Journal of Cellular Biochemistry 94:241–246 (2005)

In Search of the Molecular Mechanism by Which Small Stress Proteins Counteract Apoptosis During Cellular Differentiation

André-Patrick Arrigo*

Laboratoire stress oxydant, chaperons et apoptose, Centre de Génétique Moléculaire et Cellulaire, CNRS UMR-5534, Université Claude Bernard Lyon-I, 69622 Villeurbanne, France

Abstract Many differentiation programs are accompanied by an increase in small heat shock proteins (sHsps) level. Most of the time transient, this accumulation takes place during the early phase of the process and is correlated with the growth arrest that precedes the differentiation. Important biochemical modifications of sHsps occur, such as changes in phosphorylation and oligomerization. The fact that these proteins are induced independently of the signal that triggers differentiation, of the differentiation type, and of the cell type strongly suggests their involvement in fundamental mechanisms of cellular differentiation. Moreover, impairment of sHsps accumulation leads to abortion of the differentiation program and, subsequently, to a massive commitment to cell death. Recent advances in this field of research are presented as well as the hypothesis that should be tested to unravel the mode of action of these proteins during cellular differentiation. J. Cell. Biochem. 94: 241–246, 2005. © 2004 Wiley-Liss, Inc.

Key words: small stress proteins; differentiation; apoptosis

Small heat shock proteins (sHsps), also known as small stress proteins, form a ubiquitous group of proteins found in virtually all organisms with a molecular weight ranging between 15 and 43 kDa. sHsps were first described in *Drosophila* as proteins that displayed increased synthesis following heat shock or conditions or agents that alter protein folding [Arrigo and Tanguay, 1991; Arrigo and Landry, 1994]. sHsps are characterized by an evolutionary conserved C-terminal region, called the alpha-crystallin domain. These stress proteins also share low amino acid homology within their N-terminal region and in most cases they have a short and variable C-terminal tail. Several of the sHsps are phosphoproteins but all have in common the striking feature of forming high molecular weight oligomeric complexes that have heterogeneous native sizes ranging from 100 to 800 kDa [Arrigo et al., 1988; Arrigo and Ducasse, 2002; Garrido, 2002].

Overexpression of sHsps enhances the survival of cells in response to many stress stimuli. Several mechanisms have been proposed to account for the sHsp protective effect, such as molecular chaperoning of non-native proteins [Jakob et al., 1993], interaction with several component of the apoptotic machinery [Paul et al., 2002], modulation of the cellular detoxifying machinery to cope with oxidative stress [Arrigo, 2001], or modulation of cytoskeletal integrity [Mounier and Arrigo, 2002].

Families of sHsps have been characterized in different organisms [de Jong et al., 1998]. For example, in *Drosophila*, the sHsp group includes four heat inducible polypeptides [Arrigo and Tanguay, 1991]. To date the human family contains 10 polypeptides (Hsp27, MKBP, HspB3, αA- and αB-crystallin, Hsp20, cvHsp, Hsp22, HspB9, and ODF1) but only few of them are well characterized (Hsp27 and αB-crystallin) and are true heat shock proteins that display an enhanced expression at elevated temperature.

Grant sponsor: The Associtation pour la Recherche sur le Cancer ARC (to A.-P.A); Grant number: 4602; Grant sponsor: The Région Rhône-Alpes, France.

*Correspondence to: André-Patrick Arrigo, Laboratoire stress oxydant, chaperons et apoptose, CNRS UMR5534, Centre de Génétique Moléculaire et Cellulaire, Université Claude Bernard, Lyon-I, Bat. Gregor Mendel, 16 rue Dubois, 69622 Villeurbanne Cédex, France.
E-mail: arrigo@univ-lyon1.fr

Received 12 August 2004; Accepted 24 September 2004
DOI 10.1002/jcb.20349

© 2004 Wiley-Liss, Inc.

