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Chromosomally encoded gyrase inhibitor GyrI protects *Escherichia coli* against DNA-damaging agents

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Abstract DNA gyrase, a type II topoisomerase, is the sole supercoiling activity in the cell and is essential for cell survival. There are two proteinaceous inhibitors of DNA gyrase that are plasmid-borne and ensure maintenance of the plasmids in bacterial populations. However, the physiological role of GyrI, an inhibitor of DNA gyrase encoded by the *Escherichia coli* genome, has been elusive. Previously, we have shown that GyrI imparts resistance against microcin B17 and CcdB. Here, we find that GyrI provided partial/limited protection against the quinolone class of gyrase inhibitors but had no effect on inhibitors that interfere with the ATPase activity of the enzyme. Moreover, GyrI negated the effect of alkylating agents, such as mitomycin C and *N*-methyl-*N*-nitro-*N*-nitrosoguanidine, that act independently of DNA gyrase. Hence, in vivo, GyrI appears to be involved in reducing DNA damage from many sources. In contrast, GyrI is not effective against lesions induced by ultraviolet radiation. Furthermore, the expression of GyrI does not significantly alter the topology of DNA. Thus, although isolated as an inhibitor of DNA gyrase, GyrI seems to have a broader role in vivo than previously envisaged.

Keywords DNA gyrase · Microcin B17 · GyrI · SbmC · CcdB

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Introduction

Cells strive to conserve their genetic material by preventing, repairing, or bypassing lesions or "premutations" in the DNA, before they are set in the genome and are inherited. For instance, most organisms attempt to use a highly accurate polymerase to replicate their genome, in order to minimize changes in the genetic code. Furthermore, a large number of repair pathways are employed to detect lesions and mismatches before they are encountered by the replication machinery. However, when these mechanisms fail and the polymerase does encounter a lesion, it tends to stall or fall off the template. Such events would be lethal to prokaryotic organisms, with only a single copy of the genome. Therefore, bacteria respond to breaks in DNA by the dramatic "SOS response", in which the expression of several unlinked genes is altered in response to stress (Little and Mount 1982). In *Escherichia coli,* the SOS response includes the specific induction of over 40 unlinked genes (Courcelle et al. 2001). Some of these genes function to repair DNA lesions, either accurately or in an error-prone manner, restore replication, and prevent premature cell division (Koch and Woodgate 1989; Friedberg et al. 1995). However, despite many years of work, a number of genes with altered expression have yet to be functionally characterized.

It is believed that most replication forks encounter lesions in DNA that cause stalling in an unstressed cell (Cox et al. 2000). It is as yet unclear why such high levels of lesions occur despite the presence of preventative as well as repair mechanisms. One reason could be collision of the replication machinery with proteins that are interacting with DNA in its path. This is especially likely when the collision occurs with enzymes such as topoisomerases, since they themselves cause nicks or breaks in DNA, as intermediates in the reaction cycle. Topoisomerases are a class of enzymes that inter-convert DNA between different topological forms (Wang 1998; Champoux 2001). Topoisomerases carry out these topological transformations by creating transient nicks or breaks in DNA with concomi-

tant formation of a protein–DNA covalent complex and resealing after passing another DNA strand/duplex through it. Normally, the nicking reaction is tightly coupled to the religation reaction in order to prevent the accumulation of broken DNA. However, many natural and synthetic inhibitors of topoisomerases bring about lethality by uncoupling the two reactions (Lewis et al. 1996).

DNA gyrase, a type II topoisomerase, is the only enzyme that has the ability to negatively supercoil DNA. Since gyrase is essential for the survival of bacteria, many competing organisms and plasmids synthesize inhibitors of the enzyme. The inhibitors provide selective advantage in niche competition (Couturier et al. 1998). In addition, gyrase has also been a popular subject for antibiotic research for much the same reason. As a result, a large number of gyrase inhibitors are known – both natural and synthetic (Lewis et al. 1996; Maxwell 1999). Natural inhibitors can be categorized into two groups: (1) non-proteinaceous compounds, e.g. coumarins or cyclothialidines, that act by interfering with the ATPase activity of the enzyme; and (2) proteinaceous toxins, e.g. microcin B17 and CcdB, that function by stabilizing the gyrase–DNA covalent complex (Gellert et al. 1976; Vizan et al. 1991; Bernard et al. 1993; Nakada et al. 1993). The former toxin appears to be primarily used in interspecies or intraspecies competition while the latter is involved in plasmid maintenance. In agreement with their roles, the toxins are encoded by plasmids and are ordinarily expressed in conjunction with a labile antidote. In such a scheme, the presence of a chromosomally encoded proteinaceous inhibitor of DNA gyrase, GyrI, is puzzling. The physiological role of this protein is ambiguous. Previously, it was observed that GyrI negates the effects of other proteinaceous inhibitors of DNA gyrase (Chatterji and Nagaraja 2002). Therefore, instead of being detrimental to the cells, GyrI provides protection to the cells against toxins; a possible cause for the gene to be retained in evolution. Here, we show that GyrI also reduces the harmful effects of the quinolone class of drugs. More surprisingly, GyrI imparts resistance against DNA damaging agents that act independent of topoisomerases. Notably, these affects are observed without a concomitant change in the topology of plasmid DNA. Taken together, the above observations point towards a more general role of GyrI in protecting cells against DNA damage.

