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Single-Cell RNA-Seq Steps Up to the Growth Plate

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Single-cell RNA sequencing (RNA-seq) technology is hitting its stride and is beginning to be widely adopted. One of the major challenges faced by this rapidly growing field lies in data analysis. Li *et al.* now present Sinova, a single-cell analytical platform offering temporal, spatial, and regulatory reconstruction of developmental processes.

Recent technological advances have enabled the analysis of genome-wide mRNA expression within single cells. These developments are proving to be pivotal and broadly applicable over many biological disciplines. Previous analyses of gene expression were restricted to the population level, lacking resolution by averaging results over many cells, and obscuring signals from rare cell types. However, single-cell RNA sequencing can identify novel, rare cell types. For example, a previously undiscovered cell species within the intestine was recently uncovered via single-cell RNA-seq, a feat that would otherwise have proven impossible with previous approaches [1].

The past year has heralded a new phase in single-cell technology. Analysis of hundreds of cells within a study has become standard, although remaining limitations on ease, cost, and scale have been a barrier to the broad application of single-cell approaches. These impediments have recently been addressed via the realization of highly parallel single-cell expression profiling [2,3]. Of particular note, Macosko *et al.* [2] used a simple, cost-effective, and robust microfluidic method to capture thousands of single-cell transcriptomes from an individual experimental sample. Together with the reduced cost of sequencing, these developments are paving the way for single-cell RNA-seq to soon become a laboratory staple.

Enabled by these methodological advances, it is becoming commonplace to generate single-cell transcriptome data for tens of thousands of cells. How to appropriately and effectively analyze these data is an emerging challenge. For example, many sources of technical variation exist between transcriptomes, arising due to differences in capture efficiency, amplification biases, and sequencing depth, making it difficult to detect discrete biological differences between single cells. Moreover, in any approach, whether bulk or single cell, continuous monitoring of gene expression within the same cells over time is not possible. Finally, to capture expression profiles, single cells must be dissociated from tissue, resulting in a loss of spatial and temporal information. These issues present barriers to using single-cell RNA-seq to systematically describe biological processes and identify the regulators driving them.

In response to these challenges, Li *et al.* [4] developed a new single-cell analytical methodology, Sinova. With this platform, they interrogate the development of the mouse growth plate, a structure required for bone elongation and regeneration. The postnatal growth plate comprises cells at sequential stages of differentiation, the exact regulation of which remains largely unknown at a systematic level. In this study, the expression profiles of 217 single cells were captured from this developmental structure, using the Fluidigm C1 platform. This simultaneous capture overcomes much interexperimental technical variation, and such an unsynchronized population contains cells representing many stages of development, enabling both temporal and spatial reconstruction of differentiation trajectories from a population 'snapshot'.

The Sinova pipeline first uses statistical and computational methods to reorder cells according to their position within a differentiation process, in this instance growth plate development. To achieve this *in silico* reconstruction, Sinova takes inspiration from the Monocle platform [5]. Monocle represents the first of several emerging methods that perform cell subpopulation characterization, followed by 'pseudotemporal ordering', the application of algorithms that reorder cells according to their position within a differentiation process. As a consequence, transcriptionally similar cells are placed in close proximity, with the assumption that they are closely related within a developmental hierarchy. Sinova leverages this strategy to reorder single cells in an unsupervised manner, employing the expression of just one well-characterized growth plate gene, *Col10a*, to identify the beginning and end of the differentiation process. The result is the systematic resolution of the growth plate developmental process from single-cell RNA-seq data, with promise for broad application to unravel other poorly defined developmental processes (Figure 1A).

