Perspective

Cell Stress & Chaperones (1998) 3 (4), 221–227

Article no. csac.1998.0125

Heat shock proteins chaperoning life and death

Muriel Vayssier and Barbara S. Polla

Laboratoire de Physiologie Respiratoire, UFR Cochin Port-Royal, 24 rue du Faubourg Saint-Jacques, 75014 Paris, France

INTRODUCTION

La Nature est un temple où de vivants piliers
Laissent parfois sortir de confuses paroles ;
L'homme y passe à travers une forêt de symboles
Qui l'observent avec des regards familiers.
Charles Baudelaire 1857

Science often considers itself as a world apart from socio-economic constraints. It is, however, amazing to consider the main scientific currents of investigations in the very light of socio-economic evolution. While Europe was recovering from World War II, and during the ensuing economic boom, proliferation was a major research topic: proliferation, its control, and the mechanisms for aberrant proliferation. In contrast, since the world-wide recession of the late 1980s and early 1990s, many groups have become interested in studying cell death, concentrating on a particular form of death, i.e. programmed cell death, or apoptosis. The chaperone field has not escaped this trend, and the great enthusiasm recently generated for all possible relationships between chaperones and cell death is reflected by this thematic issue. While the symbolic content of apoptosis is quite heavy, the scientific knowledge on the precise role of each of the heat shock proteins (Hsps) and how they act in concert or in dissonance in 'chaperoning life and death', remains confusing. This perspective article will focus on Hsp70 and enlighten some of the many paths that have been taken to unravel the role of Hsp70 in this 'forest of symbols'.

APOPTOSIS

Apoptosis is a sophisticated system for programmed cell death involved in a number of fundamental biological processes such as metamorphosis, embryonic morphogenesis, development and removal of cells that are superfluous or damaged, and occurs as a counterpart of proliferation in growth regulation. Altered programmed cell death has been associated with pathological processes such as cancer, infections and chronic inflammation (Cope and Wille 1991; Zychlinsky et al. 1992; Sachs and Lotem 1993). Apoptosis can be induced by a large variety of agents, but regardless of the inducing agent, the apoptotic process is usually divided into three phases: induction, control and execution/degradation phases (Kroemer et al. 1995; Farschon et al. 1997):

1. during the induction phase, a number of signals can elicit cells to enter the apoptotic pathway, although a given death-inducing stimulus will trigger apoptosis only in a particular context (position in the cell cycle, metabolic environment...)
2. during the control phase, various physiological or pathological stimuli converge to a common molecular switch: the pro-apoptotic and anti-apoptotic proteins, notably of the bcl-2 family, define the cellular rheostat of cell death ('apopstat'; Salvesen and Dixit 1997), and determine either susceptibility or resistance to apoptosis and the fate of the cells
3. during the execution phase, cells enter the irreversible step leading to death. Cysteine proteases (caspases) initiate the degradation phase of programmed cell death, giving rise to the visible manifestations of apoptosis, including cytoplasmic and nuclear condensation, cell membrane blebbing and formation of so-called apoptotic bodies, chromatin clumping at the periphery of the nucleus and endonuclease-mediated DNA cleavage (Willye et al. 1984).

All of these processes are reviewed in more detail by Samali and Orrenius in this issue (pp. 228–236), as are the possible interventions of Hsps in apoptosis.

Received 30 June 1998; Revised 15 September 1998; Accepted 15 September 1998
Available on line 21 December 1998

Correspondence to: Barbara S. Polla, Tel.: +33 1 44 41 23 36; Fax: +33 1 44 41 23 38

Essentially two approaches are being taken to investigate whether or not Hsps play a role in apoptosis: (i) Hsp overexpressing cells or organisms, and (ii) anti-sense strategies for in vitro analysis, or *hsp* knockout animals for in vivo studies. In particular, for Hsp70, the prevention of apoptosis has been demonstrated not only in vitro (Wei et al. 1995, Samali and Cotter 1996), but also in vivo. Transgenic mice with Hsp70-overexpressing lymphocytes are prone to develop lymphoma (Seo et al. 1996), while targeted gene disruption of *hsp70–2* results in failed meiosis which coincides with a dramatic increase in spermatocyte apoptosis (Dix et al. 1996). The anti-apoptotic effects of Hsp70 may also decrease the efficiency of apoptosis-inducing anti-cancer drugs: this has been shown in human lymphoid and myeloid leukemia cells where Hsp70 accumulation is correlated with a decreased sensitivity to apoptosis (Lasunskaia et al. 1997). These studies suggest that by altering cell death, Hsp70 could actually be oncogenic.

