
Heat shock genes – integrating cell survival and death

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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- κ B 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- κ B 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 *hsr ω* 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.

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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 *hsr ω* 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

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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 *hsr ω* 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- κ B (NF- κ B) (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).

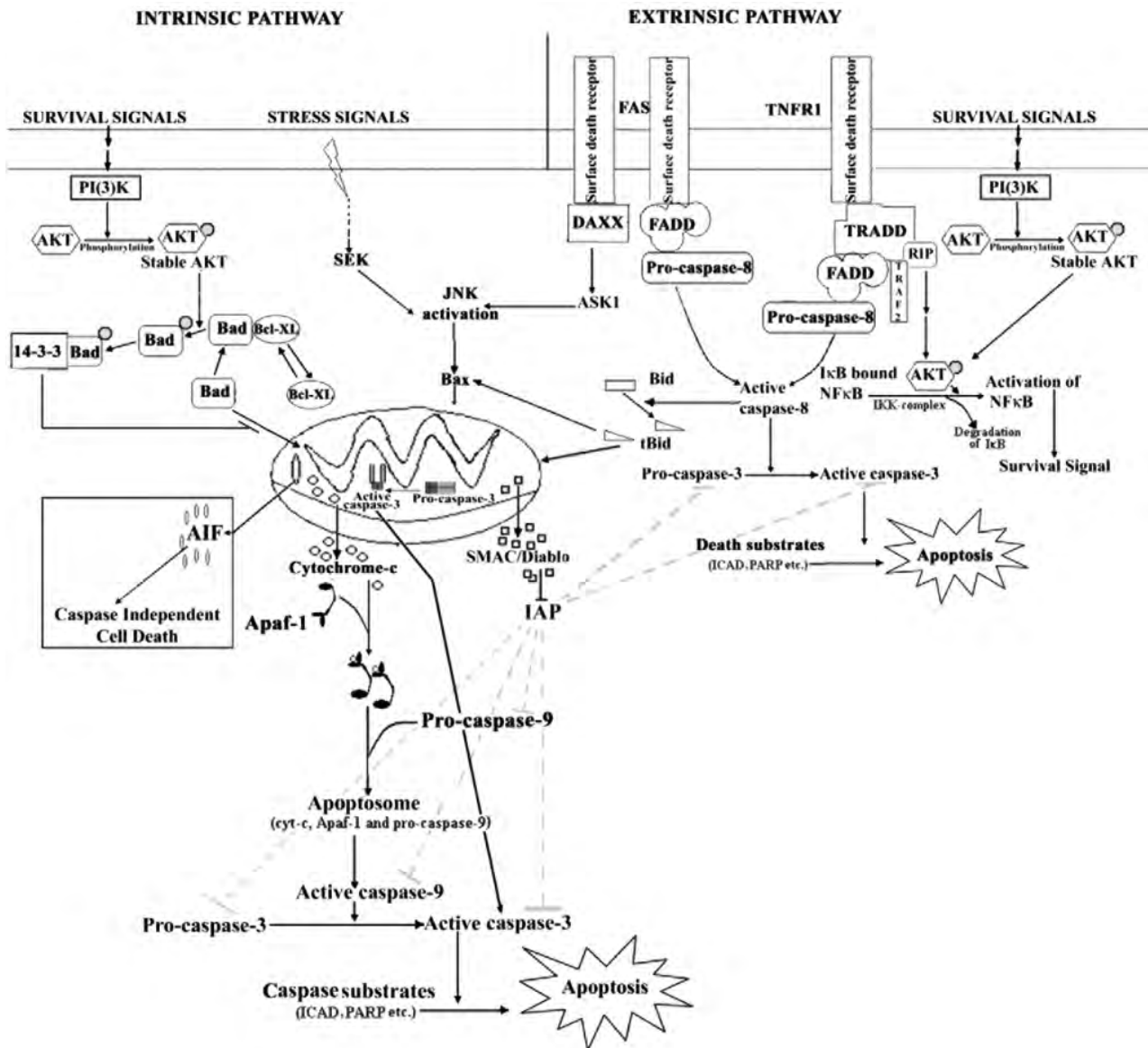


Figure 1. A schematic of intrinsic, extrinsic and AIF-mediated caspase independent pathways of apoptosis and their inter-connections with cell survival pathways. A small blue circle on a box indicates phosphorylated state of the named protein. Arrows indicate promotional while lines with small bar at 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- κ B, an important link in various biological processes