Inp1p is a peroxisomal membrane protein required for peroxisome inheritance in *Saccharomyces cerevisiae*

Monica Fagarasanu,[1] Andrei Fagarasanu,[1] Yuen Yi C. Tam,[1] John D. Aitchison,[1,2] and Richard A. Rachubinski[1]

[1]Department of Cell Biology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada
[2]Institute for Systems Biology, Seattle, WA 98103

Cells have evolved molecular mechanisms for the efficient transmission of organelles during cell division. Little is known about how peroxisomes are inherited. Inp1p is a peripheral membrane protein of peroxisomes of *Saccharomyces cerevisiae* that affects both the morphology of peroxisomes and their partitioning during cell division. In vivo 4-dimensional video microscopy showed an inability of mother cells to retain a subset of peroxisomes in dividing cells lacking the *INP1* gene, whereas cells overexpressing *INP1* exhibited immobilized peroxisomes that failed to be partitioned to the bud. Overproduced Inp1p localized to both peroxisomes and the cell cortex, supporting an interaction of Inp1p with specific structures lining the cell periphery. The levels of Inp1p vary with the cell cycle. Inp1p binds Pex25p, Pex30p, and Vps1p, which have been implicated in controlling peroxisome division. Our findings are consistent with Inp1p acting as a factor that retains peroxisomes in cells and controls peroxisome division. Inp1p is the first peroxisomal protein directly implicated in peroxisome inheritance.

Introduction

Compartmentalization of biochemical functions in membrane-bound organelles provides the eukaryotic cell a level of control unavailable to the prokaryotic cell. Because organelles must form from preexisting membranes (Nunnari and Walter, 1996; Warren and Wickner, 1996), maintenance of the advantages afforded by compartmentalization requires the accurate segregation of organelles from mother cell to daughter cell at cell division. This segregation could occur either randomly or by an ordered process requiring cellular machinery. Evidence suggests the second way is more common (Warren and Wickner, 1996; Yaffe, 1999; Catlett and Weisman, 2000), and eukaryotic cells have evolved molecular mechanisms to ensure the faithful inheritance of different organelles.

Unicellular eukaryotes, including notably the budding yeast *Saccharomyces cerevisiae*, have been used extensively and effectively to dissect the molecular pathways involved in organelle inheritance. Division in *S. cerevisiae* is asymmetrical, with the formation of a bud that is initially much smaller than its mother. A highly polarized actin cytoskeleton is needed for bud formation and for the faithful delivery of organelles to the emerging bud (Yaffe, 1991). Organelles are duplicated or fragmented within the *S. cerevisiae* mother cell and then transported along actin tracks by molecular motors to their proper location within the bud.

The class V myosins form a family of actin-associated motors that are necessary for the polarized distribution of organelles. These highly conserved myosins contain an amino-terminal motor domain that binds to actin filaments and a carboxyl-terminal tail domain that binds one or more cargos. The class V myosin, Myo2p, plays a critical role in the bud-directed transport of different organelles, including the vacuole (Hill et al., 1996; Catlett and Weisman, 1998, 2000), secretory vesicles (Govindan et al., 1995; Schott et al., 1999), late Golgi elements (Rossanese et al., 2001), mitochondria (Boldogh et al., 2004; Itoh et al., 2004), and peroxisomes (Hoepfner et al., 2001). Myo2p's involvement in the transport of many different organelles has been explained by the presence of distinct domains in the globular tail of Myo2p that bind to organelle-specific receptors in a temporal and spatial pattern characteristic for the transport of a particular organelle to the yeast bud (Catlett and Weisman, 1998, 2000; Schott et al., 1999; Itoh et al., 2004). Another class V myosin, Myo4p, has been shown to be involved in the inheritance of the cortical ER that lines the periphery of *S. cerevisiae* cells (Estrada et al., 2003).

Correspondence to Richard A. Rachubinski: rick.rachubinski@ualberta.ca

Abbreviations used in this paper: 20KgP, 20,000 *g* pellet; 20KgS, 20,000 *g* supernatant; 4D, 4-dimensional; G6PDH, glucose-6-phosphate dehydrogenase; Lat A, latrunculin A; mRFP, monomeric RFP; pA, protein A; PTS, peroxisome targeting signal; SM, synthetic minimal.

The online version of this article contains supplemental material.

The Journal of Cell Biology, Vol. 169, No. 5, June 6, 2005 765–775
http://www.jcb.org/cgi/doi/10.1083/jcb.200503083

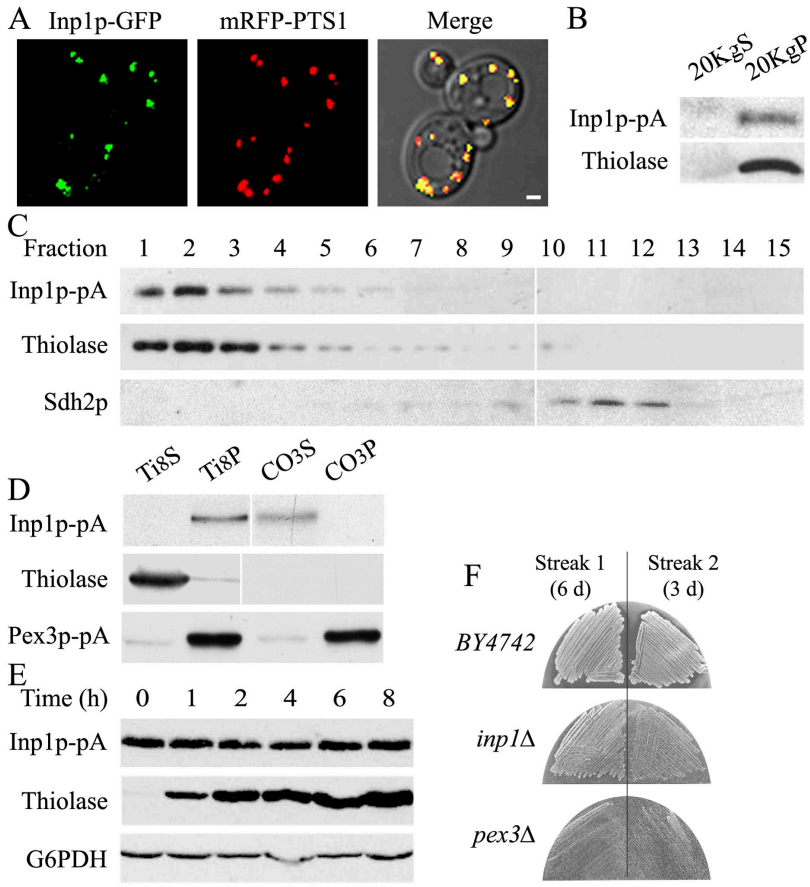


Figure 1. **Inp1p is a peripheral membrane protein of peroxisomes.** (A) Inp1p-GFP colocalizes with mRFP-PTS1 to punctate structures characteristic of peroxisomes by direct confocal microscopy. The right panel presents the merged image of the left and middle panels in which colocalization of Inp1p-GFP and mRFP-PTS1 is shown in yellow. Bar, 1 μm. (B) Inp1p-pA localizes to the 20KgP subcellular fraction enriched for peroxisomes. Immunoblot analysis of equivalent portions of the 20KgS and 20KgP subcellular fractions from cells expressing Inp1p-pA was performed with antibodies to the peroxisomal matrix enzyme, thiolase. (C) Inp1p-pA cofractionates with peroxisomes. Organelles in the 20KgP fraction were separated by isopycnic centrifugation on a discontinuous Nycodenz gradient. Fractions were collected from the bottom of the gradient, and equal portions of each fraction were analyzed by immunoblotting. Fractions enriched for peroxisomes and mitochondria were identified by immunodetection of thiolase and Sdh2p, respectively. (D) Purified peroxisomes were ruptured by treatment with Ti8 buffer and subjected to ultracentrifugation to obtain a supernatant fraction, Ti8S, enriched for matrix proteins and a pellet fraction, Ti8P, enriched for membrane proteins. The Ti8P fraction was treated further with alkali Na_2CO_3 and separated by ultracentrifugation into a supernatant fraction (CO_3S) enriched for peripherally associated membrane proteins and a pellet fraction (CO_3P) enriched for integral membrane proteins. Equivalent portions of each fraction were analyzed by immunoblotting. Immunodetection of thiolase and Pex3p-pA marked the fractionation profiles of a peroxisomal matrix and integral membrane protein, respectively. White lines indicate that intervening lanes have been spliced out. (E) The synthesis of Inp1p-pA is constant during incubation of *S. cerevisiae* in oleic acid medium. Cells grown for 16 h in YPD medium were transferred to, and incubated in, YPBO medium. Aliquots of cells were removed from the YPBO medium at the indicated times, and total cell lysates were prepared. Equal amounts of protein from the lysates were separated by SDS-PAGE, and Inp1p-pA, thiolase, and G6PDH were detected by immunoblot analysis. Antibodies against G6PDH were used to confirm the loading of equal amounts of protein in each lane. (F) *inp1*Δ cells are retarded in their growth on oleic acid medium. Cells of the wild-type strain *BY4742*, the deletion strain *inp1*Δ and the peroxisome assembly mutant strain *pex3*Δ were grown on YPD agar and then streaked onto YPBO agar (Streak 1). After 3 d of incubation, cells were sampled from Streak 1 and restreaked onto the same YPBO agar (Streak 2). Incubation was continued for a further 3 d.

