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Intern and Assistant Resident in Medicine, Royal Victoria Hospital, Montreal (1963–65)
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MRC Developmental Neurobiology Programme, Biology Department, University College London (1971–2002)
Professor of Biology, Biology Department, University College London (1979–2002)
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European Molecular Biology Organization (1974)
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President, British Society of Cell Biology (1991–1995)
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British Academy of Medical Sciences (1998)
Foreign Honorary Member of the American Academy of Arts and Sciences (1999)
Dunham Lectures, Harvard University (2001)
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Foreign Associate of National Academy of Sciences, USA (2003)
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Martin Raff

I retired in September 2002 after thirty-four years as a scientist. I was almost sixty-five, 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 ten 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 an 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 sixty-five, which immediately cheered me up.

I am now sixty-seven, 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, as I sit on many scientific advisory boards and am still a co-author 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 two years older, we shared birthdays. My childhood was generally happy and unremarkable, but I did have a number of peculiarities, which 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 eight, 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 twenties, 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, as 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 twelve 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 seventy per cent 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 twenty-six, 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 co-ed summer camp in northern Ontario called Wabi-Kon. I spent two months there every summer from age seven to twenty-one—first as a camper, then counsellor, then swimming instructor, and finally as a sailing instructor. The camp was on an island on beautiful Lake Temagami. There were no cars and no electricity in the cabins. It 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 maths. 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 not only unhelpful, they were actively destructive, as 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 four 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 four 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, which, 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 cardio-respiratory ward, which was an exciting and often terrifying experience. I had never seen anyone die before, and many of the patients I inherited 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 cardio-respiratory ward, where they frequently had more cardiac arrests and usually died. Moreover, the house officers on duty on the cardio-respiratory 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 sufficient number of babies during my rotation in obstetrics that I was well prepared when I had to deliver my own daughter, Kim, years later. She emerged unexpectedly fast at 4 a.m., 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, as the specialties that came up with the highest scores made my heart sink, until I juggled the numbers so that neurology came top, 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 off my beard before going for an interview, and was accepted.

Neurology at the MGH

I greatly enjoyed my three 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 doing neuropathology. There were no night calls, and we had ample time to read, think, and talk. At weekly "brain cutting" meetings run by EP, 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 EP 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, as there were no hints of the usual signs of aging, despite his seventy-six years.

During the neuropathology year, we each removed the brain (and, in some cases, the spinal cord, nerves, and muscle) from about fifty 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 six 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 [1]. 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-eighties, 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, D.C., 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 thirty-five, instead of twenty-six as it had been until then. The change would affect me, as I was in Boston as a landed immigrant with a green card. Checking to see if 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 US 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 US 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 fixed up.

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 was to change 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 that Mitchison wrote, which I did not understand and never actually did. I am greatly indebted to the National MS Society for funding a Canadian to work in the UK on a project with no obvious connection to 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 thirty years old. I was not planning to become a scientist; I was planning to spend two years doing experimental immunology before returning to the US 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 ways 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 [2]. 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 and lymphocytes from mice immunized with a hapten; when both lymphocyte populations were transferred into irradiated mice and immunized with the hapten coupled to the carrier protein, 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-primed cells were T cells and the hapten-primed cells, which produced the antibodies, were not [3].

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 two 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 [4, 5]. 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 labelled 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 [6], 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 [7], which began a prolonged and frustrating search by many laboratories for the antigen receptors on T cells, which were only identified as Ig-like proteins years later, after many false leads. 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 [8], 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 [9].

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" [7]. Göran Möller at the Karolinska Institute in Stockholm saw the same distribution almost ten years earlier [10]. 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 labelled 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 at one pole [11, 12]. 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, as 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 antbody-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 postdoc Philip Duffus had similar findings, and we published our fluorescence observations together [13]. 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 post-synaptic 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 eleven first-author papers in my first three years, five of them in *Nature*. (Interestingly, the one that became a Citation Classic [7] 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. Nonetheless, 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 five-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 thirty-one 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, as it allowed me to do full-time research in a university, with few teaching or administrative responsibilities. For the first twenty 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 and role model, as well as an inspiring 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. Since 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 and central nervous systems in a high proportion of rats by injecting either newborns or their pregnant mothers with the chemical carcinogen ethylnitrosourea (ENU) [14].

Kay Fields, an American phage molecular biologist who had just finished a postdoc in Geneva, took on the project of inducing the tumors, isolating cell lines from them, and raising antibodies against the lines [15]. 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 labelled the surface of Schwann cells in cultures of newborn rat sciatic nerve cells [16]. 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 five years to show that the immunological approach could work with cells of the nervous system.

