Cell Stress & Chaperones (1999) **4** (3), 153–161

Article no. csac.1999.0146

A method for the quantitative analysis of human heat shock gene expression using a multiplex RT-PCR assay

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Abstract A quantitative multiplex RT-PCR assay is described to measure the levels of messenger RNAs for eight human genes encoding the heat shock proteins (HSP) and molecular chaperones *hsp*90α, *hsp*90β, *hsp70*, *hsc70*, *mtHsp*75, *Grp*78 (BiP), *hsp60* and *hsp27*. The basis of this assay is reverse transcription of total RNA isolated from human cells followed by amplification with PCR. By the careful selection of pairs of oligonucleotide primers corresponding to unique regions of each heat shock gene, selectivity can be attained such that messenger RNAs of multiple heat shock genes can be analyzed simultaneously in a single reaction. This method provides both the absolute and relative levels of each heat shock message by including in the reaction, reference control RNAs corresponding to in vitro transcripts of heat shock gene plasmids carrying small internal deletions.

INTRODUCTION

The expression of heat shock genes reflects the needs of cells and tissues for a highly conserved family of proteins known as the heat shock proteins (HSPs) or molecular chaperones which function in the repair and protection against the stress of misfolded proteins. Although the exposure of cells and organisms to elevated temperatures has been the stress paradigm, the heat shock response is induced by conditions as diverse as viral and bacterial infection, exposure to transition heavy metals, amino acid analogues, drugs, toxic chemicals, and pathophysiologic and disease states including oxidative stress, fever, inflammation, myocardial stress, ischemia and reperfusion damage, neurodegenerative diseases, aging and cancer (Morimoto et al. 1994; Feige and Eden 1996). Many of these conditions result from or cause cell death and tissue dysfunction.

Received 13 January 1999; Revised 3 March 1999; Accepted 3 March 1999
Available on-line 12 July 1999

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Although the acute response to stress may represent a critical component in the recovery and long-term survival of affected tissues, the chronic expression of heat shock proteins and molecular chaperones may be deleterious (Feige and Eden 1996, Morimoto and Santoro 1998). During ischemia and postischemic reperfusion, heat shock proteins are rapidly induced; this has been suggested to be due to the generation of oxygen-free radicals that have also been implicated in the response to environmental agents including xenobiotics and aromatic hydrocarbons. The ability to detect and respond to oxidant damaged proteins may also be relevant to stroke and neurotransmitter toxicity. Activation of the heat shock response during myocardial reperfusion has been suggested to assist in the restoration of normal cardiac function post injury, possibly by removal of misfolded cardiac proteins and re-establishing normal cardiac protein synthesis. Likewise, the induction of *hsp70* following aortic constriction or work-overload induced cardiac hypertrophy could reflect a response to the aberrant synthesis, accumulation or degradation of

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proteins or alternatively a response to events that occur during the partial re-entry of the myocardial cell into the growth cycle. Overexpression of *hsp70* and *hsp27* in transgenic animals is sufficient to protect the myocardium against reperfusion-induced damage (Plumier et al. 1995).

An increasing number of observations associate the aberrant expression of heat shock genes with pathophysiology (Feige and Eden 1996, Favatier et al. 1997). While the majority of these studies indicate a positive correlation between elevated expression of one or more heat shock gene with a particular disease, they are often limited by sample size and methodology. The majority of studies have employed either northern blot analysis or western blot assays, which provide relative fold-induction of the respective messenger RNA or protein but are incapable of providing absolute levels. Another limitation has been the varied use of reagents which often do not distinguish among members of a gene family in contrast to gene specific reagents. The *hsp70* genes, for example, encode a large, evolutionarily conserved family which are highly related, yet localized to different subcellular compartments. While each of these *Hsp70*-related proteins shares similar function as a molecular chaperone, their distinction, however, is critical as the regulation of *hsp70, hsc70, grp78* and *mthsp75* is distinct as are the stress signals involved in regulation of each gene (Morimoto, 1998).

