Use of Polymerase Chain Reaction to Detect the Expression of the M, 70,000 Heat Shock Genes in Control or Heat Shock Leukemic Cells as Correlated to Their Heat Response

Nahid F. Mivechi and John J. Rossi
Department of Radiation Research and Molecular Genetics, City of Hope National Medical Center, and Beckman Research Institute, Duarte, California 91010

ABSTRACT

The expression of the M, 70,000 heat shock protein (HSP-70) in heat-resistant variants or heat-shocked cells has been correlated with development of thermal resistance. In these studies polymerase chain reaction (PCR) was used to detect low levels of HSP-70 mRNA present in control, unheated cells to investigate the possibility of predicting the intrinsic heat response in various leukemic cells. The expression of two human heat shock genes in control or heat-shocked cells was investigated. Synthetic primers and probes from the untranslated region of the two HSP-70 genes sequenced by Hunt and Morimoto (HSP-70A) (C. Hunt and R. I. Morimoto, Proc. Natl. Acad. Sci. USA, 82: 6455-6459, 1985) and Voellmy et al. (HSP-70B) (R. Voellmy et al., Proc. Natl. Acad. Sci. USA, 82: 4949-4953, 1985) were used in PCR reactions to follow expression in control or heat-shocked leukemic K562, KG-1, and HL-60 cells. The PCR results were correlated with heat response and patterns of protein synthesis in these cells. Results indicate that, among leukemic cells, K562 was much more resistant to killing by heat shock than either KG-1 or HL-60 cells. All control cells, however, expressed the HSP-70B gene. Of the three leukemic cells tested, K562 was the most heat resistant and constitutively expressed the HSP-70A mRNA and the heat-inducible HSP-70 protein. KG-1 and HL-60 cells did not express this gene in unheated cells. All heat-shocked cells expressed the HSP-70A mRNA and the heat-inducible HSP-70 protein. However, there was no significant increase in the mRNA level of the HSP-70B in heat-shocked leukemic cells as measured by PCR or the S1-nuclease protection assay. Other cells including normal human bone marrow and normal and tumorous tissues of the colon and breast all expressed both genes in control cells. Normal breast tissue expressed less mRNA for HSP-70B gene than the tumor tissue obtained from the same patient. In all studies the amplified β-actin mRNA expression was used as an internal standard.

These studies indicate that HSP-70B gene is expressed in all control leukemic cells. The expression of this gene did not seem to correlate with intrinsic heat tolerance. The HSP-70A expression correlated with intrinsic and transient heat resistance. These studies also indicate that both HSP-70 genes in humans may be expressed in a variety of unheated normal and tumorous tissues more so than previously reported.

INTRODUCTION

The expression of HSP-70 has been correlated with both transient heat resistance [i.e., during the development of thermotolerance (1-5)] and intrinsic heat resistance in heat-resistant variants of Chinese hamster ovary cells (6). It has also been shown recently that the microinjection of the antibody to HSP-70 rendered cells sensitive to heat killing (7). Furthermore, the competitive inhibition of the heat-inducible expression of HSP-70 mRNA resulted in sensitivity to acute heat shock (8). All of these data strongly suggest a role for HSP-70 in protection of cells against heat damage.

Two heat-inducible HSP-70 genes have been sequenced so far. Expression of the HSP-70 gene sequenced by Hunt and Morimoto (HSP-70A) (9) is inducible by heat shock and cadmium. This HSP-70 gene is also expressed in some transformed cells and as a result of the action of the adenovirus E1A gene (10) and of a c-myc gene (11). The other human HSP-70 gene was sequenced by Voellmy et al. (HSP-70B) (12) and was also found to be under heat shock control. Although the promoter segments contained several features similar to those genes which are constitutively expressed and are "housekeeping" genes (13), HSP-70B has never been shown to be present in non-heat-shocked control cells (12, 14).

