Focus on mass spectrometry in proteomics applications

Mass spectrometry is one of the most powerful technologies at hand to aid biologists in answering questions about the protein players in their system of interest. Though no other proteomic technology can now match the throughput or molecular information yield of mass spectrometry, it is still perhaps underused in cell biology. Turn to page 781 for a special series of papers that includes two Commentaries discussing the current state and outlook for the future of mass spectrometry–based proteomics in cell biology and medicine; three Reviews describing the state-of-the-art application of mass spectrometry data analysis tools, the analysis of post-translational modifications and protein interactions; and three Perspectives describing the emerging applications of top-down mass spectrometry, activity-based protein profiling and mass spectrometry imaging.

FOCUS p781–833

Genetic interaction screens in fission yeast

So far the spotlight has been on budding yeast when it comes to high-resolution genetic interaction maps. Krogan and colleagues now also bring Schizosaccharomyces pombe into the limelight—their Pombe epistatic mapper allows for fast and large-scale generation of yeast double mutants, an approach that will help determine the interdependence of genes and map their functional organization in the cell. Though similar in name, fission and budding yeast are only very distant evolutionary cousins, with fission yeast bearing more resemblance to higher eukaryotes. A comprehensive gene interaction network will therefore be of interest far beyond the yeast community.

Article p861, News & Views p777

Growing 3D tumors in vitro

Tumors are not islands of transformed cells that function autonomously, but they respond to their environment. To create tools to better understand the interplay between tumor cells and their surroundings, Mooney and colleagues developed a three-dimensional polymeric scaffold that supports the growth of a tumor in vitro that looks remarkably like its in vivo counterpart. These in vitro tumors develop a hypoxic center, their cytokine secretion resembles an in vivo profile and they respond to chemotherapeutics similarly. They should be valuable tools to study the effect of microenvironmental conditions on tumor development and malignancy.

Article p855

Many targets—one PCR

In theory, PCR lends itself to highly multiplexed reactions: each primer pair amplifies its respective targets, regardless of the total number of pairs. In practice, primers in crowded reactions start to dimerize or prime at off-target sites leading to a lot of background. Brookes and colleagues put a stop to that by designing a multiplex PCR reaction that starts with a surface-based PCR; primers are immobilized and functionally enhanced by pairing with a barrier oligonucleotide. The amplification products have common primer sites at their end, which are further amplified in a second solution-phase PCR. The researchers prove the strength of their method by amplifying 75 targets in one PCR without any preselection of primer sequences.

Brief Communication p835

Microtubules in 3D

The dynamic instability of cellular microtubules is recapitulated in cytosolic extracts and can thus be studied in vitro. This has typically been done in two dimensions, with the microtubules trapped between hard glass surfaces. But artifacts resulting from the small volume and glass-surface interactions cannot be ruled out when using this approach. Keller and colleagues now describe a method to study microtubule dynamics in three dimensions. Microtubule asters are formed in 2-mm capsules and light sheet–based microscopy is used to image only those 50 µm or more away from the capsule wall. Comparisons between two- and three-dimensional imaging show that, indeed, there are significant differences in microtubule dynamics in these two settings. Behavior in the unconstrained three-dimensional setup is more likely to mimic the situation in the cell.

Brief Communication p843