Analysis of hsp 30, hsp 70 and ubiquitin gene expression in *Xenopus laevis* tadpoles

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Summary

Heat-induced accumulation of hsp 30 mRNA (1·1 kb) during early development of *Xenopus laevis* was first detectable at the tailbud stage (stage 30–34). This contrasts with heat-induced accumulation of hsp 70 mRNA (2·7 kb) and ubiquitin mRNA (size range = 1·7–3·1 kb), which was first detectable at the mid- to late-blastula stage. Continuous exposure of tadpoles to a 33 °C heat shock resulted in a coordinate, transient accumulation of hsp 30, hsp 70 and ubiquitin mRNA. A coordinate, temporal pattern was also observed for the decay of hsp 30, hsp 70 and ubiquitin mRNA in tadpoles recovering at 22 °C following a 1 h heat shock at 33 °C. Thus, while hsp 30 genes are regulated differently during development compared with hsp 70 and ubiquitin genes, these genes all exhibit a coordinate heat-inducible pattern of expression at the tadpole stage. Levels of α-cardiac actin mRNA remained unchanged during continuous heat shock and recovery experiments.

Key words: *Xenopus laevis*, heat-shock proteins, mRNA, ubiquitin, tadpoles.

Introduction

Exposure of prokaryotic and eukaryotic cells to environmental stress such as elevated temperatures, heavy metals or arsenite results in the synthesis of a highly conserved set of proteins known as heat-shock proteins (hsps; reviewed by Novèr, 1984; Atkinson & Walden, 1985; Craig, 1985; Burdon, 1986; Lindquist, 1986). Regulation of the heat-shock response can occur at both the transcriptional and translational levels. Three families of hsps, with size ranges of 80–90, 68–70 and 15–30×10^3 M_r, are synthesized by virtually all organisms. Both the hsp 80–90 and the hsp 68–70 families exhibit a high degree of sequence and structure homology among a widely divergent range of species (Ingolia *et al.* 1982; Voellmy *et al.* 1982; Hacket & Lis, 1983; Hunt & Morimoto, 1985; Rochester *et al.* 1986). The lower molecular weight hsps are not as highly conserved, although DNA sequence data has demonstrated some degree of homology among the small hsps of *Caenorhabditis, Drosophila* and *Xenopus* (Russnak *et al.* 1983; Bienz, 1984a). Another highly conserved protein, ubiquitin, has recently been shown to be a hsp in chicken (Bond & Schlesinger, 1985, 1986), mouse (Finley & Varshavsky, 1985), and yeast (Finley *et al.* 1987; Ozkaynak *et al.* 1987).

Developmental stage-dependent expression of hsp genes occurs during early embryonic development in a variety of animal systems (reviewed by Heikkila *et al.* 1985b; Heikkila *et al.* 1986). Our laboratory has been involved in the examination of hsp gene expression during oogenesis and early embryogenesis of *Xenopus laevis*. Both oocytes and unfertilized eggs exhibit synthesis of hsps under conditions of thermal stress, a response which is terminated upon fertilization (Browder *et al.* 1987) and does not occur in early cleavage stage embryos (Heikkila *et al.* 1985a; Nickells & Browder, 1985). However, at the neurula stage of development embryos respond to heat shock by synthesizing hsps with relative molecular masses (M_r) of 87, 76, 70, 68, 57, 42, and 35×10^3 (Heikkila *et al.* 1985a; Nickells & Browder, 1985). The synthesis of at least two of these proteins, hsp 70 and hsp 87, appears to be controlled at the transcriptional level since heat-shock-induced accumulation of hsp 70 mRNA (Bienz, 1984a; Heikkila *et al.* 1985a, 1987b) and hsp 87 mRNA (Heikkila *et al.* 1987b) has been reported in midblastula and later stage embryos.
Furthermore, we have recently found that heat-induced accumulation of ubiquitin mRNA during *Xenopus* development is first detectable at the mid-blastula stage (Ovsenek & Heikkila, unpublished data).