Among the various methods to quantitate gene expression, we describe the use of quantitative RT-PCR (Gilliland et al. 1990, Ferre 1992, Siebert et al. 1992, Sperisen et al. 1992, Fandrey et al. 1993, Schwaller et al. 1997, Jensen and Whitehead 1998, O'Connell et al. 1998). The basic principle of this method is to use a modified homologous template as an internal control to normalize variations in the whole RT-PCR process, thus allowing the expression of the test gene to be determined precisely. This method is highly sensitive because it is PCR-based and requires small quantities of starting material. It also has a high specificity because the highly homologous templates can be distinguished by the design of primers based on a difference of a few nucleotides between these genes. This study presents a method for the quantitative analysis of heat shock gene expression in which we have adapted the quantitative RT-PCR method and further developed it into a multiplex quantitative PCR method. By applying this multiplex quantitative method, we are able specifically to distinguish and simultaneously to quantify the expression of eight human heat shock genes.

MATERIALS AND METHODS

Cell growth and heat shock treatment

Tissue culture cells were grown in Dulbecco's Modified Eagle's Medium (Life Technologies, Gaitherburg, MD, USA) with 10% fetal calf serum and cultured in a 5% CO_2 incubator at 37°C. For heat shock treatment, the sub-confluent plates were sealed with parafilm and maintained in a water bath at the indicated temperature and time.

RNA preparation

Total RNA was extracted from cells by the NP-40 method (Gough 1988). Briefly, 10^6 to 10^7 cells were washed with PBS and resuspended in 200 µl of buffer A (10 mM Hepes, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 2.5 mM DTT). Cells were lysed by addition of 10 µl of 10% NP-40, vigorous vortexing for 10 s and spinning for 1 min. Two hundred microliters of supernatant was transferred to a new tube, mixed with 200 µl of buffer B (7 M urea, 1% SDS, 0.35 M NaCl, 10 mM EDTA 10 mM Tris, pH 7.5). After centrifuging at full speed for 15 min, 360 µl of upper phase containing RNA were recovered. The RNA was precipitated by addition of 900 µl ethanol and spinning at full speed for 15 min. RNA pellets were washed with 70% ethanol and dissolved in DEPC-treated H_2O. Each RNA sample was further treated with 10 units of RNase-free DNase I at 37°C for 60 min to remove genomic DNA contamination. RNA was treated by phenol-chloroform, precipitated and washed as above. The final purified RNA was quantified by OD_{260} measurement and checked by agarose gel electrophoresis to insure the quality.

Preparation of control reference RNA9

For making control RNA for each gene, the cDNA fragments containing the region between the designed 5′ and 3′ PCR primers of each gene were subcloned into pGEM3zf(+). Suitable restriction sites were chosen to make an internal deletion for each gene. The restriction enzymes and digestion sites used were as follows: *hsp27* Bsu36I 529, NheI 566 (*Hickey* et al. 1986); *hsp60* HpaI 1641, PpuMI 1687 (Venner et al. 1990); *hsp70* MscI 493, BsaAI 612 (Hunt and Morimoto 1985); *hsc70* BbsI 434, 499 (Dworniczak and Mirault 1987); *mthsp75* BsmI 273–341 (Bhattacharyya et al. 1995); *grp78* PpuMI 1697, PpuII 1756 (Ting and Lee 1988); *hsp90α* NdeI 1870, AflII 1996 (Yamazaki et al. 1989); *hsp90β* XhoI 2216, 2326 (Rebbe et al 1989). To make in vitro transcripts, each control construct was linearized downstream of the 3′ antisense primer by restriction digestion. In vitro transcripts were synthesized according to the suggested protocol (Promega 1993). Briefly, the reaction mixture was prepared at room temperature in a 100 µl volume, including 20 µl of 5 × transcription buffer (Promega, Madison, WI, USA), 20 µl of 100 mM DTT, 100 units of RNase inhibitor (Promega), 20 µl of 2.5 mM NTP (Pharmacia, Piscataway, NI, USA), 2 µg of linearized plasmid, and 20 units of T7 RNA polymerase. The reaction was carried out at 37°C for

