Control of the fraction of differentiating mammalian cells by noise in the signaling network architecture

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Short Abstract — The tissue size of adult mammals is maintained by replacement of aging or damaged cells by slow, ongoing cell differentiation. Here we combine computational modeling, quantitative mass spectrometry [1] and single-cell microscopy[2] to identify the network architecture that enables fat cell precursor cells to differentiate at a very low rate of only 0.5% every 4 days. This architecture resolves two fundamental opposing requirements: to irreversibly lock cells in the differentiated state and to create large cell-to-cell signal variability to enable differentiation at very low rates. The resolution of this optimization problem by ultra-high feedback connectivity provides a generalizable mechanism for tissue size control.

Keywords — targeted mass spectrometry, selective reaction monitoring (SRM), single cell analysis, cell differentiation, cell-to-cell variability, protein expression noise

I. PURPOSE

Understanding how large populations of cells direct a very small subset to a different state is of fundamental relevance in the differentiation of precursor cells and stem cells as well as in cancer progression.

II. BACKGROUND

Adipocyte differentiation is arguably one of the most accessible experimental systems to investigate how mammalian cells make such cell fate decisions. Adipogenesis is also of fundamental importance in normal human physiology and disease associated conditions such as insulin resistance, obesity and cancer. Pre-adipocytes, the precursor cells from which adipocytes are made, are five-times less abundant than adipocytes and require four days to differentiate into adipocytes. Since 10% of mature adipocytes turnover in a year [3], pre-adipocytes differentiate very slowly at an average rate of ~0.55% in 4 days (4/365 * 5 *0.1).

Differentiation is known to be a bistable switch mechanism that involves positive feedback between two key transcription factors, CEBPA and PPARG, with a single threshold for activation in each cell. If the precursor

population were truly uniform, there should be a critical threshold above which all cells differentiate and below which all cells remain precursor cells. Nevertheless, this is not the case. Because of intrinsic noise, only a fraction of precursor cells in a population differentiates at a given stimulus intensity, and this fraction increases as the stimulus intensity increases.

However, by relying on intrinsic noise to control the fraction of differentiating cells, such a system has a fundamental problem in that the same noise must also reduce the stability of the differentiated state so that some "stably" differentiated cells will, because of the noise, revert to the undifferentiated state. What systems architecture can generate a behavior where the fraction of precursor cells that differentiate can be effectively regulated and differentiated cells can be locked in the differentiated state?

We next tested the prediction that adipocyte differentiation relies on ultra-high feedback connectivity by developing a parallel targeted mass spectrometry strategy to systematically identify positive feedback loops in a differentiation system.

III. CONCLUSION

We demonstrate computationally and experimentally that adipocyte differentiation relies on a system architecture with six positive feedbacks that resolves two fundamental opposing requirements: to irreversibly lock cells in the differentiated state and to create large cell-to-cell signal variability to enable differentiation at very low rates.

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