Crystal structure of the N-terminal domain of MukB: a protein involved in chromosome partitioning

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Background: The 170 kDa protein MukB has been implicated in ATP-dependent chromosome partitioning during cell division in *Escherichia coli*. MukB shares its dimeric structure and domain architecture with the ubiquitous family of SMC (structural maintenance of chromosomes) proteins that facilitate similar functions. The N-terminal domain of MukB carries a putative Walker A nucleotide-binding region and the C-terminal domain has been shown to bind to DNA. Mutant phenotypes and a domain arrangement similar to motor proteins that move on microtubules led to the suggestion that MukB might be a motor protein acting on DNA.

Results: We have cloned, overexpressed and crystallized a 26 kDa protein consisting of 227 N-terminal residues of MukB from *E. coli*. The structure has been solved using multiple anomalous dispersion and has been refined to 2.2 Å resolution. The N-terminal domain of MukB has a mixed α/β fold with a central six-stranded antiparallel β sheet. The putative nucleotide-binding loop, which is part of an unexpected helix-loop-helix motif, is exposed on the surface and no nucleotide-binding pocket could be detected.

Conclusions: The N-terminal domain of MukB has no similarity to the kinesin family of motor proteins or to any other nucleotide-binding protein. Together with the finding of the exposed Walker A motif this observation supports a model in which the N- and C-terminal domains come together in the dimer of MukB to form the active site. Conserved residues on one side of the molecule delineate a region of the N-terminal domain that is likely to interact with the C-terminal domain.

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Introduction

The correct partitioning of chromosomes into each of the daughter cells is a pivotal step for the faithful segregation of genetic information during prokaryotic cell division [1,2]. In most organisms, including eukaryotes, the SMC (structural maintenance of chromosomes) proteins are involved in the condensation and correct positioning of daughter chromosomes during mitosis. Although several SMC proteins have been reported in bacteria, archaea and eukaryotes, no SMC proteins have been found in *Escherichia coli* [3–8]. However, studies on the genetics of chromosomal partitioning in *E. coli* have revealed that the *muk* genes (muk stands for the Japanese word 'mukaku', meaning anucleate) are essential for the movement of nucleoids from mid-cell towards the cell quarters [9].

Mutants defective in the *mukB* gene produce normal sized anucleate cells during mitosis. The *mukB* gene codes for a multidomain protein (MukB) of 170 kDa [10]. Secondary structure prediction of MukB has indicated that it has distinct structural domains that are also found in SMC proteins, although their amino acid sequence shows only limited similarity. Both have an N-terminal globular domain, containing a nucleotide-binding sequence (Walker A motif [11]), a central region, comprising two helical coiled-coil domains that are separated by a hinge, and a C-terminal globular domain. The C-terminal domain of MukB is required for DNA binding [12], and the coiled-coil region is believed to mediate dimer formation [13,14]. Although no detailed biochemical analysis has been done, it has been shown that MukB can be cross-linked to ATP and GTP in the presence of Zn^{2+} [14]. A low level of Mg^{2+}-dependent ATPase and -GTPase activity has been reported for the N-terminal 342 residues of the MukB protein [15]. Because the domain structure of MukB has characteristics similar to that of the kinesin and myosin family of proteins, it has been suggested that MukB would be a good candidate for a force-generating enzyme in *E. coli* [16]. The N-terminal domain of MukB, containing the Walker A motif, would be the 'motor', like the heads of force-generating enzymes such as the heavy chains of myosin or kinesin. It has been shown that MukB binds to the prokaryotic tubulin homologue FtsZ, which might function as an anchor for generating force in *E. coli* [15]. However, there is no evidence yet that the protein can actually move on FtsZ polymers. Electron microscopy

has revealed that MukB, as well as *Bacillus subtilis* SMC, can form dimers in which the coiled coils are arranged in an antiparallel fashion [13]. This results in a symmetrical molecule containing two complete functional domains at each end. As pointed out by Hirano [3], the symmetrical arrangement of the SMC proteins indicates that they might actually differ from the cytoskeletal motor proteins, which have a polar structure with the ATP-binding domain located at one end of the molecule.

