Elucidating Unexpected Reporter Signals for σ^B Activity in *B. subtilis*

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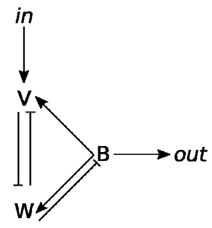
Background



• The σ^B regulon confers *B. subtilis* with the ability to respond to various stress stimuli and adapts it for future stress incidents. Metabolic expenditures are reduced, in turn, expression of about 150 protective genes is activated.

• Starvation activates the anti-anti sigma factor RsbV that inhibits RsbW by complex formation. RsbW itself is the anti sigma factor of σ^B. Hence, an inhibition of RsbW results in release of σ^B and consequently in global expression changes. RsbW can phosphorylate RsbV thus inactivating it. The proteins RsbW and RsbV are transcribed by σ^B and it is assumed that increasing levels of RsbW lead to an inactivation of RsbV and to an adaptive σ^B response.

• To monitor different levels of σ^B-dependent expression, we modulated σ^B expression in a *rsbW* mutant via IPTG induction of a P_{spac} promotor cloned upstream of the σ^B. β-Gal activity of a *ctc::lacZ* fusion was used as reporter for σ^B dependent expression. Direct activation of σ^B in this strain (BSA115) is thought to activate reporter protein synthesis and dysfunctional feedback regulation should result is lasting β-Gal activity.

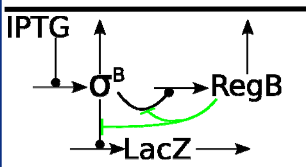
Surpise and Explanation

• Addition of IPTG induces expression of β-Gal, but activity is transient.
• Maximal β-Gal signal is independent of growth rate
• β-Gal decrease starts before transition to stationary phase for 1mM IPTG.

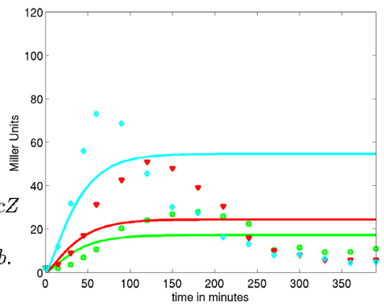
• All β-Gal signals drop to uninduced signal strength
• High σ^B activation → Strong β-Gal decrease
• Low σ^B activation → Weak β-Gal decrease

Models of the most simple three explanatory hypotheses are fitted to the experimental data to test how well each is able to reproduce the observations:

Transcription Inhibition



$$\frac{d}{dt}\sigma^B = k_{bs}\mathrm{IPTG} - k_{bd}\sigma^B$$
$$\frac{d}{dt}LacZ = k_{zs}\frac{\sigma^B}{1+regb} - k_{zd}LacZ$$
$$\frac{d}{dt}regB = k_{rs}\frac{\sigma^B}{1+regb} - k_{rd}regb.$$

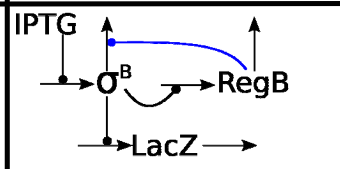


✘ transient dynamics, adaptivity

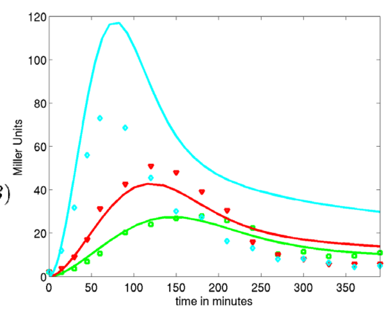
✘ shifted peak positions

✘ comparable final state level

σ^B Proteolysis



$$\frac{d}{dt}\sigma^B = k_{bs}\mathrm{IPTG} - \sigma^B(k_{bd} + k_{br}regB)$$
$$\frac{d}{dt}LacZ = k_{zs}\sigma^B - k_{zd}LacZ$$
$$\frac{d}{dt}regB = k_{rs}\sigma^B - k_{rd}regb$$

