Cell Stress & Chaperones (1996) 1 (1), 62–69
© Pearson Professional Ltd 1996

Evaluation of stress-inducible *hsp90* gene expression as a potential molecular biomarker in *Xenopus laevis*

Adnan Ali[1], Patrick H. Krone[2], Derek S. Pearson[2], John J. Heikkila[1]

[1]Department of Biology, University of Waterloo, Waterloo, Ontario, N2L 3G1, Canada.
[2]Department of Anatomy and Cell Biology, University of Saskatachewan, Health Sciences Building, 107 Wiggins Rd, Saskatoon S7N 5E5, Canada.

Abstract In this study we have evaluated stress-inducible *hsp90* mRNA accumulation as a potential molecular biomarker in *Xenopus laevis*. In order to obtain a probe for Northern blot analysis we employed a PCR-based approach using degenerate primers for the amplification and cloning of an *hsp90* gene sequence from *Xenopus laevis*. The deduced amino acid sequence is 102 amino acids in length and exhibited the highest degree of identity with zebrafish and human *hsp90β* genes. Furthermore, the putative intron and exon boundaries of this fragment are the same as *hsp90β* in chicken, mouse and human, indicating that the fragment represents a *Xenopus hsp90β*-like gene. Northern blot analyses revealed that this gene was constitutively expressed in cultured A6 cells. While heat shock and sodium arsenite exposure resulted in the increased accumulation of *hsp90* mRNA in A6 cells, treatment with cadmium chloride and zinc chloride did not. Also, exposure of A6 cells to concurrent heat shock and sodium arsenite produced a mild synergistic response with respect to *hsp90* mRNA levels in contrast to *hsp70* mRNA levels which displayed a strong synergistic effect. Finally, *hsp90* mRNA was detected constitutively throughout early embryogenesis but was heat-inducible only in late blastula and later stages of development. Given the normal abundance and limited stress-induced accumulation of *hsp90* mRNA, it may not have a great deal of potential as a molecular biomarker compared to *hsp70* and *hsp30* mRNA. However, it may be useful in conjunction with other stress protein mRNAs to establish a set of biomarker profiles to characterize the cellular response to a stressful or toxic agent.

INTRODUCTION

Heat shock proteins (Hsps) have been proposed as molecular biomarkers for toxicity associated with physical and chemical stressors (Sanders 1993; Ryan and Hightower 1994; Ovelgonne et al 1995) since the expression of their genes can by activated by a wide variety of agents including heat shock, heavy metals and arsenates (reviewed by Nover and Scharf 1991; Parsell and Lindquist 1993; Moriomoto et al 1994). Also, a number of studies have shown a relationship between the extent of *hsp* gene expression and the amount of damage inflicted on cells (Anderson et al 1987; Pipkin et al 1987; Hightower and Renfro 1988; Aoki et al 1990; Bournias-Vardiabasis et al 1990; Deaton et al 1990; Edwards et al 1990; Goering et al 1992). Since aquatic organisms such as fish are particularly susceptible to environmental toxicants these animals, as well as fish cell cultures, have been used in standardized toxicity tests (Bols et al 1985; Powers 1989). Recently *hsp* gene expression has been used as an assay to evaluate chemical and heavy metal ion toxicity in fish cells (Sanders 1993; Ryan and Hightower 1994).

Embryos of the aquatic frog, *Xenopus laevis,* have also been used for studies dealing with toxicity resulting from physical and chemical stressors because of its fecundity, ability to provide embryos year-round and the extensive information available on its developmental and molecular biology. In fact, *Xenopus* embryos have been used as

Received 16 November 1995; Revised 12 December 1995; Accepted 14 December 1995

Correspondence to: John J. Heikkila, Tel: +1 (519) 885 1211 Ext: 3076; Fax: +1 (519) 746 0614; E-mail: heikkila@sciborg.uwaterloo.ca

biomarkers in the Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX) to rapidly evaluate developmental malformations and/or lethality induced by single chemicals or complex environmental mixtures (Dawson et al 1985; Dawson and Bantle 1987; Bantle et al 1990; Dawson and Wilke 1991). Furthermore, tissue culture cell lines derived from *Xenopus* kidney, an organ involved in the excretion of toxic substances, are available for complementary studies (Rafferty 1969). Our laboratory and others have characterized *Xenopus laevis hsp* gene expression in adult tissue, cultured cells and during development (Bienz 1984a, 1984b; Heikkila et al 1985; Darasch et al 1988; Krone and Heikkila 1988, 1989; Krone et al 1992; Ali et al 1993). Most of these studies have examined heat-, or chemical-induced expression of *hsp70* and *hsp30* genes due to the fact that their cDNA and/or genomic sequences have been cloned. Little information is currently available on the pattern of stress-induced expression of another major *hsp* gene in *Xenopus laevis, hsp90.*

The *hsp90* genes are highly conserved and have been characterized in a wide range of organisms (reviewed by Nover and Scharf 1991; Parsell and Lindquist 1993; Morimoto et al 1994). In contrast to *Drosophila,* which has only a single *hsp90* gene, a number of vertebrates such as zebrafish and human have two closely related genes named *hsp90α* and *hsp90β* (Rebbe et al 1989; Krone and Sass 1994). At the protein level, numerous reports have demonstrated that Hsp90 can associate with a number of important cellular proteins including kinases, calmodulin, actin, tubulin and Hsp70 (reviewed by Craig et al 1993; Parsell and Lindquist 1993). Hsp90 has also been shown to interact with steroid hormone receptors (reviewed by Lindquist and Craig 1988; Gething and Sambrook 1992) as well as the dioxin receptor (Denis et al 1988; Wilhelmsson et al 1990). As mentioned previously, information on *hsp90* gene expression in *Xenopus* is limited. At the protein level, constitutive and heat shock-induced synthesis of an 87 kDa protein has been detected in *Xenopus* neurula embryos and in the kidney epithelial cell line, A6 (Heikkila et al 1985; Heikkila et al 1987; Darasch et al 1988). Also, in an earlier study, we found that a *Drosophila hsp83* cloned gene weakly detected the presence of *hsp90* mRNA in *Xenopus* neurula embryos (Heikkila et al 1987).

