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Mouse and *Drosophila* Genes Encoding the Major Heat Shock Protein (hsp70) Are Highly Conserved

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We used a cloned *Drosophila melanogaster* hsp70 gene to hybrid-select heat shock-induced mouse mRNA and showed that this mRNA encodes the major mouse heat shock protein. This result suggests that the sequence of the hsp70 gene(s) is highly conserved.

All living cells appear to respond to stress by synthesizing new proteins; in most organisms, one of the major stress or heat shock proteins (hsp) produced has an apparent subunit molecular weight of 70,000. These proteins (hsp70) from yeasts, *Drosophila* sp., chickens, mice, and humans are antigenically related (4), suggesting that the hsp70 genes are highly conserved. In mouse cells, we observed two heat shock-induced proteins (Fig. 1, lane 2) which were not synthesized in unstressed cells (Fig. 1, lane 1). One of these heat shock proteins (hsp89) at 89 kilodaltons migrates only slightly behind a polypeptide made at significant rates in unstressed cells, and its induction is usually not apparent, except on two-dimensional or high-porosity gels (M. D. Perry, D. G. Lowe, W. D. Fulford, and L. A. Moran, submitted for publication). The other mouse heat shock protein has an apparent molecular weight of 68 kilodaltons on the 10% polyacrylamide gel shown in Fig. 1. In *Drosophila melanogaster* cells, hsp70 is synthesized at a high rate, along with several other polypeptides, after heat shock (9, 18) (Fig. 1, cf. lanes 3 and 4). For convenience, we will also refer to the major heat shock-induced mouse protein as hsp70 since, as we show below, it shares sequence homology with the *Drosophila* hsp70.

A cross-hybridization analysis was done to determine whether the mouse genome contains sequences which are related to the *Drosophila* hsp70 gene. Southern blots of *Pst*I and *Sal*I endonuclease-digested mouse DNA (Fig. 2, lanes 2 and 3) were hybridized to a ^{32}P-labeled 2.2-kilobase *Sal*I DNA fragment containing the amino acid-coding region of a *Drosophila* hsp70 gene from plasmid 56H8 (10). *Pst*I generates two fragments, and *Sal*I generates two fragments in addition to high-molecular-weight mouse DNAs which cross-hybridize to the *Drosophila* hsp70 gene. Hybridization to *Pst*I-digested *Drosophila* DNA shows seven fragments containing hsp70 gene sequences (Fig. 2, lane 1). These fragments have been assigned to five hsp70 genes at loci 87A7 and 87C1 on chromosome 3 of *Drosophila melanogaster* (7, 8). This experiment demonstrated the existence of sequences in the mouse genome that are related to the *Drosophila* heat shock gene hsp70. However, this homology did not constitute proof that these mouse sequences were the heat shock-inducible genes, particularly in view of the fact that related but non-heat shock-inducible genes (pseudo genes?) are present in *Drosophila* (3).

To determine under what conditions, if any, the mouse homologous sequences are transcribed in cells in culture, we probed Northern blots of mouse polyadenylated [poly(A)$^+$] RNA with the *Drosophila* hsp70 gene *Sal*I fragment. Hybridization conditions equivalent to those used for the Southern blot were used to facilitate comparison. The results (Fig. 3) demonstrated that there are mouse mRNAs homologous to the *Drosophila* hsp70 probe. One of these (Fig. 3, lane 3, A) was heat shock inducible and had an apparent size of 2,700 nucleotides, slightly larger than the *Drosophila* hsp70 mRNA of 2,400 nucleotides (Fig. 3, lane 1). Other mouse RNA species were also detected by the *Drosophila* hsp70 gene probe (B and C in Fig. 3). The genes from which the three poly(A)$^+$ RNAs in Fig. 3 (A, B, and C) are transcribed were presumably contained on the mouse DNA fragments detected by Southern blotting (Fig. 2).