Here we will focus on reactive oxygen species (ROS)-related cell death, and on Hsp70, although other stress proteins, including Hsp27, Hsp90, heme oxygenase, ferritin and glucose-regulated proteins, also modulate the fate of cell death. ROS and ischaemia–reperfusion (a relevant model for increased ROS production and effects in vivo) both induce Hsp70 along with apoptosis, while their toxic effects are inhibited by Hsp70 overexpression, thus presenting a dual interest in our perspective. We will discuss the contradictory data in the literature about the effects of Hsp70 on apoptosis: essentially protective and inhibitory – though some reports suggest pro-apoptotic effects. We also propose an integrated view of the role of Hsp70 in cell death and suggest mechanisms whereby Hsp70 could act both to prevent or promote apoptosis.

ROS AS ESSENTIAL MEDIATORS OF APOPTOSIS AND HSP INDUCTION: ISCHAEMIA-REPERFUSION AS AN IN VIVO MODEL

ROS, ATP and Hsp

Major ROS include superoxide (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radical (·OH), nitric oxide (·NO) and peroxynitrites (ONOO-). ROS are produced by the cell from mitochondrial respiratory chain components as well as from a variety of enzymatic systems such as NADPH oxidases. Like heat shock, ROS induce both Hsps and apoptosis (Buttke and Sandstrom 1994; Jacquier-Sarlin and Polla 1996). The induction of Hsps by ROS is governed by dual mechanisms including the activation of heat shock factor (Hsf) and the stabilization of Hsp mRNA, leading in concert to increased Hsp synthesis and expression (Jacquier-Sarlin et al. 1995; Jacquier-Sarlin and Polla 1996).

ROS production is tightly associated with ATP depletion both in vitro and in vivo. In vitro, ATP decrease is linked to alterations in mitochondrial structure, bleb formation on cell surfaces and cytoskeletal alterations (Gabai and Kabakov 1993a). In vivo, ATP depletion during ischaemia leads to the accumulation of hypoxanthine and the irreversible, calcium-dependent, conversion of xanthine dehydrogenase to xanthine oxidase. During reperfusion, xanthine oxidase metabolizes hypoxanthine into uric acid and produces large amounts of superoxide (McCord 1985). ATP depletion may thus stimulate Hsp synthesis via multiple mechanisms: ROS-mediated Hsf activation (Knowlton et al. 1991; Nishizawa et al. 1996), ionic imbalance, organelle and cytoskeletal disruption, and/or protein aggregation.

Effects of ROS on gene activation and cell death

Many chemical, physical and biological factors able to induce apoptosis generate oxidative stress, whether infections, irradiation, or drugs that decrease the ability of cells to scavenge or detoxify ROS. At low concentrations, ROS act as second messengers and are involved in the control of gene expression, in particular the transcriptional factor NFκB which controls the inducible expression of a variety of genes involved in apoptosis (Kullik and Storz 1994; Storz and Polla 1996). At higher concentrations, ROS become toxic for cells, causing DNA single and double strand breaks, lipid peroxidation, protein denaturation and finally apoptosis or necrosis (Gabai and Kabakov 1993b, Buttke and Sandstrom 1994, Leist et al. 1997). The mechanisms for ROS-induced cell death are complex and multifactorial. They include direct DNA damage, which in turn activates poly-ADP ribose polymerase (PARP), a key enzyme for DNA repair; excessive PARP activation and poly-ADP ribosylation leads to depletion in NAD/NADH and ATP. The degree of energy deprivation then determines whether cells die by apoptosis or necrosis (Richter et al. 1996). Alternatively, ROS may oxidize arachidonic acid-derived membrane lipids implicated in tumour necrosis factor α (TNFα)-mediated apoptosis, or lead to increases in cytosolic free calcium, thus contributing to pro-apoptotic calcium-dependent endonuclease activation, further ATP depletion and necrosis.

