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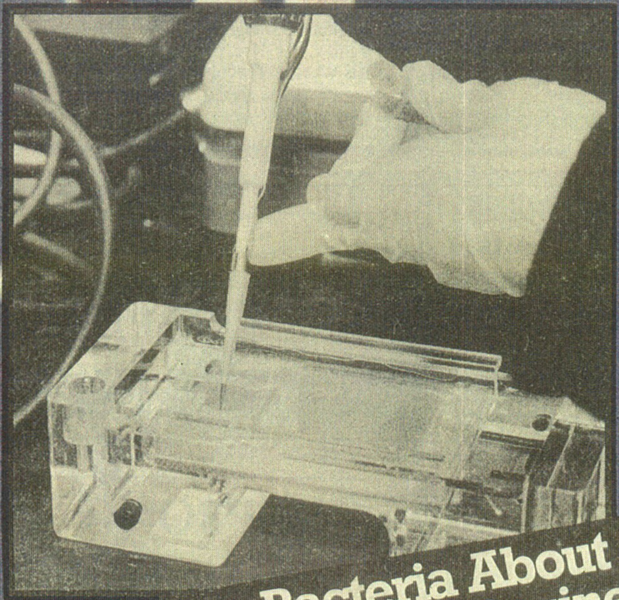
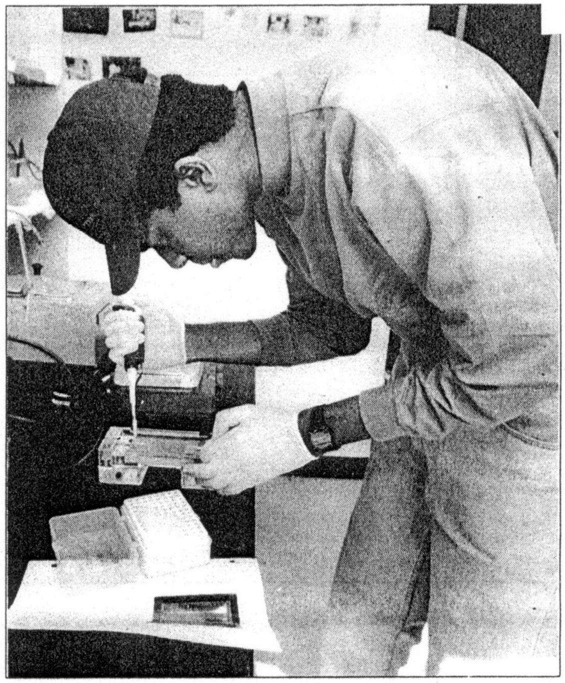


Are Bacteria About To Become Big Business at UMass?

ALSO INSIDE: Things to do from Springfield to Brattleboro

The Making of HaloGenetics Inc.



How Bacteria Are Going from the Test Tube to the Board Room at UMass

By Chris Yurko

Photos by Carol Lollis

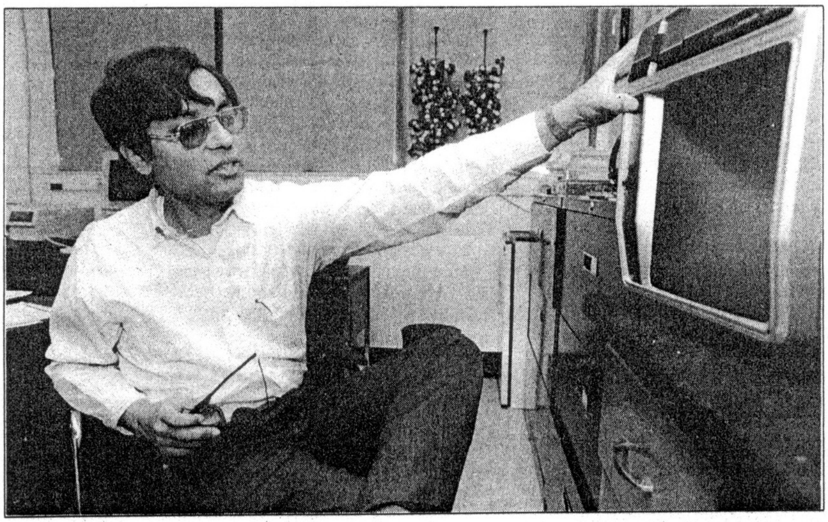
Magnified 46,000 times, these microscopic cell parts look like footballs, tapered at the ends, plump in the middle and full of air. Their function, in certain strains of aquatic bacteria, is buoyancy, keeping the cells afloat and closer to sources of oxygen and sunlight. In a few years these tiny air sacs – a.k.a. "gas vesicles" – may be helping to clean up the environment, control pesky, disease-bearing insects, and make medicine, or, perhaps, beer.

