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SPOTLIGHT

Pexophagy meets physiology

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In this issue, Xiong et al. (https://doi.org/10.1083/jcb.202503169) introduce mouse models that enable tissue-resolved mapping of peroxisome turnover and pexophagy across development, metabolism, and disease. This study reveals striking cell type–specific differences in peroxisome dynamics and establishes a versatile platform for dissecting how pexophagy integrates with mitochondrial quality control and whole-body metabolic homeostasis.

Peroxisomes are membrane bounded organelles found in nearly all eukaryotic cells. They work alongside mitochondria to control reactive oxygen species and lipid metabolism. Notably, they are solely responsible for β-oxidation of long-chain fatty acids, which mitochondria cannot handle (1). They are essential to the synthesis of plasmalogens, ether phospholipids highly expressed in myelin, with many critical roles in brain function. These lipids have also been strongly linked to the ferroptosis pathway of cell death. Like most other organelles, peroxisomes can be degraded by a selective autophagy process, in this case termed pexophagy. We need pexophagy to protect cells from oxidative damage, regulate ferroptosis sensitivity, renew peroxisomal metabolic capacity, and dynamically match peroxisome function to physiological needs. Defective pexophagy has been proposed to be a fundamental feature of Zellweger spectrum disorders that are strongly linked to peroxisome homeostasis (2).

Reporters for selective autophagy often use a standard procedure of expressing a fusion protein containing two fluorescent proteins, one pH sensitive and the other unaffected by pH, targeted to specific organelles. This approach commonly combines GFP (unstable at low pH) and RFP (low pH stable) to monitor delivery of autophagosomes to acidic lysosomes by the appearance of uniquely red puncta and can be used on fixed cells. An alternative is to use a targeted Keima fluorescent protein, which has a pH-dependent switch in its excitation wavelength that can be utilized only with live-cell imaging. Both approaches have been used in cell models to study pexophagy (3, 4, 5). Mechanistic details to emerge so far include the identification of at least two distinct pathways. One involves ubiquitylation of peroxisome proteins that recruits the autophagy adaptor protein NBR1, which can be suppressed by the deubiquitylase USP30 (3, 5, 6). The second involves direct insertion of the HIF1α-regulated gene BNIP3L, otherwise known as NIX, into the peroxisomal membrane (4, 5). Intriguingly, newly synthesized BNIP3L can insert into either mitochondria or peroxisomes to promote their autophagy. This has led to the idea of synchronized autophagy, i.e., the coordinated removal of distinct organelles by upregulation of a shared "selective" autophagy factor.

Selective autophagy reporter assays have been transferred to model organisms, which enable the study of autophagy dynamics across tissues, during development, and in disease conditions. Mitophagy reporter probes revealed that the widely studied PINK1/Parkin-dependent pathway contributes little to basal mitophagy in most tissues of flies and mice (7, 8). Initial surveys of pexophagy in *Caenorhabditis elegans* and *Drosophila melanogaster* have been made using a GFP-Cherry reporter combined with the SKL peroxisome-targeting motif (9, 10). In the current issue, Xiong et al. introduce a similar reporter, here termed PerOxisomal Lumen Tandem RFP-GFP (PO-TRG), which has been used to visualize pexophagy in mice (11) (Fig. 1). The authors find tissue-specific differences in the relative prominence of pexophagy; for example, skeletal muscle contains few peroxisomes but high pexophagy activity, whereas brown and white fat is peroxisome rich, but pexophagy poor. In the mouse liver, which is the major site of long-chain fatty acid synthesis, pexophagy is low at birth but increases steadily until adulthood. Following high-fat diet-induced obesity, the level of hepatic pexophagy decreases. The extent to which pexophagy dictates the abundance of peroxisomes in each tissue will only become clear when these mice are crossed with knockout mice for specific pexophagy adaptors, such as BNIP3L or NBR1.

