Planta (2002) 215: 127–133
DOI 10.1007/s00425-001-0723-z

ORIGINAL ARTICLE

**Gergely Molnár · Simona Bancoş · Ferenc Nagy
Miklós Szekeres**

Characterisation of *BRH1*, a brassinosteroid-responsive RING-H2 gene from *Arabidopsis thaliana*

Received: 3 August 2001 / Accepted: 13 November 2001 / Published online: 25 January 2002

Abstract Although many important aspects of plant development are controlled by brassinosteroids (BRs), the early molecular events of their hormonal action are largely unknown. Using a differential-display RT-PCR screen designed to detect early response transcripts, those regulated by BR treatment in the absence of de novo protein synthesis, we identified an *Arabidopsis thaliana* (L.) Heynh. gene (designated *BRH1*) that encodes a novel RING finger protein. As deduced from a complete cDNA clone, the 170-amino-acid sequence of BRH1 forms an N-terminal hydrophobic domain and a C-terminal RING-H2 signature. In wild-type *Arabidopsis*, the level of the *BRH1* transcript was rapidly down-regulated by brassinolide, but this effect was abolished in a BR-insensitive mutant deficient in the BRI1 receptor. *BRH1* mRNA abundance was not influenced by other phytohormones, but the pathogen elicitor chitin induced a rapid and transient accumulation of the transcript. Antisense expression of *BRH1* resulted in transgenic *Arabidopsis* plants with thicker inflorescence stems and altered leaf morphology, whereas in sense overexpression lines no phenotypic effect could be observed. Considering the potential of the RING proteins to participate in regulatory protein complexes, BR-dependent expression of *BRH1* may suggest its involvement in later hormonal effects.

Keywords *Arabidopsis* (*BRH1* gene) · Brassinosteroid · RING protein · Stress response

Abbreviations BL: brassinolide · BR: brassinosteroid · RT-PCR: reverse transcription polymerase chain reaction

G. Molnár · S. Bancoş · F. Nagy · M. Szekeres (✉)
Institute of Plant Biology,
Biological Research Center of the Hungarian Academy of Sciences, P.O. Box 521, 6701 Szeged, Hungary
E-mail: szekeres@nucleus.szbk.u-szeged.hu
Fax: +36-62-433434

Introduction

Brassinosteroids (BRs) are recently recognised phytohormones regulating cell elongation, photomorphogenesis, senescence, male fertility and various stress responses (Clouse and Sasse 1998; Altmann 1999). These physiological effects result from the specific modulation of defined gene activities through the BR signalling cascade. The active hormone brassinolide (BL) is known to be perceived by the plasma membrane-localised BRI1 leucine-rich repeat receptor kinase (LRRRK), but downstream elements required for signalling and transcriptional regulation are yet to be clarified (Li and Chory 1999; Bishop and Yokota 2001). Primary response genes, which change their activity in the absence of de novo protein synthesis, often encode protein factors coordinating further hormone-dependent changes in gene activity. Early auxin-induced genes were shown to have a role in providing and determining the availability of transcription factors mediating the auxin effect (for reviews, see: Abel and Theologis 1996; Gray and Estelle 2000).

RING finger proteins are defined by a conserved pattern of cysteine and histidine residues capable of coordinating two atoms of zinc within a characteristic cross-brace structure. Numerous members of this protein family occur in eukaryotic organisms and participate in diverse functions, such as signal transduction, vesicular transport, cell proliferation, embryonal patterning, etc. An accumulating body of evidence suggests that they act as specific organisers of regulatory protein complexes (reviewed by Saurin et al. 1996; Borden 2000). Recent studies have revealed that in many instances the RING domain, and particularly its RING-H2 variant, has a crucial function in ubiquitin-dependent protein degradation (Joazeiro and Weissmann 2000). The *Arabidopsis* genome project identified 358 distinct RING finger proteins, representing more than 1.4% of the 25,498 predicted proteins of this plant (The *Arabidopsis* Genome Initiative 2000). So far, functions have been assigned to only very few of them,

but the striking diversity suggests that they can be specificity factors in complex protein interactions.

The aim of our study was the identification of primary BR-responsive genes expected to participate in early regulatory events of hormone action. Here we report the isolation of a RING-H2 cDNA (*BRH1*) and expressional characterisation of the corresponding gene, which is down-regulated by BL in a specific and protein-synthesis-independent manner. We also show that the activity of this gene is induced by the pathogen elicitor chitin, and its antisense expression results in transgenic plants with altered leaf shape and stronger stature.

