

# Heat-shock proteins as regulators of apoptosis

Shinichi Takayama<sup>\*1</sup>, John C Reed<sup>1</sup> and Sachiko Homma<sup>1</sup>

<sup>1</sup>The Burnham Institute, La Jolla, CA 92037, USA

**Heat-shock proteins are produced in response to different types of stress conditions making cells resistant to stress-induced cell damage. Under normal conditions, heat-shock proteins play numerous roles in cell function, including modulating protein activity by changing protein conformation, promoting multiprotein complex assembly/disassembly, regulating protein degradation within the proteasome pathway, facilitating protein translocation across organellar membranes, and ensuring proper folding of nascent polypeptide chains during protein translation. When cells are stressed, a common response is to undergo cell death by one of two pathways, either ‘necrosis’ or ‘apoptosis’. Recently, both routes to cell death have been revealed to share similar mechanisms, with heat-shock proteins and their cofactors responsible for inhibiting both apoptotic and necrotic pathways. We therefore briefly summarize recent reports showing molecular evidence of cell death regulation by heat-shock proteins and their cochaperones.**

*Oncogene* (2003) 22, 9041–9047. doi:10.1038/sj.onc.1207114

**Keywords:** heat-shock proteins; Hsp27; Hsp70; Hsp90; apoptosis

## Heat-shock proteins regulate the mitochondrial pathway of apoptosis

Two major pathways for apoptosis induction have been identified: intrinsic and extrinsic. The hallmarks of the intrinsic pathway are mitochondrial involvement and the formation of the ‘apoptosome’. In the intrinsic pathway, cell death signals induce the release of cytochrome *c* (Cyt *c*) from the mitochondria, which then binds to the apoptosis protease activating factor-1 (Apaf-1), inducing oligomerization and eventual recruitment of procaspase-9. Apoptosome formation results in the processing and activation of caspase-9, which triggers the caspase pathway by activating the downstream caspase-3 (Saleh *et al.*, 2000; Jiang *et al.*, 2001).

Overexpression of Hsp27 increases the resistance of cells to various apoptotic stimuli (Mehlen *et al.*, 1996a; Rogalla *et al.*, 1999). One mechanism by which Hsp27 could interfere with apoptosis is by directly binding to cytosolic Cyt *c* and sequestering it from Apaf-1 (Garrido

*et al.*, 1999; Bruey *et al.*, 2000a). The antiapoptotic activity of Hsp27 appears to be highly dependent on its oligomeric status. Hsp27 is able to shift between different oligomeric states in a phosphorylation-dependent manner (Lambert *et al.*, 1999; Bruey *et al.*, 2000b) and only the high molecular weight Hsp27 appears competent to inhibit apoptosome formation (Bruey *et al.*, 2000a). Recently, modification of Hsp27 by methylglyoxal (a glycolysis by-product that modifies proteins at arginine and lysine residues) was reported to repress the formation of large Hsp27 oligomers and inhibit Cyt *c*-dependent apoptosis (Sakamoto *et al.*, 2002). These results support a role for Hsp27 in apoptosis by inhibition of apoptosome formation.

Two groups reported a direct interaction between Apaf-1 and Hsp70 that prevents apoptosome formation (Beere *et al.*, 2000; Saleh *et al.*, 2000). The precise mechanism by which this interaction affords apoptosis inhibition is unclear, but it appears to be mediated by the caspase recruitment domain of Apaf-1 and to require the presence of ATP, implying that the ‘foldase’ activity of Hsp70 is involved.

Recently, it was shown that Hsp70 protects serum depletion-induced cell death in cells lacking Apaf-1 (Apaf-1<sup>-/-</sup>), indicating that Hsp70 also plays a role in diminishing Apaf-1-independent apoptosis. One of the targets in this situation may be apoptosis-inducing factor (AIF), which normally resides in the intermembrane space of the mitochondria, but upon apoptosis induction translocates to the cytosol and nucleus, where it participates in caspase-independent apoptotic pathways (Ravagnan *et al.*, 2001). Hsp70 directly binds to AIF, inhibiting AIF-dependent apoptosis. This binding is mediated by the peptide domain of Hsp70, but the ATPase domain is dispensable for cell death inhibition, suggesting that this mechanism is not chaperone-activity dependent (Ravagnan *et al.*, 2001).

Hsp60 is a heat-shock protein that primarily localizes to the matrix of the mitochondria. Recently, two independent groups reported a role for Hsp60 in caspase-3 maturation (Samali *et al.*, 1999; Xanthoudakis *et al.*, 1999). In Jurkat T cells, a subpopulation of caspase-3 is found in the mitochondria in complex with Hsp60. Upon induction of apoptosis with staurosporine, mitochondrial procaspase-3 is activated and dissociates from Hsp60 prior to the release of both proteins into the cytosol (Samali *et al.*, 1999). *In vitro*, recombinant Hsp60 accelerated the rate at which procaspase-3 was activated by Cyt *c* and dATP in an ATP-dependent

\*Correspondence: S Takayama; E-mail stakayama@burnham.org

manner, suggesting that the chaperone function of Hsp60 is involved in this process (Samali *et al.*, 1999).

Cytosolic Hsp60 has also been shown to be complexed with the antiapoptotic protein Bax (Gupta and Knowlton, 2002). Under hypoxic conditions, Hsp60 and Bax dissociate, whereupon Bax translocates to the mitochondria to participate in apoptosis.

