MOLECULAR AND CELLULAR BIOLOGY, Dec. 1987, p. 4599–4602
0270-7306/87/124599-04$02.00/0
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Vol. 7, No. 12

Heat-Induced Expression of Albumin during Early Stages of Rat Embryo Development

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Received 19 June 1987/Accepted 9 September 1987

An examination of heat-induced expression of proteins in tissues from adult and embryonic liver in rats shows that albumin, which is constitutively expressed in adult liver and is not synthesized in embryos before 16 days of gestation, appears in liver cells at earlier stages of development upon heat shock. On the basis of available evidence for the expression of heat shock proteins at distinct stages of development and on the basis of our findings, it may be argued that there could be common molecular events taking place during development and as a result of heat shock. We suggest also that one of the consequences of heat shock could be an internal change of pH within the cell which, in turn, might trigger alterations in gene expression.

Cells and tissues from a wide variety of organisms respond to environmental stress, including heat, by synthesizing a small set of new proteins (15, 23). Despite their ubiquitous appearance and the conserved nature (1, 7, 10) of the gene coding for heat-induced proteins, such as hsp70, their precise function is not well understood (13, 14, 18). Heat shock proteins were also shown to appear transiently during normal development. Some of the proteins synthesized at distinct stages of development in *Drosophila melanogaster* have been reported to be identical to already known heat shock proteins (8, 29). In mouse embryos some of the first proteins that are synthesized at the two-cell stage were shown to be identical to two of the mouse heat shock proteins, hsp68 and hsp70 (2). Similar observations were made with the 16-cell-stage rabbit embryos (25) and in preblastulas of *Xenopus laevis* (4). The transient expression of heat shock proteins during normal development tends to suggest that they may have specific functional roles during development.

In the course of our studies on heat-induced proteins from rat liver, a chemically induced rat hepatoma, and also rat embryonic liver, we have made a novel observation. We have observed that a 67-kilodalton (kDa) protein, which is constitutively expressed in adult liver both at normal and at elevated temperatures but remains unexpressed at early stages of embryonic development, is expressed upon heat shock. From considerations of molecular weight it appeared to be albumin, and we have confirmed its identity and its induction upon heat shock. Albumin is an adult-liver-specific protein (22), and in rat embryos its synthesis starts from day 17 to 18 of gestation. The earliest that albumin mRNA appears in rat embryos is day 16 (24).

To characterize the heat-induced expression of the 67-kDa protein at earlier stages of development we have used embryos of either 13 or 14 days of gestation, when they were large enough to allow liver tissues to be obtained. In all the experiments reported in this communication, tissues from embryonic and adult liver or cells obtained after perfusion were incubated at 37 and 42°C for analysis of difference in protein synthesis resulting from heat shock. The incubations were conducted for 1 h unless otherwise specified. In experiments involving radiolabeling of proteins, incubations were conducted in methionine-free Dulbecco modified Eagle medium containing 100 μCi of [^{35}S]methionine per ml. After 1 h of incubation at the desired temperature, cells were pelleted (1,000 × *g*) and washed with phosphate-buffered saline (phosphate, 2 mM [pH 7.2]; NaCl, 0.2 M; KCl, 2.5 mM). The washed cells or tissues (1×10^6 to 2×10^6 cells or 60 to 70 mg of tissue) were suspended in 300 μl of Tris (1 mM, pH 7.0). The suspension was disrupted by the addition of 100 μl of 4× Laemmli (12) sample buffer (62.5 mM Tris [pH 6.8], 2.0% sodium dodecyl sulfate [SDS], 3.0% β-mercaptoethanol; 0.001% bromophenol blue), followed by mixing in a Vortex mixer (The Vortex Manufacturing Co.). The samples were kept in boiling water for 3 min and cooled, and samples (100 to 150 μl) were analyzed by SDS-10% polyacrylamide gel electrophoresis (PAGE). The gels were stained with Coomassie blue. For radiolabeled samples, the gels were dried and exposed to X-ray films (Kodak XAR-5) for 1 week at −70°C.

Identification of a 67-kDa heat-induced protein from embryonic liver by immunological methods. The results with the prominent heat shock proteins found in adult liver and in fetal liver are presented in Fig. 1. An examination of Fig. 1 shows the presence of a prominent band corresponding to a molecular mass of 67 kDa in samples of rat liver maintained at 37 and 42°C and also in embryonic liver kept at 42°C. This band is conspicuously absent in embryonic liver (13 to 14 days old) at 37°C. Figure 1 also shows the induction by heat of hsp70 in the embryonic tissues. As the molecular weight of the prominent band below that of hsp70 corresponds to that of albumin, we designed experiments to identify the protein. Extracts from heat-shocked and control cells from adult and embryonic liver were separated by SDS-PAGE, and the proteins were electroblotted (28) onto nitrocellulose paper. Purified rat serum albumin (RSA) was electrophoresed on a parallel lane as a control. The protein blots were treated with antisera against RSA in phosphate-buffered saline containing 0.25% gelatin for 5 h at 37°C. After the antiserum treatment, the blots were washed thoroughly with washing buffer (phosphate-buffered saline containing 0.1% Nonidet P-40, 150 mM NaCl, and 0.25% gelatin) and were treated with peroxidase-conjugated goat anti-rabbit immunoglobulin G for 2 h under the same conditions as for the treatment with the first antibody. The blots were washed thoroughly, as described before, and stained with diaminobenzidine (28). The data presented in Fig. 2 clearly show

