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Genetics

Heat shock locus 93D of *Drosophila melanogaster*: A spliced RNA most strongly conserved in the intron sequence

(*Drosophila hydei*/RNA processing/sequence homology)

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ABSTRACT **The *Drosophila melanogaster* heat shock locus at 93D encodes at least three overlapping transcripts, 10–12 kilobases (kb), 1.9 kb, and 1.2 kb. The abundance of the three transcripts is significantly increased during heat shock; however, all are also found in non-heat-shocked cells. The 1.2-kb transcript is found in the cytoplasm. Sequence analysis of a 1.1-kb cDNA clone representing sequences within the 1.2-kb transcript and comparison to genomic sequences indicate that it is spliced; 700 base pairs of sequence found in genomic DNA are removed from the middle of the transcript. Sequence analysis further suggests that this RNA does not encode a heat shock protein. The largest open reading frame beginning with a methionine codon would encode a polypeptide of 34 amino acids. We have not been able to detect a heat shock-induced polypeptide of this size. A DNA clone from the analogous heat shock puff of *Drosophila hydei* has been analyzed by hybridization with the small subclones used to sequence the *D. melanogaster* cDNA plus a genomic fragment containing the 700-base-pair intron. Results of this hybridization indicated strong homology of the intron fragment. Weaker homology was detected with the two small fragments flanking the intron. Other fragments of the *D. melanogaster* cDNA showed no hybridization to the cloned *D. hydei* puff DNA.**

Most of the major heat shock puffs of *Drosophila melanogaster* have been shown to code for the prominent heat shock proteins (hsps); however, one of the largest puffs, 93D, does not encode any of the known hsps. The 93D puff has a number of additional characteristics that distinguish it from the other heat shock puffs. Early experiments analyzing heat shock RNA by hybridization to polytene chromosomes indicated that, in contrast to the other puffs, a significant portion of RNA from 93D remains in the nucleus (1). Other *in situ* hybridization experiments showed cross-hybridization of sequences encoding hsps in *D. melanogaster* with heat shock puffs of *Drosophila hydei* but there was no hybridization to the large *D. hydei* heat shock puff 2-48B. Furthermore, total heat shock RNA from *D. hydei* did not cross-hybridize with puffs thought to be homologous to 2-48B in more closely related species (2). These experiments suggest that, if 2-48B of *D. hydei* is related to 93D of *D. melanogaster*, the sequence of this puff is evolving faster than are the sequences encoding the major hsps. Several other unusual features of 93D are shared by 2-48B. Each is the only puff in the genome that is also induced by benzamide and by colchicine (3, 4). During heat shock each of these puffs contains distinctive large ribonucleoprotein particles that share antigenic determinants not seen on other heat shock puffs (5). The unusual characteristics shared by these two puffs strongly suggest that the function of the locus is conserved in spite of the divergence at the nucleotide level.

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In this paper we report other features that distinguish 93D from previously studied heat shock puffs. There are multiple transcripts of parts of the 93D sequence. The most prominent cytoplasmic transcript has been sequenced and has only a few very short regions with the potential for encoding protein. This transcript is also spliced. The only other *Drosophila* heat shock transcript that has been found to be spliced is the transcript encoding hsp83 (6). We also detect a region of sequence homology between 93D and the *D. hydei* puff at 2-48B. This homology is predominantly in the sequence spliced out of the cytoplasmic transcript.

MATERIALS AND METHODS

RNA Extractions. *Drosophila* Schneider L-2 cells in logarithmic-phase growth ($3–6 \times 10^6$ cells per ml) were incubated for 90 min at the indicated temperature (25, 36, or 37°C). At the end of the incubation period the cells were centrifuged at $4000 \times g$, resuspended in phosphate-buffered saline (PBS), and centrifuged again. The cell pellet was then resuspended in an equal volume of PBS and the cells were lysed by the addition of 5 vol of guanidine hydrochloride to obtain total RNA. Cytoplasmic and nuclear fractions were prepared by the method of Fey *et al.* (7). RNA was isolated from extracts as described by Chirgwin *et al.* (8). Some aliquots of RNA were treated with RNase-free DNase prior to electrophoresis. Poly(A)$^+$ RNA was isolated as described by Lengyel *et al.* (1).

