Stochasticity and the Mechanism of Precision in the Vertebrate Segmentation Clock

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***Short Abstract* — Oscillations are prevalent in biological systems. The vertebrate segmentation clock governs the rhythmic segmental patterning of the vertebral column during embryonic development. The period of the segmentation clock dictates the number and sizes of vertebrae. Stochastic gene expression imposes a great challenge to precise embryonic development. To address this issue, we counted single RNA transcripts and determined, for the first time, the amplitude and variability of clock gene expression in an intact tissue. In contrast to previously published computational models, our results unraveled low amplitudes and high variability in oscillatory gene expression, and suggested the presence of sharp transcriptional bursts.**

***Keywords* — Vertebrate Segmentation Clock, Cell-To-Cell Signaling, Ultradian Oscillations, Stochastic Gene Expression, Single Molecule Microscopy, Time-Delayed Feedback Loops.**

I. Background

THE embryonic development relies on precise spatiotemporal patterning. Rhythmic segmentation of the precursors of vertebral column, the somites, during development is one of the most intriguing examples of spatiotemporal patterning [1-3]. Periodic segmentation of somites is controlled by the oscillatory expression of Hes/Her gene family, which is called the vertebrate segmentation clock. Several groups including ours have demonstrated that disrupting oscillations results in vertebral defects [1-2]. The segmentation clock ticks rapidly with a period of 30 minutes in zebrafish. Upon completion of each oscillation cycle, a cohort of 200 cells collectively generate a new segment in zebrafish. The rapidity of oscillations in each cell and the entrainment of oscillations at the tissue augment the challenges in achieving precision in this fascinating developmental patterning. To elucidate the underlying mechanism of precision of the vertebrate segmentation clock, we combined quantitative experimentation with computational analysis.

II. Results

Despite the unavoidable gene expression fluctuations, embryos display robust outcomes. Stochastic fluctuations in gene expression must be buffered under wild-type conditions. The amplitude of oscillations should be tightly controlled but there is no knowledge about the amplitude of oscillations and its variability in any vertebrate species. To fill this critical gap in knowledge, we quantified RNA molecules transcribed by two master duplicated segmentation clock genes (*her1* and *her7*). Previously published computational simulations reported high amplitudes and low fluctuations in oscillatory gene expression. In contrast, our results unraveled low amplitudes and high variability in oscillatory gene expression, and suggested their transcription to occur in sharp bursts. Our results further demonstrate that the intrinsic (or extrinsic) factors dominate gene expression noise at low (or high) expression levels. We propose that two extrinsic factors underlie random and sharp transcriptional bursts: 1) polymerase pausing at the proximal promoters of clock genes, and 2) fluctuations in the levels of transcriptional activators of the clock genes. We further *hypothesize* that stochastic fluctuations in gene expression must be buffered under wild-type conditions by mechanisms of redundancy, cross-regulatory feedback loops and local and long-distance cell-to-cell communication.

III. Conclusion

Oscillations of Hes/Her proteins control the temporal switch from proliferation to differentiation in various tissues [4]. Their gain-of-function correlates with cancer, while inhibition restores differentiation. Elucidating the underlying mechanism of precision in their oscillations is significant for understanding and potentially preventing vertebral malformations, for enhancing stem cell proliferation and developing therapies against cancer, and for advancing predictive modeling of cellular regulatory systems.

References

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