Volume 9 Number 20 1981 Nucleic Acids Research

Genomic organization and transcription of the αβ heat shock DNA in Drosophila melanogaster

John T.Lis*, David Ish-Horowicz† and Sheena M.Pinchin†

*Section of Biochemistry, Molecular and Cell Biology, Wing Hall, Cornell University, Ithaca, NY 14853, USA, and †Imperial Cancer Research Fund, Mill Hill Laboratories, Burtonhole Lane, London NW7 1AD, UK

Received 13 July 1981

ABSTRACT

Previous studies have shown that (i) several RNAs induced by heat shock of Drosophila melanogaster cells are homologous to tandemly repeated αβ units found in cloned segments of D. melanogaster DNA, and (ii) the αβ sequences are present both at a major heat shock locus, 87C1, and the chromocenter of polytene chromosomes (Lis, J.T., Prestidge, L. and Hogness, D.S. [1978] Cell 14, 901-919). We have used deficiencies that delete DNA from the 87C region to examine the arrangement of αβ sequences at this locus and in the centromeric heterochromatin that comprises the chromocenter, and also to determine the chromosomal location of the induced transcription. The tandemly repeated αβ units are restricted to the 87C locus. In contrast, the chromocentral αβ sequences do not form intact αβ units, and are dispersed at heterochromatic sites in some other form. Although only half of the αβ DNA is at the 87C locus, essentially all αβ transcripts (>99.5%) are derived from this locus.

INTRODUCTION

Shifting temperature of Drosophila melanogaster cells to 36°C causes a dramatic simiplication in the pattern of gene expression. This switch in transcriptional and translational specificity results in the intense synthesis of several new RNA and protein species (reviewed in 1). The loci encoding the new RNAs have been localized to sites of heat-induced puffing in polytene chromosomes. The most prominent of these sites, 87C, includes two types of heat-induced sequences. One codes for the major 70,000 d heat shock protein (hsp 70), and is also present at another heat shock site, 87A (1-4). The second yields less abundant transcripts of unknown function and is not found at 87A (5).

Sequences of this second sort have been isolated as cloned DNA segments (Dm segments), where they are contained in a tandemly repeated unit that is divisible into α and β elements, as shown in Fig. 1 (5). The αβ unit exhibits sequence homology with three size classes of heat shock, poly(A)$^+$ RNAs, one of which was shown by R-loop mapping to have an αβα structure. A

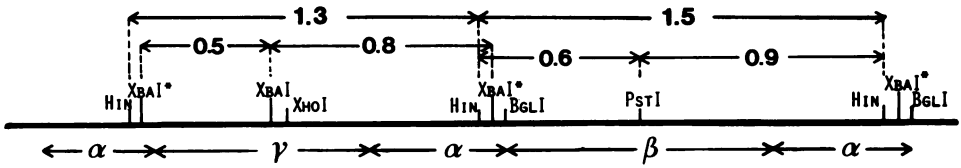

Figure 1. Map of tandemly arranged αβ and αγ units. This represents a composite of restriction and heteroduplex mapping done previously (5), of additional restriction mapping of cloned segments and of total Drosophila DNA, and of DNA sequence data (Hackett and Lis, unpublished results). The size of the fragments generated by HindIII (Hin) or by a combination of HindIII and PstI are given in kb. The XbaI* sites are found in total D. melanogaster DNA but are not detected in cloned segments Dm703 and Dm704 [some XbaI sites are known to be partially modified by E. coli (6)].

third element, γ, is found adjacent to α elements such that αγ units are interspersed among the αβ units (Fig. 1). Most of the γ element, unlike the α and β elements, is not homologous to abundant heat shock RNA.

The αβ sequences are not however confined to the 87C site; cloned αβ sequences exhibit strong in situ hybridization to the chromocenter of polytene chromosomes as well as to this site of heat-induced puffing (5). This dispersion introduces two related questions. Is the tandem repetition seen in Fig. 1 a general characteristic of all αβ chromosomal sites, or is it specific to 87C? Does heat shock induce transcription at all sites, or is that transcription restricted to 87C? We have answered these questions in this paper by the use of deficiency stocks in which the 87C locus has been deleted. The results can be simply stated: 87C is the only site containing tandemly repeated αβ units--indeed, no intact αβ units are located elsewhere; and 87C provides essentially all (≥99.5%) of the αβ heat shock RNA. The αγ units also appear to be restricted to the 87C locus.

MATERIALS AND METHODS

Isolation of Drosophila RNA

Typical preparations of D. melanogaster RNA were from fifty flies which had been heat shocked for 60 min at 36°C. The flies were ground in 1 ml of 100 mM Tris-HCl (pH 9), 20 mM EDTA, 1% SDS, 20 mg/ml polyvinyl sulfate, 1 mg/ml protease (Sigma VIII, preincubated 1 hr at 37°C) and incubated for 45 min at 37°C. The mix was extracted twice with 1 ml phenol containing 0.1% hydroxyquinoline and once with 1 ml $CHCl_3$; isoamyl alcohol (24;1). RNA was precipitated three times in 0.2 M NaCl with 2 or 2.5 volumes of ethanol.

