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*Review*

# Peroxisome diversity and evolution

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Peroxisomes are organelles bounded by a single membrane that can be found in all major groups of eukaryotes. A single evolutionary origin of this cellular compartment is supported by the presence, in diverse organisms, of a common set of proteins implicated in peroxisome biogenesis and maintenance. Their enzymatic content, however, can vary substantially across species, indicating a high level of evolutionary plasticity. Proteomic analyses have greatly expanded our knowledge on peroxisomes in some model organisms, including plants, mammals and yeasts. However, we still have a limited knowledge about the distribution and functionalities of peroxisomes in the vast majority of groups of microbial eukaryotes. Here, I review recent advances in our understanding of peroxisome diversity and evolution, with a special emphasis on peroxisomes in microbial eukaryotes.

**Keywords:** peroxisomes; glyoxysomes; glycosomes; evolution; proteome

## 1. INTRODUCTION

Peroxisomes, initially named *microbodies*, were first noted by Rhodin (1954) as part of his PhD thesis on the morphology of proximal tubule cells from mouse kidney. However, their initial characterization as a novel type of cellular organelle came years later, when Christian de Duve and his team were able to isolate peroxisomes from rat liver and study their biochemical properties (de Duve & Baudhuin 1966). de Duve's group identified the presence of several enzymes involved in the production and degradation of hydrogen peroxide and hence gave the name peroxisomes to these organelles. Since then, peroxisomes have been isolated from a variety of other organisms and it soon became evident that the specific metabolic properties of peroxisomes can differ substantially from species to species. Even at the level of a single organism, peroxisomes can display alternative enzymatic contents depending on the specific tissue or the environmental conditions considered. Indeed, some peroxisomes found in specific groups of organisms or tissues are so divergent that they were initially classified as distinct organelles and are still now commonly referred to with alternative names. For instance, peroxisomes in trypanosomatid species harbour certain glycolytic reactions and are therefore known as glycosomes (Michels *et al.* 2006), whereas some plant peroxisomes are named glyoxysomes because they mainly harbour enzymes of the glyoxylate cycle (Hayashi *et al.* 2000). In filamentous fungi, a particular type of peroxisome, referred to as the Woronin body, functions in the maintenance of cellular integrity by sealing the septal pore in response to wounding (Würtz *et al.* 2009). Some peroxisomal enzymes can only be found in a very narrow range of species. This is the case for the fluorescent luciferase in fireflies (Gould *et al.* 1987) or for several key enzymes for the production of penicillin, which are restricted to a few fungal genera such as *Penicillium* (Kiel *et al.* 2000). Other peroxisomal pathways, in contrast, show a more widespread distribution such as the $\beta$ oxidation of fatty acids or the set of enzymes responsible for oxidative stress response. Despite displaying such high levels of metabolic diversity, all peroxisomes have in common a similar set of proteins involved in their biogenesis and maintenance, as well as the use of similar targeting signals for directing the localization of proteins to the organelle. The presence of these common traits supports the idea of a single evolutionary origin for all peroxisomes, although the exact scenario still remains somewhat controversial (de Duve 2007).

In recent years, we have witnessed several breakthroughs in peroxisome research, including the elucidation of the molecular mechanisms involved in the formation and division of peroxisomes as well as the finding of novel clues about their possible evolutionary origin (van der Zand *et al.* 2006). While such findings have clearly advanced our understanding of the function and evolution of these widespread organelles, there is still little information regarding the distribution and diversity of peroxisomes across the major groups of eukaryotic organisms. Indeed, most of our knowledge about peroxisomes comes from the characterization of peroxisomal proteins in a handful of model species, including the human, rat, mouse, the yeasts *Saccharomyces cerevisiae*, *Pichia pastoris*, *Hansenula polymorpha* and *Yarrowia lipolytica*, and the model plant *Arabidopsis thaliana*. These model organisms represent only partially two of the five major groups in which eukaryotic diversity is currently classified (Keeling *et al.* 2005) (figure 1) and, with the notable exception of yeast, they represent

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One contribution of 12 to a Theme Issue 'Evolution of organellar metabolism in unicellular eukaryotes'.