In these studies we have used synthetic primers and probes specific for and complementary to one or the other of the above HSP-70 genes in order to correlate, by PCR, the expression of these genes in control or heat-shocked cells with intrinsic or transient heat resistance. We and others have been studying the possibility of the in vitro or in vivo purging of leukemic cells by hyperthermia (15-20). It would be useful if the heat response of leukemic cells could be predicted before any attempt to purge such cells was made.

These studies indicate that the expression of HSP-70A correlates with intrinsic heat resistance. However, since this gene may be expressed in many normal tissues as well as tumors in various amounts, the level of expression of this gene needs to be correlated with heat response.

MATERIALS AND METHODS

Cell Culture and Maintenance. Human bone marrow was obtained from normal donors. K562 (21), HL-60 (22), and KG-1 (23) cells are erythroleukemia and acute myelogenous leukemic cells and were obtained from American Type Culture Collection. All cell lines were maintained in Iscove's modified Dulbecco's medium plus 20% FCS and antibiotics (16).

Cell Survival Assays. The leukemic cells were grown as described previously (16). Briefly, after appropriate treatments, cells were centrifuged and plated in 35-mm Petri dishes. The growth medium containing α-minimal essential medium, 10% FCS, and 0.3% Noble agar. KG-1 cells were supplemented with 150 units/ml of recombinant granulocyte-macrophage colony-stimulating factor (24), a generous gift of the Genetics Institute of Boston, MA. Plating efficiencies for K562, HL-60, and KG-1 cells were 10 to 30%, 10 to 20%, and 5 to 15%, respectively. All cell lines were incubated at 37°C, 98% humidity, 5% CO2 for 10 to 14 days. Only colonies of 50 or more cells were counted. All experiments were repeated at least 2 times. Values are given as the mean ± SD.

Total RNA Isolation. The method of guanidinium/CsCl was used for total RNA isolation (25). Five volumes of buffer, 6 M guanidinium isothiocyanate, 5 mM sodium citrate (pH 7.0), and 0.1 M 2-mercaptoethanol were added to the cell pellet. One g of CsCl was added to each 2.5 ml of homogenate. The homogenate was then layered onto 1.2 ml of 5.7 M CsCl in 0.1 M EDTA in SW 50.1 tubes. The tubes were
centrifuged at 35,000 rpm for 16 h at 20°C. The supernatant was discarded, and the RNA pellet was dissolved in 10 mM Tris-Cl (pH 7.4), 50 mM EDTA, and 1% sodium dodecyl sulfate. The solution was then treated with DNase (4 units) for 30 min at 37°C. The solution was then extracted twice with phenol:chloroform. One-tenth volume of 3 M sodium citrate (pH 5.2) was added to the aqueous layer (0.3 M final concentration) plus 2.2 volumes of ethanol. After chilling at −20°C for 2 h, RNA was recovered by centrifugation and dissolved in H2O.

Synthetic Oligonucleotides. Oligonucleotides were synthesized by a Syntex automated DNA synthesizer in the DNA synthesis facility of the City of Hope Medical Center. The sequences of the HSP-70A and HSP-70B regions chosen to be amplified were from the 5'-end untranslated regions of the HSP genes sequenced by Hunt and Morimoto (9) and Voelmy et al. (13) to minimize cross-reactions with any other HSP-70 gene family and are shown in Fig. 1. As it is shown in Fig. 1, there are several restriction sites in the amplified segment which can be used to test the specificity of the amplified products. We have also used amplified mRNA for the β-actin (26) gene as an internal standard in our studies. For β-actin, one of the priming nucleotides is in exon 3 and spans nucleotides 1627 to 1650. The second priming nucleotide is complementary to nucleotides 2379 to 2401 and is located in exon 4. The probe is complementary to nucleotides 1652 to 1654 plus 2096 to 2115 and is located in exons 3 and 4. The amplified fragment for actin is 332 base pairs.

