Characterization of the *murF* gene of the cyanobacterium *Synechocystis* sp. PCC 6803

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The *murF* gene encodes UDP-*N*-acetylmuramoyl-L-alanyl-D-glutamyl-*meso*-2,6-diaminopimeloyl-D-Ala-D-Ala synthetase (EC 6.3.2.15), which catalyses the final step in the synthesis of UDP-*N*-acetylmuramoyl-pentapeptide, the precursor of murein. An open reading frame identified as the *murF* gene was found in the genome of the cyanobacterium *Synechocystis* sp. PCC 6803. The *murF* gene encodes a polypeptide of 454 amino acid residues with a predicted molecular mass of 48 kDa. The *murF* gene is present as a single-copy gene in the genome of *Synechocystis* sp. PCC 6803. The amino acid sequence deduced from the *murF* gene is 39% identical to that of the product of the *murF* gene of *Escherichia coli*. The cyanobacterial *murF* gene complemented a temperature-sensitive mutation in a *murF*-deficient strain of *E. coli*, restoring the mutant's ability to synthesize a cell wall and to survive at high sublethal temperatures.

Keywords: cell wall, cyanobacterium, *murF* gene, murein, *Synechocystis* sp. PCC 6803

INTRODUCTION

The biosynthesis of the bacterial peptidoglycan known as murein is a complex process that consists of at least three major steps: (1) the synthesis of UDP-*N*-acetylmuramoyl-pentapeptide from UDP-*N*-acetylglucosamine in the cytoplasm; (2) the lipid cycle reactions, in which a final pentapeptide precursor is transferred from the cytoplasm to an existing peptidoglycan; (3) the formation of cross-linked polysaccharides and the cell wall saccule (Cooper, 1991; Rogers *et al.*, 1980).

The *murF* gene of *Escherichia coli* (Parquet *et al.*, 1989) encodes UDP-*N*-acetylmuramoyl-L-alanyl-D-glutamyl-*meso*-2,6-diaminopimeloyl-D-Ala-D-Ala synthetase (EC 6.3.2.15), which adds D-alanyl-D-alanine to UDP-*N*-acetylmuramoyl-L-alanine-D-glutamyl-*meso*-2,6-diaminopimelate (Michaud *et al.*, 1987). This reaction is the last step in the chain of reactions in the synthesis of UDP-*N*-acetylmuramoyl-pentapeptide. Mutants of *E. coli* deficient in the *murF* gene cannot synthesize a properly structured cell wall and lyse at high temperatures, such as 42 °C, as a result of the impaired synthesis of peptidoglycan (Lugtenberg & van Schijndel-van Dam, 1972; Maruyama *et al.*, 1988).

The cell surface of *E. coli* has been well characterized (Cooper, 1991), but the cell surface of cyanobacteria has not been characterized to the same extent. The presence of an outer membrane in cyanobacteria (Drews & Weckesser, 1982) suggests that cyanobacterial cell walls might be similar to those of Gram-negative bacteria. However, cyanobacterial cell walls react positively in the Gram reaction (Jürgens *et al.*, 1985). The thickness of the murein layer of cyanobacterial cell walls (Weckesser & Jürgens, 1988) and the degree of cross-linkage in peptidoglycan are similar to those found in Gram-positive bacteria (Jürgens *et al.*, 1983; Jürgens & Weckesser, 1986). Jürgens *et al.* (1983) concluded that cyanobacteria have developed their own particular organization of cell walls, which differs from that of the typical cell walls of both Gram-negative and Gram-positive bacteria.

Here we present data on the structure and expression of the *murF* gene from the unicellular cyanobacterium *Synechocystis* sp. PCC 6803.

METHODS

Strains and culture conditions. Competent cells of *E. coli* JM109 for subcloning of DNA were obtained from Takara (Kyoto, Japan). A high-temperature-sensitive *murF*-deficient strain, *E. coli* JE6607 [F⁻, *murF*(Ts), Δ*pro–lac*, *thi*] (Maruyama *et al.*, 1988), was obtained from the National Institute of Genetics (Mishima, Japan). Cells of *E. coli* JE6607 were grown at 30 °C in Luria–Bertani (LB) medium (Sambrook *et al.*, 1989). The cells were rendered competent with $CaCl_2$ as described by Sambrook

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The EMBL/GenBank/DDBJ accession number for the nucleotide sequence data reported in this paper is X62437 (SSMURFA).

et al. (1989). The cyanobacterium, *Synechocystis* sp. PCC 6803, was obtained from the Pasteur Culture Collection (Paris, France). *Synechocystis* sp. PCC 6803 was cultivated at 34 °C under photoautotrophic conditions in BG11 medium (Stanier *et al.*, 1971), with continuous aeration with air containing 1% (v/v) CO_2 as previously described (Wada & Murata, 1989).

Analysis of DNA sequence. The plasmid pTZ19R/8 kbp, carrying a 7·4 kb fragment of the genomic DNA of *Synechocystis* sp. PCC 6803, was obtained as described previously (Wada *et al.*, 1990). The full-length insert was excised from the plasmid by digestion with *Eco*RI restriction endonuclease and was recloned into pBluescript II KS(+) (Stratagene). The resultant plasmid was designated pES17. The nucleotide sequence of the region downstream of the *desA* gene, which encodes the Δ12 desaturase (Wada *et al.*, 1993), was determined by the dideoxy chain-termination method (Sanger *et al.*, 1977), with deletions by exonuclease III and mung bean exonuclease (both from New England Biolabs) and with synthetic oligonucleotides as sequencing primers. The computer analysis of the sequence data was performed using the PCGene program package for personal computers (IntelliGenetics). The amino acid sequences were aligned according to the algorithm of Needleman & Wunsch (1970).