To prove that the heat shock-inducible mouse poly(A)$^+$ RNA which we detect on Northern blots does indeed encode the mouse hsp70, we did a heterologous hybridization selection experiment. Poly(A)$^+$ RNA from mouse heat-shocked cells was hybridized to denatured and filter-immobilized DNA encoding the *Drosophila* hsp70 gene. The resulting hybrids were dis-

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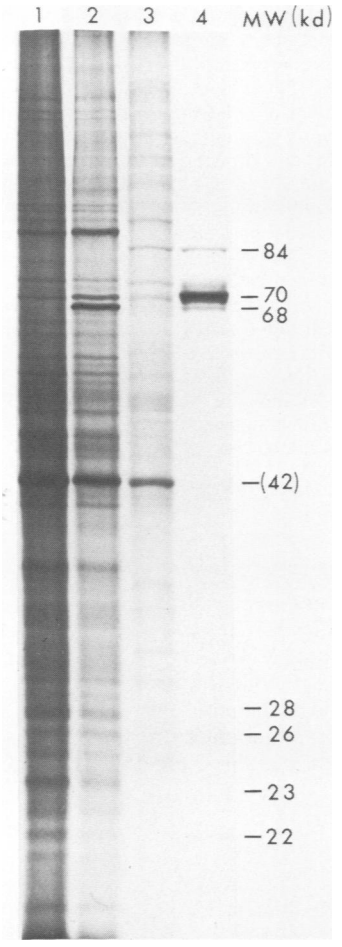


FIG. 1. Autoradiography of sodium dodecyl sulfate (SDS)polyacrylamide gel of proteins synthesized by control and heat-shocked mouse and *Drosophila* cells. Mouse L-cells (WT-4) were labeled for 1 h with L-[^{35}S]methionine at 37°C. L-cells were heat shocked by incubation at 44°C for 10 min, allowed to recover for 2 h at 37°C, and then labeled. *D. melanogaster* Schneider 2 cells were labeled for 2 h with L-[^{35}S]methionine at 25°C (control) or 37°C (heat shock) as described by Mirault et al. (9). Samples of cell lysates corresponding to approximately equal numbers of cells of each type were run on a 10% SDS-polyacrylamide slab gel (5, 16). The gel was dried and exposed to Kodak XAR-5 film at −70°C. Lanes: 1, L-cells at 37°C; 2, L-cells at 44°C; 3, *Drosophila* cells at 25°C; and 4, *Drosophila* cells at 37°C. The known molecular weights (kilodaltons [kd]) of the *Drosophila* heat shock proteins are shown on the right of the figure and that of actin is shown in parentheses.

rupted by heating, and the selected mouse mRNA was translated in vitro. The selected mRNA directs the synthesis of a polypeptide (Fig. 3, lane 6) which comigrated with the mouse hsp70 made in vivo and in vitro (Fig. 3, lanes 3 and 5). The control experiment with *Drosophila* RNA demonstrated that homologous hsp70 mRNA was efficiently selected (lane 7). The additional bands in the in vitro translation of hybrid-selected mouse mRNA were background due to labeling of reticulocyte lysate proteins by [^{35}S]methionine. We attributed the efficiency of the heterologous hybridization selection to the high degree of homology between the *Drosophila* and mouse nucleic acids encoding the major heat shock protein. This result, combined with that shown in Fig. 3, which shows only one inducible mRNA, indicates that this mouse