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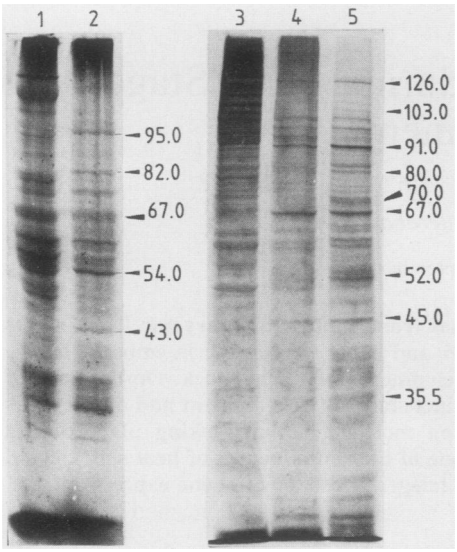

FIG. 1. SDS-PAGE profile of [^{35}S]methionine-labeled proteins of 37 and 42°C from adult and fetal liver cells. Total cellular extracts (see the text) were analyzed by SDS-PAGE (10% polyacrylamide). Gels were dried and autoradiographed as described in the text. Lanes: 1 and 2, adult liver at 37 and 42°C, respectively; 3, fetal liver at 37°C; 4, fetal liver at 42°C (30 min); 5, fetal liver at 42°C (60 min). The numbers shown with arrowheads indicate molecular sizes (in kilodaltons) of the heat-induced proteins.

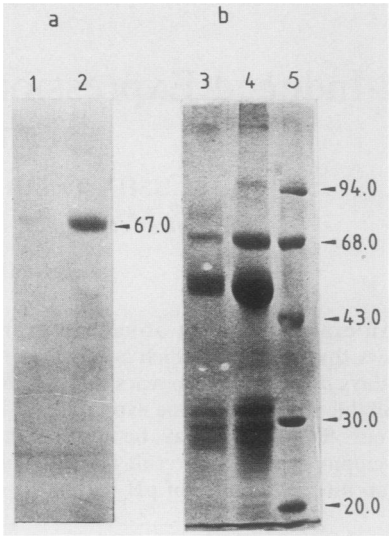

FIG. 3. Immunoprecipitation of [^{35}S]methionine-labeled proteins by using anti-RSA. Labeled proteins were immunoprecipitated with anti-RSA antibodies as described in the text. The precipitates were analyzed by SDS-PAGE, stained with Coomassie blue, destained, and subsequently dried and autoradiographed (see the text). (a) Autoradiograms. Lanes: 1, fetal liver at 37°C; 2, fetal liver at 42°C. (b) Coomassie blue-stained gel before autoradiography. Lanes: 3, fetal liver at 37°C; 4, fetal liver at 42°C; 5, molecular weight markers (Pharmacia). Molecular sizes of marker proteins are shown in kilodaltons.

that the protein corresponding to the 67-kDa band is albumin.

The antigenic identity of the 67-kDa protein was further confirmed by immunoprecipitation experiments. Extracts from [^{35}S]methionine-labeled cells ($\simeq 2 \times 10^6$) from fetal liver kept at 42 and 37°C were immunoprecipitated with antiserum against RSA. The precipitates were electrophoresed on SDS-PAGE and the gels were dried and exposed for autoradiographic analysis. In Fig. 3 are presented the results of the analysis together with the profile of the same gel stained with Coomassie blue. The anti-RSA precipitated the protein banding at a position identical to that of albumin from the lysate of heat-shocked embryonic liver cells and not from cells kept at 37°C.

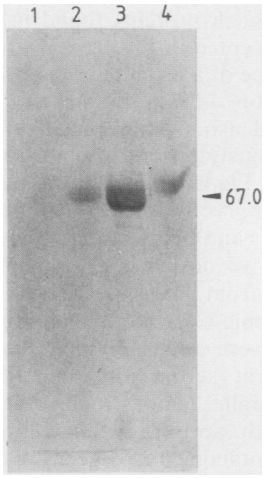

FIG. 2. Western blot (immunoblot) analysis of proteins from heat-shocked fetal and adult liver by using antiserum to RSA. Total cell lysates from normal and heat-shocked adult and fetal liver cells were analyzed on SDS-PAGE (10% polyacrylamide), transferred onto nitrocellulose paper, reacted with anti-RSA, and stained with diaminobenzidine as described in the text. Lanes: 1 and 2, fetal liver at 37 and 42°C, respectively; 3, RSA; 4, adult liver at 37°C. The number shown with the arrowhead indicates the molecular size (in kilobases) of the protein cross-reacting with anti-RSA.

