More comprehensive cell profiling to understand normal and disease states

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Single-cell multitargets and mRNA sequencing enable the simultaneous profiling of transcriptome and up to six histone modifications in individual cells.

In a multicellular organism, virtually every cell type contains an identical copy of the same genetic material, but the epigenome, defined as a set of chemical modifications to DNA and associated proteins such as modifications of histone proteins, differs substantially between cell types (*1*). The combinations of different histone modifications and chromatin-binding proteins together define the chromatin state of a cell, the disruption of which may lead to developmental defects and diseases such as cancer (*2*). Thus, understanding how the chromatin states of individual cell types differ from one another within a complex biological sample (i.e., cell type or state heterogeneity) and how chromatin states of individual cells change during development and disease progression is important for the dissection of developmental and disease mechanisms.

Single-cell genomic technologies have emerged as a powerful suite of tools to overcome the challenges of cell type or state heterogeneity present in biological samples. The advent of single-cell multimodal chromatin modification methods, in particular, has enabled simultaneous profiling of multiple histone modifications in the same cell. However, current technologies, such as scMulti-CUT&Tag (*3*), MulTI-Tag (*4*), nano-CUT&Tag (*5*), NTT-seq (*6*), and uCoTarget (*7*), have various limitations. They either require substantial hands-on time, have high risks of sample deterioration that compromises sequencing library quality and yield, or can only cocapture two to three types of histone modifications. To overcome these limitations, Wang *et al.* (*8*), in this issue of *Science Advances*, present scMTR-seq (single-cell multitargets and mRNA sequencing), a technology that can simultaneously profile transcriptome and up to six histone modifications in the same single cells with high sensitivity and high cell recovery.

To target and sequence regions of the genome near select histone modifications, the authors use Tn5 transposase fused to the antibody-binding protein A (pA-Tn5). This approach was also used in other single-cell genomics technologies (*3*, *4*, *9*). To enable simultaneous targeting of multiple histone modifications, antibodies specific for each target histone modification are preassembled with indexed pA-Tn5 and applied to cell nuclei, similar to the approach taken in Multi-CUT&Tag (*3*). Upon activation with Mg^{2+}, the Tn5 enzyme converts nearby DNA into viable sequencing library molecules to map genomic loci associated with the selected histone modifications. An in situ reverse transcription step in the intact nuclei produces paired transcriptome information (Fig. 1). The use of multiple rounds of split-pool barcoding allows the analysis of many cells without the need to isolate individual cells for processing. Compared with other similar technologies, scMTR-seq shows superior signal-to-noise ratio (SNR). This is achieved by (i) implementing a previously reported adapter switching strategy that increases the yield of sequence-able DNA fragments; (ii) adding immunoglobulin G (IgG) blocking antibodies to the unreacted, post-assembled pA-Tn5–antibody mixture to reduce off-target signals; and (iii) normalizing histone modification profiles to IgG control signals during sequencing data analyses to further suppress noises.

Using scMTR-seq, the authors first dissected chromatin state changes during human pluripotent stem cell (hPSC)–derived definitive endoderm differentiation by profiling five histone modifications and the IgG control. Using the paired transcriptome data to rank cells along the differentiation trajectory (i.e., transcriptional pseudotime), they identified patterns in the levels of histone modifications at gene promoters that correlate with the transcriptional activity of those genes. This demonstrates the application of scMTR-seq to resolve the dynamics of chromatin state changes during the induction of cell lineage fates. Next, the authors applied scMTR-seq to molecularly resolve the epiblast (EPI), trophectoderm (TE), and primitive endoderm (PE) lineages in mouse blastocysts by cocapturing six histone modifications and the paired transcriptome (Fig. 1). They uncovered asymmetrical lineage-specific patterns of chromatin states at gene promoter and enhancer regions in which TE cells have active histone modifications only at TE-associated promoters and enhancers, whereas PE cells, and, to a slightly lesser extent, EPI cells, have active histone modifications at gene regulatory regions associated with all blastocyst lineages. Furthermore, enhancer-driven gene regulatory networks in each cell lineage were inferred by coupling the histone modification data with the transcriptome data. This analysis suggested that a Gata protein Trsp1 represses TE-promoting enhancer networks in EPI cells possibly via suppressing Gata2 and Gata3.