In this study we have used multiple anomalous dispersion (MAD) methods to solve the crystal structure of the N-terminal domain of MukB at a resolution of 2.2 Å. It is a mixed α/β structure with a central six-stranded antiparallel β sheet and is not related to known nucleotide-binding proteins, including kinesin and myosin.

Results

DNA encoding the 227 N-terminal amino acids of the MukB protein from *E. coli* was cloned into the pHis17 vector and expressed as a His_6-tagged protein in C41 cells [17]. The protein was purified over a Ni^{2+}-nitrilotriacetic acid (NTA) affinity column, followed by size-exclusion chromatography. The crystal structure was determined using selenomethionine-substituted MukB and MAD methods. Selenomethionine-substituted MukB was expressed in cells non-auxotrophic for methionine, in medium inhibiting methionine biosynthesis [18]. Typically, 40 mg of purified MukB were obtained from a 12 L culture. Hexagonal crystals were grown from 0.1 M Na citrate pH 4.9, 18–22% polyethylene glycol (PEG) 600 at 4°C in sitting-drop vapour diffusion experiments. MAD X-ray structure determination was performed using four data sets collected at wavelengths on and away from the selenium absorption edge (Table 1). After solvent flattening, an almost perfect electron-density map was obtained at 2.4 Å resolution (Figure 1). Following model building and refinement, a model containing residues 4–58 and 77–224 and 75 water molecules was refined to an R factor of 0.217 (Table 2).

Table 1

Crystallographic data.

Crystal	Native	SeMet1	SeMet2	SeMet3	SeMet4
Wavelength (Å)	1.5418	0.9100	0.9790	0.9797	1.2000
f′, f″ (e) (theoretical)		1.79 3.34	–7.47 3.84	–8.08 0.50	–1.52 0.72
Resolution (Å)	2.9	2.2	2.4	2.4	2.9
I/σ(I) (last shell)	5.8 (2.4)	6.0 (2.3)	6.6 (4.5)	6.0 (3.7)	8.3 (6.2)
R_{merge}* (last shell)	0.12 (0.32)	0.09 (0.26)	0.07 (0.15)	0.07 (0.17)	0.06 (0.10)
Multiplicity (anomalous)	7.0	13.1 (6.2)	13.8 (6.8)	13.7 (6.5)	13.4 (6.5)
Completeness (%)	97.6	98.5	98.4	98.5	97.4
Phasing power†		1.06	0.53	1.48	0.85
Anomalous occupancy (e)‡		2.69	0.69	0.36	0.32
Real occupancy (e)‡		3.86	–1.56	0.00	1.95

Crystals are in space group P622 (177) with cell dimensions of a = b = 111.4 Å, c = 65.0 Å, α = β = 90.0 Å, γ = 120.0°, one molecule/asymmetric unit. *$R_{merge} = \Sigma_h\Sigma_i|I(h,i)-I(h)|/\Sigma_h\Sigma_i\, I(h,i)$, where I(h,i) are symmetry-related intensities and I(h) is the mean intensity of the reflection with unique index h. †Phasing power = mean value of the heavy-atom structure amplitudes divided by the residual lack of closure. The figure of merit was 0.53 and 0.49 for centric and acentric reflections, respectively. ‡Occupancies after refinement in MLPHARE; e, electrons.

