Traffic 2003; **4:** 512–518
Blackwell Munksgaard

ISSN 1398-9219

Review

Peroxisomes Start Their Life in the Endoplasmic Reticulum

Henk F. Tabak[1,*], Jean L. Murk[1], Ineke Braakman[2] and Hans J. Geuze[1]

[1] *Laboratory of Cell Biology, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands*
[2] *Bio-Organic Chemistry, University Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands*
* *Corresponding author: Henk F. Tabak, H.F.Tabak@umc.uu.nl*

Peroxisomes belong to the ubiquitous organelle repertoire of eukaryotic cells. They contribute to cellular metabolism in various ways depending on species, but a consistent feature is the presence of enzymes to degrade fatty acids. Due to the pioneering work of DeDuve and coworkers, peroxisomes were in the limelight of cell biology in the sixties with a focus on their metabolic role. During the last decade, interest in peroxisomes has been growing again, this time with focus on their origin and maintenance. This has resulted in our understanding how peroxisomal proteins are targeted to the organelle and imported into the organellar matrix or recruited into the single membrane surrounding it. With respect to the formation of peroxisomes, the field is divided. The long-held view formulated in 1985 by Lazarow and Fujiki (Lazarow PB, Fujiki Y. Biogenesis of peroxisomes. Annu Rev Cell Biol 1985; 1: 489–530) is that we are dealing with autonomous organelles multiplying by growth and division. This view is being challenged by various observations that call attention to a more active contribution of the ER to peroxisome formation. Our contribution to this debate consists of recent observations using immuno-electronmicroscopy and electron tomography in mouse dendritic cells that show the peroxisomal membrane to be derived from the ER.

Key words: electron tomography, endoplasmic reticulum, immuno-electronmicroscopy, organelle inheritance, peroxisome, PEX genes

Received 31 March 2003, revised and accepted for publication 29 April 2003

Introduction

The renewed interest in peroxisomes during the past 15 years has uncovered new information, particularly with regard to their formation and maintenance. Peroxisomal proteins are synthesized on free polyribosomes and, after completion of their synthesis, delivered to the cytosol (1). Here, folding starts before they are taken en route to their final destination within the cell. Peroxisomal targeting signals (PTSs) are recognized by cytosolic proteins (receptors) that guide their cargo to the peroxisomal membrane. The Pex5p receptor recognizes the PTS type 1 signal, consisting of an SKL-like consensus sequence located at the extreme C-terminal end of proteins to be imported. Most matrix proteins reach peroxisomes via this PTS1 element. A minority of matrix proteins (for instance, α-keto-thiolase) is guided by Pex7p to the peroxisomes. This receptor recognizes an N-terminally located PTS type 2 motif (2). Signal sequences in integral membrane proteins have recently been characterized (mPTS), and Pex3p and Pex19p have been proposed to support the targeting of these proteins to the peroxisomal membrane (3,4). Apart from these four primary PTS recognizing proteins, additional proteins are required to accommodate the proper targeting of proteins to peroxisomes along these routes, including cytosolic Hsp70, Djp1p (a Dna J-like protein), Pex18p and Pex21p (Pex20p in *Yarrowia lipolytica*). The last two are required for the formation of an import-competent complex containing Pex7p and α-keto-thiolase. However, we understand these processes only in rough outline.

The same is true for the translocation of proteins across the peroxisomal membrane. A number of integral and peripherally associated membrane proteins have been identified that are responsible for the correct execution of this process, but their individual contribution is still being studied and an integral picture is not yet available. This is in part due to the formidable problem that it is difficult to reconstitute protein import in an *in vitro* system. After isolation, peroxisomal membrane integrity is compromised, resulting in increased permeability to small molecules. Thus, these leaky organelles have resisted attempts to carry out in-depth analysis comparable to the protein import studies reported for mitochondria, chloroplasts and ER.

An even tougher question to answer is how and from where the lipids are acquired to accommodate the growth of peroxisomes. Although in certain mutant cells (yeast or human pex mutants with mutations in PEX genes coding for proteins with maintenance functions) peroxisomes cannot be detected, there is no obvious link between the

affected protein concerned and its possible relation to lipid biogenesis. Nevertheless, such mutants regenerate peroxisomes readily upon transformation with the corresponding wild-type gene. This observation is hard to reconcile with peroxisomes being autonomously multiplying entities. Here, we would expect: once lost, always lost! Excluding *de novo* synthesis of organelles (see below), this has led to various speculations regarding the pathway responsible for the appearance of peroxisomes.

