Brassinosteroids

Author: Clouse, Steven D.

Source: The Arabidopsis Book, 2011(9)

Published By: The American Society of Plant Biologists

URL: https://doi.org/10.1199/tab.0151

First published on November 02, 2011: e0151. doi: 10.1199/tab.0151
This chapter is an updated version of a chapter originally published on September 30, 2002, e0009. Doi: 10.1199/tab.0009

Brassinosteroids

Steven D. Clouse

Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609 USA
Address correspondence to steve_clouse@ncsu.edu

Brassinosteroids (BRs) are endogenous plant hormones essential for the proper regulation of multiple physiological processes required for normal plant growth and development. Since their discovery more than 30 years ago, extensive research on the mechanisms of BR action using biochemistry, mutant studies, proteomics and genome-wide transcriptome analyses, has helped refine the BR biosynthetic pathway, identify the basic molecular components required to relay the BR signal from perception to gene regulation, and expand the known physiological responses influenced by BRs. These mechanistic advances have helped answer the intriguing question of how BRs can have such dramatic pleiotropic effects on a broad range of diverse developmental pathways and have further pointed to BR interactions with other plant hormones and environmental cues. This chapter briefly reviews historical aspects of BR research and then summarizes the current state of knowledge on BR biosynthesis, metabolism and signal transduction. Recent studies uncovering novel phosphorelays and gene regulatory networks through which BR influences both vegetative and reproductive development are examined and placed in the context of known BR physiological responses including cell elongation and division, vascular differentiation, flowering, pollen development and photomorphogenesis.

INTRODUCTION

Brassinosteroids (BRs) comprise a class of nearly 70 polyhydroxylated sterol derivatives that appear to be ubiquitously distributed throughout the plant kingdom. Among plant hormones, BRs are structurally the most similar to animal steroid hormones, which have well-known functions in regulating embryonic and post-embryonic development and adult homeostasis. Like their animal counterparts, BRs regulate the expression of numerous genes, impact the activity of complex metabolic pathways, contribute to the regulation of cell division and differentiation, and help control overall developmental programs leading to morphogenesis. They are also involved in regulating processes more specific to plant growth including flowering and cell expansion in the presence of a potentially growth-limiting cell wall.

Numerous books (Cutler, 1991; Khripach et al., 1999; Sakurai et al., 1999; Hayat and Ahmad, 2003, 2011) and reviews covering all currently known aspects of BR biology and chemistry are available, including biosynthesis and metabolism (Fujioka and Yokota, 2003; Bajguz, 2007; Bishop, 2007; Symons et al., 2008a; Ohnishi et al., 2009), physiological effects (Clouse and Sasse, 1998; Krishna, 2003; Sasse, 2003) signal transduction (Clouse, 2002b; Vert et al., 2005; Gendron and Wang, 2007; Hardtke et al., 2007; Li and Jin, 2007; Clouse, 2008b; Kim and Wang, 2010; Li, 2010a; Clouse, 2011), natural occurrence (Fujioka, 1999; Bajguz, 2011) and research history (Steffens, 1991; Yokota, 1999).

Since the first edition of this chapter appeared in 2002, a great deal of progress has been made in understanding specific components of BR signal transduction and in clarifying mechanisms by which BR perception ultimately results in changes in the expression of genes associated with numerous developmental programs. The number of physiological processes known to involve BR action has also expanded to now include seed germination, determination of leaf shape and regulation of flowering time. These advances in molecular biology and physiology are integrated into the original version along with updates on BR biosynthesis and metabolism.

Short History of BR Research

The discovery of plant growth-promoting compounds that were subsequently shown to be steroids, was accomplished independently by research at the United States Department of Agriculture (USDA) and at Nagoya University in Japan (Yokota, 1999). In an effort to isolate new plant hormones, Mitchell and his USDA colleagues examined organic extracts of pollen from numerous species over a thirty-year period. The most active growth-promoting extracts were isolated from *Brassica napus* pollen, and were hence named “brassins”. Brassins had a pronounced effect on cell elongation and division in the bean second-internode bioassay (Mandava, 1988), and were found to increase yields when sprayed on young seedlings of radishes, leafy vegetables and potatoes. Based on this preliminary data, Mitchell et al. (Mitchell et al., 1970) somewhat prematurely, but perhaps prophetically, attributed hormonal status to the brassins “because they are spe-

cific translocatable organic compounds isolated from a plant and have induced measurable growth control when applied in minute amounts to another plant." However, they incorrectly predicted that the active component of brassins was a fatty acid ester.

The true chemical nature of the brassin active component was discovered after a major coordinated effort involving several USDA laboratories, a truckload of 227 kg of bee-collected *B. napus* pollen, pilot-plant solvent extractions, and extensive column chromatography (Steffens, 1991). The net result was 4 mg of a pure substance that was identified by single crystal X-ray analysis to be a steroidal lactone, which was named brassinolide (Grove et al., 1979). Within two years brassinolide (BL) and its stereo isomer, 24-epiBL, had been chemically synthesized, eliminating the need for such massive plant extraction procedures. With ample synthetic compound in hand, research in the 1980's focused on determination of BR physiological effects in a wide variety of biological systems and on testing greenhouse and field applications for enhanced crop yield (Cutler, 1991). Soon after the discovery of the structure of BL it was also shown in Japan that previously identified growth-promoting factors isolated from insect galls in leaves of *Distylium racemosum*, included BL and related BRs (Yokota, 1999).

A report on the effects of BR on Arabidopsis growth appeared in 1991 (Clouse and Zurek, 1991) followed by a description of a screen for BR-insensitive mutants in Arabidopsis and demonstration that BRs regulated gene expression in that species (Clouse et al., 1993). The 1990's also saw significant progress by several Japanese groups in unraveling the biosynthetic pathway to BRs from common membrane sterols as well as the identification of endogenous BRs in Arabidopsis tissues (Fujioka et al., 1996; Fujioka et al., 1998). While most chemists and biologists involved in BR research believed these compounds were indeed a new class of plant hormone (Sasse, 1991), unequivocal proof of their indispensable role in plant growth and development was not available until 1996, when a series of four independent reports described the identification and properties of three BR-deficient mutants (Kauschmann et al., 1996; Li et al., 1996; Szekeres et al., 1996) and one BR-insensitive mutant in Arabidopsis (Clouse et al., 1996; Kauschmann et al., 1996). The mutants exhibited an extreme dwarf phenotype, which could be rescued to wildtype by BR treatment of the deficient, but not the insensitive, mutants. Moreover, it was demonstrated that two of the deficient mutants resulted from lesions in genes encoding steroid biosynthetic enzymes. Thus, convincing genetic and biochemical evidence was provided that BRs were essential for normal plant growth and development. BRs were then accepted by most scientists as a new class of plant hormone and the number of researchers studying their activity began to increase.

In the decade following these discoveries, major advances were made that laid the foundation for understanding the molecular mechanisms of BR action including identification of the BR receptor and it co-receptor (Li and Chory, 1997; Wang et al., 2001; Li et al., 2002; Nam and Li, 2002; Kinoshita et al., 2005), discovery of a major negative regulator of the BR signaling pathway (Choe et al., 2002; Li and Nam, 2002; Perez-Perez et al., 2002), and characterization of two novel transcription factors that directly modulate the expression of BR-regulated genes (Wang et al., 2002; Yin et al., 2002; Zhao et al., 2002; He et al., 2005; Yin et al., 2005).

Chemical Structure

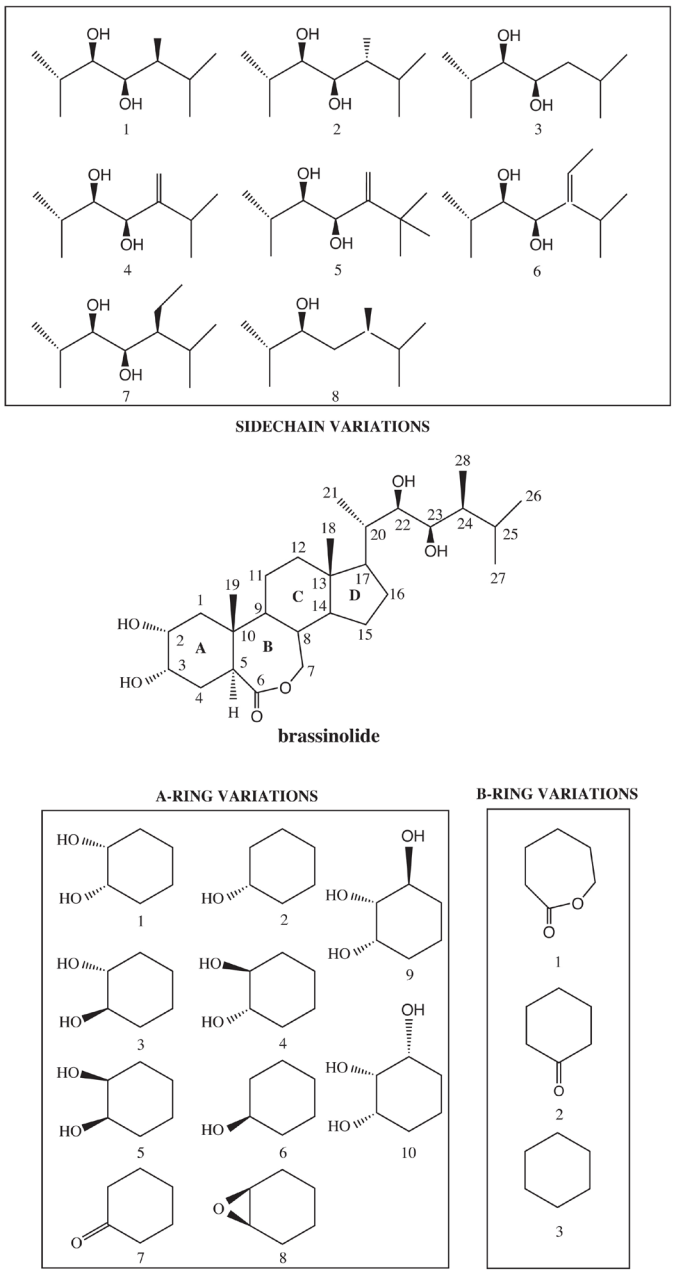
BL is a polyhydroxylated derivative of 5α-cholestan, namely (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (Figure 1). Thus, plants possess a growth-promoting steroid with structural similarity to cholesterol-derived animal steroid hormones such as androgens, estrogens and corticosteroids from vertebrates, and ecdysteroids from insects and crustacea. The BR family consists of BL and about 68 other free BRs plus several conjugates (Fujioka, 1999; Bajguz, 2011). These differ from BL by variations at C-2 and C-3 in the A ring; the presence of a lactone, ketone, or de-oxo function at C-6 in the B ring; the stereochemistry of the hydroxyl groups in the side chain, and the presence or absence of a methyl (methylene) or ethyl (ethylene) group at C-24. The conjugates are glycosylated, meristylated and laurylated derivatives of the hydroxyls in ring A or in the side chain. Many of the known BRs are biosynthetic precursors or metabolic products of BL, although castasterone, the immediate precursor of BL, is believed to have independent biological activity in some plants. The optimal structure for highest BR activity normally is that found in BL, consisting of a lactone function at C-6/C-7, cis-vicinal hydroxyls at C-2 and C-3, *R* configuration of the hydroxyls at C-22/C-23 and a methyl substitution at C-24 (Mandava, 1988).

Natural Occurrence and Distribution in the Plant Kingdom

BRs have been found in all cases in which a rigorous examination of a plant species by GC-MS or LC has been undertaken; including at least 53 angiosperms (41 dicots and 12 monocots), 6 gymnosperms, 3 algae, 1 bryophyte and 1 pteridophyte (Fujioka, 1999; Bajguz, 2011). Endogenous levels of BRs vary across plant organ type, tissue age and species, with pollen and immature seeds containing the highest levels. Young, growing shoots contain higher BR levels than mature tissue, which is not surprising in view of the greater physiological response of immature tissue to BRs. Table 1 summarizes endogenous BR levels detected in a range of species and tissue types.

Overview of Physiological Effects

Early studies of BR activity in plants depended on exogenous BR application followed by recording the observable response. From these experiments it became clear that BRs had a dramatic positive effect on stem elongation, including promotion of epicotyl, hypocotyl and peduncle elongation in dicots, and enhanced growth of coleoptiles and mesocotyls of monocots (Mandava, 1988). Exogenous BRs also stimulated tracheary element differentiation in *Helianthus tuberosus* and *Zinnia elegans*, the two primary model systems for xylogenesis (Clouse and Zurek, 1991; Fukuda, 1997). In many test systems BRs increased rates of cell division, particularly under conditions of limiting auxin and cytokinin. BRs were also shown to accelerate senescence, cause hyperpolarization of membranes, stimulate ATPase activity, and alter the orientation of cortical microtubules. Besides direct effects on growth regulation, BRs also were shown to mediate abiotic and biotic stresses, including salt and drought stress, temperature extremes and pathogen attack (Clouse and Sasse, 1998; Krishna, 2003;



brassinolide (BL)	1:1:1
castasterone (CS)	1:2:1
28-norbrassinolide (28-norBL)	1:1:3
28-norcastasterone (28-norCS)	1:2:3
6-deoxo-28-norCS	1:3:3
1β-hydroxyCS (1β-OH-CS)	9:2:1
3-epi-1α-OH-CS	10:2:1
2-epiCS	4:2:1
3-epiCS	3:2:1
2,3-diepiCS	5:2:1
2-deoxyBL	2:1:1
typhasterol (TY)	2:2:1
teasterone (TE)	6:2:1
3-dehydroteasterone (3DT)	7:2:1
6-deoxoCS	1:3:1
3-epi-6deoxoCS	3:3:1
6-deoxoTY	2:3:1
6-deoxoTE	6:3:1
6-deoxo3DT	7:3:1
secasterone (SE)	8:2:1
cathasterone (CT)	6:2:8
6-deoxoCT	6:3:8
24-epiBL	1:1:2
24-epiCS	1:2:2
3,24-diepiCS	3:2:2
6-deoxo-24-epiCS	1:3:2
dolicholide (DL)	1:1:4
dolichosterone (DS)	1:2:4
6-deoxoDS	1:3:4
28-homoCS	1:2:7
28-homoTY	2:2:7
28-homoTE	6:2:7
28-homoDL	1:1:6
28-homoDS	1:2:6
6-deoxo-28-homoDS	1:3:6
25-methylDS (25-Me-DS)	1:2:5
2-epi-25-Me-DS	4:2:5
2,3-diepi-25-Me-DS	5:2:5
2-deoxy-25-Me-DS	2:2:5
3-epi-2-deoxy-25-Me-DS	6:2:5
6-deoxo-25-Me-DS	1:3:5

Figure 1. Structure of naturally occurring brassinosteroids. The structure of brassinolide is presented with possible variations in the A and B rings and the side chain shown in boxes. Compounds to the right have been identified in plants and numbers represent structure of A-Ring: B-Ring: Side Chain. The C and D rings remain the same for all compounds.

Sasse, 2003). More recently BRs have also been demonstrated to regulate timing of flowering (Yu et al., 2008; Domagalska et al., 2010), pollen development (Ye et al., 2010) and organ differentiation (Gonzalez-Garcia et al., 2011; Hacham et al., 2011).

Many of the observed physiological responses elicited by exogenous application of BRs were shown to occur *in planta* by examination of the phenotypes of BR-deficient and -insensitive mutants in Arabidopsis, tomato, and pea. Arabidopsis BR mutants show a characteristic phenotype in the light (Figure 2), including dwarf stature, dark green, rounded leaves, prolonged life-span, reduced fertility, and altered vascular development. In the dark, they exhibit some of the features of light-grown plants including shortened hypocotyl and open cotyledons. In BR-deficient mutants, all of these phenotypic alterations are rescued to wildtype by exogenous application of BL (Altmann, 1999; Clouse and Feldmann, 1999).

Table 1. Distribution and Endogenous Levels of BRs in Selected Plants[a]

Species	Tissues	Levels (ng g^{-1} f.w.)
Arabidopsis thaliana	shoot, seed, silique	0.11-5.4
Brassica napus	pollen	>100
Brassica campestris	seed, sheath	0.00013-0.094
Raphanus sativus	seed	0.3-0.8
Helianthus annuus	pollen	21-106
Vicia faba	pollen, seed	5-628
Pisum sativum	seed, shoot	0.164-3.13
Solanum lycopersicum	shoot	0.029-1.69
Pinus thunbergii	pollen	89
Cupressus arizonica	pollen	1.0-6,400
Catharanthus roseus	cultured cells	0.047-4.5
Equisetum arvense	strobilus	0.152-0.349
Hydrodictyon reticulatum	green alga	0.3-0.4
Oryza sativa	shoot	0.0084-0.0136
Thea sinensis	leaves	0.001-0.02
Citrus sinensis	pollen	36.2
Zea mays	pollen, shoot	2.0-120
Lilium elegans	pollen	1.0-50
Typha latifolia	pollen	68

[a]Data from Fujioka (1999), Bajguz (2011) and references therein.

Figure 2. Phenotype of BR dwarf mutants. Comparison of several 5-week-old BR-deficient or – insensitive (*bri1*) mutants of Arabidopsis with a wild-type Arabidopsis plant of the same age. See the text for a description of each mutant. Adapted from a photo courtesy of S. Choe and K. Feldmann.

Biosynthesis

A clear understanding of how endogenous BR levels are regulated via synthesis and metabolism is a required component of any molecular model of BR action. The basic features of BL biosynthesis were uncovered utilizing cell suspension cultures of *Catharanthus roseus*, which were fed deuterated and tritiated putative intermediates in BL biosynthesis followed by analysis with sensitive techniques of GC-MS to monitor conversion of the labeled compounds (Fujioka and Yokota, 2003). Based on side chain structure and stereochemistry, wide distribution in the plant kingdom, and the relative biological activities in bioassays, it was predicted that the plant sterol campesterol would be converted to BL via teasterone, typhasterol and castasterone (Yokota et al., 1991). This skeletal pathway, in addition to several intermediate steps, was confirmed in *C. roseus* cells and seedlings. Moreover, it was found that C-6 oxidation could occur before (Early C-6 oxidation pathway) or after (Late C-6 oxidation pathway) hydroxylation of the side chain (Fujioka and Yokota, 2003).

While campesterol has been shown to be the BL progenitor, other BRs are likely to also be derived from common plant sterols with appropriate side chain structure such as sitosterol, isofucosterol, 24-methylenecholesterol, and 24-epicampesterol (Yokota, 1997). Plant sterols are synthesized by the isoprenoid biosynthetic pathway via acetyl-CoA, mevalonate, isopentenyl pyrophosphate, geranyl pyrophosphate and farnesyl pyrophosphate (Figure 3). Squalene is produced by condensation of two farnesyl pyrophosphate molecules, which is then converted via squalene-2,3-epoxide to cycloartenol, the parent compound of the majority of plant sterols. The conversion of squalene-2,3-epoxide to cycloartenol is unique to plants. In animals and fungi squalene-2,3-epoxide is converted to lanosterol, the precursor of cholesterol and the animal steroid hormones (Benveniste, 2004). While cycloartenol is the primary precursor of plant sterols, recent work has shown that Arabidopsis contains a functional lanosterol synthase and thus an alternate pathway to plant sterols via lanosterol appears likely (Suzuki et al., 2006). As described in detail below, several mutants have been isolated and characterized that are blocked at various steps in the biosynthesis of plant sterols, and in their conversion to BRs. Interestingly, most of the sterol and BR-deficient mutants were isolated in screens for other physiological processes, such as dwarfism, constitutive photomorphogenesis in darkness, or defects in embryogenesis, and were only later shown to be involved in sterol or steroid biosynthesis when the sequence of the cloned genes suggested such a role.

MODULATION OF ENDOGENOUS BR LEVELS IN ARABIDOPSIS

Biosynthesis of Sterol Precursors

The biosynthetic pathway to BL can be divided into general sterol synthesis (cycloartenol to campesterol), and the BR-specific pathway from campesterol to BL. Besides their role as BR precursors, plant sterols such as campesterol are integral membrane components which serve to regulate the fluidity and permeability of membranes and directly affect the activity of membrane associated proteins, including enzymes and signal transduction components (Clouse, 2002a; Lindsey et al., 2003; Schaller, 2003). Moreover, evidence from animal studies suggests that in addition to their role as steroid hormone precursors, sterols can themselves serve as ligands for nuclear receptors and interact with other transcription complexes to directly modulate signal transduction pathways (Edwards and Ericsson, 1999). In view of the multiple and critical roles sterols play, it is not surprising that mutations in genes encoding sterol biosynthetic enzymes can have

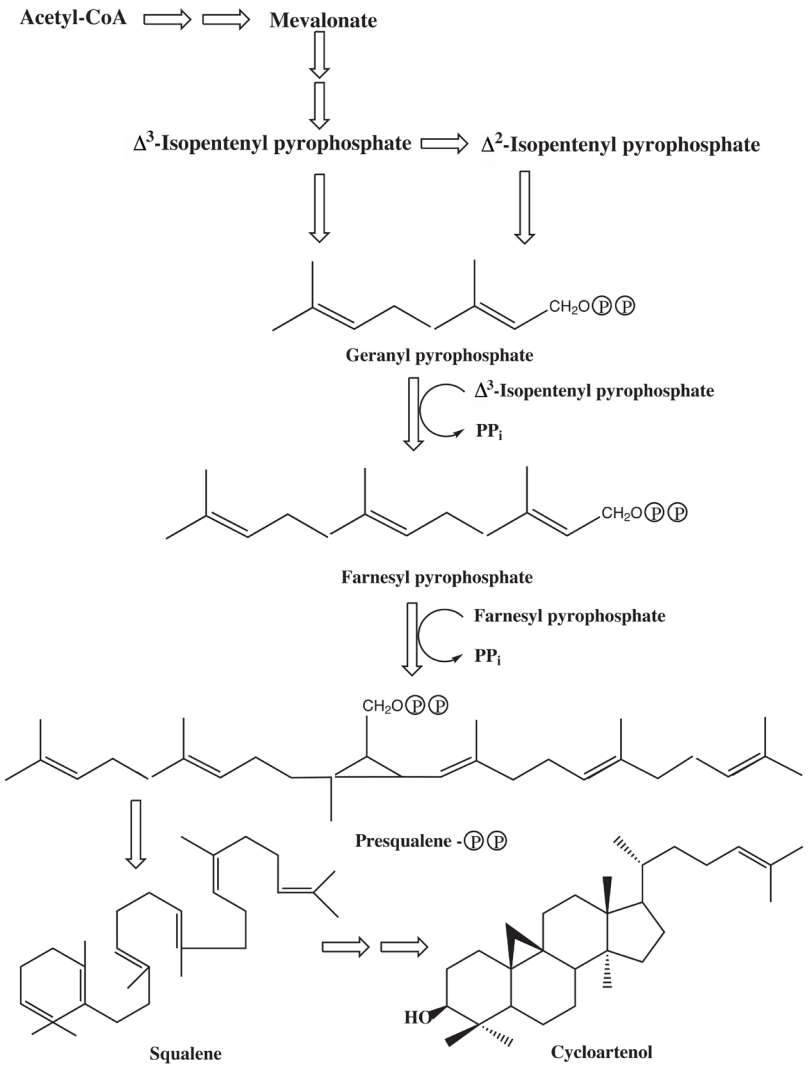


Figure 3. Biosynthetic pathway of the plant sterol precursor, cycloartenol, from mevalonate.

a dramatic affect on eukaryotic development. Mutations in genes encoding several biosynthetic enzymes in the pathway leading to plant sterols have now been identified, in addition to numerous mutants affecting the specific pathway to BRs. Comparison of the phenotype of these mutants and their response to BR application, has increased our understanding of the role plant sterols and their hormone derivatives play in controlling both embryonic and post-embryonic plant development.

