We predicted in human peroxisomal membrane proteins (PMPs) the binding sites for PEX19, a key player in the topogenesis of PMPs, by virtue of an algorithm developed for yeast PMPs. The best scoring PEX19-binding site was found in the adenine nucleotide translocator protein (ALDP). The identified site was indeed bound by human PEX19 and was also recognized by the orthologous yeast PEX19 protein. Likewise, both human and yeast PEX19 bound with comparable affinities to the PEX19-binding site of the yeast PMP Pex13p. Interestingly, the identified PEX19-binding site of ALDP coincided with its previously determined targeting motif. We corroborated the requirement of the ALDP PEX19-binding site for peroxisomal targeting in human fibroblasts and showed that the minimal ALDP fragment targets correctly also in yeast, again in a PEX19-binding site-dependent manner. Furthermore, the human PEX19-binding site of ALDP proved interchangeable with that of yeast Pex13p in an in vivo targeting assay. Finally, we showed in vitro that most of the predicted binding sequences of human PMPs represent true binding sites for human PEX19, indicating that human PMPs harbor common PEX19-binding sites that do resemble those of yeast. Our data clearly revealed a role for PEX19-binding sites as PMP-targeting motifs across species, thereby demonstrating the evolutionary conservation of PMP signal sequences from yeast to man.

Peroxisomal matrix and membrane proteins are encoded by nuclear genes, synthesized on free polysomes, and imported posttranslationally. Matrix proteins possess either of two peroxisomal-targeting signals that are conserved throughout evolution (1–3). The targeting signals for PMPs,1 the so-called membrane protein-targeting signals (mPTS), have been determined for a number of proteins (4) with some PMPs possessing more than one of them (5–7). The mPTS are distinct from the targeting signals of matrix proteins and exhibit a motif with a cluster of basic and possibly hydrophobic amino acids (8–10). For human adrenoleukodystrophy protein (ALDP) and its close homologues, a conserved motif has been described recently (11). These proteins constitute half-transporters of the ABC transporter family and are involved in the ATP-dependent transport of yet to be determined metabolites across the peroxisomal membrane. Mutations in the ALDP gene are the cause for X-linked adrenoleukodystrophy, the most common peroxisomal disease (12).

To act as a functional mPTS, the basic motif described above critically depends on one or more transmembrane domains (TMDs) in its vicinity so as to insert the PMPs into the membrane (4, 5, 13–16). For a small group of PMPs, particularly PEX19, sorting does not depend on this type of mPTS (16) indicating that these PMPs might reach their final destination in the peroxisomal membrane via a different pathway that could even involve the endoplasmic reticulum (17–19).

The largely cytosolic PEX19 is required for the topogenesis of PMPs and is supposed to escort PMPs from the cytosol to the peroxisomal membrane. PEX19 interacts with all PMPs tested so far (7, 20–23), and its down-regulation by small interfering RNA reduces import of newly synthesized PMPs but not that of matrix proteins (16). Our recent characterization of PEX19-binding sites (BS) in several PMPs of Saccharomyces cerevisiae revealed that these sites have in common a motif of about 15 amino acids and constitute an integral part of the mPTS of yeast PMPs (15). Also human PEX19 was shown to bind to targeting-competent regions of several human PMPs (6, 16); however, whether human PEX19 actually binds the targeting motif, the TMD, or yet another region within the mPTS remained elusive. Furthermore, the two human PEX19-BS that have been characterized in some detail, namely those of PEX13 and PMP34, failed to reveal any obvious similarity (7, 16).

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**EXPERIMENTAL PROCEDURES**

*Strains and Media—Escherichia coli* strain DH5α was used for all plasmid amplifications and isolations. *E. coli* strains BL21(DE3)

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1 The abbreviations used are: PMP, peroxisomal membrane protein; ALDP, adrenoleukodystrophy protein; BS, binding site; TMD, transmembrane domain; mPTS, peroxisomal membrane protein-targeting signal; PTS2, peroxisome-targeting signal type 2; GFP, green fluorescent protein; GST, glutathione S-transferase.

