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Heat-shock proteins are associated with hnRNA in *Drosophila melanogaster* tissue culture cells

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Ribonucleoprotein complexes of *Drosophila melanogaster* Kc tissue culture cells grown at 24°C or heat-shocked at 37°C were cross-linked *in vivo* by u.v. irradiation. Cross-linked heterogeneous nuclear ribonucleoprotein (hnRNP) complexes were fractionated by oligo(dT)-cellulose chromatography and CsCl density centrifugation. The hnRNP complexes of both 24°C and 37°C culture cells possess buoyant densities in CsCl between ϱ = 1.38 g/cm^{-3} and 1.43 g/cm^{-3}. The ^{35}S-labelled proteins bound to the hnRNA of 37°C culture cells correspond in mol. wt. to the so-called heat-shock proteins of 70 K, 68 K, 27 K, 26 K, 23 K and 22 K. The 70 K and 68 K proteins are also present in hnRNP complexes of 24°C culture cells. In addition, several other *Drosophila* hnRNPs of 140 K, 56 K, 45 K, 43 K, 38 K, 37 K and 34 K, whose synthesis is strongly repressed under heat-shock conditions, could be identified. The results demonstrate that the so-called heat-shock proteins possess a function as RNPs.

Key words: heat shock/u.v. cross-linking/hnRNP/protein analysis

Introduction

In response to an elevation of temperature from 24°C to 37°C, *Drosophila* cells react with an enhanced synthesis of a small number of so-called heat-shock (hs) proteins (for review, see Ashburner and Bonner, 1979). It now appears that the heat-shock response is not restricted to *Drosophila* cells alone but apparently represents a general biological reaction to environmental stress, as has become evident by the identification of hs proteins in a wide range of eukaryotes besides insects, including amphibians and mammalian cells (Bienz and Gurdon, 1982; Thomas *et al.*, 1982). Despite the recent progress in the understanding of the structure of heat-shock genes (Pelham, 1982) and the localization of the hs proteins within the *Drosophila* cell (Lindquist, 1980), very little is known about the function of these proteins.

We have investigated the heat-shock response in *D. melanogaster* tissue culture cells at the level of ribonucleoprotein (RNP) structure. Proteins which are tightly associated with heterogeneous nuclear RNA (hn RNA) and mRNA were cross-linked *in vivo* by u.v.-irradiation of intact *Drosophila* cells. Our results indicate that the so-called hs proteins possess not only a function as RNP proteins in the nucleus and the cytoplasm in heat-shocked culture cells, but possibly also in cells which are grown at 24°C.

Results

HnRNA and protein of *D. melanogaster* tissue culture cells grown at 24°C or heat-shocked at 37°C were labelled for 1 h with 5-[^{3}H]uridine and L-[^{35}S]methionine, respectively. The RNA protein complexes were cross-linked *in vivo* by u.v. irradiation of intact cells immediately after the incubation. The covalently cross-linked hnRNA-protein complexes were isolated from purified nuclei and analysed by CsCl gradient centrifugation. Prior to isopycnic gradient analysis, the isolated hnRNP complexes were treated by extraction in an SDS-high-salt-formamide solution (Mayrand *et al.*, 1981), a step which is essential for the complete separation of non-cross-linked proteins from the hnRNA, which is in turn a prerequisite for the thorough analysis of hnRNA cross-linked proteins. As shown in Figure 1, the hnRNP complexes band between ϱ = 1.38 g/cm^{-3} and ϱ = 1.43 g/cm^{-3} in CsCl corresponding to a composition of 75% protein and 25% RNA, values which are typical for hnRNP of *Drosophila* and other higher eukaryotes (Kloetzel *et al.*, 1981, 1982). A similar result was also obtained when the hnRNP were fixed with formaldehyde prior to centrifugation (data not shown). Identical buoyant densities were found for both the total hnRNP fractions of heat-shocked and non-heat-shocked tissue culture cells.

To check whether the ^{35}S-labelled protein is indeed only associated with hnRNA and not with DNA, which under our experimental conditions remains unlabelled, cross-linked hnRNP from pooled CsCl gradient fractions was incubated for 1 h at 37°C with 25 μg/ml heat-treated pancreatic RNase A and re-centrifuged (Figure 1). The result shows that the ^{35}S label is displaced from the buoyant density position of ~ϱ = 1.41 /cm^{-3} to ϱ = 1.31 g/cm^{-3}, a value expected for free protein in CsCl. Treatment of the hnRNP with DNase I, had no effect on the ^{35}S banding pattern.