A study of an allelic series of mutations in the Arabidopsis *cycloartenol synthase 1* (*CAS1*) gene, which encodes the enzyme required for conversion of squalene-2,3-epoxide to cycloartenol, showed that CAS1 is required for cell viability, plastid biogenesis and male fertility (Babiychuk et al., 2008). Other Arabidopsis mutants, with blocks in the early sterol biosynthetic pathway leading from cycloartenol to 24-methylenelophenol (Figure 4), have some of the characteristics of BR-deficient mutants in adult plants, but show unique defects in embryogenesis not previously reported in BR mutants or in sterol biosynthetic mutants later in the pathway, downstream of the step that yields 24-methylenelophenol.

The *fackel* mutant was identified in a systematic screen for mutations affecting body organization in *Arabidopsis* seedlings (Mayer et al., 1991). The *FACKEL* gene encodes a sterol C-14 reductase required for the conversion of 4α-methyl-5α-ergosta-8,14,24(28)-trien-3β-ol to 4α-methylfecosterol (Jang et al., 2000; Schrick et al., 2000), and shares significant sequence similarity to human, rat, chicken and fungal C-14 sterol reductases. Functional equivalence is demonstrated by *FACKEL* cDNA rescue of the defective sterol C-14 reductase in the yeast mutant *erg*24. Furthermore, the endogenous levels of various BRs, campesterol and sitosterol are severely reduced in the *fackel* mutant, while the substrate of the sterol C-14 reductase accumulates ten-fold. Interestingly, *fackel* also accumulates three unusual 8,14-diene sterols, similar to plant cell suspension cultures treated with the C-14 reductase inhibitor, 15-azasterol.

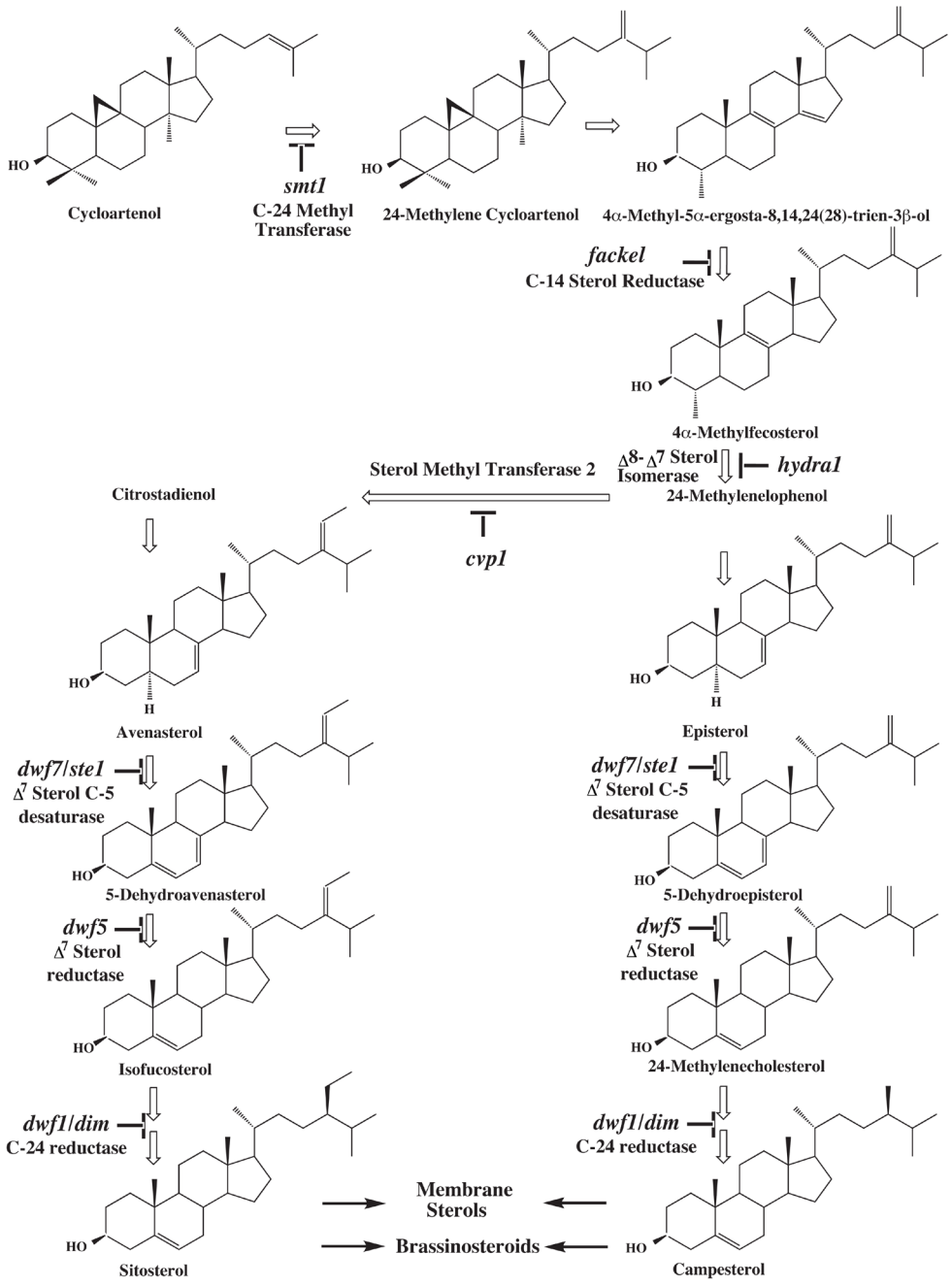


Figure 4. Biosynthetic pathways of sterols from cycloartenol. Steps in which mutants in Arabidopsis are available are indicated.

The *sterol methyltransferease1* (*smt*1) mutant of *Arabidopsis* shares many of the phenotypic properties of *fackel* and disrupts the upstream step leading from cycloartenol to 24-methylene cycloartenol (Diener et al., 2000; Schrick et al., 2002). The *SMT*1 gene encodes an enzyme capable of C-24 alkylation of the sterol side chain in the presence of S-adenosylmethionine and recombinant SMT1 catalyzes the conversion of cycloartenol to 24-methylene cycloartenol in vitro. Cholesterol accumulates in the *smt*1 mutant at the expense of sitosterol, although other sterol levels are relatively normal. The *hydra1* (*hyd1*) mutant disrupts the step immediately following FACKEL, and the *HYD1* gene encodes a Δ^8-Δ^7 sterol isomerase, converting 4α-methylfecosterol to 24-methylenelophenol (Schrick et al., 2002; Souter et al., 2002). As with *fackel*, campesterol and sitosterol are dramatically reduced in *hyd1*. Vascular development is also altered in both *fackel* and *hyd1* (Pullen et al., 2010).

Embryogenesis is severely disrupted in *fackel, hyd1* and *smt*1 mutants. Initially, a lack of asymmetrical cell division in the globular

stage embryo is observed, and while wild-type embryos progress to the heart stage, mutant embryos remain globular and disorganized. Multiple shoot apical meristems are initiated in the mutant and developing seedlings often have more than two cotyledons. Furthermore, the cotyledons appear to be directly attached to the root, with little development of the hypocotyl. Determination of mRNA levels by in situ hybridization showed prominent expression of the *FACKEL* and *SMT1* genes during wild-type embryo development (Diener et al., 2000; Schrick et al., 2000). Finally, it should be noted that while adult *fackel* and *hyd1* plants exhibit some of the features of BR-deficient mutants (and are themselves BR-deficient), they are not rescued by BR treatment, even though they retain some sensitivity to BRs (Jang et al., 2000; Schrick et al., 2000). An intriguing explanation for the lack of BR rescue of these early sterol mutants suggests that sterols themselves may serve as signaling molecules regulating embryogenesis and these sterol signals are disrupted in the *fackel, hyd1* and *smt*1 mutants. Moreover, altered sterol profiles in the mutants may affect membrane structure, which then alters the action of specific signaling proteins. Arguments in support of these hypotheses have been reviewed (Clouse, 2002a; Lindsey et al., 2003).

Immediately after 24-methylenelophenol the sterol biosynthesis pathway bifurcates into parallel pathways with one branch leading to campesterol, the progenitor of BL, while the second branch leads to sitosterol, a primary plant membrane sterol. The enzymes SMT2 and SMT3 catalyze the branch reaction from 24-methylenelophenol to 24-ethylidenelophenol and are encoded by the *COTYLEDON VASCULAR PATTERN1 (CVP1)* and *SMT3* genes in Arabidopsis (Carland et al., 2002; Carland et al., 2010). There is an accumulation of campesterol and a deficiency of sitosterol in *cvp1* mutants, accompanied by a unique vascular patterning defect not observed in BR-deficient mutants, suggesting that specific sterols play a role in vascular development that is distinct from BRs. Unlike sterol biosynthetic mutants upstream of 24-methylenelophenol, *cvp1* mutants do not display defects in embryogenesis (Carland et al., 2002).

The next step in the sterol biosynthetic pathway for which mutants are available involves the introduction of a C-5 double bond in the B ring of episterol (Figure 4). A screen of ethane-methylsulfonate (EMS) mutagenized *Arabidopsis* seedlings by gas chromatography uncovered a mutant, *ste*1 (Gachotte et al., 1995), that accumulated Δ^7 sterols with a decrease in the corresponding Δ^5 sterols. Transgenic plants expressing the yeast *ERG*3 gene, a Δ^7-C-5-desaturase, regained the normal sterol profile. The *ste*1 mutant was apparently a weak or leaky allele, since it showed no visible phenotype. Null alleles of *ste*1, *dwarf7*-1 (*dwf7*-1) and *dwf7*-2, were discovered in screens for dwarfs in a population of T-DNA mutagenized and EMS mutagenized *Arabidopsis* plants, respectively. These mutants had many of the phenotypic features of BR-deficient mutants (although not as severe), which were rescued by BR treatment (Choe et al., 1999a) and *dwf7* has been shown to have deficiencies in cell division and differentiation (Cheon et al., 2010).

The STE1/DWF7 gene is 38% identical (50% similar) to the yeast ERG3 gene and 35% identical (47% similar) to a human ortholog. All of these Δ^7-C-5-desaturases have conserved transmembrane domains and histidine clusters required for activity and both *dwf7*-1 and *dwf7*-2 are nonsense mutations resulting in premature stop codons that eliminate some of these essential domains. An apparent DWF7/STE1 homolog with 80% amino acid identity has been cloned in Arabidopsis, suggesting that duplicate genes regulate this step. Such redundancy could account for the less severe phenotype of the *dwf7* mutants when compared to mutants in the BR-specific pathway. Measurement of endogenous sterol and BR levels in *dwf7-1* by GC-SIM showed severe reduction in 24-methylenecholesterol, campesterol, campestanol and all downstream BRs. These results were confirmed by feeding experiments with ^{13}C-mevalonic acid, which showed that *dwf7* accumulated ^{13}C-episterol while ^{13}C-5-dehydroepisterol was not detected. Thus, a very thorough genetic and biochemical analysis of these mutants has rigorously identified *dwf7*/*ste*1 alleles as lesions in the gene encoding the Δ^7-C-5-desaturase of Arabidopsis sterol biosynthesis (Choe et al., 1999a). Another allele of DWF7, *boule* (*bul1-1*), shows an extreme dwarf phenotype that can be rescued by BR treatment (Catterou et al., 2001a).

The next step in the pathway, conversion of 5-dehydroepisterol to 24-methylenecholesterol by a Δ^7-sterol reductase (Figure 4), has also been thoroughly characterized. The *dwf*5 mutant of Arabidopsis was assigned to this step by similar methods to those described above for *dwf7*, including sequence analysis of the Arabidopsis homolog of Δ^7-sterol reductase in six loss-of-function *dwf*5 alleles to pinpoint the mutations, measurement of endogenous BR and sterol levels, feeding experiments with labeled intermediates, and rescue of the mutant to wildtype by applying exogenous BRs. Moreover, the *dwf*5 mutant was also complemented by transformation with the Arabidopsis Δ^7-sterol reductase gene. The phenotype of *dwf*5 is similar to *dwf7* and other BR-deficient mutants, but has some unique characteristics. The *dwf*5-1 allele has increased fertility compared to other BR and sterol-deficient mutants, but had abnormal seeds with reduced germination rates. Furthermore, *dwf*5-1 did not show the prolonged life cycle typical of BR mutants (Choe et al., 2000).

The completion of the sterol biosynthetic pathway involves the isomerization and reduction of 24-methylenecholesterol to campesterol (and isofucosterol to sitosterol by a parallel reaction). Numerous allelic dwarf mutants have been associated with this conversion. The first mutant, *dwarf*1 (*dwf*1) was originally isolated from the first population of T-DNA lines generated by seed infection (Feldmann et al., 1989). An allele of *dwf*1, *dimunito* (*dim*), was independently isolated from another T-DNA screen (Takahashi et al., 1995). However, it was not known that *dwf1/dim* were BR-deficient mutants until a third allele, *cabbage*1 (*cbb*1), was shown to be rescued by BR treatment (Kauschmann et al., 1996). Another eight alleles of *dwf*1 were identified in various screens and sequence analysis showed that *DWF*1 encoded an oxidoreductase with a flavin adenine dinucleotide-binding domain, and a putative membrane-anchoring region (Choe et al., 1999b). A majority of the alleles contained lesions in this domain, suggesting a critical role for FAD-binding in DWF1 function. Subsequent work with a green fluorescent protein-DWF1 fusion confirmed that DWF1 is an integral membrane protein, as expected for most sterol and steroid biosynthetic enzymes (Klahre et al., 1998). Two independent groups confirmed that the DWF1 protein is responsible for both steps in the conversion of 24-methylenecholesterol to campesterol, and isofucosterol to sitosterol (Klahre et al., 1998; Choe et al., 1999b).

The Classical Pathway for Biosynthesis of BL from Campesterol

The conversion of the membrane sterol campesterol to BL occurs via a series of reductions, hydroxylations, epimerizations and oxidations that have been extensively studied in several species. The discussion of this portion of the pathway is divided into the "classical" or historical view, which was in place at the writing of the first edition of this chapter, followed by the "revised" pathway based on several publications in the past seven years that have introduced numerous modifications and alternate routes from campesterol to BL.

In the classical pathway, the first four reactions lead to campestanol via reduction of the C-5 double bond in campesterol (Figure 5). One of these steps has been characterized in detail. The dwarf mutant *de-etiolated*2 (*det*2) was identified in a screen for plants that grew in the dark as if they were in the light (Chory et al., 1991). Map-based cloning of *DET*2 revealed significant sequence identity in the predicted protein (38-42%) to mammalian 5α-steroid reductases that catalyze the NADPH-dependent reduction of testosterone to dihydrotestosterone (Li et al., 1996). The mammalian reductase and the DET2 protein share 80% of conserved residues including a glutamate that is required for mammalian enzyme activity and which is mutated to a lysine in the *det*2-1 and *det*2-6 alleles. Moreover, recombinant DET2 protein expressed in human embryonic kidney cells reduced several 3-oxo, $\Delta^{4,5}$ mammalian steroids, such as testosterone and progesterone, while the mutant *det*2 protein failed to do so (Li et al., 1997). The DET2 enzyme activity was inhibited by 4-MA, a competitive inhibitor of mammalian 5α-reductases. Overexpression of human 5α-reductases in transgenic *det*2 plants resulted in wild-type phenotype without BR application, and the rescue was inhibited by 4-MA.

BRs rescue *det*2 to wildtype in the light and the dark (Li et al., 1996) and endogenous levels of campestanol are reduced about 90% in *det*2 compared to wildtype (Fujioka et al., 1997). While *det*2 has the typical BR-mutant phenotype, it is not as severe as *cpd* and *bri*1 (see below), and this may be due to the residual campestanol present in *det*2. This, in turn, could result from a second reductase catalyzing the same reaction, similar to the case of *dwf*7. Measurements of endogenous BRs and feeding wildtype and *det*2 seedlings with deuterated substrates showed that DET2 catalyzes the step involving conversion of (24*R*)-24-methylcholest-4-en-3-one (also called (24*R*)-ergost-4-en-3-one) to (24*R*)-24-methyl-5α-choestan-3-one (also called (24*R*)-5α-ergostan-3-one) (Noguchi et al., 1999a). However, subsequent work suggested that in Arabidopsis the 22-hydroxylated form of the 4-en-3-one is likely to be a primary substrate of DET2 (Fujioka et al., 2002) and 22-hydroxylation of campesterol occurs before 5-α reduction by DET2 (Fujita et al., 2006).

Another mutant, *sax*1, may also affect steps in the conversion of campesterol to campestanol. *sax*1 was identified in a screen for mutants that were hypersensitive to auxin and abscisic acid (Ephritikhine et al., 1999a). While *sax*1 shares the dwarf phenotype of BR-deficient mutants and is partially rescued by BR treatment, it does not exhibit the typical de-etiolated phenotype in the dark. *sax1* appears to block BL synthesis in a step preceeding *det2,* and feeding experiments with synthetic intermediates (Ephritikhine et al., 1999b) suggest that SAX1 acts in a putative side pathway involving conversion of campersterol to 6-deoxocathasterone via 22-hydroxylated intermediates (Figure 5). However, this pathway has not been demonstrated to occur *in planta* and the *SAX*1 gene has not been cloned. Thus the role of SAX1 in BR biosynthesis is not as clear as in the BR-deficient mutants that have been more fully characterized.

From campestanol, the classical BL biosynthetic pathway diverges into the Early and Late C-6 oxidation branches. BRs lacking a ketone or lactone in the B ring occur widely, although they have low biological activity in bioassays (Yokota, 1997). The endogenous occurrence of 6-deoxocastasterone, 6-deoxotyphasterol, 3-dehyrdo-6-deoxoteasterone and 6-deoxoteasterone has been demonstrated in numerous plants where castasterone and BL are also present including bean, wheat, rice, rye, Arizona cypress*, C. roseus* and Arabidopsis (Griffiths et al., 1995; Fujioka et al., 1996; Choi et al., 1997; Fujioka and Sakurai, 1997). The co-occurrence of 6-deoxo and 6-oxo forms of BL precursors suggests that a late C-6 oxidation pathway, in which ketone formation at C-6 follows A ring hydroxyl and side chain modification, operates simultaneously with early C-6 oxidation in a wide array of plants. Feeding a range of deuterium-labeled substrates to seedlings followed by GC-MS has clearly demonstrated that both of these pathways are functional in Arabidopsis (Noguchi et al., 2000).

The conversion of campestanol to 6-deoxocathasterone (Late C-6 oxidation) and of 6-oxocampestanol to cathasterone (Early C-6 oxidation) are both accomplished in the classical pathway by the product of the *DWF*4 gene, which encodes a cytochrome P450 (CYP90B1) with sequence similarity to mammalian steroid hydroxylases (Choe et al., 1998). The phenotype of *dwf*4 resembles that of other BR-deficient mutants and feeding experiments showed that only 22α-hydroxylated intermediates in BL biosynthesis could rescue *dwf*4 to wildtype suggesting that DWF4 functions as a C-22 steroid hydroxylase. Further details of DWF4 action are discussed below. The next step in both branches of the classical pathway also involves a side chain hydroxylation that was originally thought to be catalyzed by the product of the CPD gene, which encodes a cytochrome P450 (CYP90A1) with 43% identity (66% similarity) to DWF4. The *cpd* mutant is an extreme dwarf that is rescued only by 23α-hydroxylated BRs, suggesting that CPD acts as a C-23 steroid hydroxylase (Szekeres et al., 1996). However, this has not been confirmed biochemically and, as discussed below, CPD is unlikely to be a C-23 hydroxylase.

Characterization of steps after CPD and DWF4 in the Arabidopsis BL biosynthetic pathway took advantage of mutant studies in tomato, homology searches and biochemical analyses rather than initial genetic screens in Arabidopsis. The tomato DWARF gene encodes a cytochrome P450 monooxygenase that has been shown to catalyze the oxidation of 6-deoxocastasterone to castasterone via 6α-hydroxycastasterone (Bishop et al., 1999). Using the tomato DWARF sequence for database searches, an Arabidopsis gene, *AtBR6ox*, was cloned that encodes a cytochrome P450 (CYP85A1) sharing 68% identity (81% similarity) to the tomato DWARF P450. *AtBR6ox* was expressed in yeast, and by feeding deuterated substrates followed by GC-MS, it was shown that recombinant AtBR6ox is a steroid-6-oxidase that can convert not only 6-deoxocastasterone to castasterone, but also 6-deoxotyphasterol to typhasterol; 3-dehydro-6-deoxoteasterone

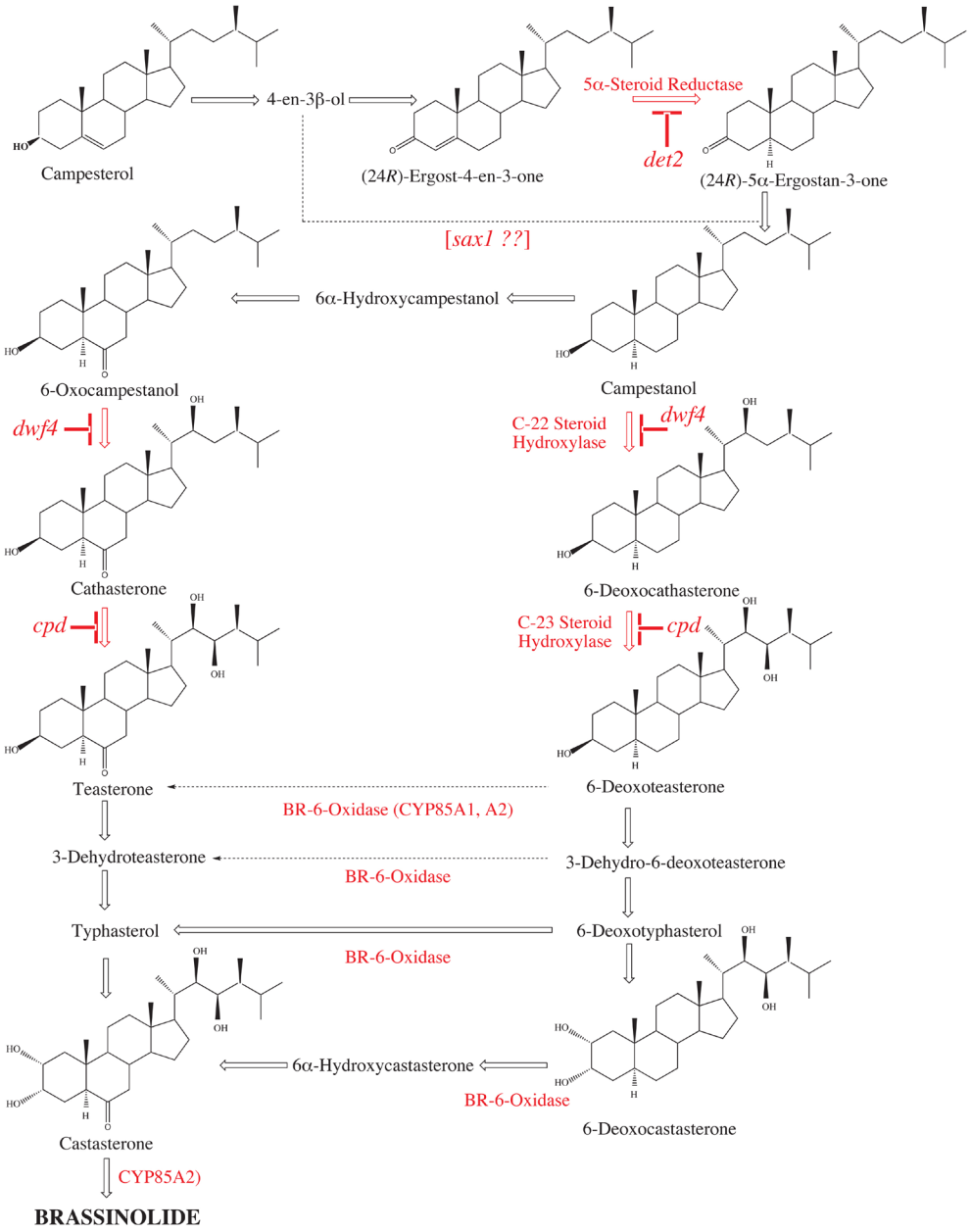


Figure 5. The classical biosynthetic pathway from campesterol to brassinolide showing known mutants in Arabidopsis that block the conversion of intermediates at the steps indicated, or prevent BR perception and/or signal transduction. The pathway on the left is termed Early C-6 Oxidation and that on the right, Late C-6 Oxidation. BR-6-Oxidase has been shown to convert 6-Deoxocastasterone and 6-Deoxotyphasterol to the corresponding 6-oxo compounds in Arabidopsis, while the conversion of 3-Dehydro-6-deoxoteasterone and 6-Deoxoteasterone has been demonstrated so far in recombinant yeast cells only. As discussed in the text, several revisions and alterations in the classical pathway have occurred over the past several years that have shortened the route from campesterol to brassinolide. Furthermore, biochemical evidence suggests CPD is unlikely to be a C-23 hydroxylase.

to 3-dehydroteasterone; and 6-deoxoteasterone to teasterone (Shimada et al., 2001). The conversion of 6-deoxocastesterone to castasterone and 6-deoxotyphasterol to typhasterol has been confirmed in Arabidopsis seedlings (Noguchi et al., 2000). Thus, BL biosynthesis in Arabidopsis appears to be more of a metabolic grid than two independent linear pathways of Early and Late C-6 oxidation.

