University of Exeter

ECMM713: Modelling Applications and Case Studies

Modelling the Response of Yeast to Osmotic Shock

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1 Introduction

Cells play a vital role in the make up and functioning of all organisms. There are many processes at work which attempt to avoid the loss (or exponential growth) of cells on a large scale. One important and very relevant example of uncontrolled cell growth is the disease cancer. The processes that regulate cell growth and multiplication break down in one cell, which in turn begins to grow and divide. This sudden increase in cells with unregulated growth destroys adjacent tissue and will sometimes spread to other parts of the body (Lodish et al. 2000).

In modern molecular cell biology it is understood that cells are only one element in a system. Organisms are made up of organs, which in turn composed of tissues. Many different cells make up these tissues and the cells in turn consist of molecules. No single element in the system can be taken apart from the others; the idea of a system is a recurring theme in modern biology. During this report the yeast *Saccharomyces cerevisiae* and its ability to adapt to osmotic shock will be explored. Yeast cells need to have many mechanisms to avoid cell loss. These cells are often exposed to a differing environment, which includes changes in pH, temperature and available water (Hohmann 2002). Adaptation to the changing levels of these factors has to be fast and effective. The activity of cells and their ability to adapt are dependent on the interactions between different system components (Alberghina 2007). The process by which yeast cells adapt to changes in water activity is called osmoregulation (Klipp et al. 2005) and helps to stabilise a cell after it has suffered osmotic shock.

Water activity inside the cell needs to be lower than in the outside medium to maintain a constant pressure gradient along which water can flow into the cell at a controlled rate (Hohmann 2002). This translates to the cell keeping it's medium at a slightly higher osmotic pressure than the external medium (Martinez de Maranon et al. 1996). When the cell suffers osmotic shock the external osmotic pressure increases rapidly and as a result the pressure gradient reverses and water starts to flow out of the cell. This outflow results in a loss of turgor pressure (loosely defined as the difference between the internal and external pressure), which needs to be stopped to prevent the cell from dying. Osmotic shock will most likely occur under a change of external conditions, either a high or low concentration of solutes. If there is a low concentration of solutes, water will rush into the cell causing it to swell and undergo apoptosis (Lodish at al. 2000). The case that is mentioned above occurs when the concentration of the solute is high and as a result water will rush out of the cell. The processes and proteins at work inside the

cell which balance the turgor pressure are of grestest interest. More details will be given on the specific processes in the next section.

The aim of this report is to develop a mathematical model which captures the interactions between different proteins in this system. By doing this we hope to recreate some of the findings from the paper (Klipp at al. 2005) and then extend the model.

2 An overview of systems biology

A major goal behind the study of systems biology is to be able to infer general principles that underly the behaviour and mechanisms of living systems (Westerhoff and Kell 2007). We wish to understand processes at work for not only special cases, but for all possible cases. This is no simple task though and systems biology draws on many other aspects of science to try to understand these processes. For the purpose of this project the mathematical aspect holds a specific interest.

Another goal of systems biology is to try and understand the workings of specific living systems. This can be done through computational and mathematical modelling. By setting constant parameters, which are either set by the system or us, and variables we can model how a cell evolves over time. Knowledge gained from this type of modeling can then inform modeling of future events. An interesting example of such a process at work in systems biology can be seen on a much larger scale. Decisions to cull organisms in population dynamics are made based upon mathematical predictions of future population sizes. At a cellular and molecular level the processes at work are no different.

The ultimate goal of systems biology is to understand every different living system in molecular terms. This understanding allows such things as biotechnology, preventive measures, diagnosis and many other important aspects of modern applied science. As such it is a very significant and interesting addition to modern scientific thought.

