

Oswald Avery and the Origin of Molecular Biology

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It is now twenty years since James Watson published his personal account of the discovery of the structure of DNA and triggered the growing scholarly study of the roots of molecular biology.¹ Watson himself was not concerned with the study of nucleic acids before he became directly involved but at least three detailed histories of the early development of molecular biology have subsequently appeared, together with books, papers and reviews from others who took part, or their partisan representatives. Of these three histories,² only one does justice to Avery's work. His surviving DNA collaborator, MacLyn McCarty, believes that only Olby in *The Path to the Double Helix* deals adequately with Avery's contribution.³

There can be little doubt that the publication in 1944 of the paper on transformation in *Pneumococcus* by Avery, MacLeod and McCarty marked a major step in the origin of molecular biology.⁴ The demonstration that the transforming substance was probably DNA provided the first clear association between a genetic phenomenon and a nucleic acid. Nine years later Watson and Crick established their successful model structure for DNA.⁵

The muted tone of Avery's conclusions and the apparent lack of response to the paper have led some to question whether Avery really understood the significance of what he had found and ask if the paper was not in some sense 'premature', or its content merely 'information' as opposed to 'knowledge'.⁶ It has been shown that Avery was certainly aware of the implications and that several people had no difficulty in seeing the paper as 'knowledge' and rapidly integrating its findings into their own research.⁷ The

1 J.D. Watson, *The Double Helix*, London, 1968.

2 R. Olby, *The Path to the Double Helix*, London, 1974; F.H. Portugal and J.S. Cohen, *A Century of DNA*, Cambridge, Mass., 1977; H.F. Judson, *The Eighth Day of Creation*, London, 1979.

3 M. McCarty, *The Transforming Principle. Discovering that Genes are Made of DNA*, New York, 1986, pp. 230–232.

4 O.T. Avery, C.M. McLeod and M. McCarty, '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, pp. 137–158.

5 J.D. Watson and F.H.C. Crick, 'A structure for deoxyribose nucleic acid', *Nature* (1953) 171, p. 737.

6 G.S. Stent, 'Prematurity and uniqueness in scientific discovery', *Scientific American* (1972) 227, p. 84; H.V. Wyatt, 'When does information become knowledge', *Nature* (1972) 235, pp. 86–89.

7 A.F. Coburn, 'Oswald Theodore Avery and DNA', *Perspectives in Biology and Medicine* (1969) 12, pp. 623–30; A.M. Diamond, 'Avery's "neurotic reluctance"', *Perspectives in Biology and Medicine* (1982) 26, pp. 132–136; McCarty, op. cit. 213–230; R. Olby, 'Avery in retrospect', *Nature* (1972) 239, p. 295.

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real problem is not that so many may or may not have failed to notice it, but how the paper came to be written in the first place. Young molecular biologists ought to know how unlikely it was that their discipline should have started how and when it did.⁸

Avery was sixty-seven years old when the transformation paper was published. His previous work had been extensive and wide-ranging, but confined to bacteriology and immunology. From the year when he was first appointed to the Rockefeller Institute until the time that sulphonamides replaced immunotherapy in the treatment of infectious diseases in the late 1930s, Avery's work was centred round the preparation, monitoring and improvement of passive immunotherapy for pneumonia patients. The complex typology of the *Pneumococcus* bacterium made this exacting work. The research conducted on transformation in his laboratory must have fitted intellectually into this immunological programme.

Avery and his group worked sporadically on the transformation problem for some sixteen years between 1928 and 1944. I have speculated recently that the biographic history and personality of scientists has a deeper influence on their science than is sometimes supposed.⁹ Avery's transformation work seems a suitable preliminary test of this idea, since it was done without the spur of competition, there seems to have been no clear theoretical objective and the isolated nature of his findings seems to preclude a sociological explanation. However, there is an irony in selecting Avery as a subject. He himself believed that the personal life of the scientist played no role whatever in scientific achievement.¹⁰ Few of his personal records have survived. We are dependent on descriptions of him provided by some of his colleagues. What follows is an extension of the views of one of his main collaborators, René Dubos.

The experimental approach which Avery adopted in the investigation of transformation fitted firmly into the pattern of his previous research. He isolated and, as far as possible, identified a chemical molecule responsible for initiating a biological process. This was his hallmark. He repeatedly tried to uncover simple chemical bases for complex biological phenomena and cajoled biochemists into helping him isolate and identify such substances. One of his central techniques was to digest away contaminating substances with enzymes, leaving a crude extract of the substance in whose biology he was interested. The transformation experiments were linear descendants of similar exercises done many times before. The same experimental style had been used throughout.¹¹ How did transformation persist for long enough in Avery's immunological programme to become the basis of a programme in molecular genetics?

