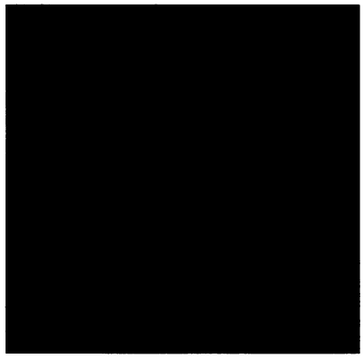
MICROBIOLOGY

Gene May Promise New Route to Potent Vaccines

For protection against an infectious disease, few things can beat a vaccine made of the living organism. But every live vaccine is a balancing act: The pathogen has to be vigorous enough to trigger an immune defense by the host, yet too weak to lead to serious illness. On page 967 of this issue, a team from the University of California, Santa Barbara, comes up with a new answer to the problem. They have found a gene that seems to orchestrate the activity of dozens of other genes needed for a full-blown infection by *Salmonella typhimurium*, a bacterium that causes food poisoning in humans and a typhoidlike disease in mice. When they knocked out the gene, the bacteria became powerless to cause disease but still elicited a fiery immune response in mice—in other words, they had apparently become the ideal vaccine.



Dam spots. A fluorescent antibody signals activity of the Dam protein in *Salmonella* cells.

Because the gene, which produces a protein called DNA adenine methylase (Dam), is shared by many other pathogens, the researchers think that easy-to-produce vaccines for a range of diseases, from meningitis to the plague, may lie within reach. For some of these, no vaccine is currently available. "We believe this may have tremendous impact in the field of infectious diseases," says geneticist and lead author Michael Mahan. Other researchers agree that the work may result in a flurry of new studies, but some are cautious. "It's an important and tantalizing clue," says John La Montagne, deputy director of the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland, "but whether or not it can work in humans is the $64,000 question."

Pathogens like *Salmonella* have many so-called "virulence genes," which are switched off when the bacteria are living on a petri dish or a chicken in the refrigerator, but spring into action once they enter the gut of a mammal, where they help the bacterium to penetrate the gut lining, travel throughout the body, and use the host's resources to grow and divide. Mahan's team had already discovered some 250 of these genes in *Salmonella*. Little was known about how they are regulated, although earlier studies had shown that a protein called PhoP controls the expression of some of them.

In their search for other regulators, the team tried many candidates. One of them was Dam, which was known to be involved in DNA repair. In *Escherichia coli* strains that cause urinary tract infections, Dam also controls the formation of pili, hairlike protrusions specialized in latching onto host cells. To see whether the protein might have a wider role, the team created an *S. typhimurium* strain that lacked the *dam* gene and found that it was utterly innocuous to mice, even in huge doses; when administered orally, the bacteria entered the mucosal tissue lining the gut but didn't colonize organs such as the liver and the spleen. Measurements of gene activity showed that the absence of Dam altered the expression of at least 20 virulence genes, and Mahan says further experiments have shown the real number to be at least 40.

Dam apparently acts as a master switch for these genes, because it can glue methyl groups to DNA strands at specific sites. By doing so, says geneticist and co-author David Low, the enzyme decreases the ability of some regulatory proteins to bind to DNA, while increasing that of others; each of these proteins can in turn crank up or slow down the transcription of one or more genes.

The authors say the finding may give scientists several new weapons in the relentless race against bacterial infections. For one, drugs that block Dam could slow down bacterial growth and possibly result in a whole new generation of antibiotics. And when the team immunized 17 mice with Dam-negative *S. typhimurium*, they found that the strain is an effective vaccine. The immunized mice all withstood terrifying doses of the normal bacterium 5 weeks later—up to 10,000 times the amount that killed nonimmunized mice.