including stress response, cell growth or death (Ghosh 1999). Under normal conditions, NF- κ B remains bound and sequestered in the cytosol by its inhibitor I κ B (DiDonato *et al* 1997; Regnier *et al* 1997). However, upon exposure to stimuli, including TNF, I κ B is degraded, resulting in release of NF- κ B, 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 I κ B

is mediated by a protein kinase complex, I κ B 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- κ B 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

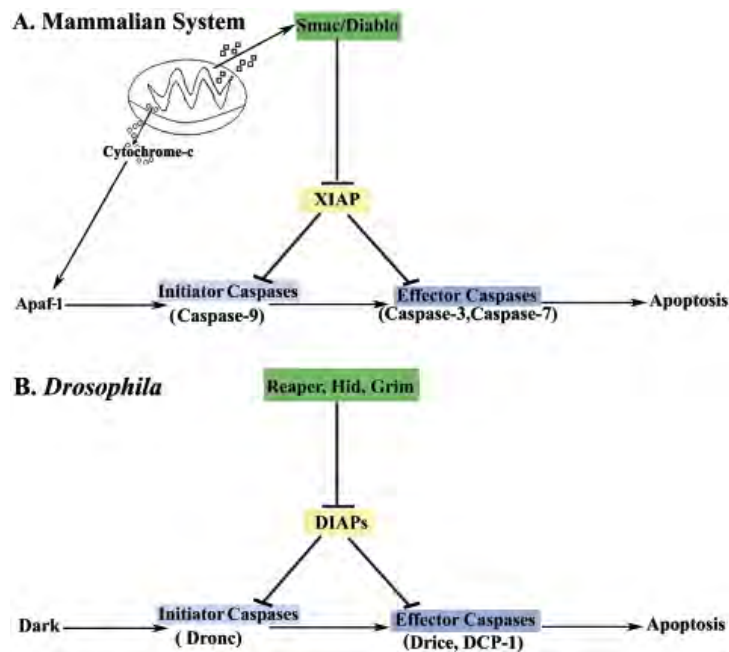


Figure 2. Comparison of the execution steps in mammalian (A) and *Drosophila* (B) apoptotic machinery. Similar colours indicate functional homology between proteins in mammals and *Drosophila*.

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 mitochondria 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- κ B (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- κ B 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 I κ B and thereby cause disassociation of NF- κ B 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 consequent inactivation of ASK-1 (figure 3), which is one of the activators of JNK (Zhang *et al* 2005).

