Stress Protein Expression Kinetics

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Abstract
In all organisms there is an elevated synthesis of a select family of “stress proteins” in response to a broad array of environmentally driven stress vectors including elevated or depressed temperature, changes in pH, treatment with many classes of chemicals, ischemia, desiccation, and UV irradiation. The presence of stress proteins, often termed heat shock proteins (HSPs), has been recognized for more than four decades, and there is an extensive literature that addresses the structure and properties of HSPs, their function in normal and injured cells and tissues, and the molecular mechanisms of HSP expression in response to stress. Owing to this substantial aggregate of research, there is a growing appreciation of the potential for manipulating the magnitude and timing of elevated HSP expression to achieve targeted therapeutic objectives. The successful realization of this potential requires an understanding of the kinetics of the HSP expression process in response to sublethal stress regimens along with the ability to model the governing events in the process to design practical protocols that could be applied in therapeutic settings. Significant progress has been made in recent years in defining and developing capabilities in these two areas.
INTRODUCTION

Stress proteins are a ubiquitous family of gene products that are expressed in higher concentrations owing to the presence of stress (1). The major class of stress proteins is called heat shock protein (HSP), often followed by a number indicating the molecular weight in kilodaltons. HSPs are present in all cells in all life forms. These proteins play a critical role in a complex defense mechanism for enhancing cell survival under adverse environmental conditions as well as in normal cellular homeostasis (2). The presence of sublethal stressful stimuli induces HSP production and can protect cells exposed to subsequent lethal insults. Initiating insults may include elevated temperature (hyperthermia) (3–5), ischemia (6), hypoxia (7), depletion of ATP (8), free radicals (9), hyperthermia (10), desiccation (11), various viruses (12), steroid hormones (13), and ethanol (14).

HSPs are present in cells under unstressed conditions for which they function as molecular chaperones (15, 16). They play a critical role in normal protein homeostasis to assist in protein folding (17–19); the assembly and disassembly of protein complexes (20); inhibition of improper protein aggregation, such as may occur owing to crowding or thermal denaturation (21, 22); direction of newly formed proteins to target organelles for final packaging; degradation or repair (23, 24); and activation of the immunological system in response to the presence of viral proteins (25, 26), resulting in the initiating mechanism for selected specific diseases (27). In response to stress, HSPs assist in refolding and repair of denatured proteins as well as facilitating synthesis of new proteins to repair damage (28, 29). The stress response is elicited primarily in response to the presence of damaged molecules as opposed to the physico/chemical nature of the insulting stress (30).

Although stress proteins occur and function endogenously, the ability to manipulate their upregulation via subjection to externally controlled stressors and pharmacological mediators provides a potential to design prophylactic and therapeutic protocols to take advantage of enhanced HSP function (31). The prior induction of HSP by a mild stress can provide protection to cells and tissues against a subsequent severe stress that would normally be damaging or lethal. The process of inducing an overproduction of HSPs in a target tissue is termed preconditioning. This technique has been demonstrated for eliciting protection against ischemic reperfusion in conjunction with cardiac surgery (32, 33). When the overexpression of stress proteins results in an enhanced ability to withstand hyperthermia, the effect is called acquired theromtolerance.

The initial documentation of these phenomena dates from more than 40 years ago (3), and it has now been described in a literature numbering in the tens of thousands of articles, including a research journal [Cell Stress and Chaperones (CSC)] published by a dedicated professional society (Cell Stress Society International). The existing literature has provided extensive insight into the structure and function of stress proteins and, to a lesser extent, into the mechanistic pathway of their expression. The field embraces a very broad perspective, with the journal CSC touted as capturing “the eclectic spirit of the cellular stress response field in a single, concentrated source of current information.” As testimony to the growing breadth of interest in HSPs,
there have been some two-dozen chapters written in various volumes over the past 20 years that have had a primary focus on the expression and function of stress proteins (see list at end of chapter). In view of such a voluminous literature relating to stress proteins, the scope of this review must be restricted to only a very limited perspective of the field, that being the kinetics of the process by which they are overexpressed under conditions of elevated environmental stress.

