PLANT HORMONES

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What are Plant Hormones?

Plants need sunlight, water, oxygen, minerals for their growth and development. These are external factors. Apart from these, there are some intrinsic factors that regulate the growth and development of plants. These are called plant hormones or "Phytohormones".

- Plant hormones are chemical compounds present in very low concentration in plants. They are derivatives of indole (auxins), terpenes (Gibberellins), adenine (Cytokinins), carotenoids (Abscisic acid) and gases (Ethylene).

- These hormones are produced in almost all parts of the plant and are transmitted to various parts of the plant.

- They may act synergistically or individually. Roles of different hormones can be complementary or antagonistic.

- Hormones play an important role in the processes like vernalisation, phototropism seed germination, dormancy etc. along with extrinsic factors.

- Synthetic plant hormones are exogenously applied for controlled crop production.

Charles Darwin first observed the phototropism in the coleoptiles of canary grass and F.W. Went first isolated auxin from the coleoptiles of oat seedlings.

What are the main functions of plant hormones?

Plant hormones control all the growth and development activities like cell division, enlargementflowering, seed formation, dormancy and abscission

Based on their action, plant hormones are categorised into two categories:

- Plant Growth Promoters
- Plant Growth Inhibitors

growth Promoters.	growth inhibitors.	Inhibitors and promoters
• Auxins.	• Abscisic acid.	• Ethylene
• Gibberellins		
• Cytokinins		

AUXINS

Auxin means "to grow". They are widely used in agricultural and horticultural practices. They are found in growing apices of roots and stems and then migrate to other parts to act.

Auxins are very widely used in plant tissue culture and usually form an integral part of nutrient media. Auxins promote, mainly in combination with cytokinins, the growth of calli, cell suspensions and Organs, and also regulate the direction of morphogenesis. The word auxin has a Greek origin: auxein means to enlarge or to grow. At the cellular level, auxins control basic processes such as cell division and cell elongation.

Since they are capable of initiating cell division they are involved in the formation of meristems giving rise to either unorganised tissue, or defined organs. In organised tissues, auxins are involved in the establishment and maintenance of polarity and in whole plants their most marked effect is the maintenance of apical dominance and mediation of tropisms (summarised in Friml, 2003).

The choice of auxins and the concentration administered depends on:

this case to have been associated with a decrease in natural auxin biosynthesis. Nakamura et al., (1998) found a correlation between decreased rhizogenic activity and the activity of a root-specific peroxidase in tobacco callus. On the other hand, in carrot cell culture, 2,4-D protryptophan-derived IA tryptophan-derived IAA (Michalczuk et al., 1992).

In cultures of wild cherry, endogenous levels of IAA are also considerably reduced by the presence of 2,4-D, although the availability of tryptophan (the precursor of IAA biosynthesis) was increased (Sung, 1979). This suggests that 2,4-D interfered directly with IAA synthesis or hastened IAA conjugation/degradation. 2,4-D inhibition of IAA synthesis has been noted in sycamore suspension cultures (Elliott et al., 1978). Conversely, reducing the external 2,4-D and NAA concentration resulted in a significant increase in internal free IAA concentration in the auxin-dependent and cytokininautonomous tobacco cell strain VBI-0 (Zažímalová et al., 1995). Maeda and Thorpe (1979) suggested that indole-based synthetic auxins might protect IAA from natural destruction by competing with it for IAA oxidase enzymes.

In carrot hypocotyl explants, neither 2,4-D nor NAA, both of which induced callus formation, had any effect on endogenous IAA concentration. This shows that in this case synthetic auxins induced morphogenesis themselves (Ribnicky et al., 1996). Inhibition of somatic embryos in the globular stage was observed during co-cultivation in growthregulator-free medium for carrot and Arabidopsis somatic embryo cultures. This was probably due to the high intracellular content of 2,4-D in Arabidopsis cultures created during preceding cultivation in 2,4D-containing medium and its release following transfer to growth-regulator-free medium (Meijer et al., 1999).

STABILITY IN CULTURE MEDIA

IAA and to some extent also IBA are heat labile and decompose during autoclaving. IAA is also unstable in culture media at room temperature. In the dark, there can be more than a ten-fold decrease in concentration over a four-week period in the absence of inocula (Campbell and Sutter, 1986; Nissen and Sutter, 1988). The rate of decrease of IAA is even more rapid in the light and is accelerated by the presence of MS salts (Dunlap et al., 1986). In liquid MS medium incubated at 25°C in a normal 16 h photoperiod, 10 µM (1.75 mg/1) IAA was reduced to less than the limit of detection (0.05 mg/l) in 14 days (Nissen and Sutter, 1988).

Because, for oxidative degradation, oxygen is required, in solid media the degradation of IAA is likely to be significantly less. IBA is more stable in solution than IAA: 75% remained unaltered after 30 days in the dark, and 40 % after 30 days in the light. Other auxins such as NAA and 2,4-D are not oxidized (Dunlap et al., 1986), but once absorbed within plant tissues, the rate of either degradation or conjugation of all auxins may be rapid, for they are then not only exposed to physical factors, but are also subjected to enzymatic conversion.

TRANSPORT OF AUXINS

Auxins seem to be the only group of plant hormones exhibiting on the level of the whole plant or its parts active transport in a polar manner in addition to long-distance movement via vascular tissues (Hopkins, 1995). Auxins appear to be transported long distances extensively through the phloem (but probably not the xylem) of higher plants. The free IAA present in the phloem sap is probably synthesised and exported from the mature leaves. This would accord with Sheldrake's hypothesis (Sheldrake, 1973) that the meristems are net importers rather than synthesisers of IAA in higher plants (reviewed by Baker, 2000) and seems to be in contradiction to the widely accepted idea that meristems are the sites of auxin biosynthesis.

