SESSION 9
PROKARYOTS

CONSERVATION AND DIVERGENCE IN SELENOCYSTEINE BIOSYNTHESIS

R.L. French [1], M. Bröcker [2], M. Simonovic [1]

[1] Univeristy of Illinois at Chicago, Biochemistry and Molecular Genetics, Chicago, United States
[2] Yale University, New Haven, United States

Selenocysteine, the 21st amino acid, is synthesized in an elaborate tRNA-dependent process, which is unparalleled in its complexity when compared to the corresponding synthetic mechanisms responsible for formation of other proteinogenic amino acids. In archaea and eukaryotes, three enzymes promote conversion of a serine precursor into selenocysteine while being attached to the selenocysteine tRNA ($tRNA^{Sec}$). The multi-step process begins with a conserved misacylation event during which seryl-tRNA synthetase couples serine with $tRNA^{Sec}$. In the subsequent step, *O*-phosphoseryl-$tRNA^{Sec}$ kinase (PSTK) phosphorylates the seryl moiety yielding the phosphoseryl-$tRNA^{Sec}$ intermediate. This intermediate is a substrate for the terminal synthetic enzyme, *O*-phosphoseryl-$tRNA^{Sec}$:selenocysteinyl-$tRNA^{Sec}$ synthase (SepSecS), which promotes the phosphoseryl-to-selenocysteinyl conversion in a reaction that requires a pyridoxal phosphate cofactor and $tRNA^{Sec}$. A number of recently determined crystal structures of the components of the selenocysteine-synthetic apparatus provide a foundation for better understanding of the mechanism of selenocysteine synthesis. Here, the most relevant findings in the last decade will be summarized. A special emphasis will be given to processes that are conserved among archaea and eukaryotes. In addition, parallels between the bacterial mechanism on one side and the archaeal and eukaryal on the other, will be drawn. Finally, a speculation that selenocysteine is formed by a series of multi-enzyme complexes acting in concert will be presented.

SELENOPROTEINS IN ARCHAEA

M. Rother

Technische Universität, Dresden, Germany

Proteins containing selenocysteine (sec) are found in all three domains of life, Bacteria, Eukarya, and Archaea. In Archaea, only members of the Methanococcales and Methanopyrales appear to use sec. The majority of archaeal selenoproteins, including sec-containing subunits of formate dehydrogenase (Fdh), formyl-methanofuran dehydrogenase, F_{420}-reducing and -nonreducing hydrogenase, and heterodisulfide reductase, are directly involved in methane formation (methanogenesis) from H_2+CO_2 or from formate. Methanogenesis is coupled to the generation of an ion motive force across the cytoplasmic membrane fueling ATP synthesis and other endergonic cellular processes. The model methanogen *Methanococcus maripaludis* further synthesizes sec-containing selenomonophosphate synthetase and the HesB-like protein of unknown function. With the exception of Fdh, all selenoproteins of *M. maripaludis* involved in methanogenesis can be substituted be isoenzymes containing cysteine (cys) at the respective position of sec. The cys-containing isoforms are induced upon selenium deprivation or when the pathway of selenoprotein biosynthesis is disrupted. This situation is thought to ensure the preferred insertion of sec during translation into Fdh over the other sec-containing proteins under these conditions. Here, an overview is given about the function of archaeal selenoproteins, about their regulation, and how *M. maripaludis* can still grow with formate as the energy source in the absence of selenoproteins.

MOLECULAR EVOLUTION OF [NiFeSe]HYDROGENASE SIMULATED BY PHYLOGENY AND MOLECULAR DYNAMICS

T. Tamura

[1] Okayama University, Okayama, Japan

Hydrogen attracts much interest in the aim of future energy resource in human society. Hydrogenase involved in anaerobic respiration generally has high 0_2-sensitivity, and it always requires strict anaerobic conditions for hydrogen production. Selenium-containing [NiFeSe]H_2ase has relatively high tolerance to oxygen. Phylogenic study on [NiFeSe]H_2ase indicated there could be at least seven more Se-containing homologs among the sulfate reducing bacteria, which are considered to have the truncated large subunit by the occurrence of opal codon. Decoding those UGA as Sec extended the C-terminal sequences, which are highly homologous to the C-terminal sequences of [NiFeSe] H2ases from *D. vulgaris*

(2wpn.pdb) and *D. baculatum* (1cc1.pdb). Among over 100 homologs of [NiFe]hydrogenases, the putative selenoeyzmes fall into the same group even when the Sec was disguised as Cys upon phylogenic study using Mixmam-likelihood method in GTR+GI model with 100 bootstrap test. Phylogenic study also suggested the sequences of possible common ancestors, which were revived *in silico* as the three-dimensional structures using homology modeling and molecular dynamics simulation. The ternary structure of the ancestor hydrogenase allowed us to speculate the evolutionary chage in the shape and volume of the gas cavity, which is the key determinant of O_2-sensitivity and efficiency of hydrogenase catalysis.

