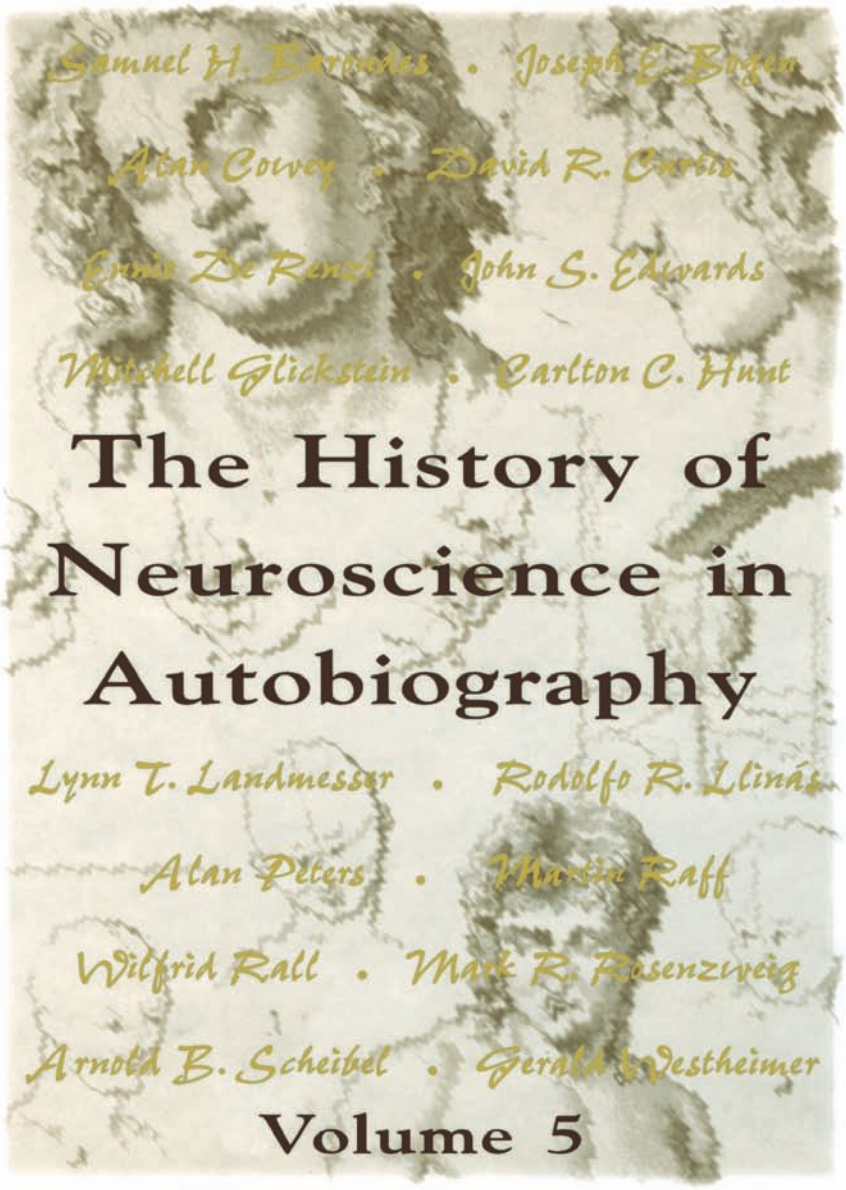

Edited by Larry R. Squire

The History of Neuroscience in Autobiography

VOLUME 5

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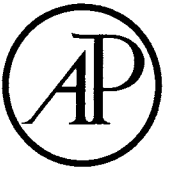

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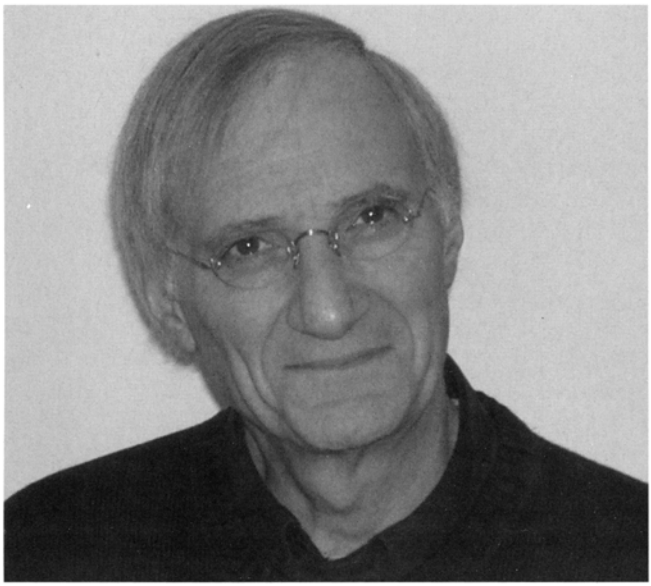

Martin Raff

Born:

Montreal, Canada
January 15, 1938

Education:

McGill University, B.Sc. (1959)
McGill University, M.D.C.M. (1963)

Appointments:

Royal Victoria Hospital, Montreal (1963–1965)
Massachusetts General Hospital (1965–1968)
Postdoctoral Fellow in Immunology, National Institute for Medical Research, Mill Hill, London (1968–1971)
MRC Developmental Neurobiology Program, Biology Department, University College London (UCL) (1971–2002)
Professor of Biology, Biology Department, UCL (1979–2002)
Scientist, MRC Lab for Molecular Cell Biology, UCL (2002–)
Emeritus Professor of Biology, Biology Department and MRC Lab for Molecular Cell Biology, UCL (2002–)

Honors and Awards (Selected):

Fellow, Royal Society of London (1985)
Academia Europaea (1988)
Feldberg Prize (1989)
President, British Society of Cell Biology (1991–1995)
British Academy of Medical Sciences (1998)
Foreign Honorary Member, American Academy of Arts and Sciences (1999)
Dunham Lectures, Harvard University (2001)
United Kingdom-Canada Rutherford Lectures (2002)
Hamdan Prize for Apoptosis in Disease and Health (2002)
Foreign Associate, National Academy of Sciences, USA (2003)
Honorary Fellow, University College London (2004)
Kuffler Lectures, UCSD, 2004
Honorary Doctorate, McGill University (2005)

Martin Raff began his scientific career as an immunologist and then brought his interest in the problem of cell identity to developmental neurobiology. He charted the origins of glia and used Schwann cells, precursors of oligodendrocytes, and retinal cells to make major contributions to understanding the regulation of cell size and number, the control of cellular differentiation and maturation, and the role of extrinsic and intrinsic mechanisms in the generation of cell diversity.

Martin Raff

I retired in September 2002 after 34 years as a scientist. I was almost 65, the standard retirement age in Europe. Although I consider my time as a scientist to have been a joy and a privilege, I had been planning my retirement and looking forward to it for more than 10 years. This was mainly because of Michael Heidelberger, an eminent immunochemist whom I had never met. When I was 51, I learned that Heidelberger had a National Institutes of Health (NIH) grant at the age of 101. I quickly calculated that, if I followed in Heidelberger's footsteps, I was not yet even a third of the way through my career. This revelation was so depressing that I promised myself I would stop at 65, which immediately cheered me up.

I am now 67, and retirement has been everything I had hoped it would be. Thanks to my kind colleagues, I have remained a member of the Medical Research Council (MRC) Laboratory for Molecular Cell Biology (LMCB) and an emeritus professor in the Biology Department at University College London (UCL). I do many of the things I did before retirement, but the pressure is off. I no longer run a laboratory or write papers or grants, which means that I can decline to referee papers and grants without guilt—a wonderful liberation. I am still connected to science, because I sit on many scientific advisory boards and am still a coauthor of the cell biology textbook *Molecular Biology of the Cell*.

Now seems a good time to look back at my research career, before I forget most of it.

Growing Up

Born and educated in Montreal, I got off to an unpromising start in school by failing kindergarten. I kept running away and returning home at recess, which suggested to both the authorities and my parents that I was not ready for school. I started again the following year, and it was relatively smooth sailing from then on.

My father was a physician and my mother a full-time housewife. My brother is my only sibling; because he is exactly 2 years older, we shared birthdays. My childhood was generally happy and unremarkable, but I did have a number of peculiarities that were much more troubling to others than to me. I frequently stole small things from friends or family

members, and I lied more often than most children. I hated losing and, as a result, had many temper tantrums. When I was 8, for example, I had a tantrum after losing a foot race. Surprisingly, they allowed me to race again three times against the winner, and this was followed by three more tantrums as I lost each time. I was also unusually fearful and could not stay alone in our house until I was in my teens. My parents, brother, and friends accepted my peculiarities with remarkable calm and good humor, and I gradually grew out of them. Looking back on this period, at what a pain in the behind I must have been, I find it inexplicable that those around me seemed not to notice and treated me with unremitting kindness.

Until my early 20s, sports dominated my life. Football, basketball, ice hockey, skiing, tennis, and sailing occupied much of the time that I was not in class. It was a great advantage having a park a few hundred yards away with a football field, two baseball diamonds, tennis courts, a ski hill, and a hockey rink. It was also advantageous having an older brother with whom I could play sports. He also stimulated me to read books and listen to music, because my parents had little interest in either.

I did well in school, and although I took all of the math and science courses available, I was not a budding scientist. I took courses in physics, chemistry, algebra, trigonometry, and intermediate algebra, but there were no biology courses available: We were taught how to brush our teeth but that was all the biology taught during my 12 years in school. It was only many years later that I realized how strange that was. What was the education board thinking? I would still not know where my heart is located if I had not eventually studied medicine.

Another oddity about growing up in Montreal in those days was that, although the city was about 70% French, I rarely saw a French Canadian. Where were they? I still wonder. We learned French from a young age, but never from a French Canadian. Soon after I left Montreal, at the age of 26, the French reclaimed their city, and it is now the great French Canadian city it should have been all along.

I spent the most enjoyable time of my youth at a coed summer camp in northern Ontario called Wabi-Kon. I spent 2 months there every summer from age 7 to 21—first as a camper, then counselor, then swimming instructor, and finally as a sailing instructor. The camp was on an island on beautiful Lake Temagami. There were no cars on the island and no electricity in the cabins. The camp focused on water sports and canoe tripping, and it had a wonderful spirit, encouraging free choice and discouraging competition. It was as close to utopia as I have experienced. I still have friends from those days, and I returned often with my children to camp on one of the many islands on the lake. Even today, I try to spend a few days on the lake every few years.

McGill Years

When studying for my B.Sc. at McGill University, I generally enjoyed the humanities more than the sciences and math. In the science courses, I spent many tedious afternoons in laboratories. Had I begun with an interest in becoming a scientist, these labs would have killed it. It was only when I started doing real science years later that I appreciated how far removed these lab classes are from experimental science: Trying to reproduce a result that the professor wants is not the same as trying to find out something new about how the world works. For me, these lab classes were more than unhelpful; they were also actively destructive, because they made me think that science was difficult and boring and that I was no good at it. I suspect that they are still deterring many students from pursuing a career in science.

I stayed on to study medicine at McGill, more out of sloth than a passion to treat disease. Many of my friends were doing it, and I found it attractive to have another 4 years without having to worry about what I would do with my life. Although I often accompanied my father on house calls (largely to ride the automatic elevators while he saw his patient), his life as a family physician was not especially appealing to me, mostly because he seemed to work too hard.