3 Osmoregulation in yeast cells

Osmotic shock leads to a quick increase in the external osmotic pressure and a sharp decrease in the turgor pressure of the cell. At the moment the idea of different osmotic pressures seems very abstract, for a better understanding of the processes at work it is necessary to define osmosis. Osmosis is the movement of water molecules from a region of lower solute concentration to

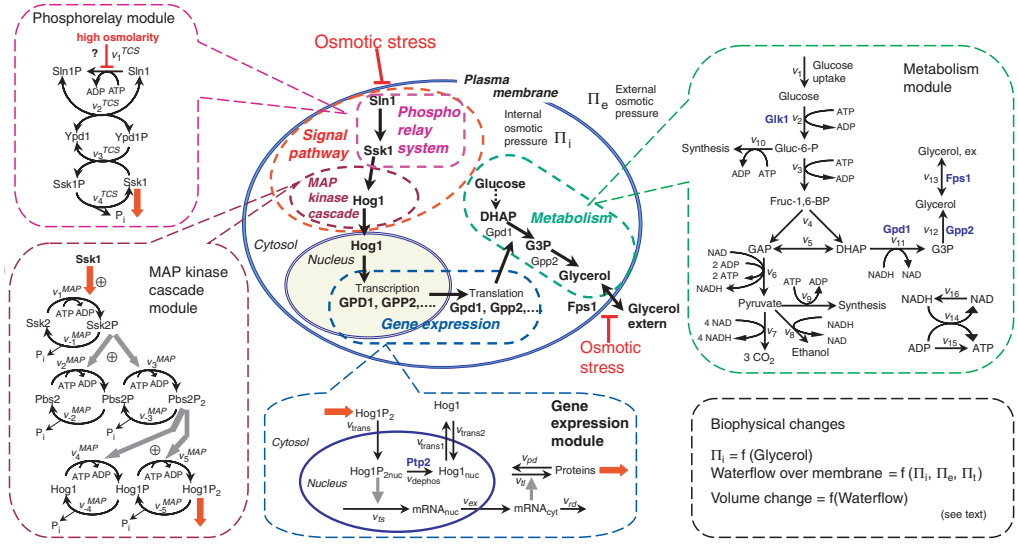


Figure 1: The response of yeast to hyperosmotic stress (Klipp 2005)

a region of higher solute concentration (Lodish et al. 2000). This flow will often occur across a layer of the cell, in the case here the flow is altered across the cell membrane. An increase in the external osmotic pressure thus relates to an increase in solute concentration outside of the cell. Water molecules will begin to move from inside the cell out through the cell membrane. To respond to this outflow, it is necessary that different proteins are activated and inhibited. Proteins are essential parts of organisms and participate in most processes inside cells since their main function is to catalyse biochemical reactions.

Generally the reactions that control the response to osmotic shock can be split into five different modules. These modules can be seen as separate entities, even though it would be difficult to draw any conclusions from each of the modules alone (Hartwell et al. 1999). Turgor pressure acts as the switch for the system and as a result is very important. It can be viewed as the amount of pressure being exerted on the cell membrane and is dependent in the internal and external osmotic pressures. For example, an outflow of water molecules will cause a cell to lose volume, which inturn will decrease the turgor pressure. The loss in turgor pressure leads to the activation of the phosphorelay module which increases the amount of the protein Ssk1. This module activates another module, called the MAP kinase module. After that the gene expression and metabolism modules are activated which leads to an increase in glycerol. This glycerol is used as an osmolyte which increases

the internal osmotic pressure (Klipp et al. 2005). The increase in internal osmotic pressure balances out the turgor pressure back to its original value, which deactivates the phosphorelay module and stops unnecessary glycerol production. From a mathematical viewpoint we are interested in how these reactions vary over time which can be solved using differential equations.

