Molecular Microbiology (1990) 4(3), 513–517

Notes

The life-cycle proteins RodA of *Escherichia coli* and SpoVE of *Bacillus subtilis* have very similar primary structures

B. Joris,[1] G. Dive,[1] A. Henriques,[2] P. J. Piggot[3] and J. M. Ghuysen[1]*

[1]*Service de Microbiologie, Université de Liège, Institut de Chimie, B6, B-4000 Sart Tilman (Liège 1), Belgium.*
[2]*Laboratorio de Genetica Molecular, Instituto Gulbenkian de Ciencia, P-2781 Oeiras, Portugal.*
[3]*Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, Pennsylvania 19140, USA.*

Summary

Comparison of the predicted amino acid sequence of the cell-cycle RodA protein with the National Research Foundation protein sequence database shows that the 370-amino-acid RodA, a protein that is essential for wall elongation in *Escherichia coli* and maintenance of the rod shape of the cell, is highly analogous, in terms of primary structure, with the *Bacillus subtilis* SpoVE protein involved in stage V of sporulation.

Introduction

There are two important morphogene clusters in the chromosome of *Escherichia coli*. One of these clusters lies in the 2-minute region of the genetic map and contains genes that are required for cell division and septum formation (Robinson *et al.*, 1984; Tashner *et al.*, 1988). Among them, *pbpB* encodes the 588-amino-acid penicillin-binding protein 3 (or PBP3) (Nakamura *et al.*, 1983), *ftsA* the 420-amino-acid FtsA protein (Robinson *et al.*, 1988), and *ftsZ* the 383-amino-acid FtsZ protein (Yi and Lutkenhaus, 1985). PBP3 is anchored in the plasma membrane by a short peptide segment at the amino terminus of the protein whereas the bulk of the polypeptide chain is in the periplasm (Bartholomé-De Belder *et al.*, 1988; Bowler and Spratt, 1989). Inactivation of PBP3 either by mutation or by selective inhibition by β-lactam antibiotics such as aztreonam, leads to filamentous growth of the cell. FtsA is thought to participate in the construction of the septum (Tormo and Vicente, 1984) and to interact with PBP3 (Tormo *et al.*, 1986). FtsA must be synthesized during a short period just before division in order for cell division to be completed (Donachie *et al.*, 1979; Tormo *et al.*, 1980). Overexpression of FtsZ leads to a hyperdivision activity displayed as the minicell phenotype (Ward and Lutkenhaus, 1985) and FtsZ is the target of the SOS-induced division inhibitor, SfiA (Jones and Holland, 1985).

The second cluster lies in the 14-minute region of the map and contains genes that are required for cell wall elongation and maintenance of the rod shape of the cell. Among them, *dacA* encodes the 403-amino-acid PBP5 (Broome-Smith *et al.*, 1988), *pbpA* the 633-amino-acid PBP2 (Asoh *et al.*, 1986) and *rodA* the highly hydrophobic, 370-amino-acid RodA protein (Matsuzawa *et al.*, 1989). PBP5 is anchored in the plasma membrane by a short peptide sequence of the carboxy-terminal region of the protein (Pratt *et al.*, 1986). Inactivation of *dacA* does not cause severe growth defects but a 10-fold overproduction of PBP5 results in spherical cells (Stoker *et al.*, 1983a). PBP2 has the same type of membrane topology as PBP3 (Adachi *et al.*, 1987) and RodA is an integral membrane protein that would possess at least nine membrane-spanning segments (Stoker *et al.*, 1983b; Matsuzawa *et al.*, 1989). Inactivation of PBP2 by mecillinam causes *E. coli* to grow and divide as round coccal forms. Mutations in either *pbpA* or *rodA* also have that effect and confer resistance to mecillinam. It has been suggested that RodA interacts with both PBP3 (Begg *et al.*, 1986) and PBP2 (Ishino *et al.*, 1986) and that the complex PBP2-RodA is linked to the ribosomes via the *lov* gene product (Bouloc *et al.*, 1989; Ogura *et al.*, 1989).