These five 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 dePetris (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 [17].

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 postdoc 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 [18]; Jeremy and Kay showed that anti-Ran-1 and anti-Thy-1 antibodies labelled non-overlapping populations of cells in cultures of newborn rat sciatic nerve cells: whereas anti-Ran-1 antibodies labelled the Schwann cells, anti-Thy-1 antibodies labelled most of the non-Schwann cells [16]. 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 [19].

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 [20] or an extract of bovine pituitary glands [21] stimulated the cells to proliferate, apparently by distinct mechanisms. These findings enabled us to produce large numbers of cultured Schwann cells [19]. 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 [22]. 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 [23]. It was around this time that Rhona began a long-term collaboration with Kristjan Jessen, a postdoc 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 postdoc 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 [24]. 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 [25]. 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 [26].

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 [27]. We obtained anti-GFAP antibodies from Bignami and found that they labelled 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 [28]. 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)—labelled these cells and no others in optic nerve cell cultures [29].

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 postdoc 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* [30], which we showed later was expressed on type-1 but not type-2 astocytes in optic nerve cell cultures. Becky Pruss, a postdoc 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 [31]. Mike Klymkowsky, a postdoc 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 [32]. Becky also made a rabbit anti-GFAP antiserum that we used for the next thirty years to identify astrocytes.

We obtained a monoclonal antibody made by Marshal Niremberg and his colleagues at the NIH called A2B5, which we also used for thirty years to help identify and purify another cell type —the oligodendrocyte precursor cell—which became my main interest until I retired. A2B5 recognizes certain gangliosides and was initially thought to label neurons specifically [33]. We found, however, that it also labelled the surface of oligodendrocyte precursor cells and type-2 (but not type-1) astrocytes in optic nerve cultures [34]. The precursor cells and type-2 astrocytes could also be labelled 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 [35].

We therefore had increasing evidence that type-1 and type-2 astrocytes are molecularly, as well as 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 fifteen-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 [34]. I like to think that I would have eventually arrived at this crucially important conclusion myself, without Adam's insight, but who knows?

Together with two postdocs, 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 [36]. Largely from the work of Bob Skoff at Wayne State University [37] and our collaboration with a close UCL colleague Barbara Fulton [38], 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 postdoc from Yale, collaborated with Mark Noble and his colleagues to show that OPCs migrate into the developing rat optic nerve some time before birth [39], 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 postdoc from the University of Wisconsin in Madison, and Simon Hughes, a postdoc 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 [40] and, together with Laura and Michael Sendtner in Munich, showed that the active protein was ciliary neurotrophic factor (CNTF) [41, 42]. 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 [43]. 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 [44]. BMPs are produced in the developing CNS, so why do type-2 astrocytes apparently not develop there? Toru Kondo, a postdoc 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 [45].

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 NIMR 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, galactocerebroside (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* [46]. Even today, I find these results remarkable, as 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 [47]. 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 [48]. Both he and our UCL colleague Bill Richardson independently showed that the mitogen is PDGF [49, 50]. 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 [51].

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 [52]. 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 fifteen 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 postdoc 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 [53]. 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 [54]. 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 postdoc from France, and Yasu Tokomoto, a postdoc from Japan, later collaborated with Björn Vennström at the Karolinska Institute to show that α1 thyroid hormone receptors mediate this effect of the hormone on OPCs [55]. Yasu also showed that one or more members of the p53 family of proteins are required for thyroid hormone to trigger OPC differentiation [56], and Nathalie, in collaboration with Gerry Melino and his colleagues in Rome, provided evidence that p53 and p73 are the relevant family members [57]; it remains to be discovered how these proteins function in OPC differentiation.

Fen-Biao Gao, a postdoc 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* [58]. 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 [59]. 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 [60]. 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 [61], which can still be stimulated by PDGF both to increase intracellular Ca^{2+} [62] and to activate the transcription of immediate-early genes in the nucleus of these differentiating cells [63].

The first clue about the molecular nature of the intrinsic timer came from Béa Durand, a postdoc 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 [64]. 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 [65]. 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 [66]. 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 [66].

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 $p27^{Kip1}$ [67]. 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 [68]. 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 [69].

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 [70]. 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 five to ten per cent of the cells in the adult optic nerve [71]. 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 [72]; 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 [73].

