Jane Mandel

Gail Mandel

Born:

Atlanta, Georgia
June 29, 1950

Education:

University of California, Los Angeles, BS (1972)
University of California, Los Angeles, PhD (1977)
University of California, Los Angeles, Molecular Biology Institute, Postdoctoral (1977–1978)
University of California, San Diego, Department of Biology, Postdoctoral (1979–1981)

Appointments:

Instructor, Department of Pathology, Harvard Medical School, Boston, MA (1982–1984)
Assistant Prof. of Molecular Medicine, Tufts-New England Medical Ctr, Boston, MA (1984–1987)
Associate Prof. of Molecular Medicine, Tufts-New England Medical Ctr, Boston, MA (1987–1989)
Associate Prof. of Neurobiology and Behavior, SUNY at Stony Brook (1989–1993)
Professor, Department of Neurobiology and Behavior, SUNY at Stony Brook (1993–2002)
Investigator, Howard Hughes Medical Institute (1997–2016)
Distinguished Professor, Dept of Neurobiology and Behavior, SUNY at Stony Brook (2002–2006)
Senior Scientist, Vollum Institute, Oregon Health and Science University, Portland, OR (2006–present)

Honors and Awards:

American Cancer Society Scholar, Harvard Medical School (1982–1984)
National Science Foundation Faculty Award for Women Scientists and Engineers (1992–1996)
Director, Program Project Grant - Institute for Cell and Developmental Biology (1996–2001)
McKnight Investigator Award in Neurosciences (1997–1999)
Jacob J. Javits Neuroscience Investigator Award, NINDS, NIH (1997–2004)
Investigator, Howard Hughes Medical Institute (1997–2016)
Chancellor's Award Honoring Research in Science, Engineering and Medicine (2001)
Chancellor's Award as First Time Patent Recipient (2002)
Fellow of the American Association for the Advancement of Science (2003–present)
Member, American Academy of Arts and Sciences (2007–present)
Member, National Academy of Sciences (2008–present)
Discovery Award, OHSU Medical Research Foundation (2011)
NIH Director's Transformative Research Award (2013–2019)
Councilor, Society for Neuroscience (2017–2022)

Gail Mandel was instrumental in launching the nascent field of molecular neurobiology through research, teaching, and mentoring. She was among the first to clone and functionally express ion channels with a principal focus on the voltage-dependent sodium channel. Her research led to identification of the genetic elements that control neuronal-specific expression and, ultimately, mechanisms that underlie the development of the nervous system. Contrary to expectations, her identification of the transcription factor, REST, showed that the acquisition of a terminally differentiated neuronal phenotype was not due to activators but rather to lack of this repressor. REST has been shown subsequently to be a master regulator for a wide cast of genes, many of which underlie human neuropathologies, including diseases external to the nervous system. She recently applied her background on gene repression to study of the transcriptional repressor, MeCP2, and its role in human diseases, showing that RETT is a disease involving both neuronal and glial dysfunction, thereby developing therapeutic approaches toward a cure.

Gail Mandel

I am a first-generation American. My mother, Judith Scheiner, was born in Berlin to Russian parents. She attended middle school in Paris and was living there at the outbreak of World War II. She, along with her parents, attempted to escape to the United States (her older sister was married to a British citizen and they had already immigrated to the United States). While her parents had Russian passports, my mother had a German passport, making it all but impossible to immigrate directly, because German Jews were flocking to enter the United States at the same time. I have the paperwork tracing her successful efforts to obtain safe passage, eventually gaining entrance through Portugal and Cuba, arriving finally in New York City in 1939. My father, Herman Mandel, the youngest of three boys, immigrated with his family from Poland when he was a toddler. Interestingly, both parents became naturalized citizens well after their immigration to the United States, with my mother filing in 1953. Dad's naturalization papers are dated 1943 as he filed to join the U.S. Army infantry in World War II. He was captured in France and was a prisoner of war until the end of the war. As he said, they woke up one morning to find all the Nazis gone and the Americans arriving. Neither parent spoke much of their struggles, but it clearly shaped their lives, as they surrounded themselves with friends who had shared similar experiences and had liberal values. This included a lot of bridge and chess, which was used as a metric for the worthiness of my boyfriends.

I was born in Atlanta, Georgia, in 1950, while my father was attending Georgia Tech. After nine years or so, we were a family of five. We moved around, first from Georgia to Lynchburg, Virginia. My mother, a talented pianist and dancer, attended Randolph-Macon Woman's College in Lynchburg, Virginia, where I had occasion as a young girl to see her perform as a ballerina in college performances. We spent several years in Glens Falls, New York, before settling in 1959 in idyllic Southern California. My dad was an engineer at Atomics International, my mother taught piano and then became a social worker, and my life centered on Malibu and Muscle Beach in Santa Monica. The beach life paid off with my first publication, featuring my friends and me in a *Life* magazine photo-op "California Girls Spangle the Beach." This lifestyle was turned upside down when, in my junior year of high school, my father was commissioned as part of a team to help research coolants in a nuclear power plant in Pinawa, a small town in Manitoba, Canada. Pinawa is a very cold place, positioned in the midst of boreal forest, beautiful lakes, and spectacular wildlife. There were activities to enjoy, like skating and canoeing the frigid waters with friends, but no beaches, I turned to

studies for the first time. My efforts landed me in the top 10 of my graduating class of 12. Still, my standardized test scores were competitive enough to be awarded a scholarship to the University of Colorado, Boulder, where I majored in journalism. I joined the Tri-Delt sorority and was having a blast when the power plant downsized in Canada, and with it, my scholarship. I moved back to the San Fernando Valley in Los Angeles with my parents and reconnected with many of my old friends. I scraped together enough money to buy a used, fire engine red Chevrolet Malibu super-sport convertible to cruise the valley and beaches, always with the top down (car that is). I was once again living the Southern California 1960s lifestyle.

My sophomore year in college was spent at what is now Cal State Northridge, where I continued to major in journalism, although an interest in biology was taking root. To investigate this new topic more thoroughly, I applied to the Eugene and Ruth Roberts summer student academy at the City of Hope Research Center in Pasadena. I didn't know it then, but this was to be the beginning of a career in science shaped by a succession of wonderful mentors with creativity in their science, alongside huge personalities that included an equally large sense of humor. As I chronicle here, they showed me that research could be both challenging and fun, and that being a good human being was, as it is in life, a necessary component for a fulfilling career.

I was accepted into the City of Hope research program for summer 1970 and assigned to the neurochemist, Bernard Haber. Bernie had an ebullient personality. We clicked right away. My project was to test acetylcholine and GABA as "putative" neurotransmitters in chick brain. While this seems like what must have been a foregone conclusion even at that time, we were very conservative in those days before stating any finding as fact. It was my first introduction to bench science with state-of-the-art techniques, such as cell fractionation and sucrose gradients. I became friends with another neurochemist, Richard Hammerschlag. Richard did beautiful work, described to me as nature offering hints to answers for questions he didn't ask. As a graduate student with Susan Leeman, Richard set out to purify corticotropin-releasing factor (CRF) from extracts of bovine hypothalami, which led not to CRF, as planned, but to the identification of substance P and neurotensin. Then, as a faculty researcher at City of Hope, he established an *in vitro* axonal transport system as a new approach to identify the elusive sensory neurotransmitter, only to be led to the early steps in the transport system itself. In addition to talking about science, Richard and I spent leisure time discussing the complexities of the Vietnam War and composing limericks. The latter groomed me for limerick compositions that were called upon as a postdoc with Bill Wickner at University of California, Los Angeles (UCLA) and much later as a member of the legendary NIH Physiology Study section. The panel's cynical sense of humor belied an ability to provide incisive, critical, but fair reviews of their peers. I benefited from their collective

wisdom, and limericks were subject to the same level of scrutiny as the grant proposals.

UCLA, 1970–1972

Despite my increasing interest in biology, I still clung to journalism when I transferred to UCLA in fall of my junior year. For one of my writing classes, I interviewed celebrities, for which there was no shortage in Westwood. This led me to coffee with comedians Gene Wilder, Phil Silvers (as Sargent Bilko), Carl Reiner, and Mel Brooks, as well as an in-depth interview with Jay Silverheels (Tonto in the *Lone Ranger* television show). My interest in biology took center stage, however, after I met UCLA graduate student, Paul Brehm, a transplant from Ferguson, Missouri. Paul was living in a small studio apartment in Beverly Hills and was having difficulty adjusting to the extroverted LA lifestyle. He made me laugh with his perfect irreverent imitations of certain UCLA faculty, and I was more than happy to help along these lines. Paul was studying bioluminescence with Jim Morin in the zoology department. Jim, along with his mentor, Woody Hastings at Harvard, was actually the first to identify and name green fluorescent protein (GFP; Morin & Hastings, 1971). They correctly surmised that GFP was an energy acceptor for the calcium-activated photoprotein, shifting light emission from blue to green. Paul was using GFP to identify and record electrophysiological responses from bioluminescent cells in the hydrozoan coelenterate where GFP was first discovered. The summer of 1971 would find us separated, with Paul at the Marine Biological Lab in Woods Hole, where Morin was an instructor in the now extinct Experimental Invertebrate Zoology Course. As for me, I was up for trying another summer with Bernie Haber. However, he had just moved his lab to the University of Texas Medical Branch, so it was summer in Galveston for me. At least there was a beach. I finalized my studies from the previous summer and presented an abstract at the annual Neurochemistry meetings, my first real scientific publication.

During the next year at UCLA, Paul and I grew close, and we moved into a small apartment at 1024 Hill Street, just blocks away from Santa Monica beach. We would walk the Venice boardwalk and eat at Al's Kitchen on the Santa Monica Pier, still home to the hippodrome that houses the merry-go-round in the movie *The Sting*. Paul and I enjoyed the same music, food, backpacking, and exploring Southern California. We would ride our bikes to and from work down Pico Boulevard into Westwood and life was great. We both grew up loving animals and just kept accumulating them without regard for the impracticalities. We started with three parakeets that lived on our pole lamp and flew wild in our small apartment and then added two cockatiels. They controlled the air space. Next, we rescued two chinchillas that were about to be sacrificed in a research lab at UCLA. Those animals were lucky there was no Institutional Animal Care and Use Committee

(IACUC) in those days. We quickly learned that they were male and female as they pumped out offspring at a nonstop pace. They controlled the ground space along with a desert packrat who nested under our refrigerator. When I was missing shiny jewelry, I knew where to find it.

During my senior year at UCLA, at the encouragement of Paul, I took all of the invertebrate courses offered, including the graduate seminars. The graduate students with faculty Jim Morin and Len Muscatine were a tight group, but I was deemed acceptable company. We made regular trips to collect invertebrates, looking especially for species that were bioluminescent. We would take clam guns to the Morro Bay mud flats and core drill for bioluminescent worms. For intertidal zones, we would carry squirt guns loaded with concentrated potassium chloride to hunt down new bioluminescent species (virtually all bioluminescent animals emit light when depolarized). Class trips included marine stations at Bodega Bay, Santa Catalina, and Puerto Penasco, Mexico. Paul introduced me to the field book *Between Pacific Tides; Sea of Cortez*, which was a field guide and the story of Ed Ricketts, a marine biologist, and author John Steinbeck, coadventurer with Ricketts. This guide colored our frequent visits to Pacific Biomarine, a collecting service headed by chemist Dr. Rim Fay who was a modern-time Ed Ricketts.

A memorable non-marine course for me was a botany course for non-majors led by Mildred Mathias, for whom the UCLA botanical garden is dedicated. With her class of about 12 students, I keyed out plants in and around LA on our field trips. Mildred walked the botanical gardens with all of us trying to keep up with her. She enthusiastically grilled us on different genus and species names for exotic plants based on our dichotomous key. I was pretty good at this, but always in the shadow of Evelyn Zwicker (now Maisel) who had a photographic memory. Evelyn became a medical doctor and talented artist. Both of us remember our time at UCLA with great warmth and were drawn back together as I researched the past for this biography.

UCLA, 1972–1978

Nearing the end of my senior year, I was looking into the prospect of graduate school. In seeking advice about applying to the UCLA Zoology program, I received conflicting recommendations, including one by a senior faculty member that I train instead for a career in dental hygiene. Somehow the liberal thinking, which then focused primarily on marijuana and protesting the Vietnam War, had not percolated down to the level of tackling gender bias in careers. This bias would take a much longer time to take center stage.

