ELSEVIER

Biochimica et Biophysica Acta 1539 (2001) 173–180

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

www.bba-direct.com

Short sequence-paper

Identification and characterisation of PEX6 orthologues from plants

Claude P. Kaplan [1,2], Josie E. Thomas [1], Wayne L. Charlton, Alison Baker *

Centre for Plant Sciences, LIBA, University of Leeds, Leeds LS2 9JT, UK

Received 8 February 2001; received in revised form 22 March 2001; accepted 26 March 2001

Abstract

The sunflower (*Helianthus annuus*) orthologue of PEX6, an AAA ATPase essential for the biogenesis of peroxisomes in yeasts and mammals, was isolated. HaPex6p is immunologically related to *Pichia pastoris* Pex6p. Like other genes involved in peroxisome biogenesis and function HaPEX6 mRNA and protein levels peak in early post-germinative growth and mRNA levels also increase in senescent tissue. HaPEX6 identifies probable orthologues in *Arabidopsis* and rice.

Keywords: Peroxin 6; PEX gene; Peroxisome biogenesis; Germination; Glyoxylate cycle

Peroxisome biogenesis is a complex and still poorly understood process that requires the function of at least 23 protein products (peroxins) encoded by the PEX genes [1]. Two of these genes, PEX1 and PEX6, encode members of the AAA family of ATPases, a large superfamily of proteins involved in the ATP-dependent rearrangement of protein complexes [2]. As is the case for many peroxins the functions of Pex1p and Pex6p are uncertain. Yeast and mammalian cells defective in either of these proteins have small peroxisomes with reduced matrix content and show reduced stability of the receptor (Pex5p) required for the targeting and import of matrix proteins with the C-terminal PTS-1 signal [3,4]. Pex1p and Pex6p interact physically and genetically [5,6] and this interaction is essential for peroxisome biogenesis [7] but there are conflicting reports concerning their cellular location and their function. In *Yarrowia lipolytica* a model of peroxisome growth by membrane fusion has been proposed in which Pex6p performs an essential priming function at an early stage of biogenesis [8]. In contrast in *Pichia pastoris*, epistatic analysis of mutants places Pex1p and Pex6p function after the translocation of matrix proteins across the peroxisome membrane [9].

Although it is presently unclear how these two very different models of Pex1p and Pex6p function can be reconciled, evidence is emerging that the details of peroxisome biogenesis and precise functions of peroxins may not be the same in all species. For example Pex16p plays an essential role in biogenesis of the peroxisome membrane in mammals but not in *Yarrowia*, and is completely lacking from the *Saccharomyces cerevisiae* genome [10]. Likewise the *Caenorhabditis elegans* genome is lacking the PEX7 gene which encodes the receptor for a second import pathway for matrix proteins [11]. Compared to yeasts and mammals, only PEX1 [12], PEX5 [13,14] and PEX14 [15] from plants have been characterised. Mutation

* Corresponding author. Fax: +44-113-233-3144; E-mail: a.baker@leeds.ac.uk

[1] The first two authors made equal contributions to this work.

[2] Present address: The Technology Partnership plc, Melbourn Science Park, Cambridge Road, Melbourn, Royston, Hertfordshire SG8 6EE, UK.

0167-4889/01/$ – see front matter
PII: S0167-4889(01)00091-X

of a putative *Arabidopsis* PEX16 orthologue SSE1 resulted in an abnormality of seed storage reserve deposition but no peroxisomal phenotype was reported [16]. Plant peroxisomes are involved in a number of roles that are essential and in some cases unique, such as photorespiration [17] and formation of the plant growth regulator indole acetic acid from indole butyric acid [18]. Given that the complement and function of PEX genes may differ in different species, and the important physiological roles of plant peroxisomes, the isolation and characterisation of plant orthologues of PEX genes will provide important insights into these processes and provide tools for their biotechnological manipulation.

Sunflower seeds (Tall single, Saunders seed merchants, Cambridge, UK) were sterilised 10 min in 0.5% hypochlorite solution and grown in vermiculite at 22–24°C in a 16/8 h light/dark cycle. RNA was isolated according to [19] except that after the first phenol/chloroform extraction DNA was selectively degraded by the addition of 3 M sodium acetate pH 3.4 followed by ethanol precipitation and washing of the pellet with sodium acetate and 70% ethanol. Poly(A)$^+$ RNA was isolated by oligo(dT) chromatography. A 2 day sunflower seedling cDNA library was prepared using the ZAP-cDNA Synthesis kit (Stratagene) following the manufacturer's instructions. Sunflower RNA was separated by denaturing formaldehyde gel electrophoresis. Nucleic acid transfer and hybridisation were according to the Hybond-N protocol book (Amersham).

