William Hayes and His Pallanza Bomb Shell

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All these things have never yet been seen
But Scientists, who ought to know,
Assure us that it must be so
Oh! Let us never, never doubt
What nobody is sure about.

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The above lines from Hilaire Belloc's poem 'The Microbe' were quoted by the author of a paper in the *Proceedings of the Cold Spring Harbor Symposium* of 1953. The author is none other than William Hayes who stands out as an example of scientists who have published very few papers in their career but whose works have revolutionised the prevailing notions and concepts and laid the foundations for monumental future developments. The classical work of William Hayes (1913–1994) on bacterial conjugation and gene transfer carried out in the early 1950's triggered major advances in bacterial genetics and, in a broad sense, molecular biology itself. Within a brief span of three years Hayes made three pathbreaking discoveries: (i) demonstrated unidirectional transfer of genes from a donor cell to a recipient cell; (ii) discovered the first bacterial plasmid, namely, the F factor (sex factor) of *Escherichia coli (E. coli)*; and (iii) independently discovered a High frequency recombinogenic (Hfr) *E. coli* strain which transferred chromosomal genes in an orderly manner and at high frequencies, enabling the realisation of a single, circular genetic map in *E. coli.* These advances have revolutionized the then prevalent notions in bacterial genetics and influenced generations of later microbial geneticists. In this brief article I will attempt to highlight the work of Hayes and its impact on bacterial genetics.

Keywords
Bacterial conjugation, fertility factor, unidirectional transfer.

Conjugation, mediated through physical contact between two cells, was discovered by Tatum and Lederberg in 1946.

Bacterial Conjugation in the pre-Hayes Era: The Work of Joshua Lederberg

There are three ways by which bacteria exchange genetic material among themselves: transformation, transduction and conjugation. These processes are now collectively called Horizontal Gene Transfer (HGT). Transformation involves transfer through free, extracellular DNA while transduction is mediated through a bacteriophage. These processes will not be dealt with in this article. The third process, conjugation, is mediated through physical contact between two cells. It was discovered by Tatum and Lederberg[1] in 1946. They observed that when two strains of *E. coli,* each requiring a specific amino acid for growth (auxotrophs) were mixed and spread on a solid medium which lacked both the required amino acids, colonies appeared at a small frequency (about 1 out of a million cells plated). This 'cross' is shown below.

[1] See *Resonance*, Vol.13, No.8, 2008.

$$\text{Strain 1}(A^-) \times \text{Strain 2}\ (B^-) \longrightarrow \text{Colonies which require neither A nor B } (A^+B^+)$$

In the above example, strain 1 requires amino acid A and strain 2 requires amino acid B, (represented as A^- and B^-, respectively). In order to rule out the possibility that either of the strains could have undergone back mutation (reversion) to A^+ or B^+, double mutants were used in the cross. The result was the same as with single mutants, as shown below.

$$\text{Strain 1}\ (A^-B^-) \times \text{Strain 2}\ (C^-D^-) \longrightarrow \text{Colonies of } A^+B^+C^+D^+$$

Since the simultaneous reversion of either pair of mutations to their respective wild-type state could be ruled out on statistical grounds, some 'mysterious' process could have facilitated exchange of genes between the two partners to generate wild-type recombinants (for more information, see [1]).

Lederberg proposed that the above process could be similar to fertilisation in higher organisms, wherein two haploid gametes fuse to form a diploid zygote. He imagined that cells of the two

mating strains could fuse to form a transient diploid entity which could segregate to haploidy after a while. He postulated that genetic exchange between the mating partners could occur during this process. This view was proved wrong by the brilliant work of William Hayes.

Counter-Selection: Unidirectional Gene Transfer

The genetic cross outlined above has been described in the literature as the 'classical cross'. The classical cross looks at the outcome of the cross rather than its mechanism. For instance, in the cross depicted above, $A^+B^+C^+D^+$ recombinants could arise by the transfer of $A^+ B^+$ genes from strain 2 into strain 1 or the transfer of C^+D^+ from strain 1 into strain 2; it is also possible that both events could occur. Scoring the products of the cross does not tell anything about the mechanism. Hayes introduced a small innovation which turned out to be a brilliant one. He set up two parallel crosses. In cross 1, he took one of the strains, say strain 1, as a streptomycin resistant strain (Str^R) and the other as streptomycin sensitive (Str^S) strain. In cross 2, the Str^R allele was reversed between the mating partners, as illustrated below.

