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Review

No peroxisome is an island — Peroxisome contact sites☆

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ABSTRACT

In order to optimize their multiple cellular functions, peroxisomes must collaborate and communicate with the surrounding organelles. A common way of communication between organelles is through physical membrane contact sites where membranes of two organelles are tethered, facilitating exchange of small molecules and intracellular signaling. In addition contact sites are important for controlling processes such as metabolism, organelle trafficking, inheritance and division. How peroxisomes rely on contact sites for their various cellular activities is only recently starting to be appreciated and explored and the extent of peroxisomal communication, their contact sites and their functions are less characterized. In this review we summarize the identified peroxisomal contact sites, their tethering complexes and their potential physiological roles. Additionally, we highlight some of the preliminary evidence that exists in the field for unexplored peroxisomal contact sites.
This article is part of a Special Issue entitled: Peroxisomes edited by Ralf Erdmann.

1. Organelle communication through contact sites

The hallmark of eukaryotic cells is compartmentalization of cellular domains into membrane-bound organelles. Organelles allow the segregation and regulation of multiple parallel processes that require unique conditions; however they cannot function in isolation. In order to promote the well-being of the cell, organelles must work in harmony with each other, exchanging information and products to coordinate cellular functions.

Numerous mechanisms have evolved to enable cross-talk between organelles including signal transduction pathways and vesicular trafficking. However, in the past few years it is becoming apparent that a common way of communication between organelles is through membrane contact sites where membranes of two organelles are tethered, facilitating close-range interactions such as transport of small molecules [1,2].

Importantly, not all membranes that come into close proximity form contact sites. True contacts are established and maintained in durable or transient states by tethering structures, which keep the two membranes in proximity while disenabling fusion. Tethering structures can form by proteins on the opposing membranes or by protein–lipid interactions. Moreover, contact sites form unique domains that harbor a defined membrane composition and are enriched with specific proteins to optimize their function [3].

2. Cellular functions of contact sites

What are the functions of contact sites? A diversity of processes has been demonstrated to rely on the formation of contact sites — some of which have been studied more extensively than others:

2.1. Arrangement of the cellular landscape

Contact sites may promote an organization such that organelles are tethered to each other, creating a dynamic but controlled map of the cell. This architecture may enable efficient targeting of molecules to organelles as well as optimize many biosynthetic pathways and responses that utilize more than one organelle to function.

2.2. Exchange of molecules

One advantage of close proximity between two organelles is the ability to exchange various molecules without relying on time-consuming extensive diffusion distances through a chaotic cytosol. Indeed, the two most studied functions of contact sites are the exchange of lipids and Ca^{2+} (reviewed in [2,4]). Notably, a recent study revealed that channels for ions other than Ca^{2+} and additional small molecules are enriched in a contact site formed between mitochondria and vacuoles (yeast lysosome) suggesting that the transfer of many additional molecules may benefit from direct contact between organelles [5].

2.3. Organelle inheritance and trafficking

Most organelles cannot form *de novo* and hence inheritance of organelles is an essential and regulated process in which contact sites

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seem to play a major role [6–10]. Dynamics of organelle movements, for other needs than inheritance, may also rely on contact sites.

2.4. Organelle fission and division

A new and unexpected role for contact sites between the endoplasmic reticulum (ER) and other organelles was recently demonstrated: The site of contact between the ER tubules and mitochondria or endosomes [11,12] marks the point in which fission of the organelles will occur. It would be intriguing to discover whether other fission events are also regulated by contacts with ER tubules.

3. Mapping peroxisome contact sites

Despite the growing body of work on contact sites and their obvious importance in coordinating organelle functions, there is still little known about even the most well-studied contacts. Some contacts have been poorly studied and some contacts have yet to be identified. Most contacts studied to date focus on those that involve one of the two biggest organelles — the ER and mitochondria. However, this review will focus on contacts formed by the peroxisome — a highly diverse and important organelle.

Peroxisomes are single-membrane-enclosed organelles that are found in almost all eukaryotes and participate in central pathways of cellular metabolism such as β-oxidation of fatty acids, amino acid catabolism and detoxification of reactive oxygen species (ROS). Peroxisomes are remarkably diverse in size, number, and the enzymes that they contain. This diversity depends on the cell type and environment and can be rapidly regulated in response to metabolic signals [13]. Like any other organelle peroxisomes must collaborate with their surroundings. Unraveling the communication of peroxisomes with the rest of the cell will enable a new level of understanding of the biogenesis, division and function of peroxisomes.

For many years electron microscopy (EM) images of peroxisomes from fungi, plants and mammals, have demonstrated that peroxisomal membranes are juxtaposed to other organelles, mainly the ER, plasma membrane (PM), lipid droplets (LDs), chloroplasts and mitochondria (reviewed in [14]) suggesting that contact sites form between these organelles. Indeed, in recent years several contact sites of peroxisomes were identified in different organisms and their functions have started to be explored. In this review we will present the known contact sites of peroxisomes, and when known, discuss their tethering proteins and functions. Additionally, we will discuss new possibilities of the cross-talk between peroxisomes and the rest of the cell.

4. Peroxisome — ER contact sites

For many years it has been known that peroxisomes can be found in close proximity to the ER. In fact EM images not only showed that these organelles are adjacent to each other, but also demonstrated that the ER membrane can wrap around peroxisomes [15–18]. Over the years several functions have been suggested for the close proximity between the two organelles including peroxisome maturation, proliferation, inheritance, dynamics and transfer of molecules.