Degenerate primers S1 5′-*GGGGAATTC*TGGGANGA(T/C)(A/G)TNGG-3′, AS2 5′-*GGGGGAT-CC*(G/C)CNA(T/C)(G/A)TACAT(G/A)TT-3′ and AS3 5′-*GGGAAGCTT*GTICCNGGNGGNCC-3′ were designed based on the sequences of *S. cerevisiae* (L20789) and *P. pastoris* Pex6p (Z22566) and a sequence reported from the 5′-UTR of the maize gene B-peru (X70791) [20]. This was identified in a DNA database search as having weak homology to several members of the AAA family, but especially PEX6. Close examination of the B-peru sequence reveals that there is a piece of DNA comprising four exons bounded by well conserved plant splice junctions that has the potential to encode 93 amino acids with high similarity to Pex6 proteins. Most likely this is a part of *Zea mays* PEX6 that has been isolated due to a cloning artefact.

Total RNA from 2 day seedling cotyledons was used for RT-PCR with primers S1 and AS2. The first five cycles were performed with an annealing temperature of 36°C followed by 35 cycles with an annealing temperature of 61°C. PCR products of the expected size were eluted from a polyacrylamide gel and re-amplified with S1 and AS3 using the same cycling conditions. The products of the second PCR reaction were purified, cloned and sequenced. The PCR product corresponding to HaPEX6 was used to screen the sunflower cDNA library.

A 20-mer peptide CAELKKYEMLRDQFEGASSK-COOH corresponding to the carboxy-terminus of HaPex6p was synthesised coupled to bovine serum albumin with *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester and used to immunise a sheep. For affinity purification of anti-HaPex6p antibodies the peptide was coupled to a Sulpholink column (Pierce) and antibodies isolated according to the manufacturer's protocol. An antibody recognising the second AAA domain was raised in rabbit by immunisation of a fusion protein comprising a functional IgG binding domain of *Staphylococcus aureus* protein A (162 amino acids) and residues 624–669 of HaPex6p. For immunoblotting 10 sunflower seedlings from each time point were ground in liquid nitrogen, resuspended in SDS sample buffer, heated for 5 min at 95°C and centrifuged at $2292 \times g$ for 15 min. 50 μl of the soluble fraction was analysed by SDS–PAGE, blotted with affinity-purified anti-HaPex6p (1:100 dilution relative to the original serum) and detected by enhanced chemiluminescence. For pre-incubation with peptide a 1:10 dilution of antibody was mixed with 500 μg/ml peptide, incubated for 30 min at 37°C then diluted 10-fold and applied to the membrane. In vitro transcription–translation and immunoprecipitation were carried out according to [21]. Sunflower cotyledon homogenates were prepared and protein and enzymes assayed as described [22].

A 135 bp fragment of HaPEX6 was amplified by semi-nested RT-PCR and used as a probe to screen a sunflower cDNA library. Two clones were isolated: Hap8.2 which contained a ca. 3.5 kb insert and Hap8.3 which contained an insert of 2.7 kb. Restriction mapping and sequencing revealed that both clones contained double inserts but that Hap8.2 contained a 2.5 kb *Eco*RI fragment corresponding to the

