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CHANGING CONCEPTS IN PLANT HORMONE ACTION

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Summary

A plant hormone is not, in the classic animal sense, a chemical synthesized in one organ, transported to a second organ to exert a chemical action to control a physiological event. Any phytohormone can be synthesized everywhere and can influence different growth and development processes at different places. The concept of physiological activity under hormonal control cannot be dissociated from changes in concentrations at the site of action, from spatial differences and changes in the tissue's sensitivity to the compound, from its transport and its metabolism, from balances and interactions with the other phytohormones, or in their metabolic relationships, and in their signaling pathways as well. Secondary messengers are also involved. Hormonal involvement in physiological processes can appear through several distinct manifestations (as environmental sensors, homeostatic regulators and spatio-temporal synchronizers, resource allocators, biotime adjusters, etc.), dependent on or integrated with the primary biochemical pathways. The time has also passed for the hypothesized 'specific' developmental hormones, rhizocaline, caulocaline, and florigen: root, stem, and flower formation result from a sequential control of specific events at the right places through a coordinated control by electrical signals, the known phytohormones and nonspecific molecules of primary and secondary metabolism, and involve both cytoplasmic and apoplastic compartments. These contemporary views are examined in this review.

Key words: phytohormone concept; plant growth substances or regulators; new naturally occurring growth and developmental regulators; hormonal balances and cross-talking; sequential involvement of different hormones; secondary messengers; hormone sensitivity; signal transduction and transport.

'Classical hormone theory confuses playing "a" role with playing "the" role'. *(Trewavas, 1986b)*

'. . .there is the air of an "unfinished project" about the phytohormone research field . . . The reasons why plants have hormones is not fully understood and doubts remain as to how they exert control over plant actions at the whole organism level'. *(Weyers and Paterson, 2001)*

Introduction

'Plant cells are constantly bombarded with information to which they must react. Signal transduction, the means whereby cells construct responses to a signal, is a recently defined focus of research in plant biology' (Trewavas, 2000). Growth regulators, in the general sense, are certainly among the external stimuli. Do internal phytohormones play only as stimuli or are they involved in the mechanisms that regulate gene expression? It is too early to give a definitive answer. What is certain is that the concepts in plant hormone action have changed dramatically from their origins. The aim of the present paper is precisely, through literature data, but mainly on the basis of personal experience, to reappraise the phytohormone concept and to show that plant hormones may act through several distinct roles, or a combination, according to the physiological phenomenon considered.

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The Phytohormone Concept

'Plant hormones play a crucial role in controlling the way in which plants grow and develop. While metabolism provides the power and building blocks for plant life, it is the hormones that regulate the speed of growth of the individual parts and integrate these parts to produce the form that we recognize as a plant. In addition, they play a controlling role in the processes of reproduction'. This sentence, beginning the preface of the book *Plant Hormones* by P. J. Davies (1995), probably summarizes in the best way what a phytohormone is. Indeed we are far from the original use of the term in the sense of the mammalian concept of a hormone. The latter denotes a chemical messenger at low concentration and involves a localized site of synthesis, transport in the bloodstream, and the control of a physiological response in a target tissue via its concentration.

Auxins and cytokinins were originally thought to produce growth responses at distances from their sites of synthesis, and thus fitted the definition of transported chemical messengers. It is now clear that none of the officially recognized five classes of phytohormones (auxins, cytokinins, gibberellins, abscisic acid, ethylene) fulfill

the requirements of a hormone in the mammalian sense. First of all, the syntheses of all plant hormones, as a rule, can occur in any type of living cell, even if certain tissues are privileged sites of synthesis and export for some hormone types (aerial meristematic tissues for auxins, growing root parts for cytokinins, for instance). Thus, phytohormones may be transported and have specific action at a distance, but it is far from being the general case; they also act in the tissue or even within the cell in which they are synthesized. Furthermore, experimental results strongly argue that phytohormone control is not only by concentration, but also by changes in sensitivity of the cells to the compounds (Trewavas and Cleland, 1983). Beyond their specific properties, the common characteristics of plant hormones are that they are natural compounds with an ability to affect physiological processes at concentrations far below those where either nutrients or vitamins would affect these processes. More simply, plant hormones are signal molecules, present in trace quantities.

Plant Growth Substances or Regulators

The lack of direct rigid parallels with animal hormones had provided arguments for abandoning the term plant hormone or phytohormone and replacing it with 'plant growth substance'. The international society for the study of plant hormones was and still is named 'International Plant Growth Substance Association' (IPGSA). The problem with the term 'substance' is that it is too vague, since growth is only one of the many processes (differentiation, development, movement) influenced. The term 'plant growth regulator' is somehow more precise, but it is still imperfect because it also does not cover the control of developmental processes in addition to growth. Moreover, it has progressively included synthetic plant growth regulators from the agrochemical industry and thus may not be used in reference to endogenous regulators. The terms 'plant hormones' or 'phytohormones' therefore remain the most appropriate.

New Naturally Occurring Growth and Development Regulators

Natural substances that have phytohormonal-like regulatory roles have been discovered more recently, such as: polyamines, oligosaccharins, salicylates, jasmonates, brassinosteroids, dehydroconiferyl alcohol glucosides, turgorins, systemin, sterols, nitric oxide, etc. (Gaspar et al., 1996b; Crozier et al., 2000; Beligni and Lamattina, 2001). There is some controversy as to whether these compounds should be classified as hormones, even within the rather broad current definition discussed above (see the arguments of Galston and Kaur-Sawhney, 1995, for polyamines, as an example). Polyamines are much more abundant in plants than are hormones such as gibberellins and cytokinins, and millimolar amounts of polyamines are required to induce a biological response. Furthermore, the polycationic character of polyamines appears to limit their translocation. For Crozier et al. (2000), polyamines do not appear to have a truly hormonal role in plants. Instead, as in animals, they appear to participate, directly or indirectly, in several key metabolic pathways essential for efficient functioning at the cellular level. Without the ability to synthesize polyamines, living cells would not survive. Polyamine concentrations correlate with cell division frequency. Polyamines stimulate many reactions involved in the synthesis of DNA, RNA, and proteins. This may explain why in some physiological responses elicited by polyamines (tuber formation, adventitious root formation, embryogenesis, flowering, fruit ripening), these regulators have been found to be indissociable from particular hormones such as auxins (Gaspar et al., 1996a, 1997; Kevers et al., 2002) and cytokinins (Bernier et al., 1993). Another question is whether the newly discovered regulators have universal effects or act in just a few special cases. The favorable justification takes into account their widespread distribution and that they can exert regulatory control over growth and development at micromolar concentrations, in a characteristic manner, which is not that of nutrients, such as amino acids or vitamins. Moreover, in plants where the content of such compounds is physiologically or genetically altered, development is affected (Davies, 1995).

Occurrence, Effects, and Roles of the Phytohormones. Questions Without Responses

With each discovery of a new plant hormone, it was first thought that its precise role in a physiological process would be elucidated when the exact location and level within the tissues is known. Even before these tasks have been achieved, questions arise about their mode of action through putative (external or internal?) receptors and second messenger pathways. The effects produced by each hormone were largely extrapolated from the effects of exogenous applications. The establishment of correlations between hormone levels and growth or development behaviors of defined genotypes or transgenic plants was needed to confirm that specific hormones fulfilled their presumed roles. In other cases, it has not been conclusively proven that an endogenous hormone acts in the same manner in different processes. For instance, endogenous auxin may regulate growth of a specific organ quite independently, while it is known that exogenous as well as endogenous balances between auxins and cytokinins can favor a developmental pattern or orient an organogenic program (Fig. 1): axillary or adventitious bud

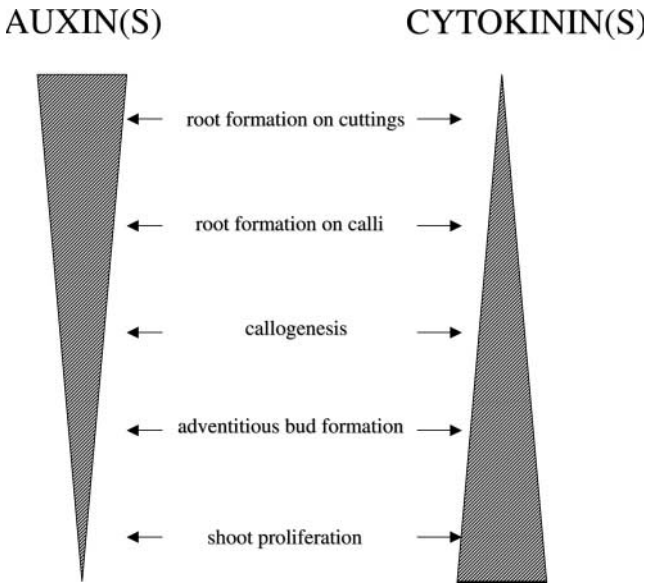

Fig. 1. The control of different organogenic programs by the balance between auxins and cytokinins.

proliferation, root or callus formation. In other words, the possibility of different hormonal receptors controlling growth and development is a new question, as well as whether different hormone types may compete for a common receptor or at least operate in separate signaling pathways (Timpte et al., 1995). The question is still more complex, in that in some cases at least, some hormones may act through others serving as intermediates (Van der Straeten et al., 1999): the control of ethylene production and emission through the balance of auxin/cytokinin is well known (Gaspar et al., 1989); the application of gibberellins rapidly enhances the endogenous auxin level (Nitsch and Nitsch, 1959; Gaspar et al., 1965); the effects of many hormonal responses are concomittant with a rise in polyamine levels (references in Altman and Bachrach, 1981, and in Bagni et al., 1981). The classical comparative auxin dose–response growth curves for roots, buds, and stems, as shown schematically in Fig. 2, still raises unanswered questions such as:

(1) Do root and stem growth responses really correspond to different levels of endogenous auxins? The comparative natural levels in these two organs have not been adequately investigated.

(2) When treated by the same exogenous auxin concentrations, do these organs absorb it in the same proportion and at the same rate ? Does their adaptive auxin-oxidase system react in the same manner?

(3) Are the different responses caused by changes in the number of receptors, changes in receptor affinity, or changes in the subsequent chain of events, including possible changes in the level of other endogenous hormones that enhance, inhibit, or mediate the response?

In addition to these questions, very importantly, it is still not clear whether the concentrations measured in extracts from whole plant parts or tissues represent active hormone pools. There is, therefore, an urgent need for the development of a method with which to differentiate between active hormone pools and pools that are not active, either because of compartmentalization or conjugation.

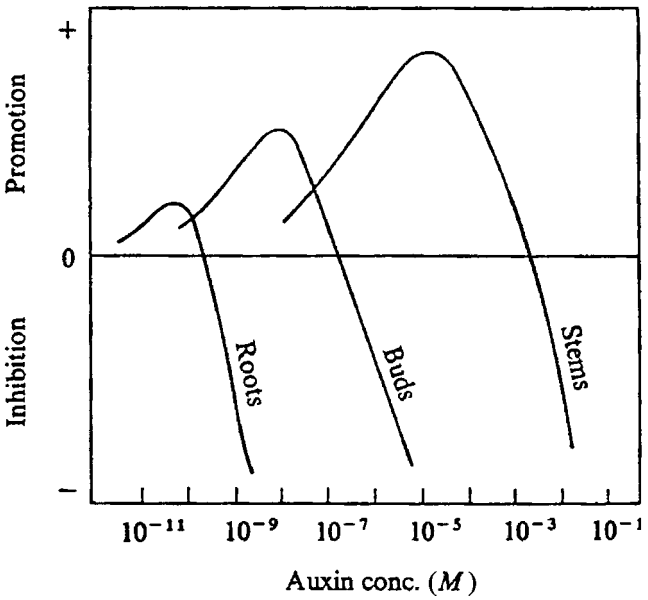

FIG. 2. Schematic representation of the growth responses of roots, buds, and stems to a range of auxin concentrations, each organ having a promotive and an inhibitory range (from Thimann, 1969. Permission McGraw-Hill Cy.)

Hormonal Balances and Cross-talking

Skoog and Miller (1957) discovered more than 40 years ago that both auxin and cytokinin were synergistically required to induce cell division and growth in plant tissue cultures. Subsequent studies on whole plants and excised tissues have demonstrated the existence of antagonistic and additive interactions between these two plant hormones. For instance, auxins and cytokinins antagonistically control lateral bud and root outgrowth (Fig. 1).

