Heat shock protein 70 binds its own messenger ribonucleic acid as part of a gene expression self-limiting mechanism

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Abstract Expression of heat shock proteins is a cellular response to a variety of stressors. HSP70, the major stress-induced heat shock protein, is involved in repair and protection after the insult. However, the prolonged presence of this protein is detrimental. Consequently, *Hsp70* expression must be tightly regulated. We have previously shown an increase in the degradation of Hsp70 messenger ribonucleic acid (mRNA) paralleling the accumulation of HSP70. Incubation of cells with transcriptional and translational inhibitors after heat shock resulted in a significant reduction in Hsp70 mRNA degradation. These observations suggest that newly synthesized, stress-induced factors might be involved in the decay of Hsp70 mRNA. We found that HSP70 binds directly to Hsp70 mRNA, as demonstrated by immunoprecipitation. This observation was confirmed by RNA gel-shift assays. These results are evidence for a novel and likely direct interaction between HSP70 and Hsp70 mRNA in cells after stress. This interaction may be part of a self-limiting mechanism to reduce HSP70 production, thus avoiding potential toxic effects of this protein in the absence of stress.

INTRODUCTION

Cells require effective systems to promote survival after severe stress conditions, such as extreme temperature, toxins, ischemia-reperfusion, and pathogens. The heat shock response is among the most primitive and highly conserved mechanisms for cell survival after a variety of insults (Morimoto 1991; De Maio 1999). The heat shock response involves expression of heat shock proteins (Hsps), which are best known as molecular chaperones. The chaperone function of Hsps is particularly important for coping with the consequences of stress, such as the appearance of denatured polypeptides, protein aggregates, and derangement of structural and functional elements (Morimoto 1991; De Maio 1999). Hsps remain in cells for several hours after stress and protect them from subsequent insults, a phenomenon called stress tolerance (Mizzen and Welch 1988; De Maio 1999). The major in-

ducible Hsp is HSP70, also known as HSP72. HSP70 is a cytosolic/nuclear member of the 70-kDa Hsp family. HSP70, which is the most abundant protein in stressed cells, plays a key role in interactions with denatured substrates and stabilization of essential metabolic pathways, such as translation (De Maio 1999). However, the presence of HSP70 is not purely beneficial and has been found to be cytotoxic in the absence of stress in *Drosophila* (Feder et al 1992) and mammalian cells (Li et al 1991; Arispe et al 2004). Thus, a mechanism to limit the expression of *Hsp70* follows the rapid and robust expression of *Hsp70* immediately after the stress. Indeed, HSP70 levels in stressed cells increase after heat shock. However, subsequent thermal insults lead to little further accumulation of HSP70 (Theodorakis et al 1999). The regulation of HSP70 levels after the stress has been postulated to be part of a self-limiting expression mechanism (Di-Domenico et al 1982; De Maio 1999). Prior studies have shown that HSP70 is likely a negative regulator of the activity of heat shock factor 1 (HSF-1), the major transactivator of the heat shock response (Shi et al 1998). Hsp70

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messenger ribonucleic acid (mRNA) half-life, which is short (1 hour) in cells after stress (Theodorakis and Morimoto 1987; De Maio 1999), is even shorter in cells already containing HSP70 protein (Theodorakis et al 1999). Similarly, changes in Hsp70 mRNA stability have been observed in prokaryotes and *Drosophila* (DiDomenico et al 1982; Simcox et al 1985; Petersen and Lindquist 1989; Ramos et al 2001).

