Heat shock genes – integrating cell survival and death

Richa Arya, Moushami Mallik and Subhash C Lakhotia*
Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221005, India
Corresponding author (Fax, 91 542 2368457; Email, lakhotia@bhu.ac.in)

Heat shock induced gene expression and other cellular responses help limit the damage caused by stress and thus facilitate cellular recovery. Cellular damage also triggers apoptotic cell death through several pathways. This paper briefly reviews interactions of the major heat shock proteins with components of the apoptotic pathways. Hsp90, which acts as a chaperone for unstable signal transducers to keep them poised for activation, interacts with RIP and Akt and promotes NF-κB mediated inhibition of apoptosis; in addition it also blocks some steps in the apoptotic pathways. Hsp70 is mostly anti-apoptotic and acts at several levels like inhibition of translocation of Bax into mitochondria, release of cytochrome c from mitochondria, formation of apoptosome and inhibition of activation of initiator caspases. Hsp70 also modulates JNK, NF-κB and Akt signaling pathways in the apoptotic cascade. In contrast, Hsp60 has both anti- and pro-apoptotic roles. Cytosolic Hsp60 prevents translocation of the pro-apoptotic protein Bax into mitochondria and thus promotes cell survival but it also promotes maturation of procaspase-3, essential for caspase mediated cell death. Our recent *in vivo* studies show that RNAi for the Hsp60D in *Drosophila melanogaster* prevents induced apoptosis. Hsp27 exerts its anti-apoptotic influence by inhibiting cytochrome c and TNF-mediated cell death. $\alpha\beta$ crystallin suppresses caspase-8 and cytochrome c mediated activation of caspase-3. Studies in our laboratory also reveal that absence or reduced levels of the developmentally active as well as stress induced non-coding hsrω transcripts, which are known to sequester diverse hnRNPs and related nuclear RNA-binding proteins, block induced apoptosis in *Drosophila.* Modulation of the apoptotic pathways by Hsps reflects their roles as "weak links" between various "hubs" in cellular networks. On the other hand, non-coding RNAs, by virtue of their potential to bind with multiple proteins, can act as "hubs" in these networks. In view of the integrative nature of living systems, it is not surprising that stress-induced genes, generally believed to primarily function in cell survival pathways, inhibit or even promote cell death pathways at multiple levels to ensure homeostasis at cell and/or organism level. The heat shock genes obviously do much more than merely help cells survive stress.

[Arya R, Mallik M and Lakhotia S C 2007 Heat shock genes – integrating cell survival and death; *J. Biosci*. **32** 595–610]

1. Introduction

As a consequence of being alive, cells of all organisms continuously suffer a variety of "damages" from internal as well as external physico-chemical and biotic factors. Therefore, living systems have evolved a variety of strategies to repair the damage and/or eliminate the damaged components. Heat shock or stress response is a cellular adaptive response, which helps maintain cellular homeostasis under stress. Among the many changes in cellular activity and physiology, the most remarkable event in stressed cells is the production of a highly conserved set of proteins, the Heat Shock or Stress Proteins (Hsps) (Schlesinger *et al* 1982) and certain non-coding RNAs, like the hsrω transcripts in *Drosophila* and the satellite III transcripts in humans (Lakhotia 2003; Jolly and Lakhotia 2006).

The Hsps are broadly classified, on the basis of their apparent molecular weights, amino acid sequences and functions (Nover 1984) into distinct families, viz., Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, small Hsps (sHsp) and Hsp10. Many members of these Hsp families are present

Keywords. Caspases; Hsp90; Hsp70; Hsp60; Hsp27; *hsr-omega*

constitutively (heat shock cognates) in cells while some are expressed only after stress. The induced and the constitutively expressed members of Hsp families are well known as molecular chaperones which i) help in normal folding of various polypeptides, ii) assist mis-folded proteins to attain or regain their native states, iii) regulate protein degradation and/or iv) help in translocation of proteins to different cellular compartments (reviewed in Hartl and Hayer-Hartl 2002).

The above functions imply that the Hsps interact with a very large variety of cellular proteins and thus are important components of cellular networks (Csermely 2004). This is also reflected by their roles, especially of Hsp90, in evolvability (Rutherford and Lindquist 1998). Another interesting example of the integrative roles of Hsps is their intervention in the apoptotic pathways. Apoptosis, one of the programmed cell death pathways, is a natural and essential developmental process, which eliminates redundant or superfluous cells to allow normal patterning (reviewed in Jacobson *et al* 1997). Stressed and damaged cells, if irreparable, also utilize this route to die. Many studies in recent years have shown that the heat shock proteins play critical roles in modulating the apoptotic cascades (Samali and Orrenius 1998; Garrido *et al* 2001; Sreedhar and Csermely 2004; Beere 2005; Kim *et al* 2006a; Didelot *et al* 2006). With a view to understand the wider roles of heat shock genes in cell regulatory pathways, in the following we briefly review how the four major classes of Hsps, viz., Hsp90, Hsp70, Hsp60 and small Hsps modulate apoptotic pathways. We also consider our own recent results relating to roles of one of the Hsp60 forms and the developmentally active and stress-inducible non-coding hsrω transcripts in apoptosis in *Drosophila*. Interlinking of the heat shock response, which primarily repairs the damage, and apoptosis, which eliminates the damaged cells, illustrates the highly integrated nature of the regulatory pathways in living systems.

2. Pathways of apoptosis

Apoptosis is a genetically regulated process of deliberate cell suicide in multicellular organisms. Characteristic features of apoptosis include nuclear condensation, DNA fragmentation, membrane blebbing and breaking of cytoplasm into apoptotic bodies that are removed by phagocytosis (Kerr *et al* 1972; Wyllie *et al* 1980). Multiple triggers (variety of stresses or developmental cues) provoke a cell to undergo apoptosis via one of the two major pathways, the intrinsic or mitochondrial death pathway and the extrinsic or receptor-mediated cell death pathway. Both of these pathways eventually activate effector caspases to execute cell death (Budihardjo *et al* 1999). A brief account of these pathways is given below (for more details, see Yan and Shi 2005). Figure 1 summarizes the major steps in the diverse apoptotic pathways and their links with cell-survival signals.

The *Intrinsic pathway* involves loss of mitochondrial membrane potential in response to death signals, leading to permeabilization of the outer membrane. This triggers release of pro-death molecules like cytochrome c and Smac/Diablo into the cytoplasm. Cytochrome c binds to the Apoptotic protease activating factor-1 (Apaf-1) helping in its oligomerization and recruitment of procaspase-9 to form a functional apoptosome. At the same time, Smac/Diablo inhibit the Inhibitor of Apoptosis Proteins (IAPs). The apoptosome complex proteolytically processes procaspase-9 to an active form, which ultimately leads to cell death by activating the effector caspase-3 (Budihardjo *et al* 1999; Zou *et al* 1999; Van Grup *et al* 2003; Yan and Shi 2005).

The *extrinsic pathway* (figure 1) transduces death signals through the binding of "extra-cellular death ligands" like TNF-α, Fas ligand [FasL]/Apo1L/CD95L, Trail/Apo2L, Apo3L to their respective cell surface receptors. A homotypic interaction takes place between the death domains (DDs) of Tumor Necrosis Factor Receptor-1 (TNFR-1) and Fas-receptors and their respective adaptor molecules, TRADD and FADD (Locksley *et al* 2001; Screaton and Xu 2000; Yan and Shi 2005). Eventually, a Death Inducing Signal Complex (DISC) is formed that activates procaspase-8, which in turn triggers caspase-3 mediated cell death events. Fas-induced apoptotic pathway can also recruit the adaptor protein DAXX instead of FADD, to activate ASK-1 or Apoptosis-Signal-Regulated Kinase-1 (Chang *et al* 1998), which activates SAPK/JNK and thereby, triggers apoptosis (Yang *et al* 1997).

The extrinsic death signals are linked to the intrinsic pathway through the Bcl-2 family of proteins (Gross *et al* 1999), which includes both pro- (e.g. Bax, Bad, Bak, Bid) and anti-apoptotic (e.g. Bcl-XL) members. It is the balance between the proteins of this family with opposing functions that actually decides the release of cytochrome c and Smac/Diablo from the mitochondria (Green and Reed 1998; Willis *et al* 2003).

Death-inducing signals are tightly coupled with survival signals (figure 1). One of the cascades that promote cell survival utilizes a serine/threonine kinase, Akt, which is activated through phosphoinositide 3 kinase, PI(3)K, by various growth factors (Staal 1987; Beere 2001; Paez and Sellers 2003). Phosphorylation stabilizes Akt (Stokoe *et al* 1997), which besides activating the Nuclear Factor-κB (NF-κB) (*see* figure 1), induces phosphorylation of Bad, which results in the latter's disassociation from Bcl-XL. Phosphorylated Bad is sequestered by the cytosolic 14-3-3 protein; this does not permit its translocation into mitochondria and consequently the downstream apoptotic events are not triggered (Zha *et al* 1996).