Materials and methods

Plant material, growth conditions and treatments

In vitro cultures of wild-type *Arabidopsis thaliana* (L.) Heynh. ecotype Columbia, and the BR-deficient *cpd* (Szekeres et al. 1996) and -insensitive *cbb2* (Kauschmann et al. 1996) mutants were grown from surface-sterilised seeds on Murashige and Skoog (MS) medium supplemented with 0.5% sucrose and 0.2% Phytagel (Sigma Chemical Co.) at 22 °C, under 14 h light/10 h dark cycles. Eight-day-old seedlings were removed from the Phytagel-supported medium and treated in MS liquid medium with 1 μM brassinolide (BL; CIDtech Research Inc, Missisauga, Ontario, Canada), 5 μM indole-3-acetic acid, gibberellic acid, 6-benzylaminopurine, abscisic acid, 50 μM jasmonic acid, 100 μM chloroethylphosphonic acid, 0.5 mM salicylic acid or 200 mg l^{-1} chitin. Cycloheximide (CHM; 100 μM) was applied 30 min before the onset of phytohormone treatment, as described in Koshiba et al. (1995). When using stock solutions prepared in ethanol, the respective control samples also contained the same amount (0.01%) of the solvent.

Isolation and characterization of the *BRH1* cDNA

Differential display reverse transcription polymerase chain reaction (DDRT-PCR, Liang and Pardee 1992) screening for BR-responsive mRNAs was performed with total RNA samples (0.2 μg per reaction) isolated from 8-day-old seedlings, using the RNAimage system (GenHunter Corp., Nashville, Ten., USA). Seedlings were pre-treated with CHM and then incubated for 1 h in the presence or absence of 1 μM BL. Out of 10 random primers, the 3 giving optimal amplification of the cDNA samples were selected empirically and used for the screening. The ^{32}P-labelled primary PCR products were separated on a 6% denaturing polyacrylamide gel, detected by autoradiography, and those showing altered abundance were isolated from the gel, re-amplified and cloned in pBluescript II vector. BR-induced changes of steady-state transcript levels were confirmed by Northern hybridisation of the original RNAs with the cDNA inserts. Five confirmed isolates were subjected to BLAST analysis (Atschul et al. 1990) and the cDNA encoding a RING-H2 protein motif of assumed regulatory function was selected for further characterisation. A clone containing the complete coding region was isolated by plaque hybridisation from a λ ZAP II cDNA library prepared from 2-week-old *Arabidopsis* (Columbia) seedlings (Stratagene). Physical mapping of the RING-H2 gene was carried out by hybridisation of the full-size cDNA to *Arabidopsis* yeast artificial chromosome (YAC) libraries (Ökrész et al. 1998).

Northern and quantitative RT-PCR analyses

Total RNA was isolated from 0.5–1 g fresh plant material by using either TRI Reagent (Sigma) or the method of Nagy et al. (1988). For determination of transcript abundance, 20-μg RNA samples were subjected to Northern hybridisation, as described earlier (Mathur et al. 1998). Quantitative RT-PCR assays were performed according to Chelly and Kahn (1994) with minor modifications. cDNA was prepared from 5 μg DNase I-treated RNA with a Ready-To-Go T-Primed First-Strand Kit (Pharmacia). One-tenth of the cDNA obtained was PCR-amplified within the linear range of accuracy by specific primers spanning 250- to 300-bp regions near the 3′ ends of the translated sequence. Signal intensities were detected by autoradiography following a single PCR cycle made with 1% of the RT-PCR products in the presence of α-[^{32}P]dCTP, using a single detection primer that was three nucleotides longer in the 3′ direction than one of the amplification primers. The constitutively expressed *UBQ10* mRNA (Sun and Callis 1997) was used as an internal control. The cDNA-specific primers used were as follows:

- *BRH1* (AF134155): 5′-CCGAAACCCGCTCACCTTTCT-3′ and 5′-GATAGAAAAGCGTGGCTTCTTCTA-3′,
- *ATL2* (L76926): 5′-CGAGTTCTAGCTCTGGATTGACGG-3′ and 5′-GGTTAACATTAAACCAATCAGACA-3′,
- *PR-3* (M38240): 5′-AGACTTCCCATGAAACTACAGGTG-3′ and 5′-GCTGAGCAGTCATCCAGAACCAAA-3′,
- *UBQ10* (L05361): 5′-GGACCAGCAGCGTCTCATCTTCG-CT-3′ and 5′-CTTATTCATCAGGGATTATACAAGGCC-3′.

The number of PCR cycles was 15 for *UBQ10*, 20 for *BRH1*, *ATL2* and *PR-3*.

