We demonstrate that transcription termination of ncRNAs is physically coupled to RNA degradation/processing performed by the nuclear exosome. The coupling is achieved by the CID of Nrd1p that recognizes a mimic of CTD (Nrd1p-interactin motif, NIM) within the TRAMP (Trf4p-Air2p-Mtr4p) complex, a cofactor of the nuclear exosome. We report the structure of Nrd1p CID bound to the Trf4p NIM. Binding assays show that Nrd1p binds Trf4p NIM with a K_D of ~ 1 M, whereas the binding affinity for CTD has a K_D of ~ 100 M. Mutational analyses of the binding surface of both Nrd1p CID and Trf4p NIM confirm our structural findings. What is more, competition experiments show that Nrd1p CID binds NIM and CTD in a mutually exclusive manner.

Since the interaction between CID, of either Nrd1p or Rtt103p, and CTD of RNAPII is crucial for the proper transcription termination, we have hypothesized that transcription termination of mRNAs may also be coupled to RNA processing/degradation performed by the nuclear exosome/TRAMP. Pull-down experiments show that Rtt103p CID also recognizes the NIM, and another region of Trf4p specific only to Rtt103p, the RIM (Rtt103-interacting motif). Specifically, we report the structure of Rtt103p CID bound to Trf4p NIM, and initial structural studies of the complex between Rtt103p CID and Trf4p RIM. We show that Rtt103p CID utilizes the same pocket to bind both CTD and the NIM. Overall, our results unveil a novel role of CID, of Nrd1p and Rtt103p, in not only transcription termination but also in RNA processing/degradation by the nuclear exosome.

Saturday, March 21, Session IX

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FUNCTIONAL ANALYSIS OF A NOVEL HALOALKANE DEHALOGENASE DsvA ISOLATED FROM THERMOPHILIC BACTERIUM *SACCHAROMONOSPORA VIRIDIS* DSM 43017

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Microorganisms able to grow in extreme conditions, including high concentration of salts, alkaline pH, low or high temperature and organic solvent medium have been an important source of robust enzymes for various practical applications [1]. Haloalkane dehalogenases (HLDs; EC 3.8.1.5) are predominantly bacterial enzymes that catalyze hydrolytic cleavage of a carbon-halogen bond in a broad range of halogenated aliphatic compounds, producing a corresponding alcohol, a halide anion and a proton [2]. These enzymes can be use in bioremediation, decontamination of warfare agents, synthesis of optically pure compounds, biosensing and cell imaging [3].

A novel member of HLD family, DsvA from *Saccharomonospora viridis* DSM 43017 isolated from peatbogs of Ireland, has been subjected to detailed biochemical characterization in this study. The enzyme was successfully expressed in *Escherichia coli* BL21(DE3) cells and purified to homogeneity by metalloaffinity chromatography. Proper folding and thermostability was assessed by circular dichroism spectroscopy. Although DsvA exhibited comparable melting temperature (Tm = 58 °C) with other HLDs isolated from mesophilic organisms, its kinetic stability determined at 45 and 60 °C was significantly higher than the kinetic stability of other HLDs. Interestingly, DsvA possesses only one, instead of two, halide-stabilizing residues in its active site previously observed in majority of characterized HLDs. Despite unusual composition of catalytic residues, DsvA exhibited clear dehalogenase activity. The highest activity of the enzyme was determined towards 1-bromoheptane, 1-iodohexane and1-bromohexane, whereas no activity was detected in the reaction with bulky and cyclic chlorinated substrates. The temperature and pH optima of DsvA were measured with 1-iodohexane. Maximal activity was detected between 45 and 50 °C and at pH 8.9. Steady state kinetic analyses were performed with 1-iodohexane, 1,2-dibromoethane, 1-iodohexane and 1,3-dibromopropane. The complex kinetic mechanism of the enzyme with 1-iodohexane was determined and revealed substrate inhibition. The kinetics of DsvA with 1,3-dibromopropane followed a simple Michelis-Menten dependence, while the kinetics of the enzyme with 1,2-dibromoethane followed a mechanism involving positive cooperative substrate binding. Crystallization of DsvA for structural analysis is currently in progress.

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L38

COMBINING NMR (NUCLEAR MAGNETIC RESONANCE) AND RAMAN SPECTROSCOPY REVEALS STRUCTURAL AND FUNCTIONAL FEATURES OF A NEW CISPLATIN DERIVATIVE

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Since its discovery some 40 years ago, cisplatin has evolved for its efficacy in one of the most used drugs in treatment of various cancer types. Huge effort was invested in understanding the action of cisplatin and development of more potent platinum, osmium, and ruthenium based drugs. These potential drugs target mainly neighboring purine bases of nuclear DNA forming covalent intra- or inter-strand cross-links that affect inhibition of replication and transcription, cell cycle arrest, and attempted repair of the damaged nucleotides. If such damage cannot be removed the cell dies [1].

Several structures determined by NMR or X-ray crystallography are now available in the PDB database containing 1,2d(GpG) cisplatin or oxaliplatin (e.g. 1AIO, 3LPV, 1A84). Common structural features of all these structures are: a significant roll (25-60°) of the guanine bases involved in the cross-link, bending and unwinding of the double helix at the site of cross-link and orientation towards the major groove. Also, the platinum-guanine plane angle varies between 19-54°. Although the experimental structures were often used as the starting models for molecular dynamics (MD) simulations [2,3], results of these MD still leave many questions unresolved. In our contribution, we will present an NMR study of a DNA cross-linked with a new highly potent cisplatin-based derivative. Details of the binding site have been cross-examined using complementary solution techniques used in modern structural biology, including Raman spectroscopy with DFT calculations aided interpretation of the obtained vibrational spectra. Moreover, the calculated structure of the DNA duplex was verified using SAXS (Small Angle X-ray Scattering) curve, that has been measured on an in-house bioSAXS.