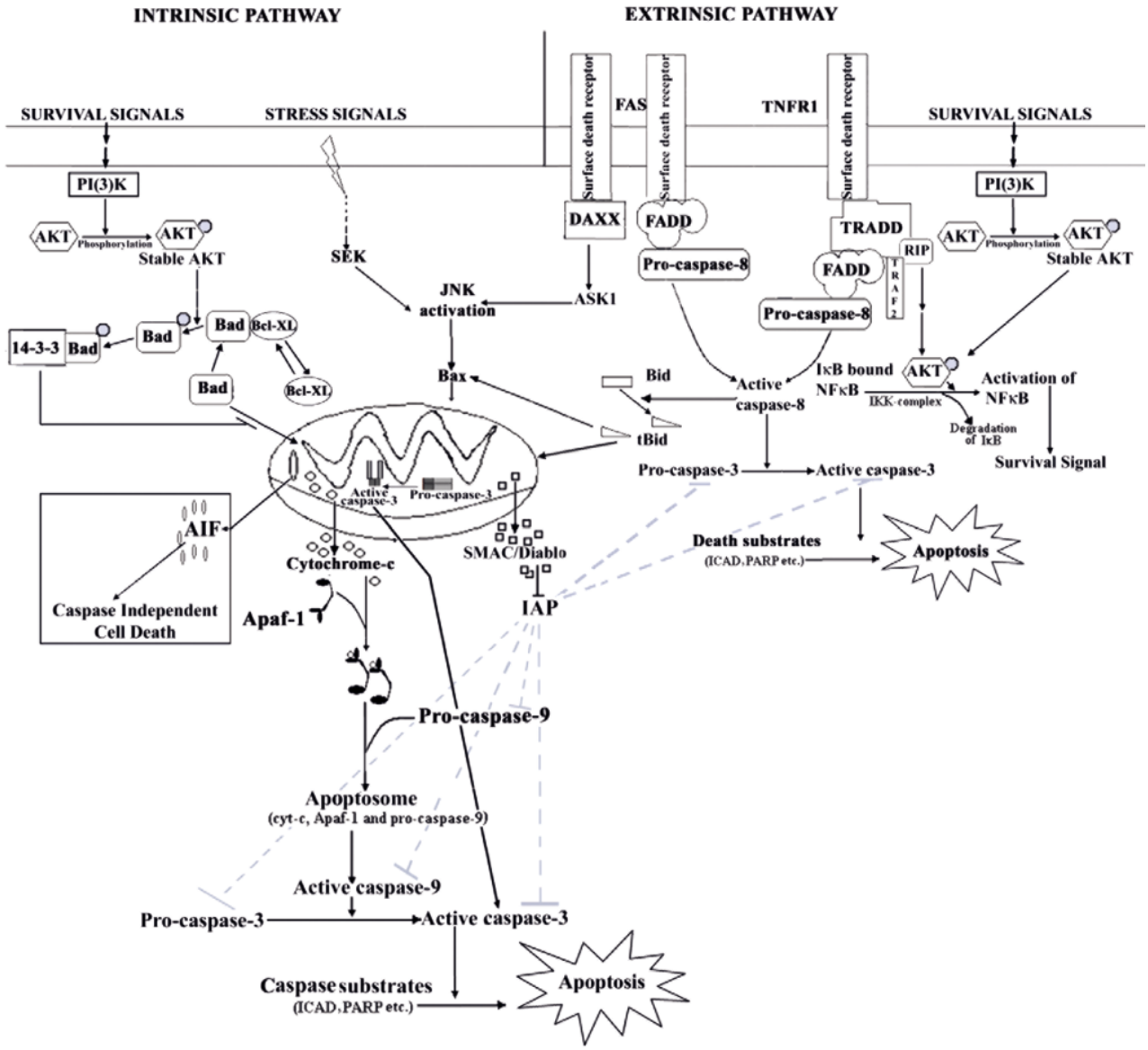

Figure 1. A schematic of intrinsic, extrinsic and AIF-mediated caspase independent pathways of apoptosis and their inter-connections with cell survival pathways. A small blue circle on a box indicates phosphorylated state of the named protein. Arrows indicate promotional while lines with small bar at the end indicate inhibitory actions. The inhibitory effects of IAPs (Inhibitor of Apoptosis Proteins) on pro-caspases and caspases are indicated by dashed lines. See text for details.

Though TNF is a potent inducer of cell death, it may also promote cell survival (figure 1) through NF-κB, an important link in various biological processes including stress response, cell growth or death (Ghosh 1999). Under normal conditions, NF-κB remains bound and sequestered in the cytosol by its inhibitor IκB (DiDonato *et al* 1997; Regnier *et al* 1997). However, upon exposure to stimuli, including TNF, IκB is degraded, resulting in release of NF-κB, which can translocate into nucleus and activate transcription of cell survival genes (Chen *et al* 1996; Wang *et al* 1998). Phosphorylation and thus, inactivation of IκB is mediated by a protein kinase complex, IκB kinase (IKK). TNF triggers activation of IKK through its association with signal transducing molecules like Receptor Interacting Protein, RIP and TRAF2 (Devin *et al* 2000; Zhang *et al* 2000). Activated Akt up-regulates kinase activity of the IKK complex leading to NF-κB mediated cell survival (Ozes *et al* 1999; Romashkova and Makarov 1999).

The core components of cell death pathways are highly conserved among *Caenorhabditis, Drosophila* and humans. *Drosophila* caspases, like those of vertebrates, are broadly grouped into initiator caspases (e.g., Dronc, Dredd and

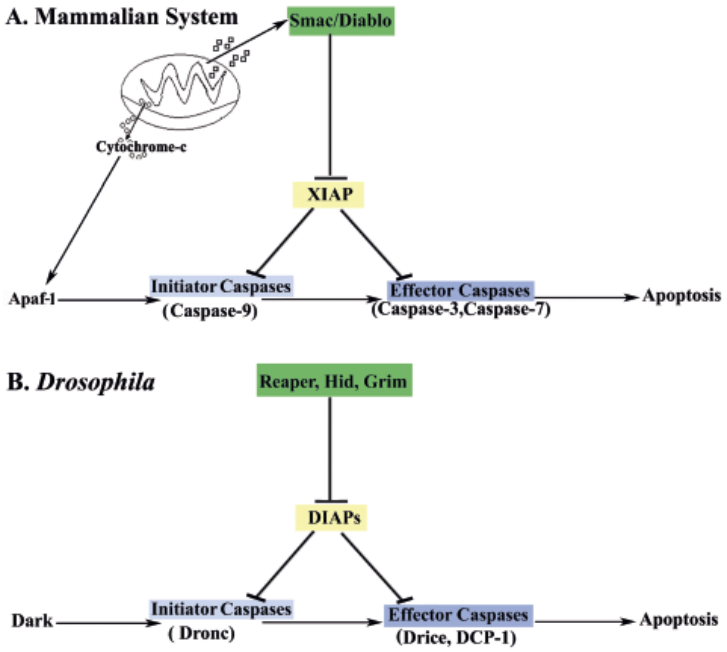

Figure 2. Comparison of the execution steps in mammalian **(A)** and *Drosophila* **(B)** apoptotic machinery. Similar colours indicate functional homology between proteins in mammals and *Drosophila*.

Strica) and effector caspases (e.g. Drice, DCP-1, Decay and Damm) (reviewed in Riedl and Shi 2004; Hay and Guo 2006). In *Drosophila* as well as in vertebrates (figure 2), both groups of caspases are negatively regulated by the IAPs (Deveraux and Reed 1999). Interestingly, in spite of the high conservation of the core cell death machinery, mammals and flies show a remarkable difference in the regulation and execution of apoptosis. In mammalian cells, caspase activation is the primary step of death control, whereas in flies, inactivation of IAPs by upstream pro-apoptotic proteins viz. Reaper, Hid and Grim (functional homologs of vertebrate Smac/Diablo) is the central event. In addition, release of cytochrome c from mitiochondria is required for caspase activation in the vertebrate system whereas role of cytochrome c in *Drosophila* cell death is debated (Kornbluth and White 2005; Hay and Guo 2006).

3. Modulation of apoptosis by different heat shock gene products

On the face of it, Hsps and apoptotic proteins serve two distinct and seemingly opposing functions, viz., survival and death of cells. However, since the cost of survival of a damaged cell or death of a potentially functional cell could be deleterious for the individual, as discussed in the following, the different heat shock proteins and members of the various apoptotic cascades indeed show multi-step networked interactions to fine tune a cell's survival or death.

3.1 *Hsp90*

Hsp90 displays a chaperoning function for unstable signal transducers to keep them poised for activation, although it is not required for their maturation or maintenance (Pratt 1998). In relation to apoptosis, Hsp90 mostly promotes cell survival through its involvement at different steps in the formation of active NF-κB (figure 3). Hsp90 is essential for the stability of RIP, which is recruited by activated TNFR-1 following binding with its ligand, TNF, for sustained NF-κB activity (Lewis *et al* 2000; Chen *et al* 2002). Hsp90 also directly interacts and maintains the activity of Akt by inhibiting its dephosphorylation (Sato *et al* 2000; Basso *et al* 2002). In addition, Hsp90 and its co-chaperone Cdc37 help in the formation of active IKK or Akt complexes, each of which can phosphorylate IκB and thereby cause disassociation of NF-κB from its inhibitor (Chen *et al* 2002).

Apart from its direct role in promoting cell survival pathways, the Hsp90-Akt complex can also indirectly promote cell survival by inhibition of JNK-mediated cell death through phosphorylation and conseuqent inactivation of ASK-1 (figure 3), which is one of the activators of JNK (Zhang *et al* 2005).

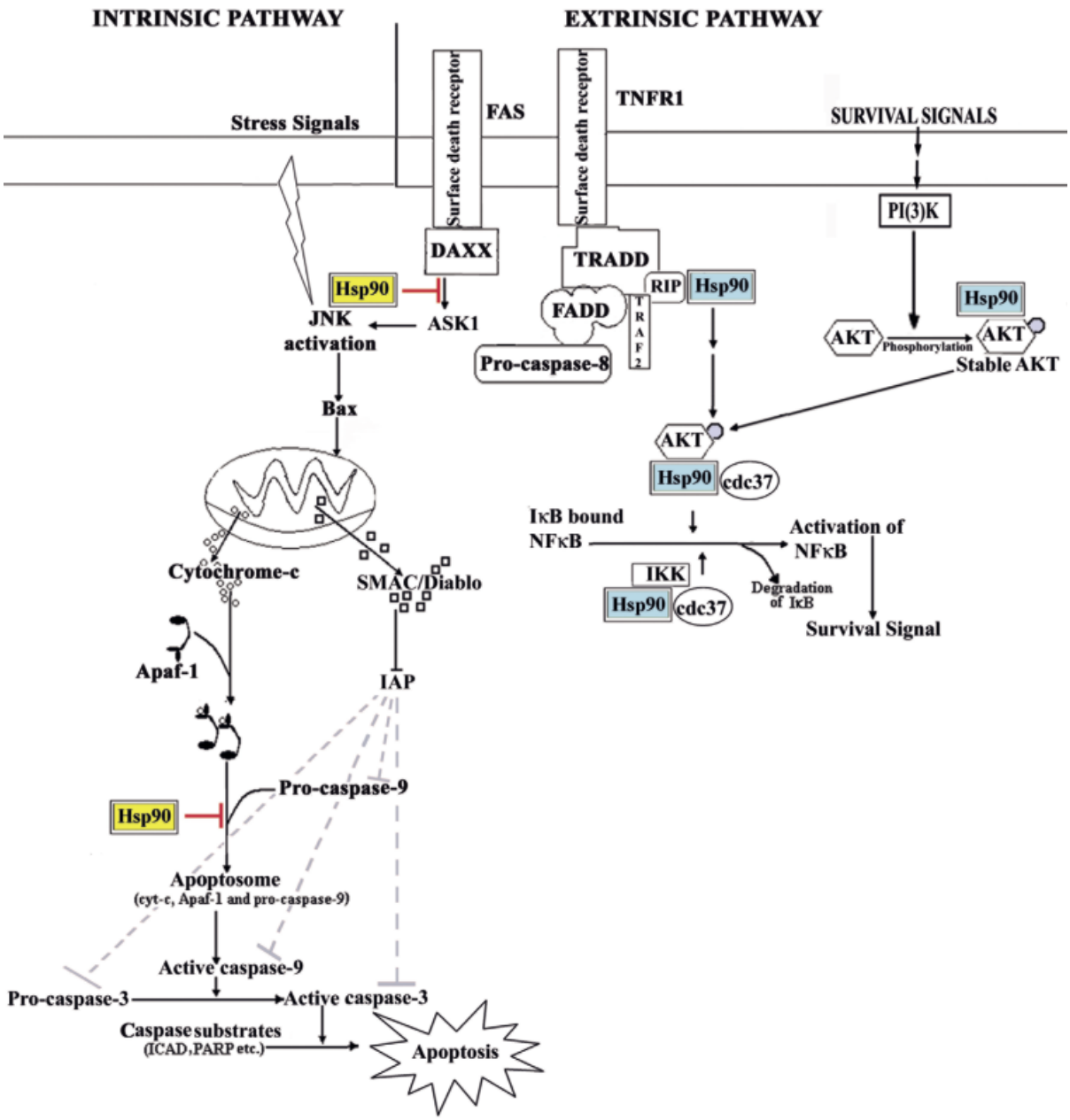

Figure 3. Anti-apoptotic roles of Hsp90 (yellow boxes) are mediated through inhibition of (red lines) activation of ASK-1 or procaspase-9. In addition, Hsp90 (blue boxes) is also an integral component of many complexes that help in cell survival. A small circle on a box indicates phosphorylated state of the given protein. Only a part of the apoptotic and cell survival network (figure 1), relevant to Hsp90 interactions, is shown.