Hybridization Conditions. RNA for transfer blot hybridization was glyoxalated and separated on 1% agarose gels as described by Thomas (9). RNA was transferred to either nitrocellulose filters or nylon membranes as recommended by the manufacturers. Probes were nick-translated by the method of Rigby *et al.* (10) to specific activities of $0.5–2.0 \times 10^8$ cpm/μg. Hybridizations were performed in 4× concentrated SET buffer (1× SET: 0.15 M NaCl/0.03 M Tris·HCl/2 mM EDTA, pH 7.0), 10× concentrated Denhardt's solution (11), and 0.5% $NaDodSO_4$ at 60°C. Filters were washed at 60°C in 0.15 M NaCl/0.015 M sodium citrate, pH 7.0, and 0.5% $NaDodSO_4$. Filters were exposed to Kodak XAR-5 film at −70°C.

Sequencing. For sequencing, restriction fragments of adm129F5 were subcloned into the M13 vectors mp18 and mp19. Sequencing was performed by the dideoxy chain-termination method of Sanger *et al.* (12).

Sources. Nitrocellulose membranes were obtained from Schleicher & Schuell. Hybond-N nylon membranes and α-^{32}P-labeled nucleotides and ^{3}H-labeled amino acids were purchased from Amersham. Sequencing reagents and enzymes were obtained from New England Biolabs. RQ1 DNase (RNase free) was obtained from Promega Biotech (Madison, WI).

Abbreviations: bp, base pair(s); kb, kilobase(s); hsp, heat shock protein.

RESULTS

The 93D Heat Shock Locus Produces Multiple Overlapping Transcripts. The heat shock locus in the puff at 93D has been localized to bands 93D5-7 by *in situ* hybridization of *in vivo*-labeled heat shock RNA (13). Genetic analyses indicate that the heat shock locus lies in the short regions of overlap of the two deficiencies *Df(3R)e*^{Gp4} and *Df(3R)GC14* (14). No other loci have been detected in this region (15). A cDNA clone, padm129F5, derived from RNA of mass-isolated salivary glands (16), has been obtained, which hybridizes *in situ* to 93D5-7 but does not hybridize to chromosomes carrying either deficiency *Df(3R)e*^{Gp4} or *Df(3R)GC14*. The cloned cDNA has allowed isolation of genomic clones covering the heat shock locus and has identified several transcripts of this locus.

Heat shock causes a significant increase in the level of at least three transcripts that show homology to the cDNA clone padm129F5. Transcripts from embryos and from cultured cells have been analyzed (Fig. 1). In both cases the same pattern of transcripts is seen. All of the transcripts are increased by heat shock and thus are heat shock RNAs; however, the transcripts are also seen at much lower levels in non-heat-shocked cells. During embryogenesis the non-heat shock transcripts become more abundant in the later stages when transcription by embryonic nuclei is active.

The largest transcript detected by the cDNA clone is some 10–12 kb in length. It is not easy to isolate this RNA intact; however, it is relatively abundant in preparations in which no degradation has occurred. The other two abundant transcripts detected by the cDNA clone are 1.9 and 1.2 kb. Genomic Southern and *in situ* hybridizations using the cDNA as probe show that the cDNA clone is homologous to a segment of single-copy DNA at 93D (data not shown). This single-copy DNA is adjacent to a stretch of 280-base-pair (bp) repeats (Asu/Taq repeats) extending over approximately 10 kb. The 1.9- and the 1.2-kb transcripts originate upstream from the repeats and do not contain any of the Asu/Taq sequence.

