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Susan Aldridge
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PART I

What is DNA?

1

DNA is life's blueprint

Take a large onion and chop finely. Place the pieces in a medium-sized casserole dish. Now mix ten tablespoons of washing-up liquid with a tablespoon of salt, and make up to two pints with water. Add about a quarter of this mixture to the onion and cook in a bain-marie in a very cool oven for five minutes, stirring frequently, and liquidise at high speed for just five seconds.

Now strain the mixture and add a few drops of fresh pineapple juice to the strained liquid, mixing well. Pour into a long chilled glass and finish off by dribbling ice-cold alcohol (vodka will do) down the side so that it floats on top of the mixture. Wait a few minutes and watch cloudiness form where the two layers meet. Now lower a swizzle stick into the cocktail and carefully hook up the cloudy material. It should collapse into a web of fibres that you can pull out of the glass. This is DNA (short for deoxyribonucleic acid).

DNA is the stuff that genes are made of. Genes carry biological information, which is translated into the characteristics of living things and is passed on down the generations. So genes determine the colour of a butterfly's wings, the scent of a rose, and the sex of a baby. DNA is just a chemical – not a more complex entity like a chromosome or a cell – and it is only in a biological context that it acquires its status as the molecular signature of an organism.

Discovering DNA

The chopping, cooking, grinding and mixing processes described above resemble those carried out every day in laboratories all

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around the world to extract DNA from living tissue. DNA dominates modern biology – yet many decades passed between its discovery and the realisation of its significance.

DNA was first isolated from human pus – a mixture of bacteria, blood plasma and white blood cells that exudes from infected wounds and abscesses – by the Swiss biochemist Friedrich Miescher in 1869. Miescher's life and work were greatly influenced by his uncle, the anatomist Wilhelm His, who was one of the founding fathers of molecular biology. After studying medicine, Miescher moved to Tübingen to work with the great chemist Felix Hoppe-Seyler in the first laboratory in the world devoted exclusively to the study of biochemistry.

The late nineteenth century was an exciting time. Although English physicist Robert Hooke had described cells as long ago as 1665 in his classic work *Micrographia*, it was only in the nineteenth century that their significance as the fundamental building blocks of all organisms was realised. Cells are tiny compartments, full of a fluid called cytoplasm and separated from their outer environment by a thin membrane of a fatty material. Organisms may consist of a single cell – like bacteria, amoebae and yeast – or they may exist as a community of different types of cell working together. These multicellular organisms range from sponges, jellyfish and tiny pond animals, which get by with just a few cell types, to humans who boast over 200 different types of cell.

In the 1860s the idea that life somehow arose spontaneously was finally overturned. Rudolf Virchow, the father of clinical pathology, developed the idea that cells – life's building blocks – could only come from other cells. Experiments carried out by the great French scientist Louis Pasteur supported this view. Pasteur showed that vessels containing broth went mouldy only when contaminated by airborne microbes. If they were heated and sealed they remained sterile – no microbial life appeared spontaneously under such conditions.

Bacteria come from other bacteria, by a simple cell division process called binary fission. This might happen as often as every 20 minutes. Given unlimited food and energy – and an idealised predator-free environment – a single bacterium would give rise to

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numbers greater than the human population (5 billion) in under 11 hours.

Binary fission is an example of asexual reproduction, in which a new organism comes from a single 'parent'. More complex creatures, such as ourselves, come from the union of a cell from each of two parents. This is sexual reproduction. The cell formed by this union grows into a complete organism (containing, in a human, at least 1000000000000 or 10^{12} cells) by a cell division process called mitosis. In multicellular organisms, cells multiply rapidly only during development and in response to tissue damage. The rest of the time there is a balance between cell death and cell renewal.

