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Review

Formation of peroxisomes: Present and past

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Abstract

Eukaryotic cells contain functionally distinct, membrane enclosed compartments called organelles. Here we like to address two questions concerning this architectural lay out. How did this membrane complexity arise during evolution and how is this collection of organelles maintained in multiplying cells to ensure that new cells retain a complete set of them. We will try to address these questions with peroxisomes as a focal point of interest.

Keywords: Peroxisome; Evolution; Endoplasmic reticulum

1. The present

An organelle can be defined as a lipid membrane, containing and surrounding proteins that are unique for this compartment [1]. Such an entity cannot be made from its individual lipid and protein components alone. As a rule organelles can only be derived from a pre-existing organelle [1,2]. Gottfried Schatz referred to this rule as the 'Third Genome', written in a lipid alphabet in contrast to the First (nuclear DNA) and Second Genome (mitochondrial DNA) written in nucleotide letters [3]. Not all organelles behave as independent entities. Two different categories can be discerned. Autonomous organelles multiply by growth and division and are inherited in much the same way as the nucleus. Here we encounter the (perinuclear) ER, mitochondria and chloroplasts. In contrast, another group of organelles is derived from an autonomous organelle and cannot take care of its individual inheritance. This group is collectively referred to as the endomembrane compartment comprising ER, Golgi, lysosomes/vacuoles, endosomes, secretory granules and plasma membrane where the ER can be viewed as the autonomous donor compartment for the others.

To multiply an autonomous organelle, two conditions must be met. First, new lipids must be synthesized and taken up to permit the membrane to grow and support the formation of new organelles. Second, new proteins must be synthesized and taken up to prevent dilution of their numbers upon multiplication. The ER is best endowed to cope with these requirements. It has the capacity to import proteins from the cytoplasm where almost all proteins are synthesized [4]. It is also the major site of lipid biosynthesis in the cell and it can supply all the members of the endomembrane family with them. This is different for mitochondria and chloroplasts, the other autonomous organelles of the cell. They are dependent on tapping the ER for most of the lipids required for membrane growth [5,6]. Of the four possible ways to traffic lipids around the cell: monomer diffusion, facilitated transport by lipid-transfer proteins, vesicle transport and transfer of lipids in contact sites of closely juxta-posed organelle membranes, only the last possibility is considered to be the most likely one for mitochondria and chloroplasts [7]. It illustrates that we are much less informed about how the delimiting membrane of an organelle is formed and enlarged

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compared to how proteins traffic within the cell and are sorted to their specific location. This is particularly true for peroxisomes. Insight into the import of proteins into the organelle has grown over the years but how the peroxisomal membrane is perpetuated received much less attention and remained an enigma for a long time.

Looking back, the question how peroxisomes multiply and how they acquire their lipid membrane has been answered in various ways. An early proposal that peroxisomes originate from the ER was based on morphological pictures taken by electron microscopy. Inspection of numerous pictures from different species occasionally showed peroxisomes in close association with ER and possible membrane continuities between them, suggesting that they originated from the ER [8]. But in the face of accumulating biochemical results this view lost its attractiveness [9]. These new data fitted much better in a concept of peroxisomes being autonomous organelles. They take up most of their proteins post-translationally utilising peroxisomal targeting signals (PTS1 and PTS2) and a peroxisome-specific protein import machinery [10]. These features seemed to give the autonomously multiplication model a rock-solid foundation.

However, peroxisomes seem to ridicule the rule of the Third Genome. Mutations in certain genes result in loss of the complete peroxisome population of a cell. Nevertheless, even after many generations of growth without peroxisomes, they reappear upon introduction of a wild type version of the gene. How can this be explained? Some have postulated the existence of a protoperoxisome as a source of regeneration of the peroxisome population [11]. This pushes the problem back and replaces one autonomously multiplying organelle, the peroxisome, with another, the protoperoxisome. Moreover, no experimental evidence has been reported to support its existence (see also below). For instance, in all the genetic screens to identify proteins involved in peroxisome biogenesis or maintenance no essential gene was found whose function could be related to the existence of a protoperoxisome. Yet, in the case of autonomous organelles such genes must exist because when such organelles are lost they can never be made again (Third Genome Rule).

Over the last 10 years many groups have found suggestive indications that ER and peroxisomes might cross paths, but for a long time the evidence remained inconclusive [12]. Many of these experiments were based on gene manipulations resulting in unbalanced protein production compared to wild type levels raising the question whether the observations were also true for a normal cell; fluorescence microscopy was applied which lacks the resolution to observe membrane contacts or biochemical approaches were used which suffer from the problem that it is often difficult to distinguish between real purification or contamination.

