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Genetic Analysis of Mutations That Compensate for Loss of *Escherichia coli* DNA Topoisomerase I

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A transposon Tn*10* insertion in *topA*, the structural gene of *Escherichia coli* DNA topoisomerase I, behaves as an excluded marker in genetic crosses with many strains of *E. coli*. However, derivative strains that accept this mutant *topA* allele are readily selected. We show that many of these *topA* mutant strains contain additional mutations that compensate for the loss of DNA topoisomerase I. Genetic methods for mapping and manipulating such compensatory mutations are described. These methods include a plate-mating test for the ability of strains to accept a *topA*::Tn*10* allele and a powerful indirect selection for transferring compensatory mutations from male strains into non-compensatory female strains. One collection of spontaneous compensatory mutants is analyzed in detail and is shown to include compensatory mutations at three distinct loci: *gyrA* and *gyrB*, the genes that encode the subunits of DNA gyrase, and a previously unidentified locus near *tolC*. Mutations at this third locus, referred to as *toc* (topoisomerase one compensatory) mutations, do not behave as point mutations in transductional crosses and do not result in lowered DNA gyrase activity. These results show that wild-type strains of *E. coli* require DNA topoisomerase I, and at least one class of compensatory mutations can relieve this requirement by a mechanism other than reduction of DNA gyrase activity.

DNA topoisomerases are enzymes that catalyze the concerted breakage and rejoining of DNA phosphodiester bonds (for reviews see references 4 and 9). *Escherichia coli* may contain four different DNA topoisomerases that could influence the supercoiling of the chromosome. These are DNA topoisomerase I (topo I) (26), DNA topoisomerase II (12), DNA topoisomerase II′ (3, 10), and DNA topoisomerase III (5, 22). Each of these enzymes can relax negatively supercoiled DNA, but only DNA topoisomerase II (DNA gyrase) can negatively supercoil relaxed DNA.

The degree of supercoiling of the bacterial chromosome is presumably determined by a balance between DNA-relaxing and DNA-supercoiling activities. The negatively supercoiled chromosome contains considerable excess free energy that is important for the processes of replication, transcription, and recombination. Hence, the enzymes that control supercoiling are expected to have a crucial role in determining the function of DNA.

The isolation of mutants that have conditionally lethal alleles of *gyrA* (13, 14) or *gyrB* (18, 20) demonstrates that these genes are essential for the viability of *E. coli*. The *gyrA* gene, located at 48 min on the *E. coli* linkage map (2), encodes the A subunits of both DNA gyrase and DNA topoisomerase II′. The *gyrB* gene, at 82 min, is the structural gene for the B subunit of DNA gyrase. The other subunit of DNA topoisomerase II′, which appears to be a fragment of the B subunit of DNA gyrase, may also originate from *gyrB*, but this has not been proved.

The structural gene for *E. coli* topo I, *topA*, is located between the *trp* operon and *cysB* at 28 min (23, 24). Sternglanz et al. (23) described *topA* deletion mutants that remained viable despite a lack of detectable topo I. Trucksis et al. (25) demonstrated that the *Salmonella typhimurium supX* gene (15) is the equivalent of the *E. coli topA* gene, encoding topo I. *S. typhimurium* mutants that have the entire *supX* gene deleted arise spontaneously and remain viable (19). Thus, at the time we began these studies, the available evidence suggested that topo I had no essential function in either *E. coli* or *S. typhimurium*.

However, more recent evidence shows that the loss of *E. coli* topo I has deleterious effects on certain strains of *E. coli* and that these effects can be abolished by extracistronic compensatory mutations. Compensatory mutations in *gyrA* and *gyrB* have been described by DiNardo et al. (7, 8), by Pruss et al. (21), and by Gellert et al. (11). The genetic mapping of these compensatory mutations required them to have an unusual associated phenotype, such as Bgl$^+$ (ability to utilize β-glucosides as a carbon source) or HimB$^-$ (inability to plate bacteriophage Mu), or to be closely linked to *gyrA* or *gyrB*. Other compensatory mutations that lacked these properties could not be mapped.

We report here the development and application of two new genetic techniques for identifying and mapping compensatory mutations. These are a direct and simple test for the compensatory phenotype in female strains and a powerful indirect selection for transferring compensatory mutations from Hfr male strains into female strains. Both techniques use a *topA*::Tn*10* insertion mutation. We have used these methods to map several compensatory mutations. These mutations occurred not only in or near *gyrA* and *gyrB* but also in a previously unidentified locus far from either of these genes. This third locus, *toc*, was linked to the *tolC* gene at 66 min.

MATERIALS AND METHODS

Bacterial strains. The strains of *E. coli* and *S. typhimurium* used in this work are described in Table 1.