RNA and DNA Amplification Procedure (PCR Assay). One μg of total RNA from normal bone marrow or other tumors or normal tissues was incubated in 1X amplification buffer [10 mM Tris-HCl (pH 8.0), 1.5 mM MgCl2 with 37 pmol of each of the two priming nucleotides, 6 mM 2-mercaptoethanol, 60 mM NaCl, 1 mM dithiothreitol, and 1 mM deoxy-nucleotide triphosphate (ATP, GTP, CTP, UTP) in a final volume of 50 μl]. The Mg2+ concentration was determined to be 1.5 mM for all genes. Samples were placed in a thermal cycler (Perkin Elmer), heated to 95°C for 5 min, and cooled to 42°C for 2 min, at which time 2 units of reverse transcriptase and 2.5 unit of Taq polymerase (Perkin Elmer Cetus) were added and incubated at 42°C for 2 min. The cycles of heating (95°C, 2 min), annealing (42°C, 2 min), and polymerization (72°C, 2 min) were continued for 30 rounds unless otherwise indicated. All enzymes were diluted in 1X amplification buffer plus 1 mM dithiothreitol (27, 28). After completion of the last cycle, samples were kept at 4°C.

Agarose Gel Electrophoresis, Blotting, and Hybridization. After PCR amplification, samples were electrophoresed in 1.8% agarose gel containing 0.5 μg/ml of ethidium bromide and 0.5X 80 mM Tris (pH 8.0):40 mM boric acid:2 mM EDTA buffer (27, 28). Following electrophoresis (1.5 V/cm), the gels were photographed and vacuum blotted on a Zeta Probe nylon (Bio Rad) membrane for 60 min using 0.4 M ammonium acetate (NH4OAc) and 0.1 M EDTA, and the solution was then washed with 1X amplification buffer plus 1 mM dithiothreitol (27, 28). After completion of the last cycle, samples were kept at 4°C.

RESULTS

Heat Response of Leukemic Cells. Heat responses of leukemic cells were compared and, as Fig. 2 indicates, K562 cells were the most resistant cells to hyperthermia. In terms of the Du of the heat survival curves (16), K5-1 and HL-60 cells were 3-fold more sensitive to heat than were K562 cells.
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Two-dimensional Gel Analysis of the Protein Synthesis of Control and Heat-shocked Cells. In order to examine whether there were differences in heat-shock protein synthesis in various sensitive and resistant cell types, cells were labeled as control or following a mild heat shock. As we have shown previously (16) on one-dimensional PAGE analysis following a mild heat shock which reduced the survival levels by 50% in all cell lines, K562 cells synthesized HSP-70 for over 24 h, and KG-1 and HL-60 cells synthesized HSP-70 for only 2 to 4 h. On two-dimensional PAGE analysis (Figs. 3 and 4), K562 and HL-60 cells showed high levels of the constitutive HSP-73 in control cells (Spot b) (2). K562, the most heat-resistant leukemic cells, showed the expression of the heat-inducible HSP-72 (Spot c) in control cells (2). All heat-shocked cells, however, show the high levels of heat-inducible HSP-72 (42°C, 60 min, and 42°C, 30 min for K562 and HL-60, respectively).

Detection of Heat-inducible Expression of HSP-70 Genes by PCR. Fig. 1 shows the sequences for the primers and probes chosen for both HSP-70A and HSP-70B genes. The amplified region for both genes was at the 5'-end untranslated region so as to minimize cross-reactions to any other HSP-70 gene family members. In order to find out whether the synthesized primers detect the mRNA for HSP-70A and HSP-70B genes, total RNA for control and heat-shocked (45°C, 20 min plus 2 h at 37°C) HeLa cells was amplified using primers specific to each, and the amplified products were detected with labeled probes (Fig. 5). Fig. 5a shows the ethidium bromide staining of the amplified fragments for HSP-70A and HSP-70B mRNA. The amplified fragments had the predicted sizes, HSP-70A and HSP-70B being 204 and 162 base pairs, respectively. With primers specific to HSP-70B, there were other bands present in the ethidium bromide staining. However, the probe always recognized the 162-base pair amplified fragments (Fig. 5b).