Hsp90 also has a role in modulating the intrinsic pathway of apoptosis (figure 3). It prevents the formation of an active apoptosome complex by inhibiting oligomerization of Apaf-1 (Pandey *et al* 2000a).

Contrary to these mostly cell survival roles of Hsp90, Kim *et al* (2006b) have shown that a direct physical interaction of Hsp90 with the hypoxia-responsive pro-apoptotic protein (HGTD-P) is essential for its translocation into mitochondria for induction of the mitochondrial death pathway.

3.2 *Hsp70*

The Hsp70 family is most diverse and includes many constitutive as well as stress-inducible proteins with

overlapping or unique functions in different cell compartments and in different cellular contexts (reviewed in Fink 1999; Mayer and Bukau 2005). Roles of individual members of the large Hsp70 family in apoptosis are not clearly delineated, since most of the experimental studies on apoptosis have examined the heat shock inducible Hsp70.

Like Hsp90, Hsp70 is also mostly anti-apoptotic. It interacts with the intrinsic and extrinsic pathways of apoptosis at a number of steps and inhibits cell death through chaperone dependent as well as independent activities (figure 4). As an anti-apoptotic protein, Hsp70 protects cells from cytotoxicity induced by TNF, monocytes, oxidative stress, chemotherapeutic agents, ceramide and radiation (Jaattela *et al* 1992; Jaattela and Wissing 1993; Simon *et al* 1995; Karlseder *et al* 1996; Mosser *et al* 1997). The apoptotic cascade stimulated by nitric oxide and heat stress triggers translocation of Bax from cytoplasm to the mitochondria, which is inhibited by over-expression of Hsp70 (Gotoh

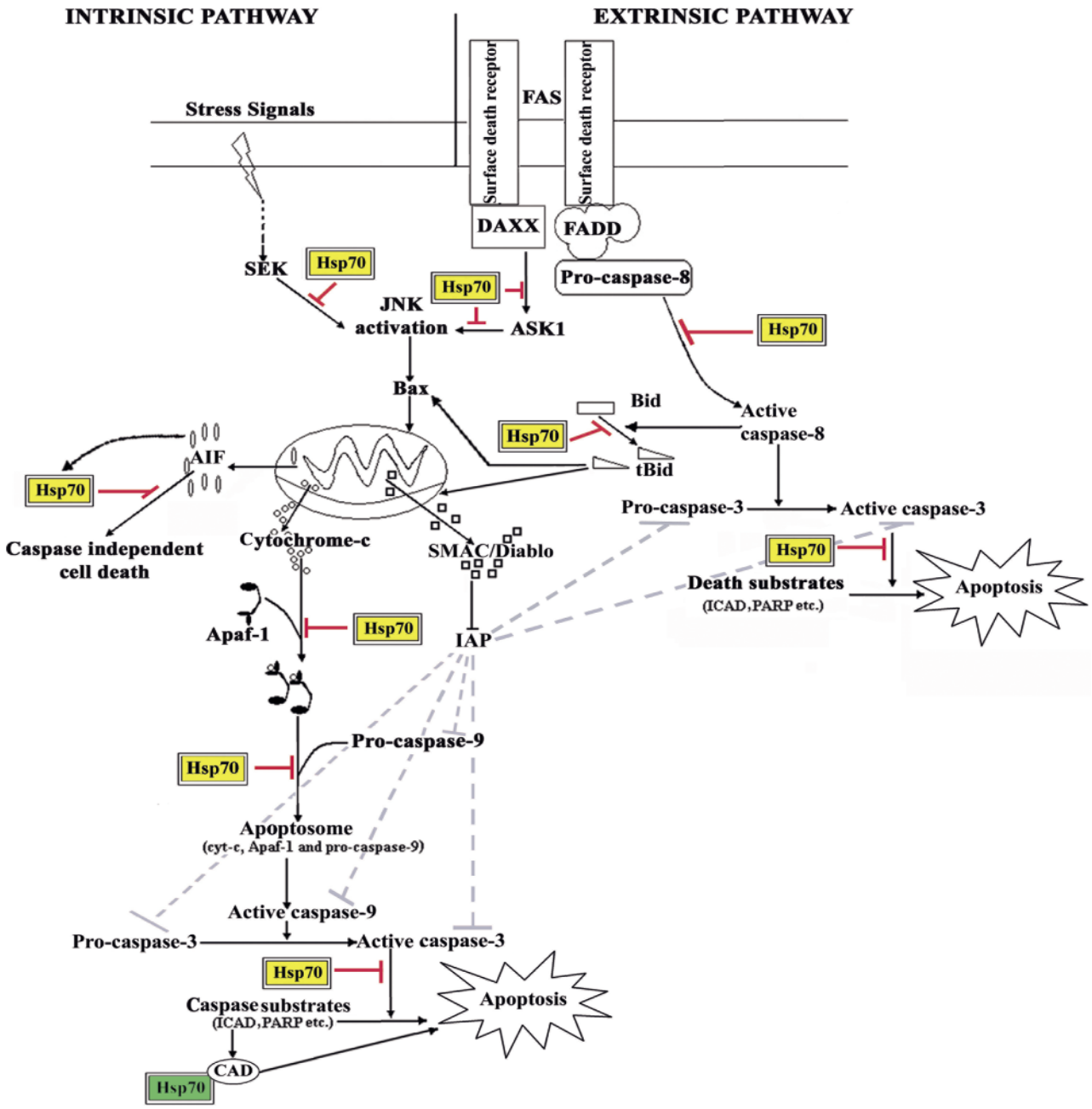

Figure 4. Hsp70 (yellow boxes) is generally anti-apoptotic since it inhibits (red lines) many steps in the apoptotic pathways. However, Hsp70 (green box) has a pro-apoptotic role also since it is required for activity of the caspase-activated DNase (CAD).

et al 2004; Stankiewicz *et al* 2005). Further downstream in the intrinsic pathway, Hsp70 inhibits formation of a functional apoptosome complex by direct interaction with Apaf-1 (Beere *et al* 2000; Saleh *et al* 2000). Hsp70 prevents late caspase dependent events such as activation of cytosolic phospholipase A2 and changes in nuclear morphology; it can also protect cells from forced expression of caspase-3 (Jaattela *et al* 1998).

Hsp70 can, independent of its chaperoning activity, inhibit JNK mediated cell death, by suppressing JNK

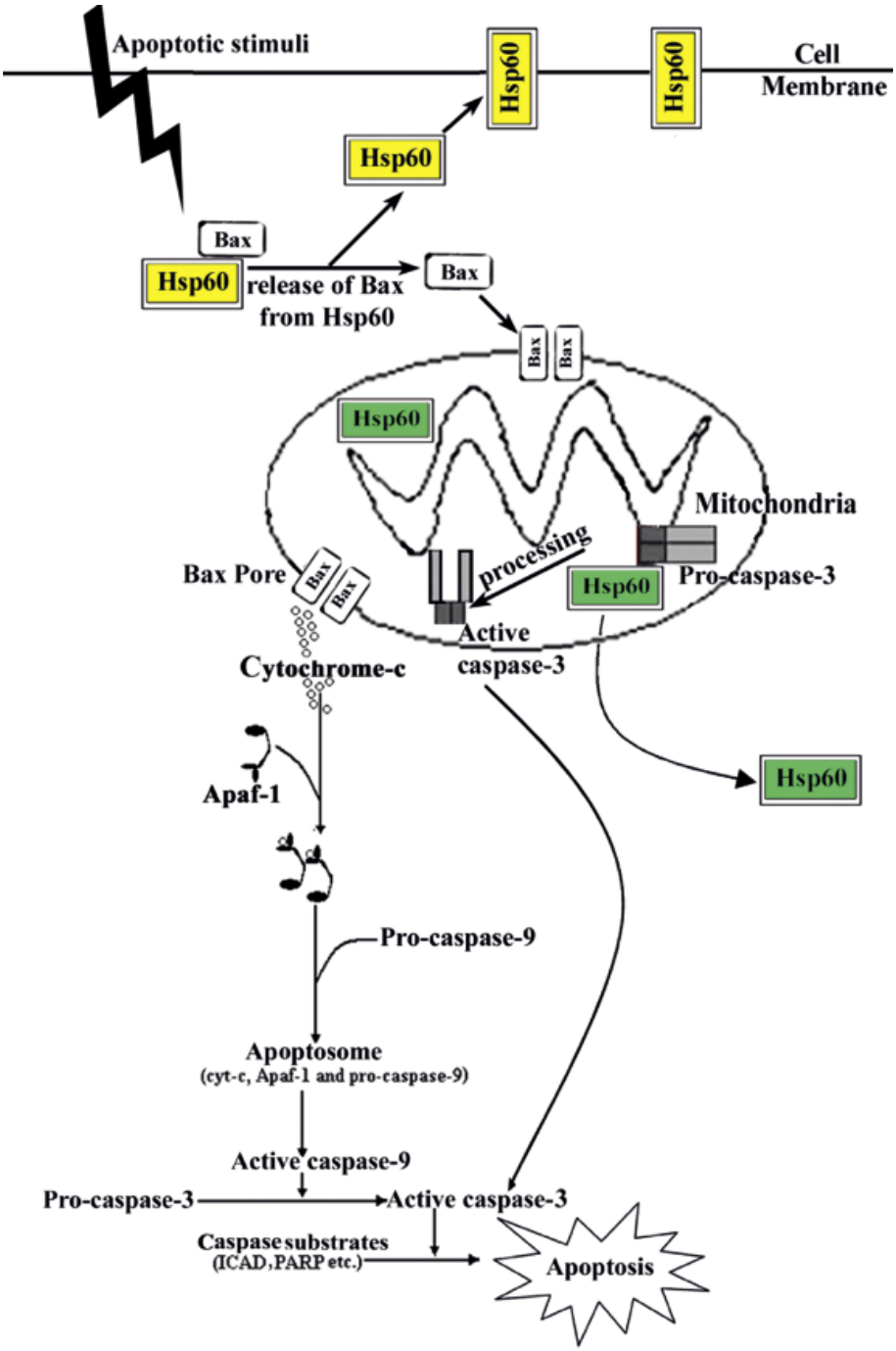

Figure 5. Anti- and pro-apoptotic roles of Hsp60. Cytoplasmic Hsp60 (yellow boxes) inhibits cell death by sequestering Bax and thereby, preventing its translocation to the mitochondrial membrane. On the other hand, mitochondrial Hsp60 (green boxes) promotes maturation of procaspase-3 and thus, has pro-apoptotic role.