A homolog of CYP85A1, Arabidopsis CYP85A2, catalyzes all of the same C-6 oxidase reactions as CYP85A1 but has the additional function, not found in CYP85A1, of conversion of castasterone to BL via Baeyer-Villiger C-6 oxidation (Kim et al., 2005b; Kwon et al., 2005; Nomura et al., 2005). Studies in which *cyp85A1/cyp85A2* double mutants were treated with exogenous castasterone or BL, clearly showed that castasterone

is an active BR in its own right, but that BL is much more efficient in promoting growth. This is consistent with many years of observation in physiological studies and with the reduced affinity of castasterone for the BR receptor compared to BL (Wang et al., 2001).

The Revised Pathway of BL biosynthesis from Campesterol

In the classical pathway of BL biosynthesis, conversion of campesterol to campestanol via DET2 precedes C-22 hydroxylation by DWF4 and this pathway has been confirmed in Arabidopsis. However, endogenous measurement of C-22 hydroxylated BRs in Arabidopsis coupled with catalytic efficiency studies of recombinant DWF4 protein, strongly suggest that the primary substrate of DWF4 is campesterol rather than campestanol, and that direct C-22 hydroxylation of campesterol by DWF4 precedes 5-α reduction by DET2 (Fujioka et al., 2002; Fujita et al., 2006). Thus, early C-22 hydroxylation plays a prominent role in BL biosynthesis in Arabidopsis and complicates the metabolic grid of possible reactions even further.

Genetic and biochemical analysis of two related cytochrome P-450s, CYP90C1 (ROTUNDIFOLIA3) and CYP90D1, showed that these enzymes were also critically involved in BL biosynthesis (Kim et al., 2005a; Ohnishi et al., 2006). Single null alleles of CYP90C1 or CYP90D1 showed little or no altered phenotype, while the double *cyp90c1/cyp90d1* mutant showed the characteristic extreme dwarf phenotype of BR-deficient mutants, which could be rescued only by C-23 hydroxylated BR biosynthetic intermediates, suggesting CYP90C1 and CYP90D1 act as redundant C-23 steroid hydroxylases in BL biosynthesis. This was confirmed by kinetic analysis of the recombinant proteins, which demonstrated C-23 hydroxylation of 6-deoxocathasterone and cathasterone by both CYP90C1 and CYP90D1, as expected. However, these classic BR intermediates were very poor substrates compared to the 22-hydroxylated 3-one product of the DET2 reaction as well as 3-epi-6-deoxocathasterone (Figure 6), which could be converted directly to 3-dehydro-6-deoxoteasterone and 6-deoxotyphasterol, respectively by CYP90C1 and CYP90D1 (Ohnishi et al., 2006). Thus, a “shortcut” pathway from campesterol to BL that bypasses campestanol, 6-deoxocathasterone and 6-deoxoteasterone was established, and biochemical experiments *in vitro* coupled with measurement of endogenous BRs suggest that this is a prominent pathway in Arabidopsis. The experiments of Ohnishi et al. also showed that CPD (CYP90A1) did not exhibit C-23 hydroxylase activity, and so while CPD is clearly a BR-deficient mutant, its position and function in the pathway needs to be redefined. A role in the pathway earlier than C-23 hydroxylation, perhaps C-3 oxidation, has been suggested for CPD (Ohnishi et al., 2006; Bishop, 2007; Ohnishi et al., 2009).

Regulation of BL Biosynthesis

Modulation of the activity and/or levels of biosynthetic enzymes in the BL pathway is an obvious way in which BR concentrations could be increased or decreased in the plant cell. Such regulation could occur at the level of transcription, mRNA stability, translation, enzyme catalytic activity and accessibility to substrates. Possible regulatory factors affecting these steps might include environmental signals and developmental cues such as light and other hormones. Homeostasis of BL could be achieved by feedback inhibition by the pathway end product. Such feedback inhibition in fact has been demonstrated in several instances. Transcription of the *CPD* gene is specifically down-regulated by BL in both dark and light, and sensitivity of the response to cycloheximide suggests a requirement for de novo synthesis of a regulatory factor (Mathur et al., 1998). *DWF*4 transcript levels are also regulated by endogenous BRs. *DWF*4 expression is very low in wildtype but increases dramatically in the *dwf*1 and *cpd* mutants. An equivalent increase is also seen in the BR-insensitive mutant, *bri*1, indicating that active BR signaling is required for repression of *DWF*4 expression in wildtype. The general importance of BR signaling in BL homeostasis is confirmed by the observation that *bri*1 mutants accumulate much higher levels of BRs than wildtype (Noguchi et al., 1999b) and also show increased metabolic flow through the pathway (Noguchi et al., 2000). Several recent studies have demonstrated the role of direct transcriptional regulation of genes encoding BR biosynthetic enzymes in regulating BR action (Mouchel et al., 2006; Guo et al., 2010; Sun et al., 2010; Chung et al., 2011; Poppenberger et al., 2011; Yu et al., 2011) and a review of the endogenous cues and environmental signals influencing BR homeostasis is also available (Hategan et al., 2011).

Regulation of transcript and protein levels of enzymes involved in rate-limiting steps of BL biosynthesis would yield the greatest impact on overall BL levels. If one assumes that levels of intermediates should be elevated immediately prior to a control point, then the reaction catalyzed by DWF4 is a candidate for a rate-limiting step in BL biosynthesis. In Arabidopsis, one substrate of DWF4, campestanol, is present in 500-fold excess over the product, 6-deoxocathasterone. Moreover, given that campesterol is more likely to be the primary substrate of DWF4 and that campesterol is present in nearly 50,000-fold excess over its product 22-hydroxycampesterol, a predominant role for DWF4 as a rate-limiting step is further supported (Fujioka et al., 2002). Another potential rate-limiting step is the conversion of 6-deoxocastasterone to castasterone, since the substrate is in ten-fold excess over the product (Nomura et al., 2001). In 26-day-old light-grown Arabidopsis plants, 6-deoxocastasterone occurs at the highest level of any BR measured (Nomura et al., 2001). Indeed, all of the 6-deoxo intermediates occur at much higher levels than the corresponding 6-oxo intermediates, suggesting that at least in the light, Late C-6 oxidation is the prominent pathway. Based on feeding experiments with *det*2 and *dwf*4, it has been proposed that 6-deoxo intermediates of the late C-6 oxidation pathway are more active in the light, while 6-oxo intermediates of the early C-6 oxidation pathway are more active in the dark (Fujioka et al., 1997; Choe et al., 1998). What biological significance this has is uncertain, particularly since endogenous BR levels in dark-grown seedlings has not yet been reported. Moreover, this is not a general phenomenon, since 6-deoxo BRs were equally effective in the light and the dark in rescuing the BR-deficient *dumpy* mutant of tomato (Koka et al., 2000).

The discovery of a specific BR-biosynthesis inhibitor provides a new tool for dissecting BR physiology and regulating endogenous BR levels at different stages in the plant growth cycle (Asami and Yoshida, 1999). Like the gibberellin biosynthesis inhibitors uniconazole and paclobutrazol, the BR biosynthesis in-

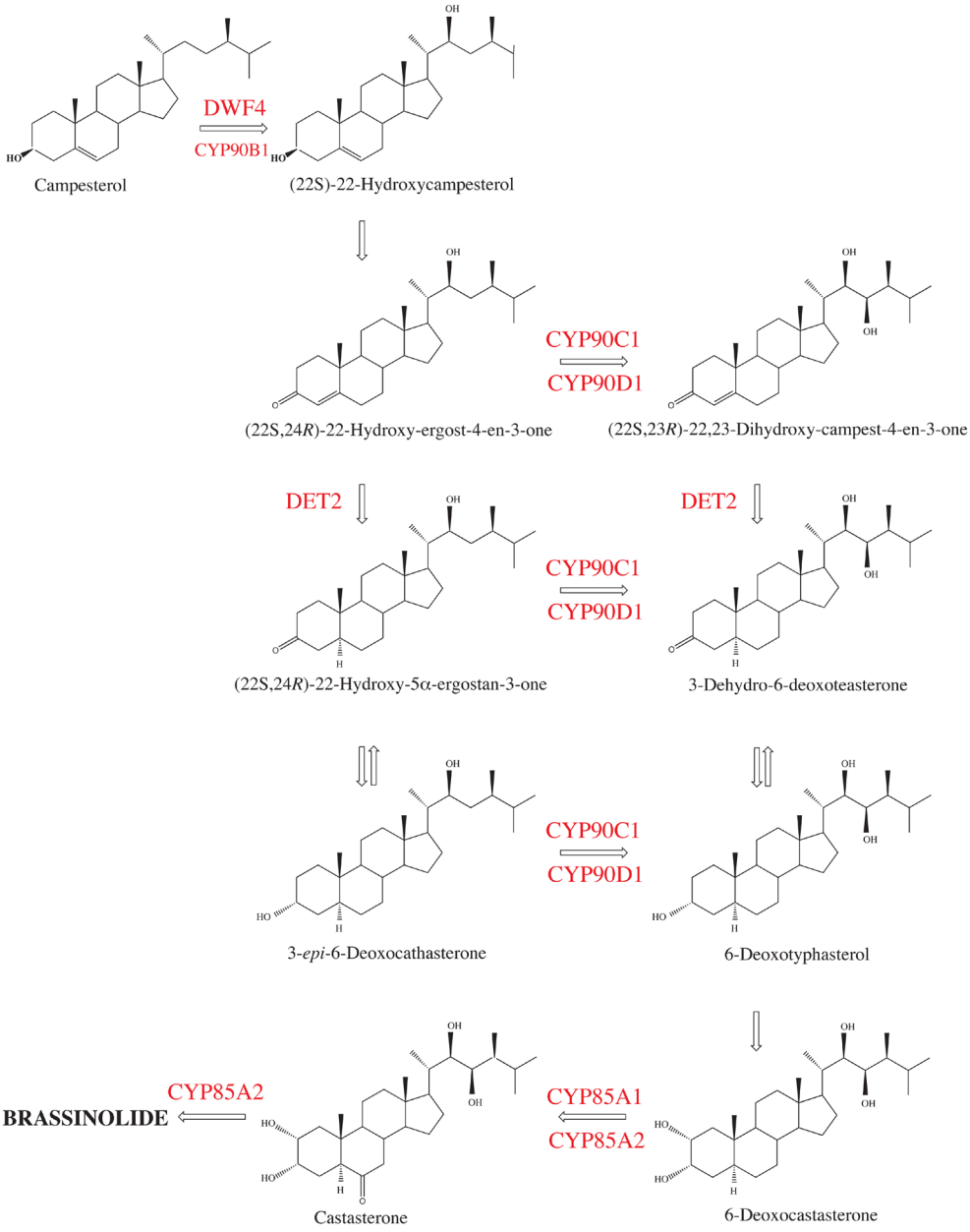


Figure 6. The revised "shortcut" pathway of BR biosynthesis from campesterol to BL beginning with direct C-22 hydroxylation of campesterol by DWF4 (Early C-22 Hydroxylation). CYP90C1 and CYP90D1 act as redundant C-23 steroid hydroxylases to convert several intermediates directly to 3-dehydro-6-deoxoteasterone and 6-deoxotyphasterol, thus bypassing campestanol, 6-deoxocathasterone and 6-deoxoteasterone. Biochemical experiments *in vitro* coupled with measurement of endogenous BRs suggest that this is a prominent pathway in Arabidopsis.

hibitor brassinazole (BRZ) is a triazole derivative which inhibits cytochrome P450 enzymes. Treatment of Arabidopsis plants with BRZ results in a similar morphology to BR-deficient mutants, and these developmental defects are rescued by BR but not GA treatment (Asami and Yoshida, 1999; Nagata et al., 2000). A modified form of BRZ, termed BRZ2001, exhibits even higher specificity in bioassays than the parent compound (Sekimata et al., 2001). These reagents augment studies using BR-deficient mutants and provide a valuable tool for clarifying BR molecular mechanisms. Treatment of Arabidopsis seedlings with either BRZ or BL showed that BR homeostasis involved regulation of multiple genes involved in sterol and BR biosynthesis including DET2, DWF4, CPD, CYP90C1, BR6OX, FACKEL, DWF5, and DWF7 (Tanaka et al., 2005).

Metabolism

Most of the early studies on BR metabolism have been in species other than Arabidopsis (Adam et al., 1999; Bajguz, 2007). In cell suspension cultures of *Solanum lycopersicum,* 25-hydroxy-24-epibrassinolide and 25-β-D-glucosyloxy-24-epibrassinolide constituted the major radioactive BRs seven days after feeding with [^{3}H]-24-epibrassinolide (Schneider et al., 1994). Further studies with [^{3}H]-24-epicastasterone showed that tomato cells metabolized this BR not only to the 25-hydroxy form but also to 26-hydroxy-24-epicastasterone and 26-β-D-glucopyranosyloxy-24-epicastasterone, and the same system was used to show that direct glucosylation could occur at either of the hydroxyls in the A ring after epimerization (Hai et al., 1996). The use of specific inhibitors suggested that one cytochrome P-450 and another hydroxylase were involved in C25 and C26 hydroxylation (Winter et al., 1997). An Arabidopsis UDP glycosyltransferase termed UGT73C5 was found to catalyze the 23-O-glucosylation of BL and castasterone and overexpression of UGT73C5 resulted in BR-deficient phenotypes, suggesting that glucosylation also regulates BR activity in Arabidopsis (Poppenberger et al., 2005).

Besides glucosylated forms, BR conjugation with a variety of fatty acid esters has been reported. Teasterone 3-laurate and teasterone 3-myristate were identified in lily pollen and studies of endogenous BR levels during pollen development suggested that conjugated teasterone may be a storage form which releases teasterone during pollen maturation to allow the biosynthesis of BL (Asakawa et al., 1996). Sulfotransferases from *B. napus* and Arabidopsis catalyze the O-sulfonation of BRs, leading to a loss of their biological activity in the bean second internode assay (Rouleau et al., 1999; Marsolais et al., 2007). This suggests sulfonation is an additional mode of BR inactivation but the implications of this have not yet been examined *in planta*.

Another important role for BR metabolism in regulating Arabidopsis growth and development has been suggested by the work of (Neff et al., 1999). The *bas*1 mutant was identified in an activation-tagging screen for the ability to suppress the long hypocotyl phenotype caused by mutations in the phytochrome B photoreceptor. The *bas*1 mutant is BR-deficient and accumulates 26-OH-BL, a proposed inactive metabolite of BL. The molecular basis of this event is the overexpression of the *BAS*1 gene, which encodes a cytochrome P450 enzyme capable of hydroxylating BR to 26-OH-BL. Thus, BAS1 and related P450s may be important regulatory proteins in controlling endogenous levels of active BL in Arabidopsis (Turk et al., 2003; Turk et al., 2005; Thornton et al., 2010). A discussion of possible factors regulating BR metabolism is available in a recent review (Hategan et al., 2011).

BR SIGNAL TRANSDUCTION

Screening for BR-Insensitive Mutants Identifies the BR Receptor

Early work on BR signal transduction focused on a single mutant, *brassinosteroid insensitive* 1 (*bri*1) which affects a gene encoding a membrane-bound leucine-rich repeat receptor-like kinase (LRR RLK) (Clouse et al., 1996; Kauschmann et al., 1996; Li and Chory, 1997). Based on the observation that most plant hormones at the appropriate concentration can inhibit primary root elongation in Arabidopsis, *bri*1 was first identified in a screen of 70,000 EMS-mutagenized seedlings by its ability to elongate roots in the presence of 0.1 μM 24-epiBL (Clouse et al., 1993; Clouse et al., 1996). The *bri*1 null allele is among the most severe of BR mutant phenotypes and exhibits extreme dwarfism, dark green downward curling leaves, male sterility, delayed flowering, altered vascular morphology and reduced apical dominance, particularly in older plants (Figure 7). The insensitivity of *bri*1 to inhibition of root elongation extends over a wide range of BR concentrations and is highly specific. The *bri*1 mutant retains sensitivity to auxins, cytokinins, GA, and ethylene, and shows hypersensitivity to ABA (Clouse et al., 1996).

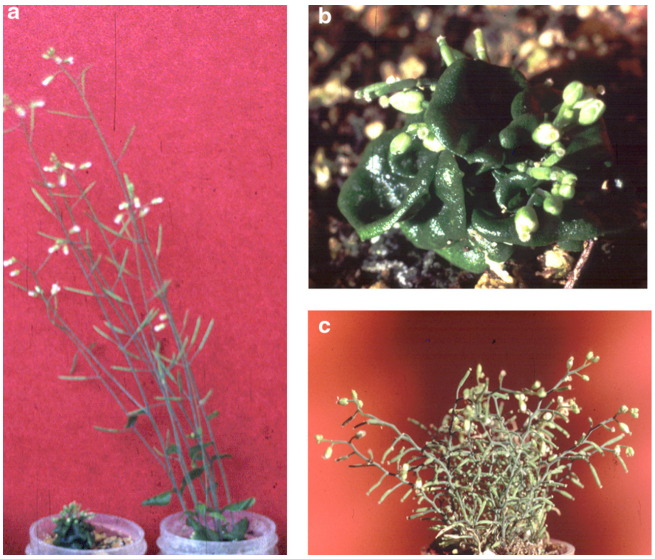


Figure 7. Phenotype of the *bri1* mutant. Panel A shows 2-month-old mutant (left) and wild type (right) plants grown in a 23 C growth chamber (16 hr light / 8 hr dark). Panel B shows a close-up view of a 2-month-old *bri1* mutant plant. Panel C shows the same plant after 4 months. Adapted with permission from figure 1 of Clouse et al. (1996).

Numerous other *bri1* alleles (cataloged in Table 2) have been identified in a variety of independent screens (Kauschmann et al., 1996; Li and Chory, 1997; Noguchi et al., 1999b; Friedrichsen et al., 2000; Bouquin et al., 2001; Jin et al., 2007; Xu et al., 2008; Shang et al., 2011). BR-insensitive mutants have also been identified in BRI1 orthologs from numerous crop species including tomato, rice, barley, and pea (Koka et al., 2000; Yamamuro et al., 2000; Montoya et al., 2002; Chono et al., 2003; Nomura et al., 2003; Holton et al., 2007). Mutational analyses in all of these species have shown conclusively that the BRI1 receptor is required for normal BR perception and plant growth.

BRI1 is an LRR RLK required for BR Perception

The *BRI1* gene was first isolated by positional cloning and verified by sequencing numerous mutant alleles (Li and Chory, 1997). The predicted protein is an 1196 amino acid LRR RLK that con-

tains the three major domains common to both animal and plant receptor kinases; the extracellular ligand-binding domain, the single-pass transmembrane domain and the cytoplasmic kinase domain. The extracellular domain contains 25 tandem copies of a 24-amino acid LRR with a unique 70 amino acid island between LRR 21 and 22 that has been shown by mutant analysis to be critical for BRI1 function. Downstream of the LRR region there is a transmembrane domain spanning amino acids 792-814 followed by a cytoplasmic juxtamembrane (JM) region from amino acids 815-882. Based on sequence alignments with many known kinases (Hanks et al., 1988), the region from Phe-883 to Phe-1155 comprises the active BRI1 kinase domain (KD), and all invariant amino acid residues and the twelve conserved kinase sub-domains are clearly present. Lastly, amino acids 1156-1196 represent the carboxy-terminal (CT) segment of the cytoplasmic domain (Vert et al., 2005).

BRI1 is localized in both the plasma membrane and early endosomes and is ubiquitously expressed in all organs of young, growing Arabidopsis plants (Friedrichsen et al., 2000; Geldner et al., 2007). Initial evidence that BRI1 was the BR receptor came from biochemical approaches using radiolabeled BL and transgenic plants overexpressing BRI1-GFP fusions which demonstrated that BL-binding activity was precipitated by antibodies to GFP, was competitively inhibited by active, but not by inactive, BRs, and was of an affinity (K_d = 7.4 +/- 0.9 nM) consistent with known physiological concentrations of BR required to regulate in planta responses (Wang et al., 2001). Subsequent experiments using a photo affinity labeled BR analog showed that BR binds directly to BRI1 through the 70 amino acid island domain in conjunction with the adjacent C-terminal LRR 22, thus defining a novel 94 amino acid steroid-binding motif distinctly different in structure from steroid binding sequences in animals (Kinoshita et al., 2005).

