Development 105, 213–222 (1989)
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# The developmental expression of the heat-shock response in *Xenopus laevis*

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## Summary

**We have investigated the *Xenopus* heat-shock response of somatic cells, oocytes and embryos. *Xenopus* defolliculated oocytes displayed a highly variable response to heat shock depending on the culture medium. Intact follicles, however, respond to heat stress by synthesizing an invariant pattern of hsps. Although a subset of the hsp70/68 complex is expressed constitutively in the absence of heat shock in oocytes and embryos (hsc70), actual induction of hsps in response to stress does not occur until the blastula stage when transcription of the zygotic genome is first activated. By gastrulation, the hsps of somatic cells, including members of the hsp30/26 complex, were expressed coordinately in response to heat shock. We further show that *Xenopus* hsps have different solubilities perhaps reflecting their different subcellular locations. The 26000–30000 $M_r$ complex (hsp30/26) was present almost exclusively in a detergent-insoluble fraction, as was 25–50 % of the hsp70/68 complex and greater than 50 % of hsp56, suggesting that these hsps may be associated with the cytoskeleton during a heat shock. In contrast, the other *Xenopus* hsps (hsp86, hsp75 and hsp61) were totally solubilized in a low-salt buffer.**

Key words: heat shock, developmental expression of heat shock, *Xenopus* heat-shock proteins.

## Introduction

The heat-shock response is a cellular response to temperature or chemical stress that most commonly involves (1) the initiation of new gene transcription; (2) a rapid and dramatic increase in the synthesis of a set of polypeptides and (3) a decrease in the rate of synthesis of most cellular proteins (Lindquist, 1986). The heat-shock response has been found in virtually every tissue or cell type that has been examined: in both animal and plant kingdoms and in both eukaryotic and prokaryotic cells. The only exceptions appear to be oocytes (King & Davis, 1987; Zimmerman *et al.* 1983), male germ cells (Zakeri & Wolgemuth, 1987; Schlesinger *et al.* 1982; Bonner *et al.* 1984), sporulating yeast cells (Kurtz *et al.* 1986), and early embryos (Dura, 1981; Muller *et al.* 1985; Roccherri *et al.* 1981; and Heikkila & Schultz, 1984). Recently, Browder *et al.* (1987) have suggested that the medium in which *Xenopus* defolliculated oocytes and eggs are heat shocked can influence their response.

Specific heat-shock proteins (hsps) are expressed independently during development in the absence of a heat shock in *Drosophila* (Zimmerman *et al.* 1983; Palter *et al.* 1986), mouse (Bensaude *et al.* 1983; and Bensaude & Morange, 1983), and yeast *S. cerevesiae* (Kurtz *et al.* 1986). However, in most if not all embryonic systems that have been investigated so far, there is a refractory period starting with the fertilized egg, during which neither transcription nor translation of heat-shock messages can be induced by heat shock. This period corresponds to the time when transcription of the zygotic genome has not yet been initiated and the embryo is utilizing maternal mRNA accumulated during oogenesis.

Coordinate expression of the heat-shock proteins (hsps) in response to a heat shock appears to be a general phenomenon. However, the heat-shock inducibility of the two major hsps of *Xenopus* (hsp70 and hsp30) has been reported to be uncoupled during embryogenesis: hsp70 is heat-shock inducible by the blastula or early gastrula stage but hsp30 has not been detected in pre-tadpole-stage embryos (Bienz, 1984). We have found that a hsp30 (actually a group of polypeptides between 26000 and 30000 $M_r$, hsp30/26) is not solubilized by the simple buffer extraction methods commonly used to examine amphibian oocyte and embryo proteins (Knowland, 1974). Only the

presence of a strong ionic detergent (sodium dodecylsulphate, SDS) in the homogenization buffer allows the extraction of these polypeptides and their detection by polyacrylamide gel electrophoresis. After re-examining the expression of hsps in *Xenopus* embryos using SDS extraction, we found that the synthesis of both a hsp30/26 group, perhaps different from the hsp30 described by Bienz (1984), and hsp70 *are* detected at gastrulation when the embryo acquires the ability to respond to heat shock. Furthermore, we report that other hsps display different solubilities, suggesting different subcellular locations for the hsps.

## Materials and methods

*Labelling of follicle cells, oocytes and embryos*

Stage-6 (Dumont, 1972) follicles (oocytes with their investing follicle layer), collagenase-treated oocytes and follicle cell layers were obtained from mature female *Xenopus laevis* (South African Snake Farm) and cultured in O-R2 (82 mM-NaCl, 2·5 mM-KCl, 1·0 mM-$CaCl_2$, 1·0 mM-$MgCl_2$, 1·0 mM-$Na_2HPO_4$, 5 mM-Hepes, pH 7·8, 3·8 mM-NaOH) (Wallace *et al.* 1973) or modified Barth's solution (MBS-H: 88 mM-NaCl, 1·0 mM-KCl, 0·33 mM-$Ca(NO_3)_2$, 0·41 mM-$CaCl_2$, 0·82 mM-$MgSO_4$, 10 mM-Hepes, pH 7·4) as described previously (King & Davis, 1987). Sometimes O-R2 and MBS-H were supplemented with 1 mM-oxaloacetic acid and 1·15 mM-pyruvic acid as indicated in the text (Eppig & Dumont, 1976). The follicle cell layer contains follicle cells as well as fibroblasts, endothelial cells and red blood cells of the ovarian theca layer, but the predominant cell type is the follicle cell. Oocytes were defolliculated by treatment with 0·15 % collagenase (Sigma Type IV) (King & Davis, 1987).

Ovulated eggs were squeezed from the body cavity of females injected 16 h earlier with 1000 i.u. of human chorionic gonadotropin (hCG). Ovulated eggs were dejellied in 5 mM-dithiothreitol in 0·05 M-Hepes (Kirschner *et al.* 1980) and incubated in either MBS-H, supplemented MBS-H or 100 % De Boer's solution (110 mM-NaCl, 1·3 mM-$CaCl_2$, 1·3 mM-KCl, pH 7·2 with $NaHCO_3$ (Katagiri, 1961) as described by Browder *et al.* (1987). Ovulated eggs were preincubated at either 22 °C or 33 °C for 15 min and then labelled for 1 h by the addition of 500 $\mu$Ci ml$^{-1}$ [$^{35}$S]methionine to the incubation medium. Temperatures higher than 33 °C resulted in the cytolysis of the eggs.

