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The new gene *mukB* codes for a 177 kd protein with coiled-coil domains involved in chromosome partitioning of *E.coli*

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An *Escherichia coli* temperature sensitive mutant which produces spontaneously normal size anucleate cells at low temperature was isolated. The mutant is defective in a previously undescribed gene, named *mukB*, located at 21 min on the chromosome. The *mukB* gene codes for a large protein (~180 kd). A 1534 amino acid protein (176 826 daltons) was deduced from the nucleotide sequence of the *mukB* gene. Computer analysis revealed that the predicted MukB protein has distinct domains: an amino-terminal globular domain containing a nucleotide binding sequence, a central region containing two α-helical coiled-coil domains and one globular domain, and a carboxyl-terminal globular domain which is rich in Cys, Arg and Lys. A 180 kd protein detected in wild-type cell extracts by electrophoresis is absent in *mukB* null mutants. Although the null mutants are not lethal at low temperature, the absence of MukB leads to aberrant chromosome partitioning. At high temperature the *mukB* null mutants cannot form colonies and many nucleoids are distributed irregularly along elongated cells. We conclude that the MukB protein is required for chromosome partitioning in *E.coli*.

Key words: chromosome/*E.coli*/new gene/*mukB*/partition

Introduction

Replicated chromosomes are spatially separated prior to cell division in bacteria, and each daughter cell contains at least one copy of the chromosome. Anucleate cells represent <0.03% of the cell population of wild-type strains (Hiraga *et al.*, 1989). The molecular mechanism of chromosome partitioning into daughter cells is unknown. In *Bacillus subtilis* nucleoid segregation does not take place progressively during the cell cycle but occurs abruptly prior to cell division, as the replicated nucleoids 'jump' to the quarter positions, which will become the centers of the daughter cells (Sargent, 1974). It has recently been reported that in *Escherichia coli*, the positioning of nucleoids at the quarter positions requires post-replicational protein synthesis (Donachie and Begg, 1989; Hiraga *et al.*, 1990). When protein synthesis is inhibited, replicating chromosomes complete their replication but remain at mid-cell, close to each other. The nucleoids can move from mid-cell to the quarter positions without detectable cell elongation upon resumption of protein synthesis. These results are inconsistent with the model proposed by Jacob *et al.* (1963) according to which the partitioning of chromosomes is carried out by a physical connection between a DNA strand and part of the cell envelope, and in which the driving force for chromosome segregation is provided by insertion of new cell envelope material at mid-cell, i.e. between the two chromosome attachment sites. This model is inconsistent with experimental results showing that during most of the *E.coli* cell cycle, growth of the cell wall takes place by random insertion over the entire surface, although during septum formation peptidoglycan precursors are inserted preferentially at mid-cell (Woldringh *et al.*, 1987).

If the positioning of chromosomes at cell quarters is actively controlled by gene products, one should be able to isolate mutants affected in genes involved in this mechanism. A hypothetical mutant defective in chromosome positioning at cell quarters should be non-lethal. In such mutants, replicated chromosomes would be normally decatenated, but they would tend to remain close to each other at the position where the chromosome was replicated. Therefore, one daughter cell with two chromosomes and one anucleate daughter cell would be produced by cell division at a non-negligible frequency (Hiraga *et al.*, 1989). To analyze genetically the mechanism of chromosome positioning, we developed techniques to isolate non-lethal mutants producing anucleate cells during cell division, and we have isolated

Table I. Bacterial strains

Strain	Relevant genotype	Reference, source or construction
W3110	Prototroph	Kohara *et al.* (1987)
SH3208	Δ*trpE5 his* (λ)	Hiraga *et al.* (1989)
SH3210	Δ*trpE5 his* (λ) (pXX747)	Hiraga *et al.* (1989)
SH3306	Δ*trpE5 his mukB106* (λ)(pXX747)	From SH3210
SH3367	Δ*trpE5 his mukB106* (λ)	From SH3306
SH3910	Δ*trpE5 his mukB106 zcb*::Tn*10* (λ)	P1*vir*/ME8307→SH3367
SH3943	*recD* (pAX804)	pAX804 DNA→FS1576
SH3929	*recD* Δ*mukB*::*kan* (pAX804)	DNA[a]→SH3943
PB103	*dadR trpE61 trpA62 tna-5*	de Boer *et al.* (1989)
GC7470	The same as PB103 except for *zcb*::Tn*10 mukB106*	P1*vir*/SH3910→PB103
GC7471	The same as PB103 except for *zcb*::Tn*10*	P1*vir*/SH3190→PB103
GC7466	*sfiA sfiC zcb*::Tn*10 mukB106*	P1*vir*/SH3910→B1686
GC7467	*sfiA sfiC zcb*::Tn*10*	P1*vir*/SH3910→B1686
GC7476	The same as PB103 except for *mukB106 sfiA*	P1*vir*/B1686→GC7470
GC7477	The same as PB103 except for *sfiA*	P1*vir*/B1686→GC7470
GC7478	*zcb*::Tn*10 mukB106 ilv*::Tn*5*	P1*vir*/GC7064→GC7470
GC7479	*zcb*::Tn*10 mukB106 ilv*::Tn*5* Δ*cya*	P1*vir*/GC7064→GC7470
GC7528	The same as PB103 except for Δ*mukB*::*kan*	P1*vir*/SH3929→PB103

[a]The *Bam*HI–*Bam*HI DNA segment of pAX922 contains the disrupted *mukB* gene (see text).