The three dimensional structure of the extracellular domain of BRI1, including the steroid binding motif, was recently solved independently in two different laboratories using x-ray diffraction of crystallized BRI1 extracellular domain derived from recombinant protein expressed in insect cells (Hothorn et al., 2011; She et al., 2011). Unlike the horseshoe structure of the LRR containing Toll-like receptors in animals, BRI1 forms a superhelix of twisted LRRs with the island domain folding into the interior of the superhelix to form a binding pocket for one molecule of BL per BRI1 monomer. BL binding likely generates a docking platform for a co-receptor, resulting in activation of the BR signaling pathway (Hothorn et al., 2011; She et al., 2011).

BRI1 Phosphorylation and Interacting Partners

Hundreds of putative receptor kinases exist in Arabidopsis and several have been shown to function in diverse physiological processes including growth and development, embryogenesis, fertilization, abscission, disease resistance and light-mediated responses (Shiu and Bleecker, 2001; Torii, 2004). Such functional diversity is likely generated by a wide range of ligands that bind to cognate extracellular domains of the receptor kinases. Mechanisms for signal transduction pathway-specific cytoplasmic components to bind to intracellular domains of receptor kinases in response to ligand binding are also required.

Table 2. *bri*1 mutant alleles in Arabidopsis

Allele	Alias	Predicted Mutation	References
bri 1-1		Ala-909-Thr	Clouse et al., 1996; Friedrichsen et al., 2000
*bri*1-2	*cbb*2	Premature Stop	Kauschmann et al., 1996; Clouse, unpublished
*bri*1-3	*dwf*2-32	Premature Stop	Noguchi et al., 1999
*bri*1-4	*dwf*2-2074	Premature Stop	Noguchi et al., 1999
*bri*1-5	*dwf*2-w41	Cys-69-Tyr	Noguchi et al., 1999
*bri*1-6, 119	*dwf*2-399	Gly-644-Asp	Friedrichsen et al., 2000; Noguchi et al., 1999
*bri*1-7	*dwf*2-WM3-2	Gly-613-Ser	Noguchi et al., 1999
*bri*1-8	*dwf*2-WM6-2	Arg-983-Asn	Noguchi et al., 1999
*bri*1-9	*dwf*2-WMB19	Ser-662-Phe	Noguchi et al., 1999
*bri*1-101	*bin*1-1	Glu-1078-Lys	Li and Chory, 1997
*bri*1-102	*bin*1-2	Thr-750-Ileu	Friedrichsen et al., 2000
*bri*1-103, 104	*bin*1-3, 4	Ala-1031-Thr	Friedrichsen et al., 2000; Li and Chory, 1997
*bri*1-105-107	*bin*1-5-7	Gln-1059-Stop	Friedrichsen et al., 2000; Li and Chory, 1997
*bri*1-108-112	*bin*1-8-12	Arg-983-Gln	Friedrichsen et al., 2000
*bri*1-113	*bin*1-13	Gly-611-Glu	Li and Chory, 1997
*bri*1-114, 116	*bin*1-14, 16	Gln-583-Stop	Friedrichsen et al., 2000
*bri*1-115	*bin*1-15	Gly-1048-Asp	Li and Chory, 1997
*bri*1-117, 118	*bin*1-17, 18	Asp-1139-Asn	Friedrichsen et al., 2000
bri1-120		Ser-399-Phe	Shang et al., 2011
bri1-201		Premature Stop	Bouquin et al., 2001
bri1-301		G-989-I	Xu et al., 2008

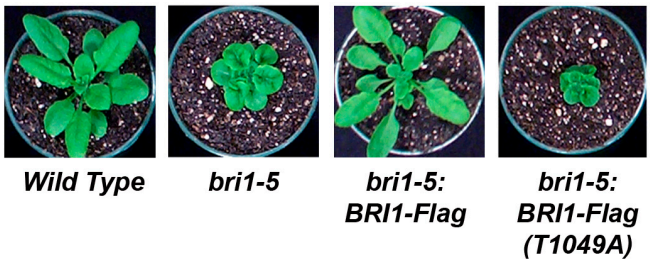


Figure 8. Functional analysis of BRI1 phosphorylation sites. The *bri1-5* receptor kinase mutant is a weak allele with semi-dwarf phenotype, altered leaf structure and shortened petioles. Expression of wild-type BRI1-Flag in *bri1-5* rescues the mutant phenotype, while expression of a mutant construct in which the critical Thr-1049 kinase domain activation loop phosphorylation site is substituted with Ala, leads to a dominant negative effect with an extreme dwarf phenotype similar to *bri1* null mutants. Adapted from figure 8 of Wang et al. (2005b) and reproduced from figure 1 of Clouse (2011) with permission.

Reversible phosphorylation of specific Ser, Thr and Tyr residues leads to critical modulation of a large number of eukaryotic signaling pathways involving receptor kinases and most often results in a cascade of protein interactions and phosphorylation events that alter the activity of specific transcription factors, leading to altered expression of hundreds of genes (Rahimi and Leof, 2007; Lemmon and Schlessinger, 2010). Ligand binding by the extracellular domain leads to oligomerization with other receptor kinase partners and a consequent activation of the cytoplasmic kinase domain of the receptor, followed by generation of docking sites for specific downstream substrates of the receptor kinase via specific phosphorylation events. Phosphorylation of these downstream substrates by the receptor kinase may alter protein stability, subcellular localization, interactions with other proteins and/or other critical protein functions, generating a phosphorelay in which initial perception of ligand by the receptor kinase extracellular domain results in transduction of the signal to the nucleus, yielding a ligand-dependent cellular response. The preponderance of evidence from several groups suggests BRI1 also follows this general paradigm of receptor kinase action (reviewed in (Kim and Wang, 2010; Clouse, 2011)).

BR initiates a cascade of cellular events by binding directly to the extracellular domain of BRI1, which leads to phosphorylation and activation of the BRI1 cytoplasmic domain and transduction of the signal via a phosphorelay through an intracellular kinase to the nucleus, where several novel BR-responsive transcription factors alter the expression of genes promoting cell elongation, division, and differentiation (Li and Nam, 2002; He et al., 2005; Kinoshita et al., 2005; Yin et al., 2005; Vert and Chory, 2006). Biochemical studies involving mass spectrometry have confirmed that BRI1 is an active kinase which autophosphorylates on specific Ser and Thr residues in the JM, KD and CT domains, while genetic analysis has demonstrated the functional significance of several of these residues in vivo including the highly conserved kinase activation loop residue T1049 (Figure 8), which is essential for BRI1 kinase function and BR signal transduction (Oh et al., 2000; Wang et al., 2005b; Wang et al., 2008). While the majority of genetic and biochemical studies have demonstrated a critical role for BRI1 kinase activity in BR signaling, the *bri1-301* allele is a kinase domain mutant that retains partial in vivo function and exhibits no in vitro kinase activity under the conditions used in the study (Xu et al., 2008). It cannot be excluded, however, that a minimal in vivo kinase activity of *bri1-301* might exists and the mechanism of the partial activity of *bri1-301* is currently unknown.

Based on sequence alignment of the kinase domain, BRI1 fits the canonical Ser/Thr kinase classification (Li and Chory, 1997). However, recent experimental evidence clearly shows that BRI1 can autophosphorylate on Tyr residues as well and that Tyr phosphorylation plays an important role in BR signaling in planta, suggesting that BRI1 is a dual function kinase (Oh et al., 2009a; Oh et al., 2009b).

BRI1 crystal studies in vitro did not provide evidence for homodimerization (Hothorn et al., 2011; She et al., 2011), but several lines of in vivo evidence suggest BRI1 can form ligand-independent homodimers in plant membranes (Russinova et al., 2004; Wang et al., 2005a; Hink et al., 2008). While the role of this homodimerization in initial activation of BRI1 is not currently clear, it has been demonstrated that full expression of BR signaling requires heterooligomerization of BRI1 with members of the SOMATIC EMBROYGENESIS RECEPTOR KINASE (SERK) subfamily of LRR RLKs (Hecht et al., 2001). BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1), also known as SERK3, interacts both in vitro and in vivo with BRI1 (Li et al., 2002; Nam and Li, 2002; Russinova et al., 2004) and this physical association, as well as the in vivo phosphorylation of BRI1 and BAK1, are promoted by BR (Wang et al., 2005b; Wang et al., 2008). Additional SERK family members including SERK4, alternatively named BAK1-LIKE (BKK1), and SERK1 also interact with BRI1 in vivo and promote BR signaling (Karlova et al., 2006; He et al., 2007). Moreover, BAK1 interacts with additional LRR RLKs to promote their function in plant defense responses (Heese et al., 2007; Chinchilla et al., 2009; Bar et al., 2010; Postel et al., 2010; Schulze et al., 2010), suggesting BAK1 functions as a co-receptor in independent pathways by enhancing the signaling output of distinct LRR RLK partners that bind different ligands (Chinchilla et al., 2009; Li, 2010b; Jaillais et al., 2011a).

A combination of mass spectrometry studies, transgene expression analyses in various genetic backgrounds and in vitro biochemical assays showed that BAK1 stimulates BRI1 kinase activity and that both BRI1 and BAK1 can transphosphorylate each other on specific residues (Wang et al., 2008). As with BRI1, mass spectrometry analysis also identified multiple in vivo and in vitro phopshorylation sites for BAK1 and functional characterization of these sites revealed that the kinase activation loop residue T455, corresponding to BRI1 residue T1049, appears essential for BAK1 function and BR signaling in planta (Wang et al., 2005b; Wang et al., 2008). These studies clarified early events of BR signaling and allowed development of a sequential transphosphorylation model of BRI1/BAK1 interaction (Figure 9) that shows plant receptor kinases share some of the properties of mammalian receptor kinases while retaining unique plant-specific features (Wang et al., 2008). Similar to BRI1, BAK1 also autophosphorylates on Tyr residues which regulates a subset of BAK1 functions in vivo (Oh et al., 2010). Dephosphorylation of BRI1 via protein phosphatase 2A (PP2A) can down regulate BR signaling and this process itself may be BR regulated through induction of a leucine

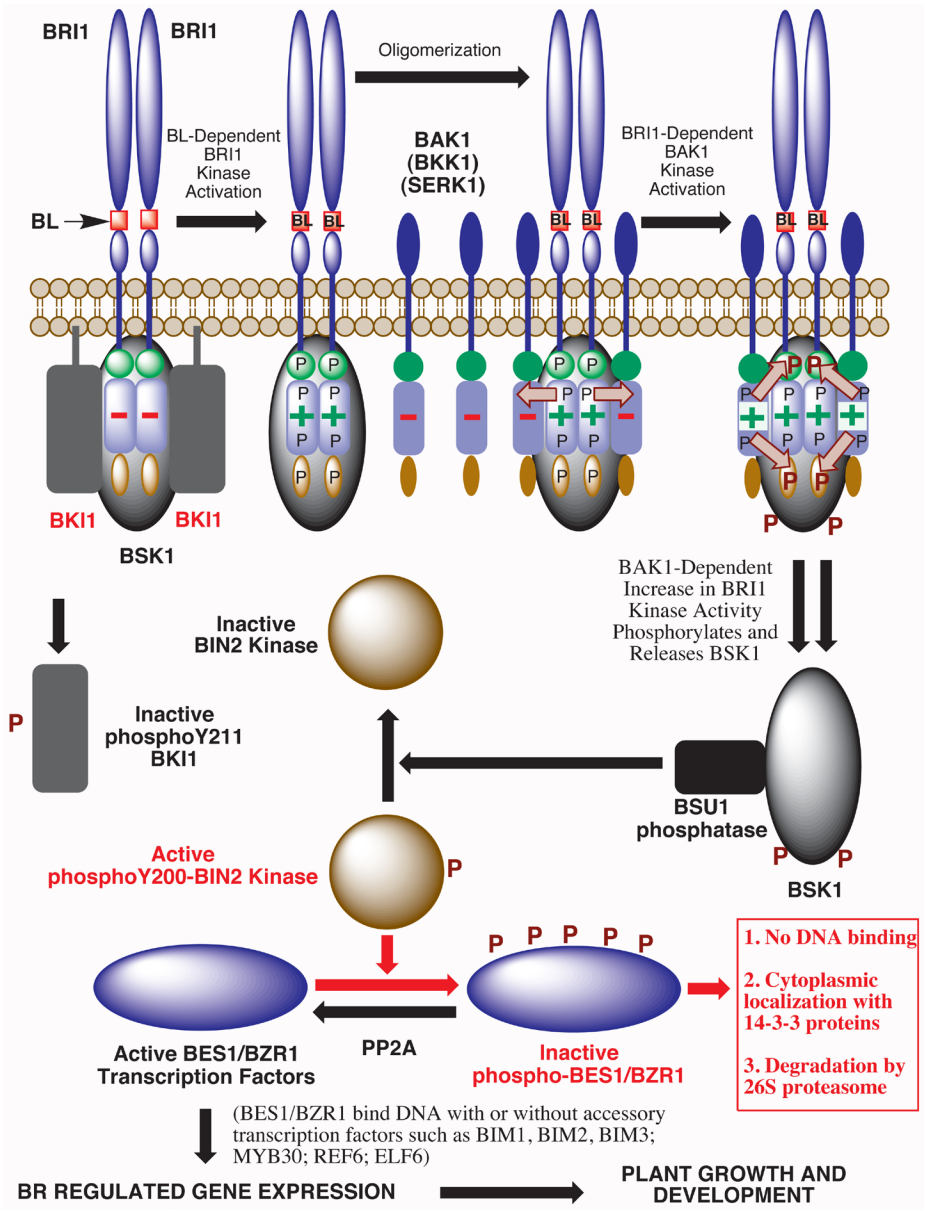


Figure 9. Current model of BR signal transduction. Brassinolide binds to the extracellular domain of the BRI1 receptor kinase, promoting BRI1 kinase activity. BRI1 then phosphorylates the negative regulator BKI1 on Y211, releasing it from the membrane and allowing BRI1 to associate with BAK1 or its homologs BKK1 and SERK1. BRI1 transphosphorylates BAK1 on KD residues and the activated BAK1 then transphosphorylates BRI1 on specific JM and CT residues, which enhances the signaling capacity of BRI1, leading to phosphorylation of BSK1 and its release from the receptor complex. Activated BSK1 associates with and activates BSU1, which dephosphorylates the BIN2 kinase on Y200, inactivating it. The unphosphorylated forms of BES1 and BZR1 transcription factors then accumulate (with aid of PP2A) and bind to the promoters of BR-regulated target genes, eliciting a specific physiological response such as cell elongation. Positive regulators of the pathway are shown in black, with negative regulators in red. For simplicity the nuclear or cytoplasmic localization of BSU1, BIN2, BES1 and BZR1 are not shown in this model. Reproduced with permission from figure 2 of Clouse (2011).

carboxymethyltransferase which methylates PP2A, facilitating its interaction with, and thus dephosphorylation of, BRI1 (Di Rubbo et al., 2011; Wu et al., 2011).

Several other proteins with critical roles in BR signaling that interact directly with BRI1 have now been identified. BKI1 (BRI1 KINASE INHIBITOR 1) is a negative regulator of BR signaling that is membrane associated in the absence of BR where it binds to BRI1 through a C-terminal 20 amino acid residue in BKI1, most likely inactivating BRI1 function by preventing association with the BAK1 co-receptor (Wang and Chory, 2006; Jaillais et al., 2011b). Several lines of in vitro and in vivo evidence suggest that BR treatment promotes BRI1 phosphorylation of BKI1 on Y211,

which causes dissociation of BKI1 from the membrane, thus allowing BRI1 to associate with BAK1 and initiate BR signaling (Jaillais et al., 2011b). This further suggests that in addition to autophosphorylation of BRI1 on Tyr residues (Oh et al., 2009b), BRI1 may also phosphorylate downstream substrates on Tyr, similar to animal RTKs (Lemmon and Schlessinger, 2010; Lim and Pawson, 2010).

A proteomic screen of BR-regulated proteins identified several members of the receptor-like cytoplasmic kinase subfamily RLCK-XII, named BR-SIGNALING KINASES (BSKs), as additional interactors of BRI1, which, in contrast to BKI1, are positive regulators of BR signaling (Tang et al., 2008b). BSK1 and BSK3 interact directly with BRI1 in vivo in the absence of ligand and in response to BR, BRI1 phosphorylates BSK1, most likely on Ser230, causing its activation and release from the receptor complex (Tang et al., 2008b). Phosphorylated BSK1 then interacts with BRI SUPPRESSOR 1 (BSU1) phosphatase (Mora-Garcia et al., 2004), promoting its interaction with the negative regulator BRASSINOSTEROID INSENSITIVE 2 (BIN2) as discussed more fully below (Kim et al., 2009). Recently, CONSTITUTIVE DIFFERENTIAL GROWTH 1 (CDG1) (Muto et al., 2004) and its homolog CDG LIKE 1 (CDL1), both members of the RLCK-VIIc subfamily of cytoplasmic kinases, were also shown to be BRI1 kinase domain substrates that activate BSU1 and its relatives to negatively affect BIN2 activity (Kim et al., 2011).

Arabidopsis TRANSTHYRETIN-LIKE (TTL) protein is a tetrameric, bifunctional enzyme with decarboxylase and hydrolase activity (Pessoa et al., 2010) that is phosphorylated by BRI1 in vitro and which appears to negatively regulate BR signaling in vivo (Nam and Li, 2004), and thus may be an additional BRI1 interacting partner. Another putative cytoplasmic substrate of BRI1 is the plant ortholog of mammalian Transforming Growth Factor-Beta Receptor Interacting Protein (TRIP-1) (Chen et al., 1995). Arabidopsis TRIP-1 is strongly phosphorylated on multiple residues by the BRI1 kinase domain *in vitro* and BRI1 and TRIP-1 co-immunoprecipitate from Arabidopsis plant extracts suggesting an *in vivo* interaction between the two proteins (Ehsan et al., 2005). Some of the morphological characteristics of transgenic lines expressing antisense TRIP-1 RNA are also consistent with a possible role for TRIP-1 in BR signaling, although this has not been definitively proven (Jiang and Clouse, 2001). TRIP-1 (also known as eIF3i) is a dual function protein that is an essential subunit of the eIF3 translation initiation complex in mammals, yeast and plants (Asano et al., 1997; Burks et al., 2001), raising the possibility that BR-dependent phosphorylation of TRIP-1 by BRI1 may affect eIF3 activity and/or assembly and thus impact protein translation in some manner, although this remains to be demonstrated by functional assays.

Downstream Components of BR Signal Transduction

A pivotal point of downstream regulation in BR signaling is the BIN2 kinase (Li and Nam, 2002), a negative regulator of the pathway with sequence similarity to Drosophila shaggy kinase and mammalian Glycogen Synthase Kinase 3 (GSK-3), which often function as negative regulators of signaling pathways controlling metabolism, cell fate determination and pattern formation (Kim and Kimmel, 2000). Arabidopsis BIN2 is a member of a small family of 10 related genes, several of which have also been implicated in BR signaling (De Rybel et al., 2009; Yan et al., 2009; Rozhon et al., 2010). BIN2 exerts its negative effect by phosphorylating specific residues in two closely related transcription factors, BRASSINAZOLE-RESISTANT1 (BZR1) (Wang et al., 2002; He et al., 2005) and BRI1-EMS SUPPRESSOR1 (BES1) (Yin et al., 2002; Yin et al., 2005), also known as BZR2 (Wang et al., 2002), which are both required for the regulation of a network of genes essential for BR response (Sun et al., 2010; Yu et al., 2011).

Evidence has been presented suggesting BIN2 is exclusively nuclear localized where its primary function is to phosphorylate the BES1 and BZR1 transcription factors, thereby inhibiting their ability to bind DNA and dimerize with other transcription factors (Vert and Chory, 2006). However, several other groups have demonstrated a BR-dependent nucleocytoplasmic shuttle in which BIN2 phosphorylation of BES1 and BZR1 results in 14-3-3 proteins binding directly to the phosphorylated transcription factors to enhance their nuclear export (Bai et al., 2007; Gampala et al., 2007; Ryu et al., 2007; Ryu et al., 2010a; Ryu et al., 2010b). An alternative model in which BIN2 phosphorylates BES1 and BZR1 primarily in the cytoplasm to enhance their cytoplasmic retention by binding 14-3-3 proteins has also been proposed (Bai et al., 2007; Gampala et al., 2007; Kim et al., 2009). BIN2 phosphorylation of BZR1 may also lead to proteasome-mediated degradation of the transcription factor (He et al., 2002). Whatever the mechanism, it is clear BIN2 negatively regulates BR signaling by phosphorylating, and thus inactivating, BES1 and BZR1, and all of the mechanisms may come into play depending on developmental stage, tissue type and BIN2 expression levels. The precise mechanism by which BIN2 phosphorylates BZR1 has been investigated, and in contrast to animal GSKs which require priming phosphorylation and/or scaffolding proteins (Harwood, 2001), BIN2 binds BZR1 directly through a 12 amino acid docking motif. Deletion of this BZR1 motif prevents BIN2-BZR1 interaction and in vivo phosphorylation of BZR1 and leads to the nuclear accumulation of BZR1-GFP in dark grown hypocotyls (Peng et al., 2010).

Until recently it was an open question how BRI1 activation by BR binding resulted in the release of the negative control of BIN2 on the pathway. It is now proposed that activated BRI1 phosphorylates BSK1 on Ser230, allowing its release from the receptor complex and promoting its association with the BSU1 phosphatase. This activates BSU1 by an unknown mechanism, which allows BSU1 to inactivate BIN2 by dephosphorylating phosphoTyr 200 (Kim et al., 2009). Moreover, BRI1 also phosphorylates CDG1 on Ser234, activating the kinase and allowing it to phosphorylate BSU1 on Ser764, which enhances the ability of BSU1 to dephosphorylate pY200 of BIN2, resulting in its inactivation (Kim et al., 2011). Another BR-regulated mechanism of BIN2 inactivation, involving proteasome-mediated degradation of BIN2 has also been proposed (Peng et al., 2008). With BIN2 inactive, the hypophosphorylated forms of BES1 and BZR1 accumulate in the nucleus where they regulate the expression of hundreds of genes required for various cellular alterations known to be induced by BR. This is aided by a cytoplasmic protein phosphatase 2A (PP2A) that is responsible for dephosphorylating BZR1 and BZR2/BES1, thus increasing the active form of these transcription factors and promoting BR signaling (Tang et al., 2011).

The PP2A holoenzyme is composed of a scaffolding subunit A, a regulatory subunit B, and a catalytic subunit C (Janssens

et al., 2008), and the PP2A B' subunit binds directly to the proline-, glutamic-acid-, serine- and threonine-rich (PEST) domain of BZR1 (Tang et al., 2011). The PEST domain is essential for both PP2A binding in vivo and for BL-dependent dephoshphorylation of BZR1 sites phosphorylated by BIN2, including Ser-173, the critical 14-3-3 binding residue. PP2A mutants showed reduced BR signaling and in some cases a typical BR-dwarf phenotype, which in conjunction with the biochemical data, suggest that PP2A is a positive regulator of BR signaling that acts by dephosphorylating BZR1, allowing the unphosphorylated and active form of BZR1 (and BZR2/BES1) to accumulate and initiate BR-regulated gene expression (Tang et al., 2011).

