Picture perfect

Medical imaging techniques are being adapted to study gene expression and other cellular activities in living animals. Corie Lok talks to the pioneers who are watching cells at work in their natural habitat.

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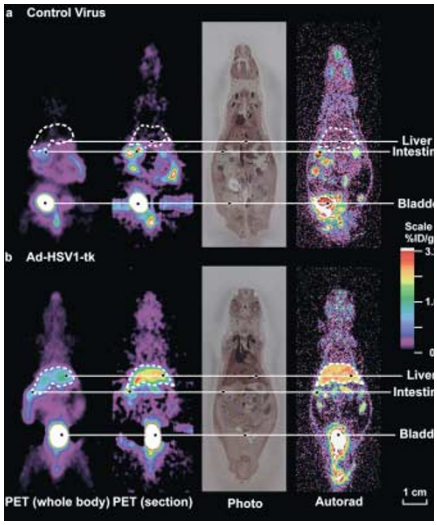
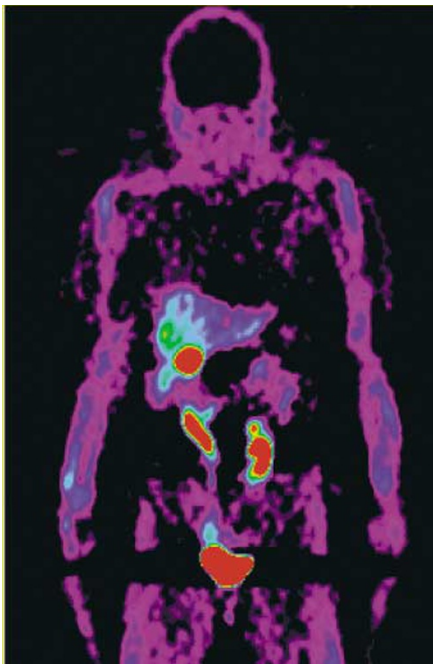




S. YAGHOUBI *ET AL. J. NUCL. MED.* (IN THE PRESS)

Colour-coded: positron emission tomography can be adapted to reveal the levels at which introduced genes are being expressed in mice (above) and human patients (right).

A decade ago, Harvey Herschman was a classic laboratory gene-hunter, focused on studies of cell cultures. His team at the University of California, Los Angeles (UCLA), was internationally respected. Yet Herschman felt he was missing something important.

"There's always something unsatisfactory about studying genes *in vitro*," he says. The same is also true for studies on laboratory animals, he adds, if all you can gain is a frozen snapshot of gene activity by doing post-mortems or biopsy studies. So in 1996, Herschman teamed up with imaging specialists, including his UCLA colleague Michael Phelps, to devise techniques to watch gene expression in living animals.

Herschman is one of a growing number of leading cell biologists, molecular biologists and geneticists who are working with experts in magnetic resonance imaging (MRI), positron emission tomography (PET) or optical imaging. "Four years ago, I couldn't spell PET," jokes Herschman. Today, he is principal investigator at UCLA's Center for *In Vivo* Imaging in Cancer Biology.

Glowing report

Advances in molecular and cellular biology have yielded a host of molecular targets, including genes and proteins, that researchers would like to study in living animals. The field of *in vivo* molecular imaging is being driven by the development of chemical probes to make these targets 'light up' in MRI, PET or optical images, and by improvements in the techniques' resolution.

The resulting images promise new insights into areas such as embryonic development, infectious disease and gene therapy. They should also allow researchers to study the subtle influences that environmental factors exert on gene expression. "Imaging brings the genome to life," says Phelps, who helped pioneer the development of PET in the 1970s. "It's powerful because it puts all the pieces together."

That power has been demonstrated in the past few years by proof-of-concept papers showing that MRI, PET and optical techniques can non-invasively visualize where, when and, in some cases, at what level a gene is being expressed in living animals.

Juri Tjuvajev, Ron Blasberg and their colleagues at the Memorial Sloan-Kettering Cancer Center in New York began exploring methods for imaging gene expression almost eight years ago. One goal was to monitor the effectiveness of gene therapy. Determining whether the genes introduced during therapy are active in the target tissues is key to judging the treatment's success, and techniques that rely on taking multiple biopsies are clearly undesirable.

