



Mary Elizabeth Hatten

BORN:

Richmond, Virginia
February 1, 1950

EDUCATION:

Hollins College, Roanoke, VA, AB (1971)
Princeton University, Princeton, NJ, PhD (1975)
Harvard Medical School, Boston, MA, Postdoctoral (1978)

APPOINTMENTS:

Assistant Professor of Pharmacology, New York University School of Medicine (1978–1982)
Associate Professor of Pharmacology (with tenure), New York University School of Medicine (1982–1986)
Associate Professor of Pathology in the Center for Neurobiology and Behavior
College of Physicians and Surgeons of Columbia University (1986–1988)
Professor of Pathology in the Center for Neurobiology and Behavior
College of Physicians and Surgeons of Columbia University (1988–1992)
Professor and Head of the Laboratory of Developmental Neurobiology, The Rockefeller University (1992–2000)
Frederick P. Rose Professor and Head of the Laboratory of Developmental Neurobiology, The Rockefeller University (2000–present)

HONORS AND AWARDS (SELECTED):

Westinghouse National Science Talent Search Award Finalist (1967)
Research Fellow of the Alfred P. Sloan Foundation (1983–1985)
Pew Neuroscience Award (1988–1992)
McKnight Neuroscience Development Award (1991–1993)
Javits Neuroscience Investigator Award (1991–1998)
National Science Foundation Faculty Award for Women Scientists and Engineers (1991–1996)
Weill Award, American Association of Neuropathology (1996)
Ph.D., Hollins College, *honoris causa* (1998)
Fellow, American Association for the Advancement of Science (2002)
Distinguished Alumna Award, Hollins University (2011)
Cowan-Cajal Award for Developmental Neuroscience (2015)
Elected to National Academy of Sciences, USA (2017)
Ralph J. Gerard Prize in Neuroscience, Society for Neuroscience (2017)

*Mary E. Hatten has used the mouse cerebellar cortex as a model to study molecular mechanisms of central nervous system (CNS) cortical neurogenesis and migration. She pioneered live imaging methods that proved that CNS neurons migrate on glial fibers and revealed a specific, conserved mode of CNS neuronal migration along glial fibers in different cortical regions. Subsequently, she used bioassays to identify key molecular regulators of neuronal migration, including the neuron-glial adhesion ligand *Astn1* and the polarity complex *mPar6*, which coordinates cytoskeletal dynamics during cerebellar granule neuron migration. Using mouse genetics and in vitro chimeras, she discovered that the cerebellar territory arises from rhombomere 1 and that the *weaver* (*Girk2*) gene acts nonautonomously in granule cells. Her lab generated the first cDNA libraries from an identified CNS neuron, the cerebellar granule neuron, which she used to identify more than 80 genes that function in cerebellar development. Her research has broad significance for human genetic studies on developmental brain disorders, such as autism, attention deficit disorder, and childhood epilepsy.*

Mary Elizabeth (“Mary Beth”) Hatten

The Early Years

I was born the middle child, between two brothers, Bobby and Jay, into a large, extended family, with deep Virginia roots and a distinguished record of community service. Although my father and grandfather, Drs. John Hatten and Waverly Payne, were prominent physicians, medicine held little attraction for me. Instead I used the laboratory of the local hospital in Newport News, Virginia, as a springboard for school science projects. Fortunately for me, that same small Virginia city was the home of NASA’s Langley Research Center, the site of biological research for the early manned space flights, where I worked during the summers in college. As a child, I was lucky to have teachers who encouraged me to do science projects as early as age 10, when Mrs. Little, one of my favorite teachers, said of course, it would be interesting to put the skeleton of the chicken we had eaten for dinner back together again! Later, wonderful teachers, who taught Advanced Placement courses in biology and chemistry in high school, would be influential, but it was my mother, Mary Lou Hatten, who inspired me to follow my interests wherever they might lead. Although my mother was a community leader, not a scientist, who helped spearhead the development of community projects like the Nature Museum and a Live Theatre for elementary school children, she had limitless enthusiasm for helping me do science projects. Thanks to her, I had incubators and microscopes to do *Drosophila* genetics in my room, and rabbits in the garden shed to generate cell surface antibodies for a project on how gravity affected the growth patterns and colony-forming characteristics of bacteria. My mother often told a story about the reaction of the local newspaper writer who, when spotting a bulletin board covered in photos of the Beatles and Rolling Stones across my room from the science experiments, said, “Thank God!” It was not unlike the reaction I got from Pamela Harriman many years later at a Rockefeller dinner, after I had come there as the first female professor. “You look so normal” she said. Being a southern girl interested in science was not the usual path.

High School Years: The Space Experiments

I went to Homer L. Ferguson High School, a large public high school in the neighborhood where we lived. Although it was not the happiest period of my

life, I was busy with sports, clubs, and activities. Two biology teachers, Henry Drudge and Bernie Freeman, encouraged me to do an independent project for the annual science fair, which led me, with my father's assistance (Dr. John Hatten), to the local hospital laboratory. Among other things, the 1960s were the age of questions about space exploration. Space captured my imagination because Hampton, the adjacent city was the home of NASA's Mercury Program. Just after his magnificent voyage, I had been able to meet John Glenn at Langley Field, a thrilling experience made all the more so as Glenn, himself a redhead, tussled my hair when I met him and asked "How are you doing, Red?"

My high school science project concerned a general question that would stay with me: how cell-cell interactions influence development. In the 1960s, it was unclear how gravity would influence the growth of bacteria, especially pathogenic bacteria, and whether uncontrolled growth would threaten the lives of the astronauts. I became interested in the proliferation of bacteria and their formation of colonies with different shapes in semisolid growth media. For my project, I grew the bacteria under two conditions—normal gravity or higher gravity generated by centrifugation. To measure differences in surface proteins of the bacteria subjected to higher gravity, I raised antibodies against the bacteria. I was remarkably fortunate that the head of the hospital laboratory, Jerry Hagelberger, seemed thrilled to have a young person interested in science in his otherwise-clinical lab. He taught me all about bacteria and provided all of the equipment I needed. I went to the hospital lab every afternoon for two years working on my project. One piece of equipment, a centrifuge, would gain fame in the article written by the local newspaper journalist mentioned previously. I told him about spinning the bacteria in the centrifuge to collect them for the immunizations and he wrote that I was spinning *rabbits* in a machine the "size of a washing machine!" That gave me a healthy suspicion of the scientific press. But the project went well, well enough to win a number of local science fairs (which my mother took me to without complaint), and well enough to become a finalist in the Westinghouse Science Talent Search.

My project "Study of the Antigenicity of a Mutant Strain of *Aerobacter Aerogenes* Produced by the Application of Centrifugal Force" also did well at the NASA Youth Science Congress in 1966, where I met Dr. Judd R. Wilkins, who ran the microbiology lab at NASA's Langley Research Center. That was especially fortunate, because he gave me a summer job in their labs for the next four years. I really loved working at NASA during the summers and was proud that my project on gravity and bacterial growth eventually flew on an Apollo mission to the moon. Watching the film *Hidden Figures* about the remarkable work of Catherine Johnson in 2016 reminded me that there were virtually no women scientists at NASA in those days (1967–1971). Just as in the movie, all of the men had white socks, pocket pen protectors, and slide rules. Fortunately, I was so excited about the science that I didn't notice their attitudes about women, and luckily Dr. Wilkins was a supportive mentor.

Hollins College

It's hard to believe now, but in 1967, when I applied to college, almost none of the colleges and universities in the East were coeducational. I ended up choosing Hollins College, not just because my mother went there, but also because the most interesting women I knew growing up were her Hollins friends. I loved Hollins for many reasons. I discovered writing and philosophy there and would later realize that it was at Hollins where I first recognized a "woman's voice," the voice missing at NASA and in the culture at large in the 1960s. I would appreciate what that meant years later at Columbia when I attended a meeting of the tenured women faculty. I was so thrilled to be meeting those amazing women, who included the great anthropologist Ruth Benedict; the historian Marjorie Nicholson; the scientists Ruth Sager, Salome Gluecksohn-Waelsch, and Sarah Ratner; the novelists Elizabeth Hardwick (*Sleepless Nights*) and Mary Gordon (*Temporary Shelter*); and the theologian Elaine Pagels (*The Gnostic Gospels*). At the meeting, 60 women were trying to fit their chairs into a circle, a stunning sight! Women's voices filled the room—a different culture, a different way of interacting with one another. The "woman's voice" that I first heard at Hollins, and recognized later at Columbia, would thankfully stay with me as I went through a career, often being the only woman or one of but a few women in the room.

My years at Hollins were deeply happy years, as I loved the academic work—biochemistry with Sandy Boatman, physical chemistry with Ralph Steinhardt, American literature with Richard Dillard, and philosophy with Larry Becker as well as the January short term when I could do research. I especially enjoyed being able to delve into the arts and humanities. I also enjoyed being involved in student governance, as we gained student representation to faculty meetings and initiated a student-run exam system while I was chair of the Student Government's Academic Legislation Committee. The times were remarkable between 1967 and 1971, because of the intensity of social change, the politics of the Vietnam War, and, of course, the incredible music! As much as anything, I enjoyed my friends at Hollins. Our long discussions across disciplines enriched how I approached my work in the lab. These conversations continue today, most frequently with my roommate Kathy Hudson, now a well-known garden writer; Jigga Gaynor Dunn, a producer of books on tape; and Nathalie Gilfoyle, general counsel for the American Psychological Association and expert on the intersection between law and psychology.

