The *Bacillus subtilis* *dnaC* gene encodes a protein homologous to the DnaB helicase of *Escherichia coli*

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Within the region of the *Bacillus subtilis* chromosome assigned to us in the genome sequencing project, we found a gene, the product of which is similar to the DnaB protein (replicative DNA helicase) of *Escherichia coli*. Three *B. subtilis* *dna* gene mutations, *dnaC30* and *ts56* causing defects in elongation and *ts199* causing a defect in the initiation of replication, were mapped in the gene by transformation and DNA sequencing. Both *dnaC30* and *ts56* have been located near the amino-terminal end of the *B. subtilis* DnaC protein. In contrast, *ts199* has been located near the carboxy-terminal of the protein. Our results indicate that the *B. subtilis* *dnaC* gene encodes a counterpart of the *E. coli* *dnaB* helicase.

Keywords: *Bacillus subtilis*, genome sequencing, *dnaC*, DNA helicase

The DnaB protein of *Escherichia coli* acts as a central replicative DNA helicase and therefore is essential for initiation and elongation in chromosome replication. In *Bacillus subtilis*, its counterpart has not yet been identified. We have recently found a gene (ORF454) near the *purA* gene in the 180 kb region assigned to us as part of the *B. subtilis* genome sequencing project, the deduced amino acid sequence of which shows 42% identity to *E. coli* DnaB helicase (Ogasawara *et al.*, 1994). The organization of ORFs around this gene is shown in Fig. 1. The *dnaC* mutation and two other groups of *dna-ts* mutations (*tsA* and *tsB*) have already been mapped near *purA* on the *B. subtilis* genetic map (Karamata & Gross, 1970; Bazill & Retief, 1969; Andersen & Ganesan, 1975; Hara & Yoshikawa, 1973). The *dnaC* and *tsA* mutants were defective in elongation, whereas *tsB* mutants were defective in initiation. Although *dnaC* and *tsA,B* were mapped at different but close loci on the *B. subtilis* genetic map (Anagnostopoulos *et al.*, 1993), the relationship between *dnaC* and *tsA,B* was not examined (Yoshikawa & Wake, 1993). To find which of the *dna* mutations were located on ORF454, three *dna* mutant strains [*ts56* and *ts199* (representatives for the *tsA* and *tsB* group, respectively) and *dnaC30*] were used as recipients for transformation. Donor DNA was derived from four λ clones (D3, D25, PL and PL2–3). As shown in Fig. 1, the

DNA of three clones (D3, D25 and PL) did not revert the temperature-sensitive phenotype of any mutant, whereas PL2–3 DNA transformed all mutants with significantly high efficiency. These results indicate that the three markers are located close to each other and in the vicinity of ORF454. To map these mutation sites more precisely, the DNA of three other clones (PL2, YS1 and YS2) was assayed for its ability to complement them. Fig. 1 shows that the DNA of all three clones transformed *dnaC30* and *ts56* mutant cells, indicating that both mutations are located on a common fragment (from an *EcoRI* site in ORF454 to a *HindIII* site in ORF66). In contrast, when a *ts199* strain was used as recipient, only YS1 DNA had transforming activity, suggesting that the *ts199* mutation is located in the 3' region of ORF454. These results suggest that all three *dna* mutations are located in ORF454.

The *B. subtilis* *purA* gene was cloned based upon its sequence similarity to the *E. coli* counterpart (Mantsala & Zalkin, 1992). In this study we have confirmed that the *B. subtilis* *purA16* mutation is situated in a sequence containing this gene (Fig. 1).

As both *dnaC30* and *ts56* were shown to be present in an *EcoRI*–*HindIII* DNA fragment of 880 bp, the nucleotide sequence of the region in both mutant chromosomes was determined to identify these mutation sites. Furthermore, the nucleotide sequence of the 2.15 kb fragment (corresponding to the insert in clone YS1) of the *ts199* chromosome was determined to identify the *ts199*

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Fig. 1. Location of the *dnaC30*, *ts56* and *ts199* mutations on the *B. subtilis* chromosome determined by transformation with cloned DNA fragments. Gene organization and a restriction map of a region containing a gene (ORF454) homologous to *E. coli dnaB* are schematically illustrated at the top left half of the figure. Boxes represent ORFs. Genes transcribed from left to right are shown above the map, and those transcribed in the opposite direction below the map. In the lower part, five λ clones (PL, PL2, PL2-3, D3 and D25) obtained from two partially *Sau3A*-digested chromosomal DNA libraries of *B. subtilis* (Ogasawara *et al.*, 1994) and two M13 subclones (YS1 and 2) derived from PL2-3 are shown as chromosomal DNA inserts in phage clones used for transformation. Abbreviations for restriction sites are as follows: E, *EcoRI*; H, *HindIII*; P, *PvuII*. (S) indicates that the *SalI* site in the λ vector portion of PL2-3 DNA was used for subcloning into M13. On the right half of the figure, the number of transformants present in 10 μ l of competent cell suspension is shown. Four mutant strains, *dnaC30* (Itaya, 1993), *ts56* and *ts199* (Hara & Yoshikawa, 1973), and *purA16* (Moriya *et al.*, 1990) were used as recipients to identify the loci of the mutations. Transformation of competent *B. subtilis* cells was performed as described previously (Haas & Yoshikawa, 1969). The transforming activity of each donor DNA (1 μ g ml⁻¹) is shown as the mean number of temperature-resistant transformants selected at 48 °C in A and *purA*⁺ transformants grown on plates lacking adenine in B.

