Peroxisomes move by hitchhiking on early endosomes using the novel linker protein PxdA

John Salogiannis,1 Martin J. Egan,1 and Samara L. Reck-Peterson1,2,3

1Department of Cell Biology, Harvard Medical School, Boston, MA 02115
2Department of Cellular and Molecular Medicine and 3Section of Cell and Developmental Biology, Division of Biological Sciences, University of California, San Diego, La Jolla, CA 92093

Eukaryotic cells use microtubule-based intracellular transport for the delivery of many subcellular cargos, including organelles. The canonical view of organelle transport is that organelles directly recruit molecular motors via cargo-specific adaptors. In contrast with this view, we show here that peroxisomes move by hitchhiking on early endosomes, an organelle that directly recruits the transport machinery. Using the filamentous fungus Aspergillus nidulans we found that hitchhiking is mediated by a novel endosome-associated linker protein, PxdA. PxdA is required for normal distribution and long-range movement of peroxisomes, but not early endosomes or nuclei. Using simultaneous time-lapse imaging, we find that early endosome-associated PxdA localizes to the leading edge of moving peroxisomes. We identify a coiled-coil region within PxdA that is necessary and sufficient for early endosome localization and peroxisome distribution and motility. These results present a new mechanism of microtubule-based organelle transport in which peroxisomes hitchhike on early endosomes and identify PxdA as the novel linker protein required for this coupling.

Introduction

Eukaryotic cells rely on the microtubule cytoskeleton to move intracellular components over long distances. Microtubules are dynamic polar structures, with “plus” ends usually located near the cell periphery and “minus” ends typically embedded in perinuclear microtubule organizing centers. Dynein motors move cargos toward the minus ends of microtubules, whereas most kinesin motors move in the opposite direction. Cytoplasmic dynein-1 (referred to here as dynein) and a relatively small number of kinesins are responsible for the transport of vesicles, organelles, proteins, and mRNAs (Vale, 2003; Cianfrocco et al., 2015).

In mammalian cells, a variety of organelles have been shown to depend on dynein and kinesin for transport, including endosomes, mitochondria, peroxisomes, Golgi, endoplasmic reticulum, autophagosomes, lysosomes, and nuclei (Harada et al., 1998; Tanaka et al., 1998; Roghi and Allan, 1999; Schrader et al., 2000; Kural et al., 2005; Maday and Holzbaur, 2012; Neuhaus et al., 2015). In some cases, the adaptors that link the molecular motors to their cargos have been identified (Kardon and Vale, 2009; Fu and Holzbaur, 2014; Cianfrocco et al., 2015). For example, in the case of mitochondria, TRAK/Milton proteins recruit kinesin-1 and dynein (Glater et al., 2006; Wang and Schwarz, 2009; van Spronen et al., 2013), and in the case of early endosomes (EEs), Hook proteins have been shown to recruit dynein and kinesin-3 (Bieliska et al., 2014; Zhang et al., 2014). This has led to the idea that each type of cargo uses distinct machinery to recruit molecular motors. Our goal is to use the model fungus Aspergillus nidulans to identify how different cargos engage the transport machinery.

A. nidulans and other filamentous fungi have proven to be excellent model systems for studying microtubule-based transport (Egan et al., 2012a). In these fungi, microtubules are used to transport cellular cargos through highly polarized, multinucleate cells, called hyphae. Furthermore, rapid forward genetics are possible, and genome engineering is fast and simple (Horio and Oakley, 2005; Nayak et al., 2006). In A. nidulans, microtubules are uniformly polarized from the hyphal tip to the tip-proximal nucleus, such that plus ends are located at hyphal tips and minus ends are embedded in the nuclear membrane (Egan et al., 2012b). As a result of this organization, defects in dynein-mediated transport generally lead to accumulation of cargo at the hyphal tip, whereas defects in kinesin-3/UncA transport can cause accumulation of cargo at the tip-proximal nucleus (Fig. 1; Egan et al., 2012a).

We took advantage of these clear organelle distribution phenotypes to conduct a forward genetic screen in A. nidulans to identify novel genes required for the microtubule-based movement of dynein and kinesin cargos (Tan et al., 2014). For our screen, we focused on three well-characterized cargos of dynein and kinesin: EEs, peroxisomes, and nuclei (Xiang et al.,...
to achieve long-range movement (Baumann et al., 2012; Higuchi et al., 2014; Guiamares et al., 2015), but the molecules mediating this behavior were not known. Our data demonstrate that PxdA is a novel linker protein mediating peroxisomal hitchhiking on EEs.