Graduate School at Princeton and in Basel: Plant Lectins and Membrane Dynamics

I arrived at Princeton in 1971 as a graduate student in biochemical sciences, one of the first years that Princeton admitted women undergraduates.

This year was also among the early years that women were admitted to the graduate program in biochemical sciences. Coming from a southern women's college, I have to say that I found Princeton to be far more conservative politically and socially than Hollins had been. That was surprising, to say the least. I enjoyed my first two years at Princeton, especially knowing Rudi Jaenish and Art Levinson during a stint in Arnie Levine's lab, and Marc Kirshner who moved into my mentor Max Burger's old lab as a new faculty member after Max left for Basel. In Max's lab, I again worked on cell surface proteins, this time using the new reagents that Max had discovered, plant lectins, to study changes in surface properties associated with cancer. After only two years at Princeton, I followed Max to the Biocenter in Basel, Switzerland, to finish my thesis research.

The Biocenter was an especially exciting place, as Werner Arber was purifying restriction enzymes across the hall and Walter Gehring was doing exciting *Drosophila* genetics downstairs. Indeed, two of Gehring's postdocs at the time, Janni Nusslein Volhard and Eric Wieschaus, went on to win the Nobel Prize for their discovery of genes that control the early development of *Drosophila*. The Burger lab was an exciting place as well, given the buzz at the time about plant lectins revealing key differences between normal and transformed cancer cells. I was remarkably fortunate to work with two talented postdocs in the lab, Rick Horwitz and Carl Scandella. With Rick, I worked out methods to remove the lipids from serum, a step that allowed us to manipulate the lipid composition of normal and transformed cell membranes. We used that approach to show that the receptors for different lectins were in different lipid domains of the membrane, a discovery that was later credited as a prelude to the concept of membrane lipid rafts. Rick, who trained with Britton Chance, was a spectacular mentor, who taught me what a good experiment was, and who pushed me to think critically, especially about my own work! After Rick went back to the United States to become an assistant professor at the University of Pennsylvania, I worked with Carl Scandella, a biochemist and biophysicist who trained with Harden McConnell and Arthur Kornberg at Stanford. With Carl, who was one of the most rigorous scientists I ever knew, I incorporated fatty acid spin labels into the cells, a method that allowed us to show that the lipids in the vicinity of the receptors for different lectins had different lipid phase properties. We also used TEMPO labeling to measure how much of the membrane was fluid in normal versus transformed cells. I really enjoyed being able to make precise biophysical measurements of the lipids around lectin receptors. Of course, at that time, we didn't realize that lectins bound to a large number of receptors. Rick and Carl were wonderful colleagues and, together with Max, they provided me with training in membrane biochemistry as well as in cancer biology.

I also enjoyed working in Europe during those years. It was exciting to be in a different culture, to be able to drive to the ski slopes in the Alps in

two hours, or to take a train to Paris or a quick flight to London, where my close Hollins friend, Jigga Dunn, was living. Through Max, I met Nicole LeDouarin, a professor at the College de France in Paris, who would introduce me to cell migrations in the developing nervous system. Indeed, it was Nicole who inspired me to work on nervous system development. Her beautiful work used genetics and developmental biology, a combination I would use in studies on the developing mouse brain.

Harvard Medical School: Cerebellar Development and Genetics

One of the reasons I studied the membrane properties of cancer cells was to understand the molecular basis of metastasis. As nearly all studies on cancer cells were carried out on transformed cell lines in culture at that time, I thought it would be important to learn about mechanisms of normal cell migrations. That took me to the nervous system, where exciting new work concerned the large-scale migrations during brain development, migrations that take young neurons from proliferative zones out to form the layers of cortical regions. To learn about CNS migration, I went to Harvard Medical School to work with Richard Sidman who, together with Pasko Rakic, was carrying out cutting-edge work on central nervous system (CNS) migrations. The lab studied the cerebellum, a region of the brain that controls motor coordination, and also used mouse genetics to analyze the development of the cerebellum of mice with spontaneous neurological mutations that Dick had identified at the Jackson Laboratories (with Hope Sweet). The mouse mutants had names like *weaver*, *reeler*, *stumbler*, and *leaner* and were the first genetic models for mammalian brain development. They were used by our lab and others to dissect the molecular mechanisms of cerebellar development.

Before joining Sidman's lab, I spent a summer at Woods Hole taking the Neurobiology Course. That was fortuitous for many reasons, including the quality of the course and the fact that it was taught by the Harvard Neurobiology Department. One of the highlights of the course was Gerry Fishbach's new method for culturing nerve and muscle cells, which provided a model system to study neuromuscular junctions. It was a great summer and provided a running start for my postdoctoral work. I also met Torsten Wiesel that summer. Torsten would be one of the most important mentors in my career, especially after I moved to Rockefeller many years later.

Learning about cerebellar development from Richard Sidman was especially exciting. Dick had assembled the first interdisciplinary neuroscience department, with neuroanatomists (Pasko Rakic), geneticists (Dick Mullen), biochemists (Mike Shelanski, Susanne Roffler-Tarlov), immunologists (Melitta Schachner), and neurologists (Verne Caviness, Dennis Selkoe). Every Tuesday, I spent the morning doing dissections of the embryonic

mouse cerebellum with Dick, an invaluable series of lessons from the master of cerebellar development. The postdocs in the department—Carla Shatz with Pasko Rakic and Jeff Noebels with Dick Sidman among others—would remain among my closest friends and colleagues. Then, too, as I had taken the Woods Hole Neurobiology Course with the Neurobiology Department faculty (a separate department at the Medical School), I often attended their seminars and came to know the neurobiology postdocs as well. They included Charles Gilbert, Josh Sanes, Tom Jessell, Jane Dodd, Story Landis, and Bill Harris, who have also been lifelong colleagues and friends. It was the golden age of Harvard neuroscience, and I was privileged to have been a part of it.

Soon after I arrived in Boston in 1975, Dick Sidman encouraged me to attend the Society for Neuroscience (SfN) meeting for the first time. The meeting was in New York and the entire event took place in the New York Hilton Hotel. There were only about 3,000 people at the SfN meeting that year!

Back at the Children's Neuroscience Department, I focused on trying to culture embryonic cerebellar neurons and test their surface properties, using lectins as probes, and on what would be the beginnings of methods to do live imaging of glial-guided migration. It was especially beneficial to work with Pasko Rakic, who was doing masterful anatomy on glial-guided migration, and to learn mouse genetics in the Sidman lab. The genetics experiments with mouse chimeras that were ongoing in the lab showed, for the first time, which genes acted autonomously and which acted nonautonomously during cerebellar development. That work changed the view that mammalian brain development involved little plasticity. Now, we know that there is extensive plasticity and that cell interactions are a major force in mammalian CNS development.

New York University and Columbia: Mechanisms of Glial-Guided Migration

Although I would have gladly stayed at Harvard for many more years, I abruptly moved to New York in 1978, because my then-husband lost his job in the Harvard History Department and moved to Wall Street. Luckily for me, Mike Shelanski, who had just become chair of the Pharmacology Department at the New York University (NYU) School of Medicine, recruited me, along with Ron Liem and Lloyd Greene, from Harvard to join the department. Mike was such a supportive chairman. Recruiting Ron turned out to be fortuitous, as it was Ron who asked me to try out his new antibody against glial fibrillary acidic protein (GFAP) in my cerebellar cultures, which was the experiment that revealed that glia provide a template for the positioning of neurons in the cultures. The ability to visualize the neurons and the glia in the cultures, and measure their interactions, led me to go back

to my original goal, to study neuronal migration. At about the same time, Mike hired Carol Mason, a wonderful neuroanatomist who had trained with Ray Guillery at Chicago, and Fred Maxfield, a biophysicist who had done important imaging work with Watt Webb at Cornell. Carol taught me neuroanatomy, and Fred opened my eyes to the power of live imaging. Indeed, Fred and I discovered optical memory disk recorder (OMDR) digital recording decks at a trade show for advertisers in New York, but I am getting ahead of myself.

Carol and I soon started a tradition of going to the SfN meeting together every year. The meetings were smaller in those years, which gave me a chance to reconnect with people I knew at Harvard, now scattered around the country, and to get to know new people, mostly through Carol. Carol's work in the visual system, along with that of Carla Shatz, took me to the talks on the visual system, where I soon met Sue McConnell, Martha Constantine Paton, and others. It was especially rewarding to connect with young women scientists.

Over the years at NYU, I focused more and more on cerebellar granule cells (GCs). Being influenced by Corey Goodman's experiments on identified neurons in the grasshopper, I developed a rapid method to purify granule neurons to image the migration of an identified CNS neuron *in vitro*. That was an important step that would open the door to molecular experiments on the mechanisms of migration and of cerebellar development. At a glance, GCs shared a lot of features with B cells of the immune system. Both were incredibly small and both had disproportionately large nuclei, a fact that made B cells "heavy" and easy to purify in Ficoll density gradients. I therefore tested Ficoll and the newer Percoll density gradients to try to purify cerebellar GCs. Fortunately, it worked as I had hoped, and we were able to purify millions of GCs based on their sedimentation rates in a step gradient. By default, we obtained a fraction of large cells that contained the glial cells (and the Purkinje cells). That enabled us to make pure cultures of GCs. Carol Mason was instrumental in our efforts to prove that we indeed had purified GCs, because of her expertise in electron microscopy (EM) and in all things anatomical. Carol was a very important influence, not the least because of her knowledge of neuroanatomy, but also because of her remarkable expertise in microscopy.