Media and chemicals. Media were prepared by using the recipes of Miller (17). Minimal A medium and LB medium were used as standard minimal and rich media, respectively. R medium was used for the preparation of bacteriophage lysates. Antibiotics from Sigma Chemical Co. (St. Louis,

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TABLE 1. Bacterial strains

Designation	Characteristics	Origin[a] or reference
2089	F⁻ Δ*lac-514 trp argA rpsL*	29
AR23	F⁻ Δ*lac-514 trp topA20*::Tn*10 argA toc-3 rpsL rpoB*	RED39 × DZ150, Tet + Rif
CC14	Hfr KL226 *rpoB*	KL226, spont. Rif-res.
CC20	Hfr KL983 *gyrA*	KL983, spont. Nal-res.
CL140	F⁻ 2089 *gyrB*	DZ156 × 2089, Cou + Str
CSH2	F⁻ *lacZ trp thi rpsL*	17
CSH57B	F⁻ *ara leu lacY tsx purE gal trp his argG rpsL xyl mtl ilv met*(*A* or *B*) *thi*	17
DZ20 [PM214(pDZ20)]	*S. typhimurium* LT2 Δ*supX13 leu-500 ara-9*/*E. coli* F'123 *trp topA20*::Tn*10*	25
DZ92	F⁻ 2089 *topA20*::Tn*10 gyrA* (compensatory)	P1:EG53 × 2089, Tet
DZ96	F⁻ 2089 *topA20*::Tn*10 gyrB* (compensatory)	P1:EG53 × 2089, Tet
DZ97	F⁻ Δ*lac-514 gyrA* (compensatory) *argA rpsL*	P1:HF4704 × DZ92, Trp⁺
DZ99	F⁻ Δ*lac-514 argA rpsL gyrB* (compensatory)	P1:HF4704 × DZ96, Trp⁺
DZ110	F⁻ CSH57B *topA20*::Tn*10*	P1:EG53 × CSH57B, Tet
DZ111	F⁻ CSH57B *topA20*::Tn*10*	P1:EG53 × CSH57B, Tet
DZ150	F⁻ Δ*lac-514 argA rpsL rpoB*	CC14 × DZ99, Rif + Str
DZ156	Hfr KL800 *gyrB*	KL800, spont. Cou-res.
DZ169	F⁻ Δ*lac-514 argA metC69 rpsL rpoB*	P1:EWH228 × JK8, DOC
DZ188	F⁻ Δ*lac-514 argA metC69 tolC rpsL rpoB*	P1:JK8 × DZ169, colicin El-res.
EG47 [CSH2(pDZ20)]	*lacZ trp thi rpsL*/F'123 *trp topA20*::Tn*10*	DZ20 × CSH2, Tet + Str
EG53	F⁻ HF4704 *topA20*::Tn*10*	P1:EG47 × HF4704, Tet
EG323	F⁻ Δ*lac-514 trp argA rpsL gyrB203 gyrB221*	P1:N4178 × 2089, Cou
EWH228 (CGSC5795)	F⁻ *hisG1 glc-11 metC69 argG6 lacY1 gal-6 rpsL8* or *9* or *104 supE44*?	B. Bachmann
HF4704	F⁻ *polA thyA uvrA phx*	13
JK8	F⁻ Δ*lac-514 argA tolC rpsL rpoB*	DZ150, spont. colicin El-res.
KL99	Hfr PO42 *thi-1 rel-1 lac-42 acrA*	16
KL226	Hfr PO2A *rel-1 tonA22*	16
KL800	Hfr PO53 *lac*	K. B. Low
KL983	Hfr PO53 *lacY1* or *lacZ4 mglP1 xyl-7*	16
RED30	Hfr KL99 *trp topA20*::Tn*10 gyrA* (compensatory)	P1:DZ110 × KL99, Tet
RED31	Hfr KL99 *topA20*::Tn*10 toc-1*	P1:DZ110 × KL99, Tet
RED32	Hfr KL99 *trp topA20*::Tn*10 toc-2*	P1:DZ111 × KL99, Tet
RED38	Hfr KL99 *trp topA20*::Tn*10 gyrA* (compensatory)	P1:DZ111 × KL99, Tet
RED39	Hfr KL99 *trp topA20*::Tn*10 toc-3*	P1:DZ111 × KL99, Tet
RED40	Hfr KL99 *trp topA20*::Tn*10 toc-4*	P1:DZ111 × KL99, Tet
RED41	Hfr KL99 *trp topA20*::Tn*10 gyrA* (compensatory)	P1:DZ111 × KL99, Tet
RED42	Hfr KL99 *trp topA20*::Tn*10 gyrB* (compensatory)	P1:DZ111 × KL99, Tet
RED44	Hfr KL99 *topA20*::Tn*10 toc-5*	P1:DZ111 × KL99, Tet
Y3	F⁻ *trpE9829 cysB*	C. Yanofsky

[a] Constructions are shown as donor × recipient and selection. Abbreviations: Cou, coumermycin A_1; DOC, deoxycholate; Nal, nalidixic acid; P1, source of transducing lysate; res., resistant; Rif, rifampin; spont., spontaneous; Str, streptomycin; Tet, tetracycline; and *toc*, third locus for compensatory mutations.

