Mathematical Modeling of Heat Shock Protein Synthesis in Response to Temperature Change

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Abstract

One of the most important questions in cell biology is how cells cope with rapid changes in their environment. The range of common molecular responses includes a dramatic change in the pattern of gene expression and the elevated synthesis of so called heat shock (or stress) proteins (HSPs). Induction of HSPs increases cell survival under stress conditions [9].

In this paper we propose a mathematical model of heat shock protein (HSP) synthesis induced by an external temperature stimulus. Our model consists of a system of nine nonlinear ordinary differential equations describing the temporal evolution of the key variables involved in the regulation of HSP synthesis. Computational simulations of our model are carried out for different external temperature stimuli. We compare our model predictions with experimental data for three different cases - one corresponding to heat shock, the second corresponding to slow heating conditions and the third corresponding to a short heat shock (lasting about 40 minutes). We also present our model predictions for heat shocks carried out up to different final temperatures and finally we present a new hypothesis concerning the molecular response to stress that explains some phenomena observed in experiments.

Keywords: Heat shock proteins; Signalling pathways; Cell stress;

1 Introduction

For many years it was believed that proteins archive their correct three-dimensional structure spontaneously once they leave the ribosome. Furthermore, proteins were assumed to maintain their native conformation until they

were degraded by specific enzymes. In the last decade this view of cellular protein folding has changed considerably. It is now known that some of the proteins require the help of other proteins to fold. These proteins are collectively called molecular chaperones because they prevent unwanted interactions between immature polypeptide chains and dissociate already existing protein aggregates [17, 21].

Heat Shock Proteins (HSPs) are encoded by genes whose expression is elevated while the cells are subjected to stress conditions, such as heat shock, oxidative stress, fever or inflammation, the presence of alcohol, inhibitors of energy metabolism and heavy metals [23].

Under stress conditions HSPs increase cell survival, protecting and disaggregating stress-labile proteins [17], as well as promoting the proteolysis of damaged proteins [22]. Most of the heat shock proteins can be referred to as "molecular chaperones" that assist the correct non-covalent assembly of other polypeptide-containing structures but which are not components of this assembled structure when they are performing their normal biological function. They bind to unfolded proteins as well as to denaturated proteins promoting their proper folding (or refolding) or targeting misfolded proteins towards degradation.

Under normal conditions HSPs have multiple "housekeeping functions" in cells. Their main function is folding new or distorted proteins into their proper shape (which is essential for their activity and function). HSPs are also involved in intracellular protein transport, activation of specific regulatory pathways, mediating activity of transcription factors, DNA replication, sorting proteins for degradation, intracellular signalling and antigen presentation.

HSPs are classified into families according to their apparent molecular mass (110, 90, 70, 65 kDa and the small HSPs). The Hsp70 family is the most conserved and the best studied class of HSPs. This family includes the stress inducible Hsp70 protein and the constitutively expressed Hsc70, which makes up 1% of the total soluble proteins. Since Hsp70s are very potent anti-apoptotic and pro-proliferation proteins [16], their induction has to be tightly controlled, since their persistent presence would adversely affect protein homeostasis and intracellular functions, leading, for instance, to inappropriate growth control. Therefore expression of HSPs is under a complex regulatory control mechanism accomplished at both the transcriptional and translational level [4]. Regulation at the transcriptional level is mediated by the interaction of a transcription factor known as heat shock factor (HSF) with heat shock elements (HSEs) which are present in promoters of the HSP genes.

Hsp70 is known to regulate protein activity involved in the cell cycle machinery (e.g. Hsp70 interacts with p53), kinases and other proteins implicated in cancer progression [23]. Indeed the appearance of cancer often arises from defects in cell apoptosis. There is now growing evidence to suggest that HSPs play an important role in many proliferative disorders of tissue such as dyspla-

sia, hyperplasia and in the first instance neoplasia (i.e. in general cancer, and specifically solid tumors). Elevated expression of Hsp70 and Hsp90 in tumour cells has been detected in several cases [6, 7]. In breast cancer, over-expression of Hsp70 has been correlated with metastasis and poor prognosis [5, 8, 20]. At least two factors contribute to this phenomenon. In normal cells the expression of the Hsp70 gene is regulated by proteins from the p53 family [2, 12]. These proteins inhibit the transcription of Hsp70 by binding to its promoter sites. In cancer cells, the mutations in p53 result in the repression of blockage of Hsp70 promoters, therefore increasing gene transcription and over-production of Hsp70 proteins. The increased expression of HSP genes is also caused by the positive regulation of oncogene signaling pathways activating the promoters of heat shock genes [23].

Despite the importance the HSPs, the literature concerning its mathematical modelling is rather limited. Previous mathematical models of heat shock proteins include those of Peper *et al.* [11], Rieger *et al.* [13, 14] and Szymańska *et al.* [18]. Apart from the latter, these papers examine the changes in Hsp70 synthesis in response to external stimuli such as heat. However, the work of Rieger *et al.* [13, 14] focusses mainly on the regulation of heat shock gene expression through the phosphorylation of heat shock factor.

In this paper we propose a model of HSP synthesis to explain some biological phenomena observed in cells exposed to stress conditions. Namely, we set out to explain the well-known experimental fact that in cells subjected to heat shock, one may observe a significant rise in HSP synthesis, whereas in cells subjected to slow heating (but up to the same final temperature as heat shock) such an increase in HSP synthesis is not visible. We also propose a possible explanation for the very high level of active transcription factor observed in cells subjected to severe heat shock (see Fig. 6 or original reference Abravaya *at al.* [1] Fig. 8).

The layout of the paper is as follows. In the next section (see Sect.2), we present the biological basis of our model i.e. we describe the process of Hsp70 regulation and synthesis. We then propose a mathematical model (see Sect.3), consisting of nine ordinary differential equations (ODEs), which describe the evolution in time of the key variables involved in the regulation of Hsp70 synthesis. We present the results of the computational simulations of our model in section 4 and in the final section 5 we provide concluding remarks including a general discussion and future directions for extensions to the current work.

