A Model of HSP-70 Synthesis in Barley Aleurone Cells During Heat Shock

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1 Introduction

Heat shock is a naturally occurring environmental stressor for plants. This stressor usually causes protein dysfunction and prevents them from properly folding, assembling, translocating and degrading. Heat-shock proteins (Hsps)/chaperones are a response protein that helps in providing assistance against the protein dysfunctions listed above. They play a critical role in protecting plants from the stress of heat-shock. They do this by re-establishing homeostasis within the cell, by either stabilizing existing structures (role of small Hsps) or allowing proteins to refold (the large Hsps). Thus, there are several different classes of heat shock proteins that help in stabilizing the cell. The one that we will be focusing on, however, is Hsp70. This Hsp interacts with a variety of co-chaperone proteins that help in refolding the unfolded proteins (Wang *et al.*, 2004). The major role that Hsp70 plays in cells are preventing aggregation, assisting refolding, protein import and translocation, signal transduction, and transcriptional activation (Wang *et al.*, 2004). It is additionally one of the first Hsps to be expressed during HSR. On top of this, some Hsp70 help to control the folded regulatory proteins, and may actually act as a negative repressor of heat-shock factor (HSF) mediated transcription (Wang *et al.*, 2004). In our particular study, we are looking at the secretion of alpha-amylase mRNA and the synthesis of Hsp70 proteins as a result of heat-shock that takes place in the aleurone layer of barley. To test whether or not these Hsps assist in the destabilized alpha-amylase mRNA or of the delamellation process of the Endoplasmic Reticulum (ER), heat shocked cells were treated with the transcription inhibitor cordycepin, which effectively inhibits the synthesis of hsps yet does not affect alpha-amylase synthesis after this enzyme has been fully induced by gibberellic acid (12 hours) (Brodl *et al.*, 1990). Although Hsps have been known to help with thermotolerance, in barley aleurone cells they do not play a major role in other heat-shock induced changes - their targets are primarily existing proteins, and so Hsp70 does not confer thermo-protection to larger structures such as the endoplasmic reticulum (Brodl *et al.*, 1990). In this paper, we explore the extant literature to help inform and contextualize our modeling project on Hsp70 and its effects in the aleurone layer in barley.

We have constructed a system of differential equations designed to model

Hsp70 synthesis in barley aleurone cells. To create this system, we have developed hypotheses about the dynamics of Hsp70 synthesis and applied the Law of Mass Action to yield a series of ordinary differential equations that model the synthesis of Hsp70 based on time and external temperature. This is not a novel approach to modeling heat shock response. PETRE *et al.* (2011), used the mass-action model as well, but focused instead on the interaction between HSPs, HSFs, and DNA binding to create a eukaryotic -and more holistic- model of heat shock response. Their simulation was validated using K562 cells under continuous heat shock. However, PETRE *et al.* simplify their model by working with the total number of HSPs in the system, regardless of their various molecular weights and roles in the cell and looked at the heat shock response under only one temperature scheme. The PETRE *et al.* model presented us with a fairly robust analysis of the HSR, however did so with the assumption that no new Heat Shock Factors are produced throughout the response, and did not attempt to account for HSP synthesis, merely assuming it is produced at a rate proportional to the amount of several other factors in the cell. .

In 2009, SZYMAŃSKA and ZYLICZ used the same approach to model Hsp70 synthesis in cancer cells, finding that there may be a correlation between Hsp70 synthesis and resistance to hyperthermia. We are fortunate that SZYMAŃSKA and ZYLICZ created a mathematical model for a system so similar to the one we have modeled. Indeed, we used insights from SZYMAŃSKA and ZYLICZ's (2009) discussion of the dynamics of Hsp70 synthesis in cancer cells to formulate our own hypotheses about how Hsp70 synthesis might occur in barley aleurone cells. Moreover, we have been able to draw conclusions about what differences exist between the synthesis processes in the two types of cells.

During experimentation, barley aleurone cells were subjected to three six-hour temperature schemes. In the fast-ramp scheme, the environment is 25 degrees centigrade for three hours, then the temperature increases 2.5 degrees every half hour for the remaining three hours. In the slow ramp scheme, the environment begins at 25 degrees and increases 2.5 degrees every hour for six hours. Finally, in the plunge scheme, the temperature is 25 degrees for three hours before increasing to 40 degrees for the final three. Our model attempts to predict the synthesis of the heat shock protein with molecular weight 70 (HSP-70) in barley aleurone cells during these three types of environmental stress. We adapt and simulate a system of ordinary differential equations developed by SZYMAŃSKA and ZYLICZ (2009) to approximate heat shock response (HSR) in the cell, with the goal of being able to predict how different temperature changes would HSP-70 synthesis.

