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A Consensus Sequence Polymer Inhibits In Vivo Expression of Heat Shock Genes

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Promoter function for *hsp70* gene expression in *Drosophila melanogaster* was studied with an in vivo competition assay. A polymer of 40 tandem copies of the pair of regulatory elements of the *hsp70* gene was constructed and cloned into a plasmid vector. Various marked genes were cotransfected with the polymer plasmid into Schneider line 2 cells, and their expression was determined by enzyme activity measurements. The polymer dramatically inhibited expression of cotransfected *hsp70*, *hsp26*, and *hsp83* genes, but not cotransfected copia and histone genes. Our results indicate that in vivo, (i) a *trans*-acting, positive regulatory factor, which can be titrated by heat shock consensus sequences, is required for activation of heat shock genes and is specific for these genes; (ii) the coordinate induction of different heat shock genes appears to be mediated by similar, but not identical, interactions of the *trans*-acting induction factor and the *cis*-acting heat shock consensus sequences; and (iii) the uninduced or basal level expression of the transfected *hsp70* gene is also due to interaction of the consensus sequence with a positively acting factor.

Specific DNA-protein interactions play key roles in the regulation of gene expression. In eucaryotes, the mechanism by which these interactions modulate expression is not well understood; however, considerable progress has been made in the identification of the *cis*-acting DNA sequences and the *trans*-acting protein factors that participate in the regulation of at least some model genes. The *hsp70* gene of *Drosophila melanogaster* is one model gene that has received considerable attention. A specific heat shock consensus sequence (HSC) has been found upstream of a variety of different heat shock genes, and it has been shown to be essential for heat induction in several non-*Drosophila* cell systems (20, 24, 25). More recent evidence has shown that a pair of these HSCs is required for the proper regulation of the *hsp70* gene in *Drosophila* transformation systems (1, 7, 32). Parker and colleagues have shown that purified heat shock transcription factor (HSTF) binds to this pair of regulatory sequences (23) cooperatively (34) and thereby stimulates *hsp70* transcription in vitro (34). Taken together, these results suggest that heat shock genes are under positive control that is mediated by a common interaction of the *cis*-acting heat shock promoter elements and the *trans*-acting HSTF.

In this study, we test this model of regulation of *Drosophila* heat shock genes by an in vivo competition assay. This approach has been used by others to formally differentiate positive and negative regulation, to identify specific control sequences, and to determine whether specific genes are regulated by distinct or common factors (4, 30, 31). We have augmented this approach with an efficient method of generating polymers of regulatory sequence candidates. We measure the expression of several marked genes in *Drosophila* cells in the presence of a cotransfected polymer of the *hsp70* regulatory sequences. The results of these studies provide in vivo support for the model that a positively acting factor recognizes the HSCs of different heat shock genes and thereby coordinates their expression. In addition, the ability of the polymer to inhibit uninduced expression of a marked *hsp70* gene indicates that even in the absence of heat shock, the expression of the *hsp70* gene requires a positively acting factor capable of binding the HSC.

MATERIALS AND METHODS

Plasmids. For the purpose of constructing plasmids containing multiple copies of the HSC, plasmid pXM1 was constructed as described in Fig. 1 and its legend. This plasmid contains a fragment from −89 to −38 of the *D. melanogaster hsp70* gene which harbors the pair of the consensus sequences required for heat shock regulation (1, 7, 32). pXM1 was digested with *Xho*I and *Sal*I, and the 51-base-pair (bp) *Xho*I-*Sal*I fragment which contains the pair of HSCs was purified from low-melting-point agarose gel. Ligation of the *Xho*I-*Sal*I fragment into polymers was done in the presence of the *Xho*I and *Sal*I enzymes in standard ligation buffer (18) at room temperature (22 to 23°C) at DNA concentrations of 100 to 200 ng/μl. The polymers thus obtained have a head-to-tail arrangement of the *Xho*I-*Sal*I fragment and are resistant to the two enzymes (Fig. 2). These polymers were then cloned into *Xho*I-*Sal*I-digested pUC13X and transformed into *Escherichia coli* HB101 (18). A plasmid, pXM4, which contains a tetramer of the pair of the HSCs, was obtained after examining restriction digests from 100 single colonies. From a polymerization with the tetramer fragment, plasmid pXM40 was obtained, which contains 40 copies of the pair of *hsp70* consensus sequences.

