Control of Outgrowth and Dormancy in Axillary Buds[1]

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The shoot system has an important role in generating a large variety of diverse plant forms (Steeves and Sussex, 1989). The overall architecture of the shoot system is derived from the activity of the primary shoot apical meristem (SAM), arising during embryogenesis, together with the activity of the additional meristems subsequently formed after seed germination. The primary SAM provides the main axis of the plant body. Plant architecture is further modified by shoot branching that results from the activity of the additional meristems. The complexity of the branching pattern depends on the temporal and spatial development of these branches. These characteristics, although they are plastic in their response to environmental cues, are genetically determined. The developmental program that specifies branching patterns in different plant species is fundamentally important for generating species-specific plant forms.

The shoot branching process generally involves two developmental stages: the formation of axillary meristems in the leaf axils and the growth of axillary buds. In many plant species, the growth of axillary meristems is inhibited by the primary shoot or primary inflorescence. This phenomenon is generally known as apical dominance. The plant hormones auxin and cytokinin are thought to have a major role in controlling this process (Phillips, 1975; Cline, 1994; Tamas, 1995; Napoli et al., 1999). Auxin has an inhibitory effect on the growth of axillary buds, whereas cytokinin promotes axillary bud outgrowth. The mechanisms of axillary bud outgrowth depend on the ratio of these two hormones rather than the absolute levels of either hormone.

A variety of experimental approaches have been used to examine the mechanisms controlling dormancy and outgrowth of axillary buds. These range from physiological studies, such as measurement and exogenous application of plant hormones, to analyses of transgenic plants overexpressing hormone biosynthetic genes to alter endogenous hormone levels. Isolation and characterization of mutations that cause alterations in shoot branching patterns are powerful approaches. These molecular genetic approaches combined with the conventional physiological studies, such as grafting experiments, revealed that not only do auxin and cytokinin function to control the growth of axillary buds, but other factors and/or signals also have important roles. More recently, the genes expressed in dormant axillary buds were isolated and characterized.

This review focuses on recent findings uncovered by physiological, genetic, and molecular studies and approaches to investigate the control of shoot branching, apical dominance, and dormancy in plants.

DEVELOPMENT AND POTENTIAL OF AXILLARY MERISTEMS

Regulation of the initiation of axillary meristems is important for controlling the overall plant form (Kerstetter and Hake, 1997; Schmitz and Theres, 1999; Sussex and Kerk, 2001). Axillary meristems are typically located on the leaf axils. In some plants like tomato, groups of meristematic cells appear to be derived directly from the SAM of the main shoot. The cells can be recognized in early developmental stages of leaf primordia in the axils. These observations suggest that axillary meristems are formed from detached parts of the primary SAM. In other plants, including Arabidopsis, axillary meristems cannot be detected in the axils during the vegetative growth phase of the primary SAM. After the primary SAM is transformed into the reproductive phase, that is, an inflorescence meristem, differentiated cells in the leaf axils undergo dedifferentiation and regain meristematic potential. Thus, axillary meristems are formed in the leaf axils.

In some plants, axillary meristems undergo immediate development to form an axillary shoot. In other plants, axillary meristems might initiate a few leaves and then become developmentally arrested or dormant because the terminal bud inhibits the growth of axillary buds to grow predominantly. These dormant axillary buds resume development at a later time depending on their developmental program or in response to environmental cues. The cycles between dormancy and growth in axillary buds were characterized using the garden pea (Stafstrom and Sussex,

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1992; Devitt and Stafstrom, 1995; Shimizu and Mori, 1998a). The second node of pea plants has four dormant axillary buds, and all of the axillary buds are different sizes. The largest bud is called the main bud and the other buds are called the accessory buds. After decapitation of the terminal bud, all four axillary buds start to grow. After 2 to 3 d, the main bud grows predominantly, and inhibits the growth of the other smaller axillary buds. Removal of the main bud promotes outgrowth of the accessory buds. The developmental stages of these axillary buds were analyzed based on the expression patterns of a ribosomal protein gene and several cell cycle-related genes. Figure 1 shows the developmental stages of axillary buds extrapolated from these studies. Axillary meristems are established in the leaf axil and form axillary buds (Fig. 1, 1). In many cases, the axillary buds enter transition stages (Fig. 1, 2). Transition stages indicate an intermediate between dormancy and growth, and axillary buds are either in temporary dormancy or temporary growth. Newly formed axillary buds in transition stages become dormant depending on the developmental program, such as suppression by the terminal bud (Fig. 1, 5). This regulation is commonly referred to as apical dominance. Alternatively, some axillary buds in transition stages undergo growth (Fig. 1, 6). Under some circumstances, the axillary buds might transit immediately to growth (Fig. 1, 2′). To respond to their developmental program or environmental signals, dormant axillary buds enter transition stages through (Fig. 1, 5), pass (Fig. 1, 3), and then undergo growth (Fig. 1, 6). In some cases, axillary buds in temporary growth at transition stages re-enter dormancy through (Fig. 1, 4) and (Fig. 1, 5). Axillary buds cycle repeatedly in the transition stages before becoming fully dormant or undergoing growth. It is possible that a set of genes that control outgrowth of axillary buds acts at any of the steps shown in Figure 1. This type of molecular study might provide the basis for understanding the regulation of axillary buds in dormancy and outgrowth.