About that time, Jim Edmondson came to my lab as my first graduate student. Jim was incredibly meticulous, a skill that enabled him to make precise cultures and later to carry out detailed video recordings. I had begun to do video recordings of migrating GCs about the time Jim arrived, having done my first successful movie the night before my son John was born! Carol and I would share another treasure during those years, our toddler sons John and Daniel. We spent many happy afternoons on the beach in Rye or Rowayton playing with the boys and talking about science.

Jim Edmondson's work would span the time at NYU and at Columbia, as Mike had decided to move our neuro group (Carol, Ron, Fred, Lloyd,

Mike, and I) from NYU Pharmacology to the Columbia College of Physicians and Surgeons. At Columbia, Eric Kandel was organizing a new neuroscience program in neurobiology and behavior with faculty across multiple departments. Eric's program was a masterstroke as it included scientists across different areas of neuroscience: Richard Axel, who was already at Columbia, in molecular neuroscience; Eric's own lab (Jimmy Schwartz, Steve Siegelbaum and others) in physiology and behavior; our group in development, neuroanatomy, and biochemistry; and young scientists he recruited from Harvard, most important for me, Tom Jessell and Jane Dodd in developmental neuroscience. Columbia became an especially vibrant and exciting environment for neurobiology.

Although we had used the best tape recording decks available to follow neuronal migration in vitro, the ones used by network news, the transit time to move the tape forward created an artifact in the videos. Fortunately, Fred Maxfield, as mentioned, and I discovered that OMDR digital recording machines would eliminate that problem and provide what were rather stunning images of GC migration. Those experiments were also facilitated by the new Nomarski optics and video-enhanced contrast differential interference contrast microscopy, with which we provided the first real-time, high-resolution digital images of CNS migration. Fred was instrumental in helping us set all of that up in the lab, but it was Jim Edmondson who made the system work for imaging migration. His work involved finding culture dishes that were flat enough to fit on the stage under the condenser and using Mary Bunge's scheme to make drop well culture dishes with a number 1 glass coverslip affixed as a false bottom. The glass coverslip enabled the use of high-magnification and low-numerical aperture (NA) objectives (Zeiss 1.5 NA 63 $\times$) to acquire high-resolution images. The optics and culture setup were absolutely necessary given the small size of the GC (4–6 microns) and the narrow diameter of the glial fiber (1 micron). The videos that Jim took in 1985–1986 were thrilling, because they proved that neurons migrate on glial fibers, a fact we showed by scribing the area we had filmed, immunostaining with antibodies against glial cellular antigen markers, and identifying the fibers that supported migration as glial fibrillary acidic protein (GFAP) GFAP+ glial fibers. Carol Mason, Jim, and Carol's postdoc Bill Gregory then teamed up to do the *tour de force* experiment—to scribe a migrating neuron and serially section that cell for EM. The correlated EM images showed that all of the morphological features that Pasko Rakic had assigned to migrating GCs in vivo were evident in cells migrating on glial fibers in our in vitro system. These features included the formation of a puncta adherens migration junction with the glial fiber just underneath the neuronal cell soma, the positioning of the nucleus in the posterior aspect of the cell, a notch in the nucleus in the direction of migration and a basal body (centrosome) just forward of the nucleus in the direction of migration. I took the images to Yale to show Pasko as soon as I could. He was as thrilled

as we were to be able to see migration in real time for the first time and thereby prove that his theory of glial-guided neuronal migration was correct.

As we were doing imaging experiments on migration, one of our main goals was to identify the receptors that guided the movement of the neurons along the glial fiber. To identify the receptor, James Edmonson had raised antibodies against GC membranes (while we were still at NYU). Trevor Stitt then used biochemistry to identify which band among the proteins recognized by the antibody was the active receptor, a protein we named Astrotactin, because it mediated neuronal touching of astroglia. Trevor showed that antibodies purified from that band in the Western blot would block the binding of radiolabeled GC membranes to astroglial cells. This demonstrated that Astrotactin was a neuron-glial adhesion protein.

Soon after we moved to Columbia, I had become a single parent. I was concerned about giving my young son John time out of New York in nature, so we started going to Woods Hole in the summer. At first, I went as an instructor in the neurobiology course, then, when that was incompatible with being a single parent, we started going to Martha's Vineyard for the month of July. That got John out of the city and helped me get out on the roads to bike or on the beaches to swim in the afternoons. Later, in the 1990s, after Gord Fishell had gotten me into sea kayaking, I also spent time out on the beautiful waters around the Vineyard and Woods Hole. Many colleagues and friends would stop to see us at the Vineyard after teaching at Woods Hole in those days. I especially enjoyed having Scott and Marianne Fraser and their children, as well as Josh Sanes and Susan Corcoran and their kids, come to visit. Chilmark had the perfect setup with an outstanding childcare program, where the cost was the same whether you had one or many children and activities ranged from tennis to arts and crafts to swimming lessons. I could work in the mornings while John was at camp. Then in the afternoons, we would go to the beach where he would play with his friends from the morning camp. We went to Martha's Vineyard for more than a decade and had wonderful times there. Although many have asked me how I managed to be a single parent and do science, I think it was a major benefit. Going home at an early hour during the school year meant I took time to "play," which I thought was great for my work, and taking so much time in the summers, before the years of 24/7 Internet access, gave me time with my son, as well as time to think about what we were doing in the lab and refuel.

At Columbia, I recruited a number of postdocs, who made critical contributions to our work on migration—Wei-Qing Gao from Moo Ming Poo's lab at Columbia, Gord Fishell from Derek van der Kooy's lab in Toronto, Renata Fishman from the Breedlove lab at Berkeley, and Urs Gasser from Switzerland. Wei-Qiang Gao and I began to work on GC proliferation, a project that we did in collaboration with Nat Heintz, a molecular biologist at Rockefeller, whom I met through Jeff Noebels at a meeting on cerebellar

genetics at the Jackson Lab. Wei-Qiang Gao would also carry out the first in vitro chimera "mix and match" experiments that proved that the *weaver* gene acted autonomously in the GC to cause deficits in migration along glial fibers. Rodolfo Rivas joined the lab about that time and developed methods to implant fluorescently labeled (PKH26) purified GCs into developing cerebellar cortex to be able to see them migrate in situ. (This work preceded genetically encoded fluorescent tags for marking cells.) When I showed Rodolfo's work at the Columbia Neurobiology retreat at Woods Hole, Eric said, "You made that up, Mary Beth!" It was a stunning achievement for that time. With Rodolfo's labeling methodology, Wei-Qing implanted labeled GCs purified from either wild-type or *weaver* cerebellum into the cerebellum of the other genotype to show that the gene acted autonomously in vivo as well. Those experiments settled a long-standing argument about whether the *weaver* defect, which was a major genetic model for CNS migration, resulted from defects in the neuron or the glial cell. Our finding that the CNS migration deficit was in the neuron, not the glial cell, provided the first indication that the navigational instructions for migration were in the neuron. This conclusion was later strengthened by Renata Fishman's beautiful experiments purifying glial membranes and coupling them to glass fibers the same diameter as the glia and showing that neurons could migrate perfectly well on "dead" glial membranes.

Gord Fishell took the molecular experiments on migration a giant step forward when he used a Berg chamber to perfuse antigen binding (FAB) FAB fragments of anti-ASTN1 antibodies onto actively migrating GCs. That work showed that antibodies against ASTN, but not integrins, which were everyone's favorite adhesion protein at the time, blocked glial-guided migration. It would become clear later that although integrins are the key adhesion proteins for nearly all fibroblast and epithelial cell migrations, and even growth cone motility, they do not function in glial-guided migration. Thus, ASTN1 appeared to be a substitute for integrins as the key adhesion receptor. Gord went on to use EM, in collaboration with Carol Mason, to show that the ASTN protein localized to the migration junction with the glial fiber in actively migrating GCs. Subsequently, Renata showed that glass fibers coated with fibronectin, another popular substrate for nonneuronal cell migration, could also support GC migration. Because fibronectin is not expressed on glial cells that support migration, we reasoned that it did not function in glial-guided migration during brain development.

The development of a culture system for purified cerebellar GCs and astroglia provided a powerful approach for studying glial-guided migration in other cortical regions. It also provided an assay system for candidate proteins that function in glial-guided migration. From Jim's early video recordings, we learned the mode of movement of CNS migration and from Carol's EM we identified the ultrastructure of migrating neurons and their junction with the glial fiber. The mode of movement was saltatory, with

the neuron cycling between adhering to the glial guide and letting go to step forward every three minutes. As the cell moved forward, the nucleus remained in the rear of the cell, and the cell body alternated between an elongated lemon-like form when the neuron stretched out along the glial fiber to a rounded form as the neuron detached and glided forward. The rhythmic movement of the neuron along the glial guide roughly resembled that of an inchworm moving along a slender tree branch. Importantly, the mode of migration that we observed in our *in vitro* model approximated what Pasko had seen in static images of cells in fixed tissue. This finding would enable us down the road to carry out a long series of experiments to test the function of specific receptors and signaling pathways in migration.

