Vaccinia DNA topoisomerase I promotes illegitimate recombination in Escherichia coli

(bacteriophage λ/lysogenic induction/prophage excision)

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ABSTRACT Vaccinia virus encapsidates a M, 32,000 type I DNA topoisomerase. Although the vaccinia gene encoding the topoisomerase is essential for virus growth, the role of the enzyme in vivo remains unclear. In the present study, the physiologic consequences of vaccinia topoisomerase action have been examined in a heterologous system, Escherichia coli. The vaccinia topoisomerase gene was inducibly expressed in an intλ lysogen BL21(DE3) using a T7 RNA polymerase-based transcription system. Expression of active topoisomerase in this context resulted in recA-dependent lysogenic induction as well as cell lysis. Surprisingly, topoisomerase expression also affected a 200-fold increase in the titer of infectious λ phage, apparently by promoting int-independent prophage excision. This effect was not observed during lysogenic induction with nalidixic acid. Restriction analysis of genomic DNA from plaque-purified excisants revealed (in 10 of 10 cases) gross alterations of the DNA structure around the att site relative to the structure of the parental phage DE3. It is construed therefore that vaccinia DNA topoisomerase I acts to promote illegitimate recombination in E. coli.

Eukaryotes possess two classes of DNA topoisomerases, types I and II, that differ in their mechanisms of strand cleavage, cofactor requirements, and sensitivity to drugs (1, 2). A role for the topoisomerases in such key cellular events as DNA replication, chromosome segregation, genetic recombination, and transcription has been suggested. Genetic experiments in yeast have evinced an essential function for DNA topoisomerase II in cell growth (3, 4); topoisomerase I, on the other hand, is dispensable for yeast viability (3–5). Thus, the assignment of a particular role to eukaryotic topoisomerase I is less than straightforward and appears to be complicated by the redundancy of function of the types I and II enzymes. For example, yeast top1 mutants that lack the type I enzyme have no readily discernable phenotype but do have profound defects in macromolecular synthesis in the context of superimposed mutations in the gene encoding type II topoisomerase (6).

A eukaryotic viral system, vaccinia, provides a useful model for the study of type I topoisomerase. Vaccinia, a cytoplasmically replicating poxvirus, contains within the infectious virion a type I DNA topoisomerase (7). The vaccinia enzyme is mechanistically similar to cellular type I enzymes but is distinguished by its smaller size, M, 32,000 (8, 9). The virus encapsidated enzyme is also encoded by the vaccinia genome (8, 10). The topoisomerase structural gene specifies a polypeptide of 314 amino acids that includes a region of sequence similarity to the type I topoisomerase of yeast (5, 8). Insertional mutagenesis studies suggest that the topoisomerase gene is essential for virus growth in cell culture (11).

Analysis of the effects on E. coli of the expression of eukaryotic type I topoisomerases may well provide insights into the functional capabilities of these enzymes in vivo, albeit somewhat out of their natural context. Bjornsti and Wang have shown that expression of yeast topoisomerase I in a temperature-sensitive topA strain of Escherichia coli confers the ability to grow at the nonpermissive temperature (12). This genetic complementation assay thus provides a phenotypic screen for topoisomerase I that is lacking in yeast itself. In the same vein, it was of interest to determine what effects vaccinia topoisomerase I expression would have on bacterial physiology. The expression strategy involved cloning the topoisomerase gene into a plasmid so as to place it under the control of a bacteriophage T7 promoter. This plasmid was then used to transform a λ lysogen of E. coli that contains an inducible T7 RNA polymerase gene inserted into the λ int gene. I report here that expression of vaccinia topoisomerase in this context leads to recA-dependent lysogenic induction, as well as int-independent prophage excision. A possible role for the eukaryotic topoisomerase I in recA is suggested.

