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Regulation of bacterial DNA supercoiling: Plasmid linking numbers vary with growth temperature

(DNA topology/gel electrophoresis)

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ABSTRACT **The level of DNA supercoiling can be altered either by breaking–rejoining reactions that change the DNA linking number or by environmental changes that alter the helical pitch of DNA. *In vitro*, temperature changes alter helical pitch and, thus, supercoiling. We find that plasmids isolated from bacteria grown at different temperatures exhibit differences in DNA linking numbers. The differences in plasmid linking numbers offset the effect temperature is expected to have on supercoiling. These results are consistent with the hypothesis that fine control of DNA topology in bacterial cells is brought about by changes in linking number to maintain a constant value for supercoiling.**

Shortly after Cairns (1) first showed that the bacterial chromosome is a circular DNA (cc DNA) molecule, Vinograd *et al.* (2) found that cc DNA molecules extracted from natural sources are more compact than their linear or nicked counterparts. The term supercoiling was introduced to signify this compaction. Supercoiling is generally described in terms of relaxed DNA, a cc DNA created by ligation of a nicked circle; supercoiling represents a state of relatively greater free energy (ΔG) in a cc DNA and arises when a cc DNA has either an excess or a deficiency of duplex turns relative to the appropriate relaxed DNA. By introducing a topological parameter called linking number (the number of times one strand in a duplex DNA molecule crosses over the other when the DNA is conceptually constrained to lie on a plane), supercoiling can be described by the relationship

$$\tau = L - L_0, \qquad [1]$$

where τ equals the number of titratable supercoils in a cc DNA molecule, L equals the linking number of the particular cc DNA in question, and L_0 equals the linking number of a comparable relaxed species (for discussion, see refs. 3 and 4).

For DNA having free ends that can rotate, the number of primary helical turns per unit length (and, therefore, per DNA molecule) is a function of environmental parameters such as temperature, ionic strength, and concentration of certain DNA-binding ligands. Consequently, relaxed topoisomers formed under different environmental conditions will have different numbers of duplex turns; the value of L_0 in Eq. 1 will vary with environmental conditions. However, no strand rotation can occur in a cc DNA molecule, so values of L are unaffected by environmental changes. Instead, they may be changed by transient strand breakage catalyzed by DNA topoisomerases (for review, see refs. 3 and 5). Thus, supercoiling can be altered either by topoisomerase activity or by environmental changes

DNA inside bacterial cells appears to be under negative superhelical tension (6, 7), a condition that arises when $L <$ L_0 (Eq. 1). Supercoiling is associated with an increase in free energy (supercoiling is lost spontaneously if a supercoiled DNA molecule is nicked or broken), and it is thought that processes involving DNA strand separation are energetically favored in negatively supercoiled DNA relative to relaxed or linear DNA. In a sense, negative supercoiling has been considered to be a means of activating DNA molecules (for discussion, see ref. 3). One manifestation of this activated state may involve the control of gene expression: changes in gene expression have been observed when levels of supercoiling have been altered *in vitro* or *in vivo* (for review, see refs. 3, 5, and 8).

In vivo, it is likely that DNA supercoiling is regulated, at least in part, by topoisomerases. Studies using antibiotic inhibitors of DNA gyrase (9–14) and mutations in genes encoding gyrase and DNA topoisomerase I (15–19) show that changes in the activities of these enzymes alter titratable supercoiling when measured *in vitro*, which presumably reflects changes in superhelical tension *in vivo*. However, these inactivation experiments have provided little information about fine control of supercoiling during normal growth. Ideally, we would like to explore fine control of supercoiling by administering a topological perturbation to cellular DNA followed by an examination of DNA linking numbers to measure the extent of topoisomerase-mediated responses. We have approximated this approach by varying growth temperature. Helical pitch of unconstrained DNA, and, therefore, L_0 for a given circular DNA, varies with temperature in a well-defined manner, increasing with decreasing temperature (20, 21). Since $\tau = L - L_0$ for cc DNA, a change in temperature (i.e., L_0) in the absence of changes in linking number (L) would be accompanied by a change in τ, assuming temperature to be the only topologically significant change in the intracellular environment when growth temperature is varied. To measure the cellular response to changes in τ, we have compared linking number (L) of plasmids extracted from cells grown at different temperatures. If (*i*) supercoiling is a regulated parameter when growth temperature is varied and (*ii*) this regulation is accomplished by changes in linking number, then plasmid DNA linking number should vary with growth temperature in a predicted way. Specifically, linking numbers should be higher at lower temperatures to compensate for the increased supercoiling that would otherwise result from the effect of temperature on helical pitch. The data presented below show this to be the case, supporting the hypothesis that topoisomerases are involved in finely controlling the levels of supercoiling in bacterial cells.

