Complex regulation of ABA biosynthesis in plants

Mitsunori Seo and Tomokazu Koshiba

Abscisic acid (ABA) is a plant hormone that plays important roles during many phases of the plant life cycle, including seed development and dormancy, and in plant responses to various environmental stresses. Because many of these physiological processes are correlated with endogenous ABA levels, the regulation of ABA biosynthesis is a key element facilitating the elucidation of these physiological characteristics. Recent studies on the identification of genes encoding enzymes involved in ABA biosynthesis have revealed details of the main ABA biosynthetic pathway. At the same time, the presence of gene families and their respective organ-specific expression are indicative of the complex mechanisms governing the regulation of ABA biosynthesis in response to plant organ and/or environmental conditions. There have been recent advances in the study of ABA biosynthesis and new insights into the regulation of ABA biosynthesis in relation to physiological phenomena.

Abscisic acid (ABA) is a plant hormone involved in various physiological processes of plants [1]. In developing seeds, ABA is necessary for inducing the synthesis of reserve proteins and lipids as well as for the onset of seed dormancy and the acquisition of desiccation tolerance. Endogenous ABA levels peak during seed maturation and the onset of primary dormancy. ABA also plays important roles in vegetative development in response to various environmental stresses such as drought and high-salinity conditions. Moreover, ABA is known to control the expression of many genes related to these phenomena. Thus, an understanding of the mechanisms underlying the regulation of plant ABA levels is a crucial part of determining ABA action in plant growth and physiological responses, which is correlated to endogenous ABA levels. Because the rates of synthesis and the rates of breakdown determine ABA levels in situ, both mechanisms should be studied in detail, although the rate of breakdown remains largely unknown [2]. The recent glut of molecular, genetic and biochemical studies on ABA biosynthesis have allowed remarkable breakthroughs towards...
**Biosynthetic pathways and related enzymes**

**Early steps: synthesis of the carotenoid precursor in plastids**

Two possible routes have been suggested for ABA biosynthesis, one direct and one indirect, in which ABA is derived from the C₁₅ compound farnesyl pyrophosphate and a C₄₀ carotenoid, respectively [1,3]. Recent studies, including the characterization of ABA-deficient mutants and the isolation of the relevant mutated genes, have shown that the indirect pathway is the main pathway (Fig. 1). Carotenoids, like other isoprenoids, are synthesized from the C₅ precursor, isopentenyl pyrophosphate (IPP). IPP is synthesized from mevalonic acid in the cytosol, whereas in plastids where carotenoid synthesis takes place, it is produced via 1-deoxy-D-xylulose-5-phosphate (DXP) from pyruvate and glyceraldehyde-3-phosphate. DXP synthase (DXS) is the enzyme that catalyzes the first step of the non-mevalonic acid IPP synthesis pathway (reviewed in [7,8]). 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. The biosynthetic pathway of carotenoids is well defined (reviewed in [9,10]).

Understanding the regulatory mechanisms associated with each gene or enzyme [2–6]. This review focuses on more recent studies of ABA biosynthetic genes and enzymes, and discusses the complex regulatory systems of ABA synthesis and their respective physiological aspects.