A Field in Disarray

During the last decade a number of groups have presented observations to challenge the concept of Lazarow and Fujiki that peroxisomes are autonomous organelles multiplying by growth and division (1). Instead, these observations all pointed to the ER as a possible contributor to their biogenesis. Biochemical experiments in which import of a peroxisomal matrix protein was followed in a rat liver homogenate indicated the existence of a maturation pathway (5). Proteins were first incorporated into structures equilibrating at a low density in a sucrose gradient and were subsequently chased into membranes positioned at the characteristic high density of mature peroxisomes. Later, observations of various sorts were reported. It is informative to read the short synopses compiled by the groups involved and appreciate the importance attached to the various arguments (6–11).

The basic problem is a lack of consistency. Experiments carried out by one group could not be repeated or corroborated by others. For instance, treatment of *Saccharomyces cerevisiae* with brefeldin A was reported to influence peroxisome biogenesis implicating the ER in this process (12). A similar effect was absent when mammalian cells were treated with this drug (13). Similarly, COPI and ARF1 were implicated in peroxisome biogenesis in mammalian cells (14), but in a careful analysis by others no role for COPI, ARF1 or SAR1 could be demonstrated (15,16). Various peroxisomal proteins have turned up in the ER, but it has been difficult to exclude mislocalization due to altered expression levels or the use of fusion proteins. Also, intimate associations between peroxisomal structures and nuclear or cortical ER have been reported, but crucial evidence for membrane continuities is still lacking (17–19).

An exception is the work carried out in the yeast *Y. lipolytica*. Here, two peroxisomal proteins (Pex2 and Pex16) were shown to move through the ER to peroxisomes in pulse-chase experiments and shown to carry N-linked glycosyl groups as evidence of their temporary ER residence (20). In addition, the Rachubinski/Titorenko group reported a series of extensive biochemical reconstitution experiments implicating vesicle fusion and a number of developmental steps in a peroxisome maturation pathway (21). This demanding set of biochemical experiments has not yet been repeated in other biological systems.

A more simple maturation pathway was formulated by the group of Gould (13). Their model is based on the well-known finding that in pex mutants the peroxisomes reappear on a relatively short time-scale of hours after reintroduction of the wild-type gene and on the functional properties of certain peroxins, particularly the ones involved in early steps of biogenesis. The enigma still remaining, however, is the nature of the 'preperoxisome' from which this putative maturation pathway starts.

Deus Ex Machina: The Mouse Dendritic Cell

By a stroke of luck, we discovered that mouse dendritic cells offer a unique opportunity to study the formation of peroxisomes (22). In these cells of the immune system, clusters of mature globular and sometimes reticulate peroxisomes with electron-dense content were often surrounded by curved electron-dense tubules or sheets (lamellae) with a highly structured appearance (Figures 1A and 2). We have previously shown by immuno-electronmicroscopy that these lamellae contain a typical peroxisomal integral membrane protein: Pex13p. Matrix proteins such as α-keto-thiolase and catalase were absent from lamellae and were exclusively located in globular/reticulate (mature) peroxisomes. The peroxisomal ABC transporter PMP70 was found in both the lamellae and peroxisomes. At certain points membrane continuities could be demonstrated between lamellae and globular/reticulate peroxisomes using electron tomography. Finally, lamellar-like structures were found extending from the rough ER (here called specialized ER). At the junction of these structures a clear separation of protein markers was evident: the rough ER contained protein disulphide isomerase (PDI), calreticulin and invariant chain but no Pex13p, while the reverse was true for the specialized ER. Furthermore, ribosomes were absent from this specialized ER.

Here, we illustrate these morphological aspects by 3-D reconstructions using electron tomography (Figure 1). Particularly striking are: (i) the rather irregular curved membranes of the rough ER vs. the straight and more electron-dense membrane of the specialized ER; (ii) the difference in luminal content, rather inconspicuous in the rough ER but highly structured with an electron-dense line running in the middle of the specialized ER; (iii) in sections the lamellae appear as tubules. But in reality they consist of interconnected lamellar sheets and (iv) the intimate envelopment of a maturing peroxisome by the surrounding lamellae (Figure 1B). Figure 1 further shows detailed examples of the membrane continuity between rough ER and specialized ER which is so crucial to our proposed model, and the continuity between a lamella and a maturing peroxisome.

