Hormonal Regulation in Higher Plants

Growth and development are regulated by interactions between promotive and inhibitory hormones.

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With the discovery of auxin some 40 years ago (*1*), Western physiologists thought they had found the key to the control of plant growth. This notion had to be revised however, with the revelation that the gibberellins, a group of compounds long-buried in the archives of Japanese science, were also native growth regulators (*2*). Further discoveries have now expanded the list to about five distinct groups of compounds, all of which appear to act in controlling specific aspects of plant growth. In addition to auxins and gibberellins, which are involved primarily in the extension growth of plant cells, the characterized plant hormones include cytokinins, abscisic acid and other inhibitors (*3*), and ethylene (*4*). Other postulated, yet uncharacterized, substances are the flowering hormone (florigen) and the wound hormone. Some evidence indicates that the former is related to gibberellin and the latter either to cytokinins or to a previously isolated compound called traumatic acid (*5*). The nature of the known plant hormones and their primary effects in controlling plant growth have been described recently (*3*). What we hope to do here is to indicate how interactions of these hormones may regulate some crucial developmental stages in the plant's life history.

It is becoming increasingly evident that hormones do not act alone in isolated systems but in an interrelated manner in the plant as a whole. Thus the proportions of various hormones present may vastly affect the growth rate or subsequent differentiation patterns of the tissue in the complete organism, while the presence of both promotive and inhibitory hormones permits a precise control of many developmental activities, in some cases, such as dormancy, on a stop-go basis. Initially then, we would like to examine how minute quantities of hormones may act to produce such large effects, and then to consider how the known hormones interact to control growth and morphogenesis in plants.

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Mode of Action of Plant Hormones

While we do not yet know the exact mode of action of any plant hormone, we know far more now than we did a few years ago. In the past, many enzyme and physiological systems have been invoked as the key points in the hormonal control of growth. However, almost all chemical correlates of hormone application have been shown to result from and not cause growth, or to have no direct relationship to the growth process. Auxins, having been known to plant physiologists for the longest time, received the most attention, and the early theories of its mode of action are well reviewed (*1, 5, 6*). It now appears that a partial answer to the action of plant, as well as animal hormones (*7*), lies in the control of the mechanism by which enzymes are made in the cell, that is, somewhere in the genetic information of the cell, which determines its ultimate potential, the messenger RNA (mRNA) which specifies the protein to be made, or the protein-synthesizing machinery involving the ribosomes and transfer RNA. Although most attention has been focused on the process of transcription of the DNA base sequence to mRNA, it is not certain that this offers the only method of control; messenger RNA's have been found to remain "dormant" in animal embryos (*8*), and control in these systems is exerted during transcription. In addition, growth is seldom an all-or-none phenomenon; rather it undergoes quantitative variations with many controlling factors. Hormonal growth control might thus involve an increase in the general protein-producing machinery rather than a triggering of the production of a particular protein. Auxin, for example, appears to cause an increase in the amount of ribosomal RNA present in the tissue (*9*).

The early work on the hormonal control of plant growth through control of nucleic acid and protein synthesis has been described (*3*). We shall therefore mention such work only briefly, and shall concentrate on the developments of the last 2 years. Some interesting advances have come from investigations of the cytokinins, which appear to form an integral part of certain transfer nucleic acids, and possibly thereby to regulate cell division and growth. Even the two "inhibitory" plant hormones ethylene and abscisic acid appear to exert their control through effects on nucleic acid metabolism, but they do so in different ways. Ethylene promotes nucleic acid and protein synthesis (*10*), which leads to the synthesis of degradative enzymes (*11*), while abscisic acid appears to shut down the entire process (*12, 13*). This enables an exact control over the protein-formation machinery to be maintained according to the levels of promotive or inhibitory substances already present in the different tissues. We shall now turn to a detailed examination of several of the hormones.

Gibberellins

In germinating barley grains the addition of gibberellin increases the hydrolysis of starch in the endosperm. This is due to the promotion of *de novo* synthesis of α-amylase by the gibberellin (*14*), which normally comes from the embryo (*15*). This enhanced enzyme synthesis also occurs with ribonuclease (*16*) and protease (*17*). The effect is possibly due to the gibberellin-stimulated formation of specific messenger RNA's in the cells of the aleurone layer which surround the endosperm (*14*).

In isolated aleurone layers (*13*) the addition of gibberellic acid caused the production of α-amylase after an ini-

tial lag period, and most of the enzyme was secreted from the aleurone layer into the medium. The hormone was required throughout the period of enzyme synthesis, as its removal caused the level of enzyme production to return to that of the aleurone layers not treated with gibberellic acid. Production of α-amylase was prevented by inhibitors of oxidative phosphorylation and protein synthesis, and by some inhibitors of RNA synthesis even when added after the gibberellin. These data are consistent with the hypothesis that the expression of the gibberellin effect requires the synthesis of enzyme-specific RNA molecules (*13*).

Further confirmation of enzyme synthesis *de novo* was obtained by incubation in $H_2{}^{18}O$ resulting in ^{18}O-labeled amino acids formed by hydrolysis of storage proteins. Use of ^{18}O-labeled amino acids in the synthesis of new protein was shown by the fact that the newly synthesized protein had a greater buoyant density in cesium chloride density-gradient centrifugation than protein from seeds incubated in unlabeled water (*17, 18*).

While the above findings provide some understanding of the action of gibberellin in germinating seeds, they provide no information on the effect of gibberellin on extension growth of the stem. Gibberellin application induces a pronounced increase in cell division in the stem apex (*19*), and this has been cited as a mechanism in gibberellin action leading to bolting in rosette plants. But growth results mainly from elongation of cells, and the role of gibberellin in this process is largely unknown. Preliminary investigations have shown that nucleic acid metabolism may be involved (*20*). Protein synthesis is required for growth induced by gibberellic acid, but experiments on the promotion of invertase synthesis by gibberellic acid in developing *Avena* internodes did not correlate with growth (*21*). Investigations of the effects of gibberellic acid on nucleic acid metabolism in isolated pea nuclei have now provided data that its presence enhanced the incorporation of tritiated cytidine triphosphate into the isolated nuclei (*22*). The addition of gibberellic acid preferentially enhanced (20 to 25 percent increase over control) the specific activity of RNA associated with DNA and of RNA tenaciously bound to methylated-albumin-kieselguhr, but had no effect on ribosomal RNA. The RNA synthesized by hormone-treated nuclei had a higher average molecular weight than that synthesized by control nuclei, and a quantitative change was found in the nucleotide composition of the RNA. It is thus clear that gibberellin is able to modify the RNA synthesized in isolated nuclei, and in this way it may exert its control over the growth and developmental processes in plants.

