Session 17: EMBO Session "Proteins and nucleic acids"

Lectures

L17.1

tRNA transcription: novel layers of regulation

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Within the past decade, there has been much progress delineating mechanisms by which RNA polymerase III (Pol III)-mediated tRNA transcription is controlled. Those studies mostly concentrated on Maf1, a general repressor, which transfers environmental signals to Pol III. Now we performed a genome-wide analysis of nascent transcripts attached to Pol III under permissive and restrictive growth conditions. For the first time Pol III transcription was mapped across the yeast transcriptome with nucleotide resolution. This identified several previously unannotated Pol III transcription units.

High-resolution, strand-specific, profiling of the distribution of transcriptionally engaged Pol III gave rise to a number of unexpected findings. Despite the short length of most Pol III transcription units, we find strikingly uneven polymerase distribution along all tRNA genes. A high density of reads over the 5´ end of the transcription unit and a weaker peak before 3´ end of the mature tRNA were detected. Two peaks appear to be localized with respect to the A and B boxes of the internal promoter suggesting that interaction of TFIIIC factor with these regions can impede transcribing Pol III, leading to transient pausing or slowing down.

Perhaps the most surprising finding was the presence of transcription termination read-through on most tRNA genes typically extending 50–200 nt beyond the expected terminator. Extended pre-tRNAs were confirmed by northern hybridization. The degree of read-through was anti-correlated with length of canonical terminator.

Finally these analyses confirmed heterogeneous effects of Maf1 or stress conditions on the expression of different tRNA genes indicated previously by microarray analysis. A subset of tRNA genes showed a low response to nutrient shift or loss of the major transcription regulator Maf1, suggesting potential "housekeeping" roles.

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L17.2

Computational prediction of RNA 3D structures and structure-based design of RNA sequences

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RNA plays a pivotal role in cell biology. RNA molecules are involved in a variety of biological processes, in particular, transmission of genetic information, protein synthesis, and regulation of gene expression on various levels. Some RNA molecules catalyze chemical reactions (ribozymes) or sense and communicate responses to cellular signals. In analogy to proteins, the function of these RNAs often depends on 3D structure and dynamics, which are largely determined by the ribonucleotide sequence. Experimental determination of high-resolution RNA structures is both laborious and difficult, and therefore the majority of known RNAs remain structurally uncharacterized. To address this problem, computational structure prediction methods were developed.

Approaches to predicting RNA 3D structures have been largely inspired by developments in the field of protein 3D structure prediction, which fall between two general categories. Bottom up approaches ('Greek science') employ a finite set of fundamental principles, usually from physics and chemistry, in the hopes of generating the bigger picture. Top down approaches start with existing knowledge ('Babylonian science': alluding to the Babylonian penchant for record-keeping) and attempt to break down very complex problems into more manageable concepts that can be solved in a knowledge-based manner.

I will present an overview of strategies and methods for computational prediction of RNA 3D structure from sequence, with emphasis on software developed in our laboratory (available at http://genesilico.pl). I will also present a method for structure-based RNA sequence design, which aims to predict RNA sequences that fold into defined 3D structures.

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L17.3

Mitochondrial signalling and protein homeostasis

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Mitochondria need more than one thousand cellular proteins to fulfill various functions. Yet, the majority of mitochondrial proteins are synthesized outside mitochondria in the cytosol and thus must be efficiently sorted into mitochondria with the help of translocation machineries. Dysfunctional mitochondrial protein import causes mitochondrial malfunctions, but also accumulation of precursor proteins in the cytosol. We aim to understand the consequences of defects in the mitochondrial protein import. Two main arms of the cellular response to mitochondrial import defects include the inhibition of cytosolic translation and activation of the major protein degradation machinery, the proteasome. The stimulation of the proteasome is driven by its more efficient assembly as a direct response to the amount of mistargeted proteins. The mechanism is beneficial for cells. Increased levels of intracellular ROS caused by the mitochondria with defective protein import serve as a signal to attenuate global protein synthesis. We propose a universal mechanism that controls protein synthesis by modulating the redox state of proteins involved in translation. These findings uncover a crosstalk between the state of mitochondria and regulatory mechanisms responsible for maintaining the cellular protein homeostasis.

