CMLS, Cell. mol. life sci. 53 (1997) 179–190
1420-682X/97/020179-12 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

HSP gene expression and HSF2 in mouse development

M.-T. Loones*, M. Rallu, V. Mezger and M. Morange

Unité de Génétique Moléculaire, Département de Biologie, Ecole Normale Supérieure, 46 rue d'Ulm, F-75230 Paris Cedex 05 (France), Fax +33 1 44 32 39 41, e-mail: morange@wotan.ens.fr

Abstract. During the pre-implantation phase of development, the mouse embryo synthesizes HSC70, and HSP90α and β at a very high rate. After implantation, the expression of HSPs appears non-coordinated and is not uniform in the different tissues. The expression of inducible HSPs appears later in development than that of constitutive members of the family. HSP25 is highly expressed early in heart and muscle development, but also in some structure of the central nervous system. HSC70 and HSP90β are expressed ubiquitously, but their expression reaches very high levels in the nervous system (neural tracks) and during bone morphogenesis (in the hypertrophic chondrocytes). The mechanisms involved in HSP expression during mouse embryogenesis are probably diverse, involving tissue-specific sequences. Although the DNA-binding activity and expression of the second heat shock transcription factor, HSF2, seems to be developmentally regulated, becoming detectable at the blastocyst stage and reaching a peak at day 10 of development, there is no obvious correlation between the level of this factor and the expression of HSPs. HSF2 might be involved in the onset of expression of HSPs, regulate (inhibit) their expression, or control the expression of other developmental genes yet to be discovered.

Key words. Chaperones; heat shock proteins; heat shock transcription factors; pre- and post-implantation development.

The mouse is used as a model in the study of mammalian (and human) organisms. This is particularly true in developmental biology where the technique of knocking-out gene function by homologous recombination has recently provided a wealth of data, and made the mouse the best known vertebrate in terms of development.

We will summarize what is known about the role and expression of HSPs and chaperones in mouse embryogenesis, reserving most space for more recent results not incorporated in previous reviews [1]. First, we will briefly add to previous articles in this multi-author review by reviewing the specific features of the heat shock response and of embryological development in the mouse.

HSPs, chaperones and HSFs in the mouse

The heat shock genes and proteins and the heat shock response are not different in mouse than in other vertebrates (see L. Nover and K.-D. Scharf in this multi-author review for a characterization of the main HSPs and chaperones). The major inducible HSP is HSP70, while the major constitutive forms of HSPs are HSP90β and HSC70 (see E. Christians in this review for a precise description of the different genes forming the HSP/HSC70 family). As in human cells, the inducible form of HSP90, HSP90α, is expressed in all organs at a constitutive level lower than that of HSP90β but is highly inducible by stress, whereas HSP90β is only weakly inducible [2]. In mouse fibroblast cell lines, in contrast to human cells, HSP70 is only expressed at a very low constitutive level and is not even detectable in most cells. However, HSP70 is expressed in vivo in different tissues and its expression increases with the age of the animal [3].

Two families of HSPs have received special attention: HSP/HSC70, additional members of which are expressed during spermatogenesis (K. D. Sarge and C. Cullen, this issue), but are also found in brain [4, 5], and the small HSP/α-crystallins. Initially, it was thought that small HSP and α-crystallins were very different proteins, the latter being specifically expressed in the lens whereas the former is expressed in other tissues. In fact, small HSP and α-crystallins are very similar (as similar as the different HSPs of *Drosophila*), and α-crystallins are present in many different tissues of the organism in addition to the lens. These proteins are expressed at high levels in the heart (and more generally in striated muscle) but also in other tissues [6–14]. In the case of αB-crystallin, thanks to the work of Joram Piatigorsky's group we have information on the sequences involved in the developmental regulation of this gene, with a precision comparable to what is known about the same family of genes in *Drosophila*. Small HSP expression is controlled by steroid hormones in mammalian tissues [15] (as it is by ecdysone in *Drosophila*).

Two heat shock factors (HSFs) have been described in the mouse [16] and are able to bind to the same sequence, the heat shock element (HSE). HSF1 is in-

* Corresponding author.

volved in the heat-inducible synthesis of HSPs. HSF2, which has been isolated from a cDNA library by homology, shares with HSF1 a very similar DNA-binding and leucine zipper domain but is quite distinct in the other domains. Both HSF1 and HSF2 polypeptides exist in two forms (differing by 22 and 18 amino acids respectively), resulting from an alternative splicing [17–19]. These two forms have different transcriptional efficiencies [19].

The mechanisms leading to the activation of HSF1 and HSF2 are different. Upon activation by heat shock and stress, HSF1 undergoes a monomer to trimer transition, concomitant with modifications in its phosphorylation state, and a nuclear translocation [20]. HSF2 is a dimer when inactive and a trimer after activation [21]. HSF2 activity is not induced by heat and other stresses but rather abolished by these treatments. Although both factors in solution are inactivated by increasing temperature, addition of the DNA target stabilizes mHSF1 whereas it destabilizes HSF2 (our own results). Therefore, the thermosensitivity of HSF2 is not consistent with a role for this factor in the response to stress. The physiological signals responsible for the activation of HSF2 are unknown. Perhaps these signals do not exist: it has been proposed that the activity of HSF2 might be simply related to its level of expression [17]. However, this relation is not linear: HSF2 mRNA is present at a low but significant level in many fibroblast cell lines, but no HSE-binding activity is detected in the same cells.

Two other factors have been described in vertebrates: HSF3 in avians [22, 23] and HSF4 in humans. HSF3 is a thermoinducible factor (at least in an erythroblast cell line), whereas the properties of HSF4 have not yet been described. It is possible, but not yet demonstrated, that similar factors exist in the mouse.

The DNA-binding properties of HSF1 and HSF2 are slightly different [24], even though the same consensus sequence (nGAAm motif repeated in inverted orientations) has been described for both of them. HSF1 binding is cooperative: this factor prefers four or five repeats of the motif nGAAm whereas three such inverted motifs constitute an optimal binding site for HSF2 [25]. The cooperativity of the thermoinducible factor is well demonstrated for *Drosophila* HSF [26]. Some similar data have also been obtained on the human factor produced in *E. coli* [27]. Since the properties of recombinant and cell-extracted heat shock factors are obviously different, these results have to be considered with caution. In our hands, the binding of mHSF1 partially purified from mouse cells is only poorly cooperative, and this cooperativity is not significantly increased by temperature. It has been demonstrated by random oligonucleotide selection that substitutions in the constant motif or in the adjacent nucleotides have different effects on the binding of HSF1 and HSF2 [25]. Moreover, HSF2 binds to the promoter of the *hsp70.2* gene as shown by gel shift assays, although the nucleotide sequence does not reveal any obvious HSE sequence in this promoter [28]. The precise nature of the binding site for HSF2 therefore remains an open question.

As for all mammals, the embryological development of the mouse (21 days) can be subdivided in two phases: the pre-implantation phase (4.5 days) which can be reproduced in vitro, and the post-implantation phase (16.5 days) which has been far less studied. In addition to the developmental studies, some data have been collected on the relative expression of the different HSPs and chaperones in adult tissues and during the differentiation processes which occur during adulthood such as in the gonads [28]. The expression of HSPs and chaperones during spermatogenesis in testes, the mechanisms responsible for this expression and its physiological significance will be documented in another article of this multi-author review (K. D. Sarge and C. Cullen).

Expression of HSPs and chaperones during early mouse embryogenesis (pre-implantation phase)

Growing oocytes spontaneously express HSC70 and HSP90 at a high level [29]. High expression of HSPs during oogenesis (and sporulation) seems to be an ancient form of developmental regulation [30]. Many studies have been devoted to the atypical expression of HSP70 during early embryogenesis: its 'spontaneous' expression at the early two cell stage, its absence of inductibility during the same period and the progressive acquisition of this inducibility between fertilization and the blastocyst stage (E. Christians et al., this multi-author review [31, 32]). These modifications in expression and inducibility are correlated with the transition from a maternal HSF1, abundant in the mature oocyte, to a newly synthesized zygotic form of this factor.

