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The life of the peroxisome: From birth to death

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Abstract

Peroxisomes are dynamic and metabolically plastic organelles. Their multiplicity of functions impacts on many aspects of plant development and survival. New functions for plant peroxisomes such as in the synthesis of biotin, ubiquinone and phylloquinone are being uncovered and their role in generating reactive oxygen species (ROS) and reactive nitrogen species (RNS) as signalling hubs in defence and development is becoming appreciated. Understanding of the biogenesis of peroxisomes, mechanisms of import and turnover of their protein complement, and the wholesale destruction of the organelle by specific autophagic processes is giving new insight into the ways that plants can adjust peroxisome function in response to changing needs.

Introduction

Plant peroxisomes possess astonishing metabolic versatility that encompasses degradation of storage oil, recycling of glycolate produced by photorespiration, pro-hormone metabolism, redox signalling and a range of biosynthetic capabilities. The biogenesis and the functions of plant peroxisomes are the subject of a recent comprehensive review to which the reader is referred [1]. In this article we highlight recent advances in the cell biology of plant peroxisomes in particular that update and extend the conclusions of the earlier article.

Update on peroxisome functions

Beta-oxidation of a wide range of substrates is one of the hallmarks of plant peroxisomes [1,2]. This central pathway integrates peroxisomes into the cellular signalling network and energy metabolism pathways. Diverse substrates for β-oxidation are imported via the PEROXISOME ABC TRANSPORTER 1/COMATOSE (PXA1/CTS) which can accept CoA esters and may cleave them upon transport [3] followed by reactivation by a peroxisomal acyl-CoA synthetase with the appropriate substrate specificity (Figure 1). The CTS/PXA1 interacting protein COMPARATIVE GENE IDENTIFICATION -58 (CGI-58) may regulate CTS/PXA1 activity in non-lipid storing tissues [4]. Peroxisomal β-oxidation participates in the synthesis of benzoic acid, which acts as a precursor to various secondary metabolites, (see [5]) and 4-hydroxybenzoic acid, an intermediate in one of two independent pathways for the ubiquinone synthesis [6] (Figure 1) . Peroxisomes also play a role in synthesis of polyamines, biotin and isoprenoid compounds [2]. Because β-oxidation is so central, mutants often have pleotropic effects. A small molecule inhibitor that blocks oil body breakdown but did not impact on pro-auxin metabolism [7] may be a useful tool separating out effects on fatty acid degradation from metabolism of other β-oxidation substrates.

Peroxisomes are one of the most significant generators of ROS in cells and their role as both signalling hubs and scavengers of ROS under normal and stress situations in plants and animals is increasingly appreciated [8,9]. Peroxisomes also produce RNS [10] and nitric oxide (NO) [11]**.** Superoxide and peroxynitrite were also shown to be produced endogenously by Arabidopsis peroxisomes and their production was increased under

conditions of Cd^{2+} stress [12]. NO can be produced in several cell compartments, and peroxisomal Xanthine oxidoreductase can reduce nitrite to NO [11]. Production of NO leads to S-nitrosylation of some peroxisome proteins including those involved in β-oxidation, photorespiration and ROS metabolism [13].

Birth of peroxisomes

Peroxisomes are classed as semi-autonomous organelles [1], as they are capable of forming de novo from the endoplasmic reticulum (ER) and they also replicate by growth and division (Figure 2). De novo peroxisome biogenesis has been studied in yeast and mammals but evidence from plants is more limited and less direct, although several plant peroxisomal membrane proteins (PMPs) have been reported to traffic via the ER [1,14].

Viruses are extremely powerful tools to study cell biology and are providing insight into possible de novo routes of peroxisome formation in plants. Tombus viruses such as Cucmber Necrosis Virus (CNV) and Tomato Bushy Stunt Virus (TBSV) are known to highjack peroxisomes for replication through the action of replication protein p33 [15]. Recently, the ER-localised vesicle transport protein Sec39p in S. cerevisiae and Sec39-like protein in tobacco were shown to be required for TBSV replication [16]. ScSec39p is required for peroxisome biogenesis from the ER [17]. ScSec39p also interacts with TBSV p33, which can replicate in yeast as a model host, and the tobacco Sec39-like protein is required for TBSV replication and leaf development [16]. In tobacco, CNV p33 localised to peroxisomes and the ER, and viral infection could initiate formation of the ER-derived aggregated membrane vesicles containing peroxisome markers [18] leading the authors to speculate that CNV may manipulate peroxisome biogenesis to increase the availability of appropriate intracellular membranes for viral replication.

