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Cloning of a gene from *Bacillus cereus* with homology to the *mreB* gene from *Escherichia coli*

(Recombinant DNA; MreB; mRNA; gene family)

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SUMMARY

We have cloned and sequenced a gene coding for a putative shape-determining protein (MreB) highly homologous to the *mreB* gene product of *Escherichia coli*. The amino acid (aa) identity was 53% and the similarity 72%. The gene is expressed early in the logarithmic phase. The aa sequence comparison showed that the protein, like the *E. coli* MreB, has structural similarity to actin and heat-shock protein Hsc70 encoded by a new super-gene family.

INTRODUCTION

A fundamental question in cell biology is how cell shape is determined. In *E. coli* two regions of the chromosome have clusters of genes involved in shape-determining processes: the *mrd* region at 14.5 min and the *mre* region at 71 min (Tamaki et al., 1980; Wachi et al., 1987; Westling-Häggström et al., 1975). Mutants of genes in these regions are spherical. The *mre* region contains the *mreB* gene, coding for a 37-kDa protein, as well as genes downstream from *mreB* coding for at least four more proteins (Doi et al., 1988; Wachi et al., 1989; 1991). We report here a nt sequence of the *B. cereus* ATCC10987 gene coding for a putative protein highly homologous to the product of the *mreB* gene of *E. coli*. This is to our knowledge the first report on the conservation of the *mre* region in Gram$^+$ bacteria.

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Abbreviations: aa, amino acid(s); *B.*, *Bacillus*; bp, base pair(s); *E.*, *Escherichia*; EtdBr, ethidium bromide; Hsc70, heat-shock protein 70; kb, kilobase(s) or 1000 bp; LB, Luria-Bertani (medium); MreB, shape-determining protein; *mreB*, murein-pathway cluster e gene encoding MreB; nt, nucleotide(s); ORF, open reading frame; PBP, penicillin-binding protein; RBS, ribosome-binding site; SDS, sodium dodecyl sulfate; SSC, 0.15 M NaCl/0.015 M Na_3·citrate pH 7.6.

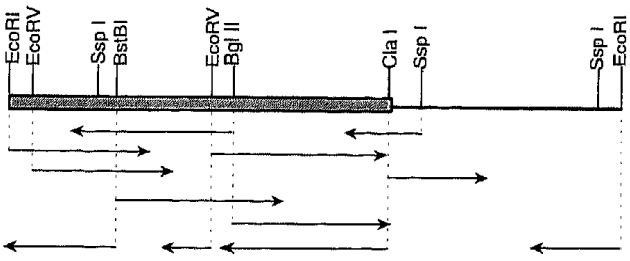

Fig. 1. Restriction map of plasmid Bc201 and strategy of nt sequencing. The *mreB* ORF is represented by a shaded box. Standard cloning methods were according to Sambrook et al. (1989). Plasmid DNA was sequenced using a fluorescence-based sequencer (Ansorge et al., 1976; Voss et al., 1989).

EXPERIMENTAL AND DISCUSSION

(a) Sequencing of Bc201

Plasmid Bc201 (AH168 in Kolstø et al., 1990) consisted of a 2.3-kb *Eco*RI fragment from *B. cereus* ATCC10987. The plasmid Bc201 had previously been used as a probe in the physical mapping of the *B. cereus* genome (Kolstø et al., 1990). The 2.3-kb *Eco*RI fragment hybridized to a 200-kb *Not*I fragment K (Kolstø et al., 1990) of the *B. cereus* ATCC10987 chromosome. Similarly, the probe hybridized to DNA from nine other *B. cereus* strains as well as to *B. thuringiensis*, but not to DNA from *B. licheniformis* or *B. subtilis* even when low-stringency washing conditions (3 × SSC/1% SDS, 65°C) were used (data not shown). The *Eco*RI fragment was sequenced (Fig. 1) and contained an ORF starting at the 5′ end of the fragment (Fig. 2). A possible RBS was observed 14 nt upstream from the first ATG in the available sequence corresponding to the 17 aa coded for by the fragment. The RBS AGGAGG fits well with the canonical *Bacillus* RBS, and the ΔG value of −16.6 kcal/mol corresponds to the average value of the strength of RBS interaction for *Bacillus* mRNAs (Hager and Rabinowitz, 1985). The putative ORF starting at the ATG after the RBS was 333 aa. A search for similar sequences

