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Hsp70 expression and function during embryogenesis

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This review focuses on the expression and function of 70-kDa heat shock proteins (Hsp70s) during mammalian embryogenesis, though many features of embryogenesis and the developmental expression of Hsp70s are conserved between mammals and other vertebrates. A variety of Hsp70s are expressed from the point of zygotic gene activation in cleavage-stage embryos, through blastulation, implantation, gastrulation, neurulation, organogenesis, and on throughout fetal maturation. The regulation and patterns of hsp70 gene expression and the known and putative Hsp70 protein functions vary from constitutive and metabolic housekeeping to stress-inducible and embryo-protective roles. Understanding the genetic regulation and molecular function of Hsp70s has been pursued by developmental biologists interested in the control of gene expression in early embryos as well as reproductive toxicologists and teratologists interested in how Hsp70s protect embryos from the adverse effects of environmental exposures. These efforts have also been joined by those interested in the chaperone functions of Hsp70s, and this confluence of effort has yielded many advances in our understanding of Hsp70s during critical phases of embryonic development and cellular differentiation.

INTRODUCTION

Mammalian embryogenesis has been well described in a number of reviews (Pedersen and Burdsal, 1994) and reference books (Theiler 1989; Kaufman *1992*; Hogan et al. 1994), and readers should look to these for detailed descriptions of the developmental processes referred to in this review. Embryonic development in mammals is readily divided into two phases. First is the relatively slow-paced preimplantation phase of development from one-cell zygote to a blastocyst which hatches from the zona pellucida and implants into the uterus. The implanting blastocyst is already composed of three distinct tissue lineages: trophectoderm, primitive endoderm, and epiblast. The second phase of post-implantation embryonic development transforms the blastocyst epiblast, the sole founder tissue of the fetus, through gastrulation and organogenesis to eventually form the mature fetus. Like embryogenesis, the vast majority of research into Hsp70 expression and function in embryos can be divided between either the pre-implantation phase of development, or the post-implantation period of organogenesis and neural tube closure. In this review we will present experimental results from several decades of work looking at expression of Hsp70s in embryos, and then try to draw some conclusions about possible functions of Hsp70s in these tissues.

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The 70-kDa heat shock proteins

A family of at least 10 mammalian hsp70 genes has now been characterized in mice, rats and humans (Table 1; Tavaria et al., 1996). All cell types (human, mouse, rat) examined express the cognate hsc70 gene (Sorger and Pelham 1987; Dworniczak and Mirault 1987; Giebel et al. 1988). However, in mouse, a bit of controversy has arisen over the identity of the original hsc70 mouse gene identified by Giebel et al. (1988), a cDNA derived from the F9 teratocarcinoma cell line. A recently characterized mRNA shares 98.9% identity with the earlier sequence and appears to be the most highly expressed hsc70 in mouse tissues (Soulier et al. 1996). It remains to be determined whether these two hsc70 mRNAs are encoded by different alleles or different genes. Hsc70 is found in both

Table 1 The Mammalian *hsp70* Family

Mouse genes	Rat homologs	Human homologs	Embryonic expression
grp75[1]		*grp75*[1]	Constitutive
grp78[2]		*grp78*[3]	Constitutive
hsc70[4]	*hsc70*[5]	*hsc70*[6]	Constitutive
hsc70t[7]		*hsp70-hom*[8]	None
hsp70–1[9–11]	*hsp70–1*[12]	*hsp70–2*[8]	Inducible, constitutive
hsp70–2[10,13]	*hst70*[14]	*hspA2*[15]	None
hsp70–3[10–11, 16]	*hsp70–2*[17]	*hsp70–1*[11,18]	Inducible, constitutive
		hsp70–6[19]	Not determined
		hsp70–7[20]	Not determined
		hsp70RY[21]	Not determined

