TRR 174
Newsletter
Spatiotemporal dynamics of bacterial cells

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Paper Report

Chromosome organization by a conserved condensin-ParB system in the actinobacterium *Corynebacterium glutamicum*

Every cell, whether we are talking about bacterial cells or human cells, needs to condense, replicate and segregate their genetic material in order to duplicate. In recent years, the ubiquitous condensin system that is dedicated to chromosome compaction gained signifycant interest. Bacterial condensin systems come in two functionally equivalent flavors, the SMC-ScpAB and MukBEF systems, respectively.

Based on early results from our lab that pointed towards a central role for chromosome organization in corynebacterial growth and morphogenesis, this new paper focuses on the characterization of the condensin system in *C. glutamicum.* Surprisingly, *C. glutamicum* encodes two types of condensin complexes, a SMC-ScpAB complex and the Muk-related MksBEFG. We were able to show that, as in *B. subtilis* and *C. crescentus*, SMC is loaded at *parS* sites in a ParB-dependent manner. In addition, chromosome conformation capture experiment (Hi-C) data, collected in collaboration with Drs. Martial Marbouty and Romain Koszul from the Institute Pasteur in Paris, revealed that the origin of replication is segregated over the existing nucleoid in a ParB-dependent manner, reinforcing the idea that chromosomes also possess a structural role.

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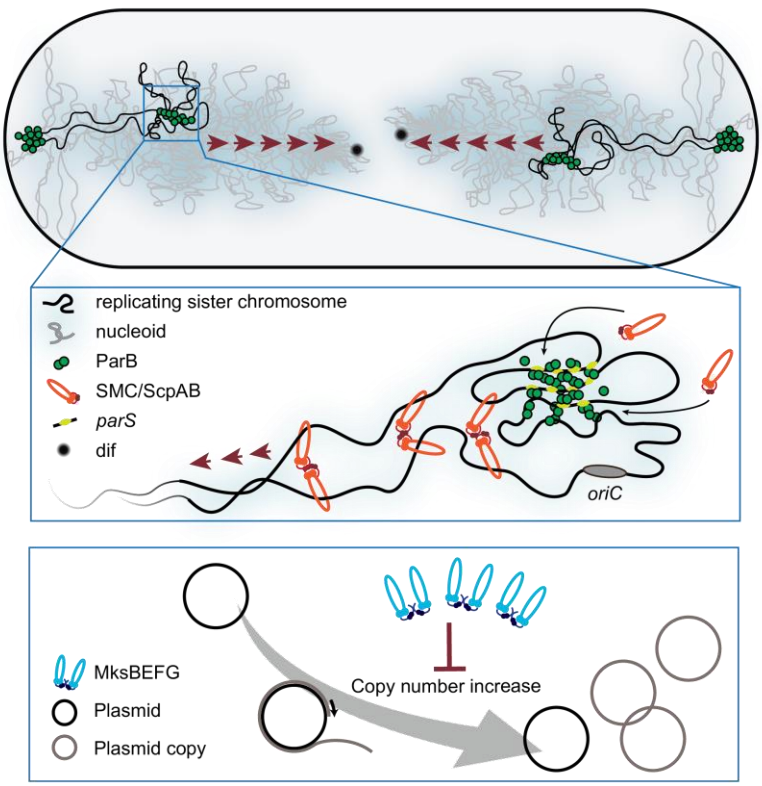

Figure 1: Cartoon of a molecular function of the *two C. glutamicum* condensin systems. A growing *C. glutamicum* cell with two polar attached chromosomes is shown in the upper panel. The ParB-*parS* nucleoprotein complex is the main organizing center of the chromosome and required to load SMC condensins (just like in *B. subtilis* or *C. crescentus*; middle panel). The alternative MksBEFG condensin complex is required to keep plasmid copy numbers in check (lower panel). One might speculate that the subunit MksG, which is absent in the homologous MukBEF system in *E. coli*, plays a crucial role in this process.

Contrarily to our expectation, the second condensin system MskBEFG had no effect on chromosome conformation. This complex localizes at the cell poles via the polar scaffold protein DivIVA, and one of its members, MksB, shows an increased fluorescence in cells containing plasmid DNA. By quantifying low/single copy plasmids enrichment in *ΔmksB* cells, we provided further data supporting the idea that the MksBEFG system is instead involved in plasmid maintenance/control. Additional support comes from the recent finding of the Sorek lab in Israel regarding the role of MksBEFG as antiviral or anti-plasmid defense systems. MksBEFG is, so far, the only known condensin system amongst pro- and eukaryotes that is not involved in chromosome organization.

