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ACC synthase and its cognate E3 ligase are inversely regulated by light

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Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; ACS, ACC synthase; ETO1, Ethylene Overproducer1; EOL, ETO1-like

1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) is the key enzyme in ethylene biosynthesis, catalyzing the conversion of S-adenosylmethionine (AdoMet) to ACC, which is the immediate precursor of ethylene. The regulation of ACS protein stability plays an important role in controlling ethylene biosynthesis. We have recently shown that 14-3-3 positively regulates ACS protein stability by both a direct effect and via downregulation of the stability of the E3 ligases regulating its turnover, Ethylene Overproducer1 (ETO1)/ETO1-like (EOL). Here, we report that treatment of etiolated Arabidopsis seedlings with light rapidly increases the stability of ACS5 protein. In contrast, light destabilizes the ETO1/EOLs proteins, suggesting that light acts to increase ethylene biosynthesis in part through a decrease in the level of the ETO1/EOL proteins. This demonstrates that the ETO1/EOLs are regulated in response to at least one environmental cue and that their regulated degradation may represent a novel input controlling ethylene biosynthesis.

The phytohormone ethylene plays an important role in many plant processes and its biosynthesis is highly regulated by a diverse array of endogenous and exogenous inputs.[1-4] This regulation of ethylene biosynthesis most often converges on ACC synthase (ACS), which catalyzes the first committed and generally rate-limiting step in ethylene biosynthesis. ACS is encoded by a multigene family in *Arabidopsis* whose transcription is differentially regulated by various developmental and environmental cues.[3,5,6] In addition to this transcriptional control, the stability of subsets of ACS proteins are regulated by cytokinin, brassinosteroid, and pathogens.[7-9]

The control of protein stability plays a key role in many plant processes, including the regulation of phytohormone signaling and biosynthesis. E3 ligases are key components regulating protein turnover, and they are encoded by a large family of genes in plants. A subset of ACS proteins have been shown to be targeted for degradation via the 26S proteasome by a small family of plant-specific E3 ligases called ETO1, ETO1-like1 (EOL1), and EOL2.[10,11] These E3 ligases, which contain a BTB/TRP domain, interact with the CULLIN3 protein to direct the ubiquitination of type-2 ACS proteins.[11] Disruption of ETO1/EOLs, CUL3, or the domain in the type-2 ACS proteins that ETO1/EOLs interact with reduces the turnover of these ACS proteins, leading to an increase in the biosynthesis of ethylene.[7,10-13]

Despite their importance, relatively little is known regarding how E3 ligases themselves are regulated in plants. Recently, we have shown that the 14-3-3 proteins bind to both the ACS proteins and to the ETO1/EOL E3 ligases to regulate their stability.[14] 14-3-3s generally interact specifically with phosphorylated proteins to control their function by changing their localization, activity or stability.[15-18] We demonstrated that 14-3-3 stabilizes ACS proteins via 2 distinct mechanisms. First, 14-3-3 binds directly to ACS proteins to reduce their turnover via an ETO1/EOL-independent mechanism. Second, 14-3-3s also bind to the ETO1/EOL E3 ligases to increase their degradation in an ubiquitin/proteasome-dependent manner, thereby increasing the stability of type-2 ACS proteins.

There are 3 classes of ACS proteins based primarily on their C-terminal domains, which impart distinct regulatory controls on the stability of the respective ACS proteins.[2] In our previous study, we demonstrated that 14-3-3 interacts will all 3 classes of ACS proteins.[14] Further, it was shown that both type-2 and type-3 ACS proteins are destabilized in response to disruption of the interaction with 14-3-3 using the R18 peptide, which acts as a strong competitive inhibitor of 14-3-3-interacting proteins.[19] In order to extend this analysis, we examined the effect of the 14-3-3 interaction on a type-1 ACS protein. Consistent with the effect on the type-2 and type-3 ACS proteins, treatment of seedlings expressing a myc-epitope tagged version of ACS2 (a type-1 ACS protein) with R18 peptide resulted in a substantial destabilization of the fusion protein (**Fig. 1**). This destabilization did not occur in response to a mutant version of the R18 peptide

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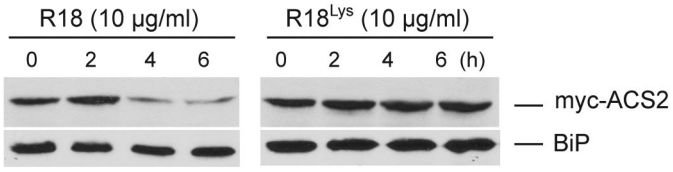

Figure 1. R18 peptide treatment results in a decrease in the steady-state level of type-1 ACS2 protein. Three-day-old etiolated seedlings expressing myc-ACS2 were treated with 10 μg/mL R18 and R18^{Lys} for the indicated times. Total protein extracts from these seedlings were analyzed by immunoblotting using an anti-myc antibody or an anti-BiP antibody as a loading control as described.14