The phenomenon of polar auxin transport was demonstrated first in the late 1920s (Went, 1928). In contrast to the movement of auxin via vascular tissues, polar cell-to-cell auxin transport is localised predominantly in parenchyma cells in the sheath surrounding differentiated vascular tissue. The polarity of auxin transport was explained (Rubery and Sheldrake, 1974; Raven, 1975) by the different permeability of opposite parts of cells for dissociated and undissociated molecules of indole-3-acetic acid (IAA and IAAH, respectively), and by an asymmetric localisation (basal in the stem cells) of a so-called auxin efflux carrier (translocating the IAA anion outside the cell). This idea was summarised by Goldsmith (1977) and named the "chemiosmotic polar diffusion theory". Now, it is believed – on the basis of biochemical, physiological and molecular biological data that at the level of the individual cell, both passive diffusion and an auxin-uptake (influx) carrier drive auxin translocation into the cell while an auxin efflux carrier drives auxin transport out of the cell. The activity of both carriers, to different extents, can be inhibited by several synthetic compounds, mainly of the phytotropin type, e.g. 1-naphthylphthalamic acid (NPA) (8) (reviews by Lomax et al., 1995; Bennett et al., 1998; Morris, 2000). Now several genes are known, coding for both putative carriers and for the NPA-binding site.

Typical representatives of these genes, AUXI (coding for the putative auxin uptake carrier), PIN gene family (coding for the putative auxin efflux carriers, expressed in different tissues and organs) and others have been identified in Arabidopsis thaliana (summarised in Galweiler et al., 1998; Morris, 2000; Friml and Palme, 2002; Morris et al., 2004). The effects of potential auxin transport inhibitors on tissue and organ culture are described below. Delbarre et al., (1996) have studied auxin accumulation at the cell level in detail. Using various radiolabelled auxins, the biochemical properties (kinetic characteristics and specificity) of both carriers (for uptake and efflux) were determined including data on interactions of carriers with potential inhibitors. On the basis of different behaviour of both auxin uptake and efflux carriers towards NAA and 2,4-D, Delbarre et al., (1996) proposed a simple method for measurement of the activity of these carriers: NAA can be used for determination of the efflux carrier, while 2,4-D can serve as a marker of uptake carrier activity. This approach has been used for identification of new auxin inhibitors of aryl and transport aryloxyalkylcarboxylic acid type (Imhoff et al., 2000). Based on studies of auxin transport inhibitors, the hypothesis arose that, unlike the auxin uptake carrier, the auxin efflux carrier has a rather complex structure. It seems to consist of at least two, probably plants like other higher organisms - have to possess intraorganismal communication system(s) working over relatively long distances.

interact with, auxin? Auxin requiring callus of tobacco has been found to accumulate more p-coumaric and p-ferulic acid (Table 5.2) than callus, (Zador et al 1985)

Other compounds, which have been suspected to be naturally-occurring inhibitors of IAA oxidase, may increase callus growth in certain circumstances. They include the quinone, juglone (Compton and Preece, 1988), some diphenolic flavonoids with antioxidant properties such as naringenin (Phillips, 1961, 1962), quercitin and its glycoside quercitrin (Furuya et al., 1962; Thimann, 1963; Feucht and Nachit, 1978), catechin and flavandiols (Feucht and Nachit, 1977; Feucht and Schmid, 1980) and chemicals of the B-inhibitor complex' (BennettClark and Kefford, 1953). Examples of the latter are coumarin (see above), scopoletin (and its glucoside scopolin) (Schaeffer et al., 1967), and various phenolic acids such as caffeic acid, chlorogenic acid, and sinapic acid (Thimann, 1963).

The phenolic vitamin riboflavin might be also of importance for plant development. It was shown to participate in the mechanism of colonization of alfalfa roots by Sinorhizobium meliloti (Yang et al., 2002), to promote induction of embryogenic callus in Zoysia japonica (Asano et al., 1996) and to protect auxin from oxidation (Brennan, 1996).

AUXIN -ETHYLENE INTERACTIONS

Higher auxin concentrations almost invariably increase ethylene production (see review by Kende, 1993). Ethylene accumulated in the tissue culture vessels may then inhibit the growth and

Development of many tissue culture grown plants. Conversely, ethylene may effect auxin transport and metabolism.

GIBBERELLINS

There are more than 100 gibberellins (GA1, GA2, GA3…..) that are known. They are acidic in nature. These are found in higher plants and fungi.

Gibberellins (Gas), a class of diterpenoid phytohormones, produced by plants and some fungi play an important role in modulating diverse processes throughout plant growth and development. So far, up to 136 different gibberellin molecules have been discovered, only a few of which are bioactive, such as GA1, GA3, GA4, and GA7. Recent studies on GA biosynthesis, metabolism, transport, and signaling, as well as cross talk between GA and other plant

hormones and environmental cues have achieved great progress along with the advancement of molecular genetics and functional genomics. Accumulating evidences suggest that the "de-repression" model makes it possible to explain signal transduction mechanisms in GA action. Bioactive Gas promote plant growth and development by promoting the degradation of the DELLA proteins, a family of nuclear growth repressors. The GA signal is perceived by the soluble receptor protein GIBBERELLIN INSENSITIVE

DWARF1 (GID1) that undergoes a conformational change and then promotes GA-GID1DELLA association with the Skp1-Cullin-F-box (SCF) E3 ubiquitin-ligase complex via the F-box protein (SLEEPY1 [SLY1] in Arabidopsis and GIBBERELLIN INSENSITIVE DWARF2 [GID2] in rice), thereby targeting the DELLA proteins for degradation via the 26S proteasome pathway. Evidence also shows that Gas act as mobile molecules that can pass through the plasma membrane for cell-to-cell transport. In this chapter, we focus on findings on GA biosynthesis, perception, and signal transduction pathways, highlighting how the evolutionary conserved GA-GID1-DELLA regulatory module is connected to developmental and environmental responses.

FUNCTIONS OF GIBBERELLINS

Gibberellins (Gas) are a large family of tetracyclic diterpenoid plant hormones that regulate many different aspects of plant growth and development through the entire life cycle of the plant, including promotion of cell division and elongation, seed germination, stem and hypocotyl elongation, root growth, and flowering induction (Daviere et al., 2008; Sun, 2011; Sun and Gubler, 2004; Vera-Sirera et al., 2016). In addition, Gas also regulate plant adaptation to biotic and abiotic stresses (Daviere and Achard, 2015; Yang et al., 2008, 2012).

The action of GA in promoting plant growth was first discovered in 1930s by studies of the rice Bakanae disease. The rice plants infected by the pathogenic fungus Gibberella fujikuroi exhibited excessive stem elongation such that they fell over easily (Silverstone and Sun, 2000). Later, the metabolite produced from the pathogenic fungus was identified as GA.

