

Synthesis of Heat-shock Proteins by Cells Undergoing Myogenesis

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ABSTRACT Subjecting 24-h-old cultures of quail myoblasts to incubation at an elevated temperature causes the pattern of protein synthesis to shift from the production of a broad spectrum of different proteins to the enhanced synthesis of a small number of heat-shock proteins. The synthesis of four major heat-induced polypeptides with M_r s of 88,000, 82,000, 64,000, and 25,000 achieve levels comparable to that of the major structural protein, actin. Two-dimensional electrophoretic separation and fluorographic analysis of these polypeptides establish that those with M_r s of 94,000, 88,000, 82,000, and 64,000 and pI s of 5.1, 5.2, 5.2, and 5.4, respectively, are synthesized by heat-shocked as well as by control (albeit not as intense) cultures. However, the synthesis of polypeptides with M_r s of 94,000, 64,000, and 25,000 and pI 's of 5.2, 5.8, and 5.4, respectively, is detectable only in myoblasts shifted to a higher temperature. Recovery of heat-shocked myoblasts, to a normal preinduction pattern of polypeptide synthesis, takes ~8 h.

Similar studies, completed in older, more differentiated myogenic cells, demonstrate that as cells progress through myogenesis their ability to respond to a similar temperature shift is diminished. The synthesis of some myoblastlike heat-shock proteins by fusing of cells or by myotubes requires that they be maintained at an elevated temperature at least twice as long as myoblasts. This observation and the demonstration that heat-shocked myotubes do not synthesize detectable levels of the 25,000-dalton polypeptide found in heat-shocked myoblasts, suggest that the synthetic response of myogenic cells to heat shock is dependent on the differentiative state of these cells.

Profound changes in the pattern of gene expression are induced in *Drosophila* cells and tissues by a mild heat "shock" (1, 2). They respond to a brief elevation in temperature by suppressing the transcription of most genes and initiating the expression of a set of previously inactive genes (1-4). The major polypeptides expressed after heat shock are referred to as *Drosophila* "heat-shock proteins" (1-4) and include approximately six to eight major different polypeptides with apparent molecular masses of 82,000, 70,000, 68,000, 36,000, 27,000, 26,000, 23,000, and 22,000 daltons (1). Similar protein-synthetic responses to brief incubations at elevated temperatures (albeit with polypeptides of slightly different molecular weights) have been reported for *Chironomus tentans* (5), chicken embryo fibroblasts (6), Chinese hamster ovary cells (7), and the slime mold (8), suggesting that this response is of a widespread occurrence and may involve the expression of a similar set of proteins with a common and potentially crucial cellular function.

Lack of knowledge concerning the effect of heat shock on

protein synthesis in differentiating cells of higher eukaryotes prompted the study reported herein. The aims of this investigation were to demonstrate the effects of heat shock on protein synthesis in primary cultures of myoblasts and to establish whether similar effects are expressed at crucial stages in the development and differentiation of these cells. The results obtained with primary cultures of quail myoblasts demonstrate that the quality and quantity of heat-shock proteins expressed by these myogenic cells are dependent upon the differentiative state of these cells.

MATERIALS AND METHODS

Cell Culture

Primary myoblasts cultures were prepared from the breasts of 9-d-old Japanese quail (*Coturnix coturnix japonica*) embryos by a modification of methods previously published (9, 10). Embryos were aseptically removed from methanol-washed eggs. Breast tissue was dissected free from epithelium and cartilage,

pooled, and dissociated by trituration for 2 min at 37°C in Hanks' Balanced Salt Solution (lacking Ca⁺⁺ and Mg⁺⁺; Grand Island Biological Co., Grand Island, N. Y. [GIBCO]) containing 0.1% trypsin (GIBCO). The cell suspension was added to HB597 culture medium (Connaught, Toronto, Canada) containing 10% fetal calf serum (GIBCO), filtered through six layers of gauze, and centrifuged at 1,000 g for 5 min. The pelleted cells were resuspended in culture medium, and 1 × 10⁶ cells (in 10 ml of culture medium) were plated in loosely capped, gelatin-coated (11) Corning T-75 culture flasks (Corning Glass Works, Science Products Div., Corning, N. Y.). One set of control cultures was grown continuously at 36.5°C in a humidified atmosphere of 5% CO₂ in air. At 24-h intervals the culture medium was changed and the cells in some flasks were fixed in methanol, stained with Giemsa's, and used for assessing the progress of cell fusion.

Heat-shock Treatment, Labeling, and Preparation of Cell Extracts

At specified times (see the figure legends) the culture medium was decanted from cells grown at 36.5°C and replaced with leucine-free minimal essential medium (MEM; GIBCO) containing 10% fetal calf serum. Cultures were subsequently incubated in a humidified atmosphere of 5% CO₂ in air at either 36.5°C or 45°C; in some cases different temperatures were used. At various times after a change in the culture medium (see individual figure legends), cultures were labeled for 2 h with 3.3 μCi/ml of L-[U-¹⁴C]leucine (342 mCi/mmol; New England Nuclear, Boston, Mass.) at 36.5°. At the end of the labeling period, the medium was decanted and the cells adhering to the flasks were washed three times with cold (5°C) saline solution and harvested (by scraping with a rubber policeman) in cold saline. Harvested cells were washed (twice) by centrifugation in cold saline, pelleted, and lysed in a solution containing 9 M urea, 5% β-mercaptoethanol, and 1 mM phenylmethyl sulfonyl fluoride (PMSF). Cell lysates were clarified by a 3-min-centrifugation in a Beckman microfuge (Beckman Instruments Inc., Spinco Div., Palo Alto, Calif.), and either used directly or stored at -70°C.

