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Review

The BAG proteins: a ubiquitous family of chaperone regulators

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Abstract. The BAG (Bcl-2 associated athanogene) family is a multifunctional group of proteins that perform diverse functions ranging from apoptosis to tumorigenesis. An evolutionarily conserved group, these proteins are distinguished by a common conserved region known as the BAG domain. BAG genes have been found in yeasts, plants, and animals, and are believed to function as adapter proteins forming complexes with signaling molecules and molecular chaperones. In humans, a role for BAG proteins has been suggested in carcinogenesis, HIV infection, and Parkinson's disease. These proteins are therefore potential therapeutic targets, and their expression in cells may serve as a predictive tool for such diseases. In plants, the *Arabidopsis thaliana* genome contains seven homologs of the BAG family, including four with domain organization similar to animal BAGs. Three members contain a calmodulin-binding domain possibly reflecting differences between plant and animal programmed cell death. This review summarizes current understanding of BAG proteins in both animals and plants.

Keywords. BAG protein, BAG domain, stress, programmed cell death, *Arabidopsis*.

Introduction

BAG genes are an evolutionarily conserved family with homologs found from yeast to animals, and including plants. The first BAG gene was discovered in a screen of a mouse embryo cDNA library using recombinant human Bcl-2 protein as bait to identify Bcl-2 interactors [1]. The gene identified in this screen was named BAG-1 – for Bcl-2 associated athanogene 1 – and was found to enhance survival synergistically with Bcl-2, suggesting involvement in programmed cell death (PCD) pathways. BAG proteins are characterized by a common conserved region located near the C terminus, termed the BAG domain (BD) that mediates direct interaction with the ATPase domain of Hsp70/Hsc70 molecular chaperones [2].

Six BAG family members have been identified in humans (Fig. 1) and shown to regulate, both positively and negatively, the function of Hsp70/Hsc70, and to form complexes with a range of transcription factors [3] modulating various physiological processes including apoptosis, tumorigenesis, neuronal differentiation, stress responses, and the cell cycle. In addition to the conserved BD, several other domains within the BAG proteins have been identified and are likely to modulate both target specificity and BAG protein localization within cells. Until recently, BAG homologs in plants were not functionally characterized and originally were only noted in comparative sequence studies [4]. Kang et al. [5] identified an *Arabidopsis thaliana* BAG protein (AtBAG-6) in a screen for calmodulin-binding proteins. Using bioinformatic approaches, our laboratory has identified seven BAG protein homologs in the *A. thaliana* genome sequence, four of which have domain organization similar to

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Table 1. A summary of human and *Arabidopsis* BAG family proteins.

Designation	Amino acids	Localization	Partners/potential role	References
Human				
BAG-1L	345	nucleus	Bcl2, Siah, Raf-1, steroid receptors, RAR, CHIP, Hip	3, 9, 13, 26–31, 45
BAG-1M	274	cytosol		
BAG-1S	230	cytosol		
BAG-2	211	cytosol	Hip, MAPKAPK2, CHIP	48–51
BAG-3	575	cytosol	PLC-γ, p65	53, 57
BAG-4	457	cytosol	TNF-R1, DR3	59
BAG-5	447	cytosol	parkin	63
BAG-6	1132	nucleus	reaper, hSGT, p53	67, 68, 71, 74
Arabidopsis				
*At*BAG-1	342	cytosol	–	–
*At*BAG-2	296	cytosol	–	–
*At*BAG-3	303	cytosol	–	–
*At*BAG-4	269	cytosol	salt tolerance	6
*At*BAG-5	215	mitochondria	–	–
*At*BAG-6	1043	nucleus	CaM/Disease resistance	5, 6
*At*BAG-7	446	nucleus	–	–

their animal counterparts. The remaining three members contain a calmodulin-binding motif near the BD (Fig. 1), a novel trait associated with plant BAG proteins indicating possible divergent mechanisms associated with plant-specific functions. Genome organization, molecular phylogeny, and comparative genomics have provided the basis for further functional studies [6]. Plant BAG family members are also multifunctional and regulate processes from pathogen attack to abiotic stress and development (Table 1), as will be discussed later.

BAG proteins in animals

The six human BAG proteins identified are BAG-1 (RAP46/HAP46), BAG-2, BAG-3 (CAIR-1/Bis), BAG-4 (SODD), BAG-5, and BAG-6 (BAT3/Scythe), and all share the signature BD near the C-terminal end, with the exception of BAG 5, which contains four of such domains. It was initially believed that the BAG domain was ~50 amino acids long, but recent crystallography studies suggest that it contains 110–124 amino acids and consists of three anti-parallel helices of 30–40 amino acids each [7]. The second and third helices represent the binding sites for the ATPase domain of Hsp70/Hsc70 [8]. BAG proteins generally differ in their N terminus suggesting that this region imposes specificity to particular proteins and pathways, and/or localization. As mentioned earlier, BAG-1 is the founding member of this family and was discovered in a mouse embryo cDNA library screen with Bcl-2 as bait [1]. Subsequently, BAG-1 was shown to interact with the plasma membrane receptor for hepatocyte growth factor [9]. Four human BAG 1 isoforms are expressed (Fig. 1) through alternative initiation sites and are designated BAG-1L (p50), BAG-1M (p46, RAP46, HAP46), BAG-1S (p36), and p29, with molecular masses of 50, 46, 36, and 29 kDa, respectively [10]. The 36-kDa isoform (termed BAG-1S throughout this review) is sometimes referred to as BAG-1 and is generally the most abundant isoform expressed in cells, followed by BAG-1L and BAG-1M. The 29-kDa isoform is expressed at low levels and is not detected consistently (reviewed in Townsend et al. [11]). Two isoforms of BAG-1 have been identified in mouse; BAG-1S (p32) and BAG-1L (p50) [12]. BAG-1 isoforms share a common BD near the C terminus as well as an upstream ubiquitin-like (UBL) domain, but differ in their N terminus depending on the translation initiation site. The function of the UBL domain remains little detailed; nevertheless, it is likely to be functionally relevant because it is conserved in BAG proteins across wide evolutionary distances and has been shown to be necessary for stress tolerance [13]. An upstream CUG codon, the first in-frame AUG codon, and the second in-frame AUG represent the translation initiation sites for the human BAG-1L, BAG-1M, and BAG-1S, respectively [14]. BAG-1L possesses a nuclear localization signal (NLS); this domain is incomplete in BAG-1M, which is cytosolic but can translocate to the nucleus through interacting

