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Lack of *S*-Adenosylmethionine Results in a Cell Division Defect in *Escherichia coli*

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The enzyme *S*-adenosylmethionine (SAM) synthetase, the *Escherichia coli metK* gene product, produces SAM, the cell's major methyl donor. We show here that SAM synthetase activity is induced by leucine and repressed by Lrp, the leucine-responsive regulatory protein. When SAM synthetase activity falls below a certain critical threshold, the cells produce long filaments with regularly distributed nucleoids. Expression of a plasmid-carried *metK* gene prevents filamentation and restores normal growth to the *metK* mutant. This indicates that lack of SAM results in a division defect.

S-Adenosylmethionine (SAM) is a central metabolite of *Escherichia coli* and other cells. Synthesized from methionine and ATP by the enzyme SAM synthetase (5), it is the major methyl donor in metabolism. It is also a precursor of spermidine, formed from decarboxylated SAM and putrescine.

SAM is an essential metabolite in yeast, where mutations causing a lack of SAM synthetase have been shown to be lethal unless SAM is provided in the medium (7). This has not been established for *E. coli*, because the cell is impermeable to SAM (13). However, SAM is generally assumed to be essential in *E. coli*, although no single SAM-dependent reaction has been shown to be indispensable.

In *E. coli*, SAM synthetase is the *metK* gene product (15). Mutants in *metK* have been isolated by virtue of their resistance to ethionine (9) and γ-glutamyl methyl ester (GGME) (19). In addition to resistance to methionine analogs, the various phenotypes reported for different (leaky) *metK* mutants include overproduction of methionine (9) and methionine or vitamin B_{12} auxotrophy or complete inability to grow on defined media (33). All such mutants show residual SAM synthetase activity. Attempts to isolate mutants totally deficient in MetK via temperature sensitivity of growth were not successful (11).

Even with some SAM synthetase activity, the leaky *metK* mutants show growth deficiencies (33) such that populations of *metK* cells quickly accumulate suppressor mutations; the only ones identified so far are in the *lrp* gene, coding for the leucine-responsive regulatory protein (21).

We show here that the unsuppressed *metK84* mutant has a complex phenotype. Growth at normal rates in glucose minimal medium requires supplementation with a high concentration of leucine (50 μg/ml). Cells grown at lower concentrations of leucine are hindered in cell division and produce long filaments.

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MATERIALS AND METHODS

Cultures and growth conditions. The strains used in this study, all derivatives of *E. coli* K-12, are described in Table 1. The plasmids used were pBR322 (3) and pBAD22 (10). Minimal medium, LB, and growth conditions were as previously described (31, 34). Carbon sources were added at 0.2%. Kanamycin was provided at 50 μg/ml, chloramphenicol was provided at 30 μg/ml, ampicillin was provided at 100 μg/ml, and tetracycline was provided at 15 μg/ml.

Genetic methods. Plasmid isolations, DNA manipulations, transductions, and transformations were performed as described by Maniatis et al. (24) and Miller (27).

Enzyme assays. β-Galactosidase was assayed in whole cells by the method of Miller and expressed in the units used previously (27). SAM synthetase assays were carried out as previously described (13).

Strain constructions. The *metK84* mutation used in this study was transferred from its strain of origin, RG62 (8), to our strain background (strain CU1008) by P1 cotransduction with *serA*$^+$, forming strain MEW30 (21). Further transfers of *metK* were made with strain MEW30 as the donor and strain MEW311 *serA*::λ*plac*Mu Δ*ara714* or strain DRN-1 *serA*::*Mud1* as the recipient. *lrp* mutants were created by using phage grown on strain MEW26 *lrp*::Tn*10*, selecting on LB medium plus tetracycline, and verifying the ability to grow with serine as the carbon source (21).

