Transcription, export and turnover of *Hsp70* and $\alpha\beta$, two *Drosophila* heat shock genes sharing a 400 nucleotide 5' upstream region

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ABSTRACT

A highly homologous 400 nucleotide sequence flanks the 5' end and extends 64 NT into the transcribed portion of all five *hsp70* and seven $\alpha\beta$ heat shock genes in *Drosophila melanogaster* (1-4). To determine the extent to which this sequence dictates coordinate regulation, we compared the total mass, continuous labeling and pulse-labeling of *hsp70* and $\alpha\beta$ RNAs at different times and temperatures of heat shock. By all these measurements, expression of both *hsp70* and $\alpha\beta$ genes increased and decreased in parallel. *Hsp70* RNA was generally synthesized at a higher rate and accumulated to a greater extent than $\alpha\beta$ RNA. As the temperature of heat shock increased, however, the rate of synthesis and accumulation of *hsp70* relative to $\alpha\beta$ RNA decreased. Another difference was that a larger fraction of *hsp70*, as compared to $\alpha\beta$ RNA was exported from the nucleus. For both RNAs, export decreased as the heat shock temperature was increased. The *hsp70* and $\alpha\beta$ genes are thus expressed in parallel, but the homologous 5' upstream sequences do not dictate equal rates of transcription or export from the nucleus.

INTRODUCTION

The heat shock response is an important example of the rapid and simultaneous activation of a group of genes by a simple stimulus. It thus provides a readily accessible system for analysis of the coordinate control of gene expression. The heat shock response has been demonstrated to occur in species which span the phylogenetic spectrum, including bacteria, yeast, slime molds, plants, insects, avians and mammals (5). An understanding of the mechanism of induction and degree of coordination of different heat shock genes should consequently be important to an understanding of coordinate gene regulation in all organisms.

The various genes involved in the heat shock response have been studied in greatest detail in *Drosophila melanogaster* (reviewed in ref. 6). Within one minute after a shift to the higher temperature, transcription at 9 heat shock loci (puffs) within the *Drosophila* polytene chromosomes is activated, while transcription at previously active sites is suppressed. Genes encoding the seven major heat shock proteins and the $\alpha\beta$ heat shock RNA (for which a

protein product has not been identified) have been cloned and localized to 5 of the heat shock puff loci.

To what extent can the coordinate induction be due to similar 5' upstream regions flanking the various *Drosophila* heat shock genes? Among the genes which have been sequenced (hsp22,23,26,27,68,70 83 and $\alpha\beta$), several regions of homology exist within the 5' upstream regions as well as within the 5' untranslated portion of the transcripts (1,2,7). The homology between 5' upstream sequences is particularly striking in the case of the hsp70 and genes. All 5 hsp70 genes (2 at chromosomal locus 87A7 and 3 at 87C1) as well as 7 $\alpha\beta$ units (at 87C1) share a 389 NT 5' upstream region which is 83-98% homologous. This region extends from -342 nucleotides upstream to +64 nucleotides in the 5' untranslated portion of the transcript (1,3,4,8).

Analysis of the inducibility of genes adjacent to *in vivo* and *in vitro* altered upstream regions has identified a 5' upstream region, from nucleotides -66 to -10, that is required for heat shock induction of hsp70 genes (9-12). More detailed examination has shown that it is the TATA box and a sequence with weak dyad symmetry at -47 to -66 which are required (10). Both of these sequences have homologs in 7 out of 8 heat shock gene upstream regions (10).

The high degree of homology over 400 nucleotides in their 5' upstream regions suggests that regulation of the expression of the hsp70 and $\alpha\beta$ genes at the transcriptional level is very similar. This seems particularly likely, as the TATA box and the -47 to -66 nucleotide sequence necessary for induction (10) are identical for both hsp70 and $\alpha\beta$ sequences (1,3,4,7).

We describe here experiments to test the hypothesis that the homologous upstream regions of the hsp70 and $\alpha\beta$ sequences dictate equal rates of transcription. We also examine the extent to which total cellular concentration, as well as export from nucleus to cytoplasm of hsp70 and $\alpha\beta$ RNA are coordinately controlled. Our results show that, while the transcription of the two sequences is regulated approximately in parallel, there are significant differences in absolute levels of transcription, in patterns of transcription during heat shock, and in export from nucleus to cytoplasm.

MATERIALS AND METHODS

Cell Culture and Labeling

Exponentially growing suspension cultures of *Drosophila* line 2-L (13) were grown to 3×10^6 ml, fed with fresh medium (13) to 2×10^6 ml, then heat shocked 1 hour later by rapid swirling in a 45°C water bath until the desired temperature (34-37°C) was attained (approximately 2 minutes). At this time,

cells were labeled with ^3H -uridine (27 Ci/mmol) at 50 $\mu\text{Ci/ml}$. Unless otherwise indicated, $t=0$ indicates the time at which the heat shock temperature was reached and when label was added.