*Xenopus* embryos were obtained from pairs that were induced to mate by injection of hCG. Embryos were dejellied and 2- to 4-cell embryos were labelled by pressure injection of 6 nl of [$^{35}$S]methionine ($5\times10^5$ cts min$^{-1}$ nl$^{-1}$) into each blastomere ($1·5–2\times10^6$ total cts min$^{-1}$ per embryo). Injected embryos were cultured individually in 0·5 ml of Steinberg's solution (33 °C) in plastic caps in a 33 °C water bath for 20 min. Heat-shock treatments exceeding 20 min resulted in the cytolysis of cleavage-stage embryos. Following heat shock, embryos were cultured an additional 60 min at 25 °C to maximize incorporation of label into any heat-shock protein. Control embryos were cultured continuously for 80 min at 22 °C. Stage-9½ to -10 embryos (gastrulae) (Nieuwkoop & Faber, 1964) were labelled with a single injection of 12 nl [$^{35}$S]methionine ($5\times10^5$ cts min$^{-1}$ nl$^{-1}$) into the blastocoel and cultured as were the 2-cell embryos, or alternatively, gastrulae were labelled for 4 h with identical results. Embryos were frozen in liquid $N_2$ and stored at −90 °C.

*Sample preparation and gel electrophoresis*

Total proteins were extracted and prepared for SDS–PAGE by homogenizing samples directly in Laemmli sample buffer (Laemmli, 1970) with a dounce fitted to an Eppendorf tube. Homogenization was carried out through three cycles using 10 $\mu$l, 10 $\mu$l, and finally 5 $\mu$l for each oocyte or embryo or follicle cell sample (follicular layers from 20 oocytes). At each cycle, samples were boiled 3 min and the 10 000 *g* supernatants pooled. For two-dimensional gel analysis, frozen samples were homogenized in 10 $\mu$l of lysis buffer (O'Farrell, 1975), 300 mM-NaCl and 0·4 mg ml$^{-1}$ protamine sulphate, incubated at room temperature for 30 min, sonicated three times for 30 s each, and the supernatants (10 000 *g* for 10 min) saturated in urea by the addition of urea crystals. Any particulate matter was removed by centrifugation (10 000 *g* for 15 min) just before loading the first dimension.

Alternatively, proteins were fractionated according to their different solubility characteristics. Soluble proteins were extracted by homogenization in 10 $\mu$l of 10 mM-Tris–HCl (pH 6·8), 100 mM-NaCl, 300 mM-sucrose, 1·2 mM-phenylmethyl sulphonylfluoride (PMSF) at 4 °C (solution SOL). Homogenates were cleared at 10 000 *g* for 5 min and supernatants removed. The above procedure was repeated through two more cycles of homogenization and the supernatants pooled and frozen in liquid $N_2$. The remaining pellets were homogenized in 10 $\mu$l per oocyte of the same buffer plus 0·5 % Triton X-100 (solution TRIT). The pellet remaining after two extractions with TRIT was homogenized in 5 $\mu$l per oocyte of a buffer composed of 2 % SDS, 20 mM-Tris–HCl (pH 8·0), 1 % 2-mercaptoethanol, 20 mM-EDTA, and 1·2 mM-PMSF (solution SDS). The homogenates were boiled for 3 min, cooled and supernatants collected as described above.

Incorporation of [$^{35}$S]methionine into protein was monitored by liquid scintillation counting of trichloroacetic acid (TCA) precipitates. Supernatants were brought to ×1 in either Laemmli sample buffer for SDS–PAGE or lysis buffer for IEF-SDS–PAGE. Quantification of gel slices and autoradiograms (Laskey & Mills, 1975) was as described in King & Davis (1987). The heat-shock proteins analysed by two-dimensional PAGE were quantified using a computer program developed by Garrison & Johnson (1982) according to procedures outlined by King & Barklis (1985).

## Results

*Heat-shock response of somatic cells: follicle cells and fibroblasts*

In order to determine whether the heat-shock response in embryonic cells is identical to the one observed in fully differentiated somatic cells, the heat-shock response of follicle cells and fibroblasts was more thoroughly characterized. At temperatures greater than 28 °C there were two noticeable effects: (1) the total rate of protein synthesis declined, and (2) a specific set of polypeptides was selectively synthesized (Fig. 1). These somatic cells incorporate about 34 600 cts min$^{-1}$ h$^{-1}$ per 1000 cells into TCA-precipitable material at 22 °C, whereas heat-shocked cells (35 °C) showed a decrease in the rate of incorporation to about 23 000 cts min$^{-1}$ h$^{-1}$ per 1000 cells. The major heat-shock polypeptide migrates as a 70 000–68 000 $M_r$ doublet after SDS–PAGE, and is resolved as a complex of five polypeptides focusing at a pI of about 5·6 after IEF–SDS two-dimensional PAGE (King & Davis,

