Uncorrelated transcription factor dynamics in sister cells

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***Short Abstract* —Single-cell microscopy experiments show that NF-κB activation and dynamics in sister cells can be uncorrelated. We use mathematical modeling to investigate the non-genetic sources of such variability.**

***Keywords* — Systems Biology, Microfluidics, Signaling, NF-KB, TNF-α, LPS, sister cells, modeling.**

SISTER cells are genetically identical, and their proteing levels are very close to each other immediately after mitosis. It is therefore reasonable to expect sister cell pairs to show very similar transcriptional activity. Indeed, several studies have shown that eukaryotic sister cells are well correlated, much more so than the rest of the cells in a population. A good example is the p53 transcription factor (TF) oscillations in sister cells that initally show high degree of correlation, and slowly loose synchrony long after cell-division [1]. We have found, through a high-throughput quantitative study of single mammalian cells, that NF-κB (p65) dynamics in sister cells can be uncorrelated even immediately after mitosis, exhibiting striking contrast to previous studies. NF-κB responds to hundreds of external stimuli (i.e. signaling molecules, virus, bacteria, physical stress and UV radiation), which in turn facilitates specific responses by translocating to the nucleus and coordinating the activation of more than one thousand genes [2]. The broad knowledge base of individual NF-κB components and their interactions makes this pathway a good candidate for systems level study of cell signaling and transcriptional activity. Here we use microfluidic time-lapse microscopy and mathematical modeling to investigate the non-genetic factors that can lead to uncorrelated activity in single mammalian cells.

Using microfluidic cell culture techniques and fluorescently labeled fibroblast cells where p65/dsRed flusion protein is under the regulation of endogenous p65 promoter [2], we measured TF activity in more than 50,000 single live cells under stimulation with various concentrations of TNF-α and LPS, with a temporal resolution of 6 minutes for duration up to 12 hours.

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We tracked and analyzed hundreds of sister cells and extracted NF-κB nuclear localization intensity. We found that sister cells show uncorrelated NF-κB activation and oscillation characteristics. Especially in cells that divide within a two hour window around the time of stimulation with TNF-α or LPS, we observed out-of-synch and variable intensity oscillations. In some cells, we observed anti-correlated oscillations, and some cells showed even non-oscillatory behavior while the other sister was oscillatory. The uncorrelated sister cells become more correlated in time and cells that divide long after stimulation tend to start out more correlated in TF dynamics.

To understand the source of such surprising behavior, we performed sensitivity analysis using a mathematical model we developed that simulates NF-κB activity in newly divided cells [2]. The analysis shows that even small variations in key regulatory proteins such as IκB and A20, and especially their mRNA transcript levels, can lead to uncorrelated behavior in sister cells.

Although the duplicated genetic material is perfectly partitioned during mitosis, the cell volume partitioning can have up to 10% variation. Using computational analysis, we found that such variation can lead to uneven partitioning of key regulatory mRNA's and proteins, especially during the early phases of NF-κB pathway activation where all IκB protein is degraded and need to be re-synthesized. If cells divide within this time period where protein and mRNA numbers are very low, sister cells can share them unevenly, which may lead to their subsequent uncorrelated behavior. We are using a black-box modeling approach to mitotic partitioning to relate NF-κB in mother cell and its progeny.

A quantitative study of sister cells is important for understanding the non-genetic origins of variation in cell populations. We show that NF-κB response can be uncorrelated, and even anti-correlated, in sister cells immediately after mitosis. The discovery of such uneven behavior in TF activation and dynamics in sister cells could have significant implications for understanding of innate immunity, stem cell differentiation and maintenance of pluripotency, and cancer metastasis via cancer stem cells.

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