Although the molecular mechanisms of inheritance of the vacuole, Golgi, ER, and mitochondria become ever more clearly defined, little is known about the inheritance of peroxisomes. Peroxisomes undergo an ordered migration during the cell cycle (Hoepfner et al., 2001). A subset of peroxisomes localizes to the presumptive bud site and is then transported to the nascent bud. Although peroxisomes in the mother cell retain fixed cortical positions, the dynamics of newly inherited peroxisomes correlate with the polarity of the actin cytoskeleton in the bud. Thus, peroxisomes cluster at the bud tip during apical growth and are distributed over the entire bud cortex during the isotropic phase. At cytokinesis, peroxisomes localize to the mother-bud junction, consistent with a reorientation of the actin cytoskeleton for septum assembly at this stage of the cell cycle. The dynamics of peroxisomes during the cell cycle appear to be dependent on Myo2p, because cells of a temperature-sensitive mutant strain of *MYO2* display a delay in, but not a halt to, the insertion of peroxisomes into the bud at the restrictive temperature (Hoepfner et al., 2001). However, to date, no protein, and in particular no peroxisomal protein, has been shown to have a direct role in peroxisome inheritance.

Systems biology approaches, including transcriptome profiling, organellar proteomics, and comparative gene analysis, have recently led to the identification of a number of novel proteins required for peroxisome assembly (peroxins) in *S. cerevisiae* (Smith et al., 2002; Rottensteiner et al., 2003; Tam et al., 2003; Vizeacoumar et al., 2003, 2004). The construction of a collection of strains expressing full-length, chromosomally tagged GFP fusions covering almost 70% of the genes of the *S. cerevisiae* genome (Huh et al., 2003) has provided another powerful systems biology tool for the identification of novel peroxisomal proteins and potentially novel peroxins. This global analysis of protein localization has led to the tentative identification of a protein of unknown function, Ymr204p, as being peroxisomal. Here, we present evidence that Ymr204p is a peripheral membrane protein of peroxisomes controlling peroxisome size and number and, notably, is required for the inheritance of peroxisomes. Ymr204p, renamed Inp1p for inheritance of peroxisomes protein 1, is the first peroxisomal protein directly implicated in peroxisome inheritance.

Results

Inp1p is a peripheral membrane protein of peroxisomes

A global analysis of protein localization in *S. cerevisiae* identified Inp1p (Ymr204p), a protein of unknown function, as a

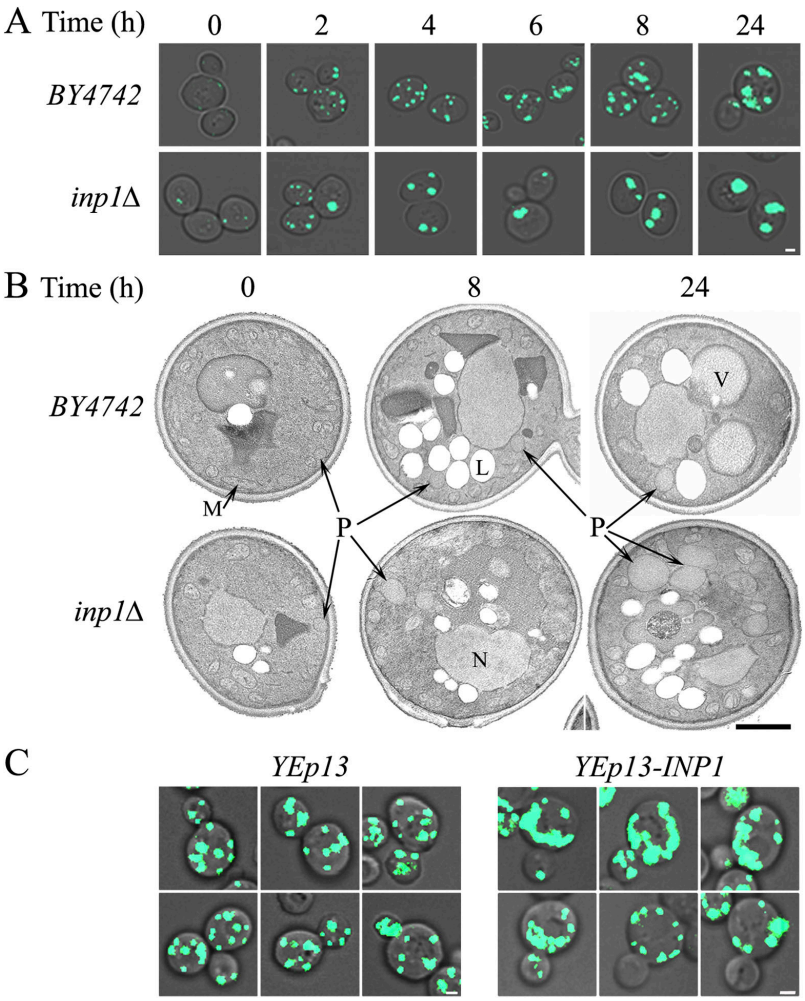


Figure 2. **Cells deleted for *INP1* exhibit an abnormal peroxisome phenotype.** (A) The wild-type strain *BY4742* and the deletion strain *inp1*Δ expressing genomically integrated *POT1-GFP* encoding peroxisomal thiolase tagged at its carboxyl terminus with GFP (Pot1p-GFP) were grown for 16 h in glucose-containing YPD medium and then transferred to oleic acid-containing YPBO medium. Fluorescent images of cells at different times of incubation in YPBO medium were captured by confocal microscopy. Bar, 1 μm. (B) Ultrastructure of *BY4742* and *inp1*Δ cells at different times of incubation in oleic acid medium. Cells were cultured as in A and then fixed and processed for EM. P, peroxisome; M, mitochondrion; N, nucleus; V, vacuole; L, lipid droplet. (C) Effects of *INP1* overexpression on the peroxisome phenotype. The strain *BY4742/POT1-GFP* was transformed with the empty multicopy plasmid YEp13 (left) or with YEp13 containing the *INP1* gene (right) for overexpression of *INP1*. Cells grown in SM medium for 16 h were transferred to and incubated in oleic acid-containing YNO medium for 8 h. Images were captured with a LSM510 META laser scanning microscope. Bars, 1 μm.

heretofore unknown peroxisomal protein (Huh et al., 2003). However, the demonstration of a peroxisomal localization for Inp1p remained tentative, because protein localization was done in strains grown in glucose medium, and peroxisomes are dispensable for growth of yeast in glucose medium. We therefore determined the localization of Inp1p in cells incubated in oleic acid medium, the metabolism of which requires peroxisomes and leads to increased numbers of peroxisomes per cell.

A genomically encoded fluorescent chimera of Inp1p and GFP (Inp1p-GFP) was localized in oleic acid-incubated cells by confocal microscopy. Inp1p-GFP colocalized with a fluorescent chimera (mRFP-PTS1) of monomeric RFP (mRFP) and the peroxisome targeting signal (PTS) 1 Ser-Lys-Leu to punctate structures characteristic of peroxisomes (Fig. 1 A).

Subcellular fractionation also showed Inp1p to be peroxisomal. A genomically encoded protein A (pA) chimera of Inp1p, Inp1p-pA, like the peroxisomal matrix protein thiolase, localized preferentially to the 20,000 *g* pellet (20KgP) fraction enriched for peroxisomes and mitochondria (Fig. 1 B). Isopycnic density gradient centrifugation of the 20KgP fraction showed that Inp1p cofractionated with thiolase but not with the mitochondrial protein, Sdh2p (Fig. 1 C).

Organelle extraction was used to determine the intraperoxisomal location of Inp1p. Peroxisomes were subjected to hypotonic lysis in dilute alkali Tris buffer, followed by ultracentrifugation to yield a supernatant (Ti8S) fraction enriched for matrix proteins and a pellet (Ti8P) fraction enriched for membrane proteins (Fig. 1 D). Inp1p-pA cofractionated with a pA chimera of the integral membrane protein Pex3p to the Ti8P fraction. The soluble peroxisomal matrix protein thiolase was found almost exclusively in the Ti8S fraction. The Ti8P fraction was then extracted with alkali Na_2CO_3 and subjected to ultracentrifugation. Inp1p-pA fractionated to the supernatant (CO_3S) enriched for peripheral membrane proteins and did not cofractionate with Pex3p-pA to the pellet (CO_3P) enriched for integral membrane proteins. These results are consistent with Inp1p being a peripheral membrane protein of peroxisomes.

The synthesis of many peroxisomal proteins is induced by incubating yeast cells in oleic acid medium. The expression level of genomically encoded Inp1-pA remained essentially unchanged during incubation in oleic acid medium (Fig. 1 E), as has been observed with some peroxisomal peroxins (Tam et al., 2003; Vizeacoumar et al., 2003, 2004). Under the same conditions, the level of the peroxisomal matrix enzyme thiolase increased considerably, whereas the level of the cytosolic enzyme glucose-6-phosphate dehydrogenase (G6PDH) remained constant and acted as a control for protein loading.

Yeast strains compromised in peroxisome biogenesis often exhibit a growth defect in medium containing oleic acid as the sole carbon source, the metabolism of which requires functional peroxisomes. Cells deleted for *INP1* were compromised in their growth on oleic acid–containing YPBO agar plates, but not to the same degree as the peroxisome assembly mutant *pex3*Δ (Fig. 1 F), consistent with a defect in some aspect of peroxisome biogenesis and/or function in *inp1*Δ cells.

