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Induced Metastable Memory in Heat Shock Response

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Abstract. We studied the dynamics of the Heat Shock Response (HSR) mechanism, and the persistence of a injury-protected state in the cell following the shocks, known as *thermotolerance*. A series of double shock experiments were performed on Chinese Hamster Ovary (CHO) cells, tracking the dynamics of some components of HSR pathway (the Hsp70 protein level and Hsp70 mRNA transcription rate). The main features of HSR dynamics were well reproduced by a simplified model of the chemical reaction pathways governing the HSR. In particular, the thermotolerance phenomenon could be well characterized by introducing a shock-dependent *switch* in mRNA halflife, that can be interpreted as a sort of primitive *memory* at the mRNA level.

Key words: heat shock response, habituation, thermotolerance, chemical reactions & chemical kinetics, analytical & numerical analysis, proteins-DNA

Introduction

The Heat Shock Response mechanism has been known for a long time [1, 2], particularly from a biological point of view [3, 4]. HSR mechanism can be seen as a sort of primitive defence system, since it protects the cells (from bacteria to humans) from external stress stimuli such as heat injury, toxic substances, ionizing and non-ionizing radiations [5]. This mechanism has been envisaged as a possible "cellular thermometer", or like a sensor of proper protein three-dimensional structure [6].

Expression of heat shock proteins protects cells from subsequent stresses inducing a protected state named "thermotolerance" that could influence the HSP trascription rate and mRNA stability [7].

The aim of this study was to investigate the HSR mechanism integrating biological results and a quantitative kinetic approach, characterizing the thermotolerance phenomenon in mammalian cells as an ancestral short-term memory in a

wide sense. In order to understand the key features of the mechanism, double heat shock experiments were performed on CHO cells. The kinetics of the total level of Hsp70, a member of the Heat Shock Protein families (HSPs) that can be induced by environmental stimuli [4], and the rate of transcription of its mRNA (Hsp70 mRNA) were monitored during the experiments by observations at different times. A model of the network of biochemical reactions that control Hsp70 level in eukaryotic cells was numerically implemented to reproduce the response of the cells to shocks.

1. Experimental Setup

1.1. CELL CULTURE

CHO cells were grown in a humidified 37 °C incubator with 5% CO_2 atmosphere in tissue culture flasks or 6-well plates (T25, Becton-Dickinson USA) in Ham's F12 medium supplemented with 10% heat-inactivated fetal bovine serum, and appropriate antibiotics and antimycotics (100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin). The cells were maintained in a logarithmic growth phase by routine passages every 2–3 days. All cell culture supplies were obtained from Life Technologies, Inc., USA. Confluent cells were trypsinized (1%), pelleted and resuspended in Ham's F12 containing 10% dimethyl sulfoxide (DMSO) at a 1:8 split ratio. The cell suspension was then aliquoted in cryogenic vials, and frozen by stepwise transferring of the vials up to –190 °C. For thawing, a vial of frozen cells was removed from the liquid nitrogen, and immersed in a 37 °C water bath. Cells were pelleted by centrifugation, resuspended in Ham's F12 complete medium culture, immediately transferred in a T-25 flask and incubated at 37 °C with 5% CO_2 atmosphere.

1.2. Hsp70 mRNA TRANSCRIPTION RATE

Cells were transfected with a plasmid containing Human Hsp70 promoter driving β-gal gene (p1730R, StressGen, Victoria, BC, Canada).

To assess HSP promoter activity, heat shock treatments were performed on cells at 70–80% of confluence. Cells were plated in a 6 multiwell plate 24 h before the transfection; 12 h after transfection the multiwell plates were sealed with parafilm and floated in a large circulating water-bath set up at 42 °C. After treatment, cells were washed with pre-warmed (37 °C) fresh medium and were allowed to recover at 37 °C for different time periods (0, 3, 5, 7 h).

The detection of HSP promoter activity was determined by measuring the enzimatic activity of the reporter gene (β-gal). Transfected cells were harvested after different periods of recovery using a lysis solution and total protein content was determined by Protein Assay Kit (Sigma Aldrich Inc. USA). The β-gal activity was assessed in 50 μg protein samples by colorimetric enzyme immunoassay (Roche

Diagnostic SpA, Milano Italy) according to the manufacturer's procedure. The results were expressed as OD/μg of proteins.