MATERIALS AND METHODS

Growth of Bacterial Strains. All strains were derivatives of *Escherichia coli* K-12. DM4100 (22) was received from R. Sternglanz, LE234 (23) was obtained from B. Bridges,

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Abbreviation: cc DNA, closed circular DNA.

LL187 (Δ*pro-lac*/*F'pro lacI*^{Q}*Z*$^{\Delta M15}$*Y*$^{+}$) was from B. Muller-Hill via L. Lindahl, and LL187-14 (*LL187recA*$^{-}$) was constructed in this laboratory by P1-mediated transduction from strain JC10240 obtained from A. J. Clark. All strains were transformed with plasmids according to the methods of Maniatis *et al.* (24). Plasmids pBR322 and pBR313 were obtained from L. Lindahl. Except where indicated, growth medium was M9 (25) supplemented with thiamine (1 μg/ml)/0.5% Casamino acids/cysteine (40 μg/ml)/0.2% glucose (wt/vol)/tetracycline (15 μg/ml). Bacterial growth was monitored by turbidity using a Klett–Summerson colorimeter. Temperature-shift experiments were done on cells in logarithmic-phase growth at a cell density of ≈1 × 10^{8} cells per ml. In these experiments, new thermal equilibria were reached within 2 min after the temperature shift.

Plasmid Purification. Bacterial cells were chilled by placing culture flasks in an ice-water bath, and they were concentrated by centrifugation at 4°C. The cells were lysed according to the method of Holmes and Quigley (26) with slight modifications. Cell lysates were centrifuged for 1–2 hr at 4°C to remove chromosomal material; plasmid DNA was precipitated from the supernatant fluid by addition of ammonium acetate to 3.75 M and cold ethanol to 70%, followed by 15 min at −70°C. Precipitates were washed with cold 70% ethanol, dried by vacuum dessication, resuspended in 10 mM Tris·HCl, pH 8/1 mM Na_2EDTA, and stored at −20°C.

Plasmid preparations were not routinely extracted with phenol. Control experiments showed that plasmid preparations extracted with phenol or heated to 95°C for 1 min exhibited the same mobility during electrophoresis as did plasmids prepared by the standard procedure.

Chloroquine/Agarose Gel Electrophoresis. Plasmid preparations were subjected to horizontal slab agarose gel electrophoresis using an Aquebogue (Aquebogue Machine Shop, Aquebogue, NY) submarine apparatus. Agarose concentrations were 0.8% (pBR313) or 1% (pBR322 and pUC9 dimers). Running buffer consisted of 45 mM Tris·PO_4, pH 7.15–7.2/0.87 mM Na_2EDTA/chloroquine phosphate (10–12 μg/ml) (Sigma). Electrophoresis was at room temperature with a voltage gradient of 2–3 V/cm applied for 15–22 hr. Running buffer was recirculated through a cooling coil immersed in H_2O at room temperature.

Staining, Photography, and Densitometric Tracing. After electrophoresis was completed, the gels were stained in 2.5 μg of ethidium bromide per ml until the plasmid bands were faintly visible. The gels were then exposed to long wavelength UV irradiation for 30 sec to introduce nicks into the cc DNAs (27). After rinsing in distilled H_2O, the gels were restained for 1–3 hr in ethidium bromide, destained in distilled H_2O or 1 mM Mg_2SO_4 (to decrease background), and photographed under UV illumination using Polaroid 667 black-and-white film. Densitometric scans of the banding patterns were made from photographic negatives using an LKB Zeineh scanning densitometer.

