Suppression of chromosome segregation defects of *Escherichia coli muk* mutants by mutations in topoisomerase I

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Escherichia coli **muk mutants are temperature-sensitive and produce anucleate cells. A spontaneously occurring mutation was found in a Δ*mukB*::*kan* mutant strain that suppressed the temperature-sensitive phenotype and mapped in or near *topA*, the gene that encodes topoisomerase I. Previously characterized *topA* mutations, *topA10* and *topA66*, were found to be general suppressors of *muk* mutants: they suppressed temperature sensitivity and anucleate cell production of cells containing null or point mutations in *mukB* and null mutations in *mukE* or *mukF*. The suppression correlated with excess negative supercoiling by DNA gyrase, and the gyrase inhibitor, coumermycin, reversed it. Defects in *topA* allow 99% of cell division events in *muk* null mutants to proceed without chromosome loss or loss of cell viability. This observation imposes important limitations on models for Muk activity and is consistent with a role for MukBEF in chromosome folding and DNA condensation.**

Accurate segregation of the *Escherichia coli* chromosome requires, among other factors, the action of the three *muk* gene products, MukB, -E, and -F (1, 2). Null *muk* mutants produce anucleate cells ≈100-fold more frequently than the wild type, and many cells have abnormal chromosome positioning and/or DNA content. All *muk* mutants are temperature sensitive (*ts*) for growth and form filaments before growth cessation (1–3). Suppressor mutations of *mukB* mutations (*smb*) have been isolated, but suppress only the temperature sensitivity, *smbA* (4), or act by overproducing the mutant *mukB* protein (5). A mutation in *era*, a gene encoding an essential GTPase involved in the *E. coli* cell cycle, was also found to suppress partially the temperature sensitivity of the *mukB* null mutation (6). Multicopy suppressors, *cspC* and *cspE*, suppress the *ts* and anucleate cell formation phenotypes of *mukB106* (3). The *cspE* effect requires overexpression of the wild-type *cspE*, *crcA*, and *crcB* genes, which have been implicated in chromosome condensation (7).

MukB resembles eukaryotic force-generating proteins, with an extended coiled-coil domain joining N-terminal ATP/GTP binding and C-terminal DNA binding domains (8). MukB was therefore suggested as a candidate for a segregation motor (1, 8). The structure (but not the sequence) of MukB is also similar to SMC proteins (7, 9), which are involved in DNA condensation and chromosome segregation in eukaryotes (10–12). Smc proteins have recently been discovered in *Bacillus subtilis* and *Caulobacter crescentus*, and *smc* mutants of these species have phenotypes similar to *mukB* mutants of *E. coli* (13–15). *E. coli* MukB and *B. subtilis* Smc share a feature not found in motor proteins such as the kinesins and myosins: the dimers are in an antiparallel configuration (9).

The 13S condensin of *Xenopus* is a five-protein complex containing two proteins in the SMC family. It introduces a global (+) writhe into DNA (12, 16) and may act by nonplanar bending of the DNA into coils that remodel the chromosome into a compact structure (16). The *E. coli* chromosome is organized into ≈50 negatively supercoiled domains (17), and its compact structure depends on bound protein and RNA (18). We present evidence suggesting that MukB, in conjunction with the MukE and MukF proteins, may play a somewhat similar role to that proposed for 13S condensin in remodeling the nucleiod structure into a more compact form as a requirement for accurate segregation.

Materials and Methods

Bacterial Strains, Phage, and Plasmids. Strains GC7528, Δ*mukB*::*kan dadR trpE61 trpA62 tna-5 imm*$^{\phi 80}$ (1); SH9019, *mukB33 zcb*::*Tn*10 (3); AZ5381, *mukF*::*kan*, AZ5450, *mukE*::*kan* (2); CAG5053, KL208: PO43 *relA1 zbc-280*::*Tn*10, CAG18455, *trpB83*::*Tn*10, CAG12094, *zcb-3059*::*Tn*10 (19); DPB923, *topA10*, DPB924, *topA*$^+$, and DPB636, *topA66 zch-2250*::*mini-kan* (20) were as described.