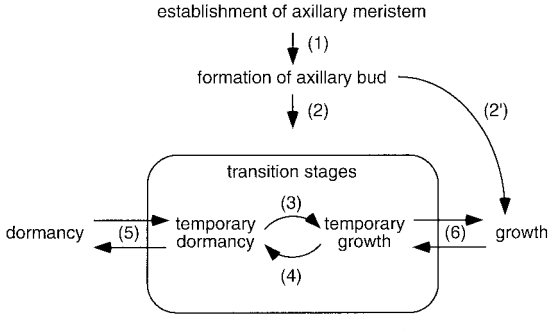

Figure 1. Extrapolated developmental stages of outgrowth and dormancy in axillary buds. Axillary meristems are initiated in the leaf axil and form axillary buds (1). The axillary buds enter transition stages (2). Transition stages indicate an intermediate between dormancy and growth. Newly formed axillary buds in transition stages become dormant, dependent on their developmental program (5). Alternatively, some axillary buds in transition stages undergo growth (6). Usually dormant axillary buds enter transition stages through (5) and (3) in response to their developmental program or environmental cue, and then undergo growth (6). In some cases, axillary buds in temporary growth at transition stages might re-enter dormancy through (4) and (5).

BIOLOGICAL FUNCTIONS OF DORMANCY AND APICAL DOMINANCE

Dormancy, broadly defined, is "the temporary suspension of visible growth of any plant structure containing a meristem" (Lang, 1987). Apical dominance is defined broadly as "the inhibitory control of the shoot apex over the outgrowth of lateral buds" (Cline, 1997; Napoli et al., 1999). Apical dominance acts as a plant survival mechanism by providing a reservoir of meristems that can replace the damaged primary shoot. This mechanism works when the primary shoot is damaged or removed through disease, herbivore grazing, or pruning. Apical dominance can also be released, depending not only on environmental cues but also on developmental programs. In some plants, dormant axillary buds start their outgrowth after the primary SAM differentiates into the determinate organ, such as a flower or an inflorescence meristem. These supplemental additional shoots are important for increasing the total number of leaves or flowers to be more fruitful.

Apical dominance controls bud growth in the vegetative developmental stages of many herbaceous plants and the juvenile stages of some trees (Cline, 2000). In woody plants, seasonal cycles of growth and dormancy occur in over-wintering buds (Powell, 1988). For example, at the end of the growing season, perennial plants cease development and assume a dormant and freezing-tolerant state, even when temperatures still favor growth. This strategy protects against the sudden arrival of winter. Later in winter, they might anticipate spring by breaking dormancy while the freezing tolerance remains high (Weiser, 1970). It is not known whether the mechanism underlying the seasonal cycle of growth and dormancy in perennial plants is the same or similar to that in axillary buds of herbaceous plants. Therefore, our discussion on dormancy and growth of axillary buds is limited to herbaceous plants.

PHYSIOLOGICAL APPROACH

Apical dominance was one of the first developmental phenomena shown to be regulated by plant hormones (Thimann and Skoog, 1934; Thimann, 1937). Auxin, derived from the terminal bud, inhibits the growth of axillary buds, whereas cytokinin derived mainly from the roots, promotes the growth of axillary buds. The role of auxin in vivo is supported by the following observations. Decapitation of *Vicia* spp. plants causes the outgrowth of axillary buds, but

application of auxin to the stump prevents the outgrowth of axillary buds. These observations have been confirmed in many plant species. Furthermore, application of the auxin-transport inhibitor, 2,3,5-triiodobenzoic acid, in lanolin to the stems of intact plants can reduce or abolish apical dominance (Snyder, 1949; Panigrahi and Audus, 1966). These data strongly support the hypothesis that apically derived auxin is transported basipetally and inhibits outgrowth of the axillary buds. In addition, direct application of auxin to axillary buds cannot prevent bud growth. Radiolabeled auxin applied to the stump is not translocated into the axillary buds. The indoleacetic acid (IAA) level of dormant axillary buds is low and that of the axillary buds after decapitation of the terminal shoot actually increases (Gocal et al., 1991).

