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REMINISCENCES ON GENETICS : FROM MENDELISM TO RECOMBINANT DNA*

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IT is a great honour that the Raman Research Institute Trust has done me by asking me to deliver on this anniversary day, the Gandhi memorial lecture. Mahatma Gandhi's ideals and dedication are badly needed in today's world, mad with violence and crushing individuality. I am going to talk about a field of science which, in my lifetime, has made enormous strides. Most I have witnessed myself. Like in any other field of science the powerful tools it now provides for mankind can be put to good or evil use. This is where Gandhi's ideals and wisdom would be most needed.

An awareness of the existence of heredity, that is of a tendency for variation not to be distributed at random between individuals of a species but to be somehow related to descent, must go back a long time in human history. The fact that offspring resemble their parents more than unrelated individuals is what, no doubt, neolithic farmers first used in improving cultivated plants and domestic animals. The simple device was that of selecting the most desirable individuals as parents of the next generation.

But the history of the attempts towards an understanding of the mechanism of heredity is full of pitfalls and false ideas. From Hippocrates and Aristotle to as recently as Darwin, for instance, the idea persisted that the features of the individual were somehow directly transmitted to its progeny through the germ cells. The pure fiction of the "homunculus", the mini-man believed to be visible through the microscope in the sperm head, was still alive 200 years ago. After all we still say "Johnny has inherited his father's blue eyes" implying something like "Johnny has inherited his father's watch". Yet in the Bible, Genesis 30, we find a garbled account which can be interpreted as showing that Jacob knew how to breed black lambs out of a white flock.

1 Gregor Mendel (1822–1884).

To find a first comprehensive scientific approach to the mechanism of heredity we have to come to Mendel in 1866. He disposed once and for all of any mechanism implying that the characters of the individual were themselves somehow transmitted through the germ cells. He showed that heredity is mediated by factors (we now call them "genes") uninfluenced by the somatic features, and that the somatic features are the consequence of the nature (we now call it "information") of such factors. He also showed that different factors are transmitted separately from each other and distinguished clearly between the genetic constitution of an

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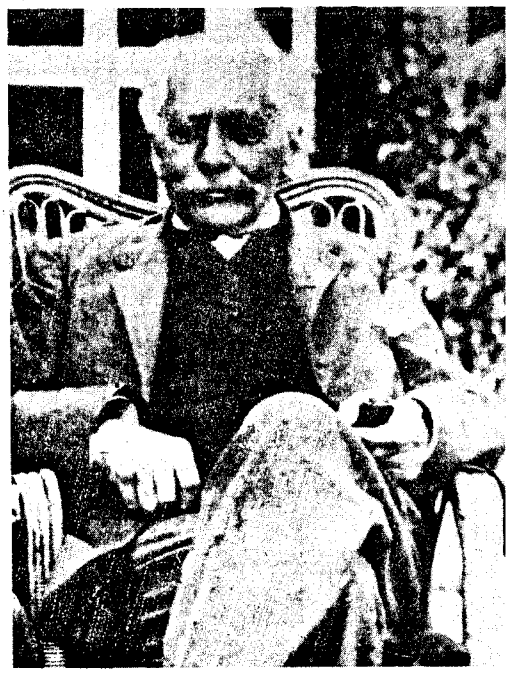
individual— its "genotype" and its characters— its "phenotype".

Mendel contribution was so far ahead of his time that it remained ignored until 1900 when Tschermak, DeVries and Correns rediscovered it. Even Darwin, whose theory of evolution by natural selection of small variations was so much in need of some knowledge of heredity, seems to have been unaware of Mendel's decisive paper.

Of the two main ideas of Mendel, *indirect inheritance* by transmission of information *via* the gametes and *particulate* nature of that information, the latter was qualifed in 1905 by Bateson's discovery of *linkage*. The qualification is that two or more genes need not be transmitted independently, but may be transmitted together in the same gamete in a higher proportion than expected by chance. Thus genes fall into "linkage groups". It is to the credit of Morgan's school at Columbia University, particularly Sturtevant, Muller and Bridges, to have shown that the number of linkage groups in a species corresponds to the number of chromosome pairs in the somatic cells of that species. Evidence of various kinds from other workers, especially that sex determination is based on the distribution of the members of a special pair of chromosomes, led Morgan's school to the generalization which goes under the title of "Chromosome theory of Mendelian inheritance".



2. William Bateson (1861-1926), at the John Innes Institute 1924.

3. H. J. Muller (1890-1967), 1959.

This generalization states that the chromosomes carry the genes in a linear arrangement and the physical distance between two genes on the same chromosome pair is correlated to the frequency with which the two genes are exchanged at meiosis before gametogenesis.

