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Visualization of Peroxisomes in Living Plant Cells Reveals Acto-Myosin-Dependent Cytoplasmic Streaming and Peroxisome Budding

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Here we examine peroxisomes in living plant cells using transgenic *Arabidopsis thaliana* plants expressing the green fluorescent protein (GFP) fused to the peroxisome targeting signal 1 (PTS1). Using time-lapse laser scanning confocal microscopy we find that plant peroxisomes exhibit fast directional movement with peak velocities approaching 10 μm s^{-1}. Unlike mammalian peroxisomes which move on microtubules, plant peroxisome movement is dependent on actin microfilaments and myosin motors, since it is blocked by treatment with latrunculin B and butanedione monoxime, respectively. In contrast, microtubule-disrupting drugs have no effect on peroxisome streaming. Peroxisomes were further shown to associate with the actin cytoskeleton by the simultaneous visualization of actin filaments and peroxisomes in living cells using GFP-talin and GFP-PTS1 fusion proteins, respectively. In addition, peroxisome budding was observed, suggesting a possible mechanism of plant peroxisome proliferation. The strong signal associated with the GFP-PTS1 marker also allowed us to survey cytoplasmic streaming in different cell types. Peroxisome movement is most intense in elongated cells and those involved in long distance transport, suggesting that higher plants use cytoplasmic streaming to help transport vesicles and organelles over long distances.

Key words: Actin — Cytoplasmic streaming — GFP — Myosin — Peroxisome — Phloem.

Abbreviations: GFP, green fluorescent protein; ER, endoplasmic reticulum; PTS1, peroxisome targeting signal-1.

Introduction

The movement of organelles is an essential function of both actin microfilament- and microtubule-based cytoskeletal systems. Cargo-associated molecular motors in the kinesin, dynein and myosin superfamilies use the energy generated from ATP hydrolysis to power movement along these cytoskeletal tracks (Kamal and Goldstein 2000). This system is used to move organelles to specific places within the cell, thus enhancing the efficiency of directional transport that would otherwise be limited by the rate of diffusion. For example, in mammalian cells, microtubules are used to transport vesicles derived from the trans-Golgi to sites of exocytosis at the plasma membrane (Wacker et al. 1997, Kreitzer et al. 2000) and both retrograde and anterograde axonal transport depends on microtubules (Kamal and Goldstein 2000). In the yeast *Saccharomyces cerevisiae*, post-Golgi secretory vesicles are targeted to a specific cortical domain of the plasma membrane through the actin cytoskeleton (Johnston et al. 1991, Karpova et al. 2000) that is also required for the inheritance of various subcellular organelles, including vacuoles and mitochondria (Catlett and Weisman 2000). Mitochondria are also frequently observed as a dynamic reticulum, and in some systems both movement and structure of mitochondria depends on the acto-myosin cytoskeleton (Suelmann and Fischer 2000).

Plant cells exhibit a rapid form of organelle movement known as cytoplasmic streaming which has been extensively studied in giant characean algae (Williamson 1993) and aquatic plants (Nagai 1993). Different cell types display different forms of cytoplasmic streaming (Allen and Allen 1978); however, in all cases movement is rotational or bidirectional. Thus, unlike other forms of cytoskeleton-dependent organelle movement, cytoplasmic streaming is not inherently unidirectional. This process is usually mediated by actin microfilaments and organelle-associated myosin motors (Williamson 1993, Nagai 1993, Staiger et al. 1994, Bradley 1973) and is considered important in large cells which need to overcome the constraints imposed by diffusion (Williamson 1993). In this case, because movement caused by cytoplasmic streaming lacks a single directionality, it is assumed to enhance directional transport by increasing the probability of interaction between organelles, proteins and solutes.

Although plant peroxisomes have been extensively studied (Hayashi et al. 2000a, Olsen 1998), little is known about their dynamic behavior. In addition to their role in fatty acid oxidation and peroxide metabolism, plant peroxisomes perform cell-specific functions; leaf peroxisomes participate in the reactions associated with photorespiration and root peroxisomes can participate in nitrogen metabolism (Olsen 1998, Hayashi et al. 2000b). Here we examine the dynamics of higher-plant peroxisomes. Unlike animal-cell peroxisomes, which move on mictrotubules (Rapp et al. 1996, Wiemer et al. 1997), we find that plant peroxisomes rapidly move on actin microfilaments. In addition, we have observed peroxisome streaming in different cell types; streaming is most intense in