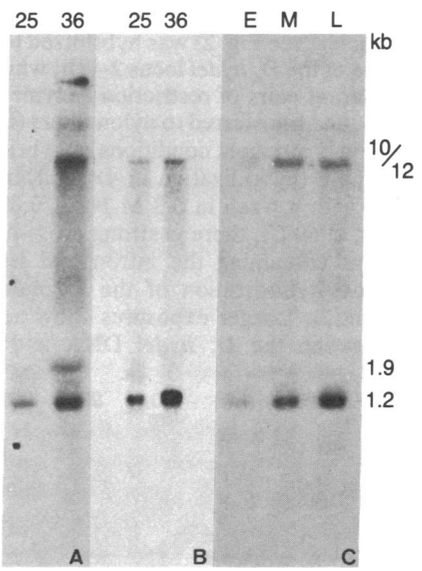

FIG. 1. Autoradiographs of control (25°C) and heat-shocked (36°C) RNA homologous to padm129F5. Three transcripts of 10–12, 1.9, and 1.2 kilobases (kb) are induced by heat shock but are also seen in non-heat-shocked cells. The constitutive level of 93D transcripts increases in later stages of embryogenesis. RNA was separated on 1% agarose gels, transferred to filters, and hybridized with ^{32}P-labeled padm129F5. Hybridizations were at 60°C in 4× concentrated SET buffer. Filters were washed in 0.15 M NaCl/0.015 M sodium citrate, pH 7.0, at 60°C. Within each panel, lanes represent equal numbers of cells. (*A*) RNA from mixed-stage embryos. (*B*) DNase-treated RNA from Schneider L-2 cells. (*C*) RNA from non-heat-shocked embryos: 0–6 hr (lane E), 6–12 hr (lane M), and 12–18 hr (lane L).

The 1.2-kb Transcript Is a Spliced Cytoplasmic RNA. The cDNA sequence cloned in padm129F5 is 1.1 kb in length, only slightly shorter than the major 1.2-kb transcript. Fragments of this cDNA clone have been subcloned into M13 and sequenced (Figs. 2 and 3). When RNA from heat-shocked cells was hybridized with the subfragments used to sequence the cDNA, each segment of the cDNA clone showed homology to the 1.2-kb and the 1.9-kb transcripts (data not shown). These results indicate that the cDNA clone provides an almost complete sequence of the 1.2-kb transcript and a large portion of the 1.9-kb RNA. Analysis of cloned genomic DNA indicated that 700 bp of sequence intervened between two portions of the sequence found in the cDNA. This 700 bp is removed from the RNA at nucleotides +401–402 bp (Fig. 2), leaving a consensus A·G splice junction (17). A subcloned fragment of genomic DNA containing the 700-bp segment hybridizes to the 1.9-kb 93D transcript but only weakly to the 1.2-kb RNA (Fig. 4*C*). Thus, the segment appears to be an intron accounting for the difference in size between the two transcripts. The sequence of the cDNA plus the 700 bp of genomic DNA in the intron should yield most of the sequence of the 1.9-kb RNA.

Most of the spliced RNAs that have been studied are exported to the cytoplasm. This is also true of the 1.2-kb 93D transcript. The 1.2-kb RNA is detected in cytoplasmic fractions of cells (Fig. 4*A*). The 1.9 kb is found only in the nuclear fractions of these cells. RNA from nuclear fractions also contains the 1.2-kb transcript. Like many other cytoplasmic RNAs, the 1.2-kb transcript is polyadenylylated. It is retained by oligo(dT)-cellulose, as is the 1.9-kb nuclear RNA (Fig. 4*B*). The cDNA sequence (Fig. 2) has a poly(A) consensus sequence and a terminal segment of 34 adenosine residues. The poly(A) consensus sequence begins 19 nucleotides upstream from the initial adenosine residue in the terminal segment, as is typical of eukaryotic poly(A) RNAs (18).

The 1.2-kb Cytoplasmic RNA Does Not Have a Large Open Reading Frame. The sequence of the cloned cDNA shows that the 1.2-kb transcript differs sharply from the other known heat shock RNAs in the amount of potential open reading frame. In the 1100 nucleotides of sequence, the largest open reading frame that begins with a methionine codon could encode a polypeptide of only 3900 daltons (34 amino acids). This open reading frame begins at nucleotide +672.

We have used gel systems that resolve polypeptides of <10,000 daltons to search for heat shock proteins predicted from the sequence of the 1.2-kb transcript. Since the accumulation of 93D RNA resembles that of the major hsp mRNAs (Fig. 5), we would expect the appearance of a translation product to parallel that of the major hsps. Because

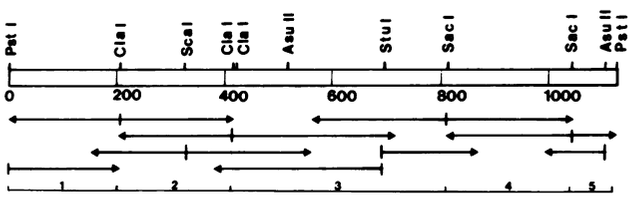

FIG. 2. Restriction map and sequencing strategy for padm129F5. Fragments obtained by digestion of the cDNA with the indicated restriction enzymes were subcloned into M13. The arrows indicate the direction and extent of sequencing. The numbers on the bottom line refer to the restriction fragments used for the hybridizations shown in Fig. 7.