4.1. Function

4.1.1. Maturation and proliferation

Peroxisomes can either be formed *de novo* from the ER or by fission of pre-existing peroxisomes [19–22]. Despite the fact that ER contacts have been shown to play an important role in fission of other organelles, currently for neither pathway is there evidence for a role of contact sites. Regardless of the mechanism of biogenesis, young peroxisomes, as well as pre-fission peroxisomes, would require a maturation step in which they are supplied with vital proteins and lipids as peroxisomes are lacking the enzymes that synthesize membrane lipids [23]. One way by which such molecules can be provided may be through vesicles demonstrated to arrive from the ER [24,25]. However, a non-vesicular transfer of ER-derived phospholipids to peroxisomes has also been described [26]. This pathway was suggested to be bidirectional and therefore is likely to provide a mechanism for the cell to rapidly regulate the amount and composition of lipids in peroxisomal membranes. Such alterations may modify the organelle's physical properties thereby supporting membrane bending or elongation during peroxisome growth and division. The fact that this transport is rapid and efficient reinforces the conjecture that it is occurring in peroxisome-ER contacts, despite the fact that specific proteins that transfer lipids to peroxisomes have not been described thus far [3,27,28].

Peroxisome-ER contact sites were suggested to have a role in controlling peroxisome proliferation in the yeast *Saccharomyces cerevisiae*. During peroxisome proliferation, Pex30 is localized primarily to ER-peroxisome contacts [29,30] where it becomes part of a complex with the ER reticulons, Rtn1, Rtn2 and Yop1, which maintain the tubular morphology of the ER (Fig. 1B). Interestingly, in cells lacking the ER reticulons or Pex30 the formation of peroxisomes is accelerated suggesting that this complex negatively regulates proliferation. Studies in the yeast *Pichia pastoris* support these findings by demonstrating that Pex30 is localized mostly to the ER interface [30]. Despite these studies suggesting that Pex30 facilitates the connection between peroxisomes and the ER, its role as a tether has not yet been proven.

4.1.2. Inheritance

During *S. cerevisiae* budding some peroxisomes remain in the mother cell while others segregate to the newly formed daughter bud. This regulated inheritance is mediated by two complexes acting at ER-peroxisome interfaces: One that allows retention of peroxisomes to the mother cell and another that promotes their segregation to the bud.

The tethering complex that is suggested to control peroxisome inheritance consists of Pex3, an integral membrane protein that resides in both peroxisomes and the ER, and the peroxisome inheritance factor, Inp1. While Pex3 provides a membrane anchor for the tether, Inp1 bridges the two compartments by acting as a molecular hinge between ER-bound Pex3 and peroxisomal Pex3. It was suggested that this tethering keeps specific peroxisomes in mother cells. Indeed, in cells lacking the ER-peroxisome tether, peroxisomes accumulate in daughter cells. Conversely, peroxisomes that are enriched in Inp2, an adaptor that connects peroxisomes to microtubules via Myo2 (class V myosin motor protein 2), are actively recruited to the daughter cell (Fig. 1A). Thus, tethering to the ER plays a critical role in selective inheritance and control of the peroxisomal population [10].

4.1.3. Dynamics

A correlation between the dynamic behavior of peroxisomes and the neighboring ER has been demonstrated as peroxisomes align with and follow the dynamic movement of adjacent ER tubules. This suggests that the two organelles are indeed connected, possibly through contact sites [31]. An example of such a contact was demonstrated to enable dynamics of peroxisomes in *Arabidopsis thaliana* plants specifically under oxidative stress [32].

4.1.4. Transfer of molecules

The peroxisome-ER cross-talk is vital for many lipid-related metabolic pathways, including the biosynthesis of ether-phospholipids, production of polyunsaturated fatty acids, cholesterol, bile acids and isoprenoids (Reviewed in [14]). For these products to be biosynthesized efficiently the ER and peroxisomes must exchange key enzymes and metabolites. Although it is not yet clear if the two organelles must be tethered together, or even adjacent, for this to occur, the existence of a contact site would definitely facilitate the efficient exchange of molecules.

In summary, although several tethering complexes have already been studied, the variety of functions that require contacts between

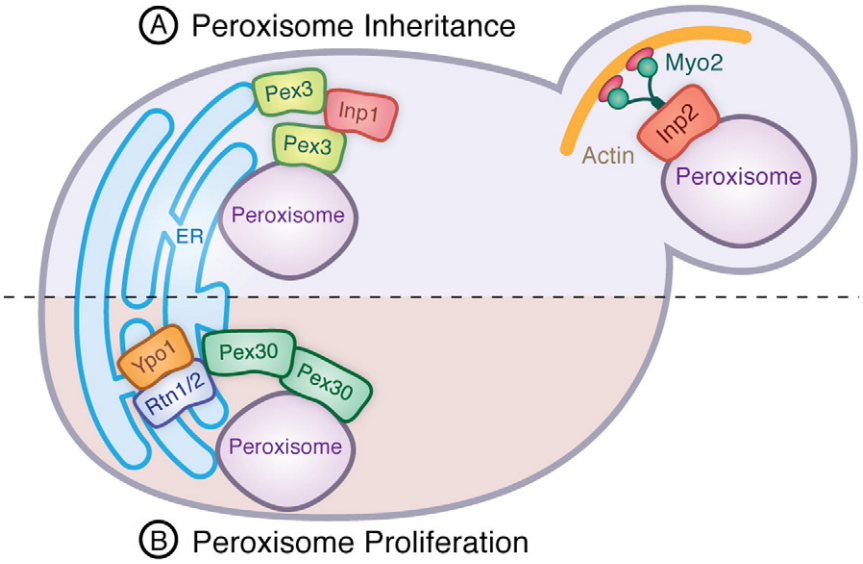

Fig. 1. Peroxisome–ER contact site. Peroxisomes have been shown to create two types of contact sites with the endoplasmic reticulum (ER): (A) Contacts required for regulated inheritance: in the budding yeast, to enable a fraction of peroxisomes to be actively retained in the mother cell, they are anchored to the cortical ER by a tethering complex consisting of ER-bound Pex3 and peroxisomal Pex3 connecting through the cytosolic Inp1. To enable active inheritance of a certain subpopulation into the bud, peroxisomes that harbor Inp2 interact with the myosin motor Myo2 triggering movement of the peroxisome along actin cables directionally to the bud. (B) Contacts required for proliferation: in the budding yeast, the membrane protein Pex30 interacts with the reticulon homology proteins Rtn1, Rtn2 and Yop1. The Pex30 protein complex acts as a hub for the regulation of peroxisome proliferation and movement.

