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Review

Stress proteins in neural cells: functional roles in health and disease

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Abstract. Heat shock proteins (HSPs) or stress proteins participate in protein synthesis, protein folding, transport and translocalization processes. Stress situations trigger a heat shock response leading to their induction. Similarly, they can be upregulated by impairment of the proteasomal degradation pathway. The upregulation of stress proteins is an important step in prevention of protein aggregation and misfolding after stress, and also is essential during development and differentiation. A number of HSPs are constitutively or inducibly expressed in the nervous system and connected to protection of nerve cells and glia. The cytoskeleton is affected by stress, and HSPs have been shown to interact with the cytoskeleton in normal cells and to assist proper assembly, spatial organization and cross-linking properties. The integrity of the cytoskeleton is disturbed in many neurodegenerative disorders, and filamentous cytoplasmic inclusion bodies, containing a variety of HSPs, are observed. This review summarizes the recent literature on the presence and induction of HSPs in neural cells, and their possible functional roles in health and disease are discussed.

Key words. Heat shock proteins; nerve cells; oligodendrocytes; inclusion bodies; neurodegenerative diseases; cytoskeleton.

Introduction

Accumulation of abnormally folded proteins as a result of a variety of stress situations, including hyperthermia, viral infection, ischemia, anoxia, oxidative stress [1] and exposure to heavy metals, triggers a heat shock response, which involves induction of heat shock proteins (HSPs) or so-called stress proteins in many cellular systems (fig. 1). Similarly, HSPs can be induced by direct impairment of the proteasomal degradation pathway by proteasome inhibitors [2]. Stress proteins may serve as biomarkers to identify stress specificity and localize pathological processes leading to cell and organelle damage. Stress-induced elevation of HSPs might play protective roles, provide tolerance against further stress situations and is involved in modulation of the proteolytic machinery, preventing protein misfolding and aggregation, thereby accelerating cellular repair processes (for review see [3]). Most HSPs are classified according to their molecular weights. The major classes comprise five families: HSP100, HSP90, HSP70, HSP60 and the small heat shock proteins (sHSPs), with molecular weights ranging from 12 to 43 kDa and which includes the α-crystallin family of proteins [4]. Also, members of the glucose-regulated proteins (Grps), e. g. Grp 78 and 94, which are induced by perturbation of the endoplasmatic reticulum (ER) (for a recent review see [5]), are markers for stress responses. Constitutively expressed HSPs function as molecular chaperones and participate in protein synthesis, protein folding, transport and translocalization processes, and upon stress prevent irreversible aggregation of proteins (fig. 2). The role of chaperones in protein

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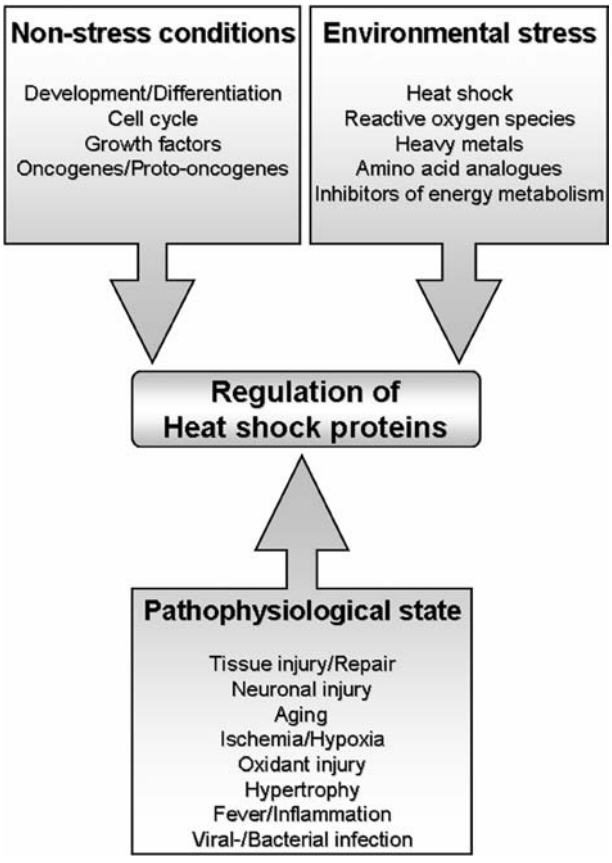

Figure 1. Physiological and nonphysiological conditions can modulate the heat shock responses and regulate the constitutive and inducible expression of HSPs.

folding has been extensively summarized in a number of excellent articles [3, 4, 6, 7], and will not be reviewed here. Chaperones not only contribute to in vivo protein folding but also prevent nonproductive interactions with other proteins and cellular components, such as the cytoskeleton and cell membranes. HSP40, HSP60 and HSP70 are the major HSPs that comprise chaperone activity.

The rapid production of HSPs after stressful insults is regulated by heat shock transcription factors (HSFs) (fig. 2). In mammalian cells, three HSFs, namely HSF1, HSF2 and HSF4, have been identified (for review see [8]), with HSF1 as the prototype in vertebrates being the major regulator of the heat shock response and other environmental stressors. Under nonstressed conditions HSF1 is maintained as an inactive monomer with little DNA binding activity. In response to stress, HSF1 acquires DNA binding activity by trimerization and phosphorylation, and mediates heat shock gene expression by binding to highly conserved heat shock elements (HSEs) in the promoter regions of heat shock genes [9, 10]. The heat shock response is attenuated by the chaperones HSP70 and HSP40, which associate with the activation domain of HSF1, and HSP90 also has been shown to play a role in maintaining HSF1 in an inert state [11]. Hence, high levels of chaperone activity are sensed by the stress- induced activator and downregulate the stress response.

Thus, upregulation of stress proteins is an important step in the prevention of protein aggregation and misfolding after stress, and is also essential during development and differentiation. It is linked to the cell cycle and controlled by mitogenic signals and growth factors (as schematized in fig. 1). The chaperone system and the proteolytic machinery closely function together and determine the fate of proteins within the cells. Misfunctions have pathogenic consequences and may lead to cell death and degeneration. In this article we review the recent literature on the presence and induction of heat shock proteins and their functional significance in neural cells, particularly in glial cells. Major emphasis is laid on the association of stress proteins with insoluble inclusions, identified in a number of neurodegenerative diseases, and their possible potential as biomedical markers of cell death and survival.

HSPs in the nervous system

A number of HSPs are constitutively expressed in the central nervous system (CNS), and their upregulation and presence has been connected to neuroprotection of nerve cells and glia [12–14]. Among these are the HSP90 family, HSP70 family, HSP60, the small HSPs including αB-crystallin, HSP32 and ubiquitin (table 1). HSPs are located in the cytosol, and in eucaryotic cells they can be associated with various cellular compartments or are translocated upon stimulation, e.g. mitochondria, ER or the nucleus. Below we briefly summarize the properties of the HSPs most relevant to this review.

HSP90 family

HSP90 is a ubiquitous cytoplasmic protein which is constitutively expressed in the mammalian brain [15]. HSP90 has chaperone activity and cooperates with HSP70 in the degradation of misfolded proteins [16]. It specifically interacts with cytoskeletal elements, such as actin and protein kinases, and might be a necessary component of fundamental cellular processes, e.g. in hormone signalling and cell cycle control [17, 18]. Another member of this family is Grp94, which is located in the ER and is induced in response to ER stress [5].

HSP70 family

The 70-kDa HSPs are most abundant and have been extensively characterized after their initial discovery (for review see [19]). They include the constitutively expressed,

