Session 14: Signalling in bacteria

Lectures

L14.1

Oncogenic signal transduction by the gastric pathogen *Helicobacter pylori*

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Many Gram-negative pathogens harbor type IV secretion systems (T4SSs) that translocate bacterial virulence factors into host cells to hijack cellular processes. The pathology of the gastric pathogen *Helicobacter pylori* strongly depends on a T4SS. This T4SS forms a needle-like pilus, and its assembly is accomplished by multiple protein-protein interactions and various pilus-associated factors that bind to host cell integrins followed by delivery of the CagA oncoprotein into gastric epithelial cells. Injected CagA becomes tyrosine-phosphorylated on EPIYA sequence motifs by Src and Abl family kinases. CagA then binds to and activates/inactivates multiple signaling proteins in a phosphorylation-dependent and phosphorylation-independent manner. A proteomic screen systematically identified eukaryotic binding partners of the EPIYA phosphorylation sites of CagA and similar sites in other bacterial effectors by high-resolution mass spectrometry. Individual phosphorylation sites recruited a surprisingly high number of interaction partners suggesting that each phosphorylation site can interfere with many downstream pathways. We now count more than 25 cellular binding partners of CagA, which represents the highest quantitiy among all yet known virulence-associated effector proteins in the microbial world. This complexity generates a highly remarkable scenario, and the involved signal tranduction pathways are currently analysed. It appears that injected CagA can act as a 'masterkey' which evolved the ability to manipulate multiple host cell signalling cascades, which include the induction of membrane dynamics, actin-cytoskeletal rearrangements and the disruption of cell-to-cell junctions as well as proliferative, pro-inflammatory and anti-apoptotic nuclear responses. However, it is not yet clear how *H. pylori* can achieve contact with its basolateral integrin receptors in polarised epithelial cells. These polarized cell monolayers are commonly sealed by tight junctions, E-cadherin-based adherens junctions and other cell-to-cell contacts. We have demonstrated that *H. pylori* secrete an active serine protease into the extracellular space, called HtrA, which can cleave E-cadherin and probably other junctional proteins. Remarkably, E-cadherin shedding results in the local disruption of adherens junctions in polarized gastric epithelial cells allowing *H. pylori* entry into the intercellular space, which is in agreement with the detection of bacteria between neighboring epithelial cells and in underlying tissues of stomach biopsies obtained from gastric cancer patients. This indicates that HtrA-mediated E-cadherin cleavage may represent an entire novel mechanism how the pathogen can promote its own paracellular transmigration. We determined the cleavage sites of HtrA in E-cadherin by Edman sequencing. Our results suggest that HtrA protease specificity is tailored to recognize an extracellular E-cadherin domain signature sequence, which reflects a most remarkable adaptation of a pathogenic enzyme to a corresponding host substrate. We propose a stepwise model for how *H. pylori* interacts with a variety of host cell receptors, components of intercellular tight and adherens junctions and integrin-based focal adhesions to disrupt the epithelial cell layer, in order to reach the basolateral integrin receptors and trigger disease-associated processes.

L14.2

Tetrameric c-di-GMP mediates effective transcription factor dimerization to control *Streptomyces* development

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The cyclic dinucleotide c-di-GMP is a signaling molecule with diverse functions in cellular physiology. We report that c-di-GMP can assemble into a tetramer that mediates the effective dimerization of a transcription factor, BldD, which controls the progression of multicellular differentiation in sporulating actinomycete bacteria. BldD represses expression of sporulation genes during vegetative growth in a manner that depends on c-di-GMP-mediated dimerization. Structural and biochemical analyses show that tetrameric c-di-GMP links two subunits of BldD through their C-terminal domains, which are otherwise separated by ~10 Å and thus cannot effect dimerization directly. Binding of the c-di-GMP tetramer by BldD is selective and requires a bipartite RXD-X_8-RXXD signature. The findings indicate a unique mechanism of protein dimerization and the ability of nucleotide signaling molecules to assume alternative oligomeric states to effect different functions.

