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Funding Source: Dudley & Virgie Alexander Gift

Functional analysis of conserved amino acid residues in the C-terminus of ACC Synthase

Ethylene is an important plant hormone that regulates growth, development, and stress response. Synthesis of ethylene from its immediate precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), is catalyzed by ACC oxidase. ACC is produced from S-adenosy1-L-methionine (SAM) in a reaction catalyzed by ACC synthase (ACS). ACS is the rate limiting enzyme of ethylene biosynthesis. Selected isoforms of ACS are substrates of MPK6 and MPK3, the two Arabidopsis stress-responsive mitogen-activated protein kinases (MAPKs). Phosphorylation of ACS6 by MPK6 stabilizes the ACS protein, thus, elevating the levels of cellular ACS activity and ethylene production. Expression of ACS6DDD, a gain-of-function ACS6 mutant that mimics the phosphorylated form of ACS6, shows constitutive ethylene production and ethylene-induced phenotypes. Analysis of Arabidopsis ACS6 and its orthologs from other species in the database revealed conserved charged amino acids (AAs) in addition to the MAPK phosphorylation sites in their C-termini. We hypothesized that these conserved residues may be involved in the regulation of ACS stability. We used site-directed mutagenesis to mutate the conserved charged residues to neutral AAs: Ala, Ile, or Leu in the ACS6WT or ACS6DDD background using the polymerase chain reaction (PCR)-based method. Mutation was confirmed by DNA sequencing. Mutated ACS6 genes were transformed into Arabidopsis plants. The stability of ACS6 protein was tested in vivo to determine if the mutation enhances or diminishes its stability. Ethylene production was used as an output reading and the levels of ACS6 protein were determined by immunoblot analysis. Mutation of positively charged AAs to neutral residues makes the ACS6 protein more stable, whereas the mutation of the negatively charged AAs which are close to the phosphorylation sites destabilizes it. Interestingly, deletion of the C-terminus stabilizes the ACS6 protein, suggesting that C-terminus is required for ACS6 degradation. We observed ethylene-induced phenotypes such as short hairy roots and epinastic leaves in ethylene-overproducing seedlings.