

Why do peroxisomes associate with the cytoskeleton?

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Abstract:

Attachment of peroxisomes to cytoskeleton and movement along microtubular filaments and actin cables are essential and highly regulated processes enabling metabolic efficiency, biogenesis, maintenance and inheritance of this dynamic cellular compartment. Several peroxisome-associated proteins have been identified, which mediate interaction with motor proteins, adaptor proteins or other constituents of the cytoskeleton. It appears that there is a species-specific complexity of protein-protein interactions required to control directional movement and arresting. An open question is why some proteins with a specific role in peroxisomal protein import have an additional function in the regulation of cytoskeleton binding and motility of peroxisomes.

Keywords:

Peroxisome motility, cytoskeleton, microtubules, actin filaments, motor proteins, peroxins.

Introduction

Peroxisomes exhibit a remarkable plasticity with respect to shape, size, abundance and metabolic function, often altered by changing environments [1]. The heterogenic appearance of peroxisomes became obvious with improvement of microscopic techniques. Electron microscopy revealed that peroxisomes are small, single-membrane bound organelles with varying diameters between 0.1 to 1.5 μm (**Fig. 1A**). Using fluorescent labelling techniques, the number of peroxisomes per cell was estimated to range between one and many hundred organelles depending on species, tissue, age and external stimuli. In mammalian cells, many peroxisomes are found in close proximity to the cytoskeleton (**Fig. 1B**). Live cell imaging of peroxisomes revealed striking dynamics of their intracellular distribution. Peroxisomes' motility involves oscillations, short range motions and long distance saltations in all possible directions (**Fig. 1C**). All organisms are supposed to require the cytoskeleton of both microtubules and microfilaments for peroxisome function, inheritance and maintenance. However, plant and yeast peroxisomes predominantly move along actin filaments, while animal cells preferentially use the microtubular network to transport peroxisomes, frequently over long distances. Recent progress has been made to understand the molecular basis of motility of peroxisomes and possible functions for each type of motion.

Myosin-driven transport of peroxisomes along actin filaments

In many organisms, the movement of organelles occurs along actin tracks by myosin motor proteins. Actin is a highly abundant and conserved protein found in virtually all eukaryotic cells. The monomeric form (G-actin) polymerizes to double-stranded, helical actin filaments (F-actin) in an ATP-dependent manner [2, 3]. Actin is a central component in muscle contraction, cell motility and organelle movement [4, 5]. Myosins are conserved motor proteins, which move along actin cables in eukaryotes [6]. The myosin superfamily is divided in as many as 37 classes [7, 8]. In metazoa and fungi, class V myosins function as motors in organelle and vesicle movement, establishing cell polarity, mitotic spindle positioning, partitioning during cell division and mRNA localization [9-12]. Plant class XI myosins are closely related to class V myosins and share most of the functions [8, 13]. While metazoan and fungi typically have one to three different class V myosins, plants usually have around a dozen different class XI myosins.

The yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) divides asymmetrically by forming a bud from the mother cell. To ensure that peroxisomes and other organelles are inherited to daughter cells, this kind of cell division requires that the transport is tightly regulated [14]. Moreover, it has to be ensured that not all organelles are transported into the bud, but some need to be retained in the mother cell. The transport of different organelles into the bud is a highly ordered process [15-17]. Different cargoes are transported at different time points of the cell cycle. For example mitochondria are transported into the bud later than peroxisomes [18, 19]. In *S. cerevisiae*, the movement of organelles to the bud occurs along actin tracks by the motor proteins Myo2 and Myo4. While Myo4 transports the cortical ER and mRNAs, most other organelles including mitochondria and peroxisomes are moved by Myo2 [20-23]. The N-terminus of the homodimeric Myo2 contains the conserved motor domain, while the C-terminus contains the cargo-binding domain [24, 25]. Specific adapter proteins have been identified for various organelles and secretory vesicles, which bind the C-terminus of Myo2 [14, 26, 27]. Like in yeast, plant peroxisomes predominantly move along actin as shown by confocal microscopy using fluorescent proteins to simultaneously visualize peroxisomes and actin [28-30]. Plant peroxisomes were found in close vicinity to actin and moved along the actin filaments [30]. Moreover, treatment of different plant cells with the actin destabilizing drugs cytochalasin and latruncilin B led to a reversible stop of peroxisomal movement [28-30].

