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

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

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?

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.

Chapter 1

Discovered!
A New Regulator of Gene Activity

The beauty of nature lies in detail.

From Wonderful Life: The Burgess Shale and the Nature of History, *by Stephen Jay Gould (Penguin Books, 1989, p. 13)*

Humans – as is all life when you really think about it – are a wonder. Imagine briefly the range of activities that your body performs on a typical day. Your heart pumping blood. The muscles that allow you to stand, walk, run and propel food through your gut. Your liver and kidneys, helping digest food, detoxifying your blood. Your immune system, providing constant surveillance of your inner health, capable of fighting known and new pathogens, recognizing what is 'you' and what is not. The electrochemistry of your brain, where over 80 billion cells are performing unfathomable computations, processing information from outside – such as sound, light, taste, smell, touch – and regulating every system inside the body. Enabling you to plan and execute, remember and forget, learn from mistakes, be creative (play a musical instrument), have empathy, feel love and sometimes act altruistically.

Our bodies perform an impossibly vast array of tasks and they do it extremely well and for a tiny amount of energy consumed, about 2,000 kilocalories a day. That's under 100 watts an hour. An electric kettle uses 30 times this amount of energy. That's a lot of 'bang for your buck', as they say. Your body does all of this through the collective actions of its basic building blocks: *cells*. Humans are made up of an estimated 30 trillion of these minuscule membrane-enclosed sacs of living chemistry. Cells work individually as well as together, teaming up to form larger structures: tissues, organs and entire systems such as the cardiovascular and nervous systems. Cells are specialised to carry out the functions of the body.

The abilities of cells to do all of this emerge from their inner biochemistry. The molecules they contain, the structures and bio-machines they form and the functions those machines carry out. In the Introduction to this book, I used the analogy of an orchestra to frame the central ideas of how precise tuning of this biological chemistry is essential for life. The sheets of music are our genes, the information needed to play the music correctly. But that is not enough for a top performance. A conductor is needed, to make adjustments to the molecular equivalent of sounds, volume and tempo, so that it all works perfectly. In between the sheets of music and the collective sound of the orchestra we have the *instruments*. The instruments we are talking about inside our cells are *proteins* and the conductor is a special form of *nucleic acid*. An information-carrying chemical cousin of DNA called ribonucleic acid (RNA).

Proteins perform vast numbers of functions in our cells. We have proteins that can read DNA. Proteins that form gates to control the movement of raw materials into our cells and let waste out. We have proteins that prompt our cells to grow and develop special features,

turning simpler cell forms into sophisticated structures such as brain cells or any one of the hundreds of other cell types in our bodies. Proteins that form nano-machines to transport other proteins. Proteins that drive the motion of the hair-like projections called cilia on the surface of cells in our lungs that waft fluid and inhaled materials past the delicate air sacs. Other proteins form mechanical rod-like structures that give certain cells the ability to change shape, a motion that allows muscle cells to contract. We have proteins that carry out chemical reactions, converting glucose into the energy currency, called adenosine triphosphate (ATP), that all cells rely on. Sometimes the cell is more or less just protein. Red blood cells are packed with about 270 million copies of the haemoglobin protein, which can bind and release oxygen. Several of the proteins you will meet in this book are able to bind to RNA. These are called RNA binding proteins (RBPs). The human genome contains instructions to make about 3,000 different RBPs. They can bind to RNA of all different shapes and sizes. Some RBPs live out their lives in the nucleus close to DNA whereas others spend their days in the cytosol, the liquid outside the nucleus that makes up the remainder of the fluid in our cells. Some find their way to far reaches of the cell, making molecular journeys the equivalent of you travelling to the moon and back. Many RBPs work to keep RNA safe, protecting it from harm. And there are enzyme versions of RBPs that can cut, breaking the chemical bonds that hold together RNA. Molecular scissors that slice off and discard chunks of RNA akin to a sculptor chipping off pieces of marble to shape the statue beneath. Two of the RBPs that have this ability are fundamental to the lives of microRNA and we will meet them in Chapter 2.

