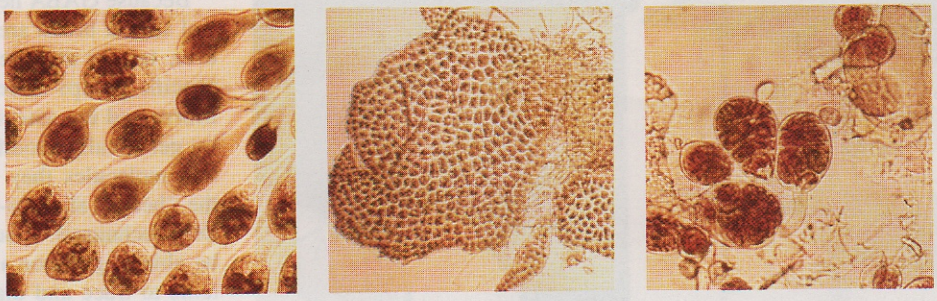
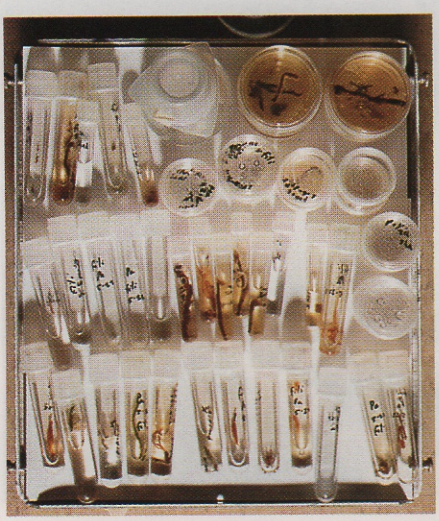


Probing Secrets of the Cell at UCSB

BY D.B. PLESCHNER



Opposite: Assistant Debi Fisher checks the progress of Aharon Gibor and Miriam Polne-Fuller's tissue cultures. Left: The Gas Research Institute funds one of the team's projects, hoping to grow vast quantities of kelp for methane fuel. Above: Polne-Fuller was the first to release seaweed cells from the tissue encasing them. She photographed (left to right) natural kelp cells under her microscope, a new plant regenerating in culture, and another well on its way at four weeks.

MICROPHOTOGRAPHY BY MIRIAM POLNE-FULLER

MY SON HAS MY EYES AND CHIN, his dad's nose, his grandfather's big toe—his inheritance is in his genes. But genes are more than a blueprint: They control life. And now, thanks to breakthroughs in molecular genetics, cell biology, and biochemistry, scientists are stripping genes of their secrets. That knowledge is revolutionizing the worlds of science, industry, health care—in fact, we're beginning a new genetic age—and the revolution is alive and well in Santa Barbara.

Genes are strands of nucleic acids, or DNA, that make up the chromosomes found in every cell nucleus, UCSB's Dan Morse tells me. "Picture them like very long, but very thin and tiny, strings of poppet beads linked together," he says. "Each gene is made of about a thousand little beads, four different kinds of beads strung together in sequence in the DNA molecule." It's the sequence that tells the cell machinery to make a unique product, which might be anything from insulin to an enzyme that can degrade petroleum.