Based on the principle that similarity of sequence indicates similarity of three-dimensional structure and function, comparison with other proteins of known primary structure can provide insights into the possible roles that FtsA, FtsZ and RodA play in the morphogenesis of *E. coli*. As shown below, a comparison of the amino acid sequence of RodA with the National Research Foundation protein sequence database (version 20; 10527 sequences; 2802055 residues) led to the conclusion that

Received 19 July, 1989; revised 14 November, 1989. *For correspondence. Tel. (41) 563395/6; Fax (41) 562355.

the 370-amino-acid RodA protein and the SpoVE protein of *Bacillus subtilis* (Bugaichuk and Piggot, 1986) are very similar in terms of primary structure.

Results and Discussion

The alignment between the published sequence of the 293-amino-acid SpoVE (Bugaichuk and Piggot, 1986) and that of the 298-amino-acid carboxy-terminal region (from E72 to V370) of the 370-amino-acid RodA (Matsuzawa *et al.*, 1989) had a score of −540, which was more than 29 standard deviations above that expected from a run of 20 randomized pairs of proteins having the same amino acid compositions as the two proteins under consideration. In addition, the aligned sequences had almost superimposable profiles of predicted transmembrane segments. Thus SpoVE essentially differed from RodA in that it lacked a 72-amino-acid amino-terminal region and two transmembrane segments. This difference prompted us to re-examine the sequence of *spoVE*, which also extended 50 bases upstream of the published one. We confirmed the sequence for all bases except that a 'T' was missed out at position 278.

This correction has several consequences. (i) It extends the amino-terminal coding region of the long open reading frame which was identified as the *spoVE* gene by the map position of *spoVE* mutations and which was shown to be read *in vivo* by studying translational *lacZ* fusions (Bugaichuk and Piggot, 1986). (ii) The most likely translational start codon for SpoVE is UUG at bases 22–24 of the published sequence. This codon is preceded by a potential ribosome binding site (at bases 7 to 12) of GGGGTG, with a free energy of base pairing (ΔG) with the 3′ end of the *B. subtilis* 16S-RNA of −16.2 kcal mol^{-1} calculated using the rules of Tinoco *et al.* (1973). (iii) The 22-amino-acid amino-terminal region of the published SpoVE sequence is replaced by a 95-amino-acid stretch. The revised SpoVE sequence extends the homology of RodA to SpoVE so that it covers essentially the entire length of both proteins. The alignment shown in Fig. 1 has a score of −636 and a standard deviation of 40. The similarity is especially significant in the ≈140-amino-acid

```
      1                                                          57
RODA  MTDNPNKKTF WDKVHLDPTM LLILLAL.LV YSALVIWSA. .SGQDIGMME RKIGQIAMGL
        ***   *      *  **    *  * ***   *                  *      *
SPOVE    MTTKKTS PDLLLVIITL LLLTIGLIMV YSASAVWADY KFDDSFFFAK RQLLFAGIGV
         1                                                          57

                                                                  115
RODA  VIMVVMAQIP PRVYEGWAPY LYIICIILLV AV..DAFGAI SKGAQRWLDL GIVRFQPSEI
       *              *    * **  ***  *     *     *   *    *    ****
SPOVE IAMFFIMNVD YWTWRTWSKL LMVICFFLLV LVLIPGVGMV RNGSRSWIGV GAFSIQPSEF
                                                                  117

                                                                  172
RODA  AKIAVPLMVA RFI.NRDVCP PSLKNTGI.A LVLIFMPTLL VAAQPDLGTS .ILVALSGLF
       * *     *  *          *       * *   *   *     ******     *
SPOVE MKLAMIAFLA KFLSEKQKNI TSFRRGFVPA LGIVFSAFLI IMCQPDLGTG TVMVGTCIVM
                                                                  177

                                                                  232
RODA  VLFLSGLSWR LIGVAVVLVA AFIPILWFFL MHDYQRQRVM MLLDPESDPL GAGYHIIQSK
                             *       *    *   *     * *  *** * *  ****
SPOVE IFVAGARIAH FVFLGLIGLS GFVGLV...L SAPYRIKRIT SYLNPWEDPL GSGFQIIQSL
                                                                  234

                                                                  292
RODA  IAIGSGGLRG KGWLHGTQSQ LEFLPERHTD FIFAVLAEEL GLVGILILLA LYILLIMRGL
       * * *** *  *     *      ***  ** **** * *** *  *    *  *   *  **
SPOVE YAVGPGGLFG MGLGQSRQ.K FFYLPEPQTD FIFAILSEEL GFIGGTLILL LFSVLLWRGI
                                                                  293

                                                                  352
RODA  WIAARAQTTF GRVMAGGLML ILFVYVFVNI GMVSGILPVV GVPLPLVSYG GSALIVLMAG
       **  *     *   * *         *  ** * * *  **  *  **  *** ** *
SPOVE RIALGAPDLY GSFVAVGIIS MIAIQVMINI GVVTGLIPVT GITLPFLSYG GSSLTLMLMA
                                                                  353

                    370
RODA  FGIVMSIHTH RKMLSKSV
       *
SPOVE VGVLLNVSRY SRY
                366
```