Finally, a number of contributions tipped the scale in favour of the ER as contributor to peroxisome formation. In *Yarrowia lipolytica* two membrane proteins involved in peroxisome biogenesis are N-glycosylated, indicating that they passed the ER en route to their peroxisomal destination [13]. For unknown reasons mouse dendritic (immune) cells show elaborate intermediate stages of peroxisome formation that are not seen in other tissues of the animal [14]. We took advantage of this biological rarity by using immuno-electron microscopy to study the location of peroxisomal marker proteins with respect to these intermediate compartments. The integral membrane protein Pex13p located in specialized regions protruding from the ER, in lamellar structures with the same morphological features (electron density and ordered substructure) and occasionally in mature, ovoid-shaped peroxisomes. The ABC transporter protein PMP70 was preferentially seen in the lamellae and mature peroxisomes while matrix enzymes such as thiolase and catalase were located exclusively within peroxisomes. Combining the functional properties of these proteins with their subcellular locations suggested a developmental pathway leading from ER via lamellae to peroxisomes. Indeed, in 3-D reconstructions using electron tomography we could observe membrane continuities between these three compartments [12].

Further proof for this proposal we obtained by real-time imaging in *S. cerevisiae*, illustrating the dynamics of the peroxisome formation process in time (Fig. 1). Here, advantage was taken of the properties of the membrane protein Pex3p which serves an essential and early role in peroxisome formation. For instance, cells lacking functional Pex3p do not contain peroxisomes or residual 'look-a-likes' anymore [15]. By appending a fluorescent marker to the protein (Pex3p-YFP, a functional derivative of Pex3p) the trafficking route of Pex3p could be delineated from its synthesis to its final location in both wild type and pex3 deleted cells. In both cases Pex3p first targets to the ER, then concentrates in foci co-localizing with the ER, which later in time lose their ER association and finally the protein is found in mature peroxisomes. Pex19p, a protein interacting with Pex3p, picks up the trail at the stage when foci are still associated with the ER and from then on follows the same route as Pex3p. When Pex3p is expressed in a pex3Δ mutant regeneration of the entire peroxisome population (appearance of multi-punctate fluorescence) is complete within 5 h [16]. In a different experimental set-up a truncated GFP-marked version of Pex3p containing only the first 46 N-terminal amino acids, which cannot support peroxisome biogenesis, was

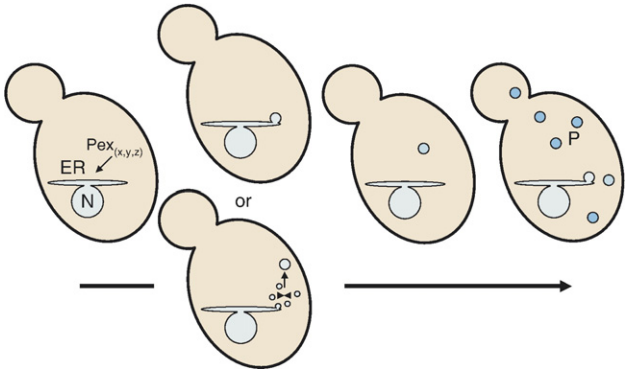

Fig. 1. Peroxisomes are sired and maintained by the endoplasmic reticulum. A number of peroxins (Pex(x,y,z)) enter or associate with the ER, concentrate in special areas of the ER in anticipation of a severing process that uncouples membrane from the ER to form precompartments that mature into peroxisomes. The nature of the severing step remains to be elucidated. Relatively large parts of the ER can develop into peroxisomal precompartments, but it is also possible that small vesicles bud from the ER that subsequently develop into mature organelles by homotypic fusion.

shown to accumulate in the ER. Upon mating with a wild type strain this truncated Pex3-GFP was chased into functional peroxisomes [17].

These new data provide a firm basis for a different concept of peroxisome formation (Fig. 1). A few Pex proteins colonize the ER and capture membrane, excluding resident ER proteins from this area. This 'specialized ER' is released from the ER donor membrane in a mechanistically still unknown way and matures into metabolically active organelles. Competence to import matrix proteins appears rather late during the maturation process arguing that after the pioneering role of a few Pex proteins others are taken up later [16]. Once all the Pex proteins building up the protein import machinery have been assembled the import of the enzymes can start to complete the maturation process. This new model can explain a few enigmas lurking around in the field:

(i) how are cells lacking peroxisomes able to regenerate the organelles? As part of the endomembrane system they are dependent on the ER as donor compartment. As long as the ER is around the 'Third Genome Rule' can be obeyed.
(ii) How do multiplying peroxisomes recruit the lipids for the necessary enlargement of the membrane? They receive their membrane from the ER, the major lipid synthesizing factory of the cell.