Before moving on to examine the molecular mechanisms of migration, we carried out further experiments on whether the navigational instructions for migration were in the neuron, this time studying migration in the hippocampus and cortex. For this work, Urs Gasser purified neurons and glia from cerebellum, hippocampus and cortex, cultured them with glia from their homotypic brain region, imaged their migrations *in vitro* and then mixed and matched neurons and glial from different brain regions. Because glial-guided migrations produced very different patterns of neuronal layers in the three brain regions, it was possible that the glial fibers encoded the navigational instructions that gave rise to structural differences among cortical regions. Urs's experiments showed a remarkable result. All of the neurons in all of the brain regions, whether on homotypic or heterotypic glia had exactly the same mode of movement, cadence, and detailed features we had shown for cerebellar neurons. We therefore called migration "riding the glial monorail," where the diameter of the fiber and receptor systems on the fiber were critical for movement, not for guidance to a certain spot. Renata Fishman's experiments confirmed this "Bauhaus" model when she showed that neurons could migrate with the same features on 1 mm glass fibers coated with glial membranes.

Neuroscience at Columbia was especially exciting in that period, with postdocs like Marc Tessier-Lavigne, Mary Hynes, Marysia Placzek, and Andy Furley in the Dodd-Jessell labs; Seth Grant in the Kandel lab; and Linda Buck in the Axel lab. At that time, the Columbia neuroscience community was small enough that we all fit into a moderate-size conference room for Friday morning progress meetings, among the Axel, Kandel, Jessell, Dodd, Mason, and other labs. I had wonderful colleagues, indeed. Beyond that, I had a number of women as close colleagues, especially Carol Mason and Jane Dodd.

As I mentioned earlier, it was a meeting of the tenured women faculty at Columbia, at which 60 women were trying to arrange their chairs into a circle, that reminded me of the women's college I had attended and of what a woman's voice in science might be. Sociological studies at the time were suggesting that little boys liked building towers and hierarchies, whereas little girls preferred circles. For me, Deborah Tannen's sociological studies

on gender differences between boys and girls was summed up in an anecdote about what a young boy versus a young girl would do when stressed. She wrote that when a young boy would become frustrated, he would say, "If you don't stop, I am going to tell Billy, and he is going to beat you up." The girl, however, would say, "If you don't stop, I am going to tell Katie, and she is going to tell all of our friends!" She termed it circles and towers. The story reminded me of the tenured women at Columbia putting the chairs into a circle. I mused about whether these gender differences also reflected different ways of thinking about science, about whether my interest in cell-cell communication and Tom Jessell's interest in hierarchical models of development were, in part, gender-based.

Although Columbia was an invigorating place with great colleagues, I soon began to realize that not knowing molecular biology was a major handicap. As mentioned, I had begun to collaborate with Nat Heintz at Rockefeller earlier during Wei-Qiang Gao's experiments on GC proliferation. Nat was a molecular biologist interested in how transcriptional regulation generated so many different cell types in the CNS. As we had developed methods to purify large numbers of GCs, it was a perfect system to generate a CNS cell-type-specific cDNA library, one that likely would be a richer library than single-cell libraries. So, Nat and I joined forces to make the GC cDNA libraries. Soon, Torsten Wiesel recruited me to Rockefeller, and I eagerly moved because of the chance to equip my lab for molecular studies and to learn molecular biology from Nat and his colleagues.

Rockefeller University: Molecular Mechanisms

When I moved to Rockefeller in 1992, I was the first female head of laboratory. Given Rockefeller's reputation as a male bastion, I might not have moved there except for the work I was doing with Nat and for Torsten, whom I had known well at Harvard. Although the science at Rockefeller was stunning, it was a bit of a shock leaving my female colleagues at Columbia and going it alone at Rockefeller. Indeed, soon after I arrived, one of the senior professors invited me to lunch to clue me in that driving a Jeep Cherokee with a sea kayak on top was not appropriate for the first female Rockefeller professor. It would be a long road to bring great women to Rockefeller, one aided by the absence of departmental structure, and in the end, by the quality of science. Today, although we would like to have more women, we have a number of world-renowned female professors, including Cori Bargmann, Titia De Lange, Elaine Fuchs, Mary Jean Kreek, Jue Chen, and Leslie Voshall, as well as a number of remarkable junior female faculty members, including Vanessa Ruta, Agata Smogorzewska, Li Zhao, and Priya Rajasethupathy.

Soon after I moved to Rockefeller, I was asked to make a scientific presentation to the board of trustees. Naturally, I focused most of the talk on our new work on neuronal migration. David Rockefeller, who was the chair

of the board, listened carefully and then asked, "But why would the young neuron want to leave the place where it was born?" Although I thought to myself that the young cell might not have left had it been born at the Rockefeller family estate in Pocantico Hills, that question still endures as one of the central mysteries of brain development.

GC cDNA Libraries: Discovery of GC Developmental Genes

My move to Rockefeller did indeed lead us into molecular experiments, starting with the generation of GC cDNA libraries with Nat that would result in the cloning of some 80 genes that functioned in cerebellar development. The first of these was Astrotactin (*Astn*), which Chen Zheng, my first Rockefeller graduate student, cloned. The characterization of *Astn* RNA, by zoo blots, which no one does anymore, showed the interesting fact that *Astn* is expressed in vertebrates down to frogs, but not in invertebrates. That was gratifying as neuronal migration along glial fibers occurs only in vertebrates. Kathy Zimmerman also joined the lab soon after my arrival, having finished a postdoc with David Anderson at Cal Tech. Kathy made critically important contributions to my understanding of molecular biology and especially of transcription factors in neural specification. Although Kathy came to work on molecular projects in my lab, it soon became evident that she should run her own lab, so she set up a small group working on transcription factors in early frog neural development. Kathy would be a critical collaborator and wonderful colleague for many years to come, until her move to Lehigh University 15 years later.

We also continued to focus on imaging CNS migrations, as Gord moved with me to Rockefeller, where he and Rodolfo Rivas finished experiments on *ASTN1* in migration and on characterizing the cytoskeleton of migrating neurons. Gord also did some nice experiments on progenitor cell movements in the ventricular zone (VZ) of the cortex, a topic that was hotly debated in response to Pasko's protomap hypothesis. Gord's live imaging of young neurons in the cortical VZ showed that progenitor cells underwent extensive lateral cell movements prior to leaving the zone via glial-guided migrations. This demonstrated that the neuroepithelial cells in the VZ were not hardwired into a given location, but, like cells in other epithelia, could freely move in the lateral plane.

Dorsal Patterning of the Cerebellum: Genetic Studies on Weaver and Dreher

Although we did a number of other migration experiments, now using gene knockout methods instead of naturally occurring neuronal mutations, Phyllis Faust's analysis of mice lacking *Pex5* among them, we turned our attention to the early development of the cerebellar anlagen. This was due

in large part to the arrival of Janet Alder, who had worked with Moo Ming Poo at Columbia. Janet and I became interested in the early steps in GC specification and whether dorso-ventral patterning mechanisms, which Janni Nusslein Volhard and Eric Wieschaus pioneered in flies and zebrafish and Tom Jessell mapped in the mouse spinal cord, might be acting in cerebellar development. After all, the puckering of the rhombic lip above the fourth ventricle, where the cerebellum emerged, displaced the anlagen from a dorso-ventral orientation to a medio-lateral orientation.

To examine whether GCs were specified by dorsal signals, Janet collaborated with Kevin Lee from the Jessell lab to probe the role of bone morphogenetic proteins (BMPs) in cerebellar development. We were interested in BMPs because Kevin and Tom had shown that roof-plate-derived BMPs induced the specification of *Math1*-positive dorsal spinal cord interneurons. As GCs expressed *Math1*, and as Huda Zoghbi showed that *Math1* was critical for GC specification and development, we reasoned that GCs were also "dorsally" specified neurons. Janet and Kevin showed that BMPs localize to the choroid plexus adjacent to the rhombic lip and to the lip itself, the site of origin of GCs. They went on to show that treatment of ventral cerebellar cells with BMPs induced a GC specification pathway. Thus, segregation of GC progenitors (GCPs) into the rhombic lip, where they would be exposed to BMPs, provided a mechanism for the specification of a GC identity. Janet went on to show that rhombic lip cells, but not VZ cells, purified from the embryonic anlagen and implanted into the postnatal cerebellar cortex, gave rise exclusively to GCs, thus showing that GCs were specified very early in development, even though clones of GCPs continued to expand to generate the phenomenal numbers of GCs seen in the adult cerebellum.

To further address dorso-ventral patterning of the cerebellum, Kathy Millen, who had trained with Alex Joyner in Toronto, and Jim Millonig, who had arrived from Shirley Tilghman's lab at Princeton, studied the spontaneously generated *Dreher* mouse mutant, a mouse genetic model in which populations of dorsal interneurons did not develop. Kathy and Jim used positional cloning to map the area around the *dr/dr* locus and to identify the *dr* gene as *Lmx1a*. They went on to show in a beautiful *Nature* paper that mice lacking *dr/dr* lack a roof plate all along the neural axis, which led to the absence of dorsal interneurons in the spinal cord and a reduction in the population of GCPs in the rhombic lip of the cerebellar system. This work extended the model of dorsal signaling in neuronal specification to rostral areas of the CNS, including the cerebellum.

Jim and Kathy then used positional cloning to identify the *weaver* gene (*wv/wv*), carrying out the laborious crosses required for cloning at that time. As the original *weaver* mutant mouse had been lost, they scraped tissue off of slides from Sidman's department to harvest DNA. Interestingly, several candidate genes in the area mapped around the *wv/wv* locus—Big Brain and the GIRK2 channel. Being a developmental neuroscience lab, we focused on

Big Brain. Lily Jan's lab, which was also working to clone *weaver*, guessed that it was the GIRK2 channel, and they turned out to have made the lucky guess. We then joined forces with Henry Lester at Caltech to study the physiology of *wv/wv* GCs in culture and were able to show that blocking the leakage through the GIRK2 channel rescued the death of the GCs, thus proving that the Jan lab's identification of *Girk2* as *weaver* was correct.