The posttranscriptional regulation of *Hsp70* expression is important even if transcription is attenuated, because the already-synthesized Hsp70 mRNAs can be further translated to continue the production of the protein. Therefore, a mechanism for the selective degradation of existing Hsp70 mRNA is crucial to limit the production of HSP70. Control of mRNA stability has been recognized as a mechanism to regulate gene expression in organisms from viruses to mammals (Grunberg-Manago 1999; Guhaniyogi and Brewer 2001), but the *cis*- and *trans*-acting elements through which it works have not been elucidated (Guhaniyogi and Brewer 2001). The potential role of HSP70 protein in this type of regulation is suggested indirectly by several pieces of evidence. Inhibiting synthesis of *Drosophila* HSP70 protein leads to stabilization and accumulation of Hsp mRNAs. When protein synthesis resumes, a threshold HSP70 level is needed to destabilize these mRNAs (DiDomenico et al 1982). HSP70 has been shown in vitro to bind AU-rich sequences, particularly AUUUA, in the 3'-untranslated region of lymphokine mRNAs; these sequences are common to many unstable mRNAs and may alter message stability (Spicher et al 1998; Henics et al 1999; Malter 2001). Finally, the Hsp70 mRNA itself contains a class III AU-rich element that lacks a defined AU-containing repeated sequence but has a generally AU-rich 3'-untranslated region (Malter 2001). However, the interaction of Hsp70 with this region has not been documented. In this context, the current study was undertaken to demonstrate an interaction between HSP70 (protein) and Hsp70 mRNA as part of a potential mechanism for limiting *Hsp70* (gene) expression. Inhibitors of transcription and translation were found to stabilize Hsp70 mRNA. Moreover, HSP70 was found by immunoprecipitation and gel-shift analysis to bind its own mRNA.

MATERIALS AND METHODS

Cell culture and heat shock

HeLa and HepG2 cells were cultured in minimal essential medium supplemented with 1× nonessential amino acids, 1 mM sodium pyruvate, 10 IU/mL penicillin, 10 µg/mL streptomycin, and 10% heat-inactivated fetal bovine serum. Prior to heat shock, cells were washed in phosphate-buffered saline (PBS) and covered in fresh medium

at 37°C. Cells were then incubated at either 43°C (heat shock) or 37°C (control) for 90 min, after which both groups of cells were transferred to 37°C for a recovery period.

Northern blot analysis

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Equal amounts of total RNA were separated by electrophoresis and transferred onto nitrocellulose membranes. After ultraviolet (UV) cross-linking, ³²P-labeled riboprobes were generated and hybridized as described previously (Theodorakis et al 1999), and the signal was detected by autoradiography or by using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA, USA).

Immunoprecipitation and slot blotting

Cells were heat shocked (43°C, 90 min) and after 2 hours of recovery (37°C), cells were rinsed with PBS and incubated for 10 minutes in ice-cold radioimmunoprecipitation assay (RIPA) buffer (20 mM HEPES, pH 7.4, 1% Triton X-100, 50 mM NaCl, 1 mM EGTA, 5 mM β-glycerophosphate, 30 mM sodium pyrophosphate, 100 mM sodium orthovanadate, 0.1 mM phenyl-methyl-sulfonyl fluoride, 10 µg/ml leupeptin, and 10 µg/ml pepstatin A) with 40 U of ribonuclease (RNase) inhibitor (RNaseOUT; Invitrogen) and 70 µL protease inhibitor cocktail (Complete Mini EDTA-free; Roche, Basel, Switzerland). Cells were then scraped and centrifuged for 15 min, and the supernatant (cell lysate) was saved for the immunoprecipitation procedure. Cell lysate containing 1 mg of total cellular protein was incubated with mouse monoclonal anti-Hsp70 antibody (StressGen Biotechnologies, Victoria, British Columbia, Canada) for 16 hours at 4°C followed by anti-mouse antibody coupled to agarose beads (Sigma, St Louis, MO, USA). The agarose beads were pelleted and washed 4 times with RIPA buffer. The bead pellets from 4 immunoprecipitations were combined and used for RNA extraction. Total RNA was extracted from the immunoprecipitated beads (supplemented with yeast transfer RNA [tRNA] used as a carrier) by the TRIzol method. The isolated RNA was slot blotted onto nitrocellulose using a Minifold II system (Schleicher & Schuell, Riviera Beach, FL, USA), UV cross-linked, and hybridized as described above. The signal was detected using a PhosphorImager. The signal quantification was performed using ImageQuant software (Molecular Dynamics).

