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Abscisic Acid as a Natural Growth Regulator [and Discussion]

P. F. Wareing and J. W. Bradbeer

Phil. Trans. R. Soc. Lond. B 1978 **284**, 483-498
doi: 10.1098/rstb.1978.0084

Phil. Trans. R. Soc. Lond. B. **284**, 483–498 (1978)
Printed in Great Britain

Abscisic acid as a natural growth regulator

By P. F. Wareing, F.R.S.
Department of Botany and Microbiology, University College of Wales, Penglais, Aberystwyth, Dyfed SY23 3DA, U.K.

Abscisic acid (ABA) is accepted as one of the five major classes of natural plant growth regulators. In many tests ABA inhibits growth and metabolism, and enhances degradative changes, as in ripening and senescence. Its sites of biosynthesis appear not to be strictly localized and it is transported within the plant both within the phloem and by cell-to-cell transport. The best authenticated example of its function as a growth regulator is in the geotropic responses of roots in which growth curvature is apparently brought about by the asymmetric distribution of ABA produced in the root cap. The evidence for a rôle of ABA in the dormancy of seeds and buds, and in the ripening and abscission of fruits, is suggestive but at present incomplete. Experiments involving both exogenous and endogenous ABA suggest that ABA may play a significant rôle in tuberization in potato and other species.

ABA inhibits hormone-induced nucleic acid and protein synthesis and there is evidence for its action both at the transcription and at the translation levels. On the other hand, ABA induces very rapid inhibition of growth which appears to be mediated via its effects on cell membrane properties. Its effects on potassium uptake may be mediated through inhibition of proton excretion by the cell.

ABA counteracts the effects of other growth promoting hormones in various tests but the nature of this interaction is unknown. It seems likely that ABA plays a general rôle in the regulation of growth and certain aspects of metabolism, through its interaction with other growth substances.

1. Introduction

As is well known, abscisic acid was originally isolated as a factor which promotes abscission in a rather specific biotest involving explants of cotton seedlings (Addicott *et al.* 1964), but with the availability of synthetic ABA its effects have been studied in a wide range of tests and a wide variety of responses have been reported, which may be summarized broadly under the following headings:

1. In the vast majority of cases where ABA is applied to growing tissues its effect is inhibitory although it is sometimes less effective when applied externally to whole plants than to isolated or excised parts.

2. With non-growing tissues, ABA frequently inhibits biosynthetic processes (e.g. nucleic acid and protein synthesis) and enhances degradative changes, such as leaf senescence.

3. In a number of instances ABA has been found to promote growth at low concentrations (McWha & Jackson 1976).

4. ABA produces formative effects, e.g. root and bud initiation, in some species.

These effects of application of exogenous ABA do not, of themselves, signify that endogenous ABA functions as a natural growth regulator, the evidence for which is based mainly upon the following considerations:

1. In many tests ABA is found to be active at low concentrations (of the order of 10^{-5} M or less), which are in the same range as that for optimal activity of other natural growth

substances. Moreover, the concentrations of endogenous ABA in plant tissues are frequently of the same order.

2. ABA is frequently found to counteract the effects of the main classes of growth promoting hormones (auxins, gibberellins and cytokinins) in various processes (§ 14).

3. In general, the inhibitory effects of ABA on growth and metabolism do not appear to be associated with toxicity and can frequently be reversed, with no deleterious after-effects, by washing ABA from the tissues.

4. There is detailed evidence for a regulatory rôle of ABA in certain processes, such as stomatal movement and the geotropism of roots.

5. ABA appears to be universally present in vascular plants.

On these various grounds, it is now generally accepted that ABA must be regarded as one of the five major classes of natural growth substances of plants. However, before ABA can be regarded as a plant 'hormone', it has to be demonstrated that it fulfils certain other criteria, namely, that it is produced only in certain localized parts of the plant and is transported to other regions, where it appears to have a regulatory function. That ABA is, indeed, transported within the plant will be shown in the following section, and the evidence for its possible regulatory function in various processes will be considered in later sections.

2. Sites of biosynthesis and transport of ABA within the plant

The present state of knowledge regarding the biosynthesis and metabolism of ABA has recently been reviewed in detail by Milborrow (1974), and hence needs to be summarized only briefly here.

Although the balance of evidence seems to support the view that endogenous ABA is synthesized directly from a C_{15} precursor, on the general route for terpenoid biosynthesis, the possibility is not excluded that some endogenous ABA may be derived from violaxanthin through xanthoxin. Various plant organs, including leaves, stems, fruits and seed tissues (endosperm and embryos) have been shown to be capable of incorporating mevalonate into ABA, so that it would appear that biosynthesis is not strictly localized in the plant (Milborrow & Robinson 1973).