[1]Domanico et al. 1993; [2]Kozutsumi et al. 1989; [3]Ting and Lee 1988; [4]Giebel et al. 1988; [5]Sorger and Pelham 1987; [6]Dworniczak and Mirault 1987; [7]Matsumoto and Fujimoto 1990; [8]Milner and Campbell 1990; [10]Hunt et al. 1993; [11]Snoek et al. 1993; [12]Lisowska et al. 1994; [13]Zakeri et al. 1988; [14]Wisniewski et al. 1990; [15]Bonnycastle et al. 1994; [16]Perry et al. 1994; [17]Mestril et al. 1994; [18]Hunt and Morimoto 1985; [20]Voellmy et al. 1985; [21]Fathallah et al. 1993

the cytosol and nucleus, and chaperones nascent polypeptides and protects against accumulation of malfolded proteins. Several different laboratories have also cloned and characterized the constitutively expressed glucose-regulated protein grp75 (Domanico et al. 1993; Michikawa et al. 1993; Bhattachryya et al. 1995) and mapped it to chromosome 18 of the mouse (Ohashi et al. 1995) and chromosome 5 of human (Kaul et al. 1995). The Grp75 protein has also been described as peptide-binding protein74 (PBP74), C3H strain-specific antigen (CSA), mortalin, and mitochondrial Hsp70. Grp75 is localized to the mitochondrial matrix and chaperones the import and folding of proteins therein. The final constitutively expressed Hsp70 is Grp78, also known as immunoglobulin heavy chain binding protein (BiP). The grp78 gene has been cloned and characterized from both mice (Kozutsumi et al. 1989) and humans (Ting and Lee 1988). Grp78 accumulates in the lumen of the endoplasmic reticulum and is required for efficient protein processing and export through this organelle.

Two additional hsp70s are expressed exclusively in spermatogenic cells of rodents and humans. Mouse hsp70–2 on chromosome 12 (Zakeri et al 1988; Hunt et al. 1993) and its rat (hst70; Wisniewski et al. 1990) and human (hspA2 on chromosome 14; Bonnycastle et al. 1994) homologs are expressed during the meiotic phase of spermatogenesis. During the post-meiotic phase of spermiogenesis mouse hsc70t on chromosome 17 (Matsumoto and Fujimoto 1990) and its human homolog hsp70-hom on chromosome 6 (Milner and Campbell 1990) are expressed. While the sequences of both spermatogenic Hsp70s are highly similar to each other, as well as to other Hsp70s, both have evolved distinct patterns of expression and unique functions in the testis which distinguish them from other Hsp70s (Dix, 1997).

In most cell types, stress induces the expression of two nearly identical, intronless hsp70 genes which have been cloned and characterized in humans (hsp70–1, Hunt and Morimoto 1985; hsp70–2, Milner and Campbell 1990), rats (hsp70–2, Mestril et al. 1994; hsp70–1, Lisowska et al., 1994) and mice (hsp70–3, Perry et al. 1994; hsp70–1, Hunt and Calderwood 1990). It is this pair of stress-inducible hsp70s that are expressed in response to a wide range of environmental stressors, in a wide range of cell and tissue types. These inducible Hsp70 proteins are believed to protect cells and help them recover from stress-induced damage. The stress-inducible hsp70 genes are clustered with the spermatid-specific hsp70 in the major histocompatibility complex of mouse chromosome 17 (Hunt et al. 1993; Snoek et al. 1993), rat (Walter et al. 1994) and human chromosome 6 (Sargent et al. 1989; Milner and Campbell 1990; Ito et al. 1998).

A second pair of stress-inducible, intronless hsp70s have been cloned, characterized and mapped to human chromosome 1 (hsp70–7, Voellmy et al. 1985; hsp70–6, Leung et al. 1990 and 1992). Similar to the stress-inducible genes on human chromosome 6 which are over 98% identical to one another, hsp70–6 and hsp70–7 are 95% identical to each other. By contrast, sequence identity between the human hsp70–6/7 and hsp70–1/2 pairs is only about 75%. While rodent homologs of human hsp70–6/7 have not been identified, a putative porcine homolog has been reported (Dezeure et al. 1993). Whereas it is well established that the stress-inducible hsp70s from the MHC region protect cells from environmental stressors, the functional significance of hsp70–6/7 expression has not been determined.