Cloning the metK gene. The *metK* gene was amplified from chromosomal DNA extracted from strain MEW1 by using primer 1, CATCCCATGGCAAA ACACCTTTTACGTCC, corresponding to 24 bp from the start of the gene preceded by 6 bases providing an *Nco*I site, and primer 2, ACGAAGCTTGAA CGCAGGTGAAGAAAGATTAC, corresponding to 24 bp at the 3′ end of the gene plus a 6-bp extension providing a *Hin*dIII site. This DNA was subcloned into pBAD22amp^r (10) by using the same two restriction enzymes. The cloned DNA was identified by sequencing with an ABI model 377 automatic sequencer with the same two primers. The sequence, read on both strands (except for the 10 codons at the 5′ end, which were read on one strand only), was identical to the *metK* sequence found in the *E. coli* genome but was significantly different from the previously published sequence (26).

Fluorescence microscopy. Cell samples were prefixed with 0.25% formaldehyde (final concentration) and stored at 4°C. Subsequently, cells were postfixed by adding OsO_4 to a final concentration of 0.1% and stained with 0.2 μg of DAPI (4′,6-diamidino-2-phenylindole dihydrochloride hydrate) per ml (final concentration) for at least 1 h. The cells were concentrated by centrifugation and immobolized on object slides coated with a dried layer of 2% agarose. The preparations were illuminated at 330 to 380 nm. Images were taken with a Princeton charged-coupled-device camera mounted on an Olympus BH-2 fluorescence microscope equipped with a 100× phase-contrast Neofluar oil immersion lens, a 3.3× photo-ocular, and an emission filter of 420 nm.

Dark-field microscopy. A wet mount of cells in liquid culture was prepared and placed in a Leitz Dialux EB20 microscope equipped with a dark-field condenser and a quartz-iodine light source. For examination under oil immersion, the no. 2 aperture could be stepped down by means of an adjustable rotating collar.

TABLE 1. *E. coli* K-12 strains

Strains	Relevant characteristic(s)	Source or reference
CU1008	*ilvA*	L. S. Williams
DRN-1	*serA*::*Mud1*	21
MEW1	CU1008 Δ*lac*	21
MEW26	MEW1 *lrp*::Tn*10*	21
MEW30	MEW1 *metK84*	21
MEW45	MEW1 *lrp*::*lacZ*	20
RG62	*metK84 lrp*	8, 21
MEW305	*serA*::λ*plac*Mu9	Z. Q. Shao
MEW311	MEW305 Δ*ara714*	6
MEW402	MEW1 *metK84*; leucine-requiring	This work
MEW403	MEW1 *metK84 lrp*::*lacZ*	This work

Photomicrographs were taken with a WildMPS45 camera, top-mounted on the microscope.

RESULTS

Leucine requirement in an unsuppressed *metK84* mutant. The *metK84* mutant RG62 was isolated by virtue of its ethionine resistance; it is also resistant to GGME and has a lower SAM pool (8). We showed that this strain at some point in its history had acquired an *lrp* mutation which conferred faster growth in glucose (21). In fact, the original RG62 isolate grew poorly on minimal glucose plates but faster in the presence of 5 mM leucine (8, 21), conditions in which Lrp exerts weaker regulation on many of its operons (29, 30). These observations suggested that a *metK84* single mutant might require L-leucine for growth; as a result, we reconstructed such a strain by cotransduction, using phage P1 grown on MEW30 *metK84* to transduce strain MEW311 (*serA*::λ*plac*Mu) to serine independence and selecting on minimal medium containing glucose, isoleucine, valine, and L-leucine (100 μg/ml). We found several leucine-requiring GGME-resistant strains and studied one further, strain MEW402. We grew strain MEW402 in liquid culture with concentrations of leucine ranging from 0 to 50 μg/ml and found that even 25 μg/ml was limiting for growth. This requirement is not likely to reflect a failure in leucine biosynthesis since biosynthetic mutants require less than 10 μg of leucine per ml (4). We suggest that this high leucine requirement reflects the need to inactivate Lrp when MetK84 activity becomes limiting, because Lrp either represses a gene whose expression becomes essential under these conditions or activates a gene whose expression becomes harmful.