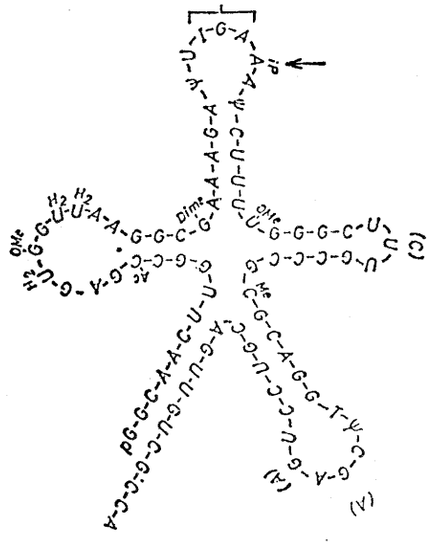

Fig. 1. The structure of serine transfer RNA showing the position of the cytokinin molecule (IPA, arrowed) adjacent to the anticodon (bracketed). [After Zachau *et al.* (*28*)]

Cytokinins

The cytokinins have been fully reviewed (*23*), but some findings on the occurrence of cytokinins in plants and contemporary thinking on their mode of action are worthy of comment. With the demonstrated presence of cytokinins in higher plants and the original derivation of kinetin from DNA, it was a natural step to examine nucleic acids for cytokinins. Several analyses of transfer RNA (tRNA) for cytokinin activity have now been undertaken. Activity occurs in hydrolyzed tRNA from yeast, *Escherichia coli*, *Corynebacterium fascians*, and calf liver, but none has been detected in hydrolyzates of ribosomal RNA (rRNA) (*24*). Fractionation of the tRNA showed activity in serine, isoleucine, and tyrosine tRNA's, but none in arginine, glycine, phenylalanine, or valine tRNA's. It was calculated that one active molecule per 20 tRNA molecules would be sufficient to account for the measured activity in the total tRNA. The presence of a cytokinin in tRNA hydrolyzates from a higher plant was first shown in RNA extracted from germinated corn grains (*25*). The cytokinin-active substance was fractionated but not identified. The first naturally occurring cytokinin [N^6-(Δ^2-isopentenyl)adenosine] (IPA) found as an integral part of a nucleic acid was identified from yeast tRNA (*26*). The same material was later identified in spinach and peas, whereas a hydroxylated derivative was isolated from the tRNA of immature sweet corn kernels (*27*). In yeast, the concentration was estimated as 0.1 mole percent of the total nucleotides, an indication that statistically it could only occur in certain tRNA molecules (*26*) and was clearly very low in concentration. Analyses of the nucleotide sequence of serine tRNA have shown that IPA is an integral part of this molecule (Fig. 1), but is absent from alanine, tyrosine, and phenylalanine tRNA's (*28*). This shows that only specific tRNA's may contain the cytokinin-active bases. In serine tRNA, IPA is adjacent to the anticodon (*28*) and is required for attachment of the tRNA to the mRNA (*29*).

Whether this might be the role of cytokinins in plants clearly depends on whether applied cytokinins are incorporated into tRNA. Experimental evidence both for and against incorporation has been presented. In one set of experiments benzylaminopurine labeled with ^{14}C was supplied to soybean and tobacco tissue cultures which required cytokinin for growth (*30*). The majority of the material was degraded, but 15 percent was detected in the tRNA extracted from the tissues, mainly as the nucleotide of 6-benzylaminopurine. Further fractionation revealed the labeled material was found in only one subfraction of tRNA, suggesting preferential incorporation of cytokinin into certain tRNA's.

Other studies contradict these results. For example, no cytokinin-requiring mutants of *Escherichia coli* have been found (*31*), despite the fact that IPA occurs in tRNA of this organism. This indicates that IPA itself is unlikely to be precursor of IPA in tRNA. In addition, radioactive 6-benzylamino-9-methylpurine, though active as a cytokinin, was not incorporated into any RNA fraction in soybean callus tissue (*31*). It has now been proposed that the IPA in tRNA results from the attachment of the isopentenyl group derived from mevalonic acid or Δ^3-isopentenyl pyrophosphate to a specific adenosine residue of preformed tRNA (*32*). This would resemble the intro-

duction of methyl groups into the bases of already formed RNA (*33*). When tRNA is catabolized, the nucleosides are released and enzyme systems have been detected which further degrade the IPA (*34*). Thus cytokinins may represent breakdown products rather than precursors of tRNA. An additional paradox is that ethanolic extracts of corn kernels contain the *trans* isomer of zeatin (*35*) while tRNA hydrolyzates from the same source yield the *cis* isomer (*27*).

Despite the occurrence of cytokinin in tRNA, it is therefore possible that the mechanism of action of these compounds lies elsewhere. Experiments with moss protonemata show that cytokinins, which cause bud formation, are only loosely attached to the "target cells" in which they act and can be easily washed out (*36*). The cytokinin did not act as a "trigger" but had to be present for the entire period of bud development, because if it was washed out during the early stages of development the buds reverted to protonemal filaments. Thus it seems as if, in this system, cytokinin may be binding to a specific site in the responding cells, and this may be the method by which they exert their control over development. The nature of the binding site and whether this type of action is common to other cytokinin responses are problems which await further investigation.

Auxin and Cell Elongation

It has long been known that there is a temperature-dependent lag phase between auxin application and the resulting growth response. This lag indicates that auxin does not act directly on growth but on some process which later alters the growth rate (*37*). The intermediate process is sensitive to inhibitors of both protein and RNA synthesis (*38*), indicating the necessity of these substances for auxin action; further, there is a parallel between the inhibition of growth and protein synthesis, as measured by the incorporation of ^{14}C-labeled amino acids.

