BRS1, a serine carboxypeptidase, regulates BRI1 signaling in *Arabidopsis thaliana*

Jia Li*, Kevin A. Lease*, Frans E. Tax†, and John C. Walker*‡

*Division of Biological Sciences, University of Missouri, Columbia, MO 65211; and †Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721

Edited by Clarence A. Ryan, Jr., Washington State University, Pullman, WA, and approved March 9, 2001 (received for review February 8, 2001)

***Brassinosteroid-insensitive 1* (*BRI1*) of *Arabidopsis thaliana* encodes a cell surface receptor for brassinosteroids. Mutations in BRI1 severely affect plant growth and development. Activation tagging of a weak *bri1* allele (*bri1-5*) resulted in the identification of a new locus, *brs1-1D*. *BRS1* is predicted to encode a secreted carboxypeptidase. Whereas a *brs1* loss-of-function allele has no obvious mutant phenotype, overexpression of *BRS1* can suppress *bri1* extracellular domain mutants. Genetic analyses showed that brassinosteroids and a functional BRI1 protein kinase domain are required for suppression. In addition, overexpressed *BRS1* missense mutants, predicted to abolish BRS1 protease activity, failed to suppress *bri1-5*. Finally, the effects of BRS1 are selective: overexpression in either wild-type or two other receptor kinase mutants resulted in no phenotypic alterations. These results strongly suggest that BRS1 processes a protein involved in an early event in the BRI1 signaling.**

Brassinosteroids (BR) are general regulators of plant growth and development. BR biosynthetic and response mutants have a similar complex phenotype characterized by dwarfed stature, round and curled leaves, reduced male fertility, and delayed flowering and senescence (1–5). The BR biosynthetic mutants can be rescued by the application of exogenous BR. The signal perception or transduction mutants, on the other hand, cannot be rescued by exogenous BR and are known as *brassinosteroid-insensitive* (*bri*) mutants (1). Interestingly, all of the over 20 *bri* mutants reported to date represent a single mutant locus, *bri1* (1–5), which suggests that *bri1* is the only nonredundant or viable mutant locus in the BR signaling pathway. *BRI1* has been cloned via a position-based strategy, which demonstrated that it encodes a leucine-rich repeat receptor-like protein kinase (LRR-RLK) (3). Whereas LRRs have been defined as a protein–protein interaction domain in many cases (6), the function of the LRRs of BRI1 remains uncertain.

The role of the BRI1 LRR domain in BR signaling has been examined by expression of a chimeric receptor, containing the BRI1 extracellular domain and the protein kinase domain of Xa21, in rice cells (7). Xa21 is an LRR-RLK from rice that functions in disease resistance. Treatment of the rice cells expressing the BRI1/Xa21 chimera with BR induced a variety of defense-related responses. These results support the hypothesis that the BRI1 extracellular domain senses BR directly.

How exactly BRI1 senses brassinolide is unknown, but two hypotheses have been proposed for the mechanism of interaction between BRI1 and brassinolide (3, 8). The first hypothesis holds that BRI1 binds BR directly, and the second hypothesis supposes that BRI1 senses BR by binding to a secreted steroid-binding protein that directly interacts with brassinolide. In either case, it is thought that BRI1 sensing of brassinolide initiates a signal transduction cascade that results in changes in growth and development. However, additional signaling components functioning with BRI1 have not been described.

To define novel genes important for BRI1 signaling, we initiated a gain-of-function genetic screen for suppressors of a weak *bri1* allele. One suppressor, which was named *brs1-1D* for *bri1 suppressor-Dominant*, was identified and predicted to encode a secreted carboxypeptidase. The suppression was found to be selective for *bri1*, and dependent on BR, a functional BRI1 protein kinase domain, and BRS1 protease activity. Because BRS1 is predicted to be secreted and suppresses extracellular domain *bri1* mutant alleles, it is likely that BRS1 acts at an early step in BRI1 signaling by proteolytic processing of a rate-limiting protein. The identification of proteolytic processing as an important regulatory element adds a new layer of complexity and contributes to a better understanding for BRI1 and other plant RLKs.

Materials and Methods

Identification of *bri1-5 brs1-1D* and Cloning of *BRS1*. The activation-tagging transgenic lines were generated in a *bri1-5* (Ws-2 ecotype; ref. 4) background with construct pSKI015 (9) via floral dipping *Agrobacterium*-mediated transformation technique (10). Plants were grown at 23°C under continuous illumination (≈100 μmol·m^{-2}·sec^{-1}). *bri1-5 brs1-1D* was identified in a screen of 2,500 transgenic plants. Homozygous *bri1-5 brs1-1D* plants were used for all of the experiments described in this paper. Genomic DNA was isolated from 2-week-old *Arabidopsis* plants with Nucleon Phytopure Plant DNA Extraction Kit (Amersham Pharmacia). For *BRS1* cloning, 10 μg of genomic DNA was digested with *Xho*I in a 100-μl volume. After digestion, the enzyme was heat-inactivated, and the reaction was passed through a CONCERT Nucleic Acid Purification System (GIBCO/BRL). A ligation was set up in a 100-μl volume, and 4 μl of the ligated solution was used as the template for inverse PCR. The ligated DNA yielded a 2-kb inverse PCR product with two inverse PCR primers (T3 long primer: 5′-AATTAACCCTCACTAAAGGGAACAAAAG; ACTRB primer: 5′-GTTTCTAGATCCGAAACTATCAGTG). For reverse transcription (RT)-PCR, 2 primers were used (BRS1cDNAForward: 5′-TCTGGTACCATGGCAAGAACCCATTTCATTTTC; BRS1cDNReverse: 5′-TCTGAGCTCCTAATAAGATCTTGGGAGTTCCTTC). The RT-PCR was carried out with the SuperScript One-Step RT-PCR System (GIBCO/BRL). The RT-PCR product (*BRS1* cDNA) was cloned into pBluescript (Stratagene) SK$^+$ at *Kpn*I and *Sac*I sites.

