Crosstalk between brassinosteroids and pathogenic signalling?

Brassinosteroids are steroid hormones that are synthesized in extremely low amounts from phytosterols in plants. Brassinolide, the most active brassinosteroid, is produced from 24-methylcholesterol via the recently characterized early and late oxidation pathways[1,2]. They show an astonishingly wide activity spectrum, stimulating leaf unrolling, xylem differentiation and cell elongation in the hypocotyl, but inhibiting root elongation, radial stem expansion and anthocyanin biosynthesis[3]. Nonetheless, the hormonal status of brassinosteroids has been questioned, because physiological data suggested that nearly all brassinosteroid-stimulated growth responses could be explained by the overlapping activity of the classical plant hormones (auxin, cytokinin, ethylene, gibberellin and abscisic acid).

Brassica sp. Photo supplied by H.D.B.

Arabidopsis dwarfs and brassinosteroids

The position recently changed with a flurry of papers describing phenotypically similar *Arabidopsis* dwarf mutants: *de-etiolated2* (*det2*)[4]; *constitutive photomorphogenic dwarf 1* (*cpd1*)[5]; and *cabbage* (*cbb1*, *cbb2* and *cbb3*)[6]. These mutants display a de-etiolated phenotype in the dark, with inhibition of hypocotyl elongation, loss of the cotyledon apical hook and activation of light-induced genes. The *DET2* gene was found to encode a steroid 5α-reductase. In human embryonic kidney cells, the DET2 protein catalysed the conversion of progesterone, testosterone and androstenedione to their 5α-reduced forms. Conversely, human type 1 and 2 steroid 5α-reductases complemented the *det2* mutation as well as an exogeneously provided brassinosteroid intermediate, campestanol. Thus, DET2 turned out to control an early step of brassinosteroid biosynthesis between campesterol and campestanol by catalysing the synthesis of (*24R*)-24-methyl-5α-cholestan-3-one from (*24R*)-24-methylcholestan-4-en-3-one[7].

The *cpd1* mutation and its *cbb3* allele resulted in a deficiency in CYP90, a cytochrome P450 sharing sequence homology with animal steroid side-chain hydroxylases. Feeding experiments with brassinolide precursors indicated that CYP90 catalyses the C23-hydroxylation of cathasterone to teasterone[5]. Brassinosteroids repress the expression of the *CPD* gene, and thus the CYP90 protein may control a rate-limiting step in brassinosteroid biosynthesis. The *cbb1* mutation proved to be allelic with *diminuto* (*dim1*) and *dwarf1* (*dwf1*), which apparently uncouple de-etiolation from the activation of light-induced genes. The DIM1 protein was shown to regulate the expression of a β-tubulin gene[8], *TUB1*, whereas *cbb1* was found to control brassinosteroid-dependent expression of *meri5* and *TCH4*, members of the *XET* (xyloglucan endotransglycosylase) gene family[6]. Brassinolide, castasterone and typhasterol restored the *dim/cbb1/dwf1* phenotype to the wild type, suggesting that *DIM1* may act early in brassinosteroid biosynthesis[5].

The unique *cbb2* class of *Arabidopsis* mutants displayed insensitivity to brassinosteroids in hypocotyl elongation assays in both the dark and the light[6]. The *cbb2* mutation proved to be allelic with *bri1*, a mutation resulting in brassinosteroid-insensitive root elongation[9]. These mutations also abolished expression of the brassinosteroid-inducible *XET* gene *TCH4* (Ref. 6), suggesting that *BRI1* may encode a brassinosteroid receptor.

BRASSINOSTEROID INSENSITIVE 1: a putative leucine-rich repeat receptor kinase

A recent paper in *Cell* provides further evidence that BRI1 may indeed function as a brassinosteroid receptor[10]. Following EMS mutagenesis of *Arabidopsis*, Li and Chory isolated 200 *det2*-like mutants, 18 of which did not respond to external brassinolide treatment[10]. Surprisingly, all 18 *brassinosteroid insensitivity* (*bin*) mutations were found to be allelic with *bri1/cbb2* and mapped to the lower arm of chromosome 4. Hybridization fingerprinting with a BAC probe identified an RFLP in the *bri1-113* allele defining the exact position of the *BRI1* gene.

