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REVIEW

Multiple hormonal control in plants

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INTRODUCTION: COMPLEXITY OF HORMONAL CONTROL

Hormones are but one class of compounds to regulate developmental processes in plants; however, they are probably the most important mediators in signal transduction.

The classic concept of a plant hormone defined by Went & Thimann (1937) as a substance being produced in one part of the organism and then transferred to another part to influence a specific physiological process is, as expected after more than 60 years of plant hormone research, inadequate to explain hormonal regulation of development in plants. The complexity of multiple hormonal regulation is illustrated by the fact that each of the hormones have been found to be able to affect nearly every phase of plant growth and development (Leopold & Noodén 1984). The seeming lack of exclusive control of any one step of development by any one of the known hormones as well as the lack of correlation between hormone concentrations and changes in developmental processes has led to doubts as to whether the plant hormones are indeed the regulators of development (Trewavas 1980, 1983). In this connection it was postulated that hormonal control in development may be due to changes in the sensitivity to the hormones (Kende & Lang 1964; Trewavas 1983). Recently, Bradford & Trewavas (1994) proposed a quantitative model based on sensitivity threshold distributions and proportional rate responses to regulatory factor levels, which can account for a wide range of phenomena in plant growth and development.

The complexity of hormonal regulation is further compounded by evolutionary plasticity which may have led to substantial differences even between related plants. One need only look at the tremendous differences in fruit structures between species to realize that development (and its underlying hormonal controls) may differ greatly even between taxonomic related plants (see Esau 1977; Coombe 1976). Plant development is the result of an intricate spatial and temporal multiple hormonal control through the

regulation and expression of multiple gene systems. However, the complexity of the pleiotropic effects of plant hormones may well result from a single primary action of the hormone, a single effect on gene expression. That is, though different things happen in different cells, the molecular basis by which the hormone acts may still be the same.

Hormone actions are studied at several different levels. Progress has been made on the physiological, biochemical, biophysical and molecular biological level. In recent years molecular genetics studies have gained more importance. The physiological and molecular analysis of hormone mutants, generated mainly in *Arabidopsis*, has contributed greatly to our knowledge of hormone biosynthesis, perception and transduction (Koornneef *et al.* 1994). Parts of the molecular approaches to studying hormonal regulation of plant development have been reviewed by Van Loon & Bruinsma (1992).

There are numerous examples of developmental processes that are influenced by more than one hormone. Multiple hormonal control may be based on different types of interaction between hormones and can very well be located at different levels: physiological, biochemical as well as molecular. This review is confined to multiple hormonal control and discusses the rapid progress made in recent years, especially through molecular-genetic studies on hormone-regulated gene expression. Although several new compounds, e.g. jasmonate (Staswick 1992) and oligosaccharides (Ryan & Farmer 1991), have been discovered in recent years and may be considered as plant hormones, the role of these compounds will not be included.

SIGNAL PERCEPTION AND TRANSDUCTION

An important aspect of hormone regulation is signal perception followed by transduction. The transduction of signals involves probably the activation of a major part of the plant cell proteins, since cells have to perceive a variety of external signals, e.g. light, temperature, nutrients, several forms of stress and so on. This perception is transduced through internal signals or messengers into an appropriate response by activating receptors through binding. This may then lead to alterations in other cellular components, resulting eventually in changes in ion conductivity, cell shape, gene activity or cellular functions. Signal transduction sequences start with signal receptors. Variation in the number of receptors and their distribution can alter the sensitivity of cells to hormones or other signals as well as sensitivity changes during development. In addition, there may be several receptors for the same signal which allows for a more complex response.

Plant hormones are considered an important class of internal signal compounds which are easily transported to target cells and have been shown to be directly involved in transcriptional or translational control of gene activity in a number of plant processes. It is still a widely held view that plant hormones exert their action through recognition by specific receptors in responsive cells which initiate the transduction of the hormonal signal into specific biochemical and physiological events. However, most major questions concerning signal transduction in plants remain unanswered. For example, do plants use signal transduction systems similar to those of microbes and animals? Are the ligand binding proteins true receptors, and if so, how do they transduce the signals? Are there signal transduction chains unique to plants?