2 Heat Shock Protein Synthesis Dynamics

Some proteins can have many possible conformations from which only one (or at most a few) can be identified as the native ones. Nevertheless, proteins can have many so-called unfolded conformations, including completely denatured or random conformations. Under stress conditions (such as heat shock), the cell physiology is profoundly altered. There is a lot of experimental evidence

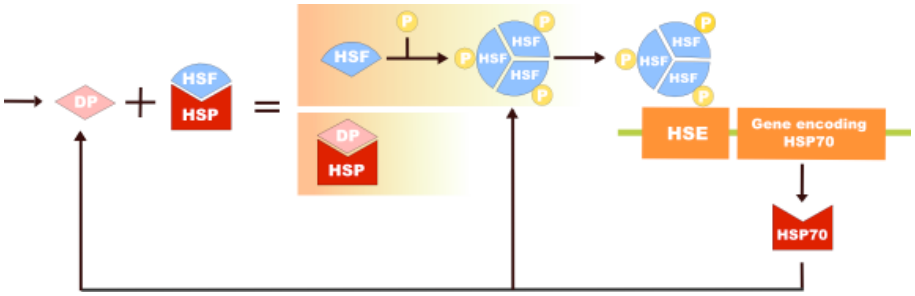


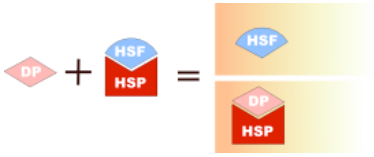
Fig. 1. A schematic diagram illustrating the key molecular events described in our model.

for the occurrence of protein denaturation within heat shocked cells [3].

A heat shock response in a cell represents an example of inducible gene expression. As we have already mentioned, in eukaryotic cells, regulation at the transcriptional level involves transcriptional activation mediated by the transcription factor HSF (heat shock factor). In an unstressed cell, HSF (present in both the cytoplasm and nucleus) is maintained in a monomeric, inactive form that has no DNA binding activity. This is due to its interaction with Hsp70. When a cell undergoes different types of environmental stress, some of the proteins denature. Hsp70 then binds to the denatured protein releasing HSF molecules that after phosphorylation assemble into trimers and accumulate within the nucleus [10]. These phosphorylated trimers constitute an active form of HSF that binds to HSE (Heat Shock Element, a specific DNA recognition sequence) and initiates the transcription. The stress dependent activation of HSF implies that HSP is negatively regulated. The increased levels of misfolded proteins induced during heat shock (as well as other stresses) sequester Hsp70, resulting in the activation of HSF. The response to heat shock is rapid. Activation and binding of HSF to HSE are detected within minutes of temperature elevation. The heat shock transcriptional response attenuates upon prolonged exposure of cells at medium heat shock (42^oC) or upon return to its physiological temperature (37^oC). This attenuation is accompanied by Hsp70-dependent conversion of the active HSF trimers to nonactive monomers and a return to their normal spatial distribution within a cell. In contrast, prolonged exposure to extreme heat shock (43^oC) results in sustained heat shock gene transcription [9].

In this paper we consider the dynamics of the synthesis of Hsp70 and its interactions with key intracellular components i.e. the kinetics of Hsp70, HSF, its substrate (mainly denatured proteins), Hsp70-HSF interacting complexes, Hsp70-substrate complexes, active HSF (trimer form), free HSE, bound HSE and finally Hsp70 mRNA. The complete process that we are modelling is illustrated in Fig. 1. We now describe each of the key components of the process in some more detail.

1. When a complex of Hsp70 and HSF meets a proper substrate (e.g. a denatured protein or a misfolded protein) the complex dissociate and Hsp70 binds to the substrate and releases the monomer of HSF, illustrated in the schematic diagram below.

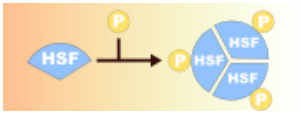


The reaction is reversible and so may be written as a rate reaction thus:

$$[\text{Hsp70} \cdot \text{HSF}] + \text{S} \underset{l_6}{\overset{k_6}{\rightleftharpoons}} [\text{Hsp70} \cdot \text{S}] + \text{HSF},$$

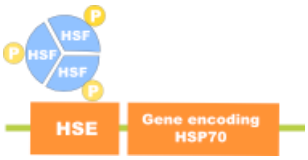
where k_6 and l_6 are the forward and backward rate constants respectively.

2. The phosphatase is assumed to be present in excess amount compared to HSF and therefore we omit the process of kinase dependent phosphorylation in our model. The monomers of HSF are nonactive, but once they are released they assemble into active trimers, with rate constant k_3:



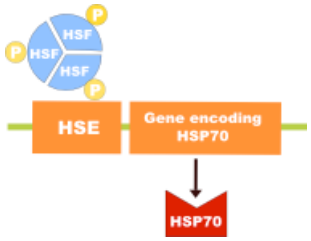
$$3 \cdot \text{HSF} \xrightarrow{k_3} [\text{HSF}_3]$$

3. Active HSF trimers bind to HSE (a specific DNA recognition sequence) and initiate transcription (a reversible reaction with rate constants k_7 and l_7). Therefore, formation of HSF trimers leads to elevated transcription, synthesis and accumulation of heat shock proteins - in particular Hsp70. We assume that HSF is present in excess compared to HSE [13, 15]. We also assume that HSE is either free or bound to $[\text{HSF}_3]$.



$$[\text{HSF}_3] + \text{HSE} \underset{l_7}{\overset{k_7}{\rightleftharpoons}} [\text{HSF}_3 \cdot \text{HSE}]$$

4. Active HSF trimers bound to HSE induce transcription and the creation of a new Hsp70 mRNA molecule (rate constant k_8):



$$[\mathrm{HSF}_3 \cdot \mathrm{HSE}] \xrightarrow{k_8} [\mathrm{mRNA}]$$

5. The dissociation of the HSF trimers occurs only in the presence of free Hsp70. When an Hsp70 molecule meets an HSF trimer, the Hsp70 molecule breaks it up and binds to one of the HSF monomers and releases two others with rate constant l_3 (this type of auto-regulation loop was described for the first time in Escherichia coli by Tilly at al. [19], and for eucaryotes similar behaviour was shown by Morimoto [9]):

$$\mathrm{Hsp70} + [\mathrm{HSF}_3] \xrightarrow{l_3} [\mathrm{Hsp70} \cdot \mathrm{HSF}] + 2 \cdot \mathrm{HSF}$$