Gene Regulation and Transcriptional Networks in BR Signaling

The majority of BR-regulated changes in cellular function appear to be driven by altered gene expression. Mutant analysis combined with microarray studies revealed that both BZR1 and BES1 regulate the expression of hundreds of genes in a BR-dependent manner (He et al., 2005; Yin et al., 2005). In response to BR activation, these transcription factors directly bind to the promoters of many of these genes at defined target sequences and the specificity and strength of promoter binding and transcriptional activation can be modulated by association with other transcription factors including those in the basic helix-loop-helix (bHLH), MYB, Interact-With-Spt6 (IWS), and Jumonji N/C domain families of nuclear proteins (Yin et al., 2005; Yu et al., 2008; Li et al., 2009; Li et al., 2010).

Besides dimerization with other transcription factors, BES1 and BZR1 regulate the expression of numerous transcription factors by binding directly to their gene promoters, thus amplifying the BR-regulated transcriptional cascade. A small family of atypical bHLH proteins containing six proteins named PACLOBUTRAZOL RESISTANT (PRE) 1 – 6, that were originally identified as positive regulators of GA signaling (Lee et al., 2006), have now been shown to also be positive regulators of BR signaling (Wang et al., 2009; Zhang et al., 2009). Overexpression of PRE1 in Arabidopsis results in increased cell elongation and BR sensitivity and PRE1 is a direct target of BZR1, with PRE1 RNA levels being enhanced by BZR1 promoter binding, as would be expected from the positive role of PRE1 in BR signaling. Dimerization with a classical bHLH protein enhances DNA binding to the E-box (CANNTG) promoter element in target genes while dimerization with an atypical, non-DNA binding bHLH, inhibits DNA binding of the partner as well (Toledo-Ortiz et al., 2003). PRE1 is thought to act by dimerizing with a classical bHLH protein, IBH1 (ILI1 Binding bHLH Protein1), which is a negative regulator of BR signaling. PRE1 association with IBH1 likely inhibits DNA binding of the negative regulator, releasing inhibition of BR signaling (Zhang et al., 2009). PRE1 is expressed at high levels in young growing tissue, while IBH1 is more highly expressed in mature organs that have ceased growth, suggesting these two BR-regulated antagonistic factors provide a growth regulating mechanism that enhances cell elongation in immature tissue that is highly responsive to BR while arresting growth in mature tissue. Interestingly, IBH1 is also a direct target of BZR1 with IBH1 RNA levels being repressed by BZR1 promoter binding (Zhang et al., 2009).

Another member of the atypical bHLH family, PRE3, which has been alternatively named ACTIVATION-TAGGED BRI1-SUPPRESSOR1 (ATBS1), is also a positive regulator of BR signaling that rescues multiple aspects of the *bri1-301* mutant allele including cell expansion, leaf shape and flowering time (Wang et al., 2009). ATBS1 interacts with all four members of another family of atypical bHLH proteins termed ATBS1 INTERACTING FACTOR (AIF) 1 – 4, and overexpression of AIF1 abolishes the suppression of *bri1-301* by ATBS1 indicating that AIF1 is a negative regulator of BR signaling (Wang et al., 2009). PRE3/ATBS1 is also a direct target of the auxin-dependent transcription factor MONOPTEROS (MP), required for embryonic root initiation and specification (Schlereth et al., 2010), and additional PRE family members are involved in light signaling pathways and photomorphogenesis (Hyun and Lee, 2006; Mara et al., 2010). Thus, this small family of atypical bHLH proteins appears to play essential roles in modulating plant growth and morphogenesis by participating in at least three hormone signaling pathways as well as light regulated growth (Li, 2010a).

Two recent independent studies employing chromatin-immunoprecipitation microarray (ChIP-chip) experiments have demonstrated the range of genes that are direct targets of BES1 and BZR1 and have uncovered extensive gene regulatory networks that modulate BR-promoted growth in Arabidopsis, perhaps through heterodimerization of BES1 and BZR1 with different factors that enhance or repress binding to specific promoters of genes involved in a particular physiological response (Sun et al., 2010; Yu et al., 2011). The function of these direct BZR1 and BES1 gene targets confirm the role of BR signaling in known BR-regulated responses such as cell elongation and division and include several genes involved in the biosynthesis, transport or signaling of auxin, GA, abscisic acid, ethylene, cytokinin and jasmonate, suggesting that besides BR-specific target genes, BR signaling affects the activity of most other plant hormones in a variety of ways that may lead to integration of hormone signals in normal growth and development (Sun et al., 2010; Yu et al., 2011).

In summary, a total of 953 BR-regulated genes were shown by ChIP-chip analysis to be direct targets of BZR1 with 450 being activated, 462 repressed, and 41 affected in complex ways by BZR1 binding to their promoters (Sun et al., 2010). For BES1, 250 direct targets that were also regulated by BR treatment were identified, with 165 being activated and 85 repressed by BES1 binding (Yu et al., 2011). Comparison of the BRZ1 and BES1 target lists shows an overlap of 120 genes, indicating that BZR1 and BES1 can regulate the same individual gene by binding to its promoter. Moreover, it is clear that both BES1 and BZR1 can function as either transcriptional activators or repressors, depending on the specific target gene promoter and dimerization partner.

MODES OF BR ACTION IN ARABIDOPSIS

Cell Elongation

The dwarf stature of BR-deficient mutants and the specific ability of BRs to rescue the dwarf phenotype, provides convincing genetic evidence that BRs are essential for normal plant growth. Examination of cell files in wild-type Arabidopsis and *cbb*, *dwf*4, *cpd* and *dim* mutant plants by light and electron microscopy has

provided direct physical evidence that longitudinal cell expansion is greatly reduced in BR mutants (Takahashi et al., 1995; Kauschmann et al., 1996; Szekeres et al., 1996; Azpiroz et al., 1998). Cell expansion, critical for growth and differentiation in all plant organs, is controlled by coordinated alterations in wall mechanical properties, cell hydraulics, biochemical processes and gene expression (Cosgrove, 1997). The primary cell walls in dicotyledenous and non-Poaceae monoctoyledenous plants are thought to consist of cellulose microfibrils tethered into a network by non-covalent attachment to hemicelluloses (primarily xyloglucans) which, in turn, are embedded in a pectic gel matrix (Carpita and Gibeaut, 1993). In order for turgor-driven cell expansion to proceed, the cell wall must transiently yield by slippage or breakage of the hemicellulose tethers, accompanied by incorporation of new wall polymers to prevent thinning and weakening of the walls. Regulation of the synthesis and activity of wall-modifying enzymes such as xyloglucan endotransglycosylase/hydrolases (XTHs), glucanases, expansins, sucrose synthase and cellulose synthase, becomes an obvious target for hormones involved in cell elongation.

In support of this model, BR regulation of genes encoding XTHs and expansins has been demonstrated in soybean, tomato and Arabidopsis (Clouse, 1997) and BRs have been shown by biophysical measurements to promote wall loosening in soybean epicotyls (Zurek et al., 1994) and hypocotyls of *Brassica chinensis* and *Cucurbita maxima* (Wang et al., 1993; Tominaga et al., 1994). Promotion of cell expansion through BR-regulated expression of genes involved in cell wall modifications, cellulose biosynthesis, ion and water transport, and cytoskeleton rearrangements has been demonstrated in genetic studies and global microarray analyses (Clouse and Sasse, 1998; Vert et al., 2005; Kim and Wang, 2010), and many of the genes in this classification have also been shown to be direct targets of the BZR1 and BES1 transcription factors (Guo et al., 2009; Sun et al., 2010; Xie et al., 2011; Yu et al., 2011), directly linking BR binding by the BRI1 receptor to altered expression of genes essential for cell elongation via BR signal transduction.

The arrangement of cortical microtubules is known to be important in determining the orientation of cell expansion and BRs have been shown to affect re-configuration of microtubules to transverse orientation to allow longitudinal growth (Mayumi and Shibaoka, 1995). Interestingly, in the *dim* mutant there is reduced expression of a specific tubulin gene thought to be involved in cell elongation (Takahashi et al., 1995). However, it has also been shown using immunofluorescence of α-tubulin in a BR-deficient mutant that BRs can promote microtubule organization and cell elongation directly without increases in tubulin gene expression (Catterou et al., 2001b). DEVELOPMENTALLY REGULATED PLASMAMEMBRANE POLYPEPTIDE (DREPP) was first identified as a BR-regulated protein in proteomic studies (Tang et al., 2008a) and the *DREPP* gene was later confirmed to be a BR-induced direct target of BZR1 (Sun et al., 2010). A gene with sequence similarity to DREPP encodes a microtubule-associated protein functioning in directional cell growth (Wang et al., 2007), suggesting that DREPP might also be involved in BR-mediated microtubule reorganization. Overexpression of DREPP enhances cell elongation in a BR-deficient mutant, indicating DREPP is a positive regulator of BR response (Sun et al., 2010).

Besides alterations in cell wall properties, BRs may also affect transport of water via aquaporins and the activity of a vacuolar H^+-ATPase, both of which are associated with cell elongation (Schumacher et al., 1999; Morillon et al., 2001). Recent studies combining fluorescence lifetime spectroscopy, confocal microscopy and electrophysiology suggest that BR promotes membrane hyperpolarization and rapid cell elongation by the BR-modulated interaction of BRI1 and an ATPase in the plasma membrane (Caesar et al., 2011).

Cell Division

Early work using the bean second internode bioassay suggested that BRs affected cell division as well as elongation (Steffens, 1991). BRs have been shown to stimulate cell division (in the presence of auxin and cytokinin) in cultured parenchyma cells of *Helianthus tuberosus* (Clouse and Zurek, 1991), and in protoplasts of Chinese cabbage and petunia (Nakajima et al., 1996; Oh and Clouse, 1998). BRs increased the mitotic index in onion root tip cells (Howell et al., 2007) and promoted cell proliferation in tobacco BY-2 cell suspension cultures (Miyazawa et al., 2003). Slower rates of cell division were observed in the *dwf7-1* mutant and results of callus induction, shoot regeneration, flow cytometry and gene expression studies supported a role for BRs in Arabidopsis cell division (Cheon et al., 2010). A promotive role for BRs in Arabidopsis cell division was also implicated by the finding that 24-epiBL treatment of *det2* cell suspension cultures increased transcript levels of the gene encoding cyclinD3 (CycD3), a protein involved in the regulation of G1/S transition in the cell cycle. CycD3 is also regulated by cytokinins and it is interesting to note that 24-epiBL could effectively substitute for zeatin in the growth of Arabidopsis callus and cell suspension cultures (Hu et al., 2000). ChIP-chip analysis further demonstrated that CycD3, in addition to other cyclins and cyclin-dependent kinases, were BR-regulated genes and direct targets of BZR1 (Sun et al., 2010). An additional connection between CycD3 expression, cell division and BR signaling was provided in a recent study demonstrating that BR mutants have reduced root meristem size and altered expression of specific cell cycle markers, which can be rescued by over expression of CycD3 (Gonzalez-Garcia et al., 2011).

Cell Differentiation

Besides the well-known role of auxins and cytokinins in vascular differentiation, a good deal of accumulated evidence supports the role of BRs in this process. Nanomolar levels of BL stimulate tracheid formation in *H. tuberosus* explants and isolated mesophyll cells of *Zinnia elegans,* the two primary model systems for study of xylogenesis (Clouse and Zurek, 1991; Iwasaki and Shibaoka, 1991). In the Zinnia system, BRs also regulate the expression of several genes associated with xylem formation (Fukuda, 1997). High levels of *BRU1* expression in paratracheary parenchyma cells surrounding vessel elements in soybean epicotyls also suggests a role for BRs, and XETs, in xylem formation (Oh et al., 1998), and it is relevant that BRs have been identified in cambial scrapings of *Pinus silvestris* (Kim et al., 1990). In Arabidopsis, it is again the microscopic analysis of BR mutants that has shown a role for BRs in vascular differentiation in this species. The BR-deficient mutant *cpd* exhibits unequal division of the cambium, producing extranumerary phloem cell files at the expense of xylem cells (Szekeres

et al., 1996). The sterol and BR-deficient mutant *dwf7* shows the same increase in phloem vs. xylem cells and the number of vascular bundles is reduced from eight in the wildtype to six in the mutant. Furthermore, the spacing between vascular bundles is irregular and two vascular bundles can be joined without a separating layer of parenchyma cells (Choe et al., 1999a). Further work in Arabidopsis demonstrated that BRs modulate vascular bundle number and together with polar auxin transport, determine the radial pattern of vascular bundles in shoots (Ibanes et al., 2009).

BRs appear to regulate the differentiation of procambial cells into xlyem elements in part through transcriptional regulation of genes required for this process, including HD-ZIP transcription factors (Ohashi-Ito and Fukuda, 2003; Fukuda, 2004; Ohashi-Ito et al., 2005; Ohashi-Ito and Fukuda, 2010). The BRI1 receptor kinase and its close BR-binding relatives, BRL1 and BRL2, which are expressed in vascular tissue, are required for this process (Cano-Delgado et al., 2004; Fukuda, 2004). BR biosynthesis increases dramatically in procambial cells prior to tracheary element differentiation in Zinnia cells (Yamamoto et al., 2001; Yamamoto et al., 2007), and BRs may also promote autophagy during programmed cell death in differentiating tracheary elements through transcriptional regulation of a small GTP-binding protein RabG3b (Kwon et al., 2010).

Reproductive Biology and Senescence

Reduced fertility or male sterility is a common characteristic of most BR-deficient and insensitive mutants. Pollen is a rich source of endogenous BRs and in vitro studies in *Prunus avium* have suggested that pollen tube elongation could depend in part on BRs (Hewitt et al., 1985). Also, pollination is often the initial step for the genesis of haploid plants, and in both Arabidopsis and *Brassica juncea*, treatment with BL induced the formation of haploid seeds which developed into stable plants (Kitani, 1994). In support of the view that BRs are important elements in male fertility, it was suggested that the male sterility observed in the *cpd* mutant was due to the inability of pollen to elongate during germination (Szekeres et al., 1996). However, in *dwf*4 the pollen appears to be viable and sterility is due to the reduced length of stamen filaments which results in the deposition of pollen (Choe et al., 1998). A recent study combining microscopic analysis with transcript profiling demonstrated that BES1 binds directly to the promoters of several genes that regulate anther and pollen development and that these genes have altered expression in BR mutants, leading to observable defects in anther and pollen morphology (Ye et al., 2010).

Interestingly, *dwf*5-1, the only BR mutant with wild-type fertility, is also the only mutant with stamens longer than the gynoecium (Choe et al., 2000). Despite its increased fertility, *dwf*5-1 has seeds that do not develop normally and that require exogenous BR application for full germination. While no other BR mutant requires BR in the germination medium, a possible endogenous role for BRs in Arabidopsis seed germination has been proposed. ABA and GA play antagonistic roles in establishing and breaking dormancy during seed development and germination. It was found that BRs can rescue the germination defect in GA biosynthetic and insensitive mutants, and that the BR mutants *det*2 and *bri*1 are more susceptible to inhibition of germination by ABA than wildtype. Thus it was concluded that BR signaling may be required to reverse ABA-induced dormancy and to stimulate germination (Steber and McCourt, 2001). Genetic studies have indicated a specific protein in ABA signaling that may also be a target of BR action in regulating germination (Xi and Yu, 2010).

Arabidopsis BR deficient and insensitive mutants exhibit delayed flowering, suggesting an additional role for BRs in regulating the timing of floral initiation (Chory et al., 1991; Li and Chory, 1997; Azpiroz et al., 1998). BRI1-dependent BR signaling appears to promote flowering by repressing the expression of FLOWERING LOCUS C (FLC), a quantitative repressor of flowering (Domagalska et al., 2007). A possible mechanism for this BR-mediated inhibition of flowering may involve RELATIVE OF EARLY FLOWERING 6 (REF6), a Jumonji N/C domain-containing transcriptional regulator that functions in chromatin modification and directly interacts with BES1 (Yu et al., 2008). REF6 is a repressor of FLC and *ref6* mutants accumulate FLC transcripts, which delays flowering. It is possible that BR regulates FLC expression through a BES1/REF6 dimer, but this requires experimental confirmation (Clouse, 2008a). Further experiments employing BR and GA deficient double mutants and Arabidopsis plants overexpressing key BR and GA biosynthetic enzymes, also suggest an interaction between BR and GA in regulating time of flowering (Domagalska et al., 2010).

The Arabidopsis *SEUSS* gene encodes a transcriptional adaptor protein involved in floral organ identity and the development of the carpel margin meristem (Bao et al., 2010). A novel role for BRs in gynoecium and ovule development has now been uncovered through genetic screens which identified CYP85A2, a cytochrome P450 involved in BR biosynthesis, as a suppressor of mutations in *SEUSS* (Nole-Wilson et al., 2010). Studies of CYP85A1, another cytochrome P450 step in BR biosynthesis, implicates BRs in megagametogenesis in Arabidopsis (Perez-Espana et al., 2011).

Besides reduced fertility, most of the BR mutants also exhibit an extended life span and delayed senescence. While a typical wild-type Arabidopsis plant senesces after appoximately 60 days, BR mutants can remain green and initiating new flowers well after 100 days. The extent of the delayed senescence is correlated with reduced fertility, with the sterile mutants such as *bri*1 having the most delayed development. It has been proposed that the inability to produce signals for the onset of senescence in the infertile mutants leads to the observed extended life span (Choe et al., 1999a), and indeed the fertile *dwf*5-1 mutant does not show delayed senescence (Choe et al., 2000). Senescence of leaf and cotyledon tissue has often been shown to be retarded in vitro by administration of cytokinins, while 24-*epi*BL accelerated senescence in such systems (Zhao et al., 1990; Ding and Zhao, 1995; He et al., 1996). Delayed senescence in Arabidopsis BR mutants would tend to support the role of BRs in accelerating senescence in normal plants, however it is not clear whether BRs play a critical function in the intrinsic program of senescence in vegetative tissue. Examination of the effect of BR application on senescence-associated mutants of Arabidopsis and study of the expression of senescence-associated genes in BR mutants should help clarify this question, and indeed an enhancer trap analysis showed that BRs enhance expression of several genes that promote leaf senescence (He et al., 2001).

Light, BR and Arabidopsis Development

Light quality, duration and intensity profoundly influence plant development throughout the life cycle and the etiolated dicot seedling is a particularly striking example that demonstrates how absence of light affects morphogenesis. Dark-grown seedlings exhibit greatly expanded hypocotyls or epicotyls, have a pronounced apical hook and contain undifferentiated chloroplast precursors. Upon exposure to light, stem elongation slows (Chory et al., 1996). While light is an essential signal influencing transition from the etiolated to de-etiolated state, plant hormones also play a critical role in regulating cell expansion, but the molecular mechanisms integrating these signals is not clear. Several de-etiolated mutants isolated in screens for plants that grow in darkness as if they were in the light, were subsequently revealed to be mutations in genes encoding BR biosynthetic enzymes (Li et al., 1996; Szekeres et al., 1996).

Numerous BR-deficient mutants in Arabidopsis, pea and tomato show defects in cell expansion in the dark. Depending on the severity of the mutant allele and the species, some of these mutants also show other characteristics of light-grown plants in the dark, such as expanded cotyledons, lack of an apical hook and aberrant expression of light-regulated genes (Clouse and Feldmann, 1999). While some of these de-etiolated characteristics have been attributed simply to the short stature of Arabidopsis BR mutants and their growth on agar plates (Azpiroz et al., 1998), it is also possible that light signal transduction pathways might impact the de novo synthesis or activity of BR biosynthetic enzymes, the metabolism of BRs, or BR signal transduction.

Some have questioned the connection between BR action and photomorphogenesis because BR levels remained the same in the dark and the light in tomato and pea (Symons and Reid, 2003; Symons et al., 2008b). However, genetic studies in Arabidopsis continue to suggest interactions between BR and light signaling pathways. The Arabidopsis *bas*1 mutant, which inactivates BL by overexpressing a C-26 BL hydroxylase, is capable of suppressing the long hypocotyl phenotype of phytochrome B mutants, pointing to a possible link between BR metabolism and phytochrome signaling (Neff et al., 1999; Turk et al., 2003; Turk et al., 2005). ChIP-chip and microarray analyses in Arabidopsis have also suggested that BR regulation of light signaling is a possible mechanism of interaction between these factors (Sun et al., 2010; Yu et al., 2011).

More than 750 genes were regulated in the same way by red light or by loss of BR signaling in the *bri1-116* mutant, which would result if red light and BR antagonistically affect seedling photomorphogenesis by regulating a common set of target genes in opposite ways (Sun et al., 2010). Further evidence supporting an antagonistic interaction between BR and light by targeting common downstream genes in the two pathways was provided by the discovery that HY5, a basic leucine zipper transcription factor that functions as a positive regulator of photomorphogenesis, binds to the promoters of 1170 genes that are also BZR1 direct targets, and these genes are generally regulated in opposite ways by BZR1 and HY5 (Sun et al., 2010). Moreover, BZR1 represses expression of the GATA2 transcription factor, another positive regulator of photomorphogenesis, by directly binding to its promoter more strongly in the dark than in the light and GATA2 induces many light-activated but BR-repressed genes while down-regulating light repressed but BR-activated genes (Luo et al., 2010).

Further connections between light and BR pathways in regulating morphogenesis can be found in the observation that the GOLDEN2-LIKE (GLK) transcription factors, required for chloroplast development (Waters et al., 2009), are direct targets of BES1, while microscopic analysis suggests that BR inhibits chloroplast development through BES1 repression of GLK1 and GLK2 transcription (Yu et al., 2011).

Conclusion and Future Prospects

The use of mutant analysis in Arabidopsis coupled with GC/MS measurements of endogenous BR levels has greatly advanced our understanding of BR physiology and biosynthesis. The biosynthetic pathway to BL is nearing saturation with mutants, and biochemical studies of substrate properties has identified redundant C-23 hydroxylases involved in a more direct biosynthetic pathway from campesterol to BL (Bishop, 2007).

With respect to signal transduction, the outline of the entire pathway is complete and the precise mechanisms by which steroid perception at the cell surface leads to significant alterations in gene expression and consequent changes in specific developmental programs has become more clearly defined (Kim and Wang, 2010; Clouse, 2011). It appears that the pleiotropic effects of BR action on plant physiology is likely due to BZR1 and BES1 mediated regulation of specific genes involved in processes such as cell elongation, flowering, senescence, leaf morphogenesis, etc., as well as regulation of sets of transcription factors that are direct targets of BZR1 and BES1, and which in turn regulate subsets of genes in a specific response to amplify the BR signal. The identification of hundreds of direct targets of BES1 and BZR1 (Sun et al., 2010; Yu et al., 2011) will allow many years of genetic and biochemical analyses of those targets, including both genes with known functions which need to be precisely placed in a BR signaling context, as well as genes with previously undefined functions, which may reveal novel physiological processes affected by BR signaling. While BES1 and BZR1 appear to be the predominant modulators of BR-regulated gene expression, it may be that additional, undiscovered transcription factors are involved in some specific BR responses and continued searches for these proteins may be productive. Moreover, the finding that BIN2 phosphorylates additional targets besides BES1 and BZR1 is intriguing and worth pursuing further (Wang et al., 2009).