Structural basis of peroxisome interactions with myosin and actin

In *S. cerevisiae*, the peroxisomal membrane Protein Inp2 plays a central role in peroxisome inheritance. Inp2 interacts directly with the tail of type V myosin Myo2, which is involved in actin based transport of organelles, and it is mostly associated with peroxisomes that are destined to be transported to the bud [31, 32]. In Inp2 deficient cells, the transport of peroxisomes to the bud is strongly reduced, while the transport of other organelles is not affected. Overexpression of Inp2 leads to a complete transfer of all peroxisomes into the bud [31]. Inp2 contains PEST sequences (rich in proline (P), glutamic acid (E), serine (S), and threonine (T), which are common to rapidly degraded proteins. Steady-state levels of Inp2 fluctuates with the cell cycle. The protein is degraded when the peroxisomes have reached the bud [31]. Moreover, several recognition sites for the cyclin-dependent kinase Cdk1, which are phosphorylated during cell cycle, have been found in Inp2. In cells, expressing Myo2

mutants unable to bind Inp2, the inheritance of peroxisomes is impaired and the amount of Inp2 is increased. In these cells, the phosphorylation of Inp2 is not affected [32]. Thus, the level of Inp2 seems to be affected by the positioning of peroxisomes rather than by the cell cycle. The increase of Inp2 levels suggests the existence of a compensation mechanism, as the cell increases the amount of the peroxisome-specific Myo2 adaptor in consequence of the lack of peroxisomes in the bud. In baker's yeast, also Pex19, a protein essential for protein insertion into peroxisomal membranes [33], is required for the proper association of peroxisomes to Myo2. It was shown that also Pex19 directly interacts with the C-terminus of Myo2 [34].

In the yeast *Yarrowia lipolytica*, Pex3B and Pex3, proteins required for the formation of peroxisomal membranes and the insertion of membrane proteins, interact with the myosin V motor and promote transport of peroxisomes to the bud [35]. In plants, it was shown by fluorescent labelling of different myosins that class XI myosins including XI-1, XI-2, XI-K and XI-1 are at least partially colocalized with peroxisomes [13, 36-39]. Moreover, mutations of either XI-2 or XI-K lead to striking, but not complete, decrease in peroxisome mobility, while mutations of XI-1 lead to a slight decrease [40]. Taken together, it seems that in plants many different class XI myosins are involved in the movement of peroxisomes, while in yeast one single myosin (Myo2) with its peroxisomal adaptor Inp2 is responsible for peroxisomal motility. So far, there is no peroxisomal protein known in plants that mediates the interaction to one or several myosins.

Peroxisome transport along microtubules

In animal cells, microtubule (MT)-mediated motility of peroxisomes was demonstrated by treatment of cultured cells with MT-depolymerizing agents, which did result in altered distribution, clustering and abnormal morphology of the organelles [41, 42]. In addition, co-localization of peroxisomes with microtubules was clearly indicated by microscopic analyses in human cells (Figure 1B and [41]), Chinese hamster ovary cells [43], CV1 cells [42], *Drosophila* S2 cells [44], and *in vitro* [41, 45]. Directional movement along microtubules is clearly distinct from the oscillatory motions of peroxisomes based on actin association [43]. Unidirectional long-range movements were observed more frequently than bidirectional movement to and away from the cell center. It has been noted that only a small percentage (10-15%) of all peroxisomes move along MT [42, 43, 46, 47]. Consistently, computational

analysis and modelling revealed that only a small fraction of fast moving peroxisomes allows uniform distribution and homogenisation of the peroxisomal compartment at optimal energy costs [48]. The maximal speed over long distances ($>1\ \mu\text{m}$) was estimated up to $10\ \mu\text{m/s}$, the averaged velocity ranging between 0.1 to $1\ \mu\text{m/s}$ [44, 49]. With respect to speed and distances, the transport of peroxisomes resembles that of other organelles like endosomes, lysosomes and mitochondria [50, 51].