METHODS

Bacteria and Plasmids. The E. coli strains used for topoisomerase expression, referred to as BL21(DE3) and HMS174-DE3, are λ DE3 lysogens of host strains BL21(F−, hsdS, gal) and HMS174 (F−, hsdR, recA, RifR). DE3 (imm21) contains the T7 RNA polymerase gene under the control of a lacUV5 promoter, inserted within the int gene of the phage (13). Additional derivatives of these lysogens contain the plasmid pLysS or pLysE. The pLys plasmids, which confer chloramphenicol resistance, contain a fragment of T7 DNA that includes the T7 lysozyme gene inserted into pACYC184. pLysE and pLysS differ only in the orientation of the lysozyme gene with respect to the tet promoter, such that higher levels of lysozyme expression are achieved with pLysE. T7 lysozyme is a specific inhibitor of T7 RNA polymerase (14) and is useful in expression studies in lowering the basal activity of T7 RNA polymerase in the DE3 lysogens. Plasmids pA9topo and pC6topo contain the vaccinia topoisomerase gene inserted into the expression vector pAR3038 (15); in the case of pA9topo, the gene is in the sense orientation with respect to the T7 promoter, while pC6topo, the vaccinia gene is in the antisense orientation. Previous studies had shown that pA9topo, but not pC6topo, could program the synthesis of active vaccinia topoisomerase in E. coli BL21 upon provision of T7 RNA polymerase by phage infection (10). These plasmids, which confer ampicillin resistance, were used to transform BL21(DE3), HMS174(DE3), and related pLys-containing strains.

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Abbreviation: IPTG, isopropyl β-D-thiogalactopyranoside.
RESULTS

Expression of Vaccinia Topoisomerase in λDE3 Lysogens.

Vaccinia topoisomerase can be expressed in active form in E. coli under the control of a T7 promoter by using λ infection to deliver T7 RNA polymerase to the cell (10). This method, which does not allow for fine control of the amount of target gene expression and which also results in killing of the infected cell, is not suitable for assessing the effects on E. coli of vaccinia topoisomerase expression. Thus, to address this point, I turned to the inducible lysogen-based expression system described by Studier and colleagues (13).

A culture of BL21(DE3)pLysEpA9topo was induced to express T7 RNA polymerase by the addition of 0.4 mM IPTG. Upon induction, the concentration of newly made T7 RNA polymerase can be expected to exceed that of the inhibiting lysozyme and thus drive the expression of vaccinia topoisomerase. The growth characteristics of the induced culture are shown in Table 1. An initial period of exponential growth was followed by lysis of the bacterium at 1.5–2 hr postinduction. Lysis required the expression of the vaccinia topoisomerase gene, since otherwise identical cells carrying the antisense topoisomerase expression plasmid pC6topo did not undergo lysis. The dramatic effects of IPTG on cell growth correlated with the appearance of active vaccinia topoisomerase, as shown by assay of cell lysates for E-DNA relaxing activity (Fig. 1). Three distinct colony isolates of BL21(DE3)pLysEpA9topo displayed a marked increase in relaxing activity postinduction. Cells bearing pC6topo manifested no increase postinduction.

Topoisomerase Expression Causes Lysogenic Induction. How might vaccinia topoisomerase cause lysis of the host bacterium? Topoisomerase expression could lead to lysogenic induction of the phage, resulting in late viral gene expression and concomitant cell lysis by either the λ-encoded or the pLysE plasmid-encoded lysozyme. Lysogenic induction depends on cleavage of the λ repressor by recA protein (19); thus, the role of lysogenic induction in topoisomerase-expression.

Table 1. Induction with IPTG

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>BL21(DE3)pLysEpA9topo</th>
<th>BL21(DE3)pLysEpC6topo</th>
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<tr>
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<td>0.267/10</td>
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<td>0.5</td>
<td>0.480/450</td>
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<tr>
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<tr>
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<tr>
<td>2.0</td>
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<table>
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<tr>
<th>Time (hr)</th>
<th>H9M174(DE3)pLysEpA9topo</th>
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<tr>
<td>2.5</td>
<td>0.353*/&lt;10</td>
<td>0.853*/&lt;10</td>
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Bacteria were grown in LB medium. Ampicillin (100 µg/ml) and/or chloramphenicol (30 µg/ml) were included to maintain the plasmids. Logarithmically growing cells (A600 0.2–0.3) were induced to express T7 RNA polymerase by addition of IPTG to 0.4 mM concentration at time 0. Further cell growth was followed at the indicated intervals by measurement of A600. To determine the phage titer of the cultures, aliquots were withdrawn, made 2% in CHCl3, and centrifuged to remove bacteria and cell debris. Serial dilutions of the supernatants were titrated on BL21 plating bacteria as described (17). pfu, Plaque-forming units.

* Lysis of cultures.

