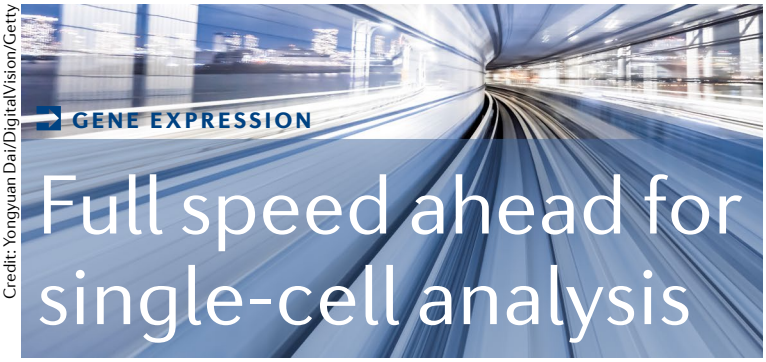

Credit: Yongyuan Dai/DigitalVision/Getty

GENE EXPRESSION

Full speed ahead for single-cell analysis

Single-cell RNA sequencing (scRNA-seq) has been used extensively to characterize the constituent cell types of various tissues in different organisms. However, the cells are destroyed during processing, so each data set represents a static snapshot in time, with no ability to resample the same cells for their transcriptional states at additional time points. A new study uses a strategy called RNA velocity that leverages nascent unspliced RNA to deduce the future transcriptional states of cells, thus providing insights into developmental trajectories.

The overarching principle of the RNA velocity method reported by La Manno et al. is that relative to a cell expressing a given gene at steady-state level, transcriptional upregulation of that gene will result in a transient excess of nascent, unprocessed transcripts compared with processed transcripts, whereas transcriptional downregulation results in a relative depletion of nascent transcripts. Calculating the ratio of unspliced to spliced transcripts for each gene might therefore be used to deduce which genes are undergoing upregulation or downregulation and hence the future state of a cell; for example, a precursor cell upregulating classic marker genes for a particular differentiated cell type would indicate its likely future cell type.

Current scRNA-seq pipelines comprise diverse methodologies, but most feature oligo(dT) primers to enrich for polyadenylated transcripts, so that sequencing bandwidth is not dominated by unwanted transcripts such as ribosomal RNAs. In principle this would hinder RNA velocity analysis because unprocessed transcripts would be typically unpolyadenylated and thus not interrogated. However, La Manno et al. showed that across diverse scRNA-seq pipelines, 15–25% of reads originated from priming in the intronic regions of unspliced molecules, and hence such data sets might indeed be amenable to RNA velocity analysis.

As proof of principle that RNA velocity analyses provide useful information on future transcriptional states, the authors analysed a time course series of mouse liver samples across the circadian cycle using bulk cell population RNA-seq, showing that RNA velocity predicts the change in RNA abundance in the subsequent time point. Furthermore,

> “Layering on RNA velocity data … allowed the multi-step differentiation process to be traced”

HUMAN EVOLUTION

The girl with Neanderthal and Denisovan parents

Neanderthals and Denisovans are distinct groups of hominins that diverged 390,000 years ago. Their DNA has been detected in both ancient and present-day individuals, indicating past gene flow between archaic and modern hominins. However, direct evidence for genetic mixing between overlapping populations is limited, and the closest reported association is a modern human (Oase 1) with a Neanderthal ancestor within four to six generations. Now, a study in *Nature* characterizes the genome of Denisova 11, a first-generation offspring of Neanderthal and Denisovan parents.

Denisova 11, a bone fragment found alongside more than 2,000 others in Denisova Cave (Russia), had previously been shown to be more than 50,000 years old and to have Neanderthal mitochondrial DNA (mtDNA). In this new study, genomic analysis indicates that Denisova 11 was a female who, based on the thickness of the bone fragment, was at least 13 years old at the time of her death. To find out more about her ancestry, her genome was compared to Neanderthal (Altai Neanderthal) and Denisovan (Denisova 3) genomes, both obtained from bones found in Denisova Cave. The Denisova 11 genome contained a similar proportion of Neanderthal (38.6%) and Denisovan (42.3%) derived alleles, indicating that both hominin groups contributed equally to her ancestry — either she belonged to a mixed Neanderthal–Denisovan population or one of her parents was Neanderthal (her mother, based on previous mtDNA analysis) and the other Denisovan.

> “mixing between hominin groups occurred frequently when populations overlapped”

Credit: EyeEm/Alamy

To distinguish between these two scenarios, the researchers estimated the heterozygosity of the Denisova 11 genome by measuring the frequency of transversion polymorphisms. It was four times more heterozygous than sequenced genomes from Neanderthals (Altai Neanderthal and Vindija 33.19) and Denisovans (Denisova 3). Further analysis of transversion sites that were homozygous for different alleles in Altai Neanderthal and Denisova 3 showed that the proportion of heterozygous sites (that is, sites with one Neanderthal allele and one Denisovan allele) in Denisova 11 was consistent with her having inherited one set of chromosomes from a Neanderthal and the other set from a Denisovan.

applying the method specifically to single-cell data, they showed that in a day 12.5 mouse embryo scRNA-seq data set, RNA velocity showed the expected developmental trajectory from Schwann cell precursors into chromaffin cells. Additionally, the authors demonstrated the ability of RNA velocity analysis to recapitulate known developmental trajectories in diverse scRNA-seq data sets collected from various mouse tissues using different types of RNA-seq pipelines.