Fractionation and analysis of hnRNP

HnRNP complexes of heat-shocked and non-heat-shocked culture cells labelled with [^{35}S]methionine and [^{3}H]uridine

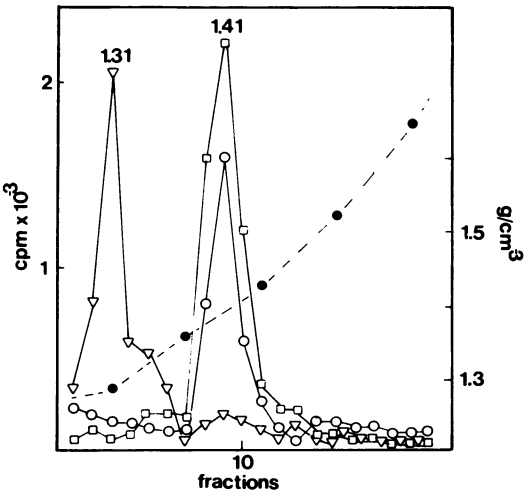

Fig. 1. CsCl density gradient analysis of [^{3}H]uridine and [^{35}S]methionine labelled u.v. cross-linked hnRNP complexes of heat-shocked *D. melanogaster* tissue culture cells. ○——○, RNA; □——□, protein; △——△, protein distribution after incubation of the isolated hnRNP in the presence of 25 μg/ml heat-inactivated RNase A.

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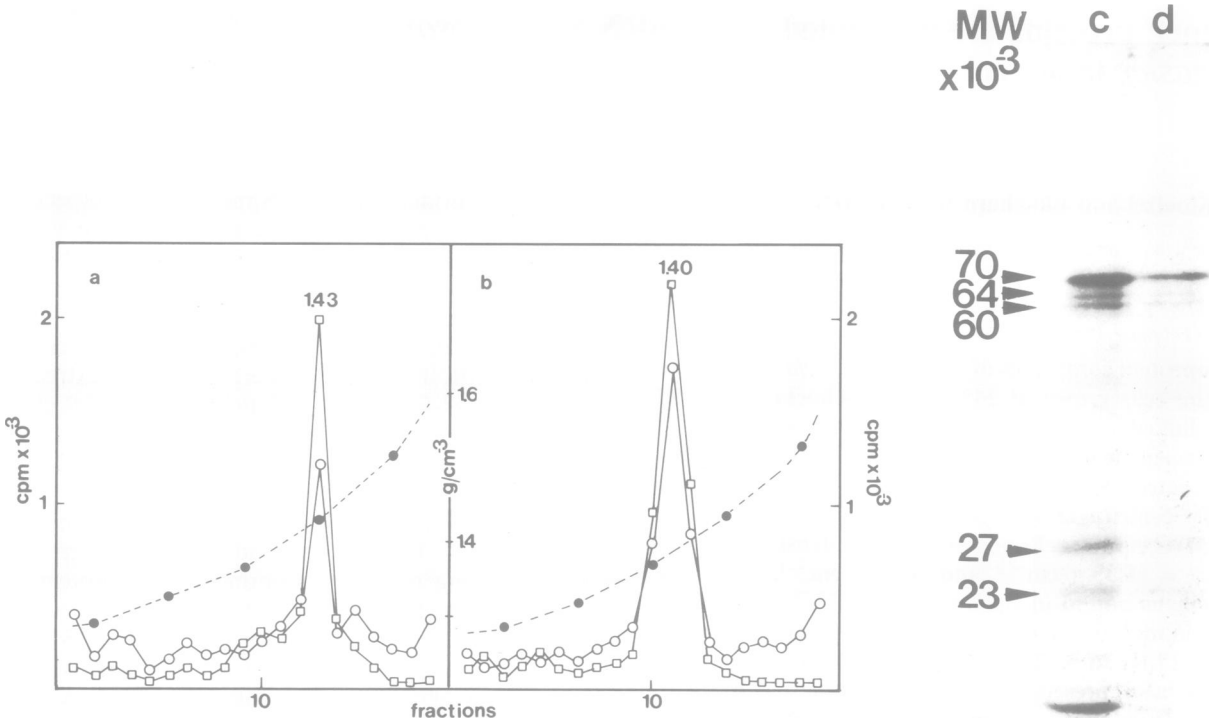