All proteins are formed by the linking together of amino acids into long chains, assembled one at a time inside giant molecular machines called ribosomes. There are about twenty different amino acids that make up all the proteins in our cells. Amino acids are simple molecules made up of two main parts attached to a central carbon atom. One side has an amine group (one nitrogen and three hydrogen atoms; NH_3). The other end has a carboxyl group (one carbon, two oxygen and one hydrogen; COOH). Inside a cell, the two ends are charged, with the amine end carrying a positive charge and the carboxyl end a negative charge. This polarity provides them with the means to bond together in long chains, just as the positive end of one magnet is attracted to the negative end of another. Each amino acid differs in the nature of the atoms that form a branch off the central carbon atom called the *side chain*. Some carry a charge; others are neutral; others have highly reactive atoms that power chemical reactions. These differences are exploited by mixing together different combinations of amino acids to generate a protein that can do a particular job. Some amino acids are good at creating bulk and shape in a protein, while others sit at the centre of the reaction core where molecules are split apart or bonded together. The simplest amino acid is glycine. That contains a hydrogen (H) as the side chain. The amino acid cysteine contains a sulphur atom in its side chain and when paired with another cysteine can form a chemical bond called a di-sulphide bridge. This helps proteins fold and maintain their correct shape. Some amino acids, including alanine and leucine, are especially good at forming twists in proteins called α-helices.

The instructions for the order in which the amino acids are placed are carried by a molecule called *messenger RNA* (mRNA). This became a household name during the Covid-19 pandemic because some of the vaccines used the mRNA that codes for the spike protein on the virus to teach the immune system what to look out for. The instructions for the mRNA sequences to assemble your proteins are encoded in your DNA. These instructions for making you were inherited from your parents. Deoxyribonucleic acid (DNA) is

a mega-molecule. Technically it is a polymer, a name given to molecules that are made up of fixed, repeating sub-units. A *gene* is commonly used to refer to a discrete section of DNA that has the instructions for making a protein. But there are many sites in the genome that this does not apply to. Indeed, less than 2 per cent of the human genome contains information to make proteins. The HUGO [Human Genome Organisation] Gene Nomenclature Committee (HGNC), which globally agrees a set of rules, defines a gene as 'a DNA segment that contributes to phenotype/function'. It is becoming increasingly clear that far more of our genome than we once thought meets this criterion. Most microRNAs are genes under the HUGO nomenclature, that is, distinct units that can be transcribed, that are book-ended by start and stop signals, that are heritable and that serve specific biological functions.

It is at a key step along the pathway from DNA to protein that microRNAs act. Indeed, while this book is about microRNAs, it is ultimately a book about proteins because the main job of microRNAs is to make sure that our cells have just the right amount of each protein. Is it important to have just the right amount of a protein in a cell? Yes. While most systems in the body can tolerate a degree of variation in the proteins that carry out specific functions, we know that without microRNAs, a key regulator of protein levels, you would not be alive to read this. If you're missing microRNAs at the start of life then you never get much further than being a ball of cells. If you remove microRNAs around the time of birth then you fail to develop much further. If you remove them when you reach adulthood, you can develop cancer or accelerated ageing. Stop them working in the brain and you develop seizures before the brain turns to mush. I am taking some liberty with the word 'you' here. This knowledge comes mainly from experiments in lab animals such as mice. But we are confident that the outcome would be more or less the same in humans. Indeed, people are born with errors in the machinery for making these molecules and this can have devastating impacts on their health. Some of the microRNA genes are so important that we never see people born without them because it is lethal at an early stage. So, this system for controlling protein levels in cells is essential for life. **Figure 1.1** provides a simple overview of the 'gene pathway'. Information flows from DNA to RNA and on to making a protein. I have highlighted the approximate position in this process where microRNAs act.