Molecular work on the links between hormone action and cell division led to the cloning of several genes that are responsive to auxins, cytokinins, or both hormones. However, the function of the majority of these genes is either not yet known or has no obvious connection with cell cycle control. A more direct link between plant hormones and cell cycle control is now being uncovered by analyzing the expression patterns and activity of proteins that are homologous to those controlling the cell cycle in yeast (Coenen and Lomax, 1997). Lateral root primordia are generally initiated through the commencement of cell divisions in the pericycle opposite the xylem poles of the root vasculature. The antagonistic effect of auxins and cytokinins on this process is paralleled by the interaction of auxins and cytokinins in regulating expression of a cdc2-like protein (Fig. 3; John et al., 1993). Although auxins increase immunologically detectable cdc2-like protein in extracts, cytokinins reduce levels of the cdc2 kinase.

Classical studies on the mechanism of suppression of lateral bud growth by the plant apex (apical dominance) have shown that auxin produced at the apex represses the outgrowth of lateral buds, whereas cytokinins applied to lateral buds stimulate growth and cause release from apical dominance (Sachs and Thimann, 1967). More recent investigations (Bangerth, 1994; Li et al., 1995) have demonstrated that decapitation, and thus removal of the endogenous auxin source, leads to a large (up to 40-fold) increase in the cytokinin content of xylem exudate. This increase can be eliminated by application of the synthetic auxin α-naphthaleneacetic acid (NAA) to the apex of decapitated plants. This effect of auxin on cytokinin concentrations in the xylem suggests that auxin can influence apical dominance via inhibition of cytokinin synthesis or export from the roots. However, bud outgrowth can also be inhibited by apically applied auxin in isolated stem segments, which indicates that this is not the only mechanism available.

Coenen and Lomax (1997) have proposed the scheme of Fig. 4 with the potential points of control of active cytokinin pools by auxin, namely through a control of cytokinin oxidase by auxin. Conversely, the auxin and cytokinin interaction on root growth (Fig. 5) has been interpreted through a cytokinin control of isoperoxidases that are supposed to function as auxin-oxidases (Darimont et al., 1971). However, increases in free indole-3-acetic acid (IAA) are observed both in cytokinin-overproducing lines of *Nicotiana glutinosa* transformed with the *ipt* gene (Binns et al., 1987) or after exogenous application of cytokinins to maize (Bourquin and Pilet, 1990) or pea (Bertell and Eliasson, 1992) roots. Although the basis for the latter cytokinin-induced changes in auxin levels is not fully understood, a putative mechanism is cytokinin inhibition of the enzymes that conjugate free IAA. These data illustrate the different possibilities of a mutual control of auxin and cytokinin abundance, even before the enzymes involved in

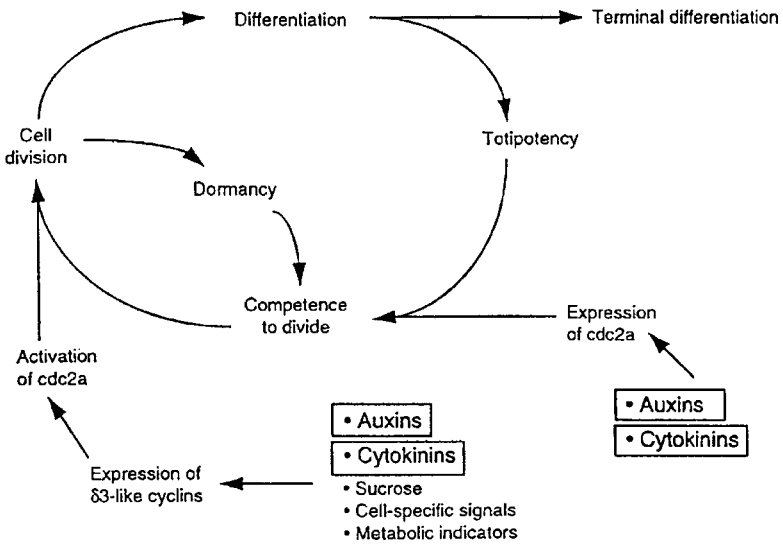

FIG. 3. Speculative model for control of the cell cycle through auxins and cytokinins. Both cytokinin and auxin have been reported to regulate the expression of cdc2 kinases and the cyclins that are required for their activation. The interaction between auxin and cytokinin in regulating the cell cycle is synergistic in undifferentiated cells, such as callus cells or protoplasts, with both auxin and cytokinin stimulating expression of the cdc2 kinase and cytokinin treatment increasing expression of a cyclin. In lateral root primordia, the interaction is antagonistic: auxins stimulate and cytokinins reduce levels of the cdc2 kinase, and the expression of at least one cyclin is increased by auxin (from Coenen and Lomax, 1997 Permission Elsevier Science).

auxin and cytokinin metabolism have been isolated and cloned, and while a debate persists on the anabolic and catabolic pathways of both hormone types. Other possibilities of mutual interactions between auxins and cytokinins have been discussed by Schmülling et al. (1997).

There are other examples of interactions of other hormone couples, whatever the types, in several physiological processes. The regulation of several types of dormancy through the couple gibberellins/abscisic acid (like substances) is among the most classical (Taiz and Zeiger, 1991); growth regulation of plant cancerous cells through the couple polyamines/ethylene is a more recent discovery (Gaspar et al., 1999; Kevers et al., 1999a, b; Bisbis et al., 2000b). Many interactions between hormone metabolism, whatever the types, have been shown. Cytokinins, for instance, can inhibit plant amine oxidases (Galuska et al., 1998). Often ethylene and polyamines negatively affect the accumulation of each other (Turano et al., 1997). Ethylene and polyamines share a common precursor *S*-adenosylmethionine (AdoMet; SAM) (Fig. 6) and there are several additional interacting possibilities, namely polyamine scavenging the free radicals necessary for the conversion of 1-aminocylopropane-1-carboxylic acid (ACC) to ethylene (Bisbis et al., 2000b). There are also interactions between polyamines and gibberellins; polyamines in any case are needed for gibberellic acid promotion of growth (Smith, 1990).

Fig. 7, as proposed by Coenen and Lomax (1997), shows the potential mutual interacting points between two hormones; but the variety of ways in which such hormones regulate physiological responses suggests that there may well be mechanisms of so-called hormonal cross-talking (Smalle and Van der Straeten, 1997; Rodrigues-Pousada et al., 1999) that are, moreover, dependent upon the plant species and tissue type (Dominov et al., 1992; Schmülling et al., 1997).

Obvious (obligatory) cross-talking between different hormones in the control of growth and development processes has been discovered while analyzing 'specific' hormone-insensitive mutants. Epistasis analysis between ethylene- and abscisic acid- (ABA)

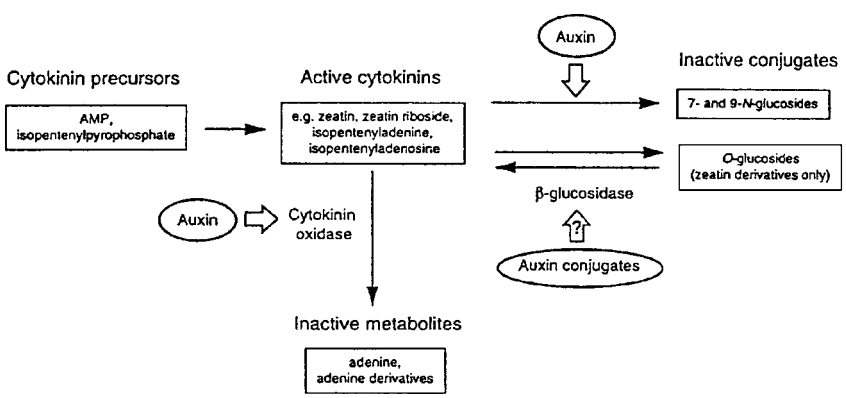

FIG. 4. Potential points of control of active cytokinin pools by auxin. *Open arrows* indicate the steps where auxin or auxin conjugates are thought to regulate enzyme activity, resulting in changes in conjugate or metabolite levels (from Coenen and Lomax, 1997. Permission Elsevier Science).

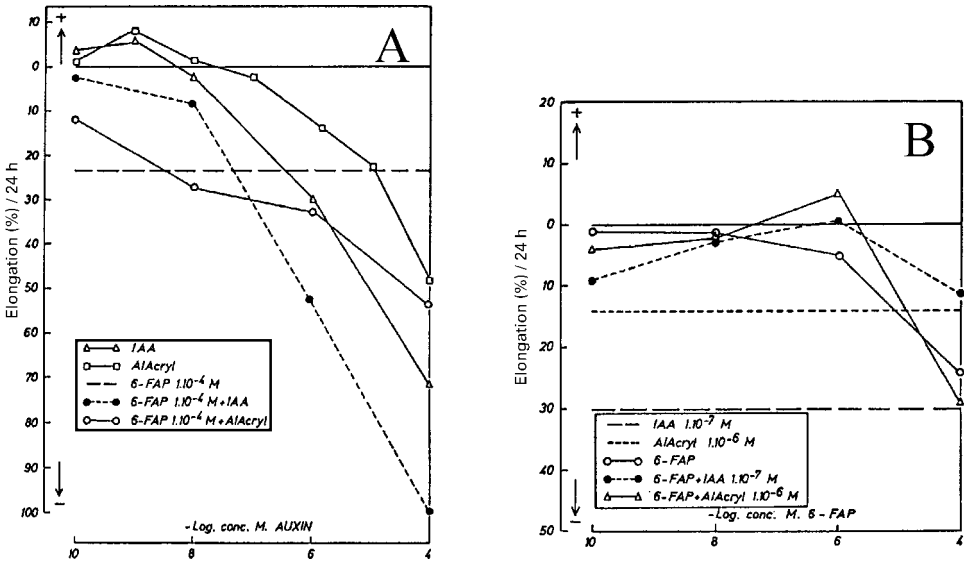

FIG. 5. Effect on the lentil root growth of (*A*) increasing concentrations of IAA or indoleacrylic acid (AIAcryl) combined with a fixed concentration of kinetin (6-FAP) and (*B*) increasing concentrations of 6-FAP combined with a fixed concentration of auxin (from Darimont et al., 1971).

insensitive mutations indicated that endogenous ethylene promotes seed germination by decreasing sensitivity to endogenous ABA. In marked contrast, ABA inhibition of root growth requires a functional ethylene signaling cascade (Beaudoin et al., 2000). Mutations in the *Arabidopsis* gene *AXR1* are characterized not only by auxin insensitivity, but also by reduced sensitivity to ethylene in seedling roots and apical hooks. The *nonphototropic hypocotyls 4* (*nph4*) mutant is impaired in auxin-induced hypocotyl binding, but this defect can be functionally complemented by the exogenous application of ethylene. The *NPH4* gene product, ARF 7 (see later for the significance of auxin response factors; ARFs) belongs to the family of auxin-response element DNA-binding proteins. Thus in the *nph4* mutant, ethylene may enhance auxin responsiveness by stimulating either the activity or the sensitivity of a redundant ARF system (Stepanova and Ecker, 2000, and references therein).