Mo.) were used at the following final concentrations where indicated: nalidixic acid, 20 mg/liter; rifampin, 100 mg/liter; streptomycin, 100 mg/liter; and tetracycline, 10 mg/liter.

Genetic methods. The procedures of Miller (17) were used with few modifications. Transductions with P1 *vir* were performed as described, except that sodium citrate was not added to the transducing mixture. Spontaneous *tolC* mutants were selected as described by Whitney (27). These mutants were selected for colicin E1 resistance and screened for sensitivity to sodium deoxycholate (1 g/liter of LB medium). Sodium deoxycholate was also used to select or score recombinants that received the wild-type *tolC* allele in genetic crosses.

Plate-mating assay for the compensatory phenotype. The plate-mating assay for the compensatory phenotype tested the ability of recipient clones to accept the *topA20*::Tn*10* allele from the Hfr strain RED31 and was based on the Low method B for rapid mapping of *E. coli* mutations (16). Master grids of clones to be tested were prepared on LB agar and incubated until significant growth was observed (but not more than 24 h at 37°C or 48 h at 30°C). A fresh culture of strain RED31 was grown in LB at 37°C to late-exponential phase (ca. 10^9 cells per ml). Selective agar plates (LB plus tetracycline and either streptomycin or rifampin) were prewarmed to 37°C. One at a time, the selective plates were flooded with 2 to 3 ml of the RED31 culture, the excess was poured off, and each flooded plate was used immediately for a print mating. A delay at this point could interfere with the transfer of tetracycline resistance. Recipient colonies were replicated from the master grid onto the damp selective agar via dry velveteen. The inoculated mating plate was quickly returned to the 37°C incubator. If the recipient colonies contained temperature-sensitive markers, the mating plates were shifted to the permissive temperature after 30 min at 37°C. After appropriate incubation (24 h at 37°C or 48 h at 30°C), the mating plates were examined for growth. Patches of nearly confluent growth were scored as indicating a compensatory phenotype. Those that gave little or no growth were scored as non-compensatory.

DNA gyrase assays. DNA gyrase activity in crude extracts was assayed by the production of supercoiled DNA from relaxed pBR322 DNA. The relaxed substrate was produced by treating supercoiled pBR322 DNA with calf thymus topo I (gift of L. Liu). Extracts were prepared from 6×10^8 cells grown exponentially in LB at 37°C, pelleted in a Microfuge (Brinkmann Instruments, Inc., Fullerton, Calif.), and sus-

pended at 0°C in 20 μl of 25% sucrose–40 mM Tris-hydrochloride (pH 8)–1 mM EDTA which contained lysozyme at 1 mg/ml. The cell suspension was left at 0°C for 5 min, and then 20 μl of 0.9% Brij 58–10 mM Tris-hydrochloride (pH 8)–10 mM $MgSO_4$ was mixed with the suspension to effect lysis. After 10 min more at 0°C, the lysate was clarified by centrifugation for 5 min at 4°C in the Microfuge, and the supernatant was used as the extract. To assay DNA gyrase activity, 3 μl of the extract or a dilution thereof (in 25 mM Tris-hydrochloride [pH 7.5], 20 mM potassium phosphate [pH 7.5], 1 mM EDTA) was added to a reaction mixture (20 μl) containing 25 mM Tris-hydrochloride (pH 7.5), 20 mM potassium phosphate (pH 7.5), 10 mM $MgCl_2$, 5 mM spermidine, 5 mM dithiothreitol, 2 mM ATP, 1 mM EDTA, 2 μg of yeast tRNA, 1 μg of bovine serum albumin, and 2 μg of relaxed pBR322 DNA. The reaction was allowed to proceed for 30 min at 30°C and was stopped by chilling on ice and then by adding 4 μl of a solution containing 24% glycerol, 60 mM EDTA, 4% sodium dodecyl sulfate, and 0.04% bromophenol blue. The extent of reaction was monitored by electrophoresis in submerged 0.8% agarose gels which were then stained with ethidium bromide and photographed (6).

RESULTS

***topA* mutant alleles behave as excluded markers in *E. coli*.** To study the physiological role of topo I in *E. coli*, we wanted to construct strains that could not produce this enzyme. Our previous attempts to introduce deletions of the chromosomal *topA* gene had failed (24), even though such deletion mutants have been reported to be viable (23). We therefore decided to use a Tn*10* insertion in the *topA* gene to construct topo I-deficient mutants. We had previously constructed plasmid pDZ20, a *topA*::Tn*10* derivative of an *E. coli* F-prime plasmid in an *S. typhimurium* host (25). The tetracycline resistance conferred by the Tn*10* insertion provided a positive selection for the *topA* allele. We transferred plasmid pDZ20, by conjugation, into a recombination-proficient *E. coli* host, strain CSH2, and attempted to find topo I-deficient haploid segregants of these merodiploid strains. We found none.