During the same time frame, I was fortunate to have Richard Wingate join the lab from Andrew Lumsden's lab at King's College in London. Richard was an expert on chick quail chimeras, pioneered by Nicole Le Douarin years earlier. Richard carried out chick quail chimera experiments to map the origin of the cerebellar cortex, using the recently discovered marker for rhombomere 1, *Hoxa2*. His fate-mapping experiments showed that the cerebellum arises entirely from rhombomere 1, not partially from the presumptive midbrain territory as had been thought earlier. Richard also showed that a population of cells in the rostral rhombic lip that had been thought to only generate the GCPs, generate precursors that migrated to the basilar pons. It was great fun to have chick quail experiments in my lab.

In that same period, Daniver Morales, a postdoc from Chile, mapped transcription factor expression in the early cerebellar anlagen of the chick and the mouse. Dani discovered markers for immature neurons of the cerebellar nuclei, for Purkinje cells and for GCPs as they migrate out of the rhombic lip. That work also showed the remarkable migration patterns of the cell that give rise to the cerebellar nuclei, which first migrate onto the surface of the anlagen and then migrate off of the surface into the deeper zone in concert with the migration of GCPs from the rhombic lip across the surface. Dani also showed that some of the *Math1*⁺ cells from the rhombic lip migrate into the cerebellar nuclei, a result that Huda Zoghbi also demonstrated in beautiful fate mapping experiments. Dani's work went on to provide the first direct evidence that immature Purkinje cells migrate along brain lipid-binding protein positive radial glial fibers from the VZ into the upper aspects of the thickening anlagen.

During the 1990s, I became involved in SfN, first as the founding chair of a committee to promote women in neuroscience, then as a member of the SfN Council, and finally as treasurer. Although I am no financial expert, as treasurer, I realized that we urgently needed a long-term financial plan that included a reserve fund to cover the possibility that a natural disaster, like a hurricane, could cancel the annual meeting, the main source of revenue. Torsten Wiesel, who was president of Rockefeller then, was a huge help in that effort as he had the investment officer at Rockefeller help me make contacts in the New York investment world and get the program going. I am proud to say that the fund is now worth many millions of dollars and serves as a critical financial support for the SfN.

In addition to studying migration in the lab at that time, we continued my long-standing interest in neuronal regulation of glial differentiation.

Kim Hunter, who joined the lab from King's College and St. George's in London, did interesting work showing that radial glial transformation is bidirectional, as factors purified from embryonic brain could transform mature astrocytes back into radial glial cells. We spent considerable effort trying to isolate the protein from the culture medium of embryonic neurons, but we were never able to get enough material to identify the protein. This project should be revisited, given the advances in mass spectroscopy. Kim's work followed my earlier work showing that the ratio of neurons to glia dramatically changed glial morphology, with higher ratios inducing the glia to form highly elongated fibers that support neuronal migration. Over the years, we have consistently observed interdependence between neuronal and glial differentiation. Studies on a number of mouse mutants with defects in neuronal migration or differentiation also revealed indirect, nonautonomous effects on radial glial differentiation.

Functional Studies of Genes in GC Development

During the mid to late 1990s, we used two approaches to discover new genes involved in GC development. The first was screening for evolutionarily conserved genes that function in axon outgrowth and migration and the second was to use GC gene expression data to identify important genes. Toshi Tomoda, a postdoc from Tokyo, was the first person in the lab to examine evolutionarily conserved genes from *C. elegans* in GC development. Toshi studied the *Unc51* gene, *Unc51.1*, in the mouse, which he showed functions in parallel fiber outgrowth. Toshi also established powerful new general methodologies for examining gene function in GC differentiation, including transducing GCPs with retroviral constructs to express genes in vitro. He further discovered that he could simply incubate neonatal cerebellar slices with retrovirus to selectively express genes in proliferating GCPs ex vivo. The use of retroviruses to express genes of interest in GCPs has been a powerful methodology for a wide variety of studies.

Toshi also worked with Rupal Bhatt, a wonderful MD-PhD student in my lab, on his *Unc51.1* project and on her thesis project to explore whether receptor tyrosine kinases (RTKs) were important for GCP differentiation. Rupal discovered an especially interesting RTK that she cloned from GCPs: discoidin domain receptor 1 (DDR1). DDR1 is an orphan receptor that contains an RTK domain and an extracellular discoidin (lectin) domain. Rupal and Toshi, working together, overexpressed a dominant negative form of DDR1 in proliferating GCPs and found that this blocked axon outgrowth in vitro. Rupal went on to show that DDR1 functions after GCs begin to extend axons, suggesting it is involved in the maintenance, not initiation, of axon outgrowth. Thus, *Ddr1* came on slightly later than *Unc51.1*, after GCPs committed to cell-cycle exit and functioned in the maintenance of parallel fiber extension rather than initiation of axon outgrowth. Rupal also

showed that DDR1 binds to collagen in the pia, suggesting the importance of the three-dimensional (3D) geometry of the cerebellum to patterning differentiation signals.

One of the nicest aspects of Rupal's DDR1 paper was her beautiful imaging of granule neurons in ex vivo slices, images that showed both the extending parallel fiber axon and the descending migration process. We processed those images in 3D with a Silicon Graphics Program Voxel View, which Nick Didkovsky, a brilliant computer specialist, set up for us in the lab. Nick would go on to make seminal contributions to our videos and later to designing the GENSAT (Gene Expression Atlas) website.

Toshi teamed up with Jee Hae Kim, a postdoc who had trained with Rick Haganir at Johns Hopkins, to demonstrate that *Unc51.1* binds to SynGAP, a Ras regulator, and also to a PDZ containing scaffolding protein, Syntenin, which binds a Ras regulator Rab5 GTPase. This was the first indication that GCP process outgrowth involved Ras-like GTPase activity as well as endocytic pathways. The latter fit well with our studies on ASTN2, described later, that showed a key role for receptor trafficking in neuronal locomotion along glial fibers. Receptor trafficking was an early step in axon extension. Toshi also initiated a collaboration with Akira Sawa at Johns Hopkins to use our methodologies to study the role of the schizophrenia-associated gene *DISC1* in brain development. Those experiments were the first in my lab that related directly to neurodevelopmental disorders.

Along the way, we continued our efforts to define the molecular mechanisms of GCP migration, especially the role of ASTN in migration, with a graduate student Gunnar Dietz generating the first knockout mouse lacking *Astn1*. Niels Adams, a wonderful neuroanatomist, who had trained with Ray Guillery at Oxford, analyzed GC migration in mice lacking *Astn1*. As we had anticipated, those experiments showed a severe deficit in GC migration along glial cells, a deficit that indirectly affected Purkinje cell development, as the Purkinje cells tilted out of the sagittal plane when GCs migrated slowly.

The GENSAT Project

About that time, Nat's lab came up with bacterial artificial chromosome (BAC) methodology, which enabled us to generate enhanced green fluorescent protein (EGFP)-labeled genes by homologous recombination. Nat had the idea to do a very large contract, which NIH called GENSAT, to map cell-type-specific gene expression for hundreds of CNS genes. My role as a co-principal investigator of GENSAT was to develop the anatomy methods to carry out large-scale brain sectioning of hundreds of lines of mice and also to image the EGFP in the CNS of the mice at cellular resolution. Assembling the large team to do this work and setting up the imaging and designing the website with Nick was a huge project. Although Nat and I spent almost all of our time on the project, we gave the mice away and published only two core

papers, a *Nature* paper describing the project and a later paper describing the use of BAC methodology to make CRE lines for specific genes. We, and many other labs, would use those lines to generate cell-type-specific conditional knockout mutations. The GENSAT project was one of many projects that I enjoyed doing with Nat, a wonderful friend and colleague. His interest in molecular mechanisms and mine in understanding gene function have dovetailed in many joint projects.

The GENSAT project revealed a large number of developmental pathways that had not been recognized previously, both in the spinal cord and in the developing brain. Hillary Osheroff, a graduate student, used two lines of GENSAT mice, *Tg(Pde1C-EGFP)* mouse embryos, in which the EGFP transgene labeled all preplate neurons, and *Tg(Girk4-EGFP)* mouse embryos, where EGFP expression was restricted to Cajal–Retzius neurons. This allowed us to examine gene expression during preplate patterning, an important transient step during early stages of cortical development. Her studies showed more than 200 genes enriched in preplate neurons, including genes involved in transcription, cortical development, cell and axon motility, protein trafficking and steroid hormone signaling. Additionally, Hillary identified 10 genes in preplate neurons that were related to degenerative diseases of the cerebral and cerebellar cortex.

Through the years, I greatly enjoyed working with wonderful postdocs in my lab and serving on the boards and review committees of three major neuroscience scholar awards for young faculty: the John Merck Scholars Board and the review committees for the McKnight Neuroscience Program and the Pew Scholars Award. Few meetings were as exciting as those meetings, hearing young scientists talk about their innovative work and, over a period of years, watching their research programs develop.

The Cerebellum and Cancer

The arrival of David Solecki, who had trained with Eckhard Wimmer at Stony Brook in the early 2000s, led to work on GCP proliferation and the role of GCPs in medulloblastomas. David and Toshi followed up on an observation by a graduate student, Xiao Lin Liu, that Notch 2 was highly represented in the GC cDNA library. Their work showed that NOTCH2 activation maintained GCP proliferation through the JAG2 ligand. Although David's finding on NOTCH2 was overshadowed by the discovery and subsequent publication that SHH promoted GCP proliferation, recent studies show a link between NOTCH2 activation and medulloblastomas.