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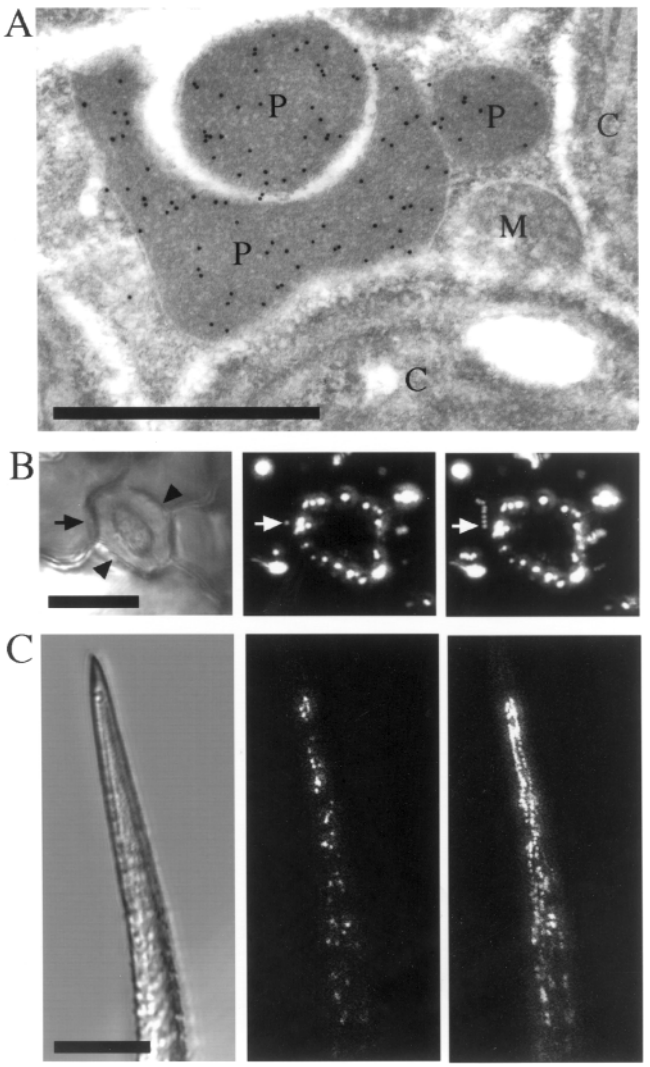

Fig. 1 Peroxisomes in transgenic plants expressing GFP-PTS1. (A) Localization of GFP-PTS1 protein to peroxisomes in a leaf mesophyll cell by immunoelectron microscopy using anti-GFP antibodies followed by 10-nm gold particles. P, peroxisome; M, mitochondria; C, chloroplast. Bar, 1 μm. (B) Peroxisomes in leaf guard and mesophyll cells. Left panel, Nomarski control; middle panel, first image of the series; right panel, seven consecutive images taken at 1-s intervals. Note that peroxisomes are stationary in guard cells (arrow heads). The arrow shows one peroxisome in a mesophyll cell that is moving. Bar, 25 μm. (C) Peroxisome streaming in a single branch of the trichome. Left panel, Nomarski control; middle panel, the first image of the series; right panel, seven consecutive superimposed images. Note that movement is most intense near the trichome tip. Bar, 25 μm.

elongated cells and those involved in long distance transport. Thus, like giant algae (Shimmen and Yakota 1994), most large cells in higher plants probably use cytoplasmic streaming as a means of transporting organelles, vesicles and solutes over long distances.

Results

Peroxisome labeling and movement in living plant cells

To examine peroxisome structure and dynamics in living plants, we generated transgenic Arabidopsis plants to express a GFP-PTS1 fusion protein from the constitutive cauliflower mosaic virus 35S promoter. All cell types examined displayed a punctate pattern of fluorescence indicative of targeting to a subcellular compartment. Previous work has shown that a GFP fusion to the PTS1 signal from hydroxypyruvate reductase (a leaf peroxisomal protein) is properly targeting to the plant peroxisome (Mano et al. 1999). To confirm that this is also the case for our GFP-PTS1 fusion protein, we used anti-GFP antibodies and immunoelectron microscopy. In transgenic but not control plants, dense labeling was observed over the matrix of leaf peroxisomes but not cytosol, mitochondria or chloroplasts (Fig. 1A). These data show that the GFP-PTS1 protein is properly targeted to peroxisomes as it is in yeast (Monosov et al. 1996) and mammalian systems (Wiemer et al. 1997).

To document the behavior of peroxisomes, laser-scanning confocal microscopy was used to gather a series of single optical sections over time. This series of images was superimposed and presented next to the first image of the sequence (Fig. 1B, C and Fig. 2A–D). Viewed in this way, moving peroxisomes exhibit tracks where the distance between dots corresponds to the instantaneous velocity over a single time interval. All cell types displayed varying degrees of peroxisome streaming and, in general, this streaming was more intense in elongated cells such as trichomes (Fig. 1C) and cortical cells in the hypocotyl of dark-grown seedlings (Fig. 2A) compared to smaller cells such as guard cells or leaf mesophyll cells (Fig. 1B). To examine the nature of peroxisome movement in more detail, we observed cortical cells in the hypocotyl and acquired 10 images at 1-s intervals (Fig. 2A). Peroxisomes show variable behavior; some are stationary (Fig. 2A, arrows) whereas others are moving rapidly (Fig. 2A, arrow heads). To quantify the behavior of moving peroxisomes, we selected several clear traces (Fig. 2E) and plotted their instantaneous velocity over nine 1-s intervals (Fig. 2F). These results show that peroxisomes undergo periods of acceleration and deceleration with a maximum and minimum velocity of 6 μm s^{-1} and 0 μm s^{-1}, respectively. Both the velocity and saltatory nature of peroxisome movement is reminiscent of recently described behavior of the plant Golgi apparatus (Boevink et al. 1998, Nebenfuhr et al. 1999), suggesting that these organelles may utilize a related mechanism of movement.

