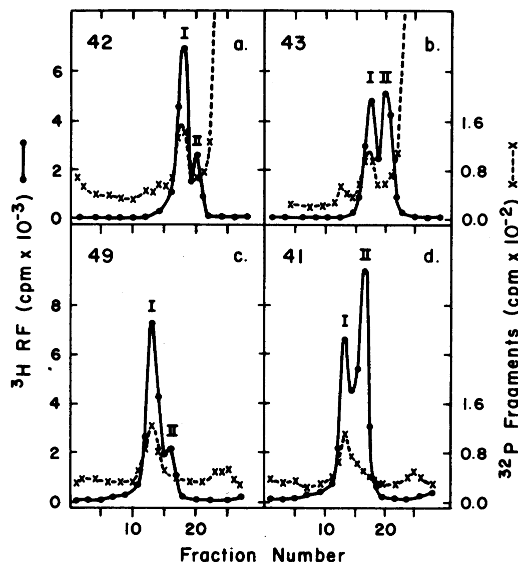

FIG. 3. Influence of superhelicity on uptake of fragments. A reaction mixture (0.14 ml) containing 20 mM sodium acetate, pH 5.5, 0.2 M NaCl, 1 mM $ZnSO_4$, 40 nmol of ϕX174 RFI [^{3}H]-DNA nucleotide (5.2 × 10^3 cpm/nmol) and 50 units of S_1 nuclease was incubated at 37° for 30 min. The reaction was terminated by addition of 0.01 ml of 2 M Tris·HCl, pH 7.5, and 0.05 ml of 5 M NaCl. To this was added 0.2 ml of ϕX174 [^{32}P]DNA fragments (1.55 × 10^4 cpm/nmol of nucleotide) containing 195 nmol of nucleotide with an average chain length of 36. The mixture was heated to 75° and cooled slowly as described in *Methods*, laid on top of 12 ml of a gradient of neutral sucrose (5–20%), and centrifuged 7 hr at 41,000 rpm in the Spinco SW 41 rotor. In a mixture that was otherwise identical, S_1 nuclease was omitted. Both gradients were collected into vials, and aliquots of 0.1 ml were removed for determination of radioactivity. The sedimentation profiles for both the undigested (a) and digested (b) samples are shown. Samples corresponding to the combined peaks of form I DNA and form II DNA were pooled in both cases and the DNA was precipitated by the addition of 2 volumes of ethanol. Each precipitate was collected by centrifugation and redissolved in 0.6 ml of 0.1 M NaCl, 10 mM Tris·HCl, pH 7.5, and 1 mM EDTA. Aliquots of 0.2 ml were laid on neutral sucrose gradients and centrifuged for 180 min at 50,000 rpm in the Spinco SW 50.1 rotor. The sedimentation profiles of the reisolated undigested and digested samples are shown in (c) and (d) respectively. The numbers in the upper left-hand corner of each frame represent residues of fragment nucleotide (^{32}P) per molecule of RFI (^{3}H).

(Fig. 3b). The untreated and treated preparations were annealed individually with homologous single-stranded fragments and sedimented through neutral sucrose gradients (Fig. 3a and b). Samples corresponding to the combined peaks of form I and form II DNA were pooled and centrifuged again, a procedure that eliminated the large excess of unassociated fragments (Fig. 3c and d). The molar ratio of ^{32}P-labeled fragment to ^{3}H-labeled RF in the faster sedimenting peak was the same in the preparation that was partially digested by S_1 nuclease as in the untreated preparation. Little or none of the fragments was associated with relaxed circular DNA. The constancy of the molar ratio of fragment to superhelical DNA supports the interpretation that the fragments were taken up specifically by superhelical DNA rather than by relaxed circles or by some minor contaminant such as single-stranded DNA.

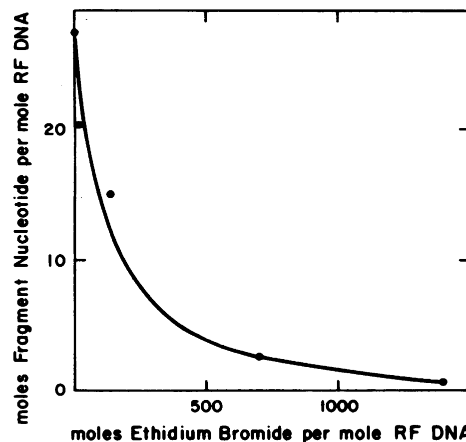

FIG. 4. Inhibition of uptake by ethidium bromide. Mixtures (0.2 ml) containing 23 nmol of ϕX174 [^{32}P]DNA fragment nucleotide (3.3 × 10^4 cpm/nmol; average chain length 27) and 24 nmol of ϕX174 RFI [^{3}H]DNA nucleotide (2.2 × 10^4 cpm/nmol) and the indicated amounts of ethidium bromide were incubated and centrifuged as in *Methods*. Moles of RF DNA for this figure were calculated by adopting the round number of 10^4 nucleotides per molecule of RF.