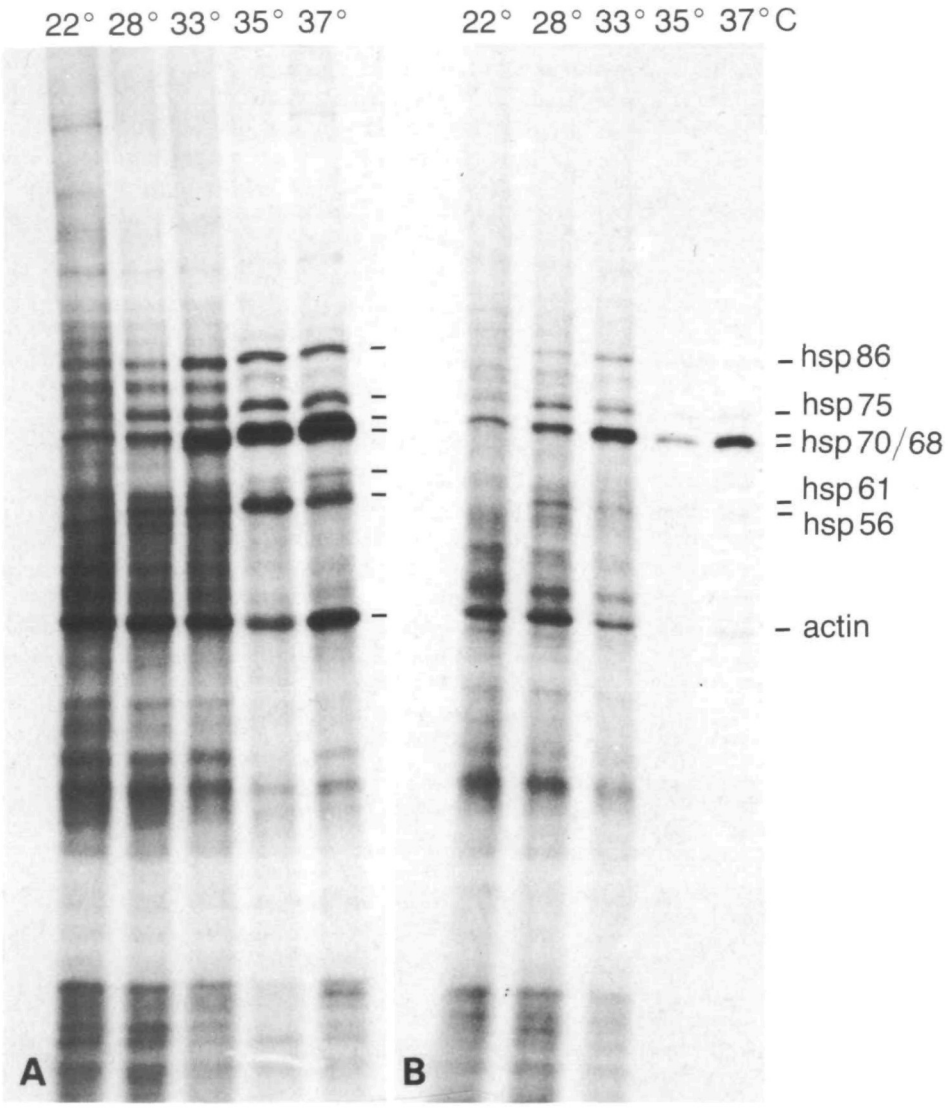


**Fig. 1.** Pattern of soluble proteins synthesized in follicles at increasing temperatures. Individual stage-6 follicles were incubated at the temperatures indicated for 20 min then labelled with [$^{35}$S]methionine (500 $\mu$Ci ml$^{-1}$) for 2 h at the respective temperatures (°C). Equal TCA precipitable cts min$^{-1}$ per lane (A) or equal protein/lane (B) were loaded.

1987). Approximately 46 % of the radioactive label was in the hsp70/68 complex.

Minor and variably expressed heat-shock polypeptides of approximate relative molecular masses of 86 000 $M_r$ (hsp86), 75 000 $M_r$ (hsp75), 61 000 $M_r$ (hsp61), and 56 000 $M_r$ (hsp56) were also observed in the somatic cells. These proteins were frequently present in cells cultured at 22 °C as well. With increasing temperature, these hsps showed only a two- to threefold increase in their relative rate of synthesis (Fig. 1A,B).

Another major hsp, hsp30, has been reported for *Xenopus* somatic cells (Bienz, 1982; 1984), but we were unable to detect it in our samples. Since approximately 50 % of the TCA-precipitable cts min$^{-1}$ were in the pellet, we examined total cellular protein by extracting heat-shocked cells directly in Laemmli sample buffer (containing 2 % SDS) or IEF sample buffer (containing 4 M-urea). Only after this treatment were a group of polypeptides of approximately 26 000 to 30 000 $M_r$ (hsp30/26) detected (Fig. 2). This complex focused between pIs of approximately 5·0 and 5·9 on autoradiograms of two-dimensional gels of heat-shocked samples (Fig. 3D).

*Different solubilities of the heat-shock polypeptides in follicles*

The results described above suggested that (1) a subset of heat-shock proteins might have different subcellular locations and (2) the failure to detect hsp30/26 prior to tadpole stages in earlier studies (Bienz, 1984; Heikkila *et al.* 1985; Nickells & Browder, 1985) should be re-examined. As a first step towards determining if the hsps found in somatic cells have different subcellular locations, we asked if the hsps could be differentially extracted from the cell. Cells were metabolically labelled with [$^{35}$S]methionine at either 22 °C or 35 °C. Proteins solubilized in solution SOL at 4 °C were extracted until no more proteins were eluted as determined by SDS–PAGE. The pellet that remained was extracted with solution TRIT (containing the nonionic detergent Triton X-100 and 100 mM-NaCl) in a similar fashion. This buffer removes most of the yolk and lipid

from the sample. After this treatment, a pellet still remained that could only be solubilized after boiling in solution SDS (see Materials and methods). [$^{35}$S]methionine-labelled proteins from each extraction were analysed by SDS–PAGE and proteins of interest quantified by densitometry. The data show that hsp70/68 is present in all three fractions and increased three- to fivefold per unit protein over constitutive levels after heat shock (Figs 2 and 3). The hsp30/26 group and hsp56 are absent in nondetergent samples. Hsp30/26 is solubilized almost exclusively by solution SDS. Per unit protein, the synthesis of hsp30/26 increased tenfold over background levels. Hsp56 is distributed equally between the TRIT and SDS fractions, increasing over 25-fold in its relative rate of synthesis in each fraction (Figs 2, 3D). The variably expressed hsps 86, 75 and 61, are seen in the SOL samples at constitutive levels (per unit protein) (Figs 1 and 2) but are not observed in TRIT or SDS samples analysed by two-dimensional gel electrophoresis (Fig. 3, only the SDS sample is shown). These results are summarized in Table 1.

*Effect of heat shock on oocytes*

Recently, we have argued that there is no convincing evidence for a heat-shock response in *Xenopus* oocytes (King & Davis, 1987). However, stage-6 oocytes that are completely defolliculated and cultured at 22 °C do synthesize polypeptides that comigrate with two of the polypeptides in the hsp70/68 complex, as determined by two-dimensional gel analysis (Fig. 4). After heat shock, these polypeptides do not increase in their rate of synthesis as does the hsp70/68 complex (compare Figs 4B and 3B). We refer to these proteins as the constitutive 70 000 $M_r$ proteins (hsc70). We examined protein synthesis in heat-shocked oocytes using the differential extraction methods already described to look specifically for the hsp30/26 complex or hsp56 in the SDS fraction or hsp86, hsp75, or hsp61 in the SOL fraction and again found no evidence for a heat-shock response in oocytes (Fig. 4). In fact, in Fig. 4 four times the amount of protein was loaded for the heat-shocked samples, underscoring the absence of a detectable heat-shock response. Unlike hsp70 in follicles, no hsc70 is evident in the SDS fraction of non-heat-shocked oocytes (Fig. 4C). All of the hsc70 labelled at ambient temperature with [$^{35}$S]methionine is apparently solubilized in the SOL and TRIT buffers. A small and variable amount of hsc70 labelled with [$^{35}$S]methionine in heat-shocked oocytes is detectable on autoradiograms of SDS samples, suggesting that hsc70 may translocate at

