DNA sequence-specific binding activity of the heat-shock transcription factor is heat-inducible before the midblastula transition of early Xenopus development

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Summary

We have examined the activity of the Xenopus heat-shock transcription factor (HSF) in extracts from stressed and unstressed embryos at various stages of development using DNA mobility shift analysis. A specific interaction between HSF and a synthetic oligonucleotide corresponding to the proximal heat-shock element (HSE) of the Xenopus HSP70B gene was greatly enhanced in heat-shocked embryos compared to controls. HSF binding was inducible at all developmental stages examined including pre-midblastula transition (MBT) stages which are incapable of expressing HSP genes. In time-course experiments with both cleavage and neurula stage embryos, the activation of HSF binding was rapid and transient. Removal of cleavage and neurula stage embryos from heat stress resulted in a rapid loss of binding activity. The molecular mass of HSF, as determined by comparative gel electrophoresis of photoaffinity-labeled factor was $88 \times 10^3$ in both heat-shocked cleavage and neurula stage embryos. These experiments suggest that maternally derived HSF is stored in pre-MBT embryos in a heat-activatable form and may function in the regulation of heat-shock genes immediately after the MBT.

Key words: Xenopus, midblastula transition, development, HSF, heat shock, transcription factor.

Introduction

During early development, Xenopus cleavage and early blastula stage embryos are transcriptionally dormant. Activation of the zygotic genome commences after twelve cleavage cycles at a crucial point in the development of the frog embryo known as the midblastula transition (MBT; Brown and Littna, 1964; Newport and Kirschner, 1982a,b). The mechanism by which Xenopus embryos remain transcriptionally dormant until the 4000-cell midblastula stage, and then activate selected genes (Sargent and Dawid, 1983; Krieg and Melton, 1985), is not fully understood. The most widely accepted model explaining the transcriptional dormancy involves an overabundance of a putative factor that prevents a G phase in the cell cycle by rapidly triggering the onset of mitosis after S phase; when a critical nucleus-to-cytoplasm ratio is reached after 12 cell divisions, this factor is titrated resulting in a slower cell cycle allowing transcription to occur (Newport and Kirschner, 1982a,b; Newport et al. 1985; Kimelman et al. 1987).

The mechanism for the activation of certain genes at the MBT has not been established. However, it is generally believed that the transcription factors responsible for gene expression at the MBT are of maternal origin. We have recently found DNA-binding proteins specific to CCAAT and ATF/API-like sequences in nuclear extracts from early blastula stage embryos, and demonstrated that they may be involved in transcription of a microinjected reporter gene at the late blastula stage (Ovsenek et al. 1990). Also, Mohun et al. (1989a,b) demonstrated that a binding activity associated with the cardiac actin CARG promoter element was present in unfertilized Xenopus eggs. These findings demonstrate that at least some transcription factors are maternally derived and may function immediately after the MBT.

Among the subset of zygotic genes that are selectively transcribed or inducible at the MBT are a number of the heat-shock protein (HSP) genes including HSP70 (Bienz, 1984a; Heikkila et al. 1985; 1987). The transcriptional activation of all heat-shock genes is mediated by the heat-shock element (HSE; Pelham, 1982; Pelham and Bienz, 1982). The presence of one or more HSEs is a common feature in the 5'-flanking sequences of HSP genes. For example, the Xenopus HSP70B promoter contains three HSEs, which confer heat inducibility to a covalently linked $\beta$-globin gene in mammalian cells and Xenopus oocytes (Bienz, 1984b; Bienz and Pelham, 1986). Heat-inducible synthesis of
HSP mRNA is thought to be mediated in part by an interaction between the HSE and a transcriptional activating protein known as the heat-shock factor (HSF). These sequence specific binding proteins have been identified in *Drosophila*, yeast, mouse and HeLa cells, and more recently in *Xenopus* XTC cells. In yeast, HSE–HSF complexes are formed in the absence of stress and transcription appears to be activated after phosphorylation of the bound factor (Sorger et al. 1987; Jakobsen and Pelham, 1988; Zimarino et al. 1990). In most studies with human and *Drosophila* cells, an HSF–HSE complex is formed following heat shock (Wu, 1984; Parker and Topol, 1984; Topol et al. 1985; Wu, 1985; Sorger et al. 1987; Larson et al. 1988). However, Mosser et al. (1988) have reported high levels of constitutive binding activity in HeLa cells. Mouse embryonal carcinoma (EC) cells also contain high levels of constitutive HSE-binding activity in contrast to murine fibroblasts, which exhibit HSF binding only following heat stress (Mezger et al. 1989). Induction of HSF binding has been demonstrated in the absence of protein synthesis in human, *Drosophila* and *Xenopus* cells indicating that a post-translational modification is involved (Kingston et al. 1987; Zimarino and Wu, 1987; Zimarino et al. 1990). To date, however, little is known about the behaviour of HSF during vertebrate embryogenesis.