Cells deleted for or overexpressing *INP1* exhibit abnormal peroxisomes

Wild-type and *inp1*Δ cells expressing the genomically integrated chimeric gene *POT1-GFP* encoding peroxisomal thiolase tagged at its carboxyl terminus with GFP (Pot1p-GFP) were incubated in YPBO medium and observed at different times of incubation by direct fluorescence confocal microscopy (Fig. 2 A). Peroxisomes increased dramatically in size during time of incubation in YPBO medium and were noticeably larger than peroxisomes of wild-type cells, particularly at longer times of incubation. There was also a dramatic decrease in peroxisome number in *inp1*Δ cells compared with wild-type cells with time of incubation. However, there was heterogeneity in the peroxisome phenotype in *inp1*Δ cells, with some cells exhibiting decreased numbers of enlarged peroxisomes and others exhibiting peroxisomes similar in size and number to peroxisomes of wild-type cells (Fig. 2 A, 2 h image). EM (Fig. 2 B) and morphometric analysis (Fig. S1 and Table S1, available at http://www.jcb.org/cgi/content/full/jcb.200503083/DC1) confirmed an overall increase in the size and decrease in the number of peroxisomes in *inp1*Δ cells with time of incubation in oleic acid medium.

The multicopy plasmid YEp13 containing the *INP1* gene was introduced into wild-type cells synthesizing Pot1p-GFP to determine the effects of *INP1* overexpression on the peroxisome phenotype. Overexpression of *INP1* in cells incubated in oleic acid medium led to the preferential localization of peroxisomes to the cortical regions of cells (Fig. 2 C), as shown by the analysis of individual optical sections in a *z*-stack. In addition, overexpression of *INP1* led to an apparent irregularity in the distribution of peroxisomes between mother cells and buds, with a significant number of buds not containing any readily evident fluorescent peroxisomes.

Deletion of *INP1* leads to increased numbers of mother cells without peroxisomes

The uneven distribution of peroxisomes in cells in which Inp1p was either absent or overproduced suggested an involvement of Inp1p in partitioning peroxisomes between mother cell and bud during cell division. To investigate this possibility, wild-type and *inp1*Δ cells synthesizing Pot1p-GFP to fluorescently label peroxisomes were incubated in SCIM-containing glucose and oleic acid to permit both the growth and division of cells and the proliferation of peroxisomes and analyzed by fluorescence confocal microscopy. Fluorescent images were collected as a stack, and all optical slices were analyzed for each field. In wild-type cells, peroxisomes were observed in essentially all mother cells and buds, irrespective of bud size (Fig. 3 A), as has been observed previ-

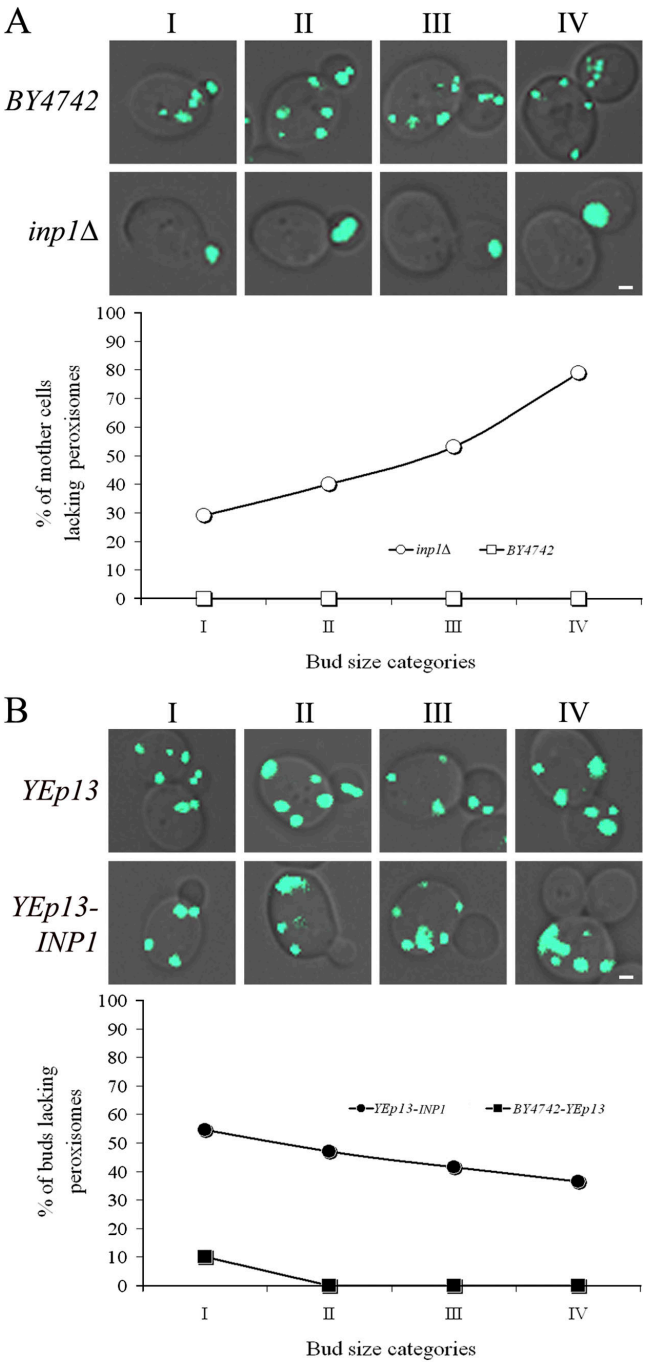


Figure 3. **Deletion or overexpression of *INP1* leads to defects in partitioning peroxisomes between mother cell and bud.** (A) Wild-type and *inp1*Δ cells expressing *POT1-GFP* to fluorescently label peroxisomes were incubated for 16 h in SCIM-containing glucose and oleic acid to allow for cell division and proliferation of peroxisomes. Fluorescent images of budded cells were acquired by confocal microscopy. Mother cells were scored for the presence or absence of fluorescent peroxisomes. Buds were sized according to four categories relative to the volume of the mother cell, expressed as a percentage of the mother cell volume (category I, 0–12%; category II, 12–24%; category III, 24–36%; category IV, 36–48%; see Materials and methods). Quantification was performed on at least 20 budded cells from each category. (B) Wild-type and *INP1*-overexpressing cells synthesizing Pot1p-GFP to label peroxisomes were incubated in SCIM and examined by confocal microscopy as described in A. Buds were scored for the presence or absence of fluorescent peroxisomes, sized and categorized, and quantification was performed, as defined in A. Bars, 1 μm.