Although plant cells show long-distance transport of peroxisomes via actin filaments, these motile organelles were frequently observed to pause at cortical MT microtubules [52, 53]. Peroxisome-MT association in plants is further supported by proteomic approaches [53].

Architecture of microtubules and associated motor proteins

Microtubules form hollow cylindrical structures build from 13 parallel protofilaments, each composed of alpha- and beta-tubulin heterodimers. Since the heterodimers stack head-to-tail, each protofilament has a minus (alpha-tubulin) and a plus (β -tubulin) end. Both subunits can bind GTP but only β -tubulin hydrolyzes and exchanges the nucleotide. Coupled to the GTP cycle, tubulin changes from a polymeric into dimeric forms. The minus ends are normally centered in a single microtubule-organizing center (MTOC), which in most cells is located at the vicinity of the nucleus. Proteins that bind to MT are collectively called microtubule-associated proteins (MAPs). In most cases, they influence assembly and disassembly of protofilaments but they can also mediate bridging to other cell components. MT-dependent movement of peroxisomes and other organelles and vesicles is mediated by two types of motor proteins, belonging to the dynein or kinesin families of microtubule-based motor proteins [54-56]. The 'hand-over-hand' motion of dimeric kinesins from one tubulin-binding site to the other is coupled to ATP hydrolysis and exchange of ADP against ATP. Dyneins are minus-end directed motor proteins, which utilize ATP hydrolysis to induce 'power-stroke' motions by binding/unbinding to MT.

It has been suggested that the bidirectional movement of peroxisomes is the result of the transfer of a peroxisome from one motor to the other [57]. During bi-directional transport both types of motor proteins are coordinated, being turned off and on in such a way that they are not simultaneously dragging the organelle [57]. It also seems that multiple dyneins or multiple kinesins work together to produce the high velocity [57].

Structural basis of peroxisome interaction with MT

Peroxisome-anchored proteins can bind directly to motor proteins or microtubules or they can use adaptor molecules to bridge the interaction. Recent research revealed that all three possibilities are realized in mammalian cells. **Table 1** summarizes peroxisomal proteins and the corresponding cytoskeletal binding partners.

Direct peroxisomal interaction with motor proteins. Kinesins and dyneins are heterogeneous classes of motor proteins with distinct cargo selectivity. In mammalian cells, two large and heteromeric cytosolic dynein complexes, dynein 1 and 2, are recognized that are composed of eleven and two different proteins, respectively [58]. The subunits of dimeric kinesins are expressed from at least 45 genes [55]. Dynein light chain Dyn2, a yeast protein subunit of cytosolic dynein has been shown to interact with constituents of the peroxisomal protein import machinery [59-61]. However, so far there is no evidence that Dyn2 is required for peroxisome motility. Instead, a direct role in peroxisome biogenesis is indicated [60]. For Dyn2 other non-motor-related functions have been assigned previously, i.e. as an organizer of the assembly of the nuclear pore complex in *S. cerevisiae* [59]. Although kinesins typically use scaffold and/or adaptor proteins to bind to cargo vesicles, direct cargo binding has also been observed. The kinesin-like protein KifC3 has been identified in a 2-hybrid screen to associate with Pex1, a component of the peroxisomal protein import machinery, which is transiently associated with the peroxisomal membrane [62]. The conserved KifC3 belongs to Kinesin-14 family of which members are characterized by a C-terminal motor domain. Typically, these motor proteins drive their cargo vesicles in minus-end direction to perinuclear regions [55]. Unexpectedly, the knockdown of KifC3 in mammalian cells led to clustering of peroxisomes at centrosomes, suggesting a function for this protein in peroxisome motility. Dietrich et al. [62] consider a regulatory role of KifC3 in minus-end directed peroxisome transport, perhaps acting antagonistically as a brake for dynein motors. However, the physiological meaning of its selectivity for the Pex1 remains unclear. Pex1 is one of the less abundant membrane-associated proteins, which is only transiently anchored by Pex26, an integral membrane protein. It is noteworthy that Pex1-deficient human fibroblast cells show minor clustering of peroxisomal remnants, but most of them still align with microtubules and normal distribution could be achieved by overexpression of the peroxisome-proliferation factor Pex11 β [63]. Further studies indicated that the peroxisomal ghosts in Pex1-deficient fibroblasts exhibit

normal peroxisome motility in terms of speed and distances [47]. This indicates that besides Pex1 other non-identified membrane receptors for motor proteins exist.

