Alteration of Protein Synthesis and Induction of Specific Protein Phosphorylation by Hyperthermia

Ira Rubin, Godfrey Getz, and Hewson Swift

Departments of Pathology [I. R. G. G., H. S. J., Biochemistry [G. G.], and Biology [H. S.], The University of Chicago, Chicago, Illinois 60637

ABSTRACT

Confluent cultures of the mouse cell line clone 1D were subjected to 1-hr hyperthermic treatments. Temperatures were increased from the control level of 37° to values ranging from 38 to 45°. Protein synthesis patterns were determined in fluorograms of sodium dodecyl sulfate-polyacrylamide gels labeled with \(^{35}\)S]methionine. Although incorporation into most proteins was either repressed or decreased by heat treatment, several proteins showed an increased label or were apparently induced de novo. Among the induced proteins was a prominent band, probably a doublet, with an estimated molecular weight of 70,000 to 69,000.

Crude cell lysates made from 37°, 41°, and 45°-treated cells were tested for kinase activity at 30° by a 10-min incubation with adenosine \(\gamma\)-\(^{32}\)P]triphosphate. Several specific proteins exhibited increased phosphorylation, while phosphorylation of other proteins decreased. The most significant increase in phosphorylation was shown by a protein with molecular weight of about 37,000. We suggest that heat treatment induces or activates one or more specific phosphokinase(s) with the ability to phosphorylate proteins with approximate molecular weights of 37,000, 36,000, 23,000, and 16,000.

INTRODUCTION

In 1866, Busch (5) reported on several cases of human tumors, as diagnosed by microscopic examination, which regressed after the patients accidentally acquired erysipelas and thus high fevers. These patients had what was reported to be "permanent cures" of their cancer. Many clinicians have made similar observations through the years, but to date hyperthermic treatment of cancer patients is still experimental and controversial (10).

A clearer understanding of the molecular effects of hyperthermia should help to define its optimal use in therapy. Research in this area is presently being conducted by many experimenters interested in radiation biology and oncology. At the same time, cell and molecular biologists have been studying temperature responses in similar systems but with emphasis on mechanisms of gene control rather than on the differential death of neoplastic cells. Most of these studies have been made with the well-defined "heat shock" system of Drosophila melanogaster (1). This alteration of cell synthesis patterns in response to heat is clearly not unique to Drosophila but in fact is shared by many organisms. Alterations of cell growth and death of neoplastic cells. Most of these studies have been made with the well-defined "heat shock" system of Drosophila melanogaster (1). This alteration of cell synthesis patterns in response to heat is clearly not unique to Drosophila but in fact is shared by many organisms.

The heat shock response of neoplastic cells. Most of these studies have been made with the well-defined "heat shock" system of Drosophila melanogaster (1). This alteration of cell synthesis patterns in response to heat is clearly not unique to Drosophila but in fact is shared by many organisms.

The heat shock response of neoplastic cells. Most of these studies have been made with the well-defined "heat shock" system of Drosophila melanogaster (1). This alteration of cell synthesis patterns in response to heat is clearly not unique to Drosophila but in fact is shared by many organisms.

The heat shock response of neoplastic cells. Most of these studies have been made with the well-defined "heat shock" system of Drosophila melanogaster (1). This alteration of cell synthesis patterns in response to heat is clearly not unique to Drosophila but in fact is shared by many organisms.

The heat shock response of neoplastic cells. Most of these studies have been made with the well-defined "heat shock" system of Drosophila melanogaster (1). This alteration of cell synthesis patterns in response to heat is clearly not unique to Drosophila but in fact is shared by many organisms.

The heat shock response of neoplastic cells. Most of these studies have been made with the well-defined "heat shock" system of Drosophila melanogaster (1). This alteration of cell synthesis patterns in response to heat is clearly not unique to Drosophila but in fact is shared by many organisms.

The heat shock response of neoplastic cells. Most of these studies have been made with the well-defined "heat shock" system of Drosophila melanogaster (1). This alteration of cell synthesis patterns in response to heat is clearly not unique to Drosophila but in fact is shared by many organisms.