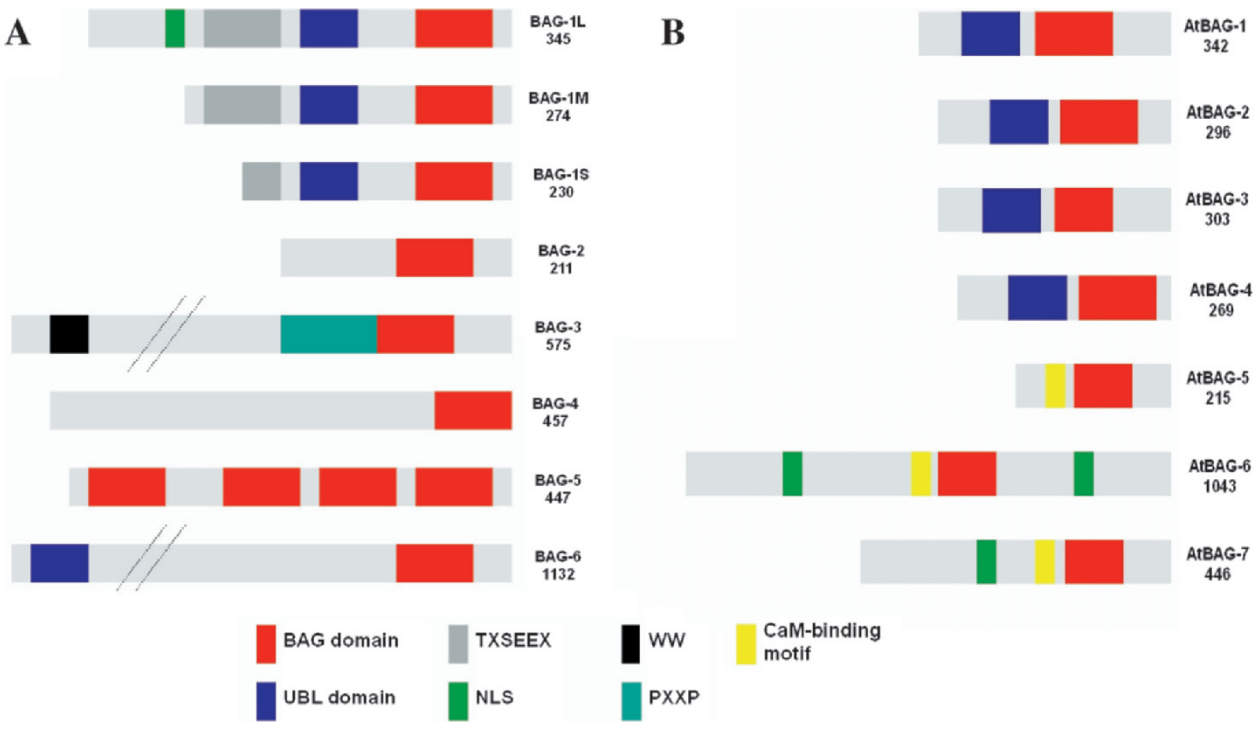

Figure 1. Structure of the human (*A*) and *Arabidopsis thaliana* (*B*) BAG family proteins, showing the conserved BAG domain, ubiquitin-like (UBL) domain, TXSEEX and PXXP repeats, nuclear localization signal (NLS), WW domain, and calmodulin-binding motif. The number of amino acids is shown on the right of each BAG protein.

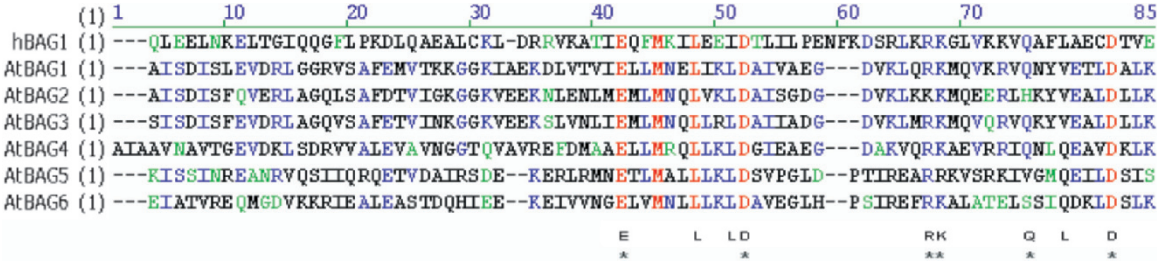

Figure 2. Sequence alignment of the BDs of the human BAG-1 and *Arabidopsis thaliana* BAG proteins. The conserved residues forming the interaction surface with the Hsc70 ATPase domain are shown in the bottom row (*); the additional leucine residues are important for packing interactions.

proteins. The N-terminal region of both BAG-1L and BAG-1M contain eight TXSEEX repeats, reportedly involved in DNA binding and transcription activation, consistent with their nuclear localization [15]. The presence of multiple isoforms indicates that BAG-1 is likely to interact with several targets, potentially expanding its regulatory functions.

The regulation of Hsp70-mediated chaperone activity in mammalian cells is complex but well studied and involves an array of accessory proteins or co-chaperones that alter, assemble, and confer target specificity to Hsp70. The 70-kDa heat shock proteins (Hsp70s) are perhaps the best characterized chaperones and perform a broad spectrum of cellular functions. Hsp70s interact with their polypeptide substrate in an ATP-dependent manner and have high affinity for unfolded proteins when bound to ADP and a low affinity when bound to ATP. The broad-spectrum activity of Hsp70s involves the recruitment of co-chaperones and other chaperone systems resulting in complex networks [16]. Generally, chaperones are proteins whose function is to mediate proper protein folding, membrane translocation of organellar and secretory proteins, and cell death [17]. New roles for chaperones continue to be discovered, such as targeted protein degradation as well as involvement in prion formation and propagation [18]. BAG-1, in the presence of ATP and Hsp40, has been shown to bind the ATPase domain of Hsp70 inducing conformational changes [2]. The regulatory effect of BAG-1 on

Hsp70 has yet to be clearly elucidated. It is believed that BAG-1 intervention reduces Hsp70 nucleotide binding affinity and therefore inhibits Hsp70-mediated chaperone activity [8]. This effect is antagonistic to that of HIP, which inhibits nucleotide exchange activity by stabilizing the ADP-bound form of Hsp70 [19–21]. However, the exact molecular role of BAG-1 has been controversial. Based on *in vitro* assays involving the refolding of artificially denatured reporter proteins, it was demonstrated that various BAG-1 isoforms regulate Hsp70 in different ways. BAG-1M was found to inhibit the refolding of denatured substrates [22, 23], while BAG-1S was shown to initially inhibit protein refolding [24], but in a later study had a stimulating effect [25]. It is unclear whether these contradicting results were due to differences in experimental conditions, or to the dual effect of BAG-1S on Hsp70-dependent chaperone activity.

The BAG-1 protein is also a binding partner to a wide range of signaling molecules including Siah [26], steroid hormone receptors [3, 9, 27–29] and the Raf-1 protein kinase [30, 31]. Siah is the vertebrate homolog of the *Drosophila* sina gene-encoded protein that is required for fly eye development [32]. The function of Siah family proteins (Siah-1A, Siah-1B, and Siah-2) is not known; however, the murine Siah-1B has been identified in a differential cDNA screen for genes induced during p53-mediated apoptosis in myeloid leukemia cells [33]. Matsuzawa et al. [26] identified Siah-1A as a BAG-1-binding partner, and demonstrated that BAG-1 functions downstream of p53-induced gene expression, inhibiting p53-mediated suppression of cell growth, most likely by suppressing the actions of Siah-1A. The interaction of BAG-1 with a number of steroid hormone and retinoic acid receptors is well documented; certain BAG-1 isoforms either stimulate or repress transcription of these receptors. BAG-1L was co-immunoprecipitated with androgen receptors (ARS) from prostate cancer cells and was shown to enhance its transcriptional activity in an Hsp70-dependent manner resulting in resistance to anti-androgen drug therapy [27]. BAG-1M, on the other hand, was shown to negatively regulate glucocorticoid-induced apoptosis by inhibiting the transactivation property of the glucocorticoid receptor [28]. BAG-1 was shown to interact with retinoic acid receptor (RAR), inhibiting its binding to various retinoic acid response elements (RAREs) [29], a finding of potential relevance to breast cancer. Another target for BAG-1 is the catalytic domain of the protein kinase Raf-1, whose activity it stimulates; Raf-1 and Hsp70 appear to bind competitively to BAG-1 due to an overlap in their binding site [30, 31]. In addition to BAG-1, Raf-1 kinase is regulated by several other proteins, including; Ras, 14-3-3 proteins, protein kinase C, tyrosine kinases, and Hsp90 [34]. Raf-1 kinase activity is important for proliferation and survival and plays a central role in connecting extracellular signals through tyrosine kinases and Ras, with downstream serine/threonine kinases [34]. BAG-1 has also been found to act as a physiological mediator of extracellular survival signals linked to the cellular mechanisms that prevent apoptosis in hematopoietic and neuronal progenitor cells [35]. BAG-1S participates in neurotrophin-mediated neurite growth in an Hsp70-dependent manner [36, 37].

All BAG-1 isoforms contain a UBL domain, suggesting involvement with proteosome-mediated protein degradation. The ubiquitin/proteosome system (UPS) functions as a regulator of targeted protein turnover by directing degradation of proteins via the proteosome. Ubiquitin is a highly conserved 76-amino-acid polypeptide present in all eukaryotes [38–40]. Proteins fated for degradation are first tagged by covalent attachment of ubiquitin molecules to a specific lysine residue; the resulting polyubiquitin chain serves as a recognition marker for the downstream 26S proteasome complex, which then degrades the 26S proteasome complex [41]. Conjugation of ubiquitin is a multistep process that requires a ubiquitin-activating enzyme, E1, the ubiquitin-conjugating enzyme E2, and the ubiquitin-protein ligase E3, which catalyzes the final step of the conjugation process [42–44]. It was shown that BAG-1 itself is a substrate of an E3 protein, CHIP. A ternary complex with Hsp70 is formed that targets proteins for degradation [13, 45]. BAG-1 binds to the N-terminus of Hsp70, while CHIP interacts with its C-terminal region [8, 46]. In all, CHIP-dependent ubiquitination of BAG-1 appears to stimulate the association of BAG-1 with the proteasome system. As mentioned earlier, BAG-1 binds Siah-1, another protein with the RING finger domain trait of E3 ligases [26, 47]. Unlike CHIP, this interaction does not involve the ubiquitin domain of BAG-1, suggesting that Siah-1 may act as a facilitator of E2 ubiquitination [47].