the two organelles suggest that perhaps more functions and specific tethers exist and are yet to be discovered. It is also intriguing to ask if the identified contacts between peroxisomes and the ER are localized to the same position along the ER and if so, are they part of the same contact site or do many differentially regulated ER-peroxisome contact sites exist?

5. Peroxisome — mitochondria contact sites

Over the years, substantial evidence has been provided for peroxisomes and mitochondria having a co-dependent relationship and exhibiting functional interplay [14]. The metabolic cooperation of peroxisomes and mitochondria in β-oxidation of fatty acids [33] and detoxification of ROS [34,35] are the best studied examples of this cross talk. Remarkably, peroxisomes and mitochondria also share key proteins of their division machinery, which suggests coordinated division under certain conditions and demands organized targeting and recruitment of those proteins [36,37]. Furthermore, peroxisomes and mitochondria co-operate in anti-viral signaling and defense [38,39] and it is well documented that mitochondria are defective in several peroxisomal disorders [40]. Despite the tight relationship, the mechanisms of communication between the two organelles are still elusive, but diffusion processes, vesicular transport, and physical contact sites have all been implicated [41–43]. Recent studies have started to shed light on possible contacts between peroxisomes and mitochondria. It was observed that in the yeast *S. cerevisiae* peroxisomes can be localized adjacent to a specific mitochondrial niche in which the ER-mitochondria contact occurs [44]. The proximity to the ER-mitochondria contacts may suggest that a three-way junction is occurring. How such a three way junction forms is unclear. Potentially it could suggest that each organelle could be tethered to the two others by either the same or different tethering structures. This possibility enables bi-directional transfer of molecules between all the three organelles simultaneously. However another possibility for a three-way junction formation is that one organelle is placed (sandwiched) between the other two. In that case the middle organelle acts as a "bridge" between the two other organelles. The understanding of the structure and function of this three-way ER-mitochondria-peroxisome contact site can have a huge impact on the way we think of contact sites.

5.1. Tether

It was recently suggested that in yeast Pex11, a key protein involved in peroxisomes proliferation, and Mdm34, one of proteins creating the ER-Mitochondria tether, interact with each other and might serve as the peroxisomes-mitochondria tether [45] (Fig. 2). Another tether that was suggested in mammals is the ATP binding cassette (ABC) transporter *ABCD1* which is located in the peroxisomal membrane. It was hypothesized that this protein facilitates the interaction between peroxisomes and mitochondria, and that it is the loss of this interaction that causes the X-linked adrenoleukodystrophy (X-ALD) associated with loss of the ABCD1 gene [46]. However further work should be done to verify the identity of the tether between the organelles.

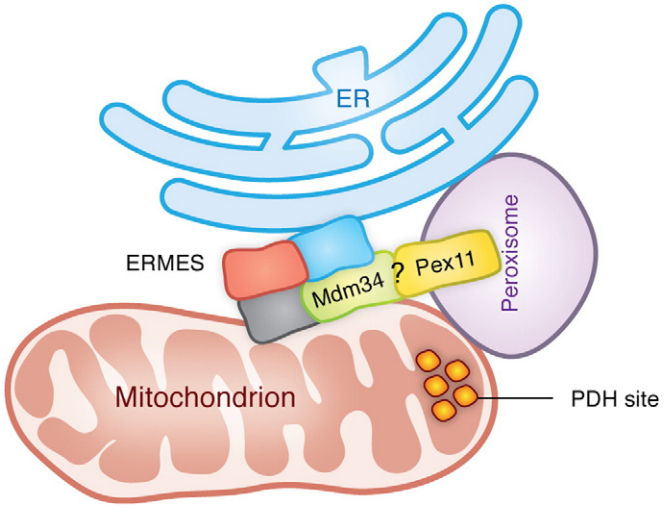

Fig. 2. Peroxisome–mitochondria contact site. Peroxisomes can be localized adjacent to a specific mitochondrial niche near the ER-mitochondria contact site proximal to where the pyruvate dehydrogenase (PDH) complex is found in the mitochondria matrix. The proximity to the ER-mitochondria contacts may suggest a function of a three way organelle junction. The peroxisome-mitochondria tether has been suggested to be mediated by the interaction between Pex11, a key protein involved in peroxisome proliferation, and Mdm34, one of the proteins creating the ER-mitochondria tether (ERMES).

5.2. Function

5.2.1. Fission and organelle turnover

A three-way junction may coordinate a more complex pathway or high-order co-regulation of fission for peroxisomes and mitochondria by the ER, especially given that peroxisomes and mitochondria share some of the fission machinery proteins. In yeast the degradation of peroxisomes by pexophagy (peroxisomal autophagy) and the degradation of mitochondria by mitophagy (mitochondrial autophagy), depends on division [47]. Intriguingly, the scaffold protein Atg11 recruits the dynamin related GTPase, Dnm1 containing fission machinery to the organelle destined for degradation and functions at mitochondria–peroxisome contacts [47].