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2 Background

Heat shock is a naturally occurring environmental stressor for plants. Generally, heat shock prevents proteins from properly folding, assembling, translocating and degrading. Heat-shock proteins (HSPs) are a response protein that help the cell cope with the protein dysfunctions above. HSPs play a critical role in protecting plants from the stress of heat-shock. They do this by re-establishing homeostasis within the cell, by either stabilizing existing structures (role of small HSPs) or allowing proteins to refold (the large HSPs). Thus, there are several different classes of heat shock proteins that help in stabilizing the cell. The one that we will be focusing on, however, is HSP-70. This HSP interacts with a variety of co-chaperone proteins that help in refolding the unfolded proteins. The major role that HSP-70 plays in cells are preventing aggregation, assisting refolding, protein import and translocation, signal transduction, and transcriptional activation. It is additionally one of the first HSPs to be expressed during HSR. On top of this, some HSP-70 help to control the folded regulatory proteins, and may actually act as a negative repressor of heat-shock factor (HSF) mediated transcription.

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3 Our Model

Our model is a modified version of Szymańska and Zylicz's (2009) model of HSR in cancer cells. In the Szymanska method, the Law of Mass Action and a series of hypotheses about the dynamics of HSP-70 synthesis under HSR yield a system of ordinary differential equations.

There is more support for this method in the literature. Petre *et al.*, used a mass-action model as well, but focused instead on the interaction between

HSPs, HSFs, and DNA binding to create a eukaryotic -and more holistic- model of heat shock response. Their simulation was validated using K562 cells under continuous heat shock. However, Petre et al. simplify their model by working with the total number of HSPs in the system, regardless of their various molecular weights and roles in the cell and looked at the heat shock response under only one temperature scheme. The Petre et al. model presents us with a fairly robust analysis of the HSR, but it does so with the assumption that no new Heat Shock Factors are produced throughout the response, and does not attempt to account for HSP synthesis, merely assuming it is produced at a rate proportional to the amount of several other factors in the cell.

The variables of the model are as follows: HSP-70 freely in the cell, HSP-70 in complex with HSF, HSP-70 in complex with substrate, the substrate in question: denatured protein (DP), which is a result of protein (P) denaturing due to temperature effects, and finally HSF, HSF trimer, and mRNA coding for HSP-70 synthesis. Finally, F is a variable that estimates the effects of temperature (T) on the rate of protein denaturation, and is a transformation of the equivalent equation in Szymańska and Zylicz model to fit our temperature range.

There are two main differences between our model and Szymańska and Zylicz. While our model incorporates a variable for amount of protein in the cell, we do not use a variable to account for the heat shock element that helps promote HSP-70 expression. We omit this variable in order to minimize the number of unknown variables and parameters. Although we have some idea of the amount of protein relative to HSP-70 from data of HSR in barley aleurone, we did not have data for the amount or strength of promotion of HSE. Furthermore, unlike Szymańska and Zylicz, we assume an HSF is produced for every molecule of HSP-70, in order to balance the amount of HSP-70 freely in the cell. Lastly, the addition of P into the model ensures that, while still not following the Mass Action Law, our model is a bit closer to doing so than Szymańska and Zylicz's (2009), as it accounts for the amount of proteins that can denature in the cell at any given time. This may complicate some of our estimates of how temperature acts in order to denature proteins, yet it seems to be an assumption that makes the model more reflective of actual biological processes. These ideas are represented schematically in Figure 1.

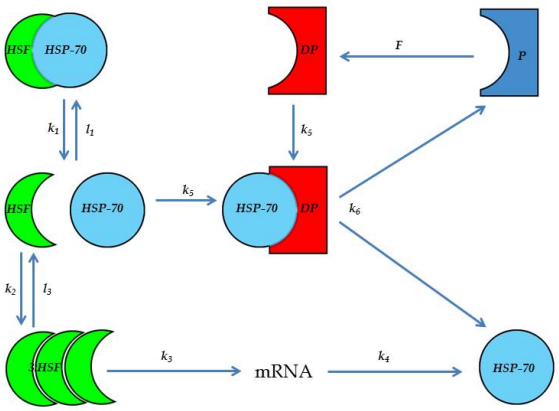

Figure 1: Schematic diagram of the theoretical model of heat shock response in Barley Aleurone Cells. While the model is constructed on mass law action principles, it does not follow a mass action law, as HSP-70 is continuously produced whenever mRNA is able to form. mRNA degrades, however, at the same rate as it creates HSP-70, so a further simplification of the model may just ignore mRNA and assume HSP-70 is produced when 3HSF is present, but at low rates.