There are at least 136 fully characterized Gas, named from GA1 to GA136, which have been identified from various bacteria, fungi, and plants (Hedden and Thomas, 2012; Silverstone and Sun, 2000). However, only a few of the Gas, such as GA1, GA3, GA4, and GA7, have biological activity as regulators of plant growth and development. The genetic evidence has revealed that GA1 and GA4 are major active Gas in most plant species although GA3 has been identified in plants. Moreover, the bioactivity of GA4 is stronger than that of GA1 in both Arabidopsis and rice (Cowling et al., 1998; Magome et al., 2013; Nomura et al., 2013; Ueguchi-Tanaka et al., 2007). GA homeostasis is tightly feedback controlled by GA metabolism and signaling (Hedden and Kamiya, 1997).

Oscps1-1 mutant with a transgene driven by the OsCPS1 promoter (Sakamoto et al., 2004; Toyomasu et al., 2015). Therefore, OsCPS1 and OsCPS2 have probably been subjected to sub-functionalization with overlapping but distinct enzymatic activities. Similarly, two pumpkin CPS genes, CmCPS1 and CmCPS2,, display different expression patterns.

Recombinant fusion CmCPS proteins have CPS activity in vitro. These CPS genes may fulfill their functions in GA biosynthesis at different developmental stages (Smith et al., 1998). Therefore, proper tissue or cell-specific expression of CPS genes is critical for their biological roles in the biosynthesis of Gas and other terpenoids; this also suggests that Gas act in specific cells.

The gene AtKS (also known as GA2) is also single copy in Arabidopsis, and was first identified as the homolog of the pumpkin KS gene with genetic confirmation by mutant complementation (Yamaguchi et al., 1996, 1998b). The ga2-1 mutant, similar to the ga1 mutants, is a severe GA-deficient mutant, with a non-germinating, extreme dwarf phenotype. By contrast, the rice genome contains a family of KS-like (OsKSL) genes, including a pseudogene OsKSL3, which are responsible for the biosynthesis of various diterpenoids (Tezuka et al., 2015; Xu et al., 2007). Only OsKS1, the closest homolog of Arabidopsis AtKS, functions in GA biosynthesis (Margis-Pinheiro et al., 2005; Sakamoto et al., 2004; Xu et al., 2007). Recently, the maize ZmKS1 gene was also isolated by a map-based cloning experiment from the GA-deficient mutant dwarf-5 (d5). The ZmKS1 locus contains a tandem array of three TPS genes, ZmTPS1, ZmKSL3, and ZmKSL5. Only the ZmKSL3 protein serves as the KS for GA metabolism in maize (Fu et al., 2016). Therefore, the genetic control in the first step of GA biosynthesis is most likely conserved in diverse plants.

In the second stage of the GA biosynthesis pathway, the conversion of entkaurene to GA12 via stepwise oxidation is catalyzed by ent-kaurene oxidase (KO) and ent-kaurenoic acid oxidase (KAO). KO belongs to the CYP701A P450 subfamily, which initiates the first cytochrome P450-mediated step in GA biosynthesis in the endoplasmic reticulum (ER) and in the plastid envelope (Helliwell et al., 1998, 1999, 2001a; Ko et al., 2008; Morrone et al., 2010). The single AtKO protein is a multifunctional cytochrome P450 that catalyzes the sequential oxidation of ent-kaurene from -CH3 → -CH2OH → -CHO → -COOH on C-19 to produce the intermediates ent- kaurenol and ent-kaurenal and the end product ent-kaurenoic acid through three steps of reactions (Helliwell et al., 1998, 1999). The AtKO protein is encoded by the GA3 gene in Arabidopsis. The loss-of-function mutant of GA3 is also GA-deficient dwarf. The AtKO gene is expressed in all examined tissues, with the highest expression levels in inflorescence tissues (Helliwell et al., 1998), consistent with high levels of bioactive Gas during plant flowering. Subsequent studies have clarified the reaction sequence, enzymatic features, and substrate specificity of KO. In the rice genome, genes for five CYP701A subfamily members, OsKO1 to

(Nomura et al., 2013). Intriguingly, 12α-hydroxy GA12 (GA111) is produced as a major product and 13-hydroxy GA12 (GA53) as a minor product, when GA12 is used as a substrate for CYP714A2 (Nomura et al., 2013). Ectopic expression of PtCYP714A3, an EUI homolog in Populus trichocarpa, in the rice eui mutant could rescue the rice excessive-shoot-growth phenotype. The overexpression of PtCYP714A3 in rice led to a semidwarf phenotype and reduced endogenous bioactive GA levels. The results indicate that CYP714A3 is likely also to be involved in the GA deactivation; however, the enzymatic functions of CYP714A3 have not yet been identified (Wang et al., 2016).

Two GA methyltransferases, GAMT1 and GAMT2, catalyze the methylation of active Gas to generate inactive GA methyl esters in Arabidopsis seeds (Fig. 4.2) (Varbanova et al., 2007). Siliques of the gamt1 gamt2 double mutant accumulate high levels of active Gas. In contrast, overexpression of AtGAMT1 reduces the level of the major bioactive GA4, resulting in typical GA deficiency semidwarf phenotypes and increased tolerance to drought stress in transgenic plants (Nir et al., 2014; Varbanova et al., 2007). Thus, the methylation of Gas is part of the mechanism that regulates the levels of active Gas in plants. However, whether the methylation of Gas is a common deactivation reaction in higher plant species remains to be determined.

In conclusion, the GA metabolism pathway, which includes GA biosynthesis and catabolism, is a complex process that is regulated by multiple genes through the entire life cycle of plant growth and development. The uncertainties regarding the mechanisms of the GA metabolism pathway require further study.