Southern blot hybridization. Genomic DNA was isolated from *Synechocystis* sp. PCC 6803 by the method of Williams (1988). The DNA was digested with *Hin*dIII, *Dra*I, *Ava*I and *Nco*I restriction endonucleases, and the DNA fragments were subjected to electrophoresis in a 0·8% agarose gel and transferred to a GeneScreen Plus nylon membrane (NEN Research Products). The copy number of the *murF* gene was determined with a DNA probe that corresponded to the region from position 408 to position 1098 of the nucleotide sequence shown in Fig. 2. The DNA fragment was labelled with [α-^{32}P]dCTP by nick translation. Hybridizations were carried out as described previously (Wada *et al.*, 1992).

Northern blot hybridization. Isolation of total RNA from *Synechocystis* sp. PCC 6803 and Northern blot hybridization were carried out as described previously (Los *et al.*, 1993). The DNA probe was the same as that used for Southern blot hybridization. The size of the *murF* mRNA was determined by reference to the mobilities of a set of RNA molecular mass markers (Boehringer Mannheim).

Complementation of a mutation in the *murF* gene in *E. coli*. Plasmids for the transformation of the *murF*-deficient strain of *E. coli* JE6607 were constructed as follows (see Fig. 4a). A plasmid designated pSA21 was obtained by subcloning the *Sma*I–*Apa*I fragment of 2837 bp from pSE17 (positions 2321–5158) into the corresponding sites of pBluescript II KS(+). Plasmids pSA216, pSA217, pSA2116, and pSA2118 were obtained after truncation of the *Sma*I–*Apa*I insert of pSA21 by exonuclease III and mung bean exonuclease. The sequences of the 5′ regions of the inserts were determined. Plasmid pAB92 was obtained by subcloning the *Apa*I–*Sma*I fragment of 2789 bp from pSE17. pEA13 was obtained by subcloning the *Apa*I–*Eco*RI fragment of 2837 bp from pSE17 into the analogous sites of pBluescript II KS(+). These plasmids were used for transformation of competent cells of the *murF*-deficient strain of *E. coli* JE6607. The resultant transformants were grown at 30 °C on LB agar plates supplemented with 50 μg ampicillin ml^{-1}. Single colonies were transferred to LB agar plates without ampicillin.

The genetic complementation of the mutation in the *murF* gene in transformants was examined by monitoring the ability of the transformants to grow at 42 °C. The cells of the *murF*-deficient strain of *E. coli* JE6607 and the transformants were grown for 14–16 h at 30 °C in liquid LB medium. Portions of the cultures were inoculated into 100 ml LB medium to give an OD_{660} of 0·015 at 30 °C, and these cultures were incubated at 42 °C. The growth of the cells was followed by monitoring OD_{660}.

RESULTS

Organization of the *murF*-containing region of the genomic DNA of *Synechocystis* sp. PCC 6803

We determined the nucleotide sequence of a 7490 bp DNA fragment that is located downstream of the *desA* gene on the chromosome of *Synechocystis* sp. PCC 6803. Fig. 1 shows the genetic map of the DNA fragment. In addition to the previously cloned *desA* gene (Wada *et al.*, 1990) five open reading frames were found in this region. ORF454 and ORF291 were located on the same strand as *desA*, whereas ORF457, ORF128 and ORF172 were located on the opposite strand. ORF172 was previously identified as the *rplI* gene, which encodes the ribosomal protein L9 (Malakhov *et al.*, 1993). ORF128 represents the *cytM* gene, which encodes a *c*-type cytochrome (Malakhov *et al.*, 1994). ORF454 showed a high degree of homology to the *murF* gene of *E. coli* (Parquet *et al.*, 1989) and was shown to be the equivalent of the *murF* gene in *Synechocystis* sp. PCC 6803, as described below. A search for sequence homology in the SWISS-PROT and PIR databases did not reveal any significant similarity between ORFs 291 and 457 and known proteins.

Southern blot analysis revealed only one band that hybridized with the probe for the *murF* gene after digestion of the genomic DNA of *Synechocystis* sp. PCC 6803 with *Hin*dIII, *Dra*I, *Ava*I and *Nco*I restriction endonucleases (data not shown). This result suggests that the *murF* gene exists as a single copy in the chromosome of *Synechocystis* sp. PCC 6803. The analysis of the copy numbers of the other ORFs in the vicinity of the *murF* gene (data not shown) revealed that they are also present as single-copy genes in *Synechocystis* sp. PCC 6803.

Structural features of the *murF* gene

The nucleotide sequence of the *murF* gene obtained from both strands and the deduced amino acid sequence are

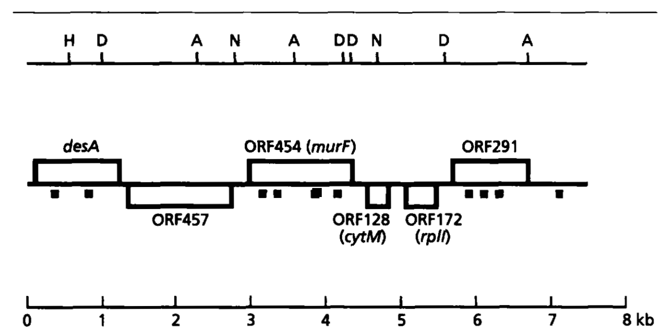

Fig. 1. A genetic map of the *murF*-containing region of the genomic DNA of *Synechocystis* sp. PCC 6803. The highly iterated palindromic sequences GGCGATCGCC (HIP1) are indicated by solid boxes. The sites of recognition by the restriction endonucleases used for Southern blot analysis are shown: A, *Ava*I; D, *Dra*I; H, *Hin*dIII; N, *Nco*I.

