Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 301 (2003) 225–230
BBRC
www.elsevier.com/locate/ybbrc

Conservation between animals and plants of the *cis*-acting element involved in the unfolded protein response☆

Dong-Ha Oh,[a] Chang-Seob Kwon,[a] Hiroshi Sano,[b] Won-Il Chung,[a,1] and Nozomu Koizumi[b,*]

[a] *Korea Advanced Institute of Science and Technology, Taejon 305-701, Republic of Korea*
[b] *Nara Institute of Science and Technology, Ikoma 630-0192, Japan*

Received 13 December 2002

Abstract

Using *Arabidopsis thaliana*, we identified the *cis*-element involved in the plant unfolded protein response (UPR). In transgenic plants, tunicamycin stimulated expression of a reporter gene under the control of the BiP promoter and promoter analysis identified a 24 bp sequence crucial to this induction. When fused with a minimal promoter, a hexamer of this sequence was sufficient for induction of a reporter gene in protoplasts treated with tunicamycin or dithiothreitol. Induction rate equivalent to original promoter was observed when the assay was conducted in transgenic plants. This 24 bp sequence contained two elements also responsible for the UPR in animals. Either of these elements was sufficient for the plant UPR, indicating conservation between animals and plants of *cis*-elements involved in the UPR.

Keywords: *Arabidopsis thaliana*; BiP; Tunicamycin; UPR; *cis*-Element; Promoter

Proteins synthesized in the endoplasmic reticulum (ER) are translocated to their proper destination by vesicle transport. Before exit from the ER, proteins need to be in correct conformation. If correct folding or assembly is prevented, a set of genes encoding molecular chaperones such as BiP are induced to maintain homeostasis of protein structures. This phenomenon is termed the unfolded protein response (UPR) [1] and is invoked following ER stress. The UPR also results in attenuation of protein synthesis and degradation of proteins [2,3]. Although the UPR is often experimentally induced by chemicals such as tunicamycin (an inhibitor of N-linked glycosylation) and dithiothreitol (DTT, a reducing agent which inhibits the disulfide bond formation) [4], it appears to be involved in nutrient sensing and differentiation [5–8].

The UPR signal pathway has been extensively characterized in yeast and recently in animals [for reviews, see 8–10]. In yeast, a 22 bp sequence in the *BiP* promoter is sufficient for UPR induction and is designated the UPRE (UPR element) [11]. Subsequent studies showed that a partial palindromic sequence with a one-nucleotide spacer (CAGCGTG) was essential for UPRE function [12]. The UPRE is also found in other ER chaperone genes. The bZIP-type transcription factor Hac1p binds UPRE and activates transcription of coding genes [12,13]. Accumulation of Hac1p is regulated by specific splicing of its mRNA catalyzed by the ribonuclease domain of Ire1p, a transmembrane protein that consists of a sensor domain in the N-terminus and kinase/ribonuclease domain in the C-terminus [13–15].

In animals, the consensus sequence (CCAAT-N9-CCACG) responsible for induction of human BiP (GRP78) and the GRP94 gene is designated ERSE (ER stress element) [16]. There are at least two bZIP transcription factors, ATF6 and XBP1, that bind ERSE [16].

☆ *Abbreviations:* ER, endoplasmic reticulum; GUS, β-glucuronidase; UPR, unfolded protein response; CaMV, cauliflower mosaic virus; DTT, dithiothreitol.

* Corresponding author. Fax: +81-743-72-5659.
E-mail addresses: wichung@mail.kaist.ac.kr (W.-I. Chung), nkoizumi@gtc.aist-nara.ac.jp (N. Koizumi).
[1] Also corresponding author. Fax: +82-42-869-5624.

0006-291X/02/$ - see front matter © 2002 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0006-291X(02)03019-X

ATF6 is a type II membrane protein that is converted by proteolysis to a soluble transcription factor upon ER stress [16,17]. XBP1 levels are regulated by splicing of its mRNA catalyzed by the product of IRE1, similar to yeast *HAC1* [5,6,18]. Since *XBP1* has an ERSE in its promoter, ATF6 and XBP1 itself regulate transcription of *XBP1*. In addition to ERSE, a sequence in the promoter of the human Herp gene (ATTGG-N-CCACG) was found to be responsible for the UPR and was designated ERSE II [19]. Another sequence, GA-TGAC GT-G(T/G), referred to as the XBP1 binding site, was also sufficient for induction of a reporter gene by tunicamycin [5,18,20]. These observations indicate that the mechanism regulating the UPR is conserved, in part, from yeast to animals, although the UPR of animals is more complex.

