SESSION 3
BIOSYNTHESIS OF SELENOPROTEINS I

MECHANISTIC ASPECTS OF Sec INCORPORATION

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The co-translational insertion of selenocysteine (Sec) into mammalian selenoproteins requires a set of novel protein factors whose functions are dedicated to this process. The efforts of our group are focused on determining the mechanistic basis for the Sec incorporation reaction. As such, we have recently begun dissecting the precise role of eEFSec as a Sec-tRNA^{Sec} delivery factor. We have found that the novel fourth domain of this protein is a multifunctional platform that is required for all of the known aspects of eEFSec function: Sec-tRNA^{Sec} binding, SBP2 binding, and GTPase activity. In parallel with this work, we have also found that all of the factors known to be required for Sec incorporation are, in fact, sufficient for promoting Sec incorporation in vitro. We added purified factors (SBP2, eEFSec and Sec-tRNA^{Sec}) to a plant based (Sec naive) in vitro translation system to establish that these factors alone are capable of supporting Sec with the important caveat that mammalian ribosomes are also required, thus establishing that reconstitution of the mammalian Sec incorporation process in heterologous organisms is limited. Moving forward, two key mechanistic questions stand out: How can Sec incorporation be efficient and in the case of Selenoprotein P (SelP) production, how can Sec incorporation be processive? We have begun to tackle the latter question by identifying the proteins that bind to the 3´ UTR of SelP, which contains two SECIS elements and a total of over 700 highly conserved nucleotides. To date we have identified eight proteins that specifically interact with the SelP 3´ UTR. Determining the role of these proteins in SelP synthesis has been hampered by the fact that this protein is not efficiently made in vitro or in transfected cells. Current efforts are centered around developing a SelP expression system and characterizing the function of SelP 3´ UTR binding proteins.

TARGETED DELETION OF SECISBP2 IN THE MOUSE

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Eukaryots have evolved a fascinating mechanism for selenocysteine insertion into selenoproteins: Selenocysteine Insertion Sequence (SECIS)-Binding Protein 2 (Secisbp2) binds to the hairpin-like SECIS element located in the 3'-UTR of the selenoprotein mRNAs and recodes the UGA codon to selenocysteine. Mutations in *SECISBP2* in humans lead to reduced selenoprotein expression thereby affecting thyroid hormone-dependent growth and differentiation processes. Some patients also display myopathy, hearing impairment, male infertility, increased photosensitivity, mental retardation, and ataxia. Mouse models are needed to investigate selenoprotein-dependent processes underlying the patients' pleiotropic phenotypes. Our group set out to create several mouse models in order to test whether Secisbp2 is essential for selenoprotein biosynthesis, in particular in organs not accessible in patients.

Homozygous *Secisbp2* embryos fail shortly after implantation. In contrast, *Secisbp2* heterozygotes have no obvious thyroid hormone, growth, or biochemical phenotypes. Hepatocyte-specific *Secisbp2* knockout mice appear normal, but show a dramatic reduction of hepatic selenoprotein expression. Interestingly, loss of *Secisbp2* reduced the abundance of many, but not all, selenoprotein mRNAs. Transcript-specific effects on selenoprotein mRNA abundance were greater in *Secisbp2*-deficient primary hepatocytes than in tRNA$^{[Ser]Sec}$-deficient cells. Despite the massive reduction of *Dio1* and *Sepp1* mRNAs, significantly more corresponding protein was detected in primary hepatocytes lacking Secisbp2 than in cells lacking tRNA$^{[Ser]Sec}$. Since phenotypes (selenoprotein expression, induction of Nrf2-dependent genes or embryonic development) were consistently milder in *Secisbp2*-deficient livers than in tRNA$^{[Ser]Sec}$-deficient livers, we conclude that Secisbp2 is required for efficient selenoprotein biosynthesis, but not essential. A novel role of Secisbp2 in selenoprotein mRNA stabilization is suggested.

REGULATION OF SELENOPROTEIN BIOSYNTHESIS BY SELENIUM, OXIDATIVE STRESS AND AGING

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Selenium is an essential trace element that, when deficient, causes physiological disorders, increases cancer risk and reduces lifespan in human. It is incorporated as selenocysteine via co-translational UGA recoding into 25 human selenoproteins, which depends on selenium bioavailability. The nature of the SECIS element located in the 3´UTR of selenoprotein mRNAs modulates UGA recoding efficiencies. Deciphering selenoprotein regulation as a function of oxidative stress and cellular senescence in defined cellular models might enlighten their roles in redox control and ageing.

First, we investigated how the selenium concentration altered the antioxidant defenses and selenoprotein expression of Hek293 cells in the context of oxidative stress. We observed that selenium deficiency caused an increased production of reactive oxygen species and protein carbonyls, concomitantly with the downregulation of several selenoproteins and lower proliferation rate. Then, we found that several selenoproteins expressed in cytosol, mitochondria and endoplasmic reticulum were upregulated by H_2O_2 treatments, a higher stimulation being observed in selenium deficient conditions. On the other hand, we studied the relationships between selenium level, selenoprotein expression and replicative lifespan of human WI-38 fibroblasts. We found that selenium concentration regulated the entry into replicative senescence and modified the cellular markers characteristic for senescent cells. We observed that several selenoproteins involved in antioxidant defense were specifically affected in response to cellular senescence.

In both cases, fine regulation of the selenoproteome was performed in response to oxidative stress and cellular senescence, which resulted from a selective tune of mRNA levels and regulation of UGA recoding efficiency. Our data reported novel selective controls of selenoprotein expression via modulation of selenocysteine insertion activity.

