Cellular organization of a metabolic pathway

Pablo Meyer [1] and Jonathan Dworkin[1]

***Short Abstract* — We studied the cellular localization of the enzymes responsible for the synthesis of peptidoglycan in *B. subtilis*. The first steps of this metabolic reaction occur in the cytoplasm and the last steps are membrane bound. We found by tagging with fluorescent proteins the enzymes of the cytoplasmic part of this reaction that the first (MurAA) and last enzyme (MurG) co-localize and are enriched at the sites of PG synthesis near the membrane. MurAA and MurG co-immunoprecipitate, showing that they are together in a complex. Finally, during sporulation, MurAA and MurG localization seems to be determined by the phospholipid cardiolipin. Taken together, these results show that the cytoplasmic part of the biosynthesis pathway has a very organized cellular distribution where the first and last enzymes of the pathway form a complex that localizes to sites of peptidoglycan synthesis.**

***Keywords* — Bacillus subtilis, sporulation, cellular organization, metabolic pathway, localization, fluorescence, cardiolipin, membrane curvature.**

I. Purpose

Although much is understood about the enzymatic cascades that underlie cellular biosynthesis, comparatively little is known about their cellular organization. That is, are enzymes in a metabolic pathway freely diffusing or are they part of a large multienzyme complex? The hypothesis of metabolic channeling proposes that reaction products in a metabolic pathway move from one active site to another within tightly associated multienzyme complexes (Srere, 1987). However experimental evidence for this hypothesis remains elusive. Metabolic reactions have been widely studied in biology and the chemical and dynamical processes that govern their function are globally understood. However, one pending question is how these biochemical reactions are temporally and spatially controlled and organized inside the cell in order to effectively perform their functions. We decided to study the cellular organization of the enzymes synthesizing peptidoglycan in the gram-positive bacteria *B. subtilis*. In sporulating cells that are responding to nutritional cues, peptidoglycan synthesis becomes restricted to a cellular compartment that can be readily distinguished by microscopy: the engulfed forespore. This restriction to the spore allows to study the question of how enzymes are targeted to the specific site where they are needed.

Acknowledgements: This work was funded by NIH grant GM83156 and a Helen Hay Whitney postdoctoral fellowship

[1]Department of Microbiology, Columbia University Medical School, 701 W. 168th St. New York, NY 100032. E-mail: pm2256@columbia.edu

II. Results

During both vegetative growth and sporulation, the first steps of PG synthesis occur in the cytoplasm and the last steps are membrane bound (van Heijenoort, 1996).

A. Co-localization of PG synthesis enzymes

We found by use of GFP fusions that the first (MurAA) and last enzymes (MurG) of the cytoplasmic part of this reaction co-localize and are enriched at the sites of PG synthesis near the membrane. Also MurAA and MurG co-immunoprecipitate, showing that they are together in a complex. A fluorescent fusion of GcaD, an enzyme that produces UDP-GlcNAc the substrate for both MurAA and MurG also co-localized with these enzymes.

B. Cardiolipin localizes PG synthesis during sporulation

During sporulation, MurAA and MurG localization seems to be determined by the phospholipid cardiolipin, since in strains where the cardiolipin-synthesizing genes (Kawai et al., 2006) were deleted, both MurAA and MurG did not localize to the forespore membrane. Furthermore, MurG localization during sporulation was rescued by addition of purified cardiolipin. Finally, point mutants of MurG demonstrated that a specific helix domain is responsible for this localization.

III. Conclusion

These results demonstrate that the cytoplasmic part of the peptidoglycan biosynthesis pathway has a very specific cellular organization where the first and last enzymes of the reaction form a complex that localizes to the sites of peptidoglycan synthesis. These results are consistent with the hypothesis of "metabolic channeling" stating that products in a metabolic reaction do not freely diffuse around the cell but are bound to a macromolecular biosynthetic complex.

References

[1] Kawai, F., Hara, H., Takamatsu, H., Watabe, K., and Matsumoto, K. (2006). Cardiolipin enrichment in spore membranes and its involvement in germination of Bacillus subtilis Marburg. Genes Genet Syst *81*, 69-76.

[2] Srere, P. A. (1987). Complexes of sequential metabolic enzymes. Annu Rev Biochem *56*, 89-124.

[3] van Heijenoort, J. (1996). Murein Synthesis, In Escherichia coli and Salmonella: Cellular and Molecular Biology, F. C. Neidhardt, ed. (American Society for Microbiology).