Polyacrylamide Gel Electrophoresis

One-dimensional (1-D) gel electrophoresis in the presence of SDS was performed according to the method of Laemmli (12) except that the separating gel consisted of a 3-15% polyacrylamide gradient. Two-dimensional (2-D) analysis of the proteins in the cell lysates followed, with minor modifications, the gel electrophoresis method developed by O'Farrell (13). Nonidet P-40 (BDH Chemicals, Ltd., Poole, England) and ampholines (pH range, 3.5-7; LKB Instruments, Inc., Rockville, Md.) were added to the samples just before isoelectric focusing. The second-dimension polyacrylamide gel slabs consisted either of 8.3% polyacrylamide or a 3-15% polyacrylamide gradient overlaid with a 3% polyacrylamide stacking gel. In all cases, molecular weight markers and a portion of the lysate applied to the first-dimensional gel were added to preformed wells in the sides of the slab gel used in the second dimension (14).

To characterize the isoelectric point (pI) and molecular weight (*M_r*) of the electrophoretically separated polypeptides, 1 coelectrophoresed marker proteins of known pI and *M_r*. In addition, the pH gradients established in electrically focused gels were determined by directly measuring the pH in intact gels with a pH probe (Bio-Rad Gel Pro-pHler; Bio-Rad Laboratories, Richmond, Calif.) and by slicing companion gels and determining the pH of the water extract (15). Isoelectric point marker proteins consisted of ovalbumin and creatine phosphokinase (purchased from Sigma Chemical Co., St. Louis, Mo.) and tropomyosin (prepared in our laboratory; 16). Molecular weight marker polypeptides consisted of actin, α-actinin, M-line protein, tropomyosin, and the heavy chain of myosin prepared in our laboratory (16-18), albumin (Sigma Chemical Co.), and standards from a low molecular weight calibration kit (phosphorylase b, 94,000; albumin, 67,000; ovalbumin, 43,000; carbonic anhydrase, 30,000; soy bean trypsin inhibitor, 20,100; and α-lactalbumin, 14,400) purchased from Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.

Fluorography and Counting of Diphenyloxazole-impregnated Gel Slices

Coomassie Brilliant Blue G (Sigma Chemical Co.)-stained slab gels were photographed, impregnated with 2,5-diphenyloxazole (19), and dried down onto Whatman 3 MM filter paper with a Bio-Rad model 224 gel slab dryer. Fluorographs were prepared by apposing dried gels at -70°C to Kodak RP Royal X-Omat film (XR-1) that was preflashed to an optical density of 0.15 (20).

For determination of the relative amounts of incorporation into individual polypeptides, regions from dried gels, corresponding to spots on the fluorograms, were excised, hydrated, and incubated with 0.5 ml of 90% NCS solubilizer (Amersham Corp., Arlington Heights, Ill.) for 3 h at 55°C (21). Radioactivity

was determined by scintillation counting of the solubilized samples in a toluene-based fluor (22) on a Beckman LS-255 scintillation counter. Similar-sized cuts from blank areas of the gel were used to determine background and efficiency (23).

Protein and Radioactivity Determination

Protein concentrations were determined by the method of Lowry et al. (24), or by a turbidimetric assay (25), with bovine serum albumin as standard. Radioactivity of ¹⁴C was routinely measured in a Beckman LS-255 liquid scintillation counter with a Triton X-100/toluene/Omnifluor (New England Nuclear) cocktail (22).

RESULTS

Primary Cultures of Quail Breast Tissue

During the first 24 h the cultures consist of single cells and, on the basis of their characteristic bipolar shape (9, 26), the majority of these cells are classified as myoblasts. It is obvious by inspection that there is an increase with time in the number of myoblasts. After 48 h, under these modified culture conditions, ~50% of the cells are multinucleated (Fig. 1). These multinucleated cells arise through the fusion of mononucleated myoblasts (27). During the ensuing days in culture, the number of nuclei within the multinucleated cells increases at the expense of the population of single cells. By the 4th d (96 h) in culture, 85-90% of the nuclei in a culture are found within the multinucleated myotubes (Fig. 1).

Effects of Incubation Temperature on Protein Synthesis in Myoblasts

General protein synthesis in primary cultures of quail myoblasts is not significantly altered by a 1-h shift from 36.5°C to a temperature between 38° and 46°C but falls sharply after a shift to higher temperatures (Fig. 2A). When myoblasts are maintained at temperatures above 46°C for 1 h or more, they begin to round up and do not recover when shifted back to 36.5°C. Cells maintained at temperatures from 36.5° to 46°C for 1-6 h are not altered in their relative rate of protein synthesis, do not generally round up, and, when shifted back to 36.5°C, fuse and form multinucleated myotubes (not shown). A 1-h incubation at temperatures above 43°C results in the enhanced synthesis of at least four polypeptides (Fig. 2B) and

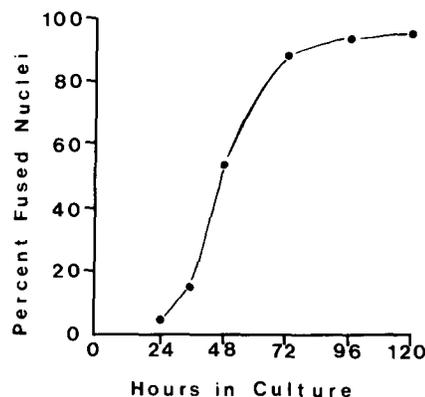


FIGURE 1 The increase in the percentage of nuclei in fused cells with time in culture. Cells in flasks from sibling cultures were fixed and stained on successive days. The surface of each flask was divided into four quadrants, and random fields were counted within each quadrant. The number of nuclei within multinucleated myotubes and the number of mononucleated myoblasts were scored. Each point represents the mean from three separate determinations.

