A Deletion of the 3' End of the *Drosophila melanogaster* hsp70 Gene Increases Stability of Mutant mRNA During Recovery from Heat Shock

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hsp40, an X-ray-induced deletion mutant of the major *Drosophila melanogaster* heat shock protein gene **hsp70**, was shown to be incorrectly regulated at the translational level. **hsp40** protein synthesis persisted at a high level after the release from heat shock, whereas **hsp70** protein production was rapidly repressed. This result was observed both in flies heterozygous for the **hsp40** gene and in tissue culture cells transfected with the truncated gene. Analysis of the transcription of the **hsp40** gene indicated that its mRNA, unlike **hsp70** mRNA, was not actively destabilized after a return to control temperatures, permitting prolonged production of the mutant protein.

Heat shock, the ubiquitous response of organisms to abnormally high temperatures, constitutes an ideal system in which to study gene regulation. In *Drosophila melanogaster*, a temperature shift to 37°C causes the rapid production of seven major proteins called the heat shock proteins (23). Upon temperature elevation, heat shock genes are actively transcribed to the exclusion of non-heat shock genes; in addition, the mRNAs corresponding to the heat shock genes are preferentially translated, whereas non-heat shock messages are sequestered from translation (1). After a return to normal growth temperatures, heat shock transcription is rapidly repressed, and the transcription of normal cellular genes resumes. Normal cellular mRNAs reenter translation as a group while the translation of the heat shock messages is turned off, although not synchronously (10, 11). The mRNA corresponding to the major heat shock protein, **hsp70**, is apparently actively destabilized such that **hsp70** protein synthesis is quickly repressed upon release from heat shock (11, 14). The synthesis of the other heat shock proteins is much more gradually switched off (14; this paper). Although a great deal is known about the transcriptional regulation of the heat shock genes at the molecular level (2, 9, 13, 19), little is known about their translational regulation.

Here we show that the rapid repression of **hsp70** protein synthesis after release from heat shock is delayed when the 3' half of the gene is deleted. This truncated gene was isolated after X-ray mutagenesis and is designated **hsp40** (7). In this study, we show that after release from heat shock, **hsp40** protein synthesis continues, whereas that of **hsp70** is rapidly repressed. Likewise, the mRNA corresponding to the **hsp40** gene is present at higher relative levels than the **hsp70** mRNA after recovery from heat shock and is apparently more stable. These data suggest that the 3' sequences of **hsp70** mRNA which are deleted the **hsp40** mRNA are involved in the active destabilization of the **hsp70** mRNA after release from heat shock.

MATERIALS AND METHODS

Fly stocks. Oregon R and *In(3R)AFA Df(3R)kar^{D2}/Sb cu kar* [hereafter referred to as *Df(3R)kar^{D2}*] (7) were maintained at

23°C in vials containing regular *Drosophila* food. *Df(3R)kar^{D2}* flies are heterozygous for a deficiency of 87A to 87D on the right arm of the third chromosome. This deficiency deletes three of the five **hsp70** genes normally present in the haploid genome, leaving one **hsp70** intact and the remaining gene truncated at the 3' end. The truncated **hsp70** gene encodes a heat-inducible protein of 40 kilodaltons and is hereafter referred to as **hsp40** (7).

Transfection of tissue culture cells. All tissue culture cells were maintained at 23°C in M3 medium (24). The shi/pMH cell line was isolated as follows. Cells from the temperature-sensitive shibire (shi) cell line were treated with wild-type *D. melanogaster* DNA and pMH10A DNA (6) in a calcium phosphate precipitate. The precipitate was left on the cells for 12 h at 23°C. The precipitate was then washed off, and the cells were given fresh medium. After 24 h, the cells were transferred to 30°C, a restrictive temperature for shi cells. After 3 weeks, a number of colonies were observed, and these were isolated. Presumably, the colonies were derived from cells that had incorporated a wild-type copy of the shi gene [others have shown a DNA-mediated rescue of shi tissue culture cells (22)]. These colonies were screened for the presence of the cotransformed plasmid pMH10A by Southern analysis. One line was shown to contain about five copies of pMH10A per haploid genome and was designated shi/pMH. pMH10A is a derivative of pBR322 with a 22-kilobase insert of the two heat shock genes from the 87A locus of the deletion chromosome *Df(3R)kar^{D2}* and thus contains the **hsp40** gene.

