ROLES OF PLANT GROWTH REGULATING SUBSTANCES

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Summary

Plant growth is regulated by several mechanisms. Five classes of plant hormones have been identified. They are auxin, cytokinins, gibberellins, abscisic acid and ethylene. In general, these hormones control plant growth and development, affectingcell division, elongation, and differentiation of plants. Auxin-induced genes fall into two categories: early and late, by repressor proteins that are degraded via a ubiquitin activation pathway.

Gibberellin acts by deactivating repressors, such as SPY, GAI, and RGA route to both an increase in cell elongation and the production of α-amylase. Cytokinins increase the abundance of several specific mRNAs. Some of these are primary response genes that are similar to bacterial two-component response regulators. A family of genes, which encode proteins similar to bacterial two-component histidine kinase, encodes the ethylene receptor. Ethylene binds to these receptors in a transmembrane domain and turns on the signal transduction pathway. The ABA response appears to be regulated by more than one signal transduction pathway.

1. Introduction

Five classes of plant hormones have been identified. They are auxins, cytokinins, gibberellins, abscisic acid and ethylene. In general, these hormones control plant growth and development, affecting cell division, elongation, and differentiation of plants. Each hormone has multiple effects, depending on its site of action, the developmental stage of the plant, and the concentration of the hormone.

Recently, transgenic technology has provided insights into the expression of genes involved in the biosynthesis of hormones. This article deals with the physiological role, metabolism, and molecular action of the five classical plant hormones, namely, indole-3-acetic acid (IAA), gibberellins (GAs), abscisic acid (ABA), cytokinins, and ethylene (Figure 1).

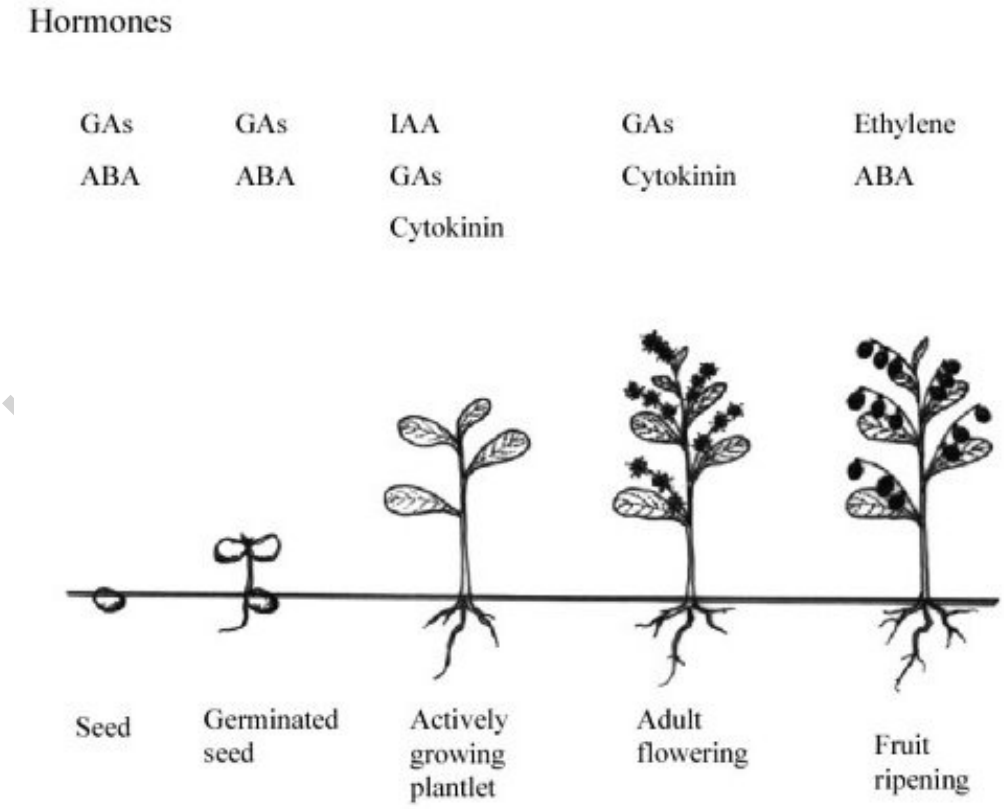

Figure 1. Key hormones regulating the germination of the seed, the growth of the plantlet and the flowering of the plant, as well as and ripening of the fruits.

2. Indole-3-Acetic Acid

The natural auxin is IAA. It can promote cell division and cell elongation. Yet it may also inhibit root elongation and lateral buds. Auxins have commercially been used in agriculture and horticulture for over 50 years, e.g. in rooting of cuttings for plant propagation, promotion of flowering, prevention of fruit fall, etc. Auxins have wide effects on plant growth and morphogenesis.