Cross 1. Strain 1. A^- B^- Str^R × Strain 2. C^-D^- Str^S

Cross 2. Strain 1. A^- B^- Str^S × Strain 2. C^- D^- Str^R

The yield of $A^+B^+C^+D^+$ Str^R recombinants from both the crosses were selected on a medium which lacked all the four supplements and contained a lethal concentration of streptomycin. The result was that only one of the crosses was fertile, that is, yielded recombinants; the other cross was sterile. This result showed that $A^+ B^+$ genes were transferred from strain 2 into strain 1 which, being streptomycin resistant, survived to give recombinants. Had there been transfer of C^+D^+ from strain 1 into strain 2, cross 2 also should have been fertile. This was not the case. By this simple yet elegant improvisation, Hayes demonstrated unidirectional gene transfer between mating partners. Therefore there is some sexual differentiation in *E. coli* as 'male' (donor) and 'female' (recipient). The males were represented as F^+ (fertility positive) and

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females as F^- (fertility negative). In the above example strain 2 was the donor and strain 1 was the recipient. In these experiments, matings (crosses) were done in the absence of streptomycin and the recombinants were scored in the presence of streptomycin. Subsequent experiments showed that the crosses were fertile even if done in the presence of streptomycin, provided the recipient was streptomycin resistant. The crosses were fertile even when the donors (Str^S) were treated with streptomycin to reduce their viability million-fold and crossed with Str^R recipients. These experiments unambiguously showed that the viability of the recipient is critical for a successful cross.

The Bomb Shell at Pallanza

Hayes published his observations as a note in *Nature* in January, 1952. He also presented them at a meeting of the Society of General Microbiology held at Oxford in April, 1952. It always happens that anything unorthodox or revolutionary is initially received with scepticism. The observations of Hayes were no exception. Interestingly, Lederberg also faced a similar problem when he reported his discovery of bacterial mating and recombination in the Cold Spring Harbor meeting of 1946 (see [1]). However, things changed dramatically when Hayes presented his data at the Second International Symposium on Microbial Genetics held at Pallanza, Italy, in September, 1952. The community of bacterial geneticists immediately grasped the significance of his observations and Hayes became an instant celebrity! James Watson who also attended the Pallanza meeting, was so impressed by the work of Hayes that several years later he wrote in his book *Double Helix* as follows: "... everyone in the audience knew that a bomb shell had exploded in the world of Joshua Lederberg". Although Lederberg did not immediately accept the conclusions of Hayes, namely, unidirectional gene transfer, he did so eventually when the observations turned out to be reproducible in many laboratories, including his own. The Pallanza bomb shell procured for Hayes an invitation from (a half- convinced and reluctant) Max Delbrück to the Cold Spring Harbor Symposium of 1953, where Hayes presented the most definitive account of his experiments

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and hypothesis. With a modesty so characteristic of him, Hayes concluded his presentation at the meeting and the paper that followed with the lines from Hilaire Belloc's poem 'The Microbe' quoted at the beginning of this article. Interestingly, it was at this meeting that James Watson presented the double helical structure of DNA.

Molecular Basis for Sexual Differentiation in *E. coli*

Even before Hayes arrived on the scene, many workers (the Lederberg couple Joshua and Esther, Cavalli-Sforza, Heslot *et al*.) had screened several isolates of *E. coli* for their ability to yield recombinants when crossed with the standard tester strain of Lederberg. It turned out that only a few strains elicited the capacity. In the light of the observations of Hayes 'maleness' is associated only with a few strains and such strains possess 'something' which others lack. A clue to the identity of this 'something' came from the work of Hayes himself and also from the independent work of Lederberg and Cavalli-Sforza. (The reports from both the groups were published back to back in the *Journal of General Microbiology*. Since this article focuses on Hayes, only his work will be highlighted below. This is not to belittle the contribution of the other two).

