CHAPTER 4

# HEAT SHOCK PROTEINS AS THERAPEUTIC TARGETS IN AMYOTROPHIC LATERAL SCLEROSIS

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**Abstract:** Amyotrophic lateral Sclerosis (ALS) is a progressive neurodegenerative disorder characterized by the loss of motoneurons in the motor cortex, brainstem and spinal cord, resulting in paralysis and death within 1–5 years of diagnosis. Although the precise etiology of ALS remains elusive, approximately 20% of cases are known to be familial and of these approximately 10%–20% are due to mutations in the ubiquitously expressed human Cu/Zn superoxide dismutase (SOD1) gene. Transgenic mice that over-express the mutant human SOD1 (mSOD1) protein exhibit a phenotype and pathology that resemble that observed in ALS patients. ALS is widely regarded as a motoneuron-specific disorder but increasing evidence indicates that non-neuronal cells also play a significant role in disease pathogenesis. Some characteristics of the disease observed in mice and patient tissue, such as the presence of insoluble protein aggregates containing heat shock proteins (Hsps) as well as the apoptotic degeneration of motoneurons, suggest that manipulation of the heat shock response (HSR) may be a successful strategy for the treatment of ALS. In this chapter evidence for the involvement of the various Hsp families in disease pathology and their therapeutic potential is reviewed based on the molecular characteristics of the Hsp sub-families

**Keywords:** Motoneuron degeneration; motor neuron disease; protein aggregation; apoptosis; co-chaperones; HSF-1

## INTRODUCTION

Motor neuron diseases (MND) are a group of progressive disorders involving the nerve cells responsible for innervating voluntary skeletal muscles. There are four main types of MND: (i) Amyotrophic Lateral Sclerosis (ALS); (ii) Progressive Muscular Atrophy; (iii) Progressive Bulbar Palsy and (iv) Primary Lateral Sclerosis, although there can be a great deal of clinical and pathological overlap between

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each form. ALS is the generic term widely used in the USA, where it is also known as Lou Gehrig's disease, whereas MND is used more widely in Europe. ALS was first described in 1869 by the French neurologist, Jean-Martin Charcot (Charcot and Joffroy, 1869). It is a fatal, adult onset neurodegenerative disease that affects the upper and lower motoneurons in the brain and spinal cord. The disease is characterized by weakness and paralysis of voluntary skeletal muscles due to the progressive loss of motoneurons, ultimately leading to respiratory failure and death, usually within 2–5 years of diagnosis. The incidence of ALS is approximately 2 per 100,000 of the population, with prevalence at any point in time of 6 per 100,000. The vast majority of ALS cases are sporadic, with around 10% of cases which are familial (inherited). Approximately 120,000 new cases of ALS are diagnosed worldwide each year, and although it can strike at any age it is more commonly found in the 40–70 year age group. Despite intensive research, particularly during the past 10 years, there remains no effective treatment for this devastating disease. It is possible that the development of an effective disease-modifying therapy for ALS has been hindered by our relatively poor understanding of the pathogenesis of ALS. A major breakthrough came in 1993, with the discovery that approximately 10%–20% of the familial forms of ALS (FALS) were due to a mutation in the Cn/Zn Superoxide Dismutase 1 (SOD1) gene (Rosen et al., 1993) and to date, more than 100 mutations in the SOD1 gene have been discovered that are linked to ALS. Shortly following the discovery of mutant SOD1(mSOD1)-linked ALS, a transgenic mouse over-expressing the same mutant form of the human SOD1 protein was developed and this has become an indispensable tool for research not only investigating the pathogenesis ALS but also as an animal model for preclinical testing of potential therapeutic agents (Gurney et al., 1994).

## MECHANISMS UNDERLYING ALS PATHOLOGY

It is now clear that ALS is a multi-factorial disease in which a number of pathological mechanisms contribute to the selective and progressive degeneration of motoneurons. The sporadic and familial forms of ALS show both phenotypical and pathological similarities and so an understanding of the mechanisms involved in the death of motoneurons in SOD1 mice, which model the familial form, are likely to also be of relevance to the majority of sporadic cases of ALS (see Shaw and Eggett, 2000; Bruijn et al., 2004; Shaw, 2005; Boillee et al., 2006). Motoneurons have some specific functional and morphological characteristics that may actually contribute to their selective vulnerability in ALS. For example, as a consequence of their high metabolic load, motoneurons are particularly susceptible to excitotoxic insults and oxidative damage (Shaw, 2005), so that even healthy motoneurons are more susceptible to activation of AMPA receptors than other neuronal populations (Carriedo et al., 1996). Motoneurons also have particularly high energy demands (Briese et al., 2005) which makes them susceptible to mitochondrial dysfunction. Indeed, altered mitochondrial morphology, increased mitochondrial $Ca^{2+}$ levels, mitochondrial deposits of mutant SOD1 protein and reduced complex IV activity

have all been observed in tissue from both ALS patients and SOD1 mice (Siklos et al., 1996; Borthwick et al., 1999; Jaarsma et al., 2001; Menzies et al., 2002; Liu et al., 2004). Motoneurons also have some of the longest axons of all neurons in the human body, so that mutations or abnormal assembly of cytoskeletal neurofilament proteins will have a great impact on the function of motoneurons and, for example, alterations in neurofilament structure in motoneurons may lead to impaired axonal transport. Indeed, in approximately 1% of ALS cases a mutation in the neurofilament heavy chain has been identified and a number of neurofilament mutations have been shown to result in ALS-like symptoms in mice (Cote et al., 1993; Xu et al., 1993; Lee and Cleveland, 1994). Furthermore, functional deficits in axonal transport have also been observed in SOD1 mice, and these can be present prior to the onset of disease symptoms (Williamson and Cleveland, 1999; Ligon et al., 2005). In fact, deficits in axonal transport are present in motoneurons even during very early development and can be detected in E13 motoneurons cultured from embryonic SOD1 mice (Kieran et al., 2005). These deficits in axonal transport represent some of the very earliest pathological changes reported in SOD1 mice to date, suggesting that axonal transport defects may play a key role in ALS pathogenesis. Another hallmark feature of ALS which is also characteristic of other neurodegenerative disorders is the presence of intracellular ubiquitinated protein aggregates (Ince et al., 1998; Watanabe et al., 2001). However, although it has generally been accepted that such aggregates are toxic to neurons, recent results have suggested that aggregates may in fact either be inert by-products of the disease or may represent an effort of the part of cell to sequester and isolate toxic proteins (Gispert-Sanchez and Auburger, 2006). In either case, the presence of aggregates is suggestive of proteasome dysfunction, a result of which is the accumulation of ubiquitinated proteins that would have been otherwise degraded by the proteasome (Puttaparthi et al., 2003; Kabashi et al., 2004; Cheroni et al., 2005). There is evidence that aggregated mSOD1 can sequester otherwise vital proteins such as anti-apoptotic Bcl-2, cytoplasmic dynein and Hsps into the aggregates and so such aggregates can have toxic effects, even if only indirectly (Pasinelli et al., 2004; Matsumoto et al., 2005, 2006; Deng et al., 2006).

Although previously regarded as a motoneuron-specific disorder, surprising experimental evidence has recently established that ALS is a non-cell autonomous disease that requires multiple cell types and multiple disease factors to act together in order for the disease to manifest. The role of non-neuronal cells in ALS pathogenesis has been established in a number of elegant experiments which showed that expression of mSOD1 in either motoneurons or glia alone does not lead to disease (Gong et al., 2000; Pramatarova et al., 2001; Lino et al., 2002; Clement et al., 2003). Furthermore, increasing the proportion of healthy glia surrounding mSOD1-expressing motoneurons can protect the motoneurons from mSOD1 toxicity (Beers et al., 2006; Boillee et al., 2006). Activated microglia and astroglia mediate activation of inflammatory pathways (Kawamata et al., 1992; Alexianu et al., 2001). Microglial activation leads to the release of potentially neurotoxic reactive oxygen species (ROS), nitric oxide and proinflammatory cytokines (Elliott, 2001; Almer et al., 2002; Hensley et al., 2002). Astrocytes also play a critical role in ALS

pathogenesis and recent results have shown that mSOD1-expressing astrocytes release soluble factors that are toxic to motoneurons (Di Giorgio et al., 2007; Nagai et al., 2007). It has been suggested that ALS consists of distinct disease phases where pathological events in neurons play a key role in initiatiating disease onset whereas surrounding microglial cells influence disease duration (Boillee et al., 2006). However, the precise role of glial cells may be even more complex than currently suggested, as these cells are capable of not only initiating neurotoxic mechanisms but can also activate neuroprotective cascades (for review see Benarroch, 2005).

Ultimately, in ALS, pathological events involving non-neuronal cell populations and intracellular abnormalities within motoneurons eventually lead to the activation of the apoptotic cascade and subsequent death of motoneurons. In view of the multiple pathological pathways and cell populations that are involved, it is likely that the most effective therapeutic strategy for the treatment of ALS will involve targeting of multiple cellular sites and pathways. Increasing attention has recently focused on the possibility that manipulating ubiquitously expressed endogenous cellular defence pathways may be a successful approach to take in the treatment of neurodegenerative disorders. The heat shock response (HSR) forms part of such a cytoprotective mechanism that is present within all cells but until recently received little attention in the field of neurodegeneration in general and ALS in particular (Muchowski and Wacker, 2005). Heat shock proteins (Hsps) are a highly conserved, ubiquitiously expressed family of stress response proteins which are expressed at low levels under normal physiological conditions. However, in response to cellular stress the expression of Hsps is dramatically increased. Hsps can function as molecular chaperones, facilitating protein folding, preventing protein aggregation, or targeting improperly folded proteins to specific degradative pathways. However, some Hsps also play a role in regulating apoptosis by interacting directly with key components of the apoptotic pathway. Hsps are classified into subfamilies according to their molecular weight. In this Chapter the current understanding of the role of individual Hsps in the progression of ALS will be summarized, with a particular focus on the possible therapeutic implications that manipulation of this pathway may have for the treatment of ALS.

## MEMBERS OF THE SMALL HEAT SHOCK PROTEIN (SHSP) FAMILY OF STRESS PROTEINS

### Intracellular Effects of Small Heat Shock Proteins

Members of the small molecular weight Hsp family are more heterogeneous in their molecular weight than any other Hsp family, ranging from 16 to 40 kDa. The defining feature of this Hsp subfamily is the α-crystallin domain which is highly conserved from prokaryotes to mammals and which is thought play a crucial role in sHsp oligomerization following stress (de Jong et al., 1998; Haslbeck et al., 2005). Features common to all sHsps are firstly, that they can all be phosphorylated at

several sites and secondly, that they tend to form large oligomeric complexes, aggregates with a size of between 300 and 800 kDa. There are several reports that suggest that the phosphorylation state of sHsps influences their oligomeric size (Ehrnsperger et al., 1997). The phosphorylation of sHsps favors the dissociation of multimers (Rogalla et al., 1999; Hollander et al., 2004) in which form they act as molecular chaperones, providing protection against the negative effects of a diverse range of stress factors (Ciocca et al., 1993; Kato et al., 1994; Lelj-Garolla and Mauk, 2006). Interestingly, although there are numerous genes which are responsible for sHsp proteins, unlike other Hsp families and subfamilies, no specific "cognate" and "inducible" isoforms have yet been identified. sHsps are also distinct from other families of Hsps in that they do not bind and hydrolyse ATP in order to chaperone client proteins. Instead sHsps act as large chaperone oligomers, stably binding unfolded proteins. However, refolding of these non-native proteins requires the contribution of additional chaperone proteins, such as Hsp70.

The neuroprotective properties of sHsps are attributed not only to their ability to chaperone large proteins but to another intracellular function that is independent of their chaperoning activity. SHsps can bind to elements of cytoskeletal proteins such as intermediate filaments and microtubular proteins, particularly under conditions of stress, thereby stabilizing their cellular structure (Head and Goldman, 2000). SHsps participate in the process of actin polymerization/depolymerization, modulating the assembly of intermediate filament proteins and inhibiting the aggregation of tubulin. Un-phosphorylated Hsp27 is thought to act as an actin-capping protein, inhibiting actin polymerization when bound (Lavoie et al., 1993). In its un-phosphorylated form, Hsp27 also binds and sequesters actin monomers, making them unavailable for further polymerisation (During et al., 2007). Upon stress, Hsp27 is phosphorylated by p38 MAPK (Casado et al., 2007) causing a conformational change in the structure of Hsp27, resulting in the dissociation of Hsp27 from the barbed ends of actin filaments as well as from actin monomers. These monomers are then available in the cytosol for polymerisation with actin filaments (Pichon et al., 2004; During et al., 2007). This interaction of Hsp27 with cytoskeletan proteins is related to its protective function in a manner that promotes reorganization of actin filaments and stabilizes fibres when cells are exposed to stress (Beck et al., 2001; Head and Goldman, 2000). Another member of this family of sHsps, αB-crystallin, has also been shown under conditions of stress to be phosphorylated by p38 MAPK and to protect cytoskeletal filaments from destruction (Singh et al., 2007).

Another key role of sHsps is their anti-apoptotic function within normal cells (Tezel and Wax, 2000). Under conditions of stress and in the absence of Hsp27, markers of the apoptotic cascade such as cytochrome c and p38 phosphorylation rapidly increase (Schepers et al., 2005). However, in the presence of Hsp27, interaction between Apaf-1 and procaspase-9 is inhibited as a consequence of the binding of Hsp27 to cytochrome c (Garrido et al., 1999; Bruey et al., 2000; Garrido, 2002). There is also evidence that suggests that Hsp27 can inhibit the release of mitochondrial cytochrome c (Gorman et al., 2005; Schepers et al., 2005) and association

with Daxx, thereby inhibiting the motoneuron-specific molecular death pathway of Fas-Ask1-p38 mediated apoptosis (Charette et al., 2000; Raoul et al., 1999, 2002, 2005, 2006).

## Distribution of Small Heat Shock Proteins in Healthy Tissues

αB-crystallin was first identified as a structural protein in the lens of the eye, but it is now known to have a wider physiological role. For example, αB-crystallin is a component in the central nervous system, where it is expressed in oligodendrocytes and astroglia (Clayton and Truman, 1968; Iwaki et al., 1989). Other members of the sHsp family, such as Hsp27 and the mouse homologue Hsp25, are also constitutively expressed in the adult nervous system, predominantly in neurons (Plumier et al., 1997; Armstrong et al., 2001; Franklin et al., 2005). During embryonic and early postnatal development, neurons gradually acquire the ability to express Hsp25 and Hsp27 and this expression has been shown to be essential for the survival of sensory and motoneurons (Gernold et al., 1993; Kalmar et al., 2002a). Indeed, in a number of models of neurodegeneration, a loss of sHsp immunoreactivity within neurons is thought to contribute to their degeneration (Pieri et al., 2001; Suzuki et al., 2007). Interestingly, this reduction in neuronal expression of sHsps is accompanied by an increased expression of sHsps in glial cells (Pieri et al., 2001; Suzuki et al., 2007).

## Role of Small Heat Shock Proteins in ALS Progression

Although there is a normal pattern of expression of Hsp25 and Hsp27 in presymptomatic SOD1 mice (Strey et al., 2004), immediately prior to disease onset there is a transient loss in Hsp25 and Hsp27 immunoreactivity in spinal cord motoneurons (Maatkamp et al., 2004; Strey et al., 2004). However, by the late stages of disease, Hsp27 and αB-crystallin are up-regulated in the spinal cord of SOD1 mice (Vleminckx et al., 2002; Strey et al., 2004) although Hsp27 is predominantly present in the cytoplasm and nuclei of glial cells and some neurons and αB-crystallin is only present in the cytoplasm of reactive glial cells (Vleminckx et al., 2002; Wang et al., 2007). The absence of αB-crystallin and Hsp27 immunoreactivity in motoneurons in the late stages of disease, together with the fact that mSOD1 protein co-immunoprecipitates with Hsp25 (Strey et al., 2004), suggests that as disease progresses in SOD1 mice, sHsps become depleted in vulnerable motoneurons. This absence of Hsp27 in motoneurons during the later stages of disease in SOD1 mice is likely to contribute to the death of motoneurons and at the very least will render them more vulnerable to the high levels of stress that motoneurons are under, particularly during the later stages of disease. Indeed, it has been shown that the expression and phosphorylation of Hsp27 is essential for the survival of sensory and motoneurons in stressed conditions (Costigan et al., 1998; Benn et al., 2002; Kalmar et al., 2002a). A reduction in Hsp27 will increase the vulnerability of motoneurons

to factors that have been implicated in ALS pathogenesis including protein aggregation, axonal transport defects and apoptosis mediated by the FasL-ASK1-Daxx pathway, all characteristic cellular processes in which sHsps have been implicated.

