Formation and breakdown of ABA

Adrian J. Cutler and Joan E. Krochko

The phytohormone, abscisic acid (ABA) is found in all photosynthetic organisms. The amount of ABA present is determined by the dynamic balance between biosynthesis and degradation: these two processes are influenced by development, environmental factors such as light and water stress, and other growth regulators. ABA is synthesized from a C_{40} carotenoid precursor and the first enzyme committed specifically to ABA synthesis is a plastid-localized 9-*cis*-epoxycarotenoid dioxygenase, which cleaves an epoxycarotenoid precursor to form xanthoxin. Subsequently, xanthoxin is converted to ABA by two cytosolic enzymes via abscisic aldehyde, but there appears to be at least one minor alternative pathway. The major catabolic route leads to 8′-hydroxy ABA and phaseic acid formation, catalyzed by the cytochrome P450 enzyme ABA 8′-hydroxylase. In addition, there are alternate catabolic pathways via conjugation, 4′-reduction and 7′-hydroxylation. As a consequence of recent developments, the mechanism by which the concentration of hormonally active ABA is controlled at the cellular, tissue and whole plant level can now be analyzed in detail.

The biosynthetic and catabolic pathways of phytohormones, including (+)-*S*-abscisic acid (ABA), have been difficult to study using conventional biochemical methods because of the low levels of phytohormones in cells and tissues. For many years it was difficult to establish even the basic framework of the ABA synthetic pathway in higher plants. In 1987, Ram K. Sindhu and Daniel C. Walton wrote that, '…ABA …has been shown to be derived from MVA [mevalonic acid]… surprisingly it is still not possible to make any other definitive statements about the biosynthesis of ABA in plants'[1]. Ironically, the only element of ABA biosynthesis that appeared certain at the time that the statement was made, has recently been shown to be incorrect[2]. Since 1987, many *in vivo* and *in vitro* biochemical studies of ABA metabolism, including studies of ABA-deficiency mutants, have established an outline of the probable biosynthesis route. A cluster of recent studies based mainly on the cloning of genes responsible for ABA deficiencies, have clarified and verified many details of ABA biosynthesis in higher plants (Fig. 1).

The ABA catabolism pathways, via phaseic acid (PA) and glucose-ester conjugation, were established before much was known about ABA biosynthesis[3], but subsequently our understanding of catabolism has proceeded surprisingly slowly. To date, no information has been obtained from studies of mutants, and only recently has it been possible to study the biochemistry of PA formation from ABA in detail[4].

ABA biosynthesis in higher plants

Early steps in the plastid

Initial research focused on two possible routes to ABA: a 'direct' pathway from farnesyl pyrophosphate (similar to the fungal route discussed later) or an 'indirect' pathway via cleavage of a carotenoid precursor[3]. In each case the ultimate precursor was thought to be mevalonic acid (MVA).

Several persuasive lines of evidence favored the 'indirect' pathway to ABA via a C_{40} carotenoid intermediate. Some reports show that corn viviparous mutants (*Vp*), defective in carotenoid biosynthesis, are also ABA-deficient (Table 1). In addition, chemical inhibitors of carotenoid biosynthesis, such as fluridone and norflurazon, also inhibit ABA accumulation (Ref. 3.) Finally, *in vivo*-labeling studies using $^{18}O_2$ are consistent with ABA formation from a large precursor molecule, probably an oxygenated carotenoid[5]. Existence of the 'indirect' pathway to ABA via carotenoids strongly suggested that the early steps of ABA biosynthesis take place in the plastids.

The well-established idea that MVA was the carotenoid and ABA precursor was unexpectedly challenged when the 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway for isoprenoid formation in bacteria was discovered. In the DOXP pathway the C_5 isoprene unit, isopentenyl diphosphate (IPP), is formed via DOXP from pyruvate and glyceraldehyde-3-phosphate rather than MVA (Ref. 6). Subsequently, this pathway has been shown to provide the precursors for carotenoid biosynthesis in unicellular algae and in higher plants[7]. Experiments in which both MVA and pyruvate are incubated with intact spinach chloroplasts confirm that pyruvate is incorporated more efficiently into ABA than MVA (Ref. 2). Pyruvate and glyceraldehyde-3-phosphate are now firmly established as the ultimate precursors of ABA.

Plastidial xanthophyll formation and cleavage

Direct evidence of the formation of an ABA precursor by *in vitro* carotenoid cleavage has been difficult to demonstrate. However, the development of a cell-free system from oranges (*Citrus sinensis*) revealed that all-*trans*-violaxanthin, all-*trans*-neoxanthin and 9′-*cis*-neoxanthin can all act as precursors for xanthoxin, ABA and related metabolites[8].

