Vaccinia virus DNA topoisomerase I preferentially removes positive supercoils from DNA

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Abstract Type I DNA topoisomerases homologous to *Escherichia coli* topoisomerase I normally only remove negative supercoils from DNA. Topoisomerases I from various eukaryotes share sequence homology and remove both positive and negative supercoils from DNA. Here we report that vaccinia virus topoisomerase I has significant difference in substrate preference from the other homologous type I topoisomerases. Vaccinia virus topoisomerase I shows a definite preference for removal of positive supercoils. In contrast, topoisomerase I from human, wheat germ and *Saccharomyces cerevisiae* has little preference between positive and negative supercoils. The vaccinia enzyme may have evolved for functions required for optimal viral growth.

Key words: Type I topoisomerases; Vaccinia virus; Positive DNA supercoil; Negative DNA supercoil

1. Introduction

DNA topoisomerases regulate DNA topology by coupling DNA strand passage with the concerted breaking and rejoining of DNA [1,2]. Type I DNA topoisomerases break and rejoin a single strand of DNA during catalysis. In *E. coli*, DNA topoisomerase I is responsible for the removal of excess negative superhelical tension from chromosomal DNA [3]. It normally only removes negative supercoils from DNA, a property also shared by other type I topoisomerases with which it shares sequence homology [4–9]. Topoisomerases I from various eukaryotic sources form another subfamily of type I topoisomerases [10–18] and relax both positive and negative supercoils. The loss of topoisomerase I function in *E. coli* due to a mutation in *topA* can be compensated by the expression of plasmid encoded topoisomerase I from *S. cerevisiae* [19] or human [20]. Although vaccinia virus topoisomerase I can also relax both positively and negatively supercoiled DNA in vitro like the human and *S. cerevisiae* topoisomerase I, its expression in *E. coli* cannot compensate for the loss of *topA* function [21]. This suggested that vaccinia topoisomerase I enzymes have significant differences in enzymatic properties when compared to these other eukaryotic enzymes, influencing in vivo function. We have also reported previously [21] that the decline in *E. coli* viability due to the overexpression of vaccinia virus topoisomerase I could be suppressed by the presence of gyrase mutations or inhibitors. Our hypothesis to be tested here is that when expressed in *E. coli*, the vaccinia virus topoisomerase I behaves more like the *E. coli* gyrase rather than the *E. coli* topoisomerase I in the relaxation of supercoiled DNA.

According to the twin-supercoiled domain model [22], translocation of RNA polymerase during transcription elongation results in positive supercoils ahead of the RNA polymerase complex and negative supercoils behind the complex. In *E. coli*, topoisomerase I can remove the negative supercoils while gyrase is responsible for the removal of the positive supercoils [3]. This model has been supported by the isolation of positively supercoiled pBR322 DNA topoisomers from *E. coli* cells treated with gyrase inhibitors [23] and the previous observation [24] that in vitro transcription in the presence of *E. coli* DNA topoisomerase I led to the accumulation of positive supercoils in plasmid DNA substrate. In vitro transcription with T3 RNA polymerase and a plasmid DNA substrate with a T3 promoter was used here to examine the effect of different eukaryotic topoisomerases I on the superhelical state of DNA during transcription. The effect of vaccinia virus topoisomerase I expression on accumulation of positive supercoils in *E. coli* was also examined to test the proposed hypothesis.

2. Materials and methods

2.1. Enzymes

E. coli and vaccinia virus DNA topoisomerase I were purified from expression clones as described previously [25,26]. *S. cerevisiae* topoisomerase I was a generous gift from Dr. J.C. Wang (Harvard University). Wheat germ topoisomerase I was purchased from Promega and human topoisomerase I was obtained from TopoGEN.

2.2. In vitro transcription reactions

Plasmid pC15 [27] was relaxed completely with wheat germ topoisomerase I and purified by phenol extraction and ethanol precipitation prior to use as substrate in the in vitro transcription reactions. The transcription reaction with T3 RNA polymerase were carried out under conditions as described previously [27]. DNA topoisomerase I from various sources was added to the reaction as indicated. The plasmid DNA was then analyzed by 2D agarose gel electrophoresis and visualized by hybridization to ^{32}P-labeled, *Mst*II digested pC15 DNA [27]. Autoradiography was carried out using Kodak BIOMAX film.