Cell Fractionation and RNA Purification

Cells were fractionated, RNA was purified and DNase treated, and poly(A)⁺ RNA was prepared as described (14,15). A slight modification of the cell fractionation protocol was that nuclei were stripped of cytoplasm by centrifugation over a pad of 0.75M sucrose in lysis buffer (14) containing 1% Tween40 and 0.5% sodium deoxycholate. This fractionation protocol leads to good separation of nuclear from cytoplasmic RNA (16). For preparation of total cellular RNA, cells were lysed in lysis buffer, then deproteinized with proteinase K after addition of an equal volume of 2X SDS buffer (14). After ethanol precipitation, nucleic acids were extracted with phenol and chloroform/isoamyl alcohol.

Quantitative Filter Hybridizations

Quantitative hybridization to excess DNA on filters (14) was used to measure the amount of radioactive hsp70 and $\alpha\beta$ RNA in ^3H -in vivo- and in ^{32}P -in vitro-labeled RNA fractions. Following the hybridization, filters were treated with RNase (2.5 $\mu\text{g/ml}$ RNase A and 2.5 units/ml RNase T1 in 2XSSC, 37°C, 1 hr). All hybridization data are the average of 2-6 separate filter hybridizations and have been corrected for background (10-30 cpm), for hybridization efficiency (25-90%; this was the range in the percent of homologous cRNA that hybridized to the probe as measured in each experiment) and, for ^3H , for efficiency of scintillation counting (14%) (14). Other hybridization corrections are described below.

Hsp70 RNA was detected by hybridization with DNA of plasmid 229.1 (17) which contains the 0.8 kb Bam HI-Sal I genomic fragment from the 3' end of an hsp70 coding sequence inserted into pBR322. $\alpha\beta$ RNA was detected by hybridization with DNA of plasmid JL1003, a subclone of the 1.5 kb $\alpha\beta$ Hind III fragment from plasmid cDm703 (18) inserted into the Hind III site of pBR322.

Since the hsp70 and $\alpha\beta$ sequences are repeated (18-20), divergence between transcript and DNA probe could lead to an underestimate of transcript abundance. Plasmid 229.1 DNA was used as a probe because it detects the more conserved portion of the hsp70 transcripts. The most divergent hsp70 genes (i.e., those at 87A7 compared to those at 87C1) differ by 15% in the first few hundred nucleotides at their 5' ends (21) but are more conserved (only 4.5% divergent) in the Bam HI-Sal I fragment insert of plasmid 229.1. Because this insert is complementary to only 0.8 kb of the 2.4 kb hsp70 mRNA, all dpm in

hybrid with 229.1 DNA were multiplied by 3 to obtain total dpm in hsp70 RNA.

Control experiments were carried out to determine if the probe or hybridization conditions underestimated the total transcripts in hybrid. The release from filters of only 7% of hybridized hsp70 RNA by a high stringency wash (60°C, 0.1XSSC) was consistent with the conclusion that hsp70 transcripts are highly homologous to the 229.1 DNA probe (data not shown). In contrast, the same high stringency wash released 26% of hybridized $\alpha\beta$ RNA. Furthermore, only 40% of hybridized $\alpha\beta$ RNA remained bound to the filter after the RNase treatment, compared to 80% for perfect hybrids (22, data not shown). On the basis of these results, we made no additional correction for hsp70 dpm in hybrid and multiplied the dpm in $\alpha\beta$ RNA hybrid by two.

Additional sequences homologous to hsp70 have been described. The heat shock cognate (hsc) genes are 25% divergent from hsp70 and are at least 10-fold less abundant than maximally induced hsp70 RNA (23,24). We therefore do not expect to detect hsc transcripts in our assay for hsp70 transcripts. The hsp68 gene is 15% divergent from hsp70 (21). After 1 hour of heat shock at 37°C, the amount of hsp68 transcript detected with an hsp70 probe is less than 5% of the hsp70 transcript itself (21). After the stringent RNase treatment, which removes 70% of hybrid from transcripts only 10% divergent (22) we do not expect to detect significant amounts of hsp68 RNA.

RESULTS

To determine the degree to which the hsp70 and $\alpha\beta$ sequences are expressed coordinately, we investigated their expression at several heat shock temperatures as a means of varying the level and duration of the heat shock response (6, 25-27).

Comparison of Hsp70 and $\alpha\beta$ Transcripts

Electrophoretic analysis of poly(A)⁺ cytoplasmic RNA from cells heat shocked at 35°C shows that hsp70 RNA migrates as a single species of 2.5 kb (Fig. 1b); this size determination is consistent with sequence analyses (1,28).