**Results and discussion**

**PxdA is a novel protein required for peroxisome distribution and motility**

In our original screen, 19 mutants were categorized as only affecting peroxisome distribution, making them excellent candidates for cargo-specific regulators (Tan et al., 2014). Whole-genome sequencing of two mutants (RPA604 and RPA628) with a strong peroxisome distribution phenotype identified independent mutations in the uncharacterized gene AN1156; both mutations were predicted to result in premature stop codons. AN1156 mutants displayed hyphal tip accumulation of peroxisomes, but normal distribution of RabA5a-positive EEs.
and nuclei (Fig. S1 A). Based on this phenotype, we named AN1156 pxdA (peroxisome distribution mutant A). To confirm that PxdA is expressed, we performed immunoblots of lysates from an HA-tagged pxdA strain and detected an ~250-kD band (Fig. S1 B), which corresponds well to the larger of two predicted open reading frames for AN1156.

To verify that pxdA plays a role in regulating peroxisome distribution, we deleted the endogenous gene in haploid strains containing fluorescently labeled EEs, peroxisomes, or nuclei. Consistent with the phenotype of RPA604 and RPA628, peroxisomes accumulated in pxdAΔ hyphae, but EEs and nuclei were distributed normally (Fig. 1 A). To quantify peroxisome and EE distribution along hyphae, we performed line scans of fluorescence micrographs and plotted the average fluorescence intensity as a function of distance from the hyphal tip. The average peroxisomal intensity increased significantly in pxdAΔ hyphal tips, but no differences were observed in the average EE signal or position of the tip-proximal nucleus (Fig. 1 B). As an independent measurement of peroxisome distribution, we found that peroxisomes labeled with PexK (a homologue of Saccharomyces cerevisiae Pex11; Hynes et al., 2008) also accumulated in the hyphal tips of pxdAΔ strains (Fig. S1, C and D). We conclude that PxdA specifically regulates the distribution of peroxisomes, but not EEs or nuclei.

Peroxisome distribution and motility in A. nidulans requires the microtubule-based motors cytoplasmic dynein/NudA and kinesin-3a/UncA (Egan et al., 2012a,b). To determine whether the loss of pxdA affects peroxisome motility and distribution, we imaged peroxisomes in wild-type (WT) and pxdAΔ strains. In WT hyphae, peroxisomes exhibit both long-range runs and oscillatory movements (Video 1). In WT hyphae, 12% (n = 486) of peroxisomes exhibit long-range movements (distances ≥3 µm), consistent with previous studies in mammalian cells in which only 10%–15% of peroxisomes are motile (Rapp et al., 1996; Bharti et al., 2011). These long-range movements are reduced by >90% in pxdAΔ hyphae (Fig. 1, C and D; and Video 2). Furthermore, the velocity and run length of the small pool of peroxisomes that move in pxdAΔ hyphae are also reduced compared with WT (Figs. 1 E and S1 E). In contrast, the flux and velocity of EEs are similar in WT and pxdAΔ strains (Fig. 1, F–H; and Videos 3 and 4).

Taken together, our findings demonstrate that PxdA is required for the proper distribution and long-range movement of peroxisomes, but it is not required for the distribution or motility of EEs or nuclei. Our data also suggest that PxdA is not a core component of the transport machinery because deletions of this type would be predicted to result in defects in the transport or distribution of EEs and nuclei as well. Consistent with this, we find that the localization of dynein, characteristically seen as “comets” near the microtubule-plus end (Xiang et al., 2000), is intact in pxdAΔ hyphae (Fig. S1 F).

Peroxisomal hitchhiking on EEs is mediated by PxdA

Because PxdA is essential for peroxisome movement, we hypothesized that it might colocalize with moving peroxisomes. To visualize PxdA, we tagged the endogenous copy at its C terminus with either TagGFP (referred to as “GFP” from here on) or the red fluorescent protein mKate. C-terminal tagging of PxdA did not disrupt its function because line-scan analysis revealed that PxdA-GFP hyphae have normal peroxisome distribution (Fig. S2 A). In time-lapse videos, PxdA exhibits rapid bidirectional movement along the entire length of hyphae (Video 5). Kymograph analysis reveals that the velocity of PxdA is consistent with that of microtubule-based movement (Fig. 2). Representative kymographs generated from a movie of PxdA-GFP show that PxdA puncta moved 5.48 ± 0.62 (SEM) per hyphae in the DMSO control (C; n = 21 hyphae), 0.33 ± 0.16 per hyphae in the presence of benomyl (Ben; n = 15), and 4.81 ± 0.57 per hyphae in the presence of latrunculin A (Lat A; n = 16). Benomyl but not latrunculin A treatment was significantly different than the control (one-way analysis of variance, Bonferroni post hoc test, *, P < 0.001).