1.3. Hsp70 PROTEIN LEVEL

To assess Hsp70 production, heat shock treatments were performed on cells at 50–60% of confluence. Cells were plated in culture flasks. For the shock, flasks were floated in water-bath setup at 42 °C. After the treatment, cells were washed with prewarmed (37 °C) fresh medium and were allowed to recover at 37 °C for 24, 48, 72 h.

To perform double thermal shock experiments, cells were allowed to recover after the first shock for 24 or 72 h. Flasks were then sealed and floated again for 1 h in a 42 °C water bath. Subsequent recovery was 0, 7, 24, 48 and 72 h.

To determine the Hsp70 content, cells were lysed in Laemli buffer without β-mercaptoethanol and bromophenol blue, and the protein concentration of each sample was measured using a Protein Assay Kit before the addition of denaturing agents. Aliquots of 10 μg of proteins were boiled for 5 min and separated on a 12%-SDS-polyacrylamide gel in a minigel apparatus (BioRad Laboratories Inc., USA). Proteins were electrophoretically transferred onto a nitrocellulose membrane (Amersham Bioscences, USA). Blots were washed two times in PBS and soaked in Ponceau Red. The efficiency of protein transfer was checked by the parallel Coomassie Blue staining of the gel. Non-specific protein binding sites on the nitrocellulose membrane were blocked with 3% milk powder in TBS-T20 (20 mM Tris pH 7.5, 500 mM NaCl 0.1% Tween-20) for 3 h at room temperature. Membranes were then probed with a 1:1000 dilution of the monoclonal antibody directed against the heat–inducible form of Hsp70 (Mouse Anti-Hsp70 monoclonal antibody SPA 810 StressGen – Victoria, Canada). After washing with several changes of PBS-T20 (PBS-0,1% Tween20) membranes were incubated with a 1:10.000 dilution of goat biotin conjugate anti-mouse IgG (SAB-103, StressGen) and then with 1:1.000 dilution of anti-biotin Horseradish Peroxidase (HRP)-linked antibody. The chemiluminescent signals were developed using the Enhanced Detection System (Pierce Technology Corp., USA) according to the manufacturer's protocol. The intensity of signals were determined by Fluor-S MultImager (BioRad Laboratories Inc., USA). The results were expressed as OD/mm^2. To confirm the correct amount of protein loaded in each well, membranes were stripped and reprobed with a 1:1000 dilution of the monoclonal antibody directed against β-tubulin (Mouse anti-β-tubulin, Santa Cruz Biotechnology Inc. USA) .

2. Model of Heat Shock Response

The HSR mechanism acts in defence of the cell from external agents that can cause protein degradation or unfolding, by controlling the production of a highly

specialized family of proteins, the HSPs, that prevent the damaged proteins from clustering, help their refolding or destine them to proteolysis [15]. Thermotolerance is a state of cell characterized by a strong resistance to heat shocks, that increases cell survival probability. This state follows a non lethal heat stimulation (*conditioning shock*), and it is characterized by a high concentration of HSPs. It can last for a time much longer than the shock itself, thus it can be seen as a *memory* of the cell past stimulation. In most cells[1], HSPs are commonly found at very low concentrations, but the production of some of them can be induced in response to external stimuli. This phenomenon could be explained by the complex regulation of Heat Shock Transcription Factor (HSF) [8] and its relationship with HSP and the misfolded proteins [3]. At the promoter site of Hsp70 is found the Heat Shock Element (HSE), that can be stimulated by the complexation with a trimerized protein, the Heat Shock Factor (HSF). In normal conditions, HSF is bound to Hsp70, preventing its trimerization and the subsequent binding to HSE [3]. When a shock occurs, the preexisting HSPs bind to the degraded proteins, so that HSF is free to trimerize and bind to HSE. This is the onset of a cascade of reactions that leads to the production of new HSPs.