1 IFAESSMVGR RKPLVLSSTK SLINSVLNSS NKLNTNGIDG APINKHSSGD

51 VDGSETTALQ LRAGILRLSD EKLGLSRQKA ISLDEAALIG LSTSLLKRLS

101 ITSGSLVIVK NVDSQIQRIS QIVALDPPNV HDVSANNELL SYNASNAMVV

151 FPSVTYPLIH HVPLASEVAY VSPLLAFNLG LHTSCLKLLL HHGEDKLASL

201 FEVKGDNESD LETNEGFSVN LDIKPLTKLP SLKIVNKKID IALNEYFTID

251 RYLSRGDVFS IRVNWSCKSA MCIACSQNNQ NSRDTNLYFK VVAAEPSEEP

301 VLRINRTETA LVLSASTPSA IPPNLLVNKK KGFSPLHQDT VKTLASIITP

351 TLCPSALSSK FRVAVLLFGL PGCGKRTVVK HVAHQLGLHV VEYSCHDLVA

401 SSEMENFCHV DSSFHCCSYS PTILLLRHFD AFSNLSSNDG SPNDQIGVNS

451 EVASVIREFT EPFTQDEDDY EDEDEAEH***M***N STRAIYTHPV LLVASADNSE

501 GLPPTIRRCF SHE***M***K***M***GPLT EDQRVELLSQ SLQRIPELLP DMSPEDLAKD

551 MVGQTSGFMP RDIRALIADA SSSLVPTNGI SFENKESQKD IQPPSKEFMS

601 KALERSKKRN ASALGTPKVP NVK**WEDVGGL EDVKKSILDT VQLPLLHKDL**

651 **FSSGLRRSSG VLLYGPPGT**G KTLLAKAVAT ECFLNFLSVK GPELINMYIG

701 ESEKNVRDIF QKARAARPCV IFFDELDSLA PARGASGDSG GVMDRVVSQM

751 LAEIDGLNDS SQ*DLFIIGAS NRPDLIDAAL LR*PGRFDKLL YVGVTTDPSY

801 RERVLKALTR KFKLHEDVSL YSIAKKCPPN FTGADMYALC ADAWFHAAKR

851 KVLAADADPT NMKDDVDSVV VEYEDFVTVL RELSPSLSL**A ELKKYEMLRD**

901 **QFEGASSK***

Fig. 1. Deduced amino acid sequence of HaPex6p. P-loop motifs are boxed, Walker B motifs underlined, AAA family signature italic. The two regions used to raise antibodies are highlighted in bold. Internal methionines that are probably used for initiation of translation in vitro are shown in bold italic.

5′ part of the HaPEX6 cDNA while Hap8.3 contained a 1.5 kb *Xho*I fragment corresponding to the 3′ part of HaPEX6. The two clones were identical in 1.1 kb of sequence overlap so were ligated together at the unique internal *Xho*I site to produce Hap8.4. Re-screening the library by nested PCR failed to isolate any longer clones. Hap8.4 (2875 bp) was fully sequenced (EMBL accession number AJ305171). A genomic Southern blot indicates that HaPEX6 is a single copy gene in sunflower (data not shown).

The HaPex6p sequence (Fig. 1) was used to search the Swall protein database with the BLASTp program. The most similar entry is a hypothetical *Arabidopsis* protein F10O3.18 on chromosome I (Swall accession number Q9SA70, $P = 3.7e{-}227$) followed by a hypothetical rice protein ZWH0005.1 (Swall accession number Q9SMA4 $P = 2.7e{-}197$). The next nine entries are peroxin-6 from mammals and yeasts with rat Pex6p being the most similar ($P = 1.2e{-}119$) and *Hansenula polymorpha* the least similar ($P = 4.7e{-}93$). The CDC48 proteins form the next most closely related members of the AAA family ($P > 7.8e{-}79$).

