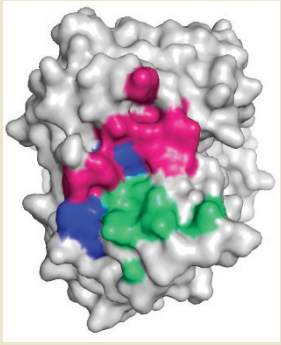
Two-in-one antibodies



The idea of simultaneously targeting more than one antigen has given rise to combination approaches involving two different antibodies or bivalent molecules. But now researchers from Genentech (S. San Francisco, CA, USA) have shown that a single antibody molecule can target two antigens. Starting with Herceptin (trastuzumab), a monoclonal antibody directed against the human epidermal growth factor HER2, the researchers introduce mutations in the light chain complementarity-determining region (CDR)—so chosen because HER2 binding involves only heavy-chain CDRs—and screen for variants that bind both HER2 and vascular endothelial growth factor (VEGF), the target of the cancer drug Avastin (bevacizumab). They isolate >100 variants that bind VEGF, many retaining the ability to bind HER2. Shotgun mutagenesis studies define the residues important for the two interactions and reveal that different CDR residues are responsible for the interaction with either HER2 or VEGF, even though the two binding region surfaces overlap. Finally, using an affinity-improved version of one dual-specific antibody, the group inhibits cancer cell proliferation both *in vitro* and *in vivo*. This effort is the first to create an antibody with a normal structure that binds two targets. (*Science* **323**, 1610–1614, 2009) *LD*

Ubiquitinylation inhibitor

Protein turnover in cells is regulated by the ubiquitin-proteasome system, dysregulation of which can lead to cancer as well as neurological and autoimmune disorders. Although the 26S proteasome has been successfully targeted by the drug Velcade (bortezomib), the search continues for small molecules that inhibit ubiquitinylation. Soucy *et al.* intervene in the ubiquitinylation pathway through a common regulator of a large subgroup of the E3 family, called cullin-RING ligases (CRLs). CRL activation is controlled by the attachment of the ubiquitin-like-protein NEDD8 by the NEDD8-activating enzyme (NAE). The authors describe a potent inhibitor of NAE, MLN4924, that abolishes CRL-mediated protein degradation. *In vitro,* cells treated with MLN4924 show defects in the regulation of DNA replication, leading to DNA damage and apoptosis. *In vivo*, the inhibitor reverses or stops tumor growth in three different mouse cancer models. This suggests that NAE is a promising target for anticancer drugs. (*Nature* **458**, 732–736, 2009) *ME*

Digital footprinting

Two studies involving *Saccharomyces cerevisiae* couple the well-documented ability of regulatory proteins to protect specific DNA and RNA sequences from nuclease digestion with the high-throughput potential of massively parallel sequencing. Whereas one enables genome-wide analysis of local chromatin structure and binding by regulatory proteins, the other provides a handle on quantifying rates of translation *in vivo*. A limitation of current genomic strategies to identify where regulatory proteins bind DNA, such as those based on chromatin immunoprecipitation, is the requirement for prior knowledge of the protein(s) of interest and reagents that specifically recognize them. Hesselberth *et al.* combine limited DNase I digestion with deep sequencing to provide an unbiased snapshot of genome-wide protein occupancy of *S. cerevisiae* genomic DNA at nucleotide-level resolution. Ingolia *et al.* use a broadly comparable approach to address the well-known inadequacy of mRNA measurements as reliable proxies for protein synthesis. In an improvement on a microarray-based approach called polysomal profiling, they identify regions of mRNAs protected by bound ribosomes and use the number of reads for a particular sequence within each mRNA type to quantify the efficiency with which it is being translated. The ability to monitor rates of protein synthesis promises to be invaluable to discerning the subtleties of translational regulation. (*Nat. Meth.* **6**, 283–289, 2009; *Science*, published online, doi:10.1126/science.1168978, 12 February 2009) *PH*

Waking dormant HIV

The ability of the HIV virus to integrate into the genome of a subset of $CD4^+$ T cells and to lay dormant for years, termed HIV latency, is a major obstacle to clearing the virus from those infected. Because of HIV latency, the discontinuation of highly active antiretroviral therapy (HAART) when plasma viral load becomes undetectable is followed by the re-establishment of high viral titers. Yang *et al.* identify a splicing variant of the transcription factor Ets-1 as a potent activator of latent HIV. Previous studies had shown that global T-cell activation wakes dormant HIV proviruses but has several toxic side effects. To find genes that can activate HIV independent of the T cell–activating nuclear factor (NF)-κB pathway, they design a cDNA expression cloning screen based on a luciferase reporter gene under the control of a mutated HIV-LTR promoter region that is unable to bind NF-κB. Screening 47,700 cDNAs identifies the ΔVII-Ets-1 splice variant, the HIV provirus–stimulating function of which is demonstrated in primary T cells derived from HAART patients with viral loads below the detection limit. In combination with HAART, the selective activation of this protein could increase clearance of infected cells either by direct cytotoxic effects of HIV replication or by selective killing of such cells by the immune system. (*Proc. Natl. Acad. Sci. USA* **106**, 6321–6326, 2009) *ME*

Profiling prion disease networks

Despite the discovery that misfolded prion proteins cause neurological disease, the broad effects of these proteins on the brain have not been systematically investigated. Hwang *et al.* take a systems biology approach to study disease progression in the brains of prion-infected mice. They first measure gene expression profiles of whole-brain tissue at about 10 time points after infection. To deal with the enormous noise present in transcriptome data, they infect eight different genotypes of mice with two different prion strains and develop new statistical approaches to integrate these data. This approach identifies a core set of 333 prion-relevant genes that are then mapped onto protein-protein interaction networks. Taken together, these interactions explain most of the pathological features of prion disease, identify connected networks that are perturbed at different times during disease progression and define six new modules of genes that were not known to play a role in prion disease. This work provides a paradigm for future studies employing a systems approach to disease in model organisms. (*Mol. Syst. Biol.* **5**, 252, 2009) *CM*

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