Occasionally, a series of plasmid DNA bands in one lane of a gel did not align perfectly with bands in an adjacent lane. This phenomenon has been described previously and has been attributed to overlap in the migration of neighboring topoisomers (28). This skewing of the topoisomer distribution does not significantly affect our estimates of differences in average linking number among the plasmid populations examined.

RESULTS

Plasmid Linking Number Varies with Growth Temperature. To determine whether linking number varies with growth temperature, we extracted pBR322 from cells growing at 17°C or at 37°C and examined the distribution of plasmid topoisomers by agarose gel electrophoresis. When appropriate concentrations of chloroquine are present during electrophoresis, topoisomers can be electrophoretically separated, and they become visible as a series of bands (Fig. 1*A*). The linking number (*L*) in each member of the series of bands differs from that of an adjacent member by one (20, 21). As a group, plasmids isolated from strain DM4100 grown at 37°C migrate more rapidly than plasmids isolated from cells grown at 17°C (Fig. 1). At these chloroquine concentrations, all plasmids are negatively supercoiled; those migrating more rapidly are more highly supercoiled and, therefore, have lower linking numbers. Plasmids from cells grown at 17°C have on average linking numbers two greater than plasmids from cells grown at 37°C (Fig. 1).

Since linking numbers can be changed only by transient strand breakage, it is likely that the increase observed in plasmids from cells grown at 17°C relative to 37°C arises from topoisomerase activity. The linking number change of two roughly offsets the effect temperature is expected to have on supercoiling (see *Discussion*).

DNA winding due to decreased temperature has been shown to be about 0.012 helical degrees/base pair·°C (20, 21); thus, we expect the effect of temperature on linking number to (*i*) be proportional to the number of base pairs in the DNA, and (*ii*) exhibit intermediate values at intermediate temperatures. A larger plasmid should show a proportionately greater change in linking number. Plasmid pBR313, from which pBR322 was derived (29), is approximately twice the length of pBR322 (29, 30). When isolated from cells grown at 17°C, the most abundant topoisomer for pBR313 has a linking number five higher than that observed in pBR313 isolated from cells grown at 37°C (Fig. 2 *A* and *B*). Furthermore, as shown in Fig. 2*C*, an intermediate temperature (30°C) leads to an intermediate distribution of plasmid linking number. Thus, the linking change due to temperature is roughly proportional to the size of the plasmid and the growth temperature used.

Since temperature has a pronounced effect on cellular growth rate, we asked whether growth rate *per se* affects DNA linking number. Cells grown at 17°C have a generation time of about 8 hr, compared to 40 min for those grown at 37°C. We approximated this difference in growth rate by substituting acetate for glucose in the growth medium; this change lengthened the generation time to 7 hr at 37°C. Plasmid pBR313 has the same average linking number when cells are grown in acetate-containing medium as when cells are grown in glucose-containing medium (data not shown). Thus, growth rate alone is not responsible for the change in linking number.

The effect of growth temperature on plasmid linking num-

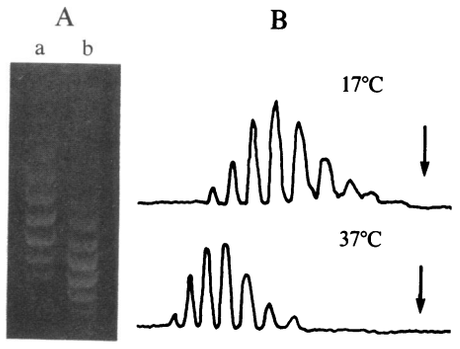

FIG. 1. Effect of growth temperature on plasmid pBR322 linking number. (*A*) Chloroquine/agarose gel electrophoresis on plasmid pBR322 obtained from cultures of DM4100/pBR322 growing exponentially at 17°C (lane a) and 37°C (lane b). Migration is from top to bottom. (*B*) Densitometric tracings of the banding patterns pictured in *A*. Migration is from right to left, and the arrow indicates the position of the relaxed plasmid bands that serve as internal markers.