## Therapeutic Potential of Small Heat Shock Proteins

There are a number of studies that describe the beneficial effects of sHsp up-regulation in models of neurodegenerative diseases including Parkinson's, Alzheimer's and Huntington's disease (Outeiro et al., 2006; Perrin et al., 2007). Since these neurodegenerative disorders are characterised by protein aggregation, it would seem likely that the beneficial effects of increased levels of sHsps in these models is due to their chaperoning capabilities. However, there is evidence that suggests that the neuroprotective effects of sHsps in models of neurodegeneration are in fact related to their anti-apoptotic and other cytoprotective capabilities including their ability to reduce neuroinflammation and to protect against oxidative damage and excitotoxicity (Wagstaff et al., 1999; Wyttenbach et al., 2002; Akbar et al., 2003; Kalwy et al., 2003; Masilamoni et al., 2006). Hsp27 is a potent promoter of regeneration and has the ability to support actin reorganization and therefore axonal growth (Hirata et al., 2003; Williams et al., 2006; Dodge et al., 2006). In vitro, up-regulation of Hsp27 is more effective at protecting SOD1 motoneurons against cellular stress when other Hsps such as Hsp70 and Hsp40 are also up-regulated (Patel et al., 2005; Batulan et al., 2006). However, in an in vitro model of mSOD1 toxicity, although transfection with Hsp27 did not provide any cellular protection, mild heat shock that resulted in massive up-regulation of Hsp27 saved these cells against a subsequent thermal stress (Krishnan et al., 2006). These controversial results are reflected in vivo, where it was found that over-expression of Hsp27 in transgenic mice prevents the extensive death of motoneurons that would normally occur following neonatal nerve injury (Sharp et al., 2006).

The majority of studies investigating the effects of increased sHsp expression employ transgenic over-expression models to increase intracellular levels of Hsps. However, genetic manipulation of Hsps may not be the most effective approach to take when attempting to develop a successful therapy for ALS. Firstly the difficulty of ensuring efficient delivery to motoneurons within the CNS in humans must be overcome. Moreover, although genetic manipulation may result in an increased expression of the protein, it may also disturb the fine balance that exists between Hsp expression and interaction with their co-chaperones and client proteins as well as, in case of sHsps, their phorphorylation level. Furthermore, for sHsps, the level of phosphorylation determines the size of intracellular oligomers and thereby determines their cellular function. In short, simply increasing the amount of sHsps such as Hsp27 within motoneurons may be futile if the sHsp is unable to carry out its cellular function. It may therefore be a more successful approach to pharmacologically manipulate or modify those intracellular functions which are critical for the survival and function of diseased neurons. An example of such a pharmacological agent is a small peptide construct called 35b that has been tested in

a model of Alzheimer's and shown to affect intracellular $Ca^{2+}$ levels, mitochondrial membrane potential, cytochrome c release as well as increasing Hsp27 expression (Faden et al., 2003, 2004). Thus, it appears that strategies that aim to simply increase levels of Hsp27 alone are likely to be less successful at rescuing motoneurons from cell death than an approach that aims to up-regulate Hsp27 in conjunction with other cytoprotective Hsps such as Hsp70.

## THE HSP70 FAMILY OF STRESS PROTEINS

### Role and Distribution of Hsp70 in the Healthy Nervous System

Members of the Hsp70 family are found in most cellular compartments including the cytosol, ER (grp78 or also known as BiP) and mitochondria (grp75). The cytosolic homologue consists of at least two isoforms: a constitutively expressed 73 kDa cognate form, called Hsc73 and an inducible 72 kDa isoform. There is little biochemical or biological/functional difference between the constitutive Hsp73 isoform and the highly inducible Hsp72 isoform, apart from different signals for their synthesis. Most Hsp70s possess ATP binding and hydrolysis activity in their N-terminal domain. Hsp70 family members bind to nascent proteins via their carboxyterminal domain (Hightower and Li, 1994). Members of the Hsp70 family differ primarily in their protein binding sites and their binding affinity to the nascent proteins. Hsp70 forms complexes with co-chaperone proteins that help to exert their activity and at the same time act as regulators of chaperone activity. The main positive regulator protein of Hsp70 is called Hip, which binds to the ATP-ase domain, upon which it is converted to the ADP-bound state. For ADP binding, the assistance of Hsp40 is necessary since Hsp70 has only a weak ATPase activity when not associated with Hsp40. Two other proteins, CHIP and Bag-1 have been described as negative regulatory proteins of Hsp70, inhibiting the ATPase-chaperoning activity of Hsp70 and instead redirecting client proteins towards the proteasome (Nollen et al., 2001; Song et al., 2001; Takayama and Reed, 2001). Thus, Hsc70 not only chaperones its client proteins but, with the help of its co-chaperones, it also assists in intracellular sorting, transport and degradation of proteins. Hence Hsc70 is also named as clathrin-uncoating ATPase, indicating its role in protein translocation within the cell. In the presence of ATP, Hsp70 protein binds to the clathrin coat of endocytotic vesicles, contributing to the removal of clathrin from the proteins. Hsp70 also capable of inducing to induce disaggregation of proteins, a widely accepted intracellular role of Hsp70 (Pelham, 1990). Another housekeeping function attributed to Hsp70 proteins relates to the translocation of proteins across membranes (Kang et al., 1990). By binding to unfolded nascent proteins, Hsp70 maintains the soluble form of the protein while being transported between organelles such as ER or mitochondria (Craig, 1990). This finding is confirmed by other studies showing that Hsp73 has a prolonged interaction with polypeptides that cannot fold properly, suggesting a role for members of the Hsp70 family in preventing aggregation of precursors and in stabilizing them in an unfolded

and therefore translocation-competent form (Becker and Craig, 1994). As expected of a housekeeping protein, Hsp70 is present in neurons and glial cells of the spinal cord throughout embryonic and postnatal development, but at relatively low levels which do not change with age (D'Souza and Brown, 1998; Loones et al., 2000; Kalmar et al., 2002a).

## Hsp70 in Cellular Stress

Among heat shock proteins, the inducible Hsp72 is thought to be the main cytoprotective protein, protecting cells against the damaging effects of a variety of stressors. Whilst Hsc73 levels are high in unstressed nervous tissue, levels of the inducible Hsp72 isoform are almost indetectable. There are a number of stress conditions that induce the expression of the inducible Hsp72 protein including hyperthermia (Brown, 1983; Manzerra et al., 1993; Satoh and Kim, 1994; Xia et al., 1998), CNS injury (Brown et al., 1989), axotomy (New et al., 1989; Tedeschi and Ciavarra, 1997), hypoxia (Kitamura, 1994), glutamate excitotoxicity (Armstrong et al., 1996), oxidative stress (Dastoor and Dreyer, 2000; Paschen et al., 2001) and ischemia (Bertrand et al., 2000). Induction of inducible Hsp70 synthesis is thought to be the most important mechanism by which mild heat shock or ischemia protects cells from subsequent and more toxic insults (Rordorf et al., 1991; Currie et al., 2000; Kelly, 2005; for review see Yenari, 2002). Following exposure to cell stress, the chances of cell survival are related to the ability of the cell to elevate Hsp70 synthesis (Chen and Brown, 2007). Indeed, in a model of Huntington's disease it has been shown that variations in the ability of different neuronal populations in the brain to increase Hsp70 expression in response to the expression of the expanded polyglutamine tract played a critical role in determining their vulnerability to cell death (Tagawa et al., 2007). For example, these authors found that Hsp70 induction was greatest in cerebellar granule cells that are resistant in Huntington's disease and that silencing Hsp70 in these cells results in an increased vulnerability of this neuronal population to extended polyglutamine tract toxicity.

In spite of the accumulating in vitro and in vivo evidence for the role of Hsp70 in cellular protection, the mechanism by which this cytoprotection is generated is still not clearly understood. Much of our current understanding about the mechanism of Hsp70 mediated neuroprotection comes from cell lines or transgenic mice that overexpress Hsp70. Evidence accumulated using a number of stress models including models of injury, ischemia, polyglutamine tract toxicity and Aβ-toxicity, all suggest that the neuroprotective effects of increased expression of Hsp70 are a consequence of increased chaperoning activity by Hsp70 (Uney et al., 1994; Amin et al., 1996; Plumier et al., 1997; Fink et al., 1997; Cummings et al., 2001; Kelly et al., 2001; Hoehn et al., 2001; Fujiki et al., 2003; Magrane et al., 2004). It is thought that most stress conditions, including pathological states, compromise the structural integrity of proteins, exposing otherwise hidden structural domains. The binding of Hsp70 may protect these functionally crucial domains, keeping the protein chaperoned and thereby preventing its aggregation (Becker and Craig, 1994). However, there is also

evidence that suggests vulnerable neurons do not necessarily benefit simply from increasing the levels of neuronal Hsp70. Studies on transgenic mice over-expressing the inducible Hsp70 have shown that Hsp70 over-expression does not protect against ischemic insults (Olsson et al., 2004). Similarly, Hsp70 over-expressing astroglia are no more resistant to oxygen–glucose deprivation injury than normal astroglia (Lee et al., 2001). However, these results do not necessarily signify that Hsp70 does not have a cytoprotective role, but rather they may highlight the likely importance of the concerted interaction of Hsp70 with a number of co-chaperones, an interaction that may be essential if Hsp70 is to exert its optimal cellular effects. Indeed, co-expression of Hsp70 in combination with its co-chaperone Hsp40 has been used successfully to reduce aggregate formation (Kobayashi et al., 2000; Bailey et al., 2002).

On the other hand, the intracellular effects of Hsp70 go beyond its ability to maintain proteins in a folding-competent state by keeping them chaperoned. It has recently been recognized that Hsp70 interacts with members of the cellular machinery that regulates the cell cycle and members of the apoptotic cascade, and therefore plays an important role in the maintenance and survival potential of cells. Thus, Hsp70 is also an anti-apoptotic protein and this function is independent from its chaperoning activity. In addition, through interaction with one of its co-chaperones, Bag-1, Hsp70 can also regulate cell growth. Bag-1 can interact with the anti-apoptotic protein Bcl-2 and the growth regulatory protein Raf-1 (Takayama and Reed, 2001). Hsp70 competitively inhibits Bag-1 and Raf-1 interaction by binding and sequestering Bag-1. This competitive inhibition can be very strong, particularly following heat shock, when levels of Hsp70 are elevated (Hohfeld and Jentsch, 1997; Townsend et al., 2003). An even more direct regulatory role of Hsp70 in cell survival and cell death decisions involves Hsp70 binding to Apaf-1 (apoptotic protease activating factor-1). Apaf-1 is normally activated by cytochrome c released from mitochondria and this activation leads to the formation of the apoptosome and the downstream activation of caspase-9 (Beere et al., 2000; Saleh et al., 2000; Li et al., 2000; Matsumori et al., 2006; for review see Beere and Green, 2001). It is therefore clear that a sufficient expression and functional activity of members of the Hsp70 family is essential for the maintenance of normal cellular homeostasis and cell survival under normal and stressful conditions.

Hsp70 also has intracellular actions which are independent of its chaperoning and anti-apoptotic roles. Increasing evidence suggests a complementary regulation between Hsp70 and inflammatory mediators such cyclooxygenase-2 (COX-2) and the transcription factor NFkappaB. In models of inflammation and stroke, exposure to a preconditioning stress or up-regulation of Hsp70 has been found to reduce the expression of COX-2 and production of nitric oxide (NO) (Feinstein et al., 1996; Van Molle et al., 2002; Ialenti et al., 2005; Jo et al., 2006; Zheng et al., 2008). These anti-inflammatory actions of Hsp70 are mediated by the binding of Hsp70 to NFkappaB and its subsequent inhibition. On the other hand, inflammation is itself a stimulus for increased Hsp70 production (Rockwell et al., 2000; Ianaro et al., 2001). A negative feedback mechanism exists between some inflammatory mediators and

Hsp70. For example, COX-2 over-expression inhibits Hsp70 synthesis following heat shock, and this inhibition of Hsp70 expression can be reversed by exposure to inhibitors of COX-2 (Ethridge et al., 1998). Moreover, in a model of cellular stress in which pharmacological inhibition of the proteasome results in Hsp70 up-regulation, the addition of NFkappaB inhibitors further increases Hsp70 synthesis, implying a negative regulatory role of NFkappaB in the machinery of the heat shock response (Rockwell et al., 2000). A number of studies using various pharmacological inducers of Hsps such as the immunosuppressant FK506, the Hsp70 inducer geranylgeranyl acetate (GGA) or the herbal drug celastrol, have all been shown to have anti-inflammatory effects (Pinna et al., 2004; Oltean et al., 2005; Sinn et al., 2007). Quite surprisingly, non-steroid anti-inflammatory drugs (NSAIDs) that inhibit COX-1 and COX-2 also increase Hsp70 synthesis. However, this action of NSAIDs is probably mediated by activation of heat shock factor 1 (HSF1) rather than by inhibition of NFkappaB (Housby et al., 1999). HSF1 is the transcriptional inducer of hsp gene expression in response to cell stress.

## Hsp70 in ALS

In the majority of familial and sporadic cases of ALS, intracellular aggregates containing ubiquitin, proteasomal proteins and Hsc70 are present in spinal cord motoneurons (Namba et al., 1991; Garofalo et al., 1991; Watanabe et al., 2001) and astroglia (Kato et al., 1997). However, the pattern of expression of Hsp70 in ALS spinal cord is somewhat controversial. For example, a recent study found no Hsp70 up-regulation in either motoneurons or astroglia of ALS patients (Batulan et al., 2003), whereas in the spinal cord of SOD1 mice, clear Hsc70 immunoreca-tivity was observed in motoneurons, localized to inclusions (Watanabe et al., 2001; Howland et al., 2002). However, overall levels of Hsp70 within the spinal cord of SOD1 micer were not generally elevated (Vleminckx et al., 2002; Batulan et al., 2003).

There is overwhelming evidence that the SOD1 mutation alters the solubility of the protein, making it more prone to aggregation (Deng et al., 1993; Shinder et al., 2001.). The increased tendency of mSOD1 to aggregate suggests that Hsps in general and Hsp70 in particular, may play a crucial role in ALS pathogenesis. A number of studies have described the association of mSOD1 with Hsps and have also demonstrated the presence of Hsp27, Hsp40 and Hsp70 within mSOD1 aggregates (Shinder et al., 2001; Howland et al., 2002; Matsumoto et al., 2005). This finding supports the hypothesis that during disease progression in ALS, the sequestration of housekeeping Hsps into protein aggregates reduces the amount of Hsps available to undertake the other cellular functions that they are normally involved in, including cytoprotection, resulting in an increase in the vulnerability of motoneurons to cell death (Okado-Matsumoto and Fridovich, 2002). Furthermore, there is also a significant reduction in chaperoning activity in the spinal cord of SOD1 mice (Bruening et al., 1999). This is significant since it has been shown in in vitro models of mSOD1 toxicity, that resistance to mSOD1 toxicity is strongly

correlated with chaperoning activity and that over-expression of Hsp70 can significantly reduce markers of disease including aggregation (Bruening et al., 1999; Koyama et al., 2006).

However, motoneurons have a surprisingly high threshold for the activation of HSF-1 and as a consequence a significantly higher threshold for the induction of Hsps than other cells (Batulan et al., 2003). This inability to respond to stressful stimuli by activating this endogenous cellular defence mechanism, may in part explain why motoneurons are particularly vulnerable. For example, motoneurons are selectively susceptible to mutations in SOD1 which are ubiquitously expressed in neural and non-neural cells, but only result in the death of motoneurons. With an impaired ability to increase Hsp70 expression in response to stress, the vulnerability of mSOD1-expressing motoneurons is exacerbated by the fact that mSOD1 binds Hsp70 much stronger than wild-type SOD1, effectively depleting mSOD1-expressing motoneurons of the little Hsp70 that they have (Batulan et al., 2003; Matsumoto et al., 2005). Furthermore, interaction between mSOD1 and Hsp70 not only depletes the cell of Hsps but also inhibits the normal anti-oxidant functions of SOD1. Thus, it has been shown that the binding between mSOD1 and Hsp70 inhibits the uptake of SOD1 into mitochondria, causing an accumulation of reactive oxygen species (ROS) in mitochondria in the absence SOD1, a key antioxidant enzyme (Okado-Matsumoto and Fridovich, 2002). However, in an in vitro model of ALS using cell lines transfected with wild-type (wt) and mSOD1, exposure to oxidative stress modifies the conformation of wtSOD1 causing it to bind to Hsp70 in the same way as mSOD1. Thus, following exposure to oxidative stress, wtSOD1 can acquire toxic properties which are similar to mSOD1, which cause Hsp70 to be sequestered. This in turn results in inhibition of Hsp70-dependent cytoprotective mechanisms, under conditions of stress when Hsp70 chaperoning actions are most needed (Ezzi et al., 2007). These recent findings may be important for our understanding of the pathological mechanisms underlying the majority of sporadic, non-mSOD1 cases of ALS and suggest that SOD-1 dysfunction might also be involved in these cases. This also suggests that strategies that target SOD1 dysfunction may well be applicable to both familial mSOD–ALS as well as the sporadic forms of ALS.

## Harnessing Hsp70 as a Therapeutic Target in ALS

Strategies that result in over-expression of Hsp70 have been shown to be neuroprotective in a number of models of stress such as thermal and ischemic damage as well as axotomy (Uney et al., 1994; Amin et al., 1996; Fink et al., 1997; Plumier et al., 1997; Hoehn et al., 2001; Kelly et al., 2001). The well known protective effects of a "preconditioning stress", in which a mild stress is applied prior to a toxic and damaging insult, is mediated by up-regulation of protective Hsps, mainly comprising Hsp70 (Sato et al., 1996; Currie et al., 2000; Ahn and Jeon, 2006). In neurodegeneration, up-regulation of Hsp70 appears to be particularly successful in models of Huntingtons, Spino-Cerebellar-Ataxia (SCA) and

Kennedy's Disease (KD) (otherwise known as Spino-Bulbar Muscular Atrophy; SBMA), where neurodegeneration is known to be associated with an expanted polyglutamine tract in a specific protein (Kobayashi et al., 2000; Cummings et al., 2001; Bailey et al., 2002; Waza et al., 2005; Tagawa et al., 2007). In these models, neuronal death is preceded by the presence of well characterized protein aggregates which decrease in both size and formation following over-expression of Hsp70. Similar protective, anti-aggregate effects of Hsp70 over-expression have also been observed in models of other neurogenerative diseases such as Aβ-Amyoid and α-synuclein induced toxicity (Magrane et al., 2004; Zourlidou et al., 2004; Sahara et al., 2007).

