INTRODUCTION

For pathogenic or non-pathogenic *Escherichia coli* to colonize human gastrointestinal tracts, it has to survive the challenge of extreme acid pH (Giannella et al., 1972; Gorden & Small, 1993). Enterohaemorrhagic *E. coli* strains cause disease at a low infectious dose, due partly to their high degree of acid resistance (AR) to pH of 2-5 or lower (Benjamin & Datta, 1995; Conner & Kotrola, 1995; Lin et al., 1996; Price et al., 2004). Three distinct AR systems are responsible for protecting stationary-phase *E. coli* cells from killing by extremely low pH (Castanie-Cornet et al., 1999; Foster, 2004). The oxidative or glucose-repressed system (system 1) is dependent on the stationary-phase sigma factor RpoS (Small et al., 1994). The GAD system requires glutamate during acid challenge, and involves glutamate decarboxylase activity (Lin et al., 1995; Hersh et al., 1996; De Biase et al., 1999). The ARG system requires arginine in the acid medium, and arginine decarboxylase activity (Lin et al., 1995; Castanie-Cornet et al., 1999). RpoS is also required for the expression of *gadA* and *gadBC*, the structural genes for glutamate decarboxylase isozymes, at neutral pH in stationary phase (Castanie-Cornet et al., 1999; Castanie-Cornet & Foster, 2001).

Topoisomerase I is the major activity that removes negative supercoils from DNA in *E. coli*, and along with DNA gyrase and topoisomerase IV, plays an important role in the regulation of both global and localized supercoiling (Zechiedrich et al., 2000; Champoux, 2001). Four promoters have been characterized for the *E. coli topA* gene, which encodes topoisomerase I (Qi et al., 1997). Promoter P1 is recognized by the heat-shock-responsive sigma factor $\sigma^{32}$ (Lesley et al., 1990; Qi et al., 1996), while promoter Px1 can be recognized by the stationary phase and stress-responsive sigma factor $\sigma^{p}$ (RpoS). The transcription of *topA* by these sigma factors in response to environmental challenge suggests that topoisomerase I has a role in the adaptation and survival of *E. coli*. This hypothesis is supported by the lower survival rates of...
the topA deletion mutants versus topA+ isogenic strains after challenges with high temperature or oxidative stress (Qi et al., 1999; Tse-Dinh, 2000). Loss of topoisomerase I activity can affect transcription initiation at promoters due to the influence of topoisomerase I on DNA topology (Steck et al., 1993; Wang & Lynch, 1993), and its action during transcription elongation, which suppresses hypernegativ supercoiling and R-loop formation (Massé & Drolet, 1999; Hraiky et al., 2000). The efficient transcription of genes required for AR may thus require topoisomerase I activity to be present in the cell. In Helicobacter pylori, topA was found by differential display PCR to be one of the genes induced by prolonged acid exposure (Dong et al., 2001), suggesting the involvement of topoisomerase I in adaptation to low pH by this organism. We now present the results of experiments that demonstrate the effect of ΔtopA mutation on E. coli AR, and on the expression of genes involved in the oxidative and GAD AR mechanisms.

