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Regulation of Heat Shock Protein Message in Jurkat Cells Cultured Under Serum-Starved and Gravity-Altered Conditions

Marian L. Lewis[1]* **and Millie Hughes-Fulford**[2]

[1]Department of Biological Sciences, University of Alabama, Huntsville, Alabama 35899
[2]Laboratory for Cell Growth and Differentiation, University of California and Veterans Affairs Medical Center, San Francisco, California 94121

Abstract Although our understanding of effects of space flight on human physiology has advanced significantly over the past four decades, the potential contribution of stress at the cellular and gene regulation level is not characterized. The objective of this ground-based study was to evaluate stress gene regulation in cells exposed to altered gravity and environmentally suboptimal conditions. We designed primers to detect message for both the constitutive and inducible forms of the heat shock protein, HSP-70. Applying the reverse transcriptase-polymerase chain reaction (RT-PCR), we probed for HSP-70 message in human acute T-cell leukemia cells, Jurkat, subjected to three types of environmental stressors: (1) altered gravity achieved by centrifugation (hypergravity) and randomization of the gravity vector in rotating bioreactors, (2) serum starvation by culture in medium containing 0.05% serum, and (3) temperature elevation (42°C). Temperature elevation, as the positive control, significantly increased HSP-70 message, while centrifugation and culture in rotating bioreactors did not upregulate heat shock gene expression. We found a fourfold increase in heat shock message in serum-starved cells. Message for the housekeeping genes, actin and cyclophilin, were constant and comparable to unstressed controls for all treatments. We conclude that gravitational perturbations incurred by centrifugal forces, exceeding those characteristic of a Space Shuttle launch (3*g*), and culture in rotating bioreactors do not upregulate HSP-70 gene expression. In addition, we found RT-PCR useful for evaluating stress in cultured cells. J. Cell. Biochem. 77:127–134, 2000.

Key words: stress response; HSP-70; RT-PCR; rotating bioreactors; space flight

Mammalian cells flown on the Space Shuttle exhibit changes in growth response, metabolism, structure, and gene expression compared with ground controls maintained in the same hardware and subjected to the same experimental design. Determining whether these cellular changes are caused by microgravity per se or by other conditions of space flight is critical to accurate interpretation of results from microgravity research. Among the responses reported for cells flown on the Space Shuttle are altered cytoskeletal morphology and reduced cellular growth in osteoblasts [Hughes-Fulford et al., 1993; Hughes-Fulford and Lewis, 1996], and growth failure, microtubule anomalies, disorganization of microtubule organizing centers, increased glucose metabolism, and a time-dependent and microgravity-related increase in the apoptosis factor, Fas/APO-1 in Jurkat cells [Lewis et al., 1998]. The blunted response of T lymphocytes to mitogen stimulation is well documented [Cogoli, 1987; Cogoli et al., 1996], yet the reason for reduced growth activation in microgravity is unknown. The present study presents significant evidence to show that stress factors must be considered in determining whether the effects observed in space-flown cells are due to stress or to altered gravity.

Because of these varied gravity-affected changes in cell function, organelle structure, and gene expression, we undertook this ground-based study, using heat shock protein regulation to investigate the possibility that stress may be a factor in the observed responses of cells to space flight. Heat shock proteins can be

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*Correspondence to: Marian L. Lewis, Department of Biological Sciences, Wilson Hall 360, University of Alabama, Huntsville, AL 35899. E-mail: lewisml@email.uah.edu

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induced by elevated temperature, chemicals, and other environmental stresses [Schlesinger et al., 1982]. First described for *Drosophila* salivary gland cells by Ritossa [1962] and subsequently discovered in bacteria [Lemeaux et al., 1978], heat shock proteins are also found in the cytosol and nucleus of mammalian cells. In mammalian cells, they function as molecular chaperones for nascent polypeptide chains and in protecting cells against accumulation of improperly folded proteins [Bhattacharyya et al., 1995]. In this study, we chose to evaluate HSP 70, as its expression is strongly induced in response to mild mechanical stress [Knowlton et al., 1991] or by heat stress [Hatayama et al., 1992; Haas, 1995].