L14.3

Cell cycle and environmental control of chromosome hypomethylation

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Heritable DNA methylation imprints are ubiquitous and underlie genetic variability from bacteria to humans. In microbial genomes, DNA methylation has been implicated in gene transcription, DNA replication and repair, nucleoid segregation, transposition and virulence of pathogenic strains. Despite the importance of local (hypo)methylation at specific loci, it is unclear how and when these patterns are established during the cell cycle. Taking advantage of bacterial genomes that are small enough for full methylome analysis, we investigate local hypomethylation patterns in synchronizable α-proteobacteria. We used genome-wide methyl-N6-adenine (m6A-) analyses by restriction-enzyme cleavage sequencing (REC-Seq) and single-molecule real-time (SMRT) sequencing to unearth a conserved m6A-based hypomethylation system operating during the (α-proteo)bacterial cell cycle: the transcriptional regulator MucR, that controls expression of virulence and cell cycle genes, occludes CcrM methylation sites in S-phase, but not in G1-phase. Constitutive expression of CcrM or heterologous methylases during the (α-proteo)bacterial cell cycle homogenizes m6A patterns even when MucR is present and controls transcription. Environmental stress (phosphate limitation) can override and reconfigure local hypomethylation patterns superimposed on the systemic constraints by the cell cycle circuitry that dictate when local hypomethylation is instated at potentially (rare) hypomethylated sites, as revealed by REC-Seq.

Posters

P14.1

Butyrolactone binding proteins in the cascade regulation of coelimycin synthesis

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Gamma-butyrolactones (GBLs) are diffusible bacterial hormones that switch on antibiotic production and morphological differentiation in *Streptomyces*. These signalling molecules are recognized by cytoplasm-localized receptors belonging to the TetR protein family. In *Streptomyces coelicolor A3(2)* GBL receptor ScbR, represses the transcription of genes coding for *Streptomyces* antibiotic regulatory proteins (SARPs) CpkO and CpkN from coelimycin synthesis cluster *cpk*. During the transition growth phase, increased gamma-butyrolactone SCB1 concentration results in its binding by ScbR and subsequent derepression of SARP-coding genes. Recent studies have provided evidence that endogenous antibiotics can also be utilized by *Streptomyces* as signalling molecules. ScbR2, so-called pseudo gamma-butyrolactone receptor, bearing 32% sequence identity to ScbR, lacks the ability to bind SCB1 but binds antibiotics actinorhodin and undecylprodigiosin instead. ScbR2 binds to the same promoters as its homologue ScbR1, however the sequences recognized by the proteins are not always overlapping. Although genes coding for ScbR and ScbR2 are localized in the coelimycin synthesis *cpk* gene cluster, their products take part in other complicated regulatory cascades by interacting with a range of metabolic and physiology regulatory proteins. As pleiotropic regulators, ScbR and ScbR2 modulate glycolysis and oxidative stress response besides antibiotic production. It has been proposed that the interplay between ScbR and ScbR2 may serve as a switch turning on and off coelimycin synthesis by enhancing or interfering with *cpkO* transcription. Here, we describe the action of those two regulatory proteins on transcription of another SARP protein - CpkN. To gain an insight into their activity, we conducted EMSA tests that led to subsequent identification of the exact ScbR and ScbR2 binding sites on *cpkN* promoter by DNAse I Footprinting. One of these sequences is common to both ScbR and ScbR2. By assessing the binding constants of ScbR and ScbR2 to this region by fluorescence polarization assay, we concluded that modulation of coelimycin synthesis through CpkN pathway is achieved not only through different ScbR and ScbR2 transcription maxima timepoints but also through different binding affinities of these regulators to *cpkN* promoter.