We then used P1-mediated transduction to transfer the *topA20*::Tn*10* allele from this merodiploid strain, EG47, into a haploid strain of *E. coli*, HF4704. Transfer of tetracycline resistance was inefficient, and few of the resistant transductants were *topA* mutants as judged by radioimmunoassays for topo I antigen (24). It appeared that strains in which the Tn*10* element had transposed to new locations were recovered more efficiently than homologous recombinants that kept the Tn*10* insertion in the *topA* gene.

Using the rare *topA20*::Tn*10* haploid recombinant strains as donors, and strain Y3, a *trpE cysB* double mutant, as a recipient, we attempted to show that the *topA20*::Tn*10* mutation was linked to the *trp* and *cysB* loci by P1-mediated transduction. None of the Trp^+ or Cys^+ transductants became resistant to tetracycline. Furthermore, the usual 50% linkage between *trpE* and *cysB* was reduced to less than 5%. For example, when strain Y3 was transduced with P1 grown on strain HF4704, 72 of 150 Cys^+ transductants became Trp^+; however, when Y3 was transduced with P1 prepared on strain EG53, only 1 of 52 Cys^+ transductants became Trp^+. These results suggested that recombinants that received the *topA20*::Tn*10* allele did not form colonies. In other words, the *topA20*::Tn*10* allele was behaving as an excluded, or lethal, marker in strain Y3.

We have tested the ability of several different *E. coli* K-12 strains to serve as recipients for the *topA20*::Tn*10* allele in P1-mediated crosses. In most strains, strain 2089 for example, $TopA^-$ homologous recombinants were infrequent. Strain CSH57B was an exception; *topA20*::Tn*10* derivatives of this strain were frequent. These results showed that the *topA* mutation behaved as an excluded marker in many strains but that strains could differ in their abilities to survive the loss of topo I. This suggested a possible explanation for the seeming paradox that *topA* mutations appeared to be lethal in many strains, but that $TopA^-$ derivatives occasionally could be recovered from these strains. Perhaps the rare clones that did accept the mutant *topA* allele had been genetically altered in their ability to survive the loss of topo I.

Topo I-deficient strains contain compensatory mutations. If a $TopA^-$ strain had acquired an extracistronic mutation that permitted it to accept the *topA20*::Tn*10* allele, this allele should not behave as an excluded mutation in that strain or in derivatives that retained the genetic alteration. Strains DZ92 and DZ96 are derivatives of strain 2089 that carry *trp* and *topA20*::Tn*10* markers. Strains DZ97 and DZ99 are Trp^+ $TopA^+$ transductants of DZ92 and DZ96. DZ97 and DZ99 were used as recipients in P1-mediated transductions to tetracycline resistance from a *trp topA20*::Tn*10* donor. These strains readily accepted the *topA20*::Tn*10* allele and showed high linkage between this allele and the *trp* marker of the donor. In contrast, a Trp^+ derivative of strain 2089 gave few tetracycline-resistant transductants, and few of these became Trp^-. We inferred that the backcrossed strains had inherited an ability to compensate for the detrimental effects of the *topA20*::Tn*10* allele and that this ability was not determined by a secondary mutation within the *topA* gene. We refer to this ability as a compensatory phenotype and to the extracistronic genetic alteration as a compensatory mutation.

Construction of topo I-deficient Hfr strains. To map the compensatory mutations, we needed a better test for the compensatory phenotype. Testing by transduction was tedious and not readily applied to screening the large numbers of clones generated in mapping experiments. An Hfr strain that donated the *topA20*::Tn*10* allele as an early marker should be a more efficient vehicle for this marker. To construct such a strain, we transduced the *topA20*::Tn*10* allele into the Hfr donor strain KL99. Strain KL99 apparently did not have a compensatory phenotype to start with, because most of the tetracycline-resistant transductants had Tn*10* insertions in places other than the *topA* gene. Of 150 tested transductants, we found 14 that were *topA* mutants. We arbitrarily chose one of these, strain RED31, as our standard test strain. This strain efficiently donated the *topA20*::Tn*10* mutation to strains such as DZ97 and DZ99 that had a compensatory phenotype. In matings with recipients that did not have a compensatory phenotype, such as strain 2089, tetracycline-resistant exconjugants were only ca. 2% as frequent.

A plate-mating assay for the compensatory phenotype. We developed a simple plate-mating assay to screen large numbers of clones for the ability to accept tetracycline resistance from strain RED31. As described above, colonies to be tested were replicated onto a selective plate that had been seeded with a culture of RED31. Those colonies that gave many tetracycline-resistant recombinants were scored as having a compensatory phenotype.