Exposure of cultured *Xenopus* A6 cells to heat shock induces the expression of hsp 70 and the other aforementioned hsp genes as well as the hsp 30 gene family (Heikkila et al. 1987a; Darasch et al. 1988). During *Xenopus* development, it has been reported that the hsp 30 gene(s) are not heat-inducible until the free-swimming tadpole stage (stage 42; Bienz, 1984a). In the present study, we have re-examined and extended the investigation of hsp 30 gene expression in *Xenopus laevis* embryos. We have found that heat-inducible hsp 30 mRNA accumulation is first detectable at the tailbud stage of development (stage 30–34), not the tadpole stage (stage 42) as was previously reported (Bienz, 1984a). Furthermore, hsp 30, hsp 70 and ubiquitin mRNA accumulate and decay in a coordinate fashion during continuous heat shock and during recovery from heat shock in free-swimming tadpoles, a stage at which all three of these genes are heat-inducible. Levels of tadpole a-cardiac mRNA remain unchanged under these conditions.

**Materials and methods**

**Embryo and tadpole manipulation**

*Xenopus laevis* eggs were obtained, fertilized and dejellied by the methods of Heikkila et al. (1985a). All of the embryos and tadpoles used in this study were maintained in Steinberg’s solution. Embryos were staged by external criteria according to Nieuwkoop & Faber (1967).

**Recombinant DNA probes**

Detection of hsp 30 and hsp 70 mRNAs was accomplished using genomic subclones of either the *X. laevis* hsp 30 gene (pXS 43B) or the entire hsp 70 gene (pXL 16P; gifts of Dr M. Bienz, MRC Laboratory of Molecular Biology, Cambridge, UK; Bienz, 1984b). Relative actin mRNA levels were measured with a *X. laevis* a-cardiac actin cDNA clone (pXLcA2; gift of Dr T. Mohun, University of Cambridge, UK; Mohun et al. 1984). The *X. laevis* ubiquitin cDNA clone used to detect ubiquitin mRNAs was a gift of Dr M. Dworkin, Department of Biological Sciences, Columbia University, New York (pXLgC20; Dworkin-Rastl et al. 1984). All probes were prepared with deoxyctydine 5'-[a-32P] triphosphate (ICN Biomedicals, Inc. California) to a specific activity of 2x10^6 cts min^-1 ml^-1 using the method of Maniatis et al. (1975).

**RNA isolation**

Total lithium-chloride-precipitable RNA was isolated according to the method of Auffrey & Rougeon (1980) as modified by Mohun et al. (1984). Briefly, frozen embryos or tadpoles were homogenized in 3 M-lithium chloride, 6 M-urea, 0.5% SDS, 70 mM-mercaptoethanol, 10 mM-sodium acetate (pH 5.0) and precipitated overnight at 4°C. The RNA was pelleted by centrifugation, resuspended in 0.2% SDS, 100 mM-sodium acetate (pH 5.0), and extracted two times with phenol/chloroform (1:1) and two times with chloroform. The aqueous phase was adjusted to 0.3 M-sodium acetate and the RNA was ethanol precipitated at -20°C overnight.

**Agarose gel electrophoresis and hybridization**

10 µg of total RNA was subjected to gel electrophoresis on a horizontal 1:2% formaldehyde–agarose gel (Maniatis et al. 1982) and transferred to nitrocellulose according to the method of Thomas (1983). RNA transcript sizes were determined by comparison with the migration of an RNA marker ladder (Bethesda Research Laboratories, Bethesda, Maryland). Prehybridization of RNA blots was performed for 12 h at 42°C in 50% formamide, 0.75 M-sodium chloride–0.075 M-sodium citrate (pH 7.0), 10 mM-sodium phosphate buffer (pH 7.0), 0.05% bovine serum albumin, 0.05% polyvinylpyrrolidone, 0.05% Ficoll and 250 µg ml^-1 denatured salmon sperm DNA. The hybridization reactions were carried out at 42°C for 48 h in prehybridization buffer containing 7.5% dextran sulphate. The labelled probe was added to a final concentration of 5x10^4 cts min^-1 ml^-1. The blots were washed four times over a 15 min period at room temperature in 0.15 M-sodium chloride–0.015 M-sodium citrate (pH 7.0), 0.1% SDS followed by two 15 min washes in 0.05 M-sodium chloride–0.0075 M-sodium citrate (pH 7.0), 0.1% SDS at 42°C and ten 10 min washes in 0.015 M-sodium chloride–0.0015 M-sodium citrate (pH 7.0), 0.1% SDS at 42°C. The blots were then exposed to Kodak XAR-5 film at -70°C using a Cronex intensifying screen.