Four poly(A)⁺ cytoplasmic RNA species, of 2.5, 1.8, 1.4 and 1.1 kb, are detected by the $\alpha\beta$ probe (Figure 1a). The hsp70 mRNA and the largest $\alpha\beta$ RNA migrate to the same position in the gel (the autoradiograms show the same gel transfer hybridized first with an $\alpha\beta$, then with an hsp70 probe; see Fig. 1 legend). This observation raises the question of whether the 2.5kb $\alpha\beta$ and hsp70 RNAs are homologous. Hybridization of Bam HI- and Sal I-digested DNA of the hsp70-encoding plasmid 56H8 (29) with ³²P-DNA of the $\alpha\beta$ -carrying plasmid JL1003, however, revealed no detectable homology between the $\alpha\beta$ and hsp70

Figure 1. Poly(A)⁺ cytoplasmic hsp70 and $\alpha\beta$ RNAs during heat shock at 35°C. Cytoplasmic RNA was prepared from aliquots taken at various times from a culture of cells which had been raised to 35°C. Poly(A)⁺ RNA, prepared from equal amounts of RNA from each sample, was electrophoresed in a formaldehyde gel (46) and transferred to nitrocellulose paper (47); the far right lane (120 min) was accidentally partially cut off. a.) Hybridization (47) was first with 2×10^7 dpm (1 μ g) of ³²P labeled JL1003 DNA, to detect $\alpha\beta$ RNA. After autoradiographic exposure (6.5 days), the JL1003 probe was boiled off the filter in 0.01X SSC, 0.1% SDS. b.) The second hybridization was with 6×10^7 dpm (1 μ g) of ³²P labeled 229.1 DNA, to detect hsp70 RNA; the autoradiographic exposure for this second hybridization was 0.25 days. Size markers in kb, determined from the parallel electrophoretic migration of Hind III-digested lambda and Hae III-digested X174 DNA fragments, are indicated between panels a and b. The time (in minutes) during heat shock at which the aliquot was taken is shown below each gel lane. The t=0 time point is from 25°C cells.

sequences (data not shown). The 64 nucleotide homology between the 5' ends of the hsp70 and $\alpha\beta$ transcripts was not detected because the JL1003 probe does not contain the γ sequence (3). The 2.5kb band in Fig. 1b cannot be a remnant of hybridization with the $\alpha\beta$ probe because it appeared in the autoradiograph so much more rapidly and because only one, not four, bands were detected by the hsp70 probe.

The pattern of multiple $\alpha\beta$ RNA species present in cytoplasmic poly(A)⁺ RNA of cultured cells (Fig. 1a) is similar to that in total RNA of adult flies (20). Its coelectrophoresis with the 2.5 kb hsp70 RNA demonstrates that the largest $\alpha\beta$ RNA is smaller than the 2.8 kb originally reported (20). The 1.8 and 1.1 kb $\alpha\beta$ RNAs appear to be the same as the 1.8 and 0.8 kb species described previously (18,20). The 1.4 kb species is probably the same as a minor species detected but not previously described (20).

Figure 2. Changing concentrations of total hsp70 and $\alpha\beta$ RNA during heat shock at different temperatures. Cells were brought to the indicated heat shock temperature at $t=0$. Aliquots of the culture were withdrawn at various times and total RNA was extracted, labeled *in vitro* by kinasing with ^{32}P (48), and hybridized to hsp70 and $\alpha\beta$ DNA on filters.

The levels of hsp70 and $\alpha\beta$ RNAs increase and decrease in a parallel manner over 2 hrs of heat shock at 35°C (Fig. 1). Neither RNA was detected at 25°C (0 min of heat shock). By 30 minutes, both RNAs reached a maximal level, which was maintained until 60 minutes of heat shock. By 2 hours, the concentration of both RNAs had decreased several-fold.

Although the time course of the changing levels of hsp70 and $\alpha\beta$ poly(A)⁺ cytoplasmic RNAs is similar, the absolute amount of each of these RNAs present during heat shock is quite different, as deduced from the difference in exposure time required to obtain autoradiographs of similar intensity for the two RNAs (see Fig. 1 legend).

Quantitative Analysis of Hsp70 and $\alpha\beta$ Expression

To make the above observations more quantitative and to extend them to different heat shock temperatures, we determined the total amount of hsp70 and $\alpha\beta$ RNA in heat shocked cells by filter hybridization (Figure 2). As in Figure 1, the hsp70 and $\alpha\beta$ RNAs increased and decreased in parallel at each temperature. There was significantly more hsp70 than $\alpha\beta$ RNA, at all times and temperatures of heat shock. The mass ratio of hsp70 RNA to $\alpha\beta$ RNA was approximately five at 34 and 35°C, but fell to two or less at 37°C.

The duration of the heat shock response increased with increasing temperature. At 34°C, levels of hsp70 and $\alpha\beta$ RNA reached a peak at 30 min and declined shortly thereafter; these same levels remained at a plateau until 60 min at 35°C and until 120 min at 37°C (Figure 2a-c).

Incorporation of Radioactivity in vivo

The measurements presented above provide only a static view of heat shock gene expression. The concentrations of cytoplasmic hsp70 and $\alpha\beta$ RNAs at different times during heat shock, however, are dynamic functions of rates of synthesis, export and turnover. Insight into the role which these processes play in controlling heat shock gene expression can be obtained by examining hsp70 and $\alpha\beta$ RNAs in continuous labeling, pulse-labeling and pulse-chase experiments during the course of heat shock.

a) Continuous Labeling To allow comparison between incorporation of radioactivity at different heat shock temperatures, we determined UTP pool specific activities during the labeling period (Fig. 3a-c). The time course of the change in pool specific activity was similar at all heat shock temperatures examined. Different temporal profiles of incorporation of label into RNA at the different heat shock temperatures (Fig. 3, g-l) are thus not due to differences in kinetics of pool equilibration. Furthermore, the total amount of RNA accumulated at each heat shock temperature was approximately the same, since both pool specific activity and total incorporation at 37 °C were half those at 34°C.

