Cell-specific Expression of Heat Shock Proteins in Chicken Reticulocytes and Lymphocytes

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ABSTRACT We have found that chicken reticulocytes respond to elevated temperatures by the induction of only one heat shock protein, HSP70, whereas lymphocytes induce the synthesis of all four heat shock proteins (89,000 mol wt, HSP89; 70,000 mol wt, HSP70; 23,000 mol wt, HSP23; and 22,000 mol wt, HSP22). The synthesis of HSP70 in lymphocytes was rapidly induced by small increases in temperature (2°–3°C) and blocked by preincubation with actinomycin D. Proteins normally translated at control temperatures in reticulocytes or lymphocytes were not efficiently translated after incubation at elevated temperatures. The preferential translation of mRNAs that encode the heat shock proteins paralleled a block in the translation of other cellular proteins. This effect was most prominently observed in reticulocytes where heat shock almost completely repressed α- and β-globin synthesis.

HSP70 is one of the major nonglobin proteins in chicken reticulocytes, present in the non–heat-shocked cell at approximately 3×10^6 molecules per cell. We compared HSP70 from normal and heat-shocked reticulocytes by two-dimensional gel electrophoresis and by digestion with *Staphylococcus aureus* V8 protease and found no detectable differences to suggest that the P70 in the normal cell is different from the heat shock-induced protein, HSP70. P70 separated by isoelectric focusing gel electrophoresis into two major protein spots, an acidic P70A (apparent pI = 5.95) and a basic P70B (apparent pI = 6.2). We observed a tissue-specific expression of P70A and P70B in lymphocytes and reticulocytes. In lymphocytes, P70A is the major 70,000-mol-wt protein synthesized at normal temperatures whereas only P70B is synthesized at normal temperatures in reticulocytes. Following incubation at elevated temperatures, the synthesis of both HSP70A and HSP70B was rapidly induced in lymphocytes, but synthesis of only HSP70B was induced in reticulocytes.

Procaryotic and eucaryotic cells respond to elevated temperatures by the synthesis of the ubiquitous "heat shock" proteins, a family of proteins of 80–90,000 mol wt, 70,000 mol wt, and smaller proteins of 20–30,000 mol wt (36). Other cellular effects of heat shock include altered cell morphology (37, 40), inhibition of DNA synthesis affecting the cell cycle and mitosis (12, 34), a generalized repression in RNA polymerase II–dependent transcription (10, 38), and the selective translation of heat shock messenger RNAs (23, 25). Heat shock proteins are also induced in various cells after exposure to diverse and apparently unrelated chemicals including heavy metals (Cd, Cu, Zn), inhibitors of energy metabolism (arsenite), amino acid analogues (canavanine, 5-methyltryptophan), chelators (disulfiram), and infection with certain viruses such as adenovirus 5 (2, 15, 17, 21, 32).

Among diverse organisms as yeast, *Drosophila*, chicken, mouse, and human, these induced proteins are highly conserved. Kelley et al. (18) have shown that heat shock proteins from different eucaryotes are antigenically related to the chicken heat shock proteins using rabbit polyclonal antibodies. The relatedness of heat shock proteins extends to the level of DNA sequence. The yeast and *Drosophila* HSP70 genes are 64–72% homologous in the derived amino acid sequences (14). Furthermore, we have observed that the *Drosophila* HSP70 gene hybridizes to genomic sequences from many different organisms including representatives from the plant and animal kingdoms. The heat shock protein (HSP)70[1]

[1] *Abbreviations used in this paper:* DME, Dulbecco's modified Eagle's medium; HSP, heat shock protein.

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gene(s) of *Drosophila*, mouse, chicken, and human are highly conserved by comparisons of the DNA sequence (Morimoto, R., C. Hunt, L. Berg, and B. Wu, manuscript submitted for publication).

Heat shock proteins have been detected in a variety of cells as components of the normal cellular repertoire. The mRNAs for the small heat shock proteins are transcribed during early development in *Drosophila* (45). Recently HSP70 synthesis has been detected during the two-cell stage of mouse embryogenesis (3) and in mouse embryonal carcinoma cells (4, 31). The implication of these results is that heat shock proteins are likely to have other essential functions in addition to their role(s) in the cellular response to acute forms of stress.

One approach to understanding heat shock proteins and their function (in a normal or stressed cell) is to identify a cell that expresses these proteins under normal conditions in the absence of heat shock. In this paper we show that chicken reticulocytes synthesize a 70,000-mol-wt protein that is identical to the major HSP70 of chicken cells. The HSP70 is present in both embryonic and adult erythrocytes at high levels. Furthermore, reticulocytes and lymphocytes respond differently to heat shock. Whereas all four heat shock proteins are rapidly induced in lymphocytes, only HSP70 is synthesized at elevated levels in reticulocytes.

MATERIALS AND METHODS

Animals and Cell Lines: White Leghorn chickens (2–3 kg) were obtained from Specific Pathogen-Free Avian Supplier (SPAFAS) Farms in Norwich, CT and Lincoln Poultry in Chicago, IL. Anemia was induced by intramuscular injection with acetylphenylhydrazine in 75% ethanol using a 4-d injection schedule (day 1, 10 mg/kg; day 2, 7 mg/kg; days 3 and 4, 5 mg/kg). The level of anemia was monitored by the loss of color in the hen's comb and examination of blood smears stained with Wright's-Giemsa (26) for the proportion of reticulocytes. Fertile eggs were incubated at 37°C in a humid atmosphere with mechanical rocking. Chicken embryo fibroblasts were prepared from decapitated and eviscerated day-11 chicken embryos. The embryo tissue was minced and incubated in PBS containing 1% trypsin to liberate single cells. Primary cell cultures were maintained in Ham's F10 containing 10% fetal calf serum at 37°C in 5% CO_2.

Cell Fractionation: Blood was drawn from the medial wing vein into NKM (140 mM NaCl, 3 mM KCl, 3 mM $MgCl_2$, 3 mM KH_2PO_4) containing 1 mg/ml heparin. Blood cells were either used directly (whole blood) or the reticulocytes and mononuclear leukocytes were washed twice in ice-cold NKM containing 1 mg/ml heparin and collected at 600 g at 4°C for 5 min. Reticulocytes and mononuclear leukocytes were separated on step Ficoll-Hypaque gradients (7). Washed blood cells were resuspended in 0.5 ml NKM and layered over 10 ml of Ficoll-Hypaque (Sigma Chemical Co., St. Louis, MO; density = 1.077 g/cm^3) and centrifuged at 600 *g* for 30–60 min at room temperature. The mononuclear leukocytes (primarily lymphocytes) appear as a homogeneous band at the interface, well separated from the pellet of erythrocytes. The homogeneity of each cell fraction was determined by light microscopic examination of Giemsa-stained smears. Typical preparations of mononuclear leukocytes contain up to 20% contamination with erythrocytes.