Despite my less than noble motives for studying medicine, I greatly enjoyed my 4 years in medical school, partly because it was my first real exposure to biology. We spent an excessive amount of time studying anatomy, however, so that my brain is still cluttered with detailed and useless anatomical facts that, unlike more useful information, I cannot seem to forget. There was an enormous amount of rote learning, but our teachers were excellent, and they prepared us well for practicing medicine. Medical school is probably not an optimal preparation for being a scientist. It trains you to look backward and compare the patient at hand with similar patients seen previously, whereas a scientist tries to see things in new ways, in order to make discoveries. This would rarely be a good way to treat a patient.

After medical school, I did a rotating internship and a year of residency in medicine at the Royal Victoria Hospital, one of the McGill teaching hospitals. I started my internship on the cardiorespiratory ward, which was an exciting and often terrifying experience. I had never seen anyone die before, and many of the patients on our ward died within the first week of my starting. In those days, hospitalized patients did not just die; they suffered a cardiac arrest, and if they were successfully resuscitated, they were transferred to the cardiorespiratory ward, where they frequently had more cardiac arrests and usually died. Moreover, the house officers on duty on the cardiorespiratory service were called to help resuscitate patients who had a cardiac arrest anywhere in the hospital, and so we rarely had an

uninterrupted meal or night's sleep. I learned a great amount of medicine fast, however.

I delivered a number of babies during my rotation in obstetrics, and so I was well prepared when I had to deliver my own daughter, Kim, years later. She emerged unexpectedly fast at 4 AM, when there were no doctors or nurses around on the obstetrics floor at University College Hospital in London, which was right across the street from where I worked.

When it came time to choose a medical specialty to pursue after my year as a medical resident, I drew up a table listing the advantages of the various possibilities, giving a value out of ten for interest, life style, earning potential, and so on. This proved invaluable, because the specialties that came up with the highest scores made my heart sink, until I juggled the numbers so that neurology came first, making it clear to me that neurology was what I really wanted to do. Paul Rosman, an old friend, encouraged me to apply to the neurology program at the Massachusetts General Hospital (MGH) in Boston where he was at the time. I applied, shaved my beard before going for an interview, and was accepted.

Neurology at the MGH

I greatly enjoyed my 3 years as a neurology resident at the MGH. I was strongly influenced by Raymond Adams, C. Miller Fisher, and E.P. Richardson, who were outstanding practitioners and teachers. I especially liked the second year of the program, which we spent in neuropathology. There were no night calls, and we had ample time to read, think, and talk. At weekly "brain cutting" meetings run by E.P., we were given a brief medical history of a patient who had died recently and were asked, in turn, to discuss what neurological disease we thought the patient had. The answer would usually be revealed when E.P. took the brain out of a pot and sliced it. This could be extremely embarrassing, as when we all failed to diagnose a classic case of poliomyelitis that had been sneakily pulled from the archives. None of us had ever seen a case of polio, but it was nonetheless humiliating to not have even mentioned it as a possibility.

On Tuesday evenings, we examined the histological slides of the nervous system and other organs of a deceased patient. Without being given any history at all, we were asked, in turn, to discuss what neurological disease we thought that the patient had. One evening, we examined slides of Einstein's brain; we all thought we were looking at the brain of a young person, because there were no hints of the usual signs of aging despite his 76 years.

During the neuropathology year, we each removed the brain (and, in some cases, the spinal cord, nerves, and muscle) from about 50 patients who had died, whether or not they had a neurological disease. Before starting,

we read the patient's medical records to see if there was anything of neurological relevance. On one occasion, I noticed that the patient, who had died of a pulmonary embolus, had had a neurological illness 6 weeks before. He was diabetic and had awakened one morning with weakness and loss of sensation in a random pattern in one leg, consistent with a diagnosis of diabetic mononeuropathy multiplex. Virgilio Sangalang, a senior fellow in neuropathology, and I suspected from the history that he may have had ischemic strokes (infarcts) in scattered nerves of his affected leg, and so we took as many nerves as we could and processed them for histology. We found just what we expected (Raff, Sangalang, and Asbury, 1968). This pathology in diabetic mononeuropathy multiplex had not been described before, although there was one report of an ischemic lesion in a cranial nerve of a diabetic patient who had had an acute episode of ophthalmoplegia. It remains a mystery how, in our case, infarcts occurred specifically in multiple nerves almost simultaneously, although it presumably reflected an underlying diabetic angiopathy.

Twenty-five years after leaving the MGH, I was invited back for several days as the Raymond Adams Visiting Professor in Clinical Neurology. Although I had not seen a patient since leaving, it was a thrill to make rounds on the neurology ward and observe that Adams and Fisher, now in their mid-80s, were as sharp as ever and indistinguishable from how I remembered them.

Exodus from America

During my time in Boston, the Vietnam War continued to escalate, and the war and the military draft dominated American politics. In the middle of my second year of residency, a lawyer friend in Washington, DC informed me that there was likely to be a change in the draft law that would make immigrant physicians eligible for the draft until the age of 35, instead of 26 as it had been until then. The change would affect me, because I was in Boston as a landed immigrant with a green card. Checking to see whether there could be a possibility of joining the National Guard, the Reserves, or the Public Health Service, I learned that these options were closed to immigrants. I quickly obtained clearance from my local draft board and the Internal Revenue Department, and my family and I flew back to Montreal and returned to Boston the next day, exchanging our green cards for exchange visitor visas at the American Immigration desk at the airport.

We were able to switch visas, because the change in the draft law was still several weeks away. The new visa, however, meant that I had to leave the United States at the end of my training, which was just over a year away. Until I changed visas, I had been planning to stay in the United States after my residency, possibly to do neuro-ophthalmology with David Cogan at the Massachusetts Eye and Ear. Now I needed a new plan.

I asked my friend Barry Arnason, a neurologist and neuroimmunologist at the MGH, if he had any suggestions. At a similar stage in his career, he had done experimental immunology with Guy Voisin at l'Hôpital St. Louis in Paris. He suggested that I do the same and offered to arrange it. I agreed, and a few hours later it was all arranged.

Within weeks, however, a physicist friend in Boston sent an article to me entitled "Immunology at Mill Hill" that had just been published in *Science*. It described the immunological research being done at the National Institute for Medical Research (NIMR) in Mill Hill, London. Although I could not understand most of it, it seemed clear that the NIMR would be an exciting place to do immunology. Besides, I had loved London when I visited years earlier, and I was worried about learning French and immunology at the same time in Paris; my poor French was especially embarrassing considering that I grew up in Montreal. Barry warned me that it would be difficult to obtain a place at the NIMR, and so I decided to write to the immunologist whose work I could understand least from the article—Avrion (Av) Mitchison. This turned out to be an immense stroke of luck, and it changed the entire course of my life.

Mitchison accepted me, and the American National Multiple Sclerosis Society awarded me a postdoctoral fellowship to work with him. My fellowship application was based on a basic immunology project, written by Mitchison, that I did not understand and never actually carried out. I am greatly indebted to the American National Multiple Sclerosis Society for funding a Canadian to work in the United Kingdom on a project with no obvious connection to multiple sclerosis (MS). I am also grateful to the society for supporting several postdocs to work with me in later years, when our research, by pure chance, was somewhat more relevant to MS.

Immunology at the NIMR

I arrived in London in the autumn of 1968. I was 30 years old. I was not planning to become a scientist; I was planning to spend 2 years doing experimental immunology before returning to the United States as an academic neurologist in a teaching hospital. I knew almost no immunology and even less about experimental science. I did not even know that there was more than one strain of mouse. It did not take me long, however, to realize that, by sheer good luck, I had chosen the right person and right place. Av was an ideal mentor, and the NIMR was the perfect environment to learn science in general and immunology in particular. It was overflowing with outstanding immunologists. Besides Av, there were Ita Askonas, Brigid Balfour, David Dresser, John Humphrey, Peter Medawar (who was the Director of the NIMR), Mike Parkhouse, Roger Taylor, Alan Williamson, and Henry Wortis, as well as those on sabbatical, including Alistair Cunningham,

John Owen, Klaus Rajewsky, Eli Sercarz, and Byron Waksman, and various American postdocs, including Harvey Cantor and Paul Plotz.

This was an exciting time in immunology. It was becoming clear that there are two functionally distinct classes of lymphocytes–thymus–derived T cells and bone-marrow-derived B cells. Because the two classes of cells look the same and occur together in the peripheral lymphoid organs such as the spleen and lymph nodes, there was an urgent need for methods to distinguish and separate them from each other. Av had recently heard the Boston immunologist Arnold Reif describe an alloantigen called *theta* (later called *Thy-1*) that was present on the surface of mouse thymus lymphocytes. As thymus lymphocytes give rise to T cells, Av thought that Thy-1 might also be present on T cells but not B cells, in which case it could serve as a useful T-cell marker.

Using an antibody- and complement-mediated cytotoxicity assay that I learned from my lab-mate Marion Ruskowicz, I first showed that Thy-1 is present on peripheral lymphocytes as well as on thymus lymphocytes. I then examined lymphocytes from mice that Sandra Nehlsen (a Ph.D. student of Medawar who worked across the hall from me) had been chronically treating with anti-lymphocyte serum to deplete T cells. I found that the spleen and lymph nodes of these mice contained normal numbers of Thy-1-negative lymphocytes but very few Thy-1-positive lymphocytes, strongly suggesting that Thy-1 is present on T but not B cells as Av had suspected (Raff, 1969). I then used the antibodies to analyze a system that Av had developed to study the collaboration between lymphocytes from mice immunized with a carrier protein A and lymphocytes from mice immunized with a hapten coupled to carrier protein B; when both lymphocyte populations were transferred into irradiated mice and immunized with the hapten coupled to carrier protein A, the mice produced large amounts of anti-hapten antibodies. Before transferring the cells, I treated one or other population with anti-Thy-1 antibodies and complement to kill the T cells and showed that the carrier-A-primed cells were T cells and the hapten-primed cells, which produced the antibodies, were not (Raff, 1970a).

When it came time to publish these findings, Av would not put his name on the papers, even though the projects were his idea and he had begun to produce the anti-Thy-1 antibodies for the project before I had arrived in London. This exceptional act of generosity had an enormous influence on my career. Thy-1 rapidly became a standard marker for mouse T cells, and the two papers gave me immediate international recognition after only 2 years in science. This was not special treatment: I was to see it repeated over and over again with subsequent generations of Mitchison students and postdocs. Av always did his own experiments, and many of his contributions have been landmarks in immunology, but, because he let his students and postdocs publish on their own, his contributions to science are far greater than is documented in the literature. Similarly, it would be

difficult to deduce our connections to Av from the literature. Had I known then what I know now, I would have insisted that his name be on those two papers.

In a series of very fruitful collaborations with John Owen, who was on sabbatical from Oxford, we used the anti-Thy-1 antibodies to study the distribution, development, and functions of T cells (Owen and Raff, 1970; Raff and Owen, 1971). In addition, to directly visualize Thy-1 on lymphocytes, I turned to fluorescence microscopy, using fluorescent anti-immunoglobulin (Ig) antibodies to detect the bound anti-Thy-1 antibodies on living T cells. Despite the primitive fluorescence microscopes of the time, the method worked well, but the experiments turned up an unexpected result: In control experiments, in which I omitted the anti-Thy-1 antibodies, the fluorescent anti-Ig antibodies on their own labeled a substantial proportion of peripheral lymphocytes. Roger Taylor, working across the hall, had independently obtained similar results using radioactive anti-Ig antibodies, and we published these findings together (Raff, Sternberg, and Taylor, 1970) as one of the first direct demonstrations of Ig molecules on the surface of lymphocytes, which immunologists had postulated would serve as receptors for antigens.

