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Article Addendum

Toward Understanding Plant Peroxisome Proliferation

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Addendum to:

The PEROXIN11 Protein Family Controls Peroxisome Proliferation in Arabidopsis

Orth T, Reumann S, Zhang X, Fan J, Wenzel D, Quan S, Hu J

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ABSTRACT

Plant peroxisomes are highly dynamic organelles that adapt to environmental variation by altering their number, but the molecular basis for plant peroxisome proliferation is largely unknown. To begin understanding how this fundamental cell biological process is controlled in plants, we recently characterized the Arabidopsis homologues of the yeast Pex11p protein, which is involved in peroxisome proliferation via an unknown mechanism. Using a combination of fluorescence microscopy, immunobiochemistry, overexpression and loss-of-function studies, and heterologous gene expression in yeast cells, we showed that all five Arabidopsis PEX11 proteins target to peroxisomal membranes and promote peroxisome proliferation with partial redundancy and specificity. A subset of the dynamin-related proteins (DRPs) is also involved with peroxisome division in plants, yeast, and mammals. Future experiments should focus on addressing the biochemical function of PEX11 and using new tools to uncover additional components of the peroxisome proliferation pathways, especially those that are unique to plants.

Plants contain a large number of structurally similar but functionally diverse peroxisomes involved in embryogenesis, lipid mobilization, photorespiration, nitrogen metabolism, hormone biosynthesis, hydrogen peroxide degradation, photomorphogenesis, and plant-pathogen interaction.[1-6] The morphology and abundance of plant peroxisomes were reported to vary at different developmental and metabolic stages and upon plant exposure to various environmental stresses.[7-13]

In yeast and possibly other eukaryotes, peroxisome abundance is controlled by at least two incompletely characterized pathways: budding off from the endoplasmic reticulum (ER) and division from pre-existing peroxisomes through peroxisome elongation and fission.[14] It is unclear which pathway plays a major role in peroxisome proliferation, defined here as an increase in the total volume or number of peroxisomes. Pex11p, Pex25p/Pex27p, Pex28p/Pex29p, and Pex30p/Pex31p/Pex32p are four classes of yeast peroxisomal proteins that are specifically involved with peroxisome proliferation via unknown mechanisms.[15] Pex11p, Pex25p, Pex27p, Pex31p and Pex32p are positive regulators of peroxisome abundance, whereas Pex28p, Pex29p and Pex30p repress peroxisome proliferation.[14,15] Among these eight PEX proteins, only Pex11p has apparent sequence homologues in plant genomes.[16] Yeast and mammals also contain transcriptional complexes that, upon activation by a variety of fatty acids and their derivatives, induce the expression of numerous genes involved in peroxisome biogenesis and function.[17,18] Sequence homologues to these nuclear proteins have not been identified in plant genomes, either.

THE FIVE-MEMBER ARABIDOPSIS PEX11 PROTEIN FAMILY PROMOTES PEROXISOME PROLIFERATION

To begin building a model for plant peroxisome proliferation, our laboratory characterized the Arabidopsis PEX11 proteins using a combination of genetic, cell biological, and biochemical approaches with whole plants.[19] Our research complemented a previous study of this gene family, in which transient assays were used with Arabidopsis and BY-2 cell cultures.[20]

The Arabidopsis PEX11 family is composed of three subfamilies: PEX11a, PEX11b, and PEX11c to PEX11e. Interestingly, rice also contains five PEX11 homologues. The diversification of plant *PEX11* genes appears to have occurred before the evolutionary split of monocots (rice) from dicots (Arabidopsis), indicating functional distinctions between subfamilies in plants. Using fluorescence microscopy and immunobiochemical analysis, we determined

the peroxisomal membrane localization of all five Arabidopsis PEX11 homologues. Consistent with their being positive regulators of peroxisome proliferation, *PEX11* genes overexpressed in Arabidopsis caused elongation and increased abundance of peroxisomes, and reducing gene expression via RNA interference (RNAi) caused a reduction in peroxisome number and partial defects in peroxisome separation in the transgenic plants. By showing that PEX11c and PEX11e partially complemented the yeast *pex11* mutant phenotype, we also provided direct evidence for conserved function between some Arabidopsis PEX11 proteins and yeast Pex11p.[19]

Studies of the Arabidopsis PEX11 homologues added plants to the list of organisms, including yeast, *Trypanosoma*, and mammals, that utilize PEX11 as a major positive regulator of peroxisome proliferation. Yet, PEX11 may function differently in yeast and plants in the regulation of peroxisome size. For example, in contrast to the yeast *pex11* cells, which contained fewer, but giant, peroxisomes,[21] reducing the expression of *PEX11a* and *-c to -e* decreased the size of the organelles in Arabidopsis.[19] Furthermore, PEX11 has amplified in plants into a family of five proteins, all of which are functional in peroxisome proliferation, suggesting that individual proteins may function in specific peroxisome subtypes and possibly conduct distinct biochemical functions. Consistent with these notions are the following observations: (1) *Arabidopsis PEX11* genes display tissue specificity and induction by specific environmental cues;[19] (2) overexpression of individual family members confers partially distinct morphological changes to the peroxisome;[19,20] (3) PEX11a has a different membrane topology from that of other PEX11 proteins;[20] and (4) complementation of the yeast *pex11* mutant was accomplished only by two of the Arabidopsis PEX11 proteins.[19] Similarly, mammals have three PEX11 isoforms that have different impacts on peroxisome proliferation and development.[22,23]

To better understand to what extent this protein family is needed for peroxisome biogenesis and plant development, current work in our laboratory focuses on determining the specific function for each PEX11 protein in Arabidopsis and creating mutants in which all five *PEX11* genes are silenced. Even though PEX11 is one of the most abundant peroxisomal membrane proteins in diverse species and has conserved function in peroxisome proliferation, its biochemical function is still elusive and also needs to be addressed. Current evidence favors the model that PEX11 proteins recruit downstream effector proteins to carry out the proliferation process; thus, future experiments should focus on identifying cytosolic or peroxisomal matrix proteins that are recruited by PEX11 to mediate the elongation and fission of peroxisomes.

PERSPECTIVES

The proliferation of peroxisomes is a highly regulated but poorly understood process. We have uncovered PEX11 proteins as crucial players in peroxisome proliferation from pre-existing peroxisomes. In yeast and mammals, members of the dynamin-related superfamily of large GTPases are also involved in the late stages of peroxisomal division.[15] Arabidopsis contains 16 dynamin-related proteins (DRPs), which are grouped into six subfamilies and exert diverse functions in the cell.[24] The DRP3 subfamily consists of two members: DRP3A and DRP3B, both of which were shown to control mitochondrial division.[25-27] The *drp3A* mutant was recently isolated from a genetic screen for mutants with aberrant peroxisome morphology (*apm*) and exhibits slightly larger peroxisomes with long string-like tails.[28] Having over 75% amino acid identity with DRP3A, DRP3B may also be involved in peroxisome division.

The function of PEX11 and DRPs in peroxisome proliferation appears to be mostly conserved across plant, fungal, and mammalian species. However, many proteins known to function in peroxisome proliferation in non-plant systems do not have obvious orthologous sequences in plant genomes. Additional forward genetic screens and proteomic analysis of the peroxisome membrane should be employed to identify plant-specific constituents of the peroxisome proliferation machinery.

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