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Integration, transcription, and control of a *Drosophila* heat shock gene in mouse cells

VICTOR CORCES*, ANGEL PELLICER†‡, RICHARD AXEL†, AND MATTHEW MESELSON*§

*Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138; and †College of Physicians and Surgeons, Institute of Cancer Research, Columbia University, New York, New York 10032

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ABSTRACT Mouse L cells were transformed with a cloned 3.6-kilobase (kb) segment of *Drosophila melanogaster* DNA carrying the 2.25-kb transcribed sequence for the *Drosophila* 70,000-dalton heat shock protein (hsp70) and 1.1 kb and 0.2 kb of 5′ and 3′ flanking DNA, respectively. Heat shock of one of three such transformed cell lines containing multiple copies of the intact *Drosophila* segment induced the abundant accumulation of transcripts of the *Drosophila* gene, with correct or nearly correct 5′ and 3′ termini. This provides evidence, in accord with earlier indications, that diverse eukaryotes, including vertebrates, have heat shock systems similar to that studied extensively in *Drosophila*. Our results suggest that the signals for heat shock transcription and the chromosomal sites with which they interact have been highly conserved in evolution and that the regulatory sequences controlling transcription of the gene for hsp70 lie within the 3.6-kb *Drosophila* segment.

Heat shock and certain other treatments induce in a wide variety of species the vigorous synthesis of a small number of characteristic proteins. The response has been studied extensively in *Drosophila melanogaster*, in which heat shock of intact animals or cultured cells induces transcription of genes for seven major proteins, while most other transcription ceases or is reduced. A corresponding shift occurs in the specificity of protein synthesis; non-heat-shock mRNA persists but is translated with greatly reduced efficiency. If the elevated temperature is not too high, or if the initial low temperature is restored, the heat shock response eventually subsides, and normal transcription and translation resume [reviewed by Ashburner and Bonner (1)]. The function of the heat shock response is not known, but its specificity and coordination and the occurrence of an apparently similar response in diverse organisms suggest that it is adaptive, preventing or counteracting deleterious effects of the inducing treatment. In accord with this view, a mild heat shock, sufficient to induce the heat shock proteins, protects *Drosophila* against a subsequent, more severe heat treatment, which by itself would cause developmental abnormalities and death (2).

Heat shock induces transcription-dependent synthesis of specific proteins not only in *Drosophila* but also in other eukaryotes, including vertebrates (3–6). Heat shock proteins of vertebrates and of *Drosophila* are also induced by arsenite and certain other agents (7). These indications of functional and perhaps structural homology between the heat shock systems of widely divergent species led us to ask whether a heat shock gene in a defined *Drosophila* DNA segment introduced into a vertebrate cell can be faithfully transcribed and regulated under heat shock control. A study of this question is made possible by the availability of cloned *Drosophila* heat shock genes and by the development of techniques for transforming tissue culture cells with heterologous DNA [reviewed by Pellicer *et al.* (8)].

We transformed mouse fibroblasts with a cloned 3.6-kilobase (kb) DNA segment containing the transcribed mRNA sequence for the 70,000-dalton *D. melanogaster* heat shock protein (hsp70). We find that increasing the temperature of a transformed line containing about 20 copies per cell of the intact *Drosophila* segment strongly induces the accumulation of transcripts of the *Drosophila* gene with the same or nearly the same 5′ and 3′ termini as the authentic *Drosophila* mRNA. This suggests that the heat shock transcriptional control signals and the sites with which they interact have been highly conserved in evolution and that the regulatory sequences for transcription of the gene for hsp70 lie within the 3.6-kb *Drosophila* segment.

