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Regulation of ascorbic acid synthesis in plants

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Keywords: ascorbic acid, L-galactose pathway, regulation, VTC1, ERF98, CSN5B

Abbreviations: AsA, ascorbic acid; VTC1, GDP-mannose pyrophosphorylase; ROS, reactive oxygen species; MDHA, monodehydroascorbate; DHA, dehydroascorbate; AO, ascorbate oxidase; APX, AsA peroxidase; MDAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase; ERF, ethylene response factor

Ascorbic acid (AsA) is a major antioxidant and plays an important role in plant growth and development. There are two aspects to improve AsA content, including de novo synthesis and recycling from its oxidized form. However, the knowledge of regulatory mechanisms of AsA synthesis pathways and metabolism still remains limited. We have determined that AsA synthesis process was modulated on both transcriptional and post-transcriptional levels in *Arabidopsis*. GDP-mannose pyrophosphorylase (VTC1) is the initial AsA biosynthetic enzyme in L-galactose pathway, we previously showed that *Arabidopsis* ERF98 transcriptionally activates gene expression of *VTC1* to improve AsA content and respond salt stress; recent discovery of the interaction between photomorphogenic factor COP9 signalosome (CSN) subunit CSN5B and VTC1 indicates that CSN5B promotes VTC1 degradation in the dark, which keeps the change of AsA content from day to night. This mini-review integrates previous reports and recent evidence to better understand the regulatory mechanisms involved in AsA synthesis.

Ascorbic acid (AsA) is a ubiquitous low molecular weight antioxidant in eukaryotes. Plants provide the major source of dietary AsA for humans because they lack the last step AsA synthetic enzyme.¹ AsA has a number of physiological roles in plants, preventing plants from reactive oxygen species (ROS) damage, cofactor of many enzymes and electron donor in electron transport chain.^{2,3} Over past decades, much progress has been made toward understanding the synthesis pathways and metabolism of AsA. There are multiple proposed AsA synthesis pathways, such as L-galactose, uronic acid, L-gulose and myo-inositol pathways and the enzymes participated in these pathways.⁴ AsA in vivo could be oxidized to monodehydroascorbate (MDHA) and dehydroascorbate (DHA) through ascorbate oxidase (AO)⁵ and AsA peroxidase (APX),⁶ then the reduced form is regenerated by the monodehydroascorbate reductase (MDAR) and dehydroascorbate reductase (DHAR).⁷ Except for the redox state transformation, AsA also undergoes degradation enzymatically or non-enzymatically.⁸ The fully reduced state of AsA is necessary for the activity, therefore, either improving de novo synthesis or

promoting recycling is effective to increase AsA content in vivo.⁹ Nevertheless, the increase of AsA content through enhancement of reductase activity is confined to substrate levels. Therefore, intending research focused on AsA synthesis regulation will be more viable. Both developmental and environmental cues make effects on AsA content, however, the molecular mechanism of these process and knowledge of related regulators remain largely unclear. Recent reports including our studies have provided evidence that regulators on transcriptional and post-transcriptional levels participate in the control of AsA synthesis.

Contributions of Transcriptional Regulation on AsA Synthesis

In the past years, many reports showed that increased AsA content results in enhanced tolerance to various abiotic stresses,¹⁰⁻¹² nevertheless, when the environmental stresses come, the plants adjust themselves to survival through a cascade of responses including activation or inhibition of gene expressions on transcriptional level. So far, there are two reported regulators involved in AsA synthesis in response to stresses. AMR1 was reported as a negative regulator of L-galactose pathway in *Arabidopsis*. The expression of all enzyme genes in the pathway decreased in *AMR1* overexpression transgenic lines and increased in *amr1* mutants. Respectively, *amr1* mutant showed higher accumulation of AsA levels and more tolerant to oxidative stress.¹³ Moreover, *AMR1* transcripts decreased with increased light intensity, resulting in the inhibition of *AMR1* expression, subsequently results in promoted AsA synthesis and higher AsA levels, which will be benefit to plants to eliminate accumulated ROS generated by high light.¹³ Conversely, a positive regulator ERF98, a member of ethylene response factor (ERF) family, which is induced by ethylene, salt and H₂O₂, transcriptionally activates AsA synthesis. This regulation enhances tolerance to salt stress through direct activation of AsA synthesis enzyme gene expression in *Arabidopsis*.¹⁴ Moreover, ERF98 transcriptionally regulates the expression of other enzyme genes in the L-galactose pathway. Thus it would be possible that ERF98 has more effects on AsA synthesis than we observed. In addition, AsA pool size is related to photosynthesis mainly due to its antioxidant role in photoprotection.¹⁵ Light-induced AsA synthesis is modulated on transcriptional level, the expression of AsA synthetic enzyme genes is light-controlled, high light and

