MOLECULAR BIOLOGY

Industrial-Style Screening Meets Academic Biology

A $100-million-a-year-effort to find chemicals for exploring cellular processes and drug discovery is about to move into production; skeptics say it is struggling to meet its goals

Parasitologist David Williams has spent his career studying *Schistosoma*, a type of snail-borne worm that kills 280,000 people a year in the tropics and leaves millions more with chronic liver and intestinal problems. By 2005, he had found a possible target for a drug—an enzyme the parasite requires for survival. But he had no easy way to find a molecule that would block it. Then he learned that the U.S. National Institutes of Health (NIH) was inviting researchers to submit material to be tested against a huge number of chemicals to find "hits," or biological interactions. Williams applied, was accepted, and last April, he and collaborators published the results in *Nature Medicine*: After screening 71,000 compounds, they found one, Compound 9, that inhibits the enzyme and killed at least 90% of the worms in schistosome-infected mice.

Williams is now seeking funds to develop it as a drug. "It would be pretty exciting if we could get something that would be effective for schistosomiasis," a disease whose devastation he first witnessed as a Peace Corps volunteer in Ghana, he says. The worm is beginning to show resistance to the existing drug, and a better drug is needed.

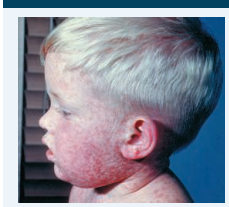
The schistosomiasis story has been touted as one of the first successes of a costly, controversial NIH program announced 5 years ago called the Molecular Libraries Initiative (MLI). It aims to bring so-called high-throughput screening, once reserved for big pharmaceutical companies, to academic scientists. Its specific goals are to develop probes for exploring cell function—small molecules that bind to protein targets—and to help find treatments for diseases that don't interest big pharma. NIH says the program, now ending a 5-year, $385 million pilot stage, has begun to pay off. Ten screening centers have produced more than 60 research probes, including a few potential drug leads. This month, NIH will move into full-scale production with grants to three large centers.

The libraries project also has a side benefit, proponents say: It has spurred scores of universities to set up their own small-molecule screening facilities (see sidebar, p. 766). "Virtually every major medical school in the country" is jumping aboard in some way, says pharmacologist Bryan Roth of the University of North Carolina (UNC), Chapel Hill.

Yet even boosters of MLI acknowledge that this more than $100-million-per-year program is still an experiment—and still struggling. The screening centers took longer than expected to set up, and some were more successful than others. MLI leaders have had trouble defining certain goals, such as how strongly a compound must bind to its target to work well as a probe. NIH's original plan for sharing results has also faltered.

As the program expands, the research community remains deeply divided about it. Believers say it is generating a valuable trove of shared data and bringing rigor to the hunt for new medicines and biochemical probes. The skeptics, including several prominent drug industry leaders, aren't convinced this is a wise use of NIH's tight budget. Some worry that it may be too diffuse. It may be "a worthwhile thing to do," says Steven Paul, executive vice president for science and technology at Eli Lilly and Co. in Indianapolis, Indiana. But he asks: "Is it realistic, and is it cost effective? How potent and selective are these probes?" The answers may not become clear, some say, until nearly a billion dollars has been spent.

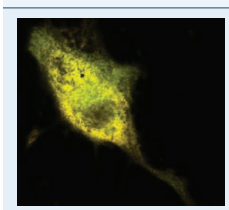
EARLY SCREENING DISCOVERIES



MEASLES
A compound that inhibits a polymerase used by the virus.

SCHISTOSOMIASIS
A chemical that killed 90% of worms in mice infected with this disease.

ANGIOGENESIS
A new inhibitor of blood-vessel formation found by screening zebrafish embryos.



GAUCHER DISEASE
Compounds that restore the ability of mutant glucocerebrosidase to process lipids in patients' cells.

Promise. NIH's molecular screening program has produced research probes and potential drug leads for several rare or neglected diseases.