**STRESS PROTEIN EXPRESSION AND REGULATION**

Stress proteins exist in cells under both normal and stressed conditions, with their concentration potentially enhanced significantly in the latter. Given that the production of stress proteins can be altered substantially in response to a wide variety of stressor processes, the regulatory feedback mechanism of the expression apparatus must be both sensitive and rapid. Briefly, environmental stress activates a specific set of heat shock genes to accelerate the preferential synthesis of HSPs. The increase in HSP concentration in the affected cells occurs with a predictable time course consisting of a progressive rise to a maximum value, followed by a temporary period of elevation, and subsequently a decline to normal levels. Thus, the overexpression of HSP is a transient phenomenon with a time constant that is typically a few days. As with all gene-to-protein processes, the pathway and associated feedback control mechanisms are complex (34–37). The details are extensive and beyond the scope of this review; only an overview germane to the presentation of subsequent topics is covered.

Indeed, developing a rigorous and general understanding of the complex regulatory process by which genes express proteins is one of the major scientific challenges of the present time. It is identified as one of the 25 big questions in science to be answered over the coming 25 years in the one hundred and twenty-fifth anniversary issue of *Science* (38).

The inducible elevated synthesis of stress proteins is known to be facilitated directly by activity of heat shock factors (HSFs) on targeted genes (39). This stress-induced transcription process requires activation of a HSF to bind to the heat shock promoter element (HSE) characterized by the specific nucleotide motif (40). Multiple HSFs (denoted as HSF1, HSF2, HSF3, and HSF4) are involved in this process in mammals, providing redundancy of function and specialization of stress control signals (41, 42). Key issues of interest are how the basal level of HSPs is maintained in nonstressed conditions, how the transient rate of HSP synthesis is upgraded transiently under stress and in proportion to the nature and magnitude of the stress loading, and how differences in the signaling and control mechanisms of the homeostatic and stress states differ to produce the differential expression rates. Details of current understanding of the activated transcription process are available in various review articles (43, 44).

It has been demonstrated that HSF1 is a positive and necessary regulator for expression of HSP under all conditions, thus with a ubiquitous function (45–49). It is the key transcription factor that mediates the stress protein response. HSF2A is not an activator in the absence of stress, but functions with HSF1 in a coupled
process under the action of an applied stress (50, 51). Unstressed cells have a low background level of HSF1, with its transcribing activity less than 5% that of severely heat-stressed cells (52). The presence of stress results in a change in the negative feedback relationship between extracellular signal-regulated protein kinase 1, 14–3–3ε and HSF1, defining a differential transcription mechanism between homeostatic and stress states (53). Imposition of stress causes a rapid reprogramming of the gene expression mechanism to the increased synthesis of HSP from the normal nonstressed state (54). Numerous factors are involved in the stress protein response via both positive and negative feedback mechanisms. HSF1 is constitutively present in cells, but under this condition its transcription initiating activity is limited by binding with a HSP90 multichaperone complex (55). Applied stress causes an accrual of denatured proteins that compete with the HSF1 for HSP90 multichaperone complexes, resulting in unbinding of HSF1 so that it becomes much more available for transcription activity after conversion to homotrimer. DAXX is a proapoptotic molecule present in both the cytoplasm and nucleus that is normally bound in that domain (56). DAXX is released from this deposit during stress and when freed has the nuclear function of enhancing the activation of HSF1 by counteracting the repression mechanism of the multichaperone complex (52).

**EXPRESSION KINETICS**

The targeted induction of stress protein overexpression presents an attractive option for manipulating this powerful endogenous defense and repair mechanism to benefit for specific prophylactic, therapeutic, and trauma scenarios. It has been long established that the level of accumulated stress protein expression is critical in achieving effective tissue effects (32). In this review, the expression process is characterized for response only to thermal stress protocols, although there are efficacious alternatives. Thus, an understanding of how the temperature and time of stress can be manipulated to control the expression process is necessary to design and elicit a thermal stress protocol propitious for inducing a desired effect.

