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Heat Shock Alters Nuclear Ribonucleoprotein Assembly in *Drosophila* Cells†

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Heterogeneous nuclear RNA is normally complexed with a specific set of proteins, forming ribonucleoprotein particles termed hnRNP. These particles are likely to be involved in mRNA processing. We have found that the structure of hnRNP is profoundly altered during the heat shock response in *Drosophila* cultured cells. Although hnRNA continues to be synthesized at a near-normal rate during heat shock, its assembly into hnRNP is incomplete, as evidenced by a greatly decreased protein content of the particles in Cs_2SO_4 density gradients. RNA-protein cross-linking conducted in vivo (Mayrand and Pederson, Proc. Natl. Acad. Sci. U.S.A. **78**:2208-2212, 1981) also reveals that hnRNA made during heat shock is complexed with greatly reduced amounts of protein. The block of hnRNP assembly occurs immediately upon heat shock, even before the onset of heat shock protein synthesis. Additional experiments reveal that hnRNP assembled normally at 25°C subsequently disassembles during heat shock. The capacity for normal hnRNP assembly is gradually restored after heat-shocked cells are returned to 25°C. Heat-shocked mammalian cells also show a similar block in hnRNP assembly. We suggest that incomplete assembly of hnRNP during heat shock leads to abortive processing of most mRNA precursors and favors the processing or export (or both) of others whose pathway of nuclear maturation is less dependent on, or even independent of, normal hnRNP particle structure. This hypothesis is compatible with a large number of previous observations.

Exposure of *Drosophila* embryos or cultured cells to elevated temperature elicits major changes in gene transcription and protein synthesis (2). Transcription of a small set of genes is activated by heat shock (4, 11), and the resulting heat shock mRNAs are rapidly exported to the cytoplasm where they are preferentially translated (19, 29, 47). Meanwhile, the appearance of new non-heat shock mRNAs in the cytoplasm ceases (46). It is not known whether this latter effect of heat shock reflects decreased rates of transcription, a reduced efficiency of mRNA processing, or both. We have previously reported that the rate of actin mRNA transcription rapidly decreases to a low level during heat shock in *Drosophila* cultured cells, suggesting that this particular mRNA may be regulated chiefly at the transcriptional level (11). However, there are other observations which indicate that transcription of heterogeneous nuclear RNA (hnRNA) continues during heat shock. First, although the rates of heat shock gene transcription are very high, these mRNAs nevertheless are found to comprise a surprisingly small fraction (2%) of the nonribosomal nuclear RNA that is synthesized during heat shock (11). Second, the size distribution of nonribosomal nuclear RNA made during heat shock is heterogeneous and is similar to that of nuclear RNA from non-heat-shocked *Drosophila* cells (23–26). In the present investigation we examined hnRNA transcription in more detail and find that it indeed continues at a nearly normal rate during heat shock in *Drosophila* cultured cells. In addition, and unexpectedly, we discovered that heat shock blocks the assembly of this hnRNA into its normal nuclear ribonucleoprotein (RNP) form. We suggest that this block in RNP assembly is related to post-transcriptional selection of mRNAs for nuclear export during heat shock.

(A brief account of this work was presented at the 22nd annual meeting of the American Society for Cell Biology, 30 November through 4 December 1982 [S. Mayrand and T. Pederson, J. Cell Biol. **95**:471a, 1982]).

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MATERIALS AND METHODS

Cell culture. *Drosophila* Kc0 cells were grown in Eschalier D-20 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 1.5% lactalbumin hydrolysate, 0.15% yeastolate, and 5% fetal calf serum

(M. A. Bioproducts, Walkersville, Md.). Before use, the serum was incubated at 56°C for 30 min. The cells were maintained at between approximately 1×10^6 and 2×10^6/ml in 150-cm^2 plastic flasks at 25 ± 0.5°C, with 50 ml of medium per flask. The precautions taken with respect to temperature control during cell harvesting and labeling are described in further detail below.

HeLa and mouse erythroleukemia cells were maintained as previously described (37, 41).

Heat shock, radioisotopic labeling, and cell fractionation. The desired number of cells were removed from flasks and transferred to a sterile 2-liter glass Erlenmeyer flask (maximum volume of cell suspension, 300 ml). 5-Fluorouridine was added to a concentration of 5 μg/ml, and the suspension was incubated in the nonstoppered flask for 1 h at 25 ± 0.5°C with occasional gentle swirling by hand. The cells were then centrifuged at 600 × *g* for 5 min at 21 to 26°C (the range of ambient temperature in the laboratory) in 600-ml conical glass bottles and resuspended at a density of 2×10^7 cells per ml in the original (i.e., supernatant) medium.

