Two closely linked transcription units within the 63B heat shock puff locus of *D. melanogaster* display strikingly different regulation

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ABSTRACT

We report the isolation and characterization of a cloned DNA of *D. melanogaster*, Dm4L, that is derived from the major heat shock puff site at 63B. This segment contains two closely linked genes that are each present once per *Drosophila* haploid genome. One of these, the hsp 83 gene, encodes an abundant heat shock mRNA that, unlike other major heat shock mRNAs, is also abundant in uninduced (23°) Kc cells. Although only a slight increase in the level of total hsp 83 RNA can be detected after heat shock in Kc cells, the level of hsp 83 poly(A)⁺ mRNA increases more than 6-fold and the level of pulse-labeled hsp 83 RNA in total cellular RNA increases 11-fold relative to uninduced cells. In contrast, the levels of total, poly(A)⁺, and pulse-labeled RNA homologous to the second gene, 63B-T2, are approximately the same in both induced and uninduced cells. Hence, even though these genes are separated by only one thousand base pairs, and, from *in situ* hybridization to polytene chromosomes, both lie within the heat shock puff, they display strikingly different regulatory properties. These results demonstrate that close linkage of a gene to a heat shock puff is not sufficient to render its expression heat inducible.

INTRODUCTION

Gene expression in *Drosophila* and in a wide variety of other organisms is dramatically altered by an increase in temperature of several degrees (1, a review). The expression of most genes is repressed, while a specific set of genes is activated. Transcriptional activation of this set of heat shock genes occurs within minutes of the temperature increase. Accompanying this activation are changes in chromosome structure that are evident from both the formation of puffs on polytene chromosomes (2) and the increased sensitivity of these genes to DNaseI in nuclei from heat shocked cells (3). However, the mechanistic relationships of these changes in structure to changes in gene expression remain to be established.

We have examined the expression and genomic arrangement of two genes that are closely linked at the 63B heat shock puff. One gene encodes the hsp 83 protein (4,5). This protein is abundant in uninduced cells and increases

in heat shock induced cells (6,7). We demonstrate here that the level of pulse-labeled hsp 83 transcript shows a comparable increase in response to heat shock. The closely linked gene, 63B-T2, has not been previously noted. Since this gene is only 1 kb from the hsp 83 gene and in situ hybridization to polytene chromosomes shows that both genes reside within the heat shock puff, we were able to test whether proximity to an activatable gene and presence within a puff are sufficient for gene activation. Measurements of the amount of RNA homologous to each of the two genes in induced and uninduced cells indicate that, despite their structural proximity, these genes have distinct regulatory properties. As controls we have also compared the levels of pulse-labeled copia and histone RNAs in induced and uninduced cells. Histone RNA levels are approximately the same before and after heat shock whereas copia RNA decreases after induction.

MATERIALS AND METHODS

a) Nomenclature.

(i) Transcripts

We have named the second small transcript that is homologous to the Dm4L cloned DNA segment 63B-T2. The "63B" designates the cytological location; "T" designates transcript; "2" designates that it is the second identified transcript derived from 63B. We propose the use of this nomenclature for Drosophila transcripts of unknown function.

(ii) Cloned segments

The term Dm is used to name cloned genomic segments of D. melanogaster, such as Dm4L, while dm is used exclusively for cloned cDNA segments made from D. melanogaster RNA. Dm4L was isolated in our laboratory and is not to be confused with pDm4 from D. Hogness (8).

b) Isolation of λ bDm4 from pooled hybrid phage.

Previously we isolated and characterized a plasmid, adm63BC.1, that contains a cDNA segment made from a heat shock induced mRNA from the 63BC locus (9). We used this DNA as a specific probe to identify the homologous genomic segment in a collection of hybrid phage provided by the laboratory of D. Hogness. This collection is available as 100 different phage lysates, each of which contains 500 independent D. melanogaster/ λ Sep6 hybrids. These hybrids contain mechanically sheared Drosophila DNA joined to the EcoRI sites of λ Sep6 by the dA·dT homopolymer technique (8).