P14.2

Comparative structural studies of OspC from *Borrelia burgdorferi sensu lato*

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Lyme disease, a tick-borne multisystem disease of high incidence, is caused by *Borrelia* spirochetes. The bacteria are transferred to mammals by *Ixodes* ticks. Several structural studies have confirmed that on the surface of the spirochete more than 100 different outer surface proteins are present, playing important roles at different stages of the infection. Some of those proteins allow *Borrelia* to avoid the host immune system in a very efficient way.
One of them is the Outer surface protein C (OspC). There are 19 types of *Borrelia* producing OspC, but not all are infectious to humans. The expression of the OspC protein is activated after the bacteria have colonized the tick, and is induced by the specific environmental conditions inside the tick body. OspC shows a high polymorphism, even in bacteria within the same geographic area; it is probably associated with variable regions present in this protein.
OspC has a high affinity for the salivary gland protein 15 (Salp15) present in the salivary glands of the tick. This affinity allows the coating of the OspC by Salp15 to prevent recognition of OspC by the host immune system. Understanding the structure and function of the OspC protein and the possibility of its modifications will contribute to better Lyme disease diagnostics, prophylactics and therapy. The present study is focused on OspC from four common types of spirochetes: *Borrelia burgorferi sensu lato*, *Borrelia garinii*, *Borrelia afzelii* and *Borrelia spielmanii*. Their amino-acid sequences are compared and searched for variable and conserved regions. The proteins are currently produced in *E. coli* for crystallization experiments. Ultimately, our comparative structural studies will elucidate the OspC function.

P14.3

Searching for new genes influencing chromosome supercoiling in *Streptomyces coelicolor*

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Bacterial chromosome must be highly compacted to fit within the small volume of the cell, but remain accessible for the dynamic processes of transcription, replication and segregation. The compaction is introduced by DNA supercoiling and DNA binding proteins such as condensins and nucleoid-associated proteins (NAPs). DNA supercoling is mostly modulated by the opposing activities of enzymes: topoisomerase I and gyrase. The chromosome topology is an important environmental sensor that coordinates adaptation of the cell to stressful conditions by controlling gene transcription.

Streptomyces are soil bacteria appreciated as the producers of broad range of secondary metabolites (i.e. antibiotics, immunosuppressants and cytostatics). They grow forming branched vegetative hyphae and undergo morphological differentiations with production of spores. The supercoiling of *Streptomyces* chromosome influences production of metabolites as well as sporulation. It was shown that *Streptomyces* possess only one topoisomerase I (TopA) and *topA* gene is essential to survival of this bacteria. Depletion of TopA leads to slower growth and inhibition of sporulation. In our study, we are looking for TopA depletion supressor mutations. Earlier, we have observed that deletion of the gene encoding segregation protein ParB partially suppressed TopA depletion [1]. We have suggested that ParB, by formation of large nucleoprotein complex, induces topological tension and separation of ParB complexes requires high level of TopA. Additionally we have shown that ParB increases TopA activity. We expected that other DNA organising proteins may also collaborate with TopA in the maintaining the chromosome topology. With use of transposon mutagenesis we search for the mutants which restore wild type phenotype in the strain with lower level of TopA, as well as mutations which are synthetically lethal in conditions of excessive supercoiling or excessive relaxation of chromosomal DNA.

Reference:

1. Szafran M *et al* (2013) *J Bacteriol* **195**: 4445.

P14.4

Mycobacterium smegmatis nucleoid dynamics during the cell cycle

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Our view on bacterial nucleoid organization has been considerably changed due to the application of modern fluorescent microscopic methods, which allow real time observations of protein complexes and particular chromosomal regions in a single cell. So far, little is known about chromosome organization of slow-growing pathogens including *Mycobacterium* genus. Here, we present the real time analysis of nucleoid dynamics during the cell cycle of *Mycobacterium smegmatis*.

Visualization of the *M. smegmatis* chromosome was performed by tagging with EGFP one of the nucleoid associated proteins – HupB (HU homolog from *E. coli*, which binds DNA unspecifically). Microscopic analysis revealed that cells of the HupB-EGFP strain contain multiple foci distributed along the entire nucleoid a similarly to the foci pattern obtained by DAPI staining. Therefore, we postulate that the strain expressing HupB-EGFP could be used for *in vivo* analysis of nucleoid dynamics. For the real time analysis, we cultured bacteria on agar plates (IBIDI) and examine using inverted fluorescence microscope. Our results indicate that HupB-EGFP foci preserved similar condensation level at all growth stages and nucleoid is localized asymmetrically inside the cell – it remains closer to the new cell pole. In further studies we investigate real time replisome localization on the *M. smegmatis* chromosome (using β-subunit of DNA polymerase III tagged with mCherry for replisome visualization). Additionally, we examined the influence of HupB on replication dynamics and we found that HupB is involved in the regulation of replication initiation in *M. smegmatis*.