Strain CC3955 is *recA56 lacZ*$_{\text{am}}$ (λ *c*I857 *recA*$^+$). CC3974 is a *rec*$^+$ recombinant derived from CC3955 that was transduced to Δ*mukB*::*kan* by a P1 lysate grown on GC7528, cured of λ by heat pulse, and lysogenized by λ *xis6 ind*$^-$. It was subsequently found to have acquired an *imm*$^{\phi 80}$ prophage from GC7528 and a spontaneous mutation linked to *topA*. This strain was transduced (21, 22) to *muk*$^+$ *zcb-3059*::*Tn*10 by selecting a tetracycline-resistant (Tc^r), kanamycin-sensitive (Km^s) colony to give CC3976, *lacZ*$_{\text{am}}$ *zcb-3059*::*Tn*10 (λ *xis6 ind*$^-$). CC3977, *lacZ*$_{\text{am}}$ *rpsL* (λ *xis6 ind*$^-$), is a Tc^s derivative made as described (23). Both CC3976 and CC3977 have the *imm*$^{\phi 80}$ prophage and putative *topA* mutation. DPB923 and DPB924 gave rise to CC4207 and CC4208, respectively, by transduction to Δ*mukB*::*kan* using a P1 lysate grown on CC3979 (MC4100 Δ*mukB*::*kan*), kindly supplied by Nancy Trun (National Cancer Institute). CC4209, CC4210, CC4213, and CC4214 are pAX804 transformants of DPB923, DPB924, CC4207, and CC4208, respectively. CC4209 and CC4210 were transduced with P1 lysates grown on AZ5381 or AZ5450 to produce CC4211, *mukF*::*kan topA10* [pAX804]; CC4212, *mukF*::*kan* [pAX804]; CC4217, *mukE*::*kan topA10* [pAX804]; and CC4218, *mukE*::*kan* [pAX804], respectively. CC4221, *mukB33 topA10 zcb*::*Tn*10; and CC4222, *mukB33 zcb*::*Tn*10 were made by transducing DPB923 and DPB924 with P1 grown on SH9019. CC4220, *mukB33 zcb*::*Tn*10, was derived from CC4208 by transduction with P1 grown on SH9019, and three independent *topA66* transductants, CC4223–4225, were made by transduction of CC4220 using a P1 lysate grown on DPB636. CC4226 is a *mukB33 zch-2250*::mini-*kan zcb*::*Tn*10 (*topA*$^+$) transductant.

λ *c*I857 *recA*$^+$ contains the wild-type *recA* gene and promoter and was kindly provided by Frank Stahl (University of Oregon). The P1 miniplasmids λ-P1:5R and λ-P1:5R*parB34* (24) were as described, as was λ-P1:5RΔ1005::pALA1952 (25).

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λ-P1:5RΔ1005::pALA1991 is similar to the latter but lacks the *parS* site. Plasmid pALA1413 produces the proper levels of ParA and ParB for accurate P1 partition (25) and pAX804 carries the wild-type *mukB* gene (1). The *mukB* alleles in wild-type or *topA* mutant strains were confirmed by using standard PCR techniques, screening for the increased length of the Δ*mukB*::*kan* gene or the *Ssp*I site introduced by *mukB*33.

Media. LB-broth and plates were as described (26) but with NaCl at 5 g/liter. Ampicillin (100 μg/ml), tetracycline (12.5 μg/ml), chloramphenicol (2.5 μg/ml or 10 μg/ml), streptomycin (100 μg/ml), kanamycin (50 μg/ml), or tetracycline (6 μg/ml) plus sodium citrate (5 mM) were used as needed. Coumermycin A_1 (coumermycin, Sigma) at 5 mg/ml in DMSO was added as needed.

Determining the *topA* Status of Cells. Alleles of *topA* were confirmed by their ability to suppress *par* mutant plasmids (27, 28). Additionally, pBR322 plasmid DNA introduced into the cells that were grown to an optical density (OD_{600}) of ≈1.0 in LB broth at 23°C. The DNA was extracted from the cells and was analyzed on 1% agarose gels containing chloroquine at 5 or 15 μg/ml. Gels were run at ≈1.6 V/cm with recirculated TA buffer (29) containing chloroquine for ≈40 hr at 4°C.

Mapping the Suppressor Mutation. Initial crosses were carried out in the presence of 5 mM sodium citrate at 42°C to prevent reinfection by the $imm^{\phi 80}$ phage (30) and were designed to remove the prophage at the φ80 attachment site. CC3977 was mated with the Hfr strain, CAG5053 (KL208: PO43 *zbc-280*::*Tn*10), selecting for Tc^r and against the donor with streptomycin resistance (19). Forty-seven of one hundred exconjugants had lost $imm^{\phi 80}$. Eight of these retained the $lacZ_{am}$ and imm^{λ} markers needed for the P1 partition assay (25). None of the eight retained the suppressor of P1 Par^-, suggesting that the suppressor locus was linked to the φ80 attachment site. P1 transduction (21, 22) of Tc^r from CAG18455 (*trpB83*::*Tn*10: a Tc^r insertion linked to *attφ*80) to CC3977 to give Tc^r at 42°C resulted in 11 of 19 transductants losing the P1*par* suppressor mutation. Five of the eleven retained $imm^{\phi 80}$. Thus, the suppressor mutation is ≈58% linked to the *trpB83*::*Tn*10 on the side opposite to *attφ*80, in the vicinity of the *topA* gene (≈51% linked to the *trpB83*::*Tn*10 marker).

