Adding time-resolution to your single-cell RNA-seq

The understanding of individual cells has enormously improved with the application of single-cell RNA-sequencing approaches, enabling researchers to map the precise expression profile of individual cells in a heterogeneous cell mixture. From this heterogeneous sample, cells with similar gene expression profiles can be grouped together, rare subpopulations can be identified, and their signatures can be compared against each other. Furthermore, the response to treatments can be investigated or the cellular differentiation trajectory can be derived from the expression profiles.

However, this conventional single-cell RNA-sequencing approach only reflects a snapshot of a given time-point. This snapshot is upgraded to a more vivid image with a newly developed kit from Singleron Biotechnologies called DynaSCOPE™ Single Cell Dynamic RNAseq Library Kit, adding a "time" dimension to the single-cell RNA-seq data. Thereby, all transcripts of the single-cell RNA-seq experiment are marked with a timestamp, which then enables the investigation of transcriptional dynamics. This allows discerning nascent transcripts from static ones over a period of time and gives insights into how fast a transcript is synthesized or metabolized.

The best part of this innovative technology is that the new workflow can be easily integrated into the conventional single-cell RNA-sequencing workflow. For this purpose, a chemical compound (4-Thiouridine) is added to the cells and will be only incorporated into freshly synthesized transcripts (nascent transcripts), but not into static ones, hence, providing metabolic labeling of the RNA as a timestamp. Then, the standard single-cell RNA-seq workflow can be followed-up by implementing only one additional step for the conversion of the incorporated 4-Thiouridine prior to standardized next-generation sequencing (**Figure 1**).

The time-resolution of DynaSCOPE™ can be explored on a global or transcriptional level. Using DynaSCOPETM on a global level, it reveals that different cell types of the same tissue (e.g., lung) have varying RNA turn-over rates (Figure 2). At the same time, on the transcript level, different genes, specifically expressed in lung tissues (e.g. Figure 2), show distinct transcription dynamics. For instance, transcripts that are freshly synthesized in a specific cell type (e.g., Lamp3 in B cells) can be distinguished from those transcripts that are more stable and long-lasting (e.g., Sfta2 in B cells and Macrophages) (**Figure 2**).

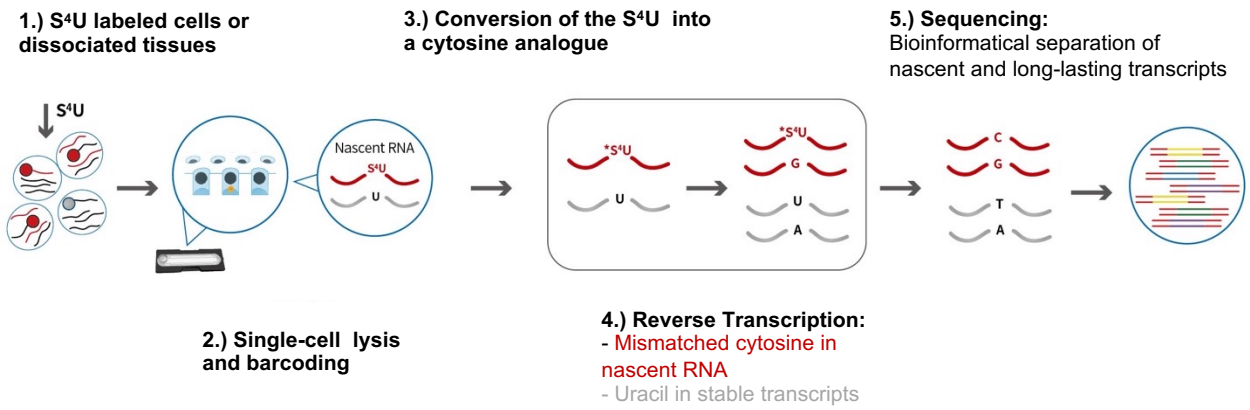

Figure 1. Schematic workflow of the DynaSCOPE™ single-cell transcriptome dynamics kit.

