A gene trap *Dissociation* insertion line, associated with a RING-H2 finger gene, shows tissue specific and developmental regulated expression of the gene in *Arabidopsis*

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Received 27 December 2001; accepted 11 March 2002

Received by B. Hohn

Abstract

Real interesting new gene (RING) finger proteins act as E3 ubiquitin-protein ligases and play critical roles in targeting the destruction of proteins of diverse functions in all eukaryotes, ranging from yeast to mammals. *Arabidopsis* genome contains a large number of genes encoding RING finger proteins. In this report we describe the identification of more than 40 RING-H2 finger proteins that are of small size, not more than 200 amino acids, and contain no other recognizable protein–protein interaction domain(s). We characterize RHA2b, one of these small RING-H2 finger genes. A gene trap line, SGT6304, was identified to contain a *Dissociation* (Ds) insertion in RHA2b gene. No RHA2b transcript was detected in the homozygous SGT6304 plants. Despite the elimination of RHA2b function, homozygous SGT6304 plants lacked detectable growth or development defects, suggesting functional redundancy of RHA2b with other RING finger genes. Expression of RHA2b was specifically active in vascular tissue and in upper pistil of inflorescence as well as in root tip and shoot apical meristem region. Potential functions of ubiquitin-proteolysis pathway in vascular formation and in fertilization are discussed. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Proteolysis; Ubiquitin ligase; Vascular formation; Transposon tagging

1. Introduction

The RING (for Real Interesting New Gene) finger motif is a small zinc-binding domain defined by the consensus sequence Cys-X2-Cys-(9–39)-Cys-(1–3)-His-X2-(2–3)-Cys/His-X2-Cys-(4–48)-Cys-X2-Cys, where X can be any amino acids and the Cys (cysteine) and His (histidine) representing zinc binding residues. RING fingers can be subcategorized into RING-HC and RING-H2 depending of whether a Cys or His occupies the fifth coordination site, respectively. The RING finger forms one integrated structural unit which binds two zinc atoms in a unique ‘cross-brace’ arrangement, differing from the tandem arrangement of zinc binding sites characteristic of zinc fingers (reviewed in Freemont, 2000; Joazeiro and Weissman, 2000).

Recent studies demonstrate that RING finger proteins play critical roles in specific ubiquitination events and it has been more generally hypothesized that all RING proteins act as ubiquitin (Ub) protein ligases (Freemont, 2000; Joazeiro and Weissman, 2000). Ub-dependent proteolysis pathway controls the selective degradation of a large number of proteins involved in diverse cellular processes that include cell cycle regulation, stress responses, signal transduction, metabolic regulation and cell differentiation (for review, see Hershko and Ciechanover, 1998). Generally, protein ubiquitination is achieved through sequential action of three types of enzymes: Ub-activating enzyme (E1), Ub-conjugating enzyme (E2) and Ub-protein ligases (E3). Ub is first attached, through its C-terminus, to the active site cysteine of E1; then transferred to an E2, again through a thiol-ester linkage. E3s, which are primarily responsible for providing specificity to Ub conjugation,
interact with E2 and substrate, facilitating formation of isopeptide linkages between the C-terminus of Ub and lysines either on a target protein or on the last Ub of a substrate-bound poly-Ub chain. Once a poly-Ub chain is assembled on a substrate, the substrate is then degraded by the 26S proteasome. The 26S proteasome appears to be similar in organization and structure, and likely functions in an analogous manner in plant as in animal cells (Parmentier et al., 1997).

RING finger motifs have been shown in many cases to be required for interactions between RING finger proteins and E2s (reviewed in Freemont, 2000; Joazeiro and Weissman, 2000). Crystal structure analysis revealed that the E2 UbcH7 binds to the RING protein c-Cbl through contacts between a groove within the RING domain of c-Cbl and two loops in the E2 fold of UbcH7 (Zheng et al., 2000). Although elements determining the specificity of RING-E2 pairs have yet to be clearly defined, RING fingers have been proposed to serve to specifically recruit and allosterically activate E2s.