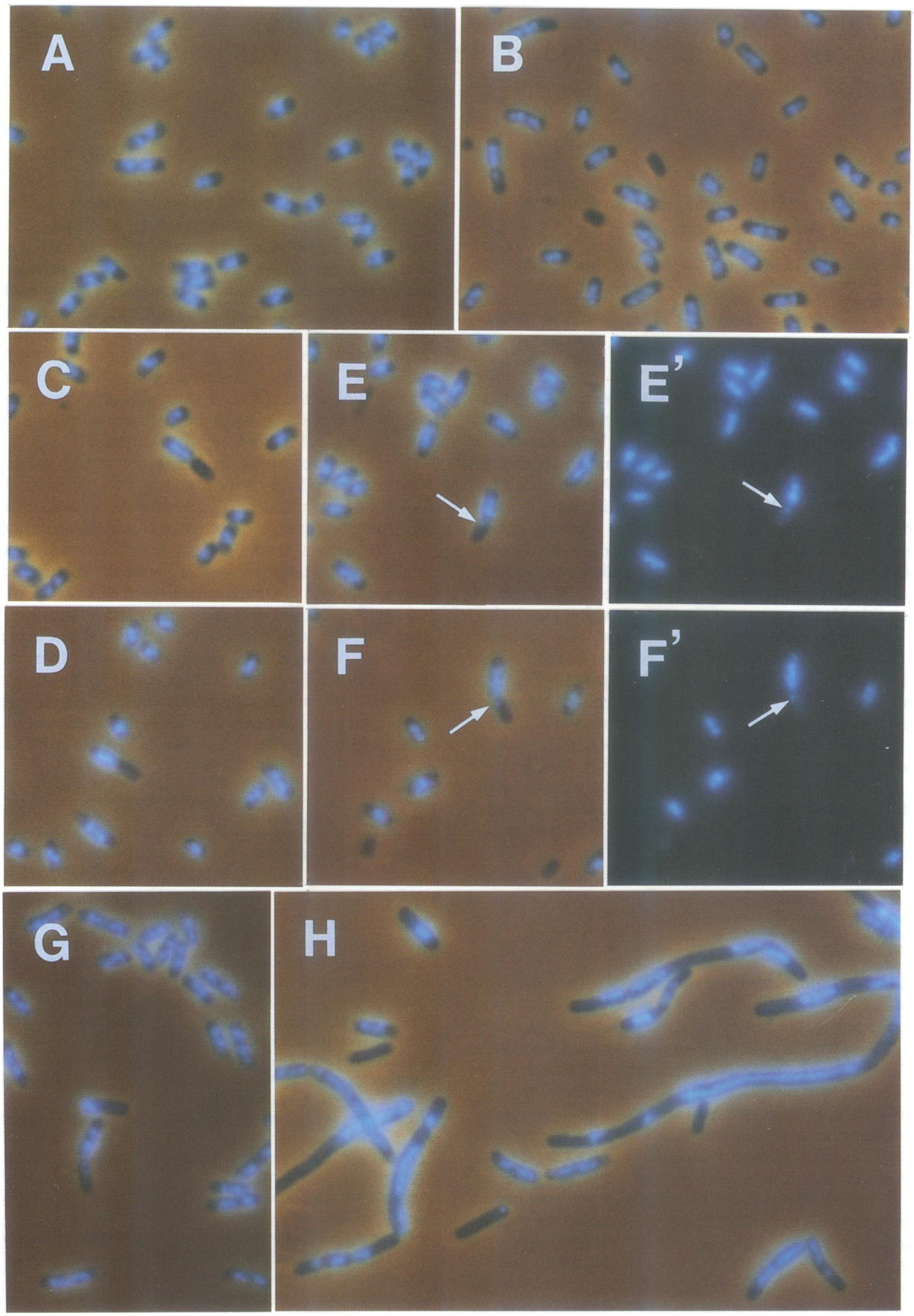

Fig. 1. Photographs of *mukB*$^+$, *mukB106* and Δ*mukB* strains. Cells grown in medium E containing glucose (0.5%), casamino acids (0.4%) and L-tryptophan (50 μg/ml) were photographed after DAPI staining by Hiraga's fluophase combined method (Hiraga *et al.*, 1989). (**A**) The *mukB*$^+$ strain SH3208 was incubated at 22°C. (B–F) The *mukB106* mutant SH3367 was incubated at 22°C. (**C** and **D**) A pair of cells: one nucleate cell and

one anucleate cell. (**E** and **F**) A pair of cells: one nucleate cell and one cell having a small amount of DNA. (**E'** and **F'**) Fluorescence profiles (using only UV light) of cells corresponding to E and F. (**G**) The *mukB* null mutant GC7528 grown at 22°C. (**H**) The *mukB* null mutant incubated for 4 h at 42°C.

many mutants showing these expected properties. One such mutant, *mukA1*, was characterized in detail; it exhibited the above properties and was located in the *tolC* gene (Hiraga *et al.*, 1989) coding for an outer membrane protein. The results suggest that the *mukA* gene product is related directly or indirectly to the mechanism of chromosome positioning; as expected, its gene product is not essential for cell division.

In the present paper, we describe another *muk* mutant defective in a new gene, named *mukB*. The results indicate that the *mukB* gene codes for a 177 kd protein. The MukB protein is required for orderly chromosome partitioning to daughter cells. Computer analysis of the amino acid sequence deduced from the nucleotide sequence of the *mukB* gene revealed that the MukB protein has distinct secondary structural domains. There is a nucleotide binding consensus sequence in the amino-terminal globular domain. The central region contains α-helical coiled-coil domains, as in rod regions of myosin heavy chain (McLachlan and Karn, 1983) and kinesin heavy chain (Yang *et al.*, 1989) in eukaryotic cells. The carboxyl-terminal domain, which is larger than the amino-terminal domain, is globular and has characteristic structures. Predicted functions of the MukB protein are discussed.

Results

Isolation and properties of the mukB106 mutant

Using previously described techniques, we isolated mutants which frequently produced anucleate cells during growth (Hiraga *et al.*, 1989). One of these, the *mukB106* mutant (SH3367; see Table I), grew nearly normally but produced spontaneously ~5% normal size anucleate cells in exponentially growing cultures in enriched minimal medium at 22 or 30°C (Figure 1B). In contrast, anucleate cells represent <0.03% of total cells in the *mukB*$^+$ control strain SH3208 (Figure 1A). In the *mukB106* mutant, in addition to anucleate cells there were pairs of cells: one anucleate cell with ~1 unit of cell length and one nucleate cell of 1−2 units (Figure 1B, C and D) as observed in the *mukA1* mutant (Hiraga *et al.*, 1989). The nucleate in a pair of this type had about twice as much DNA as did newborn nucleate cells. This is consistent with the speculation that chromosome replication is arrested for one generation in a daughter cell which received two daughter chromosomes, until the next cell division occurs (Hiraga *et al.*, 1989). There is another type of pairs of cells: one nucleate cell with about two copies of chromosomal DNA and one cell having small amounts (5 to ~20%) of chromosomal DNA at the cell pole near the division site (Figure 1E, E', F and F'), suggesting a guillotine effect by septal closure on nucleoids.

Cultures of the *mukB106* mutant growing at 37°C contain nucleate cells of normal cell size, elongated cells with many nucleoids, and anucleate cells. Anucleate cells represent 10−20% of the total cell population. The majority of anucleate cells have a length similar to that of newborn nucleate cells. When a culture of the *mukB106* mutant grown at 22°C was transferred to 42°C, cells elongated and many nucleoids were irregularly distributed along the cells. The nucleoid distribution was quite different from that of filamentous cells of temperature sensitive *ftsA* and *ftsI* mutants, defective in septum formation at 42°C. In *ftsA* and *ftsI* filaments, nucleoids were regularly located at constant distances from each other (data not shown; for a review see Donachie and Robinson, 1987). These results suggest that the *mukB106* mutant is defective in chromosome positioning from 22 to 42°C, and that it is partially defective in cell division at high temperature.

The *mukB106* mutant formed minute colonies after overnight incubation on L agar plates at 42°C. Using this temperature sensitivity, the *mukB106* mutation was mapped at 21 min on the *E.coli* chromosome by analysis of cloned chromosomal DNA segments complementing the *mukB106* mutation (see below) and by transduction with phage P1*vir*. The *mukB106* mutation was cotransduced to strain PB103 with the *zcb*::Tn*10* marker located at the 21 min region (43% cotransduction). One *mukB106* transductant, GC7470, exhibited the same properties as the original *mukB106* mutant SH3367.

A *mukB106 sfiA* strain (GC7476) and a *mukB106 sfiA sfiC* strain (GC7466), defective in the SOS dependent cell division inhibitors, produced anucleate cells at similar frequencies to the isogenic *mukB106 sfiA*$^+$ strain. In addition, a *mukB106* Δ*cya* strain (GC7479) also produced anucleate cells as well as the isogenic *mukB106 cya*$^+$ strain (GC7478), indicating that anucleate cell formation in the *mukB106* mutant is independent of cAMP. This is in contrast to anucleate cell formation following DNA synthesis blocks (Jaffé *et al.*, 1986). These results suggest that the production of anucleate cells in the *mukB106* mutant is not due to inhibition of DNA replication. Flow cytometric analysis of the *mukB106* mutant according to Boye and Løbner-Olsen (1990) showed that nucleate cells had 2, 4 or 8 chromosome origins (E.Boye, personal communication) indicating that all origins present are initiated simultaneously (Skarstad *et al.*, 1988).

