MAN MADE LIFE

A Genetic Engineering Primer

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Preface

The instructions needed to construct and maintain a living organism are written in a four-letter code. The letters are grouped into words three letters long. Behind that absurdly simple account lies a great research effort by a diverse body of scientists, a century-long enterprise that came to fruition towards the end of the 1960s when the final words of the code were deciphered. In the few years since, molecular biologists have gone way beyond those achievements. They can now read the code with ease, manufacture new bits of code that will do things as instructed, and shuffle instructions between utterly different types of organism. They can engineer genes to suit almost any purpose. This book is about the tools the genetic engineers use and the tasks they tackle with them.

The tools and the knowledge to use them have emerged from basic research, and while they will undoubtedly serve the community at large in the future, they have already made extremely important contributions to our understanding of the processes of life. Much information has been uncovered that simply could not have been known just ten years ago. The most mind-boggling of these concerns a virus called ΦX174. It multiplies within bacteria, and carries its instructions as a string of 5386 letters. ΦX174 was the first organism to have its genetic message read in its entirety. When Frederick Sanger and his colleagues in Cambridge scrutinized that 'message', they were amazed to find overlapping instructions. The three-letter words that specify one protein contain, within them, an entirely distinct message for a different protein. By shifting the starting position, so that the same letters are grouped into words differently, the virus specifies two proteins in the space for one. I do not consider it hyperbole to call this superb piece of packaging a wonder of evolution, and without the techniques of genetic engineering it would not have been discovered.

The revelations of genetic engineering are awesome and fascinating, but one cannot expect them to affect everyone in the same way. The practical benefits are closer to hand. In February 1982 an issue of the journal *Science* contained some unremarkable looking photographs. One showed a collection of bacteria, the sort of picture that is now fairly commonplace. The bacteria seemed a bit lumpy, but otherwise quite ordinary (see page 166 below). They were, in fact, insulin factories. The lumps were packages of insulin, manufactured by the bacteria in response to instructions from a human insulin gene, deliberately placed in the bacteria by genetic engineers. The other photograph showed a collection of needle-shaped crystals (see page 179). They too looked quite ordinary, until one read that they were crystals of pure interferon. This wonder drug, scourge of viruses and possibly of cancer too, is so hard to obtain that its effects have not yet been properly evaluated. It has always been too impure and too scarce to crystallize. Now here were crystals of pure interferon, and like the insulin they too were made in bacteria according to instructions from a human. In this book you will find the details of how these, and other, marvels were achieved.

The ability to create living organisms to order – 'man made life' is no exaggeration – brings with it responsibilities. The furore that surrounded the birth of genetic engineering is testimony to the strong passions that this sort of power arouses. Many of us have a deep fear of genetic meddling, a fear that Mary Shelley, for example, capitalized upon in her novel *Frankenstein*. The public response to the scientists' new abilities reflected that fear and uncertainty, and the scientists' own concern was indicated in their decision to halt experimentation while they assessed the completely unknown risks. Now, almost ten years on, genetic engineering is seen as no more dangerous than other forms of microbiology. But the issues of safety and responsibility, and the effects that the commercial aspects of genetic engineering are having on the old academic freedoms, are still cause for concern. Those worries too are discussed in this book. With the knowledge we have today there can no longer be any excuse for the uninformed debate that characterized the early stages. Back in 1977 Alfred Velluci, mayor of Cambridge, Massachusetts, asked the president of the National Academy of Sciences to investigate reports of a hairy, nine-foot creature with orange eyes. Velluci felt

that the animal might be connected in some way to genetic engineering work; how, he didn't specify. That sort of thing should not happen now that we know so much more about molecular biology.

This book is about a science, the applications of that science, and the morality of those applications. It is not complete, nor is it definitive, but I hope it is informative. In the nature of things the writing is at times technical, although I have tried to ensure that the detail is understandable. Chapter 4 in particular may seem somewhat daunting, but it describes the basic processes that the genetic engineers use; persevere, and the rest will be that much clearer.