ALTERNATIVE TRANSCRIPTS AND 3´UTR ELEMENTS GOVERN THE INCORPORATION OF SELENOCYSTEINE INTO SELENOPROTEIN S

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Selenoprotein S (SelS) is a 189 amino acid transmembrane protein that plays an important yet undefined role in the unfolded protein response. It has been proposed that SelS may function as a reductase, with the penultimate selenocysteine (Sec(188)) residue participating in a selenosulfide bond with cysteine (Cys(174)). Cotranslational incorporation of Sec into SelS depends on the recoding of the UGA codon, which requires a Selenocysteine Insertion Sequence (SECIS) element in the 3´UTR of the transcript. Here we identify multiple mechanisms that regulate the expression of SelS. The human SelS gene encodes two transcripts (variants 1 and 2), which differ in their 3´UTR sequences due to an alternative splicing event that removes the SECIS element from the variant 1 transcript. Both transcripts are widely expressed in human cell lines, with the SECIS-containing variant 2 mRNA being more abundant. In vitro experiments demonstrate that the variant 1 3´UTR does not allow readthrough of the UGA/Sec codon. Thus, this transcript would produce a truncated protein that does not contain Sec and cannot make the selenosulfide bond. While the variant 2 3´UTR does support Sec insertion, its activity is weak. Bioinformatic analysis revealed two highly conserved stem-loop structures, one in the proximal part of the variant 2 3´UTR and the other immediately downstream of the SECIS element. The proximal stem-loop promotes Sec insertion in the native context but not when positioned far from the UGA/Sec codon in a heterologous mRNA. In contrast, the 140 nucleotides downstream of the SECIS element inhibit Sec insertion. We also show that endogenous SelS is enriched at perinuclear speckles, in addition to its known localization in the endoplasmic reticulum. Our results suggest the expression of endogenous SelS is more complex than previously appreciated, which has implications for past and future studies on the function of this protein.

CAENORHABDITIS ELEGANS AS A MODEL FOR STUDYING SELENOCYSTEINE INCORPORATION AND SELENOPROTEIN FUNCTION

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C. elegans is an excellent model to study selenocysteine incorporation: possesses a single Sec residue in its proteome, in thioredoxin reductase (TRXR) active site. The SECIS-binding protein (SBP2) plays an essential role in Sec decoding. Known SBP2s possess an L7Ae domain and a Sec-incorporation domain (SID) thought to be essential for decoding. By iterative sequence analyses, we identified *C. elegans* SBP2, which lacks the SID. Moreover, the SID was absent in *C. elegans* proteome. N-terminal to the L7Ae domain we found a 40 residue-long K-rich extension, which may functionally replace the SID. A *C. elegans sbp2* KO strain ablated Sec incorporation confirming that *sbp2* is essential for Sec incorporation. An *in silico* analysis of the Sec insertion trait in the nematode lineage revealed: the absence of the SID, SBP2s consisting of the L7Ae domain preceded by the K-rich extension and the maintenance of Sec incorporation linked to TRXR. The absence of SID may be explained by the unusual nematode SECIS element or the restricted use of Sec in this lineage. We also found that plant nematode parasites lost the ability to incorporate Sec.

C. elegans encodes Cys-containing othologs of several mammalian selenoproteins being a model to study their function. We focused on SELT studies, because SELT1 and SELT2 are the only proteins of the SELT/SELW/SELH redoxin family in *C. elegans*. No phenotypes were observed for a Δ*selt1*/Δ*selt2* KO strain. Since SELT has a putative ER localization, we are subjecting the double KO strain to DTT, tunicamycin and RNAi of ER thiol oxide reductases. The dynamics of SELT evolution indicates an ancestral duplication in nematodes with subsequent SELT loss in some lineages and that Cys was the ancestral residue, but a Cys→Sec replacement occurred in one lineage.

C. elegans and the nematode lineage provide insights into Sec incorporation, selenoprotein function and the evolution of Sec utilization trait, selenoproteomes, selenoproteins and Sec residues.

THE MOLECULAR BIOLOGY OF SOME SELENOPROTEINS INVOLVED IN THE PATHOGENESIS OF ALZHEIMER´S DISEASE

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Researches on selenium and Alzheimer's disease (AD) have been systematically reviewed, in which no explicit conclusion was drawn, especially for the clinical trial. In our lab, the molecular biology of some selenoproteins, including SelM, SelP, and SelR, are studied regarding the pathogenesis of AD.

We found that the Sec-to-Cys mutant of SelM (SelM′) and the His-rich domain of SelP (SelP-H) are capable of binding transition metal ions and modulating the Zn^{2+}-mediated amyloid-β (Aβ) peptide aggregation, ROS production and neurotoxicity. Each SelM′ or SelP-H coordinated 0.5 or 2 molar equivalents of Zn^{2+}/Cd^{2+} with micromolar and submicromolar affinities, respectively. Zn^{2+} binding to $Aß_{42}$ almost completely surpressed $Aß_{42}$ fibrillization, which could be significantly restored by SelP-H and SelM′. Both SelM′ and SelP-H inhibited Zn^{2+}-$Aß_{42}$ induced neurotoxicity and intracellular ROS production in living cells. These studies suggest that SelP and SelM may play certain roles in regulating redox balance as well as metal homeostasis in the pathogenesis of AD.

We also found in brain that SelR interacts with clusterin (Clu), a chaperone protein and a newly identified AD relevant gene. The interacting domain of Clu was a dynamic molten globule structure, while that of SelR was a Zn^{2+}-bingding tetrahedral complex. Synergic effect was found between the two proteins. Co-overexpression of SelR and Clu in N2aSW cells, an AD model cell line, significantly decreased the level of intracellular ROS. Meanwhile, Clu interacted with Aβ, which suggested a potential effect of SelR and Aβ with the aid of Clu. Those results provide a novel clue for studying the function and mechanism of SelR in the pathogenesis of AD.

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