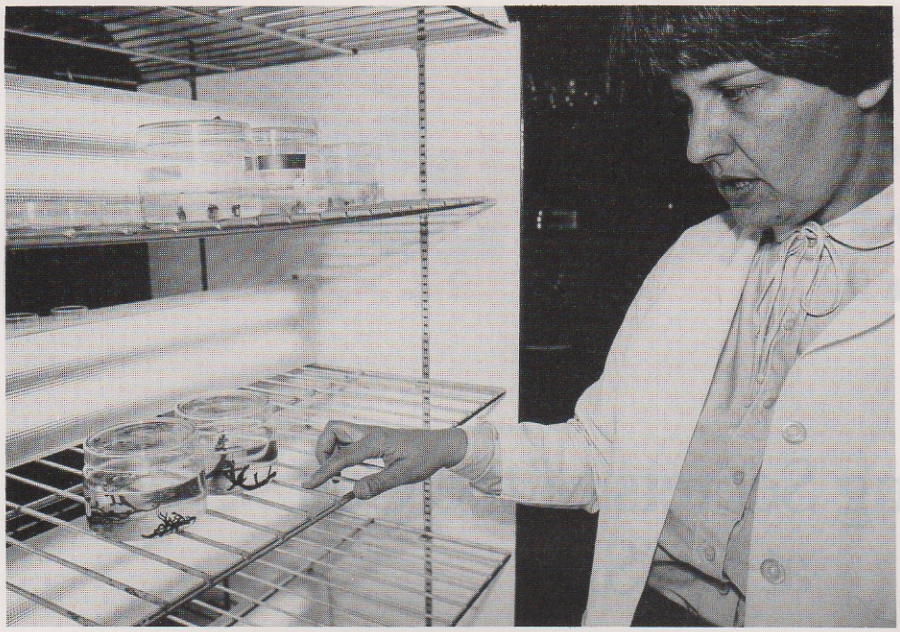
Dan Morse, formerly a Harvard biochemist and molecular biologist, now professor of molecular genetics at UCSB, emigrated west over a decade ago. He came to further his research on molecular mechanisms that influence genes.

Working with abalone, Morse and his colleagues used biochemical engineering to probe for the natural genetic catalysts that regulate reproduction, development, and growth in the mollusks. What they found earned them two World Mariculture Society first-place awards: one for discovering that spawning can be induced by adding hydrogen peroxide to seawater, a method that works as well on 29 other mollusk species. The second award was for isolating the amino acid GABA, which induces larval settlement and metamorphosis. Now looking into abalone growth requirements, Morse's studies suggest that insulin may function as a growth hormone, a discovery that has important human implications.

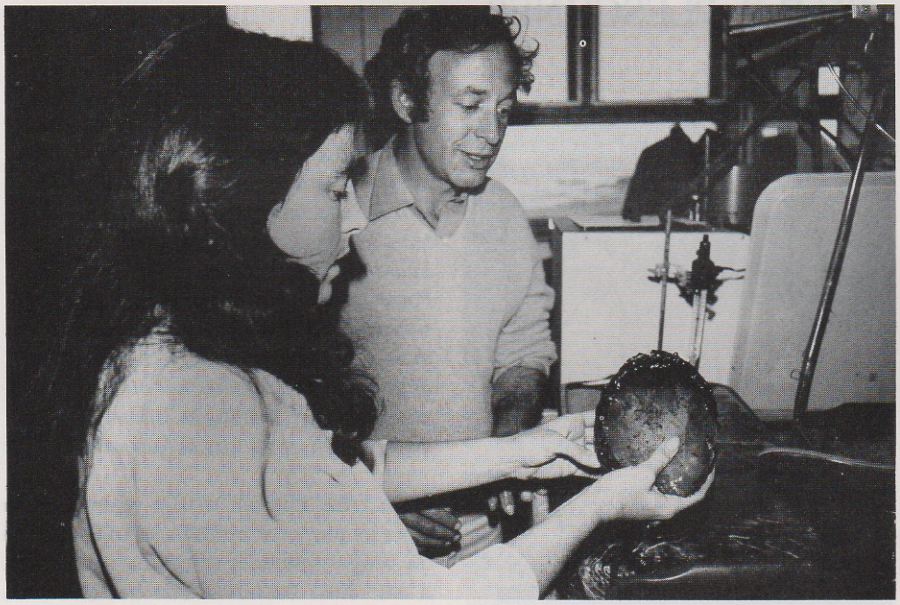
Some forms of diabetes are caused by a lack of insulin, which normally regulates the use of sugars and carbohydrates for energy and may also influence development. Diabetes patients who lack the gene that codes for insulin must receive the hormone by repeated injection. In recent years, scientists have devised ways to extract the human insulin-making gene from its place on the DNA strand—unpop the proper sequence of beads—and insert it into host bacteria. The bacteria then grow large quantities of insulin very inexpensively, multiplying from one cell to millions overnight.

