

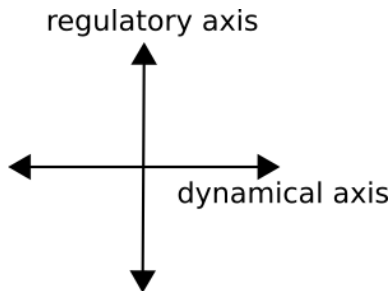
Establishing a quantitative framework for analyzing inducible gene expression in HeLa cells

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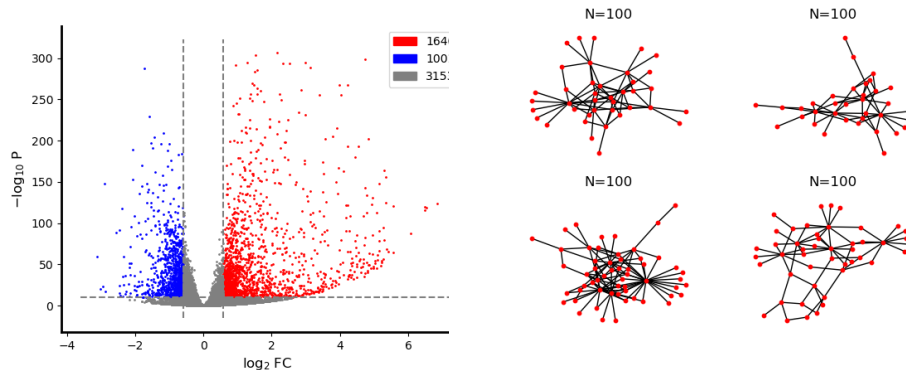
The three stages

- ▶ Select a set of genes that are differentially expressed after interferon treatment
- ▶ Mark genes which have special relevance in the literature e.g., PDL-1
- ▶ Iterative RNA FISH experiments, apply bursting models, spatial analysis



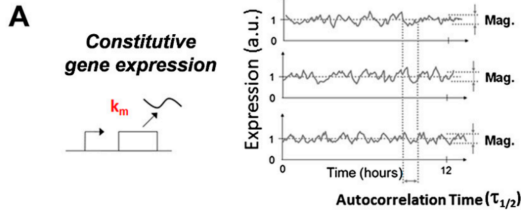
Interferon- γ induces differential expression of many genes

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



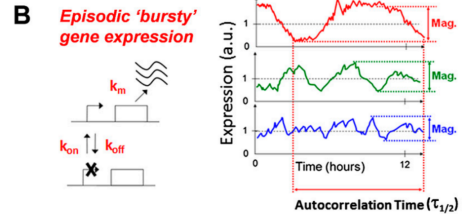
Randomly selected $N = 100$ genes with edges drawn for $I(X; Y) \geq 0.1$ using Kraskov's method: $I(X, Y) \approx \psi(k) - \langle \psi(n_x + 1) + \psi(n_y + 1) \rangle + \psi(N)$

