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Four Drosophila heat shock genes at 67B: characterization of recombinant plasmids

Elizabeth A.Craig*
Department of Physiological Chemistry, University of Wisconsin, Madison, WI 53706, and

Brian J.McCarthy
Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143, USA

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ABSTRACT

A 33 kilobase region of the 67B locus of Drosophila melanogaster genome has been isolated. The genes for the 27K, 26K, 23K and 22K heat shock induced proteins are contained within an 11 kb segment. The 27K gene, approximately 1.4 kb in length, and the 23K gene, approximately 1.0 kb in length, are separated by about 1.5 kb of spacer DNA; a third block of transcription, which encodes the 26K gene, is approximately 1.1 kb in length, and is separated from the gene for the 23K protein by 4.5 kb of spacer DNA. The 22K gene is located approximately 1.2 kb from the 26K gene. The RNAs encoding the 27K, 23K and 22K genes are transcribed from the same DNA strand. With the assignment of the four small heat shock induced proteins to 67B, genes for all seven major heat shock induced proteins have now been mapped cytologically.

INTRODUCTION

Brief exposure of Drosophila melanogaster larvae or tissue culture cells to elevated temperature or a variety of drugs results in the activation of a small number of genes, as well as the repression of most active ones (1). This activation was first observed as the induction of approximately 8 puffs on the polytene chromosomes of the salivary glands (2,3). It has been shown that RNA is transcribed in large amounts at the sites of the induced puffs (4,5,6). The heat treatment also results in the induction of synthesis of at least 7 new proteins (7,8).

Genes coding for several of the heat shock proteins have been assigned to specific puffs. Of particular interest here are the genes for the 27K, 26K and 23K proteins which have been assigned to 67B by genetic techniques (9) and in situ hybridization (10). The mRNA coding for these three proteins has been shown by sucrose gradient sedimentation and gel electrophoresis experiments to be about 12S to 13S, equivalent to about 1.1 to 1.3 kb (11,12,13).

This paper reports the isolation of segments of Drosophila melanogaster DNA which contain the genes for the 27K, 26K, 23K and 22K heat shock proteins.

The localization of the mRNA homologous regions, direction of transcription and the presence of a nearby repetitive DNA element are described.

MATERIALS AND METHODS

General Methods--Isolation and purification of DNA, transformation, colony screens and digestion with restriction enzymes were performed as previously described (14).

Preparation of Heat Shock RNA--Kc tissue culture cells were grown in D20 media without serum (15). RNA was purified using the procedure described by Boshes (16). Heat shocking of the cells was performed by immersing the culture vessels in a water bath such that the culture reached 35 to 37°C in 10 min and was subsequently incubated at that temperature for an additional 30-45 min. Typically, cells from one liter were pelleted, washed once in phosphate buffered saline and resuspended in 10 ml of 0.01M $MgCl_2$, 0.01M Tris-HCl, pH 7.6, 0.05M KCl, 0.004M DTT to which was added 1% Triton X-100 and 0.25M sucrose. The cells were homogenized and the lysate centrifuged at 10,000g for 30 min in the Sorvall SS34 rotor. The supernatant was layered onto a 0.5 to 1.5M linear sucrose gradient made in the above solution. The gradients were spun in the SW27 rotor at 25K at 4°C for 2.5 to 3 hr and then collected from the bottom through a UV flow cell. The fractions containing the large polysomes were pelleted overnight at 4°C in the SW27 rotor at 25K. The polysomes were resuspended in 100mM NaCl, 10mM Tris-HCl, pH 7.5, 1mM EDTA and 0.5% SDS, extracted three times with equal volumes of phenol and chloroform-isoamyl alcohol (24:1) and precipitated with alcohol. PolyA containing RNA was selected by hybridization to oligo(dT) cellulose. RNA was adsorbed in 0.5M NaCl, 10mM Tris-HCl, pH 7.5 and eluted in 0.01M Tris-HCl, pH 7.5.

In Vitro Translation--The mRNA dependent rabbit reticulocyte system of Pelham and Jackson (17) was used throughout. Cloned DNA molecules were cleaved with an appropriate restriction enzyme, denatured, bound to nitrocellulose and hybridized with mRNA under the conditions specified by Ricciardi et al. (18).

In Vitro Labeling of Nucleic Acid--Whole plasmid DNA was labeled using the nick translation reaction as described by Rigby et al. (19). Insert DNA which was to be nick translated was separated from the parent plasmid by electrophoresis in a 7% acrylamide gel. Subsequent to electroelution from the gel and concentration by ethanol precipitation, 100 to 500 ng of insert was nick translated as described by Maniatis et al. (20), except that 1 ng of DNAse I was added along with DNA polymerase I (Worthington) in 50mM Tris-HCl, pH 7.8, 5mM $MgCl_2$, 10mM 2-mercaptoethanol.

