Establishing a quantitative framework for analyzing inducible gene expression in HeLa cells

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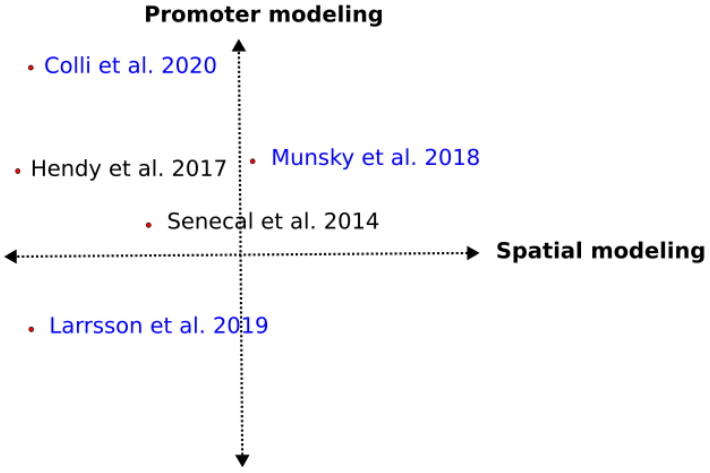
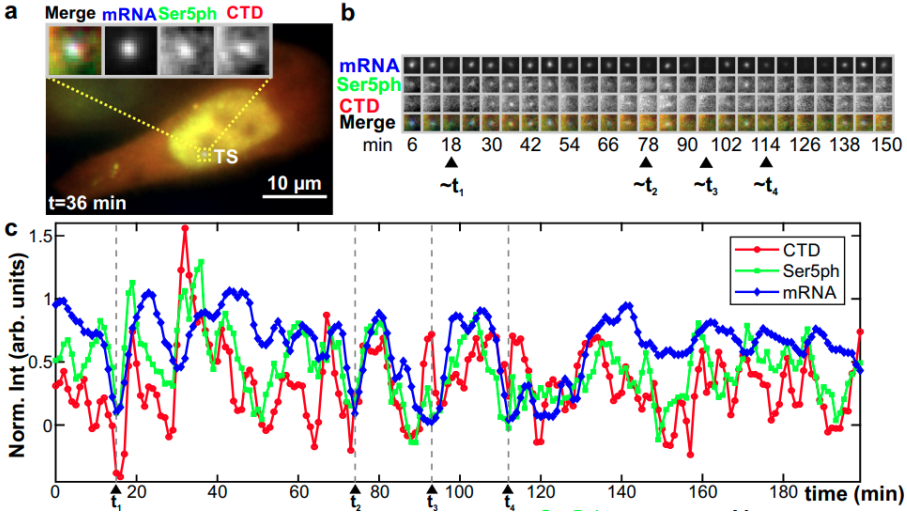
Transcriptional burst literature landscape

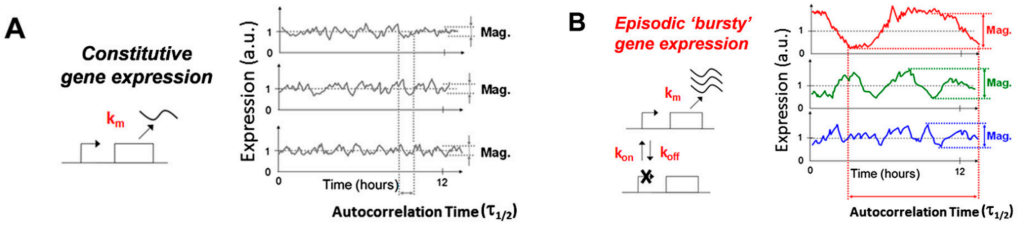


Gene expression is stochastic (live-cell MS2-MCP)



Forero-Quintero, et al. *Live-cell imaging reveals the spatiotemporal organization of endogenous RNAPII phosphorylation at a single gene*. Nat Commun 2021

Promoter models are necessary for non-constitutive gene expression



Single-state models

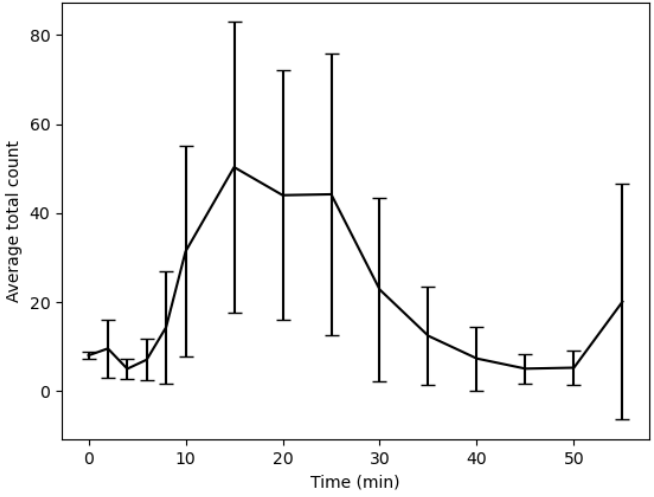
- RNAs are 'born' at a fixed rate
- RNA counts are Poisson