If peroxisome membranes bud from the ER there could be a balancing retrograde flow to return essential components of the protein sorting and vesicle budding machinery. Relocation of peroxisome membrane proteins to the ER was observed in response to TBSV infection in tobacco cells, and was inhibited by co-expression of a dominant negative ADP-ribosylation factor 1 (ARF1) mutant [15]. In Chlamydomonas reinhardtii, treatment with Brefeldin A (BFA), an inhibitor of the GTP exchange factor for ARF1, also inhibits fatty acid breakdown, a process which relies on peroxisomal β-oxidation [19]. The authors speculated that BFA disrupted peroxisome biogenesis and hence fatty acid breakdown, although studies with inhibitors should be interpreted with caution because of potential off target effects. However, this is consistent with BFA inhibiting sorting of peroxisomal ascorbate peroxidase to peroxisomes [20]. ARF1 and coatomer were also recruited to highly purified rat liver peroxisome membranes [21]. The issue of de novo peroxisome formation from the ER in plants is worth reinvestigation with the higher resolution and time resolved techniques now available.

The matrix protein import cycle

Peroxisome matrix protein import has been reviewed recently [1,22], and involves a number of sequential processes: (1) receptor-cargo binding in the cytosol, (2) docking of the receptor-cargo at the peroxisome membrane docking complex, (3) translocation into the peroxisome and cargo release into the peroxisome matrix, (4) mono-/poly-ubiquitination of the receptor, (5) receptor release from the membrane, and (6) effective recycling/degradation of the receptor (Figure 3). Proteins destined for import into the peroxisome matrix have one of two peroxisome targeting signal (PTS) sequences, PTS1 or PTS2, within their primary sequence. These proteins once synthesised and folded are recognised by their respective receptors PEX5 and PEX7 in the cytosol [23]. Receptor-bound PTS-containing proteins are recruited to the docking complex (PEX13/PEX14) at the peroxisome membrane, and a transient pore is formed which allows translocation of receptor and cargo into the peroxisome.

Subsequently, the cargo is unloaded from the receptor and PEX14 may play a role in unloading PTS1- [24] or PTS2-cargoes [23]. Mono-ubiquitination of PEX5 on a conserved cysteine by the E2 ubiquitin-conjugating PEX4, anchored at the peroxisome membrane by PEX22, and E3 ubiquitin ligase complex comprising PEX2, PEX10 and PEX12, marks the receptor for recycling from the peroxisome membrane [25]. The Arabidopsis RING (Really Interesting New Gene) peroxins also possess E3 ligase activity [26] and are essential proteins for viability [1]. Recent studies in both yeast and mammalian cells suggest the conserved Cys in PEX5 is redox sensitive and that import is reduced under oxidative stress, although there is disagreement about the possible mechanism [27,28]. Interestingly the corresponding Cys residue is conserved in plant PEX5.

The mono-ubiquitinated PEX5 is recognised by the dislocation complex comprising the AAA-type ATPases PEX1 and PEX6, which are anchored to the peroxisome membrane by ABERRANT PEROXISOME MORPHOLOGY9 (APEM9/DAYU) in Arabidopsis. Mutations in apem9/dayu lead to dysfunctional PTS1- and PTS2-dependent import [29,30]. Mono-ubiquitinated PEX5 is then removed from the membrane, de-ubiquitinated and recycled. In humans, PEX7 recycling is reported to be only possible after the release of PEX5 from the peroxisome membrane, however the recycling of the receptors takes place separately [31].

If PEX5 is not recycled, it may be poly-ubiquitinated and degraded by the proteasome by a process termed “receptor accumulation and degradation in the absence of recycling” (RADAR). In Arabidopsis, ubiquitin domain-containing protein DSK2a and DSK2b interact with PEX2 and PEX12, and may help shuttle poly-ubiquitinated protein to the proteasome for degradation [26]. Similarly, Arabidopsis PEX7 also interacts with RabE1c GTPase which is proposed to enable PEX7 degradation by the proteasome machinery in the presence of a compromised import cycle [32].

Peroxisome movement

Peroxisomes are highly mobile within cells. In plant and yeast cells, peroxisomes move on actin filaments whereas animal peroxisomes move on microtubules. Plant

peroxisomes are moved by class XI myosins that recruit organelles through an adaptor protein [33]. Characterising relationships between a specific myosin and a particular organelle has proved difficult, due to redundancy in myosin XI and its effect on the organisation of actin filaments leading to pleiotropic effects [34]. More is known about peroxisome movements in budding yeast (S. cerevisiae) [35]. Peroxisomes are tethered to the cortical ER in mother cells by Inp1p [36]. Inp2p acts as an adaptor that mediates peroxisome recruitment by the myosin Myo2 for transport to the daughter cells, where regulated degradation of Inp2p causes release of peroxisome [35]. Myo2 also transports vacuoles through binding the vacuole specific adaptor Vac17, which is degraded following ubiquitination mediated by a novel E3-ubiquitin ligase Dma1 and its paralogue Dma2, to release the cargo. Dma1 and Dma2 are also required for peroxisome movements in yeast [37]. GTP hydrolysis by associated Rabs is also proposed as a mechanism for release of exocytic vesicles from myosin V in yeast [38], so it remains to be seen if either or both mechanisms function in plants to release peroxisomes from their molecular motors. Plant peroxisomes are observed to cluster around fungal penetration sites and are linked to the plant immune response [39,40]. Clearly, understanding mechanisms of peroxisomal movement and positioning will be important to fully elucidate host-pathogen responses.