FIG. 1. Induction of vaccinia DNA topoisomerase by IPTG. Cultures (10 ml; A600 0.4–0.6) of BL21pLysEpA9topo transformants containing the topoisomerase expression plasmid in the sense (pA9-8, pA9-9, and pA9-2) or antisense (pC6-6) orientation were made 0.4 mM in IPTG and allowed to grow for an additional 2 hr. A parallel set of cultures received no IPTG. Cells were pelleted and resuspended in 1 ml of 150 mM NaCl/50 mM Tris-HCl, pH 7.5/10 mM EDTA/1 mM dithiothreitol/10% (wt/vol) sucrose. Lysis was achieved by two cycles of freezing and thawing with subsequent addition of Nonidet P-40 to 0.01%. The lysates were clarified by centrifugation at 100,000 × g for 60 min in a type 60 Ti rotor. The supernatant was removed and assayed for protein concentration (10) and EDTA-resistant topoisomerase activity. Topoisomerase assays were performed as described (8) using pBR322 DNA as substrate, activity being gauged by the conversion of superhelical DNA to the relaxed form. Each reaction mixture contained 100 ng of lyase protein; this was done to correct for the uniformly lower concentration of protein in IPTG-induced pA9-2 lysates owing to the presence of cell ghosts in harvesting the bacteria. Reaction products were analyzed by agarose gel electrophoresis (8); a photograph of the gel is shown. The symbols above each lane denote the bacterial isolate used as a source of enzyme. Induced cultures are indicated by a plus sign; uninduced cultures are indicated by a minus sign; C, control reaction from which enzyme was omitted. The positions of supercoiled (S) and relaxed (R) forms of DNA are indicated by arrows.

Dependent lysis could be tested by performing similar experiments in a recA strain, HMS174. As shown in Table 1, topoisomerase expression in this background caused arrest of growth at 1.5 hr postinduction but no discernable cell lysis. Arrest of growth was attributable to vaccinia topoisomerase, since neither the HMS174(DE3) lysogen itself nor the lysogen carrying the antisense topo plasmid pC6topo experienced a similar cessation of growth.

Topoisomerase-Dependent Prophage Excision. It was anticipated that cell lysis would not be accompanied by the generation of progeny phage, because of the int+ genotype of the integrated DE3 prophage. It was surprising, therefore, to note that IPTG induction of topoisomerase expression resulted in a 200-fold increase in the infectious phage titer (Table 1). The phage burst was not evident in the strain bearing pC6topo. This increase in A titer, like lysis of the cells, required both topoisomerase expression and a wild-type recA protein.

To explore further the basis of cell lysis and of the phage burst, a parallel series of experiments was performed with nalidixic acid, an inhibitor of DNA gyrase and a classical inducer of recA, to effect lysogenic induction of the same bacterial strains. As shown in Table 2, exposure of growing cells to nalidixic acid resulted in synchronous lysis of BL21(DE3)pLysEpE strains with a time course similar to that of topoisomerase-dependent lysis by IPTG. Nalidixic acid, unlike IPTG, caused lysis whether or not a T7 target plasmid was present. Also, as expected, nalidixic acid induced lysis only in a recA+ background. The depressed rate of growth of those strains not lysed (Table 2) was consistent with the inhibitory effects of the drug on bacterial DNA replication. In addition, we can infer from the failure of nalidixic acid to lyse BL21(DE3) lacking a pLys plasmid that it is the accumulated T7 lysozyme, rather than the λ lysozyme (gene R product), that is responsible for lysis in this case, as well as in the topoisomerase induction experiments.
Table 2. Induction with nalidixic acid

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<td>pC6topo</td>
<td>pLysE</td>
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**Experiment 1**

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**Experiment 2**

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<td>0.449/&lt;10</td>
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<td>0.579/&lt;10</td>
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Logarithmically growing lysogens were induced by addition of nalidixic acid to 50 µg/ml at time 0. Further cell growth was followed at the indicated intervals by measurement of A600. The phage titer of the cultures was determined as described in Table 1. pfu. Plaque-forming units.

Lysis of cultures.

Comparison of the phage titers after nalidixic acid-mediated lysogenic induction with those after topoisomerase-mediated induction revealed striking differences. In none of the strains tested did nalidixic acid cause an increase in phage titer. This is reflective of the lack of int function required for excisional recombination of the prophage and confirms the tightness of the int' phenotype in the DE3 lysogens. Thus, the phage burst noted in Table 1 must be due to topoisomerase-dependent int-independent excision of the phage. The burst cannot be merely a function of cell lysis (i.e., with release of preexisting intracellular phage particles) since the lysis of pA9topo-bearing BL21(DE3)pLysE by nalidixic acid caused no change in phage titer. The magnitude of the phage yield (on the order of 10⁸ pfu/ml per cell) is significantly lower than that expected from excision of wild-type prophage. We cannot readily distinguish whether this is due to a low efficiency of topoisomerase-dependent excision, or if the true potential for phage production is underestimated by the relatively rapid onset of cell lysis to T7 lysozyme.

