JOURNAL OF BACTERIOLOGY, Feb. 2007, p. 1185–1188 Vol. 189, No. 4
0021-9193/07/$08.00+0 doi:10.1128/JB.01710-06

Making a Point: the Role of DivIVA in Streptococcal Polar Anatomy▽

Miguel Vicente* and Marta García-Ovalle

Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas Campus de Cantoblanco, C/ Darwin 3, 28049 Madrid, Spain

Like the cells of other bacteria that are not rod shaped, *Streptococcus pneumoniae* cells manage to avoid potentially distressing changes in their surface-to-volume ratio as they grow. The work reported by Fadda et al. in this issue (9) on the streptococcal DivIVA protein suggests new ideas on how this protein may contribute to transformation of the midcell into two pointed poles that result in duplication of the cell volume with no net increase in cell diameter, a mechanism that prevents changes in the surface-to-volume ratio during growth and division.

Some nearly spherical bacteria, like *Staphylococcus aureus*, have circumvented the issue of surface-to-volume ratio maintenance by placing a sagittal septum at midcell while maintaining a nearly constant diameter. Once finished, the *S. aureus* septum splits in the middle, and two polar, hemispherical caps are gradually reshaped from the resultant almost flat moieties (33). In contrast, the ovoid *S. pneumoniae* cells grow and divide simultaneously by recreating two pointed polar caps at midcell. Although a septum is formed, it does not have continuity in the sagittal plane until the very end of division. In some respects the *S. pneumoniae* septation process resembles constriction, like that observed in *Escherichia coli* dividing cells, except that the resultant polar surface contributes a larger portion of the cell surface in the case of the streptococcal cell than in the case of the rod-shaped *E. coli* cell.

divIVA COLOCALIZES IN THE STREPTOCOCCAL CHROMOSOME WITH OTHER CELL DIVISION GENES

The *divIVA* gene, which for the most part is confined to gram-positive bacteria, was first identified in *Bacillus subtilis*. Cells with a mutation in this gene have a reduced septation frequency and undergo aberrant polar division, leading to the formation of anucleate minicells (17, 27). In *S. pneumoniae* a gene coding for a protein with a high level of homology to *B. subtilis* DivIVA is located in a region downstream from the cell division *ftsZ* gene (8, 16), forming part of one of the three portions dispersed from the *dcw* cluster that in rod-shaped bacteria contains most of the tightly grouped cell division and cell wall synthesis genes (18, 31).

The *divIVA* gene codes for a protein that has been implicated in selection of septum positioning at midcell in vegetative division of *B. subtilis*, where it has been proposed to play a role similar to that of the *E. coli* MinE topological specificity component of the MinCDE division site selection system (6, 34). In *B. subtilis* the DivIVA protein has an additional role during sporulation; together with Spo0J, RacA, and Soj, it is required for relocation of chromosomes and their attachment to the cell poles (2, 23, 32). In gram-positive bacteria, besides *S. pneumoniae*, the *divIVA* gene has been studied in *Enterococcus faecalis* (25) and *Staphylococcus aureus* (24). A *divIVA* gene is also present in *Streptomyces coelicolor* (10) and in other actinomycetes, like *Mycobacterium tuberculosis*, where Wag31 (antigen 84), a protein proposed to be involved in cell shape maintenance (10, 13), exhibits some sequence similarity with DivIVA from *Brevibacterium lactofermentum* (26). While many gram-positive bacteria may contain *divIVA* but lack *minE* and even the full *minCDE* system, many gram-negative bacteria have *minE* but no *divIVA*. However, there are some exceptions to this. *Myxococcus xanthus*, a gliding gram-negative bacterium in which peptidoglycan is discontinuously distributed along the wall, contains a gene, *fruD*, with homology to *divIVA* (1), but no *minE* gne has been identified. In some of the clostridia (*Clostridium perfringens*, *Clostridium beijerinckii*, *Clostridium phytofermentans*, and *Clostridium thermocellum*), which are gram-positive bacteria, *divIVA* and *minE* are both present (30). *Synechococcus elongatus*, a cyanobacterium in which the envelope contains multiple complex layers (28), contains both *divIVA* and *minE* (19). In their paper, Fadda et al. propose that in *S. pneumoniae*, a species without the MinCDE system, DivIVA plays a role in the cell division machinery (9).