METHODS

Bacterial strains, plasmids and culture media. The bacterial strains and plasmids used in this study are described in Table 1. P1 transduction was carried out according to standard protocols (Miller, 1992). The insertion mutation in rpoS and topA mutant strains selected by Tet was confirmed by Western blotting with antibodies against the targeted protein. E. coli was grown at 37°C in Luria Broth (LB), LB supplemented with 0.4% glucose (LBG), or LB buffered with either 100 mM MES (pH 5-5) or 100 mM MOPS (pH 8). Overall AR was measured by diluting the culture into LB pH 2-2 or 2-5 after overnight growth in LB buffered at pH 5-5 or 8-0 (Castanie-Cornet et al., 1999). To test the individual AR systems, survival rates were measured following treatment in E minimal medium (Vogel & Bonner, 1956) containing 0.4% glucose (EG) at pH 2-5, after overnight growth under different conditions as described (Castanie-Cornet et al., 1999). For system 1 (oxidative system), overnight growth was in LB pH 5-5, followed by treatment in EG pH 2-5 with no supplement. For the GAD AR system, an overnight culture in LBG was diluted into EG pH 2-5 supplemented with 1.5 mM glutamate. For the ARG AR system, overnight growth was in brain–heart infusion medium supplemented with glucose (BHIG), followed by treatment in EG pH 2-5 supplemented with 0.6 mM arginine. In the experiments involving hns mutants, M9 medium at pH 2-5 with 0.4% glucose was used as the acid-challenge medium (Hommais et al., 2001). After challenge for the indicated period of time, the viable counts were determined by dilutions in M9 minimal medium (Sambrook et al., 1989), and plating on LB plates supplemented with ampicillin (100 μg ml⁻¹), tetracycline (15 μg ml⁻¹) or kanamycin (25 μg ml⁻¹) where appropriate.

Northern blotting and real-time RT-PCR. Total RNA was isolated from cells grown in buffer LB to OD₆₆₀ 0-4-0.5 (EP, exponential phase) or 1-2-1.5 (SP, stationary phase), using previously published procedures (Hraiky et al., 2000). The 1-4 kb gad probe for detection of both gadA and gadBC was generated by PCR as described previously (Castanie-Cornet & Foster, 2001), and labelled with [x-³²P]dCTP using the MEGA Prime kit from Amersham. Electrophoresis and transfer of RNA samples (5 μg) were carried out as described by Sambrook et al. (1989). The PerfectHyb Plus solution (Sigma) was used for hybridization. For real-time RT-PCR analysis, RNA was treated with DNase I (Promega), prior to cDNA synthesis with random primers and AMV reverse transcriptase (Promega). Primers 5'-GAACGGGGGCTTACATGC-3' and 5'-GGCGATAGGATCTCAG-3' were used to amplify a 376 bp fragment from both gadA and gadB cDNA using the Roche Life Cycler.

Western blotting. Cells from exponential-phase or overnight cultures were collected by centrifugation, and lysed by boiling in SDS gel electrophoresis sample buffer for 5 min. The total lysates, with volumes adjusted for cell densities, were electrophoresed in 10% SDS-polyacrylamide gels. The proteins in the gel were either stained with Coomassie blue to check for equal loading, or transferred onto nitrocellulose filters for Western blotting; signals were detected with ECL Plus reagents (Amersham). The antibodies against E. coli topoisomerase I (Qi et al., 1996) and GadA/B (Castanie-Cornet et al., 1999) were described previously. Mouse monoclonal antibodies against E. coli RpoS was purchased from Neoclone. Antibodies against GadW were raised in rat using the peptide sequence