Protective effects of Hsp70 against ROS-mediated cell death

In order to protect themselves against oxidative damage, cells have developed antioxidant strategies including Hsp induction (Jacquier-Sarlin and Polla 1996). The molecular targets for Hsp protection could theoretically be DNA, cell membranes or proteins. Although partial protection of all of these compounds by Hsp70 has been shown, we and others propose mitochondria as selective targets for protection from oxidative injury by Hsps (Patriarca and Maresca 1990; Borkan et al. 1993; Polla et al. 1996). The importance of mitochondria as a target of Hsp70-mediated protection has also been shown in vivo: when rats in which cardiac Hsp overexpression was induced by in vivo heat shock (HS), myocardial mitochondria and, particularly, state three respiration, are protected from oxidative injury (Currie et al. 1988; Bornman et al. 1998).

Alternatively, Hsps could act on the cellular redox status by modulating the oxidant/antioxidant balance. During ATP depletion in vivo, Hsp70 could chaperone altered polypeptides, thus contributing to protection against ischaemia. However, while Hsp accumulation *before* ATP depletion significantly reduces necrosis resulting from protein aggregation and blebs formation, it does not prevent the effects of a moderate decrease in intracellular ATP level, suggesting that Hsps do not act directly on ATP levels (Gabai and Kabakov 1993b; Kabakov and Gabai 1995).

Still another target for Hsp70-mediated protection could be the actin network: indeed, stress-induced actin aggregation appears as a key event in promoting rapid necrotic death under ATP deprivation. Since both resistance to necrosis and the decrease of actin aggregation under ATP depletion coincide with heat-induced Hsp70 accumulation, a major role of Hsp70 could be to protect the actin network, thus preventing cytoskeletal disorganisation and necrosis (Kabakov and Gabai 1994).

The various mechanisms by which Hsp70 could prevent apoptosis are summarized in Figure 1 (see also Samali and Orrenius, this issue, pp. 228–236):

- Firstly, Hsp70 appears to act at the mitochondrial level (Polla et al. 1996): indeed, Hsp induction by HS protects mitochondria against depolarization mediated by low pro-apoptotic levels of H_2O_2.
- Secondly, the protective effects of Hsp70 may stem from chaperoning of proteins involved in the apoptotic process. Indeed, Hsp70 takes part in the recovery of partially denaturated proteins and in removal of damaged, misfolded proteins (Welch 1992; Macario 1995). Hsp70 and its constitutive homologue Hsc70 bind proteins known to take part in the regulation of apoptosis such as p53, c-myc, or Bag (Koskinen et al. 1991; Matsumoto et al. 1995; Takamaya et al. 1997). Hsp70 is able to chaperone (and to reduce; Simpkins et al. 1993) the pro-apoptotic mitochondrial co-factor of the activation of caspases, i.e. cytochrome c (Vanbuskirk 1991). Thus, Hsp70 has been suggested to inhibit caspase conversion and suppress downstream proteolytic events by its effects on cytochrome c.
- Thirdly, Hsp70 is able to modulate the activity of enzymes involved in the apoptotic process, including PARP or phospholipase A_2 (PLA_2). Hsp70-mediated protective effects against apoptosis involve a down-regulation of PARP, both in HS-induced and in NO-mediated cytotoxicity (Bellmann et al. 1995; Mosser et al. 1997). PLA_2, which is localized to the membrane and is modulated by cytosolic proteins, is another target of Hsp70: in Hsp70-overexpressing cells, the prevention of TNFα-induced apoptosis is linked to the inhibition of PLA_2 activity (Jäättelä 1993).
- Finally, the activation of kinases such as SAPK is also inhibited in cells in which Hsp70 is induced to high levels which indicates that Hsp70 can block apoptosis by inhibiting signaling events upstream of kinase activation (Mosser et al. 1997).