Tjuvajev and Blasberg turned to PET, which produces images by detecting the radiation given off by molecules 'tagged' with radioactive isotopes. In 1998, their team published the first report using PET to monitor gene expression in rats[1]. The gene that they studied, *HSV1-tk*, comes from the herpes simplex virus 1. It produces an enzyme that converts the drug gancyclovir from an inactive form into a form that kills cells. In what is dubbed 'suicide gene therapy', researchers hope to combat cancer by selectively introducing the gene into tumour cells and then administering the drug.

The enzyme produced by *HSV1-tk* adds phosphate groups to thymidine — one of the four chemical 'bases' in the genetic code — and related molecules. The addition of phosphate to a thymidine analogue called FIAU

UCLA'S JONSSON CANCER CENTER

Clear-sighted: Harvey Herschman (left) and Sanjiv Gambhir have teamed up with Michael Phelps (opposite) to develop PET technology to visualize gene activity in laboratory animals.

converts it into a form that gets trapped inside cells. So by injecting their rats with radioactively labelled FIAU, Tjuvajev and Blasberg were able to use PET to show where, and at what level, the introduced *HSV1-tk* gene was being expressed.

A year later, a team in Herschman's lab led by Sanjiv Gambhir repeated Tjuvajev and Blasberg's success in mice[2]. Rather than using a standard clinical PET machine, Gambhir and his colleagues took advantage of a scanner with higher resolution designed by UCLA colleagues led by Simon Cherry specifically for imaging small animals[3]. This device, called the microPET, is now being manufactured by Concorde Microsystems, a company in Knoxville, Tennessee.

Into the clinic

PET imaging of *HSV1-tk* expression is now being used in a clinical gene-therapy trial. Wolf-Dieter Heiss and Andreas Jacobs of the Max Planck Institute for Neurological Research in Cologne and the University of Cologne have administered the *HSV1-tk* gene, packaged inside tiny balls of lipid, into recurring malignant brain tumours in six patients. They are using PET to monitor the gene's incorporation into tumour cells.

The Sloan-Kettering and UCLA groups have also used the *HSV1-tk* system to monitor the expression of a second added gene, which should allow the gene to be used as a general marker for the success of gene therapy. By linking both genes to the same regulatory DNA sequence, they get expressed in unison. In 1999, Tjuvajev and Blasberg demonstrated the principle[4] using single-photon emission computed tomography, a technique related to PET that uses different types of radioactive probes. The following year, the UCLA researchers reported similar success using PET[5].

More recently, both groups have adapted the system to study the expression of the body's own 'endogenous' genes, rather than those added in gene-therapy experiments. The activity of a gene can be studied indirectly by taking the regulatory sequence of DNA that controls its activity and linking it to a marker gene that produces an easily detected signal. By creating transgenic mice in which *HSV1-tk* is linked to the regulatory sequence for the gene encoding the protein albumin, the UCLA team has shown that the system can monitor endogenous gene expression[6]. The Sloan-Kettering researchers, meanwhile, have created mice bearing tumours in which *HSV1-tk* is linked to a regulatory sequence that is controlled by the p53 protein, which suppresses tumour growth. Because p53 turns on a series of other genes, the resulting images can be used to monitor their expression[7].

MRI, which has a higher resolution than PET, can also be adapted to monitor the behaviour of genes and proteins. The technique measures changes in the magnetization of hydrogen protons in water molecules sitting in a magnetic field after they have been hit with a pulse of radio frequencies. Protons from different tissues react differently, giving a picture of anatomical structures. These images can be enhanced by adding 'contrast agents', which sharpen the contrast by affecting the behaviour of protons in their proximity. In standard clinical MRI scans, these agents travel through the bloodstream and tissues, heightening contrast wherever they go. To image cellular activities, researchers are tailoring the agents so that they are only 'turned on' in the presence of a specific molecule.

Two teams, one led by Ralph Weissleder at the Massachusetts General Hospital in Boston, the other by Thomas Meade of the California Institute of Technology in Pasadena, are making rapid progress.