Another characteristic feature of the early embryo is the high level of synthesis of some of the HSPs, HSC70 and HSP90α-β, at the eight cell and blastocyst stages [2, 33, 34]. This high level of expression is obvious at the eight cell stage: the rate of synthesis of HSC70 and HSP90α-β, detected by one- or two-dimensional gel electrophoresis of the neo-synthesized proteins labelled with ^{35}S-methionine, is similar to the rate of synthesis of actin, and far superior to the rate of synthesis of other cellular proteins. This level of synthesis remains high at the blastocyst stage. It leads to the progressive accumulation of these proteins (in addition to HSP60) which are revealed as strong protein spots by silver staining of two-dimensional gels run with extracts of day 8.5 embryos [35]. At the blastocyst stage, this high rate of synthesis is observed in cells of the inner cell mass as well as in the trophectoderm [33], demonstrating that, at this stage of development, there is no obvious corre-

lation between the rate of synthesis of these HSPs and the differentiation state of the cells harbouring them.

Although these observations are well established, the mechanisms involved and their physiological significance remain obscure. The fact that this high level of expression or at least a part of it is observed in all embryonal carcinoma (EC) or embryonic stem (ES) cells so far tested [34, 35] has opened the way to approaches which are out of reach of experimenters working directly on embryos. HSC70 overexpression results from a stabilization of mRNA [36] as well as from an increased rate of transcription of the gene [37]. HSP90α overexpression, which has not been reported in all studies [38], was explained by an increased rate of transcription [36]. A similar high level of HSP90β mRNA has been described [39], but no experimental data have so far been provided for HSP60. It has not been demonstrated that the same mechanisms operate in embryos. Moreover, the level of expression of small HSP/α-crystallins, or of the cytosolic chaperonin CCT (TRiC), in EC or ES cells or in the early embryo remains unknown. However, both in the embryo and in EC cells, the overexpression of HSPs is correlated with the occurrence of a constitutive HSF-like activity, which by supershifting the HSE-HSF complex with specific antibodies has been shown to be due to HSF2 [38, 40–42]. HSF2 is absent from the oocyte and the embryo immediately after fertilization and the levels of HSF2 mRNA and protein increase during pre-implantation development (Christians, unpublished data). They are high at the blastocyst stage but increase further after implantation, with peak levels reached at about day 10 of development (our own data, see below). It is an attractive hypothesis to correlate the high level of expression of HSPs to the presence of this additional transcription factor. However, this correlation remains problematic: 1) the highest levels of HSP90α-β and HSC70 synthesis are observed at the eight cell stage, when the level of HSF2 is far from maximal; 2) the form of HSF2 which is expressed during embryogenesis is not the most transcriptionally active form (our own data [19]); 3) in EC cells, HSF2 is not bound to the *hsp70* promoter [38]. The latter observation correlates well with the absence of spontaneous expression of HSP70 in these cells. It casts some doubt on the true physiological activity of the HSF2 factor present at the early stage of embryogenesis (see below).

The physiological significance of this high level of HSP expression is also obscure. One must recall that what is physiologically meaningful is the level of HSPs and chaperones and not their rates of synthesis. The former becomes significant only after implantation of the embryo [35], while the latter is high as early as the eight cell stage.

The explanations that can be proposed for the alterations (increases) in HSP expression are not specific to the pre-implantation phase of development. We shall also refer to them when, later in this review, we discuss the variations in *hsp* gene expression which occur after implantation of the embryo. It is always possible to argue that the high level of HSPs is linked to the high rate of protein synthesis during this early phase of development, and the profound modifications in cell morphology and organelle structure which affect most cells of the embryo. In particular, the cytoskeleton is extensively reorganized during early development, and it would be very useful to have some information on the expression of the different polypeptides forming the cytoplasmic chaperonin CCT [43], and on the small HSPs which are involved respectively in the synthesis of the cytoskeletal components (actin and tubulin) and in the control of their structure [44, 45]. The two isoforms of HSP90 might participate in the formation of active transcription factors belonging to the steroid receptor or HLH superfamilies (such as myoD), which would be involved in the complex changes in transcriptional regulation occurring during this early phase of development [45, 46] (see [47] and the article by P. H. Krone et al. in this multi-author review, who put forward the same hypothesis to explain the high specific expression of HSP90α in zebrafish muscle). HSP90 might also be involved in the control of protein synthesis [48] or participate in the protection of the embryo at these stages of development. Another less frequently mentioned parameter which might also affect the expression of HSPs is the modification in the energy sources and metabolic pathways used by the embryos, which occurs between the pre- and post-implantation phases of development and later at the onset of vascularization of the implanted embryo. The modification of stress protein expression by metabolic conditions was described very early [49]. Since then, there has been ample experimental support for the stress proteins localized in the endoplasmic reticulum, but this is also true for the stress proteins localized in the cytosol. It has also been shown that some HSPs are differentially expressed during the different phases of the cell cycle (see D. Walsh et al. in this multi-author review for results and a review of the literature). Therefore some of the variations in HSP levels during development might simply result from modifications in the rate of cell division (and in the duration of the different phases of the cell cycle). Finally, a collagen environment also stimulates HSP synthesis [50]. This modulation of HSP synthesis by collagen might play a major role, in particular at later stages of development.

Expression of HSPs in the post-implantation phase of mouse development

As mentioned previously, HSP levels vary in the different tissues of an organism. Even within the same organ,

different cells may have different levels of HSP synthesis and a differential sensitivity to heat shock. For instance, in the brain (especially in the hippocampus and in Purkinje layer of the cerebellum), neurons exhibit a high level of HSC70 synthesis but respond weakly to a stress, whereas glial cells (oligodendrocytes and microglia) have a weaker level of constitutive HSPs but respond strongly to stress [51]. HSP90 is also abundant in neurons and its expresson is low or absent in glial cells [52]. The highest level is found in the Purkinje layer of the cerebellum. A correlation between the level of HSP90 and that of the steroid (glucocorticoid) receptor has been demonstrated [53]. However, in contrast to what has been described for members of the HSP70 family, both forms of HSP90 are inducible in the neurons. The contrast between the high level of HSC70 and the weak inducibility of HSP70 in neurons can be easily explained if the level of active HSF is directly controlled by the level of HSP70 proteins, as proposed by Abravaya et al. [54] and Mosser et al. [55], but questioned by Rabindran et al. [56]. The inducibility of both forms of HSP90 in the same cells is more difficult to explain in this model. In fact, many data have been collected on stress protein synthesis and induction in the brain [57] and retina [58], in relation to the fact that the stress response is induced in these organs in a wide variety of physiological and pathophysiological situations and that HSPs might have a protective effect against these stresses. Many studies have also been devoted to the expression of HSPs in testes during spermatogenesis (see the article by K. D. Sarge and C. Cullen in this multi-author review). One additional objective of a study on the developmental expression of heat shock genes is to understand how these adult characteristics are progressively established during development.

We feel that the simplest organization of the data is to present successively the main findings concerning the expression of the different families of HSPs, before turning our attention to the molecular mechanisms which might be responsible for this expression. It should be recalled that the existing data on HSP expression during mouse embryogenesis are heterogeneous since they result from five different lines of research which provide only partially complementary results. First, the in vivo study of cell differentiation: interesting results were obtained very early on muscle differentiation [59] and hematopoeisis (see later). The second group of studies involves the determination of the level of mRNAs for different heat shock proteins during development, either by Northern blots or, with a higher resolution, by in situ hybridization. Additional studies were devoted to the direct localization of the HSPs during development by Western blots or immunocytochemistry. The fourth approach has been the study of transgenic animals, with reporter genes under the control of heat shock gene promoters: in so far as these transgenic animals reproduce the normal pattern of expression, they gave some clues to the expression of HSPs. Finally, we have already mentioned that a specific form of a heat shock transcription factor, HSF2, is expressed at a high level during most of embryogenesis. Therefore, we directly measured the expression (at the mRNA and protein level) and activity of this factor during mouse embryogenesis. This latter approach must be compared with the previous ones in order to assess the physiological significance of this factor in the expression of HSPs and chaperones. This point will be addressed at the end of this review.