There are several features of the pathomechanism of ALS that suggest that a therapy based on manipulation of Hsp70 may be particularly effective. Hsp70 can unload the proteasome by keeping stressed proteins in a folded state and this capability also enables Hsp70 to reduce protein aggregation. On the other hand, Hsp70 can also inhibit the apoptotic pathway that is eventually activated as a consequence of dysfunctional intracellular protein control in motoneurons in the later stages of disease. Since motoneurons have a reduced capacity to activate HSF-1 in response to stress, manipulation of Hsp-70 may be particularly effective in protecting motoneurons against stress (Batulan et al., 2003). Furthermore, manipulation of Hsp-70 levels in non-neuronal cells such as astroglia may also be effective in protecting motoneurons since it has been shown that even under normal conditions, motoneurons are supplied with essential Hsps by neighbouring astroglia (Hightower and Guidon, 1989; Guzhova et al., 2001). This hypothesis of a motoneuron–astroglia functional unit is supported by some evidence which shows that exogenously applied Hsp70 can be taken up by motoneurons (Robinson et al., 2005). Therefore, strategies that aim to increase Hsp-70 within motoneurons and astroglia may not only overcome the inherent inability of motoneurons to up-regulate Hsps in response to stress, but by increasing Hsp-70 expression within astroglia, such an approach will also support astroglial function, itself critical for motoneuron survival, and provide motoneurons with an additional source of Hsp-70 to be taken up as needed.

The experimental evidence for the beneficial effects of an Hsp70-based therapy for ALS are, however, very controversial. In in vitro models of mSOD1 toxicity, Hsp70 over-expression has been found to reduce aggregate formation, improve chaperoning activity and increase cell survival (Bruening et al., 1999; Koyama et al., 2006). These cytoprotective effects of Hsps are not restricted to Hsp-70 since over-expression of multiple Hsps, such as Hsp70, Hsp40 and Hsp27 is a more successful strategy to optimize the intracellular heat shock response in vitro (Takeuchi et al., 2002; Patel et al., 2005; Batulan et al., 2006). Unfortunately, these promising in vitro results have not always translated into successful in vivo strategies. Thus, although Hsp70 over-expression in vitro reduces aggregate formation and improves survival of primary motoneurons derived from SOD1 mice, increasing Hsp-70 expression in SOD1 mice in vivo, by crossing them with transgenic mice over-expressing Hsp70, has no effect on

disease progression or lifespan of SOD1 mice (Liu et al., 2005). Perhaps even more surprising was the finding that the Hsp70 over-expressing mice were as vulnerable to ischemic damage as wild-type mice (Olsson et al., 2004) and that astrocyte cultures from brains of Hsp-70 over-expressing mice were less resistant to hypoglycemia and oxygen–glucose deprivation than wild-type astroglia (Lee et al., 2001).

These findings suggest that transgenic models which have been generated to synthesize one particular Hsp behave differently and less effectively, from models in which the endogenous heat shock response is activated for example, following exposure to a preconditioning stress. However, all actions of Hsps involve a finely tuned interaction between Hsps and their co-chaperones and client proteins. It is therefore possibly more surprising that increased expression of a single Hsp, such as Hsp70, in the absence of targeting of co-chaperones, has any neuroprotective effects at all! Certainly, co-induction of multiple Hsps is a more successful strategy than up-regulation of individual hsps (Takeuchi et al., 2002; Patel et al., 2005; Batulan et al., 2006). Furthermore, since upregulation of most hsps is induced by HSF-1, strategies that activate this main heat shock transcription factor are likely to be particularly effective in protecting cells from stress. Under cellular stress HSF1, which normally resides in the cytosol, trimerizes and migrates to the nucleus. In the trimeric state, HSF1 has a high affinity for cis-acting DNA sequence elements, the heat shock elements (HSEs) in the promoter region of heat shock protein genes. The bound trimer forms a complex which is capable of activating transcription of the hsp gene. In vitro, transfection with a constitutively active HSF-1 offers significant protection against mSOD1-induced toxicity (Batulan et al., 2003). We have observed similar neuroprotective effects in vivo from pharmacological targeting of HSF-1 using a novel Hsp co-inducer called arimoclomol (Kieran et al., 2004). Arimoclomol is a member of a family of non-toxic hydroxylamine derivatives that have been shown to up-regulate Hsp40, Hsp70 and Hsp90, by prolonging the activation of HSF-1 (Vigh et al., 1997; Kalmar et al., 2002b; Hargitai et al., 2003; Kieran et al., 2004). We found that treatment of SOD1 mice with arimoclomol delays disease progression, increases motoneuron survival and significantly extends lifespan (Kieran et al., 2004). Celastrol is an herbal medicine that has a similar mechanism of action as arimoclomol and which has also been shown to be effective in extending the lifespan of SOD1 mice (Kiaei et al., 2005). However, although the results of these studies confirm that activation of HSF-1 can have significant neuroprotective effects, it is unlikely that these effects are simply the result of an up-regulation in the expression of specific Hsps such as Hsp70 and Hsp90. Indeed, it is likely that the neuroprotective effects of HSF-1 activation are the result of a number of diverse effects of HSF-1 that are independent of its ability to induce Hsp expression, which may help to explain why pharmacological manipulation of Hsp induction by activation of HSF-1 is more successful in protecting motoneurons than genetic manipulation of the expression individual Hsps (Liu et al., 2005).

## THE HSP90 FAMILY OF STRESS PROTEINS

Most members of the Hsp90 family are cytosolic. There are two Hsp90 isoforms, Hsp90α (Hsp84) and Hsp90β (Hsp86). Hsp90α is the more inducible isoform, whereas Hspβ, sometimes called Hsc90, is less inducible and is mainly expressed constitutively as a cognate form. The N terminal domain of Hsp90 contains an ATP binding site, a sequence that is structurally homologous with the type II topoisomerase DNA gyrase B (Pearl and Prodromou, 2000). The binding site for geldanamycin, a functional blocker of Hsp90 ATPase is also located in the N terminal domain (Prodromou et al., 1997). The primary binding site of Hsp90 for other co-chaperones is located on the C terminal region of the protein. This site binds to the partner protein containing the sequence called tetratricopeptide repeat, TPR (Pearl and Prodromou, 2000). Hsp90 exists in the cell as phosphorylated dimer (Rose et al., 1987) and this dimerisation is essential for its physiological function. The protein has an ATP binding site and is capable for autophosphorylation (Csermely and Kahn, 1991). Upon binding to ATP, the Hsp90 dimer structure undergoes substantial changes so that from an open form, in which the N terminal domains are separated, it transforms into a form that contains an associated ring formed by the two N terminal domains.

### Intracellular Functions of Hsp90

As a consequence of its own hydrophobic characteristics, Hsp90 binds positively charged or hydrophobic proteins. The client proteins of Hsp90, which can have widely different functions, include proteases, kinases and a group of nuclear hormone receptors including the glucocorticoid receptor, indicating a role for Hsp90 in differentiation and development (Pratt, 1997; Pratt and Toft, 1997). Apart from steroid receptor signalling, Hsp90 is known to play a role in the maturation and function of several tyrosine and serine/threonine kinases, such as V-Src, Wee-1, the cyclin dependent serine/threonine kinase Cdk4 and Raf and other enzymes, including nitric oxide synthase and calcineurin (Mayer and Bukau, 1999; Young et al., 2001; Pratt and Toft, 2003). It is most likely that the ability to interact with such a wide variety of substrates arises from similarities in the structural properties of these proteins rather than their cellular function. These proteins are very large, with a multi-domain structure and rely on co-chaperones such as Hsp90 to stabilize their structure while they undergo conformational changes as a consequence of the interactions with their own substrates. Due to variability in its substrates, Hsp90 plays a role in a number of cellular processes. Hsp90 interaction is required for the function of a series of oncogenic tyrosine kinases, so that inhibition of Hsp90 can disrupt this specific binding. Thus, geldanamycin, a specific Hsp90 inhibitor, is a promising candidate for anti-tumor therapy. Geldanamycin and another Hsp90 inhibitor 17-allylamino-17-demethoxy-geldanamycin (17-AAG) alter the Hsp90 complex with Raf-1 protein, which plays a significant role in MAPK pathway. Without the stabilizing interaction with Hsp90, client proteins are directed towards

the proteasome for degradation (Waza et al., 2006a). This leads to a decrease in Raf-1 levels and a disruption of the MAPK-mediated altered transcription factor signalling pathway (Bertorelli et al., 1998). Thus, Hsp90 may be an effective target for some cancer strategies (Pearl and Prodromou, 2000).

It is widely accepted that in most cases the de novo folding of proteins does not require Hsp90. However, a very restricted number of eukaryotic proteins are temporarily stabilized by Hsp90. These are molecules that have large hydrophobic surfaces that are important for binding to other functionally important substrates (Nathan et al., 1997). Hsp90 plays an important role in promoting disaggregation and refolding of denatured proteins and also has been shown to suppress the aggregation of unstable proteins (Miyata and Yahara, 1992). It binds to partially re-natured forms of proteins, maintaining their "folding-competent" state, so that they can be successfully refolded by other chaperones such as Hsc70 (Freeman and Morimoto, 1996).

Hsp90 and microtubular proteins have been shown to co-localize in a number of cell types (Fostinis et al., 1992; Liang and Macrae, 1997). Binding of Hsp90 to microtubules may explain their role in receptor trafficking, as translocation of proteins to organelles is usually mediated by the cellular microtubule-system. Hsp90 links cargo proteins to the dynein motor protein for retrograde transport along microtubules (Galigniana et al., 2004; Harrell et al., 2004). Hsp90 also contributes to the transport of some steroid hormones to the nucleus, a process that also requires some binding to the cytoskeletal system (Csermely et al., 1998). Hsp90 may also be associated with intermediate filaments although other cytoskeletal counterparts, such as microfilaments do not bind to Hsp90 (Redmond et al., 1989).

More recently, significant antiapoptotic properties have been attributed to Hsp90. In an in vitro model of apoptosis, over-expression of Hsp90, but not Hsp27 or Hsp70, provided significant protection against apoptosis (Lee et al., 2001). Hsp90 can interfere with the apoptotic cascade at multiple levels. Thus, Hsp90 can bind to Apaf-1, inhibiting the activation of the apoptotic complex and caspase-9 (Pandey et al., 2000). Through the binding to its client protein, Akt, Hsp90 phosphorylates and inactivates ASK1 (Zhang et al., 2005), which mediates Fas/Fasligand mediated apoptosis downstream from Daxx activation. This pathway has been specifically described in motoneurons during SOD1 mediated toxicity in ALS (Raoul et al., 2002). In addition, Hsp90 also interferes with inflammatory pathways by promoting NFkappaB activation through its co-chaperone cdc37 and its client IKK that phosphorylates NF-kappaB (Chen et al., 2002; Arya et al., 2007).

Grp94 is the Hsp90 isoform that is present in the lumen of the endoplasmic reticulum (ER). Similarly to Hsp90, grp94 is also an ATP binding protein that associates with a number of other proteins, including protein kinases, actin filaments and calmodulin. It can also associate with other ER chaperones such as grp78 (Melnick et al., 1992). However, unlike most Hsps, grp94 is a glycoprotein. Although Grp94 is constitutively expressed in all cell types, its expression is increased in response to various forms of stress including low glucose levels, ischaemia, low extracellular pH and viral infections (Argon and Simen, 1999).

A common feature of these stress conditions is that they all involve the accumulation of misfolded proteins within the ER. A change in the pattern of glycosylation has also been described following environmental stress, which results in an increased resistance of grp94 protein against endoglycosidase digestion (Booth and Koch, 1989). In addition to altered glycosylation, stress also induces the translocation of grp94 from the ER to the Golgi apparatus (Feldweg and Srivastava, 1995).

**Disribution of Hsp90 in Healthy and ALS Tissues**

Hsp90 is constitutively expressed in most mammalian tissues and in the nervous system it accounts 1%–2% of the total protein content (Heikkila, 1993; Loones et al., 2000). Hsp90 is up-regulated in vivo in response to various forms of stress, such as heat shock and ischaemia (Quraishi and Brown, 1995; Gasbarrini et al., 1998). Since Hsp90 is expressed at relatively high levels in neurons, up-regulation in Hsp90 levels following exposure to stress mainly occurs in astroglia and microglia. For example in mice, excitotoxic insults result in an increase in grp94 expression in astroglia and Hsp90 expression in microglia (Jeon et al., 2004). Furthermore, ischemic stress causes a more intense up-regulation of the endoplasmic grp94 than the cytoplasmic Hsp90 (Jeon et al., 2004), whereas oxidative damage causes a down-regulation of grp4 in primary neurons (Paschen et al., 2001).

In ALS tissue, weak Hsp90 immunoreactivity has been observed in sporadic and familiar ALS spinal cord sections, with expression mainly localized to inclusions (Watanabe et al., 2001). In spinal cord tissue of different strains of SOD1 mice, Hsp90 levels have been reported to be either normal ($SOD1^{G93A}$ mice; Kieran et al., 2004) or slightly elevated ($SOD1^{G85R}$ strain; Liu et al., 2005) during the late stages of disease.

**Therapeutic Potential of Hsp90 Manipulation in ALS**

Therapeutic manipulation of Hsp90 has been widely employed in cancer therapy, where inhibition of Hsp90 results in modifications in the cell cycle and selective degradation of Hsp90 client proteins that induce apoptosis in cancer cells (for review see Xiao et al., 2006; Cullinan and Whitesell, 2006). However, acting via an alternative mechanism, an Hsp90 targeted therapy may also be effective in the treatment of neurodegenerative diseases, particularly if aggregation of an Hsp90 client protein is involved. For example, in Kennedy's Disease (KD) a trinucleotide repeat expansion in the androgen receptor (AR) results in nuclear inclusions of the mutant AR with expanded polyQ in motoneurons and this eventually results in their death. In models of KD, treatment with Hsp90 inhibitors such as geranylgeranyl acetone (GGA), geldanamycin and 17-AAG have shown to be successful in reducing aggregation of the mutant androgen receptor by reducing the total amount of mutant AR (Katsuno et al., 2005; Waza et al., 2006a, b). However, this effect of Hsp90 inhibiton is not due to an enhanced heat shock response, but is instead the result of preventing Hsp90 from interacting with its disease-causing

client protein (Neckers, 2002; Zaarur et al., 2006; Waza et al., 2006b). Indeed, in some neurodegenerative diseases such as the tauopathies, it has been shown that Hsp90 maintains the mutant but not wild-type Tau protein (Luo et al., 2007). Inhibition of Hsp90 in cellular and mouse models of tauopathies leads to a reduction of the pathogenic activity of these proteins and results in elimination of aggregated Tau. Thus, in this model Hsp90 plays a crucial role in maintaining and facilitating the degenerative phenotype by binding to the aberrant protein. Thus, inhibition of Hsp90 provides a common platform for the development of therapies aimed at both cancer as well as neurodegenerative diseases (Luo et al., 2007).

An alternative hypothesis to explain the neuroprotective effects of Hsp90 inhibition involves the mechanism of activation HSF-1. It is possible that when HSF-1 activity is suppressed, an Hsp70–Hsp90 complex sequesters HSF-1. Interaction of geldanamycin with Hsp90 frees HSF-1 from the binding of Hsp70–Hsp90 complex, enabling HSF-1 activation and translocation into the nucleus where it binds to the HSE element of heat shock genes (Ali et al., 1998; Kim et al., 1999; Lu et al., 2002), whereas GGA preferentially binds to the C-terminal of Hsp70 that binds and inhibits the activation of HSF-1 (Otaka et al., 2007). In this way, inhibition of Hsp90 can ultimately lead to an up-regulation of other Hsps, such as Hsp40, Hsp70 as well as Hsp90. If this mechanism of action could be successfully harnessed in ALS, it may reduce inclusion formation in motoneurons and astroglia. Indeed, in mSOD1 expressing primary spinal cord neurons, 17-AAG results in a robust up-regulation of multiple Hsps, such as Hsp70 and Hsp40 and a reduction in inclusion formation (Batulan et al., 2006). Arimoclomol (also known as BRX-220) is a co-inducer of Hsps that has been shown to protective to motoneurons against injury-induced and mSOD1-induced cell death. Arimoclomol also up-regulates the expression of multiple Hsps in motoneurons and astroglia under stress conditions (Kalmar et al., 2002b; Kieran et al., 2004). Thus, it is possible that Hsp90 up-regulation may be beneficial in ALS but is likely to be most effective when working in conjunction with the induction of other Hsps, such as Hsp70 and Hsp40.

