SESSION 14
BIOLOGY AND FUNCTION OF SELENOPROTEINS II

REWIRING TRANSLATION FOR GENETIC CODE EXPANSION

D. Söll, M. Bröcker, M. Englert, P. O'Donoghue, J. Ho, N. Chirathivat

Yale University, New Haven, United States

At the time of its elucidation the genetic code was suggested to be universal in all organisms, and the result of a 'frozen accident' unable to evolve further (1). How do we see the genetic code today - five decades after the familiar 'alphabet' with twenty amino acids was established? There are 22 natural amino acids (2): selenocysteine, the 21st, and pyrrolysine, the 22nd, are directly inserted into growing polypeptides during translation. The incorporation of selenocysteine directed by UGA requires the action of specific RNA and protein elements. In contrast, pyrrolysine is ligated directly to a suppressor tRNA^{Pyl} and inserted into proteins in response to UAG codons. Based on the realization that protein plasticity is a feature of living cells (3), man-made expansion of the genetic code has begun by adding non-canonical amino acids (*e.g.*, *O*-phosphoserine, 4; selenocysteine, 5-7) to the standard repertoire of the cell. These developments are the underpinning for the creation of organismal variants in the realm of genetic code expansion.

References:

1. Crick (1968) The origin of the genetic code. *J Mol Biol* **38**, 367.

2. Ambrogelly et al (2007) Natural expansion of the genetic code. *Nat Chem Biol* **3**, 29.

3. Ruan et al (2008) Quality control despite mistranslation caused by an ambiguous genetic code. *Proc Natl Acad Sci USA* **105**, 16502.

4. Park et al (2011) Expanding the genetic code of *Escherichia coli* with phosphoserine. *Science* **333**, 1151.

5. Palioura et al (2009) Catalytic complex of human SepSecS-tRNA^{Sec} reveals mechanism of selenocysteine formation. *Science* **325**, 321.

6. Aldag et al (2013) Rewiring translation for elongation factor Tu-dependent selenocysteine incorporation. *Angew Chem Int Ed Engl* **52**, 1441.

7. Itoh et al (2013) The decameric SelA:tRNA^{Sec} ring structure reveals the mechanism of bacterial selenocysteine formation. *Science* **340**, 75.

SELENIUM, FUNCTIONAL GENETIC POLYMORPHISMS AND DISEASE RISK

J. Hesketh

Newcastle University, Institute for Cell and Molecular Biosciences, Newcastle upon Yyne, United Kingdom

Genomic approaches focusing on single nucleotide polymorphism (SNP) analysis are providing new insights into selenoprotein function and the relationship between their function, Se intake and disease risk. A number of SNP in selenoprotein genes appear functional: these include rs713041 in a region of the glutathione peroxidase 4 gene (*GPX4*) corresponding to the 3'untranslated region (3'UTR), and two in the selenoprotein P gene (*SEPP1*), one in the coding region (rs3877899) predicted to cause an amino-acid change and one in the 3'UTR (rs7579). Reporter gene, protein-RNA binding and over-expression studies suggest that the T and C allelic variants of a SNP in GPX4 (rs713041) alter 3'UTR function and interfere with the selenoprotein hierarchy. Both human umbilical vein endothelial cells (HUVEC) and monocytes homozygous for the T-variant showed elevated adhesion levels compared with C-variant cells. HUVEC homozygous for the T-variant showed elevated levels of VCAM-1 protein in the presence of arachidonic acid and selenium-dependant changes in lipid peroxide levels. Genetic association studies suggest various selenoprotein SNPs may be linked to cancer risk. Two studies indicate that a SNP in the promoter of *SELS* influence risk of colorectal cancer; however Se status of these populations is not known. Our ongoing analysis of EPIC samples will define the interaction of Se intake and selenoprotein SNPs with colorectal cancer risk. In addition, in a Danish cohort the risk of developing breast cancer women is modulated by both rs3877899 and rs1050450 (GPX1) and in a German population rs1050450 modified the effect of serum Se concentration on prostate cancer risk. In conclusion, in populations of sub-optimal Se status both SNPs and serum Se appear to affect cancer risk. Work in JH's laboratory has been supported by Biotechnology and Biological Sciences Rresearch Council, Food Standards Agency, NuGO, Wellcome Trust, Newcastle Healthcare Charity and World Cancer Research Fund.