Between 1972 and 1973, two major events happened. First, in 1972, I entered graduate school in the Molecular Biology Institute (MBI) at UCLA, directed by Nobel laureate Paul Boyer. The field of molecular biology was in its infancy, which excited me, and they had hired junior rising stars that

included Tom Kornberg, Mike Grunstein, Bill Wickner, and Judith Lengyel. I joined the lab of Bill Clark, by that time an MBI associate professor, working in the exciting and expanding area of immunology. Second, in 1973, Paul and I were married. At that time, it was difficult to find a rabbi who would perform a union between a Jewess and non-Jew, even in Los Angeles. At the request from my parents, we did manage to find one and tried, somewhat unsuccessfully, to keep the event low-key. Our fellow graduate students and friends were kept in the dark. The following day we set out with classmates on a collecting trip to Jade Cove. When information was leaked about our wedding, they decorated our Chevrolet Blazer, and we suffered through a weekend of jokes and pranks.

In the summer of 1974, I spent time at the Woods Hole Marine Biological Lab (MBL). Paul had returned to work with Woody Hastings to investigate the biochemistry of brittlestar bioluminescence. This would be my first experience at the MBL and one that had a major impact on my future. I was intrigued. I took advantage of the courses and seminars and was witness to the unparalleled intensity of the MBL. Much later, I would return to the MBL as an instructor in the neurobiology course, directed by Arthur Karlin, teaching the first molecular neurobiology techniques in the biochemistry section. This experience also introduced me to the unique community in Woods Hole, which prompted us to buy a cottage there and that now serves as our east-coast residence when we are not in Oregon.

My continued interest in invertebrate biology carried on in the background of mammalian immunology studies. Back in the lab, I had been working on an ambitious project aimed at identifying the T-cell receptor, using anti-idiotypic antibodies, but after three years of effort, I ultimately failed. The technology for identifying the T-cell receptor was just not there yet. Indeed, it was another nine years before cDNA cloning resolved the predicted primary structure of the T-cell receptor. By the summer of 1975, I was midstream into my second thesis project. I spent the final two years on this project, which involved electron spin resonance to measure membrane fluidity. The idea under test was whether changes in membrane fluidity in tumor cells affected their cytolysis by antibody plus complement or by killer T cells. We published a methods paper on the approach for altering membrane lipids and, in a related paper, I ruled out fluidity as an underlying mechanism for cytolysis. However, membrane fluidity did affect patching of H-2 surface antigens, suggesting involvement in other immune processes. Although not the outcome I had envisioned, the results were still a contribution in the early days when the roles for membrane fluidity were poorly understood. I published my findings in the *Journal of Immunology* (Mandel et al., 1978; Mandel & Clark, 1978), a top journal focused, like most others at the time, on just the quality of the science. My PhD experience was also teaching me an important lesson; science operates on the premise of testing, not proving, an idea.

I was learning this lesson while Paul and I were living on different coasts. Paul graduated with his PhD in 1975, just in time to be called into active duty into the Army Chemical Corps. He was located at Aberdeen Proving Grounds in Maryland and the assignment was for an indeterminate amount of time. I was left on my own to finish up my thesis work at UCLA. When the Vietnam War ended abruptly, Paul was given notice of release from active duty so he was free to look for a postdoc. Opportunities were scarce, even for those of us willing to work for little pay, just for the chance to continue a career in research. He wanted to stay at UCLA while I finished my thesis work. By chance, a spot opened up in Roger Eckert's lab. My fellow graduate student and close friend, Laurinda (Rindy) Jaffe and I wasted no time in browbeating Roger into submission. In an inebriated state, Roger called Paul in Maryland and made him an offer. Roger made the right decision. In the three years in his lab, Paul would discover calcium-dependent inactivation of calcium channels, thereby completely changing the direction of the lab. By this time, we had acquired three rescued dogs along with our other animals, forcing us to move to the San Fernando Valley where we rented a house with a fenced yard.

As my thesis project drew to a close in 1977, I started to look for a postdoc position at UCLA because Paul was still with Eckert. I happened to run into Bill Wickner on the MBI stairway, and we started talking. At conversation's end, I was to be his first postdoc. Once again, I had chosen wisely. Like my experience in the Clark lab, Bill was a hands-on mentor, a lab rat that is still doing research in his own lab. Bill came into the lab every day in a jolly mood singing the lyrics of some lame song he had in his head. "Jeremiah Was a Bullfrog" from *Joy to the World* by Three Dog Night was one of his favorites. Bill also had a gift for word play that often turned up in limerick form, something I had taken to well over the years. The Wickner lab was small, with Craig Zwizinski, Pam Silver, and a technician, Teresa Killick. Science-wise, a hot topic in the field was the emerging appreciation that proteins spanned membranes and not simply adhered to the bilayer formed by lipids. Bill is a bold scientist. Not only did I have an opportunity to learn biochemistry from first principles in his lab, just as important, Bill was a role model for applying these fundamentals to risky experiments with potential for new discovery. One question at the base of much controversy was the question of how transmembrane proteins, made up of charged amino acids, could insert into the lipid interior of the plasma membrane and be stable in the face of the hydrophobic environment. In the case of secreted and transmembrane proteins, answers were emerging from the Blobel lab at Rockefeller. Small noncharged sequences on secreted and membrane proteins, "signal peptides" would guide them during translation into the membrane and subsequently be cleaved, a discovery that earned Blobel the Nobel Prize. The labs of Wickner at UCLA and Masayori Inouye at Stony Brook had proposed different models. In the Wickner model, which I pursued

using a viral coat protein that is synthesized as a soluble protein, the heart of the idea was that transmembrane proteins, if properly folded to hide charged residues and aided by the "leader sequence," could self-assemble into a membrane. Bill named our model the "Membrane-Triggered Folding Hypothesis" (Wickner et al., 1978; Ito et al., 1979; Mandel & Wickner, 1979; Wickner et al., 1980). In the Inouye "loop model," the idea was that the signal sequence could associate with the membrane in a way (looping in) that would allow self-assembly followed by an energetically favorable environment allowing the protein to traverse the membrane by itself during translation (Inouye & Halegoua, 1980). I loved being involved directly in the excitement of being part of a competitive, discovery-driven field.

As both Paul and I reflect on the years in graduate school at UCLA, we recognize that it was a period where we really could enjoy ourselves with little responsibilities while living on a shoestring budget. Graduate stipends were rare, and we relied on salary as teaching assistants. Both Paul and I were teaching assistants in a variety of high-enrollment courses that generally involved three full days of lab exercises each week. It paid very little but was enough to live on. There were no credit cards and everything was paid by check. We were terrible at money management and routinely bounced checks. That would be our signal that we were out of money. This was a lifestyle that would come back to haunt us when we applied for our first home mortgage years later. Credit scores were other things we knew nothing about. As for science, we both had experienced initial thesis projects that failed and had found new projects with the help of our thesis advisors. We were never discouraged by failure, nor did we focus on the uncertainties of the future, which were as prevalent then as they are today. We were just focused on learning as much as we could.

Science was our first priority, so we spent long hours in the lab, typically every day of the week. However, we did a have life-work balance with quality time spent outside the lab. We scheduled that time around our experiments. Paul and I would often backpack with friends deep into the California wilderness. We hiked Sequoia, Mammoth Lakes, Yosemite, Kennedy Meadows, and Kings Canyon, to name some of our favorites. We always brought along the dogs, and we would be somewhere in wilderness for a week or so. Those backpacks were heavy from food and gear, and there was no room for a tent, just a sleeping bag. Every trip was an adventure. Hiking back into Sawtooth Pass, at 12,000 feet in January was a risk, with no provisions for a possible snowstorm and no means of communication other than a mirror. I can't imagine what we were thinking. We would sign a register declaring we were in the wilderness somewhere should we turn up missing. We would drink water along the way in streams and fill our canteens with little concern about water purity. Over the many years of backpacking, Paul was a notorious prankster. We would normally gather with our friends at our place to distribute weight among the backpacks. There were no freeze-dried foods so

we would take jars of peanut butter, crackers, fruit, pasta, and tomato sauce, all of which were pretty heavy. When no one was looking, Paul would load the backpacks with additional heavy surprises, such as bricks and, once, an antique steam iron. Often, we would backpack with our close friends, Jane and Bill Koenig. Bill was a medical student and Janey was a talented fellow graduate student in the genetics program at UCLA. She would go on to a productive postdoc with Kazuo Ikeda at the City of Hope working with the now famous *Drosophila* mutant, *Shibere*. At the time, we had a 1972 Chevrolet Blazer with four-wheel drive. We used that truck for field trips to the Puerto Penasco Marine lab in Mexico and to tropical La Paz, located at the tip of Baja. The latter trip preceded the construction of a road, so it was very risky to make that thousand-mile trip, not knowing where to obtain gas and drinking water. Fortunately, we had a fellow lab mate of Paul with us, marine biologist Jon Kastendiek, who was conducting research there. We would follow the beach much of the way down, camping on the beach and snorkeling in the tropical waters.

In total, our years at UCLA were truly "golden," as Alan Grinnell, faculty leader of the neurophysiology group, described this time period. Alan was one of the people who encouraged me to pursue a career in research and who also helped with my tennis game. Many a night was spent playing Ping-Pong in front of the Grinnell and Eckert labs, followed by free food from the medical school cafeteria. Alan was a very competitive Ping-Pong player and he was very proud of his bloopers that dribbled over the net for a point. We would call him on that, accusing him of cheating. He maintained an active research program split between synaptic competition and bat echolocation that took him all over the world. He still has probably the foremost private collection of pre-Columbian artifacts in the country, so truly a Renaissance scholar, always fun to talk to. Despite our diverse research interests, the graduate students during this period remained a large cohesive group. We would work late into the night, taking breaks for Ping-Pong and hall Frisbee. When one of us would make a breakthrough in our work, the students and postdocs would celebrate together with a bunny hop down the hall chanting "publishable results—YEAH!" Funny, those breakthroughs always seemed to happen late at night. Also, in those days, major revisions were not the norm, so we were forever optimistic of seeing our results in print.

I am a loyal Bruin. I am a member of the external scientific advisory board of the UCLA Broad Stem Cell center, directed for many years by the talented Owen Witte who hired and nurtured a terrific group of scientists. Over the years, the advisory board meetings have provided me the opportunity to hear presentations of interesting science, to reconnect with faculty, and to once again experience the hickory burger, fries, and banana cream pie from the Apple Pan in Los Angeles, which thankfully survived the pandemic.

UCSD, 1978–1981

In late 1978, my time at UCLA ended and I moved to the University of California, San Diego (UCSD) to join Paul, who had begun a second postdoc at the Salk Institute. We bought a house at 11822 Jonny Lane in Mira Mesa, a San Diego development built originally as naval housing. Thousands of homes that came in a few slightly different models dotted the landscape. We managed to score a special one, at the end of a cul-de-sac offering a panoramic view of a canyon, complete with coyotes, hawks, rattlesnakes, and tarantulas. This was a house we figured we could afford, which turned out not to be the case. Our misplaced belief stemmed from a one-time postdoctoral stipend from the Muscular Dystrophy Association to Paul that was more money than we had ever seen. We took all $16,000 and invested it in a money market that was making insane interest. Being the shrewd money managers that we were, we forgot that we had mortgage payments that amounted to more than my income. Realizing our mistake, we forfeited the money market and paid a huge penalty, but it really didn't faze us. We had learned a lesson in money management and were happy with what we had.