Table 1. E. coli strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant genotype and characteristic</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RFM443</td>
<td>rpsL galK2 Δuc74</td>
<td>Drolet et al. (1995)</td>
</tr>
<tr>
<td>RFM445</td>
<td>rpsL galK2 Δuc74 gsrB221(Cou') gsrB203(Ts)</td>
<td>Drolet et al. (1995)</td>
</tr>
<tr>
<td>RFM475</td>
<td>rpsL galK2 Δuc74 gsrB221(Cou') gsrB203(Ts) Δ(topAcysB)204</td>
<td>Drolet et al. (1995)</td>
</tr>
<tr>
<td>2111</td>
<td>W3110B, gsrB(Ts, Cou') topA20::Tn10</td>
<td>Zechiedrich et al. (2000)</td>
</tr>
<tr>
<td>YT481</td>
<td>RFM445 topA20::Tn10 P1(2111) × (RFM445), Tet'</td>
<td></td>
</tr>
<tr>
<td>GY12</td>
<td>rpsO::Tn10</td>
<td>Yamashino et al. (1995)</td>
</tr>
<tr>
<td>YT445R</td>
<td>RFM445 rpsO::Tn10</td>
<td>P1(GY12) × (RFM445), Tet'</td>
</tr>
<tr>
<td>YT475R</td>
<td>RFM445 rpsO::Tn10</td>
<td>P1(GY12) × (RFM475), Tet'</td>
</tr>
<tr>
<td>FB20271</td>
<td>MG1655 hns::Tn5</td>
<td>F. Blattner laboratory*</td>
</tr>
<tr>
<td>YT445H</td>
<td>RFM445 hns::Tn5</td>
<td>P1(FB20271) × (RFM445), Kan'</td>
</tr>
<tr>
<td>YT475H</td>
<td>RFM475 hns::Tn5</td>
<td>P1(FB20271) × (RFM475), Kan'</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pSK760</td>
<td>pBR322 derivative with the rnhA gene expressing RNase H</td>
<td>Kanaya &amp; Crouch (1983)</td>
</tr>
<tr>
<td>pSK762c</td>
<td>pBR322 derivative with mutated rnhA gene expressing inactive RNase H</td>
<td>Drolet et al. (1995)</td>
</tr>
</tbody>
</table>

*University of Wisconsin, Madison, WI, USA.
YHEQQKILHNESILC. Antibodies against GadX (Shin et al., 2001) were kindly provided by Dr J. B. Kaper. The Coomassie stained gels and exposed films were analysed by an Alpha-Imager for quantification.

RESULTS

Loss of topoisomerase I function leads to reduced AR

The increased sensitivity of the topA deletion mutant strain RFM475 to high temperature and oxidative stress challenge, when compared to its isogenic topA+ counterpart, RFM445, has been reported (Qi et al., 1999; Tse-Dinh, 2000). The effect of loss of topoisomerase I function on AR was investigated here. After overnight growth in LB buffered at either pH 8-0 or 5-5, RFM475 was found to have 20–30 fold lower AR than RFM445 (Fig. 1). The cysB gene function, also missing in RFM475 due to the ΔtopA-cysB deletion, is required for the induction of the arginine decarboxylase activity in the ARG AR mechanism (Shi & Bennett, 1994; Lin et al., 1996). This loss of cysB function is expected to lead to decreased arginine decarboxylase-dependent AR in RFM475. We therefore also tested a topA20::Tn10 insertion mutant (YT481) that is wild-type for cysB function for AR, and found a similar degree of sensitivity due to the topA mutation in the absence of the cysB mutation (Fig. 1). This indicates that topA mutations affects AR mechanisms independent of the arginine decarboxylase activity. Topoisomerase I protein was not detected in the total protein of YT481 by Western blotting (data not shown).

Loss of topoisomerase I function affects the oxidative and GAD systems of AR

To demonstrate directly which of the three AR mechanisms was affected by the loss of topoisomerase I function, the three AR systems were tested individually; the results are shown in Fig. 2. As expected from the loss of the cysB function, RFM475 was defective in arginine-dependent AR. However, the arginine-dependent AR was not affected in YT481 with the topA20::Tn10 mutation, indicating that topoisomerase I function is not required for the arginine-dependent AR mechanism. The oxidative system and glutamate-dependent systems were both affected by the loss of topoisomerase I function in the two topA mutants. However, the two topA mutants differed in the relative degree of sensitivity. Mutant RFM475 appeared to be more severely affected in the oxidative system, while mutant YT481 was more severely affected in the glutamate-dependent system. The partial loss of oxidative AR in mutant YT481 was confirmed by longer exposure to the pH 2-5 EG medium. After 6 h of exposure, the survival rate of YT481 was 0-046 % versus 7-1 % for RFM445.