After years of research and $1 million in federal grants, microbiologists at the University of Massachusetts at Amherst have unraveled the genetic code governing these air sacs, which they can now produce in previously nonfloating bacteria. They hope to turn that discovery into a multimillion-dollar corporation called HaloGenetics.

"The idea here is we're engineering genes that make cells float," says Shiladitya DasSarma, a UMass associate professor of microbiology and chief scientist on the HaloGenetics project.

HaloGenetics is not yet a company. It is a well-conceived idea, a logo, a slide show, an evolving business plan, and a continuing research project with limited financing but many prospective investors. HaloGenetics has no product to market and may not for two or three years. But the commercial potential for this kind of biotechnology is vast.

For instance, DasSarma suggests it is possible to create more efficient petroleum-eating bacteria to clean up ocean oil spills. If these bacteria could float, the theory goes, they would spend more time on the surface munching rather than sinking from their own bloated bulk. DasSarma envisions a similar scenario to control the spread of malaria, by introducing the flotation genes into bacteria naturally lethal to mosquito larvae.



Shiladitya DasSarma, above, an associate professor of microbiology at the University of Massachusetts, hopes to turn his research about floating bacteria into a multimillion-dollar business, HaloGenetics Inc. At top, Guy Balan prepares a sample of the bacteria's DNA for a process called sequencing.

Splicing the gas-vesicle genes into yeast might reduce the price of beer. Floating yeast could be removed from fermentation tanks by skimming the top, rather than more common and costly methods, such as filtration.

Some of the most interesting applications would turn the gas vesicles themselves into micromanufacturing plants. DasSarma and his colleagues anticipate being able to construct gas vesicles to their own specifications. Incorporating select proteins into their surface membranes could make the gas vesicles vehicles for the production of vaccines, pharmaceuticals or pigments for the food and clothing industries.

"I think the technology has tremendous potential," says Tripp Peake, a consultant hired by UMass to advise new businesses inspired by academic research. "I'm excited by the technology, per se, because it has some very large long-term human therapeutic potential, and it has some more short- to medium-term industrial, agricultural and other market applications."

Although the road to incorporation for HaloGenetics is paved with great expectations, developments on the business front have not advanced as quickly as DasSarma had hoped. Because the gas-vesicle research has been government-funded, the technology belongs to the university. UMass officials have agreed to license it back to HaloGenetics, but there's a hitch: The rights to HaloGenetics' first patent belong to an Arizona company that licenses patents on behalf of colleges and universities that lack their own patent offices, as UMass does. University officials are negotiating to reacquire the rights to that patent, a crucial step before HaloGenetics can work out a technology transfer agreement with UMass, finalize its business plan and begin searching for financial partners.

"There's still a degree of complexity that I didn't imagine would be existing by now," DasSarma said in early February, after postponing a proposed incorporation date for the third time.

Also complicating matters are the university's own policies. In its new role as a catalyst for economic development, UMass is rewriting the rules regulating faculty ownership in companies that do business with the university.

At stake is how much DasSarma and other UMass faculty members can profit from technology developed in their own laboratories, and what kind of incentive the university plans to offer in its pursuit of new commercial ventures.

DasSarma's lifelong ambition was to be a university professor, not an entrepreneur. But he now finds himself straddling the often conflicting worlds of business and academia.