Table 1 Primer list for RT-PCR

Gene	Sequence	Location	Size of wild-type amplicon	Size of control amplicon	Sequence references
	sense:				Hinky et al. 1986
hsp27	5′-ATGGCGTGGTGGAGATCACC-3′	396–415	350	313	
	antisense:				
	5′-CAAAAGAACACACAGGTGGC-3′	742–723			
	sense:				Venner et al.
hsp60	5′-ATTCCAGCAATGACCATTGC-3′	1444–1463	306	260	1990
	antisense:				
	5′-GAGTTAGAACATGCCACCTC-3′	1749–1729			
	sense:				Hunt et al. 1985
hsp70	5′-TTCCGTTTCCAGCCCCCAATC-3′	435–455	558	438	
	antisense:				
	5′-CGTTGAGCCCCGCGATGACA-3′	993–974			
	sense:				Dworniczak et al.
hsc70	5′-AAGTTGCAATGAACCCCACC-3′	256–265	570	509	1987
	antisense:				
	5′-TTGCGCTTAAACTCAGCAA-3′	826–808			
	sense:				Bhattacharyya
mthsp75	5′-TGGCAGTTATGGAAGGTAAA-3′	228–248	524	455	et al. 1995
	antisense:				
	5′-AGCAATGACTTTGTCTTCTG-3′	752–732			
	sense:				Ting et al. 1988
grp78	5′-GATAATCAACCAACTGTTAC-3′	1584–1603	577	523	
	antisense:				
	5′-GTATCCTCTTCACCAGTTGG-3′	2162–2142			
	sense:				Yamazaki et al.
hsp90α	5′-AAAAGTTGAAAAGGTGGTTG-3′	1803–1822	625	499	1989
	antisense:				
	5′-TATCACAGCATCACTTAGTA-3′	2426–2405			
	sense:				Rebbe et al. 1993
hsp90β	5′-AGAAGGTTGAGAAGGTGACAA-3′	1803–1822	641	531	
	antisense:				
	5′-AAGAGTAGAGAGGGAATGGG-3′	2444–2425			

2 h. After the synthesis, 20 units of RNase-free Dnase I were added and maintained at 37°C for one hour to digest the DNA templates. The transcripts were then extracted by phenol-chloroform and precipitated by addition of 50 µl of 7.5 M NH_4OAC and 200 µl of ethanol. To remove free nucleotides, the pellets were dissolved in 100 µl of 1 M NH_4OAC, and reprecipitated by adding 200 µl of ethanol. The pellets were then washed in 70% ethanol, dissolved in DEPC-treated H_2O, quantified by OD_{260}, checked on agarose gel and kept at –70°C. Direct PCR was performed with each RNA sample to confirm that the plasmid DNA templates had been completely removed.

RT-PCR Protocal

Specific oligonucleotide primers for each heat shock gene were selected based on the following considerations: (1) the primers correspond to regions which are uniquely

characteristic to a member of each heat shock gene family; (2) the availability of useful restriction endonuclease site(s) for construction of the reference plasmid templates harboring small internal deletions; and (3) to maximize differences in the size of amplicons to allow clear separation of reference and test nucleic acid species by gel electrophoresis. The oligonucleotide sequence and location of each set of primer pairs in the respective heat shock gene is indicated in Table 1.

The RT mixture in a 20 µl volume included 20 pmoles of antisense primers for each gene (group one, including *hsp27, hsp60, hsc70* and *mthsp75*; group two, including *hsp70, grp78, hsp90α* and *hsp90β*) 3.5 mM $MgCl_2$, 1 × PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3), 0.5 mM dNTP mixture (Pharmacia Piscataway, NJ, USA), 5 mM DTT, 3.5 units of RNase inhibitor (Pharmacia), 4 units of MMLV reverse transcriptase (Pharmacia), 500 ng of total RNA and defined quantity of control transcripts. The PCR mixture in 30 µl volume was composed of 20 pmoles of 5′ sense primer of each gene in group one or group two, 3.5 mM $MgCl_2$, 1 × PCR buffer, 0.5 mM dNTP mixture, 5 ng DNase-free RNase A, 2.5 units of Taq polymerase and 1 µCi of α^{32}P-dCTP (Amersham, Arlington Hights, IL, USA). Master mixtures were prepared for each experiment when possible. The RT reaction was carried out at 37°C for one hour and the PCR mixture was then added. The PCR reactions were performed in a thermal cycler (PIC-100, MJ Research, Inc., MA, USA). The conditions used for PCR were 94°C for 1 min followed by 30 cycles of 92°C for 1 min, 56°C for 1 min and 72°C for 1 min. The last extension was kept at 72°C for 10 min. Eight microliters of PCR products were dried, washed with 70% ethanol and fractionated on a 4% denaturing acrylamide gel. The gel was dried and exposed with a PhosphorImager plate (Molecular Dynamics, CA, USA).

