Peroxisome Assembly and Functional Diversity in Eukaryotic Microorganisms

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Abstract

Peroxisomes are core eukaryotic organelles that generally function in lipid metabolism and detoxification of reactive oxygen species, but they are increasingly associated with taxa-specific metabolic, cellular, and developmental functions. Here, we present a brief overview of peroxisome assembly, followed by a discussion of their functional diversification. Matrix protein import occurs through a remarkable translocon that can accommodate folded and even oligomeric proteins. Metabolically specialized peroxisomes include glycosomes of trypanosomes, which have come to compartmentalize most of the glycolytic pathway and play a role in developmental signal transduction. The differentiation of physically distinct subcompartments also contributes to peroxisome diversification; in the clade of filamentous ascomycetes, dense-core Woronin bodies bud from peroxisomes to gate cell-to-cell channels. Here, the import of oligomeric cargo is central to the mechanism of subcompartment specification. In general, the acquisition of a tripeptide peroxisome targeting signal by nonperoxisomal proteins appears to be a recurrent step in the evolution of peroxisome diversity.

Contents

INTRODUCTION

Peroxisomes are single-membrane-bound eukaryotic organelles involved in diverse metabolic functions. They generally function in lipid metabolism and detoxification of reactive oxygen species, but they are also involved in diverse taxa-specific functions. These include catabolism of very-long-chain, D-amino acids, and polyamines in mammals; biosynthesis of plasmalogens in mammals (181); photorespiration in leaves (132); the glyoxylate cycle in germinating seeds (90); and assimilation of methanol in some yeasts (172). In trypanosomes, peroxisomes called glycosomes compartmentalize components of the glycolytic pathway and are implicated in a signaling pathway associated with parasite development (158). Peroxisomes also play important roles in signal transduction, such as salicylic acid signaling in plants (183), and have been implicated as platforms for signaling in vertebrate innate immunity (18). In filamentous fungi, peroxisomes are involved in penicillin biosynthesis (63), plant pathogenicity (67), and sexual development (8), and they have evolved the capacity to develop a peroxisome subcompartment known as the Woronin body, which performs an adaptive function in gating fungal cell-to-cell channels. Here we review peroxisome biogenesis, function, and dynamics, and provide an overview of their remarkable functional plasticity in eukaryotic microorganisms.

Peroxisome: single-membrane-bound eukaryotic organelle generally associated with lipid metabolism

Glycosome: specialized peroxisome housing a subset of enzymes involved in glycolysis and playing a role in developmental signal transduction

Woronin body: a physically and functionally distinct peroxisome subcompartment involved in cellular wound healing in filamentous ascomycetes

PTS1: peroxisome targeting signal 1

PTS2: peroxisome targeting signal 2

PEROXISOME ASSEMBLY

Matrix Protein Import

More than 30 genes involved in the biogenesis of peroxisomes (PEX genes) have been identified. Mutations in genes coding for the components of the matrix protein import machinery lead to the formation of "ghosts," or empty peroxisome remnants, in which membrane proteins are still inserted into the lipid bilayer. This demonstrates that matrix and membrane proteins are imported by distinct pathways.

Cargo recognition. Peroxisome matrix proteins are synthesized on free polyribosomes in the cytosol and imported posttranslationally (29, 180). Their targeting to peroxisomes depends on short sequences known as peroxisomal targeting signal (PTS) type 1 and type 2. PTS1 is present in the majority of matrix proteins and consists of a C-terminal tripeptide (S/A/C)(K/R/H)L (38). PTS2 is a degenerated nonapeptide (R/K)(L/V/I)X_5(H/Q)(L/A) found near the N terminus that,

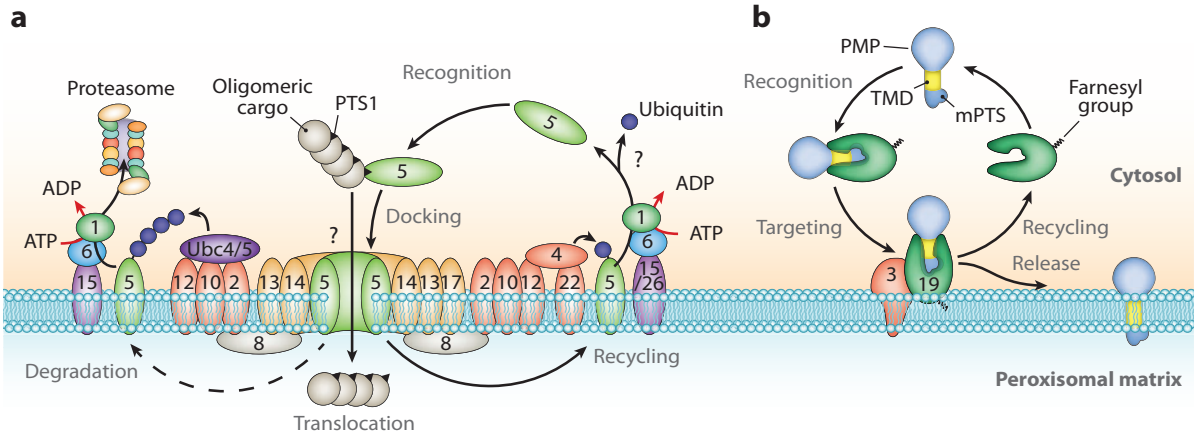

Figure 1

(*a*) PTS1 matrix import cycle. Cargo, which can be oligomeric, is recognized by Pex5 and docks with the membrane through the docking peroxins Pex13, Pex14, and Pex17. (*Right*) The cargo-receptor complex crosses the membrane via an unknown mechanism that may involve the formation of a transient pore. The Pex5 receptor is monoubiquitinated and recycled to the cytosol through the AAA ATPases Pex1 and Pex6. (*Left*) In the RADAR pathway, dysfunctional Pex5 can be polyubiquitinated and targeted to the proteasome for degradation. Adapted from Reference 145 with permission. (*b*) PMP integration. PMPs possessing a mPTS are recognized posttranslationally by Pex19p and delivered to the membrane through interaction with Pex3, where the PMP is released and integrated into the membrane. Adapted from Reference 40 with permission. Abbreviations: PTS1/2, peroxisome targeting signal 1/2; RADAR, receptor accumulation and degradation in the absence of recycling; PMP, peroxisomal membrane protein; mPTS, membrane peroxisome targeting signal; TMD, transmembrane domain.

in some species, is cleaved off after import into the peroxisomal matrix (117, 123, 157). PTS1 and PTS2 are recognized by cycling cytosolic receptors Pex5 and Pex7, respectively.

Pex5 contains a conserved C-terminal domain composed of tetratricopeptide repeats (TPRs) that directly bind the PTS1 of peroxisomal cargo (11, 33). The crystal structure of the Pex5 TPR domains shows that this protein undergoes dramatic conformational changes upon cargo binding (154). In a few cases the N-terminal half of Pex5 can also mediate the binding to cargoes lacking canonical PTS1 sequences (43, 69). This region is less conserved, disordered (154), and responsible for docking to the cytoplasmic face of the peroxisome membrane through interaction with the docking peroxins (Pex13, Pex14, and Pex17) (118, 119, 171) (**Figure 1*a***).

The targeting of PTS2-bearing proteins is mediated by Pex7 together with a coreceptor that varies from species to species: Pex18 and Pex21 in *Saccharomyces cerevisiae* (130), Pex20 in filamentous ascomycetes (82, 120, 150, 167), and Pex5L, a splicing variant of Pex5, in plants and mammals (9, 78). Thus, Pex5 is required for both PTS1 and PTS2 import in plants and mammals, whereas in fungi, Pex20 allows these two pathways to function more independently.