Contaminating DNA was removed by digestion in 100 mM NaOAc (pH 6), 10 mM MgCl for 1 hr at 26°C with 5 µg/ml DNase-I [DN-EP grade from Sigma preincubated at 80 µg/ml for 12 hrs at 25° in 0.1% diethylpyrocarbonate and 80 mM NAOAc (pH 6), to remove contaminating RNase (Elizabeth Keller, personal communication)]. The treated RNA was extracted twice with phenol; $CHCl_3$-isoamylalcohol (50:50:1).

RESULTS

Deletion Mapping of αβ Sequences within the 87C Region

In situ hybridization of Dm703, a cloned segment containing only αβ units (Fig. 1), to polytene chromosomes strongly labels both 87C and the chromocenter (5). (Weak labeling observed at 10B and 42B represents a small fraction of the total *in situ* hybridization and is not distinguished from chromocentral αβ in this study.) In this paper, we used *in situ* hybridization to map the αβ sequences at 87C relative to the endpoints of three deficiency stocks: *Df(3R)kar*$^{3\ell}$, *Df(3R)kar*^{3J} and *Df(3R)kar*^{3Q} (7). These will hereafter be referred to as *kar*$^{3\ell}$, *kar*^{3J} and *kar*^{3Q}, respectively, and the deletions they represent are defined in Fig. 2. We compared the degree of

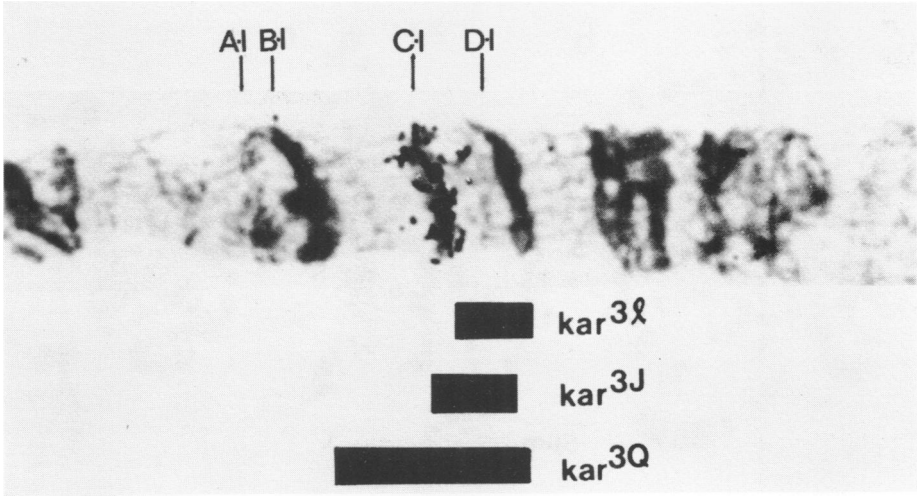

Fig. 2. Positions of the deletions in the *kar*$^{3\ell}$, *kar*^{3J} and *kar*^{3Q} deficiencies. The *in situ* hybridization autoradiograph at the top of the figure was obtained as described previously (5), using a ^{3}H-labeled probe consisting of the αβ sequences in Dm703 (Fig. 1). The silver grains are centered over the 87C1 band. The black bars below the autoradiograph represent the extent of the deletions in *kar*$^{3\ell}$ (87C2-3 to 87D3-4), *kar*^{3J} (87C1 to 87C9) and *kar*^{3Q} (87B4-5 to 87C9-D2), as previously determined (7).

hybridization to a deficiency chromosome with that to In(3R)Na, an inversion that includes 87C. Heterozygotes for In(3R)Na and a deficiency form polytene chromosomes in which the homologues are asynapsed, allowing visualization of the αβ hybridization to both the deficiency and the inversion in individual nuclei. Figs. 3a and 3b show that the 87C αβ sequences have been deleted in kar^{3J} and kar^{3Q}, whereas Fig. 3c shows that they have not been deleted in $kar^{3\ell}$. These results demonstrate that the αβ sequences at 87C map to the interval defined by the left ends of $kar^{3\ell}$ and kar^{3J} (Fig. 2).

The Tandemly Repeated αβ Units Are Confined to the 87C Locus

Fig. 1 shows that the restriction enzyme HindIII cleaves within the α element but not within the β or γ elements (5). Thus HindIII cleavage of tandem arrays of αβ and αγ units will yield 1.5 kb and 1.3 kb fragments, derived respectively from αβα and αγα series found in such arrays. The ability to detect such fragments by the method of Southern (8) provides an assay for the presence of tandem αβ and αγ arrays in total DNA of flies of various genotypes. Analysis of DNA from embryos lacking the 87C locus (kar^{3J} and kar^{3Q} homozygotes) with those retaining the locus ($kar^{3\ell}$ homozygotes and wild-type) allows the comparison of the organization of αβ