To explain how such enfeebled bacteria could provoke such a strong immune response, Mahan theorizes that knocking out *dam* actually makes the bacteria easier for the immune system to detect. Normally, he says, bacteria turn every gene on only as briefly as possible, to prevent the host's immune system from detecting and attacking foreign proteins. But with the Dam switch shut off, some genes may be expressed for a very long time, within easy sight of the host. "The bacteria become like poker players who are forced to show their cards," says Mahan. "They can't win."

Because different *Salmonella* strains probably share quite a few proteins, one Dam-negative strain may even elicit an immune response that also covers others. In as-yet-unpublished work, says Mahan, the team showed that mice immunized with *S. typhimurium* were also immune to a related *Salmonella* strain that infects chickens and eggs. The reverse was also true, and Mahan is testing whether the vaccine covers more of the 2500 *Salmonella* strains currently known. If it does, inoculating cattle and chickens with a vaccine based on the technique may help banish *Salmonella* from the food chain, he says—although it will have to compete with other vaccines in various stages of development.

Because genetic studies have shown that other gut-colonizing bacteria like *Vibrio cholera*, *Haemophilus influenzae*, *Yersinia pestis*, *Shigella*, and *Treponema pallidum* (which cause cholera, respiratory tract infections and meningitis, plague, dysentery, and syphilis, respectively) all have *dam*, perhaps they too can be crippled by knocking out the *dam* gene. "On the heels of this paper, a variety of labs will probably quickly knock out [*dam*] in their individual bugs," predicts microbiologist John Mekalanos of Harvard Medical School in Boston. Mahan, whose lab has recently gone into overdrive, has already started experiments with four of them, and the researchers have just started their own company specializing in vaccines and antimicrobials. Says Mahan: "I have a hard time believing that it's only going to work in *Salmonella*." —MARTIN ENSERINK

CREDIT: B. P. NICHOLSON, UNIV. OF UTAH

BIOMEDICAL PATENTS

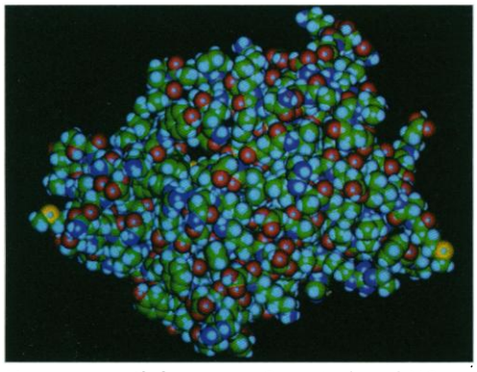
Startling Revelations in UC-Genentech Battle

Just before midnight on New Year's Eve, 1978, Peter Seeburg, then a young researcher at Genentech Inc. of South San Francisco, says he secretly removed a bacterial clone from a lab he had recently left at the University of California (UC), San Francisco, and transferred it to his new employer. That clone, Seeburg testified in court last month, helped lead to one of Genentech's early achievements as a

start-up company in 1979: a patent for the production of human growth hormone (HGH) by bacterial synthesis. The product, administered to short children to speed up growth, has earned hundreds of millions of dollars. To cover up the deed, Seeburg—who is now acting director of the Max Planck Institute for Medical Research in Heidelberg—admitted in court that he has published false "technical" data on how Genentech staffers cloned the DNA sequence it patented.

Seeburg gave this shocking testimony on 20 and 21 April in the U.S. District Court in San Francisco, appearing as a witness for UC in a civil suit against Genentech. Alleging that Genentech has been violating UC's own patent on growth hormone, the university is asking the court to award damages of about $400 million, according to a UC attorney. This is what UC would have earned, the university calculates, if Genentech had paid HGH license fees. In addition, the university is asking for triple damages, on grounds that Genentech's actions were "willful."

