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Correct Temperature Induction and Developmental Regulation of a Cloned Heat Shock Gene Transformed into the *Drosophila* Germ Line

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We have constructed a size variant of the *Drosophila* hsp28 gene by deleting 207 base pairs of the protein coding region, beginning 33 base pairs downstream of the ATG protein initiation codon. After transformation of *Drosophila melanogaster rosy* (ry^{506}) flies with this altered gene, using the P transposable element system, it was found that the transformed gene was regulated correctly both after temperature elevation and during the development of the flies. Levels of the variant mRNA were as high as those of the endogenous hsp28 during all patterns of expression, and the variant mRNA appeared in all cases to be processed correctly and to be as stable as the endogenous mRNA. Nevertheless, the chromosomal locus of the transformed gene did not puff after heat shock, suggesting that normal transcription of the gene does not require puffing of the locus. The deleted hsp28 gene retained the reading frame of the endogenous one, and a protein of the expected molecular weight of 18,500 was made after heat shock at levels comparable to those of the endogenous hsp28.

The pattern of changes in gene expression that takes place in most cells after a temperature increase of several degrees above the normal growing temperature constitutes an ideal model system to use to investigate the mechanisms underlying the control of gene expression in eucaryotes. In *Drosophila melanogaster*, a brief temperature elevation above the 30°C limiting temperature for viability induces the transcription of seven major genes, the heat shock genes, whereas transcription of most previously active genes is repressed (see reference 22 for a review). In addition to this transcriptional control, the expression of the heat shock genes is also controlled at the translational level (22). The preexisting mRNAs remain in the cytoplasm of the cell but are sequestered from translation, whereas the messages coding for the heat shock proteins are efficiently translated. In addition to this induction by temperature, a subset of the *Drosophila* heat shock genes is expressed in a tissue- and development specific fashion in the absence of heat shock. For example, the hsp28 and hsp26 genes are induced in the nurse cells during oogenesis (25), hsp23 is induced specifically in wing imaginal disks (1), and all four small heat shock genes are induced in imaginal disks by ecdysone (7).

Understanding the molecular basis for the transcriptional control of the heat shock genes will likely involve both the identification of specific sequences in the DNA and the regulatory factors with which they interact to modulate their expression during temperature elevation. In recent years, considerable effort has been made to identify the DNA sequences involved in the transcriptional control of the heat shock genes. The fact that seven different genes are coordinately induced by the same stimulus suggests that these control sequences might be common to all seven genes. Comparison of the DNA sequences of the seven genes has pointed out several conserved regions located at the 5′ end of the different *Drosophila* heat shock genes (5, 6). By using different assay systems, several investigators have tried to assign functional roles to some of these conserved sequences. These functional assays have always been based on either the stable or transient expression of *Drosophila* heat shock genes into heterologous cells and have given different results, depending on the system (14, 16; V. Corces and A. Pellicer, J. Biol. Chem., in press).

The germline transformation protocol developed by Rubin and Spradling (19) affords the possibility of stably transforming *D. melanogaster* with different cloned genes, thus enabling the study of the transcriptional and translational controls operating during heat shock in a homologous system.

In this paper we present evidence showing that the controls responsible for temperature and developmental expression of the *Drosophila* hsp28 gene can be reproduced after transformation of a cloned gene containing 2.3 kilobases (kb) of 5′ and 0.3 kb of 3′ nontranscribed sequences.

MATERIALS AND METHODS

Maintenance of stocks and *Drosophila* transformation. *Drosophila* stocks were grown at 21°C and 75% relative humidity. The transformation protocol was carried out essentially as described by Rubin and Spradling (19). Preblastula embryos were collected by placing a petri dish containing a mixture of agar and molasses on the mouth of half-pint (ca. 0.24-liter) plastic bottles containing synchronized 6-day-old *rosy* (ry^{506}) (4) flies deficient for xanthine dehydrogenase. The adults were allowed to lay eggs for 30 to 60 min. Dechorionated embryos were attached to a cover slip and injected with a mixture of plasmids pC28C at 200 μg/ml and pπ25.1 at 50 μg/ml. The cover slips with the injected embryos were moved to a petri dish containing moist filter paper, and the hatched larvae were transferred to yeast-glucose vials after 24 to 36 h at 20°C.

Isolation of nucleic acids and in situ hybridization. Plasmid DNA was isolated by standard techniques (12). Heat shock and control RNAs, and RNA from different *Drosophila* developmental stages, were prepared by lysing the tissues in 4 M guanidine isothiocyanate–0.2% *N*-lauroyl sarcosine–150 mM mercaptoethanol–12.5 mM EDTA–50 mM Tris-hydrochloride (pH 7.5) in a Dounce homogenizer. After the addition of an equal volume of 100 mM NaAc (pH 5.0) and three cycles of phenol extraction at 65°C, followed by 10 min

