METHODS IN BRIEF

STRUCTURAL BIOLOGY

Invisible excited-state RNA structures

Nuclear magnetic resonance (NMR) spectroscopy is a valuable tool for understanding the structural dynamics of proteins and RNA molecules, providing insights into their functions. To date, it has been possible to detect only relatively long-lived transition-state RNA species by NMR spectroscopy. However, short-lived, sparsely populated excited conformations of molecules are often key states for functions such as catalysis, molecular recognition or signaling. Borrowing and adapting a strategy previously developed to study protein excited states by NMR spectroscopy, Dethoff *et al.* now report a method to characterize RNA excited-state structures. The approach involves a combination of mutagenesis to stabilize candidate excited states, rotating frame carbon relaxation dispersion NMR experiments and secondary-structure prediction tools. Applying the method to three distinct RNAs, Dethoff *et al.* predict that RNA excited-state structures are common throughout the transcriptome.

Dethoff, E.A. *et al. Nature* advance online publication (7 October 2012).

SYSTEMS BIOLOGY

Genome-driven metabolome identification

The genome influences metabolite levels, and for a limited number of known metabolites, genome-wide association studies (GWAS) have shed light on this connection. A large number of metabolites, however, have not been chemically identified even though they are reproducibly detected by mass spectrometry methods. Their interaction with the genome is thus not investigated in classical GWAS. Krumsiek *et al.* seek to use the GWAS approach to identify unknown metabolites. They mapped the associations between SNPs, known metabolites and over 200 unknown metabolites to elucidate the pathways likely involved and to derive a hypothesis as to a candidate metabolite's identity that could then be experimentally tested. This approach allowed them to generate hypotheses for over 100 metabolites and confirm the identity of nine of them.

Krumsiek, J. *et al. PLoS Genet.* **8**, e1003005 (2012).

CELL BIOLOGY

Cell monolayer mechanics

Many body surfaces are lined by one-cell-thick sheets with complex intercellular junctions. Harris *et al.* describe an approach to study the mechanical properties of mature cellular monolayers in the absence of substrate. Cells are first seeded onto collagen suspended between a fixed and a flexible rod. When the monolayer has filled the space between the rods, the collagen is dissolved away. The viscoelastic properties of the monolayer are then probed by pulling the rods apart with micromanipulators and monitoring the displacement of the flexible rod. Experiments with this setup show that MDCK monolayers are almost twice as elastic as individual cells and that monolayer extension is due to cell extension without intercalation. The monolayer can be imaged during the mechanical experiments, permitting the behavior of subcellular components to be studied simultaneously.

Harris, A.R. *et al. Proc. Natl. Acad. Sci. USA* **109**, 16449–16454 (2012).

SYNTHETIC BIOLOGY

Buffered synthetic circuits

In synthetic biology, genetic parts making up circuits should ideally function equally well in any context. Unfortunately, this is not the case, as a part's function is affected by genetic and cellular environment. Therefore, one cannot generalize the performance of any part but instead needs to test its efficacy in each new context. Various insulator elements inserted at the junction between parts have recently been shown to ameliorate context dependency; Lou *et al.* now add another. They showed that insertion of a ribozyme between two elements makes the output of a circuit dependent solely on the input and eliminates any interference by other sequences at the junction of the two parts. The presence of the ribozyme ensures tight control of a NOT gate designed with a variety of inducible promoters. Interference between parts in synthetic genetic circuits is a major challenge that needs to be addressed to make full use of the ever-increasing number of parts.

Lou, C. *et al. Nat. Biotechnol.* **30**, 1137–1142 (2012).