Molecular basis of peroxisome movement

To determine the molecular basis of peroxisome movement we employed a series of drugs that disrupt specific components of the cytoskeleton. Treatment of seedlings for 30 min with the actin inhibitor latrunculin B (1 μM) abolished cyto-

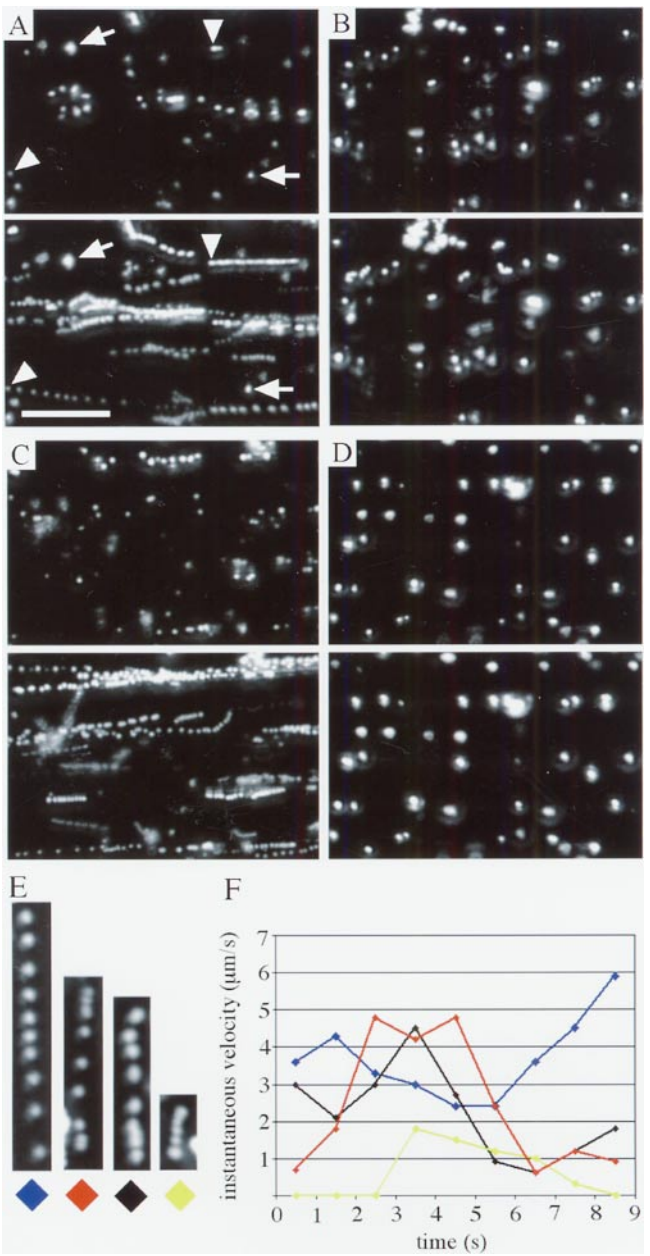

Fig. 2 Peroxisome streaming in hypocotyl cortical cells and inhibition by pharmacological agents. Observations were made at approximately the midpoint of the hypocotyl and images were rotated to place the long axis of the hypocotyl in a horizontal orientation. In A–D, first image in the time-series (upper panels) and superimposition of 10 consecutive images (lower panels) are shown for each condition. In A, arrows indicate stationary peroxisomes and arrowheads indicate moving peroxisomes. Bar, 25 µm. (A) Untreated seedlings. (B) Seedlings treated with 1 µM latrunculin B. (C) 3.3 µM nocodazol. (D) 30 mM butanedione monoxime. (E) Appearance of four peroxisome tracks extracted from A. (F) Graphical representation of instantaneous velocity at 1-s intervals. Each curve corresponds to one of the peroxisome tracks shown in E as indicated by color coding.

plasmic streaming (Fig. 2B) so that over a 10-s time interval peroxisomes displayed only Brownian motion, resulting in a slightly larger apparent diameter when ten frames are superimposed (Fig. 2B, compare upper and lower panel). Similar results were obtained when we used the myosin ATPase inhibitor butanedione monoxime (Herrmann et al. 1992) at 30 mM (Fig. 2D). On the other hand, microtubule disruption using 3.3 µM nocodozol (Fig. 2C) or 1–10 µM propyzamide (data not shown) had no effect on peroxisome streaming. Together, these data suggest that plant peroxisome movement requires intact actin filaments and is probably propelled by myosin motors.

To further demonstrate that peroxisomes move on the actin cytoskeleton, we observed peroxisomes and actin filaments simultaneously. To accomplish this, onion peel epidermal cells were co-bombarded with DNA constructs encoding GFP-talin (Kost et al. 1998) and GFP-PTS1 fusion proteins. In co-bombarded cells, actin filaments were observed as a fine network of interconnected filaments and peroxisomes were observed as spheres or short rods. Peroxisomes were also readily distinguished from actin filaments by their level of fluorescence signal that was gated to produce saturation (Fig. 3, red dots). A single image demonstrates that most peroxisomes appear associated with actin filaments (Fig. 3, upper panels) and a time series shows that they move along lines precisely defined by these filaments (Fig. 3, lower panels). As a control, we observed microtubules and peroxisomes using a Map4-GFP (Marc et al. 1998) and GFP-PTS1 fusion proteins, respectively. Microtubules were observed as a network at the cell cortex and were not observed in the transvacuolar cytosolic strands that contain microfilaments and moving peroxisomes (data not shown), further suggesting that microtubules and peroxisomes are neither functionally nor physically associated in plant cells.