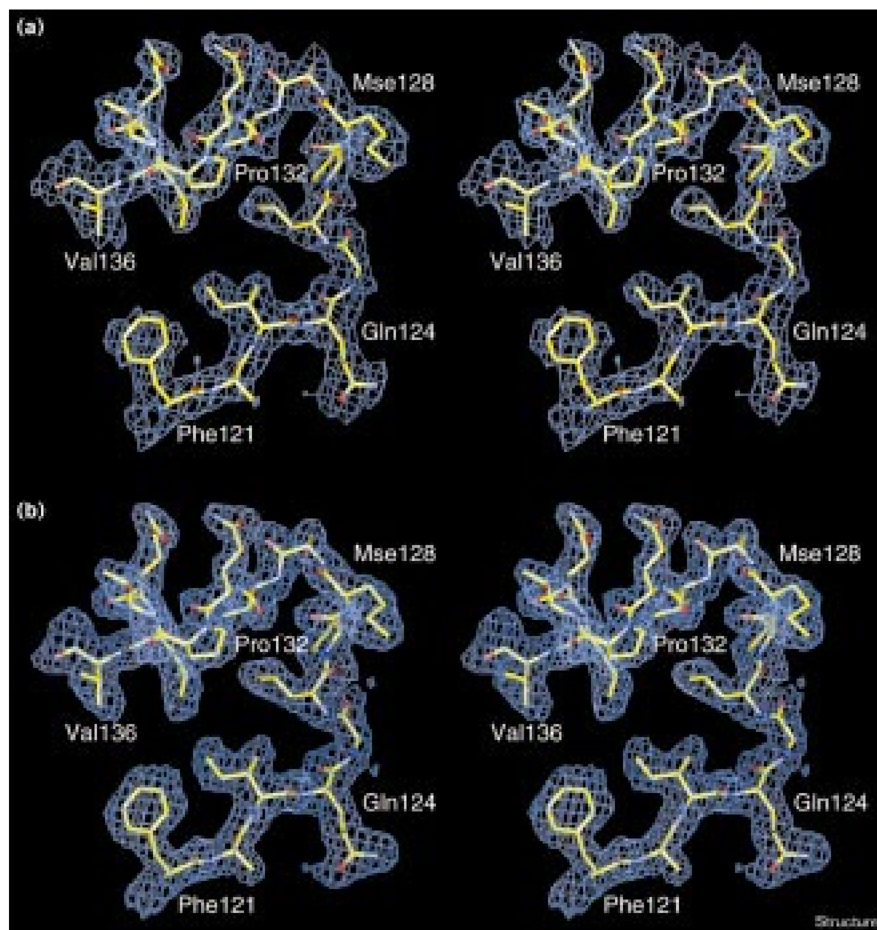
The fold of the N-terminal domain of MukB comprises a central six-stranded antiparallel β sheet with the topology 213456 surrounded by six α helices (Figure 2). Two N-terminal, antiparallel β strands, S1 and S2, are followed by α helix H1, which is part of a helical region located in the lower part of the molecule. The highly conserved glycine-rich loop (G loop) is situated between helices H1 and H2. This loop and part of helix H2 form the consensus Walker A motif that has been predicted to be involved in nucleotide binding in MukB. The second helix H2 is connected to strand S3 by a long, mostly flexible loop. Residues 59 to 76 are disordered in the crystal. Strand S3 is followed by antiparallel strands S4 and S5, which are connected by small loops containing β turns. Strand S5 leads into helix H3 via a helical loop. This loop and helix H3 are the only elements above the β sheet. A short β strand, S6, completes the six-stranded β sheet. The following helices H4, H5 and H6 are below the β sheet. In contrast to other nucleotide-binding proteins, the putative nucleotide-binding region is part of a helix-loop-helix motif, which is located on the surface of the molecule rather than in a pocket. Interestingly, another conserved glycine-rich loop in MukB (loop H5–H6) is located on the same side of the molecule.

A sequence alignment of all four known MukB sequences reveals two regions of very high conservation: the N-terminal part including residues 9–67 and the C-terminal part of this domain including residues 192–223 (Figure 3a). If mapped onto the surface in three dimensions, these regions clearly form a continuous area of 100% conservation (Figure 4), indicative of higher evolutionary pressure in this region. This area surrounds a ‘spike’ formed by helices H1, H2, H5 and H6 and their connecting loops including the G loop (Figure 4a).