We then went on to study other regulators of proliferation, including cell cycle regulators, comparing normal GCPs and medulloblastoma. In GCPs, Ink4c is expressed in normal cells, a fact that led Martine Roussel and colleagues to analyze whether a loss of Ink4c would lead to medulloblastoma. This led to a wonderful collaboration with Martine's lab, showing

that purifying the GCPs revealed a much larger change in proliferation than was evident in neuroanatomical studies alone. Sandrine Anne and Jee Hae Kim, postdocs in my lab, would go on to show that Wnt 3 is a negative regulator of GCP proliferation, and Martine would show that it also represses medulloblastoma formation. The work with Martine was gratifying because it brought me back to working on cancer again, completing the circle with my graduate school research on tumor cell regulation.

At about the same time, Enrique Salero, who came to the lab from Mexico City, began to use the developmental signals we and others had identified as functioning in GC development to develop a protocol to differentiate mouse embryonic stem cells into GCs. Recapitulating developmental signals worked very well for generating mouse GCs from embryonic stem (ES) cells, which was gratifying. Enrique went on to show that he could implant EGFP-labeled ES-derived GCs back into mouse cerebellar cortex and show that the labeled cells would migrate into place and incorporate into the cortical architecture. We are now using this approach to test whether human embryonic stem (hES) cells differentiated to a cerebellar neuron fate will integrate into mouse cerebellum. That is a large effort directed toward treatment of human neurodegenerative diseases like Ataxia-telangiectasia.

By the early 2000s, although Mary Jean Kreeke and Titia De Lange had been promoted to professor, and Elaine Fuchs (whom I had known at Princeton) had been recruited to Rockefeller from the University of Chicago, the number of female professors remained very small. Things began to change when we were fortunate enough to recruit Cori Bargmann to Rockefeller. Cori helped bring a number of young women to Rockefeller through the open search process started by Paul Nurse, and she helped promote the careers of our young female faculty, including Leslie Vosshall (Leslie is a wonderful scientist, who has been critical to the effort to bring more young women neuroscientists to Rockefeller), Vanessa Ruta, and Priya Rajasethupathy.

Cori has also been an especially insightful colleague for my work. About the time she arrived, I became interested in examining whether genes that had been identified as migration genes in *C. elegans* might also function in vertebrate CNS migrations. Early on, I had used spontaneous mouse mutations and biochemistry to discover genes and pathways involved in migration. As we did not do human genetics, it seemed that studying migration genes identified in *C. elegans* might be a way to discover novel genetic pathways in vertebrate migration. Stephanie Schneider, a postdoc from Cologne, wanted to study *mig-13*, a gene that Cynthia Kenyon had discovered in *C. elegans*. Using EGFP-tagged mice that we generated in GENSAT, Stephanie found that *Lrp12/Mig13*, a mammalian gene related to *mig-13*, was expressed in a subpopulation of preplate neurons that migrate tangentially in the preplate layer, a transient layer that forms early in cortical development, as well as in some axon pathways. She went on to show that *Lrp12/Mig13*-positive cells revealed changes in cell polarity as preplate neurons

align into a pseudocolumnar pattern before descending into the newly forming subplate layer. She also showed that these changes in cell polarity do not occur in *reeler* mice, another spontaneous mouse mutant that has been used extensively to study neuronal migration. Yung Lie, who came to the lab from Cynthia's lab, carried out some interesting experiments in *C. elegans* in my lab, with Cori's help, which was fun.

mPar6 Signaling in Glial-Guided Migration

David's gene expression profiling of GCPs mentioned earlier ushered in another wave of work on the mechanisms of neuronal migration. In the GC data sets, he noticed that the expression of a recently discovered conserved polarity gene, *mPar6*, increased as GCs left the cell cycle. Using immunocytochemistry, David showed that *mPar6* localized to the centrosome of migrating neurons. To study this in more detail, he labeled *mPar6* with Venus, a new tag that Roger Tsien had developed that was twentyfold brighter than GFP. This was important because it allowed us to express lower amounts of tagged protein than GFP, which turned out to be a critical factor. With Venus-tagged *mPar6* and spinning disc confocal imaging, David was able to prove that the signaling complex localized to the centrosome and that *mPar6* activity controlled the speed of neuronal migration along the glial fiber. This also demonstrated that the machinery for migration was perinuclear and not at the tip of the leading process.

David next looked more closely at the cytoskeletal mechanisms underlying GC migration and revealed a previously unappreciated role for actin dynamics in this process. In a wonderful set of experiments, he showed that myosin II motors and F-actin dynamics drive the coordinated movement of the centrosome and soma during GCP glial-guided neuronal migration. Those experiments used real-time imaging combined with specific activators and inhibitors of myosin II assembly to show that activation of myosin motors accelerated the speed of migration, while inhibition slowed migration. David also carried out four-dimensional volumetric mapping of F-actin in the migrating GC and showed dynamic rearrangements in the actin cytoskeleton in the proximal aspect of the leading process, near the centrosome. At the time that David was doing these experiments, Eve Govek joined the lab, after training with Linda Van Aelst at Cold Spring Harbor, and collaborated with David on studies on *Par6* regulation of the cytoskeletal dynamics during GC migration. David and Eve demonstrated that *mPar6* regulates myosin activity by regulating myosin light chain phosphorylation. Thus, acto-myosin contractility in the proximal leading process appeared to pull the migrating neuron forward along the glial guide. These experiments were the clearest indication to date of the specific role of cytoskeletal dynamics and their control by the *mPar6* signaling complex in neuronal migration.

While we were studying *mPar6* signaling in migration, Perrin Wilson, a graduate student, and Bob Fryer, a child neurologist, together with Toshi, cloned a second member of the Astn gene family, *Astn2*. Although the peptide sequences of ASTN1 and ASTN2 are very similar, their expression patterns differ, with ASTN1 being expressed only during early phases of development when migration is ongoing and ASTN2 expression continuing into adulthood. Our expectation that the two proteins would be redundant turned out not to be the case. First, far less ASTN2 is expressed on the neuronal cell surface. Second, in functional experiments, ASTN2 did not appear to function as an adhesion protein. Indeed, our imaging experiments showed that ASTN2 regulates the trafficking of ASTN1 during migration rather than as a redundant adhesion receptor. These experiments were the first to suggest that endocytosis is an important regulator of migration, by removing the adhesion receptor ASTN1 as the neuron takes a step along the glial fiber. Hourinaz Behesti, who came from Jane Sowden's lab at University College London, is now actively studying the role of ASTN2 in synaptic function in the cerebellum. The finding that human CNVs in *Astn2* are associated with autism, intellectual disability, and language disability has spurred this work. It is especially interesting to understand how a gene that is predominantly expressed in the cerebellar cortex might function in higher cognitive functions.

About that time, we also started a project with Nagi Ayad at the University of Miami, to study the role of the APC/C complex in GCP cell cycle exit. Yin Fang, my wonderful lab manager, and Eve collaborated with Clara Penas in Nagi's lab to show that Casein kinase 1 (CK1d) functions in GCP proliferation and that the ubiquitin ligase APC/C targets CK1d for degradation by the proteasome. With Martine Roussel, Nagi and I also showed that CK1d is important for medulloblastoma formation. That was our last foray into the area of the cerebellum and cancer biology until a newly established collaboration with Agata Smogorzewska, a wonderful cancer biologist here at Rockefeller. A joint graduate student, Danielle Keahi, and Samer Shalaby, a postdoc in Agata's lab, are currently studying DNA damage pathways during GCP proliferation and in medulloblastomas, a surprisingly understudied area.

Changes in Chromatin Gene Expression during Cerebellar Circuit Formation

As part of our emerging interests in stem cell biology, and continuing work on gene expression during development, we decided to use Nat's BAC TRAP methodology to carry out a large-scale examination of GC gene expression over development. One major goal in that work was to obtain a genomic fingerprint of GCs to use not just for understanding development but also for analyzing whether stem cells differentiated to a cerebellar neuron fate were very close to native cells at the level of the full transcriptome, rather

than a handful of cellular markers. Keisha John, who came from Linda Van Aelst's lab at Cold Spring Harbor, started those experiments, joined by Xiaodong Zhu, a postdoc from China, and Eve. To carry out the bioinformatics, we collaborated at the Broad with Jill Mesirov and Pablo Tamayo, who used metagene analysis, a method to use matrix factorization to cluster genes into a half dozen or so metagenes. To our surprise, those experiments revealed pivotal changes in virtually all chromatin remodeling genes during the time frame when the cerebellar circuitry forms. Among those genes, we showed that the *Tet* genes, which generate 5hmC marks, are crucial as increased 5hmC correlated with increased gene expression. Interestingly the main genes that 5hmC activated were axon guidance genes and ion channel genes, both of which function in forming the circuitry. Knockdown of the *Tet* genes impaired the transition from migrating GCPs to GCs with dendrites, suggesting a key role in forming connections with ingrowing mossy fibers. We are following up on those experiments in Purkinje cells and are testing whether knockout of specific chromatin remodeling genes impairs either synapse formation or other aspects of circuit formation.