IAA acts as a signal for cell division, elongation, and differentiation at the cellular level. Several classes of auxin-binding proteins have been identified and characterized, but it is not clear which if any of these functions as receptors in signal transduction pathway. Yet multiple types of auxin receptor and signal transduction pathways could account for some of the diversity observed in different tissues that respond to auxin. It has been reported that some primary or early auxin-responsive genes are activated or repressed within a second to three minutes after auxin application. Recent results in the IAA biosynthesis and its molecular action are extensively reviewed.

2.1. IAA Biosynthesis

Shoot apical meristems, young leaves, and developing fruits are the primary sites of IAA synthesis. Plants can synthesize IAA from L-tryptophan by three different routes: indole-3-pyruvic acid, indole-3-acetaldoxime, and tryptamine pathways. In cabbage, plasma membrane-bound enzymes convert L-tryptophan to IAA via indole-3-acetaldoxime and indole-3-acetonitrile. Indole-3-acetaldoxime is also a precursor of indole-3-methylglucosinolate, which can be metabolized to indole-3-acetonitrile. Indole-3-acetonitrile is converted to IAA by the action of nitrilases. Four genes encoding nitrilase enzymes have been cloned from *Arabidopsis*. Transgenic tobacco also expresses the nitrilase gene to hydrolyze indole-3-acetonitrile to IAA. There is a tryptophan-independent pathway of IAA biosynthesis, and indole-3-pyruvic acid is synthesized independently of tryptophan. The specific pathway of IAA biosynthesis, either tryptophan-dependent or tryptophan-independent, is developmentally controlled. Free IAA is the biologically active form, yet the majority of IAA is covalently bound and hormonally inactive. IAA is conjugated to give both high- and low-molecular-weight products, such as esters of IAA, IAA-N-aspartate, IAA-glucan and IAA-glycoproteins. Metabolism of conjugated auxin stores is a major contributing factor in the regulation of the free auxin levels. The formation of conjugated auxins may also serve other functions like protection against oxidative degradation.

Like IAA biosynthesis, the enzymatic breakdown of IAA may involve more than one pathway. Peroxidase is responsible for IAA oxidation, and this pathway leads to an oxidized product, 3-methyleneoxindole. A cationic peroxidase is able to degrade IAA, but transgenic plants with tenfold increase of anionic peroxidase do not show a change in the IAA levels. Two other oxidative pathways have also been proposed to contribute to the degradation based on isotopic labeling and metabolite indentification. IAA is also oxidized to oxindole-3-acetic acid without decarboxylation.

2.2. Physiological Role of IAA

IAA causes a fairly rapid increase in cell wall extensibility in coleoptiles and young developing stems. According to the theory, auxin causes a responsive cell to extrude protons actively into the cell wall region, and the resulting decrease in pH activates wall-loosening enzymes that promote the breakage of key cell wall bonds, increasing wall extensibility. Cell wall acidification is not the only way in which auxin induces plant cell elongation. Acid-stimulated wall loosening alone cannot cannot sustain elongation for hours, as auxin can. Auxin may also promote the synthesis of cell wall proteins, which are required for growth.

In addition to regulation of cell elongation in the normal course of plant development, auxins may mediate the effects of light and gravity on plant growth. Phototropism may be due to lateral redistribution of auxin. An unequal lateral distribution of auxin and calcium at the tip results in gravitropism in roots. IAA is synthesized in the shoot and transported to the root. In a horizontal root the statoliths settle to the side of the cap cells, triggering polar transport basipetally within the cortex. The arrival of supra-optimal IAA inhibits elongation and causes downward curvature. In young shoots the ratio of free IAA to total IAA is much higher in the rooting zone of the woody shrub *Cotinus coggygria*. IAA conjugates applied to the rooting solution do not stimulate rooting, which supports the assumption that once endogenous IAA is conjugated it becomes inactive in adventitious root initiation. Plant tissues are also capable of non-decarboxylative IAA degradation and decarboxylative IAA oxidation. Basic peroxidase localized in the cytoplasm of lupin (*Lupinus sp.*) phloem parenchyma cells is involved in the oxidation of IAA. A transient decrease of IAA in the levels of peroxidase in the first few hours following the excision of *in vitro* cutting of grapevine has been reported. This decline coincides with a transient rise in the levels of IAA in the rooting zone. A decline of peroxidase activity as well as transcripts occurs during the induction of adventitious roots in soybean hypocotyls. This supports the idea that elevated levels of IAA are important during root induction.

2.3. IAA Mode of Action

Initially, it was believed that only the slow IAA responses involved altered patterns of gene expression, but IAA can also stimulate the expression of certain genes with a lag time of only three minutes, i.e. there are early or late responsive genes. Five major classes of early auxin-responsive genes have DNA-binding motifs similar to those of bacterial repressors. Members of the Aux/IAA gene family encode short-lived transcription factors that function as repressors or activators of the expression of late auxin-inducible genes. To be induced, the promoters of the early auxin genes should contain response elements, binding to the transcription factors, which become activated in the distinct auxin response domains (AuxRDs) with the sequence TGTCTC. IAA causes the ubiquitination and dissociation of the repressor protein, then the activation of transcription factors, and initiation of the IAA-induced transcription for some early genes.

