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# The discovery of pneumococcal type transformation: an appreciation

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The remarkable paper by Fred. Griffith on the significance of pneumococcal types, reproduced in the preceding pages of this *Journal*, in which it was first published 38 years ago (Griffith, 1928), describes a series of careful and painstaking experiments which show beyond doubt that the ability to produce a polysaccharide capsule can regularly be restored to 'rough' (R) strains of pneumococci which have lost it, by the subcutaneous inoculation of mice with a mixture of a small number of the living R bacteria and an excess of heat-killed, capsulated (S) virulent bacteria. The inoculated mice frequently died from septicaemia and virulent, capsulated S organisms could be isolated from the blood. On the other hand, in control experiments, the injection of suspensions of heat-killed S bacteria alone never yielded living organisms, while recovery of virulent S organisms by (mutational) reversion, following inoculation of living R bacteria alone, was a rare event. Griffith called this phenomenon 'transformation' and, at least in the field of bacterial genetics, this name is still specifically used to describe it.

Griffith found that transformation occurred most frequently when the R strain to be transformed originated from the same capsular type as the transforming bacteria, the R bacteria being merely restored to their original capsular type. However, the main interest of the phenomenon, both then and subsequently, centred on the discovery that R pneumococci originating from one type (say, type II) could be permanently transmuted into another type (say, type I or type III) corresponding to that of the heat-killed bacteria with which it was inoculated.

Griffith's abiding interest, and his life's work, was the epidemiology of infectious disease and he believed that the proper understanding of epidemiological problems lay in more detailed and discriminating knowledge of infectious bacterial species and of the nature of bacterial variation. For him, therefore, as for nearly all medical bacteriologists of his time and for many years thereafter, the importance of transformation of pneumococcal types rested on the light it might throw on such problems as the evolution of bacterial virulence, the rise and fall of epidemics, and variations in the incidence of type infections. By any yardstick, the demonstration that such dramatic and specifically directed transmutations of both type and virulence might occur with considerable frequency, in epidemiologically well-defined types of bacteria, was a startling enough revelation.

Griffith concentrated on providing convincing evidence that the phenomenon of transformation was a fact although, as he says: 'A few years ago the statement

that a type I strain could be changed into a type II or a type III would have been received with greater scepticism than at the present day' (Griffith, 1928), largely because of his own prior studies of the mutational loss and recovery of virulence and type character. Nevertheless, he appears to have hesitated for some time before publishing his results (see Obituary, 1941). He did attempt, and failed, to demonstrate *in vitro* transformation as well as the transforming activity of cell-free extracts, later established by Dawson & Sia (1931) and Alloway (1933) respectively, but it must be admitted that he does not seem to have tried very hard; nor, so far as I can find, did he publish any further papers on the subject.

Despite his preoccupation with the epidemiological aspects of his discovery, it is strange that Griffith does not draw attention to, much less stress, what now seems to us the most striking feature of transformation, namely, that it results in an *hereditary* alteration of character wherein, of course, lies its real biological significance. The nearest he got to analysis of the phenomenon in depth was the postulation, based on the relative thermolability of the capacity of certain (type I) heated suspensions to transform, that it might be mediated by a 'specific protein structure of the virulent pneumococcus which enables it to manufacture a specific soluble carbohydrate' (Griffith, 1928), rather than by the polysaccharide itself. It is most probable that this hypothesis is simply a descriptive one, and was not intended to carry any enzymic or genetical overtones. Griffith can hardly be criticized for failing to carry his analysis further for, apart from his over-riding interest in the epidemiological aspects of transformation, the climate of knowledge of his day offered few clues which could have served him as a basis for further work or even speculation.

