Friday, March 22, Session III

L11

CONNECTING 5' TO 3' ACTIVITIES: INTERACTION BETWEEN Rtt103p AND Trf4p

Tomasz K. Kabzinski[1], Jana Lalakova[2], Viacheslav Zemljanski[1], Karel Kubicek[1], Andrea Fortova[1], Tomas Klumpler[1], Stepanka Vanacova[1] and Richard Stefl[1]

[1]CEITEC - Central European Institute of Technology, Masaryk University; Brno, Czech Republic
[2]Department of Biosciences and Nutrition, Karolinska Institute; Stockholm, Sweden

RNA polymerase II (RNAPII) transcribes protein-coding mRNAs and a subset of non-coding RNAs (ncRNAs). For both, transcription termination is regulated by specific post-transcriptional modifications of C-terminal domain (CTD) of RNAPII. The aforementioned modifications are recognized by RNA processing and transcription termination factors via CTD-interacting domains (CIDs). Termination of ncRNAs is promoted by the NNS (Nrd1p-Nab3p-Sen1p) complex via the CID domain of Nrd1p. Nrd1p CID interconnects RNAPII termination with subsequent trimming by the nuclear exosome via the interaction with Trf4p subunit of the TRAMP (Trf4p-Air2p-Mtr4p) complex, an activator of the nuclear exosome. The CID of Nrd1p recognizes the C-terminal sequence of Trf4p that mimics phosphorylated CTD diheptad (Nrd1p-interacting motif; NIM) [1].

In this work, we found that Trf4p NIM interacts with mRNA termination factor, Rtt103p, via its CID. Interestingly, we found that Rtt103p interacts with an additional region of Trf4p termed RIM (for Rtt103p-interacting motif). By using NMR studies, we demonstrate that Rtt103p CID utilizes the same binding pocket to interact with CTD and both Trf4p sequences in a mutually exclusive manner. Furthermore, we show Rtt103p dimerization is crucial for binding with both interacting motifs of Trf4p.

In vivo, trf4 deletion strains show accumulation of extended, improperly terminated mRNAs. This phenotype is rescued by wild type, but not Trf4p defective in interaction with Rtt103p CID. RNAPII ChIP analysis on selected mRNAs did not reveal any substantial read-through phenotype. Rtt103p – Trf4p interaction, therefore, appears to be important for recruitment of the TRAMP complex and potentially exosome to proofread and remove improperly terminated mRNAs.

1. Tudek, A., Porrua O., Kabzinski T. *et al.*; Mol. Cell, 2014, 55, 467-481

L12

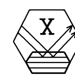
QUANTITATIVE CONFORMATIONAL ANALYSES OF INTRINSICALLY DISORDERED PROTEINS: NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY AND SMALL-ANGLE X-RAY SCATTERING IN ACTION

Vojtěch Zapletal[1,2]

[1]NCBR, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
[2]CEITEC MU, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic, vojtis@mail.muni.cz

Intrinsically disordered proteins (IDPs) are characterised by poly-peptide chains that fail to fold into stable and well defined tertiary structure. In spite of the lack of stable structure, IDPs play key roles in processes such as molecular recognition, regulation of transcription and they are related to neurodegenerative diseases. Although structural characterisation of this class of proteins is highly desirable, standard, single conformer-based approaches to structure determination necessarily fail to adequately describe such highly flexible systems. It is therefore essential to use other methods to determine local and long-range structural behaviour in IDPs from experimental nuclear magnetic resonance (NMR) and small-angle X-ray scattering (SAXS) data. In particular, we used the ASTEROIDS (**A** **S**election **T**ool for **E**nsemble **R**epresentations **O**f **I**ntrinsically **D**isordered **S**tates) algorithm that selects representative structural ensembles of IDPs on the basis of complementary experimental data sets following the evolution scheme of a genetic algorithm. We have applied these approach to study microtubule-associated protein 2c (MAP2c) and the subunit of RNA polymerase. Both proteins play important physiological roles. The ä subunit is required for rapid changes in gene expression and competitive fitness of the cell, MAP2c regulates the dynamics of microtubules in developing neurons. These biological functions require a carefully controlled balance between high flexibility and ability to form transient secondary structures and long-range contacts. Our conformational analysis revealed such structural features and showed that so-called IDPs exhibit clear structure-function relationship.

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II, project CEITEC 2020 (LQ1601), and by the Inter-Excellence Inter-Cost project LTC17078.



L13

STRUCTURAL ANALYSIS OF POSITIVE SENSE +RNA POLYMERASES

Anna Dubánková, Evžen Bouřa

Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences
Czech Republic boura@uochb.cas.cz

Positive sense single stranded RNA (+RNA) viruses are a large group of virus including dangerous human pathogens such as hepatitis C virus (HCV) poliovirus, coxsackievirus, Zika virus, and Dengue virus. The key enzyme for their replication ti the RNA-dependent RNA polymerase (RdRp) which catalyzes the formation of phosphodiester bond between RNA nucleotides and is active only after proper proteolytical processing. In picornaviruses the newly created first residue is a conserved glycine in all the RdRp enzymes that were analyzed so far. This glycine is buried in a conserved pocket which is essential for enzymatic activity. However, this glycine is not conserved in the genus kobuvirus. Instead kobuviruses (i. e. Aichi virus) have a serine residue. Intrigued by this anomaly we sought to solve the crystal structure of kobuviral RdRp enzyme. We determined the crystal structure of Aichi RdRp at 2.3 Å resolution and compared it to previously solved picornaviral RdRp enzymes. This comparison revealed a unique fold of RdRp N-terminus. Similarly we compared structures of flaviviral RdRp that, in addition, contain a methyltransferase domain.