Heat shock RNA partially digested with alkali was labeled in vitro using polynucleotide kinase. In a typical reaction 2 µg of polyA containing RNA was incubated in 40 µl of 50mM Tris-HCl, pH 9.5 at 95°C for 70 min. The reaction was then made up to 50 µl and contained 10mM $MgCl_2$, 2mM DTT and 250 µCi α-^{32}-ATP (1.5 µCi/pmol). Two units of T4 DNA polynucleotide kinase (Boehringer-Mannheim) was added and the reaction incubated for 30 min at 37°C. The reaction was stopped by addition of an equal volume of 4M NH_4Ac, 500 µg of yeast tRNA carrier was added and the mixture phenol extracted. The RNA was precipitated twice with ethanol and separated from unincorporated nucleotides by chromatography on Sephadex G50 fine. The RNA had a specific activity of approximately $1x10^7$ cpm/µg.

Hybridization Procedures--Transfer of DNA contained in gels was performed according to the method of Southern (21). After heating to 80°C for 2 hr, the filters were preincubated for 4 hr according to Denhardt (22). Hybridization was carried out in 50% formamide, 5xSSC, 0.1% SDS, 1mM EDTA, 10mM HEPES, pH 6.9 and 1X Denhardt's solution (22) at 37°C for 20 hr. After incubation the filters were washed three times in 5xSSC containing 0.2% SDS at 65°C for 45 min, followed by a 2 hr wash at room temperature in 2xSSC. Royal X-Omat film and Lightning Plus Screens (Kodak) were used.

Transfer of RNA contained in the gels to diazobenzyloxymethyl paper was performed according to the method of Alwine et al. (23). The gel was incubated at room temperature in 50mM NaOH for 1 hr, followed by neutralization in $NaPO_4$, pH 5.5, prior to transfer.

Squashes of salivary gland chromosomes were prepared for hybridization as specified by Bonner and Pardue (6). Larvae used for squashes were giants from crosses of Oregon R 236, G-1(gtw^4 homozygous) and G-x11($y^{sc}gt^{x11}$FM6). Complementary ^3H labeled RNA was synthesized using E. coli RNA polymerase (Miles) and three ^3H-ribonucleotide triphosphates; the GTP was unlabeled. Reactions were carried out in 40mM Tris, pH 7.8, 10mM $MgCl_2$, 0.1mM EDTA, 160mM KCl, 0.1mM DTT, 0.1mM GTP and approximately 0.05mM of ^3H-labeled ATP, UTP and CTP. Reactions were incubated for 60 min at 37°C. The preparations were diluted four fold in 5mM $MgCl_2$ and treated with pancreatic DNAse I (Worthington) at 100 µg/ml for 30 min at 37°C. Following phenol extraction, the cRNA was separated from unincorporated triphosphates on Sephadex G-75 columns.

Synthesis of cDNA--Primers for the synthesis of cDNA were isolated by electroelution after separation from other DNA fragments by electrophoresis in 7% acrylamide gels. Subsequent to concentration by ethanol precipitation, the DNA was mixed with 80 µg of total polysomal heat shock RNA and hybridized

under conditions described by Paterson et al. (24). The nucleic acid was precipitated twice with ethanol, before adding to the AMV reverse transcriptase reaction. In addition to the nucleic acid the reaction included 50mM Tris-HCl, pH 8.3, 10mM $MgCl_2$, 10mM DTT, 1mM TdGTP, dATP, dCTP and TTP together with 12 units of AMV reverse transcriptase in 20 µl. The product of the reactions were then analyzed on urea-polyacrylamide gels.

Gel Electrophoresis--Agarose gel electrophoresis of DNA was carried out in vertical slab gels (25) 14 x 14 x 02 cm cast with 0.8% to 2.0% agarose containing 40mM Tris-base, 20mM NaAc, 20mM NaCl and 2mM EDTA, pH 8.15. Polyacrylamide gel electrophoresis was carried out in 5% bis-acrylamide gels (1:40) in Tris-borate and EDTA as described previously (14). The DNA was stained with ethidium bromide (0.5 µg/ml) and photographed with Polaroid film using ultraviolet illumination (26).

The electrophoresis of RNA in agarose gels was carried out as described by McMaster and Carmichael (27). Nucleic acids were denatured by incubation for 1 hr at 50°C in buffer containing 1.0M glyxoal, 50% (vol/vol) Me_2SO and 10mM NaH_2PO_4/Na_2HPO_4, pH 7.0. 2% agarose slab gels containing 10mM $NaPO_4$, pH 7.0 were used.

RESULTS

Identification of a Chromosomal Recombinant Plasmid Specific for 67B

The isolation of two cDNA recombinant plasmids, pHS227 and pHS229, which are homologous to mRNA which codes for the 26K and 23K proteins, respectively, is described elsewhere (10). The cDNA inserts of pHS227 and pHS229 were labeled in vitro by nick translation and used to screen a library of recombinant plasmids containing BamHI fragments of Oregon R embryo DNA which were inserted into pBR322 (14). A single plasmid, pJ1, which hybridized with both pHS227 and pHS229 was isolated. ^{3}H-cRNA transcribed from pJ1 using E. coli RNA polymerase was hybridized in situ to squashes of salivary gland polytene chromosomes. Many grains were observed at 67B, a major heat shock locus (Fig. 1); no hybridization was detected at any other chromosomal location.