In the present study, we are interested in the mechanism of the stage-dependent and heat-inducible regulation of HSP genes during *Xenopus* development. More specifically, we have addressed the question of whether the inability to express HSF genes before the MBT is due to an absence of active HSF. Our DNA mobility shift data demonstrates that heat-activatable *Xenopus* HSF is present in extracts from early embryonic stages, before they acquire the capability to synthesize HSP mRNA. The pattern of HSF activation during continuous stress and recovery is similar in pre- and post-MBT stages indicating that the mechanism of HSF-binding modulation is also present during early development. As well, the molecular mass of HSF (88×10^3) is identical at both developmental stages. This demonstrates that a heat-activatable maternal form of HSF is stored in early *Xenopus* embryos and may be involved in the heat-inducible expression of HSP genes immediately after the MBT. Furthermore, the inhibition of HSP70 gene expression prior to the midblastula stage is not due to an absence of an activatable HSE–HSF interaction.

**Materials and methods**

**Embryo maintenance and microinjection**

*Xenopus laevis* eggs were obtained, fertilized, dejelled and maintained in Steinberg’s solution as described in Heikkila et al. (1985). Embryos were staged according to Nieuwkoop and Faber (1956). Only normally developing embryos were used for extracts.

**Whole-cell and nuclear extraction procedure**

Whole-cell embryo extracts were obtained essentially as described by Mohun et al. (1989a; Method 2). Embryos were homogenized using a Dounce homogenizer with the A pestle in buffer C (Dignam et al. 1983; TFIIIA purification from mature ovary tissue), plus 2 μg/ml aprotinin and leupeptin. For each homogenization 10 μl of buffer was used per embryo, and each sample contained at least 50 embryos. Homogenates were centrifuged at 4°C for 5 min at 14,000 revs min−1 in a microfuge after which the supernatant was carefully removed avoiding the yolk pellicle and recentrifuged as above. The resultant supernatant was frozen at −80°C.

Nuclear protein extracts were prepared from late neurula stage embryos using the method described in Mohun et al. (1989b) with minor modifications (Ovsenek et al. 1990). Embryos were washed in Steinberg’s solution and homogenized (Dounce homogenizer, B pestle) in a buffer containing 2.2 M sucrose, 10 mM Hepes (pH7.6), 15 mM KCl, 0.15 mM spermine, 0.5 mM spermidine, 1 mM EDTA, 1 mM dithiothreitol, 0.5 mM PMSF, 28 μg ml−1 aprotinin, and 10 % glycerol. After a 1 h centrifugation (SW41 rotor, 27,000 revs min−1), nuclei were resuspended in a buffer containing 10 mM Hepes (pH7.6), 100 mM KCl, 0.1 mM EDTA, 3 mM MgCl2, 0.5 mM dithiothreitol, 0.1 mM PMSF, 28 μg ml−1 aprotinin and 10 % glycerol, lysed by homogenization (Dounce homogenizer, A pestle), and incubated on ice for 30 min after addition of KCl to 0.55 M. The chromatin was pelleted (SW41 rotor, 31,000 revs min−1, 1 h), and nuclear proteins in the supernatant were precipitated by addition of 0.3 g ml−1 ammonium sulfate and incubation on ice for 1 h. Protein was pelleted (SW41 rotor, 31,000 revs min−1, 1 h), resuspended in dialysis buffer, dialyzed and frozen at −80°C.