The protective effects of Hsp70, however, cannot be extrapolated to all cases of apoptosis (Mailhos et al. 1994; DS Latchman, personal communication). Hsp70 overexpression may even enhance apoptosis as is the case for the TCR/CD3- and Fas/Apo-1/CD95-mediated apoptosis in Jurkat T cells (Liossis et al. 1997). In this model, Hsp70 overexpression results in decreased levels of protein tyrosine phosphorylation and free cytosolic Ca^{2+} responses during the early signal transduction events after ligand–TCR interactions. In the Fas-induced apoptosis models, Hsp70 increases protein phosphatases PP-1 and PP-A2 activities, which is consistent with an increase of ATPase activity, a depletion of high energy phosphates, an increase in cAMP concentrations and apoptosis. These results support the concept that ATP levels are, if not a direct target, at least a checkpoint where Hsp70 exerts its regulatory functions in apoptosis.

A PROPOSED RECONCILIATION FOR CONTRADICTORY DATA ON HSP70 FUNCTION IN APOPTOSIS: A BALANCE WITH NECROSIS

Conventionally, apoptosis has been regarded as a form of cell death morphologically and biochemically distinct from necrosis, the former being a programmed death, while the latter, a passive death, occuring after a severe and sudden injury. However, factors as diverse as heat shock, oxidative stress (e.g. ischaemia, radiation, or tobacco smoke), or infection can induce both apoptosis and necrosis and a number of events and downstream steps are common to both types of cell death (Fukuda et al. 1993; Dypbukt et al. 1994; Bonfoco et al. 1995; Shimizu et al. 1996a; Vayssier et al. 1998). Increasing evidence suggests that the two forms of cell death share similar characteristics at least in the early phase, and that they are in a continuum rather than two distinct forms of cell demise, while signaling molecules thought to be specific for apoptosis, such as caspases, also