Total RNA Isolation and Northern Analyses. Total RNA samples were isolated from 4-week-old above ground tissues with an Rneasy Midi Kit (Qiagen, Chatsworth, CA). Ten micrograms of total RNA were used for each lane in the Northern analyses. After hybridization, the blots were exposed to x-ray film for 5 days to detect the *BRS1* expression in wild-type and *bri1-5* plants.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: BR, brassinosteroid; BRI1, *brassinosteroid insensitive 1;* BRS1, *bri1 suppressor 1*; RLK, receptor-like protein kinase; LRR, leucine-rich repeat; RT-PCR, reverse transcription–PCR; T-DNA, portion of the Ti (tumor-inducing) plasmid that is transferred to plant cells.

‡To whom reprint requests should be addressed. E-mail: WalkerJ@missouri.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

www.pnas.org/cgi/doi/10.1073/pnas.091065998

Vector Construction, Site-Directed Mutagenesis, and Gene Transformation. To recapitulate the overexpression phenotype, the *BRS1* cDNA was cloned into the *Kpn*I and *Sac*I sites of pBIB-KAN (11) with a dual enhanced 35S promoter. S181F and H438A mutants were introduced with a QuickChange Site-Directed Mutagenesis Kit (Stratagene) in a pBluscript SK^+ vector. The mutagenized *BRS1*cDNA was then cloned into pBIB-KAN.

Generation of Double and Triple Mutants. *bri1-5* and *bri1-5 brs1-1D* were crossed with *dwf4-1* or *det2-101.* The F1 were allowed to self-fertilize to generate a segregating F_2. In the F_2, double and triple mutants were identified by genotyping individuals by PCR and DNA sequencing. The plants containing *brs1-1D* have a *BAR* resistance gene (9). The plants surviving application of 0.25 g/liter glufosinate-ammonium (Finale, AgrEvo Environmental Health, Montvale, NJ) were confirmed by PCR genotyping, by using a T7 primer (from the T-DNA of SKI015; T7: 5′-GTAATACGACTCACTATAGGGCGAATTG) and a 3′ end *BRS1* specific primer (*brs1*cDNA3′*Sac*I: 5′-TCTTGAGCTC-CTAGTTCAGACGTAGCTCAAGA). The plants containing an intact *brs1-1D* locus yielded a 6.7-kb PCR product, which includes four copies of the CaMV 35S enhancers, 1.1 kb of *BRS1* promoter, and 4.2 kb of *BRS1* genomic sequence (from ATG to stop codon). To genotype *bri1-5*, *BRI1* was amplified by PCR with two primers, *bri1*cDNA5′*Kpn*I (5′-TCTGGTACCAT-GAAGACTTTTTCAAGCTTC), and *bri1*cDNA3′*Kpn*I (5′-TCTGGTACCTCATAATTTTCCTTCAGGAACTTC). The PCR product was then used as the template for sequencing by using primer *bri1*cDNA5′*Kpn*I. *dwf4-1* was genotyped by PCR by using two primers flanking the T-DNA insertion as described previously (12).

Results

Identification of *brs1-1D* Locus. Genetic analyses of BR signaling have thus far failed to identify other BR perception/transduction regulatory components. We hypothesized that some components of the BR signaling pathway have not been identified in previous screens because of functional redundancy. Genetic modifier screens using activation tagging allow for the identification of new regulatory proteins that are functionally redundant (9). Taking advantage of a fertile partial loss-of-function *bri1* allele, *bri1-5* (4), we generated transgenic plants harboring an activation tagging T-DNA, designed to activate expression of genes in the vicinity of the insertion. From 2,500 transgenic plants, we identified a single plant in which the *bri* mutant phenotype was suppressed. Genetic analysis showed that the suppression is dominant and cosegregates with the single T-DNA insertion in this line. The mutant was named *brs1-1D* for *bri1 suppressor-Dominant*.

***brs1-1D* Suppresses Multiple *bri1-5* Defects.** Physiological studies demonstrated that multiple *bri1-5* defects are suppressed by *brs1-1D* (Fig. 1*A*). The primary inflorescence stems of *bri1-5 brs1-1D* plants are twice as long as those with *bri1-5* alone; the rosettes are wider, because the leaves are bigger and not curled (Fig. 1 *A* and *B*). In addition, *brs1-1D* suppresses the shortened secondary inflorescence branch length phenotype of *bri1-5* (Fig. 1*B*). Furthermore, *brs1-1D* suppresses the late flowering of *bri1-5* (Fig. 1*B*). Other traits, which are unaffected in *bri1-5* relative to wild-type plants such as flower number, coinflorescence number and branch number, were not altered in *bri1-5 brs1-1D* plants (data not shown).

