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A FCA-mediated epigenetic route towards thermal adaptation of autotrophic development in plants

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Keywords: FCA, autotrophic development, protochlorophyllide oxidoreductase, chlorophyll biosynthesis

Abbreviations: POR, protochlorophyllide oxidoreductases; pchlide, protochlorophyllide; chlide, chlorophyllide; PIF, phytochrome-interacting factor; EIN3, ethylene-insensitive 3; EIL1, ethylene-insensitive 3-like 1; ROS, reactive oxygen species

Perspective to: Jun-Ho Ha (2016) Thermo-induced maintenance of photooxidoreductases underlies plant autotrophic development. Developmental Cell, 41, 170-179. doi: 10.1016/j.devcel.2017.03.005

Abstract

Plants are able to recognize even small changes of surrounding temperatures to optimize their growth and development. At warm temperatures, plants exhibit diverse architectural adjustments, including hypocotyl and petiole elongation, leaf hyponasty, and reduced stomatal density. However, it was unknown until recently how warm temperatures affect the early stages of seedling development. In our recent study, we demonstrated that the RNA-binding protein FCA is critical for sustaining chlorophyll biosynthesis during early seedling development, which is a prerequisite for autotrophic transition, at warm temperatures. FCA plays dual roles in this thermal response. It inhibits the rapid degradation of protochlorophyllide oxidoreductases (PORs) that mediate chlorophyll biosynthesis. In addition, it induces the expression of *POR* genes at a chromatin level, which contributes to maintaining functional levels of the enzymes. Our findings provide a first molecular basis for the thermal adaptation chlorophyll biosynthesis during early stages of seedling development in nature.

Text

Plants synthesize their own energy sources by fixing carbon from atmospheric CO_2 using sun light. Consequently, plants gain autotrophic competence by inducing chlorophyll biosynthesis and concomitant chloroplast formation during early stages of seedling development. In this

physiological process, protochlorophyllide (pchlide) oxidoreductases (PORs) enzymatically convert pchlide to chlorophyllide (chlide), leading to chlorophyll biosynthesis.

In *Arabidopsis*, there are three POR enzymes, PORA, PORB, and PORC. Genes encoding POR members are differently regulated in response to light. While the expression of *PORA* gene is reduced to a basal level upon light illumination, that of *PORB* gene is not significantly influenced by light. Notably, *PORC* gene is induced after seedlings are exposed to light. Light also affects the protein stability of the POR enzymes. PORA is rapidly destabilized during de-etiolation, possibly by the action of plastid proteases. These regulatory schemes denote that POR abundance is tightly regulated by light during autotrophic development.

Temperature is another environmental cue that profoundly affects morphological and physiological events during plant growth and environmental adaptation. While extreme temperatures impose stressful effects on plants, mild fluctuations of surrounding temperatures triggers architectural modifications as well as physiological adjustments, which are distinct from stress responses. In recent years, molecular mechanisms underlying plant responses to warm temperatures have been extensively studied at the molecular level. It is known that the PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) activates auxin biosynthesis to facilitate cell growth at warm temperatures. In addition, FCA attenuates the function of PIF4 by suppressing its DNA-binding ability to prevent hypocotyl overgrowth under warm temperature conditions.

In our recent study, we have demonstrated that FCA plays a distinct role in regulating autotrophic transition at warm temperatures. While FCA-defective mutants exhibit normal greening when they were germinated and grown for a few days at normal temperatures before

exposure to warm temperatures, the *fca* mutants exhibited an albino phenotype when they were exposed to warm temperatures from the beginning of germination. Since plant autotrophic transition occurs during early seedling development, we hypothesized that FCA is involved in the establishment of autotrophic development at warm temperatures.

Our molecular studies indicate that FCA promotes the transcription of *POR* genes at warm temperatures by triggering histone acetylation in the *POR* loci (Fig. 1A). FCA is a RNA-binding protein and does not have DNA-binding domain. It has previously been reported that FCA interacts with PIF4 for its binding to the promoter regions of PIF4 target genes. We predict that as-yet unknown transcription factor(s) would be involved in the FCA-mediated regulation of *POR* expression. PIF1, ETHYLENE-INSENSITIVE 3 (EIN3), and ETHYLENE-INSENSITIVE 3-LIKE 1 (EIL1) are known to directly bind to *POR* promoters. However, we found that FCA does not interact with these transcription factors. For the elucidation of molecular mechanisms underlying FCA function, it would be required to identify upstream transcription factors that are activated by warm temperatures and interact with FCA. Proteomic analysis with anti-FCA antibodies would be helpful for this purpose.