To demonstrate directly that plasmid J1 is homologous to heat shock mRNA, polyA containing RNA isolated from the polysomes of heat shocked Kc tissue culture cells was labeled in vitro using ^{32}P ATP and T4 polynucleotide kinase and hybridized to J1 DNA. RNA was hybridized to an EcoRI restriction endonuclease digest of J1 DNA which had been fractionated on an agarose gel and transferred to nitrocellulose filters according to the method of Southern (21). Plasmid J1 contains 6 EcoRI sites; the two largest fragments generated

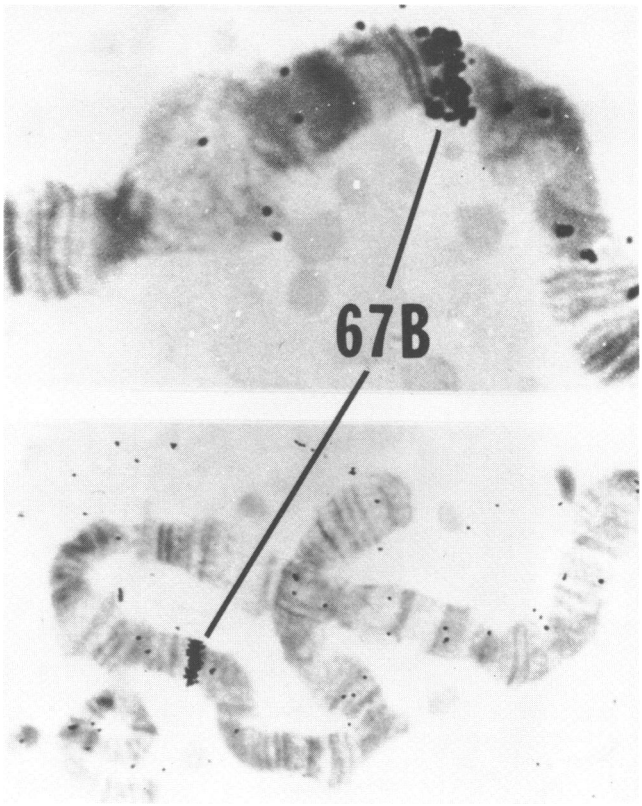

Figure 1. In situ hybridization of J1 sequences. ^{3}H-cRNA synthesized from pJ1 were hybridized to giant polytene chromosomes. 35,000 cpm per slide; 22 day exposure.

by EcoRI hybridize to heat shock mRNA (Fig. 2). The labeled inserts of pHS227 and pHS229 were also hybridized to similar digests of J1 DNA. HS227 hybridized to the largest EcoRI fragment (6.5 kb) and HS229 hybridized to the second largest fragment (4.8 kb) (Fig. 2).

Identification of mRNA Complementary Regions of J1

pJ1 DNA was cleaved with Bam H1, EcoRI, HindIII, SalI, HincII, PvuII, PstI, SacI and XbaI, and the lengths of the fragments determined by electrophoresis in agarose gels. Placement of the sites on J1, relative to the vector pBR322, was accomplished by measuring the size of the products of simultaneous digestions with two enzymes and cleavage of the subcloned EcoRI fragments of J1 (Fig. 3).

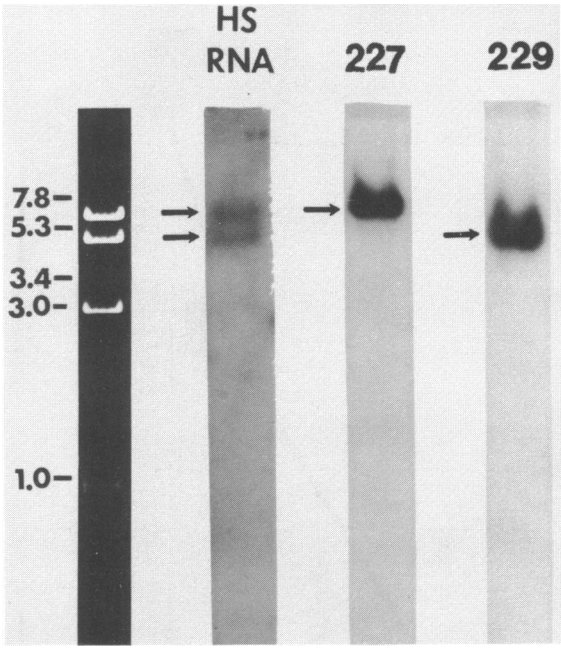

Figure 2. Hybridization of labeled poly(A)-containing RNA and HS227 and HS229 to J1 DNA. pJ1 DNA was cleaved with EcoRI and electrophoresed on three adjacent lanes of a 1% agarose gel. The gel was stained and photographed (one lane shown above, left). The DNA was transferred to nitrocellulose and hybridized; the DNA in one lane was hybridized to in vitro labeled polysomal RNA from heat shocked cells (3.2×10^6 cpm); HS227 (3×10^5 cpm); HS229 (3.5×10^5 cpm). The number on the side indicate the size in kilobases and the relative mobility of selected HindIII Ad2 fragments.