Overexpression of RNase H does not improve the AR of topA mutants

In topA mutants, the formation of R-loops during transcription elongation is driven by the accumulation of hypernegative supercoiling. The blocking of RNA polymerase by these R-loops can account at least partially for the growth defect of topA mutants (Drolet et al., 1995; Massé & Drolet, 1999; Baaklini et al., 2004). Overexpression of RNase H in topA mutants including RFM475 has been shown to correct this defect of R-loop formation (Drolet et al., 1995; Hraiky et al., 2000). The overexpression of RNase H from plasmid pSK760 has also been shown to partially alleviate the effect of topA deletion on the survival rate of RFM475 after high temperature and oxidative challenge (Cheng et al., 2003). We therefore compared the survival rates of topA mutants transformed with either pSK760 expressing wild-type RNase H, or pSK762c expressing an inactive RNase H mutant. The results (Table 2) showed that there was no significant increase in the overall AR or GAD-dependent AR of RFM475 or YT481.
that could be attributed to the overexpression of RNase H when the pSK760 plasmid was present.

**Effect of topoisomerase I function on the level of glutamate decarboxylase isoforms**

The glutamate decarboxylase activity encoded by gadA and gadB consumes an intracellular proton for the decarboxylation of each molecule of glutamate. It thus plays a major role in AR. Transcription of both gadA and gadBC is positively controlled by the transcriptional activator GadX (Shin et al., 2001; Tramonti et al., 2002). RpoS is also involved in the induction of gadA and gadBC during the stationary phase of cell growth (Castanie-Cornet & Foster, 2001). The total level of GadA and GadB proteins in RFM445, and the topA mutants RFM475 and YT481, was measured by Western blotting. The results (Fig. 3) showed that the GadA and GadB proteins were present at a significantly lower level in the topA mutants after overnight growth in both neutral (pH 8) and acidic pH (pH 5-5). The decrease of GadA/B resulting from the topA mutation was also seen under conditions with an active oxidative system (during stationary phase in LB or EG pH 5-5 medium), or repressed RpoS activity (during exponential phase in EG medium or during stationary phase in LBG medium). This decrease in GadA and GadB proteins in the topA mutants would account for the diminished AR observed under these different growth conditions.

**topA mutation diminishes the induction of gadA and gadBC genes in acidic LB during stationary phase**

In order to determine if transcription of gadA and gadBC genes was affected by the topA mutation, total RNA was prepared and used for Northern blotting. The results (Fig. 4) showed that the induction of the gadA and gadBC message levels during entry into stationary phase (OD

![Table 2. Effect of RNase overexpression from plasmid pSK760 on AR of topA mutants](Image)

Each experiment was carried out four times. Means ± SD are shown.

<table>
<thead>
<tr>
<th>Acid resistance</th>
<th>Strain</th>
<th>Percentage survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall*</td>
<td>RFM445</td>
<td>73 ± 14</td>
</tr>
<tr>
<td></td>
<td>RFM475 Δ(topAcytB)</td>
<td>0.37 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>RFM475/pSK760</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>RFM475/pSK762c</td>
<td>0.66 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>RFM445</td>
<td>8.2 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>YT481 topA20::Tn10</td>
<td>0.029 ± 0.011</td>
</tr>
<tr>
<td></td>
<td>YT481/pSK760</td>
<td>0.030 ± 0.012</td>
</tr>
<tr>
<td></td>
<td>YT481/pSK762c</td>
<td>0.043 ± 0.014</td>
</tr>
</tbody>
</table>

*Adaptation overnight in LB pH 5.5, and acid challenge in EG pH 2-5, 2 h.
†Adaptation overnight in LBG, and acid challenge in EG pH 2-5+glutamate, 2 h.