---

**Fig. 1.** The ABA biosynthetic pathway in plants. (a) Carotenoid precursor synthesis in the early steps of ABA biosynthesis. ABA is synthesized from C₄₀ carotenoids (phytoene, ζ-carotene, lycopene and β-carotene). Carotenoids are synthesized from a C₁₅ compound, IPP. In plastids, IPP is synthesized via DXP from glyceraldehyde-3-phosphate and pyruvate. (b) Formation of epoxycarotenoid and its cleavage in plastid. The first step of this specific ABA synthetic pathway is the two-step epoxidation of zeaxanthin to form all-trans-violaxanthin catalyzed by ZEP. NCED catalyzes the oxidative cleavage of a 9-cis isomer of epoxycarotenoid such as 9-cis-violaxanthin and violaxanthin. Metabolism (c) catalyzing the conversion of all-trans-violaxanthin to 9-cis-violaxanthin or 9′-cis-neoxanthin has not been identified. (c) Reactions in the cytosol for the formation of ABA. Three possible pathways are proposed. The first pathway via ABAld (1) is the most probable to function in plants, as shown by the characterization of Arabidopsis AO, which catalyzes the oxidation of ABAld. A member of SDR such as ABA2 in Arabidopsis is proposed. The second pathway via xanthoxic acid (2) might also work. In this pathway, xanthoxic acid is first oxidized to xanthoxin by AO and then xanthoxic acid is converted to ABA, presumably by SDR. Pathway (3) via acs2 alcohol oxidase appears to be a shunt pathway but is important in mutants impaired in the oxidation of ABAld. Enzymes (red) and compound names (black) discussed in the text are shown in the figure. Abbreviations: ABA, abscisic acid; ABAld, abscisic aldehyde; AO, aldehyde oxidase; DXP, 1-deoxy-D-xylulose-5-phosphate; DXS, DXP synthase; GGPP, geranylgeranyl pyrophosphate; IPP, isopentenyl pyrophosphate; NCED, 9-cis-epoxy-carotenoid dioxygenase; PDS, phytoene desaturase; PSY, phytoene synthase; SDR, short-chain dehydrogenase/reductase; ZEP, zeaxanthin epoxidase.
Specific ABA biosynthetic pathway: xanthophyll formation and its cleavage in plastids

Mutants containing a defect in carotenoid precursor synthesis exhibit a pleiotropic phenotype in addition to impaired ABA biosynthesis. The first step of the ABA-specific synthetic pathway is the conversion of zeaxanthin to all-trans-violoxanthin 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 [11]. ABA-deficient mutants such as Arabidopsis thaliana aba1 and tobacco (Nicotiana plumbaginifolia) aba2 are known to be impaired in ZEP [11–13].

The enzyme(s) involved in the conversion of all-trans-violoxanthin to 9-cis-violoxanthin or 9′-cis-neoxanthin has not been isolated. Recently, a gene encoding the enzyme with the ability to convert all-trans-violoxanthin to all-trans-neoxanthin was isolated from tomato (Lycopersicon esculentum) [14] and potato (Solanum tuberosum) [15] as a homolog of capsanthin capsorubin synthase or lycopene β-cyclase. However, the gene from tomato is identical to a novel type of lycopene β-cyclase as revealed by mutant analysis [16], indicating that the gene does not function in converting all-trans-violoxanthin to all-trans-neoxanthin in vivo [10].

The next step, the oxidative cleavage of xanthophylls, 9-cis-violoxanthin and/or 9′-cis-neoxanthin to produce xanthoxin, is catalyzed by 9-cis-epoxycarotenoid dioxygenase (NCED) (Fig. 1). The gene encoding NCED was first identified during the characterization of the maize (Zea mays) viviparous14 (vp14) mutant [17]. The recombinant VP14 protein specifically cleaves 9-cis isomers of epoxy-xanthophylls such as 9-cis-violoxanthin and 9′-cis-neoxanthin [18]. Subsequently notabilis, a tomato ABA-deficient mutant, was also shown to be defective in the Vp14-related gene (LeNCED1) [19]. NCED cDNAs have been cloned from several species, including bean (Phaseolus vulgaris) [20], cowpea (Vigna unguiculata) [21], avocado (Persea americana) [22] and Arabidopsis [23,24]. In all the species examined, NCED comprises a gene family of several related genes.