Analysis of a mutant defective in xanthophyll biosynthesis led to the first cloning of a gene associated with ABA biosynthesis. The *Nicotiana plumbaginifolia* mutant *aba2* (Table 1) has been generated by transposon-tagging: it exhibits the classic ABA-deficient phenotype of wiltiness and reduced seed dormancy[9]. Analysis of the carotenoid content in *aba2* indicates that the mutant is impaired in the epoxidation of zeaxanthin. The deduced amino acid sequence of ABA2 shows homology to various bacterial oxygenases and contains a putative N-terminal chloroplast transit-peptide. Extracts from *E. coli* expressing ABA2 protein epoxidize zeaxanthin to form antheraxanthin and violaxanthin, as predicted[9]. In addition to its role in ABA biosynthesis, zeaxanthin epoxidase (*N. plumbaginifolia*: ABA2) is an integral part of the xanthophyll cycle in plants, which is activated in leaves at high light intensities and produces zeaxanthin (for protection against photo-oxidation) by the de-epoxidation of violaxanthin[10]. *ABA2* transcripts are detected in stems and leaves (as expected) but are also detected at low levels in roots and seeds[11]. In leaves, *ABA2* transcripts appear to be linked to photosynthesis, and exhibit a diurnal fluctuation. In non-photosynthetic tissues, *ABA2* mRNA correlates with changes in ABA levels. When *Nicotiana plumbaginifolia* plants are drought stressed, *ABA2* transcripts increase in roots, but not in leaves. In

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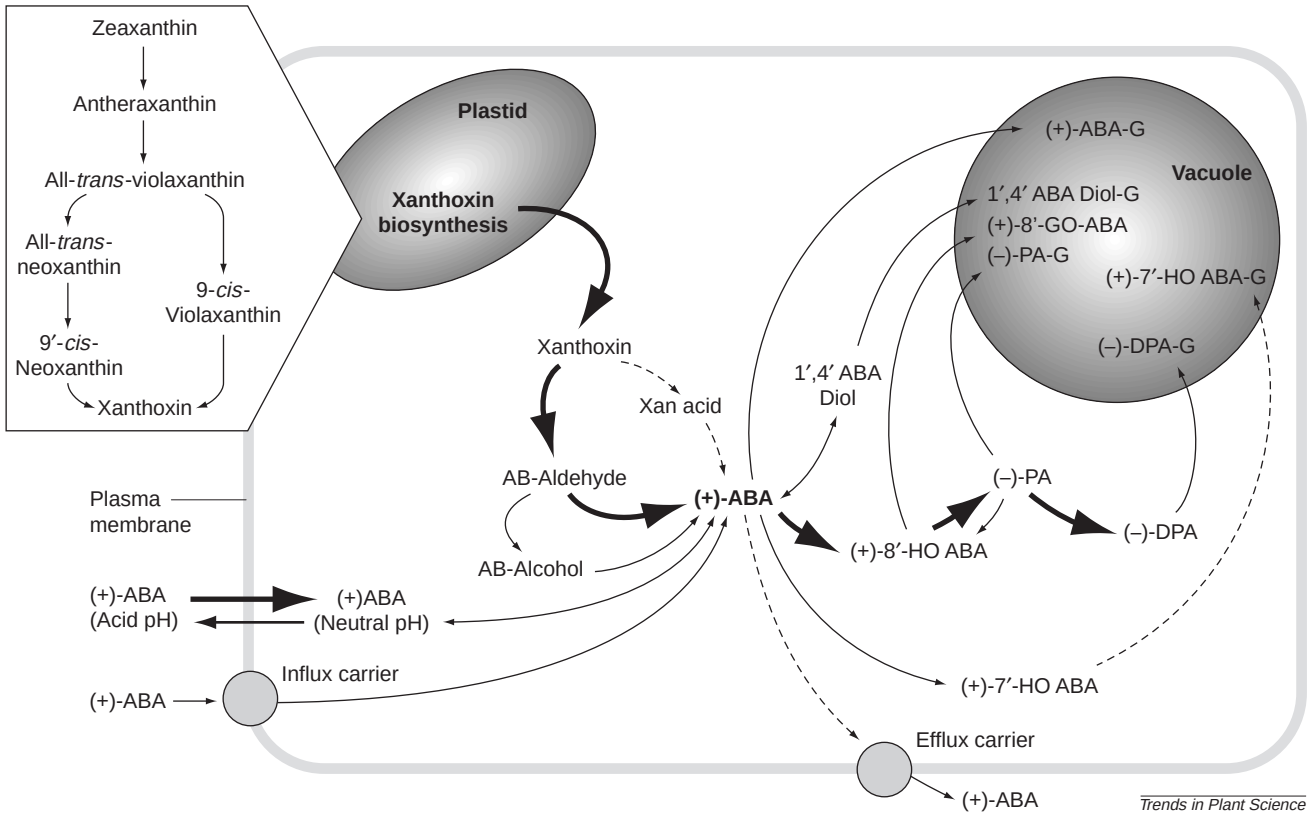