Fig. 2. CsCl gradient analysis of hnRNP of 37°C heat-shocked culture cells after oligo(dT)-cellulose chromatography; electrophoretic analysis of the protein content of the poly(A)$^+$ and poly(A)$^-$ hnRNP-complexes. **(a)** Poly(A)$^+$ hnRNP; **(b)** poly(A)$^-$ hnRNP; ○——○, [^{3}H]RNA; □——□, [^{35}S] protein; **(c)** proteins isolated from the poly(A)$^+$ hnRNP fraction of 1.43 g/cm^{-3}; **(d)** proteins isolated from the poly(A)$^-$ hnRNP fraction of 1.38–1.42 g/cm^{-3}.

were prepared for oligo(dT)-cellulose chromatography as described in Materials and methods. The poly(A)$^+$ hnRNP of non-heat-shocked culture cells contains 21% of the labelled hnRNA and 17% of the labelled protein with respect to the total nuclear RNP fraction, while the corresponding values for the poly(A)$^+$ hnRNP of heat-shocked culture cells are 29% for the RNA and 33% for the protein. The data demonstrate that the poly(A)$^+$ hnRNP fraction of heat-shocked culture cells contains not only a relatively higher percentage of labelled poly(A)$^+$ hnRNA than that of culture cells grown at 24°C but also a considerably higher percentage of ^{35}S-labelled protein. These results suggest two possible explanations. Either the poly(A)$^+$ hnRNP of heat-shocked culture cells, which includes labelled heat-shock transcripts as well as an unknown amount of unlabelled pre-existing 24°C hnRNA, is more densely packed (i.e., higher protein:RNA ratio) and hence contains a higher percentage of ^{35}S-labelled protein, or the proteins synthesized during heat-shock and transported into the nucleus become associated with poly(A)$^+$ hnRNA. Therefore, the isolated poly(A)$^+$ and poly(A)$^-$ hnRNP of both 24°C and 37°C tissue culture cells were further fractionated by CsCl density gradient centrifugation and the protein moiety analysed.

Despite the observed difference in the content of ^{35}S-labelled proteins, the isolated poly(A)$^+$ hnRNP complexes of both heat-shocked and non-heat-shocked culture cells possess identical buoyant densities of ϱ = 1.43 g/cm^{-3} (Figures 2a, 3a). Thus the observed accumulation of labelled protein in the nuclear poly(A)$^+$ hnRNP of heat-shocked cells is not due to a higher protein-RNA packaging ratio in heat-shocked hnRNP but appears to be the result of a preferential association of proteins synthesized during the heat-shock with nuclear poly(A)$^+$ hnRNA.

As shown in Figures 2b and 3b, the poly(A)$^-$ hnRNP complexes of heat-shocked and non-heat-shocked culture cells also band at identical densities of between ϱ = 1.38 g/cm^{-3} and 1.42 g/cm^{-3}, which are, however, slightly lower than those observed for the poly(A)$^+$ hnRNP complexes.

Electrophoretic analysis of hnRNP proteins

For the electrophoretic analysis of ^{35}S-labelled hnRNPs CsCl gradient fractions containing the isolated hnRNP were pooled and extensively digested with RNase A and micrococcal nuclease to release the cross-linked proteins. As control, total ^{35}S-labelled proteins of isolated nuclei and cytoplasm from 24°C and 37°C tissue culture cells were also analysed (Figure 4a–d). The most prominent hs protein of 70 K can be found in both the nuclear and the cytoplasmic fraction while the hs protein 84 appears to be almost entirely cytoplasmic. The smaller hs proteins of 27/26 K and 23/22 K are predominantly of nuclear location although minor amounts can also be detected in the cytoplasm.

Thus, the overall distribution of the hs proteins between the major cell compartments and the protein pattern itself are in good agreement with published data (Arrigo *et al.*, 1980; Lindquist, 1980; Velazquez *et al.*, 1980). Although the synthesis of these hs proteins appears to be enhanced on temperature elevation to 37°C, close analysis of the protein pattern of ^{35}S-labelled proteins in 24°C culture cells shows that proteins of identical mol. wt. are also present at the normal growth temperature and possess a similar distribution pattern between the two cell compartments.

