

Perspectives

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The Transformation of Genetics by DNA: An Anniversary Celebration of AVERY, MACLEOD and McCARTY (1944)

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THE publication of AVERY, MACLEOD and McCARTY (1944) just 50 years ago 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 (LEDERBERG 1959, 1993a).

AVERY *et al.* (1944) can be dissected into the following observations, claims and tacit extrapolations, which may be paraphrased as:

a) Certain bacteria (pneumococci) have clonally inherited attributes, notably serospecific polysaccharide capsules. These are associated with virulence and can be selected accordingly, by inoculation into mice or by serological reagents.

b) The genetic Anlage of these attributes can be transferred from clone to clone by cell-free extracts: the phenomenon of transformation. The transformed cells faithfully transmit their new phenotype to succeeding clonal generations, as had been established by GRIFFITH (1928) with crude, heat-killed cell suspensions.

c) The chemical structure of that transforming principle is DNA, to the exclusion of protein or other macromolecules.

Founded on these claims, the following radical ideas emerged:

d) Bacteria have discrete, autonomous genes analogous to those of higher life forms (*viz.* *Drosophila*).

e) The gene is DNA, and the transformation phenomenon affords the first bioassay for genes extractable *in vitro*.

f) Accordingly, bacteria might be favored subjects for genetic investigation and eventually for technological application of molecular genetic science.

I recite these principles with some nostalgia: they are precisely how they came across to me as an undergraduate already working on *Neurospora* in FRANCIS RYAN's laboratory at the Columbia University Zoology Department in Morningside Heights, New York's upper West Side. Elsewhere, I have noted how they vectored my own career aspirations into the pioneering of bacterial genetics (LEDERBERG 1987).

Studying in the academic archipelago called New York, I was uniquely well situated to observe and sometimes participate in the debate. The Rockefeller Institute was across town, overlooking the East River near the 59th Street bridge. ALFRED MIRSKY, likewise a senior member there, was a frequent visitor to Columbia to collaborate with ARTHUR POLLISTER. From 1942 on I heard a good deal of the progress in AVERY's laboratory. Reprints of the AVERY *et al.* (1944) article were circulated in the department. I borrowed one from HARRIETT TAYLOR (later EPHRUSSI), a graduate student working on yeast budding kinetics, who would shortly join AVERY's laboratory for her postdoctoral research. My personal exclamatory notes were "... unlimited in its implications, ... Direct demonstration of the multiplication of transforming factor ... Viruses are gene-type compounds [sic]. ..."

While MIRSKY was the principal herald, he was also a dogged critic of the claim that DNA, alone, had been proven to be the exclusive chemical substance of transforming activity (MIRSKY and POLLISTER 1946). That was indeed a difficult proposition: AVOGADRO's number is a formidable protagonist in that contest. My stance was sympathetic to MIRSKY's: I felt that so crucial a claim should not be impulsively grafted into the corpus of science as if by first intention. More important than doctrinal conversion was that the issue was squarely on the table and could be settled by

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overwhelming experimental analysis. Previous fiascoes had darkened the history of biopolymers: WILLS-TATTER's claim of enzymatic activity of protein-free preparations and WENDELL STANLEY's initial claim in 1935 that crystallized tobacco mosaic virus was a pure protein. AVERY himself was an epitome of caution, having had to weather similar skepticism of his conclusion that pneumococcal polysaccharide, devoid of protein, was a type-specific antigen. The main fruit of the debate was to stimulate a range of further enquiries: CHARGAFF on the base composition of DNA and my own on other modes of gene recombination in *E. coli*. And MACLYN MCCARTY, later joined by ROLLIN HOTCHKISS, added much to the repertoire of enzymatic and analytical refinements for the exclusion of protein from the DNA preparations (MCCARTY 1946; HOTCHKISS 1979). WATSON and CRICK perhaps owe some debt to MIRSKY's obstinacy. PAULING, who had collaborated with MIRSKY on protein denaturation, was led to delay entering the marathon for solving the DNA structure (WATSON 1968).

Conceptually, DNA in the 1940s was an unlikely candidate for biological specificity. The root problem was the unavailability of any homogeneous sample of DNA appropriate for detailed chemical analysis. This would have to await studies with tiny DNA viruses, and much help from precisely targeting restriction enzymes. DNA was then believed to be a monotonous structure, perhaps even merely a tetranucleotide, harkening back to PHOEBUS LEVENE's analyses. The protein-enthusiasm evoked by the successful crystallization of enzymes in the 1930s then dominated most biochemists' attention.