REGULATION OF GA BIOSYNTHESIS AND METABOLISM

FEEDBACK REGULATION OF GA HOMEOSTASIS

Gas play diverse roles in plant development. GA homeostasis must be fine-tuned with both GA biosynthesis and catabolism. Regulation of GA biosynthesis and catabolism occurs at a number of steps under different conditions (Colebrook et al., 2014; Hirose et al., 2013; Weiss and Ori, 2007; Yamaguchi, 2008). Therefore, the levels of bioactive Gas are maintained via feedback and feedforward regulation of GA metabolism (Hedden and Phillips, 2000), including regulation of the transcription of core GA signaling components, such as the GA receptor GID1 and repressor DELLA (Griffiths et al., 2006; Hedden and Thomas, 2012).

Unlike later GA biosynthesis genes, CPS genes appear not to be regulated by a feedback mechanism (Silverstone et al., 1997a). GA dynamics are mainly targeted to 2ODDs in the GA metabolism pathway to establish homeostasis. Negative feedback regulation of AtGA20ox1 expression has been demonstrated by application of bioactive Gas or a high endogenous bioactive GA concentration (Xu et al., 1999). Consistent with this model, GA20ox enzymes in Arabidopsis and rice are highly elevated in GA-deficient mutants (Phillips et al., 1995; Zhang et al., 2008; Zhu et al., 2006). The AtGA3ox1 gene, but not the AtGA3ox2 gene, is under negative feedback regulation during Arabidopsis seed germination (Yamaguchi et al., 1998a). Bioactive Gas regulate AtGA3ox1 transcript abundance in a dose-dependent manner, and downregulation of AtGA3ox1 is not triggered by the immediate precursor GA9 (Cowling et al., 1998). AtGA20ox1 and AtGA3ox1 genes were highly upregulated in both CYP714A1 and CYP714A2 overexpression plants which have lower endogenous GA4 levels (Nomura et al., 2013). Furthermore, the feedback regulation of AtGA3ox1 is modified by AGF1 (AT-hook protein of GA feedback regulation) to maintain GA homeostasis by binding to the cis-acting sequence of the AtGA3ox1 promoter (Matsushita et al., 2007). More specifically, the expression of AtGA3ox1 is repressed by the DOF transcription factor DAG1 (DOF AFFECTING GERMINATION1), which acts downstream of PIL5 (PHYTOCHROME-INTERACTING FACTOR 3-LIKE 5) in the light-mediated seed germination pathway (Boccaccini et al., 2014). Similarly in rice, expression of OsGA3ox1, OsGA3ox2, and OsGA20ox2, whose proteins catalyze formation of active Gas, was shown to be under negative feedback regulation in eui mutants that accumulate high levels of bioactive Gas (Zhang et al., 2008; Zhu et al., 2006). In contrast, the expression of the GA deactivation genes AtGA2ox1, AtGA2ox2, OsGA2ox1, and OsGA2ox3 is upregulated upon GA3 application or high endogenous bioactive GA levels (Sasaki et al., 2003; Thomas et al., 1999; Zhang et al., 2008; Zhu et al., 2006). In addition, OsGA2ox2, OsGA20ox4, and OsGA3ox2 were upregulated in the OsGA2ox6 overexpression mutant (Huang et al., 2010).

As the central GA signaling components, the GA receptor GID1, DELLA proteins, and the F-box proteins SLY1 in Arabidopsis or GID2 in rice, also function in GA homeostatic regulation (Boccaccini et al., 2014; Dill and Sun, 2001; Fukazawa et al., 2014; Sasaki et al., 2003; Ueguchi-Tanaka et al., 2007; Zentella et al., 2007). DELLA proteins affect GA homeostasis by direct feedback regulation of the GA biosynthesis genes and receptor genes (Zentella et al., 2007). Expression of AtGA3ox1 is downregulated in seeds of the DELLA double mutant gai-t6 rga28 in Arabidopsis (Oh et al., 2007). The expression of the OsGA20ox2 (SD1) gene is upregulated in the rice gid1 and gid2 mutants, and the levels of bioactive GA1 are highly elevated (Sasaki et al., 2003; Ueguchi-Tanaka et al., 2005). Moreover, the DELLA binding transcription factor GAF1 (GAI-ASSOCIATED FACTOR1) regulates AtGA20ox2 and AtGID1b and is thereby involved in feedback regulation of GA biosynthesis (Fukazawa et al., 2014). YAB1, a member of the YABBY family of C2C2 zinc finger transcription factors, as a mediator of feedback inhibition of GA biosynthesis, regulates OsGA3ox2 and OsGA2ox3 gene expression in rice (Dai et al., 2007). The precise sites and timing of GA biosynthesis and responses must be controlled for proper plant growth and development. Gas are usually present at low concentrations (0.1–100 ng/g fresh

ENVIRONMENTAL REGULATION OF GA METABOLISM

Many environmental responses are regulated through GA abundance, and GA metabolism is regulated by environmental signals, such as light, temperature, water, and nutrient status, as well as by other abiotic and biotic stresses. Light is one of the major environmental factors that affects plant growth and development (Kamiya and GarcíaMartínez, 1999). GA metabolism is sensitive to changes in light quantity, quality, or duration, which may result in increased or decreased GA content. The photoreceptor phytochrome regulates transcript levels of GA20ox and GA3ox enzymes in germinating lettuce (Lactuca sativa) (Toyomasu et al., 1998) and Arabidopsis seeds (Yamaguchi et al., 1998a). Expression of LsGA3ox1 was dramatically increased and LsGA20ox2 expression was decreased by red light ® treatment, while LsGA3ox2 and LsGA20ox2 expression were unaffected in seeds under any light conditions. These results suggest that GA1 content increases in lettuce seeds by inducing LsGA3ox1 expression via phytochrome action (Toyomasu et al., 1998). The expression of AtGA3ox1 and AtGA3ox2 genes is relatively high in germinating seeds under continuous white light and is elevated by red light, which may result in an increase in the biosynthesis of active Gas to promote seed germination (Yamaguchi et al., 1998a). Therefore, light-regulated expression of GA3ox genes via phytochrome action may be a common mechanism in plant species whose germination is dependent on a light stimulus. Cryptochromes can also regulate GA20ox and GA2ox expression in Arabidopsis seedlings (Achard et al., 2007b; Zhao et al., 2007) and rice seedlings (Hirose et al., 2012, 2013). High levels of AtGA20ox1 and low levels of AtGA2ox genes expression were found in Arabidopsis seedling hypocotyls grown in the dark. Conversely, Arabidopsis seedling hypocotyls contained low AtGA20ox1 and high AtGA2ox1 transcripts when grown in continuous light. These studies indicate that the biosynthesis and accumulation of bioactive Gas are critical to regulate the length of the seedling hypocotyls (Achard et al., 2007b).