Contrasting with the coordinate induction of all heat shock proteins following heat stress exposure, only a subset of them, including some sHsps, are expressed during cellular differentiation programs [Arrigo and Tanguay, 1991]. A similar uncoordinated induction is also observed during development where sHsps display tissue- and stage-specific expression. Unfortunately, the biological significance of this highly-regulated expression still remains a mystery as in vivo data concerning the role of sHsp during development is still unavailable. Hence, besides their role during stressful conditions, sHsps appear to be involved in cellular differentiation processes suggesting that they are more than stress-related chaperones. After some examples of cellular differentiation and biochemical modifications of sHsp during this process, this study will be centered on the possible links between sHsp functions and the differentiation program.

sHsps EXPRESSION DURING CELLULAR DIFFERENTIATION

Since *Drosophila* Hsp27 has been shown to exhibit increasing protein level during ecdysterone mediated differentiation of cultivated imaginal disks as well as during the formation of these tissues in the developing fly [Pauli et al., 1990], there has been an ever-growing number of studies involving sHsps in cellular differentiation and development of mammals or other organisms. Most of the time when a change in sHsp protein level is investigated during the course of differentiation, at least one of them is found to be induced. Furthermore, this occurrence remains true when mammalian development is considered. Concerning mammalian Hsp27 induction, one can cite the differentiation processes of Ehrlich ascite cells [Gaestel et al., 1989], embryonal carcinoma and stem cells [Stahl et al., 1992; Mehlen et al., 1997b], normal B or B lymphoma cells [Spector et al., 1992], osteoblasts and promyelocytic leukemia cells [Shakoori et al., 1992; Spector et al., 1993, 1994; Chaufour et al., 1996], normal T cells [Hanash et al., 1993], keratinocytes [Kindas-Mugge and Trautinger, 1994; Jantschitsch et al., 1998; Arrigo and Ducasse, 2002; Duverger et al., 2004], endometrium cells [Devaja et al., 1997], muscle cells [Benjamin et al., 1997], neurones [Mehlen et al., 1999; Loones et al., 2000], chondrocytes [Favet et al., 2001], and cardiomyocytes [Davidson and Morange, 2000]. In vivo observations nevertheless suggest that Hsp27 is probably not involved in all differentiation processes [Duverger et al., 2004]. For example, during myogenic differentiation αB-crystallin [Ito et al., 2001; Kamradt et al., 2002] is expressed as well as a specific complex made of MKBP/HspB2 and HspB3 [Sugiyama et al., 2000]. To date a database describing the pattern of expression and possible mutual interactions of the different members of the sHsps family in every differentiation processes is lacking.

Hsp27 up-regulation during differentiation is correlated with a strong increase in phosphorylation and in a shift of Hsp27 oligomers toward high molecular masses [Mehlen et al., 1997b]. It is noteworthy that this later phenomenon occurs concomitantly with the maximal accumulation of the protein. In contrast, phosphorylation is an early phenomenon that occurs before the increase in Hsp27 level and which is followed by a drastic dephosphorylation of the protein [Spector et al., 1993, 1994; Chaufour et al., 1996; Mehlen et al., 1997b]. The use of kinase inhibitors, especially SB203580, which inhibits the p38 MAP kinase and subsequently Hsp27 phosphorylation via MAPKAP kinase 2/3 leads to conflicting results as to whether Hsp27 phosphorylation is crucial or not for the early events of differentiation programs, such as cell growth arrest [Schultz et al., 1997; Davidson and Morange, 2000; Ito et al., 2001; Duverger et al., 2004]. While the role of the transient and early phosphorylation of Hsp27 is still unknown its later dephosphorylation may be required to favor the transient formation of large oligomers [Mehlen et al., 1997a; Rogalla et al., 1999] whose size distribution is back to normal once the differentiated phenotype has been obtained.

