January 13, 2017

Dear iScience Editor(s),

Please find attached our manuscript “Mean-Independent Noise Control via Intermediate States” to consider for publication in iScience.

In many biological systems, concentrations of signaling molecules control cell fates. However, these biochemical interactions are intrinsically noisy, raising the question of how robust fate decisions can occur. Changes to the signaling pathway are likely to change not only the amount of noise in the signal but also its mean value. Thus, the ability to control signaling noise in a general regulatory network while maintaining the correct fate decisions is difficult if not impossible. Here we identify a mechanism that functions through intermediate states that act naturally as a mean-independent noise control (MINC) mechanism. We show that this extends to multiple different models, both with and without feedback, and does not require specific parameters, demonstrating that it is potentially a general mechanism of noise control in many biological and synthetic networks.

During our analyses of MINC properties, we have developed a computational tool for relating changes between mean and variance. We use this to show that our models accurately recapitulate the mean/variance relationships we obtained in our previous experimental measurements of retinoic acid (RA) concentrations in the developing zebrafish hindbrain (Sosnik et al. Elife, 5, 2016). We demonstrate that models which have similar behavior in the mean can have distinct mean/variance relationships and develop a method to identify the dominant noise source in the RA signaling network. Additionally, this new noise control principle helps explain how sharp segmental boundaries of gene expression form despite the inherently noisy spatial gradient of RA. Previous results described a noise-induced switching mechanism for sharpening in of RA-induced gene expression boundaries in the hindbrain (e.g. Zhang et al. MSB, 8:613, 2012). Here we show that MINC mechanism regulates noise in RA to the proper levels that enable effective noise-induced switching needed to sharpen these boundaries. In particular, we show that changes in the cellular RA-binding protein, Crabp2a, directly alter the spatial noise without displacing the boundary, and we previously showed that a knockdown of Crabp2a disrupts hindbrain patterning. We bring this all together to show that a specific range of signaling noise and Crabp2a is required to achieve proper patterning. To our knowledge, this is the first direct connection between a stochastic spatial phenotype and noise, demonstrating how developmental processes have evolved to overcome inherent biochemical stochasticity and achieve complex spatial phenotypes.

**Suggested Reviewers**

William Holmes (U. of Vanderbilt, [william.holmes@vanderbilt.edu](mailto:william.holmes@vanderbilt.edu)) - an expert on modeling signaling networks, development and noise.

Wei Lin (Fudan University, [wlin@fudan.edu.cn](mailto:wlin@fudan.edu.cn)) - an expert on dynamic systems and stochastic analysis.

Jin Wang ([jin.wang.1@stonybrook.edu](mailto:jin.wang.1@stonybrook.edu)) SUNY Stony Brook. an expert on gene regulatory networks and noise

Tim Elston ([telston@ad.unc.edu](mailto:telston@ad.unc.edu)) University of North Carolina, an expert on gene regulatory network and signal transduction

We look forward to hearing from you!

Sincerely Yours



Qing Nie

Professor of Mathematics, Developmental and Cell Biology, and Biomedical Engineering