For my choice of a second postdoc mentor, I had asked Harvey Herschman, one of my thesis committee members, to recommend someone whose research he admired and would complement my training interest. He suggested Melvin Simon. Mel was yet another timely choice. He was working in a new field of gene translocation, guided by his gifted senior postdoc, Mike Silverman, and star graduate student, Janine Zieg. I was lucky to join this group. I first worked with a postdoc in the lab, Alan Boyd, to continue studying integral membrane proteins, this time focused on proteins required for bacterial motility (Boyd et al., 1982). Alan is a Brit, and between experiments we competed in crossword puzzles. I contributed to a *Science* paper on the evolution of phase variation, a gene inversion event causing a loop to form in the DNA that regulates the type of flagellum expressed by Salmonella (Simon et al., 1980; Silverman et al., 1979, 1981). This project allowed me to do some of the first DNA sequencing chemistry. It is a tribute to Zieg, Silverman, and Simon, who were able to identify the sequence of the invertible loop of DNA, the precursor to modern day three-dimensional (3D) chromatin looping, with merely conventional genetics and Maxam and Gilbert sequencing (Zieg et al., 1977). Russell Doolittle at UCSD was also a coauthor on the *Science* paper. I did some chemical syntheses in his lab, although for the life of me I cannot remember why. Russ was a scholar and competitive athlete. He gave me a copy of his favorite book, *The Decline and Fall of the Roman Empire*, by Gibbon, which I still have today. Russ regularly ran in the Boston Marathon, and he invited Paul and me to his final marathon dinner in Boston. Over dinner, he disclosed that he was also a regular participant in the debate circle with creationists. He recounted his most recent debate with proponents of intelligent design, which in simple

terms reflects antievolutionary thought. He said he had always enjoyed this sparring, but this time, he was bushwhacked by a hostile audience and an unannounced television filming. Unprepared, it became clear that this event greatly influenced his decision to leave the debate stage, which coincided with ending his marathon participation. Russ was a person of great intellect and presentation abilities, so for this to happen to him has been a constant reminder to me to be well prepared for every speaking opportunity (although Chuck Stevens, a renowned neuroscientist and great speaker, once told me that 90 percent was just showing up, helping to settle my nerves before a talk).

While at UCSD, I would reunite with Rindy Jaffe, who would continue to explore the voltage-dependent block to polyspermy, a fundamental discovery she had made as a graduate student. She still pursues this question, applying rapidly evolving optical approaches to creative preparations she develops. It was also a time we would meet another lifelong friend, Simon Halegoua. Paul was a postdoc in the newly formed Molecular Neurobiology group at the Salk Institute. He mentioned that he had befriended a fellow postdoc in the group, a quintessential New Yorker with a quick wit who was as irreverent as Paul. When he told me the name, I recognized it as an author of the loop hypothesis for membrane insertion. I was intrigued and anxious to compare notes, so Paul introduced us. We quickly became fast friends and shared the Jewish high holy holidays together, a tradition that remains. Paul's experience in the Molecular Neurobiology group would also play an unexpected role in directing my future work on the voltage-dependent sodium channel. His decision to do a physiology postdoc with Yoshi Kidokoro in the Neurobiology group had placed him squarely in the middle of a group seeking to clone an ion channel. The group, led by Steve Heinemann and his research colleague, Marc Ballivet, was in hot pursuit to obtain the first primary sequence for a subunit of the nicotinic acetylcholine receptor. This effort was among the first to apply molecular cloning techniques to ion channels and the community of ion channel biophysicists waited anxiously in the wings. My training in DNA sequencing with Mel Simon would prove to be important preparation for my eventual entry into this field.

In 1981, Paul accepted a faculty job at Tufts Medical School in Boston to join Kathy Dunlap, a fellow colleague from the Eckert lab. He missed the electrifying atmosphere of the Salk but was excited to be with Kathy and have his own lab. As well as having no one to talk to about ion channel cloning, he was one of only two single-channel "patch clamp" physiologists in Boston, David Clapham being the other. As was our custom, I stayed behind a bit to finish up my second postdoc with Mel Simon. During this 3,000-mile separation, I lived with Rindy Jaffe and Carol Vandenberg in a small beach house, since washed away I think, where I could enjoy walking the beach and seeing beautiful sunsets before heading East. Rindy, Paul,

and I were to form an enduring friendship. We still have lively discussions about science, and more recently, about interesting recipes. This friendship is easy to maintain because Rindy, and her husband Mark Terasaki, also an excellent cell biologist (and pianist), own a cottage a block away from ours in Woods Hole.

Harvard Medical School, 1981–1983

To join Paul in Boston, I accepted an instructor position in Tom Benjamin's lab in the Pathology Department at Harvard Medical School. My motivation for applying to his lab was research on polyoma virus proteins, and I thought it would be a good entrée back into eukaryotic biology. I had intended on trying to figure out how middle T antigen inserted into the host membrane, returning to my previous interests, but somehow that never came to pass. Instead, I participated in biochemistry experiments related to phosphorylation of proteins, all new to me. Although this was not my most productive time publication-wise, I owe Tom a debt of gratitude for his support, and I was fortunate to meet and work with some excellent postdocs in his group—Gordon Carmichael, Brian Schaffhausen, and Kurt Ballmer-Hofer. Both Gordon and Brian have a terrific sense of humor and a very quick wit, increasing entertainment at the lab bench. Part of my distraction around this time in Tom's lab was that Paul and I had our first child. Josh Mandel-Brehm was born in 1983. Once again, our lives were turned financially upside down. Seemingly incapable of any financial planning we had bought a house, 68 Adella Avenue in West Newton, another one we learned we could not afford. The interest rates were at 17.5 percent, a historical high, and our combined salaries were only slightly higher than the mortgage payments, forcing us to borrow more money. Our long hours in the lab were under siege with daycare adding to our commuting time and financial debt. I was feeling the pinch trying to balance science with my new life as a mother. However, a fortuitous career-changing event was about to happen.

Tufts New England Medical Center, 1983–1990

I had been thinking about projects that would bring me closer to the emerging field of neurobiology when, serendipitously, one evening, Paul spoke highly about a new faculty member at Tufts New England Medical Center who had presented a seminar on cloning neuropeptides in the Physiology Department. Neuropeptides would take me closer to my long-term interests as they played an essential, receptor-mediated role in the nervous system. The faculty member was Richard Goodman, an MD/PhD in the field of endocrinology. Dick trained with Joel Habener at Massachusetts General Hospital in Boston and was interested in cloning the cDNAs for somatostatin, vasoactive

intestinal peptide, thyroid releasing hormone, and bombesin, among others. By chance, we ran into Dick and his wife, Eve, at a movie theater, and by the end of the discussion, Dick had graciously offered me a faculty appointment. I seized this wonderful scientific opportunity. It was a move that also would mitigate the time-consuming and stressful cross-town commuting between Harvard and Tufts. I joined a large group that included Marc Montminy, Andy Leiter, Malcolm Low, Ron Lechan, Philip Stork, Thom Segerson, Steve Fink, Kevin Sevarino, and Stephanie Lee.

The years between 1984 and 1989 with Dick represented an exciting time as the lab was bursting with projects (e.g., Montminy et al., 1986; Lechan et al., 1986; Leiter et al., 1987; Cooperman et al., 1987; Fink et al., 1988; Sevarino et al., 1989; Maue et al., 1990). Cloning of neuropeptides was so successful that the lab turned toward cloning neuropeptide receptors. I was anxious to steer efforts more toward cloning of ion channels. I had the skill-set and, influenced by Paul, it was clear that cloning ion channels was an exciting new field. The major driving force was led by biophysicists who had developed models for gating that made specific structural predictions that awaited testing. The 1984 Excitable Membranes Gordon Conference sealed the deal and pointed me toward the voltage-dependent sodium channel. Traditionally focused exclusively on ion channel biophysics, Chuck Stevens had carved out a big chunk of the presentations to promote ion channel cloning and his new molecular neurobiology group at Yale. At the meeting, rumors were circulating that Shosaku Numa in Japan had already resolved the primary sequence for a voltage-dependent sodium channel, but no details were available. Oocyte expression from synthetic RNA recently had been developed so it was conceivable he had functionally expressed the first voltage-dependent ion channel. This idea really lit a fire under competitors. The ion channel biophysicists were not pulling for anyone in particular; they just wanted ion channel clones for expression, mutagenesis, and testing structural models based on physiology. It had been 30 years since Hodgkin and Huxley showed the features of the sodium channel responsible for an action potential. Since that time, some of the greatest biophysicists had generated models that could account for the voltage dependence of activation, inactivation, and selective permeability. The problem with Numa winning the race was that he was not interested in sharing when it came to his clones. With few exceptions, Numa refused to distribute his clones, even after they were published. One exception was his willingness to share the clones for the nicotinic acetylcholine receptors with the Sakmann group in Germany. Another exception was sharing the clones encoding the dihydropyridine receptor with Kurt Beam at Colorado State University (I suspect Numa had lost interest when he realized that it was a nonconducting channel). Kurt applied his strengths in biophysics and cell biology to unravel the signaling pathway between muscle depolarization and release of intracellular calcium using the cDNA clones.

Still, the dihydropyridine receptor was not on the path to delineating the pore mechanisms in voltage-dependent ion channels. Thus, using techniques developed in tandem with Dick, I set out to clone the cDNA encoding the mammalian voltage-dependent sodium channel. I say "the" channel because we had no idea about the extent of sodium channel diversity at that time. The project was launched in our lab when Paul convinced Bob Barchi (University of Pennsylvania) to collaborate with us using his antibodies to screen a lambda gt10 muscle expression library for the sodium channel alpha subunit peptide. In the midst of our muscle cloning effort, the rumors were confirmed with Numa publishing the primary sequence of the eel sodium channel (Noda et al., 1984). Once again, however, the eel sodium channel turned out to be nonfunctional when expressed in heterologous cells, which remains a mystery today. We altered our strategy in light of Numa's paper and decided to use the eel cDNA to cross species to clone the mammalian sodium channel cDNA. This experiment carried the basic assumption that mammalian and fish sodium channels were sufficiently homologous, so it remained a bit risky. Dick and I purchased an electric eel from Florida and had it flown into Boston Logan Airport. It arrived the day before Thanksgiving. We picked up the rather large box at the airport and brought it with us on the subway during rush hour. We had no problem finding a seat because the box was stamped officially in large letters, DANGER, HIGH VOLTAGE. We commissioned a marine biologist at the Boston Aquarium to help us extract the electric organ, a modified electroplax muscle. He showed up in heavy rubber boots and gloves as we gathered around in curiosity. The extraction was successful and we made a cDNA library from the eel electric organ RNA. We used this reagent to identify a large sodium channel clone from our mammalian brain cDNA library. We surmised, correctly, that Numa was already well on the way to cloning mammalian sodium channels using the same strategy as ours. Once again, Numa won the race, publishing the full-length sequence of three rat brain sodium channel isoforms. This time the channels were functional when expressed in *Xenopus* oocytes. There was, however, some pause for concern as there was abnormally slow inactivation compared with sodium currents recorded *in vivo*, a feature also seen when expressed in mammalian cell lines. I return to this curiosity later.

In addition to Numa, Dick and I were competing with several other formidable labs in the United States, including the Lester-Davidson team at Cal Tech, which also succeeded in functional expression of a full-length brain-type sodium channel (Auld et al., 1988). Catterall, Pongs, Yuh Nung, and Lily Jan also were involved in cloning ion channel cDNAs in brain. Dick and I decided to change strategy again and return to cloning the skeletal muscle sodium channel. However, there was concern that we would end up recloning the same sodium channels identified in brain, so we performed the first sodium channel molecular RNAse protection analyses to test whether the rat brain sodium channels also were expressed in muscle. They were not

(Cooperman et al., 1987)! Therefore, with Jim Trimmer and Bill Agnew at Yale, we closed in on cloning and expressing the first cDNA encoding a non-neuronal voltage-dependent sodium channel (Trimmer et al., 1989). One of the most important and unexpected revelations from our work was the discovery that the muscle sodium channel sequence was quite divergent from the brain sequences. Distinctions in tetrodotoxin (TTX) sensitivity of sodium channels were known, but this was not enough information to have drawn conclusions about protein structure. One very important contribution of our cloning effort was the first full-length cDNA clone for a voltage-dependent ion channel that would be freely available, for the first time, to all interested scientists, without stipulations of collaboration. The sharing of such an important reagent began to reap benefits for the biophysicists and provide new mechanistic insights into congenital diseases that had been entirely unexplored. One of the first studies revealed that mutations in this channel (SkM1) turned out to be the basis of hyperkalemic periodic paralysis (Cannon et al., 1995). The biophysical community would soon learn that structural diversity within the sodium channel gene family was even greater than we had imagined. At last count, there are 10 distinct mammalian sodium channel subtypes in different excitable tissues, many expressed in tissue-specific fashion. I was to investigate the tissue-specificity more intensively later.