(D) Representative kymographs generated from simultaneous time-lapse movies of peroxisomes (Pex) and PxdA-GFP. White arrows point to a co-migrating PxdA puncta and peroxisome. Bar, 5 µm. (F) Normalized intensity line scans of peroxisomes and PxdA during comigrating runs (n = 11).
Indeed, we find that PxdA accumulates at the hyphal tip in strains. HookA EE's, PxdA would accumulate at hyphal tips in Δ 2014). We reasoned that if most PxdA protein is associated with the time-lapse movies and kymographs revealed that PxdA localizes at the leading edge of moving peroxisomes with the peak PxdA signal leading by 560 ± 80 nm (mean ± SEM; Fig. 2 F). We also occasionally observed instances in which a moving PxdA puncta paused at a nonmotile peroxisome, an event that was followed by the movement of comigrating PxdA and the peroxisome (Fig. 2 D, middle and right).

Because we observed many more moving PxdA puncta than moving peroxisomes, and because PxdA motility was reminiscent of EE motility (Abenza et al., 2009; Zhang et al., 2010; Egan et al., 2012b, 2015), we next asked whether PxdA also colocalizes with EEs. To test this, we analyzed kymographs from simultaneous time-lapse movies of labeled PxdA and EEs (Video 7). Ninety percent of PxdA puncta colocalize with EEs, but only 55% of EEs colocalize with PxdA puncta (Fig. 3, A and B), demonstrating that PxdA is found on a subset of EEs.

We next sought to determine whether perturbing EE localization also perturbed PxdA localization. HookA has recently been identified as a cargo adaptor between EEs and dynein and kinesin-3 in filamentous fungi (Bielska et al., 2014; Zhang et al., 2014), and its vertebrate homolog (Hook3) is an activator of dynein/dynactin (McKenney et al., 2014). In the absence of HookA, EEs accumulate near the hyphal tip (Zhang et al., 2014). We reasoned that if most PxdA protein is associated with EEs, PxdA would accumulate at hyphal tips in hookAΔ strains. Indeed, we find that PxdA accumulates at the hyphal tip in hookAΔ compared with WT strains (Fig. 3, C and D), suggesting that PxdA is present on EEs that require HookA for motility.

The association between PxdA and EEs is somewhat surprising given that PxdA does not affect the motility or distribution of EEs (Fig. 1). Because PxdA colocalizes with a subset of EEs and moving peroxisomes, we next asked if EEs colocalized with moving peroxisomes. Kymograph analysis (Fig. 3 E) of time-lapse movies (Fig. 3 F and Video 8) reveals that 71% (n = 28) of moving peroxisomes colocalize with EEs, correlating well with the percentage of PxdA that colocalizes with moving peroxisomes (66%). In addition, similar to PxdA, EEs colocalized at the leading edge of moving peroxisomes (Fig. 3 F and Video 8). The peak EE signal was leading the peak peroxisome signal by 610 ± 170 nm (Fig. 3 G), comparable with what we observed for peroxisomes and PxdA (Fig. 2 F).

These results suggest that peroxisomes move by “hitchhiking” on EEs and that this process is mediated by PxdA. The concept that cellular components can hitchhike on EEs has been observed in U. maydis for Rrm4-containing ribonucleoprotein complexes (Baumann et al., 2012) and polysomes (Higuchi et al., 2014). In a study parallel to ours, also in U. maydis, peroxisomes, endoplasmic reticulum, and lipid droplets were observed to hitchhike on EEs (Guimaraes et al., 2015).

Endosomal localization of PxdA is required for peroxisome hitchhiking

We hypothesized that PxdA tethers EEs to peroxisomes to promote hitchhiking. If this were the case, we might be able to separate PxdA’s EE localization from its function in peroxisome motility. To test this, we constructed a series of PxdA mutants (Fig. 4 A).