In addition to the transcriptional control, FCA also suppresses the destabilization of POR enzymes at warm temperatures (Fig. 1B), although proteolytic mechanisms underlying POR degradation are yet unknown. Chemical treatments employing inhibitors against proteases and 26S proteasome did not rescue the POR degradation in *fca* mutants. It has been reported that plastid proteases are responsible for the light-mediated POR degradation. It is thus possible that the plastid proteases would mediate the thermo-induced destabilization of POR enzymes. Alternatively, the POR enzymes might be degraded through selective autophagy. It is known that denatured protein aggregates are selectively recognized by autophagic machinery for degradation. Investigation of POR protein stability in autophagy-

related mutants would be helpful to examine the hypothesis.

The albino phenotype of FCA-deficient mutants is evident only when developing seedlings are exposed to warm temperatures, suggesting that FCA function would be activated by warm temperatures. In accordance with this notion, it has been reported that FCA-mediated inhibition of PIF4 activity is promoted by increasing temperatures. Notably, it has recently been reported that the FCA-PIF4 interaction is enhanced when plants are exposed to warm temperatures. We found that the binding of FCA to the *POR* gene promoters are also thermally induced. However, it is largely unknown how FCA is activated by warm temperatures. The gene transcription and protein abundance of FCA are only marginally altered under warm temperature conditions. A possible mechanism would be that FCA is post-translationally modified. Since protein phosphorylation is a representative mechanism for the activity of regulatory proteins in most organisms, it would be worth examining whether and how FCA is phosphorylated at warm temperatures.

In de-etiolating seedlings, POR enzymes rapidly convert pchlide to chlide as pchlide absorbs light energy. However, light-absorbed, but POR-unbound pchlide acts as a photosensitizer that produces reactive oxygen species (ROS) (Fig. 1C). ROS are often considered as toxic chemicals that cause oxidative damages to DNA, proteins, and lipids. However, accumulating evidence support that ROS also play a beneficial role during plant growth and development. Particularly, ROS accumulate to high levels during early seedling development, where ROS promote radicle elongation. In our recent study, we found that POR enzymes are highly unstable at warm temperatures during autotrophic development. It is thus possible that plants would reduce POR levels to produce ROS by increasing POR-unbound pchlide levels at warm temperatures, which facilitates radicle and hypocotyl growth. The growth rate of seedlings is increased at warm temperatures to rapidly move away the leaves

and shoot apical meristemic tissues from the heat-absorbing soil. We propose that this physiological response provides an adaptation strategy, by which developing seedlings utilize ROS as a growth stimulator under warm temperature conditions.

FCA has been originally identified as a flowering time regulator. Oure recent study has identified a distinct role of FCA, which is associated with autotrophic development. Notably, the role of FCA in autotrophic transition is critical for plant survival, since its mutation causes seedling albinism under warm temperature conditions. It is apparent that FCA mediates chlorophyll biosynthesis modulating POR protein stability and its gene expression. However, detailed regulatory mechanisms are still missing. Further genetic and biochemical analyses on FCA-mediated regulation of POR function are required to fully understand autotrophic developmental events that occur at warm temperatures.

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Figure 1

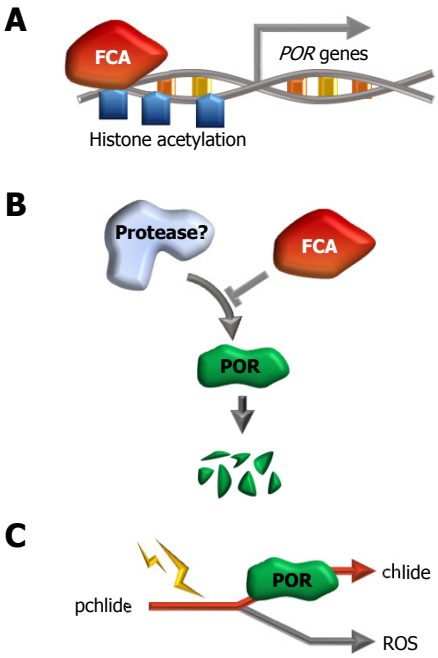

Fig. 1. Schematic diagram of FCA function in sustaining POR abundance at warm temperatures.
(A) FCA induces *POR* transcription. At warm temperatures, FCA binds to the promoter regions of *POR* genes to promote their transcription through histone acetylation.
(B) FCA inhibits POR degradation. POR proteins are highly unstable at warm temperatures. FCA stabilizes PORs possibly by suppressing plastid proteases.
(C) POR enzymes shifts the chlorophyll-ROS balance toward autotrophic development. POR abundance is maintained by FCA at warm temperatures, which leads to light-induced chlorophyll biosynthesis, while suppressing ROS production.