Real time imaging reveals a peroxisomal reticulum in living cells

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SUMMARY

We have directly imaged the dynamic behavior of a variety of morphologically different peroxisomal structures in HepG2 and COS-7 cells transfected with a construct encoding GFP bearing the C-terminal peroxisomal targeting signal 1. Real time imaging revealed that moving peroxisomes interacted with each other and were engaged in transient contacts, and at higher magnification, tubular peroxisomes appeared to form a peroxisomal reticulum. Local remodeling of these structures could be observed involving the formation and detachment of tubular processes that interconnected adjacent organelles. Inhibition of cytoplasmic dynein based motility by overexpression of the dynactin subunit, dynamitin (p50), inhibited the movement of peroxisomes in vivo and interfered with the reestablishment of a uniform distribution of peroxisomes after recovery from nocodazole treatment. Isolated peroxisomes moved in vitro along microtubules in the presence of a microtubule motor fraction. Our data reveal that peroxisomal behavior in vivo is significantly more dynamic and interactive than previously thought and suggest a role for the dynein/dynactin motor in peroxisome motility.

Key words: Peroxisome, Motility, Peroxisomal reticulum, Microtubule, Dynein, Dynactin

INTRODUCTION

Peroxisomes are ubiquitous subcellular organelles that perform several important catabolic and anabolic functions, including hydrogen peroxide metabolism, the β-oxidation of very long chain fatty acids, and the biosynthesis of ether phospholipids (reviewed by van den Bosch et al., 1992). The critical role of peroxisomes in human health and disease has been well established by the discovery of a group of severe inherited metabolic diseases in which peroxisome function is perturbed (Lazarow and Moser, 1989; Wiemer and Subramani, 1994). In contrast to other cellular compartments such as the Golgi complex or lysosomes, peroxisomes show a uniform intracellular distribution that is proposed to be mediated by the microtubular network. Several recent studies have demonstrated that peroxisomes are associated with microtubules (Schrader et al., 1996; Rapp et al., 1996; Wiemer et al., 1997). Peroxisome behavior can be studied in living CHO and CV-1 cells by microinjecting fluorescein-bound luciferase, which has a peroxisomal targeting signal (Rapp et al., 1996) or expression of a construct encoding the green fluorescent protein bearing the C-terminal peroxisomal targeting signal 1 (GFP-PTS1) (Wiemer et al., 1997).

The peroxisomal compartment is highly plastic. Several morphologically distinct types of peroxisomes including elongated tubules have been described in mammalian tissues and cell lines, first by electron microscopic studies (Hicks and Fahimi, 1977; Gorgas, 1984; Yamamoto and Fahimi, 1987; Roels et al., 1991) and recently at the light microscopic level (Schrader et al., 1994, 1995; Litwin and Bilinska, 1995). A heterogeneous and more complex peroxisomal compartment is observed under conditions of rapid cell growth, for example, after hepatectomy (Yamamoto and Fahimi, 1987) or stimulation of cultured cells with defined growth factors, fatty acids or free radicals (Schrader et al., 1998a, 1999), and in tissues actively engaged in the synthesis of special kinds of lipids (Gorgas, 1987). Tubulation and fission of elongated peroxisomes has been proposed to contribute to peroxisome proliferation (Schrader et al., 1998a,b).

Peroxisomes have been proposed to be linked, via tubular segments, into an interconnected reticulum (Lazarow and Moser, 1989; Wiemer and Subramani, 1994). Reticular structures have been found by serial section reconstructions in lipid synthesizing epithelia and in regenerating liver (Gorgas, 1987; Yamamoto and Fahimi, 1987), but a reticular network has never been observed in cultured cells or studied under in vivo conditions. Moreover, the motility and dynamic behavior of complex peroxisomal structures, such as tubular and elongated peroxisomes has not been documented in detail. In the present study, we have used high magnification video-enhanced fluorescence microscopy, at real-time video rates, to analyze the dynamic behavior of the peroxisomal compartment in living HepG2 and COS-7 cells transfected with a GFP-PTS1 fusion construct. Our data indicate that peroxisomes are engaged in transient contacts and interact with each other. We also were able to see a peroxisomal reticulum in some cells. In vitro and biochemical analysis of
peroxisome motility on microtubules suggests a role for cytoplasmic dynein and dynactin in this process.

MATERIALS AND METHODS

Cell culture

HepG2 cells (Aden et al., 1979; Knowles et al., 1980) and COS-7 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 2 g/l sodium bicarbonate, 2 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 10% fetal calf serum (FCS) (all from Gibco BRL, Gaithersburg, MD, USA) at 37°C in a humidified atmosphere containing 5% CO2. HepG2 and COS-7 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA).