We devised a rapid method of screening this collection of pools. DNAs from the 100 phage pools were prepared by a rapid method (10) and 2 μ g of

DNA from each pool denatured by heating at 95° for 10 min and spotted in a volume of 20 μ l onto nitrocellulose filters. The filters were baked under vacuum for 2 hours at 80°C and hybridized with nick-translated adm63BC.1. A small control filter onto which was spotted 7.5 ng of adm63BC.1 DNA was included in the hybridization. Autoradiography showed that the DNA of only one pool hybridized, the signal being comparable to that of the control adm63BC.1 DNA. Using plaque-hybridization (11) we screened the individual phage of this pool for hybridization with adm63BC.1 DNA. The resulting positive was called λ bDm4L.

A reconstruction experiment in which λ phage DNA was seeded with trace amounts of λ lac phage DNA showed that one λ lac phage DNA molecule in 20,000 λ DNA molecules can be detected if 2 μ g are spotted onto nitrocellulose. Thus, a particular phage could be detected even if highly under-represented in the pool (if, for instance, this phage replicated slowly). With a little care the filters can be reused repeatedly without significant loss of sensitivity. Three uses resulted in less than a 4-fold loss in sensitivity. Although nitrocellulose is relatively brittle, cracks and tears can be repaired with GE silicone glue and the filters reused; obviously, glue should not be applied over an area where DNA is spotted.

c) R-loop mapping.

R-loops were formed between 50 ng of trimethylpsoralen cross-linked λ bDm4L DNA and 2 μ g of mRNA (12) from heat shock cells as described by Kaback et al. (13). Samples were spread for electron microscopy as previously described with single- and double-stranded ϕ X174 DNA serving as size standards (14). Lengths are reported in the text $\pm$ the standard deviation; n refers to the number of molecules measured.

d) In vitro labeled nucleic acids.

DNA was 32 P- or 125 I-labeled by nick-translation as described by Rigby et al. (15).

Specific DNA fragments were labeled by: 1) cleaving the DNA with restriction endonucleases, 2) nick-translating the mixture, and 3) fractionating the fragments on an agarose gel and excising the band of interest. The agarose was dissolved by adding the band to hybridization buffer and heating the mixture to 100°C for 10 minutes.

RNA was 32 P-labeled in vitro by base hydrolyzing to approximately 100 bp length and labeling with 32 P- γ -labeled ATP (New England Nuclear, 3000 Ci/mmol) with polynucleotide kinase (New England Biolabs) (16).

e) In vivo labeled RNA.

The in vivo pulse-labeled total RNA was isolated from Kc_o cells grown in low phosphate medium in suspension culture at 23°C (17). For each preparation, 6 ml of cells at 1.4×10^7 cells/ml were transferred to a 75 cm² tissue culture flask and incubated in a water bath with gentle shaking at either 23°C (uninduced cells) or 37°C (induced cells). Five minutes after the transfer, three mCi of ³²P inorganic phosphate were added to each flask. Sixty minutes later both induced and uninduced cells were harvested by centrifugation for five minutes in a table-top centrifuge. The cell pellets were resuspended in 0.2 ml of 10 mM Tris-HCl, pH 7.6, 100 mM NaCl, 1 mM EDTA and added to a vortexing mixture of 0.4 ml of phenol, 0.25 ml of extraction buffer (100 mM NaCl, 50 mM Tris-HCl, pH 8.9, 1 mM EDTA, 1% sarkosyl), and 3 µl of diethylpyrocarbonate. After one minute of vortexing, 0.4 ml of chloroform:isoamylalcohol (20:1) was added and the mixture vortexed for an additional 10 min before separating the phases by centrifugation. The aqueous phase was extracted 2 times with phenol:chloroform:isoamyl alcohol (50:50:1) and then 2 times with chloroform:isoamyl alcohol (20:1). The nucleic acids were precipitated by adding NaOAc, pH 5, to 0.1 M and two volumes of ethanol. DNA was removed by digestion with RNase-free DNaseI (9) at 5 µg/ml in 135 µl of 100 mM NaOAc, pH 6, 10 mM MgCl for 1 hr at 25°C. The RNA was re-extracted with both phenol: chloroform:isoamyl alcohol and chloroform:isoamyl alcohol and ethanol precipitated as above. This treatment efficiently removes DNA and does not degrade RNA (E. Keller, personal communication).

f) Gel transfers and hybridization.