Materials and methods

Bacterial strains and plasmids

gyrI was cloned under the P_{trc} promoter in pTrc99C-*Dra*I plasmid (Chatterji and Nagaraja 2002). pTrc99C-*Dra*I is a derivative of pTrc-99C and has a *Dra*I site five nucleotides upstream of the SD sequence. *E. coli* strains, AP1-200-9 ((F⁻ *endA1 thi-1 supE44 hsdR17 mcrB251 mrr253 mcrA252 lacZ*::Tn10 [*dinD1*: Mu dI1734 (Kan lac)] [F′ *lacI*^{q} *lacZ*::Tn5]) (Piekarowicz et al. 1991) and DH10B were used for in vivo plate assays. AP1-200-9 was used for detection of the SOS response in cells. Both *E. coli* strains were grown in Luria Bertani broth at 37 °C on a rotary shaker at 240 rpm. Cells were grown to mid -exponential phase (0.6 OD_{600}) and induced with 0.1 mM IPTG for 1 h.

In vivo assays

The effect of GyrI on the in vivo toxicity of various inhibitors was tested by spotting different concentrations of drug on a lawn of *E. coli* AP1-200-9 cells containing either pTrc99C-*DraI* or pTrc99C-*gyrI* in the presence or absence of IPTG. The diameters of the zones of inhibition were measured as a reflection of the sensitivity of the cells to the compounds. Similar experiments were also carried out using DH10B cells. To monitor the generation of DNA breaks induced by inhibitors of DNA gyrase and chemical mutagens, AP1-200-9 cells harboring either pTrc99C-*Dra*I or pTrc99C-*gyrI* were grown to 0.6 OD_{600} and treated with drugs as indicated. After 1 h of growth at 37 °C, β-galactosidase activity in the cells was measured using a standard procedure (Miller 1992). To confirm the expression/induction of GyrI in various in vivo experiments, the crude cell extract was resolved on 1%SDS-12PAGE. All inductions were done with 0.1 mM of IPTG. The time and the duration of induction were as indicated.

Viability of cells on exposure to ultraviolet radiation

Cells transformed with pTrc99C-*Dra*I and pTrc99C-*gyrI* plasmids were grown in LB broth (in the presence or absence 0.1 mM IPTG) at 37 °C and were exposed to ultraviolet radiation as described previously (Miller 1992). Briefly, cells (mid-exponential phase culture, 0.6 OD_{600}) were harvested by centrifugation and resuspended in equal volume of 0.1 M $MgSO_4$. The cultures were split in two and one half of each culture was exposed to UV rays. Cells were poured into 10-cm-diameter glass Petri dishes and irradiated for 1 min using the UV lamp in the laminar flow hood. For this purpose, a UV source of 15 Watts was used and the distance between the UV torch and the cells was 15 cm. After irradiation, the cultures were poured back into a covered flask containing equal volume of 2X LB and kept for further growth at 37 °C. The remaining half of each culture was not irradiated but underwent similar treatment and was used as the negative control. After 40 min, viable counts as well as β-galactosidase activity were estimated.

Plasmid topology measurements

Plasmid DNA was isolated from cells harboring pTrc99C-*Dra*I or pTrc99C-*gyrI* cultured in the presence or absence of IPTG. Cells were grown at 37 °C until mid-exponential phase (0.6 OD_{600}) and induced with IPTG for 1 h. Subsequently, the cells were quickly chilled and DNA was extracted by alkaline lysis (Birnboim and Doly 1979). The purified DNA was incubated with indicated amounts of chloroquine and resolved on 0.8% agarose gels in the absence of ethidium bromide. The gel and the running buffer (40 mM Tris-acetate, pH 8.3, and 1 mM EDTA) contained the same concentration of chloroquine as present in the sample. The gels were run in the dark at room temperature at 1.0 V/cm for 16 h, washed with water to remove chloroquine, and stained with ethidium bromide. The specific linking-number change of each distribution was calculated. Specific linking number (at a particular chloroquine concentration) is defined as a modal writhe of the distribution divided by the number of helical turns. The modal writhe of each distribution was estimated by band counting with respect to the relaxed DNA, assuming that the relaxed DNA has a linking number of zero. Since the specific linking number takes into account the size of the DNA, it can be used to compare the topology of plasmids with different sizes, e.g. pTrc99C-*Dra*I and pTrc99C-*gyrI* (the nicked circular band in Fig. 3 shows the size of the plasmid). To assess the effect of novobiocin on plasmid topology, mid-exponential-phase cultures of AP1-200-9 cells transformed with pTrc99C-*Dra*I were treated with different concentrations of novobiocin as indicated. After 1 h of treatment, the cells were chilled and the DNA was extracted and resolved as described above.