The electrophoretic separation of hnRNP proteins reveals that ^{35}S-labelled proteins of 70 K/68 K, 64 K, 60 K, 27/26 K and 23/22 K are the major proteins bound to the poly(A)$^+$ and poly(A)$^-$ hnRNA of heat-shocked tissue culture cells (Figure 2c, d). While the 70 K/68 K and the lower mol. wt. hs proteins, as judged by their enhanced synthesis after heat-

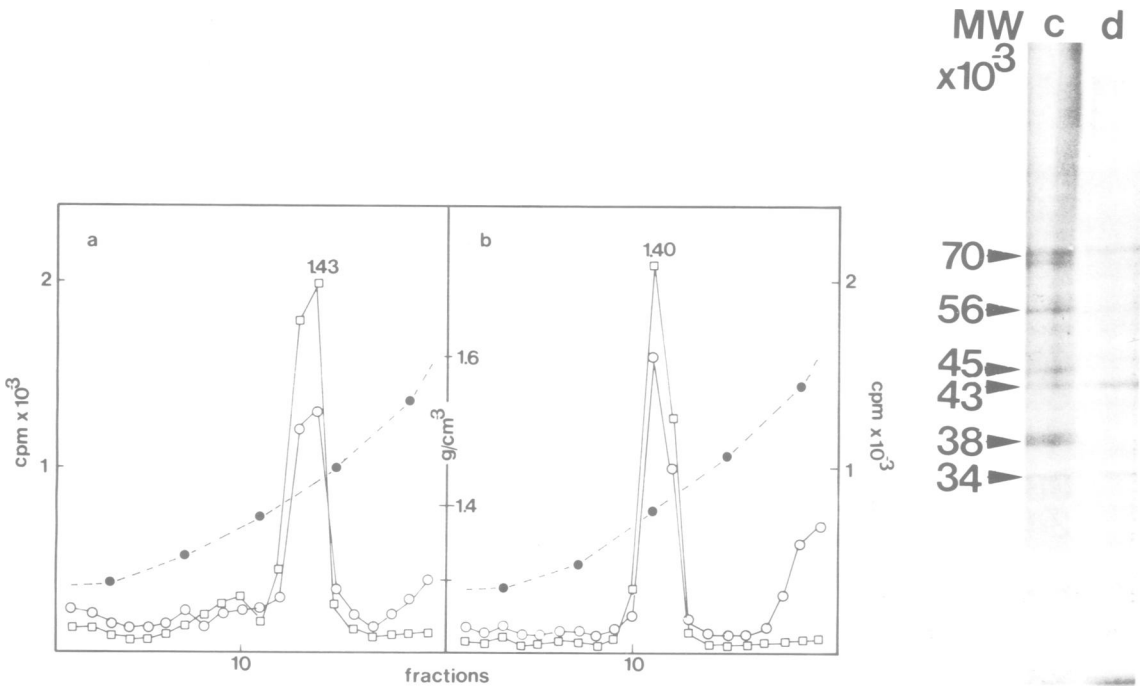

Fig. 3. CsCl gradient analysis of hnRNP of 24°C culture cells after oligo(dT)-cellulose fractionation; electrophoretic analysis of the isolated protein from the poly(A)$^+$ and poly(A)$^-$ hnRNP complexes. **(a)** Poly(A)$^+$ hnRNP; **(b)** poly(A)$^-$ hnRNP; ○——○, [^{3}H]RNA; □——□, [^{35}S]protein; **(c)** proteins isolated from the poly(A)$^+$ hnRNP fraction of 1.43 g/cm^{-3}; **(d)** proteins isolated from the poly(A)$^-$ hnRNP fractions of 1.38–1.42 g/cm^{-3}.

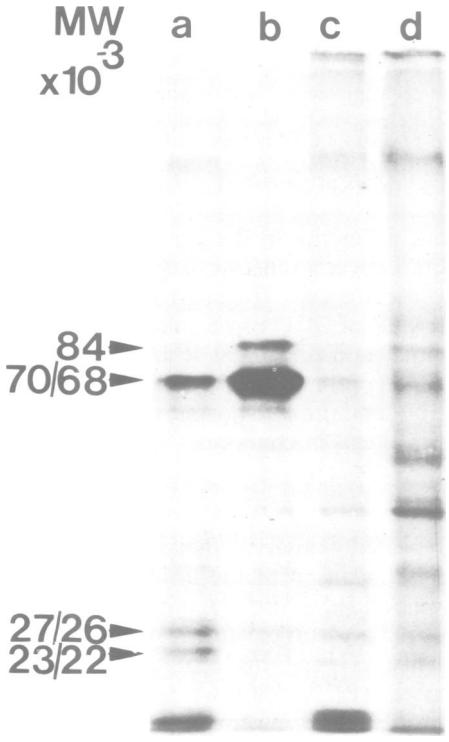

Fig. 4. Electrophoretic analysis of proteins from the nuclei and cytoplasm of heat-shocked and non-heat-shocked culture cells. **(a)** Nuclear proteins of 37°C heat-shocked cells; **(b)** cytoplasmic proteins of 37°C heat-shocked cells; **(c)** nuclear proteins of 24°C culture cells; **(d)** cytoplasmic proteins of 24°C culture cells.

