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TOPOISOMERASE FUNCTION DURING BACTERIAL RESPONSES TO ENVIRONMENTAL CHALLENGE

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1. ABSTRACT

Every living organism needs to have multiple defense mechanisms against environmental challenge for survival during evolution. Rapid changes in gene expression have been demonstrated during bacterial adaptation to environmental stress. Since DNA supercoiling varies in response to environment, and since supercoiling is known to influence strand separation in DNA, and therefore many vital cellular processes, including transcription, supercoiling could potentially serve as a molecular mechanism through which expression of many genes could be influenced by the environment.

The relationship between changes in DNA topology and a number of environmental stress conditions including high temperature, oxidative stress and extreme pH, is reviewed here. The activities of DNA gyrase and topoisomerase I are key components in determining the degree of bacterial DNA supercoiling. The specific involvement of topoisomerase function in bacterial adaptation to these environmental stress conditions, as demonstrated by the effect of mutations or inhibitors on adaptation and survival for these potentially lethal conditions, is also reviewed.

2. INTRODUCTION

DNA topoisomerases are enzymes that can alter the topological state of DNA and thus play an important role in replication, transcription, recombination and DNA repair (1-3). DNA in bacteria is maintained in an underwound or negatively supercoiled state. Changes in DNA supercoiling introduced via topoisomerase mutations or directly influence transcription and the expression of a large number of genes (4-7). The key enzymes in determining the degree of global DNA supercoiling in bacteria are DNA gyrase and topoisomerase I, with relatively minor contribution also from topoisomerase IV (8).

DNA gyrase, encoded by *gyrA* and *gyrB*, is an ATP-dependent tetrameric enzyme capable of converting relaxed DNA into negatively supercoiled DNA with energy input from ATP hydrolysis (9). Loss of gyrase activity in bacteria results in a lethal phenotype (10). Gyrase is sensitive to quinolone drugs, which trap a covalent enzyme-DNA complex formed with two A subunits of the enzyme (11-12). Coumermycin and novobiocin inhibit the ATPase activity (13) of gyrase and lead to diminished negative supercoiling of DNA.

Topoisomerase I, encoded by *topA*, is a single polypeptide enzyme that relaxes negatively supercoiled DNA in an ATP-independent mechanism (14). Mutants of *E. coli* with complete loss of topoisomerase I activity can only be isolated in the presence of compensatory mutations which usually map in the *gyrA* or *gyrB* gene so these mutations lower gyrase activity, but they can also be associated with amplification of other genetic loci (15-18). Partial loss of topoisomerase I activity results in increased negative supercoiling of DNA (15,19,20).

It is well established that bacterial promoter function *in vivo* and *in vitro* can be strongly influenced by the level of supercoiling present in the DNA template (4-7,

Figure 1. The 5' control region of the *E. coli topA* gene. The sequences of previously identified promoters P1, P2, P3, P4, and Px1 (29,37) are illustrated, as well as binding sites for IHF and FIS proteins (29,41). The starting ATG is shown in bold.

21-23). This can be at the level of transcription initiation. Although the melting of the promoter sequence is generally favored by negative supercoiling of DNA, promoters of different sequences respond to change in DNA supercoiling with a different response ratio and threshold. For example, certain promoters are strongly affected by change of supercoiling brought on by the presence of *topA* mutation or by gyrase inhibitors, while other promoters appear to be unaffected by the same treatment (4-7). The level of transcription initiation of a gene that responds to a stress condition may be sensitive to the level of supercoiling around the promoter region.

DNA supercoiling and topoisomerase functions also play important roles in transcription elongation. During transcription, positive supercoils are generated ahead of the RNA polymerase complex while negative supercoils are accumulated behind the transcribing RNA polymerase (24). Gyrase is required to remove the positive supercoils and topoisomerase I is needed to relax the negative supercoils. An absence of topoisomerase I results in accumulation of hypernegative supercoils (25) and the formation of R-loops involving the nascent transcript and the DNA template strand (26,27). The R-loops would act as road blocks for a second RNA polymerase complex that follows, preventing further transcription elongation and synthesis of the gene product. The removal of transcription-driven supercoiling by elevated topoisomerase action may be especially important during stress response because expression of a large number of genes must be induced rapidly to ensure optimal survival of the organism.