L17.4

Role of non-trivial topology for function of Methyltransferases

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Methyltransferases are enzymes that are often crucial for organisms' life, because they are responsible for important post-translational modifications of proteins or nucleic acids (tRNA, rRNA). Surprisingly these proteins perform the same biological function possessing two different folds, with their backbone being knotted or unknotted. There are over 60 distinct proteins from various organisms that possess a non-trivial topology [1] of trefoil knot, which is responsible for binding the cofactor essential for the methylation. The second surprise comes form the fact that knotted methyltransferases exist as homodimeric structures capable of binding two ligands and two substrates, while unknotted ones perform their function as monomers. Moreover, despite the symmetry in a knotted dimer, it has been shown for TrmD protein [2], that only one substrate is bound, indicating that one active site is more favorable than the other. To address this issue, we established possible function mechanism of this protein by means of molecular dynamics simulations. We were able to detect motions that lead to the asymmetrical structure in which one of the active sites is distorted. Moreover during the talk I will also discuss the role of the knotted topology in the transfer of the free energy from the substrate to tRNA based on experimental and theoretical results [2].

References:

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L17.5

Small heat shock proteins in protein aggregation and disaggregation

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Small heat shock proteins (sHsps) are an evolutionary conserved class of ATP-independent molecular chaperones. Under heat stress conditions sHsps form assemblies with misfolded proteins, preventing them from further aggregation and keeping them in a refoldable state, which facilitates subsequent solubilisation and refolding by ATP-dependent Hsp70 and Hsp100 chaperones. The sHsp-substrate solubilisation requires disruption of sHsp association with trapped misfolded proteins. Here, we will discuss a specific interplay between Hsp70 and sHsps at the initial step of the disaggregation. Hsp70 is required to displace surface-bound sHsps from assemblies during the initial phase of the reactivation process. Hsp70 acts in a passive manner by outcompeting the sHsp molecules that dynamically interact with the surface of the sHsp-substrate assemblies. The binding of Hsp70 to the sHsp-displaced surface preserves the beneficial architecture of the assembly core and allows superior substrate solubilisation upon Hsp100 recruitment.

L17.6

Structure and mechanism of reverse transcriptases

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Reverse transcriptases use their DNA polymerase and RNase H activities to convert single-stranded RNA to double-stranded DNA. This process, termed reverse transcription, is an essential step in the proliferation of retroviruses, such as human immunodeficiency virus (HIV) and retrotransposons. The latter are among the most successful mobile genetic elements, constituting nearly 50% of human genome. In order to understand the mechanism and evolution of reverse transcriptases we used a combination of structural and biochemical studies of various RTs.

For HIV-1 RT we showed that the substrate can simultaneously interact with the RNase H and polymerase active site but in doing so it adopts a new conformation not observed so far in crystal structures. These conformational changes are an essential element of fine-tuning of the amount of polymerase and RNase H activities, so that important steps of reverse transcription can be properly executed.

To understand the evolution of RTs, we solved the first crystal structure of a retrotransposon RT from the yeast Ty3 element. This element is a direct ancestor of retroviruses. The structure showed a dimeric architecture of Ty3 RT similar to HIV RT, which demonstrates that the dimeric form is ancient. There are, however, important differences between the two enzymes. Ty3 RT is a homodimer in which RNase H and polymerase reside in different subunits. HIV RT is a heterodimer – with a larger subunit with both polymerase and RNase H catalytic activities and a smaller one with altered conformation playing a structural role. The structure clearly demonstrates that the HIV RNase H domain was acquired by the enzyme from the host, while the original domain was converted to an inactive connection domain. This process occurred multiple time in retroelement evolution, but its advantages for the enzyme are not clear.

L17.7

DNA methylation and hydroxymethylation

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DNA methylation and hydroxymethylation have been known for decades. DNA methylation has always been extensively studied. Hydroxymethylation has only attracted wide interest when it was understood that this DNA modification was created enzymatically and did not result from unspecific DNA damage. 5-hydroxymethylcytosines (5hmCs) in eukaryotes appear to be always created by oxidation of methylated bases in the DNA context, and have two main roles. On the one hand, 5hmC bases are intermediates in a pathway that involves additional oxidation reactions and the base excision repair pathway and eventually leads to reversal of DNA methylation. On the other hand, 5hmC bases can also persist and act as signals in their own right. In hierarchical tissues 5hmC abundances correlate with tissue differentiation.

In the first part of my talk, I will talk about our efforts to characterize animal models of DNA hydroxymethylation. I will describe our work in zebrafish, an already accepted model for studies of DNA hydroxymethylation, and our work on insects, particularly honeybees, which also have enzymatically controlled hydroxymethylation of DNA, albeit at lower levels than vertebrates.

In the second part of my talk, I will focus on how proteins specifically detect DNA modifications, either as a prerequisite of binding, or as a feature preventing DNA binding. I will present several crystal structures from our work as well as meta-analysis of crystal structures and I will highlight principles that govern the detection of DNA modifications at the molecular level.

