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Multiple, Compensatory Regulatory Elements Specify Spermatocyte-Specific Expression of the *Drosophila melanogaster hsp26* Gene

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The *hsp26* gene of *Drosophila melanogaster* is expressed in six tissues during development and in a tissue-general response to heat shock. To be able to compare tissue-specific and heat-induced mechanisms of *hsp26* expression, we have begun an analysis of the sequences involved in the spermatocyte-specific expression of the *hsp26* gene by using germ line transformation. *hsp26* mRNA synthesized in the spermatocytes has the same start site as sites previously demonstrated for nurse cell-specific and heat-induced mRNAs. Three regions of the *hsp26* gene (nucleotides −351 to −135, −135 to −85, and +11 to +632) were able to stimulate spermatocyte-specific expression when fused with promoter sequences (nucleotides −85 to +11) that alone were insufficient to stimulate expression. These stimulatory regions appear to contain elements that provide redundant functions. While each region was able to stimulate expression independently, the deletion of any one region from a construct was without consequence as long as another compensatory region(s) was still present. There must reside, at a minimum, two independent spermatocyte-specifying elements within the sequences that encompass the three stimulatory regions and the promoter. At least one element is contained within sequences from −351 to −48. This region, in either orientation, can stimulate spermatocyte-specific expression from a heterologous promoter. A second element must reside in sequences from −52 to +632, since these sequences are also sufficient to direct spermatocyte-specific expression.

Previously, we characterized the spatial and temporal pattern of *Drosophila melanogaster hsp26* expression during development (12). *hsp26* is expressed in four somatic cell types (imaginal disks, epithelium, proventriculus, and neurocytes) and in two germ line cell types (spermatocytes and oogenic nurse cells) (12, 18, 44). In addition to being expressed in both somatic and germ line cell types, *hsp26* is expressed tissue-generally in response to heat hock (12). The opportunity to compare and contrast the mechanisms by which *hsp26* expression is stimulated in these different tissues makes it a particularly good model for the study of transcriptional regulation. In addition, the mechanism by which heat shock genes are induced to high levels of transcription has been extensively studied and is understood in considerable detail (for reviews, see reference 6 and J. T. Lis, H. Xiao, and O. Perisic, *in* R. Morimoto, A. Tissieres, and C. Georgopoulos, ed., *Stress Proteins in Biology and Medicine*, in press), providing a useful reference point with which the analysis of alternative models of regulation can be compared.

The study reported here focuses on the characterization of sequences involved in the spermatocyte-specific expression of the *hsp26* gene. Sperm development in the testis resembles a linear assembly line, with stem cells at the distal end and mature sperm at the proximal end (24). Mitotic divisions in the distal third of the testis generate 16-cell spermatocyte cysts. *hsp26* mRNA is found in these spermatocytes, and β-galactosidase activity from *hsp26-lacZ* fusion genes localizes to spermatocytes as well (12). In this study, we analyzed the spermatocyte-specific expression of 18 variant *hsp26* constructs in a total of 107 transgenic animals. The results indicate that spermatocyte-specific expression of *hsp26* is mediated by multiple elements with redundant functions.

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MATERIALS AND METHODS

S1 mapping. The 5′ endpoints of spermatocyte-specific and tissue-general heat-induced *hsp26* transcripts were determined by standard nuclease S1 mapping procedures (3). A uniformly labeled single-stranded DNA probe containing *hsp26* sequences from the *Xba*I site at nucleotide −53 to the *Bal*I site at nucleotide +393 was generated by primer extension synthesis from an M13 subclone containing the *Xba*I-*Bal*I fragment, mp26XB′, following the protocol of Akam (1) as modified by Glaser et al. (12), except that ^{32}P-labeled nucleotides were used and the probe was isolated by denaturing polyacrylamide gel electrophoresis. Five micrograms of total RNA extracted from the appropriate tissue by using guanidinium isothiocyanate was hybridized with the probe for 18 h at 52°C in 80% formamide–0.4 M NaCl–40 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), pH 6.4]–1 mM EDTA. After treatment of the RNA:DNA hybrids with empirically determined amounts of nuclease S1, protected DNA fragments were separated on an 8 M urea–4% polyacrylamide gel that was then subjected to autoradiography. The total probe length was 506 nucleotides, and the species protected from S1 digestion were 393 nucleotides long.

