Friday, March 23, Session IV

L16

ASYMMETRIC CELL DIVISION DURING SPORULATION IN *BACILLUS SUBTILIS*

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Bacillus subtilis is a Gram-positive microorganism which is able to differentiate during process called sporulation. A hallmark of sporulation in *B. subtilis* is the polar cell division. As occurs during vegetative cell division, the tubulin-like FtsZ forms a ring-like structure at mid-cell. At the onset of sporulation, however, the Z-ring migrates from mid-cell on a spiral trajectory to the two cell poles in a process that depends on the presence of SpoIIE. This protein colocalizes with the polar Z-rings. Asymmetric cell division otherwise appears to involve the same set of proteins as constitute the divisome during vegetative cell division. However, the resulting sporulation septum is much thinner. Accompanying these morphological changes is a coordinated programme of differential gene expression, involving intercellular signalling processes, that leads to the activation of the RNA polymerase sigma factors, ^{F} and ^{G} in the forespore and ^{E} and ^{K} in the mother cell [1].

Although, SpoIIE has a critical function in determining the site of formation of the sporulation septum, it is not understood (i) how it localises to the polar septum (ii) how it causes FtsZ to relocalise from mid-cell to the polar site (iii) what role SpoIIE plays in septal thinning, (iv) how its SpoIIAA~P phosphatase activity is controlled so that ^{F} acitivation is delayed until the septum is completed (v) what role SpoIIE playes in SpoIIQ-SpoIIIAH channel formation.

SpoIIE from *B. subtilis* is an 827 residue protein that consists of three regions. It has 10 putative membrane-spanning segments (region I) at its amino terminus and a PP2C-type phosphatase domain (region III) at its C-terminus. The central region II is required for localisation of SpoIIE to the divisome and its reported interaction with FtsZ. The structure of the PP2C phosphatase domain of SpoIIE as well as a part of central domain together with phosphatase domain were already solved [2,3]. In contrast, the structure of N-terminal more than one thirds of SpoIIE and the character of its interactions with partner proteins are unknown. We recently identified a new partner of SpoIIE, the cytoskeletal protein, RodZ, which is essential for cell shape determination. This interaction is additionally required for asymmetric septum formation and sporulation.

We evaluated the positioning of the asymmetric septum and its accuracy by statistical analysis of the site of septation. We also clarified the role of SpoIIE, RefZ and MinCD on the accuracy of this process. In addition, we have employed a new method of "slimfield" microscopy to study the copy number, the oligomeric state and diffusion coefficient of SpoIIE in live cells and during its different roles in asymmetric septum site recognition, activation of ^{F} and forespore engulfment.

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L17

EVOLUTIONARY UPGRADE OF STEFINS FOR SECRETION IN PARASITES

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Fasciolosis caused by the liver fluke *Fasciola hepatica* is a worldwide spread parasitic disease of ruminants and is recognized as an emerging human disease. This work is focused on FhCY2, a member of cystatin superfamily, which is present in several tissues of *F. hepatica* and its excretory/secretory products. Structural and phylogenetic analyses revealed that FhCY2 belongs to stefins (type 1 cystatins). Stefins are typically intracellular, without signal sequence and without disulfides. However, FhCY2 breaks these rules, thus resembling members of extracellular type 2 cystatins. Our work shows that FhCY2 is an evolutionary adaptation to the absence of type 2 cystatins in the *F. hepatica* genome. FhCY2 is a broad-selective inhibitor of host cysteine cathepsins as well as secreted digestive cysteine cathepsins of *F. hepatica*, suggesting a dual role of FhCY2 in the physiological regulation of exogenous and endogenous proteolytic systems.