phosphorylation either directly and/or through the upstream SEK kinase (Mosser *et al* 1997, 2000; Meriin *et al* 1999; Volloch *et al* 1999). An earlier study in our laboratory (Srivastava 2004) showed that developing eye discs of *Drosophila* expressing a dominant negative mutant form of the major constitutively expressed Hsc70.4 protein displayed high incidence of apoptosis and this was presumably mediated via the JNK pathway.

The caspase-8 mediated activation of Bid (truncated or tBid), which allows Bax to migrate onto the mitochondrial membrane to trigger release of various death factors, is a link between the extrinsic and intrinsic pathways (Li *et al* 1998; Luo *et al* 1998). Hsp70 regulates this activation of Bid independent of its chaperoning function (Gabai *et al* 2002) and thus, can influence both the pathways. In addition, various death-inducing stimuli, viz. TNF-α, Fas and many others are known to cause apoptosis via ASK-1 activation. Hsp70 hampers TNF mediated apoptosis by inhibition of ASK-1 (Park *et al* 2002).

Hsp70 plays anti-apoptotic roles in caspase independent pathway as well through its binding with the Apoptosis Inducing Factor (AIF) released from the mitochondria following death-inducing stimuli and thereby, restricting its translocation into the nucleus (Ravagnan *et al* 2001; Gurbuxani *et al* 2003). In addition, Hsp70 also impedes a lysosome mediated caspase independent cell death pathway since it maintains integrity of the lysosomal membrane and thus, prevents release of cathepsin into the cytosol (Nylandsted *et al* 2004).

In contrast to the above death inhibitory roles, a pro-death role, downstream of caspase-3, has also been ascribed to Hsp70 (figure 3). In Jurkat T cells, Hsp70 enhances TCR/CD3 and Fas/Apo-1/CD95 mediated apoptosis presumably by direct interaction with caspase activated DNase (Liossis *et al* 1997).

3.4 *Hsp60* \

The cytosolic and organellar forms of Hsp60 have anti- as well as pro-apoptotic roles (Sarkar *et al* 2006). Hsp60 and its co-chaperonin Hsp10 help prokaryotes survive severe stress (Volker *et al* 1992; Lund 1995). Both these chaperonins, in concert, prevent cell death in neonatal cardiac myocytes by maintaining the mitochondrial membrane integrity and function (Lin *et al* 2001). The cytosolic Hsp60 is mainly anti-apoptotic as it binds with pro-apoptotic Bax and Bak proteins in cardiac myocytes of rat and thus prevents triggering of the apoptotic machinery (Kirchhoff *et al* 2002). Shan *et al* (2003) found Bcl-XL also to be associated with Hsp60 in normal heart tissues. *In vitro* studies demonstrate that Hsp60 provides differential protection against intracellular beta-amyloid induced neuronal dysfunction and cell death by maintaining mitochondrial oxidative phosphorylation (Veereshwarayya *et al* 2006).

Binding of mitochondrial Hsp60 to procaspase-3 enhances its protease-sensitivity and thus makes it more susceptible to the action of cytochrome c and dATP (Samali *et al* 1999; Xanthoudakis *et al* 1999). In agreement with these observations, it was found that patients with esophageal squamous cell carcinomas with high Hsp60 showed higher apoptotic index and thus, enhanced survival (Faried *et al* 2004).

Recent studies in our laboratory suggest that one of the Hsp60 in *Drosophila melanogaster* is necessary for induced apoptosis. The Berkeley *Drosophila* Genome Project has revealed four *Hsp60* genes in *D. melanogaster*, which have been named as *Hsp60A*, *Hsp60B*, *Hsp60C* and *Hsp60D*, respectively (Sarkar and Lakhotia 2005). The *CG16954* or *Hsp60D* gene appears to be necessary for apoptosis. We have generated transgenic flies to conditionally either enhance (*UAS-Hsp60D.WT*) or ablate (*UAS-Hsp60D-RNAi*) the levels of Hsp60D under the control of a desired *Gal4* driver (Brand and Perrimon 1993, Giordano *et al* 2002). It is known that *GMR-Gal4* homozygous flies by themselves show highly disorganized ommatidial arrays in adult flies and a high level of apoptosis in the third instar eye discs (Kramer and Staveley 2003). Our studies (Arya R and Lakhotia S C, unpublished) show that *GMR-Gal4* homozygous flies co-expressing the *Hsp60D–RNAi* transgene, and thus with significantly reduced levels of Hsp60D in developing eye disc cells, have normal ommatidial arrays in adult eyes. Further evidence for essential requirement of Hsp60D in apoptosis is provided by our other (Arya R and Lakhotia S C, unpublished) observation that reduction or loss of Hsp60D protein prevents cell death (*see* figure 6) caused by directed over-expression of some of the key cell death regulators like Reaper, Hid or Grim (Grether *et al* 1995; White *et al* 1996; Hawkins *et al* 2000) in *Drosophila*. Thus it appears that availability of Hsp60D in the cell is essential for execution of induced apoptosis.

3.5 *Hsp27*

Hsp27 belongs to the family of small stress proteins that are constitutively abundant and ubiquitously present. Hsp27 regulates apoptosis through its ability to interact with key components of the apoptotic-signaling pathways (reviewed in Concannon *et al* 2003), particularly those involved in caspase activation (figure 7). Changes in the intracellular redox balance and production of reactive oxygen species initiate the apoptotic cascade through changes in the mitochondria and release of pro-apoptotic factors. Hsp27 can maintain both the redox homeostasis and mitochondrial stability in the cell. Increased expression of Hsp27 during stress response correlates with better survival from cytotoxic stress. It negatively regulates the activation of procaspase-9

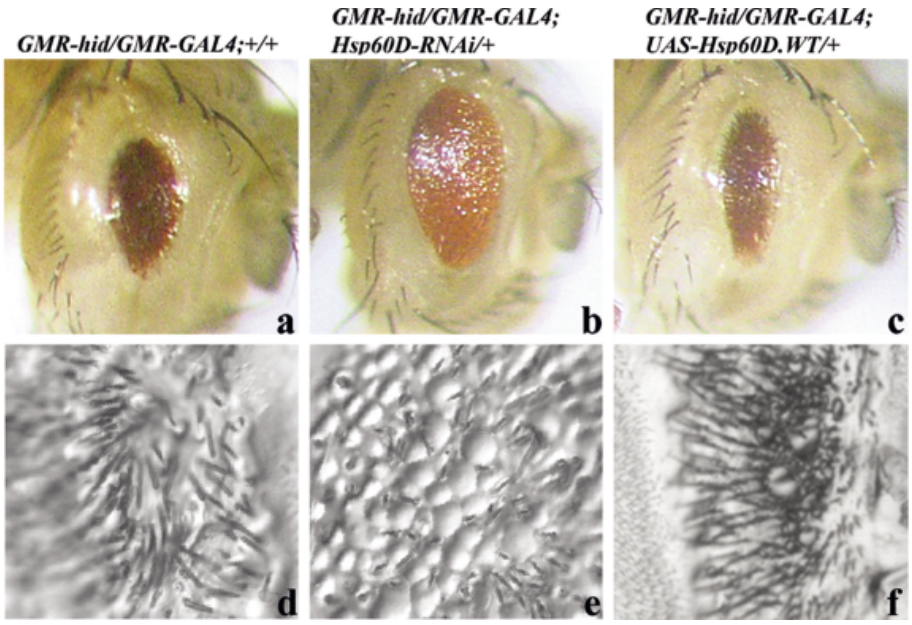

Figure 6. Hsp60D is required for cell death caused by over-expression of Hid in developing *Drosophila* eye discs. Images in **a-c** are photomicrographs while those in **d-f** are nail polish imprints (Arya and Lakhotia 2006) of adult eyes. Over-expression of Hid under *GMR* promoter in developing eye discs results in severe degeneration and reduction in size of adult eyes due to apoptosis of many of the Hid-expressing cells (**a, d**). However, *GMR* driven co-expression of *Hid* and *Hsp60D-RNAi* transgenes and consequent down-regulation of Hsp60D results in partial suppression of Hid mediated eye ablation as evidenced by the enlarged size of eyes (**b**) and formation of some ommatidial arrays (**e**). On the other hand, *GMR* driven over-expression of *HSP60D* in Hid expressing eye discs (**c, f**) resulted in smaller and more damaged eyes.

by sequestering cytosolic cytochrome c from Apaf-1, after its release from mitochondria and thus, prevents assembly of the apoptosome (Bruey *et al* 2000; Concannon *et al* 2001). Hsp27 can block release of cytochrome c from mitochondria in cells exposed to staurosporine, etoposide or cytochalasin D (Paul *et al* 2002). It also mediates inhibition of procaspase-3 activation, most likely through its ability to prevent initiator caspases like caspase-9 from gaining access to the residues whose cleavage is essential for procaspase-3 activation (Pandey *et al* 2000b). In addition, Hsp27 maintains the actin network integrity and hence, prevents translocation of pro-apoptotic factors like activated Bid (tBid) onto the mitochondrial membrane (Paul *et al* 2002).