Discussion

In this study we have solved the three-dimensional structure of the N-terminal domain of MukB (residues 1–227) from *E. coli*. The domain presented here is shorter than the N-terminal domain earlier predicted for MukB [14,15],

Figure 1



Stereoview drawings of electron-density maps for residues Phe121 to Val136 of the N-terminal domain of MukB from *E. coli*, contoured at 1.0σ. **(a)** Solvent-flattened experimental density map derived from a four-wavelength MAD experiment at 2.4 Å resolution. **(b)** Final $2F_o–F_c$ density map after refinement. Mse, selenomethionine. (The figure was prepared with the program MAIN [29].)

because the original N-terminal domain, containing residues 1–342, had very limited solubility. We have used both secondary structure [19] and coiled-coil prediction programs [20] to define a domain (residues 1–227), which does not contain residues with significant coiled-coil probability, but includes all predicted β strands in the first half of the MukB molecule. Secondary structure prediction for MukB shows very long helices after residue 227 and significant coiled coil probability for three regions between residues 227–340 (data not shown). The high solubility of this N-terminal domain, together with its high expression level, made it possible to express selenomethionine-substituted protein for crystallography in cells non-auxotrophic for methionine [18]; mass spectrometry indicated nearly 100% substitution.

The structure of the N-terminal domain of MukB shows an antiparallel six-stranded β sheet surrounded by one helix on one side and by five helices on the other side. We are not aware of any proteins showing significant similarity in topology or fold. A DALI [21] search against all currently known non-redundant protein folds showed no close or even distant homologues. Most importantly, MukB has no structural similarity to any nucleotide-binding protein and no similarity in particular to kinesin or myosin. This came as a surprise, as Lockhart and Kendrick-Jones previously showed binding of MukB to tubulin and FtsZ [15,22]. The shorter construct (containing residues 1–227) still binds to

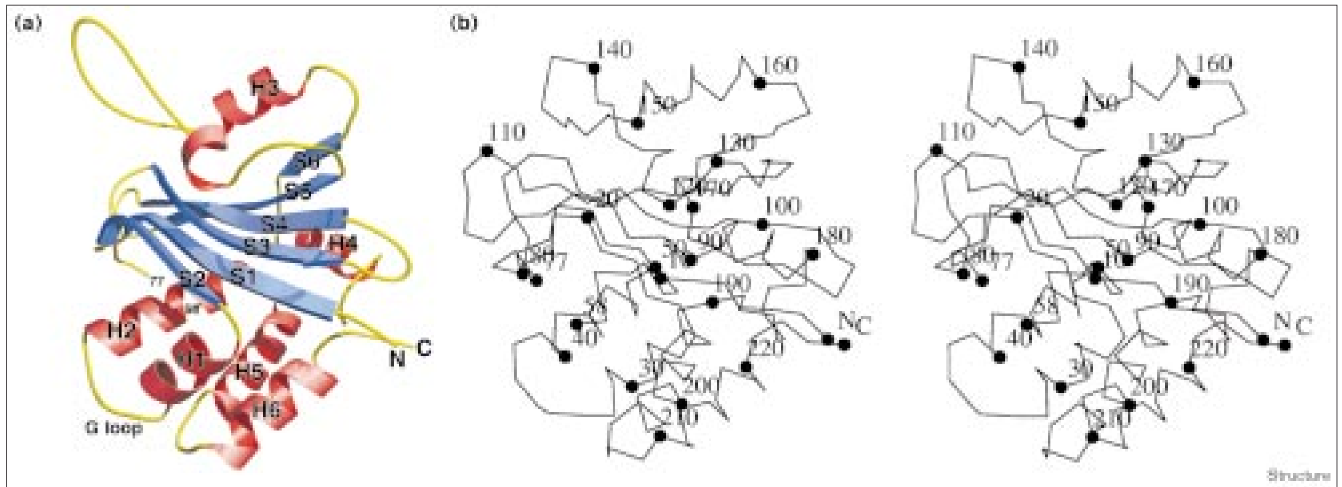
Table 2

Refinement statistics.

Model	Residues 4–58, 77–224 of *E. coli* MukB, 75 water molecules
Data	SeMet1 (0.91 Å), 10–2.2 Å, all reflections
R factor	0.217 (R_{free} 0.292)*
B factors	Average 30.3 $Å^2$, rms-bonded B factors 2.6 $Å^2$
Geometry	Rms bond lengths, 0.007 Å; rms angles, 1.49°
Backbone geometry	No Ramachandran outliers, 91% of residues in most favoured regions

*5% of reflections were randomly selected for determination of the free R factor before any refinement.