Hayes noticed that some colonies isolated from an F^+ culture which had been stored for a long time in the refrigerator, had lost the ability to act as donors while others had not. Having been unsuccessful in obtaining such a variant deliberately, Hayes wondered whether the lost fertility could be restored. In an attempt to test this notion, he co-cultured cells from the defective and normal donors overnight, re-isolated the former with the help of mutational markers introduced prior to growth, and re-tested their donor ability. About 30% of them had regained their donor ability. This amazing result showed that the donor ability is something which could be lost and regained. The loss or gain of fertility did not affect the cells in any way except their mating. Therefore it should be an autonomous and dispensable entity and was named F (fertility) factor. Subsequent work by Falkow and

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Brown showed that the *E. coli* F factor is DNA. Hirota showed that an F^+ cell could be converted to the F^- state ('curing') by treatment with the dye acridine orange that can intercalate[2] into DNA.

By this time many features of $F^+ \times F^-$ matings were brought to light. These were: (i) The transmission of the F factor from donor to recipient is very high such that a good fraction of the recipients become donors. The original donor continues to be a donor since, as was shown later, the F DNA is replicated, one copy being transferred to the recipient and the other retained in the donor. (ii) The frequency of recombinant yield is many orders of magnitude lower than that of F transfer. (iii) The recombinants obtained were also F^+ (donors). However, there was no satisfactory explanation as to why the frequency of F transfer was so high relative to recombinant yield and how F transfer brought about gene transfer. These questions got clarified after the discovery of high frequency recombinogenic (Hfr) strains, independently by Cavalli-Sforza and Hayes.

[2] Insertion between a pair of bases.

Discovery of Hfr Strains

Cavalli-Sforza (also referred to as Cavalli) isolated the first Hfr strain in 1950 but its significance was not apparent at that time. Cavalli and Lederberg reported that in Hfr $\times$ F^- crosses the yield of recombinants was very high but the recombinants remained F^- unlike $F^+ \times F^-$ matings, suggesting that the F element was not transferred. They suggested that the F factor was present in a bound form in that strain. Hayes also isolated an Hfr strain as a spontaneous variant from an F^+ population. These two were the first Hfr's to be isolated. They were subsequently called Hfr Cavalli (Hfr C) and Hfr Hayes (Hfr H). Hayes observed that in a Hfr $\times$ F^- mating only a limited number of genes were transferred into the recipient. The notion prevalent at that time was that *E. coli* has three chromosomes and Watson and Hayes reported that in Hfr strains the F factor was associated with one of them.

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Enter Elie Wollman and Francois Jacob

The direct involvement of Hayes in experiments on bacterial conjugation seems to have ended with the discovery of the Hfr strain. Subsequent advances were due to the pioneering work of Elie Wollman and Francois Jacob at the Pasteur Institute and to whom Hayes gave his Hfr H isolate. The duo exploited its potential fully and did remarkable work with it. Details of their work can be found in many text books on microbial genetics, especially the classical treatise *The Genetics of Bacteria and Their Viruses*, Editions I and II by Hayes himself. Readers should consult them and other sources cited below for detailed information. Only some salient points will be outlined here.

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1. The 'interrupted mating experiments' (see Hayes, [2]) whereby the mating was artificially interrupted by using a blender at different time points showed that there is an ordered, unidirectional transfer of genes from the donor into recipients, proceeding from an origin and progressing towards a terminus. The order and time of transfer of genes depends upon the Hfr used. (Although Hfr H and Hfr C were the only Hfr's isolated initially, many more became available later on and were tested). Theoretically, it is possible to transfer the entire donor chromosome into the recipient cells in 100 min. However it seldom happens in practice; (see below).

