The viability of the topA mutants lacking DNA topoisomerase I was thought to depend on the presence of compensatory mutations in Escherichia coli but not Salmonella typhimurium or Shigella flexneri. This apparent discrepancy in topA requirements in different bacteria prompted us to reexamine the topA requirements in E. coli. We find that E. coli strains bearing topA mutations, introduced into the strains by DNA-mediated gene replacement, are viable at 37 or 42 °C without any compensatory mutations. These topA cells exhibit cold sensitivity in their growth, however, and this cold sensitivity phenotype appears to be caused by excessive negative supercoiling of intracellular DNA. In agreement with previous results (Zhu, Q., Pongpech, P., and DiGate, R. J. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 9766–9771), E. coli cells lacking both type IA DNA topoisomerases I and III are found to be nonviable, indicating that the two type IA enzymes share a critical cellular function.

The type IA subfamily of DNA topoisomerases, some of the extensively studied examples are E. coli DNA topoisomerases I and III, yeast DNA topoisomerase III, Drosophila, and mammalian DNA topoisomerases IIIα and IIIβ, are found in all living organisms (1–3). This universal presence suggests a key cellular role of these enzymes that cannot be fulfilled by either the type IB or type II subfamily of DNA topoisomerases. In support of this notion, the viability is compromised upon inactivation of both type IA enzymes of Escherichia coli (4) or inactivation of the sole type IA enzyme of the fission yeast Schizosaccharomyces pombe (5, 6). For the budding yeast Saccharomyces cerevisiae, Δtop3 cells lacking DNA topoisomerase III are viable, but they exhibit a complex phenotype including slow growth, high level of recombination, high sensitivity to DNA-damaging agents, and inability to produce viable spores (7–10). In mouse (11), as well as in Drosophila, inactivation of DNA topoisomerase IIIα leads to embryonic lethality. Whereas viability and development of both mouse and Drosophila appear normal in the absence of DNA topoisomerase IIIβ (12, 13), mice lacking this enzyme exhibit a shortened life span and reduced fecundity and develop inflammatory responses in multiple organs (12, 14).

Insight into the molecular roles of the type IA enzymes came mostly from studies of microorganisms (reviewed in Refs. 1–3 and 15). For the two E. coli enzymes, there is strong biochemical and genetic evidence that DNA topoisomerase I has a major role in the preferential removal of negative supercoils in intracellular DNA, especially in regions behind the transcribing assembles tracking along DNA (15–19). In an in vitro plasmid replication system, DNA topoisomerase III but not I was shown to support the unlinking of the parental DNA strands to yield separate progeny DNA rings (20), implicating a role of DNA topoisomerase III in unlinking of DNA strands during replication (20). E. coli ΔtopB mutants lacking DNA topoisomerase III exhibit no growth defect (21), however, and thus it appears that copying the parental strands might precede their complete unraveling, so that the plentecenomically wound parental strands could be converted to a pair of intertwined double helices for decatenation by a type II DNA topoisomerase (22).

That the two E. coli type IA enzymes appear to play distinct cellular roles does not preclude the possibility, however, that they may also share a common function. In the yeasts, mutations in a number of genes involved in homologous recombination were shown to suppress the phenotype of yeast Δtop3 mutants, implicating a role of the topoisomerase in the resolution of a structure or structures formed in homologous recombination (22, 23). The growth defect of an E. coli topA topB double mutant was also reported to be suppressed by an additional mutation in recA, a key gene in homologous recombination (4). Based on a comparison of the mechanisms of different subfamilies of DNA topoisomerases, it has been suggested that a candidate for such a structure might be the double Holliday junction (2, 14).

