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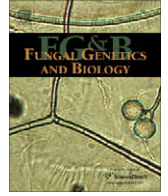
Proteins involved in microbody biogenesis and degradation in *Aspergillus nidulans*

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ABSTRACT

Fungal microbodies (peroxisomes) are inducible organelles that proliferate in response to nutritional cues. Proteins involved in peroxisome biogenesis/proliferation are designated peroxins and are encoded by *PEX* genes. An autophagy-related process, termed pexophagy, is responsible for the selective removal of peroxisomes from the cell. Several genes involved in pexophagy are also required for autophagy and are collectively known as *ATG* genes. We have re-analysed the *Aspergillus nidulans* genome for the presence of *PEX* and *ATG* genes and have identified a number of previously missed genes. Also, we manually determined the correct intron positions in each identified gene. The data show that in *A. nidulans* and related fungi the basic set of genes involved in peroxisome biogenesis or degradation are conserved. However, both processes have features that more closely resemble organelle formation/degradation in mammals rather than yeast. Thus, filamentous fungi like *A. nidulans* are ideal model systems for peroxisome homeostasis in man.

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1. Introduction

Microbodies (peroxisomes, glyoxysomes, glycosomes, and Woronin bodies) are essential organelles in mammals, plants and *Trypanosomes* (Gould and Valle, 2000; Guerra-Giraldez et al., 2002; Schumann et al., 2003). They consist of a single membrane that encloses a matrix space containing enzymes involved in various metabolic pathways (Veenhuis and Harder, 1991; van den Bosch et al., 1992). Enzymes involved in the β-oxidation of fatty acids are most likely present in all microbodies. However, in several organisms very unique and uncommon metabolic processes are catalyzed by microbody-borne enzymes (e.g. methanol metabolism in methylotrophic yeasts; photorespiration in green leaves of plants). Peroxisomes are microbodies containing at least one hydrogen peroxide producing oxidase together with catalase, whereas glyoxysomes harbour enzymes of the glyoxylate cycle. Glycosomes only occur in *Trypanosomes* and contain glycolytic enzymes, which are normally localized to the cytosol. Nevertheless, there is probably no distinction between peroxisomes, glyoxysomes and glycosomes other than their matrix enzyme contents. Currently, Woronin bodies are the only class of microbodies known to have a structural function (see below).

Microbodies also play a vital role in filamentous fungi. In addition to enzymes involved in the metabolism of various carbon and nitrogen sources, in certain fungi including *Aspergillus nidulans* and *Penicillium chrysogenum*, also the enzymes catalyzing the final steps of the biosynthesis of β-lactam antibiotics are localized to these organelles (Martín and Gutiérrez, 1995). Moreover, only filamentous ascomycetes contain a special class of microbodies, the Woronin bodies, which are required to plug the septal pore upon hyphal damage to prevent cytoplasmic leakage (Jedd and Chua, 2000; Tenney et al., 2000). The genes involved in microbody/peroxisome biogenesis, so called *PEX* genes encoding proteins designated peroxins (Distel et al., 1996), were initially identified in studies with yeast model systems (see Vizeacoumar et al., 2004 and references therein). Yeast *pex* mutants are usually characterized by the absence of morphologically normal peroxisomes, while bulk of the peroxisomal matrix proteins are mislocalized to the cytosol. As a result certain metabolic pathways (e.g. the metabolism of fatty acids) that are compartmentalized in peroxisomes, are dysfunctional in these mutants. Alternatively, certain *pex* mutants show drastically reduced peroxisome numbers, implying a role for the encoded peroxin in organelle proliferation.

Recently, the first *PEX* genes were identified in filamentous fungi (Berteaux-Lecellier et al., 1995; Kimura et al., 2001; Sichting et al., 2003; Kiel et al., 2000, 2004, 2005a; Bonnet et al., 2006; Asakura et al., 2006; Hynes et al., 2006, 2008; Managadze et al., 2007; Idnurm et al., 2007; Liu et al., 2008). Remarkably, fungal *pex* mutants show, in addition to a defect in growth on fatty acids (e.g. oleic acid), phenotypes that had not been observed before in yeast species. For instance, *Podospora anserina pex2*, *pex5* and *pex7* mutants are affected in the sexual cycle (Berteaux-Lecellier et al., 1995; Bonnet et al., 2006), the *Colletotrichum lagenarium pex6* mutant is unable to infect plant cells (Kimura et al., 2001; Asakura et al., 2006) and *P. chrysogenum pex5* cells are defective in asexual

spore formation (Kiel et al., 2004). In addition, most *Neurospora crassa pex* mutant cells lack Woronin bodies (Managadze et al., 2007; Liu et al., 2008) and, as a result, show enhanced hyphal bleeding. Finally, *pex1* and *pex6* mutants of the basidiomycete *Cryptococcus neoformans* grow poorly on glucose as carbon source (Idnurm et al., 2007). Such unexpected phenotypes of *pex* mutants in filamentous fungi are analogous to the highly complex phenotypes of human peroxisome biogenesis disorders (PBDs), *pex*$^{-/-}$ knockout mouse models or plant *pex* mutants (see Faust et al., 2001; Wanders, 2004; Hayashi and Nishimura, 2006).

In most fungi, peroxisomes are inducible organelles. Also in *A. nidulans* peroxisome numbers are enhanced when hyphae are grown on oleate as carbon source (Valenciano et al., 1996; Hynes et al., 2006) or at penicillin producing conditions (Valenciano et al., 1998). Conversely, when cells are shifted to medium in which the organelles become redundant, these are selectively degraded by an autophagy-related pathway collectively known as pexophagy (reviewed by Sakai et al. (2006)). Normally (macro-)autophagy involves the random uptake of sequestered portions of the cytoplasm (cytosol and organelles) into the vacuole/lysosome for recycling (for review see Yorimitsu and Klionsky, 2005). This process is important for cell survival, at conditions of nutrient depletion (e.g. nitrogen starvation). However, also certain processes closely related to autophagy utilize the autophagy machinery for selective uptake of proteins/organelles into the vacuole. These include pexophagy and the constitutive Cytoplasm to vacuole targeting (Cvt) pathway, a process only observed in certain yeast species that sorts precursor forms of certain resident hydrolases to the vacuole (Shintani and Klionsky, 2004; Farré et al., 2007). Genes involved in autophagy and related pathways have been designated *ATG* genes (Klionsky et al., 2003), which invariably were first identified in yeast species. Autophagy in filamentous fungi has been the subject of only few studies in *P. anserina* (Pinan-Lucarré et al., 2003, 2005), *Aspergillus oryzae* (Kikuma et al., 2006) and *Aspergillus fumigatus* (Richie et al., 2007). These studies showed that deletion of an *ATG* gene not only affects autophagy, but also results in absence of aerial hyphae and conidia, and/or accelerates cell death, suggesting that autophagy functions in differentiation in these organisms.

