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Null Mutation of the *dam* or *seqA* Gene Suppresses Temperature-Sensitive Lethality but Not Hypersensitivity to Novobiocin of *muk* Null Mutants

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***Escherichia coli mukF, mukE*, and *mukB* null mutants have common phenotypes such as temperature-dependent colony formation, anucleate cell production, chromosome cutting by septum closure, and abnormal localization of SeqA-DNA clusters. We show here that the associated *muk* null mutations cause hypersensitivity to novobiocin. Null mutation of either *dam* or *seqA* suppressed partially the temperature-sensitive lethality but failed to suppress the anucleate cell production and the hypersensitivity to novobiocin caused by *muk* null mutations.**

The *mukF*, *mukE*, and *mukB* genes are essential for faithful partitioning of sister chromosomes into both daughter cells in *Escherichia coli* (15, 17, 28). Null mutation of each *muk* gene causes the medium-dependent, temperature-sensitive, lethal phenotype and produces a significant number of anucleate cells of normal size during growth at permissive low temperature (16, 28). The three Muk proteins form a complex in vitro (29). Purified MukB protein has a DNA binding activity, an ATP and GTP binding activity (15), and a Mg^{2+}-dependent ATPase activity (12, 29). The N-terminal globular domain of MukB binds to filaments of the FtsZ protein polymer (12) and to eukaryotic microtubules in vitro (13). To analyze the in vivo function of MukB, various suppressor mutations and synthetic-lethal mutations have been identified (10, 25–27). Mutations of the *topA* gene, encoding topoisomerase I, suppress the temperature-sensitive growth and anucleate cell production caused by null mutation of each *muk* gene. The suppression correlates with excess negative supercoiling by DNA gyrase, because the gyrase inhibitor coumermycin reverses the suppression caused by the *topA* mutations, suggesting that *muk* mutations cause a defect in chromosome folding and DNA condensation (20).

DNA is fully methylated by DNA adenine methyltransferase (Dam methylase) in *E. coli* wild-type cells (1, 2, 5). Following initiation from the chromosomal origin (*oriC*), newly synthesized nascent DNA strands acquire a hemimethylated state at Dam methylation sites. The *seqA* gene is essential for control of synchronous initiation of chromosome replication (3, 14, 23). The purified SeqA protein preferentially binds GATC sequences in hemimethylated DNA (4, 22). SeqA is localized as discrete foci in exponentially growing wild-type cells of *E. coli* (7, 18). Formation of the visible SeqA foci depends on Dam methylation (7, 18) and ongoing replication (8), suggesting clusters of SeqA molecules which bind to hemimethylated nascent DNA strands. A single SeqA focus localized at midcell seems to separate into two foci, and these foci subsequently migrate rapidly in opposite directions to 1/4 and 3/4 positions of the cell (7, 8, 18). In the *mukB* null mutant, SeqA clusters are abnormal in size and subcellular localization (7, 18), suggesting that MukB may participate in separation or migration of SeqA-DNA clusters. Interestingly, *E. coli* and related bacteria possess MukF, MukE, and MukB together with SeqA, MutH, and Dam methylase (8).

Weitao et al. (24) showed that, when a *seqA* null mutation was introduced into a *mukB* null mutant by P1 transduction, a resulting transductant recovered from the temperature-sensitive growth, the anucleate cell production, and the hypersensitivity to novobiocin caused by the *mukB* null mutation. However, we report here that either a *dam* or *seqA* single mutation or a *seqA dam* double mutation suppresses partially only the temperature-sensitive growth, not the hypersensitivity to novobiocin and the anucleate cell production, of *mukF*, *mukE*, and *mukB* null mutants.

Partial suppression of temperature-sensitive growth of *muk* null mutants by a *dam* or *seqA* null mutation. To examine the effect of the *dam* null mutation on the phenotypes of *muk* null mutants, we introduced a *dam::cat* mutation into *mukB*, *mukE*, *mukF*, and *mukFEB* null mutants (Table 1) and also into the isogenic *muk*$^+$ strain YK1100 by transduction with phage P1*vir* (21), which was grown in *dam*-deficient KA468 cells. Chloramphenicol-resistant transductants were isolated after incubation for 5 days at 22°C on L agar medium (9) containing chloramphenicol (7 μg/ml) and sodium citrate (20 mM). After single-colony isolation of 10 transductants at 22°C, these transductants were confirmed for the *dam* mutation by observing the localization of SeqA with immunofluorescence microscopy (7). In all these transductants, SeqA was distributed throughout the whole nucleoid instead of displaying discrete foci, indicating the absence of Dam methylase (7).