Quantitative analysis

The signal intensity for each template was scanned and quantified with PhosphorImager system (Molecular Dynamics). The quantitations were calculated as follows.

Signal ratio (wild-type templates and control templates) = Signal value of wild-type amplicons/Signal value of control amplicons
Fold of induction = Signal ratio from heat-shocked cells/Signal ratio from control cells

Calculation of the amount of wild-type template in the testing RNA:

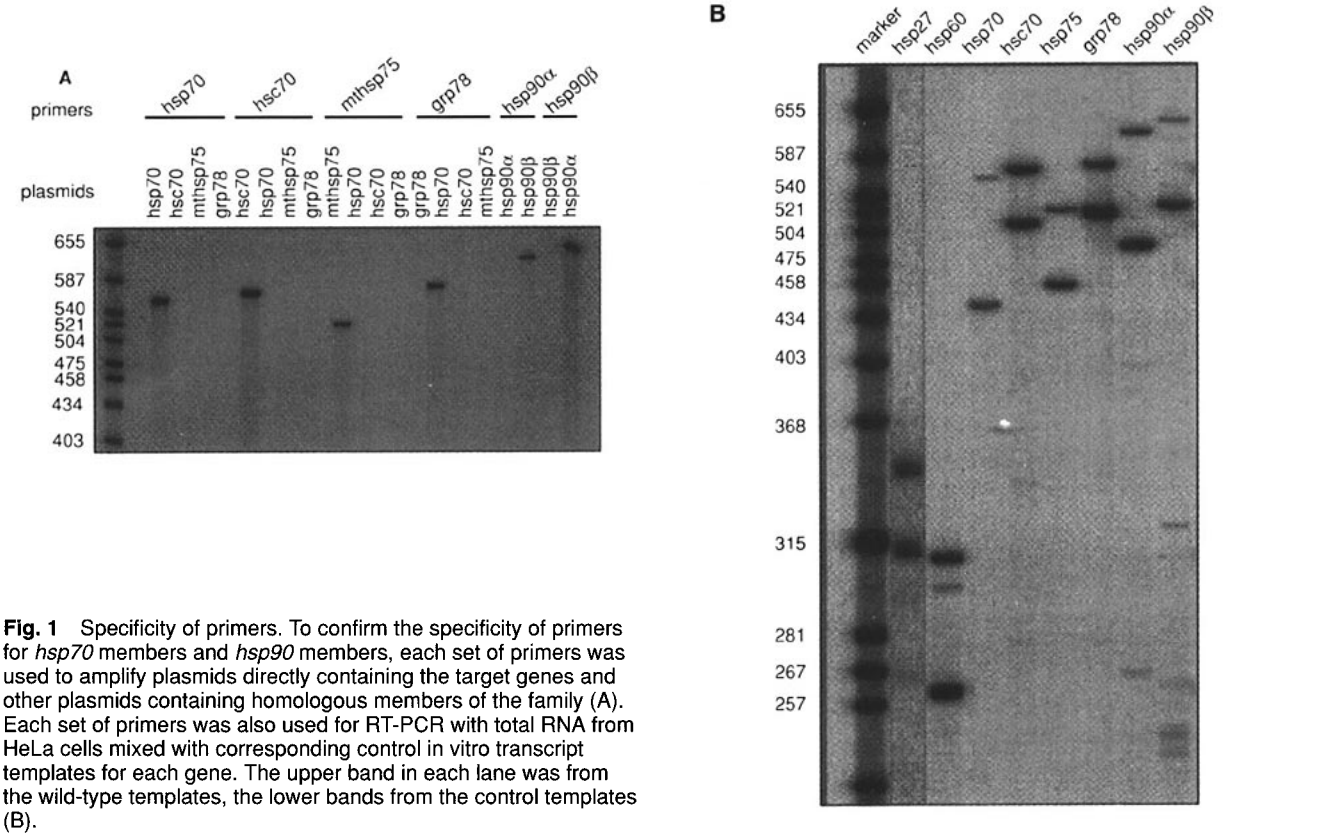

Fig. 1 Specificity of primers. To confirm the specificity of primers for *hsp70* members and *hsp90* members, each set of primers was used to amplify plasmids directly containing the target genes and other plasmids containing homologous members of the family (A). Each set of primers was also used for RT-PCR with total RNA from HeLa cells mixed with corresponding control in vitro transcript templates for each gene. The upper band in each lane was from the wild-type templates, the lower bands from the control templates (B).

