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Escherichia coli DNA Topoisomerase I Mutants Have Compensatory Mutations in DNA Gyrase Genes

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Summary

Escherichia coli deletion mutants lacking DNA topoisomerase I have been identified previously and shown to grow at a normal rate. We show that such strains grow normally only because of spontaneously arising mutations that compensate for the topoisomerase I defect. Several of these compensatory mutations have been found to map at or near the genes encoding DNA gyrase, *gyrA* and *gyrB*. DNA gyrase assays of crude extracts show that strains carrying the mutations have lower gyrase activity. Thus the mutations are in the gyrase structural genes or in nearby regulatory sequences. These results, in conjunction with DNA supercoiling measurements of others, indicate that in vivo DNA superhelicity is a result of a balance between topoisomerase I and gyrase activities. An excess of negative supercoils due to an absence of topoisomerase I is deleterious to the cell, but a moderate gyrase deficiency is not harmful.

Introduction

Covalently closed DNA isolated from any natural source is negatively supercoiled. This topological state of the DNA apparently plays a crucial role during DNA replication, transcription and recombination. In Escherichia coli the DNA is negatively supercoiled mainly as a result of the action of DNA gyrase (Gellert et al., 1976; Drlica and Snyder, 1978). This protein is one of a group of enzymes, called DNA topoisomerases, that catalyze the concerted breakage and rejoining of DNA phosphodiester bonds (Wang and Liu, 1979). Gyrase is unique among the topoisomerases in its ability to put negative supercoils into DNA. Extensive studies with inhibitors and with conditional-lethal mutants have shown that gyrase is essential for E. coli DNA replication and viability (reviewed by Cozzarelli, 1980; Gellert, 1981). Presumably, negative supercoiling facilitates unwinding of the DNA duplex during replication, although a more direct role of gyrase in replication is also possible.

It has been recognized for some time that DNA superhelicity also influences RNA polymerase–promoter interactions and the rate of transcription initiation in vitro (Wang, 1974; Richardson, 1974; Botchan, 1976). More recent studies have shown that the expression of certain bacterial operons is sensitive to inhibitors of DNA gyrase, presumably because of a change in template superhelicity (Smith et al., 1978; Sanzey, 1979; Yang et al., 1979).

E. coli has another topoisomerase, DNA topoisomerase I, previously called ω protein (Wang, 1971). This enzyme nicks and closes DNA, and in vitro, it catalyzes several topological isomerization reactions, including the relaxation of negatively supercoiled DNA, the knotting and unknotting of single-stranded DNA rings (Liu et al., 1976) and the catenation and decatenation of duplex DNA rings when at least one member of a pair of participating rings has a single-stranded scission (Tse and Wang, 1980; Brown and Cozzarelli, 1981). The absence of specific inhibitors or mutants has made it difficult to determine the in vivo role of topoisomerase I. At least with respect to in vitro supercoiling, DNA gyrase and DNA topoisomerase I have diametrically opposing effects.

Recently, mutations in *top*, the structural gene for E. coli DNA topoisomerase I, have been identified and mapped at 28 min on the chromosome, near *cysB* (Trucksis and Depew, 1981; Sternglanz et al., 1981). The latter investigators described *top* deletion mutants with no detectable DNA topoisomerase I enzymatic activity and no cross-reactive material to antibody directed against the enzyme. Yet these strains grew only slightly more slowly than wild-type strains. It was thus concluded that DNA topoisomerase I is not essential for E. coli viability.

We show that strains lacking DNA topoisomerase I grow normally only because of spontaneously arising mutations that compensate for the topoisomerase I defect. Several of these compensatory mutations have been found to map in the genes encoding DNA gyrase, *gyrA* and *gyrB*. These results, in conjunction with the DNA supercoiling measurements described by Pruss et al. (1982), indicate that DNA superhelicity is a result of a balance between topoisomerase I and gyrase activities. An excess of negative supercoils due to an absence of topoisomerase I is deleterious to the cell.

Results

top Deletion Strains Have Compensatory Mutations That Permit Viability

Although strains carrying deletions of the DNA topoisomerase I gene, Δ*top* strains, are viable (Sternglanz et al., 1981), it is very difficult to move the deletion mutations to other strains by P1-mediated transduction. Since the *top* gene is located between the *trp* and *pyrF* genes, which are only 0.7 min apart on the E. coli genetic map (Bachmann and Low, 1980), *top* should be cotransducible with either gene (Sternglanz et al., 1981). However, when a P1 lysate is prepared on a Δ*top* strain and the lysate is used to transduce any one of several *trp* or *pyrF* mutants to prototrophy, none of the Trp^+ or $PyrF^+$ transductants found after a normal 1–2 day incubation period also have Δ*top*

(<0.1%). (The criterion used to test whether or not a strain has Δ*top* is described in the Experimental Procedures.) If the transduction plates are incubated for 4–6 days, extremely slow growing colonies appear in the case of some recipient strains, and these transductants all have Δ*top*.

A similar result is seen when a Δ*top* P1 lysate is used to transduce a *trp pyrF* double mutant to prototrophy. Since the *top* gene is located between the *trp* and *pyrF* genes, it would be expected that virtually all Trp^+ $PyrF^+$ transductants would have the Δ*top* mutation. The observed result is that there are relatively few Trp^+ $PyrF^+$ transductants of normal colony size and they all lack the Δ*top* mutation. They must be the result of two independent recombinatorial events. The majority of Trp^+ $PyrF^+$ transductants grow extremely slowly and they all have the Δ*top* mutation. Upon purification and subculturing of these transductants, more rapidly growing clones are observed. These clones still have no topoisomerase I activity, but after several passages they grow almost as rapidly as wild-type strains.