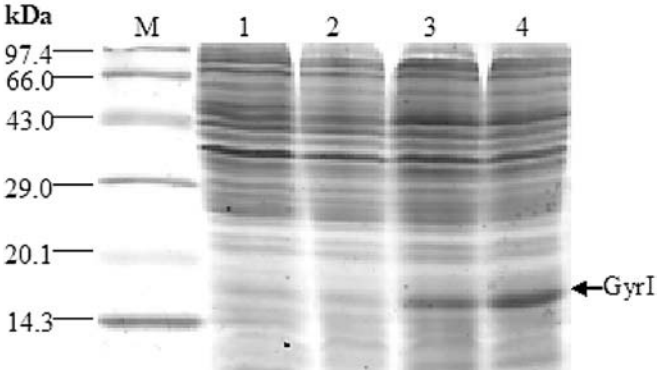

Fig. 1 Representative gel showing the levels of expression of GyrI. AP1-200-9 cells harboring the vector pTrc99C-*Dra*I (*lanes 1, 2*) or pTrc99C-*gyrI* (*lanes 3, 4*) were grown to mid-exponential phase and induced with 0.1 mM IPTG for 1 h. The crude extracts of uninduced (*lanes 1, 3*) and induced (*lanes 2, 4*) cultures were resolved by SDS-PAGE. Similar levels of expression were seen in all experiments presented here

Results

Effect of GyrI on gyrase inhibitors

Previously, GyrI was cloned and overexpressed to high levels and then purified to apparent homogeneity (Chatterji and Nagaraja 2002). Figure 1 shows the expression of GyrI under different conditions. It was observed that, in vitro, GyrI was able to prevent the formation of CcdB- and microcin-B17-stabilized gyrase–DNA covalent adducts (Chatterji and Nagaraja 2002). Furthermore, cells overproducing GyrI were able to survive at inhibitory concentrations of microcin B17. Since quinolone and its derivatives act by a mechanism similar to that of CcdB and microcin B17, the effect of GyrI on quinolone action was assessed using a plate assay. Various concentrations of ciprofloxacin (a fluoroquinolone) or nalidixic acid (a quinolone) were spotted on a lawn of cells (containing either the vector or GyrI-producing plasmid) in the presence or absence of IPTG. The diameters of the resultant zones of inhibition were then measured. For a given concentration of quinolones, slightly smaller zones of inhibition were observed in cells producing GyrI than in those containing vector alone (data not shown). This was observed for all concentrations of quinolones tested. Thus, GyrI imparts resistance against quinolones; however, unlike resistance to microcinB17, the resistance is partial (Chatterji and Nagaraja 2002).

The action of quinolones and their derivatives is known to induce the SOS system (Phillips et al. 1987). Therefore, to measure the extent of DNA damage induced by quinolones, a *lacZ* reporter gene under the control of an SOS-inducible promoter was used in AP1-200-9 cells. As expected, β-galactosidase activity was induced in the presence of 0.0035 μg ciprofloxacin/ml and 1 μg nalidixic acid/ml (Fig. 2). However, the induction was significantly higher in the presence of the former, reflecting the potency of the compounds. Notably, in the presence of GyrI-producing plasmid, β-galactosidase activity was reduced (Fig. 2), suggesting a decrease in the magnitude of the SOS response, and hence, a reduction in DNA damage. Thus, GyrI appears to diminish quinolone-mediated DNA damage in vivo. Similar experiments carried out with novobiocin revealed that GyrI did not impart any resistance against novobiocin-mediated inhibition (data not shown).

Effect of GyrI on plasmid topology

If the physiological role of GyrI is primarily to inhibit DNA gyrase, its overexpression should result in a change in DNA topology. Therefore, the effect of GyrI expression on the overall topology of DNA was tested. Plasmid DNA was isolated from cells harboring either the vector (pTrc99C-*Dra*I) or the overexpressing clone of GyrI, with or without the treatment with IPTG, which would induce the expression of GyrI in the latter construct. DNA was resolved on chloroquine agarose gels and the specific linking number (for each distribution at a particular concentration of chloroquine) was estimated. Surprisingly, there was no significant effect of overexpression of GyrI on the supercoil status of the DNA (Fig. 3), suggesting that the inhibition of gyrase by GyrI is largely dampened in vivo. It should be mentioned that the levels of GyrI used in this experiment are sufficient to confer resistance against microcin B17 (Chatterji and Nagaraja 2002). To rule out the

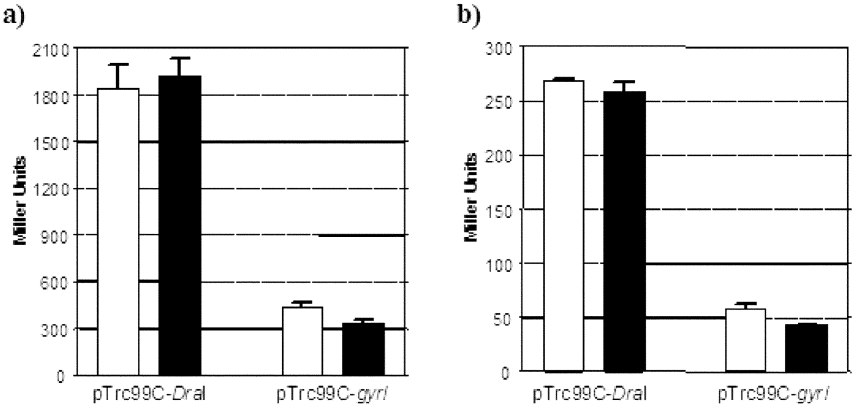

Fig. 2 Induction of the SOS system in response to nalidixic acid (**a**) and ciprofloxacin (**b**). AP1-200-9 cells were treated with 1 μg nalidixic acid/ml and 0.0035 μg ciprofloxacin/ml for 1 h and β-galactosidase activity was measured. The *white* and the *black bars* indicate cells grown, respectively, in the absence and presence of IPTG