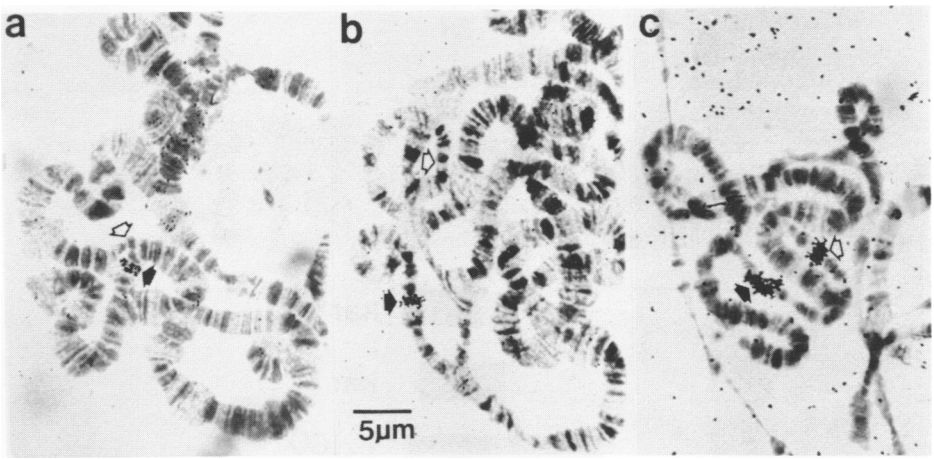

Fig. 3. In situ hybridization of αβ sequences to polytene chromosomes from larvae heterozygous for In(3R)Na and the $kar^{3\ell}$, kar^{3J} or kar^{3Q} deficiencies. [^{3}H]-cRNA transcribed in vitro from cDm703 was hybridized to the heterozygous chromosomes in situ as described previously (5). The open arrow points to the deleted region in the (a) kar^{3Q}, (b) kar^{3J} and (c) $kar^{3\ell}$ deficiency chromosomes. The solid arrow points to the 87C1 band in the In(3R)Na chromosome, in which the region between 87F and 96F has been inverted. Exposures were for (a) 15, (b) 15 and (c) 17 days.

sequences at 87C with those at the centromere. The DNA used in these experiments was isolated from embryos (9) - a necessity in the case of the deficiency homozygotes, because they fail to survive beyond this stage. Adequate numbers of such embryos can readily be obtained, since they are easily recognized by their abnormal malpighian tubules (7).

The hybridization autoradiographs resulting from these assays are given in Fig. 4a. Qualitative comparison of the tracks generated by the kar^{3J} and kar^{3Q} DNAs (E and F) with those derived from the wild-type and $kar^{3\ell}$ DNAs (A-D) indicates that deletion of the 87C heat shock locus dramatically reduces the yield of both the 1.5 kb and the 1.3 kb fragments. We have estimated the number of copies of these fragments using standard curves (Fig. 4b) generated from controls containing known amounts of the fragments (tracks G-L). Table 1 shows that the mean and range for the number of 1.5 kb αβ fragments per haploid genome is reduced from 11 (± 3) for genomes that contain the 87C locus, to $\leq$ 0.5 for those that do not. We therefore conclude that tandem arrays containing the αβ unit are confined to the 87C heat shock locus. Table 1 also shows that the copy number for the 1.3 kb fragment is reduced from 8 (± 3) to $\leq$ 1 by deletion of this locus, strongly suggesting that arrays containing αγ units are similarly confined. (The upper limit of $\leq$ 1 for the αγ fragments from the deleted genomes is greater than that for the αβ fragments because of the lower sensitivity of the αβ hybridization probe in assaying αγ fragments.)

The Chromocentral Sequences Are Dispersed and not Organized into Intact αβ Units

Given that the chromocenter lacks tandemly arranged αβ units, we asked if it contains any intact αβ units. We have settled this question by the use of a second restriction endonuclease, PstI, which cuts αβ units at a single site in the β element. Although an isolated αβ unit does not yield a characteristic fragment if cleaved only with HindIII, when cleaved with both HindIII and PstI, a single dispersed unit will yield either a 0.62 kb or a 0.92 kb HindIII/PstI fragment, depending upon whether it is in the αβ or βα configuration, respectively, and a unit within a tandem array will yield both fragments (Fig. 1). We therefore examined HindIII-PstI double digests of the above total DNAs by procedures identical to those described for Fig. 4. Fig. 5 and Table 2 show that deletion of the 87C heat shock locus eliminated both of these characteristic HindIII/PstI fragments. We conclude that all intact αβ units are confined to this locus, and consequently, that the chromocentral α and/or β sequences must be dispersed in some other