The objective of the present paper is to evaluate stress-inducible *hsp90* mRNA accumulation as a molecular biomarker in *Xenopus laevis.* We have employed a polymerase chain reaction (PCR)-based approach using degenerate primers to amplify and clone a portion of a *Xenopus hsp90β* gene. This cloned *hsp90β* DNA fragment was then used as a probe to examine basal and heat-, chemical- or metal-induced *hsp90* gene expression in *Xenopus* cultured cells and embryos.

MATERIALS AND METHODS

Xenopus embryo manipulations and A6 cells maintenance

Xenopus eggs were fertilized and cultured as described previously (Heikkila et al 1985). *Xenopus* embryos were staged according to Nieuwkoop and Faber (1967). The A6 kidney epithelial cells were grown in 55% (v/v) Leibovitz L-15 medium containing fetal bovine serum (10%; v/v), penicillin (100 IU/ml) and streptomycin (100 mg/ml). In heat shock experiments, the embryos or A6 cells were maintained at either control (22°C) or heat shock temperatures (27°C–37°C) for different lengths of time. The effect of other stresses was examined by treating the A6 cells with sodium arsenite, cadmium chloride, and zinc chloride for varying concentrations and lengths of times as previously described by Heikkila et al (1982, 1986).

Cloning and DNA sequencing

In order to obtain a *Xenopus hsp90* genomic DNA probe, a PCR-based strategy using degenerate primers was employed. This strategy has previously been used for the successful isolation of Hsp90 encoding sequences in zebrafish (Krone and Sass 1994). Amplifications were carried out using genomic DNA isolated from neurula stage embryos and amplification products were cloned into pBluescript II vector (Stratagene) using the Not I site engineered into the PCR primers. Double-stranded DNA sequencing was performed by the dideoxy chain termination procedure (Sanger et al 1977). The *hsp70B* probe (clone pXL16P) used in these experiments was a gift from Dr M. Bienz (1984b).

RNA isolation and Northern blot analysis

Total RNA was isolated from embryos and cultured A6 cells by the GIT/CsCl centrifugation method as described by Chirgwin et al (1979) with some modifications. Embryos or A6 cells were homogenized in 10 ml of 4M guanidine isothiocyanate and layered on top of 3.3 ml of 5.7M cesium chloride solution and then centrifuged for 23 h at 30 K rpm in SW-41 Ti rotor (Beckman). The RNA pellets were recovered and purified by two consecutive ethanol precipitations on dry ice. Fifteen μg of RNA was electrophoresed on a 1.2% formaldehyde agarose gel and transferred to nylon membrane (ICN, Mississauga, Ontario). The blots containing RNA were UV cross-linked in the UV cross linker (GS-Gene linker™ from Bio-Rad). The hybridization reactions were performed as described previously (Krone and Heikkila 1988) using ^{32}P-labelled *hsp90β* or *hsp70B* probes. After post-hybridization washes, the blots were exposed to XAR-5 film at −70°C overnight. Equal loading of the RNA gels was determined by visualization of the ribosomal RNA bands after staining with ethidium bromide. Furthermore, Northern blots were

© Pearson Professional Ltd 1996

```
1   TAT TCT AAT AAA GAA ATC TTC CTG CGA GAG TTA ATC TCA AAC GCC TCT GAT   51
1    Y   S   N   K   E   I   F   L   R   E   L   I   S   N   A   S   D    17
52  gtaagtcctttgctggttatgtaccagctgtgctaacttaaccagtgctctttgctggtatgtggtg  118
119 cctaattgcctctgtgtttggggaggcattgaccttttagttctgaatcagtgctgcctattatat  185
186 tgcttggggattgaaacagatctgcagctctgttgtcactggctgcacagtatagaatgagctgtgt  252
253 gtgctctgtgcagtagcgttctcccttaacaaatcatctccccatattcctgcctgcag GCC CTG  317
18                                                                A   L    19
318 GAT AAG ATT AGA TAT GAG AGC CTG ACT GAC CCA TCT AAG CTG GAC AGT GGC   368
20   D   K   I   R   Y   E   S   L   T   D   P   S   K   L   D   S   G    36
369 AAG GAC CTG AAG ATT GAC ATC ATT CCC AAC AGA CAA GAG CGC ACA CTG ACT   419
37   K   D   L   K   I   D   I   I   P   N   R   Q   E   R   T   L   T    53
420 GTG ATT GAC ACT GGA ATT GGC ATG ACG AAA GCT GAC CTC ATC AAC AAC CTG   470
54   V   I   D   T   G   I   G   M   T   K   A   D   L   I   N   N   L    70
471 GGA ACC ATT GCC AAG TCT GGC ACC AAG GCC TTC ATG GAG GCC CTC tacaggt   522
71   G   T   I   A   K   S   G   T   K   A   F   M   E   A   L            85
523 aggtagcagaggcagaatattaaaggacacttgtcccattaaagagaagccaagcacctagagtcta  589
590 tgcatactgtttctgagtggctcattttggtagaattttttgttttgagacttggttccttctctctg  656
657 a CAG GCC GGT GCG GAG ATC TCC ATG ATT GGC CAG TTC GGG GTG GGA TTC TAC 708
86     Q   A   G   A   E   I   S   M   I   G   Q   F   G   V   G   F   Y  102
```