***BRS1* Encodes a Serine Carboxypeptidase-Like Protein.** We cloned the genomic DNA flanking the *bri1-5 brs1-1D* T-DNA by inverse PCR (Fig. 2 *A* and *B*). Sequence analysis revealed that 1.1 kb from the right border of the T-DNA is a gene encoding a putative serine carboxypeptidase. This predicted protein has homology

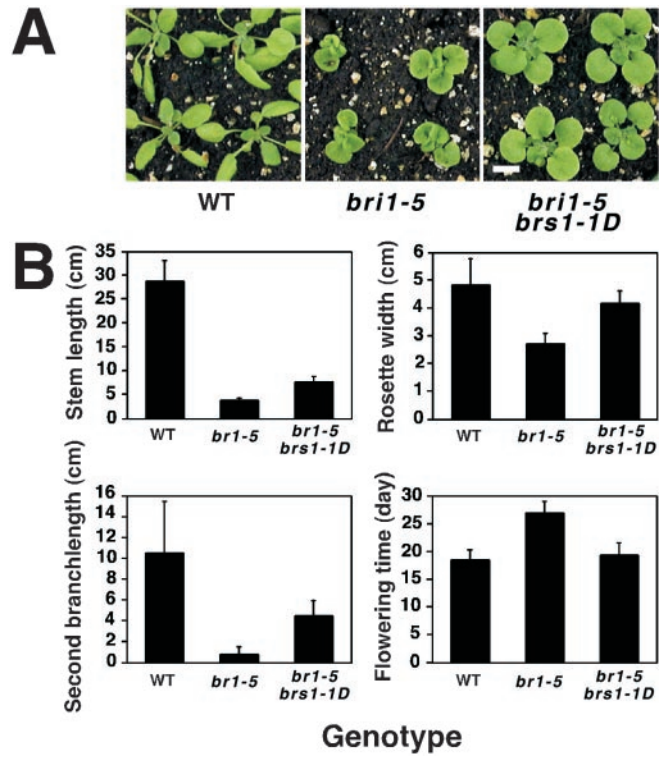

Fig. 1. BRS1 suppresses multiple *bri1-5* defects. (*A*) Comparison of plant phenotypes of wild-type plants (WT, ecotype: Ws-2), *bri1-5*, and *bri1-5 brs1-1D* 16 days after germination. Scale bar = 1 cm. (*B*) Physiological characteristics of WT, *bri1-5*, and *bri1-5 brs1-1D*. Measurements were taken 35 days after germination (except flowering time measurements). The means (± SD) were from the measurements of 40 plants per genotype.

with wheat and barley serine carboxypeptidase II proteins, and with yeast Kex1p (13–15). These proteases belong to the S10 family and are in a group named carboxypeptidase D (E.C. 3.4.16.6; ref. 16). The proteases of this group preferentially remove Arg or Lys from the C terminus of a peptide. There is a typical N-terminal signal peptide in the predicted protein that would direct the protein to the secretory pathway (Fig. 2*C*). The serine carboxypeptidase "catalytic triad," S181, D386, and H438 are conserved with other carboxypeptidase D group proteases. Sequence alignment also revealed a possible cleavage linker peptide, indicating that this serine carboxypeptidase itself may be processed within the endoplasmic reticulum (ER)-trans Golgi network to form a mature protease, similar to other plant serine carboxypeptidases (17).

Overexpression of a *BRS1* cDNA Recapitulates the Suppression Phenotype. Based on the gene annotation, we cloned the cDNA encoding this serine carboxypeptidase by RT-PCR. Northern blots showed that steady-state level of the serine carboxypeptidase mRNA in *bri1-5 brs1-1D* plants is about 30 times higher than in wild-type or *bri1-5* plants (Fig. 3). Expression of this serine carboxypeptidase mRNA in wild-type plants or *bri1-5* plants is relatively low (Fig. 3). To confirm that *bri1-5* suppression is due to the overexpression of this serine carboxypeptidase, the cDNA clone was placed under the control of a CaMV 35S dual-enhanced promoter (18, 19) and transformed into *bri1-5* plants. The resulting transgenic plants recapitulated the *bri1-5* suppression phenotype (Fig. 4) and were confirmed to overexpress *BRS1* by Northern blot analyses (data not shown). The gene was therefore designated *BRS1.*

