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

Clayton W. Seitz

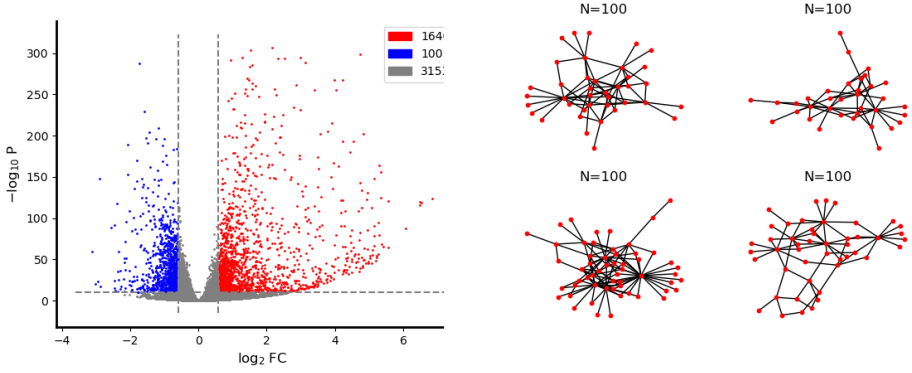
July 5, 2022

The three stages

- Select a set of genes that are differentially expressed after interferon treatment
- Estimate the bursting parameters from sequencing data
- Iterative RNA FISH experiments, apply bursting models, spatial analysis

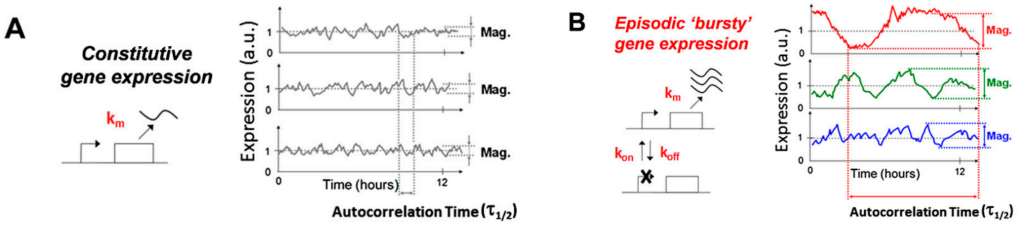
Interferon-γ induces differential expression of many genes

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



Randomly selected $N = 100$ genes with edges drawn for $I(X;Y) \geq 0.1$ using Kraskov's method: $I(X,Y) \approx \psi(k) - \langle \psi(n_x + 1) + \psi(n_y + 1) \rangle + \psi(N)$

Promoter models are necessary for non-constitutive gene expression



Single-state models

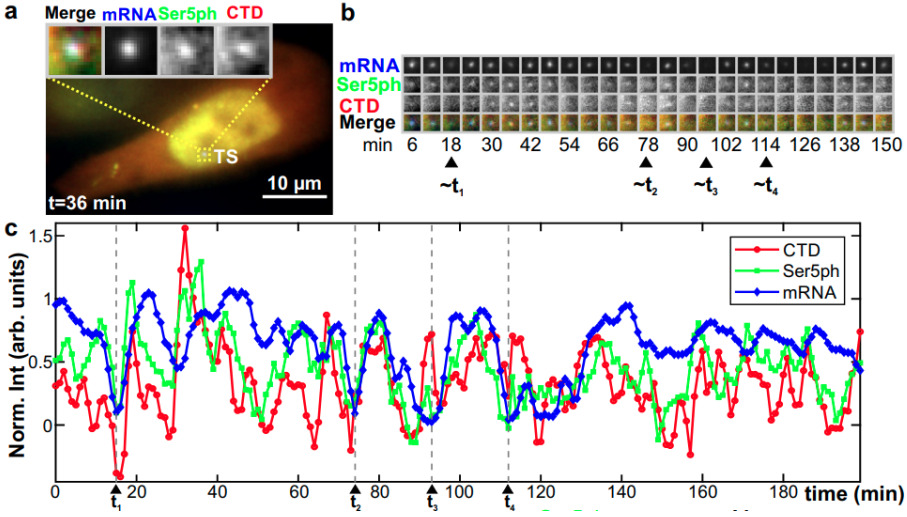
- RNAs are 'born' at a fixed rate
- RNA counts are Poisson