The above situations suggest a role for heat-shock proteins upstream of caspase activation. However, Hsp70 overexpression can also inhibit caspase-dependent events that occur much later in apoptosis, such as activation of cytosolic phospholipase A2 and changes in nuclear morphology. Hsp70 could also protect cells from forced expression of caspase-3 (Jaattela *et al.*, 1998). Thus, heat-shock proteins also inhibit events occurring downstream of caspase activation.

During the final phases of apoptosis, chromosomal DNA is digested by the DNase CAD (caspase activated DNase, also known as DFF 40, DNA fragmentation factor 40), following activation by caspase-3. Recently, the enzymatic activity and proper folding of CAD/DFF40 was reported to be regulated by Hsp70, Hsp40, and ICAD (inhibitor of CAD). ICAD appears to recognize an intermediate folding state conferred by Hsp70–Hsp40, suggesting that these heat-shock proteins may promote the formation of the CAD–ICAD complex during protein translation (Sakahira and Nagata, 2002).

### Heat-shock proteins regulate survival signaling, stress-induced apoptosis, and TNF family receptor signal transduction

The activity of survival factors, such as nerve growth factor and platelet-derived growth factor, inhibit apoptosis by activating the phosphatidylinositol 3-kinase (PI3-K) pathway. Upon stimulation with growth factors, PI3-K is activated and phosphorylates inositol lipids in the plasma membrane that attract the kinase Akt. Upon association with these lipids, Akt is activated by PDK1-mediated phosphorylation (for review, see Datta *et al.*, 1999; Vanhaesebroeck and Alessi, 2000). Akt targets multiple proteins in the apoptotic machinery, including Bad, caspase-9, and transcription factors – including the Forkhead family and IKK, the NF- $\kappa$ B regulator (Datta *et al.*, 1997; Cardone *et al.*, 1998; Biggs *et al.*, 1999; Ozes *et al.*, 1999).

A role for the heat-shock protein Hsp90 in the Akt pathway was suggested by studies using an Hsp90 inhibitor that promoted apoptosis in HEK293T as measured by caspase activation and PARP cleavage and resulted in suppressed Akt activity levels (Basso *et al.*, 2002). A direct interaction between Hsp90 and Akt was recently reported (Sato *et al.*, 2000; Basso *et al.*, 2002). When this interaction was prevented by Hsp90 inhibitors, Akt was dephosphorylated and destabilized and the likelihood of apoptosis increased (Sato *et al.*, 2000). Additional studies showed that another chaperone participates in the Akt–Hsp90 complex, namely Cdc37 (Basso *et al.*, 2002). Together, this complex protects Akt from proteasome-mediated degradation.

Hsp70 has been shown to bind to nonphosphorylated protein kinase C (PKC) via the kinase's unphosphorylated carboxyl-terminus, priming the kinase for rephosphorylation and stabilizing the protein. In a similar manner, Hsp70 binds Akt, resulting in its stabilization (Gao and Newton, 2002). Hsp27 also increases Akt activity by an unknown mechanism (Konishi *et al.*, 1997).

Stress signals are well-known targets for Hsp70. Insults such as heat shock, UV irradiation, and osmotic stress activate the c-Jun N-terminal kinase (JNK) pathway and induce phosphorylation of c-Jun following transcription through AP-1. Hsp70 inhibits stress signal-induced cell death by suppressing JNK activation (Mosser *et al.*, 2000). This suppression does not require the ATPase domain, suggesting that it is independent of chaperone activity. Recently, the JNK inhibitory effect of the inducible Hsp70 was reported to result from inhibition of JNK dephosphorylation (Meriin *et al.*, 1999).

While neither the N-terminal ATPase domain or C-terminal EEVD sequence (which is important for chaperone function) is needed for JNK inhibition, wild-type Hsp70 is necessary to suppress caspase-9 and -3 activity in stress signal-induced apoptosis. The chaperone-deficient mutant reduces only JNK activity, not apoptosis. Thus, in this system, JNK inhibition is insufficient for preventing apoptosis, rather Hsp70 prevents apoptosis through its chaperone activity (Mosser *et al.*, 2000).

TNF-family receptors are involved in the 'extrinsic' pathway for apoptosis. Upon ligation of TNF or related death receptors, a complex protein known as the death-inducing signaling complex (DISC) forms at the cytosolic C-terminus of the receptor. This complex includes adaptor proteins such as TRADD and FADD that recruit procaspase-8 to the DISC. Upon recruitment, caspase-8 becomes activated and in turn processes other downstream caspases. Recently, the Bcl-2-family protein Bid has been recognized as a mediator of the TNF receptor pathway. Bid is cleaved by caspase-8 and the N-terminal-truncated protein translocates to the mitochondria to participate in apoptosis by facilitating Cyt *c* release (Li *et al.*, 1998; Luo *et al.*, 1998; Gross *et al.*, 1999). Thus, Bid has an important role in connections between extrinsic and intrinsic pathways of apoptosis.

Hsp70 is able to inhibit TNF-induced cell death, either in its wild-type or C-terminal EEVD-deleted form (Mosser *et al.*, 2000). However, in Bid homozygous-deleted MEF cells (Bid  $^{-/-}$ ), neither EEVD-deleted nor wild-type Hsp70 afforded protection from TNF-induced cell death (Mosser *et al.*, 2000). Thus, Hsp70 appears to participate in a Bid-dependent cell death pathway. Therefore, in Bid-deficient cells, Hsp70 plays critical roles in TNF-mediated apoptosis. Hsp70 appears to affect the Bid-dependent apoptotic pathway negatively by inhibiting JNK activation by a mechanism that is not fully clear (Gabai *et al.*, 2000).