A number of early reports [Lewis, 1986; Lewis et al., 1987, 1988; Lawless et al., 1989] describe the effects on growth and differentiation in cells cultured in the rotating wall bioreactor vessels (RWV) originally developed by NASA to mimic some aspects of the microgravity environment of space. These fluid-filled, rotating bioreactors provide very low shear culture and perturb normal unidirectional gravity by randomizing the gravity vector over 360 degrees. In rat adrenal medullary PC12 cells, culture in the RWV appears to selectively activate signal transduction pathways to enhance neuroendocrine differentiation [Lelkes et al., 1998]. The locomotion of human peripheral blood lymphocytes through type I collagen in rotating-wall bioreactors was significantly reduced and phytohemagglutinin (PHA) responsiveness was almost completely negated in T lymphocytes [Pellis et al., 1997]. Activation of the cells was restored by activation of protein kinase C (PKC) [Cooper and Pellis, 1998]. Myocardial cells can be stimulated to express HSP 70 with as little as one single stretch [Knowlton et al., 1991]. Shear stress has been implicated as a possible cause of change in gene expression in cells placed under mechanical stress [Resnick et al., 1993]. These investigators identified a shear stress response element (SSRE) that is responsible for changes in cells exposed to mechanical stress. However, Kaysen et al. [1999] recently reported that the RWV causes changes in gene expression that are independent of the SSRE.

Gravity-altered environments have been shown to up- or downregulate message for a number of genes. For instance, centrifugation at a maximum of 3*g*, the gravitational force experienced during a typical Space Shuttle launch, transiently upregulated gene expression for c-*fos* and significantly decreased the mRNA level for the mineralization marker gene, osteocalcin, in mouse osteoblast cells [Fitzgerald et al., 1996]. Centrifugal elutriation, a technique often used to synchronize cells, resulted in mitotic cyclin mRNA expression and p70 S6 kinase activity in primary chick erythroblasts attributable to the presumed combination of centrifugation and lack of growth factor during the elutriation process [Mikulitis et al., 1997]. In microgravity characteristic of the Space Shuttle, gene expression in space-flown osteosarcoma cells of the line MG-63 was reduced compared with ground controls for collagen I alpha 1, alkaline phosphatase, and osteocalcin [Carmeliet et al., 1997]. In studies using reverse transcription-polymerase chain reaction (RT-PCR), Western blots, and immunofluorescence, Hughes-Fulford et al. [1999] showed that the level of fibronectin mRNA expression and protein synthesis and matrix formation was not altered by microgravity.

The present ground-based study was designed to answer the critical questions of whether space flight-related conditions, such as maintaining cells in low-serum and launch acceleration (centrifugation), as well as gravity vector-randomized culture in the RWV can induce stress response manifested as upregulation of heat shock protein 70 (HSP-70) in Jurkat cells, a human T-cell lymphoblastoid cell line. We found RT-PCR to be a useful technique for evaluating stress gene expression. We included heat treatment as a positive stressor for comparing regulation of the HSP-70 message in cells cultured under test conditions. This study provides very useful information for discriminating between stress-related factors and microgravity-induced cellular response in space-flown mammalian cells.

METHODS

Cells

The E6–1 clone of Jurkat cells was obtained from the American Type Culture Collection (ATCC) and subcultured by splitting 1:20 two to three times per week. The culture medium consisted of RPMI 1640 (Irvine Scientific, Santa Ana, CA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Summit Biotechnology, Fort Collins, CO), 2 mM glutamine, l mM sodium pyruvate, 0.1 mM nonessential amino acids, penicillin and streptomycin, 100 U

and 100 µg/ml, respectively (Life Technologies/Gibco-BRL, Grand Island, NY), and 12.5 mM Hepes buffer (Sigma Chemical Co., St. Louis, MO). Cells were tested by the ATCC and certified free of *Mycoplasma*.