Analysis of Δ Excisants. An outstanding question is whether vaccinia topoisomerase-dependent excisional recombination is site specific or illegitimate. If vaccinia topoisomerase is truly acting in place of int in a physiologic manner, it is to be expected that recombination would occur between attL and attR to regenerate attP in progeny phage (20), as depicted in Fig. 2. If recombination is illegitimate, progeny phage will have altered DNA sequences at the att locus. This issue was addressed by plaque-purifying phage isolates from IPTG-induced BL21(DE3)pLysEpA9topo, amplifying the excisants, and then analyzing the structures of their att sites.

The phage DNA of 10 such isolates was digested with several restriction endonucleases, and the digests were compared to those of the parental DE3 phage. The restriction map of DE3 (13, 21) and the expected sizes of DNA fragments after nuclease digestion are shown in Fig. 2; the results of the restriction analysis of the parental and excisant λ DNAs are shown in Fig. 3.

EcoRI digests of the topoisomerase-induced excisants resemble those of the parent DE3 except with respect to the 10.1-kilobase (kb) restriction fragment that spans attP (Fig. 3A). This region of the genome has been permuted in several cases to yield single EcoRI fragments that are either longer

Fig. 2. Structure of λ DE3 DNA in phage, prophage, and excisant forms. (a) Structure of the genomic DNA of λ DE3 is illustrated with DE3 is a derivative of λ D69 (21) containing a 4.4-kb expression cassette for T7 gene 1 inserted into the unique BamHI cloning site within the int gene (13). Solid line represents DNA derived from D69; hatched line represents the T7 cassette. The left and right cohesive ends of the phage DNA are indicated by cosL and cosR. Restriction sites in DE3 for endonucleases EcoRI (E), HindIII (H), and BamHI (B) are marked by arrows under the appropriate letter. The location of the attP site is indicated by an arrow. The borders and sizes of the EcoRI fragments (kbp) are depicted immediately below the genome. Sizes of restriction fragments (bp) expected from various endonuclease treatments are shown in tabular form. The fragments are listed in order of their position in the phage DNA, going from left to right. (b) Structure of the DE3 prophage predicted from site-specific recombination between attP and attB. The recombination junctions are indicated by attL and attR, respectively. The positions of restriction sites are marked by arrows. Internal regions of the prophage genome are indicated by the serrated line. Bacterial DNA is depicted as a thin line. (c) The sequences around attL and attR are approximated to illustrate the crossover event in prophage excision. Int-dependent excision normally involves recombination between attL and attR to reconstitute the phage genome shown in a. Topoisomerase-dependent recombination occurs at different sites around att (see text and Fig. 3). The rough locations of the crossover points (relative to restriction sites) that account for the restriction patterns of the 10 excisants (Fig. 3) are shown as connecting lines. Restriction sites in λ DNA are indicated by arrows. Predicted sites for BamHI and for EcoRI cleavage within the bacterial DNA flanking attR are indicated by the arrows below italicized letters.

(isolates 1 and 2) or shorter (isolates 3, 5, 7, and 10) than the parental att-containing fragment. In the remaining instances, a new EcoRI fragment (of ~2.5 kb in the case of isolates 4, 6, 8, and 9, and ~1.2 kb in isolate 7) is generated, along with a fragment of ~7 kb. These data indicate clearly that topoisomerase-dependent prophage excision does not reconstitute the wild-type attP site.

To localize approximately the crossover points for topoisomerase-dependent excisional recombination in the progeny phage, the DNAs were digested with endonucleases HindIII or BamHI. HindIII cleaves uniquely in DE3 at a site
FIG. 3. Restriction endonuclease analysis of λ DNAs. Two isolates of the parental DE3 virus (designated P and P') and 10 excisant progeny from an IPTG-induced culture of BL21pLysEAp9-topo (numbered 1–10) were plaque-purified and amplified in BL21 as described (13), first by growth in a 35-ml culture followed by growth in a 1-liter culture until lysis was complete. Phage was collected by polyethylene glycol precipitation and rapid isopropyl binding in CaCl2. A DNA was isolated as described (17). Restriction digests with the indicated endonucleases were performed in 20-μl reaction mixtures. After 2–3 hr of incubation, the restriction products were analyzed by electrophoresis through 0.7% agarose gels containing TBE (90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3) and 0.5 μg of ethidium bromide per ml. Photographs of the gels are shown. The source of λ DNA in each reaction mixture is indicated above the lane. Lane M contains a HindIII digest of wild-type λ DNA that was used as size marker in this analysis. The sizes of the marker fragments (in bp), in order of increasing mobility, are 23,130, 9416, 6557, 4361, 2322, and 2027.