DNA constructs. The numerical assignment of nucleotides is based on the *hsp26* sequence and transcription start site, as determined by Ingolia and Craig (17). Constructs considered wild type with respect to spermatocyte-specific expression contained upstream sequences to an *Xba*I site located at position −351. An *Xba*I-*Sal*I DNA fragment containing an *hsp26-lacZ* fusion gene with 351 base pairs of upstream sequence was subcloned from pMC1871.26 (12) into vector pΔZX (43), making pX^{u}S26Z. An *Xho*I-*Sal*I fragment from pX^{u}S26Z was then subcloned into the transformation vector Car20T (called c70T1 in reference 43), forming the transformation plasmid cP-351. The 5′ junction sequences are GTC GAGGGGGGATCCTCTAGA.

Transformation plasmids cP-272, cP-229, cP-191, cP-155,

cP-135, cP-114, cP-85, and cP-52 are all deletion derivatives of pX^{u}S26Z inserted into Car20T. The sequences 5′ of the deletion endpoints are GGATCCTCTAGGCGGCCGC NNNNN and include a *Not*I linker immediately adjacent to the deletion endpoint. A detailed description of the construction of these plasmids is reported elsewhere (R. L. Glaser, G. H. Thomas, E. Siegfried, S. C. R. Elgin, and J. T. Lis, J. Mol. Biol., in press).

Transformation plasmid cPΔCT · GA is cP-351 into which the Δ41.1 deletion of Siegfried et al. (34) was placed by first removing the wild-type *Xba*I fragment from nucleotides −351 to −48 and replacing it with an *Xba*I fragment containing the internal deletion. This internal deletion removes sequences from −134 to −85 and inserts the sequence CCTCGAG.

The transformation plasmids illustrated in Fig. 2C were derived from the deletion mutants illustrated in Fig. 2A. *hsp26* sequences downstream of the *Eco*RI site at position +11 were replaced with *hsp70* sequences. The *hsp70* sequences start at the *Pst*I site at position +89 of *hsp70*. The *hsp70* sequences are then fused at +260 in frame to *lacZ*, as described by Xiao and Lis (43). The sequences at the *hsp26-hsp70* fusion point are 5′-TCGAATTCAGCTTGGGC TGCAGTAA-3′ and include linker sequences (the *hsp26* *Eco*RI site to the left and the *hsp70* *Pst*I site to the right are underlined and are separated by linker sequence).

The transformation plasmids illustrated in Fig. 3A were constructed as follows. The *Xba*I fragment of *hsp26* was isolated, and its staggered ends were filled in by using the Klenow fragment of DNA polymerase. This fragment, containing *hsp26* sequences from nucleotides −351 to −48, was inserted into the *Nru*I site at position −50 of the *hsp70-lacZ* gene in plasmid pXM70Z (43). The *hsp70-lacZ* genes containing the *Xba*I inserts of *hsp26* sequence as illustrated were then subcloned into Car20T. For cPΔCT, the *Xba*I fragment subcloned into the *hsp70* *Nru*I site contained deletion Δ41.1 described above.

DNA sequences were compared by using the DNA Inspector IIe (TEXTCO) programs.

Germ line transformation and β-galactosidase assays. The *hsp26-lacZ* constructs were introduced into *D. melanogaster* by P-element-mediated germ line transformation (32), as described previously (35). Genomic Southern blot analyses (37) were done on all 107 transformant lines to determine the copy number of the construct inserted in each line. We found that 100 of the lines had single inserts, 6 of the lines had two inserts, and 1 line had three inserts. Only transformant lines containing autosomal inserts were used for analysis. Individual transformant lines were maintained as nonhomozygous inbred stocks.

The CPRG (chlorophenol red β-D-galactopyranoside) assays to determine levels of β-galactosidase activity were modified from methods of Simon and Lis (35). Individual males from lines to be analyzed were outcrossed to the *Adh*^{fn6}*cn*; *ry*^{502} injection stock to generate *ry*$^{+}$ progeny heterozygous for the P-element insertion. Duplicate sets of five pairs of testes were dissected from a total of 10 *ry*$^{+}$ male progeny from each outcrossed line and then homogenized in 350 μl of 50 mM K_2PO_4 (pH 8.15)–1 mM $MgCl_2$–0.25 mM phenylmethylsulfonyl fluoride. After a 2-min centrifugation at 4°C in an Eppendorf Microfuge, the supernatant was added to an equal volume of 10 mM CPRG in homogenization buffer. After incubation at 37°C for 18 h (during which time the assay was linear), the A_{574} was determined. The values for the duplicate samples were averaged, and this number was adjusted for copy number when necessary. Values are expressed as percentages of the mean level of activity observed for the appropriate control construct. Standard errors of the mean (SEM = $\sigma/\sqrt{n}$ were calculated.

