Introduction

Understanding the structure and the function of the genome has been one of the greatest and most important achievements in the history of science. An essential part of this Herculean (and ongoing) task has been understanding how the roughly 20,000 genes in the human genome are controlled. Making sure just the right amount of gene 'activity' happens.

This book is about that vital control process. There are several useful metaphors for the molecules that are the subject of this book. My favourite, and hence the title of this book, views them as nature's fine-tuning system. The conductors of the molecular orchestra. I've played a couple of musical instruments in my life, although never very well. My first was the trumpet. Our junior school had a sort of 'try-out' to find who could make a sound on it and a few of us managed a squeak and so were picked to learn it. I kept at it for another few years, getting through early grading exams. But what was really fun was being part of an orchestra. In my case, a wind band. The essence of a wind band, or indeed any orchestra, is the variety of instruments and how and when they play. This gives rise to the wondrous range of sounds that underlie a piece of music. While everyone has their own sheets of music instructions, for a piece to work properly we need a conductor. Someone at the helm, making small adjustments to the emphasis of a particular group of instruments, reminding the horn section to quieten down or the flutes to play louder. They also adjust the pace, ensuring that the timing is spot-on. Conductors are essential because they fine-tune to perfection what would otherwise be a-bit-hard-to-listen-to *noise*.

Something akin to the conductor of the orchestra is going on inside every one of the cells in your body every moment you are alive. We have genetic programmes, think music on the sheet, and we have a variety of instruments, genes and their products, that work together to do all the things that a cell needs to do to function. But this system needs a conductor. Life found a need for this oversight a long time ago. Some of the simplest organisms on Earth, comprising little more than a handful of cells, have a basic version of a conductor system. The molecules at the centre of this system and my own research are called **microRNA**. They were unknown to science until 30 years ago. But they had been there all along, tinkering away to make sure that just the right amounts of proteins are made in our cells. This book is about these conductors of the molecular orchestra.

My Road to MicroRNA

Most of us are aware of DNA as our hereditary material, the instructions for making you, me and every living thing and every thing that ever lived. An impossibly simple but sufficient set of four chemical 'letters' given the abbreviations A, C, G and T that, by arranging in different

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orders, are the instructions for how to make each protein in our cells. Errors in DNA, even a single misplaced A, C, G or T in the sequence, scramble the instructions and can cause devastating diseases.

My research concerns the chemical cousin of DNA, called ribonucleic acid (RNA). This RNA also functions as a code, a series of chemical letters that serve as instructions. But RNA is so much more. It is mobile, moving around the insides of cells. Signals from outside and inside a cell continuously adjust the amounts and types of RNA being made. It is quickly generated and just as quickly dismantled and capable of forming complex three-dimensional shapes that work as nano-machines. Many RNA sequences undergo chemical changes, additions, removals and editing, expanding our genome's information repertoire. It was messenger or 'mRNA' inside some of the vaccines that taught our immune systems how to fight back, saving millions of lives during the Covid-19 pandemic. But mRNA is just the tip of the RNA iceberg. Our genomes include instructions for making many different types of RNA that do not code for proteins. Those are called *non-coding RNAs*. Some of these have been known for decades. One type forms the large structures inside which proteins are made, while another feeds the amino acids, the building blocks of proteins, into that machine. The function of much of the remaining types of RNA was uncertain until relatively recently. Often, these were dismissed as molecular debris, leftover bits and pieces of longer RNAs. The rest was the genome's 'dark matter', an unsettlingly large portion of the genome whose role was unknown. One of these mysterious RNAs is microRNA, the subject of this book. After the discovery of microRNAs 30 years ago, scientists have been busy learning what these short RNAs are doing inside cells. They discovered that microRNAs are our genome's master controllers, making sure that the right amount of protein is made at the right time and in the right place for each cell in the body. If you could listen to the journey from gene to protein, I'd hazard a guess that it would begin with a cacophony of noise. Vast assemblies of enzymes joining together, jostling for space on DNA, generating copy upon copy of mRNA. These are spewed out to be read and translated into proteins. But this is skewed towards overproduction, the molecular equivalent of everyone playing their instruments as loudly as possible. The molecular noise needs to quieten down. This is what microRNAs do. They reduce, they sharpen and they shape the protein landscape, until the sound from the whole molecular orchestra is perfect.

I was midway through my undergraduate degree in pharmacology at the University of Bristol in England when microRNAs were discovered. Pharmacology is the study of drugs. My interests in the brain and drug discovery led me later to the University of Edinburgh in Scotland, where as a PhD student I tried to find ways to protect the brain against the effects of a stroke. My introduction to the RNA world came as a postdoctoral researcher, the first job many scientists take after finishing a PhD. I arrived in a snowy Pittsburgh, USA, at the start of December in 1997. The team I joined was looking for genes that controlled cell death after stroke. The person who hired me – a neurologist called Roger Simon – suggested I look at whether the same pathways were activated in the brain when a seizure occurred. There was evidence that prolonged or repeated seizures could harm the brain, so here was another brain disease where protective drugs might be useful. I knew very little about epilepsy, but my PhD training had taught me how to make models of what happens to the brain after an injury and I knew a few experimental methods for detecting damage to brain cells. It was these two skills that I had to offer. Developmental biology and cancer researchers had taught us that cell death is often controlled by gene programmes. We thought that if we could figure out the programme, we might find a way to interrupt the process and keep brain cells alive

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longer. I set up a model of epilepsy and then looked at the genes that became active when a seizure occurred. My first experiment looked at a gene called *GADD45*. It was known to switch on when DNA was damaged and we thought this might happen after seizures. I remember seeing the beautiful images of where the gene was active. Intense, dark patches on an X-ray film corresponding to the gene's mRNA signal, appearing in brain cells just minutes after a seizure. Seizures were causing damage to the DNA inside brain cells and the genome was fighting back, switching on this gene. I still have the original X-ray film image somewhere in my office.

