Automated Ensemble Modeling with *modelMaGe*: Analyzing Feedback Mechanisms in the Sho1 Branch of the HOG Pathway

Jörg Schaber¹,²*, Max Flöttmann², Jian Li², Carl-Fredrik Tiger³, Stefan Hohmann³, Edda Klipp²

¹ Institute for Experimental Internal Medicine, Medical Faculty, Otto von Guericke University, Magdeburg, Germany, ² Theoretical Biophysics, Department of Biology, Humboldt University, Berlin, Germany, ³ Department of Cell and Molecular Biology, University of Gothenburg, Göteborg, Sweden

Abstract

In systems biology uncertainty about biological processes translates into alternative mathematical model candidates. Here, the goal is to generate, fit and discriminate several candidate models that represent different hypotheses for feedback mechanisms responsible for downregulating the response of the Sho1 branch of the yeast high osmolarity glycerol (HOG) signaling pathway after initial stimulation. Implementing and testing these candidate models by hand is a tedious and error-prone task. Therefore, we automatically generated a set of candidate models of the Sho1 branch with the tool *modelMaGe*. These candidate models are automatically documented, can readily be simulated and fitted automatically to data. A ranking of the models with respect to parsimonious data representation is provided, enabling discrimination between candidate models and the biological hypotheses underlying them. We conclude that a previously published model fitted spurious effects in the data. Moreover, the discrimination analysis suggests that the reported data does not support the conclusion that a desensitization mechanism leads to the rapid attenuation of Hog1 signaling in the Sho1 branch of the HOG pathway. The data rather supports a model where an integrator feedback shuts down the pathway. This conclusion is also supported by dedicated experiments that can exclusively be predicted by those models including an integrator feedback. *modelMaGe* is an open source project and is distributed under the Gnu General Public License (GPL) and is available from http://modelimage.org.

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* E-mail: schaber@med.ovgu.de

Introduction

Dynamic models of complex biochemical networks have become an indispensable tool in biochemical and genetic research [1,2,3]. Despite enormous efforts in experimental research in cellular and molecular biology, there is still a substantial uncertainty in both qualitative and quantitative aspects of biochemical networks. These uncertainties need to be resolved by confronting alternative mathematical models with experimental data and by a combination of model selection and parameter fitting [4,5].

Possible combinations of uncertain structures and kinetics directly translate into alternative mathematical models. Generating and managing such candidate models poses a considerable challenge to the modeler. This is mainly because of the combinatorial complexity of model alternatives that often renders it a tedious and error-prone task to implement and handle each model individually. Currently, there is no tool that automatically generates, implements, manages and discriminates a specific user-defined set of candidate models that differ in both structure and kinetics.

Another debated issue is model documentation [6,7]. It is not only the successful models that are of interest to the research community, but also those that failed. Usually, in the course of a modeling project many unsuccessful model versions are tested but only the successful one is finally published. The unsuccessful versions, even though of interest, are never documented, because such documentation is a laborious task and unrewarding task not rewarded.

In order to handle uncertainty in kinetics and model structure, we developed the tool *modelMaGe* that automatically generates candidate models based on a single master model and specified modifications [8]. The generated models are automatically documented such that it is always apparent how they were derived from the master model, thereby keeping track of model alternatives. Finally, all generated models are automatically simulated, fitted to data (if available), and compared. At the end the user is provided with a ranking of the model fits and statistical measures that enable him to discriminate between model alternatives.

The aim of this study was to elucidate which mechanism(s) could be responsible for shutting down the response of the Sho1 branch of the high osmolarity glycerol (HOG) signaling pathway in yeast, a question that was also addressed in a recent paper [9]. In this paper, the authors compared five different models, each employing a different negative feedback mechanism. In all
models the activated Hog1 kinase exerts a negative feedback onto its own activation by deactivating upstream components. The model that fitted the data best included a Hog1-mediated desensitization of Sho1, an upstream membrane protein that interacts with the putative receptors of the pathway [10]. Subsequently, it was shown by experiments that Hog1 phosphorylates Sho1, suggesting that the phosphorylated form of Sho1 displays diminished signaling capacity. This would result in the negative feedback loop suggested by the model and rapid attenuation of Hog1 signaling.