Isotopic Labeling, Gel Electrophoresis, and Protease V8 Digestions: Equal numbers of cells (typically 5×10^7 cells) were resuspended in Dulbecco's modified Eagle's medium (DME), incubated at various temperatures for the indicated times, washed twice in 1.0 ml methionine minus (met−) DME, and labeled for 30 min at 37°C with 10 μCi/ml [^{35}S]methionine (Amersham Corp., Arlington Heights, IL) in met-DME. The cells were washed twice in 1.0 ml met-DME to remove the [^{35}S]methionine and lysed in 100–200 μl SDS lysis buffer containing 1 mM phenylmethylsulfonyl fluoride and 5% β-mercaptoethanol in preparation for gel electrophoresis. Proteins were electrophoretically separated on 10% polyacrylamide gels containing SDS (19) or on two-dimensional gels using the standard procedures described by O'Farrell (33). The isofocusing gels (4% polyacrylamide) were prepared with LKB ampholines (LKB Instruments, Inc., Gaithersburg, MD) to form linear gradients in the range of pH 5–7. We measured the pH gradient by slicing the isofocusing gel into 5-mm sections which were placed into 2 ml of degassed dH_2O. The samples were shaken and the pH was determined by measurement on a pH meter. The gels were stained with Coomassie Brilliant Blue (0.1%) in 20% methanol, 7% acetic acid at room temperature for 6 h and destained in 20% methanol, 7% acetic acid for 12–18 h. The gels were photographed with a Polaroid MP4 camera (type 55P/N film) using a Wratten 21 orange filter. Polyacrylamide gels containing radiolabeled proteins were processed for fluorography by treatment in DMSO and 2,5-diphenyloxazole as described by Laskey and Mills (20).

Total incorporation of radioactivity was measured by trichloroacetic acid precipitation of SDS lysates of equal aliquots of cells by the addition of 10% trichloroacetic acid followed by incubation at 95°C for 5 min to ensure that ^{35}S-labeled tRNA was deacylated. The protein precipitate was collected on Whatman 3-mm filters (Whatman Laboratory Products Inc., Clifton, NJ) and washed with ethanol, and the samples were counted in Liquifluor (New England Nuclear, Boston, MA). Coomassie Blue-stained gels were quantitated by densitometric scanning (Transidyne densitometer) of protein samples from blood cells electrophoretically separated by PAGE and compared with dilutions of commercially available (Sigma Chemical Co.) molecular weight standards (myosin, B-galactosidase, phosphorylase *b*, BSA, egg albumin, and carbonic anhydrase). Fluorograms were quantitated by densitometric scanning of different exposures of the x-ray film to determine relative changes in synthesis. We determined the synthesis of specific proteins by slicing gel bands from fluorograms and determined the radioactivity by counting in Liquifluor. The protease V8 digestion products of P70 or HSP70 from different cell and tissue sources was performed as described by Cleveland et al. (8). The radioactive peptides were separated on a 12.5% acrylamide gel and detected by fluorography.

RESULTS

The Pattern of Protein Synthesis in Adult Chicken Blood Cells Is Altered after Incubation at Elevated Temperatures

Incubation of chicken blood cells at temperatures above the normal body temperature (40.5°–41°C) resulted in a rapid and dramatic change in the protein synthetic pattern of ^{35}S-labeled proteins analyzed by SDS PAGE (Fig. 1*A*). In response to heat shock the protein synthetic pattern switched from the normal cellular program (synthesis of control cell proteins) to selective synthesis of only the heat shock proteins (89,000 mol wt [HSP89], 70,000 mol wt [HSP70], 23,000 mol wt [HSP23], 22,000 mol wt [HSP22]). Similar types of changes in protein synthesis have been observed for heat-shocked chicken embryo fibroblasts (17, 40) and other chicken embryonic tissues (43). The induction of heat shock proteins in blood cells parallels the rapid repression in synthesis of many control cell proteins such as actin.

Optimal synthesis of each heat shock protein was induced after incubation at 45°C. The elevated synthesis of HSP89 and HSP70 above control levels was first observed following incubation at 42°C whereas maximal levels of HSPs 23 and 22 were synthesized after incubation at 45°C (Fig. 1*A*). In blood cells heat-shocked at 45°C, the synthesis of the four heat shock proteins accounted for >90% of the de novo protein synthesis, with HSP70 accounting for 70% of total synthesis measured by the radioactivity in each protein band. Only HSP70 synthesis was detected in blood cells incubated at 47°C. The repressive effect of temperature on the overall level of control cell protein synthesis in blood cells was initially detected after incubation at 43°C and was complete by 45°C.

The rapid changes in total blood cell protein synthesis is of particular interest since the subpopulation of blood cells that are the most active in protein synthesis are the mononuclear leukocytes, most (90%) of which are lymphocytes (26). The major cell type in adult peripheral blood cells are erythrocytes, a cell that is inert in terms of protein synthetic activity.

The effects of elevated temperature on erythrocyte expression was examined by using reticulocyte-enriched blood cells from an anemic chicken. The effect of heat shock on protein synthesis in anemic blood cells (Fig. 1*B*) was found to be qualitatively similar to the changes that occur in normal blood cells (Fig. 1*A*). Protein synthesis was suppressed 70% by a shift in temperature from 41° to 45°C. This inhibitory effect