For labeling, the desired number of cells were gently pipetted into glass Erlenmeyer flasks at high cell surface area/cell suspension volume ratios. This was achieved by keeping the cell volume/flask (total) volume ratio between 0.08 and 0.16. After 30 min the control (25°C) cells were labeled for 20 min with [^{3}H]uridine (New England Nuclear Corp., Boston, Mass.) at a concentration of 100 μCi/ml. For heat shock, the cell suspension was incubated in a 50°C water bath until the cells had reached 34°C (approximately 30 s) and then transferred to a 37°C bath, which was taken as zero time of heat shock. Unless otherwise specified, the cells were labeled with [^{3}H]uridine (100 μCi/ml) between 5 and 25 min of heat shock. After labeling, both the 25 and 37°C cell suspensions were poured onto at least 20 volumes of ice-cold wash buffer (100 mM NaCl, 10 mM $CaCl_2$, 10 mM Tris-hydrochloride, pH 7.2) and centrifuged at 600 × *g* for 5 min at 21 to 26°C. (At the end of centrifugation, the temperature of the supernatant medium of the cell suspensions was 11 to 13°C.) All subsequent steps were performed at 2°C. The cell pellets were suspended in 5 volumes of ice-cold wash buffer, centrifuged again at 600 × *g* for 5 min, and then suspended in 10 volumes (relative to packed cell volume) of *Drosophila* lysis buffer (30 mM NaCl, 10 mM $CaCl_2$, 100 mM Tris-hydrochloride [pH 8.5], 1.0% Nonidet P-40 [Particle Data Laboratories Ltd., Elmhurst, Ill.]) prepared as a HeLa cell cytoplasmic extract as detailed by Findly and Pederson (11). After occasional mixing over a period of 10 to 15 min, cell lysis was monitored by phase-contrast microscopy. The lysate was centrifuged at 1,000 × *g* for 4 min, and the nuclear pellet was washed once in reticulocyte standard buffer (10 mM NaCl, 1.5 mM $MgCl_2$, 10 mM Tris-hydrochloride, pH 7.2). The nuclei were then suspended in a volume of reticulocyte standard buffer equal to four times the original packed cell volume and sonicated with two 10-pulses at 40 W (18). Nuclear disruption was monitored by phase-contrast microscopy. The nuclear sonicate was then layered on 25 ml of 30% sucrose in reticulocyte standard buffer and centrifuged for 15 min at 4,500 × *g* (5,000 rpm in a Beckman SW27 or SW28 rotor). The nucleoplasmic fraction banding at the 0:30% sucrose interface was removed for further use. As previously described for mammalian cells (31, 38, 39), this *Drosophila* nuclear fraction contains chromatin and hnRNP particles (S. Mayrand, S. George, and T. Pederson, unpublished results).

For the experiments with mammalian cells, HeLa or mouse erythroleukemia cells were harvested, pretreated with low concentrations of actinomycin (38, 41), and then incubated with [^{3}H]uridine for 20 min at either 37°C or the desired temperature (39 to 45°C). Cell fractionation and hnRNP isolation were performed as previously described (38, 41).

Cesium sulfate banding of hnRNP. Samples (0.3 to 0.5 ml) of the nucleoplasmic fractions from 25°C and heat-shocked *Drosophila* cells were layered on preformed Cs_2SO_4 gradients (1.25 to 1.75 g/cm^3) and centrifuged in a Beckman SW50.1 rotor at 34,000 rpm for 60 to 65 h at 20 ± 1°C (7). In the RNA-protein cross-linking experiments (see below), the nucleoplasmic fractions were mixed with a homogeneous Cs_2SO_4 solution having a density of 1.50 g/cm^3 (32) and then centrifuged as described above. The gradients were collected, and the density and radioactivity profiles were obtained as described previously (7).

RNA-protein cross-linking in vivo. Cross-linking experiments were performed two ways. In the first, cells were preincubated with 5-fluorouridine (5 μg/ml) for 1 h, pulse-labeled with [^{3}H]uridine at 25 or 37°C, harvested, and washed once, all as detailed above. The cells were then suspended in wash buffer at approximately 10^7 cells per ml and irradiated at 2°C with 254-nm light at 3,700 μW/cm^2 for 15 min (32). In the second approach, cells were harvested, preincubated with 5-fluorouridine as described above, and then transferred to water-jacketed chambers regulated at either 25 or 37°C. [^{3}H]uridine was then added, and after 5 min the cells were irradiated at 25 or 37°C with 254-nm light at 3,700 μW/cm^2 for 15 min. The temperatures of the cell suspensions were monitored and were constant throughout the irradiation procedure.

Nuclear RNA extraction. The nucleoplasmic fraction containing hnRNP was mixed with 10 volumes of 7 M guanidine hydrochloride (Chemical Dynamics Corp., South Plainfield, N.J.) in 0.02 M sodium acetate (pH 5.2). Portions of 30 ml were layered over 8 ml of 5.7 M CsCl in 0.1 M EDTA (pH 7.0) and centrifuged in a Beckman SW27 or SW28 rotor at 21,500 rpm for 48 h (20°C). Under these conditions RNA is pelleted while DNA and protein are not, as their buoyant densities are lower than that of 5.7 M CsCl (12). The RNA was dissolved in sterile deionized water, sodium acetate was then added to 0.2 M and pH 5.2, and the RNA was precipitated by the addition of ethanol to 67% (vol/vol). After 8 to 16 h at −20°C, the RNA was collected by centrifugation in acid-washed, sterile conical glass tubes in a Sorvall HB-4 swinging bucket rotor (10,000 rpm, 1 h, 4°C). Centrifugation in 5 to 20% sucrose gradients containing 99% dimethyl sulfoxide (DMSO) was performed as described previously (35).

Hybridization of RNA gel blots. Cytoplasmic RNA was extracted from 25°C cells or heat-shocked cells as described above for nucleoplasmic RNA. The RNA was dissolved in a small volume of water, and glyoxal and DMSO were added to final concentrations of 1 M and 50% (vol/vol), respectively. The glyoxal was deionized before use by passage over three consecutive columns of a mixed-bed ion-exchange resin [Bio-Rad Laboratories, Richmond, Calif.; AG501-X8(D)]. Sodi-

um phosphate buffer (pH 7.0) was added to 10 mM. The RNA was then denatured at 50°C for 1 h, cooled, and electrophoresed in a vertical 1.1% agarose gel. Transfer to nitrocellulose was as described previously (49). Pre-hybridization was for 16 to 20 h at 42°C in 50% formamide, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 50 mM sodium phosphate (pH 6.5), 250 μg of sonicated, heat-denatured salmon sperm DNA per ml, and 0.02% each of bovine serum albumin, Ficoll (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.), and polyvinyl pyrrolidone. The blot was then hybridized with a cloned *Drosophila* DNA fragment (pPW232.1) that contains the 5′ half of a 70,000-dalton heat shock protein gene inserted into pBR322 (30).

