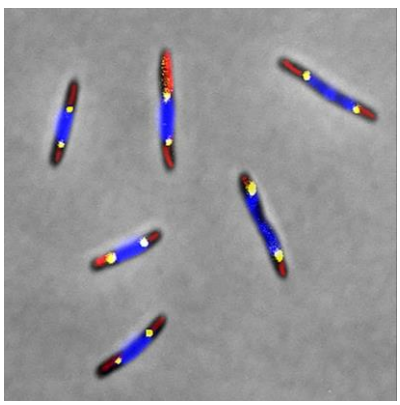
TRR 174
Newsletter
Spatiotemporal dynamics of bacterial cells

Paper Report

SMC and the bactofilin/PadC scaffold have distinct yet redundant functions in chromosome segregation and organization in *Myxococcus xanthus*

The ParAB*S* systems and the structural maintenance of chromosome (SMC) complex are important for chromosome segregation and organization in many bacteria. In some bacteria, these factors are essential, while some bacteria can survive without them. We previously showed that the rod-shaped, Gram-negative *M. xanthus* cells possess a unique arrangement of its 9.2 Mbp large circular chromosome in which the nucleoid is localized in the central part of cells leaving the large subpolar regions devoid of DNA. In *M. xanthus*, the ParAB*S* segregation machinery is essential. Moreover, Martin Thanbichler's research group has shown that the BacNOP/PadC scaffold in the subpolar regions positions the ParB·*parS* segregation complexes and the DNA segregation ATPase ParA.



Myxococcus xanthus cells expressing fluorescently tagged ParB (yellow), ParA (red) and nucleoid stained with DAPI (blue).

Because the chromosome organization in *M. xanthus* is unique, depends on the BacNOP/PadC scaffold, and the ParAB*S* segregation system is essential, we were curious about whether the SMC complex would play a role in chromosome segregation and organization in this bacterium.

We first identified the genes for the Smc and ScpAB subunits of the SMC complex in *M. xanthus*. Subsequently, using genetics we found that SMC is conditionally essential and cells lacking SMC have a growth defect at 25°C and are non-viable at 32°C. Using live-cell imaging we found that cells lacking SMC have chromosome segregation and organization defects similar to what has been observed in many other bacteria. Moreover, lack of the BacNOP/PadC scaffold not only caused a defect in chromosome organization but also in chromosome segregation. Finally, we found that inactivation of SMC is synthetically lethal in a background that lacks the BacNOP/PadC scaffold.

Altogether, we report that *M. xanthus* requires three systems for chromosome maintenance, and that ParAB*S* constitutes the essential and core machinery for chromosome segregation. At the same time, SMC and BacNOP/PadC have different yet redundant roles in this process, with SMC supporting individualization of duplicated chromosomes and BacNOP/PadC making the ParAB*S* system more robust.

Original publication

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Alumnus Report

Howdy! I'm **Karsten Miermans**. Most of you already know me from being a (former) member of the young scientists committee, where we (together with Nadia, Hanna, Muriel, John and Meike) organized several TRR 174 events.

In the period 2016-2020, I had the privilege of doing my doctorate in theoretical biophysics with Chase Broedersz at the LMU Munich. We addressed the basic question "What essential features does the bacterial chromosome need for its remarkable level of organization?". Rather than using e.g. point mutations or other empirical methods, we performed computer 'experiments'. We simulated long DNA molecules under various conditions to figure out what is crucial for the organization observed in real cells. Luckily, our simulations turned out to be useful – we found that even bacteria likely need motor proteins for genome-wide organization.

Experimentalists have frequently asked me: why theory; what is it that you do? I remember being fascinated with ideas, concepts and equations from a young age already. When I was in high-school, I remember gazing at a math textbook in a London library (one of those yellow Springer ones), fascinated with this language, which I could appreciate but not comprehend. Science is premised on both theory and experiment, but conceptual understanding was the thing that drove me. That being said, I have gotten a lot of respect for the careful, hard work that is needed for good experiments – largely through interactions I had with people in the TRR! Then, what theorists do can be broadly categorized into 'analytical' and 'computational'. There exist theoretical physicists that really use pen and paper to derive equations. I was one of the computational kind, developing tailored simulation frameworks to model certain biological systems. I think all the hours debugging my computer simulations was certainly the least fun part of my work! Whilst analyzing the results from these simulations, I discovered a passion for data and statistics. Funnily enough, I was bored by statistics before my PhD.