Genentech, which began to present its own evidence on 3 May, denies that it infringed the UC patent. Although company officials declined to comment, trial transcripts indicate that their attorneys denied that they took or encouraged anyone to take material from UC labs. And Genentech has summoned witnesses to attack Seeburg's testimony—including Seeburg's former co-worker, David Goeddel, a postdoc at Genentech in 1978 who is now chief executive of Tularik, another South San Francisco biotech firm. In opening comments to the jury, however, Genentech attorney John Kidd acknowledged that "there's no dispute that Seeburg brought some material [to Genentech]. We don't argue with that. It was his material. ... He was entitled to bring that. That's what postdocs did in those days." But Kidd insisted that "the evidence will show that we did not [use it]."



Human growth hormone. Genentech and UC are battling over rights to a genetically engineered version.

Although this trial of competing HGH patent claims has been stewing for many years, Seeburg's testimony still came as a surprise. Seeburg, a German expert in neural genetics who came to UCSF to learn about DNA cloning in the 1970s, had upheld the Genentech line on HGH until recently. On the witness stand, however, he said that for many years he has been "walking a tightrope" between truth and falsehood. When questioned under oath in the past, he said, he had "not been forthcoming" in saying that the DNA clone on which Genentech's patent is based "was obtained at Genentech." True, it came from bacterial clones grown at Genentech; but he said he failed to reveal that the source of the clones was his former lab at UCSF.

Seeburg explained on the stand that Genentech hired him in September 1978 because he was a leader in a productive HGH cloning project in Howard Goodman's lab at UCSF. UCSF failed to offer him an academic job that fall, so he went to work at Genentech for $40,000 a year, with health benefits and a chance to buy stock at 30 cents a share. Before leaving the university, he, his UC colleague John Shine, and Goodman applied with the university for a patent on a DNA sequence for human growth hormone they jointly discovered. (UC was awarded the patent in 1982; they are co-inventors.) At Genentech, Seeburg worked with Goeddel and others to replicate and extend this work, aiming to get a more complete sequence that could be put in bacteria for mass production. But he and Goeddel were frustrated by poor source tissue and many failures, Seeburg said.

To speed up the process, Seeburg testified, he decided to use the material he had developed at UC. Seeburg testified that he and a colleague made a visit to the Goodman lab at about 11 p.m. on 31 December 1978. He took many samples, including a clone containing a partial human HGH sequence, and returned quietly to Genentech, "out in the industrial district of South San Francisco." Seeburg recalled that out of the dark, "a highway patrol screeched up in front of us as we were getting out." The police officer asked what they were doing, Seeburg recalled: "We said, 'We are scientists.' But the officer laughed and said, 'You don't look like scientists.'" Still, he let them go.

Later, Seeburg testified, he and Goeddel used the UC clone in the development of Genentech's HGH sequence, for which the company obtained a patent in 1982. They also published a paper on this work in *Nature* in 1979 which, Seeburg testified, contains information on how they cloned the DNA using a plasmid (called PHGH31) which "never existed." Seeburg claimed that he remained silent about all this because he and Goeddel had an agreement not to reveal what they had done, and "I wanted to honor it."

Goeddel, who was still giving testimony as *Science* went to press, denied that he and Seeburg had ever agreed to remain silent about

ScienceScope

AIDS Windfall Microsoft CEO Bill Gates and wife Melinda have made the largest single philanthropic donation ever for AIDS research. This week, the couple announced that they will contribute $25 million over the next 5 years to the International AIDS Vaccine Initiative (IAVI). The New York–based group will use the bulk of the money to set up three new international AIDS vaccine development teams and fund two existing groups working on vaccines for Kenya and South Africa. The gift will also support applied research, advocacy, and clinical trials. The landmark gift—which will double IAVI's budget to nearly $50 million—"will allow us to significantly accelerate the scientific effort," says president Seth Berkley.

The private group's war chest is still dwarfed by the $200 million vaccine program run by the National Institutes of Health. But the iconoclastic IAVI hopes to approach the problem from a novel angle, serving as a crucible to mix academics from both wealthy and poor countries and industry members with innovative ideas.

