IN BRIEF

CELL BIOLOGY

Targeting Cas9

Rouet, R. et al. *J. Am. Chem. Soc.* **140**, 6596–6603 (2018).

A remaining bottleneck for CRISPR-mediated gene editing is cell- and tissue-specific delivery of components. Rouet et al. took advantage of cells' highly specific cell-surface-receptor-mediated cargo uptake and fused a ligand for the asialoglycoprotein receptor (ASGPr) to Cas9. This facilitated the endocytosis of the Cas9 fusion protein specifically into hepatocytes, which exclusively express this lectin receptor. No transfection reagents were necessary, but an endosomolytic agent was needed to ensure that Cas9 escaped from the endosomes after receptor-mediated uptake. The researchers showed selective activity of Cas9 in HEPG2 cells compared with that in a control cell line, but also noted that the activity in primary hepatocytes and the application of the approach in vivo will need further optimization. *NR*

https://doi.org/10.1038/s41592-018-0061-8

SENSORS AND PROBES

Sensing cellular metabolites

Sallin, O. et al. *eLife* **7**, e32638 (2018).

The nicotinamide adenine dinucleotide (NAD) metabolites $(NAD(P)^+$ and NAD(P)H) are crucial regulators of cellular processes, and the ability to sense their abundance and distribution in living cells has become important for those trying to understand their function. Several fluorescence-based, genetically encoded sensors have been developed that are specific for the various cofactors and their ratios. Sallin et al. expanded this toolkit by developing semisynthetic sensors for the quantification of NAD^+ levels and NADPH/$NADP^+$ ratios. These sensors follow the Snifit sensor design and combine an analyte-binding protein with fluorescently labeled SNAP-tags and Halo-tags. After analyte binding, a change in FRET occurs that can be read out via microscopy. These sensors are ratiometric, display large dynamic ranges, are not sensitive to pH, and are excited at longer wavelengths than most existing sensors, which makes them promising tools for cellular applications. *RS*

https://doi.org/10.1038/s41592-018-0062-7

GENOMICS

Higher-order genetic interactions

Kuzmin, E. et al. *Science* **360**, eaao1729 (2018).

Genetic interactions can go in two directions: in positive interactions, both mutations enhance the fitness of a cell to a degree beyond a simple additive effect, whereas synthetic lethality can result from the combined effects of two mutations that on their own would not have been fatal for the cell. Studies of such interactions, often performed in budding yeast for the ease of linking genotype to growth phenotype, mostly involve mutations in two genes. Kuzmin et al. expanded the view to trigenic interactions by constructing ~200,000 yeast triple mutants and quantitatively measuring negative interactions, for their greater signal-to-noise ratio. The researchers found interactions distributed over many genes, rather than concentrated around a set of highly connected genes. More than 30% of the trigenic interactions were not anticipated from double-mutant strains and provide new functional information. *NR*

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NEUROSCIENCE

Inferring neuronal activity from gene expression

Tyssowski, K. M. et al. *Neuron* **98**, 530–546 (2018).

Genes such as *Arc* have been used as reporters for neural activity; however, hundreds of other genes are induced in response to neuronal activity. Tyssowski et al. analyzed the temporal sequence of these activity-regulated genes (ARGs) in response to sustained or brief activity. The researchers grouped the ARGs into three classes: rapid primary response genes (rPRGs), delayed primary response genes (dPRGs) and secondary response genes (SRGs). rPRGs, dPRGs and SRGs are all expressed after sustained neuronal activity, but only rPRGs are induced by brief activity. On the basis of their different expression profiles, neurons can be classified as either briefly active neurons or neurons with sustained activity via a nearest-neighbor classifier. In turn, analysis of gene expression by single-cell RNA-seq allows one to infer the activity history of neurons, which the researchers demonstrate both in cultured neurons and in mouse visual cortex. *NV*

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