P14.5

The study of interaction between toxin-antitoxin system components from *Staphylococcus pseudintermedius* and *Staphylococcus aureus*

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Toxin-antitoxin systems (TA) are two-component entities widespread among bacterial species. TA are identified in the pool of plasmid and chromosomal DNA. Plasmid-located entities are mainly implicated in maintaining this pool of genetic material. The role of chromosomal TA systems are still under debate. A number of reports suggest their possible role in the stress response and virulence of bacteria. The presented study focuses on a functional analysis of chromosomally encoded TA system $PemIK_{Sp}$ of *Staphylococcus pseudintermedius* and interactions between its components with those from $PemIK_{Sa}$, the plasmid-located TA of *Staphylococcus aureus* CH91, for which the role in the regulation of gene expression and virulence was postulated [1].
In order to identify the *pemIK* system, putative TA *loci* in twenty two *S. pseudintermedius* strains were PCR amplified, sequenced and analyzed. Selected genes were cloned into expression vectors providing different antibiotic resistance and induction system. Recombinant proteins were produced in *Escherichia coli* BL21(DE3). The toxins were tested for their ribonucleolytic activity towards phage MS2 RNA. Co-expression analysis and co-purification were performed to examine the interactions between toxins and antitoxins of $PemIK_{Sp}$ and $PemIK_{Sa}$ systems.
The presence of $pemIK_{Sp}$ operon was confirmed for all tested *S. pseudintermedius* strains. Two and five variants of respectively $pemI_{Sp}$ antitoxins and $pemK_{Sp}$ toxins were identified. Ribonucleolytic activity was confirmed for the toxins, however surprisingly did not result in growth inhibition as in the case of homologous toxin from *S. aureus* CH91. *In vitro* and *in vivo* interaction with *S. aureus* CH91 toxin was demonstrated for all antitoxins, save the shortest one.
Among chromosome of *S. pseudintermedius* strains we indentified $pemIK_{Sp}$ operon, homologous to the plasmid-located $pemIK_{Sa}$ operon of *S. aureus* CH91. The functional analysis revealed that significant truncation of N-terminal part of antitoxins may cause the loss of inhibitory activity against the toxins, as shown for the shortest variant. These outcomes suggest that transmission from plasmid to bacterial chromosome may lead to TA operon sequence degradation and the loss of system's activity. However, additional studies are necessary to confirm this hypothesis and to investigate the possible physiological results of the PemIK plasmid-chromosome translocation.

References:

1. M. Bukowski *et al* (2013) *Nat Commun* **4**: no 2012.

P14.6

Unique and universal features of Epsilonproteobacteria origin of chromosome region

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Origins of chromosome replication (*oriCs*) are defined regions of bacterial chromosomes at which DNA replication starts. The general architecture of *oriC*s is universal[1,2]. However, detailed structures of the *oriC* regions and modes of orisome assembly are shared by related species while are significantly different in unrelated bacteria[3]. In this work, we characterise Epsilonproteobacterial origins of chromosome replication. We identified the putative *oriC* regions in three species of the class: *Arcobacter butzleri, Sulfurimonas denitrificans* and *Wolinella succinogenes*. We propose that *oriC* regions typically co-localize with *ruvC-dnaA-dnaN* in Epsilonproteobacteria, with the exception of Helicobacteriaceae species. The clusters of DnaA boxes localize upstream (*oriC1*) and downstream (*oriC2*) of *dnaA,* and they likely constitute bipartite origins as in *H. pylori*. In all three cases, DNA unwinding was shown to occur in *oriC2*. Unlike DnaA box pattern, which is not conserved in Epsilonproteobacterial *oriC*s, the consensus DnaA box sequences and the mode of DnaA-DnaA box interactions are common to the class. We propose that the typical Epsilonproteobacteria DnaA box consists of the core nucleotide sequence 5'-TTCAC (4-8 nt), which, together with the significant changes in amino acid residues in DNA-binding motif of their corresponding DnaAs (DnaA signature), determine the unique molecular mechanism of DnaA-DNA interaction.