Transcription of albumin mRNA. To investigate the level of expression of mRNA transcripts in embryos at early stages of development we have carried out molecular hybridization experiments using a cDNA clone (pmalb 2, 660-base-pair insert in pBR32, a gift from Shirley Tilghman). Total RNAs (6) from fetal and adult liver kept at 42 and 37°C were dot hybridized (16, 27) on nitrocellulose paper to albumin cDNA probe. The presence of mRNA hybridizing to albumin cDNA could be clearly detected in samples isolated from heat-shocked embryonic liver and in adult liver, but not in embryonic liver kept at 37°C (Fig. 4). The amount of RNA hybridized to the albumin cDNA probe is concentration dependent. In addition, we have looked at the size of the transcript that hybridized with the albumin cDNA probe. Total RNA from heat-shocked embryonic liver was electrophoresed on formaldehyde gels (26), blotted onto nitrocellulose paper, and hybridized to the albumin cDNA probe (2×10^6 to 4×10^6 cpm). The dried gels were subjected to autoradiographic analysis (Fig. 5). The RNA transcripts from heat-shocked embryonic liver that hybridized to albumin cDNA probe were found to be of the same size as the one from adult liver (corresponding to 1.8 kilobases).

Implications of heat-induced induction of albumin at an early stage of development. Although the appearance of proteins identical to heat shock proteins during early development and differentiation has been reported earlier (2, 4, 8, 25, 29), none of these proteins have been identified with any of the known cellular proteins, so far, with the exception of

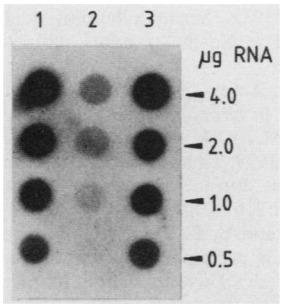

FIG. 4. Dot hybridization analysis of total RNAs from adult liver and from normal and heat-shocked fetal liver. Total RNA isolated after 30 min of incubation at 37 or 42°C was spotted (0.5 to 4 μg) onto nitrocellulose paper, vacuum dried, and probed with the nick-translated plasmid (pmalb) DNA (see the text). Lanes: 1, adult liver at 37°C; 2, fetal liver at 37°C; 3, fetal liver at 42°C.

ubiquitin, which was shown to be activated upon heat treatment (3). Our identification of the heat-induced expression of albumin, therefore, appears to be one of the first such instances. In fact, Bensaude et al. (2) have reported a 68-kDa heat-induced protein in mouse embryos, and Mirkes (17) has reported a 69-kDa heat-induced protein in rat embryos. However, in neither study was there any attempt to investigate whether these proteins designated as heat shock proteins of these systems were, in fact, already known proteins. It would be interesting to check whether these proteins are also albumin, as we have observed in rat embryos at early stages of development, although any significance of heat-induced expression of albumin is not known.

On the basis of our present observations, it is tempting to argue that common molecular events could take place during differentiation and as a result of heat shock, and it would be of interest to examine the possible relatedness of such events governing these phenomena. It remains to be seen whether the observation on the differences in the sex ratios of turtles depending on the temperature at which turtle eggs are hatched (5) is a consequence of the heat-induced induction of protein(s) playing a functional role.

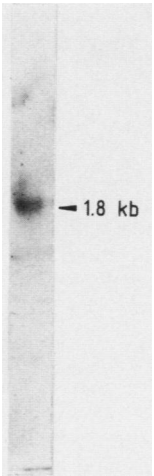

FIG. 5. Northern (RNA) blot hybridization of RNA isolated from fetal liver. RNA (15 μg) isolated after 30 min of heat shock at 42°C was denatured in formaldehyde, fractionated on 1% agarose gel, and transferred onto nitrocellulose paper. The blot was probed with albumin cDNA (see the text and the legend to Fig. 4). kb, Kilobases.

Johnson et al. (11) and Grainger et al. (9) have provided experimental evidence for the synthesis of differentiation-specific proteins as a consequence of a change in the internal pH of the cell. Also, Ober and Pardee have shown that tumorigenic Chinese hamster embryo fibroblast (CHEF) cell lines maintain an internal pH that is 0.12 unit above that of the nontumorigenic CHEF/18 parental line and that the change in pH correlates with an altered proliferative response (19). Although these are isolated observations separate from studies on heat shock proteins, it is likely that there is a relationship between the two sets of observations, in that a change in the internal pH of the cell could result from a change in temperature and that a localized change in internal pH could trigger changes in the pattern of gene expression. Experiments designed to verify this possibility are in progress.

Apart from its function, the mechanism of induction of albumin by heat shock is also presently not known. It would be of interest to check not only any similarities in the promoter regions of heat shock proteins and that of albumin, but also whether there are heat shock elements (21) present upstream of the albumin promoter that are involved in the control of expression of albumin and whether heat shock transcription factors (20) are involved in the induction of albumin.

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