These results suggested that it is deleterious for E. coli to have a deletion of the *top* gene, and that compensatory mutations can and do arise that allow Δ*top* strains to grow at normal rates. Since the Δ*top* strains described previously (Sternglanz et al., 1981) have a normal growth rate, presumably each has a compensatory mutation. To test this, we transduced three of the strains, DM700, DM750 and DM800, to Trp^- Top^+. If such strains carry a compensatory mutation, it should be possible to transduce Δ*top* back into them with high frequency. The data in Table 1 show this to be the case. When strains SD107, SD275 and SD108, Trp^- Top^+ strains derived from DM700Δ*top*, DM750Δ*top* and DM800Δ*top*, respectively, are transduced to Trp^+ with a Δ*top* donor, a high fraction of the transductants arising within 1–2 days has Δ*top*, and they have a normal growth rate. When SD104, a related strain but one that never had a *top* mutation, is used as a recipient in a similar transduction, none (0/98 and 0/144) of the Trp^+ transductants has Δ*top*.

Table 1. Transduction of Δ*top* into Strains with or without Compensatory Mutations

	P1 Donor[a]	
Recipient Strain[b]	DM800	DM700
SD104	0/98	0/144
SD107	15/15	30/35
SD275	22/37	ND
SD108	23/67	22/25

Data are given as the fraction of Trp^+ transductants that have Δ*top*. The criterion used to decide whether or not a transductant has Δ*top* is described in the Experimental Procedures.

[a] The P1 donor strains DM800 and DM700 both have Δ(*top cysB*).

[b] The recipient strains SD104, SD107, SD275 and SD108 are *trpE* Top^+ Cys^+ transductants of DM4100, DM700, DM750 and DM800, respectively, with P1 grown on PLK831 as the donor. The transductants were selected on M9 minimal medium supplemented with all 20 essential amino acids except cysteine. The full genotypes of these strains are given in Table 4.

ND: not determined.

Strain DM800Δ*top* Has a Compensatory Mutation at *gyrB*

Strain DM800Δ*top*, one of the strains described above, has an unusual phenotype that enabled us to map its compensatory mutation. This strain, unlike wild-type strains, is able to utilize β-glucosides as a carbon source; that is, it is Bgl^+. Wild-type E. coli K12 strains are Bgl^-; they are unable to use β-glucosides because the genes of the *bgl* operon, which are required for catabolism of β-glucosides, are uninducible (Prasad and Schaefler, 1974). Spontaneous activating mutations can arise that allow expression of the operon when inducer is present. These activating mutations are usually insertion mutations within the regulatory region of the *bgl* operon (Reynolds et al., 1981). In contrast, our analysis shows that the mutation that gives rise to the Bgl^+ phenotype in strain DM800 is not located within the *bgl* operon. Furthermore, the Bgl^+ phenotype is inseparable from the compensatory mutation and has been used to map the compensatory mutation.

Using P1 transduction, we showed that the Bgl^+ phenotype of DM800 was not due to a mutation within the *bgl* operon by demonstrating the presence of a wild-type *bgl* operon in this strain. The phenotypically Bgl^+ strain AE199 was used as a P1 donor, and a *bgl*$^+$ strain known to have an activating mutation in the *bgl* operon was used as a recipient. Strain AE199 is a derivative of DM800 that retains the Bgl^+ phenotype and has Tn10 inserted in its *tna* gene; this gene is 60%–80% cotransducible with *bgl*. If the Bgl^+ phenotype of DM800 had been due to a mutation in the *bgl* operon, greater than 99% of selected Tet^R transductants would have been Bgl^+. In fact, 53% of Tet^R transductants were found to be Bgl^-, indicating that a wild-type *bgl* operon was present in the DM800 donor.

Further analysis indicated that although the mutation that gives rise to the Bgl^+ phenotype is not at *bgl*, it is in fact linked to *tna*. A P1 lysate grown on strain KO635 *tna*::Tn10 was used to transduce DM800 to tetracycline resistance. Among 150 transductants, 53 were Bgl^+ and 97 were Bgl^-, showing 65% linkage between Bgl^+ and *tna*. This would be consistent with a map position for Bgl^+ near *gyrB* or near *bgl*, since *tna* is located between these genes and is about 60% cotransducible with either one. In another transduction, the Bgl^+ phenotype was found to be 3.8% cotransducible with *rbs*. (The order of relevant genes in this region of the E. coli chromosome is *gyrB tna bgl rbs*.) These mapping results, taken together, show

that the mutation of DM800 leading to a Bgl$^+$ phenotype maps at or near *gyrB*.

Since DM800 has a compensatory mutation (Table 1) and since it has an unusual Bgl$^+$ mutation mapping at or near *gyrB*, we tested the relationship between the Bgl$^+$ phenotype and the compensatory mutation. P1 transduction was used to move the *gyrB* region of AE199(DM800 *tna*::Tn10) to SD104, a strain that is Bgl$^-$ and does not have a compensatory mutation (Table 1). We used the Bgl$^+$ phenotype as a convenient way to screen for the presumptive *gyrB* mutation. Among 40 tetracycline-resistant transductants, 35 were Bgl$^+$ and five were Bgl$^-$, again showing that Bgl$^+$ is linked to *tna*. Five of these transductants (three Bgl$^+$ and two Bgl$^-$) were then tested to see if they carried the compensatory mutation, defined as the ability to receive the Δ*top* mutation in a P1 transduction. They were transduced to Trp$^+$ with a P1 lysate grown on DM800Δ*top*, and Trp$^+$ transductants were screened for Δ*top*. The results are shown in Table 2. It can be seen that in the case of the three Bgl$^+$ recipients, many of the transductants have Δ*top*, while for the two Bgl$^-$ recipients, no Δ*top* transductants are found. These data demonstrate that the SD104 Bgl$^+$ transductants now carry the compensatory mutation of DM800, whereas the Bgl$^-$ transductants do not. We also moved the *gyrB* region of DM800 to strain PLK831 and demonstrated that these transductants now also can receive the Δ*top* mutation (data not shown). It appears that a mutation in strain DM800, located at or near *gyrB* and defined by its Bgl$^+$ phenotype, can compensate for the loss of topoisomerase I.