Blue light affects bioactive GA level through regulating the expression of AtGA2ox1, AtGA20ox1, and AtGA3ox1, which requires cryptochromes (Zhao et al., 2007). In rice seedlings, phytochromes mediate the repression of GA biosynthesis capacity, including the repression of two GA20ox genes, OsGA20ox2 and OsGA20ox4, and a GA3ox gene, OsGA3ox2, and the induction of four GA2ox genes, OsGA2ox4 to OsGA2ox7 (Hirose et al., 2012). A further study indicated that independent photoreceptors in rice separately but cooperatively mediate GA metabolism, for example, blue light sensed by cryptochrome 1 (cry1a and cry1b) induced the expression of the four GA2ox genes (OsGA2ox4–OsGA2ox7) (Hirose et al., 2013). Phytochrome B, together with auxiliary action of phytochrome A, mediates the repression of GA20ox genes (OsGA20ox2 and OsGA20ox4). These independent effects cumulatively reduce active GA contents, leading to the suppression of leaf sheath elongation.

The circadian clock directly influences GA biosynthesis and signaling. Thus AtGA20ox1, but not AtGA3ox1, is activated under long-day (LD) conditions when Arabidopsis plants were

severe dwarf phenotype than that of the GA-deficient ga1-3 mutant. These genetic evidences suggest that AtGID1 isoforms are the GA receptors in Arabidopsis and have some degree of functional redundancy (Griffiths et al., 2006). Indeed, the Atgid1a Atgid1b Atgid1c triple mutant did not germinate readily and only started to grow when the seed coat was removed after imbibition. The Atgid1a Atgid1b Atgid1c triple mutant was insensitive to exogenous treatment of GA, and seedlings of this triple mutant were severe dwarfs that grew only a few millimeteres high after one month. By comparing Atgid1 multiple mutants with sly1 Atgid1 double mutants, roles of AtGID1a, AtGID1b, and AtGID1c in proteolytic and non-proteolytic GA signaling were demonstrated. Three alleles of AtGID1 were found to play different roles in germination, stem elongation, and fertility involving proteolytic and non-proteolytic GA signaling (Hauvermale et al., 2014).

Ala scanning experiments using conserved amino acid residues among the rice and three Arabidopsis GID1 proteins revealed that 12 blocks are essential for GA-binding activity and 13 blocks are important for GID1-SLR1 interaction (Ueguchi-Tanaka et al., 2007). The detailed crystal structure analysis of the GA receptor GID1 provided us with a better understanding of how Gas operate at the molecular level. The GA-binding site of GID1 protein corresponds to the active site of the HSL domain, and four helices at the N terminus and the central part of GID1 form a lid closure (Fig. 4.3). Both the lid and the binding pocket containing GA are necessary for the DELLA interaction. DELLA interacts with the GID1-GA complex at its N-terminal region, and as a result of DELLA binding, the GID1-GA complex is stabilized (Fig. 4.3) (Ueguchi-Tanaka et al., 2007). In 2008, two research groups independently demonstrated crystal structures of rice GID1 (OsGID1)-GA complex and the Arabidopsis GID1 (AtGID1a)-GA-DELLA complex (Murase et al., 2008; Shimada et al., 2008). The structures of the GA3- and GA4-AtGID1a-GAI complexes display a stocky structure with a globular AtGID1a that is bound on one side by the GAI-DELLA domain (Fig. 4.3). The AtGID1a protein is monomeric and is composed of one α/β core domain with an N-terminal extension that extends up the core surface toward the DELLA domain. The crystal structure studies provided the idea that bioactive GA is an allosteric inducer of AtGID1a, which causes conformational changes that allow the receptor to associate with DELLA proteins, but GA does not interact directly with DELLAs itself (Murase et al., 2008). This finding is consistent with the crystal structures of GA4-OsGID1 and GA3-OsGID1 (Shimada et al., 2008). Further Ala scanning experiments demonstrated that the conserved residues within plant GID1 proteins, but not among HSL proteins, are necessary for GA-binding activity, indicating that these residues have been recruited to establish a receptor for GA from the ancestral HSL structure (Shimada et al., 2008).

Thus, the GA perception mechanism differs from that of auxin, which serves as the “molecular glue” that brings together a substrate protein and an F-box protein without changing the structure of either protein or requiring the involvement of a third protein (Hedden, 2008; Tan et al., 2007). In contrast, the GA receptor can be activated by the allosteric effector GA to function

largely supported the roles established by exogenous application of cytokinins, though the precise role of cytokinins in plant development is still unclear. In one elegant experiment, the ipt gene was expressed from a senescence-specific promoter (Gan and Amasino, 1995). These transgenic tobacco plants developed normally except that senescence was greatly delayed, resulting in dry mass and seed yields 50% greater than wild-type plants.

Induction of expression of ipt under the control of a heat shock promoter in Arabidopsis leads to a large, transient rise in the level of zeatin, mostly in the riboside and ribotide forms (Rupp et al., 1999). Zeatin returned to basal levels within 72 hours after the heat shock. In contrast, the 9-glucoside conjugate of zeatin was elevated after 8 hrs and remained elevated at 72 hrs after a heat shock in these transgenics. Growth for two weeks with a daily heat shock regime resulted in plants with an altered phenotype from the controls. Overall, the biomass of the heat-shocked transgenics increased. The stem was thicker, due to increase in the pith parenchyma, and the leaf thickness increased, partially as a result of an increased number of mesophyll cell layers. The leaves of the heat- shocked transgenics were serrated at the margins, which is similar to the phenotype of Arabidopsis plants that overexpress the KNAT1 and STM genes. Indeed, the ipt transgenics had a higher steady-state level of KNAT and STM mRNA in response to heat shock as compared to the controls (see below).

CYTOKININ AND THE SHOOT APICAL MERISTEM

The shoot apical meristem is a highly specialized group of cells from which the majority of the aerial portion of the plant is derived by reiterative development (Kerstetter and Hake, 1997). The ability of cytokinins to initiate shoots from undifferentiated callus cultures and the initiation of ectopic meristems in transgenic plants engineered to overexpress cytokinins suggest a role for this class of hormones in SAM development.