shock, correspond to the so-called major hs proteins, the 64 K and 60 K proteins are probably identical to the minor hs proteins as described by Buzin and Petersen (1982). Differences in the composition of ^{35}S-labelled proteins between the poly(A)$^+$ and poly(A)$^-$ hnRNP fractions exist with respect to the 27 K, 26 K, 23 K and 22 K proteins which appear to be more prevalent in the poly(A)$^+$ hnRNP complexes of heat-shocked culture cells.

In poly(A)$^+$ and poly(A)$^-$ hnRNP complexes of 24°C culture cells, 70 K and 68 K proteins also represent major ^{35}S-labelled proteins. In some experiments, minor amounts of the 23/22 K proteins could also be detected (data not shown). The 64 K and 60 K proteins, however, cannot be detected in the hnRNP complexes of 24°C cells. In addition to the set of ^{35}S-labelled proteins which can be found in both the hnRNP complexes of 24°C and 37°C culture cells, a number of ^{35}S-labelled proteins with mol. wts. of 56 K, 45 K, 38 K, 37 K and 34 K appear to be present only in the hnRNP complexes of 24°C culture cells.

Analysis of proteins linked to ^{32}P-labelled nucleotides

In the experiments described so far we were able to show that the so-called hs proteins are tightly associated with nuclear hnRNA. However, since the elevation of temperature of culture cells to 37°C results in the repression of most of the normal protein synthesis, ^{35}S-labelling of proteins after heat-shock gives only limited information with regard to the total protein complement associated with hnRNA synthesized under heat-shock conditions.

In an attempt to overcome this problem, RNA of heat-shocked cells was labelled with [α-^{32}P]UTP and the total hnRNP isolated as described below. If RNA is labelled with [α-^{32}P]UTP and hnRNP is cross-linked *in vivo* by u.v. irradiation and then digested with nuclease, any protein that had been in direct contact with the labelled hnRNA will carry ^{32}P-labelled nucleotides due to the covalent cross-linking of RNA nucleotides which are in direct contact with protein. The ^{32}P atoms therefore mark the proteins in direct contact with hnRNA and the former can be analysed by electrophoresis and autoradiography (Möller and Brimacombe,

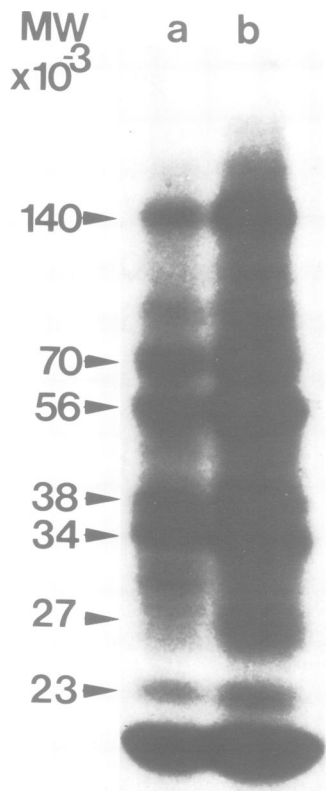

Fig. 5. Electrophoretic analysis of ^{32}P-labelled hnRNP proteins from 37°C and 24°C tissue culture cells. (a) ^{32}P-labelled protein from hnRNP complexes of 24°C culture cells; (b) ^{32}P-labelled proteins from hnRNP complexes of 37°C heat-shocked culture cells.

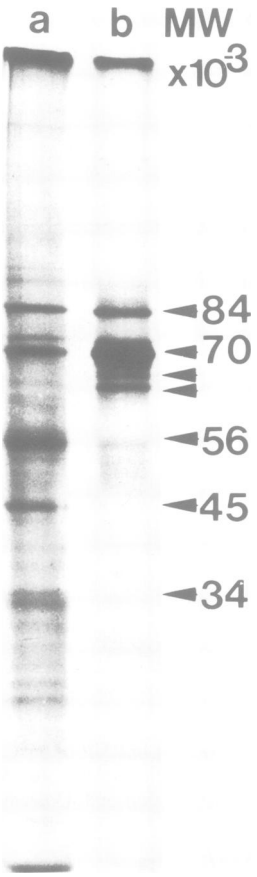

Fig. 6. Analysis of the proteins of non-ribosomal RNP complexes from the cytoplasm of 37°C heat-shock and 24°C culture cells. Cytoplasmic RNP complexes were purified by CsCl density gradient centrifugation and the proteins of RNP complexes of 1.36–1.40 g/cm^{-3} analysed. (a) RNPs of 24°C culture cells; (b) RNPs of 37°C heat-shock culture cells.

