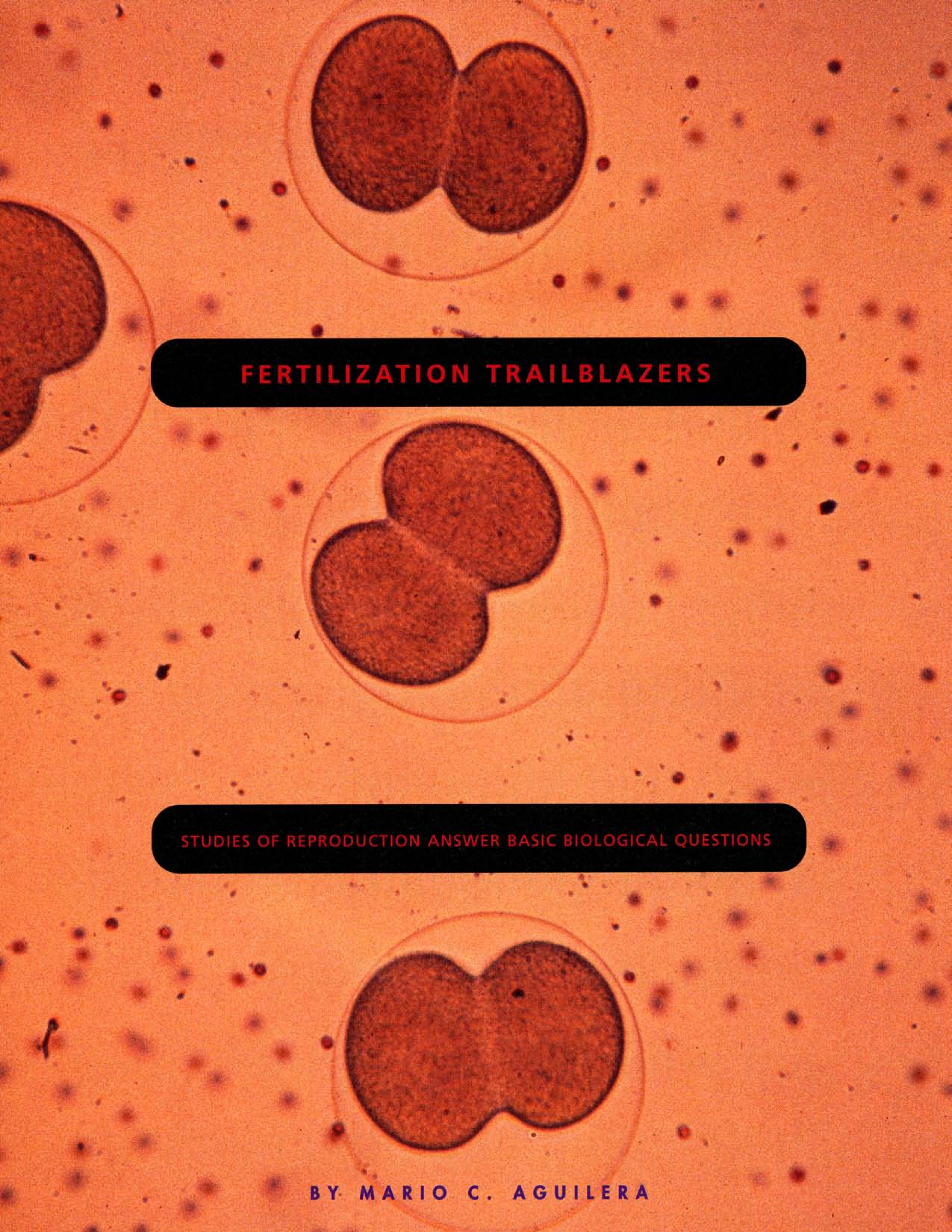
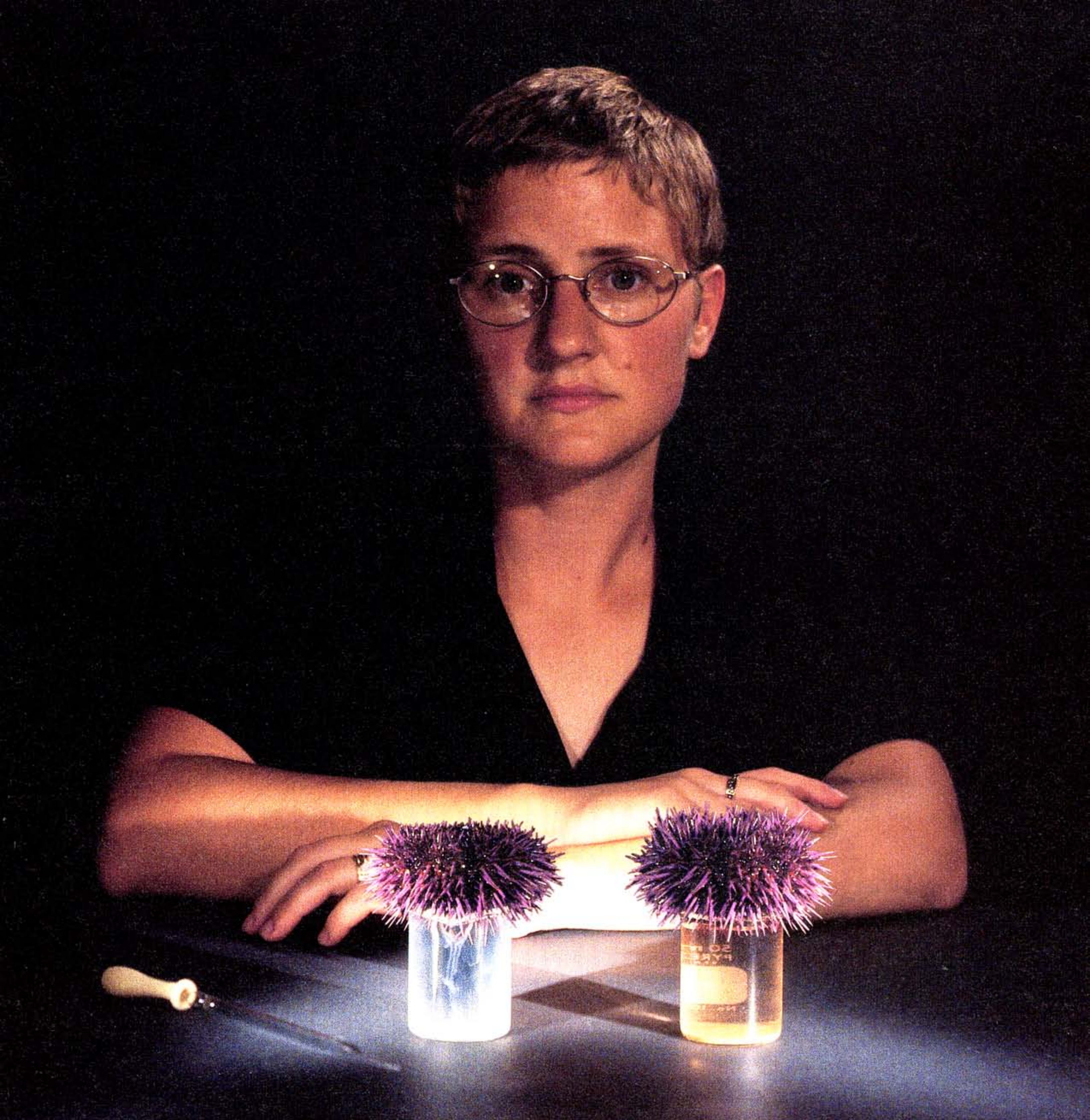