Changes in the level of β-galactosidase observed for the first 5 days after eclosion agreed well with changes in the number of spermatocyte cysts. β-Galactosidase activity fell by 24% during the first 3 days after eclosion but fell only an additional 1% from 3 to 5 days posteclosion (data not shown). The number of spermatocyte cysts decreases approximately 16% during the first 3 days after eclosion, but the number drops only approximately 1% from 3 to 5 days posteclosion (10). Testes were dissected from males 3 to 5 days old to minimize variability due to the rapid change in spermatocyte number that occurs shortly after eclosion.

Qualitative X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) assays were performed as described previously (12), except that the testes were dissected directly into the assay buffer without a glutaraldehyde preincubation.

RESULTS

Spermatocyte-specific and heat-induced expression utilize the same transcription start site. One mechanism by which some *Drosophila* genes mediate their expression in different tissues is to utilize alternative promoters (5, 27, 33), resulting in tissue-specific mRNAs with different, tissue-specific transcription initiation sites. To determine whether this was the case for *hsp26*, we compared the initiation site of *hsp26* transcripts isolated from spermatocytes with that of transcripts generated in the tissue-general heat shock response by using nuclease S1 mapping techniques. Spermatocyte-specific RNA had the same 5′ endpoint, within a resolution of 5 base pairs, as tissue-general heat-induced transcripts (Fig. 1), indicating that elements that regulate spermatocyte and heat shock expression act on the same promoter. Cohen and Meselson (8) have demonstrated that the nurse cell-specific expression of *hsp26* also utilizes this same start site.

Multiple regions of *hsp26* can mediate spermatocyte expression. To simplify the discussion of the *hsp26* mutants presented in this report, we will refer to regions of the *hsp26* gene as illustrated for construct cP-351 (Fig. 2A). The numerical assignment of nucleotides in the sequence is based on the transcription start site as determined by Ingolia and Craig (17). Region P, nucleotides −85 to +11, contains the promoter. Region D contains the downstream transcribed sequences from +11 to +632. Region A contains sequences from −351 to −135, and sequences from −135 to −85 are designated the CT · GA region because they contain multiple arrays of poly(dC-dT) · poly(dG-dA) (34).

Mutations in each of these regions were generated in chimeric genes marked by fusing *hsp26* sequences, and in some constructs *hsp70* sequences, to the *Escherichia coli* *lacZ* gene. Constructs were reintroduced into the germ line by P-element-mediated transformation. Spermatocyte-specific expression was quantitated by measuring levels of β-galactosidase activity by using the colorimetric substrate CPRG. Qualitative localization of expression was determined by incubating testes in buffer containing X-Gal (see Materials and Methods). The *Drosophila* *hsp70* gene is not expressed in the spermatocytes (7, 12), and sequences from *hsp70* were used in constructs illustrated in Fig. 2C and Fig. 3A. Finally, constructs considered wild type with respect to spermatocyte-specific expression contained sequences 351 base pairs upstream of the transcription start site (12).

The promoter alone (region P) was not able to drive detectable levels of spermatocyte-specific expression (Fig.

