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- Review -

Update on Phosphorylation-Mediated Brassinosteroid Signaling Pathways

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Protein phosphorylation is a universal mechanism that regulates cellular activities. The brassinosteroid (BR) signal transduction pathway is a relay of phosphorylation and dephosphorylation cascades. It starts with the BR-induced activation of the membrane receptor kinase brassinosteroid insensitive 1 (BRI1), resulting in the dephosphorylation of transcription factors such as BZR1/BES2 and BZR2/BES1 followed by BR-induced gene expression. Brassinosteroid signal transduction research has progressed rapidly by identifying the phosphorylation/dephosphorylation site(s) of the BR-regulated kinase and phosphatase substrates with a simultaneous pursuit of mutant phenotypes. Autophosphorylation, transphosphorylation, and serine/threonine and tyrosine phosphorylation of the receptor protein kinases BRI1 and BRI1-associated kinase (BAK1) have increased the understanding of the regulatory role of those kinases during physiological and developmental processes in plants. The phosphorylation event initiated by BR is also found in the regulation of receptor-mediated endocytosis and the subsequent degradation of the receptor. However, the basic molecular links of the BR signal transduction pathway are not well understood regarding this phosphorylation/dephosphorylation event. This review summarizes the current state of BR signal transduction research to uncover the phosphorylation/dephosphorylation networks and suggests directions for future research on steroid signal transduction to gain a more comprehensive understanding of the process.

Key words : Brassinosteroid, protein phosphorylation, leucine-rich repeat receptor-like-protein kinase (LRR-RLK), phosphatase, receptor-like cytoplasmic kinase (RLCK)

Introduction

Brassinosteroids are the only group of steroid hormones discovered in plants, which are different from steroid hormones in the animal system that has several different steroid hormones. Brassinosteroids regulate plant growth and development such as cell elongation, vascular development, senescence, stress responses, and photomorphogenesis [10,11,68]. BR-related mutants of the brassinosteroid biosynthesis pathway and signal transduction exhibited a short hypocotyl, opened cotyledons, and de-etiolation in the dark. Under light conditions, they exhibited severe dwarfism, aggregated and dark-green rosette leaves with epinasty, decreased elongation of the petiole, defects in the fertility of pollens, apical dominance, and senescence [9,69]. Therefore, brassinosteroids have pivotal roles in plant growth and differentiation [11,18]. In animal systems, steroid ligands bind to nuclear binding receptors that act as transcriptional regulators [65]. However, in plant systems, BRs bind to BRI1, an LRR-RLK on the plasma membrane, and this binding induces a signal, which is transduced to the nucleus through several signaling components in the cytoplasm [33,63].

For the past few years, information on brassinosteroid-mediated phosphorylation has increased enormously. Not only serine/threonine phosphorylation but also tyrosine phosphorylation have been found, and the significance of autophosphorylation and transphosphorylation became known. Recently, many phosphorylation sites on protein kinases in the brassinosteroid signal transduction pathway have been discovered [32].

In this review, an updated brassinosteroid signal transduction pathway is presented and new information is added on proteins related to phosphorylation such as kinases, phosphatases, and kinase-binding proteins are discussed. Abbreviated names of the proteins in the BR signal transduction pathway are summarized in Table 1.

Brassinosteroid signal transduction pathway: synopsis and update

The brassinosteroid signal transduction pathway and its molecular mechanism have been intensively studied using the yeast two-hybrid system and screening for suppressor

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mutations of BR-related mutants [59,63]. One of the best-studied LRR-RLKs is BRI1 [1]. BAK1, a phylogenetically different LRR-RLK from BRI1, is a binding partner of BRI1 [40]. In the absence of BR, BKI1 binds to BRI1 to inhibit binding between BRI1 and BAK1 and inactivates BRI1 [60]. BIN2, a cytoplasmic GSK3/SHAGGY-like kinase, which is downstream of BRI1 and BAK1, phosphorylates transcription factors, BZR1/BES2 and BZR2/BES1. These phosphorylated proteins bind to 14-3-3 proteins in the nucleus and move to the cytoplasm together to be degraded by a proteosome in the absence of BR [19,49]. In the presence of BR, BRI1 and BAK1 are activated by autophosphorylation and homo/heterodimerization and endocytized. On the other hand, the activated BRI1 phosphorylates CDG1 [30] and BSK1 [56] and then these kinases bind to BSU1, which dephosphorylates and inactivates BIN2 [62]. Recently, it was reported that BRI1 and BAK1 autophosphorylate tyrosines including the tyrosine on BKI1 so that BKI1 is released from the membrane in response to BR perception [25]. At the same time, a phosphatase, BSU1, dephosphorylates BIN2 so that BR

