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Review Article

REGULATION OF 5-AMINOLEVULINIC ACID BIOSYNTHESIS IN HIGHER PLANTS

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ABSTRACT
Chlorophyll (Chl) molecules are essential for life. Plants need Chls to harvest light energy that it can utilize in the process of photosynthesis to prepare food. Though, Chl molecules are vital, free Chl molecules are reactive in nature and produce reactive oxygen species in different environmental stress condition. Second, Chl biosynthesis pathway intermediates are important for proper functioning of other pathways like carotenoid synthesis, tochopherol synthesis etc. Third, Chl level dramatically increases during the initial stage of greening process of etiolated seedlings, where the chloroplasts and the photosystems are yet not fully developed. At this stage and after the development of matured chloroplast and photosystems, the Chl supply should be distinctly controlled to maintain a functional photosynthetic apparatus. To achieve this, plants must co-ordinately regulate the enzyme activities of the Chl biosynthesis pathway. 5-aminolevulinic acid (ALA) is the precursor of chlorophyll molecules. ALA amount in plants actually regulates the chlorophyll biosynthesis intermediates and when the latter accumulates in dark that shuts off the ALA synthesis. Plants use this technique to deal with the ALA-induced chlorophyll intermediate accumulation, which actually are very reactive in nature and produce reactive oxygen species. So, regulation of ALA is very crucial for plant development, productivity as well as for its survival. In this review, I describe how ALA synthesis gets regulated under different environmental stress conditions.

Keywords: *ALA, Chlorophyll Biosynthesis, Abiotic Stress*

INTRODUCTION
Environmental cues have a deep impact on plant growth and development. For example, light has significant effects on morphogenesis of seedlings during the transition from heterotrophic to photoautotrophic growth. Plants tend to adapt the structure of photosynthetic apparatus and pigment composition to light quality and quantity and other environmental factors. Chlorophyll pigments and its intermediates play vital roles in in regulating cell metabolism including photosynthesis and respiration (Tanaka and Tanaka, 2007; Biswal *et al.*, 2012). Plants are exposed to various abiotic stresses such as low temperature, high temperature, salinity, drought, flooding, oxidative stress and heavy metal toxicity etc. during their entire life cycle. When seeds germinate beneath the soil, their seedlings remain in near-darkness for a while. Therefore, etiolated seedlings beneath the soil do not synthesize chlorophyll and contain a special form of plastids called etioplasts. As seedlings come out of soil, they are exposed to light and light-mediated chlorophyll biosynthesis and other associated greening processes are initiated resulting in transformation of etioplasts to chloroplasts.
Chloroplast development involves the biosynthesis of components of photosynthetic apparatus involving synthesis of chlorophyll and carotenoids, lipids and proteins which is governed in a coordinated manner by chloroplast and nuclear genomes (Gray *et al.*, 2003). Biosynthesis of tetrapyrroles particularly that of Chl during early greening stages of seedlings is elucidated in detail (Tanaka and Tanaka, 2007). 5-aminolevulinic acid (ALA) is the precursor of chlorophyll. Increased amount of ALA synthesis in plants resulted in synthesis and accumulation of chlorophyll synthesis pathway intermediates. However, too much chlorophyll intermediate accumulation in dark is bad for plants as those intermediates produce reactive oxygen species in presence of light. Feeding of ALA always results in accumulation of protochlorophyllide, one of the chlorophyll synthesis ntermediates, in dark and upon transfer to light these pigments cannot be photoconverted fully and as a result they react with molecular oxygen to

produce singlet oxygen. So, plants actually try to regulate its ALA synthesis differently in different growth conditions, so that they will be protected from oxidative stress and loss of productivity. Therefore, study of ALA biosynthesis and its regulation in different environmental condition is very important for agriculture.

Biosynthesis of 5-Aminolevulinic Acid

The synthesis of 5-aminolevulinic acid (ALA) in plants requires 3 different enzymes located in the chloroplast stroma. They are glutamyl-tRNA synthetase (GluRS), glutamyl-tRNA reductase and glutamate semialdehyde aminotransferase.

Glutamyl-tRNA synthetase (GluRS), also known as aminoacyl-tRNA synthetase, ligates glutamate to $tRNA^{GLU}$ (Huang *et al.,* 1984; Kannangara *et al.,* 1984). Unlike class I aminoacyl-tRNA synthetases, GluRS avoids the aminoacyl-AMP formation in the absence of tRNA. In eukaryotic cells chloroplastic GluRS is post-translationally imported into the chloroplast where it ligates glutamate to $tRNA^{GLU}$ that contains the UUC anticodon (Schon *et al.,* 1986).

Glutamyl-tRNA reductase (GluTR) is the second enzyme of the pathway. NADPH reduces the activated α-carboxyl group of glutamyl-tRNA (Glu-tRNA) to synthesize glutamate 1-semialdehyde (GSA) (Hoober *et al.,* 1988; Schon *et al.,* 1986). This enzyme is subject to feedback regulation by heme and appears to be a major control point of porphyrin biosynthesis (Kannangara *et al.,* 1988). In *A. thaliana* GluTR interacts with FLU, a negative regulator of the Chl biosynthesis pathway (Meskauskiene *et al.,* 2001). FLU is a nuclear-encoded chloroplastic protein and the *flu* mutant has a higher level of ALA synthesis and protochlorophyllide (Pchlide) accumulation than that of wild-type plants. Probably FLU is a component of negative regulatory system for ALA synthesis when cells have high Pchlide contents.

The formation of 5-aminolevulinate from GSA is catalyzed by glutamate 1-semialdehyde aminotransferase (GSA-AT), the third and the last enzyme required for ALA biosynthesis **(**Matsumoto *et al.,* 2004). This enzyme is functionally an aminomutase, which transfers the amino group from carbon 2 of GSA to the neighboring carbon atom i.e., carbon 5 to form ALA. The enzyme is inhibited by gabaculine (Gough *et al.,* 1992).