Genetic mapping of compensatory mutations. We used two different approaches for determining the approximate map locations of compensatory mutations, depending on whether the compensatory mutation was in an F^- female strain or in an Hfr male strain. Compensatory female strains were mated

TABLE 2. Segregation of *toc* and *metC* with respect to *tolC*[a]

Donor	*toc* allele	Amt (%) of recombinant genotypes with:			
		metC69 *toc*	*metC*$^+$ *toc*	*metC69* *toc*$^+$	*metC*$^+$ *toc*$^+$
RED31	*toc-1*	33	18	33	16
RED32	*toc-2*	42	7	46	5
AR23	*toc-3*	6	31	38	25
RED40	*toc-4*	39	6	51	4
RED44	*toc-5*	5	30	39	26
KL99	*toc*$^+$	0	0	63	37

[a] P1-mediated transductions into DZ188 (*tolC metC69*). Deoxycholate-resistant (*tolC*$^+$) transductants were scored for methionine auxotrophy (*metC69*) and for the ability to compensate (*toc*).

with non-compensatory Hfr strains donating a selected marker, and the loss of the compensatory mutation was scored as an unselected marker by means of the plate-mating assay. Hfr strains that contained the *topA20*::Tn*10* allele were mated with non-compensatory female strains, and the compensatory mutations were indirectly selected for by the introduction of tetracycline resistance. These matings were allowed to proceed without interruption for several hours. The rationale for this procedure is that the recipient would not accept the *topA*::Tn*10* insertion mutation without also gaining a compensatory mutation, and the frequency of receiving the donor compensatory mutation is much higher than the rate of spontaneous mutation to a compensatory phenotype or of transposition. As demonstrated below, this indirect linkage between a *topA* mutation and a compensatory mutation can cause two distant genes to appear to be close to each other. To date, we have found three different locations for compensatory mutations as follows: *gyrA*, at 48 min on the genetic map; *gyrB*, at 82 min; and a third locus, which we designate *toc*, at 66 min. The following are specific examples for each locus.

***gyrA* compensatory mutations.** Female strain DZ92 (*trp topA20*::Tn*10*) was mated with strain CC20, a nalidixic acid-resistant derivative of the non-compensatory Hfr strain KL983. Trp$^+$ recombinants were selected and scored for nalidixic acid and tetracycline resistance. All of the nalidixic acid-resistant recombinants became sensitive to tetracycline, and none of the tetracycline-resistant recombinants became resistant to nalidixic acid. The apparent incompatibility of the donor *gyrA* marker and the recipient *topA* marker suggested that the compensatory mutation in strain DZ92 may be in the *gyrA* gene.

Strain DZ97 is a Trp$^+$ TopA$^+$ derivative of DZ92 that retained the compensatory phenotype. We used P1-mediated transduction to introduce the nalidixic acid-resistant *gyrA* allele from CC20 into DZ97. All of the nalidixic acid-resistant transductants lost the compensatory phenotype, whereas Arg$^+$ transductants selected from the same mixture of P1 and DZ97 did not. We concluded that the compensatory mutation found in strain DZ92 and its derivatives is probably an allele of *gyrA*.

The 14 TopA$^-$ Hfr strains derived from KL99 as described above were screened for *gyrA* compensatory mutations by analyzing the results of long-term uninterrupted matings with three different nalidixic acid-resistant non-compensatory female strains and selecting for the introduction of tetracycline resistance. An Hfr strain that carried a *gyrA* compensatory mutation would be expected to replace the non-compensatory nalidixic acid-resistant mutation in most of the tetracycline-resistant recombinants. Three of these Hfr strains, RED30, RED38, and RED41, showed high linkage (77 to 98%) between the acquisition of tetracycline resistance and loss of nalidixic acid resistance in all three recipient strains. One Hfr strain showed high linkage (95%) in only one recipient. The remainder of these Hfr strains showed less than 20% linkage to *gyrA*. We concluded that no more than four and perhaps only three of these strains contained *gyrA* compensatory mutations.

***gyrB* compensatory mutations.** Female strain DZ96 (*trp topA20*::Tn*10*) is sensitive to 300 mg of novobiocin per liter of LB agar, although the parental strain 2089 is resistant at this concentration. Preliminary crosses showed that the compensatory allele in DZ96 was closely linked to the *ilvB* locus and that the tetracycline resistance of the *topA* insertion was incompatible with either novobiocin- or coumermycin-resistant *gyrB* alleles.

Strain DZ99 (a Trp$^+$ TopA$^+$ derivative of DZ96) retained the compensatory phenotype and novobiocin sensitivity of DZ96. We mated DZ99 with CC14 (a rifampin-resistant *rpoB* derivative of the non-compensatory Hfr strain KL226), selecting for the introduction of the *rpoB* marker. All of the exconjugants that retained their sensitivity to novobiocin retained the compensatory phenotype, whereas those that received the wild-type resistance from the donor lost the compensatory phenotype. We concluded that the compensatory mutation in strain DZ96 is probably an allele of *gyrB* and that the wild-type *gyrB* allele is non-compensatory.

We also tested a temperature-sensitive *gyrB* mutation for its effect on compensation. A derivative of strain 2089 that carried this mutation, strain EG323, had a compensatory phenotype at 37°C but not at 30°C, according to the plate-mating assay. These results suggested that the compensatory phenotype of this strain was due to decreased DNA gyrase activity.