We have previously described the tunicamycin-stimulated co-operative induction of five genes encoding ER-resident chaperones in the leaves of the model plant *A. thaliana* [21]. This observation suggests that *A. thaliana* has a UPR pathway similar to other eukaryotes. In the present study we investigated the UPR mechanism in plants, and in doing so, identified *cis*-acting elements in the *BiP* promoter of *A. thaliana* sufficient for initiating transcription upon triggering of the UPR.

Materials and methods

Plasmid construction. For stable transformation, promoter regions of *Atbip1* and *Atbip2* were amplified by polymerase chain reaction (PCR) with primers (CCCGTCGACAAATGAGTGGTGTAATGA ACG and CCACCCGGGGAGCGAGCCTATACGGAAAC for *Atbip-1*), and (CCCGTCGACTCTTTTGCACCTATCGTACAC and CCACCCGGGGAGCGAGCCTATACGGAAAC for *Atbip-2*). Amplified fragments digested with *Sal*I and *Sma*I were inserted into the pBI101 vector (Clontech). For the transient luciferase assay, the β-glucuronidase (GUS) gene in pBI121 (Clontech) was replaced with the firefly luciferase gene derived from pGL3-Basic (Promega) using *Bam*HI and *Sac*I, and the fragment was cut by *Eco*RI and *Hind*III containing the cauliflower mosaic virus (CaMV) 35S promoter and the firefly luciferase gene and the nopaline synthase terminator was transferred to pUC18, to create the plasmid p35Lt. The appropriate region of the *Atbip2* promoter amplified by PCR was replaced with the CaMV 35S promoter of p35Lt using *Pst*I and *Bam*HI. The firefly luciferase gene of p35Lt was replaced with the *Renilla* luciferase gene derived from pRL-TK (Promega) and the resultant construct was designated p35RLt. For the linker-scanning mutant, two back-to-back primers containing five nucleotides of substitution in their 5′ ends were designed. By inverse PCR using ten sets of these primer pairs and subsequent self-ligation, a series of plasmids containing serial 10 nucleotide-substitution mutations were constructed. For gain of function analysis of the *cis*-element, the CaMV 35S promoter in p35Lt was replaced with the CaMV 35S -46 minimal promoter. A *Xho*I site was also inserted upstream of the minimal promoter and this plasmid was termed pM35Lt. Monomers, trimers, and hexamers of the 24 bp sequence containing incomplete *Xho*I and *Sal*I sites at their 5′ ends were inserted into the *Xho*I site of pM35Lt. For transgenic plants, the region containing the hexamer of the 24 bp sequence and the 35S minimal promoter was excised using *Hin*dIII and *Xho*I and inserted in pBI101 with *Hin*dIII and *Sal*I. For analysis of P-UPRE with mutations, trimers of P-UPRE and mutated P-UPREs were synthesized with *Hin*dIII and *Xho*I sites. The oligomers were annealed and inserted into the *Hin*dIII and *Xho*I sites of pM35Lt.

Dual luciferase assay. Protoplasts were prepared from three week old *A. thaliana* (ecotype Columbia) and transfected with constructs as per Shillito and Saul [22]. Protoplasts were treated with tunicamycin (10 μg/ml) or DTT (0.5 mM) and incubated at 23 °C for 18 h. Luciferase activity was measured using the Dual Luciferase assay system (Promega), according to the manufacturer's instructions.

Stable transformation and GUS enzyme assay. *Arabidopsis thaliana* (ecotype Columbia) were infected with *Agrobacterium tumefaciens* harboring chimeric constructs, as previously described [23]. Seeds were collected and selected on plates containing kanamycin (25 μg/ml). Progeny of transgenic plants were subjected to GUS analysis. Quantitative analysis of GUS activity was performed as previously described [24].

Results

Two closely related BiP promoters respond to tunicamycin

Previously we isolated two BiP genes from *A. thaliana* that were highly similar, even with regard to introns and the 5′ upstream region [25]. In order to clarify whether both genes respond to the UPR, approximately 1.5 kb of each promoter was fused with the GUS reporter gene and introduced into *A. thaliana*. When seedlings of transgenic plants were treated with tunicamycin, GUS activity was induced more than 10-fold in both cases (Fig. 1A). This result suggested that both BiP genes were responsible for the UPR.