Promoter models are necessary for non-constitutive gene expression



Single-state models

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

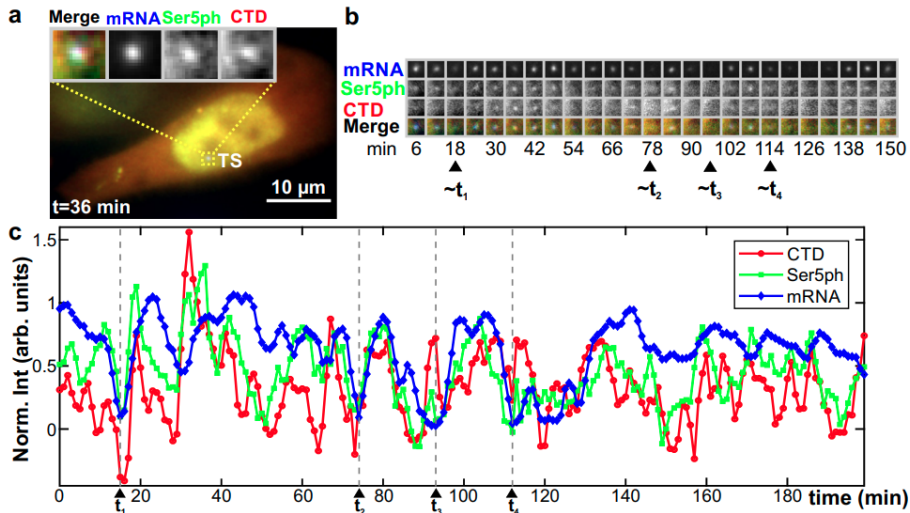


Multi-state models

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

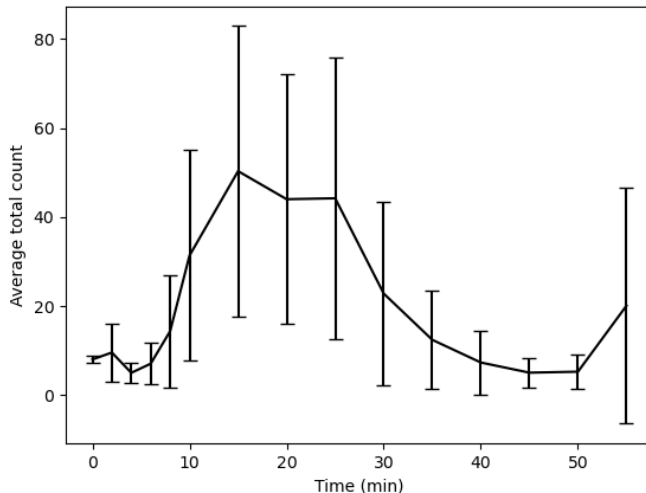
Single-state models tend to **underestimate variance in RNA counts**

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

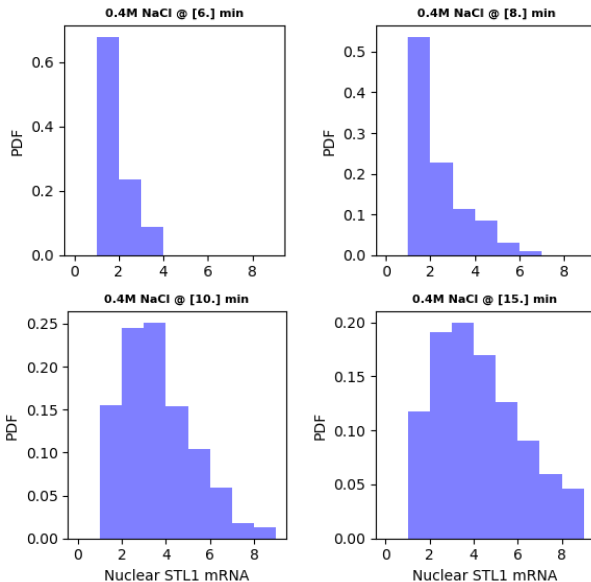


Error bars represent standard deviations from the mean

Cells marked ON for > 3 STL1 mRNA in yeast

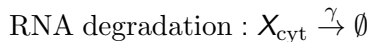
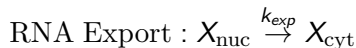
Assessing STL1 mRNA count variability at the transcription site

- ▶ Brightest spot in the nucleus defined as putative TS
- ▶ TS marked ACTIVE if $I > 2 * med$
- ▶ Nascent mRNA count is $round(I/med)$
- ▶ 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)



Raw data collected post induction can be used to infer parameters

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

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

$$\frac{dP(x, t|x_0)}{dt} = \sum_k T_k(x - \nu_k)P(x - \nu_k, t) - T_k(x)P(x, t)$$

It is possible to find $P(x, t|x_0)$ 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)