I must acknowledge the help and support given to me by several people. John Clark rekindled my enthusiasm. John Davey was a kind, thoughtful and patient editor. An unknown reader made several suggestions that improved the final product; as is customary, the errors that remain are mine alone. Colleagues at Oxford, notably Marian Dawkins, Alan Grafen and Mark Ridley, were kind enough to listen to me talking about genes when I should perhaps have been talking about behaviour. Roger Lewin is all one could want as a friend and mentor.

Acknowledgement must also be made to the following sources on which some of the figures have been based: figs. 1.2, 1.3, 4.3, 4.7 from Watson, J. D. and Tooze, J., *The DNA Story*, W. H. Freeman and Company, 1981, pp. 539, 565, 567; fig. 2.1 from Campbell, A. M., How viruses insert their DNA into the DNA of the host cell, copyright © 1976 by Scientific American, Inc., all rights reserved; fig. 1.4 from Kornberg, A., The synthesis of DNA, copyright © 1968 by Scientific American, Inc., all rights reserved; figs. 4.1, 4.2 from Cohen, S. N., The manipulation of genes, copyright © 1975 by Scientific American, Inc., all rights reserved; figs. 1.5, 5.1 from Grobstein, C., The recombinant-DNA debate, copyright © 1977 by Scientific American, Inc., all rights reserved; figs. 4.6, 4.8 from Gilbert, W. and Villa-Komaroff, L., Useful proteins from recombinant bacteria, copyright © 1980 by Scientific American Inc., all rights reserved.

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1 Beginnings

The early history and blossoming of molecular biology

It starts with a thin soup of bacteria cells, about a thousand million individuals suspended in a broth of nutrient chemicals and doubling in number every half hour or so. The soup is cloudy because the cells reflect light, and it is warm because the cells grow best at blood temperature. This culture is to be used as a source of DNA, the most vital of the many chemicals found in living cells.

The first step is to concentrate the cells a bit, remove them from the nourishing broth; the instrument to do this is a centrifuge. The test tube full of cloudy soup is whirled round at about 8000 revolutions per minute. The cells, normally kept suspended in the liquid by the random motion of the molecules around them, are forced down to the bottom of the test tube by centrifugal force, and after 30 minutes or so of this treatment the broth has separated into an absolutely clear liquid above a small pellet, dingy and greyish yellow. The pellet is made up of the compacted mass of cells, and most of the liquid can now be poured away. Now the pellet must be broken up again, the cells resuspended in a small amount of liquid, by vibrating the test tube with a mechanical shaker. Once again we have a cloudy broth, but now it is much thicker than before.

Each bacterial cell has a wall that keeps its insides inside and protected from the environment. To get at the DNA we have to destroy the cell walls and release the cytoplasm within. There are many ways to do this, but the method of choice in most laboratories involves two chemicals. The first is EDTA – ethylenediamine tetra-acetate – which removes magnesium ions (charged atoms) from the cell walls. Without the magnesium, the walls become very weak. The second chemical is SDS – sodium dodecyl sulphate. It is a detergent, and just as washing-up liquid dissolves grease, SDS dissolves the long fatty molecules that make up the cell wall. The combination of EDTA and SDS is an effective mixture, breaking

open the cell walls and releasing the chemicals inside. As the cells are dissolved they no longer reflect the light and the mixture becomes transparent once again. After half an hour at body temperature the test tube is clear.

It is also very viscous; where before the broth acted essentially like water, now it is more like egg white. The reason is that the DNA, formerly neatly packaged within the cells, has been released from its confines. It is a long thin molecule, and the swirling DNA strings intertwine to make the liquid sticky. Of course, there is a lot more inside the average bacterial cell than DNA, and the next step is to get rid of some of those other chemicals. The detergent has dealt with the fats; the major remaining impurity is protein, which accounts for about 15 per cent of the average bacterial cell. To get rid of it we add phenol – carbolic acid – though chloroform will do just as well. Carbolic acid found a use as one of the first antiseptics precisely because it does attack proteins, thereby killing the cells. It causes proteins to precipitate out of the solution, and because it is heavier than the liquid the phenol sinks to the bottom and takes the protein with it. Shaking the mixture at this stage would break the delicate DNA strands into small pieces, so we rock the test tube gently back and forth to mix the phenol with the broth. Immediately the mixture becomes even thicker, and grey. Once again, half an hour in the centrifuge separates the protein and phenol from the DNA. At the bottom of the tube is a small clear layer, phenol, and on top of that a thick and faintly repellant white band of coagulated proteins. Above the protein is another layer of clear liquid, and that is the layer that contains the DNA.