Mutation analyses of the Atbip-2 promoter

In order to identify *cis*-elements responsible for the UPR, the *Atbip2* promoter was further analyzed by transient dual luciferase assay using protoplasts. Five deletion clones were constructed starting from −652 (Fig. 1B). Although 4- to 6-fold-induction was observed in −652, −477, −267, and −209 constructs upon tunicamycin treatment, −166 did not show detectable induction. These data indicated that the *cis*-element regulating the UPR is located between −209 and −166.

In order to more finely map this *cis*-element, mutations were introduced between −264 and −164 with 10 bp linkers. We found that although most mutated promoters responded to tunicamycin in 4- to 6-fold-induction, basal luciferase activity was barely detectable in LS8 (−191 to −182) (Fig. 1C). Hence, we considered that a *cis*-element responsible for the UPR was located around the −191 to −182 region.

Identification of a 24 bp sequence responsible for the UPR

Since the sequence around −191 to −182 was considered critical for induction, a 24 bp sequence from −197 to −174 was fused with the CaMV 35S -46 minimal promoter and used in a transient transfection reporter

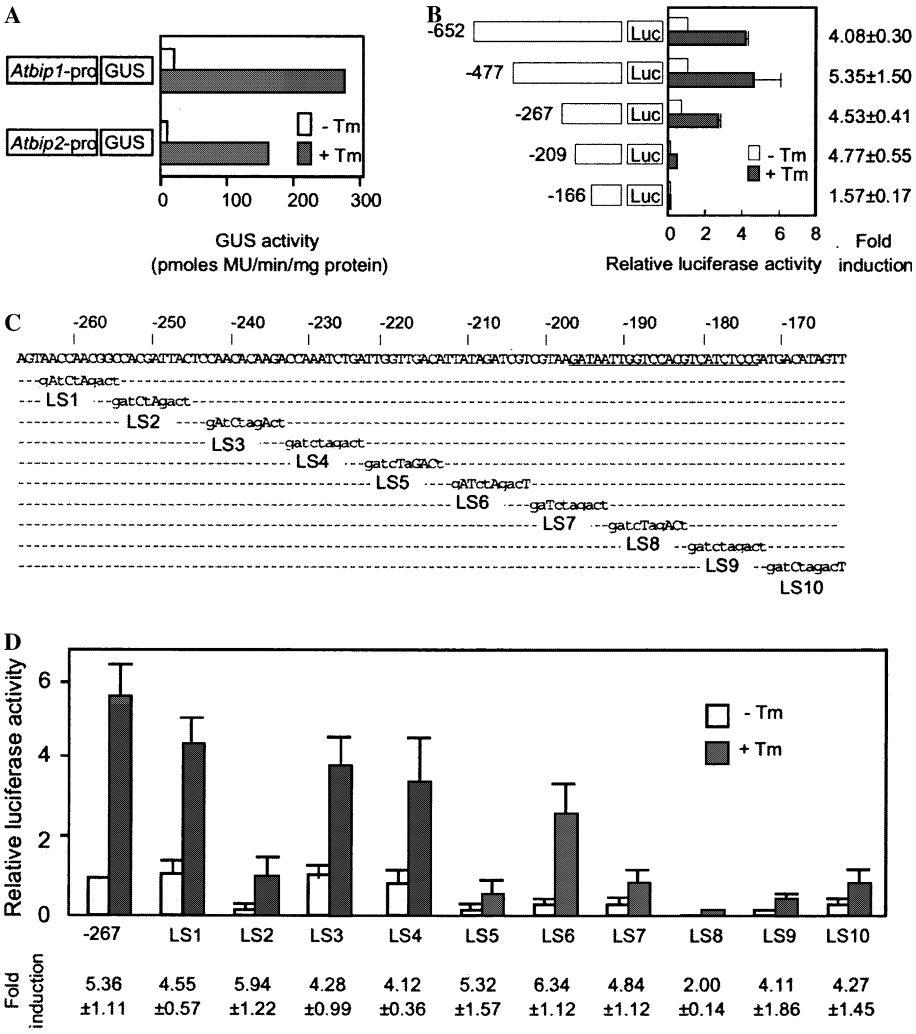

Fig. 1. (A) Analysis of transgenic plants containing a construct comprising either the *Atbip1* or *Atbip2* promoter upstream of the GUS reporter gene. Seedlings were treated with (closed bars) or without (open bar) tunicamycin (5 μg/ml) for 16 h before GUS assay. (B) Deletion analysis of the *Atbip2* promoter. Protoplasts were transfected with constructs consisting of the *Atbip2* promoter and the firefly luciferase gene. Tunicamycin (10 μg/ml) treatment was conducted for 16 h. Results were normalized for efficiency of transfection following determination of *Renilla* luciferase activity driven by the CaMV 35S promoter. 'Activity' represents activity relative to basal activity of constructs with the −652 promoter. Means and standard deviations from three independent experiments are shown. (C) Linker-scanning mutation of the *Atbip2* promoter. Ten-base mutations were introduced between −265 and −162 bp of the *Atbip2* promoter. Luciferase assays were conducted as described in (B). 'Activity' represents activity relative to the basal activity of constructs with the wild-type −267 promoter. The 24 bp sequence is underlined.

