Brassinosteroid and systemin: two hormones perceived by the same receptor

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Brassinosteroids, coordinating developmental events, and systemin, inducing systemic wound responses to attacks by insect pests, are newly recognized plant hormones that are perceived by plasma membrane-localized leucine-rich repeat receptor kinases. The recent characterization of the brassinosteroid receptor BRI1 from tomato revealed that this protein is identical to the previously isolated SR160 systemin receptor, strongly suggesting that both brassinosteroid and systemin signalling use the same surface receptor.

Brassinosteroids (BRs) are polyhydroxy-steroid phytohormones controlling important developmental functions, such as growth, photomorphogenesis, fertility, seed germination, senescence and stress tolerance [1]. Brassinolide (BL), the most active BR, was identified in 1979; several key elements of its signalling pathway have now been characterized with the help of BR-response mutants. Perception of BL by BRASSINOSTEROID INSENSITIVE 1 (BRI1), a plasma membrane-localized leucine-rich repeat (LRR) receptor kinase, probably initiates a phosphorylation cascade that deactivates the cytoplasmic GSK3/SHAGGY-like BIN2 kinase, a negative regulator of BR signalling. By phosphorylating BES1 and BZR1, two downstream components of the pathway, BIN2 can prevent their translocation to the nucleus, where they act as positive regulators of BR-responsive gene expression [2–4].

Considerable attention has been focused on the functional characterization of BRI1, the putative BR receptor. A series of elegant experiments revealed that the extracellular portion of the protein is required for BR-dependent activation of the intracellular, Ser/Thr-specific kinase domain [5,6]. Using radiolabelled BL, it was shown that BRI1 co-immunoprecipitated with BL binding activity, but hormone binding was prevented by mutations affecting the extracellular domain of the protein [7]. These data unequivocally demonstrated that BRI1 is an essential component of the BR receptor complex.

Recent cloning and sequence analysis of the tomato BRI1 gene (tBRI1) by Teresa Montoya and colleagues led to the exciting discovery that it encodes the *Lycopersicon esculentum* equivalent of the *Lycopersicon peruvianum* SR160 LRR receptor kinase [8], which had been identified as the receptor of the peptide hormone systemin [9].

**Abs1 and Cu3 encode the tomato ortholog of BRI1**

The BR-insensitive mutants of tomato, *altered brassinolide sensitivity 1* (*abs1*, from *L. esculentum*) [8] and *curl 3* (*cu3*, from *Lycopersicon pimpinellifolium*) [10] have similar phenotypic features, but *abs1* is less dwarfed, develops an elongated root and retains its fertility and partial responsiveness to BL (Fig. 1). In both *abs1* and *cu3*, reverse-transcription PCR assays detected increased
transcript levels of the BR-downregulated Dwarf (BR C-6 oxidase) gene. Furthermore, gas-chromatography mass-spectrometry analyses showed the accumulation of endogenous BRs, a characteristic feature of the BR-insensitive mutants [11,12]. Intriguingly, in spite of the nearly 30-fold accumulation of castasterone, its immediate precursor, BL could not be detected in the mutant samples. This seems to confirm the view that castasterone is a bioactive BR in tomato [13].

Allelism tests between the two mutants clarified that abs1 is a new, weaker allele of cu3 [8]. Because BRI1 is thought to be the major non-redundant component of BR signalling in Arabidopsis, cu3 and cu3 abs plants were assumed to carry mutations in the gene encoding tBRI1. Using an approach that should be useful for isolating BRI1 homologs from various plant species, a segment of tBRI1 was PCR-amplified from genomic DNA samples with degenerate primers corresponding to kinase domain regions conserved in Arabidopsis and rice BRI1. Using the sequence information obtained from the PCR product, the complete tBRI1 gene could then be isolated by inverse PCR.

Sequence analysis of the tBRI1 copy amplified from cu3 revealed a nonsense mutation (G749Z) in the 25th LRR motif, whereas the tBRI1 amplified from cu3 abs identified a missense mutation (H1012Y) within the kinase domain. Co-segregation of the mutation-specific RFLP patterns with the respective BR-insensitive phenotypes confirmed that cu3 and cu3 abs are deficient in the tomato ortholog of BRI1.

