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Article Addendum

The Arabidopsis membrane-bound transcription factor AtbZIP60 is a novel plant-specific endoplasmic reticulum stress transducer

Yuji Iwata,[1,*] Nina V. Fedoroff[1,2] and Nozomu Koizumi[3]

[1]Huck Institutes of the Life Sciences; Pennsylvania State University; University Park, PA USA; [2]Department of State; Washington DC, USA; [3]Graduate School of Life and Environmental Sciences; Osaka Prefecture University; Nakaku; Sakai, Osaka Japan

Abbreviations: bZIP, basic leucine zipper; DTT, dithiothreitol; ER, endoplasmic reticulum; RIP, regulated intramembrane proteolysis; SREBP, sterol regulatory element-binding protein; UPR, unfolded protein response

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Accumulation of unfolded proteins in the endoplasmic reticulum (ER) of eukaryotic cells triggers a protective response, termed the ER stress response or the unfolded protein response, to maintain cellular homeostasis. Recently we characterized the Arabidopsis (*Arabidopsis thaliana*) membrane-bound basic leucine zipper (bZIP) transcription factor AtbZIP60 involved in the ER stress response. We reported that AtbZIP60 is activated by regulated intramembrane proteolysis (RIP), a mechanism by which a membrane-bound transcription factor is released by proteolytic cleavage. We presented evidence that the AtbZIP60 protein resides in the ER membrane under unstressed conditions and is activated by proteolytic cleavage in response to ER stress to translocate into the nucleus where it acts as a transcription factor. Further analysis, however, showed that this cleavage is independent of the function of Arabidopsis homologs of S1P and S2P proteases, which mediate the proteolytic cleavage of the mammalian transcription factor ATF6. Thus AtbZIP60 is an ER stress transducer activated by a novel RIP mechanism that may be unique to plants.

In eukaryotic cells, secretory and membrane proteins first enter the endoplasmic reticulum (ER) as unfolded polypeptide chains. Proper folding and maturation of these proteins in the ER are a prerequisite for transport to their final destinations. Disruption of this process causes accumulation of unfolded proteins in the ER, a condition called 'ER stress', and triggers a protective response termed the ER stress response or the unfolded protein response (UPR).[1] Extensive studies conducted in yeast and mammalian cells have shown that the ER stress response plays an important role not only in regulating the expression of numerous genes that function within the secretory pathway but also in a wide variety of cellular processes.[1]

In contrast to yeast and mammalian cells, there is relatively little information about the molecular mechanisms of the ER stress response in plants. We recently characterized the Arabidopsis (*Arabidopsis thaliana*) *AtbZIP60* gene, which is strongly induced in response to ER stress caused by either tunicamycin (an N-glycosylation inhibitor) or dithiothreitol (DTT; a reducing agent inhibiting disulfide bond formation).[2] We showed that overexpression of a cDNA encoding a truncated form of AtbZIP60 lacking the C-terminal trans-membrane enhanced expression UPR marker genes *BiP* and *calnexin*, whereas overexpression of a cDNA encoding the full-length AtbZIP60 did not, suggesting that proteolytic cleavage is necessary for its activation.[2] In subsequent studies, we further characterized AtbZIP60, presenting direct evidence for cleavage and nuclear localization.[3] We showed that AtbZIP60 resides in the ER membrane under unstressed conditions and that it is activated by proteolytic cleavage in response to ER stress. We detected the cleaved N-terminal fragment of AtbZIP60 in the nucleus of tunicamycin-treated cells. We also showed that a number of ER stress-responsive genes are much less strongly induced by ER stress in *atbzip60* mutant plants than in wild-type plants. We infer that the N-terminal fragment of AtbZIP60, which contains a basic leucine zipper (bZIP) domain, translocates to the nucleus where it acts as a transcription factor for a subset of ER stress-responsive genes.

Activation of membrane-bound transcription factors by proteolytic cleavage is termed regulated intramembrane proteolysis (RIP). There is growing recognition that RIP is involved in many cellular events in both bacterial and mammalian cells.[4,5] The mammalian ER stress transducer ATF6, a RIP-regulated transcription factor, is

*Correspondence to: Yuji Iwata; Huck Institutes of the Life Sciences; Pennsylvania State University; 211 Wartik Laboratory; University Park, PA 16802 USA; Tel.: +1.814.863.5720; Email: yuji.iwata01@gmail.com

