

Transcription of a *Drosophila* heat shock gene is heat-induced in *Xenopus* oocytes

(microinjection/gene regulation)

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ABSTRACT *Xenopus* cells, like many other eukaryotic cells, respond to heat treatments by increasing the rate of synthesis of a few characteristic proteins, the heat shock proteins. Because of the generality of this response, it seemed possible to examine the expression of isolated heat shock genes in a heterologous system. Phage 122 DNA, containing two identical genes coding for the *Drosophila* 70,000-dalton heat shock protein (*hsp70* genes), was microinjected into *Xenopus* oocyte nuclei. The *Drosophila hsp70* genes are transcribed efficiently in heat-treated oocytes (35–37°C) to give RNA of the correct size and sequence content. Transcription is sensitive to low levels of α -amanitin and therefore is carried out by RNA polymerase II. At normal temperatures (20–28°C) essentially no *Drosophila*-specific RNA is formed. The isolated insert fragment of phage 122 also gives RNA of correct length in heat-treated oocytes which hybridizes to the coding segment of *Drosophila hsp70* genes only. At normal temperatures, however, its rate of transcription is variable and only RNA heterogeneous in size is formed.

Heat shock genes are present in a wide variety of eukaryotic cells (1–4). These genes are active at higher temperatures but are transcribed at low rates or not at all at normal growth temperatures. In all cells investigated so far, heat shock proteins of 70,000 and \approx 85,000 daltons were found to be made (1–6). As judged by peptide analysis, heat shock proteins from species as different as *Drosophila* and man have similar structures (ref. 6, unpublished data). These observations demonstrate that both the structure of the genes coding for heat shock proteins and their mode of regulation have been well conserved throughout evolution.

The *Drosophila* genes coding for the major 70,000-dalton heat shock protein (*hsp70* genes) have been isolated and characterized in considerable detail (7–11). The *hsp70* genes are repeated five to nine times in the haploid *Drosophila* genome (12) and are located at chromosomal regions 87A and 87C (3). Each *hsp70* gene (see Fig. 2) consists of a 2.2-kilobase (kb) mRNA coding region, Z_c , and a 0.3- to 0.4-kb-long noncoding segment, Z_{nc} (13, 14). By now, the nucleotide sequences of the *hsp70* genes and some of the more distal 5' flanking regions have been determined (15–17).

The mechanism by which heat shock genes are activated is not understood as yet. The nature and the location of DNA sequence elements involved in the transcriptional regulation of these genes are unknown. Because of the generality of the heat shock response, it seemed possible to investigate the mechanism of regulation of heat shock gene transcription in a heterologous system. If heat shock gene control sequences were conserved as well as the protein encoding portions, one might

expect that control regions associated with heat shock genes of one organism could also be recognized in cells of another organism.

We have chosen microinjection into *Xenopus* oocytes as an assay for studying the expression of *Drosophila* heat shock genes. *Xenopus* oocytes have some major advantages over many other *in vitro* or *in vivo* transcription systems. The transcription of a structural gene in the oocyte not only is dependent on the presence of T-A-T-A but also is evidently influenced by other DNA sequences as well. For instance, the expression of the histone genes is controlled by several sequence elements located in the 5' flanking extension (18). Whereas viral genes are efficiently read (19, 20), some cell type-specific genes, such as the ones coding for rabbit β -globin (21) or chicken ovalbumin (22), are not specifically transcribed in the oocyte. Moreover, in the oocytes, no crossover events are expected to bring the injected genes into the neighborhood of host DNA sequences that might affect their transcription. Thus, the regulation of the expression of an injected gene solely depends on the presence of its own control sequences.

MATERIALS AND METHODS

***hsp70* Genes.** The isolation and characterization of phage 122 carrying two *Drosophila melanogaster hsp70* genes has been described (23). Plasmid 122X14 is a derivative of phage 122 and contains one of the *hsp70* genes along with long flanking sequences (23). Plasmid Sal 0 has been constructed by inserting the coding portion of the *Drosophila hsp70* gene from plasmid 56H8 into the unique *Sal* I site of pBR322 (14). Plasmid and phage DNAs were prepared as described (7, 24). Phage 122 DNA or the 10.5-kb *Eco*RI insert fragment isolated from it was used for injection. The DNAs were circularized by incubation with T4 ligase. Routinely, about 30% of the gene fragments were found to be in a circular form as judged by electrophoresis on agarose gels.

