Modelling of Signal Transduction in Yeast – Sensitivity and Model Analysis

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1. Abstract

Experimental research has revealed components and mechanisms of cellular stress sensing and adaptation. In addition, mathematical modelling has proven to foster the understanding of some basic principles of signal transduction and signal processing as well as of sensitivity and robustness of information perception and cellular response. Here we review some modelling principles, results and open questions exemplified for a model organism, the yeast *Saccharomyces cerevisiae*.

2. Introduction

During their life span, cells face a multitude of stresses and changes in the environment. Most of those changes are normal processes that can happen more or less frequently, like temperature changes, variation in nutrient supply or appearance of a mating partner. Therefore, species had to adapt to such types of stress during evolution and to develop appropriate, specific and efficient mechanisms to cope with such typical demands.
In the last few years, a series of modelling approaches has been used and adopted to support the understanding of the complex behaviour of signalling networks. The concepts range from very abstract models that elucidate some key properties of signalling pathways (e.g. Heinrich et al., 2002, Papin and Palsson, 2004) to very detailed models that precisely monitor the dynamics of specific regulatory events (e.g. Vaseghi et al., 2001, Schoeberl et al., 2002, Yi et al., 2003, Swameye et al., 2003). Systematic overview on structural properties and dynamic features of signalling pathway models are given in (Papin et al., 2005, Tyson et al., 2003).
The complexity of biochemical networks is far from being resolved experimentally. Nevertheless there is need to understand their behaviour in a rational way, which is often hard to achieve by intuition. Establishing models of such networks supports the integration of experimental knowledge into a consistent picture, the formulation of hypotheses and cognitions in a precise language. It serves to test, support, or falsify hypotheses about the underlying

biological mechanism. Modelling may integrate different parts of the whole and thereby allow analysis of properties that only emerge upon the interaction of elements in a comprehensive network. A sound model can produce predictions that can be experimentally tested and it can simulate processes that are experimentally hidden.

3. Modelling: Mathematical Techniques and Tools

3.1. Purpose of Modelling

The development of a model serves the abstract and condensed representation of facts in order to allow for the analysis of their relations and to gain understanding about their internal organization and their communication with the environment.
Although the number of data in biological research currently explodes, such data is useless without sufficient interpretation. A computational model can on hand serve the data interpretation; on the other hand it can point to biological aspects that are still not sufficiently experimentally resolved. Within the field of Systems Biology, the view has been established that experimental research and model development should go hand in hand in an iterative manner including formulation of an initial model, hypothesis generation, experimental testing of hypotheses, model-based experimental design, model refinement upon new data, and so on.
The iterative modeling and experimentation process is hard to follow in publications, since they often only represent the final results. Model improvement with time and with accumulating experimental information is documented e.g. for yeast cell cycle (Novak et al., 1999, Chen et al., 2000, Chen et al., 2004 and others) and for signaling pathways (Bhalla, 2004, Bhalla, 2002, Bhalla and Iyengar, 2001, Bhalla and Iyengar, 1999).

3.2. Model Development

Usually, an experimental observation inspires the formulation of a hypothesis as a first step. In the second step we define what questions the model is supposed to answer, i.e. the *scope* of the model. The scope determines what components and processes the model will take into account or omit and it defines the system's boundaries. Omitting certain processes from the models even though they might play a role is based on the assumption that they have only a minor influence on the event under study, that their values remain constant in the experimental setup, or that they simply cannot be described with the currently available means. For example, the effect of regulated gene expression is usually neglected in the modelling of metabolic networks although modellers are certainly aware of production and degradation of enzymes. But the different time scales of protein turnover and metabolic reactions justify this simplification in many cases. The initial model is usually formulated as a word model. The word model itself is also subjected to a process of refinement and sophistication in the course of model development. A graphical representation of the model structure, e.g. a diagram, is also helpful.

Subsequently, the word model is translated into a mathematical model (for an overview on mathematical techniques see below). To assure that our model is in principle able to answer our initial question we must *verify* whether our model can achieve this independently of choice of specific parameter values, i.e. in a qualitative way. For example, when we want to explain an observed temporal oscillation of a cellular compound, we must test whether our model is structured in a way that it is able to produce oscillations. This might not be as trivial as it sounds in some cases. For example, until now there exists no general theorem for the existence of oscillations in chemical systems with more than three compounds (Heinrich & Schuster 1996). When no mathematical theorem is available that tells us something about the general properties of our system, verification of the proposed model behaviour is generally obtained by playing around with the model structure and its parameters, checking whether it behaves in the way we want. Verification of the model structure is an important step in the process of model development because it can save much time and effort later on. When the model is not able to fit observed data, this might be a general problem of the model structure. Having checked this in advance we can avoid validating a model in vain.
Generally, it is also desirable to learn more about general properties of the model, like e.g. steady states and bifurcation points. When we analyse metabolic systems, we can apply mathematical tools like Metabolic Control Theory to analyse the system.

