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)

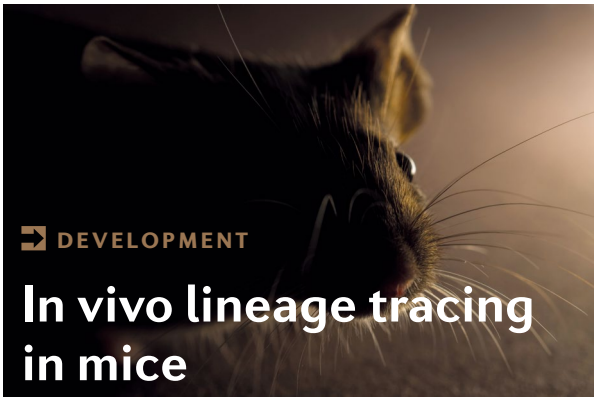
This conclusion was further supported by coalescent simulations that accounted for likely genetic differences between Denisova 11's ancestors and Altai Neanderthal and Denisova 3, and by the observation that heterozygous sites were evenly distributed throughout the Denisova 11 genome.

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)