Stressors

Temperature stress. A volume of cell suspension containing one million cells per ml was gently distributed into each of two sets of T-25 flasks. One set was placed at 37°C as a control. The other was placed in a 42°C water bath. Both sets were incubated for 30 min with caps tightened, followed by collection of cells by centrifugation at 800 rpm for 5 min. The supernatant was decanted, cells were resuspended in 1 ml of cold Dulbecco's phosphate-buffered saline (PBS), transferred to a siliconized, autoclaved microfuge tube and spun for 30 s at 12,000 rpm. The supernatant was decanted and cell pellets were immediately frozen at −80°C pending RNA extraction.

Serum starvation. Cells were serum starved by culture in medium containing 0.05% FBS. After 43 h, the cells were divided into two sets and spun at 800 rpm for 5 min. The medium was discarded and cells in one set received fresh culture medium containing 10% FBS, and the other set received fresh medium with 0.05% FBS. After 30 min, the cells in both sets were harvested by centrifugation at 800 rpm. The supernatant was discarded, and the cells were resuspended in 1 ml of cold Dulbecco's PBS, transferred to a siliconized, autoclaved microfuge tube, and spun for 15 s at 12,000 rpm. The supernatant was decanted, and cell pellets were immediately frozen at −80°C pending RNA extraction. Nonstarved controls were handled in the same manner except that they were cultured in medium containing 10% serum throughout the test.

Hypergravity. To evaluate the effect on gene expression of *g* forces in a range common to routine cell handling in the laboratory and in excess of the 3*g* characteristic of Shuttle launch acceleration, Jurkat cells were centrifuged at room temperature for 20 min at either 400 rpm or 2,000 rpm in a clinical tabletop centrifuge. The supernatant was discarded, and cells were resuspended in 1 ml of cold Dulbecco's PBS, followed by transfer to a siliconized, autoclaved microfuge tube. Cells were harvested by spinning for 15 s at 12,000 rpm in a microfuge. The supernatant was decanted and cell pellets were immediately frozen at −80°C pending RNA extraction. Controls were held in static suspension at room temperature throughout the test and otherwise processed in the same manner as test cells.

Randomized gravity vector. RWVs were rotated at either eight rpm optimized to maintain the cells in suspension or at 53 rpm, a nonoptimal rotation rate that causes cells to move toward the outer wall of the vessels. For each of the rotation speeds, cells were cultured for a total of 4 h in an RWV and then collected by centrifugation at 800 rpm for 5 min. The supernatant was discarded, and the cells were resuspended in 1 ml of cold Dulbecco's PBS, transferred to a siliconized autoclaved microfuge tube, and spun for 15 s at 12,000 rpm. The supernatant was decanted, and cell pellets were immediately frozen at −80°C pending RNA extraction as described for the other treatments. Controls consisted of cells cultured in static T-75 flasks harvested in the same manner as the test cells.

RT-PCR Methodology

Moloney murine leukemia virus reverse transcriptase, buffer, and the 100-base pair (bp) DNA molecular-weight markers were purchased from Gibco-BRL. *Thermus aquaticus* DNA polymerase (Amplitaq), RNase Inhibitor, primers, and dNTPs were purchased from Perkin-Elmer-Applied Biosystems (Norwalk, CT). Agarose and low-melting agarose were purchased from Fisher Scientific (San Francisco, CA). The human *cox-2* cDNA probe was purchased from Oxford Chemicals (Oxford, MI). Heat shock protein, HSP-70 ci (constitutive) and HSP-70i (inducible) primers were designed and tested for specificity by one of us (M.H.F.) based on published Genebank™ human sequences. The forward primer is shared by both genes. The primer sequences are as follows: HSP-70ci **F**-CCATGGTGCTGACCAAGATGAAG: -**R**-TCGTCGATCGTCAGGATGGACAC HSP-70i: -**R**-CACCAGCGTCAATGGAGAGAACC-The *HSP* primers produced the predicted 283- and 284-bp product relating to bases 850–1129 cDNA for HSPci and 620–902 for HSPi. Identity was confirmed by restriction enzyme digestion. The oligonucleotides were synthesized at the University of California Biomolecular Resource Center (San Francisco, CA).