MATERIALS AND METHODS

The mouse Ltk$^-$ cell line, the cloned herpes simplex thymidine kinase (tk) gene (phenotype tk$^+$), and the procedure for cotransformation have been described (9–11), as have methods for isolation of plasmid DNA, restriction analysis, and preparation of whole-cell RNA from *Drosophila* embryos (12, 13). *D. melanogaster* (Oregon R) embryos were grown for 6–18 hr at 25°C and heat shocked for 1 hr at 36°C. The plasmid pPW229, referred to here as 229, is described by Livak *et al.* (12). DNA and poly A-containing cytoplasmic RNA were prepared from mouse cells by the method of Wold *et al.* (14). RNA was electrophoresed in a 0.8% agarose gel containing formaldehyde (15) and transferred to nitrocellulose paper (16) by a protocol similar to that of B. Seed and D. Goldberg (personal communication). The running buffer was 20 mM 4-morpholinepropanesulfonic acid/5 mM NaOAc/1 mM EDTA, pH 7.0. Agarose was dissolved in hot water, cooled to 60°C, mixed 9:1 (vol/vol) with 10-times concentrated running buffer, and made 2.2 M in formaldehyde. RNA samples in running buffer with 50% (vol/vol) formamide and 2.2 M formaldehyde were kept 5 min at 60°C, cooled on ice, made up to 20% Ficoll, and placed on the gel. After electrophoresis, the gel was kept 1 hr in 3 M NaCl/0.3 M Na citrate, pH 7. Transfer to nitrocellulose paper, hybridization, washing, and autoradiography were done as described (16). Analysis of DNA protected against endonuclease S1 by hybridization with RNA was done as described by Holmgren *et al.* (17), except that the protected region of the 2.1-kb *Bgl* I-*Pst* I fragment of 229 was electrophoresed on a 6% acrylamide/urea gel. The cloned dihydrofolate reductase cDNA was a gift of R. Schimke.

Abbreviations: kb, kilobase(s); hsp, heat shock protein; tk, thymidine kinase.

‡ Present address: Dept. of Pathology, New York Univ. Medical Center, 550 First Ave., New York, NY 10016.

§ To whom reprint requests should be addressed.

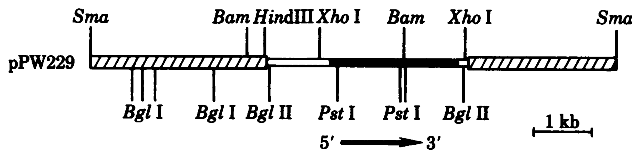

FIG. 1. Restriction map of plasmid 229 opened with *Sma*. ▬, The 2.25-kb transcribed region of the *D. melanogaster* hsp70 gene; ▨, the pMB9 vehicle; ➞, the direction of transcription; *Bam*, *Bam*HI.

RESULTS

Transformation of Mouse Cells With a *Drosophila* Heat Shock Gene. The principal heat shock protein of *D. melanogaster*, hsp70, is encoded by repeated unsplit genes at chromosome positions 87A and 87C. The *Drosophila* DNA of plasmid 229 includes the entire 2.25-kb hsp70 transcribed region, together with 1.1 kb of 5′ and 0.2 kb of 3′ flanking DNA (Fig. 1; ref. 12). The *Drosophila* segment of 229, approximately 3.6 kb in length, is inserted through A-T joints at the *Eco*RI site of pMB9. The portion of 229 whose sequence has been determined (unpublished data), approximately 400 base pairs centered on the mRNA initiation site, is identical to the corresponding region of the hsp70 genes from 87C, whose sequence has been published by Ingolia *et al.* (18) and by Karch *et al.* (19).

Earlier work has shown that cells transformed by a selectable marker DNA are efficiently cotransformed with nonselected, physically unlinked DNA present in the transformation mixture (20). We used the herpes simplex tk gene cloned in pBR322 as a selectable marker for cotransformation of mouse tk^- fibroblasts by plasmid 229 DNA. A calcium phosphate precipitate containing 1 μg of 229 cut at the *Sma* site of pMB9, 1 μg of the tk^+ plasmid cut with *Bam*HI, and 20 μg of mouse Ltk^- carrier DNA was added to each of five dishes containing 5×10^5 mouse Ltk^- cells. After a 2-wk incubation in hypoxanthine/aminopterin/thymidine medium to select for tk^+ transformants, 8 of the approximately 20 colonies that appeared were picked, grown into mass cultures, and tested for the presence of the *Drosophila* hsp70 gene. DNA extracted from each culture was digested with *Xho* I or *Bgl* II, electrophoresed on agarose gels, transferred to nitrocellulose paper, and hybridized with ^{32}P-labeled 229 DNA.