5.2.2. Shared metabolic pathways

Peroxisomes and mitochondria crosstalk is essential for numerous metabolic pathways that include the detoxification of glyoxylate and phytanic acid α-oxidation [48]. However, the coordinated cooperation of the organelles in the β-oxidation of fatty acids is perhaps the best known example for crosstalk. In animals, both peroxisomes and mitochondria cooperate in the degradation of fatty acids. This is different in some yeast species and plants, where fatty acid β-oxidation is thought to be solely peroxisomal [49]. Although the biochemical steps of fatty acid β-oxidation in both organelles are similar, each organelle harbors its specific set of substrate specificity enzymes. Furthermore, peroxisomal β-oxidation generates only chain-shortened fatty acids, and unlike mitochondria, does not result in complete degradation of fatty acids. The medium chain fatty acids obtained in peroxisomes as well as acetyl-CoA are routed to mitochondria for further oxidation and ATP production in the tricarboxylic acid (TCA) cycle. It is still not clear how molecules are exchanged between peroxisomes and mitochondria however it has been shown to involve shuttle mechanisms such as the carnitine system and membrane pores [41], vesicular trafficking [43,50], and it might be also facilitated by contacts between the organelles. Interestingly, in yeast, peroxisomes have been found to localize proximal to areas of the mitochondrial matrix where the pyruvate dehydrogenase complex is enriched [44]. The proximity to the pyruvate dehydrogenase complex may serve to either provide an alternate acetyl-CoA source for mitochondria during reduced cytosolic glycolysis (stationary phase) or to concentrate acetyl-CoA in a specific cellular spot that can be used for example to maximize entry into the TCA cycle or to build new phospho/sphingo-lipids.

The functional interplay of peroxisomes and mitochondria in different metabolic pathways as well as their oxidation-reduction (redox)-sensitive cross-talk highlight the need for uncovering the mechanisms that governs the close communication between the organelles. Future studies will help to understand the relationship between these organelles in many peroxisomal disorders.

6. Peroxisome — chloroplast contact sites

Plant peroxisomes have been intensely studied for their involvement in lipid mobilization, photorespiration, redox metabolism and hormone biosynthesis. For all of these functions, peroxisomes cooperate with chloroplasts and mitochondria [51]. EM images have shown that peroxisomes are located in close proximity to chloroplasts and mitochondria in green cotyledon cells [52,53]. Additionally, peroxisomes and chloroplasts segregate together when subjected to density centrifugation [54]. Interestingly, even in *A. thaliana chup* (chloroplast unusual positioning) mutants, where chloroplasts are mislocalized and lose their coordinated movement with other organelles (e.g. mitochondria), they remain proximal to peroxisomes [55] suggesting a very tight association between these two organelles.

6.1. Tether

Functional photorespiration requires the localization of matrix enzymes within peroxisomes as well as the association between peroxisomes, chloroplasts, and mitochondria to enable the metabolite flow between these organelles. One mechanistic suggestion as to how *A. thaliana* peroxisomes physically associate with the outer membrane of the chloroplast envelope is by the Zn RING finger of Pex10 (Fig. 3). Inactivation of the Pex10 RING finger domain reduces the peroxisomes-chloroplast interaction and eliminates transfer of metabolites between the organelles [56]. However, the involvement of Pex10 as an actual tether structure remains to be proven.

Recently a light-dependent regulation of organelle contacts has been shown whereby upon exposure to light, peroxisomes became more elliptically shaped in order to increase the area and strength of their interaction with chloroplasts [57]. These results suggest the presence of a light dependent regulatory mechanism, which promotes and maintains an interaction between peroxisomes and chloroplasts via dynamic morphological changes of peroxisomes (Fig. 3). Interestingly, when photosynthesis inhibitors or mutants defected in electron transport were used (but not when photoreceptors or photorespiration were inhibited), peroxisomes remained spherical and showed reduced interaction with chloroplasts even when exposed to light. Moreover, it was observed that mitochondria actively participate in the interaction between peroxisomes and chloroplasts, and many joined three-way organelle complexes were observed. Mitochondria interacting with peroxisomes and chloroplasts were elongated in the light but spherical in the dark, similarly to peroxisomes, and the number of three-organelle complexes was larger in light conditions. This is highly reminiscent of the ER-Mitochondria-Peroxisome three-way contact observed in yeast and suggests that maybe such interactions are more prevalent than have previously been appreciated.

6.2. Function

6.2.1. Photosynthesis and photorespiration

The fact that chloroplast–peroxisome contacts are regulated by light suggests that the peroxisome–mitochondria–chloroplast three way junction serves to exchange photosynthesis products [58]. Since leaf peroxisomes interact with chloroplasts and mitochondria during photorespiration, the relationship between these organelles was proposed to represent a requirement for efficient shuttling of metabolites through the photorespiratory pathway [59,60].

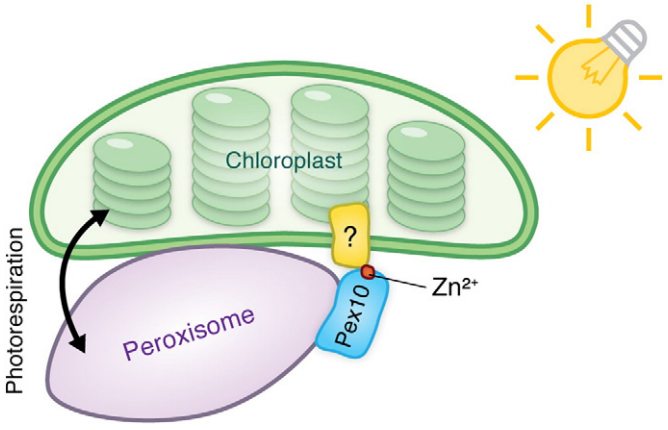

Fig. 3. Peroxisome–chloroplast contact site. In *Arabidopsis thaliana*, upon exposure to light, peroxisomes increase their contact sites with chloroplasts to facilitate the transfer of photorespiration metabolites. During light conditions, peroxisomes also change their morphology to an elliptical shape potentially to increase the area and strength of the contacts. The mechanism for tethering is through the Zn RING finger of Pex10 and an unknown counterpart on the chloroplast.