Mini-P1 Partition Assays. Mini-P1 partition assays were carried out by measuring retention of the plasmid during 25 generations of unselected growth. Tests used λ-P1:5R (Par^+) or λ-P1:5R*parB34* as described (31), or the colony color test that measures the maintenance of λ-P1:5RΔ1005::pALA1952 ($parS^+$) or λ-P1:5RΔ1005::pALA1991 (no *parS*) when P1 ParA and ParB are supplied in trans from pALA1413 (25).

Growth of Cells in Coumermycin. Fresh single colonies were inoculated into LB broth at 23°C, were grown to an optical density (OD_{600}) of 0.1, were diluted 25-fold, and were grown for 1 hour. Coumermycin or the equivalent amount of DMSO was then added at the indicated concentrations, and growth (OD_{600}) was monitored at 30-min intervals for 6–8 hours. Doubling times were determined from the growth occurring after the 4-hour time point because there appeared to be a lag period before the full effect of the drug was seen (data not shown). Cells for microscopy were grown from a single colony at 23°C with coumermycin at a concentration of 1 μg/ml. Samples were taken after 6 hours and were 4′,6-diamidino-2-phenylindole-stained as outlined below.

Microscopy. Cells for microscopy were grown for a minimum of 10 generations of balanced growth and were stained as described (7). Cells were viewed with a 100/1.25× plan objective on a Nikon Eclipse E600 microscope using a combination of UV and visible (phase contrast) light. Photographs were taken with a Nikon FDX-35 camera with Kodak 1000 color print film.

Table 1. Plasmid stability of mini-P1 in *mukB* mutant cells

Relevant genotype (strain number)	Percent retention of P1 miniplasmid, 25 generations	
	Mini-P1Par^+	Mini-P1Par^-
Δ*mukB*::kan (GC7528)	87	1
$mukB^+$ (CC3955)	86	2
Δ*mukB*::kan *topA?* (CC3974)	93	32
Δ*mukB*::kan *topA?* (CC3974)*	78	35
$mukB^+$ *topA?* (CC3977)*	90	34

*Colony color partition tests. See *Materials and Methods.*

Results

A mutation that Suppresses a P1 Plasmid Partition Defect also Suppresses *mukB* Temperature Sensitivity. Defects in the P1 plasmid *par* genes increase the plasmid loss rate some 100-fold (24). However, a Δ*mukB*::*kan* mutant strain, CC3974, maintained a *par* mutant mini-P1 plasmid considerably better than its Muk^+ progenitor, CC3955 (Table 1). The partial suppression was not due to the Δ*mukB*::*kan* allele itself because other Δ*mukB*::*kan* mutant strains did not show this effect (GC7528, Table 1). Moreover, the suppressor effect persisted on replacing the Δ*mukB*::*kan* allele of CC3974 by the wild-type $mukB^+$ allele (CC3977, Table 1). The suppressor mutation mapped in or near *topA*, the gene for topoisomerase I (*Materials and Methods*). Mutations in *topA* are known to promote the stability of partition-defective P1 and F mini-plasmids (27, 28).

As the putative *topA* mutation arose spontaneously in strain CC3974, we reasoned that it might confer an advantage on the strain and may have been inadvertently selected. Consistent with this, CC3974 grew well at 37°C on LB plates whereas other Δ*mukB*::*kan* mutant strains such as GC7528 failed to grow at

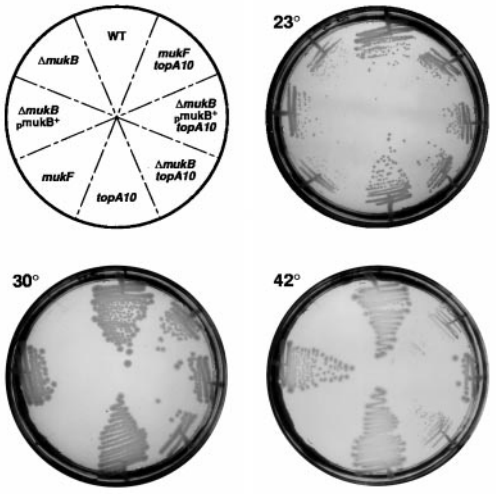

Fig. 1. Suppression of *muk* mutant temperature sensitivity. Colonies grown at 23°C were streaked onto LB-agar plates at the indicated temperatures and were grown for 24 hours. Strain numbers starting with "WT" and proceeding clockwise are DPB924, CC4211, CC4213, CC4207, DPB923, CC4212, CC4214, and CC4208. CC4211, CC4212, CC4213, and CC4214 contain the plasmid pAX804 that produces wild-type MukB protein. At 30°C, the wild-type and *topA10* and *topA10* Δ*mukB*::*kan* mutant strains had generation times of 35, 34, and 43 min, respectively, whereas the Δ*mukB*::*kan* strain failed to grow.

