SESSION 7
BIOSYNTHESIS OF SELENOPROTEINS II

SBP2-SECIS-RIBOSOME INTERACTIONS DURING SELENOPROTEIN SYNTHESIS

O. Kossinova [1,2], A. Malygin [1], A. Krol [2], G. Karpova [1]

[1] Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russian Federation
[2] Institut de Biologie Moléculaire et Cellulaire, Architecture et Réactivité de l'ARN, Strasbourg, France

The amino acid selenocysteine is encoded by UGA, usually a stop codon, thus requiring a specialized machinery to enable its incorporation into selenoproteins. The machinery comprises the $tRNA^{Sec}$, a 3'UTR mRNA stem-loop termed SECIS, which is mandatory for recoding UGA as a Sec codon, the SECIS Binding Protein 2 (SBP2) and other factors. Little is known about the molecular mechanism and in particular when, where and how the SECIS and SBP2 contact the ribosome. Previous work by others used the isolated SECIS RNA to address this question. We have developed instead a novel approach using engineered minimal selenoprotein mRNAs containing SECIS elements derivatized with photoreactive groups. With these tools in hands, new information could be gained about the SBP2 and SECIS contacts with the large ribosomal subunit at various translation steps. On the other hand, footprinting experiments on complexes formed between SBP2 and purified human ribosomes led to the identification of discrete areas of the 28S ribosomal RNA protected by SBP2 from various chemicals. Altogether, our findings led to broaden our understanding about the unique mechanism of selenocysteine incorporation in mammals.

MATURATION OF SELENOPROTEIN mRNPS

L. Wurth [1,2], A.-S. Gribling-Burrer [1], M. Leichter [1], C. Verheggen [3], E. Bertrand [3], A. Krol [1], C. Allmang [1]

[1] University of Strasbourg, CNRS, Architecture and Reactivity of RNA, Strasbourg, France
[2] CRG, Barcelona, Spain
[3] University of Montpellier, CNRS, Montpellier, France

Selenoproteins, key players of oxidative stress protection, are synthesized by co-translational recoding mechanisms whereby UGA_{Sec} codons are redefined to encode selenocysteine. In mammals, this process involves the assembly of RNA-protein (RNP) complexes to specific stem-loops located in the 3' untranslated region of selenoprotein mRNAs, called Selenocysteine Insertion Sequences (SECIS). Essential in this process is the SECIS binding protein 2 (SBP2) that binds the SECIS RNA and recruits translation and assembly factors to the mRNP. We have showed that selenoprotein mRNP assembly is similar to that of several small non-coding RNPs, such as snRNPs (involved in splicing) or snoRNPs (ribosome biosynthesis). Indeed, SBP2 shares a common RNA binding domain with core proteins of small non-coding RNPs. Furthermore, we showed that proper selenoprotein mRNP assembly in the 3'UTR, pre-requisite to their translation, relies on assembly machinery linked to the Hsp90 protein chaperone that is common to small non-coding RNPs. More recently we have demonstrated that selenoprotein mRNAs also undergo 5' cap maturation events. Mammalian mRNAs are characterized by the presence of a 7-methylguanosine (m^7G) cap structure added co-transcriptionally to the 5′ end. We have recently discovered that several selenoprotein mRNAs escape this rule and harbour at their 5' end hypermethylated m_3G cap structures in the same way as small non-coding RNAs.

Our data show that the trimethylguanosine synthase 1 (Tgs1) interacts with selenoprotein mRNAs to hypermethylate their cap. We have deciphered the cap maturation pathway and analyzed its functional impact on selenoprotein mRNA translation.