The presence of ABA in phloem sap has been demonstrated for several species (Hoad 1967, 1973). A recent study by Zeevaart (1977) suggests that ABA may be synthesized in leaves and exported from there to other parts of the plant. Using plants of *Ricinus communis*, he investigated the possible sites of ABA production and metabolism by analysing the levels of ABA, phaseic acid (PA) and dihydrophaseic acid (DPA) in the various parts of water-stressed and non-stressed plants. Water stress increased the concentration of ABA, PA an DPA in phloem exudate and increased the levels in mature leaves and in shoot tips, the latter having a very high level of DPA. The results were interpreted as indicating that ABA is synthesized and metabolized in mature leaves of non-stressed plants and that ABA and its metabolites are translocated in the phloem to the shoot tips, where it appears to be metabolized rapidly.

The evidence presented above seems to indicate that ABA is transported throughout the plant in the phloem and xylem. Studies have also been carried out on cell-to-cell transport of ABA. Earlier studies seem to indicate that the rate of basipetal transport of ABA was greater than acropetal transport (Dörffling & Böttger 1968; Milborrow 1968). However, more recent studies seem to indicate that the rate of cell-to-cell transport of ABA is low and non-polar

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(Dörffling *et al.* 1973; Veen 1975). Studies on geotropism in roots seem to indicate that there is movement of ABA from the root cap to the region of the root meristem (see below); on the other hand, in the older parts of the root, ABA transport is acropetal (Hartung & Behl 1974, 1975).

3. Abscisic acid and geotropism in roots

The problem of determining whether a given metabolite, which shows activity in various tests involving growth or other responses, plays a regulatory rôle *in vivo* has proved a singularly difficult one, not only for ABA but for all the major classes of natural growth substances. The problem cannot normally be solved by the results of a single crucial test, but by assessing the cumulative evidence obtained from a variety of approaches, involving studies both on responses to the application of exogenous growth substances and on quantitative and qualitative changes in the endogenous substances within the plant. Recent studies on the rôle of ABA in the geotropic responses of roots provide a good example of the way in which this type of problem can be approached and have yielded the best evidence to date of a growth regulatory function for ABA. These studies have recently been summarized by Audus (1975) and Wilkins (1977).

For many years the generally accepted hypothesis for the hormonal control of geotropic curvatures in roots was the well known Cholodny–Went theory, according to which the downward ('positive') curvature of horizontally placed roots is due to the lateral migration and accumulation of indoleacetic acid (IAA), produced in the root tip, to supra-optimal (inhibitory) concentrations, thus causing reduced growth on the lower side of the roots and hence downward curvature. However, recent intensive and critical studies in at least five different laboratories has led to a radical revision of views on the mechanism of geotropism and seem to indicate a clear functional rôle for ABA in this process. This new approach to the problem commenced with the demonstration that removal of the root cap eliminated the capacity of maize roots to respond to gravity (Juniper, Groves, Landau-Schachear & Audus 1966). Further experiments involving removal of half the cap demonstrated that the root always developed a curvature towards the side on which the remaining half cap was located (Gibbons & Wilkins 1970), indicating that some growth inhibitory influence arises in the cap, which is transmitted to the growing zone of the root. Further experiments strongly suggested that the root cap is the source of a growth inhibitory substance which is distributed asymmetrically to the extending zone of the root, as a result of downward lateral transport (Shaw & Wilkins 1973).

Extraction of large numbers of root caps of *Zea* demonstrated the presence of an inhibitory substance whose chromatographic properties corresponded to those of ABA (Kundu & Audus 1974), and further work demonstrated the presence of ABA in such extracts (Wilkins & Wain 1974) together with two other unidentified inhibitory fractions.

It was found that *Zea* roots do not exhibit a geotropic response if fruits are germinated and grown in total darkness, but exposure to white, red or blue light renders the roots geotropically sensitive. Wilkins & Wain (1975*a*) showed that light inhibits the elongation of *Zea* roots and that the site of light perception is the root cap. They exposed detached root caps and 'decapped' roots separately to light or dark. When a light-treated cap was placed on a dark-treated root apex the growth of the root was strongly inhibited, whereas a dark-treated cap placed on a light-treated root apex resulted in no growth inhibition. Hence light apparently leads to the production of a growth inhibitor in the root cap. Moreover, it was shown that the

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inhibitors formed in the root cap after exposure to light are required for the geotropic response (Wilkins & Wain 1974).