I began by describing Griffith's paper as remarkable, and have already given some reasons why it should have been so regarded by his contemporaries. What Griffith did not know and, sadly, did not live to see, was that his work was the fuse of a time bomb whose explosion 16 years later ushered in the greatest revolution in biological knowledge of the twentieth century. Following the demonstration by Alloway (1933) that the transforming principle was present in cell-free extracts of donor strains of pneumococci, O. T. Avery and his colleagues began a systematic analysis of its chemical nature, culminating in a convincing mass of evidence that it is composed of pure, highly polymerized deoxyribonucleic acid (DNA) (Avery, MacLeod & McCarty, 1944). Apart from the chemical evidence, it was shown that the activity of transforming preparations remains unaffected by treatment with proteolytic enzymes and ribonuclease (RN-ase), but is rapidly and completely destroyed by the enzyme deoxyribonuclease (DN-ase) (McCarty & Avery, 1946). Subsequent purification studies virtually excluded the possibility that the active agent in transforming preparations could be any substance other than DNA (Hotchkiss, 1952). Finally, in more recent years, it has been shown not only that radioactive phosphorus incorporated into transforming DNA is transferred irreversibly to the DNA of the transformed bacteria, but also that the amount of transfer is proportional to the number of transformants (Goodgal & Herriot, 1957; Lerman & Tolmach, 1957).

In 1944, when transforming principle and DNA were first equated, the chemical

nature of genetic material was still a mystery. It was known, of course, that both DNA and protein were intimately associated with the chromosomes, but only protein had been shown to display specificity and was thought to possess enough complexity of structure to carry the vast number of specifications which are the inheritance of even the simplest of creatures. Accordingly there was widespread reluctance to draw general conclusions from the facts about transformation. Progress developed along two lines. First, it soon became apparent that transformation is far from being a very special phenomenon restricted to capsule formation in pneumococci, but occurs in many bacterial genera and species, and involves as many characters as can be recognized and selected for. It should also be remembered that the occurrence of transformation may be difficult to observe. It depends on the ability of the recipient bacteria to take up the transforming DNA molecules; the state of competence, during which they can do this, may exist for only a small fraction of the growth cycle, or be dependent on subtle environmental factors, while the conditions for its expression may differ widely between different species. Nevertheless, apart from *Diplococcus pneumoniae*, the occurrence of transformation has now been reliably reported in various species of *Agrobacterium*, *Bacillus*, *Escherichia* (one strain only), *Haemophilus*, *Neisseria*, *Rhizobium*, *Staphylococcus*, *Streptococcus* and *Xanthomonas*, while a wide variety of characters, including morphological features such as filament, capsule and spore formation, the production of specific antigens, resistance to a considerable range of antibacterial agents and synthesis of a large number of specific enzymes mediating steps in amino acid synthesis and in the fermentation of sugars, have been shown to be transformable (see Ravin, 1961).

The second line of development was the increasing evidence that transformation behaves as if it were due to the transfer of fragments of genetic material (chromosome) from donor to recipient bacterium, where it pairs with the homologous region of the recipient chromosome; part of the immigrant fragment then replaces the corresponding region of resident chromosome by a more or less random process of genetic exchange or 'crossing-over' (Ephrussi-Taylor, 1951). The evidence for this is briefly as follows:

(1) Transformation can occur in both directions; thus, for example, not only can R strains of pneumococci be transformed to S, but S pneumococci yield R transformants if treated with DNA from an R donor strain.

(2) The transformed character is not simply added to the phenotype of the recipient bacteria, but replaces its corresponding, or allelic, character.

(3) Wild-type recombinant bacteria can be obtained by transformation between two parental strains which are defective in the same character. A probable instance of this was first described by Griffith (1928: table XV) who obtained smooth type II pneumococci by inoculating mice with a mixture of living R bacteria derived from type II and heat-killed R bacteria derived from type I (see below).

(4) With regard to certain pairs of characters, joint transformation is possible. In pneumococci, for example, the characters of streptomycin-resistance (or sensitivity) and mannitol fermentation (or non-fermentation) are inherited together in transformation with a very much higher probability than can be accounted for by

chance, and irrespective of which combination of alleles (*str-s.mtl*$^+$ and *str-r.mtl*$^-$, or *str-s.mtl*$^-$ and *str-r.mtl*$^+$) characterizes the donor and recipient strains (Hotchkiss & Marmur, 1954; for other examples see Ravin (1961). This means that the determinants of such pairs of transformable characters must be arranged in a fixed relationship to one another, and sufficiently closely as to be often transferred together on the same molecule of DNA. This type of genetic 'linkage' is also a feature of transformation in *Haemophilus* and *B. subtilis* although quite different kinds of characters are involved, and is irrefutable evidence that the DNA involved in transformation constitutes fragments of the genetic material itself.

