Single-cell genotyping

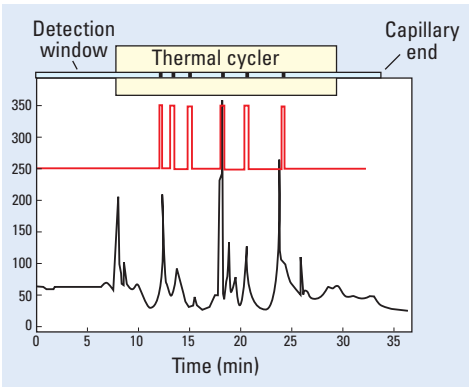
In the diagnosis of human disease, no technique has seen greater recent development than the polymerase chain reaction (PCR). In fact, the technology has reached the point where target sequences can be amplified from single cells, which is important when trying to identify defects in a few malignant cells surrounded by healthy ones. Traditional single-cell PCR, however, requires labor-intensive sample manipulation, making the technique less than optimal for high throughput. But Hanlin Li and Edward Yeung of Iowa State University (Ames) recently developed an on-line capillary tube PCR method coupled with laser-induced fluorescence as a simple detection scheme for single-cell genotyping (*Electrophoresis* **2002,** *23,* 3372–3380).

The first challenge for the researchers was to develop a method to determine the exact location and number of cells within the capillary. The researchers opted to detect the cells using partial dark-field microscopy, where no cell stain, which tended to interfere with subsequent PCR reactions, was needed, and they could clearly see each of the cells under 100× or 200× magnification.

The researchers then tested a variety of lysis conditions on cells in a PCR mixture of buffers, salts, and enzymes. A simple combination of heat (95 °C pre-PCR incubation) and osmotic pressure was found to be the most compatible with the on-line capillary PCR.

Once the conditions were optimized, the researchers tested their system by amplifying a segment of the β-actin gene from individual human lymphoblasts. They found a clear correlation between the relative locations of the cells in the capillary and the pattern of the PCR product peaks as they eluted from the column. Elution peak heights were not identical, but the researchers explained that this was probably due to the known variability of exponential amplification and inadequate cell lysis. They are enthusiastic that their method represents another step in the development of a new clinical diagnostic tool.

—RANDALL C. WILLIS



A one-for-one correlation. By lysing single cells in a capillary and then performing PCR amplification (top), researchers hope to correlate the presence of a specific gene (bottom) with individual cells (middle). (Reproduced with permission from Li, H.; Yeung, E. S. *Electrophoresis* **2002,** *23,* 3372–3380.)

Cotton: The fabric of our libraries

In the continuing effort to find a faster, more efficient way to build peptide libraries, Jarl E. S. Wikberg and colleagues (Uppsala University, Sweden) found that cotton might make a useful substrate because of its low cost, easy handling, relatively high substitution degree, and good mechanical stability.

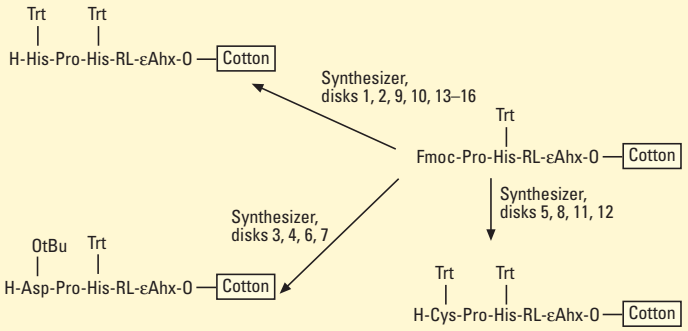
The team generated (and stored) a nonrandom library of 3- to 4-mg quantities of various hexapeptides supported on cotton fabric disks (*J. Comb. Chem.* **2003,** in press). Sixteen activated disks with 9-fluorenylmethoxycarbonyl (Fmoc)–6-aminocaproic acid "handles" were stacked in the column of a commercial continuous-flow peptide synthesizer, Fmoc Rink linker was added, and the residual amino groups of the handle were acetylated.

The first amino acid was coupled to the linker, and a C-terminal dipeptide was then attached. The next three amino acids (histidine, cysteine, and aspartic acid) were added by sorting the disks into three groups, and placing them into the column for three consecutive runs. In each case, addition of the corresponding amino acid, capping, and Fmoc removal were performed with an automated process.

Amino acids were added to various disks at the fourth position by manually placing several disks in each of eight beakers and shaking the disks in a dimethylformamide solution containing various amino acid derivatives. The disks were re-sorted, and a fifth amino acid was added in two synthesizer runs. The manual procedure was then used to attach the sixth amino acid, and then the hexapeptides were cleaved from the cotton and the side chains were deprotected.

The isolated end products were of satisfactory quality for screening studies and showed the expected mass-to-charge ratios and HPLC retention times. The authors estimate that their method could be used to perform at least 300 different amino acid couplings per day.

—NANCY K. McGUIRE



Cotton chem. Addition of first three amino acids to cotton supports. (Fmoc)–6-aminocaproic acid (εAhx) and Fmoc Rink linker (RL) are already attached.