Figure 2

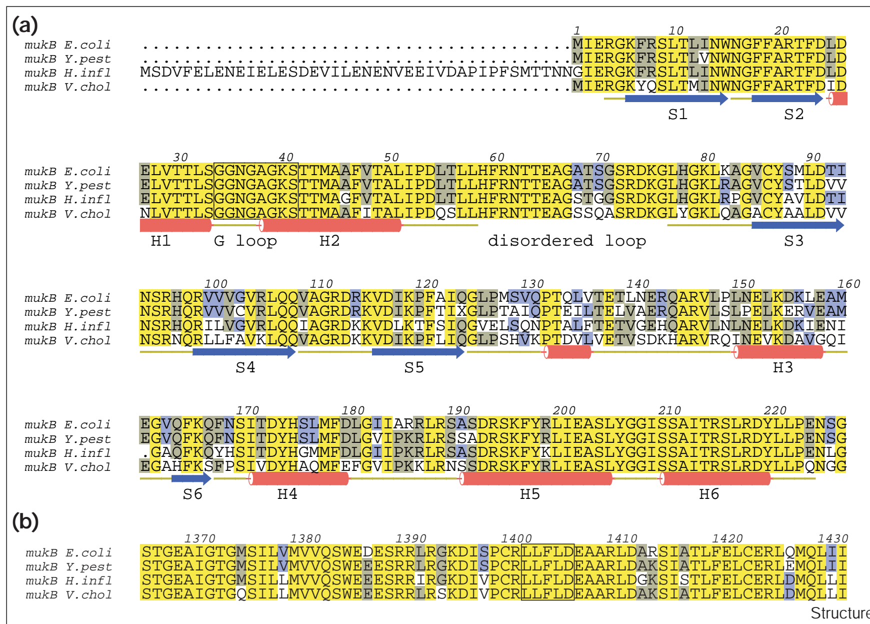


The three-dimensional structure of the N-terminal domain of MukB. The model contains residues 4–58 and 77–224; residues 59–76 are disordered in the crystal. **(a)** Ribbon representation of MukB with the secondary structure elements labelled. **(b)** Stereoview Cα trace. Every tenth residue is labelled. (The figures were prepared using the program MOLSCRIPT [32].)

tubulin (data not shown). As there is no predominantly positively charged surface region on the N-terminal domain of MukB, it is unlikely that the binding of MukB to tubulin or FtsZ is based purely on a non-specific electrostatic interaction.

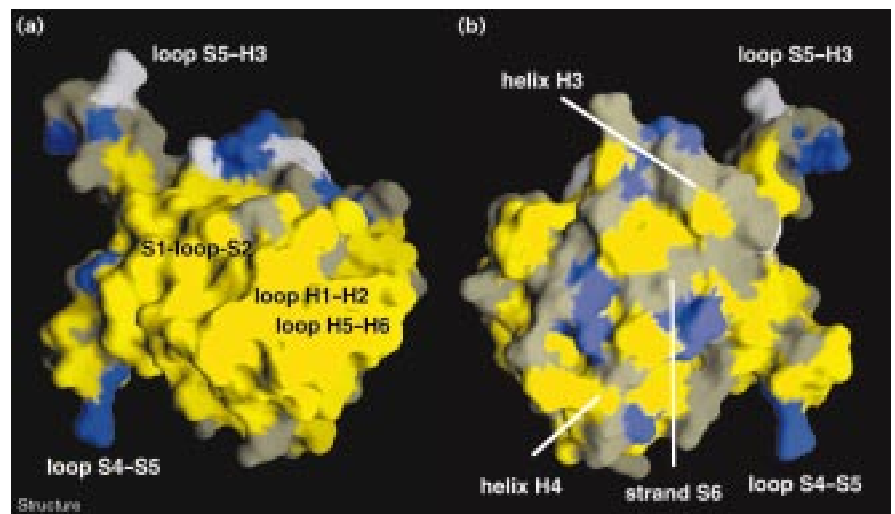
A second important finding is the location and conformation of the G loop, which has the Walker A nucleotide-binding consensus sequence. The G loop is located in a helix-loop-helix motif on the surface of the molecule. This motif and its surrounding residues are 100% conserved in all four known MukB sequences (Figure 3a); the motif shows a convincing match to the consensus Walker A motif ([AG](X)$_4$GK[ST] in single-letter amino acid code; MukB, SGGNGAGKST). In all nucleotide-binding proteins containing the Walker A motif, the glycine-rich sequence adopts the P-loop (phosphate-binding loop; for a review see [23]) conformation and is