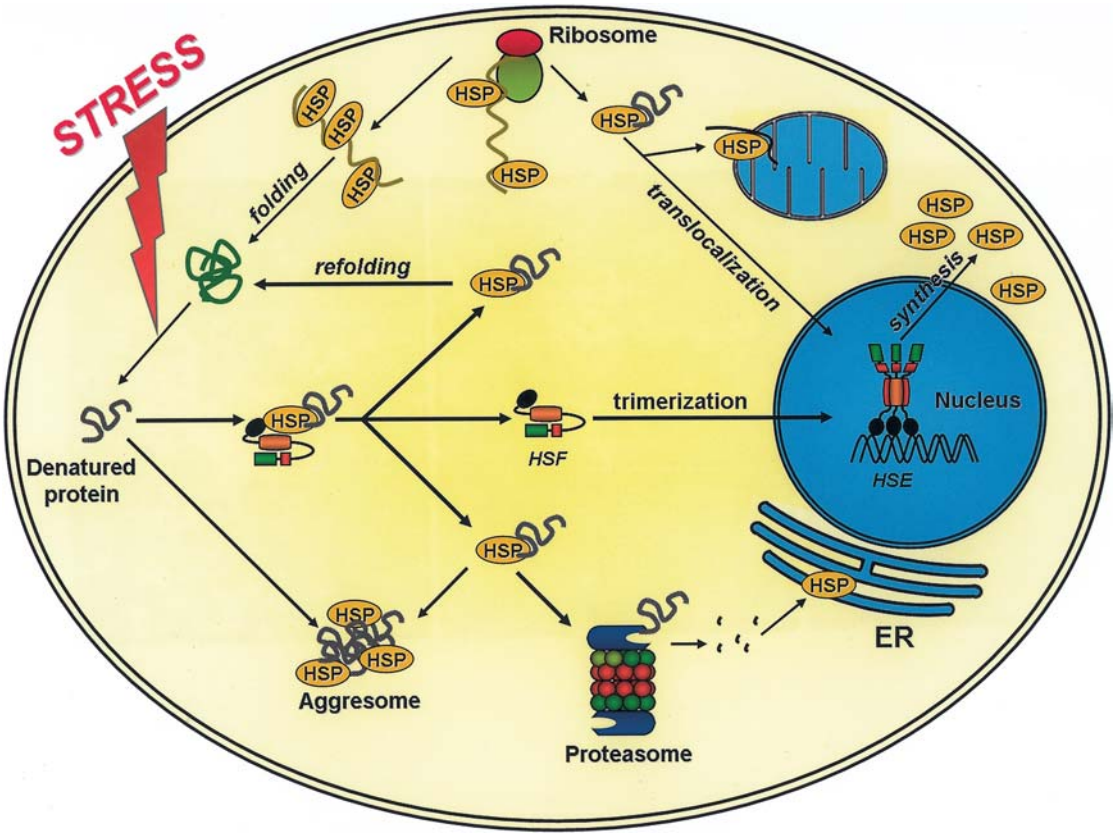

Figure 2. Illustration of the multiple functions of heat shock proteins and their induction pathway. HSPs bind to the nascent polypeptide chain emerging from the ribosome, and participate in their folding and assembly. HSPs also target proteins for translocation to the nucleus, the mitochondria and endoplasmatic reticulum (ER). Upon stress, the presence of denatured proteins induce the release of mutant heat shock transcription factor 1 (HSF1) from the HSP, followed by trimerization, phosphorylation and translocation to the nucleus, where HSF1 binds to the heat shock element (HSE) in the promoter region of heat shock genes. HSF1 induction results in an increase in HSP synthesis. HSPs help to refold denatured proteins to prevent their aggregation. Proteins that fail to properly fold again are ubiquitinated and guided to the proteasome for degradation. If the capacity of the cell to degrade or refold abnormal polypeptides is exceeded, denatured proteins accumulate, tend to aggregate and form aggresomes.

only moderately inducible, cognate protein HSC70 with an apparent molecular weight of 73 kDa, its closely related inducible form HSP70, with an apparent molecular weight of 72 kDa, and Grp78 or BiP (for binding protein), which is located in the lumen of the ER. The inducible form HSP70 is not constitutively expressed and strongly induced following neural trauma, including tissue injury and ischemia [15, 20]. After induction, HSP70 appears in the cytoplasm and also in the nucleus [21]. Grp78 is a stress-response protein which is induced by agents or conditions that adversely affect ER function [22]. This protein is essential for the proper glycosylation, folding and assembly of many membrane-bound and secreted proteins [23]. Grp78 is critical for maintenance of cell homeostasis and prevention of apoptosis [24]. Levels of Grp78 serve as a reliable biomarker to monitor hypoglycemia [25], and its protective function in neurons exposed to glutamate and oxidative stress has been described [22].

All members of this family bind to ATP and comprise chaperone activity. HSP70 members recognize and bind to nascent polypeptide chains as well as to partially folded intermediates of proteins, preventing their aggregation and misfolding. The binding of ATP triggers a critical conformational change leading to the release of the bound substrate protein [4]. HSC70 has been reported to participate in folding and assembly of nascent proteins, to maintain proteins in their unfolded state and to promote their translocation through intracellular membranes. Accordingly, HSC70 is thought to participate in protein-targeting processes to the nucleus [26], the ER [27] and the mitochondria [28]. HSP70 interacts with HSP40, a heat-inducible DnaJ homologue [29]. Together they promote cellular protein folding and repair misfolded proteins [30–32]. HSJ1, a human DnaJ homologue, has been shown to be preferentially expressed in neurons [33], and recently the DnaJ-like protein MRJ with the potential to inhibit polyglutamine aggregation and its cellular toxic-

Table 1. HSPs, their localization and potential functions.

HSP	kDa	Loc	Potential function
Grp94	94	ER	chaperone
Grp78 (BiP)	78		GRPs Ca^{2+}-binding proteins cytoprotection against ER stress
HSP90	90	C	chaperone cooperates with HSP70 interacts with cytoskeleton hormone signalling cell cycle control
HSC70 (HSP73)	73	C	chaperone
HSP70 (HSP72)	72	N	binds to unfolded proteins interacts with cochaperone HSP40 (DnaJ-homologue) neuroprotection synaptic plasticity
HSP60	60	M	chaperonin interacts with cochaperone HSP10 folding and assembly of transported proteins to mitochondria involved in apoptotic signalling pathway
HSP32/HO-1	32	C	comprises enzymatic activity sensor and regulator of oxidative stress contributes to pathological iron deposition
HSP25/27	25–27	C	chaperone phosphorylated upon stress
αB-crystallin	20		associated with cytoskeleton and centrosome stabilization of microfilaments modulator of cytoskeleton
Ubiquitin	8.5	C N	covalent attachment to proteins targets proteins to the proteasome for degradation proteolysis-independent transcription regulation signal transduction hallmark of inclusions detected in neurodegenerative diseases marker for cell damage

Abbreviations: Loc, localization; C, cytoplasm; ER, endoplasmatic reticulum; M, mitochondrial matrix; N, nucleus.

ity, a hallmark of Huntington's disease, was found to be highly enriched in the CNS [34].

In vitro, HSC70 binding to substrates prone to thermal aggregation was found to rely on the HSP40-enhanced ATP hydrolysis [30]. In addition, HSP70 activity is assisted by cochaperones such as BAG-1 and CHIP (carboxy terminus of HSP70-interacting protein), which recently have been demonstrated to provide a direct link between protein folding and targeting aberrant polypeptides to the proteasome for degradation (for review see [4, 16]).

HSP60

The proteins of this family are also called chaperonins. They are ring-shaped oligomeric protein complexes which bind nonnative proteins within a large cavity. In eukaryotes HSP60 is constitutively expressed and highly abundant, synthesized in the cytoplasm and then transported to the mitochondria. Its functions are dependent on HSP10, which binds to HSP60 and regulates its substrate binding and ATPase activity. HSP60 is associated with the mitochondrial matrix and participates in the folding and assembly of transported proteins into the mitochondrion [35, 36]. HSP60 was described to protect substrate proteins from thermally induced aggregation [37]. Recently, it was shown that HSP60 and its cochaperone HSP10 may also function in the regulation of procaspase-3 activation in mitochondria and hence participate in apoptotic signaling pathways [38]. The data imply that pro-caspase-3 is present in mitochondria in a complex with HSP60 and HSP10. Upon induction of apoptosis by staurosporine, pro-caspase-3 was activated and dissociated from the complex. HSPs were then released from the mitochondria simultaneously with cytochrome c, which mediates caspase activation in the cytosol. The authors hypothesize that these HSPs act as docking molecules and play a passive role in amplification of the caspase cascade [38]. Hence, HSPs which are constitutively expressed can act as positive regulators of apoptosis, while inducible HSPs might promote cell sur-

vival through the inhibition of apoptosis (for review see [39]).

sHSPs and αB-crystallin

The sHSPs and α-crystallin are closely related and contain a highly conserved carboxy-terminal region termed the α-crystallin domain [4, 40]. They exhibit chaperone activity, are stress inducible and confer thermoprotection [41]. Small HSPs associate with the cytoskeleton, specifically with microfilaments and intermediate filaments, have physiological roles as modulators of the cytoskeleton and might protect the cytoskeleton during stress [42, 43]. In mammalian cells only one small HSP is expressed, with a molecular weight of 25–28 kDa in rodents or 27–28 kDa in humans [36]. HSP27 is regulated by phosphorylation through serine protein kinases, and phosphorylation is increased upon various stimuli, such as heat shock, sodium arsenite, oxidative stress, mitogens, tumor promoters and calcium ionophores (for review see [44]). Nonphosphorylated HSP27 stabilizes actin filaments [45] and also modulates glial fibrillary acidic protein (GFAP) assembly [46].

α-Crystallins are the major structural proteins in the vertebrate eye lenses [47]. Large aggregates of α-crystallin (800 kDa) consist of two types of related subunits: acidic αA (173 amino acids) and basic αB (175 amino acids) [48, 49]. These two subunits have a sequence homology of approximately 57%. The αA subunit is lens specific, and αB-crystallin, with a molecular weight of 20 kDa, has been detected in a number of nonlenticular tissues, including cardiac tissue and glial cells in the peripheral nervous system (PNS) and CNS [50–52]. αB-Crystallin is a major component of glial filament inclusions (see below) and in the CNS is primarily expressed in oligodendrocytes [43, 53].