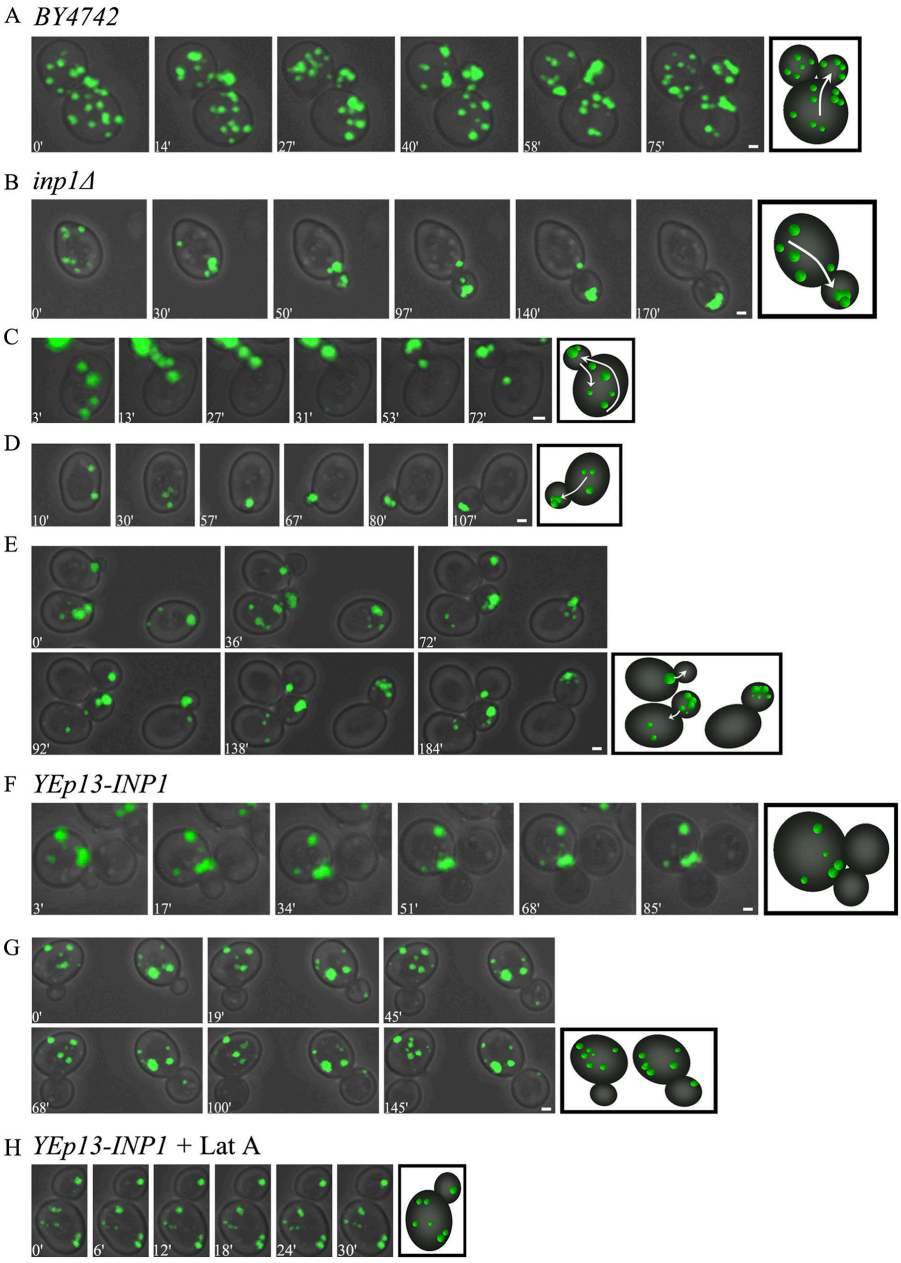


Figure 4. **Peroxisome movement during cell division as visualized by 4D in vivo video microscopy.** Peroxisomes were fluorescently labeled with genomically encoded Pot1p-GFP. Cells grown in SCIM for 16 h were placed onto a slide covered with a thin agarose pad containing SCIM. Cells were visualized at RT on a LSM 510 META confocal microscope specially modified for 4D in vivo video microscopy (see Materials and methods). Representative frames from videos show the specific movements of peroxisomes within each strain. (A) Wild-type *BY4742* cells. Some peroxisomes move directionally from mother cell to bud. A population of peroxisomes remains within the mother cell (Video 1). (B–E) *inp1*Δ cells. (B) The peroxisomes present in the mother cell before bud emergence (0′) gather at the presumptive bud site (30′). Subsequently, all peroxisomes are transported into the growing bud (30′–170′). Inside the bud, peroxisomes localize to sites of active growth, being initially clustered at the bud tip and then relocated to the bud neck region before cytokinesis (Video 2). (C) Peroxisomes present in the mother cell (3′) move into the bud (31′). One peroxisome then returns to the mother cell from the bud (72′; Video 3). (D) Initially, peroxisomes perform saltatory movements (10′–30′) and are then inserted into the growing bud (57′–107′; Video 4). (E) All peroxisomes present in the mother cells before bud emergence move into the buds (72′; Video 5). In the topmost cell, a peroxisome passes with difficulty into the bud due to its large size (0′–36′). In the cell at bottom, left, peroxisomes gather at the bud site (0′–3′) and eventually enter the forming bud. At 92′, one peroxisome returns to the mother cell. Some peroxisomes remain in the mother cell and display chaotic movements. In the cell at bottom, right, peroxisomes display chaotic movements (0′–18′) and then gather at the new bud site. Eventually, all peroxisomes move into the bud (184′; Video 5). (F and G) Wild-type *BY4742* cells overexpressing *INP1*. (F) Peroxisomes appear immobile (0′–145′). Analysis of individual optical sections from the 4D data showed the peroxisomes to be located at the cell cortex. Both first and second generation buds lack peroxisomes (Video 6). (G) Peroxisomes retain fixed cortical positions in mother cells. One peroxisome reaches the bud, keeps its mobility for a defined period of time (until 100′) and eventually becomes immobile (after 100′; Video 7). (H) Treatment of cells overexpressing *INP1* with the actin-disrupting toxin Lat A does not affect the mobility and localization of peroxisomes. Bars, 1 μm.

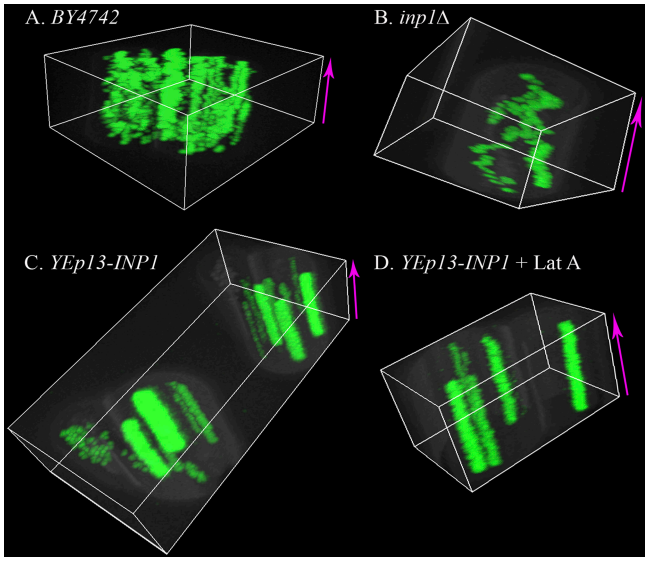
ously (Hoepfner et al., 2001). In contrast, a significant percentage of the budded cells of the *inp1*Δ strain lacked identifiable peroxisomes in the mother cell (Fig. 3 A). Quantification showed that *inp1*Δ cells exhibited an increase in the percentage of mother cells without peroxisomes as bud size increased, with 29% of mother cells with the smallest buds (category I) and 79% of mother cells with the largest bud (category IV) lacking peroxisomes (Fig. 3 A). These data suggest that the *inp1*Δ strain is defective in retaining peroxisomes in the mother cell during cell division.

Overexpression of *INP1* leads to increased numbers of buds without peroxisomes

Overexpression of *INP1* in cells led to increased numbers of buds without peroxisomes as compared with wild-type cells (Fig. 3 B). Depending on the size of the bud, from 40% to 55% of buds of *INP1*-overexpressing cells lacked peroxisomes. In contrast, 10% of only the smallest buds of wild-type cells lacked peroxisomes (Fig. 3 B). These data are consistent with an overproduction of Inp1p resulting in greater retention of peroxisomes in the mother cell, which in its turn leads to compromised peroxisome inheritance.

Impaired peroxisome inheritance in cells lacking or overexpressing *INP1*

The movement of peroxisomes between mother cell and bud was visualized by 4-dimensional (4D) in vivo video microscopy of wild-type, *inp1*Δ, and *INP1*-overexpressing cells containing genomically integrated *POT1-GFP* to fluorescently la-



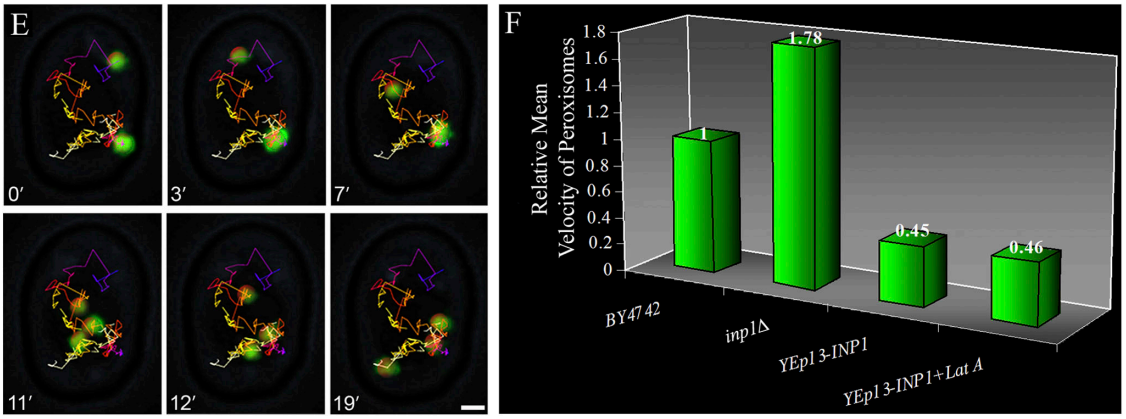


Figure 5. **Quantification of peroxisome mobility.** (A–D) 100 projections corresponding to the first 20 min of the videos corresponding to Fig. 4 (A, D, G, and H) were analyzed with Imaris 4.1 (Bitplane), and 3D models were constructed. The *z*-axis (purple arrows) represents time. A peroxisome that maintains its *x-y* position for the period of time considered and which is essentially immobile is represented by a fluorescent column parallel to the *z*-axis. A mobile peroxisome is represented by fluorescent spots that have different *x-y* positions in time. Corresponding animations are presented in Videos 8–10. (E) Tracking peroxisomes in *inp1*Δ cells. Peroxisomes in *inp1*Δ cells were tracked by analyzing the first 100 projections of Video 4 with Imaris 4.1. The trajectories of individual peroxisomes are shown as different colored lines. Bar, 1 μm. (F) Peroxisomes of *inp1*Δ cells are highly mobile. The velocities of individual peroxisomes across individual time points were measured using Imaris 4.1, and an average velocity was obtained for each peroxisome. The average velocities of individual peroxisomes in a given strain were in turn averaged to obtain the mean velocity of peroxisomes in that strain. The mean velocity of peroxisomes in a given strain are expressed relative to the mean velocity of peroxisomes of the wild-type strain, which is taken as 1.

bel peroxisomes. In wild-type cells, peroxisomes moved in a directed manner from mother cell to bud (Fig. 4 A and Video 1, available at http://www.jcb.org/cgi/content/full/jcb.200503083/DC1). A subset of peroxisomes was delivered to the growing bud, whereas there was concomitant maintenance of the peroxisome population within the mother cell. In *inp1*Δ cells, the inheritance of peroxisomes was compromised, resulting in an unbalanced distribution of peroxisomes between mother cell and bud. Frequently, all peroxisomes present in the mother cell before bud emergence were transported to the bud, resulting in a mother cell devoid of detectable fluorescent peroxisomes (Fig. 4, B, D, and E; Videos 2, 4, and 5, available at http://www.jcb.org/cgi/content/full/jcb.200503083/DC1). Once in the bud, peroxisomes accumulated at the sites of polarized growth, being initially localized to the growing tip and, before cytokinesis, relocated to the mother-bud neck. The preference of newly inherited peroxisomes for sites of active growth in the bud is therefore apparently not dependent on Inp1p (Fig. 4 B and Video 2). Interestingly, before being transported to the bud, some peroxisomes in *inp1*Δ cells performed uncharacteristic chaotic movements (Videos 4 and 5). Peroxisomes were also observed that returned from the bud to the center of the mother cell far beyond the region of the bud neck (Fig. 4 C and Video 3, available at http://www.jcb.org/cgi/content/full/jcb.200503083/DC1). Due to the larger size of many peroxisomes in *inp1*Δ cells, a delay was often observed in the passage of a peroxisome into the bud, indirectly affecting peroxisome inheritance (Fig. 4 E and Video 5). In *INP1*-overexpressing cells, peroxisomes appeared immobilized at cortical locations within the mother cell and did not passage into the bud (Fig. 4 F and Video 6, available at http://www.jcb.org/cgi/content/full/jcb.200503083/DC1). Occasionally, peroxisomes managed to pass into the bud and initially perform movements similar to those of peroxisomes of wild-type cells (Fig. 4 G and Video 7, available at http://www.jcb.org/cgi/content/full/jcb.200503083/DC1). This mobile behavior would end abruptly, and the peroxisomes would take fixed cortical positions. These observations implicate Inp1p as a factor acting in the retention of peroxisomes within cells. A possible role for actin in the retention of peroxisomes was also investigated, because actin has been shown to have a role in retaining mitochondria in cells (Yang et al., 1999). Treatment of *INP1*-overexpressing cells with the actin-disrupting toxin latrunculin A (Lat A; Rossanese et al., 2001) did not affect the localization of peroxisomes (Fig. 4 H), suggesting that actin is not involved in the retention of peroxisomes at the cell cortex.

