JOURNAL OF BACTERIOLOGY, Nov. 1992, p. 7059–7062
0021-9193/92/217059-04$02.00/0

Vol. 174, No. 21

Conditional Growth of *Escherichia coli* Caused by Expression of Vaccinia Virus DNA Topoisomerase I

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Received 18 May 1992/Accepted 25 August 1992

Active vaccinia virus topoisomerase I is expressed in *Escherichia coli* containing plasmid p1940 (S. Shuman, M. Golder, and B. Moss, J. Biol. Chem. 263:16401–16407, 1988). Growth curves showed a decline of 2 to 3 logs in the number of viable cells at 42°C after shift from 30°C because of increased vaccinia virus topoisomerase I level. Mutations in the *gyrA* and *gyrB* genes allowed cells to grow equally well at 42 and 30°C. The presence of gyrase inhibitor also improved growth at 42°C.

In *Escherichia coli*, negatively supercoiled DNA is required for many important cellular processes (7, 16, 17). Gyrase and topoisomerase I are the major enzyme activities responsible for the regulation of DNA supercoiling in *E. coli* (20). The expression of these two activities is controlled by homeostatic regulation of the transcription of their genes (10, 18, 19). Like other eukaryotic type I DNA topoisomerases, vaccinia virus topoisomerase I differs from the bacterial topoisomerase I in its ability to relax both negatively and positively supercoiled DNA (14). Its gene has been cloned into the multiple cloning site of the plasmid pUC19 (15). In the resulting plasmid p1940, the vaccinia virus topoisomerase I gene has the orientation opposite that of *lacZ*. Nevertheless, we found that Mg^{2+}-independent relaxation activity characteristic of eukaryotic topoisomerase I can be detected in the crude enzyme extract prepared from *E. coli* cells transformed with p1940. The *E. coli* strains used in this study are listed in Table 1. When we compared the growth curves of transformed MV1190 cells grown at 30 and 42°C (Fig. 1), we found that there was a decline in the number of viable cells beginning at 1 h after being shifted to 42°C. The extent of cell death depended on the strain, varying from 1 log for JD5 to 2 to 3 logs for MV1190. p1940A, constructed by filling in the *Acc*I site of p1940, has a frameshift mutation at amino acid 68 of the vaccinia virus topoisomerase I gene. It has a much smaller effect on cell growth at 42°C, probably because of read-through, since MV1190 cells transformed with the cloning vector pUC19 showed no difference in growth rate at 30 and 42°C. The loss of viability at 42°C is not due to killing by ampicillin from loss of the plasmid. Growth curves obtained in the absence of antibiotic in the medium showed the same results.

We transformed *E. coli* AS17 (2, 20) with p1940 to see whether the vaccinia virus topoisomerase I activity could

TABLE 1. *E. coli* strains

Strain	Description	Source or reference
C600	*supE44 thi-1 thr-1 leuB6 lacY1 tonA21 mcrA*	9
CKR01	C600 *galK zei*::Tn*10*	C600 P1 transduced to Tet^r (Bgl$^-$) from KVR1 (18)
CKR5	C600 *galK gyrA224 zei*::Tn*10*	C600 P1 transduced to Tet^r (Bgl$^+$) from KVR1 (12, 18)
CSD01	C600 *galK tna*::Tn*10*	C600 P1 transduced to Tet^r (Bgl$^-$) from K0635 (6, 18)
CSD5	C600 *galK gyrB225tna7*::Tn*10*	C600 P1 transduced to Tet^r (Bgl$^+$) from SD104-20 (6, 18)
JD5	JM103 *dinD1*::Mu d(Km^r *lac*)	JM103 P1 transduced to Km^r from JH140
JH140	K560 *dinD1*::Mu dI1734 (Km^r *lac*)	J. Heitman (8)
JM103	*endA1 hsdR supE sbcBC thi-1 strA* Δ(*lac-pro*) (F′ *traD36 lacI^{q}ZΔM15 proAB*)	9
HB101	*hsdS recA ara proA lacY leu galK rpsL xyl mtl supE*	4
MV1190	Δ(*lac-pro*) *thi supE* Δ(*srl-recA*)*306*::Tn*10* (F′ *traD36 lacI^{q}ZΔM15 proAB*)	Bio-Rad Laboratories
RR1	HB101 *rec*$^+$	3