Fig. 1 Nucleotide and amino acid sequence of the PCR-amplified genomic DNA fragment of the *Xenopus hsp90β* gene. The exon sequences are capitalized and the amino acid corresponding to the codon is shown immediately below it. Intronic sequences are indicated with lower case letters.

stripped of the labeled probe and rehybridized with a *Xenopus* cytoskeletal actin cDNA (Mohun et al 1983) to ensure consistent loading. In some experiments, autoradiograms were subjected to densitometric analysis using an Apple Macintosh OneScanner. The data were analyzed using NIH Image Version 1.52 software (National Institute of Health, Bethesda, MD).

RESULTS

Cloning and DNA sequence analysis of the *Xenopus hsp90β* genomic clone

Using the PCR-based approach described previously (Krone and Sass 1994), we obtained a 708 bp genomic DNA fragment corresponding to the 5′ portion of an *hsp90* gene. DNA sequence analysis of this PCR fragment and comparison with other *hsp90* gene sequences in Genbank revealed a conserved intron-exon structure to previously described *hsp90β* genes (Fig. 1; Moore et al 1989; Rebbe et al 1989; Meng et al 1995). The *Xenopus* fragment contains one entire exon and portions of two other exons. The two introns in this DNA fragment are 258 bp and 144 bp in size. The amino acid sequence deduced from the exons is 102 amino acids in length and shares the highest degree of identity with comparable regions of the human *hsp90β* (95%) and zebrafish *hsp90β* (94%) genes (Fig. 2). Lower degrees of identity to human *hsp90α* (90%; data not shown) and zebrafish *hsp90α* (91%)

```
Xhsp90    1   YSNKEIFLRELISNASDALDKIRYESLTDPSKLDSGKDLK 40
Hhsp90β   32  -------------------------------------E-- 71
Zhsp90β   1   -----------V------------------T--------- 40
Zhsp90α   1   --------------S--------------------C---- 40

Xhsp90    41  IDIIPNRQERTLTVIDTGIGMTKADLINNLGTIAKSGTKA 80
Hhsp90β   72  ------P------LV------------------------- 112
Zhsp90β   41  ------V------L------------M------------- 80
Zhsp90α   41  -EL--DQK-----I-------------------------- 80

Xhsp90    81  FMEALQAGAEISMIGQFGVGFY 102
Hhsp90β   113 ---------D------------ 133
Zhsp90β   81  ---------D------------ 102
Zhsp90α   81  ---------D------------ 102
```

Fig. 2 A. comparison of the *Xenopus* Hsp90*β* amino acid sequence (Xhsp90) derived from the cloned genomic fragment with corresponding regions of human *hsp90β* (Hhsp90*β*) and zebrafish *hsp90α* (Zhsp90*α*) and *hsp90β* (Zhsp90*β*) genes.

were observed. Thus, it is likely that the PCR-amplified fragment represents the *hsp90β* gene in *Xenopus*. This is supported by the fact that amino acid similarity analysis conducted using the neighbour-joining method as previously described (Krone and Sass 1994) placed the *Xenopus* sequence in the vertebrate *hsp90β* gene cluster (data not shown).

Stress-induced accumulation of *hsp90* mRNA in cultured A6 cells

Xenopus kidney epithelial A6 cells were used to determine the effect of different temperatures on *hsp90* mRNA

© Pearson Professional Ltd 1996

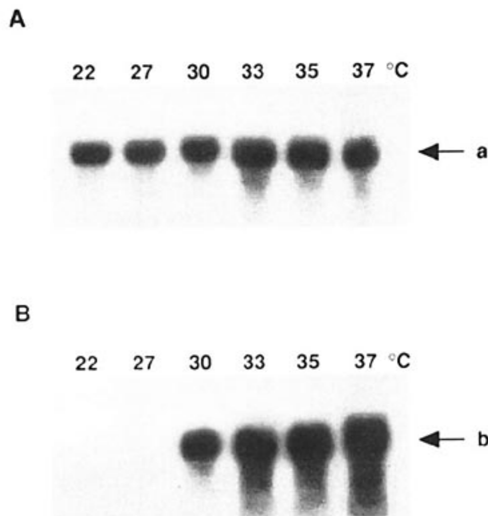

Fig. 3 Heat shock-induced *hsp90* mRNA accumulation in *Xenopus*-cultured A6 cells. A6 cells were heat shocked for 1 h at different temperatures as indicated on the top of each lane. Total RNA was isolated from A6 cells using the GIT/CsCl method and 15 μg was electrophoresed on 1.2% agarose formaldehyde gel and then transferred to nylon membrane. The blot was then hybridized against a ^{32}P-labelled *Xenopus hsp90*β (Panel A) and *Xenopus hsp70B* (Panel B) probes. a = 3.3 kb *hsp90* transcript; b = 2.7 kb *hsp70* transcript.

accumulation. Cultured A6 cells were maintained for 1 h at temperatures ranging from 22°C to 37°C. Northern blot analyses revealed a relatively high level of constitutive *hsp90* mRNA (3.3 kb) accumulation in control cells maintained at 22°C (Fig. 3A). A small increase in the level of *hsp90* message was detectable at 30°C while maximal induction occurred at 33°C–35°C and a decline in relative levels at 37°C. For comparison purposes, the same RNA samples were hybridized with a labeled *hsp70* genomic probe. A dramatic induction of *hsp70* gene was obvious after a heat shock at 30°C with maximal levels occurring at 35–37°C (Fig. 3B). Only low levels of *hsp70* transcripts were detectable at the control temperature (22°C) after longer film exposures (data not shown).

