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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The Rts1 regulatory subunit of PP2A is essential for septin organization in *Candida albicans*

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Rts1 is the regulatory subunit of protein phosphatase 2A, sharing an overall identity of 47% with S. cerevisiae Rts1, although it contains an insert of around 100 amino acids located within the B56 regulatory domain, not present in ScRts1 was common in other fungal homologs, suggesting that they might have diverged from its S. cerevisiae counterpart to fulfil specific functions in the regulation of the PP2A phosphatase.

Rts1 localizes to the nucleus throughout the cell cycle and to the bud neck in large-budded cells with divided nuclei. Time-lapse analysis with markers of the actomyosin contractile ring (CAR) indicated that Rts1 arrives to the septum when the CAR is almost contracted and remains at the cleavage plane after CAR disassembly. Rts1 localization was also analysed in cells containing a tagged septin. Interestingly, Rts1 initially localized between the two septin rings, and then it asymmetrically associated to the daughter septin ring before disappearing. During hyphal growth Rts1 also transiently associates with the apical septum.

*rts1*ΔΔ mutant cells are heterogeneous in size and often have wide bud necks, and the abnormal large cells are multinucleated, suggesting that they have defects in nuclear segregation. In addition, septin organization is also abnormal in *rts1*ΔΔ mutant cells. Due to the size heterogeneity, septin structures were also heterogeneous, being abnormal in large polynucleated cells. The abnormal septin structures included fragmented septin rings that frequently assembled as bars parallel to the longitudinal axis, small rings or septin dots outside of the bud neck.

In *S. cerevisiae*, absence of Rts1 stabilizes septin rings in cells carrying the thermosensitive *cdc12-6* allele, suppressing the growth defect of this mutant. In contrast, deletion of RTS1 in *C. albicans* did not suppressed the growth defect of the *cdc12-6* strain, suggesting that Rts1 function on septin rings is different than in *S. cerevisiae*. Indeed, septin structures are highly aberrant in the *cdc12-6 rts1*ΔΔ strain, suggesting that in *C. albicans* Rts1 is required for stabilization of septin rings during yeast growth. During hyphal growth, *rts1*ΔΔ cells produced germ tubes that were morphologically similar to wild-type cells. However, hyphae lacking Rts1 displayed a diverse variety of abnormal septin structures, including cortical bars parallel to the axis, spirals, Y-like structures, small cortical rings and faint duplicated rings, suggesting that Rts1 is essential for proper assembly of high-order septin structures in hyphae. Another interesting phenotype of *rts1*ΔΔ hyphae is that while germ tubes were similar to those of the wild-type cells after 2 hours of hyphal induction, they induced lateral branching with high frequency at later times. This suggests that Rts1 is required for inhibition of lateral budding from the subapical hyphal compartments.

Phosphorylation of different septin subunits is important during hyphal development. We analysed whether the PP2A Rts1 is involved in dephosphorylation of septin subunits. No differences in protein levels or electrophoretic mobility of Cdc3, Cdc10 and Cdc12 were observed in wild-type and mutant extracts in yeast or hyphae. However, *rts1*ΔΔ cells showed an increase in the phosphorylated forms of Sep7 in both yeast and hyphae, suggesting that the ratio of phosphorylated/non phosphorylated forms of Sep7 depends on the activity of the phosphatase PP2A Rts1.