Molecular noise facilitates NF-κB entrainment under dynamic inputs

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***Short Abstract* —A major immune pathway NF-κB exhibits feedback-driven transcriptional oscillations, but it is unclear how these internal oscillations contribute to the processing of dynamic external signals. Using high-throughput microfluidic single-cell measurements, we show that NF-κB oscillations synchronize with an oscillating inflammatory signal and become entrained in an unexpectedly wide range of frequencies, resulting in higher transcriptional output and population synchrony. Stochastic simulations show that this wide range is due to fluctuations in transcription of negative-feedback genes, suggesting that cells use intrinsic noise to achieve efficient gene expression and avoid population disorder in a dynamic signaling environment.**

***Keywords* —Single Cell Analysis, Noise, Entrainment, NF-κB, Inflammation, Dynamics, Microfluidics, Signaling**

BIOLOGICAL systems use oscillations for time-keeping and transcriptional regulation, with prominent examples in circadian rhythms, brain waves and developmental patterning. NF-κB is a signalling pathway central to immunity and many diseases that shows oscillations even under constant inputs, with significant cell-to-cell variability [1]. NF-κB oscillation dynamics help determine the specificity and timing of gene expression. Upstream oscillatory pathways or signalling waves in tissue can result in periodic inputs to cells that can lead to their entrainment, where normally out-of-phase oscillators phase-lock to the input and become synchronized. Whether NF-κB can be entrained and its implications for the population response have been unclear. Here we use high-throughput microfluidic live-cell imaging, quantitative gene expression analysis and mathematical modelling to characterize the frequency response of NF-κB at the single-cell level over 48 hours, and we find that periodic modulation of the TNF-α input readily leads to synchronization and entrainment, causing significantly reduced NF-κB and mRNA variability between cells. We measure a much broader entrainment frequency range (Arnold Tongues) than what is expected from deterministic calculations, and stochastic simulations show that intrinsic molecular fluctuations in the transcription of negative-feedback genes IKBα and IKBε cause this enhanced bandwidth. Individual cells show diverse locking responses, with cells responding to fractions of the input frequency as well as cells "hopping" between locking modes. Oscillations in the expression of early and late genes appear with NF-κB entrainment, indicating the synchronization of gene expression between individual cells. Furthermore, efficient entrainment leads to increased mRNA production at the population level.

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CONCLUSION

Our results shed light on how cell populations may operate synchronously despite significant variability in isolate cell responses, and suggest a surprising role for intrinsic noise in reducing population variability under dynamical input signals. External control of transcriptional dynamics and reducing cell-to-cell variation through microfluidic input modulation may find applications where homogenous populations need to be derived from heterogeneous ones, such as directed stem cell differentiation and immunomodulation.

REFERENCES

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