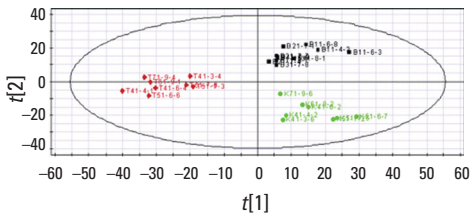
## RNA—totally, tubular

The need for rapid, cost-effective mechanisms to differentiate varying cell lines and states, especially with regard to their response to disease or drugs, has driven the movement to use automated methods of differential display related to RNA production. But this has traditionally been difficult and costly, since generally only 1–5% of total RNA is the transcription-pertinent mRNA. Now Wenwan Zhung and Edward Yeung from Ames Laboratory (*www.external.ameslab.gov*) have developed a new differential display method using capillary gel electrophoresis (CGE) that allows direct comparison of cDNA patterns between various cell types (*Anal. Chem.* **2003,** *75,* 4415–4422). As proof of concept, they analyzed commercially available total RNA isolated from three cell lines: human kidney, breast, and breast tumor.

The researchers analyzed cDNA produced from fluorescent-labeled RT-PCR amplification of the total RNA from the three cell sources using CGE-laser-induced fluorescence. Mixing high- and low-molecular-weight polyethylene oxides as a matrix helped to resolve both small and large DNA fragments. The sensitivity was such that fragments differing in only 2–3 base pairs could be resolved. Chromatograms from the gel runs were superimposed for pattern comparison by using highly reproducible starting and ending peaks, followed by computer "stretching" of the runs to account for experimental variations.

This method clearly and reproducibly distinguished overall pattern differentials for the three RNA sources (see figure). The process, say the researchers, is simple and does not require predetermined RNA probes, thus avoiding the use of expensive RNA arrays. Furthermore, highly multiplexed CE instruments are widely available, so they believe this technique is appropriate for automated high-throughput screening of RNA.

—MARK S. LESNEY

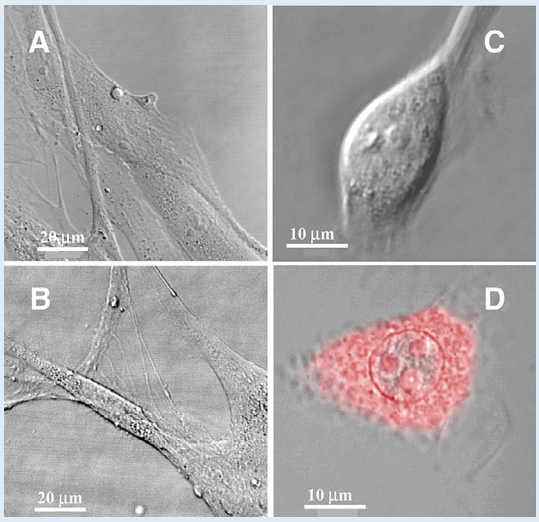



**Spotting differences.** Spots from breast tumor are red, spots from kidney are green, and spots from normal breast are black in these partial-least-squares discriminant analysis results. (Adapted with permission from Zhong, W.; Yeung, E. S. *Anal. Chem.* **2003,** *75,* 4415–4422.)

## Lytic peptides and cancer

To be effective, most anticancer drugs need to penetrate their target cell, but the therapeutic effects can be quickly lost if the cancer cells build immunity by modifying their multidrug resistance proteins.

One promising group of therapeutic molecules that might avoid this path of resistance is the cationic antimicrobial peptides, which play a central role in immunity for various organisms. These peptides are toxic to bacteria because they preferentially bind and permeate the negatively charged bacterial outer membrane rather than the more zwitterionic surface of mammalian cells. They have also been shown to be preferentially toxic to cancer cells, possibly because cancer cells have a slightly higher percentage of negatively charged phosphatidylserine than normal mammalian cells.




**A glowing success.** Researchers studied normal (left) and cancer (right) cells untreated (A and C) and treated (B and D) with rhodamine-labeled antimicrobial peptides. (Adapted with permission from Papo, N.; Shai, Y. *Biochemistry* **2003,** *42,* 9346–9354.)