3. REGULATION OF TOPOISOMERASE EXPRESSION

3.1. Regulation by change in DNA supercoiling

The regulation of both DNA gyrase and topoisomerase I by DNA supercoiling contribute to the homeostatic regulation of supercoiling of DNA in *E. coli. In vitro* transcription of plasmid-borne *topA* promoter fusions is much more efficient with negatively supercoiled DNA templates than relaxed DNA templates (28,29). However, transcription of chromosomal *topA* is relatively insensitive to fluctuations within the wild-type levels of DNA supercoiling (30). Conversely, transcription of *gyrA* and *gyrB* is stimulated by relaxation of DNA template (31,32), and it is more subject to regulation by wild-type levels of DNA supercoiling (30). The genomic response of *Haemophilus influenzae* to the gyrase inhibitor novobiocin was analyzed by microarrays, with gene expression changes resulting from change in DNA supercoiling (7). Transcription of *gyrA*, *gyrB* in this organism was also found to be upregulated while transcription of *topA* was found to be downregulated, as expected for the regulation of these topoisomerase genes by DNA supercoiling in the homeostatic control mechanism (7). The activation of gyrase synthesis by DNA relaxation was also observed in *Streptomyces sphaeroides* (33), *Mycobacterium smegmatis* (34) and Mycobacterium tuberculosis (35).

3.2. Regulation in response to environmental changes

Transcription of chromosomal *topA* in *E. coli* is directed by four promoters (Figure 1). Promoters P2 and P4 appear to be recognized by sigma-70, the major sigma factor of *E. coli* RNA polymerase. Promoter P1 is recognized by sigma-32, the sigma factor involved in heat-shock response (36); promoter Px1 is directed by sigma-s, which is active during stationary phase growth and a variety of stress conditions (37). Although the promoter P3 nucleotide sequence resembles the consensus sigma-70 recognition sequence, its utilization for *topA* transcription initiation in the chromosome has not been observed (37).

The total level of *topA* expression is thus governed by the sum of activities from these multiple promoters. In the transition from exponential to stationary phase growth, activity from promoter Px1 increases while activities from the adjacent P4 decreases, thus keeping the level of total topoisomerase I protein at a relatively constant level (37). A similar observation has been made during heat shock (38). Transcription from promoter P1 increases after a temperature shift to 42^{o}C, while activity from the other *topA* promoters decreases. Thus topoisomerase I initially did not appear to be a heat shock protein when total *E. coli* protein was analyzed by western blot technology (36). However, *topA* transcript was seen to be upregulated when *E. coli* genomic expression during heat shock was analyzed by microarray methods (39).

Transcription of *topA* in *E. coli* is also influenced by oxidative stress. Upon treatment of exponential phase *E. coli* with hydrogen peroxide or N-ethylmaleimide, transcription of *topA* from promoter P1 increases significantly (40,41). Treatment of stationary phase cells with N-ethylmaleimide also results in promoter P1 induction, while transcription from the other promoters

decreases (40). However, when stationary phase *E. coli* is treated with hydrogen peroxide, *topA* transcription increased at promoter P2 instead of P1 (40). This switch may be due to the requirement of FIS for induction of *topA* P1 promoter in response to hydrogen peroxide (41); the level of FIS protein in *E. coli* is high in exponential phase and low in stationary phase (42,43). Binding sites for FIS have been found in the 5' regulatory region of *E. coli topA* gene near promoter P1 (41). Induction of P1 by heat shock is however, FIS independent (41). Accumulation of denatured proteins from oxidative stress could also play a role in activation of sigma-32 and thereby activate P1 (40).

In *Helicobacter pylori*, an organism that is repeatedly exposed to low pH, *topA* was found by differential display PCR to be one of the genes induced by prolonged acid exposure (44). The mechanism of this regulation is not presently known.

The A subunit of DNA gyrase was identified as a cold shock protein. When exponential phase *E. coli* is shifted from 37^{o}C to 10^{o}C, GyrA synthesis increases relative to that of total protein following a lag period (45). The duration of the lag and the rate of synthesis depend on the level of the major cold shock protein CS7.4 (CspA). CspA may act by causing anti-termination of transcription or stabilization of the *gyrA* transcript (46,47).