Sequential Intervention of Different Hormones in Growth Processes

In at least four species under different treatments, we have established correlations between growth limitation, lignin accumulation, and increase of total peroxidase activity and ethylene emission (Table 1). It was found that during growth responses to several stimuli, peroxidase varied in a two-step manner, with an early variation of cationic (basic) isoperoxidases preceding changes in anionic (acidic) ones (Boyer et al., 1983; Gaspar et al., 1985a; Cuenca et al., 1989). Because there were arguments to involve basic and acidic peroxidases in auxin catabolism, in ethylene production, and in lignin polymerization, respectively, a general scheme involving sequential events, including changes in IAA level, ethylene production, and growth limitation through lignin-induced wall rigidification, was drawn (Fig. 8). This figure shows that the stimuli would cause an immediate redistribution of electrochemical potentials at the membrane level and generate different kinds of free radicals, for instance peroxide radicals, which in turn initiate lipid peroxidation. Ethylene production is a marker of this process. The degradation of cell membrane lipoprotein by lipid peroxidation by free radicals may bring about changes in ionic status and fluxes at the plasmalemma level and allow the passage of solutes such as phenolics, ascorbic acid, and even IAA, most of which are electron

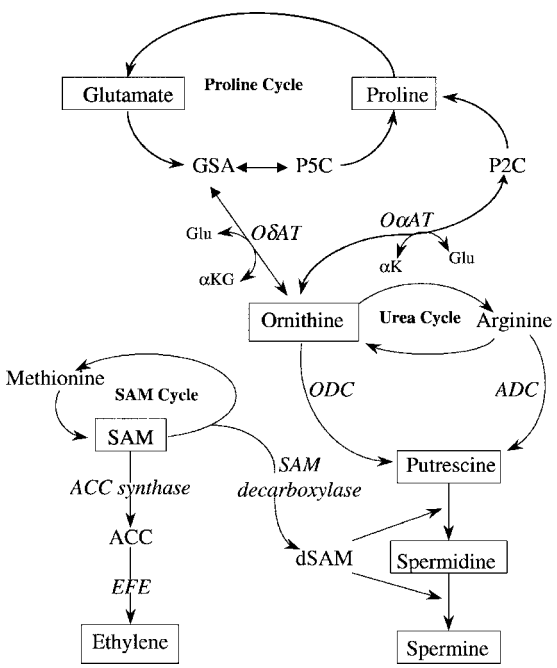

FIG. 6. Interconnection between biosynthetic pathways of ethylene, polyamines, and proline through SAM, ornithine, and glutamate. ACC, 1-aminocyclopropane-1-carboxylic acid; ADC, arginine decarboxylase; dSAM, decarboxylated SAM; EFE, ethylene-forming enzyme or ACC oxidase; Glu, glutamate; GSA, glutamate semi-aldehyde; αKG, α-ketoglutarate; P2C, Δ-pyrroline-5-carboxylic acid; SAM, *S*-adenosyl-L-methionine; OAT, ornithine aminotransferase (types α and δ); ODC, ornithine decarboxylase.

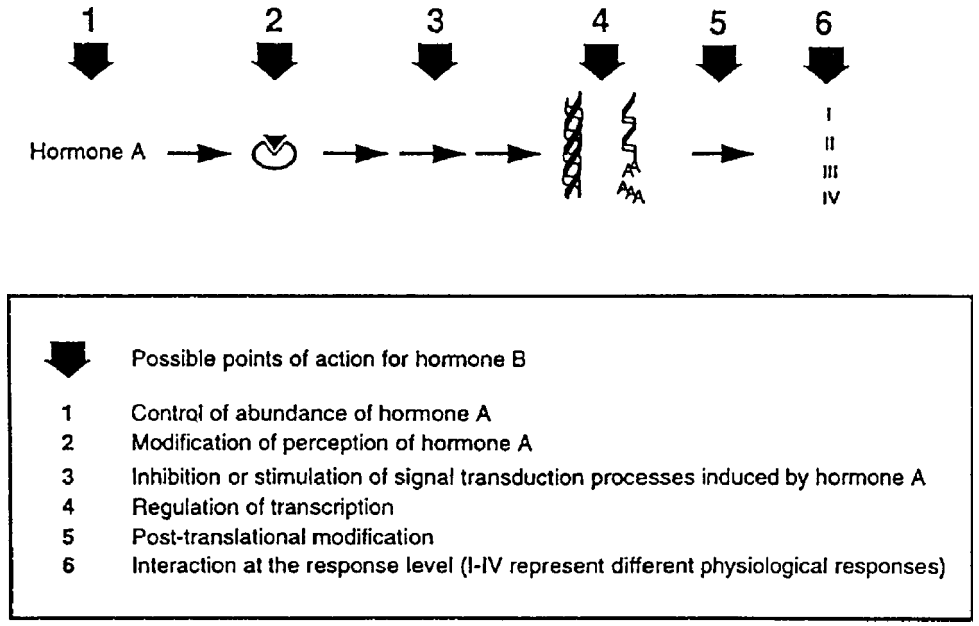

FIG. 7. Model depicting possible points of interaction between two hormones. The chain of events set in motion by hormone A may be variable in different cells and responses, and contain fewer or additional steps. Rather than influencing the depicted steps of action directly, the action of hormone B may be mediated through a similarly complicated chain of reactions. For example, a receptor that is activated by hormone B may activate a second messenger that through gene expression influences the responsiveness of the receptor to hormone A (from Coenen and Lomax, 1997. Permission Elsevier Science).

donors for peroxidase. Release of K^+ in particular, results in a modified endogenous Ca^{2+} relative level, which regulates both the secretory process and the binding of basic peroxidases to membranes. Fatty acids may contribute to peroxidase activation. Using free peroxide radicals, the basic peroxidases might attack the electron donors and among them IAA and/or ACC. As ACC-oxidases, the membrane-bound peroxidases would directly regulate ethylene production, the limiting factor being the immediately available ACC pool, although Ca^{2+} may also have a stimulating effect on ACC production. The free IAA level regulated by the so-called IAA-oxidase activity of peroxidases (whatever the mechanisms involved) would also regulate the ACC level through the control of the conversion of SAM to ACC. Ethylene, in turn, might be a feedback controller of its own synthesis and of the peroxidases involved in IAA degradation, as suggested by peroxidase changes under the effect of ethylene. However, the main role of ethylene would be to regulate phenylalanine ammonia lyase (PAL) and acidic peroxidases, thereby controlling also the lignification process. The indirect but central role of Ca^{2+} in these processes is indirectly supported by the stress-protective effect of polyamines that inhibit the Ca^{2+}-mediated binding of peroxidases to membranes. Polyamines have indeed been shown to induce release of Ca^{2+} from whole cells (references in Gaspar et al., 1991b).

In the thigmomorphogenesis process induced by mechanical stresses, the places of IAA, ethylene, and peroxidase were even more precise (Fig. 9). The speculative model presented by De Jaegher and Boyer (1990) implicates a transient rise of cytoplasmic Ca^{2+}. Based on the rapidity at which ion fluxes across the plasmalemma and accompanying action potentials are generated by external signals, it is tempting to suggest that the perception

TABLE 1

DIFFERENT MATERIALS AND PHYSIOLOGICAL PROCESSES WHERE (CAUSAL) RELATIONSHIPS WERE ESTABLISHED BETWEEN PEROXIDASE, AUXIN LEVEL, ETHYLENE PRODUCTION, LIGNIN FORMATION, AND LIMITATION OF STEM/INTERNODE GROWTH

	Growth	Lignin	Peroxidase	Ethylene	Auxin	References
Bryonia and *Bidens*						
Control	++	++	++	++	++	Boyer et al. (1979, 1983, 1986); Hofinger et al. (1979);
Rubbed or pricked	+	+++	+++	+++	+	Desbiez et al. (1981, 1987); De Jaegher et al. (1985, 1987)
Suaeda						
Control	++	++	++	++	nd	Hagège et al. (1988)
Salt-deprived	+	+++	+++	+++	nd	
Tobacco						
Wild-type	++	++	++	++	++	Faivre-Rampant et al. (1998, 2000b)
rac mutant	+	+++	+++	+++	+++	

+ and +++ less or more than the control ++. nd, nondetermind.

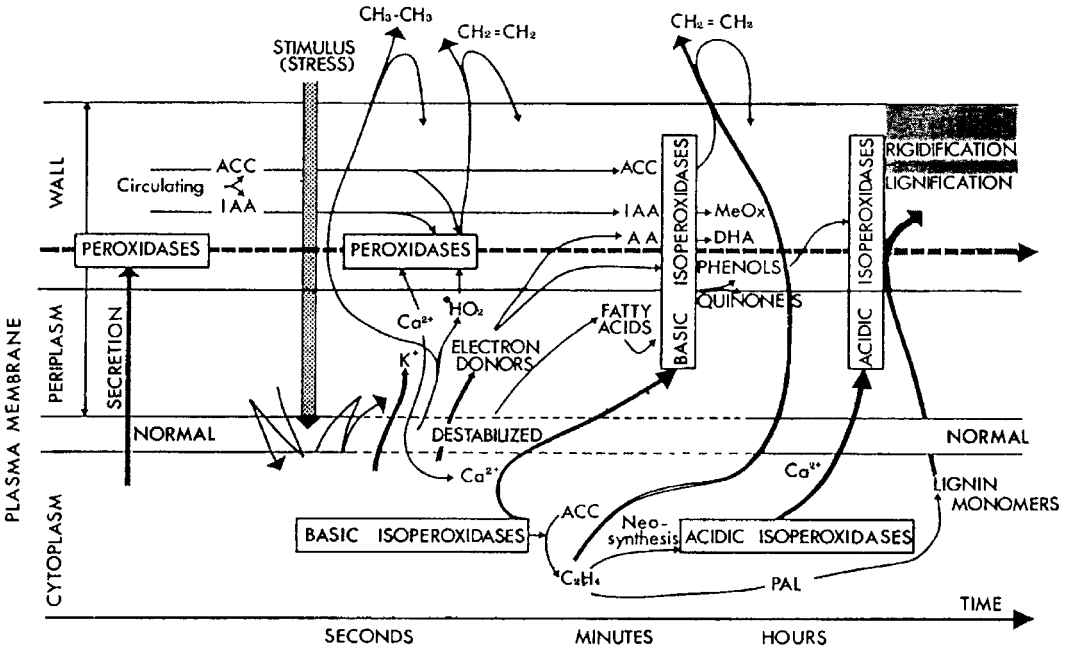

FIG. 8. Suggested common pathway of reactions in some cell compartments in response to different physical and chemical stimuli (*vertical arrow*) causing growth inhibition. It shows the two-step control of basic and acidic peroxidases and their interdependent roles in cell wall rigidification through the mediation of IAA and ethylene. *Arrow* along *bottom* of figure indicates the time spans (from Gaspar et al., 1985a, 1991b).

mechanism for the primary stimulus itself directly controls passage of external Ca^{2+} into the cytosol. While a decrease in extracellular Ca^{2+} may have a destabilizing effect on the plasmalemma and hence permits more Ca^{2+} to enter, the incoming Ca^{2+} may serve as an endogenous signal, maybe, in the first instance, for its own amplification. Ca^{2+} may activate the plasmalemma phospholipase C, and the released inositol-1,4,5-triphosphate (IP_3) in turn may mediate Ca^{2+} gating in the endoplasmic reticulum and the vacuole (see below: the sections dealing with signal perception and secondary messengers). An additional mechanism may be triggered

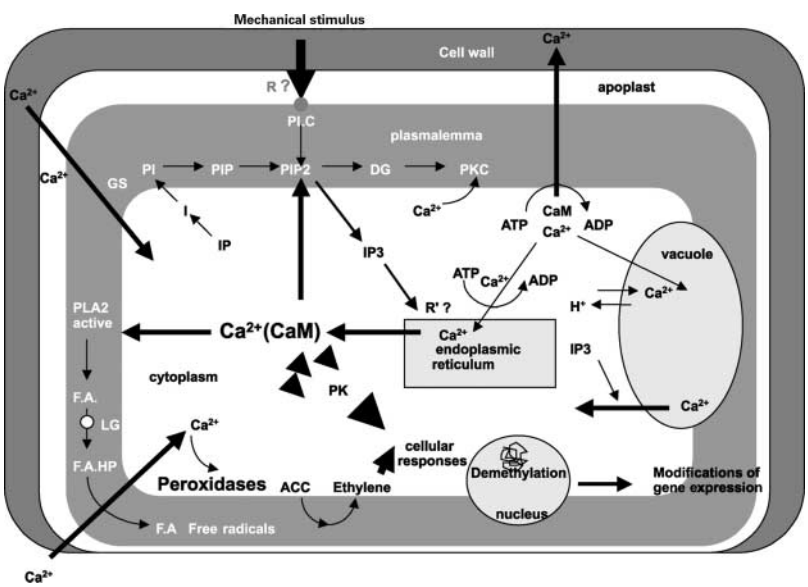

FIG. 9. Model proposed by De Jaegher and Boyer (1990), redrawn courtesy of Boyer, to explain the involvement of Ca^{2+} in mechanical stimulus reception and transduction. *Italics* represent mechanisms involved in normal Ca^{2+} equilibrium or the recovery process following stimulus-induced elevation of cytosolic Ca^{2+} level. For details, see text. ACC, 1-aminocyclopropane-1-carboxylic acid; CaM, calmodulin; DG, diacylglycerol; FA, fatty acid; I, *myo*-inositol, IP, inositol-1-phosphate; IP_3, inositol-1,4,5-triphosphate; PI, phosphatidylinositol; PIP, phosphatidylinositol-4-phosphate; PIP2, phosphatidylinositol-4,5-bisphosphate; PK, protein kinase; PKC, protein kinase C; R and R′, receptors.

by phospholipase A_2 that is also localized in the plasmalemma. Phospholipase A_2 is stimulated by Ca^{2+}/calmodulin and releases fatty acids which, hydroperoxidized by lipoxygenase, may act as a Ca^{2+} ionophore.