There are three types of IAA binding sites identified in the endoplasmic reticulum, plasma membrane and the tonoplast. The IAA receptors are found on the plasma membrane. The binding site identified in the endoplasmic reticulum satisfies also the criterion of receptor. The auxin (IAA) binding protein (ABP1) has been purified and

sequenced. The localization of ABP1 in the ER seems to contradict the original assumption that the auxin receptor is located on the plasma membrane. Studies with impermeable analogs—protein conjugation in pea epicotyl sectionssuggest that IAA does not have to enter the cell to be active. Some 2% of the total ABP1 is secreted to the surface of maize coleoptile protoplasts. This amount of ABP1 may be sufficient for it to act as an IAA receptor on the cell surface. Also, a mitogen-activated protein kinase (MAPK) is activated during auxin-induced cell division in tobacco. Moreover, the level of free calcium increases.

3. Gibberellins

In the 1930s Japanese scientists succeeded in obtaining impure crystals of two fungal growth-active compounds, which they termed gibberellin A and B. Until the mid-1950 two groups, one at Imperial Chemical Industries (ICI) in Britain, the other at the U.S. Department of Agriculture (USDA), coincidently succeeded in purifying the compound from culture filtrates, which they named gibberellic acid. More than 125 gibberellins are present in plants, but genetic analyses have indicated that only a few of them are active as plant hormones. Some of them are precursors or represent inactivated forms. Gibberellins have a variety of effects in plants, including promoting stem elongation, flowering, fruiting, growth and seed germination. The focus here is the effect of gibberellins on flowering.

3.1. GA Biosynthesis

Gibberellins are synthesized via the terpenoid pathway. Gibberellins are tetracyclic diterpenoids made up of four isoprenoid units. The GA biosynthetic pathway takes place in three stages. In stage 1, geranylgeranyl pyrophosphate (GGPP) is converted to ent-kaurene via ent-copalyl pyrophosphate (CPP). In stage 2, ent-kaurene is converted to GA_{12}-aldehyde. In stage 3, GA_{12}-aldehyde is converted to the first gibberellin. The specific step is the modification of GA_{12}, which varies from species to species. Two basic chemical changes occur in most plants: (1) hydroxylation at carbon 13 or carbon 3, or both; (2) a successive oxidation at carbon 20 (CH2→CH2OH→CHO), followed by loss of carbon 20 as CO_2. In higher plants, GA_{12} is first converted to GA_{53} by hydroxylation of carbon 13, GA_{53} is then converted to GA_{19} by successive oxidations at carbon 20, followed by the loss of carbon 20 as CO_2 to produce GA_{20}. GA_{20} is then converted to the biologically active form, GA_1, by the enzyme 3β-hydroxylase. Finally, 2β-hydroxylation reactions inactivate GA_1 by converting it to GA_8, or GA_{20} is removed from the pathway by converting it to GA_{29}. Gibberellins play an important role in mediating the effects of environmental stimuli on plant development. Environmental factors such as photoperiod and temperature can after the levels of active GA_3 by affecting specific steps in the biosynthetic pathway. When plants that require long days to flower are transferred from short days to long days, an alteration in GA metabolism is observed. A variety of gibberellin glycosides are formed by covalent linkage between GA and a monosaccharide. Glycosylation may therefore represent another form of inactivation. Glucosides may also be a storage form of gibberellins. The level of active GA can be reduced by catabolism or by conjugation to sugars. In some cases, active GA can be generated by release from the conjugated form. The transport of GA to a tissue can also affect the steady-state level of active GA.

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Biographical Sketches

Professor Zin-Huang Liu was born in Taiwan in 1950. He received his B.S. and M.S. from Tunghai University and PhD. in Simon Fraser University in Canada. He has been chairman of the Department of Biological Sciences, and director of DIVERSITAS research center in National Sun Yet-Sen University. He is chairman of Department of Life Sciences and Director of the Institute of Biotechnology in National Pingtung University of Sciences and Technology. Dr. Liu received the research award of the National Science Council (1995 to 1999).

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Professor Chang-Hung Chou was born in Taiwan in September 1942. He received his B.S. and M.S. in National Taiwan University, and a Ph D in the field of Plant Ecology from the University of California at Santa Barbara in 1971. Professor Chou has been vice president of National Sun Yet-Sen University, director of the Institute of Botany, Academia Sinica, research fellow of Institute of Botany, Councilor of Academia Sinica and Vice President of the International Union of Biological Sciences. He received many distinguished research awards from the National Science Council, Ministry of Education, Taiwan. He was elected Fellow of the Third World Academy of Science, Member (Academician) Academia Sinica, and Award of Highest Honor of Soka University. He is now the National Chair Professor and President of National Pingtung University Sciences and Technology.