Sequence analysis of the wild-type and five mutant alleles, as well as the corresponding cDNA, revealed that *BRI1* encodes a serine/threonine receptor-like kinase carrying 25 extracellular leucine-rich repeats. The BRI1 kinase shows a typical mosaic structure comprising an N-terminal endoplasmic reticulum-signal peptide, a putative leucine-zipper motif, extracellular leucine-rich repeats, a transmembrane domain and a cytoplasmic protein kinase domain. The extracellular leucine-rich repeat domain of BRI1 carries 13 potential N-glycosylation sites and has a bipartite feature including a unique island of 70 amino acids between leucine-rich repeats 21 and 22. A mutation resulting in brassinosteroid insensitivity was located within this sequence between leucine-rich repeats, whereas four other mutations were mapped to the cytoplasmic kinase domain. Within the kinase domain, BRI1 shows 37–41% sequence identity with other *Arabidopsis* receptor-like kinases, such as ERECTA (ER), CLAVATA1 (CLV1), RLK5 and TMK1, as well as to Xa21, which confers resistance in rice against *Xanthomonas oryzae* var. *oryzae*. The *BRI1* gene is expressed ubiquitously in different plant tissues in both the dark and the light, consistent with its potential role as a receptor in different cell types.

Ligand recognition

The leucine-rich repeats of transmembrane receptors identified to date recognize protein

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ligands. Extracellular leucine-rich repeats of G-protein-coupled receptors in animals are involved in the recognition of peptide hormones, such as gonadothropin, nerve growth factors and thyroid-stimulating hormones[11]. Some non-leucine-rich repeat receptors, such as the animal dioxin receptor, can bind small molecules in addition to interacting with protein ligands.

Li and Chory[10] have therefore proposed two related models for ligand-binding by BRI1. First, BRI1 may dimerize with other leucine-rich repeat receptors mediating cell-to-cell interactions (e.g. by recognizing specific Avr proteins of pathogens)[12] through their leucine-rich repeats, but may also bind small hydrophobic molecules, such as brassinosteroids, that fit in the cavity formed by the island of 70 amino acids between leucine-rich repeat domains of BRI1. As a second alternative, BRI1 may only recognize protein ligands, such as putative steroid carriers. The recently discovered sex-hormone-binding globulins, which stimulate steroid signalling events by binding to as yet unknown extracellular receptors in animals, are excellent examples in support of this model.

The model presented by Li and Chory for BRI1 may equally be applied to other plant hormones. Numerous examples in *Drosophila* and animals demonstrate that ligands can induce the synthesis of their transmembrane receptors, and it is notable that two leucine-rich repeat receptor kinases induced by gibberellin and abscisic acid have recently been identified[10]. Although the first two models are attractive, other possibilities are also conceivable. For example, it is known that cholesterol can be covalently bound to SONIC HEDGEHOG in mammals and there is a putative sterol-sensing domain in the downstream receptor PATCHED[13].

Partners in other signalling pathways

One of the most exciting aspects of modelling potential BRI1 functions concerns the similarity of BRI1 to other receptor-like kinases that control cell-fate determination, differentiation and pathogen recognition. A BRI1 homologue, CLV1, has recently been demonstrated to regulate the rate of cell proliferation and organ formation in the apical meristem. SHOOT MERISTEMLESS, a KNOTTED-type homeodomain transcription factor, opposes CLV1 activity. CLV1 may control WUSCHEL, a factor required for determination of cell identity in the centre of floral and shoot meristems[14]. According to the first model of Li and Chory[10], it is conceivable that after brassinosteroid binding, BRI1 may heterodimerize with CLV1 to trigger cell proliferation. The ER kinase may offer a similar partner for heterodimeric BRI1 interactions in other cell types. The BRI1 protein may also form heterodimers with *R*-gene-encoded leucine-rich repeat receptors that mediate the recognition of viral, bacterial or fungal pathogens.

Certain analogies between signalling pathways mediated by the *Drosophila* Toll and plant leucine-rich repeat receptor kinases have recently been highlighted[12]. In *Drosophila*, Toll mediates the recognition of Spätzle (a peptide that induces early dorso-ventral signalling), which acts through Tube and Pelle (a Pto-kinase homologue), Dorsal (an NF-κB homologue that has domain similarity to plant *R*-gene products) and Cactus (an I-κB-like inhibitor of Dorsal with ankyrin repeats that also occur in a plant defence regulatory factor, NPR1). Ultimately, this pathway controls the activation of genes encoding decapentaplegic, a regulator of embryonic patterning, and an antifungal peptide, drosomycin. Stimulation of programmed cell death by Rel-like factors, such as Dorsal and NF-κB, is also known to be negatively regulated by glucocorticoids in animals. Thus, a potential heterodimerization between BRI1 and *R*-gene-encoded leucine-rich repeat receptors may provide ample combinations for brassinosteroid-mediated control of developmental and pathogenic signalling pathways. The importance of BRI1 as a receptor will be assured if only a minor portion of these hypothetical interactions is confirmed by further studies.

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