4 Mathematical modelling

Each of the reactions that occurs within the cell can be written as an ordinary differential equation. For example, one reaction may result in an increase of Ypd1P whereas another reaction may use this protein to phosphorylate another protein. Since these reactions are happening over time with a given rate, they can just be written as first-order differential equations. On their own each of the differential equations may not be particularly complicated, however it is the combination of many of these equations that makes the system very hard to model. To model the whole system we require about 40 different differential equations, along with respective rate parameters for each of the reactions. The links between the different modules also make mistakes hard to diagnose since a problematic value at any stage will feed into the system and change many other values. Despite the difficulties when it comes to mathematical modelling, it is clear that the use of it on this system allows us to draw conclusions that once we would have been unable.

During this report we shall be using the mathematical program Matlab to run the model. Matlab provides a wealth of different differential equation solvers and correct choice can often improve the computational cost. In this situation we have an initial value problem which allows us to use inbuilt differential equation solvers ‘ode45’ and ‘ode15s’. ‘ode45’ is a routine designed for non-stiff problems and is based upon an explicit Runge-Kutta-Fehlberg formula. On the other hand ‘ode15s’ is a variable order solver which works for stiff systems. It turns out that to model this system it is better to use the stiff solver ‘ode15s’. ‘ode45’ will attempt to stay stable by reducing the time step to a very small value which will cause the solver to take a very long time. The system we are try to model is stiff as some of the variables have a rapid change in their solutions. The nature of osmotic shock will lead to these quick changes in the system, making ‘ode15s’ the correct solver to use.

5 The modular system

5.1 Volume module

The volume module works in a different way from the other modules, but is equally important when it comes to understanding what is happening in the cell. When there is an increase in the external osmotic pressure (Π_e) the osmotically changeable volume of the cell (V_{os}) decreases. This decrease in the volume leads to a decrease in the turgor pressure (Π_t) which acts as an input into the phosphorelay module. The volume of the cell is related to the different pressures by the equation

$$\frac{d}{dt}V_{os} = -G \cdot L_p(\Pi_t + \Pi_e - \Pi_i) \tag{1}$$

where G and L_p are constants. Initially the increase in the external pressure leads to a decrease in the volume of the cell. During this project we shall be using the volume and osmotically changeable volume interchangeably since

$$V_{os} = V_{total} - V_b \tag{2}$$

where V_b is the (constant) volume not affected by osmotic changes. The turgor pressure is related to the volume by the equation

$$\Pi_t = \Pi_t^0 \cdot \left(1 - \frac{V_{os}^0 - V_{os}}{V_{os}^0 - V^{\Pi_t=0}}\right) \tag{3}$$

where V_{os}^0 is the initial (constant) value for the volume and $V^{\Pi_t=0}$ is the (constant) value at which the turgor pressure collaspses. So far this module is responsible for creating a change in the turgor pressure, however for the model to work there needs to be a way in which the change of turgor pressure can be fed into the phosphorelay module. The change in turgor pressure inhibits the production of Sln1P through the parameter k_1^{TCS}

$$k_1^{TCS}(t) = k_1^{TCS,0} \cdot \left(\frac{\Pi_t(t)}{\Pi_t^0}\right)^{n_1} \tag{4}$$

which feeds directly into the phosphorelay module through the relation

$$v_1^{TCS} = k_1^{TCS}(t) \cdot \text{Sln1} \tag{5}$$

In this way the decrease in turgor pressure inhibits the rate of phosphorylation of Sln1 and the system which responds to the osmotic shock starts to function. The volume module becomes important again nearer the end of the system which will be discussed later.

5.2 Phosphorelay module

The phosphorelay module responds to the loss in turgor pressure by inhibiting the production of the protein Sln1P. Sln1P can be seen as a sensor which sits on the edge of the cell monitoring the concentration of the solute outside the cell. It is distributed evenly throughout the cytoplasmic membrane (Reiser et al. 2003). The inactivation of Sln1P leads to an decrease in the phosphorylation of the protein Ypd1 to Ypd1P, which in turn reduces the phosphorylation of Ssk1 to Ssk1P. The increase in the amount of Ssk1 turns on the MAP kinase module. An easier way to visualise the processes at work is through the reaction equations.