First, we sought to determine which domains of PxdA are necessary and sufficient for peroxisome motility. PxdA encodes a 2236-aa protein containing an ∼600-aa stretch of predicted...
tandem coiled-coil domains (CC1–3), flanked by a large N-terminal uncharacterized region (UR1) and a much smaller C-terminal uncharacterized region (UR2; Fig. 4 A). Initially, we truncated the entire N-terminal UR1 (ΔNT) or the entire C-terminal region, including CC1–3 and UR2 (ΔCT) or the UR2 region alone (ΔUR2). We performed peroxisome line scans and found that peroxisomes accumulated at the hyphal tip of ΔCT strains, but not full-length (FL), ΔUR2, or ΔNT strains (Fig. 4 B). The PxdA-ΔCT strain also had a significant reduction in the flux of peroxisome movements compared with the FL and ΔUR2 strains (Fig. 4 C). The PxdA-ΔCT strains, but not full-length (FL), ΔNT strains and found that peroxisomes accumulated at the hyphal tip of UR2 region alone (Δ UR2; Fig. 4 A). Initially, we truncated the entire N-terminal UR1 (ΔNT) and a much smaller C-terminal uncharacterized region (UR2; Fig. 4 C). This analysis suggested that only the tandem CC (CC1–3) of PxdA is required for peroxisome distribution and motility. To test this directly, we expressed PxdA’s CC1–3 alone and quantified peroxisome distribution and flux along hyphae. CC1–3 is sufficient to rescue the defects in both peroxisome distribution and peroxisome flux observed in pxdΔ hyphae (Figs. 4 D and S2 B). Furthermore, PxdA CC1–3 localizes to moving puncta that show velocity and flux similar to those of strains expressing FL PxdA (Fig. S2, C–E). These experiments show that CC1–3 is necessary and sufficient for PxdA’s function on peroxisomes.

We next sought to identify the region of PxdA required for its localization to EEs. We constructed a PxdA-mKate strain lacking CC2 and CC3 (ΔCC2/3; Fig. 4 A). Like PxdA ΔCT strains, PxdA ΔCC2/3 strains displayed an accumulation of peroxisomes at the hyphal tip (Fig. 4 E) and a severe drop in peroxisome flux (Fig. 4 F). In addition, PxdA ΔCC2/3-mKate had a diffuse signal in hyphae that was different from the EE localization observed for the FL protein, and only 3% of PxdA ΔCC2/3-mKate colocalized with EEs (Fig. 4 G). These data suggest that CC2/3 is critical for the EE localization of PxdA and that EE-linked PxdA is required for the proper distribution and movement of peroxisomes.

Overall, our study reveals that peroxisomes require PxdA to hitchhike on EEs for long-range movement. The abundance and rapid bidirectional movement of EEs in fungal hyphae make them a prime multifunctional platform for distributing a wide range of cargo (Göhre et al., 2012). In our model, HookA recruits the transport machinery to EEs. PxdA is required to tether EEs to peroxisomes and may have distinct binding partners on each organelle. The arrow depicts the direction of movement.
EEs (~150 nm; Murk et al., 2003; Griffith et al., 2011). The size of PxdA’s tandem coiled coil (CC1–3), which is predicted to be ~90 nm, could easily bridge the distance between the peroxisome and EE. Interestingly, although there is no clear homologue of PxdA in mammalian species, a region of the coiled coil contains an F-bar domain (van Weering and Cullen, 2014) and is also weakly conserved with FKBP15, a protein known to associate with EEs in mammalian cells (Viklund et al., 2009). Exciting future directions include determining whether hitchhiking is broadly conserved outside of fungi, identifying the linkers required for other cargos to hitchhike on EEs, and determining the mechanisms that initiate and terminate hitchhiking.

Materials and methods

Fungal growth conditions

A. nidulans strains were grown in yeast extract and glucose medium (Szewczyk et al., 2006) or 1% glucose minimal medium (Nayak et al., 2006), supplemented with 1 mg/ml uracil, 2.4 mg/ml uridine, 2.5 µg/ml riboflavin, 1 µg/ml para-aminobenzoic acid, and 0.5 µg/ml pyridoxine when required. Glusfonate was used at a final concentration of 25 µM as previously described (Nayak et al., 2006).