Enzymes and Isotopes. Avian myeloblastosis virus reverse transcriptase was a gift from the National Cancer Institute. *Eco*RI came from the Microbiological Research Establishment (Porton Down, England), DNA polymerase I was from Boehringer Mannheim, and collagenase grade A was from Calbiochem. RNase-free DNase was a kind gift of W. Schaffner (Zürich). All other enzymes were purchased from New England Biolabs, and the isotopes were from the Radiochemical Center, Amersham, England.

Oocyte Injection and Heat Treatment. Oocytes were isolated from the ovary and defolliculated by digestion with 0.2% collagenase (25). The injection procedure has been described

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Abbreviations: *hsp70* gene, gene coding for the 70,000-dalton heat shock protein; kb, kilobase(s).

in detail (20). Early stage 6 (26) oocytes were used and were centrifuged to visualize the position of the nuclei (27). The only modifications introduced were the use of OR2 medium (28) instead of Barth's solution and the reduction of the amount of DNA injected to 2–5 ng per oocyte nucleus. Noninjected oocytes were used as controls and treated in parallel.

After DNA injection, the oocytes were incubated for 6–20 hr in OR2 at 20°C to allow chromatin formation on the injected DNA (29, 30). Some oocytes were then injected a second time with [α - 32 P]GTP (0.1 μ Ci per nucleus; 1 Ci = 3.7×10^{10} becquerels) or α -amanitin (0.1 μ g/ml of nuclear volume, 31) or both. After 15 min, the oocytes were incubated for 2 hr at 20°C or 15°C and, in certain experiments, at 28°C.

Analysis of RNA. The RNA was isolated from oocytes as described (32) and treated with RNase-free DNase to eliminate the injected DNA. *Drosophila*-specific transcripts were analyzed by three different methods. (i) RNA, labeled *in vivo* with [α - 32 P]GTP, was hybridized to Southern blots (33) of plasmid subclone Sal 0 digested with *Sal* I. (ii) Radioactive cDNA complementary to oocyte RNA was prepared by using reverse transcriptase in the presence of [α - 32 P]dCTP (34). Random pentadeoxynucleotides (a kind gift of P. F. Spahr) or, where indicated, oligo(dT) was used to prime the reverse transcriptase reaction. Labeled cDNA was then hybridized to Southern blots of plasmid Sal 0. (iii) Transcripts of oocytes were examined directly by the RNA blotting technique developed by Thomas (35). Nick-translated (36) plasmid Sal 0 was used as hybridization probe.

Xenopus Heat Shock Proteins. Established *Xenopus laevis* epithelial kidney cells (37) were grown at 27°C in dishes in Dulbecco's modified Eagle's medium containing 15% sterile water and 10% fetal calf serum. Subconfluent epithelial cells were preincubated at various temperatures for 2 hr in methionine-free medium. [35 S]Methionine (20 μ Ci/ml) was added subsequently and the cells were incubated for 2 more hr at the same temperature. Defolliculated oocytes, prepared as above, were incubated in OR2 buffer in the presence of [35 S]methionine (1 mCi/ml) for 2 hr at different temperatures. Oocytes and epithelial cells were lysed in NaDodSO₄ sample buffer (38). Oocyte lysates were centrifuged at 15,000 \times g for 10 min to pellet yolk protein. Proteins were then analyzed on NaDodSO₄/polyacrylamide gels (38, 39). As size markers, a mixture of 14 C-labeled standard proteins or 35 S-labeled *Drosophila* heat shock proteins were used.

RESULTS

Heat Shock Response in Xenopus Cells. Because we intended to study the expression of *Drosophila* heat shock genes in *Xenopus* oocytes, it was important to determine whether *Xenopus* cells respond to heat treatments in a fashion similar to *Drosophila* cells. These experiments were performed with cultivated epithelial cells and with stage 6 oocytes.

In epithelial cells, two polypeptides of about 85,000 and 70,000 daltons were synthesized at higher rates at 35°C and 37°C than at 20°C and 27°C (Fig. 1a). An additional protein of about 55,000 daltons was found to be heat induced occasionally. The rates of formation of most other proteins did not change significantly between 20°C and 37°C.