There are, however, experimental observations and theoretical considerations that argue against such a scenario. It is well known that the HOG pathway is a perfect adaptor; following adaptation to high osmolarity the signaling pathway is shut off [2,11,12,13] and phosphorylated Hog1 levels return to the pre-shock situation. From theory it follows that perfect adaptation is impossible in a signaling pathway with a constant signal and a negative feedback of a downstream component to an upstream component. The result will always be either a non-zero steady state or oscillations, either damped or sustained [14,15,16]. In a recent study on simplified signaling networks it was shown that there are in principle two mechanisms that can bring about perfect adaptation [17], a negative integrator feedback [11,13,18] or an incoherent feed-forward loop [19]. In the HOG pathway adaptation is supposedly due to an integrator feedback control, consisting of the accumulation of intracellular glycerol, which balances the osmotic pressure gradient imposed by an osmotic shock [2,11,20]. However, most studies studying the adaptation mechanisms in baker’s yeast concentrated on the wild-type yeast [11,13] or on the Sho1-l knock-out [2].

As indicated in the introduction, the main new feature we wanted to test in order to explain the data is a negative feedback that involved an integral response instead of a transient response (P-Hog1-mediated conversion of active Sho1 (Sho1a) to desensitized Sho1 (Sho1) [Figure 1, reaction v3 in Figure S1 in Supporting Information S1]). We achieved this by assuming that phosphorylated, i.e. activated, Hog1 (P-Hog1) stimulates the production of intracellular glycerol [Figure 1, reaction v11 in Figure S1 in Supporting Information S1]. The newly introduced component Signal mimics the notion that it is the imbalance of internal and external water potential (for simplicity represented by Glycerol and OuterOsmolarity, respectively), that activates the signaling pathway, rather than just the external osmolarity. Therefore, Signal is defined as the difference between OuterOsmolarity and Glycerol. Accumulation of Glycerol can also be achieved by constitutive production of glycerol and impaired outflow through closure of the glycerol channel Fps1, which is also subject to regulation [here by Signal] (Figure 1, reactions v12 and v13 in Figure S1 in Supporting Information S1) [12,22].

We systematically tested various combinations of these different feedback mechanisms, which are depicted in a model tree in Figure 2. For simplicity, we name the generated models according to their number of species.

The candidate models in the leftmost branch are the original model published by Hao et al. (2007) (C10) and simplifications thereof. Simplifications are achieved by leaving out components and/or using simpler reaction kinetics. The two leftmost branches include the feedback where P-Hog1 mediates conversion of active Sho1 (Sho1a) into inactive Sho1 (Sho1) [Sho1 desensitization, Figure 1]. The three rightmost branches include the integral feedback, where pathway activation is regulated by Signal as described above. The three rightmost branches vary in their number of intermediate signaling components with the simplest model C5 only having five components (Figure 1). The respective simplifications of the models in the three rightmost branches
concern assumption about the glycerol accumulation. They either have a regulated glycerol efflux, including P-Hog1 activated and constitutive glycerol production, a constitutive, i.e. non-regulated, glycerol efflux, including only P-Hog1 activated glycerol production or no glycerol efflux, also including only P-Hog1 activated glycerol production. The latter corresponds to the hypothesis that the glycerol channel quickly closes and does not open again in the simulated time frame. Detailed wiring schemes of the master model and all candidate models are shown Figures S2-S13 in Supporting Information S1.

Candidate Model Generation and Discrimination

The candidates were automatically generated by modelMaGe, to which we only provided the master model (Figure 1), and the directives specifying which components should be removed for each candidate model and which kinetics should be used. The master model is formulated in Copasi-format, because the parameter estimation task also has to be specified, when the candidate models are supposed to be fitted to data (see Methods section). Model generation, fitting and ranking is then automatically performed by modelMaGe using Copasi as the simulation engine by a single command (see Supporting Information S1). The master model, the directives for modelMaGe, the data and other details are supplied in Supporting Information S1. The ranking of the candidate models according Akaike Information Criterion corrected for small sample sizes (AICc) is displayed in Table 1.

In terms of accuracy of the fit (SSR) the model by Hao et al. (2007) both in its original form as well as in the simplified version with Michaelis-Menten kinetics (C10 and C6b) performed best. In fact, the fits are even better than with the parameter set from the original publication (last line in Table 1). However, C10 is ranked lowest according to the AICc, because of its high number of parameters. Thus, in terms of parsimonious representation of the data it performed worst. Time course simulations of the original model C10 with the original parameter set from Hao et al. (2007) showed damped oscillations in the P-Hog1 concentrations (Figure 3). The C10 model with the new parameter sets converges to sustained oscillations around a steady state, which both with the original parameter as well as with the newly fitted parameters increased with increasing osmotic shock (Figure 3), as expected from theory.