Generation and characterisation of transgenic plants

Sense and antisense overexpression constructs were made by inserting the full-size *BRH1* cDNA between the cauliflower mosaic virus (CaMV) 35S promoter and the nopaline synthase (*NOS*) terminator in a T-DNA-based binary vector derived from pPCV812 (Koncz et al. 1994) by the removal of the β-glucuronidase (*GUS*) reporter gene. *Arabidopsis* plants (ecotype Wassilewskiya) were transformed by *Agrobacterium*-mediated transfer using the floral-dip method (Clough and Bent 1998). Independent transgenic plants (10 sense and 7 antisense) were isolated following selection on MS medium containing 15 mg l^{-1} hygromycin. In the T2 generation of transgenic lines the *BRH1* and *As-BRH1* transcript levels were determined by Northern hybridisation with strand-specific cDNA probes.

Results

Isolation and characterisation of a cDNA derived from a BR-responsive RING-H2 gene

The 723-bp cDNA containing the complete coding region of *BRH1* (*BRASSINOSTEROID-RESPONSIVE RING*-H2, GenBank AF134155) was isolated following its identification with a differential display RT-PCR screen for BR-regulated transcripts. It is comprised of 20-bp 5′- and 193-bp 3′-UTR sequences surrounding the 510-bp open reading frame coding for a RING-H2 protein of 170 amino acids. The chromosomal position of the *BRH1* gene was mapped to near *FUS6* within the ninth contig of chromosome 3 by colony hybridisation of yeast lines containing yeast artificial chromosome clones of *Arabidopsis* genome segments with the cDNA. The mapping results and those obtained by Southern analysis indicate that *BRH1* is a single-copy gene (data not shown). The genomic sequence (*Arabidopsis* genome

project, entry AL132962) is intron-free and features a putative TATA-box (TATAAAT) 85 bp upstream of the 5′ end position of the cDNA. The assumed translational start code is the first ATG downstream of the TATA box.

On the basis of the deduced amino acid sequence (Fig. 1), BRH1 has a calculated molecular mass of 20.06 kDa. The N-terminal part of the protein (amino acids 1 to 87) is rich in hydrophobic residues (66%), and the C-terminal region contains a characteristic RING-H2 signature C-x_2-C-x_{15}-C-x-H-x_2-H-x_2-C-x_{11}-C-x_2-C (Saurin et al. 1996). A BLAST homology search (Altschul et al. 1990) did not identify any other functional domain within the protein. Structural features of the RING-H2 motif and additional homology throughout the sequence indicate that BRH1 belongs to the RH group, RHA subgroup of RING-H2 proteins described by Jensen et al. (1998), and is closest related to the putative *Arabidopsis* proteins g9758049 and g12325012 (Fig. 1A, B).

Regulation of *BRH1* mRNA level by phytohormones

Northern analyses showed that in seedlings the abundance of the *BRH1* mRNA decreased to about 30% of the initial value within 1 h of treatment with 1 μM BL, and then remained unchanged upon longer incubation (Fig. 2C). Compared with the wild type, in the BR-deficient *cpd* mutant the transcript level was elevated, but could be down-regulated, although to a lesser extent, by the steroid hormone. The repression effect was not prevented by simultaneous application of the protein-synthesis inhibitor cycloheximide (Koshiba et al. 1995), indicating that it is mediated by protein factors already present in the cells (Fig. 2A). By contrast, in