Plasmid DNA was prepared by the method of Clewell (9) from cleared lysates on ethidium bromide-CsCl gradients generated in a vertical rotor (Beckman VTi 50). The cloned DNA was then nick translated with [α-^{32}P]dCTP to a specific activity of 5×10^7 to 9×10^7 cpm/μg. The hybridization solution consisted of the pre-hybridization mixture described above plus 10% dextran sulfate. Hybridization was for 20 h at 42°C, followed by four washes with 500 ml of 2× SSC–0.1% sodium dodecyl sulfate at room temperature and then four washes with 500 ml of 0.1× SSC–0.1% sodium dodecyl sulfate at 50°C. The blots were exposed to preflashed X-ray film at −80°C with an intensifying screen (Du Pont Cronex Lightning-Plus; Du Pont Co., Wilmingtom, Del.).

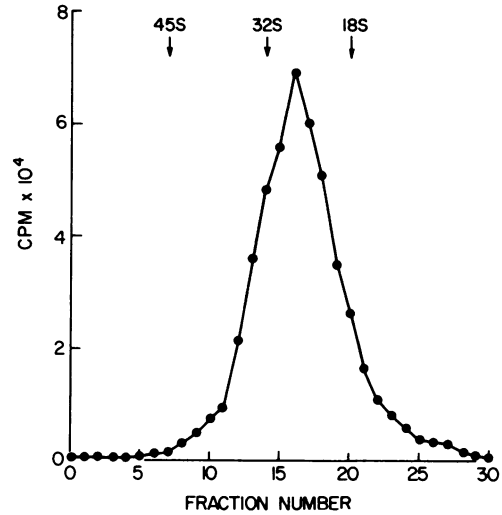

FIG. 1. DMSO-sucrose gradient of nucleoplasmic RNA labeled during heat shock. Cells were pulse-labeled for 10 min with [^{3}H]uridine between 15 and 25 min after incubation at 37°C. Nucleoplasmic RNA was extracted as detailed in the text, denatured (35), and centrifuged in a 5 to 20% sucrose gradient containing 99% DMSO (Beckman SW27 rotor, 27,000 rpm, 65 h, 28°C). The direction of sedimentation is from right to left.

RESULTS

hnRNA synthesis continues during heat shock. To examine hnRNA synthesis during heat shock, *Drosophila* Kc0 cells were exposed to 5-fluorouridine to selectively block rRNA synthesis (23) and then were pulse-labeled with [^{3}H]uridine for 20 min at 37°C. Nuclei were isolated and fractionated into nucleoplasmic and nucleolar fractions as previously described for mammalian cells (38) and as detailed above. Centrifugation in denaturing DMSO-sucrose gradients revealed that the nucleoplasmic RNA molecules labeled during heat shock were indeed heterogeneous in size, ranging from 10 to 40S (Fig. 1). Heat shock reduced the incorporation of [^{3}H]uridine into nucleoplasmic RNA of 5-fluorouridine-treated cells by an average of only 25%. These results indicate that hnRNA transcription continues at substantial rates in heat-shocked *Drosophila* Kc0 cells. Additional experiments with actinomycin (data not shown) confirmed that the high-molecular-weight nuclear RNA synthesized during heat shock is transcribed on DNA templates.

Because the persistence of a high rate of total hnRNA synthesis after heat shock was somewhat unexpected, we considered it necessary to verify that the cells were indeed undergoing a heat shock response. Therefore cells were incubated at either 25 or 37°C, and cytoplasmic RNA was analyzed by gel blot hybridization with a cloned *Drosophila* DNA fragment bearing a portion of the 70K heat shock protein gene (30). The cytoplasmic RNA of 25°C cells (Fig. 2, lane 1) did not contain detectable 70K gene transcripts under these conditions of hybridization and film exposure. In contrast, the heat-shocked cells displayed an RNA species (Fig. 2, lane 2) that hybridized with the probe and was also the size expected for the mRNA of the 70K heat shock protein (16).

hnRNP assembly is altered during heat shock. In mammalian cells, hnRNA is complexed with a specific set of nuclear proteins (39). These heterogeneous nuclear RNP (hnRNP) particles contain protein and RNA in a mass ratio of about 4:1 (7, 38, 42). A remarkable property of hnRNP particles is their ability to withstand isopycnic banding in Cs_2SO_4 without prior aldehyde fixation (7, 41). We have isolated hnRNP particles from *Drosophila* Kc0 cells (S. Mayrand and T. Pederson, unpublished data) and have found that they also are stable during centrifugation in Cs_2SO_4 and band at 1.34 g/cm^3, which indicates the same 4:1 protein-RNA composition as mammalian hnRNP (Fig. 3A). However, when hnRNP was isolated from cells pulse-labeled with [^{3}H]uridine during heat shock, the majority of the particles displayed a pronounced change in density and banded at approximately 1.63 g/cm^3. This density corresponds to a composition of about 10% protein–90% RNA. Protein-free RNA is 1.66 g/cm^3 in

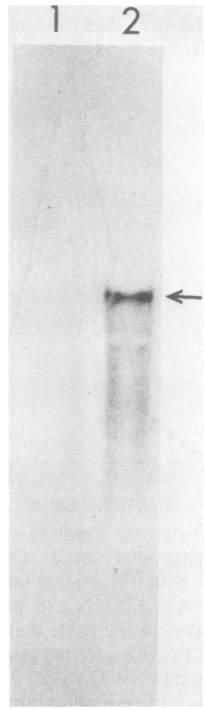

FIG. 2. Induction of mRNA for 70,000-molecular-weight heat shock protein. Cytoplasmic RNA from control (25°C) or heat-shocked (37°C, 25 min) cells was extracted, denatured, and subjected to electrophoresis as detailed in the text. After transfer to nitrocellulose, the blot was hybridized with a ^{32}P-labeled DNA probe to detect 70,000-molecular-weight heat shock protein mRNA sequences. Lanes: 1, 25°C cells; 2, heat-shocked cells.

these gradients (32, 40). This distinct shift in the density of hnRNP from 1.34 to 1.63 g/cm^3 after heat shock was consisently observed in each of many experiments. The average values for nine identical experiments were 86% of the hnRNP banding at a modal density of 1.34 g/cm^3 in control cells and 70% of the hnRNP banding at 1.63 g/cm^3 in heat-shocked cells. There was some experiment-to-experiment variability in both the control hnRNP profiles and in the proportion of hnRNP shifting to 1.63 g/cm^3 during heat shock (minimum of 31% to maximum of 100%). We suspect that this variability reflects subtle differences in the cells from one experiment to another (28). However, in no case did heat shock fail to cause a substantial increase in the proportion of hnRNP banding at 1.63 g/cm^3.