Using LC/MS/MS approaches and phosphospecific antibodies, multiple in vivo phosphorylation sites have been identified in BRI1 and BAK1 (Wang et al., 2005b; Wang et al., 2008; Oh et al., 2009b; Oh et al., 2010), as well as in downstream components BKI1, BSK1, BIN2, BES1 and BZR1 (Tang et al., 2008a; Tang et al., 2008b; Kim et al., 2009; Jaillais et al., 2011b). These detailed analyses set the stage for rigorous study of the phosphorelay initiated by BR binding to BRI1 and culminating in dephosphorylation of BES1 and BZR1. Differential phosphorylation in BR signaling components may lead to alternative downstream signaling and consequent activation of different gene sets involved in specific physiological responses (Wang et al., 2005b; Wang et al., 2008). Continued functional analysis of individual phosphorylation sites

in BRI1, BAK1 and downstream signaling components, including use of LC/MS/MS techniques that quantitatively monitoring individual phosphorylation states in response to BR (Clouse et al., 2008), will help fine-tune the mechanisms of BR signaling.

While the “island” domain in the extracellular region of the BRI1 receptor kinase was clearly shown to be involved in BR binding (Kinoshita et al., 2005), the recent solving of the three-dimensional crystal structure for the BRI1 extracellular domain was a major advance that precisely defined the unique steroid-binding motif in plants (Hothorn et al., 2011; She et al., 2011), and along with the phosphorylation studies mentioned above, brings the understanding of molecular mechanisms in BR action closer to the level of detail available for many of the well-studied mammalian receptor kinases. In fact, BRI1 is becoming one of the best understood plant receptor kinases with respect to mechanism of action and may serve as a model to approach functional studies in other members of this large family of membrane proteins regulating signal transduction pathways associated with many environmental responses and internal signals in plant development.

Acknowledgments

Thanks to S. Choe and K. Feldmann for providing photographs of BR mutants. Work in the author’s laboratory is supported by the National Science Foundation (MCB-1021363 and MCB-0742411) and the North Carolina Agricultural Research Service.

REFERENCES

Adam, G., Schmidt, J., and Schneider, B. (1999). Brassinosteroids. Fortschr. Chem. Org. Naturst. **78,** 1-46.

Altmann, T. (1999). Molecular physiology of brassinosteroids revealed by the analysis of mutants. Planta **208,** 1-11.

Asakawa, S., Abe, H., Nishikawa, N., Natsume, M., and Koshioka, M. (1996). Purification and identification of new acyl-conjugated teasterones in lily pollen. Biosci. Biotech. Biochem. **60,** 1416-1420.

Asami, T., and Yoshida, S. (1999). Brassinosteroid biosynthesis inhibitors. Trends Plant Sci.. **4,** 348-353.

Asano, K., Kinzy, T.G., Merrick, W.C., and Hershey, J.W. (1997). Conservation and diversity of eukaryotic translation initiation factor eIF3. J. Biol. Chem. **272,** 1101-1109.

Azpiroz, R., Wu, Y., LoCascio, J.C., and Feldmann, K.A. (1998). An Arabidopsis brassinosteroid-dependent mutant is blocked in cell elongation. Plant Cell **10,** 219-230.

Babiychuk, E., Bouvier-Nave, P., Compagnon, V., Suzuki, M., Muranaka, T., Van Montagu, M., Kushnir, S., and Schaller, H. (2008). Allelic mutant series reveal distinct functions for Arabidopsis cycloartenol synthase 1 in cell viability and plastid biogenesis. Proc. Natl. Acad. Sci. USA **105,** 3163-3168.

Bai, M.Y., Zhang, L.Y., Gampala, S.S., Zhu, S.W., Song, W.Y., Chong, K., and Wang, Z.Y. (2007). Functions of OsBZR1 and 14-3-3 proteins in brassinosteroid signaling in rice. Proc. Natl. Acad. Sci. USA **104,** 13839-13844.

Bajguz, A. (2007). Metabolism of brassinosteroids in plants. Plant Physiol. Biochem. **45,** 95-107.

Bajguz, A. (2011). Brassinosteroids - occurence and chemical structures. In Brassinosteroids: A class of plant hormones, S. Hayat and A. Ahmad, eds (Dordrecht: Springer), pp. 1-27.

Bao, F., Azhakanandam, S., and Franks, R.G. (2010). SEUSS and SEUSS-LIKE transcriptional adaptors regulate floral and embryonic development in Arabidopsis. Plant Physiol. **152,** 821-836.

Bar, M., Sharfman, M., Ron, M., and Avni, A. (2010). BAK1 is required for the attenuation of ethylene-inducing xylanase (Eix)-induced defense responses by the decoy receptor LeEix1. Plant J. **63,** 791-800.

Benveniste, P. (2004). BIOSYNTHESIS AND ACCUMULATION OF STEROLS. Ann. Rev. Plant Biol. **55,** 429-457.

Bishop, G.J. (2007). Refining the plant steroid hormone biosynthesis pathway. Trends Plant Sci. **12,** 377-380.

Bishop, G.J., Nomura, T., Yokota, T., Harrison, K., Noguchi, T., Fujioka, S., Takatsuto, S., Jones, J.D., and Kamiya, Y. (1999). The tomato DWARF enzyme catalyses C-6 oxidation in brassinosteroid biosynthesis. Proc. Natl. Acad. Sci. USA **96,** 1761-1766.

Bouquin, T., Meier, C., Foster, R., Nielsen, M.E., and Mundy, J. (2001). Control of specific gene expression by gibberellin and brassinosteroid. Plant Physiol. **127,** 450-458.

Burks, E.A., Bezerra, P.P., Le, H., Gallie, D.R., and Browning, K.S. (2001). Plant initiation factor 3 subunit composition resembles mammalian initiation factor 3 and has a novel subunit. J. Biol. Chem. **276,** 2122-2131.

Caesar, K., Elgass, K., Chen, Z., Huppenberger, P., Witthoft, J., Schleifenbaum, F., Blatt, M.R., Oecking, C., and Harter, K. (2011). A fast brassinolide-regulated response pathway in the plasma membrane of *Arabidopsis thaliana*. Plant J. **66,** 528-540.

Cano-Delgado, A., Yin, Y., Yu, C., Vafeados, D., Mora-Garcia, S., Cheng, J.C., Nam, K.H., Li, J., and Chory, J. (2004). BRL1 and BRL3 are novel brassinosteroid receptors that function in vascular differentiation in Arabidopsis. Development **131,** 5341-5351.

Carland, F., Fujioka, S., and Nelson, T. (2010). The sterol methyltransferases SMT1, SMT2, and SMT3 influence Arabidopsis development through nonbrassinosteroid products. Plant Physiol. **153,** 741-756.

Carland, F.M., Fujioka, S., Takatsuto, S., Yoshida, S., and Nelson, T. (2002). The identification of CVP1 reveals a role for sterols in vascular patterning. Plant Cell **14,** 2045-2058.

Carpita, N., and Gibeaut, D. (1993). Structural models of the primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. Plant J. **3,** 1-30.

Catterou, M., Dubois, F., Schaller, H., Aubanelle, L., Vilcot, B., Sangwan-Norreel, B.S., and Sangwan, R.S. (2001a). Brassinosteroids, microtubules and cell elongation in *Arabidopsis thaliana.* I. Molecular, cellular and physiological characterization of the Arabidopsis *bul1* mutant, defective in the delta 7-sterol-C5-desaturation step leading to brassinosteroid biosynthesis. Planta **212,** 659-672.

Catterou, M., Dubois, F., Schaller, H., Aubanelle, L., Vilcot, B., Sangwan-Norreel, B.S., and Sangwan, R.S. (2001b). Brassinosteroids, microtubules and cell elongation in *Arabidopsis thaliana.* II. Effects of brassinosteroids on microtubules and cell elongation in the *bul1* mutant. Planta **212,** 673-683.

Chen, R.H., Miettinen, P.J., Maruoka, E.M., Choy, L., and Derynck, R. (1995). A WD-domain protein that is associated with and phosphorylated by the type II TGF-beta receptor. Nature **377,** 548-552.

Cheon, J., Park, S.Y., Schulz, B., and Choe, S. (2010). Arabidopsis brassinosteroid biosynthetic mutant *dwarf7-1* exhibits slower rates of cell division and shoot induction. BMC Plant Biol. **10,** 270.

Chinchilla, D., Shan, L., He, P., de Vries, S., and Kemmerling, B. (2009). One for all: the receptor-associated kinase BAK1. Trends Plant Sci. **14,** 535-541.

Choe, S., Dilkes, B.P., Fujioka, S., Takatsuto, S., Sakurai, A., and Feldmann, K.A. (1998). The *DWF4* gene of Arabidopsis encodes a cytochrome P450 that mediates multiple 22alpha-hydroxylation steps in brassinosteroid biosynthesis. Plant Cell **10,** 231-243.

Choe, S., Schmitz, R.J., Fujioka, S., Takatsuto, S., Lee, M.O., Yoshida, S., Feldmann, K.A., and Tax, F.E. (2002). Arabidopsis brassinosteroid-insensitive *dwarf12* mutants are semidominant and defective in a glycogen synthase kinase 3beta-like kinase. Plant Physiol. **130,** 1506-1515.

Choe, S., Tanaka, A., Noguchi, T., Fujioka, S., Takatsuto, S., Ross, A.S., Tax, F.E., Yoshida, S., and Feldmann, K.A. (2000). Lesions in the sterol delta reductase gene of Arabidopsis cause dwarfism due to a block in brassinosteroid biosynthesis. Plant J. **21,** 431-443.

Choe, S., Noguchi, T., Fujioka, S., Takatsuto, S., Tissier, C.P., Gregory, B.D., Ross, A.S., Tanaka, A., Yoshida, S., Tax, F.E., and Feldmann, K.A. (1999a). The Arabidopsis *dwf7/ste1* mutant is defective in the delta7 sterol C-5 desaturation step leading to brassinosteroid biosynthesis. Plant Cell **11,** 207-221.

Choe, S., Dilkes, B.P., Gregory, B.D., Ross, A.S., Yuan, H., Noguchi, T., Fujioka, S., Takatsuto, S., Tanaka, A., Yoshida, S., Tax, F.E., and Feldmann, K.A. (1999b). The Arabidopsis *dwarf1* mutant is defective in the conversion of 24-methylenecholesterol to campesterol in brassinosteroid biosynthesis. Plant Physiol. **119,** 897-907.

Choi, Y.-H., Fujioka, S., Nomura, T., Harada, A.Y., T., Takatsuto, S., and Sakurai, A. (1997). An alternative brassinolide biosynthetic pathway via late C-6 oxidation. Phytochemistry **44,** 609-613.

Chono, M., Honda, I., Zeniya, H., Yoneyama, K., Saisho, D., Takeda, K., Takatsuto, S., Hoshino, T., and Watanabe, Y. (2003). A semidwarf phenotype of barley *uzu* results from a nucleotide substitution in the gene encoding a putative brassinosteroid receptor. Plant Physiol. **133,** 1209-1219.

Chory, J., Nagpal, P., and Peto, C. (1991). Phenotypic and genetic analysis of *det*2, a new mutant that affects light-regulated seedling development in *Arabidopsis*. Plant Cell **3,** 445-459.

Chory, J., Chatterjee, M., Cook, R.K., Elich, T., Fankhauser, C., Li, J., Nagpal, P., Neff, M., Pepper, A., Poole, D., Reed, J., and Vitart, V. (1996). From seed germination to flowering, light controls plant development via the pigment phytochrome. Proc. Natl. Acad. Sci. USA **93,** 12066-12071.

Chung, Y., Maharjan, P.M., Lee, O., Fujioka, S., Jang, S., Kim, B., Takatsuto, S., Tsujimoto, M., Kim, H., Cho, S., Park, T., Cho, H., Hwang, I., and Choe, S. (2011). Auxin stimulates *DWARF4* expression and brassinosteroid biosynthesis in Arabidopsis. Plant J. **66,** 546-578.

Clouse, S., and Feldmann, K. (1999). Molecular genetics of brassinosteroid action. In Brassinosteroids: Steroidal plant hormones, A. Sakurai, T. Yokota, and S. Clouse, eds (Tokyo: Springer), pp. 163-190.

Clouse, S.D. (1997). Molecular genetic analysis of brassinosteroid action. Physiologia Plantarum **100,** 702-709.

Clouse, S.D. (2002a). Arabidopsis mutants reveal multiple roles for sterols in plant development. Plant Cell **14,** 1995-2000.

Clouse, S.D. (2002b). Brassinosteroid signal transduction: clarifying the pathway from ligand perception to gene expression. Mol. Cell **10,** 973-982.

Clouse, S.D. (2008a). The molecular intersection of brassinosteroid-regulated growth and flowering in Arabidopsis. Proc. Natl. Acad. Sci. USA **105,** 7345-7346.

Clouse, S.D. (2008b). Brassinosteroid Signaling. In Plant Cell Monographs, L. Bogre and G. Beemster, eds (Heidelberger Platz 3, D-14197 Berlin, Germany: Springer-Verlag Berlin), pp. 179-197.

Clouse, S.D. (2011). Brassinosteroid Signal Transduction: From Receptor Kinase Activation to Transcriptional Networks Regulating Plant Development. Plant Cell **23,** 1219-1230.

Clouse, S.D., and Zurek, D. (1991). Molecular analysis of brassinolide action in plant growth and development. In Brassinosteroids Chemistry, Bioactivity, & Applications, H.G. Cutler, T. Yokota, and G. Adam, eds (Washington, D.C.: American Chemical Society), pp. 122-140.

Clouse, S.D., and Sasse, J.M. (1998). BRASSINOSTEROIDS: Essential Regulators of Plant Growth and Development. Ann. Rev. Plant Physiol. Plant Mol. Biol. **49,** 427-451.

Clouse, S.D., Langford, M., and McMorris, T.C. (1996). A brassinosteroid-insensitive mutant in *Arabidopsis thaliana* exhibits multiple defects in growth and development. Plant Physiol. **111,** 671-678.

Clouse, S.D., Goshe, M.B., Huber, S.C., and Li, J. (2008). Functional Analysis and Phosphorylation Site Mapping of Leucine-Rich Repeat Receptor-Like Kinases. In Plant Proteomics: Technologies, Strategies and Applications., G.K. Agrawal and R. Rakwal, eds (John Wiley & Sons), pp. 469-484.

Clouse, S.D., Hall, A.F., Langford, M., McMorris, T.C., and Baker, M.E. (1993). Physiological and molecular effects of brassinosteroids on *Arabidopsis thaliana*. Journal of Plant Growth Regulation **12,** 61-66.

Cosgrove, D. (1997). Relaxation in a high-stress environment: the molecular basis of extensible cell walls and enlargement. Plant Cell **9,** 1031-1041.

Cutler, H.G. (1991). Brassinosteroids through the looking glass. In Brassinosteroids Chemistry, Bioactivity, & Applications, H.G. Cutler, T. Yokota, and G. Adam, eds (Washington, D.C.: American Chemical Society), pp. 334-345.

De Rybel, B., Audenaert, D., Vert, G., Rozhon, W., Mayerhofer, J., Peelman, F., Coutuer, S., Denayer, T., Jansen, L., Nguyen, L., Vanhoutte, I., Beemster, G.T., Vleminckx, K., Jonak, C., Chory, J., Inze, D., Russinova, E., and Beeckman, T. (2009). Chemical inhibition of a subset of *Arabidopsis thaliana* GSK3-like kinases activates brassinosteroid signaling. Chem. Biol. **16,** 594-604.

Di Rubbo, S., Irani, N.G., and Russinova, E. (2011). PP2A Phosphatases: The "On-Off" Regulatory Switches of Brassinosteroid Signaling. Sci. Signal **4,** pe25.

Diener, A.C., Li, H., Zhou, W., Whoriskey, W.J., Nes, W.D., and Fink, G.R. (2000). Sterol methyltransferase 1 controls the level of cholesterol in plants. Plant Cell **12,** 853-870.

Ding, W.-M., and Zhao, Y.-J. (1995). Effect of epi-BR on activity of peroxidase and soluble protein content of cucumber cotyledon. Acta Phytophysiologica Sinica **21,** 259-264.

Domagalska, M.A., Sarnowska, E., Nagy, F., and Davis, S.J. (2010). Genetic Analyses of Interactions among Gibberellin, Abscisic Acid, and Brassinosteroids in the Control of Flowering Time in *Arabidopsis thaliana*. PLoS One **5,** e14012.

Domagalska, M.A., Schomburg, F.M., Amasino, R.M., Vierstra, R.D., Nagy, F., and Davis, S.J. (2007). Attenuation of brassinosteroid signaling enhances FLC expression and delays flowering. Development **134,** 2841-2850.

Edwards, P.A., and Ericsson, J. (1999). STEROLS AND ISOPRENOIDS: Signaling Molecules Derived from the Cholesterol Biosynthetic Pathway. Ann. Rev. Biochem. **68,** 157-185.

Ehsan, H., Ray, W.K., Phinney, B., Wang, X., Huber, S.C., and Clouse, S.D. (2005). Interaction of Arabidopsis BRASSINOSTEROID-INSENSITIVE 1 receptor kinase with a homolog of mammalian TGF-beta receptor interacting protein. Plant J. **43,** 251-261.

Ephritikhine, G., Fellner, M., Vannini, C., Lapous, D., and Barbier-Brygoo, H. (1999a). The *sax1* dwarf mutant of *Arabidopsis thaliana* shows altered sensitivity of growth responses to abscisic acid, auxin, gibberellins and ethylene and is partially rescued by exogenous brassinosteroid. Plant J. **18,** 303-314.

Ephritikhine, G., Pagant, S., Fujioka, S., Takatsuto, S., Lapous, D., Caboche, M., Kendrick, R.E., and Barbier-Brygoo, H. (1999b). The *sax1* mutation defines a new locus involved in the brassinosteroid biosynthesis pathway in *Arabidopsis thaliana.* Plant J. **18,** 315-320.

Feldmann, K., Marks, M., Christianson, M., and Quatrano, R. (1989).

A dwarf mutant of *Arabidopsis* generated by T-DNA insertion mutagenesis. Science **243,** 1351-1354.

Friedrichsen, D.M., Joazeiro, C.A., Li, J., Hunter, T., and Chory, J. (2000). Brassinosteroid-insensitive-1 is a ubiquitously expressed leucine-rich repeat receptor serine/threonine kinase. Plant Physiol. **123,** 1247-1256.

Fujioka, S. (1999). Natural occurrence of brassinosteroids in the plant kingdom. In Brassinosteroids: Steroidal plant hormones, A. Sakurai, T. Yokota, and S. Clouse, eds (Tokyo: Springer), pp. 21-45.

Fujioka, S., and Sakurai, A. (1997). Brassinosteroids. Nat. Prod. Rep. **14,** 1-10.

Fujioka, S., and Yokota, T. (2003). Biosynthesis and metabolism of brassinosteroids. Ann. Rev. Plant Biol. **54,** 137-164.

Fujioka, S., Takatsuto, S., and Yoshida, S. (2002). An early C-22 oxidation branch in the brassinosteroid biosynthetic pathway. Plant Physiol. **130,** 930-939.

Fujioka, S., Noguchi, T., Yokota, T., Takatsuto, S., and Yoshida, S. (1998). Brassinosteroids in *Arabidopsis thaliana*. Phytochemistry **48,** 595-599.

Fujioka, S., Choi, Y.H., Takatsuto, S., Yokota, T., Li, J., Chory, J., and Sakurai, A. (1996). Identification of castasterone, 6-deoxocastasterone, typhasterol and 6-deoxotyphasterol from the shoots of *Arabidopsis thaliana*. Plant Cell Physiol. **37,** 1201-1203.

Fujioka, S., Li, J., Choi, Y.H., Seto, H., Takatsuto, S., Noguchi, T., Watanabe, T., Kuriyama, H., Yokota, T., Chory, J., and Sakurai, A. (1997). The Arabidopsis *deetiolated2* mutant is blocked early in brassinosteroid biosynthesis. Plant Cell **9,** 1951-1962.

Fujita, S., Ohnishi, T., Watanabe, B., Yokota, T., Takatsuto, S., Fujioka, S., Yoshida, S., Sakata, K., and Mizutani, M. (2006). Arabidopsis CYP90B1 catalyses the early C-22 hydroxylation of C27, C28 and C29 sterols. Plant J. **45,** 765-774.

Fukuda, H. (1997). Tracheary element differentiation. Plant Cell **9,** 1147-1156.

Fukuda, H. (2004). Signals that control plant vascular cell differentiation. Nat. Rev. Mol. Cell Biol. **5,** 379-391.

Gachotte, D., Meens, R., and Benveniste, P. (1995). An Arabidopsis mutant deficient in sterol biosynthesis: heterologous complementation by *ERG 3* encoding a D7-sterol-C-5-desaturase from yeast. Plant J. **8,** 407-416.

Gampala, S.S., Kim, T.W., He, J.X., Tang, W., Deng, Z., Bai, M.Y., Guan, S., Lalonde, S., Sun, Y., Gendron, J.M., Chen, H., Shibagaki, N., Ferl, R.J., Ehrhardt, D., Chong, K., Burlingame, A.L., and Wang, Z.Y. (2007). An essential role for 14-3-3 proteins in brassinosteroid signal transduction in Arabidopsis. Dev. Cell **13,** 177-189.

Geldner, N., Hyman, D.L., Wang, X., Schumacher, K., and Chory, J. (2007). Endosomal signaling of plant steroid receptor kinase BRI1. Genes Dev. **21,** 1598-1602.

Gendron, J.M., and Wang, Z.Y. (2007). Multiple mechanisms modulate brassinosteroid signaling. Curr. Opin. Plant Biol. **10,** 436-441.

Gonzalez-Garcia, M.P., Vilarrasa-Blasi, J., Zhiponova, M., Divol, F., Mora-Garcia, S., Russinova, E., and Cano-Delgado, A.I. (2011). Brassinosteroids control meristem size by promoting cell cycle progression in Arabidopsis roots. Development **138,** 849-859.

Griffiths, P.G., Sasse, J.M., Yokota, T., and Cameron, D.W. (1995). 6-Deoxotyphasterol and 3-Dehydro-6-deoxoteasterone, possible precursors to brassinosteroids in the pollen of *Cupressus arizonica.* Biosci. Biotech. Biochem. **59,** 956-959.

Grove, M.D., Spencer, G.F., Rohwedder, W.K., Mandava, N.B., Worley, J.F., Warthen, J.D., Steffens, G.L., Flippen-Anderson, J.L., and Cook, J.C. (1979). Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. Nature **281,** 216-217.

Guo, H., Li, L., Ye, H., Yu, X., Algreen, A., and Yin, Y. (2009). Three related receptor-like kinases are required for optimal cell elongation in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **106,** 7648-7653.

Guo, Z., Fujioka, S., Blancaflor, E.B., Miao, S., Gou, X., and Li, J. (2010). TCP1 Modulates Brassinosteroid Biosynthesis by Regulating the Expression of the Key Biosynthetic Gene *DWARF4* in *Arabidopsis thaliana*. Plant Cell **22,** 1161-1173.

Hacham, Y., Holland, N., Butterfield, C., Ubeda-Tomas, S., Bennett, M.J., Chory, J., and Savaldi-Goldstein, S. (2011). Brassinosteroid perception in the epidermis controls root meristem size. Development **138,** 839-848.

Hai, T., Schneider, B., Porzel, A., and Adam, G. (1996). Metabolism of 24-epicastasterone in cell suspension culture of *Lycopersicon esculentum*. Phytochem. **41,** 197-201.

