Oswald Avery and the Identification of DNA as the Genetic Material

S Mahadevan

S Mahadevan works and teaches at the Indian Institute of Science, Bangalore. His areas of interest are microbial genetics, physiology and evolution.

In February 1944, when the whole world was consumed by the ravages of a World War, an important paper appeared in the rather obscure *Journal of Experimental Medicine*, entitled "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types: Induction of Transformation by a Deoxyribonucleic Acid Fraction Isolated from Pneumococcus Type III". The principal author of the paper was a short, soft-spoken physician of Canadian origin, Oswald Theodore Avery. The carefully reasoned paper of Avery and his colleagues at the Rockefeller Institute in New York, Colin MacLeod and Maclyn McCarty, was the culmination of meticulous work carried out for over a decade, following the startling results on pneumococcal transformation reported by the British physician Frederick Griffith in 1928. Though the paper was initially met with scepticism from the scientific community, it went on to pave the way for ushering in the new field of molecular genetics. It was also the inspiration for a young American graduate student by the name of James Watson, to team up with the British biophysicist Francis Crick, to solve the structure of DNA. This article attempts to highlight the triumphs and disappointments in the pursuit of the identification of the "transforming principle".

In science, monumental discoveries are seldom made in isolation. This is best exemplified by the events leading to the birth of the flourishing field now known as Molecular Biology, which can be traced back to a string of outstanding discoveries made during the mid 1940s. The one underlying thread that connects all these discoveries is the recognition that microorganisms can contribute significantly to our understanding of the fundamental principles of life.

Keywords
Oswald Avery, pneumococcal transformation, deoxyribonucleic acid.

Though microbiology as a discipline flourished during the late 19th century, thanks to the contributions of the likes of Pasteur[1] and Koch[2], the primary focus of attention at that time was the involvement of microbes in causing diseases. Their potential for unraveling the basic functioning of the cell remained mostly untapped till the 1940s. This was partly due to the mistaken notion that bacteria are notoriously unstable in terms of their morphology and physiology. For a very long time, it was even doubted whether they had genes. This was in spite of observations by microbiologists such as Massini, who showed as early as 1907, that *E. coli* which is normally capable of growing on the milk sugar lactose can spontaneously convert to become lactose-negative. But by the mid-forties, the conviction that genetic changes do occur in bacteria became stronger. The culmination of this belief was the demonstration in 1943 by Salvador Luria, a microbiologist, and Max Delbruck, a theoretical physicist, who collaborated to show that bacteria can mutate and this occurs by a spontaneous process. Using an extremely elegant strategy of analyzing the genetics of bacterial resistance to viruses, the duo for the first time found experimental evidence in the laboratory for the phenomenon of natural selection, the driving force of biological evolution. This was closely followed by the discovery of sex in bacteria by Joshua Lederberg in 1946 and the recognition of the idea that each gene is a carrier of information for the production of an enzyme (the one gene–one enzyme hypothesis) proposed by Beadle and Tatum in 1941. The announcement of the discovery by Avery, MacLeod and McCarty was sandwiched between these major historical findings. The impact of these discoveries, coming in quick succession, on the growth of the young science of genetics was phenomenal.

[1] S Mahadevan, The Legend of Louis Pasteur, *Resonance*, Vol.12, No.1, pp.15–22, 2007.
[2] Jaya S Tyagi, The Timeless Legacy of Robert Koch, *Resonance*, Vol.11, No.9, pp.20–28, 2006.

Griffith's Work on Pneumococcal Transformation

The story of Avery's discovery actually starts in the 1920s with a remarkable observation made by Griffith, working in London for the British Ministry of Health, on the organism that causes pneumonia in humans and animals – *Streptococcus pneumoniae*

By the mid-forties, the conviction that genetic changes do occur in bacteria became stronger.

Griffith made the important observation that, occasionally, cultures of the virulent bacterium throw up variants that are not harmful. Whereas the normal deadly strain forms shiny smooth colonies, the variants form colonies that have a rough appearance.

or pneumococcus for short. The effect of injecting this bacterium into experimental animals such as mice can be quite deadly - the injected animals succumb within days. Griffith made the important observation that, occasionally, cultures of the virulent bacterium throw up variants that are not harmful. This could be observed on a Petri dish. Whereas the normal deadly strain forms shiny smooth colonies, the variants form colonies that have a rough appearance (see cover picture in this issue). These were respectively called the S form and the R form. The smooth form owes its property to the presence of a polysaccharide capsule composed of alternating residues of glucose and glucuronic acid. The capsule protects the bacterium from being eaten by the immune cells of the host. The S to R conversion appeared to be a genetic change leading to the loss of the capsule, as all progeny of the R form retained this property of the strain and seldom reverted to the S form.