1975).

The analysis of the ^{32}P-marked proteins of the hnRNP complexes from heat-shocked tissue culture cells shows (Figure 5b) that the hnRNA of heat-shocked cells is not only associated with the so-called hs proteins of 70/68 K, 27/26 K and 23/22 K but also with proteins of 140 K, 56 K, 38/37 K and 34 K. The 56 K, 38/37 K and 34 K proteins can also be found as ^{35}S-labelled hnRNPs in 24°C culture cells and obviously represent RNPs which become associated with heat-shock hnRNA but whose synthesis is strongly repressed under heat-shock condition.

A similar protein pattern to that in the hnRNP of 37°C culture cells is found for the hnRNP complexes of 24°C tissue culture cells (Figure 5a), although there the 27/26 K proteins are less strongly marked by ^{32}P.

Analysis of cytoplasmic RNP

As shown in Figure 2, the 84 K protein is almost exclusively located in the cytoplasm while the 70 K hs protein can be localized in both the nucleus and the cytoplasm. When total unfractionated cytoplasmic mRNP is analysed on CsCl gradients it bands between densities of ϱ = 1.36 g/cm^{-3} and ϱ = 1.40 g/cm^{-3} and thus appears to be slightly less dense than nuclear RNP. Both the 84 K and 70 K are the main ^{35}S-labelled RNPs associated with the non-ribosomal cytoplasmic RNA of both types of culture cell, while the 64 K and 60 K proteins are only part of the heat-shocked RNP fraction (Figure 6b). However, while the 70 K protein as the major hs protein is strongly enriched in the RNP of heat-shocked culture cells, when compared with that of 24°C culture cells, no significant difference in the amount of ^{35}S-labelled 84 K protein exists between the cytoplasmic RNP of 24°C and 37°C tissue culture cells (Figure 6a). The analysis of the cytoplasmic RNP of 24°C tissue culture cells also shows that the cytoplasmic non-ribosomal RNA is associated strongly with ^{35}S-labelled proteins of 56 K, 45 K and 34 K. Thus, the nuclear hnRNP and the cytoplasmic mRNP appear to have at least several proteins in common.

Discussion

Having repeatedly observed that a considerable fraction of hs proteins sediments with the hnRNP fraction in velocity gradients, we decided to use u.v. cross-linking *in vivo* to bind RNA-associated proteins covalently to hnRNA of *Drosophila* tissue culture cells. Using this technique, which was introduced by Greenberg (1979, 1980), we can practically exclude any artificial protein rearrangement and unspecific protein association which might normally occur during cell fractionation and during the subsequent RNP isolation procedure. This is of particular importance with regard to the isolation of hnRNP and mRNP from heat-shocked *Drosophila* cells, because exposure of the cells to lower temperature during cell fractionation can result in the loss of proteins which may remain associated with hnRNA and mRNA only as a result of the heat-shock conditions.

In the present investigation we show that, independent of the incubation conditions of the *Drosophila* tissue culture

cells, proteins which correspond to the so-called hs proteins are tightly associated with nuclear hnRNA. The binding of these proteins, however, is not restricted to nuclear hnRNA. In fact the 84 K, 70 K, 68 K, 64 K and 60 K proteins were also found to be part of the cytoplasmic mRNP complexes. We have found little radioactivity after u.v. cross-linking at density positions expected to be occupied by either nucleosomes or ribosomes. Therefore, it is unlikely that the RNP association of these proteins is due to non-specific contacts caused through high *in vivo* concentrations.

Although our data are in good agreement with results showing the distribution of hs proteins between the two major cell compartments (Arrigo *et al.*, 1980; Lindquist, 1980) they appear, however, to be somewhat in contradiction to reports which suggest that the hs proteins are associated with soluble chromatin and absent from the cytoplasmic RNP fraction (Arrigo *et al.*, 1980). One possible explanation for these contradictions could be that the hs proteins serve several functions (see also Vincent and Tanguay, 1982) one of which would be that of RNP proteins. On the other hand, the different experimental approach, in which isolated chromatin possibly still containing nascent transcripts was fixed with formaldehyde, should not be neglected.

