# The Rb-E2F Pathway Serves as a Digital Counter to Enable Precise Response to Transient Growth Signals

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***Short Abstract* — Proper control of cell proliferation is critical to tissue homeostasis in multicellular organisms. Using single-cell experiments coupled with stochastic modeling, here we show that the Rb-E2F pathway regulates cell proliferation by working as a "digital counter". It precisely quantitates transient growth signals and allocates corresponding number of cells to enter proliferation. The counting algorithm is determined by inherent stochasticity and bistability of the Rb-E2F gene network, and can be modified by altering the network dynamics. We also show that the digital counting property is inherent to bistable systems in mediating the appropriate responses to transient cellular signals.**

***Keywords* — cell proliferation, transient signal, single cell quantification, stochastic modeling, signal processing.**

## I. Purpose

GROWTH signals coordinate cell proliferation in multicellular organisms. This coordination is critical to tissue homeostasis *in vivo*. In physiological conditions, growth signals exist in transient forms, produced in need-based (e.g., during wound healing) or pulsatile manners (e.g., human growth hormones). It has been observed that the growth response of an isogenic cell population to transient stimulation is rather heterogeneous [1]. Little has been characterized, however, regarding the mechanism that determines the response of cells to transient growth signals.

Our previous work has demonstrated that cell proliferation is controlled by a bistable Rb-E2F switch [2]. Cells with this switch turned-ON start proliferation following growth stimulation, while others with it shut-OFF stay in quiescence. Here we investigate how this Rb-E2F switch responds to transient growth signals, which determines the proportion of cells that enter proliferation.

## II. Results

Through quantitative analysis of the stochastic response of the Rb-E2F switch to transient growth stimulations, we show that the mechanism that controls the proliferation pool of a cell population can be described by a simple mathematical equation, which is determined by the inherent stochasticity and bistability in the Rb-E2F network.

### A. Stochastic modeling

Our previous Rb-E2F model [2] was extended to include mathematical terms corresponding to intrinsic and extrinsic noise in cell signaling. Simulation results suggested that under non-saturating conditions, the proportion of E2F-ON cells ($P$) in a given cell population follows a deterministic function of the stimulation strength ($S$) and duration ($D$), $P = k*S/(Ks+S)*D$.

### B. Single-cell quantifications

Pulses of serum (growth signals) at varying concentrations and durations were applied to stimulate quiescent rat embryonic fibroblasts. The E2F level in each individual cell was measured with a GFP reporter and the proportions of E2F-ON cells were measured by flow cytometry. It was seen, as model predicted, that 1) the proportion of E2F-ON cells ($P$) exhibited a linear response to the stimulation duration ($D$), and 2) the slope of the linear response curve follows a Hill function of the stimulation strength ($S$), with constants $k$ and $Ks$ being 0.22 and 5.6, respectively.

### C. Modification of the counting algorithm

From both stochastic modeling and experimentations, we found that perturbations of key components involved in the positive feedback regulation of E2F greatly affected the slope of the linear response curve, by modulating the network-dynamics dependent constants $k$ and $Ks$.

### D. Generalization

Our analysis using a generic bistable model suggests that the digital counter mechanism is an inherent feature of bistable systems.

## III. Conclusion

Here we suggest a linear counting mechanism by which a cell population quantitates environmental signals and allocates a corresponding proportion of cells to enter proliferation. Modifications of the counting algorithm could lead to environmental adaptation and new cell types.

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