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Influences: Sodium channel excitement

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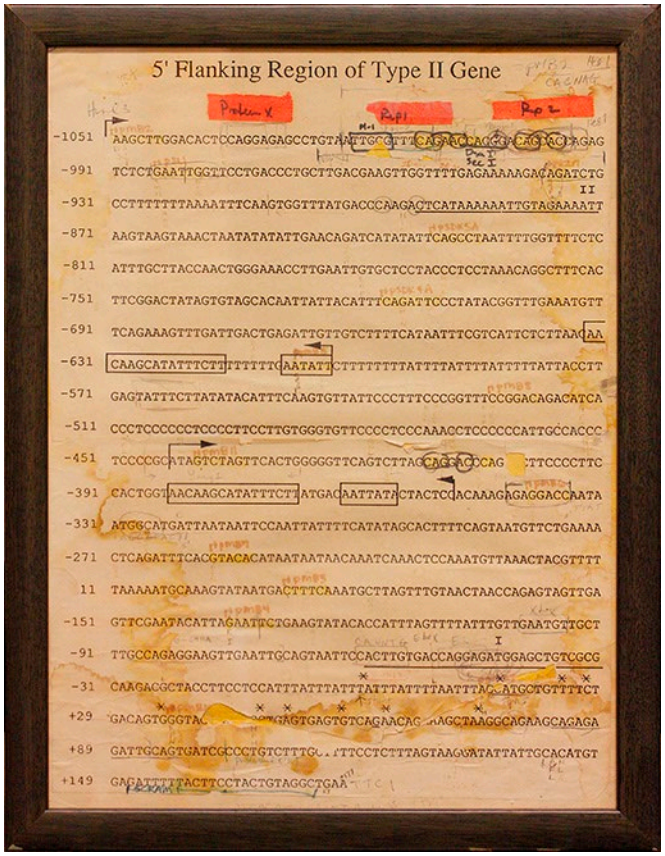
My training in southern California still shapes my sunny outlook on science. My journey began as a graduate student with Bill Clark (now an author and screenwriter) at the University of California, Los Angeles, researching T cell immunology and the phenomenon of membrane capping. Still a Los Angeles valley girl at heart, I didn't take school or science too seriously, but that began to change when I moved to Bill Wickner's laboratory as his first postdoctoral fellow. Together, we explored new ideas about the insertion of transmembrane proteins using bacteriophage as a model and I experienced my first taste of the scientific frontline when testing the membrane-folding trigger hypothesis. I subsequently elected to do a second postdoc in Mel Simon's laboratory at the University of California, San Diego to broaden my training. Mel's group was using cutting-edge phage genetics to study the switching of bacterial flagellar types by DNA translocation: another big discovery. Still living and loving the postdoc life, I ditched my boogey board and moved to Boston after my husband, Paul Brehm, accepted an Assistant Professor position in Physiology at Tufts Medical School. I joined Tom Benjamin's laboratory at Harvard Medical School as a senior postdoc/instructor. Tom was doing interesting work on mammalian viruses, and I thought that was a good entry into eukaryotic molecular biology, which was picking up steam. It was this exposure to different fields, and being mentored to think about big questions, that shaped a scientific path that would eventually lead to new discoveries in ion channels.

All of my different experiences converged in 1983 when Paul heard a seminar by Dick Goodman at Tufts New England Medical Center about cloning the genes encoding neuropeptides. Paul is an ion channel physiologist and spent his second postdoc at the Salk Institute alongside Steve Heinemann and Marc Ballivet, who were pioneering the cloning complementary DNAs (cDNAs) encoding subunits of the nicotinic acetylcholine receptor. Because Dick's laboratory was cloning neuropeptides that bound to membrane-associated receptors, Paul saw an opportunity to launch receptor and ion channel cloning at Tufts. During a chance meeting at a movie theater in Boston, we ran into Dick and his wife Eve and hit it off immediately. Dick, Paul, and I set out to express clone the receptors for bombesin, somatostatin, and vasoactive intestinal peptide in *Xenopus* oocytes. That step inched me closer to cloning voltage-dependent ion channels—an idea already ignited within the community of ion channel biophys-



Shown is the cloned genomic sequence of the 5′ flanking region of the brain type II sodium channel gene that has hung on my office wall for over 20 yr. Sequences identified by red tape identify the repressor sequences that keep the promoter region (colocalized with coffee stains at bottom) off in nonneuronal cells. The repressor protein REST was shown ultimately to bind to the repressor sequences. Boxes encompass a specialized DNA sequence caused by a transposon insertion whose function is unknown but may someday make me famous.

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icists, who wanted to test all of their hotly disputed models of selectivity and gating. At about the same time, the single channel recording technique was in high gear. I think of the confluence of cloning and single channel biophysics as the engines that drove this exciting time for ion channels, including the discoveries that filled the pages of Bertil Hille's *Ion Channels of Excitable Membranes* textbook (1).