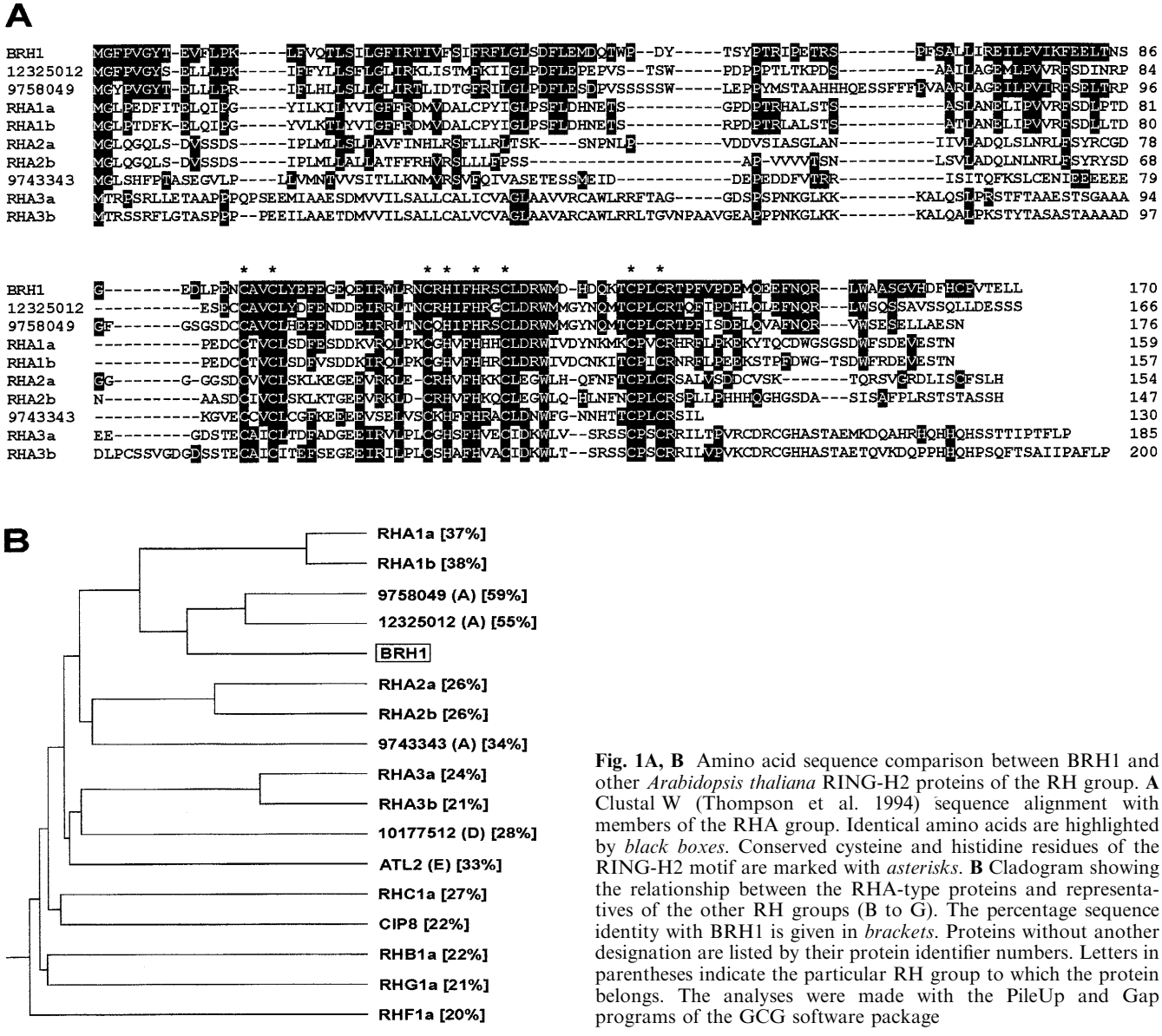

Fig. 1A, B Amino acid sequence comparison between BRH1 and other *Arabidopsis thaliana* RING-H2 proteins of the RH group. **A** Clustal W (Thompson et al. 1994) sequence alignment with members of the RHA group. Identical amino acids are highlighted by *black boxes*. Conserved cysteine and histidine residues of the RING-H2 motif are marked with *asterisks*. **B** Cladogram showing the relationship between the RHA-type proteins and representatives of the other RH groups (B to G). The percentage sequence identity with BRH1 is given in *brackets*. Proteins without another designation are listed by their protein identifier numbers. Letters in parentheses indicate the particular RH group to which the protein belongs. The analyses were made with the PileUp and Gap programs of the GCG software package

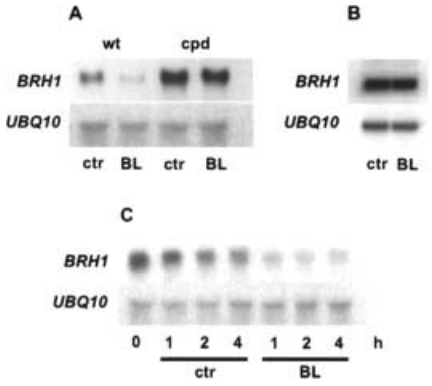

Fig. 2A–C Brassinosteroid-dependent decrease in the abundance of *Arabidopsis BRH1* mRNA. **A** Northern analysis of the transcript levels in 8-day-old wild-type (*wt*) and BR-deficient (*cpd*) seedlings following 1 h incubation with (*BL*) or without (*ctr*) 1 μM BL. **B** RT-PCR products obtained from total RNA of 8-day-old BR-insensitive cbb2 seedlings, BL-treated (1 μM, 2 h) and control. **C** Northern blot showing the mRNA levels during a 4-h incubation period in 8-day-old BL-treated and control wild-type seedlings. In all experiments *UBQ10* was used as an internal control. Each Northern blot lane contained 20 μg total RNA. In **A** and **B**, treatments were done in the presence of 100 μM cycloheximide

BR-insensitive cbb2 seedlings the de-repressed expression of *BRH1* was not influenced by BL treatment (Fig. 2B), suggesting that the BR-induced down-regulation of *BRH1* requires the participation of a functional BRI1 receptor (Li and Chory 1997).