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**FUNCTION OF THE PEX19-BINDING SITE OF HUMAN ADRENOLEUKODYSTROPHY PROTEIN AS TARGETING MOTIF IN MAN AND YEAST**

**PMP TARGETING IS EVOLUTIONARILY CONSERVED***

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**EXPERIMENTAL PROCEDURES**

*Strains and Media—Escherichia coli* strain DH5α was used for all plasmid amplifications and isolations. *E. coli* strains BL21(DE3)
(Merck, Darmstadt, Germany) and C41(DE3) (J. Walker, Medical Research Council, Cambridge, UK) were used for heterologous expression of the recombinant GST-HsPEX19 and GST-ScPex19p fusion proteins, respectively. Two-hybrid assays were performed with yeast strain PJ69-4A (P. James, Madison, WI). Localization of GFP fusion proteins was analyzed in wild-type strain UTL-7A harboring a genomically integrated copy of a peroxisome-targeting signal type 2 (PTS2)-DaRed construct (yHPR251). Standard media for the cultivation of yeast and bacterial strains were prepared as described previously (24, 25).

**Plasmids**—HsPEX19 was amplified from cDNA of human fibroblasts; ALDP and all fragments thereof were amplified from the commercially obtained cDNA clone IRAKp86110514Q2 (Resource Center and primary data base, Berlin, Germany). The primer pairs used in these PCR reactions and the vectors and restriction sites used for cloning the PCR fragments as well as the sequences of the primers are listed in Tables I and II, respectively. The sequences of all PCR-generated fragments of this study were verified by automated sequencing (MWG Biotech, Ebersberg, Germany).

**BIAcore Analysis**—Surface plasmon resonance measurements were carried out essentially as described previously (26). GST-HsPEX19 and GST-ScPex19p were coupled to CM5 sensor chips with densities of ~2,000 resonance units. In both cases, GST alone was used as a control. Interaction studies were started by injecting the peptides, dissolved in 10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, 5% MeSO, and 0.005% surfactant P20, pH 7.4, in a concentration range of 12.5–200 μM (GST-ScPex19p) and 3–50 μM (GST-HsPEX19), respectively. Data were acquired with BiacoreX equipment, and K<sub>v</sub> values were determined by applying the steady-state model using BIA-Evaluation 3.1 software (Biacore, Uppsala, Sweden).

**Morphological Analysis**—Live yeast cells were analyzed for GFP and DaRed fluorescence as described previously (15). Prior to inspection, the cells were grown for 2 days on agar plates with ethanol as sole carbon source. The human skin fibroblast (GM5756T) cell line was transfected with the pEGFP-C1-derived plasmids by using Lipofectamine (Invitrogen). The human skin fibroblast (GM5756T) cell line was transfected with the pEGFP-C1-derived plasmids by using Lipofectamine (Invitrogen) according to the manufacturer’s instructions. Cells were cultured with the pEGFP-C1-derived plasmids by using Lipofectamine (Invitrogen) according to the manufacturer’s instructions. Cells were cultured with the pEGFP-C1-derived plasmids by using Lipofectamine (Invitrogen) according to the manufacturer’s instructions. Cells were cultured with the pEGFP-C1-derived plasmids by using Lipofectamine (Invitrogen) according to the manufacturer’s instructions.

**Miscellaneous**—The prediction of PEX19-BS, the PEX19 in vitro binding assays with peptide arrays on membrane blots, including the purification of the GST-PEX19 fusion proteins from E. coli, and the yeast two-hybrid assays were carried out essentially as described previously (15, 26).