These different approaches are far from providing a complete description of HSP expression during mouse embryogenesis. Moreover, the results obtained by these different approaches are not equivalent, do not have the same degree of resolution and are open to different criticisms. The nomenclature adopted in some studies (in particular in the older ones) for the different HSPs makes the observations difficult to interpret. Moreover, some of the molecular tools (antibodies or probes) used in the different studies were specific for one form of HSPs, whereas others recognized different members of the same family.

However, some observations are common to the different techniques used. As a general rule, the most interesting results have been obtained with the HSPs expressed at highly variable levels in different tissues or with the stress proteins which are 'normally' inducible, but expressed at very low levels in the absence of stress. An increased expression of such proteins 1) is easier to observe and 2) suggests the existence of specific mechanisms regulating their expression. The interest of the different observations on the developmental expression of HSPs also depends on what is presently known about the functions of these proteins. A variation in the level of a general chaperone involved in protein folding is less informative than a variation in the level of a specialized chaperone, such as HSP25 or HSP90, for which specific, physiologically significant functions have recently been proposed (see earlier).

The first observations concern HSP70. In addition to the two cell stage, the inducible gene is spontaneously expressed during embryogenesis, early in the extraembryonic tissues (day 8.5 in the placenta and 11.5 in the yolk sac) and later, after day 15.5, in the embryo itself [60] (see also D. Walsh in this multi-author review). Surprisingly, an initial experiment with an *hsp68-lacZ* hybrid gene was unable to detect any spontaneous activity of β-galactosidase [61]. Our results obtained with a more sensitive reporter gene (*luciferase*) under the control of a *hsp70* promoter (the reporter of the *hsp70.1* gene) do not fully corroborate these early data. A weak reporter gene activity is found in the embryo, peaking at day 11, whereas no activity is present in the yolk sac.

The activity decreases very early in the anterior part of the embryo while it increases progressively in the tail, where it remains high even after birth [32]. We have shown that the protein itself is synthesized at specific locations in the embryo from day 15.5, at a low level in the epidermis and mesenchyme and at a higher level in the endothelium of the blood vessels. It is also expressed during bone formation and in the central nervous system, where antibodies against this protein label very specific structures in the telencephalon, mesencephalon and diencephalon. In vitro studies have revealed the transient expression of this protein during differentiation of human erythroleukemia K562 cells by hemin [62, 63]. This transient expression is a reflection of what occurs in vivo during hematopoiesis [64]. It is possible, although not yet demonstrated, that a similar transient expression takes place in the mouse. In K562 cells, the transient expression of HSP70 is parallel to the transient increase in the level of the HSF2 transcription factor. This increase results from an increase in the level of the corresponding mRNA, but other mechanisms might be also involved. A factor is found constitutively bound to the HSE sequences of the *hsp70* gene promoter during hemin-induced differentiation [62]: the authors have reasonably assumed that this factor is HSF2. These observations were reported before two different forms of HSF2 were characterized [17–19]. Reexamination of the published data [21] suggests that the increase in HSF2 activity might result mainly from a variation in the ratio of the spliced forms of HSF2, leading to an accumulation of the transcriptionally active form.

From these heterogeneous and fragmentary data, it is difficult to deduce a precise role for HSP70 in embryogenesis. It is however possible to establish some firm conclusions: 1) HSP70 is constitutively expressed in some tissues; this result suggests that, although HSP70 and HSC70 are structurally and functionally very similar [65], they may each have some specific properties. Their specificity may lie either in the different partners with which they interact [66] (such as the DnaJ-like proteins [67]) or in the differential recognition of their substrates [68, 69]; 2) the expression of HSP70 is limited to only some steps of embryogenesis. One possibility is that the expression of HSP70 corresponds to some stress situations, for instance to cell death which occurs during embryogenesis. However, crucial experiments to confirm this hypothesis are still lacking.

Similar observations have also been made on the inducible member of the HSP90 family, HSP90α, which is also transiently expressed during differentiation of human erythroleukemia cells by hemin [62]. Its expression is detectable by immunochemistry as soon as day 9.5 of development (our own data). Like HSP90β, HSP90α is expressed ubiquitously. Previous analyses of RNA [70] are in quite good agreement with the protein data: HSP90α mRNA appears relatively late in development (day 10.5), slightly earlier in the extraembryonic tissues, and it remains relatively constant (see however Lee [71] for contrasting results demonstrating higher expression of HSP90α at early stages of embryogenesis).

The data concerning HSP90α therefore remain very scarce: they show that the protein is spontaneously expressed at a low level during early embryogenesis, and its expression is largely parallel to the expression of HSP90β (see later). These results are therefore disappointing. The very interesting observation of Patrick Krone's group demonstrating a coexpression of myoD and HSP90α during normal muscle development in zebrafish [47] (P. H. Krone et al. this multi-author review) cannot, on the basis of currently available information, be extended to the mouse.

The expression of HSP90β is rather ubiquitous. In the adult, the lowest RNA levels are observed in the spleen, lung, intestine and pancreas [70, 71] while the gene is highly expressed in the brain (see earlier).

Our own immunocytochemical studies during mid-embryogenesis confirm the ubiquitous presence and abundance of this protein. However, two tissues exhibit a remarkably high level of labelling. The first is the central nervous system where at day 12.5 or 15.5 the protein is localized to the periphery of the neural tube in derivatives of the marginal zone, an area relatively poor in cell nuclei. In the medulla of the rhombencephalon, antibodies against HSP90 decorate tracks of nervous fibers. The second situation in which HSP90β is highly expressed is bone morphogenesis, either intramembraneous or endochondral. The cells which are the most heavily labelled are the mature hypertrophic chondrocytes (see later).

Expression of HSC70 also appears to vary only slightly between different tissues, both in the adult [3] and in the embryo (our own data). In the adult, the level of expression is particularly low in the skin and glial cells (see earlier). In the embryo, HSC70 is highly expressed during formation of the neural tube. The neural crest cells during their migration are also heavily labelled (day 9.5). At a later stage of development, HSC70 remains highly expressed in the nervous system, being more abundant in the white matter and at the level of the neuronal migratory pathways. It is also expressed in the mesenchyme, epidermis, muscles and viscerae. During bone morphogenesis, a high level of labelling is also observed in the hypertrophic chrondrocytes, even though only a fraction of this cell population is labelled by antibodies directed against HSC70, in contrast to the result obtained with HSP90β and HSP25.