The movement of peroxisomes in wild-type, *inp1*Δ, and *INP1*-overexpressing cells was also analyzed using 3-dimen-

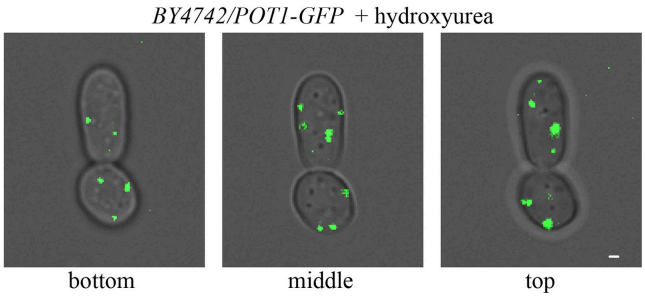


Figure 6. **Peroxisomes are actively retained in the mother cell.** Wild-type *BY4742/POT1-GFP* cells grown to mid-log phase in YPD medium were arrested in S phase by the addition of 200 mM hydroxyurea for 6 h. Fluorescent images of arrested cells were captured as a *z*-stack (bottom, middle, top) by confocal microscopy. The bottom cell is the mother cell, and the top cell is the hyperelongated bud. Bar, 1 μm.

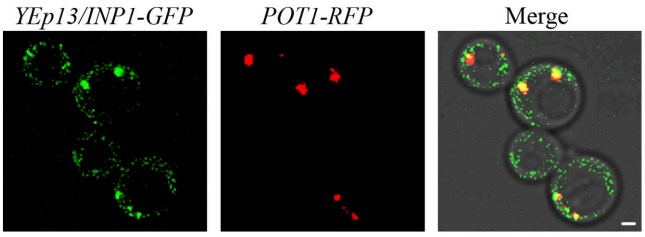


Figure 7. **Overproduced Inp1p is localized to peroxisomes and the cell cortex.** The strain *BY4742/POT1-RFP* transformed with a multicopy YEp13 plasmid construct overexpressing *INP1-GFP* were grown to mid-log phase in glucose-containing SM medium and examined by confocal microscopy. Overproduced Inp1p-GFP is localized to both peroxisomes and the cell cortex. Bar, 1 μm.

sional (3D) kymographs (Fig. 5 [A–C] and corresponding animations in Videos 8–10) that were constructed by overlapping the first 100 projections, which corresponds to 20 min of real time, of Videos 1, 4, and 7, respectively. In wild-type cells (Fig. 5 A), both static (represented by fluorescent columns) and mobile (represented by fluorescent spots that change position with time) peroxisomes were observed. In *inp1*Δ cells (Fig. 5 B), peroxisomes were highly mobile and dramatically changed their position with time. In contrast, kymographs of cells overproducing Inp1p show a large number of fluorescent columns, each representing a static peroxisome (Fig. 5 C). Treatment of *INP1*-overexpressing cells with Lat A did not destabilize the fluorescent columns, as peroxisomes maintained their positions over time (Fig. 5 D). The tracking of individual peroxisomes in *inp1*Δ cells during the first 20 min of Video 4 is presented in Fig. 5 E.

Calculation of the mean velocities of peroxisomes (Fig. 5 F) showed that, as expected, peroxisomes in *inp1*Δ cells were on average more mobile than peroxisomes of wild-type cells, with a mean velocity ~1.8 times that of peroxisomes of wild-type cells. In contrast, when *INP1* is overexpressed, peroxisomes have a mean velocity approximately half that of peroxisomes of wild-type cells. Therefore, the mean velocity of peroxisomes in *inp1*Δ cells is increased almost fourfold compared with that of peroxisomes of *INP1*-overexpressing cells. It should be noted that the difference in mean velocity between peroxisomes of *inp1*Δ and *INP1*-overexpressing cells may be an underestimate, because the peroxisomes in *INP1*-overexpressing cells preserve a certain speed due largely to localized oscillations without any pronounced displacement, whereas the peroxisomes in *inp1*Δ cells perform long-ranging movements.

Peroxisomes are actively retained in the mother cell

Our data show a role for Inp1p in retaining peroxisomes in cells. Conceptually, the distribution of peroxisomes between mother cell and bud could be a time-dependent process controlled indirectly by cytokinesis or a process in which peroxisomes are actively retained in the mother cell independently of cell cycle duration. To choose between these views, wild-type *BY4742/POT1-GFP* cells were treated with hydroxyurea to arrest cells in S phase, which leads to a protracted opening of the bud neck. This approach has been used to demonstrate an active retention mechanism for mitochondria in cells (Yang et al., 1999). After treating cells with hydroxyurea, peroxisomes remained equally distributed between the mother cell and the now hyperelongated bud (Fig. 6). In addition, the peroxisomes in the mother cell were cortically localized. These results show that peroxisomes are actively retained in the mother cell.

Overproduced Inp1p localizes to peroxisomes and to the cell cortex

If Inp1p acts to secure peroxisomes to the cell cortex during cell division, overproduced Inp1p should also associate with the cell periphery in glucose-grown cells that have few peroxisomes. To test this, Inp1p-GFP was overproduced in wild-type *BY4742/POT1-RFP* cells grown in glucose-containing medium. Inp1p-GFP showed both peroxisomal and cortical localizations, supportive of Inp1p being the link between peroxisomes and an anchoring cortical structure (Fig. 7).

The levels of Inp1p vary with the cell cycle

The accurate partitioning of peroxisomes between mother cell and bud is an ordered process that progresses in distinct steps through the cell cycle. Accordingly, it might be expected that Inp1p would be subject to some form of cell cycle–dependent regulation. To test this, cells were subjected to and released from α factor–induced G1-arrest. The levels of Inp1p varied with the cell cycle (Fig. 8), peaking 60 min after α factor release.

Inp1p binds Pex25p, Pex30p, and Vps1p

In vitro binding assays were performed to begin identifying interacting partners of Inp1p. Bacterially produced GST fused to Inp1p (GST-Inp1p) and GST alone were immobilized on glutathione resin and incubated with yeast lysates expressing TAP-tagged Pex11p, Pex17p, Pex19p, Pex25p, Pex30p, and Vps1p. Inp1p was observed to interact with Pex25p, Pex30p, and Vps1p (Fig. 9), all of which have been implicated in controlling peroxisome size and number.

Discussion

Eukaryotic cells partition their organelle populations during cell division. Here, we report that Inp1p, a protein of unknown function encoded by the *S. cerevisiae* genome, is required for

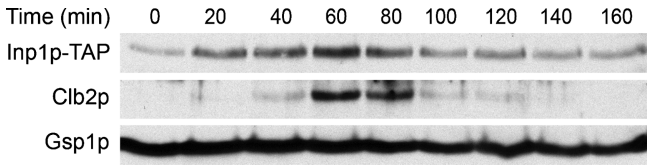


Figure 8. **The levels of Inp1p vary with the cell cycle.** Cells expressing TAP-tagged Inp1p were grown for 16 h in YPD and synchronized in G1 by addition of α factor (0 min). After removal of α factor, cells were incubated in YPD at 23°C. Samples were removed at the indicated times, and total cell lysates were prepared, separated by SDS-PAGE, transferred to nitrocellulose, and analyzed by immunoblotting with antibodies directed against the TAP tag, the cyclin Clb2p or Gsp1p (Ran). Gsp1p serves as a control for protein loading.

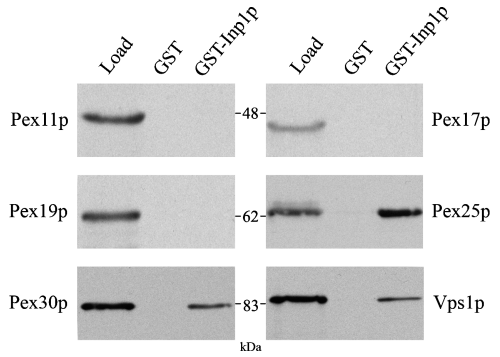


Figure 9. **Inp1p binds Pex25p, Pex30p, and Vps1p.** GST-Inp1p and GST alone were immobilized on glutathione Sepharose and incubated with whole cell lysates containing TAP-tagged peroxins or Vps1p. Lysates and bound fractions were resolved by SDS-PAGE, and TAP chimeras were detected by immunoblotting with anti-TAP antibody. Inp1p interacts with Pex25p, Pex30p, and Vps1p but not with Pex11p, Pex17p, Pex19p, or GST alone. Load represents 10% of the quantity of lysate applied to glutathione Sepharose for pull downs.

peroxisome inheritance. Inp1p is the first peroxisomal protein directly implicated in the inheritance of peroxisomes. Inp1p is not required for peroxisome assembly per se, because cells harboring a deletion of *INP1* contain readily identifiable peroxisomes by microscopic analysis and are able to import proteins targeted by either PTS1 or PTS2 (unpublished data).