Results obtained by M. Gellert (personal communication) showed that DM800 has reduced levels of active *gyrB* protomer. His assay involved complementation of partially purified extracts from DM800 with pure *gyrA* protomer and measurement of the level of reconstituted DNA gyrase activity. We developed an assay for measuring gyrase activity in crude extracts (see Experimental Procedures) and confirmed that DM800 has lower gyrase activity than a wild-type strain. We also measured gyrase levels in two of the transductants described above and in Table 2, one Bgl$^+$ and the other Bgl$^-$. Figure 1 shows that gyrase activity of the Bgl$^+$ transductant (lanes d–f) is significantly lower than that of the isogenic Bgl$^-$ transductant (lanes a–c). Thus the mutation originally present in DM800 and leading to a Bgl$^+$ phenotype lowers the gyrase activity of the cell.

It should be pointed out that DM800 remains viable even with a wild-type *gyrB* gene, as shown by the existence of Bgl$^-$ transductants in the mapping experiments described above. Apparently DM800 has at least one other mutation that can compensate for the lack of topoisomerase I.

Table 2. The Bgl$^+$ Phenotype Correlates with the Compensatory Mutation

Recipient Strain[a]	Phenotype	Fraction of Trp$^+$ Transductants That Have Δ*top*[b]
SD104-9	Bgl$^+$	31/119
SD104-12	Bgl$^+$	49/93
SD104-20	Bgl$^+$	40/80
SD104-4	Bgl$^-$	0/108
SD104-14	Bgl$^-$	0/83

[a] The recipient strains are all tetracycline-resistant transductants of SD104*trpE*, with AE199 Bgl$^+$ *tna*::Tn10 as a P1 donor. They are Bgl$^+$ or Bgl$^-$ as indicated.

[b] The five recipient strains were transduced to Trp$^+$ with DM800Δ*top* as a P1 donor. The transductants were screened for Δ*top* as described in the Experimental Procedures.

Strain DM750Δ*top* has a Compensatory Mutation at *gyrA*

Strain DM750 is an independently derived Δ*top* mutant described previously (Sternglanz et al., 1981). It has an almost normal growth rate, is phenotypically Bgl$^+$ and, as can be seen in Table 1, it also has a compensatory mutation. Mapping experiments showed that in this case, however, the Bgl$^+$ phenotype was totally unlinked to *tna*, thus indicating that it did not map at *gyrB* or at *bgl*. We therefore considered the possibility that the Bgl$^+$ phenotype still reflected a compensatory mutation but that it mapped at *gyrA*, a gene far from *gyrB*, at 48 min on the E. coli genetic map (Bachmann and Low, 1980). To test this possibility, we moved the *gyrA* region of DM750 to another strain. We took advantage of the known high linkage of *nrdA* and *gyrA* and the fact that certain *gyrA* mutants confer resistance to high levels of nalidixic acid (Bourguignon et al., 1973; Sugino et al., 1977; Gellert et al., 1977). Strain KV403*trpE*, *nrdA*(Ts) *gyrA*(Nal^R) Bgl$^-$ was constructed as described in the Experimental Procedures. It was used as a recipient in a transduction with P1 grown on a derivative of DM750 called

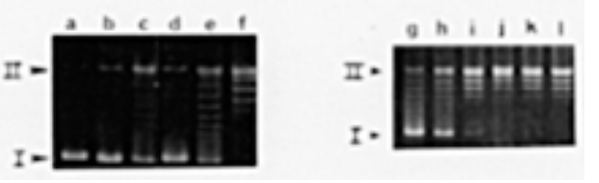

Figure 1. Assays of DNA Gyrase Activity

Extracts were prepared and assayed at three concentrations, 18 μg (lanes a, d, g and j), 6 μg (lanes b, e, h and k) or 2 μg (lanes c, f, i and l), total protein per assay, as described in the Experimental Procedures. (Lanes a–c) Strain SD104-14 Bgl$^-$; (lanes d–f) strain SD104-20 Bgl$^+$; (lanes g–i) strain JTT1; (lanes j–l) strain SD7. The unreacted fully relaxed substrate DNA migrates in this electrophoresis system as a series of topoisomers containing a few positive superhelical turns, as shown in lanes f and l (see Figure 2, lane m, for a sample with no extract added). The introduction of negative supercoils by DNA gyrase creates a ladder of DNA topoisomers migrating between nicked circular DNA (form II) and fully supercoiled DNA (form I); in the case of strong gyrase activity, the DNA migrates at the fully supercoiled position.

RS752. Temperature-resistant (*nrdA* $^+$) transductants were selected and screened for Nal sensitivity and Bgl$^+$. Of 48 transductants examined, 27 were Nal^S Bgl$^+$, 17 were Nal^S Bgl$^-$ and 4 were Nal^R Bgl$^-$. These results showed that *nrdA* and *gyrA*(Nal^R) are linked as expected; that Bgl$^+$ maps at or near *gyrA*(Nal^R); and that the relevant map order is *nrdA gyrA*(Nal^R) Bgl$^+$.

