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Nicking-closing activity associated with bacteriophage λ *int* gene product

(genetic recombination/topoisomerase)

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ABSTRACT **Integrative recombination of bacteriophage λ requires the action of the protein Int, the product of the phage *int* gene. In this paper we show that highly purified Int relaxes supercoiled DNA. The association of this nicking-closing activity with Int is shown by: (*i*) the cosedimentation of nicking-closing and recombination activities of purified Int, (*ii*) the parallel inactivation of the two activities in purified Int by both heat and a specific antiserum, and (*iii*) the alteration of both activities in crude extracts of a strain expressing a mutant *int* gene. The nicking-closing activity of Int functions in the absence of divalent cations and in the absence of an apparent source of chemical energy. The activity displays no obvious sequence specificity and is inhibited by Mg^{2+}, spermidine, and single-stranded DNA. Int relaxes positive as well as negative supercoils. We present a model for the mechanism of strand exchange that describes how the nicking-closing activity of Int might be used during recombination.**

Bacteriophage λ forms lysogens of *Escherichia coli* by integrating viral DNA into the bacterial chromosome (reviewed in ref. 1). The integration occurs as a result of a reciprocal recombination between specific genetic loci, the viral and bacterial attachment sites (*att*P and *att*B, respectively). Integrative recombination readily takes place in a cell-free system (2), and the *in vitro* reaction has been used to characterize the overall pathway of this recombination. Briefly, it has been shown that DNA supercoiling is essential for recombination: at least *att*P must be located on a negatively supertwisted circle of DNA (3, 4). Given an appropriately supertwisted substrate, the only low molecular weight substances required for full recombination activity are spermidine, KCl, and buffer. The products of integrative recombination are a reciprocal pair of continuous double-helical DNAs. This fact and the simplicity of the cofactor requirements suggest that recombination occurs by a very conservative mechanism—i.e., one that involves no degradation or resynthesis of DNA and one that retains the bond energy of the phosphodiester backbone during breakage and reunion.

In vitro integrative recombination has been used to assay the purification of the proteins involved in this reaction. We have previously reported the purification to near homogeneity of the λ *int* gene product, the only virus-coded component of the integration system (5, 6). We showed that, in addition to supertwisted substrate DNA and the cofactors described above, purified *int* gene product (Int) requires the participation of protein(s) encoded by the bacterial host in order to carry out integrative recombination. In the absence of this host component and spermidine, highly purified Int binds to DNA, forming specific stable complexes with DNA containing *att*P. Thus, Int is a specificity element for integrative recombination, recognizing at least one of the two recombining sites. We now report that, in the absence of the other components of the recombination reaction, purified Int demonstrates another activity: nicking-closing.

Nicking-closing enzymes transiently disrupt the phosphodiester backbone of DNA; i.e., they introduce a break in a strand of DNA and subsequently reseal that break (reviewed in refs. 7 and 8). No change in the covalent structure of DNA results from this activity, but the temporary disruption permits the two strands of the double helix to alter their topological linkage. A simple consequence of this activity is the conversion of supertwisted DNA to covalently closed circular DNA lacking supertwists. Such relaxation of supertwisted DNA is catalyzed by these enzymes without an obvious source of chemical energy. It has therefore been proposed that an enzyme–DNA complex conserves the energy of the phosphodiester bond during the relaxation; recent evidence supports the existence of such complexes (7, 8). It has been suggested that enzymes that carry out the concerted breakage and resealing of DNA strands be designated as topoisomerases (8). Such enzymes have been found widely in nature and, in general, their function is unknown. The exception is DNA gyrase, an enzyme that introduces negative superhelical turns into relaxed closed circular DNA by coupling a nicking-closing activity to an ATPase (9, 10). Possible roles for topoisomerases in transcription, replication, and recombination have been proposed (7, 8). In this paper we characterize the nicking-closing activity of Int and discuss its relationship to the role of Int in recombination.

MATERIALS AND METHODS

Proteins. The bacterial component of integrative recombination assays was prepared by sonication from HN356, a *recB21* derivative of *E. coli* strain N99 (constructed by R. A. Weisberg, National Institutes of Health). The clarified sonicate was dialyzed against 50 mM Tris·HCl (pH 7.4) containing 10% (vol/vol) glycerol for 2 hr and stored at −20°C.