The kinetics of Hsp70 and its mRNA were reproduced by considering the main biochemical reactions of the HSR pathway. We studied this system with the formalism of the mass action law for the reaction kinetics (see Appendix for the details of the implementation). The chemical reaction network for HSR in eukaryotic cells [3] can be summarized as follows:

$$\begin{aligned}
\mathrm{Hsp70} + U &\underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{Hsp70}U \xrightarrow{k_s} \mathrm{SINK} \\
\mathrm{Hsp70} + \mathrm{HSF} &\underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} \mathrm{Hsp70HSF} \\
3\mathrm{HSF} &\underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} \mathrm{HSF}_3 \\
\mathrm{HSF}_3 + \mathrm{HSE} &\underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} \mathrm{HSEHSF}_3 \xrightarrow{k_{i1}} \mathrm{mRNA} \\
\mathrm{mRNA} &\xrightarrow{k_{i2}} \mathrm{Hsp70}.
\end{aligned} \tag{1}$$

In this system, the production of new Hsp70s is obtained when HSF is not bound to Hsp70, and can trimerize and bind to HSE. Hsp70 can bind either to HSF or to misfolded proteins (which concentration is represented by [U]), eventually leading them to degradation (represented by the SINK term). We considered as $[U]$ only those proteins destined to degradation, and not those that are recovered by the HSR. The reaction network that we implemented numerically was simplified by neglecting the mechanisms of HSF trimerization, DNA binding and the mRNA

[1]HSPs are classified in different groups depending on their molecular weight. We concentrated on one of the most common families of about 70kDa molecular weight, namely the Hsp70 family.

production and transport. The mRNA level was thus directly related to free HSF concentration, according to the following reaction, that substitutes the last three equations of system (1):

$$\text{HSF} \xrightarrow{k_{irr}} \text{mRNA}. \tag{2}$$

The setting of the model parameters (protein concentration and reaction constants) was performed in two main steps (see Table II): first, we found reasonable baseline values in absence of shocks, with the system allowed to reach the equilibrium state; secondly, we refined the parameter settings with the data from single shock experiments. In the first part, the typical production of misfolded proteins occurring during normal protein synthesis was represented by a constant *small influx* of unfolded proteins, $[U(t)] = C \ll 1$. The simulation started with a nonzero value only for the HSF-Hsp70 complex, so that its dissociation could trigger the genetically induced production of Hsp70. In order to produce a baseline state with almost no free HSF and U present, as it is normally found in cells, we chose $k_{-1}, k_{-2} \ll 1$.

In the second part we considered the effects of a heat shock, starting from the previously calculated basal state of the cell as initial conditions. Since the main damage to the cell regards protein conformation (and thus their functionality), we introduced the shock as an increase in unfolded protein concentration [U]. We hypothesized that the main protein damage occurred during the heating phase, corresponding to the first hour of the experiment (see Appendix for the detailed kinetics of $[U]$ production). Further damages should in fact be prevented from a reduction in the synthesis of the proteins not directly involved in the HSR [11].

Finally we tested the model against the kinetics of the proteins and mRNA concentrations, collected during the double heat shock experiments performed at different time intervals.

3. Results and Discussion

Our observations showed the peak in mRNA production to occur quite rapidly after the shock (3 h), returning to baseline values soon after (see Table I), while concerning Hsp70 protein synthesis, we observed a maximum 24 h after the shock, with return to the basal level at 72 h (Figure 2, dotted line). Since Hsp70 halflife is relatively short,[2] the observed peak of Hsp70 concentration, about 24 h after the shock, should require a high level of synthesis around that time. The length of time over which the early-produced mRNA is maintained inside the cell before degradation is thus crucial for the maintenance of a high Hsp70 concentration (thermotolerance state).

[2]Typical values are about 1–2 h [7], and during shock it can increase up to 7 h [12].

Table I. mRNA production rate. C represents the mRNA concentration in cells incubated at 37 °C

Time (h)	mRNA
C	21
3	16620
5	16
7	19

The other measurements are performed at different recovery times, performed at 37 °C after 1 h shock at 42 °C.