Knowledge of pool specific activities can, in some cases, be used to obtain information about absolute rates of synthesis and decay of RNA from continuous incorporation data. This is not possible for the data in Figure 3 because rates of transcription and turnover of specific RNAs are not constant during heat shock (unpublished data, see below and 27).

The incorporation of radioactive nucleotide precursor into total nuclear RNA reached a plateau at about 90 min, at all temperatures of heat shock examined. Incorporation into cytoplasmic RNA, however, decreased significantly with increasing heat shock temperature. These results suggest that there is decreased export of RNA from the nucleus to the cytoplasm as the temperature of heat shock is increased (see below).

The incorporation of radioactivity into hsp70 and $\alpha\beta$ RNA shown in Figure 3 confirms and extends the results in Figures 1 and 2. At the three heat shock temperatures examined, the amount of radioactivity in hsp70 and $\alpha\beta$ RNAs increased and decreased in parallel (Fig. 3 g-l). The ratio of radioactivity incorporated into hsp70 RNA relative to that into $\alpha\beta$ RNA was five at 34°C, but decreased to only one-half at 37°C. Thus the synthesis of hsp70 relative to $\alpha\beta$ RNA decreases with increasing heat shock temperature; this is confirmed by pulse-labeling experiments (see below).

The data in Fig. 3(g-l) reveal that there are important effects of heat

Fig 3. Continuous labeling of hsp70 and $\alpha\beta$ RNA at different heat shock temperatures. Cells were concentrated five-fold, incubated for 30 min at 25°C, then brought to the indicated heat shock temperature and labeling was initiated. Aliquots of the culture were taken at various times for pool specific activity measurements by an RNA polymerase assay (48,49). RNA was prepared from nuclear and cytoplasmic fractions of the cell aliquots. Dpm in total and in hsp70 and $\alpha\beta$ RNA were determined by TCA precipitation and filter hybridization, respectively. The heat shock temperatures used are shown at the top of each set of four panels. The hybridized dpm in nuclear RNA are shown in open circles (o); those in cytoplasmic RNA are shown in closed circles (●).

shock on the export from nucleus to cytoplasm of the hsp70 and $\alpha\beta$ RNAs. The rapid appearance of label in cytoplasmic hsp70 and $\alpha\beta$ RNA indicates that these RNAs are exported rapidly from the nucleus. As the temperature of heat shock was increased, however, the fraction of the total dpm in both hsp70 and $\alpha\beta$ RNA which appeared in the cytoplasm decreased. In this respect the regulation of the two sequences was similar. At all temperatures of heat shock examined, however, the proportion which remained in the nucleus was less for hsp70 than for $\alpha\beta$ RNA. This indicates that hsp70 RNA is exported more rapidly from the nucleus than $\alpha\beta$ RNA; this is confirmed by pulse-labeling experiments (see below).

b.) Pulse Labeling The changing levels of hsp70 and $\alpha\beta$ RNAs during heat

Figure 4. Relative rates of incorporation of label into hsp70 and $\alpha\beta$ RNA during a brief pulse at different heat shock temperatures. Aliquots of cells were pulse-labeled for 10 min at different times during heat shock. Immediately after labeling, cells were lysed and total RNA was extracted and hybridized to hsp70 and $\alpha\beta$ DNA on filters.

shock (Figs. 1-3) are functions of both the rate of transcription of each sequence and the rate of turnover of the resultant transcripts. To estimate relative rates of transcription for the hsp70 and $\alpha\beta$ sequences, we measured the amount of radioactivity incorporated into hsp70 and $\alpha\beta$ RNA in a 10 min pulse at various times and temperatures of heat shock (Figure 4). At 34 and 35°C, pulse labeling of the hsp70 and $\alpha\beta$ RNAs reached a maximum early in the heat shock period (Figure 4a and b) and then declined rapidly. At 37°C, on the other hand, pulse labeling remained at a plateau, declining only slightly during the heat shock period (Fig. 4c). Although pulse-labeling appeared to reach a peak somewhat earlier for hsp70 than for $\alpha\beta$ RNA, the pattern of pulse-labeling of these RNAs generally changed in parallel during heat shock at different temperatures.

An important difference in these pulse-labeling patterns was that 3 to 19-fold more label was incorporated into hsp70 than into $\alpha\beta$ RNA in pulses at 34 and 35°C; this declined to 0.5 to 1.5-fold at 37°C (Figure 4; note the 5-fold difference in scale for the dpm incorporated into the two RNA species).

While the ratio of hsp70 to $\alpha\beta$ RNA decreased with increasing temperature in all the experiments described above (Figs. 2, 3 and 4), the change in the absolute amount of hsp70 RNA, in going from 34 to 37°C, was not the same in all experiments. In the experiment shown in Fig. 2, the total mass of hsp70 RNA increased in going from 34 to 37°C, while in going from 35 to 37°C, the levels of continuous labeling (Fig. 3) and pulse-labeling (Fig. 4) of hsp70

Figure 5. Distribution between nucleus and cytoplasm of pulse-labeled hsp70 and $\alpha\beta$ RNA at different heat shock temperatures. Cells were pulse labeled for 7 min at various times during heat shock at the indicated temperatures. The dpm in the cytoplasm for each species, divided by the dpm in the nucleus for that same species, at the end of each pulse, is plotted to indicate the distribution between nucleus and cytoplasm of pulse-labeled hsp70 (●) and $\alpha\beta$ RNA (○).