Multi-state models

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

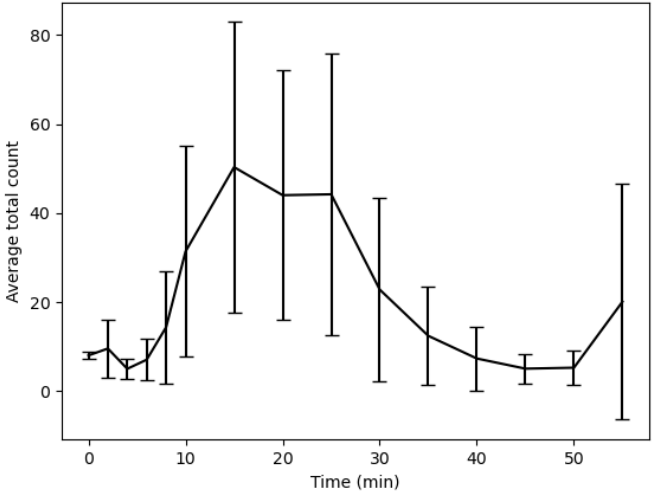
Single-state models tend to underestimate variance in RNA counts

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

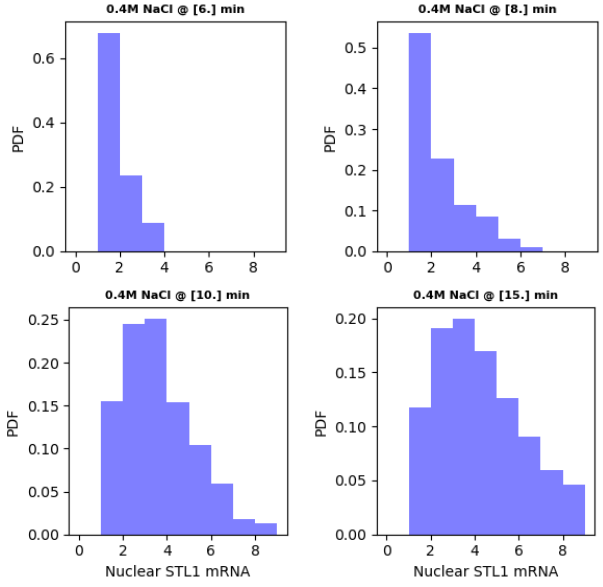
Example: variability in STL1 mRNA counts per cell at 0.4M NaCl



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



A spatial 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

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

Bayesian inference of model parameters

It is well-known that using just means and variances gives poor estimates of the model parameters (Munsky et al. PNAS 2018)

Let $\theta = (k_{on}, k_{off}, k_t, k_{exp}, \gamma)$. Using Bayes Rule:

$$P(\theta|X) = \frac{P(X|\theta)P(\theta)}{\int P(X|\theta)P(\theta)} \propto P(X|\theta)P(\theta)$$

Can infer θ if we know the likelihood $P(X|\theta)$ (the hard part) and specify a prior $P(\theta)$

Generally we have to resort to Monte Carlo methods to find $P(X|\theta)$

Kolmogorov's forward equation (chemical master equation)

To calculate the likelihood $P(X|\theta)$ one has to address the forward Kolmogorov equation

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

It is possible to find $P(\mathrm{x}, t|\mathrm{x}_0)$ in two main ways: (1) Finite state projection (2) Monte Carlo simulation

The former is exact, the latter is an approximation (see approximate Bayesian computation)