Amongst the many things I learned during my PhD is that we can be quite misguided about what we believe we find interesting. Did I find statistics boring, just the professor/book, or something else entirely? Anyhow, I'm excited to start a job as a senior data scientist & AI developer next month at a start-up (resmechanica.com). I had the pleasure and honor to meet many incredible people during the TRR events. Whenever you are in Munich, write me a message, I'd be delighted to get a beer with you!

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Science Report

Dynamics of the *Bacillus subtilis* Min system

The precise positioning of the division site is an important process to ensure correct partitioning of DNA. In many bacterial cells, proteins are known to regulate division site selection by inhibiting the assembly of the Z ring anywhere but at midcell. Two well studied model organisms are the rod-shaped bacteria *Escherichia coli* and *Bacillus subtilis,* where the Min protein system regulates division site placement.

In *E. coli*, the Min proteins MinC, MinD and MinE show a remarkable oscillation from pole to pole with a time-averaged density minimum at midcell. The Min system in *B. subtilis* lacks MinE and it is therefore believed that MinC and MinD form a static bipolar gradient decreasing towards midcell. The spatial cue for a gradient in *B. subtilis* is provided by the curvature-sensitive protein DivIVA, which is known to localize to regions of negative curvature (such as the cell poles). Even though MinC and MinD are highly conserved between *E. coli* and *B. subtilis*, it remained puzzling why these proteins are dynamic in *E. coli* but form a rather static distribution in *B. subtilis*.

A recent experimental study, performed in the Bramkamp group, shows that the Min system in *B. subtilis* relocalises from the cell poles to midcell once a septum has formed. Contrary to the picture of a static gradient, this finding suggests a dynamical process which leads to redistribution of Min proteins. In collaboration with the theoretical biophysics group of Erwin Frey, the authors propose a reaction-diffusion model, a similar approach as has been used to model Min oscillations in *E. coli*, that reproduces the experimental findings. Modeling suggests that Min localization should be understood as a (dynamic) equilibrium state rather than a static protein distribution. Furthermore, the model highlights two key factors that are required to explain Min localization in *Bacillus subtilis* consistent with experiments: The first one is the existence of an ATPase activity of MinD, which has not been shown yet experimentally, but is required to explain MinD localization. The second point is an interplay between diffusion, ATPase cycle, and the 3D cell geometry which results in a geometric effect that leads to an accumulation of MinD at midcell. Hence, MinD localization cannot be explained with simplified 1D models.

In summary, the study solves the conflict between the function of Min proteins in *E. coli* and *B. subtilis,* as they were believed to be fundamentally different (dynamic vs static).

For further reading:
Feddersen H., Würthner L., Frey E., Bramkamp M. (in review) Dynamics of the *Bacillus subtilis* Min system. *Biorxiv* (DOI: 10.1101/2020.04.29.068676v1).