Later steps in the cytosol: 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, xanthoxic acid or abscisic alcohol (Fig. 1). Typical mutants impaired in the later steps of ABA biosynthesis in the cytosol are Arabidopsis aba2 and aba3 [25], tobacco aba1 [26], and tomato flacca and sitiens [27]. The Arabidopsis aba2 mutant lacks the activity to synthesize ABA from xanthoxin, but can oxidize abscisic aldehyde to form ABA by in vitro enzyme preparations [25]. Recently, the ABA2 gene was isolated and shown to encode an enzyme related to a short-chain dehydrogenase/reductase (SDR) family, consistent with its proposed enzymatic reaction between xanthoxin and abscisic aldehyde [28]. Recombinant ABA2 protein has been shown to catalyze the conversion of xanthoxin to abscisic aldehyde in vitro (A. Endo et al., unpublished). Arabidopsis aba3, tobacco aba1 and tomato flacca lack the activity of aldehyde oxidase (AO) isoforms because of a defect in generating the sulfurylated form of the molybdenum cofactor (Moco), which is required for their activity [25,26,29,30]. However, tomato flacca retains considerable residual activity in roots, indicating organ-specific regulation of Moco sulfuration [31]. The defects in the mutants result in their incapacity to convert abscisic aldehyde to ABA. The Arabidopsis aba3 gene was cloned [32,33] and recombinant ABA3 protein was shown to have Moco sulfurase activity [33], supporting the biochemical characterization of the mutants. The sitiens tomato mutant is thought to contain a mutation in an AO gene specific for ABA biosynthesis [30], although the corresponding gene has not been identified [34]. Four members of the Arabidopsis aldehyde oxidase genes (AAO1-4) have been cloned, among them, AAO3, which encodes an AO isoform, A08, with a low $K_m$ value of 0.51 $\mu$M for abscisic aldehyde [35,36]. Furthermore, an Arabidopsis aao3 mutant with a defect in the AAO3 gene exhibits a wilty phenotype and ABA biosynthesis is impaired [37]. 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 of ABA synthesis via xanthoxic acid might also be operative [6,38]. In ripening avocado fruits, inhibition of AO activity by tungstate, a potent inhibitor of the molybdo-enzymes in plants, results in the accumulation of xanthoxin, suggesting that xanthoxin is a substrate of AO [39]. Some of the Arabidopsis AO isoforms can oxidize xanthoxin in activity gel staining after native gel electrophoresis (M. Seo and T. Koshiba, unpublished), suggesting that these isoforms might act to convert xanthoxin to xanthoxic acid. One might speculate on the possibility of the two ABA biosynthetic pathways operating in an organ and/or developmental-stage-dependent manner. Further analysis of the substrate specificity of the Arabidopsis ABA2-related enzyme and the AO isoforms for xanthoxic acid and xanthoxin, respectively, would aid in establishing the feasibility of the second pathway.

The third and last pathway, via abscisic alcohol, might be activated in some mutants. In flacca and sitiens tomato mutants, exogenously supplied abscisic acid is converted to abscisic alcohol, showing that abscisic acid is reduced to abscisic alcohol and then oxidized to ABA via a shunt pathway [40]. The shunt pathway appears to be a minor source of ABA in wild-type plants but might play a significant
role in mutants impaired in their capacity to oxidize abscisic aldehyde to ABA directly.

**Regulation during the early stages of ABA biosynthesis**

The contribution of carotenoid synthesis towards the regulation of ABA biosynthesis has not been extensively discussed. Some data indicate that the rate of carotenoid synthesis does not affect the rate of ABA biosynthesis [4] because the expression of *PDS* mRNA and its product in developing maize seeds does not correlate with endogenous ABA levels [41], and over-expression of the *PSY* gene (*PSY1*) in tomato does not result in increased ABA levels [42]. However, recent studies suggest a possible link between the early and later stages of ABA synthesis. When DXS is over- or under-expressed in *Arabidopsis*, expression levels of DXS change according to endogenous isoprenoid content in the form of chlorophylls, tocopherols or carotenoids, suggesting the possible role of DXS in the regulation of isoprenoids biosynthesis [43]. Surprisingly, endogenous ABA levels are also influenced by DXS expression. This suggests that early ABA synthesis steps, particularly the formation of DXP, might contribute, at least in part, towards the regulation of ABA biosynthesis.