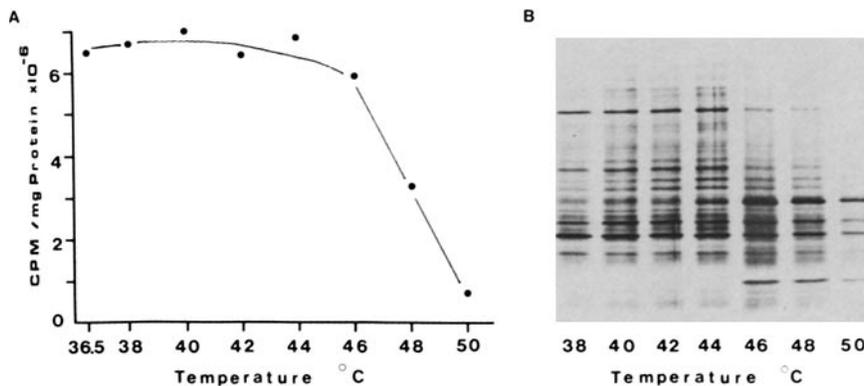


FIGURE 2 Effects of incubation temperature on protein synthesis in primary cultures of quail myoblasts. (A) Relative levels of total protein synthesis in 24-h-old myoblasts incubated for 1 h at the temperatures indicated, and subsequently labeled for 2 h at 37°C. Each point is the average of three determinations. (B) One-dimensional SDS polyacrylamide (3–15% gradient) gel electrophoretic separation of the polypeptides synthesized by the cultures used in A. The polypeptides synthesized under these conditions were detected by fluorography (19, 20) with ~30,000 cpm of acid-precipitable lysate being applied to each well (except the 50°C well, to which 15,000 cpm of lysate was added).

in the apparent depressed synthesis of many other polypeptides (Fig. 2B); this is most obvious at 48° and 50°C. The polypeptides exhibiting enhanced synthesis, after a shift of these cultures to higher incubation temperatures, achieve levels of synthesis similar to the major structural protein, actin (determined by the method of Liebermann et al. [21] but not shown).

Heat-shock-induced Polypeptide Synthesis in 24-h Myoblasts and in 120-h Myotubes

When myoblasts are grown at 36.5°C for 1–3 h in a medium lacking leucine, the polypeptides synthesized after the addition of leucine are qualitatively and quantitatively similar to those polypeptides synthesized by myoblasts not starved for leucine (lanes 1 and 2 in Fig. 3). However, when the medium is changed to a leucine-free medium and the cultures are shifted to 45°C for 1–3 h (lanes 3, 4, and 5 in Fig. 3), the polypeptides synthesized after the addition of leucine and a return to 36.5°C are qualitatively and quantitatively different from the polypeptides made by cells grown exclusively at 36.5°C. After a temperature shift the most prominent polypeptide synthetic changes evident in myoblast cultures are the enhanced synthesis and/or new synthesis of polypeptides with molecular masses of ~88,000, 82,000, 64,000, and 25,000 daltons (Fig. 3).

Similarly designed experiments with 120-h-old cell cultures from quail breast tissue (consisting primarily of myotubes [see Fig. 1]) disclosed that leucine starvation for up to 3 h did not affect the quality or quantity of polypeptides synthesized at 36.5°C (lanes 1 and 2 in Fig. 4). However, a temperature shift of these cultures to 45°C resulted in marked polypeptide synthetic changes (lanes 3, 4, and 5 in Fig. 4). Among the most notable heat-shock-induced changes are the enhanced synthesis and/or new synthesis of polypeptides with molecular weights similar to those of polypeptides synthesized by heat-shocked myoblasts (i.e. 88,000, 82,000, and 64,000; Fig. 3). However, a direct comparison of Figs. 3 and 4 discloses that heat induction of the myoblastlike polypeptides requires at least 2 h at 45°C and that the 25,000-dalton polypeptide synthesized in heat-shocked myoblasts is markedly absent as a synthetic product of heat-shocked myotubes. No detectable synthesis of a 25,000-dalton polypeptide is evident as a product of myotubes cultured at 45°C for as long as 3 h, whereas a temperature shift to 45°C for 1 h is sufficient to induce its synthesis in myoblast cultures.