Recent publications on the Hog1 dynamics upon osmotic shock in yeast with a much higher time resolution [11,13,23] imply that oscillations as well as increasing steady state concentrations are spurious effects and features that are not present in the data. Fitting spurious effects in the data is an indication of an over-fitted model. The most prominent dynamic feature of the P-Hog1 time series, i.e. a rapid increase and slower decline to the initial state, can faithfully be captured by the most simple three-parameter model C5c (Figure 4). In terms of parsimonious representation of the data (AICc) this model is ranked highest.

To challenge a critical qualitative property, we tested which of the model candidates did or did not show perfect adaptation behavior by comparing initial and steady-state simulated Hog1 activation after adaptation. Models were considered not to show perfect adaptation when their simulated steady-state value of P-Hog1 one hour after simulation was above 5% the total protein concentration. We employed the 5% threshold, because we consider this value close to the measurement error, i.e. a measured value of 5% of the maximum is practically zero. Therefore, we treated simulated values below 5% of the possible maximum as zero and therefore perfectly adapted. Strikingly, only those models that did not include an integrator feedback (C10, C5a, C6b) were not able to show perfect adaptation according to this criterion.

Model Predictions for Triple Shock

Over-fitted models, even though they tend to identify spurious effects are often better in predictions than under-fitted models [24]. We tested whether the simple C5c model was under-fitted by predicting and comparing simulations to additionally measured data of P-Hog1 time courses after repeated osmotic shock with 0.4 M KCl for both, C5c and the C10 model (Figure 5). The amount of KCl was added to the culture three times with 30 minutes intervals.

Upon triple shock, the C5c model replicated the single shock P-Hog1 profile a third time, as it is also seen in the data. The C10 model with the original parameter set showed no Hog1 activation

| Rank | Model | k | SSR | AICc | feedback | Hog1-PSS |
|------|-------|---|-----|------|----------|----------|
| 1.   | C5c   | 3 | 0.251 | -38.045 | I | <0.05 |
| 2.   | C5b   | 4 | 0.251 | -34.104 | I | <0.05 |
| 3.   | C7c   | 5 | 0.259 | -31.316 | I | <0.05 |
| 4.   | C6a   | 12 | 0.061 | -29.246 | D | >0.05 |
| 5.   | C7b   | 6 | 0.258 | -27.373 | I | <0.05 |
| 6.   | C7a   | 9 | 0.153 | -26.465 | I | <0.05 |
| 7.   | C5a   | 7 | 0.241 | -25.091 | I | <0.05 |
| 8.   | C8c   | 7 | 0.259 | -23.335 | D+I | <0.05 |
| 9.   | C8b   | 8 | 0.258 | -19.393 | D+I | <0.05 |
| 10.  | C8a   | 11 | 0.153 | -14.553 | D+I | <0.05 |
| 11.  | C6b   | 6 | 0.740 | -1.069 | D | >0.05 |
| 12.  | C10   | 20 | 0.049 | 164.842 | D | >0.05 |
|      | Hao   | 20 | 0.181 | 205.92 | D | >0.05 |

k: number of parameters. SSR: sum of squared residuals as calculated by Copasi. AICc: Akaike Information Criterion corrected for small sample size. feedback: the type of feedback employed by the model (D: Sho1 desensitization, I: integrator feedback). doi:10.1371/journal.pone.0014791.t001

**Figure 2. Model tree.** Schematic representation of the generated candidate models and their features. Models are named according to their number of species. The numbers in the subscript indicate the number of fitted parameters. doi:10.1371/journal.pone.0014791.g002
upon a third consecutive shock. This can be explained by the fact
that all activated receptor protein Sho1 (Sho1a) was already
desensitized after the second shock (Sho1i, light gray dashed line in
Figure 5) and not yet recycled again, in order to be able to react to
a third shock (dark gray dashed line in Figure 5). The C10 model
with the new parameter set was able to show a third P-Hog1
response. However, the third response was weakened and again
resulted in sustained oscillations around an even higher steady
state concentration. Interestingly, with the new parameter set the
model C10 was only able to react to a third shock at the expense of
the desensitization mechanism of activated Sho1 (Sho1a), i.e. Sho1i
showed no response at all (light gray curve in Figure 5). In fact, the
velocity of the reaction that facilitated the conversion of
Sho1a to Sho1i was at the lower boundary allowed in the parameter
estimation ($10^{-6}$, Supporting Information S1) and therefore
negligible. The time courses of Sho1a showed an oscillatory
behavior (dark gray curve in Figure 5) corresponding to the
oscillations in P-Hog1.

