SESSION 6
SELENIUM AND ROS

MsrB1 REGULATES ACTIN ASSEMBLY VIA REVERSIBLE STEREOSELECTIVE METHIONINE OXIDATION/REDUCTION AND IS REQUIRED FOR MACROPHAGE FUNCTION

B.C. Lee [1], Z. Peterfi [1], A. Kaya [1], F.W. Hoffmann [2], R.E. Moore [2], A. Avanesov [1], Y. Zhou [3], L. Tarrago [1], E. Weerapana [3], P.R. Hoffmann [2], V.N. Gladyshev [1]

[1] Harvard Medical School, Medicine/Genetics, Boston, United States
[2] University of Hawaii at Manoa, Department of Cell and Molecular Biology, Honolulu, United States
[3] Boston College, Department of Chemistry, Boston, United States

Redox control of protein function involves oxidation and reduction of amino acid residues, but specificity of such regulation is often not defined. Here, we report that methionine-*R*-sulfoxide reductase B1 (MsrB1), regulates, in conjunction with Mical proteins, mammalian actin assembly via stereoselective methionine oxidation and reduction in a reversible, site-specific manner. Two methionine residues in actin are specifically converted to methionine-*R*-sulfoxide by Mical1 and Mical2 and reduced back to methionine by selenoprotein MsrB1, supporting actin disassembly and assembly, respectively. Macrophages utilize this redox control during cellular activation by stimulating MsrB1 expression and activity as a part of the immune response, and the Mical/MsrB pair can regulate actin function when expressed in diverse eukaryotes. This study identifies the regulatory role of MsrB1 as a Mical counterpart in orchestrating actin dynamics and the immune response. More generally, our study shows that proteins can be regulated by reversible site-specific methionine-*R*-sulfoxidation in addition to importance of selenoprotein in immune function.

MAMMALIAN THIOREDOXIN REDUCTASE 1 IN CELLULAR SIGNALING PATHWAYS

E.S.J. Arnér

Karolinska Institutet, Division of Biochemistry, Dept Medical Biochemistry and Biophysics, Stockholm, Sweden

The selenoprotein thioredoxin reductase 1 (TrxR1) supports functions of Trx1, promoting cell viability and replication (via ribonucleotide reductase), antioxidant defense (via peroxiredoxins and methionine sulfoxide reductases) and redox modulation of signaling pathways [1]. Several observations suggest that TrxR1 may also modulate signaling events through reactions beyond support of Trx1. For example, rare splice variants of TrxR1 are directed to unique subcellular compartments, including nucleus [2] or the cell membrane [3, 4]. For the latter, N-terminal myristoylation and palmitoylation of the v3 variant yields membrane targeting [5]. Moreover, cisplatin targets TrxR1, promoting formation of prooxidant variants that lack Trx reducing capacity but gain toxic NADPH oxidase activities producing superoxide [6-8]. Cisplatin treatment of cells also yields covalent crosslinks of TrxR1 with both Trx1 and thioredoxin-related protein of 14 kDa (TRP14) [9]. TRP14, identified by others as a Trx-fold protein lacking activity with typical Trx1 substrates [10], was recently found to reactivate PTP1B [11], as well as being a L-cystine reductase and S-denitrosylase (unpublished). These and other aspects of TrxR1 in signaling pathways will be discussed.

References:

[1] E. S. J. Arnér, Biochim Biophys Acta 2009, 1790, 495.
[2] A. E. Damdimopoulos, et al., J Biol Chem 2004, 279, 38721.
[3] P. E. Damdimopoulou, et al., Biochim Biophys Acta 2009, 1793, 1588.
[4] P. Dammeyer, et al., J Biol Chem 2007, 283, 2814.
[5] M. Cebula, et al., J Biol Chem 2013, 288, 10002.
[6] Q. Cheng, et al., J Biol Chem 2010, 285, 21708.
[7] K. Anestål, et al., PLoS ONE 2008, 3, e1846.
[8] K. Anestål & E. S. J. Arnér, J Biol Chem 2003, 278, 15966.
[9] S. Prast-Nielsen, et al., Free Radic Biol Med 2010, 49, 1765.
[10] W. Jeong, et al., J Biol Chem 2004, 279, 3142.
[11] M. Dagnell, et al., Proc. Nat´l Acad. Sci. U.S.A. 2013, In press.