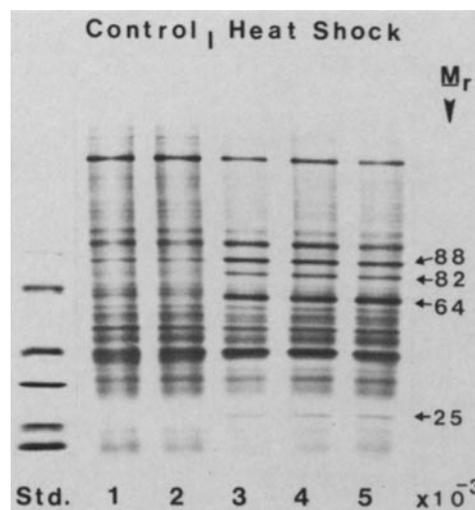


FIGURE 3 One-dimensional SDS polyacrylamide (3–15% gradient) gel electrophoretic separation of the polypeptides synthesized by control (lanes 1 and 2) and heat-shocked (lanes 3–5) 24-h-old myoblast cultures. Primary cultures of embryonic (9-d) quail breast tissue were maintained as controls at 36.5°C in a MEM containing leucine (lane 1) or lacking leucine (lane 2) for 3 h before labeling with [¹⁴C]leucine, or shifted to 45°C in a MEM lacking leucine for 1 h (lane 3), 2 h (lane 4), and 3 h (lane 5) before labeling at 36.5°C with [¹⁴C]leucine (see Materials and Methods for details). The polypeptides synthesized under these conditions were detected (40,000 cpm of acid-precipitable lysate per well) by fluorography (19, 20). The coelectrophoresed M_r marker proteins (Std.; these include phosphorylase *b*, 94,000; albumin, 67,000; ovalbumin, 43,000; carbonic anhydrase, 30,000; soybean trypsin inhibitor, 20,100; α -lactalbumin, 14,400) were visualized by staining with Coomassie Brilliant Blue (14).

Heat-shock-induced Polypeptide Synthesis during Myogenesis

The pronounced synthesis of a 25,000-dalton polypeptide in heat-shocked 24-h myoblast cultures, and the lack of a detectable induction of its synthesis in similarly treated 120-h myotube cultures, prompted an examination of the effects of heat shock on polypeptide synthesis in cells at other stages of myogenesis. A comparison of the array of polypeptides synthe-

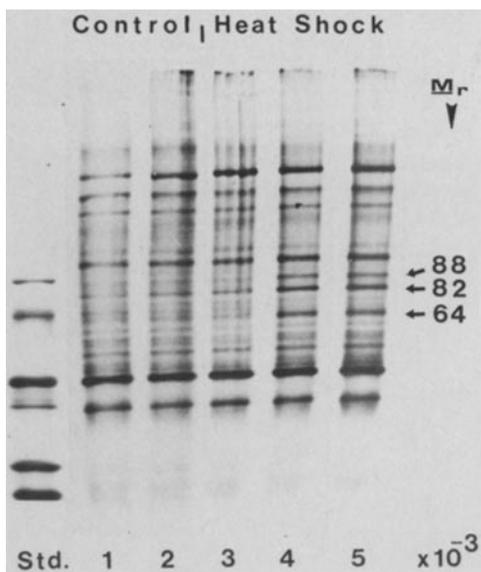


FIGURE 4 One-dimensional SDS polyacrylamide (3–15% gradient) gel electrophoretic separation of the polypeptides synthesized by control (lanes 1 and 2) and heat-shocked (lanes 3–5) 120-h-old myotube cultures. Primary cultures of embryonic (9-d) quail breast tissue were maintained as controls at 36.5°C in an MEM containing leucine (lane 1) or lacking leucine (lane 2) for 3 h before labeling with [¹⁴C]leucine, or shifted to 45°C in an MEM lacking leucine for 1 h (lane 3), 2 h (lane 4) and 3 h (lane 5) before labeling with [¹⁴C]leucine at 36.5°C (see Materials and Methods for details). The polypeptides synthesized under these conditions were detected (40,000 cpm of acid-precipitable lysate per well) by fluorography (19, 20) and the coelectrophoresed M_r marker proteins (*Std.*; for approximate M_r values, see legend to Fig. 3) were visualized by staining with Coomassie Blue.

sized at specific times during myogenesis by cells cultured continuously at 36.5°C is shown in Fig. 5A. The effects of heat shock (a 2-h incubation at 45°C just before labeling at 36.5°C with [¹⁴C]leucine) on the polypeptides synthesized in these cells (siblings of those in Fig. 5A) are shown in Fig. 5B. A comparison of Figs. 5A and B discloses that heat shock causes cells undergoing myogenesis to express an intensified synthesis and/or the new synthesis of polypeptides with molecular masses of ~88,000, 82,000 and 64,000 daltons. Fig. 5A and B also demonstrates that heat-shocked 24- and 48-h cultures synthesize a 25,000-dalton polypeptide not apparent in cells grown continuously at 36.5°C. Although synthesis of this polypeptide is also visible (barely) as a product of cells from heat-shocked 72-h cultures, it is not detectable among the polypeptides synthesized by heat-shocked 96- or 120-h cultures.