With satisfactory answers to these questions, important new ones arise. How do a few Pex proteins find their way to the ER? How are resident ER proteins excluded from the peroxisomal precompartment and peroxisomal proteins selected for entering them? How is the peroxisomal precompartment severed from the ER? Is the newly formed population of organelles still subject to fusion and/or fission processes?

At the moment there is evidence for at least three membrane proteins, Pex3p, Pex13p and Pex16p targeting and entering the ER after synthesis in the cytosol. These proteins lack obvious tell-tale features, such as signal sequences; how import is achieved is not known and attempts to implicate the ER Sec61 translocon in formation of peroxisomes failed thus far. In Pex3p important information is located in the N-terminal part comprising the single membrane span, because truncations removing substantial parts of its cytosolic domain still end up in the peroxisomes [18].

Speculations about the specificity of protein sorting taking place in the ER during precompartment assembly are intimately connected with the question how the precompartment is severed from the ER. The high resolution EM pictures taken from mouse dendritic cells suggest that relatively large parts of the ER are severed. These parts reveal a semi-crystalline substructure with intimate contact between the two membrane sheets of the precompartment. It almost suggests as if ER proteins could be passively squeezed out by the positive action of the few interacting peroxisomal proteins responsible for initiating the formation of the precompartment. The fact remains however that we are dealing with a rather extreme adaptation in these dendritic cells which we cannot without caveat extrapolate to the average situation. It is therefore equally interesting to explore the opposite possibility, in which small vesicles are pinched off from the ER membrane using the COPII machinery involved in the secretory pathway followed by homotypic fusion to form a larger organelle [19]. Although attempts to implicate COPII components in peroxisome formation have been negative thus far [20,21], it may be useful to analyse their contribution again with the more direct assays that are available now. This is underscored by the observation that a COPII-like component, Emp24p, was reported to be associated with young peroxisomes [22].

The two extreme models discussed above with regard to precompartment formation have implications for the possible existence of fission and/or fusion activities of peroxisomes. A key question here is how the future peroxisomal membrane is released from the ER. If this takes place in big chunks (as suggested from the morphology of dendritic cells) then enough membrane is released to undergo a few fission cycles in the formation of the mature organelle. If, on the contrary, the ER releases the precompartment in the form of small (COPII-like coated) vesicles, a fusion process is needed to obtain mature-sized organelles. Whatever the future has in store, there is evidence that peroxisomes are subject to fission like processes [23–26]. The dynamin-like proteins Vps1p (in yeast) and DLP1 (in mammals) have a role in peroxisome fission and remarkably DLP1 is bound in mammals to the peroxisomal membrane by Fis1p, which carries out the same job in mitochondria [27]. Unless there is still another (unknown non-ER) link to recruit lipids there is only a limited number of fissions possible until membrane becomes limiting for further division. Indeed, using a photo-reactivatable version of GFP in combination with a pulse-chase protocol, it was shown that the majority of the peroxisomes in a cell are formed from the ER and are not derived from pre-existing peroxisomes [28].

The new model that peroxisomes, as off-shoots from the ER, are part of the endomembrane system of the eukaryotic cell, has major implications about our thinking how they arose in the evolutionary past.

2. The past

To speculate about the evolution of peroxisomes one needs to have ideas about selective principles that promoted their development and facts to support them. In this respect it is difficult to take lessons from present-day peroxisomes, because one of the hallmarks of the microbody/peroxisome family is their extreme variability in enzyme content and in their contributions to cellular metabolism. Soon after their discovery research focussed on oxidative enzymes producing H_2O_2 and catalase detoxifying this product. This led De Duve already in 1969 to propose that peroxisomes evolved as a line of defense at a time, some 2.5–2.0 billion years ago, that O_2 started to appear in the earth's atmosphere [29]. A hypothesis De Duve worked out in greater detail over the years and is known as the 'O_2 disaster', because it meant exposure to a toxic compound for most organisms living at that time [30,31]. The attractiveness of this proposal is the operation of a plausible selective principle, detoxification of O_2 and its derived radicals, and the eloquence in which the proposal is phrased. But is it possible with the

wealth of genomic sequences and new insights about peroxisome function and biogenesis to underpin it with facts?