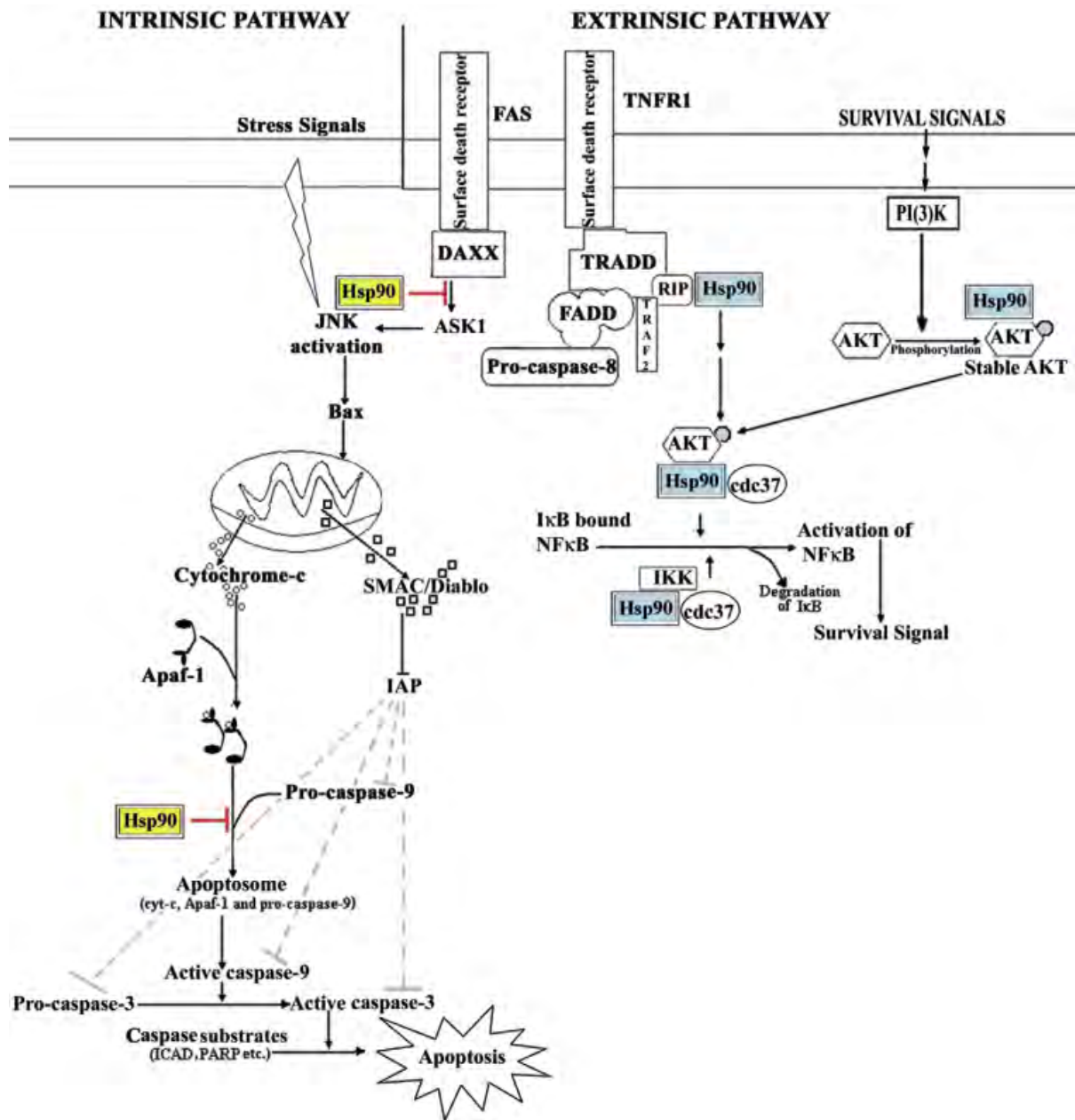


Figure 3. Anti-apoptotic roles of Hsp90 (yellow boxes) are mediated through inhibition of (red lines) activation of ASK-1 or procaspase-9. In addition, Hsp90 (blue boxes) is also an integral component of many complexes that help in cell survival. A small circle on a box indicates phosphorylated state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