Surprisingly, the PTS2 pathway seems to be absent in certain organisms including *Caenorhabditis elegans* (106), diatoms (36), and probably the red alga *Cyanidioschyzon merolae*, in which no PTS2-like sequences have been identified in silico (149). Instead, in those organisms, orthologs of PTS2-containing proteins have acquired PTS1 signals (36, 106). Thus, switching of targeting signals appears to have allowed the PTS2 pathway to be lost in certain phylogenetic lineages.

Docking and translocation. Both PTS receptor systems address their cargo to a docking/translocation platform composed of Pex13, Pex14 and, in some yeast, Pex17. Pex13 and Pex14 bind to each other (144) and interact with both PTS receptors (19, 24, 119, 155) through multiple binding sites. Remarkably, whereas Pex5 behaves like a soluble protein in the cytosol,

TPR: tetratricopeptide repeat

Peroxin: protein associated with peroxisome biogenesis and proliferation

membrane-associated Pex5 behaves like a transmembrane protein (39). Subsequent work showed that during the import cycle, both receptor and cargo cross the peroxisome membrane, and this finding has been demonstrated for Pex5 (17), Pex7 (110), and Pex20 (82). How the cargo is released has not yet been determined. However, the intraperoxisomal peroxin Pex8 interacts with Pex5 in vitro and stimulates the release of PTS1 peptides, suggesting one possible mechanism (182).

RING: Really Interesting New Gene

AAA ATPase: ATPases associated with diverse cellular activities

Recently, a new docking peroxin Pex14/17 (114), also known as Pex33 (89), has been identified in filamentous ascomycetes. This peroxin interacts with Pex5 (89) and appears to be functionally equivalent to yeast Pex17. Deletion mutants significantly reduce the efficiency of matrix import, indicating that this is a bona fide new peroxin. Interestingly, in the filamentous fungus *Podospora anserina*, *PEX14* is required for matrix import in vegetative mycelium but it is dispensable at certain developmental stages in meiocytes (121). In this specialized cell type, matrix import also occurs in a *pex14*, *pex14/17* double mutant but depends on the presence of PEX13. In *Hansenula polymorpha*, overproduction of Pex5 can stimulate residual matrix import in a *pex14* mutant (140), further suggesting that matrix import can occur in the absence of Pex14. Interestingly, in *Pichia pastoris*, the PTS2-dependent import of Pex8 requires Pex14, but not Pex13 and Pex17 (86). Together, these data suggest that the composition of the docking complex can vary in a species-, development-, and cargo-dependent manner.

Receptor recycling. Pex5 receptor recycling is initiated by monoubiquitination (128) on a conserved N-terminal cysteine residue (185). This reaction is mediated by the RING-finger E3 ligase Pex12 (127) and the ubiquitination conjugating enzyme Pex4, which is soluble and is recruited to the membrane by Pex22 (71) (**Figure 1*a***). In yeast, the intraperoxisomal peroxin Pex8 functions as a bridge between the docking receptors and the RING complex to form the overall importomer (1); Pex3 might also play a role in this process (48).

Following monoubiquitination, PEX5 is recycled back to the cytosol by the AAA ATPase peroxins Pex1 and Pex6 (103, 126), which are anchored to the peroxisomal membrane through the tail-anchored peroxin, Pex15, in yeast (6) and the orthologous function of Pex26 in animals (95) and filamentous fungi (83) (**Figure 1*a***). The N-terminal region of the PTS2 coreceptor Pex20 is similar to the N terminus of the PTS1 receptor Pex5 and contains conserved residues that are essential for its recycling from the peroxisomal membrane, suggesting that Pex20 and Pex5 are recycled through a similar mechanism (81, 82). In addition to the ATP consumed by ubiquitin activation, receptor export is believed to be the only ATP-consuming step of the matrix import cycle (103), leading to the concept of an export-driven import, which posits that receptor recycling by the AAA ATPases is mechanistically coupled to cargo translocation across the membrane (145).

When components of the receptor recycling machinery are mutated, Pex5 and Pex20 cannot be recycled back to the cytosol. Under these conditions, the receptors are polyubiquitinated at N-terminal lysine residues and directed to the proteasome for degradation (61, 81, 82, 127, 184). This process, called RADAR (receptor accumulation and degradation in the absence of recycling), appears to constitute a quality-control system that prevents obstruction of the import machinery.

Import of oligomeric cargo. Some peroxisomal matrix proteins are preassembled in the cytosol prior to import and cross the membrane as oligomers (35, 80, 98, 164, 167). Matrix proteins with mutant PTS signals can be imported by their PTS-containing binding partners, and these types of experiments provided definitive evidence for piggyback import into peroxisomes (21, 35, 98, 188). The fact that oligomeric cargo can be imported suggests a translocation machinery that can expand to accommodate substrates of variable dimensions. The presence of a large and flexible translocon is further indicated by experiments showing that 4- to 9-nm PTS1-coated colloidal gold particles can be imported (180). This remarkable feature of the matrix import pathway leads

to a central question concerning how these particles cross the membrane, and indicates that this mechanism is fundamentally different from the translocation of proteins across the endoplasmic reticulum (ER) and mitochondrial membrane.

Electron microscopy reveals the frequent occurrence of aggregates and crystals in the peroxisomal matrix (68, 175, 179), indicating that some matrix proteins form extremely high-order oligomers, and in general the functional significance of these proteins remains enigmatic. One clear case in which functions have been ascribed to these high-order matrix oligomers is found in the fungal HEX (Hexagonal peroxisome) protein, which uses a PTS1 signal and self-assembles to form the Woronin body crystalline core (189). Interestingly, HEX crosses the membrane as an oligomer, and mutants that disrupt self-assembly lead to dominant-negative effects on PTS1 import, but not on PTS2 import (83). This suggests that under normal conditions, HEX oligomers promote import efficiency by allowing each cycle of PEX5 action to import multiple HEX proteins. This provides one physiological context in which the import of oligomers is beneficial. HEX oligomers also appear to influence subcompartment specification (83), further suggesting that import of oligomers can influence peroxisome fate.

PMP: peroxisomal membrane protein

mPTS: peroxisomal membrane protein targeting signal

Nature of the translocon. Several models have been proposed to account for the ability to import folded and oligomeric proteins. These include a membrane invagination mechanism and the opening of a static pore (99, 156). Another model suggests the formation of a transient pore (156) formed by the import receptors themselves (25). Several lines of evidence support this model. Membrane-associated Pex5 behaves like an integral membrane protein (39), and patch-clamp experiments show that large-conductance channels are present in the membranes of mammalian peroxisomes (76, 79). Recently, a membrane-associated Pex5-Pex14 complex was purified, reconstituted into planar lipid membranes, and subjected to current recordings to obtain evidence for channel activity (101). PTS1-bearing peptides alone had no effect on these preparations. However, purified cytosolic Pex5 loaded with cargo induced conductance channel gating events consistent with a pore of 2.8 nm, and when a large oligomeric cargo was used this value increased up to 9.25 nm. The putative translocon present in these preparations was produced from a *pex8* mutant and was not associated with the RING complex. Thus, a major challenge for the future is to reconstitute the entire importomer complex and the full import cycle.

Membrane Targeting and Biogenesis

The majority of PEX genes are implicated in the process of matrix protein import or the regulation of peroxisome number and size. Mutants defective in matrix import typically accumulate empty peroxisomes that contain peroxisomal membrane proteins (PMPs). However, in yeast, loss of one of only two genes, *pex3* and *pex19*, leads to the complete absence of peroxisomal membrane remnants, suggesting that these functions are essential for membrane biogenesis (49).