The mammalian stress response is characterized by the

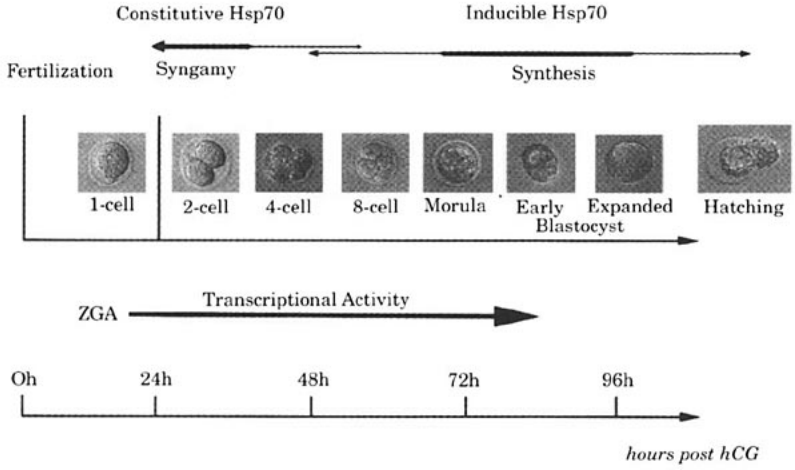

Fig. 1 Hsp70 expression during preimplantation embryogenesis has both a constitutive and a stress-inducible component. Mouse preimplantation embryogenesis is indicated in hours post human chorionic gonadotropin (hCG) for embryos obtained from super ovulated females. Zygotic genome activation (ZGA) occurs late in 1-cell stage, and includes constitutive expression of Hsc70 and Hsp70–1 and Hsp70–3 through at least the 4-cell stage. Heat and chemical-induced expression of Hsp70s begins by the 4-to 8-cell stage and is fully established in blastocysts.

Table 2 Expression of *hsp70–1* and *hsp70–3* in mouse pre-implantation stage embryos

Zygote/ embryo	hsp70–1 transgene	mRNA	Protein*	hsp70–3: transgene	mRNA	Protein*
1-cell		C[1]	N[2]			N[2]
2-cell	C[3]	C[1]	N[2]	C[4], N[5]		C[2]
4-cell	C[3]	C[1,6]	C[6]		C[6]	C[6]
8-cell	C[3]	C[1]	C[7], I[7]	C[4], N[5]		C[7], I[7]
Morula	C[6]	C[6]	I[4]	C[6]	C[6]	
Blastocyst	I[3]	C[1], I[1]	C[6–7], I[7] I[4–5]			C[6–7], I[7]
ES cells		C[8]				

*Expression of Hsp70–1 or Hsp70–3 proteins was not differentiated.; C = constitutive; I = inducible (by exposure to heat or arsenic); N = not expressed. [1]Christians et al. 1995; [2]Bensaude et al. 1983; [3]Thompson et al. 1995; [4]Bevilacqua et al. 1995; [5]Kothary et al. 1989; [6]Dix et al. 1998; [7]Edwards et al. 1995; [8]Thompson et al. 1994

rapid induction of heat shock protein (HSP) expression (Welch 1992). Stress-induced expression of HSPs, particularly the Hsp70s, occurs when cells are exposed to heat, amino acid analogues, heavy metals, metabolic poisons, oxidative stress, as well as normal cellular division and differentiation. It has also been clearly shown that the stress response and the expression of Hsp70s is often required for cells to survive environmental or developmental challenges. Thus it is presumed and has been reported that HSPs, and particularly Hsp70 expression, is a potentially quantitative indicator of environmental stress and toxicity in mouse (Fischbach et al. 1993) and human (Delmas et al. 1995) cells, as well as in transgenic mice (Sacco et al. 1997) and exposed human populations (Wu et al. 1995). Similar associations between Hsp70 expression and environmental and developmental stress are evolutionarily conserved in sea urchin (Sconzo et al. 1995), amphibian (Landsberger et al. 1995; Angelier et al. 1996; Heikkila et al., 1997) and zebrafish (Lele et al. 1997) embryos. Direct evidence of the efficacy of Hsp70 in affording protection from environmental stress has also been reported in transgenic fly larvae which overexpress Hsp70 and are thermotolerant (Feder et al. 1996). It is within this context that we present the following descriptions of Hsp70 expression controlled by constitutive, inducible and developmental mechanisms during mammalian embryogenesis. Hsp70s perform a variety of functions throughout embryogenesis which are of interest to both developmental biologists and toxicologists.