PLANT BIOLOGY

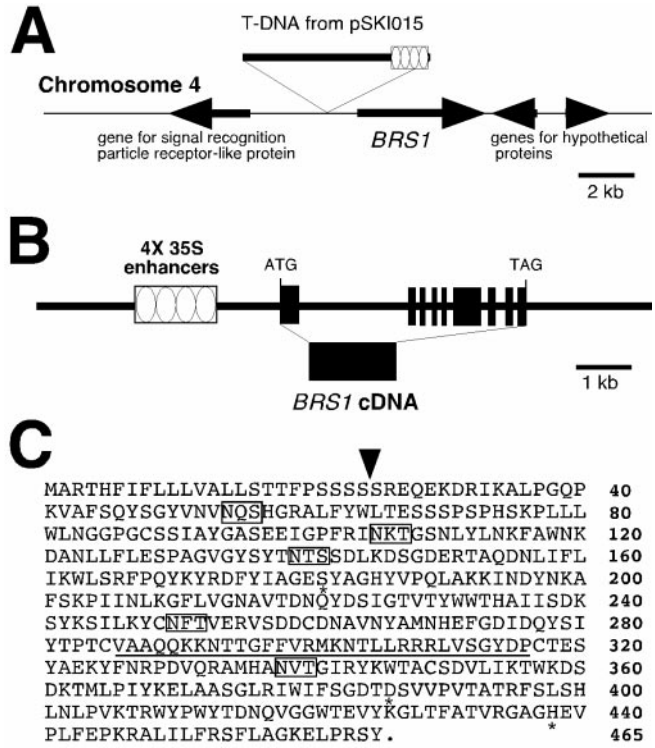

Fig. 2. The *BRS1* gene encodes a protein with homology to serine carboxypeptidases (54% and 53% identity with wheat and barley serine carboxypeptidase II proteins; 28% identity with yeast Kex1p protein). (*A*) The flanking sequence of the T-DNA was cloned via inverse PCR. The T-DNA insert localizes to the bottom part of chromosome IV. 5′ of the T-DNA, 6.5 kb from the 4 × 35S enhancers, there is a gene encoding a signal recognition particle receptor-like protein. 3′ of the T-DNA, 1.1 kb from 4 × 35S enhancers, there is a gene encoding a serine carboxypeptidase, which was subsequently confirmed as the suppressor, *BRS1*. (*B*) Comparison of the cDNA and genomic sequences indicated that *BRS1* has 9 exons and 8 introns. (*C*) Deduced amino acid sequence of *BRS1*. A possible signal peptide cleavage site is indicated by an arrow. Five potential N-linked glycosylation sites are marked in the open boxes. The asterisks below an amino acid indicate the three putative ''catalytic triad'' amino acids, S181, D386, and H438. A possible cleavage linker peptide is underlined. The BRS1 sequence was obtained from GenBank (accession no. AL161577, reference GI: 7269962).

***BRS1* May Be Functionally Redundant.** We hypothesized that *BRS1* is functionally redundant, in which case the loss-of-function *brs1* mutant would have no phenotype. The Wisconsin *Arabidopsis* T-DNA insertion lines (20) were screened, and a plant in which a single T-DNA is inserted in the first intron of *BRS1* was identified. This line lacks any detectable *BRS1* mRNA by Northern or RT-PCR analysis but is phenotypically normal (data not shown). BLAST searches (21) indicated that *BRS1* is part of a gene family. At least 36 carboxypeptidase-D-like/carboxypeptidase-II-like genes are encoded in the *Arabidopsis* genome. Only 5 of them, however, are closely related to *BRS1* and share 52% to 73% amino acid sequence identity with BRS1. Four of the five closely related gene products contain putative signal peptides. Taken together, these findings support the hypothesis that one or more genes may be functionally redundant with *BRS1*.

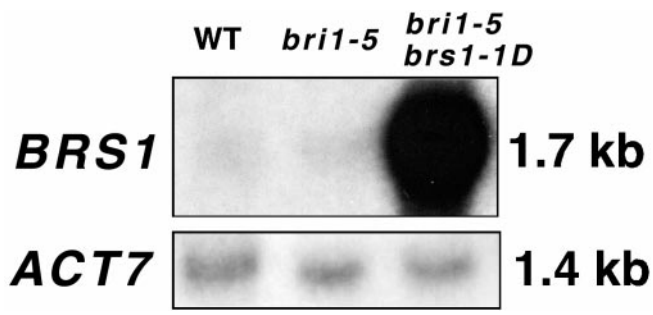

Fig. 3. A Northern blot shows that the expression of *BRS1* in *bri1-5 brs1-1D* is elevated compared with that in wild-type Ws-2 and *bri1-5* plants. Ten micrograms total RNA from 4-week-old above ground tissues was loaded in each lane. The blot was hybridized with a ^{32}P-labeled *BRS1* cDNA. *ACT7* cDNA was used as a probe to show equal loading.

***BRS1* Selectively Regulates the BRI1 Signaling Pathway, and a Functional BRI1 Kinase Domain Is Required for Suppression.** To determine whether *BRS1* acts selectively in the BRI1 signaling pathway, a *35S*::*BRS1* cDNA construct was transformed into *bri1-9* (4), *bri1-1* (5), *clv1-1* (22), *Ler* (Landsberg *erecta*; ref. 23), and wild-type plants (Table 1). The transformation results indicated that overexpression of *BRS1* suppresses a second *bri1* allele, *bri1-9*. Both *bri1-5* and *bri1-9* have missense mutations in the BRI1 extracellular domain (4). In *bri1-5*, a cysteine is replaced by a tyrosine residue (C69Y). In *bri1-9*, a serine is changed to phenylalanine (S662F). Overexpression of BRS1 in a BRI1 cytoplasmic kinase domain mutant, *bri1-1* (A909T; ref. 5) did not result in any suppression phenotype (Table 1), indicating that a functional BRI1 kinase domain is required for the regulation provided by BRS1. In addition, overexpression of *BRS1* in wild-type plants resulted in no obvious phenotypic alterations, although the expression of *BRS1* is elevated in the WT *brs1-1D* plants (Fig. 5). These data suggest that *BRS1* does not act as a general growth regulator. Transformation data also showed that the *clavata1* and *erecta* RLK mutants of *Arabidopsis* are not suppressed by *BRS1* (Table 1). Together, these results indicate that *BRS1* selectively regulates BRI1 signaling.