Sequence conservation between BRI1 orthologs reveals structural requirements of BR perception

BRI1 is a typical plasma membrane-localized receptor kinase with complex domain structure. The characteristic extracellular (sensor) part with 25 LRR motifs, interrupted by a 70-amino-acid island between the 21st and 22nd LRRs, is connected to the intracellular kinase domain through a transmembrane segment [14]. Based on the common role in BR perception, preferential conservation of the functionally important extracellular regions was expected between the BRI1 proteins of different plant species. Therefore comparing the Arabidopsis [14], rice [15] and the recently available tomato and pea BRI1 sequences offered valuable information for identifying potential ligand-binding structures. Pair-wise alignments of these sequences in seven BRI1 regions (Fig. 2) revealed the highest average amino acid identity between the kinase domains (83%). Somewhat surprisingly, among the extracellular regions, the island domain (in which the known Arabidopsis bri1 mutations are over-represented) was less conserved (61%) than the flanking LRRs on its N-terminal (72%) and C-terminal (62%) sides. These data suggest that the LRRs around the island are crucial for establishing interaction with the ligand or components of the BR receptor complex.

Unexpectedly, the amino acid sequence analyses led to the recognition that tBRI1 is almost fully (>99%) identical with the recently identified SR160 systemin receptor [9,16]. Because both LRR receptor kinases are encoded by single-copy genes and their differences seem to result from interspecific variability (tBRI1 is from L. esculentum whereas SR160 is from L. peruvianum), these proteins can be regarded as each other’s functional equivalents.

Tomato BRI1 can function as a dual ligand receptor

Systemin is a Solanaceae family-specific peptide hormone which, following its release upon wounding, initiates systemic wound responses, partly through the induction of jasmonate synthesis [17]. Isolation of the systemin
receptor was based on its high affinity toward its ligand. From a cell suspension culture that was photoaffinity-labelled with radioactive systemin, a 160-kDa plasma membrane protein was purified to homogeneity and, using its amino acid sequence, identified as the SR160 LRR receptor kinase [9]. The data presented by Montoya et al. [8] now suggest that SR160, as well as its BRI1 homolog from *L. esculentum*, participate in both BR and systemin signalling. This result is particularly interesting because to date only the oxytocin/progesterone receptor of mammals has been known to interact with more than one type of hormone ligand [18].

How can two phythohormones with such different structures and physiological functions be perceived by tBRI1/SR160? The binding of systemin to the receptor agrees with the anticipated role of LRRs in protein–protein interactions. By contrast, how BRs bind to the receptor is unclear. Even at high (1 μM) concentration, BL does not compete with systemin in ligand-binding assays, indicating that BRs and systemin are perceived by different regions of the receptor, or by different binding mechanisms. For instance, it seems possible that BRI1 perceives BRs as complex ligands formed with sterol-binding proteins [19]. In such a case, this complex could compete with systemin for the receptor ligand-binding site.

The questions regarding the ligand-specificity of tBRI1/SR160 remain to be elucidated by future experiments. Like other LRR receptor kinases, BRI1 is expected to function in a dimeric form: in a yeast expression system, *Arabidopsis* BAK1 was shown to form heterodimers and induce transphosphorylation with the structurally related *Arabidopsis* BAK1 receptor kinase [20,21]. Detailed molecular analyses should reveal whether BRI1-interacting kinase(s) can influence the ligand binding and kinase specificity of the receptor complex. Furthermore, studying systemin binding and signalling in BRI1-deficient tomato mutants, such as cu3 and cu3−/−, should be instrumental in clarifying how tBRI1 can participate in both BR and systemin perception, and how tBRI1 can selectively activate the respective signalling pathways.

Acknowledgements

I thank Gerard Bishop for the images of tomato seedlings, as well as Éva Ádám, László Koza-Bognár and Ferenc Nagy for helpful comments on the manuscript. Work in my laboratory is supported by the Hungarian Scientific Research Fund (Grant T 42639).

References