Hsp70s IN PRE-IMPLANTATION EMBRYOS

Over the past two decades numerous laboratories have examined the expression and function of Hsp70s in mammalian preimplantation embryos. Embryonic cells

express Hsp70s either constitutively, according to a developmental program, or in response to toxic stress (Fig. 1). This observation was first made by Bensaude et al. (1983), who characterized the expression of Hsp70 proteins in 2-cell mouse embryos. These Hsps were identified as the constitutive Hsc70 and the heat and sodium arsenite (NaAs) induced Hsp70s. Others have gone on to further characterize the expression of hsc70 mRNA, which is present from 1-cell stage onward (unpublished data, Dix), and the Hsc70 protein, which is expressed throughout preimplantation embryogenesis (Edwards et al. 1995). The 642 aa sequence of Hsp70–1 is nearly identical to the 641 aa sequence of Hsp70–3, differing at only two residues. Thus, even two-dimensional SDS- PAGE cannot readily distinguish between the Hsp70–1 and Hsp70–3 proteins. Because of this ambiguity, the stress-inducible Hsp70s will hereon be referred to as Hsp70–1/3. Hsp70–1/3 have been reported as heat-inducible in murine 8-cell and blastocysts (Edwards et al. 1995) and homologs are expressed in bovine 2-cell embryos (Edwards and Hansen 1996). Other groups have reported on the constitutive expression of Hsp70–1/3 from 2-cell to blastocyst stages, but did not find heat-inducible expression until the blastocyst stage (Hahnel et al. 1986). This lack of consistency in the heat-inducibility of Hsp70–1/3 did not appear linked to whether 8-cell mouse embryos were heat shocked in vivo or in vitro.

Examination of mRNA and promoter-reporter transgene expression has identified both hsp70–1 and hsp70–3 expression in preimplantation mouse embryos (Table 2). The hsp70–1 mRNA is constitutively expressed as early as the 1-cell stage (Christians et al. 1995), its expression peaks at the 2-cell stage and then diminishes until becoming heat-inducible by the blastocyst stage. Similar results were obtained with hsp70–1 (Thompson et al. 1995) and hsp 70–3 (Bevilacqua et al. 1995) promoter-reporter transgenes. However, there is evidence that a limited hsp70–3 promoter may not be sufficient to direct constitutive expression of a reporter gene in 2-cell to blastocyst stage embryos (Kothary et al. 1989). It is possible that some of this variability in results with transgene constructs is due to the absence or presence of sequences flanking the hsp70 promoters, and the role of chromatin structure in regulating hsp70 gene expression in the preimplantation mouse embryo (Thompson et al. 1995). A reasonable conclusion from the protein, mRNA and transgene data is that there is an initial, constitutive burst of Hsp70–1/3 expression during activation of the zygotic genome which peaks during the 2- cell stage (Fig. 1). This initial burst continues through the second and third cleavages and overlaps with the developing potential for inducible Hsp70 synthesis which is fully established by blastocyst stage.

This interpretation of expression data is endorsed by results with antisense oligonucleotides utilized in pre-implantation mouse embryos to inhibit hsp70–1/3 expression (Dix et al. 1998). Limiting expression of Hsp70–1 and Hsp70–3 in 4-cell embryos by oligonucleotide transfection reduced in vitro blastocyst development and heightened embryosensitivity to arsenic. These results indicate that some minimal amount of Hsp70–1 and/or Hsp70–3 is required for pre-implantation embryogenesis, and that increasing the demand for Hsp70s by toxicant exposure heightens this requirement. Similar results were obtained in studies wherein 2-cell mouse embryos were co-cultured with monoclonal antibodies against Hsp70s, which resulted in significantly diminished development of hatched blastocysts at postcoital day 5 (Neuer et al. 1998).

