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PAS10 is a tetratricopeptide-repeat protein that is essential for the import of most matrix proteins into peroxisomes of *Saccharomyces cerevisiae*

(yeast/peroxisome/protein import/snap helix)

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ABSTRACT ***pas* mutants of *Saccharomyces cerevisiae* are disturbed in peroxisome assembly (*pas*) and proliferation. Here we report the characterization of the *PAS10* gene and its product (PAS10) that is essential for the import of a large subset of proteins into the peroxisomal matrix. PAS10, a protein of 69 kDa, is a member of the tetratricopeptide repeat, or snap helix, protein family, characterized by several direct repeats of a degenerate 34-amino acid motif (Sikorski, R. S., Boguski, M. S., Goebl, M. & Hieter, P. (1990) *Cell* 60, 307–317). Other members of this family are MAS70 (*S. cerevisiae*) and MOM72 (*Neurospora crassa*), which are mitochondrial receptors for protein import. A *pas10* null mutant accumulates peroxisomal, leaflet-like membrane structures and exhibits deficient import of a number of peroxisomal matrix enzymes, particularly of proteins with an SKL-like import signal. In contrast, 3-ketoacyl-CoA thiolase associated with these membranes is resistant *in vitro* to degradation by proteinase K, indicating true protein import. These results suggest that PAS10 is an essential component of a peroxisomal import machinery which mediates the translocation of a specific subset of proteins to the peroxisomal matrix with an SKL-like import signal.**

Peroxisomes are organelles of almost all eukaryotic cells and contribute in various ways to cellular metabolism. Common properties include a β-oxidation pathway for the degradation of long-chain fatty acids, a number of hydrogen peroxide-generating enzymes, and catalase to remove the hydrogen peroxide produced. That peroxisomes are essential to cellular function is underlined by the existence of human genetic diseases resulting from peroxisomal malfunctioning, such as the cerebro-hepato-renal or Zellweger syndrome and related diseases. An understanding of these peroxisomal diseases at the molecular level is hampered by lack of knowledge about the maintenance and formation of the organelles and the still incomplete inventory of their enzymatic content (reviewed in refs. 1–3).

The current view is that peroxisomes grow by posttranslational uptake of proteins from the cytoplasm and that new peroxisomes arise by fission of preexisting organelles (4). A major breakthrough in understanding protein import was the discovery that firefly luciferase was targeted to the matrix space of peroxisomes in African green monkey cells (5). This enabled Subramani and coworkers (6) to delineate the first topogenic signal, the carboxyl-terminal Ser-Lys-Leu (SKL) motif, capable of targeting proteins into peroxisomes. This peroxisome targeting signal 1 (PTS-1) is present in many other peroxisomal proteins and is universally used in higher and lower eukaryotes ranging from animals and plants to yeast (7, 8). More recently, an amino-terminally located import signal (PTS-2) was discovered in rat 3-ketoacyl-CoA thiolase (thiolase) (9, 10), suggesting an unexpected complexity of the peroxisomal protein import system. How membrane proteins are targeted to peroxisomes and how the organelles acquire the lipid components for membrane growth are still largely unknown (for a review see ref. 11).

To identify proteins required for maintenance and assembly of peroxisomes, we and others have successfully applied the genetics of *Saccharomyces cerevisiae* to isolate mutants disturbed in peroxisome assembly (*pas* mutants) (12–14) and have used these mutants to clone the corresponding wild-type genes by functional complementation. Some *PAS* gene products are involved in the transcriptional control of genes coding for peroxisomal proteins, others contribute more directly to peroxisome biogenesis. However, for most of the PAS proteins identified thus far, precise functional roles remain elusive, even in cases where relationships with other protein families have been established.

Recently, we have described the morphology of a mutant (*pas10-1*) in which normal, round, electron-dense peroxisomes are absent but leaflet-like membranes accumulate after induction of peroxisome proliferation by oleic acid (13). These membranes are of peroxisomal origin, since they can be marked by immunogold cytochemistry using an antibody raised against thiolase. Here we report the sequence of the *PAS10* gene† and an analysis of the deficiencies of a disruption mutant. Comparison of the derived amino acid sequence with a protein data library identifies the PAS10 protein as a member of the tetratricopeptide-repeat (TPR) family, which is characterized by the presence of multiple direct repeats of a degenerate consensus sequence (15). The *pas10* null mutant is deficient in the import of proteins containing SKL-like targeting signals but not in the import of thiolase. We discuss the possible function of PAS10 in the import of proteins into the peroxisomal matrix.