THE NAME FOR THIS prospective company, HaloGenetics, derives from DasSarma's favorite microorganism, *Halobacterium halobium,* one of a variety of bacterial species that thrive in high-salt environments – the halophiles (from the Greek *halo,* for sea, or salt, and *philos,* beloved). What first attracted DasSarma to these salt-loving creatures was related only indirectly to their floating abilities.

Halobacteria have a very high mutation rate, about 1 percent, some 100,000 times the normal rate. Statistically, that means that in every batch of a hundred cells, one will have an error in its genetic code, as opposed to one in 10 million.

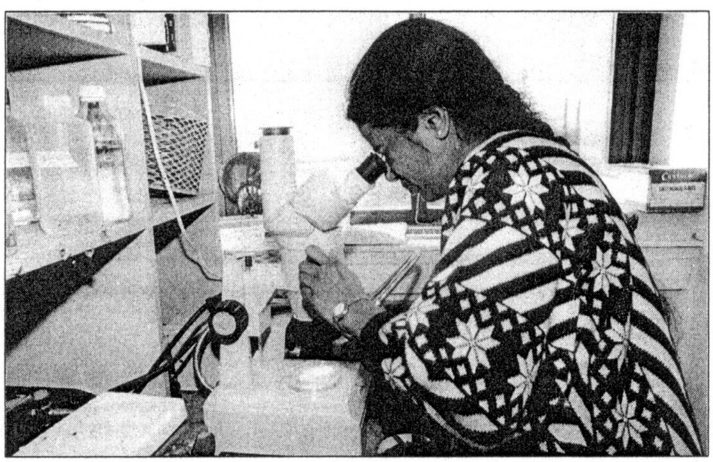
"You know genes are the elements of heredity," DasSarma explains patiently, the consummate professor. "They're an agent of information transfer from generation to generation. But at the same time, they're also agents of change – mutations. I think that one of the most important questions is how those two different functions of genes are balanced. As the environment changes, so do organisms change. It's part of evolution."

In halobacteria, these mutations are reflected in its macroscopic qualities; in other words, their appearance when grouped together in large colonies in a petri dish. A natural strain of halobacteria will have predominantly pink colonies, but also some darker, reddish-orange ones.

"I was interested in why it has that mutation rate and what the genetic mechanisms are," says DasSarma. "So you can see it had nothing to do with flotation whatsoever."

But it did, as it turns out. For the differences in the color of the colonies were due to the presence, or absence, of gas vesicles. The pink ones have them; the red ones do not. In microbiological terms these two types are "Vac plus" (the gas vesicles, also known as vacuoles, are present) and "Vac minus" (no vacuoles).

Both the Vac plus and Vac minus cells contain red and orange pigments called carotenoids. In the absence of gas vesicles the bacteria are translucent and the colonies reflect a reddish hue. When the gas vesicles are present – Vac plus cells are packed with them – they give the colonies a pink, milky appearance instead, like droplets of Pepto-Bismol.



Graduate student Vidhya Rangaswamy inspects bacteria through a microscope.

DasSarma first began studying halobacteria as a graduate student in 1980, but it was as a postdoctoral fellow at the Pasteur Institute in Paris in 1986 that he started to search for the genes responsible for gas vesicles.

A gene is a unique segment of deoxyribonucleic acid – DNA – nature's blueprint for all living things. Each gene contains the code for the construction of a specific protein, one of the major building blocks of cells, and of life.

For many years, microbiologists believed a single gene was responsible for the production of gas vesicles in bacteria. They called it the "gvp" gene, short for gas-vesicle protein gene. Although scientists had identified the gvp gene in other floating bacteria, DasSarma was the first to identify it in *Halobacterium halobium,* which he did at the Pasteur Institute.

The following September, DasSarma began teaching at UMass, where he continued his work sequencing the gas-vesicle gene, that is, determining the exact pattern of the DNA. The laboratory techniques microbiologists use for this work are similar to those that enable forensic scientists to examine and compare DNA samples in criminal investigations.

While in Paris, DasSarma had begun to suspect there was more than one gene involved in gas-vesicle production. Over the next several years in Amherst, he and his researchers discovered there were, in fact, many genes involved.