The 2.6-kb *Xho* I fragment and the 3.4-kb *Bgl* II fragment, both of which are internal to the 3.6-kb *Drosophila* segment of plasmid 229, are present in five of the eight tk^+ cell lines (Fig. 2). These are designated hs 1–hs 5. Comparison with the autoradiographic intensity of the corresponding fragments given by digestion of a known amount of 229 DNA showed the presence of approximately 20 copies of the intact *Drosophila* segment per diploid quantity of mouse DNA in hs 4 and lesser amounts in hs 1, 2, 3, and 5, with probably only one copy in hs 2. *Xho* I fragments homologous to 229 were seen also in hs 6, but there was no band at 2.6 kb. The restriction patterns were stable for more than 100 generations of propagation in hypoxanthine/aminopterin/thymidine medium. It should be noted that DNA from all of the lines, including the parental Ltk^- line, gave *Bgl* II restriction fragments homologous to 229, indicating homology between certain mouse sequences and the *Drosophila* hsp70 gene.

Induction of a *Drosophila* Heat Shock Gene in Mouse Cells. Although conditions for optimal heat shock induction of mouse L cells have not been determined, we employed conditions similar to those found by Kelly and Schlesinger (3) to induce heat shock proteins in such cells. Plate cultures of cell lines hs 1, hs 4, hs 5, and the untransformed tk^- line were trypsinized, allowed to adapt to suspension for 2 hr at 37°C, and divided into two equal portions. One was kept at 45°C for 5 min while the other served as a non-heat-shock control. After an additional 90 min at 37°C, cytoplasmic poly A-containing RNA from each of the eight cultures was electrophoresed in a formaldehyde-con-