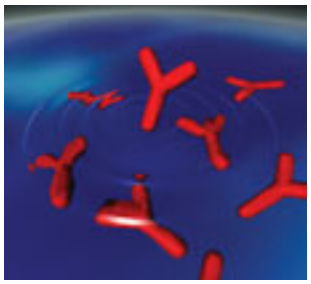
However, cationic antimicrobial peptides undergo degradation by serum components. To address this issue, Niv Papo and Yechiel Shai of the Weizmann Institute of Science (*www.weizmann.ac.il*) recently developed a series of diastereomeric peptides (containing both D- and L-amino acids) and tested their toxicity as well as their ability to selectively bind cancerlike membranes and cancer cells (*Biochemistry* **2003,** *42,* 9346–9354).

The researchers synthesized four 15- to 17-mer peptides composed of leucine and lysine. They tested the peptides for their ability to inhibit the growth of normal and cancer cell lines and found that they were quite selectively effective against the cancer cells, and unlike all-L-amino acid peptides, they had no adverse hemolytic activity.

Interestingly, the diastereomeric peptides showed little preference for cancerlike membranes but had a strong preference for binding cancer cells. These findings indicate that it is more than the phospholipid content of the membranes that causes the peptide-binding behavior.

Although the results are preliminary, the researchers believe that the simple composition of the diastereomeric peptides and their stability regarding enzymatic degradation by serum components make them excellent candidates for new chemotherapeutic drugs.

—RANDALL C. WILLIS



## Antibody Pool-EASE!

In the world of recombinant protein-based medicines and diagnostics, there is a great need to create stable cell lines that can produce significantly large pools of the desired protein. This can be difficult enough in the case of monomeric or homodimeric proteins; it can be a special challenge when trying to maximize production of heterodimeric proteins such as the critically important antibodies.

In such cases, molecular tricks that stimulate greater yield can be of benefit. For example, several cis-acting elements (such as the universal chromatin-opening element) have shown the ability to greatly facilitate gene expression in transgenics. In an attempt to increase pools of monoclonal antibodies, Arvia Morris and colleagues at Amgen (*www.amgen.com*) utilized vectors they constructed containing a cis-acting helper they named EASE (expression augmented sequence elements) to maximize production of a monoclonal chimeric test-antibody that recognizes M1 bacteriophage (*Biotechnol. Prog.* **2003,** ASAP). The mechanism of EASE is unknown, but earlier evidence suggested that it helps facilitate transgene integration into the genome.

The scientists designed four recombinant vector plasmids, each with a cytomegalovirus promoter, an adenovirus leader sequence, and an internal ribosomal entry site element enhancement element. Each vector contained either the antibody constant-chain gene (mouse origin) or the antibody variable-chain gene (rat origin, selective screen), each with or without the EASE sequences.

Chinese hamster ovary cells were transfected with the paired vectors containing the two antibody genes with or without EASE and cloned using a double-selection technique. Cell lines with EASE-containing pools showed antibody production of 350–400 mg/L, whereas those made from constructs lacking EASE showed yields of only 75–150 mg/L. EASE-transformed cells could also be cloned rapidly, without labor-intensive steps, within a three- to five-week period. The researchers believe that such manipulations, providing both cloning speed and increased pool yield, may help to create more industrially relevant cell clones for drug compounds and a host of other biologicals.

—MARK S. LESNEY

## Biologics: Generically speaking

The first round of recombinant protein therapeutics—several billion dollars worth—coming off patent over the next few years is beginning to trigger intense debate, as was evident in a panel presentation at a Food and Drug Law Institute (*www.fdli.org*) conference entitled "Issues Raised by Follow-on Versions of Biologics", held in Washington, DC, in July.

Under the 1984 Hatch–Waxman Act, a company may begin developing a generic drug with an active ingredient before it comes off patent and file an Abbreviated New Drug Application, which relies on the already-on-file data set of the originator company for safety and efficacy assurance and only requires bioequivalence tests for the generic drug. Thus, costs are much reduced.