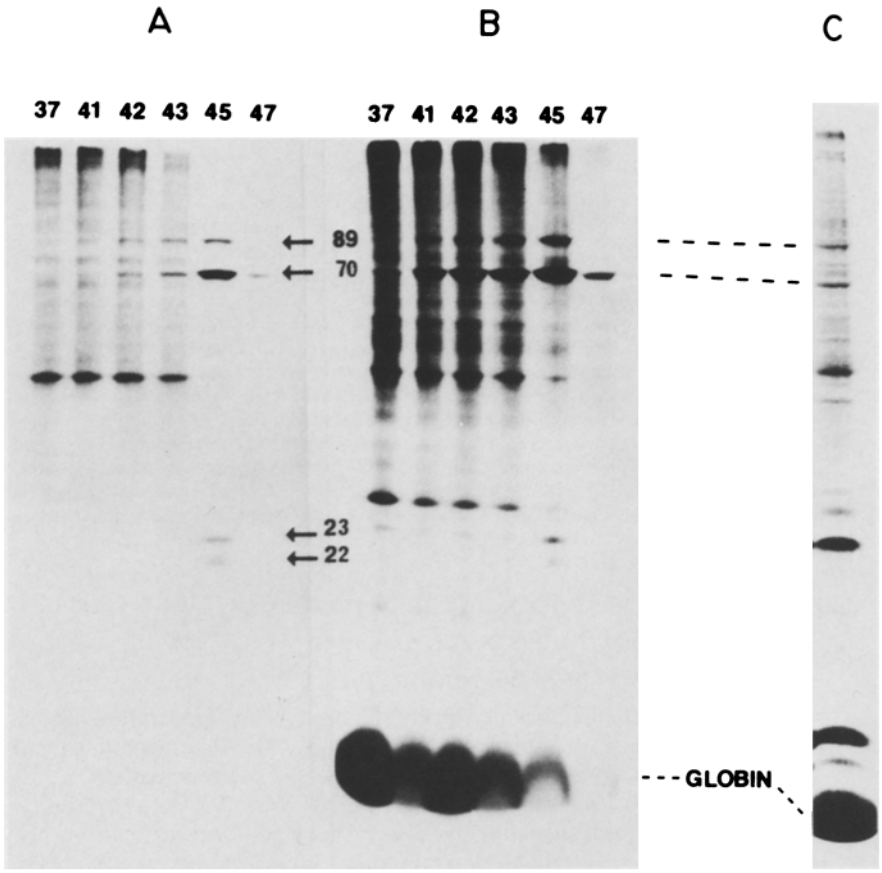

FIGURE 1 Effect of elevated temperature on protein synthesis in chicken blood cells from normal and anemic chickens. Adult blood cells from normal (*A*) and anemic (*B*) chickens were incubated at the various temperatures from 37°–47°C for 30 min and the changes in protein synthesis were monitored by in vivo labeling with [^{35}S]methionine and analysis on a 11.25% SDS acrylamide gel. Lane *C* corresponds to the Coomassie Brilliant Blue–stained pattern for proteins from blood cells of an anemic chicken. The temperature of incubation (in Celcius) is as shown above each gel lane and the molecular weights of the major chicken heat shock proteins HSP89, HSP70, HSP23, and HSP22 are noted (10^{-3}). Each lane corresponds to 3 × 10^6 cells.

of elevated temperature on protein synthesis was most dramatic for globin synthesis. In reticulocyte-enriched blood cells incubated at 45°C, globin synthesis was repressed by 80%. Incubation at 47°C resulted in almost complete repression (95%) of globin synthesis as determined by the analysis of radoactivity associated with globin proteins synthesized in heat-shocked reticulocytes.

Overall, the pattern of induction of the four heat shock proteins in anemic blood cells was similar to what was previously observed for normal blood cells. HSPs 89, 23, and 22 were synthesized at highest levels after incubation at 45°C. One striking difference between the heat shock response of normal blood cells and reticulocyte-enriched blood cells was that P70 was constitutively synthesized at control temperatures. The synthesis of P70 was induced to higher levels by incubation at temperatures from 41° to 45°C (Fig. 1*B*). Incubation at the extreme temperature of 47°C resulted in almost complete repression of globin synthesis and the continued synthesis of only HSP70 (Fig. 1*B*).

Examination of the Coomassie Blue-stained proteins from blood cells of an anemic chicken revealed that the P70 and P89 proteins are among the major blood cell proteins accounting for, respectively, 1–2% and 0.5% of total protein (Fig. 1*C*). The amount of P70 and P89 in the reticulocyte was determined by quantitation of the Coomassie Blue-stained polyacrylamide gel using scanning densitometry. From the known stoichiometry of histone H3 (16) and the size of the chicken genome (19), this corresponds to an estimate of 3 × 10^6 molecules of P70 per cell and 1 × 10^6 molecules of P89 per cell. An independent analysis using protein standards of known molecular weight yielded similar results for the amounts of P70 and P89 in the reticulocyte (data not shown). Reticulocyte proteins were electrophoretically separated on the same polyacrylamide gel with serial dilutions of molecular weight standards (described in Materials and Methods). This type of analysis assumes equal staining of proteins with Coomassie Blue and a linear response between 0.05 and 2.5 μg with the standard marker proteins. Using this approach, we found 2.7 × 10^6 molecules of P70 per cell and 1.1 × 10^6 molecules of 89 per cell, values that are in good agreement with the previous analyses.

Heat Shock Proteins Are Differentially Expressed in Lymphocytes and Reticulocytes

The elevated synthesis of P70 and HSP70 in anemic blood cells (Fig. 1*B*) as compared with normal adult blood cells (Fig. 1*A*) suggested to us that lymphocytes and reticulocytes might differ in their response to temperature elevation. To examine this, we fractionated blood cells from an anemic chicken on Ficoll-Hypaque gradients to obtain homogeneous populations of lymphocytes and reticulocytes. The homogeneity of each population of cells was determined by examination of Wright's-Giemsa–stained blood smears. Typical lymphocyte preparations were contaminated from 5 to 20% by erythrocytes.

Visual examination of the Coomassie Blue-stained protein patterns of lymphocytes and reticulocytes before and after heat shock revealed that reticulocytes have substantial levels of both P70 and P89 (Fig. 2, *A* and *B*). Incubation at the elevated temperature for up to 2 h did not have significant effects on the accumulated levels of P70 or P89. Typical preparations of lymphocytes had only slightly lower levels of P89 and P70 (Fig. 2*A*; see also Fig. 4). The contaminating levels of globin from reticulocytes in lymphocyte preparations made quantitative comparisons somewhat difficult (Fig. 2*A*).