Table 1. List of the abbreviated words of the name of the proteins involved in BR signal transduction

Abbreviation	Full name	Function
BR	BRASSINOSTEROID	Plant steroid hormone
BRI1	BRASSINOSTEROID INSENSITIVE 1	Leucine-rich repeat receptor-like protein kinase, BR receptor
BRL	BRI1-LIKE	Homologues of BRI1
BAK1	BRI1-ASSOCIATED KINASE 1	BRI1-binding protein kinase
BSK1	BR SIGNALING KINASE	protein kinase, positive regulator
BKI1	BRI1 KINASE INHIBITOR 1	An inhibitor of BRI
BIN2	BRASSINOSTEROID INSENSITIVE 2	cytoplasmic GSK3/SHAGGY-like kinase; negative regulator in BR signaling
PP2A	Protein phosphatase 2A	phosphatase that dephosphorylates phosphorylated BZR1/2
BSU1	BRI1 SUPPRESSOR 1	phosphatase that dephosphorylates phosphorylated BIN2 and deactivate it
BZR1/2	BRASSINAZOLE RESISTANT 1/2	Transcription factors involved in BR-induced gene expression; BZR1/2 are allelic to BZR2/1, respectively
BES1/2	*bri1*-EMS SUPPRESSOR 2	
CDG1	CONSTTUTIVELY DIFFERENTIAL GROWTH 1	protein kinase that phosphorylates BSU1 and activates it.
TTL1	TRANSTHYRETIN-LIKE	Substrates of BRI1. Their function is still under investigation.
TRIP-1	TGFβ RECEPTOR INTERACTING PROTEIN-1	
KAPP	KINASE-ASSOCIATED PROTEIN PHOSPHATASE	
SBI1	SUPPRESSOR OF BRI1	leucine carboxylmethyl-transferase, activates PP2A
LRR-RLK	Leucine-rich repeat receptor-like-protein kinase	Plasma membrane-located protein kinase. It acts as a receptor which binds to a specific ligand.
RLCK	Receptor-like cytoplasmic kinase	cytoplasmic protein kinase devoid of the transmembrane and extracellular domains
SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1/3/4	SERK1/3/4	Protein kinases. SERK3 is the same as BAK1,
leucine-rich repeats	LRRs	Structural domains of BRI1 (and BAK1) which have phosphorylation sites that are involved in the kinase activity and binding to other proteins
island domain	ID	
juxtamembrane region	JD	
kinase catalytic domain	KD	
carboxyl-terminal tail	CT	

stimulates cell elongation [31, 38]. This mechanism has been compared to that of the Wnt pathway in metazoans where GSK3 kinase phosphorylates β-catenin, which activates Wnt-responsive target genes [8]. It was reported that PP2A dephosphorylates phosphorylated transcription factors, BZR1/BES2 and BZR2/BES1 [57]. Finally, the dephosphorylated BZR1 /BES2 and BZR2/BES1 regulate the BR-responsive genes [9,32,69].

BRASSINOSTERROID INSENSTITIVE 1 (BRI1)

The LRR-RLK family is comprised of over 400 genes in *Arabidopsis* and over 600 genes in rice [52]. BRI1 is one of them [34]. The BR signal transduction pathway which involves BRI1-mediated RLK signaling is the best-studied pathway [32]. BR signaling involves ligand-induced BRI1 homodimerization or homo-oligomerization and hetero-oligomerization with a co-receptor kinase, BAK1 [40]. Autophosphorylation of BRI1 is also required for the regulation of its activity and binding to its interacting proteins [61].