"Now that was a big surprise because this guy in England who'd studied gas vesicles for 20 years has been saying there's a single protein so there must be a single gene," says DasSarma. "Of course, no one believed us because everybody said there should only be one gene necessary, but we found there was in fact a cluster of 13 genes necessary for synthesis of gas vesicles."

The take-home message from his study on mutation, DasSarma concluded, was that halobacteria contain an unusually high number of what he calls "selfish jumping genes." These opportunistic segments of DNA literally insert themselves into the genes required to make gas vesicles, disrupting the normal genetic sequence. When these jumping genes leap into the right spot, they block the production of gas vesicles. The cells become Vac minus instead of Vac plus. Their colonies look red. The cells sink.

Even before sequencing all of the 13 individual gvp genes, DasSarma and his collaborators were able to clone the entire group. Through a lengthy and intricate series of gene-splicing techniques (a biological cut-and-paste method called "recombinant DNA"), they inserted the gvp gene cluster into Vac minus halobacteria, transforming them into Vac plus. For the first time, scientists had created gas vesicles in cells that did not have them before – in effect, programming them to float.

SHIL DASSARMA GREW UP in Calcutta in his native India, and later in West Virginia, where his father taught chemistry at West Virginia State College. He received his Ph.D. in biochemistry from the Massachusetts Institute of Technology, and followed with postdoctoral training at Harvard Medical School and Massachusetts General Hospital.

His lifelong ambition, he says, was to be a university professor, not an entrepreneur. He teaches a first-year graduate-level course at UMass in molecular genetics and is administrator of the university's Molecular Biotechnology Computer Center. Having attained his goal, DasSarma now finds himself straddling the often conflicting worlds of business and academia.

"I wasn't hired to do anything of commercial interest," he says. "I was hired as a molecular geneticist and to do basic research. It's just a complicated issue. The technology designed by us is publicly funded at a public university and here we are saying we want to commercialize it."

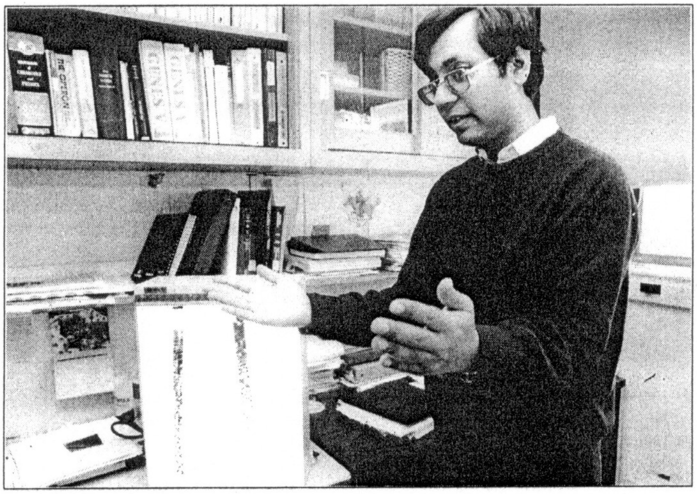
In the eight years since he arrived at UMass, the 37-year-old DasSarma has gained a reputation as one of the world's leading experts on halophilic microorganisms. He has published more than 50 papers in scientific journals based on his research, and he is the editor of a forthcoming laboratory manual – the premier lab manual, he says – for handling and manipulating halobacteria.

Writing in the university's faculty newspaper, The Campus Chronicle, in 1993, Arthur Clifford, special assistant for university relations and development, called DasSarma one of the university's youngest and most-cited scientists. Since 1986, he has been awarded grants totaling $1 million from the National Science Foundation and the National Institutes of Health, money he has used to pay research students and establish his own laboratory in the Morrill Science Center.