Int was purified to near homogeneity from *E. coli* strain WCi22642 (11) as described (6); unless otherwise stated, this material was used for all the experiments reported in this paper. For experiments comparing temperature-sensitive and wild-type *int* gene products, crude cell extracts of strains HN496 and HN497 were made by digestion with lysozyme at 0°C as described (2) except that the cells were grown at 29°C in broth containing ampicillin (Sigma) at 25 μg/ml. Strains HN496 and HN497 are derivatives of ED8654 (12); each carries a hybrid plasmid (constructed in this laboratory) in which the DNA between the lone *Eco*RI and *Hin*dIII restriction endonuclease sites of pBR322 (13) has been replaced by the 4.2-kilobase-pair *Hin*dIII–*Eco*RI fragment of λ that includes the *int* gene. The λ fragment in the plasmid of strain HN496 carries the *int* ts2004 (14) and *int*C226 (15) alleles, while that in strain HN497 carries the wild-type structural *int* gene and the *int*C226 promotor; the fragments are otherwise identical.

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Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; MalNEt, *N*-ethylmaleimide.

Antiserum to Int was prepared after intradermal injection of Int into a single New Zealand White rabbit. Serum was prepared from blood withdrawn before and 4 weeks after the injection of antigen.

DNA. Supertwisted DNAs were prepared as described (5) after chloramphenicol amplification of plasmid-containing derivatives of *E. coli* strain 204 grown in K medium containing thymidine at 5 μg/ml. Plasmid pYK100 (6) is a derivative of pBR322 (13) in which the DNA between the lone *Hin*dIII and *Bam*HI sites of the vector has been replaced by a 500-base-pair *Hin*dIII–*Bam*HI fragment of phage λ that contains *att*P. Plasmid pBB105 is a pBR322 hybrid in which the DNA between the lone *Eco*RI and *Bam*HI sites of the vector has been replaced by a 1600-base-pair *Eco*RI–*Bam*HI fragment of *E. coli* DNA that contains *att*B (constructed and kindly provided by A. Landy of Brown University). For recombination assays, pBB105 DNA was linearized with *Eco*RI restriction endonuclease (Miles). For the experiment of Fig. 2*B*, pYK100 DNA was converted to the relaxed closed circular form by incubation with DNA relaxing enzyme from *Agrobacterium tumefaciens* (Bethesda Research Laboratories, Rockville, MD).

Nicking-Closing Assays. The standard reaction mixture (20 μl) contained 50 mM Tris·HCl (pH 7.4), 5 mM sodium EDTA, 100 mM KCl, bovine serum albumin (Miles) at 3 mg/ml, *ca.* 1.0 μg of negatively supertwisted DNA, and Int. The mixture was incubated at 25°C for 60 min. For dye–buoyant density analysis, the reactions were stopped by addition of 2 μl of 10% sodium dodecyl sulfate ($NaDodSO_4$) and processed as described (5). For analysis by agarose gel electrophoresis, the reactions were stopped by addition of 3 μl of 10% $NaDodSO_4$ and 20 μl of a mixture containing 50% glycerol, 0.75 M NaCl, 0.075 M sodium citrate, and bromphenol blue. The samples were then loaded onto a 1% agarose (Sigma, Type II) slab gel in Tris/borate/EDTA buffer (16). After overnight electrophoresis at 40 V in buffer containing 0.1% $NaDodSO_4$, the gel was stained with ethidium bromide (1 μg/ml) for 1 hr, destained in water for 2 hr, and photographed in ultraviolet light as described (16).

Integrative Recombination Assay. The reaction mixtures (20 μl) contained 50 mM Tris·HCl (pH 7.4), 100 mM KCl, 5 mM sodium EDTA, 10 mM spermidine, *ca.* 1 μg of supertwisted circular pYK100 DNA, *ca.* 1 μg of linearized pBB105 DNA, *ca.* 100 μg of sonicated bacterial extract, and Int. The reaction was started by addition of Int and incubation was for 1 hour at 25°C. The reactions were stopped by addition of 3 μl of 10% $NaDodSO_4$ and the samples were prepared, electrophoresed, and photographed as described above for the nicking-closing assay.