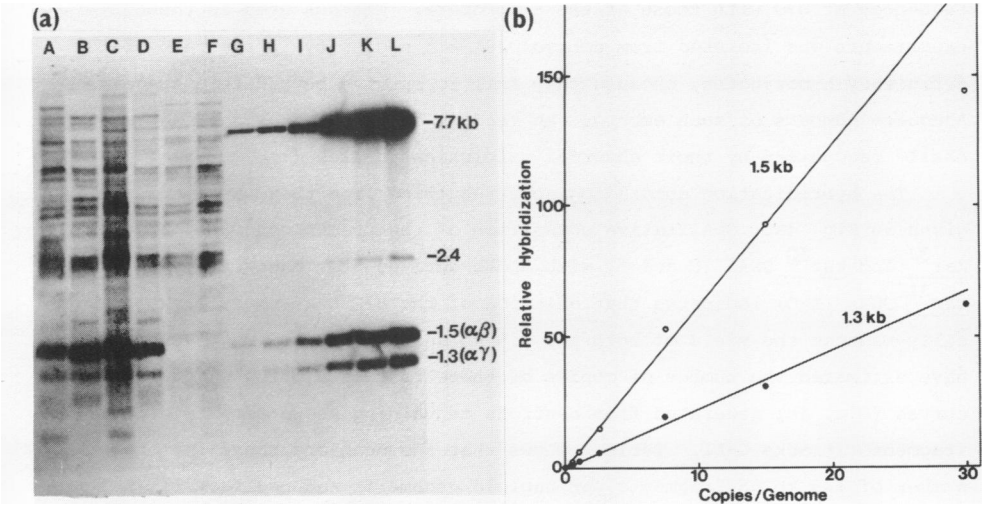

Fig. 4. Quantitative assay for tandemly arranged αβ and αγ units in wild-type and deficiency DNAs. All DNA samples were digested to completion with HindIII, electrophoresed in a 0.7% agarose gel, and the separated fragments completely transferred to nitrocellulose strips where they were hybridized with excess [^{32}P]-cDm703 DNA as previously described (5).

(a) Tracks A (Canton S), B (Oregon R [Stanford]), C (Oregon R [Mill Hill]), D ($kar^{3\ell}$), E (kar^{3J}) and F (kar^{3Q}) contain, respectively, 0.7, 1.2, 2.8, 1.4, 1.4 and 1.7 µg of DNA as determined by microdensitometric tracing of a photographic negative of the ethidium bromide stained gel. The control tracks G-L contain 0.07, 0.15, 0.36, 1.1, 2.2 and 4.4 ng of cDm704 DNA, and represent, respectively, the equivalent of 1.2 µg of genomic DNA containing 0.5, 1.0, 2.5, 7.5, 15 and 30 copies of the 1.3 kb and 1.5 kb fragments/haploid genome. The 2.4 kb fragment contains a small amount of α sequences and derives from the Dm704 segment (5), whereas the 7.7 kb fragment contains the ColE1 vector DNA of cDm704 (5). A total of 1.5 x 10^7 dpm at 4 x 10^7 dpm/µg of cDm703 DNA in 7.5 ml was used for the 40 hr hybridization; the autoradiogram shown here was exposed for 40 hr with no intensifying screen (see ref. 5 for other conditions).

(b) The amount of hybridization to the 1.5 kb and 1.3 kb fragments in each track was determined from microdensitometric tracings of the autoradiograms, all tracing being of bands that are within the linear range of the film. A plot of the relative amount of hybridization for each fragment versus the equivalent copies/haploid genome was obtained from the control tracks G-L. Comparison of the values obtained for tracks A-F to these standard curves yields the values given in Table I.

form. Further support for this conclusion comes from digestion of the above total DNA with the additional pairs of enzymes: XbaI, PstI; XbaI, BglI; and XbaI, HindIII. Each enzyme cleaves the αβ unit once (Fig. 1) and the fragments produced from tandem arrays of αβ by each pair are absent in the strain deleted of the 87C locus (Fig. 6).

Table I. Copy numbers for the 1.5kb αβ and 1.3kb αγ HindIII fragments in wild-type and deficiency strains.

HindIII Fragment	Copies per haploid genome						
	(A)	(B)	(C)	(D)	Mean	(E)	(F)
	Canton S	Oregon R (Stanford)	Oregon R (Mill Hill)	$kar^{3\ell}$	(A-D)	kar^{3J}	kar^{3Q}
1.5 kb (αβ)	13	14	10	8	11±3	≤0.5	≤0.5
1.3 kb (αβ)	10	8	8	5	8±3	≤1	≤1

Copy numbers were determined as described in the legend in Fig. 4.