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CGGAATAAAACCAGGATGTTTTACTGGTCATTACTGGCTTCCGATTTTTCTGTATCTATTTCTCC  -133
TCCAATGTAAGTGATGCAAGCACCCATGGGAGGTGATCTAGATCACAGATAAAAATTGCAAATGAC  -67
TTAACATTCCTGAGTCAATTCTAACTCAGTTTTCCTCCGCCATATTTTTCACTCCTATTCTTTCCC  -1

ATGAACTCCAAACTTTCCCTATTTGACATCCGTACATTCCTAGAAAGTTGTCTATTGTCTGTTACC   66
 M  N  S  K  L  S  L  F  D  I  R  T  F  L  E  S  C  L  L  S  V  T    22

AATCTTTCCGAGGATATACGGATAAATAATATTTGCACCGATACAAGATCTTTGGTGTCAGGGGAC  132
 N  L  S  E  D  I  R  I  N  N  I  C  T  D  T  R  S  L  V  S  G  D    44

CTATTTCTTGCCTTGCGGGGGGAAAGTTTTGATGGCCATAGCTTCATTCCCCAGGCCTTGACGGCG  198
 L  F  L  A  L  R  G  E  S  F  D  G  H  S  F  I  P  Q  A  L  T  A    66

GGGGCGATCGCCGTGGTGACGGATCGGCCAGTGGAGGGATTAGGGGAGACGGTGGCCCAATTTTTA  264
 G  A  I  A  V  V  T  D  R  P  V  E  G  L  G  E  T  V  A  Q  F  L    88

GTGGAAGATACTTTGGTGGCCTATCAACACATCGCAGCGGGATGGCGACAACGGTTTACCATTCCC  330
 V  E  D  T  L  V  A  Y  Q  H  I  A  A  G  W  R  Q  R  F  T  I  P   110

ATCATTGGGGTAACCGGTTCTGTGGGTAAGACCACCACCAAAGAATTAATTGCGGCGGTGTTGTCC  396
 I  I  G  V  T  G  S  V  G  K  T  T  T  K  E  L  I  A  A  V  L  S   132

CAGTTTGGCAATGTGCATAAAACCAGAGCTAATTACAACAATGAAATTGGGGTGCCGAAAACTTTG  462
 Q  F  G  N  V  H  K  T  R  A  N  Y  N  N  E  I  G  V  P  K  T  L   154

TTGGAACTATCCCCAGACCATGATTTTGCCATTGTGGAAATGGCCATGCGGGGTCGGGGGCAAATT  528
 L  E  L  S  P  D  H  D  F  A  I  V  E  M  A  M  R  G  R  G  Q  I   176

GCTCTTTTGGCGGATATTGCCAAGCCCACCATCGGTTTAATTACTAACGTTGGCACTGCCCACATT  594
 A  L  L  A  D  I  A  K  P  T  I  G  L  I  T  N  V  G  T  A  H  I   198

GGTCTTTTAGGCTCGGAGTTGGCGATCGCCGAAGCCAAGTGTGAATTACTAGCCCACCAACCGCCC  660
 G  L  L  G  S  E  L  A  I  A  E  A  K  C  E  L  L  A  H  Q  P  P   220

GAGAGCACCGCCATTCTCAATCGGGATAATGCTTTGTTAATGGAGACAGCCCAACGGTTTTGGCAG  726
 E  S  T  A  I  L  N  R  D  N  A  L  L  M  E  T  A  Q  R  F  W  Q   242

GGAAAAACCATTACCTATGGCCTAGAAGGGGGAGATGTGCACGGGACAGTGGATGGGGAAAATTTA  792
 G  K  T  I  T  Y  G  L  E  G  G  D  V  H  G  T  V  D  G  E  N  L   264

ATCCTTGATGGAGTTAGTTTGCCTTTGCCCCTAGCCGGTGTCCACAATGCCTCTAATTACCTAGCG  858
 I  L  D  G  V  S  L  P  L  P  L  A  G  V  H  N  A  S  N  Y  L  A   286

GCGATCGCCCTGGCCCAATGTTTAGGGTTGGACTGGCAGCAGTTACAGTCGGGGCTGACGGTGGAG  924
 A  I  A  L  A  Q  C  L  G  L  D  W  Q  Q  L  Q  S  G  L  T  V  E   308

TTACCCAAGGGGCGGGCCCGTCGTTACCAATGGGGCCAGGACGTGGTTCTATTGGATGAAACCTAT  990
 L  P  K  G  R  A  R  R  Y  Q  W  G  Q  D  V  V  L  L  D  E  T  Y   330

AACGATGGCTTGGAATCCATGTTGGCATCCTTGGATTTATTGGCCAATACCCCTGGGAAGCGACGT 1056
 N  D  G  L  E  S  M  L  A  S  L  D  L  L  A  N  T  P  G  K  R  R   352