As has been previously shown (9), our results showed that HeLa cells constitutively express the HSP-70A gene transcript (9) (Fig. 5, a and b). Furthermore, the HSP-70B gene transcript was induced upon heat shock (12) (Fig. 5, a and b). Fig. 5 also shows the autoradiograph of the HSP-70A and HSP-70B mRNA expression as detected following hybridization with the 32P-labeled probes. We have also used amplified β-actin mRNA as an internal standard for each sample and demonstrated an equal amount of amplified products in both control and heat-shocked cells (Fig. 5c).

We also tested the linearity of the PCR reactions by amplifying from various amounts of total RNA. Fig. 6A shows autoradiographs of the amplified mRNA for β-actin (332 base pairs), HSP-70A (204 base pairs), and HSP-70B (162 base pairs) using various amounts of total RNA (μg) in the starting PCR reactions. Densitometric tracings of the autoradiograph indicated the linearity of the amplified products for 1 μg and less of total RNA for β-actin and HSP-70B and for at least 0.5 μg and less for HSP-70A. It should be noted that the amplified mRNA was from heat-shocked K562 cells (45°C, 10 min plus 2 h at 37°C), which resulted in a very high level of expression of the HSP-70A gene.

In order to verify that the amplified products obtained by using the HSP-70B primers are indeed from that particular...
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Fig. 4. Two-dimensional PAGE analysis of heat-shocked leukemic cells. Methionine-labeled heat-shocked cells (1 to 2 x 10⁶) were analyzed by two-dimensional PAGE. Heat doses were 42°C/60 min and 42°C/30 min for K562 and HL-60, respectively. A, actin; a and b, M, 73,000 proteins which are constitutively expressed in human cells; c, the inducible HSP-72, the expression of which is induced or enhanced following heat shock.

gene, we used these primers to amplify the same segment from the plasmid P17, which contains all the 5'-untranslated region plus partial coding sequences of the HSP-70 gene (12). As Fig. 6B indicates, the 5'-untranslated region of P17 was indeed amplified, resulting in the predicted fragment size of 162 base pairs. As Fig. 6B also shows, the primers specific to HSP-70A did not show any amplification of P17 (Fig. 6A, Lane 1).

Detection of the Constitutive and Heat-inducible HSP-70A and HSP-70B mRNA in Leukemic Cells. Polymerase chain reaction assays were then performed in order to detect and correlate the presence of the two human HSP-70 mRNAs in control or heat-shocked cells. As Fig. 7a indicates, unlike HeLa cells, all control leukemic cells showed high levels of expression of the HSP-70B message, and its expression was not significantly enhanced upon heat shock. In order to investigate whether the expression of mRNA for HSP-70A correlates with intrinsic heat resistance in various leukemic cells, PCR was performed using total RNAs, isolated from such cells. Fig. 7b shows that K562, the most heat-resistant cell line, shows high expression of HSP-70A in unheated control cells. KG-1 and HL-60, the two heat-sensitive leukemic cells, do not show the expression of HSP-70A in unheated cells. All cells, however, showed high expression of HSP-70A following a heat shock which reduced the survival by approximately 50%. Fig. 7c shows the amplified β-actin mRNA for samples. β-Actin was used as an internal standard to make sure an equal amount of mRNA had been loaded and amplified for control or heat-shocked cells. Fig. 7d shows the ethidium bromide staining of the amplified products of HSP-70A and HSP-70B mRNA for K562, HL-60, and KG-1 cells. As the ethidium bromide staining of the amplified fragments indicates, the resulting bands are at the predicted 162 and 204 base pairs, for HSP-70B and HSP-70A, respectively.