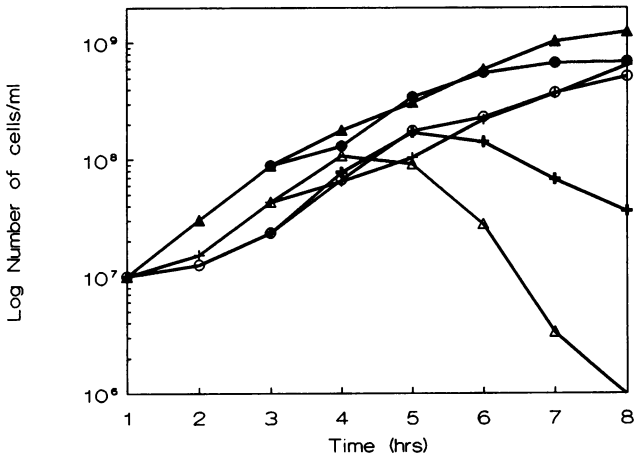

FIG. 1. Cell growth of *E. coli* MV1190 transformed with pUC19 (▲ and ●), p1940 (+ and △), and p1940A (○ and +) at 30 and 42°C, respectively. The cells were grown in LB medium (from GIBCO) with 300 μg of ampicillin per ml at 30°C for 3 h. Half of the culture was then transferred to a 42°C water bath. Samples were taken every hour, plated on an LB plate with 300 μg of ampicillin per ml, and incubated overnight at 30°C to determine viable cell count.

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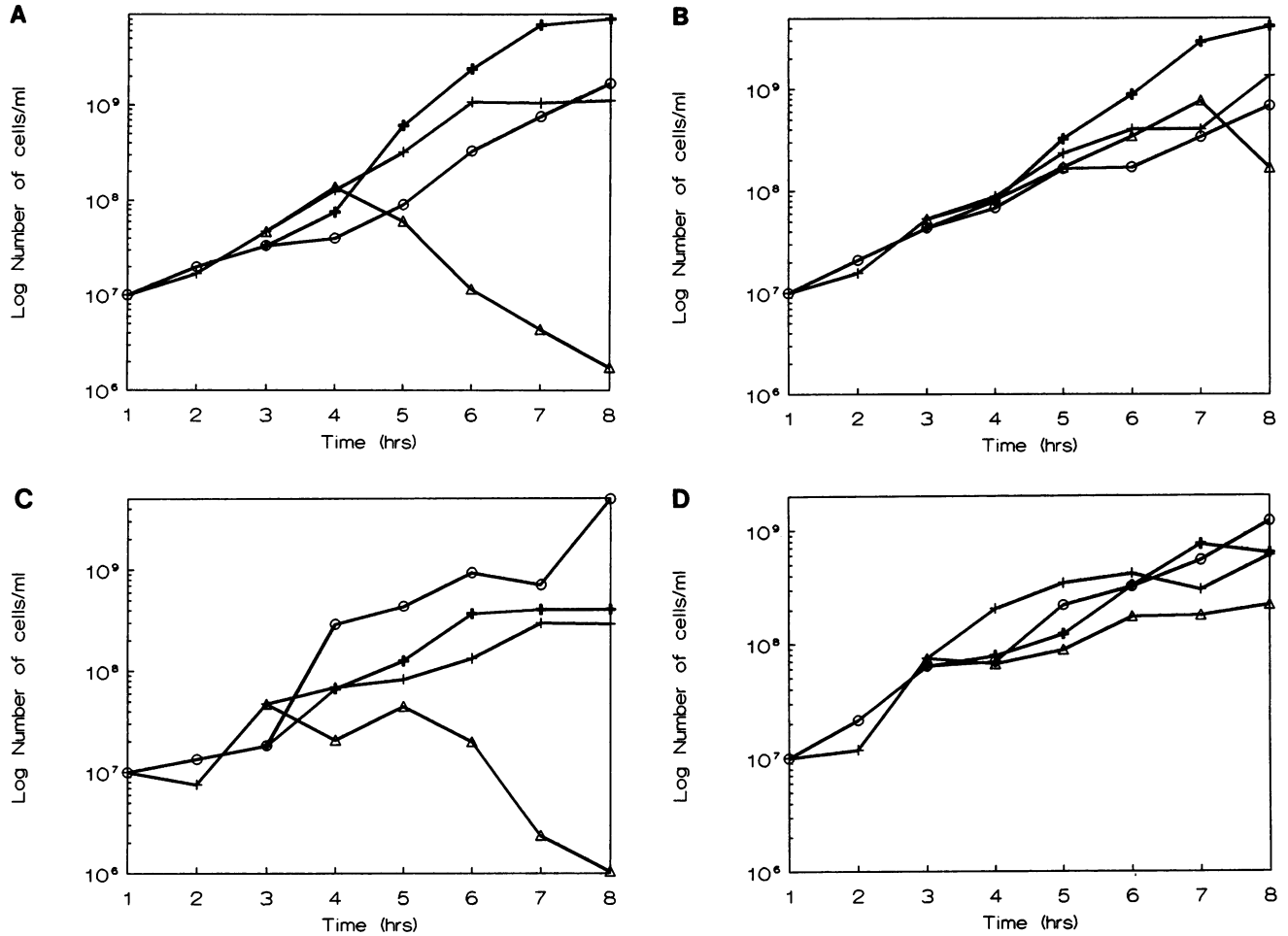