Only very recently, the first *A. nidulans pex* mutants disturbed in peroxisome biogenesis were analysed (Hynes et al., 2006, 2008). Most *A. nidulans pex* mutants are blocked in peroxisomal protein import, are unable to grow on fatty acids, have a biotin-auxotrophy and are partially defective in asexual and sexual development. So far, only a limited number of *A. nidulans PEX* genes has been identified in the fungal genome and solely the phenotype of null mutants was studied. Pexophagy has also been observed in *A. nidulans* (Amor et al., 2000), but the molecular basis of autophagy and related processes like pexophagy has not been studied yet in filamentous fungi. By manual annotation using peroxin and Atg sequences from yeast species, plants and mammals as queries we found that many more *PEX* and *ATG* genes are present in the *A. nidulans* genome than currently annotated in databases, because certain genes have either been missed or were incorrectly annotated because of improper intron splicing. In the present manuscript we list all identified *A. nidulans* peroxins and Atg proteins and discuss their function in peroxisome homeostasis.

2. Peroxins encoded by the *A. nidulans* genome (Tables 1 and 2)

2.1. Peroxins involved in the formation of the peroxisomal membrane

As yet, three *PEX* genes have been identified that are involved in the formation of the peroxisomal membrane (*PEX3*, *PEX16* and *PEX19*). In both yeast cells and human fibroblasts, deletion of *PEX3* or *PEX19* causes the complete absence of peroxisomal mem-

brane structures (reviewed in Schliebs and Kunau (2004)). Although still under debate, one of the functions of the interacting peroxins Pex19p and Pex3p is their role in targeting and insertion of newly synthesized peroxisomal membrane proteins (PMPs) into the target membrane (Schliebs and Kunau, 2004; Fig. 1). In this scheme, Pex19p is thought to function as a cytosolic receptor/chaperone that keeps the newly synthesized PMPs in an unfolded, import-competent state, while Pex3p assists in subsequent membrane insertion. As expected, both proteins are fully conserved in *A. nidulans*. Indeed, an *A. nidulans pex3* mutant was recently shown to mislocalize peroxisomal matrix proteins to the cytosol, while a Pex11p-GFP fusion protein—a peroxisomal membrane marker—no longer localized to punctate structures, suggesting the absence of peroxisomal membrane remnants (Hynes et al., 2008).

In mammalian cells, Pex16p is involved in the formation of the peroxisomal membrane (South and Gould, 1999). This protein was also identified in plants (Lin et al., 1999) and the yeast *Yarrowia lipolytica* (Eitzen et al., 1997; Guo et al., 2003), but is absent in other yeast species (Kiel et al., 2006). Remarkably, *Yl*-Pex16p appears to be involved in peroxisome proliferation, rather than membrane formation (Guo et al., 2003, 2007). Nevertheless, unlike mutants solely affected in peroxisome proliferation (e.g. *pex11*, see below), peroxisomal matrix proteins were mislocalized to the cytosol in the *Yl-pex16* mutant (Eitzen et al., 1997). Similarly, *Arabidopsis thaliana pex16* mutants have reduced numbers of enlarged peroxisomes (Nito et al., 2007), precluding an essential role for *At*-Pex16p in membrane formation.

Pex16p is also present in *A. nidulans* and other filamentous fungi. Unfortunately, so far, only limited data are available concerning the role of Pex16p in peroxisome biogenesis in these species. Recently, an *N. crassa pex16* mutant was isolated that mislocalized peroxisomal matrix proteins to the cytosol and lacked Woronin bodies (Liu et al., 2008). Unfortunately, it is not yet known whether this mutant is completely devoid of peroxisomal membrane structures, like mammalian *pex16* mutant cells.

2.2. Peroxisomal matrix protein import; the PTS receptors

Most peroxins are involved in the transport of matrix proteins from the cytosol into the peroxisome lumen. To this end, matrix proteins contain specific peroxisomal targeting signals, designated PTS1 and PTS2, that are recognized by specific receptors (for a review see Purdue and Lazarow, 2001). The PTS1 receptor Pex5p and the protein complex Pex7p/Pex20p, that together form the PTS2 receptor, are fully conserved in *A. nidulans* (Table 1). These receptors are soluble proteins that bind newly synthesized PTS1- or PTS2-containing cargo proteins in the cytosol (Fig. 1). Reports on PTS1 and PTS2 import in filamentous fungi are rather limited (see e.g. Sichting et al., 2003; Kiel et al., 2004). Recently, it was demonstrated that like yeast, *A. nidulans pex5* and *pex7* mutants are disturbed in the import of only one type of matrix protein (PTS1 or PTS2 proteins, respectively; Hynes et al., 2008). Thus, in *A. nidulans* the PTS1 and PTS2 pathways behave independently from each other unlike in mammals and certain plants, where PTS2 import requires, in addition to Pex7p, also the long isoform of Pex5p to enter peroxisomes (Braverman et al., 1998; Woodward and Bartel, 2005).

2.3. Peroxisomal protein translocation

Peroxisomal matrix proteins are usually imported into the peroxisomal lumen in a folded, enzymatically active conformation. Furthermore, during peroxisomal protein sorting entire receptor–cargo complexes become imported into the lumen of the peroxisome, whereupon the cargo dissociates from the receptor protein, which then becomes recycled to the cytosol. To assist in these pro-
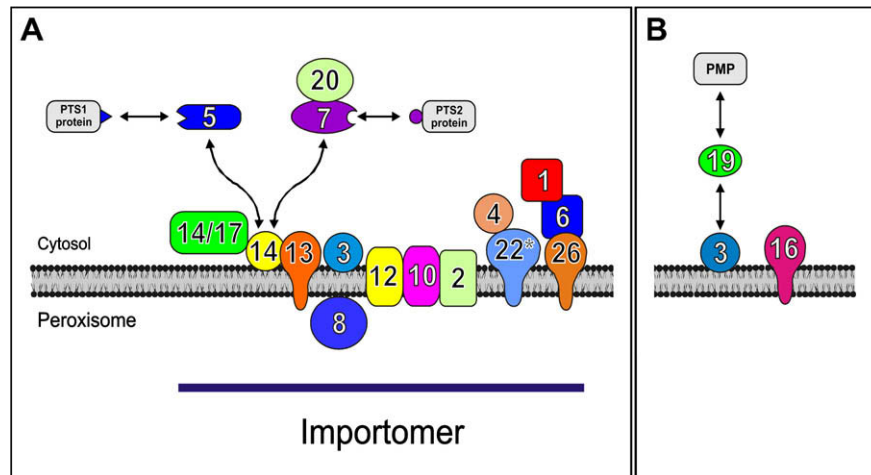
Fig. 1. Peroxisomal protein import in *Aspergillus nidulans*. (A) Schematic representation of the peroxins identified in *A. nidulans* and their putative role in peroxisomal matrix protein import (see text). Numbers refer to the respective peroxins (Pex proteins). The asterisk indicates that in *A. nidulans* and other filamentous fungi a protein with very weak similarity to Pex22p is present, that has been designated Pex22p-like protein. (B) The peroxins Pex3p and Pex19p are thought to be involved in insertion of peroxisomal membrane proteins. The role of the integral membrane protein Pex16p in formation of the peroxisomal membrane is unclear.

