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Article Addendum

The Arabidopsis peroxisome division mutant *pdd2* is defective in the *DYNAMIN-RELATED PROTEIN3A* (*DRP3A*) gene

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In plants, the division of peroxisomes is mediated by several classes of proteins, including PEROXIN11 (PEX11), FISSION1 (FIS1) and DYNAMIN-RELATED PROTEIN3 (DRP3). DRP3A and DRP3B are two homologous dynamin-related proteins playing overlapping roles in the division of both peroxisomes and mitochondria, with DRP3A performing a stronger function than DRP3B in peroxisomal fission. Here, we report the identification and characterization of the *peroxisome division defective 2* (*pdd2)* mutant, which was later proven to be another *drp3A* allele. The *pdd2* mutant generates a truncated DRP3A protein and exhibits pale green and retarded growth phenotypes. Intriguingly, this mutant displays much stronger peroxisome division deficiency in root cells than in leaf mesophyll cells. Our data suggest that the partial GTPase effector domain retained in *pdd2* may have contributed to the distinct mutant phenotype of this mutant.

In eukaryotic cells, peroxisomes are surrounded by single membranes and house a variety of oxidative metabolic pathways such as lipid metabolism, detoxification and plant photorespiration.[1,2] To accomplish multiple tasks, the morphology, abundance and positioning of peroxisomes need to be highly regulated. Three families of proteins, whose homologs are present across different kingdoms, have been shown to be involved in peroxisome division in Arabidopsis. The PEX11 protein family is composed of five integral membrane proteins with primary roles in peroxisome elongation/tubulation, the initial step in peroxisome division.[3-5] Although the exact function of PEX11s has not been demonstrated, these proteins are believed to participate in peroxisome membrane modification.[6,7] The FIS1 family consists of two isoforms, which are C-terminal tail-anchored membrane proteins with rate limiting functions at the fission step.[8,9] DRP3A and DRP3B belong to a superfamily of dynamin-related proteins, which are large and self-assembling GTPases involved in the fission and fusion of membranes by acting as mechanochemical enzymes or signaling GTPases.[10] The function of PEX11 seems to be exclusive to peroxisomes, whereas DRP3 and FIS1 are shared by the division machineries of both peroxisomes and mitochondria in Arabidopsis.[8,9,11-16] FIS1 proteins are believed to tether DRP proteins to the peroxisomal membrane,[17,18] but direct evidence has not been obtained from plants. DRP3A and DRP3B share 77% sequence identity at the protein level and are functionally redundant in regulating mitochondrial division; however, DRP3A's role on the peroxisome seems stronger and cannot be substituted by DRP3B in peroxisome division.[8,13,15]

In a continuous effort to identify components of the plant peroxisome division apparatus from Arabidopsis, we performed genetic screens in a peroxisomal marker background expressing the YFP (yellow fluorescent protein)-PTS1 (peroxisome targeting signal 1, containing Ser-Lys-Leu) fusion protein. Mutants with defects in the morphology and abundance of fluorescently labeled peroxisomes are characterized. Following our analysis of the *pdd1* mutant, which turned out to be a strong allele of DRP3A,[8] we characterized the *pdd2* mutant.

In root cells of the *pdd2* mutant, extremely elongated peroxisomes and a beads-on-a-string peroxisomal phenotype are frequently observed (Fig. 1A and B). These peroxisome phenotypes resemble those of *pdd1* and other strong *drp3A* alleles previously reported.[8,15] However, the peroxisome phenotype seems to be less dramatic in leaf mesophyll cells. For instance, in addition to the decreased number of total peroxisomes, peroxisomes in leaf cells are only slightly elongated or exhibit a beads-on-a-string phenotype (Fig. 1C and D). Previously, we reported the phenotypes of three strong *drp3A* alleles, all of which contain a large number of peroxules, long and thin membrane extensions from the peroxisome,[8] yet such peroxisomal structures are not observed in *pdd2*. On the other hand, *pdd2* has a more severe growth phenotype than most *drp3A* alleles, as it is slow in growth and has pale green leaves (Fig. 1E). Genetic analysis showed that *pdd2* segregates as a single recessive mutation (data not shown).

