Post-translational Ultrasensitive Switch Governs Pulsatile Activation of *B. subtilis* Stress-Response Sigma Factor

Abhinav Tiwari[1] and Oleg A. Igoshin[1]

***Short Abstract* —In *Bacillus subtilis* sigma factor σ^B controls a regulon of more than hundred genes whose products protect cells against a broad array of energy and environmental stress. Activity of σ^B is tightly regulated by a partner-switching network comprising anti-sigma and anti-anti-sigma factors. Recently, it was shown that under energy stress σ^B is activated in transient pulses with increasing pulses frequencies under more severe stress levels. We use a mathematical model to identify a post-translational ultrasensitive switch and other network design features responsible for pulsing.**

***Keywords* — Stress Response, Partner Switching, Sigma Factor, Pulse Regulation, Feedback Loops, Ultrasensitivity.**

I. Background

In *Bacillus subtilis* alternative sigma factor σ^B controls a general stress regulon encoding more than hundred genes whose products protect cells against a broad array of energy and environmental stress [1]. Here we examine the partner-switching network that tightly regulates the activity of σ^B.

The core components of the partner-switching network are σ^B, anti-sigma factor RsbW and anti-anti-sigma factor RsbV [2]. In the absence of stress, RsbW binds to σ^B and prevents its association with RNA polymerase, turning OFF the σ^B regulon. Under these conditions most of RsbV is in the phosphorylated form (RsbV~P) due to phosphorylation by RsbW kinase; RsbV~P has a low affinity for RsbW. In the presence of stress, a phosphatase RsbP dephosphorylates RsbV~P. Subsequently RsbV attacks the σ^B/RsbW complex to induce σ^B release, thereby turning ON the σ^B regulon. Thus, the level of active σ^B is determined by the interplay between phosphorylation and dephosphorylation of RsbV. Furthermore, genes encoding σ^B and its regulators lie within a σ^B-controlled operon, thereby resulting in positive and negative feedback loops.

Recently it was shown that energy stress resulted in pulsatile activation of σ^B (brief sudden increase in its levels) and increasing stress resulted in higher pulse frequencies [3]. We hypothesize that phosphorylation and sequestration reactions in the network give rise to a post-translational ultrasensitive switch that governs the pulsatile activation of σ^B. We extend the mathematical model of σ^B partner-switching network from [2] to investigate the design features responsible for pulsing.

II. Results

We show that excess of RsbW total concentration over that of σ^B is necessary for pulsing and negative steady-state open-loop gain. However, this constraint gives rise to two different parameter regimes with different pulse-generating mechanisms. By validating our model against experimental observations [3] we identified the relevant regime which resulted in an upper bound for RsbW total concentration. We further identify the mechanism governing rapid increase in RsbW which in turn causes σ^B levels to go down during pulsing. Initially in the absence of RsbV phosphorylation free RsbW levels are low as most of it is in the form of complex bound either to σ^B or RsbV. Later as RsbV~P levels increase due to phosphorylation, free RsbW levels also increase as now it is in excess of σ^B and RsbV. Furthermore, we find that timescale of transcriptional and post-translational processes should be comparable for pulsing to occur. We also investigate the role of σ^B pulsing on its target genes.

III. Conclusion

We identify the lower and upper bounds for RsbW total concentration – a network design feature necessary for pulsatile activation of σ^B. We also propose a mechanism responsible for generating post-translational ultrasensitivity in the network which is different from that proposed in [3].

Acknowledgements: This work was funded by NSF grant MCB-0920463 and NIH grant GM096189-01.

[1]Department of Bioengineering, Rice University, MS-142, 6100 Main St., Houston, TX 77005 USA. E-mail: igoshin@rice.edu

References

[1] Hecker M, Pané-Farré J, Völker U (2007) SigB-dependent general stress response in *Bacillus subtilis* and related gram-positive bacteria. *Annu Rev Microbiol* **61**, 215-236.

[2] Igoshin OA, et al. (2007) Distinctive topologies of partner-switching signaling networks correlate with their physiological roles. *J Mol Biol* **369**, 1333-1352.

[3] Locke JCW, et al. (2011) Stochastic pulse regulation in bacterial stress response. *Science* **334**, 366-369.