The main novel application of RNA velocity was to study mouse hippocampus development. Cells were initially plotted according to cell type on a t-distributed stochastic neighbour embedding (t-SNE) plot. Layering on RNA velocity data in the form of arrows allowed the multi-step differentiation process to be traced, from the identification of the most primitive precursor cells in the plot (from where the arrows originate) through a branching differentiation path to the various differentiated cell types at the tips of branches (where the arrows end). Furthermore, at key developmental branch points, analyses of the upregulated genes underlying the arrows pointing in alternative directions allowed inferences of potentially key developmental transition genes that are responsible for driving cell fate choices down a particular route, such as *Prox1* upregulation driving commitment to granule neuron fate.

Finally, as evidence that RNA velocity is relevant for human embryos, La Manno et al. applied scRNA-seq to the developing human forebrain, successfully identifying a differentiation path from progenitor radial glia cells through to differentiated glutamatergic neurons.

RNA velocity thus holds promise to unveil dynamics from static RNA-seq data and is widely applicable across diverse organismal systems and technical platforms.

Darren J. Burgess

ORIGINAL ARTICLE La Manno, G. et al. RNA velocity of single cells. *Nature* **560**, 494–498 (2018)
FURTHER READING Stegle, O., Teichmann, S. A. & Marioni, J. C. Computational and analytical challenges in single-cell transcriptomics. *Nat. Rev. Genet.* **16**, 133–145 (2015)

This conclusion was further supported by coalescent simulations that accounted for likely genetic differences between Denisova 11's ancestors and Altai Neanderthal and Denisova 3, and by the observation that heterozygous sites were evenly distributed throughout the Denisova 11 genome.

Next, the researchers set out to investigate the ancestry of Denisova 11's Neanderthal mother and Denisovan father. They identified at least five regions, each ~1 Mb long, that were homozygous for Neanderthal DNA, indicating that her father had some Neanderthal ancestry. The number and size of these regions, along with their high heterozygosity relative to Altai Neanderthal and Vindija 33.19, suggested that he had one or more distant Neanderthal ancestors and that they came from a different population to that of Denisova 11's mother. Analysis of Denisova 11's Neanderthal genetic heritage demonstrated that her mother was more closely related to Vindija 33.19 — who lived around 40,000 years later in Western Eurasia — than to Altai Neanderthal, who lived in the same cave 20,000 years before her. This observation suggests that Siberian Neanderthals migrated to Western Europe sometime after the existence of Denisova 11 and/or Western European Neanderthals migrated to Denisova Cave before that time.

The genome of Denisova 11 provides evidence of at least two instances of genetic mixing between hominin groups — once between her parents and at least once in her father's ancestry. Gene flow has now been detected in two of six genomes retrieved from Denisova Cave (Denisova 3 and Denisova 11) and one of three genomes retrieved from modern humans that co-existed with ancient hominins (Oase 1). The frequency of such genomes suggests that mixing between hominin groups occurred frequently when populations overlapped and that geographical and temporal barriers, rather than reproductive ones, contributed to Neanderthals and Denisovans remaining genetically distinct.

Dorothy Clyde

ORIGINAL ARTICLE Slon, V. et al. The genome of the offspring of a Neanderthal mother and a Denisovan father. *Nature* **561**, 113–116 (2018)

Credit: Redmond Durrell/Alamy

DEVELOPMENT

In vivo lineage tracing in mice

Advances in CRISPR–Cas9 genome editing have enabled the prospective tracking of cell lineages in diverse cells, tissues and lower vertebrates, such as zebrafish and axolotl, but application to mammals has been hampered by challenges arising from the complexity of mammalian development. Now, Kalhor et al. report the successful recording and reconstruction of developmental lineages in the mouse using in vivo-generated barcodes.

The team built on proof-of-principle concepts of the genome editing of synthetic target arrays for lineage tracing (GESTALT) technique, whereby lineage barcodes that consist of multiple Cas9 target sites accumulate unique mutations over time. The incrementally edited barcodes from thousands of cells are recovered by targeted sequencing, and lineages are reconstructed from the pattern of shared mutations among cells.

To apply this approach to mice, the authors generated the MARC1 (mouse for actively recording cells) founder mouse, which harbours 60 homing CRISPR guide RNA (hgRNA) loci in its genome, and crossed it with females constitutively expressing Cas9 protein. Upon Cas9-mediated activation, the hgRNA, which comprises a unique 10-base identifier and a spacer sequence, targets its own locus for mutation. In offspring, hgRNA loci begin accumulating lineage-specific mutations shortly after conception, with mutagenesis continuing throughout gestation. The hgRNA loci thus act as genetic barcodes as closely related cells exhibit greater similarity in mutational patterns within spacer sequences than more distant cells, enabling reconstruction of developmental lineages. Importantly, by targeting multiple sites simultaneously, the approach by Kalhor et al. ensures that mutations accumulate independently of each other. When combined, this creates the exponential diversity in barcodes that is required for the complex mammalian system.

Focusing on the first lineage segregation events in mouse, the authors were able to reconstruct accurate and robust lineage trees for the early developmental stages in four embryos. Finally, the team used developmentally barcoded mice to investigate axis development in the brain.

This study further highlights the potential of cumulative and combinatorial barcode editing to track cell lineages in whole organisms, including mammals. Beyond lineage tracing, this platform may be useful for recording cellular signals over time.

Linda Koch

ORIGINAL ARTICLE Kalhor, R. et al. Developmental barcoding of whole mouse via homing CRISPR. *Science* https://doi.org/10.1126/science.aat9804 (2018)
FURTHER READING Woodworth, M. B. et al. Building a lineage from single cells: genetic techniques for cell lineage tracking. *Nat. Rev. Genet.* **18**, 230–244 (2017)