BR Plays an Essential Role for BRS1's Action. Recent studies from the Chory laboratory indicated that BR regulates the perception part of the BRI1-mediated signal transduction pathway (7). To investigate the role of BR in the regulation conferred by BRS1, we created *bri1-5 dwf4-1* double and *bri1-5 dwf4-1 brs1-1D* triple mutants. *dwf4* mutants are defective in BR biosynthesis (12). The triple mutant plants did not show any phenotypic suppression compared with the double mutant plants. This result suggests BR is essential for BRS1 action (Fig. 6). Similar results were observed when another BR biosynthetic mutant, *det2-101* (24), was used to replace *dwf4-1* in the double and triple mutant analyses (data not shown).

Carboxypeptidase Activity Is Required for BRS1 Function. To test whether the serine carboxypeptidase activity is required for suppression, we created missense mutants in the "catalytic triad," known to be essential for serine carboxypeptidase enzymatic activity (16). *bri1-5* plants overexpressing these missense BRS1 mutants, S181F and H438A, did not show the suppressed phenotype as summarized in Table 1. The expression of BRS1 mutants has been confirmed by Northern analyses (data not shown). Thus, the serine carboxypeptidase activity of BRS1 is necessary for suppression of *bri1* mutant phenotypes.

Discussion

Because BRS1 overexpression suppresses multiple *bri1* defects, BRS1 might play an important role in an early stage of the BRI1 signaling pathway. The presence of an N-terminal signal peptide in BRS1 predicts that the protein should enter the secretory pathway. Sequence analysis failed to identify any endoplasmic reticulum or Golgi apparatus retention or retrieval sequences. Therefore, BRS1 is likely to be a secreted protein. In addition, BRS1 can suppress two weak BRI1 extracellular domain mu-

