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Heat shock protein gene expression during *Xenopus* development

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Abstract. Stress-induced heat shock protein gene expression is developmentally regulated during early embryogenesis of the frog, *Xenopus laevis*. For example, a number of heat shock protein genes, such as *hsp70*, *hsp90*, and *ubiquitin* are not heat-inducible until after the midblastula stage of embryogenesis. Furthermore, the family of small heat shock protein genes, *hsp30*, are differentially expressed after the midblastula stage as well as being regulated at the level of mRNA stability. Many of these stress proteins are also synthesized constitutively during oogenesis and embryogenesis during which they may act as molecular chaperones as well as being involved in sequestering proteins in an inactive state until required by the developing embryo. Furthermore the induction of these stress protein genes has been correlated with enhanced thermoresistance. During stressful conditions heat shock proteins probably prevent aggregation or misfolding of damaged proteins within the embryo.
Key words. *Xenopus*; heat shock; stress; heat shock protein; development; gene regulation.

Introduction

Heat shock-induced activation of the expression of a set of heat shock protein (*hsp*) genes giving rise to the accumulation of hsp mRNA and the synthesis of hsps has been described in essentially all prokaryotic and eukaryotic cells examined [1–3]. Furthermore, some members of the *hsp* families are expressed normally within the cell and appear to function as molecular chaperones and are involved in protein folding, assembly and transport. During cellular stress hsps may bind to and inhibit irreversible aggregation or misfolding of denatured or damaged proteins. Heat-induced activation of *hsp* gene expression is mediated by the interaction of the heat shock element (HSE) found in the 5′ upstream region of these genes with a transcriptional activating protein known as heat shock factor (HSF). HSF pre-exists within the cell as an inactive monomer which then forms an active trimer upon heat shock permitting it to bind to the HSE and facilitate *hsp* gene expression.

While a great deal of information is known about the heat shock response in somatic cells, less is known about the mechanisms involved in the developmental regulation of these genes and whether they play an active role during normal development. The following review will examine the current information available on *hsp* gene expression during *Xenopus* development. Our laboratory and others have chosen to work with *Xenopus* embryos since the eggs are easily obtainable in large quantities and can be fertilized in vitro. Also, the large size of the oocytes and eggs makes them suitable for microinjection studies [4]. Finally, a large amount of information is available regarding *Xenopus* embryonic development at both the cellular and molecular level.

Hsp genes

In 1984, Bienz [5, 6] isolated the first set of *hsp* genes from *Xenopus laevis* namely four members of the *hsp70* family (*hsp70A*, *B*, *C*, and *D*). As found previously in *Drosophila* none of the genes contained introns. DNA sequence analysis of the *hsp70A* gene revealed that it had a 74% identity with *Drosophila hsp70*. This gene also contained a heat shock element, HSE, in the 5′ regulatory region which was necessary for heat-inducibility [7]. Recently, the cDNA for the constitutively synthesized heat shock cognate 70, *hsc70.l*, has been isolated and sequenced [8]. Interestingly, *hsc70.l* has 80% similarity with *hsp70* and only 58% if one compares the carboxyl ends. In contrast, *hsc70.l* has over 92% similarity with rat, mouse and bovine *hsc70*. Furthermore, the ATP-binding domain is also conserved between rat *hsc71* and *Xenopus hsc70.l*. In the context of vertebrate evolution it is likely that the divergence of *hsp70* from *hsc70* genes occurred long before the emergence of amphibians [8]. The conservation of the *hsp70* linkage group in the major histocompatibility complex from amphibians to mammals also supports this possibility [9].

Both *hsp70* and *hsc70.l* contain a nuclear localization signal which has been reported in a number of different organisms [10–12]. This sequence appears to be functional since immunolocalization studies have documented the transolocation of both *Xenopus* hsp70 and hsc70 into the nucleus [13]. The carboxyl terminal sequence EEVD, which appears to be necessary for several essential chaperonin functions such as intramolecular coupling of ATP and substrate binding activities in

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mammalian cells [14], is also present in both *Xenopus* hsp70 and hsc70.l. The finding that members of the *Xenopus* hsp70 family contain this EEVD sequence suggests that they may also have a similar mode of chaperonin activity.

Bienz [5, 6] was also responsible for the isolation of the first pair of *Xenopus* small hsp genes, *hsp30A* and *hsp30B*. However, DNA sequences analysis determined that *hsp30A* had an insertional mutation in the coding region while *hsp30B* was a pseudogene. Nevertheless, these genes were invaluable in the isolation of additional *hsp30* genes, *hsp30C*, *hsp30D*, and *hsp30E* by Krone et al. [15]. As found for hsp70, none of the small *hsp* genes contained introns. The *hsp30C* sequence had a 97% identity with *hsp30A* but only 75% with *hsp30D*. While *hsp30A* encodes a 10 kDa protein due to its insertional mutation, *hsp30C* and *hsp30D* encode 24 kDA proteins. The upstream regulatory regions of the *hsp30* genes contain multiple HSEs. The 3′ flanking region is of interest since it contains a mRNA instability sequence which has been described for a number of other eukaryotic genes such as *c-myc* and *c-fos* [16] as well as sequence which is involved in the activation of polyadenylation during *Xenopus* oocyte maturation [17].