2.3. In vivo effect of vaccinia virus topoisomerase I expression on the accumulation of positive DNA supercoils

E. coli MV1190 cells containing either plasmid p1940 expressing vaccinia topoisomerase I [21,26] or its derivative p1940A (with frame shift mutation in the vaccinia virus topoisomerase gene) [21] were grown with 75 μg/ml of novobiocin for 7 h at 30°C. The growth temperature was then shifted to 42°C to further increase the expression of vaccinia virus topoisomerase I from p1940 [21]. After 7 h of further incubation, the cells were pelleted for plasmid DNA preparation by the Wizard system (Promega). Two-dimensional gel electrophoresis and in situ hybridization with ^{32}P-labeled, *Pst*I digested p1940 DNA were carried out as described previously [27].

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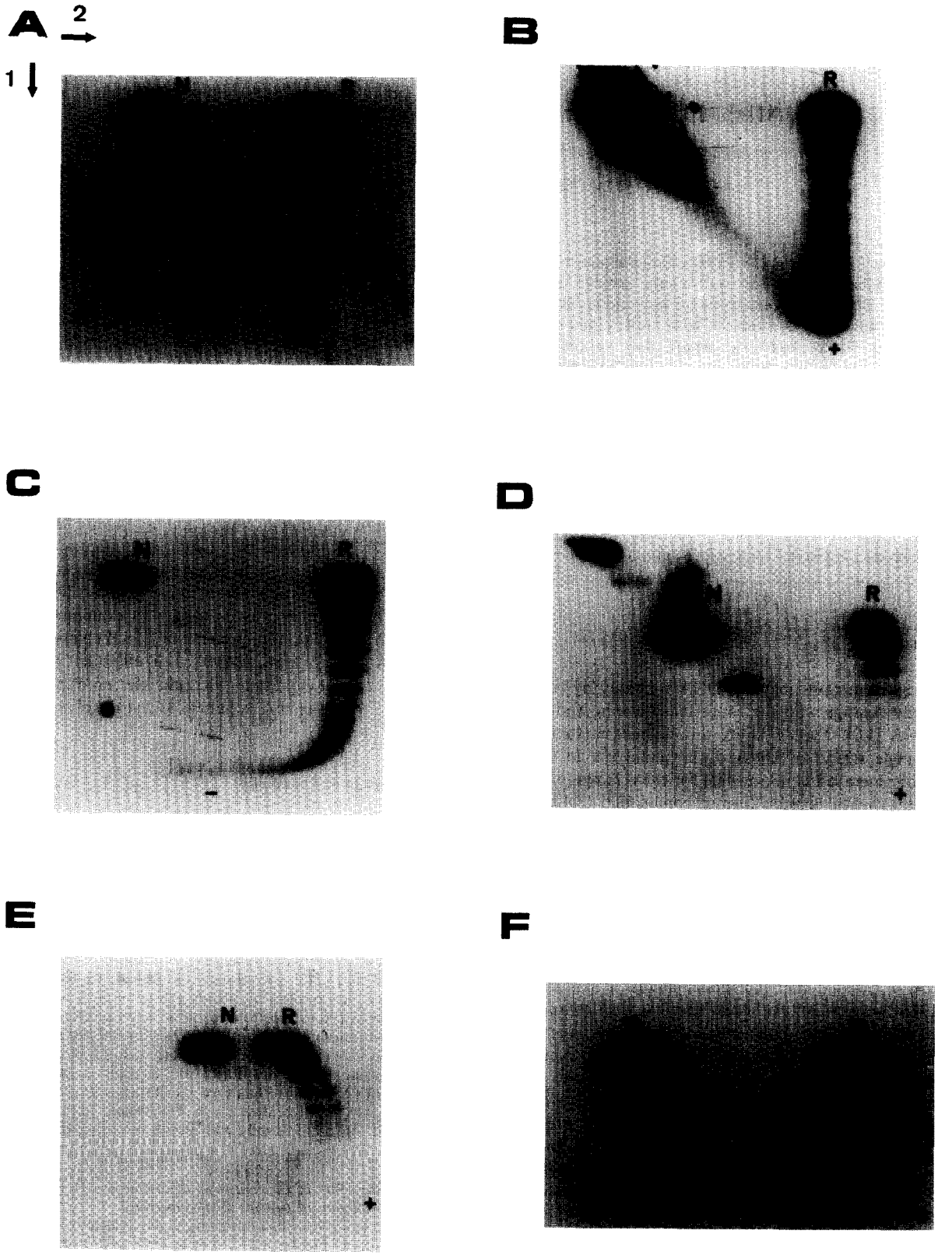