Dollars and Sense University officials will get the chance later this month to tell the White House what they think of its new draft report that attempts to clarify the sometimes strained partnership between government and academia. Although the report is filled with such paeans to research "as an investment ... guided by peer review," the real issue for universities is likely to be money. In particular, academic officials want the government to spring for a larger share of the overhead to support federally funded research on campus. "I would hope that there will be a major push for additional resources," says Nils Hasselmo, president of the Association of American Universities, who nonetheless sees the report as a vote of confidence in university-based research.

President Clinton unveiled the report (whitehouse.gov/WH/EOP/OSTP/html/rand/contents.htm) last week at a belated ceremony to honor the 1998 National Medal of Science and Technology winners. The report is even more tardy: A 1996 presidential directive set a target date of 30 April 1997; now the Administration hopes for a final report by year's end. A 25 May hearing is the first of three scheduled by the President's Council of Advisors on Science and Technology.

CREDITS: (TOP) UWE LEIN/AP; (BOTTOM) LEONARD LESSIN/FBPA

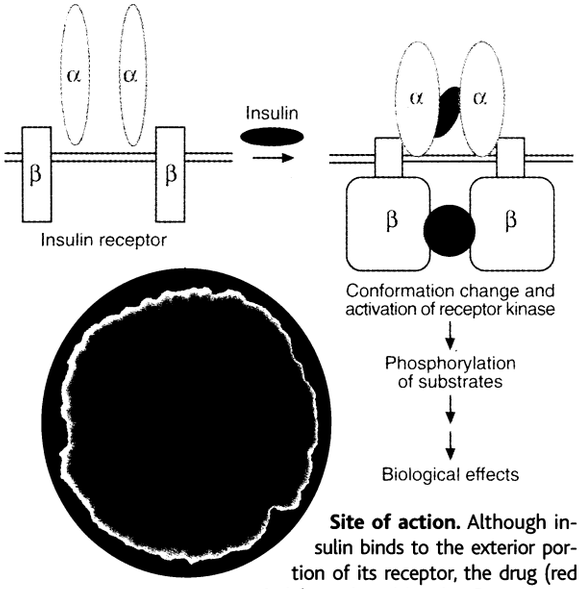
any misdeeds. "I couldn't believe that he could come up with such a story," Goeddel testified. And he denied that he used patented UC material as the basis of Genentech's discovery. Genentech's attorneys also spent a day attacking Seeburg's credibility, pointing out many inconsistencies in his testimony over the years. They reminded the jury that Seeburg—as co-inventor on the UC patent—stands to make a lot of money if UC wins this case.

Only about half the testimony has been presented so far in this trial, and there could be more surprises before the end. Barring an early settlement, this complicated dispute may go to the jury for a decision by the end of the month. **–ELIOT MARSHALL**

DIABETES RESEARCH

New Lead Found to a Possible 'Insulin Pill'

A lowly fungus that grows deep in the African forests near Kinshasa could soon be a pharmacological celebrity. Collected years ago and then analyzed by researchers from Merck Research Laboratories in Madrid, Spain, who hoped to find new drugs in rainforest flora, the fungus, called *Pseudomassaria*, attracted little notice at first. But now another Merck team, led by Bei Zhang and David Moller of the company's Rahway, New Jersey, laboratory, has found that *Pseudomassaria* produces a unique agent that could lead to a new type of antidiabetes pill. Such a treatment would be welcomed by the millions of diabetics who now must inject themselves with insulin or choose from a few orally administered drugs with serious side effects.



Site of action. Although insulin binds to the exterior portion of its receptor, the drug (red circle) produced by the *Pseudomassaria* fungus *(inset)* binds to the inner part.