Although extensive optimization steps boost the SNR, off-target signals are still detectable in scMTR-seq data especially for H3K27ac and H3K4me1. This may be due to the variable capture efficiencies and specificity of different antibodies as well as the varying degree of enrichments of different histone modification targets in the cells.

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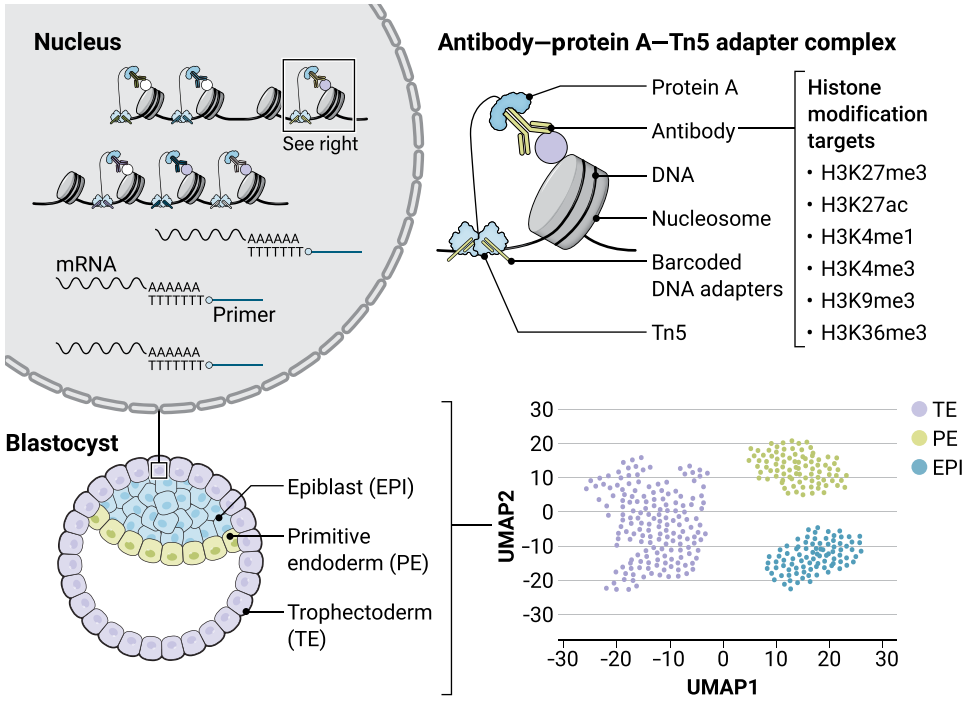

Fig. 1. Profiling transcriptome and chromatin states. scMTR-seq combines in situ reverse transcription with preassembled antibody–protein A–Tn5 adapter complexes to achieve simultaneous transcriptome and chromatin state profiling. This technology promotes our understanding of how the chromatin states of individual cell types differ from one another within a complex biological sample (such as the mouse blastocyst) and how chromatin states of individual cells change during development (such as during mouse embryonic development) and disease progression. Credit: Ashley Mastin/*Science Advances*.

Nonetheless, scMTR-seq expands our ability to characterize chromatin states and their impact on transcription. The ability to capture six histone modifications simultaneously makes it possible to define finer chromatin states (e.g., strong versus weak promoters/enhancers). This is particularly valuable for examining dynamic shifts in cell states, wherein the alignment of changes for each genomic property is critical to understanding their mechanistic relationship. Last, apart from histone modifications, it is technically feasible to adopt scMTR-seq to capture signals of chromatin-binding proteins such as transcription factor foot printing. This would further facilitate future studies on gene regulation in complex systems from development to diseases.

In summary, scMTR-seq provides a more comprehensive solution than previous methods to understand the heterogeneity in chromatin states of individual cells and how chromatin states of individual cells change during development and disease progression.

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