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   1 CTGCAGGGGGGGGGGGGGTTTGCAAGCAGTAGCTACAACCAAAAATGGAAAAGTGTAAAAATCGTGTCCCAGCAGACGAG   80
  81 CAGCAGCAGTACGAGTATTGCAAAATGCAGGGGCAAGGGCCCACGTAGTATTTTTCCACGTCGGGCATTTAATGCTCTCG  160
 161 AGTTGGAAACAAAGAAACCATACGCAAACCCCCTGGAAAAGATGTGATTAGTCATCGATTTTGCTGGTCAGCGTCGGGGA  240
 241 ATTTCGCAATGCAGCAGGCAGTTTTCTCAAATTTGGCTAGAAAGTGACCCACTAGGCAGTCTGAGGCAGTTATCCAGGAT  320
 321 GTAAGGATGTGTGCAGTACTCTCTGTGTCGAATGGCGATGGCCTGACCTGCGGATTTTGAAAGATTGAAATAGGAAGCCA  400
 401 AGTTGGGCGTTGAAAGTTGATATCGATATCGATCCGTGAAAAGTCGATACCCTGCGCAAGCATGGGGCGGCATATGGGTG  480
 481 CTGAAAACGCACTCGGCCCGATCCCGATTGCAGCGTTATTCGAAAGCTGTGTCTGCGACCGTGACTGAGATCATATGCGT  560
 561 ACATATATCTAATGTCCGGGGTCGTGGGCCAGCCAGGGTGCTCGATTCTGTCAGATTGATTGTGCGGATTGTGTTATAGG  640
 641 AACACTGGTGTATCGACTTCTCTGCTCCACTATGGGTGAAGGATACCCTACCGAAAAGGCCTTCTGTCGCTTACTATCAT  720
 721 CGAACAAGTTCCGTAAAGGGCAGACATACGTACACGTGGCAGCATATTACGTTCAATGACACATCGTCTCTGGATTAGTA  800
 801 GTTGAACCAACGAGCTCGAATATACCCTGCCAGTTGGTTGTAAATATTAATTTTGTTCTTATATTCAGTTGTAAAGTCAA  880
 881 TACGGAAGACATTTCCACACCAACCATGTGTCACTTATGTTCTTAAATACCAGAAACTGTTTAAATTTTTGTTCTTATAC  960
 961 ACTTCACCAATTAAGAATTACCTGTACTGATGTAAGTGTACCTGTTGAATCACAACAAAAATAGAAAAAATGAATAACAA 1040
1041 AAGAGCTCTATAACAGAAAAGCCACAGAAAAAGTGAATAAAAAATTCAAAAATTCAAAAAATAAATTTCGAAACATTTAA 1120
1121 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAACCCCCCCCCCCCCCCTGCAG 1171
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FIG. 3. The sequence of the coding strand of padm129F5. The coding strand was determined by hybridization of single-stranded M13 probes to RNA bound to filters. The underlined A·G at position +401–402 represents the consensus splice junction. The boxed nucleotides are the consensus poly(A) addition signal.

the sequence indicates that each of the polypeptides could have only one methionine, cultured *Drosophila* cells were heat-shocked at 36°C for 30 min and labeled for an additional 30 min in the presence of ^{3}H-labeled (uniformly labeled) amino acids to provide labeling of polypeptides regardless of amino acid sequence. The labeling period produced substantial amounts of hsps but we did not detect heat shock induction of any small polypeptides, such as would be expected from the cDNA sequence (Fig. 6). Thus, it seems likely that the 1.2-kb cytoplasmic transcript does not encode a heat shock polypeptide, although other explanations are still possible (see *Discussion*).

