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Genome-wide prediction of single-spanning inner membrane proteins

To identify further mitochondrial inner membrane proteins, we used the 5,788 verified or uncharacterized yeast protein sequences of the SwissProt database (Boeckmann et al., 2003) and selected all sequences that showed a probability of at least >85% for mitochondrial targeting according to the MitoPred algorithm (Guda et al., 2004). Out of the resulting 772 sequences, 163 are predicted to contain at least one transmembrane span according to the TMHMM prediction program (Krogh et al., 2001). A mitochondrial localization has been shown experimentally for 53 of these proteins (Andreoli et al., 2004), which contained both arrested (like Cox5a) and transferred sequences (like Oxa1). Proteins with one single transmembrane domain typically showed proline-free stretches of at least 14–16 hydrophobic residues. Most of these proteins with NH_2-terminal targeting signals and a single TMD presumably adopt a N_{in}–C_{out} topology in the inner membrane. These putative single-spanning inner membrane proteins are listed in Table S3.

Some of the proline residues in mitochondrial membrane proteins are conserved

The in vitro sorting experiments shown in this study indicated that, in *Saccharomyces cerevisiae*, the presence of a proline residue in the first TMD of Oxa1 was critical for its transfer across the inner membrane into the matrix. An alignment of the first TMD of members shows that the proline residue in this position is absolutely conserved among mitochondrial Oxa1/Oxa2 proteins of animals and fungi. In contrast, in bacterial members of the family (as well as in plants) this position is variable.

Similarly, the second TMD of mitochondrial members of the Yta10/Yta12 family contains an invariant proline residue. However, whether these residues in the TMDs of Oxa1 and Yta10 homologues are conserved to improve the efficiency of conservative sorting of these proteins or because they are required for the function of these factors is not known.

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