Novel Approach to Molecular Cloning and Polynucleotide Synthesis Using Vaccinia DNA Topoisomerase*

(Received for publication, August 15, 1994, and in revised form, October 19, 1994)

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Construction of chimaeric DNA molecules in vitro relies traditionally on two enzymatic steps catalyzed by separate protein components. Site-specific restriction endonucleases are used to generate linear DNAs with defined termini that can then be joined covalently at their ends via the action of DNA ligase. A novel approach to the synthesis of recombinant DNAs exploits the ability of a single enzyme, vaccinia DNA topoisomerase, to both cleave and religate DNA strands with extreme specificity at each step. Placement of the CCCTT cleavage motif for vaccinia topoisomerase near the end of a duplex DNA permits efficient generation of a stable, highly recombinogenic protein-DNA adduct that can religate only to acceptor DNAs that contain complementary single-strand extensions. Linear DNAs containing CCCTT cleavage sites at both ends (bivalent substrates) can be activated by topoisomerase and inserted into a plasmid vector in a simple and rapid in vitro procedure that is especially well suited to the molecular cloning of polynucleotide chain reaction-amplified DNAs. Activation of polynucleotide (e.g., branched) DNA substrates by topoisomerase offers a potentially powerful method for the synthesis of two- and three-dimensional polynucleotide networks.

Vaccinia DNA topoisomerase, a 314-amino acid virus-encoded eukaryotic type I topoisomerase (Shuman and Moss, 1987), binds to duplex DNA and cleaves the phosphodiester backbone of one strand. The enzyme exhibits a high level of sequence specificity, akin to that of a restriction endonuclease. Cleavage occurs at a consensus pentapyrimidine element 5'-C/TCCCTT1 in the scissile strand (Shuman and Prescott, 1990; Shuman, 1991a, 1991b). In the cleavage reaction, bond energy is conserved via the formation of a covalent adduct between the 3'-phosphate of the incised strand and a tyrosyl residue (Tyr-274) of the protein (Shuman et al., 1989). Vaccinia topoisomerase can religate the covalently held strand across the same bond originally cleaved (as occurs during DNA relaxation), or it can religate to a heterologous acceptor DNA and thereby create a recombinant molecule (Shuman, 1992a, 1992b). The repertoire of DNA joining reactions catalyzed by vaccinia topoisomerase has been studied using synthetic duplex DNA substrates containing a single CCCTT cleavage site. When the substrate is configured such that the scissile bond is situated near (within 10 bp1 of) the 3'-end of a DNA duplex, cleavage is accompanied by spontaneous dissociation of the downstream portion of the cleaved strand (Morham and Shuman, 1992). The resulting topoisomerase-DNA complex, containing a 5'-single-stranded tail, can religate to an acceptor DNA if the acceptor molecule has a 5'-OH tail complementary to that of the activated donor complex. Sticky-end ligation by vaccinia topoisomerase has been employed using plasmid DNA acceptors with 4-base overhangs created by restriction endonuclease digestion (Shuman, 1992b).

The specificity of vaccinia topoisomerase in DNA cleavage and religation properties of new recombinases of this type have now inspired a novel topoisomerase-mediated strategy for polynucleotide synthesis in which oligonucleotides containing CCCTT cleavage sites serve as activated linkers for the joining of DNA molecules with compatible termini. The utility of this strategy for in vitro end labeling, ligand tagging, and molecular cloning of DNA molecules is demonstrated. Additional prospects for topoisomerase-based synthesis of DNA networks are considered.

EXPERIMENTAL PROCEDURES

Enzyme Purification—Vaccinia DNA topoisomerase was expressed in Escherichia coli and purified as described (Shuman et al., 1988). The heparin-agarose enzyme fraction used in the present study was the same preparation described previously (Shuman et al., 1988). The enzyme was nearly homogeneous with respect to the 33-kDa topoisomerase polypeptide, as determined by SDS-polyacylamide gel electrophoresis. Protein concentration was determined using the Bio-Rad dye assay.

