Profile of Steven C. Hebert

Humans have been obsessed with salt for thousands of years. The quest to control it has provoked wars, and salt was once considered so valuable that it was used as currency. But salt's importance to *Homo sapiens* is far more fundamental. Salts, not only common table salt but also those containing other metals such as potassium and calcium, are essential for life. Steven Hebert, Chair of the Department of Cellular and Molecular Physiology at Yale University School of Medicine (New Haven, CT), has spent his career studying mechanisms that regulate salt in the body. Hebert discovered three families of transporters that shuttle salts in and out of cells, ensuring a balance that keeps the heart, brain, and muscles working properly. Elected to the National Academy of Sciences (NAS) in 2005, Hebert's Inaugural Article in this issue of PNAS (1) demonstrates a new paradigm for treating secretory diarrhea by activating a salt-sensing protein called the calcium-sensing receptor.

Steven C. Hebert

Starting from Sea Salt

Hebert was born in 1946 in Rockford, IL, a mid-sized industrial city 90 miles northwest of Chicago. When Hebert was 5, his father, an electrical contractor, won a subcontract from the Morton Salt Company to develop the sea salt rights to and facilities in a small Bahamian island called Great Inagua, a "fairly primitive" place, according to Hebert, within sight of Cuba. Island life was simple, says Hebert. "We had only short-wave radio contact with Nassau, and a tanker would come in once a month with supplies for the whole population of the island," he recalls.

One of Hebert's most vivid memories of Inagua was of the salt works where large, shallow areas of coral were flooded with seawater. The tropical sun evaporated the water leaving a thick white crust that bulldozers piled into sea salt mountains 100 to 150 feet high. "So maybe this is where I got my interest in salt," Hebert says. He lived on Inagua for approximately 6 years, attending school with a private tutor from 8:00 AM to 1:00 PM, Monday through Friday, year round. By the time he returned to the United States, he was four grades ahead of most children his age.

The family settled in Fort Lauderdale, FL, where Hebert attended high school and skipped two grades to enter Florida State University (Tallahassee) in 1963 at age 15. "My parents were smart people, but they didn't go to college," he says, "they began working at an early age, worked through the Depression, and they always valued education." At Florida State University, Hebert majored in mathematics and physics after developing an interest for these areas in high school. However, during his junior term, medicine piqued his curiosity, and he began taking biology classes. "I also felt that there were much broader opportunities in biology and biological research than in physics," he says.

Hebert finished college in 3 years and entered the University of Florida College of Medicine (Gainesville) in 1966, assuming he would become a practicing physician. But the universe seemed to conspire to lure him into research. For example, while in medical school, nephrologist Robert Cade was conducting research that would eventually lead to the first commercial sports drink, Gatorade, named after the school's football team. Cade investigated which electrolytes were lost in sweat and urine during exercise, and he measured body fluid spaces, salts, and salt losses in University of Florida athletes. Cade hypothesized that replacing these lost salts would revitalize the body and sustain it during long periods of exercise. This research caught Hebert's interest, as did the work of professor Thomas Maren, who became a lifelong acquaintance. Maren was internationally recognized for his work on the enzyme carbonic anhydrase, a critical component of many fluid and electrolyte transport systems. "Thus, in medical school I was surrounded by innovative scientists and educators who translated their discoveries to healthcare," says Hebert.

In 1970, just a few months before receiving his M.D., Hebert married Patricia Robertson, a registered nurse at the University of Florida. The couple moved to the University of Alabama at Birmingham (UAB) where Hebert had chosen a residency in internal medicine at the UAB Hospital. He was drawn to the hospital in large part by the opportunity to gain experience in frontline medicine and trauma care, "and the university's great football team," adds Hebert.

There he met researcher and physician Thomas Andreoli, who catalyzed Hebert's transition from physician to physician-scientist. Andreoli, who would later become president of both the American and the International Societies of Nephrology, had just moved from Duke University (Durham, NC) to direct UAB's kidney program. "He was, and is, an extraordinarily bright scientist," says Hebert, "and he excited me about all of the kinds of things going on in understanding how the kidney works." The pair struck up a relationship that has lasted more than 35 years. Says Hebert, "he became one of my important mentors, and I think he helped me solidify my interest in body salt metabolism."

The late 1960s through the early 1980s is widely considered a Renaissance in nephrology. At the time, says Hebert, it was clear that body-salt metabolism and balance were key to understanding heart failure and high blood pressure, as well as a variety of other disease-related salt imbalances, many potentially lethal. It was also clear that the kidney was an important player, if not the final arbiter, of salt balance. During this period, new methodologies enabled the isolation of various epithelial segments of the nephron, the functional unit of the kidney. These techniques allowed researchers to associate discrete regions of the nephron with the transport of particular ions. "That aspect of the kidney function was fascinating to me," says Hebert.