While BAG-1 has attracted much attention in recent years, less is known about the other members of the BAG family proteins. BAG-2 and BAG-3 were identified in a two-hybrid screen using Hsp70 as bait, and their inhibitory effect on Hsp70 chaperone activity was found to be as potent as that of BAG-1 [48]. It was also shown that Hsp70-interacting protein (HIP) antagonizes the effects not only of BAG-1 [19–21], but also BAG-2 and BAG-3 [48]. Proteomic characterization of BAG-2 identified this protein as a substrate for MAPK-activated protein (MAPKAP) kinase 2, which is known to mediate p38 MAPK-dependent functions [49, 50]. Like BAG-1, BAG-2

was shown to associate with CHIP; conversely, this association inhibits CHIP-dependent ubiquitin ligase activity, presumably by abolishing the CHIP/E2 interaction [51, 52]. BAG-3 (CAIR-1/Bis) has a unique domain organization: it contains a WW domain followed by a proline-rich region with PXXP motifs (Fig. 1). The CAIR-1 annotation occurred when CAI (calcium entry blocker)-treated cells showed increased BAG3/CAIR-1 expression levels [53]. Most proteins known to interact with SH3 domains contain at least one copy of the motif PXXP [54]. BAG-3 was shown to bind the SH3 domain of phospholipase C-γ (PLC-γ) forming a trimeric complex with Hsp70 and latent PLC-γ; this interaction thus provides a link between Hsp70 and the epidermal growth factor (EGF)-regulated-PLC-γ signaling pathway [53]. Other functions of BAG-3 relate to Hsp70-dependent protein degradation by means of the ubiquitin-mediated proteasome machinery. Overexpression of BAG-3 resulted in the inhibition of the degradation of poly-ubiquitinated Hsp70 client proteins [55]. BAG-3 has also attracted considerable attention as a potential player in both cancer and HIV therapies. In cancer cells, BAG-3 was found to sustain cell survival, impairing the response to therapy, and therefore constitutes a potential target for anti-neoplastic therapies [56]. HIV-1 relies on p65 for its genome expression; BAG-3 appears to target p65 and block its function, providing a possible tool for suppressing HIV-1 gene expression [57].

BAG-4, also known as silencer of death domains (SODD), has a similar domain organization as BAG-2 (Fig. 1) and was also identified in a screen for Hsp70-interacting proteins [48]. Structurally, the helices in BAG-4 BD bundles are three to four turns shorter than in BAG-1 and likely constitute the minimal functional fragment that is able to bind and regulate Hsp70 [58]. BAG-4 has been proposed to be a versatile regulator preventing constitutive signaling by death domain receptors [59]. PCD or its morphological equivalent, apoptosis, plays a major role in development and physiology in essentially all multicellular organisms [60]. PCD is commonly described as the suicide of a cell in a multicellular organism and is initiated by specific physiological signals. Apoptosis is a common form of PCD and several features of this process are highly conserved across broad taxonomic distances. Cells undergoing apoptosis show characteristics that include cell shrinkage, surface blebbing, chromatin condensation, and DNA fragmentation, among others. A number of genes have been identified as positive or negative regulators of cell death. Death-promoting receptors such as tumor necrosis factor receptor 1 (TNF-R1) and death receptor 3 (DR3) signal independently of ligand when overexpressed and cause inappropriate cell death. BAG-4 is thought to negatively regulate the function of TNF-R1 and DR3 by preventing self-aggregation of the death domains maintaining an inactive monomeric state [61]. This regulation is believed to involve Hsp70, which is recruited by BAG-4 to these receptors inducing conformational changes. BAG-4 dissociates from these receptors upon extracellular ligand binding. In cancer therapy and in co-operation with Hsp70 and Hsp40, BAG-4 was found to confer protection against γ-irradiation-induced effects in tumor cells [62].

BAG-5 is a unique member of the BAG family proteins in that it contains four BDs. Little is known about the role of BAG-5 in cells other than its ability to bind Hsp70. However, a recent study showed that BAG-5 plays an important role in Parkinson's disease [63]. BAG-5 was found to inhibit both parkin E3 ligase and Hsp70 chaperone activity enhancing dopaminergic neuron degeneration, and it may serve as a therapeutic target in this context.

BAG-6 (Scythe, BAT3), the longest member of the BAG family proteins, was initially isolated from the human major histocompatibility complex [64]. BAG-6 was found to be associated with the nucleus, and treatments with apoptoic inducers such as staurosporine did not alter its localization [65]. However, a recent report has shown that BAG-6 can be located in the cytosol in multiple mouse primary tissues [66]. BAG-6 was reported to associate with reaper, which plays a central role in developmental apoptosis in *Drosophila melanogaster*. Reaper-induced cytochrome c release and caspase activation in a cell-free extract of *Xenopus* eggs also required BAG-6 [67, 68]. In mice, BAG-6 function was found to be critical for both apoptosis and cell proliferation, including organogenesis [69]. The human small glutamine-rich TPR-containing protein (hSGT) is required for progression through cell division [70]. In co-immunoprecipitation studies, hSGT was shown to interact with both BAG-6 and Hsp70/Hsc70 during prometaphase, suggesting that these proteins could be involved in cell division [71]. Acetylation of p53 (the tumor suppressor protein) is essential to its biological function [72, 73]. A recent report showed that BAG-6 was necessary for p300-mediated p53 acetylation and positively regulates p53-mediated apoptosis [74].

BAG proteins have been the subject of considerable investigation due to the remarkably broad array of important cellular functions with which these molecules are associated, in particular their potential role in several cancers. It is believed that BAGs act as molecular levers that impact the state of the cell depending on whether the environmental conditions are favorable or stressful. Indeed, the competitive

binding of Hsp70 and Raf-1 to the BD of BAG-1 [30, 31] is a good example illustrating the ability of BAGs to operate as a rheostat between proliferation and stress states. Furthermore, BAG protein regulation of hormone sensitivity is of critical importance given the significance of hormones as regulatory and signaling molecules. BAGs have emerged as potential targets for cancer treatment due to their cytoprotective role in maintaining cancer cell survival. However, it remains to be seen whether these proteins have a direct causal role in cancer, and whether cell survival in this case is maintained through Bcl-2, RAF-1, chaperone collaborators, or all of these. The multiplicity of BAG targets and BAG proteins makes it difficult to functionally determine their precise function. Individual knockouts of BAG proteins in general and their putative client proteins may provide clues to BAG function; however, complex protein networks are common, and further experiments in which individual domains within the BAG proteins are evaluated and residues required for target binding are identified using specific point mutations may provide a clearer picture of the role of these proteins and the potential overlap of their targets.

BAG proteins in plants

As mentioned, BAG homologs in plants have not been characterized to nearly the same extent as in mammals and relatively little is known about the function of BAG-like proteins in plants. Blast searches for animal BAG homologs in *A. thaliana* were unsuccessful due to the low sequence identities between BDs of *A. thaliana* and animal BAG proteins. To overcome this feature, more sensitive informatic methods such as Hidden Markov Model (HMM)-based approaches and profile-profile alignment algorithms were needed [75, 76]. HMM-based protein search tools identified seven proteins containing BDs in the *A. thaliana* genome (Fig. 1) with high statistical significance, except for AtBAG-7 for which other criteria such as conservation of helix critical amino acids, similarities in electrostatic potential and hydrophobic surface residue distribution were further considered for adopting AtBAG-7 as a member of the BAG protein family.

NCBI BLAST with default parameters (substitution matrix BLOSUM62) revealed that BD amino acid sequences of the predicted *A. thaliana* BAG proteins share similarities between 35 and 90 %; identities ranged from 22 to 75 %. The *A. thaliana* BAGs appear to cluster in two groups [6]. Group I is similar to the animal BAG proteins and comprises AtBAG-1-4 with a predicted cytosolic localization. Group II, contains AtBAG-6 and 7, and are predicted to localize in the nucleus. AtBAG-5 does not belong to either group and has a predicted mitochondrial localization, a potentially novel feature since none of the human BAGs localizes to the mitochondria. This pattern of clustering could reflect closely related functions of *A. thaliana* BAG proteins within each group. Plant BAG genes are distributed among four chromosomes – I, II, III, and V. Chromosome V has four BAGs, which likely arose from local gene duplication. The other BAGs do not show any clustering within the *A. thaliana* genome. BAG-like genes appear to be widely distributed in plants. Indeed, several expressed sequence tags (ESTs) of putative BAG genes from different plants showed 42 to 79 % similarity to the BD of AtBAG-4 (Table 2). Interestingly, these ESTs were derived from plants exposed to both biotic and abiotic stresses as well as ESTs associated with tissue-specific expression. This observation suggests that plant BAG genes are involved with environmental and developmental responses.