Cloning of the chromosomal DNA segment complementing the mukB106 mutation

To clone the *mukB*$^+$ gene, a chromosomal DNA library from the wild-type strain W3110 was prepared using the cosmid vector pHSG262. After *in vitro* packaging of DNA into λ phage particles, the phage library was used to infect the *mukB106* cells (SH3367), and kanamycin resistant temperature resistant colonies were isolated. Plasmid DNA extracted from six such colonies was analyzed with restriction endonucleases. These plasmids had a common DNA segment located in the 988−1020 kb region (physical coordinate) on the restriction map of *E.coli* (Kohara *et al.*, 1987; Médigue *et al.*, 1990), consistent with the results of P1 transduction described above. One of these cosmid clones, pλH131 carrying a *Sau*3AI−*Bam*HI chromosomal segment of ~36 kb, was chosen for further experiments. The *Sau*3AI site of the chromosome became a *Bam*HI site upon insertion into the *Bam*HI site of the vector pHSG262 (Figure 2A).

To determine the precise location of the *mukB* gene, the *Bam*HI subsegments of pλH131 were cloned in plasmid pACYC184. The resulting plasmids, pAX801 and pAX804,

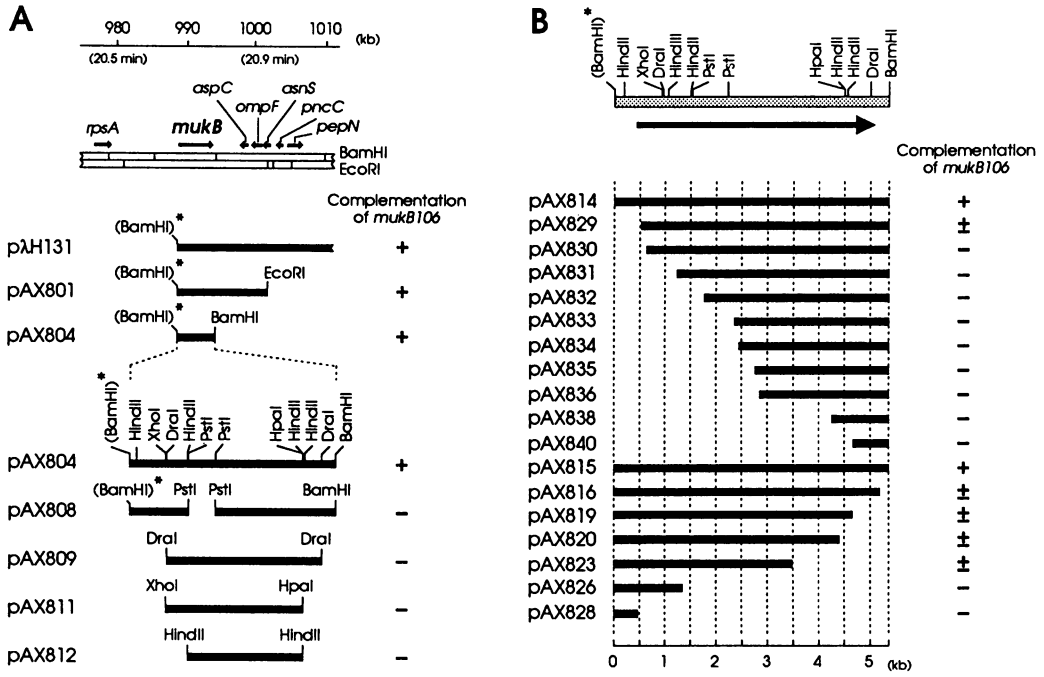

Fig. 2. Cloning of the *mukB* gene. (**A**) Upper lines represent part of the *E.coli* chromosome restriction map described by Kohara *et al.* (1987). Scales are expressed in kilobase coordinates. Cosmid pλH131 carries the *Sau*3AI–*Bam*HI chromosomal DNA segment (5.4 kb) of the wild-type strain W3110 described in the text. The *Sau*3AI site was changed to a *Bam*HI site by joining with the *Bam*HI site of the vector pHSG262. The *Bam*HI site created is represented by an asterisk in the solid bars. Plasmids pAX801 and pAX804 carry *Bam*HI chromosomal subsegments from pλH131. Plasmids pAX808, pAX809, pAX811 and pAX812 are deleted derivatives of pAX804. Each cosmid or plasmid was introduced into the *mukB106* mutant (SH3367), and transformants were tested for colony formation at 42°C on L agar plates. The open reading frame of the *mukB* gene and the direction of transcription are shown as an arrow. (**B**) Deleted derivatives of plasmids pAX814 and pAX815. Solid bars represent chromosomal DNA segments carried by the plasmids. +, ± and − represent full, partial and non-complementation, respectively. The coding frame and direction of transcription of the *mukB* gene are shown as a horizontal arrow.

complemented the temperature sensitivity of the *mukB106* mutant (Figure 2A). Plasmid pAX804 carried a 5.4 kb *Bam*HI(*Sau*3AI)−*Bam*HI DNA segment of the *E.coli* chromosome. The deleted plasmids, pAX808, pAX809, pAX811 and pAX812, derived from pAX804 did not complement the temperature sensitivity of the *mukB106* mutant (Figure 2A).

The 5.4 kb *Bam*HI(*Sau*3AI)−*Bam*HI segment was inserted into the *Bam*HI site of plasmid pUC118. The resulting plasmids, pAX814 and pAX815, carried this segment in opposite orientations, and both plasmids fully complemented the *mukB106* mutation (Figure 2B). Many deleted derivatives of pAX814 and pAX815 were isolated and tested for complementation of the *mukB106* mutation. A shown in Figure 2B, plasmid pAX829 deleted for 0.52 kb from the left *Bam*HI (*Sau*3AI) site, and plasmid pAX816, deleted for 0.17 kb from the right *Bam*HI site, no longer fully complemented the thermosensitivity of the *mukB106* mutant. These results suggested that the *mukB106* mutation lies in a large gene covering at least 4.5 kb of the 5.4 kb DNA segment carried by pAX814 and pAX815.

Detection of the mukB gene product

The *mukB* gene product was detected by the maxicell method, after [^{35}S]methionine labeling of strains carrying various plasmids. As shown in Figure 3A and B, a band of ~180 kd was detected in cells harboring the complementing plasmids. A smaller protein of ~170 kd was detected in the strain carrying the plasmid pAX820, which has a 0.95 kb deletion from the right *Bam*HI site. No high mol. wt band was detected in strains carrying the vector pUC118, pAX830 or pAX832 (see Figure 3A and B). A faint band of the 180 kd protein was detected in a strain carrying plasmid pAX804. A faint band of a smaller protein (~165 kd) was coded for by plasmid pAX820, which has an insertion of the *kan* segment in the *Hpa*I site (Figure 3A and B). No high mol. wt band protein was observed in strains carrying the vector pACYC184, pAX922 or pAX924. These results indicate that the 5.4 kb *Bam*HI(*Sau*3AI)−*Bam*HI segment encodes a protein of ~180 kd complementing the *mukB106* mutation. The mol. wt obtained from the mobility of the MukB protein is consistent with the predicted mol. wt (17,862 daltons) based on the nucleotide sequence described below. In addition, these results are consistent with the direction of transcription of the *mukB* gene deduced from the nucleotide sequence.

Characterization of a ΔmukB mutant

To analyze the properties of cells in which the chromosomal *mukB* gene is disrupted, we prepared the *Bam*HI linear DNA fragment containing the disrupted *mukB* gene (*ΔmukB::kan*) from plasmid pAX922 (Figure 3B). The DNA fragment was introduced into the chromosome of a *recD* mutant (strain FS1576) harboring the *mukB*$^+$ plasmid pAX804, taking advantage of the ability of *recD* strains to be transformed with linear DNA (Seelke *et al.*, 1987; Niki *et al.*, 1990). Kanamycin resistant transformants carrying a disrupted *mukB* gene as confirmed by Southern blot hybridization (data not shown), grew normally in the presence of the *mukB*$^+$ plasmid pAX804 in L medium at 30 and 42°C.