The cDNA clone encodes a polypeptide of 909 ami-

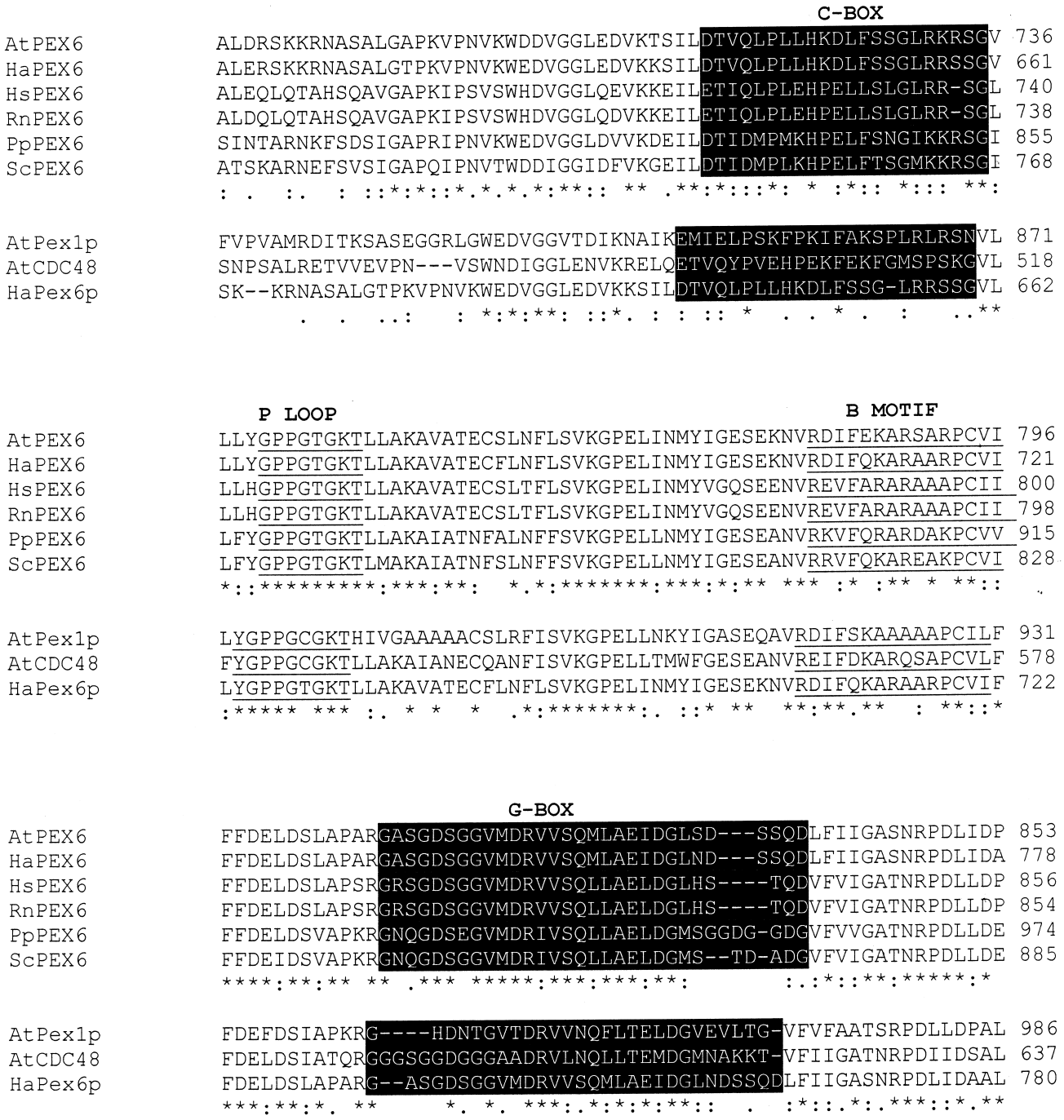

Fig. 2. Alignment of part of the second AAA domain of HaPEX6 with orthologues from plants, mammals and yeasts and compared with two other plant AAA proteins. Sequences were aligned with Clustal W. C- and G-boxes are regions in which sequence conservation is higher within AAA subfamily members than between them [23].

no acids (Fig. 1) with a calculated molecular mass of 99 574 and a p*I* of 5.75. The calculated mass is about 5% less than the experimentally determined value (Fig. 4C) and at the lower end of the size range for Pex6ps, suggesting that the clone contains most but probably not quite all of the open reading frame. The protein has 34–37% identity and 56–58% similarity to yeast and mammalian Pex6ps and like them

contains two AAA domains, the first of which is weakly conserved and has non-canonical Walker motifs. The P-loop has the consensus G/AXXXXGKT/S (boxed in Fig. 1). In the first AAA cassette only the GK are conserved in all Pex6p orthologues (not shown), while the P-loop of the second AAA cassette is perfectly conserved in all Pex6ps (Fig. 2). In contrast other AAA proteins with two AAA domains including CDC48 and Pex1p from *Arabidopsis* have two perfectly conserved P-loops. The AAA family contains hundreds of members involved in many biological functions. A detailed sequence comparison [23] identified various sub-domains within the AAA cassette, some of which are highly conserved across all AAA protein sub-families while others show higher conservation within sub-families than between them. A Clustal W alignment of part of the second AAA domain of HaPex6p, the putative *Arabidopsis* Pex6p and Pex6p from *Homo sapiens*, *Rattus norvegicus*, *P. pastoris* and *S. cerevisiae* is shown in Fig. 2. For comparison an alignment between HaPex6p and two other plant AAA proteins, *Arabidopsis* CDC48 and *Arabidopsis* Pex1p is also shown. Highlighted are two of the domains, the C- and G-boxes [23], which show more conservation within than between families. It is clear that there is much greater conservation between Pex6 proteins from all three kingdoms (7/22 and 17/32 identities in the C- and G-box respectively) than in the three plant AAA proteins HaPex6p, AtPex1p and AtCDC48 (2/23 and 11/31 identities).