The finding of Ig on some lymphocytes but not on others raised the question of which class of lymphocyte expressed the Ig. To find out, I examined lymphocytes from mice depleted of T cells, either by chronic treatment with anti-lymphocyte serum or by thymectomy, irradiation, and bone marrow transplantation. I found that all the Ig-positive cells were B cells (Raff, 1970b), which began a prolonged and frustrating search by many laboratories for the antigen receptors on T cells. After many false leads, the antigen receptors on T cells were identified as Ig-like proteins years later. Surface Ig, however, rapidly became a standard marker for B cells in all vertebrates. Later, when we moved to UCL, for example, John Owen and I collaborated with Max Cooper, who was on sabbatical from Birmingham, Alabama, and used anti-Ig antibodies and tissue explant cultures to show directly that B cells develop in the fetal liver and adult bone marrow (Owen, Cooper, and Raff, 1974), rather than in the gut as had been suggested by others (including Max), and that B cells arise from pre-B cells, which have already begun to make IgM heavy chains (Raff et al., 1976).

Cell Biology at the NIMR

When I visualized Ig on the surface of B cells with fluorescent antibodies, a remarkable feature of the staining was that it was located at one pole of the cell, forming a fluorescent "cap" (Raff, 1970b). Göran Möller at the Karolinska Institute in Stockholm saw the same distribution almost 10 years earlier (Möller, 1961). To determine what was special about the pole of

the cell where the Ig was located, I collaborated with Stefanello de Petris, an Italian scientist working at the NIMR. Nello was an expert in using ferritin-coupled antibodies to localize antigens in cells in an electron microscope. For reasons known only to him, he labeled lymphocytes with ferritin-coupled anti-Ig antibodies at two temperatures: 4°C and room temperature. The results were spectacular: At 4°C, the Ig was seen in small patches all over the B cell, whereas at room temperature, it was all in a cap at one pole (dePetris and Raff, 1972, 1973). The implications were clear: The Ig is apparently normally distributed over the entire B cell surface, and the binding of the anti-Ig antibodies induces the capped distribution, but only at the higher temperature. The finding that cell-surface Ig molecules can move in the plane of the membrane was among the earliest lines of evidence that cell membranes are two-dimensional fluids, rather than two-dimensional deformable solids, a realization that dramatically changed the way one thought about membrane structure and function.

The next few months were the most exciting of my career. Nello and I returned to immunofluorescence experiments. We quickly showed that it is the cross-linking of the surface Ig molecules by the bivalent anti-Ig antibodies that induces the Ig to cluster into patches on the B cell surface, because monovalent Fab fragments of the anti-Ig antibodies did not induce Ig patches. At room temperature, an actin-dependent process actively moves the patches to one pole of the cell. We also found that antibody binding induces the endocytosis of the surface Ig, rapidly clearing the Ig from the cell surface, providing an explanation for the phenomenon of antibody-induced antigenic modulation, previously described by Ted Boyse and Lloyd Old at the Sloan Kettering Institute in New York. A similar mechanism is now known to be responsible for the down-regulation of many types of cell-surface receptors following the binding of their extracellular signal molecules.

At this point, we discovered that our former colleague Roger Taylor, who had since moved to Bristol University, and his post doc Philip Duffus had similar findings, and we published our fluorescence observations together (Taylor et al., 1971). We tossed a coin to determine the order of authors, and, embarrassingly, I was devastated when Nello and I lost the toss. Needless to say, the results of the toss had no obvious effects on any of our careers.

Our observations on ligand-induced patching, capping, and endocytosis were undoubtedly the most important discoveries with which I have been associated. They have had implications for so much of cell biology, including membrane structure, turnover, and function, as well as for cell signaling and vesicular traffic. New implications continue to be revealed; only quite recently, for example, it was discovered that synaptic plasticity often depends on similar redistributions of neurotransmitter receptors on the postsynaptic side of synapses. These experiments with Nello transformed

me from an immunologist to a cell biologist, although, initially at least, I had still considered myself to be a clinical neurologist rather than a scientist. The transition to a scientist occurred in a single night.

In 1970, Av accepted the Jodrell Chair in the Zoology Department at UCL, a chair that his mentor Peter Medawar had occupied some years earlier. One evening, Av mentioned that, if I would consider staying in science and in the UK, he would be happy to have me join him at UCL when he moved there in 1971. That night I decided to do it, which was the best and most important decision of my life.

Looking back on my NIMR days, I am impressed at how different things were then compared to today for most people starting a career in the biological sciences. So little was known then that almost every experiment resulted in an interesting discovery. Unlike now, it was relatively easy to publish papers in good journals: I published 11 first-author papers in my first 3 years, 5 of them in *Nature*. (Interestingly, the one that became a Citation Classic [Raff, 1970b] was published in *Immunology,* a relatively low impact journal.) My rapid start had little to do with me; I was in the right place at the right time, with a brilliant mentor and outstanding colleagues. Even with luck, however, it would be hard to get off to such a rapid start today, and it is sobering to realize that those few years as a novice were to be the most productive of my career.

The Move to UCL and the Hunt for Neural Cell Markers

As my MS postdoctoral fellowship was about to run out, I had to decide how to fund my salary and research at UCL. Av suggested that we write a joint application to the MRC for a 5-year program grant. We proposed to use the antibody strategy, which had proved so useful in studying cells of the immune system, to study cells of the mammalian nervous system. Given that neither Av nor I had any track record in neurobiology, it is remarkable that the MRC funded the grant in full, including my salary. Over the next 31 years, I renewed the grant five times, so that the MRC provided my salary and research support until I retired in 2002. Although I did not have a tenured position, this was a wonderful arrangement because it allowed me to do full-time research in a university, with few teaching or administrative responsibilities. For the first 20 years or so, our group was physically embedded in Av's Tumor Immunology Unit, which was generously funded by the Imperial Cancer Research Fund (ICRF, now Cancer Research UK) until Av left UCL for Berlin. Although we were not financially part of the unit, the ICRF services were invaluable to us. Moreover, during this entire period, I had the wonderful privilege of sharing an office with Av, who was a protective patron, inspiring role model, and generous friend.

The research plan was to raise antibodies against cells of the nervous system and use them to distinguish and separate the different cell types so that we could study their development and interactions, mainly in a culture dish. This was before the monoclonal antibody revolution, and there were many formidable problems to overcome. Because neural cells are fixed in complex arrangements in the nervous system, they are much more difficult to isolate than are lymphocytes. Moreover, we would need relatively pure populations of cells for raising and testing antibodies, and so we might be defeated by the very problem we wanted to solve. To overcome this catch-22, we decided to start with cells from neural tumors; as each tumor initially arises from a single cell, its cells should be relatively homogeneous. We took advantage of the discovery by Hermann Druckrey and his colleagues at the University of Freiburg that one can induce tumors of the peripheral nervous system (PNS) and central nervous system (CNS) in a high proportion of rats by injecting either newborns or their pregnant mothers with the chemical carcinogen ethylnitrosourea (ENU) (Ivankovic, Druckrey, and Preussmann, 1966).

Kay Fields, an American phage molecular biologist who had just finished a post doc in Geneva, took on the project of inducing the tumors, isolating cell lines from them, and raising antibodies against the lines (Fields et al., 1975). After absorbing the antisera with normal non-neural tissues and different neural tumor cell lines, we hoped to obtain antibodies that distinguished one neural cell type from another. Of the various antisera that Kay produced, one specifically labeled the surface of Schwann cells in cultures of newborn rat sciatic nerve cells (Brockes, Fields, and Raff, 1977). She called the antigen *rat neural antigen 1 (Ran-1)*, which others showed years later was the low affinity NGF receptor protein, p75. This was the lab's first useful neural cell marker. It had taken 5 years to show that the immunological approach could work with cells of the nervous system.

These 5 years were difficult. I knew little neurobiology and found it hard to learn more. I remained immersed in immunology and cell biology, and it was a struggle to switch my interests. My scientific friends were immunologists; I was invited to talk mainly at immunology and cell biology meetings; and the young scientists that applied to work with me wanted to do immunology or cell biology. During this time, I continued to collaborate with Nello de Petris (who had also moved with Av to UCL) on ligand-induced redistribution of cell-surface molecules and its implications. A Ph.D. student who joined us from Scotland, Durward Lawson, used electron microscopy to study the cell biology of histamine secretion by rat mast cells; he showed, for example, that a stimulant attached to a solid bead caused exocytosis exclusively at the site of contact with the bead, indicating that the response could be localized and need not involve the cell as a whole as had been thought (Lawson, Fewtrell, and Raff, 1978).

I am not sure that I could have made the transition from immunology and cell biology to developmental neurobiology without Jeremy Brockes. He joined the laboratory as a post doc from the neurobiology department at Harvard Medical School, then the mecca of neurobiology, and he played a crucial part in both our first neurobiological success and my conversion to neurobiology. He initially collaborated with Kay to show that Ran-1 could be used as a Schwann cell marker. My first graduate student, Peter Stern, had shown earlier that Thy-1 was present on rat and mouse fibroblasts (Stern, 1973). Jeremy and Kay showed that anti-Ran-1 and anti-Thy-1 antibodies labeled nonoverlapping populations of cells in cultures of newborn rat sciatic nerve cells; whereas anti-Ran-1 antibodies labeled the Schwann cells, anti-Thy-1 antibodies labeled most of the non-Schwann cells (Brockes, Fields, and Raff, 1977). These findings allowed Jeremy to devise a strategy for purifying Schwann cells that depended on killing the contaminating non-Schwann cells with anti-Thy-1 antibodies and complement (Brockes, Fields, and Raff, 1979).

Schwann Cells

Jeremy and I went on to study some of the properties of purified rat Schwann cells. We found, for example, that either an increase in intracellular cyclic AMP (Raff, Hornby-Smith, and Brockes, 1978) or an extract of bovine pituitary glands (Raff et al., 1978a) stimulated the cells to proliferate, apparently by distinct mechanisms. These findings enabled us to produce large numbers of cultured Schwann cells (Brockes, Fields, and Raff, 1979). When Jeremy left to take up his first independent position at Cal Tech, he and his Ph.D. student Greg Lemke purified the Schwann cell mitogen in the pituitary extract (Lemke and Brockes, 1984). They called it glial growth factor (GGF), which was the first member of the Neuregulin family of extracellular signal proteins.

Rhona Mirsky, who initially came on sabbatical from Dartmouth Medical School and later returned permanently to UCL, showed that Schwann cells, unlike oligodendrocytes, require signals from axons to maintain their normal expression of myelin proteins and glycolipids (Mirsky et al., 1980). It was around this time that Rhona began a long-term collaboration with Kristjan Jessen, a post doc originally from Iceland, that continues to this day. The Mirsky-Jessen Group in the Anatomy and Developmental Biology Department at UCL rapidly became one of the world's leading laboratories studying Schwann cell development and biology.

Anne Mudge, an Australian post doc from Harvard Medical School, used purified populations of Schwann cells and embryonic sensory neurons (from dorsal root ganglia) to demonstrate that Schwann cells have a dramatic influence on the development of the neurons, inducing them to undergo their normal morphological transformation from an immature

bipolar form to a mature pseudo-unipolar form (Mudge, 1984). After Rhona left our MRC Program, Mudge took her place and has remained a close, but independent, colleague ever since.