Transient transfection by electroporation

HepG2 and COS-7 cells were grown to 90% confluency in a 75 cm2 flask, washed with 10 ml of HBS solution (21 mM Hepes, 137 mM NaCl, 5 mM KCl, 0.7 mM Na2HPO4, 6 mM dextrose), resuspended in 0.5 ml HBS, and transferred to a 0.4 cm gap, sterile electroporation cuvette containing 10 µg DNA. Electroporation was performed in a BTX ECM 600 electroporator (BTX, San Diego, CA, USA) at 230-240 V, 1500 µF, 129 Ω. After electroporation, cells were resuspended in DMEM/FCS, seeded on coverslips and processed for indirect immunofluorescence or real time imaging 24-48 hours after transfection.

Microinjection

Microinjection was performed using an Eppendorf microinjection system (Eppendorf micromanipulator 5170, and microinjector 5242, Eppendorf, Hamburg). Anti-dynein antibody DIC 70.1 (1 mg/ml) was microinjected into GFP-PTS1 transfected cells according to Burkhardt et al. (1997). 2 mg/ml of cascade blue conjugated BSA was used as a coincidence marker. Control experiments were performed using mouse anti-IgG ascites that was prepared similarly. Microinjected cells were incubated for 4 hours at 37°C before in vivo analysis.

cDNAs and antibodies

pGFP-PTS1, encoding the S65T mutant from the green fluorescent protein (GFP) and the peroxisomal targeting signal (PTS1) under the control of the CMV promotor, was a gift from Dr S. J. Gould (The Johns Hopkins School of Medicine, Baltimore, MD, USA) (Kalish et al., 1996). Dynamitin-HA in pCB6 is described by Valetti et al. (1996). Anti-dynein antibody DIC 70.1 (1 mg/ml) was a gift from Dr Völkl (University of Heidelberg, Germany). The anti-HA epitope mAb is described by Duro et al. (1996). The following antibodies were used for immunoblotting: cytoplasmic dynein IC (mAb 70.1; Steuer et al., 1990), p150Glued (mAb 150.1; Gill et al., 1991), Arp1 (mAb 45A; Schafer et al., 1994). Species-specific anti-IgG antibodies conjugated to TRITC or FITC were obtained from Jackson Immuno Research Laboratories (West Grove, PA, USA).

Nocodazole treatment

Cells transfected with the pGFP-PTS1 expression construct were treated with 33 µM nocodazole by adding the microtubule-destabilizing agent directly to the live cell motility assay (see below) and incubating for 15-30 minutes. HepG2 cells transfected with a dynamitin-HA-tagged expression construct were treated 24 hours after transfection with 15 µM nocodazole for 12-14 hours at 37°C. Such long incubations were required because peroxisome clustering was only observed after several hours in nocodazole (Schrader et al., 1996). After incubation, nocodazole was washed out to allow reorganization of microtubules. Cells were processed for immunofluorescence after 4 hours using an anti-HA monoclonal antibody. Three to five coverslips per preparation were evaluated, and 200 cells per coverslip were counted and categorized accordingly.

Indirect immunofluorescence and deconvolution

Cells grown on glass coverslips were fixed with 4% paraformaldehyde in PBS, pH 7.4, permeabilized with 0.2% Triton X-100 and incubated with primary and secondary antibodies as described (Schrader et al., 1995). Transfected cells were processed for immunofluorescence 24-48 hours after transfection. For double-labeling experiments, transfected cells were incubated with rabbit anti-PMP70, and subsequently with goat anti-rabbit IgG conjugated to TRITC. For visualization of dynamitin-HA and peroxisomes cells were double-labeled with anti-HA (FITC) and anti-PMP70 (TRITC) antibodies. Samples were examined using an Axiovert 35 TV microscope (Carl Zeiss Inc., Thornwood, NY) and photographed on Kodak TMY film or with a slow scan CCD camera (VME200A, Photometrics, Tucson, AZ, USA). Digital images were optimized for contrast and brightness using Innovision software (Innovision Corp., Durham, NC, USA). Deconvolution studies were performed with a Delta Vision (Applied Precision, Inc., Issaquah, WA, USA) using an Axiovert S100/21 microscope (Carl Zeiss Inc.) for optical sectioning. Deconvolved images in Fig. 1 were taken at 200 nm intervals with a ×100 Plan Apo objective with a 1.4 numerical aperture through focus.

