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Yeast 2-hybrid screening for proteins that recognize ACS6 C-terminus which is essential for ACS6 stability regulation

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Ethylene is an important hormone that regulates plant growth, development, and stress response. The level of ethylene is highly regulated in plants. 1-aminocyclopropane-1-carboxylic acid synthase (ACS) is the rate limiting enzyme of ethylene biosynthesis. Selected isoforms of ACS are substrates of MPK6 and MPK3, 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. We found that the C-terminus of ACS6 is important for its degradation when it is in unphosphorylated form and its stabilization by phosphorylation. Deletion of the C-terminus stabilizes the ACS6 protein. To identify additional proteins that are involved in ACS6 stability regulation, we used a LexA-based yeast 2-hybrid screen. Prey libraries in pJG4-5 vector were prepared and we made bait constructs with both full-length ACS6 and ACS6 C-terminal region in pEG202. The constructs were sequenced to confirm that the open reading frames are correct. Yeast (EGY48 strain) was transformed with bait constructs to test autoactivation and the ability of LexA-ACS6 fusion to enter the nucleus. After confirming that the bait can enter the nucleus and does not activate reporter by itself, we are going to screen the prey libraries to identify ACS6-interacting proteins.