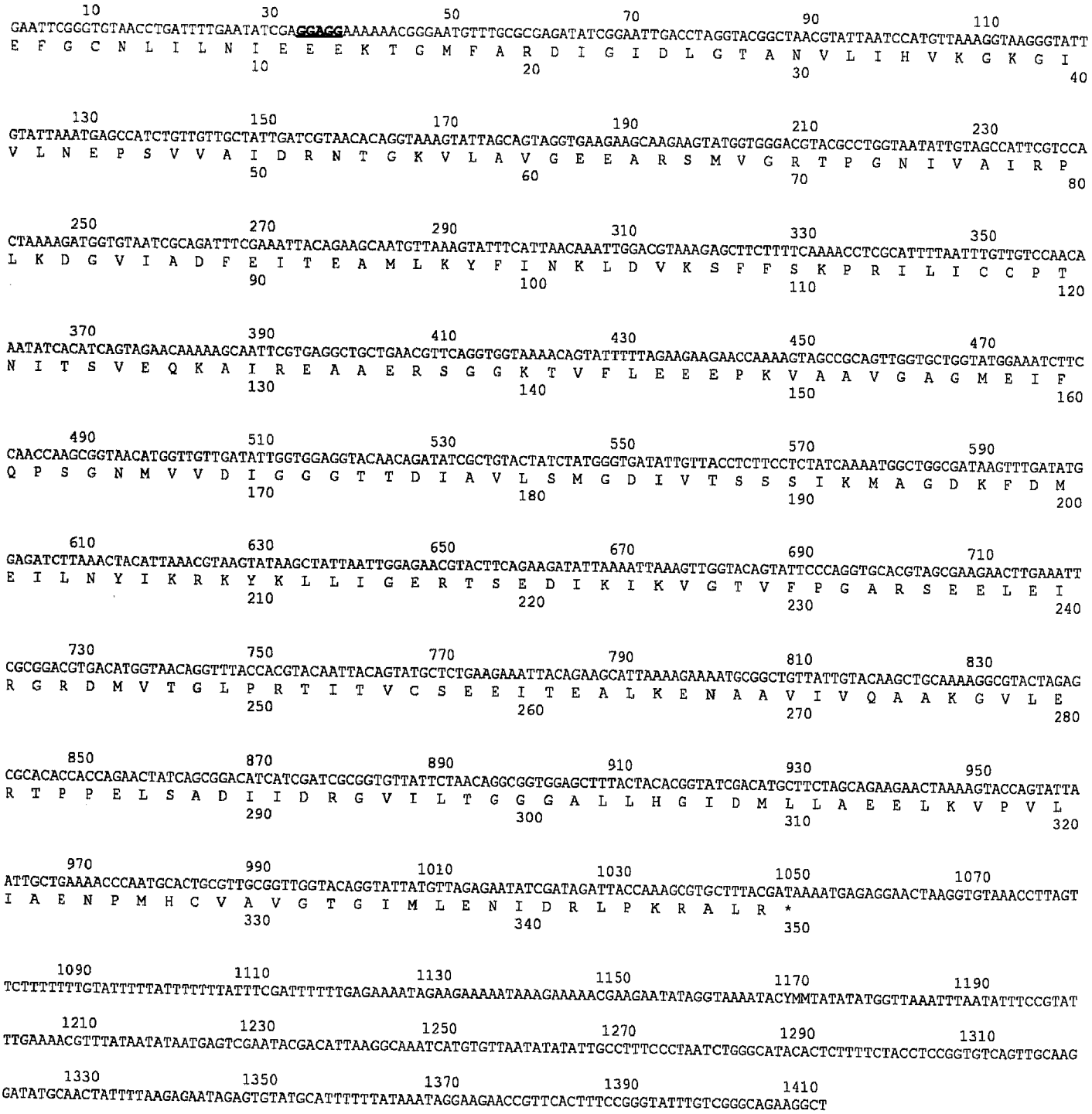

Fig. 2. The nt sequence of part of Bc201, and the deduced aa sequence of the *mreB* ORF. A putative RBS is underlined and in bold type. Asterisk represents the stop codon. The sequence has been submitted to the EMBL database under accession No. X62374.

in the databases revealed an extensive homology to MreB from *E. coli* (Doi et al., 1988) over the entire length of both proteins, with 53% aa identity and 72% similarity.