Because different types of phytohormone can act in a concerted manner or use common signalling routes, we also tested the effect of auxin, cytokinin, gibberellin, abscisic acid and ethylene on the abundance of *BRH1* mRNA. During the first 4 h of treatment the transcript level was not appreciably affected by any of these hormones (data not shown), indicating that down-regulation of *BRH1* is a specific BR effect.

Elicitor-induced activation of *BRH1*

Despite the large variety of *Arabidopsis* RH-type proteins, their biological functions are largely unknown. Because some members of this family, such as ATL2 and ATL6, were shown to be induced by chitin (Salinas-Mondragón et al. 1999), we tested the effect of this pathogen elicitor on *BRH1* expression as a possible indication of an expressional or functional relationship between *BRH1* and these *ATL* genes. Chitin caused a rapid and transient increase in *BRH1* mRNA, to about 3-fold the control level, peaking at around the 45th minute of the treatment and falling back to the initial value thereafter (Fig. 3). The induction was weaker than that of *ATL2*, but its time-course was very similar. By contrast, transcript levels of the basic chitinase (PR-3; Samac et al. 1990) remained high for several hours following an early increase in the presence of chitin. In order to determine the specificity of *BRH1* induction, we also tested other regulators of pathogen response, but

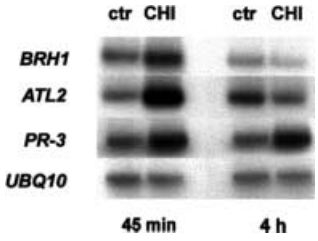

Fig. 3 Chitin-induced accumulation of *BRH1* and other pathogen-responsive transcripts. Results of RT-PCR assays with RNAs of 8-day-old wild-type *Arabidopsis* seedlings incubated with (*CHI*) or without (*ctr*) 200 mg l^{-1} chitin for 45 min or 4 h. In all assays the same cDNA preparation was used as template. *UBQ10* served as a constitutive control

salicylate, jasmonate, abscisic acid and ethylene failed to elicit appreciable transcript accumulation (data not shown).

Phenotypic effect of *BRH1* overexpression in transgenic *Arabidopsis*

Independent lines of transgenic *Arabidopsis* carrying sense or antisense overexpression constructs of *BRH1* were generated and their phenotypic traits and transgene expression levels were analysed. Compared with the wild type, antisense transformant lines AS/3 and AS/7 with high *AS-BRH1* expression level (Fig. 4A) developed into more vigorous plants with thicker inflorescence stems, enlarged rosette and cauline leaves (Fig. 4B), but their flower structure and flowering time remained unchanged. Rosette leaves had a more rounded tip, while cauline leaves were more serrate than those of the non-transformed plants (Fig. 4C). Compared with the wild type, the cotyledons and primary leaves of the AS/3 and AS/7 seedlings were less elongated. By contrast, no phenotypic effect could be observed in the antisense lines with low *AS-BRH1* and unchanged *BRH1* transcript levels, or in any of the sense overexpression plants.

Discussion

Phytohormones play an important role in coordinating developmental events at the whole-plant level. Following binding to their specific receptors, they initiate intracellular signalling events leading to well-defined changes in gene expression. While BRs are known to regulate a host of diverse physiological functions, their effect on gene activity is largely unknown. Most BR-responsive genes that have been identified so far encode biosynthetic enzymes, and affect cell expansion and proliferation, but do not participate directly in signalling processes (discussed in Bishop and Yokota 2001). Recently Jiang and Clouse (2001) have reported that a gene encoding the *Arabidopsis* homologue of TRIP1, a TGFβ receptor-interacting protein, is rapidly induced by BL and their results implicate this putative elongation factor