Previous studies have shown that, in addition to heat shock, sodium arsenite treatment also resulted in the increased synthesis of an 87 kDa protein in *Xenopus* A6 cultured cells (Darasch et al 1988). In order to examine the effect of sodium arsenite on the relative levels of *hsp90* mRNA, A6 cells were treated with this chemical stressor for 12 h followed by Northern blot analysis (Fig. 4). Exposure of A6 cells to 50 μM sodium arsenite enhanced the relative levels of *hsp90* mRNA compared to control with a reduced response at 75 μM. In contrast, treatment of A6 cells with cadmium chloride (10 and 20 μM) or zinc chloride (250 and 500 μM), conditions which have been shown to induce *hsp* gene expression in fish cells (Heikkila et al 1982), had no detectable effect on the accumulation of *hsp90* mRNA relative to control.

In a previous study, it was shown that concurrent

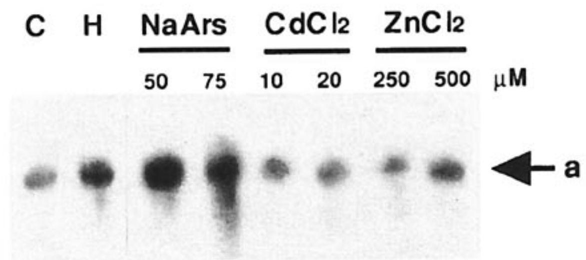

Fig. 4 Effect of sodium arsenite and heavy metals on *hsp90* gene expression. Cultured A6 cells were either heat shocked at 33°C for 1 h or treated with different concentrations of sodium arsenite (NaArs) for 12 h, cadimium chloride ($CdCl_2$) for 2h, or zinc chloride ($ZnCl_2$) for 2 h. Total RNA isolated from these cells was subjected to Northern blot analysis using ^{32}P-labelled *hsp90β* probe. C, control (22°C); H, heat shock (33°C).

treatment of mild heat shock plus low concentrations of sodium arsenite had a synergistic effect on the accumulation of both *hsp70* and *hsp30* mRNA (Heikkila et al 1987). In the present study, we examined the effect of a 27°C heat shock plus 10 μM sodium arsenite exposure for 2 h on *hsp90* mRNA levels (Fig. 5A). Densitometric analysis of the *hsp90* mRNA autoradiogram revealed that the response of the *hsp90* gene to the combined treatment of a 27°C heat shock and 10 μM sodium arsenite was approximately 1.3-fold greater than the sum of the indi-

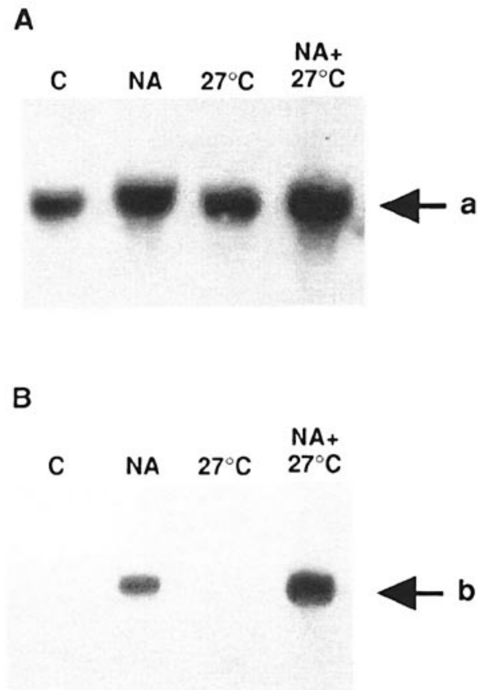

Fig. 5 Effect of concurrent heat shock and sodium arsenite treatment on the accumulation of *hsp90* mRNA in A6 cells. A6 cells were treated with sodium arsenite (10 μM) or heat shock (27°C) or both for 2 h. Total RNA was subjected to Northern blot analyses using *hsp90β* (panel A) and *hsp70* probes (panel B). C, control (22°C); NA, sodium arsenite. Transcript sizes: a = 3.3 kb *hsp90* mRNA; b = 2.7 kb *hsp70* mRNA.

© Pearson Professional Ltd 1996

vidual treatments in two separate trials. The use of higher heat shock temperatures such as 30°C in combination with sodium arsenite also produced a synergistic effect which was of the same extent (data not shown). In contrast to *hsp90* mRNA accumulation, the effect of combined treatments on *hsp70* mRNA accumulation was more than 3-fold greater than the sum of the individual stressors (Fig. 5B).

Characterization of *hsp90* mRNA accumulation in *Xenopus* embryos

Analysis of *hsp90* gene expression was also examined during early *Xenopus* development. In preliminary studies with neurula stage embryos, we found that induction of *hsp90* mRNA was first detectable in embryos exposed to 27°C for 1 h with maximal levels occurring at 30°C and 33°C, and slightly reduced levels at 35°C and 37°C (Fig. 6A). In contrast, enhanced *hsp70* mRNA accumulation was not detectable until 30°C and showed maximal accumulation at 35°C and 37°C (Fig. 6B). Also time-course studies revealed that neurulae exposed to a 33°C heat treatment displayed a small increase in *hsp90* mRNA accumulation after 15 min followed by a gradual increase peaking at 1–1.5 h followed by a decline in *hsp90* mRNA levels thereafter to 5 h (data not shown). Therefore, in order to examine heat-inducible *hsp90* gene expression during early *Xenopus* development, embryos at different developmental stages were maintained at 33°C for 1 h. The resultant Northern blot is shown in Figure 7A. Constitutive *hsp90* mRNA was present throughout development, from unfertilized egg and early cleavage to the neurula stage. However, heat-inducible *hsp90* mRNA accumulation was not detectable in cleavage or early blastula embryos but were present in heat shocked late blastula, gastrula and neurula stage embryos. Thus, heat-inducible *hsp90* gene expression like *hsp70* (Fig. 7B) is developmentally regulated during early *Xenopus laevis* development.