Taken together, these data show that plant peroxisome movement is driven by the acto-myosin cytoskeleton.

Vesicular dynamics of the plant peroxisomal compartment

We next examined the fine structure of plant peroxisomes (Fig. 4). Moving peroxisomes exhibited an array of dynamic behavior. Fig. 4A shows a time series documenting the fission of a stationary peroxisome in a cortical cell of the hypocotyl. Over the first 1-s interval, a single peroxisome is distorted into an oblong shape suggesting the presence of a pulling force. Fission of this structure occurs over the next time interval, partitioning a portion of the GFP signal into a new vesicle that accelerates as it moves away. Similar types of behavior were also observed in leaf peroxisomes (Fig. 4C); leaf peroxisomes undergo fission events and also produce fine tubular projections that appear to be pulled from the body of the peroxisome (Fig. 4C). Close examination of a budding event (Fig. 4D) shows that a bulge in the peroxisome membrane precedes vesicle formation, which is immediately followed by vesicle movement (Fig. 4D). In addition, peroxisomes could be seen moving in different directions along the same trajectory (Fig. 4B),

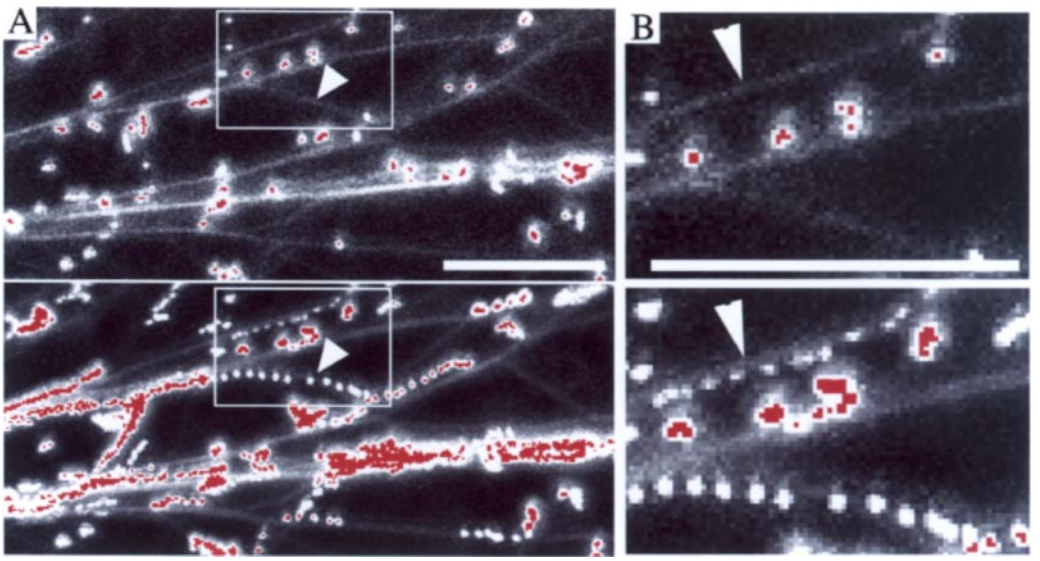

Fig. 3 Peroxisomes move along actin microfilaments. Peroxisomes are seen in association with actin filaments in onion peel epidermal cells co-bombarded with GFP-talin and GFP-PTS1 constructs. Upper panels, first frame of the time-series; lower panels, superimposition of 10 consecutive images taken at 1-s intervals. Arrowheads point to actin microfilaments along which peroxisomes are moving. Bar, 25 μm. (A) A low magnification of a region at the cell periphery. (B) A magnified view of the boxed region shown in A.

suggesting that, in Arabidopsis, actin cables contain microfilaments of opposing polarity.

Peroxisome streaming in the root phloem

During initial analysis of plants expressing *GFP-PTS1* from the 35S promoter, we obtained some transgenic lines that displayed enhanced expression of GFP-PTS1 in the root vasculature. In these lines, peroxisomes displayed the most rapid movement that we observed in seedlings, with peak velocities approaching 9 μm s^{-1} (Fig. 5). This movement was bidirectional with roughly equal numbers of peroxisomes moving up or down along the long axis of the root (Fig. 5D). Cells displaying this behavior lie between parallel xylem elements (Fig. 5A, arrows), suggesting that they correspond to the sieve tube elements in the phloem. To test this possibility, we employed the phloem-specific *SUC2* promoter which has been shown to direct GFP expression to companion cells and sieve tube elements (Truernit and Sauer 1995). Plants expressing *GFP-PTS1* from this promoter exhibited peroxisome streaming that was localized to a central cylinder of the root (Fig. 6A) and we used these seedlings to map the developmental regulation of peroxisome streaming in the phloem. This was accomplished by collecting a time-series at positions in the root phloem corresponding to different developmental stages (Fig. 6A, B). To quantify this data, a set of peroxisomes was selected at random and each time-series was animated so that peroxisome movement could be assessed. Movement was scored as positive if a peroxisome was fully displaced from its original position after 10 s and the data are presented as a percentage of the total number of peroxisomes examined in each sequence (Fig. 6C). In the meristem, peroxisomes show a low level of movement. However, in the elongation zone peroxisomes begin exhibiting vigorous movement. Cell elongation diminishes in the differentiation zone (determined by the appearance of root hair; Fig. 6A, right panel) but peroxisomes continue to show a high level of movement that is maintained for several millimeters and then gradually diminishes with distance from the meristem (Fig. 6C). These data further confirm the positive correlation of cell length and peroxisome streaming. In addition, they suggest a role for cytoplasmic streaming in functioning of the phloem.