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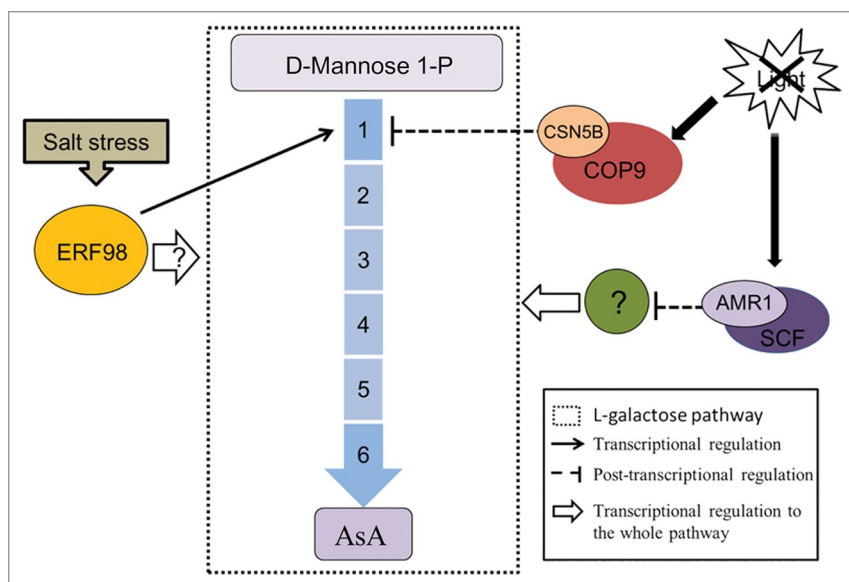


Figure 1. Overview of the reported regulators on L-galactose pathway of AsA synthesis. The broken line box shows L-galactose pathway. Enzymes: (1) GDP-Man pyrophosphorylase (VTC1); (2) GDP-Man-3',5'-epimerase (GME); (3) GDP-L-Gal phosphorylase (VTC2/VTC5); (4) L-Gal 1-phosphate phosphatase (GPP); (5) L-Gal dehydrogenase (GalDH); (6) L-Gall dehydrogenase (GallDH). Salt stress triggers *ERF98* expression and subsequently *VTC1* higher expression on transcriptional level, finally improving AsA content to respond stress, but the modulation of *ERF98* on other enzymes needs further research. Light inhibits *AMR1* expression, which further deactivates the expression of the synthesis enzyme genes mediated by an unknown protein. When the light goes out, *CSN5B* carry out function to inhibit AsA synthesis on post-transcriptional level through interaction with *VTC1* directly to promote its degradation.

continuous light upregulate transcripts of some enzyme genes^{16,17} and light responsive cis-elements in these genes were found in rice,¹⁸ however, the transcription factors involved in this process remains unknown. Further identification of transcription factors will provide us with more information about the mechanism of AsA synthesis on transcriptional level.

Proteolysis Modulation in AsA Synthesis on Post-Transcriptional Level

Ubiquitin-proteasome degradation has been involved in the plant development, phytohormone signaling and responses to abiotic stresses.¹⁹ Proteolysis is also involved in the regulation of AsA synthesis. *AMR1* might function as a component of an SCF ubiquitin ligase complex due to its typical F-box motif, to degrade its target protein that positively regulates AsA synthesis.¹³ Though the molecular mechanism of *AMR1* functions in AsA synthesis is still unclear, this finding indicates that proteolysis is possibly involved in the regulation of AsA synthesis.

It is well known that plants have circadian rhythms to respond environmental changes, photosynthesis is typically rhythmic and results in a series of periodical physiological processes, such as AsA synthesis. AsA content in plant is rhythmic over a 24 h period, AsA levels increase toward the end of the light period and decrease along with the dark period.²⁰ As human taking vitamins, plants make AsA synthesis themselves as antioxidant in the light, while AsA levels decrease when the

light goes out. Light induces the expression of AsA biosynthesis genes at transcriptional level, but it is unclear how the decrease of AsA levels in the dark is modulated. It is significant for the plant to use its limited metabolic resources most efficiently and protein degradation limiting the responses is a good way to keep metabolic cost. Similarly, proteolysis has a role in inhibition of AsA synthesis in the dark.²¹ Our most recent breakthrough finds that *VTC1* degradation results in decreased AsA levels in dark, photomorphogenic complex *CSN* inhibits AsA synthesis in darkness via direct interaction between its subunit *CSN5B* and *VTC1*.²² *CSN* functions in the ubiquitin-proteasome pathway through regulating the activity of E3 ligase.²³ Interestingly, we examined *VTC1* could be ubiquitinated and *CSN5B* promotes *VTC1* degradation via 26S proteasome pathway in the dark, loss function of *CSN5B* results in increased AsA content and enhanced tolerance to salt and oxidative stresses. Therefore, *CSN* plays a negative role in regulation AsA content on post-transcriptional level in darkness. Nevertheless, there are still some supplemental questions, the *CSN* functions exhibit in nucleus and *VTC1* is cytosol-localized, the translocation of *VTC1* is still unknown.²⁴ For the target protein degraded

through 26S proteasome pathway generally needs a special E3 ligase assistance and other components, the identification of these factors involved in *VTC1* degradation is under investigation in our laboratory.

Concluding Remarks and Perspective

L-galactose pathway has been recognized as the main AsA synthesis pathway in plants,²⁵ studies focused on the modulation of this pathway will contribute significant instruction to metabolism engineering. Based on our and previous studies, we propose a summary model to overview the current progresses on regulation of AsA L-galactose synthesis pathway in plants (Fig. 1). On the transcriptional level, *AMR1* negatively regulates transcript levels of L-galactose pathway genes, while light deactivates the expression of *AMR1*. In addition, the expression of *ERF98* is induced under salt stress and then activates *VTC1* expression to improve AsA content, resulting in increased tolerance to salt stress. On the post-transcriptional level, *CSN5B* promotes *VTC1* degradation under darkness in order to decrease AsA content, which is helpful to keep the balance of redox state in vivo. It is definitely changed that AsA content varies environmental conditions, these discoveries show that plants adjust the AsA synthesis to respond salt stress and light/dark cycles. More functions of these regulators and additional factors involved in the modulation of AsA synthesis will be identified in coming years.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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