Incomplete assembly of hnRNP confirmed by RNA-protein cross-linking in vivo. The altered buoyant density of hnRNP from heat-shocked cells (Fig. 3) could be explained in two ways. Heat shock might induce alterations in hnRNA-protein associations that would render the isolated particles less stable. According to this model, the observed shift of a major fraction of the hnRNP from 1.34 to 1.63 g/cm^3 would be due to the stripping of protein from the hnRNP by the Cs_2SO_4 gradient itself, with heat shock simply predisposing the particles to this effect. Alternatively, heat shock might actually reduce the mass of hnRNP protein complexed with hnRNA in vivo, in which case the observed Cs_2SO_4 profiles would be a reflection of preexisting differences in the compositions of the particles. To distinguish between these two possibilities, we made use of an in vivo RNA-protein cross-linking procedure.

As we previously reported (32, 33), irradiation of intact cells with 254-nm light can be used to induce the formation of covalent cross-links between hnRNA and the nuclear proteins with which it is in close contact. These in vivo-cross-linked particles can then be analyzed by Cs_2SO_4 isopycnic banding to assess the relative mass of protein cross-linked to the RNA (32). *Drosophila* cells were pulse-labeled with [^{3}H]uridine at either 25 or 37°C and irradiated at 2°C with 254-nm light. hnRNP particles were isolated and centrifuged in Cs_2SO_4. By selecting the proper

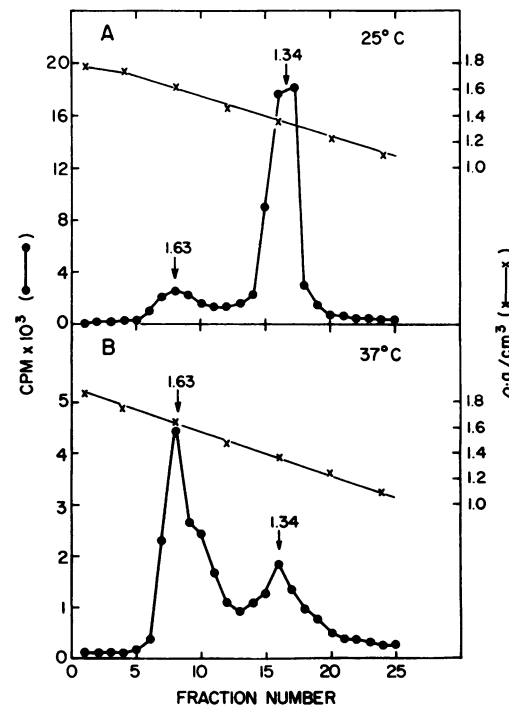

FIG. 3. Cs_2SO_4 banding of hnRNP from control and heat-shocked cells. Particles were isolated from cells labeled for 20 min with [^{3}H]uridine at 25°C (A) or 37°C (B). Details of centrifugation are given in the text.

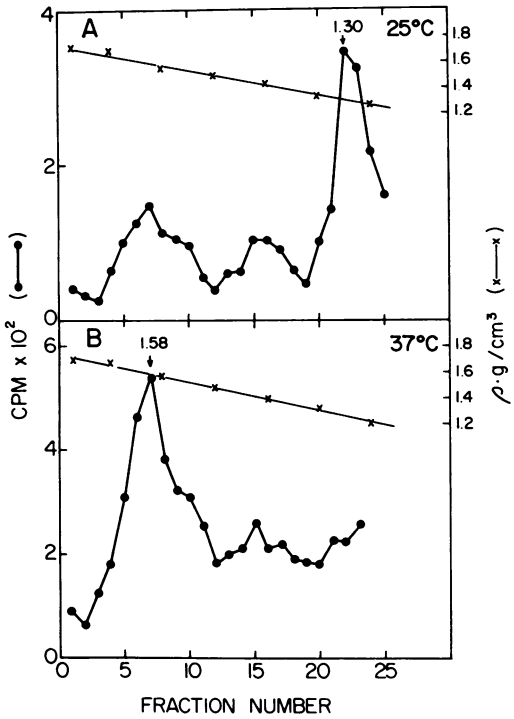

FIG. 4. In vivo RNA-protein cross-linking. Cells were pulse-labeled at 25°C (A) or 37°C (B) as in Fig. 3 and then irradiated at 2°C with 254-nm light as detailed in the text and in our previous publications (32, 33). hnRNP particles were then isolated and banded in Cs_2SO_4 gradients having an initial density of 1.50 g/cm^3.

initial Cs_2SO_4 concentration (e.g., 1.50 g/cm^3), conditions can be arranged in which, unlike the preformed gradients used in Fig. 3, non-cross-linked hnRNP is completely dissociated into RNA and protein during the centrifugation, whereas cross-linked particles withstand these conditions and band at their usual density of approximately 1.35 g/cm^3 (32). In this latter type of gradient, hnRNP from non-irradiated *Drosophila* cells (both control and heat shocked) banded as protein-free RNA at 1.66 g/cm^3 (data not shown), establishing that these centrifugation conditions completely dissociate *Drosophila* particles, as reported previously for mammalian hnRNP (32). In contrast, particles from irradiated, 25°C cells banded at 1.34 g/cm^3, verifying that cross-linking had indeed occurred (Fig. 4A). However, as shown in Fig. 4B, the majority of the particles from cells that were irradiated after heat shock displayed the same shift to higher density as had been observed in the earlier experiments with non-cross-linked particles (Fig. 3). This result demonstrates that the relatively protein-free structure of hnRNP from heat-shocked cells exists at the time of cross-linking, before cell fractionation and Cs_2SO_4 banding. This conclusion is further reinforced by the observation that the same results as shown in Fig. 4 were obtained when cells were irradiated at 25°C or during heat shock, rather than at 2°C after heat shock (see above for details). This latter result proves that hnRNA is associating with greatly reduced amounts of protein immediately after its transcription in the heat-shocked cell.