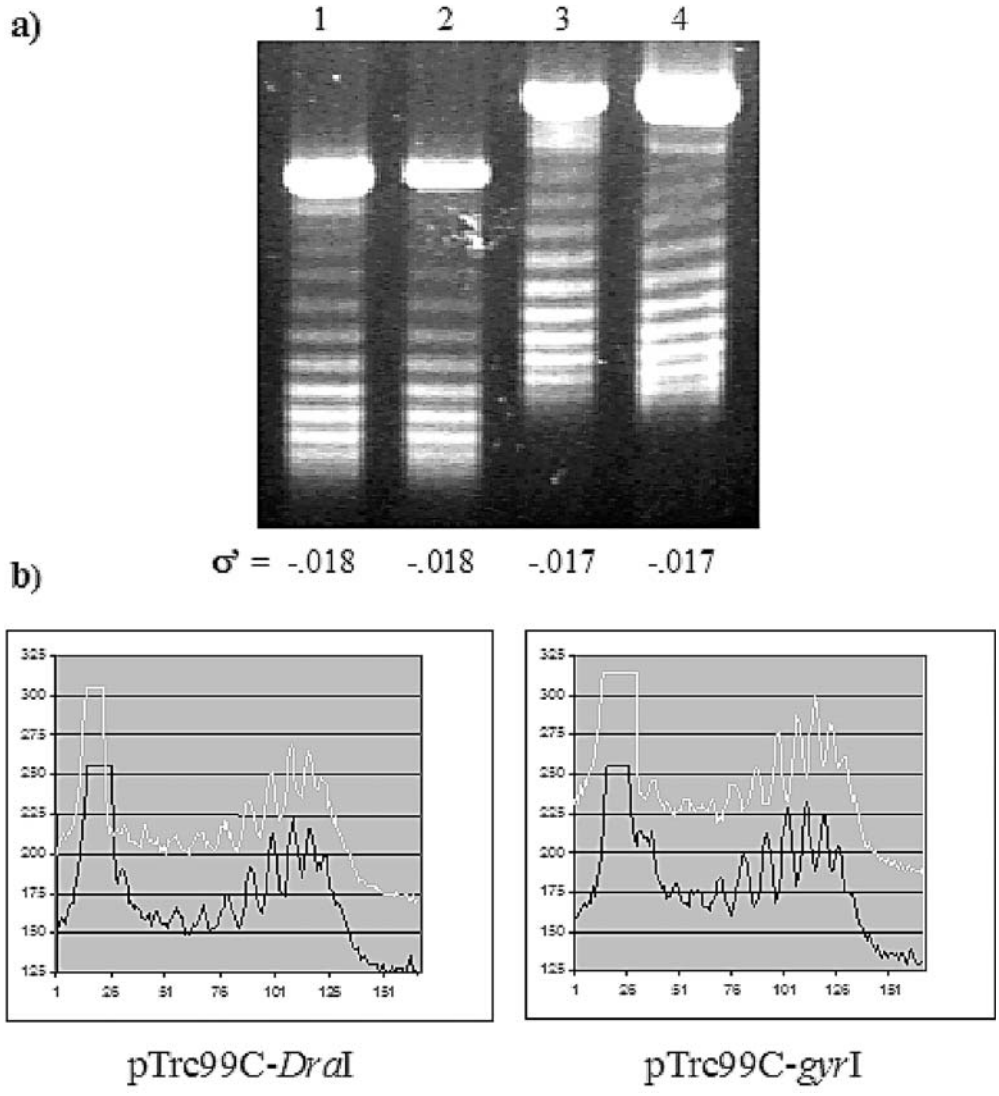

Fig. 3 **a** Visualization of plasmid topology on agarose gels containing chloroquine. Plasmids pTrc99C-*Dra*I (*lanes 1, 2*) and pTrc99C-*gyrI* (*lanes 3, 4*) were isolated from cells induced with IPTG (*lanes 2, 4*) and from uninduced cells (*lanes 1,3*) and resolved on agarose gels containing 4 μg chloroquine/ml. σ′, the specific linking number (at 4 μg chloroquine/ml), for each distribution is indicated. *NC* Nicked circular DNA. *b* Quantitation of DNA bands in **a**. The intensity of various DNA topoisomers isolated in the absence of IPTG (*black*) or presence (*white*) of IPTG is shown. Arbitrary intensity units are plotted on the *y*-axis; migration in arbitrary units is shown on the *x*-axis

possibility that the lack of a significant change in plasmid topology was due to technical reasons, the effect of novobiocin on plasmid topology was assessed. Cells transformed with pTrc99C-*Dra*I were grown until mid-exponential phase and then treated with 0, 3, or 5 μg novobiocin/ml for 1 h. In contrast to the results with GyrI, a concentration-dependent relaxation of plasmid topology was observed with novobiocin (Fig. 4), in agreement with earlier findings (Drlica and Snyder 1978; Sugino et al. 1978; Lochshon and Morris 1983).

Effect of GyrI on topoisomerase-independent chemical mutagens

As GyrI is induced in response to DNA damage, the effect of GyrI was tested on DNA lesions generated independently of topoisomerases and on their cytotoxicity. The potency of two chemical mutagens, mitomycin C (MMC) and *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG), was assessed. Plate assays were carried out with MMC and MNNG as described for quinolones. Even the uninduced level of GyrI was sufficient to confer complete resistance to MMC (Table 1). Similarly, the diameter of the zone of inhibition in the presence of MNNG was smaller for cells expressing GyrI than for cells that harbored vector plasmid (Table 1). Again, there was a small (0–18%) but consistent decrease even with uninduced levels of GyrI (Table 1). To confirm that this resistance was due to a decrease in breaks in DNA, *E. coli* AP1-200-9 cells were used to measure induction of the SOS regulon. As expected, treatment with MMC and MNNG induced β-galactosidase levels in these cells; however, as with quinolones, this induction was reduced in cells overexpressing GyrI (Fig. 5). These results demonstrate that GyrI is efficient in preventing the accumulation of breaks that are not a direct result of gyrase action.