Motor-bridging adaptors. Dynactin is a large multimeric adaptor complex, which facilitates attachment of cytoplasmic dynein to cargo [56, 64, 65]. In addition, dynactin can function as an adaptor for at least two motors of the kinesin superfamily, heterotrimeric kinesin-2 [66] and mitotic kinesin Eg-5 [67]. The multifunctional dynactin complex comprises eleven different subunits of which its largest subunit, p150, and a filament of the actin-related protein 1 (ARP1) seem to be involved in peroxisomal interaction with microtubules. This is based on the observation that overexpression of dynamitin, another subunit of dynactin, inhibited peroxisome movement in HepG2 cells and interfered with a normal re-distribution of peroxisomes after treatment with a microtubule-depolymerizing agent [46]. It has been shown earlier that overexpression of dynamitin leads to release of the p150 subunit from the dynactin complex and thereby interferes with dynein-mediated vesicle movement [68, 69]. Furthermore, it has been reported that isolated peroxisomes can physically interact *in vitro* with components of the dynactin complex (p150Glued, Arp1) (Schrader *et al.*, unpublished data, reported in [49]).

Binding of peroxisomes to microtubules. CLIPs are cytosolic linker proteins, which specifically bind organelles to microtubules [70]. It has been suggested that members of this protein family promote docking of organelles to microtubules prior to their translocation. Using an *in vitro* binding assay of peroxisomes with microtubules coated onto microtiter plates, Thiemann *et al.* showed that initial binding of peroxisomes depends on an unknown peripheral peroxisomal membrane protein and does not require motor proteins [45]. Binding could be abolished by incubation with an antibody against the conserved CLIP microtubule-binding domain.

Up to now, only one peroxisomal membrane protein, Pex14, has been identified, which directly binds to tubulin [47]. The human Pex14 is an integral membrane protein of the protein import machinery of peroxisomes [71]. Pex14 has multiple tasks. Together with the integral membrane protein Pex13, it provides the docking platform for cytosolic receptors for newly synthesized peroxisomal matrix proteins [72]. Together with one of the cytosolic import receptors, Pex5, it constitutes a highly dynamic translocation pore in the peroxisomal membrane [73]. Pex14 has also been reported to mediate dissociation of receptor-cargo

complexes in peroxisomal protein import [74]. In addition, Pex14 also binds Pex19, which is supposed to act as import receptor for peroxisomal membrane proteins [75]. All these protein-protein interactions, including the tubulin binding, are mediated through a small and highly-conserved domain at the N-terminus of Pex14 [47]. Direct binding of human Pex14 and β -tubulin was indicated by (i) proteomic identification of β -tubulin subunits also in the presence of depolymerizing drugs, (ii) physical interaction between the recombinant N-domain of Pex14 (Pex14-N) and purified porcine brain tubulin, and (iii) by decoration of the microtubular network with labelled Pex14-N in fixed cells as shown by microscopic analysis [76] (**Fig. 2**). *In vitro* assays revealed that human Pex5 and β -tubulin compete for binding to Pex14, suggesting that both proteins bind to identical or at least overlapping binding sites [47]. Dimerization of Pex14 seem to increase the efficiency to bind to microtubules [76]. This seems not to be the case for the human Pex5-Pex14 interaction where each of the eight Pex14-binding sites of Pex5 can bind one monomeric Pex14 domain with an affinity in the nanomolar range [77, 78]. Thereby, it also seems possible that Pex5 disrupts the Pex14-tubulin interaction by disassembly of Pex14 dimers. However, the physiological role of the Pex14-tubulin interaction is still speculative. Pex14-deficient cells do not any longer show long-range directional movement of peroxisomes [47]. This indicates that Pex14 rather serves as an anchor for the cytoskeleton, which then is supposed to initiate movement. This is different from the function of the above-mentioned KifC3, which binds Pex1, and deficiency in KifC3 results in peroxisome clustering [62]

Physiological role of peroxisome motility

Association with the cytoskeleton maintains the functional efficiency, biogenesis and degradation of the peroxisomal compartment. There are several plausible explanations why peroxisomes move along actin cables and microtubular filaments.