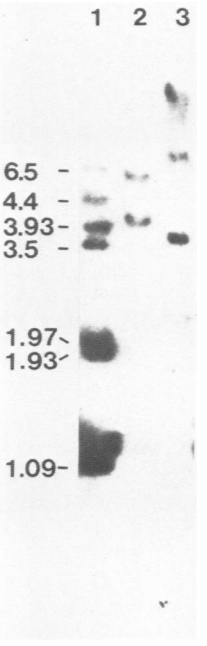


FIG. 2. Southern blot analysis of *Drosophila* and mouse DNAs. Genomic DNAs were isolated by phenol extraction from mouse L-cells and *D. melanogaster* Kc cells. DNAs were digested with restriction endonucleases, electrophoresed through a 0.8% agarose gel in 40 mM Tris base–20 mM acetic acid–5 mM sodium acetate–1 mM EDTA, and blotted to nitrocellulose (15). For probes the *Sal*I fragment from 56H8 (10) was labeled with ^{42}P by nick translation (14). Filters were prehybridized (without glycine) and hybridized as described previously (20), but with 1× rather than 5× Denhardt solution (1) in the hybridization solution. The filter was hybridized with the *Sal*I fragment in 40% formamide at 35°C for 60 h. Blots were washed at 56°C in 5× SSC (1× SSC = 0.15 M NaCl–0.015 M sodium citrate)–0.1% SDS and exposed to Kodak BB-1 film at −70°C. Lanes: 1, *Pst*I digest of 1 μg of *Drosophila* DNA; 2, *Pst*I digest of 10 μg of mouse DNA; 3, *Sal*I digest of 10 μg of mouse DNA. The sizes of the *Pst*I fragments from *Drosophila* DNA are indicated (kilobases).

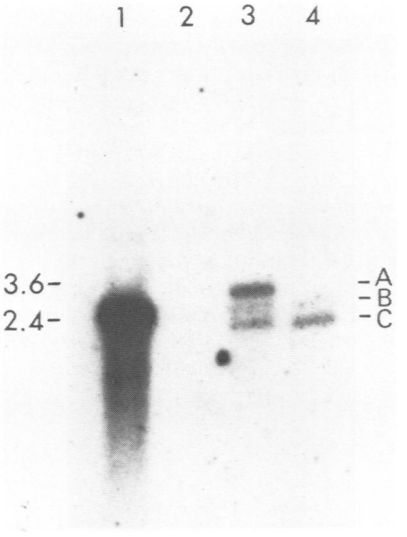
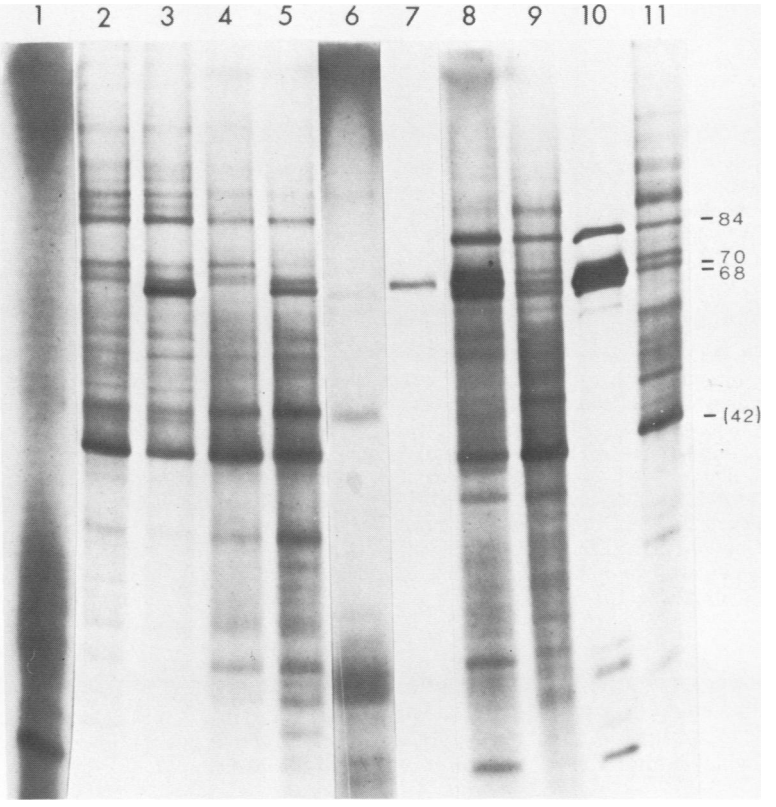