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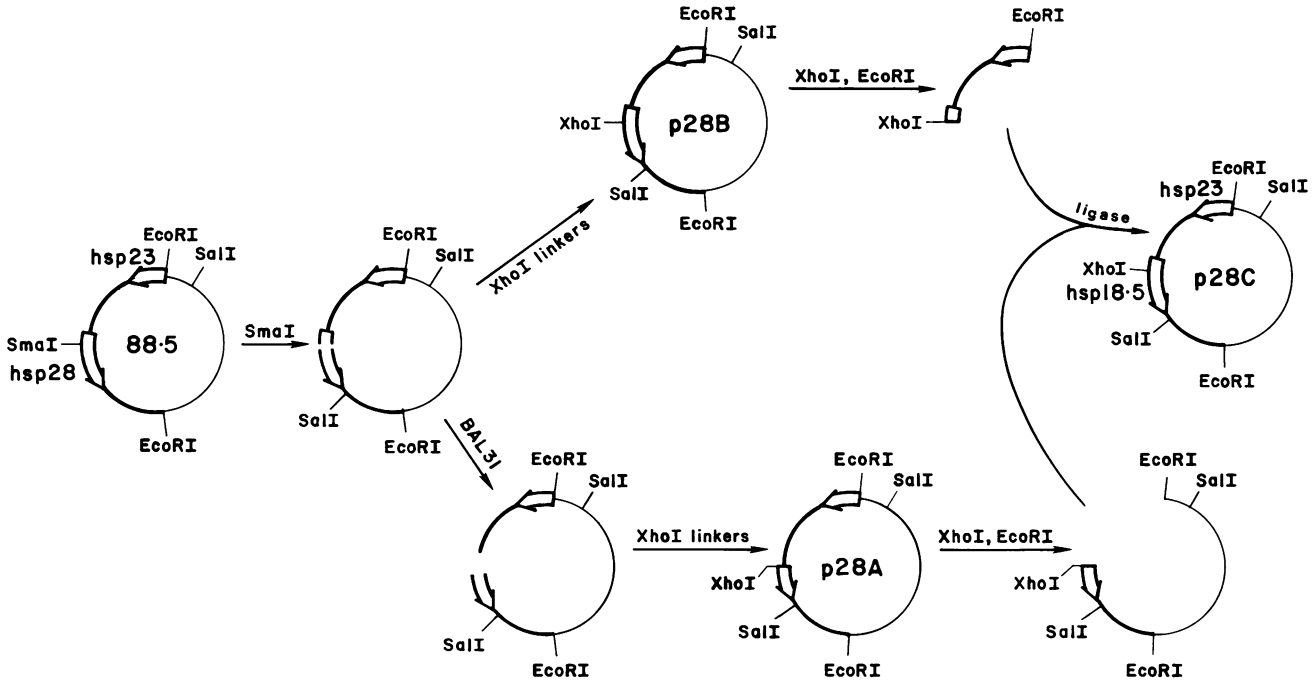

FIG. 1. Diagram showing the different steps in the construction of plasmid p28C. Plasmid 88.5 (2) was linearized at the unique *Sma*I site located in the coding region of hsp28, digested with BAL 31, ligated to a molar excess of *Xho*I linkers, and recircularized to yield plasmid p28A. Separately, plasmid p28B was constructed, containing an *Xho*I linker at the *Sma*I site of plasmid 88.5. The *Xho*I-*Eco*RI fragment from p28B containing the 5′ region of the gene was then inserted into the large *Xho*I-*Eco*RI fragment of p28A, thus yielding plasmid p28C.

on ice, the RNA was precipitated with 2 volumes of ethanol (3). In situ hybridizations were carried out by the method of Livak et al. (11). The hybridization probe was plasmid DNA labeled in vitro by nick translation with [^{125}I]dCTP (2,200 Ci/mmol; New England Nuclear Corp.) as the radioactive precursor.

DNA enzymology and gel electrophoresis. Digestion of DNA with restriction enzymes, ligation of DNA fragments, and labeling of DNA by nick translation were carried out by standard procedures (12). Digestion with BAL 31 nuclease was performed in 12 mM $CaCl_2$–12 mM $MgCl_2$–200 mM NaCl–1 mM EDTA–20 mM Tris-hydrochloride (pH 8.1) at 10°C for 5 min, with 50 U of enzyme per ml. DNA sequencing was performed by the method of Maxam and Gilbert (13). Selection of hsp28 homologous RNAs was done by covalently binding plasmid DNA containing the hsp28 gene to activated cellulose (15). The bound DNA was hybridized to polyadenylated *Drosophila* heat shock RNA in 50% formamide–0.6 M NaCl–1 mM EDTA–0.2% sodium dodecyl sulfate–30 mM Tris-hydrochloride (pH 7.4) at 37°C for 24 h. After washing the hybridized RNA, it was eluted with 90% formamide, buffered to pH 7.4 with Tris, and ethanol precipitated. In vitro translation of the selected messages was performed by the method of Pelham and Jackson (17). RNA was electrophoresed on 1% agarose-formaldehyde gels and transferred to GeneScreen (New England Nuclear Corp.) according to the instructions provided by the manufacturer. Protein electrophoresis was carried out as described by Laemmli (9).