Table II. Reaction constants for the simulation

Reaction	K	K_{INV}
Hsp70 + U $\rightleftarrows$ Hsp70U	0.3	0.1
Hsp70 + HSF $\rightleftarrows$ Hsp70HSF	0.5	0.1
HSF $\rightarrow$ mRNA	1	0
Hsp70U $\rightarrow$ SINK	1	0

In order to reproduce the observed kinetics of Hsp70, in the light of the mRNA measured production rate, we propose the hypothesis that during Heat Shock a number of processes start that, more or less directly, trigger some reactions and modify cellular behaviour, so that Hsp70 mRNA halflife is increased.

This hypothesis is consistent with previous experimental observations for which, in a normal (nonshock) condition, Hsp70 mRNA halflife is approximately 50 min, but it can drastically change following a Heat Shock treatment [11], increasing by a factor of 10 or more, thus leading to a long-lasting metastable state as compared to the time scale of the phenomenon. The cause of the halflife increase is still not known, but it has been observed in accordance with the occurrence of heat shocks or with protein synthesis inhibition [11].

This mechanism was introduced as follows: when the concentration of damaged proteins gets above a threshold related to the baseline state[3], it "switches" mRNA halflife to a 10 times higher value.

As stated before, we could fit part of our reaction constants to the dynamics of mRNA production rate. The only unobserved parameter, starting from our arbitrary baseline state, was the $[U]$ production due to the shock, but the very good agreement of our simulations with the experimental observations on mRNA (see Figure 1) shows that our assumptions are reasonable.

[3]The threshold is arbitrarily taken as 10 times the $[U]$ baseline concentration.

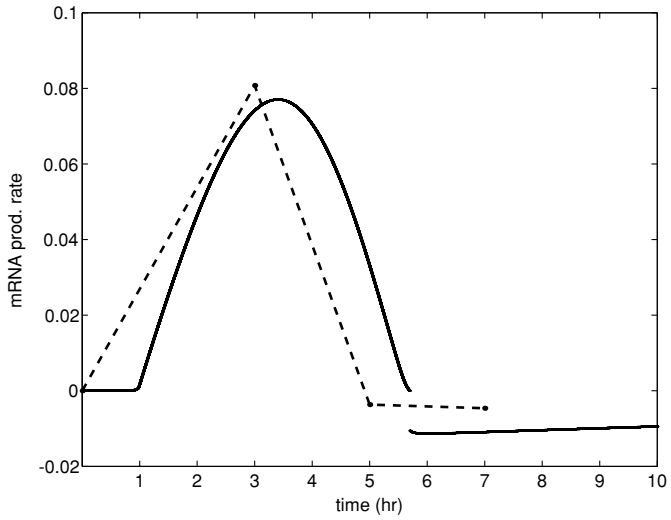

Figure 1. mRNA production rate as a function of time in the experiment (dashed line) and in the simulation (solid line). The curves have been rescaled along the y axis to allow graphical comparison.

The peak in mRNA production appears in correspondence of the early phase of heat shock, when misfolded proteins are attacked by the HSPs freed from the HSP-HSF bound, as shown in detail in Figure 1. The introduction of the switch in mRNA halflife allows the reproduction of the concentration curves also for the total Hsp70 concentration, which is the sum of free and bound Hsp70.

In Figure 2 we see the dynamics of a double heat shock experiment performed with an interval between the shocks of 72 h, that represents the case in which the cell has practically returned to the baseline state. Here the second shock induces a high response, due to the freeing of HSF from the HSP-HSF bond this can be deduced from the fast dropoff in total HSP, observed both experimentally and in our simulation at about 72 h, right after the shock (see the zoomed picture (c) in Figure 2). The agreement between observed and model kinetics is found also in the slight decrease of the second Hsp70 peak. A possible explanation is that the system is still weakly reminescent of the thermotolerance state.

The case of double shock with a shorter time interval (24 h) was also considered. In this case, the second shock occurs during the maximal thermotolerance level, while mRNA is still present. Protein misfolding and aggregation could be prevented by the high concentration of Hsp70 [15] already present in the cell. This allows a larger portion of misfolded proteins to be recovered, and in our model this is represented by a smaller number of unfolded proteins $[U]$ to be degradated (at least of a factor 100). In this case the decrease in Hsp70 at 24 h due to the sink is thus negligible, as can be seen in Figure 3, and the high level of the Hsp70 protein is maintained up to 30 h, but no HSF is freed in order to trigger further mRNA production.