The expression level of a *gyrA-lacZ* fusion was found to increase at the mid-exponential phase and to reach its peak at late exponential phase due to accumulation of cAMP and increased CRP (48). Transcription initiation of both *gyrA* and *gyrB* can also be inhibited by FIS protein as part of a complex network of homeostatic regulation of supercoiling (49). The effects of FIS and other bacterial nucleoid-associated proteins, including IHF, HU and HNS on the expression levels and activities of both gyrase and topoisomerase I can thus be important factors affecting DNA supercoiling during the response to environmental changes.

4. EFFECT OF STRESS CONDITIONS ON DNA SUPERCOILING

4.1. DNA supercoiling changes transiently during thermal stress

DNA becomes more negatively supercoiled in *E. coli* 10-15 min after a shift to higher temperature (50). However, during sudden heat shock from treatment at 42^{o}C, a transient relaxation (Linking number Lk increase) of plasmid DNA can be seen 2 min after raising the temperature (51). Experiments utilizing reporter plasmids in *Salmonella typhimuriun*, *Bacillus subtilis* and *Yersinia enterocolitica* also show that high temperature induces changes in DNA supercoiling *in vivo* (52-54). The mechanism of DNA relaxation observed in *E. coli* has been studied in detail, with both topoisomerase I and DNA gyrase activities found to be involved based on results from study with *topA* mutant and use of gyrase inhibitors (55). At later time points after the shift to 42^{o}C, the heat-shock protein DnaK stimulates the supercoiling activity of DNA gyrase, resulting in re-supercoiling of DNA (56). The histone-like protein HU is also required for the re-supercoiling of DNA, as demonstrated by the effect of mutations occurring in the genes encoding HU (57). HU probably also acts by facilitating gyrase activity (58). In contrast to the effect of heat shock, cold shock induces a Lk decrease in *E. coli* (59). This increase in negative supercoiling is also transient. DNA gyrase and HU protein are involved in this transient change in DNA supercoiling in response to cold shock (59). Studies with hyperthermophilic archaea also uncovered the same trend, with Lk increasing and decreasing during heat shock and cold shock respectively (60, 61).

The activity of some bacterial promoters regulated by temperature change is known to correlate with the degree of DNA supercoiling, suggesting that supercoiling may be utilized to link temperature changes and gene expression (55,62). However, besides DNA supercoiling, changes in growth temperature can also affect DNA conformation via other mechanisms (63). Virulence gene expression in *Yersinia enterocolitica* and *Shigella flexneri* has been shown to be regulated by temperature dependent DNA bends (64,65) and binding of H-NS protein can play an important role (65).

4.2. DNA supercoiling changes during oxidative stress response

Transient relaxation of reporter plasmid DNA can be detected as early as 2 min after treatment of *E. coli* with hydrogen peroxide, and re-supercoiling is completed 30 min later (41). It was proposed that FIS-dependent induction of the P1 promoter of *topA* accounts for this transient relaxation. When the OxyR-dependent response to hydrogen peroxide is compromised by an *oxyR* or *katG* mutation , the re-supercoiling of DNA is delayed (41). Mutations in other DNA repair genes also affect the kinetics of DNA relaxation and re-supercoiling. This shift in DNA topology may have a functional role in one of the several pathways of bacterial response to oxidative stress.

In an earlier study, relaxation of plasmid DNA was observed when *E. coli* was subject to a number of different oxidative stress conditions (66). These more severe conditions include visible light irradiation in the presence of photosensitizing dye and molecular oxygen, as well as treatment with hydroperoxides such as ter-butyl hydroperoxide, cumene hydroperoxide and hydrogen peroxide. The time course of change in DNA supercoiling was not followed. Thus it is not clear whether the change is transient. Plasmid DNA relaxation was also observed when the *topA* null strain DM800 was treated with photosensitizing dye plus light or tert-butyl hydroperoxide. Consequently, topoisomerase I is apparently not required for the DNA relaxation observed for these two oxidative stress conditions tested (66). The DNA relaxation under these more severe oxidative stress conditions may be due to depletion of cellular ATP, lowering the gyrase supercoiling activity.