2. These experiments showed that even without artificial interruption, there is spontaneous breakage of the donor chromosome during transfer as the process progresses. As a result, genes closer to the origin of transfer get transferred predominantly compared to those which lie farther away. Genes located very far from the origin are rarely transferred. This explains the gradient of transfer of genes which was observed during the early years. It also permitted the conclusion that genes could be positioned on a 'linkage map' in terms of their time of transfer in an Hfr $\times$ F^- mating.

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3. Since $F^+ \times F^-$ matings show very low frequencies of gene transfer while the frequency of F transfer was very predominant, it appeared possible that F by itself could not mobilise genes and the observed mobilisation in $F^+ \times F^-$ crosses could be due to the presence of rare Hfr cells in an F^+ population. In a sense Hfr could be looked upon as spontaneous 'mutants' in a population of F^+ cells. If so, their origin could be tested by the classical fluctuation test devised by Luria and Delbrück (see [1]). Wollman and Jacob showed that this was indeed the case. Since Hfr cells could not be cured by acridine orange unlike F^+ cells, the F factor could have integrated into the chromosome, analogous to lysogenisation of *E. coli* by a temperate phage like the lambda phage. This event (conversion of an F^+ into Hfr by chromosomal integration) is similar to the generation of spontaneous mutants in a population.

4. The linkage map initially appeared to be a linear structure but when the time and orders of gene transfer by many Hfr's were examined, it turned out that there was no fixed beginning or end. In other words, the chromosome of *E. coli* was genetically a circle. (Many years later, Cairns showed by elegant autoradiography that the *E. coli* DNA is physically also a circle). The positions of genes were fixed on the circle, but in different Hfr's the circular DNA is broken at different points and transferred in different orientations, one end forming the origin and the other the terminus. To illustrate this idea let us consider the following hypothetical example. Let us say a segment of the circular genetic map is marked as follows:

.....A-B-C-D-E-F-G-.....

The positions of the genes on the genetic map in all the Hfr's remains the same but the order in which they are mobilized during conjugation varies with the Hfr.

In Hfr 1, a break could occur between B and C and during transfer genes C-D might constitute origin-proximal genes and A-B terminal genes. In Hfr 2 the break might be between D and E and transfer could be such that E-F could be origin-proximal genes and C-D distal ones. In Hfr 3 the break might be between E and F and E-D could be proximal and G-F distal genes transferred. It should be clear from the above examples that the positions of the genes on the genetic map in all the Hfr's remains the same but the

order in which they are mobilized during conjugation varies with the Hfr. In other words there is neither an origin nor a terminus to the linkage map; therefore it is a circle. The idea of a circular linkage map (chromosome) in *E. coli* (and other bacteria) is a conceptual breakthrough that emerged with the discovery of Hfr strains. Since it was calculated that it would take 100 min to transfer the entire donor chromosome into recipients, an event not possible in practice because of spontaneous chromosome breakage during transfer (see above), the linkage map could be represented as a circle of 100 min. The position of a given gene on the circle is the time at which that gene is transferred from an Hfr into an F^- during conjugation. As a mark of recognition to William Hayes, the origin of transfer of Hfr H was chosen as the 0/100 min of the *E. coli* linkage map. If genes A, B, C ..... were mobilized at 10, 15, 25 min by Hfr H, they are assigned map locations of 10, 15 and 25 min, respectively. Currently 5000 odd genes are positioned on the linkage map of *E. coli* using many techniques of mapping besides conjugation.

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It is indeed remarkable that the pathbreaking work of Hayes was carried out within a brief span of about 2–3 years! For a long time Lederberg did not accept the conclusions of Hayes as well as Wollman and Jacob although he had himself made similar observations. Instead he tried to explain all the data in terms of his model of fusion between the male and female cells followed by genetic exchange and re-segregation to haploid cells. However, in later years (around 1957) he did accept the Hayes model.