$$\begin{aligned}
\text{Sln1} &\xrightarrow{k_1^{TCS}} \text{Sln1P} \\
\text{Sln1P} + \text{Ypd1} &\underset{k_{-2}^{TCS}}{\overset{k_2^{TCS}}{\rightleftharpoons}} \text{Sln1} + \text{Ypd1P} \\
\text{Ypd1P} + \text{Ssk1} &\xrightarrow{k_3^{TCS}} \text{Ypd1} + \text{Ssk1P} \\
\text{Ssk1P} &\xrightarrow{k_4^{TCS}} \text{Ssk1}
\end{aligned}$$

These type of reactions are called phosphorylation reactions and use other activated proteins to activate themselves and dephosphorylate the other protein. Each of these reactions will happen with certain rates (given by the values k_1^{TCS} etc.) which are determined by the amount of the proteins available. We can convert the above reactions into differential equations for each of the proteins

$$\frac{d}{dt}\text{Sln1} = v_2^{TCS} - v_1^{TCS} - \text{Sln1} \cdot V_{ratio} \tag{6}$$

$$\frac{d}{dt}\text{Sln1P} = v_1^{TCS} - v_2^{TCS} - \text{Sln1P} \cdot V_{ratio} \tag{7}$$

$$\frac{d}{dt}\text{Ypd1} = v_3^{TCS} - v_2^{TCS} - \text{Ypd1} \cdot V_{ratio} \tag{8}$$

$$\frac{d}{dt}\text{Ypd1P} = v_2^{TCS} - v_3^{TCS} - \text{Ypd1P} \cdot V_{ratio} \quad (9)$$

$$\frac{d}{dt}\text{Ssk1} = v_4^{TCS} - v_3^{TCS} - \text{Ssk1} \cdot V_{ratio} \quad (10)$$

$$\frac{d}{dt}\text{Ssk1P} = v_3^{TCS} - v_4^{TCS} - \text{Ssk1P} \cdot V_{ratio} \quad (11)$$

where v_1^{TCS} is given in equation (5) and the other v_i^{TCS} are defined

$$v_2^{TCS} = k_2^{TCS} \cdot \text{Sln1P} \cdot \text{Ypd1} - k_{-2}^{TCS} \cdot \text{Sln1} \cdot \text{Ypd1P} \quad (12)$$

$$v_3^{TCS} = k_3^{TCS} \cdot \text{Ssk1} \cdot \text{Ypd1P} \quad (13)$$

$$v_4^{TCS} = k_4^{TCS} \cdot \text{Ssk1P} \quad (14)$$

The value of V_{ratio} is very important in the model as it varies depending on the volume of the cell. This term can be viewed as a dilution of the concentrations of proteins due to growth in the cell

$$V_{ratio} = \frac{1}{V_{os}} \cdot \frac{d}{dt} V_{os} \quad (15)$$

Initially as the volume of the cell decreases the final terms of equations (6)-(11) help to activate or inhibit the production of the different proteins. As the volume starts to increase the proteins will be more spread out and as a result their concentrations will decrease. The rates v_1^{TCS} - v_4^{TCS} all depend on the concentrations of the different proteins and as such will be changing at each timestep.

5.3 MAP kinase module

The increased production of Ssk1 activates the MAP kinase module. The rapid phosphorylation in this module leads to the activation of the MAPK kinase Hog1 (Reiser at al. 2003). Some of the reactions in this module are called catalysis reactions. These are reactions where other proteins are used to catalyse the reactions between two specific proteins. In this module the protein ADP and its activated state ATP are used as catalysts in the change of Pbs2 to Pbs2P and the activation of Hog1.

$$\text{Hog1P} + \text{ATP} \underset{k_{-4}^{MAP}}{\overset{k_4^{MAP}}{\rightleftharpoons}} \text{Hog1P}_2 + \text{ADP} \quad (16)$$

Figure 1 shows the MAP kinase module in diagrammatical form in the bottom left corner. Figure 1 also shows that up until this point the reactions that have been occurring have happened in the cytosol. However as $Hog1P_2$ is obtained from this module the processes move inside the cell nucleus. $Hog1P_2$ will move in and out of the cell nucleus depending on what stage of response the cell is at. The reactions in this module can also be written as differential equations as per equations (6)-(11).