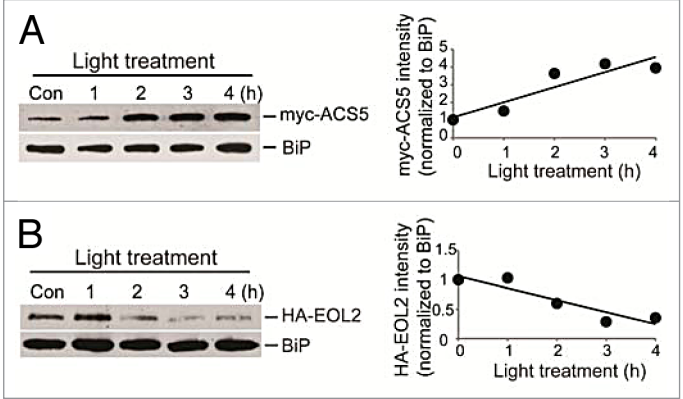

Figure 2. Light stabilizes myc-ACS5 protein, but destabilizes HA-EOL2 protein. Left: Immunoblot of protein extracts from 3-day-old seedlings expressing either a myc-ACS5 (**A**) or an HA-EOL2 (**B**) fusion protein after treatment with light. Seedlings were grown in the dark for 3 days, moved to light for the indicated times, proteins extracted and analyzed by immunoblotting using an anti-myc, anti-HA, or an anti-BiP antibody as described.14 Right: The myc and HA signal intensities were quantified and normalized to the BiP loading control.

(R18^{Lys}) that eliminates its ability to disrupt 14-3-3 interactions. Together with our previous study, this result indicates that 14-3-3s are positive regulators of all 3 classes of ACS proteins.

As the 3 classes of ACS proteins have distinct C-terminal domains, these results suggest that 14-3-3 acts through the conserved catalytic domain of the ACS proteins. Consistent with this, 14-3-3 interacts with the *eto2* version of ACS5, which disrupts the C-terminal domain of ACS5.[14] Further, the *eto2* and *eto3* versions of the type-2 ACS proteins ACS5 and ACS9, both of which lack the TOE domain necessary for interaction with ETO1/EOLs,[20] are stabilized in vivo by the phytohormones cytokinin and brassinosteroid,[8] indicating that there is a mechanism to control these proteins in a C-terminal independent manner. This alternative pathway regulating ACS stability may act through the 14-3-3 pathway.

In addition to the direct effect on all 3 classes of ACS proteins, 14-3-3 also stabilizes ACS proteins by increasing the degradation of the ETO1/EOL proteins. Disruption of the interaction with R18 caused a substantial increase in ETO1/EOL protein stability and increased ethylene biosynthesis, and conversely, overexpression of 14-3-3 resulted in decreased ETO1/EOL protein levels and reduced ethylene.[14] This suggests the level of ETO1/EOL proteins plays a role in regulating the level of ethylene biosynthesis. Consistent with this, there is a quantitative effect of disruption of the 6 copies of the *ETO1/EOL1/EOL2* genes on ethylene production in etiolated and light-grown seedlings.[10,14] If modulation of ETO1/EOL levels is a mechanism by which plants regulate ethylene biosynthesis, then the level of ETO1/EOL proteins should be regulated by a subset of factors known to regulate ethylene biosynthesis. To test this, we examined the effect of light on ETO1/EOL proteins as light has a substantial effect on ethylene biosynthesis in various plant species, including *Arabidopsis*.[21-23] Light treatment of 3-day-old etiolated seedlings caused an increase in the level of myc-ACS5 protein within 2 h (**Fig. 2A**). This increase in ACS5 protein is not associated with a change in *myc-ACS5* transcript (not shown), suggesting that light acts by increasing ACS5 stability. Light treatment had an inverse effect on EOL2 protein levels, causing a substantial decrease within 2 h after treatment (**Fig. 2B**). This result supports the hypothesis that the level of ETO1/EOL proteins is regulated in response to environmental cues, and that the altered level of the ETO1/EOL proteins likely acts as an input for the control of ACS protein stability. A subset of 14-3-3 isoforms have also been shown to be involved in a light signaling pathway regulating flowering transition, phytochrome response, and stomatal opening,[24,25] suggesting that 14-3-3 proteins may play an important role in multiple light responses.

As the 14-3-3 proteins generally act as dimers, they may increase ETO1/EOL degradation by blocking the access to the ACS substrates and promoting ETO1/EOL dimerization, leading to auto-ubiquitination. Further biochemical analyses should help clarify the mechanism by which ETO1/EOL degradation is increase by 14-3-3 and may reveal additional regulatory inputs into ETO1/EOL degradation. As 14-3-3s generally bind phosphorylated substrates, it is likely that both the ETO1/EOL proteins and the catalytic domain of ACS proteins are phosphorylated by as yet unidentified kinases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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