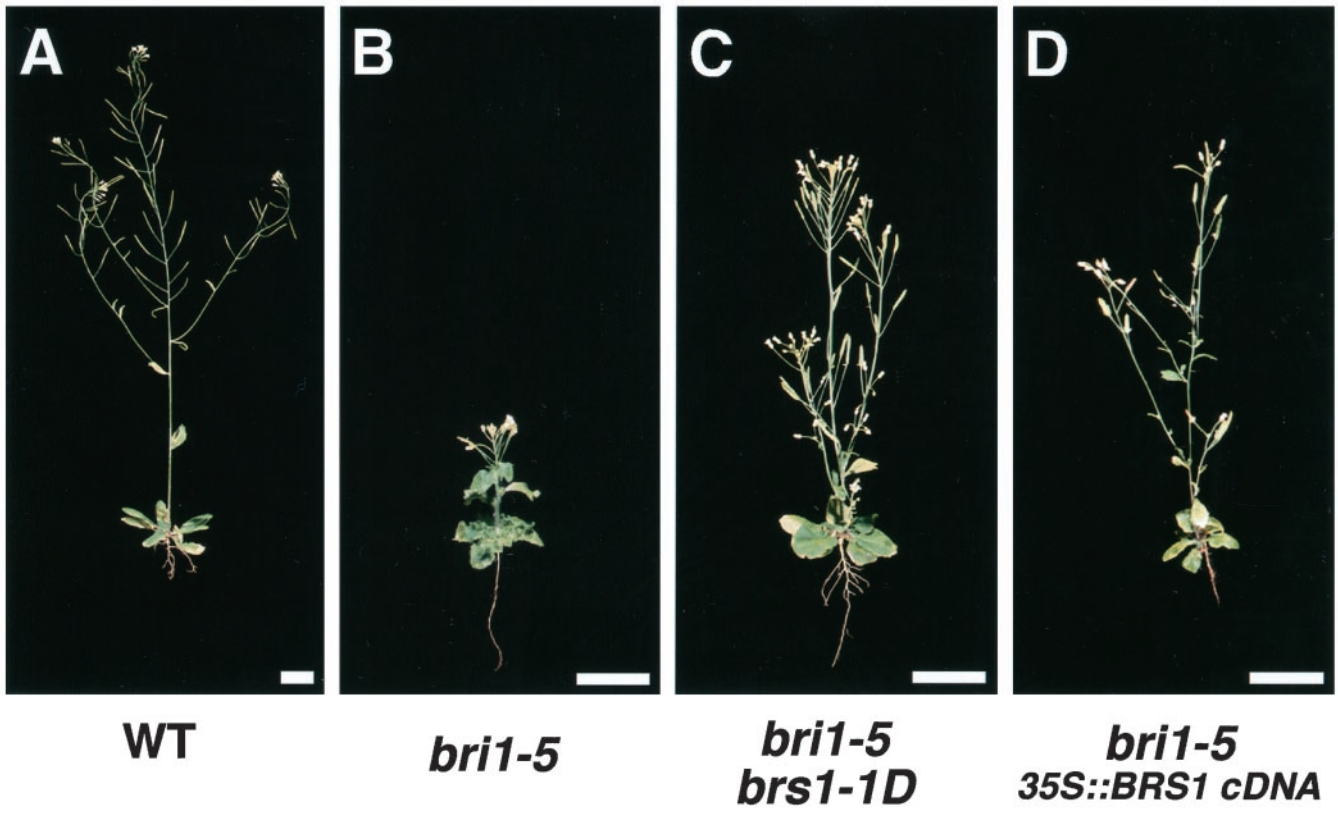

Fig. 4. Transformation of *BRS1* cDNA driven by a CaMV 35S constitutive promoter into *bri1-5* recapitulated the mutant suppression phenotype. (*A*) Wild-type Ws-2 plants. (*B*) *bri1-5* plants. (*C*) *bri1-5 brs1-1D* plants. (*D*) Transgenic plants with a *BRS1* cDNA construct confirmed that BRS1 suppresses the *bri* mutant phenotype. Plants were grown for 35 days under continuous illumination. Scale bar = 2 cm.

tants, *bri1-5* and *bri1-9*, but failed to suppress a cytoplasmic kinase domain mutant, *bri1-1*. Finally, the presence of BR is essential for BRS1's suppression activity because BRS1 cannot phenotypically affect the mutants harboring homozygous BR-deficient loci, *dwf4-1* and *det2-101*. These results are consistent with the hypothesis that BRS1 regulates an early event in BRI1 signaling. The observation that transgenic plants carrying missense mutations in the BRS1 protease "catalytic triad" failed to suppress the *bri1-5* phenotype suggests that carboxypeptidase activity is required for the suppression.

Proteases are required in many signaling pathways. For example, in yeast, both Kex1p and Kex2p are required for the excision of signaling peptides, α-mating pheromone, and K1 killer toxin, from their inactive precursors (15, 25). Kex2p is a membrane-bound endoprotease that specifically cleaves on the carboxyl side of pairs of basic amino acids (e.g., KR $\downarrow$ or RR $\downarrow$). After the action of Kex2p, Kex1p, a type D serine carboxypeptidase, selectively trims off the flanking amino acids from the C terminus of processing intermediates.

Table 1. Analysis of plants carrying different *BRS1* transgenes

Construct	Recipient	No. suppressed/total	%
35S::*BRS1* cDNA	*bri1-5*	103/199*	52
35S::*BRS1* cDNA	*bri1-9*	9/12*	75
35S::*BRS1* cDNA	*bri1-1*	0/23*	0
35S::*BRS1* cDNA	*clv1-1*	0/43†	0
35S::*BRS1* cDNA	*Ler*	0/29‡	0
35S::*BRS1* cDNAS181F	*bri1-5*	0/40*	0
35S::*BRS1* cDNAH438A	*bri1-5*	0/26*	0

*Uncurled leaves and longer stems were used to score *bri1* suppression.
†Fruit shape was used to determine the suppression of *clv1-1*.
‡Height and fruit shape were used to check *Ler* suppression.

In addition to peptide ligand processing, there are also examples of receptor proteolytic processing. One case of this processing is the insulin receptor. The insulin proreceptor is synthesized as inactive precursor. It is then processed at a RKRR site by an endoprotease in the trans Golgi network to form a mature receptor (26).

In plants, there are few reports concerning the processing of ligand-like peptides or receptor-like proteins. In response to wounding, tomato systemin is processed from its inactive form, preprosystemin (27). Also in tomato, a secreted leucine-rich repeat protein (LRP), which was thought to be involved in a plant defense response, is proteolytically processed during pathogenesis (28). It is not clear whether prosystemin is cleaved by a subtilisin-like endoprotease, but it has been found that systemin physically interacts with a subtilisin-like endoprotease (27). LRP is likely processed by a subtilisin/Kex2p-like endoprotease. Additionally, the functions of two *Arabidopsis* Kex2p-like genes have been determined: *AIR3* is involved in the regulation of auxin-induced lateral root formation (29) and *SDD1* functions in guard cell development (30). The regulatory roles of serine carboxypeptidases in plants have not yet been investigated.

Does BRS1 process the BRI1 receptor? In a recent report from He and colleagues (7), the BRI1 extracellular domain was fused with the intracellular kinase domain of Xa21 to test the hypothesis that the extracellular domain of BRI1 is the recognition domain for BR. In this study, the functional chimera was the molecular weight expected of a full-length receptor, suggesting that BRI1 is not proteolytically processed.