Fig. 1. Comparison of amino acid sequences of the *E. coli* RodA protein and the *B. subtilis* SpoVE protein. The sequences are aligned for maximal homologies. Identities between the two sequences are shown by asterisks. For more details, see text.

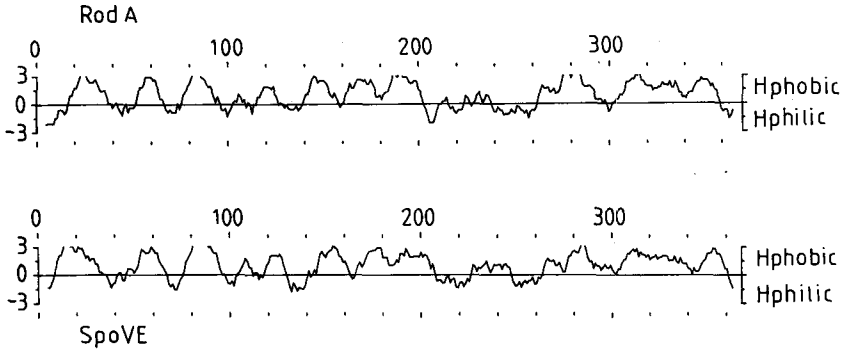

Fig. 2. Predicted transmembrane segments of the *E. coli* RodA protein and the *B. subtilis* SpoVE protein, using the program of Kyte and Doolittle (1982) (window: 9). The program of Engelman *et al.* (1986) (window: 20) generated the same profile.

region that extends from Arg212 to Gly355 in SpoVE and from Arg210 to Gly354 in RodA, both of which contain 46% of strict identities. The two proteins have identical profiles of predicted transmembrane segments (Fig. 2).

The modes of action, at the molecular level, of RodA in *E. coli* and SpoVE in *B. subtilis* are unknown but the striking similarity that exists between the two proteins suggests a common property. The *spoVE* locus is defined by mutations that cause a blockage at stage V of sporulation (Piggot and Coote, 1976). Strikingly, *spoVE* mutants appear to be grossly deficient in cortex, which consists of a sporulation-specific peptidoglycan (Coote, 1972; Piggot and Coote, 1976). Thus SpoVE may have a role in cortex synthesis, although no biochemical studies have been performed to test this possibility. The *spoVE* locus is transcribed well before cortex synthesis starts and its transcription is absolutely dependent on the *spoOH* and *spoOK* loci (Bugaichuk, 1987).

Changes in cell wall synthesis and breakdown are among the first events observed after the initiation of sporulation (Uratani *et al.*, 1983). In the course of sporulation, the PBP profile of *B. subtilis* undergoes profound remodelling, with a sporulation-specific increase in vegetative PBPs and a *de novo* synthesis of other PBPs (Buchanan and Sowell, 1983; Sowell and Buchanan, 1983; Todd *et al.*, 1983). In particular, the sporulation-specific PBP5a, an analogue of the *E. coli* PBP5 with respect to the catalysed reactions, is temporally and spatially regulated. It is detected in large amounts during spore peptidoglycan synthesis from stage II to stage V (Todd *et al.*, 1983; 1985; Buchanan and Neyman, 1986). On the basis of these observations and by analogy with RodA (see the *Introduction*), SpoVE might also link specific intracellular structures or enzymes and some PBPs at the surface of the cell.