Death of peroxisomes; protein and organelle turnover.

Cellular homeostasis requires mechanisms for the synthesis and turnover of individual macromolecules as well as organelles. In the case of peroxisomes, changes in biochemical and physiological function can take place by overlapping and complementary mechanisms; peroxisome associated protein degradation (PexAD) and by a specific autophagic process termed ‘pexophagy’ (Figure 4).

Peroxisomes contain a number of different proteases. A cysteine protease, Resistant to drought 21A-like 1 (RDL1) was identified in peroxisomes isolated from etiolated Arabidopsis seedlings and rdl1 mutants have mild peroxisome deficiency phenotypes and reduced seed viability, but RDL1 substrates are unknown [41]. Greening of etiolated seedlings is accomplished by turnover of the glyoxylate cycle enzymes isocitrate lyase (ICL) and malate synthase (MLS), and they are stabilised in mutants of pex4 and pex6, components required for PEX5 recycling (‘The matrix protein import cycle’ section). A genetic screen for mutants showing persistent GFP-ICL fluorescence (pfl mutants) identified mutants in pex14, and a novel allele of pex6 suggesting that matrix proteins must be imported in order to be degraded and are most likely ubiquitinated and re-exported in a process paralleling ER associated degradation (ERAD) [42]. Interestingly ICL has been identified as an ubiquitinated protein in a recent proteomic study [43].

Peroxisomal protein degradation or protein export for degradation by the proteasome facilitates peroxisome remodelling in response to environmental or developmental cues (Figure 4). Peroxisomes in mammalian and fungal cells can be degraded by a selective autophagic process termed pexophagy [44]. Autophagy is a process of bulk recycling of cellular components, often under conditions of nutrient limitation. Cytosol and organelles become sequestered in autophagosomes, double membrane compartments, which

originate from a phagophore assembly site (PAS) followed by fusion with and degradation in the vacuole. This process has been shown to occur in plants (Figure 2) [45,46]. Very recently four studies [47-50] have shown selective autophagy of peroxisomes also occurs in plants and intriguingly LON2, a peroxisomal protease, plays a role in both pexophagy and PexAD [47,51].

Larger but fewer peroxisomes are observed in lon2 mutants which exhibit an age-dependent defect in PTS2-protein import and peroxisome metabolism [52]. A screen for suppressors of the lon2 mutation identified multiple alleles of AUTOPHAGY RELATED (ATG) genes which encode components of the autophagic pathway [47]. This study identified atg2, atg3 and atg7 as suppressors of lon2 defects in protein import and of indole butyric acid (IBA) response, which is indicative of perturbed β-oxidation. Double mutants of lon2 atg showed stabilisation of ICL and MLS, but a single mutant did not show this phenotype. These results suggest that PexAD, mediated by LON2, and autophagy can function together to remove obsolete proteins. ICL and MLS stabilisation and increased peroxisome number was also seen in hypocotyls of atg7-2 and atg5-1 mutants [48]. Light-triggered ATG7-dependent pexophagy, and the blocking of vacuolar degradation allowed visualisation of bodies labelled with peroxisomal-targeted CFP within the vacuole in wild-type but not in the atg7-2 mutant [48]. ATG8a which marks the pre-autophagosomal structure, partially overlapped with a peroxisomal marker. Similar results were reported for peroxisomes in the leaves of atg2 mutants [50].

A screen for peroxisomal unusual positioning (peup) showed that peup1 (atg2), peup2 (atg18a) and peup4 (atg7) mutants displayed aggregated peroxisomes. Similarly, peroxisome aggregates were also induced by hydrogen peroxide treatment in wild-type plants and in the catalase (cat2) mutant suggesting that oxidatively damaged peroxisomes are cleared by pexophagy [49]. Similar results to those of [47] were reported for the apem10 mutant (an allele of lon2) which was supressed by peup1 [51]. Interestingly a mutant in the AAA domain of LON2 gave a phenotype like the apem10 peup1 double mutant leading the authors to propose that the LON2 AAA domain has a role in suppressing autophagy. [51]. Collectively these studies show that plant peroxisomes, like their yeast and mammalian counterparts, can be turned over by pexophagy but that an alternative independent pathway exists for removal of peroxisomal matrix proteins and LON2 functions in both pathways (Figure 4).