Griffith first reported *Pneumococcal* transformation in 1928.¹² He showed that if a live, non-virulent Type II strain was injected into a group of mice together with a heat

8 'I don't know who, in 1940, could possibly have foreseen that microbiology would furnish the links connecting biochemistry ever after with genetics', R.D. Hotchkiss, 'Gene, transforming principle and DNA', In: J. Cairns, G. Stent and J.D. Watson (eds), *Phage and the Origins of Molecular Biology*, Cold Spring Harbour Laboratory of Quantitative Biology, 1966, p. 182.

9 N.C. Russell, 'Breakthrough for the bloody-minded. A scientist's personality can be the key to his work', *Times Higher Education Supplement*, 25 September 1987, p. 11.

10 R.J. Dubos, *The Professor, the Institute and DNA*, New York, 1976, pp. 47-48.

11 Dubos, *op. cit.* (10), pp., 101-112, 161-179; Olby, *Double Helix*, 181-193.

12 F. Griffith, 'The significance of *Pneumococcal* types', *Journal of Hygiene* (1928) 27, pp. 113-159.

killed virulent Type I strain, some mice died of pneumonia and a live, virulent Type I strain could be isolated from them. After checking carefully that the result was not due to failure to kill the Type I bacteria properly, Griffith concluded that the co-infection had somehow caused a transformation of the avirulent Type II into a virulent Type I strain.

This was extremely surprising. Although spontaneous and stable changes in bacteriological physiology were well recognized by this date, such changes had never been observed across strains. Bacterial strains and varieties were regarded as fixed entities. Griffith's results suggested that strains were not as stable as nearly everyone had supposed.¹³

In Avery's laboratory the results were greeted with dismay. His team had recently succeeded in demonstrating that the differences between *Pneumococcus* strains were caused solely by differences in the structure of the polysaccharides in their capsules and not by more complex biological factors, that these polysaccharides were the basis of bacterial virulence and antigenic properties, and that variation in the efficiency of the host response to the different strains were also a consequence of this molecular variation. The idea that the strains were not stable created a new layer of complexity for Avery's elegant thesis that the key to the complex physiology of host-*Pneumococcus* relations lay in capsule molecular structure. The initial response at the Rockefeller was to hope that Griffith's results were wrong.¹⁴

Avery and Griffith held different views about the clinical and biological significance of Type variation in *Pneumococcus*. During the clinical progress of a pneumonia case, several *Pneumococcus* strains might be recovered. Avery's group interpreted such changes as fluctuations in the fortunes of different types within a mixed *Pneumococcal* population at different stages of the infective process. Griffith adopted the alternative view that *Pneumococcal* types might revert or mutate one to another during the course of an infection. For Avery the Types were distinct and separate forms, almost with the status of species, while for Griffith they were unstable varieties. For Avery, the differences between strains were paramount; for Griffith what mattered was their likely affinity.

Griffith therefore found the instability of Types easy to accept. He explained transformation by supposing that some substance from the dead strain had modified the live one. The process could not simply be explained by the live bacteria incorporating carbohydrates from the dead ones into their capsules, since transformation would not occur if the dead strain was subjected to temperatures higher than 80°C, implying that the transforming phenomenon was thermolabile, while carbohydrates themselves are thermostable. The carbohydrate antigens and the putative transforming principle were separate. The reversion between types suggested to Griffith that the carbohydrates had some common core structure which could perhaps be modified or rebuilt by manipulation with some sort of template material released from the dead Type. He saw the transitions between strains as minor adaptive shifts produced by changing environmental circumstances. Avery's deep commitment to the separation and specificity of Types made

13 Dubos, op. cit. (10), pp. 132–138; Hotchkiss, 1966, op. cit. (8); G.S. Stent and R. Callendar, *Molecular Genetics. An Introductory Narrative*, San Francisco, 1978, pp. 152–159.