RESULTS

Construction of plasmids used for transformation and establishment of transformed lines. To distinguish between the endogenous and the transformed hsp28 messages, a 207-base-pair (bp) deletion was made in the protein coding region of the gene, immediately 3′ to the ATG. Plasmid 88.5 (2) was linearized with *Sma*I at nucleotide +150, 33 bp downstream of the ATG, and then digested with BAL 31 nuclease such that ca. 200 bp were deleted from each end. The digested plasmid was supplied with *Xho*I linkers, then religated to circularize, and was designated p28A (Fig. 1). Since this construction deleted many of the 5′ regulatory sequences of the gene, an additional plasmid was made (p28B), which contained an *Xho*I linker at the *Sma*I site of plasmid 88.5. This plasmid was used to replace the sequences 5′ to the *Sma*I site that were deleted by BAL 31 in p28A. The resulting plasmid contained a deletion of ca. 200 bp downstream of the original *Sma*I site and will be referred to as p28C. The precise location of the deletion breakpoint in the hsp28 gene was determined by sequence analysis and was found to be from +150 to +357 (Fig. 2). With the addition of the hexameric *Xho*I linker, the resulting plasmid retained the original reading frame and was expected to encode a 950-bp mRNA and a 18,500-dalton protein, if properly expressed. This new protein will be referred to as hsp18.5. Of four methionine residues present in the original hsp28, three were expected to be present in the modified protein if translated. A 3.6-kb *Sal*I fragment of p28C containing the partially deleted coding region of hsp28, including 2.3 kb of sequences 5′ and 0.3 kb of sequences 3′ to the coding region, was then inserted into the *Sal*I site of plasmid Carnegie 20 which contains the wild-type gene for xanthine dehydrogenase (20). The resulting plasmid was called pC28C (Fig. 2). Although this plasmid contains the coding region for hsp23 in addition to that of hsp28, all the transcriptional regulatory sequences, including the TATAA box and the transcription start site for the hsp23 gene, have been removed. We therefore assumed that the hsp23 gene was nonfunctional when transformed into flies, and only the altered hsp28 was studied. ry^{506} homozygous 0- to 2-h-old embryos were

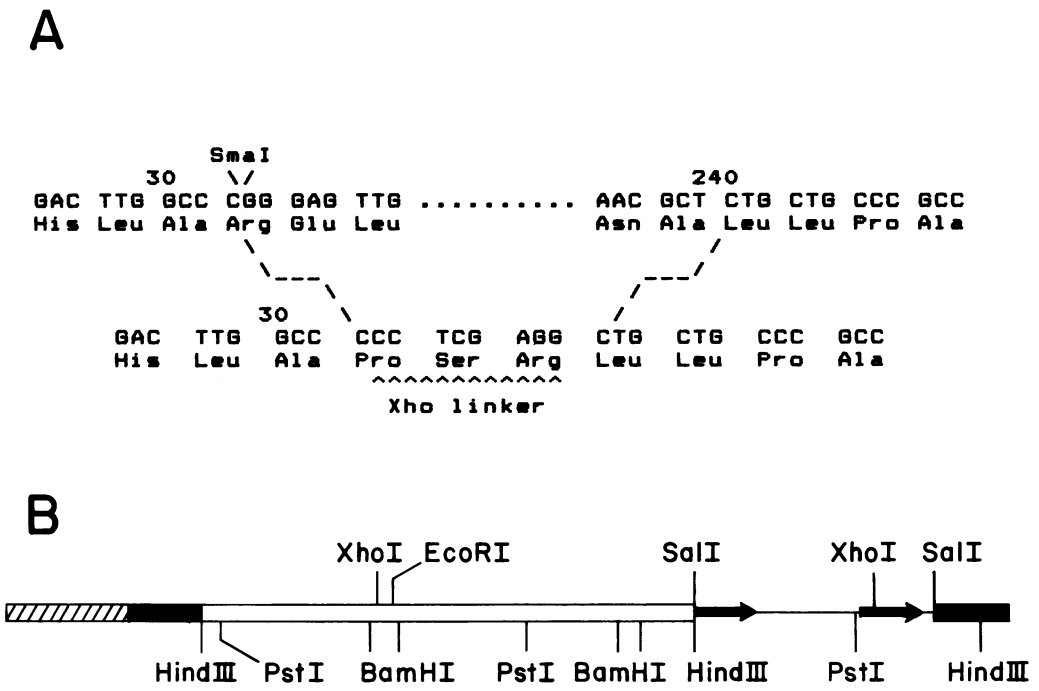

FIG. 2. Sequence analysis and restriction map of the plasmid used for transformation. (A) DNA sequence analysis of the variant hsp28 gene in the region of the deletion breakpoint, showing that the deleted gene conserves the same reading frame as the original hsp28 gene. (B) Restriction map of plasmid pC28C used in the injection experiments. Plasmid pC28C contains the *Sal*I fragment of p28C cloned into the *Sal*I site of Carnegie 20 (20). The thin line represents sequences containing the heat shock genes, and the arrows indicate the transcription units. Symbols: □, sequences from the ry locus; ■, P element sequences; □, cloning vector.

injected with this plasmid as described above, with germ line transformants being identified by the wild-type expression of the ry gene contained in pC28C. Although the majority of flies surviving the injection and emerging as adults had wild-type eyes, only 5% of the viable adults were transformed in their germ line, giving progeny with the ry^+ phenotype. Of the transformed flies obtained, two lines were fully analyzed and designated 30A and 63BC from the chromosomal location of the transformed DNA (see below). Both lines were found to contain single insertions of pC28C by Southern analysis (data not shown).