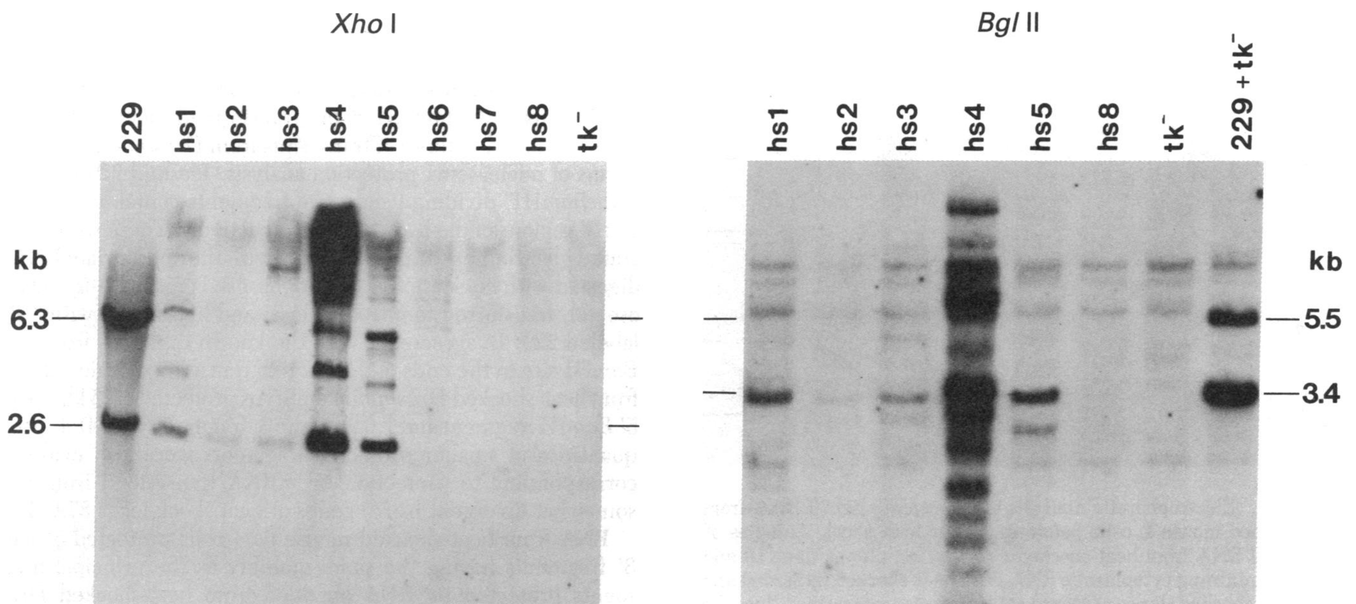

FIG. 2. Restriction analysis of DNA from transformed mouse L cells. DNA from eight cell lines (hs 1–hs 8), independently selected as tk^+ transformants after exposure to a mixture of *Sma*-cut plasmid 229, herpes tk^+ DNA, and Ltk^- mouse carrier DNA, was digested with *Xho* I (*Left*) and *Bgl* II (*Right*). Twenty-five micrograms of the digested DNA from each cell line (lanes hs 1–hs 8) and from tk^- mouse cells (lane tk^-) was electrophoresed on an 0.8% agarose gel, transferred to nitrocellulose, and hybridized with ^{32}P-labeled 229. An amount of 229 digested with *Xho* I equivalent to five copies per mouse diploid genome (lane 229) and an amount of 229 digested with *Bgl* II equivalent to 20 copies per genome were run as standards, mixed with 25 μg of Ltk^- DNA in the latter case (lane 229 + tk^-). Transformed cell lines hs 1–hs 5 are seen to contain the 2.6-kb *Xho* I and 3.4-kb *Bgl* II *Drosophila* segments of 229. Because there are no other *Xho* I or *Bgl* II sites in 229, the presence of additional bands of intensity comparable to those of the 2.6-kb and 3.4-kb *Drosophila* segments suggests that 229 DNA is integrated in various structures that are repeated.

taining agarose gel, transferred to nitrocellulose paper, and hybridized with ^{32}P-labeled 229 DNA. The autoradiograph is shown in Fig. 3. Total RNA from heat-shocked *D. melanogaster* embryos, run as a standard in the same gel, gave a single band corresponding to the 2.5-kb poly A-containing hsp70 mRNA (17). Long exposure revealed in all mouse cell lines, whether heat-shocked or not, a component homologous to 229 migrating a little ahead of the *Drosophila* hsp70 mRNA (Fig. 3 *Lower*).

The accumulation of RNA homologous to plasmid 229 was strongly induced by heat shock in cell line hs 4. The principal component of this RNA migrated indistinguishably from the authentic 2.5-kb *Drosophila* heat shock mRNA. Three additional components of lesser mobility were also present, with sizes estimated as approximately 3.2, 3.7, and 4.7 kb. None of the four components was seen without heat shock. Another transformed line containing the *Drosophila* gene, hs 1, accumulated a 3.2-kb component in response to heat shock and perhaps a 2.5-kb component as well, in quantities near the limit of detection. In the third cell line, hs 5, no transcripts of the *Drosophila* gene were detected with or without heat shock.

Hybridization of the nitrocellulose filter of Fig. 4 with ^{32}P-labeled cloned mouse dihydrofolate reductase cDNA showed no increase in dihydrofolate reductase mRNA after heat shock (data not shown). Therefore, the rapid accumulation of hsp70 mRNA observed after heat shock in line hs 4 appears not to be a general response of expressed genes in mouse L cells.

A rough estimate can be made of the amount of hsp70 mRNA induced in heat-shocked hs 4 cells. The 1 μg of poly A-containing mouse RNA placed on the gel derived from about 5 $\times$ 10^6 cells. The 1 μg of total *Drosophila* embryo RNA analyzed on the same gel derived from a quantity of tissue, including diploid, polyploid, and polytene nuclei, containing approximately 0.2 μg of DNA, which corresponds to 6 $\times$ 10^5 diploid genomes (21, 22). Therefore the nearly equal intensity of the respective 2.5-kb bands in Fig. 4 indicates that the heat-shocked mouse hs 4 line contains about one-eighth as many molecules of hsp70 mRNA per cell as are present per diploid genome in the embryos from which the *Drosophila* heat shock RNA was prepared. This number is $\approx$6000 (R. Freund, personal communication), giving an estimate of 800 molecules of *Drosophila* hsp70 mRNA per heat shocked mouse cell.