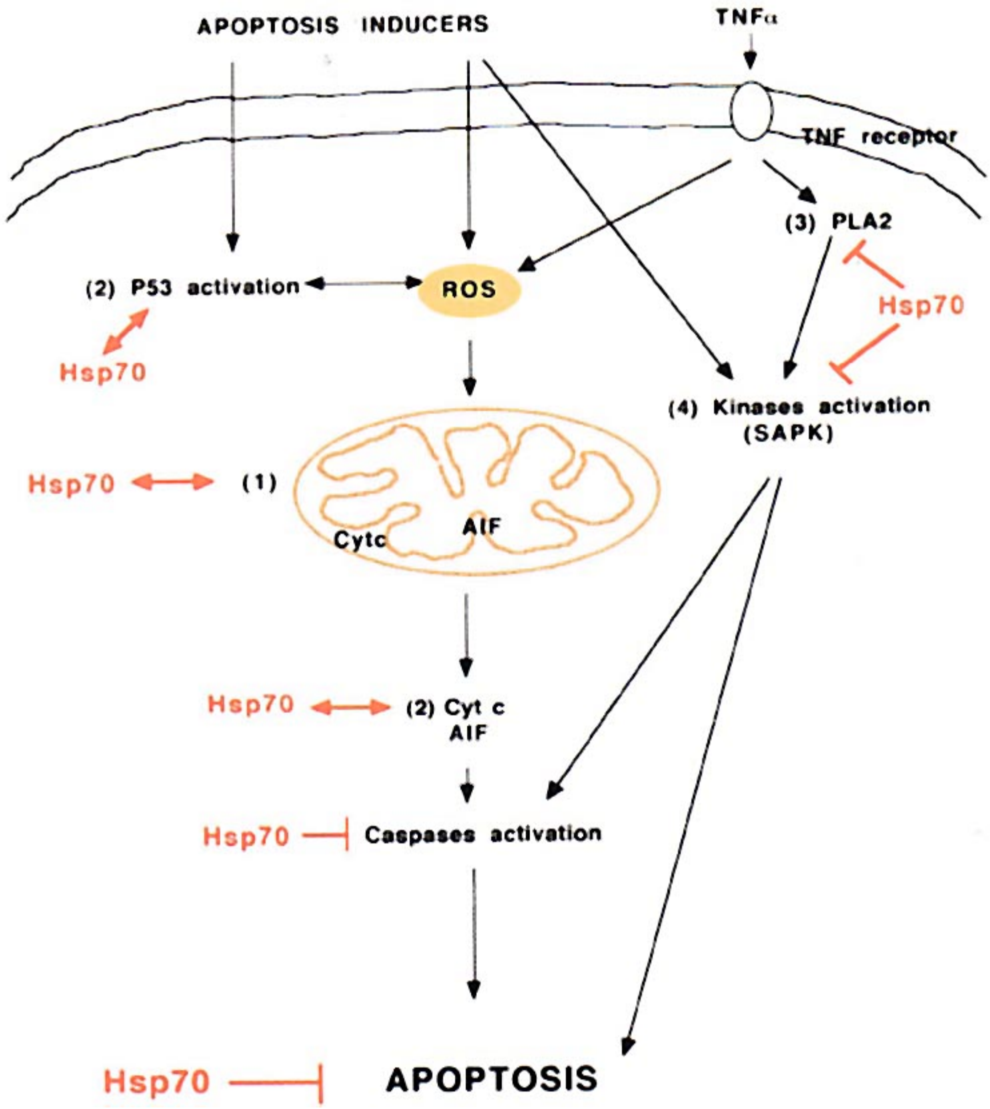

Fig. 1 Possible pathways of Hsp70 mediated protection against apoptosis. Apoptosis inducers increase intracellular ROS, leading to depolarization of mitochondrial membranes. Pro-apoptotic factors such as cytochrome c (cytc) or the apoptosis-inducing factor(s) (AIF) are released from mitochondria into the cytosol where they activate the final effectors of apoptosis, i.e. the caspases. (1) Hsp70 overexpression protects mitochondria from the deleterious effects of ROS. (2) The protective effects of Hsp70 stem from its chaperoning function, notably of proteins involved in the apoptotic process (p53). Hsp70 is also able to chaperone the pro-apoptotic mitochondrial cofactor of caspase activation, i.e. cytoc, thus inhibiting caspases conversion and supressing downstream proteolytic events. (3) Hsp70 can modulate the activity of enzymes involved in apoptosis, such as PLA_2 or PARP (the latter not shown on the scheme). (4) Hsp70 may also block apoptosis by inhibiting signaling events involved in the activation of kinases such as SAPK.

participate in necrosis (Hirsch et al. 1997; Leist and Nicotera 1997; Leist et al. 1997). The anti-apoptotic proto-oncogene product bcl-2 exerts protective functions in necrosis as well, and bcl-2 overexpression can inhibit necrotic death of various causes (Zhong et al. 1993; Kane et al. 1995; Lindenboim et al. 1995; Shimizu et al. 1996a, 1996b). Thus the theoretical opposition between an ordered execution of a death program called apoptosis and an unscheduled accidental event called necrosis is not as clear as previously suggested: cell death, whether by necrosis or by apoptosis, may be the manifestation of a distinct final execution of an initially similar program.

However, Hsps do not exert the same effects on cell death under all conditions and in all models: they may either have no effect, inhibit, or enhance apoptosis. These contrasting results have been explained previously by the different cells or apoptotic inducers used. We hypothesized that the apparently contradictory data reported on the effect of Hsp70 in promoting or inhibiting apoptosis could be reconciled if we consider their effects both in necrosis and apoptosis. As an example, we have shown that tobacco smoke induces apoptosis at low concentrations and necrosis at higher concentrations in mammalian cells (Vayssier et al. 1998). In parallel to