The most complete results obtained so far concern the small HSP/α-crystallin proteins. The variable expression of these proteins in different tissues was described very early, even before their characterization as heat shock proteins, and we now have good and reliable data

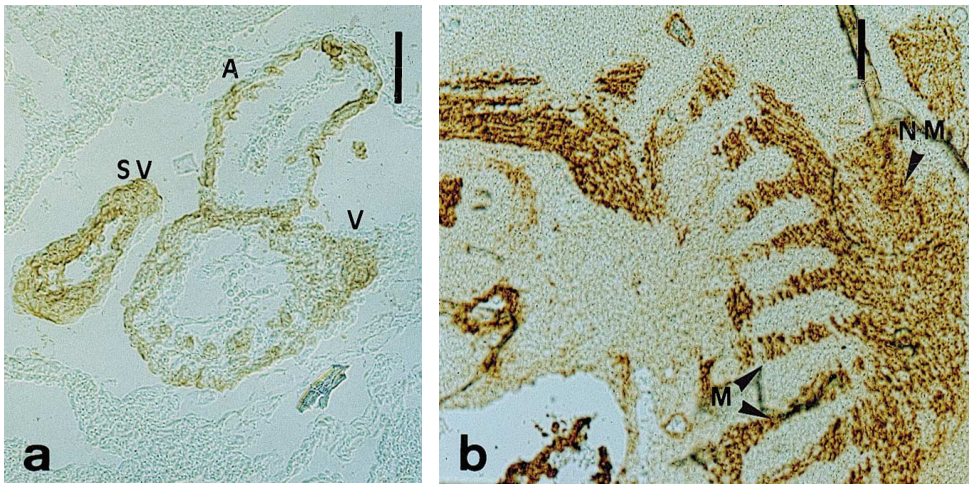

Figure 1. HSP25 is expressed at the onset of the myogenic differentiation. Indirect immunoperoxidase labelling of HSP25 on sagittal sections of day 9.5 (*a*) and day 12.5 (*b*) mouse embryos. HSP25 is localized (*a*) in the myocardium of the cardiac primordium (A: atrium, SV: sinus venosus, V: ventricle, (*b*) in the differentiating myotomes (M) and neck muscles (NM). Scale bar: 100 μm.

on their expression during embryogenesis ([72] and our own data). Moreover, as we shall see in the next section, we have some clues to the mechanisms responsible for their expression during embryogenesis. Therefore, these proteins have three characteristics which make them especially attractive for further studies: a specific pattern of expression, along with some clues to their physiological functions and to the mechanisms regulating their expression.

All three proteins (small HSP itself, αA- and αB-crystallins) share a common structural domain, called the α-crystallin domain, and they are able to form hetero-oligomers [72]. Both small HSP and αB-crystallin are heat-inducible.

The level of expression of small HSP has been shown to vary dramatically in many different in vitro differentiation systems. The variation in the expression level is linked to the nature of the differentiation pathway followed since, in the same cells, the addition of different inducers may or may not stimulate the expression of this protein [73]. A change in the phosphorylation level of this protein is frequently associated with a change in its expression. The phosphorylation of small HSP is due to MAPKAP kinase 2, which is activated by p38, a MAP kinase-like enzyme [74].

Small HSP is highly expressed in muscle during differentiation [59, 72]. We have observed that this expression increases during embryogenesis. At day 9.5, HSP25 is expressed in the mycocardium (fig. 1A). At day 12.5, it begins to be expressed in the muscular masses of the cervical region (fig. 1B). Later, at day 15.5, the labelling of all muscles is very high. Atomi [75] has provided data suggesting that HSP25 expression is controlled by the innervation (and activity) of muscles [75].

Yet small HSP is also expressed in neural tissues, in the spinal ganglia and within the neurons of the central nervous system (although this expression remains limited to a small group of neurons [72]. At day 9.5, the migration pathways of neural crest cells are decorated by antibodies targeted against HSP25 (see also D. Walsh et al. in this multi-author review for a description of HSP25 expression during neural tube closure). At day 12.5, HSP25 is present in the central nervous system, in the thalamus, as well as in different nuclei of the cranial nerves (such as the hypoglossal nucleus). This neural expression of HSP25 persists later in development, where HSP25 is present in some, but not all, structures of the central nervous system. HSP25 is also localized during embryogenesis in the regressing notochord, as well as in the lens [14, 72] (our own results).

Apart from its presence in the lens, in rat αA-crystallin is expressed in non-lenticular tissues such as spleen and thymus [13] αB-crystallin is also abundant in muscle but, most of all, in heart [7, 12], with a low expression in kidney and lung.

Recently, we have also obtained preliminary information on one of the polypeptides forming the cytosolic chaperonin CCT or TRiC (TCP1α). It is expressed in a large spectrum of tissues during mouse embryogenesis, with a pattern of expression reminiscent of that of HSC70.

The general picture of HSP and chaperone expression during mouse embryogenesis is still incomplete. We have not mentioned either the glucose-regulated pro-

teins GRP78 and GRP94, or HSP47 [76, 77], calnexin [78] or calreticulin, which are all localized in the endoplasmic reticulum. So far few data have been obtained on the expression of these proteins during mammalian embryogenesis (see however for HSP47 D. Walsh et al. in this multi-author review). The same is also true for the peptidyl-prolyl [79] and disulphide isomerases, enzymes involved in protein folding.

We have also omitted the minor members of the HSP families, or the as yet less extensively studied HSPs such as ubiquitin [80], for which no information is currently available on their expression during development: TRAP-1 and TRAP-2 which are associated with the tumor necrosis factor (TNF) receptor and which are homologous to HSP90 [81]; another new member of the HSP90 family [82]; HSP110 and HSP105, which are distant members of the HSP70 family [83, 84]; the HSP100/Clp proteins [85]; the HSP70-specific forms which would be present in the immune system [86], or the mortalins [87]. The expression of the specific forms of HSP70, HSP70.2 [88–90] and HSC70t, synthesized during spermatogenesis, is described in another article of this multi-author review (K. D. Sarge and C. Cullen). The case of HSP70.2 is very interesting: transgenesis with hybrid constructions showed that the expression of this protein was controlled at the transcriptional level [91, 92], whereas knock-out of the gene demonstrated its crucial involvement during meiosis in male germ cells [93].

Finally, we will briefly mention what is presently known about changes in the inducibility of the heat shock genes during development or cell differentiation (further information will be found in the articles of D. Walsh et al. and K. D. Sarge et al. in this multi-author review). As we saw previously, in the adult, some cells are heat-uninducible or only weakly inducible. This is the case for neurons. In some cell lines as well, the heat shock response has been described as partially defective. The *hsp70* gene is non-inducible in mouse erythroleukemia cells [94], plasmacytoma, rat hepatoma, and in murine lymphoma, as well as in some EC cell lines. This inducibility can be partially restored by in vitro differentiation in the case of EC cells [2, 33] or by growth in tumor and in situ heating [95]. The group of Benette Phillips has demonstrated that this uninducibility is due to a decreased or abolished access of transcription factors, associated with a CpG methylation [96]. Oocytes at their terminal differentiation stage are also unresponsive to stress [29]. As mentioned previously, the early embryo is fully responsive to stress only at the blastocyst stage. This uninducibility might be due to an 'immaturity' of the system responsible for the activation of HSF1 or to the ageing of the maternal HSF1 factor which has been stored in the oocyte, since the appearance of heat inducibility is parallel to the synthesis of zygotic mHSF1.

At later stages of development, D. Walsh et al. (this multi-author review) clearly demonstrate the variations in the heat shock response occurring during embryonic brain development (see also Mirkes et al. [97, 98] and Fischer et al. in the rat [99]). Despite these studies, the data on HSP inducibility in different embryonic structures are still scarce. In addition to the identification of crucial lesions [100], such information would be very useful in understanding the differential sensitivity of embryonic tissues to hyperthermia and other stresses.

Mechanisms involved in the expression of HSPs and chaperones during the post-implantation phase of embryonic development

The precise description of HSP and chaperone expression during embryogenesis outlined above does not suggest a general mechanism of regulation during embryogenesis but, on the contrary, a model analogous to the one proposed for *Drosophila* where tissue-specific expression during development is due to regulatory sequencies specific for each gene (see Michaud et al., this multi-author review).

Such seems to be the case for the best studied *hsp* genes, *α-crystallins*, where sequences specific for expression in lens, lung and muscle have been described [9–11, 101, 102]. In the lens, the master gene product Pax-6 plays a major and direct role in crystallin expression [103]. The promoter of the mouse small *hsp* gene has been also sequenced [104, 105], but tissue-specific sequencies have not yet been sought. It is true that the control of the expression of small HSPs is atypical since the induction after a stress seems to be partially independent of HSF1 [106]. Characterization of the sequences responsible for the expression of αB-crystallin gene in the heart provides a puzzling result [11]: these sequences contain, but are not limited to, an HSE sequence. The latter result opens the possibility that the tissue-specific expression of αB-crystallin might be partially due to factors binding to this HSE sequence [107].