The most surprising results were the high levels of expression of both HSP-70 genes in unheated and heated normal bone
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Fig. 7. a, PCR of control or heat-shocked leukemic cells using oligoprimers synthesized from the gene sequenced by Voellmy et al. (12). Total RNA samples were isolated from control or heat-shocked (42°C/60 min for K562 or 42°C/30 min for KG-1 and HL-60 plus 2-h incubation at 37°C) cells. Total RNA was then quantitated by A260 and electrophoresis in 6% formaldehyde/1% agarose gels, and 1 μg of total RNA was then used in an amplification (34 rounds) procedure described in “Materials and Methods.” C, control; H, heat shocked. (+) and (−) represent with or without reverse transcriptase. b, PCR of control or heat-shocked leukemic cells using oligoprimers synthesized from the gene sequenced by Hunt and Morimoto (9). Total RNA samples were isolated from control or heat-shocked (42°C/60 min for K562 or 42°C/30 min for KG-1 and HL-60 plus 2-h incubation at 37°C) cells. Total RNA was then quantitated by A260 and electrophoresis in 6% formaldehyde/1% agarose gels, and 1 ng of total RNA was then used in an amplification procedure described in “Materials and Methods.” C, control; H, heat shocked. c and d, ethidium bromide staining of the control or heat-shocked leukemic cells using oligoprimers synthesized for HSP-70A, HSP-70B, and β-actin genes. These panels show the ethidium bromide staining of the samples shown in a and b. c: C, control; H, heat shocked. 332. base pair of amplified fragments size of the β-actin mRNA, M, HaeIII digest of Phage X174. In d, Lanes 1 and 7 are control K562; Lanes 2 and 8, heat-shocked K562; Lanes 3 and 9, control HL-60; Lanes 4 and 10, heat-shocked HL-60; Lanes 5 and 11, control KG-1; Lanes 6 and 12, heat-shocked KG-1; 204 and 162 are the lengths of the amplified fragments (base pairs, bp) of HSP-70A and HSP-70B mRNA, respectively. M, as above.

It should be noted that − and + in each figure correspond to the absence and presence of reverse transcriptase, which indicates that amplification products correspond only to the mRNA present in various tissues. (+) reverse transcriptase control experiments were performed for each isolated RNA sample; however, (−) reverse transcriptase lanes have not been shown for every sample. It should also be noted that care was taken to minimize manipulations of various tissues in order to decrease the probability of stress response. Human bone marrow samples were obtained from normal donors, and within 1 h following extraction, white blood cells were separated and immediately frozen. Normal and tumorous tissues were frozen immediately following surgery.

S1-Nuclease Protection Assay Using an Amplified Segment of the P17 as a Probe. In order to confirm some of the results obtained with the PCR assay, S1-nuclease protection assays were performed using the amplified segment of the P17 5' untranslated region as a probe. The primers complementary to the HSP-70B gene were used to amplify the segment of P17. As indicated in Fig. 6B, the predicted (162 base pair) fragment was observed. The 32P-labeled amplified fragment “Materials and Methods,” was then used in an S1-nuclease protection assay. As Fig. 9 indicates, a protected segment of the HSP-70B mRNA was present in control and heat-shocked K562, HL-60, and bone marrow cells. Similar to the PCR results, the heat-shocked cells showed no significant increase in HSP-70B mRNA expression by this assay. Fig. 9 also shows a higher HSP-70B expression in breast tumor as compared to normal breast tissue (approximately 3-fold). Lanes 4 and 5 show control and heat-shocked HeLa cells. Although there was a definite enhancement of HSP-70B mRNA in heat-shocked HeLa cells,
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Fig. 8. a, PCR of control or heat-shocked normal human bone marrow and normal and tumorous tissues obtained from patients with colon and breast malignancies using oligoprimers synthesized from the gene sequenced by Voellmy et al. (12). Total RNA samples were isolated from control or heat-shocked normal bone marrow (42°C/60 min plus 2 h at 37°C) or control normal or tumorous tissues from colon or breast tissues. Total RNA was then quantitated by A260 and electrophoresis in 6% formaldehyde/1% agarose gels, and 1 μg of total RNA was then used in the amplification procedure described in “Materials and Methods.” C. control; H. heated; N. normal; T. tumorous tissue. (+) and (−) represent with or without reverse transcriptase. b, PCR of control and heat-shocked normal human bone marrow and normal and tumorous tissues from patients with malignancies using oligoprimers synthesized from the gene sequenced by Hunt and Morimoto (9). Total RNA samples were isolated from control or heat-shocked normal bone marrow (42°C/60 min plus 2 h at 37°C) or control normal or tumor from colon or breast tissues. Total RNA was then quantitated by A260 and electrophoresis in 6% formaldehyde/1% agarose gels and 1 μg of total RNA was then used in the amplification procedure described in “Materials and Methods.” C. control; H. heated; N. normal; T. tumor tissue. (+) and (−) represent with or without reverse transcriptase. c, PCR of control or heat-shocked bone marrow and other normal and tumorous tissues using oligoprimers synthesized from the β-actin gene. PCR was performed for samples shown in a and b using β-actin as primers. 332, length of the amplified fragment (base pairs, bp) of β-actin mRNA. Lanes 1 and 2, control or heat-shocked bone marrow, respectively; Lane 3, normal colon; Lane 4, colon tumor; Lane 5, normal breast; Lane 6, breast tumor.