Site of perception and receptors

The site of perception of hormones is a hardly explored area in plant physiology. However, some recent progress has been made as to probable sites of perception of auxin, recently reviewed by Jones (1994), cytokinins, recently reviewed by Binns (1994), GA and ABA in aleurone cells (Gilroy & Jones 1994) and of ABA in guard cells (Anderson *et al.* 1994).

Several auxin binding proteins (ABPs) have been identified during the last decade. They were found in several locations in the cell, like the endomembrane system from which ABP cDNA clones have been isolated (Hesse *et al.* 1989; Inohara *et al.* 1989, and Tillmann *et al.* 1989), the nucleus and soluble ABPs (overview by Jones 1994). However, the knowledge gained from this research is still too limited to explain the complex perception and action of auxin in plant cells.

For cytokinins the situation is even less clear. Although some cytokinin binding proteins have been reported (by Binns 1994), none of these were proven to be cytokinin receptors.

For ABA and GA, the results indicate that these hormones are perceived by outward-facing membrane receptors. These outward-facing membrane receptors are believed to be concerned with rapid responses of cells to transient signals (Allen & Trewavas 1994), while cytoplasmic perception may be slower with long minimal occupancy times and concerned with more prolonged but qualitatively different aspects of the response. Recent developments in the genetic approach to signal transduction use model systems, particularly *Arabidopsis*, which will allow the purification and cloning of the first putative plant receptors. In *Arabidopsis* at least two different hormone binding sites have been described. Sanders *et al.* (1991a) found two ethylene binding sites with similar binding affinities but with different association and dissociation constants.

These results indicate, and the conclusion may also hold for data obtained with other hormone binding sites, that not all these binding sites may function as a receptor and that binding to distinct sites in the same tissue may initiate different responses (Sanders *et al.* 1991b).

In addition, patch clamp studies are uncovering novel channels involved in cellular signalling (Ruck *et al.* 1993), also in relation with hormone interaction (Marten *et al.* 1991). Marten *et al.* (1991) demonstrated a direct interaction of auxins with the extracellular face of anion channels in the plasma membrane of guard cells. Furthermore, Schroeder *et al.* (1987, 1989) demonstrated that ABA induces stomatal closure by activating voltage dependent anion-channels. These studies are starting to reveal the molecular mechanisms underlying the functional properties of plant cells.

Transduction pathways

Broad similarities in the transduction pathways between plant and better studied animal systems do exist; however, there may be considerable differences in detail (see review by Verhey & Lomax 1993). For example, plants do not appear to contain a signalling pathway based on cyclic AMP; at least, cAMP is not present at a detectable level in plant cells (Trewavas & Gilroy 1991); nor do they appear to contain an enzyme regulated precisely like PKC (protein kinase c); plants do contain CDPK (Ca^{2+}-dependent protein kinases), an enzyme unique to plants that may not be utilized in animals. The involvement of Ca^{2+} and K^{+} channels as second messenger systems is well established in plants (MacRobbie 1991; McAinsh *et al.* 1991; Verhey & Lomax 1993).

A transduction cascade that is believed to be present in both animals and plants comprises the MAP (mitogen activated protein) kinases. These are serine/threonine protein kinases and mediate intracellular phosphorylation events linking extracellular signals, among them membrane depolarization (Rosen *et al.* 1994), to different cellular targets (for a review see Jonak *et al.* 1994). So far, MAP kinases (MAPK), MAP kinase kinases (MAPKK) and MAP kinase kinases (MAPKKK) have been identified (Jonak *et al.* 1994; Blumer & Johnson 1994). Such a cascade of phosphorylating enzyme is proposed for the ethylene signal transduction pathway in *Arabidopsis* (Kieber *et al.* 1993). For ABA, GA, ethylene and auxin response sequences have been found, which may be involved in the signal transduction pathway, e.g. Marcotte *et al.* (1989) and Mundy *et al.* (1990) for ABA, Souza & King (1991) and Gubler & Jacobsen (1992) for GA, Gil *et al.* (1994) and Hagen *et al.* (1994) for auxin and Ohme-Takagi & Shinshi (1994) for ethylene. These response sequences will be dealt with in more detail further on.

At this stage, however, signal transduction in plants has not yet revealed the variety in transduction systems existing in animal systems which is necessary for a finely tuned cross-talk between signalling pathways. It may be that more fundamental signalling pathways are yet to be discovered in plants.