6.2.2. Division

Similarly to mitochondria peroxisomes share some specific components (e.g. DRP5B) for their division machineries also with chloroplasts [61]. Therefore it is quite possible that contacts between peroxisomes and chloroplasts have more functions that have not been identified yet such as coordinating the division of the two organelles.

7. Peroxisome — lipid droplet contact sites

Eukaryotic cells have the ability to accumulate neutral lipids such as triacylglycerol and cholesterol ester and to store them in oil bodies/LDs. LDs are more than lipid storage containers, and evidence for their complex nature and contribution to multiple cellular functions is accumulating [62]. It was suggested that LDs interact with other organelles, among them, ER, mitochondria, vacuole/lysosomes and peroxisomes to distribute lipids and ensure cellular homeostasis [63]. In support of peroxisome-LD cross-talk it was observed that LDs undergo morphological changes due to peroxisomal malfunction or stress conditions and defects in peroxisomal fatty acid β-oxidation have been linked to enlarged LDs in the nematode *Caenorhabditis elegans* [64]. Moreover changes in the number and size of LDs have been reported in peroxisome-deficient knockout mice [65]. Conversely, peroxisomes and mitochondria have been reported to undergo changes in number and size in morphometric studies after LD depletion in mouse hepatocytes [66]. Compelling evidence for close proximity of peroxisomes and LDs has been presented in yeast, plants and mammalian cells [52, 67,68]. However, the exact nature of the connection between these organelles remains to be uncovered.

7.1. Tether

While several studies have suggested the existence of a contact site between peroxisomes and LDs, it is still not clear what is the tether that mediates the interaction between the two organelles. An interactome map of protein-protein interactions between LDs and peroxisomes has been generated in *S. cerevisiae*. The LD proteins Erg6 and Pet10 were found to interact with several peroxisomal proteins [69]. However, further experiments are required to examine if these proteins serve as tether proteins. Regardless, these findings highlight the close physical interaction between peroxisomes and LDs.

7.2. Function

7.2.1. Lipid transfer

Peroxisomes lack enzymes required for the biosynthesis of their own membrane lipids. Thus, any enlargement of peroxisomes requires the transfer of lipids to their expanding membranes from the donor membranes of other organelles. These donor membranes may be the ER (see the section on Peroxisome-ER contact sites) and/or LDs. For example, in germinated cotton seeds there is a dramatic enlargement of glyoxysomes (specialized plants peroxisomes) and it is LDs that provide the expanding glyoxysome membranes with the bulk of neutral lipids, mostly triacylglycerols, and glycerophospholipids [70]. In addition, under peroxisomal proliferation conditions, it was observed that distinct peroxisome structures accumulate in temperature-sensitive *pex3* mutant of the yeast *Yarrowia lipolytica* and wrap around the surface of LDs [71]. It is thus possible that these represent a contact site responsible for the transfer of lipids between the organelles.

In *S. cerevisiae* it was also reported that an intimate physical contact is created as peroxisomes stably adhere to LDs when cells are grown in oleic acid (where peroxisomes must proliferate) [72] suggesting direct transfer of fatty acids across organellar boundaries. Interestingly, electron microscopy revealed peroxisome protrusions, termed pexopodia, which extend into the core of the LDs. Those protrusions are a result of hemifusion of the single leaflet of the lipid droplet membrane and the outer leaflet of the peroxisome membrane. This enables direct contact of the inner peroxisomal leaflet with the core of the lipid droplet and may facilitate the transfer of fatty acids across the peroxisome monolayer. Surprisingly, pexopodia and LD inclusions are selectively enriched in peroxisomal fatty acid oxidation enzymes. This data suggest that peroxisomal contact may stimulate neutral lipid breakdown in LDs. This peroxisome-LDs interaction may serve to link lipolysis mediated by LDs to fatty acid β-oxidation within the peroxisomes. Furthermore, lipids generated by peroxisomes might move into LDs [72] (Fig. 4). Those findings reinforce the observation that extensive contacts between peroxisomes and LDs allow the efficient conversion of fatty acids to TCA cycle substrates [52]. Interestingly, in spite of the close interaction between peroxisomes and LDs the two organelle are inherited independently [9].

7.2.2. Protein transfer

Notably, lipids are not the only molecules that can be transferred between peroxisomes and LDs. It was observed that in *A. thaliana*, Sugar-Dependent 1 (SDP1), a major TAG lipase involved in lipid reserve mobilization during seedling establishment, is transferred from peroxisome membranes to LD surface through peroxisome tubulations or peroxisome-LDs contacts [73].

The collaboration between peroxisomes and LDs highlights some important questions that will be interesting to answer. Beside the identification of the contact tethers, it seems that this contact is highly regulated. As such, the regulation of the contact may uncover the extent of its role.