Cells deleted for *INP1* incubated in oleic acid medium showed a progressive decrease in the average number and increase in the average size of peroxisomes with time. However, there was heterogeneity in the peroxisome population, with some cells containing a few enlarged peroxisomes and other cells containing peroxisomes similar in size and number to peroxisomes of wild-type cells. This heterogeneity was suggestive of a defect in peroxisome partitioning. When *inp1*Δ cells were cultured in medium permitting peroxisome proliferation and rapid cell division, an imbalance in the partitioning of peroxisomes became readily apparent as mother cells without peroxisomes were observed. The overall proportion of mother cells without peroxisomes increased with increasing bud size. These observations, combined with the fact that overexpression of *INP1* led conversely to large numbers of buds without peroxisomes and relocation of peroxisomes to the cortical regions of cells, strongly suggested a role for Inp1p in peroxisome inheritance.

The inheritance of organelles in budding yeast consists of two complimentary processes: the retention of a subset population of an organelle in the mother cell and the ordered movement of the remaining portion of the organelle population to the forming bud. The close control of both processes is crucial to the successful distribution of the organelle from mother cell to bud. A retention mechanism within the mother cell has been described for mitochondria (Yang et al., 1999). Retained mitochondria accumulate at the tip of the mother cell distal to the site of bud emergence (the so called "retention zone"), a process that likely involves the actin cytoskeleton. Retention mechanisms also operate in the bud. In this study we showed that, similar to mitochondria, peroxisomes are actively retained in the mother cell. Both organelles and molecules have been shown to remain anchored to the bud cell cortex at discrete locations, as demonstrated for mitochondria (Simon et al., 1997), ASH1 mRNA (Long et al., 1997; Takizawa et al., 1997), and the protein chitin synthase 3 (DeMarini et al., 1997). Recently, the Rab-like protein Ypt11p was shown to be required for the retention of newly inherited mitochondria within buds of *S. cerevisiae* (Boldogh et al., 2004).

4D in vivo video microscopy showed that in wild-type cells, a subset of peroxisomes partitioned to the emerging bud, whereas the peroxisomes that remained in the mother cell retained fixed cortical positions. The newly inherited peroxisomes tend to concentrate at the sites of active growth inside the bud. Before cytokinesis, subsets of peroxisomes from both the mother cell and the bud redistribute to the neck region, whereas the remaining peroxisomes remain anchored to the cortices of the mother cell and bud.

Peroxisomes of *inp1*Δ cells displayed increased mobility relative to peroxisomes of wild-type cells and were never observed to be static. Moreover, in *inp1*Δ cells, there was no delay as compared with wild-type cells in the passage of peroxisomes to the emerging bud, except in those cells containing greatly enlarged peroxisomes. Therefore, Inp1p is not directly involved in the movement of peroxisomes between mother cell and bud, presumably along actin tracks. How then might Inp1p function in peroxisome inheritance? An interesting feature of the dynamics of peroxisomes in cells lacking Inp1p is that the entire peroxisome population in the mother cell first clusters at the presumptive bud site and then enters the bud, thereby depleting the mother cell of peroxisomes. At times, peroxisomes were observed that failed to be delivered to the growing bud, but they also appeared to be unattached to the mother cell cortex, performing chaotic movements within the mother cell. On occasion, peroxisomes, after having passed to the bud, returned deep into the interior of the mother cell, a phenomenon never observed in wild-type cells. Actin as a whole is apparently normal in *inp1*Δ cells (unpublished data), and thus a major reorganization of the actin cytoskeleton cannot explain why *inp1*Δ cells exhibit defects in peroxisome inheritance. In *inp1*Δ cells, peroxisomes fail to be actively retained in either the mother cell or the bud, which results in the disruption of the ordered vectorial process of peroxisome segregation during cell division. The movements of peroxisomes from buds to mother cells could be explained by proposing that peroxisomes delivered to the bud in *inp1*Δ cells have a decreased affinity for a structure that retains peroxisomes within the bud, with the possibility

that some peroxisomes actually elude the anchoring mechanism completely. Because the return of newly inherited peroxisomes usually occurred after their performance of the characteristic movements of peroxisomes in the bud observed in wild-type cells, including the initial clustering of peroxisomes at the bud tip, we would predict that other factors must also play a role in maintaining newly inherited peroxisomes in the bud, at least in the early stages. The overproduction of Inp1p results in the retention of peroxisomes in the mother cell at fixed cortical positions and prevents the distribution of a subset of peroxisomes to the growing bud. Occasionally, one peroxisome would be delivered to the bud and, after performing the usual movements in the bud, would gain a fixed position at the bud cortex. The fact that when overproduced Inp1p assumes a cortical distribution in glucose-grown cells containing few peroxisomes strengthened our conclusion that Inp1p acts to tether peroxisomes to anchoring structures localized to the periphery of cells. All in all, our data reveal a major role for Inp1p in tethering peroxisomes to anchoring structures in both mother cell and bud during cell division.

Evidence for Inp1p being regulated during the cell cycle suggests that peroxisome inheritance is tightly controlled by the cell. Increased amounts of Inp1p at certain stages of the cell cycle might be required to ensure the retention of peroxisomes in both mother cell and bud. Inp1p might increase in amount only on a subset of peroxisomes that become prone to anchoring at the cell cortex. Alternatively, Inp1p might be fairly equally distributed on all peroxisomes, and other regional regulatory mechanisms and molecules could themselves act through Inp1p to modulate the anchoring of peroxisomes to the cell cortex. The oscillation of Inp1p levels during the cell cycle correlates with the oscillation of *INP1* mRNA levels during the cell cycle (Spellman et al., 1998), suggesting that the *INP1* gene is subject to cell cycle regulatory control. It is noteworthy that Inp1p is predicted to contain a PEST sequence (a potential signal for rapid protein degradation) between amino acids 279 and 362 (Rechsteiner and Rogers, 1996). Whether this PEST sequence functions in the degradation of Inp1p during the cell cycle awaits future experimentation.

A model for Inp1p function in partitioning peroxisomes between mother cell and bud is presented in Fig. 10. A subset of peroxisomes is transported to the bud by a process dependent on Myo2p (Hoepfner et al., 2001), whereas the remaining peroxisomes are retained within the mother cell on a cortical anchor. The peroxisomal peripheral membrane protein Inp1p would link the peroxisome to the cortical anchor. It is noteworthy that overproduction of Inp1p led to a distinctly enhanced cortical distribution of peroxisomes in cells. Whether a given peroxisome will be delivered to the bud or retained in the mother may depend on a tug-of-war between Inp1p and Myo2p. Accordingly, both under- and overproduction of Inp1p would lead to impairment of normal peroxisome inheritance. Once peroxisomes are delivered to the bud, they are prevented from returning to the mother cell. Inp1p also appears to play a role also in retaining peroxisomes within the bud, probably by attaching peroxisomes to cortical anchoring structures present in the bud. Actin structures do not appear to play a role in the Inp1p-dependent anchorage of peroxisomes to the cell cortex, because the treatment of cells overproducing Inp1p with Lat A did not lead to the detachment of immobilized peroxisomes. Moreover, we did not observe a colocalization between the Sac6p-containing actin patches and peroxisomes (Fig. S2, available at http://www.jcb.org/cgi/content/full/jcb.200503083/DC1).

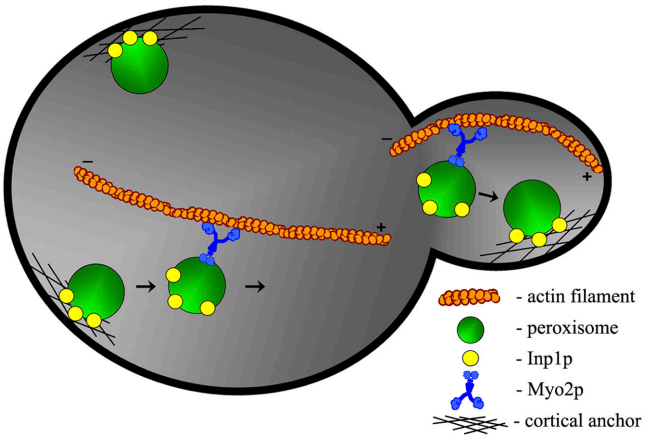


Figure 10. **A model for Inp1p function in peroxisome retention.** Peroxisomes move along polarized actin cables in a Myo2p-dependent manner from mother cell to bud. Concomitantly, a subset of peroxisomes is retained within the mother cell. Inp1p acts to link peroxisomes to a cortical anchor and retain peroxisomes in the mother cell and bud.

The phenotype of reduced numbers of enlarged peroxisomes seen for *inp1*Δ cells could conceptually arise only as a consequence of unbalanced partitioning of peroxisomes during cell division. However, the interactions of Inp1p with Pex25p, Pex30p, and Vps1p, which have all been shown previously to influence peroxisome division, support a role for Inp1p in peroxisome division. Thus, Inp1p seems to have a dual role in the division and the inheritance of peroxisomes in *S. cerevisiae*. How might these two functions be related? Other proteins are known to influence both the morphology of organelles and their distribution. Mdm10p (Sogo and Yaffe, 1994), Mdm12p (Berger et al., 1997), and Mmm1p (Burgess et al., 1994) are mitochondrial outer membrane proteins that affect mitochondrial shape and segregation. Mutation of any one of these proteins results in the presence of giant, spherical mitochondria that exhibit defects in partitioning at cell division. Recent studies (Boldogh et al., 2003) have indicated that these proteins form a complex that connects the minimum heritable unit of mitochondria (mtDNA and mitochondrial membranes) to actin, therefore functioning as a mitochondrial counterpart to the kinetochore or the "mitochore." These proteins affect the retention of mitochondria within the mother cell (Yang et al., 1999) and also Myo2p-independent mitochondrial movement (Boldogh et al., 2001).

