Controlling the Heterogeneous Quiescent State by an Rb-E2F Bistable Switch

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***Short Abstract* — Cellular quiescence is a reversible non-proliferative state that is critical to tissue homeostasis. Deregulation of the quiescent state can lead to a wide range of diseases. Control mechanisms of cellular quiescence are until now poorly understood. By combining modeling and single-cell measurements, we show that quiescent depth is determined by the activation threshold of an Rb-E2F bistable switch. We identified factors within the Rb-E2F pathway that modulate quiescent depth with different efficacy. We also show that Notch pathway and circadian rhythm pathway crosstalk with the Rb-E2F bistable switch and modulate the heterogeneous quiescent depth.**

***Keywords* — Quiescence, proliferation, heterogeneity, cellular state, Rb, E2F, bistable switch, activation threshold, Notch pathway, circadian rhythm.**

I. INTRODUCTION

QUIESCENCE is a non-proliferative state associated with many cell types (e.g., fibroblasts, lymphocytes, and stem cells) in the body. Distinct from other non-proliferative states that are irreversibly arrested such as senescence and terminal differentiation, the quiescent state is reversible. Reactivation of quiescent cells to enter the cell cycle under appropriate signals is fundamental to tissue repair and regeneration. Quiescence is often described as a "G0 phase" outside of the active cell cycle, but it is in fact not a single uniform state. Studies in the '70s and '80s have shown that when lymphocytes and fibroblasts were kept quiescent for a prolonged duration, they moved progressively "deeper" into quiescence and underwent a longer pre-replicative phase when reentering the cell cycle [1, 2].

Cells at an abnormally deeper or shallower quiescent state become hypo- or hyper-proliferative, respectively, which can lead to a wide range of diseases. It is therefore important to understand what controls the heterogeneous quiescent state and depth. Recently, Coller et al. compared the transcriptional profiles of human fibroblast cells that remained quiescent for different durations; they found that cells remaining quiescent for longer periods (at deeper quiescence) exhibited larger expression changes of a transcriptional "quiescence program" than cells remaining quiescent for shorter periods (at shallower quiescence) [3]. The transcriptional quiescence program suggests a likely regulatory mechanism of quiescent depth. However, it remains to be determined what activities in the transcriptional program were causal, instead of correlative.

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II. SUMMARY OF RESULTS AND CONCLUSION

Here we first show that as fibroblast cells go deeper into quiescent state (with prolonged serum starvation), they require stronger serum stimulation to reenter the cell cycle. On the other hand, these deep quiescent cells can still be reactivated to proliferate with sufficient serum stimulation, demonstrating that deep quiescence is distinct from senescence or cell death.

We then show that the depth of cellular quiescence can be defined by the activation threshold of an Rb-E2F bistable switch. Previously, we showed that the Rb-E2F pathway functions as a bistable switch, converting graded and transient growth signals into a binary (ON or OFF) and long-lasting E2F activity, which controls the quiescence-to-proliferation transition [4]. Here by combining modeling and single-cell measurements, we show that the degree of difficulty (i.e., threshold) to activate the Rb-E2F bistable switch accounts for the degree of difficulty to exist quiescence (i.e., quiescent depth). We identified different cellular factors within the Rb-E2F pathway with different efficacy to modulate the Rb-E2F activation threshold using computer simulations. Such model predications were further validated in experiments. We found that deep quiescent cells feature a higher Rb-E2F activation threshold and a delayed commitment to quiescence exit and cell cycle entry.

We further show that the Rb-E2F activation threshold can be modulated by the Notch pathway and circadian rhythm pathway. Such pathways crosstalk with the Rb-E2F pathway, affect the bistable region of the Rb-E2F switch, and modulate the heterogeneity of quiescence exit in response to growth signals.

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