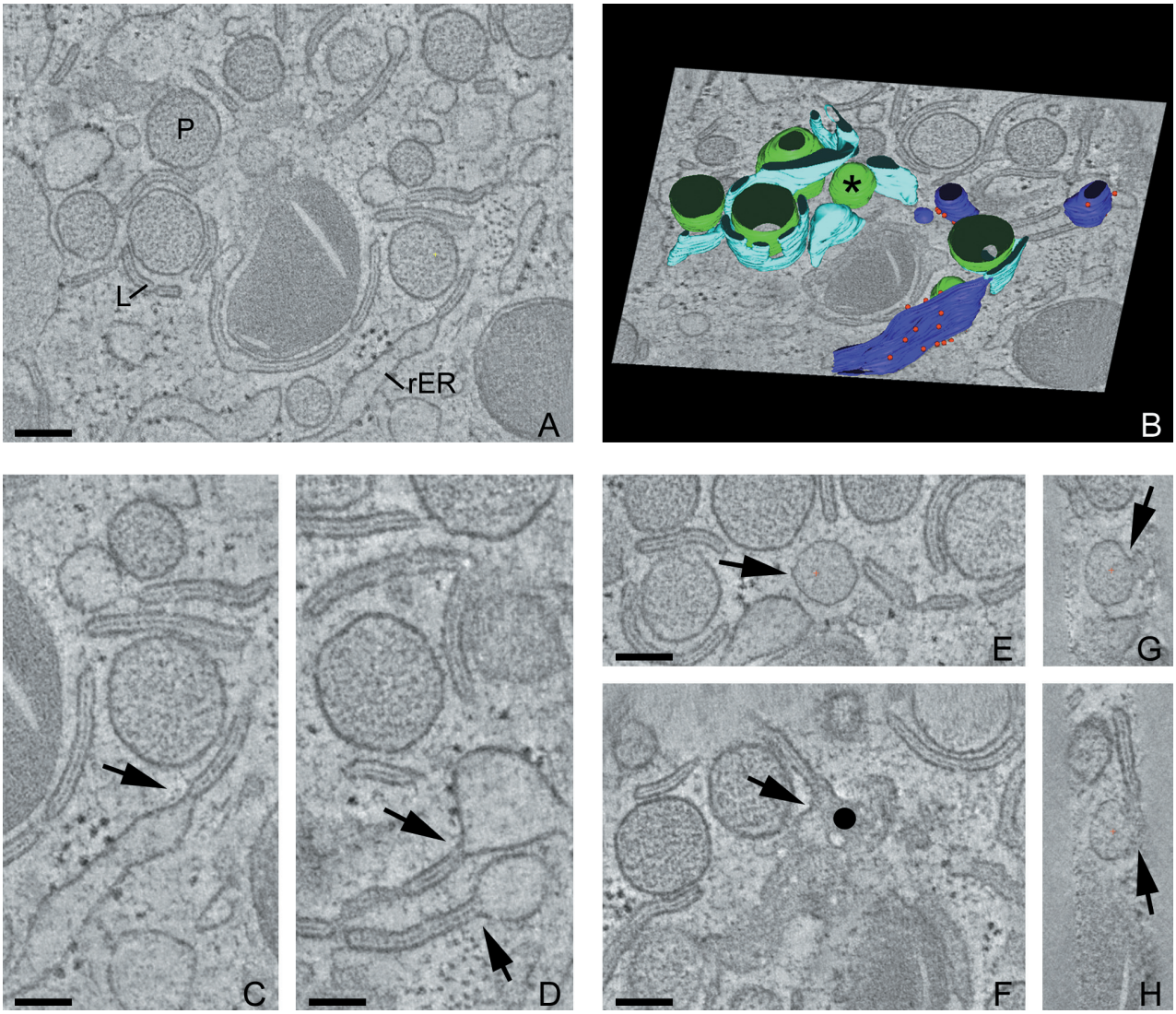

Figure 1: An electron tomographic reconstruction of a cluster of peroxisomes in a 250-nm section of a cryofixed dendritic cell showing the membrane continuities that exist between rough ER, lamellae and peroxisomes. A: overview of the cluster of peroxisomes that was selected for 3-D reconstruction. P = peroxisome, L = lamella, rER = rough endoplasmic reticulum. Bar = 200 nm. B: Model view of some of the peroxisomes, lamellae and rER from A. The model was obtained by manually tracing the membranes in 4-nm tomographic slices through the reconstructed 3-D volume. Peroxisomes are depicted in green, rER in dark blue, ribosomes in red and lamellae in light blue. The peroxisome with an asterisk is highlighted in E–H. Supplementary movie available in the Video Gallery at www.traffic.dk. C, D: Tomographic slices showing the membrane continuity (indicated by arrows) of rER with the lamellae. Bar = 100 nm. E–H: Tomographic slices showing the continuity of the peroxisome indicated by an asterisk in B with the lamellar reticulum. E–H show the same peroxisome (indicated by an arrow) in slices taken from different angles through the volume of the 3-D reconstruction. Bar = 150 nm. E: Cross-section of the peroxisome. F: Cross-section, cut in the same plane as E, through the lamellar sheet to which the peroxisome is attached. The site of attachment is indicated by a dot. The lamellar sheet is sectioned parallel through its flat side. The sagittal section in G shows the connection between the peroxisome and the underlying lamella, which appears as a bulge. H indicates that the bulge is a continuation of a lamellar sheet.

Upon stimulation, dendritic cells commit themselves to antigen presentation which involves massive production of invariant chain that moves through the secretory route (23). Interestingly, visualization of invariant chain by immuno-electronmicroscopy showed its presence not only in the organelles and vesicles of the secretory route but also in lamellae, although in much lower amounts (Figure 2). Apparently, segregation of invariant chain between rough ER and specialized ER is not completely water-tight in the face of such an abundant supply of invariant chain. The resulting spill-over of some invariant chain into the peroxisome maturation pathway is additional evidence for the continuity of preperoxisomal structures and ER. Taken together, these observations argue for the existence in dendritic cells of a single dynamic membrane-continuum encompassing rough ER,