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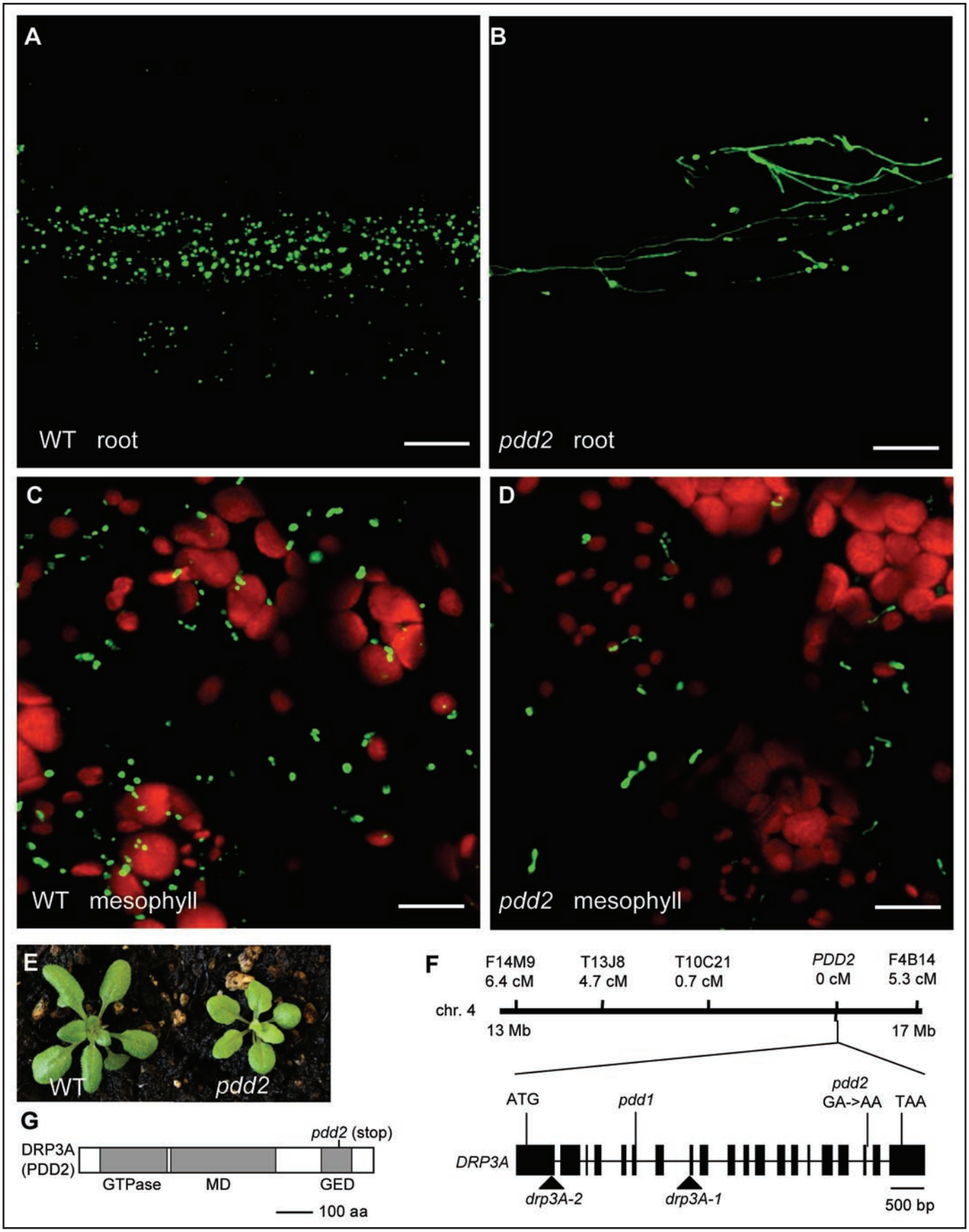
A
WT root
B
pdd2 root
C
WT mesophyll
D
pdd2 mesophyll
E
WT
pdd2
F
F14M9
6.4 cM
T13J8
4.7 cM
T10C21
0.7 cM
PDD2
0 cM
F4B14
5.3 cM
chr. 4
13 Mb
17 Mb
ATG
pdd1
pdd2
GA->AA
TAA
DRP3A
drp3A-2
drp3A-1
500 bp
G
pdd2 (stop)
DRP3A
(PDD2)
GTPase
MD
GED
100 aa

Figure 1. Phenotypic analyses of *pdd2* and identification of the PDD2 gene. (A–D) Confocal micrographs of root and mesophyll cells in 3-week-old wild type and *pdd2* mutant plants. Green signals show peroxisomes; red signals show chloroplasts. Scale bars = 20 μm. (E) Growth phenotype of 3-week-old mutants. (F) Map-based cloning of the *PDD2* gene. Genetic distance from *PDD2* is shown under each molecular marker. Positions for mutations in previously analyzed *drp3A* alleles and *pdd2* are indicated in the gene schematic. *drp3A-1* and *drp3A-2* are T-DNA insertion mutants, whereas *pdd1* is an EMS mutant containing a premature stop codon in exon 6. (G) A schematic of the DRP3A (PDD2) protein with functional domains indicated. The *pdd2* allele encodes a truncated protein lacking part of the GED domain.

The unique combination of peroxisomal and growth phenotypes of *pdd2* prompted us to use map-based cloning to identify the *PDD2* gene, with the hope to discover novel proteins in the peroxisome division machinery. A population of approximately 6,000 F_2 plants (*pdd2* x *Ler*) was generated. After screening 755 F_2 mutants, the *pdd2* mutation was mapped to the region between markers T10C21 and F4B14 on the long arm of chromosome 4 (Fig. 1F). Since this region contains *DRP3A*, we sequenced the entire *DRP3A* gene in *pdd2* and identified a G→A transition at the junction of the 18th exon and intron (Fig. 1F). Further analysis revealed that the point mutation at this junction caused mis-splicing of intron 18, introducing a stop codon in the GTPase effector domain GED near the C terminus (Fig. 1G).

DRPs share with the classic dynamins an N-terminal GTPase domain, a middle domain (MD), and a regulatory motif named the GTPase effector domain (GED) (Fig. 1G). To date, a total of 26 *drp3A* mutant alleles carrying missense or nonsense mutations along the length of the *DRP3A* gene have been isolated.[8,15] The combined peroxisomal and growth phenotype of *pdd2* and the nature of the mutation in this allele are unique among all the *drp3A* alleles, indicating that the partial GED domain retained in *pdd2* may have created some novel function for this protein. Further analysis of the truncated protein may be necessary to test this prediction.

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