SELENOPROTEOME, REDOX BIOLOGY, TGR, AND DRUG DEVELOPMENT FOR SCHISTOSOMIASIS

D. Williams

Rush University Medical Center, Chicago, United States

Schistosomiasis remains an important neglected disease with 200 million infected individuals and more than 200,000 deaths annually. Only praziquantel is available for schistosomiasis treatment. There is concern that praziquantel resistance will evolve. Enzymes in schistosome redox pathways have been found to be essential and druggable targets for schistosomiasis drug development. Of particular interest is the selenoprotein thioredoxin glutathione reductase (TGR), which plays a central role providing the activity of several distinct enzymes present in the human redox network. TGR can catalyze the reduction of a variety of substrates by NADPH. A Sec-597-Cys TGR variant had the same specificity showing that Sec is important but not the sole determinant for its broad substrate tolerance. Auranofin (AF) has been shown to inhibit TGR and to substantially reduce worm burdens in mice. We solved the structure of TGR incubated with AF and found that the actual inhibitor is free gold bound at three different sites not directly involving the Sec residue. AF inhibits Sec-containing flavoreductases (thioredoxin reductase and TGR) more effectively than non Se-containing ones (glutathione reductase). This preference has been ascribed to the high affinity of selenium for gold. Our results challenge this view; we believe that the relative velocity of the reaction rather than the relative affinity depends on the presence of Sec residues. A qHTS of chemical libraries identified oxadiazole 2-oxides as TGR inhibitors, which were able to completely cure infected animals. Development of this chemical series has been hampered by its inherent reactivity. We are determining if repositioning AF for schistosomiasis treatment is possible. AF alone does not have sufficient in vivo schistosomicidal activity, but preliminary in vitro experiments indicate that AF plus a redox sensitizing agent have greatly increased activity.

SELENOPROTEIN K AND IMMUNITY

P.R. Hoffmann

University of Hawaii JABSOM, Honolulu, United States

Selenoprotein K (Selk) has been found to play a particularly important role in immune cells related to the calcium-dependent activation of these cells. Our recent work has uncovered a key molecular mechanism by which Selk regulates calcium flux and other cellular processes through its role in the palmitoylation of certain proteins. Palmitoylation is an important post-translational modification that involves the addition of a 16-carbon fatty acid moiety onto cysteine residues on target proteins that stablizes these proteins, facilitates association with membranes, or regulates subcellular localization. We have found that several proteins crucial for immune cell activation and function require Selk for palmitoylation including the scavenger receptor CD36, the calcium channel protein IP3R, the ARF-GAP protein ASAP2, and others. For palmitoylation to occur, Selk must complex with the palmitoyl acyl transferase, ZDHHC6, at the endoplasmic reticulum membrane and together this complex completes the transfer of palmitic acid to the cysteine residues on target proteins. Impaired palmitoylation results from Selk or ZDHHC6 deficiency and these two proteins bind each other through SH3/SH3 binding domain interactions on the cytosolic side of the endoplasmic reticulum membrane. Our findings reveal a crucial role of Selk along with ZDHHC6 in the catalysis of palmitoylation, and in the absence of Selk low levels of palmitoylation of target proteins leads to low expression of the IP3R calcium channel, impaired expression and localization of CD36, and dysregulated retention of ASAP2 to phagocytic cups during macrophage uptake of opsonized particles. Overall, we propose that Selk functions as a coenzyme for ZDHHC6 dependent palmitoylation and this represents a key mechanism by which Selk, and perhaps dietary selenium levels, impact immune cell function. Moreover, this molecular role for Selk has affects on inflammation and immunity *in vivo*.

ASAP2 PALMITOYLATION IN MACROPHAGES IS SELENOPROTEIN K DEPENDENT AND PROMOTES EFFICIENT PHAGOCYTOSIS

R.L. Norton, Z. Huang, J. Fay, G. Fredericks, F.W. Hoffmann, P.R. Hoffmann

University of Hawaii at Manoa, Cell & Molecular Biology, Honolulu, United States

Selenium is a mineral micronutrient that is essential in a variety of biological systems, including immune responses. Selenium is used for biosynthesis of the amino acid selenocysteine, which is used by selenoproteins to carry out biological functions. In humans, 25 selenoproteins have been identified and Selenoprotein K (SelK) was recently identified as an endoplasmic reticulum transmembrane protein important for calcium flux during the activation of immune cells. Macrophages are leukocytes which are involved in antigen presentation and directly in pathogen defense. They play a vital role in both innate and adaptive immunity. ASAP2 is an SH3 domain containing ARF-GAP that localizes to the phagocytic cup and aids in actin remodeling when a macrophage engulfs a pathogen. Implementation of the SH3 hunter software predicted that the SH3-binding domain of SelK associates with SH3 domain containing proteins. In an unbiased screen for SelK binding partners we found that ASAP2 immunoprecipitates with SelK. Palmitoylation is a post transcriptional modification to proteins, specifically to cysteine residues, which contributes to proteins being targeted to various intracellular sites, and palmitoylation can also affect protein function. When we assessed palmitoylation states of ASAP2, we discovered that it was palmitoylated and that palmitoylation was SelK dependent. It has been established by our lab that SelK KO macrophages exhibit notably impaired phagocytosis of opsonized beads. Furthermore, in SelK KO macrophages we have determined that there is an increased ASAP2 occurrence in the phagocytic cup. Additionally cell fractionation revealed an increased ASAP2 in the membrane fraction when SelK is absent.

Studies are underway to determine if the lack of SelK impairs ASAP2 function in the phagocytic cup, and if this effect leads to an increase in ASAP2 being shuttled to the cup is a novel compensatory mechanism in macrophages.