Recently, a PCR-amplified genomic *Xenopus* DNA fragment corresponding to the 5′ portion of an *hsp90* gene has been isolated [18]. Analysis of the *Xenopus* hsp90 amino acid sequence revealed a strong identity with *hsp90β* genes from zebrafish (94%) and human (95%) and a slightly lower degree of identity with *hsp90α* genes (90–91%) from the same organisms. Therefore, the hsp90 gene fragment is likely a part of *Xenopus hsp90β* gene. In other systems, the *hsp90β* gene is expressed more strongly under normal physiological temperatures than the *hsp90α* [19, 20]. Finally, a cDNA clone encoding ubiquitin has been isolated from *Xenopus* [21]. In yeast and chicken it has been shown that some *ubiquitin* genes are heat-inducible and that they have an HSE in their 5′ promoter regions [22, 23].

Stress protein gene expression during *Xenopus* oogenesis

A number of studies have found both hsp70 and its mRNA are present constitutively during *Xenopus* oogenesis [5, 24–28]. For example, the relative levels of *hsp70A* and *hsp70B* transcripts were present at maximal levels in stage 3 oocytes and were maintained at constant levels through oocyte maturation, fertilization and early cleavage stages of embryogenesis [27]. Also later stage oocytes had relatively higher concentrations of hsp70 in mitochondria and the nucleus relative to the cytoplasm as determined by immunocytochemical and biochemical analyses [13]. In support of this finding, it has been shown that two hsp70-related proteins can recycle across the nuclear envelope in *Xenopus* oocytes [11]. *Hsp90* gene expression appears to be active during oogenesis since constitutive levels of hsp90 have been detected in oocytes while *hsp90* mRNA was present in unfertilized eggs [18, 28]. Finally, it should be mentioned that hsp30 and its mRNA have not been detected in oocytes or unfertilized eggs indicating that this family of genes is not expressed constitutively during *Xenopus* oogenesis [5, 6, 29–31].

In 1982, Bienz [24] found that exposure of *Xenopus* oocytes to an elevated temperature not only inhibited protein synthesis but also resulted in an increase in the synthesis of hsp70. Also, this finding was made in other laboratories studying hsp synthesis in *Xenopus* oocytes [24, 32, 33]. Since it was reported that the enhanced level of hsp70 production was found with both unucleated or α-amantin-injected oocytes, it appeared that the heat shock response was controlled at the translational level [24]. However, subsequent studies were unable to detect the heat shock-induced synthesis of hsp70 in oocytes and provided evidence that the earlier results may have been the result of follicular cell contamination of the oocytes [27, 34]. While the latter studies examined the effect of heat shock on *Xenopus* oocytes by analyzing either equivalent embryos or protein samples by electrophoresis, another study examined the pattern of protein synthesis in heat shocked oocytes and body cavity eggs (which are devoid of follicular cell contamination) on the basis of equivalent acid-insoluble radioactivity [25]. Since protein synthesis was extensively inhibited during heat shock in this study their detection of hsp70 synthesis may not represent a classical case of induction over control levels but rather a change in the pattern of residual protein synthesis.

While heat shock may not activate endogeneous *hsp* gene expression in *Xenopus* oocytes, a number of studies have found that heat shock factor, HSF, is present and functional. For example, a microinjected *Drosophila hsp70* gene as well as *Xenopus hsp30* gene were heat-inducible in *Xenopus* oocytes [6, 35]. In contrast, microinjection of the *Xenopus hsp70* gene resulted in constitutive expression but no heat-inducibility [6, 7]. In a re-examination of this problem, it was found that the microinjected *hsp70* gene required sufficient time for chromatin assembly before it was heat-inducible in oocytes [36, 37]. In this latter study, deletion of the CCAAT boxes inhibited promoter activity. This last study indicates that the *Xenopus* oocyte has the capacity to mount a heat shock response. Hopefully, future experiments will determine the reason for the inability to detect heat shock-induced expresssion of the endogenous oocyte *hsp70* genes.

Stress protein gene expression during early embryogenesis

A number of hsps as well as their mRNAs (*e.g. hsp90*, *hsp70* and *hsc70*) are detectable throughout early

Xenopus embryogenesis [18, 26–29, 38, 39]. The hsps and *hsp* mRNAs present in cleavage stage embryos are of maternal origin since the *Xenopus* genome is not activated until the midblastula stage of development. After the midblastula stage the constitutive levels of some of these mRNAs such as *hsc70* mRNA increase presumably due to new transcription [18]. The midblastula stage or midblastula transition (MBT) is a key point during *Xenopus* embryogenesis which is associated with an increase in the duration of the cell cycle, loss of synchronous cell division and a reduction in the rate of DNA synthesis [40, 41]. Also, at this stage the transcription of a select set of genes is activated or becomes heat-inducible. The list of *hsp* genes that are first heat-inducible after the MBT include *hsp70*, *hsp90* and *ubiquitin* [5, 6, 18, 25–27, 29, 39, 42–44) (table 1). The expression of *hsp* genes in post-MBT embryos can also be induced by other stressors such as sodium arsenite or ethanol [39]. A number of other hsps which are heat-inducible after MBT have been reported including hsp62, hsp57, hsp43 and hsp35 [43, 45, 46]. Interestingly, hsp62 and hsp35 have been identified as glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase, respectively [45, 46]. Developmental stage-dependent induction of heat-induced *hsp70* gene expression has also been observed in a number of other animal embryonic systems such as *Drosophila*, sea urchin, mouse and rabbit [47, 48]. In all these organisms the heat-inducibility of *hsp70* gene expression occurs at a comparable stage to the *Xenopus* MBT and in some cases, such as in mouse and rabbit embryos, *hsp* gene induction occurs well after the onset of zygotic genome activation. It is likely that this timing with respect to hsp gene inducibility has been conserved throughout evolution.

