Pneumococcal Transformation: Genes are Made of DNA

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MILESTONES

Avery OT, MacLeod CM, McCarty M: **Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III.**
Journal of Experimental Medicine 1944, 79:137-158

Oswald T Avery

The finding that DNA transmits heredity is considered by many to be the most important discovery in biology during the 20th century. Amazingly, the reception given the publication by Avery and colleagues announcing their discovery hardly fit such a fundamental advance. To a surprising degree the publication went unnoticed. The article appeared at the height of the Second World War — a time of hampered communication within the international scientific community — and in a journal not considered a main line periodical for geneticists and general biologists. The paper by Oswald Avery, Colin MacLeod and Maclyn McCarty[1] did catch the attention of some biochemists who generally greeted it with skepticism. The claim that genes are made of DNA challenged the established dogma that proteins were the carriers of genetic information. Almost a decade passed before the discovery by Avery and his colleagues became widely known and acknowledged as a sentinel event in genetics.

The history of the ground-breaking discovery by Avery, MacLeod and McCarty is a fascinating story filled with valuable lessons for young investigators and for the institutions that provide them the resources needed to do research. Maclyn McCarty elegantly detailed that history in a book that allows the reader to vividly experience the daily elations and disappointments of life at the laboratory bench during the first half of the 20th century[2]. Avery, the senior investigator, and his trainees MacLeod and McCarty were medical bacteriologists at the Hospital of the Rockefeller Institute in Manhattan. Avery's research focused on the pathogenesis of pneumococcal pneumonia, at the time the leading cause of death in the United States, far surpassing deaths due to heart disease and cancer. His ultimate goal of eradicating pneumococcal pneumonia always eluded him, but in the process of pursuing it for three decades he and his colleagues discovered the chemical basis of heredity.

When Avery began his work at Rockefeller in 1913, the fundamental properties of pneumococci had already been established. Pasteur had initially isolated the virulent microbe from the sputum of a patient with pneumonia. Later investigators described the organism's thick capsule and observed that a single pneumococcus injected into a mouse proved lethal. Rabbits and horses immunized with pneumococci from one patient developed antibodies that reacted with the capsule of that microbe and with the capsule of pneumococci isolated from some, but not all, other patients. The anti-capsular antibodies defined four serological types of pneumococci (I, II, III, IV). Several laboratories had shown that type-specific antibodies from rabbits could protect mice from an otherwise lethal challenge with pneumococci of the corresponding type.

Shortly after his appointment at Rockefeller, Avery discovered that the supernates of pneumococcal cultures contained a soluble component precipitated by type-specific antibodies. Michael Heidelberger, a young colleague at Rockefeller, showed that the soluble component Avery had termed "soluble specific substance" was a polysaccharide[3]. Evidence developed that the soluble polysaccharide came from the capsule of pneumococci and that the capsule was critical to the organism's virulence. Proof of the latter came from the discovery that pneumococci cultured in the presence of type-specific antibodies became nonencapsulated and totally avirulent. Mice showed no ill effect when injected with billions of unencapsulated pneumococci, but succumbed to injection of a single encapsulated microbe. Microscopic studies revealed that blood phagocytes rapidly ingested nonencapsulated pneumococci, but not the virulent encapsulated forms. However, when type-specific antibodies were present, blood phagocytes readily ingested the encapsulated pneumococci. The effect of antibody on pneumococcal virulence reinforced the rationale for using immune horse serum to treat patients with pneumococcal pneumonia.

Martin Dawson, a young associate in Avery's laboratory, initiated studies to determine if non-

encapsulated, avirulent pneumococci could undergo reversion to the encapsulated, virulent form. He found evidence for reversion and published his initial findings in 1928[4]. This launched 15 years of experiments of bewildering complexity and erraticreproducibility by Avery and his colleagues. Ultimately, they identified the mechanism of reversion and in the process discovered that genes are made of DNA. In their milestone publication they reported that when non-virulent pneumococci isolated from Strain II were cultured in the presence of DNA from virulent type III pneumococci, the Strain II microbes produced Type III capsular polysaccharide and became virulent.

Avery's findings evoked strong skepticism from several prominent biochemists who argued that protein contaminants in the DNA likely accounted for the transformation from Type II to Type III. At the time it was well established that enzymes, antibodies and other proteins exhibited high specificity, characteristics favoring their role as informational molecules. Furthermore, based on evidence at best vague but unchallenged, most biochemists considered nucleic acids totally unsuited to contain genetic information. The prevailing concept envisioned nucleic acids as monotonously similar small molecules composed of stacks of flat tetranucleotide rings that functioned to hold the chromosome together.

Avery's group went to great lengths to rule out protein contamination of their DNA preparations. Using the most sensitive analytical methods then available, they failed to detect protein in their preparations. The transforming activity in their material totally resisted the action of proteolytic enzymes and methods known to denature proteins. The recovery of transforming activity from transformed cells in amounts far in excess of that originally used to induce transformation implied replication of the transforming principle. Purified preparations of DNase did not exist so McCarty spent two years developing a procedure to isolate DNase from bovine pancreas. In a 1946 paper[5] he reported that the transforming activity was destroyed by DNase.

In spite of all the evidence presented by Avery and his colleagues that genes are made of DNA, their work continued to be criticized or ignored for almost a decade after their milestone publication. Geneticists expressed doubts about the relevance of findings in bacteria to hereditary mechanisms in higher organisms. In spite of the generally dubious reception the 1944 paper received, it did have an impact. It clearly influenced the eminent biochemist Erwin Chargaff to undertake analysis of DNA from different species, studies that eliminated the prevailing notion that nucleic acids are all alike[6]. As he pursued those studies Chargaff discovered A-T and G-C base pairing, a crucial determinant in the DNA model proposed by Watson and Crick. The 1944 paper also directly influenced the thinking of Francis Crick as he and Watson began their work[7]. Commenting on the discovery by Avery, MacCloud and McCarty forty years later, Nobel laureate Joshua Lederberg ranked its importance with the contributions of Darwin and Mendel.

It is unlikely that in today's research climate Avery's laboratory could have sustained the decades of effort that led to the groundbreaking discovery. There were long periods of slow progress, negative findings and few publications. It is often pointed out that knowledge gained through basic research advances the understanding and treatment of disease. There are fewer reminders that knowledge gained by investigating disease can advance our understanding of basic biological processes. The pneumococcal research of Avery and colleagues is a splendid example of the latter principle.

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