RNA decreased. It has been our experience that absolute amounts of heat shock mRNAs produced are not always reproducible from experiment to experiment in the cell line and medium that we use. Lindquist (30) has observed that undefined variations in the culture medium can affect the temperature of maximal heat shock expression; this is likely to have been the case in the experiments reported here. This variation in absolute levels of heat shock gene expression between experiments does not, however, affect our major point that the hsp70 and $\alpha\beta$ genes are not expressed at the same levels, and that the ratio of these levels can vary significantly as a function of different heat shock conditions.

The continuous incorporation data (Figure 3g-1) suggest that export from nucleus to cytoplasm differs for the hsp70 and $\alpha\beta$ RNAs. To obtain a quantitative estimate of this difference, we measured the ratio of radioactivity in cytoplasmic versus nuclear RNA for each species after a 7 min pulse. The results are consistent with the continuous incorporation data (Fig. 3). First, a larger portion (2-5-fold more) of total hsp70 RNA, as compared to $\alpha\beta$ RNA, is exported to the cytoplasm within 7 minutes of synthesis, at all heat shock temperatures (Fig. 5). Second, as the temperature of heat shock was increased, the export of both RNAs decreased (note the change in scale in Fig. 5c), although that of hsp70 RNA declined more dramatically than that of $\alpha\beta$ RNA. Thus hsp70 RNA is exported more rapidly than $\alpha\beta$ RNA, but this rate, relative to that of $\alpha\beta$, declines with increasing heat shock temperature.

Figure 6. Decay of $\alpha\beta$ nuclear and cytoplasmic RNA at different heat shock temperatures. Cells, concentrated fivefold, were incubated for 1 hr at 25°C with 5 mM glucosamine, to shrink the UTP pool (31). They were then shifted to either 34 or 37°C and labeled with ^3H -uridine. After the labeling period (30 min at 34°C and 90 min at 37°C), cells were diluted fivefold into medium containing 5 mM each of glucosamine and unlabeled uridine and cytidine. This regimen provides a good chase of incorporated label in *Drosophila* cultured cells (31), as indicated by the termination of further incorporation into 5S RNA (data not shown). One-half of the cells labeled at 37°C were chased at 35°C. Aliquots were taken at various times and the amount of radioactivity in nuclear (a) and cytoplasmic (b) $\alpha\beta$ RNA was determined as described in Material and Methods. Labeling and chase at 34°C (Δ), labeling at 37°C and chase at 37°C ($\square$) and at 35°C ($\circ$).

c.) Pulse-Chase Experiments The continuous incorporation and pulse-labeling data (Figs. 3 and 4), suggest that after 1-2 hrs of heat shock at 34 and 35 °C, both hsp70 and $\alpha\beta$ RNAs turn over fairly rapidly. If this were not the case, both RNAs would remain at their maximal levels throughout the labeling period, even after their rates of transcription (Fig. 5) had decreased. To demonstrate this more directly, we performed pulse-chase experiments in the presence of glucosamine (31) (Fig. 6). The results for $\alpha\beta$ nuclear RNA are consistent with the continuous incorporation data. At 34°C, $\alpha\beta$ nuclear RNA had a half-life of 20 min; this half-life increased with increasing heat shock temperature so that at 37°C, no turnover of this RNA was detected. Cytoplasmic $\alpha\beta$ RNA was more stable: the shortest half-life was 45 min at 34°C.

Since the shortest observed half-life of $\alpha\beta$ nuclear RNA was 20 minutes (at 34°C), the amount of radioactive $\alpha\beta$ RNA accumulated in a 10 min pulse should be at least 85% of the total synthesized. This means that the high ratio of radioactivity in hsp70 relative to $\alpha\beta$ RNA in a 10 minute pulse (Fig. 4a and b) is not due to a rapid turnover of $\alpha\beta$ RNA, but must be due to a higher rate of transcription of hsp70 sequences.

DISCUSSION

Aspects of Coordinate Control of Hsp70 and $\alpha\beta$ Genes

The heat shock response is a complex system of gene regulation, in which rates of transcription, turnover and translation of different transcripts are only partially coordinately regulated (27,32). Because the hsp70 and $\alpha\beta$ genes have the special feature of sharing a highly homologous upstream sequence, it is important to evaluate the extent to which these particular genes are coordinately controlled.

We have demonstrated that there is a parallel, although unequal, change in expression of hsp70 and $\alpha\beta$ genes, whether assessed in terms of total mass of RNA accumulated, total radioactivity accumulated, or label incorporated in a pulse, over the course of heat shock at different temperature. This is remarkable when one considers that there is a different temporal course of expression at each heat shock temperature. At the lowest heat shock temperature examined (34°C), expression of both sets of genes reached a brief peak at 30 min and then declined. As the heat shock temperature was increased, the expression of hsp70 and $\alpha\beta$ genes persisted longer during the heat shock period until at 37°C, the expression of hsp70 and $\alpha\beta$ genes remained maximal throughout the duration of the heat shock (120 min).

