TOOLS IN BRIEF

GENETICS

Cas9 on target

Lack of specificity and off-target cleavage are ongoing problems for the CRISPR-Cas9 gene editing system. Two groups have now made modifications to *Streptococcus pyogenes* Cas9 to increase its specificity without affecting its activity. Slaymaker *et al*. designed enhanced specificity Cas9 (eSpCas9), which has weakened interaction between Cas9 and the DNA strand that is not bound by the single guide RNA (sgRNA). eSpCas9 requires stronger base pairing between the target strand and the sgRNA-Cas9 complex to achieve cleavage. Kleinstiver *et al*. developed a high-fidelity Cas9 (SpCas9-HF) with weakened interaction between Cas9 and the phosphate backbone of the target DNA strand to decrease the energy of the complex and shift its activity to target sites only. Both approaches led to highly specific genome editing tools.

Slaymaker, I.M. *et al*. *Science* **351**, 84–88 (2016).
Kleinstiver, B.P. *et al*. *Nature* **529**, 490–495 (2016).

CHEMICAL BIOLOGY

An approach to study MARylation

Mono–ADP-ribosylation, or MARylation, is the transfer of an ADP-ribose from NAD^+ to a protein by a mono–ADP-ribosyltransferase (mono-ARTD). Most targets of this post-translational modification are currently unknown. In new work, Carter-O'Connell *et al*. describe a chemical genetic approach used to unambiguously identify specific mono-ARTD targets. Using a so-called bump-hole strategy, they engineered a specific mono-ARTD with a larger pocket (the 'hole') to accommodate a bulkier benzyl-modified NAD^+ analog (the 'bump') that cannot be used by wild-type mono-ARTDs. The NAD^+ analog additionally contains a click chemistry handle to enable ready purification of the targets of the modified mono-ARTD, for identification by mass spectrometry. The authors used this strategy to profile the MARylomes of ARTD10 and ARTD11, which led them to discover a potential function for ARTD11 in regulating the nuclear pore complex.

Carter-O'Connell, I. *et al*. *Cell Rep*. **14**, 621–631 (2016).

MOLECULAR BIOLOGY

Label-free image flow cytometry

Teasing apart complex phenotypes in single cells at high throughput is a formidable challenge. Flow cytometry can effectively detect cells with certain features if fluorescent antibodies or dyes are available; image flow cytometry, which obtains spatial images in addition to fluorescence intensities, can find more complex phenotypes such as cell cycle phases of individual cells. Blasi *et al*. now show that quantitative analysis of bright- and darkfield images collected during image flow cytometry enables cell cycle measurements without any fluorescent markers. The authors extracted measurements of cell morphology, such as size, shape and granularity, from images of either fixed or live cells and applied supervised machine learning algorithms to determine cell cycle stages.

Blasi, T. *et al*. *Nat. Commun*. **7**, 10256 (2016).

STEM CELLS

A guide to cellular transdifferentiation

A number of differentiated cell types can be forced to change identity without going through a pluripotent state, but screening for conversion factors is usually laborious. Rackham *et al*. have developed Mogrify, a computational tool that analyzes expression data from cell and tissue types in the FANTOM5 project, identifying transcription factors that are differentially expressed in each cell type and that regulate differentially expressed genes in the local regulatory network. Applied to 173 human cell types, the tool suggested sets of factors that could be used to drive the transdifferentiation of any cell type into any other cell type. A number of Mogrify predictions are validated by existing transdifferentiation protocols, and the authors experimentally verified predictions for two novel conversions.

Rackham, O.J.L. *et al*. *Nat. Genet*. doi:10.1038/ng.3487 (18 January 2016).