catalyzed by poly(ADP-ribose) polymerase and represents an immediate response of eukaryotic cells to oxidative. PARP-1 and PARP-2 display catalytic activity by contact with DNA-strand breaks and are involved in DNA base-excision repair and other repair pathways. Recent studies suggest a link between DNA damage-induced poly(ADP-ribosyl)ation and all tissues at varying levels, with the highest activity in mammalian testis, spleen and thymus. in particular, the poly(ADP-ribosyl)ation is, therefore, pivotal in the maintenance of genomic integrity as well as in the cellular responses to DNA damage. the aim of the present research has been to determine the PARP activity and to study protein expression in the testis of frog, morphological and molecular identified as *Pelophylax bergeri*, of three different polluted sites and one control site. Antioxidative Glutathione S-transferase activity has been also determined in order to correlate the poly(ADP)ribosylation with the oxidative stress. Moreover the biochemical data have been integrated with morphological evaluation, to assess the health status of the gonadal tissue.

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Differential Labeling of Reversible Protein-Oxidation and S-Palmitoylation Using the Biotin-Switch Assay

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Biotin Switch Assays are a useful molecular tool for the detection of reversible protein oxidation including S-glutathiolation, S-nitrosation, S-sulfenation and may also be used to monitor protein S-palmitoylation. These assays are indirect and the specificity solely depends on the chemistry of the "switch", which in the case of reversible protein oxidation (RPO) is reduction of the modified thiol and for S-palmitoylation hydrolysis of the thioester. All these types of modifications are reversible under physiological conditions and control cellular signaling by modulating enzyme activity and subcellular location. in addition, oxidative stress can prevent these modifications by irreversibly oxidizing highly reactive cysteine thiolates. the utilization of DTT to reduce RPO followed by labeling of freed cysteines with Biotin-HPDP in WT HRas and various HRas Cys mutants demonstrates a lack of efficiency and specificity of the assay for this type of modification. Applying mass spectrometry as well as standard Western blotting techniques, we found that DTT not only reduces RPO but also loss of the palmitoyl moiety was frequently observed during the DTT reduction step. Particularly at high pH, DTT is able to remove S-palmitoyl moieties from cysteine residues, leading to non-specific labeling. Various adaptations were introduced to the protocol, including intentional hydrolysis/ alkylation of the thioester bonds allowing accurate detection and differential assessment of RPO from S-palmitoylation when using the Biotin Switch Assay.

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Accumulation of Ptdins 4-Phosphate in Golgi Through Reversible Oxidation of the Ptdins 4-Phosphatase Sac 1 by H_2O_2

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Changes in the level of a phosphatidylinositide (PtdIns) in certain organelles often alter the function of the organelles by modifying the PtdIns-dependent recruitment of mediator and effecters. Here, we investigated if reactive oxygen species (ROS) can regulate PtdIns signaling using live cell imaging and biochemical analysis. To visualize the endogenous PtdIns-4P level, we used two PH-domain-containing fluorescent proteins that preferentially bind PtdIns-4P: FAPP1 PH-GFP and OSBP PH-GFP. Upon exposure to low levels of H_2O_2, the levels of PtdIns-4P in Golgi complex (GC) measured by the GFP probes increased dramatically in Hela, HEK293 and rat vascular smooth muscle cells (VSMCs). the increase could be reversed by exposing the cells to H_2O_2-free medium. the H_2O_2-induced increase of PtdIns-4P level was confirmed by HPLC analysis following the labeling of the cells with ^{3}H-inositol. Time lap imaging showed that transient increase of ROS by extracellular stimuli (growth factors, cytokines) in A431 and rat-VSMCs induced transient increase of PtdIns-4P level in GC. Quantitative analysis of ^{3}H-labelled PtdIns showed that total level of PtdIns-4P increased dramatically in A431 cells during EGF and its increase depends on transient accumulation of H_2O_2. We found that Sac1, a PtdIns 4-phosphatase, localized predominantly in GC, is oxidized by exogenous H_2O_2 or endogenous H_2O_2 generated after EGF stimulation in A431 cells and catalytic Cysteine-389 of human Sac1 phosphatase is sensitive to the oxidation by H_2O_2. To elucidate functional consequence of increased PtdIns-4P in Golgi, we transfected HEK293 cells with a vesicular stomatitis virus glycoprotein (VSVG)-GFP vector and applied a temperature change method to monitor protein trafficking. Fluorescence confocal imaging showed that VSVG transport from Golgi to plasma membrane (PM) is strongly increased in H_2O_2 treated cells. Based on these results, we propose that the levels of PtdIns-4P in GC increase as the result of H_2O_2-dependent reversible inactivation of Sac1, thereby enhancing protein trafficking from Golgi to PM.

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Thiol-Mediated Regulation of Epithelial Sodium Channels

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Activation of epithelial sodium channels (ENaC) is the rate limiting step for sodium reabsorption across several epithelia, including the colon, sweat glands, salivary glands, airways, and the distal nephron. Defects in the proper functioning of ENaC is responsible for nearly all of the known inherited forms of hypertension, and contribute to the pathogenesis of various lung disorders such as cystic fibrosis, and pulmonary edema. While, our work shows that reactive oxygen species (ROS) regulate ENaC activity, the mechanism by which ROS mediates these effects remains to be elucidated. Herein, we have monitored the effects of reducing and oxidizing agents on ENaC currents in kidney epithelial cells. Oxidizing agent (hydrogen peroxide), when applied at concentrations of 1mM- 500mM, potentiates ENaC-activated currents, while the reducing reagent dithiothretol (DTT) used at similar concentrations, causes inhibition. Thiol