METHODS IN BRIEF

GENE EXPRESSION

Unmixing cell lineage states with single-cell RNA sequencing

How a cell chooses between two fates is a fundamental question in development. Using single-cell RNA sequencing to identify cell states, Olsson *et al.* identified bifurcation points and inferred the regulatory genes that underlie cellular decisions. They developed an iterative clustering and guide-gene selection (ICGS) tool to find dynamically expressed genes and to identify expression patterns by clustering with pattern-specific 'guide genes'. Using RNA sequences generated from single blood cells, ICGS automatically resolved nine known myelopoiesis cell states. The authors' analysis reveals both common and rare (metastable) mixed-lineage states, as well as evidence for antagonistic relationships between regulatory genes from competing lineages. They show that a rare intermediate that gives rise to hematopoietic stem cell/progenitor and myeloid lineages could be trapped through elimination of the myeloid determinants IRF8 and GFI1.

Olsson, A. *et al. Nature* **537**, 698–702 (2016).

NEUROSCIENCE

Specific activation of dopamine neurons in macaques

Optogenetic activation is a powerful method for unraveling the functions of different cell types in the brain. However, achieving cell type specificity is challenging in organisms that are difficult to manipulate genetically. Stauffer *et al*. describe an approach for optogenetic stimulation of dopamine neurons in the macaque brain with high specificity and efficiency. The researchers injected two viruses into the target brain region: one that expresses Cre recombinase under the control of a promoter fragment specific for dopamine neurons, and one that harbors a Cre-dependent channelrhodopsin construct. This strategy allowed them to avoid the low expression levels often associated with direct promoter-fragment-driven expression of effector proteins. The researchers applied their approach to activate dopamine neurons in the macaque midbrain and studied the targeted neurons using electrophysiology and behavioral experiments.

Stauffer, W.R. *et al*. *Cell* **166**, 1564–1571 (2016).

GENETICS

Apicomplexans submit to CRISPR screening

Sidik *et al.* used CRISPR–Cas9 to carry out the first genome-wide genetic screen in apicomplexans, intracellular parasites that cause devastating diseases such as malaria and toxoplasmosis. To increase targeting efficiency over transient transfection, the authors constitutively expressed Cas9 in *Toxoplasma gondii*, in addition to a 'decoy' single guide RNA (sgRNA) to mitigate toxic overactivity. They used this strain to transfect an sgRNA library redundantly targeting each of the more than 8,000 genes in the genome, and they followed up with fitness assays. The work uncovered approximately 200 genes that were not previously known to be essential, including one encoding a broadly conserved protein they dub claudin-like apicomplexan microneme protein (CLAMP), which is required for host cell invasion.

Sidik, S.M. *et al*. *Cell* **167**, 1423–1435 (2016).

PROTEOMICS

The proteomes of excitatory and inhibitory synaptic clefts

Synapses, which come in excitatory and inhibitory flavors, are essential for communication between neurons. To gain insight into the differences between the types of synapses, Loh *et al*. developed a method to characterize the proteome of the synaptic cleft, based on an approach that they previously used to map the protein composition of organelles. They targeted horseradish peroxidase to the synaptic cleft of either glutamatergic or GABAergic synapses and then added a biotin compound, which resulted in biotinylation of resident proteins in the cleft. The biotinylated proteins could then be enriched and analyzed by mass spectrometry. Using this approach, the researchers identified 199 and 42 proteins in excitatory and inhibitory synaptic clefts, respectively, several of which they followed up on and confirmed with alternative methods.

Loh, K.H. *et al*. *Cell* **166**, 1295–1307 (2016).