To provide experimental evidence for the identity of HaPEX6 clone, immunoprecipitation was used to assess whether the protein encoded by the Hap8.4 cDNA is immunologically related to known Pex6ps. When Hap8.4 was transcribed and translated in wheat germ lysate in the presence of [^{35}S]methionine, two products of 44 kDa and 46 kDa were synthesised (Fig. 3, lane 1). Although the 7th residue from the N-terminus is methionine, the Kozak consensus is poor and the product sizes suggest the utilisation of two internal methionines at positions 479 and 514/516. This would result in the translation of a polypeptide consisting of the second (conserved) AAA cassette and the carboxy-terminus of the protein. Consistent with this both polypeptides were immunoprecipitated by antibodies raised against the C-terminal peptide of HaPex6p (lane 2), and by an antibody raised against amino acids 624–669 (lane 4) which lies within the conserved second AAA cassette just N-terminal to the P-loop and including the C-box. (Antigens are shown in bold in Fig. 1). In neither case was any product immunoprecipitated with the corresponding preimmune serum (lanes 3 and 5). Antibodies raised against *P. pastoris* (Pp) Pex6p immunoprecipitated HaPex6p (lane 6) but anti-ScCDC48 did not (lane 7). A weak precipitation was seen with anti PpPex1 (lane 8). Thus the polypeptide encoded by Hap8.4 is immunologically related to PpPex6p and more weakly to PpPex1p.

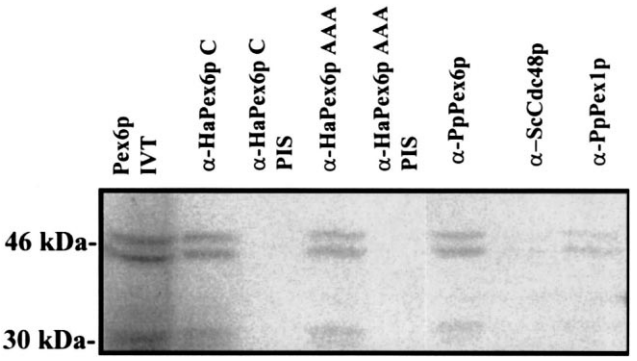

Fig. 3. HaPex6p is immunologically related to *P. pastoris* Pex6p. The Hap8.4 cDNA clone was transcribed and translated in vitro and the resulting ^{35}S-labelled polypeptides immunoprecipitated with the antibodies indicated. Precipitated proteins were separated by SDS–PAGE and radioactivity was detected by phosphoimaging.

Sequence similarity and immunological evidence strongly suggest that Hap8.4 encodes the sunflower orthologue of Pex6p. If this gene is involved in peroxisome biogenesis it should show an appropriate expression pattern. In oilseeds like sunflower and *Arabidopsis*, peroxisomal β-oxidation and the glyoxylate cycle play a crucial role in the mobilisation of reserves and the production of energy and carbon skeletons to fuel post-germinative growth and seedling establishment. During the first few days following germination peroxisomes increase in size and/or number [24,25]. Homogenates were prepared from a time course of sunflower seedling development and assayed for the two marker enzymes of the glyoxylate cycle, isocitrate lyase (ICL) and malate synthase (MS), and catalase as a general marker for glyoxysomes. Hydroxypyruvate reductase (HPR), a marker for peroxisomal photorespiration, and chlorophyll were also measured as indicators of the development of photosynthetic competence (Fig. 4A). As de-