Resulting from the pseudotemporal reordering of cells through the differentiation process, Sinova identified over 600 genes differentially expressed throughout growth plate development. Six distinct clusters of gene expression were identified based on these high-resolution pseudotemporal expression profiles. Reassuringly, this *in silico* developmental reconstruction temporally recapitulated previously reported findings on growth plate development. To generate a spatial reconstruction, cells were mapped within a cylindrical space, representing growth plate morphology and enabling visualization of single cells and associated gene expression along the proximodistal axis of the growth plate (Figure 1B). This elegant method also identified novel surface-marker expression to physically capture growth plate subpopulations via flow cytometry. While Sinova currently provides a partial spatial
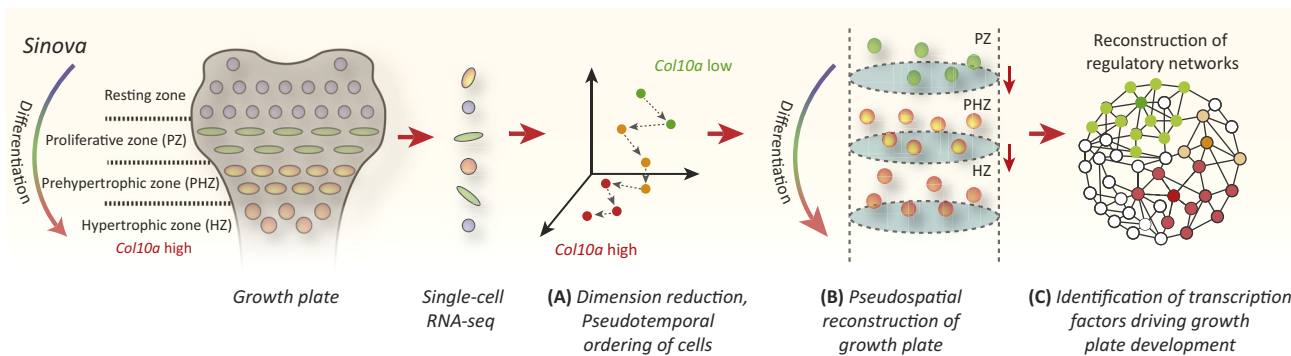
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Figure 1. Overview of the Sinova Analytical Pipeline, Enabling Temporal, Spatial, and Regulatory Reconstruction of Developmental Processes. Single-cell RNA sequencing of the growth plate, followed by pseudotemporal reordering of cells based on transcriptional similarity (A). Pseudospatial reconstruction of cells within a cylindrical space representing the growth plate (B). Reconstruction of regulatory networks to identify transcription factors driving growth plate development (C).

tissue reconstruction based on single cell similarities at the global transcriptome level, this new information on growth plate development could lead to the application of methods such as Seurat [6], where tissue reconstruction is enabled by known gene expression patterns from an *in situ* hybridization database, potentially offering higher-resolution reconstruction.

Some of the most powerful recent single-cell analytical methodologies have combined pseudotemporal ordering with the reconstruction of transcriptional regulatory networks, to infer regulatory relations responsible for driving developmental processes [7,8]. Sinova also integrates a similar approach by applying a hybrid association algorithm to estimate the regulatory potential of each transcription factor (TF) along the reconstructed growth plate pseudotemporal timeline (Figure 1C). This method identified 36 TFs with high regulatory potential in growth plate development, including factors previously not known to have a role in this process. This group of TFs was subsequently reduced to eight TFs, which were functionally validated within an *in vitro* model, where, as a pool, they promoted the expression of *Col10a*, suggesting a role for chondrocyte differentiation *in vivo*. Altogether, Sinova represents a powerful analytical toolkit, offering temporal, spatial, and regulatory reconstruction of developmental processes.

Single-cell technology continues to advance at a rapid pace and many new tools are emerging. The architects of Sinova adopted and adapted pre-existing platforms, such as Monocle, demonstrating the importance of building on and connecting current pipelines. It is of huge benefit that many of these essential tools, such as Monocle (http://cole-trapnell-lab.github.io/monocle-release/) and Seurat (http://www.satijalab.org/seurat.html), are freely available, and Sinova is no exception (https://github.com/bionova/sinova). These are extremely valuable contributions that will ensure that the single-cell community continues to grow and thrive.

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