Table 1
A. nidulans peroxins involved in microbody biogenesis

Peroxin	Locus tag
Pex1p	AN5991.3
Pex2p	AN4056.3
Pex3p	AN2281.3
Pex4p	AN10994.3
Pex5p	AN10215.3
Pex6p	AN2925.3
Pex7p	AN0880.3
Pex8p	AN6161.3
(Pex9p)	Does not exist (Kiel et al., 2006)
Pex10p	AN5681.3
Pex12p	AN10925.3
Pex13p	AN1511.3
Pex14p	AN10610.3
(Pex15p)	Functional homologue of Pex26p (Kiel et al., 2006)
Pex16p	AN5113.3
Pex17p	See Pex14/17p
Pex14/17p	AN6258.3
Pex18p	Na
Pex19p	AN4899.3
Pex20p	AN4318.3
Pex21p	Na
Pex22p	See Pex22p-like
Pex22p-like	AN0977.3
Pex26p	AN7285.3

Proteins involved in peroxisome biogenesis identified in fungal species, plants and mammals were used to identify orthologs in *Aspergillus nidulans*. Locus tag numbers are from the Broad Institute. Na, not available/identifiable.

cesses, a large protein supercomplex is present at the peroxisome membrane, that has been designated the importomer (Agne et al., 2003; Fig. 1). This supercomplex consist of three subcomplexes: (i) the receptor docking complex, consisting of the peroxins Pex13p, Pex14p and Pex17p, (ii) the RING finger complex with the Zn^{2+}-containing RING finger proteins Pex2p, Pex10p and Pex12p and (iii) the receptor recycling complex (see below). The interaction between the receptor docking complex and the RING finger complex is thought to be mediated by either Pex8p (Agne et al., 2003) or Pex3p (Hazra et al., 2002). It has been speculated that both complexes are directly involved in the translocation event, but how translocation actually takes place is still unknown. In addition, Pex8p, the sole peroxin that resides in the peroxisome matrix, is thought to also function in the release of PTS cargo proteins from their respective receptor (Wang et al., 2003).

The *A. nidulans* genome encodes orthologs of Pex2p, Pex8p, Pex10p, Pex12p, Pex13p and Pex14p (Table 1). The recent observation that an *A. nidulans* pex13 mutant mislocalizes the peroxisomal proteins malate synthase and isocitrate lyase to the cytosol (Hynes et al., 2008) confirms the essential role of this protein in peroxisomal protein import also in this filamentous fungus. Remarkably, the *A. nidulans* PEX14 gene has five exons of which exon two is only seven bp in length, a feature that is conserved in other Aspergilli. In contrast, *A. nidulans* and related filamentous fungi lack a true Pex17p ortholog. Instead, these organisms contain a protein that we have recently designated Pex14/17p (Kiel et al., 2006), which is encoded by an intronless gene. This protein has an N-terminus that is highly similar to a conserved region present in the N-terminus of Pex14p, that has been fused to a putative coiled-coil region showing weak similarity to yeast Pex17p. So far, it has however not been experimentally tested whether this filamentous fungi-specific Pex14/17 protein indeed represents a peroxin involved in peroxisomal protein transport.

The *A. nidulans* PEX2 gene and its encoded RING finger protein Pex2p show some remarkable features. The *A. nidulans* PEX2 gene contains only a single intron, while in related species (e.g. all Aspergilli, *Ajellomyces capsulatus*, *P. chrysogenum* and *Coccidioides immitis*) PEX2 has two introns, a clear case of intron loss in the *A. nidulans* lineage. In addition, during our analysis we noticed that the RING finger present in Pex2p from *A. nidulans* and other filamentous ascomycetes deviates from that observed in Pex2p's from other species. To analyse this in more detail, we have aligned Pex2p, Pex10p and Pex12p orthologs from organisms ranging from baker's yeast to man with particular emphasis on the regions comprising the RING finger domains (Fig. 2). This comparison revealed that the RING fingers of Pex2p and Pex12p are very variable in length, while that of Pex10p is highly conserved, a feature that might reflect the putative role of Pex10p in receptor recycling (see below). Typically, the RING motif is thought to consist of a $(Cys)_3His(Cys)_4$ motif and bind two Zn^{2+} ions (Barlow et al., 1994). In most organisms the RING fingers of Pex2p and Pex10p conform to this rule. However, the Pex2p RING finger in *A. nidulans* and other ascomycetous fungi contains a cysteine instead of a histidine residue at position 4, thereby creating a $(Cys)_8$ motif. It can be envisaged that this motif is still capable of binding two Zn^{2+} ions. Also baker's yeast and related species have a Pex2 protein with a deviating RING finger. However, in this case the histidine at position 4 is replaced by alanine or threonine, residues less likely to
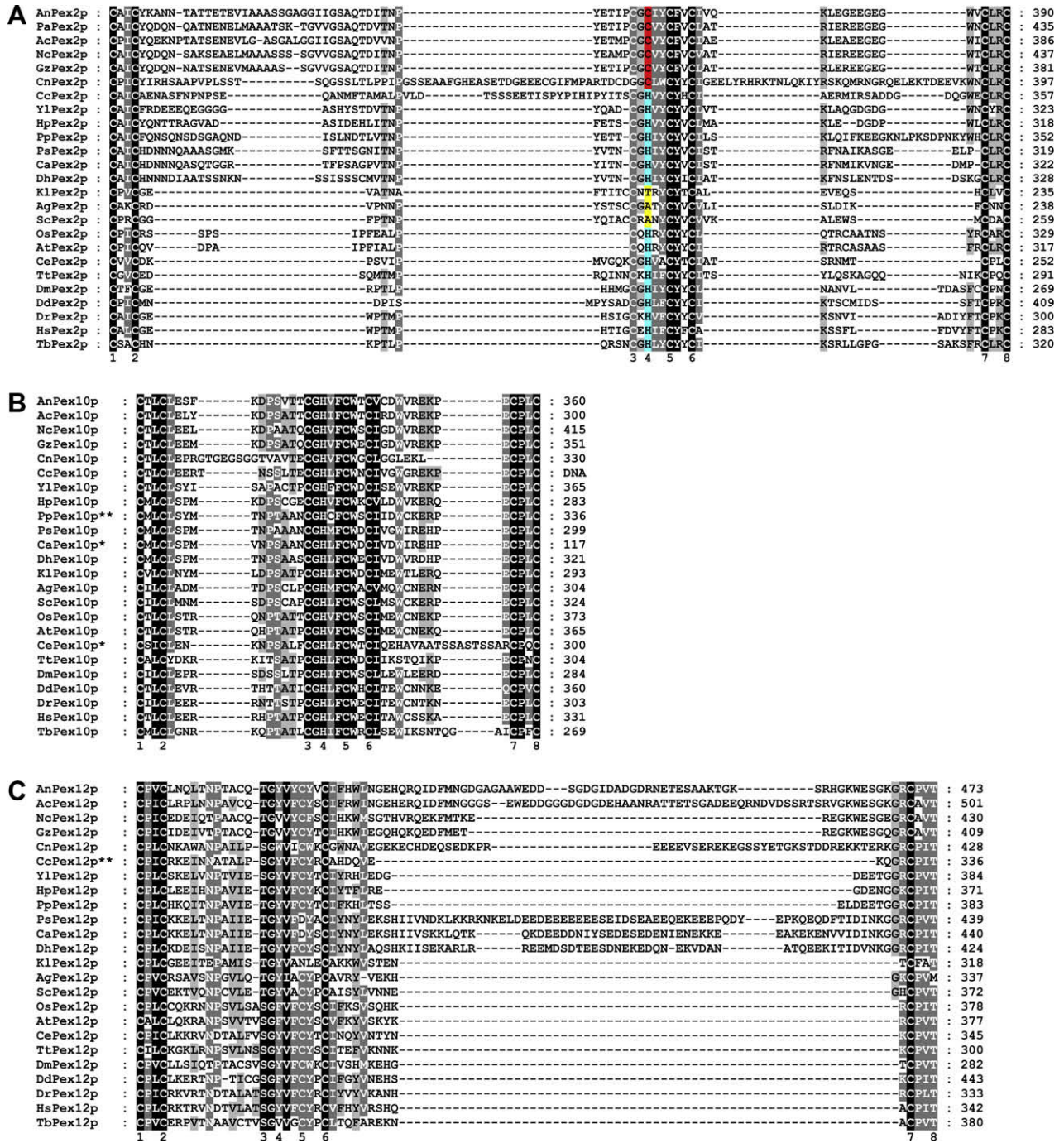
A