The shift in hnRNP buoyant density (Fig. 3 and 4) is not a direct effect of temperature per se on particle structure. Nuclei or hnRNP particles were isolated from cells pulse-labeled with [^{3}H]uridine at 25°C and were then incubated at 37°C for 25 min. Cs_2SO_4 gradient analysis revealed that normal hnRNP structure was maintained (data not shown).

Heat shock alters hnRNP structure in mammalian cells. It was of interest to determine whether heat shock of mammalian cells also leads to altered hnRNP particle assembly. Cultured human (HeLa) cells were pulse-labeled for 20 min with [^{3}H]uridine at either 37 or 43°C, and hnRNP particles were isolated and analyzed in Cs_2SO_4 gradients. hnRNP particles from non-heat-shocked HeLa cells banded at 1.34 g/cm^3 (Fig. 5A), as previously reported (7). In contrast, the hnRNP isolated from heat-shocked cells (Fig. 5B) showed a shift to a density of 1.63 g/cm^3, yielding a pattern almost identical to that observed with *Drosophila* (Fig. 3). A similar effect was observed with mouse erythroleukemia cells (C. M. Morganelli, S. Mayrand, and T. Pederson, unpublished results). Additional experiments with HeLa cells reveal that the alteration of hnRNP assembly shown in Fig. 5 begins to occur at temperatures of 39 to 41°C (C. M. Morganelli, S. Mayrand, and T. Pederson, unpublished results), which is also the temperature at which protein synthesis starts to decline in these cells (13, 34).

Further characteristics of the heat shock effect on hnRNP assembly. Heat shock apparently leads to an alteration of hnRNP structure even on hnRNA transcripts synthesized at 25°C. After *Drosophila* cells were pulse-labeled at 25°C with [^{3}H]uridine for 20 min, further transcription was blocked by the addition of high concentrations of actinomycin and 5-fluorouridine for 15 min. These inhibitors reduce *Drosophila* cell nuclear RNA synthesis by 97 to 98% (data not shown). One-half of the cells were then heat shocked (37°C) for 25 min. Cs_2SO_4 analysis revealed that a substantial fraction of the hnRNA made at 25°C banded at 1.60 g/cm^3 after exposure of the cells to heat shock conditions in the absence of further transcription (Fig. 6B). This effect was not seen in cells incubated with the inhibitors at 25°C and thus cannot be attrib-

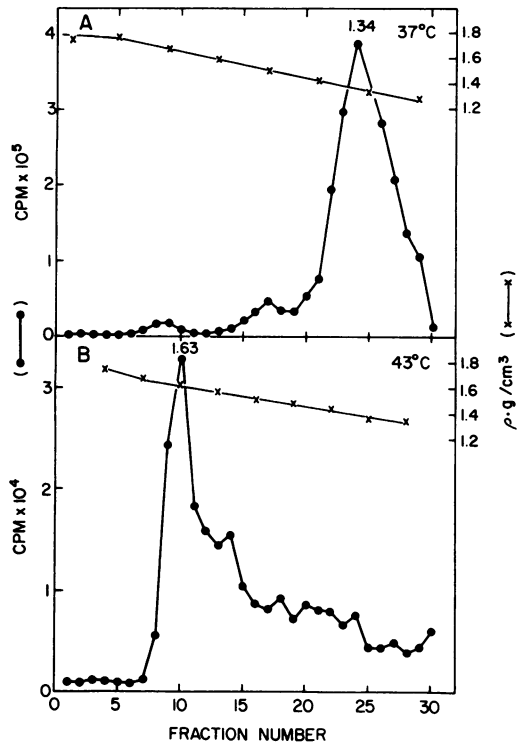

FIG. 5. Heat shock blocks hnRNP assembly in mammalian cells. HeLa cells were labeled for 20 min with [^{3}H]uridine in the presence of 0.04 μg of actinomycin per ml at 37°C (A) or 43°C (B).

uted to the inhibition of transcription per se (Fig. 6A). These results indicate that hnRNP particles assembled at 25°C subsequently become partially disassembled during heat shock.

The fate of hnRNA made during heat shock was also examined upon return of cells to 25°C. After the cells were labeled with [^{3}H]uridine for 20 min at 37°C, actinomycin and 5-fluorouridine were added, and the cells were then returned to 25°C for 20 min. Cs_2SO_4 analysis revealed that, as usual, hnRNA made at 37°C banded at approximately 1.63 g/cm^3, and that this continued to be the case after returning cells to 25°C (data not shown). Of course, this experiment is more difficult to interpret than the one shown in Fig. 6, because the restoration of normal nuclear RNP assembly after heat shock may require new transcription.

To examine the immediacy of the heat shock-induced block in hnRNP assembly, cells were pulse-labeled at different intervals after transfer to 37°C. Figure 7A shows the results obtained with the standard conditions of heat shock and labeling used throughout this investigation: 5 min at 37°C followed by an additional 20 min at 37°C with [^{3}H]uridine. As usual, the majority of hnRNP banded at about 1.62 g/cm^3. Figure 7B shows the results obtained when a 5-min [^{3}H]uridine pulse was conducted in the interval between 5 and 10 minutes of heat shock. Even at this very early time of heat shock, a pronounced block of hnRNP assembly was observed. Similar results were obtained when a 5-minute label was administered from 20 to 25 min after heat shock (Fig. 7C). Thus, the alteration of hnRNP assembly was virtually an immediate consequence of heat shock (Fig. 7B) and continued to be in effect for at least 25 min (Fig. 7C).