In closing, we have presented evidence demonstrating that the peroxisomal peripheral membrane protein, Inp1p, is directly implicated in the inheritance of peroxisomes in *S. cerevisiae*. Inp1p acts as a peroxisome-retention factor, tethering peroxisomes to putative anchoring structures within the mother cell and bud. Inp1p is the first peroxisomal protein shown to be involved in the inheritance of peroxisomes.

Materials and methods

Strains and culture conditions
The *S. cerevisiae* strains used in this study are listed in Table S2, available at http://www.jcb.org/cgi/content/full/jcb.200503083/DC1. All strains were cultured at 30°C, unless otherwise indicated. Strains containing plasmids were cultured in synthetic minimal (SM) medium. Media components were as follows: YPD, 1% yeast extract, 2% peptone, 2% glucose; YPBO, 0.3% yeast extract, 0.5% peptone, 0.5% K_2HPO_4, 0.5% KH_2PO_4, 3.3% Brij 35, 1% oleic acid; SM, 0.67% yeast nitrogen base without amino acids, 2% glucose, 1× complete supplement mixture (Bio 101) without uracil or leucine; SCIM, 0.67% yeast nitrogen base without amino acids, 0.5% yeast extract, 0.5% peptone, 3.3% Brij 35, 0.1% glucose, 0.1% oleic acid, 1× complete supplement mixture; YNO, 0.67% yeast nitrogen base without amino acids, 1× complete supplement mixture without leucine, 3.3% Brij 35, 1% oleic acid.

pA and GFP tagging of genes
Genes were genomically tagged with the sequence encoding *Staphylococcus aureus* pA or an improved version of GFP (GFP+) from *Aequoria victoria* (Scholz et al., 2000) by homologous recombination with a PCR-based integrative transformation of parental *BY4742* haploid cells (Dilworth et al., 2001). The functionality of fusion proteins was confirmed by the lack of a mutant phenotype in transformed strains.

Plasmids
pmRFP-SKL was constructed by replacing the gene for RFP from *Discosoma* species in the plasmid pDsRed-PTS1 (Smith et al., 2002) by the gene encoding mRFP (Campbell et al., 2002). Genes to be overexpressed were amplified by PCR and cloned into the plasmid YEp13. For overexpression, the *INP1* gene included 523 bp of upstream and 328 bp of downstream sequence.

Microscopy
Strains synthesizing GFP and/or mRFP chimeras were grown to mid-log phase in SM medium and then incubated in YPBO medium for 8 h or SCIM for 16 h. Images were captured on a LSM510 META (Carl Zeiss MicroImaging, Inc.) laser scanning microscope or on a microscope (model BX50; Olympus) equipped with a digital fluorescence camera (Spot Diagnostic Instruments). EM of whole yeast cells (Eitzen et al., 1997) and morphometric analysis of EM images (Smith et al., 2000) were performed as described previously.

Quantification of rates of peroxisome inheritance
The rates of peroxisome inheritance were quantified essentially as described previously for the quantification of the rates of inheritance of Golgi elements in *S. cerevisiae* (Rossanese et al., 2001). Cells synthesizing a genomically encoded chimera between GFP and the peroxisomal matrix enzyme 3-ketoacyl-CoA thiolase (Pot1p-GFP) were grown in YPD medium for 16 h, transferred to SCIM and incubated in SCIM until an OD_{600} of 0.5 was achieved. Peroxisomes were visualized by direct fluorescence confocal microscopy. For each randomly chosen field, three optical sections of 5-μm thickness were collected at a *z*-axis spacing of 1.6 μm using a high detector gain to ensure the capture of weak fluorescent signals. Because cell volume is not directly accessible, budded cells were first measured using the LSM 5 Image Browser software (Carl Zeiss MicroImaging, Inc.) and grouped into four categories according to bud cross-sectional area expressed as a percentage of mother cell cross-sectional area: category I, 0–24%; category II, 24–39%; category III, 39–50%; category IV, 50–61%. Budded cells were then assigned to four categories of bud volume, expressed as a percentage of mother cell volume, that superimpose on the aforementioned area categories, assuming a spherical geometry for all cells: (category I, 0–12%; category II, 12–24%; category III, 24–36%; category IV, 36–48%).

4D in vivo video microscopy
Cells grown in YPD medium and then incubated in SCIM for 16 h were prepared for 4D in vivo video microscopy by placing 1–2 μl of the culture on a slide with a thin agarose pad, which was covered with a coverslip and sealed with petroleum jelly (Adames et al., 2001). Cells were incubated at room temperature for image capture. Images were captured as described previously (Hammond and Glick, 2000) using a Plan-Apochromat 63×/1.4 NA oil DIC objective and an Axiovert 200 microscope equipped with a LSM 510 META confocal scanner (Carl Zeiss MicroImaging, Inc.). A piezoelectric actuator was used to drive continuous objective movement, allowing for the rapid collection of *z*-stacks. Images were collected using LSM 510 acquisition software, v3.2. The sides of each pixel represented 0.085 μm of the sample. Stacks of 14 optical sections spaced 0.45 μm apart were captured every 12 s. GFP was excited using a 488-nm laser, and its emission was collected using a 505-nm long-pass filter. The resulting images were filtered five times using a 3 × 3 hybrid median filter to reduce shot noise. Fluorescence images from each stack were projected using an average intensity algorithm that involved multiplication of each pixel value by an appropriate enhancement factor for better contrast. Correction for exponential photobleaching of GFP was performed by exponentially increasing the enhancement factor with each projection. The transmitted light images from each stack were projected using a maximum intensity algorithm. These operations were performed using NIH Image (http://rsb.info.nih.gov/nih-image/). Adobe Photoshop (Adobe Systems) was used to merge the fluorescent and transmitted light projections.

Subcellular fractionation and isolation and extraction of peroxisomes
Subcellular fractionation of oleic acid-incubated cells was done as described previously (Smith et al., 2002) and involved the isolation of a post-nuclear supernatant fraction and 20,000 *g* supernatant (20KgS) and 20KgP fractions enriched for cytosol and for peroxisomes and mitochondria, respectively. Peroxisomes were purified from the 20KgP fraction by isopycnic density centrifugation on Nycodenz gradients (Smith et al., 2002). Peroxisomes were separated into fractions enriched for matrix, peripheral, and integral membrane proteins by treatment with dilute Tris and alkali sodium carbonate as described previously (Smith et al., 2002).

Hydroxyurea arrest
BY4742 cells grown to mid-log in YPD medium were treated with 200 mM hydroxyurea for 6 h at 30°C to arrest cells before cell division as described previously (Yang et al., 1999).

In vitro binding assay
GST and the GST-Inp1p were bound to glutathione Sepharose and incubated with yeast cell lysates containing TAP-tagged proteins as previously described (Marelli et al., 2004). TAP-tagged proteins were detected by immunoblotting.

Antibodies
Antibodies to thiolase and Sdh2p have been described previously (Tam et al., 2003). Rabbit antibodies to *S. cerevisiae* G6PDH were purchased from Sigma-Aldrich. Rabbit antibodies to the TAP tag were purchased from Open Biosystems. HRP-conjugated donkey anti–rabbit IgG and HRP-conjugated goat anti–guinea pig IgG secondary antibodies were used to detect primary antibodies in immunoblot analysis. Antigen–antibody complexes in immunoblots were detected by ECL (Amersham Biosciences).

Online supplemental material
Fig. S1 presents a morphometric analysis of peroxisome size in wild-type *BY4742* and *inp1*Δ cells. Fig. S2 shows that peroxisomes do not colocalize with actin patches in cells overproducing Inp1p. Video 1 shows the movement of peroxisomes in wild-type *BY4742* cells during cell division. Videos 2–5 show the movement of peroxisomes in *inp1*Δ cells during cell division. Videos 6 and 7 show the relatively static nature of peroxisomes in cells overexpressing the *INP1* gene. Videos 8–10 present animations of 3D kymographs of peroxisome movement in wild-type and *inp1*Δ cells and in cells overexpressing *INP1*. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200503083/DC1.

We thank Dr. Benjamin Glick, Dr. Adam Hammond, and Dr. Neil Adames for help with 4D in vivo video microscopy; Dr. Xuejun Sun for help in analyzing the mobility of peroxisomes; Robert Scott and Dr. Patrick Lusk for helpful discussions; Elena Savidov, Dwayne Weber, Hanna Kroliczak, and Richard Poirier for technical help; and Honey Chan for assistance with EM.

This work was supported by grant MT-9208 from the Canadian Institutes of Health Research to R.A. Rachubinski. R.A. Rachubinski holds the Canada Research Chair in Cell Biology and is an International Research Scholar of the Howard Hughes Medical Institute. Y.Y.C. Tam is the recipient of a Studentship from the Alberta Heritage Foundation for Medical Research.

Submitted: 15 March 2005
Accepted: 3 May 2005

References

Adames, N.R., J.R. Oberle, and J.A. Cooper. 2001. The surveillance mechanism of the spindle position checkpoint in yeast. *J. Cell Biol.* 153:159–168.

Berger, K.H., L.F. Sogo, and M.P. Yaffe. 1997. Mdm12p, a component required for mitochondrial inheritance that is conserved between budding and fission yeast. *J. Cell Biol.* 136:545–553.

