NEWS

'Breeding' Antigens for New Vaccines

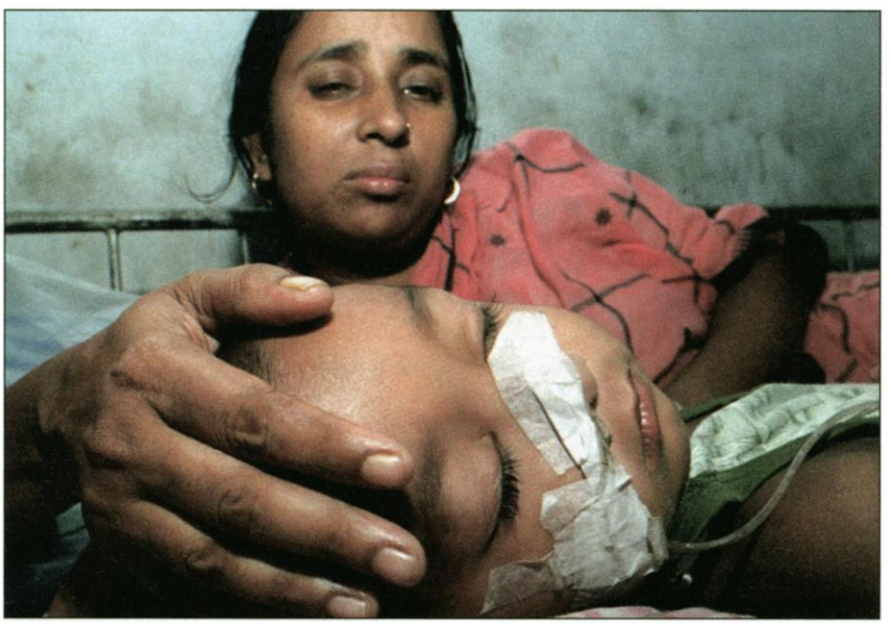
Backed by DARPA funding, a biotech company in California is hunting for better reagents with a technique known as directed molecular evolution

REDWOOD CITY, CALIFORNIA—Russell Howard is exploiting the wonders of sex to develop vaccines. For 10,000 years, explains Howard, who heads a biotechnology company called Maxygen, humans have used breeding techniques to make crops and animals with specific traits. Now Maxygen is "mating" viral genes to achieve similar ends, fashioning vaccines designed to combat AIDS, dengue, hepatitis B, and other diseases. And, Howard likes to brag, this strategy differs markedly from the standard biotech approach: Rather than carefully manipulating genes to develop a product with specific characteristics, Maxygen hunts for chance offspring that have the desired features.

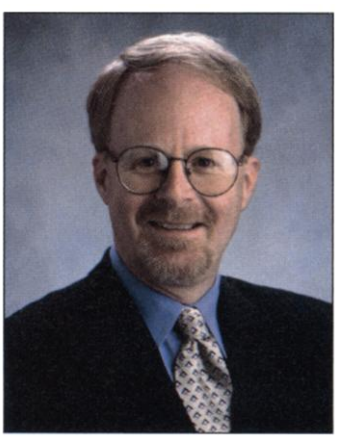
Maxygen is one of a handful of biotechs focusing on "directed molecular evolution." This approach mimics natural selection, but on a minuscule scale and with a focused purpose. These companies use a variety of techniques to modify genes, which they assemble into large libraries. They then screen the libraries for genes that produce proteins with a particular biological feature, select the best genes, modify them, and run through the process repeatedly until they get a result they like. "It's an amazingly productive way to go," says Lawrence Loeb, a researcher at the University of Washington, Seattle, who does directed evolution and consults with Maxygen.

Loeb emphasizes that nature would never select for many of the products that directed evolution allows you to create. "Nature is really limited," says Loeb, who has used directed evolution to make novel enzymes with potential medical and industrial applications. "A 'better' enzyme wouldn't help the cell."

Enzymes are a key focus of every directed evolution company, as are therapeutic proteins, antibodies, and small molecules for drugs. But only Maxygen—a company in the San Francisco Bay area that has rapidly grown in the last year (the number of employees doubled to 270)—so far has used the strategy to hunt for vaccines. Howard's rationale echoes Loeb's. The immune system evolved to respond to foreigners—which immunologists call antigens—but, he notes, "antigens were never evolved to elicit an immune response."



Complex threat. Dengue virus can be harmful if low levels of antibody are present. To reduce the risk of hemorrhagic fever, vaccinemakers attack four strains of the virus at once.



Gene shuffler. Maxygen CEO Russell Howard exploits mating strategy.