Recruitment to pexophagy requires a specific receptor (as yet unknown in plants) which interacts with ATG8 via an ATG8 interacting motif (AIM) containing the consensus amino acid sequence (W/Y/F)XX(L/V) [45]. In mammals, PEX14 is bound by the ATG8 homologue LC3-II and this binding is competitive with that of PEX5 [53]. PEX5 binds to PEX14 via closely related WX_3F/Y repeats and the LVXEF amino acid sequence in the N-terminus of PEX5 [54]. In plants Arabidopsis PEX5 and PEX14 bind one another in a similar manner [23] and it may therefore be significant that alleles of AtPEX14 were uncovered in the screen for pfl mutants [42]. Variation between species is also observed. In Pichia pastoris, the pexophagy receptor Atg30 interacts with PEX3 and PEX14 on the peroxisomal membrane and the autophagy machinery on the PAS [44]. The P. pastoris integral PMP, Atg37, an acyl-CoA binding protein, also binds Pex3p and is recruited by

Atg30 to the pexophagic receptor complex and binding to Atg30 and Acyl-CoA is competitive. The human Atg37 homologue, Abcd5, is also required for pexophagy [55]. Meanwhile in S. cerevisiae, Atg36 acts as an adapter between PEX3, ATG8 and ATG11 on the pre-autophagosomal structure, and ScPex14p is dispensable for pexophagy [56]. In mammalian cells ubiquitinated PMPs recognised by p62 or its homologue NBR1 (neighbour of BRCA1) trigger pexophagy [44,57]. However, ubiquitinated PMPs were not detected in Arabidopsis atg2 [50] mutants leading to the suggestion that oxidatively damaged membrane components could be a signal for pexophagy in plants [49,50].

Conclusions and future perspectives

Peroxisome protein import and export are linked via the process of ubiquitination and proteolysis. This allows restocking of existing organelles with new activities as proposed by the original two population hypothesis [58]. At the same time the capacity to synthesise and degrade whole peroxisomes fits to the one population hypothesis [58]. This flexibility may allow peroxisomes and their functions to adjust rapidly in response to both internal and external perturbations, which is likely to be of importance in surviving in a fluctuating environment. Recent research emphasises the central role of peroxisomes as generators of redox signalling molecules and also as the targets of oxidative damage and turnover. Future research will identify the pathways and missing components in this process. New functions are still being discovered which integrate peroxisomes into virtually all aspects of plant biology, providing potential new targets for crop improvement.

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- Using network modelling and gene knockouts two pathways for ubiquinone synthesis in arabidopsis were delineated. The peroxisomal ABC transporter PXA1/CTS and a peroxisomal p-coumarate CoA ligase are required for the phenyalanine derived branch of the ubiquinone biosynthesis pathway, identifying yet another metabolic role for peroxisomes.

*BURKHART, S. E., LINGARD, M. J. & BARTEL, B. 2013. Genetic dissection of peroxisome-associated matrix protein degradation in Arabidopsis thaliana. Genetics, 193**,** 125-41.

- This study identified mutants defective in degradation of isocitrate lyase in photosynthetic tissues. The mutants included pex14, ped1, and pex6, suggesting that proteins that need to be turned over for peroxisome transition from heterotrophy to photoautotrophy, must first enter peroxisomes before they are exported for degradation in a similar manner to the PEX5 receptor by the dislocase complex that includes PEX6.

*BUSSELL, J. D., REICHELT, M., WISZNIEWSKI, A. A., GERSHENZON, J. & SMITH, S. M. 2014. Peroxisomal ATP-binding cassette transporter COMATOSE and the multifunctional protein ABNORMAL INFLORESCENCE MERISTEM are required for the production of benzoylated metabolites in arabidopsis seeds. Plant Physiol, 164**,** 48-54.

- Through measurement of metabolite levels in a range of mutant plants the role peroxisomes in the production of benozoic acid a key intermediate in the production of secondary metabolites involved in plant defence was elucidated.

DE MARCOS LOUSA, C., VAN ROERMUND, C. W., POSTIS, V. L., DIETRICH, D., KERR, I. D., WANDERS, R. J., BALDWIN, S. A., BAKER, A. & THEODOULOU, F. L. 2013. Intrinsic acyl-CoA thioesterase activity of a peroxisomal ATP binding cassette transporter is required for transport and metabolism of fatty acids. Proc Natl Acad Sci U S A, 110,** 1279-84.

- Provides evidence that the peroxisomal ABC transporter responsible for import of diverse substrates posseses an acyl-CoA esterase activity, thereby resolving the paradox that the transporter has a preference for CoA ester substrates but peroxisomal acyl CoA synthetases are essential for further metabolism.