For imaging of germlings (for all organelle distribution experiments and CC1–3 flux experiments), spores were resuspended in 0.5 ml of 0.01% Tween-80 solution. The spore suspension was diluted at 1:1,000 in liquid minimal medium containing appropriate auxotrophic supplements. The spore and media mix (400 µl) was added to an eight-chambered Nunc Lab-Tek II coverglass (Thermo Fisher Scientific) and incubated at 30°C for 16–20 h before imaging. For imaging of mature hyphae (for velocity and flux experiments), spores were inoculated on minimal medium plates containing the appropriate auxotrophic supplements and incubated at 37°C for 12–16 h. Colonies were excised from agar plates and inverted on Lab-Tek plates for imaging. For benomyl and latrunculin A experiments, minimal media containing the drugs (2.4 µg/ml for benomyl [Sigma-Aldrich]; 12 µM for latrunculin A [Life Technologies]) or DMSO control (1.2%) were added fresh to Lab-Tek chambers containing germings and were imaged 45 min to 1 h later. For Western blots, cells were grown in yeast extract and glucose medium for 16–20 h, strained in Miracloth (Millipore), and flash frozen in liquid nitrogen. For lysis, cells were grounded in liquid nitrogen and boiled in 9 M urea denaturing buffer (125 mM Tris-HCl, pH 6.8, 9 M urea, 1 mM EDTA, pH 7.0, 4% SDS, 10 mM DTT, 10% β-mercaptoethanol, and 4% glycerol) before running on SDS-PAGE.

Strain construction

Strains of A. nidulans used in this study are listed in Table S1. All strains were confirmed by a combination of PCR and sequencing from genomic DNA isolated as previously described (Lee and Taylor, 1990). Strains were created by homologous recombination to replace the endogenous gene in strains lacking ka70 (Nayak et al., 2006) with Afribo (Aspergillus fimigatus Ribo), AfpAyG (Aspergillus fimigatus pyrG), AfpAyro (Aspergillus fimigatus pyro), or bar (Straubinger et al., 1992) as selectable markers. All linearized transforming DNA for deletion strains, including pxdA and hookA, were constructed by fusion PCR (Szewczyk et al., 2006). All PxdA DNA constructs contained a C-terminal codon-optimized fluorescent protein tag, either TagGFp2 (Subach et al., 2008) or mKate2 (Shcherbo et al., 2009), followed by pxdA’s native 3′ UTR, and were inserted into the Blue Heron Biotechnology pUC vector at 5′ EcoRI and 3′ HindIII restriction sites using isothermal assembly (Gibson et al., 2009). Plasmid constructs were confirmed by sequencing. Linearized transforming DNA from plasmid constructs was created by PCR with flanking primers located at the 5′ and 3′ ends of homologous arms.

The amino acid positions for each PxdA construct in Fig. 4 were as follows: FL, aa 1–2,236; ΔNT, aa 1,466–2,236; ΔCT, aa 1–1,465; ΔUR2, aa 1–2,116; and ΔCC2/3, aa 1–1,946. For the CC1–3 rescue experiment (Figs. 4 D and S1, B–D), the CC1–3 construct (aa 1,466–2,116) was inserted into a pxdAΔ strain at the endogenous locus.

Fluorescence microscopy

All images were collected at 22°C. For acquisition of the distribution and motility of peroxisomes (PTSI-mCherry and PexK-GFP) and for the distribution of EEs (GFP-RabA/5a), dynein (NudA-3xGFP), and PxdA, images were collected using a Plan Apo 60×/1.42 (Olympus) oil immersion objective on an epifluorescence DeltaVision Core microscope (GE Healthcare). GFP was excited with a 488-nm laser line (50 mW) and collected with a 525/50 FITC emission filter. mCherry fluorescence was excited with the 561-nm laser line (50 mW) and collected with a 594/45 emission filter. Images were acquired with a PICO edge sCMOS camera (Kelticam) and controlled with softWoRx software (GE Healthcare). For distribution images, 20 optical z-sections were collected with a step size of 0.5 µm. Hyphae (used for outlining cells) were imaged using brightfield.

Simultaneous multicolor time-lapse images were collected using a Plan Apo 60×/1.42 oil immersion objective on the DeltaVision OMX Blaze V4 system (GE Healthcare). GFP and mKate2/mCherry were excited simultaneously with 488- and 568-nm diode laser lines (100 mW), respectively. A series of dichroic mirrors were used to split emission light from fluorophores to different PCO Edge sCMOS cameras. An emission filter in front of both cameras was used to select appropriate wavelengths (528/48 and 609/37 for GFP and mKate2/mCherry, respectively). Images were aligned and registered using the OMX alignment slide and corrected for translation, rotation, and magnification differences between channels using softWoRx software. Some images were deconvolved using the enhanced ratio method (five iterations) and/or binned (2 × 2).

For EE and PxdA motility assays, time-lapse images were collected using a Plan Apochromat TIRF 100×/1.49 oil immersion objective on an epifluorescence Ti-E motorized inverted microscope (Nikon) with the Perfect focus system (Nikon) for continuous maintenance of focus, all controlled by NIS-Elements software (Nikon). GFP fluorescence was excited using a 488-nm laser line (50 mW) and collected with a 525/50 emission filter. Images were acquired with an EM-CCD camera (DU-897; Andor).