RESULTS

Characterization of nicking-closing activity

Centrifugation of DNA to equilibrium in cesium chloride gradients containing ethidium bromide showed that incubation of negatively supertwisted DNA with Int (homogeneity >95%) results in a progressive loss of superhelical turns (Fig. 1). After 20 hr of incubation virtually all of the superhelical turns have been removed; even at this longest time of incubation, no nicked circular or linear DNA is produced. Thus, under these conditions, Int carries out only concerted nicking and resealing and has no detectable net endonuclease activity.

The nicking-closing activity of Int is rather sluggish. In the experiment of Fig. 1, the DNA sample is half relaxed after 90 min of incubation. Assuming that the active species of Int is a monomer of 40,000 daltons (7) and that all the Int in our preparation is active, a turnover number of approximately 0.01

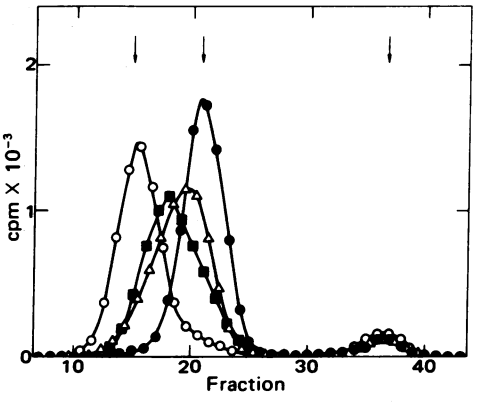

FIG. 1. Relaxation of supertwisted DNA by Int. pYK100 supertwisted [^{3}H]DNA (1.2 μg, 11,000 cpm) was incubated with Int for various times and centrifuged to equilibrium in cesium chloride/ethidium bromide gradients. A total of 54 fractions was collected from the bottom of each centrifuge tube. Starting at the left (bottom of the gradient), the arrows indicate the position of relaxed closed circular, negatively supertwisted, and nicked [^{14}C]ColE1 marker DNA used to align the four gradients. ●, Int omitted, no incubation; △, 0.3 μg of Int, 24 min; ■, 0.3 μg of Int, 90 min; ○, 0.3 μg of Int, 20 hr.

turns per molecule of Int per minute is calculated. Although inefficient, Int does indeed catalyze nicking and resealing; in an experiment in which a low Int-to-DNA ratio and a long incubation time were used, we found a single molecule of Int could, on the average, relax all the turns from at least two molecules of a 5.6-kilobase-pair plasmid (data not shown).

Electrophoresis in agarose gels separates closed circular DNA molecules of the same length according to their degree of superhelicity and is the basis of a convenient assay for relaxation of DNA (17). After incubation of negatively supertwisted DNA with Int, electrophoresis in agarose displayed a "ladder" of closed circular DNA species varying from fully supertwisted to fully relaxed (Fig. 2). Using this semiquantitative assay, we have characterized some basic features of the nicking-closing activity of Int. Relaxation of DNA is observed over KCl con-