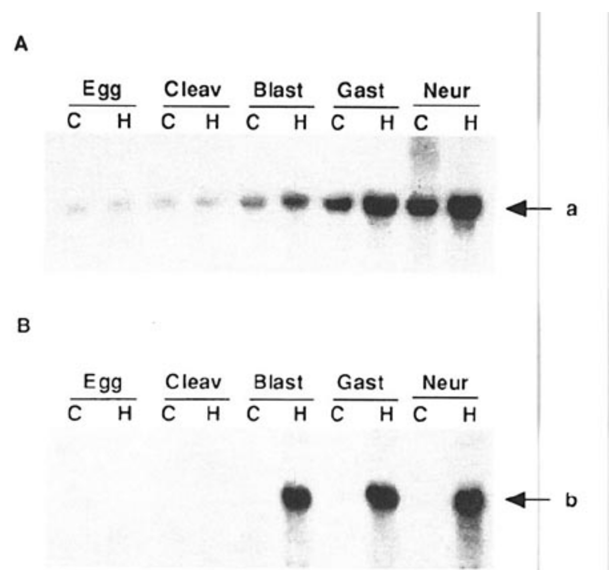

Fig. 7 Examination of *hsp90* gene expression during *Xenopus* embryogenesis. Embryos at different stages of early development were maintained either at control (C = 22°C) or heat shock temperatures (H = 33°C) for 1 h. The total RNA was subjected to Northern blot analysis using the *Xenopus hsp90β* (panel A) or *hsp70* (panel B) probe. Egg, unfertilized eggs; Cleav, cleavage (stage 4–5); Blast, blastula (stage 8); Gast, gastrula (stage 10.5); Neur, neurula (stage 17). a = 3.3 kb *hsp90* transcript; b = 2.7 kb *hsp70* transcript.

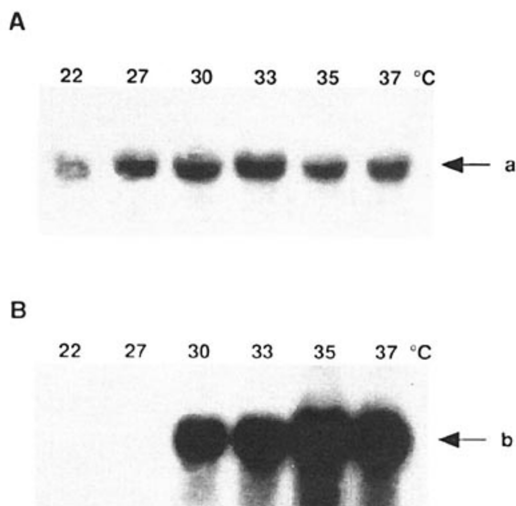

Fig. 6 Effect of temperature on *hsp90* mRNA accumulation in *Xenopus* embryos. Neurula stage embryos (stage 17) were heat shocked for 1 h at different temperatures shown at the top of each lane. Northern blot analysis of total RNA was performed using *Xenopus hsp90β* (Panel A) and *Xenopus hsp70B* (Panel B) probes. a = 3.3 kb *hsp90* transcript; b = 2.7 kb *hsp70* transcript.

DISCUSSION

In the present study, we have isolated a PCR-amplified genomic DNA fragment corresponding to the 5′ region of the *hsp90β*-like gene in *Xenopus laevis.* We have used this DNA fragment as a probe to examine the pattern of constitutive and stress-inducible *hsp90* gene expression in *Xenopus*-cultured cells and embryos and to evaluate its potential as a molecular biomarker. Using degenerate primers based on two regions of absolute amino acid identity in Hsp90 family members in a number of species (Krone and Sass 1994), we amplified a 708 bp DNA fragment from *Xenopus* genomic DNA. DNA sequence analysis of this genomic clone revealed that it has an intron/exon structure which is conserved within the comparable region of other vertebrate *hsp90β* genes and that it contains one complete exon and portions of two other exons. The deduced *Xenopus hsp90β* amino acid sequence exhibits strong identity with the *hsp90β* gene from zebrafish (94%) and human (95%) and a slightly lower degree of identity with *hsp90α* (90–91%) from the same organisms.

© Pearson Professional Ltd 1996

In mammals, both the *hsp90α* and *hsp90β* genes exhibit constitutive and heat-inducible expression (Barnier et al 1987; Legagneux et al 1989; Moore et al 1989). However, in chicken, the *hsp90α* gene is constitutively expressed and is heat-inducible whereas the *hsp90β* gene is only constitutively expressed (Meng et al 1993, 1995). In zebrafish, the *hsp90α* gene is strongly heat-inducible during early development whereas the *hsp90β* gene is expressed constitutively and appears to be only weakly heat-inducible (Krone and Sass 1994). Since *Xenopus* occupies an evolutionary position between fish and mammals, both of which contain *hsp90α* and *hsp90β* genes, it is likely that these two genes also exist in *Xenopus.* This is supported by two-dimensional PAGE analysis, which has revealed the existence of two different 87 kDa polypeptides in *Xenopus* A6 cells (Darasch et al 1987). Both of these 87 kDa proteins are heat shock- and sodium arsenite-inducible.