To determine which regions of J1 bear homology to heat shock mRNA, partially degraded polyA-containing RNA isolated from heat treated cells was labeled in vitro using T4 polynucleotide kinase and hybridized to J1 DNA. J1 DNA was cleaved with EcoRI, XbaI, PstI, HincII, and a combination of BamHI and XbaI, electrophoresed on agarose gels and transferred to nitrocellulose (Fig. 2 and 4). As shown in Fig. 2, heat shock mRNA hybridized to the two largest EcoRI fragments. The largest EcoRI fragment (the subclone J1B) includes all of J1 to the right of the last EcoRI site, and most of the vector pBR322. The second largest EcoRI fragment (subclone J1A) is the lefthand EcoRI fragment.

XbaI cleaved J1 at four sites; pBR322 contains no XbaI sites. The largest 8.9 kb XbaI fragment which includes the largest EcoRI fragment, J1B,

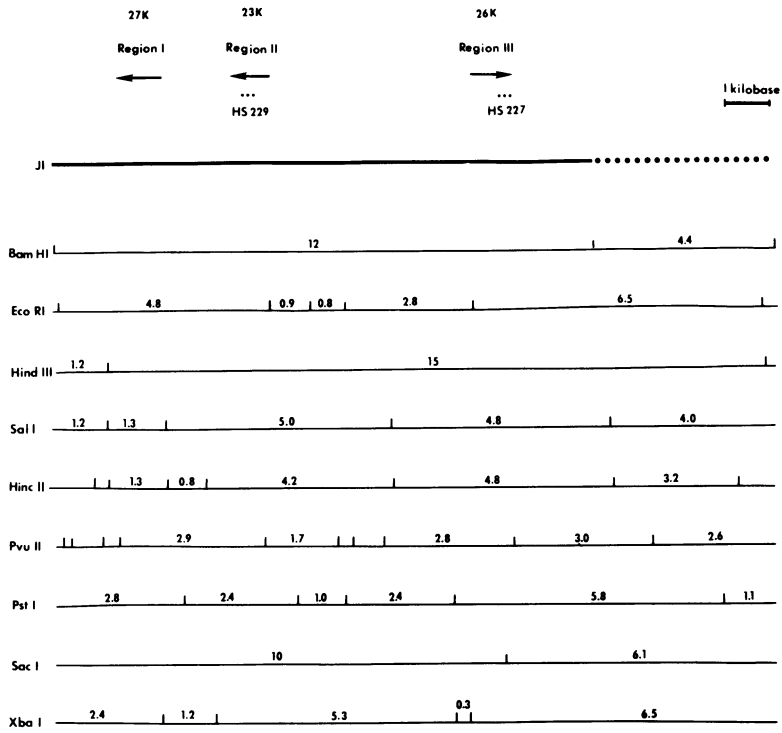

Figure 3. Map of the mRNA coding regions of J1. The distances between restriction endonuclease sites were determined by the relative rates of migration of DNA fragments to HindIII Ad2, HaeIII pBR322 and HaeIII ØX174 DNA in 1 and 2% agarose and 8% acrylamide gels. Restriction sites are indicated by vertical lines, and the size of each fragment is designated in kilobase pairs (kb). The boundaries of the mRNA coding regions, designated by the heavy solid lines, were estimated from the results of hybridization of labeled polysomal RNA to DNA fragments as described in the text and legends to Figs. 1, 4 and 7 and unpublished results. The arrows indicate the direction of transcription of the mRNAs (Fig. 9). The short dashed lines represent the limit of homology of the cDNA clones, HS227 and HS229. The vector portion of pJ1 is indicated by the heavy dashed line.

all of pBR322 and 2.4 kb of the lefthand end of J1 hybridizes to heat shock mRNA. The adjacent 1.2 kb XbaI fragment did not hybridize. However, hybridization did occur to the large internal 5.3 kb XbaI fragment. Since J1 is inserted into the single BamHI site of pBR322, simultaneous cleavage with both XbaI and BamHI separates the 4.4 kb vector from the two terminal 2.4 and 2.1 kb XbaI-BamHI fragments. Both fragments hybridize to heat shock mRNA.

Neither the small 1.2 kb XbaI fragment which separates the lefthand 2.1

kb XbaI-BamHI fragment from the 5.3 kb internal XbaI fragment, nor any of the three internal EcoRI fragments which separate J1A and J1B hybridized to mRNA. These experiments indicate that J1 contains three regions of homology to heat shock mRNA. One region (hereafter referred to as Region I) is located between the leftmost EcoRI site and the leftmost XbaI site. The position of this mRNA homologous region was localized more accurately by hybridization to HincII fragment of J1A (Fig. 4). In this region, mRNA hybridization was restricted to the 1.3 HincII (SalI) fragment. A second region (Region II) is found in the center position between the XbaI and EcoRI sites of J1. The third region (III) is localized between the EcoRI and BamHI sites of J1B.