2-D Polyacrylamide Gel Electrophoretic Characterization of the Polypeptides Synthesized by Heat-shocked Myogenic Cells

To clarify which of the major polypeptides synthesized by heat-shocked myoblasts are products of genes not normally expressed by myoblasts, the polypeptides synthesized by control (not subjected to a temperature shift) and heat-shocked myoblasts were separated by 2-D polyacrylamide gel electrophoresis (see Materials and Methods for details). A 2-D comparison of the major polypeptides synthesized by control and heat-shocked (1 h at 45°C) 24-h myoblasts is shown in Fig. 6; longer exposure of these gels (not shown) results in the ap-

pearance of numerous other minor polypeptides that will be discussed elsewhere. These 2-D separations establish that polypeptides with molecular masses of 94,000, 88,000, 82,000, and 64,000 and pIs of 5.1, 5.2, 5.2, and 5.4, respectively, appear to be synthesized by both control (Fig. 6A), albeit not so intensely, and heat-shocked myoblasts (Fig. 6B). Polypeptides with molecular masses of 94,000, 64,000, and 25,000 and pIs of 5.2, 5.8, and 5.4, respectively, were detected only in myoblasts shifted to the higher temperature. Although the heat-shock-induced synthesis of a 94,000-dalton polypeptide is not resolved on 1-D separations, its presence is quite easily detected in 2-D separations (Fig. 6B).

Although heat-shocked myotubes (120 h after plating) exhibit, by 1-D gel electrophoretic analysis, an enhanced synthesis of polypeptides with molecular weights similar to those of polypeptides synthesized by heat-shocked myoblasts (except for a 25,000-dalton polypeptide; see Figs. 3 and 4), at least 2 h at 45°C is required to induce detectable levels of their synthesis (Figs. 3 and 4). 2-D gel electrophoretic separation of the polypeptides synthesized by myotube cultures that had been

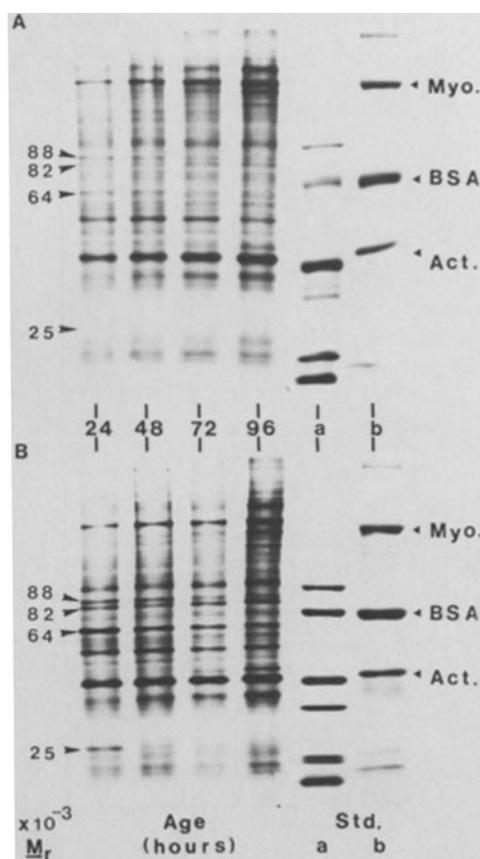


FIGURE 5 One-dimensional SDS polyacrylamide (3–15% gradient) gel electrophoretic separation of the polypeptides synthesized during myogenesis (at 24, 48, 72, and 96 h after plating) by (A) control and (B) heat-shocked cultures. Primary cultures of embryonic (9-d) quail breast tissue were maintained as controls (A) at 36.5°C or shifted (B) for 2 h to 45°C before labeling at 36.5°C with [¹⁴C]leucine (see Materials and Methods for details). Polypeptides synthesized during myogenesis (40,000 cpm/well) were detected by fluorography (19, 20). The coelectrophoresed M_r marker proteins in lanes a (for approximate M_r values, see legend to Fig. 3) and b (approximate M_r values are 200,000 for myosin [*Myo.*]; 67,000 for bovine serum albumin [*BSA*]; 43,000 for actin [*Act.*]) were visualized by staining with Coomassie Brilliant Blue.

shifted to 45° for 1, 2, and 3 h supports the 1-D gel electrophoretic analysis (Fig. 7; to emphasize particular differences, only select areas of these 2-D gels are shown). Moreover, this method of separation discloses that longer exposure (2–3 h) of myotube cultures to this elevated temperature results in the synthesis of polypeptides with the same (except for a 25,000-dalton polypeptide) isoelectric points and molecular weights as those synthesized by 1-h heat-shocked myoblasts (compare Figs. 6 and 7).

To establish whether the polypeptides synthesized by heat-shocked myogenic cells are similar throughout myogenesis and to elucidate whether they are products of genes not normally expressed during myogenesis, I made a 2-D gel electrophoretic comparison of the polypeptides synthesized by control and heat-shocked cells at various stages of myogenesis (Figs. 8 and 9). This comparison disclosed that, during myogenesis, control cultures exhibit a depression in the synthesis of a 88,000-dalton polypeptide and an enhancement in the synthesis of a 82,000-

dalton polypeptide (see control in Fig. 8). Apparently, heat shock enhances the synthesis of both polypeptides in 24- and 48-h-old cultures but primarily elevates the synthesis of the 82,000-dalton polypeptide in older, more differentiated cultures (see *Heat Shock* in Fig. 8). The results shown in Fig. 8 also demonstrate that the detectable synthesis of a 64,000-dalton polypeptide with a pI of 5.4 is common to both control and heat-shocked cultures, whereas the synthesis of one with the same M_r but with a pI of 5.8 (see arrow in Fig. 8) is restricted to heat-shocked cells. This latter polypeptide can be induced at any time during myogenesis merely by shifting cultures to an elevated temperature (45°C) for at least 2 h. At any stage of myogenesis, heat shock induces the synthesis of a 94,000-dalton polypeptide with a pI of 5.2. This polypeptide is not detectably synthesized by control myoblasts but is visibly synthesized, to a minor extent, in more differentiated control cultures. Control cultures as well as heat-shocked cultures exhibit an apparent increase, corresponding with their differentiation, in the syn-