Next we tested whether the Bgl$^+$ transductants from this experiment carried the compensatory mutation of DM750. Four isogenic Nal^S transductants, two Bgl$^+$ and two Bgl$^-$, were used as recipients for another transduction with a P1 lysate grown on DM700Δ*top*. Trp$^+$ transductants were selected and screened for Δ*top*. The results were as expected. The two Bgl$^+$ recipients had a large fraction of Δ*top* transductants (one third to one half of the total), while the two Bgl$^-$ recipients had none (less than 1 in 500). This shows that DM750 has a compensatory mutation mapping at or near *gyrA;* that this mutation can be moved to another strain (with the Bgl$^+$ phenotype to follow the mutation); and that the new strain can now accommodate the Δ*top* mutation.

DNA gyrase activity was measured in two of the transductants described above, one Bgl$^+$ and the other Bgl$^-$. Again, the Bgl$^+$ strain, KV4752, has lower gyrase activity than the Bgl$^-$ strain, KV4751 (Figure 2, compare lanes g–l with lanes a–f). Figure 2 also shows that when pure *gyrB* protomer is added to diluted extracts from the Bgl$^-$ strain, there is an increase in activity (lanes c–f). It is known that E. coli extracts contain about 10 times more *gyrA* protomer than *gyrB* protomer (Brown et al., 1979; Gellert et al., 1979). Presumably, *gyrB* protomer is limiting in the diluted extracts and its addition increases activity. However, in the extracts of the Bgl$^+$ strain the addition of pure *gyrB* protomer does not increase activity (Figure 2, lanes j–m). These results show that the low gyrase activity of the Bgl$^+$ strain is due to a deficiency of *gyrA* protomer; *gyrB* protomer is not the limiting factor as it is in wild-type strains.

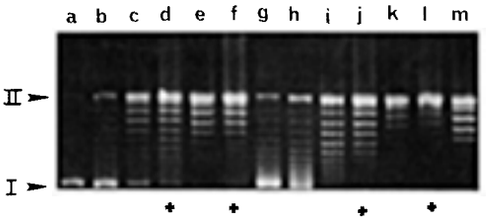

Figure 2. Assays of DNA Gyrase Activity

The conditions of the assay were the same as those described in the legend to Figure 1. (Lanes a–f) Strain KV4751 Bgl$^-$; (lanes g–l) strain KV4752 Bgl$^+$; (lane m) no extract added. (Lanes a and g) 18 μg protein per assay; (lanes b and h) 6 μg protein per assay; (lanes c, d, i and j) 2 μg protein per assay; (lanes e, f, k and l) 0.7 μg protein per assay. (Lanes +) 0.5 μg purified *gyrB* protein was added to the reaction mixture. The *gyrB* protein alone had no supercoiling activity.

Some *top10* Mutants Have Compensatory Mutations at *gyrB*

We previously reported the isolation of strains carrying a nonsense mutation in the *top* gene, the *top10* allele. Such strains have a small amount of residual topoisomerase I activity, presumably because of either the 70,000 dalton nonsense fragment or a small amount of a readthrough product (Sternglanz et al., 1981). The *top10* mutation, as opposed to the Δ*top* mutation, can be moved to other strains by P1 transduction with normal efficiency, and the *top10* transductants grow at a normal rate. However, we have noticed that not all the *top10* transductants have identical phenotypes. In particular, we found that about 30% of the Trp$^+$ *top10* transductants of strain PLK831 plated phage Mu very poorly, and this phenotype did not map in the *trp top pyrF* region. Since it is known that some *gyrB* mutants do not support the growth of phage Mu (Miller et al., 1978; M. Gellert, personal communication), we examined the possibility that the mutation causing the Mu plating defect of these *top10* mutants maps at *gyrB*.

SD7*top10,* a strain that totally blocks the growth of phage Mu (plating efficiency of $<10^{-7}$), was chosen for further study. A P1 lysate grown on SD7 was used to transduce strain N4178*gyrB*(Ts Cou^R) to temperature resistance. While N4178 plates phage Mu with a normal efficiency, 12 of 12 temperature-resistant transductants in one experiment, and 13 of 16 in another, did not plate phage Mu. Other P1 transductions with SD7 as a donor or as a recipient were also consistent with a map position for the Mu$^-$ phenotype of SD7 at or near *gyrB.* The Mu defect of an independent *top10* transductant, JTP16, was similarly found to map at or near *gyrB.*

Since SD7 (and other *top10* mutants with a Mu plating defect) seems to have a mutation at or near *gyrB,* we tested whether it has a compensatory mutation; that is, can Δ*top* transductants of SD7 grow normally? Three isogenic strains, JTT1*top* $^+$, RS2*top10* and SD7*top10 gyrB,* were all transduced to PyrF$^+$ with a P1 lysate grown on a Δ*top* strain. The results were striking. JTT1 and RS2 gave far fewer PyrF$^+$ transductants than SD7, and none of them had the Δ*top* mutation. A large fraction of the SD7 transductants (>75%) had Δ*top.* These data showed that SD7 has a compensatory mutation, and since it has a phage Mu plating defect mapping at or near *gyrB,* obviously the next experiment was to see if the two mutations were really one and the same. Two isogenic transductants generated in mapping the Mu plating defect of SD7 were used. They were derivatives of N4178, one carrying the *gyrB* (Mu$^-$) allele of SD7 and the other a wild-type *gyrB* $^+$ (Mu$^+$) gene. Each of them was converted to Trp$^-$ (see Experimental Procedures), and then transduced to Trp$^+$ with a P1 lysate grown on a Trp$^+$ Δ*top* strain. Strain N4178-15 (the *gyrB*[Mu$^-$] strain) gave a significant fraction of Trp$^+$ Δ*top* transductants (31 of 80), while strain N4178-4