**Results**

**Heat-induced, stage-dependent accumulation of hsp 30, hsp 70 and ubiquitin mRNA during Xenopus development**

In previous studies, we and others have reported that heat-shock-induced accumulation of hsp 70 (Bienz, 1984a; Heikkila et al. 1985, 1987b; Nickells & Browder, 1985), hsp 87 (Heikkila et al. 1987b) and ubiquitin mRNA (Ovsenek & Heikkila, unpublished data) occurred only after the midblastula stage of *Xenopus* development. However, *Xenopus* hsp 30 genes appeared to be regulated differently since their expression was not detectable prior to the free-swimming tadpole stage (stage 42; Bienz, 1984a).

In the present study, we have re-examined and compared the heat-induced accumulation of hsp 30 mRNA relative to hsp 70 and ubiquitin mRNA. In these experiments, total RNA from control (22°C for 1 h) and heat-shocked (33°C for 1 h) embryos was examined by Northern blot hybridization utilizing the *Xenopus* hsp 30 and hsp 70 genomic subclones and the *Xenopus* ubiquitin cDNA clone as probes. As shown in Fig. 1A, heat shock induced a dramatic
Hsp mRNA levels in tadpoles

1 234 56 789 10

Fig. 1. Stage-dependent heat-shock-induced accumulation of hsp 30 and 70 mRNA in Xenopus laevis embryos and tadpoles. Total lithium-chloride-precipitable RNA was isolated from embryos or tadpoles maintained at either 22°C or 33°C for 1 h. 10 μg of total RNA was resolved by electrophoresis on formaldehyde-agarose denaturing gels, transferred to nitrocellulose and hybridized against the labelled X. laevis hsp 30 genomic subclone to yield the autoradiogram in panel A. The radioactive signal was allowed to decay after which the blot was rehybridized against the labelled hsp 70 genomic subclone (panel B). Lane 1, neurula (stage 20) at 22°C; lane 2, neurula at 33°C; lane 3, tailbud (stage 30–32) at 22°C; lane 4, tailbud at 33°C; lane 5, 3-day tadpole at 22°C; lane 6, 3-day tadpole at 33°C; lane 7, 4-day tadpole at 22°C; lane 8, 4-day tadpole at 33°C; lane 9, 4-day tadpole at 22°C; lane 10, 4-day tadpole at 33°C.

In the accumulation of a 1.1 kb hsp 30 gene transcript in heat-shocked tailbud embryos (stage 30–32; lane 4) as well as in 3- and 4-day-old tadpoles (lanes 6, 8 and 10). However, no detectable levels of hsp 30 mRNA were present in heat-shocked neurulae (stage 20; lane 2). Heat-shock-induced hsp 30 mRNA accumulation was not detectable in preparations of either total or polyadenylated RNA isolated from blastula, gastrula or neurula stage embryos exposed to a wide range of temperatures or time regimens (data not shown). In contrast, rehybridization of the blot shown in Fig. 1A to the hsp 70 probe demonstrated that hsp 70 mRNA (2.7 kb) was present in heat-shocked neurulae as well as in heat-shocked tailbud embryos and tadpoles (Fig. 1, panel B, lanes 2, 4, 6, 8 and 10). Rehybridization of the blot to the Xenopus cardiac actin cDNA clone demonstrated the presence of actin mRNA in both control and heat-shock samples (data not shown). The developmental stage at which hsp 30 mRNA was first detectable ranged from stage 30–34 in four separate experiments. Thus, we have found that heat-shock-induced accumulation of hsp 30 mRNA occurs earlier during Xenopus development (stage 30–34) than was previously reported (stage 42; Bienz, 1984a).

We have also examined the effect of heat shock on ubiquitin mRNA accumulation during comparable stages of Xenopus development. Since Dworkin-Rastl et al. (1984) have shown that ubiquitin mRNA displays population polymorphism with respect to size, only embryos derived from a single mating were used in any one particular experiment. Four different mRNAs, ranging in size from 1.7–3.1 kb, were detected in total RNA isolated from control and heat-shocked neurulae and tailbud embryos (Fig. 2). However, the levels of these four messages were enriched in the embryos maintained at 33°C (lanes 2, 4 and 6) relative to those maintained at 22°C (lanes 1, 3 and 5). A similar heat-induced increase in ubiquitin mRNA levels was also observed in 3- and 4-day-old tadpoles (data not shown). Thus, the developmental pattern of heat-induced ubiquitin mRNA accumulation in neurula and later stage embryos is similar to hsp 70 mRNA accumulation but distinct from hsp 30 mRNA accumulation.