The protein patterns of oocytes incubated at different temperatures are shown in Fig. 1b. In both oocytes and epithelial cells, induction of heat shock proteins occurred at around 35–37°C. Similar heat shock protein patterns were observed in cells and oocytes (Fig. 1). As in the epithelial cells, a 55,000 dalton protein was found to be strongly heat-induced in some oocytes. It seems worth noting that the oocyte lysates contained some highly abundant, high molecular weight proteins (Fig. 1b,

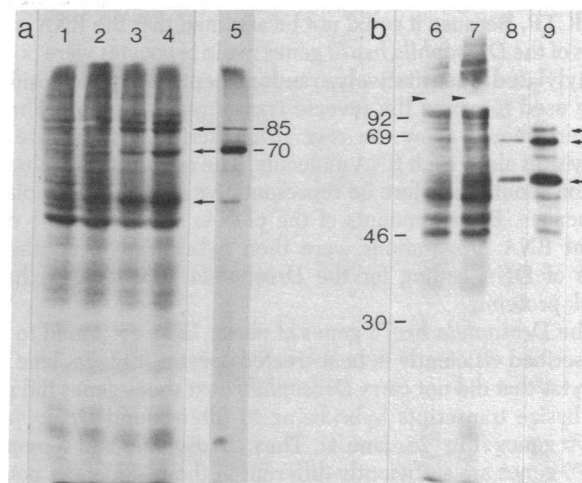


FIG. 1. Proteins synthesized at various temperatures in cultivated *Xenopus* epithelial cells (a) or in oocytes (b). (a) Epithelial cells were preincubated in methionine-free medium for 2 hr and then incubated in the presence of [35 S]methionine for another 2 hr at 20°C (lane 1), 27°C (lane 2), 35°C (lane 3), and 37°C (lane 4). Samples were prepared and electrophoresed in NaDodSO₄ gels. An extract of heat-treated *Drosophila* cells was electrophoresed in parallel (lane 5). The molecular weights of the large *Drosophila* heat shock proteins are indicated $\times 10^{-3}$. (b) Protein patterns of oocytes that had been incubated at 20°C (lane 6), 28°C (lane 7), or 37°C (lanes 8 and 9). Three times as much extract material was loaded for lane 9 than for the other lanes. The molecular weights of 14 C-labeled standard proteins are given $\times 10^{-3}$. Arrows, positions of heat-induced proteins; arrowheads in b, unlabeled abundant protein.

arrowheads) which may influence the migration properties of other proteins. We therefore did not attempt to determine the exact molecular weights of the oocyte heat shock proteins by using these gels.

In contrast to epithelial cells, most of the proteins normally made in oocytes were not synthesized anymore during heat treatments. This finding suggests that, in oocytes as in *Drosophila* cells, heat shock mRNAs are preferentially translated during heat treatment (40, 41).

Our results show that a 2-hr heat treatment at 37°C triggers the oocyte heat shock response. Heat shock genes in *Drosophila* cells are activated under the same conditions. Most of the experiments aimed at transcribing cloned *Drosophila* genes therefore were performed with oocytes treated in this fashion.

Injection of *Drosophila* Genes. Phage 122 (see map in Fig. 2) carries two identical *Drosophila hsp70* genes (each consisting of regions *Z_c* and *Z_{nc}*) arranged head-to-head around a central, asymmetrical spacer region of 0.9 kb (23). The *Drosophila* DNA segment in phage 122 derives from chromosomal region 87A.

Phage 122 DNA or the isolated 10.5-kb *Eco*RI insert fragment of it was circularized and injected into nuclei of early stage 6 *Xenopus* oocytes. Naked DNA introduced into oocyte nuclei is packed with nucleosomes within a few hours (29, 30). Oocytes were therefore routinely incubated for 6–24 hr at 20°C after the injection of *Drosophila* heat shock genes. After this incubation period the oocytes were either heat-shocked at 35–37°C for 2 hr or further incubated at 20°C. To label newly made RNA *in vivo*, [α - 32 P]GTP was injected into the oocytes prior to the final 2-hr incubation. RNAs were subsequently extracted, treated with RNase-free DNase, and then analyzed by different methods.