sHsps EXPRESSION IS ESSENTIAL FOR CELLULAR DIFFERENTIATION

Analysis of the role of Hsp27 during differentiation was assessed using an antisense approach aimed at reducing Hsp27 level of expression. The first example concerns human promyelocytic leukemic HL-60 cells that are characterized by an incomplete differentiation, which is arrested at the promyelocytic stage of myeloid development. This differentiation arrest can be reversed since these cells undergo either granulocytic or monocytic maturation in response to a number of exogenous agents, such as phorbol esters or retinoic agents. In response

to phorbol ester myristate acetate (PMA), HL-60 cells differentiate into macrophage-like cells. Hsp27 level is markedly increased (up to 10-fold) during the first 72 h of the differentiation process while cellular growth inhibition occurs [Spector et al., 1993]. After 96 h, Hsp27 is drastically decreased and returns to the level observed in non-treated cells. A similar kinetics of Hsp27 level up-regulation is observed during the retinoic acid (tRA)-mediated differentiation of HL-60 cells [Spector et al., 1994]. However, in this case, Hsp27 mRNA level is not transiently accumulated as in macrophage differentiation. It seems likely that the half-life of Hsp27 is increased during granulocytic differentiation probably as a consequence of the down-regulation of myeloblastin, a serine protease that preferentially degrades this stress protein [Spector et al., 1995]. The use of Hsp27 antisense oligonucleotides decreased Hsp27 level by about 40% and led to a less pronounced reduction in cell growth after induction with tRA and altered some parameters of granulocytic differentiation [Chaufour et al., 1996].

A more intense decrease in Hsp27 level was observed in cells transfected with a DNA expression vector that contained Hsp27 coding sequence placed in reverse orientation under the control of a constitutive promoter. This approach was used to study the differentiation of mouse embryonic stem (ES) cells. Undifferentiated mouse ES cells constitutively express a small level of Hsp27. When these cells are committed to differentiation, Hsp27 level increases and is maximal after 24 h when DNA synthesis is decreased by about 80%. After 72 h of differentiation, Hsp27 level is drastically decreased and this protein is almost undetectable. It was observed that an 80% underexpression of Hsp27, which attenuated cell growth arrest, aborted the differentiation process of ES cells because of their overall death by apoptosis [Mehlen et al., 1997b]. Similar aborting processes were observed when Hsp27 expression was impaired in rat olfactory precursor neurons [Mehlen et al., 1999]. Using the same approach it was observed that the differentiation of embryonal carcinoma P19 cell line in cardiomyocytes was impaired by low level of Hsp27 expression [Davidson and Morange, 2000]. The transient expression of Hsp27 during early differentiation is therefore not related to a specific cell differentiation program and the presence of Hsp27, probably in the form of large oligomers, is needed to counteract an aberrant differentiation or, if the decrease in Hsp27 level is important, a massive apoptosis process. This hypothesis is supported by the finding that in the population of cells committed to differentiate, Hsp27 accumulation takes place only in differentiating cells that will not undergo apoptosis [Mehlen et al., 1999]. Concerning the other sHsps, it is noteworthy that αB-crystallin negatively regulates apoptosis during myogenic differentiation by inhibiting caspase-3 activation [Kamradt et al., 2002]. Since most of the above described studies were centered on Hsp27 and αB-crystallin, future experiments in which impairment of sHsps expression is induced by RNA interference are urgently needed in order to get rid of a plausible redundancy in sHsps functions.

Overexpression of sHsps mediated by cell transfection with DNA expression vectors has been tested in several differentiation models. For example, in murine ES cells, Hsp27 overexpression enhanced the differentiation-mediated decreased rate of cell proliferation but did not alter morphological changes [Mehlen et al., 1997b]. However, during the differentiation process, Hsp27 overexpression was found to gradually vanish, hence resulting in a level of Hsp27 similar to that observed in cells transfected with a mock vector. This suggests the existence of a mechanism that inhibits a too high production of Hsp27 during ES cell differentiation. Differentiation also normally occurred in olfactive neurones overexpressing Hsp27 except that a smaller fraction of cells underwent apoptosis [Mehlen et al., 1999]. The effect was more severe during chondrocyte differentiation since Hsp27 overexpression interfered with differentiation leading to loose masses of dying cells [Favet et al., 2001]. Hence, the level of Hsp27 expression is a crucial parameter that controls the decision of the cell to undergo either growth arrest and differentiation or apoptosis.