Examination of the stress-inducible aspects of *Xenopus hsp90* gene expression were carried out in a kidney epithelial cell line, A6. These studies demonstrated that the *hsp90* gene was constitutively expressed in A6 cells and that enhanced *hsp90* mRNA accumulation was first detectable at 30°C with maximal accumulation occurring at 33°C and 35°C and lower relative levels at 37°C. In contrast, an examination of *hsp70* gene expression showed a relatively low level of constitutive *hsp70* mRNA accumulation in A6 cells which increased dramatically following heat shock. The relatively low levels of *hsp90* mRNA found at 37°C compared to *hsp70* mRNA may reflect the fact that the *hsp90β* gene contains introns, whereas the *hsp70* gene does not (Bienz 1984b). Previous studies have shown that high heat shock temperatures can interfere with RNA splicing (Yost and Lindquist 1986; Kay et al 1987; Bond 1988).

We have also examined the effect of non-heat shock stressors on the pattern of *hsp90* gene expression in *Xenopus* A6 cells. Enhanced *hsp90* mRNA accumulation was strongly induced by 50 μM sodium arsenite. This finding agrees with the results of an earlier protein study in which we found that sodium arsenite increased the synthesis of Hsp90 in *Xenopus* A6 cells (Darasch et al 1988). Sodium arsenite has also been shown to be an effective inducer of Hsp90 in *Drosophila* and chick fibroblasts (Johnston et al 1980), as well as in mammalian hepatoma cells (Wiegant et al 1994). In the present study we also found that exposure of A6 cells to 250–500 μM zinc chloride or 10–20 μM cadmium chloride, concentration ranges that have been shown to induce Hsp synthesis in a fish embryo cell line (Heikkila et al 1982), did not have a significant effect on the relative level of *hsp90* mRNA compared to control. A similar study in Reuber H35 rat hepatoma cells has shown that sodium arsenite induced the synthesis of Hsp84 protein synthesis while cadmium treatment had a very mild effect for the same duration of exposure (Wiegant et al 1994).

In this study we have also characterized the combined effect of a mild heat shock (27°C) and 10 μM sodium arsenite for 2 h on *hsp90* and *hsp70* mRNA levels in A6 cells. This concurrent exposure to heat shock and sodium arsenite had a strong synergistic effect on *hsp70* mRNA accumulation but only a slight synergistic effect on *hsp90* mRNA accumulation. This finding generally agrees with our previous protein synthetic studies in which we found that a combination of sodium arsenite and heat shock produced a significant synergistic increase in the relative synthesis of Hsp70 but not the 87 kDa heat-inducible protein (Heikkila et al 1987). Also, Rodenheiser et al (1986) have reported that ethanol and heat shock produced a synergistic effect on Hsp synthesis in mouse lymphocytes. The mechanism(s) of the synergistic activation of Hsp genes expression is not known although it is possible that the increased accumulation of the *hsp* mRNA is due to increased *hsp* gene transcription and/or increased stability of *hsp* mRNA.

Finally, we have examined the effect of heat shock on *hsp90* gene expression in *Xenopus laevis* embryos. We detected constitutive levels of *hsp90* mRNA accumulation in all of the embryonic stages examined from unfertilized eggs and early cleavage stage embryos through to neurula. The finding of constitutive levels of *hsp90* mRNA in unfertilized eggs and cleavage stage embryos indicates that these messages are maternal in origin since there is no zygotic genome transcription until the midblastula stage of development (Newport and Kirschner 1982a, 1982b; Kimelman et al 1987). Recently, it has been shown that an *hsp90* homolog, *hsc90,* was also constitutively expressed at all stages of oogenesis in the amphibian, *Pleurodeles waltl* (Coumailleau et al 1995). In the present study we have also found that heat shock-induced accumulation of *hsp90* mRNA was not detectable in cleavage stages but was inducible in late blastula, gastrula and neurula stages. Similarly, it has been shown that *hsp70* genes are not heat-inducible until the midblastula stage of development (Bienz 1984a; Heikkila et al 1985). This is the stage of development that coincides with the activation of the zygotic genome (Gerhardt 1980; Newport and Kirschner 1982a, 1982b). It is possible that the mechanism(s) associated with the developmental regulation of genes which are normally activated at midblastula also applies to the heat shock-induced expression of the *hsp90* gene. In a model proposed by Newport and Kirschner (1982a, 1982b) and Kimelman et al (1987), it was suggested that pre-midblastula embryos are transcriptionally competent (i.e. contain functional transcription factors and RNA polymerase II) but are unable to synthesize RNA due to the rapid cell cycle.

In conclusion, this study has evaluated the role of

© Pearson Professional Ltd 1996

hsp90 mRNA accumulation in *Xenopus*-cultured cells and embryos as a molecular biomarker. While *hsp90* mRNA accumulation was induced by heat shock and sodium arsenite treatment, exposure to heavy metals such as cadmium and zinc chloride did not enhance the levels of this message. Given the normal abundance and limited stress-induced accumulation of *hsp90* mRNA compared to *hsp70* mRNA, it may not, by itself, have a great deal of potential as a biomarker. However, it may prove useful in combination with other *hsp* mRNAs (e.g. *hsp70* and *hsp30*) to establish a set of biomarker profiles to characterize the response of cultured cells or embryos to physical or chemical stressors.