**Discussion**

The aim of this study was to analyze feedback mechanisms in
the Sho1 branch of the HOG pathway that are best supported by a
data set of the dynamics of P-Hog1 upon single and double shock.
The use of modelMaGe allowed us to systematically explore an
ensemble of model candidates, also documenting unsuccessful
candidates. The results are completely transparent, comprehensible
and easily communicated to the community, as the master
model, the data, as well as the directives how to generate candidate
models are described in a compact and comprehensible manner.
Moreover, the fitting and ranking procedure can be reproduced
online at http://modelmage.org using the master model and the
reduction directives provided in Supporting Information S1.

The generated models comprised the best model of Hao et al.
(2007) as well as other alternatives including several types of
transient and/or integrator feedbacks. The set of candidate models
was automatically generated and fitted to data given in Hao et al.
(2007). In addition, modelMaGe automatically generated a ranking
of the fitted models according to the Akaike Information Criterion
corrected for small sample size ($\text{AIC}_c$).

We show that according to the $\text{AIC}_c$ the three-parameter C5
model approximates the data better in terms of parsimony than
the 20-parameter C10 model. The original model seems to fit
spurious effects in the data, indicating that it was over-fitted.
Instead, our parsimonious three-parameter model could predict
the triple shock Hog1 activation profile better than the C10 model
with the original parameter set. We found also a new parameter
set for the original C10 model that fitted the data best, but was
ranked worst according to the $\text{AIC}_c$, because of its high number of
parameters. The C10 model with the new parameter set was able
to predict the triple shock Hog1 activation profile, but only at the
expense of the feedback mechanism that was actually proposed.
Therefore, we conclude that even though Hao et al. (2007) show
that Hog1 phosphorylates Sho1 and thereby dampens its own
response, the single- and double-shock data they provide do not
support the hypothesis that it is this desensitisation mechanism

![Figure 3](https://example.com/f3.png)

**Figure 3.** Time course simulations of P-Hog1 for single ($t=0$) and double ($t=0,t=30$ min) osmotic shocks of different
centrations for the C10 model, both with original parameters from Hao et al. (2008) (dashed lines, Orig.) and re-fitted
parameters (lines, New).
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![Figure 4](https://example.com/f4.png)

**Figure 4.** Time course simulations of P-Hog1 for single ($t=0$) and double ($t=0,t=30$ min) osmotic shocks of different
centrations for the C5 model.
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which leads to rapid attenuation of Hog1 signaling in the Sho1 branch. Our model discrimination analysis rather supports the hypothesis that there is a negative integrator feedback acting through glycerol accumulation. This could be tested by measuring internal glycerol concentration for the Ssk2/22 mutant as it has been done for the wild type [2], however, this is out of the scope of our study. Glycerol accumulation mediating adaptation and Hog1 de-activation probably acts via removal of the stimulus, which in turn might be volume or membrane related, e.g. turgor pressure [25]. It has been shown that for the wild type and the Sho1 branch of the HOG pathway that such an integrator feedback are probably responsible for the adaptation response. Here, we provide computational as well as experimental evidence that this is also the case for the Sho1 branch [2,11,12,13]. The rapid attenuation of the signal indicates that there is not necessarily a transcriptional-translational response involved. It has been suggested that this fast integrator feedback by fast accumulation of glycerol can be achieved by a fast activation of glycerol production that does not involve a transcriptional-translational response and/or by rapid closure of the glycerol channel Fps1 [2,11]. Indeed, the simple C5c model that does not include glycerol efflux can be interpreted with both a transcriptional-translational response and as fast closure of the glycerol channel. However, we do not refute that the proposed negative feedback of Hog1 onto Sho1 modulates the Hog1 response and may serve other functions than Hog1 deactivation, e.g. stability of the response, noise filtering, inhibiting crosstalk to other pathways or dose-response alignment, as suggested for the pheromone pathway [26].

We also conclude that modelMaGe is a useful tool that facilitates systematic testing a set of candidate models, making the modeling process and its results transparent to the community in an easy and comprehensible manner.

**Methods**

Model generation and discrimination

The main idea of modelMaGe is simple: model alternatives are generated from a master model that includes all alternatives of interest. The master model is the only place that is meant to be manipulated by the modeler, which avoids errors that are introduced by handling several models at the same time. The general workflow is depicted in Figure 6.

Generation of candidate models in modelMaGe is a two step process. The first step is to create a master model in Copasi [27] or in any other SBML [28,29] compliant editor like CellDesigner [30] or SemanticSBML [31]. The master model is a combination of all candidate models that are to be generated and simulated. Thus, the master model must include all possible species and reactions that shall be included in any of the candidate models. In the second step, the set of candidate models is generated by removing reactions, species or modifiers and combinations thereof from the master model and/or by assigning alternative kinetics to certain reactions. The removal of components and exchange of kinetics is done by giving simple logical directives to the program. Details of the usage, technology and algorithms are described in Flottmann et al. (2008) and at www.modelMAGE.org.