FIG. 3. Northern blot analysis of RNA from control and heat-shocked mouse and *Drosophila* cells. RNA was prepared from L-cells and *Drosophila* cells as described (Perry et al., submitted for publication). A 1-μg amount of poly(A)$^+$ RNA was fractionated by electrophoresis through a 1% agarose–6% formaldehyde gel (12) and transferred to nitrocellulose in 10× SSC (6). Filters were prehybridized (without glycine) and hybridized (20) to the labeled 56H8 *Sal*I fragment at 35°C in 43% formamide for 48 h and then washed at 65°C in 5× SSC–0.1% SDS (with several changes) over a 90-min period. Blots were air dried, covered with plastic wrap, and exposed to Kodak XAR-5 film with an intensifier at −70°C. Lanes: 1, *Drosophila* heat shock RNA; 2, *Drosophila* control RNA; 3, mouse heat shock RNA; 4, mouse control RNA. The numbers on the left indicate the approximate sizes of phage MS2 RNA (3.6 kilobases) from a stained gel and hsp70 mRNA (2.4 kilobases).

poly(A)$^+$ RNA encodes the mouse hsp70 and that the mouse and *Drosophila* heat shock-inducible genes have closely related nucleic acid sequences. Note that with the hybridization conditions described in the legend to Fig. 4, no mouse proteins other than hsp70 were synthesized with the selected RNA. This finding is despite detection of three RNAs on the Northern blot (Fig. 3). Under less stringent conditions, the additional RNAs can be selected and translated in vitro, but the protein products do not correspond to the mouse hsp70 (D. G. Lowe and L. A. Moran, submitted for publication).

Based on our data, together with the related



antigenicity of hsp70 from diverse organisms (4) and the cross-hybridization of a stress-inducible trout RNA and *Drosophila* hsp70 DNA (2), we conclude that the genes encoding the major stress-induced protein (hsp70) have been highly conserved throughout eucaryotic evolution. We have not determined the number of mouse sequences that are homologous to the *Drosophila* hsp70 gene, nor have we been able to construct a restriction map of the mouse genes. It is our impression from examining numerous digests of mouse DNA that the number of restriction fragments indicates less than five copies of the mouse hsp70 gene family and that the mouse genes have either no introns or very small introns (Fig. 2). It will be of interest to determine, once the mouse genes are cloned, whether they are organized in a manner similar to the *Drosophila* genes.

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FIG. 4. Fluorograph of L-[^{35}S]methionine-labeled proteins from cells and of in vitro translation of mRNAs. Mouse and *Drosophila* cells were labeled as described in the legend to Fig. 1. Lanes 2 and 3 are control and heat shock samples, respectively, from mouse L-cells. Control and heat shock samples from *Drosophila* cells are in lanes 11 and 10, respectively. mRNA populations were prepared as described in the legend to Fig. 3. Rabbit reticulocyte lysate was prepared (19), treated with micrococcal nuclease (11), and assayed with 0.5 μg of poly(A)$^+$ RNA (17). Lane 1, minus RNA translation control; lane 4, control; lane 5, heat shock L-cell mRNA translation products; lane 9, control; lane 8, heat shock *Drosophila* mRNA translation products. Hybridization selection (13) of mRNAs from heat-shocked mouse and *Drosophila* cells was preformed overnight at 42°C in 33% formamide–10 mM PIPES [piperazine-*N*,*N*′-bis(Z-ethanesulfonic acid); pH 6.4]–0.4 M NaCl–0.5% SDS–100 μg of tRNA with 80 μg of poly(A)$^+$ RNA per ml and 2 μg of denatured 56H8 *Sal*I fragment, immobilized on nitrocellulose. After hybridization filters were washed at 65°C in 5× SSC for selected mouse RNA and 65°C in 1× SSC for selected *Drosophila* RNA, with 0.1 M Tris (pH 7.6)–1 mM EDTA. Selected mRNA was eluted by boiling the filters in 150 μl of water for 80 s, followed by quick cooling. The RNA was precipitated several times, washed in 70% ethanol, and translated in vitro. Lane 6, translation of selected mRNA from mouse heat shock poly(A)$^+$ RNA; lane 7, translation of selected *Drosophila* hsp70 mRNA. Lane 7 is a shorter exposure (10 h), and lanes 1 and 6 are longer exposures (60 h), relative to the other lanes of the gel (32 h). The numbers on the right refer to the molecular weights (kilodaltons) of the three large *Drosophila* heat shock proteins and of actin (in parentheses).