The schematic diagram of the process was used to derive a series of equations according to the Law of Mass Action. However, as mentioned previously, the production of new HSP-70 breaks this law, although the addition of P as a variable balances another end of the system out.

An application of the Law of Mass Action Yields the following system.

$$\frac{dHSP:HSF}{dt} = -k_1 HSP : HSF + l_1 HSP * HSF$$

$$\frac{dHSP}{dt} = k_1 HSP : HSF - l_1 HSP * HSF - k_5 HSP * DP + k_6 HSP : DP + k_4 mRNA$$

$$\frac{dHSF}{dt} = k_1 HSP : HSF - l_1 HSP * HSF - k_2 HSF^3 + 3HSFl_3 + k_4 mRNA$$

$$\frac{dDP}{dt} = FP - k_5 HSP * D$$

$$\frac{d3HSF}{dt} = HSF^3 k_2 - 3HSFl_3 - 3HSFk_3$$

$$\frac{dmRNA}{dt} = 3HSFk_3 - l_2 x_8 - k_4 x_8$$

$$F = 0.02598^{T-25}(1 - \frac{0.39145}{e^{T-25}}) - 0.19800$$

Parameter values were obtained from Szymańska and Zylicz (2009) where possible, and estimated from the data otherwise. Initial values were set to biologically reasonable approximations, with the main interest in the ratios between the variables. The system was implemented in MATLAB using the ODE15 estimator.

4 Results

The model indicates that the quantity of HSP-70 does increase during heat shock. The quantities of HSP-70 are highest during the fast ramp temperature scheme, however these amounts equalize throughout the temperature schemes if the cells are expected to remain at 40 C^o.

These results were meant to be validate with biological data from barley aleurone cells. However, the scarcity of this data and its high error rate make validation efforts rather meaningless. In effect, at this point our analysis is limited to comparisons with theoretical expectations, rather than any comparisons with real world data. Furthermore, as the model is expected to be a continued work, and no steady state solutions have yet been found, no sensitivity or robustness analysis is presented here, though will hopefully be developed in the future.

5 Conclusion

We have developed a model for heat shock response in barley cells using a model of a very similar system in cancer cells. While our model does not currently produce results comparable to those of Szymańska and Zylicz (2009), at this point it is largely the fault of clear initial values. While running the model

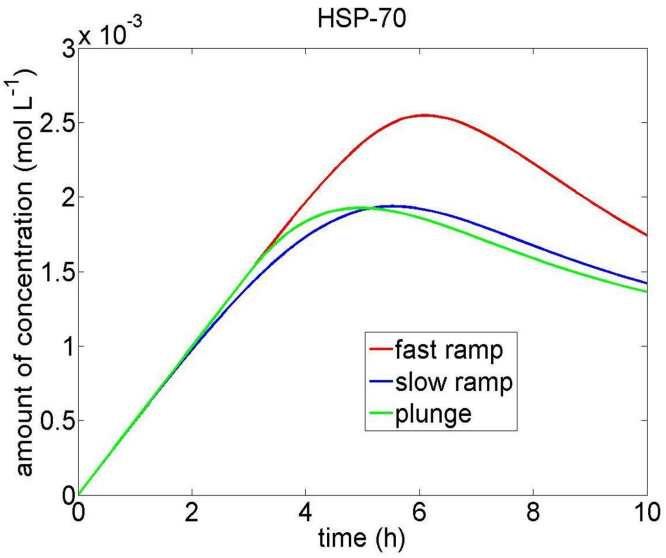

Figure 2: The change in the quantity of free HSP in the cell, during the course of a simulation. Note that initially, a small amount of HSP is released even when no denatured proteins are present (Figure 4). However, this makes sense as some free HSP-70 will be found in the cell at most times. Not changing the temperature, this amount eventually slopes off as the ratios of HSF to HSP change sufficiently.

at a set temperature until it reaches equilibrium is one possibility, even long simulation runs looking at the state of the model for > 30 hours were not able to reach a clear equilibrium. The problem largely lies with the strongest part of our model - its accounting for the quantity of proteins in the cell. This quantity should be expressed as a relative measure compared to the amount of HSP-70. However, our current efforts never produce enough HSP-70 to return the number of proteins to a somewhat balanced state. That is, once heat shock occurs, our model predicts death to the cells, under most reasonable initial parameter estimates. However, assuming that the amount of heat shock proteins is comparable to that of its substrates, the model should be able to return to an equilibrium. This would imply, however, that not all proteins are capable of denaturing, or at least not all denatured proteins can act as substrates for HSP-70. An additional concern is a slight imbalance in the amount of HSF in the cell. While HSF is produced whenever HSP-70 is in order to compensate for possible loss, the net trend is for HSF to increase far slower than the other variables. This may be an artifact of having removed HSE from the equations, which means that the model in some ways implies that HSF is converted to mRNA, as opposed to promoting its synthesis. Further work needs to be done before any clear conclusions can be drawn, however a solid background for such work has been established, and a finalization of the research should not be too