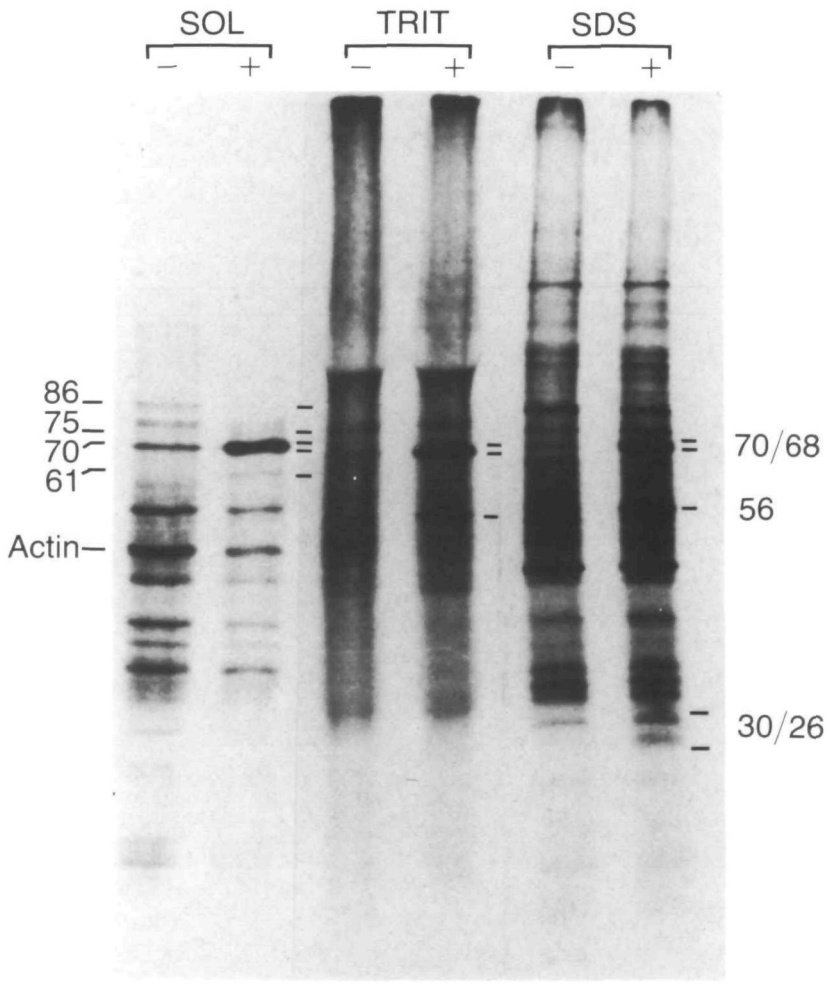


**Fig. 2.** Subcellular fractionation of *Xenopus* follicles. Follicles (follicle cells plus oocytes) were heat shocked and labelled with [$^{35}$S]methionine (500 $\mu$Ci ml$^{-1}$) for 2 h at 35 °C (+) or 22 °C (−). Follicles were processed by extraction with a series of buffers into three fractions: soluble (SOL) (10 mM-Tris–HCl, pH 6·8; 100 mM-NaCl; 300 mM-sucrose; 1·2 mM-PMSF); non-ionic detergent (TRIT) extractable (SOL plus 0·5 % Triton X-100); and sodium dodecyl sulphate (SDS) extractable (20 mM-Tris–HCl, pH 8; 2 % SDS, 1 % 2-mercaptoethanol; 20 mM-EDTA; 1·2 mM-PMSF). Equal TCA-precipitable counts per lane were loaded for each fraction (SOL, TRIT and SDS) for heat-shocked (+) and control (−) samples. For the SOL and TRIT samples this represents a twofold increase and for the SDS sample a threefold increase in the amount of protein loaded per lane for the heat-shocked (+) samples as compared to controls (−).

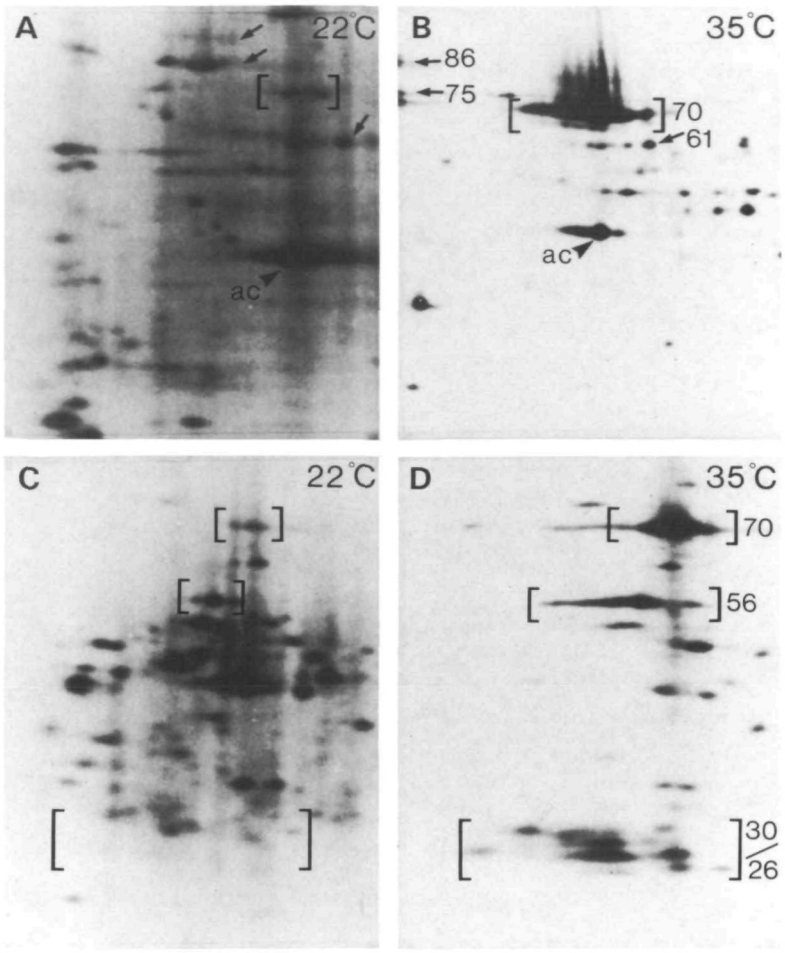


**Fig. 3.** Effect of heat shock on *Xenopus* follicle cells. Follicles were labelled with [$^{35}$S]methionine at 22°C (A and C) or 35°C (B and D) and follicle cells were isolated and processed into SOL, TRIT and SDS fractions as described in Fig. 2. SOL (A and B) and SDS (C and D) fractions were analysed by 2-D IEF–PAGE. Brackets indicate the location of the hsp70/68, hsp56 and hsp30/26 complex. Arrows indicate the location of hsps 86, 75 and 61. Equal TCA-precipitable cts min$^{-1}$ were loaded. For the SOL and SDS samples this represents a twofold increase in the amount of protein loaded per gel for the heat-shocked samples (B and D) as compared to the controls (A and C). The acid end of the gel is on the left. Only a portion of each gel is shown. *ac*, actin.