Labeled Oligonucleotide Substrates—Synthesis of DNA oligonucleotides containing the CCCTT cleavage motif was performed by the Sloan-Kettering Microchemistry Laboratory using an Applied Biosystems model 380B or model 394 automated DNA synthesizer according to protocols specified by the manufacturer. Oligonucleotides containing the CCCTT cleavage motif were labeled at the 5' end via enzymatic phosphorylation in the presence of [γ-32P]ATP and T4 polynucleotide kinase. Reaction mixtures (25 μl) typically contained 50 mM Tris-HCl (pH 8.0), 10 mM dithiothreitol, 10 mM MgCl2, 0.1 mM ATP, 100 μCi of [γ-32P]ATP, T4 polynucleotide kinase (20 units, Life Technologies, Inc.), and 500 pmol of DNA oligonucleotide (DNA was quantitated by A260). Incubation was for 60 min at 37 °C. Labeled DNA was freed of protein and radioactive nucleotide by electrophoresis through a nondenaturing 18% polyacrylamide gel. Full-sized labeled oligonucleotide was localized by autoradiographic exposure of the wet gel, and the labeled DNA was recovered from an excised gel slice by soaking the slice in 0.4 ml of H2O for 8 h at room temperature. Hybridization of labeled DNAs to complementary oligonucleotides was performed in 0.2 x Na2S by heating to 75 °C followed by slow cooling to room temperature. Annealed substrates were stored at 4 °C.

Topoisomerase-based Cloning—Reaction mixtures containing 50 mM Tris-HCl (pH 7.5), 2 μmol of topoisomerase, and either monovalent linker (0.6 pmol) or bivalent linker (0.3 pmol) were incubated for 5 min at 37 °C. A control reaction contained topoisomerase but no DNA substrate. Each mixture was then supplemented with 5'-OH HindIII-cleaved pUC18 DNA acceptor (380 fmol of ends) and incubated for another 5 min at room temperature. An aliquot (1 μl) of each sample was used to transform E. coli DH5α using a Bio-Rad Gene Pulser electroporation apparatus. Preparation of bacterial cells and electrot transformation were carried out as prescribed by the manufacturer. Aliquots of transformed bacteria were plated on LB agar containing 0.1 mg/ml ampicillin.

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‡ The abbreviations used are: bp, base pair(s); kbp, kilobase pair(s); PCR, polymerase chain reaction.
complexes were trapped by addition of SDS to 1%. Samples were then electrophoresed through a 1.2% agarose gel in TBE buffer. The extent of adduct formation was quantitated by scintillation counting of an excised gel slice containing the labeled DNA. The addition of unlabeled DNA to the topoisomerase polypeptide as detected by autoradiographic exposure of the dried gel. The extent of adduct formation was quantitated by scintillation counting of an excised gel slice containing the labeled DNA.

**RESULTS**

Sticky-end Ligation—The vaccinia topoisomerase was capable of sticky-end ligation of duplex DNAs containing only 2 bases of potential complementarity, as shown in Fig. 1. In this experiment, the “donor” was a 24-mer hairpin oligonucleotide containing a single CCCCTT motif (a “monovalent” substrate) with the scissile bond located 2 bases from the 3’-blunt end (Fig. 1A). The extent of cleavage of this substrate was proportional to enzyme concentration (Fig. 1A). The topoisomerase-DNA complex migrated as a discrete species during native gel electrophoresis (Fig. 1C). The addition of unlabeled 5’-hydroxyl-terminated Cpg-tailed linear pUC18 DNA (generated by digestion of pUC DNA with AccI followed by treatment with alkaline phosphatase) resulted in transfer of the topoisomerase-bound DNA strand to the linear DNA “acceptor.” The product of the strand transfer reaction was a radiolabeled 2.7-kbp linear form containing a hairpin end (Fig. 1C, lane 2). Accl-restricted plasmid DNA containing a 5’-phosphate terminus was inert as an acceptor (Fig. 1C, lane 3). (The requirement for a 5’-OH-terminated acceptor excluded the possibility that the reaction products might be formed by a conventional DNA ligase contaminating the topoisomerase preparation.) Linear plasmid DNA containing noncomplementary 5’-OH overhangs generated by restriction with EcoRI (5’-AATT) or HindIII (5’-AGCT) were ineffective as acceptors (Fig. 1C, lanes 4 and 6), as was 5’-OH blunt-ended linear DNA generated by restriction with Smal (lane 5).

