Cellular differentiation and cell division in bacteria

Bacteria have evolved an abundance of different life cycles and cell shapes, but the mechanisms that underlie this diversity are largely unknown. The aim of our group is to understand the molecular basis of cellular differentiation and cell division in bacteria and to elucidate how the one-dimensional information of the genetic code is translated into defined spatial and temporal regulatory patterns. To address these questions, we are using a combination of cell biological, biochemical, biophysical, and genetic approaches. Our studies focus on the model organism Caulobacter crescentus, a Gram-negative α-proteobacterium that is characterized by its unique developmental cycle (Fig. 1). C. crescentus cells can easily be synchronized and then be observed as they progress synchronously through their developmental program, which greatly facilitates the study of cell cycle-dependent processes. Moreover, owing to their asymmetric cell division, they represent an attractive model for studying bacterial differentiation.

Division site placement

A fundamental problem in cell biology is the proper temporal and spatial regulation of cell division. Our previous work has revealed the existence of a novel mechanism that is responsible for positioning of the cell division plane in C. crescentus. At its heart lies the Walker ATPase MipZ, which forms a dynamic complex with the chromosome partitioning protein ParB at the chromosomal origin of replication (Fig. 2). Upon initiation of DNA replication, the two newly synthesized origin regions are immediately re-decorated with the MipZ/ParB complex and then positioned at the two opposite cell poles. This generates an intracellular gradient of MipZ, with its concentration being highest at the cell poles and lowest at the cell center. Our analyses showed that MipZ has the ability to inhibit polymerization of the bacterial tubulin homologue FtsZ and, thereby, to prevent the assembly of the so-called FtsZ ring, which forms the foundation of the ring-shaped cell division apparatus. As a consequence, cytokinesis is limited to the midcell region and, furthermore, only initiates once chromosome segregation has started. MipZ is highly conserved among α-proteobacteria, which suggests that it represents the prototype of a new and widespread class of bacterial cell division regulators.

Fig. 1. The C. crescentus cell cycle. The C. crescentus swarmer cell bears a polar flagellum and a single, replicationally quiescent chromosome. At a certain point in the cell cycle, the flagellum is shed and a stalk is formed at the previously flagellated pole. Concomitantly, DNA replication and cell division are initiated. Cytokinesis then gives rise to a new G1-arrested swarmer cell and to a stalked cell that immediately enters the next division cycle.
MipZ thus shares similarity with small GTPases, using nucleotide binding and hydrolysis to transition between functionally distinct conformational states.

**Cell division**

The bacterial cell division apparatus (divisome) comprises a variety of different proteins, whose precise function and mode of cooperation is still poorly understood. Moreover, it is unclear to what extent the results obtained in the prototypic model system *E. coli* can be applied to evolutionarily distinct organisms. Recent work suggests that a core of essential divisome components is widely conserved among bacteria, whereas a number of accessory factors are restricted to certain lineages or a few species only.

Based on sequence similarity searches, most core components of the *E. coli* divisome have previously been identified in *C. crescentus*. One of the exceptions was FtsN, a peptidoglycan-binding protein that orchestrates the activity of cell wall synthases and hydrolases involved in cell wall invagination. Initially, the absence of this protein was not surprising, given that FtsN was thought to be a peculiarity of *E. coli* and its close relatives. However, we have now identified a *C. crescentus* protein that, despite the lack of sequence conservation, shows the structural and functional characteristics of FtsN and thus likely represents a highly diverged FtsN homologue (Möll & Thanbichler, 2009). Based on conserved features defined in our work, we were able to identify putative FtsN homologues in a large variety of species covering all proteobacterial lineages. Two of these candidate proteins were further investigated and verified to be cell division proteins, indicating that FtsN-like proteins are widespread among bacteria, albeit highly variable at the sequence level. We found that the peptidoglycan-binding (SPOR) domain of FtsN is dispensable for function but required for robust localization to the division site. Given that it is recruited to midcell in isolated form and retains its localization potential in heterologous hosts, it likely recognizes a conserved feature of the invaginating cell wall. Hence, the SPOR domain is likely to drive a positive feedback loop whereby only a small amount of FtsN is recruited to the division site by direct interaction with the divisome to initiate cell constriction. Synthesis of new cell wall material may then generate an increasing number of additional binding sites for FtsN, thus further accelerating the division process.
Searchng for interaction partners of FtsN, we have recently identified a peptidoglycan hydrolase, designated DipM, that is required for proper invagination of the cell wall during cytokinesis (Möll et al., unpublished). On deletion of the dipM gene, the outer layers of the cell envelope fail to follow the inner membrane during constriction, thus uncoupling compartmentalization of the cytoplasm from daughter cell separation. As a consequence, cells tend to form filaments or grow in chains, with branches emerging from the poles of individual cytoplasmic compartments. These results suggest that constriction of the cell may occur by deposition of additional peptidoglycan layers at the inner face of the cell wall and concomitant removal of the outer layers by lytic enzymes such as DipM. Moreover, they underscore the key role of FtsN in the organization of peptidoglycan remodeling at the division site.

