# Autobiography

**Seattle**

I was born in 1947 in Seattle, Washington, a city surrounded by mountains, forests, and the sea. My mother was the daughter of Swedish immigrants who had come to the US in the late nineteenth century while my father's family had Irish roots on one side and ancestors extending back to the American Revolution on the other. I was the second of three children, all girls. My mother was a homemaker who was exceptionally kind and witty and loved word puzzles. My father was an electrical engineer who, at home, spent much of his time inventing things and building them in our basement. It may be that my parents' interest in puzzles and inventions planted the seeds for my future affinity for science, but I never imagined as a child that I would someday be a scientist.

During my childhood, I did the things that girls often do, such as playing with dolls. I was also curious and easily bored though, so I frequently embarked on what were to me new adventures. Aside from school and music lessons, my life was relatively unstructured and I was given considerable independence. I learned to appreciate music and beauty from my mother and my father taught me how to use power tools and build things. I spent a lot of time with my maternal grandmother, who told me magical stories about her girlhood in Sweden and, to my delight, taught me how to sew clothes for my dolls. I was fortunate to have wonderfully supportive parents who told me that I had the ability to do anything I wanted with my life. They taught me to think independently and to be critical of my own ideas, and they urged me to do something worthwhile with my life, in my mother's words, to "not settle for something mediocre". I realize now that I internalized those lessons and that they have influenced my work as a scientist.

I received my undergraduate education at the University of Washington, which was only a few miles from our home. I had always wanted to have a career in which I would help others, so I initially decided to major in psychology, thinking that I would become a psychotherapist. Over time, my interests expanded and I entertained a variety of different career possibilities. However, none seemed ideal and I was reluctant to embark on something that might prove to be inappropriate. Over the next several years, I intermittently traveled, lived on a nearby island, and took more classes in Seattle. I finally found my direction when I took a course in immunology, which I found fascinating. I would be a biologist.

**Dallas**

In 1975, I began graduate school in the Microbiology Department at the University of Texas Medical Center in Dallas. The department had recently undergone an expansion in the area of immunology, making it a major center in this still young area and a stimulating place to learn. I had done a small amount of research at the University of Washington, first in psychology with Walter Makous and then in immunology with

Ursula Storb, but it was in Texas that I truly learned to be a scientist. I had a wonderful thesis advisor, Ellen Vitetta, who demanded excellence and precision in research, habits that I believe are important to learn as a student. For my thesis, I compared the functional properties of subsets of B lymphocytes that differed in the class of cell surface immunoglobulin that they used as antigen receptors. In this work and much of my subsequent work, I thought in terms of molecules and the molecular mechanisms underlying biological systems, and sought to gain insight into those mechanisms in my experiments.

**New York**

In 1980, I moved to Columbia University in New York City to do postdoctoral work in immunology with Benvenuto Pernis. As a graduate student, I had become fascinated with the unexplained requirement for major histocompatibility complex (MHC) proteins in immune responses, a mystery that was later solved. I decided to explore this puzzle, focusing on class II MHC proteins found on the surface of B lymphocytes. I found that, contrary to expectation, the MHC proteins rapidly accumulated inside these cells when they were activated. My further experiments indicated that they were being internalized from the cell surface and were probably being recycled to it. It was known that antigen is endocytosed with antigen receptors and then degraded. One possibility raised by the internalization and apparent recycling of MHC molecules was that, following internalization, they might be targeted to a specialized microenvironment where they could interact with degraded antigen. The MHC-antigen complexes might then be exported to the cell surface for corecognition by T helper cells.