Contracts don't cut it?

The contracts negotiated by medical schools with companies sponsoring multicenter clinical trials could use some improvement in promoting the integrity of the clinical research enterprise and protecting the academic independence of researchers, according to a report from Duke University (Durham, NC) researchers.

The Duke team cited guidelines set out by the International Committee of Medical Journal Editors (ICMJE) that address the industry sponsors' and academic investigators' respective roles in trial design, access to data, and publication rights. The researchers conducted a survey of contracting officers from 108 members of the Association of American Medical Colleges that take part in multicenter clinical trials to determine how the industry–academic contract agreements, which play a large part in governing trials and the handling of results, addressed the ICMJE issues (*N. Engl. J. Med.* **2002,** *347,* 1335–1341).

The survey respondents indicated that a median of 10% of each institution's contracts addressed some sort of plan for data collection and monitoring. A median of only 5% included a plan for data analysis and interpretation. It was even more rare for the agreement to require the presence of an independent steering committee or a data and safety monitoring board.

The agreements signed by the medical schools did tend to guarantee their own investigators access to data obtained at the local site, but rarely (median score of 1%) did the contracts require that authors of reports on multicenter trials have independent access to all of the trial data.

In addition, the survey reported almost no agreements that required publication of trial results or an independent writing or publications committee.

A smaller subgroup of the respondents (14) representing institutions that act as coordinating centers for industry-sponsored clinical trials generally showed somewhat better median compliance, but, especially for data access and publication requirements, several median scores were less than 50%.

This study had several limitations, including the reliance on the recollections of respondents. Another problem was the inability to address the question of whether an institution's researchers were potential authors of multicenter trial reports or simply participating investigators, which would make trialwide data access and a publication requirement less crucial.

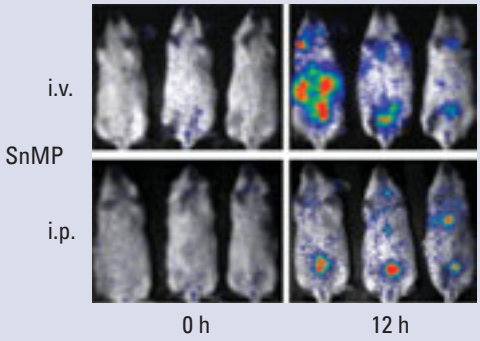
But the study does provide an important look at an issue that has received a lot less scrutiny than that of direct financial conflicts of interest by investigators. And, according to an editorial by *New England Journal of Medicine* editor-in-chief Jeffrey Drazen, "It seems unlikely that better methods will vitiate [the study's] core findings."

—DAVID FILMORE

Affecting a jaundiced view?

Physiological jaundice caused by a buildup of bilirubin, a byproduct of heme degradation, is an almost universal occurrence in the days following birth. This buildup is caused by the breakdown of the fetal hemoglobin not needed in the more highly oxygenated outside world. However, when pathological jaundice develops, the extreme buildup of bilirubin can cause neurological damage and a host of other problems. Typical treatment of pathological jaundice involves phototherapy. But in the 4% of cases where this does not work, the treatment involves exchange transfusion, which has significant associated risks, including unwanted immune reactions and heart and respiratory problems.

In a search for potential therapeutics, researchers Weisheng Zhang and colleagues from Stanford University Medical Center (Stanford, CA) and Xenogen Corp. (Alameda, CA) used a mouse model to investigate the effects of using metalloporphyrins (Mps) to inhibit the enzyme heme oxygenase (HO), which is involved in the breakdown of heme to bilirubin. In their report, they confirmed the ability of a variety of Mps to inhibit HO activity (*J. Mol. Med.* **2002,** *80,* 655–664).



Mapping Mp effect. Bioluminescent images of reporter gene expression in HO-1-luciferase transgenic mice before and 12 h after i.v. or i.p. injection of a Sn metalloporphyrin (Mp). On the other hand, a Zn Mp caused no significant change in HO-1 expression. (Reproduced with permission from Zhang, W.; et al. *J. Mol. Med.* **2002,** *80,* 655–664.)

However, the California team discovered that although these Mps did indeed inhibit HO activity, both in vitro and in vivo, many of these compounds also upregulated HO-1, the inducible isozyme, at the level of transcription. This potentially reduces or even eliminates any therapeutic effect.

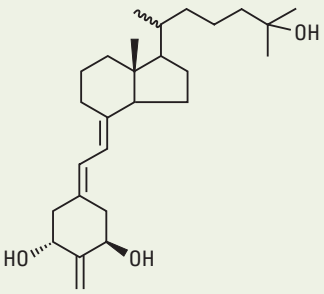
The pattern of HO-1 transcription was followed in transgenic mice with an HO-1-luciferase fusion gene, which allowed whole-body-pattern observations of two- to three-week-old mice using bioluminescence imaging. In their study, Zn bisglycol-porphyrin was the only Mp found not to alter HO-1 transcription patterns in mice while still acting as a potent inhibitor of HO activity; thus, the authors conclude, it may be suited for the prevention of pathological jaundice.