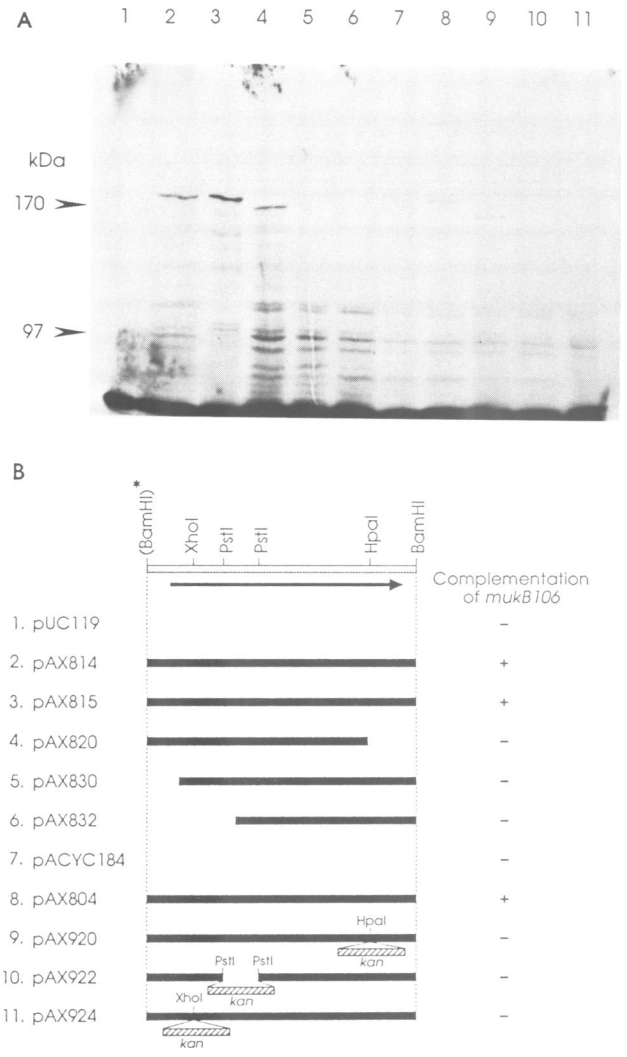

Fig. 3. Detection of the *mukB* gene product. (**A**) Detection of plasmid encoded proteins. Cells were irradiated by UV light and labeled with [^{35}S]methionine by the maxicell method described in Materials and methods. Cellular proteins were separated on SDS−polyacrylamide gels (5%) and analyzed by fluorography. The plasmids in lanes 1−11 are shown in (**B**). Molecular markers were α_2-macroglobulin (horse plasma, reduced form, 170 kd) and phosphorylase *b* (rabbit muscle, 97.4 kd). (**B**) Chromosomal segments carried by the plasmids. Plasmid pUC118 was used as vector to construct pAX814, pAX815, pAX820, pAX830 and pAX832. Plasmid pACYC184 was used as vector to construct pAX804, pAX920, pAX922 and pAX924. Solid bars represent the chromosomal segments carried by plasmids. Hatched bars represent the inserted kanamycin (*kan*) segment (1.3kb).

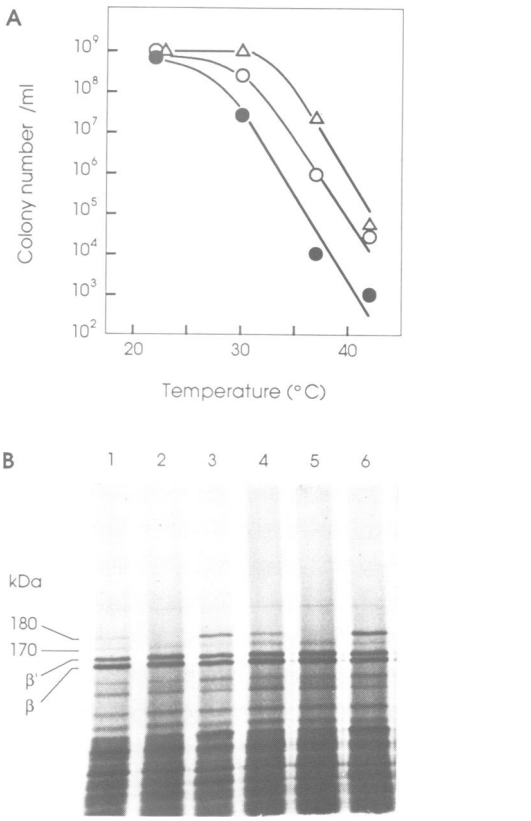

Fig. 4. Properties of the *mukB* null mutant. (**A**) Colony forming ability of the *mukB* null mutant at various temperatures. Cultures of the Δ*mukB* strain GC7528 were grown in three different media at 25°C to OD_{600} = 0.4. They were plated on the corresponding agar media and incubated at 22, 30, 37 and 42°C. ●, LB agar; ○, enriched M63 agar containing glucose (0.4%), casamino acids (0.4%) and L-tryptophan (50 μg/ml); △, M63 agar containing glucose (0.4%) and L-tryptophan (50 μg/ml). The parental *muk*$^+$ strain PB103 has a plating efficiency of 1 in all conditions. (**B**) Lack of the 180 kd protein in cell extract of the *mukB* null mutant. Growing cells were labeled with [^{35}S]methionine for 1 h at 37°C. Cell proteins were separated on an SDS−polyacrylamide gel (5%) and analyzed by autoradiography. Lanes 1 and 4, the *mukB*$^+$ strain PB103; lanes 2 and 5, the *mukB*-disrupted strain (GC7470); lanes 3 and 6, PB103 carrying pAX804. The 180 and 170 kd proteins and the β′ and β subunits of RNA polymerase are indicated by arrows.

The DNA fragment containing the Δ*mukB::kan* gene was then transduced into the plasmid-free *mukB*$^+$ strain PB103. At 22°C, kanamycin resistant transformants were obtained after 3 days incubation on L plates, but no transductants were obtained at 42°C. One transductant (GC7528) was purified at 22°C and characterized. Analysis of total [^{35}S]methionine labeled cell protein from the *mukB*$^+$ strain (PB103) and the Δ*mukB::kan* strain (GC7528) by gel electrophoresis revealed that the 180 kd protein band observed in the *mukB*$^+$ strain was absent in the cell extract of the *mukB*-disrupted strain (Figure 4B). Bands of a 170 kd protein and an ~200 kd protein were observed in both strains. These results strongly suggest that the *mukB* gene codes for a protein ~180 kd (correctly 176 862 daltons as described below). No other protein showing the same mobility as the MukB protein was detected in cell extracts of the *mukB* null mutant by SDS−PAGE.

Colony forming ability of the Δ*mukB::kan* strain GC7528 was tested on different media at different temperatures. As shown in Figure 4A, it decreased strikingly in all media as the temperature increased. The *mukB*-disrupted cells (strain GC7528) grown at 22°C in enriched minimal medium are nearly normal in size, but ~5% anucleate cells are produced in growing cultures (Figure 1G). When the *mukB*-disrupted cells grown at 22°C were transferred for 4 h to 42°C, 10−20% anucleate cells nearly normal in cell size, elongated cells with a single large conglomerated nucleoid, and elongated cells with irregularly located nucleoids were observed (Figure 1H). These results suggest that the *mukB* gene product is required for chromosome positioning from 22 to 42°C, and that the gene product is not essential at least at 22°C but it is required for cell division at higher temperatures.