I was hooked on RNA and decided I would study the RNA signals made in brain cells in epilepsy. My efforts would be aided by the development of technologies that made it possible to measure the activity of every gene at once, a method called gene profiling. One of the experiments we tried, which would later lead me to microRNAs, was to see what genes turned on or off when a brief and relatively harmless insult was given to the brain. We were trying to mimic how hibernating animals survive extremely long periods of cold and slow circulation. When the brain is stressed in a certain way, it ramps up its defences to be ready for a bigger hit in the near future. Protective molecules get turned on and sit ready for action. Other processes are switched off to conserve resources. The brain that has been forewarned can survive a stroke or prolonged seizure much better than if an insult comes out of the blue and the brain is caught unawares. We thought we could develop drugs based on this effect to protect the brain. The teams I worked with had been looking in the usual places, exploring gene activity by measuring mRNAs, when someone suggested looking at a new type of RNA. That person was Julie Saugstad, a colleague of mine at the Robert S. Dow Neurobiology Laboratories in Portland, Oregon. This was around the turn of the millennium. MicroRNAs had just been discovered in the human genome. Using an early method to measure levels of microRNAs, she found that these new types of gene also switched on or off when the brain was exposed to low oxygen. The idea sat in the back of my mind as, in 2004, I relocated from the USA to Ireland. I was there to establish an epilepsy research lab at a medical school in Dublin called the Royal College of Surgeons in Ireland, now the RCSI University of Medicine and Health Sciences. I was joining a department headed by Jochen Prehn, a world expert on the control of cell death. This opened doors to new techniques, including imaging molecules and mathematical models of how brains cells react to injury. Within a few years I was joined by a remarkable talent, a postdoctoral researcher from Madrid, Spain, called Eva Jimenez-Mateos. She and other members of my lab would lead the team into the world of microRNAs, where we have remained ever since.

How the Book Works and a Few Disclaimers

This book is the story of microRNAs. Early chapters cover their origins, who discovered them and how, why they evolved and how they do what they do. In the middle chapters, I explain how microRNAs shape the gene programmes we use during development, how they affect the properties of the brain and what happens when microRNAs fail to do their job. Finally, I look at the applications of these discoveries, and the emergence of ways to drug them and track their course as they circulate through our bodies to diagnose diseases. Much of this has been possible through remarkable developments of technology, so I touch on that as well. Finally, what is next? What are the big questions that remain and what more can we expect from these incredible molecules?

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I have written this book to be understandable to a broad readership. You will not need a degree in biochemistry or genetics to make sense of it, I hope. I have aimed to tell it as a story of discovery, how it unfurled. There is some history and I have tried to respect chronology. For some readers, I may have been too light on detail. For others, the molecular 'soup' may sometimes exceed your level of understanding or interest. To some extent, this is a personal account and memoir. I have taken a rather neuro-centric view of the role of microRNAs. Large parts of it, particularly the second half of the book, concern the actions of microRNAs in the brain, where my own research has made some contributions. I have had to be selective, and have tended to pick the discoveries that interest me the most. Throughout the book I have included light descriptions of key experiments and often mention the year and the scientific journal the work appeared in. Many examples are in the most prestigious and glamorous journals in science – *Nature*, *Science* and *Cell*. Not all of the important findings on microRNAs appear in such journals, but I hold fairly traditional views about the relative quality and importance of published research and the references I make to work that appears in such journals is a reflection of that and the respect I have for the teams that managed these feats of scientific achievement. Publishing in such top journals comes down to novelty, how far they move a field ahead, the depth and sophistication of the experiments performed, and maybe a little bit of luck with peer reviewers. Often, but not always, the biggest breakthroughs appear in these types of journal. Watson and Crick's work on the DNA helix was reported in *Nature*. But a lot of great science gets published in specialist journals that are less scientifically glamorous. For instance, a technique we and many others use to calculate the amount of protein in a given sample was reported in the *Annals of Biochemistry* and has been cited by more than 240,000 scientific studies. Watson & Crick's paper on the structure of DNA in *Nature* has been cited a tiny fraction of that number of times. I won't go further into the debate on how best to measure scientific impact and value. The intention behind explaining some of the experiments is to give the reader a sense of *how we know what we know*, the microscopes, enzyme reactions, cells, organisms and models used. I include this not simply to provide context and history but in the hope that it captures more of the life of the bench scientist. I name-check some people. These are often the heads of the labs and their name usually appears last on a research paper. The *senior author*. The first author on a paper is often the person who did most of the actual bench work. The head of a lab may no longer perform experiments themselves, but it is they that probably had the original ideas behind the experiments, the hypothesis, and they who applied for and secured the funding and managed the project team. They are often the person who writes the paper. So, I apologise to everyone else named on the papers I mention; you can find those in the References. And I apologise to the many researchers and papers that I have not singled out for specific mention but whose work has nevertheless contributed in one way or another to this field of study. Now, let us begin. MicroRNAs are profoundly important to the workings of just about every living organism, including you. This is their story; let it be told.

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