METHODS IN BRIEF

CHEMICAL BIOLOGY

Expanding incorporation of nonstandard amino acids

In a common genetic-code-expansion approach, a stop codon is reprogrammed to code for a nonstandard amino acid. But because only two of the three stop codons can be reprogrammed at a time, there is a limit to the number of different nonstandard amino acids that can be incorporated into a protein. Iwane *et al.* reasoned that because many of the 61 sense codons can be considered redundant, some of them could be reprogrammed without sacrificing the ability to code for all 20 canonical amino acids. They came up with an approach to artificially divide codon boxes, creating up to 11 vacant codons and allowing multiple different nonstandard amino acids to be incorporated into a polypeptide using an *in vitro* translation system. As a demonstration, they expressed a 32-residue peptide that incorporated all 20 canonical and 3 nonstandard amino acids.

Iwane, Y. *et al. Nat. Chem.* http://dx.doi.org/10.1038/nchem.2446 (2016).

STEM CELLS

Cellular reprogramming is not very mutagenic

A few years ago, an alarm was raised over the fact that reprogramming differentiated cells into induced pluripotent stem cells (iPSCs) can generate mutations, with potentially serious implications for their use in cell-based therapies. Although a number of subsequent publications downplayed such fears, the question needs systematic and rigorous investigation. To address the problem, Bhutani *et al.* compared nine highly controlled isogenic iPSC lines that they produced with integrating retroviral vectors, non-integrating Sendai virus or synthetic mRNAs, applying a combination of whole-genome sequencing and physical genome mapping. Their analysis of single-nucleotide variants, indels and structural variants from these data showed that only a few, mostly benign, mutations are generated during reprogramming and that mutation levels do not vary greatly between reprogramming methods.

Bhutani, K. *et al. Nat. Commun.* **7**, 10536 (2016).

IMAGING

Clearing the way for RNA visualization

Tissue-clearing approaches have made it possible to image deep inside tissues that are usually inaccessible because of light scattering. However, currently available protocols are optimized for the visualization of proteins. Sylwestrak *et al.* adapted the CLARITY clearing method to make it compatible with RNA *in situ* hybridization. The researchers included an additional fixation step in the procedure, which preserves RNA even for long-term sample storage, and found that DNA probes showed superior penetration compared to RNA probes. Amplification via the hairpin chain reaction resulted in high signal-to-noise ratios, especially for low-abundance transcripts. The modified CLARITY protocol is compatible with antibody labeling and preserves the fluorescence of expressed reporter proteins. The researchers demonstrated their method by detecting more than ten mRNA species and several microRNAs in mouse and human brain samples.

Sylwestrak, E.L. *et al. Cell* **164**, 792–804 (2016).

SYNTHETIC BIOLOGY

Printing human-scale tissues in three dimensions

Full-sized synthetic tissues and organs are an important but elusive goal for tissue transplantation and disease modeling. In addition to complex cellular interactions, macro-scale tissues require structural stability and extensive vascularization. As a solution, Kang *et al.* developed an integrated tissue–organ printer (ITOP) that lays down a mixture of cells, hydrogels and strength-imparting biodegradable polymers. Their strategy includes generating a structural hydrogel frame that can be dissolved after the printed tissue becomes rigid and depositing regular microchannels between cells that are delivered in a phase-changing hydrogel carrier by a specialized nozzle. The researchers used ITOP with detailed 3D medical images for automated bioprinting of functional, potentially transplantable human parts—skeletal muscle, ear-shaped cartilage and a section of jawbone.

Kang, H.-W. *et al. Nat. Biotechnol.* http://dx.doi.org/10.1038/nbt.3413 (2016).