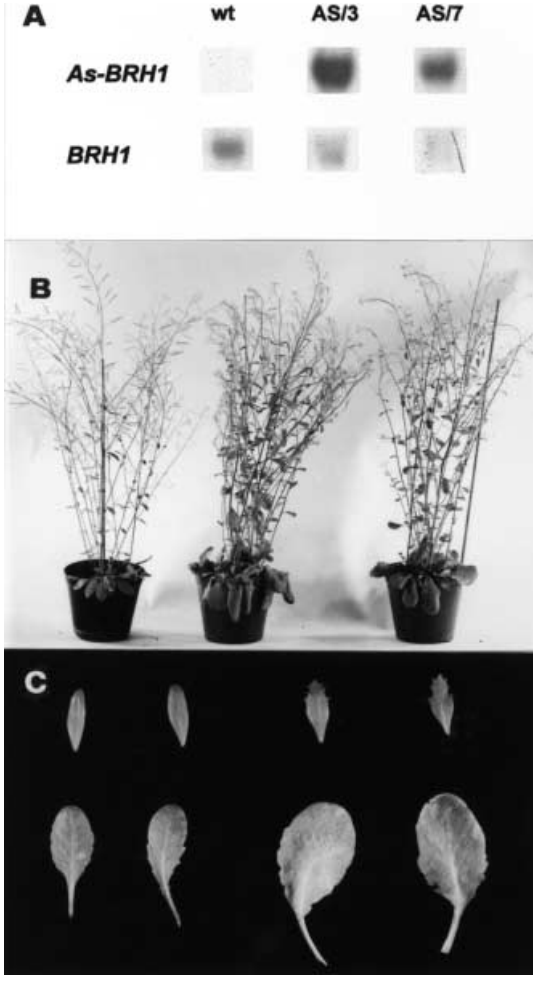

Fig. 4A–C Expression of antisense *BRH1* in transgenic *Arabidopsis*. **A** Northern analysis of antisense and sense *BRH1* transcripts from seedlings of two antisense lines (*AS/3* and *AS/7*) and the wild-type (*wt*). The blot with total RNA of 12-day-old whole seedlings was hybridised with strand-specific probes. **B** One-month-old wild-type (*left*) and antisense *BRH1*-overexpressing plants (AS/3 *centre*, AS/7 *right*). **C** Cauline (*top*) and rosette leaves (*bottom*) of one-month-old wild-type (*left*) and AS/3 transgenic plants (*right*). All plants were grown for 20 days under short days (8 h light/16 h darkness), then for 10 days under long days (12 h light/12 h darkness)

in mediating steroid response. Because BR-controlled repression of *BRH1* is independent of protein synthesis, it constitutes a primary hormone response that, in accord with the domain structure of the protein, suggests a possible involvement of BRH1 in regulatory functions.

The BR-dependent regulation of the BRH1 transcript level requires the activity of the BRH1 receptor; however, the underlying molecular mechanism is still unknown. Nonetheless, the independence of that down-regulation from de novo protein synthesis suggests that it occurs primarily at the transcriptional level.

Despite their apparent structural diversity, all RING finger proteins seem to function as molecular recognition determinants of multi-protein complexes (Borden 2000). During the past couple of years an impressive amount of information has accumulated about their participation in ubiquitination reactions that target defined proteins for S26 proteasome-dependent degradation, a regulatory mechanism conserved in eukaryotic organisms (Joazeiro and Weissman 2000). Proteins with RING motifs can function as autonomous E3 ubiquitin ligases or serve as essential domains in different classes of multi-subunit E3 complexes. In plants COP1, a RING-finger-containing central regulator of photomorphogenesis, was shown to bind the transcriptional regulator HY5 in the dark and act as an E3 by promoting its ubiquitination and concomitant proteolysis (Osterlund et al. 2000). In the case of another RING protein, the membrane-bound *Arabidopsis* RMA1, ubiquitin ligase activity was demonstrated in vitro (Matsuda et al. 2001).

Some RING-H2 proteins are essential components of multi-subunit ubiquitin ligases. In plants their best-characterised class comprises the SCF-type E3 complexes, among which *Arabidopsis* SCF^{TIR1} is an important mediator of auxin effect, presumably by initiating the degradation of inhibitors of the response. Data obtained with yeast two-hybrid assays suggest that AtRBX1, the RING-H2 subunit of this enzyme, can directly interact with components of the COP9 signalosome, the so-called "lid" subcomplex of the 26S proteasome (Schwechheimer et al. 2001). SCF ubiquitin ligases are assumed to control various other physiological responses because mutations affecting their F-box protein subunit can interfere with the circadian rhythm, flower development and jasmonate perception. The *Arabidopsis* Genome Initiative (2000) predicted 337 F-box proteins in this organism and these, as the E3 subunits interacting with the substrate, are seen as important specificity determinants (Xiao and Jang 2000). The RING-H2 elements of the SCF complexes, such as AtRBX1, are composed only of the RING and a hydrophobic domain but still can establish interaction with three other subunits, including the F-box protein. Although in plants the roles of RING-H2 proteins are little understood and may be much more diverse than participation in ubiquitination, the high number of RING-H2 genes suggests that they can contribute to the variety of E3 enzymes. On the other hand, the scarcity of recognised RING mutants in *Arabidopsis* may indicate that many of these proteins can have overlapping, partially redundant functions.