The kinetics of HSP70 expression have been measured experimentally for a number of different systems. The stress is defined by the temperature and the duration of exposure imposed on a cell or tissue specimen (57). The expression process occurs progressively from the initiation of the stress. In nearly all practical cases the duration of a stimulating stress event is significantly less than the total time required for the ultimate level of expression to be manifested, which occurs on the timescale of hours. Thus, the post-stress elapsed time is an additional important metric in describing the progressive production of stress proteins. For example, a maximum HSP70 level was found at 24 h after a cold shock on rat hearts at 4°C for 4 h (58). In a brain stroke model, the peak amount of HSP70 mRNA and HSP70 occurred between 8 to 24 h after 60-min focal cerebral ischemia (59). And, a 90-min heating duration at 42°C followed by 12 h recovery at 37°C produced a peak endogenous HSP70 expression in bovine aortic endothelial cells (BAECs) that exceeded basal levels by more than ninefold as determined by Western blot analysis (60). This latter study presents detail
sufficient to describe the interaction among the biophysical factors that govern the overexpression process.

The BAECs were subjected in an incubator to a broad matrix of thermal stress and recovery conditions that embraced elevated temperatures from 40°C to 44°C, exposure times from 0 to 5 h, and recovery periods at 37°C from 0 to 48 h. Figures 1 and 2, respectively, show the peak concentration of HSP70 achieved for combinations of exposure times and recovery periods for 42°C and for various temperatures associated with 2 h of exposure and 14 h post recovery. HSP70 concentration was normalized to actin to provide a relative calibration of the protein concentration in the specimen. The level of expression rises with increasing stress temperature. Eventually, a threshold is reached for which the level of injury is such that the cell reparative process is overwhelmed, resulting in cell death. Figure 3 shows data comparing the level of HSP70 expression with cell injury as a function of stress temperature and exposure time in a prostate cancer cell model (61). Decreases in cell viability were detected for less stressful conditions than required to achieve the largest enhancement in HSP expression. For higher stress temperatures, the cells demonstrated a greater sensitivity to injury than to HSP overexpression. Under more extreme stress conditions, as the extent of damage becomes large, the HSP expression drops precipitously because the cell function is compromised.

There is growing evidence that the overexpression process does not issue in a monotonic accrual of stress protein, but that there is a series of two temporal windows
Figure 2
HSP70 expression in BAEC for 2 h of thermal stress at the indicated temperatures followed by 14 h in a 37°C incubator. Each data point represents the average value ± SEM; n = 4 (42.25).

Figure 3
Comparison of cell viability and HSP70 expression in PC3 prostate cancer cells for incremental heating times at the indicated stress temperatures; n = 3 (42.25).
HSP70 expression in BAEC for 1.6 h of thermal stress at 42°C followed by the indicated period in a 37°C incubator. Each data point represents the average value ± SEM; n = 7 (42.25).

A stress sensitive reporter can be constructed to optically interrogate the expression of HSP over time (69). A higher temporal resolution of HSP70 expression kinetics was achieved by continuous visualization of BAECs on a microscope heating stage using green fluorescent protein (GFP) as a reporter (70). The BAECs were transfected with a DNA vector, HSP70-HSP70-GFP, which expresses an HSP70-GFP fusion protein under control of the HSP70 promoter. Expression levels were validated by Western blot analysis. Transfected cells were heated on a controlled temperature microscope stage at 42°C for a defined period, then cooled to 37°C for varied postheating times. The expression of HSP70-GFP and its subcellular localization were visualized via fluorescence microscopy as shown in Figure 5. The progressive expression kinetics were measured by quantitative analysis of serial fluorescence images captured during heating protocols from 1 to 2 hr and postheating times from 0 to 20 hr. The results show two sequential peaks in HSP70 expression at approximately 3 and 12 hr post heat shock. See Figure 6. A progressive

**Figure 4**

HSP70 expression in BAEC for 1.6 h of thermal stress at 42°C followed by the indicated period in a 37°C incubator. Each data point represents the average value ± SEM; n = 7 (42.25).