6. Free (i.e. newly synthesized) Hsp70 forms a complex with denatured proteins in a reversible reaction with rate constants k_1 and l_1 as follows:

$$\mathrm{Hsp70} + \mathrm{S} \underset{l_1}{\overset{k_1}{\rightleftharpoons}} [\mathrm{Hsp70} \cdot \mathrm{S}].$$

7. Similarly free Hsp70 also interacts with free HSF in another reversible reaction with rate constants k_2 and l_2:

$$\mathrm{Hsp70} + \mathrm{HSF} \underset{l_2}{\overset{k_2}{\rightleftharpoons}} [\mathrm{Hsp70} \cdot \mathrm{HSF}].$$

8. Although Hsp70 is a very stable protein, we assume that some degradation occurs with rate constant l_{10}.

$$\mathrm{Hsp70} \xrightarrow{l_{10}} \text{degraded Hsp70}$$

9. Finally, we assume substrate refolding processes occur with rate k_{10}, i.e.:

$$[\mathrm{Hsp70} \cdot \mathrm{S}] \xrightarrow{k_{10}} \mathrm{Hsp70}.$$

3 The Mathematical Model

We now apply the law of mass action to the previously described biochemical reactions to obtain our system of ordinary differential equations (ODEs). The variables of the mathematical model are identified by their concentration inside a cell without taking into account their (three dimensional) spatial distribution.

The first equation describes the dynamics of free Hsp70. The binding of Hsp70 to the substrate S is described by the term -k_2Hsp70·S, while the dissociation of this combination is described by the term: l_2[Hsp70·S]. Analogously, the binding of Hsp70 and HSF is described by the term -k_1Hsp70·HSF and the dissociation of this combination is described by: l_1[Hsp70·HSF]. The loss of the free Hsp70 occurring due to the dissociation of HSF trimers and a subsequent binding of one of the HSF monomers to the Hsp70 is described by the term -l_3Hsp70·HSF_3. The term k_4[mRNA] models the synthesis of new Hsp70 (for simplicity we assume that it is proportional to the concentration of Hsp70 mRNA). The terms k_{10}[Hsp70·S] and -l_{10}Hsp70 model substrate refolding and protein degradation, respectively. Thus, finally we obtain the following equation:

$$\frac{\mathrm{dHsp70}}{\mathrm{dt}} = \underbrace{-\mathrm{k_1Hsp70 \cdot HSF}}_{\substack{\text{binding of}\\ \text{Hsp70 to HSF}}} \underbrace{+\mathrm{l_1[Hsp70 \cdot HSF]}}_{\substack{\text{dissociation}\\ \text{of the Hsp70-HSF}\\ \text{complexes}}} \underbrace{-\mathrm{k_2Hsp70 \cdot S}}_{\substack{\text{binding of Hsp70}\\ \text{to the}\\ \text{substrate}}} \underbrace{+\mathrm{l_2[Hsp70 \cdot S]}}_{\substack{\text{dissociation}\\ \text{of the Hsp70-substrate}\\ \text{complexes}}}$$
$$\underbrace{-\mathrm{l_3Hsp70 \cdot [HSF_3]}}_{\substack{\text{dissociation}\\ \text{of the HSF trimers}}} \underbrace{+\mathrm{k_4[mRNA]}}_{\text{synthesis of new Hsp70}} \underbrace{+\mathrm{k_{10}[Hsp70 \cdot S]}}_{\text{substrate refolding}} \underbrace{-\mathrm{l_{10}Hsp70}}_{\text{degradation of Hsp70}} \tag{1}$$

HSF trimers originate from HSF monomers in two steps. In the first step, two monomers form one dimer. In the second step, one monomer binds to one HSF dimer forming one trimer [11]. For simplicity we do not consider the process of dimer formation in our model. Therefore, the combination of HSF monomers into trimers is described by the term -$3k_3HSF^3$, while the breakup of these trimers is described by $2l_3$Hsp70·[HSF_3]. In a similar manner to equation (1) the term -k_1Hsp70·HSF + l_1[Hsp70 · HSF] model for the interaction between HSF and Hsp70. The term k_6[Hsp70·HSF]·S describes the formation of Hsp70-Substrate complexes resulting in the release of an HSF molecule, while the term -l_6[Hsp70·S]·HSF describes the reverse reaction, i.e. the dissociation of an Hsp70-Substrate molecule and the formation of Hsp70-HSF complexes. The equation describing heat shock factor dynamics therefore reads as:

$$\frac{\mathrm{dHSF}}{\mathrm{dt}} = \underbrace{-\mathrm{k_1 Hsp70 \cdot HSF}}_{\substack{\text{binding}\\ \text{Hsp70 to HSF}}} \underbrace{+\mathrm{l_1[Hsp70 \cdot HSF]}}_{\substack{\text{dissociation}\\ \text{of the Hsp70-HSF}\\ \text{complexes}}} \underbrace{-3\mathrm{k_3 HSF^3}}_{\substack{\text{formation of the}\\ \text{HSF trimers}}} \underbrace{+2\mathrm{l_3 Hsp70 \cdot [HSF_3]}}_{\substack{\text{dissociation of}\\ \text{the HSF trimers}}}$$
$$\underbrace{+\mathrm{k_6[Hsp70 \cdot HSF] \cdot S}}_{\substack{\text{formation of Hsp70-}\\ \text{-substrate complexes and}\\ \text{release of HSF monomer}}} \underbrace{-\mathrm{l_6[Hsp70 \cdot S] \cdot HSF}}_{\substack{\text{dissociation of Hsp70-substrate}\\ \text{complexes and formation}\\ \text{of Hsp70-HSF complexes}}} \tag{2}$$