			:
AnPex2p	CAICYKANN-TATTETEVIAAASSGAGGIIGSAQTDITN	...YETIPCGCIYCGVCIVQ...KLEGEEGEG...WVCLRC	: 390
PaPex2p	CAICYDQN-QATNENELMAAATSK-TGVVGSAQTDVTN	...YETIPCGCVCGVCIAE...RIEREEGEG...WNCLRC	: 435
AcPex2p	CPICYQEKNPTATSENEVLG-ASGALGGIIGSAQTDVVN	...YETMPCGCVVCGVCIAE...KLEAEEGEG...WICLRC	: 386
NcPex2p	CAICYDQN-SAKSEAELMAAASSS-SGVVGSAQTDITN	...YEAMPCGCVVCGVCIAT...RIEREEGEG...WTCLRC	: 437
GzPex2p	CAICYDQN-NATSENEVMAAAAS--SGVVGSAQTDITN	...YETIPCGCVVCGVCIAT...RIEREEGEG...WTCLRC	: 381
CnPex2p	CPICYIRHSAAPVPLSST----------SQGSSLTLPPIGSSEAAFGHEASETDGEEECGIFMPARTDCDGGCLWCIYCGEELYRHRKTNLQKIYRSKQMRNGRQELEKTDEEVKWNCLRC	: 397
CcPex2p	CAICAENASFNPNPSE----------QANMFTAMALVLD-------TSSSEETISPYPIHIPYIITSCGHVCIHCI...AERMIRSADDG------DQGWECLKC	: 357
YlPex2p	CAICFRDEEEQEGGGG----------ASHYSTDVTN...YQAD--CGHVCYCVVT...KLAQGDGDG...WNCYPC	: 323
HpPex2p	CAICYQNTTRAGVAD-----------ASIDEHLITN...FETS--CGEVCYVCVMA...KLE--DGDP...WLCLRC	: 318
PpPex2p	CAICFQNSQNSDSGAQND---------ISLNDTLVTN...YETT--CGHIYCYVCST...KLQIFKEEGKNLPKSDPNKYWHCLRC	: 352
PsPex2p	CAICHDNNNQAAASGMK----------SFTTSGNITN...YVTN--CGHIYCYVCST...RFNAIKASGE--------ELP-CLRC	: 319
CaPex2p	CAICHDNNNQASQTGGR----------TFPSAGPVTN...YITN--CGHIYCYVCST...RFNMIKVNGE------DMP-CLRC	: 322
DhPex2p	CAICHNNNDIAATSSNKN---------SSISSSCMVTN...YVTN--CGHIVCYICIAT...KFNSLENTDS------DSKGCLRC	: 328
KlPex2p	CPVCGE----------------------VATNA...FTITCNTRVCYTCAL...EVEQS---------------HCLVC	: 235
AgPex2p	CAKCRD----------------------VPNN...YSTSCCGATCVVCVLI...SLDIK---------------FCNNC	: 238
ScPex2p	CPRCGG----------------------FPTN...YQIACCRANCVVCVVK...ALEWS---------------MCDAC	: 259
OsPex2p	CPICRS------SPS-----------IPFEAL...QHRVCYCYI...QTRCAATNS----------YRCAFC	: 329
AtPex2p	CPICQV------DPA-----------IPFIAL...QHRVCYCYI...RTRCASAAS----------FRCLRC	: 317
CePex2p	CVVCDK-------------------PSVI...MVGQKCGHVACYTCAT...SRNMT---------------CPLC	: 252
TtPex2p	CGVCED-------------------SQMTM...RQINNCKHIFCYCYCITS...YLQSKAGQQ---------NIKCPCC	: 291
DmPex2p	CTFCGE-------------------RPTL...HHMGCGHIYCYCI...NANVL---------TDASFCCPNC	: 269
DdPex2p	CPICMN-------------------DPIS...MPYSADCGHLFCYCI...KTSCMIDS---------CPVC	: 409
DrPex2p	CAICGE-------------------WPTM...HSIGCKHVFCYCV...KSNVI---------ADIYFTCPKC	: 300
HsPex2p	CAICGE-------------------WPTM...HTIGCKHIFCYFCA...KSSFL---------FDVYFTCPKC	: 283
TbPex2p	CSACHN-------------------KPTL...QRSNCGHLYCYCI...KSRLLGPG---------SAKSFRCLRC	: 320

1 2   3 4 5 6   7 8

B

			:
AnPex10p	CTLCLESF-------KDPSVTTCGHVFCWTCVCDIVREKP--------ECPLC	: 360
AcPex10p	CTLCLELY-------KDPSATTCGHIFCWTCIRDIVREKP--------ECPLC	: 300
NcPex10p	CTLCLEEL-------KDPAATQCGHVFCWSCIGDIVREKP--------ECPLC	: 415
GzPex10p	CTLCLEEM-------KDPSATQCGHVFCWSCIGDIVREKP--------ECPLC	: 351
CnPex10p	CTLCLEPRGTGEGSSGGTVAVTCGHVFCWCIGGLEKL---------ECPLC	: 330
CcPex10p	CTLCLEERT-------NSSLTECGHLFCWNCIVGXGREKP--------ECPLC	: DNA
YlPex10p	CTLCLSYI-------SAFACTPCGHLFCWDCISEXVREKP--------ECPLC	: 365
HpPex10p	CMLCLSPM-------KDPSCGECGHVFCWCVLDIVKERQ--------ECPLC	: 283
PpPex10p**	CMLCLSYM-------TNPTAANCGHCFCWSCIIDICKERP--------ECPLC	: 336
PsPex10p	CMLCLSPM-------TNPAAANCGHLFCWDCIVGIIREHP--------ECPLC	: 299
CaPex10p*	CMLCLSPM-------VNPSAANCGHLFCWDCIVDIIREHP--------ECPLC	: 117
DhPex10p	CMLCLSPM-------TNPSAASCGHLFCWDCIVDIVRDHP--------ECPLC	: 321
KlPex10p	CVLCLNYM-------LDPSATPCGHLFCWDCIMEITLERQ--------ECPLC	: 293
AgPex10p	CILCLADM-------TDPSCLPCGHMFCWACVMQICNERN--------ECPLC	: 304
ScPex10p	CILCLMNM-------DNPSCAPCGHLFCWSCIMSICKERP--------ECPLC	: 324
OsPex10p	CTLCLSTR-------QNPTATTCGHVFCWSCIMEICNEKP--------ECPLC	: 373
AtPex10p	CTLCLSTR-------QHPTATPCGHVFCWDCIMEICNEKQ--------ECPLC	: 365
CePex10p*	CSICLEN-------KNPSALFCGHLFCWTCIQEHAVAATSSASTSSARCPQC	: 300
TtPex10p	CALCYDKR-------KITSATPCGHLFCWDCIIKSTQIKP--------ECPNC	: 304
DmPex10p	CILCLEPR-------SDSSLTPCGHLFCSCLLEICLEERD--------ECPLC	: 284
DdPex10p	CTLCLEVR-------THTIATICGHLFCWCITEICNNKE--------CPVC	: 360
DrPex10p	CILCLEER-------RNTISTPCGHLFCWECITEICNTKN--------ECPLC	: 303
HsPex10p	CTLCLEER-------RHPTATPCGHLFCWDCITAICSSKA--------ECPLC	: 331
TbPex10p	CMLCLGNR-------KQPTATLCGHIFCWRCISEIKSNTQG-----AICPFC	: 269