Size of RNAs Encloded by J1

The size of the mRNAs encoded by the three regions of J1 were determined by hybridization of labeled portions of J1 to size fractionated RNA. RNA isolated from heat shocked cells was glyoxylated and electrophoresed on an agarose gel. The RNA was transferred and covalently linked to paper by the

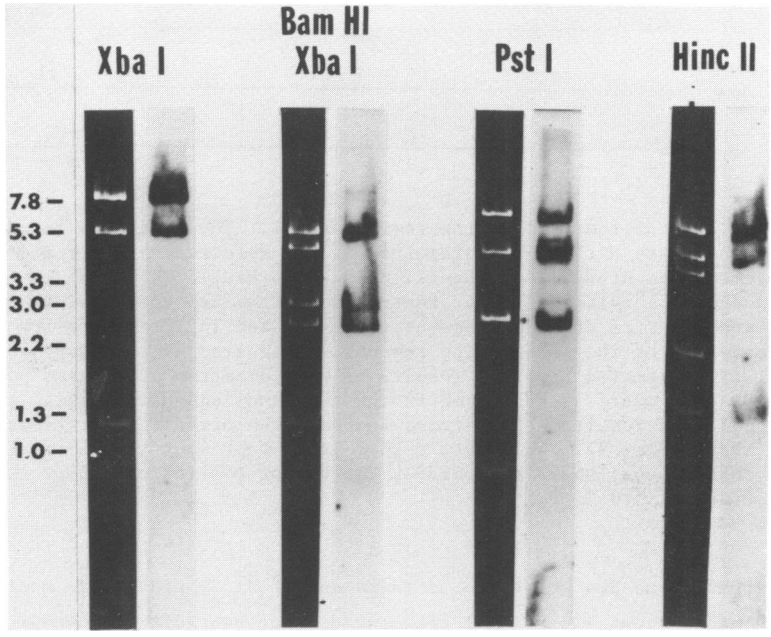

Figure 4. Hybridization of labeled polysomal RNA to pJ1 DNA fragments. pJ1 DNA was cleaved with XbaI, XbaI and BamHI together, PstI or HincII, electrophoresed on a 1% agarose gel and transferred to nitrocellulose. $5x10^6$ cpm of in vitro labeled polysomal polyA containing RNA from heat shocked cells was hybridized to the filters. The numbers indicate the size and relative migration of HindIII cleaved Ad2 DNA.

method described by Alwine et al. (23) and hybridized to labeled J1A and J1B DNA. One RNA band approximately 1.1 kb was detected after hybridization with J1B (Fig. 5); two RNAs, approximately 1.3 and 1.0 kb in size, were detected by hybridization with J1A (Fig. 5). J1A contains two blocks of mRNA homology; the 1.3 kb SalI fragment which includes Region I and the 2.1 kb PstI-EcoRI fragment which encompasses Region II. These two fragments were isolated, labeled and hybridized to immobilized RNA. The fragment encompassing Region I hybridized only to the 1.3 kb RNA; the Region II specific fragment which encodes the 23K protein hybridized only to the 1.0 kb RNA (data not shown).

Proteins Encoded by Region I, II and III

Previous results (10) demonstrated that the cDNA clones, HS227 and HS229 encoded a portion of the 26K and 23K proteins, respectively. Subsequently, it was shown that HS227 was homologous to Region III (Fig. 2) and HS229 was homologous to region II (Fig. 2 and data not shown). Further in vitro trans-

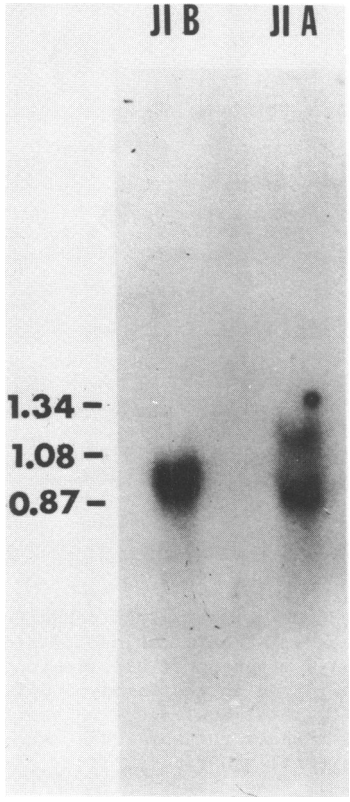

Figure 5. Hybridization of labeled J1A and J1B DNA to RNA from heat shocked cells. Total RNA from the cytoplasm of heat shocked cells (20 µg) were treated with glyoxal and electrophoresed on two lanes of a 2% agarose gel. The RNA was transferred to DBM cellulose paper and hybridized to 2.5×10^6 cpm of in vitro labeled J1B or J1A DNA. The numbers indicate the size and relative migration of ØX174 DNA cleaved with HincII and treated with glyoxal. Glyoxalated DNA molecules are accurate size markers for glyoxalated RNAs according to McMaster and Carmichael (27).

lation experiments were carried out to determine the proteins encoded by Region I and to confirm that Region II encoded the gene for the 23K protein. RNA homologous to Region I was selected by hybridization to the 1.3 kb SalI fragment of J1A; this RNA translated into a 27K protein (Fig. 6b). The Pst-I Eco RI fragment which encompasses Region II hybridized to RNA which translated into a 23K protein (Fig. 6a).