Radiolabeling and electrophoresis of proteins. Newly eclosed *Df(3R)kar^{D2}* females were selected. Ovaries were dissected and labeled for 30 min with 1 µCi of [³⁵S]methionine per ml in 15 µl of buffered Ringer solution (8). At the end of the labeling period, the ovaries were removed with forceps and dissolved in 1× sodium dodecyl sulfate (SDS) loading buffer (60 mM Tris [pH 6.8], 2% SDS, 10% glycerol, 20 mM β-mercaptoethanol, 0.024% bromophenol blue). For each sample, four ovaries were labeled. Control ovaries were labeled at 23°C. Heat-shocked ovaries were labeled during the last 30 min of a 60-min heat shock treatment at 37°C. To examine protein synthesis during recovery from heat shock, we prepared samples as follows. Three sets of two females each were heat shocked for 60 min

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at 37°C in vials with complete medium. The flies were transferred to 23°C, and the ovaries from two females were immediately dissected and labeled for 30 min at 23°C (the labeling protocol was as described above). The other females were maintained at 23°C. After 30 min, the ovaries from two more females were dissected and labeled at 23°C. After 60 min, the ovaries from the last two females were dissected and similarly labeled.

Tissue culture cells were seeded in 96-well plates at 1.7×10^5 cells per well. Individual wells were labeled for 30 min with 1 μ Ci of [35 S]methionine per ml in 15 μ l of buffered Ringer solution at consecutive intervals during a 60-min heat shock treatment at 37°C and a 90-min recovery at 23°C. Control cells were labeled for 30 min at 23°C. After labeling, the radioactive medium was removed, and the cells were lysed in 1 $\times$ SDS loading buffer. In experiments in which transcription was inhibited, actinomycin D was added to the tissue culture media at a final concentration of 1 μ g/ml (11).

Proteins were separated by SDS gel electrophoresis (15) with a 3.75% acrylamide stacking gel and either a 10% or a 15% separating gel. Gels were fixed, stained with Coomassie brilliant blue R, and dried. Autoradiography was done with Kodak X-omat AR film at -70°C.

Isolation and electrophoresis of RNA. Total RNA was prepared from Oregon R and *Df(3R)kar^{D2}* flies that had been subjected to a 45-min heat shock treatment at 37°C and flies that had been subjected to the same heat shock and then allowed to recover for 45 min at 23°C. RNA was also isolated from control flies maintained at 23°C. RNA was prepared by placing the flies in a Dounce homogenizer and lysing the tissues with lysis buffer (4 M guanidine isothiocyanate, 0.2% *N*-lauryl sarcosine, 150 mM β -mercaptoethanol, 12.5 mM EDTA, 5 mM ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetracetic acid, 50 mM Tris [pH 7.5]). Two volumes of phenol-chloroform-isoamyl alcohol-8-hydroxyquinoline (50:49:1:0.1) were added, and the mixture was vortexed and then incubated at 65°C for 10 min. One-third volume of 0.1 M sodium acetate (pH 5.0) was added, and the mixture was incubated at 65°C for 10 min and then placed on ice for 10 min. The aqueous phase was separated by centrifugation, collected, and extracted with phenol as described above but without the addition of sodium acetate. Crude RNA was precipitated with 0.2 volume of 3 M sodium acetate (pH 5.0) and 3 volumes of ethanol. The pelleted RNA was dissolved in diethylpyrocarbonate-treated water and extracted with buffered phenol-8-hydroxyquinoline, and the temperature regimen described above was repeated. The aqueous phase was extracted with chloroform, precipitated, and suspended in water. Concentrations were determined spectrophotometrically.

RNA samples were separated by electrophoresis on a 1% agarose-formaldehyde gel (17). Gels were then blotted onto Biotodyne nylon membranes, hybridized, and washed in accordance with the instructions of the manufacturer (Pall Ultrafine Filtration Corp.). The DNA probe used was nick-translated (17) plasmid pPW293, which contains the coding region of hsp70 (16).

Autoradiographs were quantitated by integral scanning densitometry with a Hewlett-Packard Integrator coupled to a Corion scanning densitometer.