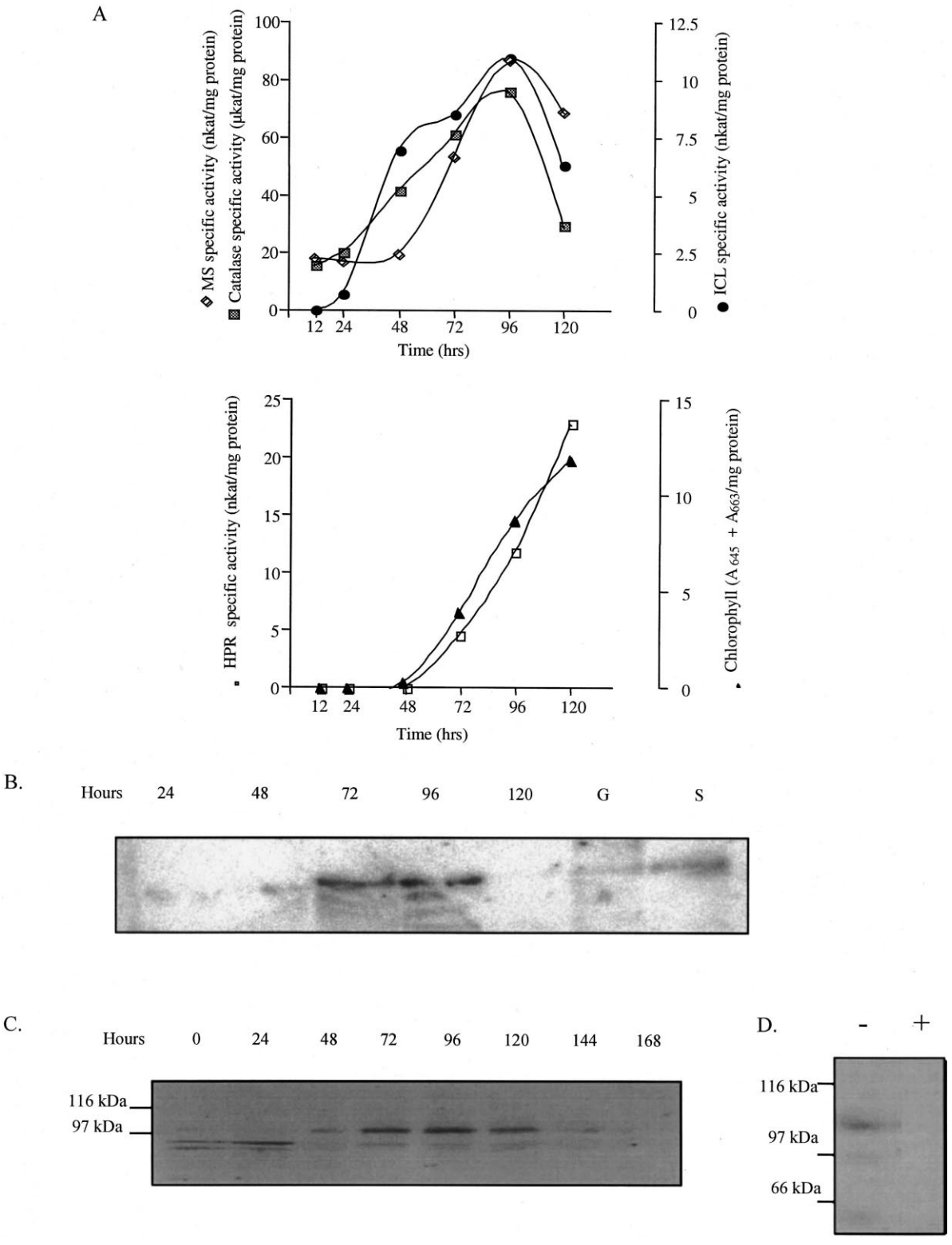
A
◇ MS specific activity (nkat/mg protein)
▩ Catalase specific activity (μkat/mg protein)
● ICL specific activity (nkat/mg protein)
Time (hrs)
▪ HPR specific activity (nkat/mg protein)
▲ Chlorophyll (A645 + A663/mg protein)
Time (hrs)
B.
Hours 24 48 72 96 120 G S
C.
Hours 0 24 48 72 96 120 144 168
116 kDa
97 kDa
D.
- +
116 kDa
97 kDa
66 kDa

Fig. 4. HaPEX6 shows a similar expression profile to peroxisomal enzymes during post-germinative growth. (A) Extracts from sunflower cotyledons grown for the time indicated were assayed for the enzymes ICL, MS, catalase, HPR and chlorophyll. (B) Total RNA was extracted from a similar developmental time course and from green (G) and senescent (S) cotyledons and probed with ^{32}P-radiolabelled Hap8.4. Radioactivity was detected by phosphoimaging. Equal amounts of RNA were loaded on each lane, based on spectrophotometric quantitation and ethidium bromide staining of a duplicate gel. (C) Protein extracts were obtained from whole seedlings at the time points indicated. Protein extract equivalent to 0.5 seedling was loaded on each lane, separated by SDS–PAGE, and immunoblotted with antibody raised against the C-terminal 20 amino acids of HaPex6p. (D) Extracts from the 72 h time point were blotted as in C except that the blot in the right-hand lane (+) was probed with antibody that had been pre-incubated with the C-terminal peptide.
←

scribed previously for sunflower [26] and cucumber [27] ICL, MS and catalase increase in specific activity over the first few days of post-germinative growth peaking, in this instance, at 96 h before declining. In contrast chlorophyll and HPR begin to accumulate after 48 h, coincident with the onset of greening as the cotyledons begin to emerge.