It was further shown that ABA is present in root caps which have been exposed to light but not in caps maintained in the dark. Finally, it was shown that the application of exogenous ABA over the concentration range 10^{-8} to 10^{-4} M to roots causes inhibition of root growth and that asymmetric application of ABA induces curvature of the roots (Pilet 1975). Moreover, horizontal, intact, dark-grown roots developed a geotropic curvature when treated with exogenous ABA whereas those in water showed little curvature (Wilkins & Wain 1975*b*). These recent detailed studies on geotropism in roots seem to point strongly to a regulatory rôle for endogenous ABA in this process.

4. Seed dormancy and germination

The term 'dormancy' is used loosely in several senses, but here it is applied to cases in which the growth of a seed or bud is temporarily arrested from causes arising within the organs themselves and not from external causes; thus it would be more accurate to speak of 'innate' dormancy. In some seeds the embryo itself is innately dormant ('embryo dormancy') and will not germinate even if the coats are removed, whereas in other types of seed the embryo will germinate if the coats are damaged or removed ('coat-imposed dormancy'), and the dormancy of the intact seed arises from the inhibitory effects of the coats.

Among the various forms of dormancy found in seeds, we need to consider here only two types, namely (1) seeds with a light requirement and (2) seeds with a chilling requirement. The former exhibit only coat-imposed dormancy, whereas among seeds with a chilling requirement both embryo dormancy and coat-imposed dormancy are found.

The idea that dormancy may involve growth inhibitory substances was first put forward by Hemberg (1949) on the basis of his observations on potato tubers and tree buds. This hypothesis was later extended to seed dormancy and evidence was presented for the possible rôle of endogenous inhibitors in various types of seed dormancy (Wareing 1965).

The discovery of ABA and the demonstration that it is a highly active inhibitor of growth and germination raised the question as to whether it plays a rôle in the regulation of both buds and seeds. Numerous studies have indicated that ABA inhibits the germination of seeds of a wide range of species. However, there appear to have been no cases reported where ABA has been found to induce dormancy of intact non-dormant seeds: inhibition of germination depends upon the continued supply of ABA and there is no carry-over effect, so that as soon as the seeds are removed from ABA they germinate. Moreover, studies with seeds of *Chenopodium* suggested that ABA prevents germination by inhibiting root growth, rather than earlier stages of germination (Karssen 1968).

Again, there is little evidence that ABA plays a regulatory rôle in the dormancy of light-requiring seeds. Extensive studies have been carried out on the rôle of ABA in the dormancy of the light-requiring seeds of lettuce but have produced little evidence that it has a regulatory rôle in these seeds (Berrie & Robertson 1976).

However, ABA may play a rôle in the dormancy of seeds with a chilling requirement, especially in those showing embryo dormancy. Thus it has been shown that the dormant immature embryos of *Taxus baccata* (which normally show a chilling requirement) may be induced to germinate by placing them in a nutrient solution which results in the leaching of an

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ABA-like fraction from the embryos (Le Page-Degivry & Garello 1973). If such non-dormant embryos are treated with exogenous ABA they are rendered dormant again and will not germinate if transferred to water, but now exhibit a chilling requirement for germination (Le Page-Degivry 1973). Thus, leaching of the immature embryos renders them non-dormant and re-application of the ABA restores the dormancy. Analogous results have been obtained with the embryos of apple, certain varieties of which have seeds which show embryo dormancy (Durand, Thévenot & Côme 1975). It was shown that embryonic axes (i.e. embryos from which the cotyledons have been removed) which have been rendered non-dormant by pretreatment at 5 °C for 20 days can be rendered secondarily dormant by treatment with 10^{-5} M ABA at 20 °C, and will not then germinate when placed in water at 20 °C unless they are again first chilled at 5 °C for 2–3 weeks. These experiments with isolated embryos of *Taxus* and apple seem to provide good evidence that application of exogenous ABA can induce embryo dormancy. Moreover, endogenous ABA is present in the seeds and embryos of several other species.

Attempts to correlate the variations in levels of endogenous ABA with the states of dormancy of seeds have given diverse and confusing results. In some species, the level of endogenous ABA is high in the dormant seed and declines during chilling treatments which break the dormancy whereas in other cases no decline is observed (Wareing & Saunders 1971; Bonamy & Dennis 1977; Bilboa-Zavala & Dennis 1977). However, there are good grounds for thinking that dormancy in seeds with a chilling requirement is regulated not only by inhibitors, such as ABA, but also by germination and growth-promoting hormones, especially gibberellins and cytokinins. Thus, not only will exogenous gibberellins and/or cytokinins stimulate the germination of many types of dormant seed, but treatments which overcome the dormancy (e.g. exposure to red light or to chilling temperatures) are found to result in concomitant increases in the levels of endogenous gibberellins and cytokinins (van Staden, Webb & Wareing 1972; Taylor & Wareing 1978). We are therefore led to the idea that seed dormancy may be regulated by an interaction between growth promoters, such as gibberellins and cytokinins, and inhibitors such as ABA. Present evidence suggests that the induction of embryo dormancy may involve endogenous ABA, whereas the breaking of dormancy during chilling may be more directly dependent upon increased release or production of gibberellins and/or cytokinins, rather than a reduction in endogenous ABA levels.