These *muk dam* double-null mutants were exponentially grown at 22°C in M9 minimal medium (19) supplemented with 0.2 mM $MgSO_4$, 0.1 mM $CaCl_2$, glucose (0.5%), Casamino Acids (0.4%; Difco), and L-tryptophan (50 μg/ml) (MCAT medium) or L medium (1% tryptone-peptone [Difco], 0.5% yeast extract (Difco), 0.5% NaCl, pH 7.4). The cultures were diluted with 0.84% NaCl and spread onto MCAT-agar and L-agar plates, respectively. These plates were incubated at 22,

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TABLE 1. Bacterial strains used

Strain	Genotype	Source or reference
YK1100	W3110 except *trpC9941*	28
AZ5372	YK1100 except Δ*mukB*::*kan*	28
AZ5450	YK1100 except *mukE*::*kan*	28
AZ5381	YK1100 except *mukF*::*kan*	28
OT7	PB 103 except Δ*mukFEB*::*kan*	29
KK347	YK1100 except Δ*mukFEB*::*kan*	This work[a]
NK7253	NK7254 except *seqA*::*tet*	14
KA468	NH5402-1 except *dam-13*::Tn*9*	T. Katayama

[a] P1*vir*[OT7]→YK1100.

30, 37, and 42°C for 1 to 4 days to allow formation of visible colonies. The *muk dam* double-null mutants were able to form colonies at 22, 30, and 37°C (Table 2), while the parental *muk* null mutants were unable to form colonies at 37°C. These double mutants also failed to grow at 42°C (Table 2). Thus the *dam* mutation partially suppressed the temperature-sensitive colony formation of these *muk* null mutants.

To test the effect of a *seqA* null mutation, the *seqA*::*tet* mutation from strain NK7253 was introduced into the *muk* null mutants by P1 transduction. After 5 days of incubation at 22°C in L-agar plates containing tetracycline (7 μg/ml) and sodium citrate (20 mM), five tetracycline-resistant transductants from each transduction experiment were isolated and the *seqA* null mutation was verified by immunofluorescence microscopy. None of the transductants were stained by the anti-SeqA antibody, indicating the *seqA*::*tet* mutation. These *muk seqA* double mutants formed colonies at 22 and 30°C as efficiently as the parental *muk* null mutants having the wild-type *seqA* gene and also as efficiently the *seqA* transductants obtained from the isogenic *muk*$^+$ strain (Table 2). In contrast to the parental *muk* null mutants, these *muk seqA* double mutants were able to form colonies at 37°C but failed to grow at 42°C (Table 2). Thus, the *seqA* null mutation also partially suppressed the temperature-sensitive growth of these *muk* null mutants. The above results were inconsistent with the phenotypes of a *seqA mukB* double-mutant strain described by Weitao et al. (24). Their double mutant was unable to grow at 25°C but was able to grow at 37°C in M9 glucose medium supplemented with Casamino Acids (24), suggesting a cold-sensitive phenotype for growth.

No suppression of anucleate cell formation in *muk* null mutants by *dam* and *seqA* null mutations. We analyzed by fluorescence and phase-contrast microscopy (9) the number of anucleate cells in exponentially growing cultures in MCAT medium at the permissive temperature of 22°C. The percentages of anucleate cells were 0.03, 4.4, 5.0, 4.2, and 5.5% for YK1100, AZ5372, KK267, KK248, and KK279, respectively, indicating that *dam* and *seqA* null mutations were unable to suppress anucleate cell formation. These bacterial cells grown exponentially at 22°C were further incubated at 37°C for 4 h. The parental *mukB* mutant showed heterogeneous lengths of elongated cells having abnormally localized nucleoids and nonseparated large nucleoids (Fig. 1B). In contrast, the *mukB dam* and *mukB seqA* double mutants and *mukB dam seqA* triple mutants showed elongated cells less frequently, indicating that the mutants had partially recovered from defects in cell division. However, anucleate cells were still frequently (10 to 15%) produced at 37°C in these genetic backgrounds (Fig. 1C to E). Thus, *dam* and *seqA* mutations were unable to suppress anucleate cell production in the *mukB* mutant. Similar results were