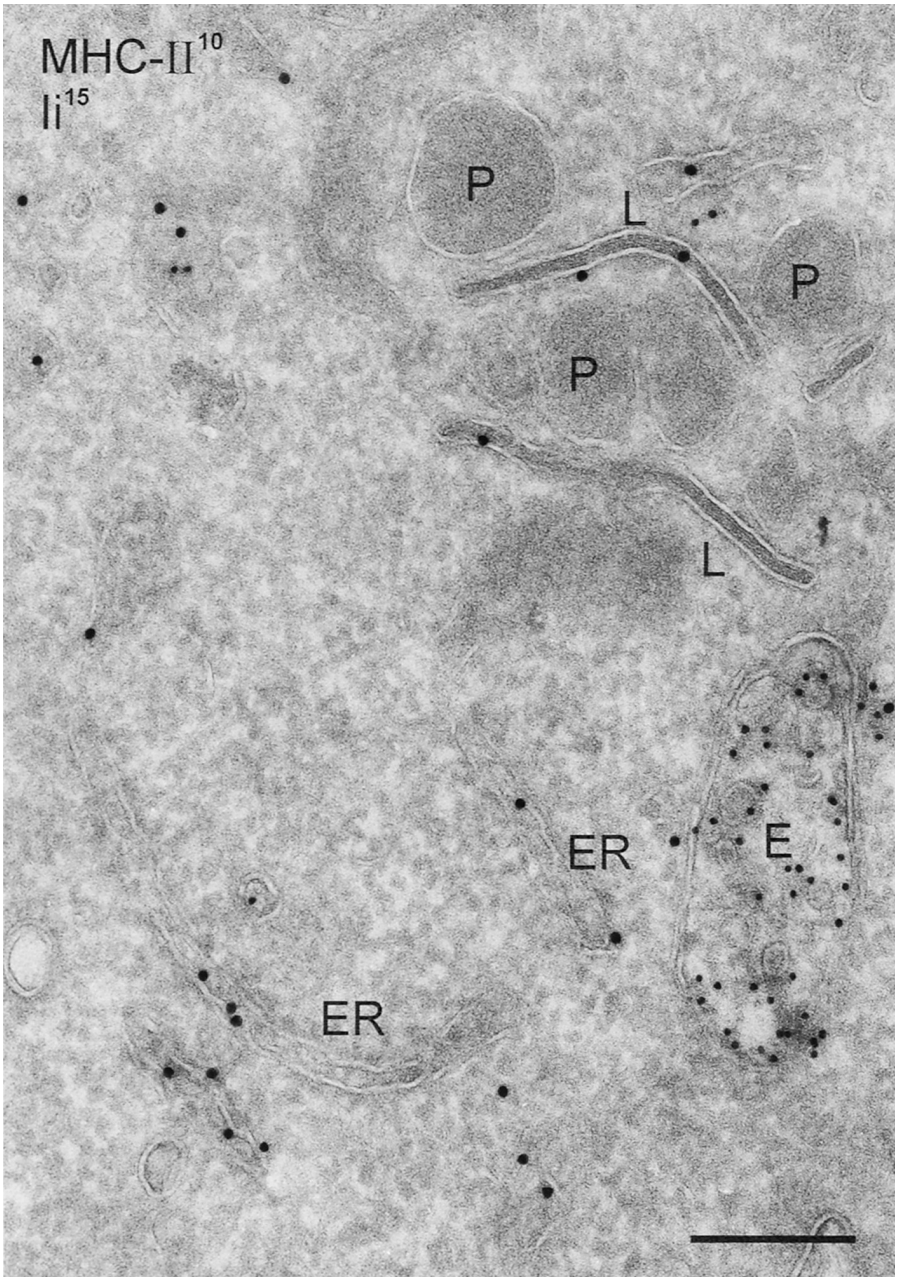

Figure 2: Illustration of spill-over of invariant chain from the ER into the peroxisomal compartment. Cryosection of a mouse dendritic cell double immunogold labeled for MHC class II with 10 nm gold, and invariant chain (Ii) with 15 nm gold as indicated on the figure. Ii is a chaperone of MHC class II and abundantly present in the ER of dendritic cells. MHC class II can be seen in a multivesicular endosome (E), also called MIIC in these cells, while Ii is present in the ER as expected, and in lower amounts in lamellae (L) that are in close association with peroxisomes (P). Bar, 200 nm.

specialized ER, lamellae and mature globular/reticulate peroxisomes.

The Past and the Present

Here, we address a basic question in cell biology: are peroxisomes autonomously multiplying organelles or are they derived from another cellular compartment? In trying to answer this question, it is of interest to start from a historical perspective. Cell growth and multiplication are dependent on the continuous production of additional macromolecular constituents, ranging from DNA, protein complexes to complete organelles. This raises the general question: how do cells make more of the same? In the case of DNA the answer is simple. The nucleotide building blocks are assembled on a DNA template strand according to the rules of complementary base-pairing. When we define an organelle as a space bounded by a lipid bilayer membrane containing (an) integral membrane protein(s), the answer to the question: how to make more of the same organelle, is less straightforward. After all, notions such as template and the complementarity principle are not immediately obvious in organelle multiplication. Summarizing a fruitful period of organelle research, G. Palade concluded in 1983: 'In all cellular systems so far investigated, new membrane components – lipids as well as

proteins – are inserted into pre-existing membranes. At present, there is no record of any type of membrane lost during cell division and regenerated post-mitosis in any daughter cell' (24). In other words, in 1983 there were no grounds for a concept of *de novo* formation of organelles. In this respect, peroxisomes are a case in point.