In general, BRI1 consists of three major domains: the extracellular domain, transmembrane domain, and intracellular kinase domain [32,68]. The extracellular domain is divided into three subdomains: amino-terminal signal peptide, 24 LRRs and an ID located between the 20^{th} and 21^{st} LRRs [59]. The intracellular domain is divided into intracellular JM, KD, and CT. The basic kinase activity resides in the kinase catalytic domain whose point mutations inactivate the receptor [17]. The phosphorylation sites on BRI1 were identified by immunoprecipitation from BR-treated *Arabidopsis* seedlings followed by liquid chromatography-tandem mass spectrometry analysis [61].

BRI1 is activated by the phosphorylation of multiple serine/threonine residues and tyrosine residues [25,42,44]. BR treatment promotes the interaction between BRI1 and BAK1 and increases their phosphorylation [61,62]. The critical phosphorylation sites in the catalytic domain, which are responsible for the kinase activity, turned out to be on T1049, S1044, and T1045 [61,62]. Since the C-terminal tail-less kinase has a stronger kinase and autophosphorylation activity, the C-terminal tail is supposed to be a negatively controlling domain to keep the BRI1 in a basal inactive state in the absence of BR [63]. Two tyrosine residues, Y956 and Y1072, are involved in the kinase catalytic activity.

When BR binds to BRI1, the kinase undergoes a conformational change and induces autophosphorylation. This releases BRI1 from the inhibition by the C-terminal tail [61]. At the same time, a specific negative regulator, BKI1, is also released from BRI1 [60]. Four autophosphorylation sites: S838, T842, T846, and S858 in the JM domain are important for the inhibition of the kinase activity [43,61,62], and S1162, S1166, S1168, and T1180 in the CT domain are important for the self-inhibition of BRI1 [61,62]. Multiple substitutions of these residues to Asp increased the BRI1 activity in the absence of BAK1 [63]. The developmentally verified phosphorylation site of BRI1 is Y831 whose mutation affects leaf development and flowering [44]. Recently, it was found that autophosphorylation could occur on the tyrosine residue in BRI1 and BAK1 [44]. The Y1052 and Y831 in BRI1 are important for BR signaling [44].

There are several BRI1-interacting substrates other than BAK1- BKI1, TTL1, BSK1, CDG1, KAPP, and TRIP-1. The first substrate BKI1 acts as a negative regulator of BR signaling [60]. An *in vitro* pull-down assay showed that BKI1 binds to BRI1 and hinders BAK1 binding to BRI1 [60]. Upon binding of BR to BRI1, BKI1 dissociates from BRI1 [60]. An *in vitro* kinase assay showed that BRI1 phosphorylates the Y211 residue of BKI1, which leads to the dissociation of BKI from BRI1 by an unknown mechanism [25,60]. BKI1 binds more strongly to the inactive BRI1 than to the active one [41].

The second substrate of BRI1 is TTL1 [41]. BRI1 phosphorylates TTL1 and TTL1 binds only to the activated BRI1 [41]. TTL1 may inhibit the activated BR receptor. The involvement of TTL1 in BR signaling by the interaction with BRI1 remains to be elucidated. Contrary to the affinity of BKI1 to the inactive BRI1, TTL1 has a greater affinity to the active BRI1 than to the inactive one [60].

The third substrate is BSK1. BSK1 belongs to the RLCK XII family [39,56]. The difference between the transmembrane RLKs and RLCK is the absence of the transmembrane and extracellular domains in the RLCK [52]. BSK1 is located in the membrane and is phosphorylated at S230 by BRI1 [56]. This phosphorylation increases the binding affinity of BSK1 to BSU1 [56]. BRI1 phosphorylates the S230 residue of BSK1 *in vitro* and it can interact with BSK1 *in vivo* [56]. BR treatment weakens the interaction between BRI1 and BSK1 [56]. Experiments using the knockdown mutant, *bsk3-1*, showed reduced BR signaling, and BSK1 overexpression partially rescued the *bri1-5* phenotype suggesting that BSK1 is one of the positive regulators of BR signaling

[56]. The precise mechanism for the activation of BSU1 by BSK1 is not clear, but the binding of the phosphorylated BSK1 to BSU1 induces the dephosphorylation of phosphorylated Y200 in BIN2, and finally, the negatively regulating BIN2 is inactivated [31].