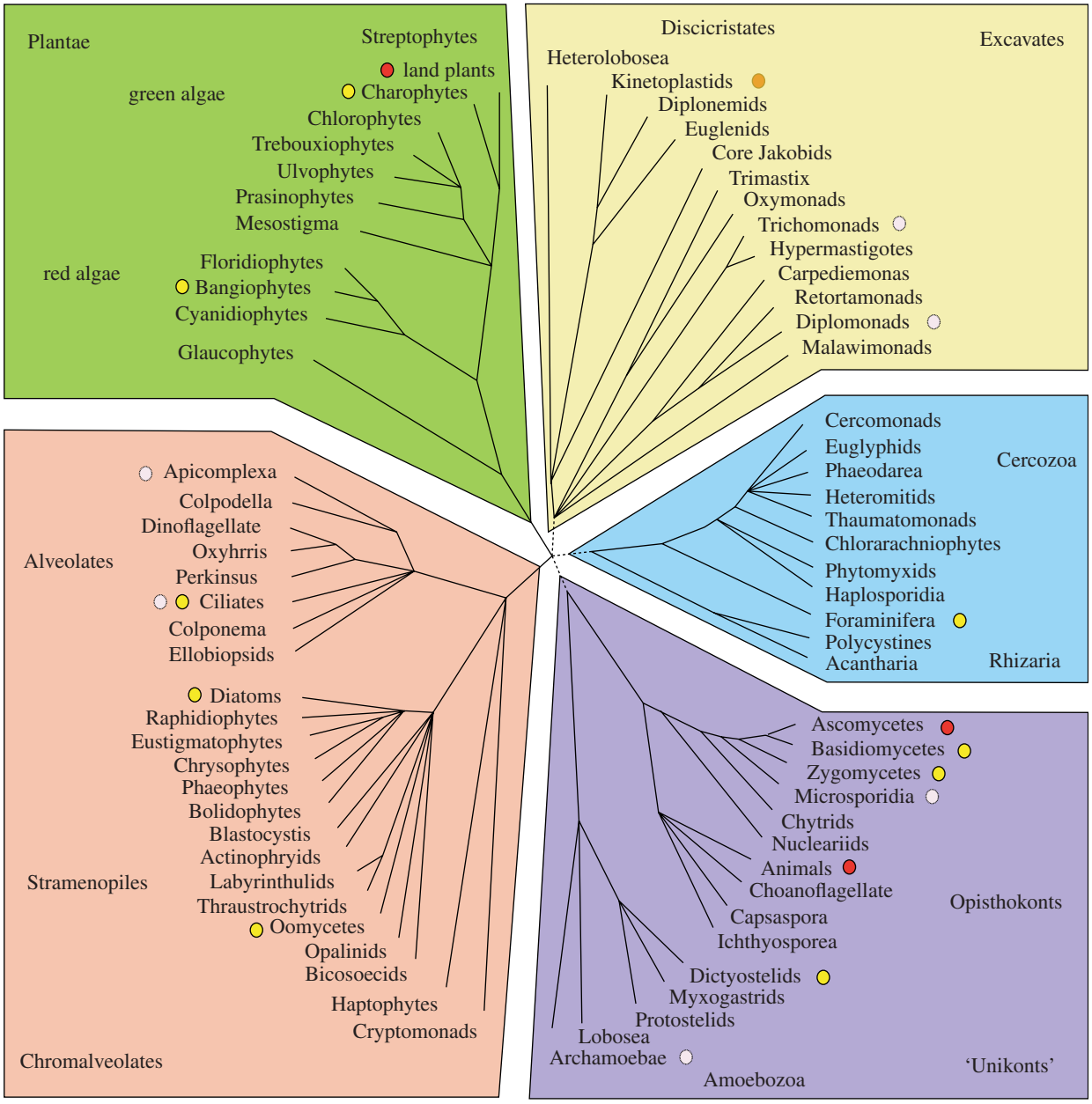
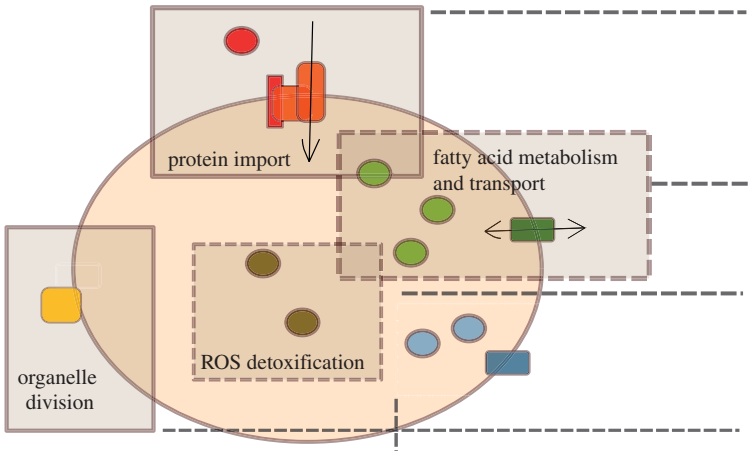



Figure 1. Our state of knowledge on peroxisome diversity across the eukaryotic tree of life is represented. The level of information of peroxisomes is based on literature searches for each taxa and their major representatives. Circles next to the different taxa indicate the type of information that is available on peroxisomes. Red circles indicates that for this group, extensive biochemical data as well as comprehensive proteomics and bioinformatics surveys are available. Orange circles indicate an intermediate level of information on peroxisomal composition, mostly based on biochemical studies of individual proteins or pathways coupled with comprehensive sequence analyses to predict peroxisomal localization. Yellow circles indicate that the presence of peroxisomes in that group is well established but that the level of the characterization of their function and diversity within this group is very scarce. White circles indicate that the presence of peroxisomes has been studied in this group revealing an apparent absence of these organelles in all the members studied (only a white circle is associated with the group) or in some of them (circles with different colours are associated with the group). Absence of a circle next to the group indicates that the presence or absence of peroxisomes or their enzymatic content in this group remains to be clearly established. (Adapted from a modified version of fig. 1 of Keeling *et al.* (2005).)