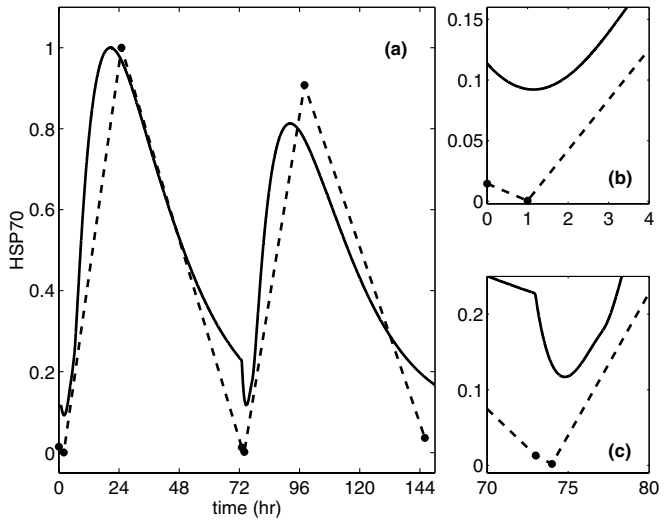

Figure 2. Total HSP concentration ([HSP] + [Hsp70 HSF] + [Hsp70U]) as a function of time during the 72 h interval double heat shock experiment. (a) measured total Hsp70 (dashed line) compared to the simulation result (solid line). The curves have been rescaled to 1 as the maximum concentration value (first peak). Figures (b) and (c) show two zoomed windows around the heat shock induction time (0–4 h and 70–80 h).

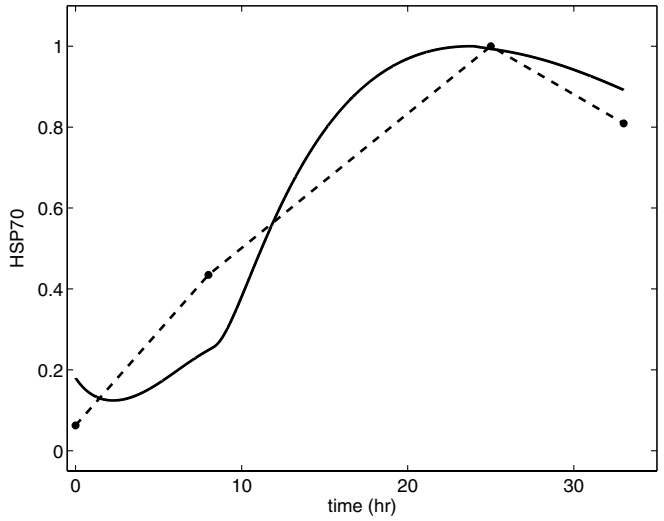

Figure 3. Total HSP concentration ([HSP] + [Hsp70 HSF] + [Hsp70U]) as a function of time during the 24 h interval double heat shock, rescaled along the y axis: experiment (dashed line) and simulation (solid line). The second heat shock is induced at the 25th hour.

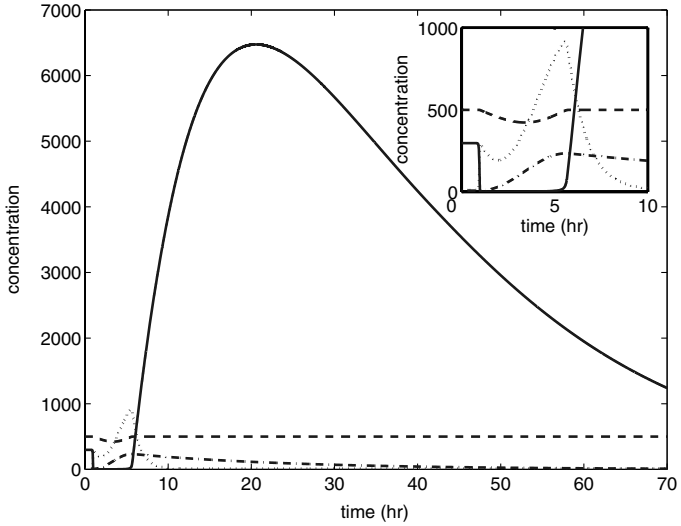

Figure 4. Reaction kinetics during single shock experiment: Hsp70 (solid line), Hsp70HSF (dashed line), Hsp$U$ (dotted line) and mRNA (dash-dotted line). The inset shows a zoom in the first 10 h interval.