(the *gyrB* $^{+}$[Mu^{+}] strain) gave no such transductants (0 of 98). These results are supported by DNA gyrase assays that show that SD7 has much lower gyrase activity than does a comparable wild-type strain (Figure 1, compare lanes g–i with lanes j–l). Taken together, these data show that SD7 has a *gyrB* mutation that can act as a compensatory mutation for the Δ*top* mutation.

himB Mutations Act As Compensatory Mutations for Δ*top*

Miller et al. (1978) have isolated and characterized a mutation called *himB114*. This mutation maps at *gyrB* and prevents the growth of phage Mu. Recently two new *himB* mutants have been isolated. These mutants have a temperature-sensitive *himB* phenotype, preventing phage Mu growth and phage lambda lysogeny at the elevated temperature (D. Friedman, personal communication). Furthermore, they both show temperature-sensitive DNA gyrase activity when assayed in vitro (M. Gellert, personal communication). Thus these strains have mutations in the *gyrB* gene. We asked whether these *himB* mutations act as compensatory mutations for Δ*top*. All three mutants, as well as an isogenic wild-type strain, were converted to Trp^{-} and then transduced to Trp^{+} with a Trp^{+} Δ*top* P1 lysate. Table 3 shows that the Δ*top* mutation can be transduced into all three *himB* mutants, but not into the *himB* $^{+}$ strain. Thus the gyrase defect of three different *himB* mutants serves as a compensatory mutation for Δ*top*.

Discussion

Compensatory Mutations Are in Gyrase Genes

Since DM700, DM750 and DM800, the Δ*top* mutants characterized previously (Sternglanz et al., 1981), grow with an almost normal growth rate, the difficulty encountered in moving the Δ*top* mutation to other strains by P1 transduction was unexpected. Newly created Δ*top* transductants are very slow to appear on transduction plates, are difficult to purify and continually segregate larger, faster-growing clones. We infer from the initially very poor growth that strains lacking topoisomerase I are at a severe growth disadvantage. Thus our previous conclusion concerning the nonessential nature of DNA topoisomerase I in E. coli needs to be amended. Apparently, deletion of the topoisomerase I gene places a heavy burden on the cell, but spontaneous mutations can and do arise that compensate for the topoisomerase I defect and allow the cell to grow at a normal growth rate.

Table 3. *himB* Mutations Act As Compensatory Mutations for Δ*top*

Strain	Genotype	Mu Plating	Trp^{+} Δ*top*/Total Trp^{+}[a]
K37	*himB* $^{+}$	+	<1%
K807	*himB114*	−	17/72
K1870	*himB*(Ts)	−	6/23
K1871	*himB*(Ts)	−	5/36

Mu plating and the Trp^{+} transduction were carried out at 37°C for strains K37 and K807 and at 42°C for K1870 and K1871. The Mu plating results confirm the results found previously by D. Friedman and M. Gellert (personal communication). The Mu plating was performed on the indicated strains. The Trp^{+} transduction was performed on *trp*::Tn10 derivatives of the indicated strains.

[a] The fraction of Trp^{+} transductants that has Δ*top*, determined as described in the Experimental Procedures.

The genetic evidence presented shows that the compensatory mutations of DM800 and SD7 map at or near *gyrB*, and that the analogous mutation of DM750 maps at or near *gyrA*. The DNA gyrase assays show that these mutations lead to lower levels of gyrase activity (Figures 1 and 2). Thus it is reasonable to conclude that the mutations are either in the gyrase structural genes or in nearby regulatory sequences, and that they serve to lower the gyrase activity in the cell.

It is noteworthy that *himB* mutations, *gyrB* mutations selected for their inability to lysogenize phage lambda and to plate phage Mu, also act as compensatory mutations; that is, the Δ*top* mutation can be transduced into these strains readily (Table 3). Strain SD7 also cannot plate phage Mu. Furthermore, strains DM800 and DM750 give small and unusually turbid Mu plaques, and this phenotype can be detected in all transductants that have received the *gyrB* region of DM800 or the *gyrA* region of DM750. Since the Mu plating defect, the compensatory mutation and the Bgl^{+} phenotype of these strains have not been genetically separated, we consider all of these phenotypes to be a reflection of the gyrase mutations.

Pruss et al. (1982) have shown that these compensatory mutations in gyrase genes lead to a lower level of supercoiling. They have found that chromosomal DNA and plasmid DNA isolated from strains DM800, DM750 and SD7 are less negatively supercoiled than DNA isolated from isogenic wild-type strains. Furthermore, they found that the various strains in which the compensatory mutations were moved to new backgrounds also have DNA that is less negatively supercoiled than normal. For example, the Bgl^{+} derivatives of SD104, in which the appropriate gyrase gene has been moved from DM800 or from DM750, have this property, while isogenic Bgl^{-} strains have normal supercoiling. Similarly, strain N4178-15 (with the *gyrB* gene from SD7) has DNA with altered supercoiling, while the isogenic strain, N4178-4 (*gyrB* $^{+}$), has normal supercoiling. Thus a lower level of DNA supercoiling can be added to the list of phenotypes associated with the compensatory mutations in gyrase genes.