Multi-state models

- Promoter can be in multiple states (switching behavior)
- RNA counts are not Poissonian

Single-state models tend to underestimate variance in RNA counts

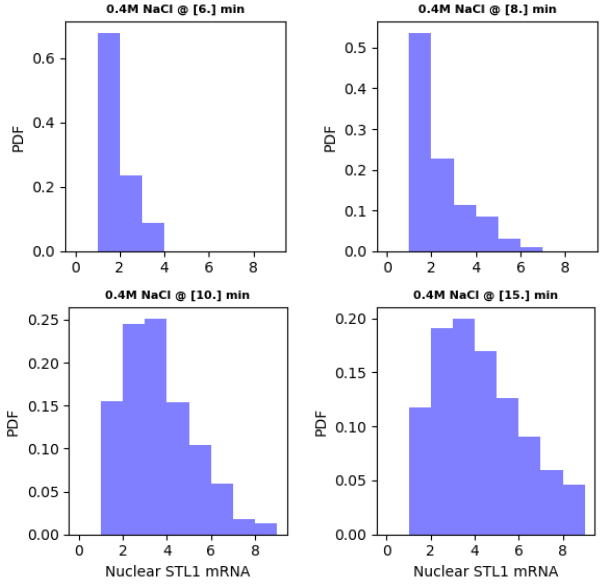
Example: variability in STL1 mRNA counts per cell at 0.4M NaCl



Error bars represent standard deviations from the mean
Cells marked ON for $>$ 3 STL1 mRNA in yeast

Assessing STL1 mRNA count variability at the transcription site

- Brightest spot in the nucleus defined as putative TS
- TS marked ACTIVE if $I > 2 * \mathrm{med}$
- Nascent mRNA count is $\mathrm{round}(I/\mathrm{med})$
- Count variability suggests asynchrony



Adding spatial information: a compartment model for induced gene expression

Let X represent an arbitrary RNA transcript of an induced gene G. Assume two promoter states (on and off)

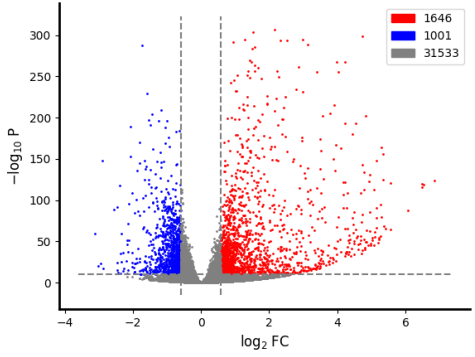
$$\begin{aligned}
\text{Gene activation} &: G_{off} \xrightarrow{k_{on}} G_{on} \\
\text{Gene inactivation} &: G_{on} \xrightarrow{k_{off}} G_{off} \\
\text{Transcription} &: G_{on} \xrightarrow{k_t} G_{on} + X_{\text{nuc}} \\
\text{RNA Export} &: X_{\text{nuc}} \xrightarrow{k_{exp}} X_{\text{cyt}} \\
\text{RNA degradation} &: X_{\text{cyt}} \xrightarrow{\gamma} \emptyset
\end{aligned}$$

Raw data collected post induction can be used to infer parameters

$$\theta = (k_{on}, k_{off}, k_t, k_{exp}, \gamma)$$

Interferon-γ induction in HeLa cells

Single cell transcriptome measurements of polyA mRNA for naïve HeLa cells (N=90), induced with interferon gamma (50ng/mL) for 24h



Siwek et al. *Activation of Clustered IFNγ Target Genes Drives Cohesin-Controlled Transcriptional Memory Cell 2020*

Interferon-γ induces transcriptional bursting

For the purpose of gene selection, let's assume the simple two-state model

Fitting and parameter selection

Kolmogorov's forward equation (chemical master equation)

Dynamics on biochemical reaction networks are inherently stochastic and the state space is discrete. We can only write probabilities over the state space

$$\begin{aligned} P(\mathrm{x}_i, t) &= \sum_j T_{ji}(\mathrm{x}_i, t|\mathrm{x}_j, t-\Delta t)P(\mathrm{x}_j, t-\Delta t) \\ &= \sum_k T_k(\mathrm{x}_i, t|\mathrm{x}_i-\nu_k, t-\Delta t)P(\mathrm{x}_i-\nu_k, t-\Delta t) \end{aligned}$$

where T_k is the probability of a reaction channel k firing in the interval $(t, t+\Delta t)$.

Taking the limit $\Delta t \to 0$ one can derive the forward Kolmogorov equation or chemical master equation (CME)

$$\frac{dP(\mathrm{x}, t|\mathrm{x}_0)}{dt} = \sum_k T_k(\mathrm{x}-\nu_k)P(\mathrm{x}-\nu_k, t) - T_k(\mathrm{x})P(\mathrm{x}, t)$$