## HSP60 OR GROEL HOMOLOGUES RESIDING IN THE CYTOSOL AND MITOCHONDRIA

Members of this Hsp family are referred to by a variety of names, most commonly by the term chaperonin, although the terminology depends on when and in which organisms these Hsps were first described. In *Escherichia coli*, a 60 kDa protein and a 10 kDa protein that form the GroE complex were first described and named GroEL and GroES, respectively. All members of the Hsp60 family are large oligomeric ring-shaped proteins, consisting of 14 subunits arranged in two heptameric rings (for reviews see Sigler et al., 1998; Thirumalai and Lorimer, 2001). They contain a large hydrophobic central cavity in which non-native proteins can bind. In the mouth region of the central cavity is the ATP binding site (Fink, 1999). The chaperone function of Hsp60 is regulated by Hsp10, which binds to Hsp60 and regulates substrate binding and ATPase activity. In the presence of ADP, two Hsp10

molecules bind to one Hsp60 molecule. It is likely that the GroE family assists in the correct folding of newly synthesized proteins within the cytosol, but at a later stage in the folding process than members of the Hsp70 family. The GroE family tend to bind to partially folded intermediates, preventing their aggregation. In addition, the GroE complex folds proteins that are imported from the cytosol into the mitochondria (Welch, 1990). Although Hsp60 and Hsp70 proteins have very similar biochemical characteristics, their physiological functions are not interchangeable and they appear to act sequentially in a common pathway to facilitate the folding and assembly of proteins.

## Distribution and Function of Hsp60 in Healthy Tissues

Constitutive expression of Hsp60 has been described in both neural and non-neural tissues (D'Souza and Brown, 1998) with most Hsp60 family members located in mitochondria. However, more recently it has been discovered that a homologue of the mitochondrial chaperonins resides in the cytosol (Gupta and Knowlton, 2002; Kirchhoff et al., 2002). It appears that of the total cellular Hsp60 content, around 80% is present in mitochondria and the remaining 20% resides in the cytosol (Soltys and Gupta, 1996) in almost all tissue types, apart from cardiac and skeletal muscle where cytoplasmic Hsp60 is even more abundant (Gupta and Knowlton, 2005). Exposure to stress, such as ischemia or heat shock, results in Hsp60 up-regulation (Naylor et al., 1996; Izaki et al., 2001).

Since the mitochondrial genome encodes only a handful of proteins, a great deal of protein transport takes place through the mitochondrial membranes, shuttling essential proteins through membranes that function in the mitochondria but are encoded by the nucleus. Because these proteins can be bulky, when passing a membrane they need to first unfold and then at their destination, they need to refold into their original conformations. Mitochondrial chaperones assist this process of refolding. The requirement for this refolding is greater during mitochondrial biogenesis and mitochondrial molecular chaperones are therefore thought to play a critical role in mitochondrial biogenesis (for review see Voos and Rottgers, 2002; Deocaris et al., 2006).

Different members of the Hsp60 family have opposing roles in the process of apoptosis. The cytosolic Hsp60 binds to pro-apoptotic Bax and Bcl-XL thereby preventing activation of the apoptotic cascade (Lin et al., 2001; Kirchhoff et al., 2002; Shan et al., 2003; Gupta and Knowlton, 2005). On the other hand, the mitochondrial Hsp60/Hsp10 complex binds to procaspase-3 and enhances its protease sensitivity, thereby promoting apoptosis (Samali et al., 1999).

## Implications for a Role of Hsp60 in ALS

To date there have been a limited number of studies investigating the role of Hsp60 in the progression of ALS. In other neuropathological conditions there is evidence

that shows an up-regulation in Hsp60 expression in astroglia and oligodendrocytes. However, Hsp60 is not up-regulated in motoneurons in ALS (Martin et al., 1993). In spite of conflicting evidence for the involvement of Hsp60 members in the process of apoptosis, it appears that increased Hsp60 expression is protective against β-Amyloid toxicity (Veereshwarayya et al., 2006) although it has been only shown to be protective in conjunction with increased synthesis of other Hsps, such as Hsp70 and Hsp90 (Veereshwarayya et al., 2006).

## THE HSP105/110 FAMILY OF STRESS PROTEINS

All members of this Hsp family possess an ATPase activity that is essential for them to exert their physiological action. The Hsp105/110 family of proteins were first described as homodimers co-precipitating with muscle actin (Koyasu et al., 1986). Members of this family also form homo-oligomers in the presence of adenine nucleotides. The heat-inducible members of this family are also known as Clp-s in prokaryotic cells and they are essential for survival following heat stress or exposure to ethanol or arsenite. Eucaryotic Hsp105/110 proteins contain two nucleotide-binding domains called NBD1 and NBD2 (Parsell et al., 1991; Schirmer et al., 1996). NBD1 is primarily responsible for the ATPase activity of this Hsp, whereas mutations in the NBD2 domain inhibit the oligomerisation of Hsp104, indicating its role in oligomer formation (Schirmer et al., 1998). Hsp104 has been described in yeast, whereas Hsp110 is the mammalian homologue (Parsell et al., 1991). Hsp105 alpha and Hsp105 beta are members of the same mammalian Hsp105/110 family.

The Hsp 105/110 family of proteins do not function to prevent the formation of aggregates but instead they mediate the re-solubilization of heat-inactivated proteins from insoluble aggregates (Parsell et al., 1994; Glover and Tkach, 2001). However, for effective protein refolding, Hsp104 requires a coordinated action with Hsp40 and Hsp70 (Glover and Lindquist, 1998). More recently it has been discovered that the nature of this cooperation between Hsps is such that Hsp110 acts as a co-chaperone of Hsp70, synergizing with Hsp40 in accelerating nucleotide exchange and thus, the ATPase (and folding) activity of Hsp70 (Dragovic et al., 2006; Shaner and Morano, 2007). On the other hand, whilst Hsp105 can strongly suppress aggregation, it can also inhibit the ATPase activity of Hsp70 (Yamagishi et al., 2003, 2004). This Hsc70-regulatory function of Hsp105 is regulated by its phorphorylation levels. Thus, suppression of Hsc70 mediated protein folding is abolished when Hsp105 alpha is phosphorylated (Ishihara, 2003).

Since Hsp105 levels are elevated in a number of malignant cells, it has been assumed that this family of Hsps can also play a role in the regulation of the cell cycle and apoptosis. Indeed, silencing Hsp105 induces caspase-mediated apoptosis (Hosaka et al., 2006). Hsp110 is also induced during developmental cell death (Evrard et al., 2000). It is thought that Hsp105/110 members negatively regulate the apoptotic pathway by inhibiting the translocation of the proapoptotic protein,

Bax into mitochondria and by inhibiting the activation of C-JUN kinase (Yamagishi et al., 2006; Hatayama et al., 2001).

## DISTRIBUTION OF THE HSP100 FAMILY OF STRESS PROTEINS: IMPLICATIONS FOR ALS AND OTHER NEURODEGENERATIVE DISEASES

Members of the Hsp105/110 family are constitutively expressed in the developing and adult nervous system, in the cytoplasm and nuclei of most neurons and glial cells (Satoh et al., 1998; Easton et al., 2000; Hylander et al., 2000; Evrard et al., 1999, 2000). Stress conditions such as heat stress and ischemia all induce Hsp110 expression in neurons and glia (Gauley and Heikkila, 2006; Gashegu et al., 2007). During embryonic development, Hsp110 expression increases just prior to caspase activation, suggesting that Hsp110 may play a proapoptotic role during embryogenesis (Evrard et al., 2000; Gashegu et al., 2007). However, increased Hsp110 levels have been shown to be protective against proapoptotic stimuli in the adult nervous system (Hatayama et al., 2001; Yagita et al., 2001; Yamagishi et al., 2006).

As would be expected from their ability to re-solubilize protein aggregates, members of the Hsp105/110 family are excellent therapeutic targets for diseases that involve toxic aggregates. Thus, effective delivery of Hsp105/110 into cells with toxic aggregates could successfully reduce inclusions even at late stages of the disease. However, to date there is limited data demonstrating the effects of Hsp105/110 in models of neurodegeneration. In a cellular model of KD, overexpression of Hsp105 alpha efficiently reduced the formation of mutant androgen receptor aggregates (Ishihara et al., 2003). In a model of Huntington's disease, also caused by expanded polyglutamine tract, lentiviral delivery of Hsp104 successfully reduced aggregate formation when delivered in conjunction with an Hsp27 lentivirus (Perrin et al., 2007). In the SOD1 mouse model of ALS, mSOD1 has been shown to bind to Hsp105 and Hsp105 levels decrease with disease progression. This finding once again supports the possibility that depletion of Hsps is a disease-causing event in ALS that results in the accumulation of toxic aggregates (Okado-Matsumoto and Fridovich, 2002; Yamashita et al., 2007). Recent results have also shown that overexpression of Hsp105 in a cellular model of ALS dramatically reduced aggregate formation (Yamashita et al., 2007). However, it is yet to be seen if increased expression of Hsp105 can successfully ameliorate disease signs in in vivo models of ALS.

## HOW CAN THE HEAT SHOCK RESPONSE BE MANIPULATED AND UTILIZED FOR THE TREATMENT OF ALS?

It is clear from this discussion of what is currently understood about the mechansism by which Hsps function, that the highest levels of neuroprotection are more likely to be achieved when several members of the heat shock response are recruited,

*Figure 1.* Heat shock proteins play an important role in the survival of motoneurons under normal conditions and during cellular stress in ALS pathology. The Figure illustrates some of the cellular events that can be modified in the CNS in motoneurons and glial cells by hsps under normal conditions and following exposure to stress. Motoneurons have an intrinsic deficit in the ability to activate the heat shock response and therefore do not up-regulate the expression of Hsps such as Hsp70. However, under normal conditions, surrounding glial cells, primarily astroglia, synthesize and release Hsps that can then be utilized by motoneurons. The Figure also summarizes the intracellular effects of Hsps. In glial cells, such as astroglia and microglia, Hsp70 acts as a negative regulator of NFkappaB activation, thereby reducing neuroinflammation, preventing the destructive process that would otherwise involve the over-production of inflammatory mediators such as Nitric Oxide Synthase (NOS) and Cycloxygenase-2 (COX-2). When Hsp levels are sufficiently high in motoneurons, they are capable of keeping aberrantly folded proteins in solution or, with the help of ubiquitinating co-chaperones, they can direct these mis-folded proteins to the proteasome for degradation. Hsps can also directly interact with different members of the apoptotic pathway thereby promoting cell survival. The Figure also indicates the cellular targets of those pharmacological compounds that have been shown to act on Hsps and the heat shock response. For example it can be seen that geranylgeranyl acetone (GGA) blocks hsp90, indirectly initiating the activation of Hsf-1, whereas arimoclomol and celastrol acts directly on the activation and trimerization of Hsf-1 (arrows) resulting in increased expression of a number of Hsps

and in the case of ALS, in multiple cell types (see Figure 1). Since all the main Hsp family members and their co-chaperones play a unique role in the machinery of the heat shock response, co-up-regulation of a number of Hsps is likely to be more successful at protecting cells than over-expression of individual Hsps (Kobayashi et al., 2000; Takeuchi et al., 2002; Patel et al., 2005; Batulan

et al., 2006). It is therefore imperative that the balance between the levels of intracellular hsps and their co-chaperones must be optimal if Hsps are to exert their protein folding and survival promoting effects. Furthermore, the state of phosphorylation (Hsp27 and Hsp105), oligomerization (Hsp27) and association with other Hsps (Hsp70 and Hsp90) must also be favourable. Thus, up-regulation of Hsps in conjunction with their assistant co-chaperones is likely to be a key feature of any therapy that aims to optimally harness the HSR for the treatment of diseases such as ALS. Furthermore, Hsps cannot exert their intracellular functions without assistance from co-chaperones which modify their function. Some co-chaperones, such as Hop, (Hsp70–Hsp90 Organizing Protein), Hip (Hsp70-Interacting Protein) and members of the Hsp40/DnaJ Hsps family, promote client binding and ATPase activity and are involved in the chaperoning and refolding function of Hsps (for reviews see Frydman and Hohfeld, 1997; Fan et al., 2004; Mayer and Bukau, 2005). Other co-chaperones, such as Bag-1 (Bcl-e Associated Athanogen-1) and CHIP, (Carboxy terminus of Hsc70 Interacting Protein) when associated with Hsp70, inhibit its ATPase activity (for reviews see McClellan and Frydman, 2001; Alberti et al., 2003). So far, the relevance of these co-chaperones in ALS has only been investigated in the case of CHIP. CHIP interacts with other proteins through its 3 tetratricopeptide (TPR) motives at its N terminal region and also possesses an E3 ubiqitin ligase activity in its C terminal U box domain as well as a chaperone activity (Ballinger et al., 1999; Murata et al., 2003; Rosser et al., 2007). This co-chaperone appears to be crucial in defining the function of the specific Hsp it is interacting with. Thus, it can inhibit the ATPase activity of Hsc70 and by ubiquitinating its client proteins, direct them towards the proteasome for degradation (Connell et al., 2001; Marques et al., 2006). Even more importantly, CHIP can initiate the heat shock response (HSR) by activating HSF-1 (Dai et al., 2003) or alternatively, after attenuation of HSF-1 activation, it can turn down the HSR. The latter occurs when the level of misfolded proteins is depleted, when CHIP ubiquitinates Hsc70 and directs it towards the proteasome (Qian et al., 2006). Up-regulation of CHIP has been described in several stress models, including α-synuclein induced toxicity, oxidative damage and proteasomal stress (Shin et al., 2005; Dikshit and Jana, 2007). CHIP also associates with mSOD1 in an in vitro model of ALS and over-expression of CHIP can reduce mSOD1 levels and its associated cytotoxicity (Choi et al., 2004). However, it appears that mSOD1 is not a substrate of CHIP, although the mSOD1-Hsc70 complex is (Urushitani et al., 2004). CHIP over-expression reduces aggregates in a cellular model of KD as well as Tau-induced aggregation (Sahara et al., 2005; Adachi et al., 2007). Over-expression of a chimeric protein containing CHIP and another ubiquitin ligase, Dorfin, also results in protection against mSOD1-mediated toxicity in neural cells (Ishigaki et al., 2007). Thus, it appears that at least in the case of CHIP, co-chaperones play a critical role in the regulation of the HSR and could therefore be more efficiently targeted in the treatment of protein misfolding diseases than Hsp70 and Hsp90 family members.

In view of the complexity of Hsp function, it is not surprising that most studies that have tested the effects of Hsp over-expression in transgenic ALS mouse models have been disappointing (Liu et al., 2005; Krishnan et al., 2006). Figure 1 summarises some of the cellular events that can be modified in motoneurons and glial cells by hsps. It is clear from this simplified representation of the complexity of Hsp interactions, that targeted up-regulation of a single member of this delicate system is unlikely to provide resistance to insults if its partner co-chaperones are not also targeted (Manzerra and Brown, 1992; Batulan et al., 2003; Taylor et al., 2007). Genetic manipulation of HSF-1 may overcome the problem of a disturbance in the balance between Hsps. Indeed, transfection of motoneurons with a constitutively active HSF-1 has overcome the intrinsic deficits in the ability of motoneurons to activate the HSR, resulting in an increase in the synthesis of multiple Hsps (Batulan et al., 2003, 2006). Transfection with activated HSF-1 is more effective in protecting motoneurons than transfection with Hsp25 or Hsp70 alone (Batulan et al., 2006). Under physiological conditions, motoneurons rely on surrounding cells such as astroglia, not only for trophic and metabolic support, but also for a supply of essential chaperones. It has been shown that astroglia are capable of releasing Hsp70 and that exogenous Hsp70 is taken up and utilized by motoneurons (Guzhova et al., 2001; Robinson et al., 2005). Therefore, for ALS it may be a more successful strategy to target spinal cord astroglia, which are naturally more responsive to stress and may transmit this increased resistance to motoneurons. Alternatively, exogenous application of a mixture of Hsps could deliver suitable amounts of protective Hsps to motoneurons. However, delivering proteins and peptides is always difficult in vivo due to the high risk of degradation by proteases before reaching their target and the problems of passing through the blood brain barrier. Small molecules that activate the general heat shock response, are easy to administer and are likely to cross the blood brain barrier could overcome this hurdle. The small hydroxylamine derivatives, Bimoclomol and Arimoclomol have been developed and act as co-inducers of the HSR in conjunction with other stressors. These compounds recruit and enhance the HSR, but only once it has been activated (Vigh et al., 1997; Kalmar et al., 2002b). The mechanism of action of these molecules is to act on the activation of HSF-1 itself, prolonging the activation of HSF-1 and thereby achieving an enhanced HSR (Hargitai et al., 2003; Kieran et al., 2004). Following promising results in a preclinical animal study, in which treatment with Arimoclomol successfully increased the lifespan and improved muscle performance of SOD1 mice (Kieran et al., 2004), this compound is now in a phase 2 clinical trial for the treatment of ALS. Celastrol, an herbal drug that has been shown to also cause activation of HSF-1 by hyperphosphorylation, has also successfully increased the lifespan of SOD1 mice (Westerheide et al., 2004; Kiaei et al., 2005). Due to the failure of a number of promising candidates for the treatment of ALS, the development of an effective disease-modifying therapy is a priority to all those who work in this field. It remains to be seen whether well designed strategies based upon manipulation of the HSR will be successful

in a clinical setting. However, in view of the multifactorial nature of ALS pathogenesis, it is likely that an effective therapy will involve targeting of a number of mechanisms. Thus, a combination of a number of drugs or drug-like agents is likely to be required if a successful therapy for the treatment of ALS is to be developed.

