Communication

Two Classes of DNA End-joining Reactions Catalyzed by Vaccinia Topoisomerase I*

(Received for publication, May 28, 1992)

Stewart Shuman
From the Program in Molecular Biology, Sloan-Kettering Institute, New York, New York 10021

The ability of eukaryotic DNA topoisomerase I to catalyze DNA rearrangements was examined in vitro using defined substrates and purified enzyme. Site-specific DNA strand cleavage by vaccinia topoisomerase I across from a nick generated double-strand breaks that could be religated to a heterologous blunt-ended duplex DNA regardless of the sequence of the internal positions could religate the bound strand to an acceptor molecule. Topoisomerase bound covalently at internal positions could religate the bound strand to an acceptor molecule. Topoisomerase bound covalently at a nick generated double-strand breaks, where the 3'-hydroxyl residue of the enzyme religated to the polarity of the nick (1). Vaccinia topoisomerase selectively binds and incises duplex DNA in vitro at a consensus pentamer sequence 5'-GATCC (6-7). Using short duplex DNA substrates containing a single enzyme binding site, I have demonstrated that vaccinia topoisomerase I can efficiently mediate intermolecular strand transfer to a heterologous acceptor (8).

Vaccinia topoisomerase, a virus-encoded eukaryotic topoi I, can catalyze sequence-specific strand transfer during genetic recombination in vivo (3, 4). The topo-mediated DNA rearrangements (i.e. prophage excision) are distinguished by the presence of an oligopyrimidine binding/cleavage motif for topo I at both recombining half-sites. Vaccinia topoisomerase selectively binds and incises duplex DNA in vitro at a consensus pentamer sequence 5'-GATCC (6-7). Using short duplex DNA substrates containing a single enzyme binding site, I have demonstrated that vaccinia topoisomerase I can efficiently mediate intermolecular strand transfer to a heterologous acceptor (8).

Further examination of the ability of the vaccinia enzyme to join DNA ends now illuminates two possible modes of topo-dependent recombination that share half-site specificity but differ in their requirement for sequence homology.

Eukaryotic DNA topoisomerase I (topo I) is thought to promote illegitimate recombination in animal cells by virtue of its ability to break and rejoin DNA strands (1, 2). Topo I binds to duplex DNA and cleaves the phosphodiester backbone of one strand. Bond energy is conserved via the formation of a covalent adduct between the 3'-phosphate of the incised strand and a tyrosyl residue of the enzyme. Religation of the covalently bound strand across the same bond originally cleaved restores the integrity of the DNA duplex (possibly accompanied by a change in linking number), whereas religation to a heterologous acceptor strand generates a recombinant molecule.

Vaccinia topoisomerase, a virus-encoded eukaryotic topo I, can catalyze sequence-specific strand transfer during genetic recombination in vivo (3, 4). The topo-mediated DNA rearrangements (i.e. prophage excision) are distinguished by the presence of an oligopyrimidine binding/cleavage motif for topo I at both recombining half-sites. Vaccinia topoisomerase selectively binds and incises duplex DNA in vitro at a consensus pentamer sequence 5'-GATCC (6-7). Using short duplex DNA substrates containing a single enzyme binding site, I have demonstrated that vaccinia topoisomerase I can efficiently mediate intermolecular strand transfer to a heterologous acceptor (8).

Further examination of the ability of the vaccinia enzyme to join DNA ends now illuminates two possible modes of topo-dependent recombination that share half-site specificity but differ in their requirement for sequence homology.

EXPERIMENTAL PROCEDURES

All experiments were performed using the heparin-agarose fraction of vaccinia DNA topoisomerase (9). Strand transfer reactions were constituted as described (8); the nature of the cleavable DNA substrate and the heterologous DNA acceptor was varied in each experiment. Preparation of 5'-32P-labeled scissile strand and hybridization to unlabeled complementary oligonucleotides were performed as described (6-8). That the nicked duplex substrate actually contained three hybridized strands was confirmed by native gel electrophoretic analysis as described (7) under conditions where single strands, two-strand-tailed molecules, and three-strand-containing duplexes were resolved. Plasmid DNA used as acceptor in strand transfer reactions was linearized quantitatively by digestion with a single-cut restriction enzyme. The 5'-phosphate terminus of the linear DNA was converted to 5'-OH ends by treatment of the DNAs with calf intestinal phosphatase. Covalent adduct formation between topoisomerase and radio labeled DNA was measured by label transfer to protein as described (7, 8).