One mechanism by which cytokinin may influence shoot apical meristem development is by regulating gene expression. Subsets of the knotted1 (kn1) homeobox family of genes are expressed exclusively in the SAM and are involved in its development and maintenance (Jackson et al., 1994; Kerstetter et al., 1994; Kerstetter and Hake, 1997). Transgenic plants that have an elevated level of cytokinin as a result of over-expression of the ipt gene have some phenotypes reminiscent of transgenic plants over-expressing kn1, such as a delay in senescence, reduced apical dominance, and ectopic shoot

formation (Kerstetter and Hake, 1997). This suggests that elevated cytokinin levels may induce kn1 expression, which is indeed the case in Arabidopsis (Rupp et al., 1999). These results suggest that cytokinins may act upstream of KNAT1 and STM in regulating shoot apical meristem development.

A similar relationship between cytokinin levels and expression of the maize kn1 gene was observed when kn1 was over-expressed in tobacco. Expressing kn1 under the control of a senescence specific promoter (SAG12) resulted in a delay of senescence, similar to the phenotype seen in plants expressing ipt under control of the SAG12 promoter (Ori et al., 1999). Intact and detached leaves stayed greener longer and displayed higher chlorophyll content than control plants. Remarkably, older SAG:kn1 leaves had cytokinin levels 15 times higher than wild-type plants, suggesting that kn1 may inhibit senescence by increasing cytokinin levels. These results suggest that the levels of cytokinin and kn1 may positively regulate each other in an interdependent fashion. Alternatively, the elevation of cytokinin in connection with ectopic expression of kn1 may not accurately reflect the endogenous relationship between cytokinin and kn1 homologs.

Further evidence linking cytokinins to apical meristems came from an analysis of transgenic tobacco lines engineered to express four Arabidopsis homologs of the maize cytokinin oxidase from the CaMV 35S promoter, which resulted in reduced endogenous cytokinin levels (Werner et al., 2001). Transgenic lines had elevated cytokinin oxidase activity and significantly reduced amounts of the cytokinins iP and zeatin, including their glycosides, and displayed severely retarded shoot development. In contrast, the growth of the root system was enhanced, indicating that cytokinins may have opposing roles in shoot and root development. These changes were the result of alterations in the rate of cell proliferation in the apical meristems: shoot apical meristems consisted of fewer cells with sizes comparable to wild-type; in contrast, there was an increased number and size of cells in the root apical meristem. Leaves were formed from a significantly decreased number of cells, which was partly compensated for by an increased cell size. Nevertheless, the transgenic leaves were about 15% of the size of their wild-type counterparts. The reduced cell proliferation in the shoot apical meristems as a result of lowered cytokinin levels is consistent with an in vivo role for this hormone in the regulation of cell division.

CYTOKININ PERCEPTION AND SIGNAL TRANSDUCTION

The downstream targets of bacterial and fungal two-component hybrid sensor kinases are histidine phosphotransfer proteins and response regulators. Similarly, the Arabidopsis response regulators (ARRs) and histidine phosphotransfer proteins (AHPs) appear to act downstream of the CRE1/AHK receptors in cytokinin signaling. Response regulators in Arabidopsis

Response regulators homologs were identified in screens for genes that are rapidly upregulated by cytokinin in Arabidopsis and maize (Brandstatter and Kieber, 1998; Sakakibara et al., 1998). The Arabidopsis response regulators form a large gene family comprised of 22 genes that fall into two main classes (type-A and type-B) based on a phylogenetic analysis of their amino acid sequences and their domain structures (reviewed in: D'Agostino and Kieber, 1999a; Figure 6; Table 4). The type-A ARRs contain a receiver domain, but lack a classic output domain. In contrast, the type-B ARRs are comprised of an N-terminal receiver domain and a fused C-terminal output domain (see below). In addition, most of the type-A ARRs are induced by exogenous cytokinins, while the steady-state level of the type-B ARRs is unaffected by cytokinin (Brandstatter and Kieber, 1998; Taniguchi et al., 1998; Imamura et al., 1999; Kiba et al., 1999; D'Agostino et al., 2000). Both the type-A and the type-B ARRs have been implicated in cytokinin signaling.

TYPE-A ARRs ARE CYTOKININ PRIMARY RESPONSE GENES

The Arabidopsis genome encodes ten type-A ARRs that fall into five pairs with highly similar amino acid sequences (Figure 6), which may reflect an evolutionarily recent duplication of the Arabidopsis genome (Vision et al., 2001). The amino acid sequences of the receiver domains of the typeA ARRs are very similar, but the sequences of the small Cterminal extensions (< 100 amino acids) are more divergent (Imamura et al., 1999; D'Agostino et al., 2000).

The levels of ARR4 and ARR5 mRNA are rapidly and specifically upregulated by cytokinin and the induction does not require de novo protein synthesis. Thus, these are cytokinin primary response genes. The increase in steadystate transcript levels of the type-A ARRs in response to cytokinin is due, at least in part, to increased transcription (D'Agostino et al., 2000), which implies that a transcription factor(s) is activated in response to cytokinin (see below). Most other type-A ARRs are also induced by cytokinin and with generally similar induction kinetics (Taniguchi et al., 1998; Kiba et

cytokinin (Hwang and Sheen, 2001). CKI1 was localized to the plasma membrane in this system. Surprisingly, overexpression of AHK2 and AHK3 had little or no effect on the induction of ARR6-LUC. Expression of mutant forms of CRE1 in which the conserved histidine and aspartate residues in the transmitter and receiver domains were mutated failed to elicit an elevated ARR6LUC response to cytokinin, supporting the model that phosphotransfer within CRE1 is required for its signaling function. As with previous results in other systems, expression of CKI1 results in an activation of ARR6-LUC that was not further increased by cytokinin treatment, suggesting that CKI1 is constitutively active or is saturated by the endogenous cytokinin in the protoplasts.