FIG. 2. Cell growth of CKR01 (*gyrA*$^+$) (A), CKR5 (*gyrA224*) (B), CSD01 (*gyrB*$^+$) (C), and CSD5 (*gyrB225*) (D) transformed with p1940 (+ and △) and p1940A (○ and +) at 30 and 42°C, respectively. Viable cell count was determined as described in the legend to Fig. 1.

compensate for the loss of the chromosomal *E. coli* topoisomerase I activity at 42°C caused by the inactivation of a *ts* suppressor. The transformant was grown in Luria broth containing ampicillin at 30°C. Serial dilutions of the culture were plated and incubated at both 30 and 42°C. We found that the presence of p1940 did not increase the viability of AS17 at 42°C (data not shown).

We then examined whether mutations in genes coding for DNA gyrase can affect tolerance for p1940 at 42°C. Strain CKR01 carrying p1940 lost viability when grown at 42°C to the same extent as did MV1190 (Fig. 2). Cells of the corresponding isogenic strain CKR5 (*gyrA224*) carrying p1940 did not die at 42°C. Identical rates of growth were obtained with CKR5 carrying p1940 and p1940A. Similar results were obtained with the isogenic strains CSD01 (*gyrB*$^+$) and CSD5 (*gyrB225*), as shown in Fig. 2C and D, respectively. These two gyrase mutations are known to reduce gyrase activities (6, 12). Gyrase activity in *E. coli* can also be reduced by the inhibitor novobiocin. In the presence of 50 μg of novobiocin per ml, the CKR01 transformants were found to have reduced growth rates at 30°C, but transformants with p1940 and p1940A showed identical growth rates at 42°C (data not shown), indicating that the presence of active vaccinia virus topoisomerase I did not lead to reduction in the number of viable cells.

Plasmid p1940 extracted from *E. coli* MV1190, HB101, RR1, CKR01, CKR5, CSD01, and CSD5 always appeared as a dimer during agarose gel electrophoresis regardless of the *recA*, *gyrA*, or *gyrB* genotype (data not shown). In contrast, monomers can readily be observed in the preparations of vaccinia virus topoisomerase frameshift mutant plasmids p1940A and p1940B (constructed by filling in the *Afl*II site in p1940).

We monitored the stability of the p1940 dimer in MV1190. Cells were grown at 30°C for 3 h and then shifted to 42°C. Samples were removed every 90 min for plasmid DNA preparation by the alkaline lysis method (1). The volume of cells used was adjusted according to the optical density at 600 nm so that approximately the same number of cells was used for each plasmid preparation. As shown in Fig. 3, p1940 was maintained as a dimer without any apparent loss of copy number up to 8 h after the shift to 42°C. However, after overnight growth at 42°C, p1940 plasmid could no longer be detected by ethidium bromide staining. The cells could still grow in medium containing 300 μg of ampicillin per ml, indicating a drastic decrease in copy number but not total disappearance of the plasmid. When these cells from the 42°C overnight culture were grown in Luria broth with 300 μg of ampicillin per ml at 30°C, the plasmid copy number did not increase to the previous level. Therefore, survival after

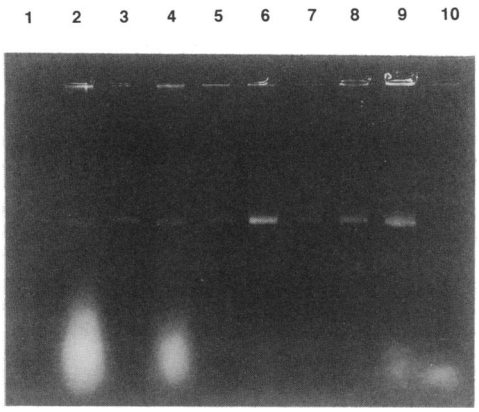

FIG. 3. Disappearance of p1940 plasmid DNA after overnight growth at 42°C. *E. coli* MV1190/p1940 was grown for 3 h at 30°C. Half of the culture was then grown at 42°C (lanes 2, 4, 6, 8, and 10), and half of the culture remained at 30°C (lanes 1, 3, 5, 7, 9). Plasmid was prepared from cells grown for an additional 3.5 h (lanes 1 and 2), 5 h (lanes 3 and 4), 6.5 h (lanes 5 and 6), 8 h (lanes 7 and 8), or overnight (lanes 9 and 10) after the temperature shift. Electrophoresis was carried out in a 1% agarose gel with TAE (40 mM Tris-acetate [pH 8.1], 2 mM EDTA) buffer in the presence of 0.5 μg of ethidium bromide per ml.

selection at 42°C is likely due to mutation in the plasmid replication region or possibly a host mutation that suppresses plasmid copy number. Plasmids p1940A and 1940B persisted at 42°C with no reduction in copy number (data not shown).