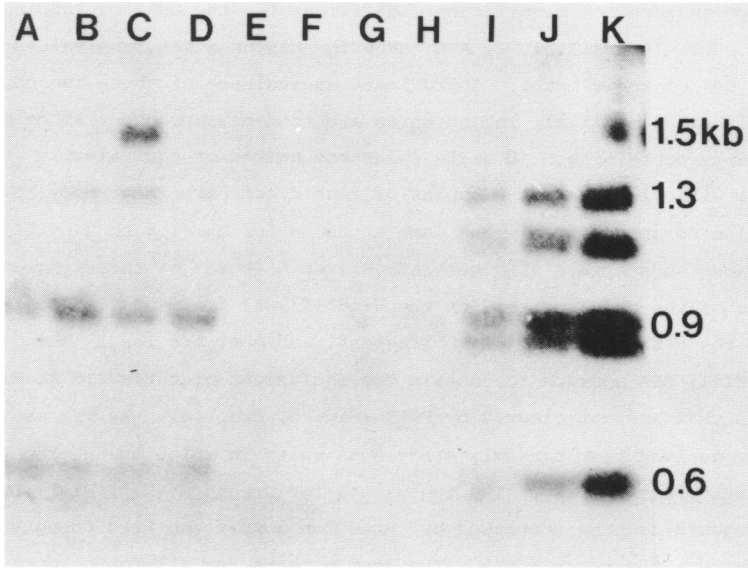

Fig. 5. Quantitative assay for intact αβ units in wild-type and deficiency DNAs. All procedures were the same as those given for Fig. 4, except that the DNA samples were double digested with HindIII and PstI, and a 1.0% agarose gel was used for the electrophoresis. Track letters represent the same DNA sources as in Fig. 4: tracks A-F contain 0.9, 1.1, 1.9, 1.5, 1.5 and 1.7 μg of the respective total D. melanogaster DNAs; control tracks G-K contain the equivalent of 1.1, 2.8, 8.3, 17 and 33 copies per 1.1 μg haploid genome. Only the relevant portion of the autoradiogram (46 hr exposure) is shown.

Table 2. Copy numbers for the 0.62 kb and 0.92 kb HindIII/PstI fragments derived from αβ units in wild-type and deficiency strains.

HindIII/PstI Fragment	Copies per haploid genome					
	(A)	(B)	(C)	(D)	(E)	(F)
	Canton S	Oregon R (Stanford)	Oregon R (Mill Hill)	$kar^{3\ell}$	kar^{3J}	kar^{3Q}
0.62 kb	13	9	4	5	0.4	0.2
0.92 kb	7	9	3	4	0.1	0.3

Copy numbers were determined as described in Fig. 4 from the data in Fig. 5.

Within each strain the copy number for the 0.62 kb and 0.92 kb HindIII/PstI fragments are not significantly different for the DNAs containing the 87C heat shock locus (Table 2, A-D; even for Canton S DNA, the values are within ± 30% of their mean). Approximate equivalence of these two copy numbers is expected if all intact units are tandemly arranged, since a tandem array containing N αβ units (plus any number of equivalently oriented αγ units) will yield N-1 or N copies of each fragment. These copy numbers should also be approximately the same as those for the 1.5 kb HindIII fragment because such arrays also generate N-1 or N copies of this fragment.

None of the copy numbers for the HindIII/PstI fragments is greater than that for the corresponding 1.5 kb fragment, and most are less. The Oregon R (Mill Hill) DNA appears to contain the equivalent of three 1.5 kb HindIII fragments that are not cleaved by PstI (Fig. 5, track C). We presume that these are derived from tandemly arrayed αβ units in which the β elements lack a PstI cleavage site. The two values for Oregon R (Mill Hill) DNA in Table 2 should each be increased by three for comparison with three values falling within the range 8 ± 2. The copy numbers for all four genomes are therefore consistent with the concept that all intact αβ units are tandemly arranged, although errors in the determination of these copy numbers clearly allow the possibility that a few units occur singly. It should be emphasized that such errors do not affect the conclusion that all intact αβ units are confined to the 87C heat shock locus.

Intact αγ fragments appear also to be restricted to the 87C locus. The enzyme XbaI cuts in both α and γ elements, thereby cleaving the tandem 1.3

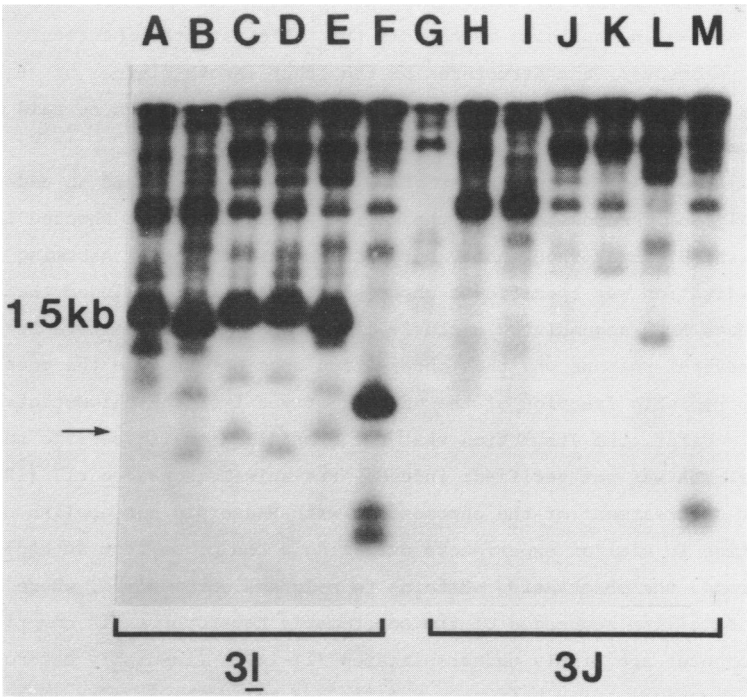

Fig. 6. Assay for tandem αβ and αγ elements using a variety of restriction endonucleases. The digested DNA was run on a 1.4% agarose gel, and the αβ-containing fragments detected essentially as for Fig. 4. Tracks A-F contain digests of kar$^{3\ell}$ DNA; tracks H-M of kar^{3J} DNA. The following digests are on each track: A and H, HindIII; B and I, XbaI and HindIII double digests; C and J, XbaI; D and K, XbaI and XhoI double digest; E and L, XbaI and BglI double digest; F and M, XbaI and PstI double digest. Track G contains molecular weight markers. The arrow indicates the 0.8 kb fragment.

kb unit to yield 0.5 kb and 0.8 kb fragments (Fig. 1). The XbaI digest of Fig. 6 reveals the presence of the 0.8 kb fragment in kar$^{3\ell}$ DNA and its absence from kar^{3J} DNA (the smaller 0.5 kb fragment possesses less than 50 base pairs of the α element and is not seen in either DNA).