Our characterization, by pulse labeling, of parallel changes in transcription rate of hsp70 and $\alpha\beta$ genes is consistent with studies on puffing of polytene chromosomes during heat shock. The heat shock puffs, although different in size (presumably as a result of different rates of transcriptional activity), increased and decreased in parallel over the course of heat shocks at various temperatures (25). We have shown that, as the temperature of heat shock increased, the level of transcription of hsp70 and $\alpha\beta$ genes remained maximal for longer periods of time. Similarly, in the cytological studies, puffs remained large for a longer period of time when the heat shock was at 37°C than when the heat shock was at 32°C (25). This agreement between two very different kinds of data suggests that the transcriptional phenomena observed in the cultured cells are similar to those which occur during the in vivo heat shock response.

The parallel expression of the hsp70 and $\alpha\beta$ genes is similar to that of the heat shock genes at 67B (hsp22, 23, 26 and 27) in that in each case a cluster of genes is activated by heat shock to form, within the resolution of the cytological analysis, a single puff. While there are also examples of transcriptional units located in heat shock puffs which do not appear to be expressed during the heat shock response (33,34), it is possible that clus-

tering of some heat shock genes, particularly hsp70 and $\alpha\beta$, plays a role in their coordinate expression.

Deviations from Coordinate Control

a.) Transcription Rates As described above, expression of the hsp70 and $\alpha\beta$ genes changes in parallel during different heat shock regimens. Regardless of the criteria used for comparison (mass of RNA, total incorporation or pulse-labeling) however, the level of expression of hsp70 genes was strikingly different from that of $\alpha\beta$ units. In a pulse-label for 10 or 7 min (Fig. 4 and data not shown), the ratio of incorporation into hsp70 RNA relative to that into $\alpha\beta$ RNA was as high as 10 at 34°C. Since pulse-labeling gives a reasonable estimate of relative rates of transcription (discussed above), these results indicate that a significant portion of the difference in accumulation of hsp70 and $\alpha\beta$ RNA was due to a difference in transcription rates of the two sets of genes. Similar parallel, but unequal rates of transcription, have been described in a comparison of hsp70 gene expression with that of the unlinked heat shock gene hsp26 (35).

An additional striking difference is that relative transcription rates of the hsp70 and $\alpha\beta$ genes change with changing temperature of heat shock. The ratio of hsp70 to $\alpha\beta$ transcription, assayed by pulse labeling in the first 10 min of heat shock, decreased with increasing heat shock temperature, from 10 at 34°C to only 1.5 at 37°C.

Pulse labeling provides estimates only of relative rates of transcription. Minimal estimates of absolute transcription rates for hsp70 and $\alpha\beta$ genes can be made from the percent of the total mass of cellular RNA which each RNA species constitutes after the first 40-70 min of heat shock (Fig. 2). We assume that the total mass of RNA per *Drosophila* 2-L cell is 6 pg (14), that each cell is approximately 3.4C (13) and that there are 5 copies of each hsp70 gene per haploid genome in the cultured cells (19, and unpublished data). Using these assumptions, we calculate that the average rate of transcription of each hsp70 gene is 20-40 molecules/min. This is within the range of the maximal rate of transcription of a eucaryotic gene at 37°C of 10-50 molecules/min (36) and is somewhat greater than the rate of 10 molecules/min/gene calculated for 36°C heat shocked *Drosophila* embryos (Freund, quoted in ref. 37). These results suggest that, during the peak of heat shock in both cultured cells and the whole animal, all hsp70 genes are transcribed at close to the maximal rate. This is consistent with studies of deletion mutants at the 87A7 and 87C1 loci, which indicate that most of the hsp70 genes are transcribed in vivo (19,38). The 5-fold repetition of hsp70 sequences in the

genome may thus be necessary to provide the required amount of hsp70 protein in a very short period of time. This is consistent with the observation that genes encoding hsp70-related proteins in yeast and mouse are also repeated (39).

To calculate the average rate of transcription of $\alpha\beta$ units, we assume that the average $\alpha\beta$ RNA is 1.8 kb in length (Fig. 1) and that only 7 $\alpha\beta$ units per haploid genome are transcribed. We made this latter assumption because only $\alpha\beta$ units at 87Cl are transcribed and, of these, only 7 are immediately downstream from the γ element which is homologous to the 5' upstream region of hsp70 (20). DNA gel blots (data not shown) indicate that the number of γ -associated $\alpha\beta$ units is the same in the cultured cells studied here as in whole flies (20). Using the above assumptions, as well as those described for calculations on hsp70 transcription, and taking into account the stability of the $\alpha\beta$ nuclear RNAs shown in Fig. 6, we obtained a minimal transcription rate of 4-18 molecules/gene/min for the 7 γ -associated $\alpha\beta$ units. We cannot determine if all $\alpha\beta$ units adjacent to γ elements are transcribed at the same average rate, or if a smaller number are transcribed at a higher rate. The $\alpha\beta$ units are transcribed at close to the maximal possible rate at 37°C (18 molecules/gene/min), but at a significantly lower rate at 34 and 35°C (4-7 molecules/gene/min).