THE MECHANISM OF tRNA-DEPENDENT SEC FORMATION IN BACTERIA

M.J. Bröcker [1], Y. Itoh [2], M. Simonovic [3], S. Yokoyama [2], D. Söll [1]

[1] Yale University, Dept. of Molecular Biophysics and Biochemistry, New Haven, United States
[2] RIKEN Systems and Structural Biology Center, Yokohama, Japan
[3] Univeristy of Illinois at Chicago, Dept. of Biochemistry and Molecular Genetics, Chicago, United States

Selenocysteine formation in bacteria is a tRNA-dependent process that commences with the seryl-tRNA synthetase catalyzed formation of Ser-$tRNA^{Sec}$ which is then converted to Sec-$tRNA^{Sec}$ by the selenocysteine synthase SelA. This enzyme forms an elaborate ring-shaped homodecamer that binds ten $tRNA^{Sec}$ substrate molecules. Productive coordination of each $tRNA^{Sec}$ molecule involves proper positioning of residues from four SelA subunits achieved only in the decameric layout of the enzyme. While the characteristic elongated extra-arm of $tRNA^{Sec}$ is not recognized, the $tRNA^{Sec}$ specific D- and T-arms are precisely bound by the SelA N-terminal region. The aminoacyl acceptor arm is directed into the active site where a 5'-pyridoxal phosphate cofactor and a set of conserved arginine residues enables the conversion of tRNA-bound Ser to Sec via a dehydroalanine intermediate.

PREDICTION OF NEW GENES INVOLVED IN SELENIUM METABOLISM IN BACTERIA

J. Lin, Y. Zhang

[1] Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Selenium (Se) is a trace element that mainly occurs in proteins in the form of selenocysteine and in tRNAs in the form of selenouridine. In the past twenty years, several genes that are essential for Se utilization have been characterized in both prokaryotes and eukaryotes. However, Se homeostasis and the associated regulatory networks are not fully understood. In this study, we conducted comparative genomics and phylogenetic analyses to identify novel genes involved in Se utilization in bacteria. Investigation of genomic context of known Se-related genes in different organisms revealed, several candidate genes, in addition to genes already known to participate in Se metabolism. Among them, a membrane protein which shows distant homology to a sulfur transporter, is exclusively found in Se-utilizing organisms, suggesting that it may be a potential Se transporter. A small protein family was widespread in Se-utilizing organisms, and might serve as an important chaperon involved in Se trafficking within the cells. In addition, a LysR-like transcription factor subfamily may be important for the regulation of Sec biosynthesis and/or other Se-related genes. In conclusion, our results revealed new genes involved in Se metabolism in bacteria and should be useful for further understanding the complex metabolism and the roles of Se in biology.

FUNCTION OF THE UNIQUE MULTIHEME SELENOPROTEIN OF THE METAL-REDUCING BACTERIUM *GEOBACTER SULFURREDUCENS*

H. Mihara [1], Y. Tani [1], E. Shinno [1], W. Zhang [2], S. Saito [1], T. Kurihara [2], N. Esaki

[1] Ritsumeikan University, College of Life Sciences, Kusatsu, Japan
[2] Kyoto University, Institute for Chemical Research, Uji, Japan

In the previous study, we identified a novel multiheme-containing selenoprotein (MESEP), which contains five hemes and one selenocysteine residue per subunit, in the dissimilatory metal-reducing bacterium *Geobacter sulfurreducens* (Fujita M, Mihara H, Goto S, Esaki N, Kanehisa M. *BMC Bioinformatics* (2007) 8, 225). MHSEP occurs in a limited number of bacterial species and exhibits no significant sequence homology to other known proteins. In this study, we investigated the structural and functional properties of MHSEP. Western blot and N-terminal amino acid sequence analyses showed that the protein is translated as a precursor carrying an N-terminal signal peptide and translocated into the periplasmic space. The UV-vis spectrum of the as-isolated 47-kDa mature MHSEP exhibited maxima at 553 nm (α-band), 525 nm (β-band), and 410 nm (Soret band), similar to those observed for *c*-type cytochromes. The protein showed peroxidase activity with guaiacol as a substrate but had no catalase activity. To address *in vivo* function, we generated an MESEP gene-disrupted *G. sulfurreducens* strain. The growth rate of the mutant strain was reduced as compared with the wild-type strain. We found that the wild-type strain was capable of transforming $Mo^{VI}O_3$, $Mo^{VI}O_4^{2-}$, and $Mo^{VI}{}_7O_{24}^{6-}$ into molybdenum blue (reduced polymolybdate complexes) under anaerobic conditions. In contrast, the mutant strain failed to produce molybdenum blue under the same conditions. The wild-type strain grew in the cultivation medium containing those molybdenum compounds, whereas the mutant strains exhibited no growth phenotype. These results suggest that MHSEP functions in the dissimilatory reduction of molybdenum compounds.