CONCLUSIONS: POSSIBLE LINKS BETWEEN sHsps FUNCTIONS AND APOPTOSIS INHIBITION DURING DIFFERENTIATION

The Chaperone Hypothesis

Intracellular modifications, structural as well as functional, of differentiating cells can lead to unfavorable conditions for some proteins or create unspecific interactions. Then cells may need chaperones to prevent them from the

toxicity of misfolded protein aggregation or from inaccurate protein interactions. Several facts are in favor of this hypothesis. First, as described above many differentiation programs correlate with the formation of large Hsp27 oligomers [Spector et al., 1993; Chaufour et al., 1996; Mehlen et al., 1997b] which represent the active form of the protein as a molecular chaperone [Rogalla et al., 1999]. Second, an intriguing observation was that once differentiated in keratinocytes, human HaCat cells can undergo a dedifferentiation-like process that re-increases Hsp27 level as well as the native size of this protein [Arrigo and Ducasse, 2002]. This suggests that the appearance of the chaperone-active form of Hsp27 is not a basic effect related to cell differentiation per se but is aimed at protecting the cells undergoing drastic changes in their protein content, structural organization, and/or localization. By decreasing their intracellular level, Hsp27 large oligomeric structures are probably overwhelmed and cannot cope with the drastic protein changes that occur during cell differentiation. Accumulation of junk protein structures may then lead to aberrant differentiation processes or apoptosis. Third, during the differentiation of murine ES cells, the transient increase of Hsp27 native size is concomitant with a cellular protection against apoptosis [Mehlen et al., 1997b].

Some proteins may also need to be protected from a transient hostile environment. On the opposite, sHsps could promote degradation of proteins that have become useless or undesirable for differentiated cells. In support of this hypothesis we noticed that the up-regulation of Hsp27 is often correlated with a stimulated activity of the 20S chymotrypsin activity of the proteasome.

The next question concerns the substrates that are recognized by Hsp27 large oligomers: are they always the same or are they specific to each differentiation process? Cytoskeletal proteins are probably among the preferred substrates. For example, during the keratinocyte differentiation of PAM212 cells, murine Hsp27 appears to drive the disassembly of the keratin network through the sequestration of keratin 5 and 14 subunits, thus permitting a switch from the keratin MK5/MK14 network to a new keratin MK1/MK10 network [Duverger et al., 2004]. The disruption of the MK5/MK14 network may involve a partial unfolding of keratin subunits that are sequestered by murine Hsp27 to prevent undesirable interactions and aggregation. It is not yet known whether Hsp27/MK5/MK14 structures are a first step toward degradation or a sequestration of keratins that can be reused once they have readopted their native state. Future studies will have to test whether sHsps involvement during other types of differentiation processes is related to their ability to drive a proper disassembly/assembly of intermediate filament networks or other protein complexes or depends on their capacity to regulate actin polymerization [Mounier and Arrigo, 2002]. An important fact to mention concerns the ability of sHsps to form tissue-specific hetero-oligomeric structures with themselves. Only few of these complex structures have been characterized yet, thus future studies will have to test whether those multiple, specific, and dynamic sHsps interactions can be seen as a highly modulatable system able to face rapidly with changes such as cellular differentiation. For example, Hsp27 and αB-crystallin, but not MKBP, are localized on well-developed actin bundles specific to myotubes, underlying the fact that two structurally independent sHsps complexes may have specific interactions with distinct molecular targets.

The Anti-Apoptotic Hypothesis

Hsp27 or αB-crystallin overexpression counteracts apoptosis induced by different agents by acting at different levels of the mitochondrial apoptotic pathway. Cell death that spontaneously occurs during normal cell differentiation or which is massive when differentiation is triggered in cells underexpressing Hsp27 is still not well characterized and further studies are needed to evaluate the protective role of sHsps in this process.

The Redox State Hypothesis

Analysis of murine ES cells differentiation revealed that this phenomenon correlates with a drastic increase in the intracellular level of reduced glutathione. This increase may initiate the metabolic modifications implicated by the differentiation program since the redox state can modulate the activity of transcription factors. On the opposite, it may protect the cell from the changes of the redox parameters that occur during the differentiation. An intriguing observation was that the kinetics of glutathione increase was similar to that of Hsp27 [Mehlen et al., 1997b]. Hsp27 and αB-crystallin

overexpression is characterized by a protective effect against oxidative stress, through the ability of these proteins to increase reduced glutathione levels and to maintain the redox parameters of the cells [Arrigo, 2001]. Though, only the large oligomers of Hsp27 modulates glutathione levels [Mehlen et al., 1997a]. Further work is therefore needed to determine the impact of Hsp27 toward the redox state modulation that can be detected during differentiation.