complex multicellular organisms. Only recently, the interest has partially shifted to peroxisomes in microbial eukaryotes. Considering their adaptation to a variety of niches and life styles, it is among these organisms, where the highest diversity in terms of metabolic properties of peroxisomes is expected. Thanks to the availability of completely sequenced genomes for a growing number of microbial eukaryotes, we are now just starting to unveil the existing diversity of peroxisomes in these organisms.

In this review, I will provide an overview of the current state of our knowledge on peroxisome diversity and evolution. For this, I will first focus on the common mechanisms shared by all peroxisomes to then survey what is known to be specific in the major groupings of eukaryotic taxa. When discussing this metabolic diversity, and to provide a logical framework, I will follow the classification of eukaryotic taxa into five major groups, namely Unikonts, Plantae, Excavates, Chromalveolates and Rhizaria, as described




*Protein import machinery.* A core set of components of the machinery used to import peroxisomal matrix proteins is shared among all peroxisomes, indicating a single origin. The core components of this machinery show homology with components of the ERAD machinery in the endoplasmic reticulum

*Fatty acid metabolism.* Almost all types of peroxisomes contain enzymes involved in fatty acid metabolism although the specific routes or enzymes are not necessarily the same.

*Reactive oxygen species detoxification.* Many oxidative reactions of the peroxisomal metabolism produce reactive oxygen species that are degraded by enzymes such as catalase or superoxide dismutase. Again, not all peroxisomes contain the same detoxifying enzymes.

*Organelle division.* The set of proteins involved in peroxisomal fusion and fission are shared accross diverse species. They are proteins of eukaryotic origin that participate in the division of other organelles such as mitochondria.

*Additional pathways.* Additionally, peroxisomes may harbour a large set of additional metabolic pathways ranging from the biosynthesis of several compounds to glycolisis or the catabolism of specific carbon or nitrogen sources. These pathways may have different evolutionary origins, including horizontal gene transfer (e.g. glycolisis of glycosomes) or re-targeting from other cellular compartments.

Figure 2. A schematic view of the peroxisome. The biogenesis and maintenance processes (full-line boxes), which comprise the proteins involved in protein import and organelle division, are present in all types of peroxisomes. The enzymatic content of the peroxisome (dashed-line boxes) is highly variable, with different enzymatic sets being present in different species. Enzymes involved in fatty acid metabolism and reactive oxygen species detoxification are widespread. Other additional pathways (in blue) might be restricted to certain groups of eukaryotes. The text at the right-hand side of the figure provides some important remarks about the diversity and evolution of each depicted process.

by Keeling *et al.* (2005). Finally, I will discuss our understanding of how the peroxisomal proteome has been shaped during evolution and the current debates on the possible evolutionary origin of this intriguing organelle. Throughout the text, an emphasis will be put on microbial eukaryotes.

## 2. DIVERSE BUT ALL THE SAME: COMMON TRAITS OF PEROXISOMES

A general description of peroxisomes that would fit most organisms will be that of a single membrane-bounded organelle with fairly conserved systems for their biogenesis and maintenance but with a highly variable enzymatic content (figure 2). The peroxisomal lumen often harbours enzymes involved in fatty acid metabolism and the detoxification of reactive oxygen species. In addition, a multitude of other anabolic and catabolic processes have been observed in certain taxa. In contrast to mitochondria or chloroplasts, peroxisomes do not possess an organellar genome. All peroxisomal proteins are therefore encoded in the nuclear genome and translated by cytosolic ribosomes. These proteins must then be incorporated into the organelle by specific import routes, which rely on the presence of targeting signals in their sequences (Brown & Baker 2008; Girzalsky *et al.* 2009; Ma & Subramani 2009). The majority of matrix peroxisomal proteins use a short peroxisomal targeting signal (PTS) at their *C*-terminus, which mainly consists of the three amino acids SKL or conservative variants thereof, although residues situated upstream seem to have an influence on the transport (Brocard & Hartig 2006). Other proteins, however, use the alternative bi-partite signal PTS2 with the consensus sequence [RK]-[LVI]-×5-[HQ]-[LA] at their *N*-terminal region. In addition, some peroxisomal proteins do not possess a recognizable targeting signal and are transported into peroxisomes associated with other domains of Pex5 or with other PTS-carrying proteins (van der Klei & Veenhuis 2006*a*). Targeting signals are recognized by a molecular machinery that carries peroxisomal proteins into the organellar matrix. This import complex, referred to as the importomer (Agne *et al.* 2003), consists of two main functional/structural modules: a membrane protein complex including the receptor docking proteins Pex13 and Pex14 and a receptor export module on the cytoplasmic side containing several RING-domain proteins, ubiquitinating enzymes and the AAA-ATPases Pex1 and Pex6 (Grou *et al.* 2009). This system is used by receptor proteins such as Pex5 and Pex7, which shuttle in and out of the peroxisome, thereby importing their cargoes into the peroxisomal matrix. Importantly, the peroxisomal import machinery has no resemblance to those of other organelles such as mitochondria and chloroplasts, and presents the particularity of being able to transport folded proteins (Walton *et al.* 1995) and even oligomers (McNew & Goodman 1994). Despite the presence of a common set of proteins involved in the import of peroxisomal proteins, this system may present particularities in the different taxonomic groups. For instance, the yeast *S. cerevisiae* possesses a set of biogenesis proteins of which homologues for 13 have not yet been found in plants or mammals (Schluter *et al.* 2006), although they are conserved among fungi (Kiel *et al.* 2006). Another mechanism that seems to be shared by all