assay. We found that chimeric constructs containing trimers and hexamers responded to tunicamycin and DTT, indicating that this sequence was sufficient for the UPR (Fig. 2A). In order to determine whether this 24 bp region was functional in intact plants, hexamers with minimal promoters were fused to the GUS gene and this construct was used to generate transgenic plants. Six independent lines with either this construct or the original full-length *Atbip2* promoter (same as Fig. 1A) were subjected to quantitative GUS assay. Upon tunicamycin treatment, induction was observed in transgenic plants with either construct (Fig. 2B). Although induction rates were similar, GUS activity was more than 5-fold higher with the full-length promoter compared to the minimal chimeric promoter, suggesting the presence of additional enhancer-like sequences in the full-length promoter. It should be noted that induction rate in transgenic plants (~16-fold) was much higher than that in protoplasts (~3-fold), indicating that the induction rates obtained from transient assay were underestimated.

Cis element of the plant UPR composed of cis elements of the animal UPR

Examination of the 24 bp plant sequence GATAATTGGTCCACGTCATCTCCG revealed that it contains two sequences responsible for the UPR in animals. ATTGGTCCACG perfectly matches the

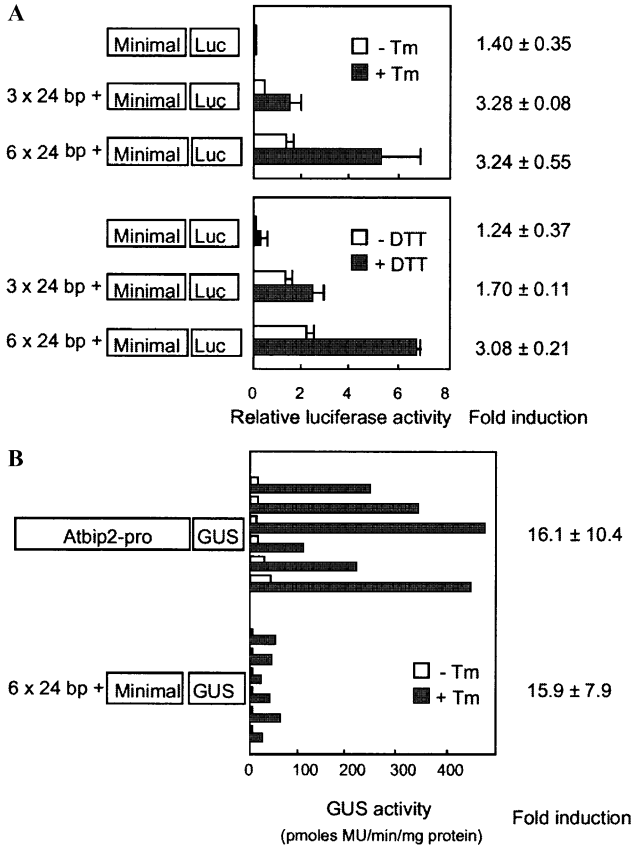

Fig. 2. Effect of the 24 bp sequence on induction of reporter genes. (A) Trimer and hexamer of the 24 bp sequence were fused with the CaMV 35S −46 minimal promoter (−46) and firefly luciferase (Luc). Protoplasts were treated with tunicamycin (10 μg/ml) or DTT (0.5 mM) for 16 h. Luciferase assays were conducted as described in Fig. 1B. (B) GUS assays were conducted as described in Fig. 1A. Data are results from six independent transformants for each construct.