The Genetic Code

The human genome is a code running to three billion letters. Despite the extraordinary information it contains, including the 20,000 or more genes that code for proteins plus many other interesting parts that code for RNAs that do important things in our cells, it has oddly straightforward chemistry. The code is made from repeats of four simple chemicals called bases: adenine, cytosine, guanine and thymine or A, C, G and T for short. Like amino acids, they are simple molecules and about the size of one of the larger amino acids. The order of the DNA bases in a protein-coding gene determine which amino acid gets picked. Three bases (a trinucleotide) form the unit of information that codes for a particular amino acid. This is known as a *codon*. Because it is a triplet code, we have 4^3 possible combinations, giving us 64 different codons. Of these, 61 code for amino acids and 3 perform another function, signalling to terminate the making of the protein. Most amino acids can be coded for by more than one codon. For example, a glycine is placed in a protein if the mRNA sequence read contains the codons GGC, GGA or GGG.

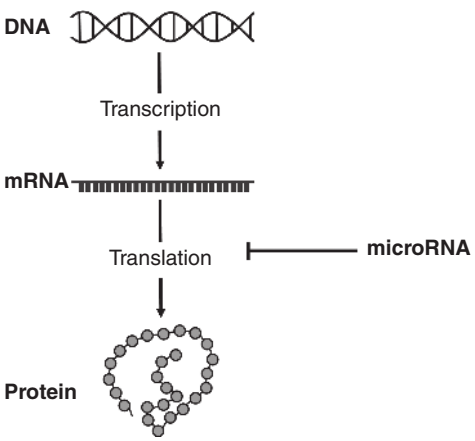

Figure 1.1 Overview of where microRNAs act on the pathway from gene to protein

In this simplified pathway from gene to protein, the first step is transcription, where an RNA copy is made from a gene encoded in the DNA. For protein-coding genes, the RNA formed is called mRNA. The nucleotide sequence of the mRNA (indicated by notches) is used as a template to generate a protein during translation. Proteins are formed by the sequential assembly of amino acids (round circles in the diagram). MicroRNAs act after the mRNA is formed but prior to the formation of the protein.

The structure of DNA slightly resembles a ladder or a spiral staircase, comprising two strands with the bases pairing to one another at regularly spaced intervals up the middle. The backbone (in the ladder analogy, the sides of the ladder you hold as you step up and down) is made up of two types of molecule. The first is a form of carbohydrate, closely related to the sugar you might add to sweeten something. Sugars are made up of carbon, oxygen and hydrogen. The one in the backbone of our DNA has five carbons and so is a pentose sugar; it is called deoxyribose. The deoxy refers to it missing a hydroxyl (OH) group. The other part is a phosphate group, a phosphorous atom with four oxygen atoms attached to it (PO_4). The deoxyribose and the phosphate are strongly bonded together and alternate as deoxyribose-phosphate-deoxyribose-phosphate and so on. The base attaches to the deoxyribose. So each rung of the ladder has a deoxyribose, a phosphate and one of the four bases. This unit – the base, the sugar and the phosphate – is called a *nucleotide*. Bases pair in a specific way. In DNA, an A is always paired across from a T and a C is always paired across from a G; Gs don't bind to Ts or As, and As don't bind to Gs or Cs. A gene sequence is simply an extremely long series of these four bases in different orders, for example A-C-T-G-C-G-T-A and so on. **Figure 1.2** provides an overview of the chemistry of DNA.