In vitro RNA transcription and electrophoretic mobility shift assays

Full-length ³²P-labeled Hsp70 sense RNA was transcribed from a pig Hsp70 complementary DNA (cDNA) using T7

Fig 1. Hsp70 mRNA after heat shock is stabilized in the presence of transcriptional and translational inhibitors. HepG2 cells were heat shocked at 43°C (1.5 hours) or maintained at 37°C (control). Cells that were stressed for 1.5 hours were allowed to recover at 37°C for different time periods (1, 2, 4, 6, and 8 hours). Act-D (10 µg/mL) or cycloheximide (CHX) (100 µM) were added to the cells immediately after heat shock and further incubated in the presence of these inhibitors for up to 8 hours. In parallel, stressed cells were incubated at 37°C in the absence of the inhibitors. Total RNA was isolated from the cells, and the levels of Hsp70 mRNA were visualized by Northern blotting using a radiolabeled probe specific for Hsp70 mRNA.

RNA polymerase (Promega, Madison, WI, USA), whereas antisense transcript was generated using SP6 RNA polymerase (Invitrogen). RNA was then extracted using an acid/guanidinium thiocyanate/phenol/chloroform protocol (Chomczynski and Sacchi 1987). Mobility shift assays were carried out as previously described (Henics et al 1999), with the following modifications: 1 µg of recombinant Hsp70 protein (StressGen) was used, reactions were incubated for 30 min at 30°C, 7.5 U of RNase T1 were used alone, and samples were analyzed by 5% non-denaturing polyacrylamide gel electrophoresis (PAGE) in Tris-borate-EDTA (TBE) buffer (0.09 µ tris-borate pH 8.0, 0.002 µ EDTA) and the signal was detected using a PhosphorImager.

RESULTS

The degradation of Hsp70 mRNA is prevented by inhibitors of transcription and translation

HepG2 cells were exposed to 43°C for 90 min (a nonlethal heat shock), followed by recovery at 37°C to allow the expression of Hsp70. The transcriptional inhibitor actinomycin D (Act-D), or the translational inhibitor cycloheximide was added immediately after heat shock, and RNA was extracted from cells at varying times following the addition of the drug. Hsp70 mRNA was highly induced after heat shock in comparison with nonstressed cells (control), as detected by Northern blotting. After the addition of Act-D or cycloheximide, Hsp70 mRNA was present at high levels for at least 8 hours after heat shock. These observations are markedly different from the rapid disappearance of Hsp70 mRNA observed in the absence of the inhibitors (Fig 1). Hsp70 mRNA was also stabilized when Act-D was added at 3 hours of recovery after heat shock, resulting in an increased Hsp70 mRNA half-life of 2.6 hours as opposed to 1.5 hours in the absence of the drug. Hsp70 mRNA levels were also stabilized upon ad-

dition of cycloheximide or puromycin at 3 hours of recovery following the stress (Fig 2). The delayed decay of Hsp70 mRNA in cells exposed to these drugs indicates that one or more thermal stress-induced factors normally play a role in promoting the degradation of this mRNA.

HSP70 is associated with Hsp70 mRNA after thermal stress

The possibility that HSP70 might be one of the stress-induced factors that destabilizes Hsp70 mRNA was further explored. HeLa cells were exposed to 43°C for 90 min and allowed to recover for 2 hours at 37°C. Control cells were maintained at 37°C for the entire experiment. Both groups of cells were lysed after the 2-hour recovery period. HSP70 was immunoprecipitated from cell lysates using an antibody specific for HSP70. RNA was extracted from the immunoprecipitate, slot-blotted, and probed for the presence of Hsp70 mRNA. Analysis of immunoprecipitates from heat-shocked cells showed greater content of Hsp70 mRNA in comparison with immunoprecipitates from control cells (Fig 3 A,B). No such increase in signal relative to control was observed when the blot was probed for β-actin (Fig 3B). Immunoprecipitation with anti-HSC70 antibodies did not result in any detectable Hsp70 mRNA (data not shown). These observations suggest that HSP70, present at greater levels in heat-shocked cells, was specifically associated with Hsp70 mRNA after heat shock.