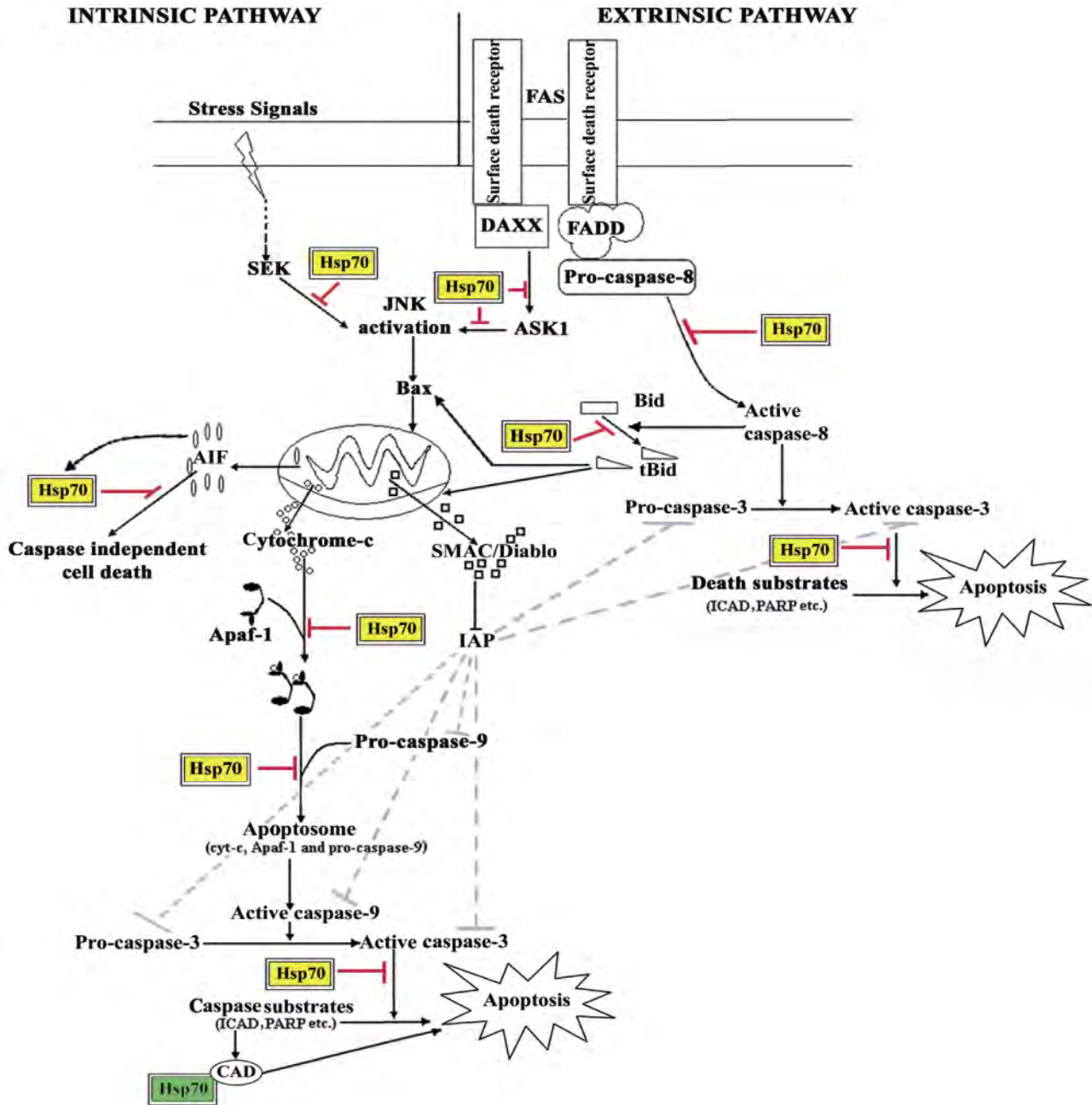


Figure 4. Hsp70 (yellow boxes) is generally anti-apoptotic since it inhibits (red lines) many steps in the apoptotic pathways. However, Hsp70 (green box) has a pro-apoptotic role also since it is required for activity of the caspase-activated DNase (CAD).