14 Dubos, op. cit. (10), pp. 132–138; McCarty, op. cit. (3), pp. 72–88; Olby, *Double Helix*, 169–179.

it impossible for him to see it that way. The existence of transformation of Type presented an intellectual threat and implied the existence of a major, as yet unexplored, phenomenon.¹⁵

Investigations into transformation began in Avery's laboratory only after confirmation from other places had occurred. Dawson, a young Canadian physician, had already been looking at the process of reversion between virulent and non-virulent forms within single strains at Avery's behest in 1926 and 1927. He confirmed the existence of transfer between strains in 1930. He and Sia then began to try to induce transformation with mixed strains *in vitro*, succeeding in this and publishing in 1931.¹⁶

Avery's active involvement with transformation in this early period was probably minimal, because the concept was so opposed to his own views on the fixed, immutable nature of *Pneumococcus* strains. Avery's name did not appear on Dawson's papers reporting the confirmation of transformation, sure signs that he had played no planning or experimental part in the work.¹⁷ Dawson left for a clinical post later in 1930 and was replaced by Alloway. The programme now began to fit more closely with Avery's approach as Alloway attempted to separate a soluble chemical principle from the dead cells which could induce transformation. He succeeded in obtaining such an extract and produced, in 1932, a crude alcohol precipitate of what was certainly DNA. Neither Dawson nor Alloway came to firm conclusions about the origin or nature of their transforming substance, but both believed it was either part of, or closely associated with, the capsular materials and was probably a protein or glycoprotein. Alloway in turn left Avery's laboratory in 1932 and for the next two years Avery continued on his own. It seems reasonable to assume that Avery became directly involved in transformation research sometime during 1930.

1933 and 1934 were intensely frustrating years. Using Alloway's techniques, the isolation of the active principle was completely erratic; sometimes an extract with a testable transforming activity was isolated but as often as not there was nothing. Avery nevertheless persisted, although there were other, more successful programmes going on in which he was heavily involved.

With Goebel he was pioneering the use of Landsteiner's artificial antigen techniques to discover precisely what features of the capsular polysaccharide were antigenic, laying down the ground rules for the discipline of analytical immunochemistry, while with Dubos he was investigating a bacterial enzyme which had the ability to digest Type III capsular antigens *in vivo* and render this particularly dangerous strain harmless. They were testing the enzyme on a variety of animal species with a view to demonstrating its safety for trials in man.¹⁸

By 1934 at the latest, Avery's thinking on transformation had become the subject of a 'red seal record', the famous monologues which he delivered to colleagues and students.

¹⁵ Dubos, *op. cit.* (10), pp. 132–138; McCarty, *op. cit.* (3), pp. 72–88; Olby, *Double Helix*, 173–179.

¹⁶ Olby, *Double Helix*, 169–179.

¹⁷ Dubos, *op. cit.* (10), pp. 80–85; Olby, *op. cit.* (2), pp. 176–179; W.F. Goebel, 'The golden era of immunology at the Rockefeller Institute', *Perspectives in Biology and Medicine*, 18, pp. 417–426.

¹⁸ Dubos, *op. cit.* (10), pp. 108–110, 116–119; R.D. Hotchkiss, 'Oswald T. Avery 1877–1955', *Genetics* (1965) 51, pp. 1–10.

with the twin objectives of clarifying his own thinking and stimulating others to subordinate their work to his programmes.¹⁹ Yet the identification of the transforming principle would not apparently have contributed much to the analysis of either capsular antigens for improved immunotherapy or anti-Type III enzyme chemotherapy.

Transformation could have been interpreted, of course, as a mutational event, with the change in Type resulting from alteration in carbohydrate structure a consequence of genetic change in the bacterium, since the biological specificity of polysaccharide structure was a thread which ran through much of Avery's work. However, there is no evidence that Avery was thinking within such a genetic framework at that time. Once he had clearly perceived the genetic possibilities some five years later, he did start reading very widely in genetics. Dubos believed that his interest in transformation had more to do with an unsuccessful programme, his investigation of the reasons for the poor antigenic performance of Type III vaccines used to raise antisera in experimental animals.

Avery believed that this was because the capsular antigens were digested off the bacterial surface, a process he called 'antigenic dissociation', either by host anti-bacterial response or by an agent released from the bacteria themselves during autolysis. There were obviously conceptual connections between a principle which seemed to assemble capsular materials at the cell surface (transformation) and a putative principle which digested the capsular materials off (antigenic dissociation). Avery evidently believed that there was some point in pursuing these phenomena in tandem, hoping that a coherent solution to the problem of the failed Type III vaccine would emerge.²⁰

MacLeod joined Avery's group in 1934, and responding to Avery's 'red seal record' induction on transformation worked for three years on the topic. He made great improvements on Dawson and Alloway's procedures, especially in the selection of suitable bacterial strains, in growing the transforming strain on a large scale and in the isolation and assay of the soluble principle. But nothing was published and even in 1937 there was little definite evidence of what the principle might be, although it seemed certain that it could not be either protein or carbohydrate.²¹

Avery's grip over the diverse research in his laboratory may have faltered at about this time because the incipient Grave's disease from which he suffered reached a crisis point. Sometime in 1934 or 1935 he underwent partial thyroidectomy and was convalescent for some time afterwards.²² MacLeod continued work on transformation, enthused by his new research career, acting as the flywheel which carried the programme through what might otherwise have been a deadspot. It seems doubtful whether Avery would have picked up the threads of this project again if MacLeod had not been occupied more or less full-time on it between 1934 and 1937.