References:

1. Kaguni JM (2011) *Current Opinion in Chemical Biology* **15**: 606-613.
2. Briggs GS *et al* (2012) *Journal of Bacteriology* **194**; 19: 5162-5170.
3. Wolański M *et al* (2014) *Front Microbiol* **5**: 735.

P14.7

Coordination of chromosome topology with cell division and differentiation of *Streptomyces venezuelae*

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The size of a bacterial cell requires significant compaction of the chromosomal DNA. Bacterial DNA is organized by DNA binding proteins: a high-molecular-weight SMC (in *E. coli* MukB functional homolog of SMC) and number of small nucleoid associated proteins (NAPs). SMC proteins affect the global chromosome topology at long range by interactions with different regions of the chromosome. NAPs are small, abundant proteins that dimerise or oligomerise and bind DNA with a low sequence specificity, inducing bending, bridging or wrapping (Wang *et al.*, 2013). Streptomycetes, Gram-positive soil bacteria, are known for their ability to produce many valuable antibiotics and other secondary metabolites. They are filamentous bacteria, growing by tip extension and hyphal branching to form a dense mycelial network of vegetative hyphae. Compartments of the vegetative hyphae contain several copies of the large, linear uncondensed chromosome. Upon nutrient depletion Streptomyces colonies differentiate to form multigenomic aerial hyphae, subsequently developing into chains of unigenomic spores. During sporulation chromosomes undergo complex topological changes. Moreover, conversion of the aerial hyphae into chains of unigenomic spores requires the segregation of multiple chromosomes and the synchronous placement of regularly spaced Z-rings (Flärdh & Buttner, 2009).
Although the *Streptomyces* life cycle have been extensively investigated not much is known about chromosome condensation and topology. There is a great variation in NAPs in different bacteria, but some NAP proteins are Actinomycetales-specific – HupB, HupS (homolog of the HU protein) and Lsr2 (H-NS like protein). We aim to answer the following question: how the nucleoid-associated proteins and SMC shape the chromosome architecture and how the changes in DNA topology may affect morphological differentiation of *Streptomyces*. To answer this question we constructed *Streptomyces venezuelae*strains with the mutations in genes encoding *smc, lsr2, hupS* and we analyzed them using time-lapse microscopy, which allows the monitoring of growth and division of single sporogenic hyphae.Additionally, we also observed the timing of sporulation septation in relation to the extension of the aerial hyphae. As a marker of Z-rings we used strains expressing FtsZ-ypet. Our studies show that chromosome condensation affects the position and time of FtsZ rings placement.

References:

Flärdh K, Buttner MJ (2009) *Nat Rev Microbiol* **7**: 36-49.
Wang X *et al* (2013) *Nat Rev Genet* **14:** 191-203.

P14.8

Target genes of an ion dependent GntR-like regulator of *Streptomyces coelicolor* A3(2) include putative zinc metalloprotease and dihydropicolinate synthase genes

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Secondary metabolism and differentiation of bacteria from the genus *Streptomyces* are controlled by a complex regulatory network. GntR-like proteins form a broad superfamily of regulators which have similar N-terminal winged helix-turn-helix DNA binding domains and various C-terminal effector binding – oligomerization domains. They respond to environmental factors such as nutrient availability. They include a number of pleiotropic regulators, which may act both as repressors and activators. Their activity is modulated by binding ligands. Here we describe a putative sensor protein from a model organism *Streptomyces coelicolor* A3(2) which belongs to FadR family of GntR-like regulators. The DNA sequence recognized by the protein in its native promoter region was identified by DNA footprinting. Promoter regions of a putative zinc metalloprotease and dihydropicolinate synthase with a very similar sequence motif are also recognized by the regulator in the electrophoretic mobility shift assay. We found that DNA binding of the recombinant protein is prevented by the addition of divalent metal ions. Results of the tryptophan fluorescence assay show the strongest effect of zinc and suggest, that it is the metal recognized by the sensor.

