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Ting Dong & Inhwan Hwang

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Contribution of ABA UDP-glucosyltransferases in coordination of ABA biosynthesis and catabolism for ABA homeostasis

Ting Dong and Inhwan Hwang*

Division of Integrative Biosciences and Biotechnology; Pohang University of Science and Technology; Pohang, Korea

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The phytohormone abscisic acid (ABA) plays a crucial role in numerous aspects of plant growth and environmental stress responses. Endogenous ABA levels are regulated by a balance between its biosynthetic and catabolic activities. This balance may occur at multiple levels and includes the expression of genes involved in these processes. ABA UDP-glucosyltransferase (UGT), the major player in the ABA conjugation pathway, has been shown to have a marginal effect on free ABA levels. However, recent studies provide new insight into the importance of the ABA conjugation pathway in contributing to the control of ABA homeostasis. Gain-of-function and loss-of-function mutant analyses have revealed that UGT71B6, an ABA UGT, and its 2 closely related homologs, UGT71B7 and UGT71B8, play a crucial role in ABA homeostasis and in adaptation to various abiotic stresses.

Introduction

The phytohormone abscisic acid (ABA) plays crucial roles in plant growth and development, and stress responses.^{1,2} Cellular ABA levels fluctuate constantly to adjust plants to changing environmental and physiological conditions.^{3,4} The level of the hormone is under the control of complex regulatory processes involving biosynthesis, catabolism, and transport.⁵⁻⁷

Since the discovery of ABA in the late 1960s, the ABA de novo synthesis pathway was extensively studied by isolating various biosynthetic mutants at every step in the biosynthesis pathway in *Arabidopsis*, maize, and tomato.^{8,9} All of the steps in the de novo ABA biosynthetic pathway occur in plastids, with the exception of the last 2 steps that occur in the cytosol.¹⁰ Another pathway for ABA synthesis is the simple one-step hydrolysis of glucose-conjugated ABA (ABA-GE) to ABA by 2 β -glucosidases, AtBG1 and AtBG2, which localize to the ER and vacuole, respectively.^{11,12}

In addition to ABA biosynthesis, catabolism is a major process for controlling cellular ABA levels. ABA catabolism is composed of 2 pathways, hydroxylation and glucose conjugation. Hydroxylation is performed by 4 members of the cytochrome P450 family, CYP707A1 to CYP707A4, which hydroxylate ABA at the 8' position to produce unstable 8'-hydroxyl ABA.⁶ In the conjugation pathway, ABA is conjugated with glucose by ABA UDP-glucosyltransferase (UGT) to produce ABA-GE, which is a storage or transport form of ABA and accumulates in the vacuole and apoplast.¹³ Although extensive work has been conducted on the hydroxylation pathway, little is known about the conjugation pathway. In particular, despite the fact that the conjugation pathway can inactivate ABA and thereby lower the cellular ABA level, its contribution in the homeostasis regulation of cellular ABA levels has been less clear.

In this review, we focus on the recent findings which improve our understanding of the role of UGT71B6 and its 2 homologs in ABA homeostasis.

The molecular function of UGT71B6 and its 2 homologs in ABA homeostasis

UGT71B6 has ABA-glucosyltransferase activity that can conjugate ABA with glucose to produce ABA-GE.¹⁴ However, its involvement in regulation of the cellular ABA level has not been fully understood. The loss-of-function mutant of *UGT71B6* does not show any noticeable phenotype, and *UGT71B6* overexpression has a marginal effect on reducing ABA content.¹⁴ In *Arabidopsis*, UGTs represent a superfamily containing more than 100 homologs.¹⁵ Thus, it is possible that multiple genes encoding UGTs glucosylate ABA, and mutation in one of these genes does not produce any phenotype because of functional redundancy. Consistent with this idea is that the amino acid sequence alignment of members in UGT sub-group E reveals 2 additional homologs (UGT71B7 and UGT71B8) that show more than 90% amino acid sequence similarity to UGT71B6. Indeed, UGT71B6 and its 2 closely related homologs, UGT71B7 and UGT71B8, are able to modulate the cellular ABA levels.¹⁶ There are also several lines of supporting evidence. First, ectopic expression of 3 UGTs (*UGT71B6*, *UGT71B7*, and *UGT71B8*) in protoplasts inhibited the ABA-induced expression of the firefly luciferase reporter gene (*LUC*) driven by ABA-responsive *RD29A* promoter, *RD29A_p*. Second, *UGT* RNAi transgenic plants which had lower levels of the *UGT* transcripts