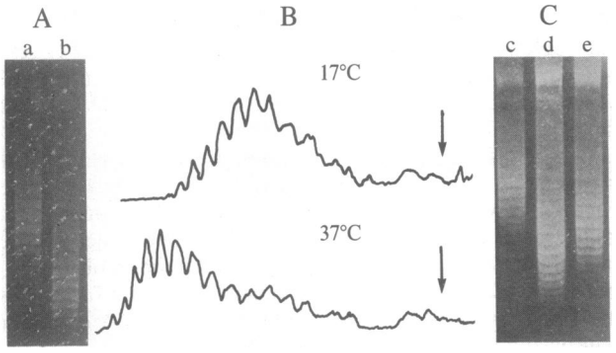

FIG. 2. Effect of growth temperature on plasmid pBR313 linking number. (*A*) Chloroquine/agarose gel electrophoresis of plasmid pBR313 obtained from cultures of DM4100/pBR313 growing exponentially at 17°C (lane a) and 37°C (lane b). Migration is from top to bottom. (*B*) Densitometric tracings of the banding patterns pictured in *A*. Migration is from right to left, and the arrow indicates the position of the relaxed plasmid band. (*C*) Chloroquine/agarose gel electrophoresis of plasmid pBR313 obtained from cultures of DM4100/pBR313 growing exponentially at 17°C (lane c), 43°C (lane d), or 30°C (lane e) as indicated. Migration is from top to bottom.

ber is likely to be a general phenomenon in *E. coli*, for we found results similar to those described above with two other strains of *E. coli* K-12 [LE234/pBR322 and LL187-14/pUC9 (dimers); data not shown]. The phenomenon is also reproducible. In the course of these studies, we carried out 15 experiments, and all gave results similar to those shown in Figs. 1 and 2 *A* and *B*.

Effect of Temperature Shifts on Plasmid Linking Number. Changes in plasmid linking number were measured after shifts in cell growth temperature to determine how rapidly cells bring about the changes in plasmid linking number. Plasmid pBR313 was extracted from strain DM4100 at various times after cultures were shifted from 17°C to 37°C. Between 10 and 30 min after the shift, the distribution of plasmid linking number reaches that of plasmids extracted from cells grown for many generations at 37°C (Fig. 3). About 30 min is required for the cell growth rate to equal that of cells growing at 37°C.

The expected plasmid linking number changes were also observed in the reciprocal experiment in which cells were shifted from 37°C to 17°C. In this case, linking number equilibration required between 1 and 2 hr (Fig. 4). About 1 hr is required for the growth rate to reach that of cells growing at 17°C.

The cell-harvesting procedure used in these experiments includes a downshift (from 37°C or 17°C to 0°C). We expected this extreme downshift to retard enzymatic activities enough to effectively eliminate linking changes that might occur during cell harvest. Nevertheless, we compared linking numbers of plasmids from cells grown at 37°C and harvested at 37°C with those grown at 37°C and chilled within seconds on ice. The topoisomer distributions were the same for the two treatments (data not shown), so it seems likely that the cell-harvesting procedure preserves the plasmid linking numbers present *in vivo*.

