Actin remodelling and autophagy meet at the peroxisome

Microscopic visualization of the ARP2/3 complex showed that it colocalizes with peroxisomes in *Arabidopsis thaliana* and tobacco cells in vivo. Colocalization with an autophagy marker and analysis of peroxisomes in autophagy-mutant or ARP2/3-mutant lines demonstrated that ARP2/3 facilitates peroxisome degradation by the autophagic pathway, that is, pexophagy.

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The question

The ARP2/3 complex is a seven-subunit protein complex that has a conserved role in actin remodelling at the actin–membrane interface[1]. In plants, loss of the active ARP2/3 complex by mutation of individual genes has multiple phenotypic manifestations, including a well-characterized disruption of the morphogenesis of cells with complex shapes, such as trichomes, or of epidermal cells[2]. ARP2/3 is also involved in other processes associated with membrane remodelling, such as host plant interactions with symbiotic organisms, apical growth and autophagy. As ARP2/3-mediated actin nucleation is involved in a number of important processes in plants, a more detailed understanding is needed of the role of actin in membrane remodelling. To address this issue, we tagged different subunits of the complex with GFP and RFP and expressed them in *Arabidopsis thaliana* and tobacco plants. Our aim was to identify where the ARP2/3 complex is found in plant cells and whether its localization is related to any of the processes disrupted in plants lacking the ARP2/3 complex.

The discovery

Surprisingly, tagged ARP2/3 subunits localized to numerous highly mobile structures in the cytoplasm. After colocalization experiments with organellar markers established that these structures are peroxisomes, we asked whether additional ARP2/3 subunits localize to peroxisomes and whether the ARP2/3 complex assembled there is active. A tagged version of NAP1 — a component of WAVE/SCAR, which activates the ARP2/3 complex — colocalized with these motile structures, indicating that the active, assembled ARP2/3 complex localizes to peroxisomes. To examine this localization at peroxisomes in greater detail, we performed Airyscan super-resolution microscopy, which revealed that ARP2/3 is found at the periphery of peroxisomes (Fig. 1a). We performed a variety of microscopy and biochemical experiments to determine whether lacking the ARP2/3 complex impaired peroxisome function, number or size in plants. These analyses revealed that mutants lacking a functional ARP2/3 complex have more and larger peroxisomes than wild-type plants. To identify proteins that interact with ARP2/3 at peroxisomes, we performed proteomic analysis of the peroxisomal fraction from plant cells. We detected all seven subunits of the ARP2/3 complex, confirming our results from the microscopy analysis. Interestingly, our microscopy experiments revealed that the autophagic marker ATG8f localized to peroxisomes, indicating that ARP2/3 might be involved in autophagic degradation of peroxisomes (pexophagy). We confirmed by confocal microscopy that ATG8f colocalizes with the ARP2/3 complex at peroxisomes (Fig. 1b). We subsequently focused our analysis on autophagic processes in ARP2/3 and autophagy mutants.

Pharmacological inhibition of autophagy resulted in a substantial increase in the abundance and colocalization of the ARP2/3 complex and ATG8-positive peroxisomal domains. Furthermore, autophagic flux was markedly decreased in ARP2/3 mutants. Thus, our data clearly link the ARP2/3 domain on peroxisomes to pexophagy.

Future directions

Our results are consistent with data from other organisms where a role for ARP2/3 complex in membrane remodelling during autophagy has been described[3]. Although loss of ARP2/3 in plants is not as lethal as it is in animals or yeast, it seems that some functions of this complex are conserved. However, the accumulation of ARP2/3 at peroxisomes was a surprise. A role for the ARP2/3 complex in pexophagy thus extends the list of its functions in plants, which is already quite diverse, and demonstrates its extensive involvement in cellular processes.

Peroxisomes are comparatively poorly studied among plant organelles, but we know that they are important in many metabolic processes[4]. Peroxisomes are small organelles with an average size of about 1 um, so studying them is difficult as they are close to the diffraction limit of standard confocal fluorescence microscopy. Consequently, with the methods we used, we were unable to distinguish whether ARP2/3 localizes to the cytoplasmic side of peroxisomes or whether it can also enter the peroxisome lumen. Similarly, we have only limited evidence for the existence of actin in this peroxisomal domain. Our future work will incorporate the use of high-resolution microscopy, including electron microscopy, to understand the precise function of ARP2/3 and actin in membrane remodelling during the early stages of pexophagy.