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## REFERENCES

Adachi H., Waza M., Tokui K., Katsuno M., Minamiyama M., Tanaka F., Doyu M., and Sobue G. (2007) CHIP over-expression reduces mutant androgen receptor protein and ameliorates phenotypes of the spinal and bulbar muscular atrophy transgenic mouse model. *J. Neurosci.* 27, 5115–5126.

Ahn T.B. and Jeon B.S. (2006) Protective role of heat shock and heat shock protein 70 in lactacystin-induced cell death both in the rat substantia nigra and PC12 cells. *Brain Res.* 1087, 159–167.

Akbar M.T., Lundberg A.M., Liu K., Vidyadaran S., Wells K.E., Dolatshad H., Wynn S., Wells D.J., Latchman D.S., and de Belleroche J. (2003) The neuroprotective effects of heat shock protein 27 over-expression in transgenic animals against kainate-induced seizures and hippocampal cell death. *J. Biol. Chem.* 278, 19956–19965.

Alberti S., Esser C., and Hohfeld J. (2003) BAG-1 – a nucleotide exchange factor of Hsc70 with multiple cellular functions. *Cell Stress Chaperones* 8, 225–231.

Alexianu M.E., Kozovska M., and Appel S.H. (2001) Immune reactivity in a mouse model of familial ALS correlates with disease progression. *Neurology* 57, 1282–1289.

Ali A., Bharadwaj S., O'Carroll R., and Ovsenek N. (1998) HSP90 interacts with and regulates the activity of heat shock factor 1 in Xenopus oocytes. *Mol. Cell Biol.* 18, 4949–4960.

Almer G., Teismann P., Stevic Z., Halaschek-Wiener J., Deecke L., Kostic V., and Przedborski S. (2002) Increased levels of the pro-inflammatory prostaglandin PGE2 in CSF from ALS patients. *Neurology* 58, 1277–1279.

Amin V., Cumming D.V., and Latchman D.S. (1996) Over-expression of heat shock protein 70 protects neuronal cells against both thermal and ischaemic stress but with different efficiencies. *Neurosci. Lett.* 206, 45–48.

Argon Y. and Simen B.B. (1999) GRP94, an ER chaperone with protein and peptide binding properties. *Semin. Cell Dev. Biol.* 10, 495–505.

Armstrong C.L., Krueger-Naug A.M., Currie R.W., and Hawkes R. (2001) Constitutive expression of heat shock protein HSP25 in the central nervous system of the developing and adult mouse. *J. Comp. Neurol.* 434, 262–274.

Armstrong J.N., Plumier J.C., Robertson H.A., and Currie R.W. (1996) The inducible 70,000 molecular/weight heat shock protein is expressed in the degenerating dentate hilus and piriform cortex after systemic administration of kainic acid in the rat. *Neuroscience* 74, 685–693.

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

Bailey C.K., Andriola I.F., Kampinga H.H., and Merry D.E. (2002) Molecular chaperones enhance the degradation of expanded polyglutamine repeat androgen receptor in a cellular model of spinal and bulbar muscular atrophy. *Hum. Mol. Genet.* 11, 515–523.

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

Batulan Z., Shinder G.A., Minotti S., He B.P., Doroudchi M.M., Nalbantoglu J., Strong M.J., and Durham H.D. (2003) High threshold for induction of the stress response in motor neurons is associated with failure to activate HSF1. *J. Neurosci.* 23, 5789–5798.

Batulan Z., Taylor D.M., Aarons R.J., Minotti S., Doroudchi M.M., Nalbantoglu J., and Durham H.D. (2006) Induction of multiple heat shock proteins and neuroprotection in a primary culture model of familial amyotrophic lateral sclerosis. *Neurobiol. Dis.* 24, 213–225.

Beck T., Delley P.A., and Hall M.N. (2001) Control of the actin cytoskeleton by extracellular signals. *Results Probl. Cell Differ.* 32, 231–262.

Becker J. and Craig E.A. (1994) Heat-shock proteins as molecular chaperones. *Eur. J. Biochem.* 219, 11–23.

Beere H.M. and Green D.R. (2001) Stress management – heat shock protein-70 and the regulation of apoptosis. *Trends Cell Biol.* 11, 6–10.

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.

Beers D.R., Henkel J.S., Xiao Q., Zhao W., Wang J., Yen A.A., Siklos L., McKercher S.R., and Appel S.H. (2006) Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 103, 16021–16026.

Benarroch E.E. (2005) Neuron–astrocyte interactions: partnership for normal function and disease in the central nervous system. *Mayo Clin. Proc.* 80, 1326–1338.

Benn S.C., Perrelet D., Kato A.C., Scholz J., Decosterd I., Mannion R.J., Bakowska J.C., and Woolf C.J. (2002) Hsp27 up-regulation and phosphorylation is required for injured sensory and motor neuron survival. *Neuron* 36, 45–56.

Bertorelli G., Bocchino V., and Olivieri D. (1998) Heat shock protein interactions with the glucocorticoid receptor. *Pulm. Pharmacol. Ther.* 11, 7–12.

Bertrand N., Siren A.L., Tworek D., McCarron R.M., and Spatz M. (2000) Differential expression of Hsc73 and Hsp72 mRNA and proteins between young and adult gerbils after transient cerebral ischemia: relation to neuronal vulnerability. *J. Cereb. Blood Flow Metab.* 20, 1056–1065.

Boillee S., Vande V.C., and Cleveland D.W. (2006) ALS: a disease of motor neurons and their nonneuronal neighbors. *Neuron* 52, 39–59.

Booth C. and Koch G.L. (1989) Perturbation of cellular calcium induces secretion of luminal ER proteins. *Cell* 59, 729–737.

Borthwick G.M., Johnson M.A., Ince P.G., Shaw P.J., and Turnbull D.M. (1999) Mitochondrial enzyme activity in amyotrophic lateral sclerosis: implications for the role of mitochondria in neuronal cell death. *Ann. Neurol.* 46, 787–790.

Briese M., Esmaeili B., and Sattelle D.B. (2005) Is spinal muscular atrophy the result of defects in motor neuron processes? *Bioessays* 27, 946–957.

Brown I.R. (1983) Hyperthermia induces the synthesis of a heat shock protein by polysomes isolated from the fetal and neonatal mammalian brain. *J. Neurochem.* 40, 1490–1493.

Brown I.R., Rush S., and Ivy G.O. (1989) Induction of a heat shock gene at the site of tissue injury in the rat brain. *Neuron* 2, 1559–1564.

Bruening W., Roy J., Giasson B., Figlewicz D.A., Mushynski W.E., and Durham H.D. (1999) Up-regulation of protein chaperones preserves viability of cells expressing toxic Cu/Zn-superoxide dismutase mutants associated with amyotrophic lateral sclerosis. *J. Neurochem.* 72, 693–699.

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.

Bruijn L.I., Miller T.M., and Cleveland D.W. (2004) Unraveling the mechanisms involved in motor neuron degeneration in ALS. *Annu. Rev. Neurosci.* 27, 723–749.

Carriedo S.G., Yin H.Z., and Weiss J.H. (1996) Motor neurons are selectively vulnerable to AMPA/kainate receptor-mediated injury in vitro. *J. Neurosci.* 16, 4069–4079.

Casado P., Zuazua-Villar P., Prado M.A., Valle E.D., Iglesias J.M., Martinez-Campa C., Lazo P.S., and Ramos S. (2007) Characterization of HSP27 phosphorylation induced by microtubule interfering agents: implication of p38 signalling pathway. *Arch. Biochem. Biophys.* 461, 123–129.

Charcot J.M. and Joffroy A. (1869) Deux cas d'atrophie musculaire progressive avec lésions de la substance grise et des faisceaux antéro-latéraux de la moelleépinière. *Arch. Physiol. Neurol. Pathol.* 2, 744–760.

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.

Chen S. and Brown I.R. (2007) Neuronal expression of constitutive heat shock proteins: implications for neurodegenerative diseases. *Cell Stress Chaperones* 12, 51–58.

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.

Cheroni C., Peviani M., Cascio P., Debiasi S., Monti C., and Bendotti C. (2005) Accumulation of human SOD1 and ubiquitinated deposits in the spinal cord of SOD1G93A mice during motor neuron disease progression correlates with a decrease of proteasome. *Neurobiol. Dis.* 18, 509–522.

Choi J.S., Cho S., Park S.G., Park B.C., and Lee D.H. (2004) Co-chaperone CHIP associates with mutant Cu/Zn-superoxide dismutase proteins linked to familial amyotrophic lateral sclerosis and promotes their degradation by proteasomes. *Biochem. Biophys. Res. Commun.* 321, 574–583.

Ciocca D.R., Oesterreich S., Chamness G.C., McGuire W.L., and Fuqua S.A. (1993) Biological and clinical implications of heat shock protein 27,000 (Hsp27): a review. *J. Natl. Cancer Inst.* 85, 1558–1570.

Clayton R.M. and Truman D.E. (1968) The structure and cross-reactivity of chick lens proteins. *J. Physiol.* 198, 72–73.

Clement A.M., Nguyen M.D., Roberts E.A., Garcia M.L., Boillee S., Rule M., McMahon A.P., Doucette W., Siwek D., Ferrante R.J., Brown R.H., Jr., Julien J.P., Goldstein L.S., and Cleveland D.W. (2003) Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science* 302, 113–117.

Connell P., Ballinger C.A., Jiang J., Wu Y., Thompson L.J., Hohfeld J., and Patterson C. (2001) The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. *Nat. Cell Biol.* 3, 93–96.

Costigan M., Mannion R.J., Kendall G., Lewis S.E., Campagna J.A., Coggeshall R.E., Meridith-Middleton J., Tate S., and Woolf C.J. (1998) Heat shock protein 27: developmental regulation and expression after peripheral nerve injury. *J. Neurosci.* 18, 5891–5900.

Cote F., Collard J.F., and Julien J.P. (1993) Progressive neuronopathy in transgenic mice expressing the human neurofilament heavy gene: a mouse model of amyotrophic lateral sclerosis. *Cell* 73, 35–46.

Craig E.A. (1990) Role of hsp70 in translocation of proteins across membranes. In: Stress proteins in biology and medicine. Eds Morimoto R.I., Tissieres A., and Georgopoulos C. Cold Spring Harbor Laboratory Press, Woodbury, NY, pp 279–287.

Csermely P. and Kahn C.R. (1991) The 90-kDa heat shock protein (Hsp-90) possesses an ATP binding site and autophosphorylating activity. *J. Biol. Chem.* 266, 4943–4950.

Csermely P., Schnaider T., Soti C., Prohaszka Z., and Nardai G. (1998) The 90-kDa molecular chaperone family: structure, function, and clinical applications. A comprehensive review. *Pharmacol. Ther.* 79, 129–168.

Cullinan S.B. and Whitesell L. (2006) Heat shock protein 90: a unique chemotherapeutic target. *Semin. Oncol.* 33, 457–465.

Cummings C.J., Sun Y., Opal P., Antalffy B., Mestril R., Orr H.T., Dillmann W.H., and Zoghbi H.Y. (2001) Over-expression of inducible HSP70 chaperone suppresses neuropathology and improves motor function in SCA1 mice. *Hum. Mol. Genet.* 10, 1511–1518.

Currie R.W., Ellison J.A., White R.F., Feuerstein G.Z., Wang X., and Barone F.C. (2000) Benign focal ischemic preconditioning induces neuronal Hsp70 and prolonged astrogliosis with expression of Hsp27. *Brain Res.* 863, 169–181.

Dai Q., Zhang C., Wu Y., McDonough H., Whaley R.A., Godfrey V., Li H.H., Madamanchi N., Xu W., Neckers L., Cyr D., and Patterson C. (2003) CHIP activates HSF1 and confers protection against apoptosis and cellular stress. *EMBO J.* 22, 5446–5458.

Dastoor Z. and Dreyer J. (2000) Nuclear translocation and aggregate formation of heat shock cognate protein 70 (Hsc70) in oxidative stress and apoptosis. *J. Cell Sci.* 113(Pt 16), 2845–2854.

de Jong W.W., Caspers G.J., and Leunissen J.A. (1998) Genealogy of the alpha-crystallin – small heat-shock protein superfamily. *Int. J. Biol. Macromol.* 22, 151–162.

Deng H.X., Hentati A., Tainer J.A., Iqbal Z., Cayabyab A., Hung W.Y., Getzoff E.D., Hu P., Herzfeldt B., Roos R.P., et al. (1993) Amyotrophic lateral sclerosis and structural defects in Cu, Zn superoxide dismutase. *Science* 261, 1047–1051.

Deng H.X., Shi Y., Furukawa Y., Zhai H., Fu R., Liu E., Gorrie G.H., Khan M.S., Hung W.Y., Bigio E.H., Lukas T., Dal Canto M.C., O'Halloran T.V., and Siddique T. (2006) Conversion to the amyotrophic lateral sclerosis phenotype is associated with intermolecular linked insoluble aggregates of SOD1 in mitochondria. *Proc. Natl. Acad. Sci. U.S.A.* 103, 7142–7147.

Deocaris C.C., Kaul S.C., and Wadhwa R. (2006) On the brotherhood of the mitochondrial chaperones mortalin and heat shock protein 60. *Cell Stress Chaperones* 11, 116–128.

Di Giorgio F.P., Carrasco M.A., Siao M.C., Maniatis T., and Eggan K. (2007) Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model. *Nat. Neurosci.* 10, 608–614.

Dikshit P. and Jana N.R. (2007) The co-chaperone CHIP is induced in various stresses and confers protection to cells. *Biochem. Biophys. Res. Commun.* 357, 761–765.

Dodge M.E., Wang J., Guy C., Rankin S., Rahimtula M., and Mearow K.M. (2006) Stress-induced heat shock protein 27 expression and its role in dorsal root ganglion neuronal survival. *Brain Res.* 1068, 34–48.

Dragovic Z., Broadley S.A., Shomura Y., Bracher A., and Hartl F.U. (2006) Molecular chaperones of the Hsp110 family act as nucleotide exchange factors of Hsp70s. *EMBO J.* 25, 2519–2528.

D'Souza S.M. and Brown I.R. (1998) Constitutive expression of heat shock proteins Hsp90, Hsc70, Hsp70 and Hsp60 in neural and non-neural tissues of the rat during postnatal development. *Cell Stress Chaperones* 3, 188–199.

During R.L., Gibson B.G., Li W., Bishai E.A., Sidhu G.S., Landry J., and Southwick F.S. (2007) Anthrax lethal toxin paralyzes actin-based motility by blocking Hsp27 phosphorylation. *EMBO J.* 26, 2240–2250.

Easton D.P., Kaneko Y., and Subjeck J.R. (2000) The Hsp110 and Grp1 70 stress proteins: newly recognized relatives of the Hsp70s. *Cell Stress Chaperones* 5, 276–290.

Ehrnsperger M., Graber S., Gaestel M., and Buchner J. (1997) Binding of non-native protein to Hsp25 during heat shock creates a reservoir of folding intermediates for reactivation. *EMBO J.* 16, 221–229.

Elliott J.L. (2001) Cytokine up-regulation in a murine model of familial amyotrophic lateral sclerosis. *Brain Res. Mol. Brain Res.* 95, 172–178.

Ethridge R.T., Hellmich M.R., DuBois R.N., and Evers B.M. (1998) Inhibition of heat-shock protein 70 induction in intestinal cells over-expressing cyclooxygenase 2. *Gastroenterology* 115, 1454–1463.

Evrard L., Vanmuylder N., Dourov N., Glineur R., and Louryan S. (1999) Cytochemical identification of Hsp110 during early mouse facial development. *J. Craniofac. Genet. Dev. Biol.* 19, 24–32.

Evrard L., Vanmuylder N., Dourov N., Hermans C., Biermans J., Werry-Huet A., Rooze M., and Louryan S. (2000) Correlation of Hsp110 expression with all-trans retinoic acid-induced apoptosis. *J. Craniofac. Genet. Dev. Biol.* 20, 183–192.

Ezzi S.A., Urushitani M., and Julien J.P. (2007) Wild-type superoxide dismutase acquires binding and toxic properties of ALS-linked mutant forms through oxidation. *J. Neurochem.* 102, 170–178.

Faden A.I., Fox G.B., Di X., Knoblach S.M., Cernak I., Mullins P., Nikolaeva M., and Kozikowski A.P. (2003) Neuroprotective and nootropic actions of a novel cyclized dipeptide after controlled cortical impact injury in mice. *J. Cereb. Blood Flow Metab.* 23, 355–363.

Faden A.I., Knoblach S.M., Movsesyan V.A., and Cernak I. (2004) Novel small peptides with neuroprotective and nootropic properties. *J. Alzheimers Dis.* 6, S93-S97.

Fan C.Y., Lee S., Ren H.Y., and Cyr D.M. (2004) Exchangeable chaperone modules contribute to specification of type I and type II Hsp40 cellular function. *Mol. Biol. Cell* 15, 761–773.

Feinstein D.L., Galea E., Aquino D.A., Li G.C., Xu H., and Reis D.J. (1996) Heat shock protein 70 suppresses astroglial-inducible nitric-oxide synthase expression by decreasing NFkappaB activation. *J. Biol. Chem.* 271, 17724–17732.

Feldweg A.M. and Srivastava P.K. (1995) Molecular heterogeneity of tumor rejection antigen/heat shock protein GP96. *Int. J. Cancer* 63, 310–314.