Filamentation in leucine-starved *metK84* mutants. We have previously shown that the doubling time of *metK* strains in minimal medium is considerably shorter in the presence of leucine (85 min) than in its absence (>120 min). Nevertheless, these strains accumulate leucine-independent derivatives (data not shown). Experiments involving strain MEW402 were therefore done with recently constructed strains. When a fresh transductant was inoculated into glucose minimal medium and examined 14 to 18 h later, little or no growth was seen without leucine present, whereas the culture with 50 μg of leucine per ml grew to the same density as the leucine-nonrequiring parent strain. Cultures grown with 25 μg of leucine per ml had lower densities than those grown with 50 μg/ml; they also showed a striking morphological change.

As shown in the photomicrographs (Fig. 1), the culture grown with 25 μg of leucine per ml contained a large proportion of very long filaments, some as long as 100 μm, i.e., 50 times the normal *E. coli* cell length; shorter filaments and cells of normal cell length were also seen. Cultures grown with 50 μg of leucine per ml consisted mainly of normal-length cells with a few filaments, occasionally very long. These filaments could also be observed in cultures grown with limiting leucine in the presence of GGME.

Linkage of the determinant for leucine requirement and filamentation to *serA*. Strain MEW402 showed three phenotypes: GGME resistance, a leucine requirement, and filamentation on starvation for leucine. GGME resistance was highly linked to *serA*. In a transduction of *metK* into DRN-1 (*serA*::*lacZ*), selecting serine independence as described above, 10 of 46 colonies tested from the transduction plates grew in the presence of GGME. This linkage is in reasonable agreement with the known map positions of *metK* and *serA* (66.4 and 65.8 min, respectively). We purified the 46 transductants on the same minimal glucose plates supplemented with leucine. After three purifications on the same medium, three of the GGME-resistant transductants required leucine. It is clear that the leucine requirement is also caused by a gene linked to *serA*.

To show that the filamentation of strains grown with leucine limitation was linked to *metK*, we verified that strain MEW402 would produce filaments even in the presence of 500 μg of GGME per ml and then inoculated the 10 GGME-resistant strains directly from the colonies on the transduction plates into liquid minimal medium with isoleucine and valine, 500 μg of GGME per ml, and a limiting concentration of leucine, 10 μg/ml. We found some long filaments in 9 of the 10 cultures tested, although most of the cells were normal. It is clear that filamentation is also linked to *serA*.

The preceding linkage data are generally consistent with leucine auxotrophy and filamentation being due to the *metK* mutation. The reason that the expected 100% linkage of GGME resistance to these phenotypes was not obtained is likely to reflect the rapid appearance of suppressors during purification of transductants. As a further test to show that filamentation is due to the *metK* mutation, we wanted to determine whether a plasmid providing *metK* function could prevent filamentation and restore normal growth.

Suppression of filamentation by a plasmid-carried *metK* gene. To determine whether a *metK*$^+$ plasmid could suppress filamentation, we grew strain MEW402(pBAD*metK*) in glucose minimal medium with 50 μg of leucine per ml and 200 μg of ampicillin per ml, subcultured the strain in the same medium for 4 h with fresh ampicillin, and then subcultured it in glucose minimal medium with ampicillin and with 0, 10, 25, and 50 μg of leucine per ml, each with and without 500 μg of arabinose per ml.

Cultures without arabinose grew well with 50 μg of leucine per ml but produced much less dense cultures consisting mainly of long filaments at lower concentrations of leucine or completely without it. All cultures with arabinose grew to the usual density of *E. coli* overnight cultures (whatever the leucine concentration used) and consisted entirely of normal-size, nonfilamentous cells. It is clear that even in the presence of glucose, 500 μg of arabinose per ml can induce sufficient *metK* gene product to restore normal growth. To verify that the arabinose cultures were still composed of mutant cells, we plated the culture grown with glucose, 10 μg of leucine per ml, and 500 μg of arabinose per ml on LB medium, replicated the culture on LB medium plus ampicillin, picked one of the very rare ampicillin-sensitive colonies, and noted the filaments it made when grown with glucose and 10 μg of leucine per ml.