MATERIALS AND METHODS

Strains. Strains used were the *S. cerevisiae* HR2 (*MATα, leu2, his4, trp1::URA3*), BJ1991 (*MATα, leu2, trp1, ura3-52, prb1-1122, pep4-3*) (16), and *pas10-1* (*MATα, leu2, his4, trp1::URA3*) (13) and *Escherichia coli* DH5α (*recA, hsdR, supE, endA, gyrA96, thi-1, relA1, lacZ*).

Culture conditions. Yeast media, except for glycerol plus glucose-containing medium, and yeast culture conditions were as described (13). Glycerol plus glucose-containing medium consisted of yeast nitrogen base without amino acids (6.7 mg/ml) (Difco), amino acids (20 μg/ml) as needed, glycerol (20 mg/ml), and glucose (1 mg/ml).

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Abbreviations: PTS, peroxisome targeting signal; thiolase, 3-ketoacyl-CoA thiolase; TPR, tetratricopeptide repeat.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. L23076).

A

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                                                5' AATATGGTCGTTAGTCGACTG ATTGTAGCTAACCAGAACGGTAGGCAGGAC ATACAATTAAGAAGCCGAGGATAACTGGAA   81
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ATGGACGTAGGAAGTTGCTCAGTGGGAAAT AATCCGCTTGCGCAGTTGCACAAACATACT CAGCAGAACAAATCGCTTCAGTTTAATCAG AAGAATAATGGGCGTCTTAATGAGTCACCT   441
1  MetAspValGlySerCysSerValGlyAsn AsnProLeuAlaGlnLeuHisLysHisThr GlnGlnAsnLysSerLeuGlnPheAsnGln LysAsnAsnGlyArgLeuAsnGluSerPro

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41 LeuGlnGlyThrAsnLysProGlyIleSer GluAlaPheIleSerAsnValAsnAlaIle SerGlnGluAsnMetAlaAsnMetGlnArg PheIleAsnGlyGluProLeuIleAspAsp

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121 SerGlnGluPheGlnGlySerAsnSerIle GlnAsnArgAsnAlaAspThrGlyAsnSer GluLysAlaTrpGlnArgGlySerThrThr AlaSerSerArgPheGlnTyrProAsnThr

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201 GlnGlnProTrpThrAspGlnPheGluLys LeuGluLysGluValSerGluAsnLeuAsp IleAsnAspGluIleGluLysGluGluAsn ValSerGluValGluGlnAsnLysProGlu

ACTGTTGAGAAGGAAGAAGGAGTATATGGA GATCAGTATCAATCTGATTTCCAAGAAGTG TGGGATAGCATACACAAGGACGCTGAAGAA GTCTTGCCATCCGAATTAGTTAATGATGAC   1161
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CTCAATCTAGGAGAAGACTACTTGAAATAT CTCGGCGGTAGAGTAAATGGGAACATCGAG TATGCTTTTCAATCTAACAACGAATATTTT AATAATCCTAATGCTTATAAAATTGGCTGC   1281
281 LeuAsnLeuGlyGluAspTyrLeuLysTyr LeuGlyGlyArgValAsnGlyAsnIleGlu TyrAlaPheGlnSerAsnAsnGluTyrPhe AsnAsnProAsnAlaTyrLysIleGlyCys

CTACTGATGGAAAACGGAGCCAAATTGAGC GAGGCAGCGCTAGCATTTGAAGCTGCTGTT AAAGAAAAACCGGACCATGTGGATGCATGG CTAAGATTGGGTCTAGTACAAACCCAGAAT   1401
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401 PheThrMetLeuAspLysTrpAlaGluThr LysTyrProGluIleTrpSerArgIleLys GlnGlnAspAspLysPheGlnLysGluLys GlyPheThrHisIleAspMetAsnAlaHis

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441 IleThrLysGlnPheLeuGlnLeuAlaAsn AsnLeuSerThrIleAspProGluIleGln LeuCysLeuGlyLeuLeuPheTyrThrLys AspAspPheAspLysThrIleAspCysPhe