In the cells of eukaryotes, organisms that include mushrooms, plants and animals, DNA is found in the nucleus, one of several organelles (another is mitochondria, which make most of our cells' ATP). The DNA in eukaryotic cells is found wrapped around the outside of proteins called histones. This provides a way to compact the genomic instructions and avoid DNA strands becoming tangled. The mix of DNA and histone proteins is called *chromatin*. This is a suitable place for a very short aside on the history of DNA. Key discoveries of what DNA is made of came from work in the early 1900s by Phoebus Levene, a Russian-American biochemist who identified the four DNA bases, the basic rules of their combinations and the presence of the ribose sugar. Most people are familiar with Watson and Crick. They are credited with solving the structure of DNA, which was

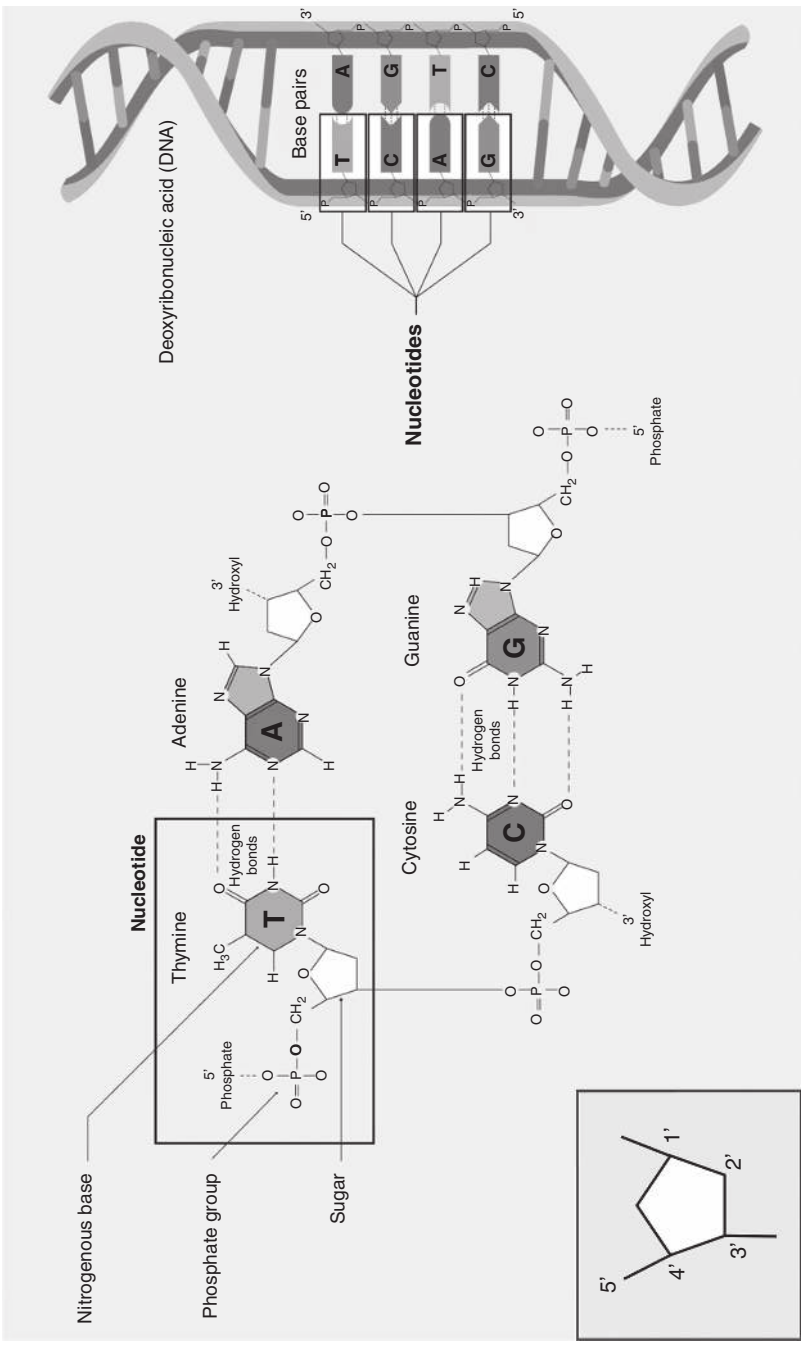

Figure 1.2 Chemical structure of DNA
This shows the basic chemical structure of the nucleotide, the basic building block of DNA, and the base-pairing rules. A nucleotide consists of a sugar molecule (deoxyribose in DNA) attached to a phosphate group and one of the four chemical bases. The bases in DNA are adenine (A), cytosine (C), guanine (G) and thymine (T). In RNA, the base uracil (U) takes the place of thymine. Molecules of DNA and RNA are polymers made up of long chains of nucleotides. The inset (bottom-left) shows the number-position labelling of carbons in the ribose sugar.
Source: Image courtesy of Darryl Leja and the National Human Genome Research Institute (www.genome.gov).

reported in the journal *Nature* in 1953, and this helped our understanding of how DNA worked (1953 is also famous for being the year that kainic acid was discovered, a key tool in neuroscience and epilepsy research – see Chapter 6). Key to confirming Watson and Crick's insights were X-ray images of a crystal of DNA. These were produced by Rosalind Franklin and shared without her knowledge. She was not initially credited with this work, but is now recognised as conducting the key experiments used in the discovery. Some accounts imply that Rosalind failed to grasp what her images revealed about the structure of DNA, but this is a misrepresentation. She was an equal contributor in the solution. She died from cancer owing to the radiation she was exposed to as part of her work; we owe much of modern scientific and medical discovery to this woman. Once the organisation of the DNA molecule was understood, with the sugar as the outside backbone and the bases pairing across from one another in the middle, how it coded could be understood. There was still a lot of work to do and Watson and Crick didn't determine what combination of bases coded to make proteins. That would