**Regulation of ABA biosynthesis in relation to physiological processes**

*Plant responses to water stress*

One of the most striking aspects of ABA biosynthesis is the drastic increase in ABA levels during dehydration. The increase in the ABA content of root, xylem sap and leaves of drought-stressed plants has been widely reported [44]. It has been shown that the expression of tobacco (*ABA2/NpZEP*) and tomato (*LeZEP1*) *ZEP* genes in leaves is not correlated to endogenous ABA levels during water stress [45,46]. Expression of *ZEP* in leaves seems to be influenced by diurnal functions. However, the expression of *ZEP* in roots is induced by dehydration [45,46]. A tobacco *aba2* mutant complemented by *NpZEP* under the control of a constitutive promoter produces normal ABA levels under well watered conditions. The expression of *NpZEP* in the transgenic plants under water-stress conditions remains constant, and accumulation of ABA is not observed, indicating the importance of drought-induced *NpZEP* expression in controlling ABA synthesis in roots [47].

A detailed study of *NCED* expression during water stress using *Phaseolus vulgaris* 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 [20]. The induction of *NCED* (*LeNCED1*/*NOTABILIS*) expression in dehydrated leaves and roots has also been observed in tomato [46]. Furthermore, the induction of *NCED* expression after water stress in leaves has been reported in cowpea and avocado [21,22], although the expression of this gene in roots has not always been determined. Over-expression of *LeNCED1* in tomato plants results in the over-production of ABA in leaves [48]. These results strongly support the idea that NCED is a key regulatory enzyme in ABA biosynthesis, at least in leaves. It has been reported that the expression of an *Arabidopsis* *NCED* mRNA (*AtNCED3*) in whole plants is induced by dehydration and that the over-expression of *AtNCED3* results in the accumulation of ABA [24].

The last two reactions in the cytosol, from xanthoxin to ABA, had not previously been considered to be a regulatory step in ABA biosynthesis because the bulk enzymatic activity is constitutive and the rate of this activity does not change during water stress [49]. However, recent studies on an *Arabidopsis* *aa03* mutant indicate that *AAO3* plays an important role in plant dehydration tolerance; the *aa03* mutant exhibits a relatively normal phenotype in well watered conditions, but wilts readily after dehydration with no significant increase in ABA levels in the dehydrated leaves [37]. The expression of *AAO3* mRNA in the leaves of wild-type plants increases rapidly in water-stressed leaves [36]. However, the level of protein or its activity in leaf extracts does not significantly change during the stress. It is possible that post-transcriptional regulation is involved in controlling the activity of *AAO3*. Because AO requires Moco for activity, the availability of the cofactor would contribute towards the regulation of its activity levels. This is supported by the observation that the expression of *Arabidopsis* *ABA3*, encoding Moco sulfatase, is induced by dehydration treatment of shoots [32,33]. It is possible that these different regulatory systems modulating AO activity at the transcriptional and post-transcriptional levels are coordinated at tissue levels during water-stress responses, and might be responsible for the insignificant effects observed on bulk AO activity under these conditions. In contrast to the effect of dehydration on leaves observed in *Arabidopsis*, the expression of *AAO3* in roots is not induced by dehydration [36]. Regulation of *Arabidopsis* *ABA2* gene expression by dehydration is not observed, although the expression is up-regulated by sugar treatment (W-H. Cheng et al., unpublished).

*Seed maturation and dormancy*

ABA plays a crucial role in seed maturation, and in the establishment and maintenance of dormancy. ABA has been suggested to induce a dormant state during the late phase of seed maturation. The expression of *NpZEP* correlates well with endogenous ABA levels during seed development, which peaks during the middle of seed development [45]. Transgenic plants over-expressing *NpZEP* cDNA accumulate ABA in seeds, resulting in enhanced dormancy, suggesting a regulatory role for *ZEP* in seeds [50]. The expression of other genes involved in
ABA biosynthesis during seed development has not been examined in detail. In ripening avocado fruits, where endogenous ABA transiently accumulates, the expression of the two NCED mRNAs (PaNCED1 and PaNCED3) fluctuates in a similar way to that observed for endogenous ABA levels [22]. Over-expression of LeNCED1 in tomato results in increased seed dormancy [48]. These results indicate that NCED might also play an important role in ABA synthesis during seed development.