Overexpression of several type-A ARRs in the protoplast system suppressed the cytokinin induction of ARR6-LUC, indicating that type-A ARRs negatively regulate their own expression. As with the type-B ARRs in this system, a mutation of the conserved phosphorylation site (Asp) did not affect the repression of ARR6-LUC, indicating that phosphorylation of the type-A ARRs is not required for this repression.

Several lines of evidence also implicate the AHPs as mediators of cytokinin signaling, linking the activation of CRE1 by cytokinin binding to the activation of the type-B ARRs. First, AHP1 and AHP2, but not AHP5, were found to transiently accumulate in the nucleus within 20 minutes of application of exogenous cytokinin, as measured by GFP fusions in Arabidopsis protoplasts (Hwang and Sheen, 2001). Secondly, overexpression of AHP2 from a CaMv 35S promoter in transgenic Arabidopsis resulted in modest hypersensitivity to exogenous cytokinin in root and hypocotyl elongation assays (Suzuki et al., 2001a). These results, coupled with the two-hybrid and in vitro phosphorylation experiments described above, indicate that these histidine phosphotransfer proteins are likely to mediate signaling between CRE1 and the type-B ARRs. Definitive demonstration of a role of these genes awaits analysis of loss-of-function alleles.

The data described above are generally consistent with the simple model for phosphorelay signal transduction in cytokinin signaling presented in Figure 7. Cytokinins bind to the CRE1 histidine kinase, and most likely also AHK2 and AHK3, in the conserved extracellular domain. This induces autophosphorylation on a histidine residue within the transmitter domain and subsequent transfer of the phosphate to an aspartate residue within the fused receiver domain. The phosphate is then probably transferred to a histidine residue on the AHPs, which then translocate to the nucleus where they activate type-B ARRs. The activated type-B ARRs bind to elements within

and may affect the cytokinin response pathway. This mutation does not affect the morphology of the adult plant.

The altered meristem program 1 (amp1) is an Arabidopsis mutant that over-produces cytokinin and several amp1 phenotypes, such as the lack of apical dominance, delayed senescence and increased shoot regeneration in tissue culture, are consistent with what a cytokinin over-producing mutant might look like (Chaudhury et al., 1993). However, other amp1 phenotypes, such as polycotyledons, precocious flowering and abnormal phyllotaxy, have not been observed in plants treated with exogenous cytokinin or in transgenic plants overexpressing ipt. The AMP1 gene has recently been cloned and found to encode a putative glutamate carboxypeptidase (Helliwell et al., 2001). This homology makes it unlikely that this gene directly affects cytokinin biosynthesis. The authors propose that AMP1 may regulate the level of an extracellular peptide signal that controls meristem function. Cytokinins and gene expression

Many changes in gene expression, in addition to the induction of type-A ARRs, have been detected in response to application of exogenous cytokinins (reviewed in Schmülling et al., 1997). Genes showing cytokinin-responsive expression are good candidates for components of cytokinin signaling and effector pathways to various cellular and developmental responses. Several genes involved in regulating meristem function and/or cell proliferation are upregulaed by cytokinin in Arabidopsis, including members of the knotted gene family (see above) and CycD3 (see below).

Additional genes responsive to cytokinin have been identified by cytokinin treatment of soybean or tobacco tissue culture cells that were starved for cytokinin. In an extensive study, 20 genes were identified whose transcripts accumulated within four hours of cytokinin application (Crowell et al., 1990). These 20 genes were also responsive to auxin. Sequence analysis revealed that two of these genes were homologous to ribosomal proteins and one was homologous to a pollen allergen protein (Crowell et al., 1990; Crowell, 1994). Several studies have also examined cytokinin-regulated gene expression during tissue differentiation, including nodulation, senescence, floral development, lateral bud induction, and various aspects of light development. Genes identified in these studies include photosynthetic genes, ribosomal protein genes, nitrate reductase as well as many novel genes. However, most of these genes are also induced by other stimuli, most notably light and auxin, and none of these genes are induced with kinetics suggestive of an immediate early response gene. Cis-acting DNA elements that are responsive to cytokinin have not yet been delineated. Additional

In eukaryotes, there are eight subfamilies of ABC transporters, namely ABCA to ABCH. Of the ABCG subfamily, several members have been identified in Arabidopsis and Medicago truncatula, including both ABA exporters such as AtABCG25, AtABCG31, and

MtABCG20, and ABA importers such as AtABCG30 and AtABCG40 (Kuromori et al. 2010; Kang et al. 2011, 2015 Kuromori et al. 2011; Pawela et al. 2019). Among these transporters, AtABCG25 functions in exporting ABA from vascular tissues to multiple sites such as guard cells. This transporter together with AtABCG31 also exports ABA from the endosperm, whereas AtABCG30 and AtABCG40 import ABA into the embryo (Kuromori et al. 2010; Kang et al. 2015). Besides ABCG transporters, AtDTX50 also acts as an exporter of ABA, and both AtNPF4.6 and OsPM1 control ABA influx (Kanno et al. 2012; Zhang et al. 2014; Yao et al. 2018). The gene encoding AtABCG22 is similar in sequence to AtABCG25. However, AtABCG22 may not transport ABA directly, although it is possibly involved in ABA efflux (Kuromori et al. 2011). Functionally, ABA transporters have been increasingly shown to be involved in transpiration, root morphology, seed germination and other processes important to stress.

STRESS-MEDIATED ChANGES IN ABA

Imaging is an important functional approach in studying ABA. With the introduction of FRET (fluorescence resonance energy transfer) marker sensors, ABACUS and ABAleon, detection of ABA at a cell level has become attainable (Jones et al. 2014; Waadt et al. 2014). Changes in ABA levels in individual guard cells and roots were detected after exposure to altered amounts of humidity and salinity (Waadt et al. 2014). The root-derived CLAVATA3/EMBRYO-SURROUNDING REGION-RELATED 25 (CLE25) peptide, perceived by BARELY ANY MERISTEM (BAM) receptors, promotes ABA biosynthesis in leaves, in response to dehydration by upregulating NCED3 expression (Takahashi et al. 2018). The flowering repressor SHORT VEGETATIVE PHASE (SVP), a central regulator of ABA catabolism, is able to decrease expression of CYP707A1/3 while enhancing expression of AtBG1 simultaneously in response to water deficits (Wang et al. 2018c). Also, NGATHA proteins (NGAs) upregulate the expression of NCED3 via direct binding to its promoter (Sato et al. 2018). The HD-ZIP transcription factor HAT1, a negative regulator in ABA biosynthesis, which suppresses the expression of both ABA3 and NCED3, can be phosphorylated and inactivated by SnRK2.3 (Tan et al. 2018). The subsequent transcriptional changes lead to the rapid release and increased synthesis of ABA, while reducing ABA catabolism under drought stress, allowing the initiation of several ABA-mediated responses that affect the growth and survival of plants.