The 14 Hfr *topA* mutant strains were screened for *gyrB* compensatory mutations by crossing them with a coumermycin-resistant non-compensatory recipient, CL140, and selecting for tetracycline resistance. Only one Hfr strain, RED42, gave high linkage (88%) between tetracycline resistance and coumermycin sensitivity. Three of the Hfr strains, RED34, RED36, and RED43, gave between 28% and 47% linkage. The remainder of these strains gave negligible linkage (2 to 10%). By this test, only one of these Hfr strains has a simple *gyrB* compensatory mutation.

A third locus for compensatory mutations. Many of the TopA$^-$ Hfr strains appeared to contain compensatory mutations that were not *gyrA* or *gyrB* mutations. We chose one of these strains, RED31, and mated it with a variety of non-compensatory recipients, selecting for the introduction of tetracycline resistance and screening for linkage to unselected markers. The greatest linkage was found with the *tolC*

TABLE 3. Segregation of *toc* and *tolC* with respect to *metC*[a]

Donor	*toc* allele	Amt (%) of recombinant genotypes with:			
		tolC *toc*	*tolC*$^+$ *toc*	*tolC* *toc*$^+$	*tolC*$^+$ *toc*$^+$
RED31	*toc-1*	1	12	68	19
RED32	*toc-2*	2	8	82	8
AR23	*toc-3*	0	20	63	17
RED40	*toc-4*	2	8	79	11
RED44	*toc-5*	1	17	65	17
KL99	*toc*$^+$	0	0	70	30

[a] P1-mediated transductions into DZ188 (*tolC metC69*). MetC$^+$ transductants were scored for deoxycholate resistance (*tolC*$^+$) and for the ability to compensate (*toc*).

mutation of strain JK8. Of the tetracycline-resistant exconjugants, 97 to 100% became TolC$^+$ (resistant to deoxycholate). Similar results were obtained with Hfr strains RED32, RED39, RED40, and RED44. These results suggested that each of these strains contained a compensatory mutation located near *tolC*. We named compensatory mutations at this locus *toc* (topoisomerase one compensatory) mutations.

Transductional analysis of *toc* mutations. To determine whether each *toc* mutation was sufficient to confer a compensatory phenotype on a non-compensatory strain, we performed transductional crosses. Strain AR23 was substituted for RED39 in these experiments, because RED39 was resistant to bacteriophage P1. Crosses from these five *toc* mutant strains into TolC$^-$ recipients, selecting TolC$^+$ recombinants, resulted in 35 to 50% of the recombinants acquiring a compensatory phenotype according to the plate assay. Similar crosses with the nearby markers *dnaG* and *metC* gave few compensatory recombinants. We concluded that each *toc* mutation is closely linked to the *tolC* gene and is sufficient to convert a non-compensatory strain into a compensatory strain.

The *toc* mutations did not behave as point mutations. Table 2 gives the results of P1-mediated crosses from a *toc* mutant and wild-type donors into a MetC$^-$ TolC$^-$ recipient, DZ188, selecting TolC$^+$ transductants. As can be seen from this table, each of the *toc* mutant alleles was cotransduced with the *tolC*$^+$ allele (from 35 to more than 50% linkage). However, four of the five *toc* mutations altered the rate of cotransduction of the *tolC* and *metC69* markers. The *toc-2* and *toc-4* alleles reduced the linkage between *metC* and *tolC* to only 10 to 12%. The *toc-3* and *toc-5* alleles increased the linkage between *metC* and *tolC* to ca. 56%. Only the *toc-1* allele had little effect on the *metC tolC* linkage.

We used previously described equations (28) to derive linkage maps from the data presented in Table 2, assuming that the *toc* mutations were point mutations. The five *toc* mutations gave three different map positions. The *toc-2* and *toc-4* alleles appeared to be between *metC* and *tolC*; the *toc-3* and *toc-5* alleles gave *toc metC tolC* as the apparent gene order; and the *toc-1* allele gave *metC tolC toc* as the most likely order of markers, although no order gave a good fit to the data for this allele. One possible explanation for the different orders of markers was that the five mutations we designated *toc* represented compensatory mutations in three different genes, each of which was highly linked to *tolC*. This, however, would not explain the altered linkage between *tolC* and *metC*.

Table 3 gives the results of crosses similar to those in the preceding table, except that Met$^+$ transductants were selected. These data were consistent with the gene order *metC tolC toc* for each of the five *toc* mutant alleles, in contrast to the results of selecting for TolC$^+$ transductants. In other words, four of the five *toc* alleles failed to demonstrate a consistent gene order. These results caused us to suspect that the *toc* mutant alleles were not point mutations but were chromosomal rearrangements such as tandem duplications. The compensatory transductants from these crosses were somewhat unstable. Of the subclones from overnight cultures, 5 to 15% lost the compensatory phenotype. Such instability is a characteristic of duplication mutations (1).

