

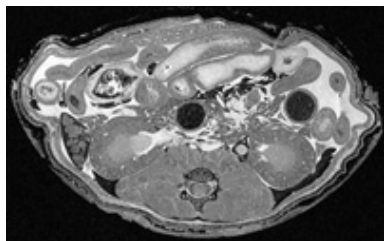
## Characterizing Targets in Model Organisms Drug Discovery & Development - November 08, 2005

Getting useful data from model organisms is often laborious, but new strategies and technologies are helping.

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On the long road from finding a new molecule in a cell to putting a pill in a bottle, one of the main obstacles is validating the target in model organisms. In essence, this



***A high-resolution magnetic resonance imaging (MRI) slice of a mouse showing internal structures for detailed phenotypic mapping. Part of the Visible Mouse Project, the image's resolution is more than 10,000 times sharper than that of a routine clinical MRI scan. (Source: Allan Johnson, PhD, Duke University)***

consists of inactivating or stimulating the target in the cells of a live animal and seeing whether that produces the desired effect. But in drug development, as in marksmanship, seeing a target is the easy part—hitting it is hard.

Like so much of modern drug development, the concept only sounds simple. Live animals are far more difficult to control and study than individual cells, and even the most straightforward questions about a target can be maddeningly difficult to answer. Using a range of intriguing new technologies, though, several research groups are starting to address some of the field's biggest challenges.

Phenotypes are the central issue in target validation: What is the model organism's normal biology and how does inactivating the target change that? Unfortunately, although molecular biology revolutionized the way researchers study genotypes, phenotyping remains a vague science where even seemingly simple questions remain unanswered. Compounding the problem, advances in medical imaging have enabled physicians to diagnose and study human diseases in patients in unprecedented detail, creating a demand for drugs to treat conditions that were previously not diagnosable. The technology for imaging model organisms, however, has lagged far behind. Identifying an early-stage tumor in a patient is important, but developing a new drug to treat it requires seeing it in a mouse.

The simplest approach to the problem involves applying clinical imaging techniques to model organisms, and indeed many researchers have done just that, especially with magnetic resonance imaging (MRI). "People do it routinely. They take their clinical MRI machine and they put a mouse in there and then they try to make an image," says Allan Johnson, PhD, director of the Center for In Vivo Microscopy at Duke University Medical Center, Durham, N.C.

But producing an image is a far cry from obtaining useful data. Just as digital cameras record fixed numbers of pixels, MRI machines record fixed numbers of voxels, so scanning an animal thousands of times smaller than a human yields an image with thousands of times less resolution. In addition, the metabolism of a mouse is an order of magnitude faster than that of a human. "So if you want to look at a mouse heart, you've got to image it 10 times faster than if you were looking at a human heart, yet your signal is 3,000 times weaker," says Johnson. Using conventional MRI, the result is like a movie downloaded over a telephone modem. The technique can reveal gross abnormalities from one animal to the next, but modern target validation demands more detail.

To address this, Johnson and his colleagues have steadily increased the bandwidth of the available imaging systems. For MRI, this means combining stronger magnetic fields, more sensitive receiver circuits, and more sophisticated image analysis software. Each technical adaptation boosts the resolution a bit, while meticulous anesthesia techniques keep the mouse still enough to scan. "You don't get that factor of 3,000-[fold improvement] by some magical little trick. You earn it," says Johnson, who adds that "each phenotype is its own challenge."

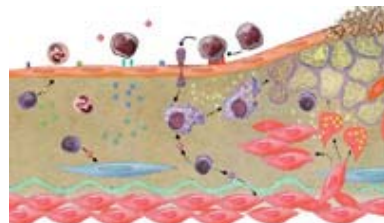
Besides imaging live animals, the new techniques can also be used on dead ones, a strategy that is especially useful for characterizing structural phenotypes like atherosclerosis or tumor size. Scanning a dead animal provides a reasonably detailed three-dimensional map of the internal structures, which can then be sectioned and studied microscopically for even greater resolution. Combining the new tools may let drug developers study mice even more thoroughly than doctors now study patients.

Other groups are also working on high-resolution phenotyping, especially for mice. Major mouse breeding facilities are trying to organize the flood of new data. Although phenotypic information is still far less abundant than genotypic data, phenotypes based on images are much harder to search and analyze than gene sequences, so the field may soon be facing a bioinformatics bottleneck.

### **A clear advantage**

Seeing tiny changes inside a mouse is challenging, but in some other model organisms the effects of inhibiting a target molecule are literally clearer. The

worm *Caenorhabditis elegans*



click the image to enlarge

***An artist's rendition of the process of atherosclerotic plaque development showing the complex interactions of multiple cell types. Medically important phenomena like this are often difficult to replicate in model organisms. (Source: Bioseek Inc.)***

is the undisputed champion of biological transparency, providing researchers with a detailed view of its innards throughout its life, along with predictable genetics and easy culturing. Because many of the key signaling pathways in human cells have homologs in worms, many drug developers already use this model for initial target validation.

There is a lot of evolutionary distance between worms and mice, though, so to test targets in a system with intermediate complexity, some companies are turning to the zebrafish (*Danio rerio*). The adult fish are opaque, but their eggs and embryos are conveniently transparent, and some biotechnology companies have been working hard to adapt this model for drug discovery. Researchers at Phylorix, Cambridge, Mass., and Zygogen LLC, Atlanta, developed extensive sets of drug activity and toxicological assays in the tiny fish. Both companies use fluorescent labels and visual screens to score phenotypes, and the organism's transparency allows screening for everything from liver toxicity to angiogenesis inhibition. Conveniently, the eggs and embryos are small enough to fit in the wells of 96-well plates.