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synthesized as a precursor form anchored in the ER membrane.[1] In response to ER stress, it translocates to the Golgi apparatus, where it is cleaved by S1P and S2P proteases.[6] The luminal domain of ATF6 contains a Golgi localization signal, which is masked by BiP binding, and dissociation of BiP in response to ER stress allows translocation of ATF6 to the Golgi.[7] S1P first cleaves the luminal part of ATF6, which makes the luminal portion of ATF6 shorter, allowing S2P access to the intramembrane region of ATF6 and triggering the second cleavage.[8] This two-step processing frees the cytosolic domain of ATF6, which contains a bZIP domain, from the membrane. Recent studies have identified OASIS and CREBH as additional trans-membrane bZIP transcription factors involved in the mammalian ER stress response that are cleaved by the S1P and S2P proteases.[9,10]

However, cleavage of AtbZIP60 was not affected by mutations in the genes coding for the Arabidopsis homologs of either the S1P or S2P proteases.[3] This is consistent with the fact that AtbZIP60 lacks a canonical S1P cleavage site. Furthermore, the luminal domain of AtbZIP60 is much shorter than those of ATF6 and the ER stress transducers IRE1 and PERK, which are thought to function as ER stress sensors.[1] This raises the possibility that AtbZIP60 itself does not function as an ER stress sensor, but that the sensor is an AtbZIP60-interacting protein or a protease that cleaves AtbZIP60. The mammalian sterol regulatory element-binding protein (SREBP), another RIP-regulated transcription factor, has accessory proteins designated SCAP and INSIG that act as sterol sensors and regulate transport of SREBP to the Golgi, where it is cleaved by S1P and S2P proteases in a similar manner to ATF6.[11-13] SCAP also functions as an escort protein for SREBP from the ER to the Golgi[11] in addition to its role as a sterol sensor. Such accessory proteins might function to regulate AtbZIP60 activation.

AtbZIP28 is another recently identified Arabidopsis RIP-regulated trans-membrane transcription factor.[14,15] Unlike *AtbZIP60* transcripts, which are strongly induced by ER stress, the *AtbZIP28* gene is only slightly activated by ER stress.[2,14] Because AtbZIP60 upregulates its own transcription, we have speculated that AtbZIP60 not only upregulates ER stress-responsive genes, but also amplifies the ER stress signaling.[2] Moreover, the AtbZIP28 and AtbZIP60 proteins show little homology, although both contain bZIP and trans-membrane domains. Indeed, AtbZIP28 shows some homology to ATF6 and the two proteins have a luminal domain of almost the same size containing a canonical S1P recognition site. These observations suggest that AtbZIP28 is regulated by a RIP mechanism similar to that of ATF6, namely the S1P/S2P system, although there is not direct evidence as yet. Thus, although both AtbZIP60 and AtbZIP28 are activated by proteolytic cleavage in response to ER stress, the underlying mechanisms are likely to be different. Taken together with the observation that AtbZIP60 homologs occur in several plant species, including rice (*Oryza sative*), soybean (*Glycine max*) and capsicum (*Capsicum annuum*), but not in yeast or mammals,[3] we conclude that AtbZIP60 is a plant-specific RIP-regulated bZIP transcription factor involved in the ER stress response. Further analysis will help us understand this plant-specific RIP mechanism.

bZIP transcription factors generally form dimers to recognize target DNA sequences.[16] The results of bioinformatic analyses indicated that AtbZIP60 and AtbZIP28 can form hetero-dimers, as well as homo-dimers.[17] Since both transcription factors activate ER-stress responsive genes, the homo- and hetero-dimers may activate different subsets of genes. Indeed, the prevailing view is that the ability of a bZIP domain to form hetero-dimers with the bZIP domains of other transcription factors serves to target them to a wide variety of genes. Hence it is a reasonable conjecture that the AtbZIP60 and AtbZIP28 homo- and hetero-dimers differ in their affinity to the cis-elements, such as the ERSE, ERSE-II and UPRE, that mediate the induction of ER stress-responsive genes.[18] Experimental analysis of the homo- and hetero-dimers formed by AtbZIP60 and AtbZIP28 during the Arabidopsis ER stress response awaits future study.

Another interesting finding is that the AtbZIP60 protein is activated in anthers and the *AtbZIP60* gene is highly expressed in pollen and tapetal cells in the absence of treatment with ER stress inducers such as tunicamycin and DTT.[3] It has been reported that pollen has high secretory capacity to support pollen tube growth after pollination.[19] Tapetal cells have also been reported to secrete large amounts of proteins and lipids to pollen surfaces.[20] Consistent with these secretory functions, such cells have an extensive ER.[21-25] Thus, it appears likely that aspects of the ER stress response are important in the development and function of such secretory cells. Furthermore, tapetal cells are known to undergo programmed cell death late in anther development, although the underlying mechanism remains to be elucidated.[21,26,27] Since ER stress-induced programmed cell death was recently described in several plant species,[28-30] it is tempting to speculate that programmed cell death of tapetal cells is mediated by ER stress. How the ER stress response regulated by AtbZIP60 (and possibly AtbZIP28) supports the development and function of such secretory cells is an interesting topic for future study.

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