By this time, it had become clear to me that to study molecular mechanisms underlying biological systems, which is what interested me, I needed to learn the recently developed techniques of molecular biology. To this end, I moved to the laboratory of Richard Axel at Columbia University. Richard had begun to work in the area of neuroscience several years earlier through collaboration with Eric Kandel, who was also at Columbia. Their collaboration had focused on molecular studies of the nervous system of *Aplysia*, a sea snail. This was the model organism that Eric had used in many of his studies of learning and memory, for which he received a Nobel Prize in 2000. Perhaps not surprisingly, I was interested in searching for genes encoding neuronal cell surface receptors. However, at that time, Richard wanted to continue studying *Aplysia*, so I agreed to a project in which I would try to develop a technique for cloning genes expressed in one *Aplysia* neuron, but not another. After spending a short time learning molecular techniques from Jim Roberts, a student in the lab, I started my *Aplysia* project. Eric Kandel's group showed me how to isolate giant *Aplysia* neurons that had been assigned names and could be identified by their locations and, within a relatively short time, I began to uncover genes that were differentially expressed among *Aplysia* neurons.

While studying a neuropeptide gene expressed in neuron number R15, I discovered that the gene was also expressed in some other neurons, but that its primary transcript was alternatively spliced in different neurons to give different polyproteins. The two polyproteins could generate two different combinations of peptides in different neurons, suggesting a way to produce physiological or behavioral programs with partially

overlapping components. While working on the neuropeptide gene, I encountered numerous technical challenges that increased my knowledge of molecular biology and honed my abilities. During this period, I learned a lot of molecular biology from Richard and other members of his lab. I also got to know Eric Kandel, who has continued to be a wonderful source of inspiration and encouragement for me over the years.

From my first introduction to neuroscience, I had been fascinated by the brain's cellular and connectional diversity. In parallel with my *Aplysia* experiments, I sporadically tried to find a way to scan the genome for genes that had undergone gene rearrangement or gene conversion in neurons, thinking that genes that showed this characteristic might be involved in the generation of neuronal diversity. One method that I devised showed promise in *Drosophila*, but was not sensitive enough for the much larger genome of a mammal, which is what interested me. Nonetheless, these efforts were a great source of creative enjoyment for me as I proceeded with the more mundane task of searching for minute alternative exons in the *Aplysia* genome.

I was grateful that Richard was tolerant of my high-risk endeavors. He was an unusual mentor in that he gave people in his lab extensive independence in charting their own course once they had established themselves. During this time, I had many colleagues at Columbia with whom I enjoyed long discussions about science. Among these were George Gaitanaris, who has remained a close friend over the years, and Tom Jessell and Jane Dodd, neuroscientists from whom I learned a great deal about neural development.

As I was nearing the end of my *Aplysia* project, I read a paper that changed my life. It was a 1985 publication from Sol Snyder's group that discussed potential mechanisms underlying odor detection. This was the first time I had ever thought about olfaction and I was fascinated. How could humans and other mammals detect 10,000 or more odorous chemicals, and how could nearly identical chemicals generate different odor perceptions? In my mind, this was a monumental puzzle and an unparalleled diversity problem. It was obvious to me that the first step to solving the puzzle was to determine how odorants are initially detected in the nose. This meant finding odorant receptors, a class of molecules that had been proposed to exist, but had not been found. I decided that this is what I had to do as soon as my neuropeptide work was completed.

In 1988, I embarked on a search for odorant receptors, staying on in Richard's lab for this purpose. In a recent commentary in the journal *Cell*, I described what was known about odor detection at that time and the approaches that I tried in the quest to find the elusive odorant receptors. In short, it was known that odorants depolarize, and thereby activate, olfactory sensory neurons in the nose. Although there were varied proposals as to what kind of molecules might interact with odorants, there was compelling evidence that olfactory transduction involved G-protein induced increases in cAMP. After trying several different approaches, I identified the odorant receptor family by designing experiments based on three assumptions. First, since odorants vary in structure and can be discriminated, there would be a family of varied, but related odorant receptors, which would be encoded by a multigene family. Second, odorant receptors would be at least distantly related to the relatively small set of G protein coupled receptors whose

sequences were known at that time. And finally, odorant receptors would be selectively expressed in the olfactory epithelium, where olfactory sensory neurons are located. It took some time to devise and develop the methods I used in my search, but in the end they succeeded. Looking at the first sequences of odorant receptors obtained from rat, I was moved by Nature's marvelous invention. This work showed that the rat has a multigene family that codes for in excess of one hundred different odorant receptors, all related, but each one unique. The unprecedented size and diversity of this family explained the ability of mammals to detect a vast array of diverse chemicals as having distinct odors. In 1991, Richard Axel and I published the identification of odorant receptors.

