several chaperones have been evaluated. The expression level of respiratory chain subunits was also measured with or without Lon depletion. The mitochondrial compartment morphology was monitored in different stress conditions, and measured using a parameter devoted to the evaluation of the mitochondrial dynamics. None of these investigations showed a significant phenotype resulting from Lon down-regulation

A possible impact of Lon depletion on oxidized mitochondrial proteins level was then sought. 1D gel electrophoresis after the derivatization of protein carbonyl groups with 2,4-dinitrophenyl hydrazine (DNPH) revealed an increase in carbonylated proteins more important in mitochondrial extracts than in total cellular extracts. 2D difference gel electrophoresis (DIGE) experiments provide results consistent with these observations with some enlightenments. Performed with fluorescent dyes labelling either proteins or their carbonyl groups, these experiments indicated proteome modifications in cells with Lon down-regulation both at the level of protein expression and at the level of protein oxidation. These variations are noted in proteins acting in different cellular activities, i.e. metabolism, protein quality control and cytoskeleton organization.

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Investigating free radical generation in HepG2 cells using immuno-spin trapping

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Abstract

Oxidative stress can induce the generation of free radicals, which are believed to play an important role in both physiological and pathological processes and a number of diseases such as cancer. Therefore, it is important to identify chemicals which are capable of inducing oxidative stress. In this study, we evaluated the ability of four environmental chemicals, aniline, nitrosobenzene (NB), *N,N*-dimethylaniline (DMA) and *N,N*-dimethyl-4-nitrosoaniline (DMNA), to induce free radicals and cellular damage in the hepatoma cell line HepG2. Cytotoxicity was assessed using lactate dehydrogenase (LDH) assays and morphological changes were observed using phase contrast microscopy. Free radicals were detected by immuno-spin trapping (IST) in in-cell western experiments or in confocal microscopy experiments to determine the subcellular localization of free radical generation. DMNA induced free radical generation, LDH release and morphological changes in HepG2 cells whereas aniline, NB and DMA did not. Confocal microscopy showed that DMNA induced free radical generation mainly in the cytosol. Preincubation of HepG2 cells with *N*-acetylcysteine and 2,2'-dipyridyl significantly prevented free radical generation upon subsequent incubation with DMNA, whereas preincubation with apocynin and dimethyl sulfoxide did not. These results suggest that DMNA induces oxidative stress and that reactive oxygen species, metals and free radical generation play a critical role in DMNA-induced cytotoxicity.

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Monitoring dynamic changes of glutathione redox state in subcellular compartments of human cells – an approach based on rxYFP biosensor

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Abstract

The kinetic and spatial separation of redox systems renders redox biology studies a particularly challenging field. Genetically encoded biosensors including the glutathione-specific redox-sensitive yellow fluorescent protein (rxYFP) provide an alternative way to overcome the limitations of conventional glutathione/glutathione disulfide (GSH/GSSG) redox measurements. In this study, the plasmids expressing respectively cytosol-, nucleus-, and mitochondrial matrix- targeted rxYFP were created and introduced to human cervical carcinoma (HeLa) cells. The rxYFP redox states were monitored by direct assessment of the oxidized to reduced rxYFP ratio via redox protein extraction, redox Western blot and signal quantification. RxYFP proteins expressed in the cytosol, nucleus or mitochondrial matrix of HeLa cells were responsive to the intracellular redox state changes induced by reducing as well as oxidizing agents. Compartment-targeted rxYFP sensors were able to detect different steady-state redox conditions between the cytosol, nucleus and mitochondrial matrix. Furthermore, rxYFP sensors were able to sense dynamic and compartment-specific redox changes caused by 100 μM hydrogen peroxide (H_2O_2). Mitochondrial matrix-targeted rxYFP displayed a greater dynamics of oxidation in response to a H_2O_2 challenge than the cytosol- and nucleus-targeted sensors, largely due to a more alkaline local pH environment. Our data provide direct evidence that mitochondrial glutathione redox state is maintained and regulated independently from that of the cytosol and nucleus. Complementary to existing redox sensors and conventional redox measurements, compartment-targeted rxYFP sensors provide a novel tool for examining mammalian cell redox homeostasis, permitting high resolution readout of steady glutathione state and dynamics of redox changes.

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Hydroxybenzoic acids and their derivatives as peroxynitrite scavengers

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Abstract

A social challenge of the 21st century is to reduce the incidence of chronic diseases. A balanced diet rich in polyphenols could contribute to reduce the risk and to the prevention of diabetes, coronary heart disease, cancer, Alzheimer's diseases and cataract[a]. Hydroxybenzoic acids (HBA) and their derivatives, which are one of the substances responsible for these beneficial properties, are known mainly due to their antioxidant properties[b]. They are effective scavengers of free radicals and reactive nitrogen species, such as peroxynitrite. Peroxynitrite is resulting from the reaction of nitric oxide with superoxide, causes lipid peroxidation and subsequent cellular