The expression of a related member of the hsp70 family, namely, glucose-regulated protein 78 (grp78) which is also called immunoglobulin heavy chain binding protein (BiP) has been examined in *Xenopus* embryos [49]. Grp78 and another glucose-regulated protein, grp98, are abundant within the endoplasmic reticulum and act as chaperones and function in the folding and assembly of new proteins [20, 50]. In *Xenopus* tissue culture cells as well as in mammalian cells *grp78* genes are expressed constitutively but can be induced by inhibitors of glycosylatin, sulfhydryl-reducing compounds and conditions of glucose starvation [50–52]. In *Xenopus* embryos, *grp78* and *grp78* mRNA are detectable throughout early development including the cleavage stages but their levels are not enhanced by tunicamycin until after the MBT [49]. Furthermore, *grp78* genes may be differentially expressed during *Xenopus* development. Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) studies have detected a total of 3 putative grp78 isoforms of which one was observed in both embryo and adult cells while another was adult-specific and the third was embryo-specific [49].

Table 1. Stress-induced accumulation of hsp transcripts during early *Xenopus* development[a]

	Developmental stages						
mRNA	Cleav	MB	Gast	Neur	Early TB	Mid-TB	TP
PTB hsp30	−	+	+	+	?	?	?
hsp30A	−	−	−	−	+	+	+
hsp30C	−	−	−	−	+	+	+
hsp30D	−	−	−	−	−	+	+
hsp30E	?	?	?	?	?	?	?
hsp70[b]	−	+	+	+	+	+	+
hsc70[b]	−	−	−	−	−	−	−
hsp90[b]	−	+	+	+	+	+	+
ubiquitin[b]	−	+	+	+	+	+	+
grp78[b]	−	−	+	+	+	+	+

[a]All embryos were heat shocked except in studies with grp78 in which embryos were treated with tunicamycin.
[b]These mRNAs were detected constitutively throughout development.
Cleav = cleavage; MB = midblastula; Neur = neurula; TB = tailbud; TP = tadpole; PTB = pre-tailbud.

Regulation of heat-inducible *HSP* gene expression at the MBT

An important issue that has been addressed concerns the mechanism(s) associated with heat-inducible expression of *hsp* genes after MBT. One approach to this problem involved the analysis of the expression of microinjected *hsp70* genes in *Xenopus* embryos [30, 36]. For example, it was reported that a microinjected *Xenopus hsp70*/*chloramphenicol acetyl transferase* (*CAT*) gene construct was not heat-inducible until after the MBT as found with the endogenous *hsp70* gene [30]. These results indicated that the transcription factors required for heat-inducible expression of the *hsp70* gene such as HSF were present in post-MBT embryos.

In order to examine the presence of HSF in *Xenopus* embryos, Ovsenek and Heikkila [53] used an oligonucleotide corresponding to the proximal HSE of the *Xenopus hsp70B* gene in a series of DNA mobility shift experiments. Interestingly, heat shock-induced HSF binding activity was detected at all stages of *Xenopus* development including cleavage stage embryos which are transcriptionally inactive and are unable to mount a heat shock response. The finding of HSF activity in unfertilized eggs and cleavage stage embryos indicate that this transcription factor is maternal in origin. The properties of HSF in pre- and post-MBT embryos were compared and found to be similar [53, 54]. For example, the kinetics of HSF activation and deactivation as well as the size of HSF (approximately 88 kDa) as determined by photoaffinity labeling were similar in both cleavage and neurula stage embryos. These studies suggest that the lack of a heat shock response in *Xenopus* cleavage stage embryos is not due to the absence of activatable HSF binding. In fact pre-MBT embryos contain not only RNA polymerase II but also a number of

other transcription factors including those binding to GC, ATF/AP-1, CCAAT and serum responsive elements [40, 41, 55, 56]. It has been proposed that the rapid cell cycle associated with pre-MBT embryos prevents RNA transcription and that the lengthening of the cell cycle at MBT allows the onset of transcription [40, 41, 57].

Recently, Stump et al. [58] have isolated a cDNA encoding the *Xenopus laevis* heat shock factor, XHSF1. Analysis of the coding region revealed that the 451 amino acid protein was of comparable size to a number of other vertebrate HSF proteins and that there was similarity in the putative DNA-binding and trimerization domains. Furthermore, DNA mobility shift analysis and DNase I footprinting studies determined that the *Xenopus* HSF synthesized in vitro specifically bound to the HSE. It was also found that HSF made in *Xenopus oocytes* from microinjected XHSF1 mRNA accumulated in the nucleus and promoted transcription of a subsequently injected *Xenopus hsp70/CAT* construct [37, 58]. Future studies examining the regulation of *hsp* gene expression during *Xenopus* development will undoubtedly be assisted by the availability of the *Xenopus* HSF cDNA.

Finally, the constitutive and tunicamycin-inducible expression of the *grp78* gene in *Xenopus* embryos has also been examined [59, 60]. A microinjected rat *grp78* gene was expressed constitutively after the MBT as well as being tunicamycin-inducible. Furthermore a series of experiments using a variety of promoter deletion mutants indicated that the regulatory elements associated with the *Xenopus* and mammalian *grp78* promoters may be conserved.