HSP70 interacts directly with Hsp70 mRNA

To further clarify the association between HSP70 and its message, an electrophoretic mobility shift assay was performed. Full-length radiolabeled sense or antisense Hsp70 mRNAs were generated by in vitro transcription. These RNA probes were incubated with recombinant

Fig 2. Hsp70 mRNA is stabilized after the addition of translational inhibitors during the recovery period after heat shock. HepG2 cells were heat shocked at 43°C (1.5 hours) or maintained at 37°C (control). After 3 hours of recovery at 37°C, cells were treated with (A) cycloheximide (CHX) (100 μ M) or (B) puromycin (1 μ g/mL) and further incubated in the presence of these inhibitors for up to 11 hours. In parallel, stressed cells were incubated at 37°C in the absence of the inhibitors. Total RNA was isolated from the cells, and the levels of Hsp70 mRNA were visualized by Northern blotting using a radiolabeled probe specific for Hsp70 mRNA.

HSP70, and the reaction mixtures were then treated with RNase. When the reactions were separated by PAGE, a band of apparent lower electrophoretic mobility (indicated as "shift" in Fig. 4) was observed in reactions incubated with HSP70. This band of lower electrophoretic migration was not observed when the radiolabeled antisense RNA transcript was used (Fig 4). Electrophoretic mobility shift reactions were also conducted in the presence and absence of 125 μ M adenosine triphosphate (ATP). Although a shift in the migration of radiolabeled sense Hsp70 transcript was observed in the presence of recombinant HSP70 and absence of ATP, the signal intensity of this band was attenuated in the presence of ATP (Fig 4). This observation suggests that ATP had an inhibitory effect on HSP70-Hsp70 mRNA interaction.

DISCUSSION

Hsps play several roles within cells, including the folding of newly synthesized polypeptides. This folding activity

is crucial during stress conditions, particularly if the insult results in the appearance of denatured polypeptides. Thus, Hsps are involved in the renaturing of these unfolded polypeptides, the resolubilization of protein aggregates, and the targeting of irreversible unfolded polypeptides for degradation after stress (Morimoto 1991; De Maio 1999). Indeed, HSP70 has been found to fold polypeptides in vitro (Skowrya et al 1990; Ziemienowicz et al 1993; Freeman and Morimoto 1996). The presence of HSP70 has also been correlated with the development of stress tolerance, which is proposed to be due to stabilization of basic cellular processes, such as translation (De Maio 1999). Despite these advantageous attributes of HSP70, the extended presence of this protein appears to accelerate cell death (Feder et al 1992). Thus, the expression of *Hsp70* needs to be tightly regulated. Expression of *Hsp70* was attenuated during the recovery period after stress (DiDomenico et al 1982; Petersen and Lindquist 1988; Abravaya et al 1992) and after conditional expression of HSP70 (Shi et al 1998). Moreover, functional mu-

Fig 3. Hsp70 mRNA is present in immunoprecipitated HSP70. HeLa cells were either heat shocked at 43°C for 1.5 hours and recovered at 37°C for 2 hours (HS), or not stressed (Ctrl). Cells were lysed and subjected to immunoprecipitation using an antibody specific for HSP70 followed by a secondary antibody conjugated to agarose beads. RNA was extracted from immunoprecipitate pellets, slot-blotted, and hybridized to a radiolabeled probe specific for Hsp70 or β-actin (A). RIPA buffer alone ("buffer") was used as a negative control. The slot blots are representative of 3 independent determi-

Fig 4. HSP70 binds to Hsp70 transcripts in in vitro conditions. Full-length radiolabeled Hsp70 mRNA was transcribed in vitro and incubated with recombinant Hsp70 protein (in duplicate) in the presence or absence of ATP. A similarly sized antisense Hsp70 transcript was also produced and used as a negative control. Reactions were then treated with RNase T1 and subjected to non-denaturing PAGE. Lanes 1–4 show the results of reactions with the sense transcript and lanes 5–8, with the antisense transcript. The reactions in lanes 1, 3, 6, and 8, labeled "+ATP," were conducted in the presence of 125 μM ATP. The arrowhead indicates the location of the "shifted" band.