HSP32

HSP32, also known as heme oxygenase 1 (HO-1), is a small stress protein with enzymatic activity that belongs to the heme oxygenase (HO) family. HO catalyzes the oxidative degradation of heme to biliverdin, which is subsequently converted to bilirubin, and equimolar amounts of carbon monoxide and iron. Heme and iron may increase the formation of reactive oxygen intermediates, and hence exacerbate intracellular oxidative stress [54]. Biliverdin and bilirubin, on the other hand, have antioxidant capacity [55, 56, for review see 57]. Three mammalian isoforms of HO have been identified: HO-1, an inducible enzyme that is synthesized in response to heat shock, heme and oxidative stress [13, 58]. HO-1 is most highly concentrated in tissues that are involved in the catabolism of heme proteins [59]; HO-2, the constitutively expressed and noninducible isoform, which is present in highest concentrations in the brain and testes and is thought to be particularly involved in signalling pathways [60]; HO-3, an isoform with low catalytic activity and nonresolved physiological roles [61]. HO-1 interacts with the mitogen-activated kinase (MAPK) cascade [58], might be an effective system to counteract oxidative stress and is considered as a sensor and regulator of many forms of oxidative stress [54, 57].

Ubiquitin

Chaperones play a key role in assisting protein folding and also participate in the degradation of misfolded proteins, which cannot be converted to the native state in the chaperone system [62, 63]. Chaperone proteins assist and enable these proteins to enter the ubiquitin-proteasome pathway, which has been extensively reviewed in the past and is referred to [64–66]. Ubiquitin is a small, evolutionarily highly conserved protein (8.5 kDa) which is covalently attached in successive steps to the target protein and degraded by the 26S proteasome [64]. Protein modification by ubiquitination is one of the most common regulatory processes in eukaryotes.

Besides its classic role in proteolytic events, ubiquitin is involved in diverse proteasome-independent regulatory processes, including regulation of transcription by directly modifiying transcription factors [67], and is an important factor in signal transduction processes in immunity [68].

Ubiquitin is a stress-induced protein, one of the smallest HSPs and expressed throughout the brain, where it is elevated under stress and a variety of pathological situations (for review see [69, 70]). An increase in ubiquitin immunoreactivity is commonly observed in many neurological disorders and a hallmark of cellular inclusions detected in neurodegenerative diseases [63, 65, 71], which will be discussed below. Hence, ubiquitin is a useful marker of cell damage.

Differential expression of stress proteins in nerve cells and glia: indicators of cytoprotection or cell death?

Numerous studies have been carried out in a variety of cell culture systems on the presence and induction of HSPs. These studies have shown that HSPs are differentially expressed in nerve cells and glia and that cell-type-specific responses to various stressors might be observed. Also, individual cell types in the brain show different susceptibilities to stress situations. Hence, depending on the level of stress, some cells remain unaffected, while others are already severely stressed and respond by induction of stress proteins. Induction of HSPs, instead of playing a protective role, might pass a certain threshold and contribute to the onset of cell death.

HSP70

HSP70 is the one most extensively studied, and it is generally accepted that its overexpression in neurons and glia leads to neuroprotection (for review see [14]). The constitutive isoform, HSC70, and its cooperator HSP40 are present in the postsynaptic structures of rat brain, where they might be related to synaptic plasticity phenomena [7]. While in nerve cells HSP70 is only weakly inducible [72–75], it is highly upregulated by hyperthermia and a variety of other stress situations in astrocytes, oligodendrocytes and microglia, respectively [53, 76–82]. In oligodendrocytes HSP70 induction was not observable after oxidative stress exerted by micromolar concentrations of hydrogen peroxide [53, 80]. Interestingly, when oligodendrocytes were treated with hydrogen peroxide and then subjected to heat shock, HSP70 was induced to a much greater extent than by heat stress alone. However, cells were not rescued and died by programmed cell death [53]. Recently it was shown that HSP70 can be released by glia cells and that exogenous HSP70 can enhance neuronal stress tolerance [83]. Another study indicates that the addition of HSP90, HSP70 or HSP32 to rat microglial cultures caused microglia activation and an increase in cytokine production, which might contribute to neuroprotective roles in the brain [84].

HSP60

HSP60 is constitutively expressed in neuronal cells [85, 86], astrocytes [87] and oligodendrocytes [53, 88, 89]. Figure 3 demonstrates its constitutive expression in rat brain oligodendrocytes, where it is present in the cell soma and the cytoplasmic extensions. After heat shock or oxidative stress it is only slightly induced in oligodendrocytes [53] and astrocytes [unpublished results] in vitro. However, elevated HSP60 expression was described as a prominent feature in acute lesions of multiple sclerosis brains, and at the height of the disease, astrocytes and oligodendrocytes displayed both mitochondrial and cytosolic immunoreactivity [90]. Similarly to HSP70, HSP60 can elicit strong immunological reactions and is a target for autoimmune attacks [36]. In this respect, it is of interest that a secreted HSP60-like protein was detected in the conditioned media derived from a human neuroblastoma cell line and rat brain astrocytes [91]. Furthermore, its increased expression in human adult astrocytes in response to proinflammatory cytokines, e. g. tumor necrosis factor α (TNF-α), interleukin (IL)-1β, IL-4, IL-6 and IL-10, points to an important role of HSP60 in the pathogenesis of autoimmune diseases [87]. Also HSP90, which is constitutively expressed at high levels in all brain cells [15, 85, O. Goldbaum and C. Richter-Landsberg, unpublished results], might be involved in immunregulatory processes, since it recently was shown to mediate macrophage activation by taxol and the bacterial lipopolysaccharide [92].

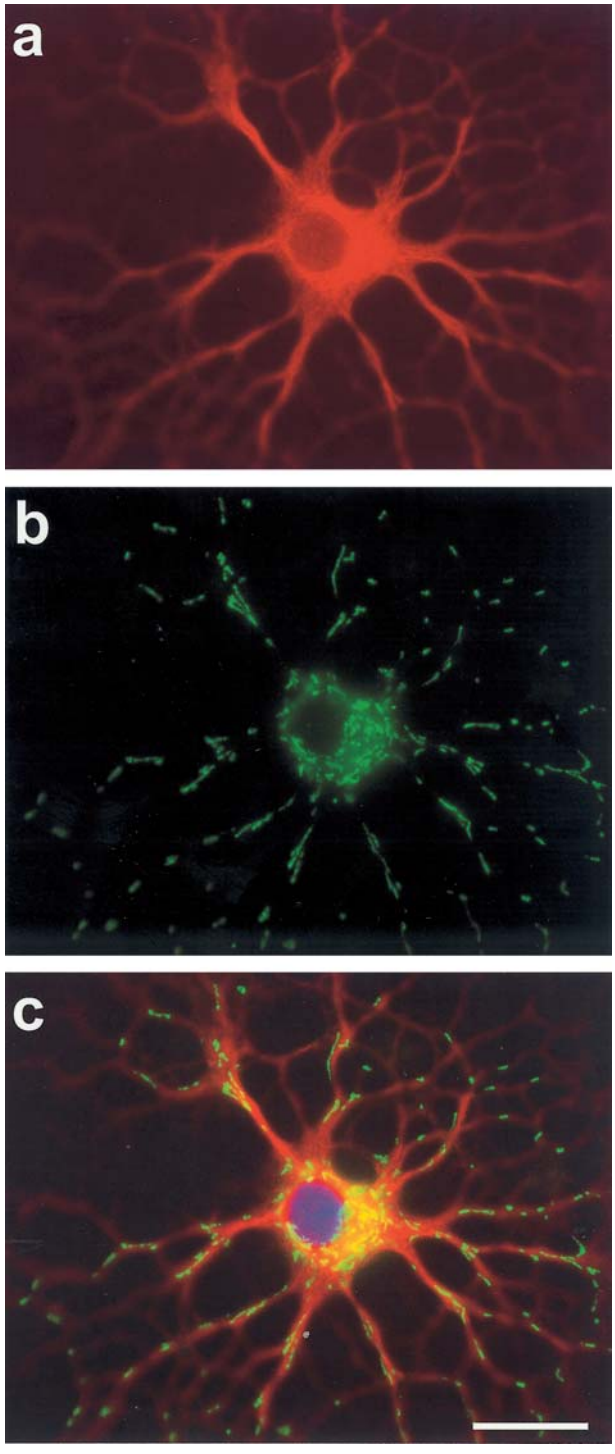

Figure 3. Distribution of HSP60 in mature oligodendrocytes derived from rat brain. Cells were fixed with paraformaldehyde (3%), and indirect immunofluorescence staining was carried out, using anti-tubulin or anti-HSP60 antibodies. (*a*) Tubulin, (*b*) HSP60, (*c*) double image of HSP60 and tubulin. HSP60 is present in the mitochondria in the cell soma and cellular extensions. Bar, 20 µm.