The recruitment of substrates by the RING finger E3s can be achieved either through additional protein–protein interaction domains present on the RING proteins themselves, such as the SH2-domain in the case of c-Cbl (Joazeiro and Weissman, 2000), or through multisubunit protein complexes. A small noncanonical RING-H2 finger protein, RBX1 (also referred to as ROC1 and HRT1), has been identified to be an essential component of SCF E3 complexes (reviewed in Tyers and Jorgensen, 2000). The SCF complex, named from the three initially identified subunits SKP1, Cullin/CDC53, F-box protein, targets a number of cell cycle regulators, transcription factors and other proteins for degradation. In this complex, the F-box protein acts as substrate-targeting subunit and interacts through the SKP1 intermediate with the N-terminal part of the Cullin/CDC53. RBX1 promotes association of the E2 CDC34 with the complex through interaction with the C-terminus of the Cullin/CDC53 (Wu et al., 2000; Furukawa et al., 2000). In a strikingly similar manner, the small RING-H2 finger protein APC11 is an essential component of the APC (or cyclosome) E3 complex, which specifically targets a number of mitotic factors such as cyclin B and securin for degradation (Gmachl et al., 2000; Leverson et al., 2000).

Sequence similarity analysis reveals that the Arabidopsis genome, the first entirely sequenced plant genome (The Arabidopsis Genome Initiative, Nature 408: 796–815), contains a large number of genes encoding RING finger proteins. Although characterization of several plant RING finger proteins including the photomorphogenesis regulators COP1 and CIP8 (Torii et al., 1999), the N-end rule E3 enzyme PRT1 (Potuschak et al., 1998), and the membrane-bound E3 enzyme RMA1 (Matsuda et al., 2001) have been reported, few molecular data are available on the expression and function of the small RING finger proteins. In this report, we describe characterization of the Arabidopsis RHA2b gene (named according to Jensen et al., 1998) that encodes a RING-H2 finger protein of 147 amino acids. A Dissociation (Ds) insertion line associated with RHA2b was initially isolated during random analysis of flanking sequences from the collection of gene trap lines (Parinov et al., 1999). Analysis of this Ds-insertion line revealed that the Ds-insertion has created a loss-of-function allele. No obvious mutant phenotype was evident at the studied experimental conditions, suggesting redundant function of RHA2b with other genes. Reporter gene expression patterns revealed that RHA2b was highly expressed in vascular tissues as well as in the transmitting tissue of the styles of flowers.

2. Materials and methods

2.1. Plant materials

The Arabidopsis plants were of the landsberg erecta ecotype. Seeds were produced under greenhouse conditions. Arabidopsis cell suspensions were maintained by weekly subculture as described by Glab et al. (1994).

2.2. Arabidopsis line SGT6304

Arabidopsis line SGT6304 containing Ds insertion in the RHA2b gene was kindly provided by Dr. V. Sundaresan (Institute of Molecular Agrobiology, The National University of Singapore). F4 seeds collected from multiple kanamycin (Km) resistant plants were surface sterilized and plated onto Km (50 mg/l)-supplemented medium (half-strength Murashige and Skoog salts, 1% sucrose, 0.9% agar, pH 5.7). Km-resistant plants were transplanted to soil and allowed to set seeds by self-fertilization. Homozygous plants, which segregated 100% for Km resistance, were identified in the F4. Ds integration in the RHA2b gene was confirmed by PCR-amplification using gene specific primers: 5'-AGCTTAGATAGATATGAAATCAGCAT-3' (corresponding to −293 to −265 bp upstream of ATG codon of RHA2b) and 5'-TGTTTCCGTCCCGCAAGT-3' (Ds3'-3a in Parinov et al., 1999) for the 5'-end, and 5'-TTGATTCTAGACAGACAGATGCGAT-3' (corresponding to +295 to +323 bp downstream of ATG codon of RHA2b) and 5'-TCCGTTCCTTTTCGTTTTAC-3' (Ds5'-2a in Parinov et al., 1999) for the 3'-end junction between RHA2b and the Ds. The PCR products were sequenced to determine the precise fusion between RHA2b and Ds.

2.3. Histochemical GUS assays

Whole-mount β-glucuronidase (GUS)-staining of Arabidopsis seedlings germinated on plates or in inflorescences from soil-grown plants were performed using the method described by Jefferson et al. (1987). For visualization, the tissues were cleared for chlorophyll by rinsing with ethanol (70–95%) and photographed under a stereomicroscope or with Nomarski optics. Thin sections of GUS-stained mate-
rial were prepared after embedding in Historesin according to the manufacturer’s recommendations (Leica Instruments GmbH, Heidelberg). Images were scanned and processed with Adobe Photoshop 3.0 (Adobe systems, Mountain View, CA).