Discussion

Plant peroxisomes move on actin microfilaments

We have investigated the dynamics of peroxisomes in living plant cells and tissues. Our results are significant in two respects. First, they provide new insights into plant-cell specific aspects of peroxisome behavior; second, the excellent signal achieved with the GFP-PTS1 fusion protein has allowed us to observe cytoplasmic streaming in hitherto unexamined cell types.

Peroxisome movement in plants depends on an intact acto-myosin cytoskeleton but not on microtubules (Fig. 2) and plant peroxisomes were further shown to associate with actin microfilaments in living cells (Fig. 3). This is in contrast to mammalian peroxisomes which move on microtubules with peak velocities in the range of 0.75 μm s^{-1} (Wiemer et al. 1997) to 2 μm s^{-1} (Rapp et al. 1996), whereas our observations of Arabidopsis peroxisomes indicate peak velocities near 10 μm s^{-1}.

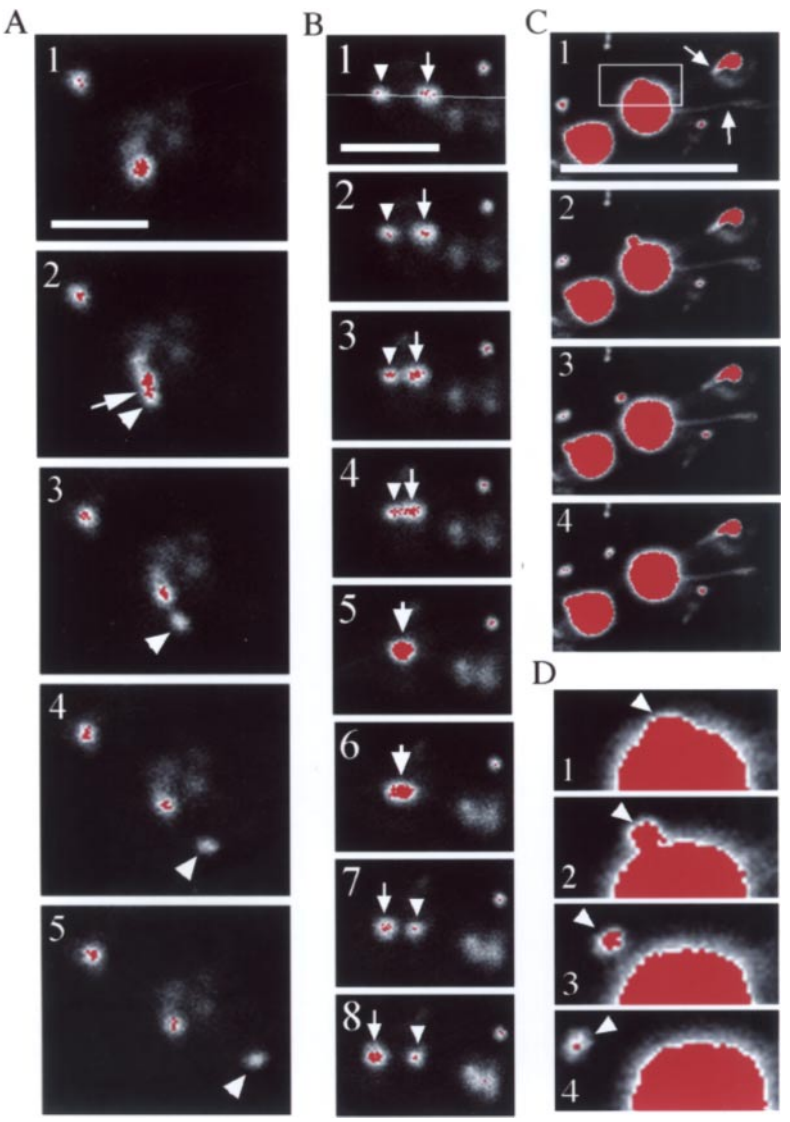

Fig. 4 Peroxisome dynamics in various cell types. (A) In a hypocotyl cortical cell a stationary peroxisome (arrow) produces a vesicle (arrowhead) which moves away following budding. Bar, 10 μm. (B) Two peroxisomal vesicles (arrow and arrowhead) in a hypocotyl cortical cell move towards one another and apparently fuse (large arrow) and then separate. The line in frame 1 shows the deduced trajectory of movement. Bar, 10 μm. (C) Structure and dynamics of leaf-mesophyll peroxisomes. A group of peroxisomes of differing size can be seen, one of them (white box) producing a vesicle that moves away. Note the fine tubules that are produced from two of the larger peroxisomes (Frame 1, arrows). Bar, 25 μm. (D) The region boxed in part C shown at a higher magnification. The arrowhead follows the process of vesicle budding. In A–D numbering of frames corresponds to single optical sections collected at 1.08-s intervals.