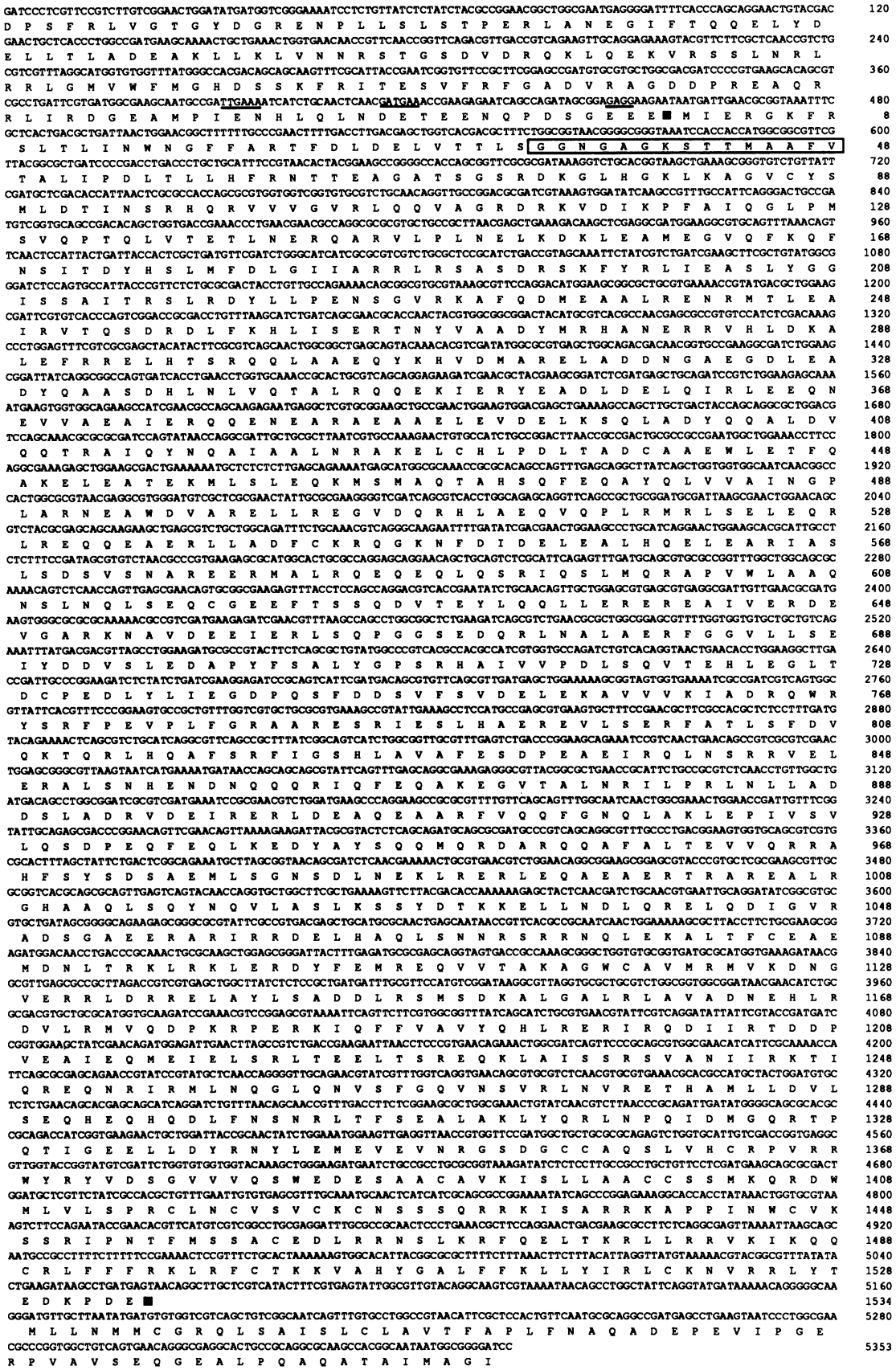

Fig. 5. Nucleotide sequence and the predicted amino acid sequence of the 5353 bp *Sau*3AI–*Bam*HI chromosomal DNA segment containing the *mukB* gene. The nucleotides and the corresponding amino acids of the MukB protein are numbered on the right. The boxed amino acids represent a possible ATP binding sequence. Solid squares represent stop codons. The putative promoter region (−35 to −10) and ribosome binding site sequence of the *mukB* gene are underlined.

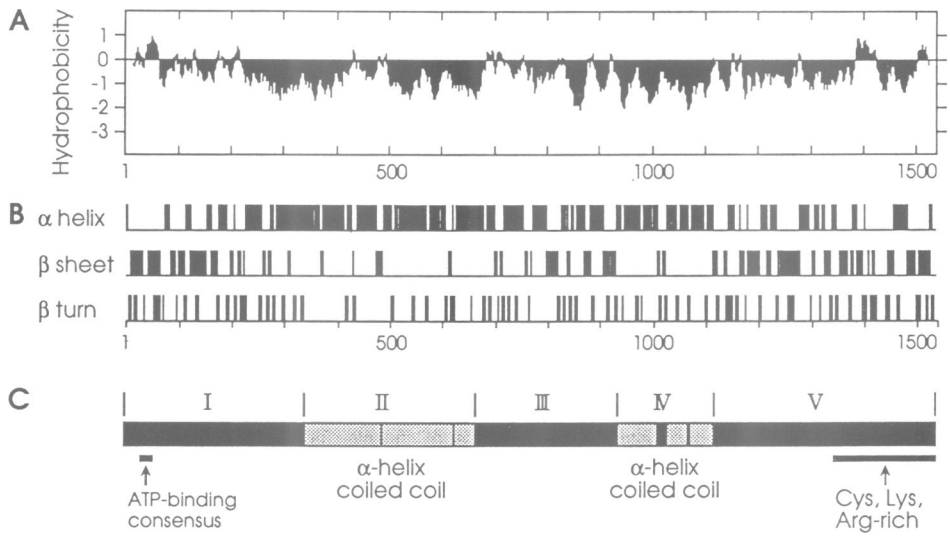

Fig. 6. Hydrophobicity profile and predicted secondary structure of the MukB protein. (**A**) A hydrophobicity profile of the MukB protein deduced by the DNA Strider program (Kyte−Doolittle Hydrophobicity, 4/23). (**B**) The possible secondary structure of the MukB protein predicted by GENETYX sequence analysis program (Chou−Fasman). Predicted structures are shown as vertical lines or solid boxes on each basal line. Random coil regions are not shown. Amino acid coordinates are numbered. (**C**) The predicted secondary structure of the MukB protein. Solid regions represent globular structure and shadowed parts represent extended α-helical coiled-coil structures with some disruptions (vertical lines). Five domains are numbered.