Transcription of *Drosophila hsp70* Genes in Heat-Treated Oocytes. RNA from heat-treated oocytes carrying phage 122 DNA or its isolated insert fragment and from heat-treated control oocytes was reverse transcribed in the presence of [α -

^{32}P]dCTP. Because it could not be assumed that the RNA products of the *Drosophila hsp70* genes made in oocytes were polyadenylated quantitatively, random pentadeoxynucleotides were used to prime the reverse transcriptase reaction. These primers should allow the reverse transcriptase to initiate at many sites along each RNA molecule. The resulting cDNA population should therefore be representative of all RNA template sequences. Equal amounts of the cDNAs made from the different RNA preparations were then hybridized to Southern blots of DNA coding for the *Drosophila* 70,000-dalton heat shock protein.

The *Drosophila hsp70* genes of phage 122 were found to be transcribed efficiently in heat-treated oocytes (Fig. 2a, lane 1). Oocytes that did not carry *Drosophila* heat shock genes did not synthesize transcripts hybridizing to filter-bound *Drosophila hsp70* genes (Fig. 2a, lane 4). Thus, *Drosophila* and *Xenopus hsp70* genes are sufficiently different in their sequences not to cross-hybridize under stringent conditions. Very little *Drosophila*-specific RNA was synthesized when phage 122 DNA had not been circularized prior to injection (Fig. 2a, lane 2). This finding confirms earlier observations that suggested that only circular DNA molecules are stable in oocyte nuclei whereas linear molecules are rapidly degraded (29). At least a fraction of the oocyte transcripts of phage 122 DNA appeared to be polyadenylated. cDNA that was made from oocyte RNA containing phage 122 transcripts with oligo(dT) used as primer of reverse transcriptase hybridized strongly to *Drosophila hsp70* genes (Fig. 2a, lane 3). The *hsp70* genes of the 10.5-kb phage 122 insert fragment were also transcribed (Fig. 2b, lane 6), indicating that transcription of the *hsp70* genes starts at an initiation site on the insert DNA and not on λ DNA.

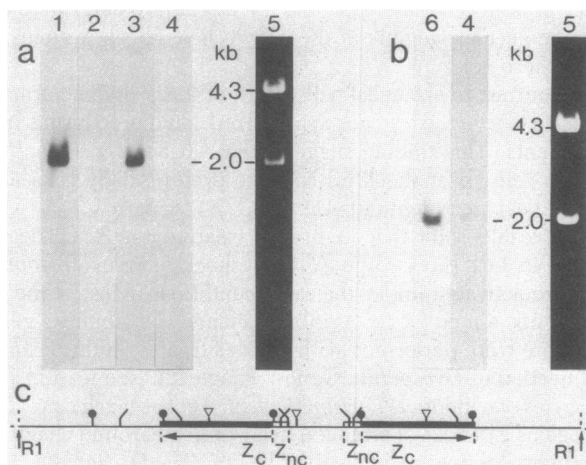


FIG. 2. Transcription of *Drosophila hsp70* genes in heat-treated oocytes. Groups of 10–15 oocytes were injected with 2 ng of phage 122 DNA (a) or of the 10.5-kb *Eco*RI insert fragment of it (b) per oocyte. Oocytes carrying *Drosophila* genes or noninjected oocytes were preincubated for 20 hr at 20°C and then heat-treated for 2 hr at 35°C. RNAs extracted from the oocytes were treated with RNase-free DNase, reextracted three times with phenol/chloroform/isoamyl alcohol and then reverse transcribed in the presence of [α - ^{32}P]dCTP with random pentadeoxynucleotides as primers (except in the experiment in a, lane 3, oligo(dT) was used). A *Sal*I digest of plasmid Sal 0 was electrophoresed on an agarose gel to separate the 2.0-kb *Drosophila hsp70* gene coding region from the pBR322 sequences (see lanes 5 in a and b) and transferred to a nitrocellulose filter according to Southern (33). The labeled cDNAs (about 5×10^6 Cerenkov cpm to filters 1, 2, 4, and 6 and 2×10^6 cpm to filter 3) were hybridized to the Southern blots. Lanes: 1, phage 122 DNA; 2, unligated phage 122 DNA; 3, phage 122 DNA, cDNA probe prepared with oligo(dT) as primer; 4, no DNA; 6, phage 122 insert DNA injected. (c) Map of phage 122 insert DNA. The symbols used to identify restriction sites are explained in Fig. 5c.