Each time a cell divides it produces two cells of the same type. A human skin cell has to make another human skin cell, for example, and leaf cells must make more leaf cells, while bacteria produce more bacteria of the same species. The problem facing Virchow, Pasteur and their contemporaries was how to build on the proof that cells come from other cells and show how the particular characteristics of each cell type were transmitted when the cells multiplied.

Most cells are too small to be seen with the naked eye, so much laboratory time was spent peering at them through microscopes. The new biochemists seized upon the intensely coloured dyes, such as Perkin's Mauve, which were being turned out by the fledgling German chemical industry. These stains helped to reveal the inner structure of cells. This, together with improvements in the optics of the microscope, showed that many cells have a central core known as the nucleus (first observed in 1831). Just prior to Miescher's discovery of DNA, the German scientist Ernst Haeckel had suggested that the nucleus was of key importance in passing on characteristics from one generation to the next.

Miescher had a particular interest in the chemical contents of cells. Every morning he would call at the local clinic to pick up used bandages. In the days before antiseptics, these would be soaked in pus and Miescher had discovered that the large nuclei of the white blood cells it contained were ideal for his studies.

It was in these nuclei that Miescher discovered a new substance in 1869. It appeared only when he added an alkaline solution to his cells. Looking under the microscope he saw that this treatment made the nuclei burst open, releasing their contents. So he called the new substance nuclein, on the assumption that it came from the nucleus.

Analysis of nuclein showed that it was an acid, and that it contained phosphorus. These findings suggested that nuclein did not fit into any of the known groups of chemicals that are found in cells, such as proteins, carbohydrates and lipids. Miescher went on to show that nuclein was present in many other cells. Later, nuclein was renamed nucleic acid, and we now know it as DNA.

Miescher became particularly interested in sperm cells from Rhine salmon, because nuclei account for more than 90% of the cell mass in that type of cell (in later life Miescher's attention shifted back to the whole organism and he became interested in the conservation of Rhine salmon). In these experiments, Miescher also extracted a simple protein, protamine, from the nucleus. Protamine is unique to sperm nuclei. In all other nuclei, a similar protein is found called histone, first identified by the German chemist, Albrecht Kossel. It was therefore established that the nucleus contained both DNA and protein – but which of them was involved in the process of inheritance?

Picking up the threads of inheritance

Meanwhile, the microscope was revealing more and more about the secret life of cells. In 1879, the German chemist Walther Flemming discovered tiny thread-like structures within the nucleus, made of a material that he called chromatin because it readily absorbed colour from the dyes used to stain cells and tissues. (Later these threads were named chromosomes.)

The stained chromosomes revealed the intimate details of mitosis to Flemming and others. They saw how the chromosomes double up, as if a copy of each has been provided by the cell. And then, just before the cell divides, the paired chromosomes split up, like a divorcing couple, with each eventually taking up residence in one of the two new cells produced by the cell division. So mitosis is accompanied by the delivery of a fresh set of chromosomes to each new cell.

Then sex was put under the microscope. Oskar Hertwig, working in the French Riviera in 1875, placed tiny drops of sea water containing eggs and sperm from the Mediterranean sea horse on a glass slide, focussed the lens on his microscope, and then sat back to watch the action. He missed the moment of fertilisation – when sperm and egg cells meet – but saw their two nuclei fuse together and then begin to divide.

Eight years later Edouard van Beneden, at the University of Liège, saw that chromosomes from sperm and egg mingled during the fertilisation of the horse threadworm. More importantly, he saw that these germ cells had half the number of chromosomes that other cells had. As we now know, germ cells are formed from a special kind of cell division called meiosis, which involves a halving of the number of chromosomes. So when the germ cells join in fertilisation, the fertilised egg – which goes on to form a new organism – has a full complement of chromosomes.

Chromatin was, evidently, the stuff of inheritance. Analysis showed that it contained nuclein, and in 1884 Hertwig stated that 'Nuclein is the substance that is responsible . . . for the transmission of hereditary characteristics', which is more or less in keeping with our current understanding of the role of DNA.