1. Number of molecules of the input control templates
   Number of molecules of the input control templates = Weight of input amount of control RNA × 6.023 × 10^{23}/MW of control RNA
2. Number of molecules in the input wild-type mRNA
   Number of molecules in the input wild-type mRNA = Number of molecules of the input control templates × Signal ratio
3. Number of molecules of the wild-type mRNA per cell
   Typical eukaryotic cells contain 1 μg RNA/10^5 cells (Sambrook et al. 1989); therefore,
   Cell numbers corresponding to the input RNA = input amount of RNA (μg) × 10^5
   Number of molecules of the wild type mRNA per cell = number of molecules of the input wild-type mRNA/cell numbers
   All final numbers were rounded to the nearest 10.

RESULTS AND DISCUSSION

Rationale for a quantitative analysis of human heat shock gene expression

Our objective was to establish a method for the quantitative analysis of human heat shock gene expression. Rather than northern blot analysis, primer extension or S1 nuclease protection assays which provide the relative message levels of individual heat shock genes, we developed an RT-PCR assay using gene-specific oligonucleotide primers to provide information on both the absolute and relative levels of expression of a large number of heat shock genes. Inclusion of internal control reference RNAs corresponding to the same wild type genes to be assessed provides a direct quantitative measure of gene expression in cells exposed to different stresses.

Specificity of oligonucleotide primers and reference control templates

The specificity of each primer was demonstrated by direct PCR amplification of the target plasmid and other plasmids of related members of the *hsp70* and *hsp90* family (Fig. 1A) and by RT-PCR amplification of the target mRNAs and corresponding control transcripts for the eight heat shock genes (Fig. 1B). Each primer set amplified only the expected target DNA templates and did not cross-amplify other closely related gene sequences to generate spurious bands. For example, the *hsp70* primer set amplified a 558 nucleotide amplicon from the *hsp70* gene, as predicted from the nucleotide sequence of the *hsp70* gene, furthermore, no signal was detected using plasmids for the *hsc70*, *mthsp75*, or *grp78* genes (Fig. 1A). These results fulfill the requirement of gene specificity.

The quantitative aspect of this assay is based on the inclusion of internal reference control transcripts. For each of the heat shock genes, we constructed plasmids with internal deletions such that PCR co-amplification would generate a reference band of a smaller size. For example, relative to the wild type *hsp70* amplicon of 558 nucleotides, the control amplicon is 438 nucleotides in size (Fig. 1B). Direct comparison of the signal generated from the wild-type to the control amplicons by phosphorimager analysis thus provides a quantitative measure of the level of expression of each heat shock message in a population of total RNA.