Many years after our initial studies on Schwann cells, my last graduate student, Ian Conlon, returned to these cells to address a fundamental, but understudied, problem in cell biology—how cell growth (enlargement) and cell-cycle progression are coordinated to ensure that proliferating cells maintain an appropriate size. He showed that GGF stimulates Schwann cells to progress through the cell cycle but does not stimulate them to grow, whereas insulin-like growth factor 1 (IGF-1) on its own does the opposite (Conlon et al., 2001). Using these two signal proteins, he provided evidence that the size of proliferating Schwann cells at division does not depend on a cell-size checkpoint as we had suspected; instead, their size at division depends on how fast the cells are growing and how fast they are going through the cell cycle, and these rates depend on the concentrations of extracellular signals that control cell growth, cell-cycle progression, or both (Conlon and Raff, 2003).

Our initial studies on Ran-1 and Schwann cells provided a much-needed proof of principle that we could use antibodies to identify and purify a neural cell type. Our goal, however, was to use this approach to study cells of the CNS, which was much more challenging.

Optic Nerve as a Model System

To begin to define markers for CNS cells, we turned to cultures of newborn rat optic nerve cells. The advantage of these cultures is that they do not contain neurons, and so they are not much more complex in terms of cell types than cultures of sciatic nerve. Fortunately, Amico Bignami and Larry Eng and their colleagues had already identified glial fibrillary acidic protein (GFAP), an intermediate filament protein expressed by astrocytes but not by oligodendrocytes, microglial cells, or neurons (Bignami et al., 1972). We obtained anti-GFAP antibodies from Bignami and found that they labeled two morphological types of astrocytes, which we later called type-1 and type-2 astrocytes; the former had a fibroblast-like morphology and the latter a process-bearing morphology (Raff et al., 1983). Other cells in the culture had multiple branching processes and were GFAP-negative, and we assumed that they were oligodendrocytes. I then tested a number of lectins and antibodies, hoping that some would label these putative oligodendrocytes. I found one that did: Antibodies against a major myelin glycolipid—galactocerebroside (GC)—labeled these cells and no others in optic nerve cell cultures (Raff et al., 1978b).

By this time, George Kohler and Cesar Milstein had developed the monoclonal antibody technology that dramatically increased the power of the antibody approach. Perry Bartlett, an Australian post doc from

Johns Hopkins University, made our first monoclonal antibody by immunizing mice with cultured brain astrocytes. He called the cell-surface antigen that the antibody recognized *Ran-2* (Bartlett et al., 1980), which we showed later was expressed on type-1 but not type-2 astrocytes in optic nerve cell cultures. Becky Pruss, a post doc from UCLA, made our second monoclonal antibody. She made it against GFAP, but it turned out to react with all intermediate filament proteins, including those from squid and a marine worm, providing the first evidence that all of these proteins were homologous, which was very surprising at the time (Pruss et al., 1981). Mike Klymkowsky, a post doc from UCSF, microinjected the antibody into 3T3 cells and showed that it caused the intermediate filaments to collapse around the nucleus. This had no effect, however, on cell shape, motility, or division, indicating that intermediate filaments are not required for these cell activities, which was also a great surprise at the time (Klymkowsky, 1981). Becky also made a rabbit anti-GFAP antiserum that we used for the next 30 years to identify astrocytes.

We obtained a monoclonal antibody made by Marshall Nirenberg and his colleagues at the NIH called A2B5, which we also used for 30 years to help identify and purify another cell type—the oligodendrocyte precursor cell—that became my main interest until I retired. A2B5 recognizes certain gangliosides and was initially thought to label neurons specifically (Eisenbarth, Walsh, and Nirenberg, 1979). We found, however, that it also labeled the surface of oligodendrocyte precursor cells and type-2 (but not type-1) astrocytes in optic nerve cultures (Raff, Miller, and Noble, 1983). The precursor cells and type-2 astrocytes could also be labeled on their surface by tetanus toxin, which Rhona Mirsky and her Ph.D. student Linda Wendon had shown previously bound to the surface of cultured PNS and CNS neurons (Mirsky et al., 1978).

We therefore had increasing evidence that type-1 and type-2 astrocytes are molecularly and morphologically distinct, which made us think that they may derive from different cell lineages. A fortuitous finding enabled us to show that both type-2 astrocytes and oligodendrocytes develop in culture from a common progenitor cell. Our colleague Jim Cohen had begun using a serum-free culture medium for his cultures of cerebellar cells that was based on a medium originally described by Jane Bottenstein and Gordon Sato. He suggested that I try it on my optic nerve cell cultures, which we normally maintained in 10% fetal calf serum (FCS). Remarkably, whereas cultures with 10% FCS contained many type-2 astrocytes and very few oligodendrocytes, those in serum-free medium contained the opposite. I suspected that this result might reflect the different survival requirements of type-2 astrocytes and oligodendrocytes. But when I showed the cultures to my 15-year-old son, Adam, he suggested an alternative possibility: Maybe the FCS induces cells destined to become oligodendrocytes to become type-2 astrocytes instead. He was absolutely right, and it was

relatively simple to demonstrate it (Raff, Miller, and Noble, 1983). I like to think that I would have eventually arrived at this crucially important conclusion myself, but who knows?

Together with two post docs, Mark Noble from Stanford and Bob Miller from UCL, I went on to show that type-1 astrocytes develop from one type of precursor cell, whereas both oligodendrocytes and type-2 astrocytes develop from a common, A2B5-positive precursor, which we therefore initially called an O-2A progenitor cell (Raff, Abney, and Miller, 1984). Largely from the work of Bob Skoff at Wayne State University (Skoff, 1990) and our collaboration with a close UCL colleague Barbara Fulton (Fulton, Burne, and Raff, 1991), it gradually became clear, however, that type-2 astrocytes are probably not present in the normal optic nerve (or possibly elsewhere in the CNS), and so we began to refer to the oligodendrocyte precursors as OPCs. Rochelle Small, a post doc from Yale, collaborated with Mark Noble and his colleagues to show that OPCs migrate into the developing rat optic nerve some time before birth (Small, Riddle, and Noble, 1987), suggesting that the neuroepithelial cells that form the optic stalk give rise only to type-1 astrocytes, whereas OPCs are immigrants from the brain.

Laura Lillien, a post doc from the University of Wisconsin in Madison, and Simon Hughes, a post doc from Cambridge University, identified signals (other than FCS) that could induce OPCs to become type-2 astrocytes in culture. Simon found that extracts of developing optic nerve could induce OPCs transiently to express GFAP (Hughes and Raff, 1987) and, together with Laura and Michael Sendtner in Munich, showed that the active protein was ciliary neurotrophic factor (CNTF) (Hughes et al., 1988; Lillien et al., 1988). Laura went on to demonstrate that CNTF plus extracellular-matrix-associated molecules made by optic nerve cells induce OPCs to become stable type-2 astrocytes in culture (Lillien, Sendtner, and Raff, 1990). Some years later, John Kessler and colleagues showed that bone morphogenic proteins (BMPs) on their own induce cultured OPCs to become stable type-2 astrocytes (Mabie et al., 1997). BMPs are produced in the developing CNS, so why do type-2 astrocytes apparently not develop there? Toru Kondo, a post doc from Japan, later showed that optic nerve glial cells, including OPCs themselves, produce BMP antagonists such as Noggin, which greatly decrease the sensitivity of OPCs to the type-2-astrocyte-inducing activity of BMPs; he also showed that BMPs in FCS are responsible for inducing OPCs to become type-2 astrocytes (Kondo and Raff, 2004a).

An Intrinsic Timer in OPCs

Remarkably little is known about how the timing of developmental processes is controlled. Erika Abney, a Mexican immunologist and a friend from my immunology days, joined our group in the late 1970s, and it was

her initial findings that first aroused my interest in the developmental timing problem. Using GFAP to identify astrocytes, GC to identify oligodendrocytes, and the presence of beating cilia to identify the ependymal cells that line the ventricles in the CNS, she determined when the first cells of each type appear in the developing rat brain. Analyzing cell suspensions from embryonic day eleven (E11) through to birth at around E21, she found that the first astrocytes appear at E15–16, the first ependymal cells at E17–18, and the first oligodendrocytes at the time of birth. Amazingly, when she isolated cells from E10 brain and cultured them in 10% FCS, the times of first appearance of these three cell types were the same as if the cells had been left in the developing brain. Moreover, when cultures were prepared from E13 brain, all these cell types appeared 3 days earlier, just as *in vivo* (Abney, Bartlett, and Raff, 1981). Even today, I find these results remarkable, because the main morphogenic cues in the developing brain are missing in these cultures: there is no anterior and posterior, ventral and dorsal, medial and lateral, or inside and outside. The findings suggested that some timing mechanisms apparently operate normally in dissociated-cell cultures, and they made me think that we might be able to understand their molecular basis. I did not think, however, that we could study them in brain cell cultures, which, in principle, could contain hundreds of different cell types. We therefore returned to optic nerve cell cultures.

We focused on the timing of oligodendrocyte development. What is it that determines when an OPC stops dividing and differentiates into an oligodendrocyte? Like oligodendrocytes, most cell types in mammals develop from dividing precursor cells that stop dividing after a limited number of cell divisions and terminally differentiate. In no case do we know why the precursor cells stop and differentiate when they do.

As in the brain, we found that the first OPCs in the developing rat optic nerve stop dividing and differentiate into GC-positive oligodendrocytes around the day of birth. Moreover, as long as there are sufficient numbers of type-1 astrocytes present, the timing is remarkably similar in cultures of embryonic optic nerve cells (Raff, Abney, and Fok-Seang, 1985). Mark Noble, who had started his own laboratory at the Institute of Neurology in London, showed that type-1 astrocytes produce a mitogen for OPCs, which is required to prevent OPCs from prematurely differentiating (Noble and Murray, 1984). Both he and our UCL colleague Bill Richardson independently showed that the mitogen is platelet-derived growth factor (PDGF) (Richardson et al., 1988; Noble et al., 1988). Together, our three laboratories showed that PDGF can bypass the need for large numbers of type-1 astrocytes and allow normal timing of oligodendrocyte development in sparse embryonic optic nerve cell cultures (Raff et al., 1988).

Sally Temple, a Ph.D. student from Cambridge University, demonstrated that OPCs have a cell-intrinsic mechanism that helps determine when they stop dividing and differentiate into oligodendrocytes (Temple

and Raff, 1986). She placed an individual OPC onto a monolayer of type-1 astrocytes in a microwell and found that the cell proliferates and that the progeny cells stop dividing and differentiate at about the same time. She then showed that if the two daughter cells of an OPC are placed on astrocyte monolayers in separate microwells, they tend to divide the same number of times before they differentiate, establishing that an intrinsic counting or timing mechanism is built into each OPC. We spent much of the next 15 years trying to determine how the intrinsic mechanism operates. This seemed a worthwhile effort, because we suspected that similar mechanisms probably operate in many types of precursor cells.