The motility of peroxisomes contributes to a uniform intracellular distribution of peroxisomes, which is required to ensure an equal distribution of peroxisomes between mother and daughter cells during mitosis [49]. This is of importance for symmetrically dividing mammalian cells, in which the inheritance strategy of peroxisomes varies depending on the cell type and organism and peroxisomes do not always associate with the mitotic spindle during cell division [42, 79, 80]. In yeast, the inheritance of peroxisomes strongly depends on the actin

cytoskeleton. Peroxisome inheritance in yeast starts at very early stages of bud formation. At least one peroxisome of the mother cell is transported to the forming bud by the myosin motor protein Myo2 via actin filaments, while other peroxisomes remain in the mother cell [16]. In the process of bud formation, formins control the formation of ordered actin cables that converge at the bud neck and the bud tip [81-83]. These actin cables are the tracks along which most organelles are transported to the bud.

Organelle movement seems to be extremely important in plants, as mutations in myosins lead to severe defects in plant development in *Arabidopsis* [84-87]. In many plant cells large quantities of cytoplasm and organelles including peroxisomes are moved by cytoplasmic streaming [29, 88]. This process is usually driven by actin and organelle-associated myosin [89-92].

Nevertheless only little is known about the specific role of organelle mobility in plant development. During interphase, the movement of plant peroxisomes, like other organelles, seems to be almost erratic and chaotic. In some plant cells, e.g. onion root tip and leek leaf epidermis cells, peroxisomes start to accumulate in the division plane in the anaphase. The cluster is then divided in two layers by the forming cell plate. It was speculated that peroxisomes may be needed at the division plane to remove hydrogen peroxide or to recycle membrane lipids [28]. The positioning could also contribute to an equal partitioning of peroxisomes to the daughter cells [28, 93]. Nevertheless, the accumulation of peroxisomes during cell cycle does not occur in all plant cells, e.g. in *Arabidopsis* root tip cells accumulation is not detectable [28].

Another obvious reason for peroxisome motility is that peroxisomes may need to be uniformly dispersed throughout the cytoplasm to protect the cell from toxic substrates that are depleted by peroxisomal metabolism. These may include reactive oxygen species, which are decomposed by peroxisomal enzymes [49] or long fatty acids, which also are degraded by peroxisomes [94]. Clusters of peroxisomes were observed in mammalian cells treated with MT-depolymerizing drugs [41, 42]. Unfortunately, due to cellular damages, the long-term effects of increased concentrations of these toxic compounds could not be analyzed. Noteworthy, cells of patients with Zellweger syndrome (a congenial disorder characterized by depletion of functional peroxisomes) survive for many generations. One possible explanation is that some detoxifying matrix enzymes could still be active in the cytosol. It has been

suggested that neurodegenerative damages of Zellweger patients and also Morbus Alzheimer are partly due to non-functionality and uneven cellular distribution of peroxisomes [63, 95].

Peroxisome motility along MT is also supposed to contribute to the peroxisome specific degradation mechanisms by pexophagy in mammalian cells [96]. One essential protein in macropexophagy is LC3. This protein is required for formation of autophagosomal membrane, which sequesters peroxisomes and fuses with vacuoles. It has been reported that mammalian Pex14 interacts with the membrane-bound form of LC3 (LC3-II) via microtubules [97]. Further studies revealed that LC3 and the peroxisomal import receptor Pex5 compete for the direct interaction with Pex14, indicating that peroxisomes that do not import proteins might be marked for disposal [98].