On the other hand, direct application of cytokinin to axillary buds promotes the outgrowth of axillary buds. As with auxin, these observations have been confirmed in many plant species. Outgrowth of axillary buds is well correlated with the cytokinin level in the buds. It is thought that auxin acts to control the concentration of cytokinin derived from the roots (Bangerth, 1994). Cytokinins are produced not only in the root, however, but also in the shoot (Chen et al., 1985). Cytokinin concentrations in bean xylem exudate of bean after decapitation increase within 16 h and gradually return to basal levels (Bangerth, 1994), and those in chickpea axillary buds after decapitation increase 7-fold by 6 h and 25-fold by 24 h (Turnbull et al., 1997), suggesting that cytokinins are necessary to initiate outgrowth of axillary buds.

Several reports indicate that the abscisic acid (ABA) content of axillary buds is closely correlated with bud dormancy. The decline of the ABA level after decapitation precedes the onset of bud outgrowth, whereas application of auxin to the stump recovers the ABA level to that before decapitation (Knox and Wareing, 1984). Moreover, the *era1* mutant, which is hypersensitive to ABA, has reduced branching (Pei et al., 1998), suggesting that ABA inhibits bud outgrowth. Little is known, however, about the relationship between ABA and auxin in growth inhibition.

A physiological approach was recently taken using a new assay system in Arabidopsis (Chatfield et al., 2000). In this assay system, plant hormones are applied to both ends of excised nodal sections including axillary buds. It was reported that cytokinin acts independently to regulate the growth of axillary buds, rather than as a second messenger for auxin. This type of experiment with hormone-signaling or -synthesizing mutants might provide new findings regarding hormonal regulation of axillary bud growth.

There is an interesting study of apical dominance using *Lupinus angustifolius*. The vegetative shoot of this plant produces approximately 20 nodes. The axillary buds at nodes 1 through 5 (counting acropetally) and at nodes 13 through 20 grow rapidly, whereas axillary buds at nodes 6 through 12 grow slowly in intact plants. Decapitation of the main shoot above node 12 promotes accelerated growth of the axillary buds at nodes 8 through 12 (Miguel et al., 1998). Thus, the axillary buds in the mid-region of this plant have the potential to grow, however they cannot grow on intact plants. The concentrations of IAA, cytokinins, and ABA were measured in the apical meristems of axillary buds at different stages of development (Emery et al., 1998). The rate of bud growth did not correlate with the absolute concentrations of IAA or cytokinins. The ratio of these two hormones, however, correlated with the rate of axillary bud growth in the early developmental stages. The ratio of cytokinins to IAA was high in the rapidly growing axillary buds at the basal and upper nodes, whereas the ratio was low in the slow growing axillary buds at the middle node. In early developmental stages, ABA concentrations did not correlate with the rate of axillary bud growth. In later developmental stages, ABA had a strong negative correlation with the rate of axillary bud growth when the cytokinins to IAA ratio did not correlate with the rate of axillary bud growth. Thus, the potential of axillary bud outgrowth, which is related to position on the main axis, appears to be determined by a balance among several hormones. This balance can be changed during developmental stages. It is possible that outgrowth and dormancy of axillary buds is regulated by the hormonal status of particular plant organs, depending on their developmental program.

Analyses of transgenic plants have also contributed to a better understanding of the role of plant hormones in the control of apical dominance. Transgenic cytokinin-overproducing plants, e.g. *ipt* (isopentenyl transferase from *Agrobacterium tumefaciens*), and transgenic plants with reduced levels of IAA, e.g. *iaaL* (indole-3-acetic acid Lys synthetase from *Pseudomonas savastanoi*), have been produced and reported to exhibit reduced apical dominance (Medford et al., 1989; Romano et al., 1991). On the other hand, transgenic plants with elevated levels of IAA, e.g. *iaaH* and *iaaM* (indoleacetamide hydrolase and Trp monooxygenase from *A. tumefaciens*), had increased apical dominance (Sitbon et al., 1992; Romano et al., 1993). In addition, transgenic plants that contain both IAA- and cytokinin-overproducing genes have an intermediate phenotype (Klee and Estelle, 1991). These results support the theory that the ratio of auxin to cytokinin might be the central factor controlling the growth of axillary buds. It is unclear, however, whether auxin is a primary signal to induce changes in growth and development of axillary buds. Auxin alone increases ethylene levels. Therefore, to uncouple auxin and ethylene effects, transgenic plants with increased levels of auxin were crossed with transgenic plants defective in ethylene biosynthesis (Ro-