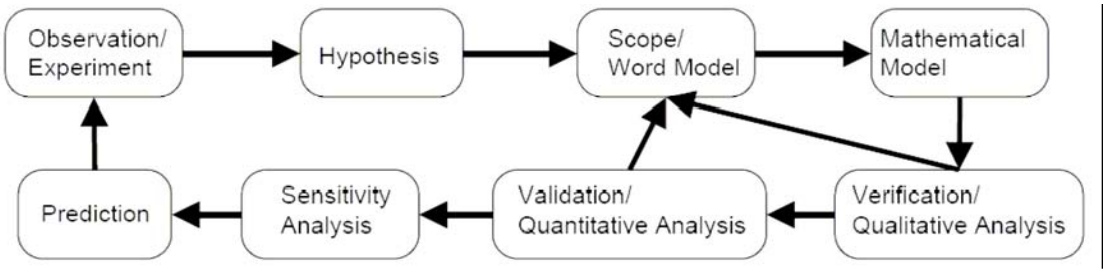

Figure 1: Model development flow chart.

Having verified that the model can principally reproduce our expectations we can now *validate* that the model can also reproduce our observations in a quantitative manner. This is generally achieved by adjusting the model parameters such that the components of the model match observational data. It is important to gain further support for our model by testing whether it is also able to reproduce independent data without changing the fitted parameters. Independent in this sense means that the data was neither used to fit the parameters nor to develop our model. We need a training data set and test data set. The test data generally describe the same phenomena but under slightly different conditions. It is a prerequisite for a sound model validation that the model is able to reproduce observed data under different conditions but with the same parameters that were used to reproduce the training data set. This is supposed to reflect the fact that our model accurately describes the intrinsic structure of the studied system and,

like nature, is able to adequately adjust its reaction to a changing environment/input without changing internal structure and interactions.
It is important to know the limits of applicability of a model. They determine to what extent possible predictions and conclusion hold. Moreover, it is important to know what parameters are sensitive, i.e. whose changes have a substantial impact on the systems behaviour, and thus have to be determined with great accuracy. To this end we must conduct a *sensitivity* analysis. Usually, this is achieved by changing one parameter value at a time and looking at the resulting change of a specific output variable. A classical measure of sensitivity is the relative sensitivity S that is defined as

$$S = \frac{\Delta O}{O} \cdot \frac{p}{\Delta p} \tag{1}$$

where $\Delta O/O$ is the relative change of some output of interest and $\Delta p/p$ is the relative parameter change, compared to the initial state of parameter, respectively. S is easy to interpret, as $S = 1$ means that a certain percentage change of a parameter yields the same percentage change of the considered output. Usually, when $|S| \leq 1$, p is considered as non-sensitive. When $|S| >> 1$, p is considered as sensitive. The range in which p is changed depends on the uncertainty with which p is determined. This can be the measurement error or some other knowledge about the range in which p can vary. With no such knowledge, it is usually a good start to change p by 50%.
Classical sensitivity analysis studies the reaction of one or more output variable to the change of one parameter at a time. Generally, it cannot be assumed that parameters have an independent influence on the considered output. In most cases the sensitivity of one parameter depends on the state of one or more other parameters. However, manipulating individual parameters can be viewed as unusual perturbation of the system by, e.g. a mutation or other kind of damage. It is reasonable to assume that under the conditions we are mostly interested in it is unlikely that many parameters change or are perturbed at the same time.
Having determined sensitive parameters gives us important information about our system. It not only tells us where small measurements errors can have drastic consequences for the system behaviour but also where additional research or measurements might be adequate. Sensitive parameters can also be interesting targets for drug developers as it makes sense to manipulate a system where it is most sensitive. Sensitivity analysis tells us something about the robustness and resilience of the system.
It is not only important to explore the sensitivity of the system to parameter changes but also to changes in the input stimuli. Biological systems are always subjected to varying environmental conditions and we must check whether our system is as flexible as we expect it to be. Moreover, a structural sensitivity analysis, i.e. not only changing parameters but also model formulas, can give valuable information what features of the model are necessary to exhibit a certain behaviour and what parts can be omitted or simplified.
The sensitivity analysis relates to and complements the two preceding steps