Effect of GyrI on lesions induced by UV irradiation

Finally, the ability of GyrI to counter the effects of UV radiation, a physical mutagen, was tested. When assessed in

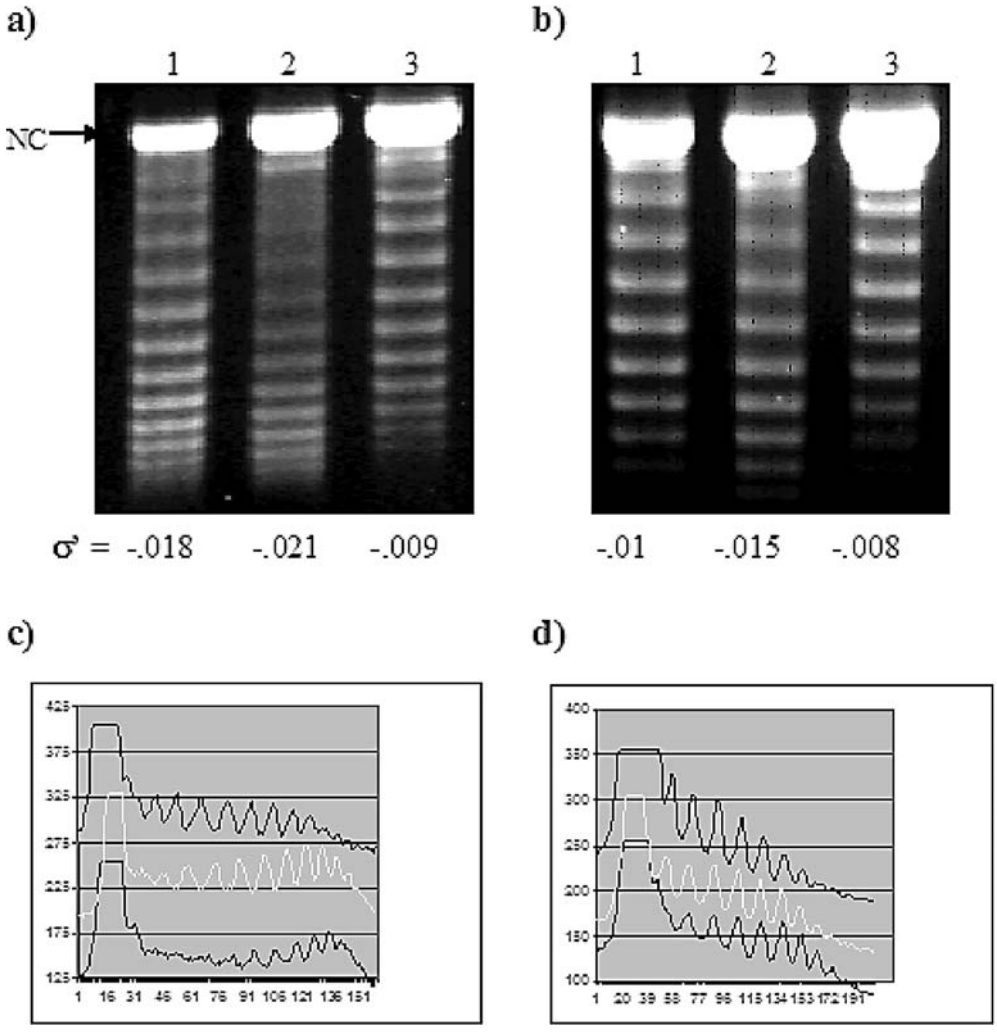

Fig. 4 Effect of novobiocin on plasmid topology. Distribution of topoisomers of plasmid DNA isolated from cells treated with 3, 0, and 5 µg novobiocin/ml, respectively, for 1 h (*lanes 1–3* in **a** and **b**). The DNA was resolved on 0.8% agarose gels in the presence of 3 and 4 µg chloroquine/ml, respectively (**a, b**). The specific linking number at the particular chloroquine concentration (σ') for each distribution is given. Quantitation of the relative intensities of bands in each lane is shown graphically (**c, d**). Arbitrary intensity units are plotted on the *y*-axis; migration in arbitrary units is shown on the *x*-axis. In each graph, the *bottom line* represents the reaction in the absence of novobiocin; the *middle* and the *top lines* represent the reaction carried out in the presence of 3 and 5 µg novobiocin/ml, respectively

Table 1 Potency of mitomycin C (*MMC*) and *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (*MNNG*) in the presence of GyrI

	Concentration (µg)	Diameter of zone of inhibition (mm)		
		pTrc99C-*Dra*I (–IPTG)	pTrc99C-*gyrI* (–IPTG)	pTrc99C-*gyrI* (+IPTG)
MMC	20	11 ±0.8	0	0
	10	9 ±0.6	0	0
	5	7 ±0.5	0	0
	2.5	4.5±0.8	0	0
MNNG	20	9.5±0.5	8.5±0.4	6.5±0.6
	10	8.5±0.4	7.0±0.6	5.5±0.5
	5	6.5±0.5	5.5±0.7	4.0±0.6
	2.5	4.0±0.9	4.0±0.4	3.0±0.4
	1.25	3.5±0.4	3.0±0.6	0

terms of the viability of cells after exposure to UV rays, no significant difference between cells harboring pTrc99C-*gyrI* and those harboring pTrc99C-*Dra*I was observed (Table 2). In addition no significant difference in β-galactosidase activity was observed irrespective of GyrI expression (Fig. 6). Hence, GyrI fails to diminish/negate the damaging effects of UV irradiation in vivo.