In the last decade, emphasis has shifted to molecular approaches to study events in hormone-regulated gene expression, especially by using single gene hormone-deficient mutants or gene introduction systems with *Agrobacterium*. Rapid advances are made by using hormone mutants, mainly isolated from *Arabidopsis* (see review by Reid 1993), which are beginning to provide details on the elements in the hormone–response pathway.

Analysis of hormone mutants in *Arabidopsis* revealed recently a number of kinases and phophatases and other compounds involved in the transduction pathways of ethylene, ABA, auxin and GA.

For example, phosphorylation–dephosphorylation is known to control the activities of a number of plant enzymes. For instance, the ethylene signal is transduced via protein phosphorylation events in plants (Raz & Fluhr 1992, 1993). Ethylene is a plant hormone involved in a plethora of abiotic and biotic environmental stresses as well as aspects of plant development and climacteric fruit ripening. Sensitivity to ethylene is presumably mediated by a specific ethylene receptor whose activation signal is then transduced via an unknown cascade pathway. Raz & Fluhr (1993) have used the plant pathogenesis response, exemplified by the induction of pathogenesis-related (PR) genes, as a paradigm to investigate ethylene-dependent signal transduction in plant cells. Ethylene application induced very rapid and transient protein phosphorylation in tobacco leaves. In the presence of the kinase inhibitors H-7 and K-252a, the transient rise in phosphorylation and the induced expression of PR genes were abolished. Similarly, these inhibitors blocked the response induced by an ethylene-dependent elicitor, α-AB. Reciprocally, application of okadaic acid, a specific inhibitor of phosphatases type 1 and type 2A, enhanced total protein phosphorylation and, by itself, elicited the accumulation of PR proteins. In the presence of H-7 and K-252a, PR protein accumulation induced by okadaic acid was blocked. In contrast to the action of ethylene and α-AB, xylanase elicits the accumulation of PR protein by an ethylene-independent pathway. Xylanase-induced PR protein accumulation was not affected by H-7 and K-252a. These results indicate that responsiveness to ethylene in leaves is transduced via putative phosphorylated intermediates that are regulated by specific kinases and phosphatases.

Kieber and co-workers (1993) cloned the *CTR1* gene which acts as a negative regulator of the ethylene response pathway in *Arabidopsis*. Plants carrying a mutated CTR1 gene show a constitutive ethylene response phenotype and the gene encodes a putative serine/threonine protein kinase that is most closely related to the Raf protein kinase family. The Raf protein clearly acts as a MAPKK (Kyriakis *et al.* 1992; Howe *et al.* 1992; Dent *et al.* 1992); therefore, it is suggested that the *CTR1* gene product acts similarly (Kieber & Ecker 1993). Furthermore, they established the position of this gene in the signal transduction chain in relation to two other ethylene-responsive mutants *ein1* and *ein3* (Guzman & Ecker 1990). The *CTR1* gene product acts at or downstream the *EIN1* gene product and at or upstream the *EIN3* gene product (Kieber *et al.* 1993). The cloning of the gene *ETR1* (which is probably allelic to *EIN1*, Kieber & Ecker 1993) from the ethylene-insensitive *Arabidopsis* mutant revealed that this gene product may act as a sensor of ethylene (Chang *et al.* 1993). It has homology with the prokaryotic two-component system and may be involved in transduction by transfer of phosphate. Its mode of interaction with ethylene and the target of the *ETR1* gene product remains unknown. Since *CTR1* codes for a protein kinase with similarity to the Raf protein kinase family and is downstream of *ETR1* action. Chang *et al.* (1993) proposed a similar interaction between CTR1 and ETR1 as has been described for Ras protein and Raf protein interaction (Avruch *et al.* 1994). It was recently found that the Ras protein codes for a GTPase (Stokoe *et al.* 1994; Leevers *et al.* 1994) and was found to regulate a protein kinase cascade (Nishida & Gotoh 1993) that may involve a MAPKK (Hall 1994).

Moreover, the *ABI1* gene, one of the three genes (the others are *ABI2* and *ABI3*) that confers ABA insensitivity in *Arabidopsis* (Koornneef *et al.* 1984) has been cloned recently by two laboratories independently (Leung *et al.* 1994 and Meyer *et al.* 1994) and its predicted function is a Ca^{2+}-modulated serine/threonine protein phosphatase 2C.