By 1915 the essentials of the mechanism of hereditary transmission were clear. Two basic points were still obscure. One, how do genes exert their effects on the characters of the individuals? Two, what are the properties of the gene material?

The first decisive contribution to the problem of gene action had come earlier from Garrod, a physician in Oxford, in 1908. He had shown that

certain "*inborn errors of metabolism*" in man, each the result of deficiency in a particular enzyme, were inherited as single Mendelian recessives. It took almost forty years, to 1945, for Beadle's generalization "*one gene one enzyme*", refined and expanded in 1956 by Benzer as "*one cistron-one polypeptide chain*".

To the second question - what are the properties of the gene material? Muller gave a brilliant and definitive answer in a paper of 1922. These are *replication* and *mutation*. Replication by itself is not unique to the gene material: crystallization, for instance, is a non-biological analogue. Mutation by itself, as a stochastic process, has also many non biological analogues. But replication *with* mutation, that is replication persisting inspite of mutation, is indeed a unique feature of the gene material. Muller asked what extraordinary structure the gene material must have to give it this *carte blanche* ability to go on replicating in the changed form whenever change has occurred. The answer to this question came in 1953, thirty years later, with Watson and Crick's double helix structure for DNA.

Muller concluded: given a material with these fateful properties, evolution would automatically follow. Remember, this was at a meeting in 1922. Osborn, the distinguished palaeontologist, was in the chair. As Muller reported, Osborn commented: "I am glad you have a sense of humour". The idea of a material, other than the whole cell, with such properties, let alone forming the building blocks for evolution, was outrageous in 1922. We now take it for granted.

In the same paper Muller compared the gene material with bacteriophages and prophetically suggested that a chemical and physical attack on them could throw light on the nature of the genes. Thus Muller qualifies as the forerunner, indeed a founder, of molecular biology.

The telling title of another paper which Muller wrote in 1924-"The gene as the basis of life-"-shows a powerful imagination capable of building models and generalising from the basis of hard experimental evidence. In biology, as distinct from the physical sciences, the combination of deduction and induction was still frowned upon. "Real" biology was supposed to be equal to careful gathering of facts. Darwin, of course, seventy years before had already been a victim of this sort of prejudice, to which he had to reply: how can you gather facts if you do not have a model to test? We can well consider Muller's ideas of 1922 as the second major milestone in genetics, taking Mendel's as the first.

The technology of genetic analysis, and for that of genetic synthesis, based on the chromosome theory made great strides between 1915 and 1950. It also opened the way to many useful applications in animal and plant breeding. But that technology required the experimental use of sexual reproduction and the classification of the kinds and proportions of gametes produced by informative individuals. It could not be applied effectively to organisms in which practical or ethical considerations stood in the way of using sexual reproduction. Man is of course one such organism. Consequently, its genetics was very poorly known in comparison with most other aspects of its biology. This was the situation up to 1968, when the new technology *via.* somatic cell genetics came on to the scene.

Two approaches converged to produce this new technology. One was the development in the early 50s by my colleagues-especially J. A. Roper - and myself of a series of procedures for answering the question: does some sort of gene segregation and recombination occur in somatic cells, albeit as a rare event, and if it does, can it be harnessed to genetic analysis and synthesis? The answer was in the affirmative and we developed procedures first with the mould *Aspergillus nidulans.*

In this mould fusion of vegetative cells occurs regularly and is followed, at a low but manageable rate, by fusion of their nuclei. The nuclei resulting from fusion may undergo during multiplication two processes of segregation and recombination. One is "somatic crossing over", as masterly discovered and analysed by Stern in Drosophila in 1936. The other is a progressive loss of chromosomes. The latter turned out to be most valuable for assigning genes to their chromosomes. This prompted me to suggest, at the CIBA Symposium of 1958, that the same

procedure should be applied to human cells in culture.

The other approach was the development, mainly by Ephrussi and his school, of the techniques for isolating hybrid cells from fusion of mammalian somatic cells in culture. The breakthrough came in 1968 when Mary Weiss and Howard Green found that in hybrids between human and mouse cells the human chromosomes were rapidly eliminated. Application of the same rationale worked out for *Aspergillus* 18 years earlier led to a procedure for identifying which human chromosome carries a given human gene. This procedure does not require mutants of the genes which one wishes to assign to their chromosomes because the differences, particularly electrophoretic, between the human form of a protein and its mouse counterpart can be used, in Ephrussi's expression, as "built-in markers".