Fig. 1. Overall scheme for (+)-abscisic acid (ABA) metabolism in a representative higher plant cell. The first biosynthetic steps from zeaxanthin to violaxanthin leading to epoxycarotenoid cleavage take place in the plastid. The cleavage product, xanthoxin, leaves the plastid (by an unknown mechanism) and is subsequently converted to ABA in the cytoplasm. The main metabolic route in the cytoplasm [xanthoxin to abscisic aldehyde (AB-aldehyde) to ABA] is shown with bold black arrows. Minor pathways are shown with non-bold arrows, and speculative or unproven steps are shown with broken arrows. The most predominant catabolic route is via 8′-hydroxylation, but there are several alternate degradative routes of which conjugate formation from ABA and ABA metabolites is the most significant. Glucose esters, glucosides and other conjugates are all represented by G, and although the sites of conjugate formation within the cell are uncertain, all accumulate in the vacuole. Other degradative processes include 7′-hydroxylation of ABA and reduction of ABA at the 4′ position, which produce both *cis* and *trans* isomers of 1′,4′ ABA diol. Because cellular ABA levels are influenced by transport, the movement of ABA by protein carriers and passive uptake are indicated. ABA tends to accumulate passively in cells because it is membrane-permeable in the protonated form (acidic pH, as in culture media or apoplastic fluid) and impermeable in the ionic form (neutral pH, as in the cytosol). Abbreviations: DPA, dihydrophaseic acid; PA, phaseic acid; AB-alcohol, abscisic alcohol.

seeds, *ABA2* mRNA reaches a maximum just before mid-development, at approximately the time when ABA concentrations are maximal. Thus, zeaxanthin epoxidase appears to be rate-limiting for ABA biosynthesis in non-photosynthetic tissues[10]. Transgenic experiments provide further evidence for zeaxanthin epoxidase involvement in regulating ABA concentrations and ABA-responses in seeds: overexpression of *ABA2* delays seed germination and antisense expression reduces seed dormancy[12].

The first direct analysis of the xanthophyll cleavage reaction was obtained by characterizing *vp14*, a viviparous and ABA-deficient maize mutant produced by transposon tagging[13] (Table 1). Analysis of carotenoid content and the fact that *in vitro* conversion of xanthoxin to ABA (these subsequent steps of ABA biosynthesis are discussed later) was not reduced in *vp14*, strongly suggested that VP14 was responsible for xanthophyll cleavage. The deduced amino acid sequence of the cloned *Vp14* gene contains an N-terminal chloroplast transit-peptide sequence, but is otherwise similar to lignostilbene dioxygenase of *Pseudomonas paucimobilis*, which catalyzes an analogous oxidative cleavage[13]. The cloned VP14 protein cleaves the 9-*cis*-isomers of both violaxanthin and neoxanthin to *cis*-xanthoxin and a C_{25} epoxy apo-carotenoid (Fig. 2), as well as the 9-*cis*-isomer of zeaxanthin at the 11–12 position to form corresponding products. Of the xanthophylls tested as substrates, none of the all-*trans*-isomers was cleaved[14]. These experiments clearly establish that the 2-*cis* geometry of ABA [*cis*-(+)-*S*-ABA] is determined by this enzymatic cleavage. The *S*-(+)-stereochemistry of ABA (at C-1′) is determined by zeaxanthin epoxidase. *Vp14* is one member of a family of related genes and is expressed constitutively in embryos and roots. Wild-type seedling leaves contain low levels of transcripts, but mRNA accumulates strongly when detached leaves are water-stressed[13]. A similar 9-*cis*-epoxycarotenoid dioxygenase has been characterized in tomato[15]. The 9-*cis*-epoxycarotenoid dioxygenase catalyzes the first step committed specifically to ABA biosynthesis, and thus is probably a key regulatory enzyme for this pathway.

Later steps in the cytosol

By 1988, several pieces of evidence indicated that xanthoxin (C_{15} intermediate) is a precursor of ABA (Ref. 3). For example, cell-free extracts from leaves and roots of bean and from leaves of pea, maize, *Cucurbita maxima* and *Vigna undulata* convert xanthoxin to ABA (Ref. 1). These activities are cytosolic, constitutive

Table 1. Mutants impaired in ABA biosynthesis

Species	Mutant	Enzyme	Ref.
Arabidopsis thaliana	*aba1*	Zeaxanthin epoxidase	28
Nicotiana plumbaginifolia	*aba2*	Zeaxanthin epoxidase	9,11
Zea mays	*vp14*	9-*cis*-Epoxycarotenoid dioxygenase	13,14
Lycopersicon esculentum	*notabilis*	9-*cis*-Epoxycarotenoid dioxygenase	15
Arabidopsis thaliana	*aba2*	Xanthoxin oxidase	19
Arabidopsis thaliana	*aba3*	AB aldehyde oxidase[a]	19
Hordeum vulgare	*nar2a*	AB aldehyde oxidase[a]	19
Lycopersicon esculentum	*flacca*	AB aldehyde oxidase[a]	22
Nicotiana plumbaginifolia	*aba1*	AB aldehyde oxidase[a]	19
Lycopersicon esculentum	*sitiens*	AB aldehyde oxidase	22

[a]Defect in the molybdenum cofactor required for aldehyde oxidase activity.