Proper transcription of the transformed gene during heat shock. To determine whether the transformed hsp28 gene was induced correctly during heat shock, and to determine whether the levels of mRNA from the transformed gene were comparable to those of the endogenous gene, total RNA, prepared from adult flies grown both at 21°C and heat shocked at 36.5°C for 45 min, was analyzed by Northern blots. The results (Fig. 3) indicate that ry^{506} homozygotes and both transformed lines accumulate similar levels of the normal hsp28 1.15-kb mRNA (2) after induction by heat shock. Both transformed lines, however, express an additional 0.95-kb mRNA corresponding to the transformed gene. The 0.95-kb mRNA, like the 1.15-kb mRNA, is evident only during heat shock and is present at levels equal to that of the endogenous message in both lines. This smaller message is the size expected from sequence analysis of the p28C plasmid and appears to be as stable as the message encoded by the endogenous gene. Polyadenylation of the 0.95-kb message also appears to be normal, since the size of the mature message is as expected if proper polyadenylation takes place and the RNA binds to an oligodeoxythymidylic acid-cellulose column (see Fig. 5C).

Chromosomal characterization of the transformed lines. Since characteristic puffs corresponding to the loci of all of the heat shock genes are induced on polytene chromosomes when subjected to high temperature, we heat shocked salivary glands in vitro to determine whether the transformed genes were capable of inducing new heat shock puffs at their respective locations. Figure 4 shows in situ hybridizations of

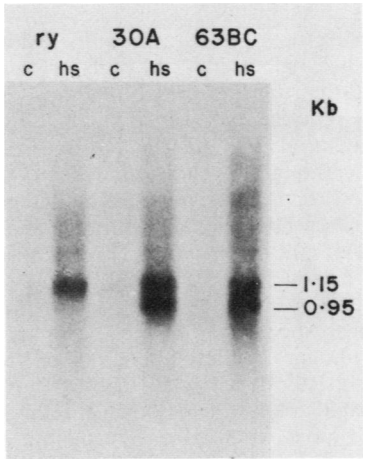

FIG. 3. Electrophoretic analysis of RNA from ry^{506} homozygous and transformed non-heat-shocked and heat-shocked flies. *Drosophila* embryos were transformed with plasmid pC28C, with the wild-type expression of the xanthine dehydrogenase gene as a selectable marker. Homozygous transformed adults (lines 30A and 63BC) were selected, and total RNA was made from flies grown at 21°C (c) and flies kept at 36.5°C for 45 min (hs). RNA samples (3 μ) were electrophoresed on a 1% agarose–formaldehyde gel, transferred to GeneScreen, and hybridized with ^{32}P-labeled 88.1 plasmid DNA (2).