Direct targeting of PMPs to peroxisomes. In the pathway for direct targeting, PMPs are believed to be synthesized on free cytosolic ribosomes and subsequently imported directly to the peroxisome membrane. This process depends on membrane peroxisome targeting signals (mPTSs) consisting of short stretches of basic amino acids associated with hydrophobic transmembrane domains (133). In this pathway, Pex19 functions as a cycling chaperone, which recognizes the mPTS of PMPs and ferries them to the membrane where PMP integration follows docking to Pex3 (28, 58, 133) (**Figure 1*b***). In vitro systems have been used to reconstitute PMP membrane integration, and these studies show that Pex19 can keep PMPs in an import-competent state before docking to the membrane through a physical interaction with Pex3 (97, 124). Consistent

with this, fluorescence resonance energy transfer (FRET) experiments suggest that the interaction between Pex3 and Pex19 occurs mainly at the peroxisomal membrane (109). Interestingly, cargo-loaded Pex19 has a higher affinity for Pex3 than does free Pex19, suggesting that the mPTS or transmembrane domains of the PMP also contribute to Pex3 binding (124). The C-terminal region of Pex19p forms a globular α-helical domain that binds the mPTS of PMPs (147), and a distinct N-terminal region is responsible for binding to Pex3 (143, 146).

Pex19 also possesses a farnesylated C-terminal CaaX box. Yeast mutants in which Pex19 cannot be farnesylated produce peroxisomes, but contain significantly reduced steady-state levels of PMPs and show defects in matrix import, which are presumably a secondary consequence of defects in PMP import (136). In addition, PMP recognition is 10 times more efficient when Pex19 is farnesylated (136). Together, these observations suggest that farnesylation promotes Pex19 function, possibly by promoting its interaction with the membrane and/or hydrophobic transmembrane domains associated with the mPTS.

PMP trafficking via the ER. N- and O-linked glycosylation occurs in the lumen of the ER, and several PMPs receive these types of modification, suggesting ER–to–peroxisome trafficking. These PMPs include Pex2 and Pex16 in *Yarrowia lipolytica* (165) and Pex15 (20, 77) and Pex3 (72) in *S. cerevisiae*. In addition, the putative mPTS signals of Pex3 and Pex22 do not interact with Pex19 (47), implying that these proteins follow a different pathway to the membrane. Furthermore, mutations that interfere with the Sec61 translocon (163, 173) and ER exit (165) also interfere with PMP trafficking. Get3 is a chaperone responsible for the integration of tail-anchored proteins into the ER, and loss of function in this system leads to mistargeting of the tail-anchored peroxin Pex15 (148, 173). Further evidence for ER trafficking comes from pulse-chase experiments that follow the reappearance of peroxisomes in peroxisome-free cells. Here, a Pex3 GFP (green fluorescent protein) fusion is first seen in the ER and later in punctate structures that mature to eventually attain the capacity to import matrix proteins (50, 72, 105, 160). In *P. pastoris*, Pex30 and Pex31 also appear to traffic via the ER (187). Although there is abundant evidence to show that PMPs can transit the ER en route to the peroxisome, more work is required to identify the machinery involved in trafficking. Interestingly, mutations in Pex1 and Pex6 delay ER exit of Pex2 and Pex16 in *Y. lipolytica* (165). Pex1 and Pex6 have also been associated with fusion of pre-peroxisomal vesicles (166), suggesting that they may be involved in ER–to–peroxisome trafficking (**Figure 2**).

Peroxisome Proliferation

Peroxisome abundance can vary dramatically depending on cellular need and environmental conditions. Peroxisomes can proliferate through growth and division of preexistent peroxisomes and they can also arise de novo from the ER.

Proliferation by growth and division. Peroxisome proliferation follows a multistep process involving the Pex11-dependent tubulation of peroxisomes, followed by dynamin GTPase-dependent scission of the elongated organelles (reviewed in Reference 116) (**Figure 2**). Pex11p is the first protein identified as being involved in peroxisome proliferation in yeast (23). Here, loss of Pex11 leads to reduced peroxisome abundance and enlarged peroxisomes (23). By contrast, its overexpression produces cells crowded with elongated clusters of peroxisomes (92). Pex25 and Pex27 are two additional *S. cerevisiae* isoforms that share weak similarities with Pex11. Both are involved in peroxisome division and appear to have partially redundant functions with Pex11p (134). The activity of Pex11p appears to be tightly regulated. In yeast, *PEX11* expression is stimulated when

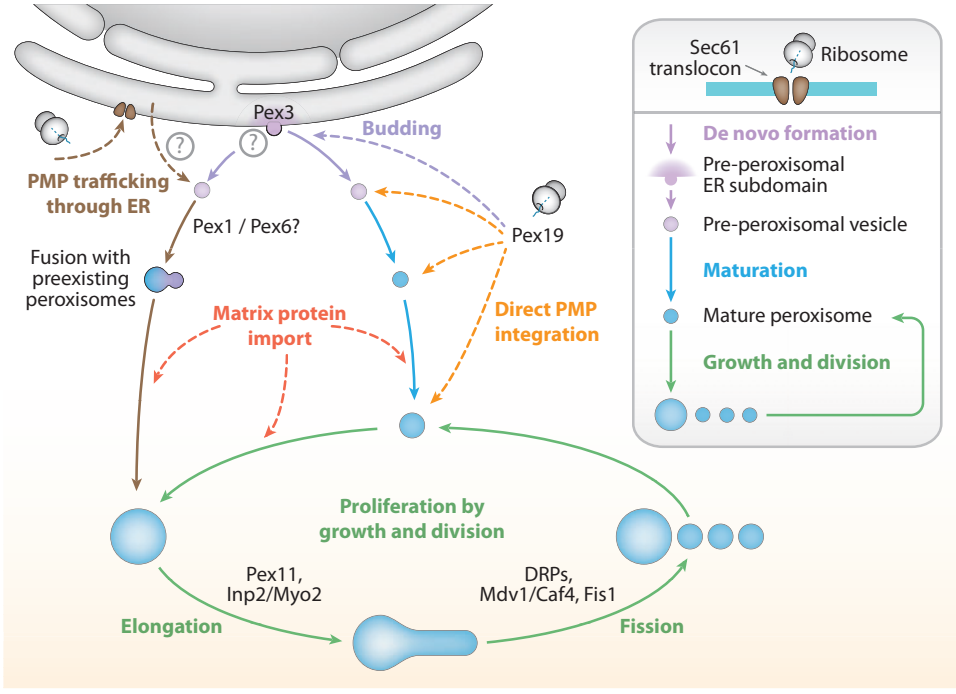

Figure 2

Peroxisome biogenesis and proliferation. Peroxisomes can multiply by growth and division or rise de novo from the ER. In the de novo pathway, Pex3 is targeted to the ER membrane and may bud in a Pex19-dependent manner to form a pre-peroxisome, which matures through the import of additional PMPs to eventually acquire the capacity to import matrix proteins. PMPs may also be inserted into the ER membrane and traffic to the peroxisome membrane. Please see text for additional information. Abbreviations: ER, endoplasmic reticulum; PMP, peroxisomal membrane protein; DRP, dynamin-related protein.

peroxisome proliferation is induced (151, 174), and the activity of Pex11 is also regulated by phosphorylation (70, 139). Interestingly, recent work shows that Pex11 has the inherent ability to sculpt membrane curvature through the insertion of a conserved N-terminal amphipathic helix into the membrane, and this provides a molecular mechanism for its mode of action (115). A number of other peroxins have been associated with peroxisome division and they display varying interactions with one another and Pex11 (168, 176, 177, 187), suggesting that the number and size of peroxisomes are controlled by a complex network of interacting peroxins. In *Y. lipolytica*, Pex16 is intraperoxisomal and functions as a negative regulator of division. As peroxisomes mature to attain a high matrix protein density, acyl-CoA oxidase (Aox) is relocalized from the matrix to the inner face of the membrane, where it inhibits Pex16 function and triggers modification of membrane lipid composition. This further promotes recruitment of the cytosolic division machinery (44, 45) and provides a means of coordinating peroxisome maturation and division. The absence of Pex16 in most other yeast suggests that this type of regulation is achieved through diverse mechanisms.

