ABSTRACT

PEROXISOME DIVISION IN ARABIDOPSIS THALIANA

By

Xinchun Zhang

Peroxisomes are versatile, single-membrane bound organelles with diverse functions in eukaryotes. Their division is controlled by at least three types of proteins, PEROXIN11 (PEX11), FISSION1 (FIS1) and Dynamin-Related Proteins (DRPs), in yeast and humans. The five PEX11 proteins promote peroxisome elongation, which initiates peroxisome division, while DRP3A plays a role in peroxisome fission, the late step of peroxisome division in Arabidopsis thaliana. To further determine the molecular architecture of peroxisome division in planta, we used forward and reverse genetic strategies to search for more players involved in this process. Four new components of the peroxisome division machinery in Arabidopsis were identified: DRP3B, DRP5B, FIS1A and FIS1B. DRP3B is a homolog of DRP3A, and both proteins are involved in mitochondrial division. DRP3B appears as puncta marking the fission sites or potential fission sites, not only on mitochondria, but on peroxisomes. Disruption of DRP3B causes defects in peroxisome fission. drp3A drp3B double mutants display stronger deficiencies than each drp3 single mutant in peroxisome abundance, seedling establishment and plant growth, suggesting that DRP3A and DRP3B are functionally redundant.

DRP5B is the only known DRP serving as a component of the chloroplast division complexes. We addressed a new role for DRP5B in peroxisome division. Subcellular localization analysis shows that DRP5B not only forms a ring on chloroplast as previously reported, but also is co-localized with peroxisomes. Mutations in the DRP5B

gene lead to aggregated peroxisomes with membrane constriction. Furthermore, impaired peroxisome functions caused by loss of DRP5B affect seedling establishment and plant growth in Arabidopsis. Taken together, DRP5B mediates peroxisome division.

FIS1A and FIS1B that are dual-targeted to peroxisomes and mitochondria function in the division of both organelles. Overexpression of each FIS1 gene increases the abundance of both mitochondria and peroxisomes, by contrast, loss of FIS1 results in number reduction of both organelles showing incomplete fission and enlarged size. Domain truncation studies show that the C-terminal transmembrane domain is required for FIS1 targeting to peroxisomes. Moreover, FIS1 silencing experiments demonstrate that FIS1A and FIS1B play rate-limiting and partially overlapping roles in promoting the fission of peroxisomes and mitochondria. In summary, FIS1A and FIS1B are involved in the fission of peroxisomes and mitochondria.

Lastly, bimolecular fluorescence complementation (BiFC) and co-immunoprecipitation (Co-IP) assays demonstrate that DRP5B interacts with itself, and also with both DRP3A and DRP3B. These physical interactions suggest that the three DRPs may assemble together to exert their functions on peroxisomal membrane fission. Additionally, FIS1 and PEX11 proteins physically interact with DRP5B, DRP3A and DRP3B in vivo and in vitro, indicating that two families of transmembrane proteins, FISls and PEXlls, might anchor DRPs to different organelles. In conclusion, our data support the view that PEX11, DRPs and FIS1 orthologs are common conserved proteins of the peroxisomal division apparatus across eukaryotic species, and plant-specific targeting mechanisms by which DRPs are recruited to different organelles may have been evolved.