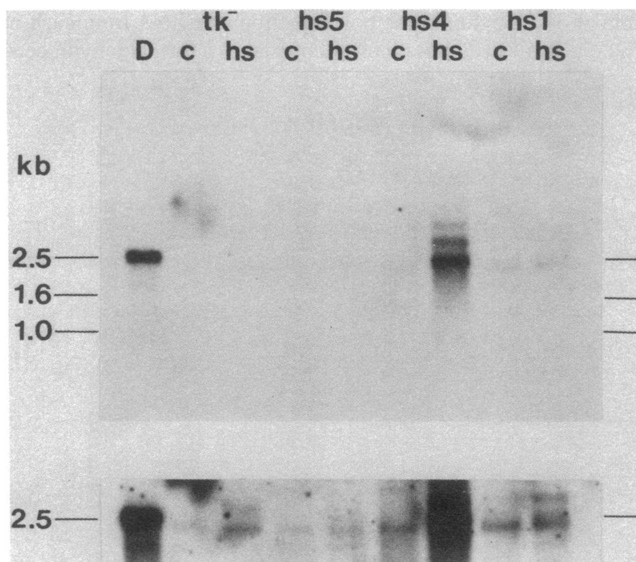

FIG. 3. Electrophoretic analysis of *Drosophila* hsp70 RNA from transformed mouse L cells before and after heat shock. Samples of whole-cell RNA from heat-shocked *Drosophila* embryos (lane D) and poly A-containing cytoplasmic RNA from heat-shocked (lanes hs) and non-heat-shocked (lanes c) mouse cells of three transformed lines (hs 5, hs 4, and hs 1) and of the untransformed tk$^-$ line were electrophoresed on an agarose gel containing formaldehyde, transferred to a nitrocellulose filter, and hybridized with ^{32}P-labeled 229. One microgram of RNA was run in each lane. The position of the 2.5-kb *D. melanogaster* hsp70 mRNA and the positions of the two mouse dehydrofolate reductase mRNAs (1.6 kb and 0.8 kb, R. Schimke, personal communication), subsequently located on the filter by hybridization with a dehydrofolate reductase cDNA clone, are indicated. (*Lower*) Longer exposure of the same gel in the 2.5-kb region.

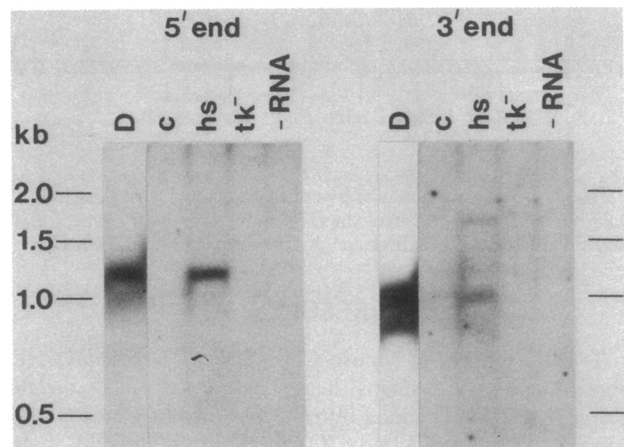

FIG. 4. Mapping of the 5′ and 3′ ends of the *Drosophila* hsp70 mRNA induced by heat shock in transformed cell line hs 4. Plasmid 229 was cut with *Bam*HI into two fragments containing the 5′ (*Left*) and 3′ (*Right*) regions of the gene, respectively. Fifty nanograms of each fragment was hybridized to 1 μg of whole-cell RNA from heat-shocked *Drosophila* embryos (lanes D), 1 μg of cytoplasmic poly A-containing RNA from mouse tk$^-$ cells (lanes tk$^-$), and non-heat-shocked (lanes c) or heat-shocked (lanes hs) cells of line hs 4. After incubation with nuclease S1, the samples were electrophoresed on a 1.5% alkaline agarose gel, transferred to nitrocellulose paper, and hybridized with ^{32}P-labeled 229. A control from which RNA was omitted was run in the last lane (−RNA) of each panel. Restriction fragments of 229 were run on the same gel as size standards.