The available evidence suggests that most plant organelles participate in cytoplasmic streaming. The plant Golgi apparatus also moves on the acto-myosin cytoskeleton and shows streaming dynamics similar to those described herein for peroxisomes (Boevink et al. 1998, Nebenfuhr et al. 1999). Since the ER is also associated with the actin cytoskeleton, it is possible that myosin-propelled Golgi movement is required for efficient ER-to-Golgi transport (Hawes et al. 2000, Nebenfuhr et al. 1999). Similarly, it will be interesting to determine if proteins involved in peroxisomal protein import or proliferation are also associated with the actin cytoskeleton. Consistent with this possibility, targeting of the peroxisomal membrane protein ascorbate peroxidase has recently been shown to proceed through an intermediate reticular network resembling a subdomain of the ER (Mullen et al. 1999).

Plant cells are frequently extremely large; cells in the hypocotyl of Arabidopsis are up to 1 mm long (Gendreau et al. 1997) and trichomes as well as root cells can achieve similar dimensions. Cytoplasmic streaming has been extensively studied in giant characean algae cells, where it is assumed to mix

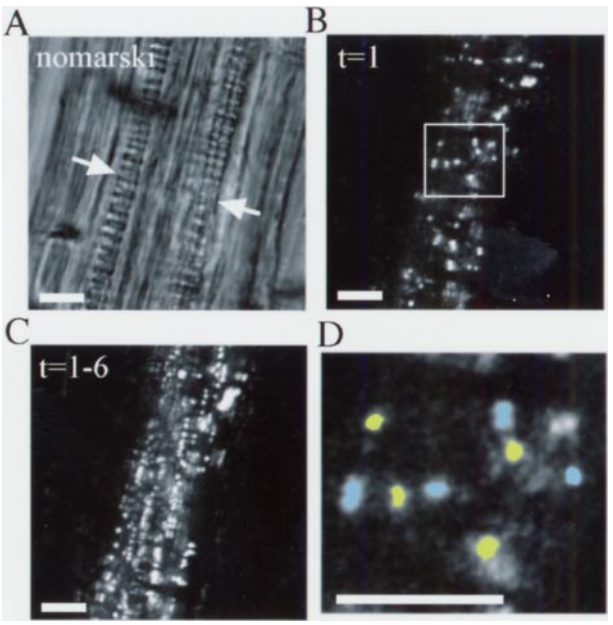

Fig. 5 Bidirectional peroxisome streaming in the root vasculature. Bar, 10 μm. (A) Nomarski image shows xylem elements. (B) First image of the time-series (t=1). (C) Superimposition of six images taken at 0.56-s intervals. (D) The boxed region shown in B is magnified and peroxisomes are color-coded according to whether they are moving toward aerial tissues (blue) or root tip (yellow).

cytoplasmic contents and thus affect the efficient distribution of organelles and solutes over large distances (Williamson 1993). Our observations of peroxisome streaming in Arabidopsis are consistent with this assumption as we observe a general correlation between the degree of peroxisome streaming and cell size (Fig. 1B, C, Fig. 6). The examination of other organelles at the whole plant level is needed to show that the relationship we observe between cell size and peroxisome streaming also applies to other organelles. A similar correlation has also been observed for Golgi movement in cultured tobacco BY-2 suspension cells, where Golgi streaming is less active in young small cells than in older large cells (Nebenfuhr et al. 1999). Organelles known to move on actin filaments or exhibit cytoplasmic streaming in plant cells include the Golgi apparatus (Boevink et al. 1998, Nebenfuhr et al. 1999), the ER (Hawes et al. 2000, Liebe and Menzel 1995), plastids (Liebe and Menzel 1995) and mitochondria (Nebenfuhr et al. 1999, Kachar and Reese 1988). Each of these organelles may have a specific associated myosin motor; alternatively, the myosin-dependent movement of the ER may provide sufficient force to move organelles that are not directly attached to the acto-myosin system (Kachar and Reese 1988). A plant-specific family of myosin motors has been isolated from Arabidopsis (Kinkema et al. 1994) and their future subcellular localization should help resolve this issue.

Actin microfilaments show a parallel orientation to the direction of growth in elongated plant cells such as trichomes (Mathur et al. 1999), pollen tubes (Taylor and Hepler 1997), root and hypocotyl cells (Kost et al. 1998). The bidirectional cytoplasmic streaming we observed in trichome and hypocotyl cells is similarly oriented, consistent with a model where cytoplasmic streaming non-specifically delivers organelles and vesicles to regions of active growth. Disruption of actin microfilaments abolishes polarized growth in many of these systems (Mathur et al. 1999, Taylor and Hepler 1997). However, because the actin cytoskeleton has other functions in addition to its role in cytoplasmic streaming, the importance of this role in polarized growth will need to be assessed using mutants specifically disrupted in this process. Plant peroxisomes can move in opposite directions along the same trajectory (Fig. 4B), suggesting that higher-plant actin cables can contain microfilaments of opposing polarity. Interestingly, we did not observe this type of bidirectional movement in onion peel epidermis (data not shown), and actin cables in giant algae are probably composed of uniformly oriented microfilaments (Palevitz and Hepler 1975). Thus, in plants, this aspect of actin architecture may be under cell-type specific control.