While studying the strains, Griffith made the following significant observation. Pneumococcus strains could be classified into Type I, II and III based on their immunological properties. When he injected a non-virulent R strain derived from Type II pneumococcus into mice, the mice survived. Similarly, injection of a heat-killed Type III S strain was ineffective as there were no living S cells in the heat-killed culture to cause an infection. *However, when he injected them with a mixture of the same R strain and the heat-killed Type III S strain, the mice died* ! When blood from the mice that died due to the injection of the mixture was examined, Griffith could isolate Type III S strains. This was quite startling; as the implication was that the Type II R strain has been genetically "transformed" into a Type III S strain! In other words, "*the R form growing under these conditions has newly acquired the capsular structure and biological specificity of Type III Pneumococci*". The information for making the Type III capsule was apparently coming from the dead cells! But what was mediating this transformation? Unfortunately Griffith did not have the right answer and could not pursue the question further. He was killed in a German bombing raid during World

The smooth form owes its property to the presence of a polysaccharide capsule composed of alternating residues of glucose and glucuronic acid.

War II. The solution to the riddle emerged from the meticulous work of Avery and his colleagues at the Rockefeller Institute.

Avery's Triumph

Avery was born in 1877 in Halifax, Nova Scotia, Canada. His father was a Baptist minister who had recently immigrated to Canada from England. When Avery was ten years old, the family again relocated to New York City and he studied at Colgate University. Upon completion of his undergraduate studies, Avery enrolled at the College of Physicians and Surgeons, Columbia University, to study medicine. After obtaining his MD, Avery was more interested in clinical research rather than practicing medicine. Therefore, in 1907, he accepted a research position at the Hoagland Laboratory in Brooklyn, New York. He became an accomplished clinical microbiologist/immunologist, working on several pathogenic strains. His work on *Mycobacterium tuberculosis* was well recognized and he was offered a position at the Rockefeller Institute, where he stayed throughout his scientific career.

When Griffith was making the startling discovery described above, Avery had already developed a strong interest in pneumococci and had made significant contributions by demonstrating that the chief antigenic component of the bacterium was its polysaccharide capsule. The subtle differences in the capsule composition are responsible for the differences in the immunological properties of pneumococcal strains. This is the basis for their serotypic classification. Avery had also demonstrated the stable nature of the three serotypes of the bacterium.

Avery had a strong interest in pneumococci and had made significant contributions by demonstrating that the chief antigenic component of the bacterium was its polysaccharide capsule.

Avery came across Griffith's work while he was at the Rockefeller Institute. The results were puzzling as he himself had demonstrated that the pneumococcal serotypes were quite stable. How was it possible for the dead cells to change the serotype of the living R cells? Even in the R to S reversion induced by continued passage through animals, the serotype of the strain remained the same. Finding the solution to this puzzle became his major mission in life.

How was it possible for the dead cells to change the serotype of the living R cells? Finding the solution to this puzzle became Avery's major mission in life.

Avery and his colleagues started a systematic approach to identify the agent mediating the transformation.

By 1929, the results of Griffith were reproduced at the Rockefeller Institute. Within two years, the requirement of experimental animals could be bypassed as transformation of the R to S type could be demonstrated by plating test tube mixtures of the two cultures on a Petri dish. Another major breakthrough was the demonstration by James Alloway, another colleague of Avery, that transformation could be mediated by a cell-free extract of the S strain prepared by lysing the cells and filtering the lysate to remove remaining cells. This was the starting point of the biochemical identification of the "transforming principle".

Avery and his colleagues started a systematic approach to identify the agent mediating the transformation. Large quantities of the deadly Type III S strain were grown and were harvested using a centrifuge chilled by circulating ice-cold water. The cell pellets were lysed by heat treatment and incubation with bile salts. Capsular polysaccharides were removed by several extractions with saline solution and proteins were removed by extracting with chloroform. Upon addition of alcohol to the partially purified extract, a viscous precipitate was obtained. The major component of the precipitate was the sodium salt of deoxyribonucleic acid or DNA. This was the first demonstration of the presence of DNA in pneumococcus. Curiously, this was the same compound, originally called nuclein, that was isolated by the German biochemist Friedrich Miescher more than fifty years ago from the nuclei of white blood cells. Their yield of DNA was about 25 milligrams from 75 litres of culture. Today we can get more than one hundred times the yield with detergents such as sodium dodecyl sulphate (SDS) to lyse cells and phenol to remove proteins. However, for these pioneers, this extraction of pure DNA was a tremendous achievement.

Just as they carry out most of the functions of the cell, proteins were also thought to be the most likely carriers of genetic information.

Though there were early speculations about DNA being the chemical carrier of genetic information, this idea was soon abandoned in favour of the more versatile cellular polymer, protein. Just as they carry out most of the functions of the cell, proteins were also thought to be the most likely carriers of genetic information. This was to a large extent contributed by the belief that

DNA is a rather monotonous polymer made of sugar, phosphate and just four variable elements – the bases, whereas proteins come in all varieties and forms. Despite this scepticism about DNA as the carrier of genetic information, Avery and his colleagues started the difficult job of obtaining very pure preparations of DNA to test its ability to cause transformation. Was this sheer genius or pure chance? We do not know for sure.

