METHODS IN BRIEF

EPIGENETICS

A high-resolution look at DNA demethylation

During DNA demethylation, 5-methylcytosine (5-mC) sites are progressively oxidized before removal by repair enzymes. Methods exist to profile the final two rare intermediates, 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC), but most approaches involve enrichment steps and cannot provide single-base resolution or absolute quantification. Bisulfite sequencing converts unmodified cytosine as well as 5-fC and 5-caC to thymine, reading out positions of 5-mC and another intermediate, 5-hydroxymethylcytosine, at single-base resolution, but not 5-fC and 5-caC. Hu *et al*. introduce M.SssI methylase–assisted bisulfite sequencing (MAB-seq), which uses the bacterial methyltransferase M.SssI to methylate unmodified cytosine within CpG dinucleotides, protecting it from bisulfite conversion. When traditional bisulfite sequence data and MAB-seq data from the same sample are compared, 5-fC and 5-caC levels can be measured at single-base resolution, thus shedding light on the dynamics of DNA methylation in the genome.

Wu, H. *et al*. *Nat. Biotechnol.* **32**, 1231–1240 (2014).

PLANT SCIENCES

A powerful haploid tool for plant genetics

Several years ago, an easy way to generate haploid *Arabidopsis thaliana* plants was reported. A 'haploid inducer' (HI) line expresses a modified centromeric histone CENH3 in a *cenh3* (*htr12*) null mutant background that causes chromosomes to degrade after fertilization, yielding haploid offspring. Ravi *et al*. have now improved the HI line by adding a seed-specific fluorescent reporter that enables rapid screening for haploid-bearing seeds. They used the line for diverse applications including cytoplasm swap, in which the organellar genotype of a female HI parent differs from the nuclear genotype of the male parent; ploidy reduction of an autotetraploid strain to diploid; haploid generation of related species in interspecific crosses; first-generation genetic screens of recessive mutants in the haploid state; production of gametophytic lethal mutants inherited through the unaffected sex; and mutation 'stacking' to rapidly generate plants with multiple desired genotypes.

Ravi, M. *et al*. *Nat. Commun.* **5**, 5334 (2014).

BIOCHEMISTRY

Proteins masquerading as DNA for efficient delivery

Exogenous proteins introduced into cells can carry out a myriad of desirable functions, but they cannot penetrate mammalian cells without assistance. One way around this is to introduce the protein in DNA or mRNA form, an approach that is widely used but bears some risk. Zuris *et al*. focused on delivering the proteins directly using cationic liposomal reagents routinely used for nucleic acid delivery. They first had to create a uniform negative charge in the protein cargo by complexing the protein with polyanionic macromolecules and then with cationic lipids to facilitate cellular uptake. This strategy allowed them to deliver Cre recombinase and gene-editing nucleases into cells as well as the inner ear of a mouse. The proteins retained high activity and showed more specific targeting than enzymes delivered by DNA transfections.

Zuris, J.A. *et al*. *Nat. Biotechnol.* doi:10.1038/nbt.3081 (30 October 2014).

STRUCTURAL BIOLOGY

Solutions for structural heterogeneity in diffractive imaging

Single-particle diffractive imaging can be used to obtain structural information for large biological entities such as viruses or organelles, without crystallization. High-resolution, three-dimensional structure determination of such objects has remained challenging, however. Hantke *et al*. describe methods that allowed them to overcome two experimental difficulties in single-particle diffractive imaging. First, they developed an efficient aerosol injector to stream single particles into an X-ray free-electron laser beam. Second, they developed a computational purification approach to sort the heterogeneous population of particles. These advances allowed them to reconstruct a three-dimensional structure for the carboxysome, a micro-organelle responsible for carbon fixation in cyanobacteria, at 18.1-nanometer resolution—the highest resolution reported to date for a biological particle imaged with an X-ray laser.

Hantke, M.F. *et al*. *Nat. Photonics* **8**, 943–949 (2014).