The in vitro catalytic properties of DNA gyrase and DNA topoisomerase I suggested that these enzymes may play counteracting roles in the cell with respect to supercoiling. Our findings that spontaneous gyrase

mutations arise to suppress the deleterious effects of a Δ*top* mutation support this notion, as do the supercoiling measurements of Pruss et al. (1982). They found that chromosomal and plasmid DNAs isolated from strain RS2, a *top10* mutant that does not appear to have a compensatory mutation, are more negatively supercoiled than those from an isogenic *top*$^+$ strain. This is the expected result if topoisomerase I relaxes DNA supercoils in vivo. On the other hand, covalently closed DNA isolated from Top$^-$ strains with compensatory *gyrA* or *gyrB* mutations is less negatively supercoiled than DNA isolated from wild-type (see above). Therefore, it seems that the compensatory gyrase mutations overcompensate for the topoisomerase I defect with respect to supercoiling.

It is noteworthy that Top$^+$ transductants of DM800 or DM750 retain the compensatory gyrase mutation. They do not seem to be under any strong selective pressure to revert to Gyr$^+$. Pruss et al. (1982) have found that chromosomes from a Top$^+$ *gyrB* strain, SD108, have identical superhelicity with that of chromosomes from the Δ*top gyrB* parent, DM800. It is not clear why the reintroduction of the *top* gene product does not further reduce the level of supercoiling in the cell. These observations may be the first hint of a more complex control of DNA superhelicity than simple competition between gyrase and topoisomerase I. If such regulation exists, it is conceivable that compensatory mutations could be found in the regulatory elements. A search for such mutations is currently under way. In this regard, it is noteworthy that DM700, a *top* deletion strain with a strong compensatory mutation (see Table 1), is Bgl$^-$, and thus far we have been unable to map its compensatory mutation.

Why Is The Δ*top* Mutation Deleterious?

As mentioned above, strain RS2*top10*, with a small amount of residual topoisomerase I activity and no known compensatory mutation, has DNA that is more negatively supercoiled than normal (Pruss et al., 1982). Presumably, a new Δ*top* mutant, having no topoisomerase I activity, would have DNA that is even more negatively supercoiled. Apparently, a cell can tolerate a slight increase in superhelicity, but a large increase is harmful. This leads to the selective pressure for compensatory mutations. It is not clear whether DNA replication or gene expression, or both, are adversely affected by a large increase in DNA negative superhelicity. On the other hand, the gyrase mutations found for strains DM800, DM750 and SD7 that result in DNA which is less negatively supercoiled than wild-type (Pruss et al., 1982) do not seem to be harmful to the cell. It should be emphasized that these gyrase mutations are stable and not deleterious, even in strains containing normal levels of topoisomerase I (Top$^+$ strains).

As described in the Results, the *top10* mutation can be moved to other strains by P1 transduction with normal efficiency, and all the *top10* mutants found after such a transduction have a normal growth rate. It is not clear why the majority of transductants have no apparent compensatory mutation (for example, RS2), while some of the transductants (30% in the case of recipient strain PLK831, as judged by Mu plating data) have a compensatory mutation at *gyrB* (for example, SD7). Perhaps all *top10* mutants evolve compensatory mutations, but we have not discovered the phenotype for some of them.

The Salmonella typhimurium *supX* gene (Dubnau and Margolin, 1972) is the structural gene for DNA topoisomerase I; it is the Salmonella equivalent of the E. coli *top* gene (Sternglanz et al., 1981; Trucksis et al., 1981). Overbye and Margolin (1981) have presented evidence that Salmonella *supX* mutants acquire secondary mutations, called modifiers, that change the phenotype of the *supX* strains. Presumably, these modifier mutations are analogous to the compensatory mutations that we have described.

Gyrase Mutations Activate the *bgl* Operon

The cryptic *bgl* operon can be activated by various mutations, including insertions in the regulatory region at the beginning of the operon (Reynolds et al., 1981). We have described mutations at *gyrB* for strain DM800 (Table 2) and at *gyrA* for DM750 that activate the *bgl* operon. The Bgl$^+$ phenotype depends on inducer and cAMP in these strains, just as it does in strains activated by mutation at the *bgl* operon. Pruss et al. (1982) have shown that the mutations at *gyrA* and *gyrB* cause a lower level of supercoiling. The simplest interpretation of all these results is that the *bgl* operon can be activated by a decrease in negative superhelicity, either because RNA polymerase binds more effectively or because a repressor binds more weakly. This is the clearest case in which an operon is activated by a decrease in DNA negative superhelicity. The *lacI* gene may be another such example (Sanzey, 1979).

We have observed that the *gyrB* mutation present in strain DM800 does not activate the *bgl* operon when it is moved to strain PLK831. Similarly, strain SD7, derived from PLK831, is Bgl$^-$, but when its *gyrB* mutation is moved into strain SD104 the Bgl$^+$ phenotype is observed. Apparently, the activation of the *bgl* operon by gyrase mutations only occurs in certain strain backgrounds.

It is important to point out that the interpretation of gene expression data in terms of template superhelicity is not straightforward. We previously reported that strain RS2*top10* has a higher rate of expression than JTT1*top*$^+$ for both the *lac* and tryptophanase operons (Sternglanz et al., 1981). This effect was attributed to the increase in superhelicity expected for RS2, an expectation that has now been demonstrated (Pruss et al., 1982). However, strain SD7 has an even higher rate of expression for the *lac* and tryptophan-

ase operons (S. DiNardo and R. Sternglanz, unpublished data), but, as has already been pointed out, SD7 is less negatively supercoiled than wild-type. Thus, in the case of the three isogenic strains JTT1*top*$^+$, RS2*top10* and SD7*top10 gyrB*, there is no correlation between superhelicity and gene expression. It is not simply the absence of the *top* gene product that affects gene expression, since various Δ*top* strains show the same rate of expression of the *lac* and tryptophanase operons as the wild-type (S. DiNardo and R. Sternglanz, unpublished data).