Getting the DNA-rich layer out of the tube and away from the protein and phenol is a problem, because any attempt to suck it out is thwarted by the viscosity of the liquid; it pulls itself back out of the end of the pipette. The answer is to use a pipette with a bent tip, and to remove the sticky liquid one drop at a time. That done, you have a test tube full of DNA in solution. What now? The most spectacular trick is to pull the DNA out of the solution and make it into a fibre. Alcohol – ordinary alcohol but absolutely pure – can be poured into the tube where, unlike phenol, it floats above the DNA. A glass rod, dipped through the alcohol and into the DNA, picks up some of the long fibres. As the rod is pulled back through the alcohol the fibres precipitate, and we can draw out of the test tube a long glistening filament, gossamer fine but quite strong, of almost pure DNA.

Suspended from the tip of a glass rod we now have hundreds of thousands of individual DNA strands, lying side by side and making up a fine thread, just as the individual hairs of a sheep are spun to make a thread of wool. What we do with it now is limited only by our imagination.

DNA discovered

DNA – deoxyribonucleic acid – is something of a miracle molecule. The word DNA is far and away the most common single word in the titles of scientific papers, so well known that I was content to launch straight in to its extraction without any further ado, confident that you would be aware of its importance, if not the reasons behind that importance.

But why is DNA so special? Because it contains, coded along its length, all the information needed to construct and maintain the complex machinery of the living cell. It also contains the information needed to reproduce itself, so that, in a very real sense, DNA is a living molecule. Living things are able to reproduce themselves, and DNA can reproduce itself; it really is fundamental to life. Indeed, there are many biologists who view the entire panoply of life, from the precise biochemical reactions guided by enzymes to societies and even ethical behaviour, simply as devices used by DNA to ensure its own survival. These considerations do not concern us here; what is important is that DNA is the primary determining force of every living thing. The information that it contains ultimately directs the manifold complexity of life.

Despite its central importance, DNA spent a long time unrecognized. Johann Friedrich Miescher, a young Swiss of 25 studying in the German town of Tubingen, first found the substance in 1869. Miescher was interested in the chemistry of the cell nucleus, a small body found within the cells of all living things except bacteria. His source of nuclei was the white blood cells that constitute the pus that oozes from surgical wounds, and he plundered the local surgical clinic for discarded dressings. White blood cells have relatively large nuclei, which Miescher diligently separated from their surrounding cytoplasm. His analyses revealed the presence of a hitherto unknown compound, which was acidic and rich in phosphorus, and was apparently organized into very large molecules. He called the compound 'nuclein', and when he moved back to his

native Basle was able to exploit a less unpleasant source of nuclei – sperm cells from the salmon that thrived then in the Rhine – to continue his studies of this fascinating compound.

Richard Altmann, a student of Miescher's, coined the phrase 'nucleic acid', and within a few years the essential chemistry of the compound had been quite thoroughly worked out. There is a sugar – a ribose – that contains five carbon atoms in a ring, and a phosphorus atom surrounded by four oxygen atoms to make a phosphate group. It is the phosphate group that makes nucleic acids acidic, and it also links the sugars together in an unending alternation of sugar and phosphate. Attached to each sugar in this chain is a third type of compound called a base; the triptych of sugar, base and linking phosphate group is called a nucleotide. But while sugar and phosphate alternated predictably along the nucleic acid molecule, the bases were altogether more perplexing. They came in five varieties, known as guanine, adenine, cytosine, thymine and uracil (usually designated simply by their initials: G, A, C, T and U); and although all forms of life contain nucleic acids, the precise ratios of the five bases vary from one to the other.

Later, by the 1920s, two different nucleic acids had been distinguished. One, found primarily inside the cell nucleus, was DNA. The other, more common in the cytoplasm around the nucleus, contained a very slightly different sugar: ribose instead of DNA's deoxyribose, which lacks a single atom of oxygen. This was called ribonucleic acid, RNA. The two nucleic acid molecules are alike in many respects, both consisting of a long chain of alternating sugar and phosphate with a base attached to each sugar. RNA and DNA both contain adenine, guanine and cytosine, but whereas in DNA the fourth base is thymine, in RNA it is the chemically similar uracil (see figure 1.1).

