A SUBSTRATE TRANSLOCATION TRAJECTORY IN THE MONOCARBOXYLATE/H+ SYMPORTER JEN1

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Abstract:
Previous mutational analysis of Jen1p, a Saccharomyces cerevisiae monocarboxylate/H+ symporter of the Major Facilitator Superfamily, has suggested that the consensus sequence 379NXX[S/T]HX[S/T]QD387, located in transmembrane segment VII (TMS-VII), is part of the substrate translocation pathway. In this work, we rationally design and analyse novel mutations concerning residues in TMS-V and TMS-XI. Our analysis identifies several residues critical for Jen1p function. Among these, F270 (TMS-V) and Q498 (TMS-XI) function as specificity determinants for the distinction of mono- from di-carboxylates, whereas N501 is irreplaceable for function. Using a novel theoretical model created on the basis of Jen1p similarity with GltP permease, we demonstrate that all polar residues in TMS-VII and TMS-XI, shown previously and herein to be critical for function and/or specificity (N379, H383, D387, Q498, N501), are perfectly aligned in a row along an imaginary axis that lies parallel to a protein pore. The model also predicts that the flexible side-chain of an additional polar residue, R188 in TMSII, faces the pore and subsequent mutational analysis showed that this aminoacid, similar to most polar residues of the pore, is irreplaceable for function. Finally, our model shows that the location of F270 and Q498 could justify their role in substrate specificity. Independent substrate docking approaches reveal a 'trajectory-like' displacement of the substrate within the Jen1p pore. In this inward-facing trajectory the flexible side-chain of R188 plays a major dynamic role mediating the orderly relocation of the substrate by subsequent H-bond interactions involving itself and residues H383, N501 and Q498.