In contrast to the dispensability of E. coli DNA topoisomerase III in terms of cell viability (21), inactivation of E. coli DNA topoisomerase I was generally thought to be lethal. Shortly after the identification of a set of viable E. coli ΔtopA mutants (25), it was found that the ΔtopA locus of these mutants could not be readily transduced into strain PLK831 (∆trpB63 pyrF287 nirA trpR72 iclR7 gal25 rpsL195) by phage P1 (26, 27). The same recipient strain became more easily transduced, however, if it had acquired a compensatory change within certain regions of the E. coli chromosome, including gyrA and gyrB, which encode the two subunits of gyrase, and a region containing tolC, which encodes an outer membrane transporter (26–29). These results suggested that ΔtopA is lethal in the absence of compensatory genetic changes. A more direct demonstration of the lethality of topA deletion was provided by experiments demonstrating the thermal sensitivity of E. coli topA amber mutants expressing a thermal sensitive amber suppressor. Presumably, suppression of the amber codon at a permissive temperature would provide an adequate level of DNA topoisomerase I and, consequently, cell growth (30, 31).
The idea that the topA gene is essential for cell growth was not reinforced, however, by studies in *Salmonella typhimurium*, a bacterium closely related to *E. coli*. Early studies indicated that null mutations in an *S. typhimurium* gene termed supX, which was later shown to be identical to topA (28, 32), led to no lethal effects; there was no indication that viability of these topA cells depended on the presence of a compensatory mutation (33, 34). More recently, Shigella flexneri topA null mutants were also found to be viable without apparent compensatory mutations (35). Thus there appears to be a difference in topA requirement in different bacteria; it is this apparent difference that prompted us to reexamine the viability of *E. coli* mutants lacking DNA topoisomerase I.

**MATERIALS AND METHODS**

**Targeted Gene Replacement and Phage P1 Transduction—**The vector pRM4-N for gene replacement in *E. coli* by chi-stimulated homologous recombination (40) was kindly provided by Dr. G. Smith (University of Washington). In this plasmid, a multiple cloning site is placed in between two triplets of *chi* sequences. DNA segments derived from a 12.1-kb Sall-HindIII fragment containing the *E. coli* cysB-topA-trpE region (42) were placed within the multiple cloning site according to routine cloning manipulations (see “Results” for a description of the three classes of derivatives, all of which have a chloramphenicol resistance marker inserted at a unique SphI site of the 12.1-kb segment). The chloramphenicol resistance marker *cam* was amplified from pACYC184 (supplied by New England Biolabs) by PCR in the presence of primers that would add SphI sites to both ends of a 1.41-kb DNA product; the inducible lac promoter used in the construction of one class of derivatives was obtained from pW312 as a HindIII-EcoRI fragment (39). Codon substitutions within the *topA* region were done by replacing appropriate DNA segments by the corresponding segments containing the alterations L5F C662H (36) or G65N W79S (37). Codon substitutions within the *topA* region were done by replacing appropriate DNA segments by the corresponding segments containing the alterations L5F C662H (36) or G65N W79S (37).

Various pRM4-N derivatives were linearized by digestion with NotI. The large linear DNA segments lacking the replication origin of pRM4-N were purified by gel electrophoresis and used for gene replacement in the *cysB*-*topA*-*trpE* region of the *E. coli* chromosome, through homologous recombination mediated by *recBCD*, as described by Dabert and Smith (40), or the phage *λ* Red recombination system, as described by Datsoenko and Wanner (41). In the latter case, the thermal sensitive plasmid expressing the *λ* Red functions *γ*, *β*, and *exo*, pKD46, was kindly provided by Dr. B. L. Wanner (Purdue University). *cam*− colonies were first selected in these experiments, and several tests were utilized to ascertain the incorporation of the expected changes in chromosomal DNA samples extracted from selected colonies. For mutants in which the *topA* promoter was replaced by a lac promoter, or mutants in which a large *topA* segment was deleted, amplification of these shortened regions by PCR was carried out to confirm the presence of these altered regions and the absence of the corresponding wild-type segments. Appropriate restriction digests were also analyzed by Southern blot hybridization for the presence of the *cam* marker and other changes, taking advantage of alterations in restriction sites in the G65N W79S mutants (37). In all cases, the selected *cam*− colonies were found to have correctly incorporated the intended changes in the *topA* region. Transduction by phage P1eir was carried out according to the protocol described by Silhavy (51), using a phage stock kindly provided by Dr. N. Kleckner (Harvard University).

**Analysis of Plasmid Linking Number Distributions—**Two-dimensional agarose gel electrophoresis and the use of Southern blot hybridization to monitor plasmid supercoiling were carried out as described previously (43).