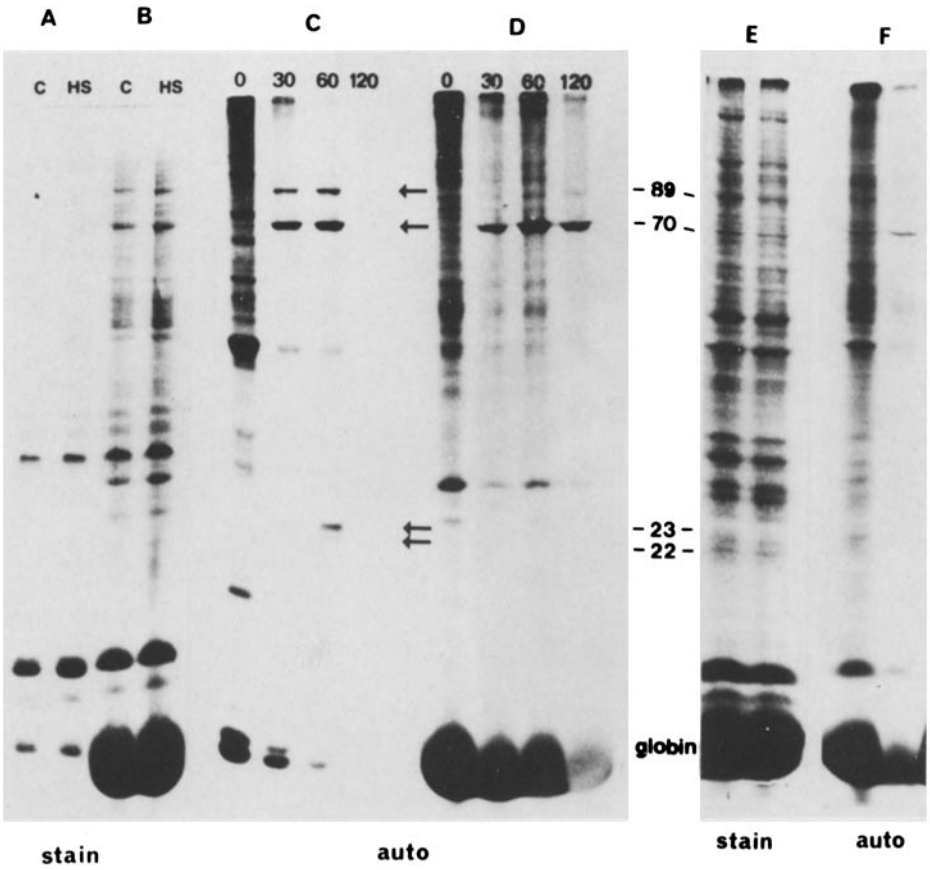

FIGURE 2 Differential expression of heat shock proteins in lymphocytes, reticulocytes and day 5–6 embryonic erythroid cells. Populations of each cell type were purified by centrifugation through a Ficoll gradient, cells were incubated at 43°C for 0 (control), 30, 60, or 120 min and labeled with [^{35}S]methionine, and the proteins were analyzed on a 11.25% SDS polyacrylamide gel. The Coomassie Brilliant Blue–stained gel is shown for (*A*) control and heat-shocked lymphocytes (10^6 cells per lane) (*B*) reticulocytes (5×10^6 cells per lane). The HSP89, 70, 23, and 22 proteins and globin are labeled. Fluorograms of the protein synthetic pattern in heat-shocked lymphocytes (lane *C*) and heat-shocked reticulocytes (lane *D*) show the induction of heat shock proteins. The size of the proteins are given (10^{-3}). The Coomassie Brilliant Blue–stained pattern for (lane *E*) control and heat-shocked day 5–6 embryonic red cells (5×10^6 cells per lane) and (lane *F*) the corresponding autoradiogram of the [^{35}S]methionine-labeled proteins of control and heat-shocked embryonic erythroid cells is shown.

Lymphocytes responded to incubation at 43°C by the induction of all four heat shock proteins and almost complete repression of pre-existing levels of control cell protein synthesis (Fig. 2*C*). The induction of the four heat shock proteins in lymphocytes was transient; overall levels of protein synthesis were almost completely repressed after a 120-min incubation at the elevated temperature. Actin synthesis was repressed to 27% of control levels after 30 min and to 1% after 60 min at 43°C (Fig. 3). We have observed similar effects of heat shock on protein synthesis in chicken MSB cells (a line of chicken leukemia cells transformed by Marek's disease virus) (Banerji, S., L. Berg, and R. Morimoto, manuscript in preparation).

Consistent with our observations using blood cells from an anemic hen, purified reticulocytes responded to incubation at 43°C by the perferential synthesis of HSP70 and almost complete repression of globin synthesis. The elevated synthesis of HSPs 89, 23, and 22 was not detected in reticulocytes as compared with lymphocytes or embryo fibroblasts, as analyzed by both one- and two-dimensional gel electrophoresis (Figs. 2*D* and 4, *F*, *I*, and *K*). The overall level of protein synthesis in heat-shocked reticulocytes was repressed approximately 20-fold with globin synthesis reduced to a level of 5% of control synthesis (Fig. 3). During the 2-hour incubation at 43°C, synthesis of HSP70 in reticulocytes was induced 20-fold above control levels.

A more detailed analysis of these proteins was obtained by a combination of isoelectric focusing gel electrophoresis and size separation on polyacrylamide gels containing SDS (33). The P70 protein(s) in total anemic blood cells, lymphocytes, or reticulocytes separated into two major protein spots, an acidic P70A (apparent pI = 5.95) and a basic P70B (apparent pI = 6.2). Both P70A and P70B have been previously detected in Coomassie Blue-stained gels of partially purified preparations from chicken myotubes, skeletal muscle, or embryo fibroblasts (44). Both proteins are antigenically related yet have distinct protease V8 peptide patterns and are translated in wheat germ lysates from separate mRNAs (R. Morimoto, unpublished data).

In total blood cells from an anemic hen, P70B was the predominant protein synthesized at normal temperatures whereas synthesis of P70A was detected at substantially lower levels (Fig. 4*B*). After heat shock the synthesis of P70B increased in parallel with the rapid induction in the synthesis of HSP89 (apparent pI = 5.6) and HSPs 22 and 23 (apparent pI = 6.0) (Fig. 4*C*). The low level of P70A synthesis in whole blood cells was due to the contribution of proteins synthesized in lymphocytes. In purified lymphocytes maintained at control temperatures, P70A was the major 70,000-mol-wt protein synthesized whereas P70B was synthesized at very low levels (Fig. 4*E*). After lymphocytes had been heat-shocked for 60 min, the synthesis of HSP70A increased three-fold while synthesis of HSP70B increased approximately 10-fold relative to the level of synthesis in non–heat-shocked cells (Fig. 4*F*). The level of induced heat shock protein synthesis in lymphocytes (Fig. 4*F*) was very similar to the changes in protein synthesis observed for embryo fibroblasts (Fig. 4, *J* and *K*). The synthesis of a 72,000-mol-wt protein (apparent pI = 5.4) was also elevated in heat-shocked embryo fibroblasts (Fig. 4*K*). The response of reticulocytes provides a striking contrast

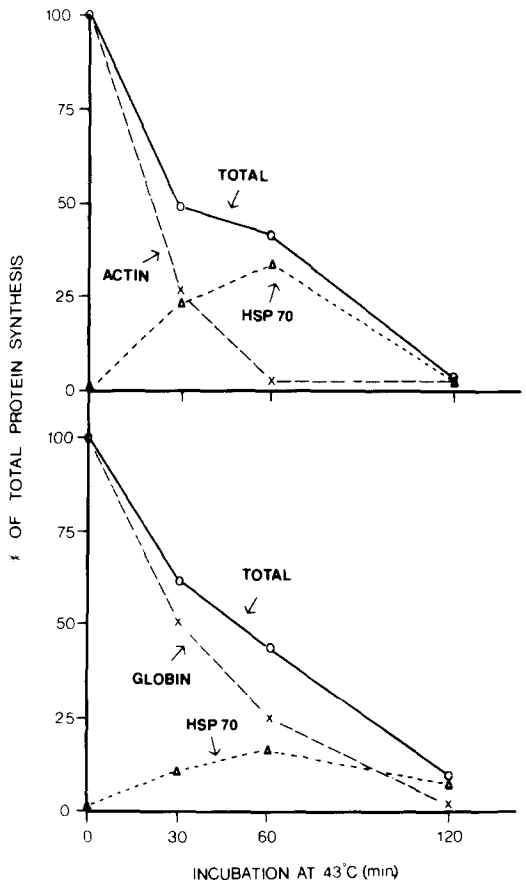

FIGURE 3 Effect of incubation at 43°C on protein synthesis in lymphocytes and reticulocytes. Total protein synthesis of lymphocytes (*A*) and reticulocytes (*B*) following incubation at 43°C for 0.5, 1, and 2 h. Proteins synthesized in equal aliquots of cells was measured in duplicate by trichloroacetic acid precipitation of [^{35}S]methionine labeled proteins as described in Materials and Methods. The synthesis of actin, globin, or HSP70 was measured by counting the radioactivity in the corresponding bands on the fluorogram. All data was normalized to the level of protein synthesis in control cells.

to lymphocytes or embryo fibroblasts in that only P70B was synthesized at control temperatures and induced by heat shock in reticulocytes (Fig. 4, *H* and *I*). Reticulocytes also differ from other chicken cells in that synthesis of only HSP70 was induced by heat shock. In contrast to lymphocytes or embryo fibroblasts where synthesis of both HSP70A and HSP70B was detected, reticulocytes synthesized only HSP70B.