The forth substrate is CDG1 [30]. CDG1 is another RLCK identified after BSK1 [30]. The identification of CDG1 has great importance that it added a new route for BRI1-mediated BR signaling from BRI1 to BSU 1 [30]. CDG1 is a plasma membrane protein, which is attached to the membrane by palmitoylation on the two cysteine residues in the N-terminal region [30]. CDG1 is phosphorylated at S234 by activated BRI1 upon BR treatment, and then, it phosphorylates BSU1 at S764 [30]. This phosphorylated BSU1 dephosphorylates phosphorylated Y200 in BIN2 to inhibit the phosphorylation of BZR1/BES2. The phosphorylation of CDG1 does not increase the catalytic activity of the BSU1 but enhances the affinity between BSU1 and BIN2 [30].

The next substrate is KAPP [50]. It contains the phosphopeptide binding domain and interacts with SERK1, BRI1, and BAK1 [13]. The increased sensitivity to BR in the *kapp-bri1-5* double mutant suggests that KAPP is a negatively regulating molecule in BR signaling [50].

The last substrate is TRIP-1. TRIP-1 interacts with BRI1 *in vitro* and *in vivo* and it is phosphorylated by BRI1 *in vitro* [16]. It is presumed that TRIP-1 might play a role in BR-mediated translational control, since TRIP-1 is an essential subunit of the eIF3 eukaryotic translation initiation factor in other systems, but there is no direct evidence [13].

BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1)

In the BR receptor complex, another receptor kinase BAK1 is involved in BR signal transduction [36, 40, 48]. BAK1 was cloned from a yeast two-hybrid screen for potential BRI1-interacting proteins [40] and from an activation-tagging screen looking for suppressors of *bri1-5* [36]. There are at least two homologs of BAK1, BRL1 and BRL3, which bind with BRI1 and their mutation rescues the *bri1* mutant [5,74]. These homologs are involved in the development of vascular tissues [5]. BRI1 and BAK1 can interact with each other *in vitro* and *in vivo* through their kinase domains [36,40]. This kinase is also called the SERK3, which is a member of SERK family of LRR-RLKs [23]. Actually, for the full expression of BR signaling, hetero-oligomerization of BRI1 with LRR-RLKs from the subfamily of SERK kinases is necessary [23]. For example, BRI1 binds to BAK1 (SERK3) [36,40], SERK4 [22], and SERK1 which is involved in embryogenesis [27].

Since BAK1 cannot bind with BR, BAK1 should be activated after the binding of BR to BRI1 [63]. Transphosphorylation increases the kinase activity of the BRI1-BAK1 complex by partial phosphorylation of BAK1 by BRI1 and BRI1 phosphorylation by BAK1 in turn [62]. BAK1 also has a critical phosphorylation site for its catalytic activity. L46 seems to be involved in the binding of BAK1 to BRI1 since its point mutation to glutamate abolished BAK1 binding to BRI1 in yeast [72].

There are six phosphorylation sites in the intracellular region of BAK1. T445, T446, T449, and T450 are in the activation loop and S290 and T312 are in the CT domain [28,62]. T445 is essential for the kinase activity [62]. Multiple mutations in T446, T449, and T450 abolished the kinase activity [62,72]. BAK1 transphosphorylates S838, T846 and S858 in the JM domain and S1166 and S1180 in the CT domain of BRI1 [62]. By these transphosphorylations, BAK1 activates BRI1 [62]. Interestingly, there is a negatively acting residue, T872, whose mutation increases the kinase activity [61]. S286 is identified as an autophosphorylation site *in vitro*, but it is not known whether it is involved in the desensitization process *in vivo* [63].

Recently, the additional roles of BAK1 have been intensively studied. BAK1 is involved in a light-dependent cell death [21], pathogenesis [4,6,24,29,46,51,54], and male sporogenesis [2,12]. These reports support the notion that BAK1 can act in a BRI1-independent way by binding to different LRR-RLKs which bind different ligands. The notion that the individual phosphorylation site in BAK1 affects different signaling outputs is supported by the fact that a mutation in T450 rescued the cell death and dwarf phenotypes of the *bak1bkk1* mutant but failed to rescue the flagellin-insensitive phenotype in the same mutant [61].