Boot Camp

After his medical residency, Hebert remained at UAB as a research fellow. He

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 9390.

www.pnas.org/cgi/doi/10.1073/pnas.0604149103

specialized in nephrology and focused his research on how the kidney regulated salt balance. But when his fellowship ended in 1975, Hebert was drafted into the military and spent the next 2 years stationed at the U.S. Naval Regional Medical Center in Portsmouth, VA, where he practiced clinical nephrology. "I saw many patients with interesting kidney disease, which intensified my interest in understanding fluid and electrolyte metabolism . . . major conditions nephrologists diagnose and treat. I learned what things were unknown and therefore what kinds of questions needed to be addressed," he says. When Hebert completed his military service in 1977, he was hired as Assistant Professor of Medicine at UAB in Andreoli's program.

In 1979, Andreoli moved to the University of Texas Medical School in Houston, and Hebert joined his team there after being hired as an assistant professor. Hebert continued to work with Andreoli until 1984. Hebert was interested in how salt and water were processed in the distal nephron. He wanted to know how salts like sodium, chloride, and potassium were absorbed, what factors regulated their absorption, and how the body fine-tuned the quantity of salt excreted to achieve the final balance. "When I got into the field as a researcher, it was extraordinarily exciting—almost anything that you found was new," says Hebert.

The distal nephron has particular clinical importance because it is where diuretics, drugs used to treat hypertension and volume overload, exert their effect. Andreoli and Hebert used a battery of techniques to define the physiology and mechanisms of salt transport and focused their studies on a region of the distal nephron called the thick ascending limb, which is a part of the Loop of Henle. The thick ascending limb is critical for absorbing a large percentage of salt back into the body and for separating salt and water required for the kidney to both dilute and concentrate urine. "This was a heady time in research, and our lab made important contributions to the understanding of the mechanism and regulation of salt transport in this region [distal nephron] of the kidney," he says.

One Decade, Three Gene Families

In 1984, Hebert accepted an Assistant Professor of Medicine position in Boston and joined Harvard Medical School and the renal section of Brigham and Women's Hospital, which was led by "nephrology giant" Barry Brenner. Hebert yearned to understand salt transporters at the molecular level and realized he would need to clone them—a daunting task with the human genome project in its infancy. At that time, only a few mammalian ion transport proteins had been cloned: the cystic fibrosis chloride channel and some voltage-gated sodium and potassium channels.

"So I took a leap of faith. I was going to give up all aspects of my lab associated with the understanding the physiology of these transport processes and focus on their cloning," says Hebert. One idea that made the challenge tractable was that genes could be cloned based on their specific function. The key was to identify a tissue expressing high levels of the transport activity of interest. In the late 1980s, Hebert began searching for the Na-K-2Cl cotransporter in the mammalian kidney. He knew from his studies with Andreoli that the transporter was highly active in the ascending limb and was vital for salt transport in the region. After a year and a half, Hebert made only limited progress.

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Around this time, however, "lightning struck," says Hebert. "My big insight was using the bladder of a bony fish, the winter flounder, *Pseudopleuronectes americanus*." The idea stemmed from Hebert's summer stints at the Mount Desert Island Biological Laboratory in Salisbury Cove, ME, just a few years earlier. He had studied the kidney of cartilaginous fish and learned from other researchers that the bladder of a winter flounder expressed considerable Na-Cl cotransport activity. Hebert hypothesized that if he could identify this Na-Cl transporter in the flounder, he might be able to find the human Na-Cl and Na-K-Cl cotransporters in the mammalian kidney by homology. Hebert isolated total mRNA from the fish bladder, fractioned the transcripts by size, and took each size pool and injected it into a *Xenopus laevis* frog oocyte as a reporter cell. If the transport assay showed the oocyte expressing the anticipated salt transport, then a cDNA library was made from that size fraction of mRNA. The cDNA library was then used to produce cRNA, and the entire procedure was repeated with decreasing fractions until only a single transcript was tied to the transporter activity.

Within 4 months, this "functional cloning" technique paid off. This fish Na-Cl transporter was the first member of a new gene family that now includes mammalian Na-Cl cotransporter (NCC), the target for thiazide diuretics, and the mammalian Na-K-2Cl cotransporter (NKCC2) that is critical for "loop diuretics" (2, 3). Subsequently, several laboratories contributed additional family members, including the K-Cl cotransporters. Many of these transporter genes are expressed not only in the kidney but also throughout the body where they play critical roles in salt transport and the regulation of cell volume and chloride activity.

Hebert then turned his attention to potassium, a critical salt regulated by the kidney. If potassium balance goes awry, the muscles, heart, and brain will not function properly because potassium concentration is essential for establishing membrane potential, which is vital for the function of these cells. Hebert successfully applied the same method he used to identify the Na-Cl and Na-K-2Cl transporters and cloned a potassium channel called ROMK that is required for normal activity of the NKCC2 in kidney. ROMK, referred to as an inwardly rectifying potassium channel, was unique. It represented a novel structural paradigm for a potassium channel because it had only two membrane spans. ROMK was also the major channel involved in potassium secretion in the distal nephron (4).