Fig. 1. Two-dimensional gel electrophoresis showing the effect of various type I DNA topoisomerases on transcription-driven supercoiling of DNA template. (A) Control, T3 RNA polymerase in vitro transcription with no topoisomerase added. (B) 1 U *E. coli* DNA topoisomerase I. (C) 2 U vaccinia virus DNA topoisomerase I. (D) 1 U *S. cerevisiae* DNA topoisomerase I. (E) 1 U wheat germ DNA topoisomerase I. (F) 1 U human DNA topoisomerase I. N, nicked DNA; R, relaxed DNA; +, positively supercoiled topoisomers; −, negatively supercoiled topoisomers.

3. Results

3.1. Vaccinia virus DNA topoisomerase I preferentially removes the positive supercoils generated during transcription

Both positive and negative supercoils were generated on the plasmid pC15 template from the transcription action of T3 RNA polymerase. *E. coli* DNA topoisomerase I can only remove the negative supercoils. The expected accumulation of positive supercoils from the action of the *E. coli* DNA topoisomerase I was observed when the plasmid DNA was analyzed by 2-D agarose gel electrophoresis (Fig. 1B). In the presence of vaccinia virus DNA topoisomerase I, accumulation of negative supercoils was observed instead (Fig. 1C). The topoisomerase I from human, wheat germ and *S. cerevisiae* gave similar results (Fig. 1D–F), with the DNA possessing a small number of positive superhelical turns, not significantly different from the control transcription reaction (Fig. 1A) where no topoisomerase was present. This demonstrated that vaccinia virus topoisomerase I acted preferentially on the positive supercoils generated during in vitro transcription while the other eukaryotic type I topoisomerases tested did not show any significant preference between the positive and negative supercoils.

3.2. The action of vaccinia virus DNA topoisomerase I on positive supercoils in vivo

Treatment of *E. coli* cells with the gyrase inhibitor novobiocin is known to lead to the presence of highly positively supercoiled plasmid DNA topoisomers [23]. Plasmid p1940 expressing vaccinia virus DNA topoisomerase I and plasmid p1940A with a frame shift mutation in the enzyme coding region were prepared from *E. coli* MV1190 cells treated with novobiocin and analyzed by 2-D agarose gel electrophoresis. Positively supercoiled p1940A could be readily observed (Fig. 2A) while the p1940 preparation had few positive topoisomers (Fig. 2B). This demonstrated that vaccinia virus topoisomerase I expressed from p1940 removed the positive supercoils efficiently in vivo.