FERTILIZATION TRAILBLAZERS

STUDIES OF REPRODUCTION ANSWER BASIC BIOLOGICAL QUESTIONS

BY MARIO C. AGUILERA



In the jungles of Asia, a single mosquito bite can transmit malaria and its payload of sweat, chills, fever, and death. Villages in South America are ravaged by parasites carrying a blood-borne disease that induces heart failure. And throughout the world, about one out of every 1,000 people is afflicted with a hereditary kidney disease, caused by a gene mutation, which often results in early death.

Meanwhile, in a building overlooking the Pacific Ocean in La Jolla, California, Victor D. Vacquier and his students at Scripps Institution are busy discovering molecules that regulate sperm and egg interactions in sea urchins and abalones.

Left, The first cell division following the combination of egg and sperm in fertilization is shown here. These dividing sand dollar cells, which are very similar to sea urchin cells, are about 100 micrometers in diameter. **Above,** Graduate student Kathryn White conducts research using spawning sea urchins.

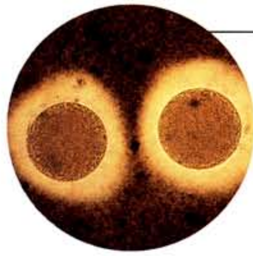
What do human diseases have to do with basic marine biology?

Vacquier, a member of Scripps's Center for Marine Biotechnology and Biomedicine, has shown that deciphering sperm and egg interactions at the cellular level in lower animals can unlock mysteries of cell interaction in higher animals.