PEPTIDOGLYCAN SYNTHESIS AND DivIVA LOCALIZATION OCCUR AT COINCIDENT SITES IN STREPTOCOCCI AND OTHER BACTERIA

Using immunofluorescence and immunogold labeling, Fadda et al. (9) found that streptococcal DivIVA localizes at sites coincident with sites at which peptidoglycan synthesis takes place (Fig. 1). Similar positions are occupied in the hyphal tip by the *S. coelicolor* homolog, where it is thought to promote hyphal growth and morphogenesis (10), and in the ends of *B. lactofermentum* cells, where it promotes apical growth (26). *B. subtilis* DivIVA, when present in *E. coli* as a heterologous protein fused to green fluorescent protein, also localizes at midcell (depending on the presence of a Z ring) and at the poles; even more remarkably, in *Schizosaccharomyces pombe* the DivIVA-green fluorescent protein fusion associates with the division septum and the growth zones (7). Interestingly, the *B. subtilis* DivIVA protein localizes in the fission yeast in the same manner as the endogenous Cdc8p tropomyosin-like protein, suggesting that a possible targeting signal for DivIVA localization is conserved in prokaryotic and eukaryotic organisms.

During spore germination in *B. subtilis* DivIVA seems to assemble at the poles even before FtsZ, the initial component

* Corresponding author. Mailing address: Centro Nacional de Biotecnología, CSIC Campus de Cantoblanco, 28049 Madrid, Spain. Phone: 34 91 585 46 99. Fax: 34 91 585 45 06. E-mail: mvicente@cnb.uam.es.

▽ Published ahead of print on 17 November 2006.

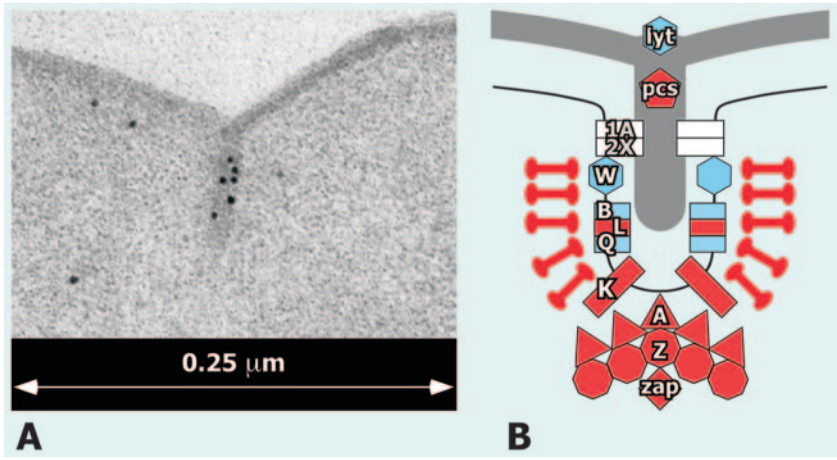

FIG. 1. Intracellular localization of DivIVA and interactions with other components of the *S. pneumoniae* divisome. (A) Immunogold staining pattern observed at the septation site of a dividing cell when an antibody against DivIVA was used. (B) Schematic representation of the interactions detected by Fadda et al. (9). Red indicates a stronger interaction, blue indicates a weaker interaction, and white indicates no interaction. The red "dog bone" structures represent DivIVA, like the structure of *B. subtilis* DivIVA polymers observed in vitro (29). Other symbols are simplified from those used for equivalent *E. coli* elements by Vicente et al. (36). Abbreviations: zap, ZapA; Z, FtsZ; A, FtsA; K, FtsK; Q, FtsQ (DivIB); L, FtsL; B, FtsB (DivIC); W, FtsW; 2X, FtsI (PBP 3); 1A, PBP 1A (high-molecular-weight bifunctional PBP); pcs, PcsB; and lyt, LytB. The PcsB and LytB peptidoglycan hydrolases are probably equivalent to AmiC and EnvC of *E. coli*. See the text for further explanation.

of the division proto-ring (35), is present, indicating that under these conditions the localization of DivIVA does not depend on FtsZ (12). In *S. pneumoniae* (and in *B. subtilis* vegetative division) this does not occur, as a 5-min delay between the assembly of a Z ring and the presence of DivIVA was observed by Fadda et al. (9); moreover, septal positioning of DivIVA depends on the presence of functional FtsZ, and together with the results of protein interactions, this suggests that DivIVA may have developed an affinity for the division ring components. Nevertheless, in the absence of results for strains with the FtsZ function impaired, these timing results have to be interpreted cautiously as the amount of FtsZ protein per cell is approximately 1 order of magnitude greater than the amount of DivIVA (O. Massidda, personal communication). If detection requires a minimum number of proteins to be located at one site, FtsZ could be visualized earlier than other lower-abundance proteins.