**DNA mobility shift assays**

The standard binding reactions for the DNA mobility shift assays (Fried and Crothers, 1981) contained 5 μl of nuclear protein, or 10 μl (representing one embryo equivalent) of whole cell extract, mixed with 1.0 ng of 32P-end-labeled oligonucleotide (Ovsenek et al. 1990). The sequence of the double-stranded synthetic HSE oligonucleotide corresponds to position −132 to −102 upstream of the transcriptional start site of the *Xenopus* HSP70B gene (5'-(GAATTCAGAACCTGCCTTCCCGAAACTTCGCGGTCG-3'). Each reaction with nuclear or whole-cell extracts contained either 5.0 μg of poly(dI)–poly(dC) (Pharmacia), or 2.0 μg of sheared herring sperm DNA (Sigma), respectively, in 10 mM Tris (pH7.8), 50 mM NaCl, 1 mM EDTA, 0.5 mM DTT, and 5 % glycerol in a final volume of 25 μl. For competition experiments, a 50-fold molar excess of HSE oligonucleotide, or CCAAT oligonucleotide (Ovsenek et al. 1990) was added to the binding reaction. After incubation at 22°C for 20 min, a dye solution (2 μl) containing 0.2 % bromophenol blue, 0.2 % xylene cyanol and 50 % glycerol was added to the reactions which were then loaded directly onto 4 % polyacrylamide gels in 6.7 mM Tris (pH7.5), 1 mM EDTA, and 3.3 mM sodium acetate. Gels were run at 150 V for 3 h, dried, and exposed overnight to Kodak XAR-5 film at −80°C with an intensifying screen. Densitometric scans on appropriately exposed films within the linear range of film density were performed using a Bio-Rad scanning densitometer (model 1650).

**Photoaffinity labeling of HSF**

A 32P-labeled bromodeoxyuridine (BrdU)-substituted HSE oligonucleotide probe was prepared by annealing a 9-base primer 5'CCGCAC(CA)-3' to the strand shown above and filling in with the Klenow fragment of DNA polymerase 1 with dATP, dGTP, [α-32P]dCTP, and a 1:1 ratio of dTTP:BrdU (Wu et al. 1987). Binding reactions using whole-cell extracts from heat-shocked (0.5 h at 33°C) cleavage and neurula stage embryos were performed as described above except that 2 ng
of BrdU substituted probe was used and the total reaction volumes were scaled up threefold to maximize the recovery of complexes. Following incubation, the reactions were irradiated for 30 min at 4°C under a short wave UV transilluminator (Fotodyne) and then loaded directly onto 4% polyacrylamide gels and separated as described earlier. Following electrophoresis, the gel was irradiated with UV for an additional 30 min at 4°C. The gel was then exposed to X-ray film for 2 h to locate the complexes, which were subsequently excised, boiled for 5 min in 10 μl of Laemmli buffer (Laemmli, 1970) and separated on a 10% polyacrylamide–SDS gel. The gel was dried and exposed to Kodak XAR-5 film at -80°C with an intensifying screen.