![Fig. 3. Effect of topA mutation on the GadA and GadB protein levels determined by Western blotting. (a) RFM445 (topA<sup>+</sup>) and YT481 (topA20::Tn10) were grown in EG pH 5-5 medium to exponential phase (EP, OD<sub>600</sub> 0.5) and stationary phase (SP, 18 h). (b) RFM445 and RFM75 (ΔtopA) were grown in buffered LB for 18 h with either no glucose or 0.4 % glucose added.](Image)

![Fig. 4. Effect of topA mutation on the gadA and gadBC mRNA levels determined by Northern blotting. RNA was extracted from RFM445 (topA<sup>+</sup>), RFM75 (ΔtopA) and YT481 (topA20::Tn10) grown in LB at pH 5-5 or 8-0 to EP or SP.](Image)
The sigma factor RpoS is central to the resistance to acidic pH by the glucose-repressed mechanism in complex medium. The AraC-like regulators GadX and GadW have also been implicated in the complex regulation of the glutamate decarboxylase system (Shin et al., 2001; Tramonti et al., 2002; Ma et al., 2002, 2003). Western blotting was used to compare the amounts of RpoS, GadX and GadW in the topA+ and topA mutant cultures, to determine if a change in the levels of these regulators accounted for the decreased gadA and gadBC expression in the topA mutants. The results showed that the levels of RpoS that accumulated in overnight stationary-phase cultures of the topA mutants RFM475 and YT481 were similar to those found in RFM445 (Fig. 5). There was also no difference observed for the level of GadX and GadW due to the topA mutation under different growth conditions (Fig. 5). Therefore, topoisomerase I function had no significant effect on the accumulated levels of these regulators.

An RpoS-independent mechanism also accounts for some of the effect of topA mutation

An RpoS-dependent promoter is involved in the transcription of topA (Qi et al., 1997), suggesting that topoisomerase I function may be important for gene expression directed by RpoS. The rpoS::Tn10 mutation was introduced into the strains RFM445 and RFM475 to determine if the effect of topA mutation on AR was entirely dependent on transcription directed by RpoS. Survival in the absence of glutamate at pH 2.5 for 1 h was 3.5% for YT445R (rpoS), and 0.098% for YT475R (rpoS topA); in the presence of glutamate at pH 2.5 for 2 h, survival was 0.1 vs 0.0029%, respectively. These results showed that the presence of topA mutation in the rpoS mutant background further decreased the survival rate. Therefore, the effect of the topA mutation on AR was not entirely due to RpoS-dependent transcription. Measurement of the level of GadA and GadB proteins by Western blotting also showed that there was a further decrease of expression from the topA mutation in the rpoS-null background (Fig. 6a).

The effect of topA deletion can be suppressed by hns mutation

H-NS protein is a negative regulator of AR (Foster, 2004). The hns mutation affecting the nucleoid protein H-NS has been shown to increase expression of the gadA and gadBC

Levels of RpoS, GadX and GadW are not altered by the topA mutation

The sigma factor RpoS is central to the resistance to acidic pH by the glucose-repressed mechanism in complex medium. The AraC-like regulators GadX and GadW have also been implicated in the complex regulation of
**Table 3.** hns mutation can suppress the effect of the topA mutation on AR

Each experiment was carried out four times. Means ± SD are shown. Adaptation overnight in M9 pH 5-5 and acid challenge in M9 pH 2-5+glutamate, 2 h.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Percentage survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFM445</td>
<td>47 ± 9</td>
</tr>
<tr>
<td>YT445H hns::Tn5</td>
<td>59 ± 9</td>
</tr>
<tr>
<td>RFM475</td>
<td>0-25 ± 0-09</td>
</tr>
<tr>
<td>YT475H hns::Tn5</td>
<td>44 ± 5</td>
</tr>
</tbody>
</table>

genies (Yoshida et al., 1993a, b; De Biase et al., 1999; Hommais et al., 2001; Tramonti et al., 2002; Ma et al., 2002; Waterman & Small, 2003). The hns::Tn5 mutation was introduced into RFM445 and RFM475. The AR of the resulting strains YT445H and YT475H was compared after using conditions previously shown to demonstrate the increase in AR from hns mutation (Hommais et al., 2001).