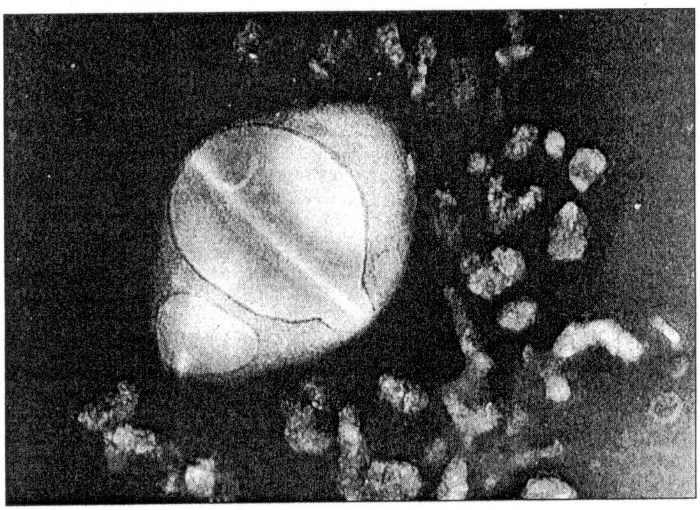
DasSarma's first two lab recruits in 1986 are now the other principal players in HaloGenetics, although the trio has met together only once in the last four years, collaborating instead through conference calls and electronic mail. John Halladay, now 32 and a postdoctoral fellow at the University of Wisconsin Medical School, was then a graduate student in microbiology. Jeffrey Jones, 38 years old and the chief architect of the HaloGenetics business plan, was DasSarma's lab technician.

For nearly four years, DasSarma, Halladay, Jones and another graduate student, Wai-Lap Ng, worked together on gas-vesicle research. Jones, who lives in Wilbraham, left the university in 1990 to take a job at EcoScience, a biotechnology company that also had its origins in a UMass laboratory.

"The first person I hired was Jeff," says DasSarma. "He really set up the laboratory and got things going. He did most of the sequencing of the gas vesicles that this whole idea is based on."



Determining the exact pattern of certain genes in Halobacterium halobium *has been essential to DasSarma's research. Here he compares two DNA samples.*



By splicing the "water-wing" *genes from* Halobacterium halobium, *HaloGenetics researchers hope to create gas vesicles, or air sacs, in nonfloating strains. Above, a gas vesicle viewed under an electron microscope.*

Halladay, who has been writing grant proposals for HaloGenetics, expects to return to Amherst later this spring. Ng is a postdoctoral fellow at the University of Washington in Seattle, and may join HaloGenetics once it is established.

In April 1992, in the scholarly journal Gene, Halladay, Ng and DasSarma announced to the scientific world that they had implanted the gas-vesicle genes into nonfloating Vac minus halobacteria cells, and turned them into the floating Vac plus type.

For a variety of reasons, DasSarma believes halobacteria could supplant *E. coli* and yeast as the principal microorganisms used in industrial biotechnology today: They are easier to grow, easier to handle and nontoxic.

Both *E. coli* and yeast require cultivation in closed, regulated fermentation systems; halobacteria grow easily in brine, and flourish naturally in vast, open-air marine salterns, the kind used by industrial salt manufacturers to distill salt from seawater.

"You can have very large amounts, vast amounts," says DasSarma. "You could never imagine growing yeast or *E. coli* in such large amounts."

Both *E. coli* and yeast have tough cell walls and are thus difficult to break open. To harvest the useful components from their bioengineered cells, it is necessary to treat them with enzymes and detergent to loosen and then rupture their cell walls. Halobacteria cells can be ruptured simply by adding tap water to their medium.

The connection between DasSarma's gene cluster and its applications first surfaced during his regular Friday afternoon meetings at UMass, brainstorming sessions in which his students discuss developments in their research and hash out new ideas and directions.

Publication of the article in the journal Gene had been preceded in late 1991 by the filing of a patent application to protect what DasSarma and his colleagues knew was an important scientific breakthrough. Lacking a patent operation of its own, the university had filed the application through Research Corporation Technologies, a nonprofit company in Tuscon, Ariz.

"The university didn't have any resources so we really basically had to sell out to RCT," says DasSarma. "We had to sell them the rights basically for about $4,000."

That money went toward further research on halobacteria, but as DasSarma notes, "That's nothing, really. For $4,000 you can support an undergraduate for three months in the summer."