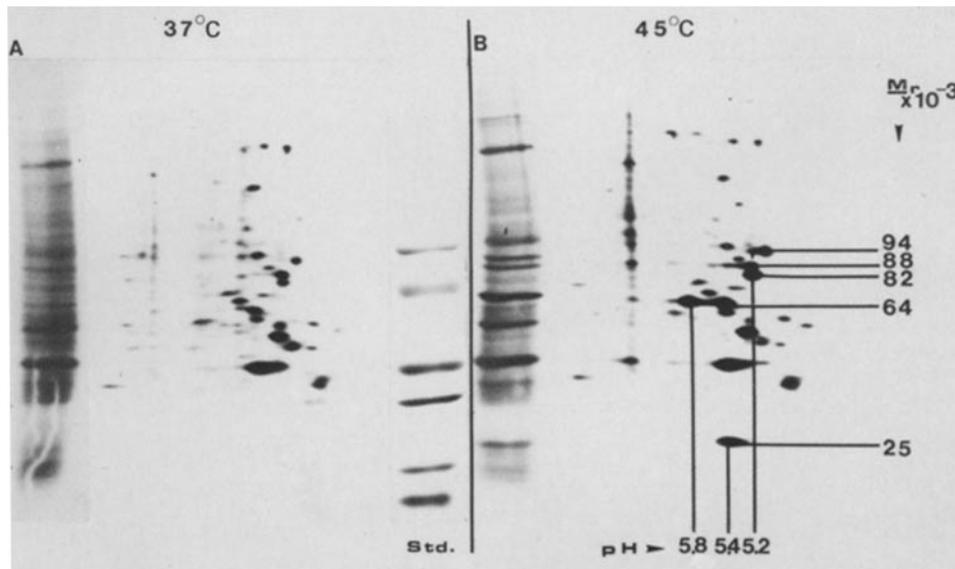


FIGURE 6 Two-dimensional (2-D) polyacrylamide gel electrophoretic (IEF/SDS PAGE) separation of the proteins synthesized by (A) control (maintained at 37°C) and (B) heat-shocked (1 h at 45°C before labeling at 36.5°C) 24-h myoblast cultures. M_r marker proteins (*Std.*; see legend to Fig. 3 for details) and aliquots (70,000 cpm of acid-precipitable lysate) of the samples used in the first-dimensional IEF gel were routinely electrophoresed in separate wells of the second-dimension polyacrylamide (3–15% gradient) gels. Inclusion of marker proteins with known pIs and direct pH measurements on the first-dimension IEF gels establish the pH reported in this figure (see Materials and Methods for details). ~150,000 cpm of acid-precipitable lysate was applied to each 2-D gel. Fluorographs in this figure were exposed for 24 h; many more polypeptides are detectable with longer exposure times.

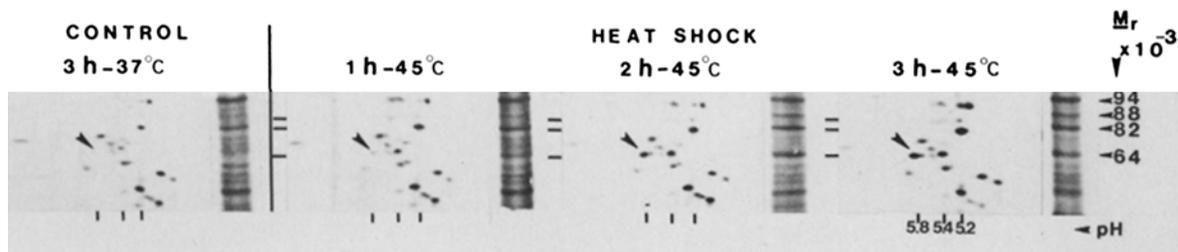


FIGURE 7 2-D polyacrylamide gel electrophoretic (IEF/SDS PAGE) separation of the proteins synthesized by control (maintained at 37°C) and heat-shocked (1, 2, and 3 h at 45°C before labeling at 36.5°C) 120-h myotube cultures. M_r marker proteins (not shown) and aliquots 70,000 cpm of acid-precipitable lysate) of the samples used in the first-dimensional IEF gels (fluorographs of these are inserted at the side of each of the 2-D fluorographs) were electrophoresed in separate wells of the second dimension (8.3% polyacrylamide) gels. Only those areas of the 1- and 2-D gels corresponding to the polypeptides of interest are emphasized by reproduction in this figure; ~150,000 cpm of acid-precipitable lysate was applied to each 2-D gel. Fluorographs were exposed for 24 h. All pH measurements were determined as described in Fig. 6. Arrowheads mark the time-dependent development at 45°C of a 64,000-dalton polypeptide having a pI of 5.8.