Hsp27 is reported to block DAXX-mediated apoptosis by preventing its translocation to the membrane and thus, inhibiting its interaction with Fas and ASK-1 (Charette *et al* 2000). Rane *et al* (2003) have suggested that Hsp27 regulates apoptosis of neutrophils through interaction with Akt (Protein Kinase B): Hsp27 is phosphorylated by Akt which results in dissociation of Hsp27 and stabilization of Akt. Disruption of interaction between Akt and Hsp27 impairs Akt activation, which leads to enhanced constitutive apoptosis of neutrophils.

The only known pro-apoptotic role of Hsp27 so far is that it enhances TNF-induced apoptosis by inhibiting IκB degradation and thereby, preventing NF-κB mediated cell survival (Kammanadiminti and Chadee 2006).

$\alpha\beta$-crystallin, a small Hsp family member closely related to Hsp27 (Ingolia and Craig 1982), is constitutively expressed in many tissues and is especially abundant in eye lens, heart and in muscles. $\alpha\beta$-crystallin interferes in the processing of the precursor of procaspase-3 (Mao *et al* 2001; Kamradt *et al* 2001; Alge *et al* 2002). Additionally, $\alpha\beta$-crystallin inhibits apoptosis through sequestration of Bax and Bcl-Xs in the cytoplasm (Mao *et al* 2004). Over expression of $\alpha\beta$-crystallin can also inhibit apoptosis caused by RAS activation (Li *et al* 2005).

3.6 *Non-coding hsrω transcripts in* Drosophila

The *hsrω* gene of *D. melanogaster* is developmentally active in most cell types and is also one of the most strongly induced genes following heat shock. This gene produces several non-coding RNAs as functional end products (reviewed in Lakhotia 2003). The >10 Kb nucleus limited hsrω-n transcript of this gene is dynamically associated with several different hnRNPs in the nucleus to form fine nucleoplasmic omega speckles and it has been suggested that the omega speckles regulate nuclear trafficking and availability of hnRNPs and other related RNA binding

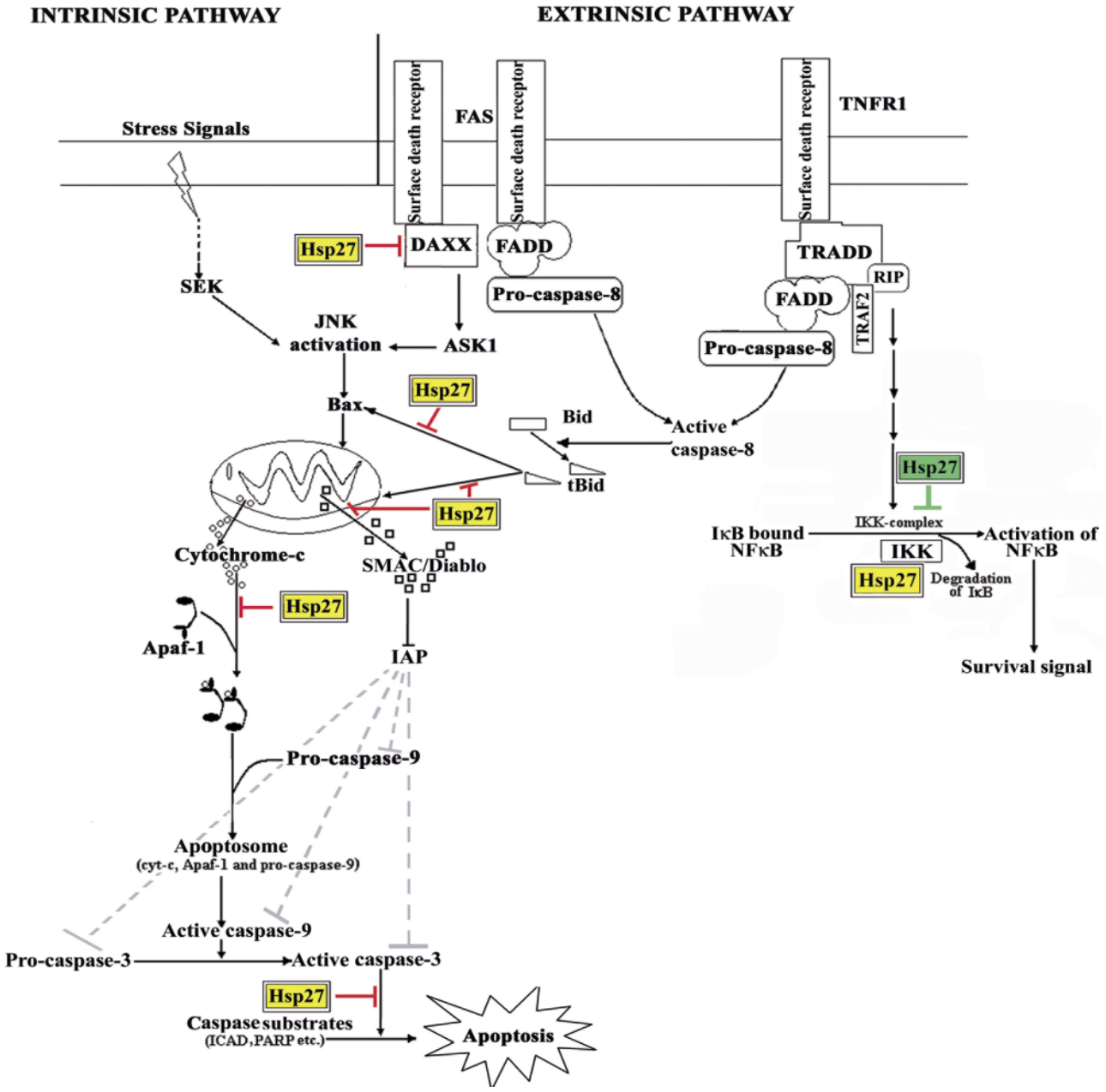

Figure 7. Anti-apoptotic (yellow) and pro-apoptotic (green) roles of Hsp27 in intrinsic and extrinsic cell death pathways (see text for details).

proteins in the cell (Lakhotia *et al* 1999; Prasanth *et al* 2000).

It is known that expression of expanded polyglutamine (polyQ) containing proteins is cytotoxic to neuronal cells, which ultimately die because of apoptosis (Warrick *et al* 1998; Faber *et al* 1999; Paulson *et al* 2000). Recent studies in our laboratory demonstrated that mis- or over-expression of the non-coding hsr*ω* transcripts enhances polyQ-induced neurodegeneration (Sengupta and Lakhotia 2006). To gain a better understanding of the physiological roles of the non-coding *hsrω* gene, we have employed GAL4 driven over-expression (EP mediated) or ablation (RNAi mediated) of hsr*ω* transcripts and examined the ensuing developmental/phenotypic abnormalities. Our results (Mallik M and Lakhotia S C, in preparation) show that ablation of the hsr*ω*-n transcripts through RNAi in developing eye disc

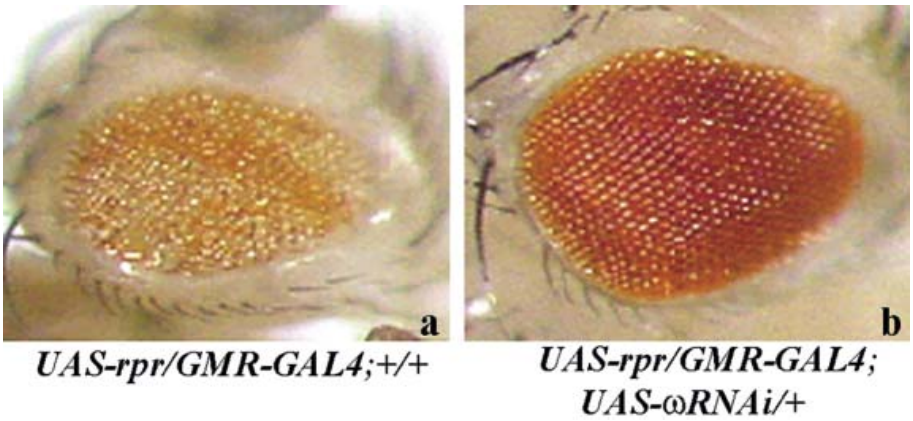

Figure 8. Absence of the non-coding hsrω transcripts suppresses induced cell death**.** Ectopic expression of the apical cell death trigger Reaper in photoreceptor neurons and accessory pigment cells of developing eye discs under control of the *GMR-GAL4* driver results in massive cell death in the developing retina and causes rough and reduced eyes in adult flies (**a**). However, reduction in the levels of the omega-n transcripts through RNAi in eye disc cells expressing Reaper restores the adult eye morphology to near wild type (**b**).

cells expressing expanded polyQ substantially rescues neurodegeneration in eyes. In order to see if this rescue is due to inhibition of apoptosis, we have examined effects of alterations in the levels of the hsrω transcripts on apoptosis following activation of Reaper and other apical cell death triggers in developing retina. It is seen (Mallik M and Lakhotia S C, in preparation) that, as expected, *GMR-Gal4* driven over-expression of Reaper in developing wild type eye discs has severe effects on morphology and pigmentation of adult eyes (figure 8a); however, RNAi of hsrω transcripts in eye discs over-expressing Reaper results in near complete absence of eye defects (figure 8b). We have observed similar inhibitory effect of RNAi of the non-coding hsrω transcripts on apoptosis triggered by other cell death stimuli (Mallik M and Lakhotia S C, in preparation). This novel involvement of a non-coding RNA in apoptosis opens new paradigm/s for integration of various cellular activities.

4. Epilogue

The above brief survey clearly shows that many of the heat shock proteins (constitutive as well as stress induced) interact with members of the apoptotic cascades to inhibit or promote cell death. It is significant that members of each of the Hsp families not only act at multiple levels but even in opposing directions.

At any given time, a living cell is a multi-tasking system in which multitudes of external and internal information/ signals are processed in parallel but the responses to any one or more of these activities have to be integrative. Hsps by the nature of their functions, appear to be significant integrators or co-ordinators of cellular signals and responses. They provide "weak" but very important links between many "hubs" and thus, help transmission of information/signal across different paths in cellular networks (Csermely 2004; Korcsmáros *et al* 2007).

The intricately networked organization of the various functional and structural compartments of a cell implies that damage or unfavourable perturbations in any one compartment can have varying consequences for other compartments as well. In view of the existence of multiple signals that can activate one or the other apoptotic pathways, any of these compartments can trigger cell death depending upon the extent of the unfavourable signals received. Excessive cell death can be very "costly" for the organism and therefore, appropriate checks need to be exercised before the apoptotic cascade is permitted to execute cell death. Hsps provide one of the check systems. The significance of the modulatory roles of the various Hsps at multiple steps in apoptotic cascades lies in the fact that as weak "links" (Csermely 2004), they can tilt the balance in subtle but precise ways in favour of cell survival or cell death in a context-dependent manner.

