Structural Similarities between Corresponding Heat-shock Proteins from Different Eucaryotic Cells*

Richard Voellmy§, Peter Bromley¶, and Hans P. Kocher¶

From the §Department of Molecular Biology, University of Geneva, CH-1211 Geneva, Switzerland and the ¶Department of Medical Biochemistry, University of Geneva Medical Center, Geneva, Switzerland

Effects of heat treatments on chick embryo fibroblasts, Drosophila embryonic cells, and human lymphoblastoid cells have been compared. Cells from all three species synthesize large heat-shock proteins (hsps) with \( M_r = 70,000 \) and 84,000-85,000. Different small hsps with \( M_r \) between 22,000 and 27,000 are made at high rates in heat-treated chicken and Drosophila cells but could not be observed in human cells. The structural features of the large hsps from cells of the different organisms were compared by three methods of peptide mapping, namely the examination of tryptic digests by two-dimensional thin layer chromatography or by high pressure liquid chromatography and of incomplete V8 digests by polyacrylamide gel electrophoresis. The \( M_r = 84,000-85,000 \) polypeptides from all three organisms are closely related, the chicken and human polypeptides having many peptides in common. The relationship between the \( M_r = 70,000 \) polypeptides of the different organisms appears to be less close; possible explanations for this latter result are discussed. Rates of synthesis of total as well as poly(A)+ RNA are much lower in heat-treated than in untreated cells of all three organisms. Heat treatments induce dramatic changes in the shape of chick embryo fibroblasts as seen by microscopic examination. Human lymphoblastoid cells do not show changes in shape.

The main consequences of heat treatments of Drosophila cells or salivary glands are a substantial reduction in the overall rate of total and poly(A)+ RNA synthesis (1, 2) and a dramatic stimulation of transcription of a small number of genes (2) including those coding for the so-called heat-shock polypeptides. These polypeptides have characteristic \( M_r = 22,000-84,000 \) and are abbreviated here as hsp\(^{122} \), 23, 26, 27, 68, 70, and 84.

Increased synthesis of specific sets of polypeptides following heat treatment has also been observed in chick embryo fibroblasts, mouse L cells, and baby hamster kidney cells (3). Chick embryo fibroblasts produce three different hsp hsps. The molecular weights that have been assigned to these proteins range from 83,000-100,000, 68,000-73,000, and 22,000-27,000 (3-6).

Hsps with sizes very similar to those of the two largest chick embryo fibroblast hsps are also found in cultured human foreskin cells (5). Several hsps with \( M_r \) between 70,000 and 100,000 are synthesized in Chinese hamster ovary cells (7), in the slime mold Polysphondylium pallidum (8), in Dictyostelium discoideum (9), Tetrahymena pyriformis (10), HeLa cells (11), plant cells (12, 13) and even in Saccharomyces cerevisiae (14).

So far, little is known about the structure and function of hsps. Drosophila larvae make large amounts of hsps at 35-37 °C (15) but not at 25 °C, and late chicken embryos make hsps at 43-44 °C but not at 37 °C (16). These experiments suggest that hsp synthesis is a physiological response occurring in intact animals at temperatures which are tolerated and which may be encountered by them in their natural environment. In endothermic animals, such elevated temperatures (44 °C in chickens) (17) may be reached during periods of fever caused by bacterial or viral infections. Mild heat treatments that allow the rapid synthesis of hsps greatly increase the ability of Drosophila melanogaster and D. discoideum cells to survive subsequent heat treatments at temperatures which are normally lethal (9, 15). Whether or not hsps are involved in the acquisition of thermal resistance is not known. Recently, it has been reported that one of the chick embryo fibroblast hsps is associated with the Rous sarcoma virus transforming protein pp60<sup>vt</sup> (hsp89, Ref. 18). It remains to be shown, however, whether this association is of physiological importance.