ACKNOWLEDGEMENTS

This research has been supported by Natural Sciences and Engineering Research Council of Canada grants to JJH and PHK. AA was the recipient of an Ontario Graduate scholarship. DSP was supported by a Graduate Scholarship from the University of Saskatchewan.

REFERENCES

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

Anderson NL, Giere FA, Nance SL, Gemmell MA, Tollaksen SL and Anderson NG (1987) Effects of toxic agents at the protein level: quantitative measurement of 213 mouse liver proteins following xenobiotic treatment. Fundam. Appl. Toxicol. **8**: 39–50.

Aoki Y, Lipsky MM and Fowler BA (1990) Alteration in protein synthesis in primary cultures of rat kidney proximal tubule epithelial cells by exposure to gallium, indium and arsenite. Toxicol. Appl. Pharmacol. **106**: 462–468.

Bantle JA, Fort DJ, Rayburn JR, DeYoung DJ and Bush SJ (1990) Further validation of the developmental toxicity of five known mammalian teratogens and non-teratogens. Drug Chem. Toxicity **13**: 267–282.

Barnier JV, 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.

Bienz M (1984a) Developmental control of the heat shock response in Xenopus. Proc. Natl. Acad. Sci. USA **81**: 3138–3142.

Bienz M (1984b) *Xenopus hsp70* genes are constitutively expressed in injected oocytes. EMBO J. **3**: 2477–2483.

Bols NC, Boliska SA, Dixon DG, Hodson PV and Kaiser KLE (1985) The use of fish cell cultures as an indication of contaminant toxicity to fish. Aquat. Toxicol. **6**: 147–155.

Bond U (1988) Heat shock but not other stress inducers leads to the disruption of a sub-set of snRNPs and inhibition of in vitro splicing in HeLa cells. EMBO J. **7**: 3509–3518.

Bournias-Vardiabasis N, Buzin C and Flores J (1990) Differential expression of heat shock proteins in Drosophila embryonic cells following metal ion exposure. Exp. Cell Res. **189**: 177–182.

Chirgwin J, Przbyla A, MacDonald R and Rutter W (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**: 5294–5299.

Coumailleau P, Billoud B, Sourrouille P, Moreau N and Angelier N (1995) Evidence for a 90 kDa heat-shock protein gene expression in the amphibian oocyte. Dev. Biol. **168**: 247–258.

Craig EA, Gambill BD and Nelson RJ (1993) Heat shock proteins: Molecular chaperones of protein biogenesis. Microbiol. Rev. **57**: 402–414.

Darasch S, Mosser DD, Bols NC and Heikkila JJ (1988) Heat shock gene expression in *Xenopus laevis* A6 cells in response to heat shock and sodium arsenite treatments. Biochem. Cell Biol. **66**: 862–870.

Dawson DA and Bantle JA (1987) Development of a reconstituted water medium and preliminary validation of the frog embryo teratogenesis assay – *Xenopus* (FETAX). J. Appl. Toxicol. **7**: 237–244.

Dawson DA and Wilke TS (1991) Evaluation of the frog embryo teratogenesis assay: *Xenopus* (FETAX) as a model system for mixture toxicity hazard assessment. Environ. Toxicol. Chem. **10**: 941–948.

Dawson DA, McCormick CA and Bantle JA (1985) Detection of teratogenic substances in acidic mine water samples using the frog embryo teratogenesis assay – *Xenopus* (FETAX). J. Appl. Toxicol. **5**: 234–244.

Deaton MA, Bowman PD, Jones GP and Powanda MC (1990) Stress protein synthesis in human ketatinocytes treated with sodium arsenite, phenyl dichloroarsene and nitrogen mustard. Fundam. Appl. Toxicol. **14**: 477–490.

Denis M, Cuthill S, Wikstrom A-C, Poellinger L and Gustafsson J-A (1988) Association of the dioxin receptor with the Mr 90 000 heat shock protein: A structural kinship with the glucocorticoid receptor. Biochem. Biophys. Res. Commun. **155**: 801–807.

Edwards MJ, Dykes PJ, Donovan MRO, Merrett VR, Morgan HE and Marks R (1990) Induction of heat shock proteins as a measure of chemical cytotoxicity. Toxicol. In Vitro **4**: 270–276.

Gerhardt J (1980) Mechanisms regulating pattern formation in the amphibian egg and early embryo, In: Biological Regulation and Development, Vol. 2, Plenum, NY, 133–316.

Gething MJ and Sambrook J (1992) Protein folding in the cell. Nature **355**: 33–45.

Goering PL, Fisher BR, Chaudhary PP and Dick CA (1992) Relationship between stress protein induction in rat kidney by mercuric chloride and nephrotoxicity. Toxicol. Appl. Pharmacol. **113**: 184–191.

Heikkila JJ, Schultz GA, Iatrou K and Gedamu L (1982) Expression of a set of fish genes following heat or metal ion exposure. J. Biol. Chem. **257**: 12000–12005.

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

Heikkila JJ, Darasch SP, Mosser DD and Bols NC (1986) Heat and sodium arsenite act synergistically on the induction of heat shock gene expression in *Xenopus laevis* A6 cells. Biochem. Cell Biol. **65**: 310–316.

Heikkila JJ, Ovsenek N and Krone PH (1987) Examination of heat shock protein mRNA accumulation in early *Xenopus laevis* embryos. Biochem. Cell Biol. **65**: 87–93.

Hightower LE and Renfro JL (1988) Recent applications of fish cell culture to biomedical research. J. Exp. Zool. **248**: 290–302.