Plasmid p83Z is a pMC1403 (5) derivative which contains a *D. melanogaster hsp83-E. coli lacZ* fusion gene in which the *hsp83* gene regulatory sequences control the expression of *E. coli lacZ* enzyme coding sequences. For the construction of p83Z, plasmid aDm4.46 (22) was digested with *Bam*HI (at −870 of *hsp83*) and *Pst*I (at +1618 of *hsp83*). The *Bam*HI-*Pst*I fragment was purified from the low-melting-point agarose gel and cloned into *Bam*HI-digested pMC1403. After 2 h of ligation, the *Pst*I sticky end at codon 110 of the *hsp83* gene and the *Bam*HI sticky end at codon 8 of *lacZ* were treated with S1 nuclease (18) and religated. After transformation into *E. coli*, a blue colony was selected on X-gal (18) for DNA preparation. Transfection into *Drosophila* tissue culture cells confirmed that it contained a functional hybrid *lacZ* coding region.

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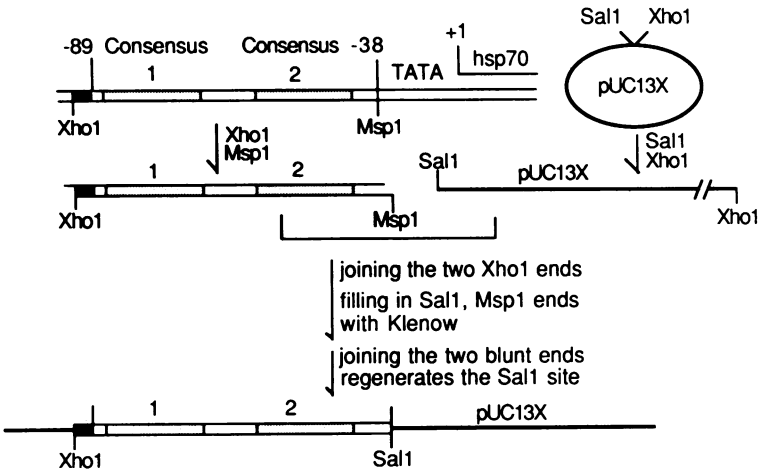

FIG. 1. Construction of pXM1. Plasmid SP1-89 (32) with an *Xho*I linker at the −89 deletion end point of the *hsp70* gene was digested with restriction enzymes *Xho*I and *Msp*I (the *Msp*I site is at −38 of *hsp70*). The relevant portion of the plasmid is illustrated in linear form. The 51-bp fragment containing the pair of consensus sequences was cloned into *Xho*I- and *Sal*I-digested pUC13X (a pUC13 derivative with an *Xho*I linker inserted into the *Sma*I site of pUC13; Claudia A. Sutton, unpublished result). After joining the two *Xho*I ends, the *Sal*I and *Msp*I sticky ends were filled in with Klenow polymerase. Ligation of the two blunt ends regenerated the *Sal*I site, producing a plasmid, pXM1, that contained the pair of HSCs between an *Xho*I and an *Sal*I site. The structure of the relevant region of the resulting plasmid was checked by DNA sequence analysis.

Plasmids p70Z and cp26Z have been described by Simon et al. (32). Plasmids hsp70-CAT and copia-CAT have been described (5) and were kindly provided by Igor Dawid. Plasmid D7 is a pMC1403 derivative which contains a *D. melanogaster* histone-*E. coli lacZ* fusion gene (Dave Gilmour, unpublished result).