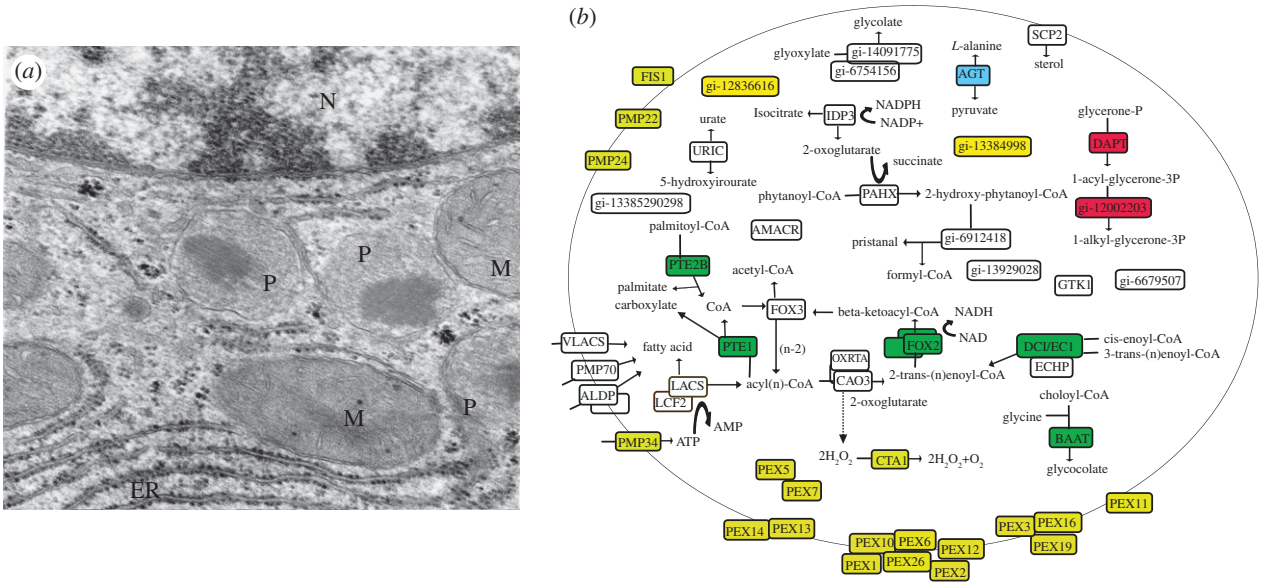



Figure 3. A schematic view of mammalian peroxisomes. (*a*) Micrograph shows part of a rat liver cell, where peroxisomes (P), can be seen surrounded by other cellular compartments such as the nucleus (N), the rough endoplasmic reticulum (ER) and mitochondria (M). Note the crystalline lattice formed inside peroxisomes, which results from tightly bound enzymatic material. Picture kindly provided by Douglas F. Bray (University of Lethbridge). (*b*) A reconstruction of the peroxisomal proteome and metabolism as inferred from proteomics data is shown. Colour codes indicate the likely evolutionary origins of the proteins as follows: green, alphaproteobacterial; yellow, eukaryotic; red, actinomycetales; blue, cyanobacterial; and white, undetermined. (Adapted from a modified version of fig. 5 of Gabaldón *et al.* (2006).)

types of peroxisomes is that responsible for the division of the organelle. In recent years, there has been a significant progress in the elucidation of this mechanism, which has been shown to be largely conserved in yeast, plant and mammalian peroxisomes. This division machinery involves, at least, a dynamin-like protein and a TPR (Tetratrico Peptide Repeat)-motif containing protein that serves as a membrane anchor. Interestingly, these proteins are also involved in mitochondrial fission, establishing a link between these organelles (Delille *et al.* 2009).

