

高等植物脱落酸生物合成途径及其酶调控^①

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摘要 脱落酸(ABA)生物合成一般有两条途径: C₁₅直接途径和C₄₀间接途径, 前者经C₁₅法呢焦磷酸(FPP)直接形成ABA; 后者经由类胡萝卜素的氧化裂解间接形成ABA, 是高等植物ABA生物合成的主要途径。9-顺式环氧类胡萝卜素氧化裂解为黄质醛是植物ABA生物合成的关键步骤, 然后黄质醛被氧化形成一种酮, 该过程需NAD为辅因子, 酮再转变形成ABA-醛, ABA-醛氧化最终形成ABA。在该途径中, 玉米黄质环氧化酶(ZEP)、9-顺式环氧类胡萝卜素双加氧酶(NCED)和醛氧化酶(AO)可能起重要作用。

关键词 脱落酸, 生物合成, NCED基因, AO基因, 酶调控

Pathways and Related Enzymes of ABA Biosynthesis in Higher Plants

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Abstract Two pathways have been proposed for the biosynthesis of ABA: the 'direct pathway' and 'indirect pathway'. In the direct pathway, which operates in some fungi, ABA is derived from farnesyl diphosphate; in indirect pathway, ABA is produced from the oxidative cleavage of carotenoids. The first committed step for ABA biosynthesis in plants is the oxidative cleavage of a 9-*cis*-epoxycarotenoid(C₄₀) to produce xanthoxin(C₁₅) and a C₂₅ by-product(Fig.2). Xanthoxin is then oxidized to a ketone by an NAD-requiring enzyme. Consequently, there is a nonenzymatic desaturation of the 2'-3' bond and opening of the epoxide ring to form abscisic aldehyde. In the final step of the pathway, abscisic aldehyde is oxidized to ABA. These endogenous enzymes such as zeaxanthin epoxidase(ZEP), 9-*cis*-epoxycarotenoid dioxygenase(NCED) and aldehyde oxidase(AO) may play key roles in the regulation of ABA biosynthesis in higher plants.

Key words Absciscic acid, Biosynthesis, NCED gene, AO gene, Enzymatic regulation

Abscisic acid (ABA) is a plant hormone involved in various physiological processes of plants. The biosynthetic pathway of ABA remains a contentious issue to date. Nevertheless, the stress-

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D-xylulose-5-phosphate(DXP) from pyruvate and glyceraldehyde-3-phosphate catalyzed by DXP synthase(DXS) (Fig.1).IPP is converted to a C₂₀ product, geranylgeranyl pyrophosphate(GGPP). Conversion of GGPP to a C₄₀ carotenoid phytoene, catalyzed by phytoene synthase(PSY), is the first committed and rate-limiting step in carotenoid synthesis. Subsequently, phytoene is converted to ζ -carotene, lycopene, β -carotene and then to a xanthophyll, zeaxanthin. Phytoene desaturase(PDS) catalyzes the conversion of phytoene to ζ -carotene and is also one of the enzymes dedicated to carotenoid synthesis (Hirschberg, 2001).

2 Epoxy-carotenoid Formation and its Cleavage in Plastids

The first step of the ABA-specific synthetic pathway is the conversion of zeaxanthin to all-*trans*-violaxanthin by a two-step epoxidation (Fig. 1). The enzyme that catalyzes the reaction is zeaxanthin epoxidase (ZEP) — the first enzyme to be identified as an ABA biosynthetic enzyme(Marin *et al.*, 1996). ABA-deficient mutants such as *Arabidopsis thaliana aba1* and tobacco (*Nicotiana plumbaginifolia aba2*) are known to be impaired in ZEP (Duckham *et al.*, 1991; Marin *et al.*, 1996). Because the level of epoxycarotenoids in green leaves is high relative to the amount of ABA synthesized, it is considered unlikely that ZEP has a regulatory role in these tissues. The expression of *ZEP* transcripts in green tissue did not increase in wild tobacco(Audran *et al.*, 1998), tomato(Thompson *et al.*, 2000a) or cowpea (Iuchi *et al.*, 2000) that were subjected to osmotic stress. In etiolated tissues, the concentration of carotenoids is significantly lower and the increased expression of *ZEP* mRNA did correlate with elevated ABA biosynthesis in roots and seeds(Audran *et al.*, 1998; Borel *et al.*, 2001). It was reported that the expression of *Arabidopsis AtZEP*(encoding epoxidase) mRNA increased in response to osmotic stress or ABA treatment in both roots and shoots(Xiong *et al.*, 2002). The osmotic induction of *AtZEP* transcript was impaired in ABA-deficient mutants and in the ABA-insensitive mutant, *abi1*. Several additional genes necessary for the later steps in ABA biosynthesis were also found to be induced by stress and ABA(Xiong *et al.*, 2001; 2002). It was suggested that ABA biosynthesis might be subjected to positive feedback regulation.