mano et al., 1993). The results indicate that auxin increases apical dominance independently of the ethylene concentration. It seems, however, that the ratio of auxin to cytokinin is not the only regulatory factor that influences apical dominance. In both transgenic cytokinin-overproducing plants and transgenic reduced-auxin plants, the axillary buds remained dormant in the vegetative stage even though the ratio of auxin to cytokinin was altered. In the reproductive stage of these transgenic plants, the axillary buds grow more rapidly than do the wild-type plants. It is possible that the axillary buds of juvenile transgenic plants are insensitive to a favorable ratio of hormones for release of apical dominance, or alternatively, some other factors are involved in these mechanisms. More recently, it was proposed that indole-3-acetaldoxime is the metabolic branch between IAA and indole glucosinolate biosynthesis in Arabidopsis (Bak et al., 2001). Loss-of-function cytochrome P450, *CYP83B1*, mutants exhibit increased apical dominance, whereas gain-of-function *CYP83B1* mutants have decreased apical dominance. In addition, CYP83B1 catalyzes the first step in indole glucosinolate biosynthesis by metabolizing indole-3-acetaldoxime. These results suggest that the level of IAA is regulated by the flux of indole-3-acetaldoxime to the glucosinolate pathway by*CYP83B1* gene activity. Thus, the hormone levels regulated by the P450 gene family might control the growth of axillary buds.

GENETIC APPROACH

To analyze the mechanisms controlling apical dominance and shoot branching, mutants were isolated and characterized from Arabidopsis, maize, tomato, petunia, and pea. These mutants were divided into three classes based on their phenotype. In the first class, the mutants had increased branching. This phenotype is caused by the release of dormant axillary buds, that is, release of apical dominance. In this category, mutations do not affect early developmental stages of the axillary meristem. In other words, the timing and the number of formed axillary meristems are normal. *teosinte branced 1*, *decreased apical dominance*, *ramosus*, *auxin resistant*, and *iaa28* mutants belong to this category. Leyser's group recently isolated novel mutants *max 1–4* (*more axillary branching 1–4*). At the 17th International Conference on Plant Growth Substances, Leyser reported that*MAX2* acts in the bud and encodes an F-box protein, which is expected to be involved in auxin signal transduction. *MAX3* acts outside the bud. *MAX4* might act outside the bud and encodes an NCED (nine-cis-epoxycarotenoid dioxygenase)-like protein, which is thought to be the rate-limiting enzyme in ABA biosynthesis. Because the data are not published, however, the details are not known. In the second class, there is an increased number of formed axillary meristems. These mutants are affected in early developmental stages of the axillary meristem. The *supershoot/bushy* mutant belongs to this category. In the third class, the mutants have decreased branching. Mutations in this class usually affect the axillary meristem initiation. The *lateral suppressor* and *torosa-2* mutants belong to this category. Some reported mutants characterized so far are described as follows.

tb1 (teosinte branced 1)

Teosinte is a wild ancestor of maize and has elongated axillary shoots with terminal male inflorescences (tassels) at most nodes and short secondary axillary shoots that bear female inflorescences (ears; Doebley et al., 1997). It seems that *TB1* acts as a repressor of axillary shoot growth and regulates the sex of the inflorescences terminating the shoot. The *TB1* gene encodes a transcriptional regulator that might function in proliferating tissues to influence growth and cell division. Based on the expression patterns of *TB1* gene, in teosinte, the *TB1* gene would be inactive or expressed at low levels in the primordia of primary branches, allowing them to develop extensively. On the other hand, in maize, the *TB1* gene would be active in lateral shoot primordia, suppressing their outgrowth.

dad1-1 (decreased apical dominance)

The recessive *dad1-1* mutant has a highly branched growth pattern resulting from a proliferation of branches (Napoli, 1996). Graft studies indicate that a *dad1-1* scion, when grafted onto wild-type stock, is converted to a phenotype similar to the wild type. Furthermore, a small wild-type inter-stock fragment inserted between a mutant root stock and a mutant scion is sufficient to convert the *dad1-1* scion to near wild type. Thus, the *dad1-1* mutant is deficient in graft-transmissible substances that inhibit branching. It is possible that the wild-type gene products act as diffusible suppressors of axillary development. To date there is no molecular information on the nature of this gene.