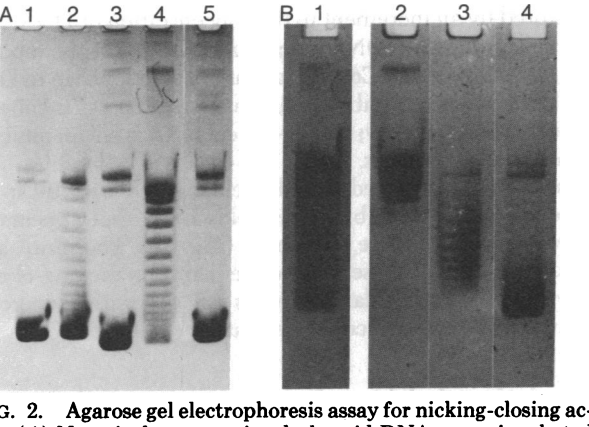

FIG. 2. Agarose gel electrophoresis assay for nicking-closing activity. (*A*) Negatively supertwisted plasmid DNAs were incubated as described in *Materials and Methods*: lane 1, pYK100, Int omitted; lane 2, pYK100, 0.2 μg of Int; lane 3, pBR322, Int omitted; lane 4, pBR322, 0.2 μg of Int; lane 5, pBR322, 0.2 μg of Int, 10 mM spermidine added. (*B*) Negatively supertwisted or relaxed pYK100 DNA (0.7 μg) was incubated with 0.3 μg of Int in a nicking-closing assay containing various amounts of ethidium bromide for 2½ hr at 25°C. Lane 1, Supertwisted DNA, ethidium bromide omitted; lane 2, relaxed DNA, ethidium bromide omitted; lane 3, relaxed DNA, ethidium bromide at 1 μg/ml; lane 4, relaxed DNA, ethidium bromide at 3 μg/ml. The ethidium bromide in samples 3 and 4 was removed by 1-butanol extraction prior to electrophoresis.

centrations from 10 to 120 mM; the reaction is inhibited at KCl concentrations above 150 mM. Although EDTA and bovine serum albumin are included in the standard incubation mixture, neither is required for or stimulates nicking-closing activity. Nicking-closing is readily observed at temperatures between 20°C and 35°C, but this activity is rapidly lost at temperatures above 40°C (see below). At 25°C and in the presence of 100 mM KCl, Int has more nicking-closing activity at pH 8.0 than at pH 7.4. The ladder pattern of partially relaxed DNA is unchanged if reaction mixtures are extracted with phenol or treated with Pronase (1 mg/ml) prior to electrophoresis.

In contrast to the specificity for attachment sites shown by Int in its role in integrative recombination and in the formation of nonfilterable complexes with DNA (6), nicking-closing by Int appears to be nonspecific. In Fig. 2A (lanes 2 and 4) it can be seen that supertwisted DNA from the plasmid pBR322 is relaxed at least as efficiently as that from pYK100, a derivative of pBR322 that carries *att*P. Supertwisted circles of pBR322 derivatives carrying *att*B, supercoils of λ*att*B–*att*P, and replicative form I of bacteriophage φX174 are also relaxed efficiently by Int (data not shown). Detailed comparisons between these various substrates must await analysis by a more quantitative assay.

The nicking-closing activity of Int is inhibited by spermidine (Fig. 2A, lanes 4 and 5). Inhibition is seen at concentrations of spermidine above 5 mM and is observed equally with substrates containing or lacking an attachment site. Our previous search for the enzymatic activities of a purified Int preparation (5, 6) was carried out under conditions suitable for recombination; as a result, spermidine (6 mM) was included in those reactions and the nicking-closing activity of Int was obscured. Magnesium ions also inhibit the nicking-closing activity of Int: when EDTA is omitted from reaction mixtures, concentrations of $MgCl_2$ greater than 5 mM are inhibitory (data not shown). It remains to be determined whether inhibition reflects an interaction between spermidine and Mg^{2+} with Int or results from an effect of these agents on DNA structure. Inhibition of the nicking-closing activity of Int is observed under two other conditions. First, relaxation of supertwists is decreased in incubations in which more than 50 Int molecules are included per plasmid DNA molecule; under these conditions, nicking-closing activity is restored by an increment in the concentration of DNA. Second, single-stranded DNA is a potent inhibitor of the topoisomerase activity of Int. Concentrations of bacteriophage fd DNA (Bethesda Research Laboratories) as low as 20 μg/ml abolish nicking-closing activity; double-helical DNA is not an inhibitor at these concentrations.

Incubation of relaxed closed circular DNA with Int in the presence of ethidium bromide yields DNA that appears supercoiled when the dye is removed (Fig. 2B). Therefore, as in the case of DNA gyrase (9) and a eukaryotic nicking-closing enzyme (17), Int can relax positive as well as negative supercoils. No apparent preference for relaxation of either species was observed.