Does BRS1 process a proteinaceous proligand or an extracellular BR binding protein? Whereas ligands that function in two other RLK-signaling pathways, CLAVATA3 (31) and SCR (32),

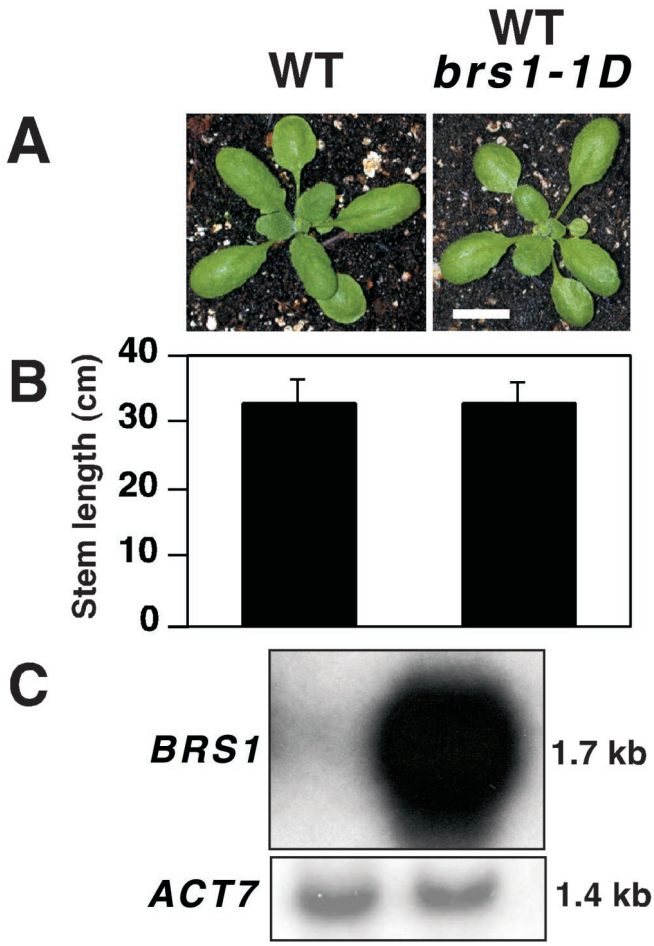

Fig. 5. BRS1 selectively regulates BRI1 signaling pathway. Wild-type plants harboring the *brs1-1D* allele (WT *brs1-1D*) do not show phenotypic alterations. (*A*) Phenotypes of a wild-type (WT, Ws-2) and a WT *brs1-1D* plant (both are 18 days old). Size bar = 1 cm. (*B*) Average main stem length of 37-day WT and WT *brs1-1D* plants under continuous illumination condition. The means (± SD) were from measurements of 28 plants per genotype. (*C*) The expression levels of *BRS1* in WT and WT *brs1-1D* plants. *ACT7* was used to show equal loading of total RNA.

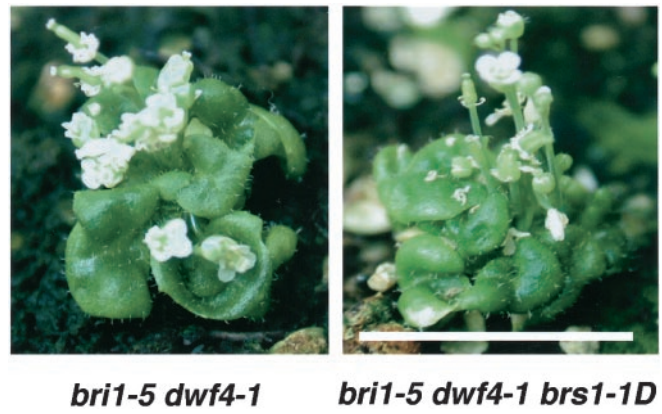

Fig. 6. Biosynthesis of BR is required for BRS1 regulation. *BRS1* failed to suppress mutants containing homozygous BR-deficient loci, *dwf4-1*. The mutants were generated through genetic crossing. The genotypes were determined by both PCR analyses and DNA sequencing. *bri1-5 dwf4-1* double mutant plants are phenotypically identical to *bri1-5 dwf4-1 brs1-1D* triple mutant plants. The plants were photographed 56 days after germination. Scale bar = 1 cm.

contain possible Kex2p-processing sites, a proteinaceous ligand for BRI1 has not yet been described. However, at least two putative steroid-binding proteins, which contain signal peptides and may be secreted, have been identified in the recently completed *Arabidopsis* genome sequence (33). These putative steroid-binding proteins, which contain potential processing sites, could be the substrates of BRS1. The proteolytic processing may resemble the actions of yeast Kex1p and Kex2p, in which an *Arabidopsis* Kex2p-like endoprotease may recognize and cleave a dibasic site in its substrate. After cleavage, BRS1 trims the intermediate and releases an active BR binding protein. The BR–BR binding protein complex (ligand complex) then binds to the extracellular domain of BRI1 and trigger a series of cellular responses. Because of the extracellular domain mutation in *bri1-5*, ligand activation of the BRI1 receptor may be rate limiting. Elevated expression of BRS1 would increase the amount of the active steroid binding protein, which would favor formation of ligand complex-BRI1 receptor binding. Increase in ligand binding would enhance BRI1-5 receptor activity and the signal transduction pathway. As a result, multiple *bri1-5* defects are suppressed.

The results reported in this study show that the serine carboxypeptidase encoded by *BRS1* is involved in the BRI1 signaling pathway. Although it is not clear whether proteases function in other RLK-signaling pathways, the identification of *BRS1* suggests that protein processing/proteolysis in plant signal transduction is important.