**Boston**

In 1991, I departed for Boston to be an assistant professor in the Neurobiology Department at Harvard Medical School. There, I was immersed in an environment in which I could broaden my understanding of the nervous system. I received excellent support from my chairman, Gerry Fischbach, as I set up my lab. I also developed many excellent colleagues, including David Hubel, whose pioneering studies of the visual system with Torsten Wiesel, for which they received a Nobel Prize in 1981, had always been an inspiration to me. In 1994, I became an investigator of the Howard Hughes Medical Institute, which has generously supported our work for the past eleven years. Over the next decade, I remained at Harvard, gradually rising through the ranks to become associate and then full professor. In 1994, I met Roger Brent, a marvelous intellect and fellow scientist who has been my partner and an important part of my life ever since.

The discovery of odorant receptors had explained how the olfactory system detects odorants. My next goal was to learn how signals from those receptors are organized in the brain to generate diverse odor perceptions. I was joined in this endeavor by a series of excellent students and postdoctoral fellows. The discoveries on the organization of the olfactory system that were cited by the Nobel Foundation were made over a period of ten years, during which I was a faculty member at Harvard.

The first question we asked was how odorant receptors (ORs) are organized in the olfactory epithelium of the nose. This work was begun by Kerry Ressler, an M.D./Ph.D. student who came to the laboratory for a few months just as the equipment and supplies I had ordered began to arrive in January 1992. I had decided to switch from rat to mouse as a model organism because of the advantage of using isogenic inbred strains for dissecting a multigene family, and the possibility of generating transgenic mice. After cloning and sequencing a series of mouse OR genes, Kerry did our first *in situ* hybridization experiments to examine patterns of OR gene expression. By June, Kerry had returned as a full time student and Susan Sullivan had joined the lab as a postdoctoral fellow. At this point, we began to precisely analyze OR expression patterns and to compare them in different individuals. Prior to the present era of digital photographs that can be stored and analyzed on a computer, this was painstaking work that involved displaying photographic slides on a desktop viewer and recording, on transparencies, the locations of individual labeled cells in different animals. Our studies showed that each OR gene is expressed in

about 1/1000 olfactory sensory neurons, that the olfactory epithelium has several spatial zones that express nonoverlapping sets of OR genes, and that neurons with the same OR are randomly scattered throughout one zone. This indicated that signals derived from different ORs are segregated in different sensory neurons and in the information they transmit to the brain. It further indicated that, in the olfactory epithelium, neurons that detect the same odorant are dispersed and those that detect different odorants are interspersed. Thus, there is a broad organization of sensory information into several zonal sets in the epithelium, but, overall, information is encoded in a highly distributed manner. We published these findings in 1993. Similar observations in rat by Richard Axel and his colleagues were also reported that year.