Two recent studies have addressed this issue following similar approaches [32,33]. In absence of a peroxisomal genome they collected a reliable core set of peroxisomal proteins and cytosolic proteins with a dedicated role in peroxisome function: the peroxisomal protein signature. For each protein an attempt was made to trace its phylogenetic origin with the hope that the ensemble of the peroxisome proteome would reveal clues about the evolutionary roots of the organelle. The most homogeneous group is formed by the Pex proteins involved in peroxisome formation and maintenance. Although also here species variability is encountered, a core set comprising Pex1p, Pex2p, Pex4p, Pex5p, Pex6p, Pex7p, Pex10p, Pex11p, Pex12p, Pex14p, Pex16p and Pex19p can be confidently shown to be present in all peroxisome containing species, including the glycosome containing species like Leishmania (Fig. 2). Moreover, some individual Pex members (Pex1p, Pex2p, Pex4p, Pex5p, Pex6p and Pex10p) show homology with proteins functioning in the ERAD pathway responsible for removing unproductive proteins from the ER. Interestingly, the peroxisomal counterparts of cdc48, Pex1p and Pex6p, retained a similar function. They support the PTS1 receptor protein (Pex5p) to recycle to the cytosol by extracting it in an ATP dependent manner from the peroxisomal membrane [34,35]. This part of the analysis provides support for the notion that peroxisomes developed within the