Cell culture and transfection. Schneider line 2 *D. melanogaster* cells (28) were grown in Schneider medium (29) with 10% fetal calf serum in T flasks at 23°C. Two to three days before transfection, 1:10 dilutions were made from a single batch of nearly confluent cultures into Falcon T75 flasks with a 50-cm^2 growth area containing 3 ml of medium. Transfection was done when the cultures grew to a density of ca. 1×10^7 cells/ml. DNA samples for the transfection were prepared by using the calcium-phosphate coprecipitation method (6, 21). Each coprecipitation mixture contained three different plasmid DNAs. (i) One of the indicator plasmids *hsp70-lacZ* (p70Z), *hsp83-lacZ* (p83Z), *hsp26-lacZ* (cp26Z), *hsp70-CAT*, histone-*lacZ*, or copia-*CAT*. These plasmids are used to monitor heat shock, histone, or copia gene expression by determining β-galactosidase or chloramphenicol acetyltransferase (CAT) enzyme activity. (ii) The competitor plasmid, pXM40, which is used to titrate out the cellular factors that may interact with the sequence elements. (iii) The carrier plasmid pUC13X, used to keep the total DNA concentration constant in each precipitation mixture, is identical to the competitor plasmid pXM40 except that it lacks the HSC polymer.

For the preparation of the coprecipitation mixture, 0.3 ml of DNA-$CaCl_2$ solution (2 to 4 μg of indicator plasmid DNA, 25 to 30 μg of competitor plus carrier DNAs, and 0.25 M $CaCl_2$) was added dropwise to 0.3 ml of 2× HeBs buffer (6) in 1.5-ml Eppendorf tubes, and the tubes were then vortexed gently for a couple of seconds. After about 30 to 60 min at room temperature, the precipitate was added to the flask. Cells were left undisturbed until harvesting about 48 h later.

Cell harvest and heat induction. A 0.5- to 1.0-ml sample from each flask was taken and heat induced by incubation in a water bath (36 to 37°C) for 2 h. The non-heat-shocked samples were kept on ice. The cells were then collected by a 1-min centrifugation in a microfuge at 4°C and washed once with PBS buffer (10 mM $NaPO_4$, pH 7.2, 125 mM NaCl). The cells were then recentrifuged and suspended either in 0.2 to 0.5 ml of PBS buffer or in β-galactosidase assay buffer (27) for β-galactosidase assay, or in 0.1 to 0.2 ml of 0.25 M Tris chloride (pH 8.0) for CAT assay.

Enzyme assays. Cells were broken by sonication, and CAT or β-galactosidase activity was assayed as described (8, 26). In some experiments, whole cells were used for β-galac-

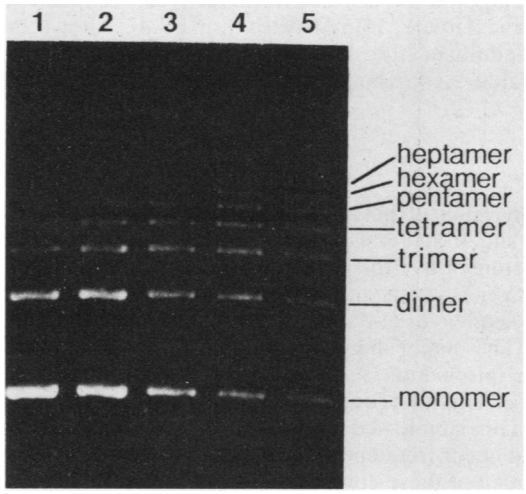

FIG. 2. An agarose gel showing the polymerization (ligation) of the *Xho*I-*Sal*I fragment in the presence of the *Xho*I and *Sal*I enzymes (for experimental details, see Materials and Methods). At different time points, a sample was taken into 10 μl of TE buffer (10 mM Tris chloride, pH 8.0, 1 mM EDTA) and heated to 65°C to inactivate the enzymes. The samples were then run on a 2.5% agarose gel. Lane 1, 10-min ligation; lane 2, 20-min ligation; lane 3, 40-min ligation; lane 4, 80-min ligation; lane 5, same as lane 4 except that a sample was taken into 10 μl of restriction buffer and digested with *Sal*I and *Xho*I for 30 min at 37°C. The ligation products were resistant to both enzymes (compare lanes 4 and 5).