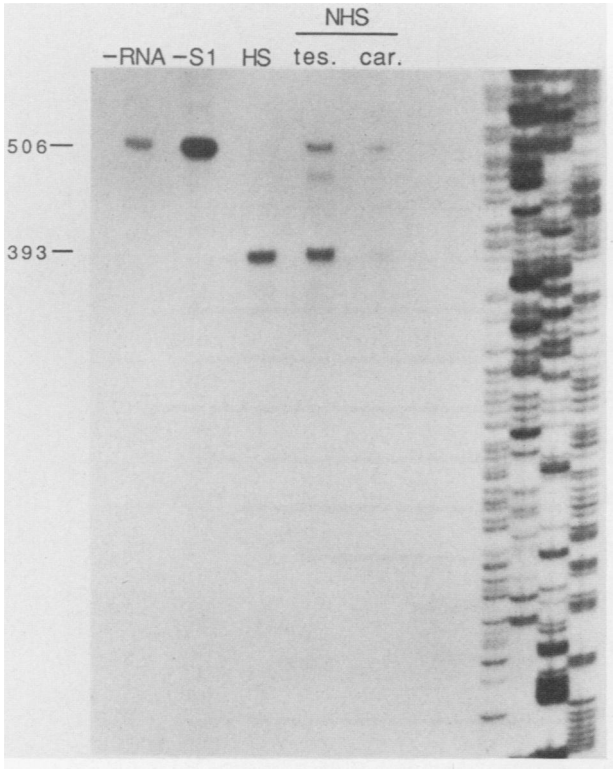
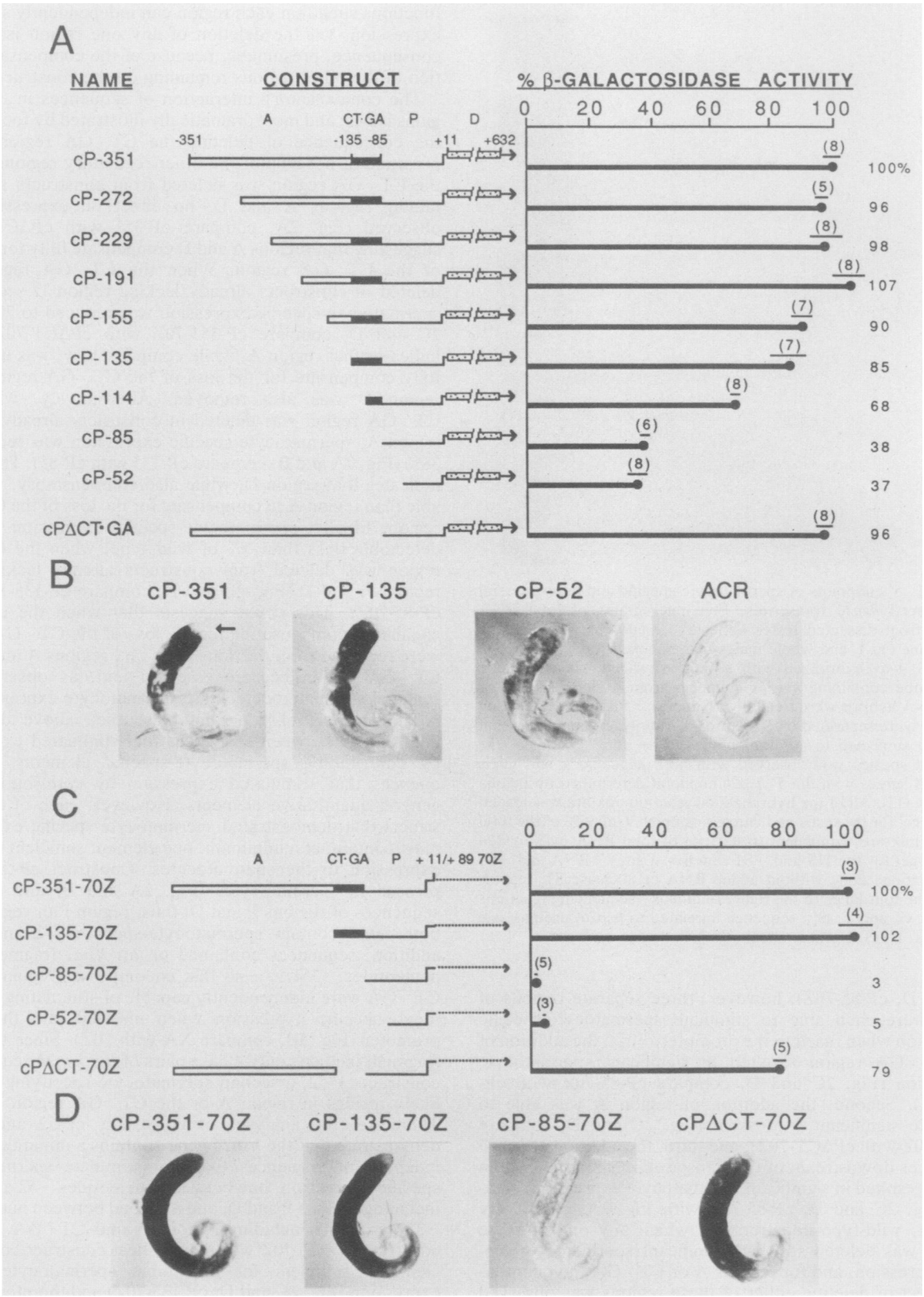


FIG. 1. 5′ endpoints of spermatocyte-specific and tissue-general heat-induced *hsp26* transcripts. Five micrograms of total RNA isolated from dissected testes (lane tes.), male carcasses lacking testes (lane car.), and whole males heat shocked for 1 h at 36.5°C (lane HS) was hybridized with a single-stranded, 506-nucleotide DNA probe containing *hsp26* sequences from −53 to +393. The RNA:DNA hybrids were treated with nuclease S1, and the resulting products were separated on a 4% urea–8 M polyacrylamide gel that was then subjected to autoradiography. The same 393-nucleotide protected species was observed with both heat shock and testis RNA and agrees with the 5′ *hsp26* endpoint determined by Ingolia and Craig (17). All of the hybridization reaction mixture was loaded onto the gel for the testis and carcass samples. Only 5% of the total reaction mixture, equivalent to 0.25 μg of total RNA, was loaded onto the gel for the HS and −S1 samples. Lanes −RNA and −S1 were reactions done without added RNA or nuclease S1, respectively. The four lanes to the right are dideoxy sequencing reactions for A, C, G, and T, of a sequence unrelated to *hsp26*, used as size standards. NHS, No heat shock.