In terms of metabolism, the picture is rather different and extremely high levels of diversity can be found across different taxa. Although most peroxisomes share the presence of some fatty acid oxidation routes, etherlipid biosynthesis and enzymes for the detoxification of reactive oxygen species, there seems to be no common set of enzymes that correlates completely with the presence of peroxisomes, that is, enzymes present in all species with peroxisomes but absent from organisms devoid of the organelle (T. Gabaldón & B. Gasse 2009, unpublished data). This view of the peroxisome as an organelle with fairly conserved biogenesis and maintenance mechanisms but with a largely variable enzymatic content shaped to the specific needs of each organism or tissue is likely to become more established as peroxisomes from novel organisms are characterized. In the following sections, I will provide a brief overview of the main metabolic characteristics of peroxisomes from the major eukaryotic groups.

## 3. PEROXISOMES IN UNIKONTS

Unikonts constitute a recently proposed taxonomic group that includes amoebozoans, metazoans and fungi (Cavalier-Smith 2002). Without any doubt, this group is the one for which we know more details about its peroxisomes. Peroxisomes of metazoans such as human, mouse or rat have been extensively characterized (Schluter *et al.* 2007). For instance, proteomic analyses of rat liver peroxisomes have reported more than 50 peroxisomal proteins (Kikuchi *et al.* 2004; Islinger *et al.* 2006). A wide range of enzymatic functions have been identified in mammalian peroxisomes including α oxidation of branched chain fatty acids, amino acid metabolism and different steps for the synthesis of purines, pyrimidines, cholesterol, ether lipids and bile acids (figure 3). Comparisons of peroxisomes from different tissues such as mouse liver and kidney (Mi *et al.* 2007) have pointed to the existence of tissue-specific specializations.

Several microbial eukaryotes belong to the Unikont group, including unicellular fungi and amoebozoans such as the slime mould *Dictyostelium discoideum*, where peroxisomes have been identified by microscopy and biochemical assays (Parish 1975). However, information regarding the enzymatic content of amoebozoan peroxisomes is very scarce. Different studies on *D. discoideum* peroxisomes have identified citrate synthase, catalase, the multi-functional enzyme of the fatty acid β oxidation and the purine metabolism enzymes phosphodiesterase and urate oxidase (Hayashi & Suga 1978).

In contrast to amoebozoan peroxisomes, those of fungi have been intensively studied. Indeed, peroxisome research has taken great advantage of the wealth of molecular tools and genomic resources for the model yeast *S. cerevisiae*. For instance, several comprehensive studies including the analysis of gene expression induced by growth in oleate (Smith *et al.* 2002), large-scale fluorescence microscopy of GFP (Green Fluorescent Protein)-fused proteins (Huh *et al.* 2003)

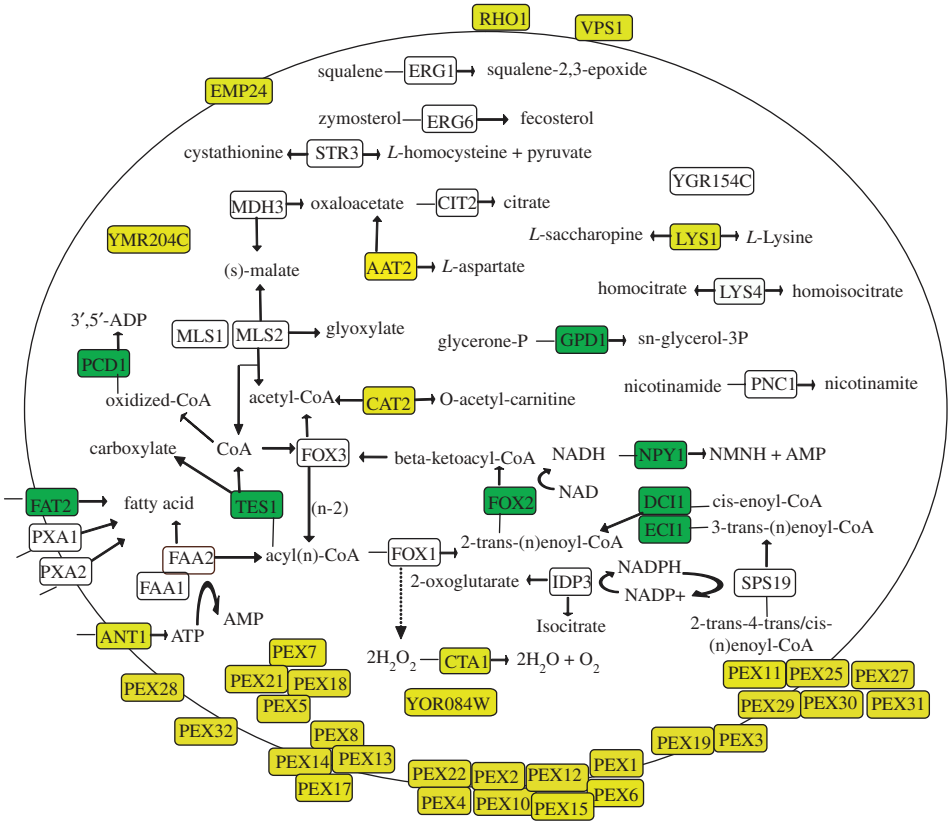



Figure 4. *Saccharomyces cerevisiae* peroxisomal proteome and metabolism as inferred from proteomics data. Colour codes as in figure 3. (Adapted from a modified version of fig. 5 of Gabaldón *et al.* (2006).)