RNA Isolation and cDNA Synthesis

Total RNA was isolated using *STAT-60* from Tel-Test (Friendswood, TX). The OD 260/280 ratio was determined with a GeneQuant spectrophotometer (Pharmacia Biotech) to obtain the RNA concentration. To ensure mRNA purity and to confirm RNA concentration calculations, RNA formaldehyde gels (1% agarose) containing ethidium bromide, were run at 120 V for about 25 min (Hoeffer mini-gel) with 1.5 mg total RNA for each sample.

Reverse Transcription Reactions

RTs were run with 1.5 mg total RNA, with 2.5×10^4 copies of pAW109 control RNA template and reverse transcriptase buffer according to the manufacturer's protocol (GeneAMP RNA PCR kit, Perkin-Elmer-Cetus). pAW109 RNA containing interleukin-1α (IL-1α) template, was added as a control for the RT and PCR reactions. All PCR reactions were run with the RT cDNA, AmpliTaq, PCR buffer, primers, $MgCl_2$, and dNTPs according to the manufacturer's protocol (GeneAMP RNA PCR kit, Perkin-Elmer-Cetus). HSP reactions were run for 28–32 cycles. PCR products were separated on a 2% agarose gel containing ethidium bromide, in order to visualize the product bands. Photograph were taken using a Polaroid DS-34 camera and 667 film. Photographs were scanned into a digitized image, using the Hewlett Packard Scanjet Iic at 600 dpi. Gene products were evaluated against actin and cyclophillin and all were from the same RT reaction. Heat shock bands were normalized to cyclophillin. Density analysis was performed on an accelerated Macintosh SE/30 computer, using the public domain NIH Image 1.55 program written by Wayne Rasband at the U.S. National Institutes of Health. Cycle curves for each of the products were run to verify that the particular cycles chosen were within the exponential phase of amplification. PCR product verification included matching appropriate base pair size and Southern analysis with appropriate DNA.

RESULTS

Effect of Temperature Elevation

The cDNA product made by reverse transcriptase using total RNA extracted from 6×10^6 Jurkat cells incubated either at 37°C or 42°C for 30 min was reacted with primer pairs for β-actin, inducible heat shock protein (HSP-70i), and inducible heat shock protein primers with low-affinity reacting constitutive HSP-70 (HSP-70ci). Resulting cDNA for actin evaluated on DNA gels showed comparable bands for heat-treated and nontreated controls, indicating that RNA message for actin was not altered by temperature elevation (Fig. 1). With both the HSP 70i and HSP-70ci primers, the cells incubated at 37°C had a single band corresponding to a 238-bp HSP-70 RT-PCR product. For RNA isolated from cells heated at 42°C, and reacted with HSP-70i primers, there were two bands corresponding to the inducible form. These primers were developed with an intron spacer; the presence of two weak bands in lanes reacted with HSP-70ci are indicative of the inducible HSP as a contaminant.

Serum Starvation

The resulting cDNA products from cells cultured in medium containing 0.05% FBS were reacted with primers for β-actin and HSP 70ci. The DNA gels are shown in Figure 2. Bands showed that message for the housekeeping gene, β-actin, was constitutively expressed in all samples, regardless of serum concentration (Fig. 2, lanes 1–3). The PCR product for HSP 70ci was evident in extracts of cells grown in low FBS-containing medium whether or not cells were incubated with 10% serum for 30 min (Fig. 2, lanes 4–6). Densitometry scans of the