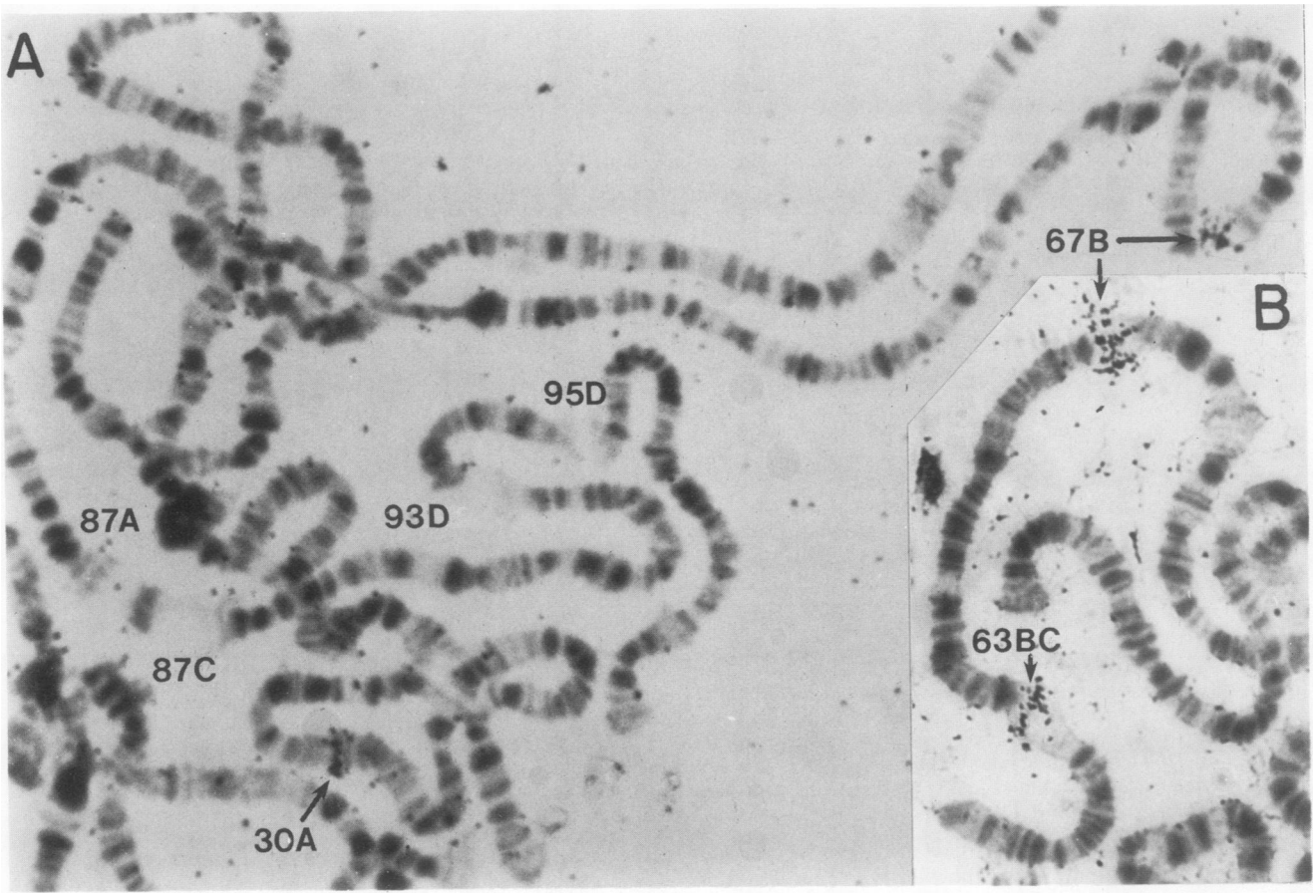

FIG. 4. In situ hybridization of the hsp28 gene to polytene chromosomes from transformed flies. Salivary gland chromosomes of both transformed lines were heat shocked at 36.5°C for 20 min, squashed, and hybridized with ^{125}I-labeled DNA from plasmid p28C. (A) Five of the six endogenous characteristic heat shock puffs are shown, labeled by their chromosomal locations. Grains are evident over both the endogenous hsp28 locus at 67B and the transformed nonpuffed locus at 30A. (B) The left arm of the third chromosome from the other tranformed line shows grains over the endogenous 67B region and the insertion site of the injected plasmid at 63BC. Both the endogenous locus and the combined hsp83-hsp18.5 locus are shown well puffed.

iodinated p28C plasmid to heat-shocked polytene chromosomes from both of the transformed lines. Five of the six characteristic heat shock-induced puffs are evident in Fig. 4A, labeled by their chromosomal locations. Grains can be seen over the endogenous hsp28 locus at 67B and the transformed locus at 30A on the left arm of the second chromosome. Although the endogenous locus is well puffed, the transformed locus does not appear detectably puffed. Figure 4B shows a heat-shocked chromosome of the other transformed line. Grains over 63BC in this chromosome, in addition to the endogenous hsp28 67B location, indicate that the transformed hsp28 has fortuitously integrated into the endogenous heat shock puff of the hsp83 locus at 63BC. Since the morphology of the combined hsp83-hsp18.5 locus puff is not consistently distinguishable from that of the endogenous hsp83 puff, the presence of the transforming DNA seems to have no effect on the puffing of this locus. The integration of one of the transformed genes into a heat shock-induced puff at 63BC, and the integration of the other into a nonpuffed 30A location, enabled us to make a correlation between puffing and RNA levels. Since there is no difference in the amount of heat shock transcript from the endogenous locus at 67B, the unpuffed 30A locus, and the puffed transformed locus at 63BC, and since the levels of the transformed transcripts are equal to the levels of the endogenous gene, it seems that puffing is neither a prerequisite nor a necessary accompaniment for the high levels of RNA produced from this heat shock gene after temperature elevation.

Translation of the transformed gene message. Since the deletion made in the coding region of the hsp28 gene retained the reading frame of the endogenous gene, and the mRNA levels of the transformed gene after heat shock were as high as those of the endogenous one, the protein product of the transformed gene was expected at levels equivalent to that of the endogenous hsp28 gene. To test this, imaginal disks from late third-instar larvae were dissected from ry and transformed flies and then labeled in vitro with [^{35}S]methionine at both 25 and 37°C. Figure 5A shows the results of these experiments carried out with the 30A transformant; the same results were obtained with the transformed line 63BC (data not shown). Although both ry and transformed heat-shocked disks show the seven characteristic heat shock proteins, the transformed flies show an additional protein with an approximate molecular weight of 18,500, which is expressed only after temperature elevation. The same result was obtained by labeling salivary glands for 5 min at both 25 and 37°C (Fig. 5B). The presence of some of the small heat shock genes in the control lanes is expected due to their developmental expression in late third-instar

larvae (1, 7, 24). In no case, however, are hsp70 or hsp68 evident in the control lanes in a shorter exposure of the same autoradiogram (data not shown), indicating that these samples were not inadvertantly heat shocked. To show that the new heat shock-induced protein is encoded by the transformed gene, the hsp28-homologous heat shock messages of both ry and transformed flies were isolated from a population of polyadenylic acid-containing RNA by hybridization to p28C plasmid DNA covalently bound to cellulose. Figure 5C shows the results of the in vitro translation of these messages, using a rabbit reticulocyte cell-free translation system. It can be seen that the hsp28 message selected from ry flies codes only for the expected 28,000-dalton protein, whereas the messages selected from the transformed flies code for an additional 18,500-dalton protein, which is the same size as the in vivo-induced protein expressed in the imaginal disks and salivary glands of the transformed flies.