GAAAGTGCGTTGAGGGTGAATCCTAATGAC GAACTCATGTGGAATAGATTAGGGGCTTCA TTGGCCAATTCCAATAGATCAGAGGAAGCA ATCCAAGCCTATCATAGGGCACTACAACTA   1881
481 GluSerAlaLeuArgValAsnProAsnAsp GluLeuMetTrpAsnArgLeuGlyAlaSer LeuAlaAsnSerAsnArgSerGluGluAla IleGlnAlaTyrHisArgAlaLeuGlnLeu

AAACCTTCTTTTGTTAGAGCTCGCTATAAT CTGGCGGTATCATCCATGAATATAGGCTGT TTCAAAGAAGCAGCAGGCTACTTATTAAGT GTTCTAAGTATGCATGAAGTGAACACTAAT   2001
521 LysProSerPheValArgAlaArgTyrAsn LeuAlaValSerSerMetAsnIleGlyCys PheLysGluAlaAlaGlyTyrLeuLeuSer ValLeuSerMetHisGluValAsnThrAsn

AATAAAAAAGGAGACGTTGGATCTCTCTTG AATACGTACAATGATACTGTTATAGAGACT TTGAAGAGAGTTTTTATAGCGATGAATAGA GATGATTTACTTCAAGAAGTGAAGCCAGGC   2121
561 AsnLysLysGlyAspValGlySerLeuLeu AsnThrTyrAsnAspThrValIleGluThr LeuLysArgValPheIleAlaMetAsnArg AspAspLeuLeuGlnGluValLysProGly

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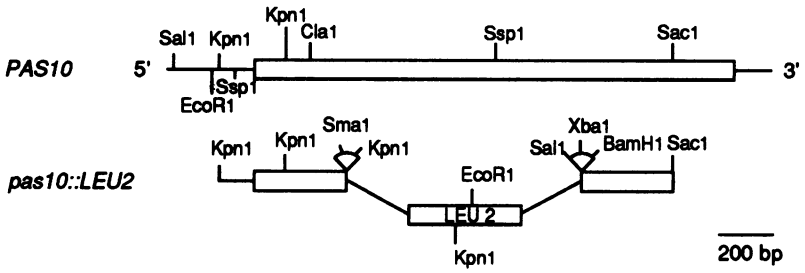
B



C Consensus sequence

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Ala Glu Ala Trp Phe Gly Leu Gly His Ile Tyr Glu Lys Leu Gly Asp Leu Glu Lys Ala Leu Asp Ala Phe Gln Lys Ala Leu Leu Leu Asp Pro Asn Asn

64  Asn Met Ala Asn Met Gln Arg Phe Ile Asn Gly Glu Pro Leu Ile Asp Asp Lys Arg Arg Met Glu Ile Gly Pro Ser Ser Gly Arg Leu Pro Pro Phe Ser
313 Pro Asn Ala Tyr Lys Ile Gly Cys Leu Leu Met Glu Asn Gly Ala Lys Leu Ser Glu Ala Ala Leu Ala Phe Glu Ala Ala Val Lys Glu Lys Pro Asp His
347 Val Asp Ala Trp Leu Arg Leu Gly Leu Val Gln Thr Gln Asn Glu Lys Glu Leu Asn Gly Ile Ser Ala Leu Glu Glu Cys Leu Lys Leu Asp Pro Lys Asn
381 Leu Glu Ala Met Lys Thr Leu Ala Ile Ser Tyr Ile Asn Glu Gly Tyr Asp Met Ser Ala Phe Thr Met Leu Asp Lys Trp Ala Glu Thr Lys Tyr Pro Glu
418 Arg Ile Lys Gln Gln Asp Asp Lys Phe Gln Lys Glu Lys Gly Phe Thr His Ile Asp Met Asn Ala His Ile Thr Lys Gln Phe Leu Gln Leu Ala Asn Asn
457 Pro Glu Ile Gln Leu Cys Leu Gly Leu Leu Phe Tyr Thr Lys Asp Asp Phe Asp Lys Thr Ile Asp Cys Phe Glu Ser Ala Leu Arg Val Asn Pro Asn Asp
491 Glu Leu Met Trp Asn Arg Leu Gly Ala Ser Leu Ala Asn Ser Asn Arg Ser Glu Glu Ala Ile Gln Ala Tyr His Arg Ala Leu Gln Leu Lys Pro Ser Phe
525 Val Arg Ala Arg Tyr Asn Leu Ala Val Ser Ser Met Asn Ile Gly Cys Phe Lys Glu Ala Ala Gly Tyr Leu Leu Ser Val Leu Ser Met His Glu Val Asn
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FIG. 1. Characteristics of the *PAS10* gene. (*A*) Nucleotide and predicted amino acid sequence of a 2.2-kb genomic DNA fragment encoding PAS10. Amino acid residues are numbered at left; nucleotide positions are numbered at right. The potential polyadenylylation signal is indicated by a single underline. (*B*) Restriction map of *PAS10* and the *pas10*::*LEU2* disruption construct. (*C*) Alignment of the eight tetratricopeptide repeats present in PAS10 with the degenerate, 34-amino acid consensus motif characteristic for TPR proteins (15). The first amino acid of each repeat is numbered on the left. Those amino acids which are highly conserved among all TPR proteins (34) are boxed; those that are conserved among only a few TPR proteins or within the repeats of PAS10 itself are circled (34).

