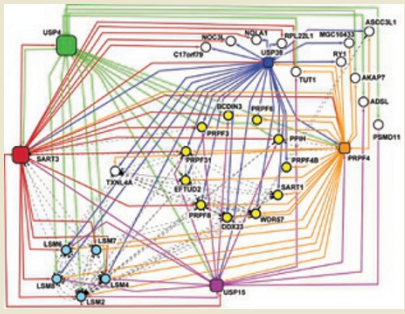
Deubiquitinase interactome



The ubiquitin-proteasome system is central to the maintenance of cellular homeostasis and has been implicated in the development of cancer and neuronal diseases. Although the molecular machinery that attaches ubiquitin residues to proteins has been well characterized, much less is known about the role of the ~95 deubiquitinases encoded by the human genome. Sowa *et al.* present a comprehensive proteomics screen for stable interaction partners of 75 deubiquitinases, which will provide leads for elucidating the cellular functions of these enzymes. They identify >600 new interacting proteins by mass spectral analysis of the complexes purified by immunoprecipitation of the deubiquitinating enzymes. Using the gene ontological terms ascribed to the interacting proteins, the deubiquitinases are assigned to cellular processes in which they are most likely to be involved. This strategy is validated by confirming the role of USP13 in endoplasmic reticulum–associated degradation that was suspected from its interaction profile. (*Cell* **138**, 389–403, 2009) *ME*

Neural disease modeling

The ink was not dry on the first report of induced pluripotent stem cells (iPSCs) before scientists began to appreciate the enormous potential of this technology for creating cellular models of disease. Several groups have already described iPSCs derived from individuals with various single-gene and genetically complex diseases; the next step is to use such cells to gain insight into pathogenic mechanisms and possible therapies. Studer and colleagues have pursued this strategy for a rare monogenic disease called familial dysautonomia. A fatal neuropathy, familial dysautonomia is associated with point mutations in the I-κ-B kinase complex–associated protein (*IKBKAP*) gene, but how these mutations lead to loss of sensory and autonomic neurons is not well understood. As primary affected neurons are not available for study, the authors reprogrammed fibroblasts from three children with familial dysautonomia and differentiated the resulting iPSCs into various neural cell types. Molecular and functional characterization of neural crest precursors revealed reduced levels of IKBKAP, downregulation of genes involved in neural development and an impaired migration capacity. The authors also used iPSC-derived cells to evaluate the ability of several drugs to correct these defects. (*Nature*, published online August 20, 2009; doi:10.1038/nature08320) *KA*

Multiplexed genetic engineering

Despite advances in genetic manipulation, engineering an organism's genome is typically still done one step at a time—by sequential gene replacement or mutation. A problem with this serial approach, however, is that it is only possible to explore a limited number of potential genetic manipulations when optimizing cellular phenotypes. Wang *et al.* describe a method for rapidly introducing millions of targeted genetic changes in a combinatorial, multiplexed fashion in a matter of hours or days. Their method takes advantage of *Escherichia coli* recombination-based genetic engineering (recombineering)—the method by which single-stranded DNA oligos that contain regions of homology to the *E. coli* genome will recombine with host cell DNA to introduce genetic changes. The authors synthesize a pool of 470,000 oligos designed to introduce all possible 11 nucleotide variants of an optimized ribosome binding site upstream of 20 endogenous *E. coli* genes previously identified to increase the yield of lycopene, an industrially important metabolite. By automating the process of growing cells and delivering the oligo pool into the cells with a brief pulse of electricity, Wang *et al.* rapidly drive the cells through many rounds of recombineering. Over three days, the cell population explores billions of genetic combinations, resulting in strains that produced fivefold more lycopene than the starting population. (*Nature* **460**, 894–898, 2009) *CM*

Written by Kathy Aschheim, Laura De Francesco, Markus Elsner, Peter Hare & Craig Mak

Antisense subverts toxic triplet repeats

Myotonic dystrophy type 1, the most common form of adult muscular dystrophy, affects individuals containing >50 CUG repeats in the non-coding regions of RNAs encoding a particular protein kinase. The ability of these repeats to sequester a splicing regulator, muscle-blind-like 1 (MBNL1), provokes muscular dysfunction by inducing the inappropriate expression of fetal splice isoforms in adult tissue and destroying the function of proteins such as the muscle-specific chloride channel 1 (ClC-1). Instead of overexpressing MBNL1 to correct this defect, Wheeler *et al.* use a 25-nucleotide antisense morpholino oligonucleotide to displace the inappropriately bound splicing regulator. As antisense morpholinos do not cleave their target RNAs, this strategy averts the risk of inadvertently degrading mRNAs containing innocuous CUG repeats. Injection of the morpholino into muscles of mice expressing a transgene bearing 250 repeats in the 3′ untranslated region of an actin mRNA restores ClC-1 activity, corrects missplicing of three other muscle transcripts and markedly reduces the muscular hyperexcitability symptomatic of myotonia. Although triplet-repeat disorders, such as Huntington's disease and spinocerebellar ataxia type 3, are caused by expanded repeats in coding regions, the approach might find application beyond diseases caused by toxic repeats in untranslated regions. (*Science* **325**, 336–339, 2009) *PH*

Promoting metastasis

The ability of immune cells to either stimulate or inhibit tumor progression confounds the understanding and treatment of certain cancers. In some breast cancers, for example, high ratios of $CD4^+$ to $CD8^+$ cells in tumors are associated with poor survival. Using a mouse mammary tumor model of breast cancer, DeNardo *et al.* now identify T-helper 2 (Th2) $CD4^+$ lymphocytes as the culprits and show that these cells direct differentiation of tumor-associated macrophages, which in turn stimulate epidermal growth factor receptor signaling. Interestingly, Th2 cells affect metastases, not primary tumor progression; $CD4^+$ T-cell double knockout mice have fewer lung metastases, an effect that is reversed when $CD4^+$ cells are replenished in the animal. Profiling the effector molecules present in the mouse tumor reveals that Th2-associated cytokines interleukin (IL)-4 and IL-13 are prevalent, and that IL-4 appears to be the primary mediator; reducing IL-4 levels through knockouts or neutralizing antibody reduces metastases both *in vivo* and in an *in vitro* model of invasiveness, a prerequisite for metastasis. The different effects of $CD4^+$ cells on primary versus metastatic tumorigenesis here and in other studies show the importance of the context of the complex tumor microenvironment on immune cell function. The identification of the bioactive molecules in mammary tumors may provide targets for therapeutic intervention. (*Cancer Cell* **16**, 91–102, 2009) *LD*