Cloned Drosophila DNAs were cleaved by the appropriate restriction endonucleases, size fractionated by electrophoresis through agarose gels and transferred to nitrocellulose (9). If the filter was to be hybridized with in vivo labeled RNA the gel was trimmed before transfer leaving only the one or two bands of interest in order to decrease the amount of hybridization buffer needed. Hybridizations with nick-translated DNA were essentially as described by Lis et al. (12). Hybridizations with labeled RNAs were as described by Wahl et al. (18). Under the conditions used the hybridization signal is proportional to RNA concentration (9).

RNAs were size fractionated on agarose gels, transferred to nitrocellulose, and hybridized with nick-translated DNA as described by Thomas (19).

g) In situ hybridization.

Cytological squashes of salivary gland chromosomes were prepared from

gt/gt^{X11} larvae that had been incubated at 37°C for 15 min. The squashes were then hybridized with aDm4.13 that had been labeled with ¹²⁵I-dCTP by nick-translation (12). The identities of the bands were based on a comparison to the maps of Bridges (20).

RESULTS

- a) The cloned genomic DNA segment, Dm4, contains two closely linked genes.

In a previous study we described the cloning and characterization of a collection of cDNA molecules made from total heat shock mRNA using reverse transcriptase (9). One cloned cDNA segment, adm63BC.1, was shown to hybridize in situ to polytene chromosomes at a single major heat shock locus, 63BC. We have used this cDNA segment as a specific probe to isolate a hybrid phage, λ bDm4L, that contains the corresponding segment of genomic DNA (Methods).

The positions of RNA homologous regions of the Dm4L segment are evident from the micrograph of RNA/DNA duplexes (R-loops) shown in Figure 1. We generated R-loops by incubating λ bDm4L with the poly(A)⁺ RNA fraction of a

Figure 1. R-loops formed between λ bDm4L and mRNA preparations from heat shock induced cells. The arrows point to the two genes.

polysome pellet from heat shock cells. A large contiguous R-loop of 2.4 ± 0.2 kb ($n = 35$) was present on most λ bDm4L DNA molecules. A second, smaller R-loop of 500 ± 150 bp ($n = 17$) is also present on 50% of the molecules that have the larger R-loop. The two RNA homologous regions are closely linked, that is, they are separated by only 1.0 ± 0.3 kb.

We generated a restriction map of the Dm4L genomic segment by analyzing the sizes of fragments produced by single and double digests of λ bDm4L with restriction endonucleases. The fine structure mapping was facilitated by subcloning into pBR322 two BamI-SalI fragments which constitute most of the Dm4L segment (Fig. 2). The restriction fragments of the resulting subclones, aDm4.13 and aDm4.46, were ordered and their maps combined to give the Dm4L map illustrated in Figure 2.

The positions of the R-loops relative to the restriction map were established by forming R-loops with XhoI cleaved λ bDm4L DNA. The large R-loop begins 615 ± 65 bp ($n = 26$) from an end of the 6.8 kb XhoI fragment. Since the original cDNA cloned segment hybridizes with restriction fragments derived from the left end of this large XhoI fragment (data not shown), we can unambiguously position the R-loops as shown in Figure 2.

No introns were detected in the main body of the RNA homologous region. However, a short leader sequence could be missed by these methods. S_1 mapping and DNA sequencing of a region preceding the 5' end

Figure 2. Restriction map of λ bDm4L and the positions of RNA homologous regions. The restriction endonucleases are BamHI (Ba), BglII (Bg), EcoRI (RI), HincII (H), KpnI (Kp), PstI (Ps), PvuI (Pl), PvuII (P2), SalI (Sa), SmaI (Sm), XbaI (Xb), and XhoI (Xh). The positions of the leader sequence and intron are based on the results of Holmgren *et al.* (21) and Hackett and Lis (unpublished results). The sites between the first BamHI and second PvuII sites have been confirmed by DNA sequencing (Hackett and Lis, unpublished results). PstI sites have been mapped only in aDm4.13.

of the main body of the hsp 83 gene provide support for a leader of 150 bp (21, and R.W. Hackett and J.T. Lis, unpublished results). Such a leader could account in part for the size difference between the large R-loop, 2.4 kb, and the mRNA which we measured to be 3.0 kb by glyoxal-agarose gel electrophoresis (9). Another one to two hundred bases could be accounted for by poly(A).