Quite apart from discovering DNA, Miescher also displayed great insight into the problems posed by the biochemicals of living things. And in addition to insight he showed great foresight. Three years before he died of tuberculosis at the age of 55, he wrote a letter to his uncle, in which he pointed out that large biochemical molecules often consist of a repetition of similar, but not identical, subunits. This, Miescher said, gave these molecules the potential to harbour the hereditary message, 'just as the words and concepts of all languages can find expression in twenty-four to thirty letters of the alphabet'.[1] Alas, as Horace Freeland Judson points out,

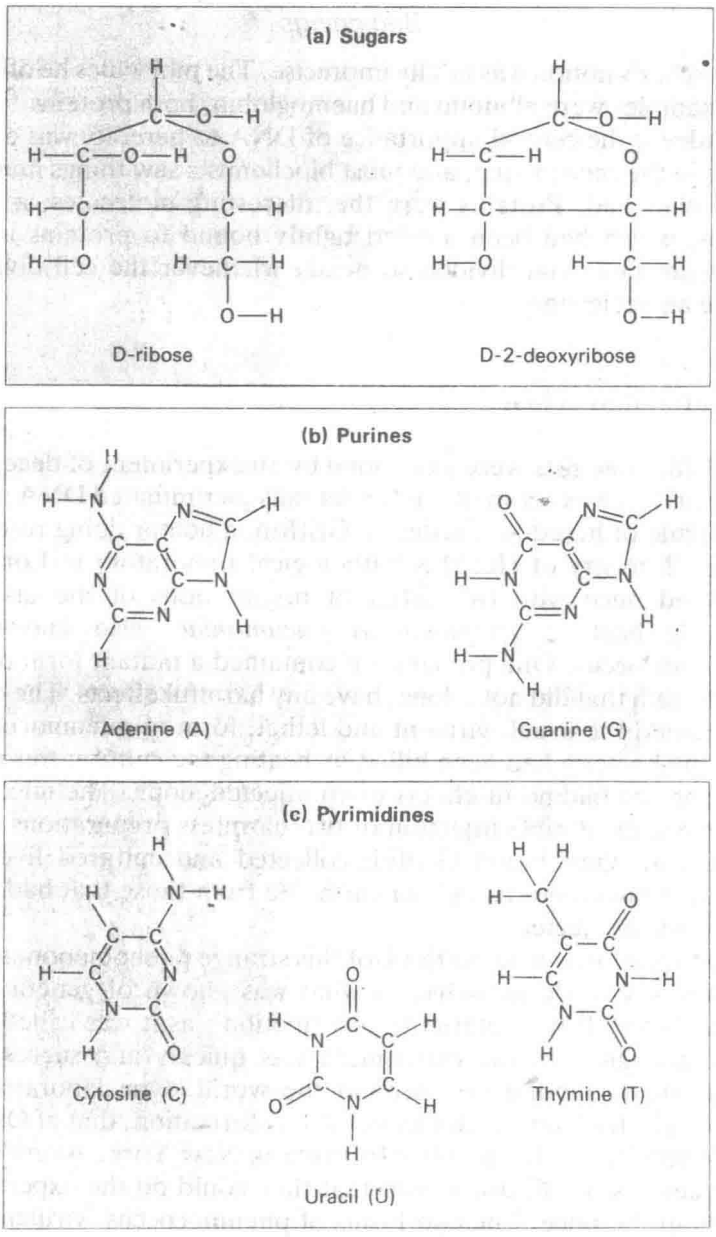

Figure 1.1
The components of nucleic acids (H = hydrogen, C = carbon, N = nitrogen, O = oxygen). Sugars are strung together with phosphate groups, and a base is attached to each sugar. DNA uses D-2-deoxyribose sugar and RNA D-ribose. Both nucleic acids use the same two purines, but thymine in DNA is replaced by uracil in RNA.

'Miescher's notion was fatally imprecise. The molecules he offered as examples were albumin and haemoglobin, both proteins.'[2]

Indeed, the central importance of DNA to heredity was a long time in the recognizing, and most biochemists saw things much as Miescher had. Proteins were the interesting molecules; nucleic acids, which had been located tightly bound to proteins in the chromosomes that divided so neatly whenever the cell divided, were an irrelevancy.

