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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A moonlighting protein in the non-conventional yeast *Yarrowia lipolytica*

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Moonlighting proteins are able to perform different unrelated functions and are not the result of gene fusion.. We have uncovered a protein in the non-conventional yeast *Yarrowia lipolytica* that shows characteristics of a moonlighting protein. This protein is the N-acetylglucosamine (NAGA) kinase, the first intracellular protein in the metabolism of NAGA. This metabolism implicates the coordinated expression of 4 genes (*NAG* genes) encoding respectively a transporter (*NGT1*), the kinase (*NAG5*), a deacetylase (*NAG2*) and a deaminase (*NAG1*). The final product of the metabolic pathway is fructose-6-P that enters the glycolytic trunk. Growth in NAGA promotes the transcription of these genes while grow in glucose abolishes it.

However, disruption of the gene *YlNAG5* that causes inability to grow in NAGA, allows transcription of the *NAG* genes during growth in glucose. This suggests that the *Yl*Nag5 protein could have two functions: one as kinase, the other as transcriptional regulator. To test this hypothesis we have expressed several heterologous NAGA kinases or modified versions of the *Yl*Nag5 protein in an *Ylnag5* mutant and examined if they complemented both functions. Expression of these proteins was checked by western blot. NAGA kinase activity was measured and transcription state of the genes was assessed by determining the enzymatic activity of *Yl*Nag1. We found that proteins with similar kinase activity behaved differently in their role as transcriptional regulator thus showing that these functions may be separated. A search for proteins that could interact with *Yl*Nag5, using a pulldown assay, showed that *Yl*Nag5 interacts with a protein similar to *Saccharomyces cerevisiae* Urp2, a component of the small (40S) ribosomal subunit.

An alternative explanation for the behaviour of the *Ylnag5* mutants could be that the deregulation of the *NAG* genes were triggered by NAGA accumulation in the mutant. Chitin hydrolysis, which takes place outside the cell, would be the source of NAGA in this case. We found that in a double mutant *Ylnag5 ngt1* that cannot internalize NAGA, the deaminase activity is derepressed.

Our results support the idea that *Yl*Nag5 is a moonlighting protein with a metabolic and a regulatory role in the transcription of the *NAG* genes.

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