8. Peroxisome – peroxisome contact sites

Cells usually harbor more than one peroxisome and these expand in number under conditions where their functions become essential. In many cases where organelles are scattered throughout the cell they create self-contacts to coordinate functions, transfer molecules and facilitate movement, inheritance and growth. Indeed, peroxisome-peroxisome interactions were observed by different methods in various organisms [73–76]. Peroxisomes were shown to be engaged in several transient, but vivid and long term contacts. Within the course of the interaction, peroxisomes separated and re-contacted several times. Some peroxisomes displayed fast, directional movements along microtubules, which resulted in an interaction with another peroxisome before movement resumed [76–78] (Fig. 5A). Although the physiological role of the inter-peroxisomal interactions is unclear, they might signal the cell to ensure that peroxisome populations are stably maintained. Potentially the formation of small peroxisome clusters with close apposition may represent functional units of peroxisomes, which interact and cooperate via contact sites. Notably, it was previously suggested that peroxisome-peroxisome interactions might be a snap-

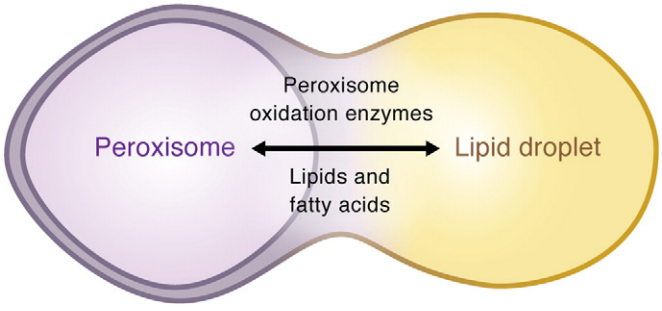

Fig. 4. Peroxisome–lipid droplet contact site. In the budding yeast, peroxisomes stably adhere to lipid droplets (LDs) thereby stimulating the breakdown and transfer of various lipids across these organellar boundaries. Peroxisome protrusions, termed pexopodia, have been seen to invade the LD core as a result of hemifusion of the single leaflet of the lipid droplet membrane and the outer leaflet of the peroxisomal membrane. Pexopodia are believed to facilitate the flux of fatty acids into peroxisomes and the transfer of peroxisomal oxidation enzymes into LDs.

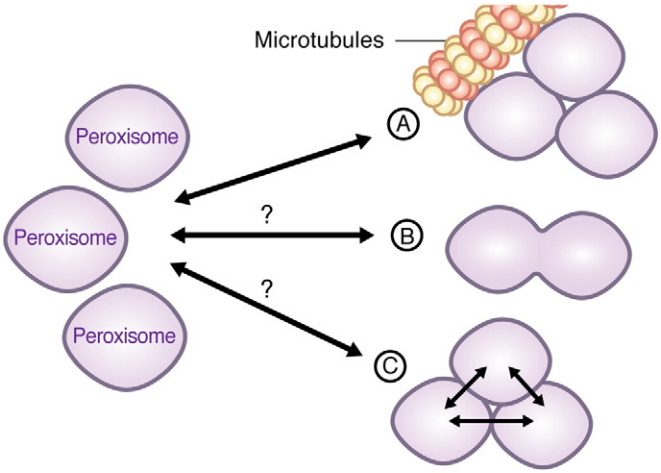

Fig. 5. Peroxisome–peroxisome interaction. In mammalian cells, peroxisomes are engaged in close self-interactions. Although such interactions are transient, peroxisomes are able to re-contact rapidly creating long-term contacts. The interaction between peroxisomes has three possible roles: (A) Movement of peroxisome populations in the cell. (B) Fusion between peroxisomes. (C) Creation of functional units to exchange metabolites or lipids.

shot of a pre-fusion event (which would nullify this interaction as a genuine contact site). Whether peroxisomes can fuse in some cell types or under some conditions is still under debate (Fig. 5B) [19,76,77,79–81]. Currently, the notion that very little fusion occurs supports the close interactions between peroxisomes to be those mediated by contact sites.

8.1. Function

8.1.1. Exchange of metabolites

The close interaction of peroxisomes, potentially forming a contact site, might serve to exchange metabolites and could also contribute to the degradation of hydrogen peroxide (H_2O_2) or other ROS (Fig. 5C). A close interaction might be beneficial for creating a tight knit web to capture all ROS species and prevent leakage. This hypothesis was reinforced by a computational model that provided evidence that a combination of fast, ATP-driven movements of peroxisomes and subsequent formation of close contacts between individual peroxisomes can principally contribute to the homogenization (intermixing) of the peroxisomal compartment on a timescale of one to several hours. However, an increase in heterogeneity among different peroxisome populations by manipulating ROS and fatty acid levels did not promote peroxisome interactions or facilitates the exchange of metabolites such as fatty acids or H_2O_2 [77].

It seems that inter-peroxisomal interactions are more complex than previously assumed and represent a new dynamic behavior of this organelle. Whether the mechanism of inter-peroxisomal interactions is achieved by the creation of tethering complexes is still unclear. If this is the case, uncovering the tether holding the organelles would dramatically propel the field forward.

9. Peroxisome–lysosome contact sites

Recently, lysosomal compartments have begun to be studied in the context of contact sites [2,3] and indeed they seem to also have important roles in the contacts formed with peroxisomes.

9.1. Tether

It was very recently shown that in mammals, the tether holding the two organelles together is the integral lysosomal membrane protein, synaptotagmin VII (Syt7), through binding to the lipid $PI(4,5)P_2$ on the peroxisomal membrane (Fig. 6) [82].

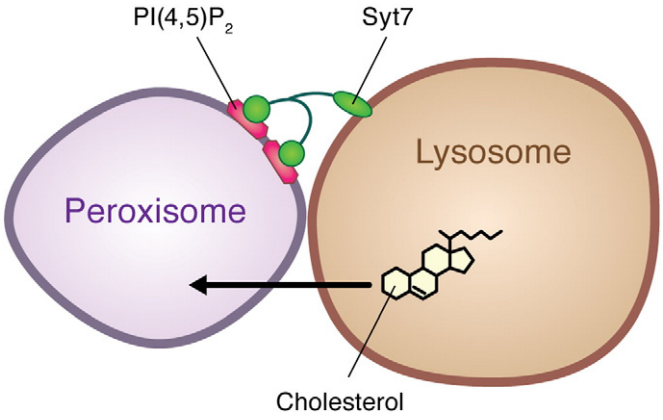

Fig. 6. Peroxisome–lysosome contact site. It was recently identified that a peroxisome–lysosome contact site occurs (termed LPMC) in human cells. The LPMC is dynamic and cholesterol dependent. The tether holding the two organelles together is the integral lysosomal membrane protein, Synaptotagmin VII (Syt7), through binding to the lipid $PI(4,5)P_2$ on the peroxisomal membrane. This contact site facilitates the transfer of cholesterol from lysosomes to peroxisomes.