***Drosophila hsp70* Genes are Heat-Activated in Oocytes.** RNA made during the 2-hr heat treatment or the control incubation in oocytes carrying phage 122 DNA or the 10.5-kb phage 122 *Eco*RI fragment was labeled *in vivo* with [α - ^{32}P]GTP. After extraction the labeled RNAs were hybridized to Southern blots of *Drosophila hsp70* genes. *Drosophila hsp70* gene transcripts were synthesized by the oocytes only during heat treatment at 35–37°C (Fig. 3a). No *Drosophila*-specific RNA was made at 20°C or at 28°C. Transcription of the phage 122 genes in heat-treated oocytes could be blocked by low concentrations of α -amanitin. Thus, the *Drosophila hsp70* genes on phage 122 are transcribed by RNA polymerase II in oocytes as in *Drosophila* cells. Transcription of the *hsp70* genes on the isolated phage 122 fragment was also heat-stimulated (Fig. 3b). However, this stimulation varied in different experiments. As is evident from the data in Fig. 3b, the isolated genes are transcribed nonspecifically to some extent because a fraction of *Drosophila*-specific RNA synthesis was resistant to α -amanitin.

That the genes on phage 122 are heat-activated in oocytes was confirmed in additional experiments. RNAs from heat-treated or untreated oocytes carrying the *Drosophila* genes were reverse transcribed in the presence of [α - ^{32}P]dCTP with random pentadeoxynucleotide primers. Equal amounts of the different cDNAs were then hybridized to filter-bound *Drosophila hsp70* genes (33). Again the phage 122 *hsp70* genes were transcribed only in heat-treated oocytes (Fig. 4b). Similar observations were made with isolated phage 122 genes. The extent of stimulation of transcription of the isolated genes varied as in the experiments described above.

Size of *Drosophila hsp70* RNA Synthesized in Oocytes. RNA from oocytes containing phage 122 DNA or the 10.5-kb gene fragment was analyzed by the blotting technique of Thomas (35). Nick-translated *Drosophila hsp70* genes were used as hybridization probes. In heat-treated oocytes, most of the *Drosophila*-specific transcripts were of the same length as *hsp70*

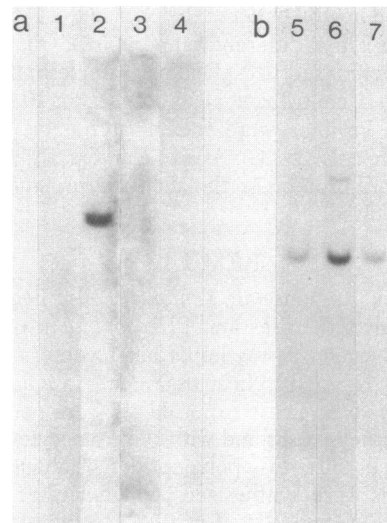


FIG. 3. *In vivo*-labeled *Drosophila hsp70* from heat-treated and untreated *Xenopus* oocytes. Groups of 20 oocytes injected with phage 122 DNA (a, lanes 1–3), with isolated 10.5-kb *Eco*RI insert fragment of phage 122 (b), or control oocytes (a, lane 4) were injected a second time with [α - ^{32}P]GTP (0.1 μCi per oocyte) alone or together with α -amanitin (0.1 $\mu\text{g}/\text{ml}$ of nuclear volume). After 2 hr of incubation at different temperatures, RNA was extracted from the oocytes and hybridized to Southern blots of *Sal*I digests of plasmid Sal 0. (a) Lanes: 1, oocytes injected with phage 122 DNA incubated at 20°C; 2, at 35°C; 3, at 35°C with α -amanitin; 4, noninjected oocytes at 35°C. (b) Lanes: 5, oocytes injected with the 10.5-kb phage 122 insert incubated at 20°C; 6, at 35°C; 7, at 35°C with α -amanitin.