(b) Analysis of mRNA

To analyze transcription of the putative gene, RNA was isolated from *B. cereus* cells (Igo et al., 1986). Aliquots of RNA were subjected to electrophoresis, blotted onto nylon filters and hybridized with the *Bgl*II-*Cla*I fragment from the coding region (Fig. 1). A transcript of about 1.2 kb was observed, suggesting that the gene is not part of a larger operon. The ORF of fragment Bc201 is 1047 bp long, the size of the message indicates that the transcribed region is not much longer. When RNA isolated from different time points of the growth curve was analyzed, a sharp fall in specific mRNA was observed (Fig. 3,a and b). Both EtdBr staining and hybridization experiments using part of the gene for *B. cereus* 23S RNA as a probe showed that RNA was present at all time points. These results suggest a predominant synthesis of the mRNA only in the early logarithmic phase. The gene appeared to be shut off during late exponential and stationary phase.

The function of the *mreB* gene product of *E. coli* has not yet been identified. It has been proposed that the *mreB* gene may function as a regulator of progression to cell division or elongation (Wachi and Matsuhashi, 1989). Overproduction of MreB after introduction of a plasmid carrying the *mreB* gene resulted in filamentous cells, indicating inhibition of cell division, while the spherical *mreB* mutants had an increased amount of PBP3 (Wachi and Matsuhashi, 1989), the septum formation-specific peptidoglycan transglycosylase-transpeptidase. Our data show that *mreB* in *B. cereus* is transcribed early in the growth phase only, indicating that the concentration of the MreB protein per cell may decline during late exponential growth even if the protein is stable. In what way the level of MreB might play a role in the regulation of cell shape is not known.

(c) The aa sequence comparison

The homology of a large 143-aa C-terminal segment of MreB from *E. coli* with a corresponding region in FtsA from *E. coli* (Doi et al., 1988; Robinson et al., 1984) is also observed in the sequence of MreB from *B. cereus*. In addition, the homology can be extended to an N-terminal part (Matsuhashi et al., 1990) (Fig. 4). Furthermore, using FASTA (Pearson and Lipman, 1988), a significant similarity between MreB and members of the Hsc70 family (Matsuhashi et al., 1990) of heat-shock proteins over the entire length is indicated (optimized scores up to 147). This is further support for the existence of a new superfamily containing functionally very different proteins such as actin, hexokinase, Hsc70 (Flaherty et al., 1990; 1991; Kabsch et al., 1990), as well as FtsA and MreB (Bork et al., 1992). Based on structural comparisons of actin, Hsc70 and hex-

