Mapping protein-DNA interactions

The physical binding of proteins to specific DNA sequences is a fundamental step in the regulation of gene expression. In this issue, authors describe improved tools for the large-scale mapping of protein-DNA interactions in several organisms. Walhout, Reece-Hoyes, Deplancke, Brady and colleagues report efficient yeast one-hybrid pipelines and near-complete, sequence-verified collections of full-length transcription factors for rapid gene-centered mapping of protein-DNA interactions of worm, fruit fly, plant roots and human. Wichterle and colleagues use chromatin immunoprecipitation followed by high-throughput sequencing on mouse embryonic stem cell lines harboring inducible, epitope-tagged transcription factors to map genomic binding of these factors during embryonic stem cell differentiation to motor neurons. These complementary approaches should provide rich datasets for more detailed analysis of gene regulation.

Articles p1059, 1065, Brief Communications p1050, 1053 and 1056, News and Views p1016

Targeting malaria genes

In many organisms, genes are relatively easy to manipulate with tools such as small interfering RNAs or bacterial artificial chromosomes. Not so in malaria parasites. *Plasmodium* species have no RNA interference machinery and their (A+T)-rich genome makes bacterial artificial chromosomes unstable. Bilker, Rayner and colleagues discovered a way around these limitations by creating a *P. berghei* library in a bacteriophage vector that stably integrated the DNA of the mouse malaria parasite and allowed genetic modification via recombineering in *Escherichia coli*. The altered genes, once released from the bacteriophage vector and transfected back into *P. berghei*, yielded the expected phenotype in the parasites. This library will help explore the function of the *P. berghei* genome.

Article p1078

Fluorescent protein design

The palette of fluorescent proteins has been expanded far beyond the original green hue of GFP. In a Perspective, Verkhusha and colleagues outline approaches for the design of improved fluorescent proteins, using red fluorescent proteins as the basis for their discussion. They describe strategies for the rational design of probes with specific properties, based on knowledge of protein structure, photochemistry and chromophore placement in existing fluorescent proteins. In addition, they discuss improvements in both the biological and instrumental aspects of screening techniques that could enable more efficient molecular evolution of fluorescent proteins with desired attributes.

Perspective p1019

Optimal proteotypic peptides

Selected reaction monitoring (SRM) mass spectrometry is a powerful approach to detect and quantify selected proteins from complex samples. 'Proteotypic' peptides that uniquely represent a protein and have good mass spectrometry response characteristics are chosen to generate SRM assays. However, relying on experimental data or prediction tools does not always yield ideal proteotypic peptides. MacCoss, Stamatoyannopoulos and colleagues describe an empirical approach for identifying optimal proteotypic peptides, using the cDNA clone collections available for human and model organisms to generate full-length proteins. By comparing the spectral intensities of different peptides from these in vitro–synthesized proteins, they identify those that offer the most sensitive SRM detection.

Brief Communication p1041

A mix of super-resolution

Interest in super-resolution microscopy is still expanding; in three papers in this issue authors describe very different methodological developments. Diaspro and colleagues overcome the prior limit of the technology to single-cell layers by combining photoactivated localization microscopy with light-sheet microscopy, allowing super-resolution imaging deep in multicellular samples. Meanwhile, Zhuang and colleagues provide valuable information to the many researchers who use stochastic optical reconstruction microscopy, by providing a detailed characterization of the performance properties of 20 different organic dyes covering a wide range of emission wavelengths. Finally, although structured illumination microscopy cannot provide resolution as high as the methods above, it continues to have value for fast high-resolution imaging of living samples. Work from Shao and colleagues in the late Mats Gustafsson's laboratory allows imaging of the entire three-dimensional volume of a mammalian cell every 5 seconds at 120-nanometer lateral and 360-nanometer axial resolution.

Brief Communications p1044, 1047, Analysis p1027