It is interesting to look, in retrospect, at Griffith's original transformation results and to interpret them in the light of genetical analysis and of what we now know of the biochemistry of pneumococcal polysaccharide synthesis (Jackson, 1962; Mills & Smith, 1962). In general it turns out that capsular synthesis involves a pathway mediated by a number of different enzymes and, therefore, of different genes, and that some at least of these genes are not only transferred on the same DNA fragment but are also very closely linked. Moreover, part of the pathway is common to different pneumococcal types so that genetic blocks there, leading to failure of polysaccharide synthesis, can be repaired by transforming DNA from the other types. In contrast, genes determining enzymes responsible for the specificity of type polysaccharides are usually mutually exclusive alternatives, or alleles, and so cannot coexist or participate in mutual repair, but can only substitute for one another.

In Fig. 1, the various categories of transformation discovered by Griffith are expressed in terms of genetic exchanges. Two genetic regions are shown, of which *A* determines that part of the synthetic pathway common to the synthesis of types I and II polysaccharide, while *B* is a region (or gene) conferring type specificity. In transformation, as indeed in all types of bacterial sexuality, because the donor genetic contribution is fragmentary, at least two (and in any case an even number) of genetic exchanges are required to yield a complete recombinant chromosome. As Fig. 1 shows, the production of a particular transformant type depends on whether the mutation in the recipient is in the *A* or *B* region as well as on the positions of the genetic exchanges in relation to these regions.

In the case of one of Griffith's strains (R 4, type II; tables VII–XII) we can be sure that the mutation leading to loss of capacity to produce type II polysaccharide involved region *A*, mediating that part of the pathway common to types I, II and III polysaccharide. The transformation of this strain to type II capsulation by heterologous types indicates that its *B* region, determining type II specificity, must have remained intact, while the concomitant production of types I and III transformants by killed, capsulated type I (table XI) and type III (table XII) donors respectively, reveals the interchangeability and, therefore, the similarity of the *A* regions of the three types. Continuing this line of reasoning, let us examine the last result shown in Fig. 1 where a single mouse, receiving a mixture of heat-killed, non-capsulated type I bacteria (R I) and living, non-capsulated type II (R II) recipients, yielded capsulated type II organisms. Let us also assume, as is likely, that this single result was due to transformation. In this case the original

loss of type II capsulation must have been due to mutation in the *A* region since type II capsule was restored, but, as Fig. 1 shows, we cannot say whether the *A* or *B* region of the R I donor strain was defective. Nor do we get much help from Griffith's paper since the particular R I mutant used is not specified and might have been any one of a number of independent R I isolates (see table XIII). If we assume that both of the non-capsulated strains under discussion were defective in the *A* region, then the production of capsulated progeny probably resulted from recombination between mutational sites in the same or two very closely linked