Discussion

DNA topoisomerases maintain the cellular DNA in a compact yet accessible form. Due to this critical function, most of these enzymes are essential to organisms. Therefore, the presence of a protein like GyrI, which inhibits DNA

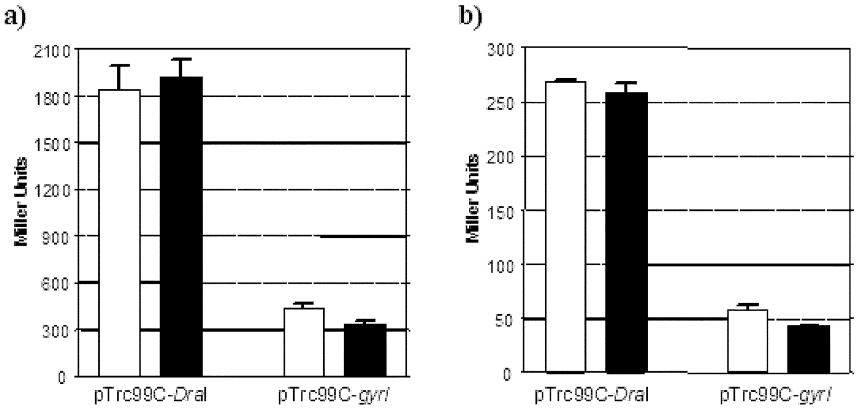

Fig. 5 Induction of the SOS system in response to mitomycin C (*MMC*) (**a**) and *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (*MNNG*) (**b**). AP1-200-9 cells were treated with 1.5 µg MMC/ml and 1 µg MNNG/ml for 1 h and β-galactosidase activity was measured. The *white* and the *black bars* indicate β-galactosidase activity in cells grown, respectively, in the absence and presence of IPTG

Table 2 Effect of UV irradiation on the viability of bacterial cells expressing GyrI. *CFU* Colony-forming units

	Viable counts (CFU/ml)		
	pTrc99C-*Dra*I (–IPTG)	pTrc99C-*gyrI* (–IPTG)	pTrc99C-*gyrI* (+IPTG)
Irradiated	2.77×10^7	1.17×10^7	1.66×10^7
Not irradiated	1.06×10^8	1.09×10^8	1.40×10^8

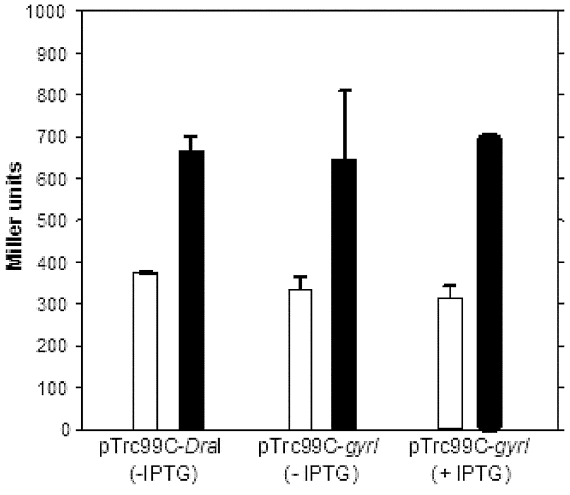

Fig. 6 Induction of the SOS system in response to UV radiation. β-Galactosidase activity was measured in AP1-200-9 cells exposed to UV light for 1 min. The *white* and the *black bars* indicate β-galactosidase activity in cells grown, respectively, in the absence and presence of IPTG

gyrase, is unexpected. This paradox was partially resolved by the observation that GyrI imparts resistance against natural toxins, namely CcdB and microcin B17 (Chatterji and Nagaraja 2002). These toxins act by targeting DNA gyrase and are members of plasmid addiction systems.

To determine whether the action of GyrI was restricted to toxins, its effect was tested on the quinolone family of compounds. Quinolones and their derivatives are gyrase inhibitors that act by stabilizing the gyrase–DNA covalent complex, a mechanism similar to that of the toxins (Lewis et al. 1996); however, quinolones are synthetic in nature and are chemically distinct. From our observations, it appears that GyrI specifically protects cells against gyrase inhibitors that act by trapping the gyrase–DNA covalent complex, e.g. CcdB, microcin B17, and quinolones. However, GyrI has little effect on the action of novobiocin, a coumarin, which inhibits DNA gyrase by interfering with ATPase activity (Gellert et al. 1976). Therefore, GyrI possibly protects only against inhibitors that act by stabilizing the gyrase–DNA complex. The extent of protection varies for different inhibitors, with the lowest level being for the quinolone family of compounds. This might be due to the fact that quinolone-mediated cell death is significantly faster than death mediated by CcdB or microcin B17, as suggested by biochemical experiments (Kampranis et al. 1999; Heddle et al. 2001). Therefore, it is possible that the cells die before GyrI can have any effect, hence resulting in a marginal change in the qualitative plate assay and a partial decrease in β-galactosidase levels (Fig. 2). It should be noted that there is significant induction of GyrI under these experimental conditions (Fig. 1).