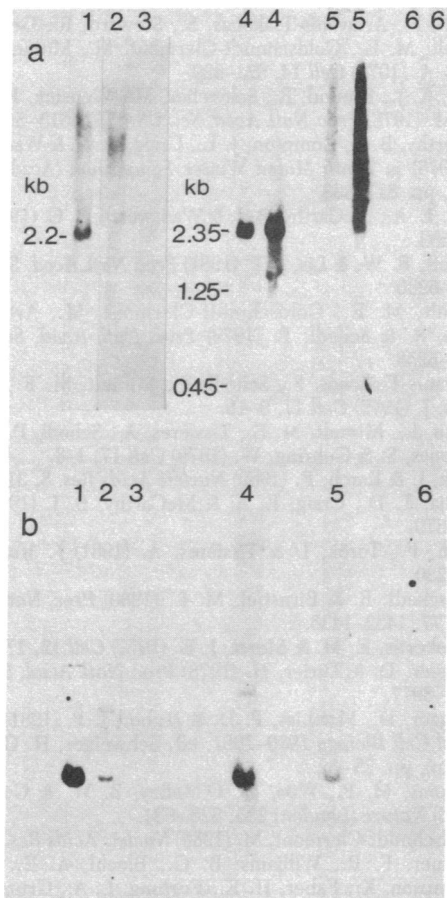


FIG. 4. (a) Size of *Drosophila hsp70* RNA made in oocytes. Total RNA was isolated from oocytes injected with phage 122 DNA after 2 hr of heat treatment at 35°C (lane 1) or incubation at 20°C (lane 2), from oocytes containing the phage 122 insert fragment after heat treatment (lane 4) or incubation at 20°C (lane 5), or from heat-treated control oocytes (lanes 3 and 6). Equal portions of the RNAs were DNase-treated, glyoxylated, and electrophoresed on a 1.4% agarose gel. As size marker, a *Bam*HI/*Bgl*I double digest of pBR322 was used. The RNAs were subsequently transferred to a nitrocellulose filter (35) and hybridized with nick-translated Sal 0. Two different exposures of filters 4-6 are shown. (b) Quantitative analysis of the heat activation of phage 122 *Drosophila hsp70* genes in oocytes. RNAs from oocytes treated as described in a were reverse transcribed as in Fig. 2. Equal amounts of the cDNAs were hybridized to Sal 0 Southern blots. Lanes are as in a.

RNA from *Drosophila* cells (2.2 kb; see Fig. 4a). The variable amounts of *Drosophila*-specific RNA that formed mainly in untreated oocytes containing the isolated phage 122 insert fragment were heterogeneous in size. These RNAs were, on the average, longer than authentic *hsp70* mRNA.

Only the Coding Regions of *Drosophila hsp70* Genes are Transcribed in Heat-Shocked Oocytes. That *Drosophila hsp70* mRNA and RNA made from injected *Drosophila hsp70* genes in heat-treated oocytes are of the same length suggested that only the RNA coding regions of these genes are transcribed in heat-treated oocytes. To confirm that injected *Drosophila hsp70* genes are transcribed specifically, RNA from heat-treated oocytes containing phage 122 DNA was isolated.

To obtain a probe representative of all RNA sequences, ³²P-labeled cDNA to oocyte RNA was prepared by the random priming procedure. The cDNA was then hybridized to Southern blots of various restriction digests (Fig. 5a) of plasmid 122X14 which contains one of the phage 122 genes together with long stretches of 5' and 3' flanking sequences (Fig. 5c). Hy-