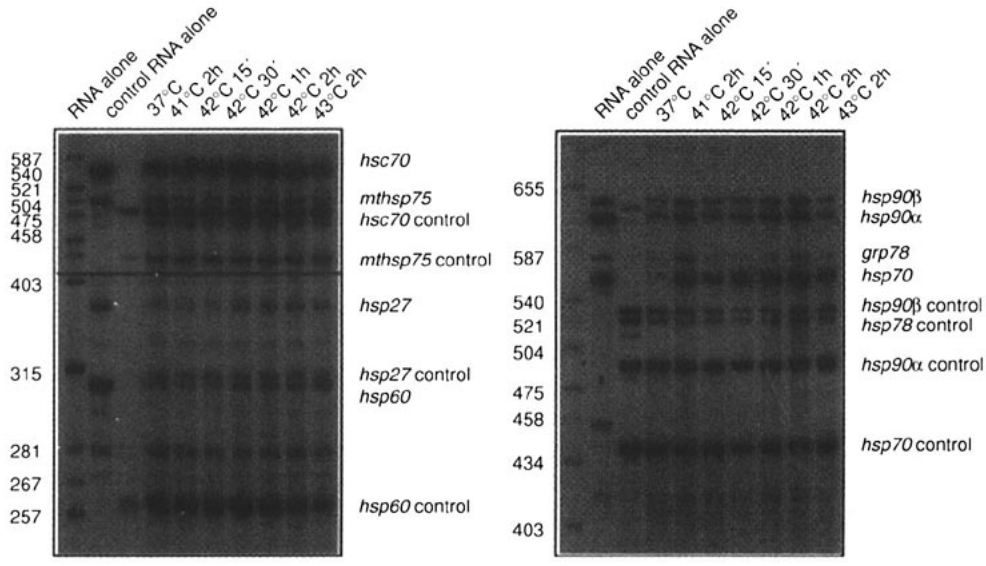

Fig. 2 Pattern of multiplex quantitative RT-PCR for heat shock genes. Total RNA from control and heat shocked K562 cells were used for the reaction. Eight genes were divided into two groups based on their sizes after amplification. The control in vitro transcripts and antisense primers were added to the total RNA for reverse transcription. PCR was performed after reverse transcription upon addition of the sense primers.

Table 2 Comparison of signal ratio of wild-type amplicons/control amplicons between single and multiplex RT-PCR*

Cycles	*hsp70*		*grp78*		*hsp90α*		*hsp90β*	
	Single	Multiple	Single	Multiple	Single	Multiple	Single	Multiple
25	0.2	0.2	0.3	0.3	0.2	0.3	0.3	0.3
30	0.2	0.1	0.3	0.4	0.2	0.3	0.4	0.3
35	0.1	0.1	0.3	0.3	0.3	0.2	0.4	0.3

*The number is the ratio between the signals of wild-type and control amplicons.

Table 3 Reproducibility of the multiplex RT-PCR method*

Experiment	*hsp70*	*grp78*	*hsp90α*	*hsp90β*
1	0.6	0.3	0.6	0.6
2	0.5	0.2	0.7	0.6
3	0.5	0.2	0.5	0.7

*The number is the ratio between the signals of wild-type and control amplicons.

The multiplex quantitative RT-PCR assay

Quantitative RT-PCR provides a valuable tool for the analysis of gene expression. However, it can become laborious to analyze the expression of multiple genes, each in separate reactions. Such repetition would require larger amounts of total RNA; additionally, comparison between experiments would introduce additional experimental variation. Therefore, to simplify the method and to minimize variation, we converted the conventional quantitative RT-PCR method into a multiplex quantitative RT-PCR method. In this process, all of the antisense primers, control transcripts and test RNAs were combined in a single reaction for reverse transcription. All of the sense primers were then combined for PCR co-amplification. Based on the length of the amplified sequences, the eight heat shock genes were separated into two sets of four genes. A typical gel pattern representative of the multiplex quantitative PCR reaction is shown in Figure 2. In each lane, eight bands corresponding to the wildtype and control templates at the expected electrophoretic positions are indicated as the PCR products of the respective test or control RNA species. Direct quantitation of these bands by phosphorimager analysis establishes a ratio for each wild-type and control signal which in turn provides a measure of the level of mRNA expression for each gene.