Therefore HSP and chaperone gene expression during embryogenesis might be dependent on a factor or factors binding to specific heat shock sequences. At the onset of zygotic genome expression, this factor might be simply the maternal factor HSF1 (see E. Christians et al. in this multi-author review). At later stages of development, HSF2 is a good candidate for such a role. In uterus, HSF2 is activated in parallel with estrogen stimulation of HSP90 expression [108]. We have shown that HSF2 is expressed early in development and is very

abundant and active (as a DNA-binding factor) until day 15.5, with a maximum at about day 10.

As discussed previously for the role of this factor in HSP expression at the blastocyst stage, a closer examination of the data casts some doubt on the physiological meaning of the abundance of this factor: 1) during embryogenesis, the most actively synthesized form is that which is least transcriptionally active; 2) the tissues with high HSF2 expression are not those with the highest rate of HSP synthesis. For instance, in the brain, where HSF2 activity persists longer than in other embryo tissues (until day 15.5), HSF2 is found in the ventricular layer of dividing neurons whereas HSC70 and HSP90β, which are also highly expressed in the central nervous system, are present in the marginal zone and concentrate in the tracks of neurofibers; 3) a comparison with the expression of a reporter gene under control of an *hsp70.1* heat shock promoter is also puzzling: it appears as if the decrease in HSF2 concentration is a prerequiste for a high rate of expression of the heat shock genes.

These observations make a case for more 'subtle' functions for HSF2. This factor might be responsible for the onset of heat shock gene expression, for instance by keeping the chromatin open. Conversely, it might have an inhibitory effect, preventing full expression of heat shock genes. Or, simply, it might act on different promoters yet to be identified. As mentioned previously, the precise nature of the promoter sequences to which HSF2 binds and of the genes controlled by this factor are currently unknown.

Future directions

We now have a wealth of data on the expression of some HSPs and chaperones during embryogenesis, and preliminary data on the expression of HSF2. It is obvious that future research has to move from a simple description of the expression of HSPs and HSF2 to a more mechanistic approach to determine the functions of HSPs in the development and the mechanisms regulating their expression. Future studies will have to focus, as a priority on: 1) HSPs for which specific functions have been proposed and 2) differentiation processes in which HSPs seem to be critically involved.

For the first requirement, small HSP/α-crystallins are good candidates. As mentioned previously, they are involved in the control of the cytoskeletal structure [44] and have a specific chaperone function [109, 110] and a protective action against oxidizing agents [111] by participating in the control of the redox state. More generally, HSP25 might have an antiapoptotic role [112]. HSP90α and β also have specific roles in the maturation of some transcription factors [45, 46] and interact with the cytoskeleton [113]. HSP90 might also control translational efficiency by modulating the activity of eIF-2α kinase [48]. HSP90s might contribute in general to the regulation of signalling pathways [114].

HSP90s and small HSP/α-crystallins have many characteristics in common. They are both regulated by steroid hormones [15, 115, 116] (the variation of HSP90 synthesis in murine mammary gland [117] might also be hormonally-controlled). The transcription of small *hsp/α-crystallin* genes is controlled in some tissues such as muscle or heart [11] by HLH proteins, which require HSP90 to be fully functional.

One of the most informative systems might be muscle differentiation, where small HSPs/α-crystallins are transiently expressed at high level [59]. Good systems exist by which the complete pathway of muscle cell differentiation can be followed in vitro. By using anti-sense oligonucleotides, it would be possible to block specifically the expression of HSPs in such systems and to observe the consequences on muscle differentiation. Moreover, embryos affected in genes (*myoD*, *myf5*) essential for muscle differentiation are available. The relationship between small HSP expression and the activity of these master genes involved in muscle formation might be followed in these systems. It might also be possible to test whether the expression of HSPs is negatively correlated with the expression of HSF2, as previously suggested.

Two other differentiation processes in which HSPs might play an important role have been revealed or confirmed by our general study of HSP expression during mouse embryogenesis. HSPs are highly expressed in neural crest cells and during bone morphogenesis. During migration, the neural crest cells are heavily labelled by antibodies targeted against HSC70 and HSP25. The hypertrophic chondocytes which appear during cartilage formation are also heavily labelled by antibodies, revealing a very high expression of HSP90β, HSC70 and CCT. The same cells express a transcription factor (Krox 20) whose deletion is reponsible for anomalies in bone morphogenesis [118]. Since in vitro systems exist in which the formation of this hypertrophic chondrocyte can be followed [119], it is possible to test the role of HSPs in this differentiation process and the relationship between *krox20* and *hsp* gene expression. This expression of HSPs during bone morphogenesis is puzzling, since it was the expression of the stress proteins localized in the endoplasmic reticulum, not of the cytosolic HSPs and chaperones, which was expected in these cells which are heavily engaged in the secretion of proteins involved in the formation of cartilage and of the bone matrix.

We can be certain that in the near future, the study of HSP and chaperone expression during embryogenesis will shift more and more towards a functional approach and make HSPs no longer curiosities, but full players in the developmental process.

Acknowledgements. We are indebted to Ronald Melki (for a gift of TCP1α recombinant protein and specific antibodies) and to Y. Gitton for support and helpful comments. This work was supported by grants from l'Association pour la Recherche sur le Cancer (ARC n° 6505), and from the Ministère de la Recherche (ACC). One of us (M.R.) was a recipient of an AMN from the Ministère de l'Education Nationale.