Not only are RNA and protein synthesis essential to auxin-induced growth (*39*), but auxin causes an increased synthesis of both compounds (*9, 40*). In some growing systems, such as etiolated pea stem sections, auxin stimulates RNA and protein synthesis, but only after a lag period of 1 hour, by which time the rate of cell extension is nearly maximum (*41*). Hybridization studies showed that the RNA synthesized as a result of auxin application consisted to a small extent of mRNA fractions which were in low concentrations in untreated tissue, but mainly of rRNA which showed up as an increase in the polysome fraction. This stimulated RNA formation occurred initially in the nucleus and later in the cytoplasm (*41*). Antiauxins counteracted the effect of auxin (2,4-D) on RNA synthesis (*42*). In other experiments, 5-fluorouracil and low concentrations of actinomycin D partially curtailed RNA synthesis without affecting auxin-induced growth (*43*). Therefore it was hypothesized that the RNA required for auxin-induced growth was unaffected by the above treatments. The RNA had many characteristics of messenger RNA (composition similar to DNA, heterogeneity of size, the rate of formation, and turnover). It was also found associated with polyribosomes in the soybean root (*44*), further supporting its designation as mRNA.

Detailed kinetic studies of the effect of actinomycin D on growth (*45*) showed that some previously stockpiled substance was used during auxin-induced growth, but not in the absence of auxin. Regardless of when the inhibitor was applied, the addition of auxin always induced additional growth before the effect of the transcription inhibitor was manifested. Taken together with the above evidence, it would appear that some mRNA specific for auxin-induced growth exists which, in the presence of auxin, is translated into protein in the ribosomes. As this mRNA is utilized, more must be made by transcription for the auxin-induced growth to continue, and it is this process which is sensitive to actinomycin D. An alternative explanation is that there are two RNA's involved, one for the cell state preparatory to auxin action, and one for the action itself (*46*).

The major action of auxin in facilitating cell extension is to increase the plasticity of the cell wall, which is then stretched by water-uptake resulting from the osmotic potential of the vacuolar sap. Plasticity is a nonreversible wall deformation thought to be caused by the breaking of crosslinks between the cellulose microfibrils of the cell wall (*47*). The increase in cell size takes place in two stages, readily demonstrated by immersing stem sections in isotonic mannitol. Initially a wall-loosening process occurs which requires the presence of auxin and oxygen; this is followed by an osmotic uptake of water and by wall expansion, which requires neither auxin nor oxygen (*48*). In oat coleoptiles plastic wall deformation increased after the addition of auxin, reached a maximum after 90 to 120 minutes and then remained constant for up to 24 hours (*49*). Wall loosening occurred at turgor pressures greater than zero, while rapid cell extension only took place when the turgor pressure exceeded a critical value (*50*).

As RNA and protein synthesis are required for growth, they should also be essential for wall loosening if the auxin acts primarily on this process. Experimental studies on the influence of inhibitors on wall extension indicate that protein synthesis is indeed required. In the presence of inhibitors such as cycloheximide, auxin fails to induce any increase in tissue deformation, and the inhibitor may even reduce the deformation of tissues treated without auxin (*51, 52*). The evidence on the requirement for RNA synthesis is however conflicting (*52, 53*). Actinomycin D has been found to prevent growth and wall extensibility when added after auxin, but wall extensibility did not revert to that in the absence of auxin as it did on the removal of auxin; and the addition of auxin after actinomycin D still permitted some increase in cell wall extensibility in response to auxin (*52*). These studies indicate that although protein synthesis appears to be required for auxin-induced wall loosening, this is not necessarily coupled with RNA synthesis, and that the RNA synthesis may be required for processes other than wall loosening.

Enzymes may be required for auxin-induced wall loosening and the other factors of cell extension (*54*), though the nature of the enzymes is uncertain. Changes in protein patterns (*55*) and increases in cellulase synthesis have been detected (*56*) in the presence of auxin. When polyribosomes were extracted from plants treated with indoleacetic acid, they incorporated radioactive amino acids into cellulase more rapidly than control preparations, differences being evident within 15 minutes (*57*). Glucanases and pectic enzymes also increased after treatment with indoleacetic acid (*58*). However, these enzymes appeared only slowly in intact tissues, often over a period of days, far longer than the time required (about 10 minutes) for auxin to affect growth (*37*). External applications of

β-1,3-glucanase promote pea stem elongation to an extent similar to auxin within 30 minutes of application, but cellulase and pectin methylesterase did not have this effect (*59*).

The requirement for enzyme synthesis in auxin-promoted cell wall extension has been questioned by the demonstration that such extensibility is reversible in the presence of respiratory inhibitors. This reversibility makes it unlikely that wall loosening is mediated by polysaccharide-degrading enzymes, because their action is essentially irreversible (*60*). In addition to the function of proteins as enzymes, proteins containing hydroxyproline may act as stiffening agents within the cell wall. Although they were shown to be essential for auxin action, they were not the site of wall loosening (*61*).

For irreversible extension of the cell wall, both breakage and resynthesis of cross links would be required. Auxin promotes cell wall synthesis but not without prior cell extension (*62*). Some evidence showed that this is due to an increased uptake of sugar and also increased enzymatic levels in polysaccharide- (*63*) and cellulose-synthetase (*64*). Auxin was also found to cause an increase in new cell wall material within the existing wall in contrast to deposition at the wall surface. Since this internal deposition consisted mainly of hemicellulose, it has been suggested that internal incorporation of hemicelluloses might play a role in the cell wall expansion involved in plant growth (*65*). Hemicelluloses have also been suggested as the possible site of turnover during auxin-induced wall extension (*59*).

In summary, protein synthesis appears to be essential for auxin action, though it is not known whether the proteins produced deal with polysaccharide metabolism. Initially RNA synthesis is not required for the synthesis of these proteins and the action of auxin, although it is for continued auxin action, indicating the involvement of either long-lived mRNA or an auxin-specific mRNA already present in the cell. The major difficulty in invoking protein synthesis is that growth is measurable before any protein changes can be detected. Auxin-stimulated growth has been observed in 10 to 15 minutes in both oat and pea tissue (*37*, *66*). The time required for protein increases is approximately four times longer than these growth changes, although not all growth changes necessarily occur that rapidly. Unless our techniques of protein detection are not yet sensitive enough, we have to conclude that either the initial action of auxin is to cause qualitative rather than quantitative changes in protein synthesis or that, in these rapid responses, the auxin must act on some preformed system. This could involve activation of preformed proteins or a direct action within the wall. The continuance of the auxin effects on growth would then depend either on a feedback control to the protein-synthesizing system or on direct action upon this system. Perhaps it is this later process which has been investigated up to now, while the initial action of auxin is still to be elucidated.