Original publication

Böhm K., Giacomelli G., Schmidt A., Imhof A., Koszul R., Marbouty M., Bramkamp M. (2020) Chromosome organization by a conserved condensin-ParB system in the actinobacterium *Corynebacterium glutamicum*. *Nature Communications* 11: 1485.

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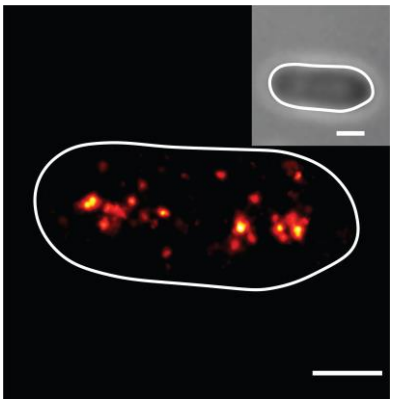

Figure 2: Gaussian rendering of ParB-PAmCherry localizations in a *C. glutamicum* wild-type background cell (functional allelic replacement) obtained via photoactivated localization microscopy (PALM). In agreement with *C. glutamicum* polyploidy, multiple origins of replication can be observed at any given time via proxies: the ParB-*parS* complexes. Scale bars: 500 nm.

Announcement: Workshop – Presenting Posters Successfully

The evaluation of our application for a second funding period will take place on 1-2 October 2020. Due to the current situation, the usual on-site visit of the reviewers will be replaced, at least in part, by online conferences. In preparation of the evaluation, we are organizing the two-day workshop *Presenting Posters Successfully*, which should be attended by every PhD student and postdoc who may potentially be involved in the presentation of a project. It is held by Dr. med. Stefanie Rummel and takes place on 3-4 August in Marburg and on 6-7 August in Munich. Of course, any other person who is interested is welcome to take part as well if slots are available.

The aim of this workshop is to develop your skills in poster preparation and presentation. This session enables you to present clearly and in a more structured and self-confident way. Before-and-after comparisons through video recording will help you to become more aware of your content creation and your presentation skills. Working on a real aim will help you to put everything you learned into practice.

In particular, you will address the following questions:

- How do I present typically?
- What am I afraid of?
- How do I use my voice?
- How do I use my body language?
- Do I send clear messages?
- How do I create good poster presentations?
- How do I visualize and structure my poster well?
- How do I present posters efficiently and effectively?
- How am I perceived? What do I wear?
- How do I deal with question and answer session?

The trainer Stefanie Rummel is specialized as a certified trainer, speaker, coach and artist on “Soft-Skills”. After completing her dissertation on nonverbal communication at Frankfurt University, she now supports her clients to develop their e.g. personal presence through better communication and presentation skills, vocal presence, nonverbal communication, and how to transfer content verbally, visually and vividly. Speaking fluently German, English and French, she works throughout Europe for clients such as universities, the EU and companies in the pharma, air & space, IT and financial sector.

More infos
www.soft-skill-seminar.de

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Alumnus Report

My name is **Devid Mrusek**. I am a chemist by training and a former member of the TRR 174. I studied at ETH Zurich and Freie Universität Berlin. During my master's studies, I oriented myself towards bioorganic chemistry and became intrigued by membrane proteins. In fact, I started a PhD project on bioorganic methodology in the context of membrane proteins with a cross-institutional approach in Berlin/Potsdam and was able to secure a PhD-scholarship. However, things developed sideways and after some time I had to stop this project. Due to networking I had already met Gert Bange. Supported by my scholarship and my future peers in Marburg, I was able to reorient my proposal. Gert gave me the opportunity to re-boot my PhD in his group and eventually supported me to join the TRR 174. On a personal level, I discovered that the interdisciplinary environment of SYNMIKRO in Marburg and the support of Gert were very rewarding. This became even more true when the TRR 174 started. It was a pleasure for me to co-organize the first international conference of the Transregio in Marburg in 2018 together with other TRR-members. The Transregio offered the opportunity to advance an understanding at the biochemical and cellular levels in different disciplines and I was able to contribute to this approach in a collaboration with the group of Kai Thormann from Giessen University. Other collaborations included the groups of Roland Beckmann and Erwin Frey. Most of these projects were so intriguing that it was tempting to dig deeper into the topics. But experimental time was limited, and the Bange group collaborated extensively also within Marburg to provide support in structural biology, which provided me with additional exciting projects. Due to my internships in two DAX-companies I knew that I wanted to start a career in a corporate environment after finishing my PhD. Those internships took place during the global financial crisis in 2008. This experience was very instructive and becomes quite relevant in the current Corona situation. In December 2018, I received an offer from Evonik, which is a specialty chemicals company with an interesting history. I started at *Creavis*, the strategic innovation unit of Evonik in 2019, where I was assigned to the general manager as a scientific advisor. Creavis is responsible for mid- to long-term high-risk research projects and offered me the chance to interact with the corporate as well as with the operative departments of the company. Because of my experience in organizing events and my writing skills, I also took care of the communicative tasks at Creavis (www.creavis.com).

