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The Single-Cell Yin and Yang of Live Imaging and Transcriptomics

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A new approach to monitoring both signaling over time and a global gene expression profile from the same cell establishes a functional role for NF-κB dynamics in transcription.

Diverse dynamics have been observed at the protein level and in localization of transcription factors in response to different stimuli (Behar and Hoffmann, 2010; Purvis and Lahav, 2013). However, the functions of these dynamics, especially at the transcriptional level, are unclear, mainly because it has not been feasible to robustly measure signaling dynamics and global gene expression profiles in the same cell. In this issue of *Cell Systems*, Lane et al. (2017) design a novel integrative approach, combining single-cell RNA sequencing (RNA-seq) with live-cell imaging to allow, for the first time, the analysis of signaling dynamics and transcriptomics from the same single cell. The authors show on a transcriptome-wide scale that different NF-κB dynamics result in differential activation of NF-κB targets. They also provide evidence for mechanisms by which transcription of different targets could be synchronized. Beyond its relevance to NF-κB, this study provides a new way to explore the functional role of signaling dynamics in other systems.

Researchers have long realized that single-cell monitoring is required to uncover heterogeneity. Early studies took advantage of advances in live-cell imaging and computational tools to watch the dynamics of transcription factor activation, as measured by the temporal fluctuations in protein levels or localization (Lahav et al., 2004; Tay et al., 2010). Later work showed that these dynamic responses can even vary between genetically identical individual cells (Lee et al., 2009; Paek et al., 2016). Such studies shed light on the temporal and spatial dynamics of transcription factor activation and, in many cases, revealed mechanisms by which different dynamics are generated.

Associating signaling dynamics with functional outcomes has been successful when clear phenotypic differences between cells, such as differences in apoptosis, proliferation, and differentiation, were readily observable (Purvis and Lahav, 2013). In contrast, associating dynamics with transcription is challenging because population-based RNA-seq techniques intrinsically mask cell-to-cell variation.

In recent years, the development of many single-cell RNA-seq technologies has allowed unprecedented investigation of cellular heterogeneity in different tissues, niches, and differentiation stages (Habib et al., 2016; Tirosh et al., 2016). Using fluorescence-activated cell sorting to isolate single cells prior to single-cell RNA-seq can reveal the cell state and specific characteristics at a given time prior to sequencing. This method of separation, however, cannot be utilized properly to address the relationship between transcription factor dynamics and the transcriptome, due to the absence of temporal information.

The transcription factor NF-κB is an ideal candidate for studying the relationship between transcription factor dynamics and transcriptomics. Its dynamics depend on the type and strength of stimulus (Nelson et al., 2004), and it has been shown that single cells display different dynamics even in response to a single stimulus (Lee et al., 2009). Whether or not this dynamical heterogeneity translates to transcriptional heterogeneity remains unresolved. With this in mind, Lane et al. (2017) integrated live cell imaging with single-cell RNA-seq (Figure 1). Although seemingly straightforward, this integration faced many challenges.

First, the identity of a cell must be maintained while it is being imaged and sequenced, but these two measurements are typically done on two different, incompatible platforms. To address this, the authors created a new experimental protocol for a Fluidigm C1 microfluidics instrument to allow rapid adherence of cells and treatment with stimuli while cells are in the microfluidics chip. The use of the Fluidigm C1 chip is an elegant way to resolve the integration challenge given the ability to visualize the cells in the microfluidic chip prior to processing them for single-cell RNA-seq (Tay et al., 2010). Additionally, the Fluidigm C1 is a commercially available platform already present in many institutions. Therefore, for many researchers, this will provide an almost off-the-shelf approach to tackle similar problems.

In addition to the technical challenge, integrating single-cell imaging and single-cell RNA-seq data requires a sophisticated bioinformatics pipeline. Analysis of single-cell imaging data is relatively labor intensive and time consuming. Single-cell RNA-seq data contain a high degree of noise and variability. This poses a challenge for highly expressed genes because a difference in expression of gene X between cells that have similar NF-κB dynamics might be real or might be a result of inter-sample variability. In addition, lowly expressed

genes might not be detected, a phenomena termed dropout. To improve statistical robustness, Lane et al. (2017) first grouped cells by NF-κB dynamics and correlated the corresponding transcriptomes, and then they grouped cells by transcriptomes and associated their NF-κB dynamics.

This unified approach (Figure 1) uncovered the relationship between NF-κB dynamics and the resulting transcriptome. After lipopolysaccharide (LPS) treatment, three subpopulations of NF-κB dynamics were observed. Two subpopulations exhibited prolonged NF-κB response, one with a broad NF-κB peak and another with a narrow initial peak followed by reoccurring activation. A third subpopulation exhibited only a narrow NF-κB peak. Transcriptomic analysis showed that >100 genes changed in expression levels, relative to a control. In general, differentially expressed genes, including negative regulators of the NF-κB response, appeared in the two subgroups that exhibited prolonged NF-κB levels. In contrast, no differentially expressed genes appeared in the subgroup that exhibited a narrow NF-κB peak. By perturbing NF-κB dynamics, the authors have convincingly showed that NF-κB dynamics and transcription are strongly associated and that variations in the expression of the cytokine TNF-α can, in part, explain the heterogeneity in NF-κB response. Additionally, the authors provide evidence for two mechanisms by which temporal dynamics of transcription factor levels could potentially synchronize activation of targets genes within cells.