RESULTS

Persistence of hsp40 protein synthesis at a high level during recovery from heat shock in *Df(3R)kar^{D2}* flies. The normal induction of hsp40 during heat shock has been shown previously (7). To see if the synthesis of hsp40 protein was

FIG. 1. SDS electrophoresis of proteins synthesized in *Df(3R)kar^{D2}* ovaries. Ovaries were labeled for 30 min at consecutive intervals during a 60-min heat shock treatment at 37°C and a 90-min recovery at 23°C. Proteins from four ovaries were loaded in each lane. Lanes: a, control, ovaries labeled at 23°C for 30 min; b, ovaries labeled for the last 30 min of a 60-min heat shock treatment at 37°C; c, ovaries labeled between 0 and 30 min of a recovery at 23°C; d, ovaries labeled between 30 and 60 min of a recovery at 23°C; e, ovaries labeled between 60 and 90 min of a recovery at 23°C. hsp83, hsp70, hsp68, hsp40, and the four small heat shock genes (hsp22, hsp23, hsp26, and hsp28) are indicated. The amount of sample loaded in each lane was not identical owing to differences in the sizes and synthesis activities of individual ovaries.

also regulated correctly during recovery from heat shock, we subjected ovaries from *Df(3R)kar^{D2}* females to heat shock (37°C for 60 min) and then allowed them to recover (90 min at 23°C). These flies contain a single copy each of the hsp70 and hsp40 genes on the deletion chromosome and five copies of hsp70 on the homologous chromosome. The proteins produced by the ovaries were radiolabeled at intervals during the temperature regimen. The proteins synthesized during this experiment are shown in Fig. 1. hsp70 protein synthesis was maximal at the end of heatshock and then rapidly decreased during recovery so that it was hardly detectable 90 min after the shift to the control temperature. In contrast, hsp40 protein synthesis persisted after the release from heat shock so that it was still high at the end of the recovery period, paralleling the protein synthesis rates of the small heat shock genes hsp22, hsp23, hsp26, and hsp28.

Translational control of hsp40 in tissue culture cells. To ensure that the genetic deletion which gave rise to the hsp40 gene specifically altered the regulation of hsp40 rather than the regulation of the heat shock genes in general, we repeated the heat shock and recovery experiment with a cell line containing introduced copies of the hsp40 gene (shi/pMH) and with the parental cell line (shi). This experiment also allowed us to discount any effect of the genetic background of the mutant fly stock and to precisely quantitate the relative amounts of hsp40 and hsp70 proteins synthesized during heat shock and recovery. To isolate the shi/pMH cell line, we transfected a plasmid containing the hsp40 gene (pMH10A) (6) into the shi cell line (21). Southern analysis of a transformed cell line revealed that it contained five copies of the transfected plasmid per haploid genome (data not shown). Individual wells containing 1.7×10^5 shi or

FIG. 2. SDS gel electrophoresis of proteins synthesized by shi and shi/pMH cells. (A) shi and shi/pMH cells were plated in 96-well plates at 1.7×10^5 cells per well. Individual wells were labeled for 30 min at consecutive intervals during a 60-min heat shock treatment and a 90-min recovery. The cells were then lysed, and the proteins were electrophoresed. Lanes a through f represent shi/pMH cells which contained transfected hsp40 genes, and lanes g through l represent the parental cell line shi. Lanes: a and g, cells labeled for 30 min at 23°C (control); b and h, cells labeled during the first 30 min of heat shock at 37°C; c and i, cells labeled during the second 30 min of heat shock; d and j, cells labeled during the first 30 min of recovery at 23°C; e and k, cells labeled between 30 and 60 min of recovery; f and l, cells labeled between 60 and 90 min of recovery. The heat shock proteins are indicated (see legend to Fig. 1). (B) Comparison of the synthesis of the hsp70 and hsp40 proteins and the small heat shock proteins. Lanes c through f of the autoradiograph shown in panel A were scanned with a densitometer. The relative synthesis rates are displayed as a percentage of hsp23 synthesis for each time point during the recovery. Symbols: □, hsp70; +, hsp40; ◇, hsp28; △, hsp26; ×, hsp23; ▽, hsp22.

shi/pMH cells were labeled with [35 S]methionine for 30 min at consecutive intervals during a 60-min heat shock treatment (37°C) and a 90-min recovery (23°C). The proteins synthesized are shown in Fig. 2A. Although the hsp40 protein was present only in the transformed cells, the patterns of synthesis of the other heat shock proteins appeared to be similar in both the transformed and parental cell lines. The relative rates of synthesis of the heat shock

proteins during the 90-min recovery period are shown in Fig. 2B. The transfected hsp40 gene showed a pattern of expression like that observed in the ovaries of *Df(3R)kar^{D2}* flies; it maintained a high rate of synthesis, paralleling that of the small heat shock genes. Again, in contrast, the expression of its parent gene, hsp70, was rapidly repressed to a small fraction of its expression at the onset of recovery. We can therefore conclude that the altered pattern of regulation of