Hsp30 gene expression during *Xenopus* development

The isolation of the genes for the different members of the *hsp30* family permitted a detailed analysis of their expression during *Xenopus* development [5, 6, 15, 29–31, 38, 61] (table 1). In a preliminary study by Bienz [5], it was found that *hsp30A* was not constitutively expressed during early embryogenesis and that it was not heat-inducible until the tadpole stage. Subsequent experiments, employing RNase protection analysis and reverse transcription-polymerase chain reaction (RT-PCR) assays were able to narrow down the developmental stage at which *hsp30A* and *hsp30C* genes were first heat-inducible to the early tailbud [15, 30, 38]. Interestingly, an analysis of the expression of *hsp30D* gene expression determined that it was first heat-inducible at the mid-tailbud stage which is approximately one day later in development than found for *hsp30A* and *hsp30C* [61]. In contrast to *hsp70* mRNA, constitutive levels of the *hsp30* mRNAs were not detected at any early developmental stage (1 cell to tadpole).

Recently, a new group of heat-inducible *hsp30* mRNAs have been detected in late blastula, gastrula and neurula stage *Xenopus* embryos [61]. These *hsp30* transcripts originally escaped our attention due to the fact that they are present in very low amounts in heat shocked embryos (approximately 50–100-fold less than heat shocked tailbud embryos). Furthermore, these transcripts were not detected with specific probes designed for *hsp30A, C* and *D* transcripts in RNase protection and RT-PCR assays. While the exact identity of these pre-tailbud (PTB) *hsp30* transcripts will have to await the cloning of their cDNAs, their sizes are comparable to the known *hsp30* mRNAs. The *PTB hsp30* mRNAs appear to be unstable transcripts since treatment of late blastula and gastrula embryos with cycloheximide greatly enhanced their levels after heat shock. This conclusion was based on the finding in a number of systems including *Xenopus* embryos that cycloheximide stabilizes unstable mRNAs [62, 63]. For example, treatment of *Xenopus* with cycloheximide has been shown to stabilize *Eg2* mRNA [64]. It has been suggested that inhibition of protein synthesis by cycloheximide may reduce the levels of a factor(s) involved in mRNA degradation [62, 63]. It is possible that the *PTB hsp30* mRNAs may contain a mRNA instability element in the 3′ untranslated region as has been shown for *hsp30C* mRNA [15]. These experiments suggest that the expression of the *Xenopus* PTB *hsp30* genes are regulated at the level of mRNA stability. Chicken *hsp23* gene expression also appears to be regulated at the level of mRNA stability [65]. Furthermore, the uniqueness of the *Xenopus PTB hsp30* mRNAs is highlighted by the fact that while other hsp mRNAs are unstable at control temperatures but stable at heat shock temperatures, the *PTB hsp30* mRNAs are unstable during heat shock as well. Future studies examining the 3′ end of these mRNAs, once their cDNAs are cloned, may aid in the understanding of this phenomenon.

The heat-induced expression of the *PTB hsp30* genes after MBT followed by *hsp30A* and *hsp30C* at early tailbud and finally *hsp30D* at mid-tailbud indicate that this gene family is differentially regulated during development. It is likely that the mechanism responsible for zygote gene activation at MBT may be responsible for the heat inducibility of the *PTB hsp30* genes. However, the mechanism(s) involved in the developmental regulation of the *hsp30A, hsp30C* and *hsp30D* genes in response to heat shock is not known. It is possible that site-specific methylation of key regulatory regions of the promoters of the *hsp30* genes may inhibit their expression in MBT embyros as found with a number of eukaryotic genes [66]. This possibility is supported by the presence of potential methylation sites in the 5′-flanking region of the *hsp30C* gene [15, 61]. However, methylation may not be involved since a preliminary study has shown that the treatment of *Xenopus* embryos

with 5-azacytidine failed to prematurely induce the expression of the *hsp30D* gene (N. Ohan and J. J. Heikkila, unpublished data).

It is also possible that alterations in chromatin structure may be involved in the sequential activation of the *Xenopus hsp30* genes since in *Caenorhabditis elegans*, the expression of a set of small hsps has been correlated with differences in chromatin structure [67]. Also, *globin* genes, which are found in clusters and sequentially expressed like the *Xenopus hsp30* genes, are regulated at the level of chromatin conformation by a globin locus control region [68]. The regulation of *hsp30* gene expression by chromatin structure would explain previous studies in which microinjected *hsp30A/CAT* and *hsp30C/CAT* constructs were heat-inducible, but prematurely expressed at the midblastula stage [30, 38]. In these latter experiments, it is possible that the microinjected DNA constructs were not assembled into the proper chromatin conformation and thus were not correctly regulated during development.

While protein synthetic studies were heat shocked *Xenopus* kidney epithelial cells revealed up to 16 small hsps, the identification of the *hsp30* gene products remained unknown [69]. Recently, the development of an antibody prepared against a 15 amino acid peptide corresponding to the caroxyl end of the putative hsp30C sequence has permitted the characterization of the hsp30 family by 2-D PAGE immunoblot analysis [31]. Neither constitutive nor heat-inducible synthesis of hsp30 peptides were detected in cleavage, blastula, gastrula or neurula stage embryos. The first detectable heat-inducible accumulation of an hsp30 protein was detected in early tailbud stage embryos. Five additional heat-inducible hsp30 polypeptides were found in late tailbud embryos while a total of 13 small hsps were observed at the early tadpole stage. Approximately 8 members of the hsp30 family were detected in *Xenopus* A6 kidney epithelial cells. A comparison of the early tadpole and A6 cells hsp30 pattern revealed a number of common and unique proteins. Given the large number of small hsps in *Xenopus* tadpoles it is possible that some of the spots on the 2-D PAGE gels may be the result of post-translational modifications such as phosphorylation. This is a modification which has been observed in small hsps in other organisms [70]. While post-translational modification may be involved it is possible that multiple genes are also involved since seven different *hsp30* genes and cDNAs have been described [5, 6, 15]. Multiple small hsps are not unique to *Xenopus* since they have been detected in a number of other organisms such as *Drosophila*, desert fish and *Caenorhabditis elegans* [71–73].