The αβ Heat Shock RNAs Are Transcribed from the 87C Locus

The level of αβ transcripts is dramatically increased in response to heatshock (5). Are these transcripts derived from the 87C heat shock puff, the chromocenter, or from both sites? Given that the 87C locus is the sole repository of tandem arrays of αβ and αγ units, and indeed, of all intact αβ units, one is tempted to conclude that the heat shock RNA which exhibits an αβα structure must be transcribed from this locus (5). However, one can

imagine that transcripts of the dispersed chromocentral sequences might yield the αβα RNA by splicing mechanisms that eliminate the intervening sequences. Moreover, the structures of the other length classes of αβ heat shock RNAs have not been determined (5), and hence, little can be said about their chromosomal origin.

Livak et al. (3) observed that αβ DNA sequences hybridized to undenatured polytene chromosomes from the salivary glands of heat shocked larvae labelling the 87C heat shock puff, but not the chromocenter. Assuming that this hybridization was specific to chromosomal RNA, they concluded that the αβ heat shock RNAs accumulated exclusively at the 87C locus. There are, however, several reasons why this observation does not exclude the possibility that an appreciable fraction of the αβ RNA derives from the chromocentral αβ sequences. First, the assumption that the hybridization is specific to chromosomal RNA was not verified; indeed, Artavanis-Tsakonas et al. (10) found that pretreatment of the chromosomes with RNase did not abolish such hybridization in similar experiments utilizing a DNA probe from an hsp70 gene. Second, the observation pertains to polytene chromosomes, where at least the satellite sequences of the centromeric heterochromatin comprising the chromocenter are highly underreplicated (11-13). Presumably heterochromatic sequences lying between satellite and fully polytene chromosomes display a polyploidy between these extremes. Finally, this technique should be sensitive to the rate of flow of RNA away from, and conceivably into, the chromosomal site, not simply to the frequency of transcription at the site.

These various indeterminacies induced us to examine this question by measuring the amount of αβ heat shock RNA produced by flies in which the sequences at the 87C locus have been deleted. $\underline{Dp(2;3)ry^{+w70h}}$ is a duplication of the 82C2-3 through to the 88 region which has been inserted into the second chromosome (W. Gelbart, personal communication). Although $\underline{kar^{3J}}/\underline{kar^{3J}}$; $\underline{Dp(2;3)ry^{+w70h}}$ individuals lack 87Cl and its αβ sequences, they develop into adult flies. We have compared the heat-induced RNA from $\underline{kar^{3J}/kar^{3J}}$; $\underline{Dp2;3)ry^{+w70h}}$ flies ($\underline{87Cl^{-}}$ RNA) with that from $\underline{kar^{3\ell}/kar^{3J}}$; $\underline{Dp(2;3)ry^{+w70h}}$ flies which retain an intact 87Cl locus ($\underline{87Cl^{+}}$ RNA). The RNAs were run on agarose gels, immobilized on activated paper and the αβ-containing species visualized by hybridizing with labelled αβ DNA. Track A on Figure 7 shows that $\underline{87Cl^{+}}$ RNA has several species of αβ-containing RNA. In contrast, $87Cl^{-}$ RNA has no detectable αβ homology (Track B). The ab sequences are still readily detectable at a 1 to 20 dilution of $\underline{87Cl^{+}}$ RNA (Track C). Microdensitometric analysis of these tracks reveals that greater