4. Conclusion

We developed a simplified chemical reaction network of the Heat Shock Response machinery to study the thermotolerance state of the cell. After characterization of the baseline state for the network, obtained in a stationary $[U] \ll 1$ regime, we considered the effect of single and double heat shocks on the production of Hsp70 and its mRNA, and reproduced with good agreement the kinetics of double heat shock experiments.

These experiments characterized two different states of the cell, one in which the thermotolerance induced by a previous shock has almost disappeared, and one in which thermotolerance is at its highest level. The key assumption was to introduce a switch for the control of mRNA halflife in relation to some shock indicators, represented in our model by the concentration of unfolded proteins $[U]$ destined for degradation. This hypothesis is consistent with previous observations of heat shock response in Drosophila [11].

We want to emphasize that these state-dependent variations in mRNA stability, well–described in Drosophila and confirmed by our observations in mammalian cells, could represent a general mechanism for keeping record of past events inside a cell. In particular, these mechanisms could represent one of the first steps in the development of memory during evolution, embedded in the context of an ancient "mRNA world", previous to the appearance of DNA as the "standard" information storage molecule in living organisms [13, 14].

Appendix

The kinetics of the system of reactions (1) has been formalized using the mass action law. The system of ordinary differential equations obtained from our set of reactions (see below) was implemented with the $O(\Delta T)$ Euler method.

In addition to the law of mass action framework (which preserves the total mass of the reaction terms) it was further introduced a *sink* term, representing the degradation by proteasomes of the Hsp70U complex, and a *production* term for the mRNA-induced increase of Hsp70 concentration. Moreover, we accounted for the finite halflife of Hsp70 and mRNA by means of two decay terms added to the equations.

The kinetics of the unfolded proteins $[U]$ was implemented as a function of three factors: a constant term U_{IN}, representing the normal production of misfolded proteins, its complexation with Hsp70 (degraded through the sink), and its production during the shock. The $[U]$ baseline value was obtained as the equilibrium concentration when only U_{IN} is present. For the shock, we supposed that the production of $[U]$ occurred only during the heating phase (that lasted 1 h in each experiment) reaching its maximum at the end of the shock. The functional form of $[U]$ accumulation in time was a sigmoidal: $U_{\mathrm{shock}}(t) = U_{\max}/(1 + e^{-\lambda t})$, with $U_{\max}$ the maximum number of damaged proteins, found at the end of the 1-h shock. In the evolution equations we thus introduced the time derivative of $U_{\mathrm{shock}}(t)$. From this initial parametrization of the shock the relation was derived between iteration steps and time, by comparison of the theoretical and the experimental kinetics of mRNA concentration: 1000 iterations corresponded to 1 h, $\Delta T = 3.6$ s.

k_{SW}, the mRNA decay constant, is the switch that changes mRNA halflife as a response to shocks. When a shock occurs, represented as $[U]$ overcoming a threshold value, k_{SW} is reduced tenfold, similarly to what observed in [11]. The threshold value for the change of k_{SW} was taken as 10 times the $[U]$ baseline value.

With the simplification introduced in Equation (2), the system is the following:

$$
\begin{aligned}
dU/dt &= U_{\mathrm{IN}} - K_1\mathrm{Hsp70}(t)U(t) + K_{-1}Hsp70U(t) \\
&\quad + U'_{\mathrm{shock}}(t) + U_{\max}/(1 + e^{-K_{\exp}t})^2 K_{\exp} e^{-\lambda t} \\
d\mathrm{Hsp70}/dt &= -K_1\mathrm{Hsp70}(t)U(t) - K_2\mathrm{Hsp70}(t)\mathrm{HSF}(t) \\
&\quad + K_{-1}\mathrm{Hsp70}(t)U(t) + K_{-2}\mathrm{HSFHsp70}(t) \\
&\quad + Kirr_2\mathrm{mRNA}(t) - Kd_{\mathrm{HSP}}\mathrm{Hsp70}(t) \\
dHsp70U/dt &= K_1 Hsp70(t)U(t) - K_{-1}Hsp70U(t) \\
&\quad - K_{\mathrm{sink}}\mathrm{Hsp70}U(t) \\
d\mathrm{HSF}/dt &= -K_2\mathrm{Hsp70}(t)\mathrm{HSF}(t) + K_{-2}\mathrm{Hsp70HSF}(t) \\
d\mathrm{Hsp70HSF}/dt &= K_2\mathrm{Hsp70}(t)\mathrm{HSF}(t) - K_{-2}\mathrm{Hsp70HSF}(t) \\
d\mathrm{mRNA}/dt &= k_{\mathrm{irr}}\mathrm{HSF} - k_{\mathrm{SW}}\mathrm{mRNA}
\end{aligned}
\tag{3}
$$

The reaction constants were estimated by obtaining a reasonable equilibrium state, with low U, mRNA and free HSF concentrations, and by fitting the single shock experimental observations (see Table II and Figure 4).

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References

1. Ritossa, F.: New Puffing Pattern Induced by Temperature Shock and DNP in Drosophila, *Experientia* **18** (1962), 571–573.
2. Tissieres, A., Mitchell, H.K. and Tracy, U.M.: Protein Synthesis in Salivary Glands of *D. melanogaster*: Relation to Chromosome Puffs, *J. Mol. Biol.* **84** (1974), 389–398.
3. Morimoto, R.I.: Regulation of the Heat Shock Transcriptional Response: Cross Talk Between a Family of Heat Shock Factors, Molecular Chaperones, and Negative Regulators, *Genes Dev.* **12** (1998), 3788.
4. Kiang, J.G. and Tsokos, G.C.: Heat Shock Protein 70 kDa: Molecular Biology, Biochemistry, and Physiology, *Pharmacol. Ther.* **80**(2) (1998), 183–201.
5. de Pomerai, D., Daniells, C., David, H., Allan, J., Duce, I., Mutwakil, M., Thomas, D., Sewell, P., Tattersall, J., Jones, D., Candido, P.: Non-Thermal Heat-Shock Response to Microwaves, *Nature* **405** (2000), 417.
6. Arnvig, K.B., Pedersen, S. and Sneppen, K.: *Thermodynamics of Heat Shock Response*. (cond-mat/9912402), *Phys. Rev. Lett.* **84:13** (2000), 3005.
7. Theodorakis, N.G., Drujan, D., De Maio, A.: Thermotolerant Cells Show an Attenuated Expression of Hsp70 After Heat Shock, *J. Biol. Chem.* **274:17** (1999), 12081.
8. Bonner, J.J., Carlson, T., Fackenthal, D.L., Paddock, D., Storey, K., Lea, K.: Complex Regulation of the Yeast Heat Shock Transcription Factor, *Mol. Biol. Cell* **11**(5) (2000), 1739–1751.
9. Morimoto, R.I., Tissières, A. and Georgopoulos, C. (eds.): *The Biology of Heat Shock Proteins and Molecular Chaperones*, Cold Spring Harbor Laboratory Press, 1994.
10. Kiang, J.G., Tsokos, G.C.: Heat Shock Protein 70 kDa: Molecular Biology, Biochemistry, and Physiology, *Pharmacol. Ther.* **80**(2) (1998), 183.
11. Theodorakis, N.G. and Morimoto, R.I.: 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**(12) (1987), 4357.
12. Li, D. et al.: *Eur. J. Biochem.* **231**(2) (1995), 454.
13. Barbieri, M.: *The Organic Codes*, Cambridge Univ. Press, Cambridge, 2003.
14. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. and Watson, J.: *Molecular Biology of the Cell III ed.*, Garland, New York, 1994.
15. Hartl, F.U. and Hayer-Hartl, M.: Molecular Chaperones in the Cytosol: From Nascent Chain to Folded Protein, *Science* **295** (2002), 1852–1858, DOI: 10.1126/science.1068408