1 2   3 4 5 6   7 8

C

			:
AnPex12p	CPVCLNQLTNPTACQ-TGYVVCYCVCIFHWINGEHQRQIDFMNGDGAGAAWEDD---SGDGIDADGDRNETESAAKTGK----------SRHGKWESGKGRCPVT	: 473
AcPex12p	CPICLRPLNNPAVCQ-TGYVFCYSCIFRWINGEHERQIDFMNGGGS--EWEDDGGGDGDGDEHAANRATTETSGADEEQRNDVDSSRTSRVGKWESGKGRCAVT	: 501
NcPex12p	CPICEDEIQTEAACQ-TGVVYCFSCIHKWISGTHVRQEKFMTKE---------------------REGKWESGEGRCAVT	: 430
GzPex12p	CPICIDEIVTETACQ-TGYIHKWIEGQHQKQEDFMET-------------------------REGKWESGQGRCAVT	: 409
CnPex12p	CPLCNKAWANNPAILP-SGVVICWKCGWNAIEGEKECHDEQSEDKPR-----------------EEEVSEREKEGSSYETGKSTDDREKKTERKGRCPIT	: 428
CcPex12p**	CPICRKEINNATALP-SGVVFCYCIAHDQVE-------------------------------KQCRCIT	: 336
YlPex12p	CPLCSKELVNPTVIE-SGYVFCYTCIYRHIEDG---------------------------DEETGGRCPVT	: 384
HpPex12p	CPLCLEEIHNPAVIE-TGYVFCYTCIYTFIRE-----------------------GDENGKRCPIT	: 371
PpPex12p	CPLCHKQITNPAVIE-TGYVFCYTCIFKHITSS-----------------------ELDEETGGRCPIT	: 383
PsPex12p	CPICKKELTNPAIIE-TGYVEDYACIYNYIEKSHIIVNDLKLKRKRNKELDEEDEEEEEESEIDSEAEEQEKEEEPQDY----EPKQEQDFTIDINKGGRCPVT	: 439
CaPex12p	CPLCKKELTNPAIIE-TGYVEDMSCIYNYIEKSHIIVSKKLQTK------QKDEEDDNIYSEDESEDENIENEKKE-------EAKEKENVVIDINKGGRCPIT	: 440
DhPex12p	CPLCKDEISNPAVIE-TGYVFCYCIYNYIAQSHKIISEKARLR------REEMDSDTEESDNEKEDQN-EKVDAN--------ATQEEKITIDVNKGGRCPIT	: 424
KlPex12p	CPLCGEEITENAMIS-TGYVANLECAKKWISTEN------------------------TCFAT	: 318
AgPex12p	CPVCRSAVSNPGVLQ-TGYIACYPCAVRYI-VEKH-------------------------CKCPVM	: 337
ScPex12p	CPVCEKTVQNPCVLE-TGYVACYPCAISYITVNNE------------------------GHCPVT	: 372
OsPex12p	CPLCCQKRKNPSVLSASGFVFCYSCIFKSISQHK-------------------------RCPIT	: 378
AtPex12p	CALCLQKRANESVVTVSGFVFCYSCVFKYISKYK-------------------------RCPVT	: 377
CePex12p	CPICLKKRVNDTALFVSGYVFCYTCINQYVNTYN-------------------------KCPVT	: 345
TtPex12p	CILCGKGKLRNESVLNSSGYVFCYSCITEFVKNNK-------------------------KCPVT	: 300
DmPex12p	CPVCLLSIQTETACSVSGYVFCWKCIVSHIKNEH-------------------------TCPVT	: 282
DdPex12p	CPLCLKERTNPN-TICGSGFVFCYPCIFGYINEHS-------------------------KCPIT	: 443
DrPex12p	CPICRKVRTNDTALATSGYVFCYRCIVYYIKANH-------------------------RCPLT	: 333
HsPex12p	CPLGRKTRVNDTVLATSGYVFCYRCVFHYIRSHQ-------------------------ACPIT	: 342
TbPex12p	CPVCERPVTNAAVCTVSGVVGCYPCLTQFAREKN-------------------------ACPVT	: 380

1 2   3 4 5 6   7 8

Fig. 2. Sequence alignment of RING finger motifs in the peroxins Pex2p (A), Pex10p (B) and Pex12p (C). For the alignments protein sequences from representative organisms were used (see below). With the exception of *A. nidulans* sequences all sequences were taken from the NCBI protein database. Entire protein sequences were aligned using the Clustal-X programme (Thompson et al., 1997). Gaps were introduced to maximize the similarity. Only the portion of the alignments comprising the RING finger domains are shown. Residues that are similar in all proteins are represented by white letters that are shaded black. Conserved residues are either shown as white letters that are shaded dark grey, or as black letters that are shaded light grey, depending on the level of conservation. A single asterisk indicates that the proteins did not align properly outside the RING finger domain. A double asterisk indicates that the protein sequence of the RING finger required correction. The cysteine and histidine residues that constitute the $(Cys)_3His(Cys)_4$ motif are indicated by the numbers 1–8. The non-conserved nature of the histidine in the Pex2p RING finger is indicated in colour (blue, histidine; red, cysteine; yellow, other amino acid). Protein sequences from the following organisms were used: Fungi: Ascomycetes: Ac, *Ajellomyces capsulatus*; Ag, *Ashbya gossypii*; An, *Aspergillus nidulans*; Ca, *Candida albicans*; Dh, *Debaryomyces hansenii*; Gz, *Gibberella zeae*; Hp, *Hansenula polymorpha*; Kl, *Kluyveromyces lactis*; Nc, *Neurospora crassa;* Pp, *Pichia pastoris*; Ps, *Pichia stipitis*; Sc, *Saccharomyces cerevisiae*; Yl, *Yarrowia lipolytica*. Basidiomycetes: Cc, *Coprinopsis cinerea*; Cn, *Cryptococcus neoformans*. Higher eukaryotes: At, *Arabidopsis thaliana*; Ce, *Caenorhabditis elegans*; Dd, *Dictyostelium discoideum*; Dm, *Drosophila melanogaster*; Dr, *Danio rerio*; Hs, *Homo sapiens*; Os, *Oryza sativa*; Tt, *Tetrahymena thermophila*. Parasite: Tb, *Trypanosoma brucei*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

be involved in Zn^{2+} binding. Nevertheless, it was recently demonstrated that *Saccharomyces cerevisiae* Pex2p can actually bind two Zn^{2+} ions (Koellensperger et al., 2007). A closer analysis of the sequence of the RING finger in Pex2p of *S. cerevisiae* and its close relatives reveals a conserved cysteine residue immediately preceding cysteine 3 of the RING finger (Fig. 2). Combined, these data indicate that the RING finger of Pex2p structurally varies in different organisms, which may affect the—as yet unknown—function of

this peroxin in peroxisome biogenesis. Recently, Bonnet et al. (2006) have suggested that *P. anserina* Pex2p might have a direct role in sexual development independent of its function as a peroxin. Possibly, the deviant RING finger of Pex2p in ascomycete filamentous fungi including *A. nidulans* is related to this putative role in sexual development.