One concern that is generic to all selective autophagy reporters is whether the measured output reflects true selective autophagy or simply reflects different extents of nonselective autophagy. This can be addressed by direct comparison with a mouse model expressing a GFP-RFP-LC3 reporter of autophagic flux. The authors have adopted a tangential comparative approach with a mitophagy reporter mouse model, expressing GFP-RFP-FIS1. This largely reports on mitophagy, although there is a fraction of FIS1 on peroxisomes. Such a comparison potentially offers a first glimpse into the extent of synchronization between mitophagy and pexophagy mediated by common adaptors, as proposed for BNIP3L. However a comprehensive analysis across tissues has not yet been carried out. In the

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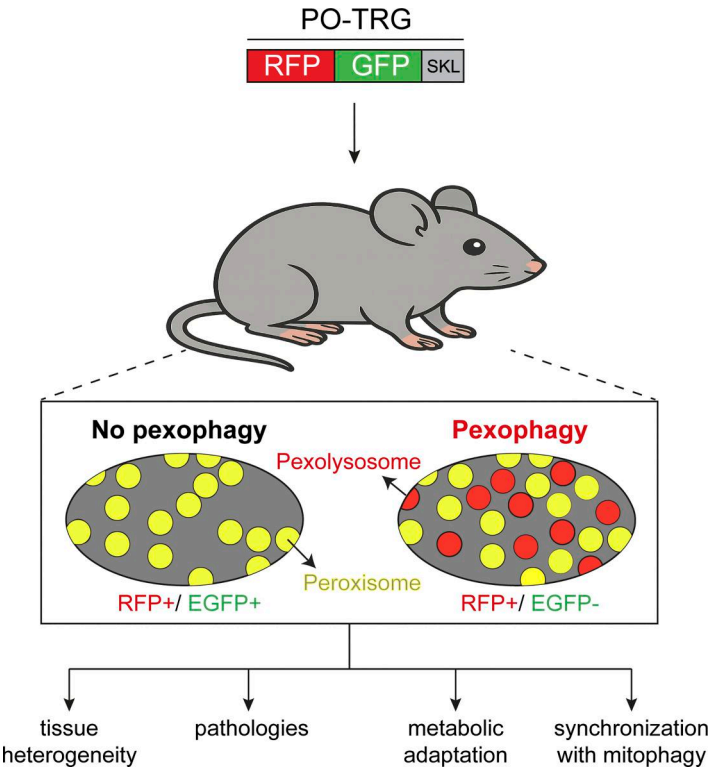

Figure 1. **A Tandem Red-Green reporter linked to a targeting signal for the peroxisomal lumen (PO-TRG) has been expressed in mice.** Material that has been delivered to lysosomes via selective autophagy shows as red punctae and provides an index of pexophagy. This opens the way for physiological studies of pexophagy in a mammalian system for the first time.

present study, it is shown that levels of pexophagy and mitophagy are both high within splenic cells. In skeletal muscle, they are both present but spatially segregated, while in Purkinje cerebellar neurons, both processes occur in the cell body, but only mitophagy occurs in the axons. This contrasts with the fly model, using neuronal specific drivers of reporter expression, in which the axonal projections of embryonic motor neurons were shown to be rich in pexophagy (10). However, it should be noted that fly axons are not ensheathed by myelin. In mammalian brains, axonal peroxisomes could contribute to supply of essential lipids for myelin function. In the fly model, oenocyte cells were observed to be highly enriched in peroxisomes, consistent with their role in producing long-chain fatty acids, but showed little evidence of pexophagy compared with neighboring cells (10). Whether this is due to the specific lack of pexophagy adaptors and if mitophagy is correspondingly suppressed in these cells remain open questions.

The authors note some concerns with the ubiquitous expression of the reporter causing peroxisome aggregation in the heart. Their solution is to generate a conditional Cre-Activated PerOxisome Tandem-Red-Green reporter mouse in which the RFP–GFP–SKL cassette is expressed only after Cre recombinase excises a STOP sequence. This enables adult-only expression and precise tissue expression. It also allows for sparser labelling, which aids visualization in single cells.

Changes in abundance of peroxisomes may reflect either the synthesis rate or the turnover rate. This new tool now provides a way of visualizing the latter, which will likely enable an appreciation of the physiological importance of pexophagy. The mito-QC mitophagy reporter mouse model first published in *Journal of Cell Biology* has bridged fundamental mechanistic cell biology studies with physiology (12). It has been highly impactful in providing insight into the likely mechanisms of mitophagy across tissues during development as well as pathological conditions. Alongside this, there is strong interest in developing therapeutics to stimulate mitophagy for a variety of conditions. Mouse mitophagy reporter models have therefore provided the "go to" model for testing mitophagy-regulating compounds "in vivo," often in collaboration with industry partners. This first mammalian reporter model for pexophagy unlocks similar opportunities for understanding and treating diseases linked to peroxisome homeostasis. In fact these two models may be examined in parallel to assess the coordination between these two selective autophagy pathways in response to metabolic demands.

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