The results described above clearly demonstrate the involvement of kinases and phosphatases in the transduction pathways of several hormones; the next step will be to identify the upstream and downstream activators of these proteins.

HORMONE-REGULATED GENE EXPRESSION: HORMONE-RESPONSIVE ELEMENTS

The involvement of plant hormones in the regulation of gene expression is now well-established (see review by Ho & Hagen 1993). A number of hormone-responsive genes and cDNAs have been isolated and characterized. These genes are proving to be valuable molecular probes to study the mode of action of plant hormones and these studies indicate that the regulatory mechanisms of these genes are quite complex. Several elements in a gene promoter, as well as multiple DNA-binding proteins, need to interact with each other in order to elicit the developmentally regulated, tissue-specific, and hormone-induced gene expression. The products of these hormone-regulated genes are supposedly involved in the physiological effects of hormones. However, although hormone-induced gene expression may play a pivotal role in hormone action, the function of many hormone-induced genes remains unclear.

Little or nothing is known about how hormone-binding proteins trigger 'downstream' events, i.e. the hormone signal transduction pathway remains largely unexplored. Another ABA responsive gene, *VP1* in maize, was cloned and it encodes a novel transcription factor (McCarty *et al.* 1991). It was suggested that the *VP1* gene product

activates the *C1* regulatory gene during seed mutation by interacting with one or more ABA-regulated transcription factors (Hattori *et al.* 1992). A similar gene, *ABI3* was isolated from *Arabidopsis* shortly after that by Giraudat *et al.* (1992). Although the *ABI3* gene may have similar functional properties to *VP1* it remains unclear whether it is the exact counterpart.

Several genes were identified that are required for a normal auxin response (Estelle 1992; Hobbie & Estelle 1994) one of these, *AXR1*, was shown to encode a protein related to ubiquitin-activating enzyme E1 (Leyser *et al.* 1993). The similarity between *AXR1* gene product and E1 suggests that the ubiquitin pathway may play an important role in plant hormone action (Leyser *et al.* 1993). Studies in vertebrate cells show that several membrane receptors are ubiquinated in response to ligand binding (Cenciarelli *et al.* 1992; Mori *et al.* 1992; Paolini & Kinet 1993). By analogy, the function of an auxin receptor might be altered by ubiquitination (Leyser *et al.* 1993).

Although not yet cloned, there are two proposed models in which the *SPY* locus in *Arabidopsis*, which confers resistance to the GA biosynthesis inhibitor paclobutrazol and therefore is hypersensitive to GA, may be involved in the GA signal perception and transduction pathway (Jacobsen & Olszewski 1993). First, the GA signal may be functionally redundant and *SPY* could regulate only one portion of the pathway while the other part functions normally. Secondly, the primary function of the SPY gene product may be to regulate cross-talk from a non-GA-regulated pathway in a negative manner.

Hormone-responsive elements

Studies by, among others, Lanahan *et al.* (1992) showed that two separate but physically adjacent elements in the promoter (of Amy32b) were essential for GA-induced transcription above a minimal level; mutation or deletion of either lowered transcription to near baseline (Rogers *et al.* 1994). A gibberellin–response complex in cereal α-amylase gene promoter, the Amy32b gene, is a representative member of a closely related family of α-amylase genes expressed under hormone control in aleurone layers of barley grains. Transcription of this gene is induced by GA and suppressed by ABA. The promoter elements of the Amy32b gene that govern the developmental and hormonal control of its expression in aleurone were defined and it showed that two functionally distinct yet physically associated elements are essential: a GA response element (GARE) mediates regulation by GA and ABA, and an Opaque-2 binding sequence (O2S) is thought to interact with a barley homologue of the maize endosperm-specific transcriptional regulator Opaque-2. An additional element, CCTTTT, which together with the O2S forms a part of a canonical 'endosperm box', is important in modulating the absolute level of expression of the Amy32b promoter, as is another separate, highly conserved element TATCCATGCAGTG.