Plasmids. Plasmids used were YCplac33KAN^R [which contains the *Bgl* II kanamycin-resistance (KAN^R) fragment of pBL2 (17) cloned into the *Aat* II site of YCplac33 (18)], pUC19 (19), and pEL54 [which contains the luciferase coding region behind the catalase promoter (14)].

Gene Cloning, Sequence Analysis, and Disruption. The *PAS10* gene was cloned by complementation of the *pas10-1* oleic acid-negative phenotype, using a *S. cerevisiae* genomic library constructed in YCp50 (20). Initial Ura$^+$ transformants were selected on plates containing glycerol plus glucose and subsequently replica plated onto plates containing minimal oleic acid medium. Complementing plasmids were rescued in *E. coli* by transformation using a Bio-Rad electroporator according to the instruction manual. The 15- to 20-kb genomic insert was reduced in size by a partial *Sau*3A1 digestion and the resulting fragments were cloned in the *Bam*HI site of the YCplac33KAN^R vector. The plasmids containing *PAS10* were selected from the recombinant ones by another round of functional complementation and subsequent rescue in *E. coli*.

The smallest complementing DNA fragment was sequenced double-stranded with the aid of several appropriate BAL-31 deletion clones with [α-^{32}P]dATP by the dideoxy chain-termination method (21). The obtained nucleic acid and protein sequences were compared with the GenBank data base by the FASTA program (22). A comparison between the *S. cerevisiae* PAS10 and *Pichia pastoris* PAS8 amino acid sequences was performed by the use of the Wisconsin Genetics Computer Group software (settings: gap weight, 6; length weight, 0.2).

A gene disruption construct was made in pUC19 from two appropriate BAL-31 deletion clones that contained parts of respectively the 5′ and 3′ ends of the *PAS10* gene between which the *LEU2* gene was cloned (Fig. 1*B*). The disruption construct, with a deletion of codons 123–420, was cut with *Pst* I and *Sac* I and subsequently transformed to BJ1991.

Enzyme Assays. Enzyme activities were measured at room temperature, or at 28°C in the case of multifunctional enzyme, by published procedures: catalase (23); cytochrome-*c* oxidase (24); 3-hydroxyacyl-CoA dehydrogenase (an activity of multifunctional enzyme) (25), and luciferase (26).

Antibodies. Antibodies used were polyclonal antibodies directed against either denatured thiolase [gift of W. H. Kunau (26)], luciferase [gift of E. Wiemer (27)], or commercially available monoclonal antibodies directed against the c-Myc epitope (Sanbio, Uden, The Netherlands).

Localization of Proteins by Subcellular Fractionation. Subcellular fractionation of a spheroplast homogenate and subsequent measurement of the distribution of the peroxisomal proteins over obtained fractions were carried out as described (13). Fractions obtained were a spheroplast homogenate, a 15,000 × *g* organellar pellet, and a supernatant representing the cytosol. Proteinase K treatments were performed on lysed spheroplasts as described (28), but with half the amount of proteinase K-agarose beads and 0.15% Triton X-100. Western blots were analyzed with a PhosphorImager (Molecular Dynamics).

Other Procedures. Published procedures were used for SDS/PAGE (29), Western blotting (30), determination of protein concentrations (31), preparation of cryothin sections of oleic acid-induced cells fixed with 2% paraformaldehyde and 0.5% glutaraldehyde (8), immunolabelling of thin cryosections (32), yeast mating (33), and yeast transformation (18). Plasmid isolation, recombinant DNA methods, and construction of the BAL-31 deletion clones of *PAS10* were performed according to ref. 19.