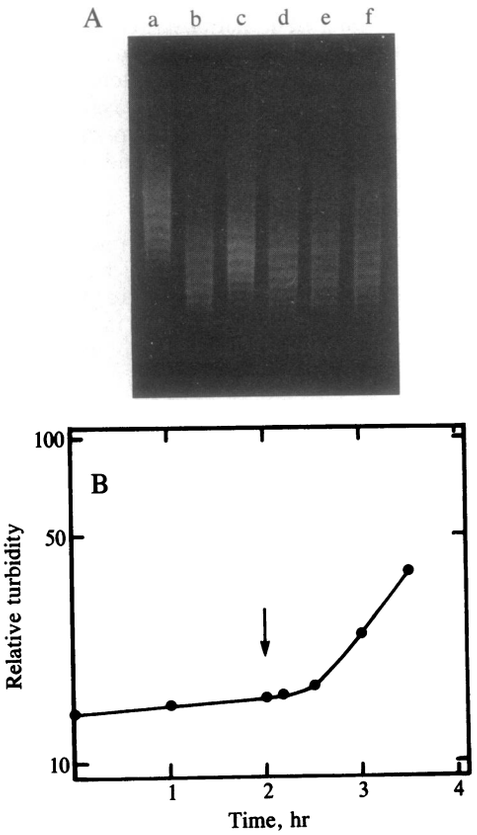

FIG. 3. Kinetics of change in plasmid linking number and cell growth rate after temperature upshift. (*A*) Chloroquine/agarose gel electrophoresis of pBR313 from a culture of DM4100/pBR313 growing exponentially at 17°C (lane a), portions of which were shifted to 37°C for 10 min (lane c), 30 min (lane d), and 60 min (lane e) prior to cell lysis. Lanes b and f represent plasmids obtained from a 37°C control culture growing exponentially. Migration is from top to bottom. (*B*) Growth of DM4100/pBR313, as followed by optical density measurements, before and after shifting temperature from 17°C to 37°C. The arrow indicates the time of temperature shift.

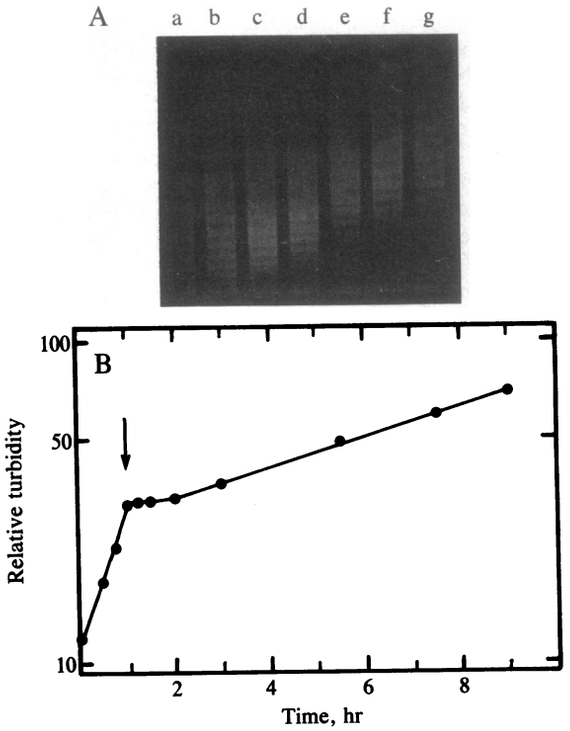

FIG. 4. Kinetics of change in plasmid linking number and cell growth rate after temperature downshift. (*A*) Chloroquine/agarose gel electrophoresis of pBR313 from a culture of DM4100/pBR313 growing exponentially at 37°C (lanes a and b), portions of which were shifted to 17°C for 15 min (lane c), 60 min (lane d), 120 min (lane e), and 180 min (lane f) prior to cell lysis. Lane g represents plasmids from a 17°C control culture growing exponentially and in parallel to the others. Migration is from top to bottom. (*B*) Growth of DM4100/pBR313, as followed by optical density measurements, before and after shifting temperature from 37°C to 17°C. The arrow indicates the time of temperature shift.

DISCUSSION

The effect of growth temperature on DNA supercoiling can be explained by examining changes that occur in the parameters in Eq. 1 ($\tau = L - L_0$). We calculate that growth at 17°C rather than at 37°C will increase L_0 for pBR322 by 2.9 and for pBR313 by 5.8 [using 0.012 helical degrees/base pair per·°C (20)]. Under steady-state conditions, we observe that values of L are higher at 17°C than at 37°C by about 2 for pBR322 and by about 5 for pBR313. Thus, within the accuracy of our measurements, τ is similar at the two temperatures due to changes in L. This result suggests that bacterial cells maintain superhelical tension at a fixed level by small changes in linking number.