TTAGCAGTGCTGGGGGCGATGAAGGAACTGGGAGATTATGGCCCGACATTTCACCAACGGGTGGGG 1122
 L  A  V  L  G  A  M  K  E  L  G  D  Y  G  P  T  F  H  Q  R  V  G   374

GCCAAAGTGAAAGCCCTAGGTTTAGACGGTCTTTTCCTATTAGCCAATGATCCCAATACCGATGCC 1188
 A  K  V  K  A  L  G  L  D  G  L  F  L  L  A  N  D  P  N  T  D  A   396

ATTGCCGCCGGGGCTAACGGTGTGGAGACCCAATCCTTCAGCGATGGGCCTAGTTTAGTGGCAGCC 1254
 I  A  A  G  A  N  G  V  E  T  Q  S  F  S  D  G  P  S  L  V  A  A   418

CTAAAAACTACCCTCCAACCAGGCGATCGCCTCCTGTTTAAAGCATCCAATTCCGTCGGTCTAGGG 1320
 L  K  T  T  L  Q  P  G  D  R  L  L  F  K  A  S  N  S  V  G  L  G   440

GCTGTGGTCAGTCAGTTGTTGGCAGAAAATCCCACTTCGGTTTAAAACGGCAAGGCTTGAGTCAGT 1386
 A  V  V  S  Q  L  L  A  E  N  P  T  S  V  *                         454

TAAAAATGGGGTTAATCTCACTCCAGCGGGATCAATCGAGATCAATAGGTGTGGGGTTGGTTGGCA 1452
GTGGCATCCAACAAATGGCCTAAAACCAGATGGTTAAGAACATGGCTAAAAACGTCTATCCAAACC 1518
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Fig. 2. The nucleotide sequence and the deduced amino-acid sequence of the *murF* gene of *Synechocystis* sp. PCC 6803. The highly iterated palindrome 1 (HIP1)-like sequences are double-underlined.

shown in Fig. 2. The *murF* gene encodes a protein of 454 amino acid residues with a predicted molecular mass of 48 kDa and a calculated pI of 4·9. These characteristics are similar to those of the protein from *E. coli*, which has a predicted molecular mass of 48 kDa and a calculated pI of 5·3. The 5′ flanking region of the *murF* gene of *Synechocystis*

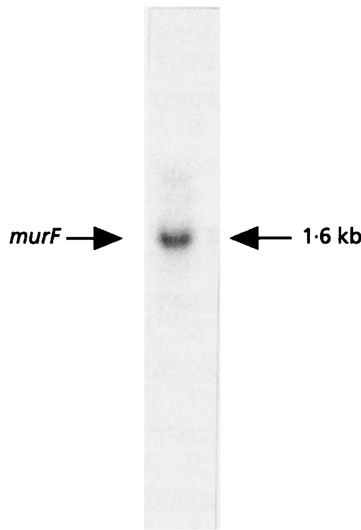

Fig. 3. Northern blot analysis of the expression of the *murF* gene in *Synechocystis* sp. PCC 6803.

sp. PCC 6803 does not contain any sequence motifs that resemble the regulatory sequences found in most genes from *E. coli*, such as the −35, −10, and Shine–Dalgarno motifs.

The details of the nucleotide sequence of the *murF* gene of *Synechocystis* sp. PCC 6803 are unique in that this gene contains four palindromic GGCGATCGCC motifs (double-underlined in Fig. 2). The *murF* gene of *E. coli* (Parquet *et al.*, 1989) does not contain such sequences. This palindromic sequence appears ten times in the 7490 bp fragment of the genomic DNA of *Synechocystis* sp. PCC 6803 (Fig. 1).

Northern blot analysis of the expression of the *murF* gene

Total RNA from *Synechocystis* sp. PCC 6803 was subjected to Northern blot hybridization with the probe for the *murF* gene. Only one specifically-hybridizing band appeared, with the mobility of a 1·6 kb fragment (Fig. 3), indicating that the *murF* gene is transcribed as a monocistronic operon. The upper bands represent nonspecific hybridization of the probe to the rRNAs. This result coincides with the finding that the *murF* gene is located as a non-clustered single gene on the chromosome of *Synechocystis* sp. PCC 6803 (Fig. 1). We examined alterations in the level of the *murF* transcript upon incubation of the cells of this strain at low and high temperatures and upon incubation in the dark and in the light at various light intensities. Under all the conditions tested, the intensity of the hybridized band that corresponded to the *murF* gene remained at a constant level (data not shown). This result implies that the *murF* gene is transcribed constitutively in the cells of *Synechocystis* sp. PCC 6803.