Association of nicking-closing activity with the *int* gene product

Several lines of evidence strongly support the identification of the nicking-closing activity present in our highly purified Int preparation with the *int* gene product itself. First, nicking-closing activity cosediments with the recombination activity of Int, a significant result because the purification of Int involves no fractionation that is based on size. Fig. 3 shows the results of the centrifugation of purified Int through a glycerol gradient. In the upper portion of the figure, we present assays of the ability of Int to carry out integrative recombination. For this

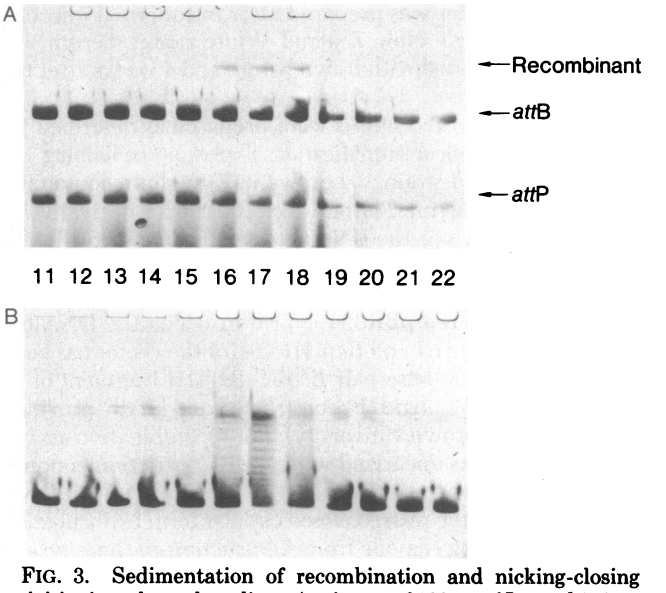

FIG. 3. Sedimentation of recombination and nicking-closing activities in a glycerol gradient. A mixture of 180 μg of Int and 360 μg of bovine serum albumin was centrifuged through a 15–30% (vol/vol) linear glycerol gradient (5 ml) containing 0.4 M KCl, 50 mM Tris·HCl (pH 7.4), 1 mM sodium EDTA, and 1 mM 2-mercaptoethanol in a Beckman SW 50.1 rotor for 21 hr at 50,000 rpm, 3°C. A total of 23 fractions was collected from the bottom of the tube. (*A*) A mixture of 0.75 μg of pYK100 supertwisted DNA and 1 μg of pBB105 linear DNA was incubated with 0.5-μl aliquots of each glycerol gradient fraction for 2 hr in a recombination assay like that described in *Materials and Methods* except that the spermidine concentration was 12.5 mM. The positions of substrate and recombinant DNAs after agarose gel electrophoresis are indicated. (*B*) pYK100 supertwisted DNA (0.75 μg) was incubated with 1-μl aliquots of each glycerol gradient fraction in a nicking-closing assay for 2 hr at 25°C. No activity was recovered in either assay from fractions 1–10.

test we have used a modification of the method of Mizuuchi and Mizuuchi (4) in which supertwisted DNA carrying *att*P is recombined with linear DNA carrying *att*B to produce a linear recombinant whose length is the sum of the two substrate species. Relative to an internal bovine serum albumin standard ($s_{20,w}$ = 4.3 S), this assay shows that recombination activity migrates with a sedimentation coefficient of 3.0 S, in agreement with our earlier results (5, 6). In addition, $NaDodSO_4$/acrylamide gel electrophoresis of aliquots of the glycerol gradient fractions showed that the 40,000-dalton Int polypeptide cosediments with recombination activity (data not shown). The lower portion of Fig. 3 presents assays of nicking-closing activity; the identity between fractions with recombination activity and fractions with nicking-closing activity is apparent. Activity is detected only in one region of the gradient; we estimate that more than half of the recombination activity and more than half of the nicking-closing activity applied to the gradient are recovered from this peak.

Thermal inactivation provides a second line of evidence supporting the identity of nicking-closing activity with the *int* gene product. Fig. 4A (lanes 1–6) shows that when Int is heated for various times at 40°C recombination activity and nicking-closing activity are inactivated in parallel. Other studies showed the inactivation of both nicking-closing and recombination activities by antiserum to purified Int. When increasing amounts of antiserum were added to a fixed amount of Int, both activities were progressively inhibited (Fig. 4B, lanes 1–3). Analogous treatment with preimmunization serum caused little change in either activity (Fig. 4B, lanes 4–6).