**RESULTS**

**Construction of E. coli topA Conditional Mutants by DNA-mediated Gene Replacement—**The introduction of methods for DNA-mediated gene targeting in *E. coli* has made it possible to construct better defined *E. coli* topA mutants. In order to minimize the possibility of inadvertently acquiring compensatory mutations in these mutants, conditional topA mutants were first constructed under permissive conditions that produced a functional mutant DNA topoisomerase I. Fig. 1 depicts the common features of the cloned DNA segments used for gene replacements in the *topA* region of the *E. coli* chromosome. A *cam* gene encoding chloramphenicol resistance was first inserted at a SphI site within a cloned 12.1-kb Sall-HindIII fragment. This insertion does not disrupt any of the open reading frames in the *topA* region; to minimize plausible interference with *cam* expression, *cam* transcription was also chosen to be directing away rather than into *topA*. Two classes of conditional *topA* mutants were constructed. The first class, *topA* ts (L5F C662H) and *topA* ts (G65N W79S), encodes two thermal sensitive DNA topoisomerase I with the indicated point mutations L5F C662H and G65N W79S, respectively. The L5F C662H mutant was derived from site-directed mutagenesis of individual codons of cysteinyl residues that are involved in Zn(II) binding (36). The mutation C662H was found to give a thermal sensitive enzyme (36), and sequencing of the mutant gene revealed the presence of a second mutation L5F in the gene, which by itself had little effect on enzyme activity. The (G65N W79S) mutant was expected to produce the same mutant DNA topoisomerase I of *E. coli* coli topA strain AS17, which harbors a supD amber suppressor (30, 31). The *topA* gene of strain AS17 has a G65N mutation and an amber codon instead of Trp-79 of wild-type *topA* (37). Originally, it was thought that the thermal sensitivity of the supD suppressor in this strain, which would incorporate a serine at the amber codon only at a permissive temperature (38), was responsible for the *topA* ts phenotype of AS17. Recent evidence indicates, however, that the protein product in the presence of the suppressor, with amino acid changes G65N and W79S relative to the wild-type protein, is itself thermal sensitive (37).

The second class of mutant *topA* segments are denoted Plac-topA ts (L5F C662H) and Plac-topA ts (G65N W79S); they were derived from the corresponding members of the first class by replacing the *topA* regulatory region (39) with a lac promoter so that expression of the mutant *topA* genes can be turned on or off by the presence or absence of the lac inducer IPTG (38) (the region replaced by the lac promoter is indicated by an X in Fig. 1). An additional DNA segment bearing the mutation Plac-topA (L5F), which encodes a protein with wild-type DNA topoisomerase I activity, was also used in the construction of a control. As will be described later, several mutants with the deletion of a large region in the *topA* region, marked by an X in Fig. 1, were also made in the later stages of the present study.

**Viability of E. coli topA Mutants Expressing a Thermally Sensitive DNA Topoisomerase I—**Replacement of the chromosomal *topA* region by *topA* ts (L5F C662H) or *topA* ts (G65N W79S), using either the *chi* site-dependent homologous recombination method (40) or the *λ* Red recombination system (41), led to no lethal effects; there was no indication that via-
Viability of E. coli topA Mutants Lacking DNA Topoisomerase I

Table I

| Strain  | Genotype                                      | Source or Ref. |
|---------|-----------------------------------------------|----------------|
| AB1369  | DEG-gpt-proA62 lacY1 tac-29 glv44(A5) galK20(oc) LAM– cysB38 Rec-9 hisG4(Oc) rfd1 | A. L. Taylor   |
| MG1655  | F’ LAM – rph-I                                | 48, 49         |
| MM28    | galK2(Oc) LAM – IN(rrnD-rrnE1) rpsL200(strR)   | M. Meselson    |
| W3110   | LAM – IN(rrnD-rrnE1) rpsL200(strR) rph-I       | J. Ledenberg   |
| BL21(DE3) | hsdS gal (sc tns57 ind1 San7 nin5 lacUV5-T7 gene1) | 50             |
| BL21(DE3)-topB | BL21(DE3) topB-Tn5 | 20             |
| BR83    | F’ topA57(Am) argA rpsL lac5Δ14 supD 43, 74 (ts 42 °C) | R. E. Depew    |
| AS17    | F’ topA17(Am) pLL1[supD 43, 74 (ts 42 °C)]      | R. E. Depew    |
| RFM480  | rpsL galK2(oc) gyrB221(oc) gyrB203(ts) topA29::Tn10 Δlac74 | 44             |

Listed are the strains previously reported.

was first carried out in two E. coli strains AB1369 and MG1655 (see Tables I and II for the genotypes of these and other strains used in this work). Strain AB1369 is a cysB– strain used in the original cloning of the E. coli topA gene (42), and strain MG1655 is a widely used laboratory strain. Transfected cell cultures were incubated at 30 °C in these experiments, except that in experiments using the λ Red recombination system the cultures were kept at 37 °C for 1 h following transfection to eliminate the thermal sensitive plasmid pKD46 bearing the λ Red genes.