Suppression of filamentation by Lrp deficiency. We showed earlier that Lrp deficiency restores a normal growth rate to the *metK* mutant. To determine whether the other phenotypes were also suppressed, we transduced *lrp*::*lacZ* from strain MEW45 into strain MEW402, producing strain MEW403 *lrp*::

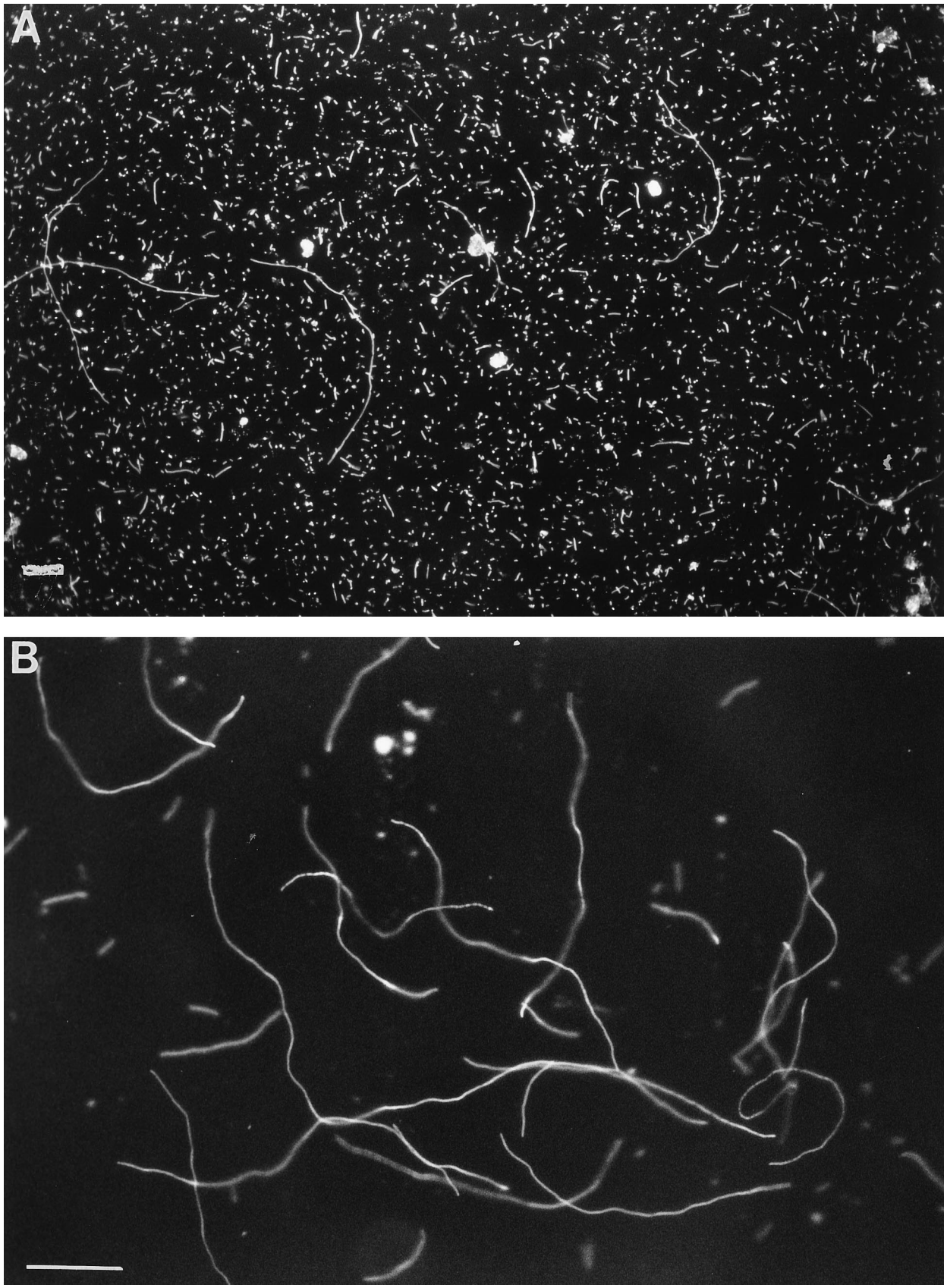

FIG. 1. Filaments of the *metK84* mutant grown under limiting leucine conditions. The photomicrographs of strain MEW402 were taken under dark-field illumination. (A) Culture growing with 50 μg of leucine per ml; bar, 20 μm. (B) Culture growing with 10 μg of leucine per ml; bar, 10 μm.