BRASSINOSTEROID INSENSITIVE 2 (BIN2)

BIN2 (allelic to *UCU1* or *DWF12*) encodes the GSK3/SHAGGY-like kinase [7,35,46]. BIN2 is downstream of BRI1 and acts as a negative regulator of BR signaling [35]. BIN2 phosphorylates BZR2/BES1 and BZR1/BES2 in the nucleus [58]. BZR2/BES1 and BZR1/BES2 are nuclear-localized transcription factors [64, 70]. Phosphorylation of these proteins decreases the affinity to the BR-responsive promoters (E-box

for BES1, CANNTG; BRRE for BZR1/BES2, BR Response element, CGTG(T/C)G and recruits the 14-3-3 protein for cytoplasmic retention of BZR2/ BES1 and BZR1/BES2 [19,49]. In the absence of BR, both BZR2/BES1 and BZR1/BES2 are found in the cytoplasm and nucleus, but, upon BR treatment, their dephosphorylated form accumulates in the nucleus [19, 49]. BZR2/BES1 and BZR1/BES2 are downstream of BIN2 and act as positive regulators of BR signaling. The consensus site of BIN2 phosphorylation is S/TXXXS/T (S/T stands for serine or threonine and X stands for any amino acid) and this is repeated 20 times in the BZR2/BES1 and BZR1/BES2 protein [64,71].

bri-1-Suppressor 1, PROTEIN PHOSPHATASE 1 (BSU1)

BSU1 is a nuclear serine/threonine protein phosphatase [31, 38]. BSU1 interacts with BZR2/BES1 in yeast, and dephosphorylates BZR2/BES1 [31, 38]. Since phosphorylated BZR2/BES1 is the inactive form, BSU1 is a positive regulator of BZR2/BES1-mediated transcription. However, there is some discord on the function of BSU1 because overexpression of BSU1 partially suppressed the *bin2-1* phenotype in one study [38] and the same overexpression did not rescue the *bin2-1* phenotype but suppressed the *bri1-5* phenotype in another study [31]. Recently, it is found that phosphorylated BSKs interact with BSU1, and BSU1 dephosphorylates the phosphorylated Y200 of BIN2 [31]. Therefore, it is proposed that both BSK1 and BSU1 are downstream of BRI1 to dephosphorylate BIN2 and inactivate it [31].

PROTEIN PHOSPHATASE 2A (PP2A)

PP2A is a heterotrimeric serine/threonine phosphatase, which consists of scaffolding subunit A, regulatory subunit B, and catalytic subunit C [26]. BZR1/BES2-dephosphorylating PP2A was identified by tandem affinity purification screening for BZR1/BES2-interacting proteins [57]. PP2AB' isoforms interact with BZR1/BES2 *in vivo* and *in vitro* [57] and bind with the PEST domain in BZR1/BES2 [57]. PEST domains are known to mediate BZR1/BES2 and BZR2/BES1 degradation [20,70]. This might explain the stability of *bzr1-1D*, which has a mutation in the PEST domain and still functions as the dephosphorylated form (active form). In addition, a loss-of-function mutant of PP2A caused the accumulation of the dephosphorylated BZR1/BES2, and overexpression of the PP2AB' component increased both the phosphorylated and dephosphorylated BZR1/BES2, which suggests that PP2A is involved in the protein degradation of BZR1/BES2. The action of PP2A is also related to the 14-3-3 protein binding to BZR1/BES2 [57]. The BZR1/BES2 homolog binds to the PP2A B', α and β subunits [57].

Interestingly, PP2A dephosphorylates the kinase domain of BRI1 *in vitro*, and it is a substrate of the SBI1, a leucine carboxylmethyltransferase [14,67]. *sbi1* was found in the process of screening for suppressors of *bri1-5*, and it showed a constitutive BR response due to the accumulation of BRI1 [67]. Carboxylmethylation of PP2A by SBI1 did not change the PP2A activity but directed its activity and localization [55]. The significance of this finding is that it is the first time showing the possibility of regulating the concentration of activated BRI1 receptors on the plasma membrane by the dephosphorylation of BRI1 through PP2A, which is under the control of SBI1. Direct dephosphorylation of the targeted phosphorylated residues can regulate the recycling of receptors by both endocytosis and degradation [3].