How can we explain the difference in transcription rates of hsp70 and $\alpha\beta$ genes, as well the changing ratio of these rates with increasing heat shock temperature? While rates of transcription of hsp70 genes are self-limited by levels of the protein product (27,32), this cannot explain the difference in rates of transcription between hsp70 and $\alpha\beta$ genes within the first 10 minutes of heat shock, when very little hsp70 can have been synthesized. Sequences believed to be necessary for heat shock induction, the TATA box and the -47 to -66 region (10), are both present 5' upstream of both the hsp70 and $\alpha\beta$ genes. It has not been demonstrated, however, that these sequences are sufficient to allow the maximal level of hsp70 gene transcription observed *in vivo*. When the hsp70 promoter was linked to the *E.coli* β -galactosidase gene and then introduced into the *Drosophila* germ line by transformation, the calculated level of expression of the hsp70- β -galactosidase hybrid protein after induction was 20-fold less than that obtained from the normal hsp70 genes *in vivo* (40). While this result could be due to a greater instability of the hybrid than the normal hsp70 protein, it is also consistent with our results which indicate that sequences in addition to the TATA box and the -47 to -66 region of dyad symmetry are involved in setting the absolute rate of transcription of the hsp70 and $\alpha\beta$ genes. Whether such control sequences are outside the

389NT homologous region, or within the region but in a region of lower homology, remains an open question.

b.) Export As the temperature of heat shock was increased, export of total RNA decreased significantly (Fig. 3). This is consistent with previous observations that precursors to both the 37S and 5S ribosomal RNA precursors are synthesized but not processed during heat shock at 37°C (41). Some of the RNA which fails to be exported from the nucleus must be the 37S ribosomal RNA precursor, which constitutes 30-40% of total rapidly labeled nuclear RNA in Drosophila cultured cells (42); we presume that there is also a failure to export some of the hnRNA.

The export of hsp70 and $\alpha\beta$ transcripts from nucleus to cytoplasm also decreased as the temperature of heat shock was increased. At all heat shock temperatures examined, a smaller fraction of $\alpha\beta$ as compared to hsp70 RNA was exported. The difference in export of hsp70 and $\alpha\beta$ transcripts constitutes a significant difference in expression of these two heat shock sequences. We have previously observed that there is less export from nucleus to cytoplasm of the total transcripts from the 93D region as compared to those from the 87A region (hsp70 RNA) (16). Thus there is variation in export among a number of heat shock RNAs. While the formation of imperfect hnRNPs has been proposed to explain the overall low processing of total RNA made during heat shock (43), the difference between hsp70 and $\alpha\beta$ transcripts responsible for their difference in processing remains unexplained.

A dramatic decrease in mRNA export has been noted for HeLa cellular mRNAs during adenovirus infection (44). This phenomenon may be similar to the results described here since it has been observed that adenovirus infection induces synthesis of at least one heat shock protein in the same cell line (45).

Conclusions

The control of expression of heat shock genes occurs at the levels of transcription, export, mRNA turnover and translation (this work, 6, 27,32). Our results show that even the possession of almost identical 5' upstream regions is not sufficient to set equal rates of transcription of the hsp70 and $\alpha\beta$ genes. There must exist control signals, in addition to the TATA box and the imperfect dyad sequences at -47 to -66, that are involved in setting absolute rates of transcription. We have also shown that there is a difference in rate of export for the hsp70 and $\alpha\beta$ RNAs, the first such demonstration for transcripts from any of the cloned heat shock genes.