Boldogh, I.R., H.C. Yang, D.W. Nowakowski, S.L. Karmon, L.G. Hays, J.R. Yates III, and L.A. Pon. 2001. Arp2/3 complex and actin dynamics are required for actin-based mitochondrial motility in yeast. *Proc. Natl. Acad. Sci. USA*. 98:3162–3167.

Boldogh, I.R., D.W. Nowakowski, H.C. Yang, H. Chung, S. Karmon, P. Royes, and L.A. Pon. 2003. A protein complex containing Mdm10p, Mdm12p, and Mmm1p links mitochondrial membranes and DNA to the cytoskeleton-based segregation machinery. *Mol. Biol. Cell.* 14:4618–4627.

Boldogh, I.R., S.L. Ramcharan, H.-C. Yang, and L.A. Pon. 2004. A type V myosin (Myo2p) and a Rab-like G-protein (Ypt11p) are required for retention of newly inherited mitochondria in yeast cells during cell division. *Mol. Biol. Cell.* 15:3994–4002.

Burgess, S.M., M. Delannoy, and R.E. Jensen. 1994. MMM1 encodes a mitochondrial outer membrane protein essential for establishing and maintaining the structure of yeast mitochondria. *J. Cell Biol.* 126:1375–1391.

Campbell, R.E., O. Tour, A.E. Palmer, P.A. Steinbach, G.S. Baird, D.A. Zacharias, and R.Y. Tsien. 2002. A monomeric red fluorescent protein. *Proc. Natl. Acad. Sci. USA*. 99:7877–7882.

Catlett, N.L., and L.S. Weisman. 1998. The terminal tail region of a yeast myosin-V mediates its attachment to vacuole membranes and sites of polarized growth. *Proc. Natl. Acad. Sci. USA*. 95:14799–14804.

Catlett, N.L., and L.S. Weisman. 2000. Divide and multiply: organelle partitioning in yeast. *Curr. Opin. Cell Biol.* 12:509–515.

DeMarini, D.J., A.E. Adams, H. Fares, C. De Virgilio, G. Valle, J.S. Chuang, and J.R. Pringle. 1997. A septin-based hierarchy of proteins required for localized deposition of chitin in the *Saccharomyces cerevisiae* cell wall. *J. Cell Biol.* 139:75–93.

Dilworth, D.J., A. Suprapto, J.C. Padovan, B.T. Chait, R.W. Wozniak, M.P. Rout, and J.D. Aitchison. 2001. Nup2p dynamically associates with the distal regions of the yeast nuclear pore complex. *J. Cell Biol.* 153:1465–1478.

Eitzen, G.A., R.K. Szilard, and R.A. Rachubinski. 1997. Enlarged peroxisomes are present in oleic acid-grown *Yarrowia lipolytica* overexpressing the *PEX16* gene encoding an intraperoxisomal peripheral membrane peroxin. *J. Cell Biol.* 137:1265–1278.

Estrada, P., J. Kim, J. Coleman, L. Walker, B. Dunn, P. Takizawa, P. Novick, and S. Ferro-Novick. 2003. Myo4p and She3p are required for cortical ER inheritance in *Saccharomyces cerevisiae. J. Cell Biol.* 163:1255–1266.

Govindan, B., R. Bowser, and P. Novick. 1995. The role of Myo2p, a yeast class V myosin, in vesicular transport. *J. Cell Biol.* 128:1055–1068.

Hammond, A.T., and B.S. Glick. 2000. Raising the speed limits for 4D fluorescence microscopy. *Traffic.* 1:935–940.

Hill, K.L., N.L. Catlett, and L.S. Weisman. 1996. Actin and myosin function in directed vacuole movement during cell division in *Saccharomyces cerevisiae. J. Cell Biol.* 135:1535–1549.

Hoepfner, D., M. van den Berg, P. Philippsen, H.F. Tabak, and E.H. Hettema. 2001. A role for Vps1p, actin, and the Myo2p motor in peroxisome abundance and inheritance in *Saccharomyces cerevisiae. J. Cell Biol.* 155: 979–990.

Huh, W.-K., J.V. Falvo, L.C. Gerke, A.S. Carroll, R.W. Howson, J.S. Weissman, and E.K. O'Shea. 2003. Global analysis of protein localization in budding yeast. *Nature.* 425:686–691.

Itoh, T., A. Toh-e, and Y. Matsui. 2004. Mmr1p is a mitochondrial factor for Myo2p-dependent inheritance of mitochondria in the budding yeast. *EMBO J.* 23:2520–2530.

Long, R.M., R.H. Singer, X. Meng, I. Gonzalez, K. Nasmyth, and R.P. Jansen. 1997. Mating type switching in yeast controlled by asymmetric localization of *ASH1* mRNA. *Science.* 277:383–387.

Marelli, M., J.J. Smith, S. Jung, E. Yi, A.I. Nesvizhskii, R.H. Christmas, R.A. Saleem, Y.Y.C. Tam, A. Fagarasanu, D.R. Goodlett, et al. 2004. Quantitative mass spectrometry reveals a role for the GTPase Rho1p in actin organization on the peroxisome membrane. *J. Cell Biol.* 167:1099–1112.

Nunnari, J., and P. Walter. 1996. Regulation of organelle biogenesis. *Cell.* 84: 389–394.

Rechsteiner, M., and S.W. Rogers. 1996. PEST sequences and regulation by proteolysis. *Trends Biochem. Sci.* 21:267–271.

Rossanese, O.W., C.A. Reinke, B.J. Bevis, A.T. Hammond, I.B. Sears, J. O'Connor, and B.S. Glick. 2001. A role for actin, Cdc1p, and Myo2p in the inheritance of late Golgi elements in *Saccharomyces cerevisiae. J. Cell Biol.* 153:47–62.

Rottensteiner, H., K. Stein, E. Sonnenhol, and R. Erdmann. 2003. Conserved function of Pex11p and the novel Pex25p and Pex27p in peroxisome biogenesis. *Mol. Biol. Cell.* 14:4316–4328.

Scholz, O., A. Thiel, W. Hillen, and M. Niederweis. 2000. Quantitative analysis of gene expression with an improved green fluorescent protein. *Eur. J. Biochem.* 267:1565–1570.

Schott, D., J. Ho, D. Pruyne, and A. Bretscher. 1999. The COOH-terminal domain of Myo2, a yeast myosin V, has a direct role in secretory vesicle targeting. *J. Cell Biol.* 147:791–807.

Simon, V.R., S.L. Karmon, and L.A. Pon. 1997. Mitochondrial inheritance: cell cycle and actin cable dependence of polarized mitochondrial movements in *Saccharomyces cerevisiae. Cell Motil. Cytoskeleton.* 37:199–210.

Smith, J.J., T.W. Brown, G.A. Eitzen, and R.A. Rachubinski. 2000. Regulation of peroxisome size and number by fatty acid β-oxidation in the yeast *Yarrowia lipolytica. J. Biol. Chem.* 275:20168–20178.

Smith, J.J., M. Marelli, R.H. Christmas, F.J. Vizeacoumar, D.J. Dilworth, T. Ideker, T. Galitski, K. Dimitrov, R.A. Rachubinski, and J.D. Aitchison. 2002. Transcriptome profiling to identify genes involved in peroxisome assembly and function. *J. Cell Biol.* 158:259–271.

Sogo, L.F., and M.P. Yaffe. 1994. Regulation of mitochondrial morphology and inheritance by Mdm10p, a protein of the mitochondrial outer membrane. *J. Cell Biol.* 126:1361–1373.

Spellman, P.T., G. Sherlock, M.Q. Zhang, V.R. Iyer, K. Anders, M.B. Eisen, P.O. Brown, D. Botstein, and B. Futcher. 1998. Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. *Mol. Biol. Cell.* 9:3273–3297.

Takizawa, P.A., A. Sil, J.R. Swedlow, I. Herskowitz, and R.D. Vale. 1997. Actin-dependent localization of an RNA encoding a cell-fate determinant in yeast. *Nature.* 389:90–93.

Tam, Y.Y.C., J.C. Torres-Guzman, F.J. Vizeacoumar, J.J. Smith, M. Marelli, J.D. Aitchison, and R.A. Rachubinski. 2003. Pex11-related proteins in peroxisome dynamics: a role for the novel peroxin Pex27p in controlling peroxisome size and number in *Saccharomyces cerevisiae. Mol. Biol. Cell.* 14:4089–4102.

Vizeacoumar, F.J., J.C. Torres-Guzman, Y.Y.C. Tam, J.D. Aitchison, and R.A. Rachubinski. 2003. *YHR150w* and *YDR479c* encode peroxisomal integral membrane proteins involved in the regulation of peroxisome number, size, and distribution in *Saccharomyces cerevisiae. J. Cell Biol.* 161: 321–332.

Vizeacoumar, F.J., J.C. Torres-Guzman, D. Bouard, J.D. Aitchison, and R.A. Rachubinski. 2004. Pex30p, Pex31p, and Pex32p form a family of peroxisomal integral membrane proteins regulating peroxisome size and number in *Saccharomyces cerevisiae. Mol. Biol. Cell.* 15:665–677.

Warren, G., and W. Wickner. 1996. Organelle inheritance. *Cell.* 84:395–400.

Yaffe, M.P. 1991. Organelle inheritance in the yeast cell cycle. *Trends Cell Biol.* 1:160–164.

Yaffe, M.P. 1999. The machinery of mitochondrial inheritance and behavior. *Science.* 283:1493–1497.

Yang, H.C., A. Palazzo, T.C. Swayne, and L.A. Pon. 1999. A retention mechanism for distribution of mitochondria during cell division in budding yeast. *Curr. Biol.* 9:1111–1114.