In hematopoietic cells, Hsp70 is able to protect against TNF-induced apoptosis. The exposure of these cells to TNF induces the activity of the proapoptotic

double-stranded RNA-dependent protein kinase (PKR). Recently, Fanconi anemia complementation group C gene product (FANCC) protein has been reported as an inhibitor of PKR. Hsp70 interacts with the FANCC protein via its ATPase domain and together with Hsp40, inhibits the induction of apoptosis through the ternary complex of Hsp70, FANCC, and PKR (Pang *et al.*, 2001, 2002).

Hsp90 also has a role in modulating TNF-receptor signaling. Upon ligation of TNFR-1, the receptor interacting protein (RIP) is recruited to the receptor and promotes the activation of NF- $\kappa$ B and JNK. Hsp90 interacts with RIP, resulting in its stabilization (Lewis *et al.*, 2000). If this interaction is disrupted, RIP is rapidly degraded and causes decreased TNF-induced NF- $\kappa$ B activity and increased vulnerability to cell (Lewis *et al.*, 2000). Another route by which Hsp90 affects TNF-induced NF- $\kappa$ B activity is via the IKK complex. This complex is composed of two catalytic and one regulatory subunit, and recently it was determined that Cdc37 and Hsp90 are also present, with the association mediated through the kinase domain of the catalytic subunits (Chen *et al.*, 2002). The antitumor agent and Hsp90 inhibitor, geldanamycin, abolishes this complex and prevents TNF-induced activation of IKK and NF- $\kappa$ B, highlighting the role of Cdc37/Hsp90 in NF- $\kappa$ B activation following TNF exposure (Chen *et al.*, 2002).

TNF induces necrosis in some cells lines. In a fibrosarcoma line, Hsp90 seems to control whether cells undergo necrotic or apoptotic cell death. When the Hsp90 function is inhibited by geldanamycin, TNF-R1 induces apoptosis rather than necrotic death, probably as a result of destabilization of intracellular proteins that interact with the TNF receptor (Vanden Berghe *et al.*, 2003).

Hsp27 also increases resistance to TNF-induced cell death through a mechanism that remains unclear. In the case of the TNF-family receptor Fas, upon ligation, the adaptor protein Daxx is reportedly recruited to the Fas receptor C-terminus, which is followed by eventual JNK activation (Mehlen *et al.*, 1996b). Hsp27 may interact with Daxx to prevent its translocation from the nucleus to the cytosol. However, these events are unique to Daxx (Charette *et al.*, 2000).

Oxidative stress-induced apoptosis is another stress signal that is regulated by Hsp27 in a manner dependent upon the oligomerization state of the protein. The inhibition of Hsp27 phosphorylation discourages the formation of high molecular weight complexes of Hsp27 and in turn decreases its protection against oxidative stress-induced cell death.

Gene ablation studies have shown that Hsp70 plays important roles in apoptosis. Recently, a mouse line lacking Hsp70.1 was generated. In these mice, TNF-induced apoptosis is not prevented when the mice are heat stressed (Van Molle *et al.*, 2002). The testis-specific isoform of Hsp70 (Hsp70.2) has also been ablated, resulting in germ cell apoptosis. Germ cells show G2M arrest and death in late pachytene cells, indicating an important role for Hsp70.2 in meiosis regulation (Dix *et al.*, 1996).

## Role of heat-shock proteins in ischemic and degenerative disorders

During focal ischemia, the cells surrounding the core infarct rapidly upregulate Hsp27, 70, and 90 mRNA levels, implying that increases in heat-shock proteins represent a stress response to ischemia-reperfusion injury (Iwaki *et al.*, 1993; Kawagoe *et al.*, 1993). The overexpression of Hsp70 in the neurons of transgenic mice or mice injected with *Hsp70*-expressing viral vectors results in cytoprotection in several different models of nervous system injury, including ischemia. In myocardial infarction models, heart-specific transgenic mice or *in vivo* gene transfer of *Hsp70*-expressing vectors have increased resistance to cell death and better functional recovery (Radford *et al.*, 1996; Suzuki *et al.*, 1997; Okubo *et al.*, 2001).

In muscle cell death, caspase-mediated cleavage of the intermediate filament desmin is associated with muscle cell death (Chen *et al.*, 2003). An important role for caspase-8 and caspase-8 antagonists in heart development has also been revealed through gene knockout studies in mice (Varfolomeev *et al.*, 1998; Yeh *et al.*, 2000), providing additional evidence for a link between caspases and muscle physiology. Overall, however, the mechanisms regulating caspase activation and apoptosis in muscle are largely unknown.

Differentiation-induced apoptosis of myogenic cells is regulated by the small heat-shock protein  $\alpha$ B-crystallin, which is closely related to Hsp27.  $\alpha$ B-crystallin inhibits the proteolytic activation of caspase-3 during myoblast differentiation (Kamradt *et al.*, 2002). This protective effect is blocked when  $\alpha$ B-crystallin carries a phosphorylation-deficient point mutation at Arg120, which is the same mutation responsible for Desmin-like myopathy (myofibrillar myopathy) (Kamradt *et al.*, 2002). The overexpression of Arg120Gly  $\alpha$ B-crystallin causes aberrant desmin function and aggregation of  $\alpha$ B-crystallin with early death and myopathy (Wang *et al.*, 2001). In muscle atrophy, phosphorylated small heat-shock proteins are recruited to aggreosomes for quality control of proteins, suggesting a role for Hsp27 in proteasome regulation (Kato *et al.*, 2002). Recently, the ubiquitin protein ligase (MAFbx/Atrogin-1) and ring finger protein (MuRF1) have been cloned as inducible genes of muscular atrophy (Bodine *et al.*, 2001; Gomes *et al.*, 2001). It would be interesting to explore the possible functional interactions between small heat-shock proteins and atrophy-specific proteasome machinery.