After tubulation, peroxisome division requires the function of dynamin-like protein (DLP) GTPases, which are involved in various cellular membrane fission events. DLPs oligomerize around membranes, forming ring-like structures that, after GTP hydrolysis, cause deformation and ultimately scission of the membranes (for review, see Reference 129). The DLPs Vps1

DLP: dynamin-like protein

and Dnm1 are involved in peroxisome fission in yeast (51, 75, 105, 178). Dnm1 is recruited to peroxisomes via Mdv1 and Caf4, which are linked to the peroxisomal membrane via the tail-anchored protein Fis1 (107). Fis1 is targeted to mitochondria, where it promotes fission through the same factors (for review, see Reference 113). This suggests that these two organelles share this fission machinery and that this might function to coordinate their biogenesis. Vps1 also functions in peroxisome fission, but it is not recruited to peroxisomes via Fis1 and instead seems to require Pex19 for peroxisome association (178). A recent study in *S. cerevisiae* shows that Pex34 interacts with Pex11, Pex25p, and Pex27p, as well as Fis1p, establishing in yeast a link between Pex11 proteins and the fission machinery (168).

De novo formation of peroxisomes from the ER. In yeast, *pex3* and *pex19* mutants are devoid of peroxisome membranes. However, their reintroduction can induce the de novo formation of peroxisomes. In this case, Pex3p traffics from the ER to punctate structures that gradually attain the capacity to import matrix proteins in a process that depends on Pex19 (50, 72, 105, 160) (**Figure 2**). On the basis of their known function in promoting PMP integration, Pex3 and Pex19 are believed to recruit additional PMPs to pre-peroxisomal vesicles to eventually reconstitute the matrix import pathway. Moreover, Pex3 targeted to mitochondria can induce the formation of import-competent peroxisomes in Pex3-deficient *S. cerevisiae* cells, further suggesting that Pex3 can initiate the de novo peroxisome biogenesis pathway from diverse membranous precursor (135). In *H. polymorpha*, the reintroduction of peroxisomes also requires Pex25, a relative of Pex11, and the Rho1 GTPase (142). Pex25 was previously shown to interact with Rho1 in *S. cerevisiae* (91). However, whether these represent conserved or taxa-specific functions required for the de novo pathway remains to be determined. In animal cells, *pex16* mutants also lack peroxisome remnants, and Pex16 is believed to be responsible for recruiting Pex3 to the membrane either directly (96) or via the ER (66). Pex16 is absent in most yeast but is present in *Y. lipolytica*, where it acts as a negative regulator of peroxisome division (45). Interestingly, Pex16 is present in all filamentous ascomycetes (64), and in *Neurospora crassa*, a *pex16* mutant is defective in the PTS1 matrix import pathway (84), suggesting a more central role for this peroxin in the filamentous ascomycetes, which warrants further investigation.

The degree to which de novo biogenesis and the division of preexisting organelles contribute to normal peroxisome proliferation remains an open question. The importance of these two pathways may vary significantly from species to species and depending on cellular physiology. In yeast, the de novo pathway was initially observed only in the absence of preexistent peroxisomes. Using pulse-chase and mating assays, it has been shown that the de novo pathway is slow and engaged only when peroxisomes are absent due to defects in inheritance (105). Interestingly, when a *pex19* mutant background is used to accumulate Pex3p in the ER and this strain is mated with a wild-type strain, Pex3 appears to rapidly traffic directly to preexistent peroxisomes rather than follow the slow de novo pathway. This, together with the rapid kinetics of ER–to–peroxisome trafficking in *Y. lipolytica* (165), conforms to the notion that the pathway for sorting PMPs from the ER to peroxisomes may be partially or fully independent of the de novo peroxisome assembly pathway (**Figure 2**).

In vitro budding assays. In vitro assays have recently been developed to examine the production of pre-peroxisomal vesicles from the ER. In *S. cerevisiae*, a modified form of the tail-anchored peroxin Pex15 (Pex15G) containing a lumenal glycosylation domain appears to traffic from the ER to peroxisomes in wild-type cells and accumulates in the ER of *pex19* mutant cells (77). Using microsomes from this strain, in vitro assays show that Pex15G together with Pex3 is packaged into vesicles in a reaction that requires Pex19, ATP, and additional cytosolic factors (77), but is

independent of the COPII (coat protein complex II) coat. Similar results have been obtained in a complementary *P. pastoris* system (2), in which both Pex11 and Pex3 are integrated into vesicles emerging from the ER, and here too the budding mechanism is cytosol, ATP, and Pex19 dependent. Surprisingly, Pex11 budding was independent of Pex3, but these vesicles are devoid of most of the PMPs present in Pex3 vesicles and might represent peroxisomal remnants (2). Nevertheless, this raises the possibility that Pex19 can promote vesicle budding independently of Pex3. The mode of action of Pex19 in the budding process is unclear. In animal cells, Pex16 lacking its mPTS is trapped in the ER, suggesting that the mPTS is required for Pex16 trafficking to peroxisomes (66). This implies that Pex19 might act in pulling out the budding vesicle via its interaction with the mPTS of PMPs (85). Pex19 alone is not sufficient for budding; thus, the identification of essential cytosolic factors should help define the budding mechanism and its ATP requirement.

COPII: coat protein complex II

FUNCTIONAL DIVERSIFICATION OF PEROXISOMES

The set of proteins involved in peroxisome biogenesis and maintenance is highly conserved, suggesting a single evolutionary origin of this cellular compartment. However, the peroxisomal enzymatic content can vary substantially in different groups of organisms and this appears to be the result of the evolutionary acquisition of PTS targeting signals. The most pronounced example of this is the peroxisomal glycosome of trypanosomes, which has evolved to compartmentalize most of the enzymes for glycolysis. Woronin bodies of filamentous ascomycetes provide another example of functional diversification through the evolution of a complex sorting machinery that enables production of a physically distinct peroxisome subcompartment.

Glycolysis in Trypanosomes

Trypanosomatids are the causal agents of sleeping sickness, leishmaniasis, and Chagas' disease. In addition to glycolysis, glycosomes also contain the canonical peroxisome functions and a variety of other pathways. Glycolysis in other eukaryotes is essentially cytosolic; thus, its compartmentalization in these cells is unique and of great interest.

Glycosomes during the trypanosome life cycle. Trypanosomes are transmitted between their mammalian hosts by blood-feeding insects and experience highly different environments within these hosts. The enzymatic content of glycosomes varies considerably during the life cycle. In the animal host, the parasite (long-slender form) is in a glucose-rich environment, where mitochondrial oxidative phosphorylation is repressed and all the ATP is generated through glycosomal glycolysis (10). At this point, glycolytic enzymes represent 90% of the protein content of the organelle (102). By contrast, in the insect host, the concentration of sugars is low. While still in the bloodstream, the parasite can differentiate into a short-stumpy form preadapted for life in the insect. This form of the parasite has a partially derepressed mitochondrial system, which allows the parasite to survive in the low-sugar environment of the insect midgut. Here, exposure to environmental signals (16) triggers differentiation into a procyclic form that is fully adapted to the insect host.

Differentiation of the short-stumpy form into the procyclic form is repressed by the activity of a tyrosine phosphatase, TbPTP1 (159). Upon ingestion by the insect host, TbPTP1 is inactivated and differentiation into the procyclic form occurs. The downstream component in this developmental signaling pathway is a Ser/Thr phosphatase, TbPIP39, which is inhibited by tyrosine phosphorylation and is thus negatively regulated by TbPTP1 (158). During differentiation, TbPIP39 is rapidly phosphorylated by a yet unidentified kinase and its RNAi-mediated depletion inhibits

development of the procyclic form, indicating a key role in promoting this developmental transition. Remarkably, TbPIP39 possesses a consensus PTS1 signal (–SRL, serine-arginine-lysine or Ser-Arg-Lys), which is required for function, and shows perfect colocalization with a glycosomal marker (158). TbPTP1 is presumably cytosolic, and this raises the question of how it can regulate TbPIP39. TbPIP39 protein is induced with differentiation, and this nascent TbPIP39 can be subject to phosphorylation/dephosphorylation in the cytosol before import into the peroxisome.