The Sequence of the Heat Shock Locus at 93D Is Conserved Most Strongly in the Intron. The evolution of the sequences of the heat shock locus at 93D differs from that of the other major heat shock transcripts. Although RNAs encoding the major hsps cross-hybridize well, experiments using *in vivo*-labeled RNAs detected no cross-hybridization of heat shock RNA from *D. hydei* to polytene puff 93D of *D. melanogaster* (2). Our subcloned fragments of the cDNA and intron sequence gave the opportunity to look more closely at smaller parts of this region to see whether any sequence conservation was detectable since the phenotypes of the two puffs suggest strongly that they are analogous.

Each of the small fragments that had been subcloned into M13 for sequencing (see Fig. 2) was hybridized to DNA from a genomic clone of the *D. hydei* locus 2-48B, which had been cut by two different pairs of restriction enzymes, separated on agarose gels, and transferred to nylon filters (Fig. 7). Even under the relatively stringent conditions of hybridization and washing used here (hybridization in 4× concentrated SET buffer at 60°C with a wash in 0.3 M NaCl/0.03 M sodium citrate, pH 7.0, at 60°C), there is strong cross-hybridization of the fragment containing the intron and less, but still significant, cross-hybridization of the fragment 5′ to the intron (fragment 2). Longer exposures show additional hybridization between the *D. hydei* DNA and fragment 3

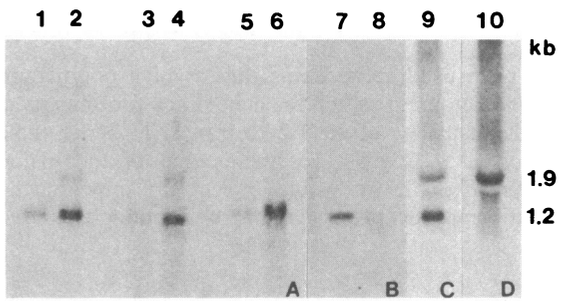

FIG. 4. Autoradiographs characterizing the 1.9- and 1.2-kb transcripts of 93D. The 1.9- and 1.2-kb transcripts are both found in the nucleus. The 1.2-kb transcript can also be found in the cytoplasm and is polyadenylylated. The 1.9-kb transcript contains a 700-bp intron that is not present in the 1.2-kb transcript. (*A*) Total (lanes 1 and 2), nuclear (lanes 3 and 4), or cytoplasmic (lanes 5 and 6) RNA fractions from control (lanes 1, 3, and 5) and heat-shocked (lanes 2, 4, and 6) Schneider L-2 cells hybridized with padm129F5. (*B*) Cytoplasmic poly(A)$^+$ (lane 7) and poly(A)$^-$ (lane 8) heat shock RNA hybridized with padm129F5. (*C*) Total embryo heat shock RNA probed with padm129F5 (lane 9) or an intron probe (lane 10). Only the region of the filters showing the 1.2- and 1.9-kb transcripts is presented.

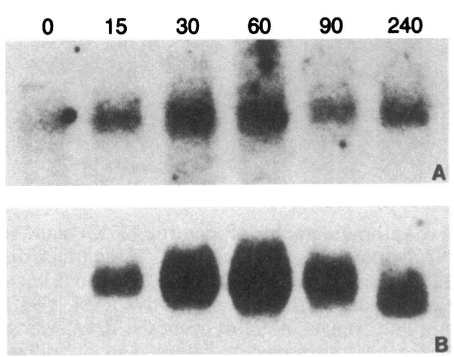

FIG. 5. Autoradiographs showing similar accumulation of the 1.2-kb transcript of 93D and the hsp22 mRNA during heat shock. Total RNA was purified from Schneider L-2 cells at 25°C (0) or after 15, 30, 60, 90, or 240 min of heat shock. RNA was separated on 1% agarose gels, transferred to filters, and hybridized with padm129F5 (*A*) or a probe for hsp22 (*B*).

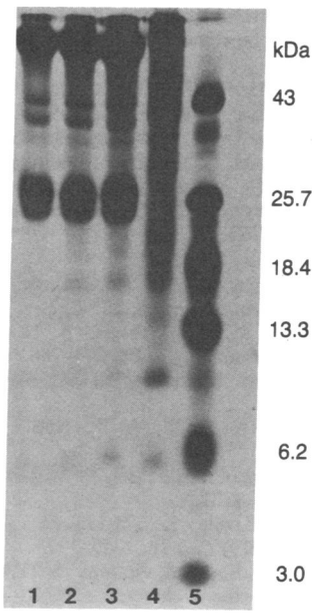

FIG. 6. Autoradiograph of low molecular mass polypeptides synthesized at 25°C or between 30 and 60 min of heat shock separated on $NaDodSO_4$/urea polyacrylamide gel (19). Polypeptides of the size predicted from the sequence of the cDNA do not show accumulation, although large amounts of the major hsps (e.g., the small hsps) are seen. Control cells (lane 4) were incubated in the presence of ^3H-labeled (uniformly labeled) amino acids for 30 min. Experimental cells (lanes 1–3) were heat-shocked for 30 min prior to labeling for an additional 30 min. Low molecular mass protein standards labeled with ^{14}C are shown in lane 5.