Comparison of the RING finger of Pex12p indicates much less conservation of the cysteine and histidine residues. Pex12p's seem to have a $(Cys)_2[Ser/Thr][Tyr/Phe](Cys)_3Thr$ motif (Fig. 2). In a typical $(Cys)_3His(Cys)_4$ motif, cysteines 1, 2, 5 and 6 form one Zn^{2+} binding site, while histidine 4 and cysteines 3, 7 and 8 form another (Barlow et al., 1994). In most species including *A. nidulans*, only the cysteine residues forming the first binding site are conserved in the Pex12p RING motif, allowing binding of a single Zn^{2+} ion. This was recently confirmed for *S. cerevisiae* Pex12p (Koellensperger et al., 2007). However, this might not be the case for *Pichia stipitis*, *Candida albicans* and *Kluyveromyces lactis* Pex12p, where cysteine 5 has been replaced by aspartate or asparagine. Whether these degenerate RING motifs can actually bind Zn^{2+} is currently unknown.

2.4. Peroxisomal receptor recycling

After the PTS receptors have released their cargo in the lumen of the peroxisome, they have to be recycled to the cytosol. This has recently been studied in detail for the PTS1 receptor, Pex5p. In baker's yeast and man, it was shown that the recycling step includes mono-ubiquitination of the receptor Pex5p (Kragt et al., 2005; Platta et al., 2007). This process involves the recycling complex, which comprises of two parts, one containing the peroxin Pex4p, a ubiquitin conjugating enzyme (E2), which becomes recruited by the integral membrane protein Pex22p, the other containing the AAA-ATPases Pex1p and Pex6p attached to their own membrane anchor (designated Pex15p in yeast and Pex26p in mammals and other eukaryotes; see Kiel et al., 2006). Recently, Eckert and Johnsson (2003) demonstrated that in baker's yeast the E2 enzyme Pex4p is located in close proximity of Pex10p, which has a highly conserved RING finger motif (see above). RING finger proteins may actually function as ubiquitin ligases (E3) in protein ubiquitination reactions (Joazeiro and Weissman, 2000). Thus, Pex10p and Pex4p might function as the E3 and E2 enzymes, respectively, in the mono-ubiquitination of the receptor Pex5p, which appears to occur at a conserved cysteine residue in the N-terminus of the protein (Carvalho et al., 2007; Williams et al., 2007). In addition, in certain yeast *pex* mutants (e.g. *pex1*, *pex4*, *pex6*) Pex5p becomes quickly degraded by the proteasome, a process that requires poly-ubiquitination at the peroxisomal membrane. This has been considered a "quality control" mechanism and is aimed to remove receptor molecules from the importomer that cannot recycle to the cytosol (cf. Kiel et al., 2005b,c). In this case one or two lysine residues in the N-terminus of Pex5p are the target for the poly-ubiquitination step, with Ubc4p functioning as the E2 enzyme instead of Pex4p (Platta et al., 2004; Kiel et al., 2005c; Kragt et al., 2005). Whether in this quality control mechanism one of the proteins of the RING finger complex functions as an E3 is currently unknown.

The function of the AAA-ATPases Pex1p and Pex6p in this recycling process appears to be analogous to that of yeast Cdc48p (and mammalian p97) in endoplasmic reticulum-associated protein degradation (ERAD) (reviewed by Platta et al. (2008)). In this scenario, the Pex1p/Pex6p complex "pulls" Pex5p out of the membrane into the cytosol to enable a new round of import. In this respect the N-terminus of Pex1p is important since it can adopt a double psi barrel fold, that is also found in the N-terminal domain of yeast Cdc48p and mammalian p97 (Shiozawa et al., 2004). It has been suggested that this domain may be involved in interactions

with ubiquitin-related molecules and may possess a putative adaptor or substrate binding site. In addition, it was demonstrated that the N-terminus of mammalian Pex1p is able to bind phosphoinositides (Shiozawa et al., 2006), but how this fits with the role of Pex1p in receptor recycling is unclear.

Orthologs of Pex1p, Pex4p, Pex6p and Pex26p are present in *A. nidulans* and other filamentous fungi. As can be expected, *A. nidulans pex6* and *N. crassa pex1*, *pex4* and *pex6* mutants mislocalize PTS1 and PTS2 proteins to the cytosol (Hynes et al., 2008; Liu et al., 2008), but data on their functional role in receptor recycling is lacking. A Pex22p ortholog has been difficult to identify because of its extremely weak conservation (cf. Zolman et al., 2005). However, extensive Blast analysis has identified a protein in filamentous fungi with very weak similarity to Pex22p, which we designated Pex22p-like protein (Kiel et al., 2006). An ortholog of this protein is present in *A. nidulans* (Table 1), but future investigations will have to elucidate whether this protein is actually involved in peroxisome biogenesis.

2.5. Proliferation of peroxisomes (Table 2)

Peroxins involved in peroxisome proliferation allow the cell to regulate the number of organelles in the cell, which may increase the efficiency of certain metabolic routes significantly. Furthermore, this process is required to provide the new cells formed at the hyphal tip in mycelia with peroxisomes and Woronin bodies. The peroxins implicated in peroxisome proliferation are integral protein components of the peroxisomal membrane. These can be classified in two families based on their (weak) similarity to either Pex11p or Pex23p (Kiel et al., 2006).

2.5.1. The pex11p family

Orthologs and paralogs of Pex11p are present in all eukaryotes including *A. nidulans*. Overproduction of Pex11p induces massive peroxisome proliferation in all organisms studied (for review see Thoms and Erdmann (2005)), including the filamentous fungus *P. chrysogenum* (Kiel et al., 2005a). Conversely, a reduction of Pex11p levels significantly reduces organelle numbers, also in *A. nidulans* (Hynes et al., 2008). Like in yeast species, *A. nidulans pex11* is induced by fatty acids. In *A. nidulans*, this induction depends on the transcription factors FarA and FarB (Hynes et al., 2006, 2008). It was demonstrated that microbody proliferation in response to oleate is greatly reduced in a *An-farA* mutant. A putative FarA/FarB binding site (CCGAGG) can be identified upstream of *An-pex11* (Table 2).

In addition to Pex11p, the genome of *A. nidulans* encodes two other Pex11p-related proteins, designated Pex11Bp and Pex11Cp, of which the latter only displays weak similarity to Pex11p. This situation is analogous to that observed in human cells that also contain three Pex11p-related proteins (PEX11α, β and γ; Thoms and Erdmann, 2005). Of these, HsPEX11α is required for peroxisome proliferation in response to external stimuli, while HsPEX11β takes care of constitutive peroxisome proliferation. *A. nidulans* Pex11p and Pex11Bp show highest similarity to both mammalian isoforms α and β, while Pex11Cp more resembles HsPEX11γ. Remarkably, putative FarA/FarB binding sites are present upstream of *pex11C*, but not *pex11B* (Table 2), suggesting that also Pex11Cp synthesis is enhanced during growth on fatty acids. How the Pex11p family proteins exactly function in stimulating peroxisome proliferation in still unknown.