Companies now produce insulin and other biochemicals using bacteria as "factories," growing them in huge vats. The products, faithful replicas of the natural substance, are licensed for medical use by the FDA. This ability to copy or "clone" genes is called recombinant DNA technology, or genetic engineering. It is making once-prohibitively expensive products affordable.

Besides some forms of diabetes, nearly a thousand human disorders are known to result from genetic mutations, Morse notes. He lists various forms of heart disease, cancer, hemophilia and other blood and neurological disorders, cystic fibrosis, and muscular dystrophy. One day it may be possible not only to inexpensively



D. B. PLESCHNER



COURTESY MARINE SCIENCE INSTITUTE

Above top: Along with tissue culture, Miriam Polne-Fuller has great hopes for seaweed's somatic hybridization, a technique now used to make monoclonal antibodies for tracking human disease. Above: Professor Dan Morse and colleague Helen Duncan are aiming their studies of genetic catalysts in abalone to more help for humans in the future.

produce naturally synthesized human-based products to counteract these mutations, but to introduce cloned "good" genes directly into patients. Clinical trials are just now beginning on volunteer patients in a few research hospitals around the country.

In Santa Barbara, Morse has found that another aspect of his abalone research may have direct bearing on human disease. GABA, the metamorphosis trigger in abalone, is also a major chemical messenger in the human brain. It seems to control about half of all the brain cells, in fact. And the GABA molecules that Morse and his colleagues identified in abalone also interact strongly with, or bind to, signal receptors in the brains of mammals. Because they do bind, Morse thinks they may be useful in a new diagnostic technique called positron emission tomography, or PET scan, which uses radioactively labeled molecules as tracers. When PET scan's tracers bind to selected areas of the brain, they generate a three-dimensional image that can pinpoint areas of malfunction, such as in Parkinsonism or epilepsy. The new diagnostic tool eliminates the need for exploratory surgery, in many cases.

Because GABA neurotransmitters control such a broad area of the brain, however, tracers are needed that can isolate specific groups of cells. And that's what Morse and his associates hope to supply by genetically modifying the small protein molecules they found—rearranging the "beads" slightly—using recombinant DNA techniques. If successful, Morse will be able to produce a new family of proteins to trace and diagnose malfunction, and, perhaps, be useful for treatment.

The largest group of prescriptions written in the United States today is for a variety of drugs that bind to cells regulated by GABA—for control of epilepsy, sleeping, psychological state, muscle tension. Morse points out that many of these drugs have unpleasant side effects. "They're not selective enough," he says. "So we feel that our discovery from marine organisms may have its most exciting applications in human medicine."

KNOWLEDGE OF MARINE ALGAE, or seaweed, is also coming of age in the biotechnology revolution. And in Bio-Sci 2, the tallest building on the UCSB campus, Miriam Polne-Fuller is helping to make it happen. When I arrive at the lab—a welter of workbenches holding seaweed-stuffed test tubes shaking in centrifuges—she is racing to finish a presentation for a conference on genetics in aquaculture at UC Davis.

Polne-Fuller came from Israel to UCSB in 1975 as a graduate student, where she began working with professors Aharon Gibor and Mike Neushul. Neushul is an ecologist and well-known aquaculturist, while Gibor specializes in cell physiology and tissue culture. Working between the two renowned scientists, Miriam Polne-Fuller found the best of two worlds.

Tissue culture is not a new field. Over 30 years ago scientists realized they could isolate single cells from different areas of a plant, manipulate the cells to improve their traits, and grow them into superior plants. Scientific tissue culture of land-based plants has been employed with increasing success ever since. Soon it may be possible to "design" new plants by introducing DNA coding for the desired characteristics. Another goal is to genetically alter plants so they provide their own fertilizer.

This is no pipe dream. Some bacteria naturally trap nitrogen from the atmosphere and turn it into nutrients that plants can use. Today genetic engineers are trying to isolate the nitrogen-fixing gene from bacteria and insert it into corn or rice, for instance. In the not-too-distant

future they may be able to select genes providing the best traits, add nitrogen-fixing genes for self-nourishment, combine them all in a single cell, and grow the new seed stock in a petri dish. Farmers could then transfer the "perfect" seeds to the field.