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

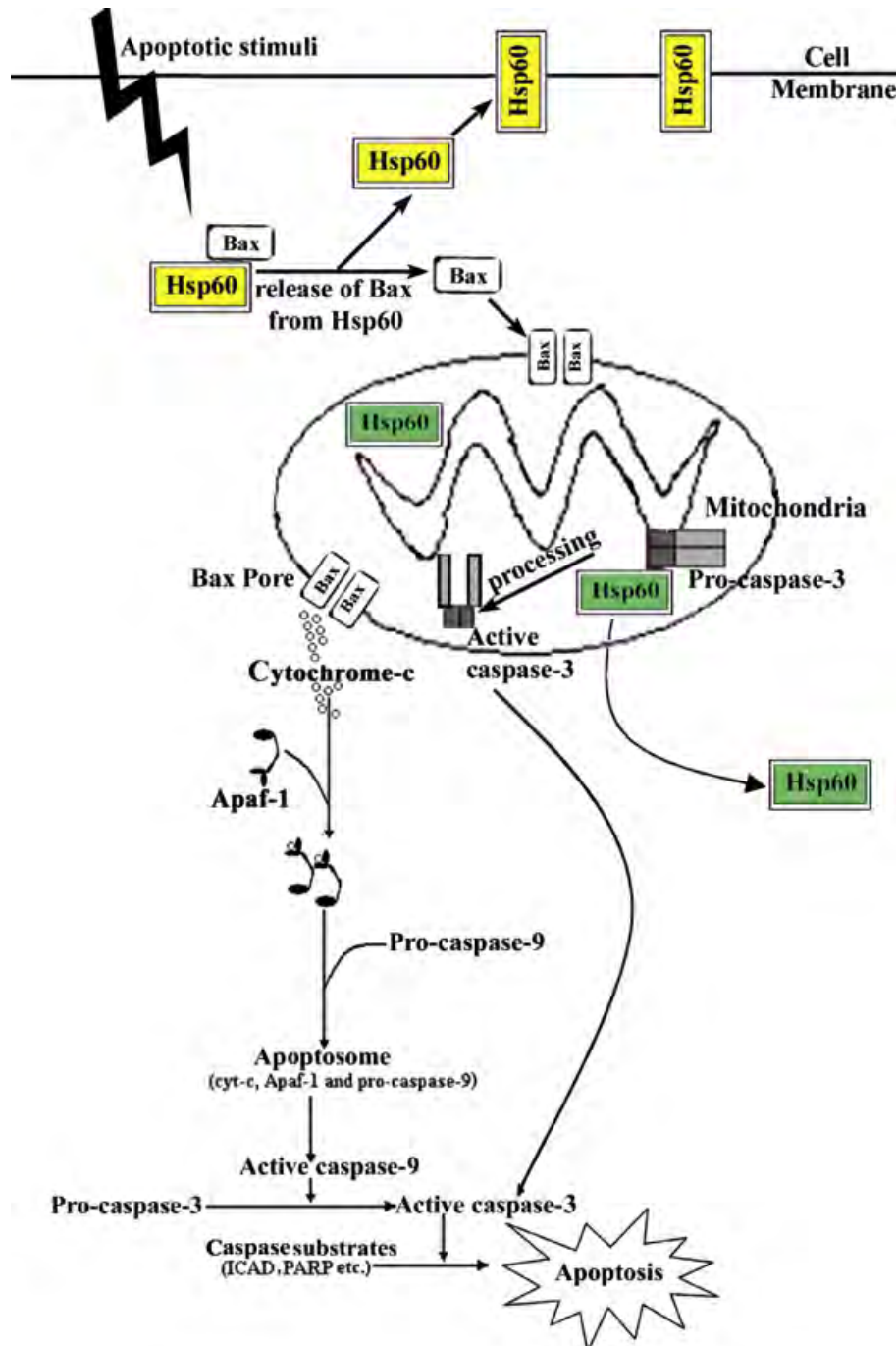


Figure 5. Anti- and pro-apoptotic roles of Hsp60. Cytoplasmic Hsp60 (yellow boxes) inhibits cell death by sequestering Bax and thereby, preventing its translocation to the mitochondrial membrane. On the other hand, mitochondrial Hsp60 (green boxes) promotes maturation of procaspase-3 and thus, has pro-apoptotic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