The effect of the topA mutation in RFM475 versus RFM445 under these experimental conditions was > 100 fold reduction in AR (Table 3). There was a modest increase in AR when the hns mutation was introduced into RFM445. The increase in AR from the hns mutation was much greater in RFM475, restoring it close to the level observed for RFM445. The increase in AR in the hns mutants correlates with the increase in expression of GadA and GadB proteins, as analysed by Western blotting (Fig. 6b).

**Effect of gyrB mutations and gyrase inhibition on AR**

The topA+ strain RFM445 contains gyrase mutations necessary for the survival of RFM475 and YT481. The AR of the gyr+ strain RFM443 was measured to determine if the gyrB221(Tou) and gyrB203(Ts) mutations in RFM445 had any effect. Overnight growth of RFM443 was also carried out in the presence of sublethal concentrations of novobiocin to determine if inhibition of gyrase activity influenced AR. Besides the conditions of adaptation in the pH 5-5 M9 medium used previously to test the effect of the hns mutation (Hommais et al., 2001), the RpoS-suppressed condition (overnight growth in LBG), with the relatively lower survival rates for the topA+ strains, was also tested, so that any positive effect from the reduced gyrase activity could potentially be detected. The results (Table 4) showed that the gyrB mutations in RFM445 resulted in a small increase (about twofold) of the RpoS-independent and glutamate-dependent AR after overnight growth in LBG. This could be due to the moderate level of reduction of gyrase activity in RFM445, compared to topoisomerase I activity being missing totally in the topA mutants. However, the gyrase mutations had the opposite effect when AR was measured after overnight growth in M9 medium at pH 5-5. The presence of novobiocin at 15 and 30 µg ml⁻¹ inhibited overnight growth of RFM443 by about tenfold (data not shown). There was a small decrease in AR after adaptation in LBG (about 2-3-fold), and a more significant decrease in AR after adaptation in pH 5-5 M9 medium (up to tenfold).

**DISCUSSION**

Glutamate decarboxylase activity plays an important role in the AR of E. coli. Data presented here show that a topA mutation resulted in significantly lower transcription of gadA and gadBC, accounting at least in part for the decreased AR of the topA mutants. Unlike other growth defects of topA mutants, including increased sensitivity to high temperature and oxidative stress, the reduced AR could not be rescued by the overexpression of RNase H. Therefore, it is unlikely for R-loop accumulation during transcription elongation to be the cause of the reduced AR, even though it could conceivably be argued that degradation of the mRNA required for AR by the overexpressed RNase H led to the low level of survival.

Expression of gadA and gadBC in stationary phase has been shown to be largely dependent on RpoS (Castanie-Cornet et al., 1999; De Biase et al., 1999). This is mediated by the RpoS-dependent transcription of GadX, an activator of gadA and gadBC (Ma et al., 2002). Our results showed that the decreased survival of the topA mutants under the RpoS-dependent mechanism of AR was not due to a reduction in the level of RpoS or GadX protein. The loss of topoisomerase I function could have affected the

**Table 4.** Effect of the gyrB mutations in RFM445 and inhibition of gyrase activity in RFM443 by novobiocin on AR

Each experiment was carried out four times. Means ± SD are shown.

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>RFM445</th>
<th>RFM443</th>
<th>RFM443+15 µg novobiocin ml⁻¹</th>
<th>RFM443+30 µg novobiocin ml⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overnight in M9 pH 5-5, challenged for 2 h in M9 pH 2-5+glutamate</td>
<td>47 ± 9</td>
<td>90 ± 10</td>
<td>25 ± 6</td>
<td>8 ± 0 ± 10</td>
</tr>
<tr>
<td>Overnight in LBG, challenged for 2 h in EG pH 2-5+glutamate</td>
<td>8 ± 2 ± 3-2</td>
<td>4 ± 4 ± 2-2</td>
<td>2-6 ± 1-4</td>
<td>1-4 ± 0-6</td>
</tr>
</tbody>
</table>
transcription of other RpoS target promoters directly, since a high level of negative supercoiling has been reported to inhibit transcription by RpoS (Bordes et al., 2003), and localized hypernegative supercoiling might be present in the topA mutants. It was found that the gyrase inhibitor novobiocin induced the RpoS-directed transcription initiation from the osmEp gene without affecting the level of RpoS accumulation (Bordes et al., 2003). However, in this study the introduction of gyrase mutations or the addition of novobiocin to RFM443 resulted in only small changes in AR, and not always in the opposite direction to topA mutation. In addition, the data from the rpoS mutants indicated that topoisomerase I function was also involved in an RpoS-independent mechanism of AR.