5′ and 3′ Termini of Induced Transcripts. The termini of the hsp70 transcripts induced in the transformed mouse line hs 4 were compared to those of transcripts from *Drosophila* itself by means of nuclease S1 protection analysis. Plasmid 229 was cut with *Bam*HI, dividing it into two fragments containing the 5′ and 3′ regions of the hsp70 gene, respectively. These were separated electrophoretically, annealed with the appropriate RNA, digested with nuclease S1, electrophoresed on an alkaline agarose gel, transferred to nitrocellulose, and hybridized with ^{32}P-labeled 229. In agreement with the known distances from the *Bam*HI site to the ends of the 2.25-kb transcribed region, RNA from heat-shocked *Drosophila* embryos protected 1.25 kb of the 5′ *Bam*HI fragment and 1.0 kb of the 3′ fragment (Fig. 4). Lesser quantities of smaller protected fragments were also evident, corresponding to protection by mRNA transcribed from the somewhat divergent hsp70 genes at heat shock locus 87A (17).

RNA from heat-shocked mouse hs 4 cells protected 5′ and 3′ fragments having the same mobility as the principal fragments protected by RNA obtained from heat-shocked *Drosophila* (Fig. 4). No protection was observed when annealing was carried out with RNA from unshocked hs 4 cells or from heat-shocked tk$^-$ mouse cells, or when RNA was omitted. Whereas only one 5′ fragment of the cloned hsp70 gene was protected by RNA from heat-shocked hs 4 cells, there were two protected 3′ fragments. The most abundant one, of size 1.0 kb, presumably derived from protection by the predominant 2.5-kb poly-A-containing induced RNA. The other 3′ protected

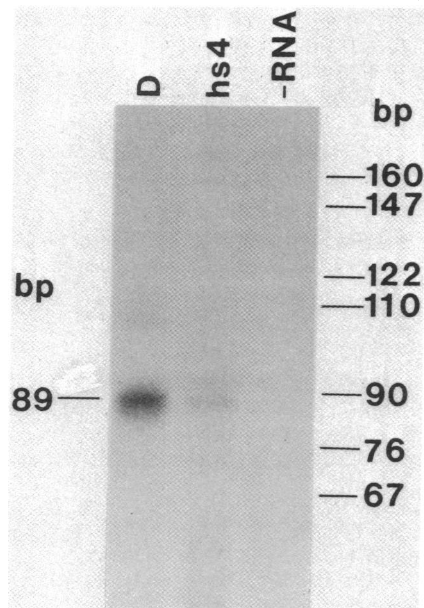

FIG. 5. High-resolution mapping of the 5′ end of *Drosophila* hsp70 mRNA induced by heat shock in mouse cell line hs 4. The 7.8-kb *Pst* I fragment of plasmid 229 was 5′-end labeled with [γ-^{32}P]ATP and polynucleotide kinase. After digestion with *Bgl* I, the 2.1-kb *Bgl* I–*Pst* I fragment was hybridized to 10 μg of whole-cell RNA from heat-shocked *Drosophila* embryos (lane D) and 1 μg of cytoplasmic poly A-containing RNA from heat-shocked hs 4 cells (lane hs 4), incubated with nuclease S1, and electrophoresed on a 6% acrylamide/urea gel. A control without RNA was run in the third lane (−RNA). *Hpa* II fragments of pBR322 run on the same gel were used as markers. bp, Base pairs.

fragment, of about 1.7 kb, would then derive from the less abundant 3.2-kb RNA. Protected fragments derived from the rarest RNAs induced in hs 4 would not have been detected. Thus, although the 5′ end of the hsp70 transcripts induced in mouse cells may be unique, there appears to be at least one minor 3′ terminus, in addition to the principal one.

