

Article Addendum

Light control of peroxisome proliferation during Arabidopsis photomorphogenesis

Jianping Hu* and Mintu Desai

Department of Energy Plant Research Laboratory; Michigan State University; East Lansing, Michigan USA

Key words: arabidopsis, light, peroxisome proliferation, PEX11b, phytochrome A, HYH

Peroxisomes are multifunctional organelles whose abundance and metabolic activities differ depending on the species, cell type, developmental stage and prevailing environmental conditions.¹ However, little is known about the signaling pathways that control these variations, especially in plants. Our laboratory recently investigated the regulatory role of light in changes in peroxisome abundance and identified a phytochrome A-dependent pathway responsible for the proliferation of peroxisomes during dark-to-light transition in Arabidopsis seedlings. Light induces peroxisome proliferation at least in part through upregulating the *PEX11b* gene, which encodes a peroxisomal membrane protein that mediates the early stages of peroxisome multiplication. Activation of *PEX11b* requires the far-red light receptor phyA, as well as the bZIP transcription factor HYH, which binds directly to the promoter of *PEX11b*. We conclude that during photomorphogenesis, both the import of leaf-peroxisome enzymes from the cytosol and the induction of peroxisome proliferation take place to prepare seedlings for photosynthesis and photorespiration. In addition to light, other plant peroxisome proliferators may also exert their functions by targeting members of the *PEX11* gene family for transcriptional activation.

Light is one of the major environmental cues that have profound impacts on plant development. It is perceived by several classes of photoreceptors, which initiate a complicated network of downstream signaling events leading to physiological responses including germination, photomorphogenesis, shade avoidance, photoperiodism, phototropism, chloroplast and stomate movement, circadian rhythm and time to flower.²⁻⁴ During photomorphogenic responses, Arabidopsis seedlings exhibit hypocotyl growth inhibition, opening of cotyledons, development of chloroplasts and expression of genes

involved in photosynthesis and related functions.⁴ Photoreceptors responsible for seedling photomorphogenesis are phytochromes, which perceive red and far-red light, and cryptochromes, which perceive blue and UV-A light.²⁻⁴

What is the role of peroxisomes in photomorphogenesis? Plant peroxisomes are composed of several metabolically specialized subtypes, including glyoxysomes in seeds and germinating seedlings, leaf peroxisomes, gerontosomes in senescent tissue, nodule-specific peroxisomes and unspecialized peroxisomes. Peroxisomes mediate photorespiration, fatty acid β -oxidation, the glyoxylate cycle, nitrogen metabolism, synthesis of plant hormones, metabolism of hydrogen peroxide, and other important physiological processes.⁵⁻⁷ Upon exposure to light, as seedlings undergo the transition from heterotrophic to autotrophic growth, organelles involved in photosynthesis and photorespiration need to develop and genes required in these processes are activated. It is believed that glyoxysomes in dark-grown seedlings are converted into leaf peroxisomes by replacing enzymes specific for lipid metabolism during germination with proteins involved in glycolate recycling during photorespiration.⁷ To determine whether changes in peroxisome abundance also occur during this process, we analyzed dark-grown Arabidopsis seedlings constitutively expressing the peroxisomal marker protein YFP-PTS1 (Peroxisome Targeting Signal type 1). Upon light treatment, these plants exhibited a strong increase in the total number of peroxisomes in cotyledon cells, which followed a multi-step process consisting of peroxisome elongation, constriction and fission.⁸

We later identified several components of the light signaling pathway that leads to peroxisome proliferation.⁸ Previous studies showed that five Arabidopsis PEX11 isoforms, PEX11a to -e, are integral membrane proteins of the peroxisome and promote peroxisome elongation and number increase.^{9,10} Our study demonstrated that a member of this gene family, *PEX11b*, was strongly upregulated by light. The RNAi mutant of *PEX11b* displayed subtle changes in peroxisome morphology and much-reduced increases in peroxisome abundance during dark-to-light transition.⁸ This finding placed PEX11b as a key peroxisomal mediator in light-induced peroxisome proliferation. To determine which wavelengths of the light spectrum specifically affect the expression of *PEX11b*, we also performed RT-PCR and searched online gene expression databases to obtain quantitative measures of *PEX11b* expression under various light conditions. Far-red light was found to confer the strongest upregulation of *PEX11b*. Consistent with this result, the light induction of

*Correspondence to: Jianping Hu; Michigan State University—Department of Energy Plant Research Laboratory; East Lansing, Michigan 48824 USA; Tel.: 517.432.4620; Fax: 517.353.9168; Email: huji@msu.edu

Submitted: 03/07/08; Accepted: 03/10/08

Previously published online as a *Plant Signaling & Behavior* E-publication: <http://www.landesbioscience.com/journals/psb/article/5876>

Addendum to: Desai M, Hu J. Light induces peroxisome proliferation in Arabidopsis seedlings through the photoreceptor phytochrome A, the transcription factor HY5 HOMOLOG, and the peroxisomal protein PEROXIN11b. *Plant Physiol* 2008; 146:1117-27; PMID: 18203870; DOI: 10.1104/pp.107.113555.

