the interaction landscape between TFs and the nucleosome."

In the second study, Lai et al developed single-cell micrococcal nuclease sequencing (scMNase-seq): DNA fragments protected from MNase digestion were isolated, sequenced and mapped to the genome to generate a genome-wide map of both chromatin accessibility (from ≤80 bp subnucleosome-sized particles) and nucleosome spacing and positioning (from 140–180 bp nucleosome-protected fragments) for each cell.

Data from three different cell types (NIH3T3 cells, mouse embryonic stem cells (ESCs) and mouse naive CD4 T cells) revealed that nucleosome organization was reflective of chromatin accessibility: nucleosomes at silent chromatin showed uniform spacing but highly variable positioning, whereas at active chromatin (such as at DNase I hypersensitive sites (DHSs)) they were precisely positioned but variably spaced. Moreover, nucleosome spacing at DHSs could be either 'wide' (~300 bp) or 'narrow' (~190 bp) for a given cell but, for more than 80% of DHSs in the genome, both types of spacing were detected in cell populations. Furthermore, the level of heterogeneity in nucleosome spacing at any given DHS was correlated with variation in chromatin accessibility, nucleosome positioning and gene expression — raising the possibility that differences in nucleosome organization reflected future differences in cell identities. Indeed, further analysis revealed population-level variability in nucleosome occupancy at lineage-specific de novo enhancers in naive T cells and mouse ESCs, suggesting that nucleosome reorganization primes some undifferentiated cells in a population for differentiation towards a specific lineage.

Taken together, these two studies paint a complex picture of how TFs and nucleosomes regulate gene expression and cell fate.

Dorothy Clyde

ORIGINAL ARTICLES Zhu, F. et al. The interaction landscape between transcription factors and the nucleosome. *Nature* **562**, 76–81 (2018) | Lai, B. et al. Principles of nucleosome organization revealed by single-cell micrococcal nuclease sequencing. *Nature* **562**, 281–285 (2018)

Using available crosslinking immunoprecipitation followed by sequencing (CLIP–seq) data sets for 76 RNA-binding proteins (RBPs), the authors determined that lead sQTLs showed significant enrichment in binding sites of 18 RBPs. sQTLs were also enriched in binding sites of several heterogeneous nuclear ribonucleoproteins (hnRNPs), including hnRNP C, which has previously been linked to Alzheimer disease, thus revealing disease-relevant changes in pre-mRNA splicing.

Transcriptome-wide association studies (TWAS) for 4,746 genes and 15,013 differentially spliced introns identified 21 genes whose mRNA expression or levels of intron excision were associated with Alzheimer disease, including genes in known loci, such as *CR1*, *PTK2B* and *SPI1*, as well as 8 novel loci, including *AP2A1*, *AP2A2* and *MAP1B*. Two complementary replication approaches were used to validate the findings.

Network analysis of known and novel Alzheimer susceptibility genes revealed an enrichment of pathways related to endocytosis and autophagy. Consistent with previous work implicating protein degradation in Alzheimer disease, proteins encoded by *AP2A1*, *AP2A2* and *MAP1B*, which have a role in the autophagy–lysosomal-related pathway, were shown to be connected with known disease genes in a protein–protein interaction network.

The authors hope that their catalogue of variants that affect RNA splicing in the ageing cortex will prove useful as a resource for "annotating the results of genetic and epigenomic studies of neurologic and psychiatric diseases". Beyond this aim, the reference map generated by Raj et al. not only increases the number of variants implicated in Alzheimer disease but also may aid the functional analysis of these susceptibility alleles.

Linda Koch

ORIGINAL ARTICLE Raj, T. et al. Integrative transcriptome analyses of the aging brain implicate altered splicing in Alzheimer's disease susceptibility. *Nat. Genet.* https://doi.org/10.1038/s41588-018-0238-1 (2018)

TRANSCRIPTOMICS

Navigating mouse cell types

Credit: ballyscanlon/Stockbyte/Getty

Advances in single-cell RNA sequencing (scRNA-seq) technologies and the coordination of multi-laboratory consortia have set the stage for the generation of transcriptome data from the many thousands of cells that are required for deep characterization of tissues or whole organisms. A new study reports the 'Tabula Muris', an atlas of >100,000 cells across 20 mouse tissues.

The Tabula Muris Consortium harvested multiple organs from the same mice to control for potentially confounding factors, such as age and environment. On the dissociated cell samples they used two scRNA-seq methods, which provide complementary data types. Microplate-based scRNA-seq was applied to all 20 organs and provided high-depth full-length transcript data. Such full-length data enable additional downstream analyses of transcript isoform features, such as alternative splicing. By contrast, the droplet-based scRNA-seq applied to 12 of the organs provides more of a transcriptome snapshot by sequencing just the 3′ ends at lower depth. However, the high scalability of cellular throughput of the droplet method facilitates data generation from thousands of cells (for example, >11,000 trachea cells analysed by droplet scRNA-seq), which enables the identification of rare cell types.

The main analyses performed were clustering of cells into putative groups of cell types on the basis of similarities in gene expression patterns, identification of those cell types from the expression of key marker genes, and then further inferences from any notable features of the clustering or gene expression patterns. Importantly, both scRNA-seq platforms performed comparably for cell-type clustering, thus highlighting the interoperability of the data types and opportunity for merged analyses.

Analysing data for single organs revealed various individual genes with putative roles in the development of those organs. However, a major strength of the project is that the methodological standardization across organs allows global clustering of cells for cross-tissue inferences. For example, the authors identified similar endothelial, mesenchymal and stromal cells across multiple organs, and could infer differentiation and activation statuses across T cells resident in different organs. Finally, they characterized the key transcription factor expression patterns that define cell types across organs, which may help to refine protocols for directed cell differentiation or reprogramming.

The Tabula Muris project complements other related efforts such as the Mouse Cell Atlas and Human Cell Atlas projects. Together, these valuable community resources will deepen our understanding of numerous phenomena, such as organ development, cell type diversity and gene regulatory networks.

Darren J. Burgess

ORIGINAL ARTICLE The Tabula Muris Consortium. Single-cell transcriptomics of 20 mouse organs creates a *Tabula Muris*. *Nature* https://doi.org/10.1038/s41586-018-0590-4 (2018)