Figure 3



Alignment of all currently known MukB amino acid sequences. **(a)** Alignment of the N-terminal domain. Highly conserved residues cluster around the G loop (indicated by a black box) and helices H5 to H6. Residues conserved in all four sequences are coloured in yellow, residues conserved in only three or two sequences are in grey or blue, respectively. The secondary structure elements of the *E. coli* structure are shown with the numbering described in this paper. **(b)** Sequence alignment of the C-terminal domain containing the putative Walker B motif (indicated by a black box). Sequences: MukB from *E. coli* (SwissProt: MUKB_ECOLI), MukB from *Yersinia pestis* (preliminary sequence data were obtained from the Sanger Centre website at http://www.sanger.ac.uk), MukB from *Haemophilus influenzae* (SwissProt: MUKB_HAEIN), and MukB from *Vibrio cholerae* (preliminary sequence data were obtained from the Institute for Genomic Research website at http://www.tigr.org). (The figure was prepared using the program ALSCRIPT [33].)

Figure 4



Mapping the degree of sequence conservation onto the surface map of the N-terminal domain of MukB. A large patch of highly conserved residues covers a region around the G loop and helices H5 to H6 on one side of the molecule. Residues coloured in yellow are conserved in four sequences, residues in grey or blue are conserved in three or two sequences, respectively. Colour coding corresponds to that in Figure 3. **(a)** Front view. The orientation is similar to that in Figure 2, but slightly rotated around the x axis to show the bottom of MukB. **(b)** Rotated by 180° around the y axis with respect to (a). (The figure was prepared using the program GRASP [34].)

part of a strand-loop-helix motif. The nucleotide-binding site in these P-loop-containing proteins is located on one side of the mostly parallel β sheet and is embedded in a conserved topology of alternating helices and β strands. None of these features of P-loop proteins can be found in the N-terminal domain of MukB. The lack of a complete nucleotide-binding site was further demonstrated by our failure, despite numerous attempts, to bind ATP or GTP to the crystals, although this could also be due to a 'wrong' conformation of the molecule being present in the crystals. The biochemical evidence for nucleotide binding and hydrolysis is not very clear at the moment. Full-length MukB can be cross-linked to nucleotides only in the presence of 10 mM Zn^{2+}, but no nucleotide hydrolysis could be detected [14]. In contrast, no nucleotide binding was found for the N-terminal 342 residues of MukB (unpublished observations), but some hydrolysis of ATP and GTP has been reported [15].

The striking similarities between the mutant phenotypes and the arrangement of globular domains separated by coiled-coil domains led others to include MukB in the SMC family of proteins [3,13]. It has been shown that the N-terminal domain of yeast SMC1 and SMC2 can be cross-linked to azido-ATP in the presence of magnesium without any need for a C-terminal domain [24]. The secondary-structure prediction for the N-terminal domain of SMCs matches the secondary structure reported here for the N-terminal domain of MukB (with the very important exception of H1 in MukB which is predicted to be a strand in SMCs). Also, the ~100 N-terminal residues around the G loop align well in SMC and MukB proteins. In SMC proteins, a consensus Walker B motif has been predicted to be present in the C-terminal domain which, in *bona fide* P-loop proteins, forms the second major site of nucleotide interaction. Although sequence similarity in the C-terminal regions of MukB and SMC proteins is very limited, we could locate a putative consensus Walker B motif in the C-terminal domain of MukB (Figure 3b) (four hydrophobic residues preceding an aspartate residue: in MukB the motif is LLFLDEAA, in the bovine ATPase β subunit it is LLFIDNIF).