TABLE 2. SAM synthetase activity of *metK* and *lrp* mutants

Medium[a]	SAM synthetase activity (nmol/min/mg of protein) in:			
	WT[b]	*lrp*	*metK84*	*lrp metK84*
−Leucine	2.3	4.6	0.04	0.28
+Leucine	2.9	6.3	0.26	0.29

[a] Cultures were grown in glucose-minimal medium with (+) and without (−) 100 μg of leucine per ml, and SAM synthetase was assayed. The cultures assayed were also examined with a microscope; all cells were short rods, except for cells of the *metK84* mutant grown without leucine, which consisted mainly of filaments. The cultures used were MEW1 (wild type [WT]), MEW45 (*lrp*::*kan*), MEW402 (*metK84*), and MEW403 (*lrp*::*kan metK84*).

lacZ metK. This strain had no leucine requirement and showed no filaments.

Effects of leucine and Lrp on SAM synthetase activity. A simple explanation of the Lrp effect on the *metK84* mutant is that Lrp represses the *metK* gene, so that the absence of Lrp or the presence of leucine in the medium results in higher levels of SAM synthetase, which, in the case of the mutant enzyme, could have a significant effect on the SAM pool. To test this hypothesis, we assayed SAM synthetase activity in extracts of the *metK* and *lrp* mutants, a *metK lrp* double mutant, and our parental strain, grown in the presence or absence of leucine.

It is clear that SAM synthetase is induced either by leucine or by an *lrp* mutation (Table 2). The effects are small in the parental strain but large in the *metK* mutant, for which either condition increased SAM synthetase sevenfold. It seems that a SAM synthetase level over 0.25 nmol/min/mg of protein suffices for normal growth and that the parental strain produces a considerable excess of this enzyme. In the *metK84 lrp* double mutant, a level of 0.28 nmol/min/mg of protein is reached without leucine and not further increased in cells grown with leucine.

Nucleoid partioning in *metK* filaments. *E. coli* populations produce filaments in response to a variety of mutations and environmental problems, although few of the filaments are as long as those shown in Fig. 1A. If the primary defect in the *metK84* mutant involves DNA replication, while protein synthesis and cell wall elongation continue, one would expect to see long filaments with few nucleoids, as in filaments in which DNA synthesis has been blocked (2, 17, 28). If DNA synthesis is normal but septum formation is specifically inhibited, one would expect to see many evenly spaced nucleoids, with or without constrictions at the presumptive division site (18, 35).

To investigate nucleoid partitioning in the *metK84* filaments, we took samples from cultures grown in glucose minimal medium with 25 or 50 μg of leucine per ml and fixed them for fluorescence microscopy. All filaments showed partitioned nucleoids (Fig. 2), indicating that DNA replication and nuclear segregation continue.

DNA methylation in the *metK84* strain MEW402. *E. coli* DNA is normally methylated at GATC sequences by the Dam methylase (25). Although DNA synthesis continues in the *metK84* mutant, when SAM synthetase activity is limiting, the DNA might be undermethylated, as has been observed when the SAM pool is lowered by expression of a SAM hydrolase (14). We tested this by transforming *metK84* cells with pBR322, a multicopy plasmid containing 22 GATC sites (3). We isolated plasmid DNA from cells grown with 25 μg of leucine per ml, verified microscopically that the culture contained filaments, and showed that plasmid DNA was cut by restriction endonuclease *Dpn*I, which cuts only methylated GATC sequences, and by *Sau*3, which cuts any GATC site, but not by *Mbo*I, which cuts only nonmethylated GATC sites. We conclude that *metK84* cells methylate most of their GATC sequences and thus are not totally starved for SAM.