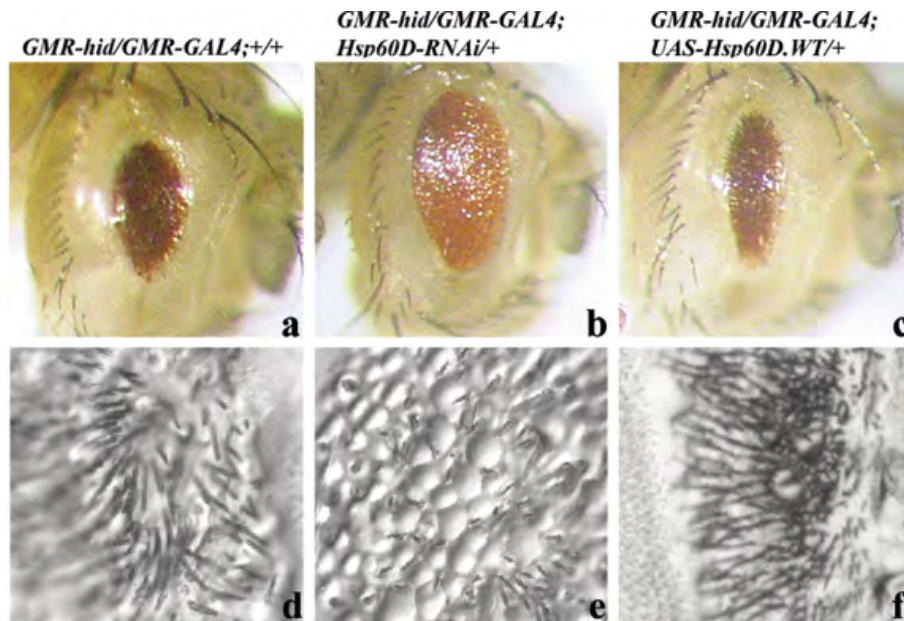


Figure 6. Hsp60D is required for cell death caused by over-expression of Hid in developing *Drosophila* eye discs. Images in **a-c** are photomicrographs while those in **d-f** are nail polish imprints (Arya and Lakhotia 2006) of adult eyes. Over-expression of Hid under *GMR* promoter in developing eye 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 I κ B

degradation and thereby, preventing NF- κ B 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 *hsr ω* transcripts in *Drosophila*

The *hsr ω* 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 *hsr ω -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