How phospho-TbPIP39 promotes differentiation from within the glycosome, and whether this relates to glycosomal metabolism or further signal transduction, remains an open question. Glycosomes are predicted to contain over 200 proteins (13), and in principle any of these could be subject to regulation by TbPIP39. Other members of this family possess lipid phosphatase activity (158), suggesting possible regulation through second messengers.

phospho-: phosphorylated form

HXK: hexokinase

PFK: phosphofructokinase

The importance of glycolysis compartmentation. Glycolysis is initiated by hexokinase (HXK) and phosphofructokinase (PFK), which phosphorylate glucose to produce fructose 1,6-bisphosphate (Fru1,6BP). These initial reactions consume two ATP molecules, while net ATP production only comes from downstream reactions. Because this ATP can fuel the activity of HXK and PFK beyond the capacity of downstream reactions, the overall reaction is autocatalytic and can result in the depletion of cellular ATP and the accumulation of hexose phosphate intermediates [glucose 6-phosphate (Glc6P), fructose 6-phosphate (Fru6P), and Fru1,6BP] to toxic levels. In most organisms, glycolysis is cytosolic and this consequence is avoided by tight negative-feedback regulation of HXK and PFK by hexose phosphates. This regulation ensures that upstream reactions do not exceed the capacity of downstream reactions. This type of regulation is absent in trypanosomes (15, 112). However, glycosomal reactions result in no net ATP synthesis, which only occurs in terminal cytosolic reactions, where this ATP is unavailable to glycosomal HXK and PFK. Thus, in trypanosomes, feedback regulation appears to be replaced by partial glycosomal compartmentation.

Several studies using RNAi to disrupt glycosomal function support this model. RNAi against Pex2 (42), Pex14 (30, 46, 60), Pex6, Pex10, or Pex12 (73) leads to the cytosolic mislocalization of glycosomal enzymes and cell death. Cell death can also be triggered by the addition of glucose (30), which results in the accumulation of Glc6P (46), and depletion of HXK has a protective effect on glycosome-deficient trypanosomes (60). The role of compartmentalization is further demonstrated in *Leishmania donovani*, another kinetoplastid parasite, in which the expression of a catalytically active PTS2-truncated HXK located in the cytoplasm also engenders glucose toxicity (74). Together, these data suggest that glycosomal compartmentation provides an alternative to allosteric regulation of HXK and PFK and protects trypanosomes from the autocatalytic nature of glycolysis (46).

Origins of glycosomal compartmentation. Analysis shows that the majority of glycolytic enzymes use PTS1 signals, whereas others utilize PTS2 and internal PTS (iPTS) signals (reviewed in Reference 14). Interestingly, functional analyses of these various PTS1 targeting signals show that glycosomes tolerate a greater degree of degeneration in the PTS1 sequence than do animal peroxisomes. For example, the signal -SSL is functional in trypanosomes but nonfunctional in animal cells (7). From an evolutionary perspective, the shift of glycolysis from a cytosolic to a glycosomal localization presents an interesting problem given that the PTS1 signal is acquired by random mutation and must have been acquired sequentially by these various enzymes. In this case, it is likely that early intermediates in these signals were partially functional and led to dual localization in peroxisomes and the cytosol as previously suggested (31, 41). This situation could sustain functional glycolysis until all the required enzymes had acquired rudimentary PTSs, at

which point these could be refined by further mutation for full functionality. Further sampling of the peroxisomal environment may have been facilitated by the loose glycosomal PTS1 consensus (7), which is due presumably to variation in the PTS1 receptor PEX5.

Glycosomes as a target for therapeutic drugs. The emergence of drug-resistant trypanosomes is becoming a major problem, making the development of new medicines critical. The identification of several known peroxins and their associated functions in *Trypanosoma brucei* clearly supports the view that membrane biogenesis (3) and protein import (32) mechanisms are conserved between peroxisomes and glycosomes. However, the sequence conservation between human and trypanosomal peroxins and glycosomal enzymes is low (14). For example, TbPex19 possesses only 20% sequence identity to human PEX19 (3), 32% between TbPex7 and its human homolog (32), and 36% between HsPex6 and TbPex6 (73). This suggests that specific inhibitors can be designed to interfere with essential glycosomal functions without affecting human peroxisomal function.

Other Taxa-Specific Functions

Biotin biosynthesis. Among the eukaryotes, only plants and some fungi are able to synthesize biotin. Although the final steps are well characterized and occur in mitochondria, the initial events leading to the biosynthesis of biotin were unknown until recently. In the filamentous fungi *Aspergillus oryzae* and *Aspergillus nidulans*, peroxisome mutants are auxotrophic for biotin (87, 161). Moreover, the biotin biosynthetic enzyme encoded by the *bioF* gene (8-amino-7-oxononanoate synthase) has a PTS1 and its peroxisomal localization is required for biotin biosynthesis (87, 161), as is an intact β-oxidation cycle (87). Biotin auxotrophies induced by defects in β-oxidation can be complemented by pimelic acid supplementation, suggesting that the substrate for BioF is pimeloyl-CoA generated via peroxisomal β-oxidation (87). Peroxisomal targeting of the BioF ortholog also occurs in *Arabidopsis*, suggesting that peroxisomes are important for biotin biosynthesis in plants.

Secondary metabolism: penicillin biosynthesis. β-lactam antibiotics such as penicillins and related cephalosporins are produced as secondary metabolites by certain actinomycetes and filamentous fungi (e.g., *Penicillium*, *Aspergillus*, and *Acremonium* species). These compounds are of particular interest in the treatment of bacterial infections and contribute to over 40% of the total antibiotic market. The principal organism used for their production is the fungus *Penicillium chrysogenum*. Penicillin biosynthesis is initiated in the cytoplasm, where the three amino acids α-aminoadipic acid, cysteine, and valine are converted to a peptide by the nonribosomal peptide synthetase, δ-(L-aminoadipyl)-L-cysteinyl-D-valine. This peptide is then cyclized by isopenicillin N synthase to form a β-lactam to produce isopenicillin N (IPN). These first two steps occur in the cytosol and the intermediate IPN is then imported into the peroxisome through action of the PMP CefP (170). The final two reactions occur in the peroxisome: Isopenicillin-*N*:acyl-CoA acyltransferase (IAT) replaces the α-aminoadipyl side chain of IPN for a hydrophobic acyl group, which is provided by activity of the phenylacetyl CoA ligase (PCL) (100), and both of these enzymes possess consensus PTS1 signals (−ARL, alanine-arginine-leucine or Ala-Arg-Leu in IAT, and −AKL, alanine-lysine-leucine or Ala-Lys-Leu in PCL). The related cephalosporins (produced by *Acremonium chrysogenum*) are also produced from the IPN precursor, and two of these enzymes appear to be peroxisomal and utilize PTS1 signals (62, 93).