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

Fink S.L., Chang L.K., Ho D.Y., and Sapolsky R.M. (1997) Defective herpes simplex virus vectors expressing the rat brain stress-inducible heat shock protein 72 protect cultured neurons from severe heat shock. *J. Neurochem.* 68, 961–969.

Fostinis Y., Theodoropoulos P.A., Gravanis A., and Stournaras C. (1992) Heat shock protein HSP90 and its association with the cytoskeleton: a morphological study. *Biochem. Cell Biol.* 70, 779–786.

Franklin T.B., Krueger-Naug A.M., Clarke D.B., Arrigo A.P., and Currie R.W. (2005) The role of heat shock proteins Hsp70 and Hsp27 in cellular protection of the central nervous system. *Int. J. Hyperthermia* 21, 379–392.

Freeman B.C. and Morimoto R.I. (1996) The human cytosolic molecular chaperones Hsp90, Hsp70 (Hsc70) and hdj-1 have distinct roles in recognition of a non-native protein and protein refolding. *EMBO J.* 15, 2969–2979.

Frydman J. and Hohfeld J. (1997) Chaperones get in touch: the Hip–Hop connection. *Trends Biochem. Sci.* 22, 87–92.

Fujiki M., Kobayashi H., Abe T., and Ishii K. (2003) Astroglial activation accompanies heat shock protein up-regulation in rat brain following single oral dose of geranylgeranylacetone. *Brain Res.* 991, 254–257.

Galigniana M.D., Harrell J.M., Housley P.R., Patterson C., Fisher S.K., and Pratt W.B. (2004) Retrograde transport of the glucocorticoid receptor in neurites requires dynamic assembly of complexes with the protein chaperone Hsp90 and is linked to the CHIP component of the machinery for proteasomal degradation. *Brain Res. Mol. Brain Res.* 123, 27–36.

Garofalo O., Kennedy P.G., Swash M., Martin J.E., Luthert P., Anderton B.H., and Leigh P.N. (1991) Ubiquitin and heat shock protein expression in amyotrophic lateral sclerosis. *Neuropathol. Appl. Neurobiol.* 17, 39–45.

Garrido C. (2002) Size matters: of the small HSP27 and its large oligomers. *Cell Death. Differ.* 9, 483–485.

Garrido C., Bruey J.M., Fromentin A., Hammann A., Arrigo A.P., and Solary E. (1999) HSP27 inhibits cytochrome c-dependent activation of procaspase-9. *FASEB J.* 13, 2061–2070.

Gasbarrini A., Esposti S.D., Di C.C., De N.S., Loffredo S., Abraham A., Simoncini M., Pola R., Colantoni A., Trevisani F., Bernardi M., and Gasbarrini G. (1998) Effect of ischemia – reperfusion on heat shock protein 70 and 90 gene expression in rat liver: relation to nutritional status. *Dig. Dis. Sci.* 43, 2601–2605.

Gashegu J., Ladha R., Vanmuylder N., Philippson C., Bremer F., Rooze M., and Louryan S. (2007) HSP110, caspase-3 and -9 expression in physiological apoptosis and apoptosis induced by in vivo embryonic exposition to all-trans retinoic acid or irradiation during early mouse eye development. *J. Anat.* 210, 532–541.

Gauley J. and Heikkila J.J. (2006) Examination of the expression of the heat shock protein gene, Hsp110, in Xenopus laevis cultured cells and embryos. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 145, 225–234.

Gernold M., Knauf U., Gaestel M., Stahl J., and Kloetzel P.M. (1993) Development and tissue-specific distribution of mouse small heat shock protein Hsp25. *Dev. Genet.* 14, 103–111.

Gispert-Sanchez S. and Auburger G. (2006) The role of protein aggregates in neuronal pathology: guilty, innocent, or just trying to help? *J. Neural. Transm. Suppl.* 70, 111–117.

Glover J.R. and Lindquist S. (1998) Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. *Cell* 94, 73–82.

Glover J.R. and Tkach J.M. (2001) Crowbars and ratchets: Hsp100 chaperones as tools in reversing protein aggregation. *Biochem. Cell Biol.* 79, 557–568.

Gong Y.H., Parsadanian A.S., Andreeva A., Snider W.D., and Elliott J.L. (2000) Restricted expression of G86R Cu/Zn superoxide dismutase in astrocytes results in astrocytosis but does not cause motoneuron degeneration. *J. Neurosci.* 20, 660–665.

Gorman A.M., Szegezdi E., Quigney D.J., and Samali A. (2005) Hsp27 inhibits 6-hydroxydopamine-induced cytochrome c release and apoptosis in PC12 cells. *Biochem. Biophys. Res. Commun.* 327, 801–810.

Gupta S. and Knowlton A.A. (2002) Cytosolic heat shock protein 60, hypoxia, and apoptosis. *Circulation* 106, 2727–2733.

Gupta S. and Knowlton A.A. (2005) HSP60, Bax, apoptosis and the heart. *J. Cell Mol. Med.* 9, 51–58.

Gurney M.E., Pu H., Chiu A.Y., Dal Canto M.C., Polchow C.Y., Alexander D.D., Caliendo J., Hentati A., Kwon Y.W., Deng H.X., et al. (1994) Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. *Science* 264, 1772–1775.

Guzhova I., Kislyakova K., Moskaliova O., Fridlanskaya I., Tytell M., Cheetham M., and Margulis B. (2001) In vitro studies show that Hsp70 can be released by glia and that exogenous Hsp70 can enhance neuronal stress tolerance. *Brain Res.* 914, 66–73.

Hargitai J., Lewis H., Boros I., Racz T., Fiser A., Kurucz I., Benjamin I., Vigh L., Penzes Z., Csermely P., and Latchman D.S. (2003) Bimoclomol, a heat shock protein co-inducer, acts by the prolonged activation of heat shock factor-1. *Biochem. Biophys. Res. Commun.* 307, 689–695.

Harrell J.M., Murphy P.J., Morishima Y., Chen H., Mansfield J.F., Galigniana M.D., and Pratt W.B. (2004) Evidence for glucocorticoid receptor transport on microtubules by dynein. *J. Biol. Chem.* 279, 54647–54654.

Haslbeck M., Franzmann T., Weinfurtner D., and Buchner J. (2005) Some like it hot: the structure and function of small heat-shock proteins. *Nat. Struct. Mol. Biol.* 12, 842–846.

Hatayama T., Yamagishi N., Minobe E., and Sakai K. (2001) Role of Hsp105 in protection against stress-induced apoptosis in neuronal PC12 cells. *Biochem. Biophys. Res. Commun.* 288, 528–534.

Head M.W. and Goldman J.E. (2000) Small heat shock proteins, the cytoskeleton, and inclusion body formation. *Neuropathol. Appl. Neurobiol.* 26, 304–312.

Heikkila J.J. (1993) Heat shock gene expression and development. II. An overview of mammalian and avian developmental systems. *Dev. Genet.* 14, 87–91.

Hensley K., Floyd R.A., Gordon B., Mou S., Pye Q.N., Stewart C., West M., and Williamson K. (2002) Temporal patterns of cytokine and apoptosis-related gene expression in spinal cords of the G93A-SOD1 mouse model of amyotrophic lateral sclerosis. *J. Neurochem.* 82, 365–374.

Hightower L.E. and Guidon P.T., Jr. (1989) Selective release from cultured mammalian cells of heat-shock (stress) proteins that resemble glia-axon transfer proteins. *J. Cell Physiol* 138, 257–266.

Hightower L.E. and Li T. (1994) Structure and function of the mammalian hsp70 family In: Heat shock proteins in the nervous system. Eds Mayer J. and Brown I. Academic Press, Harcourt Brace & Co Publishers, New York, pp 1–32.

Hirata K., He J., Hirakawa Y., Liu W., Wang S., and Kawabuchi M. (2003) HSP27 is markedly induced in Schwann cell columns and associated regenerating axons. *Glia* 42, 1–11.7.

Hoehn B., Ringer T.M., Xu L., Giffard R.G., Sapolsky R.M., Steinberg G.K., and Yenari M.A. (2001) Over-expression of HSP72 after induction of experimental stroke protects neurons from ischemic damage. *J. Cereb. Blood Flow Metab.* 21, 1303–1309.

Hohfeld J. and Jentsch S. (1997) GrpE-like regulation of the Hsc70 chaperone by the anti-apoptotic protein BAG-1. *EMBO J.* 16, 6209–6216.

Hollander J.M., Martin J.L., Belke D.D., Scott B.T., Swanson E., Krishnamoorthy V., and Dillmann W.H. (2004) Over-expression of wild-type heat shock protein 27 and a nonphosphorylatable heat shock protein 27 mutant protects against ischemia/reperfusion injury in a transgenic mouse model. *Circulation* 110, 3544–3552.

Hosaka S., Nakatsura T., Tsukamoto H., Hatayama T., Baba H., and Nishimura Y. (2006) Synthetic small interfering RNA targeting heat shock protein 105 induces apoptosis of various cancer cells both in vitro and in vivo. *Cancer Sci.* 97, 623–632.

Housby J.N., Cahill C.M., Chu B., Prevelige R., Bickford K., Stevenson M.A., and Calderwood S.K. (1999) Non-steroidal anti-inflammatory drugs inhibit the expression of cytokines and induce HSP70 in human monocytes. *Cytokine* 11, 347–358.

Howland D.S., Liu J., She Y., Goad B., Maragakis N.J., Kim B., Erickson J., Kulik J., DeVito L., Psaltis G., DeGennaro L.J., Cleveland D.W., and Rothstein J.D. (2002) Focal loss of the glutamate

transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). *Proc. Natl. Acad. Sci. U.S.A.*. 99, 1604–1609.

Hylander B.L., Chen X., Graf P.C., and Subjeck J.R. (2000) The distribution and localization of Hsp110 in brain. *Brain Res.* 869, 49–55.

Ialenti A., Di Meglio P., D'Acquisto F., Pisano B., Maffia P., Grassia G., Di Rosa M., and Ianaro A. (2005) Inhibition of cyclooxygenase-2 gene expression by the heat shock response in J774 murine macrophages. *Eur. J. Pharmacol.* 509, 89–96.

Ianaro A., Ialenti A., Maffia P., Pisano B., and Di Rosa M. (2001) HSF1/Hsp72 pathway as an endogenous anti-inflammatory system. *FEBS Lett.* 499, 239–244.

Ince P.G., Lowe J., and Shaw P.J. (1998) Amyotrophic lateral sclerosis: current issues in classification, pathogenesis and molecular pathology. *Neuropathol. Appl. Neurobiol.* 24, 104–117.

Ishigaki S., Niwa J., Yamada S., Takahashi M., Ito T., Sone J., Doyu M., Urano F., and Sobue G. (2007) Dorfin-CHIP chimeric proteins potently ubiquitylate and degrade familial ALS-related mutant SOD1 proteins and reduce their cellular toxicity. *Neurobiol. Dis.* 25, 331–341.

Ishihara K., Yamagishi N., and Hatayama T. (2003) Protein kinase CK2 phosphorylates Hsp105 alpha at Ser509 and modulates its function. *Biochem. J.* 371, 917–925.

Ishihara K., Yamagishi N., Saito Y., Adachi H., Kobayashi Y., Sobue G., Ohtsuka K., and Hatayama T. (2003) Hsp105alpha suppresses the aggregation of truncated androgen receptor with expanded CAG repeats and cell toxicity. *J. Biol. Chem.* 278, 25143–25150.

Iwaki T., Kume-Iwaki A., Liem R.K., and Goldman J.E. (1989) Alpha B-crystallin is expressed in non-lenticular tissues and accumulates in Alexander's disease brain. *Cell* 57, 71–78.

Izaki K., Kinouchi H., Watanabe K., Owada Y., Okubo A., Itoh H., Kondo H., Tashima Y., Tamura S., Yoshimoto T., and Mizoi K. (2001) Induction of mitochondrial heat shock protein 60 and 10 mRNAs following transient focal cerebral ischemia in the rat. *Brain Res. Mol. Brain Res.* 88, 14–25.

Jaarsma D., Rognoni F., van Duijn W., Verspaget H.W., Haasdijk E.D., and Holstege J.C. (2001) CuZn superoxide dismutase (SOD1) accumulates in vacuolated mitochondria in transgenic mice expressing amyotrophic lateral sclerosis-linked SOD1 mutations. *Acta Neuropathol. (Berl)* 102, 293–305.

Jeon G.S., Park S.W., Kim D.W., Seo J.H., Cho J., Lim S.Y., Kim S.D., and Cho S.S. (2004) Glial expression of the 90-kDa heat shock protein (HSP90) and the 94-kDa glucose-regulated protein (GRP94) following an excitotoxic lesion in the mouse hippocampus. *Glia* 48, 250–258.

Jo S.K., Ko G.J., Boo C.S., Cho W.Y., and Kim H.K. (2006) Heat preconditioning attenuates renal injury in ischemic ARF in rats: role of heat-shock protein 70 on NF-kappaB-mediated inflammation and on tubular cell injury. *J. Am. Soc. Nephrol.* 17, 3082–3092.

Kabashi E., Agar J.N., Taylor D.M., Minotti S., and Durham H.D. (2004) Focal dysfunction of the proteasome: a pathogenic factor in a mouse model of amyotrophic lateral sclerosis. *J. Neurochem.* 89, 1325–1335.

Kalmar B., Burnstock G., Vrbova G., and Greensmith L. (2002a) The effect of neonatal nerve injury on the expression of heat shock proteins in developing rat motoneurones. *J. Neurotrauma* 19, 667–679.

Kalmar B., Burnstock G., Vrbova G., Urbanics R., Csermely P., and Greensmith L. (2002b) Up-regulation of heat shock proteins rescues motoneurones from axotomy-induced cell death in neonatal rats. *Exp. Neurol.* 176, 87–97.

Kalwy S.A., Akbar M.T., Coffin R.S., de Belleroche J., and Latchman D.S. (2003) Heat shock protein 27 delivered via a herpes simplex virus vector can protect neurons of the hippocampus against kainic-acid-induced cell loss. *Brain Res. Mol. Brain Res.* 111, 91–103.

Kang P.J., Ostermann J., Shilling J., Neupert W., Craig E.A., and Pfanner N. (1990) Requirement for Hsp70 in the mitochondrial matrix for translocation and folding of precursor proteins. *Nature* 348, 137–143.

Kato S., Hayashi H., Nakashima K., Nanba E., Kato M., Hirano A., Nakano I., Asayama K., and Ohama E. (1997) Pathological characterization of astrocytic hyaline inclusions in familial amyotrophic lateral sclerosis. *Am. J. Pathol.* 151, 611–620.

Kato H., Liu Y., Kogure K., and Kato K. (1994) Induction of 27-kDa heat shock protein following cerebral ischemia in a rat model of ischemic tolerance. *Brain Res.* 634, 235–244.

Katsuno M., Sang C., Adachi H., Minamiyama M., Waza M., Tanaka F., Doyu M., and Sobue G. (2005) Pharmacological induction of heat-shock proteins alleviates polyglutamine-mediated motor neuron disease. *Proc. Natl. Acad. Sci. U.S.A.* 102, 16801–16806.

Kawamata T., Akiyama H., Yamada T., and McGeer P.L. (1992) Immunologic reactions in amyotrophic lateral sclerosis brain and spinal cord tissue. *Am. J. Pathol.* 140, 691–707.

Kelly K.J. (2005) Heat shock (stress response) proteins and renal ischemia/reperfusion injury. *Contrib. Nephrol.* 148, 86–106.

Kelly K.J., Baird N.R., and Greene A.L. (2001) Induction of stress response proteins and experimental renal ischemia/reperfusion. *Kidney Int.* 59, 1798–1802.

Kiaei M., Kipiani K., Petri S., Chen J., Calingasan N.Y., and Beal M.F. (2005) Celastrol blocks neuronal cell death and extends life in transgenic mouse model of amyotrophic lateral sclerosis. *Neurodegener. Dis.* 2, 246–254.

Kieran D., Hafezparast M., Bohnert S., Dick J.R., Martin J., Schiavo G., Fisher E.M., and Greensmith L. (2005) A mutation in dynein rescues axonal transport defects and extends the life span of ALS mice. *J. Cell Biol.* 169, 561–567.

Kieran D., Kalmar B., Dick J.R., Riddoch-Contreras J., Burnstock G., and Greensmith L. (2004) Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice. *Nat. Med.* 10, 402–405.

Kim D., Kim S.H., and Li G.C. (1999) Proteasome inhibitors MG132 and lactacystin hyperphosphorylate HSF1 and induce Hsp70 and Hsp27 expression. *Biochem. Biophys. Res. Commun.* 254, 264–268.

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

Kitamura O. (1994) Immunohistochemical investigation of hypoxic/ischemic brain damage in forensic autopsy cases. *Int. J. Legal Med.* 107, 69–76.

Kobayashi Y., Kume A., Li M., Doyu M., Hata M., Ohtsuka K., and Sobue G. (2000) Chaperones Hsp70 and Hsp40 suppress aggregate formation and apoptosis in cultured neuronal cells expressing truncated androgen receptor protein with expanded polyglutamine tract. *J. Biol. Chem.* 275, 8772–8778.