SELENOPROTEINS – FROM STRUCTURE TO FUNCTION

A. Dikiy

Norwegian University of Sciences and Technology (NTNU), Biotechnology, Trondheim, Norway

Structural analysis of proteins is a highly informative approach to assess protein function and regulation. It can help establish catalytic mechanisms of enzymes and visualize the structural basis for their interactions with substrates and partner proteins. The concept of determining protein structure on a genome-wide scale is called structural proteomics. Its methods include X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy on the experimental side, and molecular modeling and functional inferences on the computational side. Presently some of the mammalian selenoproteins have been structurally characterized either by X-ray or NMR. For structural analysis of most of these proteins, the catalytic selenocysteine was mutated to cysteine or glycine, allowing high protein expression. These structures and dynamic properties of selenoproteins verified the

dominance of thioredoxin fold in mammalian selenoproteins and yielded critical insights into their functions and catalytic mechanisms.

Paramagnetic NMR spectroscopy can provide an additional info on catalytic mechanism peculiarities of the protein with a paramagnetic metal ion. Expression of mammalian selenoprotein MsrB1 in Co-containing media in *E.coli* resulted in reproducible appearance of stable protein - MsrB1-Co. NMR studies allowed us to generate a structure of MsrB1-Co sharing the overall fold with native MsrB1-Zn. On the basis of the obtained data, it was proposed that the N-terminus of the protein spends significant time in the proximity of the metal-binding site, and this proximity ensures high catalytic efficiency of MsrB1 due to the short distance between the catalytic and resolving residues. Functional studies showed that both MsrB1-Zn and MsrB1-Co exhibit similar levels of activity in agreement with the performed structural studies. The proposed metal ion substitution approach may have a methodological significance in determination whether MsrB protein contains metal ion.

TRIT1 tRNA$^{[Ser]Sec}$-ISOPENTENYL-TRANSFERASE

N. Fradejas Villar [1], B.A. Carlson [2], E. Rijntjes [3], N.-P. Becker [3], R. Tobe [2], U. Schweizer [1]

[1] Institut für Biochemie und Molekularbiologie Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany
[2] Molecular Biology of Selenium Section, Laboratory of Cancer Prevention, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, United States
[3] Institut für Experimentelle Endokrinologie Charité Universitätsmedizin Berlin, Berlin, Germany

Transfer tRNA$^{[Ser]Sec}$ contains five modifications: ψ^{55} (pseudouridine ^{55}), m^1A^{58} (1-methyladenosine ^{58}), i^6A^{37} (N^6-isopentenyl-adenosine^{37}), mcm^5U^{34} (5-methoxycarbonylmethyluridine ^{34}) and its further methylated form, mcm^5Um^{34} (5-methylaminomethyl-2-thiouridine ^{34}). Although these modifications have been shown to be necessary to maintain adequate selenoprotein expression levels, most of the enzymes responsible for tRNA$^{[Ser]Sec}$ modifications are unknown.

Isopentenyladenosine is frequently found at position 37 following the anticodon in tRNAs decoding codons starting with uridine. This modification is thought to enhance translational accuracy. Isopentenylation is catalyzed by tRNA isopentenyl transferases, which add an isopentenyl group to the tRNA, using dimethylallyl pyrophosphate (DMAPP) as donor. Trit1 is an isopentenyl transferase present in mouse and human.

In this work, we demonstrate that Trit1 catalyzes the isopentenylation of tRNA$^{[Ser]Sec}$ at adenine 37. Recombinant Trit1 was able to transfer a isopentenyl group from [^{14}C]DMAPP to tRNA$^{[Ser]Sec}$. Trit1 mutants, D55G and T32A, which affect the active site of Trit1, showed a reduced activity. Mutant A37G tRNA$^{[Ser]Sec}$ was not isopentenylated in the presence of the recombinant Trit1 protein as revealed by minor base analysis. Knockdown of Trit1 expression in mouse and human cell lines exhibited a significant decrease in selenoprotein expression under selenium deficient conditions, which it was compensated in the presence of selenium in the medium or when Trit1 was overexpressed.