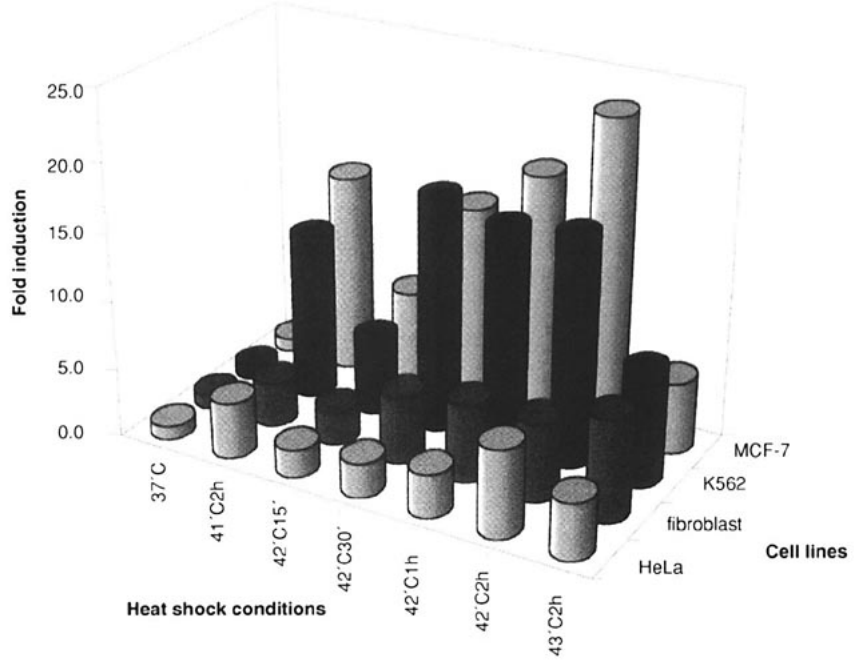

Fig. 3 Determination of the sensitivity of the method. Four different cell lines were heat-shocked at the indicated temperature and times. The expression of heat shock genes was quantified by the multiplex quantitative RT-PCR method. This figure shows the dynamic pattern of *hsp70* gene expression in these cells.

Table 4 Estimation of number of mRNA molecules per cell in MCF-7 cell line

Condition	*hsp70*	*grp78*	*hsp90α*	*hsp90β*
37°C	180	1060	880	2020
41°C 120 min	2700	720	1700	1530
42°C 15 min	1290	760	850	1360
42°C 30 min	2680	800	1690	1750
42°C 60 min	3310	750	2110	1820
42°C 120 min	4230	850	2820	2370
43°C 120 min	960	860	2770	2280

The presence of multiple primers and control templates in a complex reaction mixture raised potential concerns whether the amplification reactions for a particular set of gene templates are affected negatively by the presence of other oligonucleotides and RNAs. In principle, the interference caused by multiple components in the reaction should affect the wild-type and control templates equally, with the amplification ratio unchanged for each set of wild-type and control templates. To address this, we performed experiments in which the amplicon signal for a particular heat shock gene was compared in PCR reactions containing single or multiple genes. In these reactions, the same reagents were used with the exception of either a single set or multiple sets of primers and single control transcripts or a set of control transcripts. No significant difference in the ratio of each set of templates between single and multiplex reactions was observed (Table 2). The ratio between target templates and control templates remained linear and proportional to the number of cycles in the multiplex reaction as for reactions containing single templates, thus indicating that the ratio is cycle-independent (data not shown).

The reproducibility of the multiplex RT-PCR method was demonstrated using the same RNA sample in three separate reactions. The ratios between the target and control signals were compared and the variation observed was within 10% (Table 3). Several considerations need special attention to ensure consistency: (1) the RNA samples should be of high quality; preferably the RNA preparations should be fresh and not repeatedly frozen/thawed; (2) the control templates need to be pure and free of DNA and nucleotides for accurate quantitation for copy number, and (3) the reaction mixtures should be prepared as a master mix to minimize variation.

Quantitative analysis of human heat shock gene expression

Comparison of the expression of heat shock genes in different human tissue culture cells harvested at normal growth temperatures or following heat shock provides an assessment of the value of the multiplex RT-PCR assay.