Total RNA was prepared from a similar developmental time course and probed with a HaPEX6 probe. The size of the transcript was estimated to be between 2900 and 3200 nucleotides, in reasonable agreement with the size of the Hap8.4 cDNA allowing for polyadenylation. The HaPEX6 transcript is detectable at 48 h but increases in abundance up to 96 h before decreasing (Fig. 4B). This corresponds to the period when the radical elongates and the cotyledons just begin to emerge, and coincides with the rapid increase in glyoxylate cycle marker enzymes (Fig. 4A). The transcript was also detectable at a low level in mature green leaves, and was more abundant in senescent leaves. During senescence, glyoxylate cycle enzymes are synthesised once again and taken up into peroxisomes [28]. AtPex1p shows a similar expression pattern [12]. In good agreement with the Northern data, Western blots on total protein extracts of sunflower seeds with the anti-C-terminal peptide antibody detected a protein of 105 kDa that peaks at 96 h then declines (Fig. 4C). The detection of this polypeptide can be completely abolished by the pre-incubation of the antiserum with the peptide antigen (Fig. 4D).

In summary, we have cloned a gene encoding an AAA family member from sunflower, which, on the basis of sequence identity, immunological cross-reactivity and expression profile can be identified as sunflower Pex6p. Highly related ORFs were found in the genomes of *Arabidopsis* and rice. Interestingly the *Arabidopsis* ORF F10O3.18 is very close to the map location of two mutants, B11 and ibr4, isolated due to their resistance to the inhibitory effect of the auxin indole-3-butyric acid on root elongation. B11 falls into the same phenotypic class as another mutant, B44, which is a missense mutation in Pex5p, the import receptor for PTS1 targeted peroxisomal proteins [18]. F10O3.18 is therefore a strong candidate for the defective gene in the B11 mutant. Primers designed against F10O3.18 amplified a partial cDNA from a 2 day *Arabidopsis* library (data not shown) showing that it is expressed at this developmental stage along with other *Arabidopsis* PEX genes ([12], W.L. Charlton, unpublished data). These genes and antibodies will prove useful tools to investigate the role of Pex6p in peroxisome biogenesis in plants.

We thank Dr Andreas Beyer (Ruhr Universität, Bochum, Germany) for drawing our attention to the sequence similarity between PEX6 and the sequence in the 5′-UTR of B-peru and Prof. Suresh Subramani (Univerity of California, San Diego, CA, USA) for antibodies against *Pichia pastoris* Pex1p and Pex6p.

References

[1] L.J. Olsen, The surprising complexity of peroxisome biogenesis, Plant Mol. Biol. 38 (1998) 163–189.
[2] R.D. Vale, AAA proteins: lords of the ring, J. Cell Biol. 150 (2000) F13–F19.
[3] A. Spong, S. Subramani, Cloning and characterisation of PAS5: A gene required for peroxisome biogenesis in the methylotropic yeast *Pichia pastoris*, J. Cell Biol. 123 (1993) 535–548.
[4] T. Yahraus, N. Braverman, G. Dodt, J.E. Kalish, J.C. Morrell, H.W. Moser, D. Valle, S.J. Gould, The peroxisome biogenesis disorder group 4 gene PXAAA1 encodes a cytoplasmic ATPase required for stability of the PTS1 receptor, EMBO J. 15 (1996) 2914–2923.
[5] K.N. Faber, J.A. Heyman, S. Subramani, Two AAA family