5.4 Transcription and translation

The increase in the concentrations of Hog1P2 in the nucleus will activate gene expression, which occurs due to messenger ribonucleic acid (mRNA). The expression of two of these genes is stimulated by the active Hog1 in the nucleus. This leads to the enzymes Gpd1 and Gpp2 catalysing the conversion of dihoydroxyacetonephosphate (DHAP) via glycerol-3-phosphate (G3P) to glycerol (Klipp et al. 2005). Other genes will work to dephosphorylate the active Hog1 in the nucleus, which causes it to move back out into the cytosol.

5.5 Metabolism module

The final module in the system reacts to the increase in the Gpd1 enzyme to stimulate added glucose uptake. Through many different catalysis reactions (which again require the protein ATP) the amount of DHAP is increased which is converted to glycerol. Below the 3 main equations in this process are provided, although the module itself is much more complicated and involves many other reactions

$$\frac{d}{dt}\text{Gluc} = v_1 - v_2 - \text{Gluc} \cdot V_{ratio} \tag{17}$$

$$\frac{d}{dt}\text{DHAP} = v_4 - v_5 - v_{11} - \text{DHAP} \cdot V_{ratio} \tag{18}$$

$$\frac{d}{dt}\text{Glyc} = v_{12} - v_{13} - \text{Glyc} \cdot V_{ratio} \tag{19}$$

where all the v_i are arbitrary rate terms which vary with time. Equation (19) will lead to an increase in the amount of glycerol produced in the cell. This glycerol is then accumulated in the cell and leads to an increase in the internal osmotic pressure. To realise this change the concentration of glycerol feeds back into the volume module. For this it is necessary to define c which is the total concentration of osmotically active compounds. The rate of change of this concentration is defined as

$$\frac{d}{dt}c = v_{12} - v_{13} - c \cdot V_{ratio} \tag{20}$$

which is related to the internal osmotic pressure

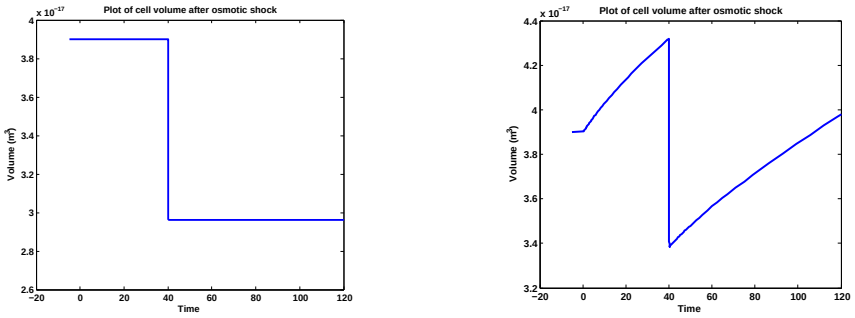
$$\frac{d}{dt}\Pi_i = \Pi_i \cdot \frac{1}{c}\frac{d}{dt}c \tag{21}$$

After osmotic shock, the concentration of osmotically active compounds will increase. This increase leads to an increase in the internal osmotic pressure which will cause the cell to regain its volume. As the cell volume increases there will be a negative feedback. Turgor pressure will begin to rise as a consequence of the increase in volume and this will activate the protein Sln1P. The reactivation of Sln1P will serve to deactivate the MAP kinase pathway and the cell will settle back to steady state. When the steady state is reached the concentrations of the proteins return back to normal levels. This occurs so that the cell does not continue to produce glycerol when it is not needed. Certain mutant cells may not have the ability to accumulate this glycerol or may me unable to stop producing glycerol.