Current Work

Currently, we still focus on mechanisms of neuronal migration as well as on the role of ASTN2 in neurodevelopmental disorders and on efforts to generate human cerebellar neurons from hES cells. Eve is using genetic approaches to study the Rho GTPase Cdc42 in GCP migration. That work, which has been submitted for publication, shows that Cdc42 regulates neuronal polarity during GCP axon formation and glial-guided migration. Remarkably, a conditional loss of *Cdc42* in GCs results in abnormalities in the foliation of the cerebellum, revealed by the new iDISCO clearing method and light sheet microscopy, which we did in collaboration with Marc Tessier-Lavigne, and a loss of a pseudocolumnar organization of GCPs in the external granular layer. In migrating GCPs, cells lacking *Cdc42* fail to form the migration junction with the glial fiber, which slows migration dramatically. By phosphoproteomics, a loss of *Cdc42* results in lower phosphorylation of targets that include the polarity protein and actin regulatory proteins PAK1/4. In a second migration project, Zachi Horn (who trained at the Karolinska in Stockholm) is using biochemical and genetic studies to identify the long-sought ligand for ASTN1, which Zachi has identified as CDH2. He is currently carrying out biophysical measurements of the binding of ASTN1 and CDH2 in collaboration with Rob Gilbert's group at Oxford. Micha Hanzel, who recently arrived from Richard Wingate's lab in London, is characterizing an *Astn2* loss of function mutant that Zachi generated with Shiaochoing Gong from Nat's lab.

As mentioned, Hourinaz Behesti's experiments show that ASTN2 modulates synaptic strength at later stages of development, by controlling the

trafficking and degradation of surface proteins, including synaptic proteins. Her work includes studies of patients that have *ASTN2* CNVs as well as detailed studies of the function of *ASTN2*. The latter shows that *ASTN2* localizes to vesicles, binds to synaptic proteins, and regulates receptor trafficking and protein degradation. These functional insights are provocative, because they offer clues on molecular pathways involved in *ASTN2*-related neurodevelopmental disorders, such as intellectual disability and autism.

To generate human cerebellar neurons, Dave Buchholz, who worked with David Clegg and Pete Coffey at the University of California, Santa Barbara on retinal stem cells, is developing methods to differentiate hES cells into human Purkinje cells. That has been far more difficult than I anticipated as it turns out that human cells are quite different from mouse cells. Dave will use the TRAP methodology to examine gene expression patterns of the hES-derived Purkinje cells and to compare gene expression patterns of mouse and human Purkinje cells. He is also using the implantation methods that we developed for mES-derived GCPs to test whether human Purkinje cells will integrate into the mouse cerebellar cortex.

In addition to these main lines of work on cerebellum, Sasa Jareb a graduate student in Bob Darnell's lab, is working on cell-type-specific 3'UTR isoform expression; Arif Kocabas, a postdoc from Mary Hynes's lab, is using CRISPR methods to knock out chromatin remodeling genes; and Isaac Marin-Valencia, who recently arrived from Joe Gleeson's lab, is working on metabolic pathways and cerebellar development. Thus, we still have plenty of exciting projects in the lab.

I also still have an active collaboration with Nat Heintz, who has been my main colleague here at Rockefeller for so many years. I am also enjoying collaborating with Court Hull, an assistant professor at Duke, who is carrying out electrophysiological studies on *Astn2* mutant mice, as well as with Mustafa Sahin at Harvard, with whom we are studying mTor in autism pathways associated with cerebellar Purkinje cells.

I am so lucky to have so many wonderful colleagues at Rockefeller, including Torsten Wiesel, Nat Heintz, Cori Bargmann, Charles Gilbert, Mike Young, Rod MacKinnon, Jeff Friedman, Vanessa Ruta, Gaby Maimon, Winrich Freiwald, Bob Darnell, Bruce McEwen, and Agata Smogorzewska, who have contributed so much to my work.

Looking Back: Reflections

Reflecting on my life in science, three themes have endured: cell migration (imaging and molecular mechanisms), cerebellar development (genetics and cell biology), and cerebellar neurogenesis (including forays into cancer). In this age of translational neuroscience, a new area is emerging—understanding how cerebellar development relates to neurodevelopmental disorders, such as autism and intellectual disability. Although neurodevelopment is not as popular as it once was, I believe there are still many major

problems to solve, and cerebellar development remains my core interest. Harvard, Columbia, and Rockefeller made important contributions to my career, through the stimulating and enduring collaborations and wonderful friendships, most of which have persisted for many years. I am grateful to Dick Sidman for teaching me about cerebellar development, to Pasko Rakic for introducing me to GC migration, to Torsten Wiesel for his unwavering support since my Harvard days, to Carol Mason for our exciting work together to image CNS glial-guided migration, to Carla Shatz for so many years of shared interests in development, and of course, to Nat Heintz, who brought so many innovative molecular approaches to my work on cerebellar development and with whom I have done so many critical experiments. Moving to Rockefeller was certainly a boon to my work for all these many years, thanks in no small part to presidents like Torsten Wiesel, Marc Tessier-Lavigne, and now Rick Lifton.

References

- Zhu X, Girardo D, Govek EE, John K, Mellen M, Tamayo P, Mesirov J, Hatten ME. 2016. Role of Tet1/3 Genes and Chromatin Remodeling Genes in Cerebellar Circuit Formation. *Neuron* 89:100–112.
- Penas C, Govek EE, Daniel M, Fang Y, Wang W, Rahaim RJ, Bibian M, Kawauchi D, Finkelstein D, Han J-H, Li B, Robbins DJ, Malumbres M, Roussel MF, William R, Roush WR, Hatten ME, Ayad NG. 2015. Casein Kinase 1d Is an APC/CCdh1 Substrate That Regulates Cerebellar Granule Cell Neurogenesis. *Cell Reports* 11:1–12.
- Anne SA, Govek EE, Ayrault O, Kim J-H, Zhu X, Murphy D, Van Aelst L, Roussel MF, Hatten ME. 2013. Wnt3 Inhibits Cerebellar Granule Neuron Progenitor Proliferation and Medulloblastoma Formation via MAPK Activation. *PLoS One* Nov 26;8(11):e81769.
- Govek E, Hatten ME, VanAelst L. 2011. The Role of Rho GTPase Proteins in CNS Neuronal Migration. *Dev Neurobiol* 71:528–553.
- Roussel MF, Hatten ME. 2011. Cerebellum Development and Medulloblastoma. *Curr Top Dev Biol* 94:235–282.
- Schneider S, Gulacsi A, Hatten ME. 2011. *Lrp12/Mig13a* Reveals Changing Patterns of Preplate Neuronal Polarity during Corticogenesis That Are Absent in *Reeler* Mutant Mice. *Cereb Cortex* 21:134–144.
- Wilson P, Fryer RH, Hatten ME. 2010. *Astn2*, a Novel Member of the Astrotactin Gene Family, Regulates the Trafficking of ASTN1 during Glial-Guided Neuronal Migration. *J Neurosci* 30:8529–8540.
- Solecki DJ, Trivedi N, Govek EE, Kerekes RA, Gleason SS, Hatten ME. 2009. Myosin II Motors and F-Actin Dynamics Drive the Coordinated Movement of the Centrosome and Soma during CNS Glial-Guided Neuronal Migration. *Neuron* 63:63–80.
- Osheroff H, Hatten ME. 2009. Gene Expression Profiling of Preplate Neurons Destined for the Subplate: Genes Involved in Transcription, Axon Extension, Neurotransmitter Regulation, Steroid Hormone Signaling, and Neuronal Survival. *Cereb Cortex* 19 Suppl 1:126–134.