Developmental regulation of the transformed gene. The mRNA of the endogenous hsp28 gene has been found strongly induced in early pupae and in the nurse cells during oogenesis. The nurse cells transport the hsp28 message to the developing oocytes, where it persists until gastrulation (25). To determine whether the transformed gene was regulated correctly during development, total RNA was isolated from different developmental stages of the 30A transformed line. The RNA was then analyzed by Northern blotting, with the endogenous hsp28 gene as the probe. As during heat shock, the transformed gene appears to be regulated correctly, being expressed at the same developmental stages and at the same levels as the endogenous one (Fig. 6). Both mRNA species are evident in 0- to 24-h-old embryos, in which they have presumably been transported from the ovarian nurse cells and stored. In addition, both genes are found coordinately expressed at the same levels in prepupae and young pupae, with young pupae giving a stronger signal than prepupae for both mRNAs. There is also a weak signal evident for both the transformed and endogenous mRNAs in the adult RNA lane, which we presume to be from the expression of the genes in the female ovaries. The same results were obtained with the 68BC transformed line, suggesting that the proper developmental regulation of the transformed gene is a consequence of sequences carried by the introduced DNA rather than a position effect due to controlling elements residing at the site of insertion.

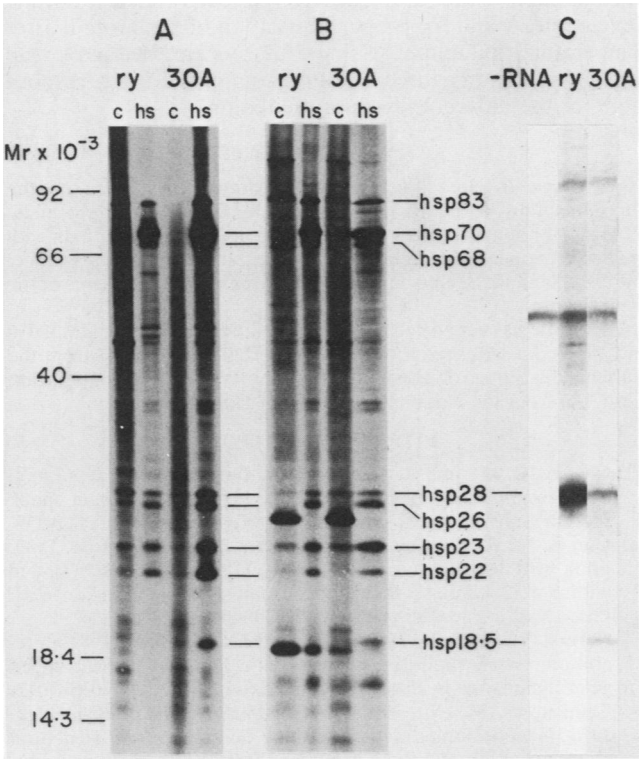

FIG. 5. Electrophoretic analysis of in vitro synthesized proteins. (A) Imaginal disks were dissected from ry^{506} (ry) and transformed flies (30A) and labeled in vitro with 20 μCi of [^{35}S]methionine for 60 min at 22°C (c) and 36.5°C (hs) in Ringer solution. The heat-shocked tissues were preincubated at the high temperature for 15 min before adding the label. The labeled tissues were then solubilized in sample buffer (9) and electrophoresed on a 15% sodium dodecyl sulfate–polyacrylamide gel. (B) Salivary glands from ry^{506} and transformed third-instar larvae were labeled with [^{35}S]methionine for 5 min in the conditions described above.The labeled proteins were then electrophoresed on a 15% sodium dodecyl sulfate–polyacrylamide gel. (C) RNA complementary to the hsp28 gene was selected from polyadenylic acid-containing RNA obtained from heat-shocked ry^{506} and 30A transformed flies. The selected RNA was then translated in vitro by a rabbit reticulocyte translation system, and the synthesized proteins were electrophoresed on a 15% sodium dodecyl sulfate–polyacrylamide gel.

DISCUSSION

We have constructed a modified hsp28 gene by deleting 207 bp of the protein coding region of the normal hsp28 *Drosophila* gene. After injection of plasmids into preblastoderm embryos, we have obtained two different transformed lines which contain single-copy insertions of the transforming gene. The variant hsp28 gene is properly regulated both after temperature induction and during development, suggesting that the information contained in the transforming DNA is sufficient to specify both patterns of control. In addition, the transformed DNA encodes an RNA that accumulates to levels comparable to those of the endogenous gene. In contrast to the normal heat shock locus, the