They propose that this type of whole-body assay should be generally useful for enhancing the utility of animal models in the development of novel therapies. The issue of potential heme toxicity, sometimes also a problem in neonates, was not addressed in this study.

—MARK S. LESNEY

Building bones

Vitamin D aids in absorption and utilization of calcium and phosphorus, is needed for normal blood clotting, and enhances immunity. But researchers at the University of Wisconsin–Madison have found a new use for a close analogue to this essential nutrient: A specialized form of vitamin D has shown effectiveness in inducing bone growth. The discovery could be significant for the estimated 44 million Americans who are at risk for or suffer from osteoporosis.



Although research has not been conducted on human subjects, a vitamin D analogue known as 2MD has been tested in rats and has shown great potential for reversing bone loss. "From where I sit, this is the most promising vitamin D compound I've seen," said author Hector DeLuca.

DeLuca and colleages removed the ovaries of laboratory rats to simulate menopause, the time in a woman's life when osteoporosis is likely (*Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 13487–13491). The rats given 2MD showed a 9% increase in total body bone mass, and vertebrae in the treated rats showed a 25% increase in mass. Spinal column bones are usually the most affected by osteoporosis. According to the researchers, this compound is the first vitamin D analogue that has increased bone mass and exhibits no apparent toxicity or side effects.

"Our results suggest that 2MD exhibits, at very low concentrations, a marked and unexpected activity in stimulating the synthesis of new bone," the team wrote.

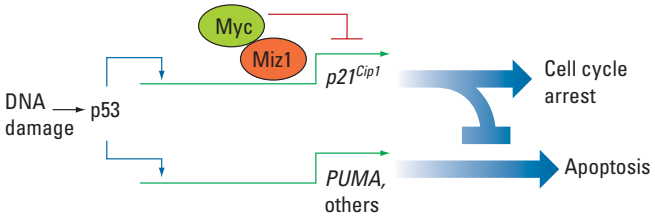
DeLuca says that even though the compound shows amazing results in the laboratory experiments, it will likely be several years before a drug using this technology reaches the market. It is expected that this compound could also become an alternative to hormone replacement therapy, which has become controversial for its detrimental side effects.

—FELICIA M. WILLIS

Signaling cell death

When chemicals or radiation damage a cell, there is a fine line between cellular repair and cell death, a line mediated by a balance of cell signaling factors. One such factor is the tumor suppressor p53, which is triggered by DNA damage to induce either cell cycle arrest (cytostasis) or cell death (apoptosis). It is hoped that by understanding the proteins that control this p53-mediated switch, scientists will be able to cause cancer cells to self-destruct.

The transcriptional activator cyclin-dependent kinase inhibitor ($p21^{Cip1}$) mediates the cytostatic effects of p53, while other transcriptional mediators such as *PUMA* and *PIG3* mediate its apoptotic effects. Thus, Joan Massagué and colleagues at the Memorial Sloan-Kettering Cancer Center (New York) looked for ways to inhibit the cytostatic effects of $p21^{Cip1}$ without interrupting apoptosis, focusing their attention on the transcription factor Myc, a known inhibitor of $p21^{Cip1}$ (*Nature* **2002**, *419*, 729–734).



When Myc levels are high, Myc is recruited to the $p21^{Cip1}$ promoter by Miz-1, inhibiting $p21^{Cip1}$ activation and promoting apoptosis.

Initially, the researchers looked at the effects of Myc on p53 and $p21^{Cip1}$ expression when DNA damage was chemically induced in carcinoma cells. In the absence of Myc, expression of both p53 and $p21^{Cip1}$ increased in the damaged cells. By contrast, damaged cells infected with an adenovirus encoding Myc showed only an increase in p53 levels, indicating that Myc somehow intervenes in the p53-mediated induction of $p21^{Cip1}$.

The researchers then performed transcriptional activation studies to determine where Myc bound in the $p21^{Cip1}$ promoter region. Myc was found to bind to the proximal promoter region of $p21^{Cip1}$, away from the upstream p53-binding site. Also, a chromatin immunoprecipitation assay indicated that Myc does not bind to the promoter alone, but rather it brings a DNA-binding partner in the form of Myc-interacting zinc finger 1 (Miz-1). Furthermore, DNA binding assays showed that Myc is incapable of binding to the $p21^{Cip1}$ promoter sequence without Miz-1. Finally, Myc expression was not shown to affect the p53-mediated expression of *PUMA*.

Thus, it appears that Myc indirectly promotes the apoptotic pathway in DNA-damaged cells by interrupting the p53-mediated induction of the cytostatic $p21^{Cip1}$. "Although it remains to be seen whether repression of $p21^{Cip1}$ would be beneficial in cancer treatment," the authors write, "the mechanism proposed here suggests ways to influence the cell's response to stresses that result in activation of p53."

—RANDALL C. WILLIS

KEY TERMS: automation (p 9), cell biology (p 9, 13), clinical (p 10), combinatorial chemistry (p 9), genomics (p 9), imaging (p 10), medicinal chemistry (p 13), regulations (p 10), screening (p 10), techniques (p 9, 10)