FIG. 3. Northern analysis of RNA synthesized by Oregon R and *Df(3R)kar^{D2}* flies during heat shock and recovery. (A) Total RNA was extracted from adult Oregon R flies maintained at 23°C (c), heat shocked at 37°C for 45 min (hs), or after recovery from heat shock for 45 min at 23°C (rec). Ten micrograms of each RNA was electrophoresed on a 1% agarose-formaldehyde gel, blotted onto a nylon membrane, and probed with ³²P-labeled hsp70 DNA. The RNA species corresponding to the hsp70 gene is indicated. The hs and rec lanes of the autoradiogram were scanned with an integrating densitometer; the output of the densitometer is shown in panel C. (B) Total RNA extracted from *Df(3R)kar^{D2}* flies harboring the hsp40 gene and electrophoresed as described for panel A. hsp70 and hsp40 RNAs are indicated; the densitometer output is shown in panel D.

hsp40 was caused by a *cis*-acting genetic defect which had no effect on the regulation of the other heat shock genes.

Maintenance of a higher relative level of hsp40 mRNA than of hsp70 mRNA after heat shock. The prolonged production of hsp40 protein relative to hsp70 protein could be the result of continued transcription of the hsp40 gene, the greater stability of the hsp40 mRNA, or the increased translational efficiency of the hsp40 message. To begin to distinguish among these possibilities, we performed Northern analysis on both wild-type Oregon R flies and flies having the hsp40 gene and determined the relative levels of the hsp40 and hsp70 mRNAs. Equal numbers of Oregon R and *Df(3R)kar^{D2}* adult flies were heat shocked at 37°C for 45 min. Half were then homogenized for RNA preparation, and the others were allowed to recover for 45 min at 23°C before RNA was extracted. As a control, RNA was also isolated from flies of each genotype maintained at 23°C. These RNAs were analyzed by Northern blotting. The filter was probed with ³²P-labeled plasmid DNA containing the coding region of

hsp70. This probe has homology to both hsp40 and hsp70 RNAs. Fig. 3 shows the results of this analysis. A 2.3-kilobase band corresponding to the normal hsp70 transcript was seen in RNA from each heat shock and recovery sample. The 1.9-kilobase RNA corresponding to the hsp40 transcript (5) was only seen in the heat shock and recovery samples prepared from the *Df(3R)kar^{D2}* flies. It was evident from the autoradiograph that the hsp40 transcript maintained a higher accumulated level after recovery than did the hsp70 transcript. Integral densitometric scans of the autoradiographs (Fig. 3) showed that the levels of hsp40 RNA relative to those of hsp70 RNA increased from 15% during heat shock to 45% after recovery. The relative RNA levels during heat shock reflected the allelic frequency of the hsp40 gene versus that of the hsp70 gene in the fly stock (1:6), whereas the relative RNA levels after recovery significantly deviated from the expected value. Thus, the prolonged synthesis of the hsp40 protein appeared not to be the result of an increased efficiency of translation of the hsp40 message but rather the normal translation of a relatively more abundant mRNA.

Greater stability of hsp40 mRNA than of hsp70 mRNA during recovery from heat shock. The higher level of hsp40 mRNA than of hsp70 mRNA could be the result of either prolonged transcription of the hsp40 gene after the release from heat shock or greater stability of the hsp40 mRNA. If continued transcription of the hsp40 gene during recovery were responsible for the extended production of hsp40 protein, it should be possible to prevent this synthesis by blocking transcription during the recovery period. To test this possibility, we repeated the heat shock and recovery experiment with the *shi/pMH* cell line while inhibiting transcription with actinomycin D. *shi/pMH* cells were treated with actinomycin D either immediately after a 60-min heat shock treatment at 37°C or after 30 min of recovery at 23°C (Fig. 4). Actinomycin D at a final concentration of 1 µg/ml was effective at blocking transcription, as control cells subjected to heat shock in the presence of the drug did not synthesize the heat shock proteins (Fig. 4). The three sets of experimental cells, those with and without actinomycin D, showed a very similar general pattern of protein synthesis after heat shock. Blocking transcription after heat shock had no detectable effect on the prolonged synthesis of hsp40 protein, showing that preexisting hsp40 mRNA, and not new message, was responsible for its synthesis. The deletion in the hsp40 gene therefore does not affect the regulation of its transcription but rather results in an mRNA that is more stable than that of the parent gene hsp70.