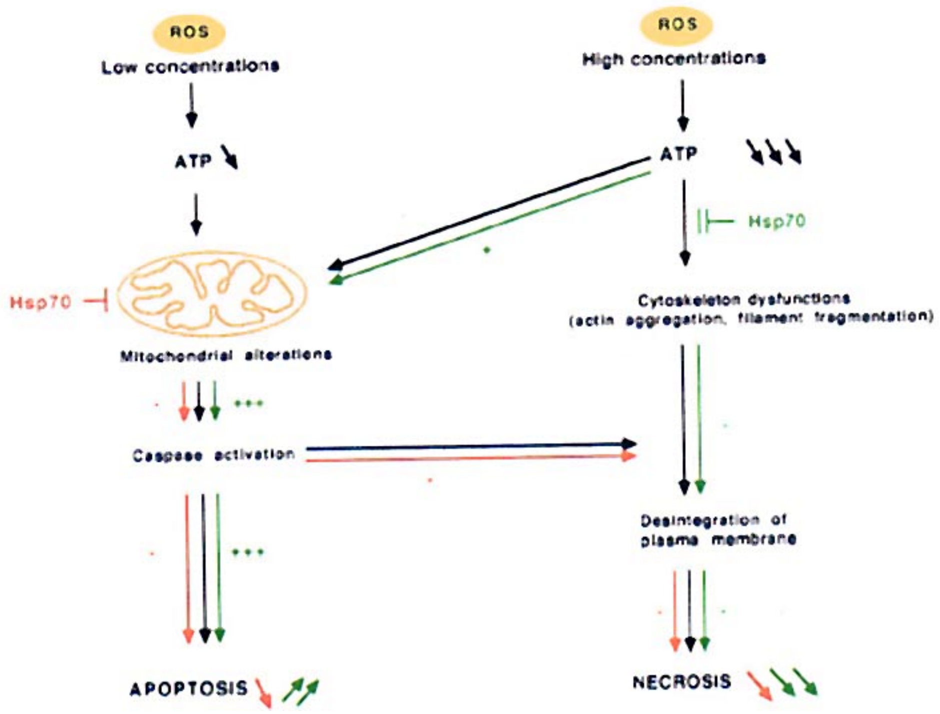

Fig. 2 Proposed explanation of the contradictory effect of Hsp70 on apoptosis. Black arrows represent the normal events happening in cells submitted to moderate or severe ATP depletion. Red arrows represent the effects of Hsp70 overexpression in the events caused by moderate ATP depletion, and green arrows represent the effect of Hsp70 overexpression under conditions of severe ATP depletion. Low ROS concentrations cause a moderate decrease in ATP level involving mitochondrial alterations and caspase activation secondary to the release of pro-apoptotic factors. In this case, Hsp70 protects cells from apoptosis at the various levels described in Figure 1(red arrows). High ROS concentrations promote severe ATP depletion leading to cytoskeletal alterations, plasma membrane disintegration and cell death by necrosis, along with mitochondrial alterations and caspase activation. In that case, Hsp70 prevents actin aggregation and filament fragmentation, thus preventing necrosis. If Hsp70 does not simultaneously prevent mitochondrial alterations due to high level of ROS, then the inhibition of necrosis will lead to an increase in apoptosis (green arrows).

apoptosis and necrosis, tobacco smoke induces in these cells an increase in Hsp70 levels. Using Hsp70-overexpressing cells, we showed that Hsp70 essentially inhibits tobacco smoke-induced necrosis, subsequently leading to an indirect increase in tobacco smoke-induced apoptosis: cells which do not die by necrosis will finally die by apoptosis (Vayssier et al. 1998). Thus, the apparent apoptosis-enhancing effect of Hsp70 (or Hsp90; Galea-Lauri et al. 1996) described in some models could be a logical, indirect consequence of necrosis protection. Accordingly, the role of Hsp70 in inducing apoptosis proposed by some authors would not relate to a direct effect of Hsp70 on apoptosis but rather would occur as a consequence of Hsp-mediated protection against necrosis.