or subcellular proteomics of highly pure peroxisomal fractions (Schafer *et al.* 2001; Yi *et al.* 2002) are approaching the full characterization of the protein repertoire of this organelle. Enzymes present in the peroxisomes of *S. cerevisiae* are mainly involved in fatty acid oxidation, amino acid metabolism and the detoxification of reactive oxygen species derived from these reactions (figure 4). Other yeast species may display quite a different metabolic repertoire in their peroxisomes, these being specialized in the metabolisms of several unusual carbon- and organic nitrogen sources used for growth (van der Klei & Veenhuis 2006*b*). For instance, methylotrophic yeast species (e.g *Candida boidinii, H. polymorpha, P. pastoris*) induce peroxisome development during growth on methanol as a sole carbon source. These peroxisomes harbour enzymes necessary for methanol metabolism such as alcohol oxidase and dihydroxyacetone synthase, which may account for up to 70 per cent of the protein content in the cell. In filamentous fungi, peroxisomes may also be involved in a number of biosynthetic roles. For instance, peroxisomes are the place for the final steps of the synthesis of the antibiotic penicillin in *P. chrysogenum* (van der Klei & Veenhuis 2006*b*). Other filamentous fungi such as *Neurospora crassa* induce a special type of peroxisome, glyoxysomes, during growth on ethanol or acetate (Kionka & Kunau 1985). Similar to glyoxisomes in plants, these organelles are characterized by their enrichment in enzymes from the glyoxylate cycle such as malate synthase and isocitrate synthase. Moreover, peroxisomes in *N. crassa* lack the enzyme catalase. Finally, as in other eukaryotic groups, peroxisomes have been lost from some fungal species such as those belonging to the Microsporidia.

## 4. PEROXISOMES IN PLANTAE

Members of the group Plantae are characterized by the presence of plastids derived by primary endosymbiosis. Besides higher plants (Viridiplantae), this group also includes photosynthetic microbial eukaryotes such as glaucophytes, green algae and red algae. Peroxisomes have been extensively studied in higher plants, where they play important roles in many processes including seed germination, leaf senescence, fruit maturation, response to abiotic and biotic stress, photomorphogenesis, photorespiration, biosynthesis of plant hormones and cell signalling by reactive oxygen and nitrogen species. Although not as extensively as in mammals and yeasts, proteomic studies have been performed on plant peroxisomes (Reumann *et al.* 2007; Eubel *et al.* 2008; Palma *et al.* 2009). These, together with computational analyses of protein sequences (Reumann *et al.* 2004), have helped to identify a wide variety of biochemical pathways present in peroxisomes. Among many other pathways, plant peroxisomes have been shown to contain enzymes involved in the pentose phosphate pathway, oxidation of fatty acids, ascorbate–glutathione cycle, biosynthesis of jasmonic acid and auxin, metabolism of nitric oxide and reactive oxygen species. Plant peroxisomes show a high degree of tissue specialization and at least four distinct types of this organelle have been

described. Undifferentiated plant peroxisomes contain mainly catalase and uricase. Glyoxysomes are enriched with enzymes of the fatty acid oxidation and the glyoxylate cycle, and their combined action allows these organelles to convert the seed storage lipids into sugar, necessary for seed germination and subsequent growth. Leaf peroxisomes, present in photosynthetic tissues, are specialized in the metabolism of glycolate and host many of the enzymes necessary for photorespiration. Finally, another type of peroxisomes has been identified in the root nodules of certain tropical legumes, in which the synthesis of allantoin is carried out.

In contrast to the relatively large amount of information available for higher plants, little is known about the diversity of functions of peroxisomes in unicellular plants. The presence of peroxisomes has been reported in several green and red algae (Codd *et al.* 1972; Shinozaki *et al.* 2009). However, their specific metabolic repertoire remains largely to be described.