By using ^{32}P-label as a protein marker we were able to identify hnRNPs in 37°C culture cells whose synthesis is repressed under heat-shock conditions. The data reveal that the hnRNA synthesized under heat-shock conditions possesses more or less the same general protein complement as does the hnRNA of 24°C culture cells, although minor differences cannot be entirely excluded at present. They also show that *Drosophila* culture cells must possess an available pool of RNP proteins.

Even more intriguing than our finding that the so-called hs proteins are tightly associated with hnRNA and mRNA in heat-shocked cells is the result that proteins of the same mol. wts. are also associated with nuclear and cytoplasmic RNA at normal growth temperature, a result which would suggest a much more general role for these proteins than previously thought.

Several control experiments, such as growing the culture cells at lower temperature, incubation in full D_{22} medium, omitting any centrifugation step before the labelling period, avoiding the use of 5-fluorouridine as well as preventing possible anoxic growth conditions in order to avoid stress conditions as far as possible, did not alter the results. In fact, low amounts of 70 K protein were recently detected in 25°C culture cells by the use of monoclonal antibodies directed aginst that protein (Velazquez *et al.*, 1983). Low levels of 70 K protein message were also reported by Findly *et al.* (1981). Thus, the heat-shock effect appears to be not a true off-on situation but rather a drastic increase in the activity of already active genes.

At present we can, however, only speculate about the function of the so-called hs proteins. However, with the knowledge that these proteins possess a function as RNP proteins and the availability of monoclonal antibodies, which under heat-shock conditions react specifically with certain heat-shock puffs (Kabisch and Bautz, 1983; Dangli *et at.*, 1983), it should be possible to elucidate further their molecular function in the near future.

Materials and methods

Cell culture

The *D. melanogaster* tissue culture cell line Kc 0 (Echalier and Ohanessian, 1969, 1970) was used. The cells were adapted to grow in suspension in D_{22} medium containing 2% foetal calf serum (FCS). The cells were grown in spinner flasks at 24°C and were adjusted to concentrations of between 2 x 10^6 and 6 x 10^6 cells/ml.

Heat-shock and labelling conditions

Tissue culture cells were harvested by centrifugation at 1000 r.p.m. for 5 min. Cells were resuspended in D_{22} medium minus yeast extract and FCS and incubated for 1 h at 24°C with 5-fluorouridine (5 μg/ml) which selectively inhibits rRNA synthesis in cultured *Drosophila* cells (Lengyl and Penman, 1978). Cells were then collected again at 1000 r.p.m. for 5 min, resuspended in the same medium plus 5-fluorouridine at a 5-fold higher cell concentration and incubated at 24°C for 30 min for recovery before further handling. In some experiments, the addition of 5-fluorouridine was avoided in order to test whether this compound has any effect on protein and RNA synthesis. For RNA and protein labelling of 24°C tissue culture cells, 25 μCi/ml 5-[^{3}H]uridine (sp. act. 40–60 Ci/mmol) and 20 μCi/ml L-[^{35}S]methionine (sp. act. >1000 Ci/mmol), respectively, were added.

For heat-shock, cells were rapidly brought up to 37°C and placed into a circulating water-bath under mild agitation. For the labelling of RNA, [^{3}H]-uridine was added 15 min and, for the labelling of protein, [^{35}S]methionine was added 20 min after the heat-shock and the cultures were labelled for the desired period of time. For ^{32}P-labelling of the RNA, cells were resuspended in D_{22} medium minus phosphate and [α-^{32}P]UTP (sp. act. 3000 Ci/mmol) added at a concentration of 10 μCi/ml.

U.v. cross-linking

U.v.-induced RNA-protein cross-linking was performed on intact *D. melanogaster* tissue culture cells in culture medium (Mayrand *et al.*, 1981; Wagenmakers *et al.*, 1980). Samples to be irradiated were transferred into sterile Petri dishes and gently shaken during irradiation. Irradiation was performed from the top using two 15 W germicidal tubes (Philips TUV). The distance between sample and lamps was 4 cm; 70% of the irradiated energy was emitted at 253.7 nm. The radiation dose received by the sample was 6800 J/m^{-2}min.