rms (ramosus)

In pea, several mutants have been isolated based on the phenotype of altered branching patterns (Arumingtyas et al., 1992). The *rms1* and *rms2* mutants have increased branching at basal and aerial nodes, reduced internode length, and elevated levels of free IAA. Graft experiments indicate that apical dominance is almost fully restored in both mutants by grafting a mutant scion onto wild-type stock (Beveridge et al., 1994, 2000; Foo et al., 2001). Genetic analyses revealed that *Rms1* and *Rms2* might control a different pathway in the regulation of the branching pattern. Measurements of endogenous hormone

levels in these mutants suggest that the endogenous levels of IAA and cytokinin are not always correlated with the degree of apical dominance, and root-exported cytokinin is not the only graft-transmissible signal regulating branching in plants. It is possible that the additional novel signal regulated by the *RMS* gene moves acropetally in shoots and acts as a branching inhibitor.

axr1 (auxin resistant)

The recessive *axr1* mutant was selected on the basis of resistance to auxin supplied exogenously in Arabidopsis. Tissues of the *axr1* mutant have reduced auxin sensitivity. The phenotype of *axr1* is pleiotropic, such as small rosettes, small crinkled leaves, shortened petioles, and increased shoot branching at maturity. These observations suggest that the *AXR1* gene is required for auxin signaling. The *AXR1* gene encodes a protein related to ubiquitin-activating enzyme E1 (Leyser et al., 1993). The development of lateral shoots in *axr1-12* plants was compared with that in wild-type plants (Stirnberg et al., 1999). The *axr1* mutation does not affect the timing of axillary meristem formation. The *axr1* mutant, however, has more rapid growth of the lateral shoots. These findings suggest that auxin functions in the stages of lateral shoot growth following formation of the axillary meristem. These findings are consistent with the analyses of transgenic plants.

iaa28

The Arabidopsis mutant *iaa28-1* has severely defective lateral root formation, shorter plant body size, and decreased apical dominance (Rogg et al., 2001). The *IAA28* gene encodes a member of the *Aux/IAA* protein family. Analyses of the gain-of function *iaa28-1* mutant suggest that *IAA28* suppresses the transcription of genes that induce lateral root initiation in response to auxin signals.

sps (supershoot)/bus (bushy)

The *sps/bus* mutant isolated from Arabidopsis generates 500 or more inflorescences in a single plant (Reintanz et al., 2001; Tantikanjana et al., 2001). Analysis of the mutant plants reveals that the primary defect is an increase in the number of meristems formed in leaf axils, together with release of bud arrest, resulting in reiterative branch formation from rosette and cauline leaves. The *SPS/BUS* gene encodes a cytochrome P450, *CYP79F1*. In *sps/bus* mutant plants, the levels of Z-type cytokinin are increased 3- to 9-fold compared with wild-type plants. These findings suggest that the *SPS/BUS* gene functions to modulate hormone levels in plants. The *SPS/BUS* gene is strongly expressed at the leaf axils. It is possible that the localized decrease in cytokinin levels at sites of bud initiation control both initiation of the axillary meristem and growth of axillary buds. IAA content and its precursor indole-3-acetonitrile, however, are also increased in the *sps/bus* mutants. Therefore, the possibility that IAA relates to these phenotypes cannot be ruled out. Transgenic Arabidopsis with cosuppression of *CYP79F1* gene has a loss of apical dominance (Hansen et al., 2001).

ls (lateral suppressor) and *to-2 (torosa-2)*

In tomato, several mutations defective in axillary meristem initiation have been isolated (Tucker, 1979). The recessive *ls* mutant prevents the initiation of axillary meristems during the vegetative phase (Malayer and Guard, 1964), although axillary buds form normally after flowering. In addition, *ls* plants have a defect in petal development leading to the absence of the second whorl of flower organs. The results of a different bioassay demonstrated that the endogenous activities of gibberellic acid (GA), auxin, and ABA in the shoot tip are drastically increased, whereas cytokinin levels are reduced. The LS protein belongs to a family of proteins of unknown biochemical function, named VHIID domain proteins (Schumacher et al., 1999). This protein family includes the Arabidopsis *GAI* (*GIBBERELLIC ACID INSENSITIVE*; Peng et al., 1997) and *RGA* (*REPRESSOR OF GA1-3*; Silverstone et al., 1998). Both genes act as negative regulators of the GA signal transduction pathway. This leads to the working hypothesis that LS protein also functions as a negative regulator in GA signaling, and GA also has a role in controlling the formation of axillary meristems. Considering the limited similarity of *LS* to *GAI* and *RGA*, however, more experimental evidence is required to support this hypothesis.