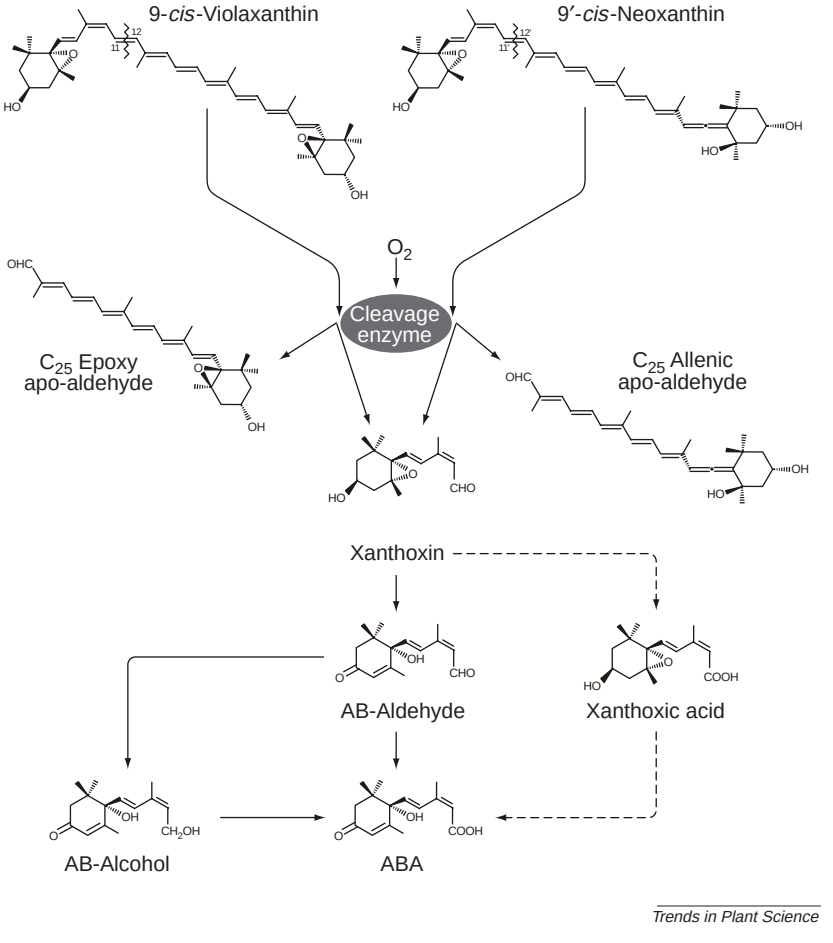

Fig. 2. Biosynthesis of (+)-ABA from carotenoid precursors in higher plants. Carotenoids are involved in photosynthesis and therefore epoxycarotenoid (C_{40})-cleavage (catalyzed by 9-*cis*-epoxycarotenoid dioxygenase) takes place in the plastid and yields xanthoxin (C_{15}) and a C_{25} derivative. The pathway showing cleavage of xanthophylls is adapted from Ref. 13. Two-step conversion of xanthoxin to ABA occurs in the cytoplasm. Broken arrows indicate minor or speculative steps. The major route is via abscisic aldehyde (AB-aldehyde), involving the enzymes xanthoxin oxidase and AB-aldehyde oxidase, but there is an alternative route via abscisic alcohol (AB-alcohol) that is especially significant when aldehyde oxidation is blocked. If AB-aldehyde oxidase can use xanthoxin as an alternative substrate, then xanthoxic acid might be an intermediate also.

and require NAD or NADP. However, the identification of intermediates, the reaction order and the relative importance of alternative routes for ABA synthesis *in vivo* have been more difficult to ascertain. There are three steps and several possible routes between xanthoxin and ABA, and, therefore, six possible intermediate compounds[16]. Three possible precursors of ABA have been tested in cell-free extracts[17]. Both bean and wild-type tomato extracts convert abscisic aldehyde (AB-aldehyde) into ABA as efficiently as xanthoxin. Although both xanthoxic acid and 1′,4′ *trans*-ABA diol are also converted to ABA, the rates of conversion are much lower than with AB-aldehyde. From these results it was concluded that, '…AB aldehyde is the penultimate compound in the conversion of Xan to ABA'[17] (Fig. 2). Recent results have shown that although 1′,4′ *trans*-ABA diol can be converted reversibly to ABA, it is an ABA catabolite and not a precursor[18]. However, the possibility that xanthoxic acid is an ABA precursor has not been completely eliminated.

Further clarification of the cytoplasmic portion of the pathway has been obtained from the biochemical characterization of two *Arabidopsis* ABA-deficient mutants, *aba2* and *aba3* (Ref. 19; Table 1). Cell-free extracts from either *aba2* or *aba3* plants show only limited conversion of xanthoxin to ABA; although *aba2* (but not *aba3*) extracts convert AB-aldehyde to ABA as efficiently as wild-type extracts[19]. These results suggest that *aba3* is deficient in the last enzyme of the pathway, AB-aldehyde oxidase, which converts the C-1 aldehyde to a carboxylic acid group to form ABA, whereas the *aba2* mutant is deficient in the enzyme required for the preceding step, xanthoxin oxidase. Xanthoxin oxidation to form AB-aldehyde involves conversion of the 4′ hydroxyl group to a ketone and the subsequent opening of the epoxide to form an allylic alcohol. Whether epoxide opening is necessarily linked to 4′ ketone formation remains uncertain. Although numerous ABA biosynthesis mutants have been characterized from many species, to date no mutants have been identified that are impaired only in their ring transformation (epoxide to allylic alcohol)[19].