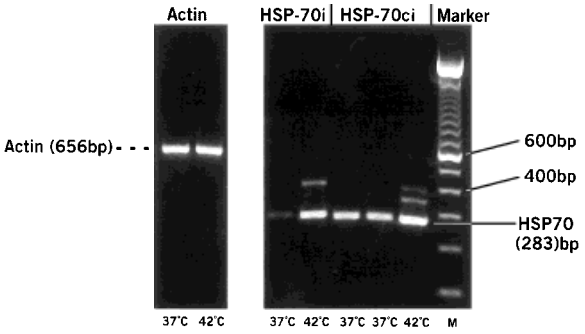

Fig. 1. Heat shock protein regulation in temperature stressed Jurkat cells. Jurkat cells (1×10^6 cells/ml) were placed either in a 37°C incubator or a 42°C water bath for 30 min. RNA was extracted; reverse transcription-polymerase chain reaction (RT-PCR) was performed with primers as described under Materials and Methods. Bands represent cell extracts reacted with primers for: β-actin (**lane 1**) at 37°C and (**lane 2**) at 42°C; inducible form of HSP-70i (**lane 3**) at 37°C, and (**lane 4**) at 42°C; inducible heat shock protein primers with low-affinity reacting constitutive HSP-70 (HSP-70ci) at 37°C (**lanes 5,6**) and 42°C (**lane 7**). Bands at 42°C (**lanes 4,7**) show that messages for both the constitutive and inducible forms of heat shock protein were upregulated.

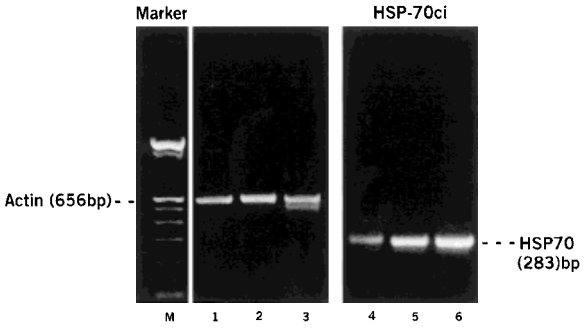

Fig. 2. Heat shock protein regulation in serum-starved Jurkat cells. Cells were cultured in RPMI-1640 medium containing 0.05% fetal calf serum (FCS). Control cells were maintained in medium containing 10% FCS. After 43 h, the serum-starved cells were centrifuged from medium; one set was resuspended in medium containing 10% FCS to facilitate production of heat shock protein message (**lane 5**). A second set of serum-starved cells was resuspended in 0.05% FCS (**lane 6**). RNA in these cells and the control (**lane 4**), maintained on 10% FCS throughout the test, was extracted and subjected to reverse transcription-polymerase chain reaction (RT-PCR), using the HSP-70ci primer set as described under Materials and Methods. Bands for the actin control primer set (**lanes 1–3**) confirmed reaction parameters and showed no significant variation under the three conditions tested. The PCR products of the HSP-70ci primer set showed an approximately fourfold elevation in serum-starved cells (cf. **lanes 5,6** with **lane 4**), regardless of serum concentration during the additional 30-min incubation.

gels showed that HSP 70ci message was elevated approximately fourfold in serum-starved cells compared with the controls.

Centrifugation and Gravity Vector Randomization

The PCR products of Jurkat cells grown in the RWV Bioreactor for 4 h at 8 or 53 rpm, or centrifuged for 20 min at either 400 or 2,000 rpm, are shown in Figure 3. Cyclophilin primers, as a control for the RT-PCR reaction, produced a single band corresponding to 628 bp characteristic of cyclophilin under all gravity conditions (Fig. 3, lanes 1–5). Bands from extracts primed with HSPci were faintly visible after 32 PCR cycles. To make certain that we would detect the possibility of HSP-70 upregulation, we subjected the reaction to an additional six cycles. As shown in Figure 3, lanes 7–10 for the 38-cycle PCR reaction, a single band representing HSP-70 ci was common to all treatments and comparable to the static culture control (lane 6), indicating that HSP-70 was not upregulated by culture in the RWV Bioreactor or by centrifugation.

