TRR 174
Newsletter
Spatiotemporal dynamics of bacterial cells

Comment

Prof. Martin Thanbichler – Spokesperson TRR 174

Dear all,
first of all, I wish you a happy, successful and healthy New Year. Let's hope that in 2021 the current efforts to overcome the COVID-19 pandemic will take effect. Interactions are the essence of human life and science, and it will be wonderful to meet people again in person without the fear of risking their and one own's live.

Interactions also represented the heart of TRR 174. In the past four years, our joint activities led to close ties between our groups and yield a series of novel findings that would have been impossible to achieve without the multifacetted expertise available in our consortium.

I was hoping that I could use this occasion to give you a preview of our joint activities in the next four years. However, as you all know, things did not turn out the way we hoped and our renewal application was not supported by the DFG. The reasons for this decision were not at all related to our scientific work. On the contrary, all aspects of the science done in the Transregio were rated to be excellent, including the relevance of the research topic, the number and quality of our publications, the international visibility of the groups, the interdisciplinarity of the research program, the extent of collaboration and the synergies between the different groups and locations. We were told that, when asked by the DFG, the reviewers had problems identifying the scientific highlight of the funding period, because there are too many excellent publications to pick from. The reviewers also appreciated the organization of the Transregio and the many joint activities we organized. One of them concluded that he/she was terribly jealous and DFG would be crazy not to fund us. I think this feedback is something we can be proud of, and I would like to thank everyone, including PhD students, postdocs, group leaders and our administrative staff, for making this possible.

Unfortunately, however, scientific excellence alone was not sufficient to warrant a renewal of our grant. The decisive problem was the lack of junior research group leaders at the two applying universities (UMR and LMU) and, to a lesser extent, the underrepresentation of women among the newly recruited PIs. We made considerable efforts to address these structural issues whenever possibe. However, in the end, it was beyond the possibilities of the Transregio to significantly change the age and gender distributions of the participating PIs within the relatively short period of time available.

Although it is extremely disappointing that our application was not successful, especially in light of the underlying reasons, we should now look forward and build on the foundation we have laid in the past years. I think we have all greatly enjoyed working with colleagues who share the same excitement about bacterial cell biology but take a different angle on the topic. It would therefore be wonderful to continue our interdisciplinary work in some way, either in the context of a new collaborative research center or in smaller collaborative projects supported by other funding sources. The final year of funding now gives us the possibility to reorient our projects and make new plans for the future.

I thank you for the time and efforts you invested in making our joint venture work and look forward to exciting new collaborations in the years to come.

All the best, Martin

Science Report

Spatiotemporal dynamics of native and engineered multi-replicon systems in *Sinorhizobium meliloti*

My name is Marcel Wagner and I am a PhD candidate in the comparative genomics group of Prof. Anke Becker at the Center for Synthetic Microbiology (SYNMIKRO) in Marburg. I started my TRR 174 research project in 2017, focusing on how the DNA in *Sinorhizobium meliloti* is coordinated in space and time during the cell division cycle, influenced by the existing genome architecture to draw conclusions on underlying molecular mechanisms.

S. meliloti possesses a tripartite genome composed of a main chromosome (3.65 Mb) and the RepABC-type secondary replicons pSymA (1.35 Mb) and pSymB (1.68 Mb), classified as megaplasmid and chromid, respectively. In course of this project I established, together with former colleagues, novel genome variants of *S. meliloti*. Beside strains with extended genome content by addition of artificial mini replicons (pABCs) based on heterologous *repABC* regions we rearranged the tripartite genome configuration and created strains with bi- and monopartite genomic DNA using a site-specific recombination (Cre/*lox*) strategy. We developed a triple fluorophore based labeling system, which enabled simultaneous tracking of three individual genomic loci. We used this system *inter alia* to show that in *S. meliloti* pABCs propagate autonomously, are single copy replicons and seem to mimic the spatiotemporal coordination of the RepABC-type secondary replicons.

