MAPPING ACTIVE GROWTH SITES IN THE CELL WALL OF ESCHERICHIA COLI BY AFFINITY LABELING AND CONFOCAL MICROSCOPY.

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The peptidoglycan layer of the bacterial cell wall (sacculus) is a covalently closed macromolecule which completely encloses the cell and exerts the function of an exo-skeleton. As such it confers resistance to the cell envelope against physical stresses as the cytoplasmic turgor pressure, and shapes the cell. Because of its chemical nature and layout, growth, shape and division of the bacterial cell are strictly conditioned by the biosynthesis of the sacculus itself. Any change in the physical dimensions of the sacculus depends upon the insertion of new precursors in a precise and defined way. Alterations in peptidoglycan biosynthesis normally trigger cell lysis or aberrant morphologies often lethal [1]. A precise knowledge of the topology, differentiation and dynamics of precursor insertion sites is clearly needed to understand bacterial morphogenesis. Although the so called "D-cysteine labeling method" previously developed by myself [2], helped to define the areas were insertion of precursors was taking place, and lead to the discovery of inert peptidoglycan domains at the poles of the bacterial cell, its temporal and spatial resolution were still unsatisfactory.

Here I report on the development, and initial results, of an alternative, and complementary, method to detect the active insertion sites by a classical combination of affinity labeling, immunodetection and confocal fluorescence microscopy.

The method is based on: i) the ability of the macrolide antibiotic vancomycin to bind with high affinity to the C-terminal D-alanyl-D-alanine dipeptide characteristic of peptidoglycan precursors, and ii) in E. coli, and other bacteria, the terminal D-ala is cleaved off from precursors at the time of, or very shortly after, the insertion reaction, therefore active insertion sites should be the only places in the sacculus able to react with vancomycin. Key factors for success were the definition of experimental condition to ensure specificity of binding and availability of an anti-vancomycin antibody (GTX19968, GeneTex Inc. USA) well suited for immunofluorescence techniques. The method showed very high specificity and good sensitivity. Under appropriate conditions it was possible to image the putative insertion sites both in mutants with a higher than normal steady state concentration of D-alanyl-D-alanine in the sacculi and in wild type strains. The results show that insertion of new precursors takes place only at a few (3-6) discrete sites on the cell surface at any time, and that newly inserted precursors are interwoven with old material in a reticular fashion. These observations are compatible with the MreB-driven helical displacement of the biosynthetic sites as proposed by Daniels [3], but other geometries could as well be accommodated.