REFERENCES

Arrigo A-P. 2001. Hsp27: Novel regulator of intracellular redox state. IUBMB Life 52:303–307.

Arrigo A-P, Ducasse C. 2002. Expression of the anti-apoptotic protein Hsp27 during both the keratinocyte differentiation and dedifferentiation of HaCat cells: Expression linked to changes in intracellular protein organization? Exp Gerontol 37:1247–1255.

Arrigo A-P, Landry J. 1994. Expression and function of the low-molecular-weight heat shock proteins. In: Morimoto RI, Tissieres A, Georgopoulos C, editors. The biology of heat shock proteins and molecular chaperones. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. pp 335–373.

Arrigo A, Tanguay R. 1991. Expression of heat shock proteins during development in *Drosophila*. Results Probl Cell Differ 17:106–119.

Arrigo AP, Suhan JP, Welch WJ. 1988. Dynamic changes in the structure and intracellular locale of the mammalian low-molecular-weight heat shock protein. Mol Cell Biol 8:5059–5071.

Benjamin IJ, Shelton J, Garry DJ, Richardson JA. 1997. Temporospatial expression of the small HSP/alpha B-crystallin in cardiac and skeletal muscle during mouse development. Dev Dyn 208:75–84.

Chaufour S, Mehlen P, Arrigo A-P. 1996. Transient accumulation, phosphorylation, and changes in the oligomerization of Hsp27 during retinoic acid-induced differentiation of HL-60 cells: Possible role in the control of cellular growth and differentiation. Cell Stress Chaperones 1:225–235.

Davidson SM, Morange M. 2000. Hsp25 and the p38 MAPK pathway are involved in differentiation of cardiomyocytes. Dev Biol 218:146–160.

de Jong WW, Caspers GJ, Leunissen JA. 1998. Genealogy of the alpha-crystallin–small heat-shock protein superfamily. Int J Biol Macromol 22:151–162.

Devaja O, King RJ, Papadopoulos A, Raju KS. 1997. Heat-shock protein 27 (HSP27) and its role in female reproductive organs. Eur J Gynaecol Oncol 18:16–22.

Duverger O, Paslaru L, Morange M. 2004. HSP25 is involved in two steps of the differentiation of PAM212 keratinocytes. J Biol Chem 279:10252–10260.

Favet N, Duverger O, Loones MT, Poliard A, Kellermann O, Morange M. 2001. Overexpression of murine small heat shock protein HSP25 interferes with chondrocyte differentiation and decreases cell adhesion. Cell Death Differ 8:603–613.

Gaestel M, Gross B, Benndorf R, Strauss M, Schunk W-H, Kraft R, Otto A, Bohm H, Stahl J, Drabsch H, Bielka H. 1989. Molecular cloning, sequencing, and expression in *Escherichia coli* of the 25-kDa growth-related protein of Ehrlich ascites tumor and its homology to mammalian stress proteins. Eur J Biochem 179:209–213.

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

Hanash S, Strahler J, Chan Y, Kuick R, Teichroew D, Neel J, Hailat N, Keim D, Gratiot-Deans J, Ungar D. 1993. Database analysis of protein expression patterns during T-cell ontogeny and activation. Proc Natl Acad Sci USA 90:3314–3318.

Ito H, Kamei K, Iwamoto I, Inaguma Y, Kato K. 2001. Regulation of the levels of small heat-shock proteins during differentiation of C2C12 cells. Exp Cell Res 266: 213–221.

Jakob U, Gaestel M, Engels K, Buchner J. 1993. Small heat shock proteins are molecular chaperones. J Biol Chem 268:1517–1520.

Jantschitsch C, Kindas-Mugge I, Metze D, Amann G, Micksche M, Trautinger F. 1998. Expression of the small heat shock protein HSP 27 in developing human skin. BrJ Dermatol 139:247–253.