Gene and domain organization

Like their mammalian counterparts, the common feature of all *A. thaliana* BAGs is the presence of the signature BD at the C-terminal region, and four of the predicted plant BAG members contain UBL domains at their N-terminus. AtBAG-5-7 possess a calmodulin-binding motif near the BD, a novel feature associated with plant BAG members [5]. Comparative sequence alignment between mammals and plants showed that most residues required for Hsp70 binding in mammals [8] are conserved in *A. thaliana* BAG proteins (Fig. 2). Therefore, it is reasonable to speculate that *A. thaliana* BAGs bind Hsp70 in a manner similar to that of their mammalian counterparts. Indeed this is the case for at least AtBAG 4; pull-down assays confirmed plant BAG/Hsp70 interaction using AtBAG-4 and *A. thaliana* Hsp70 [6]. AtBAG-4 bound to GST-Hsp70 but not to the glutathione-Sepharose beads, suggesting that AtBAG-4 may regulate Hsp70 chaperone activity in plants. Furthermore, orthologs of the Hsp70 co-chaperoning machinery such as Hsp40, HIP, and CHIP [77–79] have been identified in *A. thaliana*, consistent with the idea that their function is conserved.

Genes that encode proteins predicted to localize in the same cellular compartment, tend to have similar exon/intron organization. BAG genes in the cytoplasmic group (AtBAG-1-4) contain one intron in the UBL domain-coding region and two introns in the BD-coding region. These patterns of organization are highly suggestive of gene duplication events. BAG

Table 2. ESTs of putative BAG genes from various plants obtained from EST database searches using default parameters of NCBI BLAST.

Plant	Description	Accession number	% BD of AtBAG 4[1]
Abiotic stress			
Hordeum vulgare	seedling shoot (2 days of cold stress)	BF622329	60
		BF622327	62
Sorghum bicolor	mix of 5-week-old plants (7 and 8 days of water stress)	BE592352	67
		BE593047	67
Glycine max	drought-stressed leaf tissue of 1-month-old plants	BG436000	59
Beta vulgaris	4-day-old seedlings under stress (NaCl, mannitol)	BI073238	43
Vitis vinifera	stressed berries	CB912188	77
Biotic stress			
Hordeum vulgare	*Blumaria*-challenged seedlings	BE216889	62
Vitis vinifera	Leaves infected with *Xylella fastidiosa*	CB342561	54
Medicago truncatula	root-derived cell culture elicited with yeast cell wall extract	BF646049	59
Tissue-specific expression			
Capsicum annuum	flower bud	BM066508	73
Medicago truncatula	developing flower	BI272171	62
Sorghum bicolor	ovary	BG356714	62
	developing pre-anthesis panicles	BI140027	48
Vitis vinifera	berries (post-harvest)	CB980485	79
		CB980084	79
		CB980018	79
	fruit (green stage)	BQ799356	66
		BQ799253	65
		BQ798817	65
Gossypium arboreum	fibers	BQ403711	44
		BF268630	42
Solanum tuberosum	mature tuber	BF460358	56
	roots	BM108950	58
Triticum aestivum	roots of 5-day-old etiolated seedlings	BE405084	64
	seedling shoot	BE426477	62
Glycine max	immature seed coats	AI960691	59
Malus × domestica	cultured shoots	AU223481	63
Pinus pinaster	differentiating xylem	BX249429	61

[1]Amino acid similarity to the bag domain (BD) of AtBAG 4.

genes in the nucleus group (AtBAG-6, 7) contain one intron within the BD-coding region. AtBAG-5, the putative mitochondrial localized BAG protein, has intron/exon organization with no pattern similarity with the other BAG genes and contains an intronless BD.

Expression patterns of BAG genes in *A. thaliana*

In silico analysis of BAG gene expression in *A. thaliana* was conducted by means of EST database searches for each AtBAG gene [80]. The abundance of ESTs was used to organize these genes into three groups. The first group comprised AtBAG-2, 5, and 6 with two ESTs or less, and the EST number in this group did not differ significantly from zero. The second group comprised AtBAG-1, 4, and 7 with six to seven ESTs and therefore representing a statistically higher expression level. AtBAG-3 represented the third group and showed the highest expression level among AtBAGs with 17 ESTs. Although the conditions under which these ESTs were derived are not well defined, from this study, AtBAG-3 has the highest expression level in *A. thaliana.*

Expression levels of the predicted cytosolic-localized AtBAG-4 and the nuclear-localized AtBAG-6 were analyzed experimentally by reverse transcription-PCR [6]. Higher AtBAG-4 transcript levels were detected in roots and flowers compared to other tissues. Although AtBAG-6 transcript expression was not detected in leaves, its transcription level in other tissues was similar to that of AtBAG-4. Transcript levels of both AtBAG-4 and AtBAG-6 were much

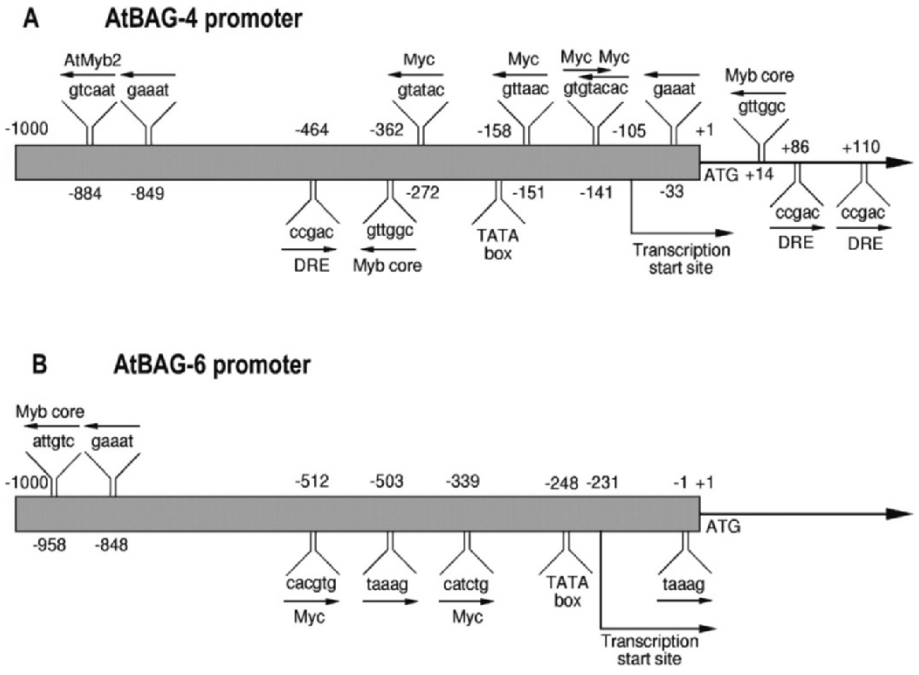

Figure 3. The *Arabidopsis thaliana* AtBAG-4 (*A*) and AtBAG-6 (*B*) gene promoters. The positions of the conserved elements, transcription and translation start sites, and TATA box are indicated.

higher at younger, actively growing stages. BAG induction was analyzed in leaf tissue of *A. thaliana* plants in response to cold and heat treatments. AtBAG-4 induction was detected 20 min after cold stress, while AtBAG-6 transcripts were not observed until 90 min of such a treatment; these results indicate more rapid induction of AtBAG-4 in response to cold compared to AtBAG-6. Conversely, AtBAG-6 expression was strongly induced in response to heat stress, with no detectable induction observed for AtBAG-4. These results suggest that the expression patterns during cold and heat stresses are distinct for these two genes.