Although the university technically owns the patent, RCT holds all the rights to it. This means the university would share in revenues should RCT license the patent for development into commercial products. But unless the university regains the patent rights, there is no guarantee DasSarma and his colleagues will do the development or have any further input at all. Chances are good UMass will be able to buy back the patent, say those involved. No matter what, DasSarma, Jones and Halladay have plans to file additional patents based on new and related research.

News of DasSarma's flotation genes has commanded international attention. There have been stories in scientific journals, as well as general academic and business periodicals such as The Scientist, Boardroom Reports and Inside R&D; a short article in Business Week touted the environmental implications of the "water-wing genes." In its July 1994 issue, Taipan, a high-tech business newsletter with a global circulation of 65,000, noted, "We believe the cloning techniques developed by Dr. DasSarma have the potential of creating new momentum in an industrial revolution that will reshape the world's key industries by the dawn of the 21st century."

"Any sector of the biotech industry that relies on the harvesting of cells will benefit," the Taipan article continued, "particularly the four major biotech growth areas: agriculture, human diagnostics, human therapeutics, and the biological (as opposed to chemical) treatment of waste products... ."

According to the Biotechnology Industry Organization in Washington, D.C., the 1,311 biotechnology companies in the United States had total sales of $7.7 billion in 1994, up 10 percent from 1993. In an executive summary prepared last August, DasSarma and Halladay estimated that revenue for HaloGenetics could, within 10 years, exceed $100 million a year.

How does one measure such potential?

"It's hard," says Jim Theroux, a visiting assistant professor in the UMass School of Management who has been helping DasSarma and Jones assemble a business plan for HaloGenetics. "One way is to look at the number of potential customers. When you're talking about producing vaccines, then you have a lot of potential customers. All of humanity."

The media attention has paid off for HaloGenetics, though not yet in actual dollars. DasSarma has received more than 60 inquiries from interested investors all over the world: Wall Street, Hawaii, Japan, England, Holland. But the HaloGenetics team still lacks the resources and formal structure to explore these opportunities or do their own outreach soliciting financial partners.

"Obviously, we don't want to just go with the first few investors who come along," says Jeff Jones. "It would be much preferable to be in a position to select who you want your investors to be."

As research continues in the laboratory, still funded by a grant from the National Science Foundation, always weighing heavily on DasSarma's mind is the issue of how university resources may be used for economic gain.

"Basically, we are in a sort of gray area of what is allowed and not, and to what extent can university employees spend their regular business day working toward a new venture," says DasSarma. "There's obviously an office of economic development where that is their primary job, so to some extent the university must be a valid place to be carrying out these kind of activities. But there's a limit. I have responsibilities to my own scholarly goals. I don't really want to give those up to do this."

LAST SUMMER, UMASS officials hosted the first in a series of meetings geared to introduce inventors to venture capitalists searching for investment opportunities. DasSarma was there and spent part of the meeting handing out business cards and making a short pitch about HaloGenetics to representatives from venture capital firms.

These "venture forums" were just one of several initiatives launched by the university in 1994 to promote economic development in the Pioneer Valley. Together with the forums' co-sponsor, the Pioneer Valley Economic Council, an association of regional business executives, the university founded Mass. Venture Corp., a nonprofit corporation that is expected to assist high-tech start-up companies in the region. A third plank in the university's economic development platform called for establishing a venture capital fund to provide seed money to new businesses; a fourth, to construct a special office facility to house Mass. Venture Corp. and other fledgling technology-oriented companies. (Plans for a privately funded research center in Hadley that will house Mass. Venture Corp. were unveiled last week.)

HaloGenetics is in many ways a test case for UMass. It is likely to benefit eventually from the services of Mass. Venture Corp. and the university's other economic development initiatives. At the moment, however, HaloGenetics is caught in the limbo of the transition, as the university works to develop its own patent and licensing operation.

"At one point the idea of developing technology transfer was not as high a priority as it is now," says Tripp Peake, managing partner of Science Park Associates of New Haven, Conn., a business-consulting firm under contract to UMass. "The university was starting to realize that this is something it needs to do by itself."