P14.9

The role of the N-terminus DnaA in assembly and regulation of *H. pylori* replication initiation complex

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DNA replication is one of the most important and complicated stage in bacterial lifetime. It has to be synchronized with a cell cycle. That is why there are many proteins involved in regulation of the process of DNA replication. DnaA is a bacterial protein which primary role is to initiate chromosome replication. DnaA binds DNA at origin of chromosome replication (*oriC*), assemblies into a helical filament that is capable of opening double stranded DNA and recruiting other proteins to a replisome, i.e. into a complex, which replicates the chromosome. The DnaA protein also controls frequency of chromosome replication at the initiation step and additionally, in some species, regulates expression of certain genes. To be able to act as a multipurpose factor, DnaA is composed of four domains, which play distinct but mutually dependent roles. The C-terminal domain IV interacts with DNA while domain III drives the proper oligomerization of the protein complex in an ATP dependent manner. Domain I also mediates interactions between DnaA molecules, but in addition, it binds other proteins that affect orisome formation. Domain II constitutes a flexible linker between domains III-IV and domain I. Out of these four domains the role of N-terminus (domains I-II) in assembly of the initiation complex is the least understood. The role of domain I of *H. pylori* DnaA in assembly and activity of the *H. pylori* replication initiation complex is still not fully revealed. It was previously shown that domain I interacts with HobA. This interaction enhances and accelerates DnaA binding to *oriC1*. Here we present that N-terminus of DnaA also mediates interactions between DnaA molecules. This interaction influences the activity of the DnaA oligomer: it affects DnaA oligomerisation upon DNA binding and changes DnaA unwinding activity. It also influences the structure of the initiation complex assembled on bipartite *H. pylori oriC*. We propose that N-terminus of DnaA similarly as in other bacteria, plays a regulatory role in initiation complex assembly by controlling DnaA oligomerization ability.

P14.10

The ParA-Wag31 interaction provides the link between chromosome segregation and other cell cycle processes in Mycobacteria

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Bacterial chromosome segregation depends on the ParA and ParB proteins. ParB binds DNA and forms large nucleoprotein complexes which organise newly replicated chromosomes. The ParB complexes are positioned in the specific locations of the cell due to interactions with dynamic ATPase, ParA.

The elimination of ParA inhibits growth of *Mycobacterium smegmatis* and disturbs chromosome segregation [1]. We have shown that mycobacterial ParA interacts with polar growth determinant Wag31, the homologue of DivIVA protein [1]. This interaction is unique to *Mycobacteria*, but its biological significance is unclear.

Here, to further explore the interaction between ParA and Wag31 in *M. smegmatis*, we have identified the point mutation in ParA protein that inhibits the interaction with Wag31. Although the identified mutation in *parA* did not visible disturb growth and chromosome segregation of *M. smegmatis* strain the careful analysis indicated that it affects ParA localisation and coordination of chromosome segregation with other cell cycle processes.

We suggest ParA-Wag31 interaction allows the coordination of the cell cycle in *Mycobacteria* and may be crucial during persistence.

Reference:

Ginda K *et al* (2013) ParA of Mycobacterium smegmatis co-ordinates chromosome segregation with the cell cycle and interacts with the polar growth determinant DivIVA. *Molecular Microbiology* **87**: 998–1012.

P14.11

Role of topoisomerase I in *Streptomyces coelicolor* chromosome organization during vegetative growth

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Topological stress is caused by supercoils formation during such processes as transcription, replication or recombination. Optimal level of DNA supercoiling in cell is maintained by topoisomerases, which can be divided in two distinct classes. Enzymes, of class one are able to remove one supercoil per reaction and don't require ATP while type II topoisomerases can introduce or remove more supercoils, but require ATP hydrolysis.

Streptomyces are filamentous, soil bacteria known as producers of wide range of secondary metabolites. Main characteristic of *Streptomyces* is their complex life cycle involving two distinct phases: vegetative, during which branched hyphae compartments can contain several uncondensed chromosomes and aerial, when chromosome condensation and segregation occurs. Interestingly, *S. coelicolor* chromosome contains only one gene encoding type I topoisomerase – *topA*. Its deletion is lethal while TopA depletion slows growth, inhibits sporulation and affects secondary metabolism.