In a number of pex mutants (in which a peroxin function is lost), no traces of peroxisomes can be detected. Yet, after introduction of the gene coding for the corresponding wild-type peroxin, peroxisomes are regained within a rather short time-span, despite the fact that mutant cells were cultivated for many generations without peroxisomes. This observation is difficult to reconcile with autonomously multiplying organelles, and the challenge is to determine the source of these new peroxisomes. Putting aside (according to Palade's rule) the possibility that they arise *de novo,* they must be derived from pre-existing structures. And if they arise from pre-existing structures, what is their nature?

A difficulty in this dilemma is that the concept of autonomous multiplication of peroxisomes rests on solid grounds. Based on a survey of the literature, Lazarow and Fujiki formulated in 1985 the concept that peroxisomes were autonomous organelles multiplying by growth and division (1). Important evidence for this was the observation that peroxisomal proteins were synthesized on free polyribosomes and post-translationally imported into the organelles. Although these observations were based on matrix proteins and only one integral membrane protein known at the time, this situation has not changed now that more examples of integral membrane proteins have been characterized. The original concept was reinforced by the discovery of peroxisomal targeting signals (PTS) for matrix proteins (PTS1 and PTS2) and integral membrane proteins (mPTS) that support their post-translational trafficking to peroxisomes with the help of cytosolic factors. The only enigma still remaining in this concept is how lipids reach the peroxisomes, allowing enlargement of the peroxisomal membrane and subsequent fission upon multiplication. Despite the consistency and beauty of the autonomous multiplication model, heretical results obtained in recent years have led researchers in the field to seriously consider a contribution of the ER to peroxisome formation (as mentioned above).