Having determined how inputs from different ORs are organized in the nose, we asked how they are arranged at the next structure in the olfactory pathway, the olfactory bulb. In the bulb, the axons of olfactory sensory neurons synapse in about 2,000 spherical structures, called glomeruli. Kerry began to use retroviral vectors to investigate how the axons of neurons expressing specific ORs are organized in the bulb, but then we inadvertently found another way to address the question. While using *in situ* hybridization to identify a number of OR genes expressed in each epithelial zone for chromosomal mapping studies, Susan found that, in one tissue section, an OR probe labeled a single spot in the bulb, which proved to be a glomerulus. Using probes that recognized single OR genes rather than subfamilies of related OR genes, we found that each probe labeled OR mRNAs in sensory axons that were confined to one or a few glomeruli at only two sites, one on either side of the bulb. Different OR probes labeled different glomeruli and those glomeruli had virtually identical locations in different individuals. I still remember a meeting with Kerry and Susan in my office in which I asked Kerry how many sections separated different labeled glomeruli in different bulbs. All of us were stunned by his answer, because it provided the first hint that the bulb might have a stereotyped map of OR inputs and we could not imagine how this could be generated given the organization of OR gene expression in the epithelium. This mystery still has not been solved. These studies indicated that while thousands of neurons expressing the same OR are highly dispersed in the epithelium, their axons all converge in a few specific olfactory bulb glomeruli. The result is a stereotyped map of OR inputs in which signals derived from different ORs are segregated in different glomeruli and in the bulb projection neurons whose dendrites innervate those glomeruli. Remarkably, Bob Vassar in Richard Axel's lab had concurrently found that different OR probes labeled different glomeruli in the rat bulb. Our two groups published these findings in 1994.

Several years later, we began to investigate how the OR family and the patterning of OR inputs encode the identities of different odorants. Using single cell RT-PCR (reverse transcriptase-polymerase chain reaction), Bettina Malnic, a fellow in the lab, had been comparing gene expression in single olfactory sensory neurons. Her work demonstrated that each neuron expresses only a single OR gene, something that we had previously suspected, but that needed to be verified. Bettina was initially focused on the identification of genes that might be involved in OR gene choice or axon targeting in the bulb, but we decided to change course when Takaaki Sato visited our lab and told us about his calcium imaging studies of odor responses in the olfactory epithelium. This was

the beginning of a highly successful collaboration in which Takaaki used calcium imaging to define the odor response profiles of individual neurons and Bettina then used RT-PCR to identify the OR expressed by each responsive neuron. These studies demonstrated that the OR family is used in a combinatorial manner. Different neurons are recognized, and thereby encoded, by different combinations of ORs, but each OR is used as one component of the combinatorial receptor codes for many different odorants. As discussed in my Nobel Lecture, these studies also provided explanations for several intriguing features of human odor perception, including how a slight change in the structure of an odorant can dramatically change its perceived odor quality.

As soon as we had determined how OR inputs are organized in the olfactory bulb, we began to explore how they are arranged at the next structure in the olfactory pathway, the olfactory cortex. Lisa Horowitz, an M.D./Ph.D. student in the lab, initially investigated connections between the bulb and cortex using classical anatomical techniques. By depositing different tracers in the dorsal and ventral bulb, she determined that these areas project axons to the same regions of the cortex. In agreement with previous findings, this indicated that there could not be a point-to-point patterning of connections between the bulb and cortex. We decided to abandon traditional approaches and to instead ask whether we could chart neural pathways genetically by expressing a gene encoding a transneuronal tracer in olfactory sensory neurons. Lisa found that this was indeed possible. When she made transgenic mice that expressed barley lectin in all olfactory sensory neurons, the lectin crossed two synapses, labeling second-order neurons in the bulb and then third-order neurons in the cortex. This work, which we published in 1999, opened the way to investigating a wide array of questions concerning neural circuits, including those that carry olfactory information.

We then went on to use the genetic tracer to examine how inputs from individual types of ORs are organized in the olfactory cortex. To do this, we used gene targeting to generate mice that coexpressed barley lectin with a single OR gene. Lisa, together with a fellow in the lab, Jean-Pierre Montmayeur, prepared the DNA constructs for gene targeting. Zhihua Zou, another fellow, then made and analyzed mice that coexpressed the tracer with different OR genes. The approach worked, but was difficult, with Zhihua investing almost a year in perfecting the conditions needed to detect minute amounts of the tracer in cortical neurons. These studies revealed that the olfactory cortex has a stereotyped map of OR inputs, but one that is radically different from that in the bulb. As I discussed in my Nobel Lecture, the segregation of OR inputs in different glomeruli and neurons in the bulb gives way in the cortex to a complex array of OR inputs in which signals from different ORs partially overlap and single cortical neurons appear to receive signals from combinations of different ORs. This offers a means by which the individual components of an odorant's receptor code could be integrated at the level of single neurons. This could serve as an initial step in the reconstruction of an odor image from its deconstructed features, which are conveyed by the OR elements of the receptor code. We published our findings on the cortex in 2001.