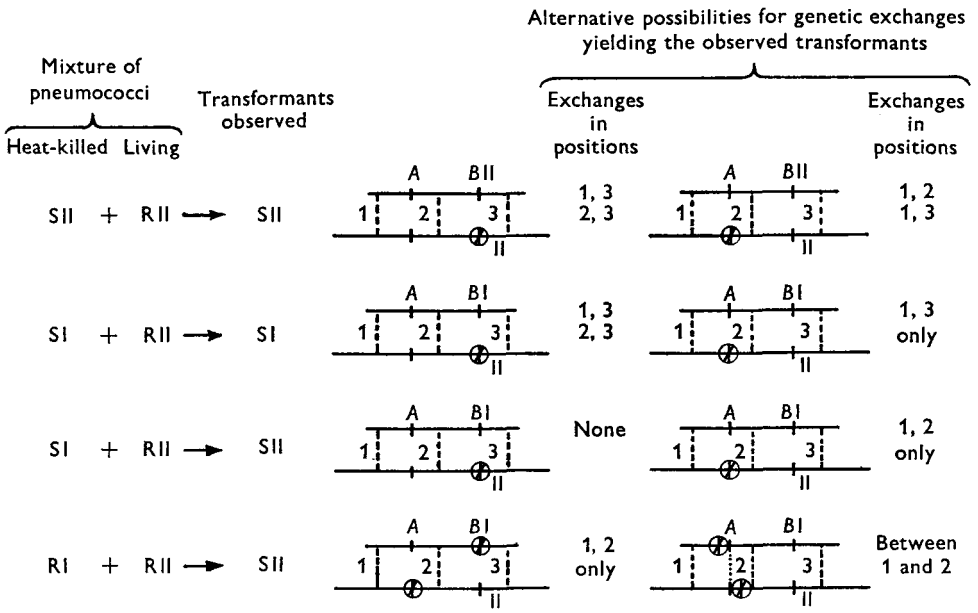


Fig. 1. An interpretation of some of Griffith's (1928) transformations of pneumococci in genetical terms. The pneumococcal strains used in each experiment, and the types of the resulting transformants, are shown on the left; S I and S II indicate capsulated strains of types I and II respectively, while R I and R II are non-capsulated, rough variants (mutants) derived respectively from types I and II pneumococci. The diagrams on the right half of the figure show, for each experiment, the positions of genetic exchanges which could yield the observed transformants. The lower and longer of each pair of lines represents the chromosome region of recipient bacteria which determines capsular polysaccharide synthesis; the upper and shorter line represents the corresponding fragment of donor chromosome (DNA). The chromosomal regions marked *A* are concerned with that part of the biosynthetic pathway common to types I and II polysaccharide; the regions marked *B* determine the type specificity of the polysaccharide, indicated by the suffix I or II. The alternatives shown for each experiment depend on whether the mutation resulting in loss of capsulation involved a gene in the *A* or the *B* region. The site of mutation is indicated by the symbol —⊕—. The interrupted, vertical lines represent the positions of genetic exchanges, of which two are necessary to produce a viable transformant. The type of transformant resulting from these exchanges depends on the particular part of the donor fragment which they incorporate into the recipient chromosome. This can be found for any pair of exchanges by tracing along the recipient (lower) chromosome from the left, then up to the donor fragment at the first exchange point and, finally, down to the recipient chromosome at the second exchange point. Further explanation in text.

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genes. Thus, in addition to being the first to perform and record the results of genetic crosses in bacteria, Griffith may also, however unwittingly, have made the first analysis in any living creature of what is now called the genetic fine structure. The order of scale involved is set by the fact that all recombination events leading to transformation of any kind occur within the dimensions of transforming fragments of DNA, which usually have a mean molecular weight of about $10^7$ and are long enough to carry some twenty genes; this is about one-hundredth the length of the whole bacterial chromosome and corresponds to about one-hundred-thousandth the total chromosomal DNA of a mouse cell.

The new certainty arising from chemical and genetical research into the nature of transformation, that the genetic material, at least of bacteria, consists of DNA, stimulated increasing study of the chemical and physical structure of this nucleic acid. Pre-eminent among these studies were the X-ray diffraction analyses of M. F. H. Wilkins and his colleagues (Wilkins, Stokes & Wilson, 1953; Franklin & Gosling, 1953). Finally, precisely a quarter century after the publication of Griffith's paper, the culminating step was taken by Watson & Crick (1953*a*), who, by a brilliant synthesis, fitted together the chemical and diffraction data into a symmetrical, double-helical structure which not only conformed to all the known facts but also possessed all the inherent properties one would expect of genetic material. Once the model of DNA was constructed, it immediately became obvious how the genetic material replicates, how it carries genetic information, and why it suffers heritable mutation (Watson & Crick, 1953*b*). This model, of an elegant and beautiful simplicity, has stood the test of time and there is now no doubt that the genetic material of all cells, whether animal, plant or bacterial, consists fundamentally of DNA. Only in the genetic material of some viruses is DNA substituted by its close but single-stranded analogue, ribonucleic acid (RNA).