Maxygen's work is still in the early stages of test tube and animal testing. But if it succeeds, it could add a novel tool to the vaccinemaker's workbench. "It's a powerful technology, there's no doubt," says Gerald Joyce, a pioneering investigator in the directed molecular evolution field who works at the Scripps Research Institute in La Jolla, California. "They've really run with it in a very pragmatic way."

DNA shuffling

Maxygen evolved from the work of Willem "Pim" Stemmer, formerly a staff scientist at the Affymax Research Institute, who in 1993 discovered a new way to direct molecular evolution. Joyce, Loeb, and researchers in other labs were creating novel DNA or RNA with a version of this technique, introducing random mutations into genes by chemically damaging them or copying them in systems that introduce errors. Stemmer took a different tack, dubbed "DNA shuffling," which resembles the way genetic material recombines through sex.

As Stemmer first reported in *Nature* in 1994, the initial step in DNA shuffling is to isolate several slightly different genes that code for the same product. Enzymes chop the genes into random fragments, and the polymerase chain reaction assay (PCR) recombines fragments from various genes. Finally, recombined fragments are reassembled into unique, full-length genes (see sidebar). "Recombination is a very gentle way of increasing diversity," says Howard.

In 1997, Stemmer, Howard (then Affymax's scientific director), and Affymax founder Alejandro Zaffaroni spun off Maxygen as an independent company. Zaffaroni, a well-connected chemist and founder of innovative biotechs, quickly put together a star-studded scientific advisory board; it includes Nobel laureates Baruch Blumberg, Arthur Kornberg, and Joshua Lederberg. Maxygen split into different divisions to work on agriculture, chemicals, pharmaceuticals, and vaccines. The vaccine division, Maxyvax, has not yet presented much data publicly, but vaccine experts are watching it closely and it is getting support from an unusual sponsor of vaccine research: the Defense Advanced Research Projects Agency (DARPA), which has kicked in $20 million from its unconven-

CREDITS: (TOP TO BOTTOM) AFP/CORBIS; COURTESY OF MAXYGEN

tional pathogen countermeasures program.

Traditional vaccines stimulate the immune system by exposing it to whole disease-causing organisms that have been weakened or killed to render them harmless. But these concoctions have sometimes caused the diseases they aim to prevent. Modern vaccines, with the help of genetic engineering, attempt to avoid this problem by delivering only parts of the bug in question—the coat proteins, for example. Maxygen is trying to improve the potency of some of these modern vaccines.

For more than a decade, vaccine researchers have mimicked the live, weakened approach by using safe bacterial or viral "vectors" to shuttle genes from the disease-causing organisms into the body. One of the most popular vectors is naked DNA, a circular piece of bacterial DNA that slips inside target cells. Yet it has its own limitation: In the body, it often produces low levels of the antigen that's required to stimulate an immune response. Maxygen says its shuffling technology has cranked up antigen yields using naked DNA. But results are wrapped in secrecy.

High on DARPA's wish list is a vaccine that could protect against dengue, a virus transmitted by mosquitoes. It causes severe fevers and, in extreme cases, hemorrhaging and death. Four related but antigenically distinct strains of dengue cause the disease, and antibodies against one often do not protect against another. Maxygen has shuffled various dengue strains to create single antigens that, Howard says, produce antibodies that work against all four strains. The U.S. Navy now is evaluating Maxygen's data.

A single-antigen dengue vaccine could have important advantages. Donald Burke, who heads the Center for Immunization Research at Johns Hopkins University and has studied dengue in Thailand, says, in theory, it could be better than one that contains antigens from each of the four strains because dengue has a peculiar feature. Low levels of antibodies can actually enhance the ability of the virus to cause hemorrhagic fever. With a vaccine composed of four independent antigens, explains Burke, "the concern is you'd get waning of immunity to one antigen, and that would sensitize you to hemorrhagic fever." A single-antigen vaccine may produce a uniform response.

Maxygen has been more open about another vaccine project, one that involves hepatitis B. The existing hepatitis B vaccine contains a genetically engineered version of that virus's surface protein. Seeking a more powerful effect, Maxygen scientists took surface antigen genes from several hepatitis B viruses, shuffled them, and put the recombined genes into naked DNA vectors. They then injected mice with these vaccines and natural (or "wild-type") hepatitis B surface proteins. Animals injected with the shuffled genes produced five-fold higher levels of antibodies than did the mice with the best wild-type antigen. Then they went a step further, reshuffling genes that produced the best antigens and reinjecting mice. They found vaccines that led to as much as a 12-fold greater antibody response than the most potent wild-type vaccine.

