Analysis of reversible and irreversible protein modifications upon oxidative stress in *Schizosaccharomyces pombe* by proteomic approaches

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Reactive oxygen species (ROS) (H_2O_2, OH^-, O_2^{-}) generated as a consequence of the oxygen metabolism in aerobic organisms, have important roles in cell signalling. However, if they reach concentrations beyond homeostatic levels they generate a situation of oxidative stress where damages to cellular components are produced. In the case of H_2O_2, even physiological levels may produce a chronic low level of stress, which progressively drives the cell into the aging process.

In order to understand the consequences of an oxidative stress situation in the cell, it is important to unravel which are the primarily biological targets of ROS and which are the consequences of such reactivity. Here we report the study of different types of modifications that ROS produce in proteins in *Schizzosacharomyces pombe*, under exogenous oxidative stress, by adding H_2O_2 to the culture media, and also under endogenous oxidative stress, using mutant strains defective for ROS scavengers.

By 1D electrophoresis, we observe that reversible disulfide bond formation is increased upon both stresses, and in the case of H_2O_2 treatment, the kinetics correlates with oxidative activation of the *S. pombe* H_2O_2-sensor Pap1. We also show how shifting the growth of the mutant strains from an anaerobic condition to an aerobic one results in differential irreversible carbonylation of proteins. We are extending these studies to the proteome level to identify the target proteins of both stresses. We have established a modification of the ICAT protocol to quantify the extent of reversible modified cysteines. In addition, we have established a 2D electrophoresis approach to identify targets of carbonyl modifications produced by oxidative stress. This would allow us to study the consequences of irreversible and reversible protein modifications and their biological relevance after situations of intrinsic and extrinsic oxidative stresses.

La lipoproteína lipasa de rata se nitra *in vivo* en respuesta a la administración de lipopolisacárido

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La lipoproteína lipasa (LPL) juega un papel central en el metabolismo lipídico hidrolizando los triacilgliceroles (TAG) plasmáticos. Una de las respuestas metabólicas características a la infección es la hipertrigliceridemia, que es consecuencia, al menos en parte, de la disminución de la actividad LPL de los tejidos. La administración de lipopolisacárido (LPS) provoca una inhibición de la actividad LPL tisular a nivel postranscripcional [1,2] y un aumento de la producción de óxido nítrico (NO). Estudios anteriores han propuesto una relación causa/efecto entre el aumento de la síntesis de NO y la disminución de actividad LPL tisular [2] aunque no se ha determinado si esta interacción es directa o está