Synthesis of large hsps appears to be universal in eucaryotic cells. The attractive possibility therefore exists that the large hsps may play identical roles in all eucaryotic cells. If these proteins were indeed to serve the same function in different cell types, their structural features should have been conserved throughout evolution. In order to test this possibility, we attempted to examine and compare the structures of corresponding hsps from three organisms which are not closely related species by various methods of peptide analysis. Since the rates of total and poly(A)+ RNA synthesis are considerably lower in heat-treated than in untreated Drosophila cells (1, 2) and since similar observations concerning the accumulation of total RNA have been made also in HeLa cells (19), the generality of these heat effects on RNA synthesis was also examined.

**EXPERIMENTAL PROCEDURES**

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§ Present address, Department of Biochemistry, University of Miami School of Medicine, P.O. Box 10129, Miami, FL 33101.

¶ Present address, Battelle, Geneva Research Centres, 7 route de Drize, CH-1227 Carouge, Switzerland.

1 The abbreviation used is: hsp, heat-shock protein.

2 Portions of this paper (including "Experimental Procedures," Table 1, and Figs. 1, 3, and 4) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 82M-1339, cite authors, and include a check or money order for $3.20 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

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RESULTS

Induction of Hsp Synthesis—In preliminary experiments, the incubation temperatures and the lengths of incubation required for strong hsp production were determined. In agreement with previous findings (3), chick embryo fibroblasts were found to synthesize hsps either only in small amounts or not at all at 41 °C (Fig. 1 in Miniprint). The rates of synthesis of hsps gradually increased with temperature. At 44 °C, the hsps are the predominant protein products made. Hsp formation occurs at a low rate in human lymphoblastoid cells at 37 °C. Strong induction of hsp synthesis was observed at 41-42 °C (Fig. 1 in Miniprint).

Cultured chick, Drosophila, and human cells all synthesize major hsps of $M_r = 70,000$ and 84,000-85,000 (Fig. 1 in Miniprint). Small hsps are made at relatively high rates in Drosophila cells (2) and in chick embryo fibroblasts (3), but could not be detected in human lymphoblastoid cells.

Structural Relationships between Hsps from Different Organisms—The sizes of corresponding hsps from chicken and human cells were found to be identical ($M_r = 70,000$ and 85,000). The largest major Drosophila hsp, however, appeared to be somewhat smaller than the corresponding vertebrate hsps (Fig. 1 in Miniprint; Drosophila hsp84 and chicken hsp85). This minor size difference was still seen when hsps purified by electroelution from acrylamide gels were compared. During such purifications, small fractions of the proteins were found to be partially degraded. This effect was most obvious with Drosophila hsp84. Small quantities of a $M_r = 70,000$ polypeptide were usually seen in hsp85 preparations from chicken and human cells and a $M_r = 68,000$ polypeptide with Drosophila hsp84 (see top arrow on the left in Fig. 4 in Miniprint, lane 1).

The tryptic peptides of corresponding hsps from the three different organisms (see "Experimental Procedures" for preparation of proteins and peptides) were compared by two-dimensional thin layer chromatography and by high pressure liquid chromatography in two different solvent systems. In addition, protease V8 digests of the various hsps were analyzed.

Typical maps obtained by two-dimensional chromatography of tryptic peptides from human hsp85, chicken hsp85, and Drosophila hsp84 are shown in Fig. 2 (a, b, and d). Major peptides are indicated and characterized by numbers. To decide whether or not two individual peptides from different digests co-migrate, mixtures containing equal amounts of radioactivity of chicken and human hsp85 digests (c) or of Drosophila hsp84 and chicken hsp85 digests (e) were also chromatographed. All chromatograms exhibit peptide spot 1 which has a very characteristic shape and does not show in the peptide maps of several other proteins including hsp70. The peptides of the three different digests which are found in spot 1 have very similar properties but do not co-migrate exactly (Fig. 2, c and e). The chicken and human peptides in spots 2, 3, 4, and 5 have identical properties (Fig. 2, a, b, and c). The chicken and human hsp85 maps differ in some of the minor peptide spots (see for example 6-11 in Fig. 2, a, b, and c).
The *Drosophila* hsp84 map contains the major peptide spots 3 and 4 in addition to the characteristic spot 1. Spot 7 is found in the *Drosophila* hsp84 and in the human hsp85 map (Fig. 2, a and d). *Drosophila* peptide 12 migrates similarly to chicken and human peptide 2. The major peptide 15 is only found in the *Drosophila* hsp84 map. Thus, the chicken and human hsp85 digests contain the same major tryptic peptides. Spot 1 and two of the four other major peptide spots are also present in the *Drosophila* hsp84 map. These results suggest that chicken and human hsp85 are very similar. *Drosophila* hsp84 has important structural features in common with chicken and human hsp85.