2.5.2. The Pex23 family

A second family of proteins thought to be involved in determining peroxisome numbers in the cell consists of the proteins Pex23p, Pex24p and certain paralogs that have been identified mainly in yeast species (Kiel et al., 2006). So far, these peroxins have been

Table 2
A. nidulans peroxins involved in microbody proliferation

Peroxin	Locus tag	Position of FarA/FarB binding sites
A	Pex11p family	
Pex11p	AN1921.3	−281
Pex11Bp	AN5428.3	None
Pex11Cp	AN7256.3	−178, −844
Pex25p	Na	—
Pex27p	Na	—
B	Pex23 family	
Pex23p	AN5692.3	None
Pex23p-like	AN4995.3	None
Pex24p	AN7677.3	None
(Pex28p)	Orthologue of Pex24p (Kiel et al., 2006)	—
Pex29p	Na	—
(Pex30p)	Orthologue of Pex23p (Kiel et al., 2006)	—
Pex31p	Na	—
Pex32p	Na	—

Proteins involved in peroxisome proliferation identified in fungal species, plants and mammals were used to identify orthologs in *Aspergillus nidulans*. Locus tag numbers are from the Broad Institute. Na, not available/identifiable; FarA/FarB binding site, CCTCGG or its complement CCGAGG.

the subject of relatively few studies, and exclusively in yeast species (*S. cerevisiae*, *Y. lipolytica* and *P. pastoris*; Brown et al., 2000; Tam and Rachubinski, 2002; Vizeacoumar et al., 2003, 2004; Yan et al., 2008). Despite this limited attention, the nomenclature of these proteins is confusing with *S. cerevisiae* and *P. pastoris* Pex30p being the orthologs of *Y. lipolytica* Pex23p, *S. cerevisiae* Pex28p that of *Y. lipolytica* Pex24p and *P. pastoris* Pex31p that of *S. cerevisiae* Pex32p. We utilize exclusively the numbers of the genes that appeared first in the literature (i.e. Pex23p, Pex24p etc.; Kiel et al., 2006; cf. Table 2). All members of the Pex23p family contain a putative DysF motif with an unknown function that was first identified in human Dysferlin (Ponting et al., 2001). In *S. cerevisiae* absence of any of the DysF family members (*Sc*-Pex28p, *Sc*-Pex29p, *Sc*-Pex30p, *Sc*-Pex31p and *Sc*-Pex32p) affect the number, the shape and/or the clustering of peroxisomes during growth on oleate (Vizeacoumar et al., 2003; 2004). Recently, it was found in the methylotrophic yeast *P. pastoris* that the absence of either of two identified Pex23p family proteins (*Pp*-Pex30p and *Pp*-Pex31p; actually representing orthologs of Pex23p and Pex32p, respectively; see Kiel et al., 2006) resulted in fewer, but larger peroxisomes only during growth on oleate, and not during growth on methanol (Yan et al., 2008). In *P. pastoris* both proteins have a dual localization, being not only peroxisomal but also located at the endoplasmic reticulum.

Aspergillus nidulans and other filamentous fungi contain orthologs of Pex23p and Pex24p (Table 2). In addition to this, we recently identified one other DysF domain protein related to Pex23p, that is conserved in filamentous fungi as well as in most yeast species (Kiel et al., 2006). However, the function of this Pex23p-like protein in peroxisome proliferation is unknown. Clearly, the lack of understanding of the function of these DysF family proteins requires further study.

3. The formation of Woronin bodies (WBs)

As indicated above, WBs are exclusively found in filamentous acomycetes and function in sealing the septal pore in response to wounding (Markham and Collinge, 1987). WB formation has been studied in most detail in *N. crassa*, because they can easily be recognized by phase contrast microscopy (see Liu et al., 2008 and the references therein). WBs contain major amounts of a single filamentous fungus-specific protein Hex1p, which self-assembles into the typical crystalline matrix of the organelle (Jedd and Chua,

2000; Tenney et al., 2000). Initially, the biogenesis of WBs was associated with large membranous sacs from which an electron-dense organelle containing a crystalline core was pinched off. The presence of a PTS1 at the C-terminus of Hex1p has directly linked WB formation with peroxisomes. Indeed, it was demonstrated in *N. crassa* that all peroxins involved in peroxisomal matrix protein import, except those involved in PTS2 import (Pex7p and Pex20p), are required for the formation of WBs (Managadze et al., 2007; Liu et al., 2008). Remarkably, in *N. crassa* not all peroxisomes are source of WBs. Rather, Hex1p crystalloids only form in one/few large peroxisomes per cell. Furthermore, a novel protein, designated the Woronin sorting complex (WSC), which is homologous to the mammalian proteins PMP22 and MPV17, was required to partition the Hex1p crystalloids from the peroxisomal matrix and to form WBs. This process invariably occurred at the cell cortex (Liu et al., 2008). WB formation also appeared to be independent of the factors required for normal peroxisome fission, implying the presence of a unique fission machinery.

Also Aspergilli contain WBs and orthologs of both Hex1p and WSC are encoded by their genomes (in *A. nidulans* the proteins AN4695.3 and AN7258.3, respectively). Aspergilli do not contain the large hexagonal WBs observed in *N. crassa*. However, studies in *A. oryzae* have shown that Hex1p requires phosphorylation to enable crystallization and WB formation (Juvvadi et al., 2007). So far, biogenesis of WBs in Aspergilli was not studied in detail, but it is likely that the same molecular mechanisms are involved in the formation of these organelles.

Table 3
A. nidulans proteins involved in autophagy and related pathways

Peroxin	Locus tag
Atg1p	AN1632.3
Atg2p	AN5491.3
Atg3p	AN11004.3
Atg4p	AN3470.3
Atg5p	AN5174.3
Atg6p	AN10213.3
Atg7p	AN7428.3
Atg8p	AN5131.3
Atg9p	AN3734.3[*]
Atg10p	AN10728.3
Atg11p	AN2887.3
Atg12p	AN1760.3
Atg13p	AN2076.3
Atg14p	Na
Atg15p	AN5919.3
Atg16p	AN0090.3
Atg17p	AN6360.3
Atg18p	AN0127.3
Atg19p	Na
Atg20p	Na
Atg21p	Na
Atg22A1p	AN7437.3
Atg22A2p	AN7591.3
Atg22B1p	AN5876.3
Atg22B2p	AN2876.3
Atg23p	Na
Atg24p	AN3584.3
Atg25p	Na
Atg26p	AN4601.3
Atg27p	AN0861.3
Atg28p	AN1701.3
Atg29p	AN4832.3
Atg30p	Na
Atg31p	Na

See also Tables 1 and 2. Proteins in bold are implicated in either macroautophagy or in all autophagy-related processes. Proteins in italics are implicated in selective transport of material to the vacuole. [*], the sequenced *A. nidulans* genome contains a frame shift in exon 1 of *ATG9* (between nt 600 and 601 in the CDS), which is presumably due to a sequencing error.

