mRNA expression fundamentals

Most research into the expression and regulation of genes has recently focused on examining the expression of the entire complement of genes in a population of cells. A parallel line of research, however, is attempting to visualize individual mRNA molecules in single cells to gain insight into the fundamental dynamics of gene expression. Three papers in this issue from the labs of Umezawa, Broude and Gerst describe different GFP-based approaches to image mRNA molecules in real time in living human, yeast and bacterial cells, respectively. These methodological advances provide useful tools for researchers attempting to understand the fundamentals of gene expression.

Brief Communication p409, Articles p413 and p421, News & Views p391

Glycan analysis in multiplex

Carbohydrate post-translational modifications, known as glycans, have important biological roles. Because they are often found in altered forms in disease states, they have great promise as clinical biomarkers. Haab and colleagues describe a multiplexed method for profiling glycans on individual proteins from complex biological mixtures. They use an antibody microarray to capture proteins of interest, followed by the use of multiple lectins as glycan detection probes. Importantly, they describe a chemical method to block lectin binding sites on the spotted antibodies, thus reducing the risk of false positive detection. By running parallel sandwich and glycan detection arrays, they simultaneously quantified protein concentration and glycan variation for two serum proteins from pancreatic cancer samples.

Article p437

Targeted chemical genetics

Chemical genetics is a powerful tool to probe cellular function. Finding small molecules that regulate the function of a target protein in a desired way with no nonspecific effects, however, is challenging. Liscovitch and colleagues describe a method they call ‘ligand interaction scan’ that overcomes some of these limitations. The method involves inserting a small ligand binding site into a protein at individual locations and screening for mutants that exhibit the desired ligand-dependent changes in activity. They demonstrate the method using a tetracysteine binding site and the biarsenical fluorescein ligand (FIAsH).

Brief Communication p393

Automating phenotype profiling

High-throughput imaging is a potentially powerful technology for assaying complex phenotypic changes in cells. It allows researchers to quantify changes in expression, localization and modification of many individual proteins as well as overall cell morphology at a single-cell level. This large amount of data, however, requires automated analysis methods. Although there has been considerable improvement in this area, there is still no truly objective method for finding and classifying phenotypes. Altschuler and colleagues describe an automated data analysis approach that greatly reduces the complexity of the data and improves human interpretability with little loss of classification accuracy.

Article p445

Targeted enrichment: now for metabolomics

Targeted chemical methods for isolating selected portions of the proteome for subsequent analysis by mass spectrometry have been extremely valuable. The metabolome, or total complement of small-molecule metabolites in a cell or organism, is even more complex than the proteome in terms of chemical diversity, not to mention sheer number. Reasoning that the field of metabolomics might also benefit from targeted enrichment strategies, Cravatt and Carlson developed a general method to enrich portions of the metabolome based on shared chemistries. By using specific chemical probes attached to a solid support, they were able to enrich various diverse classes of metabolites for liquid chromatography–mass spectrometry analysis, including polar and low-mass small molecules, which are difficult to detect with conventional metabolomics strategies. They demonstrated the utility of the method by profiling the thiol metabolome of human cancer cells.

Article p429