Analogous results were obtained when the same digests were compared by high pressure liquid chromatography. All digests were analyzed with two different solvent systems (Fig. 3 in Miniprint). Peptides were eluted from the chromatography column by increasing linearly the hydrophobicity of the solvent mixture. Major fractions of all digests did not bind to the column and appeared in column fractions 1-20 (see profile obtained with chicken hsp85 in Fig. 3 in Miniprint, left). Gradient elution started at column fraction 21. Very similar elution patterns were obtained with tryptic digests of hsp85 from chicken and human cells. Using solvent system 1, five of the six major peak fractions were found in identical positions (*peaks* 1-5 in Fig. 3, Miniprint, left). The peptides in peak fractions 1 and 5 are also present in the *Drosophila* hsp84 pattern, while peptide 8 is only found in the *Drosophila* profile. Chicken peptide 7 co-migrates with *Drosophila* peptide 9. In chromatography runs with solvent system 2, eight of the 10 major chicken and human hsp85 peptides eluted at identical positions (Fig. 3 in Miniprint, right). Peak fraction 2 is clearly separated from fraction 1 in the chicken pattern but can only be seen as a shoulder to fraction 1 in the human profile. *Drosophila* hsp84 digests also contain the peptide fractions 1-3 and 5 (Fig. 3 in Miniprint, right). The peptides in *Drosophila* peak fractions 11-14 (Fig. 3 in Miniprint, right) do not co-migrate with any of the major chicken or human peptides. These results are in agreement with the ones obtained by two-dimensional chromatography and show again that chicken and human hsp85 are indeed very similar and that *Drosophila* hsp84 closely resembles the corresponding chicken and human proteins.

Further evidence for the similarity of the $M_r = 84,000-85,000$ polypeptides from the three different organisms was provided by the examination of the polypeptide patterns produced by partial digestion of the different hsps with *Staphylococcus aureus* protease V8. The individual hsps were digested with different amounts of V8, and the digests were analyzed on 15% polyacrylamide gels (Fig. 4 in Miniprint). The partial digests of chicken and human hsp85 contain several characteristic high molecular weight degradation products with identical molecular weights (see arrows in Fig. 4, Miniprint). *Drosophila* hsp84 digests also gave similar patterns.

A similar analysis as with the $M_r = 84,000-85,000$ polypeptides was performed with the $M_r = 70,000$ polypeptides from the three organisms. The two-dimensional tryptic peptide maps of the different $M_r = 70,000$ polypeptides revealed some similarities. A group of characteristic major peptides with low mobilities was found to be present in the maps of all three hsp70 digests (data not shown).

The same tryptic digests were also analyzed by high pressure liquid chromatography. Large fractions of the hsp70 digests did not bind to the column. When this nonadsorbed material was concentrated, desalted and examined by two-dimensional chromatography, it was found to contain mainly the characteristic low mobility peptides described above. The adsorbed portions of all hsp70 digests contained several peptides with similar elution properties. Corresponding peptides in the different digests, however, greatly differed in their relative quantities. That many of the chicken and human hsp70 peptides indeed co-migrate was shown by additional experiments in which equal amounts of the individual digests were mixed before chromatography.