The work was was supported by Czech Science Foundation grant number 17-05200S and also by Project InterBioMed LO1302 from the Ministry of Education of the Czech Republic.

L14

CRYSTAL STRUCTURE AND BIOPHYSICAL CHARACTERIZATION OF SELECTED REP SEQUENCES

Jakub Svoboda[1], Petr Kolenko[1,2], Tatsiana Charnavets[1] and Bohdan Schneider[1]

[1]*Laboratory of Biomolecular Recognition, Institute of Biotechnology of the Czech Academy of Sciences, v.v.i., Prumyslova 595, Vestec, 252 50, Czech Republic*
[2]*Dept. of Solid State Engineering, FNSPE Czech Technical Univ., Brehova 7, Prague 1, 11519, Czech Rep.*

Repetitive extragenic palindromic (REP) elements are short (around 20-30 bp long) DNA non-coding sequences. In genomes of various bacterial species, they occur in a high number of copies and are often clustered into bacterial interspersed mosaic elements (BIME). These sites serve as a binding platform for a diverse group of proteins. REPs are usually characterized by having conserved GTAG sequence on its 5'-end followed by GC-rich palindrome [1]. Recently a new class of transposases (REP associated tyrosine transposase – RAYT) was discovered. It is unusual in its ability to act strictly upon single-stranded DNA. RAYT gene is usually flanked by REP [2].

We analyzed selected sequences using circular dichroism (CD) spectroscopy, differential scanning calorimetry (DSC) and X-ray crystallography. CD spectra of all analyzed oligonucleotides show signal characteristic for guanosine-quadruplex with complex topology strongly indicating its presence in solution even in higher temperatures (around 80 °C).

In order to measure the value of melting temperature, we measured absorbance at 260 nm at a temperature range between 10 and 90 °C. To determine the number of contributing species we subjected the measured spectra to single-value decomposition. We observed that there are more than one species present in solution.

Three sequences have been crystallized – Hpar1-18mer, Chom-18mer and Chom-18mer bromouracil derivative. They crystallized within 2-4 days in hanging drop setup. Phase problem was solved using anomalous diffraction on bromine in the bromouracil derivative crystal, bromouracil was placed instead of thymine in position 9 in Chom-18mer. Oligonucleotides of both sequences form a duplex structure with TT-mismatch in the center. Both structures were determined at the crystallographic resolution of 2.7 Å.

1. Di Nocera, P.P., De Gregorio, E., Rocco, F. (2013) GTAG- and CGTC-tagged palindromic DNA repeats in prokaryotes. Bmc Genomics 14, 522.
2. Nunvar, J., Huckova, T., Licha, I. (2010) Identification and characterization of repetitive extragenic palindromes (REP)-associated tyrosine transposases: implications for REP evolution and dynamics in bacterial genomes. Bmc Genomics 11, 44.

LTAUSA18197 Design, development, and testing of bioinformatic tools for validation of experimental and computer molecular models in structural biology, biotechnology and pharmacy.

CIISB4HEALTH This publication was supported by the project Czech infrastructure for integrative structural biology for human health (CZ.02.1.01/0.0/0.0/16_013/0001776) from the ERDF.

L15

WHEN AFFINITY MATTERS

Pawel Kania

NanoTemper Technologies CEE

When affinity matters

At NanoTemper Technologies, we are passionate about achieving excellence in everything we do. This has made us the partner of choice for thousands of researchers worldwide in pharmaceutical and biotechnology industries as well as in academic research institutes.

The foundation of NanoTemper Technologies is microscale thermophoresis (MST), a powerful technique to quantify biomolecular interactions. MST is a biophysical technique that measures the strength of the interaction between two molecules by detecting variations in fluorescence signal as a result of an IR-laser induced temperature change. The range of the variation in the fluorescence signal correlates with the binding of a ligand to the fluorescent target. MST signal strongly depends on a variety of molecular properties such as size, charge, hydration shell or conformation. Thus, this technique is highly sensitive to virtually any change in molecular properties, allowing for a precise quantification of molecular events independent of the size or nature of the investigated specimen. Along, MST with its unique capabilities enables researchers to work with unpurified proteins, directly in their close to native conditions, such as cell or tissue lysate and membrane fraction as well.

When screening matters

Our newest platform is a result of a constant evolution of our core technology to respond to expectations coming from NanoTemper Technologies customers- Dianthus NT.23. Dianthus NT.23 brings a breakthrough in novel biophysical tactic to high throughput screening approach and removes the complexity of binding interaction measurements for drug discovery, while providing a standardization and automation friendly 384-wellls plate format.

Dianthus NT.23 allows for:

- ✓ Working with even the most challenging targets, previously inaccessible, regardless whether they are nucleic acids, multimeric proteins without enzymatic function or any other
- ✓ Measuring affinities with the highest sensitivity spanning from pico- to millimolar range, covering all needs in the most projects
- ✓ Ensuring minimal sample consumption, saving on expensive targets as well as allowing for very small test compounds use
- ✓ Free use of literally any buffer of choice, even cell lysates or any other bioliquid, enabling the best possible scenario for studied drug discovery target corresponding to its most native state
- ✓ Work in free solution, where no immobilization required, studied molecules are available for interactions in all dimensions and no extra time to work on expensive immobilizing chemistries is necessary
- ✓ Test more than 8.000 compounds in duplicate in 24 hours and collect vast amount of meaningful data for further studies

To discuss your specific research interest we invite you to visit our company booth during the meeting.