Neurodegenerative disorders, such as Parkinson's and Huntington's disease, are caused by the deposition of misfolded proteins, which in turn cause neuronal cell death. Hsp27 is capable of protecting motor neurons from apoptosis following mechanical injury (Benn *et al.*, 2002). This protective mechanism requires the proper phosphorylation state of Hsp27 and appears to act somewhere between Cyt *c* release and caspase-3 activation. The phosphorylation requirement may be important for Hsp27 dissociation to the oligomeric form that would then be competent to interact with newly released Cyt *c* (Bruey *et al.*, 2000a; Benn *et al.*, 2002).

Hsp70 has been shown as an anticell death factor in neurodegenerative diseases. Using *Drosophila* as a model system, Hsp70 was identified as having a major role in preventing cell death and protein aggregation in polyglutamine and Parkinson's model systems. Hsp70 immunostaining was localized to the protein aggregates and overexpression suppressed the degeneration observed normally (reviewed in Bonini, 2002). The ATPase activity of Hsp70 was required for this reversal, implicating its chaperone activity in this protective effect. Hsp70 overexpression may afford this improvement because it replenishes the supply of chaperones that may be depleted by the excess proteins comprising the aggregates (Warrick *et al.*, 1999; Kazemi-Esfarjani and Benzer, 2000; Auluck *et al.*, 2002). Hsp70 association with polyglutamine proteins may suppress aggregation *in vivo*, as supported by studies using fluorescently labeled Hsp70 proteins that were found to be transiently rather than permanently associated with protein aggregates (Kim *et al.*, 2002).

Autosomal recessive juvenile Parkinsonism results from mutations in the *parkin* gene. The Parkin protein has a ubiquitin-like domain at its N-terminus, and two ring finger motifs plus an IBR (in between ring fingers) at its C-terminus. Parkin has an E3 protein ligase activity that suggests its role in the ubiquitination pathway for misfolded proteins arising from the endoplasmic reticulum (ER) (Imai *et al.*, 2002). Parkin recognizes unfolded Pael-R, which resides in the ER membrane and causes ER stress-induced cell death as a ubiquitin ligase target (Imai *et al.*, 2001). Parkin cooperates with Hsp70 and the cochaperone CHIP to inhibit Pael-R-induced neurodegenerative cell death through the ubiquitin proteasome protein degradation pathway (Imai *et al.*, 2002).

The above examples provide evidence for the importance of heat-shock and cochaperone proteins in governing ischemia-reperfusion injury and degenerative disorders in brain and muscular tissues.

### Apoptosis regulation by cochaperones

How do heat-shock proteins serve such diverse functions in apoptosis regulation? It may be that the cochaperones determine the multifunctional properties of each heat-shock protein. Heat-shock proteins may select different partners depending on guidance from various cochaperones. These cochaperone partners include three different protein families: DnaJ/Hsp40, tetratricopeptide repeat (TPR), and BAG-family proteins.

#### *DnaJ* family

The Hsp40 homolog, DnaJ, contains a ~70 amino-acid J-domain at its N-terminus, which binds to the bacterial homolog of Hsp70, DnaK. DnaJ also has three other domains: Gly-Phe-rich, Cys-rich Zn-finger, and C-terminal (for review, see Pfanner, 1999; Zhang *et al.*, 2002). J-domain-containing proteins can be grouped into two subfamilies: the DnaJ/Hsp40 family and the

J-domain-only family. The subfamily proteins that carry only the J-domain may have acquired this domain as a means of attracting Hsp70 molecules. This may then afford the specialization necessary for Hsp70 to play wide-ranging roles in cell signaling and apoptosis (Kelley, 1998). These J-domain-only proteins stimulate Hsp70 ATPase activity and target its chaperone activity in the context of cell-cycle regulation, protein translocation, exocytosis, tumor cell growth, DNA replication, apoptosis, etc.

The DnaJ-family proteins and Hsp70 are key regulators of apoptosome formation as described in the previous section. The mitochondrial matrix protein Tid1 – a homolog of a *Drosophila* tumor suppressor gene – has two isoforms, long and short, which are pro- and antiapoptotic, respectively. Tid1 is a DnaJ-family protein and interacts with mitochondrial Hsp70 (Syken *et al.*, 1999). Tid1 regulates apoptosis induced by DNA-damaging agents in a J-domain-dependent manner. The short form lacks the J-domain and is thus protective, while the J-domain carrying the long form promotes apoptosis (Syken *et al.*, 1999). MDG/Erdj4 is a DnaJ protein family member that is expressed in response to ER stress. This protein may act to stabilize ER chaperone GRP78/BiP binding to unfolded proteins in a J-domain-dependent manner, which prevents unfolded proteins from accumulating in the ER, which in turn prevents ER stress (Kurisu *et al.*, 2003).

The J-domain is well conserved throughout evolution. The SV40 large T antigen has a J-domain at its N-terminus and associates with Hsp70, implicating it in the regulation of tumorigenesis, cell cycle, and transformation. The SV40 large T antigen also associates with p53 and pRB, suggesting that it may act as a bridge between those proteins and the cell death pathway (Gjoerup *et al.*, 2000).

#### *TPR* family

The TPR is known as a protein-interacting motif that binds to Hsp70 and Hsp90. TPR domains are found in various proteins and regulate the cell cycle, protein folding, transcription, protein transport, ubiquitin-proteasomes, hormone receptor signaling, and several other pathways.