The information gained by Vacquier during his 30-year research career can be used to elucidate human disease processes and, thus, increase the ability to prevent them. Such research may clarify how disease-causing cells invade normal cells. Knowing how sperm penetrate egg cells also can help scientists understand, and perhaps alter, processes in fertilization and conception to control insect and animal pests or increase production of food animals. Possible applications also may exist for human contraception. Understanding cellular interactions also can aid in explaining the evolution of new species.

Sea urchins and abalones don't immediately grab the casual observer as trailblazers in scientific studies. Small spherical creatures housed within shells covered with long, moveable spines, sea urchins are commonly found in shallow waters. These colorful animals usually range from two to five inches (5 to 12 cm) in diameter. They move about on tiny tube-like feet; however, many species burrow into the sand or attach to rocks to protect themselves.

Abalones have a convex, single shell, which covers their body, and a muscular "foot," that the animals use to travel over the seafloor or attach to rocks. Abalones can grow to more than twelve inches (30 cm) long and feed on algae. They live primarily in tropical and temperate oceans.



DECIPHERING SPERM AND EGG INTERACTIONS AT THE CELLULAR LEVEL IN LOWER ANIMALS CAN UNLOCK MYSTERIES OF CELL INTERACTION IN HIGHER ANIMALS.

Vacquier studies these marine invertebrates because their sperm-egg interaction occurs in seawater outside the body, making the process easy to observe. Also, they are prolific breeders; a female sea urchin releases up to 40 million eggs into the sea, where they mix with swimming sperm in a matter of seconds. Therefore large numbers of cells can be obtained quickly and easily.

Sea urchin sperm and eggs exaggerate many molecular processes common to all cells. Because the sperm of one sea urchin species will attach only to eggs of that species, their interaction is a model system for studying the general biochemistry of how cells adhere to each other.

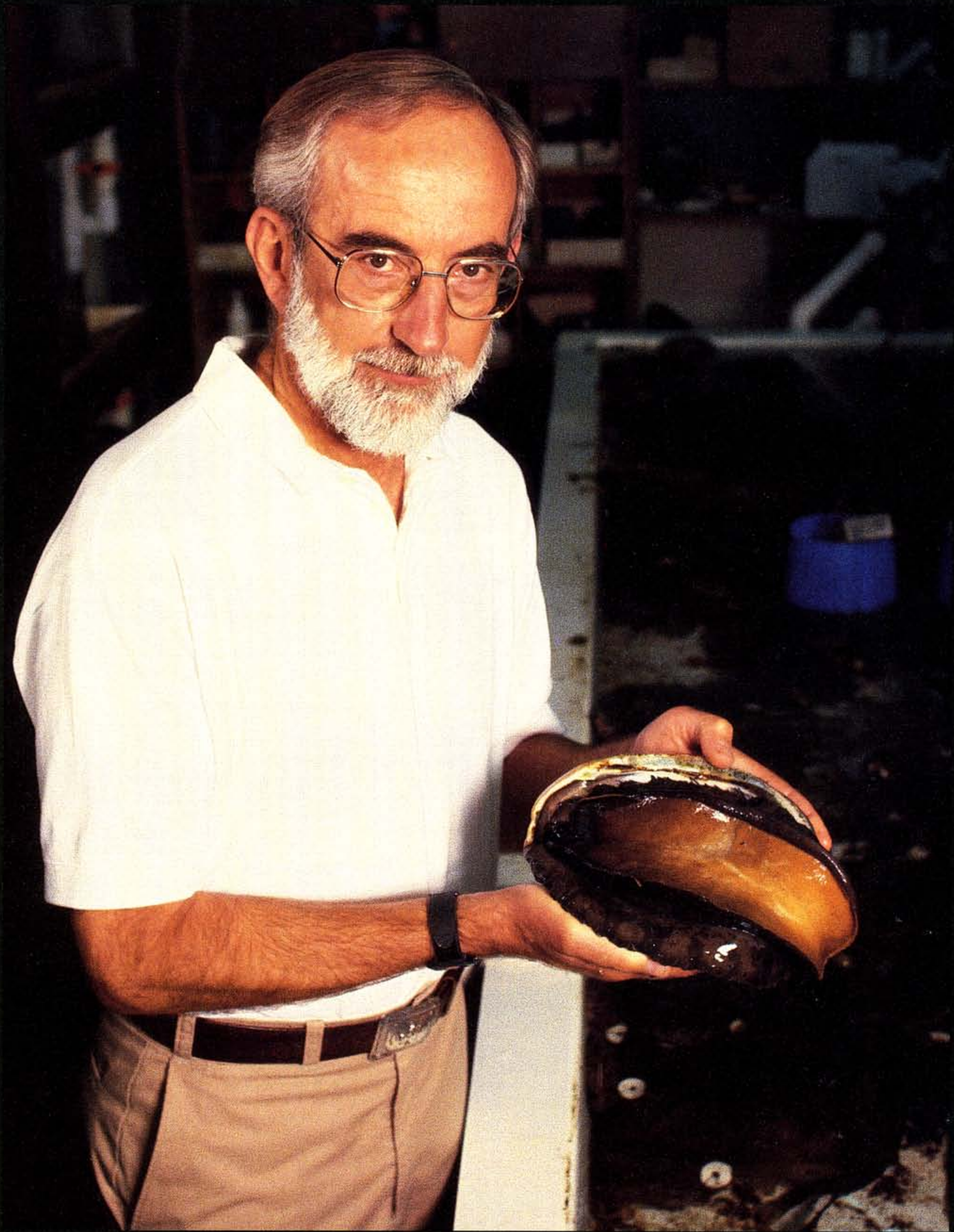
"Our main focus is cell surface proteins and how they work," said Vacquier. "If you can isolate and identify all the cell surface molecules, then you might be able to find out how they interact with each other. There are numerous examples where parasites bind to, and then penetrate other cells."