Inhibition by Ethidium Bromide of the Uptake of Homologous Fragments. Since the binding of intercalating dyes is also promoted by superhelicity, competition might be expected between the uptake of ethidium bromide and that of homologous single-stranded fragments. As illustrated in Fig. 4, low concentrations of ethidium bromide inhibited the uptake of fragments. Half-maximal inhibition was observed when 120 molecules of ethidium bromide were added per molecule of form I DNA (0.43 μg/ml or 1.1 μM ethidium bromide). If 26° is taken as the angle of unwinding per molecule of ethidium bromide bound (22), the ϕX174 RF might have been unwound, as a maximum estimate, by nine of its 41 superhelical turns. In other experiments we studied the effect of ethidium bromide on complexes. A short incubation of complexes in 0.66 mM ethidium bromide resulted in the dissociation of about half of the bound fragments (Fig. 5b). Centrifugation of complexes in 3 M CsCl, 0.76 mM ethidium bromide for 48 hr resulted in complete dissociation of the

TABLE 1. *Effect of temperature and salt on uptake of fragments*

Temp (°C)	Time	[NaCl] (M)	Relative efficiency (%)
4	48 hr	0.63	82
10	10 min	0.63	15
37	60 min	0.63	24
45	60 min	0.63	19
75*	5 min	0.63	100
75	10 min	0.63	69
75	10 min	0.10	50
75	10 min	0.0	5

The data are derived from two experiments in which uptake under standard conditions (*Methods*) was respectively 24 and 40 residues of fragment nucleotide per molecule of RFI.

* The standard condition: The sample was cooled at the rate of 0.6°/min. Other heated samples were allowed to cool at ambient temperature.

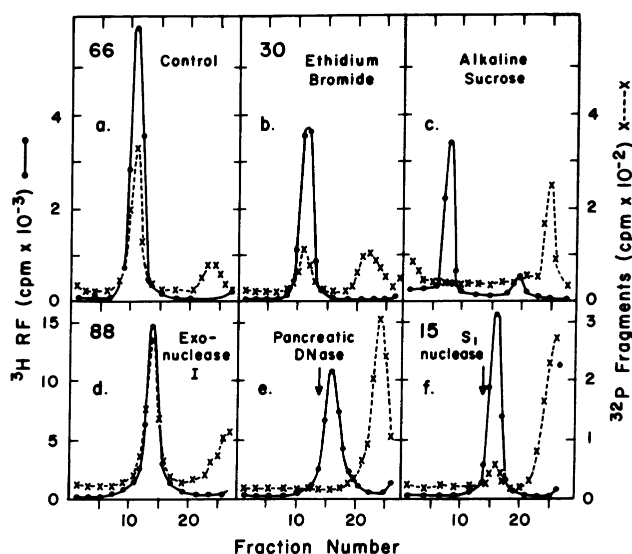

FIG. 5. Characterization of complexes. The experiments shown in a–c were done with a preparation of complexes made, as described in *Methods*, from ϕX174 RFI [^{3}H]DNA (5.2×10^3 cpm/nmol of nucleotide) and single-stranded fragments of ϕX174 [^{32}P]DNA (3.9×10^4 cpm/nmol of nucleotide) which were 36 residues long on the average. Complexes were reisolated from preparative gradients by pooling the samples corresponding to the peak of RF (compare Fig. 2a) and dialyzing against 0.1 M NaCl, 10 mM Tris·HCl, pH 7.5, and 1 mM EDTA. The preparation obtained had 20 nmol of DNA nucleotide per ml. (a) *Control.* A sample (0.2 ml) of reisolated complexes was centrifuged through a gradient of neutral sucrose. (b) *Ethidium Bromide.* To an aliquot (0.2 ml) of the isolated complex were added 6 μl of 25 mM ethidium bromide and 20 μl of 5 M NaCl. After 10 min at 22° the mixture was laid on a neutral sucrose gradient and centrifuged. (c) *Alkaline Sucrose Gradient.* To 0.1 ml of the reisolated complex were added 25 μl of 2 N NaOH and 75 μl of H_2O. The mixture was laid on top of a 5 ml linear sucrose gradient (5–20%) containing 0.3 N NaOH, 0.7 M NaCl, 1 mM EDTA, and centrifuged 120 min at 50,000 rpm in the Spinco SW 50.1 rotor.