STREPTOCOCCAL DivIVA INTERACTION NETWORK

Although mostly shown by indirect procedures (4, 14), there seem to be numerous interactions between the *E. coli* and *B. subtilis* components of the division and the cell wall synthesis machinery (for reviews, see references 11 and 36). If, as suggested by the evidence discussed above, DivIVA forms part of this machinery in *S. pneumoniae*, it should be possible to detect a number of interactions with its other components.

Fadda et al. (9) used the *E. coli* two-hybrid assay developed by Di Lallo et al. (4, 5) to probe DivIVA interactions with other components of the streptococcal divisome. They found that streptococcal DivIVA interacts with itself, which agrees with the interactions of *B. subtilis* and *Enterococcus faecalis* DivIVA in vivo and in vitro, respectively (20, 25). As expected, streptococcal DivIVA also interacts with the streptococcal homolog of the chromosome segregation protein Spo0J. In addition, Fadda et al. found that DivIVA interacts with most of the molecules involved in the early and late stages of the *S. pneumoniae* septation process, including FtsZ, FtsA, FtsK, DivIVB (FtsQ in *E. coli*), DivIC (FtsB in *E. coli*), FtsL, and FtsW. It is even able to interact with homologs of the ErzA and ZapA proteins that regulate Z-ring stability in other bacteria. Moreover, interactions were also found with proteins involved in cell wall rearrangement, like LytB, a protein with polar localization involved in daughter cell separation (3), and PcsB, a putative murein hydrolase reported to be a morphogenetic determinant (21). On the other hand, no interaction between DivIVA and two septal enzymes involved in murein biosynthesis, PBP2X and PBP1A, or between DivIVA and PrfA, an orthologue of a *B. subtilis* DNA-binding protein implicated in cell wall synthesis, chromosome segregation, and DNA recombination and repair (22), was detected. Perry and Edwards (23) performed coimmunoprecipitation assays demonstrating the association of *B. subtilis* DivIVA with FtsZ and MinD during vegetative growth and with Spo0J during sporulation; Fadda et al. (9) similarly found that streptococcal DivIVA coprecipitates with FtsZ and Spo0J but, as expected, not with PrfA.

DivIVA ROLE: POINTING AT THE SEPTUM AND SHARPENING THE POLE

Although the absence of DivIVA in *S. pneumoniae* does not result in complete cessation of cell division, it does decrease the proportion of productive FtsZ rings able to terminate septation, although not their total number, suggesting that DivIVA may contribute to Z-ring progression. This lack of closing of Z rings gives rise to 15% anucleate dead cells. As reported by Fadda et al. (9), introduction of an A78T mutation into streptococcal DivIVA in a region shown in *B. subtilis* to be related to cell division defects resulted in a partially active protein that was not fully efficient in localizing at the septal and polar positions occupied by the wild-type protein. The A78T protein was able to interact with itself and with the Spo0J and

PcsB proteins involved in segregation, and therefore, the mutant showed no salient segregation defects; however, there was no interaction with LytB, and so the mutant was not able to effect cell separation, resulting in a chainy phenotype.

Curiously, neither the morphology nor other identifiable cell growth and division processes of *divIVA* mutants of the spherical organism *S. aureus* are affected (24), while the *S. pneumoniae divIVA* null mutant described by Fadda et al. (9) is hemispherical rather than ovoid. It is possible that DivIVA is attracted to regions in which the cell envelope bears a stress associated with growth of a thick or complex peptidoglycan layer during the formation of either a lateral (elongation) or transverse (septation) wall. Once there, DivIVA may recruit a variety of other proteins involved in septation and even in DNA partition.

Is it possible that the DivIVA protein is used by gram-positive bacteria primarily as part of a cytoskeletal stress-bearing mechanism related to cell wall growth? Is it possible that other proteins took advantage of the DivIVA localization to add a topological determinant to their own function by simply establishing an interaction with DivIVA? Considering the large number of interactions with other proteins found for DivIVA, this is a possibility. If all this were correct, the acquisition of a DivIVA interacting domain could have been used frequently by proteins to acquire topological specificity. Such acquisition should have left an evolutionary marker in their sequences.

We do not know if the ancestral gram-positive bacteria (15) contained both MinE and DivIVA as topological determinants as some present-day clostridia do. In bacteria that evolved an outer membrane and consequently could streamline their peptidoglycan layer (e.g., gram-negative bacteria), the stress-bearing DivIVA protein may have become dispensable and eventually lost together with its topological determination for some activities. In the case of MinC and MinD, being attracted to the poles is a requirement, as they block FtsZ polymerization; therefore, their presence prevents abnormal polar septation. Eventual loss of MinE could be tolerated by gram-positive bacteria if MinC and MinD could be directed to the poles by an alternative interaction with DivIVA. On the other hand, gram-negative bacteria, if they were deprived of DivIVA, would be forced to retain or otherwise acquire MinE as their sole topological specifier of the septum-positioning mechanism.