Concluding remarks and perspectives

For the past few years, the regulatory roles of kinases and phosphatases in the BR signal transduction pathway have been identified by site-directed mutagenesis followed by biochemical binding assays, *in planta* transformation, immunoprecipitation of the epitope-tagged BRI1 and BAK1 proteins, tandem mass spectrophotometry, and *in vitro* and *in vivo* phosphorylation assays. After the discovery of tyrosine phosphorylation, BRI1 is now considered as a dual specificity kinase and that the initial event of BR signaling is becoming more comparable to the signaling of membrane receptor kinases in the animal system such as TGFβ [16,37,47] and EGF signaling [3,15,53]. BIN2 can be compared to Wnt signaling pathway [58,66].

Fig. 1 shows a model of the phosphorylation-mediated brassinosteroid signal transduction pathway based on published reports from the initial discovery of BRI1 to BR-dependent gene expression. CDG1 and SBI1 were added to the most recent model published by Ye *et al.* [69] and Clouse [9]. More mechanisms need to be elucidated regarding the endocytosis of BRI1 and the action of PP2A on BRI1.

Many phosphorylation sites have been identified and some of them have been verified in terms of their biological significance and authenticity in BR signaling. However,

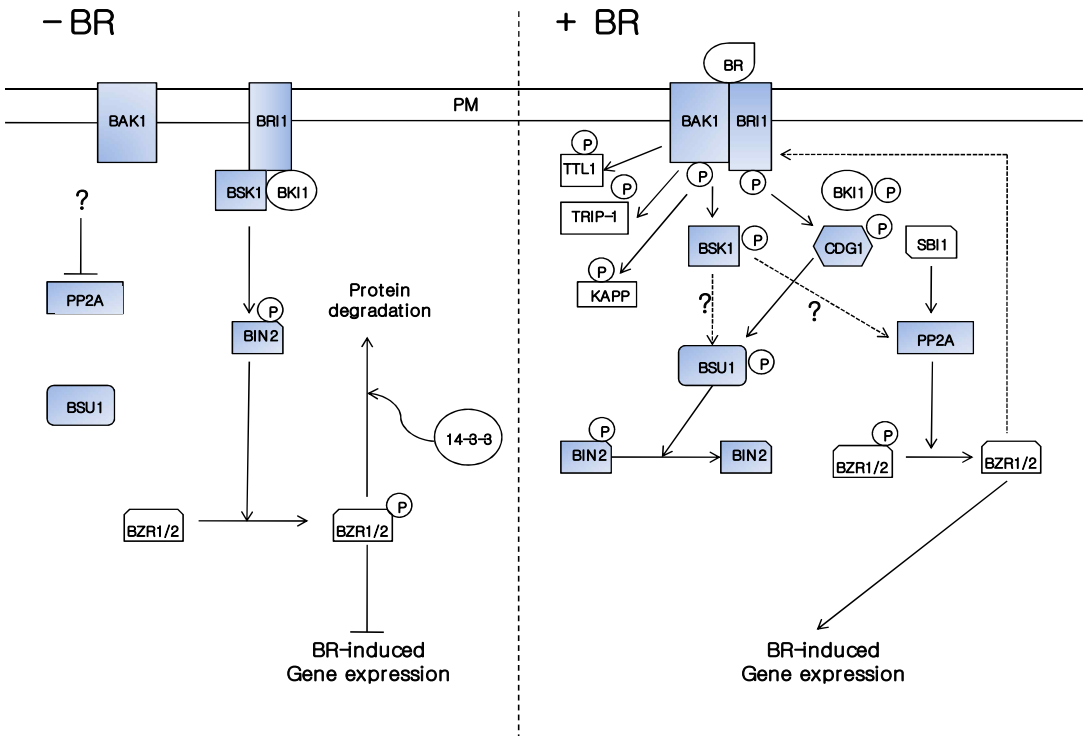

Fig. 1. Model of the BR signal transduction pathway. In the absence of BR, BKI1 inhibits BRI1 function. The negative regulator BIN2 phosphorylates BZR1/BES2 and BZR2/BES1 to inhibit BR-induced gene expression. There are three proposed mechanisms to explain the inhibition of BZR1/BES2 and BZR2/BES1 function: reduced affinity to the BR-responsive element, phosphorylation-induced BZR1/BES2 and BZR2/BES1 degradation, and binding of 14-3-3 proteins to the phosphorylated BZR1/BES2 and BZR2/BES1, which leads to the cytoplasmic retention of BZR1/BES2 and BZR2/BES1. In the presence of BR, BKI1 is phosphorylated by BRI1 and dissociates from BRI1. BAK1 is also phosphorylated by BRI1 and it binds to BRI1. At the same time, BRI1 and BAK1 are autophosphorylated, and BAK1 transphosphorylates BRI1 to enhance BRI1 activity. Activated BRI1 phosphorylates several substrates such as BSKs, CDG1, KAPP, and TRIP-1. Phosphorylation of BSK1 by BRI1 results in the association of BSK1 with BSU1, which leads to the dephosphorylation and inactivation