The effect which this discovery has had on our basic biological conceptions has been so rapid and profound as to constitute a revolution. However this is another story, which although perhaps derivative from Griffith and his work is no longer of direct relevance to it. On the other hand, it would be relevant, and might be of interest to some, to conclude this retrospective appreciation with a brief summary of a few of the ways in which transformation itself has proved of value to medical and biological research.

Speaking broadly, transformation may play two distinct types of role in biological research. First, it can be used more or less directly in the analysis of bacterial behaviour and, indeed, there are some genera and species in which it is the only method of genetic analysis. I will give two examples of the kind of information it can provide. First, when DNA from strains of penicillin-resistant pneumococci is used to transform sensitive strains to resistance, the transformants show only a fraction of the degree of resistance of the donor strain. However, the low-level resistance of the transformants can be further increased in a stepwise manner by subsequent, successive exposures to the same DNA preparation until the donor level of resistance is reached (Hotchkiss, 1951). This pattern of acquisition of resistance has been shown to be characteristic of the majority of antibiotics. It confirms what had previously been inferred about penicillin from mutation studies

(Demerec, 1945), that high resistance results from the summation of a series of independent mutations, usually in unlinked genes; in transformation these genes are carried on separate DNA molecules so that normally only one is taken up at a time by any particular recipient bacterium. In contrast, transformation to high-level resistance to streptomycin, due to a single mutation, is achieved in one step.

Transformation has recently been put to rather different use in demonstrating that replication of the chromosomal DNA of *B. subtilis* begins at a particular point on the chromosome and is polarized, that is, proceeds in the same direction in all the bacteria of a culture (Sueoka & Yoshikawa, 1963; Oishi, Yoshikawa & Sueoka, 1964). Stationary phase bacteria or spores, both strands of whose DNA have been made denser than normal by growth in a medium containing heavy nitrogen ($^{15}N$) and heavy water ($D_2O$), are seeded into light medium containing $^{14}N$ and $H_2O$. At intervals during the first division cycle thereafter, samples of the bacteria are removed and their DNA extracted. The newly synthesized DNA, having one old, heavy and one new, light strand and, therefore, of intermediate density, is then separated from the initial heavy DNA molecules in a density gradient, and tested for its ability to transform recipient bacteria with respect to a range of genes known to be distributed along the chromosome. The genes are found to appear in the newly synthesized, transforming DNA in a strict and reproducible sequence as replication of the chromosome proceeds; only at the end of the cycle can the preparation of newly synthesized DNA transform with respect to all the genes.

The second role of transformation in biological research rests on the fact that it is the only way in which the effects of defined physical or chemical alterations, or of radiations, on the biological activity of DNA can be measured. For example, the phenomenon of photoreactivation has been found to be due to an enzyme which, *in vitro* and in the presence of visible light, can restore transforming activity to DNA which has previously been inactivated by ultraviolet light (Rupert, 1961). There is no doubt that when biologically active DNA is finally synthesized in the test tube, the criterion of its activity will be its transforming ability.

In conclusion we may note the discovery of another kind of transformation, provoked by the prior knowledge that large molecules of nucleic acid can penetrate semi-permeable cell membranes. This is the ability of purified, viral nucleic acid to infect cells and promote the synthesis of both new viral nucleic acid and protein, and the liberation of complete, infective virus particles (Gierer & Schramm, 1956; Fraenkel-Conrat, Singer & Williams, 1957). Just as in the case of bacterial transformation, viral infection turns out to be a genetic phenomenon. Very recently an extraordinary collaboration between these two processes was achieved by the growth of vaccinia virus in *B. subtilis* bacteria which had been exposed, in the competent state, to the viral DNA (Abel & Trautner, 1964). Similarly, using a special technique, it has proved possible to infect *E. coli* with RNA from encephalomyocarditis virus, with the resulting formation of complete virus particles (Ben-Gurion & Ginsburg-Tietz, 1965). Although it is too early to speculate on the future implications of these astounding experiments, enough has been said to show that, in this twenty-fifth anniversary year of Fred. Griffith's death, the phenomenon which he discovered is far from being forgotten.

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