Johnston D, Oppermann H, Jackson J and Levinson W (1980) Induction of four proteins in chicken embryo cells by sodium arsenite. J. Biol. Chem. **255**: 6975–6980.

Kay RJ, Russnak RH, Jones D, Mathia C and Candido EPM (1987) Expression of intron-containing *C. elegans* heat shock genes in mouse cells demonstrates divergence of 3′ splice recognition

© Pearson Professional Ltd 1996

sequences between nematodes and vertebrates, and an inhibitory effect of heat shock on the mammalian splicing apparatus. Nucleic Acids Res. **15**: 3723–3741.

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.

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

Krone PH and Heikkila JJ (1989) Expression of microinjected *hsp70*/CAT and *hsp30*/CAT chimeric genes in developing *Xenopus laevis* embryos. Development **106**: 271–281.

Krone PH and Sass JB (1994) *Hsp90α* and *hsp90β* genes are present in the zebrafish and are differentially regulated in developing embryos. Biochem. Biophys. Res. Comm. **204**: 746–752.

Krone PH, Snow A, Ali A, Pasternak JJ and Heikkila JJ (1992) Comparison of the regulatory regions of the *Xenopus laevis* small heat-shock protein encoding gene family. Gene **110**: 159–166.

Legagneux V, Mezger V, Quelard C, Barnier JV, Bensaude O and Morange M (1989) High constitutive transcription of *hsp86* gene in murine carcinoma cells. Differentiation **41**: 42–48.

Lindquist S and Craig EA (1988) The heat-shock proteins. Annu. Rev. Genet. **22**: 631–677.

Meng X, Jerome V, Devin J, Baulieu EE and Catelli MG (1993) Cloning of chicken *hsp90β*: The only vertebrate *hsp90* insensitive to heat shock. Biochem. Biophys. Res. Comm. **190**: 630–636.

Meng X, Baulieu EE and Catelli MG (1995) Isolation of chicken *hsp90β* gene promoter. Biochem. Biophys. Res. Comm. **206**: 644–651.

Mohun TJ, Brennan S, Dathan N, Fairman S and Gurdon JB (1983) Cell type-specific activation of actin genes in the early amphibian embryo. Nature **311**: 716–721.

Moore SK, Kozak C, Robinson EA, Ullrich SJ and Appella E (1989) Murine 86- and 84-kDa heat shock proteins, cDNA sequences, chromosome assignments, and evolutionary origins. J. Biol. Chem. **264**: 5343–5351.

Morimoto RI, Tissieres A and Georgopoulos C (1994) Progress and perspectives on the biology of heat shock proteins and molecular chaperones. In: The Biology of Heat Shock Proteins and Molecular Chaperones, eds RI Morimoto, A Tissieres and C Georgopoulos, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1–30.

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

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

Nieuwkoop PD and Faber J (1967) Normal Table of *Xenopus laevis* (Daudin), 2nd ed, North Holland Publishing, Amsterdam.

Nover L and Scharf K-D (1991) Heat shock proteins. In: Heat Shock Response, CRC Press, FL, 41–127.

Ovelgonne JH, Souren JEM, Wiegant FAC and Van Wijk R (1995) Relationship between cadmium-induced expression of heat shock genes, inhibition of protein synthesis and cell death. Toxicology **99**: 19–30.

Parsell DA and Lindquist S (1993) The function of heat-shock proteins in stress tolerance: Degradation and reactivation of damaged proteins. Annu. Rev. Genet. **27**: 437–496.

Pipkin JL, Anson JF, Hinson WG, Burns ER, Casciano DA and Sheehan DM (1987) Cell cycle specific effects of sodium arsenite and hyperthermic exposure on incorporation of radioactive leucine and phosphate by stress proteins from mouse lymphoma nuclei. Biochim. Biophys. Acta **927**: 334–344.

Powers DA (1989) Fish as model systems. Science **246**: 352–358.

Rafferty KKA (1969) Mass culture of amphibian cells: Methods and observations concerning stability of cell type. In: Biology of Amphibian Tumours, ed M. Mizell, Springer-Verlag, Berlin, 52–81.

Rebbe NF, Hickman WS, Ley TJ, Stafford DW and Hickman S (1989) Nucleotide sequence and regulation of a human 90-kDa heat shock protein gene. J. Biol. Chem. **264**: 15006–15011.

Rodenheiser DI, Jung JH and Atkinson BG (1986) The synergistic effect of hyperthermia and ethanol on changing gene expression of mouse lymphocytes. Can. J. Genet. Cytol. **28**: 1115–1124.

Ryan JA and Hightower LE (1994) Evaluation of heavy-metal ion toxicity in fish cells using a combined stress protein and cytotoxicity assay. Environ. Toxicol. Chem. **13**: 1231–1240.

Sanders BM (1993) Stress proteins in aquatic organisms: an environmental perspective. Crit. Rev. Toxicol. **23**: 49–75.

Sanger F, Nicklen S and Coulson AR (1977) DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**: 5463–5467.

Wiegant FAC, Souren JEM, Rijn JV and Wijk RV (1994) Stressor specific induction of heat shock proteins in rat hepatoma cells. Toxicology **94**: 143–159.

Wilhelmsson A, Cuthill S, Denis, M, Wikstrom A-C, Gustafsson, J-A and Poellinger L (1990) The specific DNA binding activity of the dioxin receptor is modulated by the 90 kd heat shock protein. EMBO J. **9**: 69–76.

Yost HJ and Lindquist S (1986) RNA splicing is interrupted by heat shock and is rescued by heat shock protein synthesis. Cell **45**: 185–193.

© Pearson Professional Ltd 1996