Networking

Inside a nondescript building off a busy road in Rockville, Maryland's, biotech corridor, neurogeneticist Christopher Austin presides over the NIH Chemical Genomics Center (NCGC)—a 50–staff member intramural version of the 3-year pilot screening centers NIH funded at nine external sites. At its heart is a quiet room in which three state-of-the-art yellow robots are hard at work processing biological assays. They fetch plates that are each dotted with 1536 tiny wells of different small organic molecules, mix in a protein or cell solution, then run the plate through a detector that spots whether any of the chemicals on the plates has triggered some change in the protein or cells. In another room, medicinal chemists tweak these "hits" to improve the strength and specificity of the interaction.

Although drug companies have long relied on such high-throughput screening, "this is not a world that most academic [biologists] have been in," says Austin, a former Merck researcher who says he often feels like a John the Baptist, bringing small-molecule screening to academia. The time is right for this evangelism, say Austin and other NIH officials. The explosion in genomics launched by the Human Genome Project has revealed a wealth of proteins whose functions are unknown. Some are involved in disease processes. Advances in robotics have brought down costs, making it feasible for university labs to screen a protein against hundreds of thousands of compounds, looking for one that interacts with it. That compound could then be developed into a probe that researchers would use to disrupt a protein's action or explore a cell pathway. Some, such as the schistosomiasis project, might also generate new drug leads for a tiny fraction of the overall cost of drug development (see timeline).

CREDITS (TOP TO BOTTOM): CDC; DAVID BYRES, FCC-J; RAY DINGLEDINE; O. GOKER-ALPAN/NHGRI

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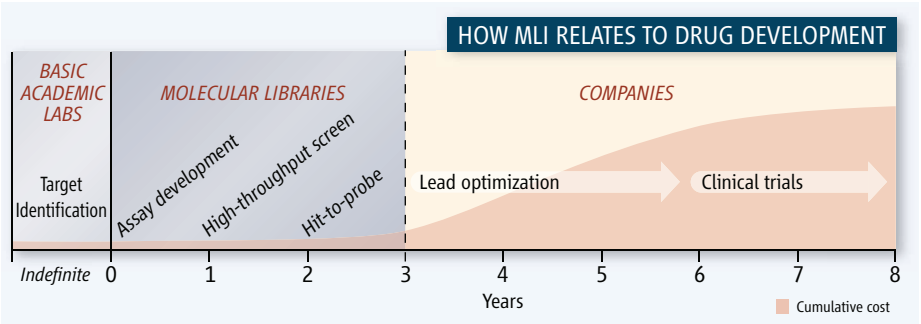

Filling a gap. NIH says that research probes developed through its Molecular Libraries Initiative could help fill the pipeline of potential drug leads, boosting research in early stages when costs are low.

In 2004, leaders described their plan to set up a huge central repository of 500,000 compounds that all centers would use for such screening (*Science*, 12 November 2004, p. 1138). They said that any biologist could propose screening a candidate protein, cell-based test, or even a novel assay based on a whole organism. The assay would then be peer reviewed and, if accepted, assigned to a screening center. Compounds that bind to the protein or modulate cell activity would be chemically modified until potent enough to work in a test tube but not necessarily in animals. The resulting probes would be "made available without encumbrance to all researchers," that is, without intellectual-property restrictions, Austin and other NIH leaders wrote.

MLI debuted in 2003 as the largest piece of NIH's Roadmap, a set of cross-institute initiatives. Some researchers argued that such top-down projects siphon funds from investigator-initiated science. But NIH Director Elias Zerhouni described it as a boost for basic research in his 2004 budget request to Congress, saying it would "help accelerate researchers' ability to prove the function of the complex biological circuits … in normal function and disease."

The start-up was slow. Equipping 10 academic centers to screen molecules entailed "a huge learning curve," acknowledges Carson Loomis, MLI program co-director. Initially, NIH hoped the scale-up would be similar to creating the first genome sequencing centers, he says. But high-throughput screening is not as straightforward. Centers wrestled with balky robotics equipment and chemicals that degraded. They soon realized that most of the biological assays would require many modifications to work properly when screened. They also faced the challenge of merging two cultures—biologists and chemists—and getting them to work together on a product, not hypothesis-driven research. "That interface is not a smooth one automatically," says Ray Dingledine, director of the center at Emory University in Atlanta, Georgia, and chair of the screening network.