Real time imaging, video analysis and image capture

Cells transfected with GFP-PTS1 cDNA were grown on 31 mm round glass cover slips (Biophysicsa Technologies, Towson, MD, USA). Samples were imaged, data recorded and the tracks of individual GFP-labeled peroxisomes were analyzed as described by Valetti et al. (1999). Still images were grabbed from the Hi8 video-cassette to the hard drive of a Sun workstation running Innovision software (Innovision Corp.), using an image acquisition subroutine written by Mr Andrew Nechkin of the Advanced Microscopy Facility at Johns Hopkins University. Four video frames (1/30 seconds per frame) were averaged to eliminate camera noise and the averaged images were optimized for contrast and brightness. The first frame number was used to calculate the times indicated on Figs 2, 3 and 5. Two or three coverslips were observed per experiment and between 5 and 10 different fields were viewed on each coverslip. Peroxisome movements continued for the entire interval of observation, indicating that the cells were not suffering considerably from photodamage. Significant differences between experimental groups were detected by analysis of variance for unpaired variables using SigmaStat for Windows (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± s.d., with an unpaired t-test used to determine statistical differences. P values <0.05 are considered as significant, and P values <0.01 are considered as highly significant. For Fig. 3B the number of movements seen in intervals of 5 minutes were calculated with n=20 intervals for each group. For each treatment, 25-35 different fields were observed.

Purification of tubulin and microtubule-based motors

Twice-cycled bovine brain tubulin was purified over a phosphocellulose column (Sloboda and Rosenbaum, 1982), frozen dropwise in liquid nitrogen, and stored at −80°C until use. The ATP-release fraction was prepared from chick embryo brain using an ATP-sensitive microtubule affinity protocol as described previously (Schröer and Sheetz, 1991).

In vitro motility assay

Peroxisomes were isolated from rat liver according to a protocol described previously (Völkl and Fahimi, 1985; Völkl et al., 1996). Briefly, one liver was homogenized (1 stroke, 2 minutes, 1000 rpm) using a Potter S homogenizer (Braun, Melsungen, Germany) in ice-cold homogenization buffer (5 mM MOPS, pH 7.4, 250 mM sucrose, 1 mM EDTA, 0.1% ethanol, 0.2 mM PMSF, 1 mM e-aminocaproic acid, and 0.2 mM DTT). After subcellular fractionation, peroxisomes
were sedimented into exponential Metrizamide gradients (Lüers et al., 1993). The peroxisomal fraction with a density of 1.23 g/cm^2 (>95% pure) was used for further experiments. Microtubules were immobilized on coverslips as described (King and Schroer, 2000). Peroxisomes were diluted in final dilution buffer (FDB; 35 mM PIPES, pH 7.2, 5 mM MgSO_4, 1 mM EGTA, 0.5 mM EDTA, 1 mM GTP, 20 μM paclitaxel), mixed with ATP-release or appropriate proteins, and brought to 25 μl with FDB supplemented with 2 mM ATP. Samples were imaged by video-enhanced DIC and data recorded on a S-VHS VCR (Panasonic AG-7300). Movements were tracked and analyzed as in living cells.

RESULTS

A variety of morphologically distinct peroxisomal structures can be visualized by a peroxisome targeted GFP fusion protein

COS-7 and HepG2 cells were transfected with a cDNA construct encoding GFP fused to a peroxisomal targeting sequence (GFP-PTS1) that was previously shown to label peroxisomes in cultured vertebrate cells (Wiemer et al., 1997; Huber et al., 1997) as well as the yeast P. pastoris (Monosov et al., 1996; Kalish et al., 1996). To show that this construct exclusively labeled peroxisomes in both COS-7 and HepG2 cells, we localized GFP-PTS1 fluorescence in comparison to a variety of peroxisomal marker proteins (PMP70, Fig. 1B; acyl-CoA oxidase, and catalase, data not shown) in fixed cells. COS-7 cells contained a heterogeneous mixture of spherical and tubular peroxisomal structures (Fig. 1C). The elongated, tubular organelles often appeared to be segmented and resembled chains of beads on a string. In addition, one to five tight accumulations of tubular peroxisomes could be observed in about 25% of COS-7 cells, similar to what was seen in untransfected cells stained with an antibody to PMP70 (data not shown). These clusters did not appear to have a preferential location within the cell (40-50% perinuclear, 50-60% peripheral). Image deconvolution of peroxisome clusters revealed a network of interconnected, reticular structures surrounded by smaller individual peroxisomes (Fig. 1D-G). Such a reticular structure was first proposed by Lazarow and coworkers (1980) and is supported by serial sectioning EM studies (Gorgas, 1987; Yamamoto and Fahimi, 1987). The present analysis verifies the existence of a peroxisomal reticulum in vivo. As previously reported (Schrader et al., 1994, 1996), HepG2 cells contained primarily elongated, tubular peroxisomes but not interconnected reticular structures (data not shown).