The third equation describes the dynamics of free substrate. Once again we have the same terms as in equations (1) and (2) which are responsible for the interactions between Hsp70, HSF and the substrate. In addition to internal reactions, the concentration of the substrate (i.e. misfolded proteins) within a cell depends on particular (external) stress factor (in our case, the temperature). We describe the inflow of substrate by a function F(T), where T denotes the temperature. Taken together the complete equation describing the dynamics of the substrate is:

$$\frac{\mathrm{dS}}{\mathrm{dt}} = \underbrace{\mathrm{l_2[Hsp70 \cdot S]}}_{\substack{\text{dissociation of the}\\ \text{Hsp70-substrate}\\ \text{complexes}}} \underbrace{-\mathrm{k_2 Hsp70 \cdot S}}_{\substack{\text{binding of}\\ \text{Hsp70 to the}\\ \text{substrate}}} \underbrace{-\mathrm{k_6[Hsp70 \cdot HSF] \cdot S}}_{\substack{\text{formation of Hsp70-}\\ \text{-substrate complexes and}\\ \text{release of HSF monomer}}}$$
$$\underbrace{+\mathrm{l_6[Hsp70 \cdot S] \cdot HSF}}_{\substack{\text{dissociation of Hsp70-substrate}\\ \text{complexes and formation}\\ \text{of Hsp70-HSF complexes}}} \underbrace{+\mathrm{F(T)}}_{\substack{\text{increase in the}\\ \text{amount of denatured}\\ \text{proteins due to stress}}} \tag{3}$$

We now describe the dynamics of the Hsp70-HSF complexes, Hsp70-substrate complexes and HSF trimers. We assume that the rate of change of each of these complexes is determined by the law of mass action. Therefore, in the equation for Hsp70-HSF complexes as previously described, we have terms responsible for: Hsp70-HSF complexes formation and dissociation, the substrate dependent dissociation of an Hsp70-HSF molecule (together with its reverse reaction) and Hsp70 dependent dissociation of HSF trimers:

$$\frac{\mathrm{d[Hsp70 \cdot HSF]}}{\mathrm{dt}} = \underbrace{\mathrm{k_1 Hsp70 \cdot HSF}}_{\substack{\text{binding of}\\ \text{Hsp70 to HSF}}} \underbrace{-\mathrm{l_1[Hsp70 \cdot HSF]}}_{\substack{\text{dissociation}\\ \text{of the Hsp70-HSF}\\ \text{complexes}}} \underbrace{-\mathrm{k_6[Hsp70 \cdot HSF] \cdot S}}_{\substack{\text{formation of Hsp70-}\\ \text{-substrate complexes and}\\ \text{release of HSF monomer}}}$$
$$\underbrace{+\mathrm{l_6[Hsp70 \cdot S] \cdot HSF}}_{\substack{\text{dissociation of Hsp70-substrate}\\ \text{complexes and formation}\\ \text{of Hsp70-HSF complexes}}} \underbrace{+\mathrm{l_3 Hsp70 \cdot [HSF_3]}}_{\substack{\text{dissociation}\\ \text{of the HSF trimers}}} \tag{4}$$

The dynamics of the Hsp70-substrate complexes depends on: the formation of Hsp70-substrate complexes (binding Hsp70 to the substrate) and its dissociation, the substrate dependent dissociation of an Hsp70-HSF molecule (together with its reverse reaction) and substrate refolding:

$$\frac{\mathrm{d}[\mathrm{Hsp70}\cdot\mathrm{S}]}{\mathrm{dt}} = \underbrace{\mathrm{k}_2\mathrm{Hsp70}\cdot\mathrm{S}}_{\substack{\text{binding of}\\ \text{Hsp70 to the}\\ \text{substrate}}} \underbrace{-\mathrm{l}_2[\mathrm{Hsp70}\cdot\mathrm{S}]}_{\substack{\text{dissociation}\\ \text{of the Hsp70-HSF}\\ \text{complexes}}} \underbrace{+\mathrm{k}_6[\mathrm{Hsp70}\cdot\mathrm{HSF}]\cdot\mathrm{S}}_{\substack{\text{formation of Hsp70-}\\ \text{-substrate complexes and}\\ \text{release of HSF monomer}}} \underbrace{-\mathrm{l}_6[\mathrm{Hsp70}\cdot\mathrm{S}]\cdot\mathrm{HSF}}_{\substack{\text{dissociation of Hsp70-substrate}\\ \text{complexes and formation}\\ \text{of Hsp70-HSF complexes}}} \underbrace{-\mathrm{k}_{10}[\mathrm{Hsp70}\cdot\mathrm{S}].}_{\text{substrate refolding}} \tag{5}$$

In the equation governing the dynamics of the HSF trimer form we have (again previously described) terms responsible for: HSF trimer formation and its Hsp70-dependent dissociation, the binding of active trimers to HSE and dissociation of HSF trimers from HSE:

$$\frac{\mathrm{d}[\mathrm{HSF}_3]}{\mathrm{dt}} = \underbrace{\mathrm{k}_3\mathrm{HSF}^3}_{\substack{\text{formation of}\\ \text{HSF trimers}}} \underbrace{-\mathrm{l}_3\mathrm{Hsp70}\cdot[\mathrm{HSF}_3]}_{\substack{\text{dissociation of}\\ \text{the HSF trimers}}} \underbrace{-\mathrm{k}_7[\mathrm{HSF}_3]\cdot\mathrm{HSE}}_{\substack{\text{binding of}\\ \text{HSF trimers to HSE}}} \underbrace{+\mathrm{l}_7[\mathrm{HSF}_3\cdot\mathrm{HSE}].}_{\substack{\text{dissociation of the}\\ \text{HSF trimers from HSE}}} \tag{6}$$