RESULTS

Cloning, Sequencing, and Disruption of the *PAS10* Gene. The *PAS10* gene was cloned by functional complementation of *pas10-1* and the nucleotide sequence of a 2.2-kb genomic fragment containing *PAS10* was determined (Fig. 1*A*). The longest open reading frame codes for a hydrophilic protein of 612 amino acids with a calculated molecular mass of 69.3 kDa. To construct a *pas10* null mutant most of this reading frame was replaced by the *LEU2* gene and the disruption construct (*pas10*::*LEU2*) was integrated into the genome of wild-type cells by homologous recombination (Fig. 1*B*). The integration site of the construct in the *PAS10* locus of the *pas10*::*LEU2* strain was verified by Southern blot analysis using a *PAS10* probe (data not shown). Crossing of *pas10*::*LEU2* with *pas10-1* yielded diploids that were unable to grow on oleic acid-containing plates (data not shown). This indicated that the cloned gene was indeed *PAS10* and not an extragenic suppressor of *pas10-1*. *PAS10* mRNA was induced in wild-type cells grown on oleic acid-containing medium (peroxisome induction) compared with cells grown on glycerol or glucose (peroxisomal derepression and repression, respectively) (Fig. 2). This result supports a direct contribution of PAS10 to peroxisome biogenesis and functioning.

Pas10 Belongs to the TPR Protein Family. The *PAS10*-encoded open reading frame was compared with the GenBank data base by the FASTA program (22). It is homologous to a number of proteins that belong to the TPR (or snap-helix) protein family. These proteins are characterized by the presence of several repeats of a degenerate 34-amino acid consensus sequence arranged as tandem arrays (15). Based on the consensus amino acid sequence defined by Sikorsky *et al.* (15) and the alignments as presented by Goebl and Yanagida (34), PAS10 contains seven tandemly arranged TPR units in its carboxyl-terminal half. On average, 11 amino acids per TPR motif (Fig. 1*C*) are similar to amino acids conserved between either (*i*) the repeats of all TPR proteins (indicated with a square) or (*ii*) the repeats of PAS10 itself (indicated with a circle according to conventions specified in ref. 34). An additional but less conserved motif is found in the amino-terminal part (Fig. 1*C*). Recently, the DNA sequence of a *PAS10* homologue in *P. pastoris* was reported, *PAS8* (35). A comparison of the two amino acid sequences shows an overall similarity of 57% and an identity of 37%. The similarity is much higher, however, in the carboxyl-terminal halves, which contain the repeated TPR motifs.

Biochemical and Electron Microscopic Characterization of the *pas10*::*LEU2* Disruption Strain. Electron microscopic characterization of the *pas10*::*LEU2* strain showed a morphology which was similar to that of the *pas10-1* mutant (13). Round, electron-dense peroxisomes, as present in wild-type cells, are absent from the *pas10*::*LEU2* strain. Instead, a peroxisomal membrane structure is present which can be stained by immunogold cytochemistry using an anti-thiolase antibody.

To investigate whether thiolase was imported into the peroxisomal membrane structures or merely adhered to its cytosolic surface, we studied the protection of thiolase against treatment with protease *in vitro*. Experiments were carried out with lysed spheroplasts from which large debris was removed by a short low-speed spin. Fractionation into supernatant and organelle-containing pellet fractions was avoided to minimize damage to peroxisomal membranes. In a homogenate of the wild-type strain BJ1991, thiolase was protected against proteinase K degradation (Fig. 3). In the presence of Triton X-100 a degradation product of thiolase accumulated within 5 min, and a substantial amount of intact thiolase had disappeared after 20 min. A pattern of protease protection identical to that of the wild type was observed in the homogenate of the *pas10* mutant. However, in lysates of the *pas7* mutant, thiolase was extremely vulnerable to attack by proteinase K, in accordance with the observed lack of import of thiolase into peroxisomes of this mutant (13, 36). The results indicate that thiolase in the *pas10*::*LEU2* strain is

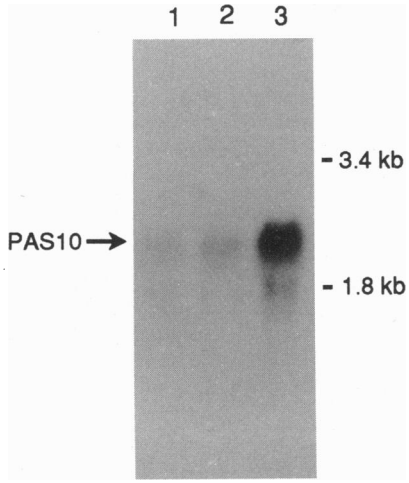

FIG. 2. Northern analysis of *PAS10* mRNA produced in wild-type yeast cells grown on medium containing glucose (lane 1), glycerol (lane 2), or oleic acid (lane 3). Ten micrograms of total RNA was loaded in each lane.

