EXTRACTING TRANSCRIPTIONAL BURSTING PARAMETERS FROM SINGLE NASCENT RNA MICROSCOPY DATA

Stefano Coppola, Heta P. Patel, Tineke L. Lenstra
The Netherlands Cancer Institute (NKI), Oncode Institute
Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands
Email: stefano.coppola87@gmail.com

KEYWORDS: live cell, yeast, transcriptional bursting, single molecule microscopy

Gene expression is a dynamic and stochastic process, resulting in heterogeneity within and between cells, which can affect essential cell fate decisions of an isogenic population [1]. Countless research efforts have been made to pinpoint the origin and consequences of such heterogeneity, but a mechanistic understanding is lacking. A simple yet experimentally validated model accounts for the production of RNA in bursts, which can theoretically be described by the existence of ON/OFF states for a gene and the stochastic transcription during the ON states [2]. Yet recent data suggest that gene regulation is more complex, characterized by more states and regulatory steps, e.g., for two neighboring genes. Although single molecule FISH (smFISH) permits detecting single nascent RNAs at the transcription site with high spatial resolution, such an approach is performed on fixed cells and, therefore, cannot access the temporal switching between ON/OFF states. Live-cell imaging technologies based on MS2/PP7 stem-loops access the temporal dimension by measuring the number of nascent RNAs over time [3, 4]. Here we present a novel analysis strategy that maximizes the spatio-temporal resolution by combining dual-color smFISH and dual-color live cell imaging of nascent RNAs data of two galactose-responsive neighboring genes in budding yeast, i.e., ***GAL1*** and ***GAL10***. A model of transcription co-regulation in which ***GAL1*** and ***GAL10*** show correlated bursts is proposed. The simultaneous fitting of smFISH and live-cell nascent RNA distributions through stochastic simulations allows us to extract kinetic parameters of transcription, such as the burst sizes and frequencies for both ***GAL*** genes. We also determined that ***GAL1*** and ***GAL10*** show highly correlated bursts, with ~70% of bursts from ***GAL10*** co-occurring with bursts from ***GAL1***.

References

[1] Tunnacliffe E, Chubb JR. What Is a Transcriptional Burst? *Trends Genet*. 2020 Apr;36(4):288-297
[2] Xu H, Skinner SO, Sokac AM, Golding I. Stochastic Kinetics of Nascent RNA. *Phys Rev Let*. 2016 Sep 16;117(12):128101
[3] Donovan BT et al. Live-cell imaging reveals the interplay between transcription factors, nucleosomes, and bursting. *EMBO J*. 2019 Jun 17;38(12):e100809
[4] Brouwer I et al. Single-Molecule Fluorescence Imaging in Living Saccharomyces cerevisiae Cells. *STAR Protocols* 2020, doi.org/10.1016/j.xpro.2020.100142