REFERENCES

1. **Akiyama, T., S. Inouye, and T. Komano.** 2003. Novel developmental genes, *fruCD*, of *Myxococcus xanthus*: involvement of a cell division protein in multicellular development. J. Bacteriol. **185:**3317–3324.
2. **Ben-Yehuda, S., D. Z. Rudner, and R. Losick.** 2003. RacA, a bacterial protein that anchors chromosomes to the cell poles. Science **299:**532–536.
3. **De Las Rivas, B., J. L. García, R. López, and P. García.** 2002. Purification and polar localization of pneumococcal LytB, a putative endo-beta-*N*-acetylglucosaminidase: the chain-dispersing murein hydrolase. J. Bacteriol. **184:** 4988–5000.
4. **Di Lallo, G., L. Castagnoli, P. Ghelardini, and L. Paolozzi.** 2001. A two-hybrid system based on chimeric operator recognition for studying protein homo/heterodimerization in *Escherichia coli*. Microbiology **147:** 1651–1656.
5. **Di Lallo, G., M. Fagioli, D. Barionovi, P. Ghelardini, and L. Paolozzi.** 2003. Use of a two-hybrid assay to study the assembly of a complex multicomponent protein machinery: bacterial septosome differentiation. Microbiology **149:**3353–3359.
6. **Edwards, D. H., and J. Errington.** 1997. The *Bacillus subtilis* DivIVA protein targets to the division septum and controls the site specificity of cell division. Mol. Microbiol. **24:**905–915.
7. **Edwards, D. H., H. B. Thomaides, and J. Errington.** 2000. Promiscuous targeting of *Bacillus subtilis* cell division protein DivIVA to division sites in *Escherichia coli* and fission yeast. EMBO J. **19:**2719–2727.
8. **Fadda, D., C. Pischedda, F. Caldara, M. B. Whalen, D. Anderluzzi, E. Domenici, and O. Massidda.** 2003. Characterization of *divIVA* and other genes located in the chromosomal region downstream of the *dcw* cluster in *Streptococcus pneumoniae*. J. Bacteriol. **185:**6209–6214.
9. **Fadda, D., A. Santona, V. D'Ulisse, P. Ghelardini, M. G. Ennas, M. B. Whalen, and O. Massidda.** 2007. *Streptococcus pneumoniae* DivIVA: localization and interactions in a MinCD-free context. J. Bacteriol. **189:** 1288–1298.
10. **Flärdh, K.** 2003. Essential role of DivIVA in polar growth and morphogenesis in *Streptomyces coelicolor* A3(2). Mol. Microbiol. **49:**1523–1536.
11. **Goehring, N. W., and J. Beckwith.** 2005. Diverse paths to midcell: assembly of the bacterial cell division machinery. Curr. Biol. **15:**R514–R526.
12. **Harry, E. J., and P. J. Lewis.** 2003. Early targeting of Min proteins to the cell poles in germinated spores of *Bacillus subtilis*: evidence for division apparatus-independent recruitment of Min proteins to the division site. Mol. Microbiol. **47:**37–48.
13. **Hermans, P. W., F. Abebe, V. I. Kuteyi, A. H. Kolk, J. E. Thole, and M. Harboe.** 1995. Molecular and immunological characterization of the highly conserved antigen 84 from *Mycobacterium tuberculosi*s and *Mycobacterium leprae*. Infect. Immun. **63:**954–960.
14. **Karimova, G., N. Dautin, and D. Ladant.** 2005. Interaction network among *Escherichia coli* membrane proteins involved in cell division as revealed by bacterial two-hybrid analysis. J. Bacteriol. **187:**2233–2243.
15. **Koch, A. L.** 2003. Were Gram-positive rods the first bacteria? Trends Microbiol. **11:**166–170.
16. **Massidda, O., D. Anderluzzi, L. Friedli, and G. Feger.** 1998. Unconventional organization of the division and cell wall gene cluster of *Streptococcus pneumoniae*. Microbiology **144:**3069–3078.
17. **Mendelson, N. H.** 1975. Cell division suppression in the *Bacillus subtilis divIC-A1* minicell-producing mutant. J. Bacteriol. **121:**1166–1172.
18. **Mingorance, J., J. Tamames, and M. Vicente.** 2004. Genomic channeling in bacterial cell division. J. Mol. Recognit. **17:**481–487.