The properties of the nicking-closing activity in purified Int preparations distinguish it from the two known *E. coli* enzymes

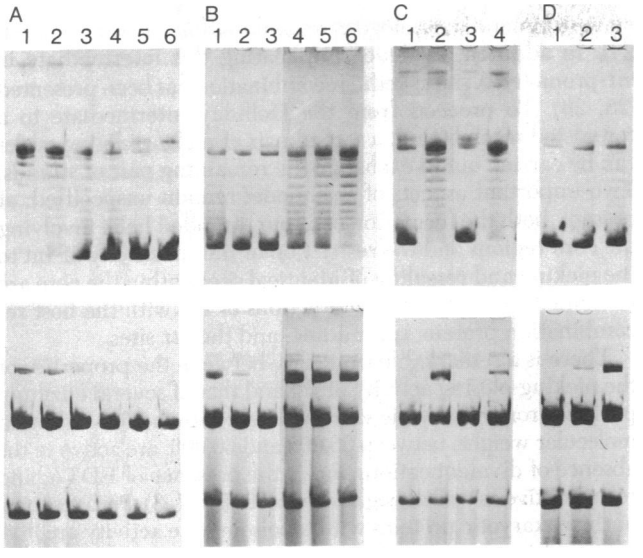

FIG. 4. Comparison between nicking-closing and recombination activities. (*A*) Int (5 μg in 10 μl) was incubated at 40°C in 50 mM Tris·HCl (pH 7.4) containing 0.6 M KCl, 25 mM potassium phosphate (pH 7.4), 10% (vol/vol) glycerol, 1 mM sodium EDTA, 1 mM 2-mercaptoethanol, and bovine serum albumin at 2 mg/ml. At various times (lane 1, 0 min; lane 2, 1 min; lane 3, 2 min; lane 4, 4 min; lane 5, 8 min; lane 6, 16 min) a portion was withdrawn and chilled. A 0.5-μl aliquot from each of the treated samples was assayed for nicking-closing activity with 0.9 μg of pBR322 supertwisted DNA (upper panel). A 1-μl aliquot from each treated sample was assayed for recombination activity (lower panel) as described for Fig. 3. (*B*) Various amounts of rabbit serum were incubated for 30 min at 0°C with 0.5 μg of Int together with 60 μg of bovine serum albumin. Identical samples were then assayed for nicking-closing activity (upper panel) after the addition of 0.9 μg of pYK100 supertwisted DNA and for recombination activity (lower panel) after addition of 0.9 μg of pYK100 supertwisted DNA and 1.6 μg of pBB105 linear DNA. Lane 1, 0.33 μl of anti-Int serum; lane 2, 1 μl of anti-Int serum; lane 3, 3 μl of anti-Int serum; lane 4, 0.33 μl of preimmunization serum; lane 5, 1 μl of preimmunization serum; lane 6, 3 μl of preimmunization serum. (*C*) Crude extracts from strain HN496, carrying the *int* ts2004 (temperature sensitive) gene, and strain HN 497, carrying the wild-type *int* gene, were assayed for nicking-closing (upper panel) and recombination (lower panel) activity for the indicated times and at the indicated temperatures, using DNA substrates as described in Fig. 3. Lane 1, int^{ts}, 20°C, 4 hr; lane 2, int^{+}, 20°C, 4 hr; lane 3, int^{ts}, 33°C, 3 hr; lane 4, int^{+}, 33°C, 3 hr. (*D*) Nicking-closing activity (upper panel) with 0.75 μg of pYK100 supertwisted DNA and recombination activity (lower panel) with 0.75 μg of pYK100 supertwisted DNA and 1.0 μg of pBB105 linear DNA were measured. Lane 1, Int omitted; lane 2, 1.5 μl of a sample of Int (fraction 17 of the glycerol gradient of Fig. 3) that had been treated with 7 mM *N*-ethylmaleimide (MalNEt) for 15 min at 25°C and then exposed to 14 mM dithiothreitol; lane 3, an identical sample of Int that had been incubated for 15 min with a solution containing 7 mM MalNEt and 14 mM dithiothreitol.