verification and validation. Verification tells us something about the theoretical properties of our model system, how the model could behave, i.e. the qualitative structure of the state space. Validation determines a concrete state of the system that reflects observed biological phenomena, i.e. tells us where our system is quantitatively located in the theoretical state space. Finally, sensitivity analysis provides us with a quantitative picture of the state space around our system.
We can then use the model to explore more systematically regions of the state space that are of particular interest, i.e. make predictions. The model ideally should be able to predict future experiments. When the model correctly predicts the experiments we gain confidence in the model and also in the original hypothesis. Moreover, the model can be used to design future experiments. In combination with the sensitivity analysis we can determine where additional measurements give us most information about the system.
In case, the model does not correctly predict the experiments it has to be checked whether the experiments still comply with the original hypothesis. If it does we have to modify the model, otherwise we have to modify the hypothesis. Both ways, we close the cycle.

3.3. Mathematical Description of Dynamic Processes

Depending on the available experimental information, the purpose of modelling, the experience and preference of the modeller, signalling pathways can be described with different techniques. In general, all approaches rely on a description of the network structure with a graph representing as edges the interaction (activation, inhibition, complex formation) between the nodes, i.e. the different signal molecules. Boolean networks or Petri nets describe the states of individual nodes in a discrete fashion and these states are updated along a discretised time axis according to the rules assigned to the edges. In their basic version, Boolean networks allow only for two states (1 or 0, i.e. active or not active). Petri nets assign individual tokens to the places (i.e. nodes). More sophisticated approaches tend to consider more different states and update rules.
The dynamics on a continuous time scale can be simulated in a stochastic manner, e.g. with one of Gillespie's methods (e.g. Gillespie, 1977) by assuming discrete state values, e.g molecule numbers. A frequent approach is the description with ordinary differential equations (ODEs), where the state space is continuous (concentrations or activities) and the time is continuous. In the following we will focus on the ODE model approach.
The dynamics of the biochemical reaction network is expressed by the balance equations

$$\frac{d\boldsymbol{S}(t)}{dt} = \boldsymbol{N}\boldsymbol{v}(\boldsymbol{S}(t), \boldsymbol{p}) \tag{2}$$

where $\boldsymbol{S}$, $\boldsymbol{v}$, and $\boldsymbol{p}$ denote the vectors of concentrations, reaction rates, and parameters of the system, respectively, and t is the time. The matrix $\boldsymbol{N}$ contains the stoichiometric coefficients. Typical expressions for the reaction rates are the so-called mass action rate law

$$v_i(S_j) = k_i \cdot S_j \tag{3}$$

or the Michaelis-Menten rate law

$$v_i(S_j) = \frac{V_{max} S_j}{K_M + S_j} \tag{4}$$

or the Hill kinetic

$$v_i(S_j) = \frac{V_{max} S_j^n}{K_{0,5}^n + S_j^n} \tag{5}$$

The mass action law implies a linear dependence of rate on substrate concentration, while hyperbolic Michaelis-Menten kinetics and sigmoid Hill kinetics show saturation. Note that more elaborated kinetic mechanisms are described, especially for more substrates and for reversible reactions (Cornish-Bowden, 2004).

In the cell, signalling pathways have to cross several boundaries: the cell membrane, the nuclear envelope, the mitochondrial membranes or others. This may make it necessary to include different compartments into the model. Moving between compartments has different effects in discrete or continuous settings: if one molecule leaves a compartment, then one molecule will arrive in the neighbouring compartment. If one µm of a substance leaves a compartment, the concentration change in the neighbouring department depends on their relative volumes.

3.4. Analysis of Models

The model can be analyzed in various ways, first to test whether its behaviour really reflects the aspects that we wanted to represent, second to deduce predictions based on a presumably appropriate description.

Purely based on the stoichiometry, i.e. on the wiring, is the analysis of the stoichiometric matrix ***N***. The linear dependence of rows of the stoichiometric matrix points to moiety conservation in the system, i.e. it reveals which compounds or moieties are neither produced nor degraded by the network in total, such as the sum of differently modified forms of a protein. In mathematical terms, one has to find a regular matrix ***G*** such that $G \cdot N = 0$. Then $G \cdot S = const.$ expresses the conservation relations. The linear dependence of columns of ***N*** ($N \cdot K = 0$ with regular matrix ***K***) reveals the dependence of fluxes in steady state, i.e. steady state fluxes are linear combinations of the columns of matrix ***K***. For example, in an unbranched pathway, all fluxes must be the same in case of steady state.