The experiments in d–f were done on a preparation of complex made from ϕX174 RFI [^{3}H]DNA (2.0×10^4 cpm/nmol of nucleotide) and single-stranded fragments of ϕX174 [^{32}P]DNA which were 63 nucleotides long on the average. Complexes were reisolated from gradients of neutral sucrose (*Methods*). The DNA was precipitated by addition of 2 volumes of ethanol and the precipitate was redissolved in 1 ml 0.1 M NaCl, 10 mM Tris·HCl, pH 7.5, and 1 mM EDTA. The complex contained 88 residues of fragment nucleotide per molecule of RF. The following samples were centrifuged through gradients of neutral sucrose (*Methods*). In frames e and f the arrows mark the expected position of RFI as judged from separate gradients in the same run. (d) *Exonuclease I.* A reaction mixture (0.2 ml) containing 67 mM glycine, pH 9.5, 6.7 mM $MgCl_2$, 16 mM NaCl, 2.6 nmol of reisolated complex (nmol of nucleotide), and 0.5 units of exonuclease I was incubated at 37°. After 30 min 5 μl of 0.5 M EDTA were added. The specific activity of the single-stranded fragments of DNA was 4.1×10^4 cpm/nmol of nucleotide. (e) *Pancreatic DNase.* A reaction mixture (0.2 ml) containing 10 mM Tris·HCl, pH 7.5, 5 mM $MgCl_2$, 16 mM NaCl, 2.6 nmol of reisolated complex (nmol of nucleotide), and 1 ng of pancreatic DNase was incubated at 22°. After 3 min, 5 μl of 0.5 M EDTA were added, and the sample was heated at 60° for 1 min. The specific activity of the single-stranded fragments of DNA was 3.9×10^4 cpm/nmol of nucleotide. (f) *S_1 Nuclease.* A reaction mixture (0.2 ml) containing 50 mM sodium acetate, pH 5.0, 1.5 mM $ZnSO_4$, 0.1 M NaCl, 2.6 nmol of reisolated complex (nmol of nucleotide), and 113 units of S_1 nuclease was incubated at 37°. After 15 min, 20 μl of 2 M Tris·HCl, pH 7.5, were added to stop the reaction by changing the pH. The specific activity of the single-stranded fragments of DNA was 3.3×10^4 cpm/nmol of nucleotide. The numbers in the upper left hand corners of the frames are residues of fragment nucleotide per molecule of RF.

homologous fragments (data not shown). Superhelical DNA isolated from gradients of CsCl containing ethidium bromide took up fragments inefficiently unless special care was taken to remove the ethidium bromide. A batch of RFI, prepared as described in *Methods* without the use of ethidium bromide, was observed to take up 23 residues of fragment nucleotide per molecule of RFI. The addition of 25 μM ethidium bromide reduced the efficiency of uptake to less than 1%. Two passages of the mixture of RFI plus ethidium bromide through columns of Dowex-50 (Na^+) restored the efficiency of uptake to 88% of its original value.

Enzymic Cleavage of Complexes. A preparation of reisolated complexes was treated with several different nucleases (Fig. 5). The ^{32}P-labeled fragment in the complex was not attacked by an amount of exonuclease I that digested 96% of the fragment from a heat denatured sample (Fig. 5d). This supports the inference that most of the fragment is held in the complex by hydrogen bonds. Acted upon by pancreatic DNase, complexes sedimented more slowly, in the position expected for relaxed circular or linear molecules (Fig. 5e). Only 7% of the ^{32}P label was made acid-soluble, but 94% of the fragments were displaced from the complexes (data not shown). The rest of the ^{32}P label was displaced by heating for 1 min at 60° in 16 mM NaCl (Fig. 5e) or in 100 mM NaCl. Fragments were not displaced from the original superhelical complexes by heating in 16 mM NaCl. S_1 nuclease also converted complexes into relaxed molecules. In the experiment shown in Fig. 5f, 17% of the original ^{32}P label was retained in the relaxed complex. Another preparation of complexes was digested with S_1 nuclease and was reisolated by passage through a column of Sepharose in order to adjust the concentrations of solutes. When heated for 1 min at 60° in 20 mM NaCl none of the fragments in the digested complex was displaced. Since the fragments are not taken up by relaxed DNA (Fig. 3) and can be displaced from complexes that are randomly nicked (Fig. 5e), the result observed upon digestion by S_1 suggests that at least some of the complexes were cleaved at the site of uptake. All or part of a fragment might not be susceptible to displacement by branch migration if at the site of uptake S_1 nuclease made two cuts in the plus strand of the recipient.