Quantitatively, the O2S and GARE, were the most important elements identified. In addition, the results indicated that other elements also participated in establishing the final level of expression from the promoter, but that quantitatively the O2S and GARE were central to the process. It was also shown that O2S and GARE will only function together in one orientation with respect to each other and with respect to the TATA box. Increasing the distance between these elements drastically decreased transcription from the promoter. Thus, interaction of elements within this gibberellin-response complex (GARC) is limited by tight positional and spatial constraints. It was further demonstrated that a different functionally defined element can substitute for the O2S

within a GARC, and thus suggest that many different sequences may be able to perform this coupling function and that the nature of the coupling element within a hormone–response complex will profoundly affect the cell type and temporal pattern of gene expression induced by GA or ABA, because factors binding to the coupling element may be regulated in different ways. This concept is substantially different from that posed, for example, by the simple animal steroid hormone receptor–response element interaction (Fuller 1991; Miner & Yamamoto 1991). It is much more consistent with data regarding interactions of the glucocortoid receptor with other transcription factors on recently described composite response elements (Fuller 1991; Miner & Yamamoto 1991). The general applicability of this concept will be tested when response elements for other phytohormones, such as ethylene and cytokinin are defined. The validity of the concept and mechanisms involved can only be tested definitely when an *in vitro* transcription system using purified factors is available for one of the hormonally regulated gene systems.

Deletion analysis of the promoter of a high-pl α-amylase gene of barley has previously shown that the major GA- and ABA-responsive elements are located −174 bp downstream from the TATA box (Jacobsen & Close 1991). Transient expression assays in barley aleurone protoplasts were used to identify sequences between −174 bp and +53 bp that confer GA and ABA responsiveness on expression of a beta-glucuronidase reporter gene (Gubler & Jacobsen 1992). The results showed that both the TAACAAA and TATCCAC boxes play an important role in GA-regulated expression. It is proposed that the TAACAAA box is a GA response element and that the TATCCAC box acts cooperatively with the TAACAAA box to give a high level of GA-regulated expression, and that together these motifs form important components of a GA response complex in high-pl α-amylase genes. The TAACAAA box also appears to be the site of action of ABA. In addition, a sequence was identified that acts as a repressor of GA action and that resembles a cAMP response element.

These results confirmed the proposal by Skriver *et al.* (1991) that the TAACAAA box plays a central role in both GA and ABA regulation of α-amylase gene expression. In addition, it was shown that TATCCAC box is important for high level expression. Revealing the precise mechanism of action of GA and ABA on α-amylase gene transcription mediated via the TAACAAA and TATCCAC motifs await the detection and characterization of hormonally regulated transcription factors that interact with these elements!

Hagen *et al.* (1994) have analysed the promoter regions of two soybean auxin responsive genes, *GH3* and *SAUR*. The auxin responsive elements were defined to a 76 bp region in the *GH3* promoter and a 30 bp region (the NDE region) for the *SAUR* promoter. Gil *et al.* (1994) has isolated the *SAUR* homologue from *Arabidopsis, SAUR-AC1* and showed that several auxin responsive boxes are highly conserved not only in soybean and *Arabidopsis* but also with the PS-IAA4/5 gene in pea (Ballas *et al.* 1993).

Mundy *et al.* (1990) have identified a highly conserved sequence motif in the promoter of an ABA-regulated rice gene, rab-16A, which was footprinted by proteins in nuclear extracts from rice shoot tissue. A similar sequence motif in the promoter of an ABA-regulated wheat gene, *Em*, was found to interact with a leucine zipper protein (Guiltinan *et al.* 1990). However, ABRE binding activity and expression of a gene encoding a specific ABRE binding protein (EmBP) are not limited to tissues that contains the relevant ABA regulated transcript (Mundy *et al.* 1990; Marcotte *et al.*

1992). Also a *cis*-regulatory element, CCACGTTGG, was isolated that is involved in ABA and water-stress responses of the maize gene *rab28* (Pla *et al.* 1993). the maize gene *rab28* has been identified as ABA-inducible in embryos and vegetative tissues. It is also induced by water stress in young leaves. The proximal promoter region contains the conserved *cis*-acting element CCAGTTGG (ABRE) reported for ABA induction in other plant genes. Transient expression assays in rice protoplasts indicated that a 134 bp fragment (− 194 to − 60 containing ABRE) is sufficient to confer ABA-responsiveness. These and other results indicated that the *rab28* promoter sequence CCACGTGG is a functional ABA-responsive element, and suggest that distinct regulatory factors with apparent similar affinity for the ABRE sequence may be involved in the hormone action during embryo development and in vegetative tissues subjected to osmotic stress.