The heat shock response of neoplastic cells. Most of these studies have been made with the well-defined "heat shock" system of Drosophila melanogaster (1). This alteration of cell synthesis patterns in response to heat is clearly not unique to Drosophila but in fact is shared by many organisms.

The heat shock response of neoplastic cells. Most of these studies have been made with the well-defined "heat shock" system of Drosophila melanogaster (1). This alteration of cell synthesis patterns in response to heat is clearly not unique to Drosophila but in fact is shared by many organisms.

The heat shock response of neoplastic cells. Most of these studies have been made with the well-defined "heat shock" system of Drosophila melanogaster (1). This alteration of cell synthesis patterns in response to heat is clearly not unique to Drosophila but in fact is shared by many organisms.

The heat shock response of neoplastic cells. Most of these studies have been made with the well-defined "heat shock" system of Drosophila melanogaster (1). This alteration of cell synthesis patterns in response to heat is clearly not unique to Drosophila but in fact is shared by many organisms.

The heat shock response of neoplastic cells. Most of these studies have been made with the well-defined "heat shock" system of Drosophila melanogaster (1). This alteration of cell synthesis patterns in response to heat is clearly not unique to Drosophila but in fact is shared by many organisms.

The heat shock response of neoplastic cells. Most of these studies have been made with the well-defined "heat shock" system of Drosophila melanogaster (1). This alteration of cell synthesis patterns in response to heat is clearly not unique to Drosophila but in fact is shared by many organisms.

The heat shock response of neoplastic cells. Most of these studies have been made with the well-defined "heat shock" system of Drosophila melanogaster (1). This alteration of cell synthesis patterns in response to heat is clearly not unique to Drosophila but in fact is shared by many organisms.
I. Rubin et al.

The amount of protein in crude lysates was determined with the Bio-Rad protein assay kit by the method of Bradford. Phosphorylation was carried out in 40-μl aliquots containing 0.5 mg protein per ml, 20 mM magnesium acetate, and 4 μCi [γ-32P]ATP (specific activity, 3000 Ci/mmol; Amersham/Searle Corp.) for 10 min at 30°. Samples were precipitated with 95% acetone at −70° overnight, resuspended, and assayed by polyacrylamide gel electrophoresis as described above. Gels were stained with Coomassie blue, dried directly on Whatman No. 3MM paper, and exposed to Kodak XAR-5 film. Radioactivity in the autoradiograms was quantitated as above.

RESULTS

Effects on Protein Synthesis. Fig. 1 shows the heat-induced synthesis of a specific set of proteins in mouse fibroblast cells by temperatures 1 to 8° above the normal level of 37°. Proteins with apparent molecular weights of 95,000, 87,000, 78,000, 70,000 to 69,000, 55,000, 35,000, and 26,000 were the most prominently induced. The most notable shift is seen at temperatures above 41°. At these higher temperatures, tubulin (apparent M, = 55,000), as well as several low-molecular-weight proteins, was completely repressed. This is shown in Fig. 1, in which at 45° almost no label was incorporated (41° samples had ~50%, and 45° samples had ~10% of total incorporated label compared to 37° controls). At ≥41°, most of the heat-induced proteins appear with the exceptions of the high-molecular-weight proteins at 95,000, and 70,000 to 69,000. These proteins are apparent at low levels at the control 37° temperature. The most significantly induced protein(s) is the doublet with molecular weight of 70,000 to 69,000, comprising at 41°, approximately 10% of the total incorporation of labeled cellular proteins synthesized compared to <1% at 37°. Thus, 41° is a significant transition temperature. This temperature has also been implicated cytologically, where cell morphology has been observed by scanning electron microscopy to change at temperatures above 41°.3