Main goal of our work was to examine how changes of TopA level affect chromosome organization during spore germination and vegetative growth. To achieve this, we have constructed a number of strains with decreased or elevated level of TopA and fluorescently labelled segrosomes or *oriCs*. Fluorescent time-lapse microscopy was used to observe the organization of chromosomes in germinating spores and vegetative hyphae. By using ImageJ and R program we were able to locate and track the movement of chromosomes present in *S. coelicolor* hyphae.

Our results show that TopA depletion affects *S. coelicolor* spore germination and increases the mean distance between chromosomes and the tip of the hypha. At the same time it decreases the distance between chromosomes. Thus, our data indicate that lower level of TopA, which leads to increased negative DNA supercoiling and compaction, affects chromosome organization within vegetative hyphae. Optimal level of TopA is also necessary for proper spore germination and growth.

P14.12

The response of *topA* promoter to the changes in DNA supercoiling and heat shock in *Streptomyces coelicolor*

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Secondary metabolites (i.e. antibiotics, immunosuppressants and cytostatics) grow forming branched vegetative hyphae. Environmental stress, particularly nutrient depletion triggers morphological differentiations and production of spores. An important environmental sensor that coordinates the adaptation of the cell to stressful conditions by controlling gene transcription is the chromosome topology. The level of DNA supercoiling is affected by a number of factors such as temperature, osmolarity or availability of nutrients. The chromosome supercoiling influences production of metabolites as well as sporulation. The topology of the DNA is mostly modulated by the opposing activities of enzymes: topoisomerase I (TopA) and gyrase. The regulation of TopA and gyrase activity can be achieved in several ways, including availability of ATP, interaction with other proteins and the direct regulation of transcription of *topA* and *gyrAB* genes. Although it was shown before that the activity of topoisomerase I influence gene expression, regulation of TopA level itself is poorly understood in *Streptomyces*.

In this study we examined activity of *topA* promoter from S. *coelicolor*, the model organism to study genus *Streptomyces*. The existence of *topA*-dedicated promoter was confirmed. It was shown that *topA* gene is essential to survival of this bacteria. With use of nuclease S1 protection assay *topA* transcription start sites were determined. It was also shown that activity of TopA regulates transcription of its own gene. The response of *topA* promoter activity to changes in DNA supercoiling was investigated. Finally, it was shown that *topA* promoter is important during cell response to heat shock by maintaining proper level of TopA and thus controlling DNA supercoiling.

Our data suggest that regulation of TopA amount in bacterial cell is crucial to survive in stress conditions and *topA* promoter is the main factor that participate in maintaining proper DNA supercoiling, which in turn regulates expression of other genes, making cell responding to environmental changes. The involvement of chromosome topology in stress response in *Streptomyces* can be critical for industrial purposes.

P14.13

Exploitation of microfluidic fluorescent microscopy to study single-cell dynamics of the chromosome replication upon treatment with antibiotics that inhibit gyrase

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The application oftime-lapse microfluidic fluorescence microscopy (TLFM) has allowed direct observation of replication dynamics in single bacterial cells in real time. Unlike replisomes (replication machinery) in eukaryotes, those in bacteria are not stationary but dynamic. Moreover, the positioning of replisomes in bacteria is species-specific and dependent on cell cycle features. The aim of our study is to compare influence of antibiotics inhibiting gyrase on dynamics of the chromosome replication in two bacteria: Gram-negative, fast growing *Escherichia coli* and Gram-positive, slow growing *Mycobacterium smegmatis*. Earlier studies revealed that in *E. coli*, replisomes move to the opposite cell halves and return to midcell at the end of replication, whereas in *M. smegmatis* the two highly dynamic replisomes remain in relatively close proximity moving in the same direction from a subpolar region toward the cell center (Trojanowski *et al.*, 2015).Moreover, *Mycobacterium* cells, in contrast to *E. coli* cells, do not exhibit overlapping replication cycles.
We analysed the cell morphology and the dynamics of chromosome replication in *E. coli* and *M. smegmatis* upon treatment with noviobiocin and nalidixic acid, which inhibit gyrase by binding the GyrB and the GyrA subunit, respectively. Our studies allowed characterizing a small fraction of cells that survived lethal conditions. The results confirm that the bacterial population is heterogeneous and consists of cells that exhibit different antibiotics tolerance.