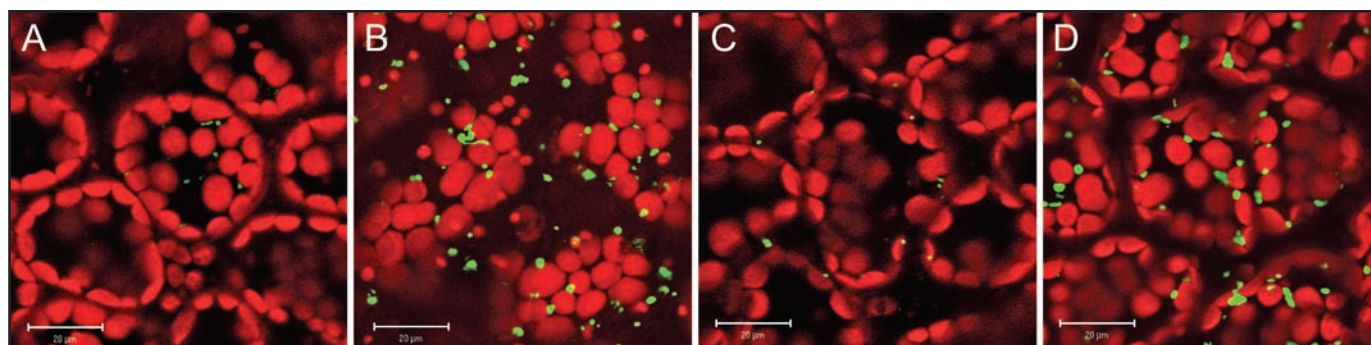


Figure 1. Overexpressing *PEX11b* in *phyA* and *hyh* mutants rescued the low peroxisome abundance phenotype. Shown are confocal microscopic images of cotyledon cells from 5-d green seedlings. (A) *phyA*; (B) *phyA* overexpressing *PEX11b*; (C) *hyh*; (D) *hyh* overexpressing *PEX11b*. Scale bars, 20 μ m. Peroxisomes are labeled with YFP-PTS1. The bigger and round organelles in the background are chloroplasts emitting autofluorescence.

PEX11b expression was strongly decreased in mutants impaired in the far-red light receptor *phyA*. While searching for downstream components of the light signaling pathway that might have an impact on *PEX11b* expression, we detected a partial but significant reduction in the transcript level of *PEX11b* in the *hyh* mutant defective in the bZIP transcription factor HYH.⁸

Further support of the role for *phyA* and HYH in inducing peroxisome proliferation via activating the expression of *PEX11b* came from our genetic and biochemical analyses. Mutants of *PHYA* or *HYH* crossed into the YFP-PTS1 peroxisomal marker line showed significantly reduced peroxisome abundance, a phenotype that was rescued by transiently overexpressing *PEX11b* in these mutants.⁸ We subsequently corroborated this transient expression data with confocal microscopic analysis of *phyA* and *hyh* mutants stably overexpressing the *PEX11b* gene (Fig. 1). Interestingly, at the young seedling stage (5d) used in this study, we did not observe many elongated peroxisomes as we did in 6-week adult leaves overexpressing *PEX11b*,¹⁰ indicating that the function of PEX11b and possibly other PEX11 proteins in peroxisome proliferation may be developmentally regulated. Furthermore, gel-shift assays demonstrated that HYH but not its homolog HY5 bound to the promoter of *PEX11b*, suggesting that *PEX11b* is one of the distinct target genes of HYH in transcriptional regulation in the light.⁸

Overall, our study showed that in the complex web of *phyA*-mediated light signaling cascade, one individual pathway, which is composed of at least the HYH and PEX11b proteins, specifically triggers peroxisome proliferation during seedling photomorphogenesis.⁸ It seems that in order to keep up with the high rate of photosynthesis and photorespiration, both the import of leaf peroxisomal enzymes into the organelles and a strong increase in peroxisome abundance are required.

Studies have shown that environmental and metabolic stress conditions such as ozone, herbicide, clofibrate and high light increased peroxisome abundance in plant cells.¹¹⁻¹⁶ However, molecular players in the signaling events underlying these phenomena are still elusive. The identification of components in the light signaling pathway that induces peroxisome proliferation in *Arabidopsis* offers a starting point for dissecting signaling pathways under other peroxisome-proliferating conditions. PEX11 orthologs across kingdoms play highly conserved roles in the rate-limiting first steps of peroxisome multiplication.¹⁷ In addition to the light-activated *Arabidopsis* *PEX11b* gene, *PEX11* from yeast *S. cerevisiae* and *PEX11 α* from

mammals are also transcriptional activation targets for peroxisome proliferation agents such as oleic acid and phenylbutyrate.¹⁸⁻²⁰ The dynamin-related protein DRP3A is another critical player in the *Arabidopsis* peroxisome proliferation apparatus, which powers the scission of the membranes once constriction occurs.^{21,22} However, DRP3A does not seem to be a rate-limiting factor and overexpressing the gene did not cause any obvious morphological or population changes of peroxisomes.²² Thus, we predict that besides light, other plant peroxisome proliferators may also use members of the *PEX11* gene family as the main entry point to implement their regulatory roles in peroxisome proliferation.

