fied several aptamers that bound the fluorophores and resulted in different spectral properties, producing a palette of colors ranging from red-orange to light blue. These aptamers can be readily fused to an RNA sequence.

In the brighter enhanced GFP (EGFP) variant, HBI exists mainly in the phenolate form (versus the phenol form which predominates for GFP). Using this principle, Jaffrey's group designed an HBI derivative exclusively in the phenolate form and selected an aptamer to it to produce Spinach, a green RNA tag with very high brightness and photostability. They tracked intracellular dynamics of 5S RNA, a small noncoding RNA that associates with the large ribosomal subunit, in living mammalian cells. The group is currently working on producing brighter variants of other colors to allow simultaneous imaging of multiple RNAs.

Jaffrey sees a potentially broad application for tags based on their design. "We think that it's possible to tag many different types of RNAs, but as with tagging proteins, you have to confirm that the tag doesn't influence the localization or behavior of the RNA," he notes. Still, he does not see any reason why other noncoding RNAs or mRNAs could not be tagged.

Jaffrey also sees many potential applications beyond trafficking, ranging from studying splicing in axons, to RNA folding by chaperones, to the action of RNA helicases critical for translation, to RNA-RNA and RNA-protein interactions, to RNA degradation. "It opens the door to imaging a lot of different molecular biology processes," he says.

Allison Doerr

RESEARCH PAPERS
Paige, J.S. *et al.* RNA mimics of green fluorescent protein. *Science* **333**, 642–646 (2011).

EDGE has some built-in advantages over RNA-seq: less sequencing per transcript means more samples can be run together, there is no bias against shorter transcripts, and quantification is straightforward. In contrast, EDGE tags exhibit weak cut site bias and are problematic in cases where they can be assigned to multiple genes, though the latter problem is mitigated somewhat by longer tags.

Working with the Cheetah Conservation Fund in Namibia, the team obtained single skin biopsies from black and yellow regions of an anaesthetized cheetah. "That was just a taste, I hope, of things to come," says Barsh, who has obtained more samples since this proof-of-concept work. They could assign tags to genes using related sequences from domestic cat—either a low-coverage genome sequence or low-coverage RNA-seq of ten tissues. This was enough to profile expression of over 14,000 genes, including a first indication that the pathway downstream of *Mc1r* is recruited in directing the cheetah's mottled pattern.

Barsh sees a unique window for tools such as EDGE, before whole-genome sequencing becomes accessible enough for RNA-seq to be applied broadly. For now, they present a great opportunity to explore the genetic basis of variation in the wild and get a peek behind the cheetah's beautiful camouflage.

Tal Nawy

RESEARCH PAPERS
Hong L.Z. *et al.* Digital gene expression for non-model organisms. *Genome Res.* advance online publication (15 August 2011).

NEWS IN BRIEF

IMAGING

A palette of calcium indicators

Single-color genetically encoded calcium (Ca^{2+}) indicators have so far only been available in a green color. Zhao *et al.* now describe improved green, blue and red Ca^{2+} indicators. To generate these tools, they screened large libraries of GFP-based Ca^{2+} indicator genetic variants for large Ca^{2+}-dependent changes in fluorescence. These tools open the door to imaging calcium signaling in multiple colors in single cells and in *Caenorhabditis elegans*.

Zhao, Y. *et al. Science* advance online publication (8 September 2011).

MOLECULAR ENGINEERING

Manipulating genomes with new recombinases

Expanding the toolset for site-specific recombination would accommodate more complex genome manipulations. Starting with predictions of yeast recombinases similar to Flp recombinase, Nern *et al.* engineered three new genes for fruit fly codon usage and short half-life. They showed that modified recombinases KD, B2 and B3, along with the previously identified R recombinase exhibited high efficiency, low toxicity and no cross-talk in the fly, and that KD and B3 recombinases functioned in mammalian cells.

Nern, A. *et al. Proc. Nat. Acad. Sci.* **108**, 14198–14203 (2011).

SINGLE MOLECULE

Freely orbiting magnetic tweezers

Magnetic tweezers are useful tools to study cellular processes such as replication, transcription, repair and protein binding to DNA, at the single-molecule level. Conventional setups, however, do not allow rotational motion to be tracked directly. Lipfert *et al.* now describe freely orbiting magnetic tweezers, which allow changes in the twist of nucleic acids to be measured, and use them to monitor assembly of recombination protein A filaments on DNA.

Lipfert, J. *et al. Nat. Commun.* **2**:439 (2011).

CELL BIOLOGY

Growth-factor immobilization in hydrogels

Wylie *et al.* describe a method to pattern growth factors in a three-dimensional hydrogel in a controllable manner. They introduced reactive thiol sites in the hydrogel with selective two-photon uncaging of coumarin-caged thiols; these sites serve as anchors for proteins such as streptavidin or barnase. Growth factors can then be immobilized at these sites by fusing them to the cognate binding partners of streptavidin, biotin, or barnase, barstar. Growth factors introduced in this manner guided cell migration in the hydrogel.

Wylie, R.G. *et al. Nat. Mater.* advance online publication (28 August 2011).

MOLECULAR ENGINEERING

Protein origami

Although nanostructures of ever-increasing complexity are being built using principles of DNA 'origami', protein molecules have been underexploited. Sinclair *et al.* now describe a general strategy to design ordered protein materials. One- and two-dimensional protein lattices can be constructed by the fusion of peptide chains derived from multisubunit protein complexes with matching rotational symmetry.

Sinclair, J.C. *et al. Nat. Nanotechnol.* **6**, 558–562 (2011).