Fig. 9. S1-nuclease protection assay of mRNA from control or heat-shocked cells using an amplified fragment from P17 as a probe. An S1-nuclease protection assay was performed on 40 μg (except normal and tumorous tissues which were 20 μg) of total RNA samples (same samples that were used for PCR). Lane 1, salmon sperm DNA plus 32P-labeled probe and S1-nuclease treated (negative control); Lane 2, 32P-labeled probe not treated with S1 nuclease; Lane 3, molecular weight marker; Lanes 4 and 5, control or heat-shocked HeLa cells; Lanes 6 and 7, control or heat-shocked K562 cells; Lanes 8 and 9, control or heat-shocked HL-60 cells; Lanes 10 and 11, control or heat-shocked bone marrow; Lanes 12 and 13, normal and tumorous tissues from the breast. 162, size of the hybridized fragment (base pairs, bp).

the control also shows small basal levels of mRNA for this gene. The ethidium bromide staining of the PCR data for control HeLa cells shown in Fig. 5a also shows a minor band (Lane 3). It is therefore possible that the HeLa cell line used in our studies expresses low levels of HSP-70B in control cells.

DISCUSSION

Two human heat-inducible HSP-70 genes have been sequenced so far. One gene sequenced by Voellmy et al. (12) shows a high degree of homology to HSP-70 isolated from other species. Expression experiments in Xenopus oocytes show high levels of transcription in heat-shocked but not in control oocytes containing the fragment of this human gene (12). Other expression experiments in mammalian cells also have shown a high rate of transcription in heat-shocked but not in control cells (12). The other sequenced HSP-70 gene is that of Hunt and Morimoto (9). This gene is induced by heat shock and cadmium, and its expression is present in some transformed cells.
PCR assays have been previously shown to detect low levels of specific mRNAs of several different genes (28, 31–33). In our studies we have taken several steps to ensure the specificity of the amplified fragments and whether they do correspond to HSP-70A and HSP-70B mRNA. (a) The sequences for the primers were chosen at the 5′-untranslated region which has been shown in many gene families to be unique to every gene. (b) The amplified fragments resulted in the predicted fragment sizes of 162 and 204 base pairs, for HSP-70B and HSP-70A, respectively. (c) Both amplified fragments contain several restriction sites which can be used to test the specificity of the fragments. (d) As Fig. 6B indicates, the primers specific to HSP-70B amplified a segment of the plasmid P17 containing HSP-70 human gene segment. Similar amplifications were not observed when primers specific to HSP-70A were used. (e) The synthesized probes specific to each gene only recognize one band corresponding to the amplified fragment of each gene. All of the above reasons, plus the fact that the expressions of these genes are similar to those of previous reports, indicate that these amplified segments correspond to HSP-70A and HSP-70B genes.