References

- Purdue PE, Lazarow PB. Peroxisome biogenesis. *Annu Rev Cell Dev Biol* 2001; 17:701-52.
- Chen M, Chory J, Fankhauser C. Light signal transduction in higher plants. *Ann Rev Genet* 2004; 38:87-117.
- Jiao Y, Lau OS, Deng XW. Light-regulated transcriptional networks in higher plants. *Nat Rev Genet* 2007; 8:217-30.
- Wang H, Deng XW. Dissecting the phytochrome A-dependent signaling network in higher plants. *Trends Plant Sci* 2003; 8:172-8.
- Beevers H. Microbodies in higher plants. *Ann Rev Plant Physiol* 1979; 30:159-93.
- Nyathi Y, Baker A. Plant peroxisomes as a source of signalling molecules. *Biochim Biophys Acta* 2006; 1763:1478-95.
- Olsen LJ, Harada J. Peroxisomes and their assembly in higher plants. *Annu Rev Plant Biol* 1995; 46:123-46.
- Desai M, Hu J. Light induces peroxisome proliferation in *Arabidopsis* seedlings through the photoreceptor phytochrome A, the transcription factor HY5 HOMOLOG, and the peroxisomal protein PEROXIN11b. *Plant Physiol* 2008.
- Lingard MJ, Trelease RN. Five *Arabidopsis* peroxin 11 homologs individually promote peroxisome elongation, duplication or aggregation. *J Cell Sci* 2006; 119:1961-72.
- Orth T, Reumann S, Zhang X, Fan J, Wenzel D, Quan S, Hu J. The PEROXIN11 protein family controls peroxisome proliferation in *Arabidopsis*. *Plant Cell* 2007; 19:333-50.
- Castillo MC, Sandalio LM, Del Rio LA, Leon J. Peroxisome proliferation, wound-activated responses and expression of peroxisome-associated genes are cross-regulated but uncoupled in *Arabidopsis thaliana*. *Plant, Cell Environm* 2008, In press.
- Nila AG, Sandalio LM, Lopez MG, Gomez M, del Rio LA, Gomez-Lim MA. Expression of a peroxisome proliferator-activated receptor gene (xPPAR α) from *Xenopus laevis* in tobacco (*Nicotiana tabacum*) plants. *Planta* 2006; 224:569-81.
- de Felipe M, Lucas MM, Pozuelo, JM. Cytochemical study of catalase and peroxidase in the mesophyll of *Lolium rigidum* plants treated with isoproturon. *J Plant Physiol* 1988; 132:67-73.
- Ferreira M, Bird, B, Davies, DD. The effect of light on the structure and organization of *Lemna* peroxisomes. *J Exp Bot* 1989; 40:1029-35.
- Oksanen E, Haikio E, Sober J and Karnosky DF. Ozone-induced H₂O₂ accumulation in field-grown aspen and birch is linked to foliar ultrastructure and peroxisomal activity. *New Phytol* 2003; 161:791-9.
- Palma JM, Garrido M, Rodriguez Garcia MI, del Rio LA. Peroxisome proliferation and oxidative stress mediated by activated oxygen species in plant peroxisomes. *Arch Biochem Biophys* 1991; 287:68-74.
- Thoms S, Erdmann R. Dynamin-related proteins and Pex11 proteins in peroxisome division and proliferation. *FEBS J* 2005; 272:5169-81.
- Erdmann R, Blobel G. Giant peroxisomes in oleic acid-induced *Saccharomyces cerevisiae* lacking the peroxisomal membrane protein Pmp27p. *J Cell Biol* 1995; 128:509-23.

19. Li X, Baumgart E, Dong GX, Morrell JC, Jimenez-Sanchez G, Valle D, Smith KD, Gould SJ. PEX11 α is required for peroxisome proliferation in response to 4-phenylbutyrate but is dispensable for peroxisome proliferator-activated receptor α -mediated peroxisome proliferation. *Mol Cell Biol* 2002; 22:8226-40.
20. Marshall PA, Krimkevich YI, Lark RH, Dyer JM, Veenhuis M, Goodman JM. Pmp27 promotes peroxisomal proliferation. *J Cell Biol* 1995; 129:345-55.
21. Hu J. Plant peroxisome multiplication: highly regulated and still enigmatic. *Int J Plant Biol* 2007; 49:1112-18.
22. Mano S, Nakamori C, Kondo M, Hayashi M, Nishimura M. An Arabidopsis dynamin-related protein, DRP3A, controls both peroxisomal and mitochondrial division. *Plant J* 2004; 38:487-98.