In a 2D mapping study we categorized and compared the topological organization of the tripartite wild type genome with other DNA architecture variants to gain insights on how the presence or absence of one or both secondary replicons or replicon fusions influence cellular positions of genomic loci. For the wild type we found that the chromosomal DNA molecule spans the whole cell with *oriC* and *terC* enriched at the old and new pole, respectively. In contrast, the data of secondary replicons suggested a distribution in only one quarter (pSymA) or to two quarters (pSymB) of the cell with an enrichment of *oriA* and *oriB* in the subpolar region of the old cell compartment. This localization pattern was changed in strains with reduced genome content or fused replicons. In strains lacking pSymA, parts of pSymB seemed to be located closer to the old pole probably filling the space formerly occupied by pSymA. In the strain with all replicons fused into a single DNA molecule (SmABC), the location of the three replication origins and the chromosomal terminus region was generally maintained, whereas other DNA segments, such as the terminus regions of pSymA, showed an altered localization.

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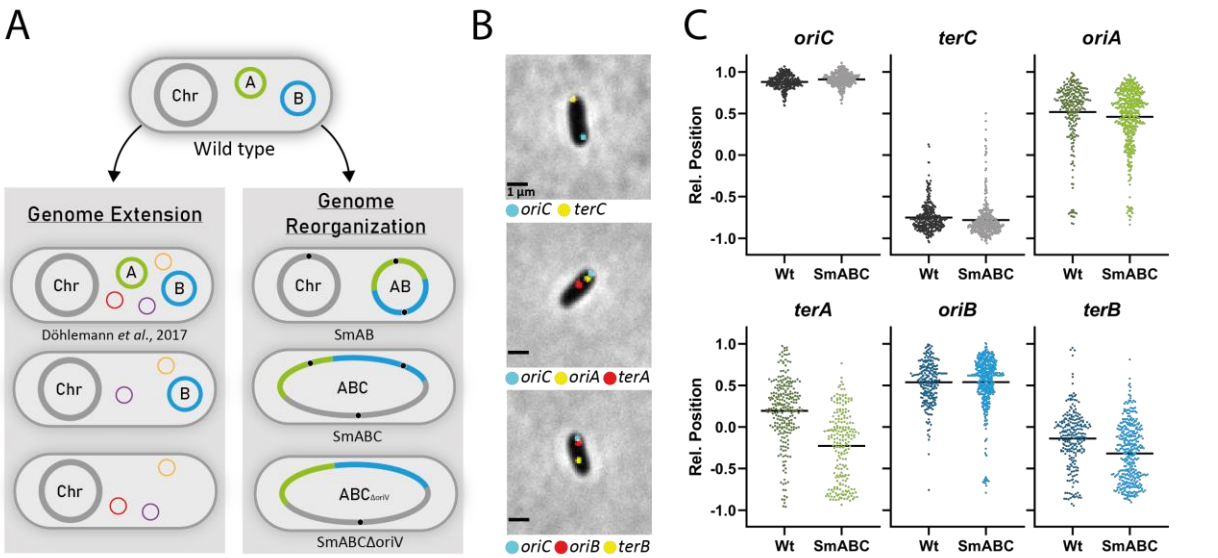

A: Strains with genome extensions and rearrangements constitute a suitable experimental platform to address questions on how replicon number, size, and architecture affect spatial organization and dynamics of the replicons in the different phases of the cell cycle. **B:** Usage of the triple-color labeling system in a snap shot analysis of *S. meliloti* cells with all replicons fused into a single DNA molecule (SmABC). **C:** Comparison of origin and terminus localization in the wild type and in SmABC. Old pole: 1, New pole: -1.

These analyses were carried out in collaboration with David Geisel from the theoretical physics group of Peter Lenz, who produced *in silico* genome configurations of wild type and genome variant strains considering altered genomic loci to be spatially constrained. Further, he supported the experimental design by analyzing which distribution of two or three fluorescent labels on one or different replicons optimizes information content of the experimental data.

The segregation process of the three replication origins in the *S. meliloti* wild type was previously found to follow a temporal order starting with the chromosome, followed by pSymA and pSymB. In a recent study we found that this segregation pattern is conserved even when the genomic content is fused into a bi- and monopartite configuration. By deletion of both secondary replicon origins from the monopartite variant we turned the multi-origin molecule into a single 6.7 Mb replicon (SmABCΔoriV). This strain showed a changed segregation order for the inactivated origin and the unchanged terminus regions of pSymA and pSymB with an altered localization of the inactivated origin regions in the midcell area.

In conclusion, these findings demonstrate the high tolerance of *S. meliloti* to comprehensive genome rearrangements and the potential of strains with altered genomic content promoting the elucidation and comprehension of prokaryotic multipartite genome biology.