Table 2. Microscopic observation of nucleoids of *muk* mutant cells

Strain: genotype	Percent anucleate cells
DPB924: wild-type	0.3
DPB923: *topA10*	<0.3
CC4208: Δ*mukB*::*kan*	12.4
CC4207: *topA10* Δ*mukB*::*kan*	1.0
CC4220: *mukB33*	14.5
CC4221: *topA10 mukB33*	<0.2
CC4223: *topA66 mukB33**	1.0
CC4218: *mukE*::*kan*	19.0
CC4217: *topA10 mukE*::*kan*	1.0
CC4212: *mukF*::*kan*	19.0
CC4211: *topA10 mukF*::*kan*	2.1

Cells were grown in LB broth at 23°C. From 375–2,500 cells were scored for each strain, and those with little or no 4′, 6-diamidino-2-phenylindole staining were considered ''anucleate.''

*Of three *topA66* mutant transductants examined, all behaved identically. One *topA*$^+$ transductant from the same cross, CC4226, gave similar results to CC4220.

30°C or above. It seemed probable that a *topA* mutation could suppress both the P1 partition defect and *mukB* temperature sensitivity.

Suppression of *muk* Mutant Temperature-Sensitivity by Known *topA* Alleles. The topoisomerase I activity of *topA10* is ≈1% of wild-type (32). Isogenic *topA10* Δ*mukB*::*kan* and Δ*mukB*::*kan* mutant cells were constructed at 23°C. The Δ*mukB*::*kan* mutant failed to grow at 30°C and above unless plasmid pAX804 (1), containing the wild-type *mukB* gene, was introduced. However, the Δ*mukB*::*kan topA10* double mutant cells grew at all temperatures between 23°C and 42°C (Fig. 1). Temperature sensitivity of a point mutation in *mukB*, *mukB*33, was also substantially suppressed by *topA10* (data not shown), as were null mutants in *mukE* (data not shown) and *mukF* (Fig. 1). The suppressed cells were fully viable at the higher temperatures but grew somewhat more slowly than wild-type cells (data not shown). The *topA66* mutation also suppressed *muk* mutant temperature-sensitivity (data not shown).

Anucleate Cell Formation of *muk* Mutants Is Suppressed by *topA10*. The Δ*mukB*::*kan* mutant cultures contained ≈12% anucleate cells at 23°C (Table 2 and Fig. 2*C*; refs. 33 and 34). Only 1% of Δ*mukB*::*kan topA10* double mutant cells were scored as anucleate (Table 2, Fig. 2*D*), representing only a small increase over the proportion of anucleate cells seen in wild-type cultures (≈0.3%, Table 2). The *topA10* mutation also suppressed anucleate cell formation of *mukB33* 15-fold (Table 2). The *mukB* null and point mutants produce a number of filamentous cells at 23°C (4–10%), and the *topA10* mutation also suppressed this phenotype (Fig. 2).

Anucleate cells and filaments are also produced by *mukF*::*kan* and *mukE*::*kan* mutants and are a direct effect of the disruption of the respective gene rather than of polarity on downstream genes (2). On transferring these mutations into *topA10* mutant cells, both anucleate (Table 2) and filamentous cell formation was suppressed. Thus, *topA10* can suppress the temperature