Hanks, S.K., Quinn, A.M., and Hunter, T. (1988). The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science **241,** 42-52.

Hardtke, C.S., Dorcey, E., Osmont, K.S., and Sibout, R. (2007). Phytohormone collaboration: zooming in on auxin-brassinosteroid interactions. Trends Cell Biol **17,** 485-492.

Harwood, A.J. (2001). Regulation of GSK-3: a cellular multiprocessor. Cell **105,** 821-824.

Hategan, L., Godza, B., and Szekeres, M. (2011). Regulation of brassinosteroid metabolism. In Brassinosteroids: A class of plant hormones, S. Hayat and A. Ahmad, eds (Dordrecht: Springer), pp. 57-81.

Hayat, S., and Ahmad, A. (2003). Brassinosteroids: Bioactivity and crop productivity. (Dordrecht: Kluwer Academic).

Hayat, S., and Ahmad, A. (2011). Brassinosteroids: A class of plant hormone. (Dordrecht: Springer).

He, J.X., Gendron, J.M., Yang, Y., Li, J., and Wang, Z.Y. (2002). The GSK3-like kinase BIN2 phosphorylates and destabilizes BZR1, a positive regulator of the brassinosteroid signaling pathway in Arabidopsis. Proc. Natl. Acad. Sci. USA **99,** 10185-10190.

He, J.X., Gendron, J.M., Sun, Y., Gampala, S.S., Gendron, N., Sun, C.Q., and Wang, Z.Y. (2005). BZR1 is a transcriptional repressor with dual roles in brassinosteroid homeostasis and growth responses. Science **307,** 1634-1638.

He, K., Gou, X., Yuan, T., Lin, H., Asami, T., Yoshida, S., Russell, S.D., and Li, J. (2007). BAK1 and BKK1 regulate brassinosteroid-dependent growth and brassinosteroid-independent cell-death pathways. Curr. Biol. **17,** 1109-1115.

He, Y., Tang, W., Swain, J.D., Green, A.L., Jack, T.P., and Gan, S. (2001). Networking senescence-regulating pathways by using Arabidopsis enhancer trap lines. Plant Physiol. **126,** 707-716.

He, Y.-J., Xu, R.-J., and Zhao, Y.-J. (1996). Enhancement of senescence by epibrassinolide in leaves of mung bean seedling. Acta Phytophysiologica Sinica **22,** 58-62.

Hecht, V., Vielle-Calzada, J.P., Hartog, M.V., Schmidt, E.D., Boutilier, K., Grossniklaus, U., and de Vries, S.C. (2001). The Arabidopsis SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1 gene is expressed in developing ovules and embryos and enhances embryogenic competence in culture. Plant Physiol. **127,** 803-816.

Heese, A., Hann, D.R., Gimenez-Ibanez, S., Jones, A.M., He, K., Li, J., Schroeder, J.I., Peck, S.C., and Rathjen, J.P. (2007). The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. Proc. Natl. Acad. Sci. USA **104,** 12217-12222.

Hewitt, F.R., Hough, T., O'Neill, P., Sasse, J.M., Williams, E.G., and Rowan, K.S. (1985). Effect of brassinolide and other growth regulators on the germination and growth of pollen tubes of *Prunus avium* using a multiple hanging drop assay. Aust. J. Plant Physiol. **1,** 201-211.

Hink, M.A., Shah, K., Russinova, E., de Vries, S.C., and Visser, A.J.

(2008). Fluorescence fluctuation analysis of *Arabidopsis thaliana* somatic embryogenesis receptor-like kinase and brassinosteroid insensitive 1 receptor oligomerization. Biophys. J. **94,** 1052-1062.

Holton, N., Cano-Delgado, A., Harrison, K., Montoya, T., Chory, J., and Bishop, G.J. (2007). Tomato BRASSINOSTEROID INSENSITIVE1 is required for systemin-induced root elongation in *Solanum pimpinellifolium* but is not essential for wound signaling. Plant Cell **19,** 1709-1717.

Hothorn, M., Belkhadir, Y., Dreux, M., Dabi, T., Noel, J.P., Wilson, I.A., and Chory, J. (2011). Structural basis of steroid hormone perception by the receptor kinase BRI1. Nature **474,** 467-472.

Howell, W.M., Keller, G.E., 3rd, Kirkpatrick, J.D., Jenkins, R.L., Hunsinger, R.N., and McLaughlin, E.W. (2007). Effects of the plant steroidal hormone, 24-epibrassinolide, on the mitotic index and growth of onion (*Allium cepa*) root tips. Genet. Mol. Res. **6,** 50-58.

Hu, Y., Bao, F., and Li, J. (2000). Promotive effect of brassinosteroids on cell division involves a distinct CycD3-induction pathway in Arabidopsis. Plant J. **24,** 693-701.

Hyun, Y., and Lee, I. (2006). KIDARI, encoding a non-DNA Binding bHLH protein, represses light signal transduction in *Arabidopsis thaliana*. Plant Mol. Biol. **61,** 283-296.

Ibanes, M., Fabregas, N., Chory, J., and Cano-Delgado, A.I. (2009). Brassinosteroid signaling and auxin transport are required to establish the periodic pattern of Arabidopsis shoot vascular bundles. Proc. Natl. Acad. Sci. USA **106,** 13630-13635.

Iwasaki, T., and Shibaoka, H. (1991). Brassinosteroids act as regulators of tracheary-element differentiation in isolated Zinnia mesophyll cells. Plant Cell Physiol. **32,** 1007-1014.

Jaillais, Y., Belkhadir, Y., Balsemao-Pires, E., Dangl, J.L., and Chory, J. (2011a). Extracellular leucine-rich repeats as a platform for receptor/coreceptor complex formation. Proc. Natl. Acad. Sci. USA **108,** 8503-8507.

Jaillais, Y., Hothorn, M., Belkhadir, Y., Dabi, T., Nimchuk, Z.L., Meyerowitz, E.M., and Chory, J. (2011b). Tyrosine phosphorylation controls brassinosteroid receptor activation by triggering membrane release of its kinase inhibitor. Genes Dev. **25,** 232-237.

Jang, J.C., Fujioka, S., Tasaka, M., Seto, H., Takatsuto, S., Ishii, A., Aida, M., Yoshida, S., and Sheen, J. (2000). A critical role of sterols in embryonic patterning and meristem programming revealed by the fackel mutants of *Arabidopsis thaliana*. Genes Dev. **14,** 1485-1497.

Janssens, V., Longin, S., and Goris, J. (2008). PP2A holoenzyme assembly: *in cauda venenum* (the sting is in the tail). Trends Biochem. Sci. **33,** 113-121.

Jiang, J., and Clouse, S.D. (2001). Expression of a plant gene with sequence similarity to animal TGF-beta receptor interacting protein is regulated by brassinosteroids and required for normal plant development. Plant J. **26,** 35-45.

Jin, H., Yan, Z., Nam, K.H., and Li, J. (2007). Allele-specific suppression of a defective brassinosteroid receptor reveals a physiological role of UGGT in ER quality control. Mol. Cell **26,** 821-830.

Karlova, R., Boeren, S., Russinova, E., Aker, J., Vervoort, J., and de Vries, S. (2006). The Arabidopsis SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE1 protein complex includes BRASSINOSTEROID-INSENSITIVE1. Plant Cell **18,** 626-638.

Kauschmann, A., Jessop, A., Koncz, C., Szekeres, m., Willmitzer, L., and Altmann, T. (1996). Genetic evidence for an essential role of brassinosteroids in plant development. Plant J. **9,** 701-713.

Khripach, V.A., Zhabinskii, V.N., and de Groot, A.E. (1999). Brassinosteroids: a new class of plant hormones. (San Diego: Academic Press).

Kim, G.T., Fujioka, S., Kozuka, T., Tax, F.E., Takatsuto, S., Yoshida, S., and Tsukaya, H. (2005a). CYP90C1 and CYP90D1 are involved in different steps in the brassinosteroid biosynthesis pathway in *Arabidopsis thaliana*. Plant J. **41,** 710-721.

Kim, L., and Kimmel, A.R. (2000). GSK3, a master switch regulating cell-fate specification and tumorigenesis. Curr. Opin. Genet. Dev. **10,** 508-514.

Kim, S.-K., Abe, H., Little, C.H.A., and Pharis, R.P. (1990). Identification of two brassinosteroids from the cambial region of Scots pine (*Pinus silvestris*) by gas chromatography-mass spectrometry, after detection using a dwarf rice lamina inclination bioassay. Plant Physiol. **94,** 1709-1713.

Kim, T.-W., Hwang, J.-Y., Kim, Y.-S., Joo, S.-H., Chang, S.C., Lee, J.S., Takatsuto, S., and Kim, S.-K. (2005b). Arabidopsis CYP85A2, a Cytochrome P450, Mediates the Baeyer-Villiger Oxidation of Castasterone to Brassinolide in Brassinosteroid Biosynthesis. Plant Cell **17,** 2397-2412.

Kim, T.W., and Wang, Z.Y. (2010). Brassinosteroid Signal Transduction from Receptor Kinases to Transcription Factors. Annu. Rev. Plant Biol. **61,** 681-704.

Kim, T.W., Guan, S., Burlingame, A.L., and Wang, Z.Y. (2011). The CDG1 kinase mediates brassinosteroid signal transduction from BRI1 receptor kinase to BSU1 phosphatase and GSK3-like kinase BIN2. Mol. Cell **43,** 561-571.

Kim, T.W., Guan, S., Sun, Y., Deng, Z., Tang, W., Shang, J.X., Burlingame, A.L., and Wang, Z.Y. (2009). Brassinosteroid signal transduction from cell-surface receptor kinases to nuclear transcription factors. Nat. Cell Biol. **11,** 1254-1260.

Kinoshita, T., Cano-Delgado, A., Seto, H., Hiranuma, S., Fujioka, S., Yoshida, S., and Chory, J. (2005). Binding of brassinosteroids to the extracellular domain of plant receptor kinase BRI1. Nature **433,** 167-171.

Kitani, Y. (1994). Induction of parthenogenetic haploid plants with brassinolide. Jpn. J. Genet. **69,** 35-39.

Klahre, U., Noguchi, T., Fujioka, S., Takatsuto, S., Yokota, T., Nomura, T., Yoshida, S., and Chua, N.H. (1998). The Arabidopsis *DIMINUTO/DWARF1* gene encodes a protein involved in steroid synthesis. Plant Cell **10,** 1677-1690.

Koka, C.V., Cerny, R.E., Gardner, R.G., Noguchi, T., Fujioka, S., Takatsuto, S., Yoshida, S., and Clouse, S.D. (2000). A putative role for the tomato genes *DUMPY* and *CURL-3* in brassinosteroid biosynthesis and response. Plant Physiol. **122,** 85-98.

Krishna, P. (2003). Brassinosteroid-Mediated Stress Responses. J. Plant Growth Regul. **22,** 289-297.

Kwon, M., Fujioka, S., Jeon, J.H., Kim, H.B., Takatsuto, S., Yoshida, S., An, C., and Choe, S. (2005). A double mutant for the CYP85A1 and CYP85A2 Genes of Arabidopsis exhibits a Brassinosteroid dwarf phenotype. J. Plant Biol. **48,** 237-244.

Kwon, S.I., Cho, H.J., Jung, J.H., Yoshimoto, K., Shirasu, K., and Park, O.K. (2010). The Rab GTPase RabG3b functions in autophagy and contributes to tracheary element differentiation in Arabidopsis. Plant J. **64,** 151-164.

Lee, S., Yang, K.Y., Kim, Y.M., Park, S.Y., Kim, S.Y., and Soh, M.S. (2006). Overexpression of *PRE1* and its homologous genes activates Gibberellin-dependent responses in *Arabidopsis thaliana*. Plant Cell Physiol. **47,** 591-600.

Lemmon, M.A., and Schlessinger, J. (2010). Cell signaling by receptor tyrosine kinases. Cell **141,** 1117-1134.

Li, J. (2010a). Regulation of the nuclear activities of brassinosteroid signaling. Curr. Opin. Plant Biol. **13,** 540-547.

Li, J. (2010b). Multi-tasking of somatic embryogenesis receptor-like protein kinases. Curr. Opin. Plant Biol.**13,** 509-514.

Li, J., and Chory, J. (1997). A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. Cell **90,** 929-938.

Li, J., and Nam, K.H. (2002). Regulation of brassinosteroid signaling by a GSK3/SHAGGY-like kinase. Science **295,** 1299-1301.

Li, J., and Jin, H. (2007). Regulation of brassinosteroid signaling. Trends Plant Sci. **12,** 37-41.

Li, J., Nagpal, P., Vitart, V., McMorris, T.C., and Chory, J. (1996). A role for brassinosteroids in light-dependent development of Arabidopsis. Science **272,** 398-401.

Li, J., Biswas, M.G., Chao, A., Russell, D.W., and Chory, J. (1997). Conservation of function between mammalian and plant steroid 5alpha-reductases. Proc. Natl. Acad. Sci. USA **94,** 3554-3559.

Li, J., Wen, J., Lease, K.A., Doke, J.T., Tax, F.E., and Walker, J.C. (2002). BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. Cell **110,** 213-222.

Li, L., Ye, H., Guo, H., and Yin, Y. (2010). Arabidopsis IWS1 interacts with transcription factor BES1 and is involved in plant steroid hormone brassinosteroid regulated gene expression. Proc. Natl. Acad. Sci. USA **107,** 3918-3923.

Li, L., Yu, X., Thompson, A., Guo, M., Yoshida, S., Asami, T., Chory, J., and Yin, Y. (2009). Arabidopsis MYB30 is a direct target of BES1 and cooperates with BES1 to regulate brassinosteroid-induced gene expression. Plant J. **58,** 275-286.

Lim, W.A., and Pawson, T. (2010). Phosphotyrosine signaling: evolving a new cellular communication system. Cell **142,** 661-667.

Lindsey, K., Pullen, M.L., and Topping, J.F. (2003). Importance of plant sterols in pattern formation and hormone signalling. Trends Plant Sci. **8,** 521-525.

Luo, X.M., Lin, W.H., Zhu, S., Zhu, J.Y., Sun, Y., Fan, X.Y., Cheng, M., Hao, Y., Oh, E., Tian, M., Liu, L., Zhang, M., Xie, Q., Chong, K., and Wang, Z.Y. (2010). Integration of Light- and Brassinosteroid-Signaling Pathways by a GATA Transcription Factor in Arabidopsis. Dev Cell **19,** 872-883.

Mandava, N.B. (1988). Plant growth-promoting brassinosteroids. Annu. Rev. Plant Physiol. Plant Molec. Biol. **39,** 23-52.

Mara, C.D., Huang, T., and Irish, V.F. (2010). The Arabidopsis floral homeotic proteins APETALA3 and PISTILLATA negatively regulate the *BANQUO* genes implicated in light signaling. Plant Cell **22,** 690-702.

Marsolais, F., Boyd, J., Paredes, Y., Schinas, A.M., Garcia, M., Elzein, S., and Varin, L. (2007). Molecular and biochemical characterization of two brassinosteroid sulfotransferases from Arabidopsis, AtST4a (At2g14920) and AtST1 (At2g03760). Planta **225,** 1233-1244.

Mathur, J., Molnar, G., Fujioka, S., Takatsuto, S., Sakurai, A., Yokota, T., Adam, G., Voigt, B., Nagy, F., Maas, C., Schell, J., Koncz, C., and Szekeres, M. (1998). Transcription of the Arabidopsis *CPD* gene, encoding a steroidogenic cytochrome P450, is negatively controlled by brassinosteroids. Plant J. **14,** 593-602.

Mayer, U., Torres-Ruiz, R., Berleth, T., Misera, S., and Jurgens, G. (1991). Mutations affecting the body organization in the Arabidopsis embryo. Nature **353,** 402-407.

Mayumi, K., and Shibaoka, H. (1995). A possible double role for brassinolide in the re-orientation of cortical microtubules in the epidermal cells of Azuki bean epicotyls. Plant Cell Physiol. **36,** 173-181.

Mitchell, J.W., Mandava, N.B., Worley, J.F., Plimmer, J.R., and Smith, M.V. (1970). Brassins: a new family of plant hormones from rape pollen. Nature (London) **225,** 1065-1066.

Miyazawa, Y., Nakajima, N., Abe, T., Sakai, A., Fujioka, S., Kawano, S., Kuroiwa, T., and Yoshida, S. (2003). Activation of cell proliferation by brassinolide application in tobacco BY-2 cells: effects of brassinolide on cell multiplication, cell-cycle-related gene expression, and organellar DNA contents. J. Exp. Bot. **54,** 2669-2678.

Montoya, T., Nomura, T., Farrar, K., Kaneta, T., Yokota, T., and Bishop, G.J. (2002). Cloning the tomato *curl3* gene highlights the putative dual role of the leucine-rich repeat receptor kinase tBRI1/SR160 in plant steroid hormone and peptide hormone signaling. Plant Cell **14,** 3163-3176.

Mora-Garcia, S., Vert, G., Yin, Y., Cano-Delgado, A., Cheong, H., and Chory, J. (2004). Nuclear protein phosphatases with Kelch-repeat domains modulate the response to brassinosteroids in Arabidopsis. Genes Dev. **18,** 448-460.

Morillon, R., Catterou, M., Sangwan, R.S., Sangwan, B.S., and Lassalles, J.P. (2001). Brassinolide may control aquaporin activities in *Arabidopsis thaliana*. Planta **212,** 199-204.

Mouchel, C.F., Osmont, K.S., and Hardtke, C.S. (2006). BRX mediates feedback between brassinosteroid levels and auxin signalling in root growth. Nature **443,** 458-461.

Muto, H., Yabe, N., Asami, T., Hasunuma, K., and Yamamoto, K.T. (2004). Overexpression of *constitutive differential growth 1* gene, which encodes a RLCKVII-subfamily protein kinase, causes abnormal differential and elongation growth after organ differentiation in Arabidopsis. Plant Physiol. **136,** 3124-3133.

Nagata, N., Min, Y.K., Nakano, T., Asami, T., and Yoshida, S. (2000). Treatment of dark-grown *Arabidopsis thaliana* with a brassinosteroid-biosynthesis inhibitor, brassinazole, induces some characteristics of light-grown plants. Planta **211,** 781-790.

Nakajima, N., Shida, A., and Toyama, S. (1996). Effects of brassinosteroid on cell division and colony formation of Chinese cabbage mesophyll protoplasts. Japanese J. Crop Science **65,** 114-118.

Nam, K.H., and Li, J. (2002). BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. Cell **110,** 203-212.

Nam, K.H., and Li, J. (2004). The Arabidopsis transthyretin-like protein is a potential substrate of BRASSINOSTEROID-INSENSITIVE 1. Plant Cell **16,** 2406-2417.

Neff, M.M., Nguyen, S.M., Malancharuvil, E.J., Fujioka, S., Noguchi, T., Seto, H., Tsubuki, M., Honda, T., Takatsuto, S., Yoshida, S., and Chory, J. (1999). *BAS1*: A gene regulating brassinosteroid levels and light responsiveness in Arabidopsis. Proc. Natl. Acad. Sci. USA **96,** 15316-15323.

Noguchi, T., Fujioka, S., Takatsuto, S., Sakurai, A., Yoshida, S., Li, J., and Chory, J. (1999a). Arabidopsis *det2* is defective in the conversion of (24R)-24-methylcholest-4-En-3-one to (24R)-24-methyl-5alpha-cholestan-3-one in brassinosteroid biosynthesis. Plant Physiol. **120,** 833-840.

Noguchi, T., Fujioka, S., Choe, S., Takatsuto, S., Tax, F.E., Yoshida, S., and Feldmann, K.A. (2000). Biosynthetic pathways of brassinolide in Arabidopsis. Plant Physiol. **124,** 201-209.

Noguchi, T., Fujioka, S., Choe, S., Takatsuto, S., Yoshida, S., Yuan, H., Feldmann, K.A., and Tax, F.E. (1999b). Brassinosteroid-insensitive dwarf mutants of Arabidopsis accumulate brassinosteroids. Plant Physiol. **121,** 743-752.

Nole-Wilson, S., Rueschhoff, E.E., Bhatti, H., and Franks, R.G. (2010). Synergistic disruptions in seuss *cyp85A2* double mutants reveal a role for brassinolide synthesis during gynoecium and ovule development. BMC Plant Biol. **10,** 198.

Nomura, T., Sato, T., Bishop, G.J., Kamiya, Y., Takatsuto, S., and Yokota, T. (2001). Accumulation of 6-deoxocathasterone and 6-deoxocastasterone in Arabidopsis, pea and tomato is suggestive of common rate-limiting steps in brassinosteroid biosynthesis. Phytochemistry **57,** 171-178.

Nomura, T., Bishop, G.J., Kaneta, T., Reid, J.B., Chory, J., and Yokota, T. (2003). The LKA gene is a BRASSINOSTEROID INSENSITIVE 1 homolog of pea. Plant J. **36,** 291-300.

Nomura, T., Kushiro, T., Yokota, T., Kamiya, Y., Bishop, G.J., and Yamaguchi, S. (2005). The last reaction producing brassinolide is cata-

lyzed by cytochrome P-450s, CYP85A3 in tomato and CYP85A2 in Arabidopsis. J. Biol. Chem. **280,** 17873-17879.

Oh, M.-H., Romanov, W., Smith, R., Sasse, J., and Clouse, S. (1998). *Bru1* encodes a xyloglucan endo-transglycosylase that is expressed in inner and outer tissues of elongating soybean epicotyls. Plant Cell Physiol. **39,** 124-130.

Oh, M.H., and Clouse, S.D. (1998). Brassinolide affects the rate of cell division in isolated leaf protoplasts of *Petunia hybrida*. Plant Cell Reports **17,** 921-924.

Oh, M.H., Clouse, S.D., and Huber, S.C. (2009a). Tyrosine phosphorylation in brassinosteroid signaling. Plant Signal Behav. **4,** 1182-1185.

Oh, M.H., Ray, W.K., Huber, S.C., Asara, J.M., Gage, D.A., and Clouse, S.D. (2000). Recombinant brassinosteroid insensitive 1 receptor-like kinase autophosphorylates on serine and threonine residues and phosphorylates a conserved peptide motif in vitro. Plant Physiol. **124,** 751-766.

Oh, M.H., Wang, X., Kota, U., Goshe, M.B., Clouse, S.D., and Huber, S.C. (2009b). Tyrosine phosphorylation of the BRI1 receptor kinase emerges as a component of brassinosteroid signaling in Arabidopsis. Proc. Natl. Acad. Sci. USA **106,** 658-663.

Oh, M.H., Wang, X., Wu, X., Zhao, Y., Clouse, S.D., and Huber, S.C. (2010). Autophosphorylation of Tyr-610 in the receptor kinase BAK1 plays a role in brassinosteroid signaling and basal defense gene expression. Proc. Natl. Acad. Sci. USA **107,** 17827-17832.

Ohashi-Ito, K., and Fukuda, H. (2003). HD-zip III homeobox genes that include a novel member, ZeHB-13 (Zinnia)/ATHB-15 (Arabidopsis), are involved in procambium and xylem cell differentiation. Plant Cell Physiol. **44,** 1350-1358.