DISCUSSION

In this report, we present a new phenotype associated with decreased SAM synthetase activity in *E. coli*, a partial cell division defect resulting in the formation of long filaments, more than 50 times the normal cell length (Fig. 1). The filaments contain nucleoids evenly dispersed along their length (Fig. 2), suggesting that they have no problem in synthesizing and partitioning DNA but they do not form cross walls. The parental phenotype (growth as small rods) is restored by expression of *metK* from a plasmid-carried gene. This suggests that SAM is involved in septation.

Regulation of *metK* expression. Leucine has a strong effect on the metabolism of the *metK84* mutant, which is partially defective in SAM synthetase. A nearly normal growth rate can be restored to the *metK84* mutant by the presence of leucine in the medium or by the loss of Lrp, as previously reported (8, 21). We show here that filamentation, too, is suppressed by exogenous leucine or by an *lrp* mutation. Growth rate, leucine independence, and a normal cell size were also restored by expression of *metK* carried on pBR322 under control of the arabinose promoter.

Slow growth and filamentation are observed in the *metK84* mutant when SAM synthetase levels are extremely low, and suppressing conditions, i.e., the presence of leucine or the absence of Lrp, increase these levels more than sixfold. From this correlation, we conclude that SAM synthetase at 0.26 nmol/min/mg of protein is sufficient for normal growth and division, whereas a level of 0.04 nmol/min/mg of protein is insufficient. Our results can thus be explained in terms of the regulation of SAM synthetase activity.

The increased SAM synthetase activity observed in *metK84* strains in the presence of leucine or in the absence of Lrp could reflect the expression, under these conditions, of a second SAM synthetase, and indeed the existence of such an enzyme, a product of the *metX* gene, has been suggested (33). However, a BLAST search (1) revealed no *metK* homolog in the *E. coli* genome, a significant result given the high conservation of SAM synthetases (e.g., *E. coli* MetK is 55.7% identical and 70% similar to the human enzyme). We conclude that there is only one *E. coli* gene coding for SAM synthetase, *metK*.

The observed variations in SAM synthetase activity must therefore reflect regulation of the *metK* gene. The simplest explanation of our results is that Lrp represses *metK* expression and leucine antagonizes Lrp, analogous to the action of Lrp and leucine on *sdaA* expression (21). This hypothesis is reinforced by the observation that loss of Lrp increases SAM synthetase activity in wild type cells as well (Table 2).

In this work, we tried to decrease SAM by decreasing SAM synthetase. This was done earlier by expression of a cloned SAM hydrolase (14). Cells became elongated and occasionally filamentous, but no leucine requirement was found. The authors concluded that SAM plays a direct or indirect role in cell division.

Role of SAM in cell division. Why a low SAM level leads to a cell division block is unclear. This could be a direct or indirect effect on cell division. One possibility involves the elongation factor Tu, discovered for its role in translation but involved in other processes as well. EF-Tu associates with the cell membrane (16). Part of the EF-Tu population is methylated at Lys56 (22), and the membrane-associated molecules are preferentially methylated under starvation conditions (36). Furthermore, certain mutants with altered EF-Tu form fila-