immediately 3′ to the intron. No hybridization of other fragments is detected. Lowering the stringency to hybridization in 4× concentrated SET buffer at 56°C with washes in 0.3 M NaCl/0.03 M sodium citrate, pH 7.0, at 56°C does not significantly increase the homology detected.

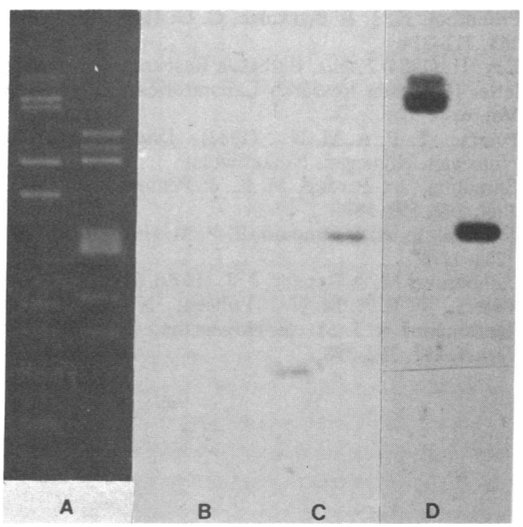

FIG. 7. Autoradiographs showing strong cross-hybridization of 93D heat shock intron sequence with *D. hydei* 2-48B sequences. Weaker cross-homology is seen with fragments 5′ and 3′ to the intron. Other regions of the cDNA show no cross-homology between the two species. *D. hydei* cosmid clone cDh171 (24) was digested with *Eco*RI and *Hin*dIII or with *Eco*RI and *Xba* I and then separated on a 0.7% agarose gel (*A* shows ethidium-stained sample of gel lanes). Restriction fragments were transferred to membranes and hybridized with ^{32}P-labeled M13 probes of the indicated fragments (see Fig. 2). (*B*) Fragment 1. (*C*) Fragment 2. (*D*) Intron sequence. Hybridizations were performed at 60°C in 4× concentrated SET buffer. Filters were washed at 60°C in 0.3 M NaCl/0.03 M sodium citrate, pH 7.0.

DISCUSSION

In spite of the early failure to show sequence homology between 93D and 2-48B of *D. hydei*, the two puffs have several characteristics (see Introduction) that suggest that they are equivalent and possibly share a function that can be maintained in spite of greater sequence divergence than occurs in the genes encoding the major heat shock polypeptides. The results of our study support the view that 93D and 2-48B are equivalent and add the surprising observation that the most conserved sequences are in the intron. The weaker interspecific homology flanking the intron may be a by-product of conservation within the intron or may reflect conservation of elements necessary to the function of the locus.

It is interesting to note that 93D and 2-48B have very long transcripts of 10–12 kb. In the *D. hydei* RNA studies, the 2-48B transcript was identified by a cloned member of a stretch of 115-bp DNA repeats (20), which appear to be analogous to the 280-bp Asu/Taq repeats at 93D. However, *D. hydei* transcripts analogous to the 1.9- and the 1.2-kb transcripts of 93D have not been reported. In *D. melanogaster* the shorter transcripts do not extend into the Asu/Taq repeats and would not be detected if those repeats were used as a probe. The analogy suggests that the *D. hydei* transcript, like the *D. melanogaster* transcript, will have a unique sequence related to our cDNA clone followed by repeat sequences. The region of the *D. hydei* DNA that shows homology to the *D. melanogaster* intron sequence is located just upstream to the segment of 115-bp repeats. This is the site that would be predicted from the studies on *D. melanogaster* and encourages us to think that a probe from this region of 2-48B will detect transcripts analogous to the 1.9- and the 1.2-kb RNAs. We now have preliminary evidence that there are smaller *D. hydei* heat shock RNAs that hybridize with the 2-48B sequence cloned in the cosmid cDh171. Subcloning the regions with homology to the 93D cDNA should give a probe that will allow us to detect these RNAs more specifically.