Biologics, by and large, are reviewed and licensed under a completely different federal law than small molecules and are not subject to this section of the act (for historical reasons recombinant insulin and several hormone and enzyme products are reviewed as drugs, not biologics). But continued motivation to cut drug costs has sparked discussion on developing a similar "abbreviated" approach for generic ("follow-on") biologics.

The controversy of the issue was on display in a roundtable discussion with Stephan Lawton, vice president and general counsel of the Biotechnology Industry Organization (*www.bio.org*); Bruce Kuhlik, senior vice president and general counsel of the Pharmaceutical Research and Manufacturers of America (*www.phrma.org*); and Steve Bende, vice president of the Generic Pharmaceutical Association (*www.gphaonline.org*).

All three agree that the situation of follow-on biologics is different from conventional generics—the molecules are much more elaborate and the recombinant processes are highly defined for each product, so even subtle changes can lead to considerable variations in safety and efficacy. There was marked disagreement, however, on whether these differences are showstoppers.

Lawton focused on the legal issues, stating that Hatch–Waxman was not intended "to be the basis for a follow-on policy that allows access to [the] confidential data" that would be needed for abbreviated development of generic biologics, whether or not those compounds are technically approved as drugs or biologics. Kuhlik agreed on this point and built on it to say that safety could not be guaranteed for products developed without the complete originator data set and manufacturing details. "Undue risk is not appropriate," he said, "until there is more science."

On the legal issue, Bende insisted that the other two were "dead wrong." He also said that current analytical technologies can characterize proteins in great detail and detect subtle changes (and progress is occurring quickly). What is necessary, he says, is a "spectrum of data needs" to establish equivalence that should vary depending on the specific biologic product. Both Lawton and Kuhlik dismissed this "case-by-case" approach as inappropriate.

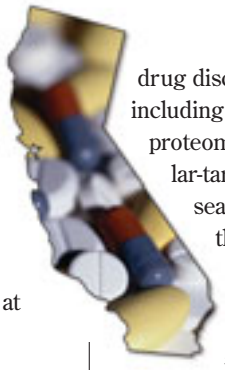
Nonetheless, an element of political inevitability framed the debate. The first speakers of the day were Trish Knight and Bruce Artim, staffers for Senator Orrin Hatch (of Hatch–Waxman), who expressed support for abbreviated follow-on biologics and said the question was one of "when, not if".

—DAVID FILMORE

## START in CA

SRI International (*www.sri.com*), a nonprofit independent research institute, committed an initial half-million dollars and more than 1000 hours of consulting time to launch a collaboration of California-based universities, research institutes, and small biotech companies designed to accelerate the translation of basic drug discovery research into clinical products.

The founding members of the new consortium, called PharmaSTART (Biopharmaceutical Support for Translating and Advancing Research and Technology, *www.pharmastart.org*), are Stanford University (*www.stanford.edu*), the University of California at San Diego (UCSD, *www.ucsd.edu*), UC-San Francisco (UCSF, *www.ucsf.edu*), and the UCSF campus of the California Institute for Quantitative Biomedical Research (*www.qb3.org*).



"Billions of government dollars have been invested in drug discovery research, including large genomics, proteomics, and molecular-targeted basic research initiatives. Yet there is still a major gap in the translation of these discoveries into new drugs," said Glenn Rice, vice president of SRI's biosciences division, in a press release. "Universities are not equipped to perform the necessary Food and Drug Administration-compliant development tasks required to start clinical trials."

PharmaSTART will offer consultation to consortium university investigators and their spin-off companies to help create drug development plans to efficiently bring early-stage breakthroughs into the clinic. Typical consulting services will hit on everything from lead development and optimization to cell line characterization and banking to manufacturing, toxicology, analytical methods, and regulatory affairs.

The collaboration will also help scientists identify potential funding sources and assist in the preparation of proposals to government grant programs such as the NIH, Small Business Innovative Research Program, and Small Business Technology Transfer Program.