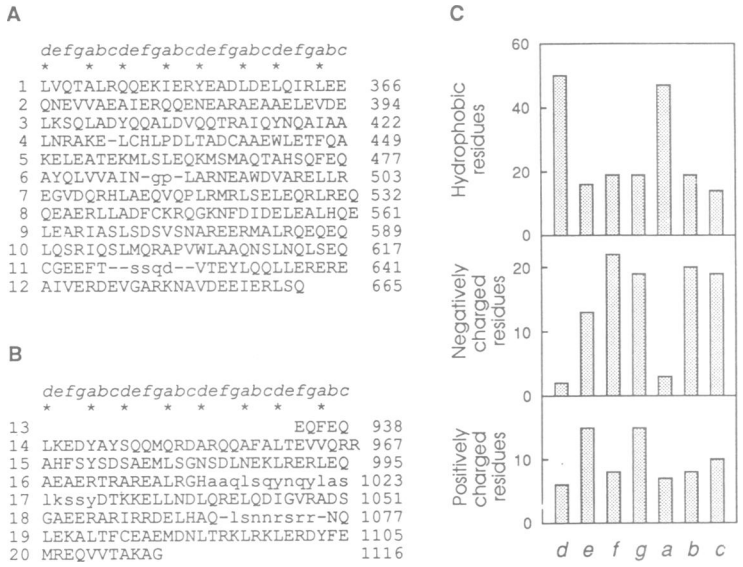

Fig. 7. Heptapeptide motifs of the MukB protein. (A and B) The regions of heptapeptide repeat sequences were at residues 339−665 (**A**) and at residues 934−1116 (**B**). The sequence is arranged with four seven-residue repeats in each row. The positions *d e f g a b c* of the seven-residue coiled-coil repeat are marked above the columns. Hydrophobic residues usually occupy positions *a* and *b*, which are marked with asterisks. The number on the right side indicates the residue number of these amino acids with respect to the protein sequence in Figure 5. There are a few disruptions of the heptapeptide repeats (small letters). Padding characters (hyphens) have been inserted in the outside of these disruptions in order to get better alignment. Extra residues have been inserted in the pattern in zones 7 and 8 in (A), and in zone 14 in (B). (**C**) Histograms of the distributions of hydrophobic residues (Ile, Val, Leu, Met, Phe, Tyr, Trp and Ala), negatively charged residues (Glu and Asp) and positively charged residues (Lys, Arg and His) in the heptapeptide repeats. The vertical axis represents the number of amino acid residues in each position of zones 1−20, except for disruptions (small letters).

Nucleotide sequence of the mukB gene

The entire nucleotide sequence (5353 bp) of the *Sau*3AI−*Bam*HI segment containing the *mukB* gene was determined (Figure 5). A large open reading frame of 4602 nucleotides initiating with methionine was found at nucleotide positions 459−5060. The deduced amino acid sequence has 1534 amino acid residues, with a mol. wt of 176 826 daltons. The most likely promoter of the gene has a −35 region beginning at nucleotide 392 and a −10 region at nucleotide 415. There is a suitable ribosome binding sequence at 448−451, upstream of the start codon.

A 456 bp open reading frame (position 1−456) lies upstream of the *mukB* gene, with the promoter proximal part outside of the sequenced segment. It is not clear at the present whether this open reading frame is translated. Another open reading frame was found downstream of the *mukB* gene. This frame has a possible start codon ATG at nucleotides 5164−5166 and potential −35 and −10 promoter sequences

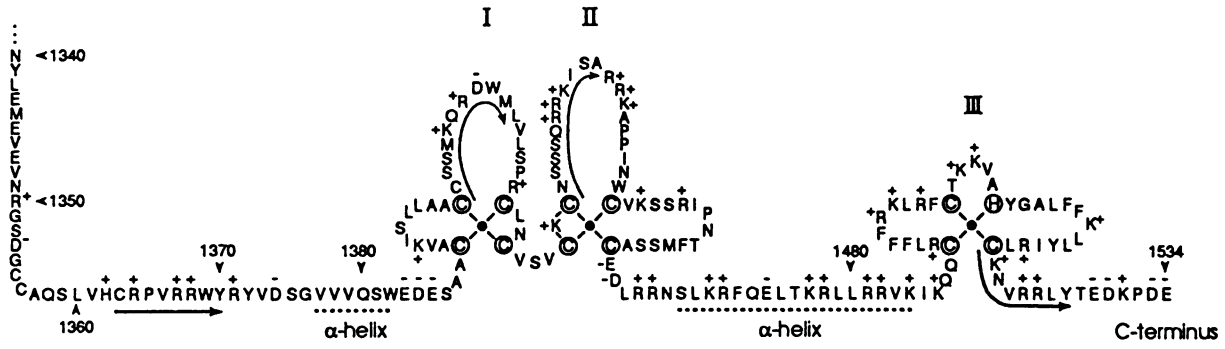

Fig. 8. Putative folding structures in the carboxyl-terminal region of the MukB protein. Each putative folding domain centered on a tetrahedral arrangement of a zinc atom or another metal atom (solid circle). The arrangement type Cys-Cys . . . Cys-Cys is used in structures I and II, and Cys-Cys . . . His-Cys is used in structure III. The two pairs of arrows represent sequence similarity (see text). Predicted α-helical regions are indicated by dotted lines.

and a suitable ribosome binding sequence at nucleotide positions 5114−5121, 5140−5145 and 5152−5154, respectively.

Molecular structure of the MukB protein

The deduced amino acid sequence of the MukB protein was analyzed by computers. A hydrophobicity profile of the MukB protein is shown in Figure 6A. The protein is hydrophilic with no highly hydrophobic region, such as those typical membrane regions of integral membrane proteins. The potential conformation and organization of the MukB protein was predicted by computer analysis (Figure 6B). The results indicate that the protein has distinct characteristic domains, as summarized in Figure 6C. First, the amino-terminal portion (~338 residues) is predicted to be globular because it is characterized by the alternation of four different conformational features (α-helix, β-sheet, β-turn and random coil). There is a possible binding consensus sequence with ATP or another nucleotide (Walker *et al.*, 1982; for a review see Houten, 1990) from residue 34 to 48, within the first globular domain (Figures 5 and 6C).

Second, a central region (approximately residues 339−1116) contains three characterized domains: two domains which have a high potential to form α-helical structures lie at approximately residues 339−665 and residues 934−1116 (Figure 6C). The remainder, predicted to be globular, is located between these α-helical domains, i.e. at residues 666−933. The two α-helical domains were found to exhibit a heptapeptide repeat motif *a b c d e f g*; hydrophobic residues were enriched at positions *a* and *d*, and periodicity of negatively charged residues (aspartic acid and glutamic acid) and of positively charged residues (lysine, arginine and histidine) was observed in these α-helical regions (Figure 7). These results are consistent with an α-helical coiled-coil conformation in these regions.

Third, the carboxyl-terminal domain (residues 1116−1534) is predicted to be globular (Figure 6C). A subregion (residues 1364−1525) is rich in positively charged residues (arginine and lysine), and relatively poor in negatively charged residues (aspartic acid and glutamic acid). The region from 1355 to 1520 is rich in cysteine residues.

Discussion

The results presented here indicate that the *mukB* new gene encodes a 177 kd protein. The *mukB*-disrupted strain can grow at 22°C. Its colony forming ability decreases as the temperature increases (Figure 4A). In growing cultures of the *mukB*-disrupted strain at 22°C, normal-sized nucleate cells and anucleate cells (~5% of total cells) were observed. This shows that the MukB protein is involved in chromosome partitioning into daughter cells, presumably via active chromosome positioning at the cell quarters. However, the MukB protein is not essential for cell division, at least at low temperatures. As the temperature increases, the *mukB*-disrupted strain becomes abnormal in cell division, suggesting that the MukB protein is directly or indirectly required for cell division at higher temperatures. One possible explanation at present is that in the *mukB*-disrupted strain another protein helps cell division at low temperatures but does not work at high temperatures.

Results of flow cytometric analysis of DNA content per cell, after incubation with rifampicin and cephalexin to inhibit initiation of chromosome replication and cell division suggested that the initiation of chromosome replication occurs normally in the *mukB* mutant (unpublished data). We have analyzed whether the *mukB* mutation affects the superhelicity of plasmid DNA. No detectable difference in the superhelicity of plasmid pBR322 was observed between the *mukB106* mutant and the *mukB*$^+$ strain by electrophoresis on agarose gels containing chloroquine phosphate (unpublished data).