Potassium channels identified in Hebert's laboratory, and fellow NAS member Lily Jan's laboratory at University of California, San Francisco, triggered a flood of interest culminating in the identification of all members of this family, including one involved in regulating insulin secretion in pancreatic β cells and others expressed in blood vessels, heart, and brain (5).

In addition to his other two cloning efforts in the late 1980s, Hebert launched a third project to investigate calcium homeostasis. Edward Brown, an endocrinologist and colleague at Brigham and Women's Hospital, was interested in how calcium regulated the secretion of a parathyroid hormone from the parathyroid glands. Hebert wondered how calcium regulated salt transport in the kidney. Hebert and Brown teamed up to identify the mechanism controlling how cells recognize and respond to changes in extracellular calcium. Brown and Hebert used the func-

tional cloning strategy that had yielded Hebert's two previous gene families.

Studies in the kidney and parathyroid suggested to Hebert and Brown that their calcium monitor might be a G protein-coupled receptor. "This was pretty much heretical," says Hebert, "because we were suggesting that the G protein-coupled receptor would recognize an inorganic ion as its primary binding partner—that was unheard of." The pair began their search in 1990, and 3 years later published the cloning and characterization of their new gene, the extracellular calcium-sensing receptor (CaSR), a member of family C of the G protein-coupled receptor superfamily (6–9). CaSR was most homologous to pheromone receptors. "So we actually think of this receptor as being the nose on a cell, and it's sniffing extracellular calcium concentration," says Hebert.

The finding triggered a paradigm shift because it meant that calcium, a structural element in bone, an important enzyme cofactor, and a cellular second messenger, was a first messenger, just like hormones. "So this was rather unique and remains really unique," Hebert says. CaSR regulates parathyroid hormone secretion, which is critical for calcium homeostasis and bone function and modeling. CaSR's discovery led to alliances with pharmaceutical companies to find compounds that would activate the receptor. For example, a receptor agonist discovered by Amgen is currently being used to treat hyperparathyroidism.

Biotech Venture: MariCal

The study of CaSR also led to another venture. In the mid-1990s, Hebert, Brown, and William Harris, a pediatric nephrologist and another Harvard colleague based at Children's Hospital, found that CaSR was the master salinity sensor in fish (10). They subsequently launched a marine biotechnology company, currently called MariCal, for marine calcium. Seawater is rich in sodium chloride but also in calcium and magnesium. Fish sense their marine environment by detecting the calcium and magnesium concentrations via these receptors. This ability is particularly important for fish to adapt from fresh to salty water, a process called smoltification.

But exactly when fish such as salmon smolt is unpredictable. "We developed a way to do this faithfully, reproducibly, and rapidly," says Hebert. "Most importantly," he adds, "we can do this by nongenetically modifying the expression of this receptor in marine fish."

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Salmon do most of their growing in salty water, and MariCal is collaborating with the aquaculture industry to match the receptor activity to specific salinity conditions.

Just as MariCal was getting off the ground, Hebert received an offer from Vanderbilt University Medical School (Nashville, TN) in 1997 to run a major clinical program in kidney medicine. Hebert accepted, but within 3 years he accepted another offer from Yale University School of Medicine to run the Department of Cellular and Molecular Physiology. The position represented a shift from clinical medicine to a new focus on basic research and teaching. "It was an opportunity to lead a world-class department well recognized for work in ion transport," says Hebert. The opportunity also allowed Hebert to continue his close collaboration with another NAS member, Gerhard Giebisch, a longtime friend and mentor, who is an expert in potassium transport and internationally recognized for decoding the mechanisms of potassium homeostasis.

Treating Secretory Disease

Since moving to Yale, Hebert has continued his work on understanding the function and regulation of all three families of transporters. Hebert's PNAS Inaugural Article (1), however, focuses specifically on CaSR. The receptor is highly expressed in the gastrointestinal system and is an important regulator of salt transport across the intestine, just as it is an important regulator of salt transport in the kidney.

What makes CaSR clinically important is its ability to stop fluid loss triggered by cholera toxin and another bacterial toxin called STa, a factor that causes travelers' and infantile diarrhea. Secretory diarrheas kill millions of people worldwide, and current treatments focus on oral rehydration. But Hebert and his colleagues showed that activating CaSR decreased cAMP and cGMP, which halts salt and water loss across the intestine. The finding suggests that CaSR may be a drug target for treating secretory diarrheas.

After discovering four genes, all of which are involved in human disease, Hebert clearly has his favorite. "I love them all . . . but if I had to pick one that has had the broadest and most direct impact in human disease, it would certainly be the calcium-sensing receptor," he says. CaSR has broken new ground and redefined the range of receptor functions present in diverse cell types from marine species to land animals.

Seeing his research work come full circle has been extraordinary, says Hebert. "Scientifically I would say I'm an integrator . . . although I want to know the molecular mechanism in the finest detail . . . I want to understand what that means at the whole organismal level," he says. "Translating one's science to human disease is very satisfying."

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