AtBAG-4 confers stress tolerance

Transgenic tobacco lines overexpressing AtBAG-4 showed enhanced tolerance to several abiotic stress stimuli [6]. The level of UV irradiation resistance was inversely proportional to the level of AtBAG-4 expression as determined by RT-PCR, with relatively low expressing lines showing higher UVB tolerance, while high-expressing lines responded similarly to the wild-type plants. Similar results were obtained when these transgenic lines were exposed to oxidants such as menadione, paraquat, acifluorfen, and H_2O_2. AtBAG-4-expressing plants were also tested for cold and drought tolerance. Following cold stress (–20 °C for 10 min), dead patches were apparent on wild-type leaves, while leaves with low levels of AtBAG-4 expression remained intact. Similarly, low-level AtBAG-4-expressing plants were extremely drought tolerant (Fig. 4). Wild-type plants severely wilted while transgenic plants maintained normal turgor pressure 29 h after water removal. A number of salt- and drought-tolerant plants adjust to water deficit by lowering their osmotic potential in an attempt to retain water, and thus these two traits are often linked in nature. To evaluate the effect of AtBAG-4 on salt stress, *A. thaliana* knock-out plants were plated on Murashige and Skoog agar medium containing normal salt levels (100 mM NaCl). Knock-out plants were severely affected by this salt concentration and died within 6 weeks while wild-type plants were able to grow and develop normally (Fig. 4). These observations suggest a role for AtBAG-4 in tolerance to salt stress. AtBAG-4 appears to protect plants from a variety of abiotic stresses by inhibiting PCD. Indeed, DNA isolated from cold-stressed wild-type tobacco plants was fragmented and formed characteristic apoptosis-like DNA ladders; TUNEL-positive nuclei were also observed [6]. AtBAG-4-expressing plants survived and did not show any of these characteristics. These results parallel animal studies observations where BAG-1 overexpression provided resistance to a wide variety of stresses, such as heat shock, hypoxia, radiation, and chemotoxic drugs [81]. Abiotic stresses such as chemical toxicity, drought, salinity, and cold cause major crop losses worldwide. These stresses are often interconnected due to the common damages they cause, such as osmotic and oxidative stress, and they often activate similar signaling pathways [82]. Therefore, similar to what is known in animals, it is not

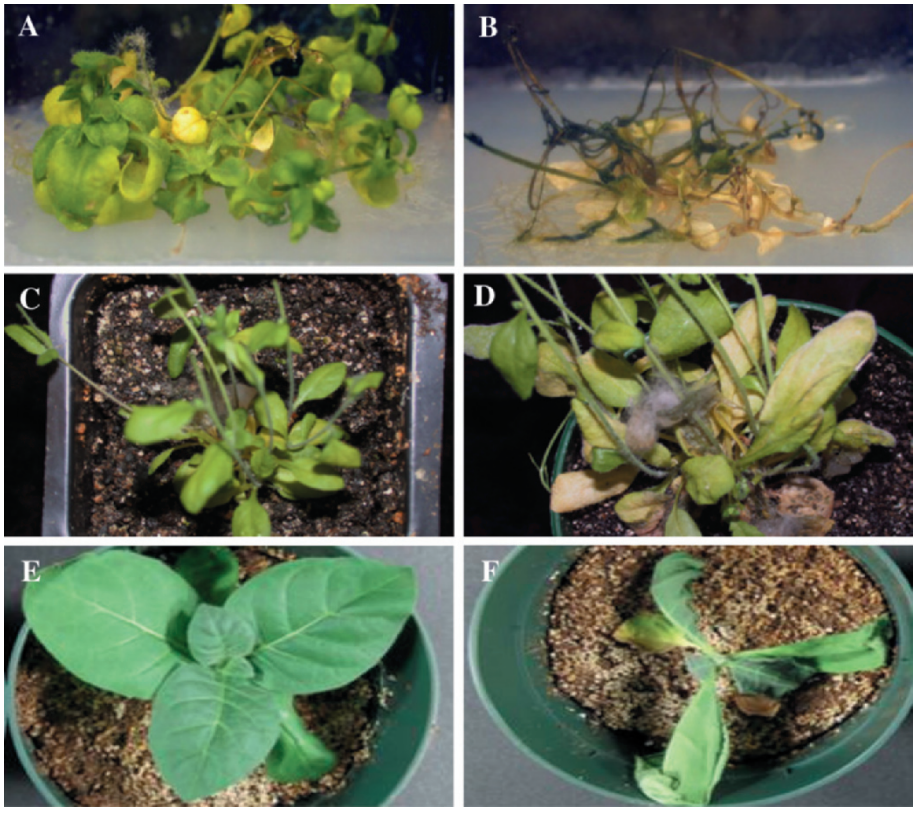

Figure 4. Cytoprotective attributes provided by AtBAG-4 and AtBAG-6 in *Arabidopsis* and tobacco against abiotic and biotic stress. Wild-type (*A*) and AtBAG-4 knock-out (*B*) *Arabidopsis* plants are shown 6 weeks following 100 mM NaCl treatment. Wild-type (*C*) and AtBAG-6 knock-out (*D*) *Arabidopsis* plants are shown 2 weeks following *Botrytis cinerea* challenge. Tobacco lines overexpressing AtBAG-4 (*E*) showed enhanced drought tolerance compared to the wild-type (*F*) 2 weeks after water removal.

surprising that plant BAG genes (AtBAG-4) confer tolerance to such a wide range of abiotic stresses.

AtBAG-6 mutants are susceptible to *Botrytis cinerea*

AtBAG-6 was first identified in a screen for calmodulin binding proteins [5]. AtBAG-6 expression in yeast and plants was shown to induce cell death, and that the calmodulin-binding motif was required. Thus, this protein appears to be regulated by calmodulin and possibly Ca^{2+}. In contrast to the finding with animal BAG proteins, yeast two-hybrid and *in vitro* assays failed to detect any interactions between AtBAG-6 and Hsc70 [5]. This observation is surprising considering that the BD of AtBAG-6 possesses the conserved residues that form the interaction surface with the Hsc70 ATPase domain (Fig. 2). Thus, the interaction of AtBAG-6 and Hsc70 *in vivo* cannot be excluded. Similarly to AtBAG-4, AtBAG-6 appears to be involved in plant growth and development: T-DNA insertion mutant plants for both of these genes senesced early and exhibited earlier flowering and shorter vegetative and reproductive phases compared to wild-type plants.

Three putative BAG ESTs derived from plants exposed to biotic stress have been identified (Table 2), suggesting that BAG proteins may also be implicated in response to biotic stresses. To test this possibility, both AtBAG-4 and AtBAG-6 knock-out *A. thaliana* plants were analyzed in response to inoculations with the necrotrophic fungal pathogen *B. cinerea*, the causative agent of gray mold on various dicotyledonous plant species [6]. While AtBAG-4 mutants were only slightly affected by this pathogen in a manner similar to that of the wild-type plants, AtBAG-6 T-DNA insertion mutants exhibited a dramatically enhanced susceptibility to this pathogen, both in terms of severity and rate of spread (Fig. 4). Furthermore, AtBAG-6 transcript levels were elevated after treatment with defense-associated signals such as salicylic acid, supporting its involvement in host defense mechanisms. These results indicate that functional differences exist between AtBAG-4 and AtBAG-6 in response to environmental stresses, and thus it is reasonable to hypothesize that the BAG family has developed specialized roles for cell regulation.

From these results, it appears that AtBAG-6 may have a role in basal resistance, by limiting disease development in *B. cinerea.* It has been suggested that disease development in necrotrophic pathogens requires host cell death pathways [83, 84; K. S. Kim, J. Y. Min and M B. Dickman, unpublisched data]; consistent with this premise is the observation that the inactivation of the cytoprotective AtBAG-6 results in a conducive

environment for *B. cinerea* pathogenic development. Furthermore, tobacco plants expressing negative regulators of PCD, such as human Bcl-2 and Bcl-xl, nematode CED-9, and baculovirus Op-IAP, demonstrated resistance to several necrotrophic fungal pathogens, including *B. cinerea* [83]. Thus, AtBAG-6 may function in an analogous manner in *A. thaliana.* However, this observation contradicts an earlier study where the calmodulin/Ca^{2+}-binding AtBAG-6 was shown to positively regulate cell death [5]. The basis for this discrepancy is not clear and additional experiments are required to clarify this matter.