At the Excitable Membranes Gordon Conference in 1984, the importance and feasibility of cloning ion channels became abundantly clear. All eyes were on the formidable Shoshaku Numa from Japan, who had been the first to clone a ligand-gated ion channel. However, Numa was very selective about who had access to his clones and the United States was generally last on his list, with the exception of Kurt Beam. After successfully cloning a muscle-type calcium channel, Numa gave Kurt the clone, which to Numa's great (and probably only) disappointment was not functional in oocytes. Kurt, however, would go on to do an elegant set of studies with Numa showing how this dihydropyridine receptor triggered ryanodine receptors to release calcium from intracellular stores within muscle (2).

Dick and I decided to tackle the voltage-dependent sodium channel, despite knowing that Numa was likely way ahead. In 1984, cloning was still a difficult proposition and, had we known just how large sodium channel messenger RNA was, we might have chosen the same path as the Jans and pursued K channels. We initially tried fancy expression-cloning strategies with Bob Barchi at the University of Pennsylvania and Bill Agnew and his postdoc Jim Trimmer at Yale, who had antibodies for muscle sodium channels. At the helm of the experiments in my laboratory was my graduate student, Sharon Cooperman (who always wore the same "Question Authority" t-shirt). In the end, the tried-and-true homology cloning method worked best, because Numa indeed published the first full-length α subunit sodium channel (NaC) sequence during the course of our experiments. Numa's clone was from eel (3), capitalizing on his successful strategy of targeting channels from tissues enriched in that particular protein. Because we couldn't get the clone, Dick and I ordered an eel from Florida, brought it back from Logan Airport on the T, then hired someone from the Boston aquarium to take out the electroplax. We made our own cDNA library and isolated a partial sequence that was a good enough probe to hybridize to mammalian cDNA. Electroplax is modified muscle and it allowed us to clone the first full-length NaC cDNA from mammalian skeletal muscle (SkM1; 4). Our paper also showed functional expression of this α subunit, although once again, Numa beat us to the punch with functional expression of a brain sodium channel (brain type II; 5) that was highly related in primary structure to the muscle channel. The following year, the same issue of *Science* featured work by Larry Salkoff and myself together with that of Lily and Yuh-Nung Jan, which together represented the first cloning of ion channel genes in *Drosophila* (6, 7, 8). This was a heady time. Unlike Numa, we distributed our NaC cDNAs without strings attached to everyone who wanted them. Looking back, this was perhaps my greatest contribution.

At this point, I returned to my gene regulation roots, having been captivated by the question of how tissue-specific expression was regulated—more specifically, how expression of brain NaCs was restricted to neurons. It was an odd turn and a bit of a risk because most people thought that gene regulation in brain would be just like it was in liver, which was then the gold standard for tissue-specific expression. However, to me it stood to reason that understanding regulation of the sodium channel, master of commerce within the nervous system, might also unlock many of the mysteries surrounding formation of the nervous system. So, I washed my hands of the pore-forming region to clone the noncoding region of the brain type II NaC gene. This was a challenging task given the embryonic state of genomic libraries, but it was in line with my molecular training. This pivotal decision came when I was about to take a faculty position in Neurobiology and Behavior at Stony Brook University. My close friend and early formidable competitor on insertion of trans-membrane proteins, Simon Halegoua, was in the department and anxious to further develop molecular neurobiology. Tufts did not share this vision so Paul and I moved, and along with my student Drew Chong and postdoc Susan Kraner, I set out on this new project. We isolated a promoter region in the brain type II gene (now Nav1.2) that, surprisingly, kept the gene completely turned off in muscle cells with no effect in neurons. This led to the new idea that neuronal specificity could simply be caused by suppression everywhere else. As seen in the image here, our sequencing analysis and bookkeeping methods, by today's standards, were pretty primitive. However, even with the limited tools at hand, we were able to manually decipher the binding site for a presumed repressor. Further, the tools were good enough to discover that the type II NaC repressor region is also found in many other neuronal genes and were sufficient to allow us to identify the repressor-binding protein.

These unexpected findings were discovered simultaneously in my laboratory and the laboratory of David Anderson (Caltech), and we named the repressor protein REST (9) and NRSF (10), respectively. Together, our findings delivered a deathblow to the more popular thinking that gene activation was the master regulator for cell lineages and set the stage for other investigators to work out the hierarchy of factors that, along with repressors, regulate different regions in central nervous system development. I figured that we had won the naming contest, but I was anxious that David's paper was going to appear in *Science* before we had a chance to publish our own. I called Ben Lewin at *Cell* and he offered, without hesitation, to publish our paper in the next issue, provided that we could submit the sequence of a full-length cDNA and that the reviews were positive. Everyone in my laboratory dropped everything else and worked 24/7 to make that happen. We prevailed, although we did make a small mistake in our rush to publish, which we have since corrected. As we prophesized, REST/NRSF turned out to be a model protein that has revealed many fundamental aspects of both nervous system development and repressor function. REST also led to the discovery of a sodium channel, PN1 (now Nav1.7), in peripheral neurons that we suggested would be involved in pain transduction (11). Our prediction was validated years later in human patients (12). Although a cliché, REST illustrates the wonderful journey that my research has taken me on, full of surprises and discovery. I credit my broad training and the inspiration I have drawn from

all of the wonderfully talented, warm, and funny people that were involved in that training, and beyond, for my continual love of experimental science.

Lesley C. Anson served as editor.

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