Another challenge has been creating the small-molecule repository itself. NIH deliberately chose a wider range of chemicals than would be standard in the drug industry to make sure nothing was overlooked. But many proved "worthless" in the screens, and the ones that panned out turned out to be pretty similar to what industry would have chosen, says Christopher Lipinski, a former Pfizer chemist renowned for his skill in predicting what works as an oral drug. NIH's Linda Brady, who helped launch MLI, says the repository is growing and has improved—"I haven't heard [the term] 'junk' in a long time," she says.

One continuing debate centers on how to define an acceptable "research probe." NIH wanted the probes to be potent and selective enough to work in vitro—but no more developed than that—so that MLI participants would feel comfortable sharing raw data and forgoing patents. "There's lots of debate about where that bar ought to be," says medicinal chemist R. Kiplin Guy of St. Jude Children's Research Hospital in Memphis, Tennessee. NIH ended up loosening its original cutoffs for potency and selectivity; now it's largely up to the center to decide when a probe is complete. That has resulted in variable quality and made some centers appear more productive than others, says one center director.

On a mission. Christopher Austin, leader of NIH's screening center, hopes academics will discover the value of small molecules.

Despite the bumps, the 10-center pilot network has screened nearly 200 biological assays (far short of the projected 400) and produced 62 probes. Among these are the schistosomiasis compound; a potential drug lead for treating Gaucher disease, a rare metabolic disorder; a molecule for exploring potassium channel receptors; and probes that have shed light on the function of a new estrogen receptor. "Every center has produced at least a couple of interesting compounds," says Brady, although three—the intramural NCGC (which began a year earlier), the Scripps Research Institute's branch in Florida, and the Burnham Institute for Medical Research in San Diego, California—have produced the majority.

Missing bridges

NIH's plan for informing the broader community about these probes hasn't worked as well, however. MLI screeners must deposit screening results in PubChem, a database created as part of MLI. But these raw data reports aren't easy to use and often contain mistakes because the data aren't curated, Lipinksi says. NIH initially asked centers to post online "probe reports," Loomis says, but took them down when journal editors complained that they were too similar to submitted papers. NIH plans to require centers to post reports after a 6-month delay.

In the meantime, at *Science*'s request, NIH produced its first-ever table of completed probes. Both the total number and details of this list drew a lukewarm response from two industry experts. Some of them look "very good," says Stephen Frye, a medicinal chemist who left GlaxoSmithKline (GSK) last year for UNC, such as a measles virus inhibitor and probes for studying SP1 receptors, which are involved in sepsis. Others, however, are not very potent, he noted. Alan Palkowitz, head of medicinal chemistry at Eli Lilly, says that, based on their structures, he believes up to

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Universities Join the Screening Bandwagon

Once shunned as too costly and industrial, high-throughput screening is becoming a hot activity at universities. An international directory put together by the Society for Biomolecular Sciences lists 55 academic molecular screening centers—some large, some small—often paid for by a university's own budget as part of a drug-discovery program.

Unlike the screening centers funded by the U.S. National Institutes of Health (NIH) (see main text), many of these facilities lack chemists to do the tweaking required to verify a "hit"—an interaction between a chemical and a protein target—and improve the strength and specificity of the interaction. Only a few schools even have a medicinal chemistry department, says Christopher Lipinski, a retired Pfizer chemist.

Some observers say this weakness shows up in talks and papers from the new screening programs. There's a "blind spot" in academia, says Edward Spack of SRI International in Menlo Park, California: "They'll get a hit, but then many can't optimize it." Ross Stein estimates that more than 10% of the hits he sees reported in journals are false positives. "There's a lot of junk in the literature," says Stein, director of drug discovery at the Harvard NeuroDiscovery Center.

Even if academics come up with a potential therapeutic molecule, a big unknown is who will take it forward. With pharma laying off employees, and venture capital for biotechs drying up, a drug lead may have to get through preclinical animal studies before a company will pick it up, says Stein. At Merck, "a whole building of people" worked on that, says neurogeneticist Christopher Austin, a former Merck staffer who heads NIH's intramural screening center. Universities have no equivalent.