In addition to the heat shock induced genes, the Heat shock transcription factors (HSFs), which primarily regulate the activation of heat shock genes following stress (Pirkkala *et al* 2001), are also now known to modulate the apoptotic cascade. Xia *et al* (2000) have shown that in HeLa cell line the activated Fas death receptor transactivates HSF1 which induces heat shock genes. Interestingly, despite the presence of activated Fas, these cells are insensitive to Fas-mediated cell death but over-expression of constitutively active HSF1 sensitizes them for cell death; this suggests novel interactions between the stress protein mediated cell survival and activated Fas-dependent cell killing (Xia *et al* 2000). Boellman *et al* (2004) demonstrated that the DAXX protein,

a modulator of apoptosis, also induces the transactivation of HSF1 under stress conditions in human cell lines. Recently HSF1 mediated cell death has been described in male germ cells (Hayashida *et al* 2006, Vydra *et al* 2006). Thus HSFs emerge as novel modulators of cell survival or cell death.

It is becoming increasingly appreciated that non-coding RNA species have important regulatory functions in eukaryotic cells (Lakhotia 1996, 2003; Eddy 2001; Mattick 2004; Prasanth and Spector 2007). Recent observations in our laboratory (Mallik M and Lakhotia S C, in preparation) that a large non-coding RNA, expressed constitutively and further activated by stress, can also modulate induced apoptosis add a new dimension to the regulation of cell death and survival. The hsrω-n transcripts are known to bind with a variety of hnRNPs, several other RNA-processing/binding proteins and Hsp90 (Lakhotia *et al* 1999; Prasanth *et al* 2000; Lakhotia 2003; Jolly and Lakhotia 2006) and thus, can potentially affect all those cellular activities in which these proteins are directly or indirectly involved. hnRNPs are known to modulate apoptosis (Jiang *et al* 1998; Charroux *et al* 1999; Shchors *et al* 2002; Hermann *et al* 2001; Schwerk and Schulze-Osthoff 2005) mostly through regulation of alternative splicing. It is likely that down-regulation of hsrω-n transcripts by RNAi, affects the nuclear hnRNP metabolism and this may affect apoptosis. Association of hsrω transcripts with Hsp90 may also be significant in this context. Association or a direct interaction of the hsrω-n transcripts with any of the caspases or the main components of the apoptotic pathways is not yet known, although our observations (Mallik M and Lakhotia S C, in preparation) indicate that reduced levels of hsrω-n transcripts prevent activation of JNK pathway and this in turn may block apoptosis. Irrespective of the mode of action, it is significant that a non-coding RNA affects a vital process. Since binding of proteins to target nucleic acids is dependent upon short motifs, we believe that RNA molecules can have multiple protein-binding potential and thus, non-coding RNA species like the hsrω-n transcripts can function as hubs in the cellular network systems with the various RNA-binding proteins providing the links with other hubs.

The high conservation of Hsp families from bacteria through higher eukaryotes shows that these are very ancient proteins and thus, had many opportunities to evolve interactions with other newly emerging proteins. Their function as molecular chaperones by itself also requires their involvement in a large variety of cellular events (Korcsmáros *et al* 2007). In addition, they participate in many cellular events in a chaperone independent manner as well. It is obvious that contrary to their generic names, the Hsps are not just reversing stress-induced cellular damage or helping nascent polypeptides to fold appropriately, but have more global roles in cell metabolism. Likewise, stress-induced non-coding transcripts also appear to have "conserved functions" (Jolly and Lakhotia 2006). An integrative understanding of the various functions of Hsps and non-coding transcripts will help us know better the networks operating in biological systems.

Acknowledgements

RA is receiving Senior Research Fellowship from the Council of Scientific and Industrial Research, New Delhi. MM is SPM Fellow (Council of Scientific and Industrial Research, New Delhi). Research work in the laboratory is supported by grants from the Department of Biotechnology and Department of Science and Technology, New Delhi to SCL.

References

Alge C S, Priglinger S G, Neubauer A S, Kampik A, Zillig M, Bloemendal H and Welge-Lussen U 2002 Retinal pigment epithelium is protected against apoptosis by alphaB-crystallin; *Invest. Ophthalmol.Vis.Sci.***43** 3575–3582

Arya R and Lakhotia S C 2006 A simple nail polish imprint technique for examination of external morphology of *Drosophila* eyes; *Curr. Sci.* **90** 1179–1180

Basso A D, Solit D B, Chiosis G, Giri B, Tsichlis P and Rosen N 2002 Akt forms an intracellular complex with heat shock protein 90 (Hsp90) and Cdc37 and is destabilized by inhibitors of Hsp90 function; *J. Biol. Chem*. **277** 39858–39866

Beere H M 2001 Stressed to death: regulation of apoptotic signaling pathways by the heat shock proteins; *Sci. STKE* **2001** RE1

Beere H M 2005 Death versus survival: functional interaction between the apoptotic and stress-inducible heat shock protein pathways; *J. Clin. Invest.* **115** 2633–2639

Beere H M, Wolf B B, Cain K, Mosser D D, Mahboubi A, Kuwana T, Tailor P, Morimoto R I, Cohen G M and Green D R 2000 Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome; *Nat. Cell Biol.* **2** 469–475

Boellmann F, Guettouche T, Guo Y, Fenna M, Mnayer L and Voellmy R 2004 DAXX interacts with heat shock factor 1 during stress activation and enhances its transcriptional activity; *Proc Natl Acad Sci USA* **101** 4100–4105

Brand A H and Perrimon N 1993 Targeted gene expression as a means of altering cell fates and generating dominant phenotypes; *Development* **118** 401–415

Bruey J M, Ducasse C, Bonniaud P, Ravagnan L, Susin S A, Diaz-Latoud C, Gurbuxani S, Arrigo A P, Kroemer G, Solary E and Garrido C 2000 Hsp27 negatively regulates cell death by interacting with cytochrome c; *Nat. Cell Biol.* **2** 645–652

Budihardjo I, Oliver H, Lutter M, Luo X and Wang X 1999 Biochemical pathways of caspase activation during apoptosis; *Annu. Rev. Cell Dev. Biol.* **15** 269–290

Chang H Y, Nishitoh H, Yang X, Ichijo H and Baltimore D 1998 Activation of apoptosis signal-regulating kinase 1 (ASK1) by the adapter protein Daxx; *Science* **281** 1860–1863

Charette S J, Lavoie J N, Lambert H and Landry J 2000 Inhibition of Daxx-mediated apoptosis by heat shock protein 27; *Mol. Cell Biol.* **20** 7602–7612

Charroux B, Angelats C, Fasano L, Kerridge S and Vola C 1999 The levels of the bancal product, a *Drosophila* homologue of vertebrate hnRNP K protein, affect cell proliferation and apoptosis in imaginal disc cells; *Mol. Cell. Biol.* **19** 7846–7856

Chen G, Cao P and Goeddel D V 2002 TNF-induced recruitment and activation of the IKK complex require Cdc37 and Hsp90; *Mol. Cell* **9** 401–410

Chen Z J, Parent L and Maniatis T 1996 Site-specific phosphorylation of IkappaBalpha by a novel ubiquitination-dependent protein kinase activity; *Cell* **84** 853–862

Concannon C G, Gorman A M and Samali A 2003 On the role of Hsp27 in regulating apoptosis; *Apoptosis* **8** 61–70

Concannon C G, Orrenius S and Samali A 2001 Hsp27 inhibits cytochrome c-mediated caspase activation by sequestering both pro-caspase-3 and cytochrome c; *Gene Expr.* **9** 195–201

Csermely P 2004 Strong links are important, but weak links stabilize them; *Trends Biochem. Sci.* **29** 331–334

Deveraux Q L and Reed J C 1999 IAP family proteins--suppressors of apoptosis; *Genes Dev.* **13** 239–252

Devin A, Cook A, Lin Y, Rodriguez Y, Kelliher M and Liu Z 2000 The distinct roles of TRAF2 and RIP in IKK activation by TNF-R1: TRAF2 recruits IKK to TNF-R1 while RIP mediates IKK activation; *Immunity* **12** 419–429

Didelot C, Schmitt E, Brunet M, Maingret L, Parcellier A and Garrido C 2006 Heat shock proteins: endogenous modulators of apoptotic cell death; *Handb. Exp. Pharmacol.* 171–198

DiDonato J A, Hayakawa M, Rothwarf D M, Zandi E and Karin M 1997 A cytokine-responsive IkappaB kinase that activates the transcription factor NF-kappaB; *Nature* (*London*) **388** 548–554

Eddy S R 2001 Non-coding RNA genes and the modern RNA world; *Nat. Rev. Genet.* **2** 919–929

Faber P W, Alter J R, MacDonald M E and Hart A C 1999 Polyglutamine-mediated dysfunction and apoptotic death of a Caenorhabditis elegans sensory neuron; *Proc. Natl. Acad. Sci. USA* **96** 179–184

Faried A, Sohda M, Nakajima M, Miyazaki T, Kato H and Kuwano H 2004 Expression of heat-shock protein Hsp60 correlated with the apoptotic index and patient prognosis in human oesophageal squamous cell carcinoma; *Eur. J. Cancer* **40** 2804–2811

Fink A L 1999 Chaperone-mediated protein folding; *Physiol. Rev.* **79** 425–449

Gabai V L, Mabuchi K, Mosser D D and Sherman M Y 2002 Hsp72 and stress kinase c-jun N-terminal kinase regulate the bid-dependent pathway in tumor necrosis factor-induced apoptosis; *Mol. Cell. Biol.* **22** 3415–3424

Garrido C, Gurbuxani S, Ravagnan L and Kroemer G 2001 Heat shock proteins: endogenous modulators of apoptotic cell death; *Biochem. Biophys. Res. Commun.* **286** 433–442

Ghosh S 1999 Regulation of inducible gene expression by the transcription factor NF-kB. Immunol.; *Immunol. Res.* **19** 183–189

Giordano E, Rendina R, Peluso I and Furia M 2002 RNAi triggered by symmetrically transcribed transgenes in *Drosophila melanogaster*; *Genetics* **160** 637–648

Gotoh T, Terada K, Oyadomari S and Mori M 2004 hsp70-DnaJ chaperone pair prevents nitric oxide- and CHOP-induced apoptosis by inhibiting translocation of Bax to mitochondria; *Cell Death Differ.* **11** 390–402

Green D R and Reed J C 1998 Mitochondria and apoptosis; *Science* **281** 1309–1312

Grether M E, Abrams J M, Agapite J, White K and Steller H 1995 The head involution defective gene of *Drosophila melanogaster* functions in programmed cell death; *Genes Dev.* **9** 1694–1708