Application and refinements of this procedure have given spectacular results in formal human genetics. Up to 1968, except for sex-linked genes, it was not known for even a single human gene which chromosome carried it. Today there are more than 300 so assigned, and for an increasing proportion of them, the analysis goes as far as the location in a chromosome band. Combined with other techniques—in situ annealing, identification or restriction enzyme markers, D.N.A. sequencing etc.—formal human genetics, based on somatic cell fusion, is now one of the frontiers of genetics. It was its Cinderella in 1968.

9. left: Jacques Monod (1919-1976); right: Boris Ephrussi (1901-1979), Cold Spring Harbor 1946.

15. Mary Weiss and Howard Green, 1983

Another extremely useful application of somatic cell genetics is the production of "hybridomas". These are clones of somatic cell hybrids which produce specific antibodies. They were first produced by Milstein and Kohler about 10 years ago. They opened a vast field both in research, for instance, for the identification and study of cell surface proteins, and as potential therapeutic agents, particularly for targeting anti-cancer agents.

Applied recently to higher plants, somatic cell genetics is a floodgate of possible practical and scientific applications. The possibility of growing whole plants from somatic hybrid cells has enormous potentialities not only in plant breeding but in the study of all sorts of basic biological problems: differentiation, evolutionary relationships and many others. Melchers has been one of the pioneers in this field, and produced whole hybrid plants from fusion of somatic protoplasts of potato with those of tomatoes.

As I mentioned before, between 1915 and 1950 Mendelian genetics made great strides in many directions, both fundamental and applied. Among these was the reconciliation of Darwinian natural selection with the new understanding of heredity and mutation. It gave birth between the 20s and 30s to population genetics. The founders of this new approach were Fisher, Haldane, Sewall Wright and Tchetverikov.

By the early 50s Mendelian genetics had produced a picture of the gene material which completely vindicated Muller's pioneer model of 1922. The gene material was viewed as a continuous linear structure of a few kinds of building blocks each capable of mutation and of reciprocal exchange with an homologous one. Individual segments of the material, the genes, determined the ability of a cell to synthesize a particular protein, or, more precisely a particular polypeptide chain. Mutation, as a change in quantity, quality or sequence in a gene, was what determined a change, qualitative or quantitative, in the relevant polypeptide chain.

5. J. B. S. Haldane (1892–1964), at the Internat. Congress of Genetics, Bellagio, 1953.

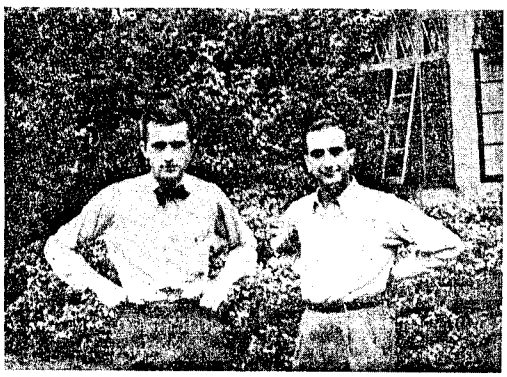
4. R. A. Fisher (1890–1962), on "Queen Mary", 1946.

6. Sewall Wright, London 1980

The fine genetic analysis carried out by E. B. Lewis and M. M. Green in *Drosophila* and by

my colleagues and myself both in *Drosophila* and in *Aspergillus nidulans* had shown that there was no discontinuity in the material of each chromosome, such as was unnecessarily implied in the old picture of genes as beads on a string. Benzer, a few years later, with his masterly analysis of fine genetic structure in bacteriophages gave a complete and definitive picture. The term "cistron", which he coined for a segment of genetic material which codes for one polypeptide chain, has now replaced in precise language the older term "gene".

The question of what the genetic material actually was in chemical terms, rather that what it had to be as deduced from its genetic properties, was still quite open throughout the 40s, in spite of Avery's demonstration in 1940 that the "transforming principle" in *Pneumococcus* was DNA. Even at the 1946 Cold Spring Harbor Symposium on "The genetics of Microorganisms", memorable for Lederberg's demonstration of recombination in bacteria and Hershey and Delbruck's in bacteriophages or for Harriet Taylor's demonstration of *linked* transformation by pure DNA, there was still dispute as to whether the gene material was DNA, nucleoprotein to protein. Even the demonstration by Hershey and Chase a few years later that the DNA of phage, and not the protein, enters the bacterial host cell and there multiplies, did not lead to general acceptance.



7. left: Max Delbruck (1906-1981); right: Salva Luria, Cold Spring Harbor 1946.

8. Max Delbruck, Salva Luria and Harriet Taylor, in the Phage Laboratory, Cold Spring Harbor 1946.

The breakthrough came with the momentous papers of Watson and Crick in 1953. The double helix structure of DNA which they proposed immediately satisfied all the requirements for the gene material which the previous 50 years of work had so precisely identified. Its impact on biology is only beginning to be felt. It is at least as great as that of Darwin a hundred years earlier. No doubt, the double helix is the particularly illuminating third major milestone in genetics.