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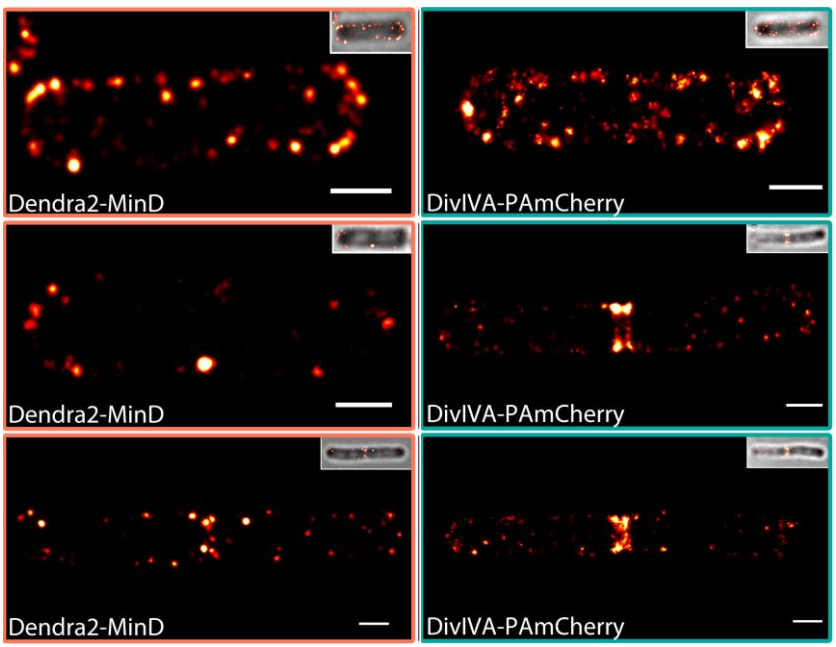

PALM imaging of strains expressing Dendra2-MinD and DivIVA-PAmCherry. Representative PALM images of Dendra2-MinD and DivIVA-PAmCherry expressing cells at different divisional states. Upon formation of a division site, DivIVA and MinD partially relocalise from the poles to the division septum, where they reside after successful cytokinesis. Scale bar 500 nm.

Have You Met …

I am Lennart Randau and started my Heisenberg-professorship at the Philipps-Universität Marburg in December 2019. My family already moved to Marburg in 2010 and I established a free-floater research group at the local MPI. My wife Jing Yuan is a project group leader at this institute and we have two sons (8 and 10 years old and growing way too fast). My research group is currently located at the MPI and we are looking forward to soon being able to move across the street into a larger lab space.

The work of my group focuses on (small) RNA biology in bacteria and archaea. During my PhD and postdoc days in the laboratories of Dieter Jahn (TU Braunschweig) and Dieter Söll (Yale University), I developed a passion for intricate RNA processing pathways in archaeal organisms growing near the upper temperature limit of life (i.e. approx 120°C), involving circularization, *trans*-splicing and other "puzzle" mechanisms. These "RNA puzzles" are likely a consequence of the positive selection of fragmented ncRNA genes. As these genes frequently serve as viral attachment sites, we hypothesized that their fragmentation protects cells from integration of harmful viral sequences.

As I worked on these pathways, the function of CRISPR-Cas clusters as antiviral immune systems was discovered and our archaeal organisms were found to contain record numbers of these systems, supporting the notion that viruses pose a special threat for some extremophilic organisms. I became fascinated by the enormous diversity of CRISPR-Cas systems and mechanisms and decided that it would be an exciting direction to pursue for my first independent position. Antiviral measures are counteracted by viral innovations that undermine these systems, which is for example visible for the enormous variety of restriction-modification systems. Similarly, viruses invented a large number of anti-CRISPR proteins and strategies that co-evolved with the diversification of CRISPR-Cas interference complexes. In the last years, we have uncovered new systems and worked with TRR 174 members Kai Thormann and Martin Thanbichler on their targeting mechanisms, and Gert Bange on the structures of CRISPR-Cas effector complexes.

One novel focus of our future research will be on the life cycle of large conjugative plasmids containing CRISPR-Cas systems. We showed that these systems target other plasmids and proposed their involvement in inter-plasmid competition. We are excited to continue and establish new collaborations within TRR 174 research center which enable us to study and visualize plasmid organization and segregation patterns for these interconnected systems.

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About TRR 174 (*www.trr174.org*)

The head office of TRR 174 is located in the New Chemistry Building of the University of Marburg, Germany.

TRR 174 is a DFG-funded Transregional Collaborative Research Center. In this consortium 16 research groups, located in the Marburg and Munich areas, have joined forces to implement a comprehensive and highly coherent research program that investigates the molecular mechanisms controlling the spatiotemporal dynamics of bacterial cells. Joint activities ensure interdisciplinary research and close ties between the groups as well as a comprehensive training program for all associated PhD students.

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