The relevance of ABA synthesis in imbibed seeds for maintaining seed dormancy has been reported recently. ABA levels increase when dormant but not non-dormant tobacco seeds imbibe; the increase in ABA content is blocked by fluridone, a carotenoid synthesis inhibitor [51]. High-temperature treatment inhibits germination of lettuce seeds [52]. The ABA content in lettuce seeds decreases rapidly during normal germination conditions, but imbibition at high temperature prevents significant changes in ABA levels. When the seeds are imbibed at high temperature in the presence of fluridone, ABA levels in the seeds decrease and hence the seeds exhibit enhanced ability to germinate due to weaker dormancy. These findings point to a specific regulatory system of ABA biosynthesis that maintains seed dormancy in imbibed seeds, although specific genes have yet to be assigned to this function.

Diurnal functions
Xanthophylls are involved in protecting the photosynthetic apparatus from photo-oxidative damage [53]. Under low or limiting light, ZEP converts zeaxanthin to violaxanthin through a two-step epoxidation. Violaxanthin is converted to zeaxanthin by the enzyme violaxanthin de-epoxidase under conditions of excessive light. In this case, the expression of ZEP is affected by a factor, light intensity, independent of ABA synthesis. In tobacco and tomato, ZEP expression fluctuates according to a diurnal rhythm during a time lapse consisting of light and dark periods [45,46]. The diurnal oscillation of ZEP expression in leaves of tobacco and tomato are probably related to its role in the xanthophyll cycle. Because leaves contain substantial amounts of xanthophylls, the fluctuation of ZEP expression and the quantities of each xanthophyll might not be limiting for ABA synthesis.

It is surprising that diurnal fluctuations of LeNCED1 expression have been observed in tomato leaves [46], because NCED is thought to be a key regulatory enzyme in leaves. The peak of NCED expression occurs at the end of the light period, whereas the expression of ZEP peaks in the middle of the light period, indicating the existence of different regulatory systems for each gene, which result in different diurnal rhythms. The influence of the diurnal oscillation of NCED on the rate of ABA synthesis needs to be examined further.

Sugar response
Interesting findings on novel ABA function have been reported from a study in which several ABA-deficient Arabidopsis mutants were isolated during the screening of sugar-response mutants [28,54–56]. The gin1, sis4 and isi4 mutants are allelic to aba2. The gin5 mutant is also likely to be an ABA-deficient mutant because the glucose-insensitive phenotype of the mutant is rescued by the application of ABA. Sugar signals are known to modulate many important processes in higher plants such as germination, seedling growth, leaf and root development, and senescence, in conjunction with the expression of the relevant respective genes [57,58]. ABA levels are increased by sugar treatment of Arabidopsis seedlings, and treatment with ABA increases sugar sensitivity, suggesting that regulation of ABA synthesis plays an important role in plant sugar responses [56]. The expression of ABA2/GIN1 is induced by the treatment of sugar but not by water stress (W-H. Cheng et al., unpublished), indicating that the expression of ABA2 is important in sugar responses. This is consistent with the observation that most of the ABA-deficient mutants screened based on sugar responsiveness were identified as aba2 alleles. The physiological role of sugar-regulated ABA synthesis has been discussed in relation to sugar-induced starch biosynthesis during early seed development [28].

Different genes of a given gene family contribute to ABA synthesis in different physiological processes
Gene families encode NCED and AO. Although it remains uncertain whether all the gene products in these families are involved in ABA biosynthesis, some might function in ABA synthesis. Three NCED-related cDNAs (PaNCED1, PaNCED2 and PaNCED3) have been isolated from avocado, two of which (PaNCED1 and PaNCED3) have the ability to convert 9-cis-violaxanthin and 9’-cis-neoxanthin to xanthoxin. PaNCED1 is highly expressed in leaves and its expression is induced by dehydration, whereas PaNCED3 is not detected in leaves [22]. The levels of both PaNCED1 and PaNCED3, expressed in ripening fruits, correlated with similar daily fluctuations of endogenous ABA levels [22]. Maize Vp14 (ZmNCED1) is highly expressed in embryos and roots, but less in leaves. ABA levels in embryos of vp14 mutants are significantly lower than in the wild type, but no significant difference is observed in ABA content in non-stressed leaves between the wild type and vp14. However, water loss occurs faster in vp14 leaves compared with wild-type leaves, and stressed leaves of vp14 accumulated lower ABA levels compared with the wild type [17]. These results indicate the presence of NCED gene(s) other than Vp14 that are responsible for ABA synthesis in at least non-stressed leaves. In Arabidopsis, there are nine NCED-related genes (AtNCEDs) on the genome database. Only four out of six gene products exhibit NCED activity when tested.
Among them, AtNCED3, whose expression is up-regulated by dehydration, is involved in ABA biosynthesis in vivo [24]. AtNCED1, the first NCED identified in Arabidopsis, lacks the targeting signal peptide to plastids and in most instances the recombinant protein cleaves a variety of carotenoids symmetrically to produce a C13-dialdehyde and two C13 products [59]. Although the expression is induced by dehydration [23], the gene product probably does not function in ABA biosynthesis. At present, detailed characterization and organ-specific expression profiles of each AtNCED gene, as well as the physiological roles of their respective gene products remain to be determined.