This period of ion channel gene discovery was an exciting time in my career. In addition to mammalian cloning efforts, there was a collaborative project with Larry Salkoff at Washington University. Around 1985, Paul ran into Larry at a Federation of American Societies for Experimental Biology (FASEB) meeting, and Larry was very anxious to apply ion channel cloning to his research. Larry had been the first to identify Shaker as a potassium channel defect and clearly understood the potential of cloning the cDNA. Paul directed him to me and working together we obtained the first genomic sequence for a fly sodium channel (Salkoff et al., 1987). In another important project, Paul's lab and mine were working together to look at sodium channel induction in a neuronal rat model, pheochromocytoma (PC12) cells. This cell line was used to investigate the signaling pathways by which nerve growth factor (NGF) led to a neuronal-like phenotype, including the ability to generate a TTX-sensitive action potential. Our objective was to be the first to link expression of a specific sodium channel isoform with native sodium current using a combination of patch clamp of the sodium current and molecular biology. Beyond identifying this link, our results would provide a direct comparison of functional properties of native and heterologously expressed sodium channels. Using RNA protection analyses, combined with whole-cell patch clamp analysis, we assigned induction of excitability, after long-term NGF treatment, to induction of the brain type II sodium channel (Nav1.2) (Mandel et al., 1988). Strikingly, the functional properties of this sodium current were markedly dissimilar to that reported by oocyte expression studies on brain type II alpha subunit. Moreover, the fast inactivation

observed for PC12 sodium current resembled that of traditional *in vivo* inactivation widely reported among different species and tissue types, which was distinct from the slow inactivation of Nav1.2 sodium channel alpha subunits expressed in the oocytes. This result put to rest the idea that the slowly inactivating sodium channel currents observed in oocytes from expressed RNA reflected function in certain native neurons, previously overlooked by electrophysiologists. Instead, the results supported the idea of the existence of an auxiliary subunit that was present in native tissue, such as the sodium channel beta subunits identified biochemically by the Catterall and Barchi labs. This proposition was later tested and proven through coexpression with a beta subunit newly cloned by others.

Paul and I submitted the PC12 sodium channel findings at the same time that Larry and I submitted the *Drosophila* sodium channel findings, both as reports to *Science*. Both papers were accepted for publication to appear in 1987, and we were told that they would likely appear in the same issue. We were excited to also learn that the *Drosophila* channel paper had been upgraded to an article. However, shortly thereafter, Paul received a call from an editor at *Science* (when journals still relied on the telephone for both good and bad news) saying that they decided to unaccept the PC12 paper. The editor told him that sodium channel papers, in particular, were no longer a priority and "were being sent to the circular file." Paul challenged him, pointing out that he had just upgraded a sodium channel paper from a report to an article. The editor asked how he knew about this, and Paul told him to check the authorship on the two papers. Clearly, there was more going on than the editor wanted to share. I think this was the instance that led to our referring to manuscripts as "under rejection" as opposed to "under review" when our work was submitted to one of the high-profile journals. In the end, the article with Salkoff was published in the same 1987 issue of *Science* as the sequence of the fly shaker K channel from the Jan labs. This was not the end, however, for the odyssey of the PC12 paper. Paul asked one of the leading ion channel biophysicists, Susumu Hagiwara, our UCLA colleague, to communicate the manuscript to *Proceedings of the National Academy of Sciences* (*PNAS*). He reviewed it and had some suggestions that were easily incorporated. Hagi called back, embarrassed after checking with *PNAS*, to say that he unwittingly had already given away his last direct communication slot. Hagi convinced a fellow NAS colleague at UCLA, George Bartholomew, a physiological ecologist, to communicate the paper (Mandel et al., 1988). After the work was published, Hagi confessed that he was in hot water with *PNAS* for transferring the paper. This was an impetus, among others, for NAS to adopt a policy for members that restricted direct communications of papers to people who are experts in the field covered by the paper.

Another ion channel cloning project that originally involved the joint Brehm/Mandel/Goodman labs was cloning cDNA that encoded the rat epsilon subunit of the pentameric muscle acetylcholine receptor (AChR). This

project was initiated shortly after Numa had identified bovine epsilon as a new AChR subunit. Epsilon was to add another member to the already four subunits (alpha, beta, delta, and gamma) known to form the AChR. The burning question became whether developmental changes in AChR conductance and gating seen in mammals and frogs could be accounted for by epsilon. The prevailing view was that the changes were due to post-translational phosphorylation of the receptor. Paul's findings with Yoshi, however, found that the changes required transcription and were consistent with the kinetics of receptor turnover and replacement. We pushed hard to obtain a rat epsilon cDNA for expression for testing, as Numa had not yet published functional properties of receptors containing the new subunit. Once again, however, by sharing his clone with the Sakmann lab, we would lose the race. Oocyte expression by this group showed that substitution of the gamma subunit by epsilon recapitulated the developmental changes in conductance and kinetics. Still, this set into motion our efforts to obtain the entire complement of *Xenopus* subunits for investigating receptor function during synapse development using the advantages offered by spinal neuron/muscle cocultures. This long-term molecular/physiology collaboration between Paul and me on AChRs would last decades, transitioning later to zebrafish.

On the home front, our daughter, Caleigh Mandel-Brehm, had been born in 1985. Now, with two children in tow, and Paul spending each summer with Kathy Dunlap at the MBL, we were, once again, stretched thin. It was worth it, however, as the MBL project was remarkably productive and fascinating, involving GFP and bioluminescence. Kathy and Paul were following up on Paul's first failed thesis project, which sought to identify the source of calcium for bioluminescence using GFP as a cell marker. Using the newly developed patch clamp technology, Kathy and Paul would be the first to discover a role played by gap junctions in chemical signaling between cells. Along with Stephen Smith from Stanford and a talented Chilean biophysicist David Naranjo, they were also the first to use bioluminescence to image calcium changes *in vivo*. This led to the discovery of intercellular propagating calcium waves, a new kind of cellular communication and a new role for gap junctions. Given the excitement of these discoveries, there was every reason to push forward with the summer separation.

Our lives would be simplified by an opportunity for me at the MBL. Arthur Karlin was the new director of the MBL neurobiology course and he invited me to join as an instructor in 1987. Summers were much more relaxed and enjoyable with the whole family at Woods Hole. As one of only a handful of ion channel molecular biologists, I was responsible for introducing molecular neurobiology to the course. Each summer we would craft a molecular biology project for the students. As one example, we set out to clone the sodium channel cDNA from the rough-skinned salamander *Taricha*, a species that produced TTX. The idea was to see whether the sodium channels in the salamander were resistant to the TTX, as first reported for puffer

fish (Kidokoro et al., 1974). If true, was the TTX resistance due to an amino acid alteration in the primary structure? It was productive research-wise for me as well, as Rindy Jaffe and I collaborated on a paper that appeared in *Science*, showing that cortical contraction in *Xenopus* oocytes could be induced by Ach following injection of RNA coding for the M1 muscarinic acetylcholine receptor (Kline et al., 1988).

Back at Tufts, our commute was steadily taking its toll and impacted negatively on my time in the lab. We tried every combination of car and rapid transit, but there was nothing rapid about the rapid transit in Boston, which included the MBTA, the bus, and the light rail. We eventually turned to driving around downtown trying to find a parking spot. We would trade off days during the week for going home while the other stayed late. Evenings involved two dinners, an early one for the kids and a much later one for us. We flipped for the weekends, with the winner going to the lab for a few hours of uninterrupted thinking and benchwork. Finally, after years of applying for a Tufts parking spot, I was approved. Still, when it came time to pick up the kids from daycare one of us would drive as in "It's a Mad-Mad-Mad-Mad World," trying to avoid the embarrassment of being the last parent to pick up their child. To this day, I see parents rushing to their cars at the end of the day in what I imagine is a fear of being late to daycare. In the final two years of Boston, we made just enough to hire a daytime nanny who would come to our house. Finally, we could take advantage of every minute in the lab and still have quality time with our kids.

In 1988, I was about to enter the most exciting time in my career, where all prior experience would come together to place me in a unique position. My background and interests were not in structure-function of ion channels or biophysics, and I was not going to follow a path of mutagenesis and functional studies. Instead, my interests were in achieving a basic understanding how the neuronal phenotype was acquired, focusing on the genetic components of the sodium channel gene that led to its neuronal-specific expression. Because the sodium channel is central to commerce throughout the nervous system, I reasoned that if I could understand what regulated sodium channel gene expression, it would lead me to more general mechanisms underlying formation of the nervous system. Indeed, the sodium channel work was going to lead me directly to the discovery of a fascinating transcription factor, the RE1 Silencing Transcription Factor (REST). The project had its roots in a set of experiments we did to isolate and clone an upstream regulatory region for the sodium channel gene, containing the promoter. We showed that this relatively small genomic fragment of about 1,200 base pairs programmed expression of a bacterial reporter gene in the PC12 neuronal cell model. Together with Bob Maue, Susan Kraner, and Dick Goodman, we designed simple deletional analyses of this genomic region in both PC12 and muscle cell lines. Unexpectedly, deleting a sodium channel gene regulatory sequence resulted in robust ectopic expression in the muscle cells with little

change in the PC12 neurons. The simplest interpretation of this result was that the regulatory region was acting as a repressor sequence in the muscle cells, and not as an activator in the neuronal PC12 cells (Maue et al., 1990). This discovery flew in the face of current dogma, holding that positively acting transcription factors were responsible for conferring tissue-specific expression. At the same time, David Anderson's group at Cal Tech published a similar story line for the neuronal SCG10 gene (Mori et al., 1990). The SCG10 gene encoded a neuronal-specific phospho-protein of unknown function, which David had identified in a screen for neuronal genes as a postdoc with Richard Axel. David and I were to do this two-step tango for the next several years as we each followed up on our initial discoveries.

Dick's and my scientific interests continued to move in different directions. His principal interests remained centered on cloning neuropeptide receptors, whereas mine were on ion channel regulation. Still, we worked together closely and toward the end of my time at Tufts, we recruited Paul to help with the neuropeptide receptor cloning, as he was routinely using oocyte expression. Several of the endocrine fellows (Tom Segerson, Eliot Spindel, and Kevin Sevarino) wanted to use RNA expression in oocytes to clone the neuropeptide receptors. Paul instructed all of them in oocyte expression and two microelectrode recordings, capitalizing on the common pathways involving intracellular calcium release used by these neuropeptides. The oscillating membrane currents would provide a faithful readout of expression and working through pools of RNA candidates ultimately would lead to identification of the single desired cDNA. The receptors included those corresponding to many of the previously cloned neuropeptides

When 1989 rolled around, I was showing signs of fatigue, reflecting the mounting demands on both scientific and personal time. Paul and I realized we could not continue making what amounted to entry-level salaries with the high cost of living in Boston. A visit by Jim Patrick, of the Salk Molecular Neurobiology group, would open our eyes to other opportunities. Jim knew Paul as a postdoc in that group and Jim was establishing the Department of Neuroscience at Baylor College of Medicine. He wanted us both to join and help guide development and Baylor rolled out the red carpet. Their offer stretched well beyond our imagination but, although greatly tempted, we ultimately turned down this opportunity in favor of Stony Brook University in New York. Our decision was driven by several factors that centered on a wonderful New York public school system for Josh and Caleigh and its seamless connection to high-quality daycare, minutes from campus. Another plus was the proximity to Woods Hole, making continued summer research at the MBL feasible and convenient. What clinched our decision, however, was our close friend and colleague, Simon Halegoua, who was working with the new chair of Neurobiology and Behavior at Stony Brook, Lorne Mendell, to recruit a person who could bring molecular neurobiology to the department. I was excited to work with Simon to develop molecular neurobiology at a

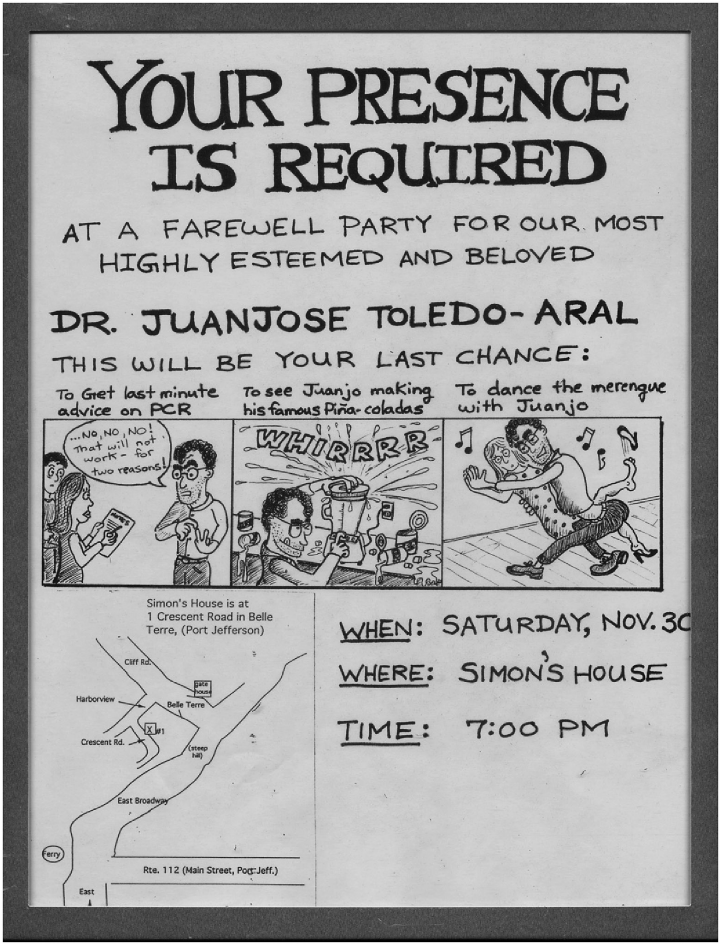
place already strong in cellular neurophysiology. As for Tufts, it was about to lose its core molecular neurobiology contingency in 1989, as Dick took Segerson, Low, Stork, and Roger Cone with him when he assumed the directorship of the Vollum Institute in Portland, Oregon. As he headed west, I would head further east with postdoc Susan Kraner, to Long Island.