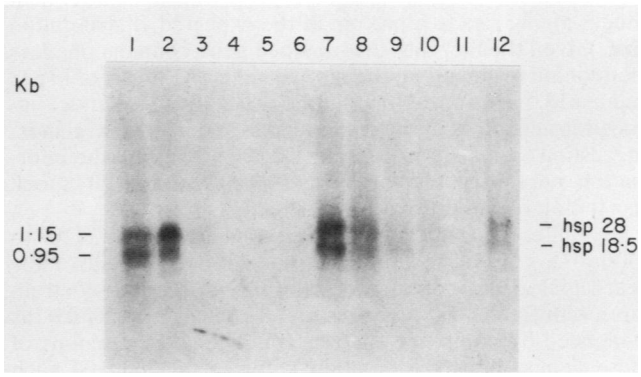

FIG. 6. Electrophoretic analysis of RNA expressed during the development of transformed flies. *Drosophila* embryos with plasmid pC28C inserted at chromosomal location 30A were grown at 21°C, and RNA from different developmental stages was prepared as described in the text. Fifteen micrograms of total RNA from each stage were electrophoresed on a 1% agarose–formaldehyde gel, transferred to GeneScreen, and hybridized with ^{32}P-labeled 88.1 DNA (2). Lanes: 1, heat shock control (1 μg of total heat shock RNA from the same flies); 2, total RNA from 0- to 12-h-old embryos; 3, 12- to 24-h-old embryos; 4, first-instar larvae; 5, second-instar larvae; 6, third-instar larvae; 7, prepupae; 8 to 11, successive stages of pupal development 24 h apart; 12, adult RNA.

chromosomal region where one of the new genes is inserted is unable to puff after heat shock induction. This result differs from previously published reports by Lis et al. (10), who were able to transform *Drosophila* embryos with a hybrid gene containing part of the *Drosophila* hsp70 gene joined to the coding region of a bacterial β-galactosidase gene and induce puffing of the transformed locus after temperature elevation. The difference in the results of these experiments could be due to the absence in the hsp28 DNA of sequences necessary for puff induction, which were present in the transforming DNA used by Lis et al. Alternatively, the inability of the hsp18.5 30A locus to puff could be due to position effects, i.e., the influence of neighboring sequences on the gene at the insertion site. Since we only have one transformant that is unable to puff, we cannot rule out this possibility. Due to the fact that the mRNA made from the hsp70-lacZ gene fusions is considerably longer than the mRNA encoded by the gene used in this study, a likely explanation for the differences in both sets of results is that the size of a puff might be dependent on the length of the transcripts from the locus. Another recent report supporting this possibility is that concerning the *Drosophila* sgs3 "glue" gene, which is one of three glue genes at the salivary gland puff 68C (18). The transformed sgs3 gene encodes a small RNA which is transcribed at levels similar to those of the endogenous gene, yet does not puff upon induction during development. Since the endogenous hsp28 resides in a 15-kb region containing three other small heat shock genes, all of which are coordinately induced during heat shock, it is possible that the combined induction of the four small heat shock genes is responsible for puffing and that a single isolated gene from this locus is not capable of inducing a puff, as is the case with the sgs3 glue gene. Nevertheless, this variant hsp28 gene insertion, although unable to puff, codes for an RNA that accumulates to the same level as the endogenous hsp28 RNA and at the same level as that of the same variant gene inserted into the endogenous heat shock puff of the hsp83 gene. This suggests that puffing may be neither a prerequisite for, nor a necessary consequence of, heavily induced transcription.

The transformed gene is also properly translated after heat shock, giving rise to a protein of the expected 18,500-dalton size. Given the fact that the modified gene contains one less methionine residue than the normal gene, the levels of hsp28 and hsp18.5 are approximately the same after heat shock-induced translation in imaginal disks and salivary glands, suggesting that the transforming DNA contains all the information necessary for the proper preferential heat shock translation of the protein.

It is interesting to note that the unusually long (110- to 240-bp) 5′ transcribed, nontranslated regions of all of the heat shock genes contain no local mRNA secondary structures with free energy changes of >-10 kcal (-41.84 kJ), as evidenced by computer analysis (8). The coding regions of these genes, however, contain between 40 and 100 such locally stable structures in their mRNA, depending on the length of the particular message. The hsp28 mRNA, for example, contains no locally stable structures in the 120C bp upstream of its AUG, whereas it contains 45 such possible secondary structures in the 1 kb downstream of its AUG. The mRNAs of the heat shock genes then show a striking difference in the number of possible local secondary structures 5′ of the AUG (none) and downstream of the AUG (many). Since all of the non-heat shock messages analyzed by computer so far contain a number of such possible locally stable secondary structures in the comparatively short transcribed, nontranslated regions of their mRNA, it is possible that the altered ribosomes of heat-shocked cells (21–23) are unable to melt or recognize the secondary structures contained in the leader sequences 5′ to the AUG of the preexisting mRNAs. The altered ribosomes are instead able to initiate only on the secondary structure-free leader regions of the heat shock messages, resulting in their preferential heat shock translation. Although the deletion we constructed in the cloned hsp28 gene used for transformation had its breakpoint very near the AUG, we fortunately did not create any locally stable secondary structures in its mRNA 5′ to the AUG. It remains to be determined whether the retention of preferential heat shock translation by our transformed gene transcript is due to the retention of a specific nucleotide sequence that interacts with a heat shock-dependent translational modulation factor or whether it instead simply retains the integrity of the leader mRNA secondary structure.

In summary, the results presented in this paper indicate that the modified hsp28 gene used in these studies contains all the regulatory sequences needed for proper transcription of the gene, both after heat shock and during normal development, and for proper translation of the protein after temperature induction. Using this system, we now can attempt to identify the control regions of the DNA responsible for the different levels of regulation.