Sometimes, the mobility of peroxisomes is deliberately blocked. Accordingly, peroxisomes inherited to yeast buds are anchored to cortical structures of the bud in a so far unknown manner [31, 99]. To ensure that some peroxisomes remain in the mother cell during the asymmetric cell division in yeast, a subset of peroxisomes is immobilized and anchored to the cell periphery of the mother cell. It turned out that these peroxisomes are retained by binding to the cortical ER via Inp1 and Pex3 as tethering proteins [18]. The role of Inp1 as a peroxisomal tether was elegantly demonstrated by two lines of evidence. In cells lacking Inp1, the tethering of peroxisomes to cortical regions was abolished and eventually all peroxisomes were transported to the bud. On the other hand, in cells overexpressing Inp1, all peroxisomes were retained in the mother cell [99]. The role of Pex3 was disclosed later as mutants of Pex3 were identified that do not recruit Inp1 to the peroxisomal membrane. Cells expressing these Pex3 mutants exhibited the same phenotype as cells lacking Inp1 [100]. Inp1 has two binding sites for Pex3 and is able to connect two Pex3 molecules. The retention of peroxisomes is achieved as Inp1 interacts with peroxisomal Pex3 as well as with ER-localized Pex3 and thus acts as a molecular hinge that tethers peroxisomes to the cortical ER, which in turn is tethered to the plasma membrane [18, 101-103].

Peroxisomes originate by division of preexisting microbodies or budding from the ER (another review this issue). Although the specific function of microtubules during biogenesis of peroxisomes is not yet fully understood, several publications suggest that microtubular association is required for both biogenetic pathways. Brocard et al. studied the early stages of biogenesis by reintroducing Pex16 in Pex16-deficient human cells, which are devoid of

peroxisomes [104]. Disruption of microtubules or dynein/dynactin complexes inhibited functional complementation, probably at the state where preperoxisomes bud from ER exit sites. Involvement of microtubule interaction in later proliferation processes was shown by Koch et al. [105]. Pex11 overproduction leads to elongated peroxisomes because of incomplete fission. Disappearance of these structures was significantly faster upon depolymerisation of the microtubular network, suggesting that MT are involved in formation of the intermediates.

It has been shown that exposure to cadmium can enhance the motility of peroxisomes in Arabidopsis leaf epidermal cells. It was speculated that this effect is an adaptation to increased reactive oxygen species production induced by cadmium [106]. However, exogenously added reactive oxygen species resulted in reduced peroxisomal movement [107]. These contrary results could be caused by the different experimental systems. Moreover, a clustering of peroxisomes was observed during pathogen invasion and wounding [108, 109]. The functional role of this clustering is not yet fully understood

Regulation of peroxisome motility

Knowledge about regulatory mechanisms of cytoskeleton-based motility is scarce. A few details have been depicted from studies on mammalian cells. It has been demonstrated that peroxisomal motility along MT is regulated by environmental changes [110]. Co-stimulation with ATP and lysophosphatidic acid (LPA) leads to arrest of movement of peroxisomes in mammalian cells, neither affecting positioning of other organelles nor modulating the integrity of microtubular network. Activation of the corresponding G-protein coupled receptors (GPCRs) modulates activities of various signal transduction enzymes including trimeric and small G-proteins, protein kinases and phospholipases [110]. A recent study reported on a role of the GTPase protein RhoA in the coordinated interplay between actin- and MT-dependent motility [111]. RhoA switches between a GTP-bound active state associating with peroxisomes and an inactive state dissociating from the organelle. Dissociation of RhoA favors bidirectional motions along microtubules whereas association of RhoA and effectors displaces peroxisomes from microtubules enabling attachment to actin cytoskeleton [111]. However, the molecular details of displacing peroxisomes from MT are not yet known. A potential effector protein in mammalian cells could be Pex14, which binds

directly to tubulin. However, the regulation of Pex14 binding to microtubules is also not understood in great detail. RhoA-GTP activates other downstream effector molecules, like protein kinases, which may switch on/off the Pex14 interaction with microtubules. Mechanism might be even more complicated since also competing peroxins of the Pex14-MT interaction, like the import receptor Pex5, might be modified by signaling cascades.

