Linking localized ERK/MAPK dynamics to transcriptional network states

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***Short Abstract* —Many genes are controlled by the activity of the ERK/MAPK pathway. Single-cell methods reveal that different ERK targets are expressed at divergent levels within the same cell. Spatial analysis indicates that these expression states are not simply the result of intrinsic noise in gene expression, but instead result from differential filtering of ERK dynamics by individual target genes. We have developed a live-cell system in which ERK activity and endogenous target gene expression can be monitored simultaneously, and we use this system to identify the dynamic signatures of ERK activity responsible for driving these spatial patterns.**

***Keywords* — Signal transduction, proliferation, cancer, live-cell imaging**

I. Background

THE ERK/MAPK pathway regulates cellular behavior by controlling the expression of transcription factors such as c-Myc, Egr-1, and Fra-1. In mammary epithelial cells, these factors control phenotypes including proliferation rate, epithelial to mesenchymal transition, and resistance to apoptosis, and their expression levels respond over a 10- to 15- fold range to the concentration of extracellular EGF. At physiological concentrations of EGF (10-100 pg/ml), these transcription factors are expressed very heterogeneously, with cell-to-cell variability spanning the full dynamic range of expression [1].

II. Results

To determine if variability in expression was correlated between ERK targets, we initially used immunofluorescence to measure the pairwise co-variance of the targets. Correlation between genes was low, revealing a wide range of expression states within the population. Although this could simply reflect noise in the process of target gene expression, spatial analysis revealed consistent patterns of expression in 2-dimensional culture on a collagen matrix: ra-1 is expressed most strongly in cells at the edge of clusters; Egr-1 is expression is highly correlated within localized patches of cells; c-Myc is expressed randomly with respect to cell position.

Our previous work has shown that ERK activity is pulsatile at the single cell level, and pulses are particularly aperiodic and asynchronous at physiological levels of EGF. Other work has suggested that these patterns of pulses may lead to differential responses at the level of target gene expression [3, 2, 4]. Taken together, these data suggested that the spatial patterns of ERK target gene expression may result from localized patterns of ERK dynamics that preferentially drive certain targets.

To test this hypothesis, we have developed a mammary epithelial cells co-expressing a reporter of ERK activity and a genomic insertion of a fluorescent protein fusion tag at the Fra-1 locus, generated by CRISPR-mediated DNA cleavage and homologous repair. Analogous cell lines for Egr-1 and c-Myc are currently under construction.

III. Conclusion

Initial results indicate that the genomic Fra-1 tag faithfully recapitulates the spatial patterns of Fra-1 expression. The preferential expression of Fra-1 at the edges of cell clusters appears to result from a pattern of sustained ERK pulses that occurs preferentially at cluster edges. We conclude that this is a viable approach for understanding how dynamic patterns of kinase activity generate a diversity of cellular expression states.

References

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