Heat-shocked *Drosophila* cells gradually resume normal patterns of gene transcription several hours after return to 25°C (11), and it was therefore of interest to examine the restoration of hnRNP assembly. Figure 8 shows the typical results of a recovery experiment. In Fig. 8A, cells were pulse-labeled for 5 min at 37°C. The expected Cs_2SO_4 pattern was observed. In panel B, at 30 min after the cells were returned to 25°C, followed by a 5-min pulse, approximately one-third of the hnRNP banded at a density (~1.41 g/cm^3) approaching that of normal hnRNP (~1.32 g/cm^3) (Fig. 8B). The resumption of normal hnRNP assembly is more pronounced in Fig. 8C where, after 60 min of recovery at

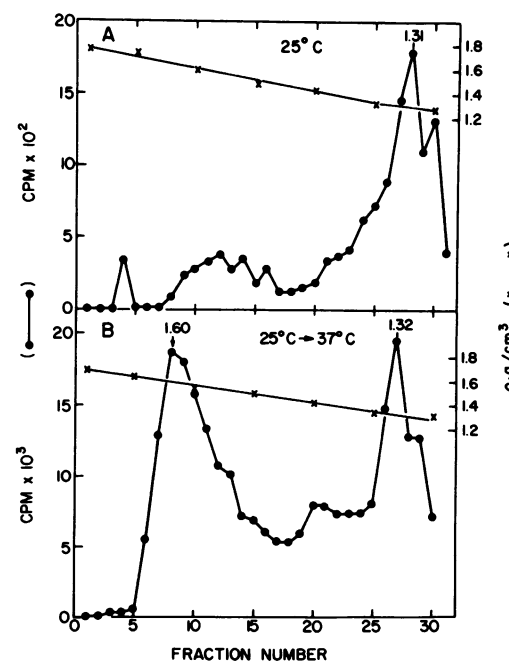

FIG. 6. Drosophila hnRNP assembled at 25°C disassembles after heat shock. Cells were labeled for 20 min with [^{3}H]uridine at 25°C in the presence of 5-fluorouridine (5 μg/ml). Actinomycin and 5-fluorouridine were then both added at 100 μg/ml, and incubation was continued at 25°C for 15 min. One-half of the culture was then heat shocked (37°C) for 20 min (B), and the other half was left at 25°C for 20 min (A).

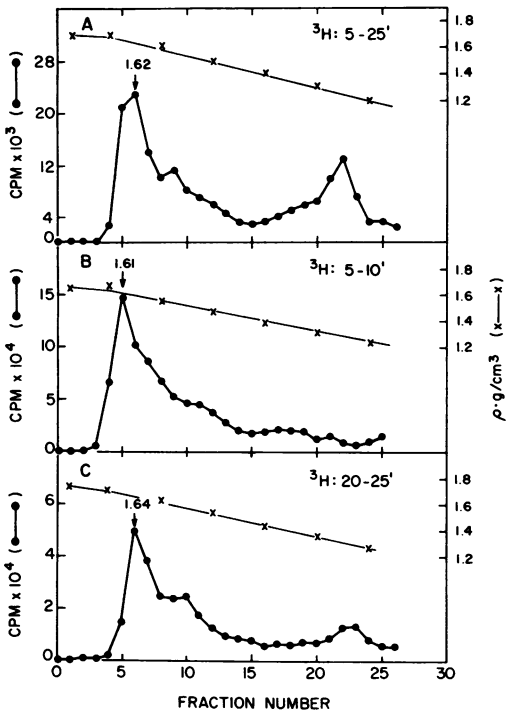

FIG. 7. Immediacy of the heat shock effect on hnRNP assembly. Cells were pretreated with 5-fluorouridine (5 μg/ml) and then labeled with [^{3}H]uridine from 5 to 25 min of heat shock (A), from 5 to 10 min of heat shock (B), or from 20 to 25 min of heat shock (C).

25°C, about 60% of the pulse-labeled hnRNP banded with a median density of 1.32 g/cm^3. However, even after 60 min of recovery, a substantial fraction (~40%) of the hnRNP still banded at the high density characteristic of heat shock. Moreover, the material banding at 1.32 g/cm^3 appeared to be considerably more heterogeneous in density than normal hnRNP (compare Fig. 8C with Fig. 3A). Therefore, normal assembly of hnRNP particles does not resume abruptly after heat shock, but does so in a gradual fashion. This is also the case for the resumption of normal transcription and protein synthesis (11, 28).

DISCUSSION

hnRNA synthesis during heat shock. We have found that synthesis of high-molecular-weight, nonribosomal nucleoplasmic RNA continues at very high rates in heat-shocked *Drosophila* cultured cells. The persistence of substantial hnRNA synthesis after heat shock raises the possibility of a post-transcriptional control of the production of 25°C mRNAs under heat shock conditions. Because of its potential importance for understanding the heat shock response, and because of its relevance to the