Koyama S., Arawaka S., Chang-Hong R., Wada M., Kawanami T., Kurita K., Kato M., Nagai M., Aoki M., Itoyama Y., Sobue G., Chan P.H., and Kato T. (2006) Alteration of familial ALS-linked mutant SOD1 solubility with disease progression: its modulation by the proteasome and Hsp70. *Biochem. Biophys. Res. Commun.* 343, 719–730.

Koyasu S., Nishida E., Kadowaki T., Matsuzaki F., Iida K., Harada F., Kasuga M., Sakai H., and Yahara I. (1986) Two mammalian heat shock proteins, HSP90 and HSP100, are actin-binding proteins. *Proc. Natl. Acad. Sci. U.S.A.* 83, 8054–8058.

Krishnan J., Lemmens R., Robberecht W., and Van Den B.L. (2006) Role of heat shock response and Hsp27 in mutant SOD1-dependent cell death. *Exp. Neurol.* 200, 301–310.

Lavoie J.N., Hickey E., Weber L.A., and Landry J. (1993) Modulation of actin microfilament dynamics and fluid phase pinocytosis by phosphorylation of heat shock protein 27. *J. Biol. Chem.* 268, 24210–24214.

Lee M.K. and Cleveland D.W. (1994) Neurofilament function and dysfunction: involvement in axonal growth and neuronal disease. *Curr. Opin. Cell Biol.* 6, 34–40.

Lee M.W., Park S.C., Chae H.S., Bach J.H., Lee H.J., Lee S.H., Kang Y.K., Kim K.Y., Lee W.B., and Kim S.S. (2001) The protective role of HSP90 against 3-hydroxykynurenine-induced neuronal apoptosis. *Biochem. Biophys. Res. Commun.* 284, 261–267.

Lee J.E., Yenari M.A., Sun G.H., Xu L., Emond M.R., Cheng D., Steinberg G.K., and Giffard R.G. (2001) Differential neuroprotection from human heat shock protein 70 over-expression in in vitro and in vivo models of ischemia and ischemia-like conditions. *Exp. Neurol.* 170, 129–139.

Lelj-Garolla B. and Mauk A.G. (2006) Self-association and chaperone activity of Hsp27 are thermally activated. *J. Biol. Chem.* 281, 8169–8174.

Li C.Y., Lee J.S., Ko Y.G., Kim J.I., and Seo J.S. (2000) Heat shock protein 70 inhibits apoptosis downstream of cytochrome c release and upstream of caspase-3 activation. *J. Biol. Chem.* 275, 25665–25671.

Liang P. and MacRae T.H. (1997) Molecular chaperones and the cytoskeleton. *J. Cell Sci.* 110(Pt 13), 1431–1440.

Ligon L.A., LaMonte B.H., Wallace K.E., Weber N., Kalb R.G., and Holzbaur E.L. (2005) Mutant superoxide dismutase disrupts cytoplasmic dynein in motor neurons. *Neuroreport* 16, 533–536.

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.

Lino M.M., Schneider C., and Caroni P. (2002) Accumulation of SOD1 mutants in postnatal motoneurons does not cause motoneuron pathology or motoneuron disease. *J. Neurosci.* 22, 4825–4832.

Liu J., Lillo C., Jonsson P.A., Vande V.C., Ward C.M., Miller T.M., Subramaniam J.R., Rothstein J.D., Marklund S., Andersen P.M., Brannstrom T., Gredal O., Wong P.C., Williams D.S., and Cleveland D.W. (2004) Toxicity of familial ALS-linked SOD1 mutants from selective recruitment to spinal mitochondria. *Neuron* 43, 5–17.

Liu J., Shinobu L.A., Ward C.M., Young D., and Cleveland D.W. (2005) Elevation of the Hsp70 chaperone does not effect toxicity in mouse models of familial amyotrophic lateral sclerosis. *J. Neurochem.* 93, 875–882.

Loones M.T., Chang Y., and Morange M. (2000) The distribution of heat shock proteins in the nervous system of the unstressed mouse embryo suggests a role in neuronal and non-neuronal differentiation. *Cell Stress Chaperones* 5, 291–305.

Lu A., Ran R., Parmentier-Batteur S., Nee A., and Sharp F.R. (2002) Geldanamycin induces heat shock proteins in brain and protects against focal cerebral ischemia. *J. Neurochem.* 81, 355–364.

Luo W., Dou F., Rodina A., Chip S., Kim J., Zhao Q., Moulick K., Aguirre J., Wu N., Greengard P., and Chiosis G. (2007) Roles of heat-shock protein 90 in maintaining and facilitating the neurodegenerative phenotype in tauopathies. *Proc. Natl. Acad. Sci. U.S.A.* 104, 9511–9516.

Maatkamp A., Vlug A., Haasdijk E., Troost D., French P.J., and Jaarsma D. (2004) Decrease of Hsp25 protein expression precedes degeneration of motoneurons in ALS-SOD1 mice. *Eur. J. Neurosci.* 20, 14–28.

Magrane J., Smith R.C., Walsh K., and Querfurth H.W. (2004) Heat shock protein 70 participates in the neuroprotective response to intracellularly expressed beta-amyloid in neurons. *J. Neurosci.* 24, 1700–1706.

Manzerra P. and Brown I.R. (1992) Distribution of constitutive- and hyperthermia-inducible heat shock mRNA species (Hsp70) in the Purkinje layer of the rabbit cerebellum. *Neurochem. Res.* 17, 559–564.

Manzerra P., Rush S.J., and Brown I.R. (1993) Temporal and spatial distribution of heat shock mRNA and protein (Hsp70) in the rabbit cerebellum in response to hyperthermia. *J. Neurosci. Res.* 36, 480–490.

Marques C., Guo W., Pereira P., Taylor A., Patterson C., Evans P.C., and Shang F. (2006) The triage of damaged proteins: degradation by the ubiquitin-proteasome pathway or repair by molecular chaperones. *FASEB J.* 20, 741–743.

Martin J.E., Swash M., Mather K., and Leigh P.N. (1993) Expression of the human groEL stress-protein homologue in the brain and spinal cord. *J. Neurol. Sci.* 118, 202–206.

Masilamoni J.G., Jesudason E.P., Baben B., Jebaraj C.E., Dhandayuthapani S., and Jayakumar R. (2006) Molecular chaperone alpha-crystallin prevents detrimental effects of neuroinflammation. *Biochim. Biophys. Acta* 1762, 284–293.

Matsumori Y., Northington F.J., Hong S.M., Kayama T., Sheldon R.A., Vexler Z.S., Ferriero D.M., Weinstein P.R., and Liu J. (2006) Reduction of caspase-8 and -9 cleavage is associated with increased c-FLIP and increased binding of Apaf-1 and Hsp70 after neonatal hypoxic/ischemic injury in mice over-expressing Hsp70. *Stroke* 37, 507–512.

Matsumoto G., Stojanovic A., Holmberg C.I., Kim S., and Morimoto R.I. (2005) Structural properties and neuronal toxicity of amyotrophic lateral sclerosis-associated Cu/Zn superoxide dismutase 1 aggregates. *J. Cell Biol.* 171, 75–85.

Matsumoto G., Kim S., and Morimoto R.I. (2006) Huntingtin and mutant SOD1 form aggregate structures with distinct molecular properties in human cells. *J. Biol. Chem.* 281, 4477–4485.

Mayer M.P. and Bukau B. (1999) Molecular chaperones: the busy life of Hsp90. *Curr. Biol.* 9, R322–R325.

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

McClellan A.J. and Frydman J. (2001) Molecular chaperones and the art of recognizing a lost cause. *Nat. Cell Biol.* 3, E51–E53.

Melnick J., Aviel S., and Argon Y. (1992) The endoplasmic reticulum stress protein GRP94, in addition to BiP, associates with unassembled immunoglobulin chains. *J. Biol. Chem.* 267, 21303–21306.

Menzies F.M., Ince P.G., and Shaw P.J. (2002) Mitochondrial involvement in amyotrophic lateral sclerosis. *Neurochem. Int.* 40, 543–551.

Miyata Y. and Yahara I. (1992) The 90-kDa heat shock protein, HSP90, binds and protects casein kinase II from self-aggregation and enhances its kinase activity. *J. Biol. Chem.* 267, 7042–7047.

Muchowski P.J. and Wacker J.L. (2005) Modulation of neurodegeneration by molecular chaperones. *Nat. Rev. Neurosci.* 6, 11–22.

Murata S., Chiba T., and Tanaka K. (2003) CHIP: a quality-control E3 ligase collaborating with molecular chaperones. *Int. J. Biochem. Cell Biol.* 35, 572–578.

Nagai M., Re D.B., Nagata T., Chalazonitis A., Jessell T.M., Wichterle H., and Przedborski S. (2007) Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. *Nat. Neurosci.* 10, 615–622.

Namba Y., Tomonaga M., Ohtsuka K., Oda M., and Ikeda K. (1991) HSP 70 is associated with abnormal cytoplasmic inclusions characteristic of neurodegenerative diseases. *No To Shinkei* 43, 57–60.

Nathan D.F., Vos M.H., and Lindquist S. (1997) In vivo functions of the Saccharomyces cerevisiae Hsp90 chaperone. *Proc. Natl. Acad. Sci. U.S.A.* 94, 12949–12956.

Naylor D.J., Hoogenraad N.J., and Hoj P.B. (1996) Isolation and characterisation of a cDNA encoding rat mitochondrial GrpE, a stress-inducible nucleotide-exchange factor of ubiquitous appearance in mammalian organs. *FEBS Lett.* 396, 181–188.

Neckers L. (2002) Hsp90 inhibitors as novel cancer chemotherapeutic agents. *Trends Mol. Med.* 8, S55–S61.

New G.A., Hendrickson B.R., and Jones K.J. (1989) Induction of heat shock protein 70 mRNA in adult hamster facial nuclear groups following axotomy of the facial nerve. *Metab. Brain Dis.* 4, 273–279.

Nollen E.A., Salomons F.A., Brunsting J.F., Want J.J., Sibon O.C., and Kampinga H.H. (2001) Dynamic changes in the localization of thermally unfolded nuclear proteins associated with chaperone-dependent protection. *Proc. Natl. Acad. Sci. U.S.A.* 98, 12038–12043.

Okado-Matsumoto A. and Fridovich I. (2002) Amyotrophic lateral sclerosis: a proposed mechanism. *Proc. Natl. Acad. Sci. U.S.A.* 99, 9010–9014.

Olsson T., Hansson O., Nylandsted J., Jaattela M., Smith M.L., and Wieloch T. (2004) Lack of neuroprotection by heat shock protein 70 over-expression in a mouse model of global cerebral ischemia. *Exp. Brain Res.* 154, 442–449.

Oltean M., Olofsson R., Zhu C., Mera S., Blomgren K., and Olausson M. (2005) FK506 donor pretreatment improves intestinal graft microcirculation and morphology by concurrent inhibition of early NF-kappaB activation and augmented HSP72 synthesis. *Transplant. Proc.* 37, 1931–1933.

Otaka M., Yamamoto S., Ogasawara K., Takaoka Y., Noguchi S., Miyazaki T., Nakai A., Odashima M., Matsuhashi T., Watanabe S., and Itoh H. (2007) The induction mechanism of the molecular chaperone HSP70 in the gastric mucosa by Geranylgeranylacetone (HSP-inducer). *Biochem. Biophys. Res. Commun.* 353, 399–404.

Outeiro T.F., Klucken J., Strathearn K.E., Liu F., Nguyen P., Rochet J.C., Hyman B.T., and McLean P.J. (2006) Small heat shock proteins protect against alpha-synuclein-induced toxicity and aggregation. *Biochem. Biophys. Res. Commun.* 351, 631–638.

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. (2000) 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.

Parsell D.A., Kowal A.S., Singer M.A., and Lindquist S. (1994) Protein disaggregation mediated by heat-shock protein Hsp104. *Nature* 372, 475–478.

Parsell D.A., Sanchez Y., Stitzel J.D., and Lindquist S. (1991) Hsp104 is a highly conserved protein with two essential nucleotide-binding sites. *Nature* 353, 270–273.

Paschen W., Mengesdorf T., Althausen S., and Hotop S. (2001) Peroxidative stress selectively down-regulates the neuronal stress response activated under conditions of endoplasmic reticulum dysfunction. *J. Neurochem.* 76, 1916–1924.

Pasinelli P., Belford M.E., Lennon N., Bacskai B.J., Hyman B.T., Trotti D., and Brown R.H., Jr. (2004) Amyotrophic lateral sclerosis-associated SOD1 mutant proteins bind and aggregate with Bcl-2 in spinal cord mitochondria. *Neuron* 43, 19–30.

Patel Y.J., Payne S., de Belleroche J., and Latchman D.S. (2005) Hsp27 and Hsp70 administered in combination have a potent protective effect against FALS-associated SOD1-mutant-induced cell death in mammalian neuronal cells. *Brain Res. Mol. Brain Res.* 134, 256–274.

Pearl L.H. and Prodromou C. (2000) Structure and in vivo function of Hsp90. *Curr. Opin. Struct. Biol.* 10, 46–51.

Pelham H.R.B. (1990) Functions of the hsp70 protein family: an overview. In: Stress proteins in biology and medicine. Eds Morimoto R.I., Tissieres A., and Georgopoulos C. Cold Spring Harbor Laboratory Press, New York, pp 287–299.

Perrin V., Regulier E., Abbas-Terki T., Hassig R., Brouillet E., Aebischer P., Luthi-Carter R., and Deglon N. (2007) Neuroprotection by Hsp104 and Hsp27 in lentiviral-based rat models of Huntington's disease. *Mol. Ther.* 15, 903–911.

Pichon S., Bryckaert M., and Berrou E. (2004) Control of actin dynamics by p38 MAP kinase – Hsp27 distribution in the lamellipodium of smooth muscle cells. *J. Cell Sci.* 117, 2569–2577.

Pieri I., Cifuentes-Diaz C., Oudinet J.P., Blondet B., Rieger F., Gonin S., Arrigo A.P., and Thomas Y. (2001) Modulation of HSP25 expression during anterior horn motor neuron degeneration in the paralysed mouse mutant. *J. Neurosci. Res.* 65, 247–253.

Pinna G.F., Fiorucci M., Reimund J.M., Taquet N., Arondel Y., and Muller C.D. (2004) Celastrol inhibits pro-inflammatory cytokine secretion in Crohn's disease biopsies. *Biochem. Biophys. Res. Commun.* 322, 778–786.

Plumier J.C., Hopkins D.A., Robertson H.A., and Currie R.W. (1997) Constitutive expression of the 27-kDa heat shock protein (Hsp27) in sensory and motor neurons of the rat nervous system. *J. Comp. Neurol.* 384, 409–428.

Pramatarova A., Laganiere J., Roussel J., Brisebois K., and Rouleau G.A. (2001) Neuron-specific expression of mutant superoxide dismutase 1 in transgenic mice does not lead to motor impairment. *J. Neurosci.* 21, 3369–3374.

Pratt W.B. (1997) The role of the Hsp90-based chaperone system in signal transduction by nuclear receptors and receptors signaling via MAP kinase. *Annu. Rev. Pharmacol. Toxicol.* 37, 297–326.

Pratt W.B. and Toft D.O. (1997) Steroid receptor interactions with heat shock protein and immunophilin chaperones. *Endocr. Rev.* 18, 306–360.

Pratt W.B. and Toft D.O. (2003) Regulation of signaling protein function and trafficking by the Hsp90/Hsp70-based chaperone machinery. *Exp. Biol. Med. (Maywood)* 228, 111–133.

Prodromou C., Roe S.M., O'Brien R., Ladbury J.E., Piper P.W., and Pearl L.H. (1997) Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. *Cell* 90, 65–75.

Puttaparthi K., Wojcik C., Rajendran B., DeMartino G.N., and Elliott J.L. (2003) Aggregate formation in the spinal cord of mutant SOD1 transgenic mice is reversible and mediated by proteasomes. *J. Neurochem.* 87, 851–860.

Qian S.B., McDonough H., Boellmann F., Cyr D.M., and Patterson C. (2006) CHIP-mediated stress recovery by sequential ubiquitination of substrates and Hsp70. *Nature* 440, 551–555.

Quraishi H. and Brown I.R. (1995) Expression of heat shock protein 90 (Hsp90) in neural and nonneural tissues of the control and hyperthermic rabbit. *Exp. Cell Res.* 219, 358–363.

Raoul C., Barthelemy C., Couzinet A., Hancock D., Pettmann B., and Hueber A.O. (2005) Expression of a dominant negative form of Daxx in vivo rescues motoneurons from Fas (CD95)-induced cell death. *J. Neurobiol.* 62, 178–188.

Raoul C., Buhler E., Sadeghi C., Jacquier A., Aebischer P., Pettmann B., Henderson C.E., and Haase G. (2006) Chronic activation in presymptomatic amyotrophic lateral sclerosis (ALS) mice of a feedback loop involving Fas, Daxx, and FasL. *Proc. Natl. Acad. Sci. U.S.A.* 103, 6007–6012.

Raoul C., Estevez A.G., Nishimune H., Cleveland D.W., deLapeyriere O., Henderson C.E., Haase G., and Pettmann B. (2002) Motoneuron death triggered by a specific pathway downstream of Fas potentiation by ALS-linked SOD1 mutations. *Neuron* 35, 1067–1083.

Raoul C., Henderson C.E., and Pettmann B. (1999) Programmed cell death of embryonic motoneurons triggered through the Fas death receptor. *J. Cell Biol.* 147, 1049–1062.