that can relax supertwisted DNA, ω protein and DNA gyrase (*E. coli* topoisomerases I and II). The nicking-closing activity of Int: does not require magnesium ions, as do gyrase and ω (7, 8); is not affected by the inhibitors of gyrase, nalidixic acid (200 μg/ml) or oxolinic acid (200 μg/ml); and is not inhibited by antiserum to ω protein (kindly provided by M. Gellert). Moreover, the subunits of DNA gyrase and ω have molecular weights of 90,000–110,000 whereas the nicking-closing activity in Int preparations appears to be associated with a polypeptide with a molecular weight of 40,000 (see above).

Finally, the behavior of a mutant *int* gene product supports the designation of nicking-closing as an activity of Int. We constructed a pair of hybrid plasmids in which the *int* gene was expressed constitutively. These plasmids were isogenic except that one carried a wild-type *int* gene and the other an *int* gene bearing a temperature-sensitive mutation. We prepared crude extracts of cells carrying one or the other of these plasmids and assayed the extracts for recombination and nicking-closing activity. In agreement with earlier observations (18), the temperature-sensitive *int* mutation yielded extracts that carried out integrative recombination rather poorly even at low temperatures (Fig. 4*C*, lane 1). Incubation at 33°C obliterated this small amount of recombination activity and had a smaller effect on recombination carried out with extracts containing wild-type *int* gene product (Fig. 4*C*, lanes 3 and 4). The nicking-closing activity in these crude extracts was commensurate with their recombination activity. Extracts containing wild-type *int* displayed substantial nicking-closing activity at both 20°C and 33°C; in contrast, extracts containing the temperature-sensitive *int* gene product showed weak nicking-closing activity at 20°C and none at 33°C. Because the *int* genotype carried by the plasmid is the only difference between the two strains used to prepare the crude extracts for this experiment, it appears that nicking-closing activity directly depends upon the *int* gene.

Not all treatments of Int affect nicking-closing and recombination identically. We have already mentioned that spermidine inhibits nicking-closing but activates recombination. Fig. 4*D* (lanes 1–3) shows that pretreatment of Int with MalNEt strongly inactivated the recombination activity of Int but had little effect on its nicking-closing activity.

DISCUSSION

The demonstration that the *int* gene product has nicking-closing activity leads to two proposals concerning this protein. First, until now there has been no evidence indicating whether Int or the host protein that is required for recombination is responsible for the breakage and reunion of DNA during integration. Because Int can break and reseal strands of DNA, parsimony suggests that it bears the catalytic site that accomplishes breakage and reunion during strand exchange and therefore that the host protein plays a more subsidiary role. This suggestion is especially attractive because both the relaxation of supertwisted DNA (7, 8) and the strand exchange of integrative recombination (3) appear to proceed by mechanisms that conserve the energy of the phosphodiester backbone. We present below one mechanism by which an activity that breaks and reseals individual strands of a double helix might be used to exchange strands between two double helices. The second hypothesis suggested by the present work is that Int is bifunctional. Our earlier studies showed that Int forms complexes with *att*P that are long-lived and stable to attack by heparin (5, 6). This heparin-resistant specific binding of DNA is inhibited by prior treatment of Int with MalNEt (unpublished results), as is the recombination activity of Int (5). In contrast, the nicking-closing activity of Int has no obvious sequence specificity and is relatively resistant to pretreatment with MalNEt. It is tempting to speculate that Int has two domains, one (MalNEt-sensitive) responsible for the recognition of *att*P and the correct placement of Int on substrate DNA and the other (MalNEt-resistant) responsible for the breakage and resealing of DNA.

