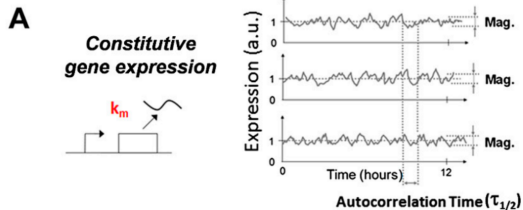


Statistical analysis for ensemble snapshots of transcriptional bursting

Clayton W. Seitz

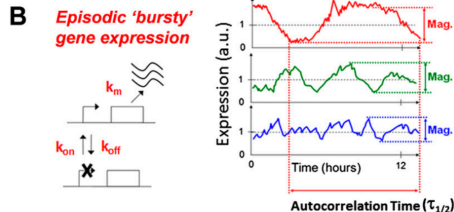
June 27, 2022

Gene expression is stochastic and non-constitutive



Single-state models

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



Multi-state models

- ▶ Promoter can be in multiple states
- ▶ RNA counts are not Poissonian

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

We would like to investigate transcriptional bursting using fluorescence in-situ hybridization (FISH)

- ▶ Our image acquisition and detection strategy is standardized (5 by 5 regions, max-intensity z-projections, LoG blob detection and 2D Gaussian fitting)
- ▶ Need to identify a set of target genes, which we have good reason to believe are under the control of our treatment condition and not expressed constitutively
- ▶ Aiming for 3000 cells (for each treatment condition?)
- ▶ Need to define a cell-line (HeLa?) and treatment condition pairing
- ▶ Need to define a set of analysis methods compatible with ensemble snapshot data