2C and D, cP-85-70Z); however, three separate regions of *hsp26* were each able to stimulate spermatocyte-specific expression when fused to the promoter. First, the addition of the CT · GA region resulted in significant spermatocyte expression (Fig. 2C and D, compare cP-85-70Z with cP-135-70Z). Second, the addition of region A was able to stimulate significant expression (Fig. 2C and D, compare cP-85-70Z with cPΔCT-70Z); and third, the addition of *hsp26* sequences downstream of the transcription start site, region D, also resulted in significant spermatocyte expression (compare Fig. 2C and D, cP-85-70Z with Fig. 2A, cP-85). By contrast, wild-type constructs in which any one of these regions was deleted still had significant spermatocyte-specific expression, and for regions A or CT · GA, no quantitative effect of deleting either of these regions was observed (region A deleted, compare cP-351 with cP-135 in Fig. 2A and B; CT · GA region deleted, compare cP-351 with cPΔCT · GA in Fig. 2A; region D deleted, compare cP-351 in Fig. 2A and B with cP-351-70Z in Fig. 2C and D). Apparently, sequences in each of these regions provide redundant functions such that each region can independently stimulate expression, yet the deletion of any one region is without consequence, presumably because of the compensatory action of the other regions remaining on the construct.

The compensatory interaction of sequences in these regions is best and most dramatically illustrated by focusing on the consequence of deleting the CT · GA region in the presence or absence of the other regulatory regions. When the CT · GA region was deleted from constructs still containing regions A and D, no effect on expression was observed (Fig. 2A, compare cP-351 with cPΔCT · GA), suggesting that regions A and D compensate fully for the loss of the CT · GA region. When the CT · GA region was deleted in constructs already lacking region D sequences, spermatocyte-specific expression was reduced to 79% (Fig. 2C and D, compare cP-351-70Z with cPΔCT-70Z). This indicates that region A, while compensatory, was unable to fully compensate for the loss of the CT · GA region when region D was also removed. Alternatively, when the CT · GA region was deleted in constructs already lacking region A, spermatocyte-specific expression was reduced to 38% (Fig. 2A and B, compare cP-135 with cP-85). This result indicates that region D, while also compensatory, was less able than region A to compensate for the loss of the CT · GA region. Finally, spermatocyte-specific expression was not detectable (less than 3% of wild type) when the CT · GA region was deleted from constructs already lacking both regions A and D (Fig. 2C and D, compare cP-135-70Z with cP-85-70Z). This result suggests that when the elements capable of compensating for the loss of the CT · GA region were removed from the construct, i.e., regions A and D, the CT · GA region became essential and, as observed, its removal eliminated detectable spermatocyte expression.

The analysis of constructs discussed above does not differentiate between sequences that stimulated expression by contributing spermatocyte-specific elements and sequences that stimulated expression by contributing only general quantitative elements. However, each of the constructs that demonstrated spermatocyte-specific expression must contain, at a minimum, one element sufficient to direct expression to the spermatocytes. Construct cP-52 is expressed in spermatocytes (Fig. 2A and B) and contains sequences of regions P and D; thus, region P or region D or both must contain spermatocyte-specifying elements. In addition, sequences contained on an *Xba*I fragment from nucleotides −351 to −48 that encompasses regions A and CT · GA were independently capable of stimulating spermatocyte-specific expression when inserted into the *hsp70* promoter (Fig. 3B, compare XA with 70Z). Since the *Xba*I fragment includes only 4 base pairs of the *hsp26* sequence in construct cP-52, a second spermatocyte-specifying element likely resides in region A or the CT · GA region or both. Therefore, the analyses of constructs cP-52 and cPXA demonstrate that the *hsp26* gene contains a minimum of two independent sequences that can stimulate spermatocyte-specific expression, one between nucleotides −52 and +632 including regions P and D, and a second between nucleotides −351 and −48 including regions A and CT · GA. Finally, construct cP-135-70Z was the smallest construct containing elements sufficient for detectable spermatocyte-specific expression (Fig. 2C and D, cP-135-70Z) and therefore represents the shortest interval of *hsp26* sequence (nucleotides −135 to +11) that must contain at least one of these spermatocyte-specifying elements.