## 5. PEROXISOMES IN EXCAVATES

Excavates are the major assemblage of protists. The group includes a broad diversity of free-living, symbiotic or parasitic forms, which often lack classical mitochondria (see paper by Embley *et al.* 2010). Some of the species from this group, such as the parasitic protozoan *Giardia lamblia*, are apparently also devoid of peroxisomes (de Souza *et al.* 2004). Others such as the kinetoplastids do possess a highly derived type of peroxisome referred to as glycosomes (Opperdoes & Borst 1977). This group of flagellate protozoa comprises important human pathogens such as the trypanosomatids of the genera *Trypanosoma* and *Leishmania*, which have recently received considerable attention. The peroxisomes of these organisms have the particularity of generally lacking catalase and harbouring a number of glycolytic enzymes. In addition, these organelles may contain additional enzymes from a variety of processes such as $\beta$ oxidation of fatty acids, the pentose-phosphate pathway, the purine salvage pathway and the biosynthesis of pyrimidines, ether lipids and squalene (Opperdoes & Michels 1993; Michels *et al.* 2006). Interestingly, the metabolism of these organelles can vary considerably during the life cycle of these parasites, which infect mammalian hosts and are transmitted by insects. For instance, glycosomes of *T. brucei* in the mammalian bloodstream are highly enriched in glycolytic enzymes, which may represent up to 90 per cent of their protein content (Michels *et al.* 2006). Apparently, the compartmentalization of this pathway into peroxisomes allows these parasites to overcome short periods of anaerobiosis during their bloodstream form. The paper by Ginger *et al.* (2010) in the present issue provides additional information on the complexity of metabolic compartmentalization in protists.

## 6. PEROXISOMES IN CHROMALVEOLATES

Chromalveolates are a eukaryotic assemblage that combines much of the diversity of algae (e.g. diatoms and dinoflagelates) with several of the major protist groups (e.g. apicomplexans and ciliates). Thanks to the availability of several genomic sequences from this eukaryotic group, we are now starting to have a glimpse of the diversity of peroxisomal metabolism of these species. For instance, *in silico* analyses of biogenesis markers have identified apicomplexans (e.g. *Plasmodium*) as the first eukaryotic group which lacks peroxisomes in the presence of classical mitochondria (Schluter *et al.* 2006). Other chromalveolates such as the ciliates of the genera *Tetrahymena* and *Paramecium* do possess this organelle (Muller 1973; Stelly *et al.* 1975). In oomycetes, peroxisomes have been detected in the genus *Phytophthora* (Philippi *et al.* 1975) and have been predicted to be present in the diatom *Thalassiosira pseudonana* (Armbrust *et al.* 2004). However, the presence of peroxisomes in other chromalveolates remains to be established. Genomic searches for core peroxisomal proteins in available sequences do suggest a patchy distribution of peroxisomes in several of these groups (T. Gabaldón & B. Gasse 2009, unpublished data).

## 7. PEROXISOMES IN RHIZARIA

Rhizaria is the only major eukaryotic super group for which no complete genome sequence has yet been obtained. It is as well one of the most recently created groupings, comprising Cercozoa, foraminifera and radiolarians (Cavalier-Smith 2002). Studies referring to peroxisomes in rhizarians are very scarce. Peroxisomes have been described as solitary organelles in several foraminiferan species, including those that inhabit the chemocline of marine sediments (Bernhard & Bowser 2008). In such anoxic environments, Foraminifera species might be associated with sulphur-oxidizing microbial mats, where micromolar levels of $H_2O_2$ are observed. Interestingly, peroxisomes of these foraminifera species have been proposed to participate in the breaking down of environmental hydrogen peroxide to produce oxygen, which would be subsequently used in aerobic pathways (Bernhard & Bowser 2008). Such a model would have important implications for the function of peroxisomes in certain environments, as their ability to produce oxygen from metabolically produced hydrogen peroxide will be important for extending the volume of sediments that is feasibly habitable by aerobic eukaryotes. As we will see below, such a function of peroxisomes is opposed to the putative ancestral role of peroxisomes as postulated by one of the evolutionary hypotheses on the origin of these organelles.

## 8. EVOLUTIONARY ORIGIN OF PEROXISOMES

The fact that the core mechanisms involved in peroxisomal division, biogenesis and maintenance are shared by peroxisomes of the most diverse organisms has fundamental implications for their evolutionary origin. In view of these data, a single evolutionary event originating a common ancestor of all existing peroxisomes seems the most plausible scenario. However, the exact nature of this evolutionary origin is more difficult to ascertain. Speculations about the possible evolutionary origin of peroxisomes began

soon after their discovery. Initial micrographs showing close interactions between peroxisomes and the endoplasmic reticulum (ER) prompted the idea that peroxisomes were formed from the endomembrane system (Novikoff & Shin 1964). But soon the alternative view that peroxisomes are independent organelles originated through endosymbiosis was proposed after it was realized that new peroxisomes are formed by the division of existing ones, and that they import proteins post-translationally (Lazarow & Fujiki 1985), two features that resemble those of bacteria-derived organelles such as mitochondria and chloroplasts. Certainly, the most elaborated and extended hypothesis on the origin of peroxisomes is the one put forward by de Duve (1982). He first proposed, and later developed over the years, a hypothesis in which peroxisomes would have been originated through endosymbiosis. In his model, de Duve (1982) provided an appealing metabolic scenario for the establishment of such an endosymbiosis that accounted for the role of enzymes in the detoxification of highly reactive oxygen species in the peroxisome. According to that scenario, the proto-peroxisome would have been acquired at a time in which the level of atmospheric oxygen was increasing and represented a toxic compound for the majority of living organisms. Perhaps boosted by the popularity of the serial endosymbiotic theory (Margulis 1970), this view has been the most widely accepted among biologists.