**RESULTS**

**Prediction of PEX19-BS in Human PMPs**—We recently characterized PEX19-BS in PMPs from *S. cerevisiae* (15). The outcome of this analysis was the development of a prediction matrix, which contains scores for each of the central nine amino acids of a 15-mer peptide. The total score of one peptide is the exponential of the sum of the individual amino acid scores and is a measure for the probability to represent a PEX19-BS. This matrix can be used to scan proteins for PEX19-BS with a 15-amino acid window, as shown for *S. cerevisiae* PMPs (15). To see whether our algorithm would also be useful for the prediction of such sites in higher eukaryotes, all known human PMPs were analyzed (Table III). For PEX11β, PEX13, PEX14, PEX16, PMP22, PMP34, ALDP, ALDP-related protein, and PMP70 the search results of our prediction were contained within fragments that had been reported in the literature to interact with PEX19 (6, 7, 16, 20–22, 28). Although most of these published fragments are rather large, two binding sites have been mapped in more detail; the core of one PEX19-binding sequence in PEX13 comprises amino acids 175–196 (7), containing the predicted amino acids 177–191 (Table III), and one site in PMP34 is delimited by amino acids 274–301 (16), which comprises most of the predicted amino acids (271–285, Table III), thereby indicating the conciseness of the applied algorithm.

**Identification and Characterization of the ALDP PEX19-BS**—The sequence that represents a PEX19-BS with highest probability was present in the ALDP (Table I). The site was predicted to reside at position 68–82 and apparently represents the only PEX19-BS of the protein (Fig. 1A). We synthesized synthetic 15-mer peptides designed to represent the first 200 amino acids of ALDP in an overlapping arrangement on a membrane and analyzed their ability to bind human PEX19. Although a control incubation of the peptide-containing cellulose membrane with purified GST did not result in significant binding to any of the peptides (not shown), one array of spots that is typical for true binding sites in peptide scan experiments emerged upon incubating the membrane with a purified GST-HsPEX19 fusion protein (Fig. 1B). The serial spots covered amino acids 67–87 of ALDP with the individual peptides sharing the nine-amino acid 73QRLLWLLRL81 region. Systematic truncations of the ALDP-(67–84) peptide, aimed to deter-

| Plasmid | Description | Primer pair | Source or Ref. |
|---------|-------------|-------------|---------------|
| pEGFP-C1 | Clontech | 34 |
| pUG35 | 35 |
| pPC97 | 35 |
| pPC86 | 36 |
| pHPR131 | ADH2pr-PTS2-DeRed | S-PEX19 in pCGX7T-2 | 15 |
| pKat61 | S-PEX19 in pCGX7T-2 | 15 |
| pHPR145 | S-PEX19 in pCGX7T-2 | 15 |
| pHPR262 | S-PEX19 in pCGX7T-2 | 15 |
| pKat82 | S-PEX19 in pCGX7T-2 | 15 |
| pHPR241 | S-PEX19 in pCGX7T-2 | 15 |
| pAH5 | HsPEX19 in pGEX4T-1 BamHI-Sall | RE734/735 |
| pAH7 | HsPEX19 (2–299) in pPC86 Sall-BglII | RE976/977 |
| pAH8 | ALDP (69–83) in pPC97 Xbal-BamHI | RE1034/1035 |
| pAH9 | ALDP (66–87) in pUG35 Xbal-BamHI | RE1065/1066 |
| pAH10 | ALDP (66–164) in pUG35 Xbal-BamHI | RE1065/1067 |
| pAH11 | ALDP (66–87) in pMS21 Xbal-BamHI | RE1065/1068 |
| pAH23 | ALDP (87–164) in pEGFP-C1 EcoRI-Sall | RE1166/1067 |
| pAH27 | ALDP (87–164) in pEGFP-C1 EcoRI-Sall | RE1198/1199 |
| pAH28 | ALDP (66–87) in pEGFP-C1 EcoRI-Sall | RE1200/1201 |
| pAH29 | ALDP (66–164) in pEGFP-C1 EcoRI-Sall | RE1200/1199 |

