

## IDENTIFICATION OF AMINO ACID RESIDUES CRITICAL FOR DISTINGUISHING MONO- AND DI-CARBOXYLATE SUBSTRATES IN JEN1

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The knowledge of the mechanisms underlying the transport of carboxylic acids is crucial towards an efficient biological production of carboxylates which have been used for many years in industry namely for the production of biodegradable polymers and as substitute for petroleum-derived chemicals. In *Saccharomyces cerevisiae*, Jen1p is major monocarboxylate H<sup>+</sup> symporter specific primarily for lactate, pyruvate and for acetate (Casal *et al*, 1999). A phylogenetic tree of ScJen1p homologues (Casal *et al*, 2008) showed the existence of two main clusters: a Jen1 group of proteins (monocarboxylate transporters) and a Jen2-like proteins (dicarboxylate transporters). In this work, we rationally design, combine and analyse novel mutations in two conserved regions located in TMS5 and TMS11 of Jen1p, which we predicted to affect more dramatically Jen1p specificity. The domain in TMS5 was identified by structure/function studies based on phylogenetic molecular comparisons among Jen1p homologues with different specificities and is critical for distinguishing mono- and di-carboxylate permeases. The conserved aminoacids in TMS11 domain pointed to the importance of this domain that was demonstrated to be involved in substrate binding. We thus identify several residues critical for Jen1p activity, among which some also function as critical specificity determinants for the distinction of mono- from di-carboxylates which constitutes a first step towards the elucidation and genetic manipulation of substrate specificity in the lactate/pyruvate:H<sup>+</sup> symporter subfamily (TC#2.A.1.12.2) and a tool for the *in silico* prediction of the function of Jen1p homologues in other fungi of industrial importance.

Casal, M., et al. (1999) J. Bacteriol. 181, 2620-2623.

Casal, M., et al. (2008) FEMS Microbiol Rev. 32, 974-994.