In recent years, however, the idea that peroxisomes originated through endosymbiosis has been challenged. Several lines of experimental evidence now point to very tight relationships between the ER and the biogenesis of peroxisomes. Among these, there is the finding that certain peroxisomal membrane proteins (PMPs) must be targeted first to the ER before they reach the peroxisomes (Tabak *et al.* 2003), and that peroxisome-less mutants in yeast can form new peroxisomes from the ER upon introduction of the wild-type gene (Erdmann & Kunau 1992). Furthermore, independent evidence for an evolutionary link between peroxisomes and the ER was provided by phylogenetic studies that showed homologous relationships between components of the peroxisomal import machinery and those of the ER-associated decay (ERAD) pathway (Gabaldón *et al.* 2006; Schluter *et al.* 2006) and raised doubts over a supposed endosymbiotic origin of matrix enzymes (Gabaldón *et al.* 2006). These findings provided support for earlier proposed models on the mechanisms of action of the import machinery of peroxisomes (Erdmann & Schliebs 2005). Altogether, these results seem to have convinced the research community of an origin of the peroxisomal membrane in the ER (Kunau 2005; de Duve 2007), but have not definitely closed the door to other speculations about a possible involvement of an endosymbiont in the origin of the peroxisome (de Duve 2007).

## 9. SHAPING THE PEROXISOMAL PROTEOME

Considering a single common ancestor for all peroxisomes, there are only two possible evolutionary scenarios to explain the current high levels of metabolic diversity. These are, namely, differential reduction from a metabolically diverse ancestor or differential acquisition of proteins and pathways. Although the first possibility was initially considered (de Duve 1969), it has been later abandoned in view of the increasing metabolic complexity of such putative ancestor (de Duve 2007). Moreover, in recent years, a growing body of evidence does suggest that differential gain of enzymes and even of complete pathways has indeed occurred in the course of peroxisomal evolution. A very illustrative case is that of alanine : glyoxylate aminotransferase, which has been re-targeted to the peroxisome in different mammalian lineages according to their dietary habits (Birdsey *et al.* 2004). There is also a clear precedent for the re-targeting of almost complete pathways to the peroxisome in the case of glycolysis and purine salvage pathways in glycosomes (Michels *et al.* 2006). Many other cases of the possible re-targeting of proteins of different sources to the peroxisomes have been reported elsewhere (Gabaldón *et al.* 2006), and the list is likely to grow as new peroxisomes from different organisms are characterized. This extensive re-targeting of proteins from different sources is not restricted to the peroxisome, as modern mitochondria also to seem have undergone a high degree of re-targeting from or to other subcellular compartments (Gabaldón & Huynen 2003, 2007). Mechanisms by which complete pathways can have been re-targeted are discussed by Martin (2010). Altogether, this highly dynamic view of the subcellular localization of proteins during evolution supports the idea that the metabolic diversity of peroxisomes is largely the result of a differential gain of proteins. Thus, the peroxisome can be regarded as a product of evolutionary tinkering, possessing a highly plastic proteome, and whose metabolic potential is shaped during evolution to adapt to the specific needs of every lineage.

## 10. CONCLUDING REMARKS

After 40 years of intensive research, peroxisomes are still mysterious organelles (Schrader & Fahimi 2008). As we get to know them, our ideas about peroxisomes are still shifting in many ways. From the concept of a simple eukaryotic organelle, containing almost exclusively catalase and some oxidative enzymes, we have moved to a picture of a cellular compartment involved in many different pathways and processes. In terms of organellar biogenesis, a new model is emerging that incorporates de novo formation of peroxisomes to the well-established growth and division of existing peroxisomes. From an evolutionary perspective, we are stepping from the view of a relict 'fossil organelle' of bacterial endosymbiotic origin towards the idea of an ER-derived organelle of the endomembrane system with a fairly conserved biogenesis but a highly adaptable enzymatic content. Evolution has shaped this enzymatic content by means of diverse processes such differential loss or acquisition of novel pathways from different sources. Remarkably, numerous studies performed on microbial eukaryotes have played an important role in these paradigm shifts. As new genomic data are made available and more research groups

are attracted to study peroxisomes in these organisms, it is likely that microbial eukaryotes will reveal to us many new clues about the function and evolution of these mysterious organelles.

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