The resulting rise in cytosolic Ca^{2+} is believed to be transient, because IP_3 is rapidly catabolized and Ca^{2+} is extruded from the cytoplasm by a calmodulin-dependent Ca^{2+}-transporting Ca^{2+}/Mg^{2+}-ATPase located in the plasmalemma, and by sequestering in organelles, the endoplasmic reticulum by a Ca^{2+}/Mg^{2+}-ATPase, and the vacuole by lesser-known mechanisms. One may also wonder to what extent the 'fatty acid hydroperoxides-Ca^{2+} ionophores' are obviated by peroxidase-catalyzed formation of fatty acid free radicals and subsequent scavenging by IAA, ACC, and wall constituents (see above). In *Bryonia dioica*, the role of ACC may be reflected by the rapid mechanical stress-induced burst of ethylene and is further supported by the fact that *in vitro* ethylene formation by a microsomal fraction, membrane-related peroxidase activity, and lipid hydroperoxide accumulation parallel the burst in ethylene (De Jaegher et al., 1985, 1987).

Several arguments may be put forward indicating that the phosphoinositide response and a rise in cytoplasmic Ca^{2+} concentration may operate in *Bryonia dioica* thigmomorphogenesis. Treatment with Ca^{2+} leads to inhibition of internode elongation and induces a cellular redistribution of peroxidases, which is in every respect comparable to that observed in rubbed internodes, including membrane association of peroxidases. Conversely, EGTA treatment prevents thigmomorphogenesis and counteracts the rubbing-induced changes in ethylene and peroxidase activity. It was further demonstrated that membrane association of purified peroxidases is Ca^{2+}-dependent and that this binding may allow ACC to ethylene conversion (see above).

A significant argument for the phosphoinositide response in *Bryonia* is the prevention of thigmomorphogenesis and exogenous Ca^{2+} effects by lithium pretreatment. Lithium is known to inhibit *myo*-inositol 1-phosphatase which is involved in the normal phosphatidylinositol turnover (see below). The effect of lithium treatment on thigmomorphogenesis might thus be explained by phosphotidylinositol (PI) and hence phosphotidylinositol-4,5-bisphosphate (PIP_2) depletion, rendering any possible activation of phospholipase C without consequence (references in Gaspar et al., 1991b).

The above schemes probably will need to be made more precise, and it is not certain that a hormone will have the same role in different events. There are even indications that a hormone may play several roles, and not necessarily in the same way, in a sequence of events for a specific process. For instance, the relationships between IAA, peroxidase, and ethylene emission are particularly difficult to establish and need very fine analyses of time-course changes; IAA can influence peroxidase activity and/or the appearance/disappearance of some isoperoxidases (references in Greppin et al., 1986); some peroxidases, from both the basic and acidic categories apparently, can destroy IAA; the IAA-induced increase of ethylene emission through membrane-bound peroxidases (Penel et al., 1990; Kevers et al., 1992) might be operative in stress situations only; the conditions where IAA can control ACC-synthase and ACC-oxidase and when ethylene retroinhibits its own biosynthetic pathways seem to vary largely from one process to another. Does the lower IAA level measured in mechanically stressed internodes (Hofinger et al., 1979; Table 1) represent the causal factor of growth inhibition or is it a consequence? Why do shorter internodes of the *rac* tobacco mutant with similar peroxidase/ethylene/lignin trends (Faivre-Rampant et al., 1998, 2000b, 2002; Table 1) contain more IAA than their normal wild counterparts? May inhibition in this case be due to the IAA excess? Through which mechanisms? Overproduction of auxin in transgenic plants also results in the overproduction of ethylene. As in the *rac* tobacco mutant, plants overproducing both auxin and ethylene display inhibition of stem growth and/or lignin deposition (Sitbon et al., 1999). However, it was finally shown that auxin overproduction can be effectively uncoupled from ethylene overproduction (Romano et al., 1993), which should enable direct manipulation of plant morphology. A 40–220% increment of lignin content was found in transgenic tomato plants overexpressing a basic peroxidase (Mansouri et al., 1999).

These examples illustrate once more that plant hormones generally do not play their role in an independent manner, but, on the contrary, in an interdependent way through a probably well-programmed series of events in each case, with shared and separate steps in the signaling pathways (Coenen and Lomax, 1998). Cytokinin and ethylene, for instance, regulate alkaloid production in periwinkle cell culture by independent pathways (Yahia et al., 1998).

Role of the Secondary Messengers

Growth of most plant cultures is improved by the addition of *myo*-inositol to the medium although there are few reports of it having a regulatory role in morphogenesis. The majority of exogenous *myo*-inositol is incorporated into phosphatidylinositol, which is considered to be an important factor in the functioning of membranes. The phosphatidyl cycle, associated with membranes, controls various cellular responses to physical and chemical, biotic or abiotic signals by generating secondary messengers such as *myo*-inositol-1,4,5-triphosphate.

Data accumulated during the past few years have indeed demonstrated that plant hormones and regulators (including toxins, elicitors, light, etc.) mediate their effects through transduction and amplification pathways, using G-proteins, as in animals. Figures 10 and 11 give a general idea of the intermediates involved.

The above-described phosphatidylinositol cycle (Fig. 12) is one of the main putative signal transduction pathways in plants. Substances that stimulate or inhibit steps in this cascade of reactions promote or decrease the responses. As an example, lithium chloride, which inhibits *myo*-inositol-1-phosphatase and thus decreases the phosphatidylinositol cycle, inhibits callus formation and growth, and the inhibition is reversed by the addition of *myo*-inositol.

Two of the principal elements in the transduction pathways of plant cells are intracellular Ca^{2+} (see above for the sequential intervention of different hormones), and protein kinases, enzymes that phosphorylate and thereby alter the activity of target proteins (Trewavas, 2000; Anil and Rao, 2001). Intracellular Ca^{2+} transduces many signals and is a prominent second messenger (i.e., a readily diffusible molecule involved in conveying information from an extracellular source to the principal target enzymes within the cell): it therefore must be maintained in the cytoplasm at concentrations many orders of magnitude lower than the Ca^{2+} concentration in the cell wall. During signaling, brief increases in Ca^{2+} concentration are often associated with initiation of responses.

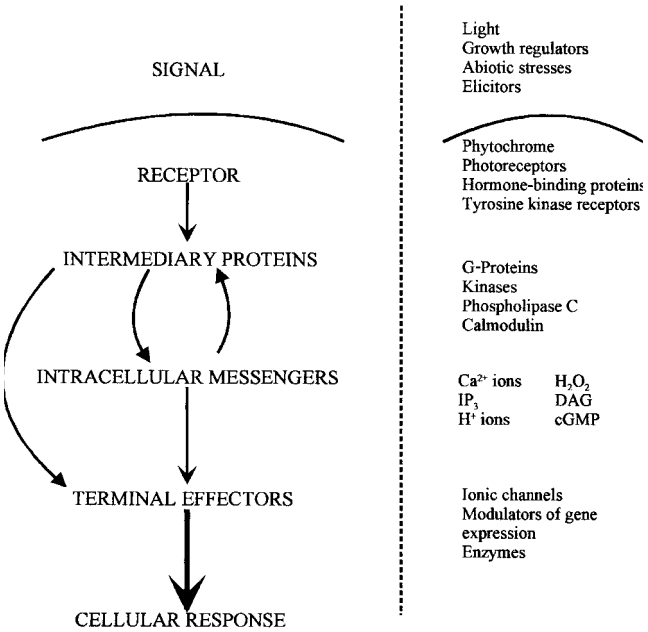

FIG. 10. General components of signal transduction pathways in plants.

This requires a well-controlled cooperation of tightly regulated Ca^{2+} channels and active Ca^{2+} transporters, such as primary Ca^{2+}-ATPases and the H^+/Ca^{2+} antiporters found both at the plasma membrane and endomembranes. Downstream events in calcium signaling involve Ca^{2+}-binding proteins (calmodulin has been functionally identified as a primary Ca^{2+} receptor, because the Ca^{2+}–calmodulin complex can activate many other enzymes), and protein kinases and phosphatases that sense, amplify, and transduce the Ca^{2+} signal further downstream. Note that feedback inhibition may involve dephosphorylation of proteins (Dominov et al., 1992). Together, the signaling pathways that utilize protein kinases and Ca^{2+} constitute a network of great complexity (see Trewavas, 2000, for more details). Note that oscillations observed in many biological processes can apparently result from a sequential filling and emptying of the Ca^{2+} stores in the endoplasmic reticulum and perhaps the vacuole.

It becomes also more and more evident that many signal transduction pathways might not be initiated without the participation of the membrane wall-associated NAD(P)H oxidase and peroxidases and the generation of active oxygen species (AOS) (Pedreno et al., 1995; Overney et al., 1998). H_2O_2, for instance, might be involved directly in the regulation of gene expression (Mehdy, 1994; Penel, 2000; Fig. 13).

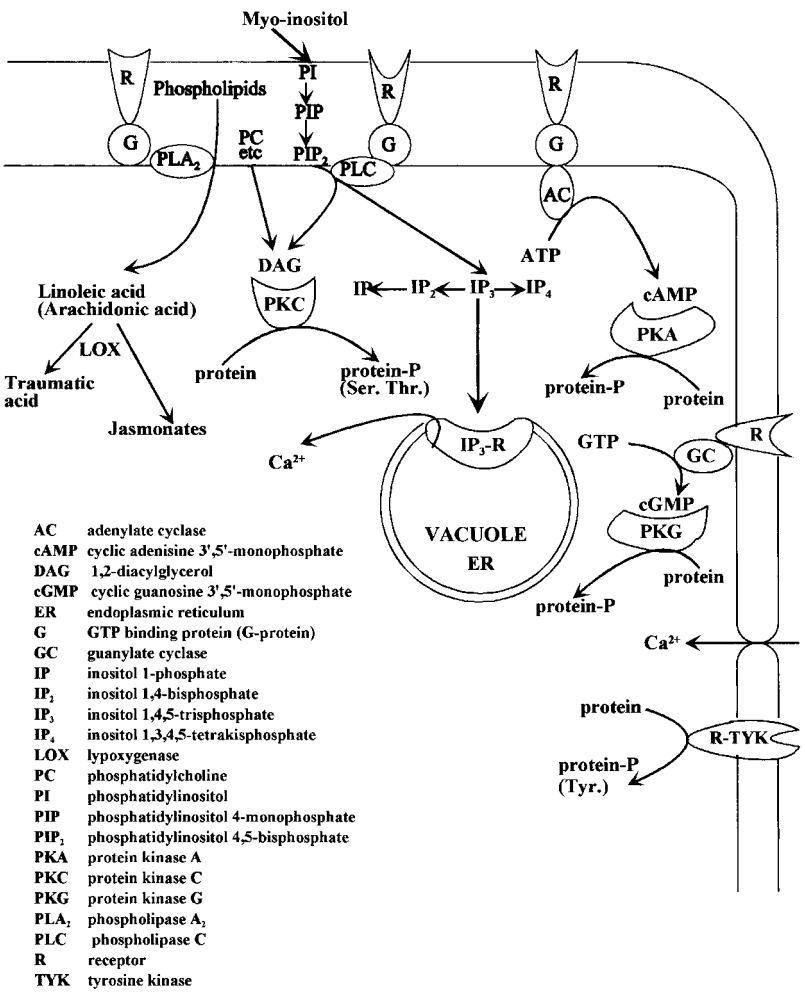

FIG. 11. Secondary messengers in signal transduction pathways.