The preceding argument rests on the assumption that no other topologically relevant parameter varies significantly *in vivo* over the temperature range examined. While this cannot be rigorously proven to be true, two points support the assumption. First, within the range in which growth rate obeys the Arrhenius equation (about 15°C–40°C) (31), it has been shown that the gross macromolecular constitution of bacterial cells is remarkably constant (32). In particular, patterns of RNA (33) and protein (34, 35) synthesis are similar within this range. However, we cannot rule out the possibility that the temperature effect is complicated by DNA-binding proteins acting in a temperature-dependent manner to effectively alter L_0 in Eq. 1. Second, it is unlikely that topologically significant ionic differences occur. Differences in intracellular cation concentrations on the order of 6- to 10-fold would probably be required to counterbalance the effect of temperature on the pitch of the DNA helix (28). Such a major difference seems unlikely in view of the sensitivity of many enzymatic systems to ionic environment. In support of this, Mg^{2+}, an abundant intracellular ion (36) having strong effects on DNA duplex rotation angle (28), exhibits little change in intracellular concentration when extracellular concentrations are varied widely (37).

The competitive action of DNA gyrase and DNA topoisomerase I probably plays an important role in regulating topological strain: mutations in the gene encoding topoisomerase I (22, 38, 39) lead to higher than normal levels of titratable supercoiling (ref. 17; G. Pruss, personal communication) and mutations in genes encoding gyrase (23, 40–42) lead to lower than normal levels of titratable supercoiling (15, 17, 18). In wild-type cells, the relative activity of each enzyme might be dictated by the superhelical tension of the substrate DNA. During a transition from one temperature to another—i.e., from one value of L_0 to another—titratable supercoiling, $L - L_0$, is expected to shift. Increasing the temperature should decrease L_0 and supercoiling; gyrase is expected to be temporarily favored until titratable supercoiling regains its preshift level. Decreasing the temperature should produce the opposite effect and temporarily favor topoisomerase I. We expect that the appropriate topoisomerase mutations will affect the thermally induced linking changes described in Figs. 3 and 4.

Several mechanisms could be involved in topoisomerase-mediated regulation of superhelical tension. As mentioned above, the balance between the topoisomerase activities may shift as the substrate topology and free energy changes. If so, results similar to those described above should be observed by using topological perturbations that do not use temperature changes. In addition, it is possible that changes in DNA supercoiling affect topoisomerase gene expression and thus alter the relative abundance of the enzymes. Examples have been found where this occurs (43). A less likely possibility is that the temperature coefficient of the enzymes changes in such a way that a constant level of supercoiling is maintained at various growth temperatures. It should be noted that our results do not rule out involvement of non-topoisomerase-mediated strand breakage and rejoining reactions, such as those generated by the combined action of DNase and DNA ligase.

The response of cells to temperature shifts is not immediate. The shift from 17°C to 37°C requires 10–30 min to reach the new steady-state level of linking number (Fig. 3) and the shift from 37°C to 17°C requires 1–2 hr (Fig. 4). Since the expression of certain genes is sensitive to changes in supercoiling (for review, see ref. 8), the transition in DNA topology that we observe may contribute to explanations of transitory changes in synthesis of some proteins associated with temperature shifts (44, 45).

In exponentially growing bacteria, the variation in DNA linking number with growth temperature supports the hypothesis that a specific level of DNA supercoiling is maintained in cells; however, under other physiological conditions the situation may be more complicated. For example, a study with cells in stationary phase indicated that DNA linking numbers are variable from one preparation to another and suggested that temperature downshifts might produce effects opposite those reported above (46). Temperature effects on DNA linking number have also been measured after infection of chloramphenicol-treated cells with bacteriophage λ (47); replicating λ DNA has a slightly lower linking number at a lower temperature. We may gain insight into topoisomerase physiology by expanding DNA linking number studies to include comparisons of DNAs extracted from cells experiencing transitions from one physiological state to another.

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