tations of *Hsp70* resulted in overexpression of this gene in yeast (Craig and Gross 1991). In part, these observations can be accounted for by the action of HSP70 as a negative regulator of *Hsp70* transcription (Shi et al 1998). A change in Hsp70 mRNA stability has been shown to be an equally important mechanism in the regulation of gene expression in humans (Theodorakis et al 1987) and *Drosophila* (Petersen et al 1988) cells. Hsp70 mRNA half-life was reduced by approximately 50% in cells containing HSP70 in comparison with naive cells after heat shock (Theodorakis et al 1999). These observations echo prior reports suggesting a self-limiting mechanism for Hsp70 expression (DiDomenico et al 1982). However, some reports suggest that functional Hsps are not required in the degradation of stress-induced mRNAs in chicken cells (White and Hightower 1984). In other studies, Hsp70 mRNA stability decreased after inhibition of translation in adenovirus-transformed cells (Kao and Nevins 1983). Thus, it is possible that the regulation of Hsp70 mRNA stability is dependent on the cell type or additional factors, such as viral infections.

In this study, we found that the rapid degradation of Hsp70 mRNA could be reduced by the addition of a transcription inhibitor, Act-D, which doubled the half-life of Hsp70 mRNA. The stabilization of Hsp70 mRNA was also observed with the addition of cycloheximide or puromycin, which block translation by different mecha-

nisms. The signal intensity for Hsp70 or β-actin mRNAs in HS or Ctrl samples were quantitated by phosphorimaging as described in Materials and Methods. The results are presented as the average of 3 independent experiments ± SEM; $P < 0.001$ by Student's *t*-test (B).

nisms. The stability of interleukin-6 mRNA, which is also a short half-life message, has likewise been reported to increase upon incubation with cycloheximide (Lutter et al 2000). The effect of transcriptional and translational inhibitors on Hsp70 mRNA stability may indicate that new gene expression was required for the rapid degradation of Hsp70 mRNA. However, we cannot discard the possibility that the effect of transcription and translation inhibitors correspond to 2 independent mechanisms. For example, the degradation of Hsp70 mRNA may require the synthesis of an RNA molecule (ie, a short interfering RNA [siRNA]), whereas the stability of the message may be modulated by the synthesis of a polypeptide, such as HSP70. An appealing hypothesis is that HSP70 signals the message for degradation. In this case, it would be expected that HSP70 interacts directly or indirectly with Hsp70 mRNA. Indeed, we found that Hsp70 mRNA coimmunoprecipitates with HSP70 in lysates from stressed cells. In addition, the interaction of HSP70 and Hsp70 transcripts was observed in *in vitro* conditions. Moreover, the presence of ATP disrupted HSP70 binding to the Hsp70 transcript. Several lymphokine and proto-oncogene mRNAs contain destabilizing AU-rich 3'-untranslated regions, which have been shown to interact with HSP70 in *in vitro* conditions (Henics et al 1999; Zimmer et al 2001). Indeed, deletion of the 3'-untranslated regions of *Drosophila* Hsp70 mRNA resulted in stabilization of the message (Simcox et al 1985; Petersen et al 1989). Thus, it has been suggested that HSP70 binds an AU-rich domain on the 3'-untranslated region of Hsp70 mRNA (Henics 2003). However, this physical interaction between HSP70 and its own message has never been demonstrated previously. The interaction of HSP70 with other transcripts has been localized to the adenosine triphosphatase (ATPase) domain of the protein (Zimmer et al 2001), which is consistent with our findings that ATP interferes with HSP70 binding *in vitro* to the Hsp70 transcript. Nevertheless, the biological significance of these *in vitro* observations needs to be interpreted with caution because the cellular concentration of ATP is very high.

