Two mutant alleles of mukB, a gene essential for chromosome partition in Escherichia coli

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Abstract The MukB protein is essential for chromosome partitioning in Escherichia coli and consists of 1484 amino acid residues (170 kDa). We have determined the base changes at the mutated sites of the mukB106 mutant and a newly isolated mutant, mukB33. These mutant mukB genes were each found to carry a single base-pair transition which leads to an amino acid substitution, a serine residue at position 33 was changed to phenylalanine in the case of mukB106, and an aspartic acid residue at position 1201 was changed to asparagine in the case of mukB33.

Key words Escherichia coli, mukB, Chromosome partition, Haemophilus aegyptius

Introduction

In Escherichia coli, replicated chromosomal DNA molecules should be spatially separated from one another during the chromosome partitioning step, after the completion of replication and before the onset of cell division [1,2]. Little is known, however, on the overall chromosome partitioning mechanism. We have isolated mutants defective in this process [1,2]. One of them, the mukB106 mutant, produced anucleate (chromosome-less) cells with normal cell size at a frequency of 10–20% to total cells and showed temperature-sensitive colony formation [3]. The mukB gene is involved in chromosome partitioning and located at 21 min on the E. coli chromosome [3]. The MukB protein consists of three domains, an N-terminal globular domain which contains a nucleotide binding motif, a middle α-helical coiled-coil domain, and a C-terminal globular domain [4]. Purified MukB protein formed homodimers and possessed the ATP/GTP binding and DNA binding activities [4]. In this report, we have obtained another mutant allele of the mukB gene, mukB33, and determined the exact nature of the mukB106 and mukB33 mutations.

Materials and Methods

Bacterial strains and medium

E. coli strains used in this study are listed in Table 1. P1 transduction was performed as de-
scribed previously [7] L medium (1% Bacto-tryptone, 0.5% Bacto-yeast-extract, 0.5% NaCl, pH 7.2) was used. Agar (1.5%) was added to medium for plates. When necessary, antibiotics were supplemented at the following final concentrations: tetracycline, 15 μg ml⁻¹; kanamycin, 30 μg ml⁻¹; and chloramphenicol, 30 μg ml⁻¹.

**Phase-contrast microscopic observation of cells and nucleoids**

Cell shape and nucleoids were simultaneously observed by the fluorescence and phase-contrast combined method as described previously [8].

**DNA cloning and sequencing**

All DNA cloning was carried out essentially by following general procedures [9]. pACYC184 [10] was used as a vector. To purify the recombinant plasmid DNAs, QIAGEN (QIAGEN Inc., Chatsworth, CA) was used according to the manufacturer's instruction. For sequencing, DNA fragments were subcloned onto M13mp19 [11]. DNA sequences were determined by the dideoxy-chain termination method [12] using Sequenase Version II (United States Biochemical Corp., Cleveland, OH). All restriction enzymes and DNA modification enzymes were purchased from Takara Shuzo (Kyoto, Japan), Boehringer Mannheim Biochemicals (Indianapolis, IN), or New England Biolabs (Beverly, MA).

**Results and Discussion**

We have previously described a strategy to isolate muk mutants, which might have a defect in the chromosome partitioning step in the E. coli cell cycle [8]. Using this strategy, we have isolated several mutants that produce anucleate cells with normal cell size at a significant frequency [1,2,3,8]. One of them, a muk-33 mutant (SH3470, Table 1), was complemented with the pAX04 plasmid [3] which carries the wild-type mukB gene. The mutation was mapped to the mukB gene locus at the 21 min region [3] by P1 transduction (results not shown). Therefore, hereafter we will call this mutation mukB33. The mukB33 mutation caused temperature-sensitive colony formation on L-agar plates containing 2% SDS, as did the mukB106 mutation (Fig 1). The temperature sensitivity is more prominent in the PB103 background than in the YK1100 background. It is also more severe in the mukB33 mutants than in the mukB106 mutants (Fig 1). Bacterial cells with the YK1100 background were grown in L medium at 30°C to mid-log phase, incubated at 42°C for 2 h, and then observed as described in Materials and Methods. The mukB33 mutant produced 29% of normal-sized, anucleate cells and 36% of elongated, nucleate cells with irregularly positioned nucleoids, while the mukB106 mutant produced 12% of anucleate cells and 24% of elongated cells.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Reference or construction</th>
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<tbody>
<tr>
<td>YK1100</td>
<td>trpC9941</td>
<td>[5]</td>
</tr>
<tr>
<td>PB103</td>
<td>dadR trpE61 trpA62 tna-5</td>
<td>[6]</td>
</tr>
<tr>
<td>GC7471</td>
<td>PB103 zcb Tn10</td>
<td>[3]</td>
</tr>
<tr>
<td>GC7528</td>
<td>PB103 ΔmukB kan</td>
<td>[3]</td>
</tr>
<tr>
<td>SH3470</td>
<td>ΔtrpE5 his mukB33 (λ)</td>
<td>This study</td>
</tr>
<tr>
<td>SH3910</td>
<td>ΔtrpE5 his mukB106 zcb Tn10 (λ)</td>
<td>[3]</td>
</tr>
<tr>
<td>SH9019</td>
<td>ΔtrpE5 his mukB33 zcb Tn10 (λ)</td>
<td>P1vac/GC7471 → SH3470</td>
</tr>
<tr>
<td>SH9071</td>
<td>YK1100 mukB33 zcb Tn10</td>
<td>P1vac/S9019 → YK1100</td>
</tr>
<tr>
<td>SH9073</td>
<td>YK1100 zcb Tn10</td>
<td></td>
</tr>
<tr>
<td>SH9075</td>
<td>PB103 mukB33 zcb Tn10</td>
<td>P1vac/S9019 → PB103</td>
</tr>
<tr>
<td>SH9077</td>
<td>PB103 zcb Tn10</td>
<td></td>
</tr>
<tr>
<td>SH9079</td>
<td>PB103 mukB106 zcb Tn10</td>
<td>P1vac/S9019 → PB103</td>
</tr>
<tr>
<td>SH9083</td>
<td>YK1100 mukB106 zcb Tn10</td>
<td>P1vac/S9019 → YK1100</td>
</tr>
</tbody>
</table>
The colony-forming ability of the mukB null cells dramatically decreased above 30°C [3]. Using this phenotype, temperature- and chloramphenicol-resistant transformants were selected at 37°C. Plasmid DNAs extracted from these transformants were analyzed with several restriction endonucleases. pKX732 (mukB106), and pKX733 (mukB33) carried the 8.5-kb BamHI chromosomal segment, which is located in the 979–987 5 kb coordinate on the E. coli map [13] and contains the mukB operon (kcb–kca–mukB) [14].

