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IN BRIEF

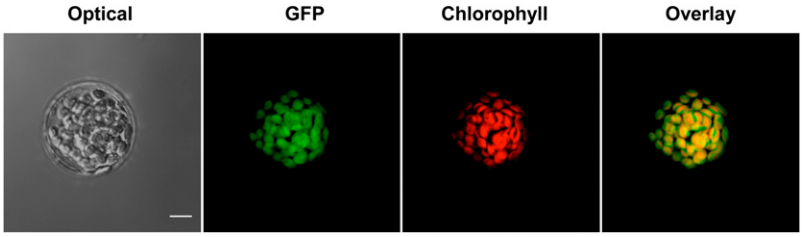
Introducing Aromatic Amino Acid Hydroxylases from Plants

In humans, a deficiency in Phe hydroxylase activity results in phenylketonuria, a metabolic disorder that is characterized by the accumulation of Phe. Phe hydroxylase belongs to the iron-containing aromatic amino acid hydroxylase (AAH) family, whose members catalyze the hydroxylation of Phe, Trp, or Tyr in a reaction that depends on the donation of electrons by tetrahydropterin (reviewed in Fitzpatrick, 2003). Whereas AAHs have been described in animals, protists, and some bacteria, their presence in plants has not been firmly established. A comparative genomics analysis identified AAH-like sequences in single-celled alga (*Chlamydomonas reinhardtii*), moss (*Physcomitrella patens*), and loblolly pine (*Pinus taeda*), suggesting that plants may indeed harbor functional AAHs (Naponelli et al., 2008).

Pribat et al. (pages 3410–3422) isolated and characterized full-length cDNA clones of AAH-like sequences from single-celled alga, moss, and loblolly pine. The catalytic domain encoded by the plant AAHs contained a conserved iron binding site. However, whereas the catalytic domain of animal AAHs is flanked by an N-terminal regulatory domain and a C-terminal tetramerization domain (reviewed in Fitzpatrick, 2003), these domains were not present in plant AAHs. In addition, the plant AAHs contained a nonconserved sequence in the N terminus that was predicted to be a plastid-targeting peptide. Functional complementation of an *Escherichia coli* Tyr auxotroph demonstrated that the plant AAHs had Phe hydroxylase activity. Furthermore, analytical size exclusion chromatography showed that, in contrast with animal AAHs, which occur as homotetramers (reviewed in Fitzpatrick, 2003), plant AAHs function as monomers. Treatment with various chelators demonstrated that the activity of plant AAHs, like that of animal AAHs, depends on iron.

Next, the authors tested a series of pterins and folates for their ability to act as a cofactor for pine and moss AAHs and identified a folate, 10-formyltetrahydrofolate, as the physiological cofactor of these enzymes. As suggested by the presence of a putative plastid-targeting peptide, targeting assays using full-length green fluorescent protein (GFP)–tagged pine and moss AAHs in *Arabidopsis thaliana* mesophyll cells (see figure), and import assays using purified pea (*Pisum sativum*) chloroplasts demonstrated that these AAHs localized to chloroplasts. Interestingly, chloroplasts are also a rich source of the 10-formyltetrahydrofolate cofactor (Orsomando et al., 2005). The authors then showed that ablation of the only AAH-like gene in *P. patens* caused Phe and caffeic acid esters, which are Phe metabolites, to accumulate but did not affect growth.

Finally, the authors present a model in which plant AAHs form a short circuit between the Phe and Tyr branches of aromatic metabolism and discuss the possible effects of AAH activity on aromatic amino acid metabolism.



Pine AAH localizes to chloroplasts. GFP-tagged *Pinus taeda* AAH was transiently expressed in *Arabidopsis* mesophyll protoplasts. Confocal planes show GFP (green) and chlorophyll (red) fluorescence, and the overlay demonstrates colocalization (yellow) of GFP-tagged AAH and chlorophyll. Bar = 10 μm.

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