At 30 °C, the mutant enzyme with the L5H C662H or G65N W79S substitutions was expected to be fully functional; at 42 °C, the activity of the mutant enzyme would be much diminished (36, 37). Surprisingly, the two topA ts derivatives of either AB1369 or MG1655 grew as well as their parent cells on agar plates at either 30 or 42 °C. To test whether DNA topoisomerase I expressed in these topA ts mutants is indeed thermal sensitive, strain AB1369 topA ts (L5F C662H) was transformed with a tetA-bearing plasmid pVS1 (43), a plasmid that has been shown to be exessively negatively supercoiled (“hypernegatively supercoiled”) in cells lacking DNA topoisomerase I (43). Plasmid samples were recovered from a randomly selected transformant grown at 30 or 42 °C. Two-dimensional gel electrophoresis of these samples showed that the degree of negative supercoiling of the plasmid is much higher at the higher temperature (Fig. 2). Thus it appeared that these cells indeed had a much reduced level of DNA topoisomerase I at 42 °C, but despite this deficiency they grew as well as their topA+ parents.

It is known, however, that DNA topoisomerase I bearing the mutations L5F C662H or G65N W79S substitutions had a significant level of residual activity at 42 °C (36, 37). To better define the requirement of DNA topoisomerase I at 42 °C, strain AB1369 and MG1655 derivatives bearing Plac-topA ts (L5F C662H) or Plac-topA ts (G65N W79S) were constructed (see Table II). In these cells, expression of DNA topoisomerase I is strongly dependent on the presence of IPTG, as demonstrated by two-dimensional gel electrophoresis of pVS1 recovered from AB1369 bearing Plac-topA ts (L5F C662H) (Fig. 3). At 30 °C, the plasmid became much more negatively supercoiled following the removal of IPTG (Fig. 3, compare lanes 1 and 2). In the absence of IPTG, the patterns at 30 and 42 °C also showed that the plasmid was more negatively supercoiled at the higher temperature, again demonstrating the thermal sensitivity of the mutant topoisomerase (Fig. 3, compare lanes 2 and 4). Plating of the mutant cells showed that even in the absence of IPTG, at 42 °C both of the Plac-topA ts derivatives grew as well as their topA+ parents, which strengthens the notion that DNA topoisomerase I is dispensable for cell growth.

Cold Sensitivity of E. coli topA Mutants Lacking DNA Topoisomerase I—At 30 °C and in the absence of IPTG, the two Plac-topA ts derivatives of either strain AB1369 or MG1655 grew very poorly relative to their respective topA+ parent; comparable growth of the isogenic topA+ and topA ts strains at this lower temperature was only seen in the presence of IPTG. This strong temperature dependence of the growth of topA-deficient cells was also seen in a derivative of strain MG1655, in which the wild-type topA locus was replaced by Plac-topA(L5F). As noted earlier, DNA topoisomerase I with an L5F mutation is indistinguishable from the wild-type enzyme, at least in terms of its enzymatic activity. It was observed that MG1655 Plac-topA(L5F) grew well at either 30 or 42 °C in the presence of IPTG; in the absence of IPTG, however, a precipitous drop in topA (L5F) expression was expected, and the cells were found to grow normally at 42 °C but very poorly at 30 °C.