**Divalent Linkers as Donors**—Two 46-mer DNA strands were annealed to form a “divalent” 46-bp substrate containing a topoisomerase cleavage site 4 nucleotides from each 3’-end (Fig. 2). Successful annealing of the constituent strands was evinced by the reduced mobility of the duplex molecule during native gel electrophoresis (Fig. 3A, lane 2) compared with that of the hairpin DNA (Fig. 3A, lane 1). Either the “flip” or “flop” monovalent hairpins were readily cleaved by vaccinia topoisomerase, resulting in the formation of a covalent protein-DNA adduct that migrated at 43 kDa during SDS-polyacrylamide gel electrophoresis (Fig. 3B, lanes 2 and 4). Incubation of topoisomerase with the divalent duplex substrate yielded two complexes of 46 and 72 kDa; the 46-kDa species represents a single.
molecule of topoisomerase bound covalently at one of the CCGT cleavage sites; the 72-kDa complex arises by cleavage at both sites on the same DNA molecule (Fig. 3B, lanes 6 and 8).

The monovalent hairpin DNA was transferred virtually quantitatively to linear pUC DNA containing a complementary 5′-OH-AGCT overhang (Fig. 4, lane 2). Incubation of the bivalent topoisomerase-DNA complex with the same acceptor yielded a complex set of products arising from ligation of the bivalent linker to two complementary ends of the linear pUC acceptor (Fig. 4, lane 4). These included circular pUC and linear pUC concatamers. A significant fraction of the pUC acceptor molecules were subject to bivalent end joining, as reflected in the distribution of EtBr-stained DNA products (Fig. 4, lane 4, left panel). All ligation events were via the radiolabeled linker DNA, which became incorporated into the reaction products (Fig. 4, lane 4, right panel).

**Molecular Cloning of DNA Using Vaccinia Topoisomerase**—The ability of topoisomerase to join both ends of a linear DNA to a complementary acceptor suggested an alternative approach to molecular cloning. In the scheme shown in Fig. 5, the “insert” was a bivalent 46-bp linker containing CCGT sites at both 3′-ends. The sequence of the linker included restriction sites for endonucleases NdeI, BglII, and EcoRV. Cleavage of the bivalent linker by topoisomerase generated a 4-base overhang complementary to a HindIII restriction site. The “vector” was pUC DNA that had been cleaved with HindIII and dephosphorylated with alkaline phosphatase. Addition of the vector to the bivalent topoisomerase-DNA donor complex should result in covalent joining of the insert to the vector. Upon transformation into E. coli, those molecules that had been circularized should be able to give rise to ampicillin-resistant colonies. It was found that the yield of ampicillin-resistant colonies from bacteria transformed with a topoisomerase reaction mixture containing linear pUC and the bivalent linker was 110-fold higher than that observed for bacteria transformed with control topoisomerase reactions containing linear pUC and either monovalent linker or no linker.

Plasmid DNA was recovered from cultures of six individual transformants and analyzed by restriction endonuclease digestion in parallel with pUC18 plasmid DNA (Fig. 5). (The restriction pattern for the recombinant clone pUC-T11 shown in Fig. 5 was indistinguishable from that of the five other clones, which are not shown.) Whereas the starting pUC18 plasmid...
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Fig. 5. Molecular cloning of DNA using vaccinia topoisomerase. Ligation reactions for topoisomerase-based cloning were performed as described under “Experimental Procedures.” The protocol is illustrated schematically at the left. The structure of the 46-bp bivalent linker is indicated at the lower right. Diagnostic restriction sites within the linker are specified above the sequence. Plasmid DNA was prepared from bacteria containing pUC18 (the parent vector) and pUC-T11 (a representative transformant from the topoisomerase ligation reaction). DNA was digested with the restriction endonucleases specified above each lane using reaction buffers provided by the vendor. Undigested plasmid DNA is shown in lane 1. Lane M contains DNA size markers. The positions and sizes (kbp) of reference fragments are indicated.