The enzyme(s) involved in the conversion of all-*trans*-violaxanthin to 9-*cis*-violaxanthin or 9-*cis*-neoxanthin has not been isolated. Recently, a gene encoding the enzyme with the ability to convert all-*trans*-violaxanthin to all-*trans*-neoxanthin was isolated from tomato(*Lycopersicon esculentum*)(Bouvier *et al.*, 2000) and potato(*Solanum tuberosum*)(Al-Babili *et al.*, 2000) as a homolog of lycopene β -cyclase. However, the gene from tomato is identical to a novel type of lycopene β -cyclase as revealed by mutant analysis, indicating that the gene does not function in converting all-*trans*-violaxanthin to all-*trans*-neoxanthin *in vivo*(Hirschberg, 2001). The xanthophylls cycle involves the light-dependent and reversible conversion of violaxanthin to zeaxanthin (Fig. 2) (Schwartz *et al.*, 2003) and is thought to be essential for the protection of the photosynthetic apparatus from photooxidative damage. Under low or limiting light, zeaxanthin is converted into antheraxanthin and violaxanthin by ZEP. In excessive light, de-epoxidation of violaxanthin into antheraxanthin and zeaxanthin is catalyzed by violaxanthin de-epoxidase(VDE). Whether the de-epoxidation leads to a reduction of violaxanthin pools in photo-

synthetic tissues, such that the availability of ABA precursors is limiting, remains to be investigated (Liotenberg *et al.*, 1999).

The committed step in ABA biosynthesis is the oxidative cleavage of xanthophylls, 9-*cis*-violaxanthin or 9'-*cis*-neoxanthin. Confirmation of the regulatory nature of the cleavage reaction was provided by the characterization of a viviparous mutant of maize, *vp14*, that exhibited a defect in ABA biosynthesis (Tan *et al.*, 1997). The *Vp14* gene was cloned and the derived protein sequence of *Vp14* is related to lignostilbene dioxygenase, a bacterial enzyme that catalyzes a double-bond cleavage reaction in ABA biosynthesis (Kamoda and Saburi, 1993). Recombinant VP14 protein catalyzes the **cleavage of 9-*cis*-xanthophylls** to form xanthoxin and a C₂₅ by-product (Fig. 2) in a reaction that requires oxygen, ferrous iron, ascorbate and a detergent for activity *in vitro* (Schwartz *et al.*, 1997b). The characteristics of the cleavage reaction in its substrate specificity and the site of cleavage (11 and 12 position) were consistent with predictions. A 9-*cis* double bond in the carotenoid precursor was necessary for activity. The product of this cleavage reaction is *cis*-xanthoxin, which is readily converted to ABA [*cis*(+)-S-ABA] by plants. Cleavage of an all *trans*-isomer would result in *trans*-xanthoxin, which is converted to biologically inactive *trans*-ABA. Northern analysis of maize leaves showed that *Vp14* expression is induced during wilting in parallel with the increase in ABA levels (Tan *et al.*, 1997). In the time since the cloning of *Vp14*, a number of genes with sequence similarity to *Vp14* have been reported. The nomenclature now used for designating genes that have homology to *Vp14* is 9-*cis*-epoxycarotenoid dioxygenase genes (*NCED*). *NCED* cDNAs have been cloned from several species, including maize, tomato (Burbidge *et al.*, 1999), bean (Qin and Zeevaart, 1999), cowpea (Iuchi *et al.*, 2000), avocado (Chernys and Zeevaart, 2000) and *Arabidopsis* (Neill *et al.*, 1998; Iuchi *et al.*, 2001). In all species examined, *NCED* comprises a gene family of several related genes. Although the *NCED*s display significant substrate plasticity *in vitro*, circumstantial evidence favors neoxanthin as the primary precursor of ABA. Neoxanthin exists almost entirely as a 9-*cis*-isomer, whereas only a small proportion of the violaxanthin is present as a 9-*cis*-isomer (Strand *et al.*, 2000). Definitive evidence of the endogenous substrate would require identification of the C₂₅ by-product in plant. Previous efforts to identify the C₂₅ compounds in vegetative tissue have been unsuccessful.