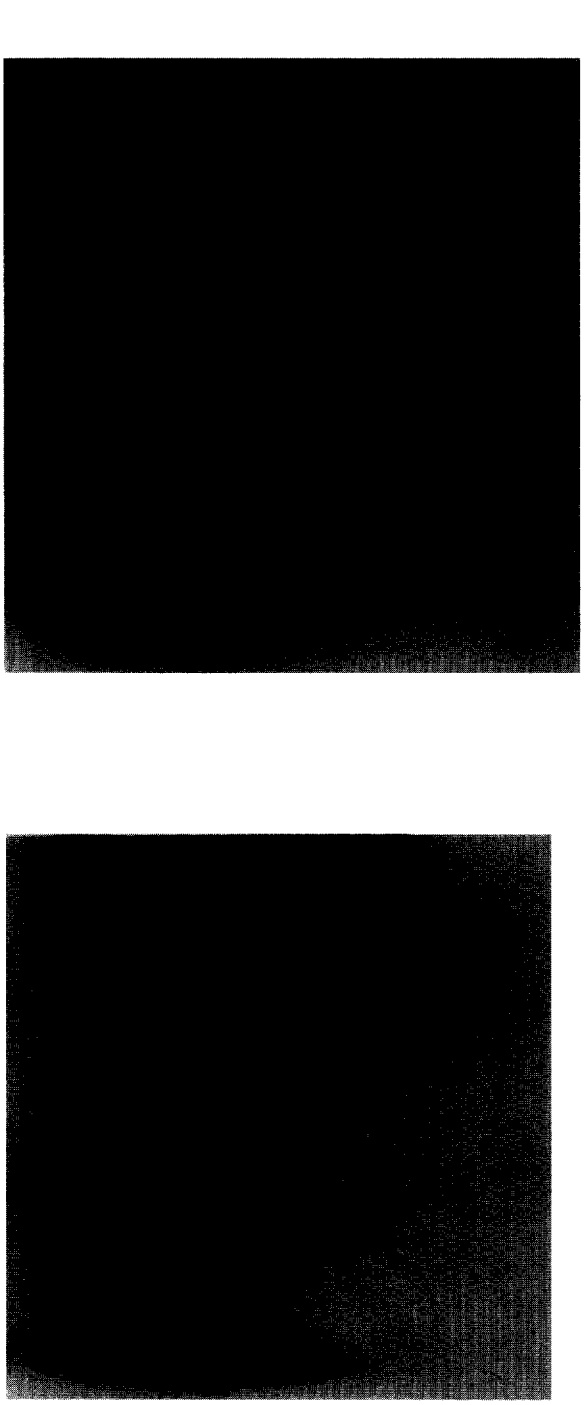

Fig. 2. Removal of positive supercoils in vivo by vaccinia virus DNA topoisomerase I expressed in *E. coli*. 2-D gel electrophoresis of (A) plasmid p1940A (with frame shift mutation in vaccinia virus DNA topoisomerase I gene) or (B) p1940 expressing vaccinia virus DNA topoisomerase I. The DNA molecules were isolated from *E. coli* cells treated with the gyrase inhibitor novobiocin to generate positively supercoiled topoisomers. Plasmid p1940 DNA was isolated in its dimeric form while p1940A DNA was mainly monomeric as observed previously [21]. N, nicked DNA; L, linear DNA; R, relaxed DNA; +, positively supercoiled topoisomers; −, negatively supercoiled topoisomers.

4. Discussion

The difference in substrate preference between vaccinia virus DNA topoisomerase I and the other type I topoisomerases characterized here can account for the inability of the vaccinia virus DNA topoisomerase I to replace the *topA* function in *E. coli*. The yeast and human enzymes could complement for the lack of *topA* function probably because they showed no preference between the negative and positive supercoils. It has been reported [28] that R-loop formation is a major problem in the absence of *E. coli* DNA topoisomerase I. Phenotypes associated with deficiency in the major RNAse H (*rnhA*) activity, as in the case of overexpression of vaccinia virus topoisomerase I [21], can also be corrected by a defect in DNA gyrase [28]. By removing positive supercoils preferentially in vivo, vaccinia virus DNA topoisomerase I would not be able to suppress R-loop formation efficiently and compensate for the loss of *E. coli* DNA topoisomerase I.

The size of vaccinia virus DNA topoisomerase I is about one third of that of the eukaryotic topoisomerase I of non-viral origins. It also differed from the non-viral eukaryotic topoisomerase I in its DNA cleavage sequence selectivity [29]. Nevertheless, regions of the enzyme believed to comprise the active site for DNA cleavage are highly conserved [30]. Our results showed that even though eukaryotic topoisomerases I can in general relax both positively and negatively

supercoiled DNA, their action on DNA may result in different superhelical states. A prokaryotic enzyme DNA topoisomerase V has been purified from the hyperthermophilic methanogen *Methanopyrus kandleri* and found to be related to eukaryotic topoisomerase I [31]. It relaxes both positively and negatively supercoiled DNA but prefers positively over negatively supercoiled substrates [32].

Vaccinia virus DNA topoisomerase I is essential for virus growth [33]. Its preference for relaxation of positive supercoils may be a requirement of its role(s) in the vaccinia life cycle. It may act as a swivel during DNA replication to remove the positive supercoils generated ahead of the replication fork. The dynamics of topoisomerase action on transcription-generated supercoiling can influence the structure and function of local DNA domains [34,35]. The localized DNA supercoiling generated by vaccinia virus topoisomerase I may be part of its functional role in the vaccinia life cycle and may also account for its lethal effect on *E. coli* cell growth.