The equation describing the dynamics of free HSE consists of the terms describing the bindings of HSF trimers to HSE and dissociation of those trimers from HSE:

$$\frac{\mathrm{dHSE}}{\mathrm{dt}} = \underbrace{-\mathrm{k}_7[\mathrm{HSF}_3]\cdot\mathrm{HSE}}_{\substack{\text{binding of}\\ \text{HSF trimers to HSE}}} \underbrace{+\mathrm{l}_7[\mathrm{HSF}_3\cdot\mathrm{HSE}]}_{\substack{\text{dissociation of the}\\ \text{HSF trimers from HSE}}}, \tag{7}$$

while the equation describing the dynamics of bound HSE is given by the same terms but with the opposite signs:

$$\frac{\mathrm{d}[\mathrm{HSF}_3\cdot\mathrm{HSE}]}{\mathrm{dt}} = \underbrace{\mathrm{k}_7[\mathrm{HSF}_3]\cdot\mathrm{HSE}}_{\substack{\text{binding of}\\ \text{HSF trimers to HSE}}} \underbrace{-\mathrm{l}_7[\mathrm{HSF}_3\cdot\mathrm{HSE}].}_{\substack{\text{dissociation of the}\\ \text{HSF trimers from HSE}}} \tag{8}$$

The dynamics of Hsp70 mRNA is governed of course simply by the transcription and translation of Hsp70:

$$\frac{\mathrm{d}[\mathrm{mRNA}]}{\mathrm{dt}} = \underbrace{\mathrm{k}_8[\mathrm{HSF}_3\cdot\mathrm{HSE}]}_{\substack{\text{transcription}\\ \text{of Hsp70 mRNA}}} \underbrace{-\mathrm{k}_4[\mathrm{mRNA}].}_{\substack{\text{translation of mRNA into}\\ \text{Hsp70 protein}}} \tag{9}$$

4 Numerical Analysis and Computational Simulation

In this section we present computational results obtained from numerical simulations of system (1-9). All simulations were carried out using the MATLAB ODE solver ode45 which uses a 4th-order Runge-Kutta method. For all the simulations we assume that in normal conditions a cell remains in a state of homeostasis and therefore we start all simulations from the steady state.

4.1 Modelling the Impact of Different Temperature Regimes

In order to carry out our numerical simulations, we must define our function F(T) which describes the substrate response to our external stimulus i.e. the temperature T. The temperature-dependent process of protein denaturation has been investigated previously and the fraction of protein denaturation (V_{den}) as a function of temperature, in the range of 37-45^{o}C, has been approximated by Peper *et al.* [11]. The precise functional form can be written as:

$$\mathrm{V_{den}} = \left(1 - \frac{0.4}{e^{T-37}}\right) \cdot 0.03 \cdot 1.4^{T-37} \text{ for } T \in [37, 45], \tag{10}$$

where T is the temperature in C^o [11]. We assume that F(T) function is proportional to the value of $\mathrm{V_{den}}$.

Moreover, there is experimental evidence that the way the temperature is changed has an essential influence on the dynamics of HSP synthesis. A significant increase of Hsp70 synthesis is observed only in the case of a fast increase in the temperature. When the temperature is increased slowly, no increase in synthesis is observed (M. Zylicz not published results).

Under normal (i.e. non-stressed) conditions the majority of Hsp70 is bound either to the substrate or to HSF. We start our simulations from the steady state of an inactive system i.e. the system with constant function F(T(t)) equal to the rate of inflow of spontaneously misfolded proteins (the substrates for Hsp70 during non-stressed conditions).

In order to test our model we study the dynamics of the system triggered by exposure to three different types of temperature regime. In two first cases the overall temperature change is from 37^{o}C to 42^{o}C, however the manner of administering the temperature is different. In the third case, the temperature is initially increased up to 42^{o}C but after 40 minutes is again switched to 37^{o}C. As a first step in testing our model, we compare these three different ways of exposing the cells to heat as follows:

- a fast temperature increase described by a step function with a large jump corresponding to a heat shock turned on at time $t = 10$ (see Fig. 2 top),
- a gradual temperature increase described by a monotonic increasing function corresponding to slow heating (see Fig. 2 middle),
- a fast temperature increase described by a step function with a large jump corresponding to a heat shock turned on at time $t = 10$ followed by fast decrease of temperature at time $t = 50$ (see Fig. 2 bottom).

As the denatured proteins act as triggers for the Hsp70 synthesis, we need to know what their dynamics are. Since the temperature (in our model) depends on time and, as we have already mentioned, the process of protein denaturation is also temperature-dependent, it follows that also the amount of the denatured proteins is time-dependent. However, the precise way in which the temperature is administered to the cells is very important.

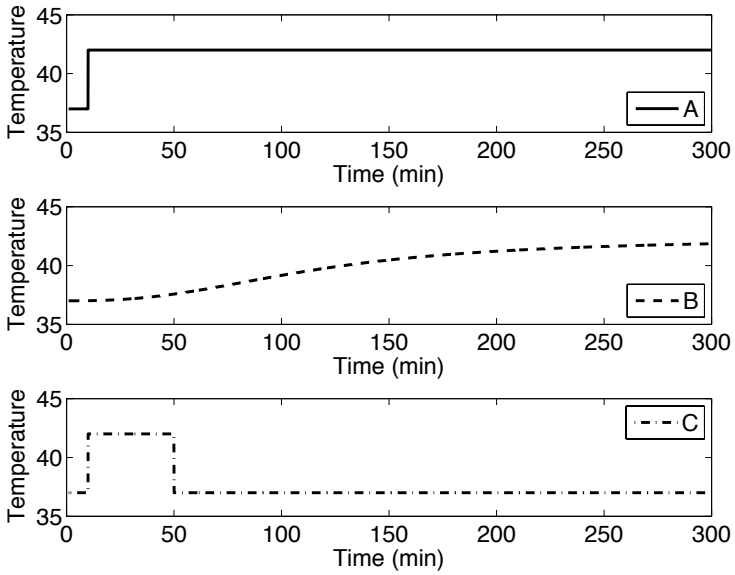


Fig. 2. Plots showing the three different types of temperature regimes that cells are exposed to considered in our work. The top plot (A, solid line) depicts the time course of the temperature change during heat shock, when temperature varies quickly from 37^oC to 42^oC, the middle plot (B, dashed line) depicts the time course of the temperature change during slow heating for the same range of temperatures, whereas the bottom plot (C, dash-dot line) corresponds to the case when temperature varies quickly from 37^oC to 42^oC and after 40 minutes is returned back to 37^oC.