DNA gyrase activity is not reduced in *toc* mutants. To determine what effect the *toc* compensatory mutations may have on the supercoiling activity of DNA gyrase, this activity was assayed in extracts of the 14 TopA$^-$ Hfr strains and in their wild-type parent KL99. Figure 1 shows representative results from an assay of several strains. Lane A shows the electrophoretic pattern of the substrate, relaxed pBR322. Lane B shows that an appropriately diluted extract of KL99 converted about half of the plasmid DNA to a more rapidly migrating supercoiled form. The strains that contained *toc* mutations showed as much DNA supercoiling activity as did KL99 within the limits of this assay, i.e., within a factor of 2. Strains identified as containing *gyrA* or *gyrB* compensatory mutations had much less supercoiling activity (e.g., lane G [RED41]) as did most of the strains that had unidentified compensatory mutations. We concluded that the mechanism by which *toc* mutations compensate for the loss of topo I does not involve substantial reduction of the supercoiling activity of DNA gyrase.

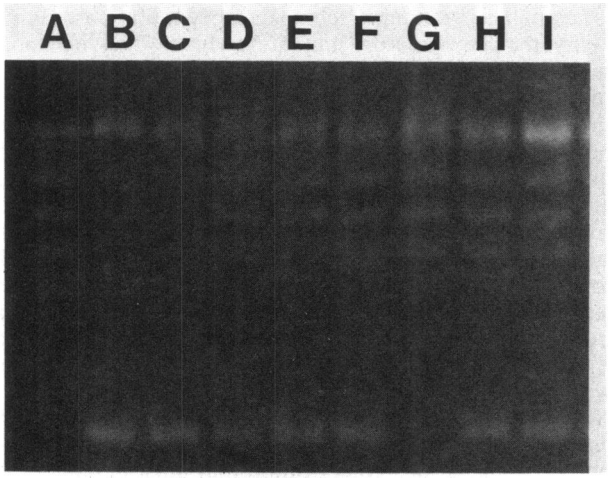

FIG. 1. DNA gyrase supercoiling activity in extracts of *toc* mutant strains and related *toc*$^+$ strains. Extracts were prepared and assayed as described in the text. This agarose gel displays the results of assays of fourfold-diluted extracts except for the first and last lanes. Lane A, relaxed pBR322 with no added extract; lane B, extract from KL99 (wild type); lane C, RED31 (*toc-1*); lane D, RED32 (*toc-2*); lane E, RED39 (*toc-3*); lane F, RED40 (*toc-4*); lane G, RED41 (*gyrA* compensatory mutation); lane H, RED44 (*toc-5*); and lane I, KL99 extract diluted eightfold. The direction of electrophoresis was from top to bottom, and the supercoiled DNA is the band with highest mobility.

DISCUSSION

Genetic methods. The *topA20*::Tn*10* mutation that we have used in these studies is as effective as *topA-cysB* deletion mutations in preventing the synthesis of topo I and has the advantage of carrying an easily selected phenotype. We have taken advantage of this phenotype in three different procedures. These include the selection of spontaneous compensatory mutations, indirect selection of transfer of compensatory mutations from one strain to another, and testing for the presence or absence of the compensatory phenotype. All three of these procedures were based on the observation that the *topA20*::Tn*10* allele behaves as a lethal marker in non-compensatory strains.

Spontaneous compensatory mutations are readily selected by transducing tetracycline resistance from *topA20*::Tn*10* donors into non-compensatory strains and screening the transductants for the loss of topo I antigen or activity. The latter step distinguishes between the desired homologous recombinants and other derivatives, such as strains in which

Tn*10* has transposed to a different site or that may contain duplications of the *topA* region. The compensatory mutations that are found by this procedure also readily accept *topA* deletions (C. W. Rinker and R. E. Depew, unpublished data), showing that the compensatory mutations are not allele-specific suppressor mutations but instead must compensate in a more general fashion for the loss of an important function of topo I. We have observed that many of the tetracycline-resistant transductants recovered from non-compensatory recipients have Tn*10* insertions that are not linked to *topA*. This unintentionally demonstrates the following new method for generating random insertion mutants: introduce a transposon that is inserted into an essential gene.

Compensatory mutations in Hfr strains that carry the *topA20*::Tn*10* allele are easily transferred into non-compensatory recipients by selecting for tetracycline resistance. The apparent linkage found between the *topA* allele and the compensatory mutation usually exceeds the linkage seen between *topA* and the nearby *trp* operon. The success of this method of indirect selection for the introduction of a compensatory mutation implies that *topA* mutants that do not have compensatory mutations are strongly counterselected.

The plate test for the presence or absence of a compensatory phenotype is a direct assay for the ability of a strain to survive with a *topA20*::Tn*10* allele. The validity of this test was confirmed with previously identified *gyrA* and *gyrB* compensatory alleles (data not shown). A disadvantage of this test is that it does not work in strains that carry certain mutations, such as *acrA* or *recA*. However, this is minor compared with the major advantage of this test, which is that it does not require that the compensatory mutations result in unexplained new phenotypes or map in expected locations.