During the ten year period at Harvard in which we did the work described above, my laboratory also investigated a number of other questions. These included studies of the

chromosomal organization of OR genes and the evolution of the OR gene family by Susan Sullivan, studies of the development of OR gene expression patterns by Susan and Staffan Bohm, and bioinformatic studies by Bettina Malnic and Paul Godfrey that defined and compared the OR gene repertoires of human and mouse. We also conducted a series of studies on the detection of pheromones in the vomeronasal organ, including studies by Emily Liman and Anna Berghard that revealed differences between transduction molecules involved in odor versus pheromone detection, the discovery of zonal patterns of transduction molecules likely to be involved in pheromone detection by Anna, analyses of vomeronasal responses to pheromones and odorants by Mehran Sam, and the discovery, by Hiroaki Matsunami, of a family of candidate pheromone receptors. During the latter part of this period, Hiroaki Matsunami, Jean-Pierre Montmayeur, and Stephen Liberles also began to explore the mechanisms underlying taste detection, in the process discovering candidate receptors for both bitter and sweet tastes, both of which were also found by other groups at about the same time.

**Seattle**

In 2002, I returned to Seattle to be a member of the Division of Basic Sciences at Fred Hutchinson Cancer Research Center and Affiliate Professor of Physiology and Biophysics at the University of Washington. I had always intended to someday return to the West Coast and had already stayed longer in Boston than I had anticipated. When Mark Groudine, then Director of the Basic Sciences Division at Fred Hutchinson, offered me a faculty position there, I gladly accepted. The Hutchinson Center had a reputation for cutting edge science as well as a high level of collegiality, both of which were important to me. In addition, by moving to Seattle, I would be closer to my partner, Roger, who lived in Berkeley, and to my family and friends in Seattle.

In Seattle, we are continuing to explore the mechanisms underlying odor perception as well as the means by which pheromones elicit instinctive behaviors. We have also become interested in the neural circuits that underlie innate behaviors and basic drives, such as fear, appetite, and reproduction. We are currently developing molecular techniques to uncover those circuits and to define their composite neurons and the genes they express. In a different vein, we have developed a high throughput approach in which we are using chemical libraries to identify genes that control aging and lifespan, our chief interest being whether there might be a central mechanism that determines lifespan and regulates the aging of cells throughout the body.

**Looking back**

Since Richard Axel and I published the discovery of odorant receptors in 1991, it has been immensely satisfying for me to see many laboratories using these receptors in a large scale effort to dissect the mechanisms that underlie the sense of smell and the developmental processes that shape the organization of the olfactory system. Molecular approaches to studying olfaction have extended to other vertebrates as well as to invertebrate species, with Cori Bargmann's group discovering a large variety of chemosensory receptors in the nematode worm, *C. elegans*, and several groups, including Richard Axel's, identifying families of odorant and taste receptors in the fruit fly, *D. melanogaster*.

Looking back over my life, I am struck by the good fortune I have had to be scientist. Very few in this world have the opportunity to do everyday what they love to do, as I have. I have had wonderful mentors, colleagues, and students with whom to explore what fascinates me and have enjoyed both challenges and discoveries. I am grateful for all of these things and look forward to learning what Nature will next reveal to us.

As a woman in science, I sincerely hope that my receiving a Nobel Prize will send a message to young women everywhere that the doors are open to them and that they should follow their dreams.

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