We also examined whether the constitutive expression of P70 in adult reticulocytes is due to the cytotoxic effects of acetylphenylhydrazine or the physiological stress of acute anemia. This was accomplished by examining the proteins synthesized in normal chicken embryonic (day 5–6) erythroid cells incubated at 37°C or after heat shock. We found that P70 and P89 were present in the Coomassie Blue-stained SDS polyacrylamide gel of both normal and heat-shocked embryonic erythroid proteins (Fig. 2*E*). Therefore, we conclude that P70 is present in the normal erythrocyte and that the constitutive synthesis of this protein is unlikely to be due to anemia. Incubation at 43°C for 60 min resulted in an overall repression of protein synthesis and the preferential synthesis of HSP70 (Fig. 2*F*). Since embryonic and adult immature erythrocytes both respond similarly to heat shock, we conclude that the selective synthesis of only HSP70 is an intrinsic property of erythroid cells. We have not examined whether the differences in the inducibility of HSP70 in embryonic erythroid cells as compared with adult reticulocytes represent underlying differences in their response to elevated temperatures. Similarly, we can conclude that acetylphenylhydrazine does not affect the ability of lymphocytes to synthesize proteins at normal or heat shock temperatures since lymphocytes in normal chicken blood cells (Fig. 1*A*) have the same response to heat shock as lymphocytes isolated from an anemic chicken (Fig. 2*C*).

Normal Cellular P70 Is Highly Related to HSP70

Within the limitations of the resolution offered by isoelectric focusing gel electrophoresis and size separation on polyacrylamide gels containing SDS, the P70B protein synthesized in normal reticulocytes was identical by charge and size to the HSP70B protein synthesized after a heat shock (Fig. 4).

A second method that allows qualitative comparison of P70B with HSP70B is one-dimensional gel electrophoresis peptide mapping by in situ digestion of gel slices containing P70B with *Staphylococcus aureus* protease V8 (8). Reticulocytes were purified, incubated at 37°C or heat-shocked at 43°C for 60 min, and the cells were labeled with [^{35}S]methionine for 60 min. The Coomassie Blue-stained band corresponding to P70 was excised from a 10% polyacrylamide gel and incubated in situ with protease V8. The peptides were separated on a 12.5% polyacrylamide gel and the partial digestion products were visualized by fluorography (Fig. 5). Although P70 from reticulocytes was the starting material, the fluorographic analysis allows comparison of only P70B to HSP70B since P70A and HSP70A are not synthesized in the reticulocytes (Fig. 4, *E* and *F*). As shown in Fig. 5, the patterns of protease V8 peptides from control and heat-shocked cells were very similar. This result is expected if the de novo synthesized HSP70B is identical to P70B. If the de novo synthesized HSP70B is a distinct protein, new peptide fragments would be detected only in the protein sample from heat-shocked cells.

Transcription Is Required for the Expression of HSP70

The elevated synthesis of heat shock proteins in total blood cells was blocked by preincubation with 10 μg/ml actinomycin D. Unfractionated blood cells from an anemic hen were heat-shocked at 43°C for 30 min and the proteins were labeled with [^{35}S]methionine. Analysis of the products on SDS acrylamide gel revealed the induction of HSPs 89, 70, and 23 + 22. Incubation with 10 μg/ml actinomycin D for 30 min before the 43°C heat shock blocked the induced synthesis of all four heat shock proteins (Fig. 6*A*). The regulation of heat shock proteins expression at the primary level of transcription has also been observed for embryo fibroblasts (43) and in transformed chicken lymphocytes (Banerji, S., L. Berg, and R. Morimoto, manuscript in preparation). The level of HSP70 synthesized in actinomycin D-treated reticulocytes that were subsequently heat-shocked was reduced relative to the level of P70 synthesized in the absence of the transcription inhibitor. This result is unexpected because actinomycin D blocked the increased synthesis of HSP70 in unfractionated blood cells. It is of interest that very little HSP70 is synthesized in heat-shocked reticulocytes that have been incubated with actinomycin D. There is no increase in the level of HSP70