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LITERATURE CITED

1. **Cheney, C. M., and A. Shearn.** 1983. Developmental regulation of *Drosophila* imaginal disc proteins: synthesis of a heat shock protein under non-heat shock conditions. Dev. Biol. **95**:325–330.
2. **Corces, V., R. Holmgren, R. Morimoto, and M. Meselson.** 1980. Four heat shock proteins in *Drosophila melanogaster* coded within a 12 kilobase region in chromosome subdivision 67B. Proc. Natl. Acad. Sci. U.S.A. **77**:5390–5393.
3. **Corces, V., A. Pellicer, R. Axel, and M. Meselson.** 1981. Integration, transcription and control of a *Drosophila* heat shock gene in mouse cells. Proc. Natl. Acad. Sci. U.S.A. **78**:7038–7042.
4. **Gelbart, W. M., M. McCarron, J. Pandey, and A. Chovnick.** 1974. Genetic limits of the xanthine dehydrogenase structural element within the rosy locus in *Drosophila melanogaster*. Genetics **78**:869–886.
5. **Holmgren, R., V. Corces, R. Morimoto, R. Blackman, and M. Meselson.** Sequence homologies in the 5′ regions of four *Drosophila* heat shock genes. 1981. Proc. Natl. Acad. Sci. U.S.A. **78**:3775–3778.
6. **Ingolia, T. D., and E. A. Craig.** 1981. Primary sequence of the 5′ flanking region of the *Drosophila* heat shock genes in chromosome subdivision 67B. Nucleic Acids Res. **9**:1627–1642.
7. **Ireland, R. C., E. Berger, K. Sirotkin, M. A. Yund, D. Osterbur, and J. Fristrom.** 1982. Ecdysterone induces the transcription of four heat shock genes in *Drosophila* S3 cells and imaginal discs. Dev. Biol. **93**:498–507.
8. **Kanehisa, M., and W. Goad.** 1982. Pattern recognition in nucleic acid sequences. II. An efficient method for finding locally stable secondary structures. Nucleic Acids Res. **10**:265–278.
9. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature (London)

227:680–685.

10. **Lis, J. T., J. A. Simon, and C. A. Sutton.** 1983. New heat shock puffs and β-galactosidase activity resulting from transformation of *Drosophila* with an hsp70-lac Z hybrid gene. Cell **35**:403–410.
11. **Livak, K. F., R. Freund, M. Schweber, P. C. Wensink, and M. Meselson.** 1978. Sequence organization and transcription of two heat shock loci in *Drosophila*. Proc. Natl. Acad. Sci. U.S.A. **75**:5613–5617.
12. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
13. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
14. **Mirault, M.-E., R. Southgate, and E. Delwart.** 1982. Regulation of heat-shock genes: a DNA sequence upstream of *Drosophila* hsp70 genes is essential for their induction in monkey cells. EMBO J. **1**:1279–1285.
15. **Moss, L. G., J. P. Moore, and C. Lawrence.** 1981. A simple efficient method for coupling DNA to cellulose. J. Biol. Chem. **256**:12655–12658.
16. **Pelham, H. R. B., and M. Bienz.** 1982. A synthetic heat shock promoter element confers heat inducibility on the herpes simplex virus thymidine kinase gene. EMBO J. **1**:1473–1477.
17. **Pelham, H. R. B., and R. J. Jackson.** 1976. An efficient mRNA-dependent translation system from reticulocyte lysates. Eur. J. Biochem. **67**:247–257.
18. **Richards, G., A. Cassab, M. Bourouis, B. Jarry, and C. Dissous.** 1983. The normal developmental regulation of a cloned sgs3 "glue" gene chromosomally integrated in *Drosophila melanogaster* by P element transformation. EMBO J. **2**:2137–2142.
19. **Rubin, G. M., and A. C. Spradling.** 1982. Genetic transformation of *Drosophila* with transposable element vectors. Science **218**:348–353.
20. **Rubin, G. M., and A. C. Spradling.** 1983. Vectors for P element mediated gene transfer in *Drosophila*. Nucleic Acids Res. **11**:6341–6351.
21. **Sanders, M. M., D. Feeney-Triemer, A. S. Olsen, and J. Farrell-Towt.** 1982. Changes in protein phosphorylation and histone H2b disposition in heat shock in *Drosophila*, p. 235–242. *In* M. J. Schlesinger, M. Ashburner, and A. Tissieres (ed.), Heat shock. From bacteria to man. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
22. **Schlessinger, M. J., M. Ashburner, and A. Tissieres.** 1982. Heat shock. From bacteria to man. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
23. **Scott, M. P., and M. L. Pardue.** 1981. Translational control in lysates of *Drosophila melanogaster* cells. Proc. Natl. Acad. Sci. U.S.A. **78**:3353–3357.
24. **Sirotkin, K., and N. Davidson.** 1982. Developmentally regulated transcription from *Drosophila melanogaster* chromosomal site 67B. Dev. Biol. **89**:196–210.
25. **Zimmerman, J. L., W. Petri, and M. Meselson.** 1983. Accumulation of a specific subset of *D. melanogaster* heat shock mRNAs in normal development without heat shock. Cell **32**:1161–1170.