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 5–10% 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 [74]. 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 [75]. They proliferate in response to injury and can produce new oligodendrocytes in response to myelin damage [76]. 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 oligodendroctytes, 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 [59]. He first compared the properties of purified E18 OPCs with P10 OPCs (which are ten 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 ten 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 postdoc 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 galactocerebroside (GC) even though they continue to proliferate and do not express other oligodendrocyte markers [77]. 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* [78]. 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 [79]. 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, Alison Lloyd and her colleagues had obtained very similar results with purified rat Schwann cells [80]. 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 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 [81].

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 madness. 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 [82]. He found that, if he treated purified OPCs with BMPs for two to three 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. 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 induces the transcription of a variety of genes that are normally expressed in neural stem cells [83]. 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 [83].

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 [84]. 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 we 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 [85]. 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 [53]. 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 twenty years earlier by Kerr, Wyllie, and Currie [86], but their insight had little impact until genes devoted to apoptosis and its regulation were identified in *C. elegans* [reviewed in 87]. 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, if not all, 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 [88]. 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 postdoc from Japan, showed that even lens epithelial cells [89] and chondrocytes [90], 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 chondocytes, respectively. Miguel Weil, a postdoc from Israel, Mike Jacobson, a postdoc 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 [91]. 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 postdoc 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 [92]. Ben now showed that fifty per cent 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 [53]. 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 [93]. Julia Burne then studied transgenic mice made by Jean-Claude Martinou 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 [94]. (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 [95].) Some years later, Pierre-Alain Fernandez, a French postdoc, showed that our old friend GGF is an important axon-associated survival signal for oligodendrocytes in the developing optic nerve [96].

Earlier, Ben had found that axons in the developing optic nerve also stimulate OPC proliferation or survival [97]. 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 [97], whereas the survival effect on oligodendrocytes does not [53].

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 [98], 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 [99]. 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 [100]. The real breakthrough, however, came from Bob Horvitz and his colleagues, who showed that apoptosis depends on special proteolytic enzymes, now called caspases [87].

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 [101]. He used the same approach to show that the loss of organelles during the terminal differentiation of keratinocytes depends on caspases [102]; Yasuki Ishizaki had shown earlier that the same is true for the loss of organelles during the terminal differentiation of lens cells [103].

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 non-professional phagocytes such as fibroblasts or astrocytes do the clearing [104]. 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 non-professional phagocyte delays for hours before engulfing apoptotic cells and then digests them very slowly. Remarkably, if apoptotic cells are first aged *in vitro*, non-professional 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, non-professionals 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 non-professional 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, as 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 [105].

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 in response to local injury and to a variety of metabolic, toxic, and inflammatory disorders, as well as during normal neuronal development. 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 postdoc from Johns Hopkins Medical School, and Alan Whitmore, a postdoc 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 [106]. Alan collaborated with Craig Thompson and his colleagues at the University of Pennsylvania to show that Wallerian degeneration occurs normally in apoptosis-deficient mice that lack both Bax and Bak, two crucial pro-apoptotic proteins of the Bcl-2 family [107].

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, in order to disconnect from their target cells to conserve resources [105]. 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 if 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 [108]. 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 postdoc 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 [109], 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 [110], Holt and Harris and their colleagues [111], and Wetts and Fraser [112], 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 labelled the DNA in proliferating E15 cells with bromodeoxyuridine (BrdU) and mixed the labelled cells with a fifty-fold excess of unlabelled newborn retinal cells. The surprising finding was that the labelled E15 cells did not give rise to rods until the equivalent of E20, just as they did when cultured alone [113]. 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 [114]. 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 [115].

Abbie Jensen, a postdoc from the University of Wisconsin in Madison, and Valerie Wallace, a postdoc from the University of Toronto, worked together to show that the signal protein Sonic Hedgehog is made by retinal ganglion cells (RGCs) and stimulates retinal precursor cells to proliferate [116]. Valerie went on to show that RGC-axon-derived Sonic Hedgehog also stimulates the proliferation of astrocytes in the developing optic nerve [117], 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 [118].

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 [119]. 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 re-specify cells fated to become rods to become bipolar cells instead [120].

We continued to work on retinal development until just before I retired. Alan Whitmore and Michel Cayouette, a postdoc from Quebec, worked together to show that some retinal precursors in the newborn rat retina divide asymmetrically [121]. 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-labelled retinal precursors in explants of newborn rat retina to follow the fates of daughter cells produced by either vertical or horizontal divisions [122]. 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 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 [123]. These unexpected results challenge the current 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° just before dividing, consistent with his previous evidence that asymmetrical divisions are important for some cell-fate choices. If this model of pre-programming is correct, one needs to determine when and how retinal precursors become pre-programmed, how many pre-programs there are, and how the programs operate.