E. coli topA Cells Are Viable at 42 or 37 °C but Not at 30 °C—In a separate series of experiments, a topA mutation, in which a large region of the topA coding sequence and the entire promoter of the gene (region indicated by Y in Fig. 1) were absent, was first introduced into strain MG1655 by DNA-mediated gene replacement at 42 °C, a temperature at which cells lacking DNA topoisomerase I are apparently viable, as described above. A transformant bearing this topA locus, as ascertained by Southern blot hybridization and PCR, was then used as the donor strain in a transduction experiment using phage P1vir. As a control, MG1655 bearing Plac-topA ts (L5F C662H), which has been shown to grow well at 42 °C in the presence or absence of IPTG, was used as the donor strain in an identical phage P1vir infection. P1vir particles obtained in these infections were then used to transform AB1369 or MG1655 recipients that expressed a plasmid-borne ampicillin resistance gene. Transductants with an altered topA gene neighboring a chloramphenicol resistance marker was then scored on plates containing chloramphenicol, ampicillin, and IPTG at 42 °C (ampicillin was included to prevent contamination of ampicillin-resistant recipient cells by the ampicillin-sensitive donor cells). As shown by results tabulated in Table III, the frequencies of topA and Plac-topA ts (L5F C662H) transductants, with either AB1369 or MG1655 as the recipient, were comparable. Thus there was no indication that the topA transductants arose as rare events in which compensatory mutations were acquired.

When these topA transductants were streaked on fresh plates and incubated at 30 °C, however, no colonies were observed after a 1-day incubation period, indicating defective growth at this temperature. Strain MG1655 topA was similarly checked for growth at 37 °C, and comparable growth was observed for the topA deletion and its topA+ parent cells. In P1vir transduction experiments using strain RFM480 as the donor, which carries a Tn10 insertion in the coding region of topA (44), cells lacking DNA topoisomerase I were again found to grow well at 42 but not 30 °C.

Cold Sensitivity of E. coli topA Mutants and Excessive Negative Supercoiling of Intracellular DNA—The above experiments show that whereas DNA topoisomerase I is dispensable for cell growth at 42 or 37 °C, its absence is detrimental at
as described previously (43). Samples analyzed in and detection of the plasmid DNA by blot hybridization were performed was used as described previously (47). Electrophoresis of plasmid DNA of the cell culture from 30 to 42 °C.

respectively isolated from cells 1 and 5 h after shifting the temperature to 30 °C (lane 1) or 42 °C (lanes 2 and 3). To minimize changes in plasmid topology during sample preparations, a rapid lysis procedure was used as described previously (47). Electrophoresis of plasmid DNA and detection of the plasmid DNA by blot hybridization were performed as described previously (43). Samples analyzed in lanes 2 and 3 were respectively isolated from cells 1 and 5 h after shifting the temperature of the cell culture from 30 to 42 °C.

30 °C. This cold sensitivity of topA nulls appears to be a direct manifestation of DNA topoisomerase I deficiency, as the introduction of a multicopy plasmid pJW312 bearing a Plac-linked topA gene, which expresses an adequate amount of the enzyme in cells in the absence of IPTG (30), was found to restore growth of the ΔtopA mutants to the same level of their topA + parents at 30 °C.

Most interestingly, growth of the topA null mutants at 30 °C was also found to be significantly improved by the introduction of a plasmid puc-hTop1 (45), which expresses human DNA topoisomerase I, a type IB enzyme that relaxes both positive and negative supercoils. Because the type IB and type IA DNA topoisomerases share few common features other than their ability to remove negative supercoils (1–3), this finding strongly suggests that the poor growth of E. coli topA mutants at 30 °C is tied to excessive negative supercoiling of intracellular DNA.

To test whether the cold sensitivity of topA mutants described so far might be a peculiar property of the genetic backgrounds of AB1369 and MG1655, similar experiments were carried out with additional topA + E. coli strains including MM28 and W3110 (see Table I for genotypes). In one series of experiments, one of the Plac-topA ts mutations in strain MG1655 was introduced into the various recipients bearing a plasmid-borne ampicillin resistance gene by P1 transduction (51). The genotypes of the donor strains are given in Table II.

![Fig. 2. Two-dimensional gel electrophoresis of a tetA-bearing plasmid pVS1 from AB1369 topA ts (L5F C662H) cells grown at 30 °C (lane 1) or 42 °C (lanes 2 and 3). To minimize changes in plasmid topology during sample preparations, a rapid lysis procedure was used as described previously (47). Electrophoresis of plasmid DNA and detection of the plasmid DNA by blot hybridization were performed as described previously (43). Samples analyzed in lanes 2 and 3 were respectively isolated from cells 1 and 5 h after shifting the temperature of the cell culture from 30 to 42 °C.]