MV1190 cells from the above experiment were also used for preparation of crude extract (20). Figure 4a showed that there was increased DNA relaxing activity in extracts prepared from cells grown at 42°C. After 8 h of growth at 42°C, a fivefold dilution of the extract had higher activity than the crude enzyme extract obtained from cells grown at 30°C.

Total RNA was extracted (13) from MV1190 cells grown at 30°C, as well as from cells grown for 3 h at 30°C and then for 3 or 7 h at 42°C. Ten micrograms of total RNA was blotted onto nitrocellulose and hybridized (9) with a ^{32}P-labeled oligonucleotide complementary to the coding sequence from amino acid 18 to 29 of the vaccinia virus topoisomerase I. Densitometer scanning of the results in Fig. 4b showed a sixfold increase of vaccinia virus topoisomerase mRNA after 7 h at 42°C.

The induction of vaccinia virus topoisomerase I at 42°C is probably due to increased transcription from a cryptic heat shock promoter. Unfortunately, our efforts in mapping the 5′ end of the vaccinia virus topoisomerase I mRNA have been unsuccessful. The DNA sequence upstream of the vaccinia virus topoisomerase I coding sequence was analyzed for the presence of the *E. coli* heat shock promoter sequence. There was no obvious match to the consensus sequence TCTC-NCTTGAAN$_{13-17}$CCCCATNTA (5).

Plasmid p1940 extracted from MV1190 grown for 3 or 7 h after being shifted to 42°C was analyzed by two-dimensional gel electrophoresis. The results in Fig. 5 showed that a substantial portion of p1940 became relaxed. In contrast, p1940 extracted from cells grown at 30°C was always negatively supercoiled. The mutated plasmid p1940B, which does not produce vaccinia virus topoisomerase I, was always supercoiled.