HSPs and αB-crystallin

A number of studies (reviewed in [43, 93]) suggest that the sHSPs are not constitutively expressed in the brain, and αB-crystallin before and after stimulation is mainly restricted to glial cells. Data from our laboratory demonstrate that in primary cultures of oligodendrocytes derived from the brains of newborn rats, HSP25 and αB-crystallin immunoreactivity is detectable only after cells have been stressed, while HSP25 could be detected in primary cultures of unstressed astrocytes [53, O. Goldbaum and C. Richter-Landsberg, unpublished observation]. In oligodendrocytes αB-crystallin was inducible by heat and oxidative stress, whereas HSP25 was induced only by heat shock and not by oxidative stress [53]. Another study showed that in organotypic brain cultures HSP25 was expressed mainly in reactive astrocytes, while αB-crystallin was predominant in oligodendrocytes. A marked induction of both proteins in astrocytes was seen after heat shock [94]. Accumulation of HSP27, αB-crystallin and ubiquitin was demonstrated to be localized to spheroid bodies in astroglial processes in old rhesus monkeys and baboons [95, 96]. An increase in αB-crystallin in astrocytes is characteristic for a wide variety of neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD) and Alexander's disease [43], and is also a consistent component of glial cell inclusions (see below).

αB-Crystallin was present in astrocytes and oligodendrocytes in multiple sclerosis (MS) lesions [97] and is discussed as an autoantigen in MS [98]. In a transgenic mouse line for αA-crystallin driven by a vimentin-promoter, large deposits of αA-crystallin were seen in astrocytes of the spinal cord, in Schwann cells of dorsal roots and sciatic nerves. These mice developed peripheral and central neuropathy, and the intracellular deposits of αA-crystallin possibly led to axonal dystrophy and demyelination [99]. Since αA- and αB-crystallin have a strong structural resemblance, it seems likely that overexpression of αB-crystallin in a transgenic model might induce similar changes. The presence of αB-crystallin in neurons was only described during neuropathological situations, e.g. in ballooned neurons in several neurodegenerative diseases and stroke [100, 101]. For example, intraneuronal αB-crystallin was found in AD [102, 103], and in telencephalic neurons without Lewy bodies in PD [104].

HO-1/HSP32

Expression of HO-1/HSP32 in the brain is normally very low [105] and restricted to select neuronal and nonneuronal cell populations in the forebrain, diencephalon, cerebellum and brainstem [106]. After heat shock or ischemia increased levels were seen in neuronal and glial cells [105, 107–109]. The prolonged expression of HO-1 after focal cerebral infarctions and traumatic human brain injury was detected mainly in microglia at the border of the lesion [110], and was transiently induced in astrocytes after cortical stab wound injury [111]. In these models HO-1 positive neuronal cells were only rarely observed. Cell culture studies further indicate that HO-1 was present in astrocytes and could be induced by hydrogen peroxide, while in cortical neurons it was barely detectable, and cells did not respond to oxidative stress by upregulation of HO-1 [112]. In rat brain oligodendrocytes, low levels of HO-1 were constitutively expressed during in vitro development, and its upregulation after oxidative stress exerted by hydrogen peroxide but not by heat stress was observed [53]. Although still debatable, growing evidence supports a role for HO-1 in protecting cells from oxidative stress and the brain against blood- and hemoglobin-mediated injury. Cerebellar granule neurons overexpressing HO-1 were protected against glutamate-mediated oxidative stress, and the accumulation of reactive oxygen species was attenuated [113]. Similarly, pretreatment of immortalized neuronal SN56 cells with hemin, an HO-1 inducer, led to elevation of HO-1 accompanied by protection against oxidative stress-induced neuronal injury [114]. Also, HO-1 antisense oligonucleotide treatment inhibited hemin-induced HO-1 upregulation and greatly increased the sensitivity of the cells to oxidative stress [114].

HO-1 is upregulated in a number of neurodegenerative diseases, including PD and AD, which points to a contribution of oxidative stress in the pathogenesis of these neurological disorders (for review see [54]). In AD brains the presence of other HSPs, e.g. αB-crystallin, HSP70 and ubiquitin, has been consistently reported. Interestingly, overexpression of HO-1 in neuroblastoma cells led to the suppression of physiologically expressed microtubule-associated protein tau, the major protein of neurofibrillary tangles (NFTs), and conferred enhanced resistance to oxidative stress injury exerted by hydrogen peroxide [115]. The possible connection of HO-1 induction and pathological changes (Alz50 epitope) of tau was demonstrated in another study by the same group. Here it was shown that oxidative stress triggers the cytoskeletal pathology in AD, and that HO-1 immunoreactivity in neurons is coincident with a conformational change in tau, recognized by antibody Alz50, while tau-hyperphosphorylation, recognized by AT8 antibody, is also found in neurons lacking HO-1 immunoreactivity [116]. The authors hypothesize that the colocalization of pathological tau and HO-1 may be a protective response of neuronal cells against oxidative stress. Again, as mentioned before, cell survival and cell death are closely linked processes, and depending on the level and duration of stress, the presence of HSPs may also be an indication of degenerative processes.

Reactive oxygen and nitrogen species play a role in inflammation and also in inflammatory demyelinating dis-

orders, such as MS [117]. Oligodendrocytes are specifically sensitive to oxidative stress and respond by the onset of programmed cell death [118, 119]. Our data show that oligodendrocytes specifically upregulate HO-1 after oxidative stress [53], and the presence of HO-1 in MS lesions could indicate that oxidative stress plays an important role in MS pathogenesis. HO-1 expression was significantly increased in experimental autoimmune encephalomyelitis (EAE), a commonly used animal model of MS, and could be localized to infiltrating monocytes [120] and reactive microglia and astrocytes, respectively [121]. The heat shock response, induced by whole body hyperthermia, reduced the severity of EAE in mice [122]. On the other hand, proinflammatory cytokines TNF-α and IL-1β, which are upregulated in MS and EAE, caused the upregulation of HO-1 in cultured rat astroctyes, followed by iron sequestration by the mitochondrial compartment [123].

Hence, prolonged HO-1 upregulation in glia might be an indicator of the pathological consequences of oxidative stress, exerted by inflammatory processes or damage to the blood brain barrier. The sustained upregulation is not cytoprotective and might promote further oxidative stress, which irreversibly leads to cell death and degenerative processes.

The data summarized above on the expression of HSPs and their individual induction in nerve cells and glia (see also table 2) demonstrate that HSPs may provide protection against subsequent stressors and sustain survival responses. On the other hand, stress responses may contribute to the onset of programmed cell death in the activated cells. HSPs are involved in regulation of the molecular pathways controlling cell survival and the cellular death programme, and the coordinated interaction of both. It has been suggested that HSPs directly modulate elements of the apoptotic pathways, such as cytochrome c, caspases, Bcl-2 and c-Jun NH_2-terminal kinase (JNK) [124–126]. Another important factor and promising candidate determining cell fate might be the transcription factor nuclear factor kappa B (NF-κB). Proteasome inhibitors, such as MG132 or lactacystin, and the heat shock response prevented NF-κB activation by accumulation of IκBα, an endogenous inhibitor of NF-κB, and in this paradigm promoted cell survival. However, depending on the state of the cells and the sequence of stressors, inhibition of NF-κB can either be cytoprotective or cytotoxic, a process which has been linked to the downstream effects of IκBα induction [127]. Thus, the complex interactions between stress responses and apoptotic cell death pathways, determining the final fate of the cells, are yet not predictable. To elucidate the mechanistic regulation of these closely linked processes is a major challenge in the field.

The cytoskeleton and cytoplasmic inclusions

The dynamic properties and spatial organization of the cytoskeletal network, consisting of microtubules (MTs), intermediate filaments (IFs) and microfilaments (MFs), is essential for establishing and maintaining cell morphology, organelle trafficking and cellular sorting processes. Nerve cells and glia can be distinguished by their IF system. In adult neurons the major IF system is composed of three subunits distinguished by their apparent molecular weights, namely NF-L (68 kDa), NF-M (160 kDa), and NF-H (210 kDa). Glia filaments, composed of GFAP, are the IFs characterisitc for astrocytes. Mature oligodendrocytes are devoid of IFs, and like neurons are characterized by their numerous MTs (for review see [128]). HSPs interact with the cytoskeleton of normal cells and might assist the proper assembly, spatial organization and cross-linking (for review see [42]). Specifically, the sHSPs, including αB-crystallin and HSP25, in-

Table 2. HSPs in nerve cells and glia and their occurrence in neurodegenerative diseases.