In our crystal structure of the N-terminal domain of MukB, the Walker A motif is exposed on the surface and forms part of a spike that contains 100% conserved residues in all four known MukB sequences (Figure 4). This observation makes it likely that this region, together with the glycine-rich loop between helix H5 and H6 and the disordered loop between H2 and S3, is functionally important. The structural difference from nucleotide-binding proteins, the exposed G loop, and a highly conserved surface region surrounding the exposed G loop all support the model proposed by Melby *et al.* where the two chains of MukB form antiparallel dimers [13]. In this model the coiled-coil domains of MukB dimerize in an antiparallel manner, producing a combined N- and C-terminal domain at each end of the molecule. We propose as an extension to this model that the G loop, as well as part of the disordered loop between H2 and S3 and the loop between H5 and H6, form the contact surface with the C-terminal domain to generate a complete nucleotide-binding site at each end of the MukB dimer.

Biological implications

During the early stages of cell division in *Escherichia coli*, replicated daughter chromosomes move from mid-cell towards the cell quarter positions, which will become the centres of the daughter cells. Correct chromosome partitioning at the quarter positions was found

to depend on the *mukB* gene [9]. Cells containing mutations in the *mukB* gene produce anucleate cells and cells with small amounts of DNA [10]. The *mukB* gene product is a 170 kDa protein with a domain structure resembling eukaryotic motor proteins like kinesin or myosin [14].

MukB contains five distinctive domains: an N-terminal domain, a coiled-coil segment, a hinge domain, a second coiled-coil segment, and a globular C-terminal domain. The N-terminal domain contains a consensus nucleotide-binding sequence (Walker A motif). On the basis of electron microscopy studies, a model has been proposed in which the N- and C-terminal domains of MukB form heads on the ends of an antiparallel coiled coil [13]; this arrangement is equivalent to that observed in the functionally analogous SMC proteins (structural maintenance of chromosomes).

In order to investigate the relationship of MukB to motor proteins like kinesin or myosin and to understand the nature of nucleotide binding in this important family of proteins, we solved the three-dimensional structure of the 227-residue N-terminal domain of MukB from *E. coli*. The structure shows no homology to P-loop-containing nucleotide-binding proteins like kinesin or myosin. The loop predicted to be involved in nucleotide binding is exposed on the surface of the domain. These findings strongly support the antiparallel dimer model for MukB and SMC proteins, which proposes a symmetrical molecule with two identical heads at each end capable of DNA and nucleotide binding.

Materials and methods

Bacterial expression and protein purification

The polymerase chain reaction (PCR) was used to amplify a stretch of DNA from the plasmid pAX814 [10] using two primers (5′-TGACTACCATATGATTGAACGCGGTAAATTTCGCTCA-3′ and 5′-AGTCTACGGATCCGCCGCTGTTTTCTGGCAACAGGTAGTC-3′). The stop codon was replaced with a *Bam*HI restriction site to produce a 706 base pair (bp) DNA fragment with unique restriction sites for *Nde*I and *Bam*HI. The PCR fragment was cloned into the pHis17 vector (B Miroux, personal communication) putting it under control of the T7 promoter and adding eight residues at the C terminus (GSHHHHHH, plasmid pHis17-ecmukb227H_6). The expressed protein contains 235 residues and has a molecular weight of 26.2 kDa. C41(DE3) [17] cells were transformed and expressed the His_6-tagged protein after addition of isopropyl-β-D-thiogalactoside (IPTG). For large-scale expression, 12 L of 2× TY medium containing 100 μg/ml ampicillin were inoculated from a 1:40 preculture and induced with 1 mM IPTG at OD_{600} = 0.6. Cells were harvested, frozen in liquid nitrogen and opened by sonication and the addition of lysozyme in 50 mM Tris pH 8.0. The lysate was loaded onto a 25 ml Ni^{2+}-NTA column (Quiagen), washed extensively with 50 mM Tris, 300 mM NaCl, 100 mM imidazole pH 6.0. Protein was eluted with the same buffer containing 400 mM imidazole. The protein was applied to a sephacryl S-200 column (Amersham-Pharmacia) equilibrated in 20 mM Tris, 200 mM NaCl, 1 mM EDTA, 1 mM Na azide pH 7.5, and eluted as a single peak. Pooled fractions were desalted over a 80 ml sephadex G25 column (Amersham-Pharmacia) into 2.5 mM Tris pH 7.5. The protein can be stored at 4°C for several months.