The order of restriction fragments in the region of the large gene agrees with the map of a 2.8 kb cloned genomic segment isolated and mapped by Holmgren *et al.* (5). Their segment is also homologous to a heat shock induced mRNA of about 3 kb and is derived from the locus 63BC.

b) Both genes of Dm4L are present as a single copy per haploid genome.

The *in situ* hybridization of the cloned hsp 83 gene to salivary gland polytene chromosomes shows that the gene is present only at the cytological locus 63BC (5,9). Here, we show that the Dm4 sequences are present only once at this site. These results indicate that both RNAs homologous to Dm4L are derived from single-copy genes that display the arrangement illustrated in the map shown in Figure 2.

Total *D. melanogaster* genomic DNA and hybrid phage λ Dm4L DNA were cleaved with the restriction endonuclease PvuII and the fragments size-fractionated by electrophoresis through agarose gels and blotted to nitrocellulose filters. The filter bound DNAs were hybridized with 32 P-labeled aDm4.13 and aDm4.46 using the quantitative experimental design described by Lis *et al.* (12). Carefully measured aliquots of plasmid DNA serve as standards to estimate the concentration of genomic restriction fragments. As shown in Figure 3, the PvuII fragments of *D. melanogaster* embryo DNA

Figure 3. Quantitative analysis of copy number of restriction fragments obtained from total *D. melanogaster* DNA. Tracks 1 and 2 contain 2.8 and 0.9 μ g of total *D. melanogaster* DNA (isolated from embryos of strain Oregon R) cleaved with PvuII. Tracks 3, 4, and 5 contain 0.35, 0.70, and 2.11 ng of PvuII cut λ Dm4L DNA. The filter bound DNA was hybridized with 5×10^6 cpm of aDm4.13 and 3×10^6 cpm of aDm4.46 (ratio of counts is equal to ratios of insert sizes) which were 32 P-labeled by nick-translation to 1×10^8 cpm/ μ g.

have mobilities identical to those present in λ Dm4L. The copy numbers of the PvuII fragments of 2.4, 1.9, 1.8, 1.3, and 0.9 kb are 0.63, 0.65, 1.0, 1.2, and 1.4, respectively, as estimated by microdensitometry. From the clustering of these values near one copy per genome we conclude that the entire Dm4L segment is represented once per haploid genome. Of course, we cannot rule out the existence of short or highly mismatched regions of repetitive DNA. An analysis of the PvuII fragments of Kc₀ DNA shows that they too are single copy (data not shown).

An analysis of the pattern of genomic restriction fragments that hybridize with an independently isolated cloned hsp 83 gene permitted Holmgren *et al.* (5) to conclude that the hsp 83 gene was either unique or embedded in a conserved repeating unit. Our quantitative analysis proves the former alternative.

c) Regulation of the hsp 83 gene and 63B-T2 gene is not coupled.

The relative concentrations of hsp 83 transcripts from induced and uninduced cells were estimated by several methods. Firstly, equal amounts of a poly(A)⁺ mRNA fraction (11) from induced and uninduced cells were size-fractionated by agarose gel electrophoresis, blotted to nitrocellulose, and hybridized with the radioactively labeled cDNA clone adm63BC.1. The autoradiogram is shown in Figure 4a. The hsp 83 transcripts are abundant in the poly(A)⁺ mRNA fraction of both preparations, as indicated by the fact that these bands are detected by relatively short exposures. However, the level of hsp 83 poly(A)⁺ mRNA is 6-fold higher in cells that were heat shock induced for one hour as estimated from the relative intensities of the hybridization signals in tracks 1 and 2. Under these conditions, hybridization signals are approximately proportional to RNA concentration as indicated from the serial dilutions of total RNA shown in tracks 4, 5, and 6.