Because hydrophobic carotenoids exist exclusively in the membranes of plastids, NCED localizes at the membrane of plastids to interact with its substrate. Bean PvNCED1 exists mainly in the insoluble fractions of chloroplasts such as the thylakoid and envelope membranes, whereas maize VP14 exists in two distinct fractions, a soluble stromal form and an insoluble thylakoid membrane-bound form in plastids [60]. The localization of VP14 inside the plastids might be under regulatory control, which, in turn, might contribute towards the regulation of ABA biosynthesis. In Arabidopsis, at least two types of NCED exist; one, such as PvNCED1, which localizes only in the thylakoid membrane, and another, such as VP14, which exits as two distinct forms [60]. It is possible that different NCEDs in a given plant species, with distinct physiological roles, are subjected to different post-transcriptional regulatory mechanisms.

The presence of AO isoforms has been reported in several plant species [31,36,61–63], and the gene family has been found in Arabidopsis [35], maize [64] and tomato [34]. Plant AO consists of two similar subunits. In Arabidopsis, at least four AO isoforms are generated as homo- and hetero-dimers by three AAO gene products; AAO1 (AAO1-AAO1), A0β (AAO1-AAO2), A0γ (AAO2-AAO2) and A0δ (AAO3-AAO3). The isoforms exhibit different substrate preferences, and are distributed differently in the organs or tissues, depending upon the expression of the corresponding genes [36,65].

The Arabidopsis aao3 mutant, impaired in the AAO3 gene, exhibits a wilty phenotype in leaves but is not affected in seed dormancy. This indicates that A0δ has a role in leaf-specific ABA synthesis, which is consistent with the high expression of AAO3 in leaves [37]. At the same time, this implies that there is another AO isoform(s) responsible for ABA biosynthesis in other organs, such as roots, stems, flowers and seeds. Although the enzymatic nature of AAO4 has not been characterized, AAO4 is a possible candidate for the AO involved in ABA production in immature seeds because AAO4 mRNA is expressed mainly in siliques and the expression in leaves is up-regulated by dehydration [36]. Although the role of A0α, A0β and A0γ remains unclear, some of these AOs probably act within the ABA biosynthetic pathway, particularly in the roots based on the dominant expression of these genes in this organ [36,65].

### Acknowledgements
We are grateful to S. Herman Lips at Ben-Gurion University and Eiji Nambara at RIKEN for their critical reading of the manuscript. T.K. is supported by a Research Fellowship of the Japan Society for the Promotion of Science for Young Scientists.

### Further prospects
Recent advances in ABA biosynthesis research have yielded substantial information on the pathways, genes and enzymes involved in the process, as well as on the regulation of each step. Table 1 represents the characteristics of ABA synthetic enzymes and the expression of the respective genes in relation to the physiological processes. The data indicate that ABA synthesis is not merely a group of reactions but