Contrast control

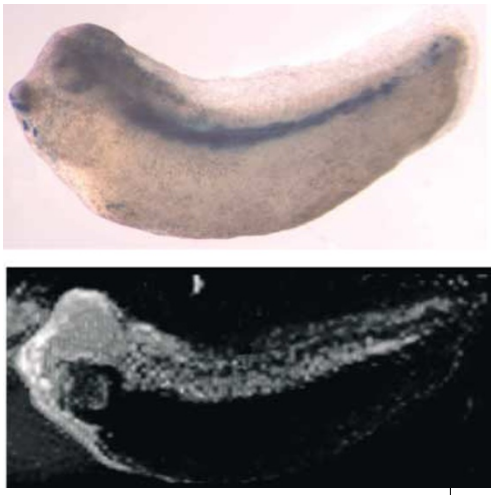
Weissleder and his colleagues have spotted the expression of a receptor gene engineered into tumour cells that were then implanted into mice. They took nanoparticles of iron oxide and chemically linked them with the protein that binds to the receptor so that only clumps of cells with the receptor on their surfaces lit up in the resulting images[8]. Contrast agents could be targeted to other cell-surface proteins in the same way, Weissleder suggests.

Meade's team lit up gene activity in frog embryos by altering another MRI contrast agent — a gadolinium ion inside a chemical scaffold — so that it was activated only by the product of the gene in question, an enzyme called β-galactosidase. By attaching sugar molecules to the contrast agent, the researchers switched off the molecule's ability to interact with nearby protons. But the β-galactosidase enzyme removed the sugar groups, turning up the contrast wherever the gene was active.

To demonstrate the technique, the researchers injected the gene for β-galactosidase, known as *lacZ*, into one of two cells in newly fertilized embryos of *Xenopus laevis*, the African clawed frog, that had undergone one cell division. Both cells were injected with the contrast agent. As the embryo grew, the side of the animal that had developed from the *lacZ*-injected cell glowed in the MRI images[9]. Scott Fraser, a developmental biologist on the team, believes that MRI will allow researchers to watch cells move, divide and specialize as an embryo grows. "Seeing where and when molecular events take place is critical in developing systems," he says.

The *lacZ* gene is often added as a 'marker' together with the gene of interest in transgenic experiments, as β-galactosidase produces a blue precipitate when it acts on a molecule called X-gal. Meade's technique should allow its role as a marker to extend to watching the incorporation of genes deep within a living animal's tissues. More generally, the Caltech team is working to develop and refine contrast agents that are activated by other enzymes, such as those produced by tumours, and by calcium ions, which act as messengers in many important cell-signalling pathways[10].

MRI and PET require expensive equipment that is beyond the reach of many labs. But in some cases, it is possible to track gene activity in lab animals using optical tech-



Magic marker: in these frog embryos, *lacZ* gives a blue colour (top) and a clear MRI signal.

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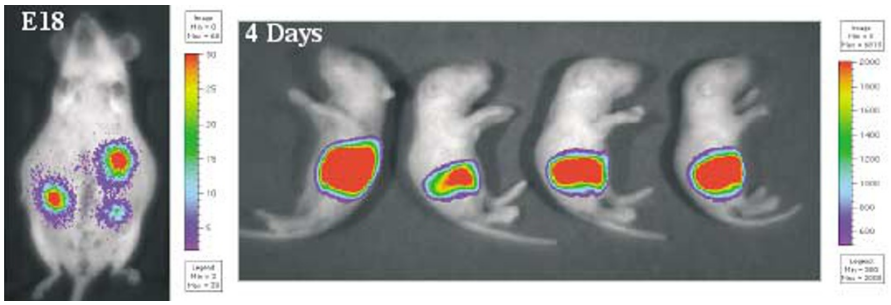
Pioneer: Ron Blasberg's team produced the first PET images of gene expression in live rats.

FROM REF. 13



In the family way: optical imaging can monitor gene expression in mice before (left) and after birth.

niques. One approach depends on genes for enzymes called luciferases, found in fireflies and certain bacteria, which generate light by oxidizing a molecule called luciferin. If this glow is sufficiently bright, light produced deep within an animal following the administration of luciferin can be detected by sensitive charged-couple-device (CCD) cameras.

Caught on camera

Researchers at Stanford University in California, led by Christopher Contag, his wife Pamela and David Benaron, have pioneered this approach, having first turned to optical imaging while trying to monitor the movement of bacteria and viruses in animals. They were frustrated with traditional techniques of analysing biopsies for the presence of pathogens, which were slow and cumbersome. "We had questions and the only way to answer them was to build new technology," says Christopher Contag.

The researchers built a prototype CCD-based camera system in 1994 and began using it to study mice infected with bacterial pathogens that had been engineered to carry the luciferase gene[11]. In 1997, Pamela Contag left Stanford to form Xenogen, a company that has further developed the technology.

