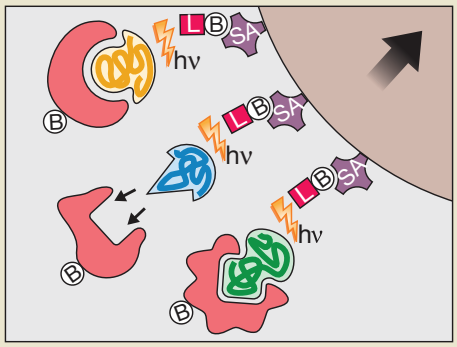
Aptamer-based proteomics arrays



Progress in the use of high-throughput proteomic analysis for biomarker discovery and diagnostics has been stymied by the challenge of quantifying tens of thousands of proteins whose abundance spans approximately twelve orders of magnitude. The use of mass spectrometry still poses technical difficulties and the inherent cross-reactivity of antibodies has limited the utility of antibody arrays. Gold *et al.* couple the use of slow off-rate modified aptamers (SOMAmers)—oligonucleotides containing functionalities that mimic amino acid side chains to enhance their specificity for targets—with the robustness of nucleotide arrays to provide an assay that can measure >800 human proteins in ~15 µl of human blood with low limits of detection (1 pM median) over a dynamic range from ~100 fM to 1 µM. First, proteins to be assayed (pink) bind tightly to their cognate SOMAmer, which is modified with biotin (B) and a fluorescent label (L), and bound protein-SOMAmer complexes are trapped on beads coated with streptavidin (SA). Then, as depicted, unbound proteins are washed away, and biotin-tagged bound proteins are released by exposure to UV light (hν). After a subsequent recovery step on SA-coated beads, the SOMAmers are eluted from their targets and quantified by hybridization to a customized DNA microarray. The fluorescent intensity of each probe spot is proportional to the amount of its target protein in the original sample, with the SOMAmers acting as both the binding agent and the quantifiable species. Gold *et al.* use this approach to identify 58 potential markers for chronic kidney disease. Ostroff *et al.* use the assay to analyze archived samples from >1,300 human subjects. From 44 candidate biomarkers, they identify a 12-protein panel with strong potential to diagnose non-small cell lung cancer. (*PLoS One* **5**, e15003, e15004, 2010) *PH*

Sequence-specific DNA-binding TALEs

Recent studies have mapped the relationships between the amino-acid sequences and DNA-binding specificities of transcription activator–like effector (TALE)-type transcription factors from the pathogenic plant genus *Xanthomonas*. Morbitzer *et al.* demonstrate that knowledge of this code allows the design of sequence-specific transcription factors that activate user-defined endogenous genes *in vivo* in plants. The researchers create custom TALEs that target a 19-bp sequence in the tomato promoter *Bs4S* or 19-bp sequences in the promoters of the *Arabidopsis thaliana* genes *EGL3* and *KNAT1*. Moreover, they show that TALEs targeting a 23-bp sequence have enhanced target specificity as compared to those targeting 19-bp sequences. Additional experiments enabled Morbitzer *et al.* to identify particular repeat units in TALE proteins that target G nucleotides specifically, an aspect of the binding code that had not been described previously. These results suggest that designer TALEs may represent an alternative to sequence-specific DNA targeting using zinc-finger domains. (*Proc. Natl. Acad. Sci. USA* **107**, 21617–21622, 2010) *CM*

Written by Laura DeFrancesco, Markus Elsner, Peter Hare & Craig Mak

Protein-sensing RNA control device

RNA-based molecules have been engineered to reprogram cells in response to externally applied small molecules or nucleic acids. To apply the same principles for modulating the effects of signaling events, RNA sensor-actuator devices need to be able to alter gene expression in response to changes in protein factors. Culler *et al.* have now constructed alternative splicing systems that either include or exclude exons based on the binding of a specific protein. The alternatively spliced exon contains a stop codon that prevents the translation of a downstream effector gene if included. The authors apply this strategy to the detection of the activity of the important cellular signaling molecules NF-κB and β-catenin using the expression of a fluorescent reporter as a readout. A potential application of this strategy is to selectively kill cells with overactive signaling pathways, for example, in cancer. To demonstrate the feasibility of such an approach, Culler *et al.* couple the NF-κB and β-catenin sensors to a gene encoding an enzyme that converts pro-drugs, causing 80% of the cells to undergo apoptosis in the presence of both pro-drug and pathway activation. (*Science* **330**, 1251–1255, 2010) *ME*

A peptide to get your GOAT

The appetite-suppressing gastric peptide hormone ghrelin is a promising therapeutic target for modulating weight gain and glucose control. As ghrelin needs to be acetylated with octanoate to be active, Barnett *et al.* set out to inhibit ghrelin *O*-acyltransferase (GOAT), the enzyme responsible for its activation. A fusion comprising ten ghrelin-derived amino acids, a stabilized octanoyl-CoA and the Tat motif (11 amino acids) inhibited GOAT *in vitro* and in cultured cells. Daily intraperitoneal doses of the peptide reduced weight gain, blood glucose and insulin-like growth factor 1 in normal, but not ghrelin-deficient, mice given a high-fat diet. These findings may open the way for new strategies to manage the growing incidence of obesity and type 2 diabetes in Western society. (*Science* **330**, 1689–1692, 2010) *PH*

Anti-inflammatory histone mimics

Exposure to pathogens results in complex responses that can both protect (immune response) and harm (inflammation cascade) the host. Nicodeme *et al.* present a new approach that could potentially prevent the deleterious effects of inflammation by inhibiting the formation of transcription complexes that upregulate inflammation-inducing genes. Targeting bromodomain and extra terminal domain (BET) proteins, which recruit proteins into transcription complexes, the researchers synthesized a histone mimic that binds BET proteins, keeping them from interacting with chromatin, which in turn prevents transcription complexes from forming. One synthetic inhibitor (I-BET), designed to bind peptides derived from acetylated histones, downregulated key inflammatory cytokines and chemokines when mouse macrophages were incubated with I-BET before treatment with lipopolysaccharide (LPS). This effect was specific for inflammation, as cytokines not induced by LPS were unaffected. I-BET treatment not only prevented complex formation but also prevented acetylation of histones on BET-sensitive promoters, though it is unclear whether I-BET inhibits acetylases directly or inhibits recruitment of acetylases to the transcription complex. Finally, the researchers showed that I-BET works *in vivo*. Injecting I-BET into mice prevented sepsis and death when given before LPS-induced shock, as well as when it was given after the signs of inflammation began to appear. In addition, I-BET prevented death in mice suffering from peritonitis and sepsis caused by cecal ligation. (*Nature*, published online, doi:10.1038/nature09589, 10 November 2010) *LD*