Coordinate, transient accumulation of hsp 30, hsp 70 and ubiquitin mRNA during continuous exposure of tadpoles to heat shock

Given the differences in the developmental regulation of expression of the hsp 30, hsp 70 and ubiquitin genes, it was of interest to examine the pattern of expression of all of these genes in heat-shocked tadpoles; a stage at which all three genes are heat-inducible. Preliminary experiments examining the effect of temperature (22–37°C for 1 h) on free-swimming tadpoles established that maximum accumulation of hsp 30, hsp 70 and ubiquitin mRNA occurred at 33–35°C (data not shown). Continuous exposure of tadpoles to 33°C induced a transient accumulation of hsp 30, hsp 70 and ubiquitin mRNA.
Fig. 2. Heat-shock-induced accumulation of ubiquitin mRNA in embryos and tadpoles of *Xenopus laevis*. Embryos and tadpoles were maintained at 22°C or 33°C for 1 h. Total RNA was electrophoresed and transferred to nitrocellulose as in Fig. 1 followed by hybridization to the labelled *Xenopus* ubiquitin cDNA clone. Transcript sizes: a = 1-7 kb; b = 2-3 kb; c = 2-5 kb; d = 3-1 kb. lane 1, neurula (stage 20) at 22°C; lane 2, neurula at 33°C; lane 3, tail bud (stage 30–32) at 22°C; lane 4, tail bud at 33°C; lane 5, late tailbud (stage 34–35) at 22°C; lane 6, late tailbud at 33°C.

(Figs 3, 4). Both hsp 30 and hsp 70 mRNA were detectable after 15 min of heat shock (Fig. 3, panels A and B, lane 2). Maximum levels of hsp 30 and hsp 70 mRNA occurred at 1-5 h (Fig. 3, panel A, lane 5) and 1 h (Fig. 3, panel B, lane 4), respectively, followed by a decline of both messages to background levels by 21 h (Fig. 3, panels A and B, lane 10). Peak levels of ubiquitin mRNA were reached after 1 h of heat shock (Fig. 4, lane 4). This was followed by a decline to control levels after a 3 h exposure to 33°C (Fig. 4, lane 7). A similar transient, coordinate accumulation of hsp 30, hsp 70 and ubiquitin mRNA was also observed in tadpoles continuously exposed to 35°C (data not shown). Very low levels of these hsp mRNAs were transiently induced in tadpoles maintained at 27°C or 30°C during similar time-course experiments (data not shown). Tadpoles maintained at 33°C exhibited no aberrant morphology throughout the 21 h time frame of the experiment. Survival rate of these tadpoles was similar to that observed in controls. However, at 35°C tadpoles became sluggish within 1/2 h and exhibited a higher mortality rate relative to controls.

**Hsp 30, hsp 70 and ubiquitin mRNA levels in tadpoles during recovery from heat shock**

Hsp 30, hsp 70 and ubiquitin mRNA levels were also examined in tadpoles during recovery at 22°C following a 1 h heat shock at 33°C. A decrease in the levels of each of these mRNAs was evident within 0-5 h of recovery (Fig. 5, panels A and B, lane 3; Fig. 6, lane 3). The levels of both hsp 30 and hsp 70 mRNA...
Hsp mRNA levels in tadpoles

Fig. 4. Relative levels of ubiquitin mRNA in tadpoles during continuous exposure to a 33°C heat shock. Total RNA was analysed by Northern hybridization as outlined in the legend to Fig. 2. Sizes of transcripts: $a = 1.7$ kb; $b = 2.3$ kb; $c = 2.5$ kb; $d = 3.1$ kb. Lane 1, control (no heat shock); lane 2, 33°C for 0-25h; lane 3, 33°C for 0-5h; lane 4, 33°C for 1h; lane 5, 33°C for 1.5h; lane 6, 33°C for 2h; lane 7, 33°C for 3h.

then remained fairly constant up to 2h (Fig. 5, panels A and B, lanes 3 to 6) after which they decayed to near background levels by 11h (lane 9).

Similarly, ubiquitin mRNA levels remained unchanged between 0-5 and 2h of recovery (Fig. 6, lanes 3–6) but decayed to control levels by 4h (lane 7). Thus, hsp 30, hsp 70 and ubiquitin mRNA showed a similar pattern of decay in recovering tadpoles. The morphology of the tadpoles remained normal over the course of these recovery experiments.