Incriminated at last

In 1928, biologists were astounded by an experiment of deceptive simplicity, an experiment that eventually incriminated DNA as the molecule of heredity. Frederick Griffith, a doctor doing research at the Ministry of Health's Pathological Laboratory in London, injected mice with two different preparations of the disease-causing bacteria *Streptococcus pneumoniae*, also known as pneumococcus. One preparation contained a mutant form of the bacterium that did not, alone, have any harmful effects. The other contained the usual, virulent and lethal, form of pneumococcus, but the bacteria had been killed by heating the culture; this preparation too had no ill-effects when injected alone. The mice that received the double injection of two harmless preparations died, and from their blood Griffith collected and cultured live and virulent pneumococci indistinguishable from those that had been dead when injected.

Nobody knew what to think of this strange phenomenon, which apparently made nonsense of what was known of genetics and inheritance. But 'bacterial transformation', as it was called, was real enough, and the experiment was quickly and successfully repeated in laboratories around the world. One laboratory in particular took up the challenge of transformation, that of Oswald T. Avery at the Rockefeller Institute in New York. Avery's colleagues first of all discovered that they could do the experiment without the mice. The two forms of pneumococcus, virulent and non-virulent, can easily be grown on artificial media, and they look very different. The virulent sort forms a smooth glistening colony, the result of a mucus coat that surrounds each cell and protects it from the defences of the hapless host. The non-virulent mutant

grows into rough crinkled colonies; it lacks the protective mucus coat and so is successfully repulsed when it tries to invade a host. Cultures of rough pneumococci, grown in the presence of heat-killed smooth pneumococci, gave rise to smooth colonies. Late in 1931 James Alloway, also a member of Avery's laboratory, went further. He ground up the virulent smooth bacteria and passed the mixture through a sieve so fine that the empty cell walls, other debris and any unbroken cells could not pass through. Even this filtered extract had the power to transform rough mutants. What is more, when Alloway added alcohol to the filtered extract, he ended up with a viscous, 'thick syrupy precipitate'.[3]

Over the next dozen or so years Avery and his team single-mindedly pursued the 'transforming factor', as it came to be known. At first, like everyone else, they thought it might be a protein. But after many years of unremitting effort the evidence was overwhelming. The transforming principle – and it took 20 gallons (75 litres) of pneumococcus culture to produce less than a hundredth of an ounce (25 milligrams) of the stuff – behaved in every respect like a nucleic acid and not a bit like a protein. It was not affected by the enzymes that digest proteins, nor did it show up in chemical tests for proteins. Enzymes known to attack DNA destroyed the transforming principle utterly, but it was not touched by those that worked only on RNA. It had to be DNA that carried the hereditary message down the generations, but how?

A second classic experiment clinched the vital role of nucleic acids in heredity. Alfred Hershey and Martha Chase, at the Cold Spring Harbor Laboratory on Long Island, were trying to unravel the reproduction of the minute viruses known as 'bacteriophages' (because they infect bacteria). These viruses consist of a protein coat surrounding a strand of nucleic acid. A single virus can infect a bacterium, which bursts open some 30 minutes later to release a shower of hundreds of new viruses, each a replica of the infecting phage. The question was whether the protein coat had anything to do with the infective process, or whether only the nucleic acid was involved. Hershey and Chase made use of the fact that DNA does not contain sulphur, which protein does, while protein does not contain the phosphorus that DNA does. They grew phage and bacteria in a culture that contained radioactive phosphorus, thus tagging the nucleic acid; or they used 'hot' (that is, radioactive) sulphur in the culture, thereby attaching the radioactive label to

the proteins. Then they infected unlabelled bacteria with the hot phage, separated the empty protein coat from the bacteria, and tried to see where the label had gone.

