Measuring transcription at a single gene copy illuminates RNA dynamics and reveals intracellular correlations

Mengyu Wang[1,2,3*], Jing Zhang[1,2*], Heng Xu[4,5], and Ido Golding[1,2,3]

***Short Abstract* — Gene regulation consists of a series of stochastic, single-molecule events, resulting in substantial randomness in RNA production between individual cells, and even between the individual copies of the same gene within a single cell. To characterize the stochastic kinetics of transcription in *E. coli* at the resolution of individual gene copies, we combine RNA and gene-locus labeling to simultaneously detect a gene of interest and measure its transcriptional activity, in individual bacteria.**

***Keywords* — Transcription, transcription kinetics, single cell, single gene copy, smFISH, RNA life history, correlation, cell cycle.**

I. BACKGROUND

Stochastic gene expression gives rise to population heterogeneity, which has been extensively studied using single-cell measurements for more than a decade [1]. However, some of the observed cell-to-cell variability may not be casued by stochasticity, but by deterministic factors such as cell size [2], gene copy number [3], correlation between gene copies in the same cell, and the cell-cycle phase [4]. Measuring these "hidden variables" will thus reveal a less random picture of transcription. Towards removing cellular hidden variables, we aimed to measure transcription from a single gene copy in individual cells.

II. RESULTS

A. Single-molecule FISH and gene locus detection

To detect individual gene copies, a set of *tetO* operators is inserted near the gene of interest in the *E. coli* chromosome [5]. The RNA transcribed from the gene is labeled by a set of fluorophore-labeled DNA oligo probes [6]. The observed co-localization of RNA signal and gene signal enables us to measure active transcription at the resolution of individual gene copies, in individual cells.

B. Kinetic model describing RNA dynamics

We developed a theoretical model describing RNA dynamics. By fitting the model (in both deterministic and stochastic formalisms) to our single-cell experimental data, we are able to obtain the kinetic parameters characterizing RNA life history: the probabilistic rate of promoter switching, transcription initiation and elongation, RNA release and degradation.

C. Correlation between different copies in the same cell

We examined the activity of individual copies of the lactose promoter P_{lac} in cells having two copies of the gene. We found that the two copies were either highly correlated or almost independent, depending on the growth conditions.

D. Correlation of gene activity to the cell cycle

We examined the transcriptional activity of P_{lac} and P_R at different times along the cell cycle, at different expression levels and growth conditions. We found that the transcription of a strong "constitutive" promoter (P_R) closely follows the gene dosage. However, for a promoter under tightly-repressed conditions (P_{lac}), we observed a transient increase of transcriptional activity upon gene replication, as has been speculated before [7].

III. CONCLUSION

The existence of these gene-copy and cell-cycle correlations demonstrates the limits of mapping whole-cell RNA numbers to the underlying stochastic gene activity and instead highlights the contribution of previously hidden variables to the observed population heterogeneity.

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[1]Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX

[2]Center for Theoretical Biological Physics, Rice University, Houston, TX

[3]Graduate Program in Quantitative and Computational Biosciences, Baylor College of Medicine, Houston, TX

[4]Institute of Natural Sciences, Shanghai Jiao Tong University, Shanghai, P.R. China

[5]School of Physics and Astronomy, Shanghai Jiao Tong University, Shanghai, P.R. China

[*]Equal contribution

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