

On-line review: Hsp90 and the small stress proteins

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Stress proteins across species

Stress proteins are fairly ubiquitous among living things, ranging from bacteria to humans. There are several families of heat shock or stress proteins, defined on the basis of molecular weight (for review, see [1]). These include

- Hsps of 100 and higher mw
- The hsp90 family
- The hsp70 family
- The hsp60 family
- The “small” hsps with mw in the 20s or 30s
- Hsp15, a nucleic acid-binding protein associated with ribosomes

We have been particularly interested in the hsp90 family and in the small hsps

Hsp90

For a review of the hsp90 family, see [2]. Within the 90kDa hsp family are a prokaryotic protein and two eukaryotic proteins. Hsp90 is a eukaryotic cytosolic protein that occurs in two isoforms (α and β); grp94 is a eukaryotic protein localized to the endoplasmic reticulum. Hsp90 is a hydrophobic [3], phosphorylated dimer [4] that can form oligomers in the presence of nonionic detergents [5]. It has a preference for binding to positively charged or hydrophobic proteins [6].

In humans, it is primarily the hsp90- α gene that is inducible. This gene is located on chromosome 14 [7]; the constitutive β gene is located on chromosome 6 [8]. Hsp90 β is unevenly distributed in the cytoplasm, but does seem to be concentrated near the nuclear membrane [9]. The presence of hsp90 on the cell surface may indicate a role in presenting proteins to immune cells. In fact, geldanamycin (a specific inhibitor of hsp90 activity which binds to the N terminal chaperone site) blocks the CD28 costimulation signal for T lymphocytes [10].

Both isoforms of hsp90 contain introns, which is unusual for hsps in general. Gene expression is regulated by binding of the heat shock factor to the heat shock element. Hsp90 levels appear to be induced during differentiation, and drop as cells reduce proliferative rates [11]. Stress produces an increase in oligomerization of the molecule [12], as well as an increase in hydrophobicity [13]. Interestingly, hsp90 is not induced during ischemia, but is during reperfusion [14].

One of the best known interactions of hsp90 is with steroid hormone receptors including glucocorticoid, progesterone, and estrogen receptors. Hsp90 binds to the hormone-binding domain of these receptors, keeping them partially unfolded in the configuration that binds the hormonal ligand with the highest affinity [15]. Steroid binding leads to dissociation of hsp90, allowing and enhancing nuclear translocation [16]. Another potential role of hsp90 is to block aggregation of unstable proteins [17] by binding to partially denatured proteins and stabilizing them for refolding by other proteins [18].

Hsp90 also binds to but is not phosphorylated by tyrosine kinases, a process which may also involve p50. It appears to be important in the functioning of development-related protein kinases [19]; [20]. Recent evidence also implicates hsp90 in the stabilization of mutant proteins in *Drosophila*, allowing mutations to accumulate in the genome [21]. Hsp90 also binds to actin [22] but not in the presence of ATP [23]. In fact, decreases in ATP levels may lead to an increase in association with actin [24]. Binding of ATP to hsp90 induces conformational changes in the protein [25]. Binding of tubulin [26] may lead to stabilization of the cytoskeleton during stress, although some studies have indicated that hsp90 inhibits tubulin polymerization [27].

Geldanamycin can deplete p185-erbB2 which is a tyrosine kinase overexpressed in some cancer cells [28]. V-SRC and Raf kinase may require hsp90 for binding to membranes [29] [30]. Hsp90 and grp94 are often upregulated in transformed cells [31], and reduced hsp90 levels appear to protect against TNF- α induced apoptosis. Recent studies indicate that hsp90 α mRNA levels are increased in cancer cells along with increased levels of cyclin D1 [32]. Hsp90 is also associated with expression of pgg [33] and is expressed constitutively in doxorubicin-resistant but not doxorubicin sensitive cell lines. *C. elegans* accumulates hsp90 in its dauer form [34].

In terms of effects of inhibitors of energy metabolism, one effect of exposure to sodium azide is a change in the ability of glucocorticoid receptors to interact with hsp90 and subsequently to bind hormone. Hu et al. [35] have demonstrated that exposing WEHI-7 cells to sodium azide causes a reduction in the state of phosphorylation of the glucocorticoid receptor, resulting in a significant reduction of the interaction between the hormone receptor and hsp90. The lack of interaction with hsp90 is the cause of the reduction in hormone binding.

In recent studies, hsp90 has been shown to mask a hidden reservoir of small mutations in the fruit fly. However, when the protein is engaged in a protective role it loses its ability to stabilize mutant proteins leading to their expression [21].