The poly(A)⁺ mRNA fraction was also assayed by the reciprocal of the above experiment. The HincII DNA fragments of plasmid aDm4.13 were size-fractionated, transferred to nitrocellulose, and hybridized with *in vitro* labeled poly(A)⁺ RNA from either induced (HS) or uninduced (23°) cells. The results in Figure 5 show that fragments containing the hsp 83 gene hybridize with 4-fold more RNA from induced than uninduced cells. Again the hsp 83 RNA is easily detected in both preparations.

A preliminary characterization of mRNA levels of several heat shock genes using cloned cDNA segments as probes showed that while most of the major heat shock mRNAs are at low levels in uninduced Kc₀ cells, the hsp 83

Figure 4. Hybridization of hsp 83 and 63B T-2 DNA to total and poly(A)⁺ RNA of heat shock and uninduced cells. The filters were hybridized with either (a) labeled hsp 83 gene DNA (adm63BC.1) or (b) labeled 63B T-2 gene DNA (3.8 kb HincII fragment of aDm4.13). Tracks 1 and 2 contain 6 µg of poly(A)⁺ RNA from uninduced (23°) and induced (37°) cells, respectively. Tracks 3 and 4 contain 20 µg of total RNA from uninduced and induced cells, respectively. In (a), tracks 5 and 6 contain 6 and 2 µg, respectively, of total RNA from induced cells. The DNAs were labeled by nick-translation to 10⁸ dpm/µg, and 2 × 10⁶ dpm of adm63BC.1 and 1 × 10⁵ dpm of the HincII fragment were used in the hybridizations. The sizes of the RNAs were estimated by comparison with glyoxal treated TaqI DNA fragments of pBR322 (22) run on the same gel. (a) is a 16 hr exposure; (b) is a 10 day exposure at -70°C with an intensifying screen.

mRNA is abundant (9). This result agrees with observations of high levels of hsp 83 protein in uninduced cells (6,7). Moreover, our results with the poly(A)⁺ containing RNA fraction may underestimate the abundance of functional mRNA in uninduced cells, since recently Storti *et al.* (7) have shown using an *in vitro* translation system that much of the hsp 83 mRNA is in the poly(A)⁻ fraction of uninduced cells and in the poly(A)⁺ fraction of induced cells.

To estimate the level of all hsp 83 transcripts we repeated the hybridization experiment shown in Figure 4a using total RNA. Track 3 shows the hybridization of the cloned hsp 83 cDNA, adm63BC.1, to size-fractionated total RNA from uninduced cells. The hybridization is approximately 80% of that of track 4, which contains RNA from induced cells. The hybridization to tracks 5 and 6, which have 1/3 and 1/10 the RNA of track 4, respectively, shows the signals are proportional to RNA concentration. This small increase

Figure 5. Relative levels of hsp 83 and 63B T-2 poly(A)⁺ containing mRNAs in uninduced and heat shock induced cells. Each track contains 0.6 μ g of HincII cleaved plasmid aDm4.13. The fragments labeled hsp 83 and 63B-T2 contain the corresponding genes. Filters were hybridized in 4 ml with 10⁷ dpm of *in vitro* labeled poly(A)⁺ mRNA fractions isolated from induced (HS) and uninduced (23°) cells.

in total hsp 83 RNA in heat shock cells contrasts with the 6-fold difference in poly(A)⁺ hsp 83 RNA. These results are summarized in Table 1.

These observations agree with those of Storti *et al.* (7), who showed that the fraction of translatable hsp 83 RNA that is polyadenylated increases dramatically during heat shock. However, these results are strikingly different from those obtained with the major heat shock gene, hsp 70. The hsp 70 RNA in these same total RNA preparations from uninduced cells is less than one-fiftieth the level of total RNA from heat shock cells (Table 1). The level of hsp 70 poly(A)⁺ RNA shows a comparable level of induction (9). The total hsp 83 RNA from induced cells represents the sum of that accumulated throughout the life of the cell plus that accumulated during one hour heat shock. Since the hsp 83 transcript is moderately abundant in uninduced cells, an increased rate of accumulation of this RNA during the one hour heat shock is difficult to detect over this high background.