HSP	Cell type	Overexpr.	Examples of HSPs in disease
HSC/HSP70	constitutive and inducible in all brain cells	prot.	AD, MS, PSP
HSP60	constitutive in all brain cells	n.d.	AD, MS PSP
HSP32/HO-1	inducible mainly in glia, less in neurons	prot.	AD, EAE, PD
HSP27	inducible mainly in glia	n.d.	Alexander's disease, PD
αB-crystallin	constitutive in astrocytes and inducible mainly in glia	n.d.	AD, Alexander's disease, CBD, MS, MSA, Pick's disease, PD

The major pathological features which are positive for HSPs are cytoplasmic inclusions in nerve cells and glia which also stain positive for ubiquitin, e. g. Lewy bodies in PD, neurofibrillary tangles in AD and Rosenthal fibers containing GFAP in Alexander's disease. Glial cytoplasmic inclusions are a major hallmark of MSA, where they are found especially in oligodendrocytes.

Abbreviations: AD, Alzheimer's disease; CBD, cortocibasal degeneration; EAE, experimental autoimmune encephalomyelitis; MS, multiple sclerosis; PD, Parkinson's disease; PSP, progressive supranuclear palsy; Overexpr., overexpression in neural cells; n.d., not determined; prot., protective.

teract with all three cytoskeletal elements (for review see [43]). The cytoskeleton is affected by stress [42, 70], and heat shock or other stressors can cause the collapse of the filament network, alter the MT properties, also through modulation of the phosphorylation of microtubule-associated proteins, including tau [129], or induce a loss of stress fibers. The integrity of the cytoskeletal network is disturbed in many neurodegenerative disorders (for review see [130]); abnormalities in the organization of the filament system often are histopathological hallmarks and may provide diagnostic markers for different stages of neurodegeneration [131].

Filamentous inclusions, which are observed in a variety of neurodegenerative disorders in nerve cells and glia [132], often contain cytoskeletal proteins and stain with antibodies against ubiquitin and a variety of HSPs (for review see [43, 70, 133, 134]). For example, tau-positive neurofibrillary lesions in AD are associated with ubiquitin and may also exhibit αB-crystallin immunoreactivity [103]. Lewy bodies in PD contain αB-crystallin and, prior to the discovery of α-synuclein, for many years were identified by immunohistochemistry using ubiquitin antibodies [135]. Glial fibrillary tangles are specifically observed in progressive supranuclear palsy (PSP), corticobasal degeneration and Pick's disease [136]. Their appearance in astrocytes is considerably variable, and they often contain ubiquitin and αB-crystallin, while in oligodendrocytes hyperphosphorylated tau and no ubiquitin was detected [137]. αB-Crystallin is a major component of Rosenthal fibers, the characteristic glial filament inclusion bodies found in astrocytes of patients with Alexander's disease [43]. Glial cytoplasmic inclusions (GCIs) in oligodendroglia cytoplasm and nucleus are a histological hallmark of multiple system atrophy (MSA), a specific adult onset degenerative disease of the nervous system, characterized by varying degrees of Parkinsonism, cerebellar ataxia and autonomic dysfunction [134, 138]. GCIs stain consistently and intensely with antibodies against ubiquitin, αB-crystallin, less intensely with antibodies against α- and β-tubulin, MAP 5 (1B), and variable reports indicate that they are tau positive [139, for review see 133, 134]. More recently the presence of α-synuclein in GCIs was shown by immunocytochemistry and in GCIs immunoisolated from MSA brain tissue [132, 140–142]. Interestingly, overexpression of α-synuclein in a neuronal cell line can promote mitochondrial dysfunction leading to oxidative stress [143]. α-Synuclein toxicity in a *Drosophila* model system could be suppressed by augmenting HSP70. HSP70 prevented dopaminergic neuronal cell loss and inclusion bodies were positive for HSP70 [144]. These studies imply that a lack of available HSPs possibly is an important factor in inclusion body formation. On the other hand, a chaperone-like activity has been attributed to synucleins in vitro [145]. It remains to be investigated whether aggregation of α-synuclein in Lewy bodies or GCIs is causally related to or a result of stress-induced upregulation.

The presence of stress proteins in cytoplasmic inclusions in nerve cells and glia suggest that stress situations, such as oxidative stress, thermal stress or multiple stressors, contribute to the formation of inclusion bodies and consequently to the pathogenesis of the respective neurodegenerative diseases (see also table 2). It might be assumed that the upregulation of HSPs in most cases is an attempt to rescue the cells, and to prevent protein denaturation and aggregate formation. The occurance of cytoplasmic inclusions indicates that the chaperone machinery either was not sufficient or defective. Furthermore, the presence of ubiquitin in most of the inclusions suggests that proteins initially were targeted for degradation by the proteasome system, though without success. Hence, severe structural changes or an impairment or overload of the ubiquitin-proteasome pathway might render the proteins inaccessible to proteolysis. Nonnative monomers might assemble into growing polymers, which eventually lead to inclusion body formation [71, 146]. The molecular mechanisms underlying aggregate formation are still not resolved. Recent studies show that inclusions in mammalian cells are formed when the proteasome activity is decreased. Individual small aggregates were delivered to the nascent inclusion body by an active, retrograde transport system on microtubules [147]. These microtubule-dependent cytoplasmic inclusions have been termed aggresomes (for review see [148]).

The question remains, whether the formation of cytoplasmic inclusion bodies is a death signal leading to neurodegeneration or a protective means. The fact that cells with inclusions can be monitored in brains of patients with neurodegenerative diseases at late stages (some examples are given in table 2), might be an indication that these cells were capable of resisting and were not phagocytosed, while others were doomed to apoptotic cell death and possibly eliminated earlier.

Concluding remarks

HSPs can prevent protein aggregation, facilitate protein repair and renaturation, and support proteolytic degradation by targeting nonreparable proteins for the ubiquitin-proteasome pathway (fig. 2). They also exhibit important functions in maintaining physiological processes, target proteins to the mitochondria and ER, and play an essential role in the establishment and functioning of the cytoskeletal network. The heat shock response and stress proteins are involved in defense mechanisms against cellular stress. Yet, when a certain threshold is passed, the stress responses cause cellular dysfunction and degenerative processes. Cell-type-specific effects are observed,

converting either neurons or glial cells or both to be the primary target. A major mystery to be solved is, What are the molecular mechanisms underlying the system-, region-, and cell-type specific responses in the nervous system that are observed after single or multiple stress situations and during neurodegenerative disorders?