Levels of cardiac actin mRNA in tadpoles during continuous heat shock and recovery

Given the coordinate, temporal pattern of hsp 30, hsp 70 and ubiquitin mRNA accumulation and decay observed in heat-shocked tadpoles, it was of interest to examine the levels of another message, namely $\alpha$-cardiac actin, which is present in tadpoles at control temperatures. During a continuous, 4h exposure of 8-day-old tadpoles to 33°C, levels of the 1.6kb $\alpha$-cardiac actin transcript remained unchanged relative to control (Fig. 7, panel A). A similar response was observed in tadpoles during recovery at 22°C following a 1h heat shock at 33°C. Levels of $\alpha$-cardiac actin message remained the same relative to control during both the 1h heat shock and the subsequent 4h recovery periods (Fig. 7, panel B).

Discussion

Previous studies have shown that heat-shock-induced accumulation of hsp 70 and hsp 87 messenger RNA during Xenopus embryogenesis does not occur until after the midblastula stage (Bienz, 1984a; Heikkila et al. 1985a; 1987; Nickells & Browder, 1985). This is also the stage at which heat-shock-induced accumulation of ubiquitin mRNA is first detectable (Ovsenek...
Fig. 6. Relative levels of ubiquitin mRNA in tadpoles during recovery following a 1 h heat shock at 33°C. Total RNA was analysed as outlined in the legend to Fig. 2. Transcript sizes: a = 1-7 kb; b = 2-3 kb; c = 2-5 kb; d = 3-1 kb. lane 1, control (no heat shock); lane 2, heat shock with no recovery; lane 3, 0-5 h recovery; lane 4, 1 h recovery; lane 5, 1-5 h recovery; lane 6, 2 h recovery; lane 7, 4 h recovery.

In contrast to the developmental pattern of hsp 70, hsp 87 and ubiquitin gene expression, we have demonstrated by Northern hybridization analysis that hsp 30 mRNA (1-1 kb) accumulation is not heat-inducible until the tailbud stage (stage 30–34). Although Bienz (1984a) reported a similar pattern of stage-dependent hsp 30 mRNA accumulation, she did not detect hsp 30 mRNA until the free-swimming tadpole stage (stage 42). The reasons for the discrepancy between the two laboratories with respect to the stage at which hsp 30 mRNA accumulation is first heat-inducible are unclear at present. The lack of any hybridizable hsp 30 messages in heat-shocked neurulae was not due to the stress being insufficient to elicit a heat-shock response since hsp 70 mRNA and increased levels of ubiquitin mRNA were detectable in these samples. The mechanism involved in the stage-dependent expression of hsp 30 genes is not clear. It has been suggested that a low level of heat-shock factor (HSF) present in *Xenopus* embryos is entirely sequestered by hsp gene promoters (e.g. hsp 70) having a higher HSF-binding affinity than the hsp 30 gene promoter (Bienz, 1984a). This seems unlikely given the recent estimate of at least 2000 molecules of HSF per eukaryotic cell (Wu et al. 1987). Another possibility is that the heat-induced expression of the hsp 30 genes is controlled by an inhibitor which is removed.

Fig. 7. Relative levels of α-cardiac actin mRNA in tadpoles during continuous exposure to a 33°C heat shock (panel A) and during recovery at 22°C following a 1 h heat shock at 33°C (panel B). Total RNA (10 μg) was analysed by Northern hybridization as outlined in the legend to Fig. 1 and then hybridized to the *Xenopus* α-cardiac actin cDNA clone. Size of transcript: a = 1-6 kb. (A), lane 1, control (22°C for 1 h); lane 2, 33°C for 0-25 h; lane 3, 33°C for 0-5 h; lane 4, 33°C for 1 h; lane 5, 33°C for 1-5 h; lane 6, 33°C for 2 h; lane 7, 33°C for 3 h; lane 8, 33°C for 4 h. (B), lane 1, control (no heat shock); lane 2, heat shock with no recovery; lane 3, 0-5 h recovery; lane 4, 1 h recovery; lane 5, 1-5 h recovery; lane 6, 2 h recovery; lane 7, 4 h recovery.
by the tailbud stage. It will be interesting to determine whether the acquisition of the ability to express hsp 30 genes is the result of, or coincides with, some major biochemical or morphological transition during development.