Several lines of evidence suggest that peroxisomal localization plays an important role in penicillin biosynthesis. A mutant in IAT lacking its PTS1 signal fails to produce penicillin (108), and a variety of mutants in *Penicillium chrysogenum* (100, 114) and *Aspergillus nidulans* (153) defective in matrix import are also defective in penicillin production. Peroxisome abundance also appears

to correlate positively with penicillin production (100). Interestingly, overproduction of either PEX14/17 (114) or PEX11 (63) leads to a twofold increase in penicillin production. The latter case is probably working through increased peroxisome abundance. However, not all conditions that increase peroxisome abundance lead to increased penicillin production (100). For example, growth on fatty acids leads to increased peroxisome abundance and decreased penicillin production. Thus, physiological factors also affect the peroxisomal contribution to penicillin production. Recently, the penicillin biosynthetic pathway has been reconstituted in the yeast *H. polymorpha*, and here too strains lacking peroxisomes produce less penicillin (34). From all these data it is clear that peroxisomal localization is important for the biosynthesis of β-lactam antibiotics. However, precisely how peroxisomes support these biosynthetic pathways remains to be determined.

GGPP: geranylgeranyl-pyrophosphate

Secondary metabolism: AK-toxin and paxilline biosynthesis. The filamentous fungus *Alternaria alternata* includes seven pathogenic variants that produce host-specific AK (*Alternaria kikuchiana*) toxins, and causes black spot disease of Japanese pear. Three enzymes responsible for AK-toxin biosynthesis (Akt1p, Akt2p, and Akt3p) are targeted to peroxisomes through their PTS1 signals (SKI, serine-lysine-isoleucine or Ser-Lys-Ile; SKL, serine-lysine-leucine or Ser-Lys-Leu; and PKL, proline-lysine-leucine or Pro-Lys-Leu) (55). In addition, a *pex6* mutant is deficient in AK-toxin production and pathogenicity, suggesting that peroxisome localization of these enzymes is essential for AK-toxin biosynthesis. Paxilline is another secondary metabolite produced by *Penicillium paxilli*. This indole-diterpene has the ability to block calcium-activated potassium channels (141). Paxilline biosynthesis requires the activity of the geranylgeranyl-pyrophosphate (GGPP) synthase, PaxG, and this enzyme is localized in peroxisomes through a PTS1 signal, which is also required for PaxG function (137). From these two examples, it seems likely that additional links between peroxisomes and other secondary metabolic pathways will emerge in the future.

Sexual development in a filamentous ascomycete. A series of studies on the filamentous ascomycete *Podospora anserina* reveals a role for peroxisomes in sexual development (5, 8, 122). In the filamentous ascomycetes, sexual development takes place in multicellular fruiting bodies in which meiocytes (asci) differentiate to support karyogamy, meiosis, and sporulation (191). In *P. anserina*, deficiency for the RING complex peroxin PEX2 was originally shown to block meiotic commitment in meiocytes just before karyogamy (5). The two other components of the RING complex, PEX10 and PEX12, are also required for this process (122), but both matrix import receptors, PEX5 and PEX7, are dispensable (8) as are the docking peroxins PEX14 and PEX14/17 (121). However, the docking peroxin PEX13 and the PTS2 coreceptor PEX20 are required for meiocyte differentiation as are both PEX3 and PEX19. The requirement of a known cycling import receptor (PEX20), a docking peroxin (PEX13), and the RING peroxins suggests that the meiocyte-specific function of the peroxisome is associated with a modified matrix import cycle. Identifying the meiotic substrates of this import pathway remains a key challenge for the future and this should help resolve whether the meiocyte-specific function of peroxisomes is metabolic or cellular in nature. In addition, contrary to the case for *Podospora*, meiosis in *Aspergillus nidulans* does not require Pex13 (54) or Pex2 (53), and understanding this difference as well as the phylogenetic distribution of the link between peroxisomes and meiocyte development is another important question for future work.

Woronin Body Subcompartment

Filamentous fungi grow through the extension of cellular filaments (hyphae), in which individual cellular compartments are interconnected through conducting channels called septal pores.

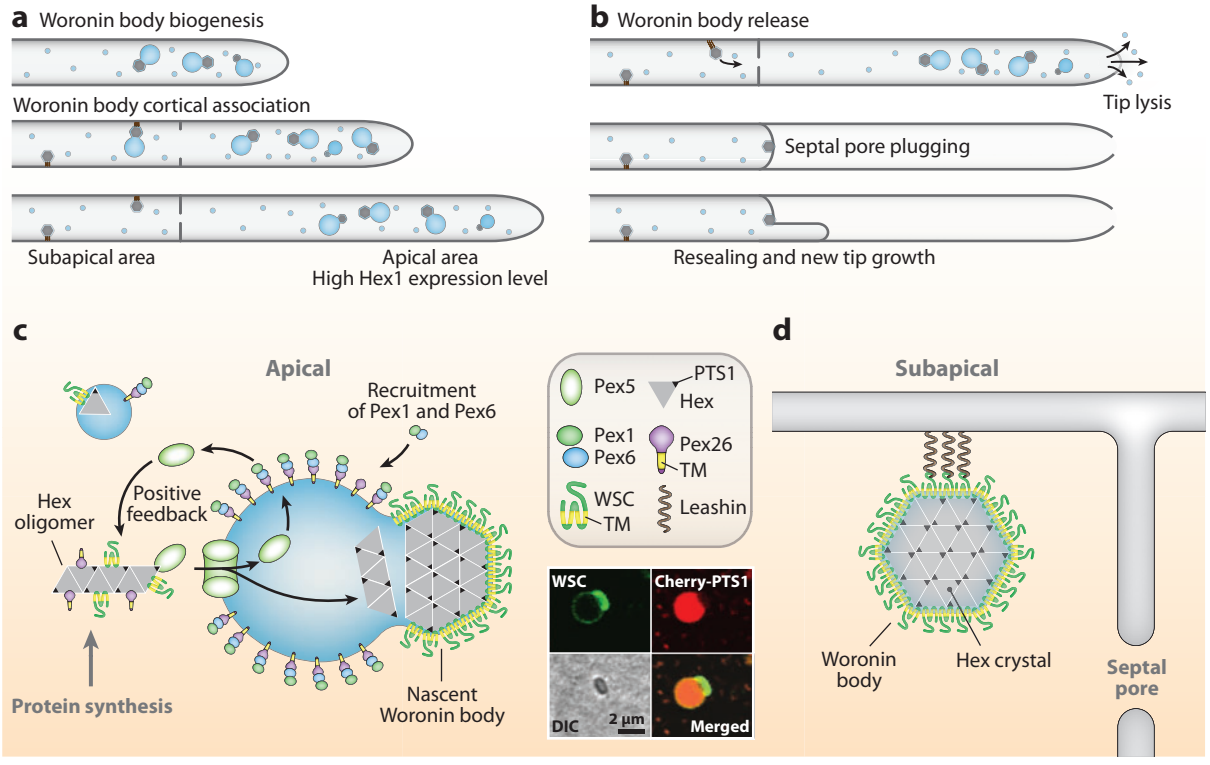

Figure 3

Woronin body biogenesis and function in *Neurospora crassa*. (*a*) Woronin body biogenesis in apical compartments. Woronin bodies are manufactured continuously in differentiated apical peroxisomes through a process determined in part by polarized *hex* gene expression. Coincident with septation, Woronin bodies attach to the cell cortex in a step that promotes their segregation into subapical compartments. (*b*) Woronin bodies in septal pore plugging, membrane resealing, and tip growth. Tip lysis triggers Woronin body release and septal pore plugging, followed by membrane resealing and the generation of new hyphal tips. Because tip lysis is likely the most common form of hyphal damage in nature, Woronin body production in apical compartments ensures that overall cell damage is minimal. (*c*) Model for differentiation of Woronin body–producing peroxisomes in *N. crassa*. Woronin bodies are produced in enlarged peroxisomes, which differentiate through the action of HEX oligomers. HEX acts by recruiting WSC (Woronin sorting complex) and PEX26 to these peroxisomes. (*Left*) HEX and PEX26 compose elements of a positive-feedback loop that promotes matrix import and the emergence of these differentiated peroxisomes. See text for additional information. (*Right*) A nascent Woronin body budding from the peroxisome. (*d*) The mature Woronin body is immobilized at the cell cortex by the tethering protein Leashin until signals from wounding trigger its release. Abbreviations: TM, transmembrane.

