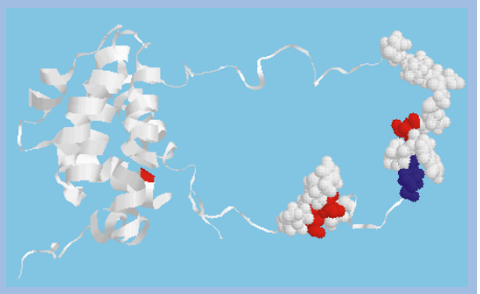
Panning for Taxol-binding proteins

New work by scientists from Florida State University, Tallahassee and the University of London's Birkbeck College provides further insight into the mechanism of action of the anticancer drug Taxol and could facilitate the design of new drugs (*J. Mol. Biol.* **285**:197–203, 1999). The cytoskeletal protein tubulin has long been known as a cellular target for Taxol, but it has been unclear how this interaction results in the death of cancer cells. Lee Makowski and his colleagues set out to identify other cellular targets of Taxol to gain better insight into how it works. The team screened a 12 amino acid phage-displayed peptide library of cellular proteins and identified over 70 peptide clones with affinity to Taxol. After sequencing the clones and searching for similarities to human protein sequences, they found that one-third had significant similarity to the disordered loop region of the BCL-2 protein, which is involved in apoptosis. ELISA-binding assays showed that the selected peptides were predictive of Taxol-binding sites in BCL-2. First author, Diane Rodi says "We are excited about this simple in vitro screen because it has the potential for identifying all molecular targets of a drug before it is tested in animals or humans." The group is characterizing additional clones identified in the screen and plans to investigate further how the drug's interaction with BCL-2 is involved in killing cancer cells.



A Crick in the elbow

If a team of researchers at New York University has its way, engineers may someday turn to DNA as a structural component for building nanometer-scale machines. They describe a robotic arm constructed from two rigid DNA "double crossover" molecules. By changing solution conditions, the sequence linking the two segments can be reversibly switched between two structural states, the B and Z forms, a transition that causes one segment to rotate relative to the other. In order to prove that the predicted motion occurs, the researchers tagged the segments with dyes whose fluorescence varies with the distance between them. "I see this primarily as a prototype. We've demonstrated both that we can make this thing and that we can demonstrate it when it [moves]," says Nadrian Seeman, a professor in the department of chemistry at New York University and senior author on the new work. Seeman adds that because the motion of the DNA arm is relatively large by nanomechanical standards—20 to 60 angstroms—it might be combined with other components capable of smaller movements: "One could imagine this as sort of the elbow or the wrist that could be attached to a robotic finger." The findings are reported in the 14 January issue of *Nature* (**397**:144–146, 1999).

Research News Briefs written by Alan Dove, Margret Einarson, and LeeAnn Leshko.

Gene therapy on demand

A new approach using two recombinant strains of adeno-associated virus (AAV) may help solve a vexing problem in gene therapy—controlling the expression of a therapeutic gene once it has been delivered to target cells. The method, reported in *Science* (**283**:88–91, 1999) capitalizes on the ability of the drug rapamycin to bind simultaneously to two different protein sequences. By attaching the two sequences to DNA binding and transcriptional activation domains, scientists have created a pair of recombinant proteins that join to form a transcriptional activator only in the presence of the drug. The two-part activator was delivered to muscle cells in one AAV vector, while a separate AAV vector carried a gene encoding erythropoietin. In immunocompetent mice and rhesus monkeys, cells co-infected with the two viruses expressed high levels of erythropoietin when animals were given rapamycin. "The properties of this system are that when it's off it's off, when you want to induce it, it induces to levels that are as high as what you achieve with a high-level constitutive promoter, [and] the extent of the induction is proportional to the dose of the drug that you give," explains James Wilson, senior author on the new study. The technique has been licensed to a joint venture between ARIAD Pharmaceuticals (Cambridge, MA) and Genovo (Philadelphia, PA).

Breathing easier with AAT deficiency

Deficiency of α-1-antitrypsin (AAT) protein is responsible for approximately 3% of all early deaths due to pulmonary disease. Gene therapy has been suggested as a possible treatment, but it has not been clear whether AAT can be delivered at therapeutic levels. Now, Terence Flotte and colleagues at the University of Florida, Gainesville have reported the development of a recombinant adeno-associated virus (rAAV) that is capable of expressing human AAT at therapeutic levels in mice (*Proc Natl Acad. Sci. USA* **95**:14384–14388, 1998). Injection of the rAAV-hAAT virus into the muscles of two different strains of mice resulted in greater than 800 μg hAAT per milliliter of serum. The human AAT protein was expressed at consistently high levels for three months. High serum levels of AAT are crucial to the success of any gene therapy approach for AAT deficiency, as protein replacement therapy requires weekly intravenous infusions to maintain high enough serum levels to combat the onset of pulmonary disease. Scaling up expression to account for the size difference between mice and humans is the next step, says Flotte.

Predicting protein function

In an important proof-of-concept experiment for structural genomics, researchers have successfully predicted the biochemical activity of a protein based on data from X-ray crystallography. The availability of complete genome sequences for many organisms has uncovered large numbers of predicted protein sequences with no homology to known proteins. In the new work (*Proc. Natl. Acad. Sci. USA* (**95**:15189–15193, 1998), researchers have expressed a protein from the hyperthermophile *Methanococcus jannaschii* and solved its crystal structure. Protein crystallization is notoriously chancy, but senior author Sung-Hou Kim explains that studying extremophiles improved the odds of success: "Because these organisms live at very high temperatures, their proteins [often] crystallize much better than their counterparts in other organisms." The structure suggested that the protein is an ATPase, a conclusion that was confirmed by biochemical assays. Kim says that the protein, called MJ0577, is the first the team has attempted to study in this way, and its ATPase domain shares homology with a number of other previously undescribed proteins, highlighting the potential of structural genomics.