The Watson-Crick model provided a full answer to what was demanded. It also provided one new idea not at all implicit in the previous work: *colinearity*, *i.e.* the linear sequence of nucleotides in the DNA of the gene corresponds to the linear sequence of aminoacids in the polypeptide chain encoded in that gene.

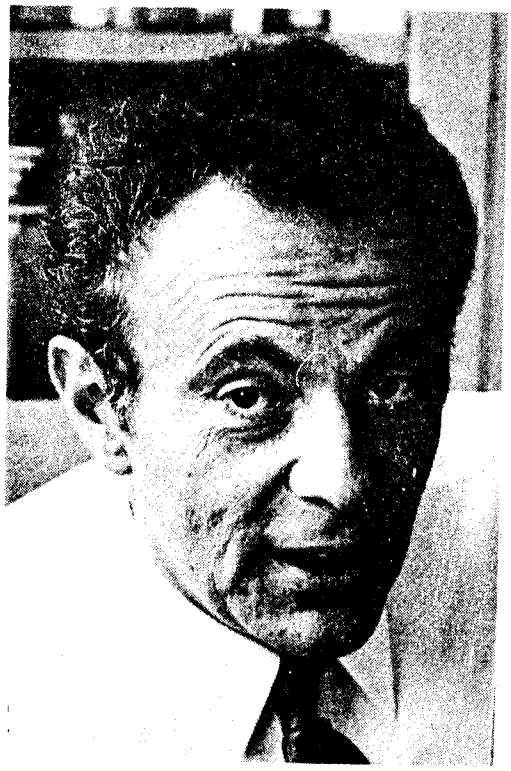
The earlier general view of the relation between a gene and the corresponding polypeptide chain was not that of colinearity. Beadle, for instance, in his important 1945 paper which produced the "one gene-one enzyme" generalization, suggested that a gene imprinted the *final specificity* on the relevant protein. We must remember that Sanger's work showing that the primary structure of insulin was a linear sequence of aminoacids was just beginning to have an impact. Globular proteins were still viewed as very complex tridimensional structures.

Long ago I asked independently Watson and Crick how they had stumbled on the idea of colinearity. The answer was identical: no other idea had ever crossed their mind. This shows how decisive ideas in science often come to those who are not too deeply steeped in a specialized field. Pasteur is a classical example of this, and in the field of DNA., Chargaff, an outstanding and brilliant biochemist, is a control example. He had produced all the data for suggesting base pairing in DNA but it was to two outsiders, far from well versed in biochemistry, to see what they meant.

The developmen's in genetics since 1953 are too well-known for me to dwelve on. I shall only mention the Jacob-Monod model for switching genes on and off. It is still the best that we have for a start on differentiation, especially now that DNA methylation seems to play a basic role in the switch.

We come now to the mid 70s when Arber's work on restriction enzymes opened the flood

10. left: Jim Watson, Swiss Alps, 1953.



14. Francois Jacob, 1982

11. Francis Crick, London, 1978.

12. Werner Arber, 1981.

gate to the latest techniques in genetic analysis and synthesis: the recombinant DNA techniques.

These varied techniques, of great versatility, are extremely powerful tools in research and applications, including the production by genetically engineered bacterial cultures of valuable substances such as human insulin and interferons, renin, growth hormones etc. In research, a striking recent result is the demonstration that the difference between a human bladder carcinoma cell and its normal counterpart stems from a single nucleotide change.

I wish only, in conclusion, to emphasize a point of great intellectual and ethical interest which seems to have been overlooked. We now understand that, *in principle*, segments of DNA of any organism, or even of synthetic origin, can

13. right: Georg Melchers, Versailles 1972.

be transferred to any other organism and there become part of the recipient's genetic make up. In nature this occurs regularly as a consequence of retrovirus infection in animals and probably of *Agrobacterium* infection in plants. In the laboratory, vectors carrying any desired segment of DNA can be constructed routinely and used for transferring it to recipient organisms. Even more simply, microsyringe injection of foreign DNA into cell seems to work. The foreign DNA, transferred by virus, plasmid, injection or cell fusion, may become active part and parcel of the recipient's genome.

Thus, the exchange of gene material, which until recently we thought would occur almost exclusively between individuals of a species, can now be viewed as possible, albeit with probabilities ranging from near zero to very high, between any two living organisms.

We come to the concept that the whole biosphere on Earth shares a common gene pool. A new view of the unity of life on Earth becomes imperative. Let us hope that this unifying view takes roots and helps in realizing Gandhi's ideals.