9.2. Function

9.2.1. Pexophagy

Peroxisomes can be degraded by two main processes, non-selective autophagy or via pexophagy. Those degradation pathways connect peroxisomes to lysosomes as both organelles need to physically interact in order to facilitate the process [83–86].

9.2.2. Cholesterol transport

A new and unexpected role of peroxisome–lysosome interaction in cholesterol transport was recently suggested. The majority of cellular cholesterol (60%–80%) is localized at the PM [87], cholesterol needs to be transported to the PM from the ER, where it is biosynthesized, and from the lysosome, where it is located after exogenous import. The molecular mechanism of cholesterol transfer from the lysosome to the PM is largely uncharacterized. Recently it was shown that peroxisomes play a critical role in the transport of cholesterol from the lysosome to the PM [82]. A peroxisome–lysosome contact site (termed LPMC) was shown to be dynamic and cholesterol dependent (Fig. 6). Notably, efficient formation of the LPMC requires Npc1 (Niemann–Pick type C), that, alongside Npc2, transports free cholesterol out of the lumen to the membrane of the lysosome [88]. Indeed, cells taken from patients suffering from peroxisomal diseases such as X-ALD, Infantile Refsum disease (IRD), and Zellweger syndrome (ZS) as well as ABCD1 KO mouse model, accumulate cholesterol in lysosomes, supporting the important role of peroxisomes in enabling cholesterol export from lysosomes.

Although the precise role of the LPMC in facilitating transport of cholesterol to the PM remains to be determined, this is a beautiful example of a peroxisomal contact sites with a suggested tether and a crucial physiological role.

10. Discussion

Peroxisomes are dynamic organelles that communicate with many, if not all, cellular organelles. Since many of the contact sites are poorly defined, the tethers are, as yet, unknown, and their functional characterization is still lagging behind. Further identification of new contact sites, their tethers and physiological functions are crucial for the understanding of contact sites in general and for better defining peroxisome functions. For example, the peroxisome–lysosome contact site and its role in cholesterol transport suggest that a contact also exists between peroxisomes and the PM however such a contact has never before been identified or studied. Peroxisomes may also have contacts with

other organelles such as endosomes and the Golgi apparatus that await discovery. Moreover, it is not clear whether only one type of contact site exists between each two organelles as evidence from peroxisome–ER contacts that suggests that several different contact sites can be established with the same organelle. Hence in the future the contact sites may have to be defined not only by the organelles participating in them but also by their exact nature (tethering complexes or function). However, beyond the characterization of peroxisomal contact sites their existence raises many questions about the function and dynamics of peroxisomes and their interplay with the rest of the cell.

10.1. Peroxisomes contact site formation

It is clear that peroxisomes physically interact with many organelles. This raises an intriguing question: how can a small organelle maintain contacts with so many other organelles? Three hypotheses as to how this is possible come to mind:

1. Generalistic peroxisomes: It is feasible that every peroxisome contains all the necessary proteins, lipids and molecules to interact with any other organelle and even with multiple organelles at once (e.g. peroxisomes–ER–mitochondria or peroxisomes–mitochondria–chloroplasts) (Fig. 7A). Under such an assumption the contact sites that would be found on each peroxisome may be random and the cell would simply regulate the existence of at least one contact site that is required between any peroxisome in the cell and its target organelle. However, as cells are often highly regulated structures and since peroxisome functions seem to be tightly regulated, this option would be surprising if it was true.

2. Condition specific contacts: The second hypothesis is that peroxisomes contacts form specifically with certain organelles for specific functions and hence are dynamic and condition dependent. For example under conditions that require β-oxidation of very long-chain fatty acids, peroxisomes might get cellular signals that promote the formation of one specific contact (e.g. with mitochondria). However, when conditions change and cross talk with another organelle becomes beneficial, the peroxisome would disconnect from its partner organelle, and connect to another organelle (Fig. 7B). Such changes in contact site partners may be enabled by specific transcription of the tethers only under the required conditions or another form of post-translational regulation. The example of peroxisomes and chloroplasts creating contact sites only under light conditions [57] or the cholesterol dependent peroxisome–lysosome contact [82] both reinforce this possibility.

3. Specialized peroxisomes: The third, non-mutually exclusive and most exiting possibility is the existence of subpopulations of peroxisomes that interact each with a specific organelle. It was previously observed that subpopulation of peroxisomes exist in the cell [13,89–91]. It would be interesting to examine if different classes of peroxisomes interact with different organelles (Fig. 7C). If this were the case, it would be intriguing to understand how this specificity is achieved. One possible mechanism that will lead to the existence of specialized peroxisomes is by biogenesis of different subpopulations of peroxisomes in the cell. If each subpopulation would contain a unique proteome, and have the ability to interact with a specific organelle then it would also most probably be responsible for a different function. Another possibility is that peroxisomes are identical when formed, and each peroxisome has the potential to interact with every organelle. However when a peroxisome interacts with a partner organelle, its protein, lipid and small molecule content are changed leading to the emergence of a specialized peroxisome.