A *cis*-element for the ethylene regulated gene expression for the tobacco class I basic chitinase genes has recently been identified (Ohme-Takagi & Shinshi 1994). It consists of a conserved 11 bp motif designated as the GCC box. A similar box has also been found in the tobacco class I glucanase gene.

Although a number of *cis*-acting elements responding to several plant hormones have been described, most of these genes are not solely regulated by hormones. This indicates that temporal and spatial regulation of these genes, that play a role in several developmental pathways and appear to be influenced by a variety of *trans*-acting factors, is much more complex.

MULTIPLE HORMONAL REGULATION AND HORMONE INTERACTION

In both plant and animal systems, a tacit assumption has been made that where hormones are present in an organism, they must be serving some regulatory function. This assumption has been drastically altered by the findings that many animal hormones occur in tissues where they do not appear to serve their usual hormonal function, and in fact are also synthesized in body parts remote from the usual synthetic sites in the gland (Roth *et al.* 1982). From the evolutionary point of view, then, the compounds which are adapted to serve regulatory roles in plants and animals may be synthesized in a wide array of cells and organisms; with the progress of specialization, organisms have developed systems in which the synthesis, translocation, physiological function, and disposal of these compounds have become adapted to involve them in regulatory roles. A consequence of this view is that extraction and identification of plant hormones from various tissues means that this hormone is serving regulatory functions in the particular tissue. The same can be said for stereo-specific attachment sites that can bind these hormones. This concept may explain the lack of correlation between hormone concentration with particular processes of growth or development and indicates that the hormone pool being sampled does not necessarily play a regulatory role.

A further complication of the hormone concept is that a hormone produced at one site is directionally transported to exert its action at the target site, and that the compound produced may not be the same as the one acting at the target site. An individual hormone system may have numerous sites for control, and regulation may be accomplished by more than one step. Multiple hormonal control may be based on different types of interaction between hormones; regulation may be achieved by a balance or ratio between hormones, by opposing effects between hormones, by alterations of the effective concentrations of one hormone by another, by alterations in

the sensitivity to one hormone by another hormone, and by sequential actions of different hormones. Further complications arise from compartmentation; developing organs and tissues consist of different cell types, which means that several hormone control systems could be operating within a tissue or organ. In addition, subcellular compartmentation of hormones does exists. Thus, the complexity of hormonal regulation of specific processes by several hormones has probably so far precluded substantial progress in our understanding of multiple regulatory controls. Hypotheses on hormone action have generally failed to present a comprehensive description of how they induce their many effects. Moreover, most studies are confined to the action of one but rarely more than two hormones.

One of the most striking features of the mutants examined to date is the similarity of phenotypes for similar mutants in widely divergent species (e.g. ABA, GA or PhyB deficiencies) which indicates similar control systems across different higher plant species. At the biochemical level, the site of action of mutants appears also similar, e.g. of the 20 well-defined mutants that block GA_1 synthesis, six block conversion of GA_{20} to GA_1, while eight appear to block the two steps between geranylgeranyl pyrophosphate and kaurene. The limited number of steps for which multiple mutants have been isolated may suggest that these are the key steps regulating the biosynthesis of the active hormone. Such steps and the genes controlling them deserve special attention.

So far, site-specific alterations in hormone levels are rarely analysed, but even crude analyses indicated (e.g. Swain & Reid 1992; Swain *et al.* 1993) that hormone synthesis genes may show marked tissue specificity. Work with mutants, however, was mainly concerned with one or a few effects of a single hormone, while each class of hormones is known to have pleiotropic effects.

The molecular-genetic approaches allow the isolation of genes whose products are, by definition, involved in a particular process. However, genetic analysis may not reveal all the components that participate in a given process, particularly those whose absence is lethal or whose function is partially redundant with that of some other gene product. It will also not allow identification of the gene products involved in the earliest stages of hormone-induced changes, since no plant hormone receptors have been purified as far as outlined earlier (Anderson *et al.* 1994; Binns 1994; Gilroy & Jones 1994; Jones 1994). We should also consider the possibility that hormone-regulated gene expression may be many steps removed from hormone action. Therefore, it remains important to continue the search for the early events in hormone action by studying the hormone directly. It is too early to tell which approach will be most fruitful in the elucidation of hormone action.