2.4. Treatments of plant tissues and suspension cultured cells

RHA2b expression in response to the plant hormone auxin was assayed according to the method described by Gil et al. (1994). Briefly, hypocotyls of etiolated seedlings of Arabidopsis that had been grown in complete darkness for 10 days were cut into 2–3-mm sections. After incubation in KPSC buffer for 4 h to deplete endogenous hormones, the segments were transferred to fresh KPSC buffer with or without exogenous hormones for 1 h at 28 °C and then frozen in liquid nitrogen for RNA preparation.

The leaf-strips experiments used to study RHA2b expression during re-initiation of cell division activity in Arabidopsis have been previously described (Genschik et al., 1994). Carboxbenzoxyl-leucinyl-leucinyl-leucinal (MG132) has been previously shown to be an efficient proteasome inhibitor that induces metaphase arrest in tobacco BY2 cells (Genschik et al., 1998). To test RHA2b expression in response to MG132, 3-day-old suspension cultured cells of Arabidopsis were incubated with 100 μM MG132. Cells were collected 1 and 3 h after the treatment and frozen in liquid nitrogen for RNA preparation.

2.5. Northern blot analysis

Total RNA was isolated from various plant tissues as well as from suspension cultured cells via the hot phenol method (Nagy et al., 1988). Aliquots of 20 μg RNA were analyzed by electrophoresis on formaldehyde/agarose gels and blotted onto Hybond-NX nylon membranes according to the manufacturer’s recommendations (Amersham Pharmacia Biotech). Hybridization was performed under standard high-stringency conditions (Sambrook et al., 1989). The RHA2b and HSP70 probes correspond to the EST clones 11A10TT (GenBank AA728515) and G5C8T7 (GenBank AAC-3′, and subsequently cloned into the EcoRI and BamHI sites of pGBKT7 vector (Clontech Laboratories Inc., Palo Alto, CA). The derived plasmid was sequenced to confirm the absence of errors from PCR-amplification and the correct in-frame fusion with the GAL4 DNA binding domain. Plasmids containing Arabidopsis Cullin cDNAs fused with the GAL4 activation domain were obtained (W.-H. Shen, unpublished). Interactions between RHA2b and Cullins were assayed according to the manufacturer’s recommendations (Clontech).

2.7. Sequence analysis

Database searches were performed using BLASTP (http://www.ncbi.nlm.nih.gov/BLAST/). Multiple alignments of protein sequences were done with the ClustalW program (http://www.ebi.ac.uk/clustalw/) and phylogenetic tree displayed using TreeViewPPC (Roderic D.M. Pase) programs.

3. Results

3.1. Homozygous SGT6304 line plants fail to express RHA2b RNA, but appear phenotypically normal

An insertional mutagenesis system through use of Ds/Ac transposable elements had been developed in Arabidopsis by Sundaresan et al. (Parinov et al., 1999). In this system, the modified Ds element carrying the GUS reporter gene as well as the NPTII gene that confers resistance to Km as a selectable marker was used as a gene trap. Random analysis of sequences flanking the Ds had revealed that the SGT6304 line contains Ds insertion associated with the RHA2b gene (Parinov et al., 1999). From F4 progeny, we selected both heterozygous and homozygous SGT6304 lines, which exhibited the characteristic segregation ratios for Km-resistance. PCR-amplification with Ds and RHA2b specific primers confirmed Ds insertion in the RHA2b gene. Sequence analysis of the PCR-amplified products revealed that the Ds is inserted at the very N-terminal coding region, between the fourth and fifth codon, of the RHA2b gene (Fig. 1A).

Northern blot analysis detected a single band with the predicted size. This transcript could be detected only in heterozygous but not in homozygous SGT6304 plants (Fig. 1B), indicating that the Ds insertion created a loss-of-function allele of RHA2b. Despite the null mutation of RHA2b, homozygous SGT6304 plants do not exhibit obvious growth or developmental defects. The mutant seeds germinate at the same time as wild-type ones, and the plants show normal morphology. Flowering time and seed production are also not affected.

3.2. RHA2b transcript accumulates at different levels in several types of plant tissues

To determine the tissue distribution of RHA2b transcript,
expression relies entirely on transcription signals of the tagged gene. In the SGT6304 line, the GUS gene was fused in the transcribed orientation with the RHA2b gene (Fig. 1A). Thus, the GUS gene represents an ideal reporter for studying the expression pattern of the tagged RHA2b gene.