Flux balance analysis (FBA) is based on the relations revealed for fluxes in steady state. To elucidate operation modes of the cell under different environmental conditions or to suggest such modes for biotechnological

processes, it calculates from all possible steady state fluxes that set of fluxes that maximizes or minimizes a certain function of these fluxes, e.g. by linear programming.

Metabolic control analysis (MCA) seeks to quantify the impact of individual rates or parameters on the steady state values of variables by calculating the respective derivative. In MCA a version of the above-defined sensitivity *S* is often applied, the response coefficient *R*, that is actually nothing else than the sensitivity *S* of the linearised system

$$R = \frac{p}{O} \cdot \frac{\partial O}{\partial p} \quad (6)$$

The theorems of MCA (Reder, 1988) establish a relation between *R*, which is a property of the whole system, and the local sensitivities of the individual rates with respect to the compound concentrations and the network stoichiometry ***N***. Especially interesting for signalling pathways is the analysis of time-dependent response coefficients $^{v_i}S_j$

$$R(t) = \frac{p}{O(t)} \cdot \frac{\partial O(t)}{\partial p} \quad (7)$$

which show the impact of a parameter value on the dynamics of a compound, not only on its steady state value (Ingalls and Sauro, 2003).

4. Modelling Cell Signalling: Concept and Examples

4.1. Components of Signalling Pathways

Despite their diversity in function and design, many signalling pathways use the same essential components, which are often highly conserved through evolution and between species. For example, proteins in yeast pathways have homologs in human pathways and G proteins or MAP kinases are conserved throughout kingdoms. Here, we will introduce the most prevalent signalling pathway modules that are frequently connected in series.

Receptors receive extracellular stimuli by ligand binding and transmit a signal to intracellular signalling molecules. Many receptors are transmembrane proteins. Upon signal sensing, they change their conformation and become active (**Figure 2A**), now being able to initiate downstream processes. Cells can regulate the number and the activity of specific receptors, e.g. in order to shut off the signal transmission during sustained stimulation. An interplay of production and degradation regulates the number of receptors (for a model involving receptor internalization in the yeast pheromone pathway see (Yi et al., 2003)). Phosphorylation of serine/threonine or tyrosine residues in the cytosolic domain by protein kinases can regulate the activity and thereby adapt the signalling system to input signals of different intensity.

A possible way of signal transmission from the receptor is the binding to and the activation of G proteins. The heterotrimeric G protein consists of the subunits α,

β, and γ (**Figure 2B**). Upon activation, a GDP bound to the α-subunit is exchanged with a GTP, and the G protein dissociates into different subunits, which transmit the signal to downstream processes. As soon as the GTP is hydrolyzed to GDP, the subunits can re-associate to form the initial heterotrimeric G protein.

The change between GTP- or GDP-bound states is also characteristic for so-called small G proteins like Ras, Rho, Rab, Ran, or Arf. They have different activities in both forms (**Figure 2C**). Transformation from the GDP state to the GTP state is catalyzed by the Guanine Exchange Factor (GEF), while the reverse process is facilitated by a GTPase-activating protein (GAP), which induces hydrolysis of the bound GTP (Schmidt and Hall, 2002).