TABLE 2. Effect of *dam* and *seqA* mutations on temperature-sensitive colony formation and hypersensitivity to novobiocin in *mukB*, *mukE*, *mukF*, and *mukFEB* null mutants

Strain	Relevant genotype	Colony-forming ability[a] in:								Novobiocin resistance[b] (μg/ml) at 22°C in:	
		MCAT medium at:				L medium at:				L medium	MCAT medium
		22°C	30°C	37°C	42°C	22°C	30°C	37°C	42°C		
YK1100	Wild type	1	0.8	1.0	0.9	1	0.7	3.1	1.9	200	200
KK266	*dam*	1	1.0	0.8	1.1	1	0.9	1.0	0.7	100	
KK259	*seqA*	1	1.0	0.9	1.1	1	1.2	0.9	0.6	100	
KK278	*dam seqA*	1	0.9	1.2	1.2	1	2.2	1.5	1.7	100	
AZ5372	*mukB*	1	0.8	2×10^{-4}	$<1 \times 10^{-5}$	1	0.3	8×10^{-4}	$<1 \times 10^{-5}$	20	20
KK267	*mukB dam*	1	1.3	0.7	$<1 \times 10^{-5}$	1	1.0	0.4	$<1 \times 10^{-5}$	20	20
KK248	*mukB seqA*	1	1.0	0.8	$<1 \times 10^{-5}$	1	1.1	1.0	$<1 \times 10^{-5}$	20	20
KK279	*mukB dam seqA*	1	1.1	0.2	$<1 \times 10^{-5}$	1	1.0	1.4	$<1 \times 10^{-5}$	20	10
AZ5450	*mukE*	1	0.8	5×10^{-4}	$<1 \times 10^{-5}$	1	1.0	2×10^{-4}	$<1 \times 10^{-5}$	20	
KK269	*mukE dam*	1	0.9	0.6	$<1 \times 10^{-5}$	1	2.6	1.8	$<1 \times 10^{-5}$	20	
KK250	*mukE seqA*	1	1.0	1.0	$<1 \times 10^{-5}$	1	0.8	0.1	$<1 \times 10^{-5}$	20	
KK281	*mukE dam seqA*	1	0.9	0.1	$<1 \times 10^{-5}$	1	1.0	4×10^{-3}	$<1 \times 10^{-5}$	20	
AZ5381	*mukF*	1	1.0	1×10^{-4}	$<1 \times 10^{-5}$	1	0.9	4×10^{-4}	$<1 \times 10^{-5}$	20	
KK268	*mukF dam*	1	0.9	0.7	$<1 \times 10^{-5}$	1	1.1	1.0	$<1 \times 10^{-5}$	20	
KK251	*mukF seqA*	1	1.5	0.9	$<1 \times 10^{-5}$	1	1.0	0.3	$<1 \times 10^{-5}$	20	
KK280	*mukF dam seqA*	1	0.9	0.1	$<1 \times 10^{-5}$	1	0.9	3×10^{-3}	$<1 \times 10^{-5}$	20	
KK347	*mukFEB*	1	0.7	5×10^{-5}	$<1 \times 10^{-5}$	1	0.9	7×10^{-5}	$<1 \times 10^{-5}$	20	
KK351	*mukFEB dam*	1	1.0	0.7	$<1 \times 10^{-5}$	1	1.1	0.5	$<1 \times 10^{-5}$	20	
KK354	*mukFEB seqA*	1	1.4	0.4	$<1 \times 10^{-5}$	1	0.8	0.1	$<1 \times 10^{-5}$	20	
KK282	*mukFEB dam seqA*	1	1.3	0.1	$<1 \times 10^{-5}$	1	1.2	0.1	$<1 \times 10^{-5}$	20	

[a] Colony-forming ability at 22°C was defined as 1.
[b] The maximum concentration of novobiocin allowing survival of more than 50% of cells.