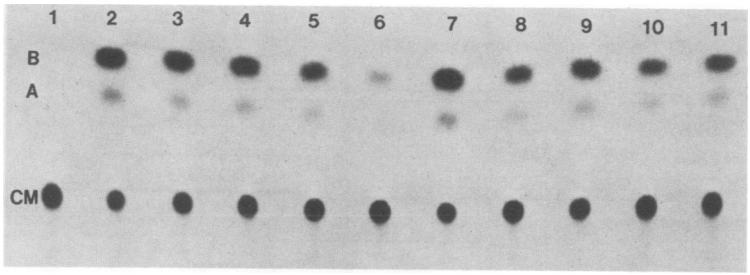

FIG. 3. Expression of heat shock and non-heat shock genes in the presence of the HSC polymer. Transfections and CAT assays were performed as described in Materials and Methods. Samples were spotted on thin-layer silica gels and run with chloroform-methanol (95:5, ascending). Chloramphenicol (CM) and its acetylated forms (in order of increasing mobility: A, 1-acetate chloramphenicol; B, 3-acetate chloramphenicol) were detected by autoradiography. Lane 1, the chloramphenicol standard. The remaining lanes show the products of chloramphenicol after incubation with extracts of cells cotransfected with a constant amount of *hsp70-CAT* (lanes 2 through 6) or copia-*CAT* (lanes 7 through 11) and increasing amounts of the HSC polymer. After autoradiography, the spots on the silica gels were cut out, and the radioactivity was counted (see Fig. 4d and 5).

tosidase assay. In this case, enzyme reaction was carried out at room temperature. After the reaction was stopped (26), samples were centrifuged to remove insoluble debris, and absorbance of the reaction mixtures was read at 420 nm.

Northern analysis of endogenous *hsp70* transcripts. Transfection was done as described above with carrier plasmid DNA only. Samples (1 ml) were taken from transfected and untransfected cells for heat induction at 36 to 37°C for 30 min. Non-heat-shocked samples were kept on ice. Cells were collected by a 1-min centrifugation in a microfuge at 4°C and washed once with PBS buffer. Cells were then lysed by suspension in 150 μl of Leder phenol (50:50:2, distilled phenol-chloroform-isoamyl alcohol) and 150 μl of FLB buffer (10 mM Tris chloride, pH 7.5, 20 mM EDTA, 1% sodium dodecyl sulfate [SDS]). After two Leder phenol and one ether extraction, RNA was collected by ethanol precipitation. Northern transfer was done essentially as described in the Schleicher & Schuell manual no. 352–54. Samples were run on a 1% agarose-formaldehyde-phosphate gel (1.1 M formaldehyde, 10 mM $NaPO_4$, pH 7.4). After transfer to nitrocellulose, the filter was probed with a ^{32}P nick-translated 2.1-kilobase (kb) *Xba*I fragment from the *hsp70* gene.

RESULTS

Generation of polymers of the *hsp70* regulatory element. Heat shock activates a relatively large family of genes (2) consisting of five to six *hsp70* genes (19), one *hsp83* and one *hsp68* gene, seven small heat shock genes (3), several copies of αβ sequences (14), and at least 20 minor heat shock genes (15). The major heat shock genes (10, 11) and the αβ transcription units (9, 16) have been sequenced and shown to possess flanking sequences that contain matches to the HSC (25). This simple sequence (with a conserved core of 8 bp) should occur frequently in the genome. Although in chromatin not all of these copies are necessarily accessible, the fact that a single copy of the consensus sequence is capable of binding HSTF (34) implies that the cellular concentration of HSTF may be relatively high.

To facilitate the in vivo competition assay, we devised a way of generating and cloning a polymer of the pair of consensus sequences that are required for expression of the *hsp70* gene (1, 7, 32). We reasoned that this polymer plasmid would permit the execution of competition assays at lower concentrations of transfected competitor DNA and would make it possible to detect competition even when a *trans*-acting factor is relatively abundant. A fragment containing the pair of consensus sequences was cloned into plasmid pUC13X (Fig. 1). This construct has the pair of consensus sequences present on a 51-bp *Xho*I-*Sal*I fragment. This fragment was purified from a low-melting-point agarose gel and ligated in the presence of *Xho*I and *Sal*I. When the *Xho*I and *Sal*I ends were ligated, the hybrid site which was generated was cleaved by neither enzyme. In contrast, the head-to-head joining of either a pair of *Sal*I ends or a pair of *Xho*I ends regenerated the respective sites, which were then cut. Thus, the irreversible formation of head-to-tail junctions drives the formation of the tandemly arrayed consensus sequence. The resulting polymers were resistant to the two enzymes (Fig. 2). These tandemly arrayed sequences were relatively stable when cloned in *E. coli*. In contrast, short, perfect inverted repeats are not stable in *E. coli* (13) and are avoided by the ligation method. Using this strategy, we generated a clone, pXM40, containing 40 copies of the pair of HSCs.