We thank Drs. J. Birchler, K. Cone, E. Liscum, J. Stone, and S. Zhang for their critical comments on the manuscript. We also thank Dr. D. Weigel for the activation-tagging construct SKI015. These studies were supported by the National Science Foundation (MCB 9809884) and the University of Missouri Food for the 21st Century Program (to J.C.W.).

1. Clouse, S. D., Langford, M. & McMorris, T. C. (1996) *Plant Physiol.* **111,** 671–678.
2. Kauschmann, A., Jessop, A., Koncz, C., Szekeres, M., Willmitzer, L. & Altmann, T. (1996) *Plant J.* **9,** 701–713.
3. Li, J. & Chory, J. (1997) *Cell* **90,** 929–938.
4. Noguchi, T., Fujioka, S., Choe, S., Takatsuto, S., Yoshida, S., Yuan, H., Feldmann, K. A. & Tax, F. E. (1999) *Plant Physiol.* **121,** 743–752.
5. Friedrichsen, D. M., Joazeiro, C. A., Li, J., Hunter, T. & Chory, J. (2000) *Plant Physiol.* **123,** 1247–1256.
6. Kobe, B. & Deisenhofer, J. (1994) *Trends Biochem. Sci.* **19,** 415–421.
7. He, Z., Wang, Z. Y., Li, J., Zhu, Q., Lamb, C., Ronald, P. & Chory, J. (2000) *Science* **288,** 2360–2363.
8. Schumacher, K. & Chory, J. (2000) *Curr. Opin. Plant Biol.* **3,** 79–84.
9. Weigel, D., Ahn, J. H., Blazquez, M. A., Borevitz, J. O., Christensen, S. K., Fankhauser, C., Ferrandiz, C., Kardailsky, I., Malancharuvil, E. J., Neff, M. M., *et al*. (2000) *Plant Physiol.* **122,** 1003–1013.
10. Clough, S. J. & Bent, A. F. (1998) *Plant J.* **16,** 735–743.
11. Becker, D., Kemper, E., Schell, J. & Masterson, R. (1992) *Plant Mol. Biol.* **20,** 1195–1197.
12. Choe, S., Dilkes, B. P., Fujioka, S., Takatsuto, S., Sakurai, A. & Feldmann, K. A. (1998) *Plant Cell* **10,** 231–243.
13. Breddam, K., Sorensen, S. B. & Svendsen I. (1987) *Carlsberg Res. Commun.* **51,** 297–311.
14. Dal Degan, F., Rocher, A., Cameron-Mills, V. & von Wettstein, D. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 8209–8213.
15. Dmochowska, A., Dignard, D., Henning, D., Thomas, D. Y. & Bussey, H. (1987) *Cell* **50,** 573–584.
16. Remington, S.J. (1998) in *Handbook of Proteolytic Enzymes*, eds. Barrett, A. J., Rawlings, N. D. & Woessner, J.F. (Academic, New York), pp. 398–401.
17. Doan, N. P. & Fincher, G. B. (1988) *J. Biol. Chem.* **263,** 11106–11110.
18. Kay, R., Chan, A., Daly, M. & McPherson, J. (1987) *Science* **236,** 1299–1302.
19. Fang, R. X., Nagy, F., Sivasubramaniam, S. & Chua, N. H. (1989) *Plant Cell* **1,** 141–150.
20. Sussman, M. R., Amasino, R. M., Young, J. C., Krysan, P. J. & Austin-Phillips, S. (2000) *Plant Physiol.* **124,** 1465–1467.

21. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) *J. Mol. Biol.* **215,** 403–410.
22. Clark, S. E., Williams, R. W. & Meyerowitz, E. M. (1997) *Cell* **89,** 575–585.
23. Torii, K. U., Mitsukawa, N., Oosumi, T., Matsuura, Y., Yokoyama, R., Whittier, R. F. & Komeda, Y. (1996) *Plant Cell* **8,** 735–746.
24. Clouse S.D. & Feldmann, K. A. (1998) in *Brassinosteroids Steroidal Plant Hormones*, eds. Sakurai, A., Yokota, T. & Clouse, S.D. (Springer, Tokyo), pp. 163–190.
25. Fuller, R. S., Brake, A. J. & Thorner, J. (1989) *Science* **246,** 482–486.
26. Barr, P. J. (1991) *Cell* **66,** 1–3.
27. Schaller, A. & Ryan, C. A. (1994) *Proc. Natl. Acad. Sci. USA.* **91,** 11802–11806.
28. Tornero, P., Mayda, E., Gomez, M. D., Canas, L., Conejero, V. & Vera, P. (1996) *Plant J.* **10,** 315–330.
29. Neuteboom, L. W., Ng, J. M., Kuyper, M., Clijdesdale, O. R., Hooykaas, P. J. & van der Zaal, B. J. (1999) *Plant Mol. Biol.* **39,** 273–287.
30. Berger, D. & Altmann, T. A. (2000) *Genes Dev.* **14,** 1119–1131.
31. Fletcher, J. C., Brand, U., Running, M. P., Simon, R. & Meyerowitz, E. M. (1999) *Science* **283,** 1911–1914.
32. Schopfer, C. R., Nasrallah, M. E. & Nasrallah, J. B. (1999) *Science* **286,** 1697–1700.
33. The *Arabidopsis* Genome Initiative (2000) *Nature (London)* **408,** 796–815.