Tissue culture and recombinant DNA technology also hold great promise for plants of the sea, a billion-dollar a year industry. About half of the world's seaweed harvest is used as food, one example being the nori wrapper that decorates sushi. The other half is used for pharmaceuticals and food additives, mainly gels and thickeners. But until a few years ago, nobody had a reliable way to free valuable seaweed cells from the gummy honeycomb of tissue encasing them. Then Polne-Fuller did it by accident.

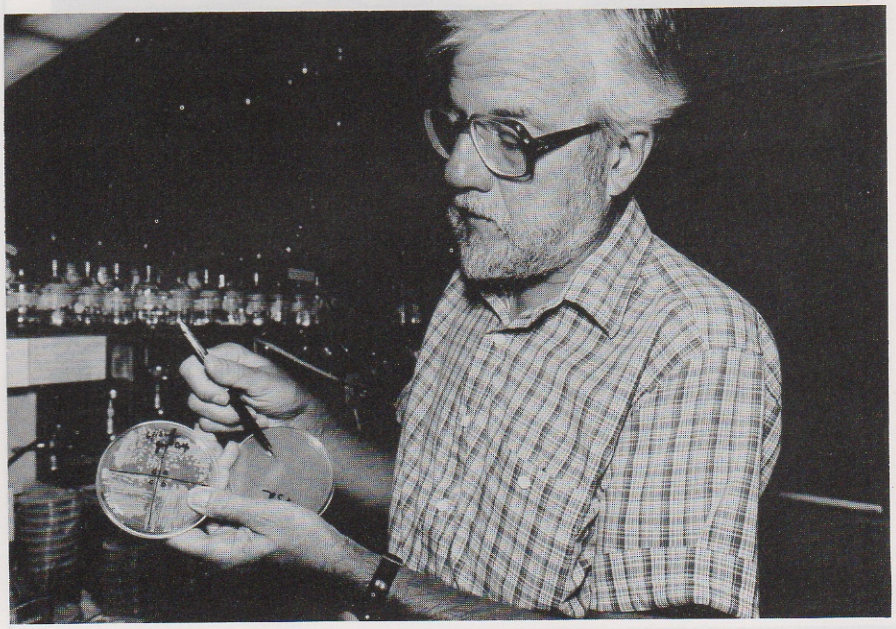
She was working with tiny seed stock, studying overwinter storage methods. One day, checking an experiment stored in calcium-free artificial seawater, she found she had "a little bag of empty outside wall, and all the cells had fallen to the bottom." Then more excitement: Each cell regenerated in culture. This was six years ago, and today the light at the end of the research tunnel gleams bright. Scientists can now isolate single cells from major seaweed species. And thanks to Gibor and Polne-Fuller, who developed techniques to remove bacteria from tissue culture, they can strip the walls from single cells—with an enzyme from abalone—to get naked cells, or protoplasts.

So where does all this lead? When seaweed single cells and protoplasts became available, the Gas Research Institute came knocking. The institute looks to open-ocean mariculture to grow volumes of kelp for methane fuel production. It is developing a long-range plan involving tissue culture, cell culture, and genetic engineering, and it funds one of Gibor and Polne-Fuller's research projects.

A promising technique that Gibor and Polne-Fuller are exploring to improve seaweeds is called somatic hybridization. When protoplasts touch each other, their membranes "pop together," Polne-Fuller explains. If the nuclei of the two cells merge as well, their chromosomes combine and the result is one big cell with the properties of two.

The same technique is used to make what's called monoclonal antibodies, new disease-diagnosing tools also being researched at UCSB. When a human can-

Above: Louise Clarke and her husband John Carbon helped pioneer the recombinant DNA revolution. Here Clarke probes for the secrets of cell division with bacteria containing cloned yeast genes. Below: Carbon, head of UCSB's biology department, points out the tiny bacterial colonies that make up one of his gene libraries.



PHOTOS BY D.B. PLESCHNER

cer cell merges with a spleen cell from mice, for instance, the hybrid cell multiplies rapidly, like a cancer cell. But it also produces the antibody of the spleen cell—only in much greater quantity than the original. Diagnostic kits are now on the market as a result of this biotechnology, and pioneering industries in Santa Barbara are using them.

