Give what you can and keep what you need!

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During cell division, peroxisomes are inherited to daughter cells but some are retained in the mother cells. Our knowledge on how peroxisome inheritance and retention is balanced and how this is regulated for each individual organelle remains incompletely understood. The new findings by Knoblach *et al* (2013) published in this issue of *The EMBO Journal* demonstrate that Inp1p functions as a bridging protein to connect ER-resident Pex3p and peroxisomal Pex3p, which anchors peroxisomes to the cortical ER for organelle retention in the mother cell. Asymmetric peroxisome division generates peroxisomes, which lack Inp1p but contain Inp2p instead, and only these peroxisomes are primed for myosin-driven transport to daughter cells.

Peroxisomes are single membrane-bound organelles found in almost all eukaryotic cells. They harbour a wide spectrum of metabolic activities that vary among different species, developmental stages and cell types (Schlüter *et al*, 2010). Eukaryotic cells have evolved elaborate mechanisms to ensure the maintenance of peroxisomes. New peroxisomes can form either *de novo* by budding from the ER or by growth and division of pre-existing organelles (Lazarow and Fujiki, 1985; Hoepfner *et al*, 2005). Despite the fact that peroxisomes can form *de novo*, yeast favours to multiply peroxisomes by growth and division (Motley and Hettema, 2007). It therefore has to be ensured that both mother and daughter cells get their share of peroxisomes during cell division. Thus, some peroxisomes need to be retained in the mother cell, while other peroxisomes are directed for transport and inheritance to daughter cells. Both processes have to be balanced to ensure a successful distribution of the organelles between the mother cell and the newly formed bud.

The molecular details of how an even peroxisome distribution of dividing cells are maintained have now been disclosed by Knoblach *et al* (2013), advancing an exciting scientific journey. This journey originally started by the finding that the partitioning of peroxisomes between mother cell and bud is dependent on actin filaments and the myosin motor protein Myo2p (Hoepfner *et al*, 2001). Inp1p and Inp2p were identified by the Rachubinski group and Inp2p turned out to function as the peroxisomal tether, which interacts with Myo2p and hooks the organelle onto the actin-track on the road to the bud (Fagarasanu *et al*, 2006). Inp1p was shown to be a peripheral peroxisomal membrane protein, which acts as a peroxisome-retention factor, tethering peroxisomes to

putative anchoring structures within the mother cell and bud (Fagarasanu *et al*, 2005). Later on, Pex3p, a multi-functional protein of the peroxisomal life cycle, was identified as peroxisomal membrane anchor of Inp1p (Munck *et al*, 2009). Until now, it was therefore known that peroxisomes hook onto Inp1p by Pex3p and Inp1p connects peroxisomes to cortical structures of unknown nature. Thus, it was an open question how peroxisomes are trapped in the mother cell and which additional factors are required for this process.

The work of Knoblach *et al* (2013) published in this issue of *The EMBO Journal* now unravelled this mystery, allowing for a more complete picture of the whole process of peroxisome retention and inheritance (Figure 1A). The authors show that peroxisomes are recruited to mitochondria that artificially expose Inp1p on their surface, clearly demonstrating that Inp1p acts as a peroxisome tether. Most importantly, they identified the mechanism of how peroxisomes are directed and anchored to the cell cortex: the ER acts as a membrane anchor for the retention of peroxisomes during cell division. *In vitro* binding assays revealed that Inp1p contains two independent binding sites for Pex3p, located at the C- and the N-terminal region of the protein, respectively. Since Pex3p exhibits a dual localization at the peroxisomal membrane and at the ER, Inp1p seems to bind to Pex3p of both compartments *in vivo* and thus link Pex3p molecules across two membranes. Indeed, it turned out that ER-located Pex3p recruits Inp1p to discrete foci in close proximity to the cortical ER. Using the split-GFP assay, the authors confirmed that Inp1p interacts not only with ER-bound Pex3p but also with Pex3p in the peroxisomal membrane. Thus, the core of the ER-peroxisome tether is generated by the Inp1p-mediated linkage of ER-bound Pex3p with peroxisomal Pex3p. The functional relevance of this ER-peroxisome tether is disclosed by the phenotype of peroxisome inheritance mutants. Accordingly, the Pex3p-V81E mutant, affected in the recruitment of Inp1p to the ER, is characterized by a defect of ER retention of peroxisomes, which drives all peroxisomes into the bud and leaves no peroxisomes in the mother cell (Figure 1B).