Hsp70s IN POST-IMPLANTATION EMBRYOS

Numerous laboratories have demonstrated the ability of a variety of agents to induce a stress response and Hsp70 expression in mammalian postimplantation embryos (reviewed in Mirkes 1997). The most extensively studied agent has been hyperthermia and it has been established that temperatures which induce heat shock response also induce abnormal development (Mirkes 1985; Kimmel et al. 1993a; Kimmel et al. 1993b; Buckiova and Jelinek 1995; Edwards et al. 1997). Malformations induced experimentally by heat likely correspond to interruption of cell proliferation and differentiation during specific stages of neurulation and organogenesis, and the closure of the neural tube is particularly sensitive to elevated temperatures. The correlation between protection from heat-induced terata and expression of heat shock proteins in animal models is clear and suggests that expression of particular heat shock proteins protect embryos from the effects of heat and toxic exposure (i.e., thermotolerance). Similar relationships between maternal hyperthermia and resultant defects in humans have been reported in several epidemiological studies (reviewed in Graham et al. 1998). A recently published prospective study confirms the association of maternal fever with increased risk of neural tube defects (Chambers et al. 1998).

Thermotolerance is elicited when cells are subjected to a mild heat shock followed by a more severe stress (hyperthermia, chemical agents, etc.). Thermotolerance protects cells from the effects of the severe stress and is presumably mediated by heat shock proteins. Accumulating evidence suggests that postimplantation embryos can be made thermotolerant by conditioning exposures to heat (Mirkes et al. 1987; Walsh et al. 1987; Kapron-Brás and Hales 1992; Finnel et al. 1993). In two recent reports, Phil Mirkes and his collaborators (Thayer

Table 3 Stress-inducible expression of heat shock proteins in post-implantation stage rodent embryos

Embryonic Species	Day	In vivo (IVV) or Exposure	In vitro (IVT)	HSPs	Reference
Mouse	8	Arsenic	IVT	Hsp70–1, Hsp70–3*	1
Mouse	8	Cadmium, heat	IVT	Hsp70–1, Hsp70–3*	2
Mouse	8	Heat, valproate	IVV	Hsp70–1, Hsp70–3*	3
Mouse	9	Arsenic, cadmium heat	IVV	Hsp70–1, Hsp70–3*, Hsp105	4
Rat	9	Heat	IVT	Hsp70–1, Hsp70–2*	5–6
Rat	10	Arsenic, heat, salicylate	IVT	Hsp70–1, Hsp70–2*	7
Rat	10	Heat	IVT	Hsp70–1, Hsp70–2*	8–10
Rat	10	Heat	IVV, IVT	Hsp70–1, Hsp70–2*, Hsp90	11

*Expression of inducible Hsp70 proteins was not differentiated (i.e. Hsp70–1 vs Hsp70–3 in mouse, Hsp70–1 vs Hsp70–2 in rat). [1]Hunter and Dix 1998; [2]Kapron-Bras and Hales 1992; [3]Finnell et al. 1993; [4]Honda et al. 1992; [5]Walsh et al. 1987; [6]Walsh et al. 1989; [7]Mirkes and Dogget 1994; [8]Mirkes 1987; [9]Fisher et al. 1996; [10]Thayer et al. 1997; [11]Fisher et al. 1995

and Mirkes 1997; Mirkes et al. 1997) demonstrated that the induction of thermotolerance in cultured rat postimplantation embryos was associated with a significant reduction in internucleosomal DNA fragmentation and associated apoptosis following acute hyperthermia. Accordingly, it was shown that the induction of inducible Hsp70s and the cytoplasmic-to-nuclear translocation of Hsp70s were correlated with the acquisition of thermotolerance. The ability of Hsp70s to prevent stress induced defects in murine embryos has also been addressed in a series of recent gain-of-function and loss-of-function experiments. Hunter and Dix (1996) employed a constitutive-promoter transgene construct to overexpress Hsp70–1 that significantly reduced embryo sensitivity to arsenite-induced neural tube defects. In the same study, antisense inhibition of Hsp70–1 and Hsp70–3 expression resulted in an eightfold higher incidence of neural tube defects in embryos exposed to subteratogenic doses of arsenite. Most recently, transgenic mouse embryos, which constitutively overexpress inducible Hsp70, were shown to be protected from the embryolethal effects of hyperthermia (Mirkes et al. 1999). These findings substantiate the causal relationship between the expression of Hsp70 and thermotolerance in embryos. The fact that Hsp70s and other Hsps (i.e. 25, 47, 90) are involved with the cellular machinery that orchestrate normal development and protective mechanisms elicited following stress is unarguable. However, the molecular mechanisms by which these proteins perform these functions is currently an area of intense research.

