MiSAC briefings 2
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DNA is the genetic material!

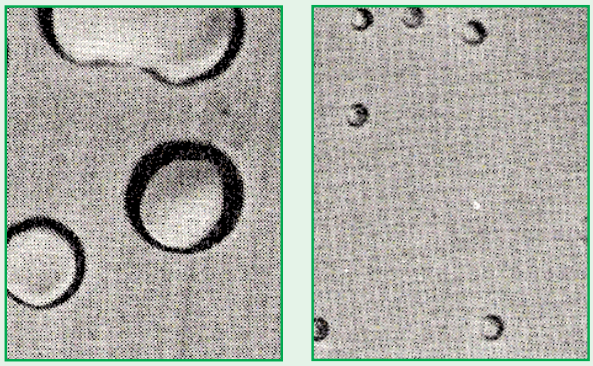
What microbiology did for Crick and Watson

James Watson came to Cambridge in 1951, age 23, determined to discover what genes are. There he met Francis Crick, age 35. They made the first public disclosure of their discovery of the secret of life to an astonished audience of fellow lunchtime drinkers in The Eagle pub in Cambridge and then announced their achievement formally and with a flourish in a brief paper published in the journal *Nature* on 25 April 1953.

The substance that was to become known as DNA (deoxyribonucleic acid) had been first isolated in 1869 by Friedrich Miescher, a biochemist, from the nuclei of dead white blood cells. He called it "nuclein", later becoming "nucleic acid" when it was shown to have the properties of an acid. A decade later, some scientists were beginning to think it highly probable that nuclein was responsible for the transmission of hereditary characteristics. However, strong doubts persisted for a very long time. It took another 50 years or more before the debate became re-focused around the finding that DNA is a major component of chromosomes and the direction of research gradually led to our current conception of DNA.

Much has been written of how Watson and Crick interacted with Maurice Wilkins, Rosalind Franklin and with their older contemporaries Lawrence Bragg and Linus Pauling and, in consequence, came to propose the double helix structure of DNA. However, it is also important to appreciate the key role that studies on microbes have played in research into the structure and role of DNA, not least in the momentous finding that DNA is the genetic material. The three major advances that progressively led to the establishment of this key concept are:

- Fred Griffith made the first breakthrough in 1928 by showing that non-virulent (i.e. non-pathogenic) strains of the bacterium *Streptococcus pneumoniae* could be changed ("transformed") into virulent strains. This crucial observation became the foundation for molecular genetics.
- In 1944, Oswald Avery, Colin MacCleod and Maclyn McCarty explained Griffith's results by demonstrating that his "transforming principle" was DNA. Although, as sometimes happens in science (cf. Gregor Mendel), the scientific community was not yet ready to appreciate the significance of their work, their discovery was to provide the historical platform of modern DNA research.



Streptococcus pneumoniae colonies:
S (left) and R (right) forms (see p. 2)
The R colonies are about 1mm in diameter

- Confirmation of DNA as the primary carrier of genetic information came in 1952 from further research by Alfred Hershey and Martha Chase using a bacteriophage (a virus that attacks bacteria; "phage" for short) that infects *Escherichia coli*. They produced conclusive evidence that the instructions for producing new bacteriophages were carried, not by protein as some still thought, but by DNA. The effect of their work was dramatic. Within a few months, speculation about the nature of the genetic code had begun, culminating in 1953 with Watson and Crick's proposal of the double-helix model for the molecular structure of DNA and heralding the modern era of molecular biology.

Research on bacterial and viral genetics continues and has been largely responsible for the continued rapid growth of molecular genetics. In consequence, the whole of biology has been revolutionised, e.g. from evolution through to genetically modified (GM) foods, gene therapy, DNA fingerprinting and cloning. Along with developments in genomics, and the Human Genome Project in particular, these various advances have much potential for continuing to provide enormous benefits.

Please turn over

But how did they do it?

Griffith discovers bacterial transformation

Cells of virulent strains of *Streptococus pneumoniae* (the pneumococcus) are surrounded by a polysaccharide capsule which gives colonies growing on an agar culture medium a shiny, smooth appearance; they are designated S. Mutant strains without a capsule are avirulent (do not cause disease) and have dull, rough colonies, designated R. The two colony forms are shown on p.1. Griffith found that mice survived inoculation with either heat-killed virulent S bacteria or living avirulent R bacteria. However, when a mixture of both dead S bacteria and living R bacteria was used, some mice died of pneumonia and their blood contained not only living R bacteria but also another living form that was capsulated and virulent, i.e. having S-type features.