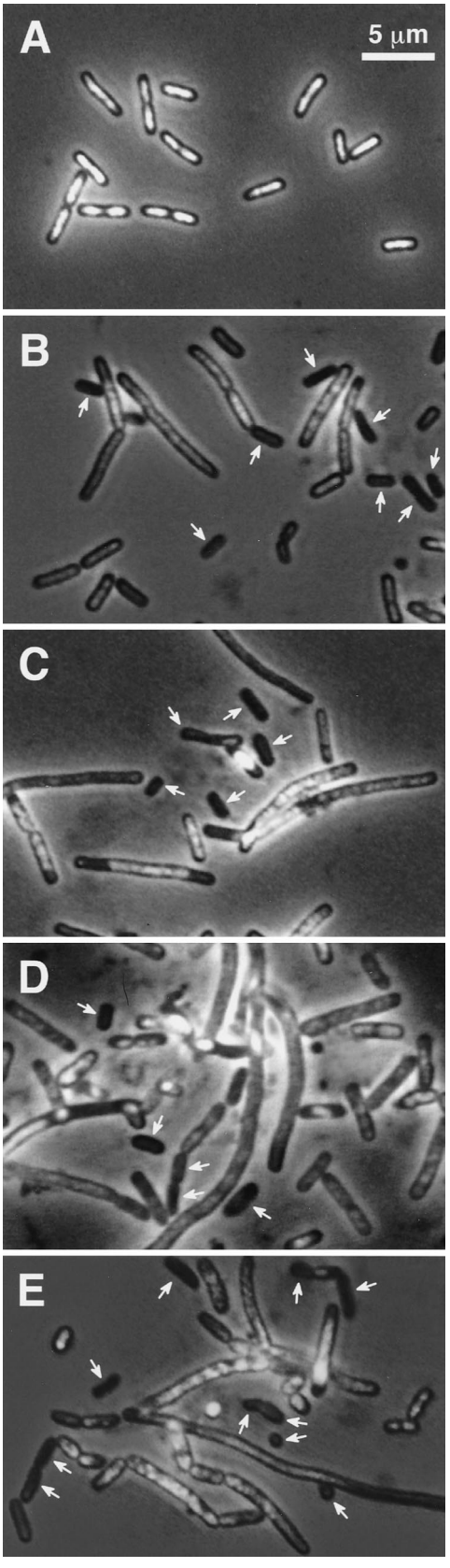

FIG. 1. Production of anucleate cells. Cells were exponentially grown at 22°C in MCAT medium and then incubated at 37°C for 4 h. Cells were fixed and stained with DAPI (4′,6′-diamidino-2-phenylindole) (9). (A) YK1100 (wild type). (B) AZ5372 (*mukB*). (C) KK267 (*mukB dam*). (D) KK248 (*mukB seqA*). (E) KK279 (*mukB dam seqA*). Arrows, anucleate cells or cells with a small amount of chromosomal DNA.

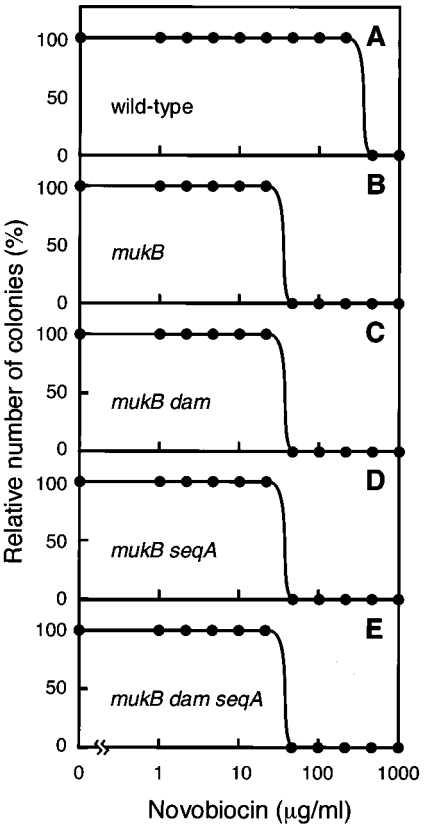

FIG. 2. Colony formation of various strains in the absence or presence of various concentrations of novobiocin. The numbers of colonies that appeared after 3 days of incubation at 22°C were scored. (A) YK1100 (wild type). (B) AZ5372 (*mukB*). (C) KK267 (*mukB dam*). (D) KK248 (*mukB seqA*). (E) KK279 (*mukB dam seqA*).