**Results**

**HSE-binding activity in heat-shocked Xenopus embryos**

To examine the DNA-binding activity of HSF in Xenopus embryos by means of a DNA mobility shift assay (Fig. 1), we used both whole-cell and nuclear extracts obtained from control and heat-shocked neurula stage embryos and a synthetic oligonucleotide corresponding to the proximal HSE of the Xenopus HSP70B gene. While low levels of HSE-binding activity were detected in extracts from unstressed embryos, increased levels were observed in extracts of heat-shocked embryos (compare lanes 2 and 3, 6 and 7). Competition experiments using both the nuclear and whole-cell extracts from neurula stage embryos demonstrated the specificity of the heat-inducible binding activity. The heat-induced HSE-binding activity was competed by the addition of a 50-fold molar excess of unlabeled HSE to the binding reaction in both types of extract (lanes 4 and 8), but not by a 50-fold excess of an oligonucleotide corresponding to the CCAAT box region of the human HSP70 gene (lanes 5 and 9). The additional faster migrating complex present in nuclear extracts was probably due to protein degradation during nuclear extract preparation. The non-specific interactions observed in assays performed with whole-cell extracts were not observed with nuclear extracts (compare lanes 2–5 to lanes 6–9), suggesting that a cytoplasmic factor was involved. The non-specific bands were not affected by heat or addition of competitor HSE DNA, and were relatively constant from sample to sample in all experiments. In subsequent experiments, we used whole-cell extracts rather than nuclear preparations because the extraction procedure was more rapid with minimal loss of material, and resulted in less degradation of transcription factors. For comparative purposes, careful measurements of extract volumes and embryo numbers were made so that we could perform each binding reaction with extract equivalent to one embryo.

**Changes in the levels of heat-induced HSE-binding activity in response to different temperatures**

In order to characterize the heat-inducible properties of HSF, we initially examined the effect of temperature on HSE-binding activity in neurula stage embryos. Embryos were exposed to a range of temperatures for 30 min prior to DNA mobility shift analysis. The effect of temperature on HSE–HSF complex formation is presented in Fig. 2. Treatment of neurula stage em-

![Fig. 1. Formation of an HSE–HSF complex is heat inducible in Xenopus embryos. DNA mobility shift assays were performed using 1 ng of 32P-labeled oligonucleotide corresponding to the proximal HSE of the Xenopus HSP70B gene promoter mixed with extracts from control or heat-shocked neurula stage embryos. Whole-cell extracts were used in lanes 2–5, and nuclear extracts were used in lanes 6–9. Lane 1, unbound probe. Lanes 2 and 6, contain extracts from control embryos. Lanes 3 and 7, contain extracts from heat-shocked embryos (33°C, 30 min). Lanes 4 and 8, contain heat-shocked extracts and 50 ng of unlabeled HSE. Lanes 5 and 9 contain heat-shocked extracts and 50 ng of CCAAT competitor (Ovsenek et al. 1990).](image)

![Fig. 2. Effect of temperature on HSF induction. DNA mobility shift analysis was performed using whole-cell extracts made from batches of embryos incubated at 22°C (control), 27°C, 30°C, 33°C, or 35°C for 30 min. The HSE–HSF complex in each lane was measured by densitometry and is expressed relative to the 33°C value (1.0).](image)
bryos at 27°C resulted in little or no increase in HSE–HSF complex formation relative to the control. Significant induction of binding activity was observed in embryos treated at 30°C, with maximal induction at 33°C and reduced levels at 35°C. At a higher temperature of 37°C, a very low level of HSF induction was observed. This may be due to a deleterious effect of this temperature on the embryos as indicated by a low survival rate in parallel experiments. Thus, the levels of HSF activation appear to be a function of the severity of the heat stress. In subsequent experiments, we chose a heat-shock regime of 33°C for 30 min to maximize the detection of HSE-binding activity.

Detection of heat-inducible HSE-binding activity in pre-MBT embryos

Given that HSP70 transcription is not heat-inducible until the late blastula stage, it was of interest to examine the onset of heat-inducible HSF-binding activity during early embryogenesis. This was determined by DNA mobility shift analysis using extracts made from control and heat-shocked unfertilized eggs, and embryos at various developmental stages (Fig. 3). Very low levels of HSE-binding activity were detected in extracts from unstressed embryos. In contrast, extracts isolated from unfertilized eggs and embryos which had been treated at 33°C for 30 min exhibited a significant increase in complex formation at each stage examined. Densitometric analysis revealed that a 2-fold increase in the control levels of the HSE–HSF complex relative to pre-MBT levels was detected in late blastula stage embryos, and a further 2-fold increase was detected in gastrula and later stage embryos (compare lanes 2,4,6,8,10 and 12). Also, the amount of heat-activated HSF per embryo remained constant during early development until the late blastula stage at which point a 2- to 3-fold increase in HSF levels relative to earlier stages was observed (compare lanes 3,5,7,9,11 and 13). Relatively high levels of heat-activated HSF in post-MBT stages of development were consistently observed in 3 separate experiments, and may reflect an increase in the proportion of HSF molecules that are activatable or an increase in HSF levels due to embryonic HSF gene expression. It was also observed that the relative migration of the HSE–HSF complex did not change significantly during development.