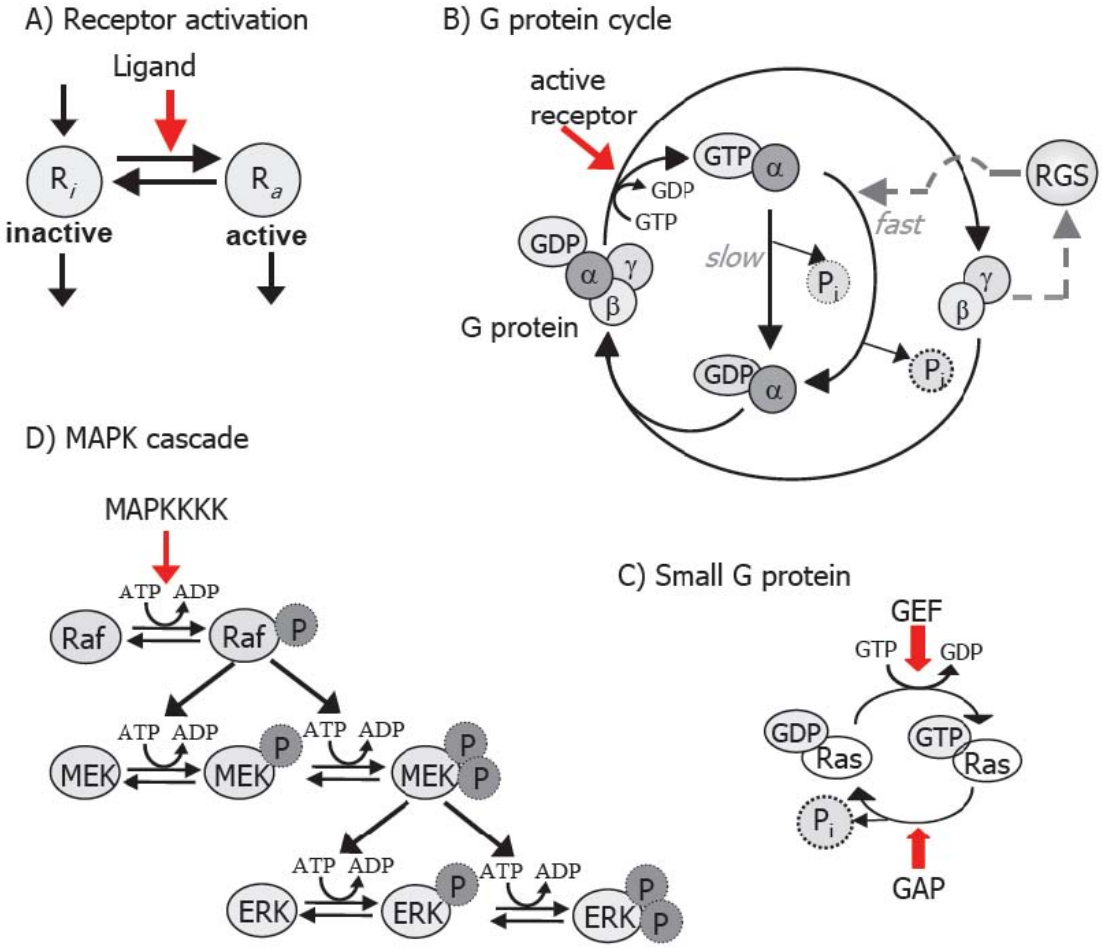

Figure 2: Building blocks of signalling pathways. A) Activation of the receptor by a ligand, B) G protein cycle including slow and fast mode; the fast mode is activated by feedback loop involving a protein (RGS), C) Small G protein switch between two states, GDP-bound and GTP-bound, D) the MAP kinase cascade involves several successive phosphorylation events.

Extracellular signal-regulated kinase (ERK) or mitogen-activated protein kinase

(MAPK) cascades consist of three or four different proteins that specifically catalyse the phosphorylation of the subsequent proteins (**Figure 2D**). According to their roles, these kinases are called MAP kinase (MAPK), MAP kinase kinase (MAPKK), and so on. The dephosphorylation is ensured by phosphatases that are often less specific, but can also be very specific to certain targets. In some cases, the MAP kinases bind to a scaffold protein forming a complex.
Several functions for such scaffold formation are discussed, such as to ensure the physical vicinity of components or their correct molecular orientation or an increase in signal amplification. Scaffolding can account for the fact that signalling pathways often appear to be decoupled although they contain common components.

4.2. Stress Response Pathways in Yeast

The response of yeast cells to external stimuli, environmental changes, nutrient supply or availability of a mating partner is ensured by a variety of signaling pathways that partly overlap by the use of common proteins (**Figure 3**).