It is clear that further experimentation is required to delineate those phenotypes due directly to the absence of the *top* gene product from phenotypes due to changes in DNA superhelicity brought about by either topoisomerase I mutations or second-site compensating mutations. For example, it was previously reported that *top* mutants show a decrease in transposition frequency (Sternglanz et al., 1981). At least some of this decrease is due to the compensatory mutations present in these strains (K. Voelkel and R. Sternglanz, unpublished data).

Experimental Procedures

Bacterial Strains

The E. coli K12 strains used are listed with their genotypes in Table 4. All strains are λ$^-$. The three Δ*top* strains, DM700, DM750 and DM800, were isolated by D. Mascarenhas, and he has communicated details of their construction to us. They were originally isolated as *supX* mutants of an F'123 episome present in a particular S. typhimurium strain, by methods described previously (Dubnau and Margolin, 1972). Specifically, S. typhimurium strain JTN83/*leu500* Δ(*trp supX cysB*)/F'123, which is Leu$^-$, was plated on a minimal medium containing cysteine but but lacking leucine. Leu$^+$ colonies were isolated and screened to find ones that were Cys$^-$. Such strains are deletions of the *supX–cysB* region of F'123 and have the genotype JTN83/*leu500* Δ(*trp supX cysB*)/F'123 Δ(*supX cysB*). The mutated F'123 episome was then mated into an E. coli *trp pyrF* strain, a P1 lysate was prepared on the resultant E. coli recombinant and the P1 lysate was used as a donor in a transduction of a *trp pyrF* strain to Trp$^+$ PyrF$^+$. This procedure moves the *supX cysB* deletion from the F' episome onto the E. coli chromosome of the final recipient strain. In the case of DM800, the recipient was JT32*trpE9829 pyrF287*. In the case of DM700 and DM750, the recipient was DM1013Δ*trpA–C pyrF287*, a strain derived from JT32 and closely related to it. It should be emphasized that DM700, DM750 and DM800 are derived from independently isolated F'123 Δ(*supX cysB*) mutants of JTN83. Sternglanz et al. (1981) subsequently presented evidence that DM700, DM750 and DM800 lack DNA topoisomerase I, and that the *supX* gene is very likely to be the same as the *top* gene. Depew and coworkers have concluded that *supX* and *top* are identical (Trucksis and Depew, 1981; Trucksis et al., 1981). We use the designation Δ*top* rather than Δ*supX* for E. coli mutants because it connotes what enzyme is missing.

R. Depew (personal communication) pointed out to us that DM800 is mitomycin C–sensitive, and that the mutation leading to sensitivity maps at or near *acrA*. We subsequently showed that DM700 and DM750 also have *acrA* mutations. This explains why all three Δ*top* strains are unusually sensitive to many drugs, including ampicillin and tetracycline. To our surprise, we found that strain JT32, from which all three deletion strains were derived, is *acrA*$^+$. Thus the possibility exists that *acrA* mutations arise spontaneously in Δ*top* strains and help them grow or survive better. However, using the methods described in the Results, we found that the *acrA* mutation of DM800 by itself is not a compensatory mutation for Δ*top* (data not shown).

Table 4. Bacterial Strains Used

Strain	Genotype	Source or Reference
DM4100	*cysB242*(Am)	D. Mascarenhas; Sternglanz et al. (1981)
DM700	Δ(*top cysB*)*217 acrA11*	D. Mascarenhas; Sternglanz et al. (1981)
DM750	Δ(*top cysB*)*218 acrA12 gyrA224*	D. Mascarenhas; Sternglanz et al. (1981)
DM800	Δ(*top cysB*)*204 acrA13 gyrB225*	D. Mascarenhas; Sternglanz et al. (1981)
SD104	DM4100 Δ*trpE63 cysB*$^+$ *pyrF287*	This work
SD107	DM700 Δ*trpE63 top*$^+$ *cysB*$^+$ *pyrF287*	This work
SD275	DM750 Δ*trpE63 top*$^+$ *cysB*$^+$ *pyrF287*	This work
SD108	DM800 Δ*trpE63 top*$^+$ *cysB*$^+$ *pyrF287*	This work
AE199	DM800 *tna7*::Tn10	This work
RS752	DM750 *tna7*::Tn10	This work
PLK831	Δ*trpE63 pyrF287 nirA trpR72 iclR7 gal25 rpsL195*	P. Kuempel
JTT1	PLK831 *trp*$^+$	Sternglanz et al. (1981)
RS2	PLK831 *trp*$^+$ *top10*	Sternglanz et al. (1981)
SD7	PLK831 *trp*$^+$ *top10 gyrB226*	Sternglanz et al. (1981); This work
JTP16	PLK831 *pyrF*$^+$ *top10 gyrB227*	Sternglanz et al. (1981); This work
K37	*galK2 rpsL200*	Miller et al. (1978)
K807	K37 *gyrB229* (*himB114*)	Miller et al (1978)
K1870	K37 *gyrB230* (*himB*, Ts)	D. Friedman
K1871	K37 *gyrB231* (*himB*, Ts)	D. Friedman
N4178	*galK2 recB rpsL gyrB203*(Ts) *gyrB221*(Cou^R)	M. Gellert
N4178-4	N4178 *gyrB*$^+$	This work
N4178-15	N4178 *gyrB226*	This work
E101	*nrdA1*(Ts) *thr1 leuB6 thyA6 thi1 deoC1 lac rpsL67 tonA21 supE44*	Fuchs et al. (1972)
KV403	SD104 *nrdA1*(Ts) *gyrA228*(Nal^R)	This work
KO635	Δ*lac3*(X74) *tna7*::Tn10	This work
SBE10	W3110 *tna trp*::Tn10	C. Yanofsky