The nucleoid protein H-NS has been shown to act as a negative regulator on E. coli genes required for AR (Yoshida et al., 1993a, b; Hommais et al., 2001). The repression of gene expression by H-NS may be due to its effect on DNA supercoiling (Tupper et al., 1994) or its binding to specific curved DNA sites in the promoter regions of these genes. The large effect of the hns mutation on strain RFM475 relative to RFM445 suggested that topoisomerase I function may be required to overcome the repression of AR genes by H-NS. It has been shown that H-NS can repress gadA and gadBC expression independent of GadX (Ma et al., 2002). The effect of hns mutation on gadA and gadBC transcription remained highly significant even in an rpoS mutant background (Waterman & Small, 2003), so this might account for the observation that the topA effect could still be seen in the rpoS mutants studied here. Topoisomerase I function may be needed to counteract the H-NS repression of gadA and gadBC expression. The role of topoisomerase I at such loci is not simply due to its relaxation activity, since the addition of novobiocin resulted in a decrease of AR.

A previous study with Salmonella typhimurium established that DNA topology can affect the environmental regulation of the pH-regulated locus antiG (Karem & Foster, 1993). The expression of antiG in mannose media is decreased in a ΔtopA mutant, but increased in two hns mutants at pH 6-0–7-0 (Karem & Foster, 1993). This is also in agreement with a possible role for topoisomerase I in relieving repression of antiG by H-NS. Many other bacterial genes, including some of the genes involved in virulence and osmotic control, are also known to be regulated by H-NS (Hommais et al., 2001; Madrid et al., 2002). The virF gene of Shigella required for invasion functions is suppressed by interaction of H-NS with its promoter, and its thermal induction was found to be inhibited in a topA mutant (Ni Bhrain & Dorman, 1993). This is also similar to the effect of topA on the induction of gad expression during stationary phase observed here. The effective DNA superhelicity at any gene promoter region is a result of the local dynamic competition between structural constraining proteins, including H-NS, RNA polymerase and the topoisomerasers (Travers & Muskhelishvili, 2005). This dynamic competition changes during growth-phase transition and adaptive response to challenge. Topoisomerase I function might have a direct role in relieving repression of H-NS at specific gene loci, but it also cannot be ruled out that topoisomerase I may be counteracting the general effect of H-NS on DNA topology in other cases (Mojica & Higgins, 1997). In a study of the E. coli protein–protein interaction network, topoisomerase I was found to interact with H-NS (Butland et al., 2005), suggesting a functional link between the two proteins. We have demonstrated previously that E. coli topoisomerase I function was required for R-loop suppression in the transcription elongation of stress genes during adaptation to high temperature and oxidative stress (Cheng et al., 2003). In this study, it was found that E. coli topoisomerase I played a different role in gene expression for AR that could involve the influence of DNA topology on the action of the positive and negative regulators of transcription. This further supports the importance of type IA topoisomerase function during environmental adaptation and its conservation in evolution (Wang, 2002). Furthermore, inhibitors of bacterial topoisomerase I activity may be useful for general suppression of adaptation of pathogenic bacteria to host environments.

ACKNOWLEDGEMENTS

This work was supported by National Institutes of Health award R01-GM54226 (to Y.T.) and R01-GM61147 (to J.W.F.). Technical assistance was provided by Kenneth Montini, Shikha Shukla and Molly Boyd.

REFERENCES