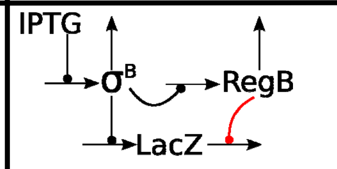


✓ transient dynamics, adaptivity

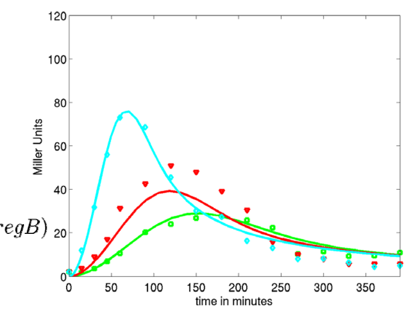
✓ shifted peak positions

✘ comparable final state level

β-Gal Proteolysis



$$\frac{d}{dt}\sigma^B = k_{bs}\mathrm{IPTG} - k_{bd}\sigma^B$$
$$\frac{d}{dt}LacZ = k_{zs}\sigma^B - LacZ(k_{zd} + k_{zr}regB)$$
$$\frac{d}{dt}regB = k_{rs}\sigma^B - k_{rd}regb$$



✓ transient dynamics, adaptivity

✓ shifted peak positions

✓ comparable final state level

Adaptive σ^B behaviour

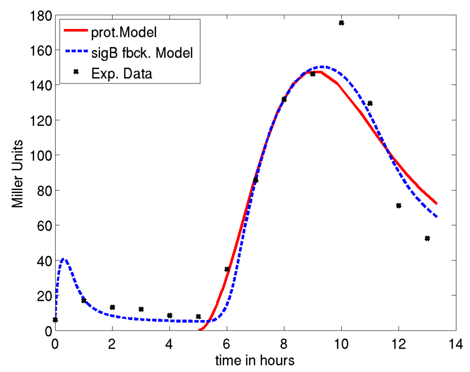
Hypothesis

Can β-Gal proteolysis contribute to the adaptive σ^B response in a wild type *B. subtilis*?:

1. Taking the β-Gal proteolysis model parameterized with BSA115 experiment as starting point.
2. Use of a previous experimental data of β-Gal in wt *B. subtilis*. σ^B response is activated by starvation at the transition to stationary phase.
3. Two parameters (IPTG, k_{zs}), that are independent of the putative protease dynamics, are allowed to vary during parameter estimation.

Result

The β-Gal proteolysis model (red) can fit a wt σ^B response with adaptive behaviour as well as a model that relies on negative feedback regulation including RsbW/V proteins (blue).



Conclusion

Therefore, the hypothesis can not be rejected and proteolysis of β-Gal can contribute to adaptivity in the wild type.

Conclusions and Perspectives

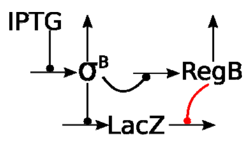
• *B. subtilis* BSA115 shows adaptive, transient dynamics of β-Gal following σ^B activation by IPTG. Modelling and Northern blot experiments suggest σ^B induced β-Gal proteolysis as the origin.

• σ^B induced proteolysis of β-Gal in BSA115 was assumed to occur also in *B. subtilis* wild type cells, raising the possibility that β-Gal instabilities might contribute to the transient nature of the σ^B response – testing the ability of the β-Gal proteolysis model to reproduce wild type adaptivity confirmed this assumption.

• Therefore, part of the adaptive dynamics of β-Gal reporter signal is due to induced protein proteolysis, questioning the applicability of β-Gal reporters.

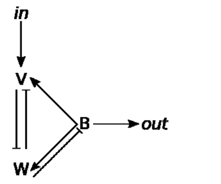
• Research focus on σ^B has been on its activation and the cause of the transient response is not well known. Our results add proteolysis to the possible mechanisms:

1.



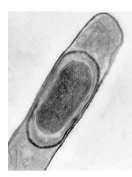
Induced proteolysis

2.



Negative feedback via anti and anti-anti sigma factors

3.



Silencing of σ^B by stress response strategies like sporulation

Affiliations, Contact & Acknowledgements

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SysMO Systems Biology of Microorganisms

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