The interactions between the effects of gibberellins, cytokinins and ABA have been studied in detail in seeds of a number of species. In seeds of lettuce and other species the inhibitory effects of ABA on germination are more effectively overcome by cytokinins than by gibberellins (Khan 1967; Sankhla & Sankhla 1968), although the germination percentage may be further increased if gibberellins are also present. These observations have led Khan (1971, 1975) to propose a general theory of hormonal control of seed dormancy, in which it is suggested that gibberellins play a primary rôle in stimulating germination, but that cytokinins are required in the presence of inhibitors such as ABA.

5. Bud dormancy

Innate dormancy is exhibited not only by many seeds, but also by the buds of various organs, including not only the winter resting buds of trees, but also the buds of stem tubers (e.g. potato), root tubers (e.g. Dahlia), corms, bulbs, rhizomes, etc. As already stated (§4), the idea that the regulation of dormancy may involve growth-inhibitory substances was first put

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forward on the basis of Hemberg's observations on dormancy in potato tubers and buds of ash (*Fraxinus*), and it was the attempt to determine the nature of the endogenous inhibitors in leaves and buds of sycamore (*Acer pseudoplatanus*) which led ultimately to the isolation and identification of ABA in extracts of these materials (Cornforth, Milborrow, Ryback & Wareing 1965). Partly purified extracts of birch leaves were capable of inducing the formation of buds when applied to birch seedlings (Eagles & Wareing 1964). When synthetic ABA became became available it was shown that bud formation can be induced in seedlings of various woody species by external application of ABA although relatively high concentrations are required and growth was arrested only after treatment for several weeks (El-Antably, Wareing & Hillman 1967). However, some subsequent attempts to repeat these results have been unsuccessful (Hocking & Hillman 1975).

On the other hand, the formation of 'turions' (winter resting buds) in duckweed, *Spirodela polyrrhiza*, can be induced by application of ABA and the turions so formed show dormancy which can be overcome by chilling or treatment with cytokinin (Perry & Byrne 1969; Stewart 1969).

In many woody species the formation of winter resting buds is promoted by short days and it was shown that under these conditions the leaves exert an inhibitory effect upon the shoot apices which appears to bring about the formation of resting buds (Wareing 1954). This observation suggested that greater amounts of endogenous ABA are produced in and exported from leaves under short days than under long days, and earlier experiments involving the bioassay of inhibitor levels in relatively crude extracts appeared to support this hypothesis (Phillips & Wareing 1959). However, when it later became possible to determine the levels by physical methods, it became apparent that the endogenous ABA levels were no higher, and sometimes lower, in short day leaves than in long day leaves (Lenton, Perry & Saunders 1972).

Again, earlier studies on changes in endogenous inhibitor levels in tree buds during the course of the winter indicated a progressive decline which appeared to be correlated with the gradual loss of dormancy (Phillips & Wareing 1958). In buds of grape (*Vitis vinifera*) a good correlation was found between the endogenous ABA levels and the depth of dormancy (During & Bachman 1975). A similar correlation was found in buds of *Betula verrucosa* (Harrison & Saunders 1975) and of blackcurrant (*Ribes nigrum*) and beech (*Fagus sylvatica*), in which free ABA levels reached a peak in the autumn with the onset of dormancy and subsequently declined (Wright 1975). On the other hand, in peach flower buds there was little change in the endogenous ABA levels during the winter until after the cold requirement had been met, when the levels declined (Corgan & Martin 1971). Dormancy breaking in flower buds of coffee (*Coffea arabica*) appears to be regulated by a balance between endogenous ABA and gibberellin levels (Browning 1973). Thus, attempts to establish the rôle of ABA in the bud dormancy in woody plants have given inconclusive results and at present the evidence for such a rôle is effectively non-existent; the same can be said for its rôle in bud dormancy in other types of organ, such as potato tubers (Rappaport & Wolf 1969).

However, before we conclude that ABA has no significant rôle in bud dormancy it is as well to bear in mind that, as in seeds with a chilling requirement, the regulation of dormancy is likely to involve not only inhibitory factors, such as ABA, but also gibberellins and cytokinins, and the normal induction of bud dormancy by short days may involve a shift in the promotor/inhibitor balance by a decline in the levels of promoters, rather than by an increase in ABA.