Theoretically the *Xenopus* hsp30 peptide antibody should detect a number of hsp30 proteins having a similar carboxyl end to hsp30C. Of the genes that have been sequenced this antibody should detect hsp30C, hsp30D as well as the protein product of an *hsp30* cDNA, *X4* [6, 15]. Identification of the hsp30C protein was achieved by means of microinjection and immunoblot technology [31]. Basically a chimeric *hsp30C* gene, under the control of a constitutive promoter was microinjected into fertilized eggs and allowed to develop to the gastrula stage. Since the gastrula stage does not synthesize hsp30 even under heat shock conditions, the *hsp30* product should be detectable. In fact, 2-D PAGE immunoblot analysis permitted the identification of the hsp30 gene product in gastrula embryos. A series of protein mixing experiments were able to pinpoint the first heat-inducible synthesis of the hsp30C protein at the early tailbud stage which is consistent with the earlier studies examining heat-inducible *hsp30* mRNA accumulation [38]. In the future, this novel approach to the identification of the *hsp30* gene product may permit the determination of the *hsp30D* gene product.

The previously mentioned possibility that the *hsp30* genes are differentially regulated during *Xenopus* development is supported by the above study. In other developmental systems such as *Drosophila*, some of the small *hsp* genes are expressed constitutively and display a very complex temporal and spatial pattern of gene expression [70, 74, 75]. To date, the constitutive expression of small *hsp* genes has not been detected during early *Xenopus* development (from egg to tadpole). However, in a recent study by Helbing et al. [76] constitutive expression of an *hsp30* gene has been detected in the liver tissue of *Rana catesbeiana* tadpoles undergoing metamorphosis. Thus, it is possible that a similar situation may exist in *Xenopus* tadpoles.

Stress protein function during *Xenopus* development

A number of studies have shown that members of the *Xenopus hsp* family are expressed constitutively. Additionally, immunocytochemical analysis has detected an increased concentration of hsp70 in the nucleus and perinuclear region of involuted cells of the marginal zone in gastrula embryos suggesting that there are differences in the pattern of synthesis of these proteins during development [13]. While chaperonin activity studies of isolated *Xenopus* hsps have not been carried out it is likely that these stress proteins function as chaperones during development. Stressful conditions in *Xenopus* embryos may induce hsps to bind and prevent irreversible aggregation or misfolding of damaged or denatured proteins. Numerous studies have shown that hsp synthesis was correlated with the acquisition of thermotolerance [1–3]. Interestingly, during *Xenopus* embryogenesis the acquisition of heat-inducible *hsp* gene expression at the midblastula stage coincides with enhanced thermoresistance [42, 43]. The presence of maternal HSF in pre-MBT embryos may be advantageous for the *Xenopus* embryo given the possible role of

hsps in stress resistance since the embryos would acquire the ability to transcribe HSP mRNA at the earliest possible time after the MBT. Additionally, the marked elevation in *hsc70* gene expression after the MBT may also contribute to the thermoresistance of the embryo [8]. It is possible that exposure of embryos to heat shock could lead initially to the interaction of hsc70 with denatured and misfolded proteins prior to an increase in effective hsp70 levels. A protective role for hsc70 during thermal stress has been suggested since the microinjection of bovine hsc70 into *Xenopus oocytes* reduced the response of a co-injected heat shock reporter plasmid [77].

Other hsps may also be involved in allowing the embryo to cope with a stressful situation. As mentioned previously, two of the hsps synthesized in response to heat shock were glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase [45, 46]. In these studies, it was suggested that heat shock might place an energy demand on *Xenopus* embryos which may be alleviated in part by increasing the levels of specific glycolytic enzymes. Furthermore, *ubiquitin* gene expression which is also enhanced during heat shock in *Xenopus* embryos may aid in the ATP-dependent proteolysis of abnormal or extensively denatured protein [78]. Finally, while hsp30 synthesis is not heat-inducible until the tailbud stage of development, these small hsps may also act as chaperones and participate in stress resistance since it has been shown that overexpression of a small *hsp* gene in mammalian cells can confer thermotolerance [70].

Constitutive hsps may be involved in sequestering proteins during early *Xenopus* development. This possibility is based on the recent finding in which co-immunoprecipitation experiments using a monoclonal anti-centrin antibody detected a complex consisting not only of centrin but also hsp70 and hsp90 in cytostatic factor-arrested *Xenopus* oocytes [28]. Upon oocyte activation centrin was released from the cytoplasmic complex presumably due to an increase in the levels of cytoplasmic free calcium. In this study, it was suggested that this phenomenon involving complex formation with hsp70 and hsp90 may be a general mechanism by which oocyte protein is stored in an inactive state until required by the embryo.

Future directions

The relatively recent availability of a variety of *Xenopus* hsp genes or cDNA as well as the HSF cDNA will enable researchers to address a number of issues relating to the regulation and function of stress proteins during *Xenopus* development. Future studies should examine the spatial distribution of constitutively synthesized stress proteins in *Xenopus*. Also, experiments designed to overproduce or inhibit the expression of stress proteins in *Xenopus* embryos may allow the determination of whether they have developmentally-related functions during development.