Hormone interaction

Two or more hormones which affect the same process may act independently or may interact in some fashion. The term interaction is commonly used in reference to any type of interplay of hormones with regard to their effect. Hormones may influence the same process in an additive fashion (independent interaction), or in a non-additive fashion (interdependent interaction), or in a synergistic promotive or synergistic inhibitory fashion, or such that one hormone promotes while the other inhibits the same process (opposite or antagonistic interaction). Much of our information on hormonal regulation has been derived from experiments in which hormones are applied to tissues or whole plants. There is much evidence that exogenous application of hormones affects the levels of endogenous hormones, which means that the interpretation of the observed effects

should take into account the evidence that the uptake, metabolism, range of secondary effects and magnitude of direct effects of the hormone action may be dependent on the presence of other hormones.

Finally, it has become clear that plant hormones not necessarily influence plant responses exclusively via changes in their concentration, but that hormonal regulation may also be exercised via changes in sensitivity of the target cells. Thus, interaction of two hormones may also mean that one hormone may change the sensitivity, and thereby the responsiveness, of tissue to another hormone bringing about the response.

Hoffman & Kende (1992) studied the role of ABA and GA in the regulation of growth in deepwater rice. Submergence induces rapid elongation of rice coleoptiles and of deepwater rice internodes. This adaptive feature helps rice to grow out of the water and to survive flooding. Earlier it was found that the growth response of submerged deepwater rice plants is mediated by ethylene and GA. Ethylene promotes growth, at least in part, by increasing the responsiveness of the internode tissue to GA. The results indicate that the growth rate of deepwater rice internodes is determined by the ratio of a growth promoter GA and a growth inhibitor ABA.

Similarly, in our laboratory it was shown for *Rumex* species growing in frequently flooded river sites that the shoot elongation response of flood tolerant *Rumex* species is crucial for maintaining or re-establishing air contact and, consequently, for survival of this species in river habitats. This elongation response was shown to be ethylene-mediated. Submergence causes ethylene entrapment whereby the ethylene concentration rises by continued production, which leads to a sufficiently high endogenous ethylene concentration to saturate the petiole elongation response in the flood tolerant *Rumex palustris*. *Rumex acetosa*, a flood-sensitive species, also accumulate ethylene upon submergence, however, without an elongation response. This difference in behaviour between *Rumex palustris* and *Rumex acetosa* is attributed to the greater responsiveness of *Rumex* palustris towards ethylene. Recent experiments indicate that GA can enhance the ethylene-mediated elongation response in *Rumex*, whether this is also due to an increase to sensitivity to GA caused by ethylene, like in deepwater rice, remains to be investigated (Voesenek *et al.* 1993; Voesenek & Van der Veen 1994).

Souza & King (1991) isolated two non-allelic, monogenic recessive mutations, *aus1* and *aus2*, with auxin hypersensitivity in a *Nicotiana plumbaginifolia* mutant. At relatively low IAA or NAA concentrations, the elongation growth of mutant seedling hypocotyls is more inhibited than in the case of the wild type; at high auxin concentrations, mutant seedlings are killed. The mutant is also more sensitive to ethylene and the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC), but not to either 6-benzyladenine or abscisic acid.

Another aspect of hormone interaction is that it may occur at different levels in the sequential cascade of processes initiated by the hormone, which includes interaction at distance (spatial) or interaction in time (temporal).

Peeters *et al.* (1991) have studied the interactive and temporal effect of two hormones on flower bud formation of each hormone during *in vitro* culture. By studying the *in vitro* flower bud formation on thin cell layers on tobacco it was observed that the interaction between auxin and cytokinin concerns both the speed of flower bud initiation and the numbers of buds formed. Flower buds were formed at particular hormone concentration combinations, whereby increased auxin concentrations could compensate for low cytokinin concentrations with respect to early bud formation. The auxin and cytokinin effect on flower bud initiation could be clearly separated in time. It

was shown that application of BA for a short period before IAA application leads to a higher number of buds formed compared to reverse application. Cytokinin determines in particular whether flower buds are formed, whereas auxin mainly determines the position of the bud on the explants. In contrast to the prevailing conceptions (Guern 1987), it was found that the hormone effects are separate in time and thus each hormone exerts its action independently; also, their interaction with respect to flower bud formation takes place beyond the level of hormone uptake and metabolism in the cascade of sequential processes that they initiate. The molecular follow-up of this work led to the isolation of an extensin-like gene that is expressed specifically during flower bud initiation. This gene is probably not directly activated by one of the hormones, although the expression of the gene is stimulated by application of each hormone individually during the first 2 days, but is activated by compounds further in the cascade of events (Peeters *et al.* 1994).

