Lectures - Saturday, March 20, morning, Session VII

L31

STRUCTURE AND DYNAMICS OF RNA POLYMERASE DELTA SUBUNIT FROM *Bacillus subtilis* DETERMINED BY NMR SPECTROSCOPY

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RNA polymerase is an essential multisubunit enzyme responsible for transcription of genetic information from DNA into RNA. The architecture of the RNA polymerase from *Bacillus subtilis* differs from its analogue from gram-negative bacteria in a presence of two additional subunits – omega1 and delta. Their role in the transcription machinery is still not fully clear. Recent results of our collaborators (L.Krásný et al., Inst. of Microbiology, ASCR, Prague) indicated that the presence of delta subunit increases the transcription specificity and the efficiency of RNA synthesis. Some reports also showed the importance of the delta subunit for the virulence of *Staphylococcus aureus* and *Streptococcus agalactiae*. Therefore, we focused on the delta subunit to reveal its structure and related dynamics. Because the C-terminal domain of the delta subunit is unstructured and its sequence is highly repetitive, we started a systematic investigation of the protein with a shorten construct, corresponding to the well-structured N-terminal part.

The N-terminal domain of the delta protein was prepared using a standard protocol of overexpression in the *E.coli* system to produce a ^{15}N, ^{13}C-uniformly labeled sample. All spectra, including a standard set of triple resonance NMR experiments, 3D TOCSY, and 3D NOESY, were measured on a 600MHz spectrometer. The secondary structure was predicted based on the chemical shifts of backbone nuclei in program TALOS, three-bond scalar couplings, and medium range NOEs. The distance restrains were extracted and assigned from NOESY spectra using program ARIA 2.1. The additional RDC restraints from two aligning media (bacteriophage Pf1, 5% polyacrylamide gel) and anisotropic contributions to the ^{13}C chemical shifts were used in the final refinement, employing the SCULPTOR CNS module. The quality of the calculated structures were checked by programs CING, PROCHECK, and WHATIF. The determined structure allowed us to identify unexpected structure homology with some proteins from the "Forkhead DNA-binding domain" SCOP family.

Fast ns-ps backbone motions of the N-terminal domain of delta subunit was observed using the standard set of ^{15}N relaxation experiments (R1, R2, NOE) performed at two magnetic field (500MHz, 600MHz). The relaxation experiments were acquired at two temperatures (300K, 280K). Slow ms-us motions were investigated by CPMG and T1rho relaxation dispersion experiments.

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THE ROLE OF NRD1-NAB3 COMPLEX IN TRANSCRIPTION TERMINATION

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Biogenesis of functional RNAs, such as small nuclear and small nucleolar RNAs, intergenic and anti-sense RNAs, involves different factors than those used at biogenesis of messenger RNA. Termination of these functional transcripts requires the nuclear pre-mRNA down- regulation (Nrd)1, the nuclear polyadenylated RNA-binding (Nab)3 proteins, and the RNA helicase Sen1. Nrd1 complex and associated factors initiate transcription termination despite of early elongation phase of transcription cycle of RNA polymerase II. To fully understand the structural basis behind this mechanism, we use multidimensional NMR spectroscopy to determine the three- dimensional structures of important components involved in this mechanism and their complexes with the termination elements that are present in the nascent transcript.

L33

STRUCTURAL INSIGHTS INTO RECRUITMENT AND DISSOCIATION OF RNA POLYMEARSE II TERMINATION FACTORS

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RNA Polymerase II recruits many accessory factors via its C-terminal domain (CTD). During the transcription cycle, CTD is dynamically phosphorylated and dephosphorylated that promote binding of required protein factors. Transcription termination occurs either with CTD phosphorylated at Ser2 or Ser5 via poly(A)-dependent or independent termination pathways, respectively. Using NMR and other biophysical methods, we aim at understanding of structural determinants that define binding and dissociation of accessory protein factors to specifically phosphorylated CTD species.

Lectures - Saturday, March 20, morning, Session VIII

L34

STRUCTURAL INSIGHT INTO REGULATION OF THE WNT/B-CATENIN SIGNALLING PATHWAY BY SCLEROSTIN: IMPLICATIONS FOR OSTEOPOROSIS TREATMENT

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The secreted glycoprotein sclerostin has recently emerged as a key negative regulator of Wnt signalling in bone and has stimulated considerable interest as a potential target for therapeutics designed to treat conditions associated with low bone mass, such as osteoporosis. The structure determination of sclerostin resulted in the identification of a previously unknown binding site for heparin, which is suggestive of a functional role in localising sclerostin to the surface of target cells. The conserved N and C-terminal arms of sclerostin were found to be unstructured, highly flexible and unaffected by heparin binding, which suggests a role in stabilising interactions with target proteins. The ability of sclerostin to specifically bind heparin was confirmed by NMR titration experiments. Similarly, mapping of the binding site for an antibody that antagonises the effects of sclerostin on Wnt signalling in vitro, and stimulates bone formation in vivo, has identified a functionally important region of sclerostin, which is clearly involved in the modulation of Wnt signalling (1).

1. V. Veverka et al., J. Biol. Chem., 284, (2009), 10890.

L35

HIGH-AFFINITY LECTINS FROM PATHOGENS: THE POTENTIAL TARGETS FOR ANTIADHESIVE DRUG DESIGN

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Protein-carbohydrate interactions play an important role in many biologically relevant processes including differentiation, signalling, fertilisation as well as cancerogenesis or metastasis development. Glycoproteins on cell surfaces are important for communication between cells, form binding sites for bacteria and viruses and are also involved in recognition process by the immune system. Currently, the recently heavily investigated area of protein-saccharide interactions has a great potential of use in the field of drug design. Understanding of protein-carbohydrate interactions enables the glycomimetics development, e.g. small-molecule drugs that can inhibit interaction between he carbohydrte-binding protein and its receptor.

The contribution will be focused on lectins from human opportunistic pathogens, which display sub-micromolar range affinity towards their carbohydrate ligands. The combination of binding experiments (isothermal titration microcalorimetry, surface plasmon resonance,...) and X-ray crystallography approaches is used to decipher the thermodynamical and structural basis for high affinity binding of these lectins to host carbohydrates. Several examples of different approaches how to proteins from pathogens can reach the high affinities even toward monosaccharides will be discussed. This knowledge is important for the molecular design of potent anti-adhesion therapeutics.

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FROM STRUCTURAL BIOLOGY TO THE DRUGS AGAINST INFLAMMATORY BOWL DISEASES

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To attract the attention of not only audience at lectures and talks, it is quite common practise to emphasize the importance of studied system by its involvement in diseases. It touches hidden conviction that once the key biological processes are characterised and understood, the development of appropriate drug against the disease is straightforward. Unfortunately, this is not true. How complicated and complex is the road from basic ideas to usable medicament will be demonstrated on the development of drug against inflammatory bowl diseases. At the beginning the aim was clear and target processes – signalling of Rac1 GTPase and glucocorticoid receptors – identified. What followed is the subject of presented work.

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2. M. Löwenberg, A.P. Verhaar, G.R. van den Brink, D.W. Hommes, *Trends Mol. Med.* **13**, (2007), 158.