Hormonal Interactions in the Regulation of Development

Strict control of growth processes can be maintained in the plant through the combined action of several regulatory substances. For example, various proportions of mobile promotive hormones can produce different ratios of cell division and cell enlargement, thus regulating the overall process of growth, or of tissue and organ differentiation. Further, the localized presence of nonmobile inhibitors can restrict the growth of certain plant organs such as buds, but other similar organs without the inhibitor can continue growth. Environmental factors, particularly light and temperature, can affect growth by controlling the type and amount of the various hormones present in any tissue through regulation of hormonal synthesis, transport, or destruction. Also, the ability of one hormone to elicit the production or destruction of another may lead to a chain of sequential growth-regulatory events, shown most clearly in the interactions of auxin and ethylene (*4*). A few of the more recently elucidated interactions will be discussed below.

Control by Relative Levels of Promotive Hormones

In the actively growing plant, form appears to be controlled mainly by the relative amounts of the promoting hormones present. Apical dominance and the monopodial growth habit are governed by an interplay of hormones and by the states of the various buds or shoots involved. Auxin, produced by the apical bud, is transported down the stem and prevents the enlargement of lateral buds by the production of ethylene at the buds (*67*). The localized application of kinetin directly to lateral buds has been found to overcome the inhibitory effect of native or applied auxin and enables the buds to commence development (*68*). Once the buds have started growing, auxin application is no longer inhibitory but enhances their growth (*69*). In some cases, however, auxin alone seemingly cannot be the controlling factor in lateral bud inhibition. For example, when the apical bud is replaced by enough auxin to give "exact substitution" for other growth phenomena, the lateral buds are not inhibited (*70*). It is possible that this is due in part to the lack of continuing auxin production when the bud is removed and replaced by an auxin application. In addition the removal of the sink effect of the apical bud on cytokinins, which are known to be produced by the roots (*71*), might enable cytokinins to accumulate in the stem and initiate the growth of the lateral buds.

In the culture of plant tissues the ratios of auxin to cytokinin also determine the type of growth. High relative concentrations of auxin promote root growth, high relative concentrations of kinetin promote shoot growth, while high concentrations of both lead to the continued growth of undifferentiated callus (*72*). Both auxin and cytokinin are required for secondary vascular tissue formation in radish roots in culture (*73*). Applied cytokinins can induce the mobilization of various substances (*74*) including auxin (*75*) to their site of application in leaves.

Gibberellic acid and auxin frequently produce a synergistic effect on growth. In dwarf pea stem sections this appears to be a direct interaction, not mediated through an effect of the former on auxin synthesis (*76*). Though gibberellin application does appear to raise auxin production (*77*), this is undoubtedly only one of many consequences of its application, and the numerous growth effects of gibberellin cannot be explained solely in terms of enhanced auxin production. The development of the secondary vascular system is influenced by the concentrations of both auxin and gibberellin. Applications of auxin have been found to stimulate cambial division and to cause the differentiation of xylem elements, while with gibberellin only the differentiation

of phloem tissue occurred (*78*). In *Ailanthus altissima*, the production first of xylem and later of phloem during the growing season has been correlated with the endogenous concentrations of auxin and gibberellin (*79*). Gibberellin, in addition to auxin, may also participate in the control of apical dominance in some species (*80*).

Abscisic Acid and Promotive Hormones

Abscisic acid (previously known as both dormin and abscisin II) was the first inhibitory hormone found to be involved in the control of growth together with growth promoters. Originally it was shown in the leaves of woody plants under short-day conditions (*81*) and also in maturing or senescing cotton fruit (*82*). More recently, its presence has been shown in several other species (*83*), and there are indications that it is ubiquitous in plants. Inhibitor fractions extracted from *Betula* were found to induce dormancy in actively growing *Betula* buds (*84*), and those from cotton to cause abscission of cotton petioles (*82*). Studies with synthetic abscisic acid (*85–87*) have shown that applications can cause the inhibition of germination, the cessation of extension growth, leaf senescence, and the formation of resting buds in woody species.

Gibberellin is known to cause flower initiation in some long-day plants (*88*), yet the hormonal control of flowering in short-day plants has remained obscure. Though the effect is far from universal, abscisic acid has been shown not only to counter the effect of gibberellin in long-day plants but also to cause flowering in some short-day plants under noninductive cycles (*85*). This indicates that either abscisic acid or some related hormonal compound may be a flowering hormone in short-day plants. The position of phytochrome in the system is not clear but it may, through control of differential membrane permeability (*89*), be directing the synthesis of these compounds. The fact that the compound which is promotive in some systems (short-day plants) is inhibitory in others (long-day plants) is contrary to the current ideas of Chailakhian (*90*), who envisages florigen to consist of gibberellin and a hypothetical compound called anthesin, both of which are required for floral initiation. It now seems better to envision a divergent phytochrome-controlled isoprenoid pathway, producing predominantly gibberellic acid in long photoperiods and abscisic acid in short photoperiods. Such a system could explain the control of bud dormancy (*84*). In reproduction, however, flowering could occur under either light regime, depending on the photoperiodic nature of the plant.

Initial indications that dormancy could be controlled by the interaction between endogenous inhibitors and promoters were obtained from the effects of inhibitor (abscisic acid) and gibberellic acid in *Betula* buds (*84*). Here it was found that emergence from dormancy relied not so much on a decrease in the concentration of inhibitors as on an increase in the concentration of gibberellin which promoted growth, overcoming the inhibitor-induced dormancy.