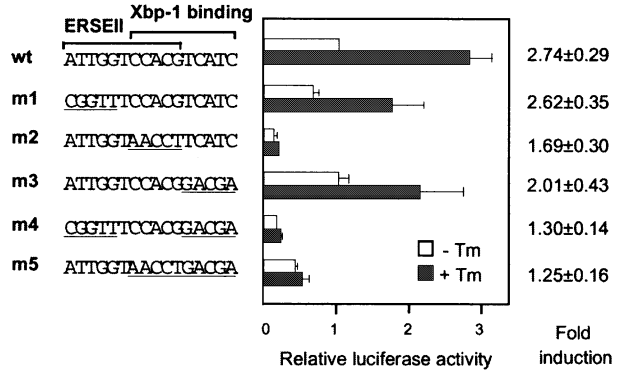

Fig. 3. Characterization of the P-UPRE using mutations. Trimers of original P-UPRE (wt) and those with mutations were fused with the CaMV 35S −46 minimal promoter (−46) and firefly luciferase (Luc). Luciferase assays were conducted as described in Fig. 1B. 'Activity' represents activity relative to basal activity of the wt trimer.

P-UPRE like

Atbip-2	-194	ATTGGT CCACGTCAT	-169
Atbip-1	-169	ATTGGT CCACGTCAT	-154
Spinach BiP	-373	ATTGGT CCACGTCAT	-358
	-212	ATTGGTTCCACGTCAT	-196
Soybean BiP	-185	ATTGGT CCACGTCAT	-170
AtCNX2	-290	ATTGGGCCCAGGTCAG	-274

ERSE like

AtPDI	-326	CCAAT-gtacaagcg-ACACG	-344
	-146	CCAAT-tagagatag-CCACG	-128
AtCNX1	-219	CCAAT-cataacatt-CCACG	-237
AtCRT1	-129	CCAAT-tagttaaat-ACACG	-147
AtCRT2	-160	CCAAT-aggtaacog-ACACG	-181
AtGRP94	-166	CCAAT-acaaaacta-CCACG	-148

Xbp-1 binding site like

AtPDI	-353	AA-TGACGT-GG	-362
AtCNX2	-147	AA-TGACGT-TC	-138
AtGRP94	-244	CT-TGACGT-GG	-253

Fig. 4. Putative *cis*-elements for the UPR found in the promoter regions of plant genes. The GenBank Accession Nos. for each gene are Atbip1: D89342, Atbip2: D89341, Spinach BiP: L23551, Soybean BiP: AF335282, AtCNX2: U08315, AtPDI: NM_102024, AtCNX1: Z18242, AtCRT1: U66343, AtCRT: NM_100791, and AtGRP94: NM_118549.

ERSEII ATTGG-N-CCACG, while CCACGTCATC is complementary to the XBP1 binding site GA-TGA CGT-G(T/G) [5,18,19]. Considering this fact, the 24 bp sequence was further analyzed using nucleotide substitutions. We found that a sequence comprising just two mammalian sequences, ATTGGTCCACGTCATC, was sufficient for induction (Fig. 3, wt) and it was designated P-UPRE (plant UPR element). If either the ERSEII or XBP1 binding site was intact, distinct induction was observed (m1 and m3 in Fig. 3). In contrast, mutation that disrupted both elements abolished induction (m2, m4, and m5 in Fig. 3). These data indicate that either of the ERSEII or XBP1 binding sites is essential and sufficient for the UPR.

Sequences of other genes induced upon the UPR

The P-UPRE identified in *Atbip2* was completely conserved in *Atbip1*. P-UPRE analogous sequences were found in the BiP promoter of spinach and soybean (Fig. 4). A similar sequence was also found in the promoter of the calnexin2 gene of *A. thaliana* (Fig. 4). The promoters of other ER-resident chaperone genes, such as calreticulin and GRP94, and the gene for protein disulfide isomerase in *A. thaliana*, also contain sequences homologous to the ERSE and XBP-1 binding sites (Fig. 4).

Discussion

Although there have been reports describing the UPR in plants [26–29], the molecular mechanism has yet to be determined. It was possible that the plant mechanism may be similar to that in yeast and animals. In fact, Ire1 homologs have been isolated from *A. thaliana* and rice [21,30,31], although their involvement in the UPR has not been demonstrated. Since the UPR is more complicated in animals than in yeast, plants may also have distinct machinery.