1 Heikkila J. J. (1993) Heat shock gene expression and development. II. An overview of mammalian and avian developmental systems. Dev. Genet. **14:** 87–91
2 Barnier J. V., Bensaude O., Morange M. and Babinet C. (1987) Mouse 89 kD heat shock protein. Exp. Cell Research **170:** 186–194
3 Tanguay R. M., Wu Y. and Khandjian E. W. (1993) Tissue-specific expression of heat shock proteins of the mouse in the absence of stress. Dev. Genet. **14:** 112–118
4 Murashov A. K. and Wolgemuth D. J. (1996) Distinct transcripts are recognized by sense and antisense riboprobes for a member of the murine HSP70 gene family, HSP70.2, in various reproductive tissues. Mol. Reprod. Dev. **43:** 17–24
5 Murashov A. and Wolgemuth D. J. (1996) Sense and antisense transcripts of the developmentally regulated murine *hsp70.2* gene are expressed in distinct and only partially overlapping areas in the adult brain. Mol. Brain Res. **37:** 85–95
6 Bhat S. P. and Nagineni C. N. (1989) αB-subunit of lens-specific α-crystallin is present in other ocular and non-ocular tissues. Biochem. Biophys. Res. Comm. **158:** 319–325
7 Dubin R. A., Wawrousek E. F. and Piatigorsky J. (1989) Expression of the murine αB-crystallin gene is not restricted to the lens. Mol. Cell. Biol. **9:** 1083–1091
8 Frederikse P. H., Dublin R. A., Haynes II J. I. and Piatigorsky J. (1994) Structure and alternate tissue-preferred transcription initiation of the mouse αB-crystallin/small heat shock protein gene. Nucleic Acid Res. **22:** 5686–5694
9 Gopal-Srivastava R. and Piatigorsky J. (1993) The murine αB-crystallin/small heat shock protein enhancer: identification of αBE-1, αBE-2, αBE-3 and MRF control elements. Mol. Cell. Biol. **13:** 7144–7152
10 Gopal-Srivastava R. and Piatigorsky J. (1994) Identification of a lens-specific regulatory region (LSR) of the murine αB-crystallin gene. Nucleic Acids Res. **22:** 1281–1286
11 Gopal-Srivastava R., Haynes J. I. II and Piatigorsky J. (1995) Regulation of the murine αB-crystallin/small heat shock protein gene in cardiac muscle. Mol. Cell. Biol. **15:** 7081–7090
12 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
13 Kato K., Shinohara H., Kurobe N., Goto S., Inaguma Y. and Ohshima K. (1991) Immunoreactive αA crystallin in rat non-lenticular tissues detected with a sensitive immunoassay method. Biochim. Biophys. Acta **1080:** 173–180
14 Klemenz R., Andres A.-C., Fröhli E., Schafer R. and Aoyama A. (1993) Expression of the murine small heat shock proteins hsp25 and αB crystallin in the absence of stress. J. Cell Biol. **120:** 639–645
15 Ciocca D. R., Adams D. J., Edwards D. P., Biercke R. J. and McGuire W. L. (1983) Distribution of an estrogen-induced protein with a molecular weight of 24 000 in normal and malignant human tissues and cells. Cancer Res. **43:** 1204–1210
16 Sarge K. D., Zimarino V., Holm K., Wu C. and Morimoto R. I. (1991) Cloning and characterization of two mouse heat shock factors with distinct inducible and constitutive DNA-binding ability. Genes Dev. **5:** 1902–1911
17 Fiorenza M. T., Farkas T., Dissing M., Kolding D. and Zimarino V. (1995) Complex expression of murine heat shock transcription factors. Nucleic Acids Res. **23:** 467–474
18 Goodson M. L. and Sarge K. D. (1995) Regulated expression of heat shock factor 1 isoforms with distinct leucine zipper arrays via tissue-dependent alternative splicing. Biochem. Biophys. Res. Comm. **211:** 943–949
19 Goodson M. L., Park-Sarge O.-K. and Sarge K. D. (1995) Tissue-dependent expression of heat shock factor 2 isoforms with distinct transcriptional activities. Mol. Cell. Biol. **15:** 5288–5293
20 Sarge K. D., Murphy S. P. and Morimoto R. I. (1993) Activation of heat shock gene transcription by heat shock factor 1 involves oligomerization, acquisition of DNA-binding activity, and nuclear localization and can occur in the absence of stress. Mol. Cell. Biol. **13:** 1392–1407
21 Sistonen L., Sarge K. D. and Morimoto R. I. (1994) Human heat shock factors 1 and 2 are differentially activated and can synergistically induce hsp70 gene transcription. Mol. Cell. Biol. **14:** 2087–2099
22 Nakai A. and Morimoto R. I. (1993) Characterization of a novel chicken heat shock transcription factor, heat shock factor 3, suggests a new regulatory pathway. Mol. Cell. Biol. **13:** 1983–1997
23 Nakai A., Kawazoe Y., Tanabe M., Nagata K. and Morimoto R. I. (1995) The DNA-binding properties of two heat shock factors, HSF1 and HSF3, are induced in the avian erythroblast cell line HD6. Mol. Cell. Biol. **15:** 5268–5278
24 Kroeger P. E., Sarge K. D. and Morimoto R. I. (1993) Mouse heat shock transcription factors 1 and 2 prefer a trimeric binding site but interact differently with the HSP70 heat shock element. Mol. Cell. Biol. **13:** 3370–3383
25 Kroeger P. E. and Morimoto R. I. (1994) Selection of new HSF1 and HSF2 DNA-binding sites reveals difference in trimer cooperativity. Mol. Cell. Biol. **14:** 7592–7603
26 Xiao H., Perisic O. and Lis J. T. (1991) Cooperative binding of Drosophila heat shock factor to arrays of a conserved 5 bp unit. Cell **64:** 585–593
27 Wang Y. and Morgan W. D. (1994) Cooperative interaction of human HSF1 heat shock transcription factor with promoter DNA. Nucleic Acids Res. **22:** 3113–3118
28 Sarge K. D., Park-Sarge O.-K., Kirby J. D., Mayo K. E. and Morimoto R. I. (1994) Expression of heat shock factor 2 in mouse testis: potential role as a regulator of heat-shock protein gene expression during spermatogenesis. Biology of Reproduction **50:** 1334–1343
29 Curci A., Bevilacqua A., Fiorenza M. T. and Mangia F. (1991) Developmental regulation of heat-shock response in mouse oogenesis: identification of differentially responsive oocyte classes during graafian follicle development. Dev. Biol. **144:** 362–368
30 Kurtz S., Rossi J., Petko L. and Lindquist S. (1986) An ancient developmental induction: heat-shock proteins induced in sporulation and oogenesis. Science **231:** 1154–1157
31 Christians E., Campion E., Thompson E. M. and Renard J.-P. (1995) Expression of the HSP70.1 gene, a landmark of early zygogic activity in the mouse embryo, is restricted to the first burst of transcription. Development **121:** 113–122
32 Thompson E. M., Christians E., Stinnakre M. G. and Renard J. P. (1994) Scaffold attachment regions stimulate HSP70.1 expression in mouse preimplantation embryos but not in differentiated tissues. Mol. Cell. Biol. **14:** 4694–4703
33 Mezger V., Legagneux V., Babinet C., Morange M. and Bensaude O. (1991) Heat shock proteins synthesis in preimplantation mouse embryo and embryonal carcinoma cells. In: Heat Shock and Development, pp. 153–166, Hightower L. and Nover L. (eds), Results and Problems in Cell Differentiation, Vol. 17, Springer-Verlag, Berlin, Heidelberg
34 Morange M., Diu A., Bensaude O. and Babinet C. (1984) Altered expression of heat shock proteins in embryonal carcinoma and mouse early embryonic cells. Mol. Cell. Biol. **4:** 730–735
35 Bensaude O. and Morange M. (1983) Spontaneous high expression of heat shock proteins in mouse embryonal carcinoma cells and ectoderm from day 8 mouse embryo. EMBO J. **2:** 173–177
36 Legagneux V., Mezger V., Quélard C., Barnier J.-V., Bensaude O. and Morange M. (1996) High constitutive transcription of HSP86 gene in murine embryonal carcinoma cells. Differentiation **41:** 42–48

37 Giebel L. B., Dworniczak B. P. and Bautz E. K. F. (1988) Developmental regulation of a constitutively expressed mouse mRNA encoding a 72-kDa heat shock-like protein. Dev. Biol. **125:** 200–207

38 Murphy S. P., Gorzowski J. J., Sarge K. D. and Phillips B. (1994) Characterization of constitutive HSF2 DNA-binding activity in mouse embryonal carcinoma cells. Mol. Cell. Biol. **14:** 5309–5317

39 Levine R. A., La Rosa F. J. and Gudas L. J. (1984) Isolation of cDNA clones for genes exhibiting reduced expression after differentiation of murine teratocarcinoma stem cells. Mol. Cell. Biol. **4:** 2142–2150

40 Mezger V., Bensaude O. and Morange M. (1989) Unusual levels of HSE-binding activity in embryonal carcinoma cells. Mol. Cell. Biol. **9:** 3888–3896

41 Mezger V., Renard J.-P., Christians E. and Morange M. (1994) Detection of heat shock element-binding activities by gel shift assay during mouse preimplantation development. Dev. Biol **165:** 627–638

42 Mezger V., Rallu M., Morimoto R. I., Morange M. and Renard J.-P. (1994) Heat shock factor 2-like activity in mouse blastocysts. Dev. Biol. **166:** 819–822

43 Kubota H., Hynes G., Carne A., Ashworth A. and Willison K. (1994) Identification six *Tcp-1*-related genes encoding divergent subunits of the TCP-1-containing chaperonin. Current Biology **4:** 89–99

44 Arrigo A.-P. and Landry J. (1994) Expression and function of the low molecular weight heat shock proteins. In: Heat Shock Proteins: Structure, Function and Regulation, Morimoto R., Tissières A. and Georgopoulos C. (eds), pp. 335–373 Cold Spring Harbor Laboratory Press, Cold Spring Habor, NY

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

46 Shue G. and Kohtz D. S. (1994) Structural and functional aspects of basic helix-loop-helix protein folding by heat-shock protein 90. J. Biol. Chem. **269:** 2707–2711