Griffith concluded that dead S bacteria possessed a substance that enabled R bacteria to gain the ability to form a capsule and become virulent. He thought that the substance, which he called the "transforming principle", was possibly the capsule polysaccharide or a protein but he did not consider that transfer of an hereditary property might be involved.

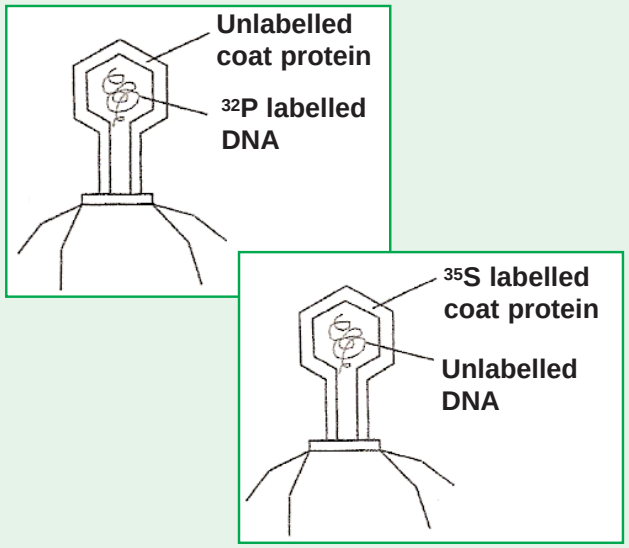


Diagram of a T4 phage showing the head and tail and radioactive labelling. The tail fibres attach to the bacteria at receptor sites on the cell surface.

Avery, MacCleod and McCarty reveal that DNA carries the genetic information

Avery and his colleagues were using transformation to develop techniques for investigating the role of DNA in heredity. It took them some 10 years before they concluded that Griffith's transforming principle was DNA. They showed that the activity of the substance involved in transmitting genetic characters (i.e. transforming R→S) was not affected by treatment with enzymes that inactivated protein and RNA. However, it had all the properties of DNA and, crucially, its activity was completely and specifically destroyed by deoxyribonuclease. They also used improved methods of purification to exclude the possibility that a minor impurity in their DNA preparations might have been responsible.

Hershey and Chase show that DNA is exclusively needed for replication of phage

The use of bacteriophages to study inheritance in bacteria was introduced in the 1940s by Max Delbrück and the influential Phage Group which included, notably, Hershey and Salvador Luria and, interestingly, Watson himself. Bacteriophages that infect *Escherichia coli* consist of a "head" containing DNA and a "tail" consisting of hollow tube surrounded by a protein sheath. The tail attaches to the outside of a susceptible bacterial cell and the DNA is injected. In consequence, hundreds of phage progeny are replicated within the cell and released when the cell lyses (ruptures).

Hershey and Chase worked with a particular type of this phage called T2 (T stands for type). They made two phage preparations, one with the DNA radioactively labelled with phosphorus (^{32}P) and the other with the protein labelled with sulphur (^{35}S). On infection, about 80% of the phage DNA (^{32}P) entered the bacterial cell and about 50% was detected in the phage progeny. In contrast, about 80% of the phage protein (^{35}S) remained outside the cell and less than 1% appeared in the phage progeny. This finally confirmed that DNA carries the genetic information.

Crick and Watson discover the Double Helix

Watson went to Cambridge with an understanding of bacterial metabolism and genetics, and research experience with phage. Crick knew a lot about proteins and X-ray crystallography and had the mathematical approach that came with this knowledge. Both had some knowledge of classical genetics. They sought the structure of DNA not through experimental work with DNA but, taking Pauling's approach, by building structural models, some made of cardboard. After many attempts, some mistakes and using clues gleaned from others, they suddenly came up with the solution in 1953.

Yet, once again, a momentous advance in science caused no more than a ripple of interest at the time. It took nearly 20 years before its significance started to be fully appreciated by the wider scientific community and the term "the double helix" began to reach the ears of the general public - and then another decade before Alec Jeffreys and his group realized in 1984 that each person has their own identifiable DNA "fingerprint".

The Nobel Prize in Physiology or Medicine was awarded to Crick, Watson and Wilkins in 1962 and in 1969 to Delbrück, Hershey and Luria. Rosalind Franklin died in 1958, aged 37.

(Note: A Nobel Prize is shared between not more than three recipients and is not awarded posthumously.)

MiSAC*briefings* provide resource material, procedures and investigations for practical microbiology for teachers, technicians and students. **MiSAC***briefings* are produced by the Microbiology in Schools Advisory Committee, Marlborough House, Basingstoke Road, Spencers Wood, Reading RG7 1AG. E: *microbe@misac.org.uk*. W: *www.misac.org.uk*.