POTENTIAL FUNCTIONS OF Hsp70s IN EMBRYOS

Hsp70s are considered chaperones which interact with other proteins to prevent aggregation and insure proper folding and cellular localization. Accordingly, previous reports provide evidence of Hsp70s regulatory role in protein synthesis (Matts and Hurst 1992; Takenaka and Hightower 1992; Gross et al. 1994; Brostrom et al. 1996) and their pivotal role in maintaining proteins in proper configuration to ensure appropriate biological activity (Morimoto et al. 1997). Considering these functional properties, the fact that constitutive Hsp70s are present at specific embryonic stages, and that synthesis of inducible Hsp70s can be elicited by stress at certain stages of development, it seems likely that Hsp70s play essential roles in both normal development and protection against damage from stressors at vulnerable stages of embryonic development.

Balance between cell cycle regulation and programmed cell death (apoptosis) is essential in embryogenesis for maintaining appropriate cell numbers during differentiation and development (Walsh et al. 1997; Weil et al. 1997). Evidence implicating HSP involvement in the regulation of the developing mammalian embryo cell cycle has been demonstrated (Walsh and Morris 1989; Walsh et al. 1993; Walsh et al. 1994). Lethal heat shock results in both G_1/S and G_2/M arrested cells in the neuroectoderm of day 9.5 rat embryos. In addition to these cell cycle blocks, vast amounts of cell death occurred in the neural plate and resulted in malformations of the developing forebrain and eye. However, induction of thermotolerance by a mild heat shock is highly effective at protecting against the aforementioned cell death. This protection is strongly associated with the expression of Hsp25, Hsp70s, and Hsp90s and a delay in the progression of the cell cycle (Walsh and Morris 1989; Walsh et al. 1993). Similar results indicating involvement of HSPs in cell cycle regulation of numerous cell types suggests this might be a fundamental property of HSPs. Milarski and Morimoto (1986) and Milarski et al. (1989) demonstrated that the synthesis, intracellular distribution and protein–protein associations of stress-inducible

human Hsp70 is tightly controlled during the cell cycle. Furthermore, Kwak et al. (1998) found that the overexpression of Hsp70 corresponds to a G_0/G_1 block in the cell cycle following treatment with phorbol 12-myristate 13-acetate (PMA). It has also been determined that overexpression of Hsp70 is involved in controlling the duration of the G_2 arrest during doxorubicin treatment in murine fibrosarcoma WEHI-S cells (Karlseder et al., 1996). Based upon these studies it seems that HSPs are involved in the regulation of the cell cycle. However, limited data addressing the mechanism(s) by which HSPs accomplish this regulation are available. Zhu et al. (1997) provide evidence for a link between Hsp70–2 and CDC2 kinase activity essential for the meiotic cell cycle in mouse spermatogenesis. In this report, a specific association between Hsp70–2 and CDC2 was demonstrated, and it was determined that Hsp70–2 was required for the complexation of CDC2/cyclin B1 and for CDC2 kinase activity. It has also been demonstrated that the heat-induced expression of Hsp70 is correlated with the induction of p21, a cyclin-dependent kinase inhibitor, and a subsequent p53-independent G_1 cell cycle arrest (Fuse et al. 1996). These results suggest Hsp70s may interact with other proteins which regulate the cell cycle, thereby providing a protective mechanism to the embryo from heat and chemical teratogens. However, because of the broad chaperone activity of HSPs, it remains a difficult task to identify specific HSP associations with cell cycle regulators.