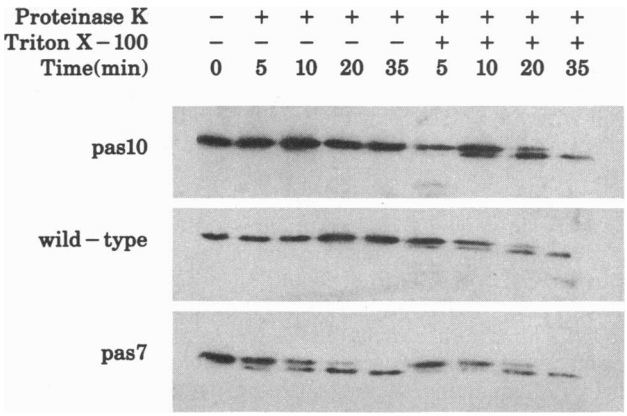

FIG. 3. Proteinase K protection analysis of thiolase in spheroplast homogenates of wild-type cells and *pas* mutants. Homogenates of oleic acid-induced cells were treated with proteinase K coupled to agarose beads for various times in the absence or presence of Triton X-100. Remaining proteins were blotted to nitrocellulose and thiolase was detected with a specific antibody.

protected from degradation and prove that the peroxisomal remnants are still competent to import thiolase.

Other matrix proteins such as multifunctional enzyme, catalase A, and heterologously expressed luciferase, although present in (almost) normal amounts, were predominantly located in the supernatant fraction after subcellular fractionation of *pas10::LEU2* homogenates. This is in contrast to their normal presence in the organellar pellet fraction obtained from wild-type cells (Fig. 4). These biochemical results indicate a differential protein-import incompetence caused by the lack of PAS10.

Is PAS10 Associated with Peroxisomes? To study the subcellular location of PAS10, we have added extensions to the carboxyl-terminus of the complete PAS10 protein that can be recognized immunologically: the c-Myc epitope or the last 105 amino acids of luciferase containing a defective import signal. Introduction of these PAS10 fusion constructs on low- or high-copy-number expression vectors in the *pas10-1* or the null mutant resulted in functional complementation. The corresponding proteins could be observed on Western blots when cells were broken with glass beads in the presence of trichloroacetic acid. However, during subcellular fractionation of spheroplast homogenates the PAS10 derivatives were rapidly degraded. In electron microscopic analysis using antibodies recognizing the added epitopes, gold labeling could be observed only after overexpression of the proteins, complicating the interpretation of these results.

DISCUSSION

Two amino acid sequence motifs, PTS-1 and PTS-2, have been identified that serve to target proteins to the peroxisomal matrix. PTS-1 consists of the carboxyl-terminal tripeptide SKL or a variant thereof and was first identified in firefly luciferase (6). The other, PTS-2, is found in the amino-terminal part of rat thiolase (9, 10), but its precise composition remains to be further established. Recently we described a peroxisome assembly mutant of *S. cerevisiae, pas10-1*, that is unable to translocate a typical PTS-1-containing protein such as luciferase across the peroxisomal membrane (13).

We have now cloned the *PAS10* gene by functional complementation of the mutant and determined its sequence. The protein of 612 amino acids encoded by *PAS10* is a member of the TPR (or snap-helix) protein family. TPR proteins are characterized by several direct repeats of a degenerate 34-amino acid motif (15). PAS10 contains seven of those tandemly arranged repeats in the carboxyl-terminal half and possibly one repeat located near the amino terminus of the protein. Members belonging to this family are involved in, for instance, mitosis (CDC23, CDC16, nuc2$^+$, and BimA), transcription regulation (SSN6 and SKI3), splicing (PRP6), stress response (STI3), and mitochondrial protein import (MAS70 and MOM72) (34). Computer graphic modeling has shown that TPR domains could be involved in the formation of a specific tertiary structure (37). In this model, the knob and hole domains formed by the TPR backbone associate with each other intra- or intermolecularly, resulting in coiled-coil or double-helix-like interactions.