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References

[1] Wang, J.C. (1985) Annu. Rev. Biochem. 54, 665–697.
[2] Maxwell, A. and Gellert, M. (1986) Adv. Protein Chem. 38, 69–107.
[3] Drlica, K. (1992) Mol. Microbiol. 6, 425–433.
[4] Tse-Dinh, Y.-C. and Wang, J.C. (1986) J. Mol. Biol. 191, 321–331.
[5] DiGate, R.J. and Marians, K.J. (1989) J. Biol. Chem. 264, 17924–17930.
[6] Wallis, J.W., Chrebet, G., Brodsky, G., Rolfe, M. and Rothstein, R. (1989) Cell 58, 409–419.
[7] Confalonieri, F., Elie, C., Nadal, M., Bouthier de La Tour, C., Forterre, P. and Duguet, M. (1993) Proc. Natl. Acad. Sci. USA 90, 4753–4757.
[8] Fouet, A., Sirard, J.-C. and Mock, M. (1994) Mol. Microbiol. 11, 471–479.
[9] Bouthier de la Tour, C., Kaltoum, H., Portemer, C., Confalonieri, F., Huber, R. and Duguet, M. (1995) Biochim. Biophys. Acta 1264, 279–283.
[10] Shuman, S. and Moss, B. (1987) Proc. Natl. Acad. Sci. USA 84, 7478–7482.
[11] Thrash, C., Bankier, A.T., Barrel, B.G. and Sternglanz, R. (1985) Proc. Natl. Acad. Sci. USA 82, 4374–4378.
[12] Uemura, T., Morino, K., Uzawa, S., Shiozaki, K. and Yanagida, M. (1987) Nucl. Acids Res. 15, 9727–9739.
[13] D'Arpa, P., Machlin, P.S., Ratrie III, H., Rothfield, N.F., Cleveland, D.W. and Earnshaw, W.C. (1988) Proc. Natl. Acad. Sci. USA 85, 2543–2547.
[14] Hsieh, T., Brown, S.D., Huang, P. and Fostel, J. (1992) Nucl. Acids Res. 20, 6177–6182.
[15] Kieber, J.J., Tissier, A.F. and Signer, E.R. (1992) Plant Physiol. 99, 1493–1501.
[16] Upton, C., Opgenorth, A., Traktman, P. and McFadden, G. (1990) Virology 176, 439–447.
[17] Gerhold, D., Thiyagarajan, M. and Kmiec, E.B. (1994) Nucl. Acids Res. 22, 3773–3778.
[18] Klemperer, N., Lyttle, D.J., Tauzin, D., Traktman, P. and Robinson, A.J. (1995) Virology 206, 203–215.
[19] Bjornsti, M.-A. and Wang, J.C. (1987) Proc. Natl. Acad. Sci. USA 84, 8971–8975.
[20] Bjornsti, M.-A., Benedetti, P., Viglianti, G.A. and Wang, J.C. (1989) Cancer Res. 49, 6318–6323.
[21] Fernandez-Beros, M.-E. and Tse-Dinh, Y.-C. (1992) J. Bacteriol. 174, 7059–7062.
[22] Liu, L.F. and Wang, J.C. (1987) Proc. Natl. Acad. Sci. USA 84, 7024–7027.
[23] Lockshon, D. and Morris, D.R. (1983) Nucl. Acids Res. 11, 2999–3017.
[24] Tsao, Y.-P., Wu, H.-Y. and Liu, L.F. (1989) Cell 56, 111–118.
[25] Tse-Dinh, Y.-C. and Beran-Steed, R.K. (1988) J. Biol. Chem. 263, 15857–15859.
[26] Shuman, S., Golder, M. and Moss, B. (1988) J. Biol. Chem. 263, 16401–16407.
[27] Wu, H.-Y., Shyy, S., Wang, J.C. and Liu, L.F. (1988) Cell 53, 433–440.
[28] Drolet, M., Phoenix, P., Menzel, R., Massé, E., Liu, L.F. and Crouch, R.J. (1995) Proc. Natl. Acad. Sci. USA 92, 3526–3530.
[29] Shuman, S. and Prescott, J. (1990) J. Biol. Chem. 265, 17826–17836.
[30] Morham, S.G. and Shuman, S. (1990) Genes Dev. 4, 515–524.
[31] Slesarev, A.I., Stetter, K.O., Lake, J.A., Gellert, M., Krah, R. and Kozyavkin, S.A. (1993) Nature 364, 735–737.
[32] Slesarev, A.I., Lake, J.A., Stetter, K.O., Gellert, M. and Kozyavkin, S.A. (1994) J. Biol. Chem. 269, 3295–3303.
[33] Shuman, S., Golder, M. and Moss, B. (1989) Virology 170, 302–306.
[34] Rahmouni, A.R. and Wells, R.D. (1992) J. Mol. Biol. 223, 131–144.
[35] Bagga, R., Ramesh, N. and Brahmachari, S.K. (1990) Nucl. Acids Res. 18, 3363–3369.