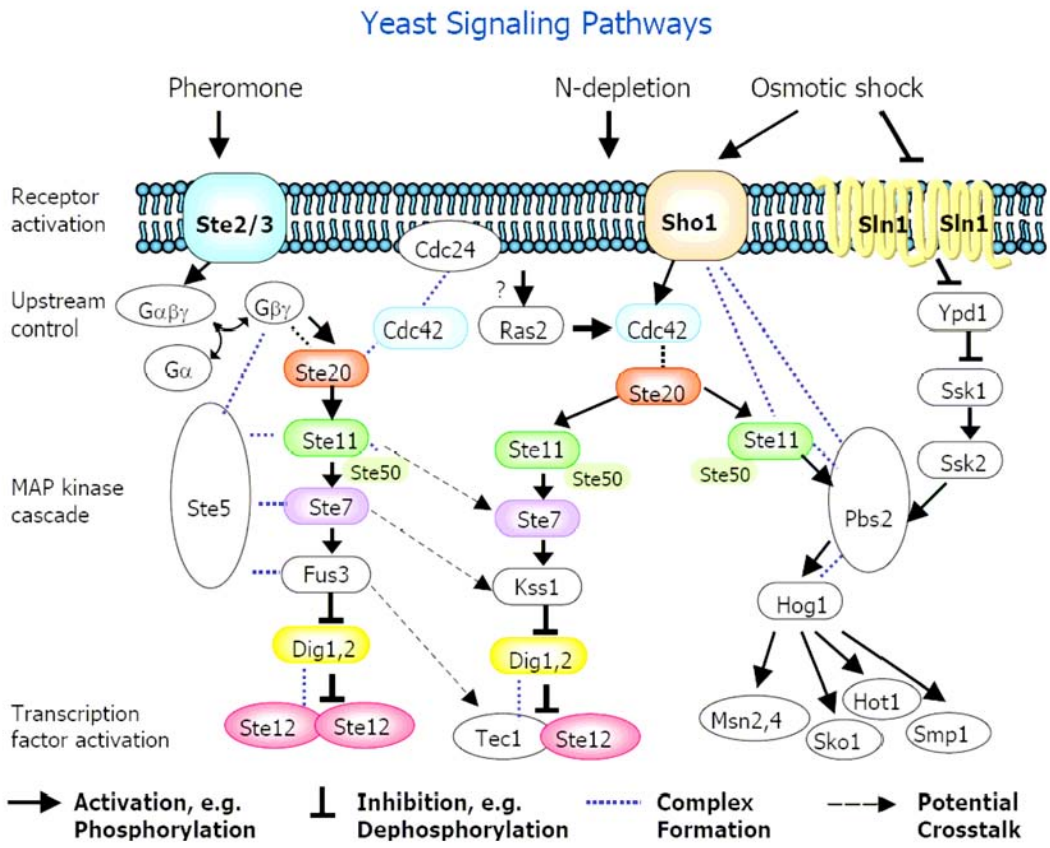

Figure 3: Selected signalling pathways of the yeast Saccharomyces cerevisiae. Shown are the pheromone pathway, the filamentous growth pathway (responding to starvation signals) and high osmolarity glycerol (HOG) pathway. These pathways share several components, and mechanisms for ensuring signal specificity and appropriate signal integration are still under investigation.

Signal transduction in yeast has been studied thoroughly; an overview is given for example in (Hohmann, 2002). Several quantitative models have been published so far and some of them are collected in databases like JWSonline (Snoep and Olivier, 2003). Yi and colleagues (Yi et al., 2003) presented a first model of the G protein activation within the pheromone pathway. This model takes into account G protein activities that have been measured using fluorescence resonance energy transfer (FRET). It comprises the production, degradation and activation of the G protein coupled α-receptor (Ste2), the activity cycle of the G protein and its regulation by the regulator of G protein (RGS) Sst2 (compare **Figure 3**).

This model has been adapted and incorporated into a more comprehensive model of the pheromone pathway (Kofahl and Klipp, 2004), which includes downstream processes of the activation of Gβγ. As shown in **Figure 3**, the components of the MAP kinase cascade bind to the scaffold protein Ste5. Binding of Ste5 to Gβγ and the MAP KKKK Ste20 brings Ste20 into the vicinity of Ste11, the MAP KKK, permitting its activation. Furthermore, a cycle of binding, phosphorylation and release of the MAPK Fus3 is considered. Phosphorylated Fus3 triggers the following events including the activation of the transcription factor Ste12, the activation of the cell cycle regulator Far1 and the activation of the RGS Sst2.

The pheromone pathway model includes several feedback loops that help to downregulate the pathway after successful signal transduction. First, the activation of Fus3 leads to a repeated phosphorylation of more Fus3 molecules. Secondly, the activation of Sst2 itself depends on the activation of Fus3. It accelerates the closing of the G protein cycle by enhancing the rate of hydrolysis of Gα-bound GTP. Yi et al. (Yi et al., 2003) studied strains with either constitutively active or inactive Sst2. Third, the transcription factor Ste12 enhances the expression of the protease Bar1, which is exported, and cleaves the α-factor, and thereby counteracts the input signal. Hence, the pathway design ensures the long-term downregulation of the pathway after successful activation of target processes.