A Brief Biography of William Hayes

Hayes was born on January 18, 1913 at Rathfarnham, County Dublin, Ireland, as the only son of William Hayes (Sr) and Miriam Hayes. He graduated in the Natural Sciences from Trinity College, Dublin, in 1936 and obtained his medical degree from the University of Dublin in 1937. After a few years of stay at the Bacteriology Department of Trinity College, under JW Bigger, he joined the Royal Army Medical Corps in 1941 with the rank of a Major. During World War II, he worked in India, first at Kasauli

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(Himachal Pradesh) and later at Poona (now Pune). This was the time when he developed an interest in bacterial genetics. After being discharged from the army in 1946, he returned to Trinity as a Lecturer in Bacteriology and obtained his ScD degree two years later. In 1950, he moved to London, accepting a Senior Lecturership in Bacteriology at the University of London Post Graduate Medical School at the Hammersmith Hospital. During this period his interests were in the study of phase variation in *Salmonella* species. The decisive turning point in his career came a little later.

While attending a meeting on bacterial chemistry, organized by EF Gale at Cambridge, Hayes met Cavalli-Sforza who was a visitor at the Genetics Department at Cambridge at that time. He learned about conjugation in *E. coli* through Cavall-Sforza who also provided the basic *E. coli* strains used in his work with Joshua Lederberg. Hayes began his work on *E. coli* conjugation in early 1951. Within a year he came up with the Pallanza bomb shell! After his debut at the Cold Spring Harbor Symposium of 1953, Hayes' reputation spread among the comity of microbial geneticists. In 1957, Hayes was invited to form a Microbial (later to become Molecular) Genetics Research Unit at Hammersmith Hospital, London. This Unit became a 'Mecca' for many established as well as aspiring microbial geneticists in later years. Hayes was elected to the Fellowship of the Royal Society in 1964. He began to write his now classic treatise *Genetics of Bacteria and Their Viruses* in 1963. The first edition of the book appeared in 1964. The second (and last) edition was published in 1968. It is indeed a loss to science that he did not update his book beyond the second edition. It remains a classic in the field even today. William Hayes and Martin Pollock (National Institute of Medical Research, Mill Hill, London) started the Department of Molecular Biology at the University of Edinburgh (the first of its kind in the UK) in 1968. Six years later Hayes moved to Canberra to occupy the Chair of Genetics at the Australian National University. After retirement in 1978, he spent a sabbatical year at CalTech with Max Delbrück, returned to Canberra and moved to Sydney in 1986. During the last years of life, Hayes was tor-

mented by progressive Alzheimer's disease and died of cardiac arrest on January 7, 1994.

William Hayes was a remarkable scientist who is said to have been more at home at the lab bench, doing experiments himself, than at the desk of the Director of an institute. The working conditions in post-war Britain at the time in which he did his pioneering studies were unlike the technology-driven research laboratories of today. On seeing the appalling conditions of his early laboratory at Hammersmith Hospital, Wollman is reported to have felt that their own primitive set-up in Paris was indeed a luxury! Hayes always liked to do things himself rather than getting them done by somebody else. It is said that he typed the first version of his book himself. All through his career Hayes had published hardly 10 or 12 papers but what an impact they had in the field of Molecular Genetics! People who had known him closely admired his paradoxical demeanour. He looked very much an army officer with his upright posture and close-cut hair but his non-hierarchical attitude, informal manners like walking around in sandals, unbuttoned shirt collar, etc., gave away the true, an inwardly gentle human being that he really was.

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Suggested Reading

[1] **R Jayaraman, Joshua Lederberg's Legacy to Bacterial Genetics, *Resonance*, Vol.13, No.8, pp.716–729, 2008.**

[2] **William Hayes, *The Genetics of Bacteria and Their Viruses*, Editions I and II. John Wiley & Sons, Inc. New York, USA. 1964, 1968.**

[3] **G S Stent and R Calender, *Molecular Genetics*: *An Introductory Narrative*. W H Freeman and Company, San Francisco, USA, 1978.**

[4] **T D Brock, *The Emergence of Bacterial Genetics*, Cold Spring Harbor Press, Cold Spring Harbor, New York, 1990.**

[5] **S Silver, J Shapiro, N Mendelson, P Broda and J Beckwith, 'William Hayes: Pioneering Contributions Remembered'. *ASM News*, Vol.61, pp.17–20, 1995.**

(In addition to the above, many informative write-ups on Hayes are available on the net).

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