CHIP was originally cloned as a protein that interacted with Hsc70 and carries both a TPR motif and U-box (Ballinger *et al.*, 1999). CHIP regulates cell death in some types of Parkinson's disease as described above.

TPR2 was identified as a protein that interacts with Rad9, a cell-cycle checkpoint protein that may also control apoptosis by interacting with the antiapoptotic Bcl-2 protein (Xiang *et al.*, 2001). TPR2 carries both a J-domain and TPR motif, the latter being responsible for interacting with Rad9, which suggests that TPR2 may have a role in regulating either cell-cycle control and/or apoptosis. The activity of transcription factor p53 is regulated by p300/CBP coactivator complex, which includes the TPR protein Strap (Demonacos *et al.*,

2001). Strap increases the stability of p53 and in turn regulates apoptosis by modulating the activity of MDM2, a protein that impairs p53 transcription activation by impeding its interaction with transcription machinery and targeting p53 for ubiquitination (Oliner *et al.*, 1993).

### BAG family

BAG-family proteins contain a conserved ~80 amino-acid domain near their carboxyl-termini, which binds the ATPase domain of Hsp70 and Hsc70 with high affinity (Takayama *et al.*, 1997, 1999). The N-terminal domains in these proteins are divergent and target Hsp70/Hsc70 to different proteins and to different locations within cells. BAG-family genes are evolutionarily conserved, with homologs identified in *C. elegans*, yeast, and other organisms. To date, six members of the BAG family have been identified in humans. Four of them have been reported as anti-apoptotic proteins.

The BAG1 protein was originally identified as a novel regulator of apoptosis by virtue of its ability to bind Bcl-2 (Takayama *et al.*, 1995). Later, BAG1 was shown to interact with Hsp70 via its ATPase domain *in vitro*. BAG1 could inhibit Hsp70-mediated refolding of an unfolded substrate, possibly by preventing release of the substrate from the Hsp70 complex (Takayama *et al.*, 1997; Bimston *et al.*, 1998).

BAG1 collaborates with Bcl-2 in suppressing apoptosis induced by Fas, kinase inhibitors, withdrawal of growth factors, and chemotherapeutic drugs (Takayama *et al.*, 1995; Wang *et al.*, 1996; Clevenger *et al.*, 1997; Danen-van Oorschot *et al.*, 1997; Terada *et al.*, 1997). The overexpression of BAG1 alone inhibits or delays cell death caused by growth factor deprivation, heat shock, and p53. Recently, BAG1 was shown to be an important inhibitor of brain ischemic injury, as transgenic mice overexpressing *bag1* in the brain display increased resistance against ischemia reperfusion injury of hippocampal neurons (Kermer *et al.*, 2003). BAG1 in combination with Bcl-2 also suppresses photoreceptor cell death in the retina of mice expressing a cytotoxic mutant of rhodopsin (Eversole-Cire *et al.*, 2000).

BAG3 is reported to be a Bcl-2-binding protein and is also called Bis, for 'Bcl-2 interacting death suppressor' (Lee *et al.*, 1999). In gene transfer experiments where BAG3 was overexpressed, BAG3/Bis displayed little or no antiapoptotic activity, but could synergize with overexpressed Bcl-2 to prevent Bax-induced and Fas-mediated apoptosis (Lee *et al.*, 1999). Thus, it is possible that BAG3 can modulate the function of Bcl-2, at least when overexpressed. Recently, it was reported that the upregulation of *bag3* gene expression is observed in cerebral infarction (Lee *et al.*, 2002). BAG3 also binds PLC- by PXXP motif located next to the BAG domain of BAG3 (Doong *et al.*, 2000). BAG3 mRNA levels increase under various stress conditions, including heat and heavy metal exposure (Liao *et al.*, 2001; Pagliuca *et al.*, 2003). BAG3 protein has a diffuse cytosolic distribution, but upon heavy metal treatment, the

protein translocates to the ER. Thus, BAG3 may regulate stress-induced cell death through various interacting proteins either in the cytosol, ER, or other cellular compartments. B-CLL cells express high levels of BAG3. Reduction of BAG3 by antisense results in the increase of apoptotic sensitivity to chemotherapeutic drugs (Romano *et al.*, 2003).

BAG4 (also known as Sodd, silencer of death domain) contains a unique N-terminal domain that reportedly allows it to associate with the death domains of TNFR1 and DR3 (Jiang *et al.*, 1999). BAG4 is speculated to recruit Hsp70/Hsc70 to unliganded TNF-family death receptors, preventing them from clustering and signaling in the absence of a ligand (Jiang *et al.*, 1999). BAG4 binds to TNF-R1 by an ATP-dependent mechanism (Miki and Eddy, 2002). Recent reports postulate a possible ATPase function of TNFR1, since BAG4 binds both Hsp70 and TNFR1 by its BAG domain. This ATPase activity may promote a conformational change in the receptor that could regulate TNF signaling (Miki and Eddy, 2002).

BAG4 was also recently shown to bind to Bcl-2 (Antoku *et al.*, 2001). A BH3-like sequence was identified in the BAG domain, which may be important for its interaction with Bcl-2, given that BH3 domains are involved in many interactions between Bcl-2 family protein members (for review see Chittenden, 2002). BH3-like domains are common to BAG1, BAG3, and BAG4 proteins, suggesting that these proteins may all interact with Bcl-2-family proteins.