Kamradt MC, Chen F, Sam S, Cryns VL. 2002. The small heat shock protein alpha B-crystallin negatively regulates apoptosis during myogenic differentiation by inhibiting caspase-3 activation. J Biol Chem 277:38731–38736.

Kindas-Mugge I, Trautinger F. 1994. Increased expression of the M(r) 27,000 heat shock protein (hsp27) in in vitro differentiated normal human keratinocytes. Cell Growth Differ 5:777–781.

Loones MT, Chang Y, 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.

Mehlen P, Hickey E, Weber L, Arrigo A-P. 1997a. Large unphosphorylated aggregates as the active form of hsp27 which controls intracellular reactive oxygen species and glutathione levels and generates a protection against TNFα in NIH-3T3-ras cells. Biochem Biophys Res Commun 241:187–192.

Mehlen P, Mehlen A, Godet J, Arrigo A-P. 1997b. hsp27 as a switch between differentiation and apoptosis in murine embryonic stem cells. J Biol Chem 272:31657–31665.

Mehlen P, Coronas V, Ljubic-Thibal V, Ducasse C, Granger L, Jourdan F, Arrigo A-P. 1999. Small stress protein Hsp27 accumulation during dopamine-mediated differentiation of rat olfactory neurons counteracts apoptosis. Cell Death Differ 6:227–233.

Mounier N, Arrigo A-P. 2002. Actin cytoskeleton and small heat shock proteins: How do they interact? Cell Stress Chaperones 7:167–176.

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

Pauli D, Tonka C-H, Tissieres A, Arrigo A-P. 1990. Tissue-specific expression of the heat shock protein HSP 27 during *Drosophila melanogaster* development. J Cell Biol 111:817–828.

Rogalla T, Ehrnsperger M, Preville X, Kotlyarov A, Lutsch G, Ducasse C, Paul C, Wieske M, Arrigo AP, Buchner J,

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.

Schultz H, Rogalla T, Engel K, Lee JC, Gaestel M. 1997. The protein kinase inhibitor SB203580 uncouples PMA-induced differentiation of HL-60 cells from phosphorylation of Hsp27. Cell Stress Chaperones 2:41–49.

Shakoori AR, Oberdorf AM, Owen TA, Weber LA, Hickey E, Stein JL, Lian JB, Stein GS. 1992. Expression of heat shock genes during differentiation of mammalian osteoblasts and promyelocytic leukemia cells. J Cell Biochem 48:277–287.

Spector NL, Samson W, Ryan C, Gribben J, Urba W, Welch WJ, Nadler LM. 1992. Growth arrest of human B lymphocytes is accompanied by induction of the low molecular weight mammalian heat shock protein. J Immunol 148:1668–1673.

Spector NL, Ryan C, Samson W, Levine H, Nadler LM, Arrigo A-P. 1993. Heat shock protein is a unique marker of growth arrest during macrophage differentiation of HL-60 cells. J Cell Physiol 156:619–625.

Spector NL, Mehlen P, Ryan C, Hardy L, Samson W, Levine H, Nadler LM, Fabre N, Arrigo A-P. 1994. Regulation of the 28 kDa heat shock protein by retinoic acid during differentiation of human leukemic HL-60 cells. FEBS Lett 337:184–188.

Spector NL, Hardy L, Ryan C, Miller WH, Jr., Humes JL, Nadler LM, Luedke E. 1995. 28-kDa mammalian heat shock protein: A novel substrate of a growth regulatory protease involved in differentiation of human leukemia cells. J Biol Chem 270:1003–1006.

Stahl J, Wobus AM, Ihrig S, Lutsch G, Bielka H. 1992. The small heat shock protein hsp25 is accumulated in P19 embryonal carcinoma cells and embryonic stem cells of line BLC6 during differentiation. Differentiation 51: 33–37.

Sugiyama Y, Suzuki A, Kishikawa M, Akutsu R, Hirose T, Waye MM, Tsui SK, Yoshida S, Ohno S. 2000. Muscle develops a specific form of small heat shock protein complex composed of MKBP/HSPB2 and HSPB3 during myogenic differentiation. J Biol Chem 275: 1095–1104.