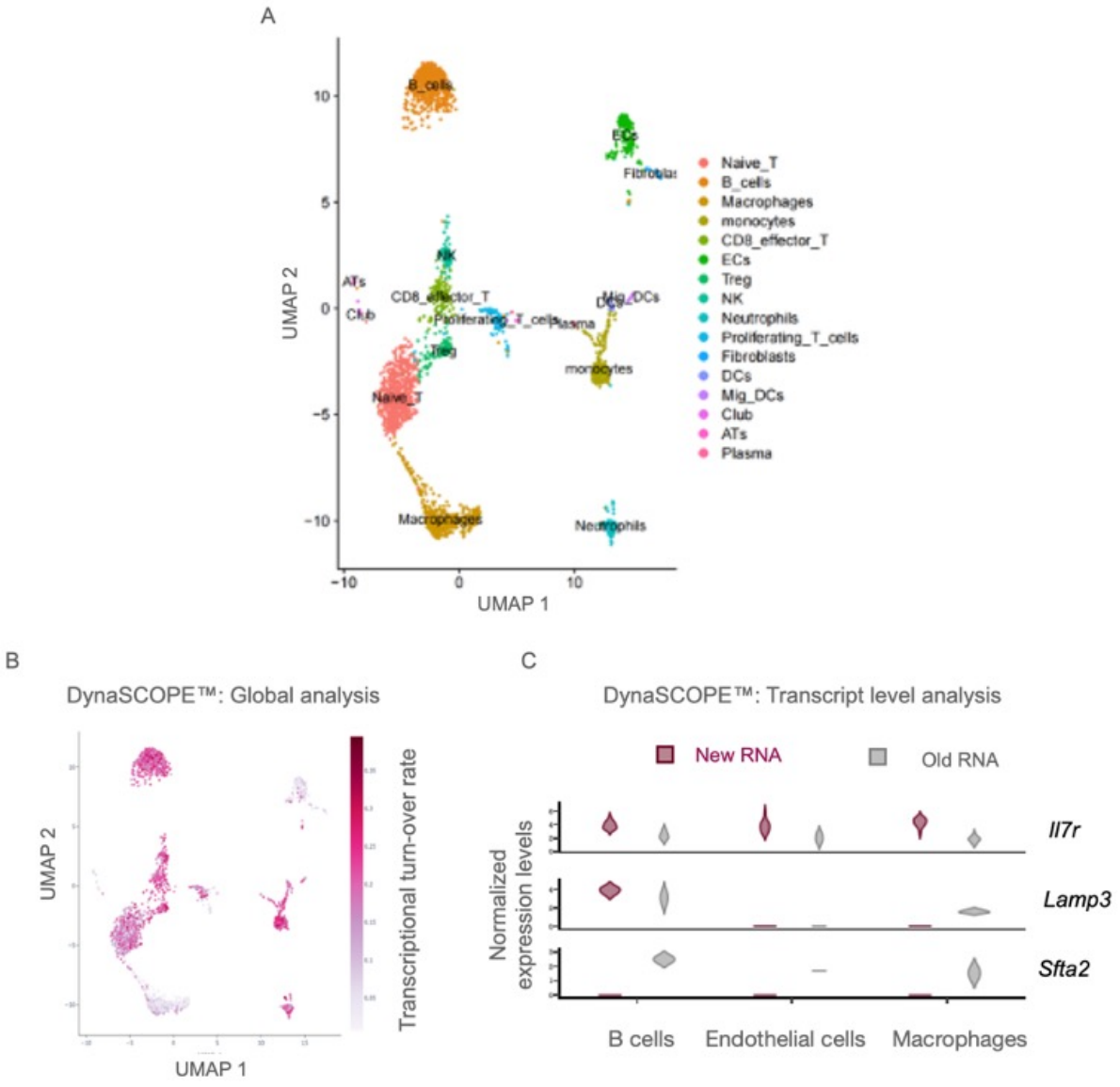

Figure 2. UMAP plot and the annotation of cell type clusters from murine lung tissue (top). With a DynaSCOPE™ data set, the time-resolution is added on a global level as turn-over rate per cell, darker clusters showing a high RNA turn-over rate (bottom, left), and for individual transcripts within each cluster (bottom, right).

One might envision that DynaSCOPE™ could help to discern primary and secondary effects of a treatment on a transcriptional level. This would shed light into signaling cascades and will facilitate the identification of potential new druggable target genes. Furthermore, it can support the prediction of developmental stages from individual cells in a time-dependent manner. This information is of particular interest to researchers of developmental biology, neuroscience (e.g. real-time analysis of massive transcript synthesis in the brain) and drug development.

The new method could also be used to investigate mechanisms of infection by bacteria or virus and how individual cells and tissues respond to an antigen or to therapeutic treatment. Thus, it opens the door for more precise medication and might improve patient outcomes in the future.

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