**Table 1.** *Subcellular fractionation of hsps in* Xenopus *follicles after heat shock*

| | SOL* | TRIT† | SDS‡ |
|---|---|---|---|
| *Major hsps*: | | | |
| hsp70/68 | 46% | 36% | 18% |
| hsp30/26 | N.D.§ | N.D. | 100% |
| *Minor and variable hsps*: | | | |
| hsp86 | 100% | N.D. | N.D. |
| hsp75 | 100% | N.D. | N.D. |
| hsp61 | 100% | N.D. | N.D. |
| hsp56 | minor & variable | 41% | 59% |

* *Xenopus* follicles were first homogenized in SOL buffer (10 mM-Tris–HCl, pH 6·8; 100 mM-NaCl; 300 mM-sucrose; 1·2 mM-PMSF). Samples were analysed by SDS–PAGE and bands corresponding to hsps were quantified by densitometry and expressed as a % per unit protein.

† Material pelleted after extraction with SOL buffer was homogenized in TRIT buffer (SOL buffer plus 0·5% Triton X-100).

‡ Material pelleted after extraction with SOL and TRIT buffers was homogenized in SDS buffer (20 mM-Tris–HCl, pH 8; 2% SDS; 1% 2-mercaptoethanol; 20 mM-EDTA; 1·2 mM-PMSF) and analysed by SDS–PAGE.

§ N.D. = not detected.

higher temperatures (Fig. 4D).

In contrast to our results and to those of Horrell *et al.* (1987), Browder *et al.* (1987) have reported a heat-shock response in oocytes and eggs cultured in MBS supplemented with the organic acids pyruvate and oxaloacetate. We have repeated their experiments and have loaded equal protein per lane for a more direct comparison. We find that, indeed, changing the culture conditions can lead to different and variable responses in defolliculated oocytes. However, intact follicles, regardless of the culture conditions, respond in an identical fashion to heat stress (Fig. 5). Defolliculated oocytes cultured in O-R2 supplemented with organic acids show a very minor (twofold) increase in the synthesis of a 70000 $M_r$ protein. Contamination of our oocyte sample with just thirty follicle cells (there are approximately 6000 ± 1000 per oocyte; Horrell *et al.* 1987) would give the same result (King & Davis, 1987). If oocytes from the same female are cultured in supplemented MBS, they show a threefold increase in the synthesis of hsp56 and hsp75 over control levels per unit protein, but now there is no increase in the 70000 $M_r$ protein (Fig. 5). Microinjection of organic acids into intact follicles has no effect on the pattern of protein synthesis (data not shown).

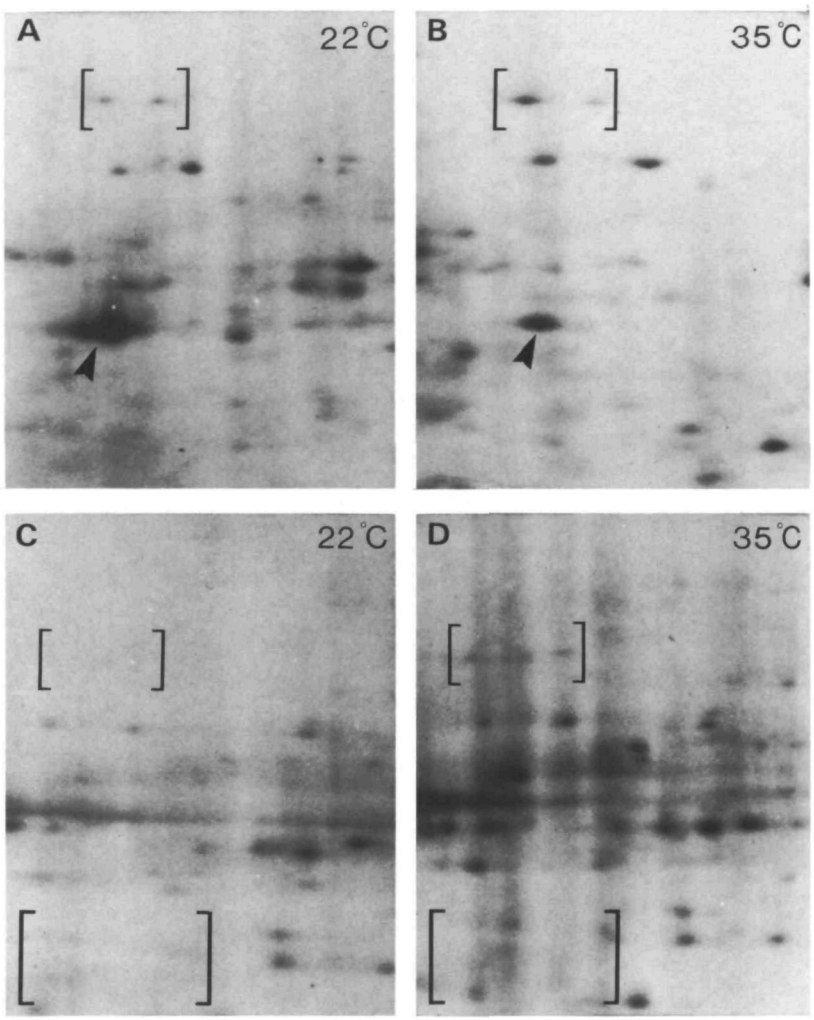


**Fig. 4.** Effect of heat shock on defolliculated oocytes. Defolliculated oocytes (collagenase-treated) were labelled with [$^{35}$S]methionine at 35°C (B and D) or 22°C (A and C) and processed into SOL, TRIT and SDS fractions as described in Fig. 2. SOL (A and B) and SDS (C and D) fractions were analysed by 2-D IEF–PAGE. Equal TCA-precipitable cts $min^{-1}$ were loaded in each case. Fourfold more protein was loaded in B and D than in A and C. The acid end of the gel is on the left. Arrows indicate the position of actin. Brackets show the positions of hsp70/68 and hsp30/26.

*Heat-shock response of embryos*

Early-cleavage-stage embryos are much less thermotolerant than oocytes, perhaps because these embryos are dividing every 30 min. Preliminary experiments showed that temperatures above 33°C were lethal to early-cleavage-stage embryos even for time points as short as 15 min. After 30 min at 33°C, cytolysis and discolouration of embryos were evident. Therefore 2- to 4-cell embryos were heat shocked at 33°C for 20 min and allowed to recover at 25°C for an additional 60 min. Under these conditions, embryos developed normally. Although temperatures above 28°C will induce hsps in somatic cells in less than 10 min, the patterns of protein synthesis in heat-shocked and control ovulated eggs and cleavage-stage embryos were indistinguishable, even though three to five times as much protein was loaded per lane for the heat-shocked samples (Fig. 6). Using culture conditions identical to those described for spawned eggs (Browder *et al.* 1987, see Materials and methods), we again failed to detect a heat-shock response (data not shown). None of the follicle cell hsps was detected in cleavage-stage embryos heat shocked for 20 min at 33°C. In fact, no heat-shock response was detected prior to stage 8 (blastula), regardless of the culture conditions used.