A NEWLY CHARACTERIZED FORM OF SELENOPROTEIN P HAS THIOREDOXIN ACTIVITY

S. Kurokawa [1], S. Eriksson [2], K.L. Rose [1], A.K. Motley [1], E.S.J. Arner [2], K.E. Hill [1], R.F. Burk [1]

[1] Vanderbilt University Medical Center, Nashville, United States
[2] Karolinska Institutet, Stockholm, Sweden

Selenoprotein P (Sepp1) is made up of an N-terminal domain (residues 1-240) that contains a selenocysteine (U) at residue 40 within a proposed -UYLC- redox-active motif and a C-terminal domain (residues 241-361) that contains 9 selenocysteine residues. Full-length Sepp1 produced by the liver supplies extra-hepatic tissues with selenium via apoER2-mediated endocytosis. A previously uncharacterized form of Sepp1 is filtered by the glomerulus and taken up by renal proximal convoluted tubule cells via megalin-mediated endocytosis. We purified Sepp1 from the urine of *megalin*$^{-/-}$ mice using a monoclonal antibody to the N-terminal domain. The purified material, Sepp1$^{1-207}$, contained 18% of the selenium in dialyzed urine.Characterization of Sepp1$^{1-207}$ by mass spectrometry revealed that it consisted of N-terminal fragments terminating at 11 different sites between amino acid residues 183 and 208. Because it has a thioredoxin fold as do other forms of Sepp1, Sepp1$^{1-207}$ was compared to full-length Sepp1, Sepp1$^{\Delta 240-361}$, and Sepp1^{U40S} as a substrate of thioredoxin reductase-1 (TrxR1). All forms of Sepp1 except Sepp1^{U40S}, which contains a serine in place of the selenocysteine at residue 40, were efficient TrxR1 substrates when coupled with H_2O_2 and with *tert*-butyl hydroperoxide as terminal electron acceptors. These results are compatible with full-length Sepp1 being proteolytically cleaved between residues 183 and 208, freeing Sepp1$^{1-207}$ to function as a redox enzyme in the extracellular space, with the catalytic activity being dependent upon the selenocysteine residue located in its N-terminal thioredoxin fold domain. Ultimately, Sepp1$^{1-207}$ is filtered by the glomerulus and its selenium is salvaged by proximal convoluted tubule cells for synthesis of Gpx3.

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GLUTATHIONE PEROXIDASE 4 IS REQUIRED FOR THE MATURATION OF PHOTORECEPTOR CELLS

H. Imai [1], T. Ueta [2], T. Inoue [2], T. Furukawa [3], Y. Tamaki [2], Y. Nakagawa [1], Y. Yanagi [2]

[1] Kitasato University, School of Pharmaceutical Sciences, Tokyo, Japan
[2] University of Tokyo School of Medicine, Department of Ophthalmology, Tokyo, Japan
[3] Osaka Bioscience Institute, Department of Developmental Biology, Osaka, Japan

Oxidative stress is implicated in the pathology of photoreceptor cells, and the protective role of antioxidant enzymes for photoreceptor cells have been well understood. However, their essentiality has remained unknown. To clarify the role of GPx4 during retina development, In this study we generated photoreceptor-specific conditional knockout (CKO) mice of glutathione peroxidase 4 (GPx4) and showed the critical role of GPx4 for photoreceptor cells. By immunohistochemical analysis, GPx4 was abundantly expressed in the retina, especially in the inner segments (IS) of photoreceptor cells while there was no detectable expression of GPx4 protein in the outer segments (OS) of the retina from 8-weeks-old mice. In the wild-type retina the dominant GPx4 expression was in the mitochondria, indicating the mitochondrial variant was the major GPx4 in the retina. In the GPx4-CKO mice, although photoreceptor cells developed and differentiated into rod and core cells by P12, they rapidly underwent drastic degeneration and completely disappeared by P21. Consistent with the deletion of GPx4 in photoreceptor cells, significant amount of acrolein, a marker of lipid peroxidation, was found in IS of photoreceptor cells in CKO mice at P12 compared with the control. The photoreceptor cell death in the GPx4-CKO mice was associated with the nuclear translocation of apoptosis-inducing factor and TUNEL-positive cells. Photoreceptor cells before undergoing apoptosis (P11) exhibited decreased mitochondrial biomass, decreased number of connecting cilia, as well as disorganized structure of outer segments. These findings indicate that GPx4 is a critical antioxidant enzyme for the maturation and survival of photoreceptor cells.