A
NAME
CONSTRUCT
% β-GALACTOSIDASE ACTIVITY
A
CT·GA
P
D
-351
-135 -85
+11
+632
0 20 40 60 80 100
cP-351
cP-272
cP-229
cP-191
cP-155
cP-135
cP-114
cP-85
cP-52
cPΔCT·GA
(8)
(5)
(8)
(8)
(7)
(7)
(8)
(6)
(8)
(8)
100%
96
98
107
90
85
68
38
37
96
B
cP-351
cP-135
cP-52
ACR
C
A
CT·GA
P
+11/+ 89 70Z
0 20 40 60 80 100
cP-351-70Z
cP-135-70Z
cP-85-70Z
cP-52-70Z
cPΔCT-70Z
(3)
(4)
(5)
(3)
(5)
100%
102
3
5
79
D
cP-351-70Z
cP-135-70Z
cP-85-70Z
cPΔCT-70Z

The *Xba*I fragment encompassing nucleotides −351 to −48 was also able to stimulate expression of an *hsp70-lacZ* gene when inserted in an inverted orientation, albeit at a reduced level (Fig. 3A and B, compare cPXA with cPXB). This ability suggests that elements within this region have characteristics of a spermatocyte-specific enhancer. The *Xba*I fragment from which the CT · GA region was deleted was still able to stimulate spermatocyte-specific expression, although at a lower level (Fig. 3A and B, compare cPXA with cPXΔCT). This result is consistent with the compensatory functions of regions A and CT · GA, both of which are contained within the *Xba*I fragment (see above), and also implicates region A as containing sequences sufficient for spermatocyte expression. A dimer of the *Xba*I fragment drove expression only slightly better than a single copy (data not shown).

DISCUSSION

We analyzed sequences involved in the spermatocyte-specific expression of the *Drosophila hsp26* gene by using germ line transformation. Three different regions of the gene were able to stimulate spermatocyte-specific expression when fused with promoter sequences that alone were insufficient to stimulate expression. Two of the regions are located upstream of the promoter, while the third is located downstream of the transcription start site in transcribed sequences. These three regions were able to compensate for each other and likely contain elements that provide redundant functions. Within the sequences that encompass the promoter and the three stimulatory regions reside, at a minimum, two independent elements capable of directing expression to spermatocytes.

Several other *Drosophila* genes have been isolated that are expressed in spermatocytes: *mst(3)gl-9* (21), the β2 tubulin gene (26), and the α2 tubulin gene (42). Sequences sufficient to mediate spermatocyte-specific expression of the β2 tubulin gene and *mst(3)gl-9* have been identified by germ line transformation, and for the β2 tubulin gene, a 14-base-pair element was implicated as playing an essential role in spermatocyte expression, although no matches to it are found in *hsp26* (26). We compared the sequences of *hsp26*, the β2 tubulin gene, and *mst(3)gl-9*, including in the search the upstream and intragenic untranslated sequences demonstrated by germ line transformation to be sufficient to mediate expression of each gene in spermatocytes. The only homologies common to all three genes that might represent candidates for spermatocyte-specifying elements were dTTP- or dATP-rich regions, in particular the sequence AAAAAAAGAAA or its complement. In addition, searching for homologies within the *hsp26* gene between the regions that could independently stimulate spermatocyte-specific expression (nucleotides −351 to −48, compared with nucleotides −52 to +632) revealed an eight-base homology, TCAAACGA, found starting at −158 and also at +62. However, no matches better than five of these eight bases were found in the β2 tubulin gene or *mst(3)gl-9*. A more precise description of sequences required for the spermatocyte-specific expression of the *Drosophila hsp26* gene and eventually identification of individual regulatory elements will require the analysis of constructs specifically designed to focus on a single regulatory region freed from the complications introduced by the presence of compensatory elements.

The complexity of regulatory components involved in the expression of *hsp26* in spermatocytes has similarity to the regulatory structure of another developmentally regulated *Drosophila* gene. Jongens et al. (19) characterized sequence elements involved in the salivary gland-specific expression of the *Drosophila Sgs-4* gene. Within a 294-base-pair region upstream of the transcription start, they identified three separate elements that are functionally redundant. Any two of the three elements were sufficient for salivary gland-specific expression, so that deletion of any one of the regions was without consequence because of the compensatory action of the remaining two regions. Redundancy of elements involved in tissue-specific gene expression has also been demonstrated for the yellow gene of *Drosophila melanogaster* (11) and in genes from other organisms (13, 39).