Examination of the pattern of total proteins synthesized at temperatures 33°C and above revealed that all of the hsps detected in follicle cells are also detected in stage-10 to -13 embryos (gastrula stage; compare Figs 7B and D to Figs 3B and D). However, the hsp30/26 follicle cell set does not appear to be identical to that expressed in the gastrulating embryo. First, the complex pattern of polypeptide spots induced in adult somatic cells overlaps with the embryonic pattern but is not identical to it. Second, some members of the gastrula hsp30/26 complex are soluble in the SOL buffer in contrast to the follicle cell hsp30/26 family that is extracted only in the SDS buffer.

Both hsp70/68 and hsp30/26 are being synthesized by gastrula-stage embryos at six and fivefold higher rates, respectively, per unit protein when compared to control embryos. This means that hsp70/68 and a hsp30/26 set are expressed coordinately in development (data summarized in Table 2). Some constitutive synthesis of 70 000 $M_r$ polypeptides (apparently the same two polypeptides we refer to as hsc70 in oocytes) occur in non-

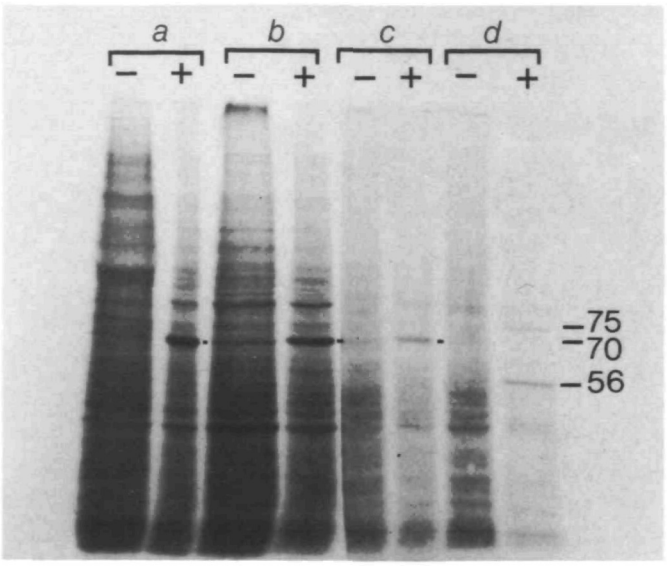


**Fig. 5.** Effect of the culture medium on the heat-shock response. Proteins synthesized in heat-shocked (+) and control (−) follicles (*a* and *b*) and defolliculated oocytes (*c* and *d*) cultured in supplemented O-R2 (*a* and *c*) or in supplemented MBS-H (*b* and *d*). Equal protein was loaded per lane.

heat-shocked gastrulae. As in oocytes, these constitutively synthesized 70 000 $M_r$ polypeptides are not found in the SDS fraction of non-heat-shocked embryos (Fig. 7C). These constitutively expressed 70 000 $M_r$ polypeptides are immunologically related to the *Xenopus* hsp70 (manuscript in preparation).

## Discussion

*Xenopus* somatic cells respond to elevated temperatures by inducing the *de novo* synthesis of two major proteins: a 70 000 $M_r$ protein (hsp70) (Ketola-Pirie & Atkinson, 1983; Wolffe *et al.* 1984) and a 30 000 $M_r$ protein (hsp30) (Bienz, 1982; 1984). By one- and two-dimensional gel analysis we find that these are actually complexes of proteins migrating at 68 000 and 70 000 $M_r$ (hsp70/68) (Fig. 3B; King & Davis, 1987) and 26 000 to 30 000 $M_r$ (hsp30/26) (Fig. 3D), respectively. Other hsps apparently respond to heat shock in a more variable manner in both somatic and embryonic cells. In addition to hsp70/68 and hsp30/26, we sometimes see hsps at 86 000, 75 000, 61 000, and 56 000 $M_r$. Heikkila *et al.* (1985) and Nickells & Browder (1985) report variably expressed hsps of similar $M_r$s in *Xenopus* embryos of 87 000, 76 000, 57 000, 42–43 000, and 35 000.

Heat-shock proteins are believed to provide protection for the cell from thermal stress or chemical insult by an as yet unknown mechanism. There is evidence that heat-shock proteins may function in a structural capacity and that some of them are specifically associated with the cytoskeleton during heat-shock (reviews by Lindquist, 1986; Pelham, 1986; Schlesinger, 1986). Most researchers working with *Xenopus* use some modification of a procedure described by Knowland (1974) to prepare their protein samples. Homogenization buffers generally contain Tris–HCl (10–150 mM, pH 6·8–7·0), RNase A, sucrose, 50–100 mM-NaCl, DNase I and/or EDTA, which would not solubilize cytoskeletal-related proteins (Fey *et al.* 1984). We examined the yolk pellet and found that as much as 50 % of the hsp70/68 and virtually all of the hsp30/26 and hsp56 are pelleted even after repeated extractions with such homogenization buffers. A buffer containing the non-ionic detergent Triton X-100 and 100 mM-NaCl will remove about half of the remaining hsp70/68 and hsp56 but very little, if any, of the hsp30/26. This suggests that some fraction of the hsp70/68 and hsp56, and all of the hsp30/26, may be associated with the cytoskeleton during a heat shock in adult somatic cells. In this regard, it is interesting to note that hsc70 was not found in the SDS fraction in non-heat-shocked oocytes and embryos while there is a small and variable amount of it detected in the SDS fraction of heat-shocked oocytes. Hsp70 and the lower molecular weight hsp24 of chick embryo fibroblasts (which is probably analogous to the *Xenopus* hsp30/26) alternate between soluble and insoluble fractions during recovery from heat-shock and restress (Collier & Schlesinger, 1986). We did not examine the solubility of *Xenopus* hsps in cells that were allowed to recover from heat-shock, but it is possible that a subset of the *Xenopus* hsps (hsp70/68, hsp56, hsp30/26, and perhaps the hsc70 as well) behave dynamically in response to recovery and restress in a similar fashion. The other *Xenopus* hsps (hsp86, hsp75 and hsp61) were totally solubilized in nondetergent buffers, suggesting that they are not associated with any insoluble structures within the cell.

The oocyte 70 000 $M_r$ polypeptides that comigrate with hsp70/68 are most probably constitutively synthesized proteins analogous to the cognate hsps (hsc70) of *Drosophila* (Craig *et al.* 1983). Evidence for a constitutively expressed hsc70 mRNA in *Xenopus* oocytes and embryos has been reported (Heikkila *et al.* 1987; Horrell *et al.* 1987). *Drosophila* hsp synthesis is not induced during oogenesis or early embryogenesis (Zimmerman *et al.* 1983), but a hsc70 is abundant in ovaries and embryos (Palter *et al.* 1986). This expression is very similar to that seen in yeast cells in which a hsp70-related protein (hsc70) is induced by sporulation but is not affected by heat shock (Kurtz *et al.* 1986).

