Targeted validation of microRNA targets

MicroRNAs crucially regulate gene expression by blocking translation or marking target mRNA for degradation. Computational algorithms designed to predict microRNA target genes have generated large candidate lists, but high-throughput experimental methods for stringent validation of the candidate targets have been lacking. Hengartner, Aebersold and colleagues now describe a targeted proteomics approach to follow up on predicted microRNA targets in Caenorhabditis elegans. They use a highly sensitive detection technique, selected reaction monitoring (SRM) mass spectrometry, in combination with a quantitative method, isotope-coded affinity tagging (ICAT), to compare the changes in protein levels of genes predicted to be targets of the microRNAs let-7 and miR-58 in normal versus mutant worms with reduced microRNA expression. The approach should be adaptable to predict the targets of any microRNA in any organism.

Article p837, News and Views p795

Mapping the MAP kinase interactome

The MAP kinase signaling pathway is essential for many fundamental cellular processes. Ideker, Sahasrabudhe and colleagues use yeast two-hybrid screening to map physical interactions between components of this pathway and other proteins of the cell, yielding a network of 2,269 interactions among 1,468 proteins. Refinement of the network based on evolutionary conservation with yeast and on selection of multiply identified interactions improved network quality and yielded a core MAP kinase network with 641 high-confidence interactions. Functional testing of the network with small interfering RNA knockdown indicated that several candidate interactors affect MAP kinase signaling. The researchers also used the network to identify putative MAP kinase scaffold proteins.

Resource p801, The Author File p771

Two-photon excitation of ChR2

Optogenetics can be used to modulate the activity of specific cells by selectively expressing light-activated proteins using genetic approaches followed by wide illumination, or alternatively, by directing focused light onto selected cells of a larger population expressing the light-activated proteins. Two-photon microscopy offers unique advantages for excitation of channelrhodopsin-2 (ChR2)-expressing neurons, but previous attempts had limitations in terms of the axial and temporal resolution. Emiliani and colleagues now use a scanless approach that combines generalized phase contrast and temporal focusing to shape two-photon excitation patterns and trigger single action potentials or trains of action potentials in cultured neurons and in mouse cortical slices. These patterns of light can be used to selectively and flexibly activate single neuronal processes as well as simultaneously excite multiple neurons or multiple dendrites of one cell.

Article p848, News and Views p798

Analyzing bugs

The true diversity of microbial communities is difficult to assess but offers important insights into many fields of research, from ecology to human disease. High-throughput sequencing has allowed an unprecedented foray into the depth of microbial diversity, at both the genome and the transcriptome level, but the generated data need careful analysis. Knight and colleagues compare several ordination methods for their ability to reveal gradients and clusters in 16S rRNA reads generated by pyrosequencing. Hugenholtz and co-workers explore the effectiveness of rRNA removal methods in the study of metatranscriptomes. Both analyses give recommendations as to which approach is most effective for analyzing the respective data.

Analyses p807, p813

Single-molecule switchable FRET

In its typical implementation, single-molecule fluorescence resonance energy transfer (FRET) is used to measure a single distance between a single fluorescent donor-acceptor pair. Methods to monitor multiple distances using multiple spectrally distinct fluorophores have been developed, but these approaches remain technically challenging to use. Kapanidis and colleagues now report the development of switchable FRET, which allows multiple distances to be measured in a single molecule, using a single donor and multiple spectrally identical acceptors that are switched on and off between a fluorescent state and a dark state. This method allowed Kapanidis and colleagues to probe the dynamic motions of the DNA Holliday junction.

Article p831