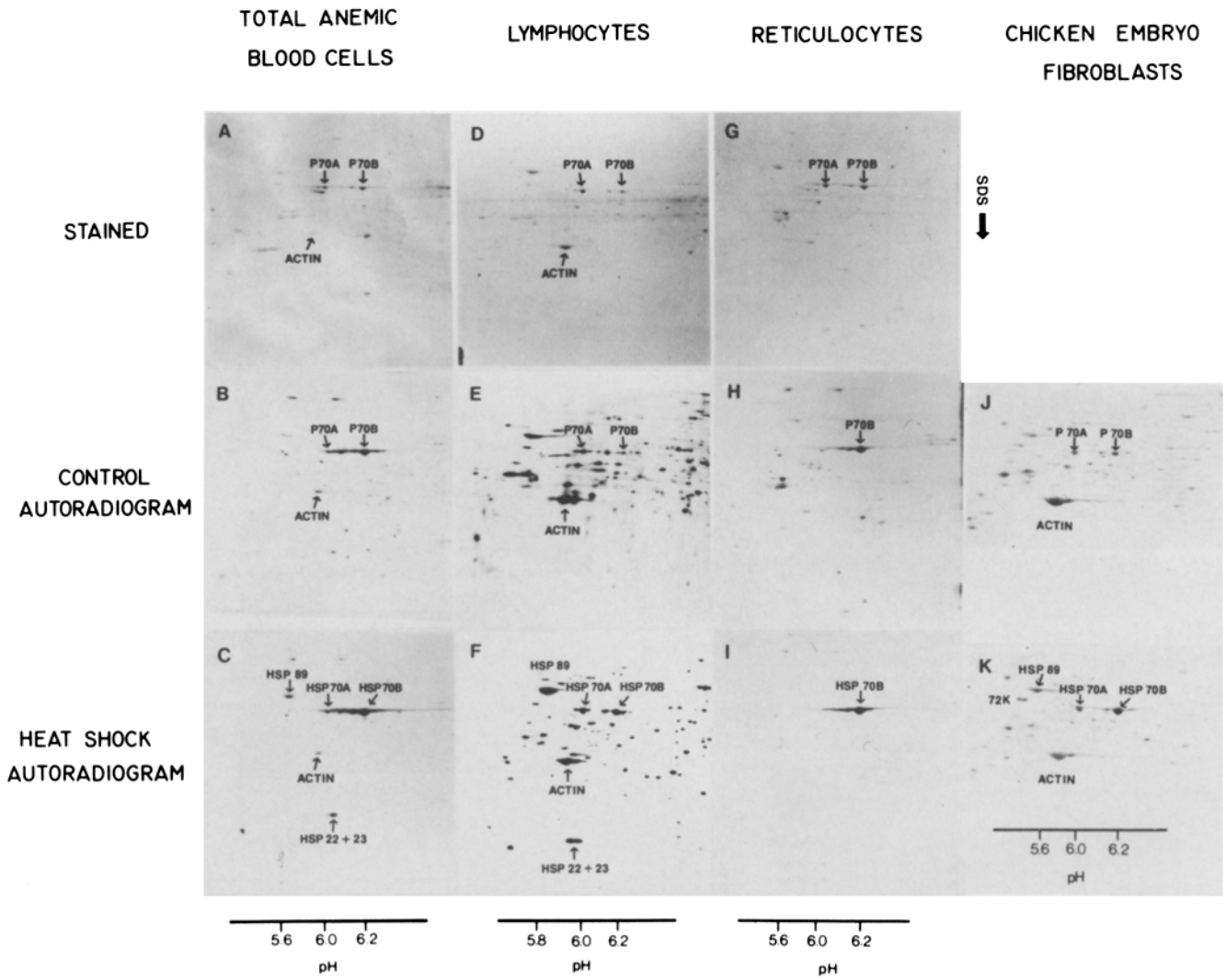

FIGURE 4 Two-dimensional gel electrophoresis of proteins from total anemic blood cells, lymphocytes, reticulocytes, and embryo fibroblasts. (*A*) Coomassie Blue staining pattern of total anemic blood cell proteins (37°C sample from Fig. 1*A*). (*B*) ^{35}S-laeled protein samples from control anemic blood cells (37°C sample from Fig. 1*B*). (*C*) ^{35}S-labeled proteins from heat-shocked (45°C) anemic blood cells. The induction of HSPs 89, 70, 23, and 22 are indicated in *C*. (*D*) Coomassie Blue staining pattern of control lymphocyte proteins. (*E*) ^{35}S-labeled proteins from control lymphocytes at 37°C. (*F*) ^{35}S-labeled proteins from heat shocked lymphocytes (43°C for 30 min). (*G*) Coomassie Blue staining pattern of control reticulocyte proteins. (*H*) ^{35}S-labeled proteins from control reticulocytes at 37°C (non-heat-shocked sample in Fig. 2*D*). (*I*) ^{35}S-labeled proteins from heat-shocked reticulocytes (43°C for 120 min). (*J*) ^{35}S-labeled proteins from control embryo fibroblasts and (*K*) ^{35}S-labeled proteins from heat-shocked (43°C for 60 min) embryo fibroblasts. Each gel contains the equivalent of 5×10^6 cells. Molecular weights are given (10^{-3}), and the measured pH values for the isofocusing gel electrophoresis are noted.

mRNA in heat-shocked reticulocytes assayed by Northern blot analysis using the cloned chicken HSP70 gene (Banerji, S., N. Theodorakis, and R. Morimoto, manuscript submitted). Two possible explanations are that actinomycin D pretreatment results in a depletion of translatable P70 mRNA, owing to mRNA turnover, or that actinomycin D has blocked the transcription of a factor required for the efficient translation of P70 (HSP70) mRNA on heat shock polysomes.

DISCUSSION

We have demonstrated that erythroid and lymphoid cells respond differently to elevated temperature in the expression of heat shock proteins. The heat shock proteins induced in chicken lymphocytes are similar if not identical to the proteins induced by heat shock in other chicken cells or tissues (17, 43, 44, and Banerji, S., L. Berg, and R. Morimoto, manuscript in preparation), whereas in reticulocytes, HSP70 is the only protein synthesized at increased levels. Reticulocytes also differ from other chicken cells in that only the basic HSP70B protein is synthesized at low levels in cells at normal temperatures and is induced by heat shock. The inability of the other heat shock proteins (HSP89, HSP70A, HSP23, and HSP22) to be induced in reticulocytes may be a consequence of gene inactivation through chromatin condensation during erythroid differentiation. Lymphocytes and reticulocytes share common ancestors (11), are both terminally differentiated and typically nondividing, and differ in that lymphocytes can be stimulated to proliferate and have different cellular functions.

Although this is the first example in which preferential synthesis of a subset of heat shock proteins has been described in a mature differentiated cell, it has been shown that heat

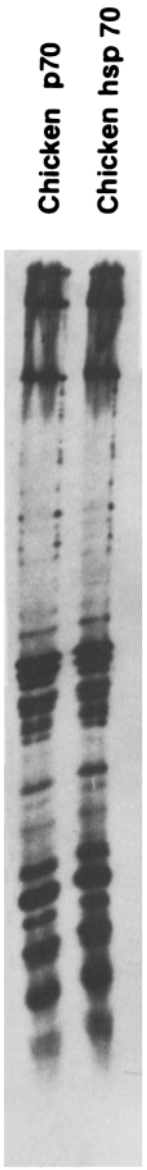

FIGURE 5 Comparison of the protease V8 digestions of P70 and HSP70 from control and heat-shocked reticulocytes. Total cell proteins from chicken reticulocytes were labeled with [^{35}S]methionine after incubation at the ambient temperature or following a heat shock. Proteins were electrophoretically separated by SDS PAGE and lightly stained with Coomassie Blue and the band corresponding to the 70,000-mol-wt protein was sliced out for in situ protease V8 digestion. The products were analyzed on a 12.5% acrylamide gel and the bands were detected by fluorography.

