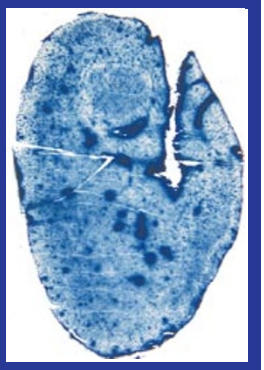
Proteins break on through to the other side



Work published in *Science* (**285,** 1569–1572, 1999) may open new possibilities for both protein replacement therapies and drug delivery in general. Until now, the plasma membrane has prevented drugs larger than 600 Da in size from entering cells, making most intracellular targets inaccessible to bulky protein treatments. Now Howard Hughes Investigator Steven Dowdy and his colleagues at the Washington University School of Medicine in St. Louis have successfully transferred into mice a protein almost 200 times larger than the size threshold. This feat was accomplished by tagging a protein of interest with a unique 11 amino-acid protein transduction domain (PTD) from HIV Tat protein and subsequently denaturing the resultant fusion with urea. According to Dowdy, denaturation uncovers hydrophobic residues that help the fusions traverse cell membranes "like parting the Red Sea." Previous in vitro studies by his group had already proved the approach successful for 60 proteins (ranging in size from 15 kDa to 120 kDa). But the results obtained in mice were better than they could have dreamed: "We were blown away," says Dowdy. Fusions with fluorescein and β-galactosidase injected into the peritoneum penetrated all tissues, including the brain (see inset), in under 25 minutes. What's more, once inside the cell, β-galactosidase both refolded and regained its activity. "The system also has obvious utility for basic studies of protein function," says Dowdy. "Over 400 laboratories around the world are now using it." Currently, the technology has been licensed to Life Technologies (Rockville, MD) for basic research applications and to IDUN Pharmaceuticals (La Jolla, CA) for therapeutics.

Electric mismatch detection

Differences in the electrical current generated when guanine is paired with different bases have been exploited by scientists at the University of North Carolina at Chapel Hill to develop a mutation detection method (*Chem. Biol.* **6,** 599–605, 1999). Holden Thorp and Patricia Ropp have used cyclic voltammetry to measure changes in the oxidation of nucleobase 8-oxo-guanine in the presence of thymine, guanine, adenine, and cytosine as a means of assessing changes in DNA secondary structure. Using osmium (2,2'-bipyridine)$_3^{3+/2+}$ as an oxidizing agent, the researchers first demonstrated that all the possible mismatches to 8-oxo-guanine in double-stranded oligonucleotides could be distinguished on the basis of electrical current. They went on to show that the common cystic fibrosis TTT deletion can be detected by probing the deletion site with an 8-oxo-guanine selectively placed in the probe strand, the mutant showing a significantly higher current enhancement than the wild-type sequence. Ultimately, Thorp believes the technology could be adapted for genomic mutation detection: "Electrochemistry is good for multiplexing," he says. A version of the approach applied to expression analysis is currently being commercialized by Xanthon (Research Triangle Park, NC).

Research News Briefs written by Alan Dove and Andrew Marshall

Double dose cancer treatment

By combining two cancer immunotherapy approaches that show only modest antitumor activity individually, researchers at the University of California, Berkeley, have developed a powerful new treatment that may be useful against several common types of tumors. In earlier work, Howard Hughes Investigator James Allison and his colleagues had targeted CTLA-4, a protein present on both cancer and normal cells that apparently inhibits T-cell-mediated destruction of tumors. Although a monoclonal antibody (Mab) against CTLA-4 caused the rejection of tumors in some animal models, "It did not work particularly well in anything but the least aggressive types of tumors," says Allison. In the new work (*Journal of Experimental Medicine* **190,** 355–366, 1999), he decided to augment the anti-CTLA-4 Mab with a vaccination approach. In addition to anti-CTLA-4 antibodies, his team injected irradiated melanoma cells, genetically engineered to secrete granulocyte–macrophage colony stimulating factor, into a mouse model of melanoma, with impressive results: the combined treatment eradicated tumors in 80% of the mice. "The effect seems specific for those T cells that respond to what are considered foreign antigens as opposed to self antigens," says Allison, adding that human trials in patients with prostate cancer are scheduled to begin by the end of the year.

Mustard plant passes the salt

In a recent issue of *Science* (**285:**1256–1258, 1999), botanists at the University of Toronto (Toronto, Canada) report that overexpression of a native ion transport protein in *Arabidopsis* confers tolerance to high levels of soil salinity, a finding with significant implications for agriculture. The protein, called the Na^+/H^+ antiport, efficiently sequesters excess Na^+ inside vacuoles, allowing the plants to overcome osmotic stress induced by salt in the environment. Plants that express high levels of the antiport were able to grow in a medium with 200 mM salt, conditions that withered wild-type controls. Eduardo Blumwald, senior author on the paper, explains that "Although it is possible that some extra energy will be required [for growth in saline soil], the plants are well suited for this task. . .I do not think that salt sequestration will require any extra nutrient uptake." In recent years, irrigated land has experienced serious declines in productivity because of increasing salinity, and Blumwald's team plans to use antiport overexpression to develop salt-tolerant crop plants, which could enter field tests within three years.

Noisy protein sorting

Modern chip fabrication techniques, and an idea initially proposed by Nobel physicist Richard Feynman, have led to the development of a device that can separate membrane-attached proteins in their native state (*Science* **285**, 1046–1048, 1999). By attaching a lipid membrane to the surface of a chip, then erecting microscopic physical barriers on the chip in a repeating pattern, researchers have created a two-dimensional "Brownian ratchet" that sorts membrane proteins. The device works by applying an electric field to proteins introduced at one corner of the chip, which while migrating through the electric field make random lateral movement due to Brownian forces. When a protein encounters one of the diagonal barriers, it is shifted a short distance across the electric field before continuing its journey. Like game pieces in a pachinko machine, proteins are sent along different paths by the barriers. As the proteins can travel through the ratchet in their native membrane-associated state, the technique may simplify many types of biochemical analysis. "Molecules in a ratchet-type device can also be used to measure alterations in properties associated with binding [and] clustering," as clustered proteins would migrate differently, explains Steven Boxer, senior author on the paper.