In addition to the pair *rodA-spoVE*, *B. subtilis* also contains homologues of the *E. coli* cell-division genes *ftsA* and *ftsZ* and expression of these *B. subtilis* homologues in *E. coli* results in filamentation and cell death (Beall *et al.*, 1988). Note also that a significant similarity has been observed over a 60-amino-acid region between the *E. coli* FtsA and the yeast cell-cycle proteins CDC28 and CDC2 (Robinson *et al.*, 1987). It is intriguing that in *B. subtilis*, *spoVE* maps very close to *ftsA* and *ftsZ*, and indeed all three loci were identified on the same λ clone (Beall *et al.*, 1988).

Experimental procedures

Comparison of the primary structure of RodA with the protein sequence database was made using the Wordsearch algorithm of the software package of the Genetics Computer Group (GCG) (University of Wisconsin Biotechnology Center, Madison, WI, USA; Devereux *et al.*, 1984). Sequences similar to a query sequence were identified with the procedure of Wilbur and Lipman (1983) using, in the present case, a word size of one. The amino-acid alignment between RodA and SpoVE was optimized with the local homology procedure of Smith and Waterman (1981) (Bestfit program in the GCG package; gap weight: 5.0; length weight: 0.3), and the significance of the homology found between the two alignments was estimated with the seqdp program of Goad and Kanehisa (1982). Prediction of transmembrane segments was made using the procedures of Kyte and Doolittle (1982) and Engelman *et al.* (1986).

Notes added in proof

While this paper was in press, Ikeda *et al.* showed that the *E. coli* FtsW was also similar to RodA and SpoVE: Ikeda, M., Sato, I., Wachi, M., Jung, H.K., Ishino, F., Kobayashi, Y., and Matsuhashi, M. (1989) Structural similarity among *Escherichia coli* FtsW and RodA proteins and *Bacillus subtilis* SpoVE protein, which function in cell division, cell elongation, and spore formation, respectively. *J Bacteriol* **171:** 6375–6378.

Acknowledgements

The work in Liège was supported by the Fonds de la Recherche Scientifique Médicale (contract no. 3.4507.83), an Action concertée with the Belgian Government (convention 86/91-90), a convention with the Région wallonne (C2/C16/Conv. 246/20428), the Fonds de Recherche de la Faculté de Médecine ULg, and a contract with the EEC (BAP-0197-B). G.D. is Chercheur qualifié of the Fonds National de la Recherche Scientifique, A.H. was

supported in part by the NATO Collaborative Research Grants Programme (SA.5-2-05(RG.0900/87)1285/87/AHJ-NA.1), and P.J.P. was supported by Public Health Service Grant AI23045 from the National Institute of Allergy and Infectious Diseases.

References

Adachi, H., Ohta, T., and Matsuzawa, H. (1987) A water-soluble form of penicillin-binding protein 2 of *Escherichia coli* construction by site-directed mutagenesis. *FEBS Lett* **226:** 150–154.

Asoh, S., Matsuzawa, H., Ishino, F., Strominger, J., Matsuhashi, M., and Ohta, T. (1986) Nucleotide sequence of the *pbpA* gene and characteristics of the deduced amino acid sequence of penicillin-binding protein 2 of *Escherichia coli* K12. *Eur J Biochem* **160:** 231–238.

Bartholomé-De Belder, J., Nguyen-Distèche, M., Houba-Herin, N., Ghuysen, J.M., Maruyama, I.N., Hara, H., Hirota, Y., and Inouye, M. (1988) Overexpression, solubilization and refolding of a genetically engineered derivative of the penicillin-binding protein 3 of *Escherichia coli* K12. *Mol Microbiol* **2:** 519–525.

Beall, B., Lowe, M., and Lutkenhaus, J. (1988) Cloning and characterization of *Bacillus subtilis* homologs of *Escherichia coli* cell division genes *ftsZ* and *ftsA*. *J Bacteriol* **170:** 4855–4864.

Begg, K.J., Spratt, B.G., and Donachie, W.D. (1986) Interaction between membrane proteins PBP3 and RodA is required for normal cell shape and division in *Escherichia coli*. *J Bacteriol* **167:** 1004–1008.

Bouloc, P., Jaffé, A., and D'Ari, R. (1989) The *Escherichia coli lov* gene product connects peptidoglycan synthesis, ribosomes and growth rate. *EMBO J* **8:** 317–323.