The involvement of intragenic sequences in the transcriptional regulation of RNA polymerase II-transcribed genes has been reported for numerous genes (2, 30, 31, 36, 41), including *hsp22* of *D. melanogaster* (16). Apparently, the transcribed sequences in region D of *hsp26* can stimulate spermatocyte-specific expression (compare cP-85-70Z and cP-52-70Z in Fig. 2C and D with cP-85 and cP-52 in Fig. 2A and B). This stimulation is likely due to the presence in

FIG. 2. Spermatocyte-specific expression from *hsp26-lacZ* constructs in transgenic flies. Germ line transformants containing *hsp26-lacZ* genes with the various mutations illustrated were analyzed for levels of spermatocyte-specific β-galactosidase activity. Regions A, CT · GA, P, and D of the *hsp26* gene and the nucleotide positions that delineate each region are shown. The transcription start point at +1 is where the line bends up. In panel A, *hsp26* at +632 is fused in frame to *lacZ*. In panel C, *hsp26* at +11 is fused to *hsp70* at +89, which in turn is fused at +260 in frame to *lacZ*. (A and C) Relative levels of spermatocyte-specific β-galactosidase activity observed in transgenic animals containing the construct illustrated on the left. β-Galactosidase activity was measured by using the colorimetric substrate CPRG. Activity is expressed as a percentage of the mean level of the control construct that is shown at the top of each series. The standard error of the mean for each determination is indicated as an error bar above the line, with the number of independent transformant lines used for each determination shown in parentheses. Actual mean percentages for the constructs are listed at the right. β-Galactosidase activity from the injection stock $Adh^{fn6}cn$; ry^{502} was not subtracted and was at 2 to 3% of wild type; thus, the level of β-galactosidase activity observed for cP-85-70Z in panel C was not significantly above background. (B and D) Localization of β-galactosidase activity within the testes of selected lines transformed with the indicated constructs. Testes from males heterozygous for the appropriate P-element construct were incubated in a buffer containing X-Gal. The time of incubation at 25°C was 6 (B) or 2 (D) h. Spermatocyte cysts are located in the distal third of the testis. For orientation, the distal end of the first testis in panel B is marked with an arrow. The location of β-galactosidase activity in lines transformed with constructs we originally analyzed (12) and those shown in panel A was confined to the spermatocyte cysts (B), consistent with the localization of endogenous *hsp26* message. When sequences downstream of +11 were replaced with sequences from the *hsp70* gene, however, β-galactosidase activity was no longer restricted to spermatocytes and was also detected in postmeiotic stages of spermatogenesis (D). While we do not know the source of this added expression, perhaps the removal of *hsp26* sequences leads to the increased stability of either the new fusion mRNA or the new fusion protein. No staining was observed in the proximal half of testes from animals transformed with constructs shown in panel A. The darkened areas in the proximal regions of the testes shown in panel B are a consequence of dense sperm bundles that appear as dark areas in black and white photographs. X-Gal assays on testes from animals transformed with constructs cP-85 and cPΔCT · GA gave results identical to those for constructs cP-52 and cP-351 (B), respectively. The length of an uncoiled testis is approximately 2 mm.