Complementation of a temperature-sensitive mutation in the *murF* gene of *E. coli* by the *murF* gene of *Synechocystis* sp. PCC 6803

To characterize in further detail the product of the *murF* gene of *Synechocystis* sp. PCC 6803, we transformed cells of

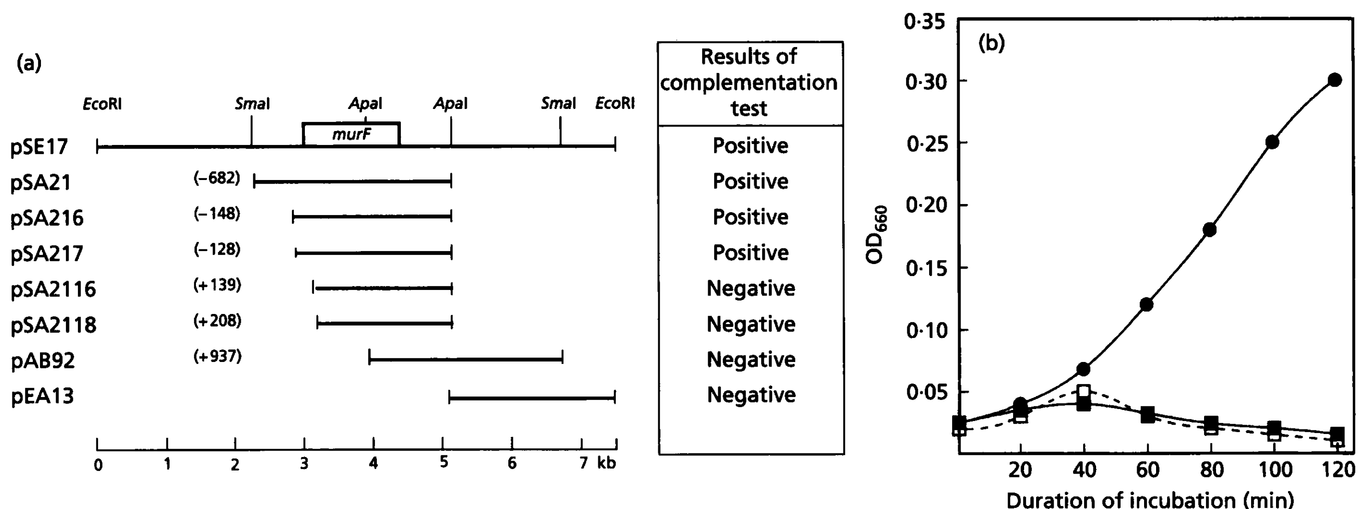

Fig. 4. Complementation of mutation in the *murF* gene in *E. coli* strain JE6607 by the *murF* gene from *Synechocystis* sp. PCC 6803. (a) Deletion map of the *murF*-containing chromosomal region of *Synechocystis* sp. PCC 6803, indicating the fragments of DNA used for genetic complementation of the temperature-sensitive *murF* mutant of *E. coli*. Numbers in parentheses indicate positions from the initiation codon (+1) of the *murF* gene. 'Positive' and 'Negative' indicate positive and negative results of complementation. The sites recognized by the restriction endonucleases that were used for subcloning are shown. (b) Growth at 42 °C of various transformants of *E. coli* JE6607. □, *murF*-deficient mutant cells of *E. coli* JE6607 (control); ■, cells transformed with pSA2116; ●, cells transformed with pSA217. Cells transformed with pSA2118, pAB92 and pEA13 gave essentially the same results as cells transformed with pSA2116. Cells transformed with pSE17, pSA21 and pSA216 gave essentially the same results as cells transformed with pSA217.

a *murF*-deficient mutant of *E. coli* JE6607 (Maruyama *et al.*, 1988) with plasmids that carried a full-length or truncated *murF* gene from *Synechocystis* sp. PCC 6803. Since the cells of this strain of *E. coli* are unable to synthesize intact cell walls, they form colonies with mucoid shape at 30 °C, and they lyse at 42 °C.

The plasmids used for the transformation of *E. coli* JE6607 are shown in Fig. 4(a). The control plasmids pBluescript II KS(+) and pEA13, which contained none of the *murF* sequence, did not restore the ability to grow at 42 °C (Fig. 4b). The transformation of the *E. coli* mutant with plasmids pSE17, pSA21, pSA216 and pSA217 which carried the full-length *murF* gene resulted in colonies with regular morphology. The transformed cells grew at 42 °C as did the wild-type cells (Fig. 4b). Plasmids pSA2116, pSA2118, and pAB92 with truncated *murF* genes did not complement the temperature-sensitive phenotype of the mutant cells (Fig. 4b). All these results indicate that the *murF* gene of *Synechocystis* sp. PCC 6803 was recognized and expressed by the transcriptional and translational machinery of *E. coli*, and that the product of the *murF* gene catalysed the ligation of D-Ala-D-Ala to UDP-*N*-acetylmuramoyl-L-Ala-D-Glu-*meso*-diaminopimelate (Michaud *et al.*, 1987). Although the *murF* gene of *Synechocystis* sp. PCC 6803 does not have any sequence motifs that resemble the regulatory elements found in the analogous gene from *E. coli* (Fig. 2), it was expressed in *E. coli* without a requirement for any additional regulatory sequence.

DISCUSSION

We cloned the *murF* gene, which encodes UDP-*N*-acetylmuramoyl-L-alanyl-D-glutamyl-*meso*-2,6-diaminopimeloyl-D-Ala-D-Ala synthetase (EC 6.3.2.15), the enzyme that participates in the synthesis of murein, from the cyanobacterium *Synechocystis* sp. PCC 6803. The organization of the genomic region that includes the *murF* gene in *Synechocystis* sp. PCC 6803 differs from that of the *murF*-containing fragment of the *E. coli* chromosome.

The clustered organization of genes involved in the synthesis of murein and cell division has been well documented in *E. coli* and in other eubacteria (Kröger *et al.*, 1991; Mengin-Lecreulx *et al.*, 1989). However, the chromosome of *Synechocystis* sp. PCC 6803 seems to be organized in a different way. We have not found any other genes for the enzymes that are involved in the synthesis of murein in the vicinity of the *murF* gene of *Synechocystis* sp. PCC 6803 (Fig. 1). The *rplI* gene was also found to be a single-gene operon in *Synechocystis* sp. PCC 6803 (Malakhov *et al.*, 1993), whereas the *rplI* gene is a member of a cluster of genes for ribosomal proteins of *E. coli* (Schnier *et al.*, 1986).