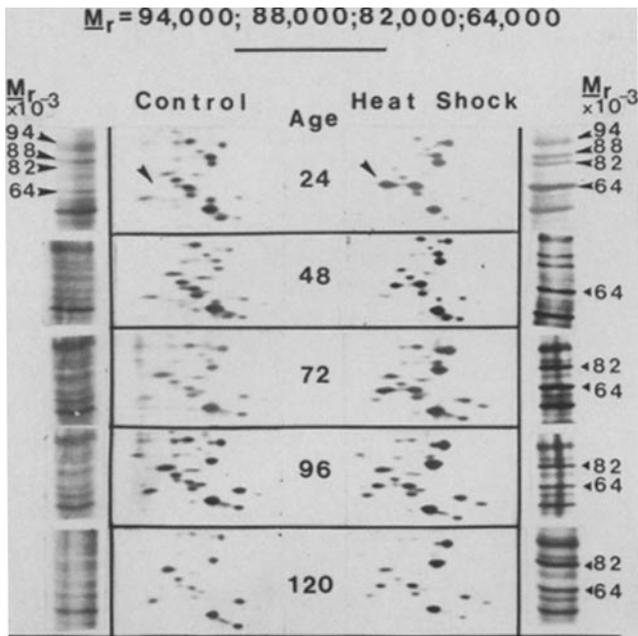


FIGURE 8 2-D polyacrylamide gel electrophoretic (IEF/SDS-PAGE) separation of the 94,000-, 88,000-, 82,000-, and 64,000-dalton polypeptides (see arrowheads) synthesized during myogenesis (at 24, 48, 72, 96, and 120 h after plating) by control and heat-shocked (2 h at 45°C before labeling at 36.5°C) cultures. M_r marker proteins (not shown) and aliquots (70,000 cpm of acid-precipitable lysate) of these are inserted at the side of each 2-D fluorograph (fluorographs of these are inserted at the side of each 2-D gel) corresponding to the polypeptides of interest are emphasized by reproduction in this figure; ~150,000 cpm of acid-precipitable lysate was applied to each 2-D gel. Fluorographs were exposed for 24 h.

thesis of low molecular weight polypeptides having M_r s of 23,000, 22,000, 21,000, and 18,000 (Fig. 9; labeled as *a*, *b*, *c*, and *d*). However, the detectable synthesis of a 25,000-dalton polypeptide is restricted to heat-shocked 24-, 48-, and 72-h myogenic cultures (Fig. 9); neither control nor older, more differentiated heat-shocked cultures synthesize detectable levels of this polypeptide.

Analysis of the Polypeptides Synthesized during Recovery from Heat Shock

To demonstrate a reversible change in the polypeptide synthetic pattern during recovery from heat shock, cultures of myoblasts and myotubes were incubated at 45°C for 2 h, returned to 36.5°C, and labeled at varying time periods after their return to 36.5°C. After heat shock, myoblasts and myotubes require at least 6–8 h of recovery at 36.5°C to return to their normal preinduction patterns of polypeptide synthesis (not shown).

DISCUSSION

The response of primary cultures of quail myoblasts to a brief incubation at an elevated temperature involves a dramatic increase in the relative rate of synthesis of a small number of polypeptides and a depression in the synthesis of most proteins normally made at 36.5°C. The synthesis of the major heat-induced polypeptides with M_r s of 94,000, 88,000, 82,000,

64,000, and 25,000 achieves levels, in quail myoblasts, comparable to that of the major structural protein, actin. This synthetic response by myoblasts is temperature-dependent. It is first detectable after 1 h at an incubation temperature of ~43°C and becomes more pronounced with increasing temperature. Myoblasts subjected to a temperature shift above 46°C for 1 h or more exhibit a decrease in their relative rate of protein synthesis, begin to round up, and do not recover when shifted back to 36.5°C, whereas myoblasts shifted to temperatures up to and including 46°C, for 1–6 h, are not altered in their relative rate of protein synthesis, do not generally round up, and, when shifted back to 36.5°C, fuse and form multinucleated myotubes. After a 1-h heat shock at 45°C, myoblasts require at least 8 h of recovery at 36.5°C to return to their normal preinduction patterns of polypeptide synthesis.

The general protein synthetic response of myoblasts is similar to that reported for invertebrate and other vertebrate cells subjected to heat shock (5–8, 28, 29). In each case, a rapid shift to an elevated temperature results in a dramatic increase in the relative rate of synthesis of a small number of polypeptides and a depression in the synthesis of proteins previously being