Testing dataset: STL1/CTT1 induction with NaCl in yeast

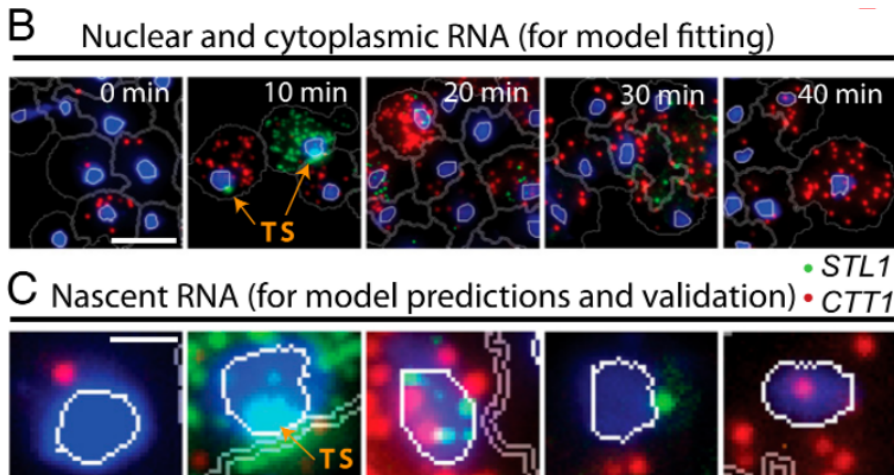


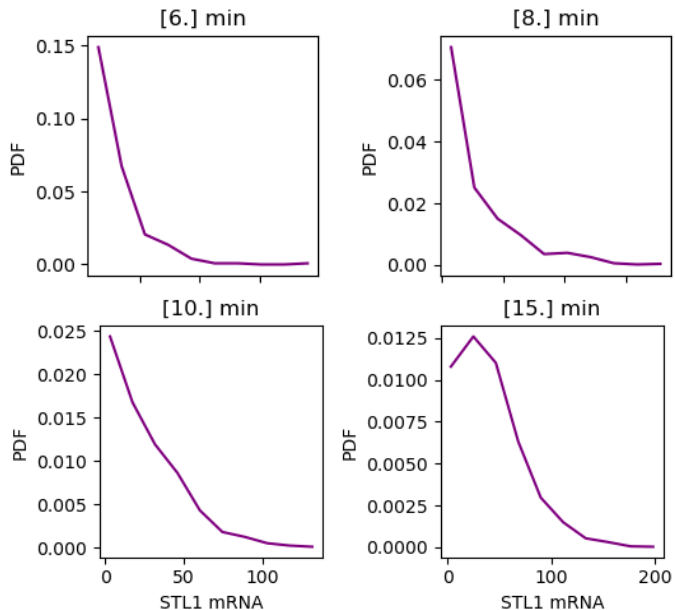
Figure 1: Munsky et al., PNAS 2018

Testing dataset: STL1/CTT1 induction with NaCl in yeast

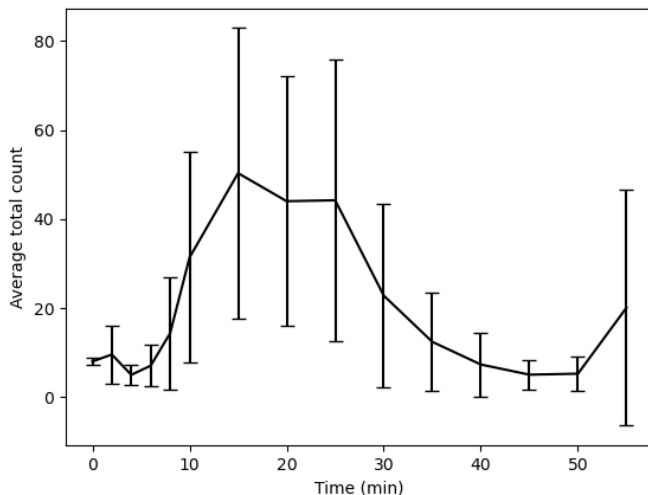
Useful as a guide when designing our own experiments:

- ▶ 0.2M NaCl
 - ▶ 2 biological replicates
 - ▶ 15 time points per replicate
- ▶ 0.4M NaCl
 - ▶ 3 biological replicates
 - ▶ 15 time points per replicate

STL1 mRNA counts per cell at 0.4M NaCl

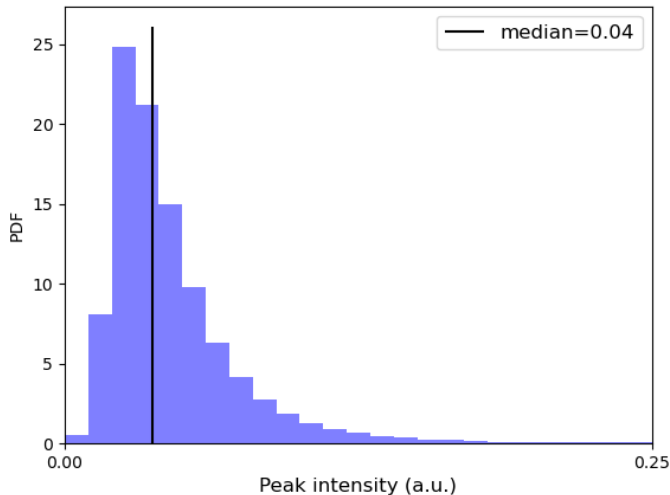


STL1 mRNA counts per cell at 0.4M NaCl



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

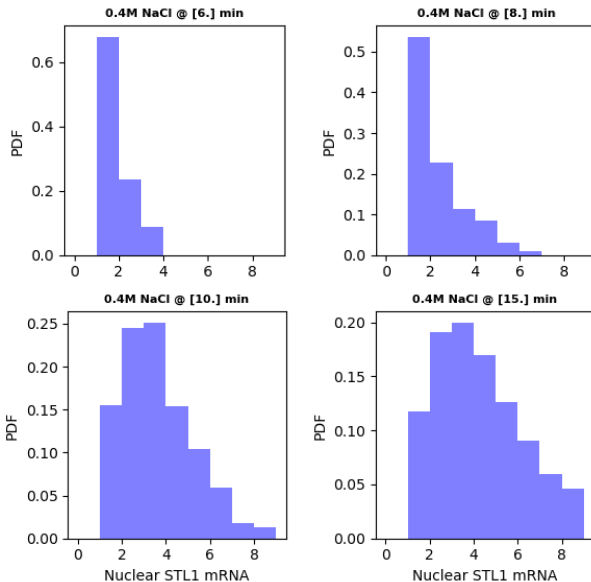
Statistically defining the unit intensity



The median of the mRNA intensity distribution is used to determine the number of nascent RNA at the transcription site (TS)

Nascent mRNA counts at the transcription site

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



Comments

- ▶ Average counts show a 'transcriptional burst', but variability is very high
- ▶ Cells are not necessarily bursting synchronously
- ▶ Ensemble averages may not accurately represent the underlying dynamics
- ▶ How to correlate transcriptional bursting with spatial organization of transcripts?