HSC polymer inhibits heat-inducible *hsp70* gene expression. Plasmids containing an *hsp70-lacZ* fusion gene or an *hsp70-CAT* fusion gene were cotransfected into *D. melanogaster* cells with various amounts of plasmid pXM40 containing the HSC polymer. The heat-inducible expression of the marked *hsp70* gene was dramatically reduced by increasing the polymer DNA concentration (Fig. 3, lanes 2 to 6, and Fig. 4a). Plasmids with a single or only a few copies of the pair of HSC sequences were much less effective at inhibiting expression of the *hsp70-lacZ* tester gene (data not shown). These results demonstrate that the HSC polymer competes for a cellular factor(s) which is necessary for *hsp70* gene expression. The consensus sequence exerts its effect in a positive rather than a negative manner, since the induced level of heat shock expression was reduced by additional copies of the consensus sequence. In addition, the transfected *hsp70* expression in uninduced cells was not increased by the presence of additional consensus sequences, as would be expected if a negative regulatory factor were being titrated from the tester gene (Fig. 4f).

To test whether the factors that are titrated by the *hsp70*-derived HSC polymer are specific for heat shock gene expression or are also used by non-heat shock genes, plasmids containing a histone-*lacZ* fusion gene or a copia-*CAT* fusion gene were cotransfected with the polymer-containing plasmid pXM40 into *D. melanogaster* tissue culture cells. As can be seen in Fig. 3 (lanes 7 to 11) and 4d and e, there was no reduction of β-galactosidase activity or CAT activity in