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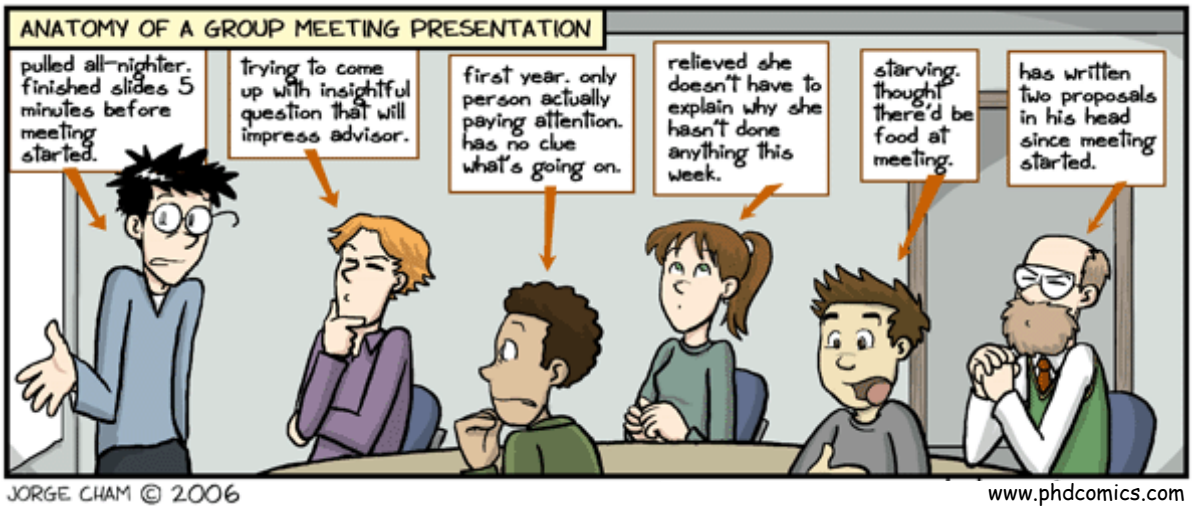

Science Report

How do bacteria restrict the assembly of a single polar flagellum?

Flagella are organelles of locomotion and multiprotein complexes whose positioning, number and assembly requires complex spatiotemporal control. In many bacterial species, the MinD-type ATPase FlhG is central to the numerical control of flagella. The deletion of *flhG* in polarly flagellated bacteria leads to polar hyperflagellation (see figure). One part of my PhD is to understand the molecular mechanism of this numerical control of flagella in *Shewanella putrefacien*s CN-32 as a general model organism. This is done in cooperation with the groups of Gert Bange, Roland Beckmann and Morgan Beeby.

To limit the number of flagella to a single one, FlhG must reach the cell pole by passive transport with the flagellar switch complex protein FliM to the nascent flagellum. Preventing FlhG-FliM interaction, results in a *flhG* deletion phenotype, i.e. polar hyperflagellation. Furthermore, electron cryotomography images of wild-type and *flhG* deletion strains showed that FlhG is not required for the assembly of flagella. But how does FlhG regulate the number of flagella?

Besides the switch complex protein FliM, FlhG also interacts with the flagellar transcriptional master regulator FlrA. While FlrA and FliM share the same binding site on FlhG, their binding depends on the ATP-dependent dimerization state of FlhG. FliM interacts with the ATP-independent FlhG monomer and FlrA interacts only with the ATP-dependent FlhG dimer. Interaction of FlrA and FlhG inhibits the FlrA transcriptional activity and therefore the production of flagellar components. An overproduction of FlhG means that the cells are no longer able to form a flagellum and an overproduction of FlrA results in a hyperflagellation phenotype as observed upon *flhG* deletion. The FlhG-FlrA interaction is interrupted ($FlrA_{L400E}$), the strain assembles multiple flagella localizing at apparently random positions over the cell body (see figure). Furthermore, FlrA stimulates the ATPase activity of FlhG, suggesting that FlrA promotes a shift from dimeric to monomeric state of FlhG. In addition, a burst of FlrA-dependent gene expression can take place to start the production of a new flagellum.