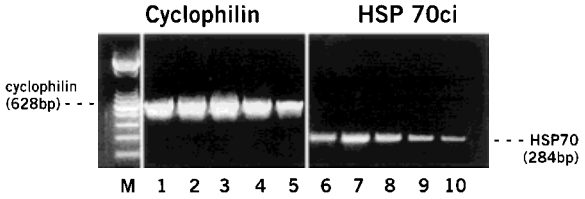

Fig. 3. Effect of altered gravity on gene expression of heat shock protein. Jurkat cells were grown in an RWV Bioreactor for 4 h or centrifuged for 20 min at 400 or 2,000 rpm. **Lanes 1–5**, bands of polymerase chain reaction (PCR), using cyclophilin primer sequences; **lanes 6–10**, results using the HSP-70ci primer sequences. Gravity treatment of the cells was as follows: **lanes 1,6**, static cultured control cells; **lanes 2,7**, RWV Bioreactor rotated at 8 rpm for 4 h; **lanes 3,8**, RWV Bioreactor rotated at 53 rpm for 4 h; **lanes 4,9**, cells centrifuged at 400 rpm for 20 min; **lanes 5,10**, cells centrifuged at 2,000 rpm for 20 min. There was no evidence of upregulation of the HSP-70 message in Jurkat cells subjected to gravity-altered conditions. Single bands for cyclophilin, as the PCR control, were present in all samples.

DISCUSSION

To determine whether the changes observed in space-flown cells are gravity (microgravity) induced, the effects of potential stresses characteristic of space flight must be considered. For instance, installation of experiments in the Shuttle Middeck at launch minus 24 h requires loading cells into flight hardware long before initiation of the experiment. In addition, crew time limitations may prevent initiation of experiments on orbit for several h or days. To accommodate these time constraints, it may be necessary to retard cell growth to optimize cell density for the experiment in microgravity. Cells can be growth retarded by reducing the serum content in the medium and lowering culture temperature. Space-flown cells may respond to this stress, in addition to the mechanical stresses of launch acceleration and vibration, making it difficult to separate the real effects due to altered gravity from effects of stresses due to space flight. This study describing the effect of low-serum, centrifugal force, and altered gravity culture in the RWV on the expression of HSP-70 in Jurkat cells adds significantly to our understanding of stress response and can facilitate interpretation of effects observed in cells during space flight.

This ground-based investigation was undertaken to determine whether altered gravity environment (culture of cells in the RWV) and potential stressors characteristic of Shuttle flight can upregulate message for heat shock proteins. Very low stress has been demon-

strated to upregulate the HSP gene [Knowlton et al., 1991]; therefore, evaluation of this gene provided a sensitive means to test stress response in cells subjected to hypergravity (centrifuge) and cultured under low-stress conditions provided in the RWV. We chose the human lymphoblastoid suspension culture cell line, Jurkat, because our previous space flight results showed that cell growth is decreased, the microtubule cytoskeleton is disrupted, and apoptosis and the secretion of the apoptosis-related factor, Fas/APO-1, are increased in Jurkat cells as a result of flight on the Space Shuttle [Lewis et al., 1998]. The information from this ground-based study provides a basis for evaluating the role of stress in assessing effect of space flight on cells. Presence of stress gene message can alert the investigator that the responses observed in space-flown cells may not be due to microgravity alone.

Routine assay for stress gene message can indicate whether cells may be regulating genes in response to stress as well as in response to microgravity. A relationship between gene expression and microgravity was partially described by de Groot et al. [1990] by demonstrating that a number of responses of A431 cells to epidermal growth factor (EGF) are affected by microgravity. The responses include a decreased c-*fos* and c-*jun* induction and serum element activity. In these sounding rocket experiments in which cells experienced microgravity for 6 min, serum and/or EGF-induced expression of the proto-oncogenes c-*fos* and c-*jun* were reduced almost 4 fold in microgravity. The decreased gene inductions were attributed to possible alterations in the response of the c-*fos* promoter-enhancer regions. The nuclear proto-oncogenes c-*jun* and c-*fos* are known regulators of DNA synthesis [Murakami et al., 1991] and are required for entry of cells into S-phase DNA synthesis. In later studies, de Groot et al. [1991] demonstrated that the nuclear responses to PKC signal transduction were sensitive to gravity changes. Phorbol ester (TPA)-induced c-*fos* and c-*jun* gene expressions were affected by microgravity, while there were no changes in the calcium response, implicating the diacylglyceride (DAG) portion of the PKC signal transduction. Rijken et al. [1993] reported that EGF-induced c-*fos* and c-*jun* expression was not due to an effect on the EGF-receptor interaction, since normal EGF receptor redistribution occurred in microgravity. This lends credibility to the idea that microgravity influences EGF-induced signal transduction, not at the EGF binding and receptor redistribution level, but upstream of early-immediate gene [de Groot et al., 1991, de Laat et al., 1993]. Finally, Peppelenbosch et al. [1993] demonstrated that EGF-induced actin cytoskeleton changes are affected by the arachidonic acid pathways through cyclooxygenase (prostaglandin) and 5-lipoxogenase (leukotriene).