RESULTS AND DISCUSSION

Two models have been invoked to account for recombination by vaccinia topo I. According to the "recombination" model (4), topo I forms a covalent intermediate at two sites destined to recombine. The bound-proteins are approximated, and DNA single strands are transferred reciprocally to form a Holliday intermediate, which is then resolved by a separate endonuclease. Nonreciprocal recombination is explained by invasion or uptake of a single strand containing a 5'-hydroxyl terminus that serves as an acceptor for religation. Strand transfer via this pathway (shown in Fig. 1A) is predicted to be facilitated by (if not entirely dependent on) homology of the incoming acceptor strand to the noncovalently bound segment of the scissile strand. Nonreciprocal strand transfer has been demonstrated in vitro using model substrates and either purified vaccinia topo I or human topo I (8, 10). Indeed, transfer to a single-strand acceptor by the vaccinia enzyme required 4 bp of homology 3' of the CCCTT motif (8).

The "deletion" model (1) posits topo I cleavage opposite preexisting nicks or gaps in the nonscissile strand. Such a reaction at two sites on the same DNA strand would generate double-strand breaks at recombining half-sites with liberation of a linear molecule containing one covalently bound topo molecule at the 3' end. Intramolecular recombination of the linear segment yields an extrachromosomal circle; the parental DNA, containing a gapped segment with one covalently activated terminus, can resolve across the gap (with deletion formation) or religate to an unrelated DNA (analogous to a heterologous acceptor strand). Two possible pathways for DNA strand transfer by topo I are illustrated in Fig. 1B (for discussion). Two models are depicted by the filled circle.

* This work was supported by National Institutes of Health Grant GM 46330, American Cancer Society Grant JFRA-274, and a scholarship from the Pew Charitable Trusts. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: topo I, topoisomerase I; bp, base pair(s); kb, kilobase pair(s).

FIG. 1. Two possible pathways for DNA strand transfer by topo I. See "Results and Discussion" for discussion. Topo I is depicted by the filled circle.
translocation event). Strand transfer via this pathway (illustrated in Fig. 1B) might be predicted to be independent of the sequence of the acceptor molecule.

The question of whether vaccinia topo I can induce double-strand breakage and heterologous end joining was examined using synthetic model DNA substrates. A 5'-labeled 24-mer scissile strand containing a single CCCTT motif was hybridized to two 12-mer oligonucleotides complementary to the labeled strand to create a duplex molecule with a strand discontinuity across from the scissile phosphodiester bond; a control duplex substrate contained a continuous nonscissile strand (Fig. 2). Purified topo I formed a covalent adduct with either substrate with similar yield and enzyme concentration (Fig. 2B). Religation of the covalently bound 5'-labeled 12-mer segment of the scissile strand to a heterologous 18-mer acceptor was manifest by the creation of novel 30-mer labeled strand that could be resolved from the original 24-mer substrate by denaturing gel electrophoresis (Fig. 2B). Note that labeled DNA covalently linked to topo did not enter the gel and was therefore not seen in the autoradiogram. In the case of the continuous duplex substrate, strand transfer occurred only with an acceptor strand homologous to the original strand (Fig. 2B, lane 6). (Strand transfer to a homologous single-strand acceptor is described in detail in a recent report from this laboratory (8).) Completely different specificity was observed for the substrate containing an opposing nick in the noncleaved strand. The nicked strand acted as an efficient donor in strand transfer to a blunt-ended duplex acceptor whose sequence was entirely unrelated to that of the 3' segment of the original scissile strand (Fig. 2B, lane 9). Blunt-end ligation to duplex DNA was relatively efficient insofar as recombinant products were also observed at 10-fold lower concentrations of acceptor (i.e. at a 2:1 ratio of acceptor to input donor molecules; data not shown). Individual single strands were entirely inert as acceptors (Fig. 2B, lanes 10 and 11), even when added in vast excess (>100-fold) over input substrate (not shown).