*Correspondence to: Inhwan Hwang; Email: ihhwang@postech.ac.kr
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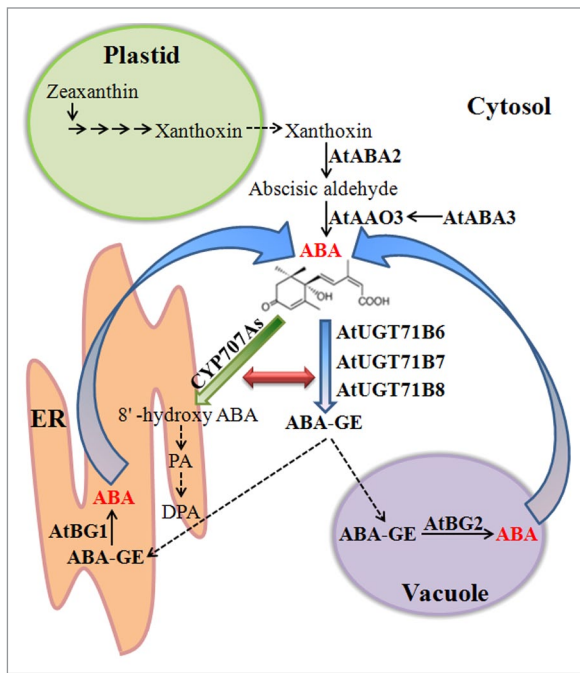


Figure 1. Coordination of ABA biosynthesis and catabolism in ABA homeostasis in *Arabidopsis*. The steps of ABA de novo biosynthesis from zeaxanthin are shown. Hydrolysis of ABA-GE is shown with the corresponding enzymes. In the catabolic pathways, ABA is inactivated by hydroxylation or glucose conjugation. Postulated pathways are shown in broken lines, and confirmed pathways are shown in solid lines. Arrows represent the activity of each enzyme. ER, endoplasmic reticulum; ABA-GE, ABA glucosyl ester; PA, phaseic acid; DPA, dihydrophaseic acid.

displayed multiple growth defective phenotypes, such as smaller rosette leaves, shorter roots and pale green leaves and also a 2-fold increase in the cellular ABA levels. Moreover, *UGT* RNAi transgenic plants showed hypersensitivity to exogenous ABA during germination and post-germination growth, and displayed enhanced resistance to osmotic and dehydration stresses. Thus, suppression of 3 *UGTs* leads to a defect in ABA-mediated responses to abiotic stresses and disturbs ABA homeostasis.

The glucose-conjugated form of ABA is biologically inactive.¹⁷ Therefore, it is reasonable that overexpression of *UGT71B6* that can glucosylate ABA to ABA-GE causes typical ABA-deficient phenotypes. However, *UGT71B6*-overexpressing plants produced essentially no observable ABA-deficient phenotype. Recent work shows that in addition to the de novo ABA biosynthesis, ABA can also be produced by 2 β -glucosidases, AtBG1 and AtBG2, through the hydrolysis of ABA-GE, the product of ABA glucosyltransferase.^{11,12} When *UGT71B6* was introduced into the *atbg1* mutant plants, *UGT71B6:GFP/atbg1* plants showed more significant ABA-deficient phenotypes than *UGT71B6:GFP*-overexpressing transgenic plants or *atbg1* mutant plants, suggesting that in *UGT*-overexpressing plants the *UGT71B6*-mediated reduction of ABA levels was compensated by ABA generated through AtBG1- and/or AtBG2-mediated ABA-GE hydrolysis, which results in no change in overall ABA levels. As a long-distance transport form of stress hormone,¹⁸ ABA-GE also provides a source of ABA via subsequent hydrolysis, indicating that a close connection exists between the

conjugation of glucose to ABA by *UGT71B6* and the hydrolysis of ABA-GE to ABA by AtBG1 and/or AtBG2. Therefore, *UGTs*, AtBG1/2, and long distance transport together play crucial roles in the homeostasis of cellular ABA levels (Fig. 1).