V8 digestions of the $M_r = 70,000$ polypeptides from the three organisms gave complicated gel patterns. Several polypeptide bands with identical sizes but of very different relative

**Fig. 5.** The shapes of heat-treated and untreated chick embryo fibroblasts. Cells in secondary culture were incubated at 41 °C (e) or at 44 °C for 2 (b), 4 (d), or 6 h (f). Cells which had been heat-treated for 2 or 4 h were incubated further overnight at 41 °C and then photographed (a and c, respectively).
intensities were found with the three hsp70 digests. Many additional bands were only seen with one or two but not with all hsp70 digests (data not shown).

RNA Synthesis Occurs at Reduced Rates in Heat-shocked Cells—Heat treatments reduce the rates of accumulation of total and poly(A)^+ RNA in Drosophila cells (1, 2). Heat-treated HeLa cells have also been reported to synthesize total RNA at lower rates than untreated cells (19). To learn more about the generality of these heat effects on RNA synthesis, rates of total and poly(A)^+ RNA accumulation were measured in chick embryo fibroblasts and human lymphoblastoid cells before and during heat treatment. To label RNA, cells were exposed to [3H]uridine for 1 h. Total RNA was extracted from the different cell samples (see "Experimental Procedures") and purified by gel filtration on Sephadex G-75. Since the cells had been exposed to the radioactivity for only a short period, incorporation of [3H]uridine into RNA was calculated per A~260 unit of total RNA. This method of calculation allowed us to eliminate errors caused by losses of cells during heat treatment or of RNA during extraction.

Chick embryo fibroblasts which had been heat-treated for 3 h synthesized 36% less RNA/h than untreated cells (Table IA, Miniprint). Even lower rates of RNA synthesis were observed after 5 h of heat treatment. The effects of 3-h heat exposures on RNA accumulation in chick embryo fibroblasts could be reversed by incubating the heat-treated cells overnight at normal temperature (41 °C). Longer heat treatments appeared to damage the cells irreversibly (see Table IA, Miniprint, and below). Analogous results were obtained with human lymphoblastoid cells (Table IB, Miniprint).

To estimate the relative rates of accumulation of poly(A)^+ RNA in heat-treated and untreated cells, aliquots of the different total RNA samples were fractionated on oligo(dT)-cellulose. The relative amounts of radioactivity in poly(A)^+ and poly(A)^− RNA were then determined for each sample. Identical fractions of the total radioactivity incorporated into RNA were found to be associated with poly(A)^+ RNA in heat-treated and untreated cells (Table I, A and B, Miniprint). These results suggest that heat treatments affect the rates of total and poly(A)^+ RNA synthesis similarly.

Heat Treatments Induce Changes in the Appearance of Chick Embryo Fibroblasts—The shapes of chick embryo fibroblasts which had been heat-treated at 44 °C for 2 (Fig. 5b), 4 (d), or 6 h (f) were found to differ from those of untreated cells (e). Almost all cells which had been heat-treated for 2 h regained their original shapes after overnight incubation at 41 °C (a). Already incomplete reversion was observed with cells which had been heat-treated for 4 h (c). Six-h heat treatments led to the death of many cells (f).

Analogous experiments were also performed with human lymphoblastoid cells. No similarly obvious changes in the cell shapes could be seen. Our observations with chick embryo fibroblasts suggest that heat treatments induce changes in the cytoskeleton structure.

DISCUSSION

We have compared several aspects of the response to heat treatment of cultured cells from three distantly related organisms: Drosophila, chicken, and man. Several apparently independent parts of this response have been conserved throughout evolution. Rates of total and poly(A)^+ RNA synthesis are reduced upon heat treatment in all three cell types (Refs. 1 and 2 and this paper).

Heat treatments have also been noted to induce changes in the cytoskeleton structure of Drosophila cells. Falkner et al. (32) have been able to demonstrate, using immunofluorescent techniques, that cytoskeletal proteins accumulate at the nuclear membrane of heat-treated cells. Our observation, that the shapes of heat-treated chick embryo fibroblasts are visibly different from those of untreated cells, suggests that analogous changes of the cytoskeleton architecture also occur in these cells upon heat treatment. That similar morphologic changes had never been reported for cultured Drosophila cells and that we were equally unable in this study to find them in human lymphoblastoid cells may be explained by the fact that the latter cell types, unlike chick embryo fibroblasts, grow in suspension, where such observations may be difficult to realize.