Defects in the synthesis of a molybdenum cofactor (required by several enzymes, including AB-aldehyde oxidase) are responsible for several ABA-deficient mutants[19] (Table 1). It is thought that the impaired transfer of a sulfido ligand into the molybdenum cofactor is responsible for the *Arabidopsis aba3* mutation[19]. Aldehyde oxidases are not highly substrate-specific, and AB-aldehyde oxidase does not distinguish

between the (+)- and (−)- enantiomers of AB-aldehyde. Both (+)- and (−)-AB-aldehyde are oxidized, respectively, to (+)-ABA (the natural hormone) and (−)-ABA (which is not found in plants)[19,20]. In *Arabidopsis*, there is an aldehyde oxidase multigene family comprising at least four members[21], one of which appears to be AB-aldehyde oxidase (M. Seo *et al*., XVI International Botanical Congress, 1999, St. Louis, MO, USA; Abstract 454).

Alternative pathways to ABA

Some observations suggest that there are other distinct, minor and perhaps also redundant ABA synthesis pathways that have not been fully elucidated. For example, ABA2 (zeaxanthin epoxidase) exists as a single copy locus in *N. plumbaginifolia*, although the ABA content in the presumed null *aba* mutant remains 23–48% of wild type[9].

An alternative route from AB-aldehyde to ABA was elucidated as a result of the observation that AB-aldehyde does not accumulate in some mutants defective in aldehyde oxidation. In the tomato *flacca* and *sitiens* mutants, *trans*-abscisic alcohol (*trans*-AB alcohol) accumulated following water stress, and exogenously supplied AB-aldehyde was converted to a mixture of *cis*- and *trans*-AB alcohol (Ref. 22). It has been found subsequently that *flacca* and *sitiens* synthesize most of their residual ABA via this alternative or 'shunt' pathway, which involves the oxidation of AB alcohol directly to ABA (Ref. 23; Fig. 2). It appears that the 'shunt' pathway from AB-aldehyde to ABA via AB alcohol might be important in mutants that are impaired in aldehyde oxidation, and that it is a minor source of (+)-*cis*-ABA in wild-type plants[23–25].

Another possible biosynthetic route has been suggested by the tentative identification of xanthoxic acid in cell-free extracts of *Citrus sinensis* exocarp, which form ABA from xanthophylls. In these extracts AB-aldehyde is not detected[8]. A xanthoxic acid pathway could occur if xanthoxin is also a substrate for aldehyde oxidase[26]; the lack of substrate specificity exhibited by aldehyde oxidases makes this an alternative that cannot be completely discarded (Fig. 2). The second step, conversion of xanthoxic acid to ABA, has been observed *in vitro*[17], as noted earlier.

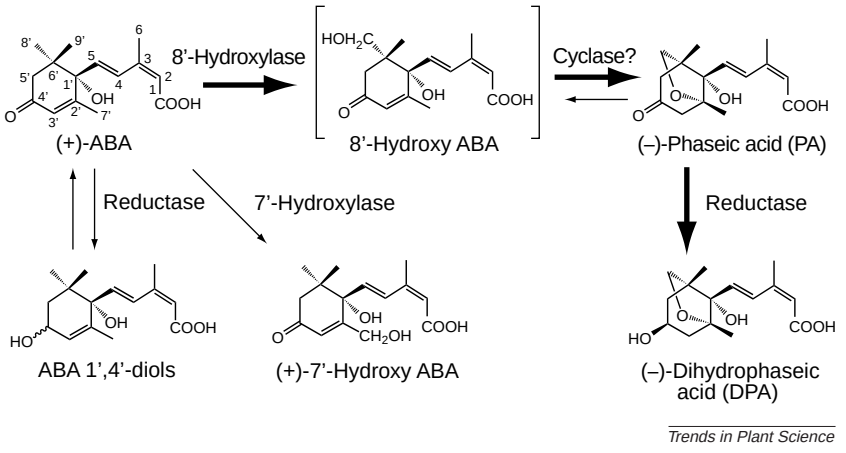

Fig. 3. Oxidative and reductive pathways for catabolism of abscisic acid (ABA). The major pathway for ABA breakdown is via the cytochrome P450 enzyme ABA 8′-hydroxylase, which produces 8′-hydroxy ABA that cyclizes to phaseic acid (PA), which is then further reduced by phaseic acid reductase to dihydrophaseic acid. Minor routes involving 7′-hydroxylation and reduction of the ketone at 4′ are also shown. Curiously, the unnatural isomer (−)-ABA is predominantly metabolized via 7′-hydroxylation (not shown). Formation of glucose esters and other conjugates with ABA and the metabolites shown, is widespread but the nature and range of products made, the site of formation and the transport and relationship to the oxidative and reductive pathways needs a lot more study. Conjugate structures are not shown here. The formation and structures of conjugates are summarized in Ref. 27. There is no evidence yet that conjugates act as a storage reserve of free ABA. The catabolites 8′-hydroxy ABA, PA and 7′-hydroxy ABA all have some hormonal activity, and so might play a role in mediating ABA-like effects.

