NOBEL PRIZE 2004

A Discerning Obsession

The study of smell brings two HHMI investigators the Nobel Prize.

The scent of verbena. Lemony and delicate in small whiffs, the fragrance can be overwhelming in clouds. Drusilla, William Faulkner's implacable Civil War widow in The Unvanquished, *wore sprigs of the flower in her hair, saying it was the only scent strong enough to be detected above the smell of horses and courage and so the only one worth wearing.*

Smell is powerful. In an instant, it can transport us to another time and place, warn us of danger, plunge us into despair, or lift us to rhapsody. Of all our senses, smell is the one we tend to take for granted. Yet humans and all other animals rely on it in countless practical and even primal ways to identify our food, our predators, our mates.

The olfactory mechanics that make possible this exquisite ability to discern smells from the most subtle to the blatant have been the subject of study for Richard Axel and Linda B. Buck for much of their research careers. Now, the two HHMI investigators—Axel at Columbia University College of Physicians and Surgeons and Buck at the Fred Hutchinson Cancer Research Center—are enjoying, as newspaper headlines have noted, "the sweet smell of success." On October 4th of this year, Axel and Buck received early-morning calls from Sweden informing them they had won the 2004 Nobel Prize in Physiology or Medicine for their discoveries of "odorant receptors and the organization of the olfactory system."

Before 1991, when Axel and Buck published their seminal discovery of odorant receptors, little was known about the mechanics of smell. Researchers—Axel and Buck among them—were essentially groping in the fog, trying to find some solid information as to the biological underpinnings. Perseverance—and a good dose of ingenuity on Buck's part—finally yielded the breakthrough that revolutionized the field.

ELEGANT EXPERIMENTS

In 1991, Buck was a postdoctoral fellow in Axel's lab. She had embarked on a search for odorant receptors three years earlier, after having already completed a postdoctoral project in Axel's lab on the neuronal system of the sea slug *Aplysia.*

Buck's background was in immunology, and she had also been trying to develop a method to identify rearranged genes in the mammalian nervous system. "I was intrigued by the possibility that gene rearrangement or gene conversion might be involved in the generation of a varied set of odorant receptors or regulate their expression, as with antigen receptors in the immune system," she says. "I became obsessed with finding the odorant receptors and stayed on in Richard's lab to look for them."

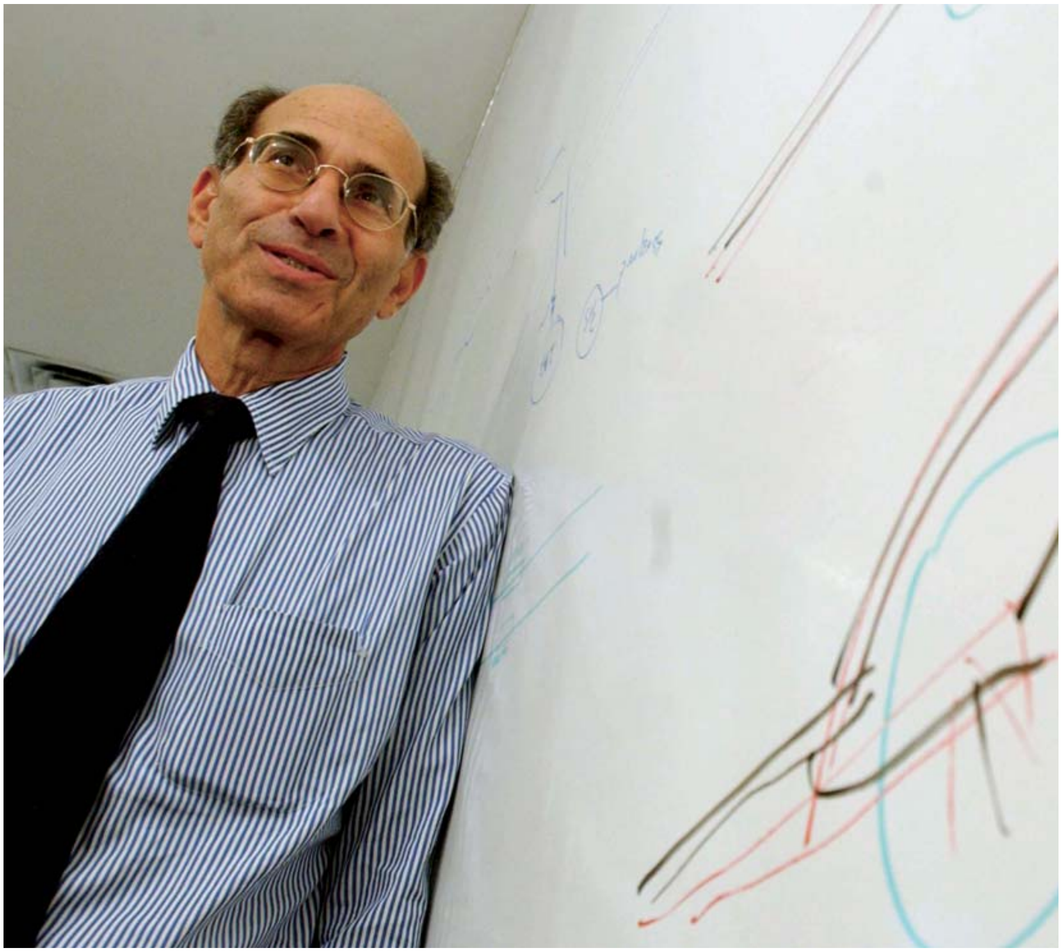
Buck and Axel initially adopted an "unbiased approach" with regard to the structure of odorant receptors, choosing to focus on two assumptions: that the receptor proteins would be selectively expressed by olfactory sensory neurons and, given the structural diversity of odorants, there would be a family of related, but varied, odorant receptors that would be encoded by a family of related genes.