An important advance in understanding the intrinsic mechanism came from Ben Barres (who was Barbara Barres at the time). He joined us as a post doc from Harvard Medical School, where, as a Ph.D. student, he did important work on the physiology of glial cells. He made two critical contributions to the analysis of the intrinsic counting or timing mechanism in OPCs. First, he developed a sequential immunopanning method to purify OPCs from neonatal rat optic nerves (Barres et al., 1992). He used Perry Bartlett's monoclonal anti-Ran-2 antibody to eliminate astrocytes, a monoclonal anti-GC antibody (made by Barbara Ranscht in Tübingen) to eliminate oligodendrocytes, and the A2B5 antibody to select positively for OPCs. His purification procedure revolutionized our ability to study these cells. Second, he showed that the intrinsic mechanism depends on thyroid hormone as well as on PDGF (Barres, Lazar, and Raff, 1994). Without PDGF, the cells prematurely stop dividing and differentiate. In the presence of PDGF but without thyroid hormone, most OPCs fail to stop dividing or differentiate; if thyroid hormone is added after the time when most OPCs would have differentiated had the hormone been present all along, the cells quickly stop dividing and differentiate. These findings suggested that the cells can count divisions or measure time in the absence of the hormone but that the hormone is required for the cells to withdraw from the cell cycle and differentiate when the intrinsic mechanism indicates it is time. Nathalie Billon, a post doc from France, and Yasu Tokomoto, a post doc from Japan, later collaborated with Björn Vennström at the Karolinska Institute to show that $\alpha 1$ thyroid hormone receptors mediate this effect of the hormone on OPCs (Billon et al., 2002). Yasu also showed that one or more members of the p53 family of proteins are required for thyroid hormone to trigger OPC differentiation (Tokumoto, Tang, and Raff, 2001), and Nathalie, in collaboration with Gerry Melino and his colleagues in Rome, provided evidence that p53 and p73 are the relevant family members (Billon et al., 2004); it remains to be discovered how these proteins function in OPC differentiation.

Fen-Biao Gao, a post doc from Duke University, used the Barres method to purify OPCs from embryonic rat optic nerve and showed that, in serum-free cultures containing PDGF and thyroid hormone, the purified

cells stop dividing and differentiate on the same schedule as they do *in vivo* (Gao, Apperly, and Raff, 1998). He also showed that young OPCs proliferate for longer than older ones, suggesting that the reason OPCs from the same age optic nerve go through a variable number of divisions before they differentiate is because they vary in their stage of maturation (Gao and Raff, 1997). Most importantly, he provided strong evidence that the intrinsic mechanism that helps control how long OPCs proliferate before they differentiate does not depend on counting cell divisions but instead measures time in some other way (Gao, Durand, and Raff, 1997). But what is the other way?

Ian Hart, a training neurologist doing a Ph.D., had earlier collaborated with Bill Richardson to show that the intrinsic mechanism probably does not depend on changes in either the number of PDGF receptors on the OPC cell surface or the signaling pathways that the receptors activate. He showed that OPCs that have stopped dividing and begun to differentiate still retain large numbers of PDGF receptors (Hart et al., 1989a), which can still be stimulated by PDGF both to increase intracellular Ca^{2+} (Hart et al., 1989b) and to activate the transcription of immediate-early genes in the nucleus of these differentiating cells (Hart, Richardson, and Raff, 1992).

The first clue about the molecular nature of the intrinsic timer came from Béa Durand, a post doc from Strasbourg. She showed that the amount of the cyclin-dependent protein kinase inhibitor $p27^{Kip1}$ progressively increases in the nucleus of purified OPCs as they proliferate in the presence of PDGF and the absence of thyroid hormone (Durand, Gao, and Raff, 1997). The amount of the protein reaches a plateau at the time when most of the cells would have stopped dividing if thyroid hormone had been present; without the hormone, the cells continue to proliferate, despite the high levels of $p27^{Kip1}$. Béa then collaborated with Jim Roberts in Seattle to show that, in cultures containing PDGF and thyroid hormone, mouse OPCs that are deficient in $p27^{Kip1}$ divide for a day or two longer than wild-type OPCs before they differentiate, suggesting that $p27^{Kip1}$ is one component of the timer (Durand et al., 1998). Jim Apperly, a Ph.D. student, showed that over-expression of $p27^{Kip1}$ accelerates the timer, providing further support for a role of $p27^{Kip1}$ in the timing process (Tokumoto et al., 2002). As most of the organs in $p27^{Kip1}$-deficient mice contain more cells than normal, it seems likely that $p27^{Kip1}$-dependent timers operate in many types of precursor cells. Yasu Tokumoto went on to show that *p27^{Kip1}* mRNA levels remain constant as the protein increases in proliferating OPCs, suggesting that the increase in the protein over time depends on post-transcriptional mechanisms that remain to be identified (Tokumoto et al., 2002).

Toru Kondo showed that the inhibitor of differentiation (Id) protein Id4 is also a component of the timer, although it works in the opposite way from $p27^{Kip1}$ (Kondo and Raff, 2000a). Id proteins inhibit basic helix-loop-helix

gene regulatory proteins that are required for differentiation in many types of precursor cells; in this way, they promote proliferation and inhibit differentiation of the precursors. Toru found that Id4 protein decreases as purified OPCs proliferate in the presence of PDGF and the absence of thyroid hormone and that, in this case, the mRNA and protein decrease in parallel, suggesting that a transcriptional mechanism is probably responsible for the progressive decrease in Id4 protein. Over-expression of Id4 prolongs proliferation and inhibits differentiation, consistent with the idea that the normal fall in Id4 helps determine when OPCs stop dividing and differentiate.

The OPC timer, like other intracellular timers, is still poorly understood. It is clear that it is complex and depends on the progressive increase of some intracellular proteins like $p27^{Kip1}$ and the progressive decrease of others like Id4. Both transcriptional and post-transcriptional controls have roles, but how these controls operate remains a mystery.

Adult OPCs

Charles ffrench-Constant, a medically trained Ph.D. student, found that there are small numbers of OPCs in cell suspensions prepared from adult rat optic nerves (ffrench-Constant and Raff, 1986). Like their neonatal counterparts, these adult OPCs are A2B5-positive and develop into type-2 astrocytes when cultured in 10% FCS and into oligodendrocytes when cultured in serum-free conditions, although they differentiate more slowly than do neonatal OPCs. Mark Noble and his colleagues independently found these cells and characterized them in more detail; most important, they showed by time-lapse recording that adult OPCs can develop from neonatal OPCs in culture (Wren, Wolswijk, and Noble, 1992).

We had great difficulty using immunocytochemistry to identify OPCs in the intact developing and adult optic nerve. Julia Burne, a technician turned Ph.D. student, in collaboration with Barbara Fulton, used a technique developed by Becky Pruss (after she had left London) to label cells, including OPCs, that have Ca^{2+} (and cobalt)-permeable glutamate-activated ion channels (Pruss et al., 1991). The method used the glutamate agonist quisqualate to stimulate such cells to take up cobalt, which could then be precipitated, enhanced with silver, and visualized by either light or electron microscopy. In this way, Julia and Barbara could specifically label OPCs in the intact rat optic nerve. They found that OPCs acquire progressively more complex cell processes as the nerve matures and that they constitute about 5% to 10% of the cells in the adult optic nerve (Fulton, Burne, and Raff, 1992). They also found that the processes of these cells end on nodes of Ranvier. Previously, Bob Miller and Barbara had injected horseradish peroxidase into individual cells in the adult optic nerve and found that some cells extend processes exclusively to nodes (Miller, Fulton,

and Raff, 1989); we originally thought that these cells were type-2 astrocytes, but it is now clear that they are OPCs, as was later confirmed by Arthur Butt and his colleagues at King's College London (Butt et al., 1999).

Adult OPCs are among the most interesting cells in the mammalian CNS. They are among the few cell types that Cajal missed, although they are 5% to 10% of the cells throughout the CNS (excepting the retina), as assessed by staining with antibodies against the NG-2 proteoglycan, which Joel Levine and Bill Stallcup at the Salk Institute originally showed recognize OPCs (Levine and Stallcup, 1987). In white matter, OPCs send their processes to nodes of Ranvier, and in both white and grey matter, they receive excitatory synapses, as convincingly shown by Dwight Bergles and his colleagues at Johns Hopkins Medical School (Lin and Bergles, 2002). They proliferate in response to injury and can produce new oligodendrocytes in response to myelin damage (Levine, Reynolds, and Fawcett, 2001). Given their abundance in both white and grey matter, their complex morphology, and the low rate of oligodendrocyte turnover in the normal CNS, it seems unlikely that the normal function of adult OPCs is simply to replace lost oligodendrocytes, but their other functions remain a mystery. There is a pressing need to study the consequences of eliminating these cells in the adult CNS.

An Intrinsic Maturation Program in OPCs

Why do OPCs become progressively more complex during development? Is it because their environment changes, because they intrinsically mature over time, or both? Fen-Biao Gao showed that perinatal OPCs have an intrinsic developmental program that changes many aspects of the cell over time (Gao and Raff, 1997). He first compared the properties of purified E18 OPCs with P10 OPCs (which are 10 days older), using time-lapse video recording of individual clones in culture. He found that, in cultures containing PDGF and thyroid hormone, the embryonic OPCs have a simpler morphology, divide and migrate faster, and divide more times before differentiating than do P7 OPCs. Remarkably, when he cultured purified E18 OPCs in serum-free medium in PDGF (without thyroid hormone, to prevent their differentiation) for 10 days, he found that the embryonic cells acquire the properties of the P10 cells, indicating that developing OPCs have an intrinsic maturation program that progressively changes many properties of the cell.

Dean Tang, a Chinese post doc with a Ph.D. from Wayne State University, showed that purified P7 OPCs can proliferate in culture for more than a year in PDGF without thyroid hormone. After many months in culture, the OPCs start to express GC even though they continue to proliferate and do not express other oligodendrocyte markers (Tang, Tokumoto, and

Raff, 2000). This is not a culture artefact, for Ben Barres and his colleagues at Stanford showed earlier that OPCs in the rat optic nerve start to express GC after many months *in vivo* (Shi, Marinovich, and Barres, 1998). It seems that the intrinsic OPC maturation program continues to change the cells for months, which is remarkable. Intracellular programs that change developing cells over time are among the most mysterious processes in development and deserve much more attention than they have received.

We were surprised to find that OPCs did not undergo replicative cell senescence and permanently stop dividing, even after more than a year of proliferation in culture. The cells remain diploid, and unlike genetically immortalized cells, they maintain p53-dependent and Rb-dependent cell-cycle checkpoint mechanisms (Tang, Tikumoto, and Raff, 2000). Dean could rapidly induce them to acquire a senescent phenotype by culturing them in FCS or by treating them with DNA-damaging drugs. At the same time, our neighbors Alison Lloyd and her colleagues had obtained very similar results with purified rat Schwann cells (Mathon et al., 2001). Together, these studies showed that, unlike human cells, some normal rodent cells can apparently proliferate indefinitely in culture: They continue to express telomerase and therefore do not undergo progressive telomere shortening and uncapping, which cause most normal human cells to stop dividing after a limited number of divisions, a process logically called replicative cell senescence. So-called replicative senescence in rodent cells, by contrast, results from "culture shock" caused by high concentrations of serum and/or oxygen or by other stresses, rather than from a telomere-dependent cell-division counting mechanism (Wright and Shay, 2000).

Conversion of OPCs to Neural Stem-Like Cells

The isolation of human embryonic stem (ES) cell lines and embryonic germ (EG) cell lines in 1998 triggered a cascade of hope, hype, and hysteria that continues to amplify to this day. A surprising observation by Toru Kondo dropped us into the middle of this stem cell storm. We had supposed that OPCs were committed to becoming oligodendrocytes (and possibly type-2 astrocytes), but Toru showed that this was not the case: They could be reprogrammed by extracellular signals to become multipotential cells that can produce both neurons and glia (Kondo and Raff, 2000b). He found that, if he treated purified OPCs with BMPs for 2 to 3 days and then with basic fibroblast growth factor (FGF-2), the OPCs convert to a phenotype that closely resembles CNS neural stem cells. They can proliferate indefinitely in FGF-2, and depending on the signal proteins in the culture medium, they are able to give rise to type-1 astrocytes and neurons, as well as to oligodendrocytes and type-2 astrocytes, just as CNS neural stem cells do.