A novel structural feature of the *murF* gene of *Synechocystis* sp. PCC 6803 is the presence of four palindromic sequences, GGCGATCGCC, in its coding region (Figs 1 and 2). The *murF* gene of *E. coli* does not contain any copies of this sequence. We performed computer analysis to examine the appearance of such sequences in the EMBL prokaryotic database, and we found that the palindrome RGCGATCGCY appears in cyanobacterial genomes ten times more frequently than in genomes of other prokaryotes. We suspect that this structural feature has a specific function in cyanobacteria and that it is probably relevant to DNA restriction and DNA modification systems. Recently, Gupta *et al.* (1993) suggested that the octanucleotide GCGATCGC, i.e. the highly iterated palindrome 1 (HIP1), is involved in recombination in *Synechococcus* sp. PCC 6301.

Alignment of the amino acid sequences of the *murF* genes from *Synechocystis* sp. PCC 6803 and *E. coli* revealed 39% identity at the amino acid level (Fig. 5). Three highly homologous regions were found in the sequences: at positions 35–55, 115–155 and 273–290 (boxed in Fig. 5). The function of the first region between positions 35 and 55 is unknown. The second region between positions 115 and 155 includes a typical ATP-binding domain GxxGKT/S (Parquet *et al.*, 1989) and it may possibly be the active site of the enzyme. The region corresponding to amino acid residues 273–290 is also conserved to a considerable extent in UDP-*N*-acetylmuramoyl-dipeptide synthetase, which is the product of the *murD* gene of *E. coli* (Ikeda *et al.*, 1990; Mengin-Lecreulx *et al.*, 1989; Mengin-Lecreulx & van Heijenoort, 1990). This region may represent the binding site of such proteins to the UDP-*N*-acetylmuramoyl-monopeptide or -tripeptide.

The transformation of *murF*-deficient cells of *E. coli* JE6607 with the cyanobacterial *murF* gene restored the normal phenotype of *E. coli* cells and allowed them to survive and proliferate at high temperature. The cyanobacterial *murF* gene was expressed in *E. coli*, although no part of the *murF* gene of *Synechocystis* sp. PCC 6803 exhibited any sequence similarity to prokaryotic regulatory motifs.

Moreover, no sequence homologous to a ribosome-binding site (Shine–Dalgarno motif) was found in the vicinity of the initiation codon of the *murF* gene of *Synechocystis* sp. PCC 6803, while the *murE–murF* operon of *E. coli* does contain a typical Shine–Dalgarno motif (Mengin-Lecreulx *et al.*, 1989; Tao & Ishiguro, 1989; Michaud *et al.*, 1990). Nevertheless, the cyanobacterial gene was effectively expressed in *E. coli*.

It has been reported that several cyanobacterial genes lack a typical Shine–Dalgarno motif (McCarn *et al.*, 1988; Tandeau de Marsac & Houmard, 1987). The absence of such sequences cannot be explained by the unusual structure of the mRNA-binding region of the 16S rRNA in cyanobacteria. Indeed, the mRNA-binding region of the 16S rRNA of a filamentous cyanobacterium, *Anabaena* sp. PCC 7120, is very similar to that of *E. coli* (Ligon *et al.*, 1991). The fact that the *murF* gene from *Synechocystis* sp. PCC 6803 can be expressed in *E. coli* suggests that a Shine–Dalgarno sequence is not a prerequisite for the translation of some mRNAs, and that some other factors may be able to substitute for this sequence. However, there are several reports of fruitless attempts to express cyanobacterial genes in *E. coli* under the control of their

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                                                      I
6803 MNSK-LS-LFDIRTFLESCLLSVTNLSEDIRINNIC TDTRSLVSGDLFLALRGESFDGHSF IPQALTA   65
     * *  ** * **   *   *        **  .. . **** *  * **.**.** ** * *   **
E.c. MISVTLSQLTDI---LNGEL-----QGADITLDAVT TDTRKLTPGCLFVALKGERFDAHDF ADQAKAG   59

6803 GAIAVVTDRPVEGLGETVAQFLVEDTLVAYQHIAAGW-RQRFTIPIIGV TGSVGKTTTKELIAAVLSQF  132
      **..  **..  .  .  . *..* ** .*.  .** * **      .. . *** ***  **. **.***
E.c. AAGALLVSRPLD-I-D-LPQLIVKDTRLAFGELAA-WVRQQVPARVVAL TGSSGKTSVKEMTAAILSQC  123

      II
6803 GNVHKTRANYNNEIGVPKTLL ELSPDHDFAIVEMAMRGRGQIALLADIAKPTIGLITNVGTAHIGLLGS  199
     **   *  * **.**** *** * *. *.*..*.     *.**     . .*   *. *.  **.  .**
E.c. GNTLYTAGNLNNDIGVPMTLL RLTPEYDYAVIELGANHQGEIAWTVSLTRPERALVNNLAAAHLEGFGS  190