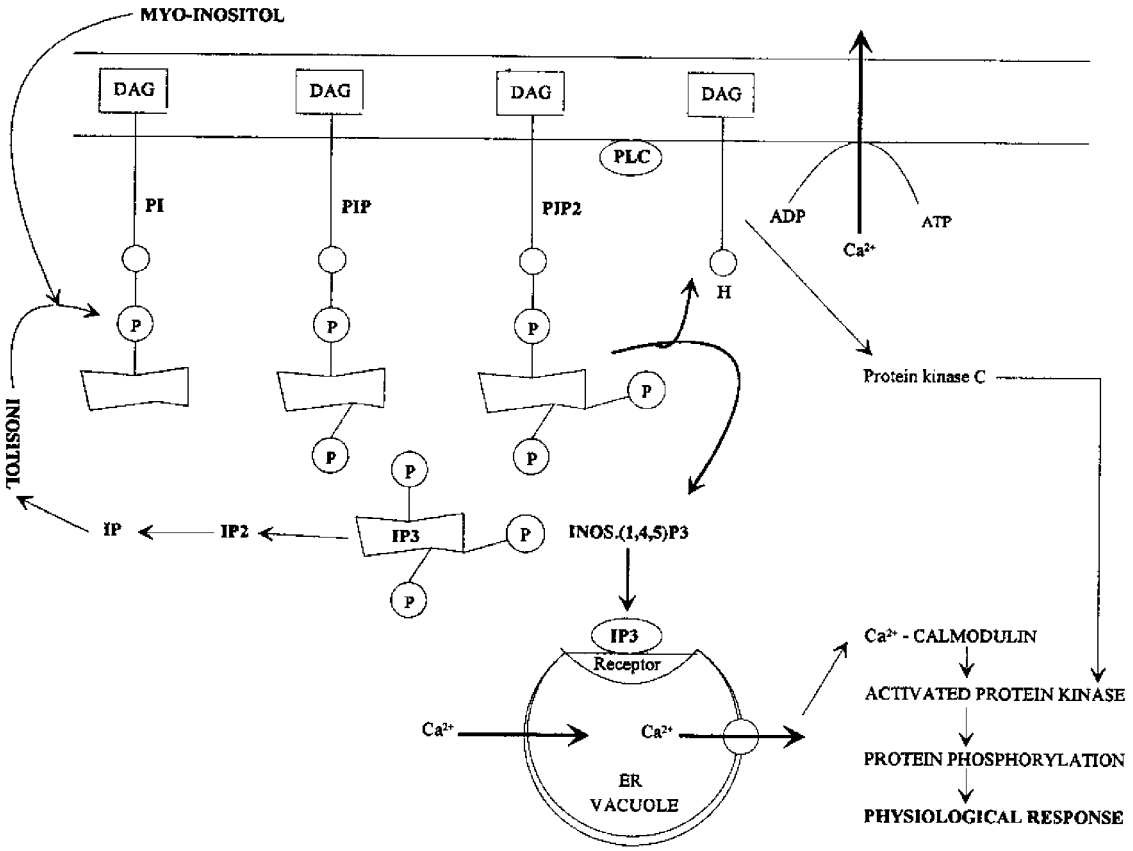

FIG. 12. *myo*-Inositol in the phosphatidylinositol cycle as part of a signal transduction pathway.

As wisely recalled by Trewavas (2000), a distinction must be made between the classical expression of 'Mendelian' genes (DNA → RNA → protein) assuming the invariable expression of inherited phenotypic characters (flower color, seed morphology), and the phenotypic characterics strongly modified by the environment under epigenetic control, that is resulting from the above complex web of interacting signal transduction networks enmeshed with products from epistatic genes (inherited independently) and pleiotropic genes (single genes that influence multiple traits). This also indirectly means that different signals may affect the transduction network in different ways and at different places by modifying gene expression. Two different phytohormones, such as auxin and cytokinin, may regulate plant growth and development through both shared and separate signaling pathways (Coenen and Lomax, 1988).

Hormone Sensitivity, Signal Transduction, and Transport

Response to a chemical signal implies sensing by the responding cells. There is until now no unifying theory behind the sensitivity concept, the term being capable of many interpretations (detailed in Weyers and Paterson, 2001). One of the main problems is that there are many examples of spatial and temporal differences and gradients which are indicators of developmental changes. To initiate transduction, a signal must in any case first be sensed by a receptor. Extracellular signals bind either to receptors on the cell surface or to receptors inside the cytoplasm or nucleus. Many hydrophilic molecules, such as peptides and carbohydrates, and osmotic signals cannot easily pass through the plasma membrane and therefore are perceived on the cell surface. Amphiphilic and hydrophilic molecules, such as growth regulators, can pass through the plasma membrane and may be perceived either by surface receptors or inside the cell (Trewavas, 2000). Most identified receptors have turned out to be proteins. Regulation of receptor concentrations, of course, can change the sensitivity of cells to signals.

The membrane potential can also act as a receptor. The plasma membrane uses pumps and proteinaceous pores, called channels, to control the flux of ions into and out of the cell. Selective discrimination against certain ions results in the establishment of a potential difference of -80 to -200 mV. Modifications of membrane potential open a group of voltage-gated channels that allow Ca^{2+} to enter and thus activate a transduction sequence. Ca^{2+} influx, in turn, can cause the subsequent opening of many potassium and chloride channels, resulting in very rapid changes in turgor. Growth regulators and phytohormones, among other signals such as red and blue light, and fungal elicitors, are known to modify membrane potential (Trewavas, 2000).

To give some examples, as far as IAA and ABA are concerned, experiments revealed that their receptors lie on the outside of the plasmalemma (Allan and Trewavas, 1994) which implies that, at least in certain cases, the apoplasm may be an important compartment (see also above and Penel, 2000). Many auxin-binding proteins have been detected, but whether they represent receptors for different auxin-mediated processes is still uncertain.

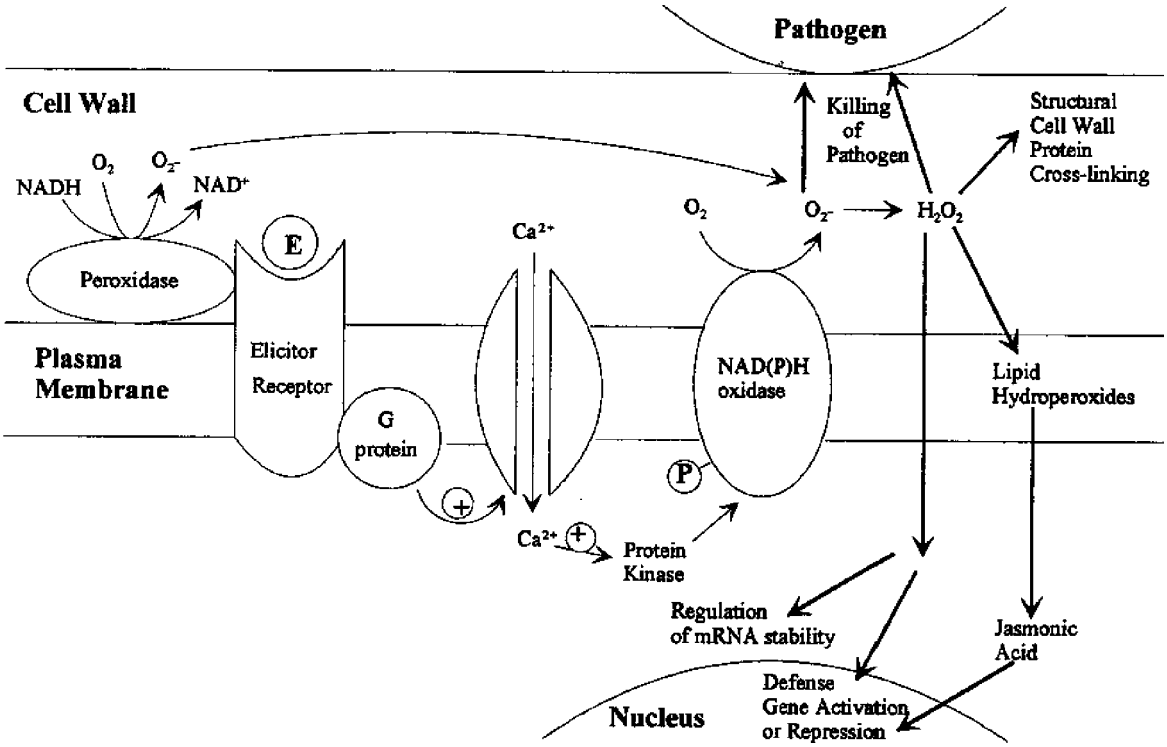

FIG. 13. A speculative model showing possible components involved in active oxygen species (AOS) generation and effects of AOS. Elicitor receptors may be coupled to active oxygen synthesis via G-proteins, increased intracellular Ca^{2+} due to Ca^{2+} channel opening, and activation of a protein kinase that activates a membrane-bound NAD(P)H oxidase by phosphorylation. Alternatively, occupation of elicitor receptors may stimulate a membrane-associated peroxidase by unknown mechanisms, which results in the production of O_2^-. O_2^- spontaneously dismutates to H_2O_2, which is membrane permeable. O_2^- and H_2O_2 contribute to killing of the pathogen, whereas H_2O_2 also participates in oxidative cross-linking of cell wall proteins and regulation of the host gene expression (From Mehdy, 1994. Permission American Society of Plant Biologists).

Nevertheless, most phytohormones have been shown to be recognized by specific receptors. These receptors activate a signal transduction pathway that either induces or inhibits cellular functions, or controls gene expression. Identification of differentially expressed genes will probably bring valuable information in the future on both the effect of a single hormone and the interaction between two or several regulatory substances. It is known that the promoter region of many genes contains *cis*-acting elements that confer hormone responsiveness. This is the case, in particular, for auxins (Guilfoyle et al., 1998), abscisic acid (Marcotte et al., 1992), gibberellins (Gubler and Jacobsen, 1992), and ethylene (Meller et al., 1993). Phytohormone action on the genome is mediated by transcription factors, encoded themselves by several multigene families (Liu et al., 1999). For instance, auxin response factors (ARFs) are transcription factors that bind with specificity to TGTCTC auxin response elements (AREs) (Fig. 14) found in promoters of primary/early auxin response genes. ARFs are encoded by a multi-gene family, consisting of more than 10 genes (Guilfoyle, 1998; Ulmasov et al., 1999). Auxin/IAA proteins function as active repressors by dimerizing with ARFs bound to AREs. Early auxin response genes are regulated by auxin-modulated stabilities of auxin/IAA proteins (Tiwari et al., 2001).

The formation of regulatory complexes of transcription factors on the promoter region of genes may depend on the respective levels of several different phytohormones. This can provide an explanation for the complex interactions observed between various regulators. The interaction between ABA and gibberellins provides a relatively simple example of such interactions (Rodgers and Rodgers, 1992; Singh, 1998). The relations between auxin and cytokinins may also be explained by regulation at the promoter level. This has been shown for the control of cell division through the expression of the *cdc2a* gene encoding a protein involved in cell cycle control (Hemerly et al., 1993). A recent example of complex gene regulation has been provided by a Korean radish peroxidase gene promoter that responds to the ratio of cytokinin to auxin (Lee et al., 2002).