mutation site. In each case, a single base pair change was found in comparison with the wild-type sequence and the change caused a missense mutation in the coding region of ORF454. Thus, aspartic acid (residue 42) was replaced by glycine (codon change from GAT to GGT) in *ts56*, threonine (residue 69) was replaced by isoleucine (ACA to ATA) in *dnaC30* and alanine (residue 350) was replaced by valine (GCG to GTG) in *ts199*. These results demonstrate that ORF454, encoding a protein similar to *E. coli* DnaB, is identical to the *dnaC* gene. Furthermore, both *ts56* and *ts199* are mutant alleles of *dnaC*. Therefore, hereafter, we call *ts56* and *ts199*, *dnaC56* and *dnaC199*, respectively. It was also shown that two types of mutants defective in elongation (*dnaC30* and *dnaC56*) and in initiation (*dnaC199*) can be isolated, both affected by the *B. subtilis* *dnaC* gene. Similarly, two types of *dna* mutants were reported for the *dnaB* gene of *E. coli* (Wechsler & Gross, 1971; Zyskind & Smith, 1977).

Nakayama *et al.* (1984a) determined that the *E. coli* DnaB protein could be divided by trypsin digestion into two relatively stable domains, a 12 kDa amino-terminal (fragment III) and a 33 kDa carboxy-terminal domain (fragment II). About 45 amino acids between fragments II and III serve as a hinge region. In addition, 14 amino acids of the amino-terminal end are also lost during the cleavage. In Fig. 2, the amino acid sequence of *B. subtilis* DnaC helicase is compared with three other helicases, *E. coli* and *Salmonella typhimurium* *dnaB* helicases and *B. subtilis* phage SPP1 replicative DNA helicase. In previous studies,

the carboxy-terminal portions of bacterial and bacteriophage primase-associated helicases were found to be conserved (Ilyina *et al.*, 1992; Pedre *et al.*, 1994). The DnaC protein of *B. subtilis* also showed significant similarity in its carboxy-terminal half with the three other helicases, in that the A and B motifs involved in purine NTP-binding and hydrolysis (Walker *et al.*, 1982) were conserved (Fig. 2). Significant similarity was also found in the remaining portion among bacterial helicases, except that the amino-terminal 14 amino acids were missing in *B. subtilis* (Fig. 2).

The *dnaC199* mutation identified in this study was found to be located near the carboxy-terminal. Since the *dnaC199* mutant is defective in the initiation of replication, it is unlikely that ATP-binding and/or ATPase activity are also affected in this mutant, since ATP hydrolysis was shown to be essential for the helicase action of *E. coli* DnaB (LeBowitz & McMacken, 1986) and the deficiency would create mutants defective in elongation, not initiation. The carboxy-terminal domain of *B. subtilis* DnaC seems to be participating in protein-protein interaction, analogous to the situation in *E. coli* where this domain in *E. coli* DnaB is able to form a hexamer (Nakayama *et al.*, 1984a), and probably interact with the λ P protein (Chang *et al.*, 1991). The mutant DnaC199 protein might not be able to interact with a carrier protein like the *E. coli* DnaC protein, although the amino-terminal domain of DnaB is essential for this interaction in *E. coli* (Nakayama *et al.*, 1984a).

Fig. 2. Alignment of the amino acid sequences of the *B. subtilis* DnaC, *E. coli* DnaB (Nakayama *et al.*, 1984b), *S. typhimurium* DnaB (Wong *et al.*, 1988) and *B. subtilis* phage SPP1 G40 DNA helicases (Pedre *et al.*, 1994). The alignment of the amino acid sequences was performed with the GENEWORKS program (IntelliGenetics). Identical amino acids are boxed. Identities between two of the four proteins were as follows: 93% between *E. coli* DnaB and *S. typhimurium* DnaB, 42% between *E. coli* DnaB and *B. subtilis* DnaC, 41% between *B. subtilis* DnaC and phage SPP1 G40 and 32% between *E. coli* DnaB and SPP1 G40 proteins. Thick lines labelled A and B indicate the putative motifs for NTP-binding (Walker *et al.*, 1982). Open arrowheads indicate the amino and carboxyl ends of fragments II and III produced by trypsin digestion of *E. coli* DnaB (Nakayama *et al.*, 1984a). Closed arrowheads indicate the mutation sites identified in this study on the amino acid sequence of the wild-type *B. subtilis* DnaC. The mutations were determined as follows. Various DNA fragments from the region identified as carrying the *dna* mutations were prepared by PCR using chromosomal DNA extracted from the mutants as template, followed by direct sequencing of the PCR products as described previously (Ogasawara *et al.*, 1994).

In contrast to the high similarity of the carboxy-terminal domain in the four replicative DNA helicases, the amino-terminal domain showed less similarity (Fig. 2). The *dnaC56* and *dnaC30* mutations, defective in elongation, were located in this domain. The amino acids in the wild-type sequence corresponding to those changed in the mutants were conserved in the bacterial helicases. Nakayama *et al.* (1984a) suggested that the amino-terminal domain of *E. coli* DnaB is essential for interaction with primase and DnaC. Therefore, an interaction of *B. subtilis*

DnaC with primase might be impaired in both *dnaC56* and *dnaC30* mutants, resulting in a deficiency in elongation.

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