The present study sought to identify the *cis*-element required for the plant UPR. Although we did not postulate that plants used the same *cis*-elements as animals, given *cis*-elements are not conserved between yeast and animals, we found that the P-UPRE ATTGGTCCACG TCATC contained two animal UPR *cis*-elements, the ERSE II and XBP1 binding sites. Since either element was sufficient for the UPR, we concluded that P-UPRE consists of two overlapping functional elements identical to *cis*-elements involved in the animal UPR. Consistent with this conclusion, analogous sequences were found in genes induced upon the UPR. Since the functions of ERSE and ERSE II are considered to be analogous in animals [19], it is possible that ERSE is also functional in plants. In support of this hypothesis, ERSE-like sequences were found in promoter regions of other chaperone genes induced upon the UPR (Fig. 4). Thus, although we have not proven that all of these sequences are functional, we suggest that it is likely they are involved in the plant UPR.

At this time there is no information about transcription factor binding to the P-UPRE. Since the *cis*-element is conserved between animals and plants, binding proteins may also be analogous. Both XBP1 and ATF6 are classified as bZIP-type transcription factors, even though their structures are barely similar. In addition, Hac1p in yeast is also a bZIP protein. Thus, it could be assumed that a bZIP transcription factor binds to the P-UPRE involved in the plant UPR. However, a simple database search did not identify genes similar to XBP1 or ATF6 among the 75 genes encoding putative bZIP proteins in *A. thaliana* [32]. Identification of a functional XBP1 or ATF6 homolog is the next challenge.

Acknowledgments

This work was supported in part by a grant (KRF-015-DP0541) from the Korea Research Foundation to W-I Chung and a grant from the Research for the Future Program (JSPS-RFTF00L01604) of the Japan Society for the Promotion of Science.

References

[1] Y. Kozutsumi, M. Segal, K. Normington, M.J. Gething, J. Sambrook, The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins, Nature 332 (1988) 462–464.

[2] H.P. Harding, Y. Zhang, D. Ron, Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase, Nature 397 (1999) 271–274.

[3] R. Friedlander, E. Jarosch, J. Urban, C. Volkwein, T.A. Sommer, A regulatory link between ER-associated protein degradation and the unfolded-protein response, Nat. Cell Biol. 2 (2000) 379–384.

[4] A.S. Lee, Mammalian stress response: induction of the glucose-regulated protein family, Curr. Opin. Cell Biol. 4 (1992) 267–273.

[5] X. Shen, R.E. Ellis, K. Lee, C.Y. Liu, K. Yang, A. Solomon, H. Yoshida, R. Morimoto, D.M. Kurnit, K. Mori, R.J. Kaufman, Complementary signaling pathways regulate the unfolded protein response and are required for *C. elegans* development, Cell 107 (2001) 893–903.

[6] M. Calfon, H. Zeng, F. Urano, J.H. Till, S.R. Hubbard, H.P. Harding, S.G. Clark, D. Ron, IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA, Nature 415 (2002) 92–96.

[7] M. Schroder, J.S. Chang, R.J. Kaufman, The unfolded protein response represses nitrogen-starvation induced developmental differentiation in yeast, Genes Dev. 14 (2000) 2962–2975.

[8] R.J. Kaufman, D. Scheuner, M. Schroder, X. Shen, K. Lee, C.Y. Liu, S.M. Arnold, The unfolded protein response in nutrient sensing and differentiation, Nat. Rev. Mol. Cell Biol. 3 (2002) 411–421.

[9] K. Mori, Tripartite management of unfolded proteins in the endoplasmic reticulum, Cell 101 (2000) 451–454.

[10] R.J. Kaufman, Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls, Genes Dev. 13 (1999) 1211–1233.

[11] K. Mori, A. Sant, K. Kohno, K. Norminton, M.J. Gething, J.F. Sambrook, A 22 bp cis-acting element is necessary and sufficient for the induction of the yeast KAR2 (BiP) gene by unfolded proteins, EMBO J. 11 (1992) 2583–2593.

[12] K. Mori, T. Kawahara, H. Yoshida, H. Yanagi, T. Yura, Signalling from endoplasmic reticulum to nucleus: transcription factor with a basic-leucine zipper motif is required for the unfolded protein-response pathway, Genes Cells 1 (1996) 803–817.

[13] J.S. Cox, P. Walter, A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response, Cell 87 (1996) 391–404.

[14] T. Kawahara, H. Yanagi, T. Yura, K. Mori, Endoplasmic reticulum stress-induced mRNA splicing permits synthesis of transcription factor Hac1p/Ern4p that activates the unfolded protein response, Mol. Biol. Cell 8 (1997) 1845–1862.