The *Drosophila* protein Scythe was first identified through its binding to the potent apoptotic inducing protein Reaper. Scythe possesses a BAG domain and inhibits the refolding activity of Hsp70. Therefore, we have termed this protein BAG6. BAG6 (Scythe) purportedly sequesters an AIP, which it releases upon binding to Reaper, resulting in an exodus of this unidentified factor into the cytosol, where it induces the release of Cyt *c* from the mitochondria (Thress *et al.*, 2001).

### Conclusions

Heat-shock proteins have been reported to regulate apoptosis and cell death. Heat-shock proteins can inhibit or aid the apoptotic machinery through their chaperone functions by affecting protein assembly and folding, ubiquitin degradation pathways, and protein translocation. Chaperone-independent regulation of apoptosis by heat-shock proteins is also reported in the case of the JNK and AIF proteins. In some cases, the multifunctional properties of heat-shock proteins can be explained by different interactions with cochaperones. Research on cochaperone function as well as on heat-shock proteins themselves in regulating apoptosis may yield more precise information on mechanisms of apoptosis and cell death regulation.

## References

- Antoku K, Maser RS, Scully Jr WJ, Delach SM and Johnson DE. (2001). *Biochem. Biophys. Res. Commun.*, **286**, 1003–1010.
- Auluck PK, Chan HY, Trojanowski JQ, Lee VM and Bonini NM. (2002). *Science*, **295**, 858–865.
- Ballinger CA, Connell P, Wu Y, Hu Z, Thompson LJ, Yin LY and Patterson C. (1999). *Mol. Cell. Biol.*, **19**, 4535–4545.
- Basso AD, Solit DB, Chiosis G, Giri B, Tschlis P and Rosen N. (2002). *J. Biol. Chem.*, **277**, 39858–39866.
- Beere HM, Wolf BB, Cain K, Mosser DD, Mahboubi A, Kuwana T, Taylor P, Morimoto RI, Cohen GM and Green DR. (2000). *Nat. Cell. Biol.*, **2**, 469–475.
- Benn SC, Perrelet D, Kato AC, Scholz J, Decosterd I, Mannion RJ, Bakowska JC and Woolf CJ. (2002). *Neuron*, **36**, 45–56.
- Biggs III WH, Meisenhelder J, Hunter T, Cavenee WK and Arden KC. (1999). *Proc. Natl. Acad. Sci. USA*, **96**, 7421–7426.
- Bimston D, Song J, Winchester D, Takayama S, Reed JC and Morimoto RI. (1998). *EMBO J.*, **17**, 6871–6878.
- Bodine SC, Latres E, Baumhueter S, Lai VK, Nunez L, Clarke BA, Poueymirou WT, Panaro FJ, Na E, Dharmarajan K, Pan ZQ, Valenzuela DM, DeChiara TM, Stitt TN, Yancopoulos GD and Glass DJ. (2001). *Science*, **294**, 1704–1708.
- Bonini NM. (2002). *Proc. Natl. Acad. Sci. USA*, **99**, 16407–16411.
- Bruey JM, Ducasse C, Bonniaud P, Ravagnan L, Susin SA, Diaz-Latoud C, Gurbuxani S, Arrigo AP, Kroemer G, Solary E and Garrido C. (2000a). *Nat. Cell. Biol.*, **2**, 645–652.
- Bruey JM, Paul C, Fromentin A, Hilpert S, Arrigo AP, Solary E and Garrido C. (2000b). *Oncogene*, **19**, 4855–4863.
- Cardone MH, Roy N, Stennicke HR, Salvesen GS, Franke TF, Stanbridge E, Frisch S and Reed JC. (1998). *Science*, **282**, 1318–1321.
- Charette SJ, Lavoie JN, Lambert H and Landry J. (2000). *Mol. Cell. Biol.*, **20**, 7602–7612.
- Chen G, Cao P and Goeddel DV. (2002). *Mol. Cell.*, **9**, 401–410.
- Chen F, Chang R, Trivedi M, Capetanaki Y and Cryns VL. (2003). *J. Biol. Chem.*, **278**, 6848–6853.
- Chittenden T. (2002). *Cancer Cell.*, **2**, 165–166.
- Clevenger CV, Thickman K, Ngo W, Chang WP, Takayama S and Reed JC. (1997). *Mol. Endocrinol.*, **11**, 608–618.
- Danen-van Oorschot AAAM, den Hollander A, Takayama S, Reed JC, van der Eb AJ and Noteborn MHM. (1997). *Apoptosis*, **2**, 395–402.
- Datta SR, Brunet A and Greenberg ME. (1999). *Genes Dev.*, **13**, 2905–2927.
- Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y and Greenberg ME. (1997). *Cell*, **91**, 231–241.
- Demonacos C, Krstic-Demonacos M and La Thangue NB. (2001). *Mol. Cell.*, **8**, 71–84.