The possible importance of abscisic acid in the regulation of plant growth has led to numerous studies on its interaction with promotive hormones and to some speculation concerning its mode of action. Gibberellins have been found to overcome the effect of abscisic acid in the inhibition of elongation of genetically tall corn leaf sections and *Avena* coleoptiles (*91*); germination of seeds of *Fraxinus* sp. (*92*), *Corylus avella* (*93*), and lettuce (*86*); the sprouting of buds in potato (*94*); and the production of α-amylase by barley grains (*13*). From the opposing activities of gibberellin and abscisic acid it was suggested that the inhibitor might act as a gibberellin antagonist in vivo (*91*) or in some cases as an inhibitor of gibberellin biosynthesis (*12*). It is now clear, however, that, though this may be true in some instances, abscisic acid can also interact with other hormones. For example, cytokinins have been found to counteract abscisic acid in lettuce seed germination (*86, 95, 96*), radish leaf senescence (*86*), and the growth of cultures of *Lemna minor* (*97*).

In some cases abscisic acid is inhibitory only in the presence of a promoter, as in the germination of light-requiring Grand Rapids lettuce seed. Gibberellin will substitute for the light requirements, and its action is inhibited by abscisic acid. This inhibitory effect of abscisic acid cannot, however, be removed by increasing concentrations of gibberellin and is opposed only by the addition of kinetin, which alone has no effect (*96*). This indicates that gibberellin and abscisic acid must be acting on different systems; and, while kinetin addition is antagonistic to the inhibitor, at the same time it permits the gibberellin stimulation of germination. In a similar kind of interaction, abscisic acid also causes the inhibition of auxin-mediated growth of *Avena* coleoptile (*82*), and this is counteracted not by auxin but by gibberellin (*91*).

With this wide spectrum of interactions it is apparent that neither the process nor the growth promoter involved is specific to the type of abscisic acid interaction, different species displaying different reactions. The conflicting results with lettuce seed germination (*86, 95, 96*) indicate that even different varieties may behave differently, and also that the relative and absolute concentration of the hormones under consideration may be of importance. Whether the hormones display a competitive or noncompetitive interaction appears to be as much a characteristic of the biological system under consideration as of the precise inhibitor-hormone combination. Thus, in Great Lakes lettuce abscisic acid and gibberellic acid interact competitively in the control of seed germination and noncompetitively in hypocotyl extension (*86*).

The actual point of action of abscisic acid has not been elucidated, although as with several other hormones, some role in the control of nucleic acid and protein synthesis is indicated. Abscisic acid inhibits the synthesis of α-amylase in barley grains and is antagonistic to gibberellin in this process (*13*). The kinetics of inhibition of enzyme synthesis in this system resemble kinetics effected by the RNA synthesis inhibitors 8-azaguanine and 6-methylpurine. This suggests that abscisic acid may exert its action by inhibiting the synthesis of enzyme-specific RNA molecules or by preventing their incorporation into an active enzyme-synthesizing unit. Inhibition of RNA synthesis has also been found in *Taraxacum officinale* leaves (*12*), while in *Lemna* abscisic acid inhibits and cytokinin promotes DNA synthesis (*97*). Interaction between abscisic acid and promotive hormones could occur at many different points between the site of hormone action and the ultimate effects. With the effect of abscisic acid on nucleic acid metabolism and protein synthesis, it is not surprising that it interacts unspecifically with several promotive hormones.

Auxin and Ethylene

Ethylene, although known for many years to have a profound effect on plant growth, has only relatively recently, with the development of sensitive measuring techniques such as gas chromatography, been shown to be produced by plants. Some argument has developed as to whether the term "hormone" should be applied to the substance in that its "translocation" in the gas phase seems nonspecific, but there can be no doubt that it is a natural, mobile plant-growth regulator. It plays a role in such diverse processes as the onset of ripening in fruits (*98*), the production of the apical hook in etiolated seedlings (*99*), the production of transverse rather than longitudinal expansion of cells (*98*), and possibly in the abscission of leaves and fruits (*100*).

The response of plant tissues to auxin varies with the concentration of auxin applied. In each tissue, an optimum auxin concentration is found, with lower concentrations being promotive and higher concentrations inhibitory. The optimum promotive concentration is low for roots, intermediate for buds, and high for stems (*100*). The reason for the growth inhibition was for a long time unknown. It has now been shown (*4*), however, that in some plant systems auxin itself only promotes growth, and that it alone is never inhibitory. At certain critical concentrations of auxin, which are different for each tissue, the production of ethylene is induced. In etiolated pea stems, the start of ethylene production coincides with the auxin concentration yielding optimum growth promotion in the tissue; thereafter the evolved ethylene negates the promotion due to auxin. In the presence of constant external ethylene, increasing auxin concentrations never cause a growth inhibition, and it was calculated that the inhibition observed after treatment with high concentrations of auxin could be completely accounted for by the auxin-stimulated ethylene production. A similar situation was found to exist for etiolated sunflower stems. In corn and oat coleoptiles, however, though auxin did stimulate ethylene production, this did not correlate with the growth inhibition caused by auxin application. Thus, it seems that auxin-induced growth inhibitions must occur for basically different reasons in coleoptiles and seedlings such as sunflower and peas (*4*).

The role of endogenous auxin-induced ethylene production is uncertain. As described earlier, applied auxin inhibits the development of lateral buds, and this is opposed by cytokinin. It is now evident that this is at least in part mediated by ethylene (*67*), since ethylene production is stimulated by auxin application to buds, and the gas inhibits bud growth. Kinetin, which counteracts the action of auxin, was also found to counteract the effect of applied ethylene.

The maintenance of the apical hook in shoots grown in the dark also appears to be due to the production of ethylene (*99*). Ethylene production has been found to occur primarily in the hook and first node and is inversely related to the capacity of these tissues to destroy auxin (*101*). Application of ethylene will keep the hook closed in the light while CO_2, which antagonizes ethylene, promotes hook opening. Once germinating seedlings reach the light, the hook opens due to a light-promoted decline in ethylene production and increase in CO_2 production.

It has been proposed that the response of pea roots to gravity is due to the ethylene production and, therefore, to growth inhibition on the lower sides of the roots caused by concentrations of auxin greater than the optimum for growth promotion (*102*). While it is evident that, as in stems, applied auxin elicits ethylene production and that this gives a growth inhibition, the theory has been criticized on the basis that growth responses in pea roots caused by supraoptimal auxin concentrations are different from those caused by ethylene (*103*). Auxin appears to reduce growth more rapidly than ethylene; the auxin effect is reversible while that caused by ethylene is not, and even in the presence of ethylene the addition of high concentrations of auxin (10 to 200 micromoles per liter) further inhibits growth. The enhanced ethylene production might be contributory to the auxin inhibition of root growth (calculations indicated that 20 to 30 percent of the inhibition could be caused by ethylene), but it is evident that in roots, as in coleoptiles, the growth inhibition by high concentrations of auxin cannot be entirely explained by ethylene.