4.3. Effect of pH on DNA supercoiling

Plasmid DNA extracted from *E. coli* and *S. typhimurium* grown in acidic conditions (pH 5 –6) has

reduced level of negative supercoiling compared to DNA obtained from cells grown in higher pH (pH 7 – 8) (67). This change in DNA supercoiling can alter pH-regulated expression as demonstrated by a *S. typhimurium* mutant with *topA* deletion mutation which causes a 31% increase in negative supercoiling versus wild type and diminished the pH-regulated *aniG* promoter activity at pH 6 (67). The addition of the gyrase inhibitor novobiocin also affected pH regulated gene expression by decreasing negative supercoiling (67,68). As in the case of gene regulation by osmolarity (69), the H-NS protein also plays a prominent role in pH-regulated gene expression in *E. coli* and *S. typhimurium* (68,70,71). The proteins synthesized in the acid tolerance response to the non-lethal acid stress conditions (pH not lower than 4) allow *E. coli* and *S. typhimurium* to be more resistant to subsequent challenge for survival in more lethal pH conditions (pH 3 or lower) (72).

5. ROLE OF TOPOISOMERASE FUNCTION IN SURVIVAL DURING ENVIRONMENTAL CHALLENGES

5.1. Effect of *topA* mutations

The *E. coli* strain RFM475 with deletion of the *topAcysB* grows reasonably well at 37°C in rich medium when compared to an isogenic strain RFM445 (26) that lacks the *topAcysB* deletion but also contains the *gyrB221*(cou^R)*gyrB203*(Ts) compensatory mutations necessary for viability of RFM475. The colonies formed by RFM475 are smaller in size than the RFM445 colonies, and there is increased lag time for RFM475 compared to RFM445 before exponential growth begins (26). However, during exponential growth, the doubling time of RFM475 is about the same as RFM445 and there is no difference in the viable counts between the two strains after overnight growth at 37°C in rich medium. Therefore, under standard laboratory conditions at 37°C, the compensatory gyrase mutations in found in both RFM445 and RFM475 can alleviate problems in global or local supercoiling that may arise due to the lack of topoisomerase I function.

The lack of topoisomerase I function, however, becomes a great liability for survival when *E. coli* is challenged with extreme environmental conditions. Deletion of *topA* gene in *E. coli* strains RFM475 and GP203, two different genetic backgrounds having different compensatory gyrase mutations resulted in 10-100 fold higher rate of killing by the high temperature challenge of 52°C (73). These *topA* mutant strains are also more sensitive than their *topA*$^+$ counterparts to oxidative challenge by hydrogen peroxide and to the toxic electrophile N-ethyl maleimide by 10 to >1000 fold, depending on the treatment conditions (40). Results from recent experiments in our laboratory have demonstrated that loss of topoisomerase I function affects both the RpoS-mediated and the glutamate decarboxylase (*gad*) systems of acid resistance of *E. coli* (72,74), resulting in >100 fold lower percent of survival upon challenge with pH of 2.5 or less.

The need for topoisomerase I function under different growth conditions is suggested by the presence of multiple *topA* promoters recognized by the sigma factors required for heat shock and general stress response (Figure 1). The importance of the ability to synthesize *topA* gene product under different conditions was illustrated by experiments (38, 73) with *E. coli* mutants that have either the sigma-32 directed promoter P1 of *topA* deleted (mutant Q1) or with P1 as the sole remaining promoter for *topA* (mutant Q2). Mutant Q1 fails to develop thermotolerance after brief exposure to 42°C prior to challenge at 52°C (38). Thermotolerance is restored if plasmid-borne topoisomerase I, under the control of the *lac* promoter is induced by IPTG (38). Although mutant Q2 has a low level of topoisomerase I protein at 30°C, it can develop thermotolerance and has higher percent of survival than mutant Q1 at 52°C because promoter P1 is induced by exposure to 42°C (73).

Promoter P1 is also induced by hydrogen peroxide treatment during exponential growth phase (40,41). Consistent with the role of FIS in P1 promoter induction by hydrogen peroxide exposure, a *fis* mutant strain was found to be more sensitive to killing by hydrogen peroxide by about 10 fold (41).

5.2. Effect of gyrase mutations and inhibitors

Characterization of mutations in *E. coli* and *S. typhimurium* that allow growth at 48°C revealed mutations in the *gyrA* gene (75-77). The addition of the gyrase inhibitor nalidixic acid to the growth medium after prolonged incubation of *E. coli* and *S. typhimurium* at 48°C in one such study selected for *gyrA* mutants that are in addition to high temperature resistant, nalidixic acid-dependent for growth (75,76). In a second study, a spontaneous nalidixic acid-resistant and thermoresistant *gyrA* mutant of *E. coli* was isolated and found to have approximately 12% less negative DNA supercoiling than the parental strain (77). In contrast, *topA* mutants are more thermosensitive (73), suggest that as temperature increases, the cells may require a higher minimal level of twist in DNA for growth (77). Alternatively, the DNA relaxation caused by the gyrase mutations (78-80) could be increasing the expression level of the heat shock proteins since the treatment of *E. coli* with gyrase inhibitors has been found to enhance the synthesis and stability of sigma32 (80-81).