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1 Birnbaum G. (1995) Stress proteins: their role in the normal central nervous system and in disease states, especially multiple sclerosis. Springer Sem. Immunopathol. **17:** 107–118
2 Lee D. H. and Goldberg A. L. (1998) Proteasome inhibitors: valuable new tools for cell biologists. Trends Cell Biol. **8:** 397–403
3 Mathew A. and Morimoto R. I. (1998) Role of heat shock response in the life and death of proteins. Ann. N. Y. Acad. Sci. **851:** 99–111
4 Fink A. L. (1999) Chaperone-mediated protein folding. Physiol. Rev. **79:** 425–449
5 Lee A. S. (2001) The glucose-regulated proteins: stress induction and clinical applications. Trends Biochem. **26:** 504–510
6 Hartl F. U. (1996) Molecular chaperones in cellular protein folding. Nature **381:** 571–579
7 Ohtsuka K. and Suzuki T. (2000) Roles of molecular chaperones in the nervous system. Brain Res. Bull. **53:** 141–146
8 Pirkkala L., Nykänen P. and Sistonen L. (2001) Roles of heat shock transcription factors in regulation of the heat shock response and beyond. FASEB J. **15:** 1118–1131
9 Morimoto R. I. (1993) Cells in stress: transcriptional activation of heat shock genes. Science **259:** 1409–1410
10 Wu C. (1995) Heat shock transcription factors: structure and regulation. Annu. Rev. Cell Dev. Biol. **11:** 441–469
11 Zou J., Guo Y., Guettouche T., Smith D. F. and Voellmy R. (1998) Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. Cell **94:** 471–480
12 Dwyer B. E. and Nishimura R. N. (1994) Heat shock proteins and neuroprotection in CNS culture. In: Heat Shock Proteins in the Nervous System, pp. 101–121, Mayer J. and Brown I. (eds), Academic Press, New York
13 Sharp F. R., Massa S. M. and Swanson R. A. (1999) Heat shock protein protection. Trends Neurosci. **22:** 97–99
14 Yenari M. A., Giffard R. M. and Steinberg G. K. (1999) The neuroprotective potential of heat shock protein 70 (HSP70). Molecular Med. Today **5:** 525–531
15 Brown I. R. (1994) Induction of heat shock genes in mammalian brain by hyperthermia and tissue injury. In: Heat Shock Proteins in the Nervous System, pp. 31–53, Mayer J. and Brown I. (eds), Academic Press, New York
16 McClellan A. J. and Frydman J. (2001) Molecular chaperones and the art of recognizing a lost cause. Nature Cell Biol. **3:** E51–E53
17 Scheibel T. and Buchner J. (1998) The Hsp90 complex – a super-chaperone machine as a novel drug target. Biochem. Pharmacol. **56:** 675–682
18 Yahara I. (1998) Structure and function of the 90-kDa stress protein Hsp90. In: Molecular Chaperones in the Life Cycle of Proteins, pp. 183–192, Fink A. L. and Goto Y. (eds), Dekker, New York
19 Hightower L. E. and Li T. (1994) Structure and function of the mammalian HSP70 family. In: Heat Shock Proteins in the Nervous System, pp. 1–30, Mayer J. and Brown I. (eds), Academic Press, New York
20 Nowak T. S., Suga S. and Saito N. (1994) The heat shock response and gene expression in brain after ischaemia. In: Heat Shock Proteins in the Nervous System, pp. 55–81, Mayer J. and Brown I. (eds), Academic Press, New York
21 Tavaria M., Gabriele T., Kola I. and Anderson R. L. (1996) A hitchhiker's guide to the human Hsp70 family. Cell Stress Chaperones **1:** 23–28
22 Yu Z., Luo H., Fu W. and Mattson M. P. (1999) The endoplasmic reticulum stress-responsive protein GRP78 protects neurons against excitotoxicity and apoptosis: suppression of oxidative stress and stabilization of calcium homeostasis. Exp. Neurol. **155:** 302–314
23 Mote P. L., Tillman J. B. and Spindler S. R. (1998) Glucose regulation of GRP78 gene expression. Mech. Ageing Dev. **104:** 149–158
24 Yang G. H., Li S. and Pestka J. J. (2000) Down-regulation of the endoplasmic reticulum chaperone GRP78/BiP by vomitoxin (Deoxynivalenol). Toxicol. Appl. Pharmacol. **162:** 207–217
25 Barnes J. A., Smoak I. W. and Branch S. (1999) Expression of glucose-regulated proteins (GRP78 and GRP94) in hearts and fore-limb buds of mouse embryos exposed to hypoglycemia in vitro. Cell Stress Chaperones **4:** 250–258
26 Imamoto N., Matsuoka Y., Kurihara T., Kohno K., Miyagi M., Sakiyama F. et al. (1992) Antibodies against 70-kD heat shock cognate protein inhibit mediated nuclear import of karyophilic proteins J. Cell Biol. **119:** 1047–1061
27 Rapoport T. A. (1990) Protein transport across the ER membrane. Trends Biochem. Sci. **15:** 355–358
28 Deshaies R. J., Koch B. D., Werner-Washburne M., Craig E. A. and Schekman R. (1988) A subfamily of stress proteins facilitates translocation of secretory and mitochondrial precursor polypeptides. Nature **332:** 800–805
29 Ohtsuka K., Masuda A., Nakai A. and Nagata K. (1990) A novel 40-kDa protein induced by heat shock and other stresses in mammalian and avian cells. Biochem. Biophys. Res. Commun. **166:** 642–647
30 Minami, Y., Höhfeld, J., Ohtsuka, K. and Hartl F. (1996) Regulation of heat-shock protein 70 reaction cycle by the mammalian DnaJ homolog, Hsp40. J. Biol. Chem. **271:** 19617–19624
31 Michels A. A., Kanon B., Konings A. W. T., Ohtsuka K., Bensaude O. and Kampinga H. H. (1997) Hsp70 and Hsp40 chaperone activities in the cytoplasm and the nucleus of mammalian cells. J. Biol. Chem. **272:** 33283–33289
32 Frydman J., Nimmesgern E., Ohtsuka K. and Hartl F.-U. (1994) Folding of nascent polypeptide chains in a high molecular mass assembly with molecular chaperones. Nature **370:** 111–117
33 Cheetham M. E., Brion J. P. and Anderton B. H. (1992) Human homologues of the bacterial heat-shock protein DnaJ are preferentially expressed in neurons. Biochem. J. **284:** 469–476
34 Chuang J. -Z., Zhou H., Zhu M., Li S. -H. and Li X. -J. (2002) Characterization of a brain-enriched chaperone, MRJ, that inhibits Huntingtin aggregation and toxicity independently. J. Biol. Chem. **277:** 19831–19838
35 Itoh H., Kobayashi R. and Waui H. (1995) Mammalian 60 kDa stress protein (chaperonin homolog). Identification, biochemical properties, and localization. J. Biol. Chem. **270:** 13429–13435
36 Brosnan C. F., Battistini L., Gao Y. L. and Aquino D. A. (1996) Heat shock proteins and multiple sclerosis: a review. J. Neuropath. Exp. Neurol. **55:** 389–402
37 Martin J., Horwich A. L. and Hartl F.-U. (1992) Prevention of protein denaturation under heat stress by the chaperonin Hsp60. Science **258:** 995–998

38 Samali A., Cia J. Y., 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

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

40 Jakob U. and Buchner J. (1994) Assisting spontaneity: the role of Hsp90 and small Hsps as molecular chaperones. Trends Biochem. Sci. **19:** 205–211

41 Muchowski P. J., Bassuk J. A., Lubsen N. H. and Clark J. I. (1997) Human alphaB-crystallin. Small heat shock protein and molecular chaperone. J. Biol. Chem. **272:** 2578–2582

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

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

44 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

45 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

46 Nicholl I. D. and Quinlan R. A. (1994) Chaperone activity of α-crystallins modulates intermediate filament assembly. EMBO J. **13:** 945–953

47 Carver J. A., Aquilina J. A., Cooper P. G., Williams G. A. and Truscott R.J. (1994) Alpha-crystallin: molecular chaperone and protein surfactant. Biochim. Biophys. Acta **1204:** 195–206

48 Horwitz J. (1992) Alpha-crystallin can function as a molecular chaperone. Proc. Natl. Acad. Sci. USA **89:** 10449–10453

49 DeJong, W. W., Leunissen J. A. M. and Voorter C. E. M. (1993) Evolution of the alpha-crystallin/small heat-shock protein family. Mol. Biol. Evol. **10:** 103–126

50 Iwaki T., Iwaki A. and Goldman J. E. (1990) Cellular distribution of αB-crystallin in non-lenticular tissues. J. Histochem. Cytochem. **38:** 31–39

51 Kato K., Shinohara H., Kurobe N., Inaguma Y., Shimizu K. and Ohshima K. (1991) Tissue distribution and developmental profiles of immunoreactive αB-crystallin in the rat determined with a sensitive immunoassay system. Biochim. Biophys. Acta **1074:** 201–208

52 Lowe J., Mcdermott H., Pike I., Spendlove I., Landon M. and Mayer R. J. (1992) αB-crystallin expression in non-lenticular tissues and selective presence in ubiquitinated inclusion bodies in human disease. J. Pathol. **166:** 61–68

53 Goldbaum O. and Richter-Landsberg C. (2001) Stress proteins in oligodendrocytes: differential effects of heat shock and oxidative stress. J. Neurochem. **78:** 1233–1242

54 Schipper H. M. (2000) Heme oxygenase-1: role in brain aging and neurodegeneration. Exp. Gerontology **35:** 821–830

55 Clark J. E., Foresti R., Green C. J. and Motterlini R. (2000) Dynamics of haem oxygenase-1 expression and bilirubin production in cellular protection against oxidative stress. Biochem. J. **348:** 615–619

56 Doré S., Takahashi M., Ferris C. D., Hester L. D., Guastella D. and Snyder S. H. (1999) Bilirubin, formed by activation of heme oxygenase-2, protects neurons against oxidative stress injury. Proc. Natl. Acad. Sci. USA **96:** 2445–2450

57 Dennery P. A. (2000) Regulation and role of heme oxygenase in oxidative injury. Curr. Topics Cellular Regulation **36:** 181–199

58 Elbirt K. K. and Bonkovsky H. L. (1999) Heme oxygenase: recent advances in understanding its regulation and role. Proc. Assoc. Am. Phys. **111:** 438–447

59 Shibahara S., Muller R., Taguchi H. and Yoshida T. (1985) Cloning and expression of cDNA for rat heme oxygenase. Proc. Natl. Acad. Sci. USA **82:** 7865–7869

60 Maines M. D., Trakshel G. M. and Kutty R. K. (1986) Characterization of two constitutive forms of rat liver microsomal heme oxygenase. Only one molecular species of the enzyme is inducible. J. Biol. Chem. **261:** 411–419

61 McCoubrey W. K. Jr, Huang T. J. and Maines M. D. (1997) Isolation and characterization of a cDNA from the rat brain that encodes hemoprotein heme oxygenase-3. Eur. J. Biochem. **247:** 725–732

62 Slavotinek A. M. and Biesecker L. G. (2001) Unfolding the role of chaperones and chaperonins in human disease. Trends Genet. **17:** 528–535

63 Sherman M. Y. and Goldberg A. L. (2001) Cellular defenses against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. Neuron **29:** 15–32

64 Schwartz A. L. and Ciechanover A. (1999) The ubiquitin-proteasome pathway and pathogenesis of human diseases. Annu. Rev. Med. **50:** 57–74