If these hypotheses hold true, Hsps probably act on some important factor that determines the fate of a given dying cell between necrosis and apoptosis. Along with Richter (1996), we propose that cellular ATP levels are the essential parameter of the balance between the two forms of death. Indeed, apoptosis proceeds only when ATP is avalaible, while under severe ATP depletion, controlled cell death evolves towards rapid necrosis (Chou et al. 1995; Richter et al. 1996; Eguchi et al. 1997; Leist et al. 1997; Tsujimoto 1997).

The in vitro evidence supporting the above hypothesis extends to in vivo models: an example for this is ischaemic brain damage. In the core of ischaemic regions, necrotic cell death is prevalent, whereas towards the border regions, where ATP depletion is less severe, apoptotic neuronal death is observed (Charriaut-Marlangue et al. 1996). If ATP levels are indeed the checkpoint between apoptosis and necrosis, then Hsp70, although being unable to rescue ATP levels, would prevent necrosis by limiting the effects of ATP depletion (Gabai and Kabakov 1993b, 1997; Kabakov and Gabai 1995).

The contrasting effects of Hsp70 on necrosis and apoptosis are schematically represented in Figure 2. If we consider that under ATP depletion caused by severe injuries, two different events are induced: apoptotic signals on the one hand (which would be common to necrosis: mitochondrial depolarization, caspase activation...) and cytoskeleton destabilization on the other, which causes preferentially necrotic death. In Hsp70-overexpressing cells, actin aggregation is prevented, the cytoskeleton stabilized and necrotic death avoided. If under the same conditions, Hsps are unable to simultaneously exert their protective effects on the signals favouring apoptosis, then cells will die by apoptosis. Upcoming studies on the effects of Hsps on cell death should thus always consider both apoptosis and necrosis, since their effects on the latter modulate the former.

FURTHER STUDIES

Chaperones/chaperone inducers are theoretically attractive as a novel strategy towards prevention of oxidative injury and are being studied for their therapeutic applications in humans. In this perspective, one should be well aware of the two-edge sword effect of Hsp70 (and potentially other stress proteins): cells overexpressing these proteins become resistant to stresses that should lead to death, and thus persist beyond their natural lifespan, allowing for the development of conditions such as chronic inflammation (Polla et al. 1998) or cancer (Seo et al. 1996). These issues should be explored in detail before chaperone inducers are considered for clinical applications in chronic diseases (Vigh et al. 1997).

ACKNOWLEDGEMENTS

The authors are grateful to Alexandre Mignon, Paris, to Patrick Arrigo, Lyon, and to Christoph Richter, Zürich, for stimulating discussions and critical review, and to Sarah Kreps, Harvard University, for reading the manuscript. MV is supported by the Société de Secours des Amis des Sciences, Paris, and Electricité de France (EDF). BSP is supported by INSERM. Work mentioned here was supported in part by the CICS.

REFERENCES

Bellmann K, Wenz A, Radons J, Kleemann V and Kolb H (1995) Heat shock induces resistance in rat pancreatic islet cells against nitric oxide, oxygen radicals and streptozotocin toxicity in vitro. *J Clin Invest* **95**: 2840–2845

Bonfoco E, Krainc D, Ankarcrona M, Nicotera P and Lipton SA (1995) Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. *Proc Natl Acad Sci USA* **92**: 7162–7166

Borkan SC, Emami A and Schwartz JH (1993) Heat stress protein-associated cytoprotection of inner medullary collecting duct from rat kidney. *Am J Physiol* **265**: F333–F341

Bornman L, Christiaan ML, Steinmann CM, Gericke GS and Polla BS (1998) In vivo heat shock protects rat myocardial mitochondria. *Biochem Biophys Res Comm* **246**: 836–840

Buttke TM and Sandstrom PA (1994) Oxidative stress as a mediator of apoptosis. *Immunol Today* **15**: 7–10

Chant ID, Rose PE and Morris AG (1996) Susceptibility of AML cells to in vitro apoptosis correlates with heat shock protein 70 (Hsp70) expression. *Br J Haematol* **93**: 898–902

