Transcription factors modulate c-Fos transcriptional bursts[1]

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***Short Abstract* — Signal-activated transcription often occurs in episodic bursts, whose mechanisms are not fully understood. We quantified proto-oncogene c-Fos activity with single mRNA resolution at individual endogenous alleles in human osteosarchoma (U2OS) cells. During MAPK induction, c-Fos mRNA synthesis occurs in bursts with transcription factor (TF) modulated frequencies, providing a simple but versatile mechanism to adapt transcription to different stimuli. Using single-cell analyses, synthetic TFs, and simple gene regulation models, we illustrate how TFs can alter responses by tuning the duration, frequency, and intensity of mRNA bursts[1].**

***Keywords* — Stochastic gene regulation; MAPK induction; Transcriptional bursting; Transcription factors.**

I. Introduction

Transcription plays a central role in myriad biological events, including stress responses and cell fate decisions. Advances in single-molecule experiments and computational analyses are rapidly advancing our understanding of transcriptional regulation[2,3,4]. Mammalian transcription often occurs as stochastic, pulsatile bursts[5], whose dynamics can be major regulatory knobs with which to adjust responses[6]. Only a few endogenous genes have been studied in mammalian cells, and major roles affecting bursts have been attributed to chromatin accessibility and chromatin modifications[5]. Fewer studies have established quantitative links between upstream TF signals and downstream transcriptional bursts[4,7]. Here, we integrate experiments and computation to understand the interplay of MAPK signal dynamics and bursting of c-Fos transcription.

II. Methods

Experiment: We examined the spatiotemporal dynamics of ERK1/2 signaling and c-Fos transcription in U2OS cells following induction with fetal calf serum. We used immunofluorescence (IF) to quantify the nuclear translocation of the phosphorylated kinase (p-ERK) over time in individual cells. We used single molecule fluorescence *in situ* hybridization (smFISH)[8] to measure the downstream c-Fos transcriptional activity at single mRNA resolution. We quantified the number of active transcription sites (TS), the number of nascent mRNA per active TS, and the total number of mature mRNA in each cell.

Computation: We proposed a discrete stochastic model and used a modified Finite State Projection[9] analyses to compute the distributions for active TS, nascent mRNA and mature mRNA versus time and fluctuating p-ERK levels. We identified parameters for simple 2- and 3-state gene regulatory models[3,4], which accurately capture the correlated spatiotemporal dynamics of p-ERK and the c-Fos transcriptional activity.

III. Results

We observed that non-induced cells contained no detectable active TS and expressed an average of only 4 mature mRNA per cell. After 30 min of serum induction, cells contained an average of 90 mature mRNA with a large variability (some have only a few mRNA, while others contain hundreds). The number of active TS and mature mRNA had correlated temporal dynamics, but the distribution of nascent mRNA on activated alleles was found to be largely independent of condition.

To capture these phenomena and match the active TS number, nascent mRNA, and mature mRNA distributions over time, our models suggest a relatively short time for c-Fos processing compared to the longer TS activation window. This leads to an effective saturation of bursts at the TS and explains why the nascent mRNA distributions are uncorrelated with the number of TS or the mature mRNA levels in most conditions. However, at maximal activation (20 min following serum stimulus), we observe an additional transcriptional mode. This high-activity mode, which is well captured by the 3-state model, leads to a temporary increase in the number of nascent mRNA per TS in the more activated cells. We observed similar dynamics in the p38 activation of c-Fos during heavy metal stress.

Beyond matching ERK1/2 and p38 induction of c-Fos, our model also suggests mechanisms by which TFs tune the duration, frequency, and intensity of mRNA bursts. We validated these mechanisms, using synthetic TFs based on the transcription activator-like effector (TALE) approach[10].

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