[15] C. Sidrauski, J.S. Cox, P. Walter, tRNA ligase is required for regulated mRNA splicing in the unfolded protein response, Cell 87 (1996) 405–413.

[16] H. Yoshida, K. Haze, H. Yanagi, T. Yura, K. Mori, Identification of the *cis*-acting endoplasmic reticulum stress response element responsible for transcriptional induction of mammalian glucose-regulated proteins. Involvement of basic leucine zipper transcription factors, J. Biol. Chem. 273 (1998) 33741–33749.

[17] K. Haze, H. Yoshida, H. Yanagi, T. Yura, K. Mori, Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress, Mol. Biol. Cell 10 (1999) 3787–3799.

[18] H. Yoshida, T. Matsui, A. Yamamoto, T. Okada, K. Mori, XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor, Cell 107 (2001) 881–891.

[19] K. Kokame, H. Kato, T. Miyata, Identification of ERSE-II, a new *cis*-acting element responsible for the ATF6-dependent mammalian unfolded protein response, J. Biol. Chem. 276 (2000) 9199–9205.

[20] Y. Wang, J. Shen, N. Arenzana, W. Tirasophon, R.J. Kaufman, R. Prywes, Activation of ATF6 and an ATF6 DNA binding site by the endoplasmic reticulum stress response, J. Biol. Chem. 275 (2000) 27013–27020.
[21] N. Koizumi, I.M. Martinez, Y. Kimata, K. Kohno, H. Sano, M.J. Chrispeels, Molecular characterization of two *Arabidopsis* Ire1 homologs, endoplasmic reticulum-located transmembrane protein kinases, Plant Physiol. 127 (2001) 949–962.
[22] R.D. Shillito, M.W. Saul, Protoplast isolation and transformation, in: C.H. Shaw (Ed.), Plant Molecular Biology: A Practical Approach, IRL Press, Oxford, UK, 1988, pp. 161–186.
[23] S.J. Clough, A.F. Bent, Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*, Plant J. 16 (1998) 735–743.
[24] S.R. Gallagher, Quantitation of GUS activity by fluorometry, in: S.R. Gallagher (Ed.), GUS Protocols, Academic Press, San Diego, CA, 1992, pp. 47–59.
[25] N. Koizumi, H. Sano, Isolation of two genes (Accession Nos. D89341 and D89342) encoding luminal binding proteins from *Arabidopis thaliana*, Plant Physiol. 113 (1997) 664–665.
[26] R.S. Boston, E.B. Fontes, B.B. Shank, R.L. Wrobel, Increased expression of the maize immunoglobulin binding protein homolog b-70 in three zein regulatory mutants, Plant Cell 3 (1991) 497–505.
[27] J. Denecke, M.H.S. Goldman, J. Demolder, J. Seurinck, J. Botterman, The tobacco luminal binding protein is encoded by a multigene family, Plant Cell 3 (1991) 1025–1035.
[28] N. Koizumi, Isolation and responses to stress of a gene that encodes a luminal binding protein in *Arabidopsis thaliana*, Plant Cell Physiol. 37 (1996) 862–865.
[29] L. D'Amico, B. Valsasina, M.G. Daminati, M.S. Fabbrini, G. Nitti, R. Bollini, A. Ceriotti, A. Vitale, Bean homologs of the mammalian glucose-regulated proteins: induction by tunicamycin and interaction with newly synthesized seed storage proteins in the endoplasmic reticulum, Plant J. 2 (1992) 443–455.
[30] Y. Okushima, N. Koizumi, Y. Yamaguchi, Y. Kimata, K. Kohno, H. Sano, Isolation and characterization of a putative transducer of endoplasmic reticulum stress in *Oryza sativa*, Plant Cell Physiol. 43 (2002) 532–539.
[31] S.J. Noh, C.S. Kwon, W.I. Chung, Characterization of two homologs of Ire1p, a kinase/endoribonuclease in yeast, in *Arabidopsis thaliana*, Biochim. Biophys. Acta 1575 (2002) 130–134.
[32] M. Jakoby, B. Weisshaar, W. Droge-Laser, J. Vicente-Carbajosa, J. Tiedemann, T. Kroj, F. Parcy, bZIP transcription factors in *Arabidopsis*, Trends Plant Sci. 7 (2002) 106–111.