Coordination of biosynthesis and catabolism to maintain ABA homeostasis

Hydroxylation and conjugation are 2 ABA catabolic pathways. However, these 2 pathways may differ in their consequences for plant physiology. Hydroxylation of ABA by *CYP707As* produces 8'-hydroxyl ABA, which is then converted to phaseic acid by spontaneous isomerization, leading to an irreversible degradation of ABA. In contrast, conjugation of ABA with glucose produces ABA-GE, which can be converted back to ABA by the hydrolytic activity of AtBG1 and AtBG2. Thus, the conjugation pathway can be considered as part of the rapid ABA biosynthetic pathway through the activity of AtBG1 and AtBG2. This may underlie the difference in the phenotype between transgenic plants overexpressing *UGT71B6* and *CYP707As*. Whereas *UGT71B6* overexpression had a marginal effect on reducing ABA levels; overexpression of *CYP707A3* effectively reduced the endogenous ABA levels and resulted in a remarkable ABA-deficient phenotype.¹⁹ Suppression of *UGTs* induced the expression of *CYP707A1* to *CYP707A4*; in parallel, overexpression of *UGT71B6* suppressed *CYP707As*. These results suggest that the catabolic pathways are finely coordinated by a certain regulatory circuit at the transcription level, and the loss of one pathway is compensated for by the transcription change of genes in other pathways (Fig. 1). A similar phenomenon was observed in the *cyp707a1 cyp707a3* double mutants, which accumulated higher levels of ABA-GE than WT plants.²⁰ Therefore, it is likely that active regulatory mechanisms are involved in coordinating the activities of the catabolic pathways for ABA homeostasis. Similarly, the multiple biosynthetic pathways are closely coordinated by a regulatory circuit. The loss of ABA production in the *atbg1* mutant plants was compensated by the overexpression of *NCED3* or *AtBG2*,¹² indicating that the multiple biosynthetic pathways are coordinated to determine the endogenous ABA levels in plant cells.

Finally, the next question would be how the biosynthetic and the catabolic pathways of ABA are coordinated to maintain homeostasis of cellular ABA levels. The biosynthetic and catabolic pathways are localized to multiple types of organelles. All steps in the de novo biosynthetic pathway take place in plastids, except for the last 2 steps that occur in the cytosol. By contrast, the hydrolysis of ABA-GE to ABA by AtBG1 and AtBG2 takes places in the ER and vacuole, respectively. Regarding the catabolic pathways, the 3 *UGTs* localize to the cytosol, whereas *CYP707As* localize in the ER membrane.²¹ Therefore, the localization of all key enzymes involved in ABA metabolism in multiple organelles raises an intriguing possibility of a complicated regulatory network(s) involving multiple organelles to maintain the cellular ABA levels. For example, in plants, ABA-GE is stored in the vacuole and apoplasmic space, whereas AtBG1 localizes to the ER. In response to abiotic stresses, ABA-GE should be imported in to the ER to rapidly meet the plant requirement for ABA. This pathway may be under a fine and precise control, as AtBG1 and its substrate are stored separately in the cell, and only brought together when

plants need to increase ABA levels. Moreover, compared with the lengthy and complex *de novo* biosynthetic pathway, hydrolysis of ABA-GE to ABA is a one-step process. Thus, this pathway seems to be another elegant mechanism for plants to increase ABA levels robustly in a short time upon stresses.

The presence of active ABA pools in 3 different compartments raises the possibility that the local concentration of ABA, rather than the overall cellular ABA content, is critical for initiating ABA-mediated signaling in particular processes. Furthermore, ABA produced in different subcellular compartments may require different ABA receptors, which coincides with the fact that plant cells contain multiple types of ABA receptors in different subcellular compartments, including the cytosol, chloroplasts and plasma membrane.²²⁻²⁴ According to this hypothesis, it is possible that ABA receptors that perceive different pools of ABA can induce different physiological responses. Currently, the most prevailing model for ABA perception and signal transduction is based on the cytosol and nucleus-localized PYR/RCAR-type ABA receptors, which interact with protein phosphatase 2Cs and SNF1-related protein kinase 2 (SnRK2). However, exactly how ABA produced in the ER and vacuole initiates ABA-mediated signaling in plant cells is still unknown.

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Conclusions and Perspectives

Based on the fact that processes involved in ABA metabolism are compartmentalized in multiple types of organelles, we propose that a complex and flexible regulatory mechanism is required to coordinate these multiple biosynthetic and catabolic pathways as well as transport to achieve the desired cellular ABA levels. Moreover, this compartmentalized ABA metabolism should also be incorporated into the ABA-mediated signaling circuit so as to adjust the plants in response to changing physiological and environmental conditions. A direction of future research would be to define the exact role of active ABA pools produced in different compartments and to determine how ABA produced in different compartments leads to ABA signaling and physiological responses.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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