Recent support for an ER-peroxisome connection came from an unexpected biological source: mouse dendritic cells (22). Based on the characterization of peroxisome formation in these cells and preliminary work in *S. cerevisiae* (D. Hoepfner, unpublished experiments), we present the following model (Figure 3). A limited number of integral membrane proteins reach the ER and contribute

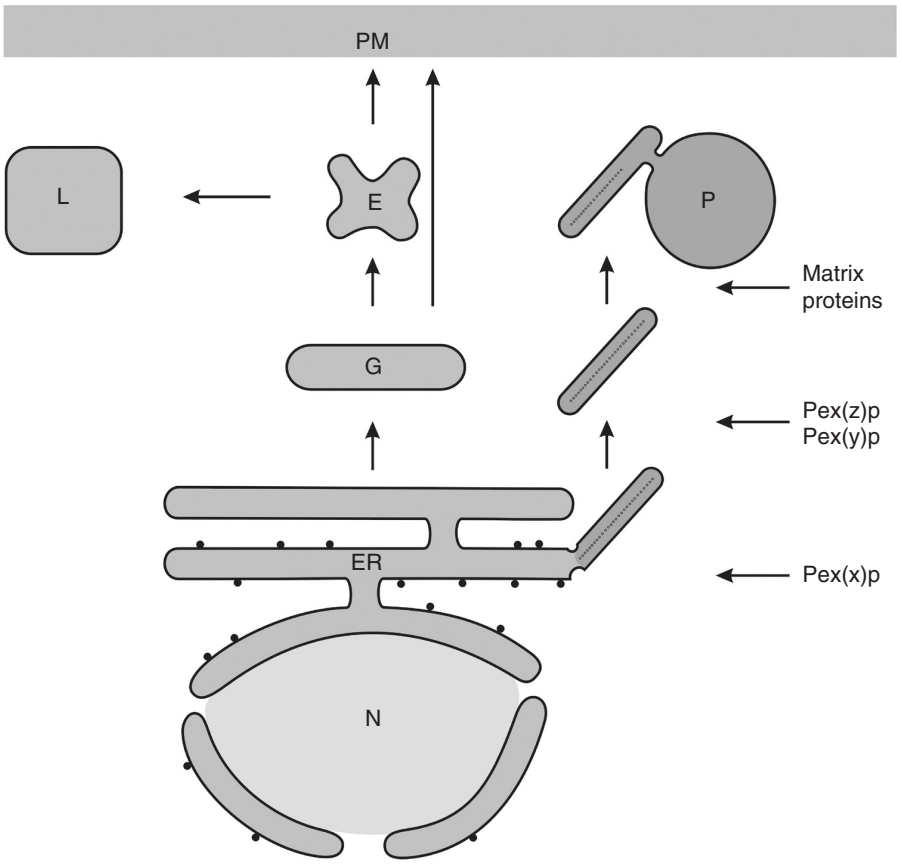

Figure 3: Biosynthetic membrane and protein traffic in the vacuolar compartment. A few integral membrane proteins [Pex(x)p] enter the ER and give rise to the formation of the specialized ER. Insertion of additional proteins [Pex(y)p] results in the build-up of a competent protein import machinery. At that moment matrix proteins start to be imported and lamellae give rise to globular, mature peroxisomes. Membrane proteins with a function in metabolism insert into the lamellar sheets once the maturation pathway is underway [Pex(z)p]. PM, plasma membrane; L, lysosome; E, endosome, P, peroxisome; G, Golgi; ER, endoplasmic reticulum; N, nucleus.

to the formation of a specialized extension from the rough ER [Pex(x)p]. Apart from Pex13p, which we found in the specialized ER, other candidates for this group are Pex3p, Pex16p and Pex19p. In Δpex3 and Δpex16 cells no residual peroxisomes can be detected, and Pex19p has been shown to recognize mPTSs and has been implicated in integral membrane protein delivery. After reaching a considerable size compared to, for instance, budding vesicles of the secretory route, this specialized ER is severed from the rough ER. Candidate proteins to operate in this severing process are the dynamins: yeast Vps1p (25) and human Dlp1p (26,27). Additional integral membrane and peripheral proteins are recruited [Pex(y)p] until the protein import machinery has been assembled and the import of matrix proteins can start as the last step in the peroxisome maturation pathway.