Furthermore, GyrI imparts resistance against chemical mutagens such as MMC and MNNG. MMC and MNNG alkylate DNA and replication of DNA past these lesions is believed to result in DNA breaks. Thus, the action of GyrI does not seem to be limited to gyrase inhibitors. Instead, it appears to have a more general function in reducing DNA damage. In agreement with our results, it was observed recently that *gyrI*, when present in a multicopy plasmid, imparts resistance against mitomycin C (Wei et al. 2001). The broad-spectrum action of GyrI probably explains the presence of this protein as part of the SOS regulon (Baquero et al. 1995). However, it is noteworthy that GyrI does not protect against all DNA lesions, such as those caused by UV light, as assessed by cell viability. Moreover, since UV radiation triggers the SOS response, there was increased β-galactosidase activity in AP1-200-9 cells; however, the levels were unchanged in the presence or absence of GyrI (Fig. 6). The mutagenic and cytotoxic effects of UV radiation are primarily due to intra-strand linkage of adjacent pyrimidines, usually thymines and thus resulting in the formation of thymine dimers. In turn, these distort the helix thereby creating a barrier for replication and transcription. It is possible that the effect of GyrI is not

sufficient to protect against UV-induced mutagenesis of DNA. Alternately, UV-induced lesions in the cell may be rectified by mechanisms distinct from those of other lesions, rendering GyrI ineffective.

The intracellular supercoil status of DNA is a balance between the relaxing activity of topoisomerases I and IV and the supercoiling activity of DNA gyrase (Zechiedrich et al. 2000). If the physiological role of GyrI is to inhibit DNA gyrase, it would lead to relaxation of DNA. Overexpression of GyrI has no significant effect on plasmid topology, unlike other inhibitors of DNA gyrase, e.g. novobiocin, that bring about relaxation of DNA in vivo. This might be due to the ability of cells to compensate for perturbations in DNA topology up to a certain extent. Two strategies used by the cells to attain such a homeostasis are alteration of the expression of other topoisomerases (Pruss et al. 1982; Tse-Dinh and Beran 1988) and stimulation of gyrase expression in response to relaxation of DNA (Menzel and Gellert 1983). Although GyrI inhibits gyrase activity in vitro, inside the cell the effect of this inhibition on steady-state levels of DNA topology is transient or marginal due to various compensatory mechanisms. On the other hand, a few single-stranded breaks in the DNA have been demonstrated to be lethal to cells (Miguel and Tyrrell 1986). Therefore, the physiological role of GyrI appears to be reducing the number of otherwise lethal double-stranded breaks formed as a result of the action of various DNA-damaging agents.

Our results clearly demonstrate an important and general function of GyrI in the cellular response to DNA damage. However, they do not reveal the underlying mechanism of action of the protein. *gyrI* and the genes present around it do not show significant sequence similarity to any genes of known function. GyrI could directly be involved in the repair of double-stranded breaks, or it could confer resistance to chemical mutagens through an indirect route, for example, by slowing down tracking machines in the cell. Most DNA-damaging agents alter the DNA, and the subsequent passage of the replication fork across these lesions causes a loss of the genetic information along with the generation of breaks. Inhibition of gyrase would slow down the replication machinery in the cell, thereby allowing the cell enough time to repair the lesions. This would reduce collisions with the replication fork, increasing the chances of cell survival. Therefore, in such cases, overexpression of GyrI would prevent the accumulation of breaks in the cell. An analogous scenario was observed by Gari et al. wherein mutations in RNA polymerase that reduce its rate of fork progression suppress the lethality of a gyrase allele that stabilizes the gyrase–DNA adduct (Gari et al. 2001).

Since GyrI has the potential to inhibit DNA gyrase, its expression is expected to be under elaborate control in vivo. Indeed, it has been observed that the promoter driving GyrI has multiple regulatory elements. The promoter is also RpoS-dependent and hence the protein is induced in stationary phase (Baquero et al. 1995; Oh et al. 2001). Moreover, it is also under the control of LexA as part of the SOS regulon. A putative CAP-CRP binding site is located in the promoter region (Baquero et al. 1995), and a recent study suggested that GyrI is further regulated by H-NS (Oh et al. 2001). These features of the *gyrI* locus hint towards a complex regulation of GyrI expression in vivo, possibly reflective of its more general function.

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References

Baquero MR, Bouzon M, Varea J, Moreno F (1995) *sbmC*, a stationary-phase induced SOS *Escherichia coli* gene, whose product protects cells from the DNA replication inhibitor microcin B17. Mol Microbiol 18:301–311

Bernard P, Kezdy KE, Van Melderen L, Steyaert J, Wyns L, Pato ML, *et al*. (1993) The F plasmid CcdB protein induces efficient ATP-dependent DNA cleavage by gyrase. J Mol Biol 234:534–541.