Thus, OPCs are specified to become oligodendrocytes, but they are clearly not irreversibly committed to do so.

As mentioned earlier, the BMPs induce OPCs to become type-2 astrocytes, and Toru showed that this step is required for the conversion of purified OPCs to stem-like cells. He found that the BMP treatment rapidly induces the transcription of a variety of genes that are normally expressed in neural stem cells (Kondo and Raff, 2004b). He focused on the induction of the *sox-2* gene, which encodes a gene regulatory protein required for neural stem cell proliferation and normal CNS development. He identified an enhancer sequence in the *sox-2* promoter that is required for the gene to be expressed in neural stem cells. He showed that both the tumor-suppressor protein Brca-1 and the chromatin-remodeling protein Brahma are recruited to the enhancer when OPCs are treated with BMPs for 2 days and that both proteins are required for the induction of *sox-2*. He also showed that the histone H3 associated with this enhancer is progressively modified during the conversion process (Kondo and Raff, 2004b).

There is evidence that adult OPCs can also convert in culture to neural stem-like cells that can give rise to both glia and neurons (Palmer et al., 1999). Given their abundance, their wide distribution in the CNS, and their ability to proliferate in response to injury, the endogenous OPCs in the adult CNS should be ideally suited for repairing the damaged CNS. The more one can learn about how to control their behavior the better.

A Diversion into Cell Death

Interest in cell death reached almost hysterical levels in the 1990s, although it never quite reached the intensity of the current preoccupation with stem cells. We became involved in cell death by chance in the late 1980s. There was a controversy about how OPCs differentiate into oligodendrocytes. My colleagues and I had proposed that it occurs by default when OPCs stop dividing and that it therefore does not require instructive signals in the way that type-2 astrocyte differentiation does. Arthur McMorris at the Wistar Institute, however, proposed that oligodendrocyte differentiation is induced by IGF-1 (McMorris et al., 1993). To try to resolve this controversy, Ian Hart cultured single OPCs in microwells as Sally Temple had originally done, only he omitted FCS and all signal proteins from the culture medium. To his dismay, the cells always died; if, however, he added either IGF-1 or high enough concentrations of insulin to activate the IGF-1 receptor, the cells lived and quickly differentiated into oligodendrocytes. Ben Barres and I repeated these observations with purified OPCs, and the results were the same: No matter how densely packed the cells, without appropriate signal proteins, such as IGF-1, the cells rapidly died, and they did so with the typical features of apoptosis (Barres et al., 1992). We concluded that IGF-1 is a survival signal for OPCs rather than a differentiation signal.

Around this time, I attended a Dahlem Conference in Berlin on neurodegenerative disorders. It was during this meeting that I first paid attention to evidence that apoptosis is an active process: a form of cell suicide. This had been suggested 20 years earlier by Kerr, Wyllie, and Currie (1972), but their insight had little impact until genes devoted to apoptosis and its regulation were identified in *Caenorhabditis elegans* (reviewed in Horvitz, 2003). I started wondering why OPCs in culture should need extracellular signals to avoid killing themselves, especially as the culture medium contains all the nutrients cells require. It was known that some developing neurons require signal proteins (neurotrophic factors) secreted by their target cells to survive, and there was strong evidence that the neurons competed for limiting amounts of these proteins. This arrangement provides a powerful way to match the number of neurons to the number of target cells they innervate. It occurred to me that the same mechanism might operate for most animal cells, which might require continuous signaling from other cells to avoid suicide; this might be the simplest strategy to ensure that animal cells only survive when and where they are needed (Raff, 1992). We spent a number of years testing this idea, and we were unable to kill it.

All of the normal cell types we tested behaved in the predicted way: When cultured alone without extracellular signals, they died by apoptosis. Yasuki Ishizaki, a post doc from Japan, showed that even lens epithelial cells (Ishizaki et al., 1993) and chondrocytes (Ishizaki, Burne, and Raff, 1994), whose normal neighbors *in vivo* are all cells of the same type, require extracellular signals to avoid apoptosis in culture. For these cells, the signals are autocrine factors secreted by other lens epithelial cells or chondrocytes, respectively. Miguel Weil, a post doc from Israel, Mike Jacobson, a post doc from UCSF, Harriet Coles, a Ph.D. student from Oxford, and my daughter Kim, who spent time in the lab while at university, together showed that all of the nucleated cells in various explanted mouse organs can be induced to undergo apoptosis if they are treated with a high concentration of the protein kinase inhibitor staurosporine in the presence of the protein synthesis inhibitor cycloheximide (Weil et al., 1996). These findings suggested that all nucleated mammalian cells can undergo apoptosis and that they constitutively express all the proteins required to do so.

Ben Barres studied the role and regulation of apoptosis during oligodendrocyte development *in vivo*. Many years earlier, Sam David, a post doc from Montreal, obtained indirect evidence that axons may promote the survival of oligodendrocytes and/or their precursors in the developing optic nerve. He found that transection of the neonatal rat optic nerve results in a dramatic decrease in both oligodendrocytes and OPCs (David et al., 1984). Ben now showed that 50% or more of the oligodendrocytes produced in the normal developing rat optic nerve die by apoptosis, apparently in a competition for limiting amounts of survival signals provided by the axons

(Barres et al., 1992). He showed that transection of the rat optic nerve at the end of the first week of postnatal life causes most of the newly formed oligodendrocytes in the nerve to die by apoptosis, as expected if these cells require signals from axons to survive (Barres et al., 1993). Julia Burne then studied transgenic mice made by Jean-Claude Martinou and colleagues in Geneva that over-express the *bcl-2* gene in neurons, including retinal ganglion cells (RGCs). Because Bcl-2 suppresses apoptosis, it decreases the RGC death that occurs during normal retinal development and thereby increases the number of axons in the transgenic optic nerve. She showed that, as a consequence of the increase in axons, oligodendrocyte cell death in the developing transgenic optic nerve is greatly reduced, so that the number of oligodendrocytes is increased to match the increase in axon numbers (Burne, Staple, and Raff, 1996). (Julia later showed that the increase in axons is also associated with an increase in astrocyte numbers in the nerve, but in this case it is because the axons stimulate astrocyte proliferation rather than astrocyte survival during development, [Burne and Raff, 1997].) Some years later, Pierre-Alain Fernandez, a French post doc, showed that our old friend GGF is an important axon-associated survival signal for oligodendrocytes in the developing optic nerve (Fernandez et al., 2000).

Earlier, Ben had found that axons in the developing optic nerve also stimulate OPC proliferation or survival (Barres and Raff, 1993). Thus, his work indicated that axons increase oligodendrocyte numbers by promoting both oligodendrocyte survival and the proliferation or survival of their precursors. He showed that the proliferation or survival effect on OPCs depends on electrical activity in the axons (Barres and Raff, 1993), whereas the survival effect on oligodendrocytes does not (Barres et al., 1992).

When we first started working on cell death, very little was known about the nature of the intracellular apoptotic program. In addition to showing that the death machinery is ubiquitously and constitutively expressed, our contributions to understanding the nature of the program were largely to show what is not required. Mike Jacobson showed that it does not require the presence of a nucleus (Jacobson, Burne, and Raff, 1994), which was surprising at the time, given that nuclear condensation and fragmentation are central features of apoptosis. Others had reported that the Bcl-2 protein is located in the inner mitochondrial membrane, raising the possibility that both apoptosis and its inhibition by Bcl-2 might depend on oxidative phosphorylation. Mike excluded these possibilities by showing that cells without mitochondrial DNA, which are incapable of oxidative phosphorylation, can still undergo apoptosis and that Bcl-2 can still protect them (Jacobson et al., 1993). Similarly, it had been suggested that the apoptotic program depends on the generation of reactive oxygen species and that Bcl-2 inhibits apoptosis by blocking their generation. Mike largely excluded these possibilities by showing that both apoptosis and Bcl-2 protection occur in anaerobic conditions, where reactive oxygen species are

unlikely to be generated (Jacobson and Raff, 1995). The real breakthrough, however, came from Bob Horvitz and his colleagues, who showed that apoptosis depends on special proteolytic enzymes, now called caspases (Horvitz, 2003).

There are many cases in animal development where large-scale apoptosis occurs but its function is unknown. One example is the folding and fusion of epithelial sheets, as occurs in the formation of the neural tube. Miguel Weil used peptide caspase inhibitors in chick embryo cultures to show that neural tube closure depends on apoptosis (Weil, Jacobson, and Raff, 1997). He used the same approach to show that the loss of organelles during the terminal differentiation of keratinocytes depends on caspases (Weil, Raff, and Braga, 1999); Yasuki Ishizaki had shown earlier that the same is true for the loss of organelles during the terminal differentiation of lens cells (Ishizaki, Jacobson, and Raff, 1998).

Our last foray into cell death was to study the clearance of apoptotic cells by phagocytosis. Rahul Parnaik, a Ph.D. student from Cambridge University, collaborated with our UCL colleague John Scholes to show that the rate of clearance depends on whether macrophages or nonprofessional phagocytes such as fibroblasts or astrocytes do the clearing (Parnaik, Raff, and Scholes, 2000). They showed that, when a macrophage (or microglial cell) encounters apoptotic cells, it immediately engulfs them and digests them in less than an hour, whereas a nonprofessional phagocyte delays for hours before engulfing apoptotic cells and then digests them very slowly. Remarkably, if apoptotic cells are first aged *in vitro*, nonprofessional phagocytes ingest them immediately, suggesting that the ingestion depends on late-appearing properties of the apoptotic cell. This clever arrangement presumably enables professional phagocytes to do the clearing if they are present in adequate numbers; if they are scarce, however, nonprofessionals will ingest the apoptotic cells before the dead cells spill their intracellular contents. It is still unknown what these late changes in apoptotic cells are that are needed to signal to nonprofessional phagocytes.

The most interesting question, however, is not how apoptotic cells signal to phagocytes, but rather how live cells inhibit macrophages from eating them. A live cell is about the only thing that macrophages do not ingest, because they happily ingest plastic or glass beads, bits of wood, lipid droplets, and so on. Presumably, the putative "don't eat me" signals produced by healthy cells are lost when the cells undergo apoptosis, but such signals remain to be identified.

Axonal Degeneration

Much effort has been devoted to understanding the nature of the neuronal cell death that occurs in various neurodegenerative diseases. Does it occur

by apoptosis or by some other mechanism? It is likely, however, that neuronal death in these diseases occurs too late to be clinically important, because degeneration of the axon probably disconnects a diseased neuron from its target long before the neuron dies (Raff, Witmore, and Finn, 2002). Axonal "dying back," for example, occurs in many neurodegenerative diseases. In this process, there is a progressive degeneration of the axon over weeks or months, beginning distally and spreading toward the cell body. The selective degeneration of an axon, without the death of the parent neuron, can also occur during normal neuronal development and in response to local injury and to a variety of metabolic, toxic, and inflammatory disorders. Some forms of axonal degeneration, including Wallerian degeneration, seem to depend on an active and regulated program of self-destruction, rather than a passive "wasting away," and in this respect resemble apoptosis. John Finn, a post doc from Johns Hopkins Medical School, and Alan Whitmore, a post doc from the Institute of Ophthalmology in London, investigated how similar the mechanisms of apoptosis and axonal degeneration actually are.