The recombinant molecules generated by topoisomerase-mediated end joining were analyzed further by digestion with restriction endonucleases that cleave once within the pUC sequence. In Fig. 7, the anticipated products of bivalent end joining by topoisomerase are shown, along with the restriction fragments expected for each product upon digestion with SspI and XmnI. The products of trivalent end joining are illustrated in Fig. 8. Experimental results showing the spectrum of strand transfer products after digestion with SspI and XmnI are shown in Fig. 9. In this analysis, each linker, which upon cleavage generated a tailed donor complex compatible with a HindIII restriction site, was tested with two acceptor molecules, one bivalent and one monovalent. The bivalent acceptor was linear pUC19 containing 5'-OH HindIII overhangs on both ends. Strand transfer of a polyvalent linker to the bivalent acceptor allows for the formation of circular and linear concatamers in a head-to-head, tail-to-tail, or head-to-tail fashion, as shown in Fig. 7. The monovalent acceptor was pUC19 containing a 5'-OH HindIII site at one end and a 5'-phosphate AclI site at the other end. Transfer of the linker by topoisomerase to the AclI terminus is precluded completely on two grounds: first, because the ends are not complementary, and second, because topoisomerase cannot religate to a 5'-phosphate strand. A monovalent acceptor will react with the topoisomerase donor complex at available compatible termini but will not be able to form circles or concatameric arrays. The structures of the various species can thus be inferred by direct comparison of the restriction digests from reactions in which monovalent, bivalent, and trivalent linkers were reacted with monovalent and bivalent acceptors.

The SspI digests of topoisomerase strand transfer products in Fig. 9A. The monovalent linker was joined to either end of the bivalent pUC19 acceptor but could not support circularization or dimerization. Hence the products were cleaved by SspI to yield two fragments derived from linear monomers (Fig. 9A, lane 1; see Fig. 7). Ligation of the bivalent linker to bivalent acceptor yielded three additional products: a 4.1-kbp fragment diagnostic of head-to-head multimer formation, a 1.3-kbp fragment indicative of tail-to-tail ligation, and a 2.7-kbp species that derived from a circular molecule (Fig. 9A, lane 3).
The annealed product was analyzed by electrophoresis through a native 7.5% polyacrylamide gel. The bivalent substrate (S304, trivalent substrate is in monovalent and gel purified. The Y-branched substrate was generated by annealing equimolar amounts of the three strands (S300, S303, S304).

pUC18 DNA acceptor (570 fmol of ends) as indicated and incubated for another 5 min at room temperature. Samples were adjusted to 0.2 and 0.5% SDS, then electrophoresed through a 1.2% agarose gel in TBE. The EtBr-stained gel is shown at the right, and gel purified. The Y-branched substrate was generated by annealing equimolar amounts of the three strands (S300, S303, S304).

After incubation for 5 min at 37 °C, the reactions were supplemented with 5'-OH HindIII-cut pUC acceptors and anticipated ligation restriction sites for Y-linker and bivalent acceptor yielded two novel high molecular weight products not observed for the bivalent linker (Fig. 9A, lane 5). The length of each arm is predicted to be 2 kbp. The electrophoretic mobility of this species was anomalously slow, as expected for a branched DNA. The higher order complex unique to the bivalent acceptor was presumed to be a Y-branched product containing pUC19 DNA ligated in a mixed head-to-head and head-to-tail orientation.

Digestion of the strand transfer products with XmnI confirmed and extended these findings (Fig. 9B). The digest of a reaction containing labeled bivalent linker and unlabeled bivalent pUC acceptor yielded diagnostic linear fragments of 3.7 kbp (head-to-head multimer), 1.7 kbp (tail-to-tail multimer), and 2.7 kbp (circle). These products were detected by EtBr staining and by autoradiography (Fig. 9B, lane 1). The 1.7-kbp species indicative of tail-to-tail ligation migrated just ahead of a 1.85-kbp fragment (derived either from end-tagged linear

**Fig. 6.** Topoisomerase-mediated joining of two ends via a trivalent linker. Panel A, each strand of the trivalent substrate (Fig. 2) was 5'-labeled and gel purified. The Y-branched substrate was generated by annealing equimolar amounts of the three strands (S300, S303, S304). The annealed product was analyzed by electrophoresis through a native 7.5% polyacrylamide gel. An autoradiograph of the gel is shown. The trivalent substrate is in lane 3. Component strands were analyzed in parallel (S303 in lane 1; S304 in lane 2). The structures of the labeled species are indicated at the right. Panel B, reaction mixtures (20 pl) contained 50 mTris-HCl (pH 7.5), 1 pmol of topoisomerase, and either 5'-32P-labeled monovalent substrate (S304, lanes 1 and 2) or 5'-32P-labeled trivalent linker (+S300/-S303/+S304, lanes 3 and 4). Each reaction contained 350 fmol of input substrate (expressed as cleavable ends). After incubation for 5 min at 37 °C, the reactions were supplemented with 5'-OH HindIII-cut pUC18 DNA acceptor (570 fmol of ends) as indicated and incubated for another 5 min at room temperature. Samples were adjusted to 0.2 M NaCl and 0.5% SDS, then electrophoresed through a 1.2% agarose gel in TBE. The EtBr-stained gel is shown at the left. The positions and sizes (kbp) of marker DNA fragments (lane M) are indicated at the left. The same gel was dried and exposed for autoradiography (right panel). The positions of the radial-labeled topoisomerase-DNA donor complex and the strand transfer products are indicated at the right by arrows and brackets.

