Using viral-mediated noisy gene expression to probe signaling dynamics in mammalian cells

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***Short Abstract* — Coupling single-cell measurements with the inherent variability in viral-mediated gene expression provides an efficient means to quantify signaling dynamics in cells. Here, we used this approach to map the *E2f1* response to MYC, serum stimulation, or both. Our results revealed an unexpected mode of regulation: *E2f1* expression first increased then decreased with increasing MYC input. A mathematical model of the network successfully predicted that normal growth signals in serum expand the range of MYC capable of activating E2F. The use of noise to probe signaling dynamics reveals how the MYC/RB/E2F pathway discriminates aberrant and normal levels of growth stimulation.**

***Keywords* — Cell cycle, E2F1, MYC, noise, adenovirus, fluorescent reporter.**

I. Background

c-*Myc* encodes a nuclear protein that mediates extracellular growth signals by coordinating events related to metabolism, protein synthesis, and DNA replication (1-2). Expression of MYC is deregulated in a wide spectrum of cancers. The physiological and pathological consequences associated with c-*Myc* expression presents a fundamental challenge surrounding how cells distinguish normal and aberrant growth signals. Upon growth factor stimulation, increases in MYC modulate the expression and activity of E2F which regulate the transcription of genes required for S-phase. Previous work demonstrated that interactions between MYC and E2F manifest in a bistable-switch that governs an all-or-none E2F response to growth serum (3). Here, we couple experiments with mathematical modelling in order to provide a detailed understanding of the E2F response to direct MYC stimulation.

II. Results

To introduce and quantify MYC in single cells, we generated a replication-defective recombinant adenovirus expressing MYC tagged with yellow fluorescent protein. Infection of REF52 fibroblasts with adenoviruses expressing MYC-EYFP resulted in broad cell-cell variability with fluorescence that spanned three orders of magnitude. We compared the regulation of *E2f1* to serum and a broad range of deregulated MYC established with adenoviral delivery. Unlike the response to serum stimulation, overexpression of MYC did not result in a detectable increase in average E2F1 protein and mRNA measured across a population. However, single cell measurements revealed that the response to MYC was biphasic: Low levels of MYC input induced *E2f1* to levels at least as high as serum however, in cells that express higher levels of MYC, *E2f1* was gradually suppressed to near-baseline levels. Surprisingly, a biphasic expression pattern was also observed in expression of *p19Arf* and the *miR-17-92* cluster - loci that are transcriptionally regulated by MYC and E2F and are implicated in the negative regulation of E2F.

A mathematical model of the MYC/RB/E2F network including direct suppression of E2F by MYC was sufficient to explain biphasic E2F expression by MYC and montonic induction of E2F by serum. Furthermore, our model predicted that serum stimulation would shift the E2F dose response upwards with its biphasic character maintained. Specifically, by reducing RB activity, serum signals greatly expand the window of deregulated MYC that propels E2F into a supraphysiological range. Consistent with model predictions, addition of serum led to a shift in the biphasic pattern: except for very high MYC levels, *E2f1* was induced in all MYC-expressing cells to a greater extent than that observed with MYC alone

III. Conclusions

Our study underscores an underappreciated application of noise that is particularly well-suited for the use of viral vectors: when coupled with quantitative single-cell measurements, drastic cell-to-cell variability associated with viral-mediated gene expression can provide an input with a wide dynamic range that facilitates analysis of cell signalling dynamics in a high-throughput manner. Our noise-driven approach revealed a hitherto unknown biphasic induction of *E2f1* by MYC which provides an intuitive explanation for the variable results in previous studies regarding MYC-mediated induction of *E2f1*. Finally, a mathematical model describing direct suppression of E2F by MYC can explain the biphasic response and successfully predicts that serum expands the range of MYC capable of inducing *E2f1*. This work represents a foundation for further computational and experimental exploration of key events in cell cycle progression.

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