Images were brightness and contrast adjusted using ImageJ (version 2.0; National Institutes of Health) and Photoshop CS3 (version 10.0; Adobe), and figures were compiled in Illustrator CC (2015.2; Adobe).

Image and data analysis

For peroxisome, EE, and PxdA line-scan distribution measurements, maximum-intensity projections of fluorescence micrographs and brightfield images (for hyphae) were obtained using ImageJ. Brightfield images of hyphae were traced using the segmented line tool (line width 25), starting from the hyphal tip and ending at a point indicated in the x-axis of respective line-scan graphs. Each trace was overlaid onto the corresponding fluorescence micrograph and used to project the average fluorescence intensity. For peroxisome normalization (Fig. 4), average intensity values at each point along hyphae were normalized against that particular condition’s baseline average (5–10 µm). For PxdA normalization (Fig. 3 D), average intensity values at each point along hyphae were normalized against that particular condition’s baseline average (5–10 µm). For flux measurements, the number of puncta crossing a line 10 µm perpendicular to and from the hyphal tip was calculated (Egan et al., 2012b).
EE, peroxisome, and PxdA velocities and run lengths were measured using ImageJ. Maximum-intensity projections were generated from time-lapse sequences to define the trajectory of particles of interest. The segmented line tool was used to trace the trajectories and map them onto the original video sequence, which was subsequently resliced to generate a kymograph. The instantaneous velocities of individual movements (for runs ≥2 μm in length) were calculated from the inverse of the slopes of kymograph traces, and run-length measurements were obtained from each of these traces. For measurements of normalized intensity during co-migrating runs between PxdA or EE s with peroxisomes (Figs. 2 E and 3 F), peroxisomes and associated puncta (PxdA or EEs) were traced using ImageJ at a point along the trajectory of the path (in the direction of movement) that was clear and distinct from other puncta on the micrograph. Line intensity profiles for each channel individually were generated. The “O” along the x axis corresponds to the position of peak intensity for the peroxisome signal. Intensities for each channel were normalized to that channel’s peak intensity for each comigrating run.

Data visualization and statistical analyses were performed using GraphPad Prism (6.0d; GraphPad Software), Excel (version 14.5.8; Microsoft), and ImageJ (2.0). Analysis of variance followed by Bonferroni post hoc statistical tests were used for all distribution comparisons, and nonparametric Mann-Whitney or Kolmogorov-Smirnov tests were used for all flux and velocity comparisons, respectively. All experiments were analyzed from at least three independent replicates, except for ΔCC2/3 experiments (Fig. 4, E–G) and CC1–3 flux/velocity measurements (Fig. 52, B, D, and E), which were analyzed from two independent replicates.

Online supplemental material

Fig. S1 shows that peroxisomes accumulate in the hyphal tip in PxdA mutant and deletion strains, but EEs, nuclei, and cytoplasmic dynein are unaffected. Fig. S2 shows that tagging PxdA does not affect its function and that expressing its CC alone moves similarly to the FL protein. Video 1 shows peroxisome dynamics in a WT hypha. Video 2 shows peroxisome dynamics are perturbed in a pxdAΔ hypha. Video 3 shows EE dynamics in a WT hypha. Video 4 shows that EE dynamics are unaffected in a pxdAΔ hypha. Video 5 shows PxdA-GFP dynamics in a hypha. Video 6 shows PxdA-GFP comigrating with a moving peroxisome. Video 7 shows PxdA-GFP colocalizing with EEs. Video 8 shows an EE comigrating with a moving peroxisome. Table S1 lists the A. nidulans strains used in this study. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201512020/DC1. Additional data are available in the JCB DataViewer at http://dx.doi.org/10.1083/jcb.201512020.dv.

Acknowledgments

We thank the Reck-Peterson laboratory and Andres Leschziner for critical feedback on experiments and the manuscript. We thank Jennifer Waters, Talley Lambert, and The Nikon Imaging Center at Harvard Medical School for helpful technical advice and imaging training.

Early stages of this project were supported by a National Institutes of Health New Innovator award (OD004268) to S. Reck-Peterson and subsequent work by RO1 GM 107214 to S. Reck-Peterson. The authors declare no competing financial interests.

Submitted: 4 December 2015
Accepted: 5 January 2016

References