47 Sass J. B., Weinberg E. S. and Krone P. H. (1996) Specific localization of zebrafish *hsp90α* mRNA to myoD-expressing cells suggests a role for hsp90α during normal muscle development. Mech. Dev. **54:** 195–204

48 Rose D. W., Welch W. J., Kramer G. and Hardesty B. (1989) Possible involvement of the 90-kDa heat shock protein in the regulation of protein synthesis. J. Biol. Chem. **264:** 6239–6244

49 Lanks K. W. (1986) Modulators of the Eukaryotic heat shock response. Exp. Cell Res. **165:** 1–10

50 Souren J. E. M., Van Schoot M. C. C. and Van Wijk R. (1995) HSP synthesis of neonatal rat heart myocytes is regulated by a collagen environment. Int. J. Hyperthermia **11:** 257–266

51 Brown I. R. (1994) Induction of heat shock genes in the mammalian brain by hyperthermia and tissue injury. In: Heat Shock Proteins in the Nervous System, pp. 32–54, Mayer J. and Brown I. (eds), Academic Press, London

52 Itoh H., Tashima Y., Eishi Y. and Okeda R. (1993) Localization of HSP90 in rat brain. Int. J. Biochem. **25:** 93–99

53 Izumoto S. and Herbert J. (1993) Widespread constitutive expression of hsp90 messenger RNA in rat brain. J. Neurosci. Res. **35:** 20–28

54 Abravaya K., Myers M. P., Murphy S. P. and Morimoto R. I. (1992) The human heat shock protein hsp70 interacts with HSF, the transcription factor that regulates heat shock gene expression. Genes Dev. **6:** 1153–1164

55 Mosser D. D., Duchaine J. and Massie B. (1993) The DNA-binding activity of the human heat shock transcription factor is regulated in vivo by Hsp70. Mol. Cell. Biol. **13:** 5427–5438

56 Rabindran S. K., Wisniewski J., Li L., Li G. and Wu C. (1994) Interaction between heat shock factor and hsp70 is insufficient to suppress induction of DNA-binding activity in vivo. Mol. Cell. Biol. **14:** 6552–6560

57 Nowak T. S. Jr., 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, London

58 Tytell M. (1994) Heat shock proteins in the retina and optic nerve. In: Heat Shock Proteins in the Nervous System, pp. 83–100, Mayer J. and Brown I. (eds), Academic Press, London

59 Atkinson B. G. (1981) Synthesis of heat shock proteins by cells undergoing myogenesis. J. Cell. Biol. **89:** 666–673

60 Kothary R., Perry M. D., Moran L. A. and Rossant J. (1987) Cell-lineage specific expression of the mouse hsp68 gene during embryogenesis. Dev. Biol. **121:** 342–348

61 Kothary R., Clapoff S., Darling S., Perry M. D., Moran L. A. and Rossant J. (1989) Inducible expression of an *hsp68-lacZ* hybrid gene in transgenic mice. Development **105:** 707–714

62 Sistonen L., Sarge K. D., Phillips B., Abravaya K. and Morimoto R. I. (1992) Activation of heat shock factor 2 during hermin-induced differentiation of human erythroleukemia cells. Mol. Cell. Biol. **12:** 4104–4111

63 Theodorakis N. G., Zand D. J., Kotzbauer P. T., Williams G. T. and Morimoto R. I. (1989) Hemin-induced transcriptional activation of the *HSP70* gene during erythroid maturation in K562 cells is due to a heat shock factor-mediated stress response. Mol. Cell. Biol. **9:** 3166–3173

64 Sing M. K. and Yu J. (1984) Accumulation of a heat shock-like protein during differentiation of human erythroid cell line K562. Nature **309:** 631–633

65 Brown C. R., Martin R. L., Hansen W. J., Beckmann R. P. and Welch W. J. (1993) The constitutive and stress inducible forms of hsp70 exhibit functional similarities and interact with one another in an ATP-dependent fashion. J. Cell Biol. **120:** 1101–1112

66 Rassow J., Voos W. and Pfanner N. (1995) Partner proteins determine multiple functions of Hsp70. Trends Cell Biol. **5:** 207–212

67 Cyr D. M., Langer T. and Douglas M. G. (1994) DnaJ-like proteins: molecular chaperones and specific regulators of Hsp70. TIBS **19:** 176–181

68 Fourie A. M., Sambrook J. F. and Gething M.-J. H. (1994) Common and divergent peptide binding specificities of hsp70 molecular chaperones. J. Biol. Chem. **269:** 30470–30478

69 Gragerov A. and Gottesman M. E. (1994) Different peptide binding specificies of hsp70 family members. J. Mol. Biol. **241:** 133–135

70 Gruppi C. M., Zakeri Z. F. and Wolgemuth D. J. (1991) Stage and lineage-regulated expression of two HSP90 transcripts during mouse germ cell differentiation and embryogenesis. Mol. Reprod. Dev. **28:** 209–217

71 Lee S.-J. (1990) Expression of *HSP86* in male germ cells. Mol. Cell. Biol. **10:** 3239–3242

72 Gernold M., Knauf U., Gaestel M., Stahl J. and Kloetzel P.-M. (1993) Development and tissue-specific distribution of mouse small heat shock protein hsp25. Dev. Genet. **14:** 103–111

73 Minowada G. and Welch W. (1995) Variation in the expression and/or phosphorylation of the human low molecular weight stress protein during in vitro cell differentiation. J. Biol. Chem. **270:** 7047–7054

74 Rouse J., Cohen P., Trigon S., Morange M., Alonso-Llamazares A., Zamanillo D., Hunt T. et al. (1994) A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP Kinase-2 and phosphorylation of the small heat shock proteins. Cell **78:** 1027–1037

75 Atomi Y., Yamada S. and Nishida T. (1991) Early changes of αB-crystallin mRNA in rat skeletal muscle to mechanical tension and denervation. Biochem. Biophys. Res. Comm. **181:** 1323–1330

76 Ferreira L. R., Norris K., Smith T., Hebert C. and Sauk J. J. (1994) Association of Hsp47, Grp78 and Grp94 with procollagen supports the successive or coupled action of molecular chaperones. J. Cell. Biochem. **56:** 518–526