obtained with the other *muk* mutants having the *mukF* or *mukE* null mutation (data not shown).

No suppression of the novobiocin hypersensitivity of *muk* null mutants by *dam* and *seqA* mutations. Novobiocin inhibits DNA supercoiling reactions by blocking the B subunit of DNA gyrase (for reviews, see references 6 and 11). We anticipated that novobiocin might have an additive lethal effect in such *muk* mutants. Bacterial cultures grown exponentially at 22°C in MCAT or L medium were diluted and spread onto MCAT-agar or L-agar plates, respectively, containing various concentrations of novobiocin (0 to 1,000 μg/ml). The plates were incubated at 22°C for 2 days, and the colonies were counted. The maximal concentration of novobiocin allowing growth of the wild-type strain was 200 μg/ml; the corresponding concentration was 20 μg/ml for all *muk* null mutants (Fig. 2 and Table 2). Thus, *muk* null mutants are hypersensitive to novobiocin as expected. Introduction of either the *dam* or *seqA* null mutation or both the *dam* and *seqA* null mutations into *muk* null mutants failed to suppress the novobiocin hypersensitivity of these *muk* null mutants (Fig. 2, Table 2). *dam* or *seqA* single mutants with the muk^+ genetic background were slightly more sensitive to novobiocin (maximum concentration of novobiocin allowing survival of more than 50% of cells, 100 μg/ml) than the wild-type strain (Table 2). The absence of SeqA and Dam is thus not sufficient to suppress the novobiocin hypersensitivity of *muk* null mutants. It is therefore unlikely that the lack of SeqA restores novobiocin hypersensitivity in the *mukB* null mutant

to the level of the *muk*$^+$ strain as described by Weitao et al. (24).

Discrepancy between our results and the previously reported results on the effect of the *seqA* null mutation. Weitao et al. (24) previously described a *seqA mukB* double-null mutant that showed cold-sensitive growth in minimum glucose medium supplemented with Casamino Acids, no production of anucleate cells, and resistance to novobiocin similar to that of the wild-type strain. By contrast, our present results revealed that all the isolated *seqA mukB* double mutants were able to grow at 22, 30, and 37°C in MCAT medium; however, these double mutants produced anucleate cells and were still as hypersensitive to novobiocin as the *mukB* single mutant on the *seqA*$^+$ genetic background. Probably, the *seqA mukB* double mutant isolated and analyzed by Weitao et al. (24) had a spontaneous third mutation that was able to suppress the production of anucleate cells and novobiocin hypersensitivity.

Role of the MukFEB complex in dynamic localization of SeqA-DNA clusters. The medium-dependent lethality of *muk* null mutants may be primarily due to cutting chromosomal DNA by septum closure, the so-called "guillotine effect," resulting from abnormal localization and structure of nucleoids (16, 28). In the absence of the MukFEB complex, the SeqA-DNA clusters are distributed irregularly and sometimes they seem to fuse to each other due to entanglement of DNA strands in the clusters (7, 18). The MukFEB complex appears to participate in the reorganization of replicated sister chromosomal strands to form two separated, folded sister chromosomes localized at the 1/4 and 3/4 positions. A high degree of entanglement between sister chromosomes likely occurs frequently in *muk* null mutants. The absence of SeqA-DNA clusters in the *dam* or *seqA* null mutants may get rid of the worst condition and rescue partially the viability of *muk* null mutants. Alternatively, *dam* and *seqA* null mutations may cause abnormal expression of various genes and restore indirectly the temperature-sensitive lethality of *muk* null mutants.

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