Promoter analysis of *A. thaliana* BAG genes

Representatives from each of the nuclear and cytosolic groups, AtBAG-4 and AtBAG-6, were chosen to evaluate promoter sequences using the PLACE database [85]. Analysis of the AtBAG-4 promoter identified three core sequences of the drought-responsive element (DRE)/C-repeat (CRT) *cis* element (CCGAC), four Myc recognition elements (CANNTG), one AtMyb2 recognition sequence (TAACTG), two core sequences of the Myb recognition element (CGGTTG), and two copies of TAAAG motifs (Fig. 3). Numerous cold- and dehydration-responsive genes contain one or more copies of the DRE/CRT *cis* element. CBF or DREB1 cold-induced transcription factors bind to this element and activate transcription of cold- and dehydration-responsive genes [86]. *A. thaliana* Myc and Myb core element-containing promoters respond to water stress [87, 88]. The TAAAG motif is related to the target site of the *trans*-acting Dof1 protein, which is involved in controlling guard cell-specific expression [89]. Collectively, these motifs are conserved elements commonly found in promoters that respond to abiotic stresses. The relatively high incidence of these recognition elements in AtBAG-4 suggests a rapid induction of this gene in response to stress. In contrast, analysis of the AtBAG-6 promoter sequence revealed only two Myc recognition elements, one core sequence Myb recognition element, and three copies of TAAAG motifs (Fig. 3). No DRE/CRT *cis* elements were identified, consistent with a weak and slower induction of AtBAG-6 in response to abiotic stress. The analysis of AtBAG-4 upstream sequence identified three heat shock promoter elements (HSEs) upstream of the TATA box, a CCAAT box upstream of the HSEs, and one scaffold attachement region (SAR). Even though AtBAG-4 possesses three HSE sites, no induction was observed in response to heat treatment. This could be explained by either an insufficient quantity of HSE sites and/or the requirement for additional sequences, or by post-translational regulation of AtBAG-4 transcript. In contrast, analysis of AtBAG-6 revealed 12 HSE sites upstream of the TATA element, three CCAAT boxes, and two SARs. The high frequency of conserved promoter elements associated with heat and other stresses suggests that at least some of these elements are functionally relevant in these BAG genes.

Conclusions

BAG proteins from humans to plants have been associated with stress responses and modulation of cell death. Our work has focused on identifying and functionally analyzing BAG proteins in *A. thaliana.* We have established that by using higher-order bioinformatic approaches distantly related genes can be identified by structure in highly divergent sequences. In plants such prediction can be easily addressed experimentally. The function of AtBAG1–3, 5, and 7 is not yet known, and even though roles for AtBAG-4 and 6 have been indicated, the localization of these proteins under various cell states is of considerable interest. Moreover, the identification of binding partners and the pathways they mediate are of critical importance. Human BAG proteins are known to interact with transcription factors, the proteasome, and cell cycle regulators. Initial yeast two-hybrid data using both full-length AtBAG-4 and the BD of AtBAG-4 alone as baits revealed several potential AtBAG-4-interacting proteins including those involved in cellular detoxification such as glutathione S-tranferases and peroxiredoxins, transcription factors that include F-box and squamosa promoter binding proteins, as well as signaling molecules such as protein kinases and GAPDH. The yeast two-hybrid test also confirmed the interaction of AtBAG-4 and Hsp70. T-DNA insertional-mutagenesis lines of the entire BAG family have been obtained and will serve as the basis for future studies. AtBAG-5 is of particular interest due to its unique mitochondrial-predicted localization in plants. The functional versatility of the BAG family noted in mammals appears to be maintained in the model plant *A. thaliana.* Furthermore, BAG proteins appear to be widely distributed in plants as indicated by EST database searches of putative BAG genes from various plants. Through a variety of methods, the plant BAG genes appear to function in cytoprotection under stress conditions and can inhibit plant PCD, supporting the idea that at least some mechanisms for apoptotic-like cell death regulation are conserved between plants and animals. The current challenge is to position plant BAG genes in their biochemical pathways and further define their

functional roles. BAG genes from humans and plants may offer new solutions for therapeutic design against related diseases and stresses involving inappropriate regulation of the PCD machinery.

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1 Takayama, S., Sato, T., Krajewski, S., Kochel, K., Irie, S., Millan, J. A. and Reed, J. C. (1995) Cloning and functional analysis of BAG-1: a novel Bcl-2-binding protein with anti-cell death activity. Cell 80, 279–284.
2 Brive, L., Takayama, S., Briknarova, K., Homma, S., Ishida, S. K., Reed, J. C. and Ely, K. R. (2001) The carboxyl-terminal lobe of Hsc70 ATPase domain is sufficient for binding to BAG1. Biochem. Biophys. Res. Commun. 289, 1099–1105.
3 Zeiner, M. and Gehring, U. (1995) A protein that interacts with members of the nuclear hormone receptor family: identification and cDNA cloning. Proc. Natl. Acad. Sci. USA 92, 11465–11469.
4 Takayama, S. and Reed, J. C. (2001) Molecular chaperone targeting and regulation by BAG family proteins. Nat. Cell Biol. 3, E237–241.
5 Kang, C. H., Jung, W. Y., Kang, Y. H., Kim, J. Y., Kim, D. G., Jeong, J. C., Baek, D. W., Jin, J. B., Lee, J. Y., Kim, M. O., Chung, W. S., Mengiste, T., Koiwa, H., Kwak, S. S., Bahk, J. D., Lee, S. Y., Nam, J. S., Yun, D. J. and Cho, M. J. (2006) AtBAG6, a novel calmodulin-binding protein, induces programmed cell death in yeast and plants. Cell Death Differ. 13, 84–95.
6 Doukhanina, E. V., Chen, S., van der Zalm, E., Godzik, A., Reed, J. and Dickman, M. B. (2006) Identification and functional characterization of the BAG protein family in *Arabidopsis thaliana*. J. Biol. Chem. 281, 18793–18801.
7 Briknarova, K., Takayama, S., Brive, L., Havert, M. L., Knee, D. A., Velasco, J., Homma, S., Cabezas, E., Stuart, J., Hoyt, D. W., Satterthwait, A. C., Llinas, M., Reed, J. C. and Ely, K. R. (2001) Structural analysis of BAG1 cochaperone and its interactions with Hsc70 heat shock protein. Nat. Struct. Biol. 8, 349–352.
8 Sondermann, H., Scheufler, C., Schneider, C., Hohfeld, J., Hartl, F. U. and Moarefi, I. (2001) Structure of a Bag/Hsc70 complex: convergent functional evolution of Hsp70 nucleotide exchange factors. Science 291, 1553–1557.
9 Bardelli, A., Longati, P., Albero, D., Goruppi, S., Schneider, C., Ponzetto, C. and Comoglio, P. M. (1996) HGF receptor associates with the anti-apoptotic protein BAG-1 and prevents cell death. EMBO J. 15, 6205–6212.
10 Yang, X., Chernenko, G., Hao, Y., Ding, Z., Pater, M. M., Pater, A. and Tang, S. C. (1998) Human BAG-1/RAP46 protein is generated as four isoforms by alternative translation initiation and overexpressed in cancer cells. Oncogene 17, 981–989.
11 Townsend, P. A., Cutress, R. I., Sharp, A., Brimmell, M. and Packham, G. (2003) BAG-1: a multifunctional regulator of cell growth and survival. Biochim. Biophys. Acta 1603, 83–98.
12 Brimmell, M., Burns, J. S., Munson, P., McDonald, L., O'Hare, M. J., Lakhani, S. R. and Packham, G. (1999) High level expression of differentially localized BAG-1 isoforms in some oestrogen receptor-positive human breast cancers. Br. J. Cancer 81, 1042–1051.
13 Alberti, S., Demand, J., Esser, C., Emmerich, N., Schild, H. and Hohfeld, J. (2002) Ubiquitylation of BAG-1 suggests a novel regulatory mechanism during the sorting of chaperone substrates to the proteasome. J. Biol. Chem. 277, 45920–45927.
14 Packham, G., Brimmell, M. and Cleveland, J. L. (1997) Mammalian cells express two differently localized Bag-1 isoforms generated by alternative translation initiation. Biochem. J. 328, 807–813.
15 Zeiner, M., Niyaz, Y. and Gehring, U. (1999) The hsp70-associating protein Hap46 binds to DNA and stimulates transcription. Proc. Natl. Acad. Sci. USA 96, 10194–10199.
16 Mayer, M. P. and Bukau, B. (2005) Hsp70 chaperones: cellular functions and molecular mechanism. Cell. Mol. Life Sci. 62, 670–684.
17 Hartl, F. U. and Hayer-Hartl, M. (2002) Molecular chaperones in the cytosol: from nascent chain to folded protein. Science 295, 1852–1858.
18 Chernoff, Y. O. (2007) Stress and prions: lessons from the yeast model. FEBS Lett. 581, 3695–3701.
19 Hohfeld, J. (1998) Regulation of the heat shock conjugate Hsc70 in the mammalian cell: the characterization of the anti-apoptotic protein BAG-1 provides novel insights. Biol. Chem. 379, 269–274.
20 Hohfeld, J. and Jentsch, S. (1997) GrpE-like regulation of the hsc70 chaperone by the anti-apoptotic protein BAG-1. EMBO J. 16, 6209–6216.
21 Hohfeld, J., Minami, Y. and Hartl, F. U. (1995) Hip, a novel cochaperone involved in the eukaryotic Hsc70/Hsp40 reaction cycle. Cell 83, 589–598.
22 Gebauer, M., Zeiner, M. and Gehring, U. (1997) Proteins interacting with the molecular chaperone hsp70/hsc70: physical associations and effects on refolding activity. FEBS Lett. 417, 109–113.
23 Zeiner, M., Gebauer, M. and Gehring, U. (1997) Mammalian protein RAP46: an interaction partner and modulator of 70 kDa heat shock proteins. EMBO J. 16, 5483–5490.
24 Takayama, S., Bimston, D. N., Matsuzawa, S., Freeman, B. C., Aime-Sempe, C., Xie, Z., Morimoto, R. I. and Reed, J. C. (1997) BAG-1 modulates the chaperone activity of Hsp70/Hsc70. EMBO J. 16, 4887–4896.
25 Luders, J., Demand, J., Papp, O. and Hohfeld, J. (2000) Distinct isoforms of the cofactor BAG-1 differentially affect Hsc70 chaperone function. J. Biol. Chem. 275, 14817–14823.
26 Matsuzawa, S., Takayama, S., Froesch, B. A., Zapata, J. M. and Reed, J. C. (1998) p53-inducible human homologue of *Drosophila* seven in absentia (Siah) inhibits cell growth: suppression by BAG-1. EMBO J. 17, 2736–2747.
27 Froesch, B. A., Takayama, S. and Reed, J. C. (1998) BAG-1L protein enhances androgen receptor function. J. Biol. Chem. 273, 11660–11666.
28 Kullmann, M., Schneikert, J., Moll, J., Heck, S., Zeiner, M., Gehring, U. and Cato, A. C. (1998) RAP46 is a negative regulator of glucocorticoid receptor action and hormone-induced apoptosis. J. Biol. Chem. 273, 14620–14625.
29 Liu, R., Takayama, S., Zheng, Y., Froesch, B., Chen, G. Q., Zhang, X., Reed, J. C. and Zhang, X. K. (1998) Interaction of BAG-1 with retinoic acid receptor and its inhibition of retinoic acid-induced apoptosis in cancer cells. J. Biol. Chem. 273, 16985–16992.
30 Wang, H. G., Takayama, S., Rapp, U. R. and Reed, J. C. (1996) Bcl-2 interacting protein, BAG-1, binds to and activates the kinase Raf-1. Proc. Natl. Acad. Sci. USA 93, 7063–7068.
31 Song, J., Takeda, M. and Morimoto, R. I. (2001) Bag1-Hsp70 mediates a physiological stress signalling pathway that regulates Raf-1/ERK and cell growth. Nat. Cell Biol. 3, 276–282.
32 Carthew, R. W. and Rubin, G. M. (1990) seven in absentia, a gene required for specification of R7 cell fate in the *Drosophila* eye. Cell 63, 561–577.
33 Amson, R. B., Nemani, M., Roperch, J.-P., Israeli, D., Bougueleret, L., Le Gall, I., Medhioub, M., Linares-Cruz, G., Lethrosne, F., Pasturaud, P., Piouffre, L., Prieur, S., Susini, L., Alvaro, V., Millasseau, P., Guidicelli, C., Bui, H., Massart, C., Cazes, L., Dufour, F., Bruzzoni-Giovanelli, H., Owadi, H., Hennion, C., Charpak, G., Dausset, J., Calvo, F., Oren, M., Cohen, D. and Telerman, A. (1996) Isolation of 10 differentially expressed cDNAs in p53-induced apoptosis: activation of the vertebrate homologue of the *Drosophila* seven in absentia gene. Proc. Natl. Acad. Sci. USA 93, 3953–3957.
34 Morrison, D. K. and Cutler, R. E. (1997) The complexity of Raf-1 regulation. Curr. Opin. Cell Biol. 9, 174–179.