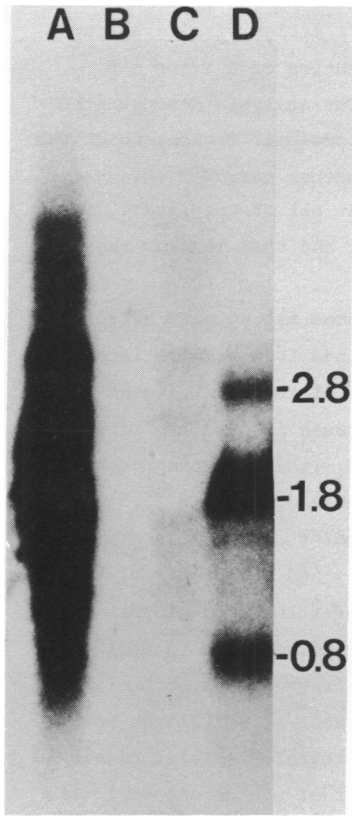

Fig. 7. Estimating the fraction of αβ RNA derived from 87C1. Preparations of total RNA were isolated from Drosophila adults. An aliquot of each RNA preparation was denatured by glyoxal treatment and fractionated by electrophoresis on a 1.5% agarose gel (14), transferred to diazobenzyloxymethyl-paper and hybridized with 3 x 10^6 dpm of ^{32}P-labeled cDm703 at 10^8 dpm/μg essentially as described by Alwine et al. (15). Poly(A) and tRNA were added at 100 μg/ml to the hybridization mix and SDS was added to 0.2%. Track A contains 17 μg of RNA from kar^{3J}/kar^{3J}; $Dp(2;3)ry^{+}w70h$ homozygotes ($87C1^{+}$), B contains 17 μg of RNA from kar^{3J}/kar^{3J}; Dp homozygotes ($87C1^{-}$), and C contains a mixture of 16.2 μg of RNA from kar^{3J}/kar^{3J}; Dp flies seeded with 0.85 μg of RNA from kar^{3J}/kar^{3J}; Dp flies. Track D has 0.4 μg of polyA-containing polysomal RNA isolated from KC cells which contains αβ RNAs of 2.8, 1.8, and 0.8 kb (5). Observation of the ethidium bromide stained gel with UV light before transfer to diazo paper showed the RNA to be intact and to be present in equal concentrations in tracks, A, B and C.

than 99.5% of the αβ RNA of normal flies is derived from 87C1. Hybridization to cloned DNA encoding other heat shock genes (hsp70 and hsp82) shows that their RNAs are still present after deleting 87C1 (data not shown). We have also assayed these and other RNA preparations by direct attachment to activated paper discs and hybridization to αβ probes. Although this assay is less sensitive, the results are qualitatively the same; heat-induced RNA preparations from flies lacking 87C1 show essentially no homology to αβ sequences.

DISCUSSION

The probes provided by cloned Dm segments have allowed us to combine DNA hybridization and deletion mapping techniques to analyze the arrangement of the αβ sequences in the D. melanogaster genome. These sequences can be divided into two topographic classes. The tandem class is originally de-

fined by the cloned segments and accounts for about one half of the αβ sequences (5). Such sequences are organized into a series of alternating α and β elements, which, with the occasional substitution of a γ for a β element, form tandem arrays of αβ and αγ units. Our analysis demonstrates that this topography is restricted to a single chromosomal region, which was mapped to 87C1-3. (Subsequent experiments have further narrowed this region to band 87C1 [16].) We have also shown that all of the intact αβ units in the genome are concentrated at this site, and that most of these units are in tandem arrays (Tables 1 and 2).

In contrast, the dispersed class of αβ sequences are at many sites as indicated by the different HindIII fragments obtained from genomes lacking 87C1 (Fig. 4a, tracks E and F). The vast majority of these sites must be located within the centromeric heterochromatin because in situ hybridization reveals that most non-87C αβ sequences are located within the chromocenter, which, in polytene chromosomes, consists of this heterochromatin (3,5). Little is known about the organization of the α and/or β sequences at these sites, except that it does not include contiguous α and β elements of the sort defined in our cloned Dm segments (5). Whatever the form of that organization, the two classes of αβ sequences appear to be closely homologous, since the hybridization of cloned αβ sequences to fragments of both classes (Fig. 4a) is resistant to stringent washing (9 mM NaCl at 65°C) that would melt poorly matched hybrids (data not shown). We therefore prefer models in which the dispersed chromocentral sequences consist of unaltered, or little altered, parts of the 87C1 αβ units (e.g., individual α and/or β elements), rather than models involving complete but diverged units that lack the restriction sites characteristic of the cloned α and β elements. Yet another explanation is that the chromocentral α elements have modified HindIII sites that no longer are cleaved to the fragments characteristic of tandem arrays. As BglI and XbaI digests give the same results as HindIII (Fig. 6), this theory is unlikely.

Hybridization and deletion mapping techniques were also used to demonstrate that essentially all (≥99.5%) of the αβ heat shock RNAs are transcribed from the 87C1 αβ sequences. Since most 87C1 αβ units are in tandem arrays, this suggests that the αβ transcription is limited to such arrays. We previously suggested that sequences in the γ element might provide initiation signals for heat-induced transcription, both because they are appropriately located relative to the 5' end of the αβα RNA sequence and because γ sequences are found at 87A as well as 87C (5), both of which

contain the gene for hsp 70 (1-4). Support for this suggestion derives from the recent observation that several hundred base pairs of the γ element are found upstream from the hsp 70 coding sequences (17) extending 64 base pairs into the hsp 70 transcription unit (18).

Differences in chromatin structure between the heterochromatic sites and 87C1 might also contribute to the localization of αβ transcription. For example, promoters could be associated with both classes of sequences, but only promoters of the tandem class might become available to RNA polymerase because of a change in chromatin structure at 87C1 that is induced by heat shock. Indeed, one can imagine that such an induced change in chromatin structure results from sequences associated with the hsp 70 genes at 87C, where these hsp 70 genes occupy positions at both ends of the stretch of DNA containing the αβ sequences (16). According to this notion, any gene inserted into this region might then be induced by heat shock, providing it was associated with an appropriate promoter.