For even easier processing of fish eggs or any other model system smaller than about a millimeter in diameter, companies are increasingly turning to large-particle flow cytometry, a technique being pioneered by researchers at Union Biometra Inc., Holliston, Mass. Like an ordinary flow cytometer, the system rapidly sorts particles from a mixed solution into individual wells while providing quick measurements of each specimen's fluorescence or light-scattering characteristics.

Union Biometra's system permits these analyses on samples that would clog an ordinary flow cytometer. Though it was initially applied to *C. elegans*, the large-particle sorter has now been adapted to handle everything from small plant seeds to whole fruit fly and fish embryos. "We've seen a number of different groups that are using *C. elegans*, we've seen quite a few using *Drosophila*, and more recently . . . zebrafish. I think there's a kind of popularity right now for using zebrafish for toxicology studies," says Rock Pulak, director of biology at Union Biometra. The system might also be useful for sorting clusters of human embryonic stem cells, embryoid bodies, and complex primary cell cultures. High-throughput systems based on these types of cellular aggregations could become especially useful as drug developers try to bridge the gap between nonhuman model organisms and humans.

Researchers usually turn to automated techniques simply to accelerate their current research, Pulak says that increasing the throughput of an animal assay can also enable entirely new types of studies. For example, many mutant animal strains develop particular disease phenotypes, but the genes that contribute to the disease remain unknown. With a high-throughput animal-handling system, a researcher could screen an entire library of inhibitory RNA molecules against the animal's expressed genes to find the ones that ameliorate the phenotype. "Any RNA interference [RNAi] that would come up in such a screen is almost immediately identifying a [drug] target," says Pulak.

#### **RNAi precision**

Of course, Pulak is not the first to suggest using RNAi technology to accelerate drug discovery. Indeed, the very existence of RNAi and its ability to do researchers'

#### **Virtual Biology Monitors Regulation**

There are seldom good explanations for compounds that look wonderful in animal tests but turn out to be costly failures in humans, especially since most of our understanding of drug metabolism comes from the same animal systems that failed to predict the problem. Because clinical trials are so expensive, the gap between humans and model organisms remains one of the greatest risks in drug development. "When you start comparing the biology of animals and people, certainly while core pathways and mechanisms might be conserved . . . what turns out to be critically different is the regulation," says Ellen Berg, PhD, chief scientific officer of Bioseek Inc., Burlingame, Calif. To address that problem, Bioseek is turning humans into model organisms—virtually.

With complex mixtures of primary human cells from blood and tissue samples, Berg and her colleagues are creating miniature versions of specific biological environments. One system incorporates endothelial cells, B cells, T cells, monocytes, and other components of a blood vessel. The culture responds appropriately to changes, such as the addition of inflammatory and anti-inflammatory signals.

By combining the primary cell cultures with a sophisticated gene expression profiling algorithm, the company can map the responses that human cells exhibit to different drugs. Comparing known and novel drugs in multiple systems produces an impressively accurate two-dimensional diagram of relationships; drugs that are near each other in the diagram tend to have very similar effects and side effects in real humans. There are shortcomings, however: "What you do miss is certainly the whole physiology, the fact that organ systems are talking to one another."

bidding still strikes many biologists as an uncommonly generous gift from nature. Though RNA researchers are still teasing out the mechanistic details (see "Opening a Portal: The Strange New World of MicroRNA," *Genomics & Proteomics*, June 2005, page 14), stopping the expression of individual proteins with RNAi already forms the backbone of many target discovery and validation efforts.

The precision of RNAi-based techniques is impressive, but there are still drawbacks. One limitation is that complex organisms have complex physiology, and the dynamics of artificial RNAi metabolism are still murky. Even if the inhibitors can be stabilized and delivered efficiently to model animals, there are some tissues, especially in the central nervous system, that remain difficult for any noninvasive technique to penetrate.

Because many of the most promising drug targets are proteins the RNAs code for, RNAi and other techniques to stop gene expression, such as conditional gene deletion, are also one step removed from the real target. What target validation really needs is a way to selectively inactivate proteins in intact tissues. By cleverly exploiting physical chemistry, Kimberly Hamad-Schifferli, PhD, and her colleagues at the Massachusetts Institute of Technology (MIT), Cambridge, Mass., are trying to develop just such a tool.

The technique entails attaching iron or iron alloy particles a few nanometers in size to the biomolecule of interest. "Then we just set it in a magnetic field [which] will heat the nanoparticle," says Hamad-Schifferli, an assistant professor of mechanical engineering. The hot nanoparticle heats the protein, denaturing and inactivating it, while other proteins that are not attached to nanoparticles remain unaffected. Having tested the approach on purified proteins, the investigators are now hoping to apply it to intact cells and eventually to tissues.

Remote-control switches for natural proteins would be wonderful, but a more likely application in drug development would be to attach the nanoparticles to artificial molecules that bind particular targets. Once the drug is bound to its target in a model system, either the drug or the target might then be activated or inactivated at will. The idea is elegant, but the implementation will take a lot of work, a situation that remains painfully familiar to drug developers. No matter what model organism a researcher uses, progressing from a promising target to a worthwhile drug is still an arduous process. With a few new tools and a bit of luck, though, drug developers may eventually be able to hit the bull's-eye.

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