![Fig. 3. Two-dimensional gel electrophoresis of pVS1 isolated from strain AB1369 Plac-topA ts (L5F C662H) cells grown under different conditions. Lane 1, cells grown at 30 °C in the absence of IPTG; lane 2, cells grown at 30 °C in the presence of 0.5 mM IPTG; lane 3, cells initially grown at 30 °C in the absence of IPTG and then shifted to a growth temperature of 42 °C for 1 h; lane 4, cells initially grown at 30 °C in the presence of 0.5 mM IPTG and then shifted to a growth temperature of 42 °C for 1 h in the same medium.]

| Laboratory designation | Strain background | topA locus | Method of construction |
|------------------------|-------------------|-----------|-----------------------|
| VS100a                 | AB1369            | topA ts (L5F C662H) | b                     |
| VS100b                 | MG1655            | topA ts (L5F C662H) | b                     |
| VS101                  | AB1369            | Plac-topA ts (L5F C662H) | b,c                  |
| VS102                  | AB1369            | Plac-topA ts (L5F C662H) | b,c                  |
| VS105                  | MG1655            | Plac-topA ts (L5F C662H) | b                     |
| VS106                  | MG1655            | Plac-topA ts (L5F C662H) | b,c                  |
| VS107                  | AB1369            | Plac-topA ts (G65N W79S) | b,c                  |
| VS108                  | MG1655            | Plac-topA ts (L5F) | b                     |
| VS109                  | MG1655            | Plac-topA ts (L5F) | b,c                  |
| VS111a                 | AB1369            | ΔtopA | c                     |
| VS111                  | MG1655            | ΔtopA | c                     |
| VS112                  | AB1369            | topA ts | c                     |
| VS113                  | MG1655            | top20 ∶Tn10 | c                     |
| VS114                  | AS17              | Plac-topA ts (L5F C662H) | c                     |
| VS115                  | BR83              | Plac-topA ts (L5F C662H) | c                     |

*a The strains VS111, VS102, VS105, and VS106 have been deposited in the CGSC.

*b Gene replacement (40, 41).

*c P1vir transduction (51).

**Table II**

*Lists of E. coli strains used in this study*

| Laboratory designation | Strain background | topA locus | Method of construction |
|------------------------|-------------------|-----------|-----------------------|
| VS100a                 | AB1369            | topA ts (L5F C662H) | b                     |
| VS100b                 | MG1655            | topA ts (L5F C662H) | b                     |
| VS101                  | AB1369            | Plac-topA ts (L5F C662H) | b,c                  |
| VS102                  | AB1369            | Plac-topA ts (L5F C662H) | b,c                  |
| VS105                  | MG1655            | Plac-topA ts (L5F C662H) | b                     |
| VS106                  | MG1655            | Plac-topA ts (L5F C662H) | b,c                  |
| VS107                  | AB1369            | Plac-topA ts (G65N W79S) | b,c                  |
| VS108                  | MG1655            | Plac-topA ts (L5F) | b                     |
| VS109                  | MG1655            | Plac-topA ts (L5F) | b,c                  |
| VS111a                 | AB1369            | ΔtopA | c                     |
| VS111                  | MG1655            | ΔtopA | c                     |
| VS112                  | AB1369            | topA ts | c                     |
| VS113                  | MG1655            | top20 ∶Tn10 | c                     |
| VS114                  | AS17              | Plac-topA ts (L5F C662H) | c                     |
| VS115                  | BR83              | Plac-topA ts (L5F C662H) | c                     |

*a The strains VS111, VS102, VS105, and VS106 have been deposited in the CGSC.

*b Gene replacement (40, 41).

*c P1vir transduction (51).

**Table III**

*Frequencies of transducing topAD and topA ts mutations into E. coli AB1369 and MG1655 strains*

| Donor | Recipient |
|-------|-----------|
| VS111[topAΔ] | 80 | 54 |
| VS106 [Plac-topA ts (L5F C662H)] | 54 | 52 |
Viability of strain AS17 and BR83 cells at 42 °C, however, apparently requires DNA topoisomerase I, as reported previously (30, 31). Transduction of Plac-topA ts (L5F C662H) into either AS17 or BR83 in the presence of IPTG showed that the resulting cam− transductants failed to form colonies on plates devoid of IPTG at either 30 or 42 °C.