### Table 1. Characteristics of ABA synthetic enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Enzyme reaction</th>
<th>Gene expression (plant species)</th>
<th>Gene family</th>
<th>Mutants (plant species)</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZEP</td>
<td>Conversion of zeaxanthin to violaxanthin</td>
<td>Induced by dehydration in roots but not in leaves (N.p., L.e.)</td>
<td>No</td>
<td>aba2 (N.p.)</td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Developing seeds (L.p.)</td>
<td></td>
<td>aba1 (A.t.)</td>
<td>[12]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diurnal fluctuation in leaves (N.p., L.e.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCED</td>
<td>Oxidative cleavage of 9-cis-epoxycarotenoid</td>
<td>Induced by dehydration in leaves (Z.m., P.v., V.u., P.a.), roots (P.v., L.e., V.u.) or whole plants (A.t.)</td>
<td>Yes</td>
<td>vp14 (Z.m.)</td>
<td>[17]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ripening fruits (P.a.)</td>
<td></td>
<td>notabilis (L.e.)</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diurnal fluctuation in leaves (L.e.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDR</td>
<td>Oxidation of xanthoxin (Oxidation of xanthoxic acid?)</td>
<td>Induced by sugar in seedlings, but not by dehydration of leaves (A.t.)</td>
<td>No</td>
<td>aao3 (A.t.)</td>
<td>[37]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diurnal fluctuation in leaves (N.p.)</td>
<td></td>
<td>sitiens (L.e.)</td>
<td>[27,30]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Developing seeds (N.p.), ripening fruits (L.e.), immature seeds</td>
<td></td>
<td>aba3 (A.t.)</td>
<td>[25,28]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Developing seeds (N.p.), ripening fruits (L.e.), immature seeds</td>
<td></td>
<td>flacca (L.e.)</td>
<td>[27,30,31]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Developing seeds (N.p.), ripening fruits (L.e.), immature seeds</td>
<td></td>
<td>aba1 (N.p.)</td>
<td>[26,29]</td>
</tr>
<tr>
<td>AO</td>
<td>Oxidation of abscisic aldehyde (Oxidation of xanthoxin?)</td>
<td>Induced by dehydration in leaves but not in roots (A.t.)</td>
<td>Yes</td>
<td>aao3 (A.t.)</td>
<td>[37]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Developing seeds (N.p.), ripening fruits (L.e.), immature seeds</td>
<td></td>
<td>sitiens (L.e.)</td>
<td>[27,30]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Developing seeds (N.p.), ripening fruits (L.e.), immature seeds</td>
<td></td>
<td>aba3 (A.t.)</td>
<td>[25,28]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Developing seeds (N.p.), ripening fruits (L.e.), immature seeds</td>
<td></td>
<td>flacca (L.e.)</td>
<td>[27,30,31]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Developing seeds (N.p.), ripening fruits (L.e.), immature seeds</td>
<td></td>
<td>aba1 (N.p.)</td>
<td>[26,29]</td>
</tr>
</tbody>
</table>

AO, aldehyde oxidase; NCED, 9-cis-epoxycarotenoid dioxygenase; SDR, short-chain dehydrogenase/reductase; ZEP, zeaxanthin epoxidase.

1A.T., Arabidopsis thaliana; L.e., Lycopersicon esculentum; N.p., Nicotiana plumbaginifolia; P.a., Persea americana; P.v., Phaseolus vulgaris; V.u., Vigna unguiculata; Z.m., Zea mays.
2The existence of a homologous gene in tomato has been proposed [46].
4There are several related genes in the Arabidopsis database, but their deduced amino acid sequence share <50% homology with ABA2.
5Defective in the synthesis of molybdenum cofactor. Arabidopsis ABA3 has been shown to encode Moco sulfurase.

http://plants.trends.com
Box 1. Factors that contribute towards the complexity of abscisic acid biosynthesis

Expression of each gene is regulated in a different manner
See Table 1.

Post-transcriptional regulation
NCED - conversion between membrane-bound and stromal forms in plastid; in contrast to membrane-localized carotenoids.
AO - sulfuration of Moco controls the AO activity (Fig. 1).

Key regulatory steps differ depending on organs or developmental stage
• Stress response in leaves (enzymes involved: NCED, AO).
• Stress response in roots (enzymes involved: ZEP, NCED).
• Seed development (enzymes involved: ZEP, NCED).