While there are large RING-H2 gene families in higher plants (Jensen et al. 1998; Salinas-Mondragón et al. 1999), our knowledge regarding their biological role is very limited. Torii et al. (1999) found that in yeast two-hybrid tests *Arabidopsis* CIP8 interacts with COP1 in a way that can influence its intracellular partitioning. Also in *Arabidopsis*, ATL2 was shown to be induced in an early response to pathogen elicitors. ATL2 and other members of the ATL family are small (less than 350 amino acids) bipartite proteins expressed differentially upon pathogen elicitor treatment (Salinas-Mondragón et al. 1999). BRH1 is also a small RING-H2 protein, composed of an

N-terminal hydrophobic domain and a C-terminal RING-H2 signature that shows 33% amino acid sequence identity with ATL2. Presently the role of these proteins is unclear. On the basis of its size and structural features, BRH1 may function as part of a multi-subunit E3 complex and influence selective degradation of proteins, including regulators, in a BR-dependent manner. Indeed, selective proteolysis controls important developmental processes, such as tracheary element differentiation, senescence, apoptosis, and pathogen response (Estelle 2001), phenomena also known to be controlled by BRs (Mandava 1988; Clouse and Sasse 1998).

The phytohormones ethylene, jasmonate and salicylate are crucial mediators of pathogen responses, but other hormones can also modulate the response and the level of resistance. Suppression of gibberellin synthesis results in increased tolerance to both abiotic and biotic stress factors (Vettakkorumakankav et al. 1999), and BRs can influence the transcript levels of stress- and pathogenesis-related genes (Szekeres et al. 1996). If BRH1 participates in selective degradation of regulatory proteins, it seems feasible that BRs could alleviate, while the pathogen elicitor chitin could enhance, this effect. The rapid, chitin-induced accumulation of *BRH1* mRNA was very similar to those of *ATL2* and *PR-3*. Sequence analysis of the *BRH1* promoter revealed the presence of four W-box elements, two of them in a closely spaced tandem arrangement, within a 500-bp segment upstream of the translational start. The role of these *cis*-regulatory elements in *BRH1* expression has yet to be clarified, but W-box sequences were shown to be sufficient to evoke transcriptional response to fungal elicitors (Raventos et al. 1995).

Overexpression of *BRH1* in transgenic *Arabidopsis* gave no visible phenotype, a result that might be consistent with the high basal activity of this gene. By contrast, from the seven antisense lines, two of strong *AS-BRH1* expression had vigorous stature and enlarged leaves. The basis of these morphological changes is unclear, but they may result from a role of BRH1 in mediating the elongation effect of BRs or a BR-dependent interference with the signalling of other elongation-promoting hormones, such as auxin and gibberellins. Because *BRH1* is member of a gene family, the possibility of antisense interference with the function of some related gene(s) sharing significant local homology cannot be entirely excluded.

Acknowledgement The authors are thankful to Csaba Koncz (Max-Planck-Institut für Züchtungsforschung, Cologne) for his helpful discussion during the preparation of the manuscript. This work was supported by a research grant from the Hungarian National Research Foundation (OTKA, grant No. T 32432).

References

Abel S, Theologis A (1996) Early genes and auxin action. Plant Physiol 111:9–17

Altmann T (1999) Molecular physiology of brassinosteroids revealed by the analysis of mutants. Planta 208:1–11

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Bishop GJ, Yokota T (2001) Plants steroid hormones, brassinosteroids: current highlights of molecular aspects on their synthesis/metabolism, transport, perception and response. Plant Cell Physiol 42:114–120

Borden KLB (2000) RING domains: master builders of molecular scaffolds? J Mol Biol 295:1103–1112

Chelly J, Kahn A (1994) RT-PCR and mRNA quantification. In: Mullis KB, Ferré F, Gibbs RA (eds) The polymerase chain reaction. Birkhäuser, Boston Basel Berlin, pp 97–109

Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J 16:735–743

Clouse SD, Sasse JM (1998) Brassinosteroids: essential regulators of plant growth and development. Annu Rev Plant Physiol Plant Mol Biol 49:427–451