Assuming that the fraction of the denatured proteins is approximated by formula (10) we may obtain the courses of the fraction of denatured proteins when a cell is subjected to heat shock, when a cell is subjected to slow heating and when a cell is subjected to short heat shock by superposition of appropriated functions.

4.2 Parameter values

In the absence of reliable empirical data, we chose our parameters based on the expected reaction times and reaction equilibriums although our simulations show that the qualitative behaviour of the solutions is similar for a large range of parameters. Therefore, we present here results obtained for the particular choice of parameters (see Tab. 1).

Parameter	Description	Value
k_1	rate of Hsp70 Substrate association	0.42
l_1	dissociation rate of Hsp70 Substrate complexes	0.005
k_2	rate of Hsp70 HSF association	0.42
l_2	dissociation rate of Hsp70 HSF complexes	0.005
k_3	trimers formation rate (HSF activation rate)	0.023
l_3	trimers dissociation rate (HSF inactivation rate)	0.00575
k_4	Hsp70 translation rate	0.035
k_6	rate of HSF dissociation and formation of Hsp70 Substrate complexes	0.023
l_6	rate of Substrate dissociation and formation of Hsp70 HSF compexes	0.00036
k_{10}	refolding rate	0.014
l_{10}	rate constant of Hsp70 degradation	0.013
k_8	translation rate	0.035
k_7	rate of active HSF HSE association	0.035
l_7	rate of active HSF HSE dissociation	0.035

Table 1: Estimated values of the model parameters. We note that the parameters have dimensions $(\text{Time})^{-1}$ $(\text{M})^{-n+1}$, where M is the (molar) concentration and n is the number of molecules formed in a complex.

4.3 Computational Simulation Results

Fig. 3 shows the dynamics of Hsp70 mRNA resulting from a computational simulation of equations (1-9) where the temperature varies from 37^oC up to 42^oC in case of heat shock (solid line plot, corresponding to the temperature profile shown on Fig. 2 (A)), slow heating (dashed line plot, corresponding to the temperature profile shown on Fig. 2 (B)) and short heat shock (dash-dot line corresponding to the temperature profile shown on Fig. 2 (C)). In the case of heat shock we obtain the well-known profile of an initial rapid increase in Hsp70 synthesis (measured in mRNA synthesis) followed by a slower attenuation and finally a return to its physiological level. In the case of slow heating, concerning the Hsp70 synthesis initially we see no increase, followed by a slow increase and then finally we observe a slow attenuation. In the case of short heat shock we obtain a rapid increase in mRNA Hsp70 synthesis (similar to those in the first case), followed by a return to its physiological level, which is faster then in the first case. These results are in good qualitative agreement with the data obtained experimentally by Abravaya *et al.* [1] who measured (i) the transcription rate of Hsp70 mRNA of cells that were shocked at 42^oC for 250 minutes and (ii) the transcription rate of Hsp70 mRNA of cells that were shocked at 42^oC for 40 minutes and then were shifted back to 37^oC (see plots shown in Fig. 4).

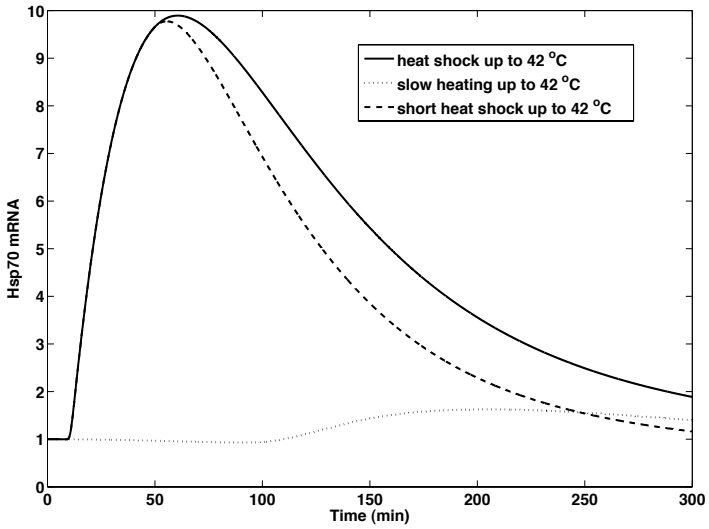


Fig. 3. Three different Hsp70 mRNA synthesis profiles obtained for the three different temperature inputs considered in our work. The solid-line plot corresponds to heat shock (see Fig. 2a); the dotted-line plot corresponds to slow heating (see Fig. 2b) and the dashed-line plot corresponds to the case where a short heat shock is applied (see Fig. 2c).

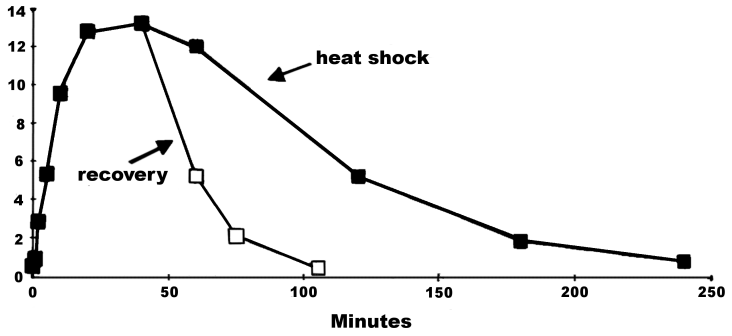


Fig. 4. Transcription rate of Hsp70 mRNA if the cells were shocked at 42^{o}C for 250 minutes (solid line) or were shifted back to 37^{o}C at the peak of heat-shock response (dashed line). Figure reproduced from Abravaya at al. [1] courtesy of the publisher. See original reference for materials and methods.