Gross A, McDonnell J M and Korsmeyer S J 1999 BCL-2 family members and the mitochondria in apoptosis; *Genes Dev.* **13** 1899–1911

Gurbuxani S, Schmitt E, Cande C, Parcellier A, Hammann A, Daugas E, Kouranti I, Spahr C, Pance A, Kroemer G and Garrido C 2003 Heat shock protein 70 binding inhibits the nuclear import of apoptosis-inducing factor; *Oncogene* **22** 6669–6678

Hartl F U and Hayer-Hartl M 2002 Molecular chaperones in the cytosol: from nascent chain to folded protein; *Science* **295** 1852–1858

Hawkins C J, Yoo S J, Peterson E P, Wang S L, Vernooy S Y and Hay B A 2000 The *Drosophila* caspase DRONC cleaves following glutamate or aspartate and is regulated by DIAP1, HID, and GRIM; *J. Biol. Chem.* **275** 27084–27093

Hay B A and Guo M 2006 Caspase-dependent cell death in *Drosophila*; *Annu. Rev. Cell Dev. Biol.* **22** 623–650

Hayashida N, Inouye S, Fujimoto M, Tanaka Y, Izu H, Takaki E, Ichikawa H, Rho J and Nakai A 2006 A novel HSF1-mediated death pathway that is suppressed by heat shock proteins; *EMBO J.* **25** 4773–4783

Hermann R, Hensel F, Muller E C, Keppler M, Souto-Carneiro M, Brandlein S, Muller-Hermelink H K and Vollmers H P 2001 Deactivation of regulatory proteins hnRNP A1 and A2 during SC-1 induced apoptosis; *Hum. Antibodies* **10** 83–90

Ingolia T D and Craig E A 1982 Four small *Drosophila* heat shock proteins are related to each other and to mammalian alpha-crystallin; *Proc. Natl. Acad. Sci. USA* **79** 2360–2364

Jaattela M and Wissing D 1993 Heat-shock proteins protect cells from monocyte cytotoxicity: possible mechanism of self-protection; *J. Exp. Med.* **177** 231–236

Jaattela M, Wissing D, Bauer P A and Li G C 1992 Major heat shock protein hsp70 protects tumor cells from tumor necrosis factor cytotoxicity; *EMBO J.* **11** 3507–3512

Jaattela M, Wissing D, Kokholm K, Kallunki T and Egeblad M 1998 Hsp70 exerts its anti-apoptotic function downstream of caspase-3-like proteases; *EMBO J.* **17** 6124–6134

Jacobson M D, Weil M and Raff M C 1997 Programmed cell death in animal development; *Cell* **88** 347–354

Jiang Z H, Zhang W J, Rao Y and Wu J Y 1998 Regulation of Ich-1 pre-mRNA alternative splicing and apoptosis by mammalian splicing factors; *Proc. Natl. Acad. Sci. USA* **95** 9155–9160

Jolly C and Lakhotia S C 2006 Human sat III and *Drosophila* hsr omega transcripts: a common paradigm for regulation of nuclear RNA processing in stressed cells; *Nucleic Acids Res.* **34** 5508–5514

Kammanadiminti S J and Chadee K 2006 Suppression of NF-κB activation by Entameba histolytica in intestinal epithelial cells is mediated by heat shock protein 27; *J. Biol. Chem.* **281** 26029–26080

Kamradt M C, Chen F and Cryns V L 2001 The small heat shock protein alpha B-crystallin negatively regulates cytochrome c- and caspase-8-dependent activation of caspase-3 by inhibiting its autoproteolytic maturation; *J. Biol. Chem.* **276** 16059–16063

Karlseder J, Wissing D, Holzer G, Orel L, Sliutz G, Auer H, Jaattela M and Simon M M 1996 HSP70 overexpression mediates the escape of a doxorubicin-induced G2 cell cycle arrest; *Biochem. Biophys. Res. Commun.* **220** 153–159

Kerr J F, Wyllie A H and Currie A R 1972 Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics; *Br. J. Cancer* **26** 239–257

Kim H P, Morse D and Choi A M 2006a Heat-shock proteins: new keys to the development of cytoprotective therapies; *Expert Opin. Ther. Targets* **10** 759–769

Kim J Y, Kim S M, Ko J H, Yim J H and Park J H 2006b Interaction of pro-apoptotic protein HGTD-P with heat shock protein 90 is required for induction of mitochondrial apoptotic cascades; *FEBS Lett.* **580** 3270–3275

Kirchhoff S R, Gupta S and Knowlton A A 2002 Cytosolic heat shock protein 60, apoptosis, and myocardial injury; *Circulation* **105** 2899–2904

Korcsmáros T, Kovács I A , Szalay M S, and Csermely P 2007 Molecular chaperones: The modular evolution of cellular networks.; *J. Biosci.* **32** 441–446

Kornbluth S and White K 2005 Apoptosis in *Drosophila*: neither fish nor fowl (nor man, nor worm); *J. Cell. Sci.* **118** 1779–1787

Kramer J M and Staveley B E 2003 GAL4 causes developmental defects and apoptosis when expressed in the developing eye of *Drosophila melanogaster*; *Genet. Mol. Res.* **2** 43–47

Lakhotia S C 1996 RNA polymerase II dependent genes that do not code for protein; *Indian J. Biochem. Biophys.* **I33** 93–102

Lakhotia S C 2003 The noncoding developmentally active and stress-inducible *hsrω* gene of *Drosophila melanogaster* integrates Post-transcriptional processing of other nuclear transcripts; in *Noncoding RNAs: Molecular biology and molecular medicine* (eds) J Barciszewski and VA Erdmann (New York: Kluwer Academic/ Plenum Publishers) pp 209–219

Lakhotia S C, Ray P, Rajendra, T K and Prasanth K V 1999 The non-coding transcripts of hsr*ω* gene in *Drosophila*: Do they regulate trafficking and availability of nuclear RNA processing factors?; *Curr. Sci.* **77** 553–563

Lewis J, Devin A, Miller A, Lin Y, Rodriguez Y, Neckers L and Liu Z G 2000 Disruption of hsp90 function results in degradation of the death domain kinase, receptor-interacting protein (RIP), and blockage of tumor necrosis factor-induced nuclear factor-kappaB activation; *J. Biol. Chem.* **275** 10519–10526

Li D W, Liu J P, Mao Y W, Xiang H, Wang J, Ma W Y, Dong Z, Pike H M, Brown R E and Reed J C 2005 Calcium-activated RAF/MEK/ERK signaling pathway mediates p53-dependent apoptosis and is abrogated by alpha B-crystallin through inhibition of RAS activation; *Mol. Biol. Cell.* **16** 4437–4453

Li H, Zhu H, Xu C J and Yuan J 1998 Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis; *Cell* **94** 491–501

Lin K M, Lin B, Lian I Y, Mestril R, Scheffler I E and Dillmann W H 2001 Combined and individual mitochondrial HSP60 and HSP10 expression in cardiac myocytes protects mitochondrial function and prevents apoptotic cell deaths induced by simulated ischemia-reoxygenation; *Circulation* **103** 1787–1792

Liossis S N, Ding X Z, Kiang J G and Tsokos G C 1997 Overexpression of the heat shock protein 70 enhances the TCR/CD3- and Fas/Apo-1/CD95-mediated apoptotic cell death in Jurkat T cells; *J. Immunol.* **158** 5668–5675

Locksley R M, Killeen N and Lenardo M J 2001 The TNF and TNF receptor superfamilies: integrating mammalian biology; *Cell* **104** 487–501

Lund P A 1995 The roles of molecular chaperones in vivo; *Essays Biochem.* **29** 113-123

Luo X, Budihardjo I, Zou H, Slaughter C and Wang X 1998 Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors; *Cell* **94** 481–490

Mao Y W, Liu J P, Xiang H and Li D W 2004 Human alphaA- and alphaB-crystallins bind to Bax and Bcl-X(S) to sequester their translocation during staurosporine-induced apoptosis; *Cell Death Differ.* **11** 512–526

Mao Y W, Xiang H, Wang J, Korsmeyer S, Reddan J and Li D W 2001 Human bcl-2 gene attenuates the ability of rabbit lens epithelial cells against H2O2-induced apoptosis through down-regulation of the alpha B-crystallin gene; *J. Biol. Chem.* **276** 43435–43445

Mattick J S 2004 RNA regulation: a new genetics? *Nat. Rev. Genet.* **5** 316–323

Mayer M P and Bukau B 2005 Hsp70 chaperones: cellular functions and molecular mechanism; *Cell Mol. Life Sci.* **62** 670–684

Meriin A B, Yaglom J A, Gabai V L, Zon L, Ganiatsas S, Mosser D D and Sherman M Y 1999 Protein-damaging stresses activate c-Jun N-terminal kinase via inhibition of its dephosphorylation: a novel pathway controlled by HSP72; *Mol. Cell Biol.* **19** 2547–2555

Mosser D D, Caron A W, Bourget L, Denis-Larose C and Massie B 1997 Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis; *Mol. Cell Biol.* **17** 5317–5327

Mosser D D, Caron A W, Bourget L, Meriin A B, Sherman M Y, Morimoto R I and Massie B 2000 The chaperone function of hsp70 is required for protection against stress-induced apoptosis; *Mol. Cell Biol.* **20** 7146–7159

Nover L 1984 *Heat shock response of eucaryotic cells* (Berlin: Springer-Verlag)

Nylandsted J, Gyrd-Hansen M, Danielewicz A, Fehrenbacher N, Lademann U, Hoyer-Hansen M, Weber E, Multhoff G, Rohde M and Jaattela M 2004 Heat shock protein 70 promotes cell survival by inhibiting lysosomal membrane permeabilization; *J. Exp. Med.* **200** 425–435

Ozes O N, Mayo L D, Gustin J A, Pfeffer S R, Pfeffer L M and Donner D B 1999 NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase; *Nature (London)* **401** 82–85

Paez J and Sellers W R 2003 PI3K/PTEN/AKT pathway. A critical mediator of oncogenic signaling; *Cancer Treat. Res*.**115** 145–167

Pandey P, Saleh A, Nakazawa A, Kumar S, Srinivasula S M, Kumar V, Weichselbaum R, Nalin C, Alnemri E S, Kufe D

and Kharbanda S 2000a Negative regulation of cytochrome c-mediated oligomerization of Apaf-1 and activation of procaspase-9 by heat shock protein 90; *EMBO J.* **19** 4310–4322