Sally Temple, now at Albany Medical College, and her colleagues have provided compelling evidence for similar pre-programming of mouse cortical precursor cells [124]. 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 [125].

Molecular Biology of the Cell

I have spent almost thirty years as a co-author 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 co-authors 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, postdocs, 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 postdocs, 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 postdoc, this is not intuitive behavior, as 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 before they were ready for publication. Instead, we benefited greatly from the

feedback we received, as well as 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, as it helped the students and postdocs 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, which have much to tell. 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, as 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 a powerful new way 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 thirty-one years. We rarely did what we proposed to do in our five-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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Table 1 (ammended Jan. 2018)

Name	Dates	Came From	Present Location
Postdocs			
Henry McFarland	72–73	Johns Hopkins Med.	Emeritus Scientist, NIH.
Ed Thompson	72–74	NIH	Retired
Colin Stolkin	73–76	Inst. Psychiatry, London	KCL, London
Sue Martin	73–74	Harvard Med.	USA
Richard Hughes	74	UCH, London	KCL, London
Rick Riopelle	74–76	Queens U., Ontario	Ontario Neurotrauma Foundation, Toronto
Gillian Humphries	76	Stanford U.	USA
Sue Cotmore	76–78	Yale Med.	Yale Laboratory of Medicine
Jeremy Brockes	76–78	Harvard Med.	UCL, London
Mark Noble	77–81	Stanford	U. Rochester Medical Center
Perry Bartlett	78–79	Johns Hopkins Med.	Queensland Brain Institute
Becky Pruss	78–80	UCLA	Windover Biomed, London
Durward Lawson	78–81	Aberdeen U.	Retired
Steve Helfand	79	Albert Einstein Med.	Brown University
John Roder	79	Karolinska Inst.	Deceased
Steve Burden	79–80	Stanford	Skirball Institute, NYU Med.
Anne Mudge	80–81	Harvard Med.	Retired

Mike Klymkowsky	80–82	UCSF	University Colorado, Boulder
Justin Fallon	80–83	U. Penn.	Brown University
Kristjan Jessen	80–81	Anatomy, UCL	UCL, London
Mark Gurney	81–82	Cal. Tech.	Tetra Discovery, Grand Rapids
Andreas Faissner	82	U. Heidelberg	Ruhr University, Bochum
Sam David	82–83	McGill U.	McGill University
Bob Miller	82–85	UCL	George Washington University Med, D.C.
Deborah Niehoff	83–84	Johns Hopkins Med.	USA
Rochelle Small	84–86	Yale Med.	Retired, USA
Simon Hughes	85–87	Cambridge U.	KCL, London
Laura Lillien	85–89	U. Wisconsin, Madison	University of Pittsburgh
Takashi Watanabe	87–90	Tokyo U.	Kyorin University, Tokyo
Jim Voyvodic	89–92	Washington U., St. Louis	Duke University
Ben Barres	90–93	Harvard Med.	Deceased
Huseyin Mehmet	90–93	ICRF, London	Atlas Venture, Cambridge, MA
Mike Jacobson	91–97	UCSF	Cambridge Biostrategy, MA
Yasuki Ishizaki	91–93	Tokyo U.	Gumma University, Japan
Abbie Jensen	92–96	U. Wisconsin, Madison	U. Massachusetts
Sarah Ahlgren	93–96	UCSF	Northwestern University
Valerie Wallace	94–97	U. Toronto	Toronto Western Research Institute

Miguel Weil	94–98	Hebrew U.	Tel-Aviv University.
Béa Durand	94–97	Inst. de Chimie Biol., Strasbourg	Institut Curie, Paris
Fen-Biao Gao	95–97	Duke U.	University Massachussets Med
John Finn	97–99	Johns Hopkins	US Dept. Homeland Security
Yasu Tokumoto	97–02	Tsokuba U.	Saitama Medical University, Tokyo
Toru Kondo	98–01	Osaka Biosci. Inst.	Hokaido University, Japan
Dean Tang	98–00	Wayne State U.	Roswell Park Cancer Institute
Pierre-Alain Fernandez	98–99	Hôpital Mont Perrin, Paris	Hopitaux du Leman, Thonon- les-bains
Rahul Parnaik	98–01	Cambridge U.	Physician, UK
Nathalie Billon	98–02	Ecole Normale Supérieure de Lyon	Institute of Biology of Valrose, Nice
Michel Cayouette	99–02	Laval U., Quebec	Montréal Clinical Research Institute.
Alan Whitmore	99–02	Inst. Ophthalmology, London	e-Therapeutics PLC, Bath
Ian Conlon	01–02	Cambridge U.	European Commission, Brussels
Ph.D. Students			
Peter Stern	72–75	UCL	Paterson Institute, Manchester
Durward Lawson	74–77	Aberdeen U.	Retired, Scotland
Linda Wendon*	76–79	Cambridge U.	Northwestern Med.