65 Hirsch C. and Ploegh H. L. (2000) Intracellular targeting of the proteasome. Trends Cell Biol. **10:** 268–272

66 Hershko A., Ciechanover A. and Varshavsky A. (2000) The ubiquitin system. Nat. Med. **6:** 1073–1081

67 Conoway R. C., Brower C. S. and Conoway J. W. (2002) Emerging roles of ubiquitin in transcription regulation. Science **296:** 1254–1258

68 Ben-Neriah Y. (2002) Regulatory functions of ubiquitination in the immune system. Nature Immunol. **3:** 20–26

69 Wilkinson K. D. (1994) Cellular roles of ubiquitin. In: Heat Shock Proteins in the Nervous System, pp. 191–234, Mayer J. and Brown I. (eds), Academic Press, New York

70 Dickson D. W. and Yen S. H. (1994) Ubiquitin, the cytoskeleton and neurodegenerative diseases. In: Heat Shock Proteins in the Nervous System, pp. 235–262, Mayer J. and Brown I. (eds), Academic Press, New York

71 Alves-Rodrigues A., Gregori L. and Figueiredo-Pereira M. E. (1998) Ubiquitin, cellular inclusions and their role in neurodegeneration. Trends Neurosci. **12:** 516–520

72 Nishimura R. N., Dwyer B. E., Clegg K. B. and de Vellis J. (1991a) Comparison of the heat shock response in cultured cortical neurons and astrocytes. Mol. Brain Res. **9:** 39–45

73 Nishimura R. N., Dwyer B.E., Vinters H.V., deVellis J. and Cole R. (1991b) Heat shock in cultured neurons and astrocytes: correlation of ultrastructure and heat shock protein synthesis. Neuropathol. Appl. Neurobiol. **17:** 139–147

74 McCabe T. and Simon R. P. (1993) Hyperthermia induces 72kDa heat shock protein expression in rat brain non-neuronal cells. Neurosci. Lett. **159:** 163–165

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

76 D'Souza D. D., Antel J. P. and Freedman M. S. (1994) Cytokine induction of heat shock expression in human oligodendrocytes: an interleukin-1-mediated mechanism. J. Neuroimmunol. **50:** 17–24

77 Satoh J., Yamamura T., Kunishita T. and Tabira T. (1992) Heterogeneous induction of 72-kDa heat shock protein (HSP72) in cultured mouse oligodendrocytes and astrocytes. Brain Res. **573:** 37–43

78 Satoh J. and Kim S. U. (1995) Constitutive and inducible expression of heat shock protein HSP72 in oligodendrocytes in culture. Neuroreport **6:** 1081–1084

79 Dwyer B. E., Nishimura R. N., de Vellis J. and Clegg K. B. (1991) Regulation of heat shock protein synthesis in rat astrocytes. J. Neurosi. Res. **28:** 352–358

80 Foster J. A. and Brown I. R. (1997) Differential induction of heat shock mRNA in oligodendrocytes, microglia and astrocytes following hyperthermia. Mol. Brain Res. **45:** 207–218

81 Almazan G., Liu H. N., Khorchid A., Sundarajan S., Martinez-Bermudez A. K. and Chemtob S. (2000) Exposure of developing oligodendrocytes to cadmium causes HSP72 induction, free radical generation, reduction in glutathione levels and cell death. Free Radic. Biol. Med. **29:** 858–869
82 Juurlink B. H. J. (1994) Type-2 astrocytes have much greater susceptibility to heat stress than type-1 astrocytes. J. Neurosi. Res. **38:** 196–201
83 Guzhova I., Kislyakove K., Moskaliova O., Fridlanskaya I., Tytell M., Cheetham M. et al. (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
84 Kakimura J., Kitamura Y., Takata K., Umeki M., Suzuki S., Shibagaki K. et al. (2002) Microglia activation and amyloid-β clearance induced by exogenous heat-shock proteins. FASEB J. **16:** 601–603
85 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
86 Dwyer D. S., Liu Y., Miao S. and Bradley R. J. (1996) Neuronal differentiation in PC12 cells is accompanied by diminished inducibility of Hsp70 and Hsp60 in response to heat and ethanol. Neurochem. Res. **21:** 659–666
87 Bajramovic J. J., Bsibsi M., Geutskens S. B., Hassankhan R., Verhulst K. C., Stege G. J. et al. (2000) Differential expression of stress proteins in human adult astrocytes in response to cytokines. J. Neuroimmunol. **106:** 14–22
88 Selmaj K., Brosnan C. F. and Raine C. S. (1992) Expression of heat shock protein-65 by oligodendrocytes in vivo and in vitro: implications for multiple sclerosis. Neurology **42:** 795–800
89 Freedman M. S., Buu N. N., Ruijs T. C., Williams K. and Antel J. P. (1992) Differential expression of heat shock proteins by human glial cells. J. Neuroimmunol. **41:** 231–238
90 Raine C. S., Wu E., Ivanyi J., Katz D. and Brosnan C. F. (1996) Multiple sclerosis: a protective or a pathogenic role for heat shock protein 60 in the central nervous system? Lab. Invest. **75:** 109–123
91 Bassan M., Zamostiano R., Giladi E., Davidson A., Wollman Y., Pitman J. et al. (1998) The identification of secreted heat shock 60-like protein from rat glial cells and a human neuroblastoma cell line. Neurosci. Lett. **26:** 37–40
92 Byrd C. A., Bornmann W., Erdjument-Bromage H., Temps P., Pavletich N., Rosen N. et al. (1999) Heat shock protein 90 mediates macrophage activation by Taxol and bacterial lipopolysaccharide. Proc. Natl. Acad. Sci. USA **96:** 5645–5650
93 Arrigo A. P. and Mehlen P. (1994) Expression, cellular location and function of low molecular weight heat shock proteins (Hsp20s) during development of the nervous system. In: Heat Shock Proteins in the Nervous System, pp. 145–167, Mayer J. and Brown I. (eds), Academic Press, New York
94 Bryzska M., Stege G. J. J., Renkawek K. and Bosman G. J. (1998): Heat shock, but not the reactive state per se, induces increased expression of the small stress proteins hsp25 and αB-crystallin in glial cells in vitro. Neuroreport **9:** 1549–1552
95 Schultz C., Dick E. J., Cox A. B., Hubbard G. B., Braak E. and Braak H. (2001) Expression of stress proteins alpha B-crystallin, ubiquitin, and hsp27 in pallidro-nigral spheroids of aged rhesus monkeys. Neurobiol. Aging **22:** 677–682
96 Willwohl D., Kettner M., Braak H., Hubbard G. B., Dick E. J., Cox A. B. et al. (2002) Pallido-nigral spheroids in nonhuman primates: accumulation of heat shock proteins in astroglial processes. Acta Neuropathol. **103:** 276–280
97 Bajramovic J. J., Lassmann H. and Van Noort J. M. (1997) Expression of αB-crystallin in glia cells during lesional development in multiple sclerosis. J. Neuroimmunol. **78:** 142–151
98 Van Noort J. M., Van Sechel A. C., Van Stipdonk M. J. and Bajramovic J. J. (1998) The small heat shock protein alpha B-crystallin as key autoantigen in multiple sclerosis. Prog. Brain Res. **117:** 435–52
99 De Rijk E. P. C. T., Van Rijk A. F., Van Esch E., De Jong W. W., Wesseling P. and Bloemendal H. (2000) Demyelination and axonal dystrophy in alpha A-crystallin transgenic mice. Int. J. Experim. Pathol. **81:** 271–282
100 Lowe J., Errington D. R., Lennow G., Pike I., Spendlove I., Landon M. et al. (1992) Ballooned neurons in several neurodegenerative diseases and stroke contain αB-crystallin. Neuropathol. Appl. Neurobiol. **18:** 341–350
101 Kato S., Hirano A., Umahara T., Kato M., Herz F. and Ohama E. (1992) Comparative immunohistochemical study on the expression of αB-crystallin, ubiquitin and stress- response protein 27 in various disorders. Neuropathol. Appl. Neurobiol. **18:** 335–340
102 Jellinger K. A. and Stadelmann C. (2001) Problems of cell death in neurodegeneration and Alzheimer's disease. J. Alzheimer's Disease **3:** 31–40
103 Mao J. J., Katayama S., Watanabe C., Harada Y., Noda K., Yamamura Y. et al. (2001) The relationship between αB-crystallin and neurofibrillary tangles in Alzheimer's disease. Neuropathol. Appl. Neurobiol. **27:** 180–188
104 Braak H., Del Tredici K., Sandmann-Keil D., Rüb U. and Schultz C. (2001) Nerve cells expressing heat-shock proteins in Parkinson's disease. Acta Neuropathol. **102:** 449–454
105 Ewing J. F., Haber S. N. and Maines M. D. (1992) Normal and heat-induced patterns of expression of heme oxygenase-1 (HSP32) in rat brain: hyperthermia causes rapid induction of mRNA and protein. J. Neurochem. **58:** 1140–1149
106 Vincent S. R., Das S. and Maines M. D. (1994) Brain heme oxygenase isoenzymes and nitric oxide synthase are co-localized in select neurons. Neuroscience **63:** 223–231
107 Takeda A., Onodera H., Sugimoto A., Itoyama Y., Kogure K. and Shibahara S. (1994) Increased expression of heme oxygenase mRNA in rat brain following transient forebrain ischemia. Brain Res. **666:** 120–124
108 Takeda A., Kimpara T., Onodera H., Itoyama Y., Shibahara S. and Kogure K. (1996) Regional difference in induction of heme oxygenase-1 protein following rat transient forebrain ischemia. Neurosci. Lett. **205:** 169–172
109 Koistinaho J., Miettinen S., Keinanen R., Vartiainen N., Roivainen R. and Laitinen J. T. (1996) Long-term induction of haem oxygenase-1 (HSP-32) in astrocytes and microglia following transient focal brain ischaemia in the rat. Eur. J. Neurosci. **8:** 2265–2272
110 Beschorner R., Adjodah D., Schwab J. M., Mittelbronn M., Pedal I., Mattern R. et al. (2000) Long-term expression of heme oxygenase-1 (HO-1, HSP-32) following focal cerebral infarctions and traumatic brain injury in humans. Acta Neuropathol. **100:** 377–384
111 Dwyer B. E., Nishimura R. N., Lu S. Y. and Alcaraz A. (1996) Transient induction of heme oxygenase after cortical stab wound injury. Mol. Brain. Res. **38:** 251–259
112 Dwyer B. E., Nishimura R. N. and Lu S. Y. (1995) Differential expression of heme oxygenase-1 in cultured cortical neurons and astrocytes determined by the aid of a new heme oxygenase antibody. Response to oxidative stress. Mol. Brain. Res. **30:** 37–47
113 Chen K., Gunter K. and Maines M. D. (2000) Neurons overexpressing heme oxygenase-1 resist oxidative stress-mediated cell death. J. Neurochem. **75:** 304–313
114 Le W. D., Xie W. J. and Appel S. H. (1999) Protective role of heme oxygenase-1 in oxidative stress-induced neuronal injury. J. Neurosci. Res. **56:** 652–658
115 Takeda A., Perry G., Abraham N. G., Dwyer B. E., Kutty R. K., Laitinen J. T. et al. (2000) Overexpression of heme oxygenase in neuronal cells, the possible interaction with tau. J. Biol. Chem. **275:** 5395–5399