Conservation of PMP Targeting
matches with our yeast-based prediction matrix. show that ALDP-(1–200) possesses a single PEX19-BS that peptide was recognized by human PEX19. The combined data whereas expression of (GAL4 AD) fusion of (GAL4 BD) and coexpressed with a Gal4p activation domain (69–83) fragment was fused to the Gal4p DNA-binding domain two-hybrid assay was carried out. The 15-amino acid ALDP—the interaction of the ALDP fragment with PEX19, a yeast described above (Fig. 1). To obtain independent evidence for PEX19 with the ALDP site more accurately, ALDP-(67–84) assay (Fig. 2, A). To compare the binding of human and yeast PEX19 with the ALDP site more accurately, ALDP-(67–84) peptides with single proline substitutions at each position were synthesized and subjected to PEX19 interaction assays. As a control, wild-type ALDP-(67–84) peptides were synthesized. For HsPEX19, leucine residues 75, 76, 78, and 79 were sensitive to the proline exchange; all other substitutions retained HsPEX19 binding at least to some extent (Fig. 2B). Incubation of an identical blot with the yeast protein revealed that ScPEX19p also bound the peptide of human origin (Fig. 2C). To compare the binding of human and yeast PEX19 with the ALDP site more accurately, ALDP-(67–84) peptides with single proline substitutions at each position were synthesized and subjected to PEX19 interaction assays. As a control, wild-type ALDP-(67–84) peptides were synthesized. For HsPEX19, leucine residues 75, 76, 78, and 79 were sensitive to the proline exchange; all other substitutions retained HsPEX19 binding at least to some extent (Fig. 2B). Incubation of an identical blot with the yeast protein revealed that ScPEX19 also bound the peptide of human origin (Fig. 2B). Furthermore, from the proline substitutions a similar yet slightly larger core sequence (LLWLLRLF) emerged. Thus, the PEX19 proteins from both organisms are likely to

**Table II**

| PMP | Position of central 15-mer | Sequence | Score<sup>a</sup> |
|-----|--------------------------|----------|------------------|
| PEX2 | 147–161 | KLGGGLNFLIFLQRG | 7714 |
| PEX2 | 199–213 | lwGFAEFLIPLLPLNV | 7014 |
| PEX10 | 52–66 | FPFFVEKACLVWWLFTIY | 3522 |
| PEX10 | 238–252 | sGRLLGVSSLHHYLVMLGLG | 6736 |
| PEX10 | 157–171 | cGRRALEAVFVRQGLL | 4882 |
| PEX11α | 173–187 | eETWVLQGSPLLFLRS | 13,473 |
| PEX11β | 236–250 | GLCGLVSSILSITLTSypwrl | 9095 |
| PEX11β | 186–200 | pGALRLKLQVLLLVARlg | 6931 |
| PEX11γ | 126–140 | nWTLSLLMLWALLG | 3714 |
| PEX11γ | 141–155 | VARSMLWMLKLQRQL | 3024 |
| PEX12 | 58–72 | WDFDEIFTLDDLQLQQny | 10,723 |
| PEX13 | 177–191 | lkhltfKVFKFAVLRVTRIYyrllqrmlgrgrs | 2437 |
| PEX14 | 24–38 | PREDILATAVKFLQNS | 6559 |
| PEX14 | 119–133 | vwegevrvzialigkRAKVLMLWMLWPK | 6749 |
| PEX26 | 276–290 | aspsSLHFYKXLALQFWRKcacsfs | 8507 |
| PEX26 | 253–267 | fslesKSSLLAILCLVVVfDpa | 8439 |
| PEX26 | 252–267 | PDLAKRLRLLLLRLVFa | 6974 |
| PM22 | 103–117 | PRRPALLQTYFLRLYp | 4105 |
| PM22 | 18–32 | HPEPLIATAVKFLQN | 6399 |
| PM24 | 127–141 | NMYLLSRVLFALSRL | 4384 |
| PM24 | 155–169 | PPFFLTAVWVLWLFyeh | 4384 |
| PM24 | 6–20 | appQRLAVLVVNALLRK | 3550 |
| PM24 | 271–285 | lEAQILQTVTLaALMFVye | 7875 |
| PM69 | 561–585 | SLKRFHLSVLRLCGG | 8221 |
| PM69 | 316–330 | vsknaVCLVLVSCPTQLDILqett | 6342 |
| PM69 | 39–53 | smqNAMLFILTLCuLTLLeqf | 4828 |
| PM69 | 20–34 | rprlidGFLQLRQLKVLFLPSwss | 3624 |
| PM70 | 82–96 | fcKETGVLVLAVHLVR | 10,110 |
| PM70 | 59–73 | VDRVFPFLSILILIKKmvpr | 7314 |
| ALDP | 68–82 | MRWVLQRLLMLRLerpvrlic | 37,393 |
| ALDRP | 80–94 | VNADFFPQELILRKi | 19,872 |