The specificity of the pre-MBT embryo HSE-binding activity was also tested by DNA mobility shift competition experiments. The HSE–HSF complex formed by heat-shocked cleavage stage extracts was competed specifically by the addition of competitor HSE oligonucleotide to the binding reaction, but not by an excess of a non-complementary probe (data not shown).

The pattern of HSF activation is similar in pre- and post-MBT stages during continuous stress and recovery

The formation of HSE–HSF complexes by extracts made from heat-shocked pre-MBT embryos (Fig. 3) is consistent with the idea that a maternal form of HSF is stored in eggs and cleavage stage embryos and may be utilized after the activation of the embryonic genome.

In order to assess the similarity of HSF-induction in pre-MBT and post-MBT stages, a series of continuous stress and recovery experiments were carried out. In time-course experiments, cleavage and neurula stage embryos were continuously exposed to a 33°C heat stress. DNA mobility shift assays revealed that in neurula stage extracts, HSE-binding activity was induced within 5 min (Fig. 4A). HSE-binding activity was maximal between 15 and 90 min, declined by 120 min and approached control levels by 180 min. This rapid and transient pattern of HSF activation in neurula stage embryos resembles the time course of induction in cleavage-stage embryos (Fig. 4A). For example, levels of HSE–HSF complex increased within 5 min, were maximal between 30–90 min, and declined to control levels by 180 min. In parallel band shift experiments using a CCAAT box oligonucleotide, there was no decrease in the levels of CCAAT box binding activity in embryos incubated at 33°C for 3h (data not shown).

Fig. 3. Profile of HSF-binding activity during early embryogenesis. DNA mobility shift assay performed with 1 ng of labeled HSE mixed with whole-cell extracts from control (22°C) and heat-shocked (33°C, 30 min) embryos at different developmental stages (stages according to Nieuwkoop and Faber, 1956). Lane 1, unbound probe; lane 2, control unfertilized eggs; lane 3, heat-shocked unfertilized eggs; lane 4, control cleavage (stage 6); lane 5, heat-shocked cleavage; lane 6, control early blastula (stage 8); lane 7, heat-shocked early blastula; lane 8, control late blastula (stage 9); lane 10 heat-shocked late blastula; lane 11, control gastrula (stage 10); lane 11, heat-shocked gastrula; lane 12, control neurula (stage 19); lane 13 heat-shocked neurula. The HSE–HSF complex is indicated by an arrow.
Fig. 4. (A) Time course of HSF induction in cleavage and neurula stage embryos. Whole-cell extracts were made from cleavage and neurula stage embryos incubated at the control temperature of 22°C (time 0) or continually heat-shocked at 33°C for 5, 10, 15, 30, 60, 90, 120 and 180 min. Densitometric scans were performed on autoradiograms of DNA mobility shift gels. Relative intensities of the HSE-HSF complexes are expressed as a fraction of the value (1.0) assigned to the maximal HSE-HSF signal on the respective autoradiograms. (B) Time course of HSF induction during recovery. Cleavage and neurula stage embryos were treated at 22°C (time 0), or at 33°C for 30 min, and then allowed to recover at 22°C for 5, 10, 15, 30, 60 or 90 min. HSE-HSF levels were measured by densitometry performed on autoradiograms of DNA mobility shift gels, and are expressed relative to the value (1.0) assigned to the most intense band in each experiment.

Also, over 90% of the embryos survived to the swimming tadpole stage indicating that the decline in HSF levels was not due to decreased viability of the embryos.