In order to correlate HSP-70 gene products with intrinsic thermal resistance, PCR was used to detect the mRNA from both of the above HSP-70 genes in control or heat-shocked leukemic cells. As we and others have previously reported, some leukemic cells are manifold more sensitive to killing by heat than normal bone marrow progenitors (15–20). Therefore, we intended to examine whether heat-resistant leukemic cells express high levels of the heat-inducible HSP-70 mRNA. High levels of the heat-inducible HSP-70 protein or mRNA have been correlated with intrinsic thermal resistance in cell lines such as HeLa (34) and 293 cells or during the development of thermotolerance (1–4).

Contrary to previous reports (12, 14), using PCR and S1-nuclease protection assays, we have found the HSP-70 gene sequenced by Voellmy et al. (12) is expressed in most control cells. HSP-70B mRNA is not significantly induced by heat shock (except in HeLa cells), and the levels of mRNA expression may be greater in some tumors than in corresponding normal tissues. These findings indicate that the gene sequenced by Voellmy et al. (12) may be constitutively expressed in many tissues. The HSP-70B gene seems to be heat inducible in HeLa cells and also a colon carcinoma cell line (LS174t; data not shown). In both HeLa and LS174t cells, there is no or a very small amount of HSP-70B expression in control cells. It is conceivable, therefore, that HSP-70B is only induced by heat when no or a very small amount of a basal level of HSP-70B mRNA is present in control cells. In contrast, the gene sequenced by Hunt and Morimoto (9) seems to be induced by heat shock, and it is not expressed in heat-sensitive KG-1 or HL-60 cells. High levels of this mRNA, however, were expressed in heat-resistant K562 cells. Surprisingly, this heat-inducible HSP-70 was also found to be expressed in normal bone marrow and normal and tumorous tissues from colon and breast. Although the lack of expression of HSP-70A in KG-1 and HL-60 cells correlates with their increased sensitivity to heat, it is not entirely clear why these cell lines do not express this gene. It is possible that some leukemic cells originating from particular differentiating stages in the bone marrow may not express this gene. Among leukemic cells, the AML have been shown to be more sensitive to heat (15, 17). Both KG-1 and HL-60 cell lines are also AML. At present, we are correlating the heat response of AML obtained from patients with the amount of HSP-70A and HSP-70B mRNA present in such cells in order to find out whether the level of mRNA expressed in these cells correlates with or can be used to predict their thermal response.

The S1-nuclease protection assay using a labeled probe from the amplified segment of P17 which contains the human HSP-70 gene segment (same as HSP-70B) confirmed the data obtained with PCR. Similar to our PCR data, the S1-nuclease protection assay also showed expression of HSP-70B in K562, HL-60, bone marrow, and normal and tumorous tissues from the breast. Furthermore, HSP-70B gene expression was not significantly increased by heat shock, and as PCR and S1-nuclease protection assays also showed, a higher level of HSP-70B message is expressed in the breast tumor than the corresponding normal tissue. HeLa cells showed an elevated expression of HSP-70B message following heat shock as has been previously reported. However, using the S1-nuclease protection assay (as well as ethidium bromide staining of control HeLa cells shown in Fig. 5a, Lane 3), we observed some basal level of HSP-70B mRNA in control HeLa cells. It is possible that the HeLa cell line used in our studies expresses low levels of the HSP-70B gene in control cells.

In conclusion, PCR has been used to detect low levels of expression of HSP mRNA present in some cells. The PCR results were also confirmed by S1-nuclease protection assays. Furthermore, the expression of heat-inducible HSP-70 genes needs to be examined in organisms other than humans by PCR in order to find out whether this gene is also expressed at low levels in unheated cells in other species.

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Use of Polymerase Chain Reaction to Detect the Expression of the \( M_r \) 70,000 Heat Shock Genes in Control or Heat Shock Leukemic Cells as Correlated to Their Heat Response

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