These findings indicate that irradiation at a high-dose rate is effective in inhibiting FST-induced immobility and oxidative stress in mice.

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## NC108

### Redox regulation of autophagy by thioredoxin o1 and its involvement in tobacco BY-2 cell viability under oxidative conditions

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Autophagy is a process by which damaged or non-useful components are degraded in a lytic cell compartment for recycling or elimination. Autophagy is essential for physiological function and stress defense of plant cells although the mechanisms involved in its regulation are less known than in animal systems. Redox regulation of autophagy components is emerging as a key mechanism and thioredoxins (TRXs) have been proposed as regulators. In a previous work, we described that mitochondrial/nuclear PsTRX*o*1 had a protective role increasing cell viability and delaying cell death after treating over-expressing PsTRX*o*1 tobacco BY-2 cells with $H_2O_2$ (Ortiz-Espín et al., 2015, Ann. Bot. 116:571-), however the link of TRX with the autophagy process was not studied. In this work, the *in vitro* interaction of autophagy related protein ATG4 and TRX*o*1 is shown by dot-blot trap analysis and the redox regulation of its activity by the thioredoxin system (NADPH/thioredoxin reductase/thioredoxin *o*1) is demonstrated. Moreover, taking into account all these results, we hypothesize an additional functional role for TRX*o*1 and autophagy in the oxidative stress response of the over-expressing TBY-2 cells, collaborating to increase cell viability. For that, we analyze ATG4 and ATG8 expression by qPCR, western blot as well as ATG4 activity in parallel to cell viability, autolysosomes visualization and immunolocalization of ATG8 by fluorescence microscopy using known autophagy inhibitors. The results indicate that overexpression of PsTRX*o*1 could be influencing the establishment of an autophagic process in the response of TBY-2 cells to $H_2O_2$, collaborating in the observed increased cell survival. The TRX*o*1 role could be through the regulation of key target proteins as ATG4. [Supported by Seneca Foundation (Excellence 19876/GERM/15) and MEC-FEDER, Spain (BFU2017-86585-P). SB-V, OL-V and MCM were supported by MEC-FPI, AECID (México) and MICINN-Ramón y Cajal, respectively. Authors acknowledge the technical support of Sandra Correa in microscopy analysis.

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## NC109

### In vitro cytoprotective effect and antioxidant capacity of salmon, mackerel and herring hydrolysates in Caco-2 clone (TC7) cells

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The fish frame contains remarkable amounts of muscle proteins. To date, the use of fractionation of heads, backbones or other fish organs in the production of protein supplements there is not, or only very limited. Thus, protein hydrolysates are a good candidate as food ingredients for nutritional supplement. In this study, the in vitro cytotoxicity of salmon, mackerel and herring hydrolysates was evaluated by MTT essay in the concentration range from 1 to 1:32 dilution in Human colon adenocarcinoma cells (Caco-2/TC7). The induction of oxidative stress, as a possible mechanism of toxicity, was determined by lipid peroxidation (LPO) and reactive oxygen species (ROS) generation. The protective effect of fish hydrolysates against oxidative stress was evaluated using $H_2O_2$-stressed human intestinal differentiated Caco-2/TC7 cells. All hydrolysates showed a hormetic effect when these cells were exposed to 1:16 dilution, preventing a decrease in cell viability. Pure hydrolysates decreased the LPO production in these cells. The highest cytoprotective effect was obtained with HSV hydrolysate with 2.5-fold. The intracellular reactive oxygen species (ROS) accumulation induced by $H_2O_2$ was suppressed by all pure hydrolysates. Due to the importance of the bioavailability of hydrolysates, their in vitro gastrointestinal digestion in Caco-2/TC7 cell were carried out. The viability of bioavailable fraction was compared with pure hydrolysates. The results suggest that HMH, HSV, HSB, HMB, HSH and Collagen have an adequate bioavailability due to the bioavailable fraction of each hydrolysate shows the same viability as pure hydrolysate. So, all hydrolysates were non-cytotoxic and prevented the propagation of oxidative stress by LPO and ROS generation in Caco-2/TC7 cell. Thus, they can be beneficial ingredients with antioxidant properties and can have protective effects against ROS mediated intestinal injuries.
Keywords: fish hydrolysates, cytotoxicity, oxidative stress, cytoprotective effect, bioavailability

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## NC110

### Detection of active cell death markers in rehydrated lichen thalli and the involvement of nitrogen monoxide (NO)

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Lichen desiccation/rehydration cycles lead to an increased oxidative stress modulated by the multifaceted mediator nitrogen monoxide (NO). Active cell death, frequently triggered by oxidative damage with NO participation, has been confirmed even in unicellular organisms. This adaptive mechanism has not been studied in lichens and no specific experimental protocols exist. Hoechst 33342 enters viable cells and DNA binding increases its fluorescence, particularly intense in condensed apoptotic chromatin. YO-PRO-1 can only permeate the altered membrane of apoptotic P2X7-positive cells. Proteolytic caspases are activated upon different types of active cell death. Our objectives are to determine if these markers indicate active cell death in *Ramalina farinacea* after desiccation/rehydration and to study the effect of NO scavenging. YO-PRO-1, Hoechst 33342 and Caspase 3/7 Green DNA binding were assessed in thalli rehydrated with deionized water and with a cocktail of apoptosis inducers. A 24 h kinetics and a microscopical analysis were performed. YO-PRO-1 fluorescence was not detected, Hoechst 33342 staining abruptly decreases during the first hours, while caspase-like activity associated to phycobionts steadily increases. Whereas the apoptosis inducers cocktail 1x significantly increased caspase-like activity affecting both symbionts, Hoechst staining was only affected at 10x. NO scavenging diminishes caspase-like activation and seems to accelerate Hoechst abrupt decrease during thallus rehydration. In conclusion, the demonstration of caspase-like activity in *R. farinacea* and its Trebouxia phycobionts point to the presence of active cell death but other methods assessing cell effective death or DNA irreversible fragmentation (i.e. TUNEL assay) are necessary to confirm this feature.
Keywords: Apoptosis, Caspases, Hoechst 33342, Oxidative stress, Programmed cell death and YO-PRO-1.

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## NC111

### Immunomodulation of melanoma in vitro and *in vivo* using reactive oxygen species

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Background: Immunogenic cancer cell death (ICD) delivers inflammatory stimuli to elicit anti-tumor immunity. Release of find-me (HMGB1, ATP) and eat-me (calreticulin; CRT) signals enables dendritic cell maturation and presentation of tumor antigen to T-cells. Effective ICD-inducing therapies are anthracyclines, ionizing irradiation, and photodynamic therapies. Intriguingly, these antitumor strategies show concomitant generation of reactive oxygen species (ROS) that can drive cell death and immunogenic signaling responses. In cancer cells, the importance of ROS is underappreciated in the onset of ICD.
Methods: We employed a novel antitumor modality capable of releasing a several types of tumor-toxic ROS simultaneously.