The recessive *to-2* mutant in tomato lacks the axillary meristem in many leaf axils. This defect is correlated with reduced levels of cytokinin in the mutant plants compared with the wild type (Mapelli and Lombardi, 1982). In both *ls* and *to-2* mutants, the primary SAM is smaller than normal. It is possible that the defects in the primary SAM restrict the initiation of axillary meristems. In tomato, axillary meristems are directly derived from the primary SAM. The primary SAM in these mutants might not produce the axillary meristems because of the smaller size.

MOLECULAR APPROACH

Several molecular approaches have been used to characterize the biochemical events associated with the outgrowth and dormancy of axillary buds. The protein composition of pea axillary buds before and after decapitation was investigated using two-dimensional PAGE (Stafstrom and Sussex, 1988). Unique sets of proteins are synthesized in the dormant-to-growing stages. In addition, the dormant

axillary buds incorporate labeled amino acids at a rate similar to that of growing buds. This indicates that dormant axillary buds are as metabolically active as growing buds. Based on these observations, sets of genes expressed specifically in dormant axillary buds might have a role in the maintenance of dormant stages in axillary buds. Therefore, several dormancy-associated genes, such as *PsDRM1*, *PsDRM2*, *PsAD1*, and *PsAD2*, were isolated and characterized from pea plants (Stafstrom et al., 1998; Madoka and Mori, 2000a, 2000b). The deduced amino acid sequence of *PsDRM1* is similar to that of an auxin-repressed strawberry clone, whereas that of *PsDRM2* has similarity to that of cold- and ABA-stimulated clones from alfalfa. On the other hand, a search of the protein databases failed to produce any sequences whose functions are well known that were similar to PsAD1 and PsAD2 proteins. Both PsDRM2 and PsAD1 proteins are rich in Gly residues, although the amino acid sequences are not similar. *PsDRM1* and *PsAD1* mRNAs accumulate mainly in dormant axillary buds on intact plants. The amount of both mRNAs rapidly decreases after decapitation of the terminal buds, whereas it rapidly accumulates when axillary buds become dormant again (refer to Fig. 1). The temporal and spatial distribution patterns of PsAD1 protein were investigated using immunocytochemical analyses (Madoka and Mori, 2000b). PsAD1 proteins localize in the procambia, leaf primordia, and apical meristem in dormant axillary buds. After decapitation, PsAD1 protein acropetally disappears in the axillary buds. These acropetal changes occur in a manner similar to the way in which the procambium differentiates into vascular tissue. Taken together, these observations suggest that PsAD1 protein has a role in the inhibition of growth and differentiation, or in the maintenance of the dormant stages in axillary buds.

The anatomy of axillary buds following removal of the terminal bud was examined to understand the release from apical dominance. Analyses of the mitotic index indicated that removal of the terminal bud rapidly promotes cell division in axillary buds (Martin, 1987). In pea axillary buds, the proliferating and quiescent (phase of the cell cycle in which the dormant bud cells are arrested) cell cycles are characterized by mRNA accumulation patterns of several cell cycle-related genes (Devitt and Stafstrom, 1995; Shimizu and Mori, 1998a). These gene transcripts accumulate in a cell cycle-specific fashion. For example, the transcripts of *histoneH4*, *cycB1;2* (B-type cyclin), *cycD3;1* (D-type cyclin), and *PCNA* (proliferating cell nuclear antigen) accumulate predominantly during the S phase, the late G_2 and M phases, the G_1 phase, and late G_1 and S phases, respectively. The mRNA levels of all the genes were very low in the dormant axillary buds on intact plants. When the axillary buds were stimulated to grow by decapitation, mRNA levels increased remarkably. Moreover, the mRNA accumulation patterns of each of the genes were different. *PCNA* and *cycD3;1* mRNA accumulates first, followed by *histoneH4* mRNA, and then *cycB1;2* mRNA. These results suggest that most cells in dormant axillary buds are arrested at the G_1 phase in the cell cycle. In mammals, the progression and arrest of the cell cycle are controlled during the G_1 phase in response to the developmental program and environmental signals. Positive and negative regulators interact, and the protein complex controls the progression and arrest of the cell cycle (Sherr and Roberts, 1995). Using immunoaffinity column chromatography, the protein complex of cell cycle regulators was specifically detected in dormant axillary buds of pea plants. The complex was immediately dissociated in axillary buds after decapitation (Shimizu and Mori, 1998b). In mammals, one of the most important key regulators for the G_1 arrest is the product of the retinoblastoma tumor suppressor gene (*RB*; Sherr and Roberts, 1995). The functions of RB protein are regulated by phosphorylation in a cell cycle-dependent manner. The isolation and characterization of some RB-related proteins from plants were recently reported (Durfee et al., 2000). Using immunoprecipitation with an antibody against an RB-related protein of pea plants, the phosphorylation state of the RB-related protein was investigated in axillary buds during the dormancy-to-growth transition. After decapitation of the terminal bud, the plant RB-related protein was immediately phosphorylated (S. Shimizu-Sato and H. Mori, unpublished data). Taken together, these results suggest that the dormancy-to-growth transition in pea axillary buds is controlled by mechanisms similar to those regulating the cell cycle in mammals.