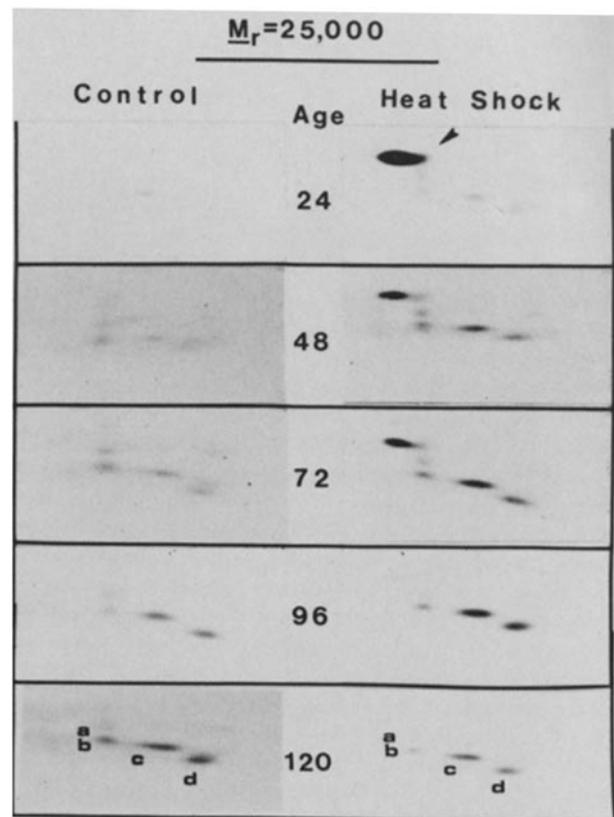


FIGURE 9 2-D polyacrylamide gel electrophoretic (IEF/SDS PAGE) separation of the 25,000-dalton polypeptide (see arrowhead) synthesized during myogenesis (at 24, 48, 72, 96, and 120 h after plating) by heat-shocked (2 h at 45°C) cultures. Only those areas of the 2-D gels corresponding to the low molecular weight polypeptides synthesized by control and heat-shocked cultures are emphasized by reproduction in this figure; ~150,000 cpm of total acid-precipitable lysate was applied to each 2-D gel. Inclusion of M_r marker proteins in the second-dimension polyacrylamide (3–15% gradient) gels, as described in the legend to Fig. 6, established that the polypeptides labeled *a*, *b*, *c*, and *d* have molecular weights of ~23,000, 22,000, 21,000, and 18,000, respectively. All fluorographs were exposed for 48 h.

synthesized. Thus, the pattern of protein synthesis during heat shock appears very similar in both invertebrate and vertebrate systems, which suggests that this response is of a widespread occurrence and may involve the expression of a similar set of proteins.

The response of *Drosophila* to a brief increase in temperature is known to involve new gene activity that results in a unique set of puffs in several chromosomal bands and in an increase in the synthesis of six (*D. hydei*) to eight (*D. melanogaster*) major proteins directed by newly made messenger RNAs (1-4, 30). Whether the enhanced relative rate of synthesis of heat-shock proteins in myoblasts results from the expression of normally quiescent genes, from enhanced and/or preferential transcriptions of mRNAs normally present, from an enhanced translation of particular mRNAs, from posttranslational chemical modifications, or from various combinations of these or other regulatory factors influencing gene expression is currently under investigation and remains to be established. However, results from 2-D gel separations of the polypeptides synthesized by control and heat-shocked myoblasts suggest that several different levels of control might be involved in regulating the synthesis of these heat-shock proteins. For example, four of the seven major polypeptides synthesized by heat-shocked myoblasts (those with M_r s of 94,000, 88,000, 82,000, and 64,000 and pIs of 5.1, 5.2, 5.2, and 5.4, respectively) coelectrophore on 2-D gels with polypeptides synthesized by control cultures, whereas three of the polypeptides synthesized by heat-shocked cells (those with M_r s of 94,000, 64,000, and 25,000 and pIs of 5.2, 5.8, and 5.4, respectively) are not detectably synthesized by control myoblasts. This simple observation reveals that the protein synthetic response by myoblasts to heat shock is complex and, unlike that in *Drosophila*, involves more than just an enhanced expression of quiescent genes.

Although the synthesis of heat-shock proteins in *Drosophila* is not considered to be tissue specific (1, 2, 31), some tissue differences have been detected (32). This discrepancy in the invertebrate studies, coupled with a lack of knowledge concerning the effects of heat shock on protein synthesis in differentiated tissue or in differentiating cells of higher eukaryotes, prompted studies aimed at determining the effects of heat shock on differentiating vertebrate myogenic cells. Results from these investigations establish that cultured, terminally differentiated myogenic cells exhibit the same temperature-dependent cytological response to heat shock (rounding up at temperatures above 46°C) as myoblasts, but their protein synthetic response to heat shock is different. The synthesis of detectable levels of heat-shock proteins in fused, multinucleated, myogenic cells (myotubes) requires more time at an elevated temperature than is required for a synthetic response from similarly treated mononucleated myoblasts. Moreover, myotubes respond to heat shock by synthesizing enhanced amounts of the same 94,000-, 88,000-, 82,000-, and 64,000-dalton polypeptides as synthesized by heat-shocked myoblasts but, unlike myoblasts, do not synthesize detectable amounts of a polypeptide having an M_r of 25,000 and a pI of 5.4. Heat induction of this polypeptide is dramatically depressed during cell fusion and is not detectable once 80-90% of the cells have fused.

Though neither this study nor any other report clearly establishes a function for heat-shock proteins, the ability of a variety of cell types from different animals to respond to rapid elevations in temperatures in a similar synthetic manner suggests that these proteins may have a common and perhaps

crucial intracellular function. The single, major detectable difference among the polypeptides synthesized by undifferentiated and differentiated heat-shocked myogenic cells raises the possibility that these polypeptides may serve more than one function in undifferentiated cells, and that differentiated and/or fused cells no longer require the service of a full complement of heat-shock proteins. Alternatively, the process of differentiation in myogenic cells may involve the establishment of regulatory mechanisms that prohibit or repress transcription of information not intimately involved with muscle protein synthesis and, as such, prevent the expression of at least one myoblastlike heat-shock polypeptide. The ability to environmentally control the expression of a few genes, in a reversible and dramatic fashion, in cultured, differentiating cells of higher eukaryotes makes further study of the heat-shock response in this system attractive.

The author would like to express his gratitude to Jan Fletcher and Tanya Cunningham for their excellent technical assistance.

This research was partially supported by an Academic Development Grant from the University of Western Ontario, London, Ontario, Canada.

Received for publication 9 February 1981, and in revised form 10 March 1981.

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