In addition, a major goal of PharmaSTART will be to promote collaborations between members to tackle complicated and expensive developmental tasks.

"PharmaSTART is a first-of-its-kind drug translation consortium," said Jerrold Olefsky, UCSD professor of medicine and a member of the PharmaSTART steering committee. "UCSD's faculty will benefit from this program, and we look forward to leveraging this new collaboration into large and productive inter-institutional drug development initiatives."

David Martin, founder and CEO of Eos Biotechnology (*www.eosbiotech.com*), has agreed to serve as chairman of the PharmaSTART steering committee, which includes representatives of each of the founding institutions.

—DAVID FILMORE

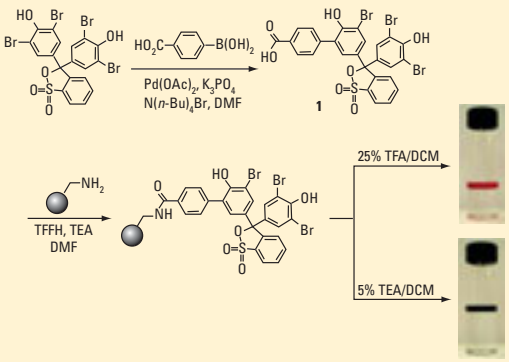
## Self-indicating synthesis

A significant challenge to solid-phase combinatorial chemistry is being able to identify and monitor the reaction history of each of the resin beads. Although several colorimetric methods have been developed, each assay results in the destruction of a small percentage of the resin material. Furthermore, methods that have been developed for real-time reaction monitoring rely on the reaction of dyes like bromophenol blue with synthesis constituents, a process that can require a lot of front-end experimentation to identify ideal conditions.

Recently, Mark Bradley and colleagues at the University of Southampton (*www.soton.ac.uk*), Merck Biosciences (*www.merckbiosciences.de*), Pfizer Global R&D (*www.pfizer.com*), and GlaxoSmithKline (*www.gsk.com*) developed a self-indicating resin by attaching a bromophenol blue derivative onto a solid support (*J. Comb. Chem.* **2003,** ASAP). Conveniently, the absorption wavelength and intensity of the bound dye differed only slightly from those of the parent dye, such that under acidic conditions, the resin turned red, and in basic solution, it turned blue.

The researchers coupled Fmoc-Leu-OH to the resin beads, which remained blue until 98% of the available coupling sites were occupied, whereupon it turned green and then greenish yellow. The researchers then used fresh beads to synthesize two peptides, determining the degree of completion via the color change from blue to greenish yellow. Using this method, they prepared peptides with high yield and purity without resorting to time-consuming chromatographic techniques.

They also used the modified beads to monitor the solution-phase synthesis of a library of ureas, using six amines and four isocyanates as building blocks. When they added beads to the wells, the beads immediately turned blue to indicate the excess amines, but when they added methylisocyanate as an amine scavenger, the beads slowly turned to yellow.




**Dyeing to succeed.** By conjugating a bromophenol blue derivative to beads, researchers have developed a resin that can colorimetrically monitor combinatorial reactions. (Adapted with permission from Cho, J. K.; et al. *J. Comb. Chem.* **2003,** ASAP.)

The researchers are confident that their self-indicating resin will facilitate the monitoring of other solid- and solution-phase syntheses. They also suggest alternative uses, including "the possibility of monitoring the release of free amines on the resin during chemical or biological screening."

—RANDALL C. WILLIS

PHOTO: PHOTODISC

## A web of health

A study conducted by the Pew Internet and American Life Project, a nonprofit, nonpartisan think tank, reports that gathering health or medical information is one of the most popular online activities—third only to sending and receiving e-mail, and researching products before buying—but, at the same time, sporadic (*www.pewinternet.org/reports/toc.asp?Report=95*). Eighty percent of those surveyed have accessed the Internet to do health-related research, but 8 out of 10 of those say they do so every few months or less frequently than that.