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Hence application of exogenous ABA under long day conditions, when high endogenous gibberellins have been found in some species (Railton & Wareing 1973), may not be sufficient to induce bud formation and dormancy, but may require a concomitant reduction in gibberellin levels. Thus it may be significant that in *Myriophyllum verticillatum* ABA will induce the formation of dormant turions under marginal daylengths but not under long days (Weber & Nooden 1976). Moreover, there is much evidence that externally applied ABA is rapidly metabolized in leaf tissues and it may be difficult to achieve higher ABA levels in the shoot apices by applying ABA through the leaves. It would seem significant that in those instances in which ABA has been effective in inducing dormancy (i.e. in isolated embryos of *Taxus* and apple, and in *Lemna*) the plant material was totally immersed in ABA solution.

6. Correlative inhibition of buds

It has been claimed that certain observations support the view that ABA may have a rôle in the correlative inhibition of lateral buds. Thus, higher levels of ABA were observed in inhibited lateral buds of *Xanthium* than in those released from inhibition (Tucker & Mansfield 1973). Application of ABA directly to released lateral buds caused little inhibition with *Xanthium*, although a greater degree of inhibition has been reported for *Pisum*. In both *Xanthium* and tomato, the inhibition of lateral buds is affected by far-red light which appears to affect the balance of IAA and ABA within the shoot apices and lateral buds (Tucker 1976, 1977).

A recent investigation of the rôle of ABA in bud inhibition in *Phaseolus* showed that ABA caused little inhibition except at high concentrations (White & Mansfield 1977). Moreover, the levels of endogenous ABA were similar in the main shoot apices, and in both inhibited and released apical buds. Complex interactions between the effects of IAA and ABA in apical dominance in *Phaseolus* were reported by Hartung & Steigerwald (1977). Thus there is little evidence at present that ABA plays any significant rôle in lateral bud inhibition (Dörffling 1976).

7. Tuberization

Studies on the effects of ABA on tuberization in potato have given conflicting results. El-Antably *et al.* (1967) reported that although application of ABA directly to stolon tips did not induce tuberization, application to the leaves of plants of *Solanum andigena* increased the number of tubers formed under long day conditions. These results could not be repeated by Claver (1970), and Palmer & Smith (1969) reported that ABA inhibited tuberization in isolated stolons of *Solanum tuberosum* grown in aseptic culture. However, we have recently obtained experimental results which seem strongly to indicate an involvement of ABA in the tuberization process in *S. andigena*.

If one-node cuttings, with the associated leaf intact, are taken from fully induced plants which have been exposed to 20 short-day cycles and are maintained under moist conditions, the axillary bud rapidly develops into a small tuber. If the leaf is removed from such one-node cuttings the axillary bud grows into an elongated shoot instead of a tuber. However, if the bases of leafless cutting are placed in a 10^{-5} M solution of ABA the bud again develops into a tuber. Hence the effect of the leaf can be replaced by exogenous ABA. The possibility thus arises that the effect of the leaf in promoting tuberization can be attributed to endogenous ABA exported from the leaf to the bud. Further experiments (A. Jennings & P. F. Wareing,

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unpublished) have indicated that (1) ABA is indeed exported from the leaf and is accumulated by the bud of one-node cuttings; (2) the amounts exported from induced and non-induced leaves are not significantly different; and (3) if a non-induced leaf is grafted on to a stem section from an induced plant, tuberization occurs. Thus, in one-node cuttings the effect of the leaf on tuberization appears to be mediated through the endogenous ABA which it produces and exports. Further work is necessary to determine whether ABA plays an essential rôle in the normal tuberization of intact plants.

Exogenous ABA has also been reported to promote the formation of tubers in Dahlia (Biran *et al.* 1972) and in Jerusalem artichoke (*Helianthus tuberosus*) (Charnay & Courduroux 1973).

8. Abscission

ABA was originally discovered as the result of a search for a naturally occurring abscission-promoting substance (Okhuma, Addicott, Sinden & Thiessen 1965). One of the biological assays used in this work was the acceleration of abscission of petiolar stumps in explants of cotton seedlings in which test the ABA proved to be highly active and it has subsequently been shown to be active in similar tests with a number of other species. However, ABA appears to have little effect in promoting leaf abscission when applied to the intact plant, except at very high concentrations (Milborrow 1974).

On the other hand, there is considerable evidence that ABA may be involved in the regulation of flower and fruit abscission. Earlier studies on abscission of immature fruits of yellow lupin pointed to the involvement of a specific substance (van Steveninck 1959) which was later identified as ABA (Cornforth *et al.* 1966). Application of exogenous ABA accelerates the abscission of mature fruits of peach, olive, citrus and apple and of the flowers and young fruits of grape (Milborrow 1974). A detailed study of the variations in endogenous levels of ABA in developing cotton fruits (Davis & Addicott 1972) showed that there were two peaks in the ABA levels, the first occurring after 8 days from anthesis and corresponding to a period of dropping of immature fruits, and a second occurring during the final stages of maturation (30–50 days) which is terminated by the final dehiscence of the fruits. These observations suggest that endogenous ABA may play a regulatory rôle in the abscission of young fruits and in the development of the mature fruits, but such a rôle cannot yet be said to have been unequivocaly established.