**GFP:** green fluorescent protein
Figure 5
Phase and fluorescence images before heating at 42°C for 100 minutes and for a 12 hour recovery at 37°C.

translocation of HSP70 from the cytoplasm to the nucleus was observed from 6 to 16 hrs. These experiments demonstrate a technique to measure HSP70 expression kinetics quantitatively in living cells in real time. This method could be a particularly useful way to study the expression of proteins which are toxic to cells when constitutively overexpressed.
MODELS FOR EXPRESSION

There are two primary classes of models that provide a quantitative description of elevated HSP expression in response to imposed environmental stress: empirical and mechanistic. Empirical modeling consists of a top-down approach in which a mathematical expression is identified that provides a reasonable match to experimental data that describe the time course and magnitude of HSP overexpression in response to graded thermal stress (or stress from other sources). Mechanistic modeling consists of a bottom-up approach in which the individual, serial, and parallel steps in the expression process plus cross-coupled signaling and modulation are described by appropriate mathematical expressions that can be integrated into a whole model that predicts behavior of the expression system in response to graded environmental stress. Empirical models can be used with confidence only for processes that fall within the state bounds of the experimental data on which they are based. Mechanistic models can be considered more general and potentially robust for extrapolation as long as the governing process mechanisms are not violated. Empirical models require much less detailed experimental information and constitutive property values than do mechanistic, but are less robust and are more limited in their domain of application. Typically, as a new field is being defined and developed, the initial modeling is empirically oriented, and as a more mature and complete understanding of the governing physico-chemical processes emerges, mechanistic-based models can then be constructed. At the present time, the field of stress protein expression kinetics is in the former stage in which empirical models are becoming available as the requisite high-level experimental data are acquired. With time and considerable experimental and theoretical work, a more complete picture and corresponding model of the stress protein expression process should emerge that can be applied as a design tool for
therapeutic process design. For example, mechanistic models are now emerging to describe protein-folding kinetics (71, 72).

Modeling of the mechanistic structure and functioning of gene regulatory networks is a fundamental goal of systems biology (73–77). Qualitative representations of gene regulatory networks have been developed recently (78), and quantitative models have just begun to be developed (79). In the latter work, the quantitative relationship between transcription factor concentrations and the rate of protein production is addressed. This relationship is defined as the gene regulation function (GRF), which designates the dependence of the rate of expression of downstream gene products through transcription and translation on the concentration of transcription factors in a cell. The magnitude of GRF is demonstrated to fluctuate temporally in living cells. The implication is that genetic transcription circuit amplification factors can vary over time and/or state. Identification of the factors that regulate the coefficients of the transcription process is critical for quantitative modeling of the coupling between level of applied stress and the pattern of upregulation of stress protein expression. Of particular relevance to the present review are the time constants associated with the various components of the process and how they act in concert to define the GRF for stress proteins (79, 80). As different processes and feedback loops operate on varying temporal scales relative to that of the cell cycle (81), they will persist in influencing expression.

There are very few quantitative models that describe the gene expression process. Diaz et al. (82, 83) have developed a mechanistic model to predict the expression dynamics of *Xenopus*. Baltimore, Hoffmann, and colleagues have developed techniques for description of cell response regulation to diverse stimuli in terms of the temporal activation of the transcription process (84–87). Specific signaling pathways are identified with the underlying molecular mechanisms and are represented by a set of ordinary differential equations. This model was built to describe the serial biochemical reactions involved in signal transduction between IkB kinase complex and nuclear transcription factor NF-κB activity. The model includes the effects of tumor necrosis factor (TNF)-induced inhibitor protein IkB activity, with provision for more than 70 process steps, including positive and negative feedback loops. The concentrations of two molecular species, x and y, are assumed to undergo a temporal rate response to an external stimulus, S. The interaction between x and y is represented by a pair of linear ordinary differential equations that are readily solved by standard numerical techniques:

\[
\frac{dx}{dt} = S - \alpha y - \beta x
\]

\[
\frac{dy}{dt} = \chi x - \delta y.
\]

Both x and y have negative self-regulation, whereas x is positively regulated by y and y is positively regulated by x. The four coefficients are rate constants that must be measured via appropriately controlled experiments. Although this model does not present an analysis for the transcription and translation of stress proteins, it
illustrates an approach that could be adapted to guide future investigations to acquire the requisite constitutive data for the mechanistic process steps and interactions.