Since gyrase activity is essential for cell viability under normal laboratory growth conditions, it may be difficult to correlate loss of gyrase activity due to mutation or inhibition with survival of the organism under stress challenges. The *gyrA*(Nal^r) and *gyrB225* mutations were present as compensatory mutations for strain GP203, one of the *topA* deletion mutants found to have reduced survival rate after high temperature and oxidative challenges (40,73). Comparison of the wild-type strain DM4100 with strain GP202 (*topA*$^+$ *gyrA*(Nal^r) *gyrB225*) showed that these two strains are equally susceptible to treatment with hydrogen peroxide and N-ethyl maleimide (40). While these two mutations in the gyrase genes reduce gyrase activity enough to compensate for a *topA* deletion under normal laboratory conditions, this reduction in gyrase activity by itself did not affect the viability during the oxidative challenges.

Gyrase inhibitors and strains with mutations in gyrase genes have been used in many previous studies to correlate gene expression during stress response with DNA supercoiling. The results for example have demonstrated that reduced gyrase activity can have considerable effects on the expression level of certain genes involved in pH adaptation (67,68,70) and aerobic-anaerobic transition (83-86). However, it is not clear from these results if the gyrase mutation or inhibition has any significant effect on the survival rates of the organism under stress challenges in a mechanism that may be different from that expected from the lethal effect of gyrase inhibition on DNA replication.

6. CONCLUDING REMARKS

Several possible mechanisms have been found through which topoisomerase function can affect gene expression during stress responses. Topoisomerase I may be needed to prevent hypernegative supercoiling and formation of R-loops during transcription elongation at the stress response gene loci, similar to what is observed for the transcription of the *rrnB* operon of *E. coli* (87). RNAse H overproduction in the *topA* mutants corrects defects in transcription elongation during rRNA synthesis (88) and partially alleviates the effect of a *topA* null mutation on survival upon challenge of the *topA* deletion mutant RFM475 with high temperature or NEM treatment (unpublished results from our laboratory), indicating that transcription-driven local supercoiling and R-loop formation can account for at least part of the effect of the *topA* under those stress conditions.

At the level of transcription initiation, DNA supercoiling not only affects the energy required for the separation of the DNA strands, but it also affects the twist of the promoter nearby regulatory sequence (89,90). DNA twist will influence not only the interaction between the promoter and RNA polymerase, but can also modulate the binding affinity of regulatory proteins. If DNA bending, looping or writhe are involved in the regulation of transcription initiation, these structural features of DNA will also be altered by change in DNA supercoiling (90-92). Different promoters are therefore affected differently by a given degree of change in DNA supercoiling depending on their sequence composition, strength and interaction with transcription factors or nucleoid-associated proteins (4-7). The overall pattern of gene expression under a given set of environmental conditions is expected to change significantly when global supercoiling is perturbed by topoisomerase mutation or gyrase inhibition. The ability of the organism to survive stress may be compromised by this change in gene expression pattern. It has been demonstrated that when sigma-32 induction is mediated by IPTG induction of a *lac* promoter fused to the gene instead of by the natural means of shifting to higher temperature, the pattern of heat shock protein induction is altered and thermotolerance is lost (93). The overall pattern of heat shock protein induction is also altered by the deletion of the *topA* P1 promoter in strain Q1, with some heat shock proteins expressed at a lower level, and others at a higher level compared to the parent strain (38), and this perturbation of heat shock protein induction pattern could also account partially for the loss of thermotolerance observed for this mutant.

The potential presence of DNA bends or other unusual DNA structures in the regulatory regions of stress responsive gene, and the demonstrated roles of many nucleoid-associated proteins in stress response combine to add to the complexity of the picture. While it is easy to demonstrate that DNA supercoiling and topoisomerase function can alter stress response and survival, detailed analysis is needed to elucidate the molecular interactions being affected.

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