Their efforts produced nothing at first. The tide turned when they added an additional assumption to their search. Based on scattered evidence from other labs, Buck made the decision to narrow her search to a family of proteins called G protein-coupled receptors (GPCRs), many of which were known to be involved in cell signaling. Making use of the recently developed gene amplification technology called PCR, or polymerase chain reaction, Buck says, she "decided to conduct an exhaustive search for GPCRs in the olfactory epithelium by taking a novel approach that involved using a number of different degenerate primers in a combinatorial fashion."

Further analysis of the PCR products narrowed the search to one candidate. "When I cloned this PCR product and sequenced five of the clones, I found precisely what we had been looking for," says Buck.

Buck's go-for-broke instincts obviated the need to sort through thousands of genes and, according to Axel, "saved several years of drudgery."

Scientific perseverance and a dose of ingenuity led to Linda Buck and Richard Axel's discovery of odorant receptors.



JENNIFER ALTMAN; OPPOSITE PAGE: DAN LAMONT

"I had tried so many things and had been working so hard for years, with nothing to show for it," says Buck. "So when I finally found the genes in 1991, I couldn't believe it. None of them had ever been seen before. They were all different, but all related to each other. That was very satisfying."

Buck found that, in contrast to the immune system, where genetic rearrangement and mutation create antibody diversity, each of the olfactory genes faithfully encodes a single receptor protein. The radical finding opened up the field of olfactory biology, providing the tools necessary to explore the mechanisms underlying odor perception at the molecular level. In the ensuing years, researchers—including Axel and Buck, who have worked independently since 1992—have filled in many of the blanks in our understanding of how we smell.

At every inhalation, air currents swirl up through the nostrils and past a thumbprint-sized patch of packed cells—some five million olfactory neurons, each neuron topped with hair-like cilia, each cilium lined with receptors. In the mouse, about a thousand genes encode an equivalent number of different receptor types. Humans have over 600 odorant receptor genes—representing a remarkable 2 to 3 percent of the entire genome—but perhaps because we no longer need a nose as keen as that of a mouse, about one-half of those genes are nonfunctional pseudogenes.

Still, with our 350 different receptor types, humans can detect an estimated 10,000 to 100,000 different odorants. "It is remarkable that we can smell so many different chemicals," says Buck. "And it's also amazing that even a slight change in the structure of the chemical can totally change the way it smells to us."

With so few receptor types, how are we able to recognize the thousands of different odorant molecules of various shapes and sizes that we encounter? And how is it that in some cases a faint whiff is enticing while a strong burst is repellent? Somewhere in the arrangement by which odorants bind to receptors and initiate an electrical signal that travels along axons to the brain's olfactory bulb (just above the back of the nose) and then on to the cortex, says Axel, lies "an intricate logic that the brain uses to identify the odor, distinguish it from others, and trigger an emotional or behavioral response."

TEASING OUT THE LOGIC

In the years since their discovery of the receptor genes, Buck and Axel have made progress in teasing out that logic. In 1993–1994, Axel, at Columbia, and Buck, then at Harvard University, reported that neurons expressing the same kind of odorant receptor are scattered throughout the olfactory epithelium (surface tissue), but that their axons all converge at specific points in the olfactory bulb that are similar among individuals. This arrangement could, Buck theorized, not only enhance sensitivity but also ensure continued olfactory function should some part of the epithelium be damaged by infection.

By 1999, Buck and colleagues had uncovered "combinatorial receptor codes," a finding that explains how we can distinguish a seemingly unlimited number and variety of odorants. The researchers showed that the odorant receptor family is used combinatorially to detect odorants and encode their unique identities. They found that a

To Learn More

More information on Linda B. Buck and Richard Axel's work may be found at the Nobel Prize site (http://nobelprize.org/medicine/laureates/2004/) and at these other Web sites:

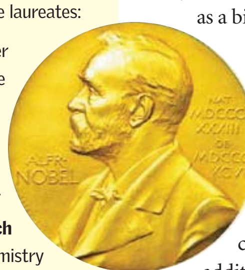
LINDA B. BUCK
www.hhmi.org/research/investigators/buck_bio.html
www.fhcrc.org/visitor/nobel/buck/