L17.8

Replication initiation proteins and strand-specific replisome assembly

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Replication initiators (Reps) bind dsDNA within the origin of replication causing local destabilization of the DNA unwinding element region (DUE). That exposes ssDNA DUE for replication initiator-dependent helicase loading. Although the contribution of Reps to the early steps of DNA replication initiation is described in literature for many replicons, it is not known what is the exact mechanism for replication complex *de novo* assembly nor how the directionality of replication is determined. By using broad-host-range plasmid system we demonstrated evidence for direct involvement of a replication initiation protein in the process of polymerase recruitment. Through interaction with 13-mer sequences on one strand of initially unwound DNA (Wegrzyn K, Fuentes-Perez ME, Bury K, Rajewska M, Moreno-Herrero F, Konieczny I (2014) *Nucleic Acids Res* **42**:7807-7818) and interactions with the subunits of DNA polymerase (Wawrzycka A, Gross M, Wasaznik A, Konieczny I (2015) *Proc Natl Acad Sci USA* **112**: E4188-E4196) the initiation protein facilitates strand specific replisome assembly at the plasmid replication origin. This step determines the direction of DNA replication. Interaction of replication initiator with β-subunit of DNA polymerase has been identified as an essential contribution of plasmid replication initiation protein to the process of the replisome assembly. We propose models of the plasmid replication initiation protein and its complex formed with single stranded DNA of replication origin. Our study provides new insights into the understanding of replication initiators activities.

.Posters

P17.1

Desulfuration of nucleoside 5'-*O*-phosphorothioates with releasing of H_2S within the catabolism of phosphorothioate drugs and prodrugs

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Phosphorothioate oligonucleotides (PS-oligos) are used in the antisense therapy because of increased resistance to endo- and exo-nucleases present in body fluids, compared to unmodified oligonucleotides. PS-oligos contain sulfur atom(s) in a non-bridging position in each or selected internucleotide bond(s). The biodistribution of PS-oligos and their pharmacokinetics have been widely reported, but little is known about their subsequent decay inside the organism. Nucleoside 5'-O-phosphorothioates are formed *in vivo* as primary products of hydrolysis of PS-oligos. Our *in vitro* study revealed that the Hint1 enzyme is able to hydrolyze different nucleoside 5'-O-phosphorothioates and during this reaction H_2S is released [1]. Our earlier experiments with cellular lysates (containing active proteins) confirmed that Hint1 could be involved in desulfuration of NMPS *in vivo* [2].
The aim of this study was to determine if NMPSs could be intracellularly desulfurated to NMP and if this process was accompanied by the release of H_2S, what would be important for the confirmation that Hint1 participates in this process. It is important issue since hydrogen sulfide is an gaseous mediator in mammalian cells that participates in multiple physiological processes.
Our studies have shown the AMPS and GMPS can serve as a donor of H_2S in biological systems. H_2S production was detected in HeLa, A549, and K562 cells transfected by AMPS or GMPS. We have observed that GMPS generated higher level of H_2S than AMPS. The measurements were performed using a fluorogenic compounds SF4 and SF7 [3] which were added to the cellular medium after the transfection. The compounds easily enter into the cells and offer a unique capability to identify H_2S generated at the physiological signaling levels. One cannot exclude that controlled delivery of (d)NMPSs into cells could offer a new way to change the levels of this gaseous signaling molecule.

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P17.2

Buried water molecules distribution in protein kinases

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Protein kinases are one of the largest families of proteins found in the living cells. Their main function, i.e. controlling multiple cellular processes, is strictly regulated by a precise activation process, during which the protein undergoes a structural transformation from inactive to active form. Such a conversion enables kinases to play the role of molecular switches. Malfunctioning of the regulatory mechanism leads to a number of serious diseases. Worth emphasizing is the fact that in spite of little sequence homology between different kinase subfamilies, their structures show highly conserved character. The well defined structure of a catalytic subunit determines the similarity of the activation mechanism. Interestingly, the analysis of crystallographic structures of different members of kinase subfamilies reveals the presence of several uniformly preserved water molecules located within regions directly involved in the activation process. The role of those buried water molecules for kinase function, dynamics, and, in particular, activation remains unknown.
Considering the difficulties in experimental characterization of bound solvent molecules, computational approaches appear as an important tool to investigate their properties. We focus on answering for the following questions: (1) Where are conserved hydration sites in kinases structure dependening on different states (active/inactive)? (2) How tightly are the localized water molecules bound? (3) What is the influence of the considered hydration sites on conformational mobility of kinase catalytic subunits?

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