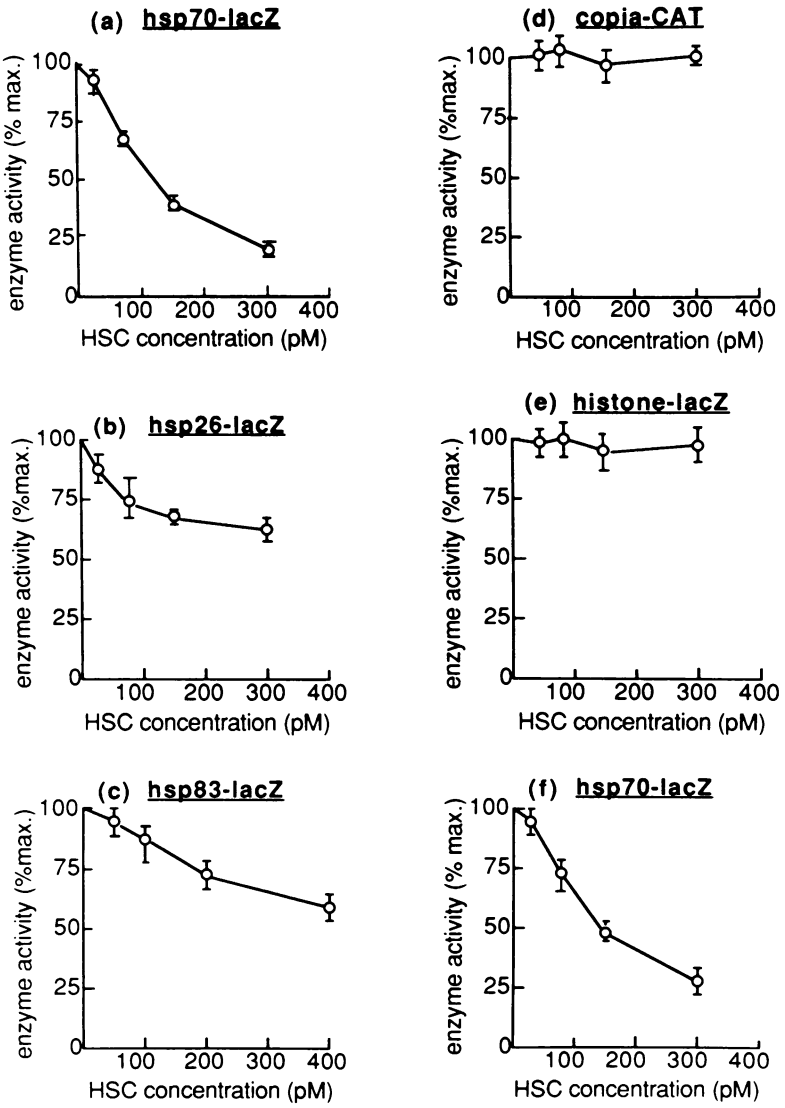

FIG. 4. Expression of marked genes in the presence of the HSC polymer. Transfections and enzyme assays were carried out as described in Materials and Methods. The background activity measured in the nontransfected cells was substracted from that of transfected cells. The values shown in the graphs represent mean values with standard deviation from two to three independent experiments, each done in triplicate. The enzyme activity without competitor present is set at 100%. (a) *hsp70-lacZ*; (b) *hsp26-lacZ*; (c) *hsp83-lacZ*; (d) copia-*CAT*; (e) histone-*lacZ*; (f) non-heat-shock-induced expression of *hsp70-lacZ*. The induction of *hsp70* was ca. three- to fivefold in our experiments.

these cases. These results demonstrate that the titrated factors do not influence the activity of the histone or copia genes.

To further demonstrate the specificity of the factor interactions, two additional experiments were carried out in which either (i) an *hsp70-lacZ* plasmid plus an *hsp70-CAT* plasmid and the polymer-containing plasmid or (ii) an *hsp70-lacZ* plasmid plus a copia-*CAT* plasmid and the polymer-containing plasmid were cotransfected. In the first experiment, both β-galactosidase activity and CAT activity were reduced to the same extent (Fig. 5a), since both *lacZ* and *CAT* are under *hsp70* control, while in the second experiment (Fig. 5b), only β-galactosidase activity was reduced when increasing amounts of polymer-containing plasmid were used. These results demonstrate that the reduction of *hsp70* gene expression is not due to a general inhibition of gene expression by the polymer plasmid.

Coordinate induction of heat shock genes is a result of the presence of HSCs. It was proposed, based on previous sequence analysis (9–11, 16, 27), that the presence of the HSCs could be responsible for the apparent coordinate induction of heat shock genes in *D. melanogaster*. In the following experiments, we tested this hypothesis, again by using the transfection-competition assay. For this purpose, a *D. melanogaster hsp83* gene was fused in phase to the *E. coli lacZ* gene (see Materials and Methods). Again, a constant amount of the *hsp83-lacZ*-containing plasmid was cotransfected with increasing amounts of the polymer-containing plasmid. As can be seen in Fig. 4c, the β-galactosidase activity was reduced as increasing amounts of the polymer DNA were used. A similar result was also obtained with a plasmid containing an *hsp26-lacZ* fusion gene (Fig. 4b). These results indicate that the polymer of the HSCs from the *hsp70* gene competes for certain factors required for the

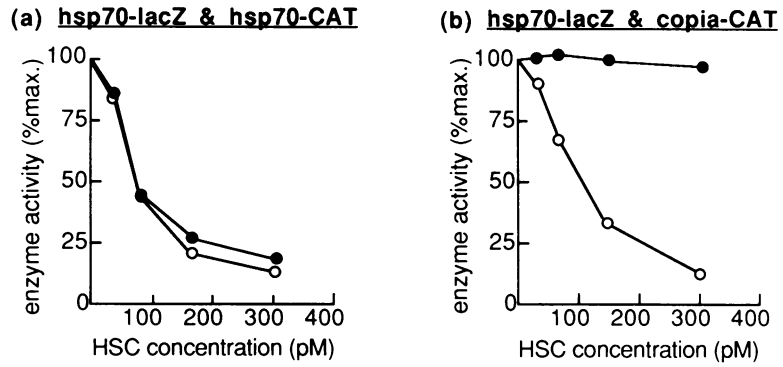

FIG. 5. Selective inhibition of heat shock gene expression by the HSC polymer. Differentially marked heat shock genes or non-heat shock genes were cotransfected with the HSC polymer into *Drosophila* S2 cells and assayed as described in Materials and Methods. Results are plotted as described in the legends to Fig. 3 and 4. Open circles, *lacZ* activity; closed circles, CAT activity.