RICHARD AXEL
www.hhmi.org/research/investigators/axel_bio.html
http://cpmcnet.columbia.edu/dept/neurobeh/axel/overview.html

HHMI and the Nobel Prize

Ten current HHMI investigators have won the Nobel Prize. In addition to Richard Axel and Linda B. Buck, the list includes these laureates:

- **Günter Blobel**, at the Rockefeller University, won the 1999 Nobel Prize in Physiology or Medicine for his discovery that certain proteins use intrinsic signals to govern their transport and localization in the cell.
- HHMI President **Thomas R. Cech** shared the 1989 Nobel Prize in Chemistry for the discovery that RNA in living cells is not only a molecule of heredity but can also function as a biocatalyst.
- **Johann Deisenhofer**, at the University of Texas Southwestern Medical Center at Dallas, shared the 1988 Nobel Prize in Chemistry for work that used x-ray crystallography to describe the structure of a protein involved in photosynthesis.
- **H. Robert Horvitz**, at the Massachusetts Institute of Technology, shared the 2002 Nobel Prize in Physiology or Medicine for identifying key genes that regulate organ development and programmed cell death.
- **Eric R. Kandel**, at Columbia University College of Physicians and Surgeons, shared the 2000 Nobel Prize in Physiology or Medicine for discoveries concerning signal transduction in the nervous system.
- **Roderick MacKinnon**, at the Rockefeller University, shared the 2003 Nobel Prize in Chemistry for research on the structure and function of cellular ion channels.
- **Susumu Tonegawa**, at the Massachusetts Institute of Technology, won the 1987 Nobel Prize in Physiology or Medicine for discovering the genetic principle for generation of antibody diversity.
- **Eric Wieschaus,** at Princeton University, shared the 1995 Nobel Prize in Physiology or Medicine for identifying 15 genes of key importance in determining the body plan and the formation of body segments of the fruit fly, *Drosophila melanogaster.*



single receptor can recognize multiple odorants and that a single odorant is typically recognized by multiple receptors, but that different odorants are recognized by different combinations of receptors. By virtue of this strategy, Buck says, the system has the potential to generate as many as a billion different codes.

The group also found that odorants with nearly identical structures have different receptor codes, a finding that explains why a slight change in its structure can alter a chemical's smell. They also found that raising the concentration of an odorant recruits additional receptors into the response. This would explain why the appeal of verbena and other odorants varies with concentration.

More recently, Buck and her colleagues devised a genetic method for tracing neural circuits that allowed them to visualize how signals from different odorant receptors are organized in the olfactory cortex. They found that inputs from one receptor type are mapped onto clusters of cortical neurons at specific locations that differ for different receptors, but are the same among individuals. Surprisingly, while signals derived from different receptors are segregated in the olfactory bulb, signals from different receptors partially overlap in the cortex, and single neurons may receive combinatorial inputs from different receptors. Buck speculates that this could allow an initial integration of the components of an odorant's receptor code and thus be important in the "reconstruction" of an odorant's identity from its deconstructed features.

At the same time, Axel and his colleagues have turned to the fly brain to explore how olfactory information is encoded in higher brain areas. "There is a remarkable conservation of much of the logic of olfactory perception between insects and mammals, such that the basic principles of odor discrimination, we believe, have been conserved over 500 million years," says Axel.

By studying the fruit fly in particular, Axel hopes to learn more about how odor can influence behavior. Fruit flies rely largely on scent to assess their surroundings and guide their social interactions. Certain odors, for example, play a critical role in flies' mating behavior, as is also true in rodents and even in humans. By studying how the flies process olfactory cues and how those cues are translated into behavior, Axel's group hopes to gain a better understanding of the neural circuitry involved. Visualizing the axonal projections of single neurons in the fly's antennal lobe, which is analogous to the mammalian olfactory bulb, they found that the patterning of projections from the antennal lobe to the mushroom body (a cluster of neurons where learning is centered) is conserved among individuals, but differs for different antennal lobe neurons.

After hearing from the Nobel committee on their selection, Axel was quick to credit the many students and fellows in his lab who contributed to the work. "As scientists, we don't work in a vacuum," he says. "We work together toward often common goals with a passion and an intensity. I've been blessed over the years with a remarkably strong and exciting group of students who did much of the work towards this end."

I had tried so many things and had been working so hard for years, with nothing to show for it. So when I finally found the genes in 1991, I couldn't believe it. —LINDA BUCK

It's the process of collaboration and discovery, Axel says, that gives his life's work meaning. "The joy of science," he says, "is in the process, not in the end."

Buck, who is one of only a dozen women to have won a Nobel Prize in the sciences, also appreciates the deep satisfaction that a career in research can give. She says she was "hooked" early on by the "monumental problem and wonderful puzzle" of olfactory perception, and she encourages her students to seek that same fascination in their own work, whatever that may be. "You want to do something that you're obsessed with, that you just have to understand," Buck says. "That's where the joy comes from. That's where the great discoveries come from."

—MARY BETH GARDINER