We propose that the HSP70-mRNA interaction is a signal for the rapid degradation of Hsp70 mRNA. The interaction of HSP70 with its own mRNA could be coupled with the translation process. In this regard, HSP70, but not HSC70, has been found associated to the 40S subunit of ribosomes during translation (Cornivelli et al 2003). Thus, it is possible that the presence of HSP70 within ribosomes is necessary to facilitate its interaction with Hsp70 mRNA. On the other hand, the rapid degradation of Hsp70 mRNA may be related to the accumulation of the protein within the cell. This possibility is consistent with the fact that the degradation of Hsp70 mRNA is only observed several hours after stress, when cells are fully recovered from the stress (Theodorakis et al 1999). A pos-

sible hypothesis is that Hsp70 mRNA is targeted for degradation when HSP70 exceeds denatured substrates. Thus, there is a rapid increase in *Hsp70* transcription when cells are exposed to stress. Immediately after the insult, HSP70 protein in the cell is largely associated with denatured polypeptide substrates, in accordance with the chaperone function of this stress protein. Over time, the number of polypeptide substrates is reduced due to refolding of these polypeptides, and the proportion of free vs substrate-bound HSP70 is increased. This free HSP70 protein is then able to affect further HSP70 production by attenuating transcription or accelerating the degradation of its own mRNA. Prior studies have shown that binding of HSP70 to a target polypeptide causes a conformational change in the protein, reducing its RNA binding capacity (Henics et al 1999). The binding of HSP70, directly or indirectly, to its own message during recovery after stress may be related to the decay process. This event may be part of the self-limiting mechanism for Hsp70 expression previously proposed (DiDomenico et al 1982). The molecular mechanisms by which HSP70 interacts with its own message affecting its stability remain to be explored.

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REFERENCES

- AbraVaya K, Myers MP, Murphy SP, Morimoto RI. 1992. The human heat shock protein hsp70 interacts with HSE, the transcription factor that regulates heat shock gene expression. *Genes Dev* 6: 1153-1164.
- Arispe N, Doh M, Simakova O, Kurganov B, De Maio A. 2004. Hsc70 and Hsp70 interact with phosphatidylserine on the surface of PC12 cells resulting in a decrease of viability. *FASEB J* 18: 1636-1645.
- Chomczynski P, Sacchi N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162: 156-159.
- Cornivelli L, Zeidan Q, De Maio A. 2003. Hsp70 interacts with ribosomal subunits of thermotolerant cells. *Shock* 320-325.
- Craig E, Gross CA. 1991. Is hsp70 the cellular thermometer? *Trends Biochem Sci* 16: 135-140.
- De Maio A. 1999. Heat shock proteins. Facts, thoughts, and dreams. *Shock* 11: 1-12.
- DiDomenico BJ, Bugaisky GE, Lindquist S. 1982. The heat shock response is self-regulated at both the transcriptional and post-transcriptional level. *Cell* 31: 593-603.
- Feder JH, Rossi JM, Solomon J, Solomon N, Lindquist S. 1992. The

