

## Identification of amino acid residues critical for the substrate translocation in lactate permease Jen1p of *Saccharomyces cerevisiae*

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Lactic, acetic and propionic acids have been used for many years in industrial and pharmaceutical companies. In *Saccharomyces cerevisiae*, Jen1p is a major monocarboxylate:H<sup>+</sup> symporter specific primarily for lactate, pyruvate and for acetate (TC # 2.A.1.12.2) (Casal *et al.*, 1999). A phylogenetic tree of ScJen1p homologues (Casal *et al.*, 2008) showed the existence of two main clusters: a Jen1 group (monocarboxylate transporters) and a Jen2-like (dicarboxylate transporters).

Structure-function relationships in Jen1p have been approached by using a rational mutational analysis of conserved amino acid residues (Soares-Silva *et al.*, 2007). Analysis of the conserved sequence <sup>379</sup>NXX[S/T]HX[S/T]QDXXX<sup>391</sup>, located in transmembrane segment seven (TMS-VII), showed that residues N379, H383 or D387 are necessary for function and specificity, while Q386 is important for the kinetics of Jen1p-mediated transport.

In this work, we rationally designed and analyzed novel mutations in conserved regions located in TMS-II, TMS-V and TMS-XI of Jen1p, which we predicted to affect Jen1p specificity (distinction between mono and dicarboxylates) and function. Among the residues studied, F270 (TMS-V) and Q498 (TMS-XI) are specificity determinants for the distinction of mono- from dicarboxylates, and N501 (TMS-XI) is critical for function.

Using a model based on Jen1p similarity with the GlpT permease, we show that all polar residues critical for function within TMS-VII and TMS-XI are aligned along the protein pore and substrate docking studies reveal a potential substrate translocation trajectory consisting mostly of the polar residues genetically identified as important for function.

Overall, our results constitute a first step towards the genetic manipulation of substrate specificity in the lactate/pyruvate:H<sup>+</sup> symporter subfamily and a tool for the *in silico* prediction of the function of Jen1p homologues in other fungi (Soares-Silva *et al.*, 2011).

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