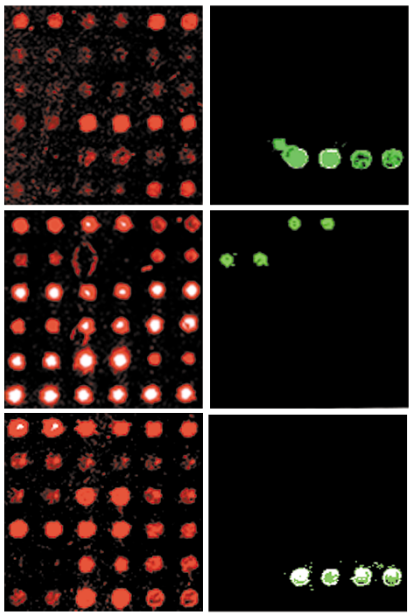
Chipping away at the proteome's mysteries

As the number of sequenced genomes increases, deciphering the function of the newly identified open reading frames becomes a more overwhelming prospect. Even for model organisms like yeast, whose genome has been sequenced, a significant portion of open reading frames encode proteins whose functions are not known. While analysis of gene function has been facilitated by DNA chip technology, similar high-throughput methods for looking directly at proteins have been much more difficult to develop. Current methods include random expression library screening, pooling strategies, and yeast two-hybrid screens. However, within the goal of rapid and comprehensive screening for function, these techniques have limitations, such as incomplete sampling, difficulties in deconvoluting data for common functions carried out by many different proteins, and limitations in the conditions and types of interactions that can be assayed.

An approach that may overcome these difficulties involves the construction of a protein chip, much like the oligonucleotide chips used in analyzing RNA expression profiles and protein–DNA interactions. But do such microarrays work for proteins? Is it feasible to produce and purify the large number of proteins representing an entire proteome? Can proteins with diverse chemistry be immobilized onto a single surface while retaining their native conformations and exposing their active sites?

The answer to these questions, according to Snyder and colleagues, appears to be 'yes'. As recently published online in *Science* (Zhu *et al.*, 26 July 2001; 10.1126/science.1062191), these authors constructed a microarray representing 80% of the yeast proteome. They cloned 5,800 yeast open reading frames, fused to GST-His_6 tags to facilitate purification, microarray binding, and detection. Proteins were overexpressed in yeast, purified in 96-well plates using glutathione-agarose beads, and spotted in duplicate onto a nickel-coated microscope slide. The resulting proteome chip was probed with anti-GST antibodies to reveal a highly successful and reproducible protein spotting efficiency.

The real test of the proteome chip, of course, is whether it can be used to assay protein function. In one of the first experiments with the chip, the authors studied protein–lipid interactions. Shown here are three different sections of the chip probed either with anti-GST antibodies (left) or with biotinylated liposomes containing specific phosphotidylinositides (PI(4)P for the top, $PI(4,5)P_2$ for the middle, and PC for the bottom) and detected with fluorescently labeled streptavidin (right). A total of 150 positive signals such as those shown here were seen for proteins binding to different types of phospholipids; many of these were for proteins whose functions were completely unknown. In addition, several proteins involved in glucose metabolism tested positive for phospholipid binding; this unexpected result was confirmed using more conventional binding assays on nitrocellulose membranes, and has led to the speculation that phospholipids may regulate some aspects of glucose metabolism or that steps in metabolism may be carried out on membrane surfaces.



Overall, the work by Snyder and colleagues demonstrates that proteome chips are indeed feasible and can provide a glimpse into protein function. The chips offer an exciting new high-throughput technology that can be used not only for identifying binding partners of uncharacterized proteins, but also for screening drug interactions, deducing networks of interactions in biochemical pathways, identifying binding motifs, and a wide variety of other analyses.

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