Analysis of a Model of σ^B Activation following Glucose Starvation

Ulf W. Liebal†, Praveen Kumar Sappa‡, Hendrikje Hildisch‡, Thomas Millat†, Leif Steil‡, Michael Hecker‡, Uwe Völker‡, Olaf Wolkenhauer†

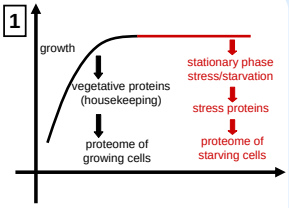
†University of Rostock, Systems Biology and Bioinformatics Group, Germany;
‡Ernst-Moritz-Arndt-University Greifswald, Institute for Microbiology and Institute for Genetics and Functional Genomics, Germany;

The BacII-SysMO project

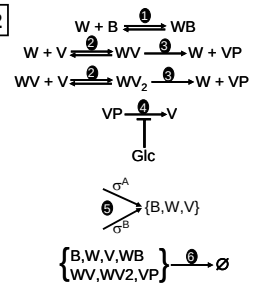
The SysMO project is a European transnational funding and research initiative on "Systems Biology of Microorganisms". The goal pursued by SysMO is to record and describe the dynamic molecular processes going on in unicellular microorganisms in a comprehensive way and to present these processes in the form of computerized mathematical models.

The objective of this project is an integrated understanding of the metabolic and genetic network that controls the transition from growth to glucose starvation, as shown in ***Fig.*** 1. This transition is a fundamental ecophysiological response that serves as a scientific model for environmental signal integration and is pivotal for industrial fermentations of Bacillus that occur predominantly under nutrient starvation.

1



2

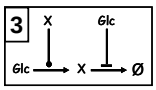


The σ^B regulon (***Fig.*** 2) confers *B. subtilis* with the ability to respond to stress stimuli and adapts it for future stress incidents.

- Anti-sigma factor W binds B thereby precluding formation of RNA-polymerase holoenzyme. (*React.* 1)
- The affinity of V towards W (*React.* 2) is reduced by phosphorylation of V by W (*React.* 3).
- Following Glc-starvation VP dephosphorylation rate is increased resulting in V increase. (*React.* 4)
- V associates with W thereby reducing free W level. (*React.* 2)
- Reduced W level stimulates dissociation of WB complex. (*React.* 1)
- Increased levels of B (σ^B) associates with RNA-polymerase to induce expression of genes. (*React.* 5)
- Proteins and complexes are degraded with a first order mechanism (React 6)

Direct Parameter Estimation Approach

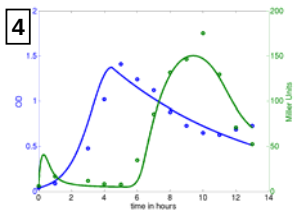
3



Glucose(Glc)-Biomass(X) model

Simulation of Glucose Starvation:
- *ctc::lacZ* reporter gene construct provides information on the transcriptionally active B level.
- Glc concentration is derived from the OD with a fitted model shown in ***Fig.*** 3

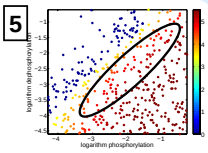
4



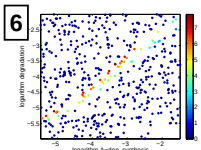
Interpretation of Simulations:
Parameter estimation process (***Fig.*** 4) using sequentially particle swarm and simulated annealing optimization still allows for large possible parameter realizations with good fitting. The model-experiment relationship is therefore non-identifiable.
The simulations agree with the principles of general stress response outlined in the introduction.

Parameter Fitness Correlations

5



6



Goal:
determining parameter ranges that satisfy pre-defined observations for the fitness of stress response.

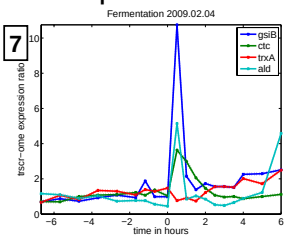
Fitness: low level of free B, high level of WB complex.

Procedure:
1. select two parameters & corresponding boundaries
2. randomly combine the two parameter values
3. evaluate the model fitness for each combination

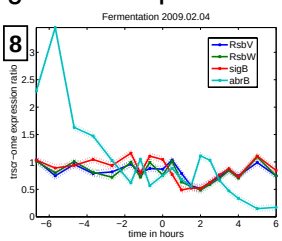
Results:
phosphorylation and dephosphorylation: Antagonistic reactions, only a narrow parameter region is physiological feasible. (***Fig.*** 5)
protein expression and degradation: Antagonistic reactions, but fitness is very sensitive on the balance of both reactions. (***Fig.*** 6)

Transcriptomic Results for Regulon and Operon

7



8



Transcriptome:
- activation of expression of the σ^B regulon during entry into starvation (***Fig.*** 7).
- expression of the operon fails to increase (***Fig.*** 8) despite the positive transcriptional feedback loop of σ^B on its operon.

Possible reason: sigma factor competition on the operon.

Conclusions and Perspectives

A model was developed that reproduces the available experimental data. However, the models are non-identifiable meaning that non-unique parameter sets can reproduce the data. To render the model identifiable, model reduction processes will be conducted to lump parameters and combine components. Additionally, we will gather more diverse data including concentrations of components in the regulation upstream of B-activation.

The fitness of parameter combinations shows high robustness of the system against changes in WV association rate. Considering pre-stress steady state fitness conditions will help to determine missing parameter values. Similar investigations will be conducted for possible steady state conditions during long lasting stress conditions.

Transcriptome results show that gene-specific sigma factor competition needs to be implemented in the models and that unknown post-transcriptional events modulate protein concentrations.

Universität Rostock Traditio et Innovatio

Contact: *Thomas.Millat@uni-rostock.de*

Systems Biology & Bioinformatics group
Department of Computer Science
University of Rostock, 18051 Rostock, Germany

SPONSORED BY THE Federal Ministry of Education and Research