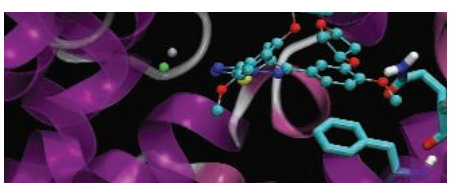
But would-be drug developers in academia note that, as part of a new push for translational research, NIH, the Wellcome Trust in the U.K., and other foundations are giving investigators money to contract out steps such as animal testing and medicinal chemistry. "If the target is important and the molecule is important, we will find a way to move it along," says molecular pharmacologist David Scheinberg of Memorial Sloan-Kettering Cancer Center in New York City.

Despite the gaps, small-molecule screening in academia is here to stay, say supporters of the approach. But there will be a shakeout. "People will either learn and get better, or they will not survive," says pharmacologist P. Jeffrey Conn of Vanderbilt University in Nashville, Tennessee. **–J.K.**

one-third of the probes might reflect spurious activity in the screens or be problematic for other reasons. He sees mostly "potential starting points" for useful probes. At the same time, both praised the list of 200-some submitted assays as including some innovative contributions such as zebrafish and tests of signaling pathways.

Arguments about quality aside, the true test of MLI will be if the broader community orders probes and starts publishing papers using them, notes Paul of Eli Lilly. However, that test may not come soon. Researchers may not have ready access to the compounds, which are often not available off the shelf. NIH is relying on center investigators to provide small amounts to the community but is not yet tracking requests in a systematic way, says Loomis. He adds, however, that a growing number of citations suggests that some probes are being used widely.

Some industry leaders question whether this massive effort is worth the time and money. If the goal is to study gene function, there are easier ways, says Peter Kim, president of Merck Research Laboratories, such as using RNAi to block gene expression and monoclonal antibodies to inhibit proteins. Small molecules are best for testing in vivo hypotheses that can lead to potential therapies, he and others say. For this, the probes usually need to be optimized to function in animals. But MLI doesn't plan to fund in vivo studies. And, says Peter Schultz of Scripps in San Diego, if academics try to do it on their own, they may face the need for the extensive medicinal chemistry and pharmacology of drug discovery. "I don't want to say the community has been swindled, but [creating selective in vivo agents is] a lot harder than it appears," says Schultz, who also oversees drug discovery as head of the Genomics Institute of the Novartis Research Foundation. (He is not involved with the Florida screening center.)



Molecular Libraries by the Numbers

Cost to date	$385 million
Pilot screening centers	10
Compound collection	~300,000
Accepted assays	268
Assays screened	191
Probes	62

Note: Data are for FY 2004–2008.

First fruits. About $385 million spent for pilot screening centers, a compound repository, a database, and technology has yielded 62 molecular probes.

MLI's leaders are used to defending against such criticism. They say small molecules are uniquely useful because they modulate the target protein directly, rather than through its gene, and can have subtle effects. "It's critical to have tools that act at the level that Mother Nature does," says Austin.

Growing investment

Despite the skepticism, reviewers who examined MLI in early 2006 concluded that it showed enough promise to continue. A project this ambitious may need 10 years to prove itself, says chemist Catherine Peishoff of GSK. "To say it's a success or failure would be unfair at this point," she says.

This month, NIH will move into what it calls "full-scale production" by funding three "comprehensive" centers for up to 6 years that will each screen 25 assays a year and have larger staffs of chemists to improve the hits. (NIH also plans to work with chemical vendors to make the probes available.) The top contenders for full-scale awards appear to be the intramural center; Scripps of Florida; Burnham; and the Broad Institute at Harvard University, which until now has had separate NIH funding for high-throughput screening. A handful of smaller centers will work on specialized screens or chemistry.

It may be expensive and risky, but MLI is important because many drug companies are abandoning high-throughput screening and shedding chemists, argues Frye, whose division at GSK was dissolved in 2007. "If the NIH doesn't pull this off, I think it's a big step backwards for drug discovery," he says.

Guy says its value will become clear over time: "It's true that people are relearning a lot of lessons," but now the data will be formally tested and widely shared. Guy says that, like the Human Genome Project, the results will be a vast expansion in public knowledge about biological systems, including targets that companies wouldn't touch before.

–JOCELYN KAISER

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