The Stanford team has shown that the system could also be used to monitor the expression of endogenous genes indirectly. Using transgenic mice carrying a luciferase gene linked to a regulatory sequence that is activated by the chemical dimethyl sulphide, the researchers confirmed that luciferase can be used as a marker to study gene expression deep within a mouse's body[12]. Having proved the principle, it should be possible to study endogenous gene expression using marker-gene strategies similar to those used by groups working with PET.

The Stanford researchers have also joined forces with Karin Gaensler of the University of California, San Francisco, to use luciferase in tests of a viral vector being developed for gene therapy on fetuses. Gaensler put a luciferase gene into the virus, and injected it into mouse fetuses *in utero*. For months after the pups were born, CCD cameras detected luciferase activity in their tissues[13].

In Boston, meanwhile, Weissleder has developed another optical-imaging technology to study tumour biology. Near-infrared fluorescence uses longer wavelengths, as these can penetrate through greater depths of tissue. Weissleder and his team have designed molecular probes that fluoresce at near-infrared wavelengths only in the presence of specific protein-digesting enzymes produced at high levels by tumours. These enzymes are thought to be involved in tumour growth and spread, plus the formation of the blood vessels that supply growing tumours with nutrients and oxygen.

The researchers linked fragments of proteins that would be digested by the tumour enzymes to the fluorescent molecules in a way that turned off their near-infrared glow. They then injected these probes into mice suffering from cancer. The tumour enzymes got to work on the protein fragments, releasing the fluorescent molecules and lighting up the tumours[14].

More recently, Weissleder's team injected a probe activated by matrix metalloproteinase-2 (MMP-2), a key protein-digesting tumour enzyme, into mice with tumours and showed that the near-infrared fluorescence signal decreased after an inhibitor of the enzyme was administered[15]. MMP-2 inhibitors are being investigated as cancer drugs, and Weissleder suggests that near-infrared imaging could provide feedback about their efficacy.

Optical techniques have so far been limited to producing two-dimensional images, rather than the three-dimensional reconstructions that are possible with MRI or PET. But Weissleder is now working on computer techniques to generate three-dimensional near-infrared images[16].

As molecular imaging techniques continue to advance, other researchers are becoming excited about the field's potential. Stem-cell biologists, for instance, are intrigued by the possibility of watching stem cells migrate to repair damaged tissues, whereas immunologists are tagging immune cells and watching them move inside lab animals.

Probing ahead

One major factor determining the field's progress is the development of new probes to home in on particular molecular targets. An important goal is to design probes that home in on the messenger RNA and proteins produced by particular genes, to allow the expression of endogenous genes to be studied directly. Other probes in the works include luciferases that give off different wavelengths of light, which could be used in combination to study the simultaneous expression of different genes; MRI contrast agents that can readily penetrate cell membranes; and PET probes that target specific tumour proteins.

As new probes emerge, enthusiasts such as Herschman are keen to get them into the hands of as many researchers as possible. He hopes to open the doors to his imaging centre to other scientists at UCLA and elsewhere. "They can start thinking about doing experiments in animals that they couldn't do before," he says. ■

Corie Lok has just completed an internship with *Nature*.

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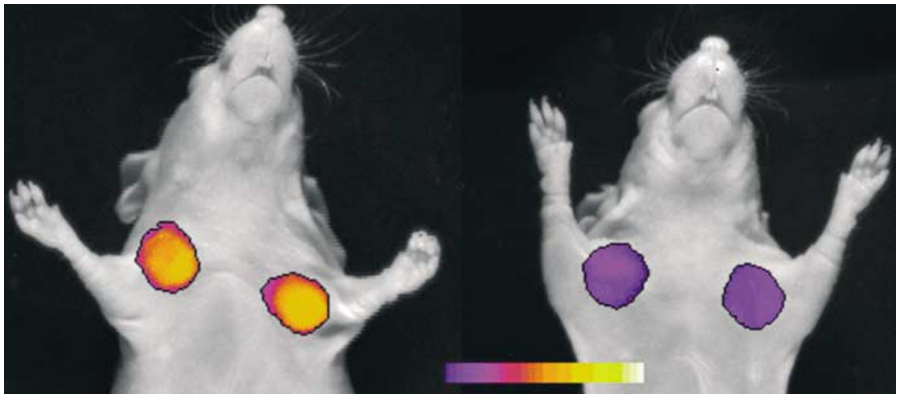

The drugs do work: near-infrared fluorescence shows the effects of a cancer drug (treated, right).