**PROCESS DESIGN BASED ON EXPRESSION KINETICS MODELS**

There are progressively more areas of medicine in which the ability to control the expression or synthetic introduction of molecular chaperones will be of benefit in a therapeutic setting (88, 89). Diverse application areas span cancer (90–91), immunology (92–94), surgical preconditioning (34), recovery of thermally injured tissues (95–97), and restoration of membrane integrity (98, 99).

An inherent feature of induced overexpression of stress proteins for therapeutic purposes is that the causative stress itself may issue in damage that will cancel the desired benefit of the protocol design. Conversely, some therapeutic protocols are designed to produce targeted cell and tissue death, such as in tumor hyperthermia. An undesirable side effect is HSP induction that can subvert the objective of tumor eradication by conditioning the cells to better withstand the therapeutic insult. Thus, it is important to be able to measure (100–102) and model (100, 103) the simultaneous effects of cell death and HSP induction in the tissues of interest.

For procedures based on in situ stimulation stress protein upregulation via the temporal manipulation of the environmental stress state, it is necessary to have access to a predictive model to apply in-process design. The goal of the model is simulation of the time course and magnitude of stress protein upregulation, which is dependent on the nature and strength of the stress. This problem must be addressed using inverse solution techniques for which the input is identified that elicits a specified output. In most instances a two-layer inverse solution is required. The upregulation process is the consequence of an imposed hyperthermic regimen, which itself is produced by an applied heating protocol defined temporally in three spatial dimensions, plus the three-dimensional constitutive properties of the subject biological material. Thus, the inverse model solution must embody a direct coupling among the parameters that define an external heating source, the biological target system, and the temporal process for expression of stress proteins. In general, the system geometry is complex and is defined in three dimensions, and the tissue properties are nonisotropic and nonlinear. As a consequence, finite element methodology is the simulation tool of choice.

The finite element approach to inverse modeling for defining thermal stress protocols to drive explicit HSP expression profiles within a targeted three-dimensional volume has been developed and applied successfully by Rylander and colleagues (100, 103). The inverse modeling process has been illustrated for the thermal and induction constitutive properties of prostate cancer and normal cells undergoing hyperthermia coagulation and sensitizing with a laser heating source. Hyperthermia can elicit HSP expression in regions where temperatures are insufficient to coagulate tissue, providing enhanced tumor viability. While it is well known that hyperthermia can be used in conjunction with radiotherapy, chemotherapy, and gene therapy to increase therapeutic efficacy, the effectiveness of these therapies can also be substantially
hindered owing primarily to HSP expression when hyperthermia is applied prior to these procedures. In regions of the tumor experiencing only moderate temperatures, HSP elevation can increase tumor cell survival. Alternatively, the induction of protective HSP in regions of normal tissue adjacent to the tumor may allow for these tissues to survive higher thermal doses, thereby allowing the delivery of more energy to the target volume. Therefore, in planning thermal therapies it is efficacious to be able to predict the HSP response of the tumor and surrounding normal tissues, leading to optimization of the therapy delivery process and a better prognosis for the overall tissue response to hyperthermia treatment.

A single, integrated computational model has been applied to predict the coupled three-dimensional transient temperature field, pattern of tissue damage, and distribution of elicited HSP27 and HSP70 expression (100). The light absorption and temperature fields during laser heating were modeled using adaptive finite element methods, which are capable of minimizing numerical error to a specified precision. A symmetric quarter sphere solid tumor grid was generated and then implemented in a model via an adaptive finite element program. The model allows specification of the thermal distribution during laser heating, analysis of the sensitivity of the thermal behavior to manipulation of individual laser source parameters, and optimization of the parameter evaluation to develop target irradiation parameter values. The model was validated by comparison with experimental data for external laser irradiation of tumors grown on the back of SCID mice.

An accurate representation of the tissue response to thermal stress requires incorporation of the governing physical and physiological phenomena into the model. To this end, the mathematical representation of the temperature distribution in tissue during laser hyperthermia includes both the Pennes bio-heat equation term (104, 105) for the thermal effects of local blood perfusion and an expression for light energy absorption:

$$\rho c \frac{\partial T}{\partial t} = \nabla (k \nabla T) + \omega_b \text{c_b}(T - T_a) + Q(x, y, z)$$

(3)

where the rate of absorbed laser energy per unit volume distributed within the tissue is given by $Q(x, y, z) = \mu_a \phi(x, y, z)$; $\rho$, $c$, and $k$ are the density and temperature-dependent specific heat and thermal conductivity of the tissue, respectively. The blood perfusion rate, arterial blood temperature, irradiation absorption coefficient, and fluence are defined as $\omega_b$, $T_a$, $\mu_a$, and $\phi$, respectively, with property values and boundary conditions specified (100).