The bacterial cell can apparently tolerate a low level of the

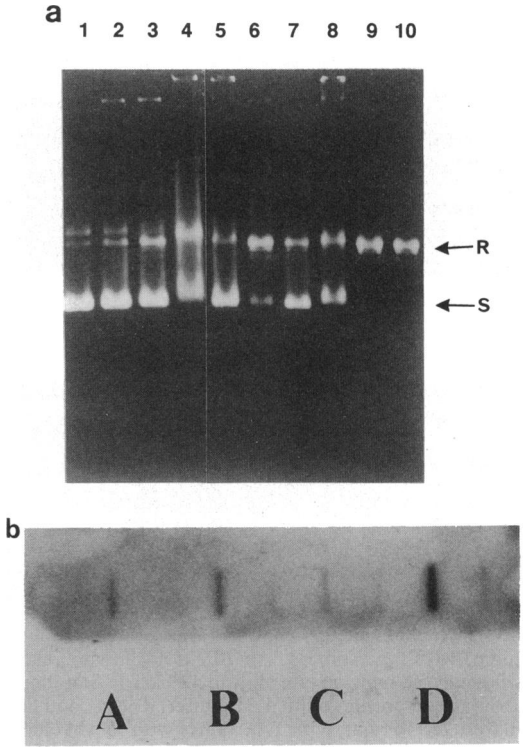

FIG. 4. Induction of vaccinia virus topoisomerase activity and mRNA at 42°C. (a) Relaxation of supercoiled DNA by vaccinia virus topoisomerase I activity in the crude extract. One microliter of crude enzyme extract, prepared as described previously (20), was incubated with 300 to 500 ng of plasmid pBR322 DNA in 20 μl of 40 mM Tris-HCl (pH 7.5)–100 mM NaCl–2.5 mM EDTA at 37°C for 30 min. The reaction was stopped by the addition of sodium dodecyl sulfate to 1%, and the mixture was heated at 60°C for 20 min and then electrophoresed in a 0.8% agarose gel with TAE buffer. The gel was stained with ethidium bromide and photographed under UV light. Lane 1, control, DNA with no extract added. The extracts added to lanes 2 to 4 were obtained from overnight cultures of MV1190 with plasmid p1940B grown at 30°C (lane 2), with plasmid p1940 grown at 30°C (lane 3), or with plasmid p1940 grown at 42°C (lane 4). Extracts added to lanes 5 to 10 were obtained from an experiment in which MV1190/p1940 was grown first for 3 h at 30°C, and then half of the culture was grown at 42°C (lanes 6, 7, 9, and 10) while the other half of the culture remained at 30°C (lanes 5 and 8). The cells were removed for extract preparation at 3.5 h (lanes 5, 6, and 7) or 8 h (lanes 8, 9, and 10) after the shift. A 1:5 dilution of the extract was used in lanes 7 and 10. Relaxed DNA (R) and supercoiled DNA (S) are indicated on the right. (b) Blotting of RNA from MV1190/p1940 by using a vaccinia virus topoisomerase-specific probe. RNA was extracted from MV1190/1940 cells grown at 30°C for 6 h (lane A), at 30°C for 3 h and then at 42°C for 3 h (lane B), at 30°C for 10 h (lane C), or at 30°C for 3 h and then at 42°C for 7 h (lane D).

eukaryotic topoisomerase I expressed at 30°C. However, when vaccinia virus topoisomerase I expression was induced at 42°C, the cell lost its viability. This is in agreement with the toxic effect previously reported for overexpression of the yeast and vaccinia virus topoisomerase I in *E. coli* (2, 11). The toxicity is likely due to the effect of the enzyme on DNA supercoiling.

The expression of yeast topoisomerase I has been reported to complement the conditional lethal *topA* mutation in AS17 (2). Previous studies have shown that the in vitro activity of the vaccinia virus topoisomerase I is not de-

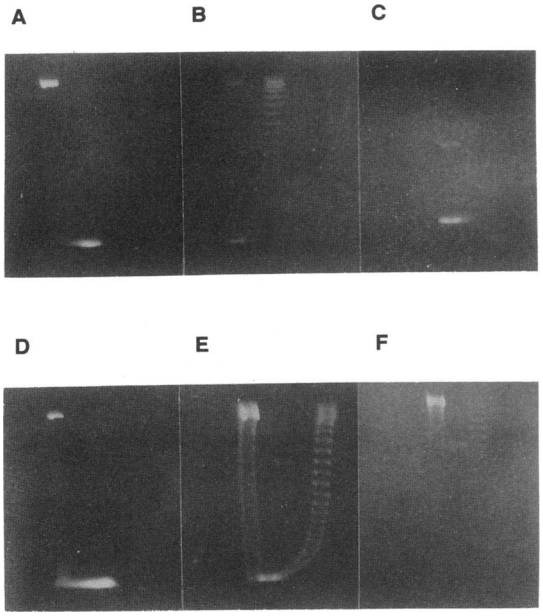

FIG. 5. Two-dimensional agarose (1%) gel electrophoresis of p1940 and p1940B topoisomers from MV1190 showing relaxation of p1940 following the temperature shift to 42°C. The first dimension is shown from top to bottom, with TAE buffer. The second dimension is shown from left to right, with TAE buffer with 15 μM chloroquine. The upper topoisomer on the left in each panel corresponds to the open circle form of the plasmid. (A) p1940 extracted from cells grown for 6 h at 30°C. The intense band near the bottom corresponds to negatively supercoiled topoisomers. (B) p1940 extracted from cells grown for 3 h at 30°C and then at 42°C for 3 h; (C) p1940B extracted from cells grown for 3 h at 30°C and then at 42°C for 7 h. The DNA is in monomeric form instead of the dimeric form for 1940. (D) p1940 extracted from cells grown for 10 h at 30°C; (E) p1940 extracted from cells grown for 3 h at 30°C and then at 42°C for 7 h; (F) p1940 relaxed in vitro with calf thymus topoisomerase I. The negatively supercoiled topoisomers have been converted to the covalently closed relaxed topoisomers, shown near the top on the right.

creased by preincubation or assay at 42°C (11). The two-dimensional gel electrophoresis analysis also showed that the vaccinia virus topoisomerase I expressed from p1940 is active in vivo at 42°C. Therefore, apparently, the harmful effects of expression of vaccinia virus topoisomerase I and the lack of *E. coli* topoisomerase I do not counteract each other to allow viability of AS17 at 42°C. In contrast, reduction of gyrase activity by either mutations in the *gyrA* and *gyrB* genes or the presence of the gyrase inhibitor novobiocin allowed growth of *E. coli* with p1940 at 42°C. The explanation for this compensatory effect is not clear and will be further investigated in the future.

This research was supported by a research grant (CH478) from the American Cancer Society.

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