Varying the culture conditions can have an effect on the pattern of proteins being synthesized under heat stress as reported by Browder *et al.* (1987). The relative rate of synthesis of some proteins (hsp70, hsp75 and hsp56) in heat-shocked defolliculated oocytes could be increased two- to threefold under certain culture conditions (Fig. 5). We believe that the most likely explanation for these results is that components in the medium can more easily enter oocytes stripped of their follicle cells thus changing the physiological milieu of that cell. These changes influence mRNA translation rates at ambient temperatures, and such effects are likely to be exaggerated during heat stress. Particular

**Table 2.** *Developmental expression of the major* Xenopus *hsps and hsp70-related polypeptides*

| | | Somatic cells | Oocytes | Ovulated egg | Cleavage stage | Gastrula stage |
|---|---|---|---|---|---|---|
| hsp70/68 | rt* | – | – | – | – | – |
| | hs† | +++ | –? | – | – | +++ |
| hsc70 | rt | + | + | +/– | +/– | ++ |
| | hs | + | + | +/– | +/– | ++ |
| hsp30/26 | rt | – | – | – | – | – |
| | hs | ++ | – | – | – | ++ |

* Synthesis of polypeptides at ambient temperature (22°C) as detected by labelling with [$^{35}$S]methionine followed by SDS–PAGE and fluorography. Symbols (–/+/++/+++) represent relative synthesis of the polypeptides and were assigned based on visual comparison of fluorographs.

† Synthesis of polypeptides at heat-shock temperatures (33°C or above).

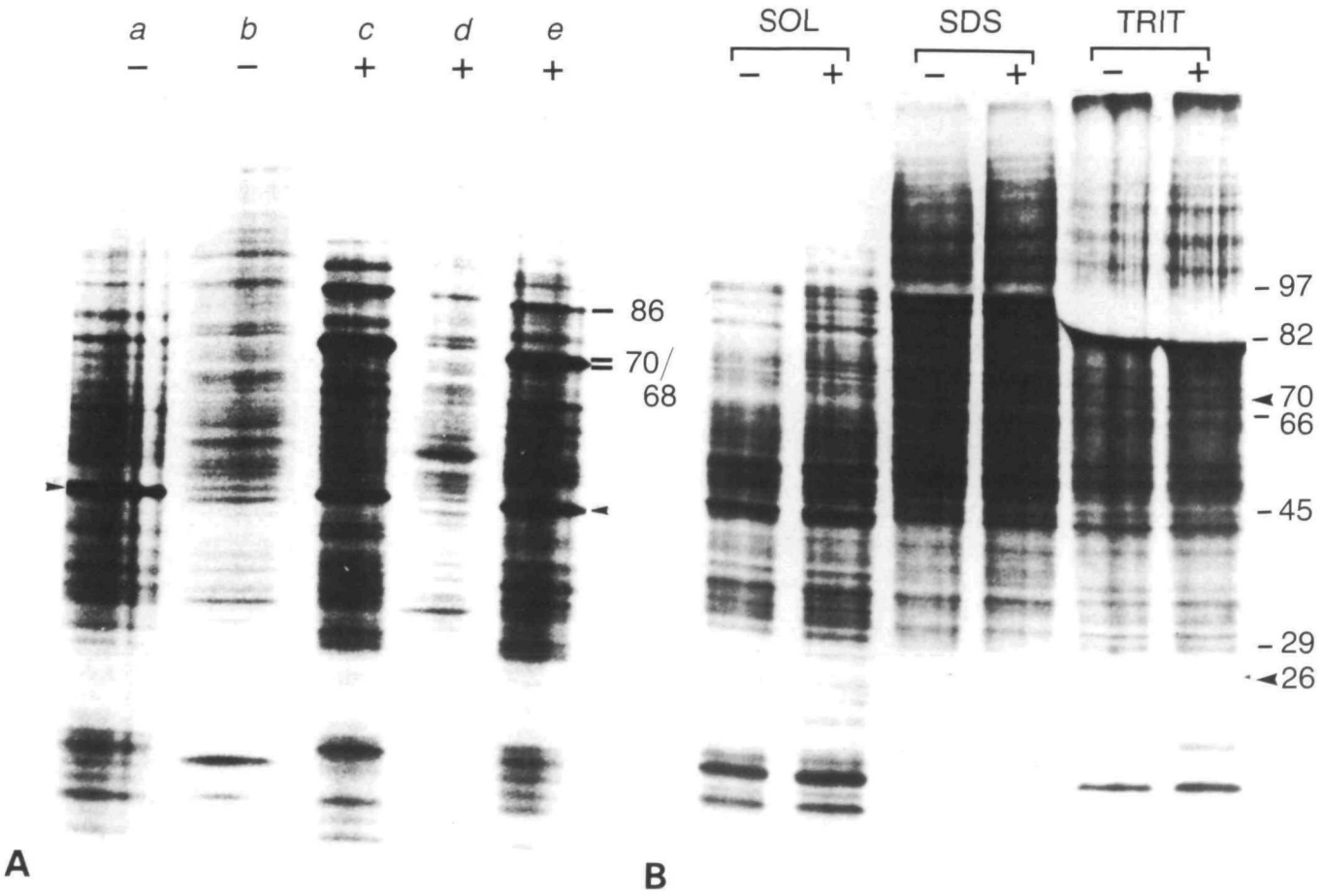


**Fig. 6.** Effect of heat shock on protein synthesis in ovulated eggs (A) and cleavage-stage embryos (B). (A) Ovulated eggs (*b* and *d*) and follicles (*a*,*c* and *e*) were labelled with [$^{35}$S]methionine as described in Materials and methods at 33°C (+) or 22°C (–). Equal TCA-precipitable cts $min^{-1}$ per lane were loaded for *b* and *d*, and for *a*, *c* and *e*. This represents threefold more protein in *d* (+) than in *b* (–) and twofold more protein in *c* (+) and *e* (+) than in *a* (–). Arrows indicate the position of actin. (B) Cleavage-stage embryos were labelled with [$^{35}$S]methionine at 33°C (+) or 22°C (–) and processed into SOL, TRIT and SDS fractions as described in Fig. 2. Equal TCA-precipitable cts $min^{-1}$ per lane were loaded. This represents fivefold more protein in SOL (+) than in SOL (–) and fourfold more protein in SDS (+) and TRIT (+) than in SDS (–) and TRIT (–). Locations of relative molecular mass markers are shown at the right $\times 10^{-3}$.