ABA catabolism in higher plants

There are several metabolic pathways by which ABA can be removed or degraded in plant tissues as a means of further regulating ABA concentrations. In the simplest case, ABA is exported by passive or carrier-mediated efflux from the cells. In the majority of plant tissues, catabolic inactivation of (+)-ABA proceeds via hydroxylation at the 8′ position to form the unstable intermediate, 8′-hydroxyABA, which subsequently cyclizes spontaneously (and/or enzymatically) to form (−)-PA (Ref. 3). The (−)- PA is sometimes further reduced at the 4′ position to form dihydrophaseic acid (DPA). In addition, minor amounts of several other metabolites, (+)-7′-hydroxyABA, *trans*-ABA and the *cis*- and *trans*-1′,4′-diols of ABA, have been detected in some plant species[3,4,27,28] (Fig. 3). Several conjugates of ABA and its metabolites have been reported, of which ABA glucose ester appears to be the most widespread[3,27,28]. These glucose conjugates have little or no biological activity and are not considered to be a reserve or storage form of ABA. In some tissues, the formation of ABA glucose ester or of other conjugates appears to be a major pathway for the inactivation of ABA (Refs 3,28).

The early oxidation products [8′-hydroxyABA, (−)-PA and (+)-7′-hydroxyABA] retain and exert significant ABA-like activity in some bioassays[29,30]. For example, 8′-hydroxyABA is as active as (+)-ABA in stimulating long-chain fatty-acid biosynthesis in *Brassica* embryos[29]. In wheat seedling roots, 8′-hydroxyABA and (−)-PA are both potent inducers of group *3 LEA* mRNA, and (+)-7′-hydroxyABA inhibits wheat embryo germination[30]. In contrast with the activities of these initial ABA-metabolites, DPA does not exhibit ABA-like activity in any of the standard protocols[27], and in this sense DPA is the fully inactivated form of ABA. Although the intermediates in oxidative catabolism have been established for some time[3], neither the enzymes involved in ABA catabolism nor their cDNA clones have been isolated. This is somewhat surprising considering that extensive screens have been conducted for *Arabidopsis* mutants that are affected in ABA sensitivity. One of these screens should have disclosed at least the first enzyme in the pathway. The failure to detect mutations in ABA catabolism suggests that either they are lethal or there is considerable redundancy in copy number or expression.

The enzyme (+)-ABA 8′-hydroxylase catalyzes the first step in the oxidative degradation of ABA, and is considered the pivotal enzyme in controlling the degradation rate of ABA. Recently, (+)-ABA 8′-hydroxylase has been characterized extensively using an *in vitro* assay, the results or which confirm that it is a membrane-associated cytochrome P450 mono-oxygenase[4]. Experimental evidence implicating ABA turnover (and ABA 8′-hydroxylase) as a modulating factor in controlling ABA responses *in planta* has been hitherto indirect and circumstantial because of difficulty in assaying the enzyme *in vitro*. Some of the metabolic

markers used to identify tissues that apparently express (+)-ABA 8′-hydroxylase include the disappearance of internal or applied ABA, accumulation of PA and DPA or their glucose conjugates, and reduced sensitivity to applied ABA. On the basis of such evidence, ABA 8′-hydroxylase activity is expressed at high levels in plant tissues recovering from abiotic stresses (e.g. drought), as well as in roots, tubers, leaves, developing and germinating seeds and young seedlings[3]. The enzyme phaseic acid reductase, which converts PA to DPA, is not as well characterized, although there are reports that it is a soluble cytoplasmic enzyme[27].

Metabolism and physiology

There is substantial biochemical and physiological evidence that ABA concentrations in plant cells are maintained dynamically by continual synthesis and degradation. Therefore, measurements of extracted ABA reflect only a 'snapshot' of the net effect of these processes and provide little information about flux, cellular or subcellular distribution and/or the potential for rapid change. By perturbing either ABA synthesis or degradation, it has been possible to gain some sense of the relative contribution of each of these processes in maintaining ABA concentrations, and consequently in the downstream physiological processes in plant tissues. For example, treating lettuce seeds with fluridone (an inhibitor of phytoene desaturase in the carotenoid pathway) shows that continued ABA synthesis is essential for both maintaining ABA concentrations and preventing germination at high temperatures[31]. Similarly, the addition of tetcyclacis (a general inhibitor of cytochrome P450 enzymes) reveals the contribution of ABA catabolism in modulating ABA concentrations and stomatal behavior in leaves[32].