The picture that is emerging from the studies of the cloned sequences from the *D. melanogaster* heat shock 93D fits well with results that have been obtained by other means. Hybridization with the cloned sequence has identified a large transcript of 10–12 kb that compares well with the estimate of 9.6 kb obtained from saturation analysis of *in situ* hybridization of heat shock RNA to polytene chromosomes (1). The *in situ* hybridizations also found that a large portion of the 93D transcript was in the nucleus while most heat shock mRNAs moved rapidly to the cytoplasm. The nuclear RNA detected by the cloned cDNA is relatively abundant. There is one discrepancy between data obtained with the cDNA clone and previous work. The early studies found that much of the cytoplasmic RNA (about 75%) transcribed during heat shock that hybridized to the polytene puff at 93D was not retained on oligo(dT) columns and by that test lacked poly(A). The sequence of the cDNA clone shows a 34-bp oligo(A) sequence at the 3′ end and a consensus poly(A) addition sequence 13 nucleotides upstream from the poly(A). Thus, the cDNA sequence strongly suggests that the cytoplasmic RNA is polyadenylylated and oligo(dT) binding confirms this for both the 1.2- and the 1.9-kb transcripts. The sequence does not give evidence on the length of the poly(A) tail since the protocol for cloning cDNA might yield either a shorter or a longer stretch of adenosine residues than existed on the RNA. Earlier studies analyzing the length of poly(A) segments on heat shock RNAs from *Drosophila* showed that, though most of the segments were of the 100- to 200-nucleotide length typically associated with mRNAs, there

was a second peak of much shorter poly(A) segments (21). If these shorter segments are associated with the 93D transcripts, the RNA might have failed to bind to oligo(dT) under the conditions of the earlier experiments.

It is noteworthy that the major 1.2-kb RNA is derived from a transcript in which a 700-nucleotide intron is removed by splicing. Most heat shock RNAs do not appear to undergo this form of processing. The mRNA for hsp83 is the only other *Drosophila* mRNA known to be spliced, although other intron-containing heat shock genes have been identified recently in *Caenorhabditis elegans* (22). It was also surprising to find that the intron sequence is the most strongly conserved portion of the 93D heat shock locus. The conservation suggests that the intron may have a function, either alone or as part of larger nuclear molecules. Although it is tempting to consider the larger nuclear RNAs simply as precursors for the cytoplasmic RNA, the relative abundance of the nuclear RNA and the conservation of the intron lead us to reserve judgment on this issue.

Although the puff at 93D is usually as large or larger than the puffs at 87A and C, which encode the predominant hsp70, no one has identified a hsp encoded at 93D. Genes for all of the major *Drosophila* hsps have been identified and mapped to other heat shock puffs (23). Our sequence analysis of the major cytoplasmic transcript from 93D suggests that it may not function as a mRNA. The transcript is 1200 nucleotides in length, but the longest open reading frame beginning with a methionine codon could only encode 34 amino acids.

It seems likely that the presence of the potential coding regions in the transcript is not significant since we have failed to detect the polypeptide predicted from this reading frame. Our experiments thus far have analyzed heat shock polypeptides made during the first 60 min of heat shock. This is a time when there is significant accumulation of the major heat shock polypeptides. It is also a time when the 1.2-kb 93D transcript is abundant in the cytoplasm and thus we would expect to find polypeptides if they are made. There are still two possible explanations for the failure to find 93D polypeptides. First, the polypeptides may turn over very rapidly and not accumulate to levels that we can detect. Second, the translation may take place at a different point in the heat shock response. At this point we can conclude that, if the 93D transcript is translated at all, it differs significantly from the other heat shock RNAs in either its translational control or in the stability of the polypeptide.

These results have not yet told us what is the function of 93D but they do begin to define the transcripts as a first step toward determining the product of the gene. In addition, the comparisons with *D. hydei* begin to give insight into the evolutionary constraints necessary to maintain the function of this locus.

Note Added in Proof. The adenosine residue indicated at position 400 is an error and should be removed.

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