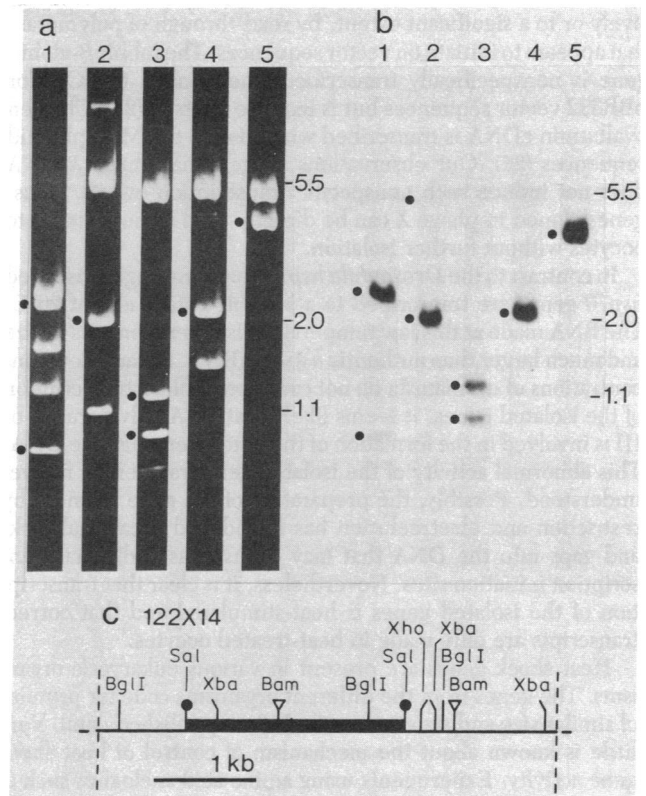


FIG. 5. Southern blot analysis of *Drosophila hsp70* RNA from heat-treated oocytes. Plasmid 122X14 (a *Drosophila* fragment obtained by incomplete digestion of phage 122 with *Xho* I has been inserted into the *Sal* I site of pBR322) was digested with *Bgl* I (lane 1), *Xba* I (lane 2), *Bam*HI and *Sal* I (lane 3), *Eco*RI and *Sal* I (lane 4), and *Eco*RI and *Xho* I (lane 5). The digests were electrophoresed on a 0.9% agarose gel (a shows the ethidium bromide-stained gel) and subsequently transferred to nitrocellulose (33). RNA isolated from heat-treated oocytes carrying phage 122 DNA was digested with DNase, extracted three times with phenol/chloroform/isoamyl alcohol, and then reverse transcribed in the presence of [α -³²P]dCTP as in Fig. 2. The labeled cDNA was hybridized to the 122X14 blot (b). Black dots identify DNA fragments in a and b that contain segments of the RNA coding region of the 122X14 *Drosophila hsp70* gene. (c) Restriction map of plasmid λ 22X14.

bridization occurred only to 122X14 fragments that contained segments of the RNA coding region (Fig. 5b). That different parts of the RNA coding sequence were labeled equally well (Fig. 5b, lane 3) supports our earlier finding that oocytes make full-length transcripts of *Drosophila hsp70* genes.

The same result was obtained when cDNA prepared from RNA of oocytes containing the isolated insert fragment of phage 122 was used for hybridization.

These experiments show that *Drosophila hsp70* genes are specifically transcribed in *Xenopus* oocytes. The expression of these genes which are heat-activated *in situ* is also heat-controlled in the oocytes.

DISCUSSION

Heat activation of injected DNA as described here seems to be specific for *Drosophila hsp70* genes. By contrast, polyoma virus DNA is less efficiently transcribed at 35°C than at 20°C (unpublished data). We have used both phage 122 DNA and the isolated insert fragment. The presence of phage λ segments does not induce nonspecific transcription of the *hsp70* genes. This is of practical interest because genes linked to various plasmid vectors have been shown to be transcribed, either exclu-

sively or to a significant extent, by read-through of polymerase that appears to initiate on vector sequences. The rabbit β -globin gene is nonspecifically transcribed when linked to pCR1 or pBR322 vector sequences but is inactive by itself (21). Chicken ovalbumin cDNA is transcribed when linked to pMB9 plasmid sequences (22). Our observations suggest that phage λ DNA does not induce such nonspecific transcription events. Thus, genes cloned in phage λ can be directly used for injection into oocytes without further isolation.

In contrast to the *Drosophila hsp70* genes in phage λ , isolated *hsp70* genes are transcribed to a variable extent also at 20°C. The RNA made at this low temperature is heterogeneous in size and much larger than authentic *hsp70* mRNA. Because low concentrations of α -amanitin do not completely block transcription of the isolated genes, it seems likely that RNA polymerase I or III is involved in the formation of the heterogeneous-size DNA. This abnormal activity of the isolated gene fragment is not yet understood. Possibly, the preparation of the gene segment by restriction and electroelution has introduced occasional nicks and gaps into the DNA that may be used as additional transcription initiation sites. Nevertheless, it is clear that transcription of the isolated genes is heat-stimulated and that correct transcripts are only made in heat-treated oocytes.

Heat shock genes are present in various eukaryotic organisms. The genes from the different organisms code for proteins of similar size and structure (refs. 1–6; unpublished data). Very little is known about the mechanism of control of heat shock gene activity. Experiments using amino acid analogues such as canavanine suggest that a labile protein is involved in the activation of the heat shock genes (1). The exact role, however, of this hypothetical protein has not yet been established. Whatever the mechanism of heat activation of heat shock genes may be, it must be based on the interaction of proteins or RNA with a DNA signal sequence unique to heat shock genes. Our experiments on the expression of *Drosophila* heat shock genes in *Xenopus* oocytes demonstrate that the heat shock genes from two different organisms are activated by similar mechanisms and that the injected *Drosophila* DNA contains the relevant signal sequence(s). Thus, the *Xenopus* oocyte system can be used as an assay for identifying the *Drosophila* heat shock gene control sequence(s).

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