Stony Brook, 1990–2007

With our move to Stony Brook University, we could finally afford a house that provided for all our needs. We bought a contemporary house at 7 Emmet Way that was located on two wooded acres. This would be the house that the kids would grow up in and the one that they would turn into the ultimate party house, much to our chagrin. The public school, only minutes away, offered great after school daycare, which included help with homework, social fun time, and putting on plays until 5 p.m. We were able to better balance family life with life in the lab. My lab was located next door to Simon Halegoua's on the fifth floor of the Life Sciences Building, and Paul was in the basement with fellow physiologist Gary Matthews. As per our tradition, Paul moved first and I sent Susan Kraner to set up the lab. Unlike Tufts, there was a graduate program and both Paul and I each accepted our first graduate students after 10 years of being faculty. Drew Chong was my first student and was exceptionally talented. He would play a major role in shaping the future direction of the lab.

In the summer of 1991, I was an instructor in the Neurobiology course at the Stanford University Hopkins Marine Station. Fellow instructors were my friends Bill Gilly and Rock Levinson. Both of them were highly entertaining and made for a great summer back on the west coast. Rock would later play a major part in my studies on sodium channel expression by providing years of experience with immunohistochemistry and antibody production. This course took on particular personal importance for two reasons. First, Hurricane Bob struck Long Island while I was away. Paul had no electricity or water for days, with our two young kids and our elderly dog, Shadow, who sadly died during the storm. Evidently, Paul tried for days to contact me, but there were no cell phones then and we were used to going long stretches without communication. I never heard the end of this one. Second, I made strong connections with two exuberant students in the course: Juan Jose Toledo-Aral, (Juanjo) from Seville, Spain, and Maurice Kernan, a fly geneticist with an interest in mechanoreceptors. Both ended up at Stony Brook, Juanjo as my postdoc, and Maurice as a faculty colleague. Juanjo would join me to explore the mechanisms underlying sodium channel expression in PC12 cells. This would set the stage for a three-way collaboration among Paul, Simon, and myself. My group at that time was brimming with talent and, with spirited Juanjo, afterwork hours seemed like one big party at our house. Juanjo would organize the rest of the lab to spend many an evening

at our house where he made authentic Sangria cocktails and taught us to dance the Merengue. My technician, David Kennedy, was a cartoonist and immortalized many of our after-hours parties as shown in the following illustration. Another talented postdoc, Yasushi Okamura, would also join in the fun both inside and outside the lab. Before joining my lab, Yasushi had been an MD/PhD student in the lab of an exceptionally creative scientist, Kuni Takahashi, at Tokyo Medical School. Yasushi led a project on protochordates in my lab and identified a contact-mediated induction mechanism of sodium channel expression at the gastrula stage (Okamura et al., 1994). In his independent lab, Yasushi later discovered the voltage sensitive phosphatase, which has revealed new functions for voltage sensors. With the core of Kraner, Chong, Okamura, and Juanjo, I was well positioned to take on new research challenges.



Thus, 1992 was a critical year for my group. Together, with Susan Kraner, Drew Chong, and Huey-Jen Tsay in the lab, we published the second part of the REST story (Kraner et al., 1992). Here, we identified Repressor Element 1 (RE1) as a 23 base pair sequence in the sodium channel promoter region. We showed that this sequence recruited a protein-binding complex only in terminally differentiated non-neuronal cell types such as fibroblasts. Furthermore, we devised a "repressor trap" by transfecting many copies of the RE1 sequence into cells, thereby preventing the complex from binding to the RE1. This trap resulted in derepression of the promoter in non-neuronal cells. The same year, in the same issue of *Neuron*, David's group published a similar sequence finding for SCG10 (Mori et al., 1992). And away we go.

The next step was obvious to both of our groups. We set out to identify the repressor protein that bound to the RE1/NRSE sequences. I recall being very nervous because David's group seemed like it was also moving into sodium channel regulation, and I was at a disadvantage having just established my lab at Stony Brook. Moreover, our son and daughter were getting to ages where they needed more time with us. We seemed to be failing at establishing the simple structured family life that was expected. No sit-down dinners together, no restaurants or movies, and no traditional family vacations. The NY schools piled on the homework with the expectation that the parents would have to play an integral part, competing with one another to help with take-home science projects, dioramas, and writing projects. It seemed as if a project was always in play, so the table saw was churning away and there was a chest of art supplies. Fast-drying plaster was a life-saver. It was getting harder to trade off with each other for weekend days in the lab as both kids became social butterflies and freely offered our house for the frequent sleepovers that invariably turned into all night parties. Then there was soccer, starting with modest competition and ascending to travel teams, select teams, and eventually school teams. I would take one and Paul would take the other to the games and tournaments. Mix in school plays, clubs, orchestra, wrestling, baseball, lacrosse, and basketball, and we had no free time left. We entertained all of these activities through grade school, junior high, and high school. Paul finally had too much with commuting between Stony Brook and Woods Hole. He ended his very productive stint with Kathy Dunlap at the MBL in 1993 and turned in their National Science Foundation grant. We needed a reprieve and found it in overnight Camp Half Moon for kids. Located in the Berkshires, the camp would take both Caleigh and Josh for eight consecutive weeks. Thankfully, cell phones had not been invented, and they were allowed only to write letters that, not surprisingly, didn't come that often. Both children went to this camp for the next six summers, providing them with new friends and adventures and freeing us to once again to live like postdocs.

As 1995 rolled around, I learned of an impending *Science* paper from the Anderson lab announcing the identification of neural restrictive

silencing factor (NRSF), which mediated repression from the NRSE site. We were close to identification of the binding factor ourselves, using a one-hybrid screening approach, and to lose at this point was unimaginable. Encouraged by Simon, I phoned Ben Lewin, then editor at *Cell*, and he said he would reserve a place in the next issue if we could show function with a full-length cDNA for REST. The entire lab pitched in for an intense stretch of 24/7 experiments. Susan Kraner had already left for a faculty position in Kentucky, but we drew her back in to run a critical experiment to show biochemical evidence for the binding of REST to the RE1 sequence *in vitro*. Late one night Drew Chong phoned me at home and said, "Gail, it worked"! The full-length REST cDNA in the PC12 cells repressed the endogenous sodium channel gene promoter present on an accompanying mini-gene. We had REST in time for publication in *Cell*. In the end, both my lab and David's lab prevailed (Chong et al., 1995; Schoenherr & Anderson, 1995). Together, we had discovered the basis for neural specificity, and it was due not to activation but to repression. If you wanted to be anything but a neuron, you needed to suppress neuronal genes with REST. I figured we had won in the name game with REST but, in fact, it now turns out to be difficult to use REST as a key word in a literature search. Google REST and you come up with any word that contains the letters r e s t. On the flip side, REST has provided an endless source of humorous seminar titles like "Despite Jet Lag, Stress and Grant Deadlines, Adults Get Plenty of REST" (thanks to Gary Westbrook); "The Rise and Fall of REST: Creating the Nervous System"; "Repression in the Nervous System; How to Quiet Your Nerves"; and "Advice to Neurons, Lose the REST."

It was during this same period that Simon, Paul, and I began our three-way collaboration on NGF-induced sodium channel excitability in PC12 cells. Simon had a long history with oncogenic signaling underlying growth-factor actions, beginning from his postdoc at the Salk Institute. He was an expert with signaling pathways in PC12 cells, while I had the molecular expertise with sodium channels, and Paul had the electrophysiological skills. We knew from previous molecular analysis that the Nav1.2 sodium channel was highly inducible by NGF in PC12 cells (Mandel et al., 1988). However, quite unexpectedly, Simon and I identified a second sodium channel type that was also NGF-induced. As this channel type was expressed specifically in peripheral neurons, we named it peripheral nerve sodium channel type 1, PN1 (D'Arcangelo, 1993), later changed to Nav1.7. At the time of publication in 1993, we were able to pin down the induction of Nav1.2 to a cAMP-dependent pathway. Juanjo Toledo Aral joined in on the hunt for NGF mechanisms that would account for PN1 induction and scored an immediate hit. He found that a brief exposure to NGF, as short as one minute, resulted in long-lasting induction of PN1 expression (Toledo-Aral, et al., 1995). The physiology would go on to show that the sodium current induction was as high as that from long-term NGF action, but short-term exposure

was selective to PN1. It worked through an immediate-early pathway that converged with an interferon-gamma signaling pathway. Simon, Juanjo, and I enlisted Rock Levinson to perform immunohistochemistry with a pan neuronal sodium channel antibody. Consistent with our initial predictions of this being a peripheral-type sodium channel, we found it was present in a subset of neurons in cultured dorsal root ganglia (DRG) neurons. Based on the distribution, we proposed that it had a specific function in nociceptors, rendering it central to pain sensation (Toledo-Aral et al., 1997). This idea was largely dismissed based on its TTX sensitivity, but when families were identified subsequently as unable to sense pain, molecular analysis assigned the pathophysiology to PN1. It was gratifying that our idea was eventually validated in humans.

In 1997, Max Cowan, then vice president of the Howard Hughes Medical Institute (HHMI), announced that I was selected as an HHMI investigator. Simon and I had already relocated to the basement of the Life Sciences building to form a new Center, joining Gary Matthews and Paul. We designed newly renovated labs from classrooms and organized the labs like a giant maze with common core facilities. There were many interconnected rooms, with people going in and out of different labs all day, and I have to say this arrangement and the personalities represented the most interactive group of scientists I have encountered in my career. Jim Trimmer, my collaborator and friend from the original sodium channel cloning days, also joined us in the basement Center from the biochemistry department. Jim built a big lab that was churning out antibodies for ion channels and dissecting the targeting elements for neurons. In addition to our individual grants, I was directing a program project from the NIH awarded to our group. I was fortunate to have Karen Warren as my HHMI administrative assistant. She would also keep all five of our labs on the rails as well. Her equally talented mother, Pat Leavy, would later join the group as my Society for Neuroscience (SfN) editorial assistant while I was on the editorial board. In short, the years in the basement would be special for all of us, but when offered luxurious labs in a brand-new building at Stony Brook, we moved. Unfortunately, it would never be the same. We were split between just floors 3 and 5, but it was enough to have a negative impact on our group interactions. Jim Trimmer would leave for a new position at UC Davis, and our NIH program project eventually terminated because of my desire to shed administrative duties. Publications continued, but we would never feel the excitement that was generated by the basement group.