Ligation of the bivalent linker to a monovalent acceptor yielded the 4.1-kbp head-to-head fragment but no fragments indicative of tail-to-tail or circular products (Fig. 9A, compare lanes 3 and 4). This was precisely as expected, because the AccI "tail" was inert for strand transfer. Reactions containing the trivalent Y-linker and bivalent acceptor yielded two novel high molecular weight products not observed for the bivalent linker (Fig. 9A, lane 5). The largest product (indicated by the arrowhead in Fig. 9A), which was also observed with trivalent linker and monovalent acceptor (lane 6), must correspond to a Y-branched recombinant containing three pUC molecules ligated in head-to-head fashion. The length of each arm is predicted to be 2 kbp. The electrophoretic mobility of this species was anomalously slow, as expected for a branched DNA. The higher order complex unique to the bivalent acceptor was presumed to be a Y-branched product containing pUC19 DNA ligated in a mixed head-to-head and head-to-tail orientation.

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**DISCUSSION**

The present study demonstrates the utility of vaccinia topoisomerase in both end modification and end joining of DNAs. The basic principle is that topoisomerase can covalently activate any DNA end containing a 3'-proximal CCCTT element and transfer the activated strand to a complementary acceptor DNA. The potential applications of topoisomerase as a synthetic tool are numerous and differ according to the valence of the ligation product. Monovalent and bivalent acceptors, a property that can be exploited for molecular cloning.

In this study, the topoisomerase cleavage sites were placed on the insert (a synthetic bivalent substrate) and cloned the digested DNA into a plasmid vector, which is compatible with the restriction site into which the PCR product will ultimately be cloned. The amplification procedure will generate duplex molecules containing the sequence GCCTTXXX-3' at both 3'-ends (where XXX is the complement of XXXX). Incubation of the PCR product with topoisomerase will result in cleavage at both termini and allow the covalently activated PCR fragment to be ligated to vector DNA, essentially as described in Fig. 5.

Topoisomerase-based cloning has several advantages over conventional ligation-based cloning of PCR products. First, the topoisomerase procedure circumvents any problems associated with the addition of noncomplementary nucleotides by DNA polymerase at the 3'-end of the amplified DNA (Clark, 1988). Any nontemplated base (N) at the 3'-end of a PCR product destined for topoisomerase-based cloning (GCCCTTXXX-3') will dissociate spontaneously upon covalent addition and will therefore have no impact on the ligation to vector. Second, in topoisomerase-mediated cloning, the only molecule that can possibly be ligated is the covalently activated insert, and the insert can only be transferred to the vector. There is no potential for in vitro covalent closure of the vector itself, which ensures low background. There is also no opportunity for the inserts to ligate to each other (this can be guaranteed by using 5'-phosphate-terminated PCR primers), which precludes cloning of concatameric repeats. Third, there is no need to consider the sequence of the DNA being amplified in designing the PCR primers. It is commonplace in standard cloning to introduce a restriction site into the PCR primer and to cleave the PCR products with that restriction enzyme to facilitate joining by ligase to vector. In cases in which the sequence between the primers is not already known, it becomes problematic to choose a site for the primer which is not present in the amplified segment. This issue becomes even more relevant as PCR methodology advances and very long targets (10–40 kbp) are amplified routinely (Cheng et al., 1994). The issue of internal topoisomerase cleavage sites (CCCTT or related pentapyrimidine elements) is not a significant impediment to topoisomerase-
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based cloning. This is because the cleavage-religation equilibrium at internal sites strongly favors the noncovalently bound state (Shuman and Prescott, 1990; Stivers et al., 1994), and at those sites that are incised, only one strand of the duplex is nicked. Internal cleavage sites can be induced to religate by raising the salt concentration, which serves to dissociate noncovalently bound topoisomerase and drive the reaction equilibrium to the left (Shuman and Prescott, 1990). In contrast, cleavage at sites near the 3'-end is virtually quantitative and is essentially irreversible until an acceptor DNA is provided.