We recently isolated specific genes other than *PsAD1* and *PsAD2* that were expressed in dormant axillary buds. This screening indicated that the *PsAD1* gene accounted for more than 90% of the genes that were expressed in dormant axillary buds. Many of these isolated genes were homologous ABA-inducible genes, e.g. *LEA* (late embryogenesis abundant protein), *rd29B* (Yamaguchi-Shinozaki and Shinozaki, 1993), and *PsDRM2*, indicating that the ABA level of dormant axillary buds was higher than that of growing axillary buds after decapitation (Knox and Wareing, 1984; Gocal et al., 1991). The ABA response element sequence was present in the *PsAD1* promoter (Y. Madoka and H. Mori, unpublished data), suggesting that ABA promotes dormancy in axillary buds or acts to maintain the dormancy in axillary buds, as with seed dormancy. Auxin-repressed genes were also expressed in dormant axillary buds, e.g. *AGR10* (Hashimoto and Yamamoto, 1998) and *PsDRM1*, indicating that the auxin level in dormant axillary buds is lower than that in growing axillary buds after decapitation (Gocal et al., 1991) and that direct application of auxin to axillary buds after decapitation cannot prevent bud outgrowth.

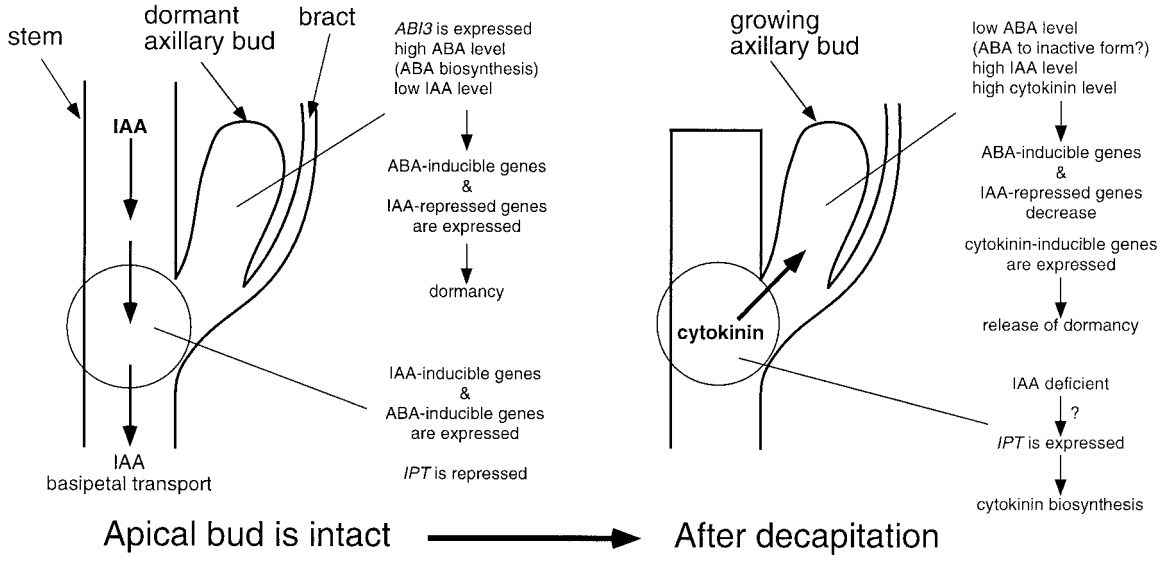

Figure 2. Proposed model of control mechanism of apical dominance by plant hormones.