<sup>a</sup> Arbitrary units.

**Table III**

| Conservation of PMP Targeting | Oligonucleotides used (5-3') |
|------------------------------|-----------------------------|
| RE 734 | AAAAGCTGATATCCGTCGCCCGTGAGG |
| RE 735 | AGTCGACTCCATGATCGACACTGTC |
| RE 976 | AAACGCGGCGCCGCGCTAGAAGAG |
| RE 976 | AAACGCGGCGCCGCGCTAGAAGAG |
| RE 1034 | TGGACGGCCTATCTTCGCCGCTGCTGCTCCTGGTCTCCTGGCAGATACGGG |
| RE 1035 | GATCTTAGCTGATGCGTACAGCAGAGCGGCCAGCGAGCGTACAGCAGAGCG |
| RE 1065 | AGCTTAGATTGCGTACAGCAGAGCGGCCAGCGAGCGTACAGCAGAGCG |
| RE 1066 | CGGATCAGCTGCAGCTGACAGCAAGOG |
| RE 1067 | CGGATCAGCTGCAGCTGACAGCAAGOG |
| RE 1166 | AGCTTAGATTGCGTACAGCAGAGCGGCCAGCGAGCGTACAGCAGAGCG |
| RE 1167 | CGGATCAGCTGCAGCTGACAGCAAGOG |
| RE 1167 | CGGATCAGCTGCAGCTGACAGCAAGOG |
| RE 1198 | AGCTTGAACCTATGCTGACAGCAGAGCGGCCAGCGAGCGTACAGCAGAGCG |
| RE 1199 | AGGTATGCAGCTGCAGCTGACAGCAAGOG |
| RE 1200 | AGGTATGCAGCTGCAGCTGACAGCAAGOG |
| RE 1201 | AGGCTTCACTACAGGACCGCAGGGAGAGAG |