Compensatory mutations. To date, we have found compensatory mutations in three distinct loci, *gyrA*, *gyrB*, and *toc*. Compensatory mutations in *gyrA* or *gyrB* are not surprising because DNA gyrase and topo I have antagonistic effects on the supercoiling of DNA and because such mutations have been described previously (7, 8, 11, 21). These previous studies characterized compensatory mutations that lower the supercoiling activity of DNA gyrase, implying that the role of topo I is to counterbalance the apparently excessive activity of DNA gyrase. DiNardo et al. (6) demonstrated lowered DNA gyrase activity in both *gyrA* and *gyrB* compensatory mutants and showed that *himB* mutants, which are *gyrB* mutants that are defective in plating phage Mu, also compensate for the loss of topo I. Pruss et al. (21) demonstrated that certain compensatory mutations result in decreased supercoiling of chromosomal and plasmid DNA, proving that compensatory mutations need not restore precisely the wild-type degree of supercoiling. Gellert et al. (11) showed that the expression of both the *gyrA* and *gyrB* genes is reduced in the absence of topo I, at least in strains that have compensatory mutations in *gyrA* or *gyrB*. If the same regulatory mechanism operates in wild-type cells, the reduction in DNA gyrase activity must be insufficient to compensate for the loss of the relaxing activity of topo I.

Our results with compensatory gyrase mutations are consistent with previous reports and add more alleles to the list of such mutations. Our finding that a thermosensitive allele of *gyrB* behaves as a compensatory mutation at a semipermissive temperature, but not at the permissive temperature, prompted a search for spontaneous conditionally compensatory mutations (C. S. Laufer and R. E. Depew, Fed. Proc. **43**:1542, 1984). However, the present results show that the *gyrA* and *gyrB* classes of compensatory mutations account for only a minority of spontaneous compensatory mutations, at least in certain genetic backgrounds.

We have presented evidence that compensatory mutations frequently arise at a third locus, *toc*, near *tolC*. We have also demonstrated that *topA* mutant strains that have *toc* mutations produce wild-type levels of DNA gyrase, unlike strains that have compensatory gyrase mutations. These results show that the *toc* mutations compensate for *topA* mutations by a mechanism other than that found in the *gyrA* and *gyrB* mutants. There may be several different ways to restore the balance of DNA-supercoiling and DNA-relaxing activities in *topA* mutant cells. For example, *himA* mutations reduce the expression of the *gyrA* gene (11). Another method of compensation would be to replace the lost DNA-relaxing activity of topo I by stimulating the production or activity of a different topoisomerase that has relaxing activity. DNA topoisomerases II′ and III are possible candidates for such a substitution. The *toc* mutations are especially interesting in this regard because they have properties that are characteristic of tandem duplication mutations (A. Raji, V. Purcell, E. Gamliel, D. J. Zabel, and R. E. Depew, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, H172, p. 120). Tandem duplications may increase the expression of a gene by fusing that gene to a strong promoter (1). Whatever their structure may be, the *toc* mutations identify a previously unknown locus that may code for or control the activity of a DNA topoisomerase.

We are not at all sure that we have exhausted the catalog of compensatory loci. We have not yet been able to map the compensatory mutations in several of our strains. In some cases, the unmapped compensatory mutations appeared to be unstable and could not be maintained in a strain in the absence of a *topA* mutation. In other cases, the compensatory phenotype appeared to be "weak" and did not give sufficiently clear results with our plate assay for this phenotype to allow confidence in the results. Strain CSH57B is such a strain. We have observed that some strains with weak compensatory phenotypes occasionally developed subclones that contained "stronger" compensatory mutations. By necessity, the compensatory mutations which we have mapped are those that gave clear tests for the compensatory phenotype.

We are also not certain that each of the compensatory mutations we have studied can function as a compensatory mutation in the absence of other mutations. Most of our studies have been restricted to the genetic backgrounds provided by strains KL99 and 2089. Preliminary attempts to transfer various compensatory mutations into different backgrounds have met with different degrees of success. Perhaps some of the compensatory mutations themselves are lethal or at least deleterious in some other strains. This would not be too surprising, because two of the loci involved, *gyrA* and *gyrB*, are themselves essential.

Most of the *topA* insertion or deletion mutant strains of *E. coli* that we have examined contain a compensatory mutation. This is a serious complication that must be taken into account in studies designed to determine the effect of *topA* mutations on physiological processes, because the compensatory mutations themselves have significant effects. We will report elsewhere our attempts to construct strains in which these effects can be studied separately.

Our results add to the rapidly growing evidence that the control of DNA supercoiling is important to the survival of *E. coli* and that this control involves the participation of several different genes and gene products. Understanding how DNA-supercoiling and DNA-relaxing activities are bal-

anced and what causes cells to die when this balance is upset is likely to be of fundamental importance to understanding how cells function.

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