Experimental data for both thermal history and cell injury are applied to determine the constitutive parameter values for an Arrhenius damage model (106, 107):

$$\Omega(\tau) = \ln \left( \frac{C_0}{C_\tau} \right) = A \int_0^\tau e^{-\left(\frac{E_a}{\mathcal{R}}T(t)\right)} dt,$$

(4)

where $\Omega$ is a dimensionless injury function defined as the logarithm of the ratio of the initial concentration of healthy cells, $C_0$, to the concentration of healthy cells remaining after thermal stimulation, $C_\tau$, for a stimulation duration of $\tau$ (seconds). $A(1/s)$ is a scaling factor, $E_a$ (Jmol$^{-1}$) is the injury process activation energy, $\mathcal{R}$ (Jmol$^{-1}$K$^{-1}$)
is the universal gas constant, and $T(K)$ is the instantaneous absolute temperature of the cells during stress, which is a function of time, $t$ (seconds). Measured HSP27 and HSP70 expression kinetics data in normal and prostate cancer cells (59) were integrated into the finite element model to enable prediction of the thermally induced HSP response.

**Figure 7** illustrates a coordinated finite element model prediction of the temperature, damage, and HSP expression in the symmetric quarter section tumor for a surface laser irradiation of 2W at 810 nm wavelength and a pulse duration of 3 min (100). Significant elevations in temperature occurred at the tumor boundary.
in close proximity with the laser source, but a substantial portion of the tumor experienced minimal temperature increase. Nominal damage was induced throughout the majority of the tumor volume, with the greatest injury occurring near the laser probe and diminishing with increasing distance from the laser source. All HSP expression was normalized, with the basal level of expression represented as 1. There are three observable zones of interest with regard to HSP expression in the tumor. The topmost blue region represents a zone where significant damage was induced, causing denaturation of all proteins and rendering the cell machinery incapable of HSP expression. The middle region with HSP expression greater than 1 represents the thermal regime where temperatures caused considerable induction of HSP expression. This is the region of concern where HSP expression must be minimized to prevent tumor cell viability following treatment. The bottommost region represents a zone where temperatures were insufficient to elicit HSP expression, with expression levels at or below the basal level. Although this analysis is for a relatively simple system, it illustrates the integration of thermal engineering modeling with molecular biology mechanistic process understanding and constitutive data to produce a tool that can be adapted for therapeutic protocol planning and could be adapted to be patient specific based on MRI tumor geometry information.

### SUMMARY POINTS

1. The expression of stress proteins is enhanced in response to elevated, but nonlethal, levels of environmental stress.
2. Within bounds, the magnitude of overexpression is proportional to the applied stress.
3. The elevation of protein expression in response to environmental stress follows a time course that is dictated by the genetic machinery and that is nonlinear.
4. Multiple peaks in expression are noted subsequent to the initiation of stress.
5. Applied environmental stress can be used to manipulate the stress protein expression up to an order of magnitude above normal levels.
6. Overexpression of stress proteins is a transient event with a time constant that can last up to a few days.
7. Integrated models can be applied to simulate the coupled energy source distribution, transient temperature field, cell injury effected, and HSP overproduction for defined thermal stress protocols.
8. The integrated models can be solved by inverse methods to optimize targeted output(s).
FUTURE ISSUES

1. Models are needed to explain HSP expression kinetics based on an understanding of the governing genetic mechanisms, including signaling pathways, positive and negative feedback loops, and factors that affect their temporal manifestation.

2. Constitutive data are missing for use in models of transient expression function. The models should be able to drive the design of experiments for data acquisition.

3. Integration of patient-specific data into models will enable the planning and guidance of therapeutic protocols to achieve optimal outcomes in individual cases.

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LITERATURE CITED


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