Modulation of ALA Biosynthesis by Environment

1. *Light Regulation of ALA Biosynthesis:* In cucumber and *A. thaliana* plants, the *HEMA1* gene is expressed in photosynthetic tissues and is induced by illumination, but no transcripts were detectable in roots. Gene expression of *HEMA1,* and the corresponding protein abundance, increases in response to light treatment of dark-grown seedlings suggesting that increased demand for Chl biosynthesis stimulates its expression and the gene promoter may have light-responsive elements (Mohanty *et al.,* 2006). On the other hand, *HEMA2* is preferentially expressed in non-photosynthetic tissues, and its expression is light-independent (Tanaka *et al.,* 1996; Nagai *et al.,* 2007). A third *HEMA* gene, *HEMA3*, has been identified in *A. thaliana*, but its expression is low (Matsumoto *et al.,* 2004).

In *A. thaliana* light stimulates transcription of *gsa* (Ilag *et al.,* 1994). Its gene expression is also activated by the hormone, kinetin (Yaronskaya *et al.,* 2006). The gene expression of *gsa* and protein abundance of GSA-AT increases when etiolated seedlings are transferred to light demonstrating that it is a light-inducible gene and significantly contributes to Chl synthesis (Mohanty *et al.,* 2006). In soybean also, the *gsa* is light inducible. It contains a light-regulated cis element (containing GAGA) that is found to be involved in transcriptional control (Frustaci *et al.,* 1995). Its message abundance is high in soybean leaves (Sangwan and O'Brian, 1993) whereas it is absent in roots (Frustaci *et al.,* 1995).

2. *Modulation of ALA Biosynthesis by Temperature:* The environmental factors such as chill or heat-stress influence gene expression, translation and post-translational modification of proteins involved in chloroplast biogenesis (Tewari and Tripathy, 1998; Mohanty *et al.,* 2006; Abdelkader *et al.,* 2007a, b; Dutta *et al.,* 2009). When five-day old etiolated wheat seedlings grown at 25ºC are transferred to 7ºC (chill-stress), 42ºC (heat-stress) or 25ºC (control) and exposed to cool white fluorescent light (50µmoles m^{-2} s^{-1}) for 24 h, the Chl content gradually increases in control seedlings. In chill- and heat-stressed seedlings Chl biosynthesis is severely down-regulated. A lag period up to 12 h is observed, both in chill- and heat- stressed wheat seedlings before Chl accumulation accelerates. ALA synthesis in the presence of

LA is almost linear up to 6 h of illumination in control and heat-stressed seedlings. For the first 3 h, ALA synthesis is completely inhibited in chill-stressed cucumber seedlings. As compared with the controls, the net synthesis of ALA is severely reduced in chill- and heat-stressed seedlings, respectively. Among ALA biosynthetic enzymes, the gene expression of *HEMA* is light-inducible in cucumber i.e., its expression increases in response to light in cucumber seedlings. Its expression was down-regulated both in chill- and heat-stressed seedlings.

The expression of *gsa* increases upon light exposure of etiolated control seedlings. However, heat-stressed etiolated seedlings display a higher *gsa* level than etiolated control seedlings. *GSA* expression further increases in illuminated heat-stressed seedlings and is significantly reduced in cold-treated cucumber seedlings.

3. *Regulation of ALA Biosynthesis by Salinity:* The Chl biosynthesis and chloroplast biogenesis are substantially regulated by salt-stress. ALA content was reduced in sunflower leaves due to treatment of salt stress (Santos, 2004) that may be due to reduction in the ALA precursor glutamate (Santos *et al.*, 2001; Santos and Caldera, 1999).

4. *Water-Stress and ALA Biosynthesis:* In response to water-stress, Chl biosynthesis is down-regulated. The reduced Chl synthesis in water-stressed seedlings is mostly due to down-regulation of early intermediates of Chl biosynthesis i.e., GSA and ALA (Dalal and Tripathy, 2012), almost similar to their down-regulation in chill- and heat-stressed rice/maize/cucumber/Pinus seedlings **(**Hodgins and Van Huystee, 1986, Tewari and Tripathy, 1998; Hodgins and Oquist, 2006).

Reduced GSA synthesis in water-stressed rice seedlings is due to down-regulation of *HEMA1* transcript abundance. The protein/ transcript abundance of GSA-AT increased in water-stressed rice seedlings, however the ALA contents declined suggesting that the GSA-AT, the next enzyme involved in ALA biosynthesis may be inactivated by post-translational modification. These results show that Chl biosynthesis pathway is down-regulated at the early steps under stress conditions to prevent the accumulation of harmful singlet oxygen generating tetrapyrroles.

Conclusion

Plant pigments play an important role in plant development, growth, productivity and modulation of their biosynthesis protects them from environmental stresses. ALA is the precursor of Pchlide and Chl. Pchlide regulates its own accumulation via feedback inhibition of ALA synthesis. Therefore, synthesis of ALA declines within 1 h after transfer of seedlings from light to dark and correlates with an immediate accumulation of Pchlide in darkness. Controlled regulation of ALA synthesis prevents accumulation of tetrapyrrolic metabolic intermediates and avoids photo-oxidative damage. On the other hand, ALA could be used as selective commercial herbicide. Transgenic plants having enhanced activities of chlorophyll biosynthesis pathway enzymes when sprayed with ALA can efficiently convert all the chlorophyll biosynthesis pathway intermediates, so they will be protected from oxidative damage. But, the weeds those grow with the transgenic plants in the field cannot metabolize the exogenous ALA and as a result will perish. This application oriented research area still has many open questions.

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