Birnboim HC, Doly J (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res 7:1513–1523

Champoux JJ (2001) DNA topoisomerases: structure, function, and mechanism. Annu Rev Biochem 70:369–413

Chatterji M, Nagaraja V (2002) GyrI: a counter-defensive strategy against proteinaceous inhibitors of DNA gyrase. EMBO Reports 3:261–267

Courcelle J, Khodursky A, Peter B, Brown PO, Hanawalt PC (2001) Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient *Escherichia coli*. Genetics 158:41–64

Couturier M, Bahassi el- M, Van Melderen L (1998) Bacterial death by DNA gyrase poisoning. Trends Microbiol 6:269–275

Cox MM, Goodman MF, Kreuzer KN, Sherratt DJ, Sandler SJ, Marians KJ (2000) The importance of repairing stalled replication forks. Nature 404:37–41

Drlica K, Snyder M (1978) Superhelical *Escherichia coli* DNA: relaxation by coumeromycin. J Mol Biol 120:145–154

Friedberg EC, Walker GC, Siede W (1995) DNA repair and mutagenesis. ASM Press, Washington DC

Gari E, Bossi L, Figueroa-Bossi N (2001) Growth-dependent DNA breakage and cell death in a gyrase mutant of Salmonella. Genetics 159:1405–1414

Gellert M, O'Dea MH, Itoh T, Tomizawa J (1976) Novobiocin and coumeromycin inhibit DNA supercoiling catalyzed by DNA gyrase. Proc Natl Acad Sci USA 74:4774–4478

Heddle JG, Blance SJ, Zamble DB, Hollfelder F, Miller DA, Wentzell LM, Walsh CT, Maxwell A (2001) The antibiotic microcin B17 is a DNA gyrase poison: characterisation of the mode of inhibition. J Mol Biol 307:1223–1234

Kampranis SC, Howells AJ, Maxwell A (1999) The interaction of DNA gyrase with the bacterial toxin CcdB: evidence for the existence of two gyrase-CcdB complexes. J Mol Biol 293:733–744

Koch WH, Woodgate R (1998) The SOS response. In: Nicholoff JA, Hoekstra MF (eds) DNA damage and repair: DNA repair in prokaryotes and lower eukaryotes. Humana, Totowa, New Jersey, pp 107–134

Lewis RJ, Tsai FTF, Wigley DB (1996) Molecular mechanisms of drug inhibition of DNA gyrase. Bioessays 18:661–671

Little JW, Mount DW (1982) The SOS regulatory system of *Escherichia coli*. Cell 29:11–22

Lochshon D, Morris DR (1983) Positively supercoiled plasmid DNA is produced by treatment of *Escherichia coli* with DNA gyrase inhibitors. Nucleic Acids Res 11:2999–3017

Maxwell A (1999) DNA gyrase as a drug target. Biochem Soc Trans 27:48–53

Menzel R, Gellert M (1983) Regulation of the genes for *E. coli* DNA gyrase: homeostatic control of DNA supercoiling. Cell 34:105–113

Miguel AG, Tyrrell RM (1986) Repair of near-ultraviolet (365 nm)-induced strand breaks in *Escherichia coli* DNA. The role of the *polA* and *recA* gene products. Biophys J 49:485–491

Miller JH (1992) A short course in bacterial genetics: a laboratory manual and handbook for *Escherichia coli* and related bacteria.Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, p.71

Nakada N, Shimada H, Hirata T, Aoki Y, Kamiyama T, Watanabe J, Arisawa M (1993) Biological characterization of cyclothialidine, a new gyrase inhibitor. Antimicrob Agents Chemother 37:2656–2661

Oh TJ, Jung IL, Kim IG (2001) The *Escherichia coli* SOS gene *sbmC* is regulated by H-NS and RpoS during the SOS induction and stationary growth phase. Biochem Biophys Res Commun 288:1052–1058

Phillips I, Culebras E, Moreno F, Baquero F (1987) Induction of the SOS response by new 4-quinolones. J Antimicrob Chemother 20:631–638

Piekarowicz A, Yuan R, Stein DC (1991) A new method for the rapid identification of genes encoding restriction and modification enzymes. Nucleic Acids Res 19:1831–1835

Pruss GJ, Manes SH, Drlica K (1982) *Escherichia coli* DNA topoisomerase I mutants: increased supercoiling is corrected by mutations near gyrase genes. Cell 31:35–42

Sugino A, Higgins NP, Brown PO, Peebles CL, Cozzarelli NR (1978) Energy coupling in DNA gyrase and the mechanism of action of novobiocin. Proc Natl Acad Sci USA 75:4838–4842

Tse-Dinh YC, Beran RK (1988) Multiple promoters for transcription of the *Escherichia coli* DNA topoisomerase I gene and their regulation by DNA supercoiling. J Mol Biol 202:735–742

Vizan JL, Hernandez-Chico C, del Castillo I, Moreno F (1991) The peptide antibiotic microcin B17 induces double-strand cleavage of DNA mediated by *E. coli* DNA gyrase. EMBO J 10:467–476

Wang JC (1998) Moving one DNA double helix through another by a type II DNA topoisomerase: the story of a simple molecular machine. Quart Rev Biophys 31:107–144

Wei Y, Vollmer AC, LaRossa RA (2001) In vivo titration of mitomycin C action by four *Escherichia coli* genomic regions on multicopy plasmids. J Bacteriol 183:2259–2264

Zechiedrich EL, Khodursky AB, Bachellier S, Schneider R, Chen D, Lilley DM, Cozzarelli NR (2000) Roles of topoisomerases in maintaining steady-state DNA supercoiling in *Escherichia coli*. J Biol Chem 275:8103–8113