Based on the results of the present study, it is unlikely that microgravity would upregulate heat shock stress response. After 32 PCR cycles, the band corresponding to the HSP 70 message was barely detectable for cells cultured in the RWV. We attributed this to the fact that the primers we designed had a low affinity for the constitutive form of HSP-70, however; to rule out the possibility of upregulation of HSP-70 in the RWV cultures, we continued the PCR cycles for an additional six reaction sequences. The PCR gel shown in Figure 3 is the result of 38 PCR cycles. Only a single band, corresponding to the constitutive form, is visible. Compared with the control, message for HSP-70 was not upregulated in cells subjected to the randomized gravity environment of the RWV Bioreactor or the centrifugal speeds that we tested.

The lack of induction of stress message in rotating bioreactors confirms one of the primary tenants in operation of these devices. The rotating wall bioreactors were developed by NASA for the purpose of providing a low-shear culture environment and to mimic some of the aspects of microgravity. The preliminary work of Lewis and colleagues with several different cell types indicates that cell growth is enhanced [Lewis, 1986; Lewis et al., 1987, 1988; Lawless et al., 1989] and the fluid mechanical characteristics of these bioreactors predict that the culture conditions in the RWVs would be nonstressful [Tsao et al., 1991]. However; whether randomization of the gravity vector would, in and of itself, cause shear stress and upregulation of the heat shock proteins has been undetermined. The use of horizontally rotating culture systems, clinostats, for low-gravity simulation is well documented [Deldoph and Dipert, 1971; Briegleb, 1983]. Studies by Kaysen et al. [1999], demonstrated that the RWV can induce gene expression in the absence of the shear stress response element (SSRE) (gagacc consensus sequence), which is not contained in the HSP-70 promoter region. Whether, and at what rotation

rate, the fast-rotating clinostats and other bioreactor systems may upregulate stress protein gene expression has not been determined.

Reduction of serum in the culture medium to 0.05% for 43 h, caused an approximate fourfold elevation in message for HSP-70 compared with the control as shown in Figure 2. Placing the serum-starved cells in medium containing 10% serum for 30 min to enhance recovery after culture in low-serum medium did not reduce expressed HSP-70 message and indicated that recovery from serum starvation is not immediate. These results appear to agree with those of Iyer et al. [1999] in that gene expression in human fibroblasts deprived of serum for 48 h continued to express message for a heat shock cognate 71kD protein 24 h after increasing serum concentration to 10%.

Even routine culture of cells in the laboratory can subject them to significant stress from nutrient depletion, temperature excursions, and mechanical insults such as centrifugation, shaking, and pipetting. These perturbations could confound the interpretation of test results. Our results showed that centrifugation at the speeds tested (400 and 2,000 rpm) and routine handling and pipetting do not upregulate HSP-70 message and thus would not be expected to induce artifactual cellular responses. On the basis of our investigations, Shuttle launch acceleration at a maximum of 3*g* would not be sufficient to upregulate HSP-70 stress genes. As expected, heating the cells to 42°C for 30 min significantly increased message for both constitutive and inducible heat shock protein (Fig. 1) and temperature stress provided a positive control for induction of the HSP-70i-inducible form. Temperature elevation had no effect on message for β-actin included as a control for the PCR reaction.

We have demonstrated the value of RT-PCR in assessing changes in heat shock protein gene expression in cells subjected to environmental perturbations including centrifugation, temperature excursions, and serum starvation. This is the first study of which we are aware to use primers for HSP 70 to assess the randomized gravity environment of the NASA rotating wall bioreactors for potential as a cellular stressor. Information gained from this study adds significantly to our understanding of environmentally induced cell-level stress response and has relevance to interpretation of the impact of launch acceleration and Shuttle flight-related stresses, versus effects of microgravity per se, on growth, gene expression, and aging responses of human cells during space flight.

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