expression of the *hsp83* and *hsp26* genes. However, at a given polymer concentration, the inducible expression of *hsp70* was most inhibited and *hsp83* expression was least inhibited (Fig. 4a, b and c). This difference was not large, but it was significant and may reflect different affinities of the genes for the transcription factor (see Discussion).

Basal level expression of heat shock genes is reduced by the HSC polymer. It is not known whether the uninduced or basal expression of heat shock genes is a result of transcription that is dependent on the HSCs. Cotransfection of a marked *hsp70* gene and the HSC polymer caused reduction of the *hsp70* basal level expression, as shown in Fig. 4f. This implies that basal-level expression results from interaction of the consensus sequence with a positively acting factor.

As seen by others, the induction of the transfected *hsp70* gene was much less than that of the endogenous *hsp70* gene or an *hsp70* gene introduced by germline transformation (1, 6, 7, 32). Either basal-level expression is higher from the gene on the transfected plasmid than from the endogenous chromosomal gene, or the induced level is lower from the gene on the plasmid, or both. We cannot distinguish between these possibilities due to the uncertainty in the number of functional plasmid copies per cell. However, the basal level observed from the marked *hsp70* gene used here was not due to stress-induced activation of the heat shock response by our transfection conditions. Northern blot analysis revealed that the uninduced level of the endogenous *hsp70* RNA in both nontransfected and transfected cells was approximately 1/500 of the induced level (Fig. 6). This level of induction is consistent with that seen previously (35). Approximately 10 to 20% of the cells took up functioning *hsp70-lacZ* plasmid DNA as estimated by X-gal staining of a transfected heat-induced culture (H. Xiao, unpublished result). Even if only this small fraction of cells were induced by our transfection conditions, the level of RNA produced by endogenous *hsp70* gene would be significantly higher than that in the nontransfected control. Thus, the high basal level of expression from plasmid-borne copies of the gene is not a consequence of stress-induced response during transfection.

DISCUSSION

A DNA consensus sequence, HSC, which is found in the regions upstream of different heat shock genes is an essential element of the heat-induced response (1, 7, 24, 25, 32). In vitro studies show that this sequence binds to a purified protein fraction that contains the HSTF, and this protein fraction also stimulates *hsp70* transcription in vitro (34). A pair of the HSCs are positioned upstream of the TATA box of the *hsp70* gene and are centered at nucleotides −78 and −55. Deletion of the distal (1, 7, 32) or the proximal copy (Simon and Lis, unpublished results) is sufficient to dramatically reduce the heat-inducible expression of an *hsp70* gene introduced by germ line transformation. Here we show that a polymer of 40 copies of the pair of the consensus sequences from the *hsp70* gene can inhibit heat-inducible expression of transfected *hsp70* gene in cultured *D. melanogaster* cells. We propose that the polymer of the HSC can compete with the *hsp70* gene for binding of a positively acting factor. This factor may be HSTF described by Parker and Topol (23). The addition of the polymer specifically inhibits heat shock gene expression and not expression of