In conclusion, Trit1 is the tRNA$^{[Ser]Sec}$ A37-isopentenyl transferase. Impaired tRNA$^{[Ser]Sec}$ isopentenylation reduces selenoprotein expression in cell lines.

TRANSLATIONAL REDEFINITION OF UGA CODONS IS RATE-LIMITING *IN VIVO* AND REGULATED BY SELENIUM AVAILABILITY

M.T. Howard [1], B.A. Carlson [2], C.B. Anderson [1], D.L. Hatfield [2]

[1] University of Utah, Human Genetics, Salt Lake City, United States
[2] National Institutes of Health, Molecular Biology of Selenium Section, Laboratory of Cancer Prevention, Center for Cancer Research, National Cancer Institute, Bethesda, United States

Incorporation of selenium into ~25 mammalian selenoproteins occurs by translational recoding whereby in-frame UGA codons are redefined to encode the selenium containing amino acid, selenocysteine (Sec). We have applied ribosome profiling (deep-sequencing of ribosome protected fragments) and RNA-Seq to examine the effect of dietary selenium levels on the mechanisms controlling selenoprotein synthesis in mouse liver. Dietary selenium levels were shown to control gene-specific selenoprotein expression primarily at the translation level by differential regulation of UGA redefinition and Sec incorporation efficiency, although effects on translation initiation and mRNA abundance were also observed. Direct evidence is presented that Sec incorporation is rate-limiting in vivo and that increasing dietary selenium causes a vast increase in ribosome density downstream of UGA-Sec codons for a subset of selenoprotein mRNAs by a process that is mediated in part by the degree of Sec-tRNA[Ser]Sec Um34 methylation. Further, we find evidence for translation in the 5´ UTRs for a subset of selenoproteins and for ribosome pausing near the UGA-Sec codon in those mRNAs encoding the selenoproteins most affected by

selenium availability. These data illustrate how dietary levels of the trace element selenium can alter the readout of the genetic code to affect differential expression of the selenoproteins.

MOLECULAR AND CELLULAR FUNCTIONS OF SELENOPHOSPHATE SYNTHETASE 1

B.J. Lee

Seoul National University, Interdisciplinary Program in Bioinformatics, Seoul, Korea, Republic of

There are two selenophosphate synthetases (SPSs) in higher eukaryotes, SPS1 and SPS2. Of these two isotypes, SPS1 was found to be essential for cell growth and embryogenesis in Drosophila. The function of SPS1, however, has not been well determined. Microarray analysis and combined gene ontology analysis showed that gene ontology terms related to vitamin B6 biosynthesis were significantly affected at the early stage and then genes related to defense and amino acid metabolism were affected at a later stage following knockdown. Levels of pyridoxal phosphate, an active form of vitamin B6, were decreased by SPS1 knockdown. Surprisingly, the targeted removal of SPS1 mRNA led to megamitochondrial formation in an intracellular glutamine level dependent manner. The levels of intracellular glutamine were increased by SPS1 knockdown by increasing the levels of glutamine synthetase and l(2)01810 which was found to be a novel type of glutamate transporter. Further studies showed that the treatment of SL2 cells with an inhibitor of pyridoxal phosphate synthesis resulted in both a similar pattern of expression as that found by SPS1 knockdown and the formation of megamitochondria suggesting SPS1 regulates vitamin B6 synthesis, which in turn impacts various cellular systems such as amino acid metabolism, defense and other important metabolic activities. Although Drosophila has only one type of SPS1, mammals have multiple splice variants (in human, there are 5 splice variants). Among the splice variants, major type consists more than 80% of total SPS1 mRNAs in most of human tissues. Interestingly, the major type was localized in both the nuclear and plasma membranes, and the others in the cytoplasm.

All variants form homodimers, and in addition, the major type forms a heterodimer with ΔE2, and ΔE8 with +E9. The level of expression of each splice variant was different in various cell lines. The expression of each alternative splice variant was regulated during the cell cycle.