9. Senescence and ripening

Exogenous ABA markedly accelerates the senescence of detached leaves and excised disks of leaf tissue, but has relatively little effect on the senescence of the intact leaves of whole plants (Milborrow 1974). By contrast, ABA would seem to have a possible rôle in the ripening and maturation of fruits. Although application of exogenous ABA to mature fruits does not appear to accelerate ripening in apples, pears or citrus, studies on endogenous ABA have demonstrated that there is a marked rise in the levels during the ripening of strawberries (Rudnicki, Pieniazek & Pieniazek 1968) and tomatoes (Dörffling 1970). In grape there is a good correlation between endogenous ABA levels and the phases of fruit ripening (Coombe & Hale 1973). Moreover, application of ABA to young fruits accelerated ripening.

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10. Chilling and frost resistance

There is increasing evidence that ABA may play an important rôle in resistance to both freezing temperatures in temperate species and to chilling temperatures in tender plants. Thus, application of exogenous ABA has been reported to increase frost hardiness in *Acer negundo* (Irvine & Lanphear 1968), apple seedlings (Holubowicz & Boe 1969) and *Medicago sativa* (Rikin, Waldman, Richmond & Dovrat 1975). Similarly, chilling injury in cucumber seedlings is ameliorated by application of ABA (Rikin & Richmond 1976; Rikin, Blumenfeld & Richmond 1976). These observations would seem to merit further investigation to determine whether endogenous ABA is involved in the natural hardening processes.

11. Effects on nucleic acid and protein synthesis

The effects of ABA on nucleic acid and protein synthesis have been studied in a wide variety of systems, involving both growing and mature tissues, and there are reports of its inhibitory effects on both processes. However, it is still not clear whether ABA acts primarily at the transcription or at the translation stage.

The effects of ABA on nucleic acid metabolism have recently been reviewed by Jacobsen (1977). There is some evidence that, in maize coleoptiles, ABA can reduce the activity of soluble RNA polymerases (Bex 1972) and there is also evidence that ABA can inhibit polysome formation, possibly by reducing the synthesis of mRNA (Wareing *et al.* 1968*b*; Villiers 1968; Poulsen & Beevers 1970). However, the available evidence suggests that ABA does not exert a general inhibition of transcription, but may inhibit the synthesis of specific species of mRNA. Thus, in the barley aleurone ABA has little effect on total incorporation of labelled precursors into RNA, although it does inhibit GA-induced labelling of poly(A)RNA (Ho & Varner 1974). Moreover in this tissue ABA inhibits α-amylase synthesis and studies involving *in vitro* translation of α-amylase mRNA show that the inhibition of GA-induced amylase synthesis is associated with a reduction in level of amylase mRNA (Higgins, Zwar & Jacobsen 1977).

On the other hand, there is good evidence from studies on other systems that ABA inhibits protein synthesis not through effects on mRNA transcription but by affecting some later stage, such as mRNA processing or translation. Thus, studies on cotton embryos (Ihle & Dure 1970) have shown that during the early stages of germination, synthesis of protease and isocitratase is not inhibited by Actinomycin D and hence is apparently dependent upon 'stored' mRNA. Studies carried out at various stages during embryo development showed that immature embryos which have attained 60% or more of their final mass can form protease in the presence of Actinomycin D, whereas younger embryos can do so only very slowly. It would appear therefore that the mRNAs for protease and isocitratase are formed during the latter stages of embryo development, and yet translation is apparently inhibited until after germination. It was shown that washing of larger embryos will allow the synthesis of protease, but that synthesis of this enzyme in washed embryos is inhibited by either ABA or partially purified extracts of the ovule wall which would contain endogenous ABA. It is suggested, therefore, that ABA plays an essential rôle in preventing the translation of mRNA formed during the later stages of development until germination occurs. Although there is little evidence in general that the inhibitory effects of ABA on growth and metabolism are exerted primarily through the inhibition of nucleic acid or protein synthesis, these studies on cotton embryos suggest that in this system ABA may indeed sometimes function through inhibition of protein synthesis.

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Effects of application of exogenous ABA on various aspects of metabolism, including amino acid and protein metabolism (Huber, Kreutmeier & Sankhla 1977) and photosynthesis (Bauer, Huber & Sankhla 1976; McLaren & Smith 1976, 1977) have been reported but there is no evidence, as yet, that these aspects of metabolism are directly regulated by endogenous ABA.