They found that the mechanisms are molecularly distinct. In contrast to apoptosis, John found that in both Wallerian degeneration and distal axonal degeneration induced by the local withdrawal of neurotrophic factors caspases are not activated and caspase inhibitors do not block the degeneration (Finn et al., 2000). Alan collaborated with Craig Thompson and his colleagues at the University of Pennsylvania to show that Wallerian degeneration occurs normally in mice that lack both Bax and Bak, two crucial pro-apoptotic proteins of the Bcl-2 family required for most forms of apoptosis (Whitmore et al., 2003).

To explain why so many different types of chronic insults to neurons can result in the same axonal "dying back" response, we proposed that neurons insulted in these ways may activate a self-destruct program in their axons, beginning peripherally, to disconnect from their target cells to conserve resources (Raff, Whitmore, and Finn, 2002). We also suggested that a similar program may be used by developing neurons to eliminate unwanted axonal branches in the axonal pruning process that occurs normally in neural development. It is still not known whether either of these hypotheses is correct, but I suspect that neurodegenerative disease research would benefit if more of it shifted its emphasis from neuronal death to axonal degeneration.

Cell Diversification in the Retina

We mainly studied the sciatic and optic nerves because they are relatively simple neural systems. From time to time, however, we dipped our toes into the more complex terrain of the retina. Charles ffrench-Constant, for

example, showed that the rat retina does not contain OPCs or oligodendrocytes partly because there is a barrier where the optic nerve joins the eye that prevents OPCs from migrating from the nerve into the retina (ffrench-Constant et al., 1988). We principally used the retina, however, to address one of the big questions in developmental biology: How do cells diversify in developing animals? The question is especially challenging in the CNS, where there are probably more molecularly distinct cell types than in the other parts of the body put together. The retina is an especially attractive part of the CNS in which to approach the problem.

Takashi Watanabe, a neurosurgically trained post doc from Tokyo University, devoted himself to this project in the late 1980s. He first showed that the astrocytes in the rat retina migrate into the developing retina from the optic nerve head (Watanabe and Raff, 1988), as had been suggested by Jonathan Stone in Australia (Jonathan had earlier spent a sabbatical period with us). This finding fit well with lineage tracing experiments of others that had recently shown that multipotential retinal precursor cells can give rise to the photoreceptors, neurons, and Müller glial cells of the retina, but not to the astrocytes. These landmark lineage studies, performed independently by Turner and Cepko (1987), Holt and Harris and their colleagues (Holt et al., 1988), and Wetts and Fraser (1988), identified the central question in retinal cell diversification: How do the multipotential precursors decide what type of retinal cell to become?

Takashi began to address this question by studying the timing of rod development. Using anti-rhodopsin antibodies to unambiguously identify rods, he found that the first rods appear in small numbers in the rat retina at E20 and then increase rapidly for the next week or so. Although rhodopsin-positive rods failed to develop in dissociated-cell cultures of E15 retina, they did develop right on schedule if the E15 cells were centrifuged into a pellet that was then cultured on a floating polycarbonate filter. The pellet culture system allowed him to mix cells from different developmental ages. He labeled the DNA in proliferating E15 cells with bromodeoxyuridine (BrdU) and mixed the labeled cells with a 50-fold excess of unlabeled newborn retinal cells. The surprising finding was that the labeled E15 cells did not give rise to rods until the equivalent of E20, just as they did when cultured alone (Watanabe and Raff, 1990). Thus, the presence of the newborn cells, which were producing large numbers of rods from the start of the culture, did not affect the timing of rod development by the E15 cells. The result was unexpected because all three laboratories that had done the lineage tracing studies had concluded that retinal precursor cells remain uncommitted until around the last cell division, when extracellular signals dictate what the daughter cells become. (They came to this conclusion largely because they found two-cell clones of mixed cell type.) Takashi's results, however, suggested that retinal precursors change their intrinsic developmental potential as development progresses. On the other hand,

although the presence of neonatal cells did not alter the time at which the E15 cells first gave rise to rods, they did increase the proportion of rods that the E15 cells generated, apparently by producing a short-range, rod-promoting, diffusible signal (Watanabe and Raff, 1992). We concluded from these studies that a combination of cell-cell interactions and an intrinsic developmental program in the precursor cells that changes a cell's developmental potential over time contributes to retinal cell-type diversity, a view that is widely held today (Livesey and Cepko, 2001).

Abbie Jensen, a post doc from the University of Wisconsin in Madison, and Valerie Wallace, a post doc from the University of Toronto, worked together to show that the signal protein Sonic Hedgehog is made by RGCs and stimulates retinal precursor cells to proliferate (Jensen and Wallace, 1997). Valerie went on to show that RGC-axon-derived Sonic Hedgehog also stimulates the proliferation of astrocytes in the developing optic nerve (Wallace and Raff, 1999), while Abbie developed a clonal-density culture system in which she could follow the proliferation, differentiation, and death of individual retinal precursor cells. Using this system, Abbie showed that, even when cultured in a homogeneous and constant environment where the cells cannot contact cells outside their own clone, the precursor cells vary in their proliferative capacity, cell-cycle time, and the cell types that they generate (Jensen and Raff, 1997).

At around the same time, Costas Neophytou, a Greek Ph.D. student from Cambridge University, provided an answer to a question raised by Takashi's earlier experiments: Why do rhodopsin-positive rods develop in high-density, dissociated-cell retinal cultures only if FCS is not present? He showed that Müller cells in culture secrete leukemia inhibition factor (LIF), which acts to arrest rod development at a stage just before rhodopsin is made (Neophytou et al., 1997). By stimulating Müller cell proliferation, FCS indirectly arrests rod development. Interestingly, FCS, for unknown reasons, does not stimulate Müller cell proliferation in pellet cultures, which is why rhodopsin-positive rods develop in these cultures even in the presence of FCS. Costas's findings corrected earlier findings that were interpreted to show that CNTF and LIF respecify cells fated to become rods to become bipolar cells instead (Ezzeddine et al., 1997).

We continued to work on retinal development until just before I retired. Alan Whitmore and Michel Cayouette, a post doc from Quebec, worked together to show that some retinal precursors in the newborn rat retina divide asymmetrically (Cayouette et al., 2001). They found that most precursors in the retinal neuroepithelium divide horizontally, with their mitotic spindle oriented in parallel to the plane of the epithelium, but a minority divide vertically, with their spindle oriented at right angles to the epithelium. They also found that the mNumb protein, a mammalian homologue of the *Drosophila* cell-fate determinant Numb, is located at the

apical pole of the precursor cells, so that in vertical divisions only the apical daughter cell inherits mNumb.

Michel then used time-lapse video recording of GFP-labeled retinal precursors in explants of newborn rat retina to follow the fates of daughter cells produced by either vertical or horizontal divisions (Cayouette and Raff, 2003). He showed that the two daughters of horizontal divisions almost always become photoreceptors, whereas the two daughters of vertical divisions almost always become different—usually one becoming a rod and the other either an interneuron or a Müller cell. Thus, at this stage of retinal development, the plane of division along the apical-basal axis clearly influences cell-fate choice, and Michel provided indirect evidence that the asymmetrical segregation of mNumb in vertical divisions might be involved possibly by inhibiting Notch signaling in the apical daughter cell, as has been shown for Numb in *Drosophila.*

To study the relative importance of cell-intrinsic mechanisms and extracellular signals in cell-fate choice in the developing retina, Michel extended Abbie's observations by developing a serum-free and extract-free clonal-density culture system to follow the fate of individual clones of proliferating E16–17 rat retinal precursor cells. Remarkably, he found that the precursors behave similarly in these cultures and in explant cultures of E16–17 retina in 10% FCS, both in the number of times the precursors divide before differentiating and in the cell types the precursors generate (Cayouette, Barres, and Raff, 2003). These unexpected results challenged the prevailing view (mentioned earlier) of how cells diversify in the developing vertebrate retina. They suggest that positive inductive signals are unlikely to be important in determining cell-fate choices, at least from E16-17 onwards. We suspect that the precursors are variously programmed before this time and then step through their particular developmental program independently of instructive extracellular signals. Also surprisingly, Michel found that some retinal precursors in dissociated-cell culture rotate their mitotic spindle through 90 degrees just before dividing, consistent with his previous evidence that asymmetrical divisions are important for some cell-fate choices. If this model of preprogramming is correct, one needs to determine when and how retinal precursors become preprogrammed, how many preprograms there are, and how the programs operate.

Sally Temple, now at Albany Medical College, and her colleagues have provided compelling evidence for similar preprogramming of mouse cortical precursor cells (Qian et al., 2000). It remains one of the great challenges for developmental biologists to discover how intracellular programs alter the developmental potential of precursor cells over time. Encouragingly, Chris Doe at the University of Oregon is making rapid progress in analyzing the molecular mechanisms involved when *Drosophila* neuroblasts change their developmental potential as they undergo a stereotyped sequence of asymmetrical divisions (Grosskortenhaus et al., 2005).

Molecular Biology of the Cell

I have spent almost 30 years as a coauthor of the cell biology textbook *Molecular Biology of the Cell*. Jim Watson convinced me and the other authors to join the project by pointing out that whatever we do in science others will do either before or soon after, but writing this book could be a unique contribution. I think he was probably right, but he was dead wrong when he said that it would take us just two summers to write the book. Instead, it took six summers and two-to-three other meetings each year. It is now in its fourth edition, and we are currently writing the fifth. The book has consumed a large part of my life, but it has been great fun, and I learned an enormous amount from my coauthors and the many scientists who generously helped us over the years. I am not sure whether it helped my science, but it greatly broadened my interests in cell biology.

Summing Up

I have been inordinately lucky in my career. My choices to become a scientist, to start at the NIMR, and to work with Av all depended on chance rather than on careful thought. Unlike many scientists, I have received more credit than I deserved, especially during my first few years in science.

I have been especially lucky in the students, post docs, and research assistants I have had as colleagues. They have been a privilege and joy to work with, and their successes are a continuing source of pleasure and pride. Our group was relatively small, usually consisting of one or two Ph.D. students, four or five post docs, a research assistant (who doubled as lab mother), and frequently a senior scientist on sabbatical. In Table 1, I list the lab members over the years (I apologize if I have left anyone out).

Although we worked on diverse biological problems, the questions were quite consistent. How does a developing cell decide whether to survive or die, to grow or stop growing, to proliferate or stop proliferating, or to differentiate into one cell type rather than another? To what extent does the decision depend on intrinsic mechanisms, signals from other cells, or both? We tried to focus on subjects that were unfashionable at the time, such as intracellular developmental programs, developmental timing, cell survival control, animal and cell size control, and axonal degeneration.