Isolation of Adjacent Regions

To extend the analysis of the 67B locus, segments of DNA adjacent to that represented by pJ1 were isolated from both Oregon R embryos and Schneider tissue culture cell DNA. Figure 7 summarizes the relevant fragments obtained and their relationship to pJ1 and each other. These adjacent segments were hybridized with RNA isolated from heat shocked cells. Two additional regions of transcriptional activity were detected. A region of approximately 1 kb,

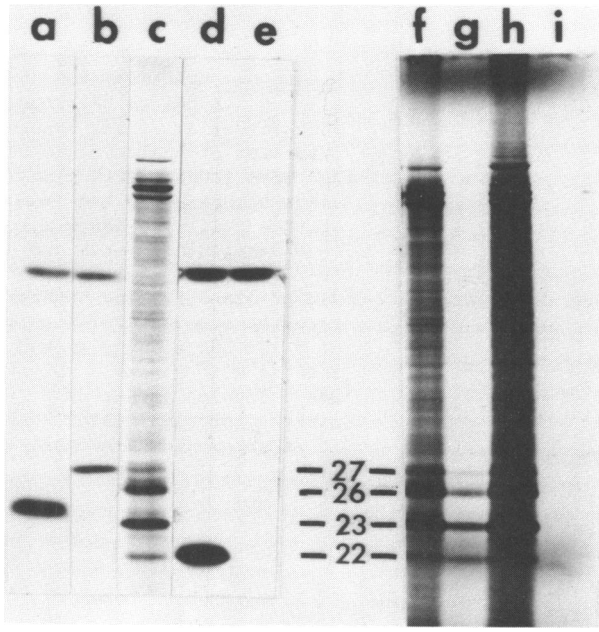

Figure 6. Cell free translation of RNA selected by hybridization to DNA from the 67B locus. (a) RNA hybridized to the 2.1 kb PstI-EcoRI subclone of J1A (Region II); (b) RNA hybridized to the 1.3 kb SalI fragment of J1A (Region I); (c) total heat shcok polysomal RNA; (d) RNA hybridized to the BamHI-HindIII fragment of T5 (Region IV); (e) no RNA added; (f) total heat shock polysomal RNA; (g) RNA hybridized to T3 DNA; (h) total heat shock polysomal RNA, and (i) RNA hybridized to T4 DNA. The heterogeneous material observed in lanes g and i is probably amino acyl tRNAs; it is sensitive to RNAse and is present in the translation products when no exogenous RNA is added.

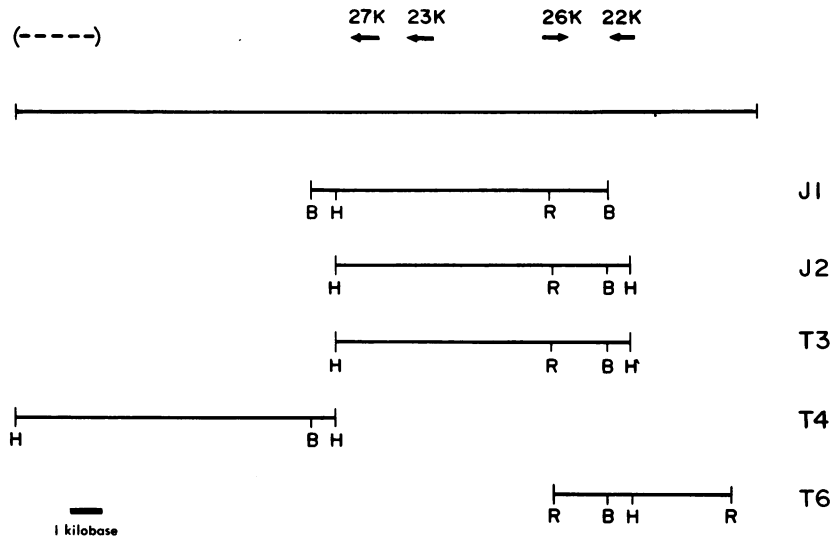

Figure 7. Cloned DNA fragments used in the analysis of gene organization at 67B. J1 and J2 were isolated from libraries of Oregon R embryo DNA fragments inserted into the BamHI and HindIII-sites of pBR322 respectively, by hybridization to HS227 and HS229. T3, T4 and T6 are fragments of DNA from Schneider's cells. T3 and T6 were identified by hybridization with a HS227 DNA probe. T4 was isolated by means of a 1.2 kb BamHI-HindIII probe from J1. The information summarized at the top of the figure was obtained as described in the legend to Fig. 3. The arrows represent the size and direction of transcription of the heat shock mRNAs. The dashed line indicates the repetitive DNA region that is transcribed into abundant RNA.

between the BamHI and HindIII sites, immediately to the right of J1 displayed homology with RNA. A fifth region of transcriptional activity was mapped to the left of J1 approximately 10 kb from the 27K gene.

RNA homologous to these two regions, carried on plasmids T3 and T4 was translated in vitro. T3 selected RNA which translated into proteins of 27K, 26K, 23K and 22K molecular weight (Fig. 6g). As T3 overlaps regions I, II and III of J1 the synthesis of the three larger proteins was to be expected. But T3 also contains an additional 1 kb of DNA from the BamHI to the HindIII site, not present in the plasmid J1, which must contain the gene for the smallest protein. In fact, heat shock mRNA does hybridize strongly to this BamHI-HindIII DNA fragment and RNA recovered from such hybrids directs the translation of the 22K protein (Fig. 6d).

The transcribed region to the left of Region I was also analyzed. When RNA was hybridized with T4, no synthesis of a protein was detected (Fig. 6i).