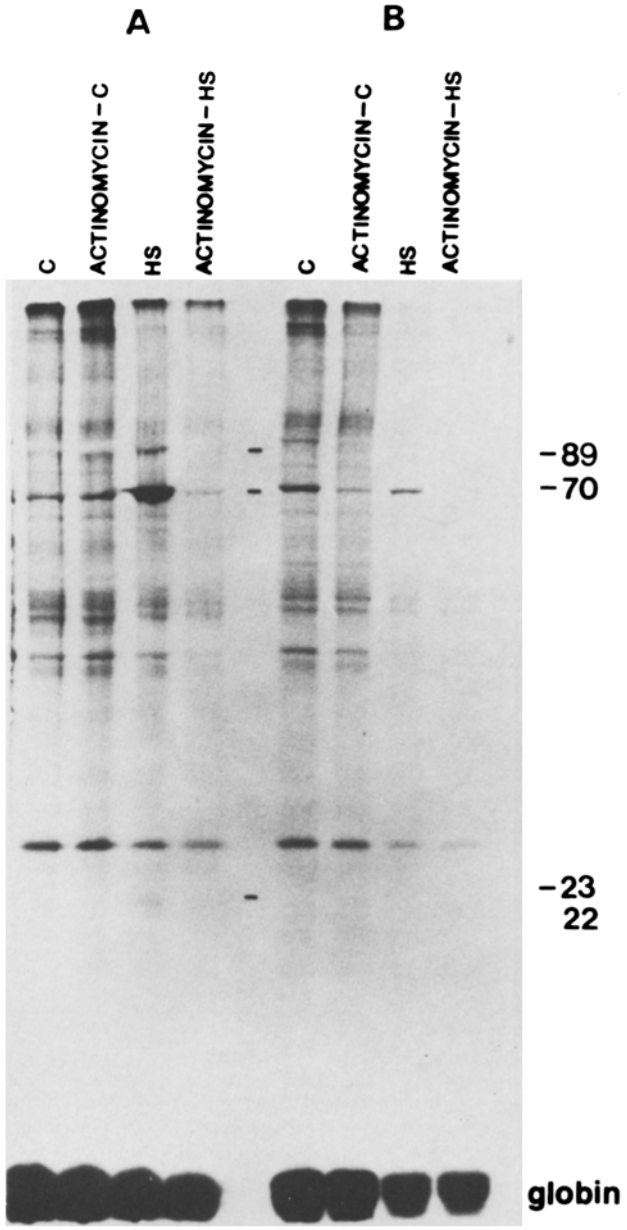

FIGURE 6 Induction of heat shock proteins in chicken blood cells is under transcriptional control. (*A*) Total blood cells (1.5×10^6 cells per lane) or (*B*) reticulocytes (10^6 cells per lane) were incubated at 37° or 43°C with or without preincubation in 10 μg/ml actinomycin D for 30 min at 37°C and labeled with [^{35}S]methionine for 30 min and the proteins were analyzed by SDS PAGE. The molecular weights of the proteins are noted (10^{-3}).

shock proteins cannot be induced in embryonic cells of *Drosophila* (13) or mouse (3). The differential expression of subsets of heat shock proteins has been observed for the small heat shock protein in chicken embryos (43) and for HSP70 during the infection of human cells with adenovirus 5 (32) or in chicken embryo fibroblasts treated with amino acid analogues (17) or arsenite (44). Presumably this reflects the underlying complexity in the regulation of expression of the genes that encode heat shock proteins.

P70 is in embryonic and adult erythrocyte as one of the major nonglobin proteins. The constitutive synthesis of several heat shock proteins at substantially lower levels has been detected in early mouse embryos (3) and in early *Drosophila* embryos (13). Chicken erythrocytes are not atypical in the high levels of P70 in these cells. We have found similar levels of P70 (HSP70) in reticulocytes from mouse, rabbit, and human but not in the erythrocytes from these organisms (Morimoto, R., unpublished data). Consistent with the data presented here for chicken erythrocytes, other nucleated erythrocytes (from frogs and lizards) also maintain high levels of P70 (HSP70). The presence of high levels of P70 in reticulocytes or nucleated erythrocytes and its absence in enucleated erythrocytes could suggest a function for P70 associated with the nucleus of the erythrocyte. However, the function of P70 is not obvious because most P70 is found in the erythrocyte cytoplasm and not associated with the nucleus even after heat shock (Morimoto, R., and A. Freiberg, manuscript in preparation). However, in reticulocytes an appreciable amount of P70 (20%) was associated with the nucleus. The association of HSP70 with nuclei of heat-shocked cells has been demonstrated by biochemical subcellular fractionation of *Chironomus* or *Drosophila* tissue culture cells (1, 42) and by autoradiographic localization of radiolabeled heat shock proteins over *Drosophila* nuclei (41).

Presumably the accumulation of P70 in erythrocytes and its constitutive expression in reticulocytes serves an essential erythrocyte function unrelated to acute stress. The high levels

of P70 in reticulocytes are not due to the physiological stress of acute anemia since both P89 and P70 are present in normal embryonic and adult erythrocytes. If the heat shock response is necessary for reticulocytes in response to acute stress, only HSP70 is synthesized at elevated levels. Perhaps P89 and P70 are abundant proteins in the erythrocyte to provide cellular protection to this terminally differentiated cell, similar to the suggested role of heat shock proteins in thermal protection (22, 27, 29). It is unlikely that the role of P89 and P70 is unique to blood cells as these proteins are synthesized in various other chicken tissues (44). Except for differences in the levels of P70 in different tissues, there are no obvious functions for P70 that can be suggested solely from the unique properties or function of a specific cell type. Heat shock is also likely to have effects on the cellular functions of lymphocytes that may have an effect on the immune response (35).

The heat shock-induced repression of synthesis of normal cellular proteins occurs in both lymphocytes and reticulocytes and is similar to the translational control of protein synthesis observed in *Drosophila* tissue culture cells and *Xenopus* oocytes (5, 23, 24, 39). The rapid repression of existing protein synthesis in lymphocytes and the even more dramatic repressive effects on globin synthesis are striking considering that the cells are responding to a 2°–4°C increase in temperature. Rabbit reticulocytes respond to elevated temperatures by the rapid repression in protein synthesis that is accompanied by polysome disaggregation (30). This is apparently due to a block at the level of initiation owing to the presence of the hemin-regulated inhibitor (6). It should now be possible to examine the mechanism of translational regulation of protein synthesis in reticulocytes using heat shock as a tool to control expression.