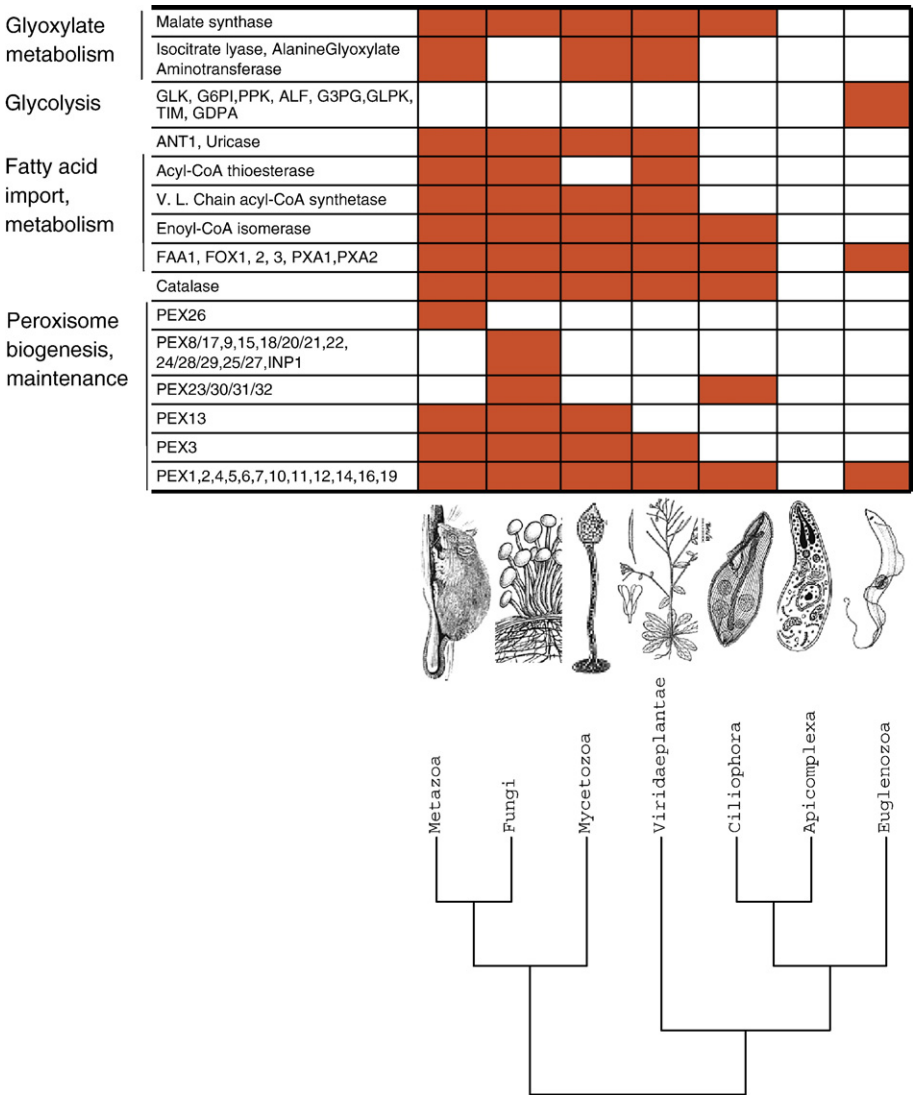

Fig. 2. Phylogenetic distribution of peroxisomal proteins among the major eukaryotic taxa. A taxon is considered to have a certain type of peroxisomal protein if there is (1) direct experimental evidence, or (2) if the taxon has a protein that is orthologous to a peroxisomal protein from another taxon and either has a PTS1 or a PTS2 signal or has a non-consensus PTS1 signal that is identical to the non-consensus PTS1 signal from its experimental peroxisomal ortholog. Definitions of PTS1 and PTS2 signals were obtained from [50]. Orthology was determined by best-bidirectional hits in Blast searches, or, in the case of the large mitochondrial/peroxisomal carrier family that includes ANT1, by phylogenetic analysis. Apicomplexa are devoid of peroxisomal proteins [33] and were included for completeness. All peroxisomal biogenesis proteins were included in the analysis, furthermore representative proteins from beta-oxidation, the glyoxylate cycle and glycolysis, and two widespread peroxisomal proteins catalase, uricase and ANT1. Proteins separated by slashes, e.g. "25/27", represent cases of taxon specific expansions of gene families or cases where orthologous proteins have been given different gene names. Glycosomal glycolysis proteins from the Euglenozoa are based on [51]. Species illustrations are from BIODIDAC (biodidac.bio.uottawa.ca). The phylogeny is based on [52].

eukaryotic lineage from an endomembrane system of ever increasing complexity.

The situation is somewhat more shaded for the enzymes comprising the peroxisomal proteome. Here a significant portion of 17–18% of the enzymes can be traced to an alpha-proteobacterial origin (Fig. 3) [32]. This is similar to the fraction of alpha-proteobacterial proteins in the proteome of mitochondria and is taken as strong support for mitochondria being derived from domesticated alpha-proteobacteria [36,37]. Does this imply an endosymbiotic origin for peroxisomes, too? We do not think so for the following reasons: (i) it would be a surprising coincidence if the peroxisome would be an independent endosymbiont originating from the same group of purple bacteria that gave rise to mitochondria considering the fact that the only other organelle of endosymbiotic origin, the chloroplast, has totally different roots, the cyanobacteria; (ii) it is remarkable that quite a number of peroxisomal enzymes have mitochondrial counterparts, which is in line with retargeting of gene products originally brought in by the purple bacteria in the host organism. Indeed, analysis of the mitochondrial proteome shows ample evidence for DNA shuffling between mitochondrial and nuclear genomes and readdressing of proteins to other cellular compartments (Fig. 4) [36,37]. Such recruitment of proteins of endosymbiotic origin to peroxisomes is not exceptional. Some