The sequence of signal transmission events so far elucidated for ethylene (Bleecker and Kende, 2000) involves first, ethylene binding to a two-component receptor, with a copper ion at its active site, that acts in concert with a 'RAF-like' kinase, CTR1, to negatively regulate a membrane protein with a possible metal transport function. Consequent to this is the activation of a family of primary nuclear transcription factors that themselves promote a second transcription factor that activates responses through mechanisms at present unknown. An especially intriguing feature is the negative regulation, implying that ethylene binding switches a functioning pathway off, so that an increase in the number of ethylene receptors would actually reduce ethylene effects. Bleecker and Kende (2000) noted that receptor isoforms with different affinities for ethylene might act to extend the dynamic ranges of ethylene action. The situation is also complicated by the existence of several transduction pathways. The specific cascades of hormone

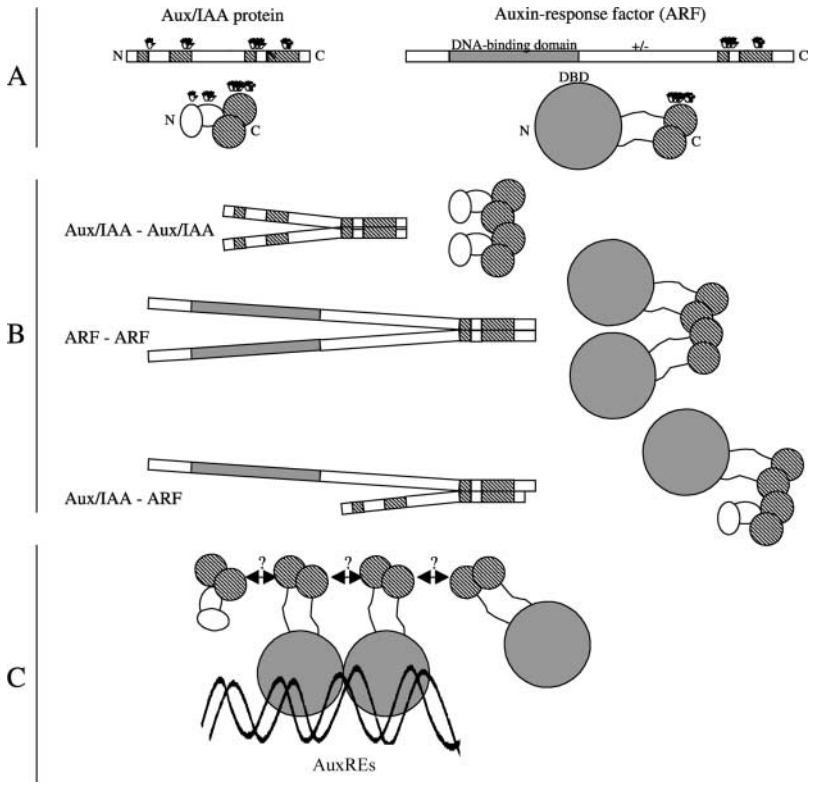

FIG. 14. *A*, Schematic diagrams for an auxin/IAA (Aux/IAA) protein and an auxin response factor (ARF). The ARF DNA-binding domain (DBD) is represented by the large, circular structure. The +/ − symbols indicate the transcriptional activation or repression domain of the ARF. *B*, Possible associations between carboxyterminal domains of ARF and Aux/IAA proteins. *C*, Associations between ARF and Aux/IAA proteins might also occur when ARFs are bound to auxin response elements (AuxREs). From Guilfoyle (1998) and Guilfoyle et al. (1998).

response systems in plants may indeed result from the combination of modules of cellular signal networks from bacteria and animals. The genes for the ethylene receptors may have a cyanobacterial origin, with no homology evident in animal or fungal genomes, while those for downstream components do have similarities to other eukaryote genes (Weyers and Paterson, 2001).

The problems of hormone transport(s) are also complicated (see Weyers and Paterson, 2001). We refer to Trewavas (1986a) who explicitly cited transport as an integral part of hormone action. Most putative roles for hormones indeed require transport, while others do not. Two difficulties associated with this problem are (1) in isolating the real sites of synthesis and effect and (2) in identifying whether a specific chemical is transported itself or as a metabolite (see Fig. 15). In some cases, hormone conjugates have been hypothesized as the transport forms. Some gibberellins are transported and some not, while some are physiologically active and others are not. It is also theoretically feasible for a precursor of an active hormone to be translocated and converted into an active compound at or near to the site of action (see Fig. 15). It would not be obvious in these cases which molecule might have true hormone status, the transported compound or the one which is active in the responsive tissue.

Finally, it must also be noted that cells communicate by means of integrins and plasmodesmata. Cellular receptors in the extracellular matrix probably bind to integrins that span the plasma membrane and then connect to the cytoskeleton through other proteins. Transduction through integrins is initiated by integrin clustering and results in activation of the Ca^{2+} pathway. The passage of signals through the plasmodesmata is thought to be regulated directly by calcium concentration. Although it might be difficult for Ca^{2+} waves to move directly from one cell to another, Ca^{2+} could generate action potentials as an alternative form of communication capable of moving from cell to cell. Plasmodesmata may play an important role in dissemination of chemical signals over short distances, including macromolecules and transcription factors, thereby allowing the formation of a supracellular network of interactions (Oparka and Santa Cruz, 2000; Trewavas, 2000; Ormenèse et al., 2001). This intercellular communication route between all plasmodesmatally connected plant cells furthermore comprises a membrane continuity, which implies a possible electrical (i.e. nerve-like) form of control.

These elements allow us to reappraise the role of phytohormones, certainly not any more through specific sites of synthesis and action, but as integrating and smoothing agents in growth and development (see also below), counteracting existing gradients, and controlling physiological resource allocation and their timing (Weyers and Paterson, 2001).

'Specific' Developmental Hormones: The Trap

It so happened that the first natural growth phytohormone, the auxin indoleacetic acid, was shown, early on, to promote

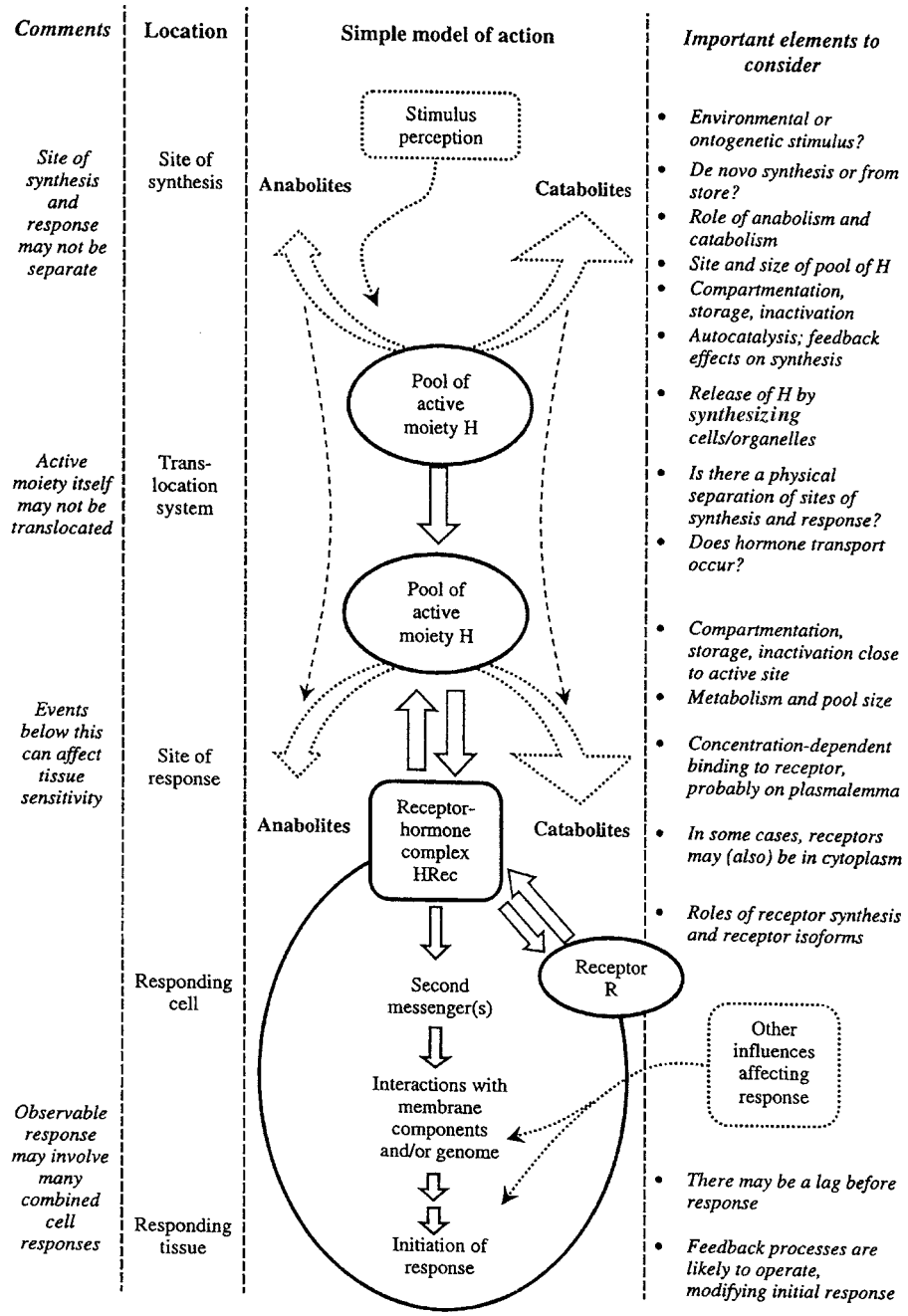

FIG. 15. Potential complexity in plant hormone responses. The diagram is divided into four *columns*. A 'simple' model of action is provided in *column 3*, while *columns 1* and *2* indicate the location of events and some spatial issues. Possible complications are illustrated both in the diagram and are noted alongside each component in *column 4*. For each of these, there is at least some evidence from published results (From Weyers and Paterson, 2001. Permission New Phytologist).

adventitious root formation (Thimann and Went, 1934). Synthetic auxins had the same effect. Hence the first idea that a single phytohormone could control both growth and developmental processes, and the still ongoing discussion about the right term to be used for such a regulator (see Introduction). The pioneer works of Bouillenne and Bouillenne-Walrand (1947) also showed, early on, that adventitious root formation did not only involve auxin but 'simultaneously' sucrose (at least sugars), an oxidase (independently of necessary respiration), and (at least) one phenolic compound. In the ambient atmosphere of the hormone discoveries, it was proposed that these components formed a unique complex(ed) molecule with a development-specific function and it was called rhizocaline (Bouillenne and Went, 1933; Went, 1934; Bouillenne and Bouillenne-Walrand, 1948). Rhizocaline could never be isolated nor identified, and Bouillenne himself (Bouillenne, 1964) claimed that adventitious root formation resulted from multifactorial control.

In the meantime, it had been shown that the developmental process necessarily involved transport of native auxin(s). It was further shown that the exogenously applied auxin may have a dual (stimulatory or inhibitory) role in rooting (Davis et al., 1988; Blakesley et al., 1991). Attempts to correlate rooting with a steady high level of endogenous auxins were contradictory (Gaspar and Coumans, 1987; Gaspar and Hofinger, 1988). Phytohormones discovered later, gibberellins for instance, were first reputed to be rooting inhibitors but it was further shown that pulse treatments at the 'right' moments might be promotive. The participation of cytokinins (as promoters of cell division and/or through the auxin/cytokinin ratio), abscisic acid, and ethylene in root formation is still debated but not excluded (Davis and Haissig, 1994; Altman and Waisel, 1997).

Our knowledge of the hormonal control of rhizogenesis increased when the process was considered to be a sequence of interdependent physiological phases (acquisition of competence and/or dedifferentiation, induction, initiation, expression: see Blakesley et al., 1991; Hausman et al., 1997a; Kevers et al., 1997) with specific requirements. The availability of suitable methods for time-course determinations of (free and conjugated) auxins (among other hormones and compounds) (see Gaspar et al., 1994) has also contributed to our understanding of the process. The main discovery that adventitious rooting obligatorily required a very temporary peaking of free auxin(s) at induction, an exhaustion at initiation, followed by a slower increase at expression phase, led to an understanding of many earlier discrepancies. The successive phases, of course associated with different cytological and histological events, have opened the doors to locate sites of action for each of the actually known hormones. Thus it was shown that the variation of polyamines could not be dissociated from that of auxins in the inductive phase of rooting (Gaspar et al., 1996a, 1997; Faivre-Rampant et al., 2000a), where peroxidase activity obligatorily undergoes a reverse pattern. Polyamines do not intervene as such: a precise regulation of their anabolism and catabolism, with roles for the catabolic products, namely γ-aminobutyrate (GABA) and H_2O_2, is needed (Hausman et al., 1997b; Martin-Tanguy et al., 1997; Aribaud et al., 1999). The same might be true for auxin (Blakesley, 1994). These progressions partly explain the involvement of secondary messengers such as calcium (Bellamine et al., 1998) and H_2O_2 (Rugini et al., 1997).