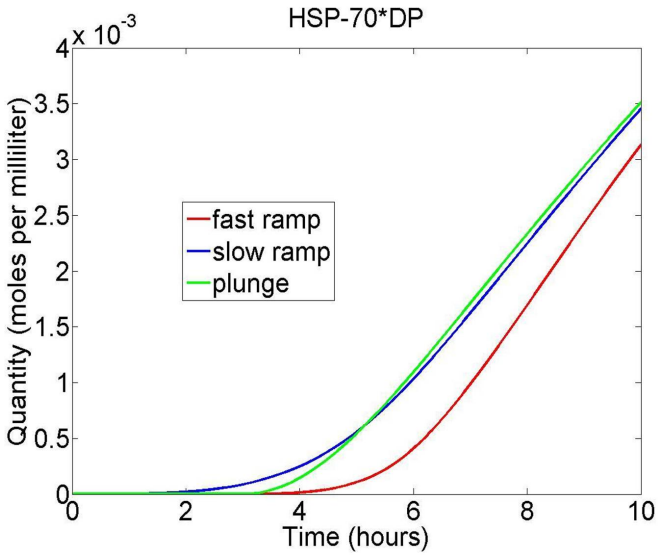

Figure 3: Change in the quantity of HSP-70 bound to Denature Protein under the different schema. Note that these numbers eventually converge to a similar limit, when in all three environments all the available HSP has been recruited to repairing denature proteins. One of the main problems with the model as currently phrased is the eventual loss of HSF in production of HSP, not evident in this figure, which limits the total number of HSP-70 that can be produced. Running the simulation for longer equilibrates all three schema to the same quantity.

difficult.

References

Brodl, M., F. Belanger, and T. Ho, 1990 Heat shock proteins are not required for the degradation of α-amylase mrna and the delamellation of endoplasmic reticulum in heat-stressed barley aleurone cells. Plant physiology **92**: 1133–1141.

Petre, I., A. Mizera, C. Hyder, A. Meinander, A. Mikhailov, *et al.*, 2011 A simple mass-action model for the eukaryotic heat shock response and its mathematical validation. Natural Computing **10**: 595–612.

Szymańska, Z., and M. Zylicz, 2009 Mathematical modeling of heat shock protein synthesis in response to temperature change. Journal of theoretical biology **259**: 562–569.

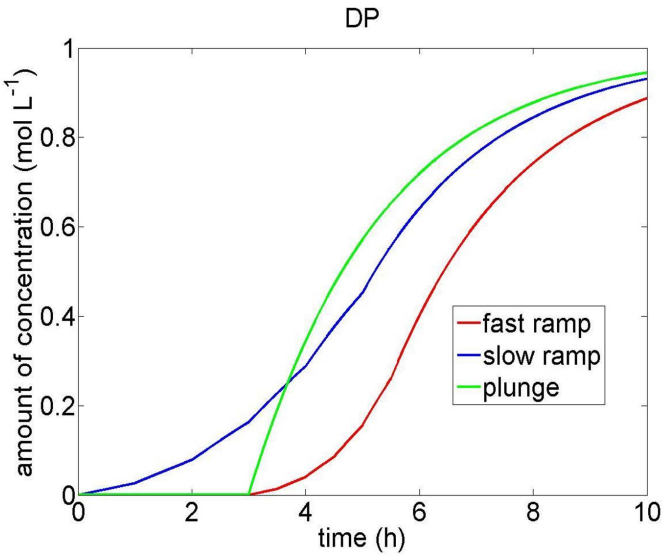

Figure 4: The increase in denatured protein, dependent on the scheme of temperature increase. Note that no proteins denature in the case of the plunge model until after the 3 hour mark, which corresponds clearly with the sudden decrease of free HSP-70 and sudden increase of HSP-70:DP at the same time. Running this simulation for longer, these values eventually even out at .98, when HSP is reconstructing the proteins at its maximal capability.

Wang, W., B. Vinocur, O. Shoseyov, and A. Altman, 2004 Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends in plant science **9**: 244–252.