Marker Rescue by Transfection with Heterozygous Complexes. Spheroplasts of a strain of *E. coli* K12 that is Su^+ were transfected by complexes containing RFI from ϕX174*am3* and fragments from *wild type*. The phage produced were assayed on Su^- and Su^+ strains of *E. coli* C (see *Methods*). Rescue of the *wild-type* allele of *am3* from the complexed fragments was shown by a 5- to 10-fold increase in the fraction of am^+ particles (Table 2). The controls were RFI *am3*, and RFII *am3*, the latter reisolated from the same mixture of RF and fragments that produced the complex RFI *am3*/ fragment am^+ (see legend to Table 2).

TABLE 2. *Marker rescue by transfection with heterozygous complexes of DNA*

	Plaques per genome equivalent		
	am+ (×10⁸)	Total (×10⁴)	*am*+/total (×10⁵)
Exp. 1			
RFI *am3*	1.47	9.5	1.5
RFI *am3*/*am*+ complex	1.7	1.94	8.8
Exp. 2			
RFI *am3*	0.36	1.78	2.0
RFI *am3*/*am*+ complex	1.72	0.96	18.0
RFII *am3* annealed with *am*+ fragments	5.0	14.9	3.4

Single-stranded fragments of [^{32}P]DNA (60 residues average chain length) from ϕX174 *wild type* were incubated under the conditions of the uptake reaction (*Methods*) with a preparation RF [^{3}H]DNA from ϕX174*am3* consisting of 80% form I and 20% form II. The mixture (1.2 ml) was centrifuged in two gradients of neutral sucrose in the Spinco SW 41 rotor at 30,000 rpm for 18 hr. Samples corresponding to the peaks of closed circular and open circular DNA, respectively, were pooled and dialyzed against 0.1 M NaCl, 10 mM Tris·HCl, pH 7.5, and 1 mM EDTA. The sample of closed circular DNA contained 67 residues of fragment nucleotide per molecule of RF. The sample of relaxed DNA contained little if any of the fragments. Transfection was done as described in *Methods*. The phages produced were diluted in 50 mM sodium borate and assayed on exponentially growing strains of *E. coli* that were Su$^+$ and Su$^-$.

DISCUSSION

We conclude that superhelical DNA spontaneously takes up homologous single-stranded fragments, forming a structure which is akin to that of D loops (8). ϕX174 RFI took up as much as 90 residues of fragment nucleotide per molecule of RFI. If we assume that the fragments are wound in a helix with 10 turns per base pair, the observed uptake is 22% of the maximum that might be accommodated by RFI, which has some 41 superhelical turns (22, 23).

We were led to do these experiments by speculations about the initiation of general genetic recombination (1, 24). According to one version of such speculations, an early event in recombination is the uptake by a recipient double-stranded molecule of a redundant strand displaced from a homologous donor. Furthermore, the intermediate resulting from uptake of the redundant strand must become the focus of a specific enzymic attack that opens the recipient molecule (Fig. 1 and ref. 1). The experiments described in this paper suggest that both uptake and enzymic attack can be realized *in vitro*, much as supposed. The observations strengthen the hypothesis that a homologous single strand might be taken up actively by the recipient molecule in a reaction that is promoted by the energy of superhelix formation. (*In vivo* such a reaction presumably would be catalyzed by specific binding proteins.) This notion need not be limited to closed circular genomes such as those of mitochondrial and various viral DNAs. The chromosome of *E. coli* can be isolated as a single continuous circle (25) or as a more compact structure which behaves as if it were composed of some 50 independent superhelical loops (10). Although the basis is not known, the observation of these superhelical loops suggests the existence of a kind of topological constraint other than that imposed by the covalent joining of the ends of DNA. As to enzymic cleavage of the triple-stranded complex, the present experiments show that an endonuclease that is highly specific for single-stranded DNA can stabilize the association of the donated strand with relaxed recipient DNA. More direct experiments are required to support our interpretation of the stabilization.

From other experiments we know that the functionally single-stranded regions of ϕX174 are distributed throughout the molecule (7, †). Since in the present experiments we observed the rescue of one genetic marker chosen at random, it is likely that strand uptake was not limited to a unique site in ϕX174. But the idea that strand uptake might initiate genetic recombination does not require that uptake occur equally often at all sites in DNA, since strand transfer might terminate anywhere (1). Factors that might influence the efficiency of marker rescue in the experiments shown in Table 2 include the frequency of uptake at the site of the mutation *am3*, the frequency and direction of mismatch repair, and the asymmetric replication of ϕX174 (26). If uptake were random and if information transfer from fragment to progeny strands were completely efficient, one might have observed 1% *am*+ particles instead of 0.01–0.02% (Table 2). It should be possible to determine if the generation of recombinants from heterozygous complexes depends upon any of the known genes of nucleic acid metabolism in *E. coli*.

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