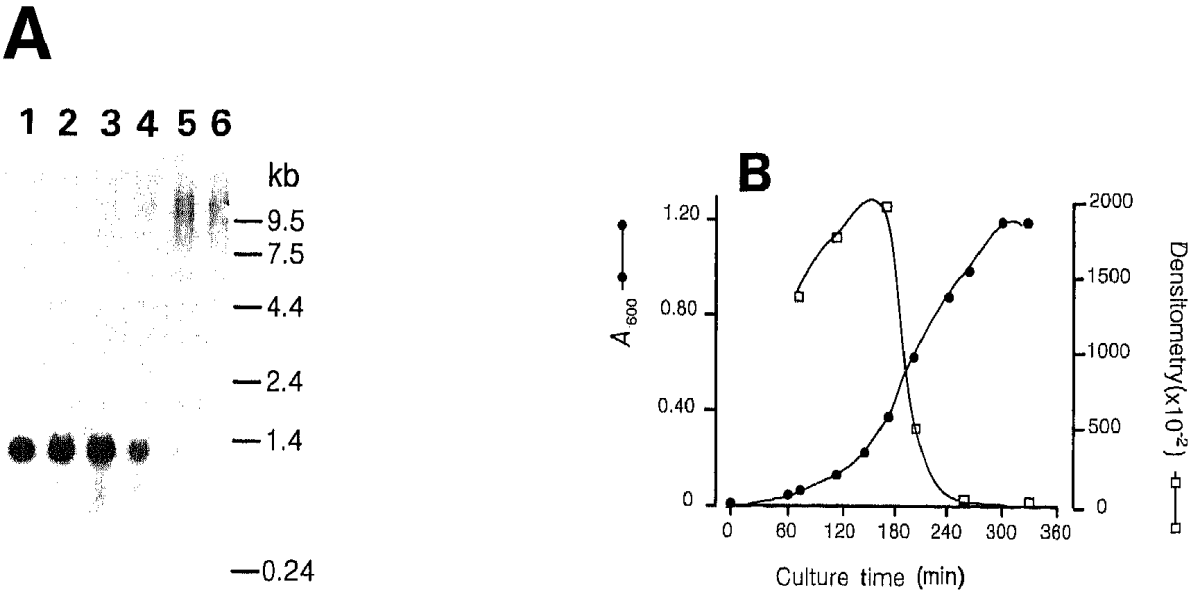

Fig. 3. Level of mRNA hybridizing with a probe from the putative *mreB* gene from *B. cereus* and the growth kinetics. (**A**) Autoradiogram of a Northern blot of 10 μg total RNA isolated at (lane **1**) 70, (lane **2**) 120, (lane **3**) 170, (lane **4**) 220, (lane **5**) 280 and (lane **6**) 350 min after inoculation. (**B**) Growth curve (A_{600nm}) ●——●, and densitometry readings (Shimadzu scanner) (arbitrary units) of Bc201 *Bgl*II-*Cla*I probe hybridized to Northern blot of total *B. cereus* RNA □——□. **Methods**: *B. cereus* was grown in LB medium at 30°C. Growth was measured by monitoring A_{600nm}, and total RNA was isolated from 10-ml samples after 60–320 min as described by Igo and Losick (1986). Electrophoresis of 10 μg aliquots of RNA was performed in 1% agarose gels containing 2.2 M formaldehyde according to Sambrook et al. (1989). The gels were stained with EtdBr, and RNA was transferred to nylon membranes (Schleicher & Schüll) by capillary blotting in 20 × SSC. Probes were labeled by incorporating [α-^{32}P]dATP/dCTP by random priming (Feinberg and Vogelstein, 1983; 1984) and hybridizations performed at 65°C in 3 × SSC/1% SDS/10 × Denhardt solution/10% dextran sulfate/100 μg per ml of denatured salmon sperm DNA. Filters were washed at 65°C in 3 × SSC/1% SDS, 1 × SSC/1% SDS, 0.3 × SSC/1% SDS, for 2 × 30 min in each solution. After autoradiography, the films were subjected to densitometry on a Shimadzu CS-9000 instrument at 600 nm.