Real time imaging of the peroxisomal compartment

To analyze the dynamic properties of peroxisomes in living cells, COS-7 and HepG2 cells expressing the GFP-PTS1 fusion
protein were imaged in real time. The majority of individual peroxisomes (approx. 90%) in COS-7 and HepG2 cells exhibited short-range oscillatory movements, similar to the microtubule-independent movements described in previous reports (Rapp et al., 1996; Wiemer et al., 1997; Huber et al., 1997, 1999). These non-directed movements were seen for all peroxisomal structures, including tubules, and were not inhibited by nocodazole treatment (data not shown). Short range peroxisome movement was most common in the cell periphery.

The remaining subset (approx. 10%) of individual peroxisomes underwent fast, directional movement. As previously described (Rapp et al., 1996; Wiemer et al., 1997; Huber et al., 1997), these movements could be blocked completely by nocodazole indicating that they were microtubule dependent (see below). Single, unidirectional saltations were most common but bi-directional movements were sometimes observed. Both spherical and tubular peroxisomes could be seen to move, and although the movements of spherical structures were more common, the velocities (range: 0.2-1.1 μm/second, mean: 0.6±0.3 μm/second) and run lengths (range: 0.6-9.7 μm, mean: 2.8±1.6) of individual movements were similar for the two populations. Elongated peroxisomes were observed to translocate over relatively long distances which is also typical for other tubular organelles, such as tubular late endosomes in COS-7 cells (Valetti et al., 1999). Segmented organelles could also move along directed or curved trajectories, whereas highly elongated tubules (>3 μm) and interconnected peroxisomes did not. The motile peroxisomes in HepG2 cells translocated at similar velocities (mean: 0.6 μm/second) and over comparable distances (ranged up to 10 μm) as in COS-7 cells. Peroxisome velocities and distances traveled were comparable to the motile properties of other intracellular organelles, for example endosomes and lysosomes in COS-7 cells (Valetti et al., 1999) or WIF-B hepatocytes (M. Schrader et al., unpublished).

Peroxisome movement could be accompanied by drastic shape changes emphasizing the transient nature and variability of individual organelles. Elongated peroxisomes were able to shorten, collapse into round structures and then elongate again (Fig. 2A). Moving peroxisomes also appeared to interact with other peroxisomes. A small fraction (approx. 10-15%) of fast, directed motile events observed within each focal plane led to an apparent interaction with another peroxisome. Some peroxisomes passed close by others and appeared to engage in transient contacts before continuing movement (Fig. 2A,C).

Peroxisomal aggregates in COS-7 cells (Fig. 3A) underwent robust short-range movement, giving the impression of a peroxisomal ‘snake pit’. When analyzed in greater detail we

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**Fig. 2.** Images of the motile behavior of individual peroxisomes in vivo. Peroxisomal movements can be accompanied by drastic changes in peroxisomal shape. (A) A peroxisome (arrowhead) moves to the right of the image before it elongates to interact with another peroxisome (*, see 9.9 panel). Afterwards it retracts and collapses into a round structure (15.2 panel) before it starts to move again showing a segmented appearance. (B) A moving peroxisome ceases motility when it comes into close proximity with a group of peroxisomes (arrow). (C) A rod-shaped peroxisome (arrowhead) moves toward a small spherical peroxisome (arrow) and interacts with it. The two peroxisomes stay in contact while moving (17.3 panel to 49.6 panel) before separating again (72.7 panel). After separation, the rod-shaped peroxisome continues movement to the right of the image. Elapsed time is in seconds. Bar, 5 μm for all panels.
observed the formation and/or scission of thin tubules of the peroxisomal network (Fig. 3A). These images provide the first insight into the dynamic behavior of the peroxisomal reticulum.