**Table III**

Prediction of PEX19-binding sites in human PMPs

An algorithm developed for PEX19-BS in *S. cerevisiae* (15) was applied to published human PMPs. Shown are the position and the sequence (in bold) of each peak scoring peptide within a detected BS. Adjoining amino acids in lower-case letters reflect BS with additional hits in close proximity to peak scoring peptides.
Human PEX19 Binds a Yeast PEX19-BS—We then asked whether a PEX19-BS from a yeast PMP would likewise be recognized by human PEX19. For that matter, two previously identified ScPex19p-binding fragments were analyzed for interaction with HsPEX19 by using the yeast two-hybrid assay. Indeed, a ScPex13p-(173–258) fragment and a ScPex11p-(1–50) peptide (not shown) tested positive in combination with HsPEX19 and, as control, with ScPex19p (Fig. 2C). Moreover, the L207P point mutation, which specifically abolishes the ScPex13p-(173–258) interaction with ScPex19p (15), also caused a loss of HsPEX19 binding (Fig. 2C). To estimate the strength of the interaction of PEX19 with its BS, the 15-mer peptide ScPex13p-(199–213) (GIFAIMKFLKKILYR) representing a yeast PEX19-BS (15) was synthesized in chemical amounts, and its affinity for both PEX19 proteins was determined by means of surface plasmon resonance. Both proteins bound the 15-mer peptide with $K_D$ values in the micromolar range ($ScPex19p, K_D = 12 \times 10^{-6}$ M; $HsPEX19, K_D = 3.2 \times 10^{-6}$ M), whereas neither protein showed significant affinity to the unrelated peptide sequence KPTTPPKPSHLKP (Ynl020cp-(608–620)). This result indicated that the interactions of PEX19 proteins with their BS are of intermediate strength.
here to bind PEX19 largely overlaps with a recently identified motif (71–84) that is required for targeting of ALDP (11). We corroborated that in the homologous human system this motif (i.e. the PEX19-BS), when appended to two TMDs (ALDP-(66–164), is indeed sufficient for peroxisomal targeting (Fig. 3, A and B) (11). Thus, our unbiased approach for finding PEX19-BS provides strong affirmation for the role of PEX19-BSs as human PMP targeting motifs.

Because ScPex19p did bind to the PEX19-BS of ALDP, we reasoned that in yeast the mPTS of ALDP should be correctly

FIG. 3. Pex19p-dependent targeting of a human mPTS in yeast. A, graphic representation of the mPTS fragment of ALDP with the positions of the TMDs according to Ref. 11 and of the PEX19-binding site as identified here. B, targeting of ALDP in human fibroblasts. Cell line GM5756T was transiently transfected with plasmids designed to express GFP fusions of the indicated ALDP fragments. Thereafter, cells were processed for indirect immunofluorescence using polyclonal anti-HsPEX14 antibodies. Bar = 10 μm. C, PEX19-binding site-dependent peroxisomal targeting of human ALDP in yeast. GFP fusions of the indicated ALDP fragments were expressed in the S. cerevisiae wild-type strain yHPR251 that harbors an integrated copy of the synthetic peroxisomal marker PTS2-DsRed. The ability of a chimera containing the ALDP PEX19-BS (ALDP-(66–87) in the context of a yeast mPTS (Pex13p-(221–310)) to direct GFP to peroxisomes was also analyzed. The transformed strains were grown on plates containing ethanol as sole carbon source for 2 days and inspected for GFP and DsRed fluorescence. The merged images reveal eventual colocalization of the GFP fusion proteins with peroxisomal PTS2-DsRed. The position of individual cells is visualized by Nomarski images. Bar = 2 μm. DIC, differential interference contrast.
targeted to peroxisomes in a PEX19-BS-dependent manner also. To investigate whether this is really the case, a GFP fusion of an ALDP-(66–164) fragment was coexpressed with the synthetic peroxisomal marker protein PTS2-DsRed in S. cerevisiae, and cells were subjected to fluorescence microscopy. Both fluorescent proteins gave rise to one or a few rather large spots per cell that were clearly colocalized as judged from the merged image (Fig. 3C). As expected for a PMP targeting motif, upon removal of the PEX19-BS from the mPTS, targeting was compromised (ALDP-(87–164)). Expression of the isolated PEX19-BS (ALDP-(66–87)) did not lead to peroxisomal targeting of GFP, likely because this fragment could not be inserted into the peroxisomal membrane. Consistent with this interpretation, appending the ALDP PEX19-BS to a yeast Pex13p-(221–310) fragment that contains a TMD but is not able to target to peroxisomes by itself (15) restored the peroxisomal localization of the protein (Fig. 3C). These data clearly demonstrate that a human mPTS is also functional in yeast because the human PEX19-BS was recognized by yeast Pex19p.