77 Nagata K. (1996) Hsp47: a collagen specific molecular chaperone. TIBS **21:** 23–26

78 Hammond C., Braakman I. and Helenius A. (1994) Role of N-linked oligosaccharide recognition, glucose trimming, and

calnexin in glycoprotein folding and quality control. Proc. Natl. Acad. Sci. USA **91:** 913–917
79 Schmid F. X. (1995) Prolyl isomerases join the fold. Current Biol. **5:** 993–994
80 Bond U. and Schlesinger M. J. (1985) Ubiquitin is a heat shock protein in chicken embryo fibroblasts. Mol. Cell Biol. **5:** 949–956
81 Song H. Y., Dunbar J. D., Zhang Y. X., Guo D. and Donner D. B. (1995) Identification of a protein with homology to hsp90 that binds the type 1 tumor necrosis factor receptor. J. Biol. Chem. **270:** 3574–3581
82 Chen C.-F., Chen Y., Dai K., Chen P.-L., Riley D. J. and Lee W.-H. (1996) A new member of the hsp90 family of molecular chaperones interacts with the retinoblastoma protein during mitosis and after heat shock. Mol. Cell. Biol. **16:** 4691–4699
83 Lee-Yoon D., Easton D., Murawski M., Burd R. and Subjeck J. R. (1995) Identification of a major subfamily of large Hsp70-like proteins through the cloning of the mammalian 110-kDa heat shock protein. J. Biol. Chem. **270:** 15725–15733
84 Yasuda K., Nakai A., Hatayama T. and Nagata K. (1995) Cloning and expression of murine high molecular mass heat shock proteins, HSP105. J. Biol. Chem. **270:** 29718–29723
85 Schirmer E. C., Glover J. R., Singer M. A. and Lindquist S. (1996) HSP 100/Clp proteins: a common mechanism explains diverse functions. Trends Biochem. Sci. **21:** 289–296
86 DeNagel D. C. and Pierce S. K. (1993) Antigen processing in B lymphocytes: a role for stress proteins. Crit. Rev. Immunol. **13:** 1–11
87 Wadhwa R., Kaul S. C., Sugimoto Y. and Mitsui Y. (1993) Induction of cellular senescence by transfection of cytosolic mortalin cDNA in NIH 3T3 cells. J. Biol. Chem. **268:** 22239–22242
88 Allen R. L., O'Brien D. A. and Eddy E. M. (1988) A novel hsp70-like protein (p70) is present in mouse spermatogenic cells. Mol. Cell. Biol. **8:** 828–832
89 Allen R. L., O'Brien D. A., Jones C. C., Rockett D. L. and Eddy E. M. (1988) Expression of heat shock proteins by isolated mouse spermatogenic cells. Mol. Cell. Biol. **8:** 3260–3266
90 Zakeri Z. F., Wolgemuth D. J. and Hunt C. R. (1988) Identification and sequence analysis of a new member of the mouse *HSP70* gene family and characterization of its unique cellular and developmental pattern of expression in the male germ line. Mol. Cell. Biol. **8:** 2925–2932
91 Dix D. J., Rosario-Herrle M., Gotoh H., Mori C., Goulding E. H., Barrett C. V. et al. (1996) Developmentally regulated expression of HSP70-2 and a HSP70-2/lacZ transgene during spermatogenesis. Dev. Biol. **174:** 310–321
92 Wisniewski J., Malezewski M., Krawczyk Z. and Gedamu L. (1993) An upstream region of the rat spermatogenesis-specific heat shock-like Hst70 gene confers testis-specific expression in transgenic mice. Eur. J. Biochem. **212:** 137–143
93 Dix D. J., Allen J. W., Collin B. W., Mori C., Nakamura N., Poorman-Allen P. 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
94 Hensold J. O., Hunt C. R., Calderwood S. K., Housman D. E. and Kingston R. E. (1990) DNA binding of heat shock factor to the heat shock element is insufficient for transcriptional activation in murine erythroleukemia cells. Mol. Cell. Biol. **10:** 1600–1608
95 Davidson S., Hoj P., Gabriele T. and Anderson R. L. (1995) In vivo growth of a murine lymphoma cell line alters regulation of expression of HSP72. Mol. Cell. Biol. **15:** 1071–1078
96 Gorzowski J. J., Eckerley C. A., Halgren R. G., Mangurten A. B. and Phillips B. (1995) Methylation-associated transcriptional silencing of the major histocompatibility complex-linked hsp70 genes in mouse cell lines. J. Biol. Chem. **270:** 26940–26949
97 Mirkes P. E., Grace R. H. and Little S. A. (1991) Developmental regulation of heat shock protein synthesis and hsp70 RNA accumulation during postimplantation rat embryogenesis. Teratology **44:** 77–89
98 Mirkes P. E. and Doggett B. (1992) Accumulation of heat shock protein 72 (hsp72) in postimplantation rat embryos after exposure to various periods of hyperthermia (40°–43° C) in vitro: evidence that heat shock protein 72 is a biomarker of heat-induced embryotoxicity. Teratology **46:** 301–309
99 Fisher B. R., Heredia D. J. and Brown K. M. (1995) Induction of hsp72 in heat-treated rat embryos: a tissue-specific response. Teratology **52:** 90–100
100 Welte M. A., Duncan I. and Lindquist S. (1995) The basis for a heat-induced developmental defect: defining crucial lesions. Genes Dev. **9:** 2240–2250
101 Cvekl A., Kashanchi F., Sax C. M., Brady J. N. and Piatigorsky J. (1995) Transcriptional regulation of the mouse αA--crystallin gene: activation dependent on a cyclic AMP-responsive element (DE1/CRE) and a Pax-6-binding site. Mol. Cell. Biol. **15:** 653–660
102 Haynes J. I. II, Gopal-Srivastava R., Frederikse P. H. and Piatigorsky J. (1995) Differential use of the regulatory elements of the αB-crystallin enhancer in cultured murine lung (MLg), lens (αTN4-1) and muscle (C2C12) cells. Gene **155:** 151–158
103 Cvekl A. and Piatigorsky J. (1996) Lens development and crystallin gene expression: many roles for Pax-6. Bioessays **18:** 621–630
104 Fröhli E., Aoyama A. and Klemenz R. (1993) Cloning of the mouse *hsp25* gene and an extremely conserved *hsp25* pseudogene. Gene *128*: 273–277
105 Gaestel M., Gotthardt R. and Müller T. (1993) Structure and organisation of a murine gene encoding small heat-shock protein Hsp25. Gene **128:** 279–283
106 Johnston R. N. and Kucey B. L. (1988) Competitive inhibition of *hsp70* gene expression causes thermosensitivity. Science **242:** 1551–1554
107 Sax C. M. and Piatigorsky J. (1994) Expression of α-crystallin/small heat shock protein/molecular chaperone genes in the lens and other tissues. Adv. Enzymol. Relat. Areas Mol. Biol. **69:** 155–201
108 Yang X., Dale E. C., Diaz J. and Shyamala G. (1995) Estrogen dependent expression of heat shock transcription factor: implications for uterine synthesis of heat shock proteins. J. Steroid Biochem. Molec. Biol. **52:** 415–419
109 Horwitz J. (1992) α-crystallin can function as a molecular chaperone. Proc. Natl. Acad. Sci. USA **89:** 10449–10453
110 Nicholl I. D. and Quinlan R. A. (1994) Chaperone activity of α-crystallins modulates intermediate filament assembly. EMBO J. **13:** 945–953
111 Mehlen P., Kretz-Remy C., Préville X. and Arrigo A.-P. (1996) Human hsp27, *Drosophila* hsp27 and human αB-crystallin expression-mediated increase in glutathione is essential for the protective activity of these proteins against TNFα-induced cell death. EMBO J. **15:** 2695–2706
112 Mehlen P., Schulze-Osthoff K. and Arrigo A.-P. (1996) Small stress proteins as novel regulators of apoptosis. J. Biol. Chem. **271:** 16510–16514
113 Koyasu S., Nishida E., Kadowaki T., Matsuzaki F., Iida K., Harada F., Kasuga M., Sakai H. and Yahara I. (1986) Two mammalian heat shock proteins, HSP90 and HSP100, are actin-binding proteins. Proc. Natl. Acad. Sci. USA **83:** 8054–8058
114 Rutherford, S. L. and Zuker C. S. (1994) Protein folding and the regulation of signaling pathways. Cell **79:** 1129–1132
115 Olazabal U. E., Pfaff D. W. and Mobbs C. V. (1992) Estrogenic regulation of heat shock protein 90 kDa in the rat ventromedial hypothalamus and uterus. Mol. Cell. Endocrin. **84:** 175–183
116 Shyamala G., Gauthier Y., Moore S. K., Catelli M. G. and Ullrich S. J. (1989) Estrogenic regulation of murine uterine 90-Kilodalton heat shock protein gene expression. Mol. Cell. Biol. **9:** 3567–3570
117 Catelli M. G., Ramachandran C., Gauthier Y., Legagneux V., Quelard C., Baulieu E. E. et al. (1989) Developmental regulation of murine mammary-gland 90 kDa heat-shock proteins. Biochem. J. **258:** 895–901

118 Levi G., Topilko P., Schneider-Maunoury S., Lasagna M., Mantero S., Cancedda R. et al. (1996) Defective bone formation in Krox-20 mutant mice. Development **122:** 113–120
119 Poliard A., Nifuji A., Lamblin D., Plee E., Forest C. and Kellermann O. (1995) Controlled conversion of an immortalized mesodermal progenitor cell towards osteogenic, chondrogenic, or adipogenic pathways. J. Cell Biol. **130:** 1461–1472

.