Charriaut-Marlange C, Margaill I, Represa A, Popovici T, Plotkinen M and Ben-Ari Y (1996) Apoptosis and necrosis after reversible focal ischemia: an in situ DNA fragmentation analysis. *J Cereb Blood Flow Metab* **16**: 186–194

Chou CC, Lam CY and Yung BY (1995) Intracellular ATP is required for actinomycin D-induced apoptotic cell death in Hela cells. *Cancer Lett* **96**: 181–187

Cope FO and Wille JJ (1991) Carcinogenesis and apoptosis: Paradigms and paradoxes in cell cycle and differentiation in apoptosis: the molecular basis of cell death. Cold Spring Harbor Laboratory Press: 61–86

Currie RW, Karmazyn M, Kloc M and Mailer K (1988) Heat shock response is associated with enhanced postischaemic ventricular recovery. *Circ Res* **63**: 543–549

Dix DJ, Allen JW, Collins et al. (1996) Targeted gene disruption of Hsp70-2 results in failed meiosis, germ cell apoptosis, and male infertility. *Proc Natl Acad Sci USA* **93**: 3264–3268

Dypbukt JM, Ankarcrona M, Burkitt M, Sjoholm A, Strom K, Orrenius S and Nicotera P (1994) Different prooxidant levels stimulate growth, trigger apoptosis, or produce necrosis of insulin-secreting RINm5F cells. The role of intracellular polyamines. *J Biol Chem* **269**: 30553–30560

Eguchi Y, Shimizu S and Tsujimoto Y (1997) Intracellular ATP levels determine cell death fate by apoptosis or necrosis. *Cancer Res* **57**: 1835–1840

Farschon DM, Couture C, Mustelin T and Newmeyer DD (1997) Temporal phases in apoptosis defined by the actions of Src homology 2 domains, ceramide, Bcl-2, interleukin-1β converting enzyme family proteases and a dense membrane fraction. *J Cell Biol* **137**: 1117–1125

Fukuda K, Kojiro M and Chiu JF (1993) Demonstration of extensive chromatin cleavage in transplanted Morris hepatoma 7777 tissue: apoptosis or necrosis? *Am J Pathol* **142**: 935–946

Gabai VL and Kabakov AE (1993a) Rise in heat-shock protein level confers tolerance to energy deprivation. *FEBS Lett* **327**: 247–250

Gabai VL and Kabakov AE (1993b) Tumor cell resistance to energy deprivation and hyperthermia can be by the actin skeleton stability. *Cancer Lett* **52**: 3648–3654

Gabai VL and Kabakov AE (1997) Heat Shock Proteins and Cytoprotection: ATP-Deprived Mammalian Cells. Heidelberg: Springer-Verlag

Galea-Lauri J, Richardson AJ, Latchman DS and Katz DR (1996) Increased heat shock protein 90 (Hsp90) expression leads to increased apoptosis in the monoblastoid cell line U937 following induction with TNF-α and cycloheximide. A possible role in immunopathology. *J Immunol* **157**: 4109–4118

Hirsch T, Marchetti P, Susin SA et al. (1997) The apoptosis-necrosis paradox. Apoptogenic proteases activated after mitochondrial permeability transition determine the mode of cell death. *Oncogene* **15**: 1573–1581

Jäättelä M (1993) Overexpression of major heat shock protein Hsp70 inhibits tumor necrosis factor-induced activation of phospholipase A2. *J Immunol* **151**: 4286–4294

Jacquier-Sarlin MR, Jornot L and Polla BS (1995) Differential expression and regulation of Hsp70 and Hsp90 by phorbol esters and heat shock. *J Biol Chem* **270**: 14094–14099

Jacquier-Sarlin MR and Polla BS (1996) Dual regulation of heat-shock transcription factor (Hsf) activation and DNA-binding activity by H_2O_2: role of thioredoxin. *Biochem J* **318**: 187–193

Kabakov AE and Gabai VL (1994) Heat shock proteins maintain the viability of ATP-deprived cells: what is the mechanism? *Trends Cell Biol* **4**: 193–196