Ironically Miescher, who speculated long and hard on the biological role of nuclein, could never accept Hertwig's ideas. He did, however, believe that information could be handed on from one cell to the next as a chemical code, stored in large molecules such as proteins. In 1892 he wrote to his uncle, Wilhelm His, that repetition of chemical units in such large molecules could act as a language 'just as the words and concepts of all languages can find expression in the letters of the alphabet'.

He remained devoted to the study of nuclein and worked long hours at the low temperatures he believed gave the best results. He was right about this – time has shown that DNA is a fragile molecule, and this is why chilling is so important in the extraction of DNA from the onion, described at the beginning of this chapter (and why a bucket of ice is a vital accessory for all self-respecting molecular biologists). In the end his intense efforts took their toll and his health, always delicate, broke down, leading to his death at the age of 51.

Dissecting DNA

So, between them, Hertwig and Miescher were right about DNA. But it has taken nearly a century of chemistry to turn their ideas into the central concept of molecular biology.

The chemistry of life is based on the element carbon. There are probably millions of different carbon compounds in nature. Most of them have never been identified and some – like the potential drugs made by plants on the verge of extinction – never will be. The chemical investigation of natural products, such as DNA, will never be over. Specialist chemical journals overflow with reports of exciting new compounds extracted from sponges sitting on the sea bed, common weeds, insects, and human tissue – to name but a few of their sources. Some of these compounds are interesting in their own right, because they have a novel arrangement of atoms, while others have an immediately obvious application to human health and welfare.

The goal of a chemist who extracts a natural product is to find out its structure; that is, the arrangements of atoms in the molecules of the new compound. Structure usually points to the properties and function of a substance. For example, the structure of diamond – which is a giant network of carbon atoms, each of which is linked to four others surrounding it – gives it the property of hardness. Thus, diamonds can be used to make hard-wearing cutting tools. The link between structure and function that was eventually found in DNA is without doubt the most significant in the history of chemistry.

The first step on the road to structure is to find out what elements the new compound contains and, from this, a crude chemical formula can be worked out. Miescher found a formula for DNA: $C_{29}H_{49}O_{22}N_9P_3$ (that is, for every 29 carbon atoms there are 49 hydrogen, 22 oxygen, 9 nitrogen and 3 phosphorus atoms).

The structures of simple molecules such as water are easy to

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solve, because a formula like H_2O (two hydrogen atoms and one oxygen) suggests only one possible arrangement for its atoms, according to the laws of chemistry. But Miescher's formula for DNA (which turned out to be incorrect – it was too simple) suggested thousands of possibilities. The next step, undertaken by Miescher and his contemporaries, was to break up the DNA molecule and find out what distinct groupings of atoms it contained.

One of the things that make people think chemistry is both boring and confusing is a lack of appreciation of the rules that make sense out of the pages of equations and formulae. There are 92 elements that occur in nature, but they do not just combine together at random to make billions of unrelated compounds. The rules about chemical combination, which were worked out in the nineteenth century, have led to a huge database of chemical families, each containing distinct groups of atoms. Compounds in the same family tend to behave in a similar, predictable way. For example, chlorides, such as sodium chloride and magnesium chloride, will always give a tell-tale white precipitate if they are mixed with silver nitrate.

Once the formula of a compound is known, the next step is to assign it to a family by doing a series of tests on it (like the silver nitrate test, described above). Complex substances such as DNA tend to have the attributes of more than one chemical family, because they contain more than one distinct group of atoms.

By 1900, chemists had worked out that DNA contained components from three different families of chemicals. It contained phosphate, a sugar – and a 'base'.