- Dix DJ, Allen JW, Collins BW, Mori C, Nakamura N, Poorman-Allen P, Goulding EH and Eddy EM. (1996). *Proc. Natl. Acad. Sci. USA*, **93**, 33264–33268.
- Doong H, Price J, Kim YS, Gasbarre C, Probst J, Liotta LA, Blanchette J, Rizzo K and Kohn E. (2000). *Oncogene*, **19**, 4385–4395.
- Eversole-Cire P, Concepcion F.A, Simon M.I, Takayama S, Reed JC and Chen J. (2000). *Investigative Ophthalmology and Visual Science*, **41**, 1953–1961.
- Gabai VL, Yaglom JA, Volloch V, Meriin AB, Force T, Koutroumanis M, Massie B, Mosser DD and Sherman MY. (2000). *Mol. Cell. Biol.*, **20**, 6826–6836.
- Gao T and Newton AC. (2002). *J. Biol. Chem.*, **277**, 31585–31592.
- Garrido C, Bruey JM, Fromentin A, Hammann A, Arrigo AP and Solary E. (1999). *FASEB J.*, **13**, 2061–2070.
- Gjoerup O, Chao H, DeCaprio JA and Roberts TM. (2000). *J. Virol.*, **74**, 864–874.
- Gomes MD, Lecker SH, Jagoe RT, Navon A and Goldberg AL. (2001). *Proc. Natl. Acad. Sci. USA*, **98**, 14440–14445.
- Gross A, Yin XM, Wang K, Wei MC, Jockel J, Milliman C, Erdjument-Bromage H, Tempst P and Korsmeyer SJ. (1999). *J. Biol. Chem.*, **274**, 1156–1163.
- Gupta S and Knowlton AA. (2002). *Circulation*, **106**, 2727–2733.
- Imai Y, Soda M, Hatakeyama S, Akagi T, Hashikawa T, Nakayama KI and Takahashi R. (2002). *Mol. Cell.*, **10**, 55–67.
- Imai Y, Soda M, Inoue H, Hattori N, Mizuno Y and Takahashi R. (2001). *Cell*, **105**, 891–902.
- Iwaki K, Chi SH, Dillmann WH and Mestril R. (1993). *Circulation*, **87**, 2023–2032.
- Jaattela M, Wissing D, Kokholm K, Kallunki T and Egeblad M. (1998). *EMBO J.*, **17**, 6124–6134.
- Jiang J, Ballinger CA, Wu Y, Dai Q, Cyr DM, Hohfeld J and Patterson C. (2001). *J. Biol. Chem.*, **276**, 42938–42944.
- Jiang Y, Woronicz JD, Liu W and Goeddel DV. (1999). *Science*, **283**, 543–546.
- Kamradt MC, Chen F, Sam S and Cryns VL. (2002). *J. Biol. Chem.*, **277**, 38731–38876.
- Kato K, Ito H, Kamei K, Iwamoto I and Inaguma Y. (2002). *FASEB J.*, **16**, 1432–1434.
- Kawagoe J, Abe K, Aoki M and Kogure K. (1993). *Brain Res.*, **621**, 121–125.
- Kazemi-Esfarjani P and Benzer S. (2000). *Science*, **287**, 1837–1840.
- Kelley WL. (1998). *Trends Biochem. Sci.*, **23**, 222–227.
- Kermer P, Digicaylioglu MH, Kaul M, Zapata JM, Krajewska M, Stenner-Liewen F, Takayama S, Krajewski S, Lipton S.A and Reed JC. (2003). *Brain Pathology*, in press.
- Kim S, Nollen EA, Kitagawa K, Bindokas VP and Morimoto RI. (2002). *Nat. Cell. Biol.*, **4**, 826–831.
- Konishi H, Matsuzaki H, Tanaka M, Takemura Y, Kuroda S, Ono Y and Kikkawa U. (1997). *FEBS Lett.*, **410**, 493–498.
- Kurusu J, Honma A, Miyajima H, Kondo S, Okumura M and Imaizumi K. (2003). *Genes Cells*, **8**, 189–202.
- Lambert H, Charette SJ, Bernier AF, Guimond A and Landry J. (1999). *J. Biol. Chem.*, **274**, 9378–9385.
- Lee MY, Kim SY, Shin SL, Choi YS, Lee JH and Tsujimoto Y. (2002). *Exp. Neurol.*, **175**, 338–346.
- Lee JH, Takahashi T, Yasuhara N, Inazawa J, Kamada S and Tsujimoto Y. (1999). *Oncogene*, **18**, 6183–6190.
- Lewis J, Devin A, Miller A, Lin Y, Rodriguez Y, Neckers L and Liu ZG. (2000). *J. Biol. Chem.*, **275**, 10519–10526.
- Li H, Zhu H, Xu CJ and Yuan J. (1998). *Cell*, **94**, 491–501.
- Liao Q, Ozawa F, Friess H, Zimmermann A, Takayama S, Reed JC, Kleeff J and Buchler MW. (2001). *FEBS Lett.*, **503**, 151–157.
- Luo X, Budihardjo I, Zou H, Slaughter C and Wang X. (1998). *Cell*, **94**, 481–490.
- Mehlen P, Kretz-Remy C, Preville X and Arrigo AP. (1996a). *EMBO J.*, **15**, 2695–2706.
- Mehlen P, Schulze-Osthoff K and Arrigo AP. (1996b). *J. Biol. Chem.*, **271**, 16510–16514.
- Meriin AB, Yaglom JA, Gabai VL, Zon L, Ganiatsas S, Mosser DD and Sherman MY. (1999). *Mol. Cell. Biol.*, **19**, 2547–2555.