Original publication:

Döhlemann J, Wagner M, Happel C, Carrillo M, Sobetzko P, Erb TJ, Thanbichler M & Becker A (2017) A family of single copy repABC-type shuttle vectors stably maintained in the Alpha-Proteobacterium *Sinorhizobium meliloti*. *ACS Synthetic Biology*, **6***:* 968–984.

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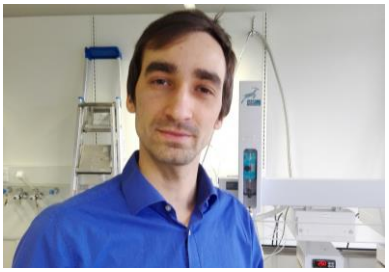
Have You Met … Wieland Steinchen

Back in late 2013, I joined the group of Gert Bange, at that time a junior research group and part of the SYNMIKRO Research Center in Marburg, as a PhD student working on nucleotide-based bacterial second messengers. It was not before 2015 when I began to intensively employ an analytical technique called hydrogen-deuterium exchange mass spectrometry (**HDX-MS**) in my own studies and in a number of collaborative projects. Shortly after obtaining my PhD in 2017, I took responsibility for HDX-MS as a staff scientist in the DFG-funded Marburg core facility for Interactions, Dynamics and Biomolecular Assembly Structure (MIDAS), which offers expertise in the extensive study of proteins by HDX-MS.

The function of proteins is based on their three-dimensional structures and dynamic properties, which allow for conformational changes between structural ensembles. The detailed analysis of protein dynamics is thus crucial for the molecular understanding of biological processes. HDX-MS is highly versatile and can be applied to study the topology of protein complexes, protein/protein interactions or interactions of proteins with small molecules and nucleic acids. Hereby, HDX-MS does not only allow assessing whether a protein changed its conformation under a certain condition. Instead, the areas of a protein that underwent a conformational change can be dissected up to the level of a few amino acids by evaluating their specific HDX pattern, facilitating localization of, for example, ligand binding sites. This information gathered by HDX-MS, either alone or in combination with other biochemical and/or structural data, allows deciphering the molecular details of a proteins' function.

In recent years, the HDX-MS methodology could contribute to a number of great projects in the framework of the Collaborative Research Center TRR 174 and I am thus looking forward to participating again in plenty of intriguing topics of bacterial cell biology.



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Alumnus Report

Hi everyone! My name is Isabella Graf and I'm a former member of the TRR 174. By training, I am a theoretical physicist and after some small excursions into mathematics and string theory I was very fortunate to become a member of Erwin Frey's group at the LMU in Munich. Working at the interface between physics and biology is both challenging and great fun for me. Biophysics as a rather new field of research has few established "unifying" theories and there is a lot of room for exciting new ideas, approaches and insights.

In my PhD I had the chance to investigate several biological questions ranging from the collective motion of filaments in networks to the self-assembly of heterogeneous structures. In particular, in a still ongoing collaboration with the groups of Kai Thormann and Gert Bange, we investigated a molecular counting mechanism for the regulation of flagellum assembly. Based on experimental data, we developed a stochastic model for the first steps of flagellum assembly in order to identify important features of the robust regulation pathway. This collaborative project and the TRR retreats have been a wonderful opportunity for me to broaden my horizon and to get to know different perspectives on biophysical questions.

After finishing my PhD in February (and managing to move to the U.S. during a pandemic), I now work as a postdoc with Ben Machta at Yale University. In collaboration with Sarah Veatch at the University of Michigan, we hope to better understand membrane critical behavior and, more generally, phase behavior in mixtures of many components.

Should any of you be in New Haven in the not too distant future (presumably post-Covid ☺), let me know – I would be happy to meet and show you around!