Concluding remarks and future perspectives

During the last years, our knowledge on the peroxisome interaction with the cytoskeleton has dramatically increased. The mode of interaction between these cellular structures turned out to be highly regulated and different species evolved distinct strategies to transport peroxisomes within one cell or from one cell to another. There are multiple reasons why peroxisomes move along the cytoskeleton. The physiological relevance of peroxisome motility is emphasized by its contribution to the biogenesis and proliferation of the organelles, their cellular distribution, inheritance and autophagic degradation. Among the proteins, which are involved in cytoskeletal interaction are strikingly often peroxins, which are also required for protein import into peroxisomes. This might suggest that peroxisome motility and protein import are correlated processes in the cell. These processes might act antagonistically as indicated by direct competition of the import receptor Pex5 and tubulin for binding to the multifunctional peroxisomal membrane protein Pex14. It is suggestive that import-active Pex14, which is bound by Pex5 cannot hook on microtubules and thereby peroxisomes will be stalled. Less import active areas in the cell, as indicative by lack of the PTS1-receptor, might favor binding to MT and therefore trigger directional movement. Such a working hypothesis would be in line with previous observations, indicating correlation of the import activity of a plant peroxisomal enzyme with the intactness of microtubular integrity [52].

It is obvious that further details of peroxisome-cytoskeleton interaction require advanced observation methods, characterized by a high temporal and spatial resolution. While methods such as fast three-dimensional particle tracking [112] allow direct observation of labelled peroxisomes relative to actin or microtubule, novel super-resolution optical techniques such as STED or PALM/(d)STORM/GSDIM microscopy [113] have the potential to highlight the exact spatial positioning of potential linker molecules between peroxisomes and cytoskeletal structures (**Fig. 3**).

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Table 1. Peroxisomal proteins and corresponding cytoskeletal binding partners

Peroxisome	Cytoskeleton	Organism (Ref)
Inp2	Myo2 (actin)	<i>S. cerevisiae</i> [31]
Pex19	Myo2 (actin)	<i>S. cerevisiae</i> [34]
Pex3, Pex3B	Class V myosin	<i>Y. lipolytica</i> [35]
Pex1	KifC3 (microtubules)	Mammals [62]
Pex14	Tub β (microtubules)	Human [47]

Legends

Figure 1: Microscopy of Peroxisomes

(A) Electron micrograph of a *Saccharomyces cerevisiae* cell. Peroxisomes are marked with an asterisk. Scale bar: 1µm. (B) Fluorescence microscopy of a human fibroblast labeled with DAPI (nucleus, blue), Pmp70 antibodies (peroxisomes, red) and TubStain-GFP (Microtubules, green). Scale bar: 10 µm. Note that all peroxisomes are in close proximity to microtubules. (C) Live cell fluorescence microscopy showing movement of peroxisomes in a human fibroblast cell. Peroxisomes are labelled with GFP. Different time points are represented by different colours. Static peroxisomes are white, moving peroxisomes are visible as a row of dots with different colours (Arrow). Scale bar: 10µm.

Figure 2: Labelling of microtubules with Pex14 (TubStain).

The figure shows the specific binding of the conserved N-domain of human Pex14 to microtubules. TubStain, a fusion protein consisting of recombinant human Pex14 fragment, GST and GFP [76], was incubated with Formalin-fixed fruit-fly S2R+ cells and cells were imaged by confocal fluorescence microscopy (green). The actin-cytoskeleton was stained with phalloidin-TRITC (red) and nuclei are labelled with DAPI (blue). Scale bar: 10µm.

Figure 3: Super-resolution microscopy of peroxisomes and microtubules.

Super-resolution GSDIM (ground state depletion microscopy followed by individual molecule return) image of immunostained microtubules (green) and peroxisomes (Pmp70, red) of fixed PtK2-cells. Conventional image is shown in the inner circle. Scale bar: 1µm. Adapted from [114].

