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The *Arabidopsis* thiamin deficient mutant pale green1 lacks thiamin monophosphate phosphatase of the vitamin B1 biosynthesis pathway

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Thiamin diphosphate (TPP, vitamin B1) is an essential coenzyme present in all organisms. Animals obtain TPP from their diets, but plants synthesize TPP de novo. We isolated and characterized an *Arabidopsis* pale green1 (pale1) mutant that contained higher concentrations of thiamin monophosphate (TMP) and less thiamin and TPP than the wild type. Supplementation with thiamin, but not the thiazole and pyrimidine precursors, rescued the mutant phenotype, indicating that the pale1 mutant is a thiamin-deficient mutant. Map-based cloning and whole-genome sequencing revealed that the pale1 mutant has a mutation in At5g32470 encoding a TMP phosphatase of the TPP biosynthesis pathway. We further confirmed that the mutation of At5g32470 is responsible for the mutant phenotypes by complementing the pale1 mutant with constructs overexpressing full-length At5g32470. Most plant TPP biosynthetic enzymes are in the chloroplasts and cytosol, but At5g32470-GFP localized to the mitochondrion of the root, hypocotyl, mesophyll and guard cells of the 35S:At5g32470-GFP complemented plants. The subcellular localization of a functional TMP phosphatase suggests that the complete vitamin B1 biosynthesis pathway may involve the chloroplasts, mitochondria and cytosol in plants.

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