Tom Vulliamy*	78–81	Oxford U.	Queen Mary University, London
Peter Kennedy	78–81	UCH, London	Glasgow Med.
Janet Winter*	79–82	Liverpool U.	Retired
Jack Price**	80–83	Open U., UK	Institute Psychiatry, KCL, London
Brenda Williams	81–84	Warwick U.	Institute Psychiatry, KCL, London
Sally Temple	82–85	Cambridge U.	Neural Stem Cell Institute, Albany, NY
Charles Jennings	83–86	Oxford U.	McGovern Institute, Cambridge, MA
Ian Hart	87–90	Glasgow Med.	Deceased
Harriet Coles	90–94	Oxford U.	Retired from Nature Journals
Julia Burne	90–94	St. Thomas's Hospital, London	Retired, UK
Rahul Parnaik	93–97	Cambridge U.	Physician, UK
Costas Neophytou	94–97	Cambridge U.	Emergo Wealth Group, Cyprus
Jim Apperly	96–99	Oxford U.	Weaveworks, London
Ian Conlon	98–01	Cambridge U.	European Commission, Brussels
*Supervised by Rhona Mirsky; **Supervised by Anne Mudge			

Associate Scientists

(Independent scientists supported by our MRC Programme Grant)

Kay Fields	72–78	U. Geneva	Retired
Joan Feldman	73–79	MRC NIMR, Mill Hill, London	Psychiatrist, London

Erica Abney	77–82	U. Mexico	Homeopathic practitioner, London
Jim Cohen	78–85	Open U., UK	Retired, London
Rhona Mirsky	79–81	Dartmouth Med.	Emeritus, UCL
Anne Mudge	81–95	Harvard Med.	Retired
Bob Miller	85–87	Case Western Reserve U.	George Washington U. Med, D.C.
Steve Moss	91–93	Johns Hopkins Med.	Tufts University
Research Assistants			
Mary Megson	72–76	USA	USA
Anne Hornsby-Smith	76–78	South Africa	South Africa
Jelena Gravilovic	78–81	UCL	U. East Anglia, UK
Julia Burne	85–98	St. Thomas's Hospital, London	Retired, London
Michele Binder	99–02	Melbourne U.	Florey Institute, Australia
Christine Jolicoeur	99–02	Laval U., Quebec	Montréal Clinical Research Institute
Sabbatical Visitors			
David Samuel	74–75	Weizmann Inst.	Deceased
Murray Freedman	74–75	U. Toronto	Deceased
Sonoko Habu	74–76	Keio U.	Juntendo U., Japan
Fred Frankel	74–75	U. Penn.	University Penn.
Rhona Mirsky	75–76	Dartmouth Med.	Emeritus, UCL
Harvey Herschman	75–76	UCLA	UCLA

Jerry Gross	76	Harvard Med.	Deceased
Terje Lømo	77	U. Oslo	University of Oslo
Reg Kelly	78	UCSF	UCSF
Bob Lisak	78–79	U. Penn.	Wayne State Med.
Peter Spencer	79	Albert Einstein Med.	Oregon Health & Sci. U.
Guy McKhan	80–81	Johns Hopkins Med.	John Hopkins Med.
Darwin Berg	80–81	UCSD	UCSD
Genevieve Rougon	81–82	Immunology Center, Marseille	Inst. Dev. Biol., Marseille
Richard Hynes	82–83	M.I.T.	M.I.T.
Jim Watson	83	Cold Spring Harbor Lab.	Cold Spring Harbor Lab.
Jonathan Stone	84	U. Sydney	University of Sydney
Herb Geller	84–85	Rutgers Med.	NIH
Monique Dubois-Dalcq	85	NIH	NIH
Robert Janzer	85–86	U. Zurich	Deceased

Emily Friedman	87	Columbia Med.	Neurosurgeon, Oklahoma City
Isabelle Suárez	88	U. de Alcala, Madrid	Retired, Spain
Claas Hildebrand	89	U. Linkoping, Sweden	University of Linkoping
Bruce Kruger	91–92	U. Maryland	University of Maryland
Ron Vale	01	UCSF	UCSF