This RNA homologous region was labeled in vitro and hybridized to EcoRI and BamHI digested Drosophila genome DNA. More than 15 bands were detected (Craig, unpublished data) suggesting that this sequence is repeated in the Drosophila genome. Furthermore RNA transcribed from this region is not heat shock specific; it is abundant at the normal growth temperature of 22°C.

Determination of the Direction of Transcription of the mRNAs

The ability of AMV reverse transcriptase to transcribe DNA from single stranded RNA using a free 3' OH end of a DNA fragment as primer (28,29) was used as a means of determining the direction of transcription of the four mRNAs (see Fig. 8). Isolated DNA fragments, terminally labeled using ^{32}P-ATP, were used as primers for synthesis of cDNA from heat shock mRNA. The labeled primer was hybridized to mRNA under conditions where DNA:RNA hybrids are more stable than DNA:DNA duplexes. The hybridized RNA was used as a template for AMV reverse transcriptase, and the labeled products were analyzed on a denaturing acrylamide gel. For each mRNA, two primers, labeled on opposite strands, were used. Figure 8 depicts the sequence of reactions if the labeled DNA strand hybridizes to mRNA. If the opposite strand were labeled then the unlabeled DNA strand would hybridize and act as primer; no extension of the labeled fragment would be detected.

Primers from Region I were isolated from the 1.3 SalI fragment of J1A (see Fig. 9a). The 125 bp SalI-XbaI fragment was end labeled at the SalI site; the 185 bp AvaII-SmaI fragment was labeled at the AvaII end. After hybridi-

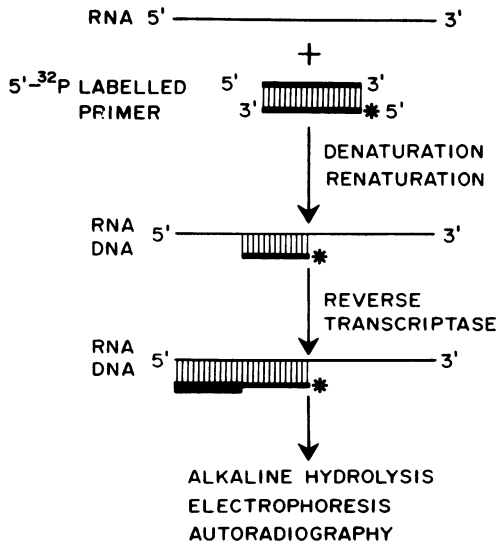

Figure 8. Diagram of the protocol used for determination of the direction of transcription.

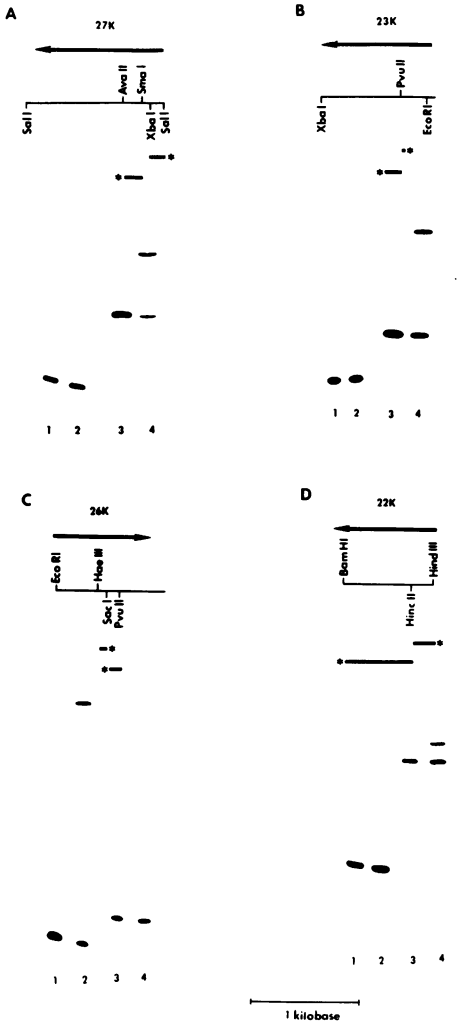

Figure 9. Determination of the direction of transcription of the 4 mRNAs. DNA fragments were used as primers for reverse transcriptase as described in Fig. 8. The asterisk indicates the end of the fragment which is labeled. Lanes 1 and 2 show experiments of fragments end-labeled at the "right hand" end of the fragment as drawn, lanes 3 and 4, labeled on the left end. The reaction products were subjected to electrophoresis and the gel was autoradiographed. Lane 1 and 3 show products synthesized in the absence of heat shock mRNA, lanes 2 and 4, in the presence of heat shock RNA.

zation with heat shock RNA, the products were analyzed by electrophoresis. The SalI-XbaI fragment did not prime labeled cDNA synthesis, whereas the AvaII-SmaI fragment extended to approximately 350 bp. This result indicates the 5' end of the mRNA is to the right of the SmaI site, and transcription is from right to left as drawn in Figs. 3, 7 and 9.