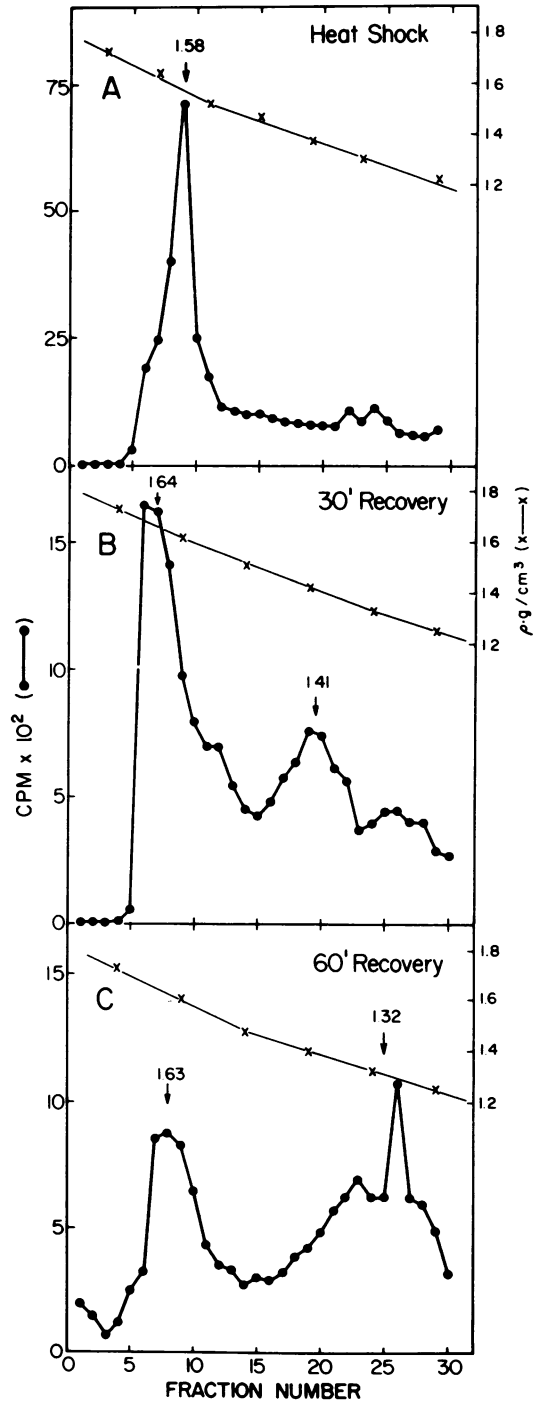

FIG. 8. Recovery of hnRNP assembly after heat shock. Cells were incubated with 5-fluorouridine (5 μg/ml) for 1 h at 25°C and then heat shocked for 25 min. One-third of the cells were pulse-labeled with [^{3}H]uridine from 20 to 25 min of heat shock (A). One-third of the cells were returned to 25°C and after 30 min were pulse-labeled for 5 min (B). One-third of the cells were returned to 25°C and after 60 min were pulse labeled for 5 min (C).

interpretation of the altered hnRNP assembly described here, we review what is known regarding nuclear RNA synthesis during heat shock in *Drosophila*. We also examine the validity of the prevalent belief that heat shock dramatically inhibits total chromosomal (hnRNA) transcription.

Levis and Penman (26) and Lengyel et al. (24) analyzed pulse-labeled, nonribosomal nuclear RNA made during heat shock in a line of *Drosophila* Schneider cells adapted to grow in mammalian cell culture medium. This RNA was found to sediment heterogeneously in (nondenaturing) sucrose gradients, with the majority of the molecules at 15 to 35S. This is very similar to the gradient profiles we observed in heat-shocked Kc0 cells when the RNA was analyzed in totally denaturing DMSO-sucrose gradients (Fig. 1).

Heat shock has been studied for the most part in *Drosophila* larval tissue rather than cultured cells (2). Therefore it is possible to argue that the persistence of hnRNA synthesis after heat shock (Fig. 1; 24, 26) is a specific feature of rapidly growing cultured cells and does not occur in the nondividing larval polytene tissues in which heat shock is commonly studied. A detailed analysis of nuclear RNA in heat-shocked larvae was made by Henikoff and Meselson (14). They found that this RNA, too, was of very high molecular weight, migrating heterogeneously from 15S to approximately 40S in denaturing (methyl mercuric hydroxide) gels. Second, they hybridized this RNA to polytene chromosomes and, although detailed grain counts were made only over two heat shock loci, 87A and 87C, the nuclear RNA labeled during heat shock was observed to also hybridize at many additional sites (14). A similar finding was reported by Lengyel et al. (Fig. 5d in reference 24). This point is also established by direct autoradiographic analysis of [^{3}H]uridine labeling in larval salivary gland chromosomes after heat shock. Although labeling is especially pronounced over heat shock loci, numerous grains are observed throughout the chromosomes which, when added up, constitute a major fraction of the total [^{3}H]uridine labeling (for clear examples see Fig. 7b and 7c in reference 3, Fig. 4a in reference 4, and Fig. 2a in reference 21). However, it is to be noted that some chromosomal loci that display especially high rates of transcription at 25°C do show a pronounced reduction in [^{3}H]uridine labeling after heat shock. These may represent 25°C transcription sites that are especially temperature sensitive. Actin gene transcription may be an example of such a case: its rate declines by 95% after heat shock, whereas total hnRNA synthesis is much less affected (11). These considerations indicate that there is not a contradiction between the biochemical observation that hnRNA synthesis continues at substantial rates after heat shock and the overall picture that emerges from [^{3}H]uridine labeling of polytene chromosomes and in situ hybridization with heat shock nuclear RNA.

Heat shock block on hnRNP assembly. The central conclusion from this study is that despite the continued synthesis of hnRNA, these transcripts do not assemble into complete hnRNP particles during heat shock. The in situ RNA-protein cross-linking results (Fig. 4) establish that the altered hnRNP structure observed by isopycnic banding of the particles occurs in vivo and not during hnRNP isolation or Cs_2SO_4 centrifugation. The hnRNA made during heat shock apparently does assemble with some protein, however, because its average density in Cs_2SO_4 is consistently 1.60 to 1.63 g/cm^3, whereas the density of completely protein-free RNA is 1.66 g/cm^3 in these gradients (32, 40).