35 Gotz, R., Wiese, S., Takayama, S., Camarero, G. C., Rossoll, W., Schweizer, U., Troppmair, J., Jablonka, S., Holtmann, B., Reed, J. C., Rapp, U. R. and Sendtner, M. (2005) Bag1 is essential for differentiation and survival of hematopoietic and neuronal cells. Nat. Neurosci. 8, 1169–1178.

36 Frebel, K. and Wiese, S. (2006) Signalling molecules essential for neuronal survival and differentiation. Biochem. Soc. Trans. 34, 1287–1290.

37 Frebel, K., Wiese, S., Funk, N., Puhringer, D. and Sendtner, M. (2007) Differential modulation of neurite growth by the S- and the L-forms of bag1, a co-chaperone of Hsp70. Neurodegener. Dis. 4, 261–269.

38 Hershko, A. and Ciechanover, A. (1998) The ubiquitin system. Annu. Rev. Biochem. 67, 425–479.

39 Kaiser, P. and Tagwerker, C. (2005) Is this protein ubiquitinated? Methods Enzymol. 399, 243–248.

40 Pickart, C. M. (2001) Mechanisms underlying ubiquitination. Annu. Rev. Biochem. 70, 503–533.

41 Glickman, M. H. and Ciechanover, A. (2002) The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol. Rev. 82, 373–428.

42 Jentsch, S. and Schlenker, S. (1995) Selective protein degradation: a journey's end within the proteasome. Cell 82, 881–884.

43 Jentsch, S. and Pyrowolakis, G. (2000) Ubiquitin and its kin: how close are the family ties? Trends Cell Biol. 10, 335–342.

44 Varshavsky, A. (1997) The ubiquitin system. Trends Biochem. Sci. 22, 383–387.

45 Demand, J., Alberti, S., Patterson, C. and Hohfeld, J. (2001) Cooperation of a ubiquitin domain protein and an E3 ubiquitin ligase during chaperone/proteasome coupling. Curr. Biol. 11, 1569–1577.

46 Ballinger, C. A., Connell, P., Wu, Y., Hu, Z., Thompson, L. J., Yin, L. Y. and Patterson, C. (1999) Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. Mol. Cell Biol. 19, 4535–4545.

47 Lorick, K. L., Jensen, J. P., Fang, S., Ong, A. M., Hatakeyama, S. and Weissman, A. M. (1999) RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. Proc. Natl. Acad. Sci. USA 96, 11364–11369.

48 Takayama, S., Xie, Z. and Reed, J. C. (1999) An evolutionarily conserved family of Hsp70/Hsc70 molecular chaperone regulators. J. Biol. Chem. 274, 781–786.

49 Ueda, K., Kosako, H., Fukui, Y. and Hattori, S. (2004) Proteomic identification of Bcl2-associated athanogene 2 as a novel MAPK-activated protein kinase 2 substrate. J. Biol. Chem. 279, 41815–41821.

50 Zu, Y. L., Ai, Y., Gilchrist, A., Labadia, M. E., Sha'afi, R. I. and Huang, C. K. (1996) Activation of MAP kinase-activated protein kinase 2 in human neutrophils after phorbol ester or fMLP peptide stimulation. Blood 87, 5287–5296.

51 Arndt, V., Daniel, C., Nastainczyk, W., Alberti, S. and Hohfeld, J. (2005) BAG-2 acts as an inhibitor of the chaperone-associated ubiquitin ligase CHIP. Mol. Biol. Cell 16, 5891–5900.

52 Dai, Q., Qian, S. B., Li, H. H., McDonough, H., Borchers, C., Huang, D., Takayama, S., Younger, J. M., Ren, H. Y., Cyr, D. M. and Patterson, C. (2005) Regulation of the cytoplasmic quality control protein degradation pathway by BAG2. J. Biol. Chem. 280, 38673–38681.

53 Doong, H., Price, J., Kim, Y. S., Gasbarre, C., Probst, J., Liotta, L. A., Blanchette, J., Rizzo, K. and Kohn, E. (2000) CAIR-1/BAG-3 forms an EGF-regulated ternary complex with phospholipase C-gamma and Hsp70/Hsc70. Oncogene 19, 4385–4395.

54 Lim, W. A. and Richards, F. M. (1994) Critical residues in an SH3 domain from Sem-5 suggest a mechanism for proline-rich peptide recognition. Nat. Struct. Biol. 1, 221–225.