The 200 bp insert of the cDNA plasmid HS229 overlaps and extends 35 bp to the right of the right most Pvull site in J1A. The excised HS229 insert was end labeled and cleaved with PvuII. The isolated fragments were used as primers. The small labeled primer was not extended; the larger was extended

(Fig. 9b). The extension of the large primer indicates that the direction of transcription of the 23K gene is from right to left as drawn in Figs. 3, 7 and 9.

The direction of transcription of Region III was determined using primers labeled at the SacI site of subclone J1B. A 80 bp HaeIII-SacI fragment; primed cDNA synthesis; a 100 bp SacI-PvuII fragment did not (Fig. 9c). The extension of the labeled HaeIII-SacI fragment indicates the transcription of the 26K gene is from left to right.

The direction of transcription of the 22K gene was again determined by using reverse transcriptase. The BamHI-HindIII fragment of T5 was isolated and terminally labeled using ^{32}P-ATP. The labeled fragment was then cleaved with HincII yielding a 620 bp BamHI-HincII piece and a 220 bp HincII-HindIII piece. Both fragments were used as primers for reverse transcriptase. The smaller fragments did not prime DNA synthesis whereas the larger fragment was extended. This result indicates that the 5' end of the mRNA is to the right of the HincII site and transcription is from right to left.

DISCUSSION

We have isolated an 11 kb region of the Drosophila melanogaster genome which includes the 27K, 26K, 23K and 22K heat shock proteins, as shown directly by in vitro translation experiments. Region I, which contains the gene for the 27K protein, encodes a 1.3 kb mRNA. A 1.0 kb mRNA is transcribed from Region II, which encodes the gene for the 23K protein; Region III, containing the 26K gene, encodes a 1.1 kb mRNA. Region I and II are separated by approximately 1.5 kb of spacer DNA; the spacer between Regions II and III is approximately 4.5 kb in length. The 22K gene (Region IV) is situated 1 kb from the 26K gene. The 27K, 23K and 22K RNAs from Regions II and III and IV are transcribed from the same strand; the 26K RNA from Region III is transcribed in the opposite direction. The fact that two pairs of adjacent RNAs are transcribed from different strands indicates that RNA transcription initiates at three or four points on this DNA segment.

Within the 33 kb of contiguous DNA analyzed in this study, an additional region of RNA homology was detected. In the DNA isolated from Schneider's cells a transcribed region is found 10 kb from the 27K gene. This region exhibits characteristics in common with dispersed multigene families described by others (for example, 30) in that it encodes an abundant RNA for which no protein product has been demonstrated and contains moderately repeated DNA sequences. We do not know whether this repeated element also occurs in the

genome of our Oregon R stock at the same location.

Spradling et al. (11) reported the identification of two heat shock mRNAs which hybridized in situ to 67B. These RNAs migrated in formamide gels as 13S molecules, which would be equivalent to 1.3 kb, certainly in reasonable agreement with size estimates for the mRNAs of the 27K, 26K and 23K mRNAs made by electrophoresis of glyoxylated RNA and reported here. The sizes of the mRNAs are also in good agreement with the size of the mRNA homologous regions defined by hybridization of labeled mRNA to restriction endonuclease generated fragments of J1 DNA. This correspondence implies that no extremely large intervening sequences exist within the coding regions for these proteins. Of course, the delineation of the exact structure of the coding unit awaits further experimentation.

Earlier studies of the heat shock response suggested a one to one correspondence between puffs and heat shock proteins. However, recent data demonstrates that a more complex situation exists. For example, it is now evident that both 87A and 87C harbor copies of the gene for the 70K protein (see Ashburner and Bonner (1) for review). Data summarized here and elsewhere (9,10) demonstrates that 67B is also a complex locus, containing at least four genes which encode 27K, 26K, 23K and 22K proteins. Genes for the 68K and 83K proteins have been localized to 95D and 63BC respectively (31). Thus the loci for all of the seven major heat shock proteins have been determined.

The 27K, 23K and 22K genes are transcribed from the same strand, while the 26K gene is transcribed from the opposite strand. This gene orientation contrasts with that of some of the 70K heat shock induced genes. A cluster of 70K genes with the three genes aligned in the same orientation and separated by two grossly identical 1.4 kb spacers has been isolated (14). Another example of linked genes transcribed from the same strand are the β-like globin genes (32). The orientation of the smaller heat shock genes is more reminiscent of the organization of the histone genes of Drosophila (33). Genes for histones H1, H3 and H2a are transcribed in the same direction, whereas the interdispersed H4 and H2b genes are transcribed in the opposite direction. This head to head and tail to tail orientation differs from the head to tail organization found in the histone genes of the sea urchin (34).

It is also of interest to consider what is known of the structure of the heat shock genes in terms of the one band-one gene hypothesis (35). According to this hypothesis, each chromomere, with an average DNA content of 28 kb, represents a single complementation group and a gene for a single protein. Information obtained thus far indicates that the genes for both the histones

and the smaller heat shock proteins do not appear to fit this model. The former exception may be reconciled with the thesis that each chromomere contains a gene for a single function by recalling that the five histone genes are coordinately expressed and their products interact as subunits of a single functional unit, the nucleosome. Since the genes for the four small heat shock proteins are also closely linked and coordinately expressed, it would not be surprising if these four proteins interact to form a functional unit.

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*To whom reprint requests should be addressed

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