Before HaloGenetics can incorporate, DasSarma, Jones and Halladay need to finalize a technology transfer agreement with the university – a step that hinges on return of the patent. Under consideration are what resources UMass will supply in terms of equipment, offices and lab space, and what economic benefit the university will receive in return.

"We think it would be in HaloGenetics' best interest to remain intimate with the university for several years," says Jones. "It is the degree of intimacy that has to be worked out."

Last year, UMass officials also began reexamining the university's intellectual-property rules, which have significant implications for HaloGenetics and other UMass spin-off ventures.

"Under the current policy, the university's own patents cannot be licensed back to the researcher who developed them, due to conflict-of-interest laws," says Jaymie Chernoff, director of the university's office of economic development, which opened in January 1994.

Chernoff has been one of the principal advocates of amending the university's intellectual property rules. The rules, in effect since 1986, were established in response to a 1981 federal law that gave universities ownership of technology created in their laboratories using federal funds. As written, the university's guidelines restrict to 1 percent faculty ownership in any company with which the university transacts business.

"Someone like Shil is not allowed to own a large stake in a company that does business with the university," says Jeff Jones. "That kind of policy discourages people from starting up enterprises such as we're trying to do."

University officials agree. Proposed revisions would boost the ownership cap to 5 percent while allowing for still more following a committee review.

"It's going to be a big breakthrough," says Chernoff. "We just couldn't have invested much in technology transfer without it."

For a time it seemed HaloGenetics would have to wait for the university to sort out the new guidelines before incorporating. DasSarma and Jones said this would be necessary to settle questions about how company shares would be divided and who the corporate officers would be. DasSarma says they now intend to abide by the current rules and amend HaloGenetics' constitution if and when changes are approved.

"The primary goal of this has never been my personal profit," says DasSarma. "I'm convinced the technology can benefit society and I've always looked at it also as an opportunity for John (Halladay) and Jeff (Jones) to develop their careers."

The policy changes could bear on two other UMass faculty members, possible partners who have been instrumental in the founding stages of HaloGenetics: Samuel Black, an assistant professor of veterinary and animal science, and James Theroux, from the School of Management.

Black has been investigating the possibilities of using the gas vesicles in halobacteria to carry vaccines. Besides advising DasSarma and Jones on the business side of HaloGenetics, Theroux said he may invest his own money in DasSarma's venture. He is himself a former entrepreneur who four years ago sold a cable television company he founded in Cleveland, Ohio.

"I have to say that there are a lot of ideas that have big potential," says Theroux. "Probably my main reason for being so interested in helping out and volunteering my time is because of Shil himself. I have a sense of his integrity, his passion for science and his commitment to do more than just make money."

For any new business, however, raising capital is a critical concern. DasSarma and Jones estimate they need $200,000 for the first six months of research and development, and $2 million more for the next 12 to 18 months. DasSarma and Jones are now looking forward to incorporating by April, so HaloGenetics will be eligible in 1995 for Small Business Innovative Research grants from the federal government.

"We spent eight years and $1 million to get to this point," says DasSarma. "But we are not at the point where we have something to sell. What we have are ideas that are technically feasible and have great potential, but we still need to do a period of two or three years of R&D, we imagine, in order to bring these ideas to market."

Jeffrey Jones, the chief architect of the HaloGenetics business plan, was once DasSarma's lab technician.

Meanwhile, in DasSarma's lab, the research on halobacteria proceeds.

Present impediments aside, people with experience in starting high-tech ventures know HaloGenetics will face many more challenges as it moves ahead – tremendous challenges on both the scientific and business fronts.

"Every step of the way is difficult," says Theroux. "One Mount Everest after another."

Perhaps the biggest challenge, says Tripp Peake, will be for HaloGenetics to make the transition from a science project to a business, commercializing the technology rather than just furthering their research.

"This is tough going," says Peake. "This is tough stuff. It requires tremendous resources and the right timing, additional management and financial partners. No technology in the early stages of a business is a slam dunk."❑