Topoisomerase-based cloning strategies need not be limited to covalent activation of the insert. By designing a plasmid polylinker such that CCCCTT sites are situated in inverted orientation on either side of a restriction site, one can generate a linear vector with topoisomerase sites at both 3'-ends. Once covalently activated by topoisomerase, the vector donor can be used to clone any complementary insert acceptor (which must have 5'-OH termini). In this scenario, religation of vector without insert is precluded. It is worth noting that the donor complex formed upon cleavage by topoisomerase at a 3'-proximal site is extremely stable. The donor molecule can be transferred nearly quantitatively to a complementary acceptor even after many hours of incubation of the covalent topoisomerase-DNA complex at room temperature. Indeed, the topoisomerase-ligamer complex can be denatured with 6 M guanidine HCl and then renatured spontaneously upon removal of guanidine with complete recovery of strand transferase activity. Thus, a topoisomerase-activated vector can be prepared once in quantity and used as many times as needed for molecular cloning.

Finally, the intent in constructing the trivalent linker was to determine if topoisomerase could be used to synthesize a novel DNA structure, in this case a Y-branched nucleic acid with pUC "arms." That such molecules were indeed produced in the strand transfer reaction containing the trivalent linker was demonstrated by diagnostic restriction digestion of the reaction products. The yield of Y-branched products was relatively low because no attempt was made in the initial study to eliminate residual bivalent and monovalent linkers from the substrate preparation or to ensure that all trivalent linkers were saturated with three bound topoisomerase molecules. Both conditions can be met by gel purifying the linker and by purifying the tricovalently activated species by sedimentation. As with bivalent ligation, the orientation of the Y-branched products can be controlled by manipulating the design of the linker or by using asymmetric acceptors. Any head-to-head-to-head type Y-branched product of trivalent strand transfer can, in theory, be organized into a trivalent lattice by adding a second trivalent donor complex that is complementary to the tail of the original acceptor DNA. There is no inherent reason that donor substrates of higher order valence cannot be used to achieve topoisomerase-based synthesis of three-dimensional lattices and polyhedra from DNA. Chen and Seeman (1991) have demonstrated the assembly of a DNA cube structure from synthetic oligonucleotides using DNA ligase. Topoisomerase-based synthesis offers a potentially powerful alternative strategy for building complex biopolymers.

REFERENCES

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FIG. 9. Restriction endonuclease digestion of end joining reaction products. Panel A, reaction mixtures (20 μl) contained 50 μM Tris-HCl (pH 7.5), 1 pmol of topoisomerase, and either monovalent substrate (lanes 1 and 2), divalent linker (lanes 3–5), or trivalent linker (lanes 5 and 6). After incubation for 5 min at 37 °C, the reactions were supplemented with either 5'-OH HindIII-cut pUC19 bivalent DNA acceptor (600 fmol of linear DNA, lanes 1, 3, and 5) or 5'-OH HindIII/PstI Accl-cut pUC19 monovalent acceptor (500 fmol of linear DNA, lanes 2, 4, and 6) and incubated for another 5 min at room temperature. The mixtures were adjusted to recommended restriction conditions by the addition of 10 mM buffer concentrate (NEB2), and the samples were digested with SstI (10 units; New England BioLabs) for 60 min at 37 °C. Samples were adjusted to 0.5% SDS and electrophoresed through a 1.2% agarose gel in TBE. An EtBr-stained gel is shown. The positions and sizes (kb) of marker DNA fragments (lane M) are indicated at the left. Panel B, cleavage reactions containing radiolabeled bivalent linker (lanes 1 and 2) or trivalent linker (lanes 3–5) were supplemented with divalent pUC19 acceptor (lanes 1 and 3) or monovalent pUC19 acceptor (lanes 2 and 4). A control reaction received no acceptor (lane 5). The strand transfer reaction products were digested with XhoI (40 units) for 2 h at 37 °C and then analyzed by agarose gel electrophoresis. The EtBr-stained gel is shown at left. The positions and sizes (kb) of marker DNA fragments (lanes M) are indicated at the left of the photograph. The same gel was dried and exposed for autoradiography (right panel). The positions of the radiolabeled topoisomerase-DNA donor complex and the strand transfer products are indicated at the right by arrows and brackets.