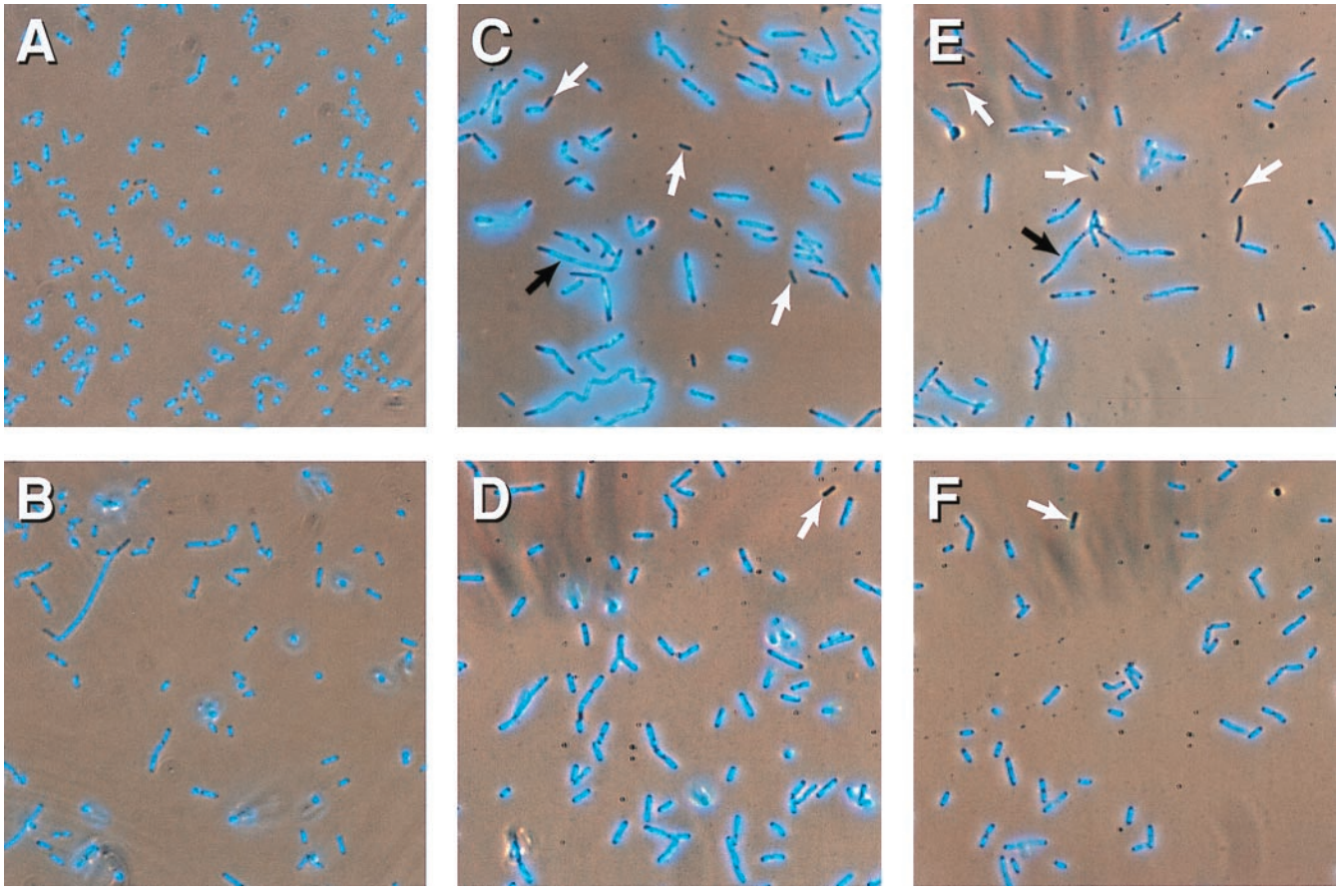

Fig. 2. Anucleate cell formation in *muk* mutant cells. Cells were grown in LB broth at 23°C. (*A*) DBP924 wild-type. (*B*) DPB923, *topA10*. (*C*) CC4208, Δ*mukB*::*kan*. (*D*) CC4207, *topA10* Δ*mukB*::*kan*. (*E*) CC4218, *mukE*::*kan*. (*F*) CC4217, *topA10 mukE*::*kan* mutant cells. White arrows highlight some anucleate cells. Black arrows highlight filamentous cells with ''DNA-free bays.''

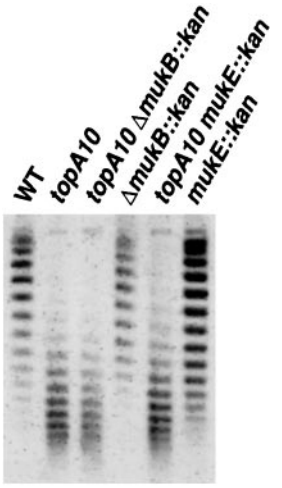

Fig. 3. Supercoiling of plasmid pBR322 isolated from wild-type and *topA10* mutant cells. A 1% agarose gel containing 5 μg/ml chloroquine was used to separate topoisomers, as outlined in *Materials and Methods*. More highly supercoiled species migrate further than less supercoiled species. The change in the topoisomer ladders seen when 15 μg/ml chloroquine was used (data not shown) indicates that all of the topoisomers produced from the *topA* and *topA muk* mutant cells are negatively supercoiled species in this gel. Likewise, most if not all of the bands produced by the *top*$^+$ cells are negatively supercoiled. Thus, the *topA* suppressed cells produce plasmid DNA that contains on average nine or more additional negative supercoils relative to their *top*$^+$ parents. DNA was isolated from pBR322 transformants of DPB924, DPB923, CC4207, CC4208, CC4215, and CC4216, from left to right, respectively.

sensitivity, anucleate cell production, and filamentation of all *muk* mutations tested.