**Effects of dynamitin overexpression on peroxisome behavior in vivo**

To examine the roles of microtubules and the minus end-directed, microtubule-based motor, cytoplasmic dynein, in peroxisome movement, we disrupted microtubule and cytoplasmic dynein function singly and in combination. As expected (Rapp et al., 1996; Wiemer et al., 1997; Huber et al., 1997), peroxisomes in control cells exhibited fast, directional movements that were inhibited by nocodazole treatment (Fig. 3B), confirming that these movements are microtubule-based. We next examined the role of cytoplasmic dynein based motility in peroxisomal movements. Cytoplasmic dynein function was disrupted by overexpressing the dynamitin subunit of the dynein activator, dynactin, (Echeverri et al., 1996; Wittmann and Hyman, 1999). When COS-7 cells were cotransfected with plasmids encoding the GFP-PTS1 fusion protein and HA-tagged dynamitin, we found that more than 90% of cells expressing one protein also expressed the other. Over 75% of the dynamitin expressing cells were found to have dispersed Golgi complexes (not shown), which is indicative of dynactin disruption (Burkhardt et al., 1997; Valetti et al., 1999). When these cells were imaged in real time, linear movements of peroxisomes were reduced to approximately 20% of control levels (Fig. 3B). Similar results were obtained when a function-blocking antibody to the cytoplasmic dynein intermediate chain was microinjected into GFP-PTS1 expressing COS-7 cells. Microinjected control antibodies used at the same concentration had no effect (Fig. 3B.

We used HepG2 cells to further study the effects of dynamitin overexpression on peroxisome shape and distribution. The cells were transfected with HA-tagged dynamitin and examined using antibodies to HA and the peroxisomal membrane protein PMP70. In both control cells and cells overexpressing dynamitin, peroxisomes showed the normal range of morphologies and appeared to be uniformly distributed throughout the cytoplasm (Fig. 4A,B). In a small fraction (15%) of dynamitin overexpressing cells the peroxisomes appeared to be more concentrated than normal near the center of the cell.

As described previously, HepG2 cells treated for long periods of time with microtubule-depolymerizing agents can develop peroxisome aggregates (Schrader et al., 1996, 1998a). When treated with nocodazole, many dynamitin overexpressing cells also developed peroxisomal clusters (Fig. 4C,D). When nocodazole was removed, all of the peroxisomal clusters redistributed into the normal pattern in the control cells. In contrast, only about 50% of the cells with peroxisomal clusters showed redistribution into the normal pattern when dynamitin was overexpressed (Fig. 4E,F). This suggests that dynamitin function (and by extension, cytoplasmic dynein based motility) is not required to maintain uniform peroxisome distribution under normal circumstances, but is required for the reestablishment of a uniform peroxisome distribution after microtubule depolymerization and regrowth.

**Isolated peroxisomes move along microtubules in vitro**

Previous efforts to reconstitute peroxisome movement on microtubules in vitro were unsuccessful; in the presence of ATP and cytosol from various cell types, peroxisomes could be observed to bind microtubules but did not move (Schrader et al., 1996). Microtubule binding appeared to be dependent on proteins that were peripherally associated with the peroxisome membrane. When a motor-enriched fraction prepared from chick embryo brain (the so-called ‘ATP release’, see Schroer...
and Sheetz, 1991) was used instead of cytosol, isolated peroxisomes could be seen to bind and translocate along microtubules (Fig. 5) at a velocity of 0.3±0.1 μm/second. The reduction in velocity of in vitro movements relative to in vivo movements likely reflects the fact that these experiments were performed at 25°C in vitro versus 37°C in vivo. Bidirectional movements were sometimes observed (Fig. 5). Purified dynein alone, or the combination of purified dynein plus dynactin isolated from bovine brain did not support peroxisome movement, suggesting that other cellular components were required. When peroxisomes were incubated with the enriched motor fraction and re-isolated by centrifugation, they contained detectable amounts of dynactin but not dynein or kinesin (data not shown). This observation, in conjunction with the motility assay, suggests that components of the dynactin complex can bind to isolated peroxisomes in vitro and support dynein-dependent motility.

**DISCUSSION**

In this study, we examined the dynamic behavior of complex peroxisomal structures labeled with GFP-PTS1 by high magnification video-enhanced fluorescence microscopy at real time video rates. We have shown that the peroxisomal compartment is heterogeneous as well as very dynamic in live cells and provide first evidence for the existence of a peroxisomal reticulum in vivo. Our data indicate that peroxisomes are engaged in transient contacts and are likely to interact with each other. Furthermore, our in vitro and biochemical analysis of peroxisomal motility on microtubules suggests a role for dynein and dynactin in this process.