6 Recreating results from the paper

Having discussed the biological and mathematical problems concerned with this paper, it is now necessary to test how well the model performs. To do this it will be useful to compare our output with that from the paper itself. If there are no mistakes then hopefully it should be possible to see the same patterns. A full set of differential equations have been provided which need to be coded up into Matlab using the 'ode15s' solver discussed earlier.

In reality, recreating the findings of the paper turned out to be much harder than was originally thought. Complications in modelling the volume module seemed to provide results that were not quite what was expected. In this section we shall look at some of the issues found when implementing the model. Creating an osmotic shock that was the same size as the paper was relatively straightforward, however it would be expected that the volume and turgor pressure of the cell would gradually increase over time to the level that it was at steady state before any shocks were introduced. The volume of the cell dropped as was expected, but would then not increase over time. This meant that the turgor pressure was also stuck at a very low value which led to uncontrolled increase in the amount of glycerol produced.



(a) Using concentration c

(b) Using glycerol concentration

Figure 2: The effect on volume from two different methods

However, this increase in the amount of glycerol did not seem to increase the internal osmotic pressure at all which contributed to the system staying in the incorrect state.

To improve the results it was suggested that equation (19) should be used instead of equation (20) in the volume module. This equation is very similar and involves the same rates v_{12} and v_{13}. The only difference is the smaller initial condition which hopefully would make the osmotic volume more susceptible to changes in glycerol production. To a certain extent this worked (See figure 2(b)), however this alone has provided some problems. After an osmotic shock has been applied, the volume does increase, but there is a time at which the cell starts to become bigger than it was originally and it still seems to increase from there. There is also a problem with finding steady state before the osmotic shock. Each of the proteins are initialized at some value, however this value may not be in steady state. It is necessary to let the system get into steady state before performing any osmotic shocks. Using equation (19) does not seem to allow the volume to ever reach a steady state and thus giving it an osmotic shock is likely to produce odd results.

Since it appears difficult to improve the volume module it would not be useful to try and draw inferences from the model as a whole. Instead it would be better to take one of the modules separately and look at its reaction to an osmotic shock. The phosphorelay module seems the best for this type of analysis since this module takes the input and activates a protein which starts the other modules running. Equations (3) and (4) related the osmotic shock to a change in the turgor pressure which entered the system by reducing the rate at which Sln1 was converted to Sln1P. It is possible to reduce the rate at a certain time like a switch, thus negating the requirement of turgor pressure.

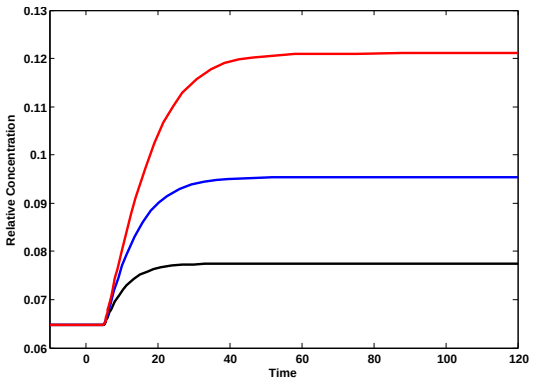


Figure 3: Effects of different size rates for k_1^{TCS} on Ssk1 concentrations: $k_1^{TCS,0}/2$ (black), $k_1^{TCS,0}/3$ (blue), $k_1^{TCS,0}/4$ (red)

It will also be unnecessary to include values for V_{ratio} since there will be no dilution due to cell volume growth. As the volume has been removed we would also expect different behaviours in the proteins. When an osmotic shock occurs proteins should be activated or inactivated, but they will not return to steady state for this experiment. This occurs since the input is just a switch like function and not related to the turgor pressure. The idea of this testing is to see how the strength of the increase in concentrations differs, dependent on a number of factors. When plotting concentrations of proteins We shall look at the percentage of the total concentration in a dephosphorylated state. This allows the relative change to be identified after the osmotic shock has occurred.

