Las levaduras: en la intersección entre la Biología de sistemas y la Biomedicina
En memoria del Profesor Julio Rodríguez Villanueva

Yeasts: at the cross-roads of Systems biology and Biomedicine
In memory of Professor Julio Rodríguez Villanueva

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When protein phosphatases go mad: the molecular bases of the toxicity of fungal phosphatase Ppz1

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Protein phosphorylation is a major mechanism for the control of cellular processes. The status of phosphorylation of a given protein is the result of the balance between the activity of more or less specific protein kinases and protein phosphatases. Alterations in such balance often leads to functional disorders.

Ppz1 is a type 1-like protein phosphatase that is only found in fungi. In *S. cerevisiae*, this phosphatase is involved in the influx of K^+ and the efflux of Na^+ cations. Remarkably, Ppz1 is the most toxic yeast protein when overexpressed: it does not kill the cells, but leads to a severe cell cycle blockage at G1 phase [1].

The activity of Ppz1 is inhibited by two partially redundant proteins (Hal3 and Vhs3). These proteins have moonlighting activity, since they also participate with essential functions in the CoA biosynthetic pathway [2]. We have demonstrated that Ppz1 toxicity derives from its phosphatase activity and not from a possible impact on CoA biosynthesis resulting from sequestration of the regulatory subunits.

The toxic effect of Ppz1 overexpression seems unrelated to alterations in cation homeostasis. To investigate the molecular basis of this phenomenon, we are developping several approaches *i*) a screen for high-copy number suppressors, *ii*) identification of proteins that co-purified with affinity-purified GST-Ppz1, *iii*) a genome-wide transcriptomic profiling of the effect caused by of Ppz1 overexpression, and *iv*) a phoshoproteomic analysis of the impact of increased Ppz1 activity. These studies suggest that the effect of Ppz1 over-dosage on the cell is multifaceted and involves, at least, alteration in protein translation, generation of oxidative stress, and widespread changes in the phosphorylation pattern of near 400 proteins (mainly dephosphorylated) including numerous proteins involved in mitotic cell cycle and bud emergence. We are in the course of investigating

possible changes in the phosphorylation pattern that might be responsible for the observed cell cycle blockage. The identification of such changes might reveal new aspects of the regulation of cell cycle.

References cited:

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2. Ruiz A, Gonzalez A, Munoz I, Serrano R, Abrie JA, Strauss E, and Arino J (2009). Moonlighting proteins Hal3 and Vhs3 form a heteromeric PPCDC with Ykl088w in yeast CoA biosynthesis. Nat Chem Biol. 5: 920–928.