How does the nicking-closing activity of Int fit in with its role in recombination? It seems clear that extensive relaxation of supertwists is not a significant feature of integrative recombination. An earlier study of the circular products of intramolecular integrative recombination showed that no major change in the degree of supertwisting need occur as a result of recombination (3). This is not surprising, because recombination assays are routinely carried out in the presence of spermidine, a potent inhibitor of the nicking-closing activity of Int. Although this

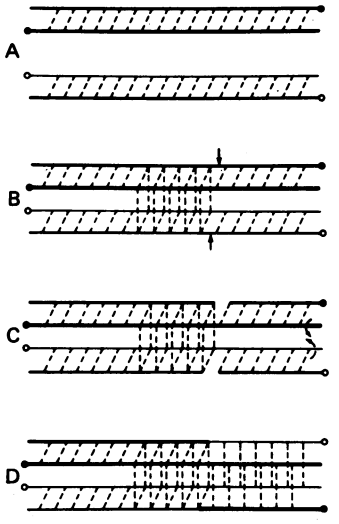

FIG. 5. Model for strand exchange by topoisomerases. (*A*) Two segments of double helix containing *att*P and *att*B are diagramed; the broken lines indicate hydrogen bonding of Watson–Crick pairs and the circles mark the polarity of each strand. (*B*) Some or all of the homologous portions of the two DNAs form a four-stranded helix; the broken lines indicate hydrogen bonds connecting sister and nonsister bases. Arrows indicate the points of attack by the nicking-closing activities of two Int molecules. (*C*) The transiently disrupted strands swivel as indicated around their intact sister strands. (*D*) After 270° rotations the disruptions are sealed, creating a pair of recombined strands.

polyamine limits the nicking-closing activity of Int, we think it likely that in complete recombination mixtures the catalytic function of Int that breaks and reseals DNA remains active but is used in a more selective way. We now suggest two different ways in which breakage and resealing of DNA strands can be used in recombination.

First, transient disruption of DNA strands can facilitate the pairing of two double helices. Such a role for topoisomerases was proposed by Champoux (19), and evidence has been presented for the *in vitro* synapsis of homologous DNA by this mechanism (20); it will not be discussed further here. The second possible role for the topoisomerase activity of Int addresses the problem of strand exchange after synapsis has occurred. Specifically, we assume that, within the limits of the short stretch of DNA that is identical in *att*B and *att*P (21), a structure involving all four strands of two attachment sites has formed (Fig. 5). McGavin (22) has constructed one such four-stranded helix; in his model, the four bases in each step of the helix are positioned around a nearly perfect square and are stabilized by a network of hydrogen bonds. Recently, Wilson (23) has clarified the topological requirements of four-strand helices and has demonstrated that a somewhat different model can also be built. We imagine that *two* Int molecules act simultaneously at one boundary of a four-stranded structure. Nicks are introduced at identical positions into strands of the same polarity and each nicked strand swivels around its partner strand. In contrast to the situation with isolated double helices, before a 360° rotation is complete, each nicked strand from one parent is brought adjacent to an Int covalently attached to the nicked strand of the other parent. If ligation occurs at this point, the result is a pair of strand exchanges.* Such a crossed strand exchange, or Holliday structure, has been widely postulated as an intermediate in general, homologous recombination (reviewed in ref. 24). In addition, evidence implicating this intermediate in *int*-promoted λ site specific recombination has been presented (25, 26). To proceed from the Holliday intermediate to a completed recombinant, a second pair of nicking-closing cycles can be carried out to exchange the remaining pair of strands. Two important aspects of this model remain unspecified; at present, both the formation of a four-stranded helix involving the core regions and the restriction of the specificity of Int to the nicking and resealing of identical sites within the core are presumed to result from interactions of Int with the host recombination protein, spermidine, and the *att* sites.

There is a remarkable similarity between the properties of the nicking-closing activity of Int and that of several enzymes purified from eukaryotic sources. These enzymes have subunit molecular weights between 30,000 and 60,000, are active in the absence of divalent cations and in the presence of EDTA, and relax positive as well as negative supertwists (7, 8). Perhaps some of the eukaryotic proteins with topoisomerase activity are, like Int, used in more complex ways as part of a recombination pathway.

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* J. Wilson (personal communication) and C. W. Chen (personal communication) have independently pointed out that strand exchange could follow from the combined action of a nuclease and a ligase on four-stranded DNA.