The boost in funding provided by the HHMI appointment allowed me to expand the lab. New members adapted rapidly, evolving molecular approaches toward understanding how REST functioned as a repressor, as these were the early days of studying repressor complexes. We were able to make important contributions to our general understanding as well as to the understanding of nervous system development. For example, our findings

pointed to REST recruitment to the chromatin of two distinct corepressors, one of which was completely novel. Postdoc Julia Grimes showed that the amino terminal repressor domain of REST recruited the already identified general corepressor, Sin3, to neuronal gene chromatin (Grimes et al., 2000). Three other postdocs in the lab, Elena Battaglioli, Maria Estella Andres, and Pepe Tapia-Ramirez, identified a corepressor that mediated repression from the carboxyl terminal REST repressor domain (Tapia-Ramirez et al., 1997; Andres et al., 1999). That corepressor we named CoREST, and it turns out to be one of the major corepressors in cells of all types, even in the blood lineage, as one of my later graduate students Huilan Yao went on to show (Yao et al., 2014). Further interrogation of CoREST function directly linked REST repression to the chromatin modifier, histone deacetylase, thereby indicating a prime mechanism for its corepressor function (Ballas et al., 2001; You et al., 2001). A problem that had us stumped for a while involved the evolution of the REST repression mechanism. We turned to the fly model with the idea that we could make faster progress using the genetic advantages offered by this system. We were to learn that while CoREST is present from yeast to humans, REST evolved with the vertebrates. Based on her fly work (Dallman et al., 2004), my postdoc, Julia Dallman, showed that a tram-track protein, with no homology to REST, could still serve the same repressor function in neurodevelopment by coopting CoREST. This idea, of nonhomologous gene replacement for REST in invertebrates, has since been supported by studies in other laboratories.

I was also fortunate to recruit Nurit Ballas to my group. Nurit had published beautiful molecular work on plants, and she and I initially thought it would be fun to look at repressor function in plants. Ultimately, however, Nurit switched to mammalian molecular biology in the nervous system. This was an excellent decision on her part. Exploiting her earlier biochemistry skills, Nurit demonstrated, using mouse embryonic stem cells, that REST repressed neuronal genes not just in non-neuronal fibroblasts, but also in pluripotent stem cells and dividing neural progenitors. Using this ideal cell culture system, she went on to become the first person to illuminate how the REST repressor complex controlled chromatin status during the different stages of neuronal differentiation, including the differentiation to mature neurons (Ballas et al., 2005; Ballas & Mandel, 2005). For example, Nurit found that REST had to depart neuronal gene chromatin in neural progenitors before terminal neuronal differentiation, and that when REST left the chromatin, its loss caused the disengagement of the entire repressor complex, specifically primary corepressors, CoREST and histone deacetylase 2 (Ballas et al., 2001; Ballas & Mandel, 2005; Ballas et al., 2005). Furthermore, persistent expression of REST during early development delayed neuronal differentiation *in vivo* (Mandel et al., 2011). Taken together, these studies provided a prototype for the epigenetic function of repressors in the developing central nervous system. I still

have students and postdocs new to the lab refer to these early papers to measure REST function in the context of differentiating cells. Nurit and I also initiated studies of the cellular basis of the neurological disease Rett syndrome, caused by sporadic mutations in the gene encoding the transcriptional repressor, Methyl CpG binding protein 2 (MeCP2). We were the first to provide experimental support for the idea that glia, as well as neurons, were affected in this neurodevelopmental disorder (Ballas et al., 2009). This idea was met with some skepticism initially, but after my move to Vollum, we strengthened the proposal, in collaboration with Nurit, through *in vivo* studies (Lioy et al., 2011). Nurit continues elegant studies elucidating the role of human astrocytes in Rett syndrome in her independent lab at Stony Brook.

In addition to revealing more about repressor function, REST was poised to make another major contribution. In a chance meeting with John Dunn (Brookhaven National Laboratory in Long Island) around 2003, I became aware of his recently developed Genome Signature Tag method for profiling genomic DNA (Dunn et al., 2002). John was a very creative scientist who openly shared his ideas with those around him. He had used this technique for genomic profiling of the *Yersinia pestis* genome, and I asked him about adapting his method for identifying REST binding sites genome-wide. John agreed it was a reasonable idea, and we agreed to work together on the experimental design and analysis. We recruited a talented bioinformatics colleague of John's at Brookhaven, Sean McCorkle, who had worked on the Yersinia project. We invited my previous collaborator, Dick Goodman, to participate in the project, surmising that it would be beneficial to use two mammalian transcription factors with completely different binding site motifs. Although now separated by 3,000 miles, we worked together again, with Dick working on CREB at the Vollum with his group, and my group working on REST at Stony Brook, with open communication among us. Graduate student Stephanie Otto led the effort in our group. Our collective efforts were successful (Impey et al., 2004; Otto et al., 2007). For us, the results represented a major contribution toward understanding REST function. We showed, unequivocally, that REST occupied, and presumably repressed, thousands of neuronal genes. For the first time, we were seeing the entire scope of REST-dependent regulation. We proposed that "neural-ness" was actually a default pathway in non-neuronal cells, which was prevented by the REST repressor mechanism. Remarkably, our REST paper was published the same year that the Wold group at Cal Tech used REST as the proof of concept for high-throughput chromatin immunoprecipitation technology (ChIP seq; Johnson et al., 2007). Although our two groups used different species and different non-neuronal cell types for the genome-wide studies, and different approaches for the binding analyses, both studies converged on the same REST binding site motifs on thousands of neuronal genes. Years earlier, David Anderson and I had predicted that

vast numbers of neuronal genes would share the REST binding site, simply using our eyes to pick out the REST binding sites in the neuronal sodium channel and SCG10 genes, so it was a rewarding denouement to our first findings.

Simon Halegoua was not the only colleague at Stony Brook who heavily influenced my research. Joe Fetcho was an engine driving both Paul and me to collaborate on zebrafish physiology and genetics. Joe had abandoned goldfish, turning to zebrafish as a model for circuitry analyses. Around 1997, he invited Michael Granato for a seminar. Granato introduced his cool motility mutants that he was using to identify central circuits involved in swimming. Paul and I thought that it was more likely that the phenotypes instead reflected defects in the final motoneuron pathway. Indeed, our intuition was correct and the mutants would pave the way to identifying key players in synaptic transmission and human congenital myasthenic syndrome. The work would involve synaptic physiology and zebrafish behavioral analysis performed in the Brehm lab and molecular analysis in my lab. One of the first mutants we explored was intriguing. The *twitch once* line was unable to mount swimming due to fatigue setting in after the first or second tail bend. Paul's postdoc, Fumi Ono, found that the acetylcholine receptors were present in the muscle membrane but in the wrong location, being distributed and not clustered at the synapse. Rapsyn was a protein known to be involved in AchR clustering and, by sheer coincidence, a postdoc in my lab, Shinichi Higashijima, had created a rapsyn-GFP transgenic zebrafish line in Japan before coming to my lab. On a hunch, Fumi crossed the rapsyn-GFP transgenic line with *twitch once*. When in-crossed, all of the fluorescent fish were able to mount normal swimming and the receptor clustering phenotype was rescued. We had our first *bona fide* hit without having to do positional cloning, which was tedious at that time. There was more to come. Fumi noticed that the fatigue associated with the mutant phenotype was similar to a very common phenotype in humans afflicted with a variety of myasthenic syndromes. When he administered cholinesterase inhibitors used to treat humans, he observed greatly improved swimming in the mutant line. On this basis, he proposed that mutations in rapsyn might represent a currently unidentified form of myasthenic syndrome. Our announcement at the 2001 SfN meeting spawned the discovery of human rapsyn deficiency (Ono et al., 2001; Ono et al., 2002). The stage was now set for our collaborative exploration into zebrafish motility mutants as a platform for identifying and studying myasthenic syndromes in humans.

My lab members who participated in the Mandel/Brehm collaboration included Julia Dallman, Michelle Gleason, and Becky Mongeon. Through combined patch clamp, cloning, and imaging, our group assigned functional defects to mutations that represent human myasthenic syndromes. A mutation in muscle calcium ATPase in the *accordion* line informed us about contractile defects in Brody disease (Gleason et al., 2004). A mutation in

the pore of the AchR in the *twister* line provided key insights as to pharmacologic treatment of slow channel syndrome (Walogorsky et al., 2012), as did the *bajan* line, a mutation in choline acetyltransferase responsible for episodic apnea (Wang et al., 2008). Other important studies involved mutations of the glial glycine transporter (*Shocked* line; Mongeon et al., 2008) and Eaton Lambert syndrome, a mutation in the P/Q calcium channel (*tb204a* line; Wen et al., 2013). In addition to resolving these neuromuscular phenotypes, we made other fundamental findings together. Led by Hua Wen in Paul's lab, we were the first to identify synaptotagmin 7 as the calcium sensor mediating asynchronous transmitter release (Wen et al., 2010). There were also rewarding collaborations with Joe Fetcho's group on zebrafish that were among the first to track activity in spinal circuits using *in vivo* imaging (Gleason et al., 2003; Higashijima et al., 2003; Armisen et al., 2007). My collaborations with Paul and Joe on zebrafish have been one of the truly fun epochs in my career, persisting over a 20-year span.

Meanwhile, back at home, our kids were growing up and becoming competitive New Yorkers, much to the trepidation of a Midwest father and West Coast mother. Pressure was on in every aspect of their lives, from sports to parties to academics and to the uncertainties about which college would admit them. That last concern was driven more by the parents than the kids as competition spread through Long Island like wildfire. Both kids were captains of their school soccer teams, and Josh was on a select travel team as well. Caleigh was traveling to Europe to compete. Josh was also captain of his wrestling team, and Caleigh was captain of the charter female golf team at her school. We managed to squeeze in a bit of cultural heritage in the form of Hebrew school. They never complained, but they clearly had a healthy skepticism of many teachings, like why miracles only happened in Biblical times and whether these people really existed. Speaking frankly, the kids stuck it out for one reason: the Bar and Bat Mitzvah parties following graduation from Hebrew School. On Long Island, these parties take on mammoth proportions, rivaling weddings. When Josh held his party at the Stony Brook Yacht club, the drapes mysteriously caught on fire and was on the fringe of becoming out of control. The Commodore informed us that for the first time in their 100-year history, the Yacht Club would not be returning a security deposit. Not to be outdone, Caleigh held her Bat Mitzvah at the historical Stony Brook Three Village Inn and restaurant, forcing closure because of noise and smoke cannons that the DJ was firing into the crowd.

All in all, we had managed to make career choices that would balance opportunities for the kids, while still providing a good opportunity for each of us to pursue research. Of course, the kids continued to get into trouble in college, but at least they were a thousand miles away and for the most part it was "good trouble." Caleigh protested for human rights of the homeless in the unforgiving winters of Madison, Wisconsin, and Josh formed the charitable Linus Foundation with members of his fraternity. They devised

an idea that involved raucous fundraising parties as a means to provide sporting equipment for poor intercity kids. They both made it through; Caleigh with a degree in zoology from the University of Wisconsin and Josh with a degree in neuroscience from Washington University. Caleigh took advantage of a wonderful opportunity to be a technician in the lab of Huda Zoghbi at Baylor College of Medicine. This would be her gateway into graduate school at Harvard Neurobiology, under the mentorship of friend and colleague, Mike Greenberg. Josh accepted a position at Hydra Bioscience in Boston, founded by David Clapham. Although Josh started off doing patch clamp screening for drug compounds, he decided he liked the business end better and was off to the University of Michigan to earn an MBA. The kids were set for the time being and we could, for the first time in many years, think more selfishly, opening the last chapter in our careers.

Vollum Institute, 2006

In what Dick Goodman refers to as his seventeen-year recruitment, Paul and I returned to the west coast to join the faculty at the Vollum Institute in Portland, Oregon. The Department of Neurobiology and Behavior at Stony Brook had become very senior and our leaving would provide them with the opportunity to recruit new junior faculty. Our star colleague and close friend, Joe Fetcho, who introduced us to zebrafish, had already left for Cornell University in Ithaca, so our lunch party was down to Simon, Paul, and me. Our decision meant we would be leaving Simon, our close friend and scientific colleague, but the west coast was calling. I had been eager to return to the west coast, but finding jobs for two very senior faculty, neither of whom was interested in being a departmental chair, had greatly narrowed the possibilities.