The parameters of this model have been estimated from literature values. The impact of individual values has been tested by sensitivity analysis. Although this model is not based on data specifically measured to support it, its predictions for graded response to increasing concentration of α-factor or for the behaviour of mutant cells match very well with experimental observations.

The response of yeast to osmotic stress has been described by a model (Klipp et al., 2005) that comprises the high osmolarity glycerol (HOG) pathway, transcriptional regulation, the effect on metabolism and the change in the production of glycerol and an additional model describing regulation of volume and osmotic pressure. The HOG pathway consists of two input branches, the Sln1 branch and the Sho1 branch (which is not considered in the model). The receptor Sln1 is a membrane protein that regulates a phosphorelay system. Under normal conditions, it is continuously phosphorylated and transmits its phosphate

group to Ypd1, which in turn passes it on to Ssk1. In this way, Ssk1 is kept phosphorylated and inactive. Upon osmotic stress, phosphorylation of Sln1 is interrupted and Ssk1 switches to a non-phosphorylated, active state. In this form, it triggers the HOG MAP kinase cascade, which involves the redundant proteins Ssk2 and Ssk22 as well as Pbs2 and Hog1. Phosphorylated Hog1 can enter the nucleus and regulate the transcription of a series of genes.

An interesting feature of this pathway is that it is downregulated despite sustained activation by external osmolarity. This cellular response could not be explained by modelling the signalling pathway in isolation. It was argued that the cells sense turgor pressure instead of the external salt concentration. The turgor pressure is partially regulated by glycerol. Active Hog1 activates the expression of genes coding for enzymes that are involved in the production of glycerol.

The parameters for this model have been determined on the basis of a standard experiment applying 0.5M NaCl to wild type cells and have been tested for various experimental scenarios with mutant cells and different salt concentrations.

Model simulations have revealed details of the signalling process, enlightening the role of the glycerol channel Fps1 in glycerol accumulation, and the feedback control exerted by protein phosphatases in the MAP kinase pathway. It turns out that Fps1 is responsible for the immediate control on the internal glycerol concentration, while the stimulated expression of GPD1/2 and GPP1/2 and the resulting increased glycerol production preserves a high level of glycerol during growth in high osmolarity. The model implies that the HOG pathway is shut off by to glycerol accumulation, cell re-swelling, and turgor increase rather than by enhanced expression of phosphatases. This result has been confirmed by the experimental fact that the pathway can be fully reactivated by a second osmotic stress.

4.3. Studied Phenomena

4.3.1. Relative Importance of Kinases and Phosphatases

MAP kinase cascades are regulated by the activity of kinases that phosphorylate the proteins, and by phosphatases that in turn ensure the dephosphorylation. While kinases activate and phosphatases deactivate, both partners are necessary to determine the basic level of activation in absence of external stimuli, but also strength and duration of activation in its presence. It has been discussed that kinases are responsible for the amplitude of the signal, while phosphatases determine its duration [Hornberg, 2005]. Interestingly, this holds only for weakly activated cascades [Heinrich, 2002], while strongly activated cascades show the tendency of prolonged activation upon increase of stimulus. This is based on conservation of MAP kinase proteins on each level, which limits the increase of the active form upon strong activation (**Figure 4**).

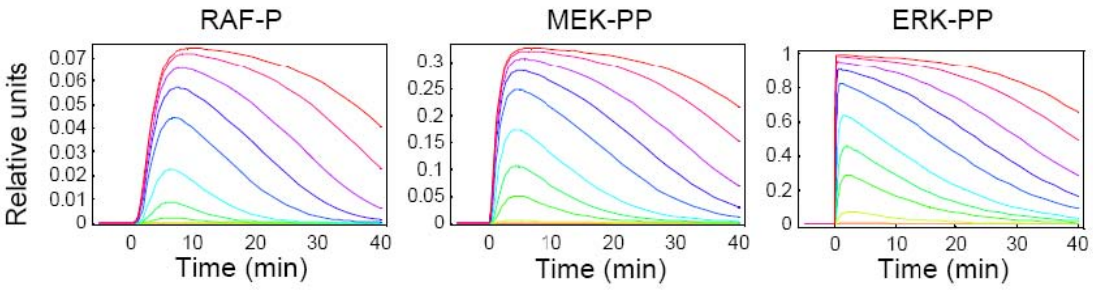

Figure 4: Time courses of the concentration of the phosphorylated forms of three kinases (Raf, MEK, ERK) in the MAP kinase cascade as in Figure 2D, i.e. their activation profiles over time: low activation of the receptor leads to an increase of the amplitude, stronger receptor activation causes longer activation. All rate laws are mass action kinetics with rate constants of kinases and phosphatases equal to 1 and the initial concentration values of the phosphorylated proteins were 0 and of the non-phosphorylated proteins were 1.