Maxygen's Robert Whalen, who works on the hepatitis B project, says the company recognizes that the existing vaccine can prevent infection. But Whalen notes that it can do little for the more than 250 million people who are infected with hepatitis B and are at risk for fatal liver disease. He's hoping that a more potent vaccine might work as a treatment by knocking down viral levels in patients with a chronic infection.

The unknowns

Improving DNA vectors, making a multivalent dengue vaccine, and boosting the potency of the existing hepatitis B vaccine represent three novel ways that DNA shuffling might help vaccine developers. But researchers often face a more fundamental, perplexing dilemma: They remain in the dark about the immune responses that a vaccine needs to trigger. "There are very few diseases where you really know what you want," says Howard.

HIV is a case in point. AIDS researchers still debate the relative importance of antibodies, which prevent cells from becoming infected, and cellular immunity, the arm of the immune system that clears infected cells. Maxygen thinks directed evolution might shine some light here, too.

Taking a page from the hepatitis B story, many AIDS vaccine researchers in the 1980s banked on the idea that an antibody to HIV's surface, or envelope, protein could be protective. But by the early 1990s, it became clear that antibodies triggered by these envelope proteins failed to stop infections in test tube experiments. Many groups abandoned antibodies altogether, focusing on cellular immunity instead.

Dennis Burton of the Scripps Research Institute steadfastly stuck with the antibody idea. After screening thousands of envelope antibodies from infected people, Burton found one that powerfully stops HIV in vitro. But Burton got only half way: He did not find a version of the envelope protein that

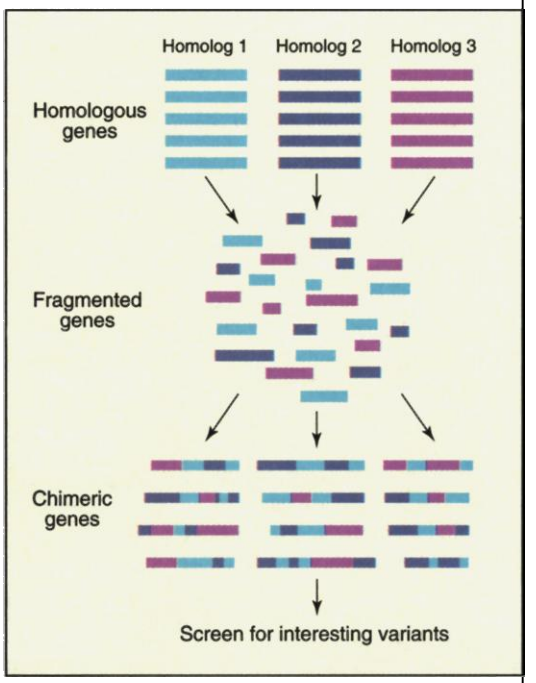
How DNA Shuffling Works

The core technology Maxygen uses in vaccine research is the polymerase chain reaction (PCR), the "molecular photocopier" that's used to amplify small amounts of DNA. But Maxygen scientists have given it a special tweak to produce what they call "DNA shuffling."

In the PCR process, DNA is heated to a temperature that separates the double helix into two strands, or templates. Researchers then add short, synthetic stretches of DNA, called primers, which complement portions of the templates, attach to them, and jump-start the copying process. This builds new double-helical DNA structures, as one base (A, T, G, or C) after another attaches to its complement on the templates.

DNA shuffling, in contrast, is PCR without synthetic primers. In this process, a family of related genes—say, the ones that codes for the surface protein of three different HIV isolates—are first chopped up with enzymes. The gene fragments then are heated up to separate them into single-stranded templates. Some of these fragments will bind to other fragments that share complementary DNA regions, which in some cases will be from other family members. Regions of DNA that are non-complementary hang over the ends of the templates (see illustration). The PCR reaction then treats the complementary regions as primers and builds the new double-helical DNA. But PCR also adds bases to the overhanging piece of the primer, forming a double helix there, too. This ultimately creates a mixed structure or chimera. In the final step, PCR reassembles these chimeras into full-length, shuffled genes.

–J.C.



CREDIT: COURTESY OF MAXYGEN

triggers production of the antibody.

Maxygen has begun to hunt for this elusive protein. Its scientists plan to shuffle envelope genes from several different isolates of HIV and test whether any of the proteins expressed by these reshuffled genes bind to Burton's antibody. They will then inject the most promising genes into mice to see whether they produce a strong antibody response. This strategy, Burton notes, differs markedly from rational gene design, which aims to fashion new HIV envelopes by deleting parts of the known structure. "One strategy says you work everything out and design a change in the protein," says Burton. "Here, you just randomize and select. And that's what nature does. It doesn't design."