Dynamic behavior of plant peroxisomal vesicles

Plant peroxisomal vesicles exhibit an array of dynamic behaviors. Peroxisomes undergo budding events that are followed by vesicle movement, suggesting an involvement of the cytoskeletal in the mechanism of vesicle budding. In this case, the force exerted by a peroxisome-associated myosin motor might pull vesicles from stationary (anchored) peroxisomes (see Fig. 4A, C, D). Peroxisomes also produce dynamic tubular projections that can extend up to several micrometers from the body of the peroxisome (Fig. 4C). These types of structures have also recently been reported for GFP fusions to full-length peroxisome markers (Cutler et al. 2000). The function of these projections in peroxisomes is currently unknown. One possibility is that they increase the surface-to-volume ratio of the peroxisome and thus enhance access to cytosolic metabolites. Alternatively, they may represent intermediates in peroxisome proliferation. Indeed, the peroxisome fission that we observe suggests that fission of pre-existent peroxisomes is one mechanism for the proliferation of plant peroxisomes.

Cytoplasmic streaming in the root phloem

The phloem functions as a conduit for the directional translocation of photosynthetic assimilates and various other metabolites from source to sink tissues. Translocation in the phloem is presumed to occur by a bulk-flow mechanism that is driven by an osmotically generated pressure gradient (Munch 1930, Oparka and Santa Cruz 2000). However, the operation of additional mechanisms has not been excluded. Our observations using GFP-PTS1 fusion protein show that peroxisomes are maintained in sieve elements well after their terminal differentiation. We find that peroxisomes in the phloem meristem are relatively stationary; however, as cells begin to elongate,

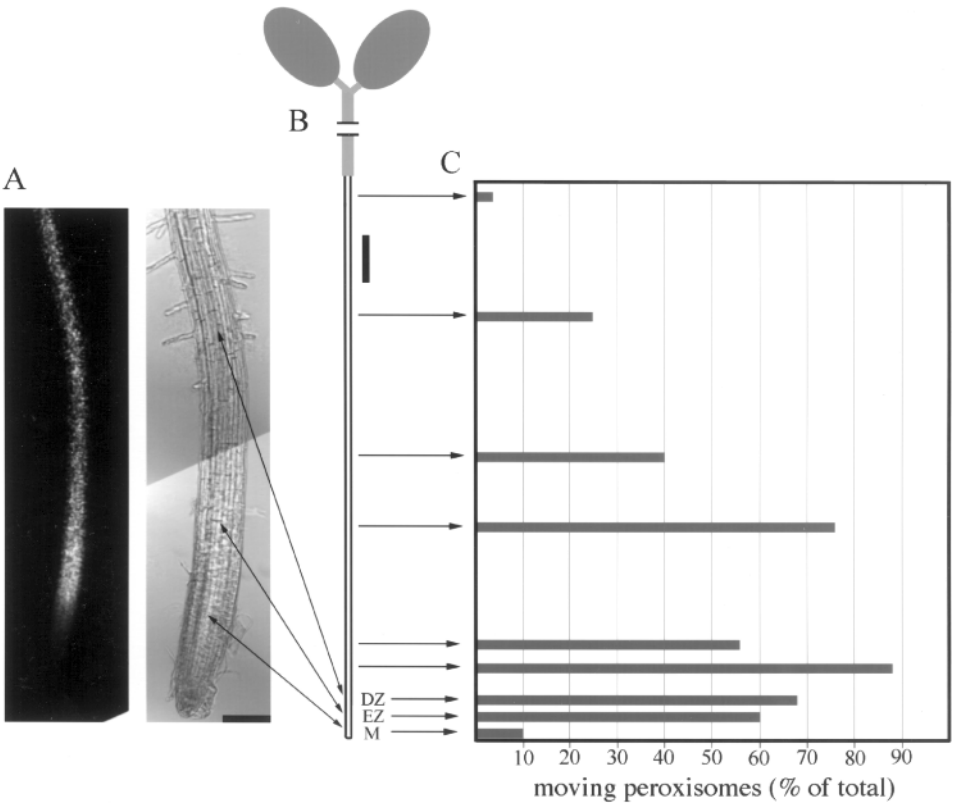

Fig. 6 Developmental regulation of peroxisome movement in the root phloem. Peroxisome movement was examined at various points along the root of a 10-day-old seedling expressing the *GFP-PTS1* cassette from the phloem specific *SUC2* promoter. (A) Fluorescence (left panel) and Nomarski (right panel) views of the root in the meristem (M), elongation (EZ) and differentiation zones (DZ). Bar, 50 μm. (B) A scale cartoon of the 10-day-old seedling examined. Bar, 1 mm. (C) Peroxisome movement in the phloem. Graph shows the percentage of peroxisomes which moved during a 10-s time-series at each of the positions of B as indicated by arrows. The data shown were derived from the observation of one seedling and are typical of several seedlings that were investigated.

peroxisomes begin rapid bidirectional movement along the long axis of the root. In this case peroxisome streaming is not a simple consequence of rapid cell elongation since vigorous streaming is maintained well after cell elongation ceases (Fig. 6). Because sieve elements are very narrow in the Arabidopsis root (Dolan et al. 1993), it is currently unclear whether peroxisome movement is unidirectional or bidirectional in individual cells. The degree of cytoplasmic streaming is not uniform along the length of the root (Fig. 6); therefore, this process alone cannot account for assimilate translocation. However, cytoplasmic streaming in the root phloem may enhance the efficiency of symplastic phloem unloading by mixing assimilates in the region of a steep concentration gradient associated with their unloading into the root meristem. In addition, various cytoplasmic organelles may function as carriers in this system. In the future, the isolation of mutants in root-phloem specific components of the acto-myosin cytoskeleton may provide a test of this model; specific disruption of cytoplasmic streaming in the root phloem is expected to diminish but not abolish assimilate transport from aerial tissues to the root meristem.