Dominov *et al.* (1992) found that both BA and 2,4-D stimulate the accumulation of a mRNA, represented by the cDNA pLS216 in *Nicotiana plumbaginifolia* suspension culture cells. The kinetics of RNA accumulation were different for the two hormones; however, the response to both was transient, and the magnitude of the response was dose-dependent. Run-off transcription experiments showed that the transient appearance of the RNA could account for the feedback regulation of transcription and not by the induction of a RNA degradation system. The feedback mechanism appeared to desensitize the cells to further exposure to the hormone. In particular, cells became refractory to the subsequent addition of 2,4-D after the initial RNA accumulation response subsided. A very different response was observed when the second hormone was added to cells that had been desensitized to the first hormone. Under such conditions, BA produced a heightened response in cells desensitized to 2,4-D and vice versa. These findings support a model in which cytokinin further enhances the auxin response or prevents it feedback inhibition. Results further suggest that hormone activation involves phosphorylation of critical proteins on the hormone signalling pathway, whereas feedback inhibition may involve dephosphorylation of these proteins. This sequence of pLS216 is similar to genes in other plants that are stimulated by multiple agonists, such as auxins, elicitors, and heavy metals, and the gene encoding the stringent starvation protein in *Escherichia coli*. It is proposed that this gene family in various plants be called the multiple stimulus response (msr) genes.

Mutants with multiple hormone resistance may be of great help into understanding the integration of hormone signals during growth and development. These have been described for auxin and ethylene (the *AUX1* locus, Picket *et al.* 1990), auxin, ethylene and ABA (the *AXR2* locus, Wilson *et al.* 1990) in *Arabidopsis* and to both auxin and ABA (the *IBA1* locus, Bitoun *et al.* 1990) in tobacco. Cloning and characterization of these loci may help to further elucidate the process of interaction between hormones.

CONCLUDING REMARKS

The pleiotropic effects of hormones in plant development have so far hampered major progress in elucidating their control mechanisms.

Although plant hormones are recognized as important signal compounds directly or indirectly involved in control of gene activity, the signal transduction pathways, site(s) of perception and receptors of these hormones remain largely unknown. However,

recent molecular-genetic studies with hormone mutants, especially in *Arabidopsis*, have demonstrated the involvement of kinases and phosphatases in the transduction pathways of some plant hormones. This indicates that signal transduction pathways in plants resemble those in animals.

In addition, many binding sites of hormones, which may possibly act as receptors, have been identified. For auxin, there are indications that perception may take place directly at the membrane by changing the activation of anion channels. There is also some evidence that hormones may interact directly with DNA and thus influence transcription.

With regard to hormone-regulated gene expression an increasing number of hormone-responsive genes are being identified and cloned. Their gene products are presumed to be involved in the physiological effects of hormones and therefore these genes serve as valuable molecular probes to elucidate the action of plant hormones. Recent studies indicate that the regulatory mechanisms of hormone-responsive genes is rather complex. Several hormone-responsive elements, i.e. for GA, ABA, auxin and ethylene, but not yet for cytokinin, have been characterized and were shown to be crucial for hormone regulation. The mechanisms involved in these regulatory genes are as yet unknown and can only be studied when *in vitro* transcription systems based on purified factors become available in the near future.

At present, a number of *cis*-acting elements which respond to several hormones have been described. However, these genes are not exclusively regulated by hormones and may be influenced by a variety of *trans*-acting factors depending on the developmental pathway being regulated. This illustrates that also on the molecular level multiple hormone regulation is very complex.

The molecular studies lead to the identification and cloning of an increasing number of hormone-regulated genes. However, the lack of purified hormone receptors precludes at present the identification of those gene products related to the early steps in hormone regulated processes. Although the molecular-genetic approach has been a major factor in the recent rapid progress in plant hormone research, molecular studies will not be able to answer all the questions regarding hormone regulation. Therefore, studies concerning direct hormonal effects remain indispensable in the search for the early events of hormone action. Also, the development of new concepts of hormone action like the one proposed by Bradford & Trewavas (1994) will be fruitful in exploring new approaches to elucidate the mechanisms of multiple hormonal control.

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