A phosphate group consists of a phosphorus atom surrounded by four oxygen atoms. The place you are most likely to see phosphate mentioned these days is in the supermarket, where rows of 'green' detergents boast of being 'phosphate-free'. Phosphate is an essential nutrient – just look on any packet of garden fertiliser – for all living things because it is an essential component of DNA (and of bone). If phosphates from detergents make their way into the water supply, they seem to encourage the luxuriant growth of algae in rivers, at the expense of other organisms, introducing an imbalance into the ecosystem. The reason why phosphates are present in detergents is that they stop dirt settling back onto fabric once it has been removed.

The sugars are a subgroup within a big biochemical family known as the carbohydrates. As the name suggests, they contain carbon and the elements of water – hydrogen and oxygen. It is easy to confirm this. Heat a teaspoon of table sugar over a flame and within minutes the white crystals give off clouds of steam, leaving a black puffy mass of carbon behind on the spoon. Or just watch toast burning – another example of the decomposition of carbohydrate! The 'D' in DNA stands for deoxyribose, the name of the sugar that was eventually identified as being part of the DNA molecule.

But it is the 'bases' that are the most significant part of the DNA molecule. Bases are chemicals that react with acids to neutralise them. One example of a base is ammonia, which is often an ingredient of household cleaners. Another is baking soda (sodium bicarbonate). If you add this to vinegar (an acid), neutralisation takes place, accompanied by effervescence, which is the evolution of carbon dioxide gas as a by-product. Something similar happens when you drop a tablet of the antacid Alka-Seltzer into a glass of water – the dry tablets contain citric acid and sodium bicarbonate; these react together when they come into contact with water.

The bases in DNA are a bit more complicated than those mentioned above, but, as they are so important in the storage and transmission of biological information, it is worth looking at them in some detail. They belong to two families called the purines and the pyrimidines. The purines in DNA are adenine (A) and guanine (G), while the pyrimidines are cytosine (C) and thymine (T). Like many biologically active molecules, such as vitamins and barbiturates (sedative drugs), the purines and pyrimidines contain carbon and nitrogen atoms arranged in a ring. Purines contain a hexagonal and a pentagonal ring, fused together, while the pyrimidines have just a single hexagonal ring. These family loyalties of the four bases in DNA – and, in particular, the relationships between the two families – were to be of immense importance in working out the detailed structure of the molecule. Put simply, purines and pyrimidines link up in DNA, providing the linchpin of the molecule and the key to its biological significance.

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This, however, is getting ahead of the story: around the turn of the century, all the chemists had was the pieces of the DNA jigsaw – phosphate, deoxyribose and the bases. They then had to work out how these were linked together.

Much of the credit for this has to go to a brilliant and productive biochemist called Phoebus Levene. He studied under the chemist and musician Alexander Borodin at the Chemical Institute in St Petersburg before emigrating to New York with his family in 1891. There he settled at the new Rockefeller Institute for Medical Research. Levene, who was continually in trouble with the Rockefeller's director, Simon Flexner, for overspending his budget, set to work to analyse DNA more closely.

He showed that the three components were linked together by chemical bonds in the order phosphate–sugar–base, with the sugar forming a kind of bridge between the phosphate and the base. He called this unit a nucleotide, and argued that DNA was made of several nucleotides strung together like beads on a necklace. He went on to prove that the string of chemical bonds that linked the nucleotides together – the thread of the necklace – ran through the phosphate groups, not the bases.

By this time another kind of nucleic acid had been identified – in the cytoplasm. This is ribonucleic acid or RNA. RNA is similar in its chemical makeup to DNA, but a base from the pyrimidine family called uracil (U) is found in place of T, while, as the name suggests, the sugar ribose replaces deoxyribose.

DNA or protein?

Unfortunately, what was not appreciated until well into the twentieth century was the sheer length of the DNA molecule. If you were to extract the DNA from the chromosomes of a single human cell and piece it together as one molecule it would have a length of over two metres. In one of the simplest organisms, the bacterium *Escherichia coli (E. coli)*, the DNA molecule is just over a millimetre long – a thousand times longer than the diameter of the bacterial cell itself. DNA molecules are different lengths in