The *Xenopus* ubiquitin cDNA probe detected a total of four transcripts, with sizes of 1-7, 2-3, 2-5 and 3-1 kb, which were present in all developmental stages examined. As well, the levels of all four of these transcripts were enhanced under heat-shock conditions. Since ubiquitin transcripts were detected in control embryos, the increased accumulation of this mRNA in response to heat shock may be the result of increased mRNA stability rather than increased gene transcription. This may be unlikely given the findings of Bond & Schlesinger (1986) who have shown that in chicken embryo fibroblasts the half-life of constitutive ubiquitin mRNA is unchanged during heat shock whereas the heat-inducible species is less stable than actin or hsp 70 mRNA.

Although the hsp 30 genes display a different developmental regulation than the hsp 70 and ubiquitin genes, their pattern of expression in free-swimming tadpoles during continuous heat-shock and recovery experiments was similar. Continuous exposure of tadpoles to 33°C resulted in a transient, coordinate accumulation of hsp 30, hsp 70 and ubiquitin mRNA. Peak levels of accumulation occurred after 1-5 h for hsp 30 mRNA and 1 h for hsp 70 and ubiquitin mRNA. Also, a coordinate, temporal pattern of hsp 30, hsp 70 and ubiquitin mRNA decay was observed during recovery of tadpoles at 22°C following a 1 h heat shock at 33°C. We have observed similar, coordinate patterns of hsp 30 and hsp 70 mRNA accumulation and decay during continuous temperature stress and recovery experiments in *Xenopus* cultured kidney epithelial cells (Darasch et al. 1985). Furthermore, the transient pattern of hsp 30 and hsp 70 mRNA accumulation observed in tadpoles at 33°C is very similar to the pattern of hsp 70 gene expression reported in *Xenopus* neurulae (Heikkila et al. 1987b). Thus, once the hsp 30 genes become heat inducible at the tailbud stage, their expression appears to be regulated in a fashion similar to hsp 70 and ubiquitin gene expression.

The coordinate patterns of ubiquitin, hsp 30 and hsp 70 mRNA accumulation and decay observed in tadpoles during continuous heat-shock and recovery experiments suggests that the genes encoding these mRNAs are regulated by a common heat-inducible control mechanism. Indeed, the chicken (Bond & Schlesinger, 1986) and yeast (Finley & Varshavsky, 1985; Finley et al. 1987) polyubiquitin genes, both of which are heat-inducible, have been shown to possess the heat-shock consensus element (HSE; C--GAA--TTC--G; Pelham, 1982) in their upstream promoter sequences. This sequence, which is also present in the *Xenopus* hsp 30 and hsp 70 gene promoters (Bienz, 1984b), is probably the binding site of the *Xenopus* heat-shock factor (HSF; Bienz & Pelham, 1982; Pelham & Bienz, 1982; Wu et al. 1987). In *Drosophila*, HSF has been shown to stimulate the transcription of the hsp 70 gene(s) (Wu et al. 1987). Thus, the transient pattern of hsp 30, hsp 70 and ubiquitin mRNA accumulation observed in heat-shocked tadpoles may be mediated at the transcriptional level by a decrease in the cellular levels of active HSF. Alternatively, the decrease in hsp mRNA levels may be due to a decrease in mRNA stability. Such a decrease in stability could also act in unison with decreased hsp gene transcription since it has been proposed that hsp 70 synthesis in *Drosophila* is auto-regulated by a decrease in hsp 70 gene transcription as well as a destabilization of hsp 70 mRNA (Didomenico et al. 1982a,b).

The levels of α-cardiac actin mRNA in tadpoles during time course and recovery experiments were found to be unaffected by heat shock. Uniform levels of α-cardiac actin message (1-6 kb) were maintained in tadpoles during continuous exposure at 33°C as well as during recovery at 22°C following a 1 h heat shock at 33°C. Constant levels of actin mRNA throughout heat shock and recovery have also been reported for cultured *Drosophila* cells (DiDomenico et al. 1982a). However, transcription of actin genes is reduced by 85-95 % shortly after cells are exposed to elevated temperatures and continues at such a rate for up to 3 h (Findly & Pederson, 1981). Thus, existing actin mRNA in *Drosophila* cells is stably maintained during heat shock. Heat-shock-induced stabilization has also been reported for control messages other than actin in *Drosophila* cultured cells (Storti et al. 1980), *Drosophila* pupae (Peterson & Mitchell, 1982), and cultured tomato cells (Nover & Scharff, 1984). The unchanging levels of α-cardiac actin mRNA observed during heat shock and recovery of *Xenopus* tadpoles in the present study may well be due to a similar type of messenger RNA stabilization.

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