Since the regulation of cell cycle and apoptosis are closely linked, it seems plausible that Hsp70s are associated with both cell cycle regulation and apoptotic pathways in embryos. Several recent reports correlate the expression of Hsp70s induced by mild hyperthermia or by transfection of heat shock protein genes with the ability to prevent apoptosis induced by a variety of toxic agents (Strasser and Anderson 1995; Samali and Cotter 1996; Gabai et al. 1997; Mosser et al. 1997; Buzzard et al. 1998; de la Rosa et al. 1998). Accordingly, similar thermotolerance (Mirkes 1987; Walsh et al. 1987) and chemotolerance (Kapron-Bras and Hales 1991) have been reported in embryos. The protection provided by Hsp70s against diverse apoptosis-inducing agents argues that the HSP family may represent a class of anti-apoptotic genes. The mechanism(s) by which HSPs prevent apoptosis may involve modulating interactions with other proteins known to regulate apoptosis (i.e., bcl-2 family members, p53, caspase proteases) and/or with stress-induced signal transduction pathways (i.e. SAPK/JNK and p38). Mosser et al. (1997) demonstrated that transient expression of Hsp70s prevents stress-induced apoptosis by inhibiting two effectors of the apoptotic pathway: SAPK/JNK activation and pro-caspase-3 cleavage. In another recent report, Lee and Corry (1998) suggest that Hsp70 expression is itself a consequence of SAPK/JNK activation and overexpression of Hsp70 inhibits the SAPK/JNK pathway through negative feedback regulation. In addition, the anti-apoptotic protein BAG-1 may modulate the chaperone activity of both Hsc and Hsp70 (Takayama et al. 1997; Höhfeld and Jentsch 1997) by acting as a nucleotide exchange factor in the Hsc and Hsp70 ATPase cycle. The observed anti-apoptotic function of BAG-1 may be exerted by modulating the chaperone activity of Hsc70 on specific protein folding and maturation pathways. For example, the association between BAG-1 and Hsp/Hsc70 may afford BAG-1 the opportunity to adopt different confirmations which enhance interactions between BAG-1 and different partner proteins (Bcl-2, Raf-1, HGF-R, PDGF-R, and steroid hormone receptors) involved in cell survival and growth regulation. These data provide further evidence of complex links between apoptosis and Hsp70s.

It has also been suggested that Hsp70s may directly block apoptosis by a mechanism similar to that suggested for the Bcl-2 family. Bcl-X_L prevents disruption of the mitochondrial membrane potential and release of apoptosis-inducing proteins into the cytosol (Vander Heiden 1997). Additionally, Bcl-2 blocks release of cytochrome *c* from mitochondria following apoptotic stimuli to prevent interaction between cytochrome *c* and Apaf-1, and the subsequent caspase activation and apoptosis (Yang et al. 1997; Kluck et al. 1997). As discussed in Buzzard et al. (1998), Hsp70 may also inhibit cytochrome *c* release from mitochondria, or bind directly to cytosolic cytochrome *c* to prevent activation of the caspases. The latter mechanism may be more likely since Hsp70 is known to bind peptides derived from cytochrome *c* (Greene et al. 1995).

Based upon the chaperone properties of Hsp70s and their roles in numerous pathways influencing cell fate, it is likely that HSPs perform similar functions in developing embryos to orchestrate development and differentiation. Jurisicova et al. (1998) have demonstrated that apoptosis in 1-cell mouse embryos is regulated by cell death factors (e.g. MA-3, p53, Bcl-2 family members) either inherited as maternal proteins or transcribed as genes from the embryonic genome. Thus, apoptosis may occur by default at the end of the first cell cycle if the embryo fails to execute essential developmental events, and it remains to be determined if Hsp70s are involved in chaperoning or regulating the apoptotic machinery at this initial step of embryogenesis. However, this potential for HSP interaction is intriguing since the end of the first cell cycle also corresponds to the onset of Hsp70 expression.

It remains a priority to continue to bridge the gaps between descriptive information on the expression of the Hsp70s with their underlying biological function and relationship to cellular physiology. This can be addressed

by applying gene-knockout and transgenic mice to investigate the functions of Hsp70s in mammalian reproduction, development and physiological adaptation. It will also be of significant value to generate combinations of transgenic and geneknockout animals to address the potential redundancy and interactions of various Hsp70s. The application of DNA microarray technology to this field promises to be another useful approach to investigate significant biological pathways involving Hsp70s.

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