Cleavage by topo I to yield a covalently activated blunt end could also occur on a 3'-tailed duplex substrate, provided that the duplex region encompassed the CCCTT motif (Fig. 3A). Such cleaved molecules could function as donors for religation to a nonspecific blunt-ended duplex acceptor, but not to single strands (Fig. 3B). The ability of 3'-tailed DNAs to be cleaved depended on the length of the single-strand tail; duplex molecules with 6-nucleotide tails were inactive as cleavage substrates, whereas DNAs with 12-mer tails were utilized by topo I. 5' Tailed molecules in which CCCTT was single-stranded were inert for covalent adduct formation (Fig. 3A).

The specificity of vaccinia topoisomerase for blunt end joining was examined further using linear plasmid DNA as acceptor. Incubation of radiolabeled tailed donor DNA with topo I resulted in the formation of a protein-DNA complex that was resolved from free DNA during native agarose gel electrophoresis (Fig. 4, lane 2). This complex was eliminated completely by digestion of the reaction products with proteinase K (not shown). Addition of unlabeled 5'-hydroxyl-termi-
Topo I Catalyzes Two Classes of DNA End-joining Reactions

Homology-dependent (sticky-end) ligation to plasmid DNA. A, strand cleavage of 5' 32P duplex 12-mer substrates (200 fmol/reaction) was plotted as a function of input enzyme. The structures of the substrates are shown. B, strand transfer reactions were performed as described in Fig. 4. Reaction mixtures included 400 fmol of the AATT-containing 12-mer (open circle) and 1.2 pmol of topo. Control reactions contained no enzyme (lanes 1 and 6). Acceptor DNAs were included as follows: 5'-OH EcoRI-cut pUC18 (lanes 3 and 6), 5'-OH HindIII-cut pUC18 (lanes 4, 5'-PO4, EcoRI-cut pUC18 (lane 5). A control reaction received no exogenous acceptor (lane 2). C, reaction mixtures included 400 fmol of the AGCT-containing DNA (closed circle). Control reactions contained no enzyme (lanes 1 and 6). Acceptor DNAs were included as follows: 5'-OH EcoRI-cut pUC18 (lanes 3), 5'-OH HindIII-cut pUC18 (lanes 4 and 6), 5'-PO4, HindIII-cut pUC18 (lane 5). A control reaction received no exogenous acceptor (lane 2).
pentamer cleavage motif (4). Finally, the site specificity of
the vaccinia topo I in DNA cleavage coupled with its versatil-
ity in end joining suggest that this enzyme may be useful in
molecular cloning, end labeling, or ligand tagging of DNA
molecules in vitro.

REFERENCES
1. Bullock, P., Champoux, J. J., and Botchan, M. (1985) Science 230, 954–
956.
2. Wang, H., and Rogler, C. E. (1991) J. Virol. 65, 2381–2392.
3. Shuman, S. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3489–3493.
4. Shuman, S. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 10104–10108.
5. Shuman, S., and Prescott, J. (1990) J. Biol. Chem. 265, 17,926–17,936.
6. Shuman, S. (1991) J. Biol. Chem. 266, 1796–1803.
7. Shuman, S. (1991) J. Biol. Chem. 266, 11372–11379.
8. Shuman, S. (1992) J. Biol. Chem. 267, 8620–8627.
9. Shuman, S., Golder, M., and Moss, B. (1998) J. Biol. Chem. 263, 16401–
16407.
10. Svejstrup, J. Q., Christiansen, K., Gromova, I. L., Andersen, A. H., and
Westergaard, O. (1991) J. Mol. Biol. 222, 669–678.