Selenomethionine-substituted MukB

Selenomethionine (SeMet)-substituted protein was expressed from the pHis17-ecmukb227H_6 plasmid in the C41(DE3) host which is non-auxotrophic for methionine and methionine biosynthesis was inhibited by the conditions of growth [18]. A 1:1000 preculture in 2 × TY medium was used to inoculate 240 ml M9 minimal medium supplemented with 0.4% glucose, 2 mM $MgSO_4$ and 100 μg/ml ampicillin. After growth overnight (OD_{600} = 0.6) the culture was 1:50 diluted into 10 L prewarmed M9 minimal medium as above. After 10 h (OD_{600} = 0.3) 100 mg/l of DL-selenomethionine (Sigma), 100 mg/l lysine, threonine and phenylalanine, and 50 mg/l of leucine, isoleucine and valine were added as solids. IPTG (1 mM) was added after 15 min and cells were grown for 13 h (OD_{600} = 1.1). Methods of protein purification was identical to that for the unsubstituted protein with all Ni^{2+}-NTA buffers supplemented with 5 mM β-mercaptoethanol and all other buffers supplemented with 1 mM dithiothreitol (DTT). The unsubstituted and selenomethionine-substituted MukB proteins were checked by electrospray mass spectrometry (MukB observed 26,177.6 Da, calculated 26,180.0 Da; SeMet-MukB observed 26,457.6 Da, calculated 26,461.4 Da)

Crystallization and data collection

Crystals were grown by the sitting-drop vapour diffusion technique using 0.1 M Na citrate pH 4.9, 18–22% PEG 600 as the crystallization solution. Droplets composed of one part 50 mg/ml protein solution and one part crystallization solution were equilibrated at 4°C for at least a week. Crystals grew from a film of clear precipitate and belong to space group P622 with cell dimensions a = b = 111.4 Å, c = 65.0 Å, $\alpha = \beta = 90.0°$, $\gamma = 120.0°$ and one molecule per asymmetric unit. Crystals were harvested in crystallization solution and soaked for 1–2 min in cryobuffer (0.1 M Na citrate pH 4.9, 35% PEG 600) and frozen in liquid nitrogen. A native data set from a frozen crystal was collected using a MAR345 image-plate detector (MAR-Research, Hamburg, Germany) mounted on a Rigaku RU-300 generator with focusing mirrors and a spot size of 0.3 × 0.3 mm. MAD data sets from a single frozen selenomethionine-substituted crystal were collected on the MPG/GBF Wiggler beamline BW6 at DESY (Hamburg, Germany) using a MAR-CCD detector (MAR-Research, Hamburg).

Structure determination and refinement

All data were indexed and integrated with the MOSFLM package [25] and further processed using the CCP4 suite of programs [26]. Four out the six possible selenium atoms could be located in the isomorphous difference Patterson map of the native and SeMet3 data sets using SHELXS90 [27]. Phases were calculated using MLPHARE with SeMet3 as the 'native' data set. Phases were improved using ten cycles of solvent flipping with SOLOMON [28] and an almost perfect electron-density map at 2.4 Å resolution allowed the complete tracing of the N-terminal domain of MukB with MAIN [29]. A loop between residues 58 and 77 is disordered and has not been included in the model. Crystallographic refinement was performed using REFMAC and X-PLOR3.8 [30] with all data of data set SeMet1 from 10.0–2.2 Å resolution and idealized geometry according to Engh and Huber [31]. The current model contains residues 4–58, 77–224, and 75 water molecules. The model shows excellent stereochemistry (Table 2) and no Ramachandran outliers. Individual temperature factors have been refined using tight restraints.

Accession numbers

The coordinates and structure factors have been deposited in the Protein Data Bank with accession codes 1QHL and 1QHLSF, respectively.

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