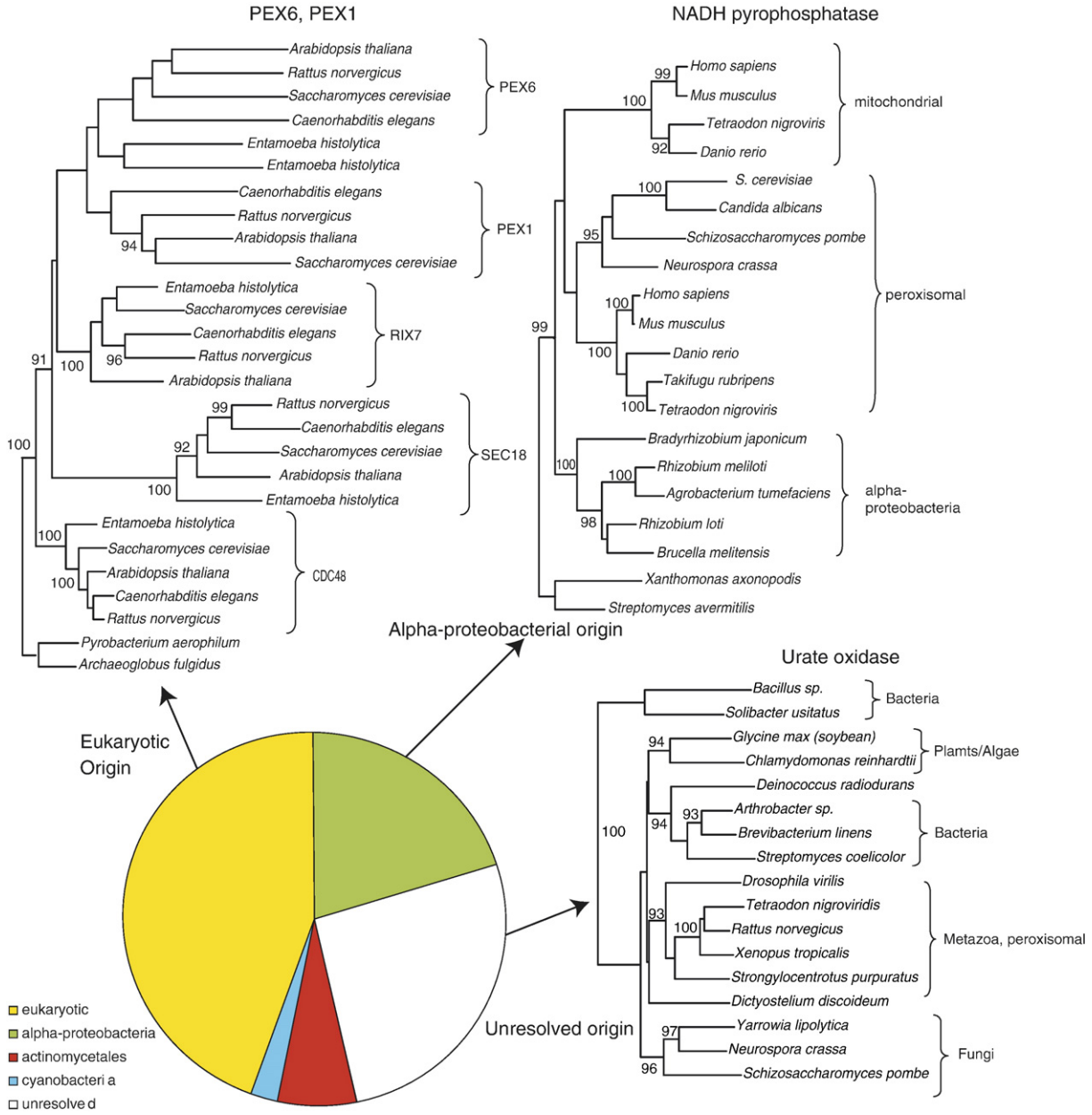

Fig. 3. The dual phylogenetic origin of the peroxisomal proteome: eukaryotic and alpha-proteobacterial. Proteins are considered to be of eukaryotic origin when they either have no Bacterial or Archaeal homologs, or when their phylogeny indicates that they were present in the common ancestor of Archaea and Eukaryotes, as in the case of Pex1 and Pex6. Peroxisomal proteins are considered to be of Bacterial origin if they cluster "within" a clade of the Bacteria, as in the case of NADH pyrophosphatase. Proteins are classified as "unresolved" when they do have Bacterial homologs but their phylogeny does not support their origin from a specific Bacterial clade, as in the case of urate oxidase, data from Gabaldon et al. [32].

proteins in the glycosomes of Trypanosomatids can be traced back to cyanobacteria, the other group of bacteria giving rise to endosymbionts [38].

Also enzymes that do not have a (detectable) alpha-proteobacterial origin show dual mitochondrial/peroxisomal locations such as NADP dependent iso-citrate dehydrogenase (Idp3), catalase (Cta1p), fatty acid CoA synthetases (Faa1p and Faa2p) and citrate synthase (Cit2p). Which location came first is not always clear but in the case of Cit2p the phylogenetic analysis suggests an ancestral mitochondrial location and subsequent retargeting to the peroxisome as the orthologs of Cit2p are mitochondrial in other species [32]. Alanine:glyoxylate aminotransferase (ATG) presents an extreme case of variability in organelle location. In mammals it is found in mitochondria or peroxisomes depending on species and diet [39]. The ease with which subcellular locations can be changed during evolution may be related to the relative simplicity of particularly the major peroxisomal targeting type 1 signal (PTS1), which is primarily determined by a weakly conserved sequence comprising the last three carboxy-terminal amino acids of a protein.

Considering the fact that the majority of the peroxisomal proteome (56% in yeast, 38% in rat) is of eukaryotic origin and that the core consists of Pex proteins contributing to peroxisome biogenesis and maintenance we favour the idea that peroxisomes arose in the eukaryotic lineage during evolution. This is supported by the phylogenomics attempt to reconstruct the minimal ancestral eukaryotic peroxisome (Fig. 5). It contains a core set of Pex proteins and enzymes which function in the degradation of fatty acids. At least one of these enzymes, 3-hydroxy-acyl-CoA dehydrogenase (Fox2p), is of alpha-proteobacterial origin [32], which one could construe as an indication that the capacity to breakdown fatty acids in peroxisomes followed the endosymbiosis of mitochondria.