Several gene products in a gene family catalyze the same step
• NCED, AO.

Alternative pathways in the last steps
(1) Xanthoxin → ABAld → ABA
(2) Xanthoxin → xanthonic acid → ABA
(3) Xanthoxin → ABAld → abscisic alcohol → ABA

Abbreviations: ABA, abscisic acid; ABAld, abscisic aldehyde; AO, aldehyde oxidase; Moco, molybdenum cofactor; NCED, 9-cis-epoxy-carotenoid dioxygenase; ZEP zeaxanthin epoxidase.

rather a sequence of multiple steps mediated by different types of gene sets that determine a complex set of mechanisms. This multi-step mechanism controls ABA biosynthesis at each stage of plant growth and development as well as in response to environmental changes. Box 1 summarizes the potential factors leading to the complexity of ABA biosynthesis. At least four steps, catalyzed by the enzymes ZEP, NCED, SDR and AO, have been investigated for their regulation and control of ABA biosynthesis. This new approach toward the elucidation of the complex regulatory mechanisms that control endogenous ABA levels might answer key questions about the molecular mechanisms of ABA function:

(1) How is the expression of each gene involved in the different parts of the biosynthetic pathway controlled in such diverse and specific manners? cis and trans factors that function in each response mode must be identified.

(2) Are the gene products expressed simultaneously in a given group of cells? This would preclude the possibility that the ABA signal is transported not as ABA itself but as an earlier product in the pathway of ABA biosynthesis.

(3) What is the site(s) of ABA biosynthesis? The second and third questions emphasize the importance of ABA movement between plant organs or tissues; several investigations on drought-induced ABA production have focused on ABA transport between roots and leaves, as well as within a leaf.

The main cDNA sets for Arabidopsis, AtZEP, AtNCED3, ABA2 (encoding SDR) and AAO3 are now available, and specific antibodies and/or transgenic plants expressing, for example, promoter-reporter gene systems, should aid in the precise cellular and histochemical localization of ABA-synthesizing enzymes. Finally, there are some other questions concerning ABA synthesis in seeds, such as whether the ABA required during seed development and maturation is synthesized in the developing seed itself or in the mother plant.

Several other aspects must also be taken into account in the elucidation of the regulation of ABA synthesis. The endogenous ABA content in a given cell is determined as the sum of the rates of biosynthesis, catabolism and transportation. Hence, the components and molecular mechanisms involved in ABA perception and signal transduction, as well as the regulation of gene expression by ABA, should be studied in parallel with the regulation and site determination of ABA synthesis and mobilization.

References
11 Marin, E. et al. (1996) Molecular identification of zeaxanthin epoxidase of Nicotiana plumbaginifolia, a gene involved in abscisic acid biosynthesis and corresponding to the ABA locus of Arabidopsis thaliana. EMBO J. 15, 2331–2342


Borel, C. et al. (2001) N. plumbaginifolia zeaxanthin epoxidase transgenic lines have unaltered baselineABA accumulations in roots and xylem sap, but contrasting sensitivities of ABA accumulation to water deficit. J. Exp. Bot. 52, 427–434


Molybdenum cofactor mutants, specifically impaired in xanthine dehydrogenase activity and abscisic acid biosynthesis, simultaneously overexpress nitrate reductase. Plant Physiol. 107, 1427–1431

Taylor, I.B. et al. (1998) The wilty tomato mutants flava and niten are impaired in the oxidation of ABA-aldehyde to ABA. Plant Cell Environ. 11, 739–745


Sagi, M. et al. (1999) Aldehyde oxidase and xanthine dehydrogenase in a flavo tomato mutant with deficient abscisic acid and wilt phenotype. Plant Physiol. 120, 571–577


Bittner, F. et al. (2001) ABA5 is a molybdenum cofactor sulfurlase required for activation of dehydrogenase and xanthine dehydrogenase in Arabidopsis thaliana. J. Biol. Chem. 276, 40381–40384


Schwartz, S.H. et al. (1997) Biochemical characterization of the aba2 and aba3 mutants in Arabidopsis thaliana. Plant Physiol. 114, 161–166