One factor to test is how the strength of the osmotic shock affects the increase in Ssk1 production. Figure 3 shows that a stronger osmotic shock will increase the concentration of Ssk1 in the cell. The concentration has been given as the percentage of the total Ssk1 and Ssk1P in the cell. In steady state 6.5% of the available Ssk1 is dephophorylated compared to 93.5% which is. After an osmotic shock which cuts the phosphorylation rate of Sln1 by a quarter, the percentage of Ssk1 increases to 12%. This occurs since a stronger osmotic shock reduces the phosphorylation rate of Sln1 more which increases the amount of Sln1 available which leads to the higher concentration of Ssk1. It is necessary that this occurs since in the full model stronger osmotic shocks will require a larger and quicker response.

In equation (12) there is a rate k_{-2}^{TCS} which may be set at a higher level in the paper than is realistic biologically. In the reaction k_2^{TCS} and k_{-2}^{TCS} are given as the same value $50(\mu \mathrm{M}s)^{-1}$. This would suggest that the phospho-

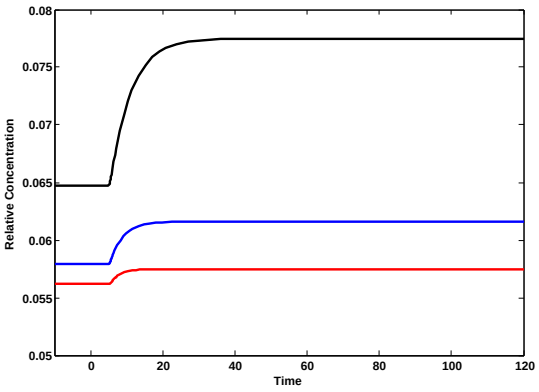


Figure 4: Effects of different k_{-2}^{TCS} $((\mu \mathrm{M}s)^{-1})$ on Ssk1 concentrations: 50 (black), 10 (blue), 0.1 (red)

rylation reaction is occurring in both directions with the same rate. Figure 4 shows what happens to the concentration of Ssk1 if the rate of k_{-2}^{TCS} is decreased. The new rate parameter affects the steady state concentration obtained and also affects the size of the reaction to the osmotic shock. The concentration turns out to be not as strong if the rate parameter is decreased, a fact that suggests that there is no reason to change this rate from a modelling perspective. For this reason for our other experiments the rate k_{-2}^{TCS} will be left at $50(\mu \mathrm{M}s)^{-1}$.

7 Adding in Skn7 protein

The model that we have been studying from Klipp does not give all of the reactions that occur within a yeast cell when osmotic shock occurs. There are other proteins at work to help the cell recover after osmotic shock. We shall focus on adding a second response regulator to the phosphorelay module. Skn7 is phosphorylated in the same way as Ssk1 and it is a transcription factor that regulates cell wall integrity and oxidative stress response (Sato et al. 2003). The modified phosphorelay module with Skn7 added in can be seen in figure 5.

For the purpose of this study, the most interesting question is how the concentration of Ssk1 is affected by the new protein added to the system. The initial concentration of Skn7 has been increased to different values, but no Skn7P has been added initially to the system. Ypd1P is now having to be used to phosphorylate both Ssk1 and Skn7. As a result it would be intuitive that concentrations of Ssk1 would be larger than they would be if

