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Title: Spatial reconstruction of cellular and RNA regulatory interactions

The main project in our group is to develop single-cell genomics and imaging tools to investigate the molecular basis of transcriptional competency in morphogen signaling during early ESC development. We have shown that cell-cell and cell-ECM interactions lead to significant changes in the single cell state that might reprogram the transcriptional elongation machinery genome-wide. We hypothesizes that the regulation of cell state (i.e. cell cycle) is a much more responsive, robust, and scalable mechanism for sensing the tissue population composition, location, and topology in vivo, rather than simply relying on morphogen gradients. The ultimate goal is to create a 'periodic table' that matches individual cell states for major signaling pathways (i.e. WNT, BMP, FGF) with state-dependent cell fate decision programs in early ESC development. The ability to spatial map and compare the fate of individual cells, their states, and their differences in interpreting common signaling pathways could have a major implication for understanding the precision of microenvironment-dependent morphogenesis and tissue patterning, whose defect may lead to tumor initiation.

To identity single-cell state variants as a function of spatial position, cellular neighborhood, and morphology/orientation during ESC organoid development, our group is using high-throughput single-cell genomics technologies to investigate their transcriptional competency genome-wide. To spatially map single-cell variants, Dr. Lee's group is developing a powerful computational strategy to combine optical imaging of ESC organoids to reconstruct their transcriptome signature in 3D. Conceptually, their approach is very similar to the way cryoEM works for reconstructing the molecular topology. In order to reconstruct the RNA transcriptome topology, Dr. Lee and Dr. George Church (Harvard) together developed a powerful method to convert three-dimensional cells or tissues whose DNA or RNA can be sequenced genome-wide *in situ* (Fluorescent In Situ RNA Sequencing, or FISSEQ).

The spatial co-localization of the single-cell identity with the environmental context is insufficient to establish their causal role in shaping cell fate specification. For this, the Lee lab is developing new in situ RNA sequencing chemistry (Heuristic *In Situ* Oligopaint sequencing, HISTO-seq) that will sequence the RNA barcode in situ with high sensitivity. Here, uniquely barcoded single-cells and their somatic mutations (i.e. Cas9-induced) can be tracked in space and used for temporal phylogenetic reconstruction. Many available RNA imaging methods lack the single-nucleotide specificity, sensitivity, or throughput for studying RNA processing or alterations, and the high-sensitivity detection of numerous somatic mutations remains difficult. Here, HISTO-seq could have big clinical implications for detecting extremely rare cell types and their molecular identities for tumor somatic mutation pathology or in sorting circulating tumor cells based on somatic mutational signatures.