Both in vitro- and greenhouse-grown plants were analyzed for GUS activity by histochemical staining. No significant difference in GUS staining pattern was noticed between in vitro-grown and greenhouse-grown plants. At juvenile phase, GUS staining was consistently localized in the vascular cylinder throughout the entire seedling (Fig. 3A). The strongest GUS staining was found in meristematic regions of shoots (Fig. 3B), in hypocotyl-root transition zones (Fig. 3C), and in tips of primary and lateral roots (Fig. 3D,F). A relatively strong staining was also observed in the vascular cylinder regions where lateral roots emerged (Fig. 3E,F).

At the adult phase, vasculature-localized GUS staining could still be observed but preferentially in young organs, such as upper axillary shoots and stems close to inflorescences (data not shown). In inflorescences, the strongest GUS staining was detected in the upper pistil, from floral stage 11 (Smyth et al., 1990) onward (Fig. 3G). Particular strong staining was localized to the lower stigmatic zone and the upper transmitting tract, which is composed of the longitudinal files of cells, of the pistil (Fig. 3H,I). The outer papillae of the stigma was barely stained. With prolonged incubation time, GUS staining was also visible on sepals of flowers (data not shown). Upon fertilization the strong GUS staining extended from the upper pistil to the carpel walls, and localized mainly in the valve margin and replum of the walls (Fig. 3J). The staining of carpel walls disappeared after the developmental time corresponding to heart embryo stage. In ovaries, GUS staining was detected in embryo-developing areas at early stages, up to early heart stage (Fig. 3K). GUS staining in embryos was observed at mature stage (Fig. 3L), with strongest staining in the tip of radicle (Fig. 3M).

Together these GUS staining patterns reveal that the expression of RHA2b is particularly active in shoot and root tips, in vascular tissues of various organs, and in upper pistils of inflorescence. This is consistent with the previous Northern data and further suggests that RHA2b functions in a highly tissue-specific and developmental regulated manner.

3.4. Accumulation of RHA2b transcript in response to auxins and MG132

It is well established that the phytohormone auxin play essential role in lateral root primordium initiation (Celenza et al., 1995; and references therein). The strong GUS staining detected in root tips and around lateral root initiation sites in SGT6304 plants promoted us to evaluate the regulation of RHA2b expression by auxin. We chose the experimental system of using hypocotyls of etiolated seedling because of its rapid response to auxin (Gil et al., 1994).
The level of **RHA2b** transcript remained unchanged 1 h after the treatment with auxins whereas the positive control **SAUR** gene responded positively to the treatments (Fig. 4A). It is thus unlikely that **RHA2b** belongs to auxin-responsive gene family.

**RHA2b** expression during re-initiation of mitotic activity was evaluated by using the leaf-strips experimental system (Genschik et al., 1994). In this system the differentiated leaf cells were stimulated to re-enter the cell cycle by wounding and auxin stimulation. The level of **RHA2b** transcript decreased significantly at 24 h, and then recovered to the initial value 48 and 72 h after the leaf strips culture (Fig. 4B). The cell cycle gene histone H4 mRNAs, however, accumulated to the highest level 24–48 h after the culture (Fig. 4B), which parallels DNA synthesis detected by [³H]dTTP incorporation (data not shown). Thus **RHA2b** behaved distinctly from the histone H4 gene in exhibiting a transient down-regulation upon re-enter the cell cycle.

To evaluate **RHA2b** expression in response to proteolysis inhibition, we performed Northern analysis on **Arabidopsis** suspension cultured cells that had been treated with MG132, an efficient and highly specific proteasome inhibitor (Genschik et al., 1998). An increased accumulation of **RHA2b** transcript was observed 1 h after MG132 treatment (Fig. 4C). This effect seems transient since **RHA2b** transcript level returned to the initial value 3 h after the treatment. Polyubiquitin transcripts also accumulated, but with a different pattern that increased with prolonged MG132 treatment. In contrast, the level of the heat shock **HSP70** transcript remained unchanged upon MG132 treatment.

### 3.5. **Arabidopsis** genome contains a large number of genes encoding small RING-H2 finger proteins

We searched in the database for sequence similar to the RING-H2 domain of **RHA2b**. At least 101 **Arabidopsis** proteins show significant matches with the RING-H2 domain. Among them 40 proteins are with a size of no longer than 200 amino acids, a characteristic previously used to define RHA group (Jensen et al., 1998). Four