Table 5 Heat shock gene expression in human cell lines

Gene	Cell line	mRNA molecules/cell		Induction
		Basal (37°C)	Heat shock (42°C 2 h)	
hsp27	MCF-7	4300	5760	1.3
	K562	4210	5800	1.4
	HeLa	6190	7320	1.2
	Fibroblast	2860	3240	1.1
	Jurkat	2090	2200	1.1
	Bjab	3520	4060	1.2
	U937	1840	2940	1.6
hsp60	MCF-7	5310	10290	1.9
	K562	950	1320	1.4
	HeLa	2960	4320	1.5
	Fibroblast	1830	3300	1.8
	Jurkat	1810	3410	1.9
	Bjab	2240	3200	1.4
	U937	880	2210	2.5
hsc70	MCF-7	860	1610	1.9
	K562	510	640	1.3
	HeLa	1540	2330	1.5
	Fibroblast	1190	1500	1.3
	Jurkat	570	1270	2.2
	Bjab	130	270	2.1
	U937	680	650	1.0
hsp70	MCF-7	180	4230	23.5
	K562	260	4340	16.7
	HeLa	1120	7110	6.3
	Fibroblast	390	2270	5.8
	Jurkat	330	1940	5.9
	Bjab	90	1380	15.3
	U937	10	170	17.0
mthsp75	MCF-7	1080	1250	1.2
	K562	380	320	0.8
	HeLa	840	610	0.7
	Fibroblast	1110	780	0.7
	Jurkat	920	520	0.6
	Bjab	1190	670	0.6
	U937	1220	1450	1.2
grp78	MCF-7	1060	850	0.8
	K562	410	1480	3.6
	HeLa	1130	1920	1.7
	Fibroblast	920	1330	1.4
	Jurkat	4140	1740	0.4
	Bjab	5320	4260	0.8
	U937	2020	2090	1.0
hsp90α	MCF-7	880	2820	3.2
	K562	350	1130	3.2
	HeLa	2520	5150	2.0
	Fibroblast	440	940	2.1
	Jurkat	2940	5540	1.9
	Bjab	2460	3870	1.6
	U937	1530	2920	1.9
hsp90β	MCF-7	2020	2370	1.2
	K562	820	2330	2.8
	HeLa	2860	3820	1.3
	Fibroblast	760	910	1.2
	Jurkat	440	570	1.3
	Bjab	780	570	0.7
	U937	170	380	2.2

hsp70 message levels in HeLa cells, primary fibroblasts, K562 cells and MCF-7 cells were quantified and the data presented in a histogram (Fig. 3) or tabulated as number of RNA molecules/cell (Tables 4 and 5). Among these human cell lines, the fold-induction of heat shock mRNAs following exposure to either 42°C or 43°C varies. The heat shock response of K562 cells and MCF-7 cells were the most robust relative to primary fibroblasts or HeLa cells. Whereas *hsp70* message levels increase over 23-fold in MCF-7 cells (Table 4), a 6-fold induction is measured in HeLa cells. By comparison, *hsp70* levels change only 1.9 and 1.5-fold and *hsp27* levels changed 1.3 and 1.2-fold in MCF-7 and HeLa cells (Table 5). The levels of *hsp70*, *grp78*, *hsp90α*, and *hsp90β* mRNAs in MCF-7 cells at 37°C or following heat shock at different temperatures and durations of incubation is presented in Table 4. Whereas the levels of *grp78* decrease by 15–25% upon heat shock, *hsp90β* increases by 10–15% only upon prolonged heat shock, and only *hsp70* and *hsp90α* are induced significantly upon heat shock (Table 4).

In addition to providing a direct comparison of the relative levels (fold-induction) of heat shock gene transcripts in control and stress cells, the multiplex RT-PCR method provides an accurate measure of basal expression. For tissue culture cells, this refers to the level of expression in cells grown at normal physiologic conditions (Table 5). The levels of *hsp70* mRNA varies among tissue culture cell lines from 10 to 1120 copies per cell and the levels of *hsp90α* varies from 350 to 2940 copies per cell. It is worth noting that there is a nearly 110-fold range in basal *hsp70* expression among these tissue culture cells maintained under the same growth conditions.

Although this method was developed and optimized using RNA samples from human tissue culture cell lines, application of this assay to the expression of heat shock genes in humans may reveal differences among individuals, between tissues, during development with an emphasis on aging, and with reference to pathophysiology. Our initial data from the analysis of human tissues reveals that the basal expression of the classical inducible heat shock genes, *hsp70* and *hsp90α*, varies dramatically (over 500-fold) in mononuclear cells obtained from different age-matched individuals whereas the expression of the *grp78*, *hsc70*, *mthsp70*, and *hsp60* genes are nearly identical in these human isolates (Wang et al., in preparation). Establishing the populational polymorphisms for heat shock gene expression is essential to an understanding of the role of heat shock genes and the heat shock response in human disease.

ACKNOWLEDGMENTS

The authors would like to thank members of the Morimoto laboratory for their assistance and encouragement and Anu Mathew for critical review of the manuscript. This work was supported by grants to R.I.M. from the NIH and the Carol Gollob Foundation for Breast Cancer Research and generous support from Mr Marrin Gollob. For a more detailed protocol, readers are referred to: http://x.biochem.nwu.edu/ibis/morimoto/protocols/rmprotocols.html

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