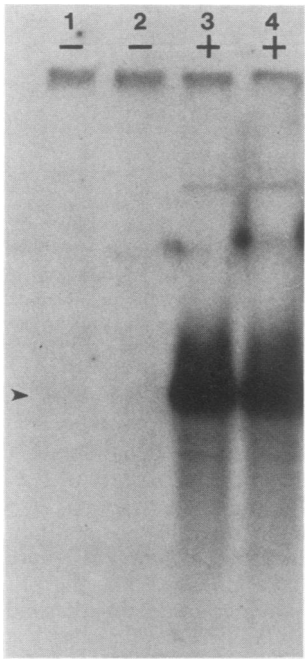

FIG. 6. Expression of endogenous *hsp70* gene in transfected cells. Northern analysis was performed as described in Materials and Methods. Each lane was loaded with 20 μg of total RNA. Lanes 1 and 3, RNA samples from nontransfected cells; lanes 2 and 4, RNA samples from transfected cells. +, RNA samples from heat shock-induced cells; −, RNA samples from uninduced cells. Arrow indicates *hsp70* mRNA. Even this level of overexposure revealed little *hsp70* signal in the track with RNA samples from the transfected but uninduced cells (compare lanes 1 and 2).

other genes, such as the copia and histone genes, indicating that a diffusible factor specific to heat shock genes is titrated by the HSC polymer.

The HSC polymer inhibited the induced expression of at least three different heat shock genes. These data strongly support the long-held assumption that the interaction of the consensus sequences of these genes with a common binding factor coordinates their response to heat shock. Nonetheless, the inhibition curves for the three different genes were quantitatively distinct. As can be seen in Fig. 4a, b, and c, the strength of the inhibition by the HSC polymer, which is derived from *hsp70*, was greater for *hsp70* than for *hsp26* and *hsp83*. We hypothesize that the relative affinity of the positive factor for the different genes varies and is greatest for *hsp83*. Wu has proposed (36) that the differential temperature requirements for the maximum induction of different heat shock genes could be a consequence of differential factor-binding affinities of the HSCs of different heat shock genes. When heat shock factor is limiting, as in the case of a low-temperature heat shock, then the genes with the highest affinity for the factor may be preferentially activated. Consistent with this hypothesis is the observed temperature maximum for the induction of different heat shock genes, which is 33°C for the *hsp83* gene and 37°C for *hsp70* (13).

The polymer competition results reported here are restricted to genes introduced by transfection as plasmids. The inducibility of a plasmid-borne *hsp70* gene in the transient transfection assay is much less than that seen for genomic copies, either *hsp70* genes introduced by germ line transformation (7, 17, 32) or endogenous copies. Nonetheless, the heat shock induction of the *hsp70* gene on a plasmid or in the genome shows the same requirement for the upstream pair of the HSCs (1, 7, 32). Thus, the effects reported here for the transfected, plasmid-borne genes probably reflect properties of the endogenous copies as well. We have not studied the inhibition of endogenous copies since only a small fraction of the cells in a culture are transfected by the added plasmid. Thus, the untransfected cells produce a high background from endogenous gene expression which would make it difficult to detect the polymer inhibition effects. In contrast, in the cotransfection procedure used here, cells receiving the tester plasmid also receive the HSC polymer-containing plasmid, since cells take up DNA as calcium phosphate coprecipitates.

The uninduced or basal-level expression of the transfected *hsp70* gene was reduced by the presence of the HSC polymer (Fig. 4f), indicating that the basal-level expression of heat shock genes is a result of interaction of the HSCs with a positively acting induction factor. If such interaction is not required, the basal level should be unaffected by the HSC polymer. Although the basal-level expression of transfected heat shock genes was high relative to induced levels (1, 6, 22, this work), the cell cultures did not appear to be heat shock-induced by our transfection conditions. The levels of endogenous *hsp70* gene expression examined in Fig. 6 revealed a normal low basal level in transfected cells.

The generation of polymers of potential regulatory sequences presented here should be generally applicable in the study of other promoters. Any sequence that can be inserted between a pair of different restriction sites that share common sticky ends, like *Xho*I and *Sal*I or *Bam*HI and *Bgl*II, can be polymerized to yield a stable tandem polymer. A variation in this approach has been independently derived by Simpson et al. (33) using *Ava*I fragments whose ends are nonsymmetric, thereby forcing head-to-tail ligation. An advantage to our approach is that the hybrid restricition sites are destroyed during polymerization; thus, the polymer can be excised as a unit for an additional round of polymerization. In addition to applications in competition experiments and in the study of chromatin structure (33), sequence polymers that contain protein-binding sites could be useful in affinity purification of interacting proteins.

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