Nonviability of topA topB Double Mutants—We have confirmed an earlier report that E. coli cells lacking both type IA DNA topoisomerases I and III are nonviable (4). A disrupted topB gene [topB::Tn5] was first transduced from strain BL21(DE3) topB::Tn5 (20) into strain AB1369 Plac-topA ts (L5F C662H), at 30 °C and in the presence of IPTG. On plates without IPTG, however, the resulting Plac-topA ts topB::Tn5 double mutant was found to form no colonies at either 30 or 42 °C. When grown in a liquid culture, highly filamented double mutant cells were seen following the removal of IPTG, especially at 42 °C. By contrast, in the presence of IPTG these cells exhibited wild-type morphology at 42 °C.

The above results indicate that the topA ts (L5F C662H) gene product retains a low level of enzymatic activity at 42 °C, as mentioned earlier, and that this low level of topA activity is sufficient to sustain cell viability in a topB− background at 42 °C. A drastic reduction of Plac-topA expression upon IPTG removal, however, results in cessation of cell growth because of the depletion of all type IA DNA topoisomerase activities (4).

DISCUSSION

Our results show that mutant E. coli lacking DNA topoisomerase I is viable in the absence of any compensatory mutation at 42 or 37 °C. We believe that earlier studies of strain AS17, and the strain BR83 derived from AS17, led to the opposite conclusion because of a bias in the construction of the original strain. Strain AS17 was selected from a screen of mutagenized cells for topA amber mutations that exhibited thermal sensitivity in the presence of a thermal sensitive amber suppressor. This selection of thermal sensitivity could bias the screen toward the selection of topA amber mutants that had acquired additional mutations that exacerbate the dependence of their growth on DNA topoisomerase I activity. We have not tested the E. coli strain PLK831 that was used in other studies implicating a requirement of topA for growth (26, 27). Based on the results reported in this work, however, it is most likely that PLK831, which has a number of known genetic changes including an altered trpE region adjacent to topA, may also carry a mutation that makes its growth critically dependent on the presence of a functional DNA topoisomerase I.

The results reported here indicate that at a temperature of 30 °C or lower, the growth of E. coli topA nulls is much compromised. Why does topA inactivation lead to cold sensitivity? There is strong evidence that E. coli DNA topoisomerase I plays a significant role in the removal of negative supercoils behind the transcription machinery, especially in cases when nascent transcripts are anchored to the cell membrane through insertion of nascent peptides into the membrane (15–19); this conclusion is best demonstrated by experiments showing the hypernegatively supercoiled of intracellular DNA upon topA inactivation, including the results depicted in Figs. 2 and 3. Whereas the finding that expression of a type IB DNA topoisomerase in E. coli topA mutants improves cell growth suggests a close link between the growth defect of the mutants and DNA hypernegatively supercoiling, a molecular interpretation of the cold sensitivity of topA null mutants has yet to emerge. In general terms, a lower temperature could exacerbate a detrimental cellular reaction favored by an increase in DNA negative supercoiling. It has been suggested previously, for example, that topA inactivation might lead to defective growth through R-loop formation between a nascent transcript and the hypernegatively supercoiled DNA template behind the transcribing polymerase (44). In such a scenario, a lower temperature could directly exacerbate R-loop formation by preferentially stabilizing the RNA-DNA duplex relative to the DNA-DNA duplex, or exert an indirect effect determined by the temperature coefficients of the various steps in R-loop formation or elimination. Interpretations unrelated to R-loop formation could also be invoked. A lower temperature, for example, could further reduce the low activity of DNA topoisomerase III in negative supercoil removal, which could otherwise partially complement the action of DNA topoisomerase I. It should be interesting to carry out a comprehensive genetic screen to identify the various mutations that affect synthetic lethality in a topA null background at 42 °C.

The findings that E. coli DNA topoisomerase I is dispensable at higher growth temperatures (this work), but at least one of the type IA DNA topoisomerases is required for growth at any temperature (Ref. 4 and this work), also bring into focus that the two type IA enzymes DNA topoisomerase I and III are likely to share a key cellular function. As suggested previously, the essentiality of a minimal level of a type IA DNA topoisomerase activity is probably closely tied to the proper resolution of certain DNA structures, of which the double Holliday junction appears to be an attractive candidate (2, 14).

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