Two of Avery's major research themes, the preparation of a suitable antiserum and an enzyme chemotherapeutic agent against Type III *Pneumococcus*, were rendered obsolete by the appearance of sulphonamides in the mid-1930s.²³ In 1937 MacLeod's

19 Dubos, op. cit. (10), pp. 80–85; C. MacLeod, 'Oswald Theodore Avery 1877–1955', *Journal of General Microbiology* (1957) 17, pp. 539–549; Hotchkiss, 'Avery'.

20 Dubos, op. cit. (10), pp. 113–123.

21 McCarty, op. cit. (3), pp. 89–100.

22 McCarty, op. cit. (3), pp. 86–88; Dubos, op. cit. (10), pp. 65–68.

23 MacLeod, 1957, op. cit. (19), pp. 539–549.

work on transformation stopped abruptly and for the next three years he was involved, amongst other things, in testing the effectiveness of sulphonamides in the treatment of pneumonia. Between 1937 and 1940 Avery was also diverted away from transformation. The problem lost its attraction. He concentrated on the isolation of a host–response substance, the C-reactive protein, perhaps deliberately diverting his attention from the dying fields of bacterial typology and passive immunotherapy to his other lifelong interest, the host response to pathogens. So fascinated did he become by this substance that he diverted the young Hotchkiss away from transformation, about which he was enthused, onto C-reactive protein, a rare example of Avery overtly directing one of his junior colleagues.²⁴

The transformation programme was suddenly revived in 1940. MacLeod and Avery made a concerted effort to purify and identify the transforming principle.²⁵ Whereas in 1937 neither of them seems to have had any theoretical insight to drive the work forward, beyond a vague desire to know more about capsular antigen behaviour in order to improve or modify vaccine production for antibody preparation, by 1940 it seems clear that Avery had realized the possibility that transformation was a form of mutation. The specificity of *Pneumococcus* Types resided in their capsule carbohydrate molecules. The separate transforming molecule, since it apparently controlled these carbohydrate structures, was behaving like a gene. The grander implication was that the transforming substance might not merely act like a gene in the local example of *Pneumococcal* typology, but be a molecule with more widespread, even universal, genetic properties.

First with MacLeod and then, from 1941, with McCarty the programme of eliminating molecular species from the principle and positively correlating the remaining component to the known behaviour and properties of purified DNA went ahead steadily. By late 1942 the identity of the transforming substance as DNA and the genetic consequences which followed were freely discussed in Avery's laboratory as essentially established facts.²⁶ Early in 1944 the work was published and the reaction, or lack of it, among the biochemical and genetic communities is usually the point at which historians of molecular biology become interested.

The most difficult period to understand in the tortuous story of *Pneumococcus* work in Avery's laboratory is from 1932–1937, when the presence of a chemical transforming agent was a possibility and its isolation and identification were the objectives of Avery's research on transformation within his immunological programme. Transformation research was abandoned in 1937 and then revived in 1940 when he saw that it might be a genetic phenomenon. This was a bold theoretical step on his part. Even by that date, few believed that bacteria showed genetic phenomena parallel to those found in higher organisms.²⁷

What kept Avery pushing the programme forward for so long through a period of illness when the goal of his work seemed so vague? Despite his notorious reluctance to enter into argument or speak in public, Avery was both a self-confident and an optimistic

²⁴ Dubos, *op. cit.* (10), pp. 97–99.

²⁵ McCarty, *op. cit.* (3), pp. 101–116.

²⁶ McCarty, *op. cit.* (3), pp. 134–135.

²⁷ Hotchkiss, 1966, *op. cit.* (8), pp. 183–184.

man,²⁸ both personality traits necessary to continue down a path with no immediate goal. In addition he was a man of great persistence.²⁹ Once he had started a project he was reluctant to leave it while there was no clear dénouement.

Avery was not an initiator of fundamental research programmes. He always allowed the ultimate targets of his work to be set externally.³⁰ At the Rockefeller Institute his brief was to investigate pneumonia organisms with the object of improving therapy. Under these circumstances the lack of any clear theoretical point to the transformation experiments might not have worried him; the work could proceed, somewhat aimlessly if need be, under cover of the laboratory's overall objectives. He only abandoned transformation, or at least put it on the backburner, when the whole direction of the laboratory was called into question by the arrival of sulphonamides, confirming Dubos' opinion that the place of the transformation work was in the immunotherapy programme. Avery perhaps lost his way in this period, only recovering his momentum with the insight that transformation might be a genetic process.