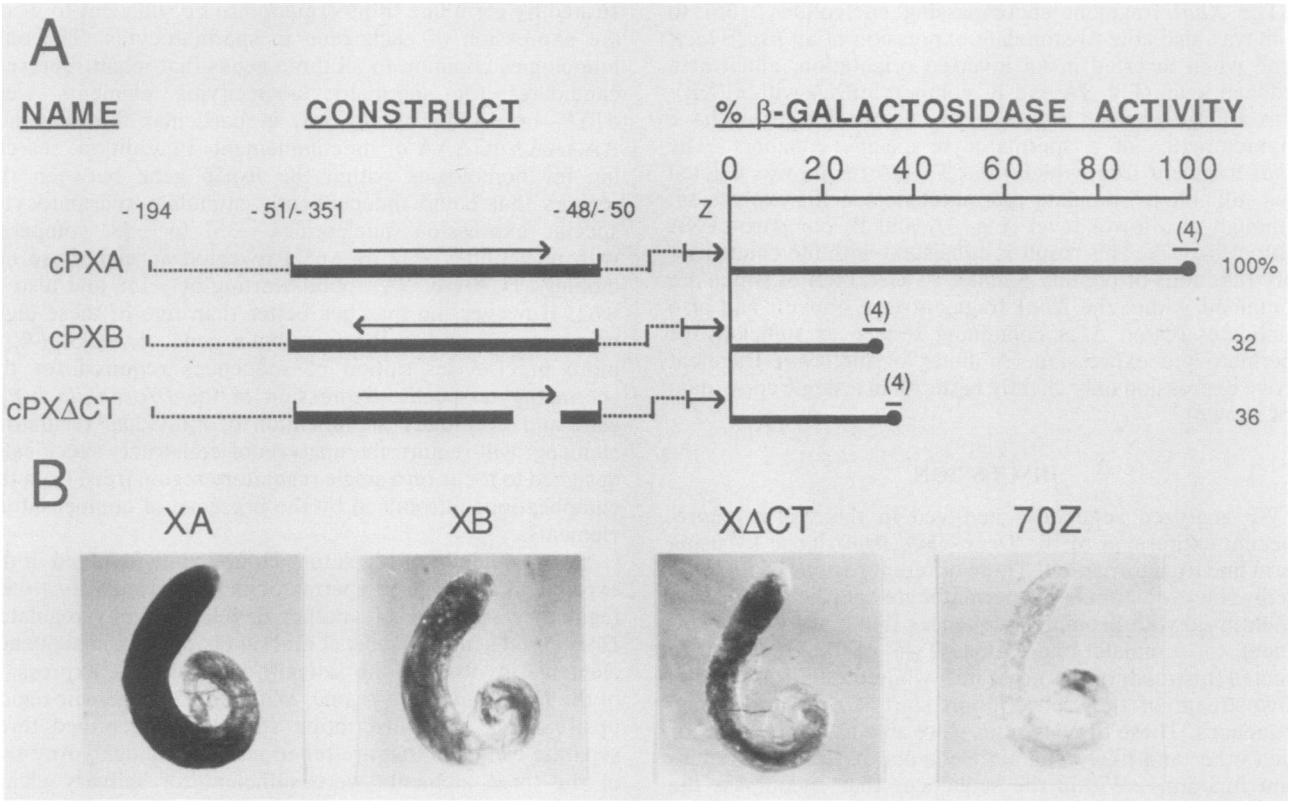


FIG. 3. Upstream region of *hsp26* drives *hsp70* promoter in spermatocytes. (A) An *Xba*I fragment from −48 to −351 of *hsp26* (■) was inserted into the *Nru*I site at −50 of an *hsp70-lacZ* gene. The *hsp70* (– – –) and *lacZ* (——) sequences are indicated. Arrows above the *hsp26* inserts indicate the normal orientation of these sequences, with the arrow pointed in the direction of the transcription start point. cPXΔCT contains a deletion of *hsp26* sequences from −85 to −134. Germ line transformants containing these constructs were analyzed for levels of spermatocyte-specific β-galactosidase activity, and the data are presented as described for Fig. 2A and C. (B) Staining patterns observed when selected lines transformed with the constructs indicated were incubated in a buffer containing X-Gal are illustrated the same way and at the same magnification as in Fig. 2B and D. The time of incubation at 25°C was 3 h. The X-Gal staining pattern of testes from flies transformed with an *hsp70-lacZ* gene without an *hsp26* insert is illustrated as construct 70Z. With this control, no staining was observed in the spermatocytes; however, some activity was observed at the very proximal end of the testis. Staining of nontransformed $Adh^{fn6}cn$; ry^{502} is illustrated in Fig. 2B.

region D of distinct regulatory elements. Recall that removal of region D results in the level of spermatocyte expression becoming considerably more dependent on the upstream CT · GA region (compare cP-135 and cP-85 in Fig. 2A and B with cP-135-70Z and cP-85-70Z in Fig. 2C and D). This result suggests that region D contains elements that, in some way, affect transcription rather than transcript or enzyme stability or translational efficiency.

Analysis of numerous *Drosophila* genes indicates that the expression of a single gene in multiple tissues involves, at least in part, distinct elements unique to each mode of expression (4, 9, 11, 14, 15, 22, 29). The mutational analyses of spermatocyte-specific (12; this report), nurse cell-specific (8, 12), and heat-induced (8, 28; Glaser et al., in press) modes of *hsp26* expression are consistent with the conclusion that each of these modes of *hsp26* expression is mediated, at least in part, by unique regulatory elements. Tissue-specific gene expression often involves the action of more general regulatory factors that act in many tissues and presumably on many different genes (20, 23, 25, 40). The action of sequences of the CT · GA region of *hsp26* may be mediated via general regulatory elements. We have studied the CT · GA region with respect to heat shock expression and found that this region is required for optimal heat-induced expression, even though it contains no heat shock elements (Glaser et al., in press). The CT · GA region may provide the same general quantitative element to both heat-induced tissue-general and spermatocyte-specific modes of expression. Alternatively, the action of sequences within this interval could be mediated by binding to both tissue-general and tissue-specific factors (38).

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