Bowler, D.L., and Spratt, B.G. (1989) Membrane topology of penicillin-binding protein 3 of *Escherichia coli*. *Mol Microbiol* **3:** 1277–1286.

Broome-Smith, J.K., Ioannidis, I., Edelman, A., and Spratt, B.G. (1988) Nucleotide sequences of the penicillin-binding protein 5 and 6 genes of *Escherichia coli*. *Nucl Acids Res* **16:** 1617.

Buchanan, C.E., and Neyman, S.L. (1986) Correlation of penicillin-binding protein composition with different functions of two membranes in *Bacillus subtilis* forespores. *J Bacteriol* **165:** 498–503.

Buchanan, C.E., and Sowell, M.O. (1983) Stability and synthesis of the penicillin-binding proteins during sporulation. *J Bacteriol* **156:** 545–551.

Bugaichuk, U.D. (1987) Studies of transcriptional regulation of the *Bacillus subtilis* developmental gene *spo*VE. *J Gen Microbiol* **133:** 2349–2357.

Bugaichuk, U.D., and Piggot, P.J. (1986) Nucleotide sequence of the *Bacillus subtilis* developmental gene *spo*VE. *J Gen Microbiol* **132:** 1883–1890.

Coote, J.G. (1972) Sporulation of *Bacillus subtilis*. Characterization of oligosporagenous mutants and comparison of their phenotype with those of asporogenous mutants. *J Gen Microbiol* **71:** 1–15.

Devereux, J., Haeberli, P., and Smithies, O. (1984) A comprehensive set of sequence analysis programs for the VAX. *Nucl Acids Res* **12:** 387–395.

Donachie, W.D., Begg, K.J., Lutkenhaus, J.F., Salmond, G.P.C., Martinez-Salas, E., and Vicente, M. (1979) Role of the *ftsA* gene product in control of *Escherichia coli* cell division. *J Bacteriol* **140:** 388–394.

Engelman, D.M., Steitz, T.A., and Goldman, A. (1986) Identifying nonpolar transbilayer helices in amino acid sequences of membrane proteins. *Annu Rev Biochem Biophys Chem* **15:** 321–353.

Goad, W.B., and Kanehisa, M.T. (1982) Pattern recognition in nucleic acid sequences. I. A general method for finding local homologies and symmetrics. *Nucl Acids Res* **10:** 247–263.

Ishino, F., Park, W., Tomiako, S., Tamaki, S., Takase, I., Kunugita, K., Matsuzawa, H., Asoh, S., Ohta, T., Spratt, B.G., and Matsuhashi, M. (1986) Peptidoglycan synthetic activities in membranes of *Escherichia coli* caused by overproduction of penicillin-binding protein 2 and RodA protein. *J Biol Chem* **261:** 7024–7031.

Jones, C.A., and Holland, I.B. (1985) Role of the SfiB (FtsZ) protein in division inhibition during the SOS response in *E. coli*: FtsZ stabilizes the inhibitor SfiA in maxicells. *Proc Natl Acad Sci USA* **82:** 6045–6049.

Kyte, J., and Doolittle, R.F. (1982) A simple method for displaying the hydropathic character of a protein. *J Mol Biol* **157:** 105–132.

Matsuzawa, H., Asoh, S., Kunai, K., Muraiso, K., Takasuga, A., and Ohta, T. (1989) Nucleotide sequence of the *rodA* gene, responsible for the rod shape of *Escherichia coli*: *rodA* and the *pbpA* gene, encoding penicillin-binding protein 2, constitute the *rodA* operon. *J Bacteriol* **171:** 558–560.

Nakamura, M., Maruyama, I.N., Soma, M., Kato, J., Suzuki, H., and Hirota, Y. (1983) On the process of cellular division in *Escherichia coli*: nucleotide sequence of the gene for penicillin-binding protein 3. *Mol Gen Genet* **191:** 1–9.

Ogura, T., Bouloc, P., Niki, H., D'Ari, R., Hiraga, S., and Jaffé, A. (1989) Penicillin-binding protein 2 is essential in wild-type *Escherichia coli* but not in *lov* or *cya* mutants. *J Bacteriol* **171:** 3025–3030.

Piggot, J.J., and Coote, J.G. (1976) Genetic aspects of bacterial endospore formation. *Bacteriol Rev* **40:** 908–962.