55 Doong, H., Rizzo, K., Fang, S., Kulpa, V., Weissman, A. M. and Kohn, E. C. (2003) CAIR-1/BAG-3 abrogates heat shock protein-70 chaperone complex-mediated protein degradation: accumulation of poly-ubiquitinated Hsp90 client proteins. J. Biol. Chem. 278, 28490–28500.

56 Rosati, A., Ammirante, M., Gentilella, A., Basile, A., Festa, M., Pascale, M., Marzullo, L., Belisario, M. A., Tosco, A., Franceschelli, S., Moltedo, O., Pagliuca, G., Lerose, R. and Turco, M. C. (2007) Apoptosis inhibition in cancer cells: A novel molecular pathway that involves BAG3 protein. Int. J. Biochem. Cell Biol. 39, 1337–1342.

57 Rosati, A., Leone, A., Del Valle, L., Amini, S., Khalili, K. and Turco, M. C. (2007) Evidence for BAG3 modulation of HIV-1 gene transcription. J. Cell Physiol. 210, 676–683.

58 Briknarova, K., Takayama, S., Homma, S., Baker, K., Cabezas, E., Hoyt, D. W., Li, Z., Satterthwait, A. C. and Ely, K. R. (2002) BAG4/SODD protein contains a short BAG domain. J. Biol. Chem. 277, 31172–31178.

59 Jiang, Y., Woronicz, J. D., Liu, W. and Goeddel, D. V. (1999) Prevention of constitutive TNF receptor 1 signaling by silencer of death domains. Science 283, 543–546.

60 Ellis, R. E., Yuan, J. Y. and Horvitz, H. R. (1991) Mechanisms and functions of cell death. Annu. Rev. Cell Biol. 7, 663–698.

61 Tschopp, J., Martinon, F. and Hofmann, K. (1999) Apoptosis: silencing the death receptors. Curr. Biol. 9, R381–384.

62 Gehrmann, M., Marienhagen, J., Eichholtz-Wirth, H., Fritz, E., Ellwart, J., Jaattela, M., Zilch, T. and Multhoff, G. (2005) Dual function of membrane-bound heat shock protein 70 (Hsp70), Bag-4, and Hsp40: protection against radiation-induced effects and target structure for natural killer cells. Cell Death Differ. 12, 38–51.

63 Kalia, S. K., Lee, S., Smith, P. D., Liu, L., Crocker, S. J., Thorarinsdottir, T. E., Glover, J. R., Fon, E. A., Park, D. S. and Lozano, A. M. (2004) BAG5 inhibits parkin and enhances dopaminergic neuron degeneration. Neuron 44, 931–945.

64 Banerji, J., Sands, J., Strominger, J. L. and Spies, T. (1990) A gene pair from the human major histocompatibility complex encodes large proline-rich proteins with multiple repeated motifs and a single ubiquitin-like domain. Proc. Natl. Acad. Sci. USA 87, 2374–2378.

65 Manchen, S. T. and Hubberstey, A. V. (2001) Human Scythe contains a functional nuclear localization sequence and remains in the nucleus during staurosporine-induced apoptosis. Biochem. Biophys. Res. Commun. 287, 1075–1082.

66 Kislinger, T., Cox, B., Kannan, A., Chung, C., Hu, P., Ignatchenko, A., Scott, M. S., Gramolini, A. O., Morris, Q., Hallett, M. T., Rossant, J., Hughes, T. R., Frey, B. and Emili, A. (2006) Global survey of organ and organelle protein expression in mouse: combined proteomic and transcriptomic profiling. Cell 125, 173–186.

67 Thress, K., Evans, E. K. and Kornbluth, S. (1999) Reaper-induced dissociation of a Scythe-sequestered cytochrome c-releasing activity. EMBO J. 18, 5486–5493.

68 Thress, K., Henzel, W., Shillinglaw, W. and Kornbluth, S. (1998) Scythe: a novel reaper-binding apoptotic regulator. EMBO J. 17, 6135–6143.

69 Desmots, F., Russell, H. R., Lee, Y., Boyd, K. and McKinnon, P. J. (2005) The reaper-binding protein scythe modulates apoptosis and proliferation during mammalian development. Mol. Cell Biol. 25, 10329–10337.

70 Winnefeld, M., Rommelaere, J. and Cziepluch, C. (2004) The human small glutamine-rich TPR-containing protein is required for progress through cell division. Exp. Cell Res. 293, 43–57.

71 Winnefeld, M., Grewenig, A., Schnolzer, M., Spring, H., Knoch, T. A., Gan, E. C., Rommelaere, J. and Cziepluch, C. (2006) Human SGT interacts with Bag-6/Bat-3/Scythe and cells with reduced levels of either protein display persistence of few misaligned chromosomes and mitotic arrest. Exp. Cell Res. 312, 2500–2514.

72 Brooks, C. L. and Gu, W. (2003) Ubiquitination, phosphorylation and acetylation: the molecular basis for p53 regulation. Curr. Opin. Cell Biol. 15, 164–171.

73 Appella, E. and Anderson, C. W. (2001) Post-translational modifications and activation of p53 by genotoxic stresses. Eur. J. Biochem. 268, 2764–2772.

74 Sasaki, T., Gan, E. C., Wakeham, A., Kornbluth, S., Mak, T. W. and Okada, H. (2007) HLA-B-associated transcript 3 (Bat3)/Scythe is essential for p300-mediated acetylation of p53. Genes Dev. 21, 848–861.
75 Bateman, A. and Haft, D. H. (2002) HMM-based databases in InterPro. Brief Bioinform. 3, 236–245.
76 Rychlewski, L., Jaroszewski, L., Li, W. and Godzik, A. (2000) Comparison of sequence profiles: strategies for structural predictions using sequence information. Protein Sci. 9, 232–241.
77 Miernyk, J. A. (2001) The J-domain proteins of *Arabidopsis thaliana:* an unexpectedly large and diverse family of chaperones. Cell Stress Chaperones 6, 209–218.
78 Webb, M. A., Cavaletto, J. M., Klanrit, P. and Thompson, G. A. (2001) Orthologs in *Arabidopsis thaliana* of the Hsp70 interacting protein Hip. Cell Stress Chaperones 6, 247–255.
79 Yan, J., Wang, J., Li, Q., Hwang, J. R., Patterson, C. and Zhang, H. (2003) AtCHIP, a U-box-containing E3 ubiquitin ligase, plays a critical role in temperature stress tolerance in Arabidopsis. Plant Physiol. 132, 861–869.
80 Yan, J., He, C. and Zhang, H. (2003) The BAG-family proteins in *Arabidopsis thaliana.* Plant Sci. 165, 1–7.
81 Townsend, P. A., Cutress, R. I., Sharp, A., Brimmell, M. and Packham, G. (2003) BAG-1 prevents stress-induced long-term growth inhibition in breast cancer cells via a chaperone-dependent pathway. Cancer Res. 63, 4150–4157.
82 Wang, W., Vinocur, B. and Altman, A. (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218, 1–14.
83 Dickman, M. B., Park, Y. K., Oltersdorf, T., Li, W., Clemente, T. and French, R. (2001) Abrogation of disease development in plants expressing animal antiapoptotic genes. Proc. Natl. Acad. Sci. USA 98, 6957–6962.
84 Lorang, J. M., Sweat, T. A. and Wolpert, T. J. (2007) Plant disease susceptibility conferred by a 'resistance' gene. Proc. Natl. Acad. Sci. USA 104, 14861–14866.
85 Higo, K., Ugawa, Y., Iwamoto, M. and Korenaga, T. (1999) Plant cis-acting regulatory DNA elements (PLACE) database: 1999. Nucleic Acids Res. 27, 297–300.
86 Shinozaki, K. and Yamaguchi-Shinozaki, K. (2000) Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. Curr. Opin. Plant Biol. 3, 217–223.
87 Abe, H., Urao, T., Ito, T., Seki, M., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2003) *Arabidopsis* AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. Plant Cell 15, 63–78.
88 Chinnusamy, V., Ohta, M., Kanrar, S., Lee, B. H., Hong, X., Agarwal, M. and Zhu, J. K. (2003) ICE1: a regulator of cold-induced transcriptome and freezing tolerance in *Arabidopsis.* Genes Dev. 17, 1043–1054.
89 Plesch, G., Ehrhardt, T. and Mueller-Roeber, B. (2001) Involvement of TAAAG elements suggests a role for Dof transcription factors in guard cell-specific gene expression. Plant J. 28, 455–464.