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1 Morimoto R. I., Tissieres A. and Georgopoulos C. (1994) The Biology of Heat Shock Proteins and Molecular Chaperones. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, NY
2 Nover L. (1991) The Heat Shock Response. CRC Press, Florida
3 Parsell D. A. and Lindquist S. (1993) The function of heat shock proteins in stress tolerance: degradation and reactivation of the damaged proteins. Annu. Rev. Genet. **27:** 437–496
4 Heikkila J. J. (1990) Expression of cloned genes and translation of messenger RNA in microinjected *Xenopus* oocytes. Int. J. Biochem. **22:** 1223–1228
5 Bienz M. (1984) Developmental control of the heat shock response in *Xenopus*. Proc. Natl. Acad. Sci. **81:** 3138–3142
6 Bienz M. (1984) *Xenopus* hsp70 genes are constitutively expressed in injected oocytes. EMBO J. **3:** 2477–2483
7 Bienz M. (1986) A CCAAT box confers cell-type-specific regulation on the *Xenopus* hsp70 gene in oocytes. Cell **46:** 1037–1042
8 Ali A., Salter-Cid L., Flajnik M. and Heikkila J. J. (1996) Isolation and characterization of a cDNA encoding a *Xenopus* 70 kDa heat shock cognate protein, *Hsc70.l*. Comp. Biochem. Physiol. (in press)
9 Salter-Cid L., Kasahara M. and Flajnik M. F. (1994) *Hsp70* genes are linked to the *Xenopus* major histocompatibility complex. Immunogenetics **39:** 1–7
10 Dang C. V. and Lee W. M. F. (1989) Nuclear and nucleolar targeting sequences of c-*erb-A*, *c-myb*, *N-myc*, p53, HSP70, and HIV tat proteins. J. Biol Chem. **264:** 18019–18030
11 Mandell R. B. and Feldherr C. M. (1992) The effect of carboxyl-terminal deletions on the nuclear transport rate of rat hsc70. Exptl. Cell Res. **198:** 164–169
12 Rensing S. A. and Maier U. G. (1994) Phylogenetic analysis of the stress-70 protein family. J. Molec. Evol. **39:** 80–86
13 Herberts C., Moreau N. and Angelier N. (1993) Immunolocalization of hsp70-related proteins constitutively expressed during *Xenopus laevis* oogenesis and development. Int. J. Dev. Biol. **37:** 397–406
14 Freeman B. C., Myers M. P., Schumacher R. and Morimoto R. I. (1995) Identification of a regulatory motif in Hsp70 that affects ATPase activity, substrate binding and interaction with HDJ-1. EMBO J. **14:** 2281–2292
15 Krone P. H., Snow A., Ali A., Pasternak J. J. and Heikkila J. J. (1992) Comparison of the regulatory regions of the *Xenopus laevis* small heat-shock protein encoding gene family. Gene **110:** 159–166
16 Brawerman G. (1987) Determinants of messenger RNA stability. Cell **48:** 5–6
17 McGrew L. L., Dworkin-Rastl E., Dworkin M. and Richter J. D. (1989) Poly (A) elongation during *Xenopus* oocyte maturation is required for translational recruitment and is mediated by a short sequence element. Genes Dev. **3:** 803–815
18 Ali A., Krone P. H., Pearson D. S. and Heikkila J. J. (1996) Evaluation of stress-inducible hsp90 gene expression as a potential molecular biomarker in *Xenopus laevis*. Cell Stress and Chaperones **1:** 62–69
19 Barnier J. V., Bensaude O., Morange M. and Babinet C. (1987) Mouse 89 kDa heat shock protein: two polypeptides with distinct developmental regulation. Exp. Cell Res. **170:** 186–194
20 Legagneux V., Mezger V., Quelard C., Barnier J. V., Bensaude O. and Morange M. (1989) High constitutive transcription of *hsp86* gene in murine carcinoma cells. Differentiation **41:** 42–48

21 Dworkin-Rastl E., Shrutkowski A. and Dworkin M. B. (1984) Multiple ubiquitin mRNAs during *Xenopus laevis* development contain tandem repeats of 76 amino acid coding sequence. Cell **39:** 321–325

22 Bond U. and Schlesinger M. J. (1985) Ubiquitin is a heat shock protein in chicken embryo fibroblasts. Mol. Cell Biol. **5:** 949–956

23 Ozkaynak E., Finley D., Solomon M. J. and Varshavsky A. (1987) The yeast *ubiquitin* genes: a family of natural gene fusions. EMBO J. **6:** 1429–1439

24 Bienz M. and Gurdon J. B. (1982) The heat shock response in *Xenopus* oocytes is controlled at the translational level. Cell **29:** 811–819

25 Browder L. W., Pollock M., Heikkila J. J., Wilkes J., Wang T., Krone P. et al. (1987) Decay of the oocyte-type heat shock response of *Xenopus laevis*. Dev. Biol. **124:** 191–199

26 Davis R. E. and King M. L. (1989) The developmental expression of the heat-shock response in *Xenopus laevis*. Development **105:** 213–222

27 Horrell A., Shuttleworth J. and Colman A. (1987) Transcript levels and translational control of hsp70 synthesis in *Xenopus* oocytes. Genes Dev. **1:** 433–444

28 Uzawa M., Grams J., Madden B., Toft D. and Salisbury J. L. (1995) Identification of a complex between heat shock proteins in CSF-arrested *Xenopus* oocytes and dissociation of the complex following oocyte activation. Dev. Biol. **171:** 51–59

29 Krone P. H. and Heikkila J. J. (1988) Analysis of *hsp30* and *hsp70*, and *ubiquitin* gene expression in *Xenopus laevis* tadpoles. Development **103:** 59–67