Based on these results, we further examined the expression of the ABA-related genes, *ABI3* and *NCED*. Although ABI3 is thought to be a seed-specific transcription factor of ABA signal transduction, recent reports demonstrated that ABI3 also acts outside of the seed (Rohde et al., 1999, 2000). *PsABI3* was expressed in dormant axillary buds and in the accessory buds that were repressed by the growth of the main buds 3 to 4 d after decapitation. In addition, some *NCED* isogenes were expressed in dormant axillary buds and the mRNA levels gradually decreased 1 d after decapitation (A. Nakako and H. Mori, unpublished data). These results strongly suggest that ABA is also involved in dormancy of axillary buds. Furthermore, we isolated the specific genes that were expressed in the second nodes before and after decapitation. In the nodes before decapitation, the expressed genes were as follows: the auxin-related genes including the *AUX/IAA* family, auxin efflux carrier protein (*PIN*), ubiquitin conjugating enzyme E2, cullin homolog, and the ABA-related genes including *LEA* and zeaxanthin epoxidase, which is an ABA biosynthesis enzyme. On the other hand, in the nodes 3 h after decapitation, the expressed genes were as follows: adenylate isopentenyltransferase (*IPT*), several IAA-amino acid hydrolases, GA 2-oxidase (Ross et al., 2000), hydroxymethylglutaryl-CoA synthase, gene-related triterpenoid biosynthesis, including squalene synthase and squalene epoxidase, the gene-related jasmonate biosynthesis including lipoxygenase and allene oxide cyclase, and some cytochrome P450 (M. Tanaka and H. Mori, unpublished data). Of these genes, *IPT*, which is the key enzyme in cytokinin biosynthesis, is especially noteworthy. Plant IPT proteins were recently characterized (Kakimoto, 2001; Takei et al., 2001). Our data suggest that cytokinin is synthesized in the stem, at least, at the node after decapitation and that *IPT* genes are repressed by auxin, which is supported by recent report that auxin might repress cytokinin biosynthesis (Eklöf et al., 1997). These findings are consistent with evidence that the axillary buds grow in the excised nodal stem segments in the absence of auxin (Tamas et al., 1989; Chatfield et al., 2000) even in the absence of roots. On the other hand, some P450 whose function is unknown, is expressed after decapitation. A membrane-bound cytochrome P450 monooxygenase catalyzed the conversion of ABA to 8′-hydroxy-ABA, inactive ABA (Krochko et al., 1998). Although ABA 8′-hydroxylase has not been identified at the molecular level, P450 could be ABA 8′-hydroxylase. Taken together with our findings, we propose the control mechanism of apical dominance by plant hormones illustrated in Figure 2. If the apical buds are intact, IAA derived from apical buds promotes the expression of IAA-inducible genes and the repression of *IPTs* in the nodes, and might indirectly promote ABA biosynthesis in the nodes and axillary buds. ABA then promotes the expression of ABA-inducible genes. As a consequence, the outgrowth of the axillary buds is inhibited. After decapitation, because there is no IAA supplied from the apical buds, the IAA in the nodes becomes deficient. As a result, *IPT* is expressed in the nodes and produces cytokinin, and cytokinin derived from the nodes enters axillary buds and promotes the outgrowth of axillary buds. The decrease in IAA levels might indirectly promote the decrease in ABA levels; one possible mechanism is that ABA 8′-hydroxylase is induced by the IAA deficiency and degrades ABA to its inactive form. As a result, the expression levels of ABA-inducible genes decline. This proposed model is still speculative. Further studies are needed to validate the model.

CONCLUSIONS

This review focuses on recent advances in understanding the control of axillary bud outgrowth and dormancy. Various approaches have been used to analyze the molecular mechanisms of the regulation of the growth of axillary buds. The conventional plant physiological approaches, such as exogenous application of plant hormones, indicated that plant hormones have an important role in regulating axillary bud growth. Analyses of transgenic plants with altered endogenous hormone levels confirmed the relationships of plant hormones. The isolation and characterization of mutations that cause alterations in shoot branching patterns have become powerful approaches. In addition, molecular genetic approaches combined with grafting experiments have provided new concepts. Several plant hormones control outgrowth and dormancy of axillary buds. Additional factors must also have a role in regulating axillary bud outgrowth and dormancy. This complex phenomenon of apical dominance will be better understood by combining the findings from various approaches.

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