**The reticular network of peroxisomes**

Our observations with GFP-PTS1 labeled COS-7 cells revealed the existence of tubulo-reticular networks of peroxisomes in living cells that are of moderate frequency, highly dynamic, undergoing morphological changes, and transient in nature. Such networks were first proposed by Lazarow et al. (1980) and have been described by serial section reconstruction in lipid synthesizing epithelia (Gorgas, 1987) and regenerating rat liver (Yamamoto and Fahimi, 1987), but a reticulum had not been observed so far under culture conditions. The reticulum in COS-7 cells represents an accumulation of interconnected
Dependent manner, but typical fission events like the segmentation of tubular structures (Schrader et al., 1996, 1998b) were not detected. A role in the regulated degradation of peroxisomes seems unlikely, because lysosomes and endocytic structures were not observed to colocalize or to interact with reticular peroxisomes. If the main function is indeed a metabolic one, it seems reasonable to assume that the reticulum is able to exchange matrix- and/or membrane components via membrane fusion events.

**Do peroxisomes move to interact with each other?**

With approximately 10% of the total peroxisome population of a given cell exhibiting microtubule-based movements, peroxisomal motility in COS-7 and HepG2 cells is a low frequency event. This is consistent with other studies on CHO and CV-1 cells (Rapp et al., 1996; Wiemer et al., 1997) and is likely to be a general feature of microtubule-based peroxisomal motility. What could be the physiological relevance of these motile events that occur with low frequency but allow translocation over long distances? Surprisingly, moving peroxisomes appeared to interact with other peroxisomes via both transient and long term contacts. It is well known that the peroxisomal population in a given cell shows some degree of biochemical heterogeneity (Yamamoto and Fahimi, 1987; Heinemann and Just, 1992; Lüers et al., 1993; Schrader et al., 1994; Wilcke et al., 1995). Therefore, peroxisomal translocation might be important to reach new ‘neighborhoods’ of organelles in other areas of the cytoplasm. Peroxisomes could then interact to exchange substrates or other proteins and adapt their metabolic functions accordingly. Recent findings even support the possibility of membrane fusion acting in the assembly of peroxisomes in yeast (Titorenko et al., 2000). Further studies are required to elucidate the nature of the peroxisomal interactions and to identify components of a putative fusion machinery.

**The role of microtubules in the positioning and formation of peroxisomes**

Peroxisomes bind to and move along microtubules as demonstrated by our in vitro assay and supported by colocalization and drug treatment studies (Schrader et al., 1996; Rapp et al., 1996; Wiemer et al., 1997). It is therefore very likely that movement along and binding to microtubules guarantees the uniform distribution of peroxisomes throughout the cytoplasm. In contrast to microtubule depolymerization, dynemin inactivation has little effect on the intracellular distribution of peroxisomes, which is consistent with recent results using Hela cells (Burckhardt et al., 1997). This indicates the involvement of microtubule-associated proteins (MAPs) other than motor proteins in the tethering of peroxisomes to microtubules. It should be emphasized that microtubules are not essential for the formation, maintenance and dynamics of tubular (Schrader et al., 1996, 1998a) or reticular peroxisomes. This is quite different from other organelles exhibiting tubular or reticular shapes such as mitochondria (Johnson et al., 1980) or lysosomes (Swanson et al., 1987) which collapse or fragment after microtubule-depolymerization.

**The role of dynactin in peroxisomal movement**

At present, the molecular basis of peroxisomal movement is poorly understood. We could inhibit peroxisomal motility by the overexpression of the dynamitin subunit of dynactin, which...
causes the destruction of dynactin and the concomitant loss of cytoplasmic dynein function (Echeverri et al., 1996), and by microinjecting a function-blocking antibody to DIC. Furthermore, overexpression of dynamin interfered with the reestablishment of a uniform peroxisomal distribution after recovery from nocodazole-based microtubule depolymerization. These data suggest a role for dynemin and dynein in peroxisome motility. However, the involvement of other motor proteins, e.g. kinesin, is likely, since dynamin overexpression does not inhibit peroxisomal movements completely, and about 50% of nocodazole-induced peroxisomal clusters redistribute to some degree in dynamin (p50) overexpressing cells. Alternatively, redistribution in overexpressing cells could be due to tethering of peroxisomes to repolymerizing and spreading microtubules. Additional factors, most likely regulatory microtubule-associated proteins and cytoplasmic linker proteins (CLIPs), might be required to switch from static microtubule binding to microtubule based movement. It will be a great challenge for future investigations to characterize further components of the tethering and translocation machinery and to determine how they are regulated.

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Dynamic behavior of the peroxisomal compartment