come later. A couple of other key discoveries on the journey are worth a mention. Swiss scientist Friedrich Meischer gets credit for the 1869 discovery of DNA in the nucleus of cells. In 1944 we have Oswald Avery and colleagues, who proved that DNA is the hereditary material in our cells. They added DNA from a dangerous strain of bacteria to a normally harmless one, causing it to become deadly. In 1950, Erwin Chargaff reported that the amounts of Gs and Cs and the amounts of As and Ts were always the same, but the ratios were different in different species. Towards the end of that decade and in the first years of the 1960s, Marshal Nirenberg is credited with understanding how the code actually works in practice. That is, how sets of bases encode the amino acids in proteins. In the years since, the specific sequence of bases that code for each amino acid has been determined.

The Journey from Gene to Protein

Let us go briefly through the full process from start to finish: the start, the reading of the DNA code, through to the generation of mRNA that later gets read to form a growing amino acid chain that ultimately becomes a protein. Our depth of understanding of this process is now remarkable. I will cover enough detail to understand where and how microRNAs participate in this process. The first stage of the journey from DNA to protein is *transcription*. This is the process of reading a particular stretch of DNA, the gene, and making a copy of that sequence out of RNA. If we are making a protein, then the RNA made is an mRNA. It is common to refer to mRNAs as 'transcripts'. Other sections of DNA can also be read and an RNA copy made. But these other RNAs do not code for a protein and are *non-coding RNA*. There are many different types of non-coding RNA. Some, such as the RNA found inside the ribosome protein factories, are far more abundant in our cells than mRNAs. MicroRNAs are an example of such non-coding RNAs. **Figure 1.3** provides an overview of the basic chemistry of RNA and some of the different forms of RNA in cells.

Reading and copying DNA to make mRNA is a highly regulated, multi-step process. An enzyme called an RNA polymerase binds to a section of DNA. The two strands of DNA are unzipped from one another. The bonds holding the Cs to Gs and the As to Ts are broken and the base pairs are separated. The RNA polymerase now makes an RNA, with each base being inserted according to what it reads from the DNA strand. The strand of DNA that is read is called the *antisense* strand. The other ignored section of DNA is called the coding or *sense* strand because its sequence is the same as the mRNA that is generated. This reading process occurs in one direction only, analogous to how you read this sentence, from left to right.