The entire nucleotide sequence of the cloned 5353 kb DNA segment carrying the *mukB* gene was determined. It includes an open reading frame encoding a protein whose predicted mol. wt is 176 862 daltons. Computer analysis of the deduced amino acid sequence of the gene product revealed that the protein has distinct structural domains: a globular head containing a possible binding sequence with ATP or another nucleotide, and two extended α-helical domains, and a carboxyl-terminal globular domain larger than the amino-terminal globular domain. The two α-helical domains have motifs consistent with the ability to form a coiled-coil. Long coiled-coil structures (McLachlan and Karn, 1983) have been described in eukaryotic filamentous proteins such as myosin heavy chain (Molina *et al.*, 1978; McLachlan and Karn, 1983), paramyosin (Kagawa *et al.*, 1989) and kinesin heavy chain (Yang *et al.*, 1989). Two MukB molecules presumably form a homodimer in these long coiled-coil regions. The predicted secondary structure of the MukB protein suggests that the protein is the first candidate in eubacteria for force-generating enzymes able

to move nucleoids from mid-cell towards the cell quarters. The head domain, containing the nucleotide binding consensus sequence, would be expected to act as a 'motor' domain, like heads of other force-generating enzymes (for a review see Vale and Goldstein, 1990) such as myosin heavy chain (Molina *et al.*, 1978), mini-myosin (Jung *et al.*, 1987) and kinesin heavy chain (Yang *et al.*, 1989).

In *Dictyostelium*, the *mhcA* myosin null mutant (Manstein *et al.*, 1989) and the *hmm* mutant (De Lozanne and Spudich, 1987), which has a truncated form of myosin, are viable but the cell size varies from one to 10 times the normal size, and DAPI staining reveals that half of the cells contain more than two nuclei with cells sometimes 'pinching off' an anucleate piece of cytoplasm. Similarly, mutants of yeast with a disrupted *MYO1* gene (Watts *et al.*, 1987) are viable but budding is incomplete, with some cells showing several clustered nuclei and many cells which do not contain nuclei, suggesting that nuclear migration is also affected. Some proteins belonging to superfamilies of force-generating enzymes in eukaryotic cells have different carboxyl-terminal globular domains interacting with other proteins, vesicles, or organelles (for a review see Vale and Goldstein, 1990). For example, kinesin heavy chain is speculated to interact with other proteins, vesicles, or organelles via the carboxyl-terminal region associated with the light chain (Yang *et al.*, 1989). The single-headed myosin I (mini-myosin) of eukaryotes (Jung *et al.*, 1987) is responsible for the movement of membranes along actin filaments; *in vitro* it binds to membranes and to pure lipid vesicles (Adams and Pollard, 1989).

The carboxyl-terminal region of the MukB protein is rich in cysteine, arginine and lysine. There are three putative 'zinc finger' like structures (Miller *et al.*, 1985; for a review see Klug and Rhodes, 1987) in the cysteine-rich portion of the MukB protein (Figure 8). Loops may be formed around the central Zn atom (or another metal atom). Four cysteine residues provide the linkers between consecutive loops in structures I and II. On the other hand, three cysteine residues and one histidine residue provide the linkers in structure III. These loops are rich in positively charged residues and hydrophobic residues, like loops of previously described 'zinc finger' (Miller *et al.*, 1985). Most of the negatively charged residues are located on the outside of these zinc finger like structures of the MukB protein. There are α-helical regions, one between structures II and III, another upstream of structure I. The region Cys1363−Tyr1370 is homologous to the region Cys1520−Tyr1527 (Figure 8). In addition, the region Cys1399−Leu1410 is similar to the region Cys1425−Ala1436. We speculate that these different characteristic domains in the carboxyl-terminal region may be involved in specific interactions with other proteins and/or DNA.

Computer searches revealed no known protein sequences sufficiently similar to MukB to suggest common ancestry. However, nucleotide positions 4623−4856, which sequence codes for the carboxyl-terminal region of the MukB protein, is highly homologous (68%) to the nucleotide sequence of the flanking region upstream of the *haeIIIM* gene (*Hae*III methyltransferase) of *Haemophilus aegyptius* (Slatko *et al.*, 1988).

Recently, Casaregola *et al.* (1990) described an *E.coli* protein of ~180 kd which cross-reacted with a monoclonal antibody raised against myosin heavy chain encoded by the *MYO1* gene of yeast *Saccharomyces cerevisiae*. It is not clear at present whether the MukB protein is identical to the protein which cross-reacts with the antibodies directed against the yeast myosin heavy chain. Apart from the nucleotide binding consensus sequence, obvious sequence homology which suggested a common ancestor protein was not found between the MukB protein and the head of yeast myosin heavy chain of the *MYO1* gene (Watts *et al.*, 1987) by computer analysis.

Biochemical data showed temporary membrane association of DNA at the replication origin of the chromosome (*oriC*) (Hye *et al.*, 1976; Yoshimoto *et al.*, 1986a,b; Ogden *et al.*, 1988). However, it is doubtful that the membrane attachment of the *oriC* region actually plays an essential role in partitioning chromosomes to daughter cells *in vivo*. It has been shown that *oriC* plasmids (or mini-chromosomes) do not have a partition mechanism and are partitioned essentially at random into daughter cells. In contrast, an *oriC* plasmid carrying the *sopA, sopB* and *sopC* genes of F plasmid is stably maintained even in non-selective media (Ogura and Hiraga *et al.*, 1983; Hiraga *et al.*, 1985; Mori *et al.*, 1986). This suggests that *oriC* does not play an essential role in chromosome partitioning.

Analysis of the biochemical functions of the MukB protein should help us to understand the molecular mechanism of active chromosome partitioning in bacteria.

Materials and methods

Bacterial strains and media

All bacterial strains used were *E.coli* K-12 derivatives. The principal bacterial strains are listed in Table I. Other strains are as follows: the *recD* strain FS1576 (Stahl *et al.*, 1986), the *uvrA recA* strain CSR603 (Sancar *et al.*, 1972), the *sfiA* (*sulA*) *sfiC* strain B1686 derived from B1654 (Jaffé *et al.*, 1986) and the *ilv*::Tn*5* Δ*cya* strain GC7064 derived from GC2700 (D'Ari *et al.*, 1988). SH392 (Jaffé *et al.*, 1985) was used as host for plasmid construction. The *zcb*::Tn*10* strain ME8307 was obtained from the National Institute of Genetics, Japan. L medium [1% Bacto-tryptone (Difco Laboratories), 0.5% yeast extract, 0.5% NaCl, pH 7.4], LB medium (1% Bacto-tryptone, 0.5% yeast extract, 1% NaCl, pH 7.2), M63 medium (Miller, 1972) and medium E (Vogel and Bonner, 1956) were used for cultivation of cells after the addition of glucose and other requirements, as necessary. P medium containing X-Gal (5-bromo-4-chloro-3-indolyl-*b*-D-galactopyranoside) was used for selection of anucleate cells forming mutants (Hiraga *et al.*, 1989). Agar (1.4%, w/v) was added to prepare agar media. When necessary, antibiotics were added to the media as follows: kanamycin (20 μg/ml), chloramphenicol (20 μg/ml), tetracycline (15 μg/ml) and ampicillin (25 μg/ml for low copy number plasmids, 50 μg/ml for high copy number plasmids).