In February, Maxygen's AIDS vaccine project received a boost when the nonprofit International AIDS Vaccine Initiative, in collaboration with the Rockefeller Foundation, both of in New York City, agreed to fund the work in exchange for a royalty-free license to distribute any resulting vaccine to poor countries. David Ho of the Aaron Diamond AIDS Research Center in New York City became a part of this collaboration, too, providing envelope genes from Asian isolates of HIV. "The concept is obviously novel," says Ho. "It's a fishing expedition: The more you toss the line out, the more chances you have."

Novel as the HIV vaccine project is, it also has a serious constraint: Maxygen's screening assays currently ignore cellular immunity. And that's true for all of their vaccines under development. "They've got a ways to go before they maximally exploit the technology," says Burke of Johns Hopkins. Maxygen's Howard agrees. "If we have an inferior assay, we're going to get inferior products," he says. And he says they eventually plan to develop assays for cellular immunity.

Whether Maxygen's experiments lead to new and improved vaccines remains to be seen. But already it's clear that they are introducing vaccine researchers to a sexy new technology. **–JON COHEN**

NEWS

Closing of Basel Institute Scatters Immunologists

Hoffmann–La Roche's celebrated center—an experiment originally run by basic researchers—experiences a traumatic change

BASEL, SWITZERLAND—Hoffmann–La Roche shocked Europe's immunology community last year when it pulled the plug on its renowned Basel Institute of Immunology (BII). For more than 30 years, the drug manufacturer had supported the institute's self-directed program of basic immunology research. In return, the institute gave Roche access to first-rate science and talented biologists. It was an idyllic relationship, but it came to an end on 5 June 2000 when Roche announced that it was converting the institute into a center for medical genomics. The BII's board of directors voted to dissolve itself, and Roche took direct control.

Paterfamilias. Fritz Melchers, the institute's former chief.

The institute has been slowly dispersing since then. Fritz Melchers, the BII's director for 20 years, retired this past April. Twenty-seven of the 48 members of the scientific staff have left, reportedly with generous severance deals. More are expected to leave by the end of July, when additional contracts expire, and a few who have longer contracts will depart over the next 18 months.

Roche's publicly stated rationale for the transition was that it needed to position itself at the "cutting edge of the biosciences." But employees paint a more complicated picture. Some BII alumni worry that the institute's demise indicates that Europe is losing interest in basic biology that doesn't involve genomics. Others say it has long been in a precarious position. It seemed as though the BII "was always closing," says Christopher Paige, who spent 8 years there in the 1980s and now directs research at the Ontario Cancer Institute. "Even when I left," says Brigitte Askonas, an immunologist at Imperial College, London, who was at the institute for a short period in the early 1970s, "there was uncertainty about the longer term future of the BII." The company's ambivalent support diminished, some say, following the death in 1999 of Paul Sacher, a Swiss conductor and avid patron of the arts and sciences who married into the Roche family. Observers say the BII was Sacher's pet project and that his departure tipped the balance.

The transition has been bruising for some, particularly researchers past middle age like Louis Du Pasquier, whose early retirement means an end to 31 years of studying amphibian immunology. Yet by and large the institute is "successful even in its death," says Melchers, pointing to the number of employees who have found good jobs. The big loss, say the people who knew the institute well, is the closing of an extraordinary opportunity for young scientists. Du Pasquier says it was "a paradise for creativity at the single-person level"—one that's not likely to be replaced.

The Valium windfall

Roche conceived the idea of an independent academic institute in the late 1960s, flush with money from sales of benzodiazepines. The company hired Niels Jerne, then almost 60, to direct the new center and gave him $22 million a year and a free hand in planning it. Although Roche got a first look at research coming out of the institute, by all accounts, commercial payback was never the focus. Askonas remembers how Jerne called several meetings of the new institute's first recruits, and "we had a wonderful time discussing how an institute should be run to encourage original research."

The structure that emerged was simple but unique. Administration was nominal. Groups were kept small to discourage empire building. Although the BII hired a handful of researchers on a permanent basis, most scientists came on 2-year, rolling contracts. "It was not a place to stay," says Du Pasquier, adding that the turnover kept it young. According to Polly Matzinger, an immunologist at the National Institutes of Health who spent 6 years at the BII, the advantage was the freedom and support for researchers to do what they wanted without any questions and without having to write grants. "The only limitation was your own brain," says Michael Julius, a one-time member of the institute and now vice president for research at the Sunnybrook and Women's Health Sciences Center in Toronto.

Jerne tried to encourage interaction by connecting the labs with a famous network of spiral staircases and putting the cafeteria in a central location. "We were forced to

CREDIT: BASEL INSTITUTE OF IMMUNOLOGY