12. Rapid effects of ABA

Although there is good evidence that all classes of plant hormone have marked effects upon nucleic acid and protein metabolism, it has become apparent that certain types of hormone, especially IAA, also have very rapid effects on growth and other processes. ABA has also been shown to have such rapid effects and under certain conditions can be shown to inhibit the extension growth of coleoptiles (Nissl & Zenk 1969; Rehm & Cline 1973) and of decapitated pea seedlings within 5 min of application (Warner & Leopold 1971). Similar rapid effects have been demonstrated for stomatal closure. These responses would seem to occur too rapidly to be accounted for by direct effects on nucleic acid and protein synthesis and it is generally held that they must be mediated via effects on cell membrane properties.

ABA has been shown to increase the permeability of carrot root cells to water (Glinka & Rhinhold 1971) and to inhibit the uptake of K^+ ions by various tissues (see below).

A remarkable effect of ABA on membrane properties is shown by its modification of the electrical properties of barley and mung bean root tips (Tanada 1973*a*, *b*). Exposure of mung bean root tips to red light causes them to adhere to glass charged with phosphates, an effect which is reversed by far-red light, indicating the involvement of phytochrome. A very low concentration (2×10^{-10} M) of ABA increases the attachment and this effect is antagonized by IAA. Comparable, though opposite, effects are obtained with barley root tips, where ABA promotes detachment and IAA attachment, to the glass. These effects, which apparently depend upon changes in surface electrical charges of the root cells, occur within 2 min of addition of ABA or IAA and seem best explained in terms of modifications of the cell plasmalemma by the hormones.

13. Ion uptake

Apart from the possible rôle of ABA in regulating K^+ exchange in stomatal guard cells (Mansfield & Wellburn 1978, this volume) the effects of ABA on K^+ uptake have been demonstrated in other plant responses. Thus, ABA inhibits K^+ uptake in slices from expanding *Vicia faba* leaves (Horton & Bruce 1972) and in beetroot tissue disks, ABA delayed the initial development of the uptake capacity for K^+, Na^+, and Fe^- (van Steveninck 1972), but later stimulated the uptake of these ions.

The effects of ABA on K^+ and Cl^- by *Avena* coleoptiles has also been investigated (Reed & Bonner 1974). ABA reduced K^+ uptake within 30 min after application, and Cl^- uptake was also inhibited though to a smaller degree than K^+ uptake. The effect did not appear to be the result of a general change in membrane permeability, since the uptake of organic molecules was influenced to a much smaller extent than that of K^+. Moreover, the effects of ABA on K^+ uptake did not appear to be due to the inhibition of growth by ABA, but there were close parallels between the effects of ABA concentration on K^+ uptake and growth and in the initial response curves after ABA application. It is currently held that auxin action in cell growth

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involves stimulation of a membrane-bound proton pump with passive influx of K^+ ions, and it is possible that ABA interferes with the proton-excretion process (Raschke 1977) or the related charge-balancing uptake of K^+.

14. Interactions between effects of ABA and those of other hormones

In considering the regulatory rôles of growth-promoting hormones (auxins, gibberellins, cytokinins) we find two apparently contrasting situations, namely (1) situations in which a process or response appears to be regulated by a single hormone, as in the control of coleoptile growth or cambial activity by IAA, and (2) situations in which control is determined by the relative levels of two different types of growth substance, as in the interaction between the effects of IAA and cytokinins in the regulation of lateral bud inhibition and regeneration of buds and roots in tobacco callus tissue.

The same two contrasting situations occur with respect to the effects of ABA in the regulation of growth and metabolism as seen (1) in the geotropism of roots, in which endogenous ABA appears to be the primary controlling factor, although other growth substances must no doubt be present at non-limiting levels; and (2) in the control of bud and seed dormancy, which appears to involve an antagonistic interaction between the effects of ABA and growth promoters, such as gibberellins and cytokinins (§§ 4 and 5).

Control systems which contain 'positive' and 'negative' elements are likely to be more effective and precise than those depending only on one such element; hence the notion that ABA may act in opposition to growth-promoting hormones as part of a complex hormonal control system seems attractive and plausible.

A number of examples of the apparently opposite and mutually antagonistic effects of ABA and the other main classes of growth-promoting hormones have already been mentioned and may be summarized as follows:

(*a*) Interactions with auxins: cell extension growth; cell surface electrical charges in roots (§ 12); abscission.

(*b*) Interactions with gibberellins: synthesis of α-amylase in barley aleurone (§ 11); dormancy of seeds and buds.