The first appearance of PMP70 in lamellae suggests that integral membrane proteins that play a role in peroxisomal metabolism, such as transporters of small molecules, also show up early in peroxisome development [Pex(z)p]. Finally, it is remarkable that the mature peroxisomes seem to balloon out of the lamellar structures rather than the lamellae themselves being filled up with matrix proteins. The neck regions connecting globular peroxisomes and lamellae may be possible targets for severing by a dynamin-related protein. In this respect, older morphological observations obtained by freeze-fracture electron-microscopy which show catalase-containing vesicles budding off from sheet-like structures are also of interest (28).

The attractiveness of this scenario is that it leaves much of the original autonomous multiplication model intact, and in addition explains how peroxisomes obtain their membranes: i.e. from the ER. A crucial aspect of the model that still remains unexplained is how the first proteins that are responsible for the formation of the specialized ER reach the ER. It is important to note in this respect that Pex13p is an integral membrane protein with two membrane-spanning regions. Therefore, although ER membrane demarcation for peroxisome formation could start with peripherally associated proteins, at a certain stage insertion of proteins into the ER membrane is required too. Attempts to show the involvement of the ER protein import machinery in peroxisome formation in yeast using cold-sensitive sec61 mutants or an ssh1 mutant have met with failure, thus far (29). Here, we are still faced with an important, unexplained feature of our proposal. Also, studies aimed at demonstrating the involvement of components needed for vesicle formation from the ER in the secretory route (Sar1, COPI and COPII) were not successful. This might be understandable in the light of our new observations. The removal of large parts of ER that are dedicated to peroxisome formation probably requires a completely different set of components than those required for the formation of the small, size-defined secretory vesicles.

This new model of peroxisome formation can explain why mutant cells can recruit new peroxisomes on short notice when the wild-type gene is reintroduced into the mutant cell. Moreover, the model builds nicely onto the views formulated in 1983 by G. Palade (24). One or a few integral membrane proteins can prime an ER membrane to become a template for the development of a distinct organelle. These initial membrane proteins recruit by specific protein–protein interactions (the complementarity principle) the remainder of the proteins that characterize a mature peroxisome in a dynamic process of self-assembly. In this view, peroxisomes are a final product of a membrane-sorting route that starts in the ER. It is in essence not different from the pathway that leads to formation of lysosomes whose origin can also be traced to the ER.

We wish to emphasize that the concept of a contribution of the ER to peroxisome formation was voiced already more than 30 years ago by Phyllis and Alex Novikoff (30), pioneers in the period of organelle characterization based on electronmicroscopic pictures taken of intestinal cells from the rat and guinea pig. It exemplifies once more that innovative concepts are not always accepted at the time they are first put forward. Finally, we believe that proposals suggesting that the evolutionary origin of peroxisomes goes back to an early endosymbiont are open to reconsideration in view of our findings.

Acknowledgments

We would like to thank Bruno M. Humbel, Abraham J. Koster and Arie J. Verkleij (Laboratory of Cell Biology, University Utrecht) for their excellent help and support in preparing 3-D reconstructions using electron tomography.

References

1. Lazarow PB, Fujiki Y. Biogenesis of peroxisomes. Annu Rev Cell Biol 1985;1:489–530.
2. Purdue PE, Lazarow PB. Peroxisome biogenesis. Annu Rev Cell Dev Biol 2001;17:701–752.
3. Dyer JM, McNew JA, Goodman JM. The sorting sequence of the peroxisomal integral membrane protein PMP47 is contained within a short hydrophilic loop. J Cell Biol 1996;133:269–280.
4. Sacksteder KA, Jones JM, South ST, Li Y, Gould SJ. PEX19 binds multiple peroxisomal membrane proteins, is predominantly cytoplasmic, and is required for peroxisome membrane synthesis. Proc Natl Acad Sci USA 2000;148:931–944.
5. Heinemann P, Just WW. Peroxisomal protein import: *in vivo* evidence for a novel translocation competent compartment. FEBS Lett 1992;300:179–182.
6. Erdmann R, Veenhuis M, Kunau WH. Peroxisomes: organelles at the crossroads. Trends Cell Biol 1997;7:400–407.
7. Titorenko VI, Rachubinski RA. Dynamics of peroxisome assembly and function. Trends Cell Biol 2001;11:22–29.
8. Mullen RT, Robb Flynn C, Trelease RN. How are peroxisomes formed? The role of the endoplasmic reticulum and peroxins. Trends Plant Sci 2001;6:256–261.