DISCUSSION

The transcriptional regulation of the *D. melanogaster* heat shock genes has been studied by a number of investigators, resulting in the elucidation of the DNA sequences responsible for the heat induction of the major heat shock protein gene hsp70 (9, 13, 19). Although the translation of the hsp70 gene has also been shown to be very tightly regulated (10, 11, 26), little is known about this regulation at the molecular level.

In this paper, we studied the translational regulation of the hsp40 gene, which is a 3' deletion mutant of hsp70. Using both flies containing the hsp40 gene and tissue culture cells transfected with a plasmid encoding the mutant gene, we found that the regulation of hsp40 protein synthesis during recovery from heat shock was dramatically different from that of hsp70 protein synthesis. Whereas hsp70 protein synthesis was seen to be actively repressed after the release

FIG. 4. SDS gel electrophoresis of proteins synthesized by shi/pMH cells in the presence of a transcription inhibitor. shi/pMH cells were plated in 96-well plates at 1.7×10^5 cells per well. Control cells were labeled for 30 min at 23°C (lane a) and for 60 min at 37°C in the presence of 1 μ g of actinomycin D per ml (lane b). Heat-shocked (HS) cells were labeled during the last 30 min of a 60-min heat shock treatment (lane c). Three sets of experimental cells were heat shocked for 60 min and then labeled at 30-min intervals during a 90-min recovery. The first set (lanes d through f) was labeled in the absence of actinomycin D, the second set (lanes g through i) was labeled in the presence of actinomycin D (actD) added at the end of heat shock, and the third set (lanes j through l) was labeled in the presence of actinomycin D (actD) added after 30 min of recovery. The heat shock proteins are indicated (see Fig. 1 legend).

from heat shock, hsp40 protein synthesis remained high. This prolonged production of hsp40 protein relative to that of hsp70 protein could be due to differences in the efficiency of translation of the corresponding mRNAs, differences in transcriptional regulation, or altered mRNA stability. We excluded the first possibility by performing an RNA analysis on wild-type flies and on flies having the hsp40 gene. This experiment showed that during recovery after heat shock, hsp40 mRNA maintained a higher relative level of protein synthesis than did hsp70 mRNA. Thus, the rapid decline in hsp70 protein synthesis during recovery was due to a reduction in the mRNA level and not to the inefficient translation of a constant amount of mRNA. The rate of hsp40 protein synthesis during recovery did not drop dramatically, as its message maintained a relatively high level. This relatively high hsp40 mRNA level could have been due to the second possibility, namely, that of prolonged transcription of the hsp40 gene during recovery while transcription of the hsp70 gene had ceased. Altered transcriptional regulation of the hsp40 gene relative to that of the hsp70 gene is unlikely because the sequences responsible for the transcriptional regulation of the hsp70 gene reside completely within a 100-base-pair region immediately upstream of the RNA initiation site (9, 13) and are therefore unaltered by the 3' deletion of the hsp40 gene. Nevertheless, we tested this possibility directly by studying the synthesis of hsp40 protein when transcription was blocked during the recovery period. We observed no change in the pattern of synthesis of hsp40 or hsp70 protein, suggesting that neither gene was transcribed during recovery and that the transcriptional regulation of the hsp40 gene was identical to that of the hsp70 gene. Thus, continued transcription of the hsp40 gene was not responsible for the elevated hsp40 mRNA and

protein levels observed. The remaining possibility, namely, that of altered mRNA stability, accounts for the abundance of hsp40 mRNA observed during the recovery period and is consistent with the other data presented. Since the message corresponding to the hsp70 gene is apparently destabilized at a much faster rate than the other heat shock messages (10, 11, 14; this paper), some cellular factors are probably responsible for selectively recognizing the hsp70 message and mediating its degradation after the release from heat shock. Since the hsp40 mRNA appears to possess a half-life which is much longer than that of its hsp70 parent, we hypothesize that the RNA sequences recognized by the putative hsp70 RNA-destabilizing factor(s) are absent from the hsp40 mRNA. The specific sequences responsible for mediating the translational regulation of hsp70 could be determined by constructing deletion mutants of the hsp70 gene in vitro and introducing these constructions into whole flies by P-element transformation (20) or into tissue culture cells by transfection (3, 4, 12, 18, 25).