of BIN2. At the same time, the phosphorylated CDG1 also promotes the binding of BSU1 to BIN2. The inactivation of BIN2 and the subsequent dephosphorylation of BZR1/BES2 and BZR2/BES1 lead to the accumulation of BZR1/BES2 and BZR2/BES1 in the nucleus and to the regulation of BR-responsive gene expression. PP2A dephosphorylates phosphorylated BZR1/BES2 and BZR1/BES1. The leucine carboxyl methyltransferase, SBI1, acts on PP2A to control its activity. The effect of PP2A on BRI1 remains to be elucidated. P in a circle indicates phosphate residue. Kinases and phosphatases are drawn in the light blue color. Dots line with an arrowhead indicates that it needs further verification. PM: plasma membrane.

phosphorylation sites shown by *in vitro* methods need to be tested to determine if they are significant sites in BR signaling *in vivo*. In the case of BRI1, there are multiple phosphorylation sites in the CT domain leading to several questions. Are individual phosphorylation sites phosphorylated in a sequential (hierarchal) manner? There is one report that BAK1 can enhance BRI1 activity quantitatively by transphosphorylation [62]. However, the order of phosphorylation is not known. Do the phosphorylation sites act as a bar code for reaction specificity? For example, if there are four multiple phosphorylation sites, does the phosphorylation of the first and third sites have a different reaction specificity from that of the first and fourth sites? Do the phosphorylation sites have tissue and/or developmental specificity? Phosphorylation research related to BR signaling is still a widely open area of study.

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초록 : 단백질 인산화에 의해 매개되는 브라시노스테로이드 신호전달 연구의 최근 상황

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단백질 인산화는 세포의 활동을 조절하는 보편적인 과정이다. 브라시노스테로이드(brassinostreoid)에 의해 매개되는 신호전달은 브라시노스테로이드에 의해 활성화된 세포막상의 protein kinase 로부터 인산화되어 있는 전사인자들을 탈인산화하는 연속적인 인산화/탈인산화 과정이다. 브라시노스테로이드에 의해 매개되는 신호전달의 연구는 인산화에 관여하는 kinase 기질상의 아미노산을 밝히고, 그와 관련된 돌연변이체의 표현형을 알아봄으로써 급속하게 발전하였다. BRI1과 BAK1의 자기인산화(autophosphorylation), 상호인산화(transphosphorylation), 타이로신 인산화(tyrosine phosphorylation)를 밝힘으로써 그들의 조절작용을 식물의 생리학적, 발생학적 과정을 더 이해할 수 있는 장이 열렸다. 브라시노스테로이드에 의한 인산화는 수용체에 의해 매개되는 세포 내 함입(endocytosis)과 그에 뒤따르는 수용체의 파괴현상에서도 볼 수 있다. 인산화/탈인산화 과정에 관련하여 브라시노스테로이드에 의해 매개되는 신호전달은 더 연구할 여지가 많이 남아 있다. 이 총설은 단백질의 인산화/탈인산화 과정을 통한 브라시노스테로이드의 신호전달 연구의 최근 상황을 기술하였다.