The difficult part was separating phage from bacterium; all sorts of grinding arrangements failed to do the trick, and the final breakthrough came with the loan of a liquidizer or blender. A few moments in the liquidizer easily broke the phage coats away from the cells, as revealed by electron micrographs, and a centrifuge separated the bacterial cells from the culture liquid and discarded viral protein coats. Labelled protein always stayed in the liquid; none came down with the cells. Labelled DNA, by contrast, stayed with the cells, which eventually released fully infective newly made viruses. It was not as rigorous as Avery's series of studies, but the work of Hershey and Chase – remembered today as the 'Waring

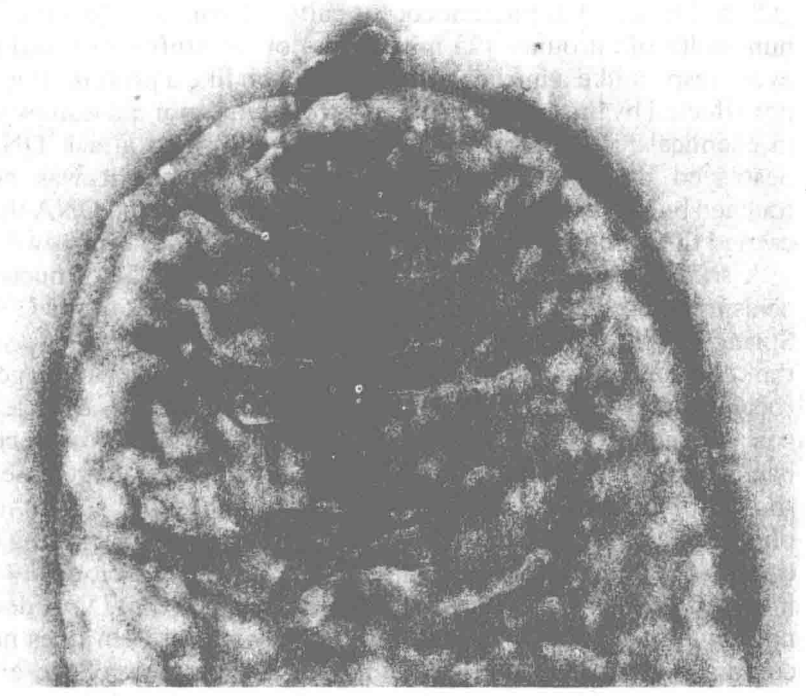

Plate 1.1
An empty lambda bacteriophage, which has just inserted its DNA into the *Escherichia coli* it is attached to. (*Photograph by Maria Schnoss, supplied by Jack D. Griffith*)

blender experiment' – confirmed that the transforming principle, the source of hereditary information, was DNA. But it got no nearer to the tantalizing problem: what was it about DNA that enabled it to fulfil the functions of a memory bank?

Two gloved hands: the golden helix

The story of the delicious solution to the problem of DNA's structure needs no retelling here.[4] James Watson and Francis Crick devised their model in a flurry of activity in the spring of 1953. They introduced it to the world with an opening paragraph remarkable for its understatement: 'We wish to suggest a structure for the salt of deoxyribose nucleic acid (DNA). This structure has novel features which are of considerable biological interest.'[5] The structure was the renowned double helix, and it permitted DNA to fulfil its assigned role with great simplicity.

The key to the double helix is the jigsaw-like fit of the the bases. Guanine and adenine, two of the bases, are composed of two linked rings of carbon and nitrogen. They are members of the class of compounds known as 'purines'. Cytosine and thymine have but a single carbon–nitrogen ring; they are called 'pyrimidines'. What Watson and Crick realized was that guanine could join with cytosine, by means of links called 'hydrogen bonds', to form a double structure that had a shape exactly that of adenine linked to thymine. The only difference is that guanine and cytosine join at three places while adenine and thymine link at only two places; aside from this, the joined purine–pyrimidine pairs are all but identical (see figure 1.2). And because of the difference in the number of available links, guanine will link only with cytosine and adenine will link only with thymine. (Occasionally the 'wrong" linkages will occur, but not often; joining thymine to guanine is like trying to put a two-pin plug into a three pin socket – it can be done, but it isn't easy.)

Watson and Crick's notion of linking purine to pyrimidine made sense of an observation that had been around, more or less unheeded, for five years or more. Erwin Chargaff had measured the amount of each base in DNAs from several different sources: bacteria, man, ox, pig, sheep and yeast. He discovered that these various DNAs differed in the overall composition of their bases,