(*c*) Interactions with cytokinins: seed and bud dormancy (§§ 4 and 5).

(*d*) Interactions with ethylene: senescence and abscission.

In a number of examples of such 'interactions' the overall response depends upon the relative concentrations of the two growth substances, and frequently the effect of one substance can be overcome by increasing the concentration of the other. Moreover, we have seen that in a number of instances in which ABA antagonizes the effects of a growth-promoting hormone it does so very specifically and not as part of a more generalized inhibition. Thus, in barley aleurone, in which the production of α-amylase is stimulated by gibberellic acid and inhibited by ABA, neither of these growth substances produces its effects by causing gross changes in metabolism (respiration, phosphorylation, total protein synthesis, total RNA synthesis, etc.), but the effect of ABA is specifically to inhibit GA-enhanced processes such as GA-induced RNA synthesis, GA-enhancement of lecithin-synthesizing enzymes, membrane synthesis and hydrolase synthesis (Jacobsen 1973). Similarly, examples could be quoted for interactions of ABA with auxins and cytokinins.

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The nature of these various interactions between ABA and other types of growth substance is obscure. Since the action of growth substances is generally assumed to involve binding with specific 'receptor' molecules, the simplest model for a mutually antagonistic interaction between two types of growth substance would seem to be one in which ABA competes with a growth-promoting hormone for the same receptor site, so that the concepts of enzyme kinetics would be applicable. Attempts have been made to determine the interaction between the effects of ABA and IAA in the growth of coleoptile sections (Rothwell & Wain 1964) and between ABA and gibberellic acid in the production of α-amylase in the barley aleurone system (Kaufman & Jones 1974), and in both cases the results appeared to indicate a 'non-competitive' type of interaction. This result is not surprising, considering (1) the complexity of the biological system in which these two processes were investigated and (2) that the ABA molecule would be unlikely to compete equally with such dissimilar molecules as those of auxins, gibberellins and cytokinins at their respective binding sites.

On the other hand, in functional terms it does appear that ABA counteracts specific effects of IAA, gibberellins and cytokinins in different sytems, but how this is achieved is entirely unknown. However, it is not difficult to envisage how this may be achieved; for example, ABA may regulate the synthesis or inactivation of a growth-promoting hormone, and indeed there is some evidence that ABA may affect gibberellin levels in this way (Wareing *et al.* 1968*a*; Stolp, Nadeau & Rappaport 1973). There is also evidence that ABA can affect IAA synthesis in *Avena* coleoptiles (Anker 1975). Moreover, observations on the 'wilty' mutants of tomato (Tal & Imber 1970) indicate that they are deficient in endogenous ABA, but show symptoms of abnormally high levels of IAA, suggesting that ABA may play a rôle in the regulation of IAA levels in normal tomato plants.

15. Conclusions

It now seems clear that ABA plays an important rôle in the control of stomatal movement and in the geotropic responses of roots, and these two examples are in themselves sufficient to indicate that ABA does play a regulatory rôle in the plant. Thus, we seem to be justified in regarding ABA as one of the major types of endogenous plant growth substances. Although the present evidence for the rôle of ABA in other aspects of growth and metabolism is much less complete, it seems likely that it will ultimately be shown to have a wide functional rôle. This conclusion is based upon the following general considerations:

(1) Many aspects of growth and differentiation appear to involve two or more types of growth substance and, conversely, each class of growth substance appears to be involved in a wide range of processes.

(2) Application of exogenous ABA evokes a wide spectrum of responses, in which its effects interact specifically with those of other types of growth substance.

(3) Endogenous ABA appears to be present in all vascular plants and in a wide variety of tissues, suggesting that it has a wide functional rôle beyond the control of stomatal movement and root geotropism.

Although it may prove difficult to establish that specific processes are 'controlled' by ABA, it seems likely that it plays a rôle as an essential component of the general complex of plant growth substances.

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Discussion

J. W. Bradbeer (*Department of Plant Sciences, University of London King's College, 68 Half Moon Lane, London SE24 9JF, U.K.*). I consider that the most noteworthy feature of abscisic acid in the imposition of seed dormancy is that it is commonly found in quite high concentrations in the dead tissues which surround the embryo, namely in the testa and the pericarp. During imbibition the water which enters the embryo carries with it abscisic acid, from the testa and pericarp, and this abscisic acid contributes to the imposition of dormancy.

P. F. Wareing. Such a mechanism could hardly account for the dormancy of weed seeds which are known to remain in the soil in a dormant condition for as long as 50 years. It is likely that abscisic acid would be leached out in a much shorter time.

J. W. Bradbeer. My proposal refers to dormancy mechanisms by which seed germination is delayed until an appropriate time in the growing season following seed formation.

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