- Salero E, Hatten ME. 2007. Differentiation of ES cells into Cerebellar Neurons. *Proc Natl Acad Sci USA* 104:2997–3002.
- Morales D, Hatten ME. 2006. Molecular Markers of Neuronal Progenitors in the Embryonic Cerebellar Anlage. *J Neurosci* 26:12226–12236.
- Kamiya A, Kubo K, Tomoda T, Takaki M, Youn R, Ozeki Y, Sawamura N, Park U, Kudo C, Okawa M, Ross CA, Hatten ME, Nakajima K, Sawa A. 2005. A Schizophrenia-Associated Mutation of DISC1 Perturbs Cerebral Cortex Development. *Nat Cell Biol* 7:1067–1078.
- Uziel T, Zindy F, Xie S, Lee Y, Forget A, Magdaleno S, Rehg JE, Calabrese C, Solecki D Eberhart CG, Sherr SE, Plimmer S, Clifford SC, Hatten ME, McKinnon PJ, Gilbertson RJ, Curran T, Sherr CJ, Roussel MF. 2005. The Tumor Suppressors Ink4c and P53 Collaborate Independently with Patched to Suppress Medulloblastoma Formation. *Genes Dev* 19:2656–2667.
- Hatten ME, Heintz N. 2005. Large-Scale Genomic Approaches To Brain Development and Circuitry. *Annu Rev Neurosci* 28:89–108.
- Solecki DJ, Model L, Gaetz J, Kapoor TM, Hatten ME. 2004. Par6a Signaling Controls Glial-Guided Neuronal Migration. *Nat Neurosci* 7:1195–1203.
- Millen KJ, Millonig JH, Hatten ME. 2004. Roof Plate and Dorsal Spinal Cord DL1 Interneuron Development in the Dreher Mutant Mouse. *Dev Biol* 270:382–392.
- Tomoda T, Kim JH, Zhan C, Hatten ME. 2004. Role of Unc51.1 and Its Binding Partners in CNS Axon Outgrowth. *Genes Dev* 18:541–558.
- Gong S, Zheng C, Doughty ML, Losos K, Didkovsky N, Shambra U, Nowack N, Joyner A, Lablanc G, Hatten ME, Heintz N. 2003. A Gene Expression Atlas of the Central Nervous System Based on Bacterial Artificial Chromosomes. *Nature* 425:917–925.
- Ozeki Y, Tomoda T, Kleiderlein J, Kamiya A, Bord L, Fujii K, Okawa M, Yamada N, Hatten ME, Synder SH, Ross CA, Sawa A, et al. 2003. Disrupted-in-Schizophrenia-1 (DISC-1): Mutant Truncation Prevents Binding to Nude-Like (NUDEL) and Inhibits Neurite Outgrowth. *Proc Natl Acad Sci USA* 100:289–294.
- Adams NC, Tomoda T, Cooper M, Dietz G, Hatten ME. 2002. Mice That Lack Astrotactin Have Slowed Neuronal Migration. *Development* 129:965–972.
- Solecki DJ, Liu XL, Tomoda T, Fang Y, Hatten ME. 2001. Activated Notch2 Signaling Inhibits Differentiation of Cerebellar Granule Neuron Precursors By Maintaining Proliferation. *Neuron* 31:557–568.
- Bhatt RS, Tomoda T, Fang Y, Hatten ME. 2000. Discoidin Domain Receptor 1 Functions in Axon Extension of Cerebellar Granule Neurons. *Genes Dev* 14:2216–2228.
- Millonig JH, Millen KJ, Hatten ME. 2000. The mouse Dreher Gene Lmx1a Controls Formation of the Roof Plate in the Vertebrate CNS. *Nature* 403:764–769.
- Tomoda T, Bhatt RS, Kuroyanagi H, Shirasawa T, Hatten ME. 1999. A Mouse Serine/Threonine Kinase Homologous To C. Elegans UNC51 Functions in Parallel Fiber Formation of Cerebellar Granule Neurons. *Neuron* 24:833–846.
- Wingate RJ, Hatten ME. 1999. The Role of the Rhombic Lip in Avian Cerebellum Development. *Development* 126:4395–4404.

- Alder J, Lee KJ, Jessell TM, Hatten ME. 1999. Generation of cerebellar granule neurons in vivo by transplantation of BMP-treated neural progenitor cells. *Nat Neurosci* 2:535–540.
- Faust PL, Hatten ME. 1997. Targeted Deletion of the PEX2 Peroxisome Assembly Gene in Mice Provides a Model for Zellweger Syndrome, a Human Neuronal Migration Disorder. *J Cell Biol* 139:1293–1305.
- Millonig JH, Millen KJ, Hatten ME. 1996. A High-Density Molecular Genetic Map around the Weaver Locus. *Mamm Genome* 7:616–618.
- Alder J, Cho NK, Hatten ME. 1996. Embryonic Precursor Cells From The Rhombic Lip Are Specified to a Cerebellar Granule Neuron Identity. *Neuron* 17:389–399.
- Kofuji P, Hofer M, Millen KJ, Millonig JH, Davidson N, et al. 1996. Functional Analysis of the Weaver Mutant GIRK2 K⁺ Channel and Rescue of *Weaver* Granule Cells. *Neuron* 16:941–952.
- Zheng C, Heintz N, Hatten ME. 1996. CNS Gene Encoding Astrotactin, Which Supports Neuronal Migration along Glial Fibers. *Science* 272:417–419.
- Hunter KE, Hatten ME. 1995. Radial Glial Cell Transformation to Astrocytes Is Bidirectional: Regulation by a Diffusible Factor in Embryonic Forebrain. *Proc Natl Acad Sci USA* 92:2061–2065.
- Rivas RJ, Hatten ME. 1995. Motility and Cytoskeletal Organization of Migrating Cerebellar Granule Neurons. *J Neurosci* 15:981–989.
- Gao WQ, Hatten ME. 1994. Immortalizing Oncogenes Subvert the Establishment of Granule Cell Identity in Developing Cerebellum. *Development* 120:1059–1070.
- Feng L, Hatten ME, Heintz N. 1994. Brain Lipid-Binding Protein (BLBP): A Novel Signaling System in the Developing Mammalian CNS. *Neuron* 12:895–908.
- Baptista CA, Hatten ME, Blazeski R, Mason CA. 1994. Cell-Cell Interactions Influence Survival and Differentiation of Purified Purkinje Cells In Vitro. *Neuron* 12:243–260.
- Fishman RB, Hatten ME. 1993. Multiple Receptor Systems Promote CNS Neural Migration. *J Neurosci* 13:3485–3495.
- Gao WQ, Hatten ME. 1993. Neuronal Differentiation Rescued By Implantation of *Weaver* Granule Cell Precursors into Wild-Type Cerebellar Cortex. *Science* 260:367–369.
- Fishell G, Mason CA, Hatten ME. 1993. Dispersion of Neural Progenitors within the Germinal Zones of the Forebrain. *Nature* 362:636–638.
- Kuhar SG, Feng L, Vidan S, Ross ME, Hatten ME, Heintz N. 1993. Changing Patterns of Gene Expression Define Four Stages of Cerebellar Granule Neuron Differentiation. *Development* 117:97–104.
- Gao WQ, Liu XL, Hatten ME. 1992. The *Weaver* Gene Encodes a Non-Autonomous Signal for CNS Neuronal Differentiation. *Cell* 68:841–854.
- Fishell G, Hatten ME. 1991. Astrotactin Provides a Receptor System for CNS Neuronal Migration. *Development* 113:755–765.
- Gao WO, Heintz N, Hatten ME. 1991. Cerebellar Granule Cell Neurogenesis Is Regulated by Cell-Cell Interactions In Vitro. *Neuron* 6:705–715.
- Stitt TN, Hatten ME. 1990. Antibodies That Recognize Astrotactin Block Granule Neuron Binding to Astroglia. *Neuron* 5:639–649.

- Gasser UE, Hatten ME. 1990. Central Nervous System Neurons Migrate on Astroglial Fibers from Heterotypic Brain Regions In Vitro. *Proc Natl Acad Sci USA* 87:4543–4547.
- Hatten ME. 1990. Riding the Glial Monorail: A Common Mechanism for Glial-Guided Neuronal Migration in Different Regions of the Developing Mammalian Brain. *Trends Neurosci* 13:179–184.
- Gasser UE, Hatten ME. 1990. Neuron-Glia Interactions of Rat Hippocampal Cells In Vitro: Glial-Guided Neuronal Migration and Neuronal Regulation of Glial Differentiation. *J Neurosci* 10:1276–1285.
- Gregory WA, Edmondson JC, Hatten ME, Mason CA. 1988. Cytology and Neuron-Glial Apposition of Migrating Cerebellar Granule Cells In Vitro. *J Neurosci* 8:1728–1738.
- Edmondson JC, Liem RK, Kuster JE, Hatten ME. 1988. Astrotactin: A Novel Neuronal Cell Surface Antigen That Mediates Neuron-Astroglial Interactions in Cerebellar Microcultures. *J Cell Biol* 106:505–517.
- Edmondson JC, Hatten ME. 1987. Glial-Guided Granule Neuron Migration In Vitro: A High-Resolution Time-Lapse Video Microscopic Study. *J Neurosci* 7:1928–1934.
- Hatten ME. 1985. Neuronal Regulation of Astroglial Morphology and Proliferation In Vitro. *J Cell Biol* 100:384–396.
- Hatten ME, Liem RK, Mason CA. 1986. Weaver Mouse Cerebellar Granule Neurons Fail to Migrate on Wild-Type Astroglial Processes In Vitro. *J Neurosci* 6:2676–2683.
- Hatten ME, Mason CA, Liem RK, Edmondson JC, Bovolenta P, Shelanski ML. 1984d. Neuron-Astroglial Interactions In Vitro and Their Implications for Repair of CNS Injury. *Cent Nerv Syst Trauma* 1:15–27.
- Hatten ME, Rifkin DB, Furie MB, Mason CA, Liem RK. 1982. Biochemistry of Granule Cell Migration in Developing Mouse Cerebellum. *Prog Clin Biol Res* 85 Pt B:509–519.
- Hatten ME, Liem RK. 1981. Astroglial Cells Provide A Template for the Positioning of Developing Cerebellar Neurons In Vitro. *J Cell Biol* 90:622–630.
- Hatten ME, Sidman RL. 1978. Cell Reassociation Behavior and Lectin-Induced Agglutination of Embryonic Mouse Cells from Different Brain Regions. *Exp Cell Res* 113:111–125.
- Hatten ME, Scandella CJ, Horwitz AF, Burger MM. 1978. Similarities in the Membrane Fluidity of 3T3 and SV101-3T3 Cells and Its Relation to Concanavalin A- and Wheat Germ Agglutinin-Induced Agglutination. *J Biol Chem* 253:1972–1977.
- Hatten ME, Horwitz AF, Burger MM. 1977. The Influence of Membrane Lipids on the Proliferation of Transformed and Untransformed Cell Lines. *Exp Cell Res* 107:31–34.
- Horwitz AF, Hatten ME, Burger MM. 1974. Membrane Fatty Acid Replacements and Their Effect On Growth and Lectin-Induced Agglutinability. *Proc Natl Acad Sci USA* 71:3115–119.
- Wilkins JR, Hatten ME, Salmirs S. 1969. Effect of Gravity on the Colonial Morphology of Staphylococci in Soft Agar. *Appl Microbiol* 18:680–681.

