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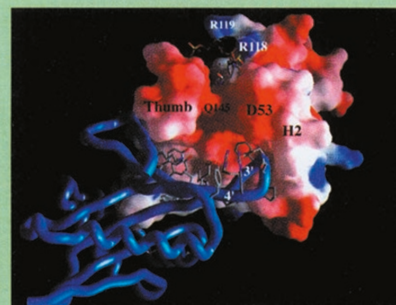
Tied down protein engineering

In vitro evolution—the process by which molecules are selected for desired activities—works well for creating novel RNA enzymes. Proteins, however, present a greater challenge, not least because no easy way exists for amplifying products. Now, a team from the University of California, Berkeley led by Peter Schultz has devised an elegant new solution (*PNAS* 95:10523–10528, 1998). By attaching substrate molecules to the surfaces of bacteriophages, they have developed a general approach for evolving or isolating protein enzymes based on their activities. As a model system, the group used streptococcal nuclease (SNase), which cleaves RNA and DNA, and tethered phages expressing SNase or a control protein to a solid support with a short piece of DNA. Only the phage expressing SNase can cleave their tether and fall off the support, separating them from phage with the control protein. "This method links genotype with phenotype outside the cell, whereas normally the only way you could do that is with an in vivo system," explains Schultz. Successive rounds of selection from an initial pool could produce enzymes with improved efficiency or entirely new activities. "Now you can do any reaction, either a biological reaction or an abiological reaction," he says.

AAT structure solved

A group of scientists at the Rockefeller University (New York) and the National Institutes of Health (Bethesda, MD) has solved the three-dimensional structure of aminoglycoside-3-*N*-acetyltransferase (AAT), a bacterial enzyme contributing to the growing problem of aminoglycoside antibiotic resistance. Heavy clinical use of aminoglycosides has increased the frequency of resistant bacterial strains containing AATs or other aminoglicosyde-inactivating enzymes. The new AAT structure, reported in *Cell* (94:439–449, 1998), opens the door for the design of drugs that directly combat aminoglicosyde resistance by inactivating AAT. "The enzyme resembles a cupped hand wrapped around a narrow cavity that can accommodate the gentamicin molecule," says HHMI investigator and team leader Stephen Burley. "The concave surface also contains a notch occupied by a molecule of acetyl coenzyme A." Once in position, coenzyme A (recognized by Arg119/Arg118 residues in notch) donates the acetyl group, which is then transferred to gentamicin (middle of dimer), resulting in its inactivation. Structure-based sequence alignments of representative members of the GCN5-related *N*-acetyltransferase superfamily suggest that all acetyltransferases share the same catalytic core—a finding confirmed by the crystal structure of a histone acetyltransferase reported in the same issue of *Cell*.



Microarrays map matings

Researchers at the University of California, Berkeley report in *Science* (281:1194–1197, 1998) the rapid and simultaneous mapping of five yeast genes, validating a technique that could have broad applications in genetics. The chips, manufactured by Affymetrix (Santa Clara, CA), contain precisely arrayed snippets of genomic DNA from either of two yeast strains. When DNA from one strain is placed on the chip representing the other strain, sequences that differ between the two fail to hybridize, and the locations of the variations can then be determined from the yeast genome sequence. Screening the progeny of a cross between the two strains, the scientists were then able to use the array data to determine which segments of the genome had been contributed by which parent, thereby mapping the locations of DNA crossovers that occurred during meiosis. As the DNA segment that segregates with a particular phenotype should contain the gene responsible for that phenotype, the researchers were able to precisely map both known and unknown genes. While cautioning that considerable hurdles remain before the technique can be applied to humans, lead author Elizabeth Winzeler is optimistic about its potential: "One could use the arrays to look for regions where the genome of a virulent strain [of bacteria] differed from an avirulent one," she suggests, which in turn could reveal new targets for antibacterials.

Research News Briefs written by Alan Dove, Margret Einarson, and Regina Raz.

Antibody snuffs cocaine

An antibody that speeds the degradation of cocaine in the bloodstream of laboratory animals may find applications in combating addiction and treating overdoses, paving the way for a new generation of drugs to block the action of other neurotoxins. In a recent issue of *PNAS* (95:10176–10181, 1998), researchers at Columbia University (New York) and the University of Michigan Medical School in Ann Arbor describe a catalytic antibody that breaks cocaine molecules into nontoxic, nonaddictive compounds. The antibodies stopped drug-seeking behavior in a rat model of cocaine addiction and protected against an overdose that was lethal to controls. In contrast to vaccines, which induce the production of cocaine-binding antibodies, the catalytic antibodies start to work immediately, making them a potentially life-saving treatment for cocaine overdoses. The team, led by Don Landry, a physician in the Division of Nephrology at Columbia, is currently working to produce similar antibodies to inactivate the nerve gases Sarin and VX.

Long-life bioreactor

A new type of bioreactor could allow extended culture of mammalian cells, optimizing recombinant protein production. At the national meeting of the American Chemical Society in Boston, Shang-Tian Yang, a professor of chemical engineering at Ohio State University, (Columbus), described a novel fibrous bed bioreactor (FBB) for large-scale mammalian cell culture. The FBB contains a three-dimensional matrix of dacron fabric with a large pore size that allows the removal of dead cells, and the continuous flow of nutrients. In a pilot study for Progenitor (Menlo Park, CA), a recombinant human osteosarcoma cell line was continuously cultured in the FBB over a four-month period, producing more than 100 milligrams of recombinant developmental endothelial locus-1 (DEL-1). Yang claims his group has now successfully cultured many other cell types in the FBB, including hybridomas, fibroblasts, and human trophoblast. "The unique characteristic of the FBB compared with hollow fiber bioreactors is the length of time the cells can be continuously cultured," he says. "This is months compared with weeks." The structure of the FBB also allows high cell density and viability to be achieved simultaneously, he adds.