After being hired at the Vollum, we bought a charming house in Portland that had been part of the Lewis and Clark Centennial Exposition in 1905. The house was moved by mule from the original site of the Exposition along the Willamette River to its final resting place at 1717 NW 33rd Avenue. We found the small city to be very lively, blending rural and urban lifestyles, good music, and good food. Still missing the northeast, however, we eventually purchased and renovated a 1907 house at 10 Orchard Street, in the heart of Woods Hole, very close to the MBL. We needed sanctuary from Portlanders and their obsession to be unique, which is tantamount to their doing the opposite of whatever New York and California were doing. Oregon had dramatic (but not warm) beaches, mountains, and wineries to explore, and our wonderful dog, Blue, benefited from the lack of leash laws at the beach. She loved chasing birds and playing in the waves. All things considered, we have enjoyed Portland and doing research in the Vollum.

I was able to move my HHMI lab to the Vollum, which certainly helped in the transition. There was space available for Paul and me to have labs

across from each other, as we had enjoyed at Stony Brook. Also, as in Stony Brook, when students and postdocs in my lab needed imaging or physiology technology, they migrated into Paul's lab, and when Paul's group needed molecular biology, they had dedicated benches in my lab. Thus, we continued to be able to collaborate on projects, for example, using zebrafish, but we also continued to pursue our individual research interests. Happily, Hua Wen, Paul's extremely talented long-term research professor and the mainstay of zebrafish physiology and molecular biology in his lab, decided to move with us to the Vollum. Even with a skeleton crew, the move was very good for me as well. I was elected into the American Academy of Arts and Sciences and, with Dick's support, also was elected into the National Academy of Sciences. With the move to the Vollum, I took the opportunity to move my research into new projects and technologies, which are still unfolding.

Our original discovery of REST opened up areas beyond what we had imagined in 1995. Because of its regulation of thousands of neuronal genes, REST became a widely studied transcriptional repressor in the central nervous system (CNS), both during neurodevelopment and in the adult. We continued our work on REST during early neurogenesis in mice and found that REST not only repressed expression of neuronal genes until cell cycle exit, as predicted, but also protected the genome from DNA damage during the cell cycle (Nechiporuk et al., 2016). Premature deletion of REST in mice resulted in DNA breaks during the cell cycle that led ultimately to depletion of the progenitor pool and microcephaly in the adult. This result added more support, from a different perspective, to previous work by others of a link between REST and neurogenesis related to human microcephaly (Yang et al., 2012). We also identified sequences in REST that regulated its stability, with consequences on axonal integrity in peripheral neurons, as well as a signaling pathway that was important in transcriptional regulation at REST-regulated promoters (Cargnin et al. 2014; Nesti et al., 2014).

Most recently, in an unexpected turn of events, we found that by studying mice exclusively we had missed an important function in REST that was specific to primates. REST levels in mouse brain decrease during aging, while in human brain they begin to increase during aging. This difference coincides with a completely distinct set of target genes in human hippocampus that represent genes encoding inflammatory and immune pathways rather than the stereotypical "synaptic" genes (McGann et al., 2021). It had been reported that REST regulates human longevity and that REST levels are lower in Alzheimer's disease. Our findings support the idea of a new role for REST in aging, and the results have opened the doors to new functions for REST in protecting human brain health. Finally, we have identified newly evolved motifs in the primate REST protein, absent in lower mammals, which we hypothesize play important roles in the expansion of

the numbers of neural progenitors in the cortex in primates. In addition to providing a model for basic mechanisms of transcriptional repression, REST dys-regulation recently has been implicated in studies by other investigators in diverse abnormal CNS pathologies, including epilepsy, cancer and hearing loss.

We continue to focus on gene repression in our studies of MeCP2, and its function in different CNS cell types. Because *MECP2* is on the X chromosome, females are mosaic for loss of MeCP2 function due to dosage compensation in mammals. A clever physiology study, led by graduate student Benjamin Rakela, comentored by both Paul and me, exploited a female Rett mouse model generated in Adrian Bird's lab (University of Edinburgh). In this model, wild-type cells in the brain can be distinguished by their GFP fluorescence from cells lacking MeCP2 expression due to mutations. In cortical slice recordings using this Rett mouse model, and by testing every combination of wild-type and MeCP2 mutant astrocytes and neurons, Benjamin found a perturbation in synaptic communication that was manifest as decreased astrocyte: neuronal excitatory signaling (Rakela et al., 2018). This central role played by astrocytes confirmed a proposal we had made earlier using cre-lox technology and the astrocyte effects on mouse Rett-like behaviors (Lioy et al., 2011). We also pursued high-resolution microscopy experiments, stimulated by the creativity of Stephen Smith. We adapted his technique of array tomography, created specifically to investigate synapses, to instead investigate architectural changes in chromatin. One of the advantages of this technique, which uses extremely thin sections, is that cells within the same tissue section are treated identically for direct side-by-side direct comparisons. Furthermore, multiple rounds of immuno-labeling can be performed. A research assistant professor in the lab, Mike Linhoff, exploited this technology, again using the female Rett mouse model in which only the wild-type neurons are GFP labeled. Mike found robust changes in the 3D-chromatin structure in the mutant MeCP2-deficient neurons in the hippocampus of these female mice. The chromatin in mutant neurons was more compact, which would be predicted to inhibit gene activation (Linhoff et al., 2015). We propose that the chromatin structural changes might make the mutant neurons less able to adapt normally to stimuli within a circuit. While this proposal awaits confirmation, independent studies by others have confirmed the chromatin structural changes that we first reported.

Because no single gene target has been identified to explain Rett syndrome, in our most recent endeavor, we have bridged basic science and translational medicine by designing experiments to repair MeCP2 mutations at the level of RNA, using a targeted RNA editing approach (Sinnamon et al., 2017; Sinnamon et al., 2020). Using this approach, John Sinnamon, a research assistant professor, led a team to show the first efficient reversal of a Rett patient mutation in nondividing cells (neurons) in culture, as well as the first effective editing of the same patient mutation across different

hippocampal neuronal types in mice (Sinnamon et al., 2020). Studies of the consequences of Rett patient mutation reversal by targeted RNA editing, on Rett-like behaviors in mice, are ongoing and promising.

Reflections on My Career

I did not know from the beginning what I wanted to do as a profession. I cannot say I always wanted to peer into a microscope, although I did have an innate curiosity about how things worked. My future direction decision came late in college, fueled by the scientists around me. From them I was to learn what it was to make a discovery, a revelation that changes the way we think about a biological process or problem. Most of science is spent trying to piece together a puzzle, but those of you who have been the very first to solve a puzzle know the thrill. To the many extraordinary colleagues who helped guide and inspire me to set the bar high, I owe my heart-felt gratitude. I have always enjoyed being in the lab, sharing pivotal moments in a project with my students and postdocs. Let discovery serve as their ambition. In closing, I have also been lucky to be part of a wonderful and interesting family, especially our two children, who are in science in one way or the other; their spouses; and our lively and inquisitive grandchildren, all of whom are the primary reason for writing this autobiography.

Selected Bibliography

Andres ME, Burger C, Peral-Rubio MJ, Battaglioli E, Anderson ME, Grimes J, Dallman J, Ballas N, Mandel G. (1999) CoREST: a functional corepressor required for regulation of neural-specific gene expression. *Proceedings of the National Academy of Sciences of the United States of America* 96(17): 9873–8.

Armisen R, Gleason MR, Fetcho JR, Mandel G. (2007) Tracking tagged molecules in single neurons in intact zebrafish. *CSH Protocols* 2007: pdb prot4780.

Auld VJ, Goldin AL, Krafte DS, Marshall J, Dunn JM, Catterall WA, Lester HA, Davidson N, Dunn RJ. (1988) A rat brain Na+ channel alpha subunit with novel gating properties. *Neuron* 1(6): 449–61.

Ballas N, Battaglioli E, Atouf F, Andres ME, Chenoweth J, Anderson ME, Burger C, Moniwa M, Davie JR, Bowers WJ, Federoff HJ, Rose DW, Rosenfeld MG, Brehm P, Mandel G. (2001) Regulation of neuronal traits by a novel transcriptional complex. *Neuron* 31(3): 353–65.

Ballas N, Grunseich C, Lu DD, Speh JC, Mandel G. (2005) REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. *Cell* 121(4): 645–57.

Ballas N, Lioy DT, Grunseich C, Mandel G. (2009) Non-cell autonomous influence of MeCP2-deficient glia on neuronal dendritic morphology. *Nature Neuroscience* 12(3): 311–7.

Ballas N, Mandel G. (2005) The many faces of REST oversee epigenetic programming of neuronal genes. *Current Opinion in Neurobiology* 15(5): 500–6.

Boyd A, Mandel G, Simon MI. (1982) Integral membrane proteins required for bacterial motility and chemotaxis. *Symposia of the Society for Experimental Biology* 35: 123–37.

Cannon SC, Hayward LJ, Beech J, Brown RH, Jr. (1995) Sodium channel inactivation is impaired in equine hyperkalemic periodic paralysis. *Journal of Neurophysiology* 73(5): 1892–9.

Cargnin F, Nechiporuk T, Mullendorff K, Stumpo DJ, Blackshear PJ, Ballas N, Mandel G. (2014) An RNA binding protein promotes axonal integrity in peripheral neurons by destabilizing REST. *Journal of Neuroscience* 34(50): 16650–61.

Chong JA, Tapia-Ramirez J, Kim S, Toledo-Aral JJ, Zheng Y, Boutros MC, Altshuller YM, Frohman MA, Kraner SD, Mandel G. (1995) REST: a mammalian silencer protein that restricts sodium channel gene expression to neurons. *Cell* 80(6): 949–57.

Cooperman SS, Grubman SA, Barchi RL, Goodman RH, Mandel G. (1987) Modulation of sodium-channel mRNA levels in rat skeletal muscle. *Proceedings of the National Academy of Sciences of the United States of America* 84(23): 8721–5.

D'Arcangelo G, Paradiso K, Shepherd D, Brehm P, Halegoua S, Mandel G. (1993) Neuronal growth factor regulation of two different sodium channel types through distinct signal transduction pathways. *Journal of Cell Biology* 122(4): 915–21.

Dallman JE, Allopenna J, Bassett A, Travers A, Mandel G. (2004) A conserved role but different partners for the transcriptional corepressor CoREST in fly and mammalian nervous system formation. *Journal of Neuroscience* 24(32): 7186–93.

Dunn JJ, McCorkle SR, Praissman LA, Hind G, Van Der Lelie D, Bahou WF, Gnatenko DV, Krause MK. (2002) Genomic signature tags (GSTs): a system for profiling genomic DNA. *Genome Research* 12(11): 1756–65.

Fink JS, Verhave M, Kasper S, Tsukada T, Mandel G, Goodman RH. (1988) The CGTCA sequence motif is essential for biological activity of the vasoactive intestinal peptide gene cAMP-regulated enhancer. *Proceedings of the National Academy of Sciences of the United States of America* 85(18): 6662–6.

Gleason MR, Armisen R, Verdecia MA, Sirotkin H, Brehm P, Mandel G. (2004) A mutation in serca underlies motility dysfunction in accordion zebrafish. *Developmental Biology* 276(2): 441–51.

Gleason MR, Higashijima S, Dallman J, Liu K, Mandel G, Fetcho JR. (2003) Translocation of CaM kinase II to synaptic sites in vivo. *Nature Neuroscience* 6(3): 217–8.

Grimes JA, Nielsen SJ, Battaglioli E, Miska EA, Speh JC, Berry DL, Atouf F, Holdener BC, Mandel G, Kouzarides T. (2000) The co-repressor mSin3A is a functional component of the REST-CoREST repressor complex. *Journal of Biological Chemistry* 275(13): 9461–7.

Higashijima S, Masino MA, Mandel G, Fetcho JR. (2003) Imaging neuronal activity during zebrafish behavior with a genetically encoded calcium indicator. *Journal of Neurophysiology* 90(6): 3986–97.

Impey S, McCorkle SR, Cha-Molstad H, Dwyer JM, Yochum GS, Boss JM, McWeeney S, Dunn JJ, Mandel G, Goodman RH. (2004) Defining the CREB regulon: a genome-wide analysis of transcription factor regulatory regions. *Cell* 119(7): 1041–5.

Inouye M, Halegoua S. (1980) Secretion and membrane localization of proteins in Escherichia coli. *CRC Critical Review Biochemistry* 7(4): 339–71.

Ito K, Mandel G, Wickner W. (1979) Soluble precursor of an integral membrane protein: synthesis of procoat protein in Escherichia coli infected with bacteriophage M13. *Proceedings of the National Academy of Sciences of the United States of America* 76(3): 1199–203.