Measurement of *in vivo* pulse-labeled RNA provides a more direct and sensitive measure of the changes in the rate of accumulation of RNA species during a one hour heat shock. The label was incorporated into RNA from ³²P-inorganic phosphate present during a 60' heat shock treatment or during

Table 1

The effect of heat shock on the expression of hsp 83, 63B-T2, hsp 70, copia, and histone genes.

hybridization
method:

nick-translated DNA to filter bound RNA

<u>gene</u>	<u>total RNA</u>	<u>Induction</u> <u>poly(A)⁺ RNA</u>
hsp 83	1.2	6
63B T-2	0.38	0.56
hsp 70	> 50	N.D.

in vitro - labeled poly(A)⁺ RNA to filter-bound DNA

<u>gene</u>	<u>Induction</u>
hsp 83	4
63B T-2	1.1

in vivo - labeled total RNA to filter-bound DNA

<u>gene</u>	<u>Induction</u>
hsp 83	11
63B T-2	1.6
copia	0.16
histone	1.2

The numbers are the ratio of the hybridization signal of induced RNA to the hybridization signal of uninduced RNA determined by microdensitometry.

an equal interval in uninduced cells. Total in vivo labeled RNA was hybridized with HincII cleaved adM4.13 that had been size-fractionated by agarose gel electrophoresis and transferred to nitrocellulose. The fragment containing the hsp 83 gene shows an 11-fold higher hybridization signal with RNA from induced cells (Fig. 6) as revealed by microdensitometric tracings of autoradiograms of shorter exposure than that shown (values of 10.2- and 11.7-fold were obtained in two independent cell labelings). RNA from equal numbers of induced and uninduced cells were used in each pair of hybridizations (the amount of label incorporated into RNA under the two conditions was also approximately equal).

In contrast to hsp 83 RNA, the 63B-T2 RNA was essentially at the same level in induced and uninduced cells when assayed by this same battery of techniques (Table 1). The ratio of 63B-T2 poly(A)⁺ mRNA levels in induced to uninduced cells was measured to be 0.56 by using the 3.8 kb HincII DNA fragment containing 63B-T2 as a hybridization probe (Fig. 4b) and 1.1 using

Figure 6. Relative level of pulse-labeled total RNA homologous to the hsp 83 and 63B-T2 genes in uninduced and heat shock induced cells. Each track contains 3 μ g of HincII cleaved plasmid aDm4.13. The filters were hybridized in 2 ml with 2×10^7 dpm of *in vivo* labeled total RNA isolation from induced (23°) and uninduced (HS) cells. The hybridizations were done in duplicate.

in vitro labeled RNA as probe (Fig. 5). This ratio was measured to be 0.38 in total RNA using the HincII DNA fragment as probe (Fig. 4). The most sensitive measure of the change in expression induced by heat shock, measurement of pulse-labeled 63B-T2 RNA, revealed the ratio to be 1.6 (Fig. 6).

The same pulse-labeled RNA was also hybridized with other cloned genes as controls. The histone genes have been reported to be active in both normal and heat shock induced cells (4). Equal amounts of pulse-labeled RNA from both sources hybridize with cDm500, a plasmid containing the cloned histone genes (23) (Table 1). In contrast, a *copia* DNA segment, cDm1142 (24) hybridizes 6-fold less with RNA from induced cells, indicating the expression of *copia* RNA is repressed by heat shock (Table 1).

The sizes of the hsp 83 mRNA appear to be the same in induced and uninduced cells in that they are indistinguishable by electrophoresis on agarose gels (Fig. 4a). The major peak in both cases is at 3 kb. Clearly a difference of less than 100 bp, such as due to polyadenylation, would not be detected. Although this result implies that the same transcriptional start and processing signals are used for the expression of the hsp 83 gene in both induced and uninduced cells, we have not directly addressed the question of whether the RNAs in each case are initiated precisely at the same site.