L18

A GTP-DEPENDENT SWITCH THAT CONTROLS G-QUADRUPLEX MULTIMER FORMATION

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G-quadruplexes are four-stranded nucleic acid structures typically made up of stacked GGGG tetrads connected by short loops [1]. Although most studies investigating potential biological roles of G-quadruplexes have focused on monomeric structures, recent work suggests that multimeric G-quadruplexes could also be important. We recently identified mutations in the central tetrad of a monomeric G-quadruplex that induce formation of higher-order structures [2-4]. Here we show that both DNA-DNA and DNA-RNA G-quadruplexes containing a guanosine to adenosine mutation at a specific position in this tetrad behave like molecular switches in which the equilibrium between monomeric and multimeric G-quadruplex is controlled by GTP concentration. Analysis of the nucleotide specificity of inhibition and characterization of the mechanism of binding by NMR suggest that GTP stabilizes the monomeric form of the G-quadruplex by becoming incorporated into one of the tetrads. Hundreds of sequences with the potential to form such GTP-dependent switches are present in the human genome, including some that are evolutionarily conserved. Our experiments provide new insights into the small molecule-mediated control of G-quadruplex multimerization, and raise the possibility that a GTP-dependent switch controls G-quadruplex multimer formation in cells.

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L19

INSIGHTS INTO THE STRUCTURE AND CONFORMATION OF BIOMOLECULES TO UNDERSTAND THEIR BIOLOGICAL FUNCTION FOR NEW DRUG SYSTEMS USING SAXS IN THE LABORATORY

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The development of new and better drugs, to overcome diseases like Alzheimer's disease, Parkinson or antibiotic resistance, are a main focus of research in the biological, pharmaceutical and medical field. In the last decades the interest in biological macromolecules and complexes providing properties to treat and cure diseases has tremendously increased. To understand their biological function in vitro especially small-angle X-ray scattering (SAXS) gains increasing attention offering complementary information to the traditionally used biological techniques. SAXS enables to study the structure and dynamics of biomolecules in solution where physiological key parameters can be tuned and tested.

SAXS is a versatile technique used for shape and size characterization of nanostructured materials between 1 nm and 200 nm. Biological samples, like proteins, peptides, monoclonal antibodies or viruses are already well known to be investigated with SAXS. Furthermore drug delivery systems like drug loaded vesicles, where size and shape parameters of the vesicle and the drug are found or granulate powders, where the internal surface obtained by SAXS correlates with the tablet hardness, are interesting examples of applications.

In the present contribution we show selected applications of biological macromolecules, using a multifunctional laboratory Small and Wide Angle X-ray Scattering (SWAXS) system, the SAXSpoint 2.0. The SAXSpoint 2.0 system is a compact lab-scale system with dedicated point collimation, which enables SAXS, WAXS and grazing-incident (GISAXS) scattering studies under ambient and non-ambient conditions, in-situ tensile SWAXS experiments, and RheoSAXS studies. It satisfies the advanced user with a wide range of dedicated sample stages, full experimental flexibility to meet the right environment for each sample, and highest resolution. The system provides simple operation, short measurement times and excellent angular resolution, enabled by a smart beam formation concept while maintaining a laboratory-friendly compact size and small footprint.

Different SAXS and WAXS studies on biological macromolecules and pharmaceutically relevant samples were performed on the presented SAXSpoint 2.0 system. Some of the samples required high resolution (very low minimum scattering angle) in order to resolve large structural dimensions. The unique sample-positioning mechanism enabled WAXS measurements to determine crystallinity without re-aligning any part of the SWAXS system. The presented studies clearly show that high-resolution and high-quality SWAXS data can be obtained for biological macromolecules and complexes investigated in their native state with a laboratory SWAXS system.

Saturday, March 23, Session V

L20

STRUCTURE AND DNA DELIVERY MECHANISM OF GENE TRANSFER AGENT OF *RHODOBACTER CAPSULATUS*

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Gene transfer agents (GTAs) are extracellular particles that enable high-frequency horizontal gene transfer among prokaryotes and thus accelerate their evolution. GTAs are derived from phages that were independently acquired by several bacterial and archaeal lineages. In spite of their importance for adaptation and diversification of prokaryotes, the structure and mechanism of DNA delivery of GTA are unknown. Here we used cryo-electron microscopy to show that GTA of *Rhodobacter capsulatus* resembles bacteriophage from the family *Siphoviridae* with several unique features. The DNA-containing head of the GTA is shortened in the direction of GTA tail relative to the regular