An important distinction to be evaluated is whether the effect on hnRNP assembly is a macromolecule-regulated aspect of the heat shock response. For example, it is conceivable that free hnRNP proteins present in the nucleus just before heat shock undergo modifications that greatly reduce their affinity for hnRNA. One obvious possibility is hyperphosphorylation, which might reduce electrostatic interactions of hnRNP proteins with hnRNA. The effect could also be due to a sudden cessation of hnRNP protein translation upon heat shock, so that the preexisting nuclear pool of free hnRNP proteins (which appears to be small in the first place [41]) becomes rapidly depleted after heat shock. We do not think that the effect on hnRNP assembly is actually dependent on the heat shock proteins themselves, because it can be observed within 10 min after heat shock (Fig. 7), which is before appreciable translation of the heat shock proteins has occurred (2, 28).

In contrast to this category of possibilities, it may simply be that normal binding equilibria between hnRNA and the hnRNP proteins are shifted by some change in physicochemical parameters inside the nucleus of the heat-shocked cell. These could include changes in nuclear water content, pH, or other ion activities, to mention only three of many possibilities. However, if such changes do occur after heat shock, it is clear that they do not lead to disruption of all nucleic acid-protein interactions in the nucleus. Nuclease digestion studies reveal that bulk DNA in the chromatin of heat-shocked *Drosophila* cells retains normal nucleosome structure (51), and we recently reported that the RNP particles that contain small nuclear RNA U1 are assembled normally during heat shock in *Drosophila* Kc0 cells (50).

hnRNP structure and mRNA processing. The findings reported here demonstrate that hnRNP structure is profoundly altered during heat shock. If normal hnRNP particle structure is important in mRNA processing, how then can the heat shock mRNAs be processed under this condition of blocked hnRNP assembly? One obvious possibility is that hnRNP assembly is blocked only for non-heat shock mRNA precursors, with transcripts of the heat shock genes still assembling into normal particles. Because the heat shock gene transcripts comprise only a few percent of the pulse-labeled nuclear RNA (11), they could be overlooked in the Cs_2SO_4 profiles of total hnRNP from heat-shocked cells, where there is almost always some material still banding at the usual hnRNP density of 1.35 g/cm^3. However, preliminary results suggest that heat shock gene transcripts band at 1.60 g/cm^3, along with the bulk hnRNP of heat-shocked cells (S. Mayrand and T. Pederson, unpublished data). This leads us to propose an alternative possibility.

As a working hypothesis, we speculate that certain gene transcripts do not use a complete hnRNP particle stage altogether during their nuclear processing and maturation. Instead of forming the usual 1.34-g/cm^3 RNP structures (80% protein–20% RNA), such transcripts could simply combine with a small set of proteins needed for their (different) pathway of nuclear maturation. This RNP structure might be more like that of polyribosomal messenger RNP, which has a much lower protein/RNA ratio than hnRNP (20, 42). The most obvious candidates for this hypothetical class of different RNP are the transcripts of genes that lack intervening sequences. These may have to undergo only a fairly limited number of modifications, perhaps just polyadenylation, before export to the cytoplasm. The lack of splicing, a process which now appears to be extremely complex, might obviate the need for these transcripts to be packaged into an extensive hnRNP particle structure.

This hypothesis is compatible with a number of observations. All but one of the heat shock mRNA-coding genes lack intervening sequences (10, 16). Moreover, the one heat shock gene that does contain an intron (15) is expressed at substantial levels in non-heat-shocked cells (8, 28) and could therefore be regulated at the translational level. The possibility that the heat shock mRNAs may have a different nuclear maturation pathway is further supported by 5′-methylated nucleotide analysis, which reveals a different cap structure than most 25°C mRNA (26). The hypothesis is also compatible with the continued entry into the cytoplasm of newly made histone mRNA during heat shock (45), as these genes also lack intervening sequences (27). Moreover, in mammalian cells histone mRNA sequences have a very short nuclear residence time and reach the cytoplasm faster than most other mRNAs (1). This could also reflect a nuclear maturation pathway that circumvents hnRNP formation.

It may be that evolution has strongly restricted the interruption by introns of those genes coding for proteins that must be produced for cell survival during environmental stress if, as we suggest, the block of hnRNP assembly under such circumstances leads to abortive nuclear processing of those mRNA precursors that do require splicing. In this context it is intriguing that the genes for both α- and β-interferons lack intervening sequences (17, 22, 36, 48). These genes must produce functional mRNAs under the stress of viral infection, which, interestingly, is usually accompanied by fever in the intact organism. Indeed, clinical studies have shown that interferon production parallels the course of fever (43), and our results demonstrate that, in HeLa cells, hnRNP assembly is blocked at temperatures (39 to 40°C; ca. 102 to 104°F) which are well within the range of fever in humans. In addition, as predicted by our model, interferon production by virus-infected cells in culture is not blocked by heat shock (44).

It may not be splicing per se (or the presence of introns in the primary transcript) that distinguishes hnRNAs that assemble fully into hnRNP particles from those that do not. Perhaps transcripts that undergo splicing require an extensive hnRNP particle structure not for the steps of intron removal and RNA ligation themselves, but for subsequent events that are obligatory for the further maturation of spliced mRNAs. This might involve, for example, a route of nuclear export different from that used by intron-lacking mRNAs. We also enter the qualification that heat shock need not necessarily favor the processing and nuclear export of all intron-lacking mRNAs, or not favor that of all intron-containing mRNAs. There may well be other factors, for example, interactions with various small nuclear RNAs (5, 6), that might allow some intron-containing mRNAs to be processed at a rate superior to most others during heat shock. The mRNA precursor for the 83,000-molecular-weight heat shock protein, which contains an intron (15), may be an example of such a case.

Although the actual significance of the block in hnRNP assembly during heat shock remains unclear, the hypothesis we have presented does appear to be congruent with all the available information and, moreover, makes several specific and readily testable predictions. In addition, quite apart from possibly providing future insights into mRNA processing during the heat shock response, the block in hnRNP assembly

we have described may be useful in studying the normal process of hnRNP formation itself. For example, it may be possible to exploit this effect to define steps in hnRNP assembly in the same way that conditional lethal (i.e., temperature-sensitive) mutants have been used to elucidate the morphogenesis of bacteriophage and animal viruses.

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