The sizes of the 63B-T2 transcripts are also the same in induced and uninduced cells. Although the major species is 0.85 kb, a second species of 0.62 kb is reproducibly detected in both poly(A)⁺ and total RNA fractions (Fig. 4b). The precise relationship of these RNAs has not been determined. However, as they both hybridize to the same 1.3 kb PvuII-PstI fragment (data not shown), their complementary DNA sequences are either extremely close or overlapping. The later arrangement may explain the large standard deviation associated with the measurement of the 63B-T2 R-

loop in section a. Polyadenylation could account for the RNA species appearing larger when measured by gel electrophoresis than by R-loop mapping.

d) Both genes appear to be contained within the 63B heat shock puff.

Heat shock induces dramatic changes in polytene chromosomes at the major heat shock loci (2). Within 2 minutes of initiating a heat shock treatment, puffs are visible at these loci (25). Since only one of the two linked genes at 63B is activated, we examined the cytological location of the genes within the puff by in situ hybridization to polytene chromosomes. We wondered if the spacer between the genes might define the junction between a condensed and a puffed region, in which case the induced gene would reside in the puff and the uninduced gene would reside in the condensed and the uninduced gene would reside in the condensed region. Our results show that this is not the case.

The radioactively labeled plasmid aDm4.13 (which contains one half of the hsp 83 gene and the entire 63B-T2 gene) was hybridized in situ to polytene chromosomes of heat shock induced larvae. The tight clustering of grains shown in Figure 7 demonstrates that both genes are within the 63B puff. Even long exposures producing greater than 100 grains in the puff (Fig. 5b) fail to label the adjacent band. Thus, at the resolution of cytological analysis the chromosome regions harboring each gene appear equivalent after heat shock. This contrasts markedly with their very different transcriptional responses to heat shock.

DISCUSSION

a) The hsp 83 is an unusual major heat shock gene.

Unlike other major heat shock proteins, the hsp 83 protein can be abundant in uninduced cells (6,7). In support of this finding we show here that the hsp 83 RNA is also abundant in Kc_o cells under conditions where other heat shock RNAs are not (9). Although hsp 83 RNA is abundant in Kc_o cells, it may not be expressed at a high level in all cell types. We recently examined the level of hsp 83 transcripts in total RNA isolated from whole flies and find a dramatic 20-fold induction upon heat shock (compare to the 20% increase in Kc_o cells) (O'Connor, Lis, and D. Ish-Horowicz, unpublished results). The basal level in uninduced flies appears at least several fold lower than that shown in Figure 4.

The cytological location of hsp 83 protein is also distinct from the other heat shock proteins. While hsp 83 protein is restricted to the cytoplasm, hsp 70 and the small heat shock proteins appear to be tightly as-

Figure 7. In situ hybridization of aDm4.13 to heat shock induced polytene chromosomes. The exposure times are 16 days (a) and 100 days (b). The hybridization appears to be within the interval 63B6 to 63B12.

sociated with nuclei (26,27).

These results imply that both the function and regulation of the hsp 83 gene have features that are distinct from other major heat shock genes, a conclusion previously drawn from the analysis of the syntheses of hsp 83 and other heat shock proteins under a wide variety of conditions (6).

- b) The gene arrangements at 63B in the fly and *Kc* cell genome are the same as that of Dm4L.

The Dm4L segment that served as the substrate for these mapping studies is derived from *D. melanogaster* embryos. *Kc* cell cultures were the source of all RNA preparations since both RNA isolation and in vivo labeling of RNA to high specific activity is technically easier in *Kc* cells than in embryos (17). To insure that the cells used to measure RNA expression have the same gene arrangement as Dm4L, we have examined the pattern of Dm4L - homologous

restriction fragments from the Kc_o cell genome using the approach described in Figure 3. Although polymorphisms of certain restriction endonuclease sites exist between Dm4L and genomic DNA from both flies and cell culture (data not shown), some enzyme digests of genomic DNA produced the same pattern of fragments as from the two Dm4L transcribed regions, indicating that the genomic arrangements of DNA from all three sources are very similar at this locus. In addition, quantitative analysis of the hybridizations shown in Fig. 3 demonstrates that the Dm4L segment is present as a single copy per genome, thereby obviating ambiguities associated with assigning transcriptional activity to each gene of a repeated set.

c) Gene linkage is not correlated to gene expression at the 63B heat shock puff site.