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**Fig. 3.** Histochemical localization of GUS activity in the gene trap line SGT6304. (A) One-week-old seedling. (B) Shoot apical meristem region. (C) Hypocotyl-root transition zone. (D) Primary root tip. (E) Lateral root initiation site (indicated by arrow). (F) Lateral root. (G) Inflorescences and early developing siliques. (H) Longitudinal section of a GUS-stained upper pistil. (I) Close-up image of the upper transmitting tract of the pistil. (J) Close-up image on the carpel wall of a young silique. (K) Embryo sac. (L) Mature embryo. (M) Close-up image on the tip of embryonic root. The same pattern was observed in more than ten independent individuals.
more Arabidopsis proteins were also identified to exhibit significant sequence similarities with human and yeast RBX1/APC11 proteins. Alignment of the entire sequence of these small RING-H2 proteins revealed that some of them form distinct phylogenetic groups, but a large number of them are only distantly related (Fig. 5A). This sequence diversity suggests that these small RING-H2 proteins display a large spectrum of function in Arabidopsis.

The RING-H2 domain of RHA2b showed highest homologies with the RING-H2 domains of RHA2a and the mammalian proteins Arkadia and RLIM (Fig. 5B). This group of RING-H2 domains differs from that of RBX1 and to a less extend of APC11 by a remarkable shortened distance between Cys2 and Cys3, which forms the loop between the two zinc binding sites. They resemble closer to the canonical RING fingers of several structure-determined proteins (Brzovic et al., 2001, and references therein), indicating that they may associate with E2 enzyme(s) through a similar structure interaction.

In the region outside of the RING-H2 domain, RHA2a was the only protein from the current databases that shows significant homologies with RHA2b (Fig. 5C). Although RBX1 and APC11 proteins also do not contain recognizable protein–protein interaction domain(s) beside the RING-H2 finger, their interaction with Cullin/CDC53 proteins had been demonstrated in the yeast two-hybrid system. To test potential interaction of RHA2b with Cullin, we performed the yeast two-hybrid assays. No significant interaction between RHA2b and AtCUL1 could be detected (data not shown). It is very unlikely that RHA2b functions in SCF E3 ligases.

4. Discussion

In this study we report the characterization of a small RING-H2 finger gene from Arabidopsis. By sequence comparison, the RING-H2 finger of RHA2b shows good conservation to the canonical RING fingers, suggesting that RHA2b may associate with an E2 enzyme through a similar structure interaction.

Despite the fact that RING finger proteins play diverse cellular functions, including gene expression, signal transduction, peroxisome biogenesis, DNA repair and recombination, and membrane vesicle sorting (Freemont, 2000; Joazeiro and Weissman, 2000), elimination of RHA2b function does not result in an obvious mutant phenotype in Arabidopsis plants. In gene trap line SGT6304 the Ds insertion in the N-terminal region of the RHA2b coding sequence, and RHA2b transcript could not be detected in the homozygous SGT6304 plants, suggesting that the Ds insertion has disrupted transcription of the RHA2b gene. Homozygous SGT6304 plants, however, do not exhibit any growth or development defects. This failure to observe a mutant phenotype is likely due to the redundancy of RHA2b with other RING finger genes. From a large number of RING-H2 finger genes identified in Arabidopsis, the gene most similar to RHA2b is RHA2a. Using RT–PCR amplification, Jensen et al. (1998) had shown that RHA2a transcript is present in seedlings, stems, flowers, leaves, roots and siliques. However, it is presently unknown if RHA2a expression overlaps RHA2b expression in a tissue- or cell-type specific fashion.

A high level of RHA2b expression was found in vascular
Fig. 5. Sequence analysis of Arabidopsis small RING-H2 finger proteins. (A) A phylogenetic tree of Arabidopsis proteins together with RBX1 and APC11 of Saccharomyces cerevisiae, and Homo sapiens. (B) Sequence alignment of the RING-H2 fingers. (C) Sequence comparison between RHA2b and RHA2a. The Cys/His residues forming the two zinc-binding sites are indicated by bold letters. Consensus symbols on top of the alignments indicate: ‘*’ for the identical or conserved residues in all aligned sequences, ‘.’ and ‘:’ for the conserved and semi-conserved substitutions, respectively. Accession numbers in the database: At4g11370 for RHA1a, At4g11360 for RHA1b, At1g15100 for RHA2a, AF078823 for RHA2b, NP_010276 for ScAPC11, NP_014508 for ScRBX1, NP_057560 for HsAPC11, NP_055063 for HsRBX1, AAD34209 for MmRLIM, NP_291082 for MmArkadia.
tissue of various organs, especially of developing or young organs including root tips and hypocotyls. The E2 enzyme AtUBC1-3 gene as well as some Ub genes have been previously reported to be highly expressed in vascular tissue (Plesse et al., 2001 and references therein). However, RHA2b is the first potential E3 enzyme showing specific expression in vascular tissue. Immunohistochemical localization studies reveal that Ub/Ub-conjugates and the proteasome are present at high levels in vascular tissue (Ingvarsdotn et al., 2001). Together these observations imply an important role of Ub-dependent pathway in vascular bundle formation. In supporting this assumption, overexpression of a Ub variant, with Lys48 replaced by Arg, had been reported to induce marked abnormalities in vascular tissue (Bachmair et al., 1990). Moreover, Woffenden et al. (1998) had shown that tracheary element differentiation from Zinnia mesophyll cell cultures requires proteasome activity.