30 Krone P. H. and Heikkila J. J. (1989) Expression of microinjected *hsp70*/*CAT* and *hsp30*/*CAT* chimeric genes in developing *Xenopus laevis* embryos. Development **110:** 217–281

31 Tam Y. and Heikkila J. J. (1995) Identification of members of the hsp30 small heat shock protein family in *Xenopus laevis* embryos. Dev. Genet **17:** 331–339

32 Baltus E. and Hanocq-Quertier J. (1985) Heat shock response in *Xenopus* oocytes during meiotic maturation and activation. Cell Differentiation **16:** 161–168

33 Rojas C. and Allede J. E. (1983) Amphibian oocytes respond to heat shock after induction of meiotic maturation by hormones. Biochem. Intl. **6:** 517–525

34 King M. L. and Davis R. E. (1987) Do *Xenopus* oocytes have a heat shock response? Dev. Biol. **119:** 532–539

35 Voellmy R. and Runggeer D. (1982) Transcription of a *Drosophila* heat shock gene is heat-induced in *Xenopus* oocytes. Proc. Natl. Acad. Sci. USA **79:** 1776–1780

36 Landsberger N., Ranjan M., Almouzni G., Stump D. and Wolfe A. P. (1995) The heat shock response in *Xenopus* oocytes, embryos and somatic cells: a regulatory role for chromatin. Dev. Biol **170:** 62–74

37 Landsberger N. and Wolffe A. P. (1995) Role of chromatin and *Xenopus laevis* heat shock transcription factor in regulation of transcription from the *X. laevis hsp70* promoter in vivo. Mol. Cell. Biol. **15:** 6013–6024

38 Ali A., Krone P. and Heikkila J. J. (1993) Expression of endogenous and microinjected *hsp30* genes in early *Xenopus laevis* embryos. Dev. Genet. **14:** 42–50

39 Heikkila J. J., Ovsenek N. and Krone P. H. (1987) Examination of heat shock protein mRNA accumulation in early *Xenopus laevis* embryos. Biochem. Cell Biol **65:** 87–94

40 Newport J. and Kirschner M. (1982) A major developmental transition in early *Xenopus* embryos: I. Characterization and timing of cellular changes at the midblastula stage. Cell **30:** 675–686

45 Marsden M., Nickells R. W., Kapoor M. and Browder L. W. (1993) The induction of pyruvate kinase synthesis by heat shock in *Xenopus laevis* embryos. Dev. Genet. **14:** 51–57

41 Newport J. and Kirschner M. (1982) A major developmental transition in early *Xenopus* embryos: II. Control of the onset of transcription. Cell **30:** 687–696

42 Heikkila J. J., Kloc M., Bury J., Schultz G. A. and Browder L. W. (1985) Acquisition of the heat shock response and thermotolerance during early development of *Xenopus laevis*. Dev. Biol. **107:** 483–489

43 Nickells R. W. and Browder L. W. (1985) Region-specific heat-shock protein synthesis correlates with a biphasic acquisition of thermotolerance in *Xenopus laevis* embryos. Dev. Biol. **112:** 391–395

44 Ovsenek N. and Heikkila J. J. (1990) Heat shock induced accumulation of *ubiquitin* mRNA in *Xenopus laevis* is developmentally regulated. Dev. Biol. **12:** 582–585

45 Marsden M., Nickells R. W., Kapoor M. and Browder L. W. (1993) The induction of pyruvate kinase synthesis by heat shock in *Xenopus laevis* embryos. Dev. Genet. **14:** 51–57

46 Nickells R. W. and Browder L. W. (1988) A role for glyceraldehyde-3-phosphate dehydrogenase in the development of thermotolerance in *Xenopus laevis* embryos. J. Cell. Biol. **107:** 1901–1909

47 Heikkila J. J. (1993) Heat shock gene expression and development. l. An overview of fungal, plant, and poikilothermic animal developmental systems. Dev. Genet. **14:** 1–5

48 Heikkila J. J. (1993) Heat shock gene expression and development. II. An overview of mammalian and avian developmental systems. Dev. Genet **14:** 87–91

49 Winning R. S., Bols N. C. and Heikkila J. J. (1991) Tunicamycin-inducible polypeptide synthesis during *Xenopus laevis* embryogenesis. Differentiation **46:** 167–172

50 Gething M. J., Blond-Egluindi S., Mori K. and Sambrook J. F. (1994) Structure, function, and regulation of the endoplasmic reticulum chaperone, BiP. In: The Biology of Heat Shock Proteins and Molecular Chaperones, pp. 111–135, R. I. Morimoto Tissieres A. and Georgopoulos C. (eds), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

51 Lee A. S. (1992) Mammalian stress response: Induction of the glucose-regulated protein. Curr. Opin. Cell Biol. **4:** 267–273

52 Winning R. S., Heikkila J. J. and Bols N. C. (1989) Induction of glucose-regulated proteins in *Xenopus laevis* A6 cells. J. Cell. Physiol. **140:** 239–245

53 Ovsenek N. and Heikkila J. J. (1990) DNA sequence-specific binding activity of the heat-shock transcription factor is heat inducible before the midblastula transition of early *Xenopus* development. Development **110:** 427–433

54 Karn H., Ovsenek N. and Heikkila, J. J. (1992) Properties of heat shock transcription factor in *Xenopus* embryos. Biochem. Cell Biol. **70:** 1006–1013

55 Mohun T. J., Garret N. and Gurdon J. (1989) Temporal and tissue-specific expression of the proto-oncogene *c-fos* during development in *Xenopus laevis*. Development **107:** 835–846