Figure 1

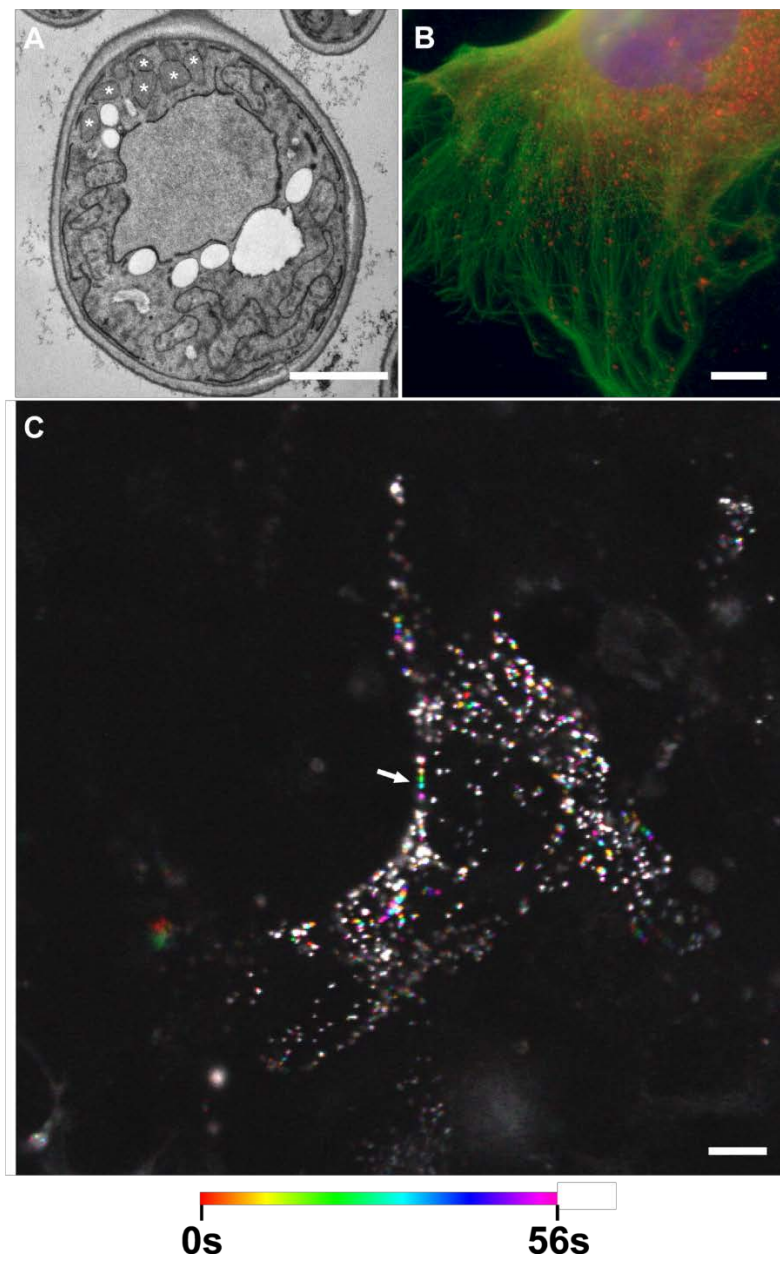


Figure 2

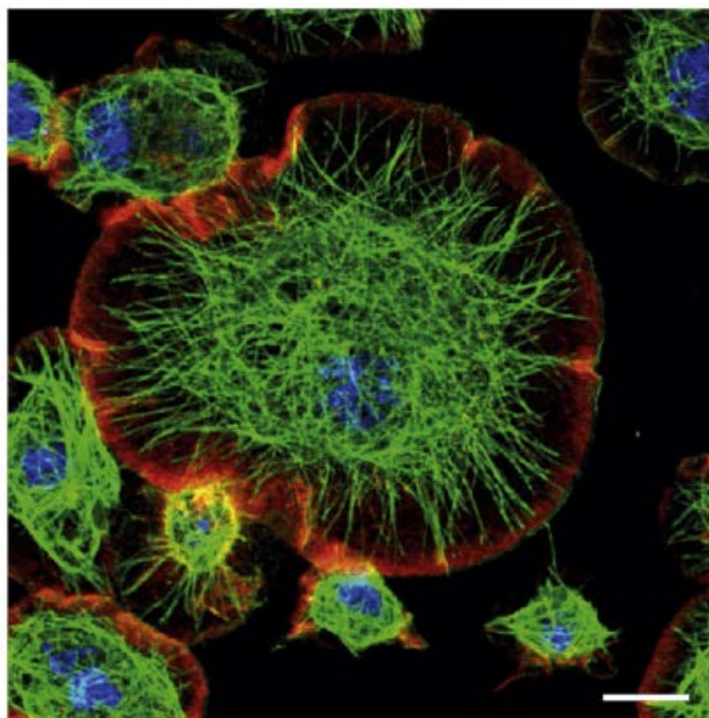


Figure 3