In fact, DNA was more popular at the turn of the century: "A tempting hypothesis, suggested by Mathews on the basis of Kossel's work, is that nuclein, or one of its constituent molecular groups, may in a chemical sense be regarded as the formative centre of the cell which is directly involved in the process by which food-matters are built up into the cell-substance" (WILSON 1906, p. 340).

By 1925, WILSON was discouraged and misled by the apparent loss of chromatin (basophilia) in the nucleus of the growing oocyte:

These facts afford conclusive proof that the individuality and genetic continuity of chromosomes does not depend upon a persistence of 'chromatin' in the older sense (*i.e.*, basichromatin). It is the expression of a morphological organization that is not destroyed by those chemical and physical transformations that lead to a netlike structure and a change from the basophilic to the oxyphilic condition (WILSON 1925, p. 351).

Just as these words were being written, ROBERT FEULGEN developed the fuchsin-bisulfite cytochemical reaction that offered the first authentic cytochemical indicator for DNA and restored confidence in the

continuity of the DNA content of the chromosome. (CLARK and KASTEN 1983).

The biological interpretation of the pneumococcus transformation was also fraught with uncertainty. DOBZHANSKY, and later BOIVIN, persisted in describing the phenomenon as a "directed mutation," and it was given overtones of "cytoplasmic inheritance" by SONNEBORN—these were all rhetorical devices intended to seal off a vaguely understood phenomenon from the sureties of chromosomal inheritance. Nothing was known of chromosomes or genes in bacteria at that time: a certain leap of faith was required to relate the transformation (and therefore, in turn, DNA) to mendelizing genes. For many years, the only marker studied was the capsular polysaccharide. In that setting, even HARRIETT TAYLOR (1951), reporting from the Rockefeller Institute, remarked, "No bridge can be seen leading over into classical genetics," and in private correspondence criticized my own efforts to do precisely that. Among early comments from geneticists, MULLER's (1947) was the closest to the mark:

... the most probable interpretation of these ... pneumococcus results then becomes that of [a] type of crossing over, though on a more minute scale ... [involving] viable bacterial 'chromosomes' or parts of chromosomes [penetrating] the capsuleless bacteria and in part at least taken root there ... However, unlike what has so far been possible in higher organisms, viable chromosome threads could also be obtained from these lower forms for *in vitro* observation, chemical analysis, and determination of the genetic effects of treatment.

In a retrospection over prior hypothetical interpretations of the transforming principle, seven alternatives could be listed (LEDERBERG 1956):

1. It was a specific mutagen with a special ability to direct a particular gene to mutate in a definite direction.
2. It was a polysaccharide autocatalyst (perhaps as a complex with DNA) that primed an enzymatic reaction for polysaccharide synthesis.
3. It was a bacterial virus, which on infecting the bacteria provoked capsular synthesis as a host reaction.
4. It was an autonomous cytoplasmic gene or a morphogenetic inducer.
5. It acted at a distance without penetrating the bacterium.
6. It was a fragment of the genetic makeup of the bacterium, the only one to have been tested to that time.
7. It was an element *sui generis* for which no general conception should be adduced.

Some of these were not logically distinguishable, but were no less strongly held semantic strongholds. The notion that the transformation was indeed a gene transfer by DNA was eventually solidified by new

work with markers other than the capsule, and especially by the linkage of mannitol fermentation and streptomycin resistance (HOTCHKISS and MARMUR 1954). It was also bolstered by other phenomena of gene transfer, such as conjugal exchange in *E. coli* (LEDERBERG 1947) and virus-mediated transduction in *Salmonella* (ZINDER and LEDERBERG 1953). Finally, the monopoly of the pneumococcus on transformation—and this was a notoriously difficult experimental system—was broken by ALEXANDER and LEIDY's (1951) report on *Hemophilus*, so that a stream of other workers could provide mutual confirmation and reinforcement about the biological interpretations.