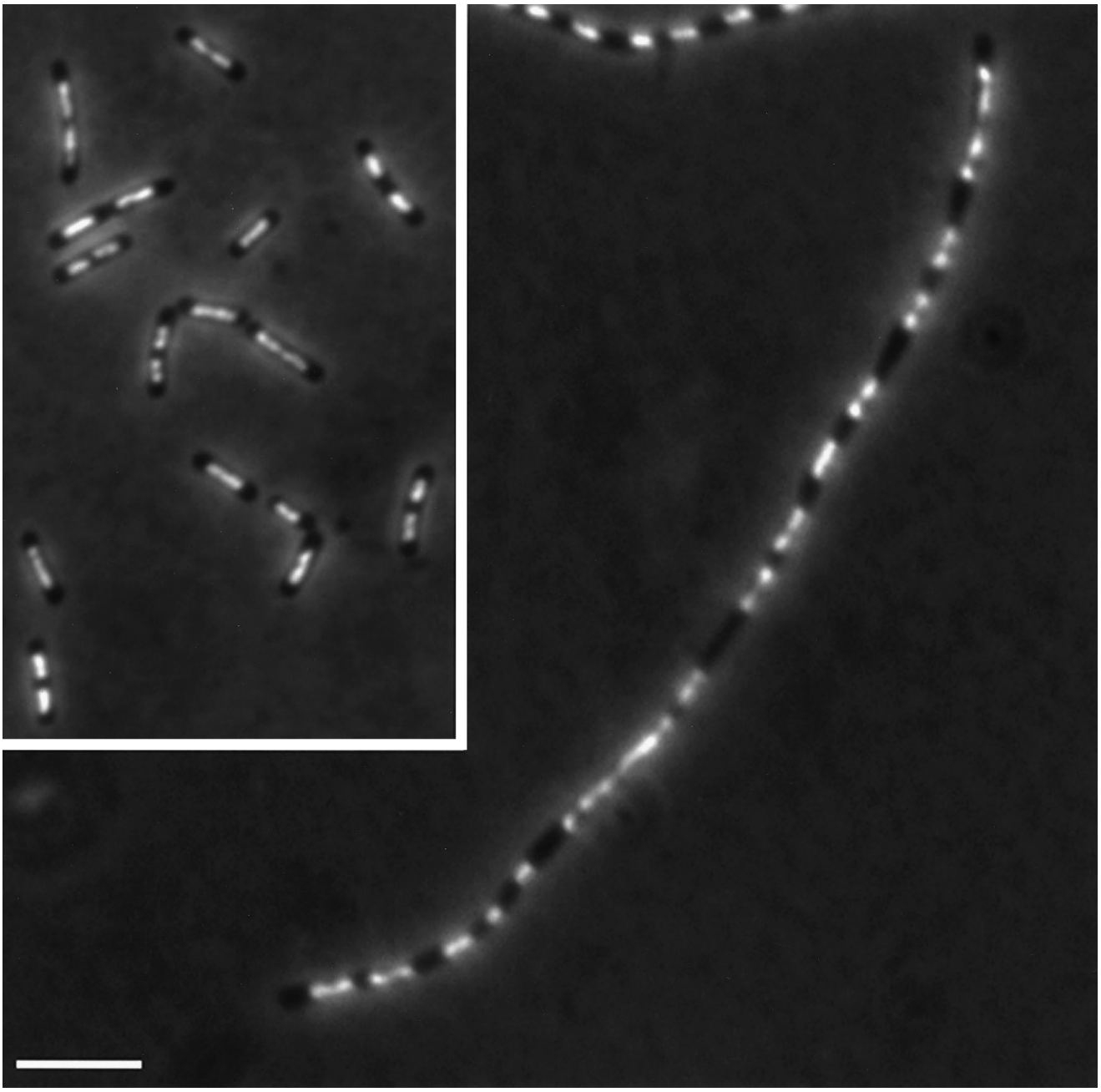

FIG. 2. Nucleoid segregation in *metK* filaments. A culture of strain MEW402 was grown for 19 h with 25 μg of leucine per ml and prepared for fluorescence microscopy as described in Materials and Methods; control cells grown with 50 μg/ml are shown in the inset. It can be seen that the nucleoids are segregated uniformly along the length of the filament. Most nucleoid regions are in the form of a dumbbell, which presumably represents two not fully separated chromosomes. Analysis of the relative amount of DNA per individual nucleoid region by integrated density measurement shows that this filament contains 16 nucleoid equivalents. Bar, 5 μm.

ments (37). A role of methylated EF-Tu in cell division is conceivable.

Another possibility for a role of SAM in cell division is as methyl donor at some particular step of the division process. The *ftsJ* gene product has a SAM-binding motif and might be a methyl transferase involved in cell division (32). The early steps in septation involve the formation of rings of the tubulin-like FtsZ protein, in association with the membrane protein ZipA and a number of other division proteins, leading to constriction and separation of the daughter cells (12, 23). Any of these division proteins might be activated by methylation, making a SAM-dependent step in division. While many cell division functions have been identified genetically in *E. coli*, at present there are no data on their methylation status. We are trying to determine the precise division step at which the *metK84* filaments are blocked, and we are investigating the possibility that some division protein may be methylated.

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