The generated models come as a set of both SBML and Copasi files that can readily be simulated by appropriate tools, e.g. Copasi or CellDesigner (Figure 6). When data for certain components is available, modelMaGe can automatically fit the models to the data by estimating parameters. For simulation and parameter estimation ModelMaGe utilizes the COPASI simulation engine CopasiSE. The parameter estimation task is most conveniently defined in Copasi’s graphical user interface. The user has to set up the parameter estimation task only once for the master model. modelMaGe automatically defines the parameter estimation task for all generated candidate models. Using the results of the parameter estimation, modelMaGe computes the Akaike Information Criterion corrected for small sample sizes ($AIC_c$) [24] for each candidate model:

$$AIC_c = 2k + n \left( \ln \left( \frac{2\pi SSR}{n} \right) + 1 \right) + \frac{2(k+1)}{n-k-1}$$

where $SSR$ is sum of squared residuals, $k$ the number of parameters and $n$ the number of data points. The $AIC_c$ is an information-theory based measure of parsimonious data representation that incorporates the goodness of the fit ($SSR$) as well as the complexity of the model ($k$) and is used to rank the candidate models, thereby giving an objective measure for model selection and discrimination. There also exists a web-based version of modelMaGe (http://modelimage.org). For a detailed discussion on the AIC and its usage in model discrimination please refer to [24].

Comparison between model simulation and data

The measured data is scaled relative to maximal measured value + standard deviation and therefore has arbitrary units. Accordingly, for the simulated values of phosphorylated Hog1 an assumption has to be made what percentage of the total Hog1 is...
phosphorylated upon maximal phosphorylation. For simplicity, we assumed that maximally 100% of the total Hog1 can be phosphorylated. As can be seen in Figure 3 and 4 this assumption fits nicely to that data. The measured maximum scaled value of $P$-Hog1 was 0.92 (Figure 3 and 4) and it is known that a) only the phosphorylated form enters the nucleus and b) upon strong stimulation almost all Hog1 enters the nucleus [32]. Therefore, it is a reasonable model result that upon stimulation with 1 M KCl around 90% of the total Hog1 becomes phosphorylated.

**Western blotting**

*Saccharomyces cerevisiae* cells BY4741 ssk1Δ (BY4741; Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ssk1::kanMX4, from the Saccharomyces Genome Deletion Project) were grown in synthetic complete medium (1x Difco™YNB base, 1x Formedium™Complete Supplement Mixture, 0.5% ammonium sulfate, 2% glucose) on a rotary shaker at 225 rpm at 30°C until reaching an optical density of 1.0 measured at 600 nm. Cells were osmotically shocked as noted with KCl from a 4M stock solution. Samples of 1 ml were taken and cells harvested by centrifugation at 14000 rpm for 30 s and the pellet frozen in liquid nitrogen. Times given in the data are the times of freezing. Total protein extracts were made from the frozen cell pellets by boiling for 6 min in 60 μl extraction buffer (Tris-HCl 75 mM, pH 6.8, Glycerol 15%, DTT 150 mM, SDS 3%, NaF 8 mM, Na$_3$VO$_4$ 75 μM, β-mercaptoethanol 0.11%). Protein samples were separated using SDS-PAGE (Tris-Cl) and transferred to nitrocellulose. Phosphorylated and total amounts of Hog1 protein were detected using antibodies #9211(Cell-Signaling Technology) and #yC-20(Santa Cruz Biotechnology) respectively. The membranes were processed for infrared fluorescent detection using secondary antibodies #926-32223(LI-COR biosciences) and #926-32214(LI-COR biosciences) respectively, and scanned for both fluorescent channels using an ODYSSEY IR-scanner(LI-COR biosciences). The signal from phosphorylated Hog1 was divided with the total Hog1 protein signal. The measurements were repeated three times with independent cell cultures (Figure S14 in Supporting Information S1).

**Supporting Information**

*Supporting Information S1* The supporting information, including supplementary figures. Found at: doi:10.1371/journal.pone.0014791.s001 (0.74 MB DOC)

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**Author Contributions**

Conceived and designed the experiments: JS MF SH. Performed the experiments: JS MF CFT SH. Analyzed the data: JS MF JL SH. Wrote the paper: JS SH EK.

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