

**Saturday, March 19, Session VIII****L32****STRUCTURE AND DYNAMICS OF SIGMA SUBUNIT OF RNA POLYMERASE FROM BACILLUS SUBTILIS****M. Zachrdla^{1,2}, L. Židek^{1,2}, A. Rabatinová³, H. Šanderová³, L. Krásný³**¹*NCBR, Faculty of Science, Masaryk University, Kamenice 5, 62500 Brno, Czech Republic*²*CEITEC, Masaryk University, Kamenice 5, 62500 Brno, Czech Republic*³*Department of Molecular Genetics of Bacteria, Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague 14220, Czech Republic
324489@mail.muni.cz*

RNA polymerase of gram-positive bacteria contains several unique subunits in comparison to RNA polymerase of gram-negative bacteria. Sigma subunits play a critical role in recognition of DNA promoter sequence. We focused on sigma A factor (SigA) from *Bacillus subtilis*. SigA belongs to group 1 transcriptional factors. SigA is composed of four domains, $\sigma_{1.1}$, σ_2 , σ_3 , and σ_4 that are connected by flexible linkers. SigA is capable of DNA recognition without interaction with other partner, therefore a regulatory mechanism to prevent DNA binding in inappropriate times has to exist. $\sigma_{1.1}$ domain is responsible for auto-regulation of SigA.

Solution state nuclear magnetic resonance was used to solve the structure of $\sigma_{1.1}$. Although $\sigma_{1.1}$ is relatively small in size, 9.3 kDa, the resonance frequency assignment of $\sigma_{1.1}$ is not a trivial task because it contains 23 glutamine or glutamic acid residues. As a result, one third of amino acids have very similar chemical shifts and therefore the standard set of NMR experiments 2D ^1H - ^{15}N HSQC, 3D HNCA, 3D HN(CO)CA, 3D HNCACB, 3D HN(CO)CACB for sequential resonance frequency assignment can lead to ambiguous assignment. This problem was solved using 3D HCCCONH experiments. Side-chain assignment was done using additional 3D HCCH-TOCSY for aromatic and aliphatic spectral regions and 3D HSQC-TOCSY. We obtained backbone assignment for all but two N-terminal res-

idues. Side-chain assignment of 97% nuclei was obtained not including His-tag. All experiments were performed on 700 MHz and 850 MHz spectrometers.

Set of ^{15}N -edited 3D HSQC-NOESY experiment and ^{13}C -edited 3D HSQC-NOESY experiments for aromatic and aliphatic spectral regions was acquired to obtain proton-proton distances from experiments based on nuclear Overhauser effect (NOE). The NOESY cross-peak assignment was done using program CANDID. 3-bond scalar couplings, residual dipolar couplings, and NOE restraints were utilized by program CNS to calculate the structure. There is structure of one homologue protein from *Thermotoga maritima* available in the PDB database. However, even though the secondary structure prediction reflects a very similar pattern, our structure of $\sigma_{1.1}$ exhibits significant differences in comparison.

In order to describe the internal motions of $\sigma_{1.1}$, we analysed auto-relaxation rates R_1 and R_2 , longitudinal and transverse cross-correlated relaxation rates, and steady-state Nuclear Overhauser enhancement. Data was obtained on 600MHz, 850MHz, and 950MHz spectrometers. Obtained relaxation rates were used for spectral density mapping and model-free analysis.

This work was supported by Czech Science Foundation, grant number GA 13-16842S.

RECOGNITION OF PTYR1-CTD BY THE ELONGATION FACTOR Spt6

Pavel Brazda¹, Karel Kubicek¹, Patrick Cramer² and Richard Stefl¹

¹CEITEC – Central European Institute of Technology and Faculty of Science, Masaryk University, CZ-62500 Brno, Czech Republic

²Gene Center and Department of Biochemistry, Ludwig-Maximilians-Universität München, Feodor-Lynen-Strasse 25, D-81377 Munich, Germany

The C-terminal domain (CTD) of RNA polymerase II (RNAPII) consists of heptad repeats with the consensus motif Y1-S2-P3-T4-S5-P6-S7. Dynamic post-transcriptional modifications of the CTD led to the formulation of the so-called “CTD code” more than a decade ago (1). It encodes recruitment of distinct processing factors for each specific position of RNA polymerase II (RNAPII) within the transcriptional cycle (2). However, how the CTD of RNAPII recruits, (de)activates, and displaces relevant processing factors, remains still poorly understood. Recent genome-wide mapping of CTD phosphorylation patterns revealed that Y1 and S2 phosphorylation levels are increased simultaneously during early elongation. Increased Y1 phosphorylation releases factors associated with RNAPII at the beginning of genes, and blocks recruitment of termination factors. In addition, phosphorylated Y1 mark stimulates binding to the tandem

SH2 (tSH2) domain of elongation factor Spt6, consistent with Spt6 occupancy within Tyr1-phosphorylated region of genes in vivo (3). We will show our structural data, acquired via a combination of structural methods, supported by biochemical assays on the study of recognition Y1-phosphorylated CTD by the tSH2 domain of the elongation factor Spt6.

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