Analysis of trypic digests of isolated hsp84/85 by two-dimensional chromatography or high pressure liquid chromatography and of protease V8 digests by sodium dodecyl sulfate-acrylamide gel electrophoresis showed that the polypeptides from chicken and human cells are structurally closely related. As expected (33), the similarities between Drosophila hsp84 and chicken or human hsp85 are less striking than the ones between the two vertebrate polypeptides. Still, considerable homology between Drosophila hsp84 and the corresponding vertebrate hsp5 clearly exists and could be demonstrated by all three methods of peptide analysis. These results indicate that the structure or at least parts of the structure of hsp84/85 have been conserved well throughout evolution. This hsp may therefore play an identical role in all eucaryotic organisms.

Even though the similarities are much less obvious than in the case of hsp84/85, Drosophila, chicken, and human hsp70 also appear to be related. Two-dimensional chromatography revealed that a group of major hydrophilic peptides is common to the trypic digests of all three M_r = 70,000 proteins. Furthermore, high pressure liquid chromatography indicated that in addition to the common characteristic peptides other peptides with similar properties are present in all hsp70 digests, although in different quantities.

Both hsp84/85 and hsp70 are ubiquitous polypeptides (3, 5, 7-14). One therefore wonders why the structures of the two polypeptide species apparently have not been conserved equally well. In Drosophila cells, the hsp70 gene is present in 5-9 copies/haploid genome (34). In contrast, Drosophila hsp84 is encoded by a single gene (27). The sequences of some of the hsp70 gene copies have been determined (26, 35, 36). From these data, it can be deduced that polypeptides made from different gene copies may exhibit as many as 16 amino acid differences (26). If multiple hsp70 genes were also present in chick and human cells, the finding of a larger apparent diversity among the M_r = 70,000 than among the 84,000-85,000 hsp5 could be explained by differences in the relative rates of expression of the various hsp70 genes in the three different organisms. This explanation is consistent with the finding that different hsp70 digests do indeed contain peptides with similar properties but in different relative amounts.

Our observations of structural similarities between Drosophila hsp84 and vertebrate hsp85 and also between different M_r = 70,000 polypeptides led us to investigate whether cloned Drosophila heat-shock genes (25, 27, 37, 38) hybridize with RNAs from heat-treated chicken or human cells. In several independent experiments using relatively stringent conditions, however, no specific hybridization could be observed between the Drosophila genes and vertebrate heat-shock RNAs. In addition it has also been shown recently that Drosophila hsp70 genes do not cross-hybridize efficiently with the corresponding mouse (39) or Xenopus genes (40) under stringent conditions. These results are not too surprising. Selective pressure acts on maintaining protein structure and function. Many third base substitutions do not affect the protein sequence (silent substitutions). Thus, identical or very similar
proteins can be encoded by gene sequences which have diverged considerably.

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Heat-shock Protein Structural Similarities

Supplementary Materials to: Structural Similarities Between Corresponding Heat-Shock Proteins from Different Eukaryotic Cells. Richard Victorino, Peter Brodsky and Hans F. Kiefer.

EXPERIMENTAL PROCEDURES

Cell cultures

The Drosophila melanogaster cell line Kc167 was obtained from G. Scholer. The cells were grown in suspension at 25°C in the 10% medium of Scholer and Chambon (1980) with 2% fetal calf serum to densities of about 5 × 10^5 cells/ml. Embryonated chicken eggs were purchased from Yago eggs, SPAFAS Co. (Chesapeake, Virginia) (10 per 120 eggs) and kept at 37°C. Primary and secondary cultures of chick embryo fibroblasts were prepared as described previously (Ishizaki et al., 1979) and were grown in culture at 37°C in Dulbecco’s modified Eagle’s medium containing 5% fetal calf serum. The human lymphoblastoid cell line 92D1947 was kept in RPMI 1640 medium (GIBCO, NY) with 10% decomplemented fetal calf serum. The cells were inoculated at 5 × 10^4 cells/ml and grown in suspension at 37°C to densities of about 1 × 10^7 cells/ml.