Thus, FlhG partner switching between the flagellar switch complex protein FliM and flagellar master regulator FlrA underlies the mechanism of flagella numerical restriction, in which the transcriptional activity of FlrA is down-regulated through a negative feedback loop. Not only the cellular copy number of FlhG but also its subcellular localization is critical for its function in the numerical regulation of flagella. This study demonstrates another level of regulatory complexity underlying the spatio-numerical regulation of flagellar biogenesis, and implies that flagellar assembly transcriptionally regulates the production of initial building blocks.

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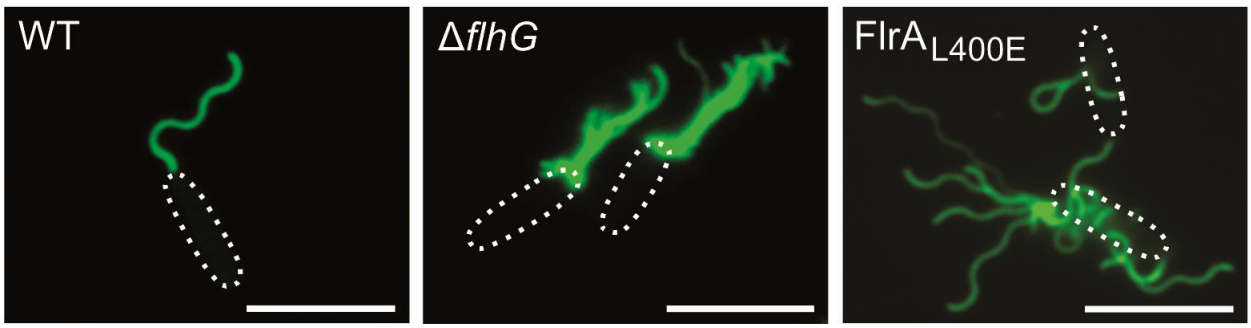

Number of flagella is regulated by MinD-type ATPase FlhG. Scale bars: 5 µm.

Have You Met …

My name is **Seán Murray** and I am a Research Group Leader at the MPI in Marburg. I have been associated to the TRR174 since March 2019. My path into biology was somewhat unusual. During my PhD and first postdoc, I worked on developing the mathematical ingredients necessary to study quantum field theories on non-commutative spacetimes, making a short detour into cosmology along the way. I switched fields in 2011 to join the group of Martin Howard in Norwich, UK, where I started applying physics and mathematics to problems in bacterial cell biology, which I have been doing ever since. So, you could say that I went from studying the very small (10^{-35} m, the Planck length) to the very big (10^{26} m, the observable universe) before settling on something in between (10^{-6} m, a bacterial cell)!

My group uses bacteria as tractable model systems in which to uncover the principles and mechanisms underlying spatiotemporal organisation in bacteria. To this end, we use mathematical modelling and stochastic simulations combined with live-cell experiments and genetics in a multi-disciplinary and systems approach. A recent focus was the self-positioning of the Structural Maintenance of Chromosomes (SMC) complex MukBEF in *Escherichia coli* and its role in chromosome segregation. In short cells, MukBEF forms a dynamic nucleoid-associated cluster that is specifically positioned at mid-cell. During cell growth the cluster splits into two with the resulting clusters re-positioned to the cell quarter positions. We developed a mathematical model to explain this behaviour based on Turing pattern formation. This is where spatial patterns arise spontaneously in a reaction-diffusion system in spite of, or rather due to, the presence of diffusion. We found that peaks in the MukBEF concentration form and are positioned even in the presence of stochastic effects. In essence, the proteins can sense the geometry of the nucleoid and position themselves appropriately. MukBEF has also been implicated in the positioning and segregation of chromosomal origins (*ori*), which are similarly positioned throughout the cell cycle. Indeed, we recently showed experimentally that *ori* display an attraction to MukBEF foci and we proposed a model in which interactions between MukBEF and *ori* lead to a mutual attraction that results in *ori* positioning and partitioning as an emergent property of the system.

In the future, I look forward to integrating my group more deeply into the Transregio. As microbiology becomes more and more quantitative, I believe that interdisciplinary and systems approaches will become evermore important and see the consortium at the forefront of that trend.

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

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

TRR 174 is a DFG-funded collaborative research center. 16 research groups, located in the Marburg and Munich areas, have joined forces to establish a Transregio-CRC with a comprehensive and highly coherent research program to investigate 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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