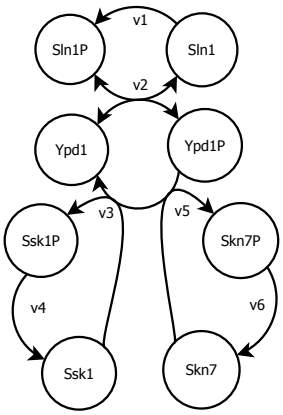


Figure 5: Modified phosphrelay module

Skn7 was not included in the process. There will be less Ypd1P available relative to what there was so less of the Ssk1 will be converted to Ssk1P. This intuition is proven correct by figure 6. An increase in the amount of Skn7 protein will lead to a increase in the concentration of Ssk1 compared to if there was no Skn7 there. As discussed in relation to figure 4 the initial conditions are being altered which leads to a different steady state value. As the concentration of Skn7 in the system is increased figure 6 shows that the steady state value the concentration settles to a larger value. Figure 6 also shows that the size of the jump in concentration after the osmotic shock is also larger with more Skn7 is present. This is a double effect which makes the response to osmotic shock faster and stronger with the introduction of Skn7. It is also significant that when there is no Skn7 the concentration is comparable to that obtained in figure 4 with $k_{-2}^{TCS} = 50(\mu \mathrm{M}s)^{-1}$. This check shows that the new model is working in line with the previous model.

During this study we have set the rates at which Skn7 is interacting with Ypd1 to be similar to those given for Ssk1, whilst only varying the initial total concentration of Skn7. More biologically realistic results could be obtained by setting the rate at which Skn7P dephosphorylates to a lower rate. This would not change the fact that Ssk1 concentrations will be increased by the presence of Skn7, but it might effect the strength of the jump in concentration after the osmotic shock. Results obtained (not shown) seem to suggest that larger rates lead to a larger concentration of Skn7 in the system which will in turn increase the concentration of Ssk1 in the system as well. This is a process that could be explored more in future work.

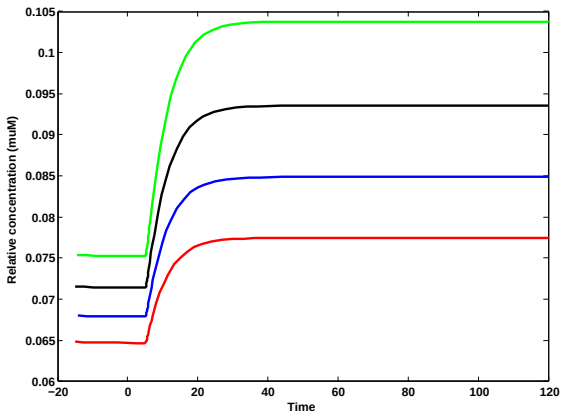


Figure 6: Effects of Skn7 totals on Ssk1 concentrations: 0.015μM (green), 0.010μM (black), 0.005μM (blue), 0μM (red)

8 Conclusion

The aim of this report was to develop a mathematical model which captured the interactions of yeast cells once osmotic shock has occurred. The results obtained have been patchy and at time often hard to derive, however they give an idea of the workings of some of the proteins within yeast cells. When not worried about the cell volume the results derived for the phosphorelay module alone are very interesting. Osmotic shock requires a clear change in the system, however this change is very much affected by the rates in which reactions are occurring within the cell. The strength of the osmotic shock also plays an important role in determining the size of response. The model being interpreted (Klipp at al. 2005) is also by no means the complete system for osmoregulation. We have shown that adding in other proteins such as Skn7 does have an effect on the output concentrations from the system as well.

Given further time it would have been interesting to try and get the whole module fully-functional. Difficulties with the volume module have somewhat restricted the scope of the results which is disappointing. However working without given code it is maybe understandable that there would be some hitches. It would also be possible to add in different reactions into the system which have been omitted. One protein that could be important is Sho1P which helps with osmoregulation (Sato et al. 2003). Adding more and more complexity to this starting point could lead to a very realistic model of osmoregulation in yeast cells.

Whilst working on this it has become clear that in mathematical research

there are no simple answers to modern research problems. The concept of systems biology is a very important one that has and will continue to have a large scientific significance. This report has concentrated on the mathematical modelling of biological systems yet with such breadth and depth it would be possible to look at the problem for many different perspectives. It is this idea that makes sysems biology unique and a great interdisciplinary challenge both now and many years into the future.

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