Genetic Methods

Bacteria were grown in LB broth or M9 minimal medium as described previously (Sternglanz et al., 1981). P1 transductions with P1*vir* were carried out essentially as described by Miller (1972). Specifically, overnight cultures of the recipient strain were pelleted and resuspended in an equal volume of 0.1 M $MgSO_4$, 5 mM $CaCl_2$. Cells were then shaken for 20 min at 37°C and infected with a P1 lysate prepared on the appropriate donor strain. The ratio of volume of cells to volume of P1 lysate ranged from 10:1 to 1:1, depending on the transducing power of the P1 lysate. The phage–cell mixture was incubated at 37°C for 20 min without shaking, an equal volume of 1 M sodium citrate was added and 0.2 ml was plated on appropriate selective medium containing 10 mM sodium citrate. In the case of transduction to tetracycline resistance, the infected cells were first pelleted, resuspended in LB medium with 10 mM sodium citrate, incubated with shaking for 1 hr and then plated on LB medium containing 10 mM sodium citrate and either 10 μg/ml tetracycline for *top*$^+$ recipient strains or 2 μg/ml tetracycline for Δ*top* strains.

Strains were converted to Trp$^-$ by P1 transduction with a lysate

grown on SBE10*trp*::Tn10. Selection for tetracycline-resistant transductants led to Trp$^-$ colonies.

Strain KV403 was constructed as follows. First, a spontaneous mutant of strain E101 was isolated that was resistant to 40 μg/ml nalidixic acid. A P1 lysate was prepared on this derivative of E101, and it was used to transduce strain SD104 to nalidixic acid resistance at 30°C. Transductants were purified and screened for temperature sensitivity. KV403 is one such Nal^R *nrdA*(Ts) transductant.

Screening for Bgl$^+$ was usually carried out on plates containing MacConkey agar base, 0.1% bromothymol blue and 0.5% salicin. Strains DM750 and DM800 and their derivatives do not grow well on MacConkey agar. For these strains, Bgl$^+$ colonies were detected on M9 minimal medium supplemented with 0.075% yeast extract (Difco), 0.02% bromothymol blue and 0.5% salicin. For both kinds of plates, salicin fermentors (Bgl$^+$) are orange and nonfermentors (Bgl$^-$) are whitish-gray colonies. In some experiments 5-bromo-4-chloro-3-indolyl β-D-glucoside (Bachem) at 40 μg/ml in LB was used as the indicator; in that case fermentors(Bgl$^+$) are blue and nonfermentors (Bgl$^-$) are white colonies.

The ability of bacterial strains to plate phage Mu was determined with exponential cultures grown in LB supplemented with 10 mM $MgSO_4$, 5 mM $CaCl_2$. Cells and appropriately diluted Mu phage were mixed with LB, 10 mM $MgSO_4$, 5 mM $CaCl_2$ and soft agar, and the mixture was poured onto LB plates. Plates were incubated overnight at 37°C and examined for Mu plaques.

Screening for Δ *top* Transductants

Since the deletion mutations (Δ*top*) used here cover not only the *top* gene but also the *cysB* gene (see above), all Δ*top* transductants will be phenotypically Cys$^-$. This property enabled us to screen large numbers of transductants for their *top* genotype. Transductions were carried out as outlined above with a P1 donor grown on a Trp$^+$ Δ(*top cysB*) strain (DM800 or DM700). The recipient to be tested for a compensatory mutation was always Trp$^-$ Top$^+$ Cys$^+$. Trp$^+$ transductants were selected on plates containing M9 minimal medium supplemented with 0.5% acid-hydrolyzed casamino acids, 40 μg/ml cysteine and other supplements as required. Plates were incubated at 37°C (or 42°C in the case of *himB*[Ts] recipients) for 1–2 days. Transductants were screened for cysteine auxotrophy by replica-printing colonies onto minimal medium plates with and without cysteine. Cys$^-$ colonies were assumed to have the Δ*top* mutation. Enzymatic assays for topoisomerase I have confirmed that this screening procedure is valid.

DNA Gyrase Assays

The substrate for the gyrase assays was fully relaxed pBR322 DNA. The DNA was relaxed by calf thymus DNA topoisomerase II as described by Miller et al. (1981). Twenty milliliter cultures of cells to be assayed were grown exponentially in LB to a density of 4×10^8 cells/ml, spun down, resuspended in 0.5 ml of 50 mM Tris (pH 7.7), 50 mM KCl, 0.5 mM EDTA and lysed by sonication. The lysate was centrifuged in a desk-top centrifuge at 4°C for 10 min, and the supernatant was used directly. In a typical assay, 1–3 μl extract or diluted extract was incubated at 30°C for 30 min in a total volume of 15 μl containing 25 mM Tris (pH 7.5), 20 mM potassium phosphate (pH 7.5), 5 mM spermidine, 100 μg/ml E. coli tRNA, 10 mM $MgCl_2$, 1 mM trisodium EDTA, 5 mM dithiothreitol, 1 mM ATP, 50 μg/ml bovine serum albumin and 15 μg/ml relaxed pBR322 DNA. The reaction was stopped by the addition of 3 μl of a solution containing 24% glycerol, 60 mM trisodium EDTA, 4% SDS and 0.04% bromophenol blue. The extent of reaction was monitored by electrophoresis as described previously (Sternglanz et al., 1981).

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