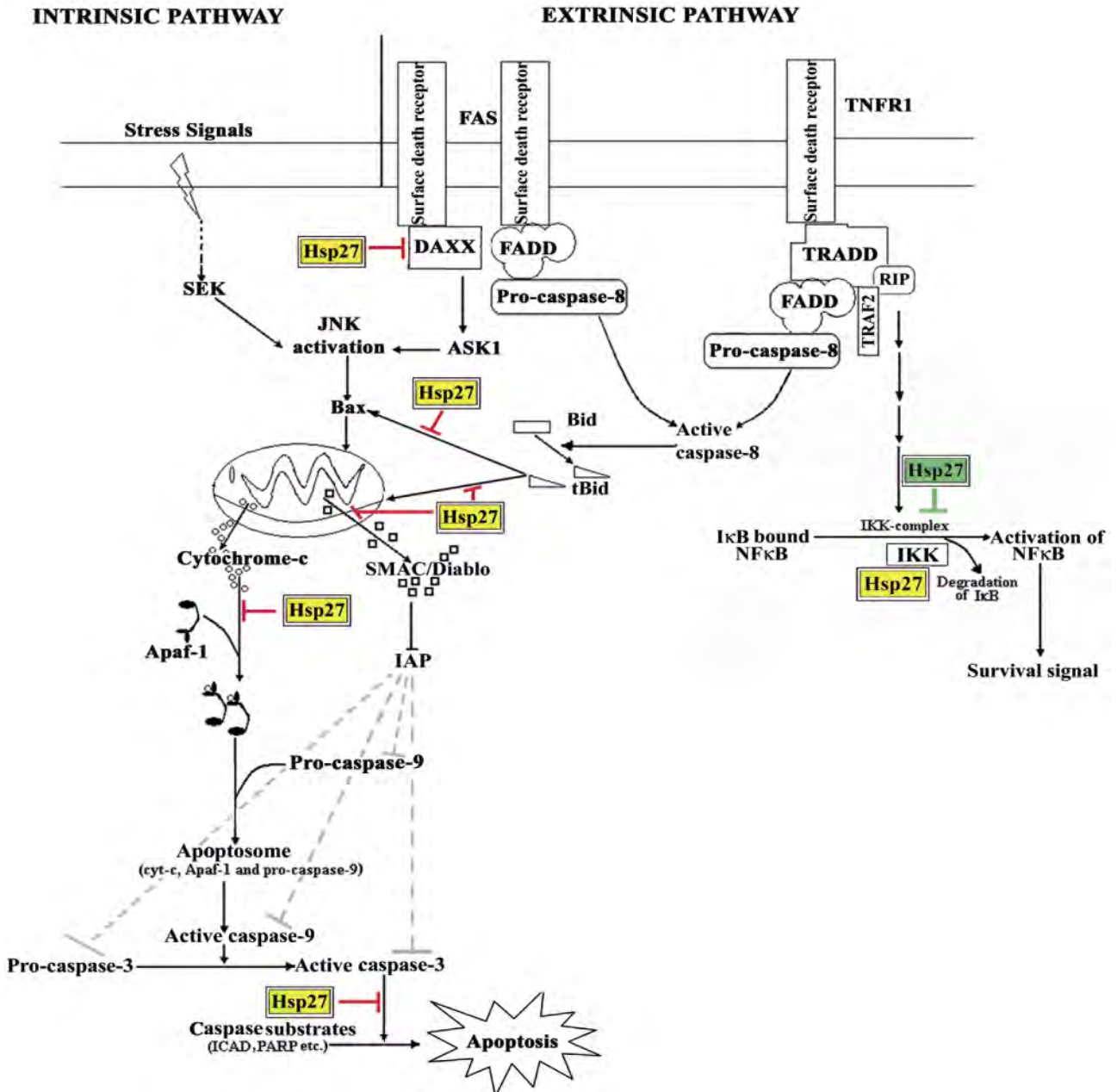


Figure 7. Anti-apoptotic (yellow) and pro-apoptotic (green) roles of Hsp27 in intrinsic and extrinsic cell death pathways (see text for details).

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 *hsw* transcripts enhances polyQ-induced

neurodegeneration (Sengupta and Lakhotia 2006). To gain a better understanding of the physiological roles of the non-coding *hsw* gene, we have employed GAL4 driven over-expression (EP mediated) or ablation (RNAi mediated) of *hsw* transcripts and examined the ensuing developmental/phenotypic abnormalities. Our results (Mallik M and Lakhotia S C, in preparation) show that ablation of the *hsw-n* transcripts through RNAi in developing eye disc

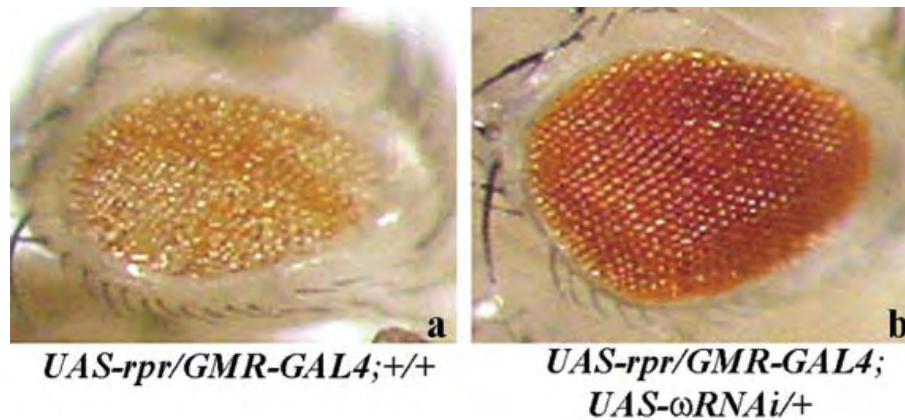


Figure 8. Absence of the non-coding *hsr ω* transcripts suppresses induced cell death. Ectopic expression of the apical cell death trigger Reaper in photoreceptor neurons and accessory pigment cells of developing eye discs under control of the *GMR-GAL4* driver results in massive cell death in the developing 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 *hsr ω* transcripts on apoptosis following activation of Reaper and other apical cell death triggers in developing retina. It is seen (Mallik M and Lakhota 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 *hsr ω* 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 *hsr ω* transcripts on apoptosis triggered by other cell death stimuli (Mallik M and Lakhota 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 *hsr ω -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 *hsr ω -n* transcripts by RNAi, affects the nuclear hnRNP metabolism and this may affect apoptosis. Association of *hsr ω* transcripts with Hsp90 may also be significant in this context. Association or a direct interaction of the *hsr ω -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 *hsr ω -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 *hsr ω -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.

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