It is suggested that there is a family of differentially regulated *NCED* genes that contributes to environmental and developmental control of ABA biosynthesis in plants. A detailed study of *NCED* expression during water stress using *Phaseolus vulgaris* (Qin and Zeevaart, 1999) and *Nicotiana glauca* (Qin and Zeevaart, 2002) showed a clear correlation between *NCED* (*PvNCED1*) mRNA expression, *NCED* protein levels, and ABA levels in dehydrated leaves and roots, indicating the regulatory role of *NCED* in ABA biosynthesis during water stress. The induction of *NCED* (*LeNCED1*/*NOTABILIS*) expression in dehydrated leaves and roots has also been observed in tomato (Thompson *et al.*, 2000a). Furthermore, the induction of *NCED* expression after water stress in leaves has been reported in cowpea (Iuchi *et al.*, 2000) and avocado (Chernys and Zeevaart, 2000), although the expression of this gene in roots has not always been determined. Over-expression of *LeNCED1* in tomato plants results in the overproduction of ABA in leaves (Thompson *et al.*, 2000b). These results strongly

support the idea that NCED is a key regulatory enzyme in ABA biosynthesis, at least in leaves. Multi-gene families often encode enzymes with related functions, but in cases where the function is the same, differential regulation ensures that distinct genes are activated in response to different environmental and other stimuli. Ultimately, the distinction between which gene is induced in response to a given stimulus lies in the promoter region.

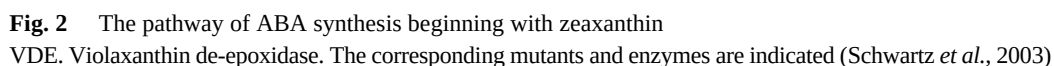
3 From Xanthoxin to ABA

After the cleavage of 9-*cis*-epoxycarotenoids, xanthoxin is converted to ABA in the cytosol. Three possible pathways have been proposed via abscisic aldehyde(ABAld), xanthoxic acid or abscisic alcohol (Fig. 1 and Fig. 2). The first pathway via ABAld is the most probable to function in plants, as shown by the characterization of *Arabidopsis* AO, which catalyzes the oxidation of ABAld. The *aba2* mutant in *Arabidopsis* was impaired in the conversion of xanthoxin to abscisic aldehyde(Schwartz *et al.*, 1997a). The gene corresponding to *aba2* has recently been cloned and the gene product was found to be similar to short-chain dehydrogenases/reductases(SDRs)(Cheng *et al.*, 2002; Gonzalez-Guzman *et al.*, 2002). As expected, the ABA2 protein was able to catalyze the conversion of xanthoxin to abscisic aldehyde utilizing NAD as a cofactor. It has not yet been reported whether the over-expression of ABA2 would have any effect on ABA level. All these studies support the pathway whereby xanthoxin is first converted to abscisic aldehyde by an enzyme of the type encoded by the *Arabidopsis* ABA2 gene, and then an AO isoform(s) catalyzes the oxidation of abscisic aldehyde to produce ABA. The second pathway via xanthoxic acid might also work. In this pathway, xanthoxin is first oxidized to xanthoxic acid by AO and then xanthoxic acid is converted to ABA, presumably by SDR. Pathway 3 via abscisic alcohol appears to be a shunt pathway but is important in mutants impaired in the oxidization of ABAld (Seo and Koshiba, 2002).

4 Further Prospects

Significant progress has been achieved in recent years with the cloning of genes encoding for the enzymes involved in the ABA biosynthetic pathway. The biochemical aspects of ABA synthesis, such as the intermediates in the pathway and the sequence of reactions, have become well established. The genes that encode most of the enzymes in the pathway have now been cloned (Fig.1 and Fig. 2). This will contribute to a better understanding of the regulation of ABA levels and should make their manipulation possible in transgenic plants.

However, there are several steps in ABA biosynthesis preceding the cleavage reaction that are not well characterized. The epoxycarotenoid precursor must have a 9-*cis* configuration to be cleaved by an NCED and for subsequent conversion to ABA [*cis*-(+)-S-ABA]. The formation of these 9-*cis* isomers has not yet been established. An enzyme that catalyzes a similar reaction, the *cis/trans* isomerization of prolycopene to lycopene, has recently been identified (Isaacson *et al.*, 2002; Park *et al.*, 2002). This isomerase appears to be necessary only in non-photosynthetic tissue. In light-grown tissue, photo-isomerization of lycopene is sufficient. It has not been established whether the 9-*cis*



The mechanisms of drought response have been investigated most extensively in a model plant, *Arabidopsis*. On the contrary, the response of crops to drought stress has not been extensively studied. Our program is focused on improving the drought tolerance of peanut by gene manipulation of *NCED* and *AO* genes involved in ABA biosynthesis, and exploring the mechanisms of drought response in crops. Molecular analysis of drought tolerance of peanut may be useful to elucidate the mechanism of drought response, improving drought tolerance of crops by using transgenic plant technique.

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