The recent advances should enable the regulation of ABA metabolism to be investigated in more depth. For example, ABA 8′-hydroxylase is induced rapidly by (+)-ABA in corn suspension cells, reaching a maximum at 16 h, followed by an equally rapid decline to low levels after 24 h (Ref. 4). Such data imply both precise and rapid changes in ABA catabolism during the course of normal plant growth and development, and suggests that ABA concentrations might be modulated not only on a whole plant or tissue level but also in individual cells. It is likely that ABA biosynthesis and catabolism are coordinately regulated and respond to environmental signals. As noted earlier, water stress increases the expression of the biosynthetic enzymes zeaxanthin epoxidase (in roots of *N. plumbaginifolia*[10]) and 9-*cis*-epoxycarotenoid dioxygenase (in detached leaves of corn seedlings[12]), whereas induction of ABA 8′-hydroxylase by ABA is blocked under analogous conditions in corn cells[33]. There is also increasing evidence that light, via phytochrome, increases ABA catabolism[25] and influences ABA levels[34].

ABA metabolism in lower plants and photosynthetic procaryotes

ABA is ubiquitous among green plants and pre-dates the evolution of seed-bearing landforms. It has been identified in ferns, bryophytes (mosses and liverworts) and all algal classes, including oxygenic photosynthetic procaryotes[35] (cyanobacteria). In many lower plants, it has been unclear until recently whether ABA is a hormone or a secondary metabolite. However, there is mounting evidence that ABA has a hormonal function in mosses, liverworts and algae[35].

Little is known about the metabolic pathways of ABA synthesis and degradation in many of these lower plants, although there is considerable circumstantial evidence for substantial conservation in these processes. As noted earlier, carotenoids in all photosynthetic organisms are synthesized in plastids or photosynthetic compartments by the DOXP pathway[6,7]. Enzymes for β-carotene synthesis are functionally and structurally conserved throughout the plant kingdom, and neoxanthin is present in all plants except for some algal families and a parasitic higher plant, *Cuscuta reflexa*[36–38]. In some algae (e.g. cyanobacteria, Dinophyta and Rhodophyta), the absence of known xanthophyll precursors of ABA (neoxanthin, violaxanthin), and the ineffectiveness of carotenoid synthesis inhibitors on ABA accumulation, suggest that ABA is not derived from a carotenoid precursor in all algal species[35,38]. In these cases, ABA is either synthesized by a more circuitous route from an unknown carotenoid precursor, or is derived via a 'direct' route from IPP precursors, similar to that found in fungi.

Oxidative catabolism of ABA to PA (and DPA) has only been demonstrated conclusively in a liverwort, *Riccia fluitans*[39] and a green alga, *Dunaliella* spp. (Ref. 40); however, one can probably generalize to other lower plant species from these findings. The inhibition of ABA degradation by tetcyclacis in these lower plants is consistent with the involvement of a cytochrome P450 monooxygenase in this process[35,39]. In addition, glucose esters of ABA have been found in some algae and bryophytes[35]. However, evidence of ABA catabolism has been difficult to document in many bryophytes, algae and lichens, perhaps reflecting that there is considerable release of ABA into the surrounding environment in some of these organisms, and that catabolic enzymes are therefore not well-developed.

To date, ABA has not been found in bacteria, although some phytopathogenic, mycorrhizal and saprophytic fungi synthesize ABA and might excrete large quantities of this compound into the surrounding medium or soil[35]. In fungi, ABA is synthesized 'directly' from a farnesyl pyrophosphate intermediate derived from a MVA precursor[35], but for the later steps each species has a different but characteristic set of ABA precursors[41]. In general, fungal cell growth is not inhibited by high concentrations of ABA and synthesis is greatest under stress or in stationary phase cultures[35,42]. This production pattern resembles secondary metabolism more than it resembles the synthesis of a regulatory molecule. Oxidative catabolism of ABA within fungal cells has not been described in the literature, although one species reduces *S*-(+)-ABA to (1′*S*, 2′*R*)-(−)-2′,3′-dihydro ABA (Ref. 43). ABA degradation probably occurs after it has been released into the medium (e.g. soil or host) by the enzymes of the affected host plant or other microorganisms.

Future prospects

The description of metabolic pathways and the identification of intermediates is mostly complete with respect to ABA (Fig. 1). However, several questions remain. Firstly, there is a significant deficiency in our knowledge of the xanthophyll *trans*-to-*cis*-isomerization reaction. This is a potentially important step in ABA biosynthesis, but to date, no mutants with defects in the isomerization of carotenoids have been isolated in plants[13], and no biochemical information has been obtained. Secondly, there is uncertainty about the existence and the role of minor and alternate pathways of ABA biosynthesis and catabolism, which require further study. Thirdly, the dominance of the DOXP pathway in the synthesis of IPP intermediates for carotenoid synthesis in non-photosynthetic tissue, such as in roots or cultured cells, has not been established experimentally. This is important in light of recent evidence that there is considerable bidirectional and developmentally controlled movement of IPP across plastid membranes[44]. Finally, in lower plants, questions remain regarding pathways and intermediates in organisms that apparently lack neoxanthin or violaxanthin.