4.3.2. Dynamic Behaviour and Parameters

The specific behaviour of a biochemical network is determined by (i) its wiring, expressed by the stoichiometric matrix ***N***, (ii) by the kinetic laws of the individual reactions including the involvement of modifiers that are not substrate or product of this reaction, (iii) by the values of the kinetic parameters and (iv) by the concentrations involved, like initial concentrations and conserved moieties.

In order to obtain a satisfactory picture of the studied object, all four aspects must be appropriate. The wiring scheme is frequently (but not always!) sufficiently well known from experimental information. For some metabolic reactions, the kinetic mechanism is also determined together with the respective parameters. However, kinetic laws and parameters are often not well-defined by experimental information, whereas concentration or number of molecules involved are often known to a satisfactory extent.

To develop models with predictive value, high-quality data is necessary. Time series data must cover the regions, in which the dynamics of the pathways take place. Moreover, for sound model validation and parameterization it is necessary to have a measure of uncertainty for the measured data, as standard deviations, for instance. This requires measurement repetitions to be done that are unfortunately often not available.

4.3.3. Signalling: Network Versus Pathway

The original perception of signalling pathways stems from the experimental analysis that could connect a stimulus of the cell with a measurable effect and could trace the path connecting both. Nowadays, it becomes obvious that cells possess a comprehensive arsenal of signalling molecules that may interact in various combinations giving rise to the transmission of various signals, but also to the integration and separation of diverse types of information.

It is now a matter of taste whether modelling starts immediately with the

complete signalling network, or whether one starts with the individual traditional pathways that are sometimes well understood and then tries to integrate them. Coupling of pathways may be performed in the same way as modelling individual pathways: pathway structure is merged and individual reaction rates are adopted using a mixture of handcrafted rules and intuition. Approaches for systematic model integration are rare. A starting point is SBMLmerge, which combines models implemented in SBML.

4.3.4. Crosstalk Between Pathways

There are many different ways in which signalling pathways can interact with each other, a phenomenon often called crosstalk. For example, different pathways can be triggered by the same receptor or they can share components that, once activated by one pathway, leak into another pathway and thereby activate it. For an overview of different ways of pathway crosstalk see (Schwartz & Baron 1999, Schwartz & Madhani 2004, Cowan & Storey 2003). In modelling crosstalk there has been the issue of quantifying the amount of crosstalk. Some studies analysed the topological and structural properties of signalling networks by, e.g., classifying modes of interaction (Papin & Palsson 2004) or by counting the theoretically possible interactions between pathways (Binder & Heinrich 2004).

As signalling is a transient process one can argue that it is the dynamic behaviour of interacting pathways that is important rather than the static features. Two recent studies address the dynamic features of pathway crosstalk. By analysing the activation of pathways by a so-called intrinsic and an extrinsic stimulus, respectively, one study defined measures for pathway specificity and fidelity (Komarova et al., 2005). These measures give useful insights how pathways interact with each other. However, it is important to note that these measures refer to responses to one stimulus at a time. However, it can be assumed that cells usually process multiple information in parallel and these measures give no clue how signals interact while being transmitted concomitantly, It can be expected that signals amplify or inhibit each other, when transmitted at the same time. Thus, it does not suffice to study each signal in isolation but also to study the cell's response to multiple stimuli at the same time. Schaber et al. (under review) proposed crosstalk measures that include parallel multiple pathway activation called the intrinsic and extrinsic specificity that yield a better understanding of how the pathways dynamically interact.

4.3.5. Modelling and Standards

The purpose of modelling is to provide an abstract description of an instance that fosters the understanding/representation of specific aspects of this instance. Such a model must neglect other aspects for the sake of simplicity, and these neglected aspects will change with a change of the specific question to be answered by the model. Therefore, one cannot establish fixed rules for a model that are valid once and forever. On the other hand, the growing modelling community and the need to communicate with experimental researchers make it necessary to establish some rules how specific aspects should be expressed in a model of a certain type.

A prominent approach for the development of such a standard is the Systems Biology Markup Language (SBML) (Hucka et al., 2003), which serves as a unified exchange language for the description of biochemical network models. Another standard is the Minimal Requirements in the Annotation of Models (MIRIAM) (Novere et al., 2005), a standard for the description and documentation of models in a publication.

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