To analyse the function of PAS10, we have studied the biochemical properties of the null mutant and have found that PAS10 contributes to the import of a subset of matrix proteins into peroxisomes, some of which are PTS-1-containing proteins. At least one matrix protein, thiolase, is still imported in the *pas10::LEU2* mutant as revealed by protease protection experiments. Although the targeting signal of yeast thiolase has not been identified, the sequence similarity with rat thiolase suggests that it is a PTS-2-containing protein (9). Thus, the absence of PAS10 in a gene-disruption strain gives rise to a differential block in peroxisomal protein import. A phenotype opposite to the *pas10* phenotype is observed in

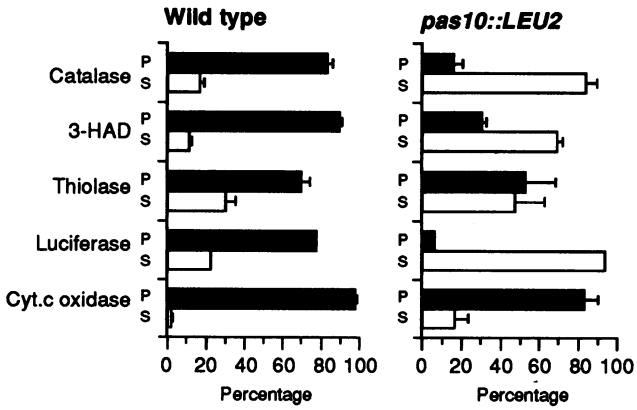

FIG. 4. Distribution of the peroxisomal matrix enzyme thiolase, catalase A, 3-HAD (the 3-hydroxyacyl-CoA dehydrogenase activity of the multifunctional enzyme), and luciferase and the mitochondrial enzyme cytochrome-*c* oxidase (control) over the organellar pellet (P) and supernatant (S) fractions which were obtained from wild-type and *pas10::LEU2* cells. Wild-type and *pas10::LEU2* cells were transformed in some cases with a luciferase reporter plasmid.

pas7 (13, 36). In this mutant, thiolase is located in the cytosol while all other matrix proteins tested are imported into peroxisomes which are morphologically similar to wild-type organelles. These differential import deficiencies correlate well with the presence of the two different classes of PTS, PTS-1 and PTS-2. We conclude therefore that at least two independent pathways exist to import matrix proteins into peroxisomes and that in the *pas10* mutant a major import pathway, involving PTS-1-containing proteins, is disturbed.

In considering a possible function of PAS10, a comparison with the only other extranuclear TPR proteins MOM72 (*N. crassa*) and MAS70 (*S. cerevisiae*) is attractive. MAS70 and MOM72 proteins are anchored via a hydrophobic membrane-spanning region in the mitochondrial outer membrane with their TPR domains sticking into the cytoplasm. They are proposed to function as receptors or facilitators for the import of some mitochondrial proteins (38, 39). A receptor-like function requiring a position on the outside of the peroxisomal membrane cannot simply be deduced from the primary amino acid sequence of PAS10. In contrast to MAS70 and MOM72, the amino acid sequence of PAS10 does not show the presence of putative membrane-spanning regions, nor does it show carboxyl-terminal amino acid sequence motifs required for prenylation which could lead to membrane association. It has been suggested that the TPR repeats themselves may form α-helices resulting in hydrophobic surfaces which could lead to membrane association (40), although this is certainly not the case for MOM72 and MAS70. Alternatively, PAS10 could interact with a partner protein located in the peroxisomal membrane. In this context it is remarkable that on the basis of direct physical interaction studies and genetic evidence, some TPR representatives cooperate in combination with a protein of the so called β-transducin protein family (34). Conceivably, PAS10 also can interact with such a partner protein located in the peroxisomal membrane. Recently, a homologue of *PAS10* was isolated from *P. pastoris* by McCollum *et al.* (35), called *PAS8*. The encoded protein subfractionated with peroxisomal membranes and interacted with an SKL-containing peptide, supporting a receptor-like function in protein import.

In summary, we have shown here that *PAS10* codes for a component of the machinery responsible for the import of peroxisomal matrix proteins, especially those targeted by an SKL-like signal. How it associates with the peroxisomal membrane and to which face of the membrane it is bound remain to be established, to define its precise role in the protein import process.

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