within the 10.1-kb EcoRI fragment located 252 base pairs (bp) to the left of attP. Digestion with EcoRI and HindIII can be expected to yield a 1.37-kb fragment from the left end and an 8.7-kb fragment from the right end of the att-containing EcoRI fragment of DE3 (Fig. 3B). All excisants except excisant 3 retain the HindIII site in the genomic DNA, as evinced by the liberation of 1.37-kb fragment with a concomitant decrease in the size of the att-containing fragment. Thus, the crossover points in these cases must lie to the right of the HindIII site. Excisant 3 has lost the HindIII site (note the lack of shift in mobility of the att-containing fragment), thereby localizing the crossover point to the region between the EcoRI site and the HindIII site.

BamHI cleaves DE3 at two sites flanking the 4.4-kb T7 insert in the int gene; the leftward BamHI site is 241 bp to the right of attP. Many of the excisants (nos. 3, 4, 6, 8, and 9) have deleted one of the BamHI sites, as manifest by the lack of a 4.4-kb BamHI fragment and the presence of only two large BamHI fragments (Fig. 3C). Other excisants (nos. 2, 5, and 10) appear to have substituted a BamHI site for one of the parental sites, as shown by the appearance of internal BamHI fragments of novel size. Combined digestion with BamHI and EcoRI localizes the BamHI sites within the excision junction-containing EcoRI fragment. As shown in Fig. 3D, BamHI liberates from DE3 a 1.8-kb fragment from the left end of the EcoRI fragment, a central 4.4-kb T7 cassette, and a 3.8-kb fragment from the right end of the EcoRI fragment. In the excisants, the novel fragments generated by either enzyme alone (e.g., the 2.5- and 1.2-kb EcoRI fragments in Fig. 3A, and the 5.4- and 1.8-kb BamHI fragments in Fig. 3C) are not altered by treatment with the other enzyme. In 9 of 10 cases (no. 3 excepted), the leftward EcoRI/BamHI fragment has been replaced with a fragment of ~2.2 kb. The rightward 3.8-kb BamHI/EcoRI fragment has been preserved in isolates 1, 2, 3, 5, and 10 but is replaced in isolates 4, 6, 7, 8, and 9 with a fragment of ~5.2 kb. The restriction patterns can be accounted for by postulating in almost all cases (except no. 3) that excision arose by crossing over between bacterial sequences upstream of attR and phage sequences lying between the leftward BamHI site and the EcoRI site (Fig. 2c).

To account for the creation of novel restriction fragments, we posit the presence of a BamHI site in the bacterial DNA flanking attR (located 0.8 kb to the right of the λ HindIII site) and an EcoRI site flanking attR (located 5.2 kb to the right of the BamHI site; see Fig. 2c). These novel recognition sites have indeed been reported (ref. 22 and references therein). The exceptional case is phage 3, which has apparently recombined among sequences in the leftward EcoRI/HindIII fragment and sequences in the BamHI/BamHI fragment of DE3. The proposed crossovers are illustrated in Fig. 2c. The essential point of this analysis is that in no case was excision accomplished by site-specific recombination between attL and attR; thus, topoisomerase I-dependent recombination is apparently illegitimate.