This syncytial multicellular organization allows the transport of cytoplasm and organelles between cells, permits cellular cooperativity, and is especially suited to support the invasive growth of saprotrophs and pathogens alike. However, this organization also carries the risk of uncontrolled cytoplasmic bleeding when hyphae are damaged. Woronin bodies evolved over 400 mya in the common ancestor of filamentous ascomycetes; they perform an adaptive function to seal septal pores in response to cellular wounding (56, 169) (**Figure 3*a*,*b***). Their biogenesis requires a dedicated machinery that promotes budding from the peroxisome and tethering to the cell cortex for segregation (**Figure 3*c***). Interestingly, the import of oligomeric PTS1-bearing cargo is central to this mechanism of subcompartment differentiation.

The self-assembled Woronin body dense-core. The Woronin body dense-core is composed of the PTS1-containing HEX protein, which is imported into peroxisomes (57, 88), where it self-assembles into solid micrometer-scale assemblies. The size of these structures appears to always exceed the diameter of the septal pore and this relationship relates to the pore-plugging function of the Woronin body (169). The deletion of *hex* abrogates Woronin body formation and leads to protoplasmic bleeding from septal pores in response to cell lysis (57, 94, 152). Moreover, expression of *HEX* in yeast leads to the formation of Woronin body–like dense-cores inside peroxisomes (57, 186), and recombinant HEX spontaneously crystallizes in vitro (57). Together, these data show that HEX is necessary and sufficient for Woronin body dense-core formation. The HEX crystal structure reveals three intermolecular contacts that promote formation of the HEX protein matrix. Mutants disrupting this assembly produce a soluble core, and despite attaining normal dimensions, these Woronin bodies are nonfunctional because they deform and pass through the septal pore during wound-induced plugging (189).

The overall fold of HEX is highly similar to that of eIF5a proteins, which are ancient cytoplasmic proteins that play a role in polypeptide chain elongation during translation (138). In addition, HEX and eIF5a share primary sequence homology, suggesting that they are related through ancestral gene duplication (57). Interestingly, the sequence at the C terminus of contemporary eIF5a proteins appears to be a single amino acid substitution away from attaining a PTS1 signal (56), and eIF5a has been shown to reversibly form tetramers and hexamers (12). This leads to the hypothesis that following *eIF5a* gene duplication, one copy acquired a PTS1 signal and was capable of undergoing a degree of self-assembly in the condensing environment of the peroxisome. This could have provided rudimentary Woronin body function that was improved over time through extensive mutation.

Apical biogenesis and budding. Woronin body biogenesis occurs in the growing apical compartment, where peroxisomes producing nascent Woronin bodies move in the cytoplasm in a generally tip-directed manner (**Figure 3*a***). At this point HEX assemblies appear to be in the process of budding from the peroxisome; with a timing that roughly coincides with septation, these can be observed to associate with the cell cortex, where they are immobilized (104, 162). Following cortex association, these hybrid organelles undergo fission, which separates the Woronin body from its mother peroxisome, and this event depends on the action of Pex11 (26). The elastic hyphal tip is easily ruptured by hypotonic shock and this is likely to occur frequently in nature. Thus, apical biogenesis ensures that the first subapical compartment has functional Woronin bodies, and as a result the outcome of tip-lysis is usually pore-plugging at the first subapical septum (94) (**Figure 3*b***). The expression of *HEX* mRNA is maximal in apical hyphal compartments and decreases rapidly in subapical regions of the colony (162). When the *HEX* expression pattern is respecified by swapping its promoter for one that is active subapically, Woronin body formation is redetermined to subapical compartments (162). Thus, the spatial pattern of *HEX* gene expression is a key determinant of the apical localization of Woronin body biogenesis. Many other transcripts are spatially regulated within the colony (59), and understanding the molecular basis for this control is an interesting area for future research.

Woronin body cores can be observed to bud from the peroxisome matrix at the level of both light and electron microscopy, and this process requires the Woronin body–specific membrane protein, WSC (Woronin sorting complex) (84). In *wsc* mutants, instead of budding, HEX assemblies vibrate randomly in the matrix of apical peroxisomes. Furthermore, these structures fail to associate with the cell cortex as they do in wild-type cells, resulting in their accumulation in the apical compartment. Moreover, this failure in segregation explains why *wsc* mutants display Woronin body loss-of-function phenotypes despite producing HEX assemblies. WSC is a

four-pass transmembrane protein and in wild-type cells, it accumulates over budding HEX assemblies in nascent Woronin bodies and coats mature Woronin bodies. However, it is found at very low levels in the majority of peroxisomes, indicating that it is a Woronin body–specific membrane protein. WSC physically associates with HEX and together these data suggest that WSC is a membrane receptor for HEX assemblies. Interestingly, WSC behaves like a generic PMP in a *hex* deletion, and this was the first evidence that HEX might play a regulatory role in subcompartment specification (see below).

Overproduction of *WSC* in a *hex* deletion mutant results in the cortical association of peroxisomes that contain elevated levels of WSC. This suggests that cortex association depends on appropriate levels of WSC in the membrane. As WSC levels in nascent Woronin bodies depend on HEX, this provides a mechanism to ensure that segregation of Woronin bodies occurs only when HEX assemblies have reached appropriate dimensions. In yeast, peroxisome segregation is controlled by a balance between retention in mother cells through cortex binding and actomyosin-dependent transport into daughter cells. In this case, cortex association depends on the peroxisomal Inp1 protein, and the proportion of peroxisomes retained in mother cells can be increased by increasing levels of Inp1 (reviewed in Reference 27). Thus, protein-level-dependent cortex association may be widely used to control peroxisome segregation in fungal systems.

WSC is related to the PMP22 family of PMPs, which are also four-pass transmembrane PMPs. *PMP22* is lost in some fungal species, suggesting that it does not execute an essential function, and this conforms to the notion that *WSC* could have evolved through co-option of the PMP22 function. The nearest *PMP22* homolog of *WSC* is unable to complement a *wsc* deletion despite being targeted to peroxisomes, and this further supports the idea that WSC has new and divergent activities.

A tether for Woronin body segregation. In most of the Pezizomycotina, Woronin bodies are found in the vicinity of the septal pore. Upon being pulled away from the septum with laser tweezers (4), these recoil to their original position, suggesting attachment to the septum through an elastic tether. A mutant screen in *Neurospora crassa* identified the *leashin* locus and these large (~500-kDa) cytosolic proteins appear to encode the Woronin body tether (111). *leashin* mutants accumulate WSC-enveloped nascent Woronin bodies in the apical compartment, indicating that Leashin is required for cortex association. An N-terminal domain of Leashin physically associates with WSC for localization to the Woronin body and the C terminus appears to be required for cortex binding. Central regions of Leashin proteins are not conserved but retain a similar character: they are acidic and enriched for the amino acids, PELS (proline–glutamic acid–leucine–serine or Pro-Glu-Leu-Ser). Similar PEVK repeats in the muscle protein titin adopt a random-coil structure that forms an elastic filament and this may also be true of Leashin. In addition, Woronin bodies are tethered to the pore at a distance of about 200 nm, and this spacing is in reasonable agreement with predicted dimensions of Leashin monomers.