Materials and Methods

Plant growth

Surface-sterilized seedlings were grown in 1× Murashige and Skoog (MS) media plates in the absence of sucrose. For experiments examining the hypocotyl, seedlings were grown for 3–4 d in the dark and then transferred to a drop of 1× liquid MS medium on a coverslip for observation. For Fig. 6, the root was further sandwiched between a second coverslip to bring the entire length of the root to the same focal plane. For the examination of trichomes and leaf cells, seedlings were grown for 10–14 d in white light and then inverted onto a coverslip, maintaining the roots in a drop of MS medium.

Plasmids and plant transformation

The *GFP-PTS1* cassette was made using the primers eGFP.n1 (GCT AGC GCT ACC GGA CTC A) and eGFP.n1.PTS1 (CGC GAA TTC TTA GAG GCG GGA CTT GTA CAG CTC GTC CAT GCC) the latter of which engineers the addition of a DNA sequence encoding PTS1 (underlined, SRL) at the amino terminus of GFP. These primers were used to amplify a *GFP* cassette from the plasmid pEGFP-N1 (Clontech, cat #6085-1) and the PCR product was cloned into the cloning vector pBluescript SK+ using the restriction enzymes *Bam*HI and *Eco*RI. This insert was sequenced and subsequently cloned into the *Bgl*II and *Eco*RI sites of the plant binary vector VIP96a. The Arabi-

dopsis *SUC2* promoter was cloned using PCR and the primers SUC2.5prime (GCG AAG CTT AGG TGA TTA TAT GCA TGC AA) and SUC2.3 prime (GCC TCT AGA ATT TGA CAA ACC AAG AAA G). These primers amplify a 1 kb minimal *SUC2* promoter (Imlau et al. 1999) and introduce *Hin*dIII and *Xba*I restriction sites. These sites were used to clone the *SUC2* promoter into pBluescript SK+ and the promoter was subsequently cloned into VIP96A using *Hin*dIII and *Xba*I, thereby replacing the 35S promoter. This plasmid was transformed into *Agrobacterium tumefaciens* strain ABI and *Arabidopsis thaliana* (ecotype Landsberg erecta) plants were transformed as previously described (Clough and Brent 1998). T1 transformants were obtained using kanamycin resistance as a selectable marker and subsequently selected on the basis of uniform punctate GFP fluorescence. The T2 generation was used in all experiments shown.

Drug treatments

Drugs (from Sigma, St. Louis) were prepared and used as previously described (Nebenfuhr et al. 1999). Latrunculin B was used at a final concentration of 1 μM 2,3-butanedione monoxime was used at a concentration of 30 mM, nocodazol was used at a final concentration of 3.3 μM and propyzamide was used at a concentration of 1–10 μM. Seedlings were treated with these inhibitors for 30–45 min prior to observation.

Biolistic transformation

Biolistic bombardment using the GFP-PTS1 vector and GFP-talin vector was carried out as previously described (Kost et al. 1998).

Confocal microscopy

A Zeiss LSM 410 microscope was used throughout this study and all images are derived from single optical sections. For the examination of peroxisome dynamics we used the time scan function at a resolution of either 512 or 256 dpi, resulting in an interval between frames of 1.08 (referred to in the text as 1 s) and 0.56 s, respectively. Figures in which a series of consecutive images are overlaid were constructed using the topography from sequence function. In Fig. 3 and 4 signal saturation is shown in red. In Fig. 5, peroxisomes were color-coded using Abobe Photoshop software. To determine the percentage of peroxisomes displaying streaming behavior (Fig. 6), the first frame of a 10-frame time-series was loaded to a video monitor and individual peroxisomes were randomly identified. The time-series was then animated and the selected peroxisomes were scored for movement. These peroxisomes were scored as positive if they were fully displaced from their original location after the 10.8-s interval.

Immunoelectron microscopy

For the localization of GFP-PTS1 by immunoelectron microscopy, transgenic seedlings were germinated in darkness for 3 d, transferred to white light for 1 d and then fixed in 4% paraformaldehyde, 0.1% glutaraldehyde for 30 min, followed by dehydration and embedding in LR white resin. Anti-GFP antibodies (Clonetech) were used at a concentration of 1 : 100 and followed by incubation with 10-nm gold particles.

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Note added in proof

While this manuscript was under review, Mano et al. published a paper reaching similar conclusions concerning the role of the actin cytoskeleton in plant peroxisome movement (Mano, S., Nakamori, C., Hayashi, M., Kato, A., Kondo, M. and Nishimura, M. (2002) Distribution and Characterization of Peroxisomes in Arabidopsis by Visualization with GFP: Dynamic Morphology and Actin-Dependent Movement. *Plant Cell Physiol.* 43: 331–341).

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