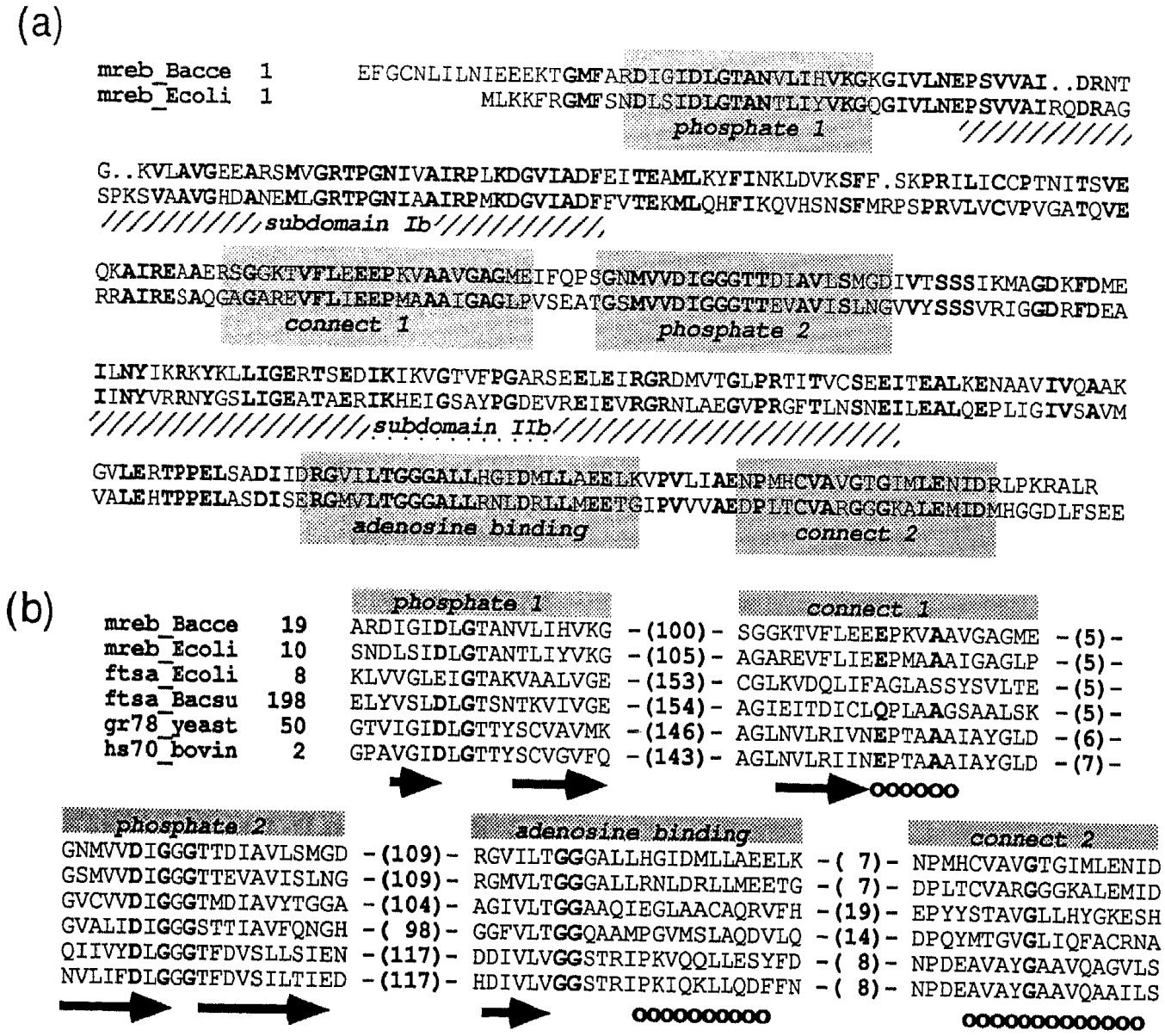

Fig. 4. The aa sequence alignments for MreB. (a) Alignment between MreB from *B. cereus* and that from *E. coli*. Conserved aa are in bold face. The shaded boxes represent structurally (and probable functionally) conserved segments in actin, Hsc70 and hexokinase, for which sequence patterns can be derived. The regions are located in subdomains Ia and IIa (for nomenclature see Flaherty et al., 1990) which are predicted to be common to all members of the superfamily. They are scattered throughout the sequence, but comprise nearly 70% of the common structural core (Bork et al., 1992). Subdomain Ib is topologically different in all known structures (actin, Hsc70 and hexokinase). MreB has a considerable deletion in this region, but within the remaining part of subdomain Ib both MreB proteins have conserved sequences. Subdomain IIb is also structurally variable in the proteins for which the 3D structure is known. Therefore, conserved regions within these subdomains represent candidates involved in specific MreB functions. These subdomains are indicated (hatched areas). (b) Multiple alignment of the regions boxed in part a. Functionally and structurally important residues, as identified by structural contact analysis, are shown in bold face. The numbers of aa between the boxes are also given. Secondary structure elements are indicated (arrows: β-strands, circles: α-helices).

okinase (for review see Holmes et al., 1992) five regions of structural as well as sequence similarity have been identified which are also present in *E. coli* MreB and FtsA (Bork et al., 1992), and in the *B. cereus* MreB (Fig. 4b). Since three of these regions are directly involved in ATP binding and the other two are located in a hinge region important for the ATP-binding mechanism both ATPase activity and an overall 3D-fold similar to the other proteins of this family is suggested. The detailed functional mechanisms of cell-shape determination including interactions of MreB with other proteins remains to be elucidated.

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