Stochastic Nuclear Localization Bursts Coordinately Regulate Target Genes

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***Short Abstract* — In the presence of extracellular calcium, Crz1, the calcineurin responsive zinc finger transcription factor of *Saccharomyces* cerevisiae, is dephosphorylated and translocates into the nucleus [1]. By observing the localization of a Crz1-GFP fusion protein using time-lapse microscopy, we found that Crz1 exhibited bursts of nuclear localization with a characteristic nuclear residence time of ~2 minutes. These bursts occurred in a stochastic fashion in individual cells and propagated to the expression of downstream genes, contributing significantly to fluctuations in gene expression. Strikingly, calcium concentration controlled the frequency, but not duration of nuclear localization bursts. Using natural and synthetic Crz1 target promoters, we find that the observed stochastic frequency modulation (FM) of localization bursts can enable cells to proportionally coordinate expression levels of multiple target genes by regulating the fraction of time a promoter is active, rather than tuning the level of activity itself. Furthermore, we observe that another stress response transcription factor, Msn2, exhibits similar localization bursts under calcium stress, but its bursts are largely uncorrelated with Crz1 bursts. These results suggest that FM localization bursts may be a general control strategy utilized by the cell to coordinately regulate multi-gene responses to external signals.**

I. Background/Motivation

Cells respond rapidly to many stresses by mobilizing transcription factors to the nucleus, where they can activate the expression of a multitude of target genes [2]. In yeast, the extracellular calcium stress affects Crz1 transcriptional activity exclusively through changes in its nuclear localization, which is regulated by phosphorylation and dephosphorylation [1]. It remain unclear how the post-translational regulation of this transcription factory operates dynamically at the single-cell level to control expression of ~100 genes that are necessary for calcium adaptation [3]. To address this issue, we acquired time-lapse movies of Crz1 localization dynamics, using a strain in which the Crz1 protein was tagged with GFP. In each movies, we tracked the response of Crz1 localization in individual cells to step changes in the extracellular calcium concentration.

II. Summary of Results

A. Nuclear Localization Occurs in FM Bursts

In the absence of calcium, Crz1 was cytoplasmic in all cells. Upon the addition of calcium, individual cells exhibited a rapid burst of Crz1 nuclear localization. This was followed by sporadic bursts of relocalization, typically lasting ~2 minutes and persisting throughout the course of the movie. We observed that the fraction of cells with nuclear-localized crz1 increased with calcium concentration. Because Crz1 localizes in bursts, this calcium dependence could result from increases in burst frequency or duration. Strikingly, movies revealed that only the burst frequency increased while the distribution of burst durations remained constant at all calcium concentrations.

B. Bursts Enable Coordinate Regulation of Target Genes

We used an analytic model to show that FM regulation of nuclear localization bursts could allow transcription factors to modulate the expression of multiple target genes in concert, keeping their relative abundances fixed over a wide dynamic range, irrespective of their individual promoter architectures. This model was verified experimentally by measuring expression levels of synthetic and natural Crz1 target promoters under varying amounts of calcium stress.

C. Bursts Appear to be More General

We examined Msn2, a general stress response transcription factor that was previously reported to exhibit nuclear localization oscillations [4-6]. We found that Msn2-GFP localization is induced by calcium stress and localizes in short bursts with similar statistics to those of Crz1. However, bursts of the two proteins were largely uncorrelated when observed simultaneously in the same cell. Evidently the cell employs multiple transcription factor localization burst systems that operate in a largely independent manner.

References

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