Hormones and Abscission

One of the interesting theoretical developments of the last few years has been the emergence of the concept that aging is an active process, controlled largely by hormonal interactions. In bean (*Phaseolus vulgaris* L.) endocarp (*104*), for example, both auxin and kinetin delay senescence through promotion of RNA and protein synthesis. In excised leaves, kinetin delays senescence and stimulates the synthesis of nucleic acids and protein (*105*).

A complex of hormonal interactions involved in the control of leaf abscission is reviewed in detail in an entire recent issue of *Plant Physiology* (*106*). A general symptom of senescence in plant tissues is a decline in the rates of nucleic acid and protein synthesis. Abscission, however, has been shown to involve an active rejection of tissues distal to the abscission zone (*11*), not just a passive break. Evidence suggests that, during leaf abscission, products of senescent tissues exported from the leaves regulate the activities of enzymes in cells of the abscission zone, particularly the enzymes involved in the breakdown of the cell wall. Thus the separation at the abscission zone has been found to result from enhanced, localized RNA synthesis and enzyme activity. An increase in cellulase occurred in the cortical cells at the pulvinar-petiole tissue interface only when senescence took place in cells distal to the interface. Ethylene, which promotes abscission, also enhanced localized RNA and protein synthesis (*10, 107*), while inhibitors of RNA and protein synthesis prevented abscission in the presence of ethylene. When senescence was retarded in pulvinar cells by auxin, abscission was also retarded, and any treatment accelerating senescence (for example, ethylene) stimulated abscission (*11*).

The aging of tissues is important not only in the production of senescence factors by the leaves but also in the response of abscission zone tissues to a given substance. Ethylene, for example, promoted petiole abscission only if applied 48 hours or more after removal of the leaf blade (*108*). Auxin generally inhibits abscission but can have promotive effects depending on the age of the tissue to which it is applied (*109*). When applied to petioles from which the leaf blades have been excised, auxin was shown to delay abscission only if given during the first few hours after deblading; thereafter it promoted abscission. It appears that, as the leaf approaches senescence, the inhibitory effect of auxin is lost and abscission

becomes increasingly responsive to the promotive effects of other factors. It has been suggested, therefore, that abscission results in part from an increase in sensitivity to ethylene which is constantly produced (*110*). This theory fails, however, to account for the promotion of abscission by auxin under some conditions.

Abscission is therefore clearly the result of a complex interaction of hormones—senescence factors, ethylene, auxin, and possibly also gibberellins (*111*). Although it is apparent that applied ethylene will cause abscission in detached aged tissue, the actual participation of ethylene in natural abscission remains uncertain. Auxin produced in young leaves inhibits abscission, and the decline in auxin production as the leaf ages has been implicated in abscission, especially since applied auxin may inhibit abscission. However, a burst of auxin production has been noted during senescence of detached leaves (*112*). The promotion of abscission by auxin is probably due to auxin-stimulated ethylene production, since the promotion can be decreased by the removal of the accumulated ethylene (*108*). This process may well bring about abscission in abscission zone tissue already aged through the action of senescence factors diffusing from the leaves. Alternatively it has been hypothesized that senescence factors themselves are responsible for the rise in the enzyme formation involved in abscission (*11*). This would eliminate abscisic acid as a senescence factor because abscisic acid has been shown to inhibit RNA and protein synthesis (*12*), and it is also ineffective in promoting abscission when applied to intact leaves (*85*).

Genetic, Hormonal, and Environmental Interaction: Peroxidase Activity

A recurrent theme in our discussion of the mechanism of action of hormones and environmental controls has been their regulation of the synthesis of particular enzymes which may be involved in growth and development. In this connection, it is instructive to consider the enzyme peroxidase, since the interaction of the various factors which control growth and development is paralleled by their interaction in the control of the activity of this enzyme in cells. While the exact biological function of this versatile heme enzyme is not known (*113*), morphogenetic roles are suggested by its action in producing (*114*) and inactivating auxin (*115*), in converting hydroxyphenylpropanes such as coniferyl alcohol to lignin-like materials (*116*), and in oxidizing such important metabolic compounds as reduced nicotinamide-adenine dinucleotide and its phosphate (*117*).

The peroxidase activity of plants is dependent on their genotype. Thus, many genetic dwarfs have abnormally high peroxidase activity (*118*). When certain dwarfs, especially single-gene mutants, are treated with gibberellin to relieve dwarfism, their peroxidase activity falls precipitously as growth is promoted (*119*). Conversely, when normal genotypes are stunted by application of compounds which prevent gibberellin biosynthesis, the peroxidase activity rises markedly (*120*). From these reciprocal effects, it would appear that low gibberellin is conducive to high peroxidase and low growth rate. they do in the control of growth. In The application of external gibberellic acid depresses peroxidase synthesis and speeds growth, while gibberellin antagonists stimulate peroxidase synthesis and retard growth. On the basis of such results, it has been suggested that dwarfism is due to the abnormally high peroxidase, which might act through auxin destruction. Although external auxin application does produce growth enhancement in some dwarf genotypes (*121*), this is small in comparison with the gibberellin effect. Presumably, the elevation of peroxidase in gibberellin-deficient plants is so great as to permit ready oxidation of any concentration of applied auxin.

Within any given plant organ, the development of peroxidase activity is a specific and orderly process, progressing steadily from the young to the old cell (*122*). In etiolated pea stems, the peroxidase activity is inversely proportional to the growth rate and to the ability of the cells to respond to auxin (*123*). In such tissue, the development of peroxidase activity may be considered a part of the normal aging process. In peas, as in many other plants, the peroxidase activity can be resolved into a number of isozymes electrophoretically separable on starch gel (*122*). When the peroxidase isozymes of green pea stems are examined as a function of the aging process, it appears that aging is associated with the formation of particular isozymes, and that when auxin is applied to the tissue, these isozymes fail to appear (*124*). The inhibitory effect of gibberellin on the development of the peroxidase activity of dwarfs is also exerted on specific isozymes (*125*).