- consequences of expressing hsp70 in *Drosophila* cells at normal temperatures. *Genes Dev* 6: 1402–1413.
- Freeman BC, Morimoto RI. 1996. The human cytosolic molecular chaperones hsp90, hsp70 (hsc70) and hdj-1 have distinct roles in recognition of a non-native protein and protein refolding. *EMBO J* 15: 2969–2979.
- Grunberg-Manago M. 1999. Messenger RNA stability and its role in control of gene expression in bacteria and phages. *Annu Rev Genet* 33: 193–227.
- Guhaniyogi J, Brewer G. 2001. Regulation of mRNA stability in mammalian cells. *Gene* 265: 11–23.
- Henics T. 2003. Extending the “stressy” edge: molecular chaperones flirting with RNA. *Cell Biol Int* 27: 1–6.
- Henics T, Nagy E, Oh HJ, Csermely P, von Gabain A, Subject JR. 1999. Mammalian Hsp70 and Hsp110 proteins bind to RNA motifs involved in mRNA stability. *J Biol Chem* 274: 17318–17324.
- Kao HT, Nevins JR. 1983. Transcriptional activation and subsequent control of the human heat shock gene during adenovirus infection. *Mol Cell Biol* 3: 2058–2065.
- Li GC, Li L, Liu Y-K, Mak JY, Chen L, Lee WMF. 1991. Thermal response of rat fibroblasts stably transfected with the human 70-kDa heat shock protein-encoding gene. *Proc Natl Acad Sci USA* 88: 1681–1685.
- Lutter R, Loman S, Snoek M, Roger T, Out TA, Jansen HM. 2000. IL-6 protein production by airway epithelial(-like) cells disabled in IL-6 mRNA degradation. *Cytokine* 12: 1275–1279.
- Malter JS. 2001. Regulation of mRNA stability in the nervous system and beyond. *J Neurosci Res* 66: 311–316.
- Mizzen L, Welch WJ. 1988. Characterization of the thermotolerant cell. I. Effects on protein synthesis activity and the regulation of heat-shock protein 70 expression. *J Cell Biol* 106: 1105–1116.
- Morimoto RI. 1991. Heat shock: the role of transient inducible responses in cell damage, transformation and differentiation. *Cancer Cells* 3: 295–301.
- Petersen R, Lindquist S. 1988. The *Drosophila* hsp70 message is rapidly degraded at normal temperatures and stabilized by heat shock. *Gene* 72: 161–168.
- Petersen R, Lindquist S. 1989. Regulation of HSP70 synthesis by messenger RNA degradation. *Cell Regul* 1: 135–149.
- Ramos JL, Gallegos MT, Marques S, Ramos-Gonzalez MI, Espinosa-Urgel M, Segura A. 2001. Responses of Gram-negative bacteria to certain environmental stressors. *Curr Opin Microbiol* 4: 166–171.
- Shi Y, Mosser DD, Morimoto RI. 1998. Molecular chaperones as HSF1-specific transcriptional repressors. *Genes Dev* 12: 654–666.
- Simcox AA, Cheney CM, Hoffman EP, Shearn A. 1985. A deletion of the 3' end of the *Drosophila melanogaster* hsp70 gene increases stability of mutant mRNA during recovery from heat shock. *Mol Cell Biol* 5: 3397–3402.
- Skowrya D, Georgopoulos C, Zylicz M. 1990. The *E. coli* dnaK gene product, the hsp70 homolog, can reactivate heat-inactivated RNA polymerase in an ATP hydrolysis-dependent manner. *Cell* 62: 939–944.
- Spicher A, Guicherit OM, Duret L, et al. 1998. Highly conserved RNA sequences that are sensors of environmental stress. *Mol Cell Biol* 18: 7371–7382.
- Theodorakis NG, Drujan D, De Maio A. 1999. Thermotolerant cells show an attenuated expression of Hsp70 after heat shock. *J Biol Chem* 274: 12081–12086.
- Theodorakis NG, Morimoto RI. 1987. Posttranscriptional regulation of hsp70 expression in human cells: effects of heat shock, inhibition of protein synthesis, and adenovirus infection on translation and mRNA stability. *Mol Cell Biol* 7: 4357–4368.
- White CN, Hightower LE. 1984. Stress mRNA metabolism in canavanine-treated chicken embryo cells. *Mol Cell Biol* 4: 1534–1541.
- Ziemienowicz A, Skowrya D, Zeilstra-Ryalls J, Fayet O, Georgopoulos C, Zylicz M. 1993. Both the *Escherichia coli* chaperone systems, GroEL/GroES and DnaK/DnaJ/GrpE, can reactivate heat-treated RNA polymerase. Different mechanisms for the same activity. *J Biol Chem* 268: 25425–25431.
- Zimmer C, von Gabain A, Henics T. 2001. Analysis of sequence-specific binding of RNA to Hsp70 and its various homologs indicates the involvement of N- and C-terminal interactions. *RNA* 7: 1628–1637.