P14.14

AdpA – a key regulator for metabolite production, differentiation and chromosome replication in *Streptomyces*

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Streptomyces are filamentous soil bacteria mainly known because of their complex life cycles and the ability to produce a wide variety of secondary metabolites including antibiotics. *Streptomyces coelicolor*, a model species for *Streptomyces* genus, grows as a multicellular consortium made up of two distinct types of mycelium, the vegetative (substrate) and the aerial (reproductive) hyphae. The later differentiate upwards from the substrate mycelium and converts into chains of spores, in the process of sporulation, to ensure cell propagation and survival. Before onset of differentiation the substrate mycelium produces antibiotics to protect nutrient resources for the growing aerial hyphae. Metabolite production and differentiation processes are globally coordinated and strictly controlled by number of transcriptional factors responding to environmental changes. AdpA protein is one of the most important transcriptional regulators that directly controls genes involved in these two events. Additionally, recent studies has shown that AdpA also negatively regulates chromosome replication at the initiation stage in *S. coelicolor* and *S. griseus*.
To investigate further role of AdpA protein in differentiation and metabolite production we employed a new model species, *S. venezuelae*. The new model organism significantly facilitates the study of cell cycle as it exhibits ability to differentiate in liquid medium and grows much faster than used so far models *S. coelicolor* and *S. griseus*. To study biological role of the AdpA we constructed the *ΔadpA* strain in *S. venezuelae*. The mutant strain exhibit impaired sporulation and grows faster in comparison to the wild-type strain. Interestingly, in contrast to *S. coelicolor* and *S. griseus* the AdpA is not involved in the regulation of chromosome replication in *S. venezuelae*. Additionally, we observed that intracellular level of AdpA in *S. venezuelae* is subjected to a posttranslational regulation that was not reported previously in other *Streptomyces* models. We have also performed transcriptional and reporter gene expression analyses to study role of AdpA in its own gene expression. Here, we compare the role and the mode of regulation of important cellular processes such as differentiation and chromosome replication for AdpA protein in three *Streptomyces* species.

P14.15

Protease MfpA from *Microbacterium* sp. degrades fibrinogen and inhibits biofilm formation by *Staphylococcus aureus*

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Biofilm formation is a complex process modulated by bacteria at a metabolic level as a survival strategy in hostile environment. Biofilms consist of bacterial cells embedded in self-secreted extracellular polymeric substances such as extracellular DNA, proteins and polysaccharides. Biofilm-associated infections are difficult to treat, due to the poor penetration of antibiotics through bacterial layer, therefore the issue is currently the subject of intensively developing research. It is known that proteins present in blood can support bacterial biofilm formation. The effect was observed, for instance, for fibrinogen [1]. On the other hand, extracellular bacterial proteases can prevent of biofilm formation and disrupt already established bacterial biofilms [2]. Recently we have identified an extracellular protease, produced by *Microbacterium* sp. strain JK6B, that has fibrinolytic properties. The MfpA (Microbacterial fibrinolytic protease) is able to degrade fibrinogen and dissolves fibrin clots *in vitro*.

The aim of the study is to investigate the MfpA protease influence on biofilm formation by *Staphylococcus aureus*, a dangerous opportunistic pathogen of humans and animals. Tests were performed on 96-well polystyrene plate coated with fibrinogen, albumin and human plasma solution. *S. aureus* strain LS-1 cells were cultivated on the plate in the presence of MfpA protease. After removing of bacteria, biofilm was fixed, and stained with crystal violet. Acetic acid-released stain has been quantified spectrophotometrically at 570 nm.

The inhibitory effect of MfpA protease on biofilm formation by *S. aureus* was shown. An increase in MfpA concentration resulted in greater biofilm inhibition, whereas thermal denaturation abolished the anti-biofilm activity of the protease. Substantial reduction of fibrinogen-driven biofilm formation occurred in presence of MfpA at 1 μg/ml, whereas at least ten times higher concentrations were needed to inhibit biofilm formation at uncoated as well as plasma and albumin coated supports. This indicates that although MfpA preferentially digests fibrinogen, as a binding support, it also degrades staphylococcal proteins involved in biofilm formation.

References:

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