FARMER, L. M., RINALDI, M. A., YOUNG, P. G., DANAN, C. H., BURKHART, S. E. & BARTEL, B. 2013. Disrupting autophagy restores peroxisome function to an Arabidopsis lon2 mutant and reveals a role for the LON2 protease in peroxisomal matrix protein degradation. Plant Cell, 25,** 4085-100

- A genetic screen for suppressors of a lon2 mutant which encodes a defective peroxisomal matrix protease identified multiple genes involved in autophagy. Analysis of double mutant phenotypes showed LON2 to be involved in peroxisomal matrix protein degradation. In the absence of LON2, pexophagy takes place and peroxisome number and function is reduced. Accumulation of glyoxylate cycle enzymes in atg mutants suggests that pexophagy and LON2 work in parallel to maintain peroxisome function and protein/peroxisome degradation.

*KIM, J., LEE, H., LEE, H. N., KIM, S. H., SHIN, K. D. & CHUNG, T. 2013b. Autophagy-related proteins are required for degradation of peroxisomes in Arabidopsis hypocotyls during seedling growth. Plant Cell, 25**,** 4956-66.

- This study showed autophagy related proteins ATG7 and ATG5 are required for peroxisome degradation in the vacuole of Arabidopsis hypocotyl cells. This was further reinforced by co-localisation of ATG8 with peroxisome markers in wild type but not in autophagy defective mutants. Autophagy defective atg7 and atg5 mutants also accumulated peroxisomal enzymes involved in the glyoxylate cycle but they were degraded in older seedlings.

SHIBATA, M., OIKAWA, K., YOSHIMOTO, K., KONDO, M., MANO, S., YAMADA, K., HAYASHI, M., SAKAMOTO, W., OHSUMI, Y. & NISHIMURA, M. 2013. Highly oxidized peroxisomes are selectively degraded via autophagy in Arabidopsis. Plant Cell, 25,** 4967-83.

- A screen for mutants with unusually positioned peroxisomes lead to identification of alleles of autophagy related genes ATG2, ATG18 and ATG7. In these mutants peroxisomes contained aggregated inactive catalase and were often clustered together. The peroxisome clustering effect and cellular oxidation could be mimicked in the cat2 mutant or by addition of exogenous H_2O_2, suggesting oxidative damage may signal autophagic destruction of peroxisomes.

*ROCHON, D., SINGH, B., READE, R., THEILMANN, J., GHOSHAL, K., ALAM, S. B. & MAGHODIA, A. 2014. The p33 auxiliary replicase protein of cucumber necrosis virus targets peroxisomes and infection induces de novo peroxisome formation from the endoplasmic reticulum. Virology, 452-453**,** 133-42.

- CNV replication protein p33 was shown to be targeted to the peroxisomes in tobacco. This study also reports that upon p33 infection, dramatic re-modelling of the ER is observed, which results in formation of compartments similar to peroxisomes that co-localises with proteins containing PTS1 signal. CNV p33 was reported to be targeted to the induced peroxisome-like structures.

Figure Legends

Figure 1. Peroxisomal β-oxidation is a central process in plant biology.

The substrates shown at the top of the figure are imported into peroxisomes via the ABCD transporter CTS/PXA1. They are activated by different acyl activating enzymes

(AAEs), which adds Co-enzyme A, and then enters into the β-oxidation pathway. The products leave the peroxisome and may be further metabolised.

Figure 2: Protein trafficking, peroxisome biogenesis and pexophagy.

Proteins are transported from the ER en route to Golgi, trans-Golgi network (TGN) and the pre-vacuolar compartment (PVC) before reaching the vacuole. Vesicles termed as pre-peroxisome vesicles also bud off from the ER that may contain peroxisomal membrane proteins. The fusion of these pre-peroxisomal vesicles causes the formation of de novo synthesised peroxisomes. Extra membrane materials and phospholipids are also obtained from the ER for the growth of peroxisome. Peroxisomal matrix proteins with peroxisome targeting signal (PTS) 1 and PTS2 are imported into the peroxisome to generate a fully functional mature "peroxisome". These peroxisomes undergo division, which involves elongation, constriction and finally fission of peroxisomes. Obsolete, damaged or dysfunctional peroxisomes can be degraded by autophagic process termed pexophagy. Autophagy-related protein 8 is found in the PAS and is generally used as an autophagosome marker. In macro-pexophagy, a double membrane structure engulfs the dysfunctional peroxisome to generate a compartment known as autophagosome, which fuses with the vacuole releasing engulfed peroxisomes to be degraded by the vacuolar proteases. Alternatively, peroxisomes can also be surrounded by vacuolar membrane and subsequently degraded in the vacuole lumen.

Figure 3: Matrix protein import into plant peroxisomes.