The mutant mukB genes from pKX732 (mukB106) and pKX733 (mukB33) were subcloned onto M13mp19 and sequenced. These mutant mukB genes were found to each carry a single base-pair transition (G C to A T) which leads to an amino acid substitution, Ser-33 to Phe in the case of mukB106, and Asp-1201 to Asn in the case of mukB33 (Fig 2). Transition mutations of G C to A T are consistent with the use of ethylmethane sulfonate as a mutagen for isolation of these muk mutants [8].

Purified MukB protein possesses the ATP/GTP binding and DNA binding activities [4]. The MukB protein has three distinct domains, an N-terminal globular domain (Met-I to Asn-338), a middle α-helical coiled-coil domain (Leu-339 to Gin-665), and a C-terminal globular domain (Pro-666 to Ser-1484) [4] (Fig 2). Since Ser-33 is the amino acid residue immediately upstream of the nucleotide binding consensus motif, GXXGXG-KS (amino acid residues 34–41), in the N-terminal globular domain (Fig 2), the mutant MukB106 protein might have a defect in the nucleotide binding activity. On the other hand, Asp-1201 is in the C-terminal globular domain (Fig 2). When the C-terminal half of the C-terminal globular

Fig 1 Colony-forming ability of the mukB33 and mukB106 mutations at various temperatures. Cells grown in L medium at 22°C were diluted appropriately, plated on L-agar plates containing 2% SDS, and then incubated overnight at 30, 37, and 42°C, and for 2 days at 22°C (A) Strains with the genetic background of YK1100, SH9073 (mukB+), ○, SH9071 (mukB33), Δ, SH9083 (mukB106) (B) Strains with the genetic background of PB103, SH9077 (mukB+), ○, SH9075 (mukB33), Δ, SH9079 (mukB106)

Fig 2 Sequence analysis of two mukB alleles. Parts of the amino acid sequence of MukB are shown. The boxed sequence represents the nucleotide binding motif.
domain was deleted, the resulting truncated MukB protein lost the DNA binding activity (Saleh, A Z M and Hiraga, S, unpublished data) This suggests that the mutant MukB33 protein probably has a defect in the DNA binding activity

During sequencing, we have found some mistakes in the previously reported mukB gene (3, accession no M38402) The nucleotide sequence data of the revised mukB gene have been deposited to the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence databases under accession number D31701 From the revised mukB gene sequence, the MukB protein was predicted to consist of 1484 amino acid residues (170 kDa) (Fig 2)

Homology search of the predicted amino acid sequences of the revised mukB gene was carried out with BLAST [15] As shown in Fig 3, we found a striking homology between the MukB protein and the C-terminal region of a truncated open reading frame (ORF), located immediately upstream of the haeIIIM gene of Haemophilus aegyptius [16] It is possible that the putative ORF encodes an MukB homologue in H aegyptius

In conclusion, we have characterized two mutant alleles, mukB106 and mukB33, both of which caused a defect in chromosome partitioning Their mutations were mapped to different domains in the mukB gene mukB106 in the N-terminal globular domain and mukB33 in the C-terminal globular domain Comparative biochemical analyses with MukB proteins purified from the wild-type and mutant strains will provide further information about the function of each domain of MukB and insights into the cellular functions of MukB

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