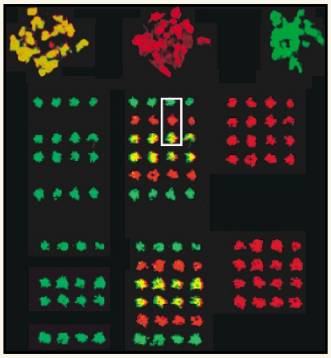
Microarrays come alive!



There is a new tool for the proteomics era—a cell-based microarray that can be used as a living screen of gene function. Researchers at the Whitehead Institute for Biomedical Research (Cambridge, MA) grew human embryonic kidney (HEK 293) cells on a slide printed with an array of complementary DNAs (*Nature* **411**, 107–110, 2001). Cells growing on each "spot" take up the cDNA vector and express the encoded protein. The researchers could then use standard detection techniques such as autoradiography or *in situ* hybridization to study the expression, and even the subcellular location, of the protein. The cell microarrays have many advantages over existing protein chips: there is no need for time-consuming protein purification, and the cDNAs are more stable than proteins. The cells also provide an ideal environment in which to study membrane-bound proteins, and can be used to monitor the transient expression of proteins. Eventually, when cDNAs are available for the estimated 30,000 genes in the human genome, a pan-genomic cell array could fit on just a handful of cell chips. David Sabatini, the lead author, says that the team's next goal is to expand the number of cell lines that will work in the system, and to make arrays involving a loss of gene function—for example, using antisense technologies. *AB*

Convincing cancer models

Transgenic mice carrying single cancer-generating genetic mutations are useful models for inherited cancers, but do not adequately model cancers that arise spontaneously. Tyler Jacks and colleagues at the Howard Hughes Medical Institute (Cambridge, MA) and Massachusetts Institute of Technology (Boston, MA) have now applied the so-called hit-and-run gene-targeting approach to generate a mouse prone to sporadic cancers such as lung cancer (*Nature*, **410**, 1111–1116, 2001). The researchers generated constructs of a "silent" *K-ras* gene—an oncogene known to be associated with lung, pancreatic, and colon cancers. The silent gene was incorporated at its normal genomic location in mouse stem cells, creating a hybrid gene (the "hit"). In the adult transgenic animals, spontaneous recombination events take place (or "run"), shuffling mutant and wild-type *K-ras* DNA segments, and occasionally giving rise to mutant *K-ras* that promotes cell proliferation. Recombination events are rare and random, and so *K-ras* is expressed diffusely in cells scattered around the body. In particular, *K-ras* mutations gave rise to lung cancers, although lymph and skin cancers were also common. The mouse could provide a useful test model for chemotherapeutics and improved understanding of lung cancer pathophysiology. *LF*

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Weighing up proteins

Current means of measuring proteins in cells are relatively insensitive and do not provide a quantitative measure of the proteins present. To address this problem, researchers at the University of Pennsylvania (Philadelphia, PA) have developed a technique that is sensitive enough to detect the presence of a protein in a single cell (*Proc. Natl. Acad. Sci.* **8**, 5497–5502, 2001). The technique—called immunodetection by T7 RNA polymerase or IDAT—combines the specificity of antibody-based detection of proteins with the amplification possible with PCR. The researchers linked Roche's (Basel, Switzerland) Herceptin antibody to a double-stranded oligonucleotide bearing a promoter region for the enzyme T7 RNA polymerase. The antibody–oligonucleotide complex binds to the target antigen, and then RNA polymerase generates multiple transcripts. The RNA polymerase amplifies the signal in a linear manner, so the concentration of the transcript produced is directly proportional to the amount of target antigen present. IDAT detected target antigen with a billion times greater sensitivity than existing techniques, and could even be used to distinguish between the different phosphorylation states of a specific protein. James Eberwine, lead author, says that the challenge will be to develop a bioinformatics platform that can marry data from IDAT, messenger RNA expression, and other biological assays. *JB*

Islet transplants?

In practice, persuading embryonic stem cells to differentiate into specific cell types has not proved straightforward. Researchers at the National Institutes of Health (Bethesda, MD) have now hit on a means of differentiating stem cells into the insulin-secreting cells of the pancreas, potentially providing material for islet transplants for people who have diabetes (*Science* **292,** 1389–1394, 2001). Using a selected subpopulation of stem cells that had previously been thought to give rise to only neurons, the researchers chemically induced stem cells to differentiate into the pancreatic cells of the islets of Langerhans, which secrete the hormones insulin, somatostatin, glucagon, and pancreatic polypeptide. The islet cells released insulin in a glucose-sensitive manner and responded appropriately to insulin-modifying drugs. When injected into diabetic mice, the differentiated cells aggregated into structures not dissimilar to those created *in situ* in the pancreas. The islet cells secrete around 50 times less insulin than normal islets and so did not help lower the blood sugar concentration, but they controlled the animals' body weight and prolonged their life span. *LF*

Enzymes with a sweet tooth

The nature of the sugars attached to many drugs (e.g., the antibiotics erythromycin and vancomycin) can influence their biological activity, but researchers have few means of manipulating glycosylation. In cells, the first step in the glycosylation pathway is the priming of a sugar with a nucleotide diphosphate, which facilitates its attachment to the substrate. However, most of the enzymes involved in this process work with a restricted repertoire of sugars. To broaden this repertoire, scientists at the Memorial Sloan–Kettering Cancer Center (New York) and Brookhaven National Laboratory (Brookhaven, NY) have created an enzyme that is more promiscuous in its preference for sugars (*Nat. Struct. Biol.* **8**, 545–549, 2001). The researchers first solved the three-dimensional structure of the Ep enzyme, a nucleotidylyltransferase from *Salmonella*. They then engineered Ep to create a variant capable of liaising with sugars not naturally targeted by the wild-type enzyme. Dimitar Nikolov, senior author, says: "Our aim is to use completely *in vitro* steps with engineered enzymes, the final result of which will be libraries of modified or randomized compounds based on naturally glycosylated compounds." Some of these variants could have superior properties as medicines. *AD*