(1) Peroxisomal matrix proteins with a PTS1 and PTS2 targeting signals bind to their respective receptors PEX5 and PEX7. (2) Cargo bound receptor PEX5 is recruited to the PEX13/PEX14 docking site. (3) Transient pore is formed and the receptor-cargo is translocated to the peroxisome matrix, where cargo is unloaded from the receptor protein. (4) The receptor is then ubiquitinated by E2 ubiquitin-conjugating PEX4, anchored to peroxisome membrane by PEX22, and E3 ubiquitin-ligase RING peroxins PEX2, PEX10 and PEX12. (5) ATP-dependent removal of PEX5 from the peroxisome membrane is mediated by AAA-type ATPases PEX1 and PEX6, anchored to peroxisome by APEM9/DAYU, which also interacts with PEX16. (6a) The receptor can then be recycled for another round on import if it is mono-ubiquitinated but in the event of poly-ubiquitination (6b), it is directed to proteasome for degradation. Ubiquitin receptor protein DSK2a and DSK2b, which interact with PEX2 and PEX12 and partially with PEX10, may also promote protein degradation by the proteasome complex.

Figure 4: Model for Peroxisome remodelling and turnover

A. On the left hand side PEX5 (brown square) imports cargo proteins (grey hexagon) via the docking and translocation machinery (DTM) and is recycled by the receptor export module (REM). LON2 and other peroxisomal proteases may turnover proteins within peroxisomes or they may be exported for cytosolic degradation analogous to ER associated degradation (ERAD) using components of the REM such as PEX4 and PEX6. Signals arising from environmental, developmental or metabolic cues may promote turnover of obsolete proteins, while the import of new proteins with different functions

(orange hexagon) allows remodelling of peroxisome capabilities such as in the transition from heterotrophy to photoautotrophy.
B. Proteins are imported as in A, and may undergo turnover by PexAD (not shown). However when peroxisomes age or become otherwise damaged, (represented here by catalase aggregation, although catalase aggregation is not necessary or sufficient since pexophagy is induced in the cat2 mutant) they can be destroyed by pexophagy. Autophagy genes shown to be involved in pexophagy in plants are shown. ATG7 (PEUP4) acts as an E1 ligase for activation of ATG8, an ubiquitin-like protein. ATG3 is the corresponding E2 and ATG5 is needed to conjugate phosphatidylethanolamine (PE) to ATG8. ATG2 (PEUP1) and ATG18a (PEUP2) are thought to be involved in the recruitment of membrane to the PAS. LON2 is required for suppression of pexophagy via its AAA domain although the mechanism is not known.

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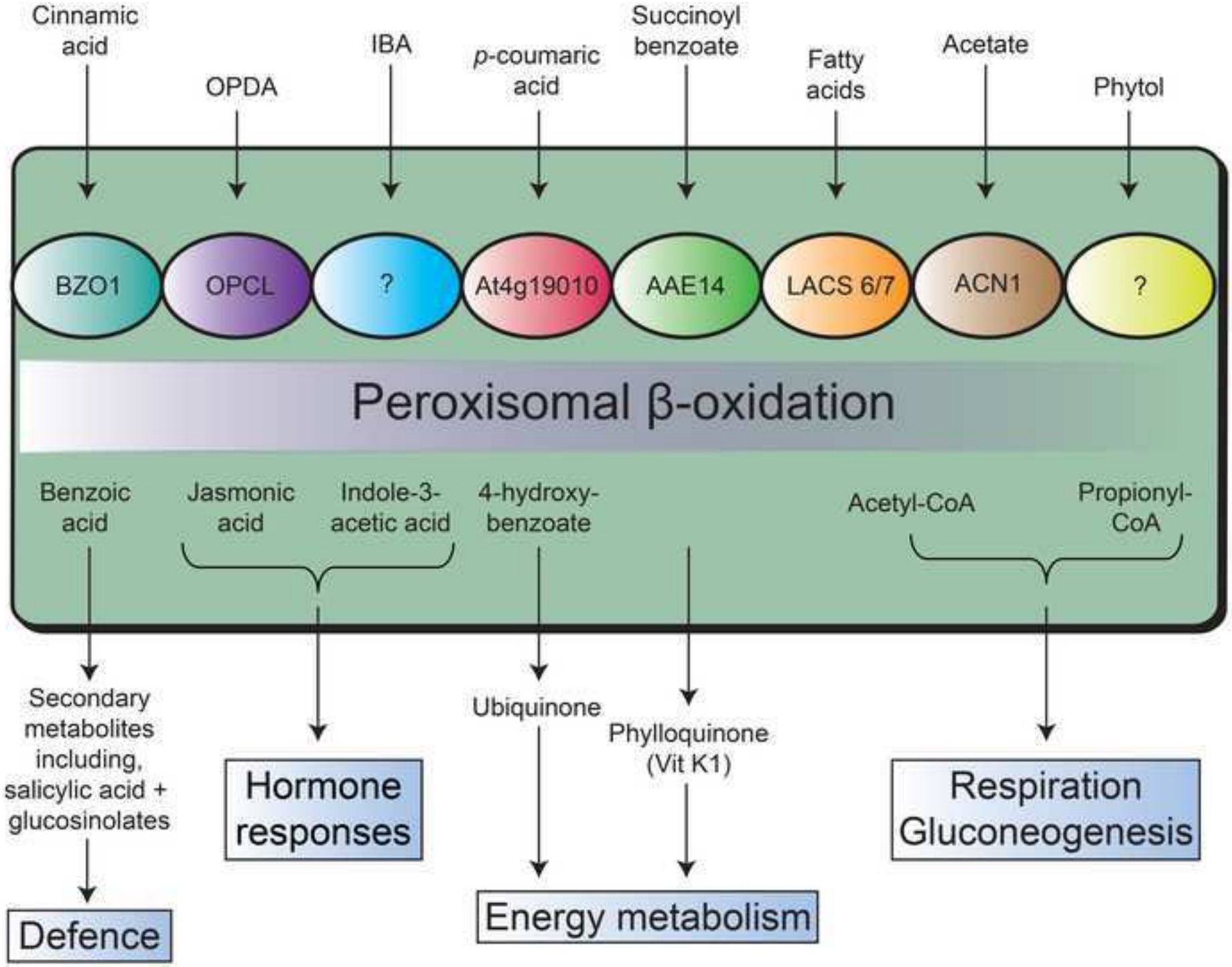