116 Takeda A., Smith M. A., Avila J., Nunomura A., Siedlak S. L., Zhu X. et al. (2000) In Alzheimer's disease, heme oxygenase is coincident with Alz50, an epitope of tau induced by 4-hydroxy-2-nonenal modification. J. Neurochem. **75:** 1234–1241

117 Smith K. J., Kapoor R. and Felts P. A. (1999) Demyelination: the role of oxygen and nitrogen species. Brain Pathol. **9:** 69–92

118 Richter-Landsberg C. and Vollgraf U. (1998) Mode of cell injury and death after hydrogen peroxide exposure in cultured oligodendroglia cells. Exp. Cell Res. **244:** 218–229

119 Vollgraf U., Wegner M. and Richter-Landsberg C. (1999) Activation of AP-1 and NF-kappaB transcription factors is involved in hydrogen peroxide-induced apoptotic cell death of oligodendrocytes. J. Neurochem. **73:** 2501–2509

120 Schluesener H. J. and Seid K. (2000) Heme oxygenase-1 in lesions of rat experimental autoimmune encephalomyelitis and neuritis. J. Neuroimmunol. **110:** 114–120

121 Emerson M. R. and LeVine S. M. (2000) Heme oxygenase-1 and NADPH cytochrome P450 reductase expression in experimental allergic encephalomyelitis: an expanded view of the stress response. J. Neurochem. **75:** 2555–2562

122 Heneka M. T., Sharp A., Murphy P., Lyons J. A., Dumitrescu L. and Feinstein D. L. (2001) The heat shock response reduces myelin oligodenrocyte glycoprotein-induced experimental autoimmune encephalomyelitis in mice. J. Neurochem. **77:** 568–579

123 Mehindate K., Sahlas D. J., Frankel D., Mawal Y., Liberman A., Corcos J. et al. (2001) Proinflammatory cytokines promote glial heme oxygenase-1 expression and mitochondrial iron deposition: implications for multiple sclerosis. J. Neurochem. **77:** 1386–1395

124 Punyiczki M. and Fésüs L. (1998) Heat shock and apoptosis. Ann. N. Y. Acad. Sci. **851:** 67–74

125 Wójcik C. (1999) Proteasomes in apoptosis: villains or guardians? Cell. Mol. Life Sci. **56:** 908–917

126 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

127 DeMeester S., Buchman T. G. and Cobb J. P. (2001) The heat shock paradox: does NF-κB determine cell fate? FASEB J. **15:** 270–274

128 Richter-Landsberg C. (2001) Organization and functional roles of the cytoskeleton in oligodendrocytes. Microsc. Res. Tech. **52:** 628–636

129 Goldbaum O. and Richter-Landsberg C. (2002) Activation of PP2A-like phosphatase and modulation of tau phosphorylation accompany stress-induced apoptosis in cultured oligodendrocytes. Glia, in press

130 Brandt R. (2001) Cytoskeletal mechanisms of neuronal degeneration. Cell Tissue Res. **305:** 255–265

131 Braak H. and Braak E. (1991) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. **82:** 239–259

132 Goedert M., Spillantini M. G. and Davies S. W. (1998) Filamentous nerve cell inclusions in neurodegenerative diseases. Curr. Opin. Neurobiol. **8:** 619–632

133 Chin S. S.-M. and Goldman J. E. (1996) Glial inclusions in CNS degenerative diseases. J. Neuropathol. Exp. Neurol. **55:** 499–508

134 Lantos P. L. (1998) The definition of multiple system atrophy: a review of recent developments. J. Neuropath. Exp. Neurol. **57:** 1099–1111

135 Hashimoto M. and Masliah E. (1999) Alpha-synuclein in Lewy body disease and Alzheimer's disease. Brain Pathol. **9:** 707–720

136 Komori T. (1999) Tau-positive glial inclusions in progressive supranuclear palsy, corticobasal degeneration and Pick's disease. Brain Pathol. **9:** 663–679

137 Ikeda K., Akiyama H., Aral T. and Nishimura T. (1998) Glial tau pathology in neurodegenerative diseases: their nature and comparison with neuronal tangles. Neurobiol. Aging **19:** 585–591

138 Dickson D. W., Lin W. I., Liu W. K. and Yen S. H. (1999) Multiple system atrophy: a sporadic synucleinopathy. Brain Pathol. **9:** 721–732

139 Murayama S., Arima K., Nakazato Y., Satoh J., Oda M. and Inose T. (1992) Immunocytochemical and ultrastrutural studies of neuronal and oligodendroglial cytoplasmic inclusions in multiple system atrophy. 2. Oligodendroglial cytoplasmic inclusions. Acta Neuropathol. **84:** 32–38

140 Tu P. H., Galvin J. E., Baba M., Giasson B., Tomita T., Leight S. et al. (1998) Glial cytoplasmic inclusions in white matter oligodendrocytes of multiple system atrophy brains contain insoluble α-synuclein. Ann. Neurol. **44:** 415–422

141 Wakabayashi K., Yoshimoto M., Tsuji S. and Takahashi H. (1998) α-Synuclein immunoreactivity in glial cytoplasmic inclusions in multiple system atrophy. Neurosci. Lett. **249:** 180–182

142 Gai W. P., Power H. A. T., Blumbergs P. C., Culvenor J. G. and Jensen P. H. (1999) α-synuclein immunoisolation of glial inclusions from multiple system atrophy brain tissue reveals multiprotein components. J. Neurochem. **73:** 2093–2100

143 Hsu L. J., Sagara Y., Arroyo A., Rockenstein E., Sisk A., Mallory M. et al. (2000) α-synuclein promotes mitochondrial deficit and oxidative stress. Am. J. Pathol. **157:** 401–410

144 Auluck P. K., Chan H. Y. E., Trojanowski J. Q., Lee V. M.-Y. and Bonini N. M. (2002) Chaperone suppression of α-synuclein toxicity in a *Drosophila* model for Parkinson's disease. Science **295:** 865–868

145 Souza J. M., Giasson B. I., Lee V. M.-Y. and Ischipoulos H. (2000) Chaperone-like activity of synucleins. FEBS Lett. **474:** 116–119

146 Johnson W. G. (2000) Late-onset neurodegenerative diseases-role of protein insolubility. J. Anat. **196:** 609–616

147 Johnston J. A., Ward C. L. and Kopito R. R. (1998) Aggresomes: a cellular response to misfolded proteins. J. Cell Biol. **143:** 1883–1898

148 Kopito R. R. (2000) Aggresomes, inclusion bodies and protein aggregation. Trends Cell Biol. **10:** 524–530