We also compared HSE-binding activity in cleavage and neurula stage embryos recovering from a 30 min exposure at 33°C (Fig. 4B). At both developmental stages, HSE-binding activity was lost rapidly after removal of the embryos from heat stress. The level of HSF induction decreased dramatically after only 5 min of recovery, and returned to control levels within 30 min. These data imply that the mechanism that modulates HSF-binding activity is functional in pre-MBT stages, and similar to that found in transcriptionally competent post-MBT embryos. Slight variations in the pattern of HSF activation between these embryonic stages probably reflects physiological differences.

Fig. 5. Comparative sodium dodecyl sulphate–polyacrylamide gel electrophoresis analysis of photoaffinity-labeled HSF from heat-shocked cleavage (lane 1) and neurula stage (lane 2) embryos. Heat-shocked extracts from embryos were UV-crosslinked to a α-32P-labeled BrdU-substituted HSE oligonucleotide. The molecular size of Xenopus HSF was determined by comparison to the relative mobility of Coomassie blue stained molecular weight markers (indicated on the left x10^-3) which were run on the same gel.

Comparison of the molecular size of HSF from pre- and post-MBT embryos

The similarity in the electrophoretic mobility of HSE-HSF complexes formed by extracts of both pre- and post-MBT embryos suggested that HSF present in cleavage and early blastula stages may be identical in size to the post-MBT factor. To investigate this possibility, the molecular mass of HSF in cleavage and neurula stage embryos was compared by photoaffinity labeling. A protein with an apparent molecular mass of 88x10^3 was observed after crosslinking extracts made from heat-shocked cleavage stage and neurula stage embryos to BrdU-substituted HSE oligonucleotide (Fig. 5, compare lanes 1 and 2). This finding is consistent with the idea that HSF is stored in a heat-inducible form in cleavage embryos and is involved in the heat-shock response at the MBT.

Photoaffinity labeling of the non-specific complex revealed that two proteins, with apparent molecular masses of 72 and 65x10^3 were involved (data not shown).

Discussion

In previous studies, it was found that the expression of a number of heat-shock genes, including HSP70, was
heat-inducible shortly after the midblastula transition of *Xenopus* embryogenesis (Bienz, 1984a; Heikkila et al. 1985; 1987). Thus, HSP70 is among a select group of genes that are activated or inducible at this point in the development of the frog embryo. The main objective of this study was to determine whether the developmental stage-dependent expression of HSP genes could be related to HSF availability. Using DNA mobility shift assays, we have shown that sequence-specific HSE-binding activity is heat-inducible in *Xenopus* embryos. The heat-inducibility of HSF binding in *Xenopus* embryos is in agreement with previous studies done with human, mouse and *Drosophila* cells (Kingston et al. 1987; Sorger et al. 1987; Goldenberg et al. 1988; Mosser et al. 1988; Mezger et al. 1989), and more recently with *Xenopus* XTC cells (Zimarino et al. 1990).

Interestingly, we detected the presence of HSF in heat-shocked unfertilized eggs and cleavage stage embryos that are incapable of expressing HSP genes. Since pre-MBT embryos are transcriptionally inactive, we were interested in comparing the properties of HSF in cleavage stage and neurula stage embryos. In time-course experiments, in which both cleavage and neurula stage embryos were continuously exposed to heat-shock temperatures, the activation of HSF was rapid and transient, returning to control levels by 2–3 h. Also, during recovery from a brief heat shock, HSF binding declined rapidly in both cleavage and neurula stage embryos. It should be noted that the effect of temperature on HSF activation as well as the temporal pattern of HSF activation and deactivation during continuous heat shock in *Xenopus* neurulae closely correlates with our previous studies examining HSP70 mRNA levels (Heikkila et al. 1987). This finding further supports the possibility that the pattern of HSP70 mRNA accumulation in heat-shocked *Xenopus* neurulae is regulated primarily at the transcriptional level.

The electrophoretic co-migration of HSE–HSF complexes formed with extracts obtained before and after the MBT provided indirect evidence that the maternal form of HSF was similar in size to the zygotic form. We confirmed this by using a UV-crosslinking technique and found that the molecular mass of *Xenopus* HSF is $88 \times 10^3$ in both cleavage and neurula stages. The molecular mass of *Xenopus* HSF is similar to that reported for HSF in HeLa cells (93×10³; Larson et al. 1988), (83×10³; Goldenberg et al. 1988), but smaller than in *Drosophila* SL-2 cells (110×10³; Wu et al. 1987).