The problem of the localization of αβ transcription is thus related to the question of the function of the αβ RNAs. Although present in polysomal RNA preparations (3,5), the αβ RNAs do not appear to code for any of the major heat shock proteins, as indicated by the lack of detectable differences in the pattern of heat-induced protein synthesis in embryos lacking 87C (7), and by hybrid arrested translation experiments (3). In D. simulans, a sibling species whose hsp 70 genes are also distributed at 87A and 87C, the αβ sequences are present at the chromocenter but not at 87C, and may not be transcribed upon heat shock (3,19). These observations do not exclude a function for the αβ RNA in the heat shock response; they could code for a minor heat shock protein or exhibit noncoding structural or regulatory functions. D. melanogaster might utilize these RNAs to produce a more sophisticated response than that in D. simulans, or the same function might be provided by other sequences in D. simulans.

The hsp 70 loci of other closely related species also lack αβ sequences, suggesting that their presence at the 87C1 locus of D. melanogaster is due to a series of insertions (19). Mobile sequence elements occur with surprising frequency in D. melanogaster (20-23). The αβ sequences may also be mobile. This raises the possibility that the αβ sequences may not be relevant to the heat shock response and that their transcription might be the accidental result of the insertion between the hsp 70 genes at 87C1. The availability of viable flies deleted for the 87C1 locus will provide the basis for testing functions of the heat-inducible αβ sequences.

ACKNOWLEDGEMENTS

We thank Deborah O'Connor for thoughtful assistance, and Mariana Wolfner for advice and assistance with the *in situ* hybridization experiments. We thank J. Hirsh for DNA from the Canton S strain of *D. melanogaster*, W. Gelbart for the *Dp(2;3)ry*$^{+w70h}$ strain, and J. de Banzie and R. Rothstein for comments on the manuscript. We also thank D. Hogness for support and comments on the manuscript.

This work was supported in part by NIH grant GM 25232 to J.T.L.

REFERENCES

1. Bonner, J.J., and Ashburner, M. (1979) Cell 17, 241-54.
2. Schedl, P., Artavanis, S., Steward, R., Gehring, W., Mirault, M.E., Goldschmidt-Clermont, M., Moran, K., and Tissieres, A. (1978) Cell 14, 921-929.
3. Livak, K.J., Freund, R., Schweber, M., Wensink, P.C., and Meselson, M. (1978) Proc. Natl. Acad. Sci. USA 75, 5613-5717.
4. Ish-Horowicz, D., Pinchin, S.M., Gausz, J., Gyurkovics, H., Bencze, G., Goldschmidt-Clermont, M., and Holden, J.J. (1979) Cell 17, 565-571.
5. Lis, J.T., Prestidge, L., and Hogness, D.S. (1978) Cell 14, 901-919.
6. Zain, B.S., and Roberts, R.J. (1977) J. Mol. Biol. 115, 249-255.
7. Ish-Horowicz, D., Holden, J.J., and Gehring, W.J. (1977) Cell 12, 643-652.
8. Southern, E.M. (1975) J. Mol. Biol. 98, 503-517.
9. Ish-Horowicz, D., Pinchin, S.M., Schedl, P., Artavanis-Tsakonas, S., and Mirault, M.E. (1979) Cell 18, 1351-1358.
10. Artavanis-Tsakonas, S., Schedl, P., Mirault, M.E., Moran, L., and Lis, J.T. (1979) Cell 17, 9-18.
11. Rudkin, G.T. (1972) in Developmental Studies on Giant Chromosomes, Results and Problems in Cell Differentiation, Vol. 4 (ed., Beermann, W.), Springer-Verlag, New York, pp. 59-85.
12. Spear, B.B., and Gall, J.G. (1973) Proc. Natl. Acad. Sci. USA 70, 1359-1363.
13. Hennig, W., and Meer, B. (1971) Nature New Biol. 233, 70-72.
14. McMaster, G.K., and Carmichael, G.C. (1977) Proc. Natl. Acad. Sci. USA 74, 4835-4838.
15. Alwine, J.C., Kemp, D.J., and Stark, G.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5350-5354.
16. Ish-Horowicz, D., and Pinchin, S.M. (1980) J. Mol. Biol. 142, 231--245.
17. Lis, J., Neckameyer, W., Mirault, M.E., Artavanis-Tsakonas, S., Lall, P., Martin, G., and Schedl, P. (1981) Dev. Biol. 83, 291-300.
18. Hackett, R.W. and Lis, J.T. (1981) Proc. Natl. Acad. Sci. USA (in press).
19. Leigh Brown, A.J., and Ish-Horowicz, D. (1981) Nature 290, 677-682.
20. Ilyin, Y.V., Tshurikov, N.A., Ananiev, E.V., Ryshov, A.P., Yenikolopov, G.N., Limborska, S.A., Maleeva, N.E., Gvozdev, V.A., and Georgiev, G.P. (1977) Cold Spring Harbor Symp. Quant. Biol. 42, 959-969.
21. Potter, S.S., Brorein, W.J., Jr., Dunsmuir, P., and Rubin, G.M. (1978) Cell 17, 415-427.
22. Strobel, E., Dunsmuir, P., and Rubin, G.M. (1979) Cell 17, 429-439.
23. Young, M.W. (1979) Proc. Natl. Acad. Sci. USA 76, 6274-6278.