An *S. cerevisiae* Peroxisomal Transporter, Orthologous to the Human Adrenoleukodystrophy Protein, Appears to Be a Heterodimer of Two Half ABC Transporters: Pxa1p and Pxa2p

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The adrenoleukodystrophy protein (ALDP) and the 70-kDa peroxisomal membrane protein (PMP70) are half ATP binding cassette (ABC) transporters in the peroxisome membrane. ALDP is defective in X-linked adrenoleukodystrophy (ALD), a neurodegenerative disorder with defective peroxisome oxidation of very-long-chain fatty acids.[1] Mutations in the PMP70 gene were found in two patients with Zellweger syndrome,[2] an inborn error of peroxisome biogenesis, though the role PMP70 plays in this disease is yet unclear. The functions and possible interactions of ALDP and PMP70 in the peroxisomal membrane are not known. To develop a system in which these questions could be addressed, we sought to clone and investigate their yeast orthologue.

Using RT/PCR with degenerate primers, we cloned a yeast gene (*PXA1*) that encodes a protein (Pxa1p) with 28% amino acid identity to the human ALDP. We showed that Pxa1p is localized to the peroxisomes, that it is involved in fatty acid β-oxidation and that, like ALDP, it is not required for peroxisome biogenesis.[3] Given the amino acid sequence similarity, peroxisomal localization, role in fatty acid oxidation and lack of an effect on peroxisomal integrity, we hypothesized that Pxa1p is the yeast orthologue of ALDP. Alignment of amino acid sequences from PMP70, ALDP, Pxa1p and other ABC transporters, reveals several blocks of amino acids which are highly conserved both in sequence and location in these proteins. We characterized two of these protein motifs; the EAA-like motif,[3] and a newly recognized, Loop 1 motif. EAA motifs were originally identified only in prokaryotic ABC transporters as conserved sequences located in the region between the fourth and fifth putative membrane-spanning domains of the protein.[4] Missense mutations in a central glycine residue, which is exceptionally conserved, were shown to cause loss of transporter function in bacteria.[5] We have reported recently that eukaryotic ABC transporters possess similar motifs, the EAA-like motifs.[3] Mutations in a conserved glutamic acid

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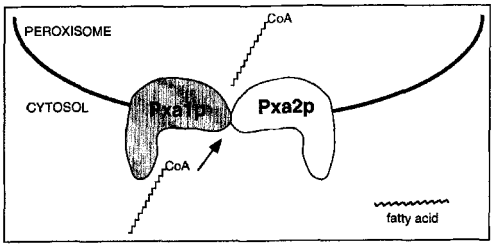

FIGURE 1. A model of the PXA transporter in the peroxisomal membrane.

residue in the EAA-like motif of the human ALD gene have been reported in four unrelated patients with ALD[5,6] (K. D. Smith, personal communication). The second region chosen for investigation is located at the end of the first putative transmembrane domain, extending into the first loop of the protein, and was named the Loop 1 motif. To analyze their importance for ABC-transporter function, we used a *PXA1* expression system; pxa1 mutant yeast exhibits impaired growth on oleic acid as a sole carbon source. Wild type growth can be restored by expressing the wild type *PXA1* gene in the *pxa1::URA3* knock-out yeast.[3] We used this expression system to access the effect of missense mutations in the two protein motifs. We found that both protein motifs are important for ABC-transporter function since missense mutations in conserved residues cause transporter dysfunction. Moreover, missense mutations in both the EAA-like and the Loop 1 motifs cause ALD in humans and Pxa1p dysfunction in yeast, indicating that the yeast protein Pxa1p is a useful system for studying the molecular basis of ALD.

PXA2, a second yeast half ABC-transporter gene, was identified by the yeast genome sequencing project.[8] We found that Pxa2p is also localized to the peroxisomes and that *PXA1* and *PXA2* have identical and nonadditive phenotypes. Furthermore, we have shown that Pxa1p is less stable in a *PXA2* mutant. These results suggest that Pxa1p and Pxa2p are two halves of the same peroxisomal ABC transporter. Based on the ALDP and Pxa1p mutation phenotype we hypothesize that the PXA transporter in yeast is involved in the transport of long- or very-long-chain fatty acids into the peroxisome. FIGURE 1 shows a model of the PXA transporter in the peroxisome membrane. This newly discovered ABC transporter may be a useful model for understanding the molecular basis of adrenoleukodystrophy and other ABC-transporter related genetic diseases.

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