9. Tabak HF, Braakman I, Distel B. Peroxisomes: simple in function but complex in maintenance. Trends Cell Biol 1999;9:447–453.
10. Veenhuis M, Van der Klei IJ. Peroxisomes: surprisingly versatile organelles. 2002;1555:44–47.
11. Gould SJ, Valle D. Peroxisome biogenesis disorders. genetics and cell biology. Trends Genet 2000;16:340–345.
12. Solomos FA, Van der Klei IJ, Kram AM, Harder W, Veenhuis M. Brefeldin A interferes with peroxisomal protein sorting in the yeast *Hansenula polymorpha*. FEBS Lett 1997;411:133–139.
13. South ST, Gould SJ. Peroxisome synthesis in the absence of pre-existing peroxisomes. J Cell Biol 1999;144:255–266.
14. Passreiter M, Anton M, Lay D, Frank R, Harter C, Wieland FT, Gorgas K, Just WW. Peroxisome biogenesis: involvement of ARF and coatomer. J Cell Biol 1998;141:373–383.
15. South ST, Sacksteder KA, Li XL, Liu Y, Gould SJ. Inhibitors of COPI and COPII do not block PEX3-mediated peroxisome synthesis. J Cell Biol 2000;149:1345–1359.
16. Voorn-Brouwer T, Kragt A, Tabak HF, Distel B. Peroxisomal membrane proteins are properly targeted to peroxisomes in the absence of COPI- and COPII-mediated vesicular transport. J Cell Sci 2001;114: 2199–2204.
17. Mullen RT, Lisenbee CS, Miernyk JA, Trelease RN. Peroxisoma membrane ascorbate peroxidase is sorted to a membranous network that resembles a subdomain of the endoplasmic reticulum. Plant Cell 1999;11:2167–2185.
18. Faber KN, Haan GJ, Baerends RJ, Kram AM, Veenhuis M. Normal peroxisome development from vesicles induced by truncated *Hansenula polymorpha* Pex3p. J Biol Chem 2002;277: 11026–11033.
19. Bascom RA, Chan H, Rachubinski RA. Peroxisome biogenesis occurs in an unsynchronized manner in close association with the endoplasmic reticulum in temperature-sensitive *Yarrowia lipolytica* Pex3p mutants. Mol Biol Cell 2003;14:939–957.
20. Titorenko VI, Ogrydziak DM, Rachubinski RA. Four distinct secretory pathways serve protein secretion, cell surface growth, and peroxisome biogenesis in the yeast *Yarrowia lipolytica*. Mol Cell Biol 1997;17: 5210–5226.
21. Titorenko VI, Rachubinski RA. The life cycle of the peroxisome. Nat Rev Mol Cell Biol 2001;2:357–368.
22. Geuze HJ, Murk JL, Stroobants AK, Griffith JM, Kleijmeer MJ, Koster AJ, Verkleij AJ, Distel B, Tabak HF. Involvement of the endoplasmic reticulum in peroxisome formation. Mol Biol Cell 2003;in press.
23. Kleijmeer MJ, Morkowski S, Griffith JM, Rudensky AY, Geuze HJ. Major histocompatibility complex class II compartments in human and mouse B lymphocytes represent conventional endocytic compartments. J Cell Biol 1997;139:639–649.
24. Palade GE. Membrane biogenesis: an overview. Meth Enzymol 1983;96:xxix–lv.
25. Hoepfner D, Van den Berg M, Philippsen P, Tabak HF, Hettema EH. A role for Vps1p, actin and the Myo2p motor in peroxisome abundance and inheritance in *Saccharomyces cerevisiae*. J Cell Biol 2001;155:979–990.
26. Koch A, Thiemann M, Grabenbauer M, Yoon Y, McNiven A, Schrader M. The dynamin-like prottein DLP1 is involved in peroxisomal fission. J Biol Chem 2003;278:8597–8605.
27. Li X, Gould SJ. The dynamin-like GTPase DLP1 is essential for peroxisome division and is recruited to peroxisomes in part by PEX11. J Biol Chem 2003;278:17012–17020.
28. Ohno S, Fujii Y. Three-dimensional and histochemical studies of peroxisomes in cultured hepatocytes by quick-freezing and deep-etching method. Histochem J 1990;22:143–154.
29. South ST, Baumgart E, Gould SJ. Inactivation of the endoplasmic reticulum protein translocation factor, Se61p, or its homolog, Ssh1p, does not affect peroxisome biogenesis. Proc Natl Acad Sci USA 2001;98:12027–12031.
30. Novikoff PM, Novikoff AB. Peroxisomes in absorptive cells of mammalian intestine. J Cell Biol 1972;53:532–560.