Isolation of anucleate cell forming mutants

Anucleate cell forming mutants were isolated as blue colonies grown on X-Gal plates at 42°C from the parental strain SH3210, which carried plasmid pXX747, after treatment with ethylmethansulfonate, as described previously (Hiraga *et al.*, 1989). The mutants isolated were used for further experiments after segregation of the pXX747 plasmid.

Microscope observation of cells and nucleoids

Cell shape and nucleoids were simultaneously observed by Hiraga's fluophase combined method (Hiraga *et al.*, 1989). Cells grown in liquid media were washed with saline, dried on a glass slide, fixed with methanol, bound tightly to the slide by poly-L-lysine, and then stained with DAPI (4′,6-diamino-2-phenyl-indole). Nucleoids shine brightly with light blue fluorescence, other cytoplasmic portions of cells are dark blue, and the background is orange or light brown.

Molecular cloning of the mukB gene

A cosmid library of chromosomal DNA extracted from the wild-type strain W3110 was constructed in the cosmid vector pHSG262 (Brady *et al.*, 1984) according to Ish-Horowicz and Burke (1981). Chromosomal DNA was partially digested with *Sau*3AI and ligated with *Bam*HI-digested pHSG262

DNA. The DNA sample was packaged *in vitro* into λ phage particles using a Gigapack kit (Stratagene, San Diego, CA) and used to infect the *mukB106* mutant (SH3367). Kanamycin resistant temperature resistant colonies were isolated at 42°C on L agar containing kanamycin (20 μg/ml). The colonies were inoculated and cultivated in L broth containing kanamycin. Recombinant cosmid DNA was extracted and analyzed with restriction endonucleases. One of these cosmid clones, pλH131, was used for subcloning (Figure 2A). Plasmid pACYC184 (Chang and Cohen, 1978) was used for the subcloning as a vector.

Deletion derivatives from pAX814 and pAX815 were prepared by using a Deletion Kit for Kilo-Sequence (Takara Shuzo Co., Kyoto, Japan) as follows: digestion with *Sma*I and *Sca*I, treatment with exonuclease III and Mung Bean nuclease, then with T4 DNA ligase. Bacterial strain SH392 (Jaffé *et al.*, 1985) was used as host in plasmid construction. The plasmids constructed were extracted and introduced into the *mukB106* strain SH3367. Cm^r transformants selected at 30°C were analyzed for colony forming ability at 42°C on L agar.

Specific labeling of plasmid-encoding proteins by the maxicell method

To detect plasmid-encoded proteins, UV light irradiated cells of the *uvrA recA* mutant CSR603 carrying a plasmid were prepared and labeled according to a modification of the maxicell method described by Sancar *et al.* (1972). Growing cells were irradiated with UV light, incubated at 37°C for 1 h, then further incubated in the presence of cycloserine (100 μg/ml) at 37°C for 8–12 h. The cells were collected and labeled with [^{35}S]methionine (37 TBq/mmol) for 1 h at 37°C. The labeled cells were collected by centrifugation, suspended in SDS–PAGE sample buffer (75 mM Tris–HCl, pH 6.8, 25% glycerol, 0.00125% SDS, 2.5% 2-mercaptoethanol, 0.001% Bromphenol Blue), and boiled for 3 min. The sample was analyzed by electrophoresis on SDS–polyacrylamide (5%) gels (Laemmli, 1970). After electrophoresis the gels were soaked in EN^{3}HANCE (New England Nuclear, Boston, MA), dried and analyzed by fluorography.

Electrophoresis of proteins in cell extracts

Bacterial cells were grown exponentially in M63 medium containing glucose and tryptophan and incubated with [^{35}S]methionine (37 TBq /mmol) at 29°C for 1 h. The cells were chilled, collected by centrifugation, washed and suspended in SDS–PAGE sample buffer. The sample was boiled for 3 min and analyzed by electrophoresis. After electrophoresis, the gels were analyzed by autoradiography using X-ray film.

DNA sequence analyses

To determine the nucleotide sequence of the chromosomal segment (~5.4 kb) carried by pAX804, subsegments of this segment were cloned into M13mp11 and sequenced by the dideoxy technique (Sanger *et al.*, 1977). The sequencing reactions were carried out using a kit of Sequenase (US Biochemicals, Cleveland, OH). Deletion derivatives of pAX814 and pAX815 (Figure 2B) were also used as templates for denatured plasmid DNA (Hattori and Sakaki, 1986). Specific internal primers were synthesized with a DNA synthesizer and used for sequencing. Computer analysis of the nucleotide and amino acid sequences was done with the SDC-GENETYX programs (Software Development Co., Tokyo, Japan) and DNA Strider (Marck, 1988). Homology search in amino acid and nucleotide sequences was done in GeneBank and the EMBL database.

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Note added in proof

The GenBank accession number for the *mukB* gene sequence is M38402. We recently found that the amino acid sequence of the amino-terminal part (460 residues) of the MukB protein is homologous with that of the microtubule-associated mechanochemical enzyme dynamin (D100) of rat brain [Obar,R.A., Collins,C.A., Hammarback,J.A., Shpetner,H.S. and Vallee,R.B. (1990) *Nature*, **347**, 256–261] as shown in the following figure.

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MukB   MIERGKFRSLTLINW--NGF--FARTFDLDELVTTLSGGNGAGKSTTMAAFVTA--LIPD    54
D100   MGNRGMEDLIPLVNRLQDAFSAIGQNADLDLPQIAVVGGQSAGKSSVLENFVGRDFLPRG    60

MukB   LTLLHFRNTTEAGATSGSRDKG-LHGKLKAGVCYSMLDTINSRHQRVVVGVRLQQVAGRD   113
D100   SGIVTRRPLVLQLVNSTTEYAEFLHCKGKKFTDFEE--------------VRLEIEAETD   106

MukB   RKVDI-KPFAIQGLPMSVQPTQLVTETLNERQARV-LPLNELKDKLEAMEGVQFKQFNSI   171
D100   RVTGTNKGISPVPINLRVYSPHVLNLTLVDLPGMTKVPVGDQPPDIEFQIRDMLMQF--V   161

MukB   TDYHSLMFDLGIIARRLRSASDRSKFYRLIEASLYGGISSAITRSLRDYLLPENSGVRKA   231
D100   TKENCLILAVSPANSDL-ANSDALKIAKEVDPQ--GQRTIGVITKLD--LMDEGTDARDV   219

MukB   FQDMEAALRENRMTLEAIRVTQSD--RDLFKHLISERTNYVAADYMRHANERR--VHLDK   287
D100   LENKLLPLRRGYIGVVNRSQKDIDGKKDITAALAAERKFFLSHPSYRHLADRMGTPYLQK   279

MukB   ALEFRRELHTSRQQLAAEQYKHVDMARELADDNGAEGDLEADYQAASDHLNLVQTALRQQ   347
D100   VLN-QQLTNHIRDTLPGLRNKLQSQLLSIEKEVDEYKNFRPD-DPARKTKALLQ--MVQQ   336

MukB   EKIERYEADLDELQIRLEEQNEVVAEAIERQQENEARAEAAELEVD--ELKSQLADYQQA   405
D100   FAVD-FEKRIEGSGDQIDTYELSGGARINRIFHERFPFELVKMEFDEKELRREIS-YAIK   393

MukB   LDVQQTRAIQYNQAIA-ALNRAKELCHLPDLTADCAAEWLETFQAKELEATEKMLSLEQK   464
D100   -NIHGIRTGLFTPDLAFEATVKKQVQKLKEPSIKCVDMVVSELTSTIRKCSEKLQQYPRL   452
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