Polysaccharides and proteins that remained in the extract were got rid of by treatment with enzymes that digest them away. The remaining extract showed all the properties of DNA. Biochemical and more sensitive immunological tests showed no traces of proteins or carbohydrates. The chemical composition of nitrogen and phosphorous in the sample again indicated DNA. So did spectroscopic analysis in the ultraviolet range. The absorbance pattern was that of DNA. To cap it all, dilutions of one in six hundred million parts of the extract, when added to a culture of the R type, still produced transformation. Addition of proteases did not affect the result. But addition of DNAse totally destroyed the transforming activity. This was indeed a *tour de force*. Their

Box 1. An Excerpt from Avery's Letter to his brother Roy

Though Avery was far more guarded in his conclusions in their 1944 publication, he was much more candid about the results in private. The following is an excerpt from his letter, written in 1943, to his brother Roy who was working at that time as a bacteriologist at the Vanderbilt University:

"If we are right, and of course that is not yet proven, then it means that nucleic acids are not merely structurally important, but functionally active substances in determining the biochemical activities and specific characteristics of the cell – and that by means of a known chemical substance, it is possible to induce *predictable* and *hereditary* changes in cells. This is something that has long been a dream of geneticists…..But with mechanisms I am not now concerned – one step at a time. Of course, the problem bristles with implications. It touches genetics, enzyme chemistry, cell metabolism and carbohydrate synthesis. But today it takes a lot of well documented evidence to convince anyone that the sodium salt of deoxyribonucleic acid, protein free, could possibly be endowed with such biologically active & specific properties and that evidence we are trying to get. Its lots of fun to blow bubbles, – but it is wiser to prick them yourself before someone else tries to."

(Quoted from H F Judson's *The Eighth Day of Creation*)

1944 publication, announcing these results in meticulous details, is a model of scientific logic and reason (see Classics section in this issue). Avery was 67 years old when the paper was published.

In spite of the overwhelming evidence in favour of DNA as the transforming agent, Avery and his colleagues were guarded in their conclusions.

In spite of this overwhelming evidence in favour of DNA as the transforming agent, Avery and his colleagues were guarded in their conclusions. In their words, "*It is, of course, possible that the biological activity of the substance described is not an inherent property of nucleic acid, but is due to minute amounts of some other substance absorbed to it or so intimately associated with it so as to escape detection. If however, the biologically active substance isolated in highly purified form as the sodium salt of deoxyribonucleic acid actually proves to be the transforming principle, as the available evidence strongly suggests, then nucleic acids of this type must be regarded not merely as structurally important, but as functionally active in determining the biochemical activities and specific characteristics of pneumococcal cells.*" This caution was obviously in deference to the prevailing views about nucleic acids.

As expected, the results were greeted with a healthy dose of scepticism. Many, including biochemist colleagues such as Alfred Mirsky at the Rockefeller Institute, openly questioned the results and suggested that the activity may be associated with minute quantities of protein present in the extract even at one in six hundred million dilutions! Disappointed by the reception to his life-time achievement, Avery retired from science in 1948 and spent his remaining life peacefully with his brother Roy in Nashville, Tennessee. By then, he was 71 years old. Avery was considered for the Nobel Prize, but the committee deferred the decision awaiting more evidence. A life long bachelor, Avery died in 1955, two years after Watson and Crick's discovery of the double helix.

Avery belongs to the rare breed of scientists who have not only made outstanding discoveries, but have also inspired others to follow in their footsteps.

Avery belongs to the rare breed of scientists who have not only made outstanding discoveries, but have also inspired others to follow in their footsteps. The identification of DNA as the

transforming agent galvanized Erwin Chargaff to drop all his biochemical work and focus on DNA. This ultimately resulted in his 1949 publication of the molar equivalence of purines and pyrimidines, the chemical basis of the complementarity of the two DNA strands. Chargaff also showed that DNA is not monotonous as it was believed to be and its base composition varies from source to source. Avery's work also stimulated Joshua Lederbeg to undertake genetic analysis of bacteria that resulted in the discovery of sex in bacteria. This discovery was of enormous significance in the study of several other phenomena such as gene regulation. More than anything else, his discovery was the stimulus that inspired the Watson–Crick duo to crack the structure of DNA within a decade. Like Gregor Mendel, whose work founded the field of genetics, Avery, who laid the foundations of molecular genetics, was also regrettably recognized only belatedly. Chargaff's tribute to Avery was befitting: "*He was a quiet man and it would have honoured the world more had it honoured him more.*"

Address for Correspondence
S Mahadevan
Department of Molecular Reproduction, Development and Genetics
Indian Institute of Science
Bangalore 560012, India
E-mail:
mahi@mrdg.iisc.ernet.in

Suggested Reading

[1] G Stent and R Calendar, *Molecular Genetics: An Introductory Narrative*, W H Freeman and Co, San Francisco, 1978.

[2] H F Judson, *The Eighth Day of Creation*, Simon and Schuster, New York, 1979.

The publication of Avery, MacLeod and McCarty (1944) marked the opening of the contemporary era of genetics, its molecular phase. The reverberations continue, now dominating large sectors of biomedical science and biotechnology, and have established the centrality of genetics in biological thought.

Joshua Lederberg