Analysis of heat-shock polypeptides

Drosophila cells were seeded 3 times with 400 mg/ml cycloheximide to reach the same density. After 20 min of preincubation at 35°C, 10% DMSO was added and the cells were incubated further for 1.5 - 5 hrs at the same temperature. Drosophila cell nuclei were isolated by centrifugation and were then resuspended in the same medium at 4°C for 10 min. All chick embryo fibroblast secondary cultures were grown several times with nonpH-sensitive medium. After 4 h of preincubation at 43-45°C, a nonpH-sensitive medium was replaced by 100 μl of fresh medium containing 1% methionine (O & O Chemicals) and the cells were incubated further for 1-2 hrs at the same temperature. Human cells were washed with nonpH-sensitive medium by centrifugation and were then resuspended at 45°C for 3-5 hrs at the same medium. Incubation with 1% methionine was for 2 hrs at 43°C (31°C) or 4°C (31°C) and for 2 hrs of culture at 5°C-18°C or 30°C/ml.

Cells were rapidly cooled to 0°C after incubation, taken up in electrophoresis sample buffer (I2) and stored at 5°C. The different cell types were then electrophoresed on 10-15% urea gel using a UREA-ACETATE gel system (II). The labeled standard proteins were coelectrophoresed and served as size markers. The gel was subsequently dried and scanned for 14-18 hrs. Autoradiographs were then re-exposed to the dried gel. Proteins from the gel lanes by electrophoresis. The 3H-labeled proteins and 50 μg of unlabeled bovine serum albumin were incorporated into 20% trichloroacetic acid protein precipitation. The protein pellets were washed with ether-water (1:1) and dried.

Proteinase K and tryptic digestion

For proteinase K digestion, protein samples were resuspended in sodium dodecyl sulfate buffer as described by Chumakov et al. (1973) and incubated at 37°C for 1 hr with 1 μg of proteinase K/ml. After digestion, the gel samples were subjected to fluorography as described above for 1-15 days. For tryptic digestion, protein samples were resuspended in performic acid and incubated in ice for several hrs. The gel was then removed by serum treatment. The resulting proteins were taken up in 100 μl of 1% trichloroacetic acid and digested with 20 μg of trypsin (Worthington) for 4 hrs at 37°C. The digests were then lyophilized and resuspended in 20 μl of 5% acetic acid. Aliquots of 2 μl were spotted onto Polygram cellulose II P60 sheets (Merck and Co., Darmstadt, Germany). Chromatography was performed in pyridine-4-methylammoniyl-4H-1,8-boronine-acetic acid-water (25:7:30:1). The dried sheets were exposed to x-ray film for 1-3 days.

High pressure liquid chromatography

A Waters Associates System (Milford, Mass.) was used. Trypsin digests were reconstituted in the initial eluent and loaded on a C-18 uBondapack reverse-phase column (3.8 x 300 mm). Solvent system 1 was the same as applied in 0.1% formic acid. The column was then reconstituted in the initial eluent and loaded with the same amount of sodium acetate and the column was washed with the same amount of sodium acetate. A total of 10 μl were spotted onto Polygram cellulose II P60 sheets (Merck and Co., Darmstadt, Germany). Chromatography was performed in pyridine-4-methylammoniyl-4H-1,8-boronine-acetic acid-water (25:7:30:1). The dried sheets were exposed to x-ray film for 1-3 days.