56 Ovsenek N., Williams G. T., Morimoto R. I. and Heikkila J. J. (1990) *Cis*-acting sequences and *trans*-acting factors required for constitutive expression of a microininjected *HSP70* gene after the midblastula transition of *Xenopus laevis* embryogenesis. Dev. Genet. **11:** 97–109

57 Kimelman D., Kirschner M. and Scherson T. (1987) The events of the midblastula transition in *Xenopus* are regulated by changes in the cell cycle. Cell **48:** 399–407

58 Stump D. G., Landberger N. and Wolffe A. P. (1995) The cDNA encoding *Xenopus laevis* heat-shock factor 1 (XHSF1): nucleotide and deduced amino-acid sequences, and properties of the encoded protein. Gene **160:** 207–211

59 Vezina C., Wooden S. K., Lee A. S. and Heikkila J. J. Constitutive expression of a microinjected *glucose-regulated protein* (*grp78*) fusion gene during early *Xenopus laevis* development. Differentiation **57:** 171–177

60 Winning R. S., Bols N. C., Wooden S. K., Lee A. S. and Heikkila J. J. (1992) Analysis of the expression of a *glucose-regulated protein* (*GRP78*) promoter/*CAT* fusion gene during early *Xenopus laevis* development. Differentiation **49:** 1–6

61 Ohan N. W. and Heikkila J. J. (1995) Involvement of differential gene expression and messenger RNA stability in the developmental regulation of the *Hsp30* gene family in heat shocked *Xenopus laevis* embryos. Dev. Genet. **17:** 176–184

62 Nanbu R., Menoud P-A., and Nagamine Y. (1994) Multiple instability-regulating sites in the 3′ untranslated region of the urokinase-type plasminogen activator mRNA. Mol. Cell. Biol. **14:** 4920–4928

63 Sheu J-J., Jan S-P., Lee H-T. and Yu S-M. (1994) Control of transcription and mRNA turnover as mechanisms of

metabolic repression of alpha-amylase gene expression. Plant J. **5:** 655–664
64 Duval C., Bouvet P., Omilli F., Roghi C., Dorel C., LeGuellec R., Paris J. and Osborne H. B. (1990) Stability of maternal mRNA in *Xenopus* embryos: role of transcription and translation. Mol. Cell. Biol. **10:** 4123–4129
65 Edington B. V. and Hightower L. E. (1990) Induction of a chicken small heat shock (stress) protein: evidence of multilevel posttranscriptional regulation. Mol. Cell. Biol. **10:** 4886–4898
66 Adams R. (1990) DNA methylation, the effect of minor bases on DNA-protein interactions. Biochem. J. **265:** 309–320
67 Dixon D. K., Jones D., and Candido E. P. (1990) The differentially expressed 16-kD heat shock genes of *Caenorhabditis elegans* exhibit differential changes in chromatin structure during heat shock. DNA Cell Biol. **9:** 177–191
68 Lowrey C. H., Bodine D. M. and Nienhuis A. W. (1992) Mechanisms of DNase I hypersensitive site formation within the human globin locus control region. Proc. Natl. Acad. Sci. USA **89:** 1143–1147
69 Darasch S., Mosser D. D., Bols N. C. and Heikkila J. J. (198) Heat shock gene expression in *Xenopus laevis* A6 cells in response to heat shock and sodium arsenite treatments. Biochem. Cell Biol. **66:** 862–868
70 Arrigo A-P. and Landry J. (1994) Expression and function of the low-molecular-weight heat shock proteins. In: The Biology of Heat Shock Proteins and Molecular Chaperones, pp. 335–373, Morimoto R. I., Tissieres A. and Georgopoulos C. (eds), Cold Spring Harbor Laboratory Press. New York.
71 Hockertz M. K., Clark-Lewis I. and Candido E. P. M. (1991) Studies of the small heat shock proteins of *Caenorhabditis elegans* using anti-peptide antibodies. FEBS Lett. **280:** 375–378
72 Ingolia T. D. and Craig E. A. (1982) Four small *Drosophila* heat shock proteins are related to each other and to mammalian α-crystallin. Proc. Natl. Acad. Sci. USA **79:** 2360–2364
73 White C. N., Hightower L. E. and Schultz R. J. (1994) Variation in heat-shock proteins among species of desert fishes (*Poeciliidae*, *Poecilipsis*). Mol. Biol. Evol. **11:** 106–119
74 Cheney C. M. and Shearn A. (1989) Developmental regulation of *Drosophila* imaginal disc proteins: Synthesis of a heat shock protein under non-heat-shock conditions. Dev. Biol. **95:** 325–330
75 Marin R., Valet J. P. and Tanguay R. M. (1993) Hsp23 and hsp26 exhibit distinct spatial and temporal patterns of constitutive expression in *Drosophila* adults. Dev. gen. **14:** 69–77
76 Helbing C., Gallimore C. and Atkinson B. G. (1996) Characterization of a *Rana catesbeiana* hsp30 gene and its expression in the liver of this amphibian during both spontaneous and thyroid hormone-induced metamorphosis. Dev. Genet. **18:** 223–233
77 Mifflin L. C. and Cohen R. E. (1994) Hsc70 moderates the heat shock (stress) in response in *Xenopus laevis* oocytes and binds to denatured protein inducers. J. Biol. Chem. **269:** 15718–15723
78 Ciechanover A., Finley D. and Varshavski A. (1984) The ubiquitin mediated proteolytic pathway and mechanisms of energy-dependent intracellular protein degradation. J. Cell Biochem. **24:** 27–53

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