Validation of the PEX19-BS Prediction—To check whether our predictions for human PMPs more generally reflect true PEX19-BS, peptides designed to represent most of the sites listed in Table III were synthesized. Each site was covered by an array of nine overlapping 15-mer peptides covering the putative binding region with the peak scoring sequence at the central position. 20 of the 22 analyzed predicted sites did bind HsPEX19, albeit to varying extents (Fig. 4). Thus, whether all of the weakly interacting peptides (e.g. PEX11β-(185–199)) are also recognized by HsPEX19 in vivo remains to be shown. For some sites, a shift of the peak interacting peptide from the central position was noted (e.g. PEX11α-(173–187)), whereas other sites appeared to possess two distinct peaks (e.g. PEX13-(177–191)), suggestive of multiple, neighboring PEX19-BS. In summary, the PEX19-BS of human PMPs possess a common motif that is predictable by our algorithm, which in turn indicates that the basic principle of PEX19-based signal recognition is conserved from yeast to man.

DISCUSSION

In this study, we identified PEX19-BS in human PMPs by applying an in silico search developed for yeast PEX19-BS (15) in combination with an in vitro peptide binding assay. This approach not only allowed us to provide a comprehensive picture of PEX19-BS in known human PMPs with relative ease, but it also revealed a remarkable conservation of these sites between yeast and man.

Strikingly, the PEX19-BS of ALDP turned out to be identical to its targeting motif (11). Also the published targeting motifs found in the ALDP homologues ALDR and PMP70 as well as those in the unrelated HsPMP22 and HsPEX2 (6, 8) were predicted by our algorithm to coincide with PEX19-BS, which was also proven experimentally by the PEX19 in vitro binding assays (Fig. 4). As a consequence, we propose that previously described targeting motifs of human PMPs can be embedded in the more global PEX19-BS motif. This conclusion significantly extends the postulate of PEX19-BS being present within mPTS (15, 16), as the rather large mPTS additionally possesses the information for insertion into the peroxisomal membrane.

Formation of the peroxisomal compartment is a remarkably conserved process. Most genes affected in human peroxisome biogenesis disorders have been isolated based on their similarity to the orthologous yeast genes. Furthermore, the two known targeting signals of peroxisomal matrix proteins function in basically all eukaryotic cells (29). Here we provide strong evidence that the targeting signals of PMPs are also conserved among species. A few previous reports had shown the correct insertion of PMPs into heterologous peroxisomal membranes, such as ScPex13p in human fibroblasts (30, 31). Our demonstration of the capability of HsPEX19 to recognize the PEX19-BS in ScPex13p (Fig. 2C) now provides the molecular basis for the observed targeting of this PMP to mammalian cells. We estimated the affinities of both yeast and human PEX19 for the PEX19-BS of ScPex13p by means of surface plasmon resonance. Binding of the two proteins was comparable and of intermediate strength with dissociation constants in the micromolar range. The slightly higher affinity of human PEX19 for the yeast-derived peptide is probably not significant in terms of function of the PMP receptor as both determined KD values are in the range expected for an import factor that only transiently interacts with PMPs en route to peroxisomes. The obtained values are also in agreement with a previous report in which the HsPEX19-HsPMP14 interaction was roughly quantified based on competition experiments (20).

Most interestingly, we demonstrate the ability of the mPTS of human ALDP to target the otherwise cytosolic GFP to yeast peroxisomal membranes. Also in this context, the PEX19-BS of ALDP was critical for correct targeting. Furthermore, the ALDP PEX19-BS was able to substitute a yeast PEX19-BS in the mPTS of ScPex13p and was bound by the yeast PEX19 protein in vitro and in vivo. Thus, human PMPs can be substrates for the yeast PMP targeting machinery. This is notable insofar as S. cerevisiae lacks PEX16, a component shown in higher eukaryotes to be required for PMP topogenesis (32, 33). In summary, our data are clear in that the molecular mechanisms of PMP targeting and especially the binding of PEX19 to PMP targeting signal motifs are evolutionarily conserved from yeast to man.

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Conservation of PMP Targeting

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