- Miki K and Eddy EM. (2002). *Mol. Cell. Biol.*, **22**, 2536–2543.
- Mosser DD, Caron AW, Bourget L, Meriin AB, Sherman MY, Morimoto RI and Massie B. (2000). *Mol. Cell. Biol.*, **20**, 7146–7159.
- Okubo S, Wildner O, Shah MR, Chelliah JC, Hess ML and Kukreja RC. (2001). *Circulation*, **103**, 877–881.
- Oliner JD, Pietenpol JA, Thiagalingam S, Gyuris J, Kinzler KW and Vogelstein B. (1993). *Nature*, **362**, 857–860.
- Ozes ON, Mayo LD, Gustin JA, Pfeffer SR, Pfeffer LM and Donner DB. (1999). *Nature*, **401**, 82–85.
- Pagliuca MG, Lerose R, Cigliano S and Leone A. (2003). *FEBS Lett.*, **541**, 11–15.
- Pang Q, Christianson TA, Keeble W, Koretsky T and Bagby GC. (2002). *J. Biol. Chem.*, **277**, 49638–49643.
- Pang Q, Keeble W, Christianson TA, Faulkner GR and Bagby GC. (2001). *EMBO J.*, **20**, 4478–4489.
- Pfanner N. (1999). *Curr. Biol.*, **9**, R720–R724.
- Radford NB, Fina M, Benjamin IJ, Moreadith RW, Graves KH, Zhao P, Gavva S, Wiethoff A, Sherry AD, Malloy CR and Williams RS. (1996). *Proc. Natl. Acad. Sci. USA*, **93**, 2339–2342.
- Ravagnan L, Gurbuxani S, Susin SA, Maise C, Daugas E, Zamzami N, Mak T, Jaattela M, Penninger JM, Garrido C and Kroemer G. (2001). *Nat. Cell. Biol.*, **3**, 839–843.
- Rogalla T, Ehrnsperger M, Preville X, Kotlyarov A, Lutsch G, Ducasse C, Paul C, Wieske M, Arrigo AP, Buchner J and Gaestel M. (1999). *J. Biol. Chem.*, **274**, 18947–18956.
- Romano M, Festa M, Pagliuca G, Lerose R, Bisogni R, Chiurazzi F, Storti G, Volpe S, Venuta S, Turco M and Leone S. (2003). *Cell Death Differ.*, **10**, 383–385.
- Sakahira H and Nagata S. (2002). *J. Biol. Chem.*, **277**, 3364–3370.
- Sakamoto H, Mashima T, Yamamoto K and Tsuruo T. (2002). *J. Biol. Chem.*, **277**, 45770–45775.
- Saleh A, Srinivasula SM, Balkir L, Robbins PD and Alnemri ES. (2000). *Nat. Cell. Biol.*, **2**, 476–483.
- Samali A, Cai J, Zhivotovsky B, Jones DP and Orrenius S. (1999). *EMBO J.*, **18**, 2040–2048.
- Sato S, Fujita N and Tsuruo T. (2000). *Proc. Natl. Acad. Sci. USA*, **97**, 10832–10837.
- Suzuki K, Sawa Y, Kaneda Y, Ichikawa H, Shirakura R and Matsuda H. (1997). *J. Clin. Invest.*, **99**, 1645–1650.
- Syken J, De-Medina T and Munger K. (1999). *Proc. Natl. Acad. Sci. USA*, **96**, 8499–8504.
- Takayama S, Bimston DN, Matsuzawa S, Freeman BC, Aime-Sempe C, Xie Z, Morimoto RI and Reed JC. (1997). *EMBO J.*, **16**, 4887–4896.
- Takayama S, Sato T, Krajewski S, Kochel K, Irie S, Millan JA and Reed JC. (1995). *Cell*, **80**, 279–284.
- Takayama S, Xie Z and Reed J. (1999). *J. Biol. Chem.*, **274**, 781–786.
- Terada S, Fukuoka K, Fujita T, Komatsu T, Takayama S, Reed JC and Suzuki E. (1997). *Cytotechnology*, **25**, 17–23.
- Thress K, Song J, Morimoto RI and Kornbluth S. (2001). *EMBO J.*, **20**, 1033–1041.
- Van Molle W, Wielockx B, Mahieu T, Takada M, Taniguchi T, Sekikawa K and Libert C. (2002). *Immunity*, **16**, 685–695.
- Vanden Berghe T, Kalai M, Van Loo G, Declercq W and Vandenaabeele P. (2003). *J. Biol. Chem.*, **278**, 5622–5629.
- Vanhaesebroeck B and Alessi DR. (2000). *Biochem. J.*, **346**, 561–576.
- Varfolomeev EE, Schuchmann M, Luria V, Chiannilkulchai N, Beckmann JS, Mett IL, Rebrikov D, Brodianski VM, Kemper OC, Kollet O, Lapidot T, Soffer D, Sobe T, Avraham KB, Goncharov T, Holtmann H, Lonai P and Wallach D. (1998). *Immunity*, **9**, 267–276.
- Wang X, Osinska H, Klevitsky R, Gerdes AM, Nieman M, Lorenz J, Hewett T and Robbins J. (2001). *Circ. Res.*, **89**, 84–91.
- Wang HG, Takayama S, Rapp UR and Reed JC. (1996). *Proc. Natl. Acad. Sci. USA*, **93**, 7063–7068.
- Warrick JM, Chan HY, Gray-Board GL, Chai Y, Paulson HL and Bonini NM. (1999). *Nat. Genet.*, **23**, 425–428.
- Xanthoudakis S, Roy S, Rasper D, Hennessey T, Aubin Y, Cassady R, Tawa P, Ruel R, Rosen A and Nicholson DW. (1999). *EMBO J.*, **18**, 2049–2056.
- Xiang SL, Kumano T, Iwasaki SI, Sun X, Yoshioka K and Yamamoto KC. (2001). *Biochem. Biophys. Res. Commun.*, **287**, 932–940.
- Yeh WC, Itie A, Elia AJ, Ng M, Shu HB, Wakeham A, Mirtsos C, Suzuki N, Bonnard M, Goeddel DV and Mak TW. (2000). *Immunity*, **12**, 633–642.
- Zhang X, Beuron F and Freemont PS. (2002). *Curr. Opin. Struct. Biol.*, **12**, 231–238.