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REFERENCES

1. Karch, F., Török, I. and Tissieres, A. (1981) *J. Mol. Biol.* 148, 219-230.
2. Holmgren, R., Corces, V., Morimoto, R., Blackman, R. and Meselson, M. (1981) *Proc. Nat. Acad. Sci. USA* 78, 3775-3778.
3. Hackett, R.W. and Lis, J.T. (1981) *Proc. Nat. Acad. Sci. USA* 78, 6196-6200.
4. Mason, P.J., Török, T., Kiss, I., Karch, F. and Udvardy, A. (1982) *J. Mol. Biol.* 156, 21-35.
5. Schlesinger, M.J., Ashburner, M. and Tissieres, A., eds. (1982) *Heat Shock: from Bacteria to Man*. Cold Spring Harbor Laboratory Press, New York.
6. Ashburner, M. and Bonner, J.J. (1979) *Cell* 17, 241-254.
7. Ingolia, T.D. and Craig, E.A. (1981) *Nucl. Acids Res.* 9, 1627-1642.
8. Török, I. and Karch, F. (1980) *Nucl. Acids Res.* 8, 3105-3123.
9. Udvardy, A., Stmegi, J., Toth, E.C., Gausz, J. and Gyurkovics, H. (1982) *J. Mol. Biol.* 155, 267-280.
10. Pelham, H.R.B. (1982) *Cell* 30, 517-528.
11. Corces, V., Pellicer, A., Axel, R., Mei, S.-Y. and Meselson, M. (1982) *Ref. 5*, pp. 27-34.
12. Mirault, M.-E., Delwart, E. and Southgate, R. (1982) *Ref. 5*, pp. 35-42.
13. Lengyel, J.A., Spradling, A. and Penman, S. (1975) in *Methods in Cell Biology*, Prescott, D. Ed., Vol. 10, pp. 195-208, Academic Press, New York.
14. Falkenthal, S. and Lengyel, J.A. (1980) *Biochemistry* 19, 5842-5850.
15. Falkenthal, S., Graham, M.L., Korn, E.L. and Lengyel, J.A. (1982) *Devel. Biol.* 92, 294-305.
16. Lengyel, J.A., Ransom, L.J., Graham, M.L. and Pardue, M.L. (1980) *Chromosoma* 80, 237-252.
17. Livak, K.J., Freund, R., Schweber, M., Wensink, P.C. and Meselson, M. (1978) *Proc. Nat. Acad. Sci. USA* 75, 5613-5611.
18. Lis, J.T., Prestidge, L. and Hogness, D.S. (1978) *Cell* 14, 910-920.
19. Ish-Horowicz, D. and Pinchin, S.M. (1980) *J. Mol. Biol.* 142, 231-245.
20. Lis, J.T. Ish-Horowicz, D. and Pinchin, S.M. (1981) *Nucl. Acids Res.* 9, 5297-5310.
21. Holmgren, R., Livak, K., Morimoto, R., Freund, R. and Meselson, M. (1979) *Cell* 18, 1359-1370.
22. Kunkel, N.S. and Weinberg, E.S. (1978) *Cell* 14, 313-326.
23. Craig, E.A., Ingolia, T.D. and Manseau, L.J. (1983) *Dev. Biol.* 99, 418-426.
24. Craig, E., Ingolia, T., Slater, M., Manseau, L. and Bardwell, J. (1982) *Ref. 5*, pp. 11-18.
25. Ashburner, M. (1970) *Chromosoma* 31, 356-376.

26. Spradling, A., Pardue, M.L. and Penman, S. (1977) *J. Mol. Biol.* 109, 559-587.
27. Lindquist, S. (1980) *Devel. Biol.* 77, 463-479.
28. Ingolia, T.D., Craig, E.A. and McCarthy, B.J. (1980) *Cell* 21, 669-697.
29. Schedl, P., Artavanis-Tsakonas, S., Steward, R., Gehring, W.J., Mirault, M.-E., Goldschmidt-Clermont, M., Moran, L. and Tissieres, A. (1978) *Cell* 14, 921-929.
30. Lindquist, S. (1980) *Dev. Biol.* 77, 463-479.
31. Levis, R. and Perman, S. (1977) *Cell* 11, 105-113.
32. DiDomenico, B.J., Bugaisky, G.E. and Lindquist, S. (1982) *Cell* 31, 593-603.
33. O'Connor, D. and Lis, J.T. (1981) *Nucl. Acids Res.* 9, 5075-5092.
34. Sirotkin, K. and Davidson, N. (1981) *Dev. Biol.* 89, 196-210.
35. Findly, R.C. and Pederson, T. (1981) *J. Cell Biol.* 88, 323-328.
36. Kafatos, F.C. (1972) *Curr. Topics Dev. Biol.* 7, 125-191.
37. Corces, V., Pellicer, A., Axel, R. and Meselson, M. (1981) *Proc. Nat. Acad. Sci. USA* 78, 7038-7042.
38. Caggese, C., Caizzi, R., Morea, M., Scalenghe, F. and Ritossa, F. (1979) *Proc. Nat. Acad. Sci. USA* 76, 2385-2389.
39. Moran, L.A., Chauvin, M., Kennedy, M.E., Korri, M., Lowe, D.G., Nicholson, R.C. and Perry, M.D. (1983) *Can. J. Biochem. Cell. Biol.* 61, 488-499.
40. Lis, J.T., Simon, J.A. and Sutton, C.A. (1983) *Cell* 35, 403-410.
41. Jacq, B., Jourdan, R. and Jordan, B.R. (1977) *J. Mol. Biol.* 117, 785-795.
42. Lengyel, J.A. and Penman, S. (1974) *Cell* 5, 281-290.
43. Mayrand, S. and Pederson, T. (1983) *Mol. Cell. Biol.* 3, 161-171.
44. Babich, A., Feldman, L.T., Nevins, J.R., Darnell, J.E., Jr. and Weinberger, C. (1983) *Mol. Cell. Biol.* 3, 1212-1221.
45. Kao, H.-T. and Nevins, J.R. (1983) *Mol. Cell. Biol.* 3, 2058-2065.
46. Rozek, C.E. and Davidson, N. (1983) *Cell* 32, 23-34.
47. Thomas, P.S. (1980) *Proc. Nat. Acad. Sci. USA* 77, 5201-5205.
48. Anderson, K.V. and Lengyel, J.A. (1980) *Cell* 21, 717-727.
49. Maxson, R.E. and Wu, R.S. (1976) *Eur. J. Biochem.* 62, 551-554.