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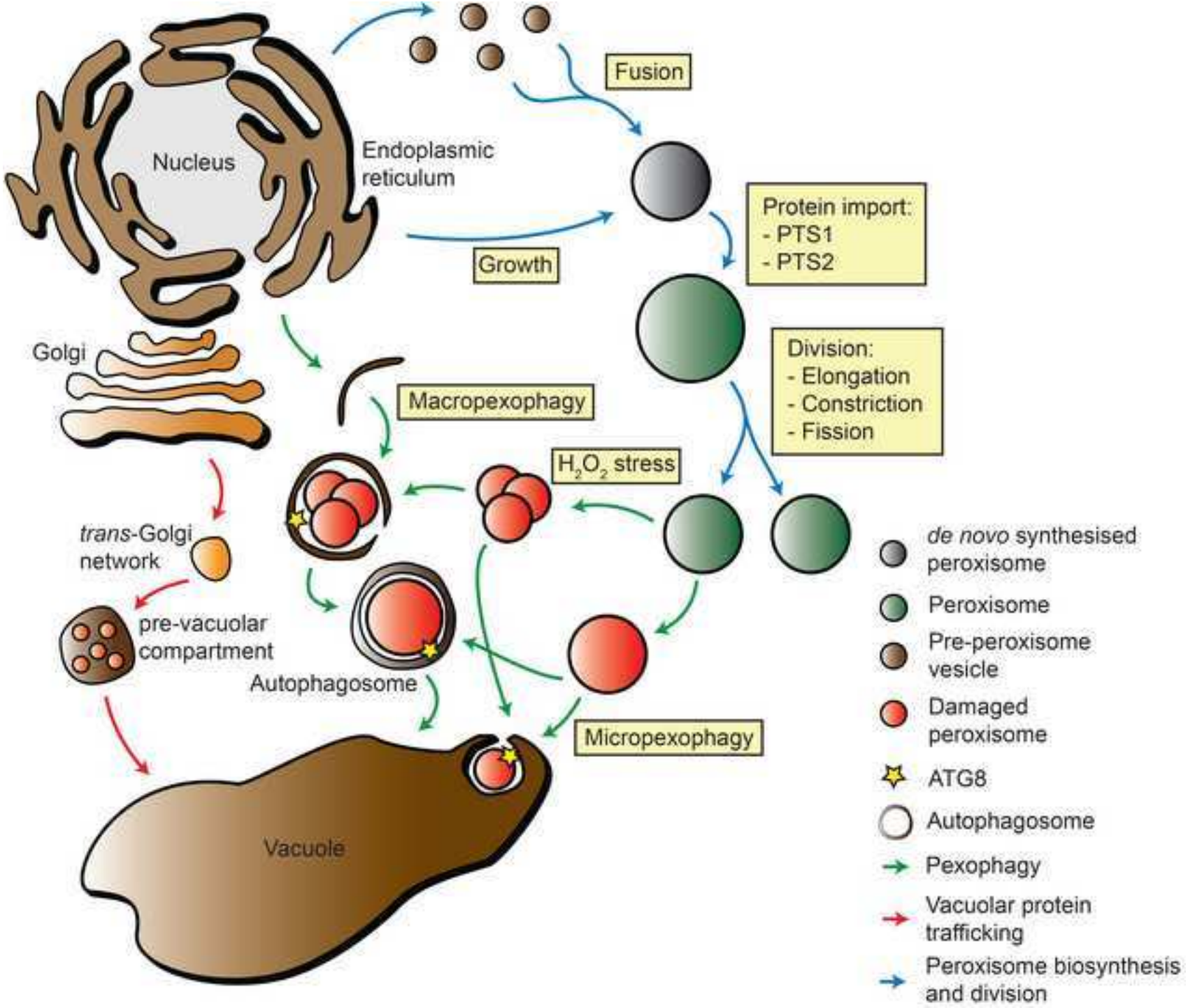