The cloned genomic DNA segment, Dm4L, contains the gene homologous to the major heat shock induced hsp 83 mRNA from the 63B locus and also a second smaller gene, 63B-T2, which maps 1 kb away. Although the 63B-T2 transcript binds oligo(dT)-cellulose and is homologous to single-copy DNA we have not proven here that it is translated. Nonetheless, the hsp 83 and 63B-T2 are independent transcripts and are not simply related via processing of a large precursor. Firstly, although we detect a transcript larger than the hsp 83 mRNA in our total cellular RNA preparations, the extra RNA is homologous to fragments from the region 5' to the main body of the hsp 83 gene while the 63B-T2 transcripts map 1 kb from the 3' end of the hsp 83 gene (21, and O'Connor and Lis, unpublished results). Secondly, no large RNA species is labeled by hybridizing cloned 63B-T2 gene to total RNA preparations even at longer exposures than those shown in Figure 4b.

Although both genes are closely linked, they show very different regulation. The amount of hsp 83 transcript that accumulates during 1 hr in heat shock induced cells is 11-fold greater than that in uninduced cell culture. In contrast, the amount of the 63B-T2 transcript is essentially unaltered by heat shock.

The amount of a given RNA species that accumulates during a pulse labeling is a function of the rates of transcription and degradation of that RNA. For RNA degradation to be an important factor in this instance, a half-life of significantly less than one hour would be required. Although we have not addressed this question here, others have presented strong evidence that the accumulation of heat shock RNA is transcriptionally regulated (25,28,1).

Both genes appear to be decondensed on formation of the 63B heat shock puff. However, the resolution of cytogenetic analyses is such that micro-

structural heterogeneity may not be detected. Preliminary studies of the DNaseI sensitivity of this region in cell cultures suggest that both genes become part of a single decondensed domain on heat shock (Costlow and Lis, unpublished results).

Another major heat shock puff site, 87C, also contains two closely linked, different transcription units. One of these codes for the most abundant heat shock protein, hsp 70; the other, $\alpha\beta$, has no known translation product (16, 29). Like the 63B-T2 gene, $\alpha\beta$ DNA is present within the heat shock puff (29). However, in contrast to the 63B-T2 gene, transcription of $\alpha\beta$ is dramatically induced by heat shock (12). Although $\alpha\beta$ transcription may be simply a consequence of its close association with the hsp 70 gene, the results presented here for the 63B locus demonstrate that the close linkage of the 63B-T2 gene to a major heat shock gene is not sufficient to render it heat inducible. A more likely explanation for the coordinate activation of the hsp 70 and $\alpha\beta$ genes is revealed from studies of sequence arrangement. $\alpha\beta$ contains a sequence that is homologous to one beginning several hundred nucleotides upstream from and extending 64 nucleotides into the 5' end of the hsp 70 transcribed region. This region of homology includes the transcriptional start sequence and presumed cis-acting regulatory elements (30,31).

In response to heat shock, many normally active genes are repressed (1). We find that copia RNAs accumulate at a 6-fold lower rate during heat shock. Likewise the level of pulse-labeled actin RNA is also dramatically lower in heat shock induced cells (30).

Not only do many transcripts accumulate at a lower rate during heat shock, many normal chromosomal puffs concomitantly regress (32,33). Moreover, RNA polymerase II appears to dissociate from these puffs and associates predominantly with heat shock puff sites (34,35). Chromosomal condensation (the puff to band transition) could participate in gene repression or simply be a consequence of gene repression. If the former is true, then the close association of 63B-T2 with the 63B puff may play a role in preventing turnoff of this gene.

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