Small stress proteins

For a review of the small stress proteins see Arrigo and Landry [36] and Ciocca et. al. [37]. These proteins have molecular weights ranging from around 15 kDa to 30 kDa, and have divergent structures. What they have in common is an alpha-crystallin domain with several highly conserved segments. Most are strongly inducible, some are also constitutively expressed. Control of expression is most likely similar to control of expression of other hsps, involving binding of the heat shock transcription factor to the heat shock element.

Small hsps occur constitutively in a variety of tissues, including reproductive tissues, skin, muscle, and blood cells [38]. A 23 kDa stress protein has also been tentatively identified in macrophages [39]. In rats, hsp25 has a strong presence in the pituitary, gastrointestinal smooth muscle, vascular smooth muscle, and other smooth muscle types [40]. In terms of subcellular distribution, hsp27 has been shown to associate with the mitochondrial fraction [41]

Activation of one small stress protein, hsp27, involves phosphorylation [42], probably by a kinase whose activity is itself regulated by phosphorylation [43]. In fact, experiments have indicated that this kinase can be activated by IL-1 or TNF [44] [45] as well as p38 [46], and enhanced expression of hsp27 in transfected cells protected against TNF-mediated cytotoxicity as well as against oxidative stress [47]. Phosphorylation of hsp27 has been noted following heat stress, and dephosphorylation was observed to

follow ATP depletion [48]. There is evidence that phosphorylation following heat stress involves either a MAPK-activated protein kinase [49] or, in a separate pathway, an IL-1-stimulated protein kinase [50]

Hsp27 has been shown to be induced by hydroxyurea [51]; hsp25 is induced by some, but not all anticancer drugs [52]. In tumor cells, overexpression of hsp27 correlates with resistance to doxorubicin [53]. But, while expression of hsp27 has been associated with thermoresistance and chemoresistance, interestingly it does not confer radioresistance [54].

Several studies have elucidated the role of hsp27 in cell death. Inhibition of hsp27 induction potentiates release of cytochrome c following exposure to apoptotic agents, inhibiting caspase activation [41]. Hsp27 may also play a role in the mechanism by which cytotoxic T lymphocytes induce cell death. Hsp27 binds to the protease granzyme A, and low levels of hsp27 may prove to confer some degree of resistance to CTL attack [55]. Phosphorylated hsp27 also binds to Daxx blocking one Fas-related apoptotic pathway [56]. Hsp27 has also been shown to bind to cytochrome c and prevent interaction of Apaf-1 with procaspase-9 [57]. Evidence also exists that elevated expression of hsp27 leads to a rise in intracellular glutathione concentration, which then results in a decrease in the intracellular level of reactive oxygen species [58]. This may be mediated through increase in glucose-6-phosphate dehydrogenase activity, leading to increase in the levels of reduced GSH [59]

Hsp27 has also been shown to inhibit protein synthesis through binding to the factor eIF4F and preventing the formation of a cap-binding initiation sequence [60]. Hsp27 has also been observed to associate with actin microfilaments. Hsp28 appears to be a substrate of the serine protease myeloblastin, and as such may play a role in retinoid-induced differentiation [61].

The relationship between expression of small hsps in cancer cells and prognosis is not entirely clear. Overexpression of hsp27 in breast cancers has been associated with increase risk of relapse, but may in fact render those cells more susceptible to being targeted by some immune system cells [62]. Hsp27 has been detected in several, but not all different types of brain tumors, with expression roughly correlating with degree of malignancy [63] [64]. Expression of hsp27 has been linked to thermoresistance and drug

resistance in transformed cells [65] [66] [67], and tumors expressing MDR1 also tend to coexpress hsp27 [68]. Fibrosarcoma cells transfected with mouse hsp25 show enhanced growth, however, cells transfected with human hsp27 show less aggressive growth than nontransfected cells [69].

Interestingly, mammalian cell lines adapted to growth at low pH also show elevated hsp27 levels [70].

Other hsps in apoptosis and cancer

Other hsps have also been implicated in apoptosis, growth control, and cancer. Hsp70 has been implicated in oncogenic transformation [71], and increased hsp70 expression can protect cells from necrosis resulting from energy deprivation [72]. Hsp70 together with HSF1 have been shown to suppress Ras-induced transcription of the *c-fos* gene [73], and hsp72 has been shown to interfere with caspase activation [41]. In addition, overexpression of mtHSP70 has been shown to induce differentiation of HL-60 promyelocytic leukemia cells [74].

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