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EXPERT OPINION

"Using a variety of inhibitors, live-cell imaging analyses and co-immunoprecipitation experiments, the authors report numerous connections between ARP2/3, peroxisomes and autophagosomes. The authors are uncovering a new area of biology that will have a broad impact on the field of cell biology." **Daniel Szymanski, Purdue University, West Lafayette, IN, USA.**

FIGURE

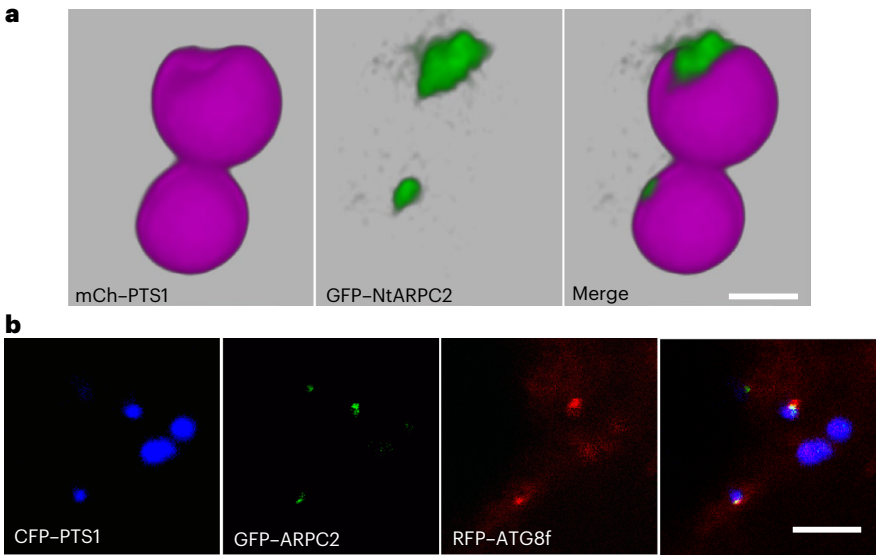

Fig. 1 | Colocalization of ARP2/3 with peroxisomes in plant cells. a, Airyscan super-resolution imaging of the GFP-tagged ARP2/3 subunit *Nt*ARPC2 (green) and the mCherry-tagged peroxisome marker PTS1 (magenta) in *Arabidopsis* hypocotyl epidermal cells. The two channel Z-stack was displayed in Zen black 3D view and rendered in Transparent mode. Bar 1 µm. **b**, Confocal microscopy images of CFP–PTS1, GFP–ARPC2 and RFP–ATG8f in *Arabidopsis* hypocotyl epidermis, showing that the ARP2/3 complex and the autophagy marker ATG8f colocalize to peroxisomes. Bar 5µm. © 2023, Martinek, J. et al.

BEHIND THE PAPER

When we found that tagged components of ARP2/3 colocalized with peroxisome markers in vivo, we were a bit disappointed because dysfunction of this organelle was least likely to explain the known phenotypes of plant ARP2/3 mutants. A possible association of ARP2/3 with peroxisomes was suggested by the presence of more and larger peroxisomes in mutant plants. However, after this first observation, the research project stalled because we could not link ARP2/3 to any peroxisome-specific process. In contrast, the discovery that an autophagy marker colocalizes with the ARP2/3 peroxisomal domain quickly got the project going again. Experiments with inhibitors of autophagic processes were a key confirmation that ARP2/3 function in the peroxisomal domain is associated with autophagic degradation of peroxisomes. **K.S.**

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FROM THE EDITOR

"The ARP2/3 complex is a well-known and conserved protein complex involved in actin nucleation. This work shows that the ARP2/3 complex forms domains at the surface of *Arabidopsis* peroxisomes that colocalize with autophagosomes and are required for peroxisome turnover, making a link between membrane remodelling and organellar degradation in plants." **Raphael Trösch, Associate Editor, *Nature Plants*.**