I learned much from Av, my only mentor—too much to summarize here. One of the most useful lessons he taught me by his example was the importance and benefits of sharing—even with your competitors—ideas, reagents, and results of experiments. For a student or post doc, this is not intuitive behavior, because one's instinct is to be protective for fear of being scooped. The benefits of sharing therefore need to be taught. To my knowledge, we never suffered from sharing reagents or results long

Table 1. Past Members and Associates of the Raff Lab

Name	Dates	Came From	Present Location
POST DOCS			
Henry McFarland	1972–1973	Johns Hopkins Medical School	Johns Hopkins Medical School
Ed Thompson	1972–1974	National Institutes of Health	Institute of Neurology, London
Colin Stolkin	1973–1976	Institute of Psychiatry, London	King's College, London
Sue Martin	1973–1974	Harvard Medical School	USA
Richard Hughes	1974	University College Hospital, London	Guys Hospital
Rick Riopelle	1974–1976	Queens University, Ontario	Montreal Neurological Institute
Gillian Humphries	1976	Stanford University	USA
Sue Cotmore	1976–1978	Yale Medical School	Yale Medical School
Jeremy Brockes	1976–1978	Harvard Medical School	University College London
Mark Noble	1977–1981	Stanford University	Rochester Medical School
Perry Bartlett	1978–1979	Johns Hopkins Medical School	University of Queensland
Becky Pruss	1978–1980	UCLA	Trophos, Marseille
Durward Lawson	1978–1981	Aberdeen University	Scotland (retired)
Steve Helfand	1979	Albert Einstein Medical School	Brown University
John Roder	1979	Karolinska Institute	Lunenfeld Institute, Toronto
Steve Burden	1979–1980	Stanford University	Skirball Institute
Anne Mudge	1980–1981	Harvard Medical School	Laboratory for Molecular Cell Biology, University College London
Mike Klymkowsky	1980–1982	University of California, San Francisco	University of Colorado, Boulder
Justin Fallon	1980–1983	University Pennsylvania	Brown University
Kris Jessen	1980–1981	Anatomy, University College London	Anatomy, University College London
Mark Gurney	1981–1982	Cal. Tech.	de Code Genetics, Inc.
Andreas Faissner	1982	University of Heidelberg	Ruhr University
Sam David	1982–1983	McGill University	McGill University
Bob Miller	1982–1985	University College London	Case Western Reserve University
Deborah Niehoff	1983–1984	Johns Hopkins Medical School	USA
Rochelle Small	1984–1986	Yale Medical School	National Institute Dental Research Council

Table 1. (Continued)

Name	Dates	Came From	Present Location
Simon Hughes	1985–1987	Cambridge University	King's College, London
Laura Lillien	1985–1989	University of Wisconsin, Madison	University of Pittsburgh
Takashi Watanabe	1987–1990	Tokyo University	Kyorin University, Tokyo
Jim Voyvodic	1989–1992	Washington University, St. Louis	Duke University
Ben Barres	1990–1993	Harvard Medical School	Stanford University
Huseyin Mehmet	1990–1993	Imperial Cancer Research Fund, London	Imperial College, London
Mike Jacobson	1991–1997	University of California, San Francisco	Boston, MA
Yasuki Ishizaki	1991–1993	Tokyo University	Gumma University, Japan
Abbie Jensen	1992–1996	University of Wisconsin, Madison	University of Massachusetts
Sarah Ahlgren	1993–1996	University of California, San Francisco	Northwestern University
Valerie Wallace	1994–1997	University of Toronto	Ottawa Health Research Institute
Miguel Weil	1994–1998	Hebrew University	Tel-Aviv University
Béa Durand	1994–1997	Institute de Chimie Biology, Strasbourg	Pasteur Institute
Fen-Biao Gao	1995–1997	Duke University	Gladstone Institute, University of California, San Francisco
John Finn	1997–1999	Johns Hopkins University	U.S. Department of Homeland Security
Yasu Tokumoto	1997–2002	Tsokuba University	National Research Institute of Cell Engineering, Tokyo
Toru Kondo	1998–2001	Osaka Bioscience Institute	Riken Center for Developmental Biology, Kobe
Dean Tang	1998–2000	Wayne State University	M.D. Anderson Cancer Center
Pierre-Alain Fernandez	1998–1999	Hôpital Mont Perrin, Paris	Centre Hospitalier de la Polynesie Française, Tahiti
Rahul Parnaik	1998–2001	Cambridge University	University of Chicago
Nathalie Billon	1998–2002	Ecole Normale Supérieure de Lyon	University of Nice
Michel Cayouette	1999–2002	Laval University, Quebec	Montreal Clinical Research Institute
Alan Whitmore	1999–2002	Institute of Ophthalmology, London	Institute of Ophthalmology, London
Ian Conlon	2001–2002	Cambridge University	Department of Transport, London

Continued

Table 1. (Continued)

Name	Dates	Came From	Present Location
PH.D. STUDENTS			
Peter Stern	1972–1975	University College London	Paterson Institute, Manchester
Durward Lawson	1974–1977	Aberdeen University	Scotland (retired)
Linda Wendon*	1976–1979	Cambridge University	Northwestern Medical School
Tom Vulliamy*	1978–1981	Oxford University	Imperial College, London
Peter Kennedy	1978–1981	University College Hospitals, London	Glasgow Medical School
Janet Winter*	1979–1982	Liverpool University	University College London
Jack Price†	1980–1983	Open University, UK	Institute of Psychiatry, King's College, London
Brenda Williams	1981–1984	Warwick University	Institute of Psychiatry, King's College, London
Sally Temple	1982–1985	Cambridge University	Albany Medical School
Charles Jennings	1983–1986	Oxford University	Harvard Medical School
Ian Hart	1987–1990	Glasgow Medical School	Walton Centre, for Neurology, Liverpool
Harriet Coles	1990–1994	Oxford University	Nature Journals, London
Julia Burne	1990–1994	St. Thomas's Hospital, London	London
Rahul Parnaik	1993–97	Cambridge University	University of Chicago
Costas Neophytou	1994–1997	Cambridge University	Cyprus
Jim Apperly	1996–1999	Oxford University	London
Ian Conlon	1998–2001	Cambridge University	Department of Transport, London
*Supervised by Rhona Mirsky; †Supervised by Anne Mudge.			
ASSOCIATE SCIENTISTS			
(Independent scientists supported by our MRC Program Grant)			
Kay Fields	1972–1978	University of Geneva	National Institutes of Health
Joan Feldman	1973–1979	Medical Research Council National Institute for Medical Research, Mill Hill, London	Institute of Psychiatry, London
Erica Abney	1977–1982	University of Mexico	London
Jim Cohen	1978–1985	Open University, UK	King's College, London
Rhona Mirsky	1979–1981	Dartmouth Medical School	Anatomy, University College London

Table 1. (Continued)

Name	Dates	Came From	Present Location
Anne Mudge	1981–1995	Harvard Medical School	Laboratory for Molecular Cell Biology, University College London
Bob Miller	1985–1987	Case Western Reserve University	Case Western Reserve University
Steve Moss	1991–1993	Johns Hopkins Medical School	U. Pennsylvania
RESEARCH ASSISTANTS			
Mary Megson	1972–1976	USA	USA
Anne Hornsby-Smith	1976–1978	South Africa	South Africa
Jelena Gravilovic	1978–1981	University College London	University of East Anglia, UK
Julia Burne	1985–1998	St. Thomas's Hospital, London	London
Michele Binder	1999–2002	Melbourne University	Walter & Eliza Hall Institute, Melbourne
Christine Jolicoeur	1999–2002	Laval University, Quebec	Montreal Clinical Research Institute
SABBATICAL VISITORS			
David Samuel	1974–1975	Weizmann Institute	Israel (retired)
Murray Freedman	1974–1975	University of Toronto	Deceased
Sonoko Habu	1974–1976	Keio University	Tokyo University
Fred Frankel	1974–1975	University of Pennsylvania	University of Pennsylvania
Rhona Mirsky	1975–1976	Dartmouth Medical School	University College London
Harvey Herschman	1975–1976	UCLA	UCLA
Jerry Gross	1976	Harvard Medical School	Harvard Medical School
Terje Lømo	1977	University of Oslo	University of Oslo
Reg Kelly	1978	University of California, San Francisco	University of California, San Francisco
Bob Lisak	1978–1979	University of Pennsylvania	Wayne State Medical School
Peter Spencer	1979	Albert Einstein Medical School	Oregon Health & Science University
Guy McKhan	1980–1981	Johns Hopkins Medical School	John Hopkins Medical School
Darwin Berg	1980–1981	University of California, San Diego	University of California, San Diego
Genevieve Rougon	1981–1982	Immunology Center, Marseille	Institute of Developmental Biology, Marseille

Continued

Table 1. (Continued)

Name	Dates	Came From	Present Location
Richard Hynes	1982–1983	Massachusetts Institute of Technology	Massachusetts Institute of Technology
Jim Watson	1983	Cold Spring Harbor Laboratory	Cold Spring Harbor Laboratory
Jonathan Stone	1984	University of Sydney	University of Sydney
Herb Geller	1984–1985	Rutgers Medical School	National Institutes of Health
Monique Dubois-Dalcq	1985	National Institutes of Health	Pasteur Institute
Robert Janzer	1985–1986	University of Zurich	University of Lausanne
Emily Friedman	1987	Columbia Medical School	Kaiser Permanente, Oklahoma
Isabelle Suárez	1988	University de Alcala, Madrid	University de Alcala, Madrid
Claas Hildebrand	1989	University of Linkoping, Sweden	University of Linkoping
Bruce Kruger	1991–1992	University of Maryland	University of Maryland
Ron Vale	2001	University of California, San Francisco	University of California, San Francisco

before they were ready for publication. Instead, we benefited greatly from the feedback we received and from the many reagents we obtained from other scientists.

I also learned from Av the value of reporting back on the scientific meetings I attended. I attended many meetings and felt guilty being away so much. The most compelling justification was that I sometimes learned things at a meeting that saved us months or years of work. Thus, whenever I turned down an invitation to a meeting, I had nightmares that I would miss something of overwhelming importance to us. Reporting back on meetings diminished my feelings of guilt, because it helped the students and post docs keep up to date. It had the added advantage that I had to take detailed notes at meetings and review them before reporting back, which helped me digest and remember what was presented.

During my immunology years, Niels Jerne had an important influence on me. He later won a Nobel Prize for his conceptual contributions to immunology. When I first met him at the Basel Institute for Immunology, which he directed, he told me that young scientists (like me) run so quickly that they see only the large objects, whereas older scientists (like him) walk slowly behind, paying attention to small

objects that have much to tell us. I was not sure exactly what he meant, but I tried to slow down anyway. He also advised me to discourage my children from becoming scientists, because it would put them under excessive pressure. This seemed sensible advice, and I did not encourage my children to become scientists. I did not entirely succeed, however. My oldest son, Jordan, studied biochemistry at Bristol University and is a successful *Drosophila* cell biologist at the Gurdon Institute at Cambridge University. It used to be that scientists asked Jordan if he was my son; now they ask me if I am his father. His younger brother, Adam, studied computer science at Edinburgh University and is setting out in business with his wife, having invented powerful new ways to search the Web. Daughter Kim, the youngest, studied biology at Cambridge University and became an intellectual property lawyer before becoming a mother. They are all happily married and thriving. They have produced five adorable grandchildren and, luckily for me, live close by.

I have also been extremely fortunate to have the MRC support my salary and research for 31 years. We rarely did what we proposed to do in our 5-year grant applications. As far as I know, no one ever compared our original proposals with our progress reports, which meant that we had complete freedom to change directions at will, which we did frequently. Will the public and governments continue to support the kind of curiosity-driven science that we did? It would be unwise to assume so.

It would also be unwise to assume that our species has the will and collective intelligence to preserve life on Earth. After all, most people on the planet know little more about how the world works than our ancestors did thousands of years ago. How can we expect them to understand the problems that threaten the biosphere and to take appropriate action, especially if they think it is all in the hands of God?

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