19. **Miyagishima, S. Y., C. P. Wolk, and K. W. Osteryoung.** 2005. Identification of cyanobacterial cell division genes by comparative and mutational analyses. Mol. Microbiol. **56:**126–143.
20. **Muchova, K., E. Kutejova, D. J. Scott, J. A. Brannigan, R. J. Lewis, A. J. Wilkinson, and I. Barak.** 2002. Oligomerization of the *Bacillus subtilis* division protein DivIVA. Microbiology **148:**807–813.
21. **Ng, W. L., H. C. Tsui, and M. E. Winkler.** 2005. Regulation of the *pspA* virulence factor and essential *pcsB* murein biosynthetic genes by the phosphorylated VicR (YycF) response regulator in *Streptococcus pneumoniae*. J. Bacteriol. **187:**7444–7459.
22. **Pedersen, L. B., and P. Setlow.** 2000. Penicillin-binding protein-related factor A is required for proper chromosome segregation in *Bacillus subtilis*. J. Bacteriol. **182:**1650–1658.
23. **Perry, S. E., and D. H. Edwards.** 2006. The *Bacillus subtilis* DivIVA protein has a sporulation-specific proximity to Spo0J. J. Bacteriol. **188:**6039–6043.
24. **Pinho, M. G., and J. Errington.** 2004. A *divIVA* null mutant of *Staphylococcus aureus* undergoes normal cell division. FEMS Microbiol. Lett. **240:**145–149.
25. **Ramírez-Arcos, S., M. Liao, S. Marthaler, M. Rigden, and J. A. Dillon.** 2005. *Enterococcus faecalis divIVA*: an essential gene involved in cell division, cell growth and chromosome segregation. Microbiology **151:**1381–1393.
26. **Ramos, A., M. P. Honrubia, N. Valbuena, J. Vaquera, L. M. Mateos, and J. A. Gil.** 2003. Involvement of DivIVA in the morphology of the rod-shaped actinomycete *Brevibacterium lactofermentum*. Microbiology **149:**3531–3542.
27. **Reeve, J. N., N. H. Mendelson, S. I. Coyne, L. L. Hallock, and R. M. Cole.** 1973. Minicells of *Bacillus subtilis*. J. Bacteriol. **114:**860–873.
28. **Samuel, A. D., J. D. Petersen, and T. S. Reese.** 2001. Envelope structure of *Synechococcus* sp. WH8113, a nonflagellated swimming cyanobacterium. BMC Microbiol. **1:**4.
29. **Stahlberg, H., E. Kutejova, K. Muchova, M. Gregorini, A. Lustig, S. A. Muller, V. Olivieri, A. Engel, A. J. Wilkinson, and I. Barak.** 2004. Oligomeric structure of the *Bacillus subtilis* cell division protein DivIVA determined by transmission electron microscopy. Mol. Microbiol. **52:** 1281–1290.
30. **Stragier, P.** 2001. A gene odyssey: exploring the genomes of endospore-forming bacteria, p. 519–525. *In* L. Sonenshein, R. Losick, and J. A. Hoch (ed.), *Bacillus subtilis* and its relatives: from genes to cells. American Society for Microbiology, Washington, DC.
31. **Tamames, J., M. González-Moreno, J. Mingorance, A. Valencia, and M. Vicente.** 2001. Bringing gene order into bacterial shape. Trends Genet. **17:**124–126.

32. **Thomaides, H. B., M. Freeman, M. El Karoui, and J. Errington.** 2001. Division site selection protein DivIVA of *Bacillus subtilis* has a second distinct function in chromosome segregation during sporulation. Genes Dev. **15:**1662–1673.
33. **Touhami, A., M. H. Jericho, and T. J. Beveridge.** 2004. Atomic force microscopy of cell growth and division in *Staphylococcus aureus*. J. Bacteriol. **186:**3286–3295.
34. **Vicente, M., and J. Löwe.** 2003. Ring, helix, sphere and cylinder: the basic geometry of prokaryotic cell division. EMBO Rep. **4:**655–660.
35. **Vicente, M., and A. I. Rico.** 2006. The order of the ring: assembly of *Escherichia coli* cell division components. Mol. Microbiol. **61:**5–8.
36. **Vicente, M., A. I. Rico, R. Martínez-Arteaga, and J. Mingorance.** 2006. Septum enlightenment: assembly of bacterial division proteins. J. Bacteriol. **188:**19–27.

The views expressed in this Commentary do not necessarily reflect the views of the journal or of ASM.