The improving knowledge of the sites of synthesis and sources of export of the different hormones involved in root formation has led to the concept that such a developmental process (the same will be true for flowering) involves nearly all the plant parts; in other words, most of the plant parts are 'informed' more or less directly of a specific developmental process ongoing somewhere in the organism. The examination of the rooting process, as a whole, from a genetic point of view, led to the conclusion that correlated effects should be expected, whether genomic modification of rooting ability is achieved by mutation, genetic engineering, or selective breeding for naturally variant alleles (Riemenschneider, 1994). The search for specific rooting genes (not necessarily associated with the specific root meristems) is nevertheless under way (Hackett et al., 1997; Duroux et al., 1998; El Euch et al., 1998; Ermel et al., 2000); but those actually identified were related to key enzymes or molecules (of phenolic metabolism, mainly) implicated in the process. Naturally, knowledge about the so-called auxin genes, i.e., genes related to auxin synthesis, transport, and signal transduction is being gained (Palme et al., 1994; Faivre-Rampant, 2002).

Flowering, as a developmental process, differs from rooting in that the sites of induction (leaves, at least in photoperiodic plants) and initiation (called evocation, at the shoot apical meristem) are distinct. Another difference lies in its dependence on or relationship with the biological clock (time perception) and the associated circadian rhythms. But changing concepts in the hormonal control of flowering followed exactly the same pattern as with rooting. Among other experiments, grafting had shown that a floral stimulus was firstly envisaged as a unique molecule with a specific function, and it was called florigen (Chaïlakhyan, 1937). After the discovery of gibberellins and their florigenic effect (at least for some plant types), Chaïlakhyan himself (Chaïlakhyan, 1985) admitted that florigen should be made of at least two components. Sachs and Hackett (1983), in the meantime developed the 'trophic diversion' hypothesis of flowering, where the vegetative shoot apical meristem should be evoked to flowering in response to the arrival of much more sucrose as a result of a modification of the source–sink distribution of the 'assimilates'. Bernier's group confirmed the florigenic effect of sucrose, but also, in a time-course, multifactorial control of flowering, including cytokinins, calcium, polyamines, amino acids, and bi-directional transport (Bernier et al., 1993). Greppin-Penel's and Krekule-Machackova's groups, in parallel, showed that auxin and ethylene were also involved but, in addition, that flowering implicated electrical signals (probably through the membrane continuum) between different plant parts (see above) (Greppin et al., 1978, 1990, 1995; Gaspar et al., 1985b; Crèvecoeur et al., 1986; Krekule and Machackova, 1986; Machackova and Krekule, 1991; Krekule et al., 1998; Greppin and Simon, 2000; see also Altamura and Tomassi, 1991). As a result, as for rooting, this means that all plant parts participate and thus are more or less simultaneously informed of the flowering process occurring at a specific place (Penel et al., 1985; Gaspar et al., 1987). Along the same lines, Wagner et al. (1998) questioned 'florigen' as being simply a frequency-coded signal. Indeed, the different organ systems most likely display their periodic organization in electrochemical oscillations, which could be the basis for communication between the tissues via frequency-coded signals. The perception of a flowering-inducing dark period possibly leads to a change in the electrochemical signaling between leaves and the apex. The involvement of action potentials is anticipated, which might trigger cytoplasmic changes in pH or Ca^{2+} as secondary messengers in photoperiodic signal transduction. There is evidence that systemic responses of plants might depend on electrochemical hydraulic controls operating at the level of membranes. Turgor-dependent volume changes, stretch-activated membrane channels and correlated changes in membrane potential might be an essential part of the 'hardware' for signal transduction at the cellular and organismic level (see above). The 'software' could involve frequency-coded signals at the cellular, the tissue, and the organismic level.

The conclusion is actually that flowering, as well as rooting, do not require a specific developmental hormone, but a sequence of specific events at the right places through coordinated control by the known phytohormones and unspecified molecules of both primary and secondary metabolism. There might even be several independent ways of flowering control (Koorneef et al., 1998; Levy and Dean, 1998; Samach et al., 2000; Araki, 2001). The use of mutants has allowed the identification of several types of gene (under the control of the above factors) concerned with flowering induction/

evocation/timing: genes coding for photoreceptor (phytochromes and cryptochromes) proteins, genes related to the biological clock and to the flowering time control, mediators between photoreceptors and the biological clock, and genes modulating sensitivity to hormones and hormonal (namely gibberellins) signaling pathways (Samach and Coupland, 2000; Périlleux and Bernier, 2002).

The time has also passed for caulocaline, a hypothesized phytohormone causing shoot development: it is now parcel of a multihormonal controlling mechanism (Leshem, 1973). Thus the formerly supposed 'specific' developmental hormones went in the trap and simultaneously it appeared that the same (unspecific) phytohormone can be involved several times in the sequential pathways of different physiological specific processes. ABA, as another example, has been proposed to have roles in stomatal regulation, dormancy, and abscission, as well as an involvement in responses to flooding, pathogen attack, and wounding (Weyers and Paterson, 2001). Similarly, gaseous ethylene serves as a hormone in plants to regulate a diverse and complex range of responses throughout plant growth and development. Ethylene is well known as the fruit-ripening hormone. Other responses to ethylene include flower and leaf senescence, leaf abscission, promotion or inhibition of cell elongation, promotion or inhibition of flowering, and breaking of seed dormancy. Environmental stresses such as wounding, chilling, pathogen invasion, and flooding enhance ethylene biosynthesis. Adaptive responses to this stress-induced ethylene include acceleration of senescence, abscission of infected organs, and induction of specific defense proteins. A number of ethylene-induced genes have been isolated and characterized (Abeles et al., 1992; Chang, 1996). Biosynthesis of ethylene in plants is highly regulated and is under both positive and negative feedback control. As for auxin(s), ethylene-inducible DNA-binding proteins interact with (an) ethylene-responsive element(s) (Ohme-Takagi and Shinshi, 1995).

Phytohormone Metabolism Integrated with Primary Biochemical Pathways

Interrelationships and cross-talking between the different phytohormone types in growth and development processes have been discussed above and detailed more in specific papers (Gaspar et al., 2000a, b). Figure 6 shows how the biosynthetic pathways of ethylene and polyamine are interconnected and dependent upon the SAM, urea, and proline cycles.

While studying the comparative physiology of habituated callus and of hyperhydrated shoots in *in vitro* cultures, we came to the conclusion that the processes of habituation and of hyperhydricity were steps of neoplastic progressions leading to true cancerous states in the absence of introduced and detected pathogens (Gaspar et al., 1991a). The auxin- and cytokinin-autonomy of habituated tissues could not be explained simply by an overproduction of these hormones (Kevers et al., 1999b). A shift to a greater sensitivity to the couple ethylene/polyamines was shown (Kevers et al., 1999a; Bisbis et al., 1998, 2000b). On the other hand, we were very early confronted with three main characteristics of both habituated calluses and vitrified tissues: an overproduction of polyamines, a lower emission of ethylene, and a deficiency in tetrapyrole-containing compounds (references in Gaspar et al., 2000a). The factors and metabolism contributing to polyamine accumulation in neoplastic cells were established:

(1) A deviation of nitrogen metabolism as a result of ammonia detoxication, through glutamate, which implicated the Kreb's cycle and the proline cycle (Fig. 16).

(2) The continuous deviation of α-ketoglutarate from the Kreb's cycle to the glutamate–proline–polyamines pathway was rendered possible through an anaplerotic pathway: replenishment of the Kreb's cycle with oxaloacetic and malic acids resulting from a higher nonphotosynthetic fixation of CO_2 to phosphoenolpyruvate (Fig. 17) (Bisbis et al., 1997).

(3) An activation of the pentose phosphate pathway providing the NAD(P)H surplus needed for the reduction of nitrate to nitrite, and supporting the functioning of malate dehydrogenase (MDH) as indicated in Figs. 17 and 18.

(4) An operative GABA-shunt through glutamate and polyamine degradation, supplying the necessary succinate for the turnover of the Kreb's cycle where the link ketoglurate–succinate is deficient (Fig. 18; references in Gaspar et al., 2000a).

(5) A higher alternative respiratory pathway (Bisbis et al., 2000c) (see Fig. 18).

The lower emission and retention of ethylene by habituated tissues has been shown to be related to polyamine metabolism, not only through competition for a common substrate, but also through mutual interaction on their metabolism, depending, as seen above, on primary biochemical pathways (Bisbis et al., 2000a; Gaspar et al., 2000a).

The deficiency of tetrapyrrole-containing compounds in non-chlorophyllous habituated cancerous tissues was shown to result from the unique functioning (at a limited rate) of the lower organism and animal mitochondrial Shemin pathway, the common higher plant Beale pathway (depending on oxoglutarate and glutamate supply) being inefficient in the achlorophyllous cells (Fig. 19) (Gaspar et al., 1999; Bisbis et al., 2000a). Polyamines and ethylene are not the sole phytohormones whose metabolisms (and action) are related to disturbed nitrogen and carbon primary biochemical pathways in the above examples. Figure 20, from Le Dily et al. (1998), shows how the biosynthesis of auxins and cytokinins might also be affected. Accumulation and translocation of cytokinins in

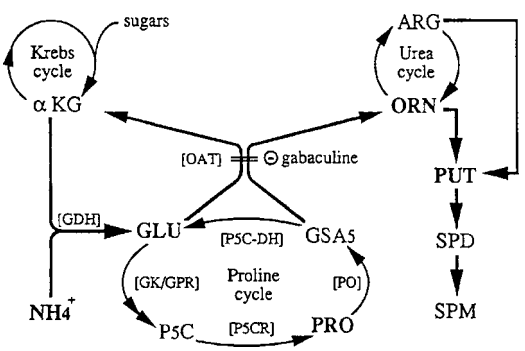

FIG. 16. Proposed scheme of disturbed nitrogen metabolism in the fully habituated callus of *Beta vulgaris* L. The *bold arrows* indicate the tight linkage between glutamate, proline, and ornithine which leads to polyamine synthesis. ARG, arginine; GDH, glutamate dehydrogenase; GK, glutamyl kinase; GLU, glutamate; GPR, glutamylphosphatase reductase; GSA_5, glutamate-5-semialdehyde; αKG, α-ketoglutarate; OAT, ornithine aminotransferase; ORN, ornithine; P_5C, pyrroline-5-carboxylate; P_5CR, pyrroline-5-carboxylate reductase; PO, proline oxidase; PRO, proline; PUT, putrescine; SPD, spermidine; SPM, spermine (adapted from Gaspar et al., 1998).

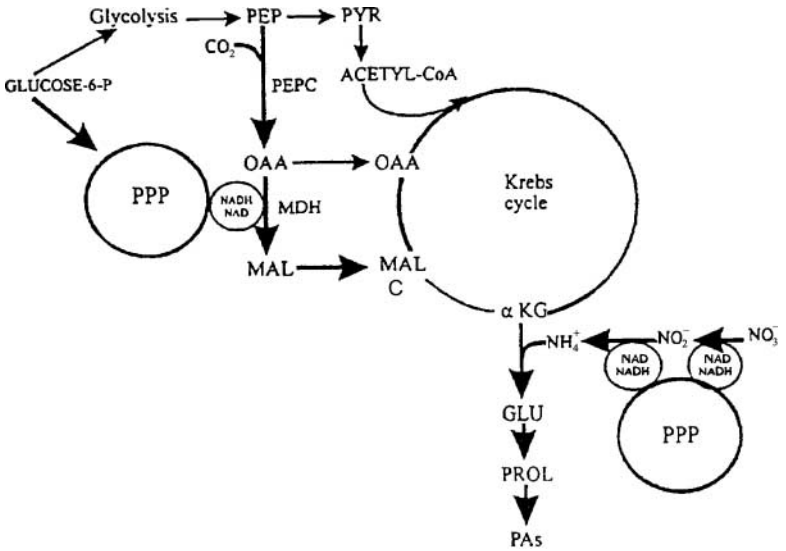

FIG. 17. Scheme showing the privileged glucose respiratory pathway of fully habituated cells (*heavy lines*) and the relationships with the nonphotosynthetic CO_2 fixation to contribute, together with ammonia detoxification, to the overproduction of polyamines. MAL, malate; OAA, oxaloacetic acid; PPP, hexose monophosphate and pentose phosphate pathway; PYR, pyruvate (adapted from Gaspar et al., 1998).

maize is mainly dependent upon nitrogen availability (Takei et al., 2001).