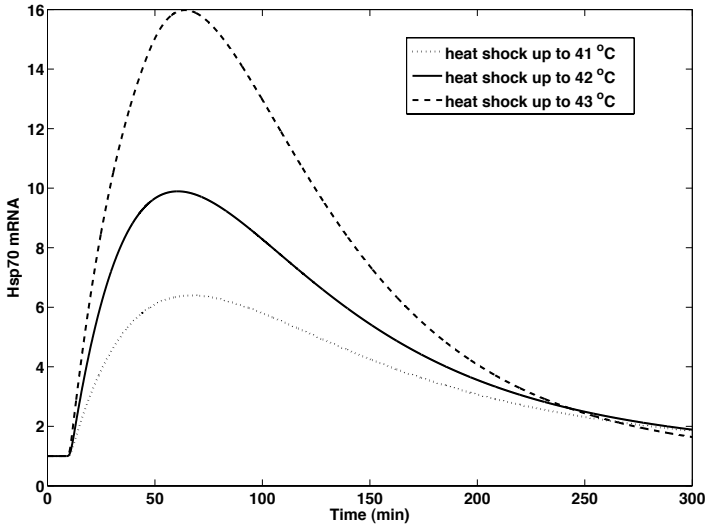


Fig. 5. Different Hsp70 synthesis profiles (Hsp70 mRNA) obtained for the temperature signals corresponding to heat shocks from 37^oC up to 41^oC (dotted line), 42^oC (solid line) and 43^oC (dashed line).

Fig. 5 shows the dynamics of Hsp70 mRNA resulting from computational simulations of equations (1-9) where the temperature varies from 37^oC up to 41^oC (dotted line), 42^oC (solid line) and 43^oC (dashed line). From these simulations we obtain the well-known profile of an initial rapid increase in Hsp70 synthesis (measured in mRNA synthesis) followed by a slower attenuation and finally a return to a value close to its physiological level. Once again these results are in very good qualitative agreement with those of Abravaya *et al.* [1] shown in Fig. 6 (or original reference page 2126, Fig. 8 A).

In their paper Abravaya at al. [1] present the results of experiments showing a persistent high level of Heat Shock Factor after a heat shock of 43^oC (see Fig. 6 or original reference page 2126, Fig. 8 A). This result was also presented by Rieger at al. [13] with a comment that at 43^oC the model by prediction of the attenuated phase of the heat shock response is not in perfect agreement with the experimental observations (See Fig. 3 therein). This phenomenon may be explained as follows: during normal conditions inactive HSF is bound not only to Hsp70 but also to Hsc70 or Hsp90. During acute hyperthermia (i.e. a heat shock 43^oC) a large amount of denatured proteins appears and therefore all HSF monomers are freed. As the reactions of transcription and translation are relatively slow in comparison to the reaction of freeing HSF monomers, active HSF trimers remain until new Hsp70 is synthesised. Fig. 7 shows the dynamics of HSF trimers resulting from the computational simula-

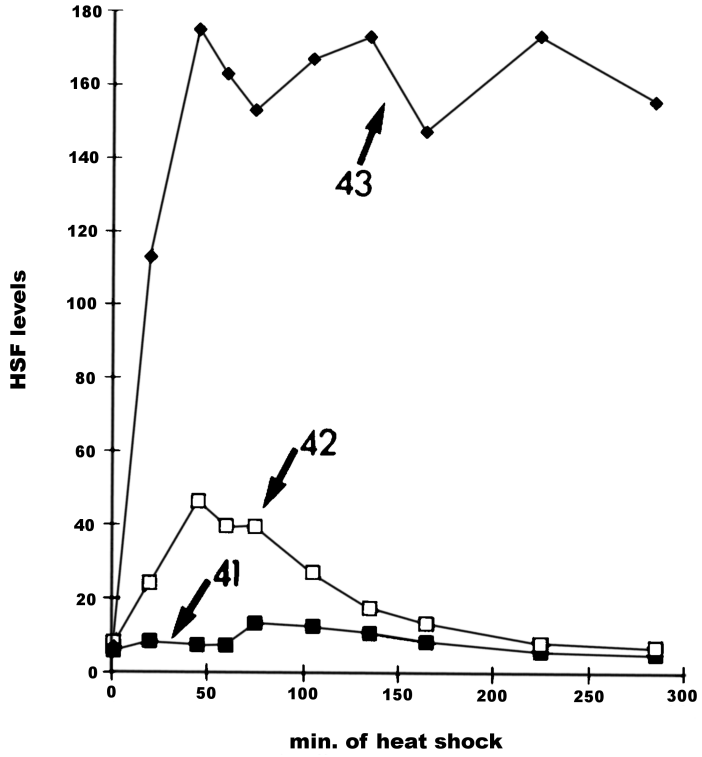


Fig. 6. Effect of heat shock and growth temperatures on the activation and deactivation of HSF. Levels of activated HSF at the indicated times of heat shock were measured by gel - shift assay. The dried gels were scanned and quantified by using a Molecular Dynamics 400A Phosphorimager. Values are machine counts, plotted on an arbitrary scale. Cells were grown for 4 days at 37^{o}C prior to heat shock at 41^{o}C, 42^{o}C and 43^{o}C. Figure reproduced from Abravaya at al. [1] courtesy of the publisher. See original reference for materials and methods.

tion corresponding to experimental heat shocks from 37^{o}C up to 41^{o}C (dotted line), 42^{o}C (solid line) and 43^{o}C (dashed line). In our simulations we can see the aforementioned plateau. However active HSF deactivates more quickly. This might be caused by the fact that at higher temperatures there are also processes such as the formation of protein aggregates and dissipative effects which we do not consider here in this model.

Another phenomenon we may see in our model predictions is the increased level of heat shock proteins subsequent to the heat shock. Therefore more

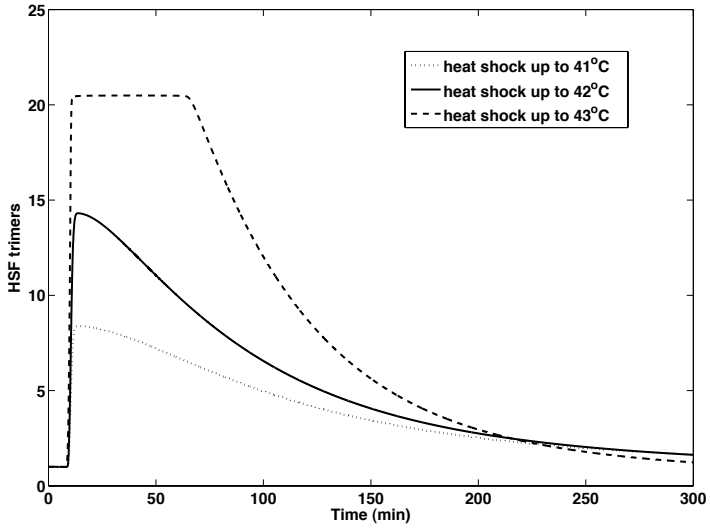


Fig. 7. Different active HSF (HSF trimers) profiles obtained for the temperature signals corresponding to heat shocks from 37^oC up to 41^oC (dot line), 42^oC (solid line) and 43^oC (dashed line).

proteins remain inside the cell and since these proteins are very stable they act in an anti-apoptotic manner for a longer period of time and therefore they are responsible for the cell's adaptation for the next stress.