Pandey P, Farber R, Nakazawa A, Kumar S, Bharti A, Nalin C, Weichselbaum R, Kufe D and Kharbanda S 2000b Hsp27 functions as a negative regulator of cytochrome c-dependent activation of procaspase-3; *Oncogene* **19** 1975–1981

Park H S, Cho S G, Kim C K, Hwang H S, Noh K T, Kim M S, Huh S H, Kim M J, Ryoo K, Kim E K, Kang W J, Lee J S, Seo J S, Ko Y G, Kim S and Choi E J 2002 Heat shock protein hsp72 is a negative regulator of apoptosis signal-regulating kinase 1; *Mol. Cell Biol.* **22** 7721–7730

Paul C, Manero F, Gonin S, Kretz-Remy C, Virot S and Arrigo A P 2002 Hsp27 as a negative regulator of cytochrome C release; *Mol. Cell Biol.* **22** 816–834

Paulson H L, Bonini N M and Roth K A 2000 Polyglutamine disease and neuronal cell death; *Proc. Natl. Acad. Sci. USA* **97** 12957–12958

Pirkkala L, Nykanen P and Sistonen L 2001 Roles of the heat shock transcription factors in regulation of the heat shock response and beyond; *Faseb J.* **15** 1118–1131

Prasanth K V and Spector D L 2007 Eukaryotic regulatory RNAs: an answer to the 'genome complexity' conundrum; *Genes Dev.* **21** 11–42

Prasanth K V, Rajendra T K, Lal A K and Lakhotia S C 2000 Omega speckles - a novel class of nuclear speckles containing hnRNPs associated with noncoding hsr-omega RNA in *Drosophila*; *J. Cell Sci.* **113** 3485–3497

Pratt W B 1998 The hsp90-based chaperone system: involvement in signal transduction from a variety of hormone and growth factor receptors; *Proc. Soc. Exp. Biol. Med.* **217** 420–434

Rane M J, Pan Y, Singh S, Powell D W, Wu R, Cummins T, Chen Q, McLeish K R and Klein J B 2003 Heat shock protein 27 controls apoptosis by regulating Akt activation; *J. Biol. Chem.* **278** 27828–27835

Ravagnan L, Gurbuxani S, Susin S A, Maisse C, Daugas E, Zamzami N, Mak T, Jaattela M, Penninger J M, Garrido C and Kroemer G 2001 Heat-shock protein 70 antagonizes apoptosis-inducing factor; *Nat. Cell. Biol.* **3** 839–843

Regnier C H, Song H Y, Gao X, Goeddel D V, Cao Z and Rothe M 1997 Identification and characterization of an IkappaB kinase; *Cell* **90** 373–383

Riedl S J and Shi Y 2004 Molecular mechanisms of caspase regulation during apoptosis; *Nat. Rev. Mol. Cell Biol.* **5** 897–907

Romashkova J A and Makarov S S 1999 NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling; *Nature (London)* **401** 86–90

Rutherford S L and Lindquist S 1998 Hsp90 as a capacitor for morphological evolution; *Nature (London)* **396** 336–342

Saleh A, Srinivasula S M, Balkir L, Robbins P D and Alnemri E S 2000 Negative regulation of the Apaf-1 apoptosome by Hsp70; *Nat. Cell. Biol.* **2** 476–483

Samali A and Orrenius S 1998 Heat shock proteins: regulators of stress response and apoptosis; *Cell Stress Chaperones* **3** 228–236

Samali A, Cai J, Zhivotovsky B, Jones D P and Orrenius S 1999 Presence of a pre-apoptotic complex of pro-caspase-3, Hsp60 and Hsp10 in the mitochondrial fraction of jurkat cells; *EMBO J.* **18** 2040–2048

Sarkar S and Lakhotia S C 2005 The Hsp60C gene in the 25F cytogenetic region in *Drosophila melanogaster* is essential for tracheal development and fertility; *J. Genet.* **84** 265–281

Sarkar S, Arya R and Lakhotia S C 2006 Chaperonins: In life and death; in *Stress Response: A Molecular Biology Approach* (eds) A S Sreedhar and U K Srinivas (Kerala, India: Research Signpost) pp 43–60

Sato S, Fujita N and Tsuruo T 2000 Modulation of Akt kinase activity by binding to Hsp90; *Proc. Natl. Acad. Sci. USA* **97** 10832–10837

Schlesinger M J, Ashburner M and Tissiers A 1982 *Heat shock proteins: from bacteria to man*; (New York: Cold Spring Harbor Laboratory Press)

Schwerk C and Schulze-Osthoff K 2005 Regulation of apoptosis by alternative pre-mRNA splicing; *Mol. Cell* **19** 1–13

Screaton G and Xu X N 2000 T cell life and death signalling via TNF-receptor family members; *Curr. Opin. Immunol.* **12** 316–322

Sengupta S and Lakhotia S C 2006 Altered Expressions of the Noncoding hsromega Gene Enhances poly-Q-Induced Neurotoxicity in *Drosophila*; *RNA Biol.* **3** 28–35

Shan Y X, Liu T J, Su H F, Samsamshariat A, Mestril R and Wang P H 2003 Hsp10 and Hsp60 modulate Bcl-2 family and mitochondria apoptosis signaling induced by doxorubicin in cardiac muscle cells; *J. Mol. Cell. Cardiol.* **35** 1135–1143

Shchors K, Yehiely F, Kular R K, Kotlo K U, Brewer G and Deiss L P 2002 Cell death inhibiting RNA (CDIR) derived from a 3'-untranslated region binds AUF1 and heat shock protein 27; *J. Biol. Chem.* **277** 47061–47072

Simon M M, Reikerstorfer A, Schwarz A, Krone C, Luger T A, Jaattela M and Schwarz T 1995 Heat shock protein 70 overexpression affects the response to ultraviolet light in murine fibroblasts. Evidence for increased cell viability and suppression of cytokine release; *J. Clin. Invest.* **95** 926–933

Sreedhar A S and Csermely P 2004 Heat shock proteins in the regulation of apoptosis: new strategies in tumor therapy: a comprehensive review; *Pharmacol. Ther.* **101** 227–257

Srivastava P 2004 *Studies on the constitutively expressed members of Hsp60 anf Hsp70 gene families in Drosophila melanogaster*, PhD. Dissertation, Banaras Hindu University, Varanasi, India

Staal S P 1987 Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma; *Proc. Natl. Acad. Sci. USA* **84** 5034–5037

Stankiewicz A R, Lachapelle G, Foo C P, Radicioni S M and Mosser D D 2005 Hsp70 inhibits heat-induced apoptosis upstream of mitochondria by preventing Bax translocation; *J. Biol. Chem.* **280** 38729–38739

Stokoe D, Stephens L R, Copeland T, Gaffney P R, Reese C B, Painter G F, Holmes A B, McCormick F and Hawkins P T 1997 Dual role of phosphatidylinositol-3,4,5-trisphosphate in the activation of protein kinase B; *Science* **277** 567–570

Van Gurp M, Festjens N, van Loo G, Saelens X and Vandenabeele P 2003 Mitochondrial intermembrane proteins in cell death; *Biochem. Biophys. Res. Commun.* **304** 487–497

Veereshwarayya V, Kumar P, Rosen K M, Mestril R and Querfurth H W 2006 Differential effects of mitochondrial heat shock protein 60 and related molecular chaperones to prevent intracellular beta-amyloid-induced inhibition of complex IV and limit apoptosis; *J. Biol. Chem.* **281** 29468–29478

Volker U, Mach H, Schmid R and Hecker M 1992 Stress proteins and cross-protection by heat shock and salt stress in Bacillus subtilis; *J. Gen. Microbiol.* **138** 2125–2135

Volloch V, Gabai V L, Rits S and Sherman M Y 1999 ATPase activity of the heat shock protein hsp72 is dispensable for its effects on dephosphorylation of stress kinase JNK and on heat-induced apoptosis; *FEBS Lett.* **461** 73–76

Vydra N, Malusecka E, Jarzab M, Lisowska K, Glowala-Kosinska M, Benedyk K, Widlak P, Krawczyk Z and Widlak W 2006 Spermatocyte-specific expression of constitutively active heat shock factor 1 induces HSP70i-resistant apoptosis in male germ cells; *Cell Death Differ.* **13** 212–222

Wang C Y, Mayo M W, Korneluk R G, Goeddel D V and Baldwin A S, Jr. 1998 NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation; *Science* **281** 1680–1683

Warrick J M, Paulson H L, Gray-Board G L, Bui Q T, Fischbeck K H, Pittman R N and Bonini N M 1998 Expanded polyglutamine protein forms nuclear inclusions and causes neural degeneration in *Drosophila*; *Cell* **93** 939–949

White K, Tahaoglu E and Steller H 1996 Cell killing by the *Drosophila* gene reaper; *Science* **271** 805–807

Willis S, Day C L, Hinds M G and Huang D C 2003 The Bcl-2-regulated apoptotic pathway; *J. Cell Sci.* **116** 4053–4056

Wyllie A H, Kerr J F and Currie A R 1980 Cell death: the significance of apoptosis; *Int. Rev. Cytol.* **68** 251–306

Xanthoudakis S, Roy S, Rasper D, Hennessey T, Aubin Y, Cassady R, Tawa P, Ruel R, Rosen A and Nicholson D W 1999 Hsp60 accelerates the maturation of pro-caspase-3 by upstream activator proteases during apoptosis; *EMBO J.* **18** 2049–2056

Xia W, Voellmy R and Spector N L 2000 Sensitization of tumor cells to fas killing through overexpression of heat-shock transcription factor 1; *J Cell Physiol* **183** 425–431

Yan N and Shi Y 2005 Mechanisms of apoptosis through structural biology; *Annu. Rev. Cell Dev. Biol.* **21** 35–56

Yang X, Khosravi-Far R, Chang H Y and Baltimore D 1997 Daxx, a novel Fas-binding protein that activates JNK and apoptosis; *Cell* **89** 1067–1076

Zha J, Harada H, Yang E, Jockel J and Korsmeyer S J 1996 Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-X(L); *Cell* **87** 619–628

Zhang R, Luo D, Miao R, Bai L, Ge Q, Sessa W C and Min W 2005 Hsp90-Akt phosphorylates ASK1 and inhibits ASK1-mediated apoptosis; *Oncogene* **24** 3954–3963

Zhang S Q, Kovalenko A, Cantarella G and Wallach D 2000 Recruitment of the IKK signalosome to the p55 TNF receptor: RIP and A20 bind to NEMO (IKKgamma) upon receptor stimulation; *Immunity* **12** 301–311

Zou H, Li Y, Liu X and Wang X 1999 An APAF-1.cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9; *J. Biol. Chem.* **274** 11549–11556

*e*Publication: 22 March 2007