All results obtained in the present study suggest that the maternal HSF may be similar to or identical to the embryonic form of HSF. Also, the mechanism by which HSF binding is activated is operative throughout early *Xenopus* development, even in the absence of transcriptional activity. It is possible that a simple mechanism in which heat acts directly to enhance HSF-binding activity functions similarly at early and later stages of development. Alternatively, if additional factors are involved in a more complex mode of HSF activation, then cleavage embryos likely contain a full complement of regulatory components. At present, however, little is known about the induction of HSF binding in eukaryotes except that a post-translational mechanism is likely involved (Kingston et al. 1987; Zimarino and Wu, 1987; Larson et al. 1988; Zimarino et al. 1990). Given our findings, it appears that the mechanism involved in the stage-dependent transcription of heat-shock genes, such as HSP70, is not due to the absence of activatable HSF prior to the MBT. However, it is tenable that additional factors may be required or that a post-translational modification of HSF which may be necessary for transcriptional activation subsequent to HSE binding is blocked prior to the MBT (Larson et al. 1988). It is also possible that the mechanisms associated with the developmental regulation of genes normally activated at MBT also applies to the heat-shock genes. In the model proposed by Newport and Kirschner (1982a,b) and Kimelman et al. (1987), it was suggested that pre-MBT 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. Assuming that HSF found in pre-MBT embryos is functional, it is likely that maternal HSF could be involved in a heat-shock response at the MBT. The obvious advantage of such a mechanism, given the role of HSPs in the acquisition of thermostolerance (Ribewol et al. 1988; Johnston and Kucey, 1988; Heikkila et al. 1985), is that embryos would acquire the ability to transcribe HSP mRNA at the earliest possible time during development.

Since the genes expressed at the MBT are the first to be transcribed, it was often predicted that the transcription factors controlling their expression were of maternal origin. It was unclear, however, if maternal factors were present as mRNAs or as mature proteins. The experiments presented here are among the first direct demonstrations of the presence of the DNA-binding activity of an RNA polymerase II transcription factor prior to the MBT. In an earlier study, we demonstrated that transcription factors which specifically bind to CCAAT and ATF–API-like sequences of the human HSP70 promoter are present in nuclear extracts from early blastula stage embryos and may be involved in the expression of the reporter construct pHb–CAT at the late blastula stage (Ovsenek et al. 1990). In related studies, a transcription factor homologous to the human serum responsive factor (SRF), which interacts with the serum responsive elements (SREs) of the cytoskeletal actin and *c-fos* genes and the CarG element of the muscle-specific cardiac actin gene, has been found in unfertilized eggs (Mohun et al. 1989a,b). Thus, it is clear that pre-MBT embryos contain a variety of transcription factors that may be used later in development.

Since the pool of cellular mRNAs that are stored in the oocyte are gradually replaced as *Xenopus* embryos become transcriptionally active, an intriguing question arises as to the time during development that the zygotic form of HSF replaces the maternal form. Because of the limited quantity of maternal factors, it is probable that HSF derived from transcription of the zygotic genome becomes active at some time shortly after the MBT. We detected an increase in the level of
activated HSF by late blastula stage. However, it is unknown if these increased levels are due to (1) a more efficient activation of maternal HSF; (2) an increase in the translation from maternal mRNA encoding HSF or (3) the activation of the zygotic HSF gene. Undoubtedly, this question and others will be answered with the eventual cloning of the Xenopus HSF gene.

We thank Dick Mosser and Pat Krone for their helpful suggestions, Rick Morimoto for supplying human HSP70 CCAAT box oligonucleotide, and Alan Wildeman for supplying HSF primer. This work was supported by a Natural Sciences and Engineering Research Council of Canada grant to J.J.H., N.O. was supported by an Ontario Graduate Scholarship.

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(Accepted 31 May 1990)