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LITERATURE CITED

1. Ashburner, M., and J. J. Bonner. 1979. The induction of gene activity in *Drosophila* by heat shock. *Cell* 17:241-254.
2. Ayme, A., R. Southgate, and A. Tissieres. 1985. Nucleotide sequences responsible for the thermal inducibility of the *Drosophila* small heat-shock protein genes in monkey COS cells. *J. Mol. Biol.* 182:469-475.
3. Benyajati, C., and J. F. Dray. 1984. Cloned *Drosophila* alcohol dehydrogenase genes are correctly expressed following transfection into *Drosophila* cells in culture. *Proc. Natl. Acad. Sci. USA* 81:1701-1705.
4. Bourouis, M., and B. Jarry. 1983. Vectors containing a prokaryotic dihydrofolate reductase gene transform *Drosophila* cells to methotrexate-resistance. *EMBO J.* 2:1099-1104.
5. Burke, J. F. 1984. High-sensitivity S1 mapping with single-stranded 32 P-DNA probes synthesized from M13mp templates. *Gene* 30:63-68.
6. Burke, J. F., and D. Ish-Horowicz. 1982. Expression of *Drosophila* heat shock genes is regulated in Rat-1 cells. *Nucleic Acids Res.* 11:6341-6351.
7. Caggese, C., R. Caizzi, M. Morea, F. Scalenghe, and F. Ritossa. 1979. Mutation generating a fragment of the major heat shock-inducible polypeptide in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 76:2385-2389.
8. 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.
9. Cohen, R. S., and M. Meselson. 1984. Inducible transcription and puffing in *Drosophila melanogaster* transformed with hsp70-phage hybrid heat shock genes. *Proc. Natl. Acad. Sci. USA* 81:5509-5513.
10. DiDomenico, B. J., G. E. Bugaisky, and S. Lindquist. 1982. Heat shock and recovery are mediated by different translational mechanisms. *Proc. Natl. Acad. Sci. USA* 79:6181-6185.
11. DiDomenico, B. J., G. E. Bugaisky, and S. Lindquist. 1982. The heat shock response is self-regulated at both the transcriptional and posttranscriptional levels. *Cell* 31:593-603.
12. Di Nocera, P. P., and I. B. Dawid. 1983. Transient expression of genes introduced into cultured cells of *Drosophila*. *Proc. Natl. Acad. Sci. USA* 80:7095-7098.

13. **Dudler, R., and A. A. Travers.** 1984. Upstream elements necessary for optimal function of the hsp70 promoter in transformed flies. *Cell* **38**:391–398.
14. **Kruger, C., and B.-J. Benecke.** 1982. Translation and turnover of *Drosophila* heat-shock and nonheat-shock mRNAs, p. 191–197. In M. J. Schlesinger, M. Ashburner, and A. Tissieres (ed.), *Heat shock: from bacteria to man*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**:680–685.
16. **Livak, J. K., R. Freund, M. Schweber, P. C. Wensink, and M. Meselson.** 1978. Sequence organization and transcription at two heat shock loci in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **75**:5613–5617.
17. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Morganelli, C. M., and E. M. Berger.** 1984. Transient expression of homologous genes in *Drosophila* cells. *Science* **224**:1004–1006.
19. **Pelham, H. R., 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.
20. **Rubin, G. M., and A. C. Spradling.** 1982. Genetic transformation of *Drosophila* with transposable element vectors. *Science* **218**:348–353.
21. **Sang, J. H.** 1981. *Drosophila* cells and cell lines. *Adv. Cell Cult.* **1**:125–182.
22. **Sang, J. H., J. M. Sinclair, J. F. Burke, and D. Ish-Horowicz.** 1984. Rescue of a *Drosophila* temperature sensitive mutant cell line by DNA transfection. *Somatic Cell Mol. Genet.* **10**:573–577.
23. **Schlesinger, M. J., M. Ashburner, and A. Tissieres (ed.).** 1982. *Heat shock: from bacteria to man*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Shields, G., and J. H. Sang.** 1977. Improved medium for culture of *Drosophila* embryonic cells. *Drosophila Information Serv.* **52**:161.
25. **Sinclair, J. M., J. H. Sang, J. F. Burke, and D. Ish-Horowicz.** 1983. Extrachromosomal replication of copia-based vectors in cultured *Drosophila* cells. *Nature (London)* **306**:198–200.
26. **Storti, R. V., M. P. Scott, A. Rich, and M.L. Pardue.** 1980. Translational control of protein synthesis in response to heat shock in *D. melanogaster* cells. *Cell* **22**:825–834.