Suppression of *mukB* in *topA* Mutant Strains Correlates with Increased Negative Supercoiling. Defects in *topA* increase negative DNA supercoiling (32) because of the unrestrained activity of DNA gyrase. As this can limit growth, mutants such as *topA10* can acquire compensating DNA gyrase mutations upon propagation (35). Thus, the resulting strains may not have excess negative supercoils. Plasmid pBR322 was introduced into the relevant strains and was reisolated from cells grown at 23°C, and the degree of supercoiling was estimated by electrophoresis in agarose gels containing chloroquine (*Materials and Methods*). The wild-type strain and its Δ*mukB*::*kan* derivative showed indistinguishable levels of plasmid supercoiling (Fig. 3). However, plasmid DNA from the suppressed Δ*mukB*::*kan topA10* strain, CC4207, showed evidence of considerably increased negative supercoiling and was indistinguishable from that of the *topA10* parent, DPB923 (Fig. 3). Virtually identical results were obtained on analysis of the *mukE*::*kan* mutant strain and its suppressed derivative (Fig. 3) and of *mukB33* strains suppressed by *topA66* (data not shown). Thus, suppression of the *muk* mutants correlates with increased negative supercoiling. Presumably, suppression is a direct effect of the increase in negative supercoiling induced by the defects in *topA*.

The *topA66* allele does not readily acquire secondary mutations (20). When this *topA* allele was introduced into *mukB33* mutant cells by generalized transduction, it suppressed the *ts* phenotype (data not shown) and anucleate cell production (Table 2) of the *mukB33* strain equally well in three separate isolates. This result is also consistent with suppression being a direct effect of the *topA* mutation rather than the acquisition of secondary mutations.

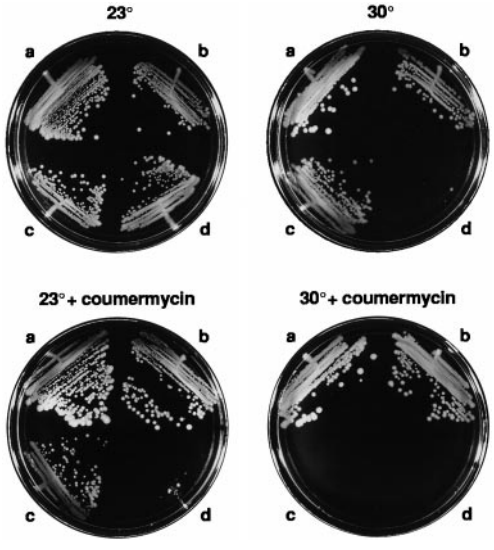

Fig. 4. Temperature-resistance of *topA10* Δ*mukB*::*kan* is reversed by coumermycin. Cells were streaked out on prewarmed plates containing DMSO (controls) or coumermycin at 1 μg/ml at the indicated temperatures. 30°C plates were incubated for 24 hours, and 23°C plates were incubated for 48 hours. Strains were DPB924, wild type (*a*), DPB923, *topA10* (*b*), CC4207, *topA10* Δ*mukB*::*kan* (*c*), and CC4208, Δ*mukB*::*kan* (*d*).

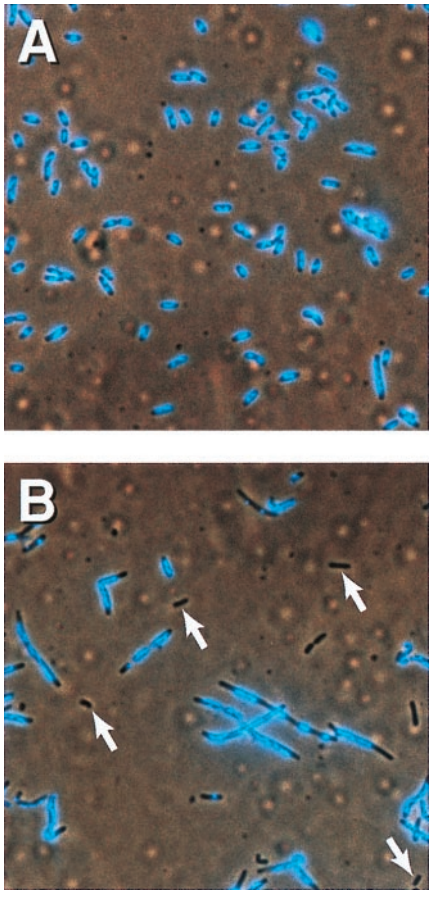

Fig. 5. Coumermycin reverses suppression of Δ*mukB*::kan by *topA10*. Cells were grown with 1 μg/ml coumermycin for 6 hours and were 4′,6-diamidino-2-phenylindole-stained for microscopy. (*A*) DPB923, *topA10*. (*B*) CC4207, *topA10* Δ*mukB*::*kan*. White arrows highlight some examples of anucleate cells.