Johnson DS, Mortazavi A, Myers RM, Wold B. (2007) Genome-wide mapping of in vivo protein-DNA interactions. *Science* 316(5830): 1497–502.

Kidokoro Y, Grinnell AD, Eaton DC. (1974) Tetrodotoxin sensitivity of muscle action potentials in pufferfishes and related fishes. *Journal of Comparative Physiology* 89:59–72.

Kline D, Simoncini L, Mandel G, Maue RA, Kado RT, Jaffe LA. (1988) Fertilization events induced by neurotransmitters after injection of mRNA in Xenopus eggs. *Science* 241(4864): 464–7.

Kraner SD, Chong JA, Tsay HJ, Mandel G. (1992) Silencing the type II sodium channel gene: a model for neural-specific gene regulation. *Neuron* 9(1): 37–44.

Lechan RM, Wu P, Jackson IM, Wolf H, Cooperman S, Mandel G, Goodman RH. (1986) Thyrotropin-releasing hormone precursor: characterization in rat brain. *Science* 231(4734): 159–61.

Leiter AB, Toder A, Wolfe HJ, Taylor IL, Cooperman S, Mandel G, Goodman RH. (1987) Peptide YY. Structure of the precursor and expression in exocrine pancreas. *Journal of Biological Chemistry* 262(27): 12984–8.

Linhoff MW, Garg SK, Mandel G. (2015) A high-resolution imaging approach to investigate chromatin architecture in complex tissues. *Cell* 163(1): 246–55.

Lioy DT, Garg SK, Monaghan CE, Raber J, Foust KD, Kaspar BK, Hirrlinger PG, Kirchhoff F, Bissonnette JM, Ballas N, Mandel G. (2011) A role for glia in the progression of Rett's syndrome. *Nature* 475(7357): 497–500.

Mandel G, Clark W. (1978) Functional properties of EL-4 tumor cells with lipid-altered membranes. *Journal of Immunology* 120(5): 1637–43.

Mandel G, Cooperman SS, Maue RA, Goodman RH, Brehm P. (1988) Selective induction of brain type II Na+ channels by nerve growth factor. *Proceedings of the National Academy of Sciences of the United States of America* 85(3): 924–8.

Mandel G, Fiondella CG, Covey MV, Lu DD, Loturco JJ, Ballas N. (2011) Repressor element 1 silencing transcription factor (REST) controls radial migration and temporal neuronal specification during neocortical development. *Proceedings of the National Academy of Sciences of the United States of America* 108(40): 16789–94.

Mandel G, Shimizu S, Gill R, Clark W. (1978) Alteration of the fatty acid composition of membrane phospholipids in mouse lymphoid cells. *Journal of Immunology* 120(5): 1631–6.

Mandel G, Wickner W. (1979) Translational and post-translational cleavage of M13 procoat protein: extracts of both the cytoplasmic and outer membranes of

Escherichia coli contain leader peptidase activity. *Proceedings of the National Academy of Sciences of the United States of America* 76(1): 236–40.

Maue RA, Kraner SD, Goodman RH, Mandel G. (1990) Neuron-specific expression of the rat brain type II sodium channel gene is directed by upstream regulatory elements. *Neuron* 4(2): 223–31.

McGann JC, Spinner MA, Garg SK, Mullendorff KA, Woltjer RL, Mandel G. (2021) The genome-wide binding profile for human RE1 silencing transcription factor unveils a unique genetic circuitry in hippocampus. *Journal of Neuroscience* 41(31): 6582–95.

Mongeon R, Gleason MR, Masino MA, Fetcho JR, Mandel G, Brehm P, Dallman JE. (2008) Synaptic homeostasis in a zebrafish glial glycine transporter mutant. *Journal of Neurophysiology* 100(4): 1716–23.

Montminy MR, Sevarino KA, Wagner JA, Mandel G, Goodman RH. (1986) Identification of a cyclic-AMP-responsive element within the rat somatostatin gene. *Proceedings of the National Academy of Sciences of the United States of America* 83(18): 6682–6.

Mori N, Schoenherr C, Vandenbergh DJ, Anderson DJ. (1992) A common silencer element in the SCG10 and type II Na+ channel genes binds a factor present in nonneuronal cells but not in neuronal cells. *Neuron* 9(1): 45–54.

Mori N, Stein R, Sigmund O, Anderson DJ. (1990) A cell type-preferred silencer element that controls the neural-specific expression of the SCG10 gene. *Neuron* 4(4): 583–94.

Morin JG, Hastings JW. (1971) Energy transfer in a bioluminescent system. *Journal of Cellular Physiology* 77(3): 313–8.

Nechiporuk T, McGann J, Mullendorff K, Hsieh J, Wurst W, Floss T, Mandel G. (2016) The REST remodeling complex protects genomic integrity during embryonic neurogenesis. *eLife* 5: e09584.

Nesti E, Corson GM, McCleskey M, Oyer JA, Mandel G. (2014) C-terminal domain small phosphatase 1 and MAP kinase reciprocally control REST stability and neuronal differentiation. *Proceedings of the National Academy of Sciences of the United States of America* 111(37): E3929–36.

Noda M, Shimizu S, Tanabe T, Takai T, Kayano T, Ikeda T, Takahashi H, Nakayama H, Kanaoka Y, Minamino N, et al. (1984) Primary structure of Electrophorus electricus sodium channel deduced from cDNA sequence. *Nature* 312(5990): 121–7.

Okamura Y, Ono F, Okagaki R, Chong JA, Mandel G. (1994) Neural expression of a sodium channel gene requires cell-specific interactions. *Neuron* 13(4): 937–48.

Ono F, Higashijima S, Shcherbatko A, Mandel G, Brehm P. (2001) A mutation in zebrafish rapsyn disrupts ACh receptor clustering and promotes synaptic depression. *Society for Neuroscience Abstract* 27:918.1.

Ono F, Shcherbatko A, Higashijima S, Mandel G, Brehm P. (2002) The zebrafish motility mutant *twitch once* reveals new roles for rapsyn in synaptic function. *Journal of Neuroscience* 22(15): 6491–98.

Otto SJ, McCorkle SR, Hover J, Conaco C, Han JJ, Impey S, Yochum GS, Dunn JJ, Goodman RH, Mandel G. (2007) A new binding motif for the transcriptional

repressor REST uncovers large gene networks devoted to neuronal functions. *Journal of Neuroscience* 27(25): 6729–39.

Rakela B, Brehm P, Mandel G. (2018) Astrocytic modulation of excitatory synaptic signaling in a mouse model of Rett syndrome. *eLife* 7: e31629.

Salkoff L, Butler A, Wei A, Scavarda N, Giffen K, Ifune C, Goodman R, Mandel G. (1987) Genomic organization and deduced amino acid sequence of a putative sodium channel gene in Drosophila. *Science* 237(4816): 744–9.

Schoenherr CJ, Anderson DJ. (1995) The neuron-restrictive silencer factor (NRSF): a coordinate repressor of multiple neuron-specific genes. *Science* 267(5202): 1360–3.

Sevarino KA, Stork P, Ventimiglia R, Mandel G, Goodman RH. (1989) Amino-terminal sequences of prosomatostatin direct intracellular targeting but not processing specificity. *Cell* 57(1): 11–9.

Silverman M, Zieg J, Hilmen M, Simon M. (1979) Phase variation in Salmonella: genetic analysis of a recombinational switch. *Proceedings of the National Academy of Sciences of the United States of America* 76(1): 391–5.

Silverman M, Zieg J, Mandel G, Simon M. (1981) Analysis of the functional components of the phase variation system. *Cold Spring Harbor Symposia on Quantitative Biology* 45(Pt 1): 17–26.

Simon M, Zieg J, Silverman M, Mandel G, Doolittle R. (1980) Phase variation: evolution of a controlling element. *Science* 209(4463): 1370–4.

Sinnamon JR, Kim SY, Corson GM, Song Z, Nakai H, Adelman JP, Mandel G. (2017) Site-directed RNA repair of endogenous Mecp2 RNA in neurons. *Proceedings of the National Academy of Sciences of the United States of America* 114(44): E9395–E402.

Sinnamon JR, Kim SY, Fisk JR, Song Z, Nakai H, Jeng S, McWeeney SK, Mandel G. (2020) In vivo repair of a protein underlying a neurological disorder by programmable RNA editing. *Cell Repair* 32(2): 107878.

Tapia-Ramirez J, Eggen BJ, Peral-Rubio MJ, Toledo-Aral JJ, Mandel G (1997) A single zinc finger motif in the silencing factor REST represses the neural-specific type II sodium channel promoter. *Proceedings of the National Academy of Sciences of the United States of America* 94(18): 1177–82.

Toledo-Aral JJ, Brehm P, Halegoua S, Mandel G. (1995) A single pulse of nerve growth factor triggers long-term neuronal excitability through sodium channel gene induction. *Neuron* 14(3): 607–11.

Toledo-Aral JJ, Moss BL, He ZJ, Koszowski AG, Whisenand T, Levinson SR, Wolf JJ, Silos-Santiago I, Halegoua S, Mandel G. (1997) Identification of PN1, a predominant voltage-dependent sodium channel expressed principally in peripheral neurons. *Proceedings of the National Academy of Sciences of the United States of America* 94(4): 1527–32.

Trimmer JS, Cooperman SS, Tomiko SA, Zhou J, Crean SM, Boyle MB, Kallen RG, Sheng Z, Barchi RL, Sigworth FJ, Goodman RH, Agnew WS, Mandel G. (1989) Primary structure and functional expression of a mammalian skeletal muscle sodium channel. *Neuron* 3(1): 33–49.

Walogorsky M, Mongeon R, Wen H, Mandel G, Brehm P. (2012) Acetylcholine receptor gating in a zebrafish model for slow-channel syndrome. *Journal of Neuroscience* 32(23): 7941–8.

Wang M, Wen H, Brehm P. (2008) Function of neuromuscular synapses in the zebrafish choline-acetyltransferase mutant bajan. *Journal of Neurophysiology* 100(4): 1995–2004.

Wen H, Linhoff MW, Hubbard JM, Nelson NR, Stensland D, Dallman J, Mandel G, Brehm P. (2013) Zebrafish calls for reinterpretation for the roles of P/Q calcium channels in neuromuscular transmission. *Journal of Neuroscience* 33(17): 7384–92.

Wen H, Linhoff MW, McGinley MJ, Li GL, Corson GM, Mandel G, Brehm P. (2010) Distinct roles for two synaptotagmin isoforms in synchronous and asynchronous transmitter release at zebrafish neuromuscular junction. *Proceedings of the National Academy of Sciences of the United States of America* 107(31): 13906–11.

Wickner W, Ito K, Mandel G, Bates M, Nokelainen M, Zwizinski C. (1980) The three lives of M13 coat protein: a virion capsid, an integral membrane protein, and a soluble cytoplasmic proprotein. *Annals of the New York Academy of Sciences* 343: 384–90.

Wickner W, Mandel G, Zwizinski C, Bates M, Killick T. (1978) Synthesis of phage M13 coat protein and its assembly into membranes in vitro. *Proceedings of the National Academy of Sciences of the United States of America* 75(4): 1754–8.

Yang YJ, Baltus AE, Mathew RS, Murphy EA, Evrony GD, Gonzalez DM, Wang EP, Marshall-Walker CA, Barry BJ, Murn J, Tatarakis A, Mahajan MA, Samuels HH, Shi Y, Golden JA, Mahajnah M, Shenhav R, Walsh CA. (2012) Microcephaly gene links trithorax and REST/NRSF to control neural stem cell proliferation and differentiation. *Cell* 151(5): 1097–112.

Yao H, Goldman DC, Nechiporuk T, Kawane S, McWeeney SK, Tyner JW, Fan G, Kerenyi MA, Orkin SH, Fleming WH, Mandel G. (2014) Corepressor Rcor1 is essential for murine erythropoiesis. *Blood* 123(20): 3175–84.

You A, Tong JK, Grozinger CM, Schreiber SL. (2001) CoREST is an integral component of the CoREST- human histone deacetylase complex. *Proceedings of the National Academy of Sciences of the United States of America* 98(4): 1454–8.

Zieg J, Silverman M, Hilmen M, Simon M. (1977) Recombinational switch for gene expression. *Science* 196(4286): 170–2.