Ohashi-Ito, K., and Fukuda, H. (2010). Transcriptional regulation of vascular cell fates. Curr. Opin. Plant Biol. **13,** 670-676.

Ohashi-Ito, K., Kubo, M., Demura, T., and Fukuda, H. (2005). Class III homeodomain leucine-zipper proteins regulate xylem cell differentiation. Plant Cell Physiol. **46,** 1646-1656.

Ohnishi, T., Yokota, T., and Mizutani, M. (2009). Insights into the function and evolution of P450s in plant steroid metabolism. Phytochemistry **70,** 1918-1929.

Ohnishi, T., Szatmari, A.M., Watanabe, B., Fujita, S., Bancos, S., Koncz, C., Lafos, M., Shibata, K., Yokota, T., Sakata, K., Szekeres, M., and Mizutani, M. (2006). C-23 hydroxylation by Arabidopsis CYP90C1 and CYP90D1 reveals a novel shortcut in brassinosteroid biosynthesis. Plant Cell **18,** 3275-3288.

Peng, P., Yan, Z., Zhu, Y., and Li, J. (2008). Regulation of the Arabidopsis GSK3-like Kinase BRASSINOSTEROID-INSENSITIVE 2 through Proteasome-Mediated Protein Degradation. Mol. Plant **1,** 338-346.

Peng, P., Zhao, J., Zhu, Y., Asami, T., and Li, J. (2010). A direct docking mechanism for a plant GSK3-like kinase to phosphorylate its substrates. J. Biol. Chem. **285**, 24646-24653.

Perez-Espana, V.H., Sanchez-Leon, N., and Vielle-Calzada, J.P. (2011). CYP85A1 is required for the initiation of female gametogenesis in *Arabidopsis thaliana*. Plant Signal Behav. **6,** 321-326.

Perez-Perez, J.M., Ponce, M.R., and Micol, J.L. (2002). The UCU1 Arabidopsis gene encodes a SHAGGY/GSK3-like kinase required for cell expansion along the proximodistal axis. Dev. Biol. **242,** 161-173.

Pessoa, J., Sarkany, Z., Ferreira-da-Silva, F., Martins, S., Almeida, M.R., Li, J., and Damas, A.M. (2010). Functional characterization of *Arabidopsis thaliana* transthyretin-like protein. BMC Plant Biol. **10,** 30.

Poppenberger, B., Rozhon, W., Khan, M., Husar, S., Adam, G., Luschnig, C., Fujioka, S., and Sieberer, T. (2011). CESTA, a positive regulator of brassinosteroid biosynthesis. EMBO J. **30,** 1149-1161.

Poppenberger, B., Fujioka, S., Soeno, K., George, G.L., Vaistij, F.E., Hiranuma, S., Seto, H., Takatsuto, S., Adam, G., Yoshida, S., and Bowles, D. (2005). The UGT73C5 of *Arabidopsis thaliana* glucosylates brassinosteroids. Proc. Natl. Acad. Sci. USA **102,** 15253-15258.

Postel, S., Kufner, I., Beuter, C., Mazzotta, S., Schwedt, A., Borlotti, A., Halter, T., Kemmerling, B., and Nurnberger, T. (2010). The multifunctional leucine-rich repeat receptor kinase BAK1 is implicated in Arabidopsis development and immunity. Eur. J. Cell Biol. **89,** 169-174.

Pullen, M., Clark, N., Zarinkamar, F., Topping, J., and Lindsey, K. (2010). Analysis of vascular development in the hydra sterol biosynthetic mutants of Arabidopsis. PLoS One **5,** e12227.

Rahimi, R.A., and Leof, E.B. (2007). TGF-beta signaling: A tale of two responses. J. Cell. Biochem. **102,** 593-608.

Rouleau, M., Marsolais, F., Richard, M., Nicolle, L., Voigt, B., Adam, G., and Varin, L. (1999). Inactivation of brassinosteroid biological activity by a salicylate-inducible steroid sulfotransferase from *Brassica napus*. J. Biol. Chem. **274,** 20925-20930.

Rozhon, W., Mayerhofer, J., Petutschnig, E., Fujioka, S., and Jonak, C. (2010). ASKtheta, a group III Arabidopsis GSK3, functions in the brassinosteroid signalling pathway. Plant J. **62,** 215-223.

Russinova, E., Borst, J.W., Kwaaitaal, M., Cano-Delgado, A., Yin, Y., Chory, J., and de Vries, S.C. (2004). Heterodimerization and endocytosis of Arabidopsis brassinosteroid receptors BRI1 and AtSERK3 (BAK1). Plant Cell **16,** 3216-3229.

Ryu, H., Cho, H., Kim, K., and Hwang, I. (2010a). Phosphorylation dependent nucleocytoplasmic shuttling of BES1 is a key regulatory event in brassinosteroid signaling. Mol. Cells **29,** 283-290.

Ryu, H., Kim, K., Cho, H., and Hwang, I. (2010b). Predominant actions of cytosolic BSU1 and nuclear BIN2 regulate subcellular localization of BES1 in brassinosteroid signaling. Mol. Cells **29,** 291-296.

Ryu, H., Kim, K., Cho, H., Park, J., Choe, S., and Hwang, I. (2007). Nucleocytoplasmic shuttling of BZR1 mediated by phosphorylation is essential in Arabidopsis brassinosteroid signaling. Plant Cell **19,** 2749-2762.

Sakurai, A., Yokota, T., and Clouse, S.D. (1999). Brassinosteroids: Steroidal Plant Hormones. (Tokyo: Springer).

Sasse, J.M. (1991). The case for brassinosteroids as endogenous plant hormones. In Brassinosteroids Chemistry, bioactivity, & applications, H.G. Cutler, T. Yokota, and G. Adam, eds (Washington, D.C.: American Chemical Society), pp. 158-166.

Sasse, J.M. (2003). Physiological Actions of Brassinosteroids: An Update. J. Plant Growth Regul. **22,** 276-288.

Schaller, H. (2003). The role of sterols in plant growth and development. Prog. Lipid Res. **42,** 163-175.

Schlereth, A., Moller, B., Liu, W., Kientz, M., Flipse, J., Rademacher, E.H., Schmid, M., Jurgens, G., and Weijers, D. (2010). MONOPTEROS controls embryonic root initiation by regulating a mobile transcription factor. Nature **464,** 913-916.

Schneider, B., Kolbe, A., Porzel, A., and Adam, G. (1994). A metabolite of 24-epibrassinolide in cell suspension cultures of *Lycopersicon esculentum*. Phytochem. **36,** 319-321.

Schrick, K., Mayer, U., Martin, G., Bellini, C., Kuhnt, C., Schmidt, J., and Jurgens, G. (2002). Interactions between sterol biosynthesis genes in embryonic development of Arabidopsis. Plant J. **31,** 61-73.

Schrick, K., Mayer, U., Horrichs, A., Kuhnt, C., Bellini, C., Dangl, J., Schmidt, J., and Jurgens, G. (2000). FACKEL is a sterol C-14 reductase required for organized cell division and expansion in Arabidopsis embryogenesis. Genes Dev. **14,** 1471-1484.

Schulze, B., Mentzel, T., Jehle, A.K., Mueller, K., Beeler, S., Boller, T., Felix, G., and Chinchilla, D. (2010). Rapid heteromerization and phosphorylation of ligand-activated plant transmembrane receptors and their associated kinase BAK1. J. Biol. Chem. **285,** 9444-9451.

Schumacher, K., Vafeados, D., McCarthy, M., Sze, H., Wilkins, T., and

Chory, J. (1999). The Arabidopsis *det3* mutant reveals a central role for the vacuolar H(+)-ATPase in plant growth and development. Genes Dev. **13,** 3259-3270.

Sekimata, K., Kimura, T., Kaneko, I., Nakano, T., Yoneyama, K., Takeuchi, Y., Yoshida, S., and Asami, T. (2001). A specific brassinosteroid biosynthesis inhibitor, Brz2001: evaluation of its effects on Arabidopsis, cress, tobacco, and rice. Planta **213,** 716-721.

Shang, Y., Lee, M.M., Li, J., and Nam, K.H. (2011). Characterization of *cp3* reveals a new *bri1* allele, *bri1-120*, and the importance of the LRR domain of BRI1 mediating BR signaling. BMC Plant Biol. **11,** 8.

She, J., Han, Z., Kim, T.W., Wang, J., Cheng, W., Chang, J., Shi, S., Yang, M., Wang, Z.Y., and Chai, J. (2011). Structural insight into brassinosteroid perception by BRI1. Nature**,** 472-776.

Shimada, Y., Fujioka, S., Miyauchi, N., Kushiro, M., Takatsuto, S., Nomura, T., Yokota, T., Kamiya, Y., Bishop, G.J., and Yoshida, S. (2001). Brassinosteroid-6-oxidases from Arabidopsis and tomato catalyze multiple C-6 oxidations in brassinosteroid biosynthesis. Plant Physiol. **126,** 770-779.

Shiu, S.H., and Bleecker, A.B. (2001). Receptor-like kinases from Arabidopsis form a monophyletic gene family related to animal receptor kinases. Proc. Natl. Acad. Sci. USA **98,** 10763-10768.

Souter, M., Topping, J., Pullen, M., Friml, J., Palme, K., Hackett, R., Grierson, D., and Lindsey, K. (2002). *hydra* Mutants of Arabidopsis are defective in sterol profiles and auxin and ethylene signaling. Plant Cell **14,** 1017-1031.

Steber, C.M., and McCourt, P. (2001). A role for brassinosteroids in germination in Arabidopsis. Plant Physiol. **125,** 763-769.

Steffens, G.L. (1991). U.S. Department of Agriculture Brassins Project: 1970-1980. In Brassinosteroids Chemistry, bioactivity, & applications, H.G. Cutler, T. Yokota, and G. Adam, eds (Washington, D.C.: American Chemical Society), pp. 2-17.

Sun, Y., Fan, X.Y., Cao, D.M., Tang, W., He, K., Zhu, J.Y., He, J.X., Bai, M.Y., Zhu, S., Oh, E., Patil, S., Kim, T.W., Ji, H., Wong, W.H., Rhee, S.Y., and Wang, Z.Y. (2010). Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in Arabidopsis. Dev. Cell **19,** 765-777.

Suzuki, M., Xiang, T., Ohyama, K., Seki, H., Saito, K., Muranaka, T., Hayashi, H., Katsube, Y., Kushiro, T., Shibuya, M., and Ebizuka, Y. (2006). Lanosterol Synthase in Dicotyledonous Plants. Plant and Cell Physiology **47,** 565-571.

Symons, G.M., and Reid, J.B. (2003). Hormone levels and response during de-etiolation in pea. Planta **216,** 422-431.

Symons, G.M., Ross, J.J., Jager, C.E., and Reid, J.B. (2008a). Brassinosteroid transport. J. Exp. Bot. **59,** 17-24.

Symons, G.M., Smith, J.J., Nomura, T., Davies, N.W., Yokota, T., and Reid, J.B. (2008b). The hormonal regulation of de-etiolation. Planta **227,** 1115-1125.

Szekeres, M., Nemeth, K., Koncz-Kalman, Z., Mathur, J., Kauschmann, A., Altmann, T., Redei, G.P., Nagy, F., Schell, J., and Koncz, C. (1996). Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in Arabidopsis. Cell **85,** 171-182.

Takahashi, T., Gasch, A., Nishizawa, N., and Chua, N. (1995). The *DIMINUTO* gene of *Arabidopsis* is involved in regulating cell elongation. Genes Dev. **9,** 97-107.

Tanaka, K., Asami, T., Yoshida, S., Nakamura, Y., Matsuo, T., and Okamoto, S. (2005). Brassinosteroid homeostasis in Arabidopsis is ensured by feedback expressions of multiple genes involved in its metabolism. Plant Physiol. **138,** 1117-1125.

Tang, W., Deng, Z., Oses-Prieto, J.A., Suzuki, N., Zhu, S., Zhang, X., Burlingame, A.L., and Wang, Z.Y. (2008a). Proteomics studies of brassinosteroid signal transduction using prefractionation and two-dimensional DIGE. Mol Cell Proteomics **7,** 728-738.

Tang, W., Kim, T.W., Oses-Prieto, J.A., Sun, Y., Deng, Z., Zhu, S., Wang, R., Burlingame, A.L., and Wang, Z.Y. (2008b). BSKs mediate signal transduction from the receptor kinase BRI1 in Arabidopsis. Science **321,** 557-560.

Tang, W., Yuan, M., Wang, R., Yang, Y., Wang, C., Oses-Prieto, J.A., Kim, T.W., Zhou, H.W., Deng, Z., Gampala, S.S., Gendron, J.M., Jonassen, E.M., Lillo, C., Delong, A., Burlingame, A.L., Sun, Y., and Wang, Z.Y. (2011). PP2A activates brassinosteroid-responsive gene expression and plant growth by dephosphorylating BZR1. Nat. Cell Biol. **13,** 124-131.

Thornton, L.E., Rupasinghe, S.G., Peng, H., Schuler, M.A., and Neff, M.M. (2010). Arabidopsis CYP72C1 is an atypical cytochrome P450 that inactivates brassinosteroids. Plant Mol. Biol. **74,** 167-181.

Toledo-Ortiz, G., Huq, E., and Quail, P.H. (2003). The Arabidopsis basic/helix-loop-helix transcription factor family. Plant Cell **15,** 1749-1770.

Tominaga, R., Sakurai, N., and Kuraishi, S. (1994). Brassinolide-induced elongation of inner tissues of segments of squash (*Cucurbita maxima* Duch.) hypocotyls. Plant Cell Physiol. **35,** 1103-1106.

Torii, K.U. (2004). Leucine-rich repeat receptor kinases in plants: structure, function, and signal transduction pathways. Int. Rev. Cytol. **234,** 1-46.

Turk, E.M., Fujioka, S., Seto, H., Shimada, Y., Takatsuto, S., Yoshida, S., Denzel, M.A., Torres, Q.I., and Neff, M.M. (2003). CYP72B1 inactivates brassinosteroid hormones: an intersection between photomorphogenesis and plant steroid signal transduction. Plant Physiol. **133,** 1643-1653.

Turk, E.M., Fujioka, S., Seto, H., Shimada, Y., Takatsuto, S., Yoshida, S., Wang, H., Torres, Q.I., Ward, J.M., Murthy, G., Zhang, J., Walker, J.C., and Neff, M.M. (2005). BAS1 and SOB7 act redundantly to modulate Arabidopsis photomorphogenesis via unique brassinosteroid inactivation mechanisms. Plant J. **42,** 23-34.

Vert, G., and Chory, J. (2006). Downstream nuclear events in brassinosteroid signalling. Nature **441,** 96-100.

Vert, G., Nemhauser, J.L., Geldner, N., Hong, F., and Chory, J. (2005). Molecular mechanisms of steroid hormone signaling in plants. Ann. Rev. Cell Dev. Biol. **21,** 177-201.

Wang, H., Zhu, Y., Fujioka, S., Asami, T., and Li, J. (2009). Regulation of Arabidopsis brassinosteroid signaling by atypical basic helix-loop-helix proteins. Plant Cell **21,** 3781-3791.

Wang, T.W., Cosgrove, D.J., and Arteca, R.N. (1993). Brassinosteroid Stimulation of Hypocotyl Elongation and Wall Relaxation in Pakchoi (*Brassica chinensis* cv *Lei-Choi*). Plant Physiol. **101,** 965-968.

Wang, X., and Chory, J. (2006). Brassinosteroids regulate dissociation of BKI1, a negative regulator of BRI1 signaling, from the plasma membrane. Science **313,** 1118-1122.

Wang, X., Li, X., Meisenhelder, J., Hunter, T., Yoshida, S., Asami, T., and Chory, J. (2005a). Autoregulation and homodimerization are involved in the activation of the plant steroid receptor BRI1. Dev. Cell **8,** 855-865.

Wang, X., Zhu, L., Liu, B., Wang, C., Jin, L., Zhao, Q., and Yuan, M. (2007). Arabidopsis MICROTUBULE-ASSOCIATED PROTEIN18 functions in directional cell growth by destabilizing cortical microtubules. Plant Cell **19,** 877-889.

Wang, X., Kota, U., He, K., Blackburn, K., Li, J., Goshe, M.B., Huber, S.C., and Clouse, S.D. (2008). Sequential transphosphorylation of the BRI1/BAK1 receptor kinase complex impacts early events in brassinosteroid signaling. Dev. Cell **15,** 220-235.

Wang, X., Goshe, M.B., Soderblom, E.J., Phinney, B.S., Kuchar, J.A., Li, J., Asami, T., Yoshida, S., Huber, S.C., and Clouse, S.D. (2005b).

Identification and functional analysis of in vivo phosphorylation sites of the Arabidopsis BRASSINOSTEROID-INSENSITIVE1 receptor kinase. Plant Cell **17,** 1685-1703.

Wang, Z.Y., Seto, H., Fujioka, S., Yoshida, S., and Chory, J. (2001). BRI1 is a critical component of a plasma-membrane receptor for plant steroids. Nature **410,** 380-383.

Wang, Z.Y., Nakano, T., Gendron, J., He, J., Chen, M., Vafeados, D., Yang, Y., Fujioka, S., Yoshida, S., Asami, T., and Chory, J. (2002). Nuclear-localized BZR1 mediates brassinosteroid-induced growth and feedback suppression of brassinosteroid biosynthesis. Dev. Cell **2,** 505-513.

Waters, M.T., Wang, P., Korkaric, M., Capper, R.G., Saunders, N.J., and Langdale, J.A. (2009). GLK transcription factors coordinate expression of the photosynthetic apparatus in Arabidopsis. Plant Cell **21,** 1109-1128.

Winter, J., Schneider, B., Strack, D., and Adam, G. (1997). Role of a cytochrome P450-dependent monooxygenase in the hydroxylation of 24-epibrassinolide. Phytochemistry **45,** 233-237.

Wu, G., Wang, X., Li, X., Kamiya, Y., Otegui, M.S., and Chory, J. (2011). Methylation of a phosphatase specifies dephosphorylation and degradation of activated brassinosteroid receptors. Sci. Signal **4,** ra29.

Xi, W., and Yu, H. (2010). MOTHER OF FT AND TFL1 regulates seed germination and fertility relevant to the brassinosteroid signaling pathway. Plant Signal Behav. **5,** 1315-1317.

Xie, L., Yang, C., and Wang, X. (2011). Brassinosteroids can regulate cellulose biosynthesis by controlling the expression of *CESA* genes in Arabidopsis. J. Exp. Bot. **62,** 4495-4506.

Xu, W., Huang, J., Li, B., Li, J., and Wang, Y. (2008). Is kinase activity essential for biological functions of BRI1? Cell Res. **18,** 472-478.

Yamamoto, R., Fujioka, S., Demura, T., Takatsuto, S., Yoshida, S., and Fukuda, H. (2001). Brassinosteroid levels increase drastically prior to morphogenesis of tracheary elements. Plant Physiol. **125,** 556-563.

Yamamoto, R., Fujioka, S., Iwamoto, K., Demura, T., Takatsuto, S., Yoshida, S., and Fukuda, H. (2007). Co-regulation of brassinosteroid biosynthesis-related genes during xylem cell differentiation. Plant Cell Physiol. **48,** 74-83.

Yamamuro, C., Ihara, Y., Wu, X., Noguchi, T., Fujioka, S., Takatsuto, S., Ashikari, M., Kitano, H., and Matsuoka, M. (2000). Loss of function of a rice brassinosteroid insensitive1 homolog prevents internode elongation and bending of the lamina joint. Plant Cell **12,** 1591-1606.

Yan, Z., Zhao, J., Peng, P., Chihara, R.K., and Li, J. (2009). BIN2 functions redundantly with other Arabidopsis GSK3-like kinases to regulate brassinosteroid signaling. Plant Physiol. **150,** 710-721.

Ye, Q., Zhu, W., Li, L., Zhang, S., Yin, Y., Ma, H., and Wang, X. (2010). Brassinosteroids control male fertility by regulating the expression of key genes involved in Arabidopsis anther and pollen development. Proc. Natl. Acad. Sci. USA **107,** 6100-6105.

Yin, Y., Vafeados, D., Tao, Y., Yoshida, S., Asami, T., and Chory, J. (2005). A new class of transcription factors mediates brassinosteroid-regulated gene expression in Arabidopsis. Cell **120,** 249-259.

Yin, Y., Wang, Z.Y., Mora-Garcia, S., Li, J., Yoshida, S., Asami, T., and Chory, J. (2002). BES1 accumulates in the nucleus in response to brassinosteroids to regulate gene expression and promote stem elongation. Cell **109,** 181-191.

Yokota, T. (1997). The structure, biosynthesis and function of brassinosteroids. Trends Plant Sci. **2,** 137-143.

Yokota, T. (1999). The History of Brassinosteroids: Discovery to Isolation of Biosynthesis and Signal Transduction Mutants. In Brassinosteroids: Steroidal Plant Hormones, A. Sakurai, T. Yokota, and S.D. Clouse, eds (Tokyo: Springer), pp. 1-20.

Yokota, T., Ogino, Y., Suzuki, H., Takahashi, N., Saimoto, H., Fujioka, S., and Sakurai, A. (1991). Metabolism and biosynthesis of brassinosteroids. In Brassinosteroids Chemistry, Bioactivity, & Applications., H. Cutler, T. Yokota, and G. Adam, eds (Washington, D.C.: American Chemical Society), pp. 86-96.

Yu, X., Li, L., Guo, M., Chory, J., and Yin, Y. (2008). Modulation of brassinosteroid-regulated gene expression by Jumonji domain-containing proteins ELF6 and REF6 in Arabidopsis. Proc. Natl. Acad. Sci. USA **105,** 7618-7623.

Yu, X., Li, L., Zola, J., Aluru, M., Ye, H., Foudree, A., Guo, H., Anderson, S., Aluru, S., Liu, P., Rodermel, S., and Yin, Y. (2011). A brassinosteroid transcriptional network revealed by genome-wide identification of BESI target genes in *Arabidopsis thaliana*. Plant J. **65,** 634-646.

Zhang, L.Y., Bai, M.Y., Wu, J., Zhu, J.Y., Wang, H., Zhang, Z., Wang, W., Sun, Y., Zhao, J., Sun, X., Yang, H., Xu, Y., Kim, S.H., Fujioka, S., Lin, W.H., Chong, K., Lu, T., and Wang, Z.Y. (2009). Antagonistic HLH/bHLH transcription factors mediate brassinosteroid regulation of cell elongation and plant development in rice and Arabidopsis. Plant Cell **21,** 3767-3780.

Zhao, J., Peng, P., Schmitz, R.J., Decker, A.D., Tax, F.E., and Li, J. (2002). Two putative BIN2 substrates are nuclear components of brassinosteroid signaling. Plant Physiol. **130,** 1221-1229.

Zhao, Y.-J., Xu, R.-J., and Luo, W.-H. (1990). Inhibitory effects of abscisic acid on epibrassinolide-induced senescence of detached cotyledons in cucumber seedlings. Chin. Sci. Bull. **35,** 928-931.

Zurek, D.M., Rayle, D.L., McMorris, T.C., and Clouse, S.D. (1994). Investigation of Gene Expression, Growth Kinetics, and Wall Extensibility during Brassinosteroid-Regulated Stem Elongation. Plant Physiol. **104,** 505-513.