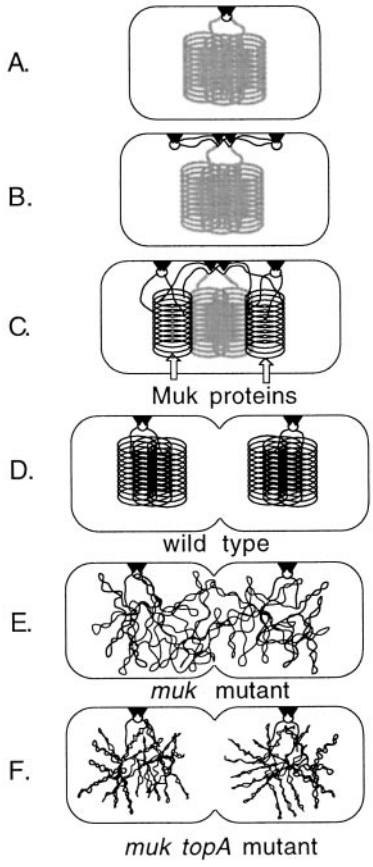

Fig. 6. Directed condensation model for *E. coli* chromosome segregation. The model is based on that of Lemon and Grossman (43). (*A*) The nucleoid is supercoiled and further condensed to some unspecified compact form (represented as stacked loops). During initiation, the origin (circle) is attached to a site (triangles) at the cell center. (*B*) The new origins (circles) attach to new sites at the 1/4 and 3/4 positions. The DNA replication forks remain attached to the central site. (*C*) Newly replicated DNA (black lines) is supercoiled by DNA gyrase and remodeled into the compact form by the action of the Muk proteins. As the origins are tethered in opposite halves of the cell, the nascent nucleoids form as separate masses. (*D*) On completion of replication, the daughter nucleoids are sufficiently separated to allow cell division. (*E*) In the absence of the Muk system, the supercoiled loops of the completed nucleoids are disorganized and the resulting masses fail to separate. (*F*) The additional negative supercoiling caused by unrestrained DNA gyrase activity in *topA* mutant cells increases superhelical density and allows for a more reliable separation.

A DNA Gyrase Inhibitor Reverses the Suppression of Δ*mukB*::*kan* by *topA10*. If increased negative supercoiling is responsible for *muk* mutant suppression, inhibition of DNA gyrase should reverse it. The effect of low levels of the DNA gyrase inhibitor, coumermycin (36), on the Δ*mukB*::*kan* suppressed cells was tested. With no coumermycin, the Δ*mukB*::*kan* mutant grew slowly and only at 23°C (110-min doubling time) whereas the Δ*mukB*::*kan* *topA10* double mutant had a similar growth rate to wild-type cells (89- vs. 82-min doubling time at 23°C) and was not temperature-sensitive (Fig. 4). Coumermycin at low concentrations (e.g., 5 μg/ml) had no significant effect on the growth or temperature resistance of wild-type or *topA10* mutant cells, and they grew at all temperatures from 23°C and 42°C (data not shown). However, the drug strongly inhibited the growth of *topA10* Δ*mukB*::*kan* double mutant cells (>170-min doubling time at 23°C), and the cells failed to grow above 23°C even at coumermycin concentrations as low as 1 μg/ml (Fig. 4). Thus, when DNA gyrase is partially inhibited, the suppression by *topA* is reversed, and the cells show the same low growth rate and temperature sensitivity as the unsuppressed Δ*mukB*::*kan* mutant cells. Note also that Δ*mukB*::*kan* mutant cells are hypersensitive to coumermycin (Fig. 4), such that they fail to grow at 23°C with only 1 μg/ml coumermycin. This suggests that these cells are critically dependent on supercoiling levels for survival.

As shown above, ≈1% of *topA10* Δ*mukB*::*kan* cells are anucleate in the absence of the drug. However, in the presence of 1 μg/ml coumermycin, many of the cells were anucleate, and the cells showed the frequent filamentation and nucleoid disorganization typical of the parent Δ*mukB*::*kan* strain (Fig. 5*B*). The *topA10* cells were unaffected by coumermycin at this concentration (Fig. 5*A*), as were wild-type cells (data not shown). We conclude that partial inhibition of DNA gyrase reverses *topA*-mediated suppression of *mukB* mutant cells for all three of the measured MukB phenotypes. This strongly suggests that the excess negative supercoiling in the *topA* mutants is responsible, directly or indirectly, for the suppression.

