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ORGANELLE BIOGENESIS

When two become one

Peroxisome biogenesis is unconventional compared with other organelles, occurring by a two-step process. First, peroxisomal membrane proteins (PMPs) and lipids from endoplasmic reticulum (ER)-derived preperoxisomal vesicles drive the formation of an 'immature' peroxisome and the peroxisomal translocon. Second, the translocon mediates the import of matrix proteins from the cytosol to produce a fully functioning peroxisome. But how the translocon itself assembles has been the source of some debate. Van der Zand and colleagues find that multiple trafficking routes are used to ensure that the full complement of translocon components only comes together in the peroxisomal membrane, perhaps ensuring additional control over where and when new peroxisomes arise.

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The translocon consists of two halves: a docking complex formed by the PMPs Pex13 and Pex14; and a RING finger complex composed of Pex2, Pex10 and Pex12. Van der Zand and colleagues set out to determine how these PMPs traffic from the ER to establish the initial preperoxisome and mediate the formation of the translocon. First, they labelled individual endogenous PMPs in live yeast cells and confirmed that the proteins were functional and formed the expected subcomplexes. Next, they examined different Pex mutants that disrupt peroxisome biogenesis at distinct steps to track when and where PMPs interact with each other. Cells carrying mutations that prevent either PMP exit from the ER or that allow only immature ER-derived preperoxisomal vesicles to form contained translocon subcomplexes but not the full translocon. Therefore, although subcomplexes could form in the ER and in preperoxisomal vesicles, something was preventing the formation of a full translocon on these membranes.

This led the authors to ask whether PMP subcomplexes leave the ER in distinct membrane carriers, ensuring their separation until a new peroxisome is formed. Indeed, biochemical characterization of the membrane fractions from Pex mutants that accumulate immature ER-derived preperoxisomal vesicles showed that RING finger PMPs and docking PMPs concentrate in different fractions. Moreover, co-immunoprecipitation and colocalization analyses verified that docking PMPs and RING finger PMPs are kept apart in different membrane carriers.

To show that this separation of the two half-translocons is not specific to Pex mutants, the authors tracked fluorescently labelled newly synthesized PMPs by pulse-chase analysis. They found that, whereas PMPs from a common subcomplex interacted at each trafficking step, PMPs from the two halves of the translocon did not coincide until a later time point. They thus conclude that docking and RING finger PMPs traffic from the ER in distinct preperoxisomal membrane vesicles and come together only in the peroxisomal membrane.

How do these vesicle precursors come together to form mature peroxisomes? It has previously been proposed that this might occur by heterotypic fusion of preperoxisomal vesicles, in a process that depends on the peroxisome proteins Pex1 and Pex6. By combining fluorescence pulse-chase analysis and a cell fusion assay, van der Zand and colleagues confirmed that only preperoxisomal vesicles underwent heterotypic fusion and that mature peroxisomes could not fuse. They also confirmed that fusion required Pex1 and Pex6, which localized to distinct vesicle fractions.

Finally, the authors asked whether the subcomplexes that form in cells expressing Pex1 or Pex6 mutants have the potential to form full translocons and are thus functional intermediates. They showed that mating the two mutants allowed fusion of the preperoxisomal vesicles to resume and, ultimately, led to the import of the matrix protein PTS1 (peroxisomal targeting signal receptor 1) labelled with cyan fluorescent protein and the formation of a mature peroxisome. As this was not the result of preperoxisomal vesicle fusion with existing peroxisomes, the authors conclude that heterotypic fusion allows the formation of new translocons and, consequently, of active peroxisomes.

Thus, by sorting the translocon subcomplexes into distinct vesicles and allowing translocon formation only after their heterotypic fusion, the ER provides an additional layer of control over peroxisome biogenesis. Working out how PMP sorting at the ER and subsequent vesicle fusion occur will therefore be key for understanding peroxisome biogenesis.

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ORIGINAL RESEARCH PAPER van der Zand, A. *et al.* Biochemically distinct vesicles from the endoplasmic reticulum fuse to form peroxisomes. *Cell* **149**, 397–409 (2012)