ABA effects in plants have been detailed using ABA-deficient or -insensitive mutants, confirmed by the application of ABA and/or reverse genetics, and quantified by whole-tissue extractions.

Such studies have been useful for establishing the central role of ABA in seed development and germination, stress adaptations, transpiration and desiccation tolerance, and for outlining signal transduction pathways. Comparable progress in unraveling the upstream events that affect the homeostatic control of ABA concentrations in plant tissues has been much slower. However, with the successful cloning of genes involved in ABA biosynthesis, the development of improved enzyme assays, newer and more specific inhibitors of ABA catabolism, and technical improvements in the quantification of ABA, it should be possible soon to dissect the involvement of ABA-flux changes in plant development.

Genetic manipulation of ABA flux will provide significant opportunities for biotechnology applications. More fundamentally, transgenic studies and localization of sites of biosynthesis and degradation in plants should greatly enhance our understanding of how ABA mediates adaptive phenotypic plasticity. Because many aspects of plant growth are controlled by synergistic or antagonistic interactions between phytohormones, it will be especially interesting to elucidate how ABA metabolism is affected by other phytohormones and *vice versa*[45]. However, a clear picture of how ABA levels are controlled will only emerge when the contribution that long-distance and transmembrane ABA transport make to the cellular ABA levels (especially with regard to protein-mediated influx and efflux) are better understood (Fig. 1).

The hormonal activity of ABA catabolites has raised the intriguing possibility that they might play a role in mediating hormonal effects hitherto associated with ABA alone[29,30]. The possibility exists that sub-sets of ABA-like responses are controlled by specific metabolites. Further studies on this subject should be aided by the development of new reagents that inhibit specific catabolic steps[46], or are chemically stable mimics of specific catabolites. Such highly specific reagents should allow us to determine the specific role of metabolites and metabolic enzymes as mediators of ABA-like effects.

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Adrian Cutler* and Joan Krochko are at the Plant Biotechnology Institute, National Research Council of Canada, Saskatoon, Canada S7N 0W9.

*Author for correspondence (tel +1 306 975 5581; fax +1 306 975 4839; e-mail adrian.cutler@.nrc.ca).

Nucleolar dominance and silencing of transcription

Craig S. Pikaard

Nucleolar dominance is a phenomenon in plant and animal hybrids whereby one parental set of ribosomal RNA (rRNA) genes is transcribed, but the hundreds of rRNA genes inherited from the other parent are silent. The phenomenon gets it name because only transcriptionally active rRNA genes give rise to a nucleolus, the site of ribosome assembly. Nucleolar dominance provided the first clear example of DNA methylation and histone deacetylation acting in partnership in a gene-silencing pathway. However, the sites of chromatin modification and the ways in which one set of rRNA genes are targeted for repression remain unclear. Another unresolved question is whether the units of regulation are the individual rRNA genes or the multi-megabase chromosomal domains that encompass the rRNA gene clusters.

Nucleolar dominance was first discovered as a reversible change in chromosome morphology[1–3]. It was noted that the metaphase ‘D’ chromosomes in the root-tip cells of pure (non-hybrid) diploid species of the plant genus *Crepis* always had a ‘satellite’: a distal portion of the chromosome attached to the rest of the chromosome by a thin secondary constriction (the primary constriction being the centromere). However, in 13 out of 21 different F1 hybrid combinations a satellite and a secondary constriction formed on the D chromosome inherited from one species but not the other, a phenomenon called ‘differential amphiplasty’ (Fig. 1). Importantly, the same species’ satellite was suppressed (under-dominant) regardless of whether this species was the maternal or paternal parent in the cross. Suppressed D chromosomes could form satellites again in the next generation if the hybrids were backcrossed to the under-dominant parent, which suggested that the chromosome was not being damaged by passage through the hybrid. It was concluded that differential amphiplasty is a reversible phenomenon that is brought about by interactions between the parental genomes[1].

Coincident with the studies on *Crepis*, Barbara McClintock demonstrated that nucleolus formation and secondary constriction formation are causally related[4]. Convincing evidence was obtained from a maize line that had undergone a reciprocal chromosome translocation resulting from X-ray-induced chromosome breakage. One break occurred within the region on chromosome 6 where the nucleolus is associated (a locus McClintock named the nucleolar organizer); the other occurred within chromosome 9. Instead of the usual single nucleolus and secondary constriction observed in wild-type maize, two nucleoli and secondary constrictions were formed precisely at the sites where the pieces of chromosomes 6 and 9 were fused. McClintock concluded that to be divisible the chromosomal information at the nucleolus organizer region (NOR) must be redundant. She was correct, and decades later NORs were shown to be multi-megabase loci where rRNA genes are repeated[5,6], sometimes in thousands of copies (Fig. 2). Based on the relative nucleolus-forming ability of the translocated maize chromosomes (alone or in the presence of wild-type chromosomes) McClintock hypothesized that dominant and under-dominant NORs differed in their ability to organize nucleoli. Considering the *Crepis* data, McClintock suggested a simple dominance hierarchy based on the functional capacity of *Crepis* NORs (Ref. 4). Consistent with this prediction, it was

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