Biotic stresses such as pathogen infection can also modulate ABA homeostasis in host plants. Some biotrophic pathogens such as wheat rust fungi can promote increases in ABA that lead to elevated cytoplasmic sugar accumulation by enhancing TaSTP6 expression (Huai et al. 2019).

SnRK2.2/3/6 from inhibition. SnRK2s may then be activated by autophosphorylation and/or transphosphorylation by several other kinases, such as the Raf-like MAKKKs, RAF10, and ARK (for ABA and abiotic stress-responsive Raf-like kinases) (Figure 2). These kinases appear to be critical for the activation of SnRK2s and subsequent responses to ABA and abiotic stresses in Arabidopsis and Physcomitrella patens (Huang et al. 2014; Lee et al. 2015; Saruhashi et al. 2015; Stevenson et al. 2016; Hwang et al. 2018; Nguyen et al. 2019; Shinozawa et al. 2019). BRASSINOSTEROID INSENSITIVE 2 (BIN2), the Glycogen synthase kinase 3s (GSK3s)/Shaggy-like kinases (ASKs) repress brassinosteroid (BR) signaling, whereas they enhance ABA signaling through specifical phosphorylation of SnRK2.2/3 at Thr180 on SnRK2.3, but not SnRK2.6 (Cai et al. 2014). Moreover, NO also represses ABA signaling through S-nitrosylation of SnRK2.6 at Cys137 that inactivates SnRK2s (Wang et al. 2015b). Although PYLs are essential for ABA-mediated activation of SnRK2s, they are also involved in an antagonistic regulation of activation of SnRK2s by osmotic stress (Zhao et al. 2018). Overall, SnRK2s can be activated by abiotic stresses and repressed by growthpromoting signals such as NO and BR.

ABSCISIC ACID-INDUCED STOMATAL CLOSURE

Stomata are pivotal for gas exchange and transpiration of plants, and the closure of stomata can be induced by numerous environmental factors such as drought, pathogen attack, darkness, low humidity, high CO2 concentrations and so on (Bauer et al. 2013; Assmann and Jegla 2016; Martin-StPaul et al. 2017; Su et al. 2017). Abscisic acid plays an important role in the closure of stomata by regulating guard cell ion fluxes. Stomatal closure is the major process controlling the transpirational water loss of plants. ABA affects stomatal pore size by both Ca2+-dependent and Ca2+-independent pathways.

ABA induces stomatal closure by both Ca2+-dependent and Ca2+-independent pathways. ABA-induced Ca2+ signal involves the induction of reactive oxygen species (ROS) and inositol-1-4-5-triphosphate (IP3), and may be decoded by CPK3/4/6/10/11/21/23 through activation of SLOW ANION CHANNEL-ASSOCIATED 1 (SLAC1) and SLAC1 HO-

MOLOG 3 (SLAH3) to promote efflux of Cl−, which is also regulated by GHR1. CPK21 activates the K+ outward + flux channels. CIPK23, CPK8-CAT3 and rectifying channel GOAK, while CPK13 inactivates KAT1 and KAT2, two K in CPK11-Di19-PR1/2/5 modules also regulate stomatal closure. The ABA activated SnRK2.6/OST1 is a key regulator of Ca2+-independent stomatal closure. OST1 activates SLAC1, KUP6, and QUAC1 to promote efflux of Cl−, K+, and malate2−, and inhibits KAT1 to reduce influx of K+. OST1 can also phosphorylate and inhibit AKS1 to reduce expression of KAT1. Together, ABA induces stomatal closure by regulating guard cell ion fluxes.

et al. 2009; Miao et al. 2018). However, ABA biosynthesis and signaling mutants aba1 and abi3 display normal embryo growth, indicating that FUS3, LEC1, and LEC2 control embryo growth arrest independent of ABA signaling (Raz et al. 2001).

During seed maturation, the ABI3/FUS3/LEC2 (AFL) subfamily of B3 transcription factors, together with LEC1 and LEC1-LIKE (L1L), compose a transcription control network called LAFL (Figure 4) (Kwong et al. 2003; Jia et al. 2014). Hormone signaling, some metabolic pathways and other transcriptional control networks are targeted by LAFL and mediate the embryogenesis process but the LAFL components have distinct temporal patterns of development (Jia et al. 2013). The core LAFL network functions upstream of several genes that modulate seed development including zinc finger factor PEI1, APETALA2 (AP2), BABY BOOM (BBM), FLOWERING LOCUS C (FLC), and two genes encoding seed storage proteins (SSP) including 2S albumin storage protein 1 (At2S1) and CRUCIFERIN C (CRC) (Jia et al. 2014). BBM has also been reported to regulate expression of most members of the LAFL network during somatic embryogenesis (Horstman et al. 2017). Further, the LAFL network can be regulated by the sister subgroup of AFL type B3 transcription factors such as VIVIPAROUS1/ABI3-LIKE1/2/3 (VAL1/2/3), which repress the LAFL network during germination but do not affect seed maturation (Figure 4) (Jia et al. 2013, 2014; Zhou et al. 2013).

LAST PHASE OF SEED MATURATION

After the suspension of cell division during embryogenesis, plant seeds begin to accumulate storage components and begin to desiccate. This final stage results in a metabolically quiescent or dormant state, enabling seeds to survive severe stress environments. Abscisic acid also functions in this final developmental stage and affects several important traits of the dormant seed.

RESERVE PRODUCT ACCUMULATION

Seeds mature by metabolically producing and then accumulating several reserve components needed for germination and initial seedling growth and development. The initiation of reserve accumulation is mediated by several processes such as gene expression, posttranslational modulation, strengthening the activity of enzymes and ATP production (Bewley et al.

2013a).