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L39

NMR STRUCTURAL STUDY OF SELF-PROCESSING MODULE

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The 179 amino acid long self-processing module (SPM) is present in a disordered form in the bacterial cell and folds only in calcium rich environment. The structural change induces autocatalytic cleavage of the Asp-Pro peptide bond. An almost complete NMR resonance assignment of the intrinsically disordered and folded SPM was obtained. Non-uniformly sampled multi-dimensional spectra were recorded in order to resolve poorly dispersed resonance frequencies of the disordered protein. The assignment was used to evaluate secondary structure propensities of the disordered form of SPM. The results show that disordered SPM exhibits a tendency to form helical structures in two regions.

The standard set of NMR experiments was used to obtain the resonance assignment of the ordered form of SPM except a region between alanine A15 and glycine G36, that

exhibits different motional behavior causing a signal disappearing. The ^{1}H - ^{1}H distance restraints were obtained from NOE cross peaks observed and unambiguously assigned in ^{15}N-edited and ^{13}C-edited NOESY spectra by the program ARIA 2.1. The structure was calculated by running restrained molecular dynamics in the program CNS 1.2 using the standard protocol.

The amount of bound calcium ions to SPM was determined by titration with EDTA as a chelating agent. Concentration of free and calcium-bound EDTA was monitored by 1D proton NMR spectroscopy. The possible calcium-binding sites were predicted from SPM sequence.

L40

LEDGF/P75 AS A TARGET FOR TREATMENT OF MLL-DEPENDENT LEUKEMIA AND HIV INFECTION

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LEDGF/p75 is an epigenetic reader important for HIV integration and mixed lineage leukemia (MLL1) fusion-driven leukemia development and is considered an attractive therapeutic target for drug development [1]. The LEDGF/p75-MLL1-MENIN complex was structurally characterized, but only partially [2].

Using NMR spectroscopy, we identified and mapped an additional MLL-LEDGF/p75 interface. Colony forming assays in MLL-AF9+ leukemic cells expressing MLL interaction-defective LEDGF/p75 mutants revealed that this additional interface is essential for leukemic transformation. Interestingly, the newly defined interface overlaps with the binding site of known LEDGF/p75 interactor, the HIV integrase (HIV IN). Overexpression of a LEDGF/p75 binding peptide CP65, originally developed to inhibit the LEDGF/p75-HIV integrase interaction, impaired the clonogenic growth of primary murine MLL-AF9 expressing leukemic blasts. [1]

Since LEDGF/p75 contributes to HIV integration and leukemic transformation and has become a new therapeutic target for drug development, it is crucial to study its physiological interactions. In addition to HIV IN and MLL1-menin, the LEDGF/p75 integrase binding domain (IBD) also interacts with JPO2 and PogZ proteins [3, 4].

Our recent data (manuscript in review) revealed structural details of LEDGF/p75 interactions with physiological binding partners, lead to identification of novel interaction partners and explained how HIV IN outcompetes these cellular proteins. The detailed mapping of interaction interfaces on the IBD revealed a notable overlap with the region involved in interaction with HIV integrase. This represents a challenge for selectivity of the recently developed IBD-interaction inhibitors.

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L41

COMPLEX BINDING SCHEME BETWEEN 14-3-3ZETA AND PHOSPHOPROTEINS ELUCIDATED BY NMR AND COMPUTATIONAL STUDIES

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14-3-3 proteins, found in all eukaryotic cells, are known to be important in cell-cycle regulation, apoptosis, and regulation of gene expression. They are also associated with oncogenic and neurodegenerative amyloid diseases. 14-3-3 proteins are active as homo- or heterodimers and bind more than 300 diverse target phosphoproteins, thereby forcing conformational changes or/and stabilizing active conformations in their target proteins.

Our recent ^{31}P NMR data showed much more complex binding mode between 14-3-3 and doubly phosphorylated peptide of human tyrosine hydroxylase 1 (hTh1) than was originally thought [1]. Analysis of the binding data revealed that the 14-3-3 dimer and the S19- and S40-dou-

bly phosphorylated hTh1 peptide interact in multiple ways, with three major complexes formed: (1) a single peptide bound to a 14-3-3 dimer via the S19 phosphate with the S40 phosphate occupying the other binding site; (2) a single peptide bound to a 14-3-3 dimer via the S19 phosphorous with the S40 free in solution; or (3) a 14-3-3 dimer with two peptides bound via the S19 phosphorous to each binding site.

Experimental determination of the binding affinities and binding modes between 14-3-3 dimer and their phosphorylated protein partners is very tedious therefore we have decided to address this problem also by computational techniques. Binding/unbinding pathways and the corresponding absolute binding affinities of the selected phosphopeptides with respect to the 14-3-3 have been studied by Hamiltonian Replica Exchange Molecular Dynamics (H-REMD) combined with a novel reaction coordinate approach (distancefield), and potential-of-mean-force (PMF) methods [2]. Our preliminary computational results are compared with the available experimental data.

The combination of the advanced NMR and computational approaches deepened our understanding about the binding mechanism of 14-3-3 proteins with their phosphorylated protein partners. The presented approaches have general applicability for almost any 14-3-3/ phophopeptide compex.

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