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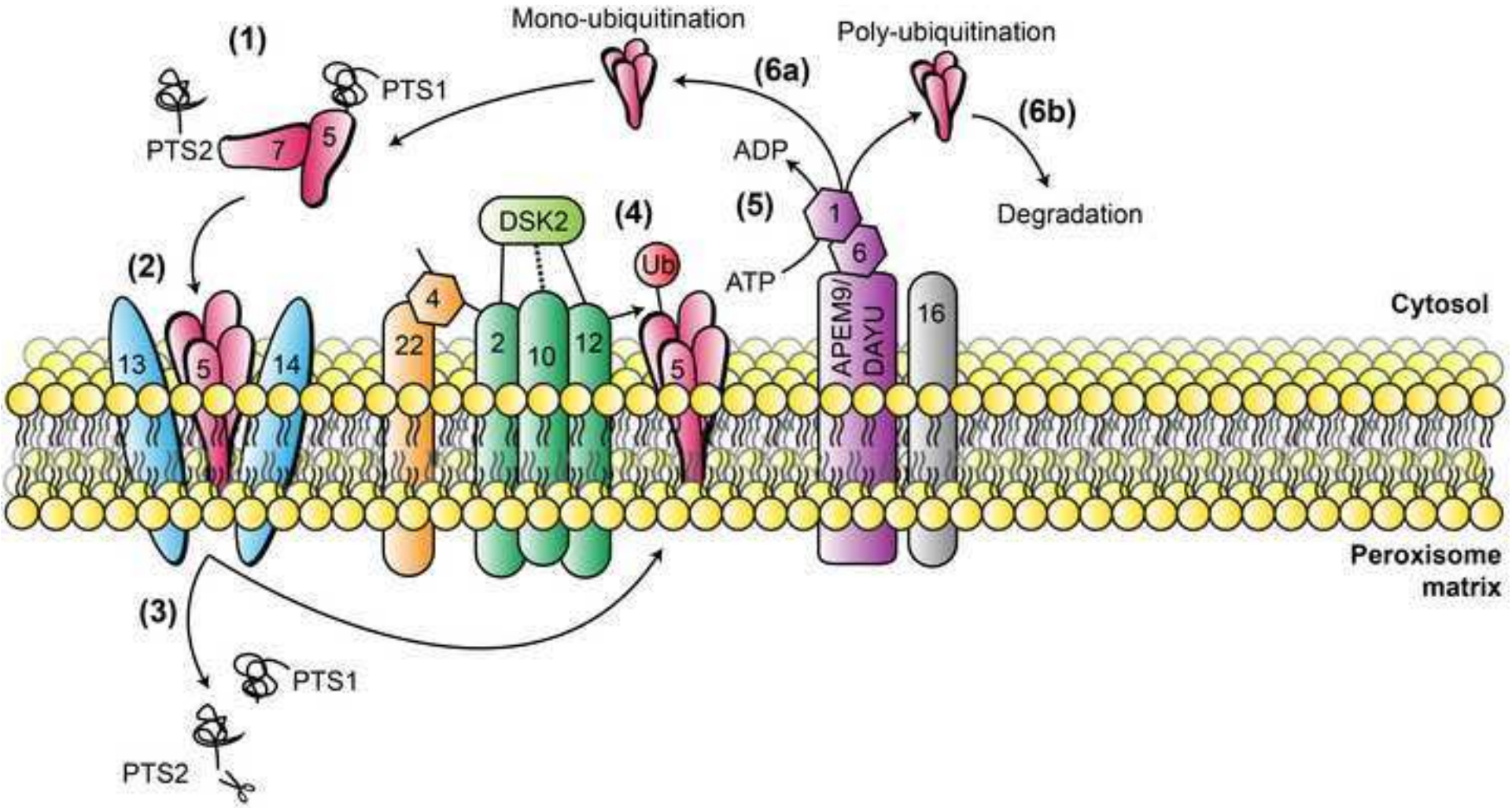

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