Estelle M (2001) Proteases and cellular regulation in plants. Curr Opin Plant Biol 4:254–260

Gray WM, Estelle M (2000) Function of the ubiquitin-proteasome pathway in auxin response. Trends Biochem Sci 25:133–138

Jensen RB, Jensen KL, Jespersen HM, Skriver K (1998) Widespread occurrence of a highly conserved RING-H2 zinc finger motif in the model plant *Arabidopsis thaliana*. FEBS Letters 436:283–287

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

Joazeiro CAP, Weissmann AM (2000) RING finger proteins: mediators of ubiquitin ligase activity. Cell 102:549–552

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

Koncz C, Martini N, Szabados L, Hrouda M, Bachmair A, Schell J (1994) Specialized vectors for gene tagging and expression studies. In: Gelvin SB, Schilperoort AR (eds) Plant molecular biology manual, vol B2. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 1–22

Koshiba T, Ballas N, Wong L-M, Theologis A (1995) Transcriptional regulation of *PS-IAA4/5* and *PS-IAA/6* early gene expression by indoleacetic acid and protein synthesis inhibitors in pea (*Pisum sativum*). J Mol Biol 253:396–413

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

Li J, Chory J (1999) Brassinosteroid actions in plants. J Exp Bot 50:275–282

Liang P, Pardee AB (1992) Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 257:967–971

Mandava NB (1988) Plant growth-promoting brassinosteroids. Annu Rev Plant Physiol Plant Mol Biol 39:23–52

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

Matsuda N, Suzuki T, Tanaka K, Nakano A (2001) Rma1, a novel type of RING finger protein conserved from *Arabidopsis* to human, is a membrane-bound ubiquitin ligase. J Cell Sci 114:1949–1957

Nagy F, Kay SA, Chua N-H (1988) Analysis of gene expression in transgenic plants. In: Gelvin SB, Schilperoort AR (eds) Plant gene research manual. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 1–29

Ökrész L, Máthé C, Horváth É, Schell J, Koncz C, Szabados L (1998) T-DNA trapping of a cryptic promoter identifies an ortholog of highly conserved *SNZ* growth arrest response genes in *Arabidopsis*. Plant Sci 138:217–228

Osterlund MT, Hardtke CS, Wei N, Deng XW (2000) Targeted destabilization of HY5 during light-regulated development of *Arabidopsis*. Nature 405:462–466

Raventos D, Jensen AB, Rask M-B, Casacuberta JM, Mundy J, San Segundo B (1995) A 20 bp *cis*-acting element is both necessary and sufficient to mediate elicitor response of a maize PRms gene. Plant J 7:147–156

Salinas-Mondragón R, Garciduenas-Pina C, Guzmán P (1999) Early elicitor induction in members of a novel multigene family coding for highly related RING-H2 proteins in *Arabidopsis thaliana*. Plant Mol Biol 40:579–590

Samac, DA, Hironaka, CM, Yallaly, PE, Shah, DM (1990) Isolation and characterization of the genes encoding basic and acidic chitinases in *Arabidopsis thaliana*. Plant Physiol 93:907–914

Saurin, AJ, Borden KLB, Boddy MN, Freemont PS (1996) Does this have a familiar RING? Trends Biochem Sci 21:208–214

Schwechheimer C, Serino G, Callis J, Crosby WL, Lyapina S, Deshaies RJ, Gray WM, Estelle M, Deng X-W (2001) Interactions of the COP9 signalosome with the E3 ubiquitin ligase SCF^{TIR1} in mediating auxin response. Science 292:1379–1382

Sun C-W, Callis J (1997) Independent modulation of *Arabidopsis thaliana* polyubiquitin mRNAs in different organs and in response to environmental changes. Plant J 11:1017–1027

Szekeres M, Németh K, Koncz-Kálmán Z, Mathur J, Kauschmann A, Altmann T, Rédei PG, Nagy F, Schell J, Koncz C (1996) Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in *Arabidopsis*. Cell 85:171–182

The *Arabidopsis* Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408:796–815

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Torii KI, Stoop-Myer CD, Okamoto H, Coleman JE, Matsui M, Deng X-W (1999) The RING finger motif of photomorphogenic repressor COP1 specifically interacts with the RING-H2 motif of a novel *Arabidopsis* protein. J Biol Chem 274:27674–27681

Vettakkorumakankav NJ, Falk D, Saxena P, Fletcher RA (1999) A crucial role for gibberellins in stress protection of plants. Plant Cell Physiol 40:542–548

Xiao W, Jang J-C (2000) F-box proteins in *Arabidopsis*. Trends Plant Sci 5:454–457