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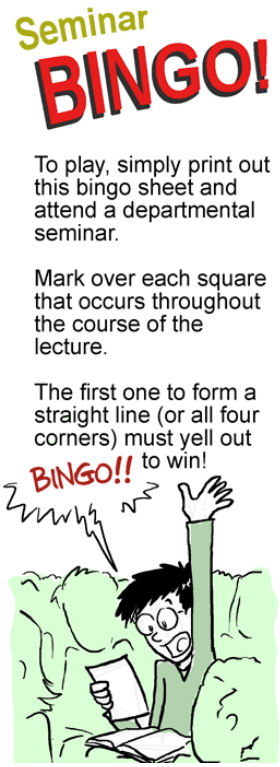

SEMINAR BINGO

Speaker bashes previous work	Repeated use of "um…"	Speaker sucks up to host professor	Host Professor falls asleep	Speaker wastes 5 minutes explaining outline
Laptop malfunction	Work ties in to latest buzz-words in your field	"…et al."	You're the only one in your lab that bothered to show up	Blatant typo
Entire slide filled with equations	"The data *clearly* shows…"	FREE Speaker runs out of time	Use of Powerpoint template with blue background	References Advisor (past or present)
There's a Grad Student wearing same clothes as yesterday	Bitter Post-doc asks question	"That's an interesting question"	"Beyond the scope of this work"	Master's student bobs head fighting sleep
Speaker forgets to thank collaborators	Cell phone goes off	You've no idea what's going on	"Future work will…"	Results conveniently show improvement

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Workshop Report: Virtual Company Visit

After a very successful virtual company visit last June, the Transregio offered again the opportunity to take part in the virtual company visit *Behind the Scenes of CSL Behring Vol II* on December 14. This event was planned for everyone, who is interested and thinking about a career in the industrial sector.

During the 2 hours visit, experts from different divisions took the participants behind the scenes of a global biopharmaceutical company. Amongst others, they gave experience reports, informed about early career programs and gave recruiting tips and tricks. The workshop ended with an extensive Q&A session, although the participants were encouraged to ask questions at any time. In future, CSL is going to offer "Virtual Company Visits - Reality Tours", "One to One CV Checks", and "Networking Workshops" for doctoral and postdoctoral researchers.

A huge *Thank You* for a well-organized, informative and great event to the initiator Daria Schüpbach (University Relations & Graduate Programs Recruiter EMEA, CSL Switzerland), as well as Lutz Bonacker (Senior Vice President & General Manager EU, CSL Marburg), Alexander Spielberg (Senior Talent Acquisition Consultant, CSL Plasma Marburg), Fabio Hopf (Project Engineer, CSL Marburg), and Maximilian Koch (Pharmacist Intern, CSL Marburg). Looking forward to another virtual company visit in spring!

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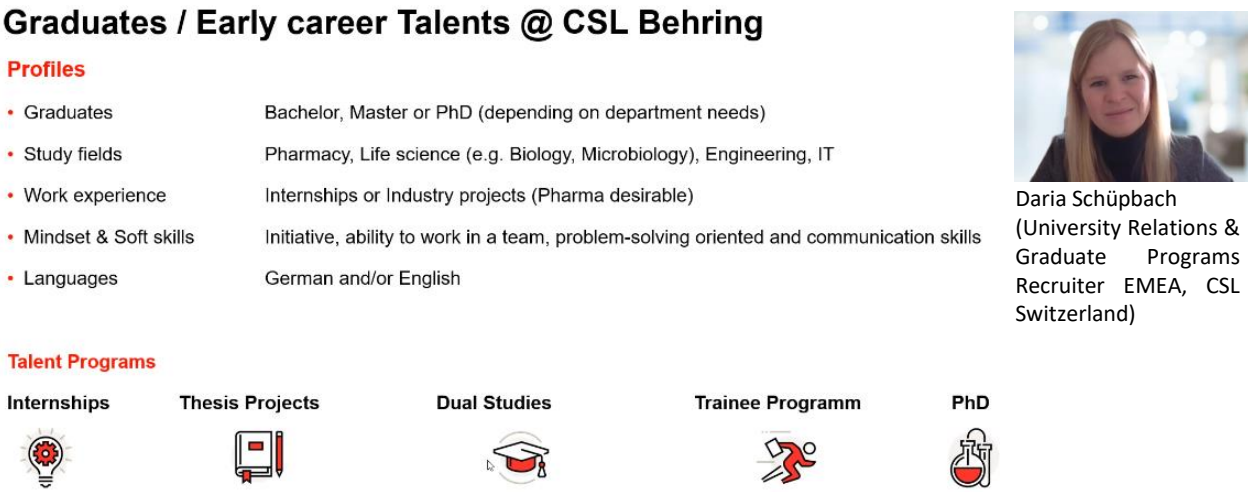

Daria Schüpbach (University Relations & Graduate Programs Recruiter EMEA, CSL Switzerland)

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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