To piece together the puzzle, a final gap had to be filled. How is the peroxisomal fraction remaining in the mother cell discriminated from those ferried to the bud during cell division? In budding wild-type cells, Inp1p exhibits a striking asymmetry along the cell division axis. Knoblach *et al* (2013)

Figure 1 Peroxisome retention and inheritance (A) free peroxisomes in the mother cell (stage I) are anchored to cortical ER by a tethering complex consisting of two molecules Pex3p, one located at the ER and the other associated with the peroxisomal membrane and Inp1p, which connects the ER-bound and peroxisome-bound Pex3p (stage II). Accordingly, Inp1p contains two Pex3p-binding domains, allowing the protein to function as a bridge between the two Pex3p-containing organelles. Peroxisomes elongate and divide, and Inp2p is loaded onto peroxisomes with an asymmetric distribution (stage III). The peroxisomal population that lacks Inp2p is anchored to the cortical ER, whereas the population of cytosolic peroxisomes containing Inp2p is destined for the transport to the bud (stage IV). To this end, Inp2p interacts with Myo2p and thus triggers the movement of the peroxisome along actin cables to the bud. The process is completed when the peroxisome is released from Myo2p in the bud (stage V). In wild-type cells, the described retention and inheritance process leads to an equal distribution of peroxisomes between mother cell. The described molecular mechanism results in a regulated balance of retention and inheritance of peroxisomes, ensuring that both the mother cell and the newly formed bud gain their share of peroxisomes. (B) However, when the endogenous Pex3p is replaced by a Pex3p-mutant (Pex3p-V81E), which lost its strong binding capacity to Inp1p, peroxisomes are not anchored to the cortical ER anymore, with the consequence that during cells' division the entire organelle population is transported to the bud and peroxisomes are not retained in the mother cell.

show that most peroxisomes of the mother cell contain Inp1p, while peroxisomes that are ferried towards the bud contain little or no Inp1p. Live-cell video microscopy of individual peroxisome revealed that Inp1p-containing peroxisomes were mostly immobile and retained in the mother cell, while highly mobile peroxisomes contained Inp2p and were predominantly found in the bud. The question remains of how peroxisomes lacking Inp1p but containing Inp2p are formed? To tackle this question, the authors took advantage of the fact that cells defective in peroxisome division contain single enlarged peroxisomes and project a tubular extension into the bud upon cell division (Kuravi *et al*, 2006).

Remarkably, Knoblach *et al* (2013) show that Inp1p and Inp2p localized to opposite ends of the giant peroxisome. Inp1p was confined to the part of the peroxisome that was retained in the mother cell, while Inp2p enriched at the tubule that protruded into the bud.

In summary, Knoblach *et al* (2013) discovered the ER as the site for peroxisome binding to the cell cortex that is responsible for the retention of peroxisomes in the mother cells during cell division and identified Inp1p as a molecular hinge connecting Pex3p of peroxisomal and ER membranes. Furthermore, peroxisome division is shown to result in an asymmetric distribution of inheritance factors with

Inp1p-containing organelles remaining tethered to the ER in the mother cell, while Inp2p-containing peroxisomes hook onto myosin motor proteins for movement to the bud. These remarkable discoveries disclose the molecular mechanism of peroxisome retention and inheritance during cell division. Moreover, this study adds to other known functions of Pex3p, which besides its newly discovered role as ER-tether for peroxisomes is also known as an initiator of *de novo* formation of peroxisomes, a docking factor for the transport

of peroxisomal membrane proteins and a tether for the regulated degradation of peroxisomes. This study adds more complexity to the network of regulated processes in peroxisome biogenesis that all merge at Pex3p, and will certainly provide the ground for further exploration.

Conflict of interest

The authors declare that they have no conflict of interest.

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