messages will be favoured over others and the pattern of protein synthesis will change accordingly. It is important to remember that defolliculated oocytes do not represent the natural condition of oocytes. Furthermore, exactly what culture medium best mimics the ionic environment *in vivo* is not known. Finally, contamination with as little as thirty follicle cells could account for the apparent increase in hsp70, hsp75 and hsp56 that we observed (Fig. 5). Taken together, we believe the significance of such increases (two- to

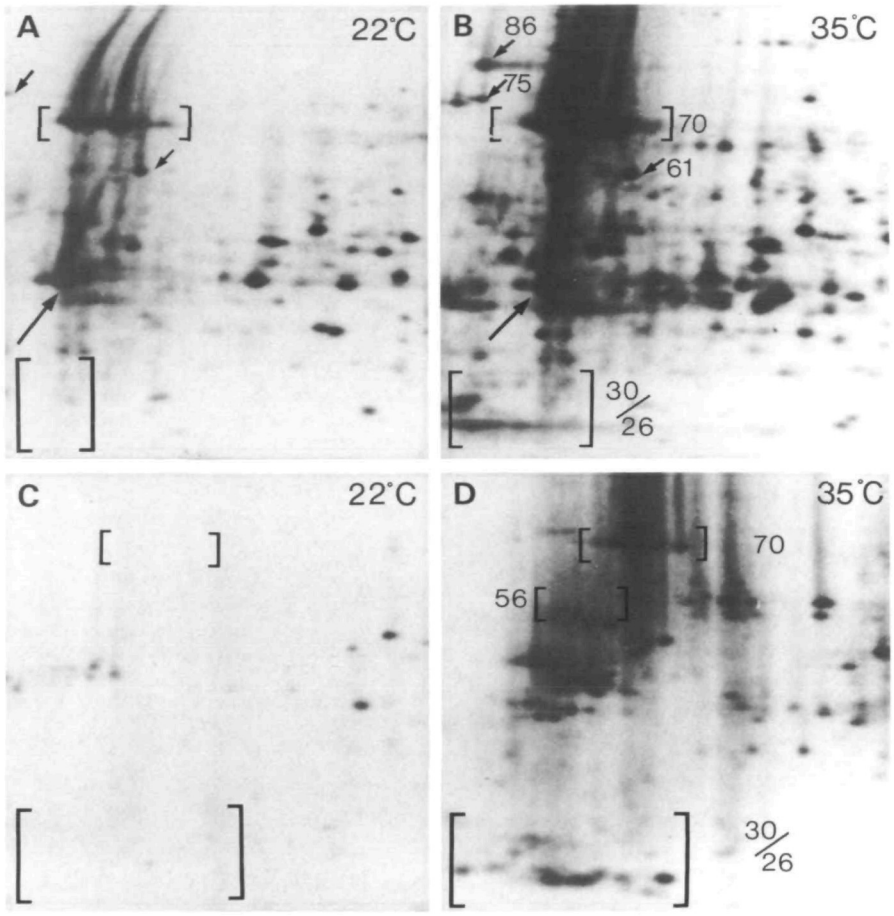


**Fig. 7.** Effect of heat shock on *Xenopus* gastrula-stage embryos. Gastrula-stage embryos were labelled with [$^{35}$S]methionine at 35°C (B and D) or 22°C (A and C) and processed into SOL, TRIT and SDS fractions as described in Fig. 2. SOL (A and B) and SDS (C and D) fractions were analysed by 2-D IEF–PAGE. Twofold more protein was loaded in B than A, and fivefold more protein was loaded in D than C. Brackets show the position of hsp70/68, hsc70, hsp56 and hsp30/26. The acid end of the gel is on the left. Arrows indicate the position of actin, hsp86, hsp75 and hsp61.

threefold) remains doubtful, especially in light of the observation that oocytes isolated from follicles *after* heat shock do not show a heat-shock response (King & Davis, 1987; Horrell *et al.* 1987). Interestingly, oocytes apparently do accumulate hsp70 mRNAs during oogenesis and hsp70 transcripts microinjected into oocytes are selectively translated during a heat-shock (Horrell *et al.* 1987).

In our hands, *Xenopus* ovulated eggs and cleavage-stage embryos are refractory to hsp-induced synthesis regardless of the culture medium used or whether cells were labelled by microinjection or by incubation. The discrepancy between our results and those of Browder *et al.* (1987) may only be apparent and not real since their results are not expressed per unit protein but as equivalent cts $min^{-1}$. The rates of protein synthesis in eggs after heat shock are very low, often five- to tenfold less than controls. Therefore, as much as 10 times more protein may be loaded for their heat-shock samples than for their controls. We find that when expressed per unit protein the rate of the 70 000 $M_r$ protein actually declines two- to threefold after heat shock (Fig. 6).

*Xenopus* embryos initiate transcription of the zygotic genome at the midblastula stage (stage 8) (Newport & Kirshner, 1982). Hsp70 synthesis can be induced by heat shock at this stage for the first time (Heikkila *et al.* 1985; Bienz, 1984; Nickells & Browder, 1985) and by stage 9 or later, the other soluble hsps can be detected (Heikkila *et al.* 1985). The expression of hsp30 has been examined at the mRNA level using a cloned sequence isolated from a cDNA library made with heat-shocked tissue culture cell mRNA. No signal was detected by nuclease S1-mapping analysis of RNA isolated from heat-shocked or control pre-tadpole-stage embryos leading to the conclusion that hsp70 and hsp30 are not coordinately regulated in *Xenopus laevis* development (Bienz, 1984). Previous analyses of heat-shock protein synthesis in amphibian oocytes and embryos may have missed identifying members of the hsp30/26 complex because of their insolubility in the buffers used. Our analysis shows that as early as the gastrula stage (stage 10–13) (Table 2), both the hsp26/30 and hsp70/68 complex are synthesized in response to a heat shock. Perhaps the reason for the discrepancy between our protein synthesis data and Bienz's RNA accumulation data is simply that we are examining the products of different genes and that the adult hsp30 cDNA probe does not recognize the embryonic hsp30/28 mRNAs.

Of course, our results do not rule out the possibility that hsps are discoordinately expressed for a brief period between the blastula stage when hsp70 is first detected and the gastrula stage (stage 10). Nickells & Browder (1986) have shown that *Xenopus* embryos acquire a heat-shock response in a regionally specific manner, with hsp70 being the earliest detectable heat-shock protein. This has also been found to be the case

for chick embryos (Zagris & Matthopoulos, 1986), possibly reflecting the different rate of development of cells in different parts of the embryo. Clearly, the developmental regulation of the *Xenopus* heat-shock response merits further careful investigation using a combination of immunological and highly specific nucleic acid probes.

We thank Luanne Walters and Barth Grant for their expert technical assistance. This work was supported by grants from the National Science Foundation (PCM-8112215) and the March of Dimes (Basil O'Connor Research Award) to M. L. King.

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*(Accepted 14 October 1988)*