A more exact comparison of the 5′ termini was obtained by performing a similar S1 protection experiment with the 2.1-kb *Bgl* I–Pst I fragment, which includes 89 nucleotides at the 5′ end of the hsp70 transcribed region (23). The isolated fragment, labeled with ^{32}P at the *Pst* I site, was annealed with heat shock RNA from *Drosophila* embryos and from hs 4 cells. The mobilities of the two protected fragments were indistinguishable (Fig. 5). We conclude that the principal hsp70 transcripts induced by heat shock in the transformed mouse line hs 4 have 5′ and 3′ termini close to or identical to those of authentic *Drosophila* hsp70 transcripts.

DISCUSSION

We found that a cloned *Drosophila* heat shock gene introduced by transformation into cultured mouse cells is expressed in apparently faithful response to heat shock. The cloned *Drosophila* segment consists of the 2.25-kb transcribed region of the *D. melanogaster* hsp70 gene, with 1.1 kb and 0.2 kb of 5′ and 3′ flanking DNA, respectively. In one transformed line, containing approximately 20 copies of the *Drosophila* segment, a brief heat shock followed by 90 min at normal growth temperature induces the accumulation of several hundred molecules of *Drosophila* hsp70 mRNA per mouse cell. Most of the transcripts have correct, or nearly correct, 5′ and 3′ ends. Much smaller quantities of additional induced transcripts that appear to extend beyond the normal 3′ end are also observed. No transcripts of the *Drosophila* gene are seen without heat shock.

A second transformed line, containing approximately three copies of the *Drosophila* segment per cell gives a much weaker response to heat shock and accumulates mainly or exclusively a species about 0.7 kb larger than the normal transcript. No response was detected in a third transformed line, containing approximately five copies of the *Drosophila* segment. Thus, the intensity and fidelity of the response differ among different transformed lines, as is generally observed for the expression of genes introduced into mouse cells by transformation. The results of restriction analysis of the transforming DNA in the various lines make it unlikely that the different responses to heat shock are due to loss of any substantial portion of the *Drosophila* segment, although the possibility of more subtle sequence alterations is not ruled out. Alternatively, the different responses to heat shock may reflect differences in the pattern of DNA methylation or in the environment of the transforming *Drosophila* segment, either within the transforming concatenate, or in the location of the *Drosophila* genes within the L-cell genome (11, 24).

Induction of specific mRNAs has been observed in mouse cells transformed with several other genes. Mouse mammary tumor, rat α_{2u} globulin and human growth hormone genes have been introduced into Ltk$^-$ cells by cotransformation. In each case, cell lines have been obtained in which accumulation of specific mRNA is induced in response to the glucocorticoid hormone analogue dexamethasone (refs. 26–28; unpublished data). It is not certain that the induction of mRNA accumulation in the transformed cells by the administration of dexamethasone or by heat shock results from control at the level of transcription. However, the induction of hsp70 mRNA by heat shock in *Drosophila* is accompanied by striking and almost immediate induction of puffs, incorporation of RNA precursors, and accumulation of RNA polymerase II at the chromosomal heat shock loci (1). Therefore, it seems very likely that the heat shock control of hsp70 mRNA we observe in the transformed mouse cells occurs at the nuclear level and results from the induction of transcription.

The simplest interpretation of our results is that the heat shock regulatory signals and the chromosomal sites with which they interact have been highly conserved in evolution and that the regulatory sequences controlling transcription of the hsp70 gene lie on the 3.6-kb *Drosophila* segment of 229. However, we cannot exclude the alternate possibility that the *Drosophila* gene is integrated in a manner placing it under the control of mouse heat shock regulatory sequences.

Conservation of heat shock regulatory sequences in evolution may have been favored by the existence of several essential heat shock genes, each interacting, for example, with a common regulatory protein. Regulatory sequences common to such a gene family should diverge more slowly than a sequence controlling only one gene, due to epistatic interactions between them and the regulatory protein. A different possibility that could also impose great evolutionary stability is that heat shock genes are controlled autonomously, directly sensing some change in chromatin structure or metabolism caused by the inducing stress. In that case, evolution of heat shock control sequences would be constrained by the conservation of the structure of chromatin itself. In any event, the ability to obtain heat shock control of a *Drosophila* gene introduced by transformation into mouse cells makes possible further experiments to locate and characterize sequences involved in heat shock regulation.

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