Cell fractionation and isolation of RNP

After u.v. irradiation, labelled culture cells were transferred to 10 volumes of ice-cold wash buffer [100 mM NaCl, 10 mM $CaCl_2$, 10 mM Tris/HCl, pH 7.2, 0.5% diethylpyrocarbonate (DPC), 1 mM phenylmethyl sulfonylfluoride (PMSF)]. Cells were washed twice and pelleted at 1000 *g* for 5 min. Cells were then resuspended in lysis buffer (30 mM NaCl, 10 mM $CaCl_2$, 100 mM Tris/HCl, pH 8.5, 0.5% Nonidet P-40 (NP-40), 1 mM PMSF) and kept on ice for 10 min with occasional vortexing. Nuclei were pelleted at 1000 *g* for 5 min. The supernatant representing the cytoplasmic fraction was decanted and centrifuged again at 20 000 *g* for 30 min to remove mitochondria and insoluble material. The supernatant was then concentrated by ethanol preicpitation at −20°C.

The nuclei were resuspended in RSB (10 mM NaCl, 10 mM Tris/HCl, pH 7.2, 1.5 mM $MgCl_2$, 0.5% DPC, 1 mM PMSF) and layered on top of an 8% sucrose cushion made up in RSB. Nuclei were pelleted at 1500 *g* for 10 min and the nuclear preparations were checked for purity under the microscope. For the isolation of hnRNP, nuclei were homogenized using a Dounce homogenizer and sonicated for 2 x 30 s in RSB plus 5 mM EDTA. Undisrupted material and most of the chromatin was pelleted by low speed centrifugation for 5 min at 500 r.p.m. in a Sorvall HB-4 swing out rotor. The supernatant was layered on top of a 30% sucrose cushion in RSB plus EDTA and centrifuged at 6000 *g* for 20 min to remove insoluble material and residual chromatin still present in the preparation. The hnRNP on top of the cushion, which forms an opalescent band, was removed and used for further analysis.

Oligo(dT)-cellulose fractionation of cross-linked RNP

Column chromatography of cross-linked RNP particles on oligo(dT)-cellulose was performed by a method similar to that described previously (Kloetzel *et al.*, 1982). The samples were adjusted to 0.5 M KCl, 10 mM Tris/HCl, pH 7.4, 10 mM EDTA, 0.2% NP-40 and applied to the columns. The samples were passed over the columns three times. After extensive washing with column buffer, bound poly(A)$^+$ RNP was eluted with 60% formamide in 10 mM KCl, 100 mM Tris/HCl, pH 7.4 and 10 mM EDTA at 45°C. The collected poly(A)$^-$ and poly(A)$^+$ RNP fractions were concentrated by ethanol precipitation at −20°C.

Determination of RNP buoyant density

For density determination, isolated u.v. cross-linked RNP complexes were applied to 20–55% preformed CsCl gradients in 10 mM Tris/HCl, pH 7.5, 5 mM β-mercaptoethanol, 10 mM EDTA and centrifuged at 33 000 r.p.m. for 23 h at 20°C in a Beckman SW 40 Ti rotor. To prevent artificial association of non-cross-linked proteins, RNP samples were solubilized in 60% formamide, 0.5 M NaCl, 100 mM Tris/HCl, pH 7.2, 0.2% SDS and in-

cubated for 3 min at 60°C (Mayrand *et al.*, 1981) prior to centrifugation. Labelled material was detected after precipitation of gradient fractions with TCA and collecting the precipitates on cellulose filters. Radioactivity was determined by counting washed and dried filters in toluene-based scintillation cocktail.

Analysis of proteins

Samples for SDS-polyacrylamide electrophoresis were prepared as follows. Pooled CsCl gradient RNP fractions were concentrated and dialysed against RSB by vacuum dialysis and ethanol precipitation. The precipitated RNP was incubated for 3 h at 37°C with 500 U/ml micrococcal nuclease and 30 μg/ml RNase A in 20 mM Tris/HCl, pH 7.5 and 1 mM $CaCl_2$. Free protein was precipitated with 10% TCA. The precipitates were pelleted, washed twice with acetone and vacuum dried. The samples were dissolved in sample solution (125 mM Tris/HCl, pH 6.8, 5% glycerol, 5% SDS, 2% β-mercaptoethanol) and heated for 5 min at 95°C. SDS-polyacrylamide gel electrophoresis on 8–15% linear polyacrylamide gel gradients was performed using the Laemmli gel system (Laemmli, 1970). The gels were stained, incubated for fluorography and dried. Kodak XRP-5 film was used for exposure at −70°C.

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