Pratt, J.M., Jackson, M.E., and Holland, I.B. (1986) The C terminus of penicillin-binding protein 5 is essential for localisation of the *E. coli* inner membrane. *EMBO J* **5:** 2399–2405.

Robinson, A.C., Kenan, D.J., Hatfull, G.F., Sullivan, N.F., Spiegelberg, R., and Donachie, W.D. (1984) DNA sequence and transcriptional organization of essential cell division genes *ftsQ* and *ftsA* of *Escherichia coli*: evidence for overlapping transcriptional units. *J Bacteriol* **160:** 546–555.

Robinson, A.C., Collins, J.F., and Donachie, W.D. (1987) Prokaryotic and eukaryotic cell-cycle proteins. *Nature* **328:** 766.

Robinson, A.C., Begg, K.J., Sweeney, J., Condie, A., and Donachie, W.D. (1988) Mapping and characterization of mutants of the *Escherichia coli* cell division gene, *ftsA*. *Mol Microbiol* **2:** 581–588.

Smith, J.F., and Waterman, M.S. (1981) Comparison of biosequences. *Adv Appl Math* **2:** 482–489.

Sowell, M.O., and Buchanan, C.E. (1983) Changes in penicillin-binding proteins during sporulation of *Bacillus subtilis*. *J Bacteriol* **153:** 1331–1337.

Stoker, N.G., Broome-Smith, J.K., Edelman, A., and Spratt, B.G. (1983a) Organization and subcloning of the *dacA-rodA-pbpA* cluster of cell shape genes in *Escherichia coli*. *J Bacteriol* **155:** 847–853.

Stoker, N.G., Pratt, J.M., and Spratt, B.G. (1983b) Identification of the *rodA* gene product of *Escherichia coli*. *J Bacteriol* **155:** 854–859.

Taschner, P.E.M., Huls, P.G., Pas, E., and Woldringh, C.L. (1988) Division behavior and shape changes in isogenic *ftsZ*, *ftsQ*, *ftsA*, *pbpB*, and *ftsE* cell division mutants of *Escherichia coli* during temperature shift experiments. *J Bacteriol* **170:** 1533–1540.

Tinoco, I., Borer, P., Dengler, B., Levine, M., Uhlenbeck, O.,

Crothers, D., and Gralla, J. (1973) Improved estimation of secondary structure in ribonucleic acids. *Nature New Biol* **246:** 40–41.

Todd, J.A., Bone, E.J., Piggot, P.J., and Ellar, D.J. (1983) Differential expression of penicillin-binding protein structural genes during *Bacillus subtilis* sporulation. *FEMS Microbiol Lett* **18:** 197–202.

Todd, J.A., Bone, E.J., and Ellar, D.J. (1985) The sporulation-specific penicillin-binding protein 5a from *Bacillus subtilis* is a DD-carboxypeptidase *in vitro*. *Biochem J* **230:** 825–828.

Tormo, A., and Vicente, M. (1984) The *fts*A gene product participates in formation of the *Escherichia coli* septum structure. *J Bacteriol* **157:** 779–784.

Tormo, A., Ayala, J.A., De Pedro, M.A., Aldea, M., and Vicente, M. (1986) Interaction of FtsA and PBP3 proteins in the *Escherichia coli* septum. *J Bacteriol* **166:** 985–992.

Tormo, A., Martinez-Sala, E., and Vicente, M. (1980) Involvement of the *fts*A gene product in late stages of the *Escherichia coli* cell cycle. *J Bacteriol* **141:** 806–813.

Uratani, B., Lopez, J.M., and Freese, E. (1983) Effect of decoyinine on peptidoglycan synthesis and turnover in *Bacillus subtilis*. *J Bacteriol* **154:** 261–268.

Ward, J.E., and Lutkenhaus, J.F. (1985) Overproduction of FtsZ induces minicells in *E. coli*. *Cell* **42:** 941–949.

Wilbur, W.J., and Lipman, D.J. (1983) Rapid similarity searches of nucleic acid and protein data bank. *Proc Natl Acad Sci USA* **80:** 726–730.

Yi, Q.M., and Lutkenhaus, J.F. (1985) The nucleotide sequence of the essential cell division gene *ftsZ*. *Gene* **36:** 241–247.