The young pith cells of the stems of geranium and tobacco lack peroxidase activity completely. In the geranium plant, pith cells never form peroxidase *in situ*, but they do so within several hours after excision (*126*). Whereas the nature of this control is not known, injury appears to derepress the genes controlling peroxidase formation. There seems to be but a single geranium peroxidase component in the usual electrophoresis experiment; its formation is inhibited by auxin and promoted by kinetin. These two substances interact in the control of peroxidase activity as they do in the control of growth. In tobacco pith, peroxidase appears progressively as the cells age, and there is a strict inverse correlation between peroxidase activity and growth of the explant in pure culture. All of the activity can be resolved into two isozymes (A_1 and A_2) migrating to the anode at *p*H 9.0 (*127*). When such tissue is excised and placed in a culture medium, it immediately starts to form two new isozymes (C_1 and C_2) which migrate toward cathode (Fig. 2). Auxin represses and kinetin promotes the synthesis of these isozymes (Fig. 3). After about 120 hours, still another isozyme (C_3) can be detected, but only if the medium contains auxin. In view of (i) the delay in this induction effect, (ii) the known stimulation of ethylene production by auxin, and (iii) the induction of peroxidase activity by ethylene (*128*), we believe that C_3 appearance is elicited by ethylene. Thus it appears that four different plant hormones, gibberellin, auxin, cytokinin, and ethylene can control the level of peroxidase activity in different tissues. This further serves to illustrate the complexity of hormonal interactions during development.

But this is still not the entire story, for the synthesis of an enzyme protein is no guarantee that it will act in the cell. When peroxidase destroys auxin, it functions as an oxidase, and requires Mn^{++} (*129*) and a monosubstituted phenolic cofactor (*130*). In completely etiolated pea stems, the cofactor is the flavonoid kaempferol, which has a 4′-OH group and is present in low concentration. When the plant is illuminated with red light, transforming the red absorbing form of phytochrome to the far-red absorbing form, kaempferol synthesis is increased in stems, and the resultant increase in indoleacetic acid

oxidase activity could account for the decline in stem growth rate after red irradiation (*131*). In leaves, the same red light induces the formation of a related 3′,4′-dihydroxy flavonoid, quercetin (*132*), which serves as an inhibitor of indoleacetic acid oxidase (*133*) and could account for the promotion of leaf growth. The light-induced formation of these compounds is made possible because of the induction of the enzymes phenylalanine ammonia lyase (*134*) and cinnamic hydroxylase (*135*) which cooperatively produce coumaric acid from the phenylalanine precursor.

Thus, the enzyme peroxidase exemplifies the interaction of genetic, developmental, hormonal (gibberellin, auxin, ethylene, and cytokinin), and external (wounding, light) influences in the control of a single chemical activity. Similar schemes could undoubtedly be produced for other enzymes, and, when all these are put together, they will furnish some basis for an understanding of plant development.

Summary

Summing up the status of our knowledge of plant hormones 2 years ago, van Overbeek (*3*) concluded that much of our information was contradictory and many important gaps existed. While the same statements can be made today, some progress has occurred and some insight into the mode of action of several plant hormones has been obtained. In addition, characterization of the wide spectrum of the action of "inhibitory" hormones and their interactions with the "promoting" hormones gives us a clearer understanding of the regulation of plant growth, and of interactions between plant and environment.

With the embryo plant in the seed, development is kept in check by the presence of natural inhibitors. A germination stimulus such as light or temperature may reduce the content of inhibitors and also increase the gibberellin level in the seeds (*136*). The increased gibberellin might then overcome the effects of the inhibitors, and promote germination by enhancing the enzymatic digestion of the stored food reserves. The young growing tips of the plant's organs respond to gravity and light as a result of the redistribution of auxin and possibly growth inhibitors though the reason for the inhibition of growth in roots is uncertain.

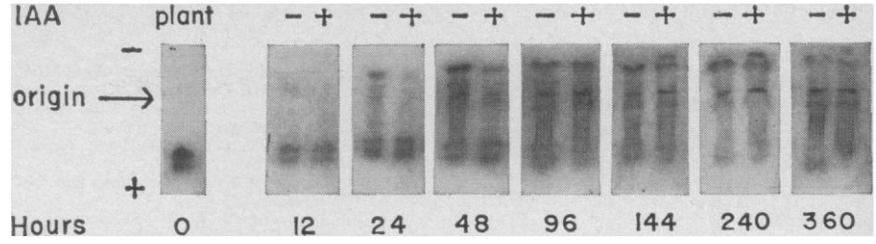

Fig. 2. The effect of the auxin indoleacetic acid [indoleacetic acid (IAA); 2.0 μg/ml] on the isoperoxidase pattern of tobacco stem pith aseptically cultured on modified White's medium. Electrophoresis on a starch gel: 10 volt/cm; NaOH-borate buffer, *p*H 9.0, 5°C; 70 minutes. Development with 5 m*M* guaiacol and H_2O_2. The original pith had two anodic isozymes; within 12 hours in culture, new cathodic isozymes develop, which are repressed by IAA. By 96 hours of culture, this repression has disappeared, and beyond this point a unique, rapidly moving isozyme appears only in cultures containing IAA.

Abscisic acid may also be involved in phototropism (*137*). Once the apical hook is above ground its opening is regulated by a phytochrome-mediated cessation of ethylene production. The rate and kind of growth accomplished by the plant is then determined by the proportions and positions of the auxins, gibberellins, and cytokinins. With the advent of adverse conditions a change in auxin content and a rise in inhibitor concentrations brings about abscission of the leaves. A decreasing supply of cytokinins from the roots has also been suggested to operate in leaf abscission by removing the previous block to senescence and the previous counteraction of any inhibitors (*95*). Short days cause a rise in inhibitor concentrations, which results in dormancy of the buds. Chilling of buds causes an increase in gibberellin content (*84*) which counteracts the inhibitors present and results in resumed growth on the return to clement conditions. Photoperiods favorable to flowering bring about changes in the hormonal balance, still uncharacterized, but possibly in the ratio of gibberellins to abscisins plus other inhibitors. The hormonal control of flowering is still poorly understood, despite the fact that the concept of a flowering hormone, "florigen," is over 30 years old.