4. Pexophagy, the selective degradation of peroxisomes

The autophagic degradation of peroxisomes requires Atg proteins. A recent review has described in detail the function of the various Atg proteins in the different steps of (macro-) autophagy and their conservation in yeast species and filamentous fungi (Meijer et al., 2007). Here we will mainly focus on the Atg proteins involved in pexophagy. Table 3 lists the identified *A. nidulans* Atg orthologs with in bold face those required either for macroautophagy or for all autophagy-related processes. Almost without exception these proteins can be identified in *A. nidulans*. Those that can not have only been found in *S. cerevisiae* and related species (e.g. Atg14p, Atg31p), and are only weakly conserved (Meijer et al., 2007). Nevertheless, we have observed that *A. nidulans* Atg15p, a lipase that is thought to be required for recycling of membrane components inside the vacuole (Epple et al., 2001), contains a novel repeat at its C-terminus (Fig. 3). This motif is preceded by a Ser/Thr rich region and is only found in filamentous fungi. In *S. cerevisiae* Atg15p is transported via the secretory pathway to the vacuole (Epple et al., 2001, 2003) and the C-terminus of this protein has been shown to be located in the lumen of the ER. Thus, the motif may be undergoing post-translational modification (e.g. *O*-glycosylation) when the protein is en route to the vacuole.

Another vacuole-specific protein is Atg22p, an integral vacuolar membrane protein with similarity to permeases of the major facilitator superfamily, which has been suggested to function in export

A

Repeat #	# of Thr+Ser prior to motif	motif
Anid-1	18/20	CKTPG-WWG-CLDD
Anid-2	13/16	CKTPG-WFG-CKDP
Anid-3	13/17	CKDPG-WFG-CRDP
Anid-4	9/13	CETPGFFWG-CYDT
Ater-1	19/21	CKTPG-WWG-CLDE
Ater-2	17/18	CKTPG-WFG-CKDP
Ater-3	14/23	CAHPG-WFG-CRDP
Ater-4	10/18	CESPGFFWG-CWDP
Afum-1	17/20	CKTPG-WWG-CLDE
Afum-2	21/21	CKTPG-WFG-CKDP
Afum-3	18/29	CKDPG-WFG-CRDP
Afum-4	12/16	CDDPGFFWG-CYDE
Anig-1	18/20	CKTPG-WWG-CLDQ
Anig-2	14/15	CKTPG-WFG-CKDP
Anig-3	15/24	CKTPG-WFG-CKDE
Acla-1	15/19	CETPG-WWG-CLDE
Acla-2	16/18	CKTPG-WFG-CKDS
Acla-3	15/28	CKDPG-WFG-CRDP
Cimm-1	11/14	CETPG-WWG-CLDK
Cimm-2	13/17	CKTPG-WFG-CKDP
Cimm-3	17/29	CETPG-WFGGCNDP
Cimm-4	12/17	CTRPGFFWG-CWDP
Pchr-1	16/18	CKTPG-WWG-CLDE
Pchr-2	18/20	CMTPG-WFG-CNDP
Pchr-3	16/24	CHDPG-WFG-CRDE
Pchr-4	10/18	CHSPGIFWG-CWDE
Pchr-5	11/20	CHSPGIFWG-CWDE
Pchr-6	11/20	CHIPGIFWG-CWDE

B

Fig. 3. A repeated region in Atg15p in filamentous fungi. (A) Comparison of amino acid sequences of the repeats present in the C-termini of Atg15p orthologs in seven related filamentous ascomycetes. Anid, *Aspergillus nidulans* (locus tag AN5919.3); Ater, *A. terreus* (Genbank: EAU38245); Afum, *A. fumigatus* (EAL93385); Anig, *A. niger* (CAK44586); Acla, *A. clavatus* (EAW13957); Cimm, *Coccidioides immitis* (EAS33299) and Pchr, *Penicillium chrysogenum* (ABO31085). Protein sequences were aligned using Clustal-X. Gaps were introduced to maximize the similarity. The number of serine + threonine residues in the region directly preceding the repeat is also indicated. (B) Weblogo graphic (http://weblogo.berkeley.edu/logo.cgi) showing the distribution and amino acid composition of the repeat in Atg15p. For the graphic the sequences shown in (A) were used. Residues with similar properties are indicated in the same colour. Polar amino acids (G, S, T, Y, C, Q, N) are green, basic amino acids (K, R, H) blue, acidic amino acids (D, E) red and hydrophobic amino acids (A, V, L, I, P, W, F, M) are black. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of amino acids generated by vacuolar proteases from the vacuole to the cytosol (Suriapranata et al., 2000; Yang et al., 2006). Unlike yeast species, *A. nidulans* contains multiple paralogs of Atg22p, designated Atg22A1p, Atg22A2p, Atg22B1p and Atg22B2p. Data from *S. cerevisiae* suggest a dual subcellular location for *Sc*-Atg22p, at the vacuole (Suriapranata et al., 2000) and at peroxisomes (Marelli et al., 2004). This might imply that the *A. nidulans* Atg22p paralogs localize to different organelles, where they perform their putative transporter function.

Table 3 also lists the Atg proteins involved in the selective types of autophagy (pexophagy and the Cvt pathway; in italics). Atg30p is thought to represent a receptor protein that, via the scaffold protein Atg11p, links the peroxisomes to be degraded to the autophagic machinery (Farré et al., 2008). In the methylotrophic yeast *Hansenula polymorpha* two peroxisomal membrane proteins were identified (the peroxins Pex3p and Pex14p, see above) that are required for pexophagy (Bellu et al., 2001, 2002). Indeed in the related yeast *P. pastoris* Atg30p was shown to interact with both Pex3p and Pex14p (Farré et al., 2008). Also Atg25p, Atg26p and Atg28p are specifically required for pexophagy (Monastyrska et al., 2005; Oku et al., 2003; Stasyk et al., 2006), but their exact function is not very well understood. Remarkably, with the exception of Atg11p and Atg26p, these proteins only show weak conservation, or are absent in *A. nidulans*. This suggests that in *A. nidulans* selective transport of peroxisomes to the vacuole may utilize another recognition mechanism to link the organelles to be degraded to Atg11p and the autophagy machinery.

5. Concluding remarks

Our analysis of the presence of *PEX* genes in the *A. nidulans* genome suggests that most peroxins involved in peroxisome biogenesis and proliferation are conserved in this filamentous fungus, stressing the importance of peroxisomes in cellular metabolism. The major difference between filamentous fungi like *A. nidulans* and other organisms with respect to the importomer appears to be the RING structure of Pex2p. Remarkable is also the presence of orthologs of Pex16p and the novel protein Pex14/17p. Pex16p has been identified in higher eukaryotes including plants and mammals, but it is absent in most yeast species. Conversely, the putative ortholog of Pex14/17p, Pex17p, has so far only been identified in yeast species. Thus, matrix protein import in filamentous fungi like *A. nidulans* may actually utilize features from both worlds.

With respect to autophagic degradation of peroxisomes in *A. nidulans*, the autophagy machinery appears to be fully conserved in this filamentous fungus. However, the receptor required to link the organelles to be degraded to this machinery has not been identified, which is also the case in higher eukaryotes. Nevertheless, *A. nidulans* contains an Atg11p ortholog, that appears to be only required for selective transport to the vacuole.

These observations and the notion that *pex* mutants in filamentous fungi better resemble human PBDs than yeast *pex* mutants, imply that filamentous fungi like *A. nidulans* may represent better model systems for studies on peroxisome homeostasis than the yeast species currently used as such.

Acknowledgments

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