In work reported on page 974, the team gave the compound, a small, five-ringed molecule of the quinone family, to mutant mice with symptoms similar to those of patients suffering from adult onset or type 2 diabetes. These include high blood sugar, defects in insulin production, and also a decreased ability of the tissues to respond to insulin. The agent reduced these symptoms in the animals, the researchers found, apparently by tweaking the same cellular receptor that insulin acts on. But, unlike insulin, the fungal compound is not a protein and, thus, could likely withstand the body's potent digestive juices. "This is an insulin mimetic molecule which could become a drug that may be able to be given by mouth," says endocrinologist Arthur Rubenstein, a diabetes expert at Mount Sinai Hospital in New York. "The potential is enormous."

To find the new compound, Zhang and Moller took advantage of the known activity of the insulin receptor, which is embedded in the cell membrane. The portion protruding to the exterior spots and attracts insulin molecules, while an inner portion is a kinase enzyme, which responds to insulin's nudging by tacking phosphate groups onto various proteins in the cell. This leads to changes in the activities of those proteins, which in turn allow cells to take up and use the sugar glucose, thereby lowering its blood levels. The insulin-triggered upswing in the receptor's phosphate-adding activity is also useful to researchers hunting for antidiabetes drugs, because they can use it to pinpoint chemicals that mimic insulin's effects.

For their drug-fishing expedition, the Merck team used hamster ovary cells engineered to produce the human insulin receptor. Then, following a strategy frequently used to search for new drugs, the researchers set up a screening assay in which they divided the cells among thousands of miniature petri dishes. After trying some 50,000 mixes of synthetic chemicals and natural extracts on these cells, the investigators scored a major hit with an extract prepared from *Pseudomassaria* culture broth. Zhang and Moller then sent the extract to Merck chemist Gino Salituro, who set about purifying the active agent, a daunting task, as the fungal extract contained hundreds of compounds.

When Salituro finally pulled out the active ingredient, chemical analysis showed that it is a quinone. That was a surprise, Moller says, because none of the other antidiabetes drugs currently in use or under investigation belongs to that class of compounds. "From looking at its chemical structure, it does not have any obvious biological activity," he says. Yet in tests on cultured cells, the *Pseudomassaria* product, known as L-783,281, stimulated the phosphorylating activity of the insulin receptor by up to 100 times more than other natural compounds tested.

And its effects appear to be specific. L-783,281 does not spur the activity of receptors with similar protein-phosphorylating ability, including the receptors for epidermal growth factor, platelet-derived growth factor, and insulin-like growth factor. L-783,281 apparently diffuses through the cell membrane and binds directly to the kinase portion of the insulin receptor, activating it.

Achieving such specificity has always been "an elusive goal," says Zhang. Other antidiabetes drugs work in various ways, such as increasing insulin production by the pancreas or binding to the outer portion of the insulin receptor, but they may have other effects as well. This can lead to serious side effects such as excessively low blood sugar or blood pH, gastrointestinal problems, or in the case of the recently controversial antidiabetic agent Rezulin, liver failure.

Preliminary animal tests with L-783,281 also look promising. The Merck team tested the compound in two mutant mouse strains that have classic diabetes symptoms. In both strains it suppressed the skyrocketing blood sugar levels by up to 50%—comparable to the reduction seen with current oral antidiabetic therapies, Moller says. The compound also reduced the elevated insulin levels seen in one strain, presumably because blood sugar levels dropped, causing the pancreas to lower its insulin production.

If further animal trials confirm that L-783,281 or chemical variants resembling it are both effective in lowering blood sugar concentrations and safe, Merck says clinical trials might be feasible. People have been talking about making an insulin-replacement pill for a long time, Zhang says, and "now we have shown it's possible." **–TRISHA GURA**

Trisha Gura is a science writer in Cleveland, Ohio.

SWEDEN

Academics Applaud Renewed Support

STOCKHOLM—Think of Thomas Östros as the calm after the storm. Following years of turmoil and distrust between scientists and policy-makers, Sweden's youthful minister of education and science has spent his 7 months in office reassuring academic scientists that the government values their contribution and has no intention of letting outsiders call the shots. And that empathy, combined with the promise

CREDIT: ZHANG *ET AL.*