What about combining tissue culture with recombinant DNA techniques? "That's the future," Polne-Fuller says and smiles, eager to begin. "Tissue culture has been applied successfully on land plants, but genetic engineering is still in a learning state. Researchers don't quite know where all the genes are or how to transfer them into cells. With seaweed, we hardly have a hint yet." Eyes twinkling, she adds, "But we feel it's possible." A new program and lab is slated to join UCSB's renowned marine studies before long. Called the Biotechnology Seawater Lab, it surely will help provide some answers.

RECOMBINANT DNA TECHNOLOGY—cloning genes—it sounds fascinating. And it was an overnight sensation—like an actor who gets "discovered" after playing bit parts for 20 years.

Some date the recombinant revolution from 1854, when Gregor Mendel, an Augustinian monk and the father of modern genetics, began tinkering with peas. A hundred years later, scientists were grappling with the question of how the DNA of genes transmits directions to make a complete organism. Then, in 1956, DNA was first synthesized in the laboratory. Each breakthrough added a new piece to the puzzle, but left another hole to be filled. And so it went until the early 1970s, when the revolution really took off.

That's when scientists began developing "cut and paste" techniques to isolate specific genes by inserting foreign DNA into bacteria, transforming it genetically. Two pioneers in developing the methods now used worldwide are UCSB's John Carbon and Louise Clarke.

Carbon, a professor of biochemistry, heads the Biological Sciences Department. Clarke graduated from Smith College and began graduate studies at UCSB in 1968. She won her doctor's degree in 1973, married John Carbon in 1975, and this morning finds her bent over a row of petri dishes transferring tiny colonies of yeast from one plate to the next. Exotic animal prints line the walls of her office; her desk overflows with photographic maps of genes and genetic charts; and a counter of tubes and plates and odd little bottles evince the workaday world of a

research scientist.

"What *do* we do?" she repeats with a laugh, searching for layman's words to describe her work. Among the couple's achievements is the establishment of the first genomic library for *Escherichia coli* bacteria, now distributed by the *E. coli* Genetic Stock Center at Yale. The library consists of some 2,000 bacterial colonies, each the cloned progeny of a cell that Carbon and Clarke genetically transformed by cutting a plasmid (a little circle of extra DNA found naturally in bacteria), adding a piece of *E. coli* DNA, and inserting the plasmid into host bacteria.

The collection of colonies contains nearly 5,000 genes found in *E. coli*. To isolate them Carbon and Clarke made billions of minute plasmid "vectors," each containing a different piece of DNA with a few genes. They used the same procedure to construct libaries for two species of yeast, one of them common baker's yeast with roughly 10,000 to 15,000 genes. Clarke says that in the 1970s, building libraries was "reasonably difficult." But they proved it could be done, enabling other researchers to easily capture virtually any gene from organisms they were studying.

Once the DNA is inserted, the bacterial host can be cultured indefinitely and always carry that one segment of genes. "A key question you need to ask about libraries," Carbon puts in, entering Clarke's office, "is how do you find which colony in this bunch of thousands has the gene you're looking for?"

Carbon and Clarke worked out a way to identify clones by using a bacterial host with a known genetic defect. "We have all these pieces of DNA from another organism," Carbon says, "and one gene there is responsible for making the product the bacteria is missing. When we put all the DNA into bacteria, none of them will grow except for the cell that picks up the DNA that corrects its defect. That's called complementation of mutations."

Carbon and Clarke were the first to use this technique to screen libraries and isolate genes. The team was also responsible for developing a cloning system for yeast, a system more and more scientists are adopting because yeast has all the advantages of bacteria plus one extra: yeast cell structure is more like human. Constructing yeast libraries, a lab team member discovered that genetic defects in bacteria could be cured by yeast genes. "No one thought that could be," Carbon remarks. "It was the first demonstration that genes could cross species lines." That knowledge—that genes from one organism can function in another—is the backbone of today's biotechnology revolution.

