A sequence similarity between proteins involved in initiation and termination of bacterial chromosome replication

We report an amino acid sequence relationship between two proteins that have different and distinct roles in the chromosome replication cycle of *Bacillus subtilis*. One, DnaB, is a 55 kDa protein involved in initiation of replication (Ogasawara *et al.*, 1986; Hoshino *et al.*, 1987) and the other, replication terminator protein (RTP), is a 14.5 kDa protein involved in termination of the cycle (Smith & Wake, 1989). The sequence similarity suggests that these proteins possess a common functional domain, and raises the possibility of a link between the initiation and termination phases of the replication cycle.

A comparison of the amino acid sequence of RTP (Carrigan *et al.*, 1987) with that of DnaB (Ogasawara *et al.*, 1986; Hoshino *et al.*, 1987) revealed a 34-residue stretch in DnaB (residues 90–123) containing 16 identities and three conservative replacements common to a 33-residue stretch in RTP (residues 17–49) when a single gap between residues 43 and 44 was included. Fig. 1 shows a comparison of these two regions; there is 47.1 % identity between them and a score of 6.8 standard deviation units was calculated for this comparison using the ALIGN program (Dayhoff *et al.*, 1983). When the NBRF NEW databank was screened with either the RTP or DnaB sequences shown in Fig. 1 using FASTA (Pearson & Lipman, 1988), the best match with the RTP sequence was DnaB, and vice versa. When the consensus sequence in Fig. 1 was used to screen the NBRF PIR and NEW databanks (~ 17700 sequences) the best two matches were RTP and DnaB and there were no other significant sequence similarities. Therefore the sequence similarity between these regions appears to be unique and may constitute a common functional domain in these proteins which derived from a common ancestral protein.

The significance of the sequence similarity between RTP and DnaB is strengthened as these proteins originate from the same organism, *B. subtilis*, and both are involved in chromosomal replication. That they play quite different roles in the replication process makes the similarity particularly interesting. DnaB is essential for initiation, as has been demonstrated with two classes of temperature sensitive *dnaB* mutants, *dnaBI* and *dnaBII* (Imada *et al.*, 1980). RTP, however, is essential for replication fork arrest at the chromosome terminus (Smith & Wake, 1989).

One of the functional possibilities for this domain is that it may be involved in interactions with DNA. Binding to the origin of the chromosome has been ascribed as one of the functions of DnaB (Winston & Sueoka, 1980), and RTP has been shown to bind to the terminus region (Lewis *et al.*, 1989, 1990). In fact, the 33-residue region from DnaB is part of a putative helix–turn–helix DNA-binding motif (residues 80–99) of DnaB) suggested by Ogasawara *et al.* (1986) on the basis of sequence comparisons with other known helix–turn–helix DNA-binding proteins. However, there is currently no experimental evidence to indicate that DnaB binds to DNA (H. Yoshikawa, personal communication).

Another possibility is that the putative DnaB/RTP homologous domain is involved in interactions with replicative helicases. In *Escherichia coli* the DnaB helicase (not to be confused with DnaB of *B. subtilis*) has a central role in the initiation of chromosome replication at *oriC* (Bramhill & Kornberg, 1988), and termination of replication is effected by the Tus protein (the *E. coli* analogue of RTP) impeding the DNA-unwinding activity of DnaB helicase (Khatri *et al.*, 1989; Lee *et al.*, 1989). It is very likely that the major replicative helicase of *B. subtilis* is similarly involved in the initiation and termination phases of DNA replication. Thus, the 'homologous' domain could be responsible for specific interactions between the replicative helicase of *B. subtilis* and both RTP and DnaB.

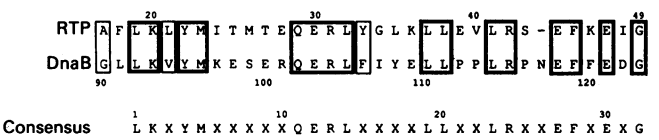

Fig. 1. Alignment of residues 17–49 of RTP with residues 90–123 of DnaB using the program ALIGN (Dayhoff *et al.*, 1983)

Heavy and lighter outlined boxes indicate identical amino acids and conservative substitutions, respectively. The ALIGN score for this comparison is 6.8 standard deviations higher than that obtained with 1000 comparisons of randomized sequences of these proteins (when a bias of +6 was added to the mutation data matrix and a gap penalty of 6 was used). Only the 16 identities were included in the consensus sequence used for searching the databases.

A final possibility concerns the suggestion that the origin and terminus of the *B. subtilis* chromosome are bound to a common replication apparatus (Sueoka, 1969). Thus, although DNA replication is now known to be a bidirectional process, it is still quite feasible that *oriC* and *terC* are closely associated within the cell. The common 'domain' in DnaB and RTP might have a role in achieving this.

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Bramhill, D. & Kornberg, A. (1988) Cell **52**, 743–755
Carrigan, C. M., Haarsma, J. A., Smith, M. T. & Wake, R. G. (1987) Nucleic Acids Res. **15**, 8501–8509
Dayhoff, M. O., Barker, W. C. & Hunt, L. T. (1983) Methods Enzymol. **91**, 524–545
Hoshino, T., McKenzie, T., Schmidt, S., Tanaka, T. & Sueoka, N. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 653–657
Imada, S., Carrol, L. E. & Sueoka, N. (1980) Genetics **94**, 809–823
Khatri, G. S., MacAllistair, T., Sista, P. R. & Bastra, D. (1989) Cell **59**, 667–674
Lee, E. H., Kornberg, A., Hidaka, M., Kobayashi, T. & Horiuchi, T. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 9104–9108
Lewis, P. J., Smith, M. T. & Wake, R. G. (1989) J. Bacteriol. **171**, 3564–3567
Lewis, P. J., Ralston, G. B., Christopherson, R. I. & Wake, R. G. (1990) J. Mol. Biol. **214**, 73–84
Ogasawara, N., Moriga, S., Mazza, P. G. & Yoshikawa, H. (1986) Nucleic Acids Res. **14**, 9989–9999
Pearson, W. R. & Lipman, D. J. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 2444–2448
Smith, M. T. & Wake, R. G. (1989) Gene **85**, 187–192
Sueoka, N. (1969) Proc. XII Int. Congr. Genet. **3**, 115–125
Winston, S. & Sueoka, N. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 2834–2838

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