It has been observed that pericycle cells of the vascular cylinder divide and lateral root primordia form without intervening mitotic quiescence (Dubrovsky et al., 2000). Consistently, a subset of cell cycle genes, such as A-type CDKs, A- and D-type cyclins, have been described to highly express in vascular tissue (Bursens et al., 2000; Swaminathan et al., 2000 and references therein). It is generally viewed that cyclin proteins are degraded through Ub-dependent proteolysis pathway, which represents a key point of control of cell division (Koepf et al., 1999). Plant cyclins appear also submitted to similar type of control (Genschik et al., 1998; Criqui et al., 2000). The findings of components of proteasome pathway in the same type of tissue thus fulfill the requirement of cell division control. In addition to developmental control, lateral root formation can be initiated from pericycle cells by environmental cues. Auxin is essential for lateral root primordium initiation (Celenza et al., 1995). It is well documented that the SCF E3 pathway is involved in auxin signaling (del Pozo and Estelle, 1999). Although strong GUS staining was found in root tips and the region of lateral root initiation, RHA2b expression was not induced by auxin. It is likely that RHA2b plays rather a role in later steps, such as vasculature initiation from meristematic cells.

RHA2b expressed very actively in upper pistils, which localized to the lower stigmatic zone and the upper transmitting tract. This observation suggests that RHA2b may play a role in fertilization. Function of Ub-dependent proteolysis in fertilization is poorly documented. Nevertheless, it has been reported that Ub-dependent mechanisms are involved in the recognition and elimination of defective spermatocytes in mammals (Sutovsky et al., 2001). In plants, inhibition of proteasome activity has been shown to inhibit pollen growth in vitro (Speranza et al., 2001). Moreover, the D-type cyclin CYCD3;2, representing potential substrate of Ub-dependent proteolysis, has also been found to express, similar to RHA2b, in the carpel walls, in the stigma and in the transmitting tract of the pistils (Swaminathan et al., 2000). Recently, we demonstrated that loss-of-function of the cullin AtCUL1 gene reduced inheritance in both male and female gametophytes (W.-H. Shen et al., submitted). Interestingly, both male and female gametogenesis of the mutant are morphologically normal, suggesting that AtCUL1-based E3 ligases may involved in fertilization process.

Our two-hybrid data revealed that RHA2b did not interact with AtCUL1, suggesting that RHA2b functions independently from the SCF E3 ligases. Consistently, the RING-H2 domains were only distantly related between RHA2b and RBX1 proteins. Besides the RING finger motif, no other conserved protein–protein interaction domain(s) could be identified from the sequences of RHA2b. Partners of RHA2b, therefore, remain to be identified.

It has been reported in mammalian as well as in yeast cells that proteasome inhibition leads to induction of heat shock proteins through likely stabilization of transcriptional regulators (Zhou et al., 1996). In contrast, such induction was not observed for the Arabidopsis HSP70 gene. However, in agreement with our previous observation in BY2 cells (Genschik et al., 1998), MG132 treatment induced Ub transcript accumulation in Arabidopsis suspension cultured cells. RHA2b responded to MG132 treatment by also an increased mRNA accumulation, but with a transient pattern that distinct from the continuous accumulation of Ub mRNA. Although functional significance of this induction as well as mechanism by which this induction occurred is unknown, RHA2b provides, in addition to Ub, an other gene of the Ub-dependent proteolysis pathway that respond to proteasome inhibition.

Acknowledgements

We are grateful to V. Sundaresan for providing the SGT6304 line. We thank S. Grava for yeast two-hybrid experiments, A. Capron and M.-C. Criqui for help in Northern analysis, P. Hammann for DNA sequencing, and M. Bergdoll for help in sequence similarity analysis. E.L. is supported by the Association Franco-Israélienne pour la Recherche Scientifique et Technologique (AFIRST).

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