Redmond T., Sanchez E.R., Bresnick E.H., Schlesinger M.J., Toft D.O., Pratt W.B., and Welsh M.J. (1989) Immunofluorescence colocalization of the 90-kDa heat-shock protein and microtubules in interphase and mitotic mammalian cells. *Eur. J. Cell Biol.* 50, 66–75.

Robinson M.B., Tidwell J.L., Gould T., Taylor A.R., Newbern J.M., Graves J., Tytell M., and Milligan C.E. (2005) Extracellular heat shock protein 70: a critical component for motoneuron survival. *J. Neurosci.* 25, 9735–9745.

Rockwell P., Yuan H., Magnusson R., and Figueiredo-Pereira M.E. (2000) Proteasome inhibition in neuronal cells induces a proinflammatory response manifested by up-regulation of cyclooxygenase-2, its accumulation as ubiquitin conjugates, and production of the prostaglandin PGE(2). *Arch. Biochem. Biophys.* 374, 325–333.

Rogalla T., Ehrnsperger M., Preville X., Kotlyarov A., Lutsch G., Ducasse C., Paul C., Wieske M., Arrigo A.P., Buchner J., and Gaestel M. (1999) Regulation of Hsp27 oligomerization, chaperone function, and protective activity against oxidative stress/tumor necrosis factor alpha by phosphorylation. *J. Biol. Chem.* 274, 18947–18956.

Rordorf G., Koroshetz W.J., and Bonventre J.V. (1991) Heat shock protects cultured neurons from glutamate toxicity. *Neuron* 7, 1043–1051.

Rose D.W., Wettenhall R.E., Kudlicki W., Kramer G., and Hardesty B. (1987) The 90-kilodalton peptide of the heme-regulated eIF-2 alpha kinase has sequence similarity with the 90-kilodalton heat shock protein. *Biochemistry* 26, 6583–6587.

Rosen D.R., Siddique T., Patterson D., Figlewicz D.A., Sapp P., Hentati A., Donaldson D., Goto J., O'Regan J.P., Deng H.X., et al. (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 362, 59–62.

Rosser M.F., Washburn E., Muchowski P.J., Patterson C., and Cyr D.M. (2007) Chaperone functions of the E3 Ubiquitin ligase CHIP. *J. Biol. Chem.* 282, 22267–22277.

Sahara N., Maeda S., Yoshiike Y., Mizoroki T., Yamashita S., Murayama M., Park J.M., Saito Y., Murayama S., and Takashima A. (2007) Molecular chaperone-mediated tau protein metabolism counteracts the formation of granular tau oligomers in human brain. *J. Neurosci. Res.* 85, 3098–3108.

Sahara N., Murayama M., Mizoroki T., Urushitani M., Imai Y., Takahashi R., Murata S., Tanaka K., and Takashima A. (2005) In vivo evidence of CHIP up-regulation attenuating tau aggregation. *J. Neurochem.* 94, 1254–1263.

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., 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.

Sato K., Saito H., and Matsuki N. (1996) HSP70 is essential to the neuroprotective effect of heat-shock. *Brain Res.* 740, 117–123.

Satoh J. and Kim S.U. (1994) HSP72 induction by heat stress in human neurons and glial cells in culture. *Brain Res.* 653, 243–250.

Satoh J., Yukitake M., and Kuroda Y. (1998) Constitutive and heat-inducible expression of HSP105 in neurons and glial cells in culture. *Neuroreport* 9, 2977–2983.

Schepers H., Geugien M., van der T.M., Bryantsev A.L., Kampinga H.H., Eggen B.J., and Vellenga E. (2005) HSP27 protects AML cells against VP-16-induced apoptosis through modulation of p38 and c-Jun. *Exp. Hematol.* 33, 660–670.

Schirmer E.C., Glover J.R., Singer M.A., and Lindquist S. (1996) HSP100/Clp proteins: a common mechanism explains diverse functions. *Trends Biochem. Sci.* 21, 289–296.

Schirmer E.C., Queitsch C., Kowal A.S., Parsell D.A., and Lindquist S. (1998) The ATPase activity of Hsp104, effects of environmental conditions and mutations. *J. Biol. Chem.* 273, 15546–15552.

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.

Shaner L. and Morano K.A. (2007) All in the family: atypical Hsp70 chaperones are conserved modulators of Hsp70 activity. *Cell Stress Chaperones* 12, 1–8.

Sharp P., Krishnan M., Pullar O., Navarrete R., Wells D., and de Belleroche J. (2006) Heat shock protein 27 rescues motor neurons following nerve injury and preserves muscle function. *Exp. Neurol.* 198, 511–518.

Shaw P.J. (2005) Molecular and cellular pathways of neurodegeneration in motor neurone disease. *J. Neurol. Neurosurg. Psychiatr.* 76, 1046–1057.

Shaw P.J. and Eggett C.J. (2000) Molecular factors underlying selective vulnerability of motor neurons to neurodegeneration in amyotrophic lateral sclerosis. *J. Neurol.* 247(Suppl 1), I17–I27.

Shin Y., Klucken J., Patterson C., Hyman B.T., and McLean P.J. (2005) The co-chaperone carboxyl terminus of Hsp70-interacting protein (CHIP) mediates alpha-synuclein degradation decisions between proteasomal and lysosomal pathways. *J. Biol. Chem.* 280, 23727–23734.

Shinder G.A., Lacourse M.C., Minotti S., and Durham H.D. (2001) Mutant Cu/Zn-superoxide dismutase proteins have altered solubility and interact with heat shock/stress proteins in models of amyotrophic lateral sclerosis. *J. Biol. Chem.* 276, 12791–12796.

Sigler P.B., Xu Z., Rye H.S., Burston S.G., Fenton W.A., and Horwich A.L. (1998) Structure and function in GroEL-mediated protein folding. *Annu. Rev. Biochem.* 67, 581–608.

Siklos L., Engelhardt J., Harati Y., Smith R.G., Joo F., and Appel S.H. (1996) Ultrastructural evidence for altered calcium in motor nerve terminals in amyotropic lateral sclerosis. *Ann. Neurol.* 39, 203–216.

Singh B.N., Rao K.S., Ramakrishna T., Rangaraj N., and Rao C. (2007) Association of alphaB-crystallin, a small heat shock protein, with actin: role in modulating actin filament dynamics in vivo. *J. Mol. Biol.* 366, 756–767.

Sinn D.I., Chu K., Lee S.T., Song E.C., Jung K.H., Kim E.H., Park D.K., Kang K.M., Kim M., and Roh J.K. (2007) Pharmacological induction of heat shock protein exerts neuroprotective effects in experimental intracerebral hemorrhage. *Brain Res.* 1135, 167–176.

Soltys B.J. and Gupta R.S. (1996) Immunoelectron microscopic localization of the 60-kDa heat shock chaperonin protein (Hsp60) in mammalian cells. *Exp. Cell Res.* 222, 16–27.

Song J., Takeda M., and Morimoto R. (2001) Bag1-Hsp70mediates a physiological stress signalling pathway that refulates Raf-1/ERK and cell growth. *Nat. Cell Biol.* 3, 276–282.

Strey C.W., Spellman D., Stieber A., Gonatas J.O., Wang X., Lambris J.D., and Gonatas N.K. (2004) Dysregulation of stathmin, a microtubule-destabilizing protein, and up-regulation of Hsp25, Hsp27, and the antioxidant peroxiredoxin 6 in a mouse model of familial amyotrophic lateral sclerosis. *Am. J. Pathol.* 165, 1701–1718.

Suzuki A., Yamaguchi Y., Hoshi T., Hayashi A., and Baba H. (2007) Differential inductions of small heat shock protein 27 and 1-Cys peroxiredoxin in reactive astrocytes in sulfatide-deficient mouse spinal cord. *J. Neurosci. Res.* 85(9), 1921–1932.

Tagawa K., Marubuchi S., Qi M.L., Enokido Y., Tamura T., Inagaki R., Murata M., Kanazawa I., Wanker E.E., and Okazawa H. (2007) The induction levels of heat shock protein 70 differentiate the vulnerabilities to mutant huntingtin among neuronal subtypes. *J. Neurosci.* 27, 868–880.

Takayama S. and Reed J.C. (2001) Molecular chaperone targeting and regulation by BAG family proteins. *Nat. Cell. Biol*. 3, 237–241.

Takeuchi H., Kobayashi Y., Yoshihara T., Niwa J., Doyu M., Ohtsuka K., and Sobue G. (2002) Hsp70 and Hsp40 improve neurite outgrowth and suppress intracytoplasmic aggregate formation in cultured neuronal cells expressing mutant SOD1. *Brain Res.* 949, 11–22.

Taylor D.M., De K.P., Minotti S., and Durham H.D. (2007) Manipulation of protein kinases reveals different mechanisms for up-regulation of heat shock proteins in motor neurons and non-neuronal cells. *Mol. Cell Neurosci.* 34, 20–33.

Tedeschi B. and Ciavarra R.P. (1997) Differential effects of axotomy on the in vivo synthesis of the stress-inducible and constitutive 70-kDa heat-shock proteins in rat dorsal root ganglia. *Brain Res. Mol. Brain Res.* 45, 199–206.

Tezel G. and Wax M.B. (2000) The mechanisms of Hsp27 antibody-mediated apoptosis in retinal neuronal cells. *J. Neurosci.* 20, 3552–3562.

Thirumalai D. and Lorimer G.H. (2001) Chaperonin-mediated protein folding. *Annu. Rev. Biophys. Biomol. Struct.* 30, 245–269.

Townsend P.A., Cutress R.I., Sharp A., Brimmell M., and Packham G. (2003) BAG-1: a multifunctional regulator of cell growth and survival. *Biochim. Biophys. Acta* 1603, 83–98.

Uney J.B., Staley K., Tyers P., Sofroniew M.V., and Kew J.N. (1994) Transfection with Hsp70i protects rat dorsal root ganglia neurones and glia from heat stress. *Gene Ther.* 1(Suppl 1), S65.

Urushitani M., Kurisu J., Tateno M., Hatakeyama S., Nakayama K., Kato S., and Takahashi R. (2004) CHIP promotes proteasomal degradation of familial ALS-linked mutant SOD1 by ubiquitinating Hsp/Hsc70. *J. Neurochem.* 90, 231–244.

Van Molle W., Wielockx B., Mahieu T., Takada M., Taniguchi T., Sekikawa K., and Libert C. (2002) HSP70 protects against TNF-induced lethal inflammatory shock. *Immunity* 16, 685–695.

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.

Vigh L., Literati P.N., Horvath I., Torok Z., Balogh G., Glatz A., Kovacs E., Boros I., Ferdinandy P., Farkas B., Jaszlits L., Jednakovits A., Koranyi L., and Maresca B. (1997) Bimoclomol: a nontoxic, hydroxylamine derivative with stress protein-inducing activity and cytoprotective effects. *Nat. Med.* 3, 1150–1154.

Vleminckx V., Van Damme P., Goffin K., Delye H., Van Den B.L., and Robberecht W. (2002) Upregulation of Hsp27 in a transgenic model of ALS. *J. Neuropathol. Exp. Neurol.* 61, 968–974.

Voos W. and Rottgers K. (2002) Molecular chaperones as essential mediators of mitochondrial biogenesis. *Biochim. Biophys. Acta* 1592, 51–62.

Wagstaff M.J., Collaco-Moraes Y., Smith J., de Belleroche J.S., Coffin R.S., and Latchman D.S. (1999) Protection of neuronal cells from apoptosis by Hsp27 delivered with a herpes simplex virus-based vector. *J. Biol. Chem.* 274, 5061–5069.

Wang J., Martin E., Gonzales V., Borchelt D.R., and Lee M.K. (2007) Differential regulation of small heat shock proteins in transgenic mouse models of neurodegenerative diseases. *Neurobiol. Aging.* In press.

Watanabe M., Dykes-Hoberg M., Culotta V.C., Price D.L., Wong P.C., and Rothstein J.D. (2001) Histological evidence of protein aggregation in mutant SOD1 transgenic mice and in amyotrophic lateral sclerosis neural tissues. *Neurobiol. Dis.* 8, 933–941.

Waza M., Adachi H., Katsuno M., Minamiyama M., Sang C., Tanaka F., Inukai A., Doyu M., and Sobue G. (2005) 17-AAG, an Hsp90 inhibitor, ameliorates polyglutamine-mediated motor neuron degeneration. *Nat. Med.* 11, 1088–1095.

Waza M., Adachi H., Katsuno M., Minamiyama M., Tanaka F., and Sobue G. (2006a) Alleviating neurodegeneration by an anticancer agent: an Hsp90 inhibitor (17-AAG). *Ann. N.Y. Acad. Sci.* 1086, 21–34.

Waza M., Adachi H., Katsuno M., Minamiyama M., Tanaka F., Doyu M., and Sobue G. (2006b) Modulation of Hsp90 function in neurodegenerative disorders: a molecular-targeted therapy against disease-causing protein. *J. Mol. Med.* 84, 635–646.

Welch W.J. (1990) The mammalian stress response: cell physiology and biochemistry of stress proteins. In: Stress proteins in biology and medicine. Eds Morimoto R.I., Tissieres A., and Georgopoulos C. Cold Spring Harbour Laboratory Press, New York, 223–279.

Westerheide S.D., Bosman J.D., Mbadugha B.N., Kawahara T.L., Matsumoto G., Kim S., Gu W., Devlin J.P., Silverman R.B., and Morimoto R.I. (2004) Celastrols as inducers of the heat shock response and cytoprotection. *J. Biol. Chem.* 279, 56053–56060.

Williams K.L., Rahimtula M., and Mearow K.M. (2006) Heat shock protein 27 is involved in neurite extension and branching of dorsal root ganglion neurons in vitro. *J. Neurosci. Res.* 84, 716–723.

Williamson T.L. and Cleveland D.W. (1999) Slowing of axonal transport is a very early event in the toxicity of ALS-linked SOD1 mutants to motor neurons. *Nat. Neurosci.* 2, 50–56.

Wyttenbach A., Sauvageot O., Carmichael J., Diaz-Latoud C., Arrigo A.P., and Rubinsztein D.C. (2002) Heat shock protein 27 prevents cellular polyglutamine toxicity and suppresses the increase of reactive oxygen species caused by huntingtin. *Hum. Mol. Genet.* 11, 1137–1151.

Xia H., Ikata T., Katoh S., Rokutan K., Saito S., Kawai T., and Kishi K. (1998) Whole body hyperthermia selectively induces heat shock protein 72 in neurons of the rat spinal cord. *Neurosci. Lett.* 258, 151–154.

Xiao L., Lu X., and Ruden D.M. (2006) Effectiveness of Hsp90 inhibitors as anti-cancer drugs. *Mini. Rev. Med. Chem.* 6, 1137–1143.

Xu Z., Cork L.C., Griffin J.W., and Cleveland D.W. (1993) Increased expression of neurofilament subunit NF-L produces morphological alterations that resemble the pathology of human motor neuron disease. *Cell* 73, 23–33.

Yagita Y., Kitagawa K., Ohtsuki T., Tanaka S., Hori M., and Matsumoto M. (2001) Induction of the Hsp110/105 family in the rat hippocampus in cerebral ischemia and ischemic tolerance. *J. Cereb. Blood Flow Metab.* 21, 811–819.

Yamagishi N., Ishihara K., and Hatayama T. (2004) Hsp105alpha suppresses Hsc70 chaperone activity by inhibiting Hsc70 ATPase activity. *J. Biol. Chem.* 279, 41727–41733.

Yamagishi N., Ishihara K., Saito Y., and Hatayama T. (2003) Hsp105 but not Hsp70 family proteins suppress the aggregation of heat-denatured protein in the presence of ADP. *FEBS Lett.* 555, 390–396.

Yamagishi N., Ishihara K., Saito Y., and Hatayama T. (2006) Hsp105 family proteins suppress staurosporine-induced apoptosis by inhibiting the translocation of Bax to mitochondria in HeLa cells. *Exp. Cell Res.* 312, 3215–3223.

Yamashita H., Kawamata J., Okawa K., Kanki R., Nakamizo T., Hatayama T., Yamanaka K., Takahashi R., and Shimohama S. (2007) Heat-shock protein 105 interacts with and suppresses aggregation of mutant Cu/Zn superoxide dismutase: clues to a possible strategy for treating ALS. *J. Neurochem* 102, 1497–1505.

Yenari M.A. (2002) Heat shock proteins and neuroprotection. *Adv. Exp. Med. Biol.* 513, 281–299.

Young J.C., Moarefi I., and Hartl F.U. (2001) Hsp90: a specialized but essential protein-folding tool. *J. Cell Biol.* 154, 267–273.

Zaarur N., Gabai V.L., Porco J.A., Jr., Calderwood S., and Sherman M.Y. (2006) Targeting heat shock response to sensitize cancer cells to proteasome and Hsp90 inhibitors. *Cancer Res.* 66, 1783–1791.

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.

Zheng Z., Kim J.Y., Ma H., Lee J.E., and Yenari M.A. (2008) Anti-inflammatory effects of the 70 kDa heat shock protein in experimental stroke. *J. Cereb. Blood Flow Metab.* 28, 53–63.

Zourlidou A., Payne S., and Latchman D.S. (2004) HSP27 but not HSP70 has a potent protective effect against alpha-synuclein-induced cell death in mammalian neuronal cells. *J. Neurochem.* 88, 1439–1448.