An important question is how to find clues about the timing of peroxisome appearance in evolution. This question is intimately associated with the way the first endomembrane structures arose. Here, we have a plethora of persuasive ideas but unfortunately not much evidence to go by. In classic models loss of a rigid cell wall followed by increment in size and development of a phagocytic lifestyle were considered to be the basis for membrane expansion and vesiculation leading to formation of the eukaryotic cell [40–42]. The capacity of phagocytosis is an important concept in these models to explain the acquisition of endosymbionts. Two recent studies suggest, however, that formation of secretory endomembrane systems originated very early in evolution and preceded phagocytosis and that such membranes were already present before endosymbiosis of mitochondria or chloroplasts even started. This is inferred from phylogenetic analysis combined with structural analysis of conserved protein motifs of seven proteins forming a building block of the nuclear pore complex (WD-40 and TPR) and of the Ras-like group of small GTPases (Sar1p, Arf1p, SRb, Rab, Ras and Rho) all of which have membrane sculpting properties in present-day cells [43,44]. Because no orthologs of Ras-like GTPase family members exist in bacteria, even such highly sophisticated analyses combining protein sequence and structural similarities do not reach far enough back in evolutionary time to tell something about the possible architecture of cells existing 2.5–2.0 billion years ago. It illustrates the problem of rooting the eukaryotic lineage within the prokaryotic world and questions whether at the time at which De Duve's proposal of an 'O_2 disaster' is positioned intracellular compartments even existed. In addition, why would a cell stow away its protective enzymes, oxidases and catalase, in a small confined space instead of having them patrolling all the holes and crevices of the cell?

The 'O_2 disaster' proposal also assumes that earth completely changed from anoxic to oxic conditions, which is probably not

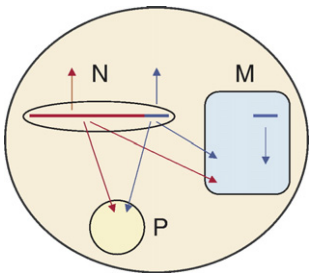

Fig. 4. Evolutionary origin of organellar proteins. Part of the alpha-proteobacterial DNA (blue) has been transferred to the nucleus and integrated onto the nuclear DNA (red). Proteins originally specified by alpha-proteobacterial DNA are not only addressed to mitochondria but also to other locations of the cell, among which peroxisomes. Proteins encoded in the DNA of the acceptor cell go to various locations including mitochondria.

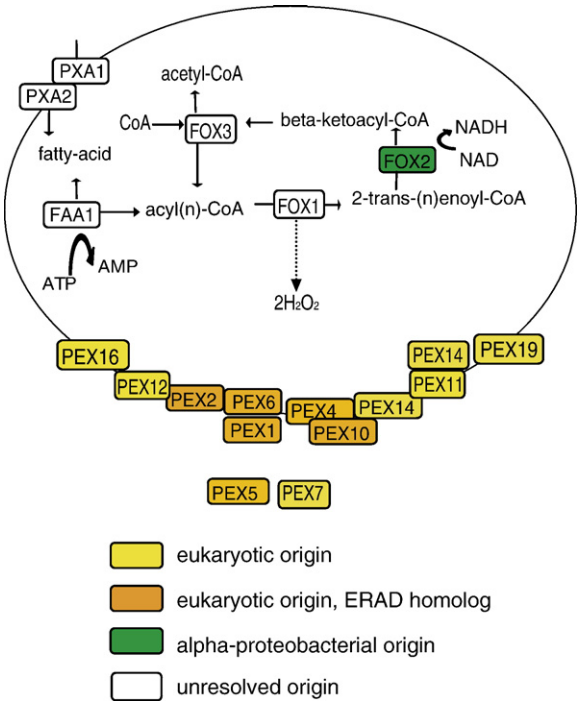

Fig. 5. Reconstruction of the universal peroxisome. Universal peroxisomal proteins and deduced metabolism, based on the analysis in Fig. 2, were included. The metabolic scheme and the phylogenetic origin of the proteins are based on Gabaldon et al. [32]. Do note that even though these proteins occur in all taxa of Fig. 2, they do not necessarily occur in all the species in those taxa. Peroxisomal proteins have been repeatedly lost in evolution, e.g. in adaptation to an anaerobic lifestyle.

true. Even to this day there are numerous habitats in which anoxic conditions prevail leaving niches for primitive cells to escape from the changes taking place in the earth's atmosphere [45]. However, examples of such cells with an elementary endomembrane system but lacking mitochondria (or family members as hydrogenosomes or mitosomes) have not (yet) been found. On closer inspection it is now clear that a-mitochondriate organisms previously thought to lack mitochondria have DNA or organellar remnants indicating that mitochondria were secondarily lost, possibly as adaptations to parasitic lifestyles [45].