Cytoskeletal elements
The cytoskeleton has a vital function in the temporal and spatial organization of both prokaryotic and eukaryotic cells. Our work has revealed the existence of a new class of polymer-forming proteins, designated bactofilins, that are widely conserved among bacteria (Kühn et al., 2009). In C. crescentus, two bactofilin paralogues cooperate to form a sheet-like structure that lines the cytoplasmic membrane in proximity of the stalked cell pole. These assemblies mediate polar localization of a peptidoglycan synthase involved in stalk formation, thus complementing the function of the actin-like cytoskeleton and the cell division machinery in the regulation of cell wall biogenesis. Myxococcus xanthus, by contrast, synthesizes four bactofilin paralogues. Three of them are encoded in an operon located in a cluster of motility-related genes, assembling into a massive, rod-shaped structure that occupies the mid-third of the cell. Deletion of this operon resulted in a severe defect in pilus-mediated gliding motility, suggesting direct or indirect involvement in locomotion. Yet a different situation is observed for Shewanella oneidensis, whose single bactofilin homologue shows the typical localization pattern of a cell division protein. These findings indicate that bactofilins can adopt a wide range of functions in the cell. Biochemical analyses revealed that bactofilins polymerize spontaneously in the absence of additional cofactors in vitro, forming stable ribbon- or rod-like filament bundles. These structures might have evolved as an alternative to intermediate filaments, serving as versatile molecular scaffolds in a variety of cellular pathways.

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Cell polarity
In cooperation with Patrick H. Viollier (Case Western Reserve University, Cleveland, Ohio), we have identified a novel protein, SpmX, that plays a vital role in controlling the differential fate of stalked and swarmer cells in C. crescentus (Radhakrishnan et al., 2008). SpmX is a membrane protein that is targeted to the stalked pole at the early predivisional stage of the cell cycle. Polar localization is dependent on a periplasmic domain with...
similarity to phage lysozymes, suggesting that the protein recognizes specific structural features of the polar cell wall. SpmX acts as a targeting factor that tethers and thereby activates the histidine kinase DivJ. Activated DivJ, in turn, launches the stalked-cell-specific developmental program in the stalked sibling once cell division has occurred. The swarmer cell, by contrast, lacks the SpmX/DivJ complex and thus expresses a different set of genes which encodes swarmer-specific functions.

Publications


Structure of the group (12/2009)

Group leader: Jun.-Prof. Dr. Martin Thanbichler

PhD students: Andrea Möll, Daniela Kiekebusch, Juliane Kühn, Anne Raßbach, Susan Schlimpert

MSc student: Alexandra Jung

BSc students: Oliver Leicht, Wolfgang Strobel

Technical assistant: Stephanie Wick
External funding

Human Frontier Science Program: Young Investigator Award (RGY 69/2008)

Invited lectures


108th General Meeting of the American Society for Microbiology, Boston, USA, Session 085: “Spatial Regulation of Cytokinesis: From Bacteria to Eukaryotes” (1–5/6/2008)

8th Graduate Retreat of the MPI for Biochemistry, Tegernsee, Germany (1–2/7/2008)

Fakultät für Biologie, Bereich Mikrobiologie, Ludwig-Maximilians-Universität München (8/7/2008)

Departement für Chemie und Biochemie, Universität Bern (8/12/2008)

Instituto de Bioquimica Vegetal y Fotosintesis, CSIC/ Universidad de Sevilla, Spain (16/4/2009)

Lehrstuhl für Mikrobiologie/Organismische Interaktionen, Eberhard-Karls-Universität Tübingen, Germany (28/5/2009)

Centre de Genetique Moleculaire, CNRS, Gif-sur-Yvettes, France (20/11/2009)

Address

Jun.-Prof. Dr. Martin Thanbichler
Max-Planck-Institut für terrestrische Mikrobiologie
Karl-von-Frisch-Straße 10
35043 Marburg/Germany

Phone: +49 6421 178-300
Fax: +49 6421 178-209
E-mail: thanbichler@mpi-marburg.mpg.de
The Max Planck Research Group "Prokaryotic Cell Biology". From left to right: Stephanie Wick, Oliver Leicht, Wolfgang Strobel, Alexandra Jung, Martin Thanbichler, Juliane Kühn, Anne Raßbach, Andrea Möll, Susan Schlimpert, Daniela Kiekebusch