peroxins, PpPex1p and PpPex6p interact with each other in an ATP-dependent manner and are associated with different subcellular membranous structures distinct from peroxisomes, Mol. Cell. Biol. 18 (1998) 936–943.
[6] J.A.K.W. Kiel, R.E. Hilbrands, I.J. van der Klei, S.W. Rasmussen, F.A. Salomans, M. van der Heide, K.N. Faber, J.M. Cregg, M. Vennhuis, *Hansenula polymorpha* Pex1p and Pex6p are peroxisome associated AAA proteins that functionally and physically interact, Yeast 15 (1999) 1059–1078.
[7] B.V. Geisbrecht, C.S. Collins, E. Reuber, S.J. Gould, Disruption of a PEX1-PEX6 interaction is the most common cause of the neurologic disorders Zellweger syndrome, neonatal adrenoleukodystrophy and infantile Refsum disease, Proc. Natl. Acad. Sci. USA 95 (1998) 8630–8635.
[8] V.I. Titorenko, R.A. Rachubinski, Peroxisomal membrane fusion requires two AAA family ATPases, Pex1p and Pex6p, J. Cell Biol. 150 (2000) 881–886.
[9] C.S. Collins, J.E. Kalish, J.C. Morrell, J.M. McCaffrey, S.J. Gould, The peroxisome biogenesis factors Pex4p, Pex22p, Pex1p, and Pex6p act in the terminal steps of peroxisomal matrix protein import, Mol. Cell. Biol. 20 (2000) 7516–7526.
[10] S.T. South, S.J. Gould, Peroxisome synthesis in the absence of preexisting peroxisomes, J. Cell Biol. 144 (1999) 255–266.
[11] A.M. Motley, E.H. Hettema, R. Ketting, R. Plasterk, H.F. Tabak, *Caenorhabditis elegans* has a single pathway to target matrix proteins to peroxisomes, EMBO Rep. 1 (2000) 40–46.
[12] E. Lopez-Huertas, W.L. Charlton, B. Johnson, I.A. Graham, A. Baker, Stress induces peroxisome biogenesis genes, EMBO J. 19 (2000) 6770–6777.
[13] C. Wimmer, M. Schmid, M. Veenhuis, C. Gietl, The plant PTS1 receptor: similarities and differences to its human and yeast counterparts, Plant J. 16 (1998) 453–464.
[14] F. Kragler, G. Lametschwandtner, J. Christmann, A. Hartig, J.J. Harada, Identification and analysis of the plant peroxisome targeting signal 1 receptor NtPEX5, Proc. Natl. Acad. Sci. USA 95 (1998) 13336–13341.
[15] M. Hayashi, K. Nito, K. Toriyama-Kato, M. Kondo, T. Yamaya, M. Nishimura, AtPex14p maintains peroxisomal functions by determining protein targeting to 3 different kinds of plant peroxisomes, EMBO J. 19 (2000) 5701–5710.
[16] Y. Lin, L. Sun, L.V. Nguyen, R.A. Rachubinski, H.M. Goodman, The Pex16p homolog SSE1 and storage organ formation in *Arabidopsis* seeds, Science 284 (1999) 328–330.
[17] N.E. Tolbert, Metabolic pathways in glyoxysomes and peroxisomes, Annu. Rev. Biochem. 50 (1981) 133–157.
[18] B.K. Zolman, A. Yoder, B. Bartel, Genetic anlaysis of indole-3-butyric acid responses in *Arabidopsis thaliana* reveals four mutant classes, Genetics 156 (2000) 1323–1337.
[19] C. Castresana, I. Gacia-Luque, E. Alonson, V.S. Malis, A. Cashmore, Both positive and negative regulatory elements mediate expression of a photoregulated CAB gene from *Nicotiana plumbaginifolia*, EMBO J. 7 (1988) 1929–1936.
[20] J.P. Radicella, D. Brown, L.A. Tolar, V.L. Chandler, Allelic diversity of the maize B regulatory gene: different leader and promoter sequences of 2 B alleles determine distinct tissue specificities and anthocyanin production, Genes Dev. 6 (1992) 2152–2164.
[21] E. Lopez-Huertas, J. Oh, A. Baker, Antibodies against Pex14p block ATP-independent binding of matrix proteins to peroxisomes in vitro, FEBS Lett. 459 (1999) 227–229.
[22] M. Pool, E. Lopez-Huertas, J.-T. Horng, A. Baker, NADPH is a specific inhibitor of protein import into glyoxysomes, Plant J. 15 (1998) 1–14.
[23] A. Beyer, Sequence analysis of the AAA protein family, Protein Sci. 6 (1997) 2043–2058.
[24] C.M. Kunce, R.N. Trelease, C. Doman, Ontogeny of glyoxysomes in maturing and germinated cotton seeds – a morphometric analysis, Planta 161 (1984) 156–164.
[25] S.G. Mansfield, L.G. Briarty, The dynamics of seedling and cotyledon cell development in *Arabidopsis thaliana* during reserve mobilisation, Int. J. Plant Sci. 157 (1996) 280–295.
[26] C. Schnarrenberger, A. Oeser, N.E. Tolbert, Development of microbodies in sunflower cotyledons and castor bean endosperm during germination, Plant Physiol. 48 (1971) 566–574.
[27] R.N. Trelease, W.M. Becker, P.J. Gruber, E.H. Newcomb, Microbodies (glyoxysomes and peroxisomes) in cucumber cotyledons: correlative biochemical and ultrastructural study in light and dark grown seedlings, Plant Physiol. 48 (1971) 461–465.
[28] H. Gut, P. Matile, Apparent induction of the key enzymes of the glyoxylate cycle in senescent barley leaves, Planta 176 (1988) 548–550.