Ady2p is essential for the acetate permease activity in the yeast *Saccharomyces cerevisiae*

Sandra Paiva*, Frederic Devaux*, Sónia Barbosa, Claude Jacq and Margarida Casal

Several yeast species, including *Saccharomyces cerevisiae*, are able to utilize acetate as a sole carbon and energy source under aerobic conditions (Barnett et al., 1990). Acetate being a normal and frequent end-product of fermentation (Flores et al., 2000). When cells of *S. cerevisiae* are grown on respiratory substrates, activity for at least two monocarboxylate proton symporters are found, with differences in their mechanisms of regulation and specificity (Casal et al., 1996). A lactate–pyruvate-acetate-propionate transporter, induced in lactic or pyruvic acid-grown cells, is encoded by the gene *JEN1* (Casal et al., 1999). In addition to Jen1p, another permease, which accepts acetate, propionate or formate, is present in cells grown in non-fermentable carbon sources (Casal et al., 1996; Makuc et al., 2001). To identify new genes involved in acetate uptake in *S. cerevisiae*, an analysis of the gene expression profiles of cells shifted from glucose to acetate acid was performed. This strategy allowed us to identify the membrane protein Ady2p as an essential component of the acetate active transport in these conditions and a valuable candidate as a new acetate transporter in yeast.

**Acetate is transported by a mediated transport system**

Glucose-grown cells ➔ Shift to YNB-acetic acid medium, pH 6.0

- The activity of the acetate permease was present only after 6 h of induction.
- The deletion of *JEN1* did not affect the ability of the cells to transport acetic acid by a mediated mechanism.

**ADY2/YCR010c is an essential gene for the mediated transport of acetic acid in *S. cerevisiae***

- *ADY2*-deleted strain (a) exhibited a dramatic decrease in the activity of the acetate permease when compared to the wt strain (A).
- In the *ADY2*-deleted strain acetic acid crossed the plasma membrane by a simple diffusion mechanism.

**Adaptation of *S. cerevisiae* W303-1A and ady2Δ strains to acetic acid (0.5%, pH 6.0)**

Cells were cultivated in glucose and transferred to a medium containing acetic acid (0.5%, pH 6.0).

- Cells of the ady2Δ have a normal growth in acetic acid when compared with the wild type strain.

**Expression changes in genes encoding known or putative transporters after 4h in acetic acid**

- Genes YCR010c/ADY2, YNR002c/FUN34 and YDR384c/ATO3, homologous to the OPR1 gene of *Yarrowia lipolytica* were clearly activated.

**Comparison of gene expression profiles between W303-1A and ady2Δ strains**

After 4 hours in acetate, the ady2Δ strain does not have a dramatic difference in the global transcriptional response compared to the wild type suggesting that *ADY2* is not a key regulator of the transcriptional response to acetic acid.

**Expression of *ADY2* in different carbon sources**

Northern-blot analysis of *JEN1* and *ADY2* transcripts from W303.1A strain. Total cellular RNA was prepared from cells growing exponentially in YNB media containing different carbon sources.

**The transcriptional adaptation of yeast cells to a shift from glucose to non-toxic conditions of acetic acid was characterized. These conditions of metabolic shift revealed to be very powerful for functional assignment of unknown proteins and for the study of mechanisms like translation regulation or mitochondria biogenesis.**

**ADY2 gene is an essential component of acetate transport system in *S. cerevisiae***

*Sandra Paiva received a PhD grant from the Portuguese Government (SFRH/BD/20895/2005) and received a PIBIC short-term fellowship for the work carried out in Paris. Branco Babes received a Technical Grant from a project (PCT/PMI/EM/00030/2003) financed by the Portuguese Government. This work was supported by the project POCTI/1999/36625.*