"Health searches are not an everyday activity for most Americans," says the director of the project, Lee Rainie, "but we have noticed that once an Internet user has been successful in an online endeavor, she will return to it the next time she has a similar problem or question, no matter how much time has lapsed between the searches."

The study found that women are more likely than men to seek health care and health information. It also found that more than half of those who recently conducted searches did so on behalf of a spouse, child, friend, or other loved one. One survey respondent noted, "I spend at least an hour a day helping others with their medical concerns."

A large percentage of adult Internet users have searched for at least 1 of 16 major health topics. The most common health topics searched online are specific diseases or medical problems (63%), specific treatments or procedures (47%), "diet, nutrition, vitamins, or supplements" (44%), and exercise and fitness (36%).

Some of the biggest users of Internet health sites are patients with chronic or rare diseases and their caregivers, many of whom take full advantage of online newsgroups and e-mail list servers. These "power users", says Susannah Fox, Pew director of research, "are very enthusiastic about using e-mail to keep in touch with their doctors." Ninety-three percent of e-mail users in the study indicated that it is a useful way to communicate their medical concerns to doctors or other health professionals.

The report was based on findings of a daily tracking survey on Americans' use of the Internet and an online survey on health resources. Telephone interviews were conducted among a sample of 2038 adults.

—FELICIA M. WILLIS

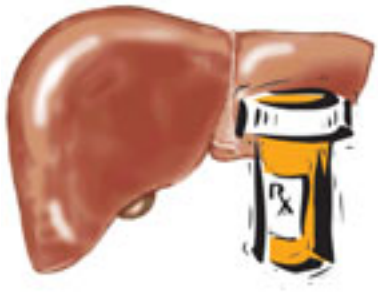
## Liver toxicity attention

Closer scrutiny is required of potential cases of liver injury resulting from adverse drug reactions by doctors, says physician and liver disease researcher William Lee from the University of Texas Southwestern Medical Center (*www.utsouthwestern.edu*).

In general, drug-induced liver injury is a rare, idiosyncratic event, but according to the FDA, such injuries are actually the leading cause of liver failure in the United States and the most common single cause for withdrawal of drugs from the market (*www.fda.gov/cder/livertox*). Furthermore, more than 75% of cases of idiosyncratic drug reactions result in liver transplantation or death.

In a review article, Lee says that the statistical infrequency of drug-related hepatotoxicity is an important part of the problem (*N. Eng. J. Med.* **2003,** *349,* 474–485). The reactions to many drugs may occur in less than 1 in 10,000 patients. But a typical Phase III clinical trial involves only about 3000 patients, and detecting a single case of an adverse reaction to a drug with 95% confidence, writes Lee, requires that the number of patients studied be 3 times the incidence of reaction, or about 30,000. Therefore, many drugs are approved before liver effects can be identified. This puts the responsibility on postmarket monitoring.

Hepatotoxic drug reactions are characterized by rapid onset of malaise and jaundice in association with elevated aminotransferase levels.




Monitoring aminotransferase levels is seldom performed consistently, according to Lee, which could help reduce serious liver-related adverse effects. But because of the abruptness of many drug reactions, this would not be a catchall. Of key importance for a physician, indicates Lee, is to keep track of a patient's diet and other medicines and discontinue use of a drug at the first sign it is causing liver damage.

"The condition is often reversible if it is caught early, but patients that believe they should continue to take the medication and are not concerned when they turn yellow get in big trouble for obvious reasons," said Lee in a UT Southwestern press release.

Besides individual patient safety, it is vital for doctors to pick up on occurrences of drug-induced liver damage to provide an accurate record to the FDA, so that regulators can access the significance of the adverse response and weigh it against the drug's benefits.

—DAVID FILMORE

**KEY TERMS:** automation (p 9), combinatorial chemistry (p 13), genomics (pp 9, 10), high throughput (pp 9, 13), medicinal chemistry (p 9), regulations (pp 10, 14), screening (p 9), technique (p 9)

PHOTO: PHOTODISC