As a corollary of his lack of interest in setting his own targets his dominant motivation was not in the solution of problems or in making new discoveries, which were for him almost epiphenomena. His real joy lay in the development of experimental procedures to resolve externally set questions. The process of solving the problem was far more interesting to him than the ultimate outcome. The design of elegant experimental solutions using the minimum of data to provide maximum information was where his true creativity lay.³¹ This concern with economy of means may have been the mirror of his personal economy of effort, which took the form of studiously rationing his enthusiasm for the job in hand and ruthlessly excluding wider scientific concerns or administrative and social chores. This was especially true after his thyroidectomy in the mid-1930s. Only in this way could he possibly have remained a productive scientist actively planning work and engaged with his junior colleagues at the bench, rather than a figurehead or administrator, to such a great age.

Several factors were significant in Avery's career and therefore, in a sense, causes of the transformation breakthrough. The Rockefeller Institutes's main scientific philosophy, as noisily dispensed by Jacques Loeb, was that complex biology was resolvable to simple physics and chemistry. Avery was already predisposed to this point of view from working for the chemist Benjamin White in the Hoagland laboratories before he arrived at the Rockefeller.³² The search for simple causes for apparently complex processes was a leitmotif of his experimental style.

Dubos sees Avery's career as a paradigm of the swing in emphasis of medical and social research away from epidemiology and the clinical analysis of infections towards investigating the fundamental biology and chemistry of the causative organisms and the host response. Nevertheless, Avery remained wedded to the notion that his overall objective should be the improvement of medical therapy, but the change in research emphasis

28 Hotchkiss, 1965, (18), pp. 1–10.

29 Dubos, *op. cit.* (10), pp. 69–85.

30 Dubos, *op. cit.* (10), pp. 87–90.

31 MacLeod, 1957, *op. cit.* (19), pp. 539–549; Dubos, *op. cit.* (10), pp. 173–176.

32 Dubos, *op. cit.* (10), pp. 35–68.

allowed physicians of Avery's generation and the one following to pursue laboratory rather than clinical careers for the first time. The shift in the framework, the existence of places like the Rockefeller Institute and the reductionist attitudes there are clearly sociological influences on Avery's work.³³

But it does not seem to stretch the point too far to claim that the continuation of the transformation work in Avery's laboratory, especially between the early 1930s and early 1940s when no-one else seemed to regard it as interesting, owes a great deal to Avery's unique research style. The driving force was his persistent urge to challenge the ingenuity of experimental design skills, either his own or those of his younger colleagues whose procedures he influenced in his subtle and unassuming way. The work was not, perhaps, central to his major research programmes in practical immunology and yet, despite discouragement and black spots, the work was not abandoned and it continued long enough to be 'transformed' into the basis for a search for a molecule with genetic properties. Only his laboratory kept on with it. Perhaps the best analogy is the studio of a Renaissance artist. The master himself designed and planned the works, putting his own hand to the important areas and encouraging and training his apprentices to both emulate his style and eventually stand on their own feet. This influence persisted despite Avery's physical absence and non-participation in the programme when both Dawson and MacLeod did their most important work, while Avery himself initiated very little experimental work after 1935.

If it is true that the transformation work was a direct consequence of his unique style it seems reasonable to see this style as a reflection of the kind of individual he was. This has to remain a reasonable assumption rather than a demonstrated link because the materials which might prove it, the records of his personal life, have not survived. This should alert us all to the need to collect archival material on science and scientists in the modern era³⁴ if we are ever to understand the motor of this most significant component in the history of the twentieth century. I propose that we ascribe this critical step in the origin of molecular biology, the discovery that transformation is caused by DNA, to the influence of a specific personality in a particular scientific context, giving more weight here to the personal character and history of the scientist than to such alternatives as the internal logic or opportunism of evolving research fields (the discovery and purification of nucleic acid digesting enzymes or the refining of the chemical basis of immuno-specificity in the 1930s, for instance) or the influence of social and institutional forces operating upon Avery and his laboratory from a higher plane of organization.

³³ Dubos, *op. cit.* (10), pp. 5–12.

³⁴ N.C. Russell, 'Towards a history of biology in the twentieth century. Directed autobiographies as historical sources', *British Journal for the History of Science* (1988) 21, pp. 77–89.