Hybridizations

RNA from heat-treated chick embryo fibroblasts or human cells was prepared by lysing in sodium dodecyl sulfate buffer followed by phenol-chloroform extraction. Aliquots of these RNAs were then reverse transcribed with AMV reverse transcriptase (gift from the National Cancer Institute, NIH) in the presence of 1 x - actinomycin D and phenol-chloroform extraction. Phenol RNA was prepared as described previously (25). Phenol RNA containing the probe sequence coding for Drosophila hsp70 RNA (the full gene fragment from 500 base pairs into the clone pDEN7, p300, 500 base pairs, was digested with PstI, a restriction enzyme specific for Drosophila hsp70 RNA. The digestion was performed with EcoRI. Digests of the two clones were electrophoresed on 0.8% agarose gels and transferred to nitrocellulose filters (18). After hybridization of the RNA to heat-treated chick embryo fibroblasts and human cells was hybridized to the RNAs at 5°C with 100 μl of 0.1% sodium dodecyl sulfate and 50% formamide. The filters were then washed in 2XSSC at room temperature after an initial 30 min wash at 80°C in hybridization buffer. In a second type of experiment (Pamplin), the RNA was spotted on paper, dried, and transferred to nitrocellulose filters (18). After hybridization of the immobilized filter was used as the hybridization probe.

Measurement of RNA synthesis

To label RNA, cells were incubated for 6 hrs in medium containing [3H]uridine (5 μCi; New England Nuclear). The same medium was used for the analysis of RNA synthesis. The same medium was then added at 45°C (30°C) and incubated for 1 hr. After incubation, the RNA was collected and analyzed as described in Materials and Methods. The results are expressed as cpm/μg RNA or as percentage of total RNA synthesis.

Table 1. RNA synthesis in heat-shocked cells

| Incubation Temperature (°C) | Incubation Time (hr) | [3H] Uridine Incorporation a | Total RNA | % of total RNA synthesis in poly (A)-RNA | poly (A)-RNA RNA | poly (A)-RNA RNA |
|----------------------------|---------------------|----------------------------|-----------|-----------------------------------------|-----------------|-----------------|
| 45                         | 1                   | 9870 106                     | 6300 44   | 27                                      | 4                          | 0.1              |
| 44                         | 3                   | 6300 64                      | 4103 26   | 27                                      | 4.1                         | 0.4              |
| 44                         | 5                   | 10925 111                    | 4103 26   | 27                                      | 4.1                         | 0.4              |
| 41                         | 5                   | 6225 63                      | 6225 63   | 10                                      | 4.1                         | 0.4              |

a) Cells were incubated in the presence of [3H] uridine for 1 hr

References (for Experimental Procedures)

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**Fig. 3** Analysis of hsp64/95 digests by high pressure liquid chromatography. Tryptic digests of human or chicken hsp64 or Drosophila hsp94 were subjected to high pressure liquid chromatography. The experiments were performed with two different solvent systems (see Experimental Procedures). After application of the samples, the columns were washed with the initial eluent and the nonabsorbed material was collected in fractions 1-10, as shown for the experiment in which chicken hsp64 peptides were chromatographed with solvent system 1. Gradient elution started at fraction 11. Fractions of 0.4 or 0.75 ml were collected, desalted in Amicon and analyzed in a liquid scintillation counter. Major peptide fractions are identified in the Figure by numbers.

**Fig. 4** Partial proteolytic mapping of hsp64/95. Heat-treated HeLa cells (lanes 1-3) and hsp95 from human cells (lanes 4-6) or from chick embryo fibroblasts (lanes 7-9) were incubated in 0.1 M sodium dodecyl sulfate buffer (pH 11.0) for 1 h at 20°C with 10 μl of 2.5 or 8.8 M of B. amyon protease V8 and were subsequently electrophoresed on 15% acrylamide gels. The undigested protein samples are shown in lanes 1, 4 and 7. Characteristic V8 digestion products are indicated by arrows. The top arrow on the left points out the major degradation product of Drosophila hsp94 which accumulates during purification of this polypeptide.
Structural similarities between corresponding heat-shock proteins from different eucaryotic cells.
R Voellmy, P Bromley and H P Kocher

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