Hormonal action, even of the naturally occurring inhibitor, abscisic acid, appears to be connected with the control of nucleic acid metabolism. Thus, several hormones appear to control development by regulating the ultimate expression of genes, that is, the coding for enzymes which modulate the different phases of plant growth. But as the relation between hormones and the genetic control of enzyme formation becomes more firmly established, it becomes equally clear that this mechanism does not supply a complete answer to the problem. The rapid effects of auxin on growth indicate that we still have to search for an alternate primary mode of action of this class of hormones; the interaction with the nucleic acids might then account for the continuation of the response. Even so, it is striking that we do not understand the

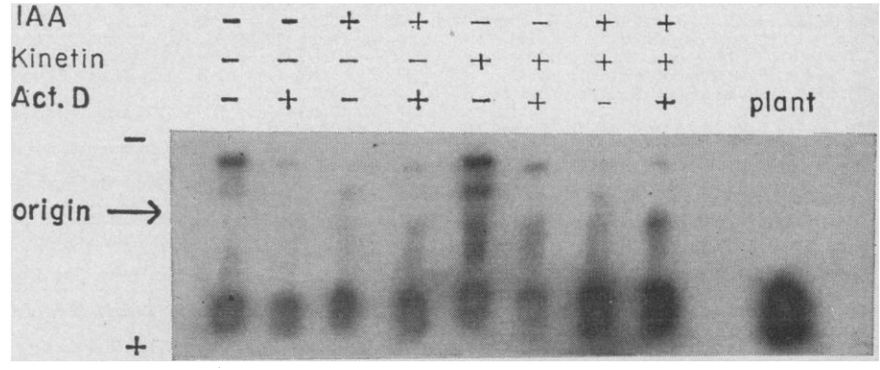

Fig. 3. The effect of IAA, kinetin and actinomycin D on early isoperoxidase patterns of cultured tobacco pith. Conditions as in Fig. 2. Kinetin alone promotes enzyme formation, while IAA and actinomycin D inhibit the appearance of the new activity. IAA, 2.0 μg/ml; kinetin, 0.2 μg/ml; actinomycin D, 4 μg/ml; 24 hours in culture.

details of the control of nucleic acid action by hormones. While we have made some recent progress, a coherent answer, as investigators of animal hormones are also finding, is still some distance away.

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Enzymatic Mechanism of Steroid Hydroxylation

Reduced nicotinamide-adenine dinucleotide phosphate serves two distinct roles in hydroxylation.

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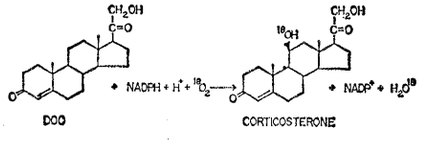
Enzymes that incorporate one atom of molecular oxygen into substrates while concomitantly reducing the other atom to water are termed mixed-function oxidases (*1*) or monooxygenases (*2*). The stoichiometry of this type reaction may be represented as follows:

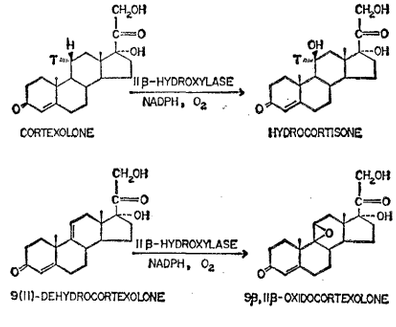
$$RH + O_2 + H_2A \longrightarrow R\text{–}OH + A + H_2O$$

RH is the substrate, and in most instances, the reductant; H_2A is either reduced nicotinamide-adenine dinucleotide phosphate (NADPH) or reduced nicotinamide-adenine dinucleotide (NADH). The importance of monooxygenases in metabolism is evident from the wide variety of substances they attack—such as, carbohydrates, lipids, amino acids, drugs, and hormones. Although considerable efforts have been devoted in the past decade to defining the enzyme components, coenzymes, and cofactors required for oxygenation, the mechanism of action of monooxygenases on a molecular level remains poorly understood. Studies on the properties of monooxygenases have been complicated by the particulate nature of the enzyme components and their relative instability. However, some progress has been made in recent years with the steroid 11β-hydroxylase (E.C.1.14.1.6) system of adrenal cortex mitochondria. In this article, the mode of action of monooxygenases with special reference to the functional roles of the electron donor NADPH is discussed.

The hydroxylation of deoxycorticosterone (DOC) at carbon-11β by adrenocortical mitochondria requires NADPH (*3*) and has been studied by the incorporation of ^{18}O from molecular oxygen into the substrate molecule, DOC (*4*). Although the stoichiometry for NADPH in this reaction is not established, there is nearly a one-to-one correlation between oxygen consumed and DOC hydroxylated (*5*). Therefore, the 11β-hydroxylase of adrenal cortex mitochondria resembles a classical mixed-function oxidase.



Enzymatic hydroxylation of steroids is known to proceed with retention of configuration (*6*), a course reminiscent of electrophilic displacement reactions. Steroid hydroxylases appear to obey the rule of Bloom and Shull (*7*) that a hydroxylase which is capable of introducing an axial hydroxyl at a given carbon atom (C-n) is also capable of converting a dehydro-substrate into an oxide also "axial" at C-n. For example, incubation of 9(11)-dehydrocortexolone with the 11β-hydroxylase of adrenal cortex mitochondria in the presence of NADPH and O_2 results in the formation of 9β,11β-oxidocortexolone (*8*). Furthermore, it has been shown that the dehydrosteroid and the corresponding saturated steroid compete for the same enzyme site (*9*). On the basis of these observations, it is likely that the so-called "active oxygen" in enzymatic hydroxylations may be the electrophilic OH^+ species.



Although the requirement of several components for steroid hydroxylation had been reported (*10*), the functional role of these components remained unknown until the studies of Omura and co-workers (*11–13*) and of Kimura and his group (*14–16*). The mitochondrial 11β-hydroxylase system has now been resolved into three components: flavoprotein or adrenodoxin reductase; nonheme iron protein or adrenodoxin; and hemoprotein or cytochrome P-450 (P-450).

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