Can this new approach be applied to other transcription factors? The use of a microfluidic device to visualize cells and process them for single-cell RNA-seq is convenient when dealing with relatively short timescales, such as the few hours required for NF-κB. The dynamics of other transcription factors, such as p53, are on a slower timescale (Lahav et al., 2004) and will therefore require that cells be monitored for longer periods of time (many hours to days). The effect of microfluidic devices on the survival and behavior of cells for a prolonged period of time is still unclear. Moreover, the isolation of cells in single wells prevents the ability to measure intra-cellular effects, such as the autocrine response in the case of NF-κB.

Another limitation, which is not unique to this study, but general and intrinsic to single-cell RNA-seq, is the low efficiency of capturing RNA that leads to loss of lowly to moderately expressed transcripts. This, in turn, leads to increase in technical variation (noise). Today, most approaches for determining technical variation rely on either spike-in of naked RNA (used in this study) or accounting for noise by using modeling. In addition to spike-in controls, Lane et al. dealt with statistical variation in single-cell RNA-seq by grouping cells, either by their NF-κB dynamics or by their transcriptomes. Improving the sensitivity and the capture efficiency of single-cell RNA-seq, together with new computational tools to distinguish noise from low-level signals, will be essential for overcoming this technical limitation.

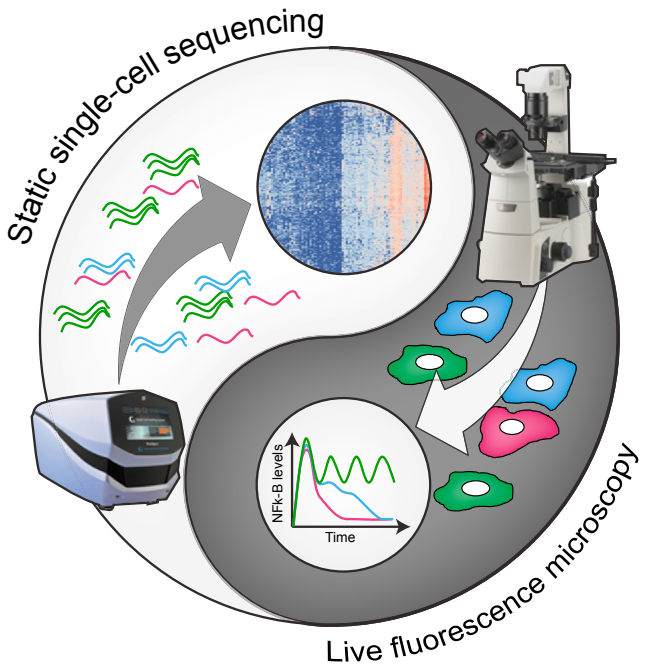

Figure 1. Yin and Yang: Integrating Live Imaging with Transcriptomics to Make Single Cells Whole

Combining live-cell imaging with single-cell RNA-seq allows researchers to develop a comprehensive understanding of cellular responses. Here, Lane et al. capture single cells on a Fluidigm C1 microfluidic chip. Cells are tracked via live-cell imaging for several hours and then processed for single-cell RNA-seq. This allows the integration of a dynamic approach (live-cell imaging) with a static measurement (single-cell RNA-seq) while retaining the identity of the cells, yielding an unprecedented view of dynamic processes leading up to the time of RNA collection.

Live-cell imaging and transcriptomics can be thought of as opposing techniques. The study of signaling dynamics using live-cell imaging focuses on cellular and molecular details of one or more pathways but misses a wider view of other potential effects and outcomes. Transcriptomics, on the other hand, produces a view that shows the big picture but lacks mechanistic details (Figure 1). The present study offers one approach to bridging the gap between these two techniques. Like yin and yang, these approaches are actually complementary, and integrating them promises to shed light on the broad cellular programs that are effected by, and that control, the dynamics of transcription factors in single cells, which is critical for understanding cellular behaviors and responses.

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REFERENCES

Behar, M., and Hoffmann, A. (2010). Curr. Opin. in Genet. Dev. *20*, 684–693.

Habib, N., Li, Y., Heidenreich, M., Swiech, L., Avraham-Davidi, I., Trombetta, J.J., Hession, C., Zhang, F., and Regev, A. (2016). Science *353*, 925–928.

Lahav, G., Rosenfeld, N., Sigal, A., Geva-Zatorsky, N., Levine, A.J., Elowitz, M.B., and Alon, U. (2004). Nat. Genet. *36*, 147–150.

Lane, K., Van Valen, D., DeFelice, M.M., Macklin, D.N., Kudo, T., Jaimovich, A., Carr, A., Meyer, T., Pe'er, D., Boutet, S.C., and

Covert, M.W. (2017). Cell Systems *4*, this issue, 458–469.

Lee, T.K., Denny, E.M., Sanghvi, J.C., Gaston, J.E., Maynard, N.D., Hughey, J.J., and Covert, M.W. (2009). Sci. Signal. *2*, ra65.

Nelson, D.E., Ihekwaba, A.E.C., Elliott, M., Johnson, J.R., Gibney, C.A., Foreman, B.E., Nelson, G., See, V., Horton, C.A., Spiller, D.G., et al. (2004). Science *306*, 704–708.

Paek, A.L., Liu, J.C., Loewer, A., Forrester, W.C., and Lahav, G. (2016). Cell *165*, 631–642.

Purvis, J.E., and Lahav, G. (2013). Cell *152*, 945–956.

Tay, S., Hughey, J.J., Lee, T.K., Lipniacki, T., Quake, S.R., and Covert, M.W. (2010). Nature *466*, 267–271.

Tirosh, I., Izar, B., Prakadan, S.M., Wadsworth, M.H., 2nd, Treacy, D., Trombetta, J.J., Rotem, A., Rodman, C., Lian, C., Murphy, G., et al. (2016). Science *352*, 189–196.