Effects on Phosphorylation. Initial studies on inorganic phosphate labeling with 32P revealed no significant changes in total cellular proteins. However, if crude control cell lysates from cells heat treated at 37, 41, and 45° are incubated with [γ-32P]ATP, several proteins are phosphorylated, as shown in Fig. 2. The major proteins phosphorylated are at apparent molecular weights of 100,000, 95,000, and approximately 36,000 to 38,000. At about 37,000, 3 closely spaced proteins are phosphorylated with apparent molecular weights of 36,000, 37,000, and 38,000. Table 1 compares their distribution and relative amounts, based on Joyce-Loebl densitometer scans of Fig. 2A as shown. The most significant change was with the protein with molecular weight of 37,000, which increased with temperature and is the only one detectable at 45°. The protein with molecular weight of 38,000 was detectable only at 41° where it is the most significant protein phosphorylated. The protein with molecular weight of 38,000 decreased with temperature and 45° is not detectable. Although the overall amount of protein phosphorylation decreases with increasing temperature treatments, the relative amount of phosphorylation at the protein with molecular weight of 37,000 significantly increases, as demonstrated in Fig. 2B. There are less pronounced modifications of other proteins. For example, at 41°, 2 proteins at apparent molecular weights of 23,000 and 16,000 are also

3 I. Rubin, G. Getz, and H. Swift, personal observations.
phosphorylated. These temperature-specific phosphorylations may reflect specific kinase activities at these respective temperatures, although other controlling factors such as substrate accessibility may be involved (23).

DISCUSSION

Certain neoplastic cells have been found to be selectively killed by hyperthermia in both the cancer patient and tissue culture. This is doubtlessly associated with numerous changes in metabolism and cell architecture. Studies on mammalian cells have implicated among many possibilities significant changes in membrane permeability and patterns of translation. Thus far, the best-studied heat-induced responses have been made in cell cultures of D. melanogaster (1). Normal transcription has been shown to be strongly inhibited when cells are raised from 25 to 35°C, and when new transcripts for 6 specific proteins are induced, while at the translational level, preexisting polysomes disappear, leaving intact messages for cell recovery. The function of these proteins is unclear, except that they have been shown to increase the resistance of the cell to elevated temperatures (14, 19).

Detailed studies have provided evidence for an immediate cessation of polypeptide chain initiation during the inhibition of protein synthesis observed after hyperthermia treatments (18). Other systems that control the overall level of protein synthesis through selective translation are the hemin control of protein synthesis in reticulocytes (17) and interferon double-stranded RNA-mediated inhibition of protein synthesis in cell cultures infected with virus (2). In these cases, the translational control involves the induction of a specific protein kinase (M, ≈ 67,000) which phosphorylates the α-subunit of Eukaryote Initiation Factor 2 (M, ≈ 37,000). Other studies have associated heat-induced differences in nuclear phosphoproteins with the control of transcription (6, 7, 20).

Interferon has been shown recently to induce a specific set of proteins including a protein with molecular weight of 67,000 thought to be this kinase (8). We speculate that our heat-induced altered cell protein synthesis and posttranslational modification (phosphorylation) of certain proteins are similar if not equivalent to those observed with interferon. Heat shock may be only one way of influencing a complex interacting network or cascade of cell reactions associated with mechanisms for homeostasis and cell survival. This concept is supported by the fact that heat shock proteins are known to be induced by a wide variety of agents (1).

ACKNOWLEDGMENTS

We would like to thank Dr. M. M. Elkkind for stimulating discussions and Dr. S. L. Lindquist for commentary on the manuscript.

REFERENCES

Fig. 2. Coomassie-stained gels and autoradiograms of cell lysates from heat-treated mouse cells. Proteins in cell lysates of heat-treated cells, at the temperature indicated, were labeled with [γ-32P]ATP and separated by sodium dodecyl sulfate-polyacrylamide (10%) electrophoresis, as described in "Materials and Methods." Coomassie-stained gels (left) are given as a quantitative measure of the amount of protein in autoradiograms (right). Arrows, proteins at apparent molecular weights of 38,000, 37,000, 36,000, 23,000, and 16,000. In A, equal amounts (20 μg) of protein were separated; gel was exposed to film for 12 hr. In B, equal amounts (100,000 cpm) of 32P-labeled proteins were separated; gel was exposed to film for 15 hr. In C, densitometer tracing is given of the regions of the gels with molecular weight of 38,000 to 36,000, in A; arrows indicate the position of major bands seen (p38, p36, and p37 at 37, 41, and 45°, respectively). Lower molecular weights are to the left.
Alteration of Protein Synthesis and Induction of Specific Protein Phosphorylation by Hyperthermia

Ira Rubin, Godfrey Getz and Hewson Swift


Updated version Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/42/4/1395

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/42/4/1395. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.