Discussion

When topoisomerase I activity was limited by mutation, the chromosome segregation defect of all *muk* mutants tested was suppressed. This suppression appears to be the result of the excess negative supercoils in the DNA because it is completely reversed by partial inhibition of DNA gyrase. In suppressing conditions, chromosome segregation proceeded successfully in 99% of cells without Muk function. This imposes important limitations on the possible roles for the Muk proteins. The resemblance of MukB to eukaryotic motor proteins and the phenotype of mutant cells suggested that the MukB protein might actively segregate *E. coli* chromosomes (8). However, it is difficult to imagine how an increase in negative supercoiling could so effectively compensate for loss of such a function. We favor an alternative idea: that the Muk system is required for condensation and organization of the chromosome into a compact structure that is important for proper segregation, and that this activity can be substituted for, in large part, by increased negative supercoiling. Consistent with this, we found that Muk mutants are hypersensitive to coumermycin. This suggests that, in the absence of the Muk system, the supercoiling density of the chromosome is in a critical range such that an increase is beneficial for segregation but a decrease is lethal.

Recent views of the mechanism for bacterial chromosome segregation are largely based on microscopic observations (37–43). Studies on the localization of origins and their associated proteins suggested a polar attachment and condensation mechanism for chromosome segregation (42). The probable location of the chromosome replication forks at the cell center further suggested a modified model in which segregation is concurrent with replication, and origins are attached to new cell division sites rather than at the cell pole (Fig. 6; ref. 43). Attachment of the replication forks to the cell center and transport of the newly replicated origins to the 1/4 and 3/4 positions in the cell should be specific and fundamental elements of the segregation process (Fig. 6). Without these events, chromosome segregation should fail, and restoration of segregation by increased negative supercoiling seems unlikely. Therefore, we do not regard the Muk proteins as good candidates for the essential effectors of these processes. The Muk system does, however, seem to be a good candidate for the condensation and folding of the nucleoids that provide the motive force for moving the bulk of the DNA during segregation in these models. The action of DNA gyrase in forming independently supercoiled loops in the newly synthesized DNA (17) is presumably critical for this step. Certain mutants defective in DNA gyrase activity are blocked for chromosome segregation (44), likely as a result of reduced negative supercoiling (45). We suggest that the MukBEF proteins are also involved in DNA condensation and folding.

Perhaps they aid in the higher order organization of DNA into the compact nucleoid structure, somewhat along the lines proposed for the 13S condensin of *Xenopus* (16). Coiling or some other form of condensation could be necessary to provide sufficient space between the daughter nucleoids to permit cell division (Fig. 6). An increase in negative supercoiling is expected to make the DNA considerably more compact (46). Suppression of *muk* mutations by *topA* mutations could be explained if the excess negative supercoiling found in *topA* mutant cells results in sufficient compaction of the nucleoid to make further organization by the Muk system largely unnecessary (Fig. 6*F*). However, we have not ruled out that the change in supercoiling in *topA* mutant cells increases the expression of other genes involved in nucleoid condensation, thus achieving the same effect.

Apart from the resemblance of MukB to SMC proteins, additional observations suggest a chromosome folding or DNA condensation role for Muk proteins. First, *muk* mutant chromosomes appear decondensed by microscopy (refs. 1 and 8; Fig. 2), and the presence of the suppressing *topA* allele appears to favor a more compact form (Fig. 2). Second, simultaneous overexpression of *crcA*, *crcB*, and *cspE* suppresses at least one *mukB* point mutation, and these small proteins are implicated in chromosome condensation (7).

If *muk* mutant cells lack a DNA condensation activity, how can the anucleate cell production of these mutants be explained by the model in Fig. 6? Tangling of the nascent daughter nucleoids might occasionally hinder the attachment of origin regions to their appropriate division sites, thus causing sporadic missegregation. Additionally, lack of proper DNA condensation might leave some of the daughter nucleoids overlapping and blocking the plane of cell division (Fig. 6*E*). Anucleate cells might then arise by breakage of one or both chromosomes during septation (''guillotining''), followed by exonucleolytic digestion of the broken chromosome. Some cells in *muk* mutant cultures do appear to have partial nucleoids (refs. 1 and 2; data not shown). In other cases, the failure of chromosomes to separate properly might inhibit normal cell septation, leading to the production of the filamentous cells that are seen in cultures of all *muk* mutants (Fig. 2; Table 2). These often contain DNA-free bays, usually at one end (Fig. 2). Abnormal divisions near these ends would produce anucleate cells. Recent observations of the location of FtsZ cell division rings in *mukB* mutant cells suggest that at least some anucleate cells may be produced by divisions near the DNA-free ends of filamentous cells (33).

In conclusion, our results are consistent with a chromosome condensation or folding role for the Muk proteins. Increased negative supercoiling of the DNA appears to provide a Muk-independent route to adequate chromosome condensation, either as a direct effect on DNA topology or via some change in gene expression that aids condensation.

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