But cloning bacteria and yeast is simple compared to cloning sophisticated human genes. "Genes in DNA are arranged just like songs on a tape," Carbon explains. "There's a control spacer between each gene." In this control region are signals used by the cell's machinery to specify where the gene starts and stops. Putting human genes into bacteria or yeast didn't work at first because the host's cell machinery couldn't recognize the right place to start processing the gene. The problem was solved by placing a control region from the host in front of the human gene before cloning it. "You're simply fooling the cell," Carbon says with a smile. "That's the whole principle of modern biotechnology."

The team's recent work involves cell division and how the chromosomes are transferred into each new cell. Division takes place at a spot on the chromosome called the centromere. A fibrous spindle attached to the centromere and each set of chromosomes pulls the requisite parts into their proper places—like a cellular stage director. "That's an absolutely essential part of cell division," Clarke declares. "If that didn't happen faithfully, one cell would come up with less DNA, and it would be dead. Life simply can't go on without it."

Clarke has isolated centromeres from yeast; some of them are in the little dots of colonies she is moving around this morning. It's tedious work, from a lay perspective, especially when some of the puzzle pieces are too small even to be seen under a light microscope. But it has its exciting moments, like when the team found they could substitute a centromere from one chromosome into another and it still functioned. They also discovered they could put a centromere in a plasmid and cause it to segregate, that is, act like a mini-chromosome. In essence they had constructed an artificial chromosome. "Now that we know the nature of DNA in this region," Clarke adds, "we would like to know what proteins bind to it, what apparatus actually sets it up, and, basically, why does it move chromosomes?"

The answers may provide clues for conquering diseases like cancer. "Cell division is regulated," Carbon interjects. "Cells don't divide willy-nilly. And what cancer is, really, is a problem in cell division."

Cancer research occupies a major portion of the scientific community, UCSB being no exception, and the recombinant revolution may be the key to success. Some labs are studying interferons. Discovered only a few years ago, these protein hor-

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mones help the body's immune system fight viral disease and some forms of cancer. Cloned interferon-making genes now provide large quantities of the substance for research at an affordable cost.

In higher animals, "onco" genes control cell division, and a few years ago researchers discovered that yeast cells and these genes work in a similar fashion. This explains why many scientists are switching to studying yeast. "Suddenly scientists are realizing that all living forms are much, much closer to each other than we ever thought," Carbon says. "Evolution has changed morphology—in the sense that humans don't look like rats—but it hasn't changed the basic life processes much at all." The genetic code is universal: DNA is DNA is DNA.

This has pricked the moral consciousness of some people in a couple of ways. For one, it plays havoc with a literal biblical interpretation of creation and man's uniqueness. Then there's the question of just how much tinkering with life is too much? That question spurred Congressional hearings at the beginning of the recombinant revolution, back in the 1970s. Congress resolved to impose no restrictions on research which makes it easy for an imaginative mind to foresee the cloning of whole complicated organisms—including people.

How do scientists feel about the issue? I posed the question and got a repeating response: There's cloning, and there's cloning. "I don't see anything wrong with cloning bacteria or yeast," Carbon declares. "Once you start cloning horses or humans, then you have a problem. But it doesn't have to extend that far. We can draw the line. I'm certainly not in favor of cloning humans."

Within this marriage of science and industry, biotechnology is beginning to copy, even improve, nature in ways that can offer enormous benefits: in new cheaper chemicals, new food products, the prevention and cure of diseases. Science is also challenging our definition of life itself, and concepts will undoubtedly continue to change as scientists probe closer to life's essence. The moral implications of that final revelation may be left for future generations to decide. But it's likely that then, as now, pioneering Santa Barbarans will play an important part. ■

Santa Barbara writer and photographer D.B. Pleschner has a special interest in environmental issues and biopolitics. She is a contributing editor of Pacific Fishing *magazine.*

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Cover photo by Jürgen Hilmer: A classic adobe archway leads to John and Brooke Bacon's old-fashioned hacienda in the Santa Ynez Valley. Meet the old-timers and the new—the Bacons, the Cassidys, and the Houtzes—starting on page 34.

Model: Mrs. John Woerner Photo: Tom Skiffington