DISCUSSION

Excision of an integrated λ prophage from the E. coli chromosome occurs via site-specific recombination between attL and attR sequences (reviewed in ref. 20). This reaction involves two phage-encoded proteins, the products of the int and xis genes, respectively, and a host protein, IHF, consisting of two subunits encoded by the E. coli himA and his genes (26). The requirement for IHF and xis proteins in excisive recombination is not absolute in that mutations affecting the int gene can relieve the strict need for the other proteins (27). Int protein, on the other hand, is absolutely required for site-specific excision. The most remarkable finding of the present study is that vaccinia topoisomerase promotes prophage excision in the absence of functional int protein. This is reflected in the 200-fold increase in infectious phage titer after IPTG treatment of topoisomerase-expressing lysogens. The magnitude of this effect is even greater (2000-fold) when comparison is made between topoisomerase-containing and non-topoisomerase-containing lysogens (Table 1). Therefore, the eukaryotic topoisomerase I is capable of complementing int function in vivo. The int protein, a M, 40,000 polypeptide, is itself a type I DNA topoisomerase that can relax positive as well as negative supercoils in vitro without obvious sequence specificity (28). Int-catalyzed DNA relaxation proceeds via a covalent DNA–protein intermediate involving a 3' phosphoryl linkage to tyrosine 342 of the int protein (29, 30). Int topoisomerase is thus similar in its biochemical properties to the family of eukaryotic type I enzymes that includes vaccinia topoisomerase. The topoisomerase activity of int protein is required for int action as a recombinase (30).

It is inferred from the present results that the vaccinia topoisomerase is capable of promoting excisive recombination in E. coli. Topoisomerase-dependent prophage excision
appears to occur via illegitimate, rather than site-specific, recombination and is thereby distinguished from exciscive recombination catalyzed by int protein. The specificity of int recombinase for the att sites is a function of binding of the protein to specific DNA sequences within the att regions (20). The degree of specificity, if any, of the vaccinia topoisomerase-mediated recombination will be revealed by finer mapping and sequencing of the recombination junctions. At first glance, however, the process would seem to be nonrandom, insofar as: (i) 9/10 of the junctions lie to the right of the HindIII site in DE3, and (ii) several of the exciscsents have remarkably similar restriction patterns. Whether these observations are due to bias inherent in the assay (i.e., in some peculiarity of DE3 that selects for viable progeny that have recombined in a particular manner) or to the specificity of topoisomerase-mediated strand cleavage remains to be determined. While the former model cannot be dismissed, the nonessential nature of the genomic region surrounding attP in either direction makes this alternative less likely.

Previous studies have implicated the DNA topoisomerases in illegitimate recombination. E. coli DNA gyrase and bacteriophage T4 topoisomerase (both type II enzymes) as well as calf thymus topoisomerase II can promote illegitimate recombination in vitro (31–33). A possible role for the eukaryotic type I topoisomerase in illegitimate recombination has also been invoked (34). The latter proposal is sustained by the finding that nucleotide sequences surrounding exchange sites for illegitimate recombination in vivo correlate with the preferred recognition sequences of topoisomerase I in vivo. The present study provides compelling support for the view that a eukaryotic topoisomerase I can promote illegitimate recombination in vivo by demonstrating the possible relationship between the presence of topoisomerase I activity and the occurrence of illegitimate recombination. The in vivo assay for recombination—the generation of infectious excisant phage—while extremely sensitive, may well underestimate the overall extent of recombination events, either in remote areas of the chromosome, or in regions of the prophage that reconstitute noninfectious phage.

It is not clear from this work whether vaccinia topoisomerase per se is sufficient to mediate recombination or whether bacterial components contribute to the effects presently reported. In this regard, relaxation of the bacterial chromosome (a probable sequela to the expression of vaccinia topoisomerase in E. coli) has been shown to increase the synthesis of DNA gyrase (35). Gyrase could then be the agent of illegitimate prophage excision, with vaccinia topoisomerase playing only an indirect role. If this were the case, however, it is expected that prophage excision would also be augmented by nalidixic acid, since illegitimate recombination catalyzed in vitro by prokaryotic type II topoisomerases is stimulated by the quinolone drugs (16, 31, 32). Moreover, nalidixic acid induces a 2- to 3-fold increase in the synthesis of the GyrA and GyrB subunits (35). The failure to observe an enhancement of phage titer by nalidixic acid (Table 2) suggests that DNA gyrase is not responsible for vaccinia topoisomerase-dependent excision. The possible participation of other E. coli proteins in this process remains to be evaluated.

Finally, the observation that vaccinia topoisomerase participates in recombination in E. coli suggests a similar function for this enzyme during vaccinia infection of eukaryotic cells. Indeed, vaccinia virus induces high levels of homologous recombination in the cytoplasm of infected cells (23–25). The strand cleavage-rejoining activity of the topoisomerase might be remodeled specific for regions of DNA homology by interaction of the topoisomerase with some other vaccinia protein. Further clarification of the role of the topoisomerase I in viral replication, and recombination in particular, will be contingent on the isolation of conditional topoisomerase mutants and on the development of in vitro systems for the study of viral recombination.

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