Neurospora and its close relative *Sordaria* are unique within the clade Pezizomycotina in that they do not tether Woronin bodies to the septal pores, but rather localize them to the cell cortex in a dispersed pattern. Phylogenetic analysis suggests that this pattern is derived from the pore-associated pattern, and variation in Leashin appears to account for this difference. In *Neurosopora*, the *leashin* locus encodes two adjacent genes. The 5′ gene (*leashin-1*) encodes the N-terminal half of ancestral Leashin and maintains a function in Woronin body segregation, and the 3′ gene (*leashin-2*) encodes the C-terminal region and is localized to the septal pore. Remarkably, in *Neurospora*, a chromosomally encoded fusion of *leashin-1* and *leashin-2* can reproduce the ancestral pattern, suggesting that splitting of ancestral *leashin* indeed underlies the evolutionary transition in organelle localization. Interestingly, deletion of *leashin-2* does not interfere with Woronin body

segregation but produces a defect in hyphal growth, suggesting that Leashin plays additional roles in controlling cellular development. Extensive protoplasmic streaming occurs through septal pores in both *Neurospora* (131) and *Sordaria*, and the emergence of this cellular physiology may have provided selective pressures for evolution of the delocalized pattern of cell cortex association (125).

NAD: nicotinamide adenine dinucleotide

FAD: flavin adenine dinucleotide

HEX oligomers promote functional peroxisome differentiation. HEX and WSC use consensus peroxisome targeting signals (PTS1 and mPTS, respectively), indicating that Woronin bodies are not differentiated from peroxisomes by special targeting signals. Moreover, Woronin bodies comprise a minor fraction of the total peroxisome population (84), and this leads to the question of how the abundance and composition of the subcompartment are controlled. This problem was resolved in part by experiments showing that enlarged Woronin body–producing peroxisomes are hypercompetent for matrix import and receive the majority of nascent PTS1 proteins. Thus, a disparity in import competence can account for the difference between Woronin body and peroxisome abundance (83).

Remarkably, differentiation of this subpopulation is self-organized by HEX. When HEX is absent, PTS1 trafficking becomes uniformly distributed to abundant small peroxisomes. Moreover, HEX is imported as an oligomer and mutations that abolish oligomer formation also abolish functional peroxisome differentiation. The activation of matrix import in a subset of peroxisomes suggested that HEX oligomers act by influencing the activity or localization of a key component of the matrix import pathway. Indeed, the tail-anchored peroxin PEX26, which functions to promote AAA ATPase membrane recruitment for receptor recycling (**Figure 1**), is associated with differentiation. PEX26 is found at elevated levels in the membrane of differentiated peroxisomes, and as with WSC (84), deletion of *HEX* results in uniform targeting of PEX26 to all peroxisomes. PEX26 physically interacts with HEX through its major cytoplasmic domain, suggesting that HEX might directly influence PEX26 localization. A *pex26* hypomorph that can still support matrix import but shows defects in differentiation results in increased Woronin body abundance, reduced size, and diminished organelle function. Together, these data suggest that HEX and PEX26 compose a positive-feedback loop to promote functional peroxisome differentiation and control subcompartment abundance (83) (**Figure 3*c***). In this model, stochastic variations in the level of HEX import are amplified by the ability of HEX to recruit a key component of the import machinery, resulting in the import of more HEX proteins.

More work is required to validate this model and determine whether other peroxins are required for differentiation. In most cell types such as animal tissue culture cells, mature peroxisomes are uniform in size and composition (52), and this type of system can be used to reconstitute HEX-dependent peroxisome differentiation and define its minimal machinery. In the preimplex hypothesis, mutually multivalent interactions between nascent oligomeric matrix proteins and components of the import machinery were proposed to play an important role in the import process (37). Although the data concerning HEX do not suggest that its oligomerization is essential for import, they are consistent with a role in increasing import efficiency and promoting functional organelle differentiation. Examining how multivalent interactions between components of the import machinery and oligomeric cargo influence these processes will be an interesting area for future investigation.

Additional functions for Woronin bodies? A number of diverse proteins involved in signaling and development have been associated with the Woronin body. TmpL is a multipass transmembrane protein containing N-terminal AMP-binding domain and C-terminal NAD(P)/flavin adenine nucleotide (FAD)-binding, which appears to segregate specifically to the membrane of Woronin bodies (65). TmpL is required for virulence of plant and animal pathogens; in *Alternaria brassicicola*, *tmpL* mutants are hypersensitive to oxidative stress and produce an excess of reactive

oxygen species during plant infection, suggesting a role in redox homeostasis or signaling. In *Sordaria macrospora*, PRO40 is a WW domain protein required for fruiting body formation. PRO40 does not have putative transmembrane domains, but it localizes to Woronin bodies (22). Finally, in *Aspergillus nidulans*, the ApsB (anucleate primary sterigmata) protein is a component of the spindle pole body and a unique fungal septum-associated microtubule-organizing center. ApsB interacts with HEX in the yeast two-hybrid system and localizes to a subset of peroxisomes using a PTS2 signal (190). Although it remains unclear how HEX influences ApsB function, these data provide further evidence for heterogeneity of fungal peroxisomes and imply a possible role in cytoskeletal regulation.

In the case of TmpL and PRO40, the functional significance of Woronin body localization remains to be determined. A network of protein-protein interactions is required for Woronin body specification. HEX physically associates with WSC and PEX26 and Leashin interacts with WSC. These types of interactions could also account for the localization of other proteins to the Woronin body membrane or surface. Woronin bodies are physically distinct from the overall peroxisome population and localize to cell-to-cell channels near the plasma membrane. It is tempting to speculate that this unique organelle environment is suited especially to support functions in redox regulation and the control of multicellular development.

The clade Pezizomycotina is estimated to comprise 90% of ascomycetes and half of all fungi, and harbors the majority of plant and animal pathogens. On the basis of the phylogenetic distribution of *HEX*, *WSC*, and *leashin*, Woronin bodies were fully evolved at the origin of this group (56). In the future, it will be interesting to learn to what extent Woronin body function was further diversified to support additional functions and promote evolutionary radiation within this diverse group.

SUMMARY POINTS

1. The peroxisome can import oligomeric cargo. The mechanism of import may involve a transient aqueous pore composed of components of the docking complex and the cycling PTS receptors themselves.
2. PEX19 and its receptor PEX3 are believed to direct nascent PMPs directly to the peroxisome membrane, but they have also been associated with budding of pre-peroxisomes from the ER.
3. Peroxisomes proliferate by growth and division but can also form de novo from ER-derived precursors and are thus bona fide components of the endomembrane system.
4. Peroxisome function has diversified through the acquisition of new metabolic pathways or through subcompartment development, as exemplified by glycosomes and Woronin bodies, respectively.
5. A key step in the evolution of peroxisome diversity occurs when nonperoxisomal proteins attain PTS1 signals. The simplicity and degeneracy of this signal are likely to promote peroxisome evolvability.
6. Both glycosomes and Woronin bodies have been associated with signal transduction and cellular development.
7. The import of oligomeric HEX promotes both import efficiency and subcompartment differentiation. The latter depends on the ability of HEX import oligomers to influence the localization of specific PMPs.

FUTURE ISSUES

1. Pex19 and Pex3 have been associated with direct targeting of nascent PMPs to the peroxisome membrane and with budding of pre-peroxisomal vesicles from the ER. Further studies are required to understand whether these activities are distinct or mechanistically related.
2. In the yeast *Saccharomyces cerevisiae*, the major pathway for peroxisome proliferation is growth and division. However, the extent to which de novo formation contributes to peroxisome renewal in other microorganisms and during developmental transitions remains to be determined.
3. PMPs appear to traffic both directly to the peroxisome and via the ER. Understanding the intrinsic targeting signals, chaperones, and membrane integrases that differentiate these pathways will help clarify the mechanisms involved in peroxisome membrane biogenesis.
4. Peroxisomes have been associated with signal transduction and developmental decisions, but more work is required to determine their precise roles in these processes.
5. As genomes are sequenced in diverse eukaryotes, bioinformatic searches for PTS1 signals in predicted proteomes can be used to further explore peroxisome diversity.

DISCLOSURE STATEMENT

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Contents

Index

Errata

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