6803 ELAIAEAKCELLAHQPPESTAILNRDNA-LLM-ET--AQR-FWQ-G-KTITYGLEGGDVHGTVDGENLIL  260
       .* ** *..   *    **.* **   *   .     * *             .   ..* *  *  . *
E.c. LAGVAKAKGEIFSGLPENGIAIMNADNNDWLNWQSVIGSRKVWRFSPNAANSDFTATNIHVTSHGTEFTL  258

                    III
6803 D---G-VSLP LPLAGVHNASNYLAAIAL AQCLGLDWQQLQSGLT-VELPKGRARRYQWCQDVVLLDET  320
     .   * * .  *** * **  * *** **    .*   . .  **  .     **     *   .. .***.
E.c. QTPTGSVDVL LPLPGRHNIANALAAAAL SMSVGATLDAIKAGLANLKAVPGRLFPIQLAENQLLLDDS  323

6803 YNDGLESMLASLDLLANTPGKRRLAVLGAMKELGDYGPTFHQRVGAKVKALGLDGLFLLANDPNTDAIAA  390
     **   . ** *  ...**  ** * * *.* * ***      *  **    ** *.* ..  .  .  .
E.c. YNANVGSMTAAVQVLAEMPGYRVL-VVGDMAELGAESEACHVQVGEAAKAAGIDRVLSVGKQSH--AIST  391

6803 GANGVETQSFSDGPSLVAALKTTL--QPGDRLLFKASNSVGLGAVVSQLLAENPTSV              454
      * **   . * *   *:  **   .  *    .* * * * *   .  **  *  ** *
E.c. -ASGVGEH-FADKTALITRLKLLIAEQQVITILVKGSRSAAMEEVVRAL-QENGT                451
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Fig. 5. **Alignment of the amino acid sequences of the products of translation of the *murF* genes of *Synechocystis* sp. PCC 6803 (6803) and *E. coli* (E.c.; Parquet *et al.*, 1989). Asterisks indicate identical amino acids and dots indicate homologous amino-acid replacements. Highly conserved regions are boxed.**

own regulatory sequences (Tandeau de Marsac & Houmard, 1987). Such problems were overcome by use of artificial promoters and ribosome-binding sites (Wada *et al.*, 1993).

The deletion analysis of the plasmids that carried the *murF* gene of *Synechocystis* sp. PCC 6803 showed that the 128 bp 5′ upstream region of the gene (positions −128 to +1, counting from the first ATG codon) contains all the necessary elements for the expression of the *murF* gene in *E. coli* (Fig. 4).

ACKNOWLEDGEMENTS

We are grateful to Dr T. Sakamoto for the subcloning of the fragment of *Synechocystis* sp. PCC 6803 genomic DNA from pTZ19R into the pBluescript vector and to Dr I. N. Maruyama for help in obtaining the *murF*-deficient mutant of *E. coli*. This work was supported, in part, by Grants-in-Aid for Scientific Research on Priority Areas (nos 04273102 and 04273103) to N.M. from the Ministry of Education, Science and Culture, Japan. D.A.L. was supported by a post-doctoral fellowship from the Japanese Society for the Promotion of Science.

REFERENCES

Cooper, S. (1991). Synthesis of the cell surface during the division cycle of rod-shaped, gram-negative bacteria. *Microbiol Rev* **55**, 649–674.

Drews, G. & Weckesser, J. (1982). Function, structure and composition of cell walls and external layers. In *The Biology of Cyanobacteria*, pp. 333–357. Edited by N. G. Carr & B. A. Whitton. Oxford: Blackwell Scientific Publications.

Gupta, A., Morby, A. P., Turner, J. S., Whitton, B. A. & Robinson, N. J. (1993). Deletion within the metallothionein locus of cadmium-tolerant *Synechococcus* PCC6301 involving a highly iterated palindrome (HIP1). *Mol Microbiol* 7, 189–195.

Ikeda, M., Wachi, M., Ishino, F. & Matsuhashi, M. (1990). Nucleotide sequence involving *murD* and an open reading frame ORF-Y spacing *murF* and *ftsW* in *Escherichia coli*. *Nucleic Acids Res* **18**, 1058.

Jürgens, U. J. & Weckesser, J. (1986). Polysaccharide covalently linked to the peptidoglycan of the cyanobacterium *Synechocystis* sp. strain PCC6714. *J Bacteriol* **168**, 568–573.

Jürgens, U. J., Drews, G. & Weckesser, J. (1983). Primary structure of the peptidoglycan from the unicellular cyanobacterium *Synechocystis* sp. strain PCC6714. *J Bacteriol* **154**, 471–478.

Jürgens, U. J., Golecki, J. R. & Weckesser, J. (1985). Characterization of the cell wall of the unicellular cyanobacterium *Synechocystis* PCC6714. *Arch Microbiol* **142**, 168–174.

Kröger, M., Wahl, R. & Rice, P. (1991). Compilation of DNA sequences of *Escherichia coli* (update 1991). *Nucleic Acids Res* **19** (Suppl.), 2023–2043.

Ligon, P. J. B., Meyer, K. G., Martin, J. A. & Curtis, S. E. (1991). Nucleotide sequence of a 16S rRNA gene from *Anabaena* sp. strain PCC 7120. *Nucleic Acids Res* **19**, 4553.

Los, D. A., Horvath, I., Vigh, L. & Murata, N. (1993). The temperature-dependent expression of the desaturase gene *desA* in *Synechocystis* PCC6803. *FEBS Lett* **318**, 57–60.