Such considerations led to formulation of radically new scenarios that position the development of the eukaryotic cell at a later stage even after entry of the alpha-proteobacteria as progenitors of the mitochondria. Central to these ideas is first, the notion that no eukaryotes without mitochondria exist and second, the remarkable radiation at the base of the eukaryote tree, which together suggest that the origin of mitochondria is placed very close to, if not coincident with the origin of the eukaryotic cell itself [46]. Martin and Koonin give this order of events more weight by providing some selective principles how this could have taken place [47]. They assert that the introns of eukaryotic split genes originated from the self-splicing group II introns introduced by the alpha-proteobacteria in an (archaebacterial) host. With the transfer of purple bacterial split genes to the gene pool of the host a problem arose. The rate of translation is much faster than the rate of splicing, resulting in the formation of mostly meaningless products. One way to avoid this dilemma was the physical separation of transcription and RNA splicing from translation resulting in the compartmentalisation of DNA, transcription and splicing in a future nucleus. Note that the invasion of introns upon symbiosis of a proteobacteria with a methanogen is also one of the rationales of the origin of the nucleus in a different theory about the origin of the eukaryotic cell which has been put forward by Lopez-Garcia and Moreiro [48]. This might have been a troublesome transition period during which most of the population died but the few survivors could make a new start and elaborate on this new compartmentalization strategy.

The coordinated development of nuclear pore complexes and small GTPases discussed before would then suggest that the endomembrane system developed during or after the invasion of the alpha-proteobacteria, which would make it much younger then in classic proposals of eukaryote development. As a consequence also peroxisomes would be a rather new invention and may have arisen during this transition period when gene pools of alpha-proteobacteria and host were mixed and sorted. Is the significant fraction of alpha-proteobacterial enzymes that we find in peroxisomes a reflection of that period and could the segregation of lipid biosynthesis in the ER from degradation in the peroxisomes have been a selective force to create a new organelle? Even then it remains difficult to understand why peroxisomes developed a protein import system of their own and why a more simple solution was not chosen. For instance, by importing the fatty acid degrading enzymes as inactive proenzymes into the ER and sort them via the secretory pathway to a separate organelle as happens in the routing of lysosomal enzymes and the formation of the lysosomal compartment.

An intriguing aspect of the peroxisome protein import machinery is that its components are all of eukaryotic origin, including the core group of Pex proteins. This contrasts with the situation in mitochondria and chloroplasts. Here the protein import complexes are built up mainly from eukaryotic proteins as well as some proteins of bacterial origin [49]. One could explain this as an indication that the peroxisomal protein import complex was already firmly established before the invasion of purple bacteria took place and that the mitochondrial protein import system still had to be shaped, this time from products of a mixed gene pool.

We can conclude that the phylogenetic analysis using the wealth of new genomic sequence data has enriched the menu with new dishes to choose from. We not only have an 'O_2 disaster' hypothesis in which peroxisomes are very ancient but also an 'intron disaster' scenario in which peroxisomes feature as youngsters in evolution and various scenarios proposing in-between situations. New insights into peroxisome formation hopefully will be a fruitful area for further research and new findings. Particularly, the mechanistic aspects of and proteins involved in severing of preperoxisomal vesicles from the ER is of major interest. Does it, for instance, make use of the proteins known to be involved in sculpting the ER, the family of small Ras-like GTPases? In that case it would be possible perhaps to link the peroxisome sculpting proteins to the already explored phylogeny of this group of proteins.

3. Concluding remarks

We have a new concept of how peroxisomes are formed and maintained in the cell. Instead of being autonomous organelles that multiply by growth and division, they are formed from the ER and are part of the endomembrane family of organelles. This has major implications for the formulation of scenarios how they arose during evolution. Although speculation is hampered by lack of knowledge about plausible selective principles and formation of the eukaryotic cell as a whole, prerequisite conditions have narrowed. Their formation must be tightly linked to the evolution of the endomembrane system and an endosymbiont origin can be excluded.

4. Note added in proof

Our attention was called to early work that glycosomes of plants originate from the ER [53].

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