These results illustrate another changing concept in plant hormone studies: that the hormonal biochemical pathways cannot be dissociated from the primary ones, and thus necessarily operate in an integrated manner, as already stated by Siedow and Stitt (1998).

CONCLUSION

The strategy of the co-actions between the genetic biosphere and the environmental ecosphere, in higher plants, is very different in comparison with animal organization and responses. Autotrophy and immobility have as a consequence a direct and permanent insertion (light, water, temperature) in the environmental constraints (Greppin

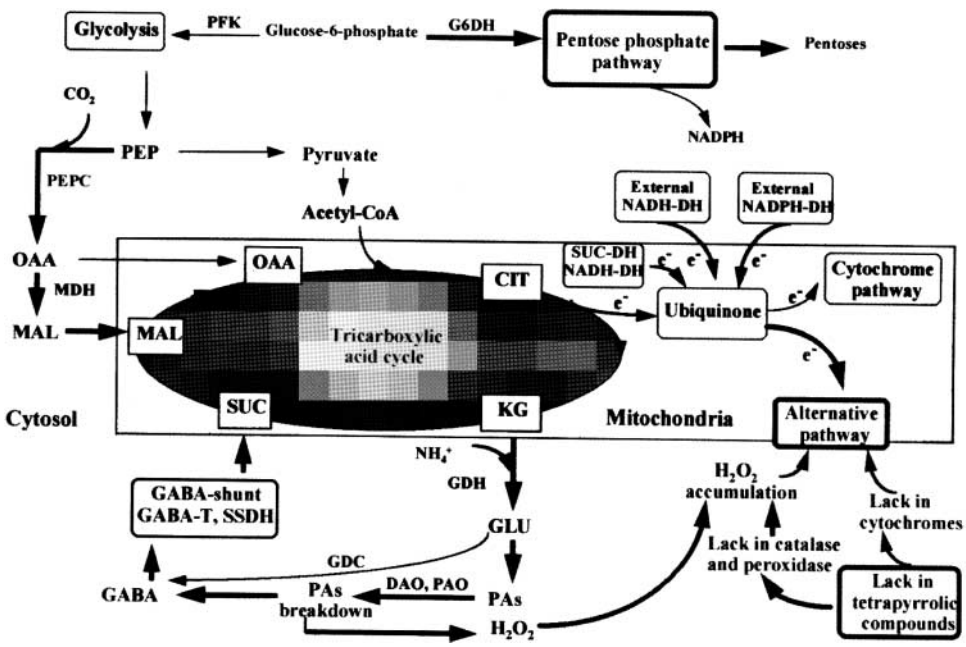

FIG. 18. Illustration of the favored metabolisms (*heavy lines*) and their contributions in the induction of the alternative respiratory pathway and in the functioning of a GABA-shunt in fully habituated neoplastic sugarbeet cells. 6-PGDH, 6-phosphogluconate dehydrogenase; G-6PDH, glucose-6-phosphate dehydrogenase; PEPc, phosphoenolpyruvate carboxylase; MDH, malate dehydrogenase; GABA, γ-aminobutyrate; GDH, glutamate dehydrogenase; GDC, glutamate decarboxylase; GABA-T, γ-aminobutyrate transaminase; SSDH, succinic semialdehyde dehydrogenase; Δ′-pyrDH, Δ′-pyrroline dehydrogenase; CoA, coenzyme A; NAD, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide (reduced form); NADPH, nicotinamide adenine dinucleotide phosphate (reduced form) (according to Bisbis et al., 2000c).

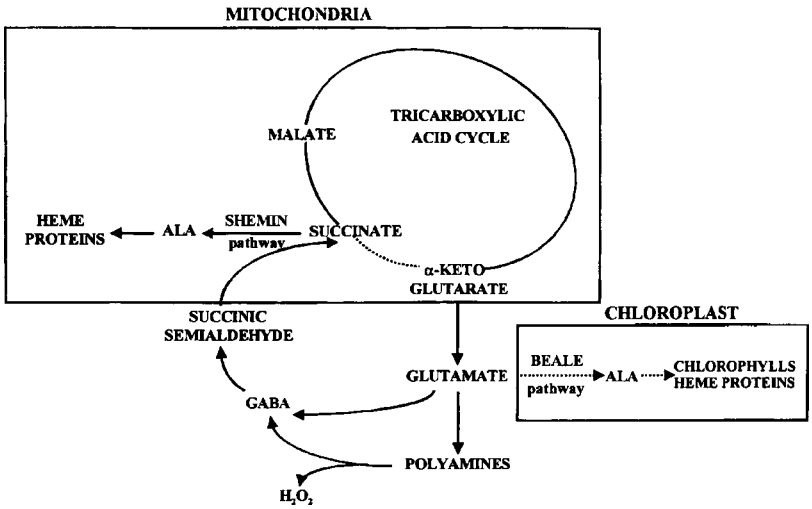

FIG. 19. GABA-shunt, through glutamate and through polyamines, in the tricarboxylic acid cycle, with indications of the Beale (from glutamate) and Shemin (from succinate) pathways of aminolevulinic acid (ALA) and porphyrin (including peroxidase) biosynthesis (according to Gaspar et al., 2000a).

et al., 1998). There is no doubt that a battery of endogenous hormones and other known and unknown factors regulate plant growth and development, as responses to environmental stimuli. But the concepts in plant hormone action have changed dramatically with the progressive discovery of new phytohormones. It can now hardly be claimed that a single hormone is responsible for one growth or development process. Growth and development processes have been dissected into successive interdependent physiological phases with different requirements. Moreover, in many cases, it was shown that the control of these events is due to the simultaneous interaction of different plant hormones, acting synergistically or antagonistically, rather than to the effect of a single hormone. Distinct cell types respond differentially to various signals. It is clearer now that the hormonal controls act in a developmental and tissue-dependent manner. Thus, the formerly claimed specificity of one hormone may simply be the result of this preponderance in a balance with another one; for example, the ratio of auxins to cytokinins in determining growth and development. In neoplastic tissues, the sensitivity to this couple of hormones is shifted to the tandem polyamine/ethylene (Kevers et al., 1999b; Gaspar et al., 2000a). The possibility that different hormonal receptors control growth and development (De Klerk et al., 1997) or that different hormones compete for a common receptor or operate in separate signaling pathways (Timpte et al., 1995) is being investigated.

Apparently, different hormones play in very tightened sequential events in the control of cell division cycles (cf. John et al., 1993; Ormrod and Francis, 1993). Moreover, the level of any one hormone affects the levels of the others by affecting their biosynthesis, degradation, conjugation, or transport (Itai and Birnbaum, 1991). This is called hormonal cross-talking (Smalle and Van der Straeten, 1997; Rodrigues-Pousada et al., 1999). It seems, therefore, that determining the level and effect of one hormone may yield information with rather limited value. No single hormone has an overriding role.

Hormone metabolism and action cannot be dissociated from the primary metabolic pathways with reciprocal influences (Gaspar et al., 1998, 2000a, b). This means that the effects of an externally applied hormone, or of an analog, cannot be interpreted simply through an increase of its endogenous bulk (Pilet, 1996), but that

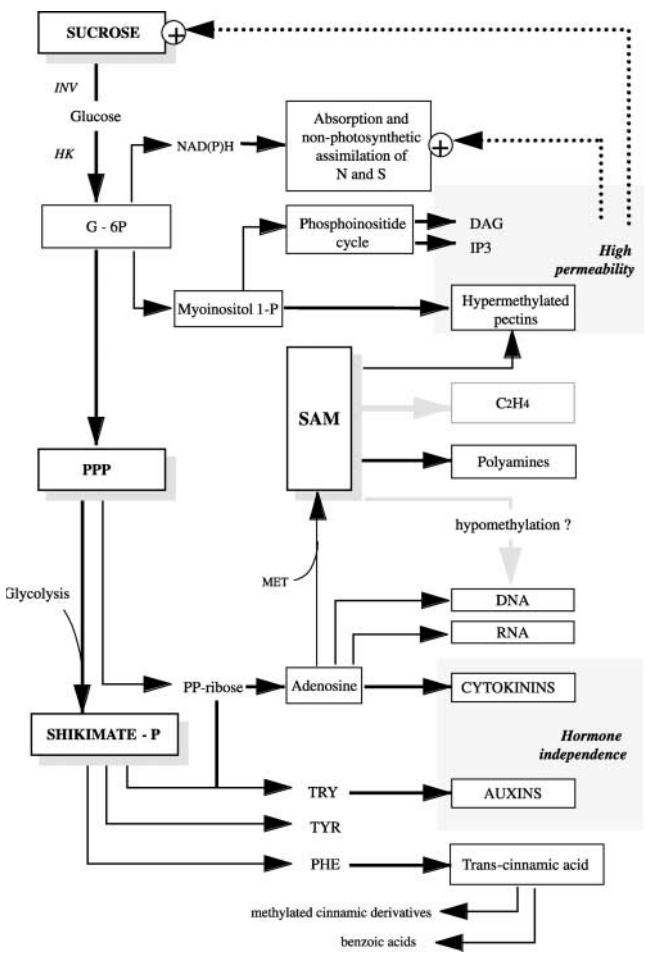

FIG. 20. Deviation of carbon metabolism and consequences of enhanced sucrose hydrolysis, pentose phosphate pathway, and shikimate pathway on hormonal autonomy of fully habituated neoplastic sugarbeet cells (according to Le Dily et al., 1998). For abbreviations see other figures.

changes in metabolism and its role as an exterior signaling molecule have to be considered.

Auxins and cytokinins were originally thought to produce growth responses at distances from their sites of synthesis, and thus to fit the definition of transported chemical messengers. It is now clear that none of the recognized five main classes of phytohormones (auxins, cytokinins, gibberellins, abscisic acid, ethylene) fulfil the requirements of a hormone in the mammalian sense, i.e., chemical messengers at low concentration, involving a localized site of synthesis, transport to a target tissue, and control of a precise physiological response in a target tissue via the concentration. The synthesis of all plant hormones, as a rule, occurs, or can occur, in any type of living cell, even if certain tissues are privileged sites of synthesis and export for some hormones (e.g., aerial meristemic tissues for auxins or growing root parts for cytokinins). Thus, phytohormones may be transported and participate in some precise physiological processes at a distance. However, it is far from being the general case; they also act in the tissue or even within the cell in which they are synthesized. Furthermore, experimental results strongly argue that phytohormone control is not only by concentration but also by changes in sensitivity of the cells to the compounds (Trewavas and Cleland, 1983). Thus, the responses evoked by plant hormones are rarely proportional to their concentration. Furthermore, the countercurrents of hormones between aerial and underground plant parts create varying gradients and continuously change organ cross-talk.

Thus, while animals and plants appear to have co-inherited homologous intracellular signaling systems, at the whole organism level, modes of hormone action may diverge. The classical 'synthesis–transport–action' mechanism of action, as a distance signal, where hormones are overriding controllers (giving 'the' executive control message for a response residing in a complicated array of stimuli), may be just one of several possible ways that phytohormones could control physiological processes. Hormonal involvement in physiological processes can appear in several other distinct manifestations: plant hormones can act as enabling substances (when sensitivity dominates and competence to respond dictates the response), stimulus integrators (by triggering a single reaction where multifold signal inputs may operate), response coordinators, temporal messages (hormones are seen as enduring messages), environmental sensors and homeostatic regulators, resource allocators and spatio-temporal synchronizers, biotime adjusters and inter-plant signalers (Weyers and Paterson, 2001).

Discerning how the different hormones interact and how their quantities are affected by environmental factors will be important and necessary to improve traits of agricultural (and economic) importance in crop plants. Preliminarily, sequencing of plant genomes and the associated genomic technologies (such as the use of mutants) will have a great impact on the knowledge and understanding of the genes and enzymes involved in the regulation of plant hormone homeostasis (Crozier et al., 2000).

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