The debate about DNA chemistry petered out by sheer exhaustion of the critics and by the conceptual plausibility of DNA as gene, introduced by WATSON and CRICK's double helix model (1953). HERSHEY and CHASE's (1952) study of the injection of phage DNA into *E. coli* lent further support to the "DNA only" view; however, this was quantitatively less rigorous than MCCARTY and HOTCHKISS' prior work on the pneumococcus. Even after 1953, HERSHEY himself was still referring to something more than DNA as a possibility. It might be said that rigorous proof was concluded only with the enzymatic and chemical synthesis *in vitro* of biologically active DNA (KORNBERG 1960; KHORANA 1969).

AVERY *et al.* (1944) was originally published in a medical journal of The Rockefeller Institute that was not habitually read by geneticists of that time. This has led some commentators to compare the launching and reception of AVERY *et al.*'s claims to the so-called prematurity of MENDEL's ideas in the last third of the 19th century (STENT 1972; WYATT 1975). Mendel was little known and for the most part ignored by his contemporaries. But I would argue that the critical reception initially given to AVERY *et al.* (1944) exemplifies the critical scientific method at its most functional (MERTON 1973). Far from being ignored, the paper enjoyed almost 300 citations between 1945 and 1954 (Science Citation Index 1945–1954), not to mention many more earned by MCCARTY's elaborations (1946). The first in GENETICS was LEDERBERG (1947). The *Annual Review of Genetics* did not exist at that time, but SEWALL WRIGHT (1945) reviewed the work in the *Annual Review of Physiology* and it was also noted by no less than three reviewers (GULLAND, MUELLER and KALCKAR) in the *Annual Review of Biochemistry* that same year. It was so well known during that decade that, as I can tell from my own experience, it was often cited by indirection, without specific reference (*e.g.*, LEDERBERG and TATUM 1946; LEDERBERG 1959).

To return, then, to attributions of "prematurity," this might mean either that the data do not exist to explain all of the paradoxes and challenges of a new

discovery, and the claims then meet critical resistance, or that the audience is incapable of understanding the challenge. The touchstone is plainly the operational reaction. For AVERY *et al.* (1944), and MCCLINTOCK (1953) as well, this comprised open controversy and active inquiry. For MENDEL, this was oblivion and a long delay before rediscovery. Happily, such examples are few and far between. In the long run of scientific advance, for a work to be ignored is perhaps only slightly worse than to be swallowed whole. A lot of revision looms ahead even for our well established dogmas (LEDERBERG 1993b).

That AVERY and his colleagues failed to win the Nobel Prize has repeatedly been a subject of critical remark. WENDELL STANLEY (1970) openly apologized for not having been more attentive to that lack of recognition, after he had won his own prize in 1946. In 1958, it came to me to plan my own Nobel lecture, the first in the field of genetics since MULLER in 1946. Rather than recite my own work on bacterial recombination, I thought it more important to acknowledge how genetics had been totally transformed by these discoveries: this was embodied in the lecture entitled "A view of genetics" (LEDERBERG 1959). AVERY had consummated this research at the very end of his career and died in 1955 before a full round of recognition could be fulfilled. The survivor of that team, MACLYN MCCARTY, has written a vibrant memoir (1985) that is a model for expert and methodical tackling of very difficult technical problems. It displays the highest ideals of the scientific personality and leaves no doubt of the importance of his role, together with that of his colleagues, in the pivotal discovery of Twentieth Century biology.

Spanning more than a decade of often frustrating effort, that discovery is an outstanding example of the feedback of clinically motivated inquiry to the most basic issues of fundamental biomedical science (BEECHER 1960). Genetics, especially as we explore the human genome, will be fraught with many more like opportunities, and precisely because of their pervasive applications with commensurate dilemmas. Many institutional arrangements today nurture such transdisciplinary and vertically integrated research, which is often the arena of the most revolutionary advances. Before the federalization of biomedical research financing since World War II, The Rockefeller Institute was very nearly the only site where this could have taken root.

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A vast effort of scholarship and erudition on the history of DNA offers easily accessible guides to the primary sources; see: OLBY (1974, 1990), JUDSON (1979), PORTUGAL and COHEN (1977), CLARK and KASTEN (1983), MOORE (1985), SAPP (1990), WOLFF and LEDERBERG (1944) and WATSON and TOOZE (1981). In addition, memoirs by DUBOS (1976), HOTCHKISS (1979), MCCARTY (1985), CHARGAFF (1978), KORNBERG (1989), WATSON (1968) and CRICK (1988)

add indispensable personal perspectives. I have referred to primary sources primarily to document or accent particular items under debate.

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