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REFERENCES

1. Arrigo, A. P., Fakan, S., and A. Tissieres. 1980. Localization of the heat shock induced proteins in Drosophila melanogaster tissue culture cells. *Dev. Biol.* 78:86–103.
2. Ashburner, M., and J. J. Bonner. 1978. The induction of gene activity in Drosophila by heat shock. *Cell.* 17:241–254.
3. Bensaude, O., Babinet, C., Morange, M., and F. Jacob. 1983. Heat shock proteins, first major products of zygotic gene activity in mouse embryo. *Nature (Lond.).* 305:331–333.
4. Bensaude, O., and M. Morange. 1983. Spontaneous high expression of heat shock proteins in mouse embryonal carcinoma cells and ectoderm from day 8 mouse embryo. EMBO (*Eur. Mol. Biol. Organ.*) *J.* 2:173–177.
5. Bienz, M., and J. Gurdon. 1982. The heat shock response in Xenopus oocytes is controlled at the translational level. *Cell.* 29:811–819.
6. Bonanou-Tzedaki, S., Sohi, M., and H. Arnstein. 1981. Regulation of protein synthesis in reticulocyte lysates: characterization of the inhibitor generated in the postribosomal supernatant by heating at 44°C. *Eur. J. Biochem.* 114:69–77.
7. Boyum, A. 1968. Separation of leukocytes from blood and bone marrow. *Scand. J. Clin. Lab. Invest.* 21(Suppl. 97):77.
8. Cleveland, D. W., Fischer, S. G., Kirschner, M. W., and U. K. Laemmli. 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. *J. Biol. Chem.* 252:1102–1106.
9. Eden, F. C., and J. P. Hendrick. 1978. Unusual organization of DNA sequences in the chicken. *Biochemistry.* 17:5838–5844.
10. Findly, C., and T. Pederson. 1981. Regulated transcription of the genes for actin and heat shock proteins in cultured *Drosophila* cells. *J. Cell Biol.* 88:323–328.
11. Fleischman, R., Custer, R. P., and B. Mintz. 1982. Totipotent hematopoietic stem cells: normal self-renewal and differentiation after transplantation between mouse fetuses. *Cell.* 30:351–359.
12. Gerner, E., and D. Russell. 1977. The relationship between polyamine accumulation and DNA replication in synchronized Chinese hamster ovary cells after heat shock. *Cancer Res.* 37:482–489.
13. Grazoli, G., Micali, F., Marzari, R., DeCristini, F., and A. Savoini. 1981. Variability of response of early Drosophila embryo to heat shock. *J. Exp. Zool.* 214:141–145.
14. Ingolia, T., M. Slater, and E. Craig. 1982. Saccharomyces cerevisiae contains a complex multigene family related to the major heat shock inducible gene of Drosophila. *Mol. Cell. Biol.* 2:1388–1398.
15. Johnston, D., Oppermann, H., Jackson, J., and W. Levinson. 1980. Induction of four proteins in chick embryo cells by sodium arsenite. *J. Biol. Chem.* 255:6975–6980.
16. Joffee, J., Keene, M., and H. Weintraub. 1977. Histones H2A, H2B, H3 and H4 are present in equimolar amounts in chicken erythroblasts. *Biochemistry.* 16:1236–1238.
17. Kelley, P. M., and M. J. Schlesinger. 1978. The effect of amino acid analogues and heat shock on gene expression on chicken embryo fibroblasts. *Cell.* 15:1277–1286.
18. Kelley, P. M., and M. J. Schlesinger. 1982. Antibodies to two major chicken heat shock proteins cross-react with similar proteins in widely divergent species. *Mol. Cell. Biol.* 2:267–274.
19. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.
20. Laskey, R. A., and A. D. Mills. 1974. Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. *Eur. J. Biochem.* 56:335–341.
21. Levinson, W., H. Oppermann, and J. Jackson. 1980. Transition series metals and sulfhydryl reagents induce the synthesis of four proteins in eukaryotic cells. *Biochem. Biophys. Acta.* 606:170–180.
22. Li, G., and Z. Werb. 1982. Correlation between synthesis of heat shock proteins and development of thermotolerance in Chinese hamster fibroblasts. *Proc. Natl. Acad. Sci. USA.* 79:3218–3272.
23. Lindquist, S. 1980. Varying patterns of protein synthesis in Drosophila during heat shock: implications for regulation. *Dev. Biol.* 137:151–158.
24. Lindquist, S. 1980. Translational efficiency of heat induced message in Drosophila melanogaster. *J. Mol. Biol.* 137:151–158.
25. Lindquist, S., B. DiDomenico, G. Bugaisky, S. Kurtz, L. Petko, and S. Sonoda. 1982. Regulation of the heat shock response in Drosophila and yeast. *In* Heat Shock: From Bacteria to Man. M. Schleisinger, A. Ashburner, and A. Tissieres, editors. Cold Spring Harbor Laboratory, New York. 165–175.
26. Lucas, A., and C. Jamroz. 1961. Atlas of Avian Hematology. Agriculture Monograph 25, U.S. Department of Agriculture.
27. McAlister, L., and D. B. Finkelstein. 1980. Heat shock proteins and thermal resistance in yeast. *Biochem. Biophys. Res. Commun.* 93:819–824.
28. McKenzie, S., and M. S. Meselson. 1977. Translation in vitro of Drosophila heat shock messages. *J. Mol. Biol.* 117:279–283.
29. Mitchell, H. K., G. Moller, N. S. Peterson, and L. Lippps-Sarmiento. 1979. Specific protections from phenocopy induction by heat shock. *Dev. Genet.* 1:181–192.
30. Mizuno, S. 1975. Temperature sensitivity of protein synthesis inactivation in the reticulocyte system. Reduced formation of the 40S ribosomal subunit-met-tRNA complex at an elevated temperature. *Biochem. Biophys. Acta.* 414:273–282.
31. Morange, M., A. Diu, O. Bensaude, and C. Babinet. 1984. Altered expression of heat shock proteins in embryonal carcinoma and mouse early embryonic cells. *Mol. Cell. Biol.* 4:730–735.
32. Nevins, J. 1981. Induction of the synthesis of a 70,000 dalton mammalian heat shock protein by the adenovirus E1A product. *Cell.* 29:913–919.
33. O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* 250:4007–4021.
34. Palzer, R. J., and C. Heidelberger. 1973. Influence of drugs and synchrony on the hyuperthermic killing of HeLa cells. *Cancer Res.* 33:422–427.
35. Roberts, N. J., and R. T. Steigbigel. 1977. Hyperthermia and human leukocyte functions: effects on response of lymphocytes to mitogen and antigen and bactericidal capacity of monocytes and neutrophils. *Infect. Immun.* 18:673–679.
36. Schlesinger, M. J., M. Ashburner, and A. Tissieres, editors. 1982. Heat Shock: From Bacteria to Man. Cold Spring Harbor Laboratory, New York. 440.
37. Simard, R., Y. Langelier, R. Mandeville, N. Maestracci, and A. Royal. 1974. *In* The Cell Nucleus. H. Busch, editor. Academic Press, Inc., NY. 447–487.
38. Spradling, A., M. L. Pardue, and S. Penman. 1977. Messenger RNA in heat shocked Drosophila cells. *J. Mol. Biol.* 109:559–587.
39. Storti, R., M. P. Scott, A. Rich, and M. Pardue. 1980. Translational control of protein synthesis in response to heat shock in D. melanogaster cells. *Cell.* 22:825–834.
40. Thomas, G. P., W. J. Welch, M. Matthews, and J. R. Feramisco. 1980. Molecular and cellular effects of heat shock and related treatments of mammalian tissue culture cells. *Cold Spring Harbor Symp. Quant. Biol.* 46:985–996.
41. Velazquez, J. M., B. DiDomenico, and S. Lindquist. 1980. Intracellular localization of heat shock protein in Drosophila. *Cell.* 20:679–689.
42. Vincent, M., and R. M. Tanguay. 1979. Heat shock induced proteins present in the cell nucleus of Chironomus tetams salivary gland. *Nature (Lond.).* 281:501–503.
43. Voellmy, R., and P. A. Bromley. 1982. Massive heat shock polypeptide synthesis in late chicken embryos: convenient system for study of protein synthesis in highly differentiated organisms. *Mol. Cell. Biol.* 2:479–483.
44. Wang, C., R. H. Gomer, and E. Lazarides. 1981. Heat shock proteins are methylated in avian and mammalian cells. *Proc. Natl. Acad. Sci. USA.* 78:3531–3535.
45. Zimmerman, L., W. Petri, and M. Meselson. 1983. Accumulation of a specific sub-set of D. Melanogaster heat shock mRNAs in normal development without heat shock. *Cell.* 32:1161–1170.