Lugtenberg, E. J. J. & van Schijndel-van Dam, A. (1972). Temperature-sensitive mutants of *Escherichia coli* K-12 with low activity of the L-alanine adding enzyme and the D-alanyl-D-alanine adding enzyme. *J Bacteriol* **110**, 35–40.

Malakhov, M. P., Wada, H., Los, D. A., Sakamoto, T. & Murata, N. (1993). Structure of a cyanobacterial gene encoding the 50S ribosomal protein L9. *Plant Mol Biol* **21**, 913–918.

Malakhov, M. P., Wada, H., Los, D. A., Semenenko, V. E. & Murata, N. (1994). A new type of cytochrome *c* from *Synechocystis* PCC6803. *J Plant Physiol* **144** (in press).

Maruyama, I. N., Yamamoto, A. H. & Hirota, Y. (1988). Determination of gene products and coding regions from the *murE-murF* region of *Escherichia coli. J Bacteriol* **170**, 3786–3788.

McCarn, D. F., Whitaker, R. A., Alam, J., Vrba, J. M. & Curtis, S. E. (1988). Genes encoding the alpha, gamma, delta, and four F_0 subunits of ATP synthase constitute an operon in the cyanobacterium *Anabaena* sp. strain PCC7120. *J Bacteriol.* **170**, 3448–3458.

Mengin-Lecreulx, D. & van Heijenoort, J. (1990). Nucleotide sequence of the *murD* gene encoding the UDP-MurNAc-L-Ala-D-Glu synthetase of *Escherichia coli. Nucleic Acids Res* **18**, 183.

Mengin-Lecreulx, D., Parquet, C., Desviat, L. R., Pla, J., Flouret, B., Ayala, J. A. & van Heijenoort, J. (1989). Organization of the *murE-murG* region of *Escherichia coli*: identification of the *murD* gene encoding the D-glutamic-acid-adding enzyme. *J Bacteriol* **171**, 6126–6134.

Michaud, C., Blanot, D., Flouret, B. & van Heijenoort, J. (1987). Partial purification and specificity studies of the D-glutamate-adding and D-alanyl-D-alanine-adding enzymes from *Escherichia coli* K12. *Eur J Biochem* **166**, 631–637.

Michaud, C., Parquet, C., Flouret, B., Blanot, D. & van Heijenoort, J. (1990). Revised interpretation of the sequence containing the *murE* gene encoding the UDP-*N*-acetylmuramyl-tripeptide synthetase of *Escherichia coli. Biochem J* **269**, 277–280.

Needleman, S. B. & Wunsch, C. D. (1970). A general method applicable to the search for similarities in the amino acid sequence of two proteins. *J Mol Biol* **48**, 443–453.

Parquet, C., Flouret, B., Mengin-Lecreulx, D. & van Heijenoort, J. (1989). Nucleotide sequence of the *murF* gene encoding the UDP-MurNAc-pentapeptide synthetase of *Escherichia coli. Nucleic Acids Res* **17**, 5379.

Rogers, H. J., Perkins, H. R. & Ward, J. B. (1980). Biosynthesis of peptidoglycan. In *Microbial Cell Walls and Membranes*, pp. 239–297. London: Chapman & Hall.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sanger, F., Nicklen, S. & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* **74**, 5463–5467.

Schnier, J., Kitakawa, M. & Isono, K. (1986). The nucleotide sequence of an *Escherichia coli* chromosomal region containing the genes for ribosomal proteins S6, S18, L9 and an open reading frame. *Mol & Gen Genet* **204**, 126–132.

Stanier, R. Y., Kunizawa, R., Mandel, M. & Cohen-Bazire, G. (1971). Purification and properties of unicellular blue-green algae (order *Chroococcales*). *Bacteriol Rev* **35**, 171–202.

Tandeau de Marsac, N. & Houmard, J. (1987). Advances in cyanobacterial molecular genetics. In *The Cyanobacteria*, pp. 251–302. Edited by P. Fay & C. van Baalen. Amsterdam: Elsevier.

Tao, J.-S. & Ishiguro, E. E. (1989). Nucleotide sequence of the *murE* gene of *Escherichia coli. Can J Microbiol* **36**, 1051–1054.

Wada, H. & Murata, N. (1989). *Synechocystis* PCC6803 mutants defective in desaturation of fatty acids. *Plant Cell Physiol* **30**, 971–978.

Wada, H., Gombos, Z. & Murata, N. (1990). Enhancement of chilling tolerance of a cyanobacterium by genetic manipulation of fatty acid desaturation. *Nature* **347**, 200–203.

Wada, H., Gombos, Z., Sakamoto, T. & Murata, N. (1992). Genetic manipulation of the extent of desaturation of fatty acids in membrane lipids in the cyanobacterium *Synechocystis* PCC6803. *Plant Cell Physiol* **33**, 535–540.

Wada, H., Avelange-Macherel, M.-H. & Murata, N. (1993). The *desA* gene of the cyanobacterium *Synechocystis* PCC6803 is the structural gene for Δ12 desaturase. *J Bacteriol* **175**, 6056–6058.

Weckesser, J. & Jürgens, U. J. (1988). Cell walls and external layers. *Methods Enzymol* **167**, 173–188.

Williams, J. G. K. (1988). Construction of specific mutations in photosystem II photosynthetic reaction center by genetic engineering methods in *Synechocystis* 6803. *Methods Enzymol* **167**, 766–778.

Received 18 July 1994; accepted 19 September 1994.