In the example of malaria, the parasite injected by the mosquito attaches specifically to red blood cells, targeting proteins of the membrane. In the South American disease, certain blood parasites exclusively attack heart cells.

In another development that may apply to biomedicine, Vacquier's group made a surprising discovery when analyzing the sperm protein REJ, or receptor for egg jelly. This protein on the surface of sea urchin eggs helps trigger fertilization by sending a signal inside the sperm cell that lets calcium into the sperm membrane.

As Vacquier's students studied the sequence of REJ, they were astonished to find that it was related to only one other known protein, human polycystin, a protein of unknown function that, when mutated, causes polycystic kidney disease (PKD). PKD is a widespread illness that can cause suffering and early death, and costs millions of dollars in medical expenses.

The similarity between REJ and PKD has led Vacquier to speculate that human polycystin is a type of regulator of membrane permeability in human cells. Since Vacquier and his team published their results in 1996, other laboratories have begun gathering evidence in support of polycystin's role as a regulator of cell permeability.



Victor D. Vacquier exposes the muscular "foot" of a red abalone. Abalones contain some of the fastest evolving proteins known, making them useful subjects for biological research.

Graduate student Silvia Armitano Mah loads a gel during electrophoresis of abalone DNA.

Vacquier's laboratory also has conducted pioneering work identifying and analyzing sperm and egg cell proteins involved in fertilization. A major discovery was the protein they named bindin.

"If you understand animal fertilization, you might be able to stop reproduction in insects that destroy crops," said Vacquier. "Another example can be seen with rodents. Half the grain supply in developing countries is eaten by rodents; if you could control fertilization you could control the rodent population. There's an economic payoff to being able to either restrict or foster greater reproduction."

HALF THE GRAIN SUPPLY IN DEVELOPING COUNTRIES IS EATEN BY RODENTS; IF YOU COULD CONTROL FERTILIZATION YOU COULD CONTROL THE RODENT POPULATION.

When the male sea urchin releases sperm, dilution in seawater activates the sperm's motility and respiration. When the swimming sperm contacts the egg, the point of the rounded sperm head undergoes a transformation into a rodlike tip.

Vacquier and his students described a fundamental sea urchin sperm protein, which unites sperm and egg. This protein is bindin. When a sea urchin sperm hits an egg, an anterior compartment opens in the sperm, releasing bindin that unites the sperm and egg. A few seconds later the membrane covering the tip of the rod fuses with the egg plasma membrane.

Other discoveries in the sperm-egg interaction process followed. In abalone, the sperm swims up to the egg and docks against its coating. To fuse with the egg, the sperm dissolves a hole in the egg surface by using a protein Vacquier and his coworkers isolated and named lysin.

Vacquier believes that basic research on the proteins involved in sperm-egg interaction could lead to the development of new methods of birth control in higher species. One of the thrusts of the National Institutes of Health, which has funded Vacquier's work for 24 years, is to develop novel methods of nonhormonal contraception. In humans this knowledge could be the forerunner of new methods for blocking sperm attachment.

Understanding the mechanisms of fertility also can be vital in areas where population increases are desired. In aquaculture, for example, these ideas could be applied in efforts to increase populations of depleted fishes, lobsters, shrimp, and shellfishes.

Studies in Vacquier's laboratory also have shed light on evolution and the biological formation of species, otherwise known as speciation.