We carried out a basic parameter sensitivity analysis in order to determine which parameters (if any) the model was sensitive to. In order to carry this out, we varied each parameter separately by changing it by a factor of 10 and then comparing these results with our baseline case. In all cases there was no significant quantitative difference between the two sets of results except for the three parameters k_4, k_8 and k_7. For the parameters k_4 and k_8 this is unsurprising since these parameters control, respectively, the transcription and translation rates of Hsp70 mRNA. However, in the case of the parameter k_7 we noticed a decrease in Hsp70 synthesis by around 25% from the baseline case (see Fig. 8). This parameter controls the rate of binding of HSF trimers to HSE and as such plays an important role in the dynamics of the system. These sensitivity results give an indication that disrupting the binding of HSF trimers to HSE is perhaps an effective way of targeting cancer cells and thereby reducing their resistance to apoptosis.

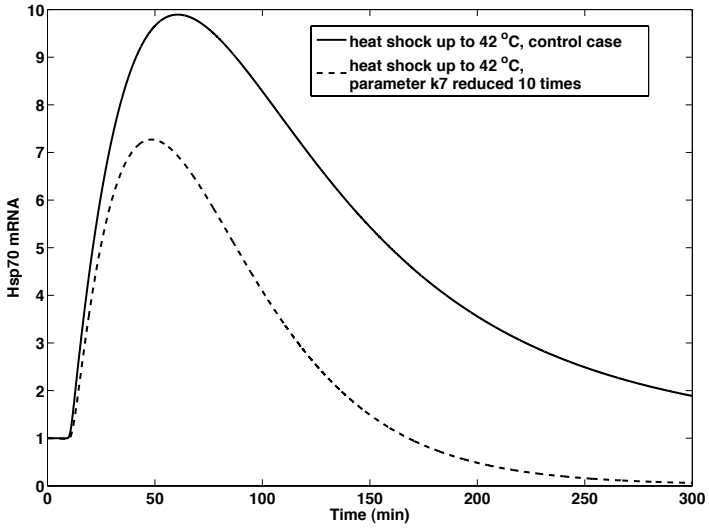


Fig. 8. Different Hsp70 synthesis profiles (Hsp70 mRNA) obtained for the temperature signal corresponding to heat shock from 37^{o}C up to 42^{o}C control case (solid line) and with the parameter k_7 reduced 10 times with all other parameters being the same as for control case (dashed line).

5 Discussion and Conclusions

In this paper we have formulated a mathematical model of heat shock protein synthesis induced by an external temperature stimulus. We focussed attention on the dynamics of the synthesis of one of the most important heat shock proteins, Hsp70, and its interactions with key intracellular components i.e. Heat Shock Factor (HSF), its substrate (mainly denatured proteins), Heat Shock Element (HSE, a specific DNA recognition sequence) and various complexes that are formed in the kinetic reactions. Using the Law of Mass Action, we converted the biochemical rate reactions into a system of 9 ordinary differential equations.

It is known from experimental data that the process of protein denaturation is temperature-dependent. We used our mathematical model to predict the outcome of Hsp70 synthesis when a cell was exposed to three different types of temperature regime. The first externally administered temperature regime we considered was a heat-shock, where an initial temperature of 37^{o}C was raised sharply to 41^{o}C, 42^{o}C and 43^{o}C. The second regime we considered represented a slow heating over a much longer period of time, but with the overall change in temperature the same from 37^{o}C to 42^{o}C. The third regime we considered here was a short heat shock where an initial temperature of

37^oC was raised sharply to 42^oC, maintained for about 40 minutes and then the temperature was decreased back to 37^oC.

The computational results of the model showed that for the administration of the heat-shock, a significant rise in Hsp70 synthesis was initially observed followed by a slow decrease back to its original steady state value. In the case of slow-heating, only a small rise in the synthesis of Hsp70 was observed i.e. almost no change in the original level. In case of a short heat shock the same significant rise in Hsp70 synthesis was initially observed as in the case of heat shock, but the following decrease in Hsp70 synthesis was faster.

Overall, our computational simulation results demonstrate that cells can adapt to and survive the application of a moderate heat shock. This result has implications for cancer treatment (i.e. chemotherapy) since many cancer therapies are based on the idea of putting some kind stress on cancer cells. The fact that Heat Shock Proteins (HSPs) prevent apoptosis induced by different modalities of cancer treatment explains how these proteins could limit the efficacy of cancer therapy and contribute to an impaired patient treatment. After a heat shock more heat shock proteins remain inside the cell and since these proteins are very stable they can act in an anti-apoptotic manner for a longer period of time and therefore prevent the efficacy of any subsequent anti-cancer treatments e.g. chemotherapy drugs and readiotherapy treatment.

In light of our model's predictions and knowing that: (i) some cancer cells are more sensitive to hyperthermia then others, and (ii) elevated expression of Hsp70 and Hsp90 in tumour cells has been detected in several cases [6, 7], we may question whether this hyperthermia sensitivity and elevated expression of heat shock proteins are not correlated i.e. whether cancer cells with over-expression of Hsp70 and Hsp90 are more resistant to hyperthermia than cancer cells without over-expression of Hsp70 and Hsp90. This hypothesis may be experimentally tested.

Future development of this work will be to consider a spatio-temporal model of heat-shock treatment of cancer cell colonies and tissue.

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