10.2. Peroxisomes contact site dynamics

Since peroxisomes are highly dynamic organelles that can change their number, size, shape, and protein content to adapt to changes, it is intriguing to explore whether peroxisomes can alter their physical contacts with other organelles. Are contact sites dynamic? Or once they form do they last for the entire life of an organelle?

The formation of peroxisomal contact sites seems responsive to environmental and cellular changes, however little is known about the dynamics of the contacts after the environmental condition is changed

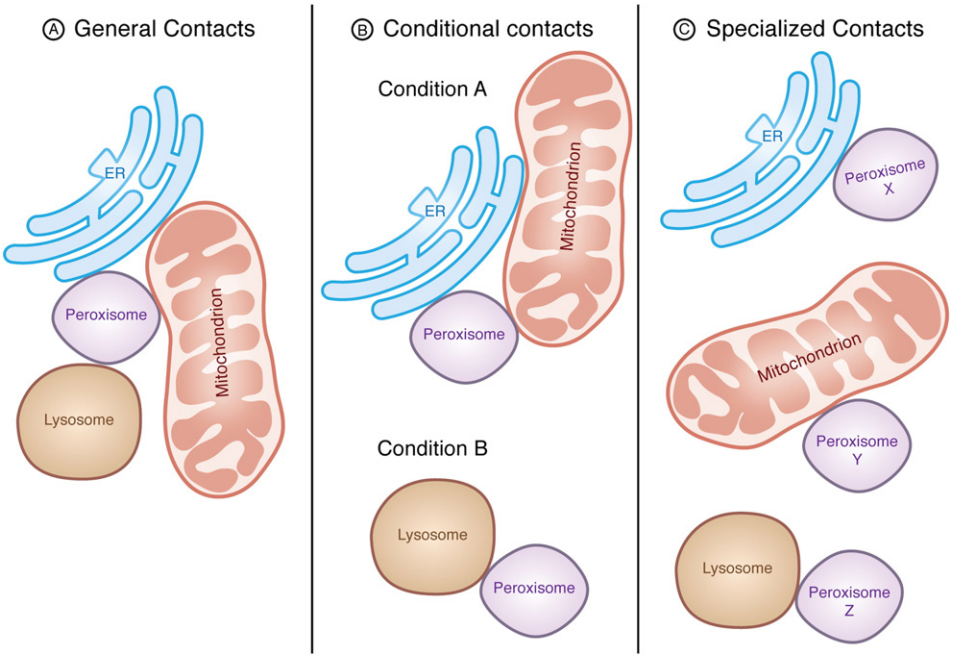

Fig. 7. Peroxisome contact site formation. Three hypotheses as to how peroxisomes maintain such diverse contacts with other organelles: (A) Random encounters: peroxisome may contain all the necessary proteins, lipids and molecules to interact with any other organelle and even with multiple organelles at once. Random encounters enable the creation of contact sites between peroxisomes and other organelles. (B) Conditional contact sites: peroxisome contact sites are condition dependent hence forming specifically with certain organelles for specific functions. Changes in contact site partners are enabled by specific signals for the regulation of the tether/proteins/lipids. (C) Specialized peroxisomes: peroxisome subpopulations exist in the cell, each tailored to interact with a specific organelle. Each subpopulation could contain a unique proteome and has the ability to interact with a specific organelle for a different function.

and whether they can get remodeled. Some interactions seem to indeed be dynamic and can detach and reconnect in a short time period [57,77], but others may be more stable and constant [29]. Nevertheless the mechanism that controls this dynamics as well as the destiny of the disconnected peroxisomes are not clear. It could be that after the peroxisome is detached from a specific organelle the protein and lipid content of the peroxisome changes. Another option is that the detached peroxisome, which is no longer needed for a specific function, undergoes pexophagy and is degraded. Further investigation of the dynamics of the contacts, their tethers and regulators may give a better understanding for the dynamics of the interaction.

10.3. Peroxisomes contact site tethers

One way to understand contact sites is to investigate the way in which the two membranes interact; many times the membranes are physically connected by tethering structures, which can be made of two membranal proteins or a complex of proteins. This tethering structure may be directly involved in the exchange of molecules between the organelles such as in the case of the ERMES complex, holding together the ER-mitochondria junction in yeast [92]. However in some cases the tether only holds the organelles in proximity while other proteins are responsible for the exchange of molecules.

Most contact site studies tried to identify the tether structure by looking for protein interactions on the membranes. However recent studies identify tethering structures that involve the interaction of a protein localized to one membrane and a lipid localized on the other [82,93]. Interestingly, in both cases the tethered lipids were Phosphatidylinositol phosphates (PIPs), which are highly regulated by remodeling enzymes. This new role for lipids in contact sites is intriguing because it opens new opportunities to identify tethers between organelles and characterize contacts. Such protein–lipid tethers might enable a more dynamic contact, as ability to change lipid content by enzymatic remodeling is faster than the ability to change protein content.

It is important to note that our own observations show that when organelles are in close proximity many of the outer membrane proteins may look like they are interacting (by various methods) when in fact they are only found in close proximity. Suggesting that simply showing that two proteins interact on opposing membranes is not enough to define a tether and finding the real tether may be more complicated and needs to be verified by demonstrating loss of the contact site upon deletion of the tether.

10.4. Concluding remarks

The study of peroxisomal contact sites in the past years has enriched the knowledge and understanding of both peroxisome functions as well as the function of contact sites between organelles, shedding light on the roles of these contacts in metabolism, signaling, dynamics, inheritance and lipid biosynthesis. However, the unknown is still much greater than the known and many open questions await to be addressed. It will be the opportunity for future investigations to identify and characterize the underlying molecular mechanisms and the physiological roles of the various peroxisomal contacts, and how they contribute to the etiology of different peroxisomal disorders.

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