"Speciation of the California abalones has occurred extremely rapidly," said Vacquier. "When we look at the red, white, flat, and pinto abalones, we think there are only one to two million years of divergence between those four species. Their sperm proteins mediating fertilization are some of the fastest evolving proteins yet discovered."

Vacquier and his students suggest that the receptors for the sperm proteins on the egg surface are the cause of this accelerated evolution. They believe that because these receptors are themselves evolving rapidly, the sperm protein lysin must adapt to its fast-changing egg receptor protein counterpart.

"Lysin essentially has to 'hit a moving target,'" said Vacquier, "because the receptor is evolving and changing very fast. The lysin proteins are being selected for their ability to adapt to changes in the receptor."

Willie Swanson, a graduate student in Vacquier's lab, made one of the laboratory's banner discoveries, which relates to macrophages, the scavenger white blood cells of the immune system that protect the body against infection. In 1997, as Swanson compared the sequence of an abalone protein to a database of genes, he found an abalone protein that is related to a protein in mouse macrophage cells. The discovery was startling because of the evolutionary distance in the animal kingdom between rodents and abalones. Its presence in the abalone and the mouse indicated for the first time that the protein

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Willie Swanson uses protein gel electrophoresis to examine sea urchin proteins.

must exist in every animal in between.

"This protein was only thought to be in mammals, and here it is in abalone," said Vacquier. "That means that it exists throughout the animal kingdom. There's so much evolutionary distance between the two that it's remarkable."

While he has pushed marine invertebrate sperm-egg interaction studies further than anyone else, Vacquier is the first to say that, like the unexplored vastness of the oceans, plenty of mysteries lie ahead.

"Fertilization is still mostly an unknown process," said Vacquier. "It is one of the least understood basic biological phenomena."

And at 58, nothing will slow him down from trying to solve these mysteries for many more years.

"I want to keep involved with science because I enjoy it so much. I love doing it. I want to know the answers to the questions nature poses."

FAMILY ENVIRONMENT KEY TO LAB SUCCESS

Victor D. Vacquier has two loves in life: science and family. He applies a youthful enthusiasm equally to marine biology, the family he nurtures at home, and the second family he parents in his Scripps laboratory.

"I have no formal ways of dealing with students, but I hope they'll get excited about working in the lab because what they're doing is discovering the secrets of nature," said Vacquier. "You let them get excited about research and don't give them too much direction. You provide for them by having everything here and all the equipment they will need. And you talk to them... you just talk to them about doing research."

Whether discussing why an experiment isn't working or making mellow chit-chat at his home during occasional laboratory barbecues, Vacquier creates an atmosphere of comradery vital to the running of the laboratory.

"Every day he keeps us going," said graduate student Silvia Armitano Mah. "He's excited about his work and he's excited about everybody else's work. He will come in the lab in the morning and say 'Oh this is a great morning,' and you feel like you're on top of the world, and there's nothing you can't do."

Vacquier's philosophy shows in his technique for writing grants and papers. Research proposals written by Vacquier are tossed onto the middle of a conference table, where each member of the lab painstakingly dissects and analyzes each paragraph. Everyone's input counts. The "all-lab proposals" have resulted in a prestigious Merit Award from the National Institutes of Health in 1996 and impressive scores, including a number one ranking.

"When we write papers, we write the first draft and we sit down with him and we go over it, line by line and word by word," said Willie Swanson, who earned his Ph.D. this past summer. "I learned how to write a grant and how to get funding, and I plan to use the same technique if I'm lucky enough to get my own lab."

Vacquier talks to each of his students for at least a few minutes per day; sometimes for hours. Open communication is one of the keys to his laboratory's success.

"Vic wants production, which is an interesting thing because he creates an environment in which the students want to get those results as badly as he does, rather than feeling forced or fearing the consequences," said Ed Metz, a former postdoctoral researcher and a member of the laboratory's historical "family." "He's a master at constructing a creative atmosphere. He builds enthusiasm. He constantly emphasizes the importance of the work and how unique the systems are and the problems that we study."

According to Vacquier, it's the professor who gains much from the professor-student relationship.

"I've learned most of what I know about science from the students and postdoctoral researchers in the lab," said Vacquier. "The most wonderful part about all this is the interaction between the professor and the student and how it builds through the years. A student comes to the lab knowing almost nothing and leaves six years later as a true colleague in every sense of the word. They're as familiar with the literature as the professor, and they often have better ideas. I help them, and they in turn help me enormously. I couldn't do it alone; the students are the reason to be here."

Following Swanson's successful defense of his Ph.D. thesis, he is toasted by Victor D. Vacquier.