12. The Postgraduate Medical School,London University. The Head of the Bacteriology Department at the PGMS(later the Royal PGMS) was Professor Lord(Trevor) Stamp who did'nt seem to bother what research I did so long as I did something. My closest colleague was Dr.Denny Mitchison(Lecturer),one of the three biologist sons of authoress Naomi Mitchison and nephew of J.B.S.Haldane,whose main interest was typing tubercle bacilli under MRC auspices. In addition there were at least two non-tenured medical graduates on the staff. My only departmental responsibilities were to take my turn at clinical bacteriology which amounted to the mornings of one week in every three or four,and to share in teaching the bacteriology course for the Diploma in Clinical Pathology which occupied me for not more than a few weeks in the year. There was,in fact,a positive obligation to spend at least half one's time at research.

I decided to return to the mechanism of somatic phase variation in *S.enteritidis*,but had begun to realise the potentialities of genetic analysis inherent in the recent discovery by Lederberg and Tatum(1946) of conjugation in *Escherichia coli* ,and wondered if Salmonella also could conjugate. But it was clear that I should first become acquainted with the phenomenon in *E.coli*. Towards the end of 1950 I went on a bacterial chemistry course at Cambridge,organized by Prof.E.H.Gale,and there met Luca Cavalli(later Cavalli-Sforza) who had worked with Joshua Lederberg on conjugation at Wisconsin and,being mainly interested in population genetics,was currently a visitor in Prof.R.A.Fisher's Department of Mathematics. Cavalli gave me the basic *E.coli* K12 auxotrophic parental strains and I began to play with them early in 1951.

Conjugation in *E.coli*: One way partial genetic transfer,and the sex factor. After confirming that prototrophic recombinants arose at low frequency(c.10^{-6}) when suspensions of the two auxotrophic parents were mixed and plated on minimal agar,I thought it might be interesting to study the kinetics of the process; i.e. at what time recombinant cells were formed after mixing and plating the parents. I therefore spread a mixture of a streptomycin(Sm)-resistant mutant of one parent(A) and a sensitive strain of the other(B) on several minimal plates,and at intervals thereafter respread the plates,in turn,with a lethal

concentration of streptomycin. I anticipated that no colonies would appear until resistant prototrophic recombinants began to arise. The experiment worked beautifully; there were no colonies when Sm was added prior to two hours after mating but thereafter they increased in number with time. Just to be sure,the experiment was repeated but now the resistance pattern was reversed so that a sensitive A strain was mated with a resistant mutant of B. This time the results were quite different. About the same number of recombinant colonies emerged from all the samples,even when Sm was added immediately after plating the mixture.

I discussed these results with Denny Mitchison and I think it was he who first suggested that one of the parents,A,might be acting as a gene donor and the other,B,as a recipient. This was confirmed by treating each sensitive parent with Sm to a survival of less than 10^{-6} colony-forming cells,and then mating with an untreated suspension of the other. The upshot was that the crosses in which strain B had been treated were invariably sterile while treated A suspensions always generated recombinants although their numbers might be markedly reduced as compared with normal crosses.

It followed that parent B was the recipient or "female" whose continued viability was essential for the whole process of recombination and segregation,while the A donor(or "male") cell was dispensible once genetic transfer had been effected. I suggested that the male cell extruded a surface "gamete" which was taken up by the female cell on contact,so that blocking male protein synthesis by Sm did little to inhibit its fertility.

This hypothesis,and the experiments supporting it,were published in a letter to Nature entitled "Recombination in Bact. coli K12: unidirectional transfer of genetic material"(Publ.16), and was presented in a paper to the June 1952 meeting of the Society for General Microbiology in London. The meeting featured a symposium on "Virus Replication" and was attended by Drs.André Lwoff and Gunther Stent from the Institut Pasteur,Paris,and the young James Watson who had recently come to work on DNA structure with Francis Crick at Cambridge. My first impressions of Jim suggested a naive gaucherie tinged with arrogance. Aged 22,he had only recently completed his PhD with Salvador Luria who, because of his liberal political views at the time of Senator McCarthy's heyday,was refused an American visa to attend the

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symposium. Instead,Jim's lanky figure occupied three seats of the front row of the theatre,his legs resting on two of them, from which he arose on one occasion to read a letter from Luria (or possible Hershey) recounting the result of the Hershey-Chase experiment. I later got to know Jim well and found him highly intelligent and enjoyed his company despite,or perhaps because of, his lack of maturity.

My next step in the E.coli story was based on a report four years previously,that UV irradiation of a mating mixture markedly increased the number of recombinants. Was this due to stimulation of the male or of the female? Experiment showed that exposure of the male,before mating,to a dose of UV permitting about 50% survival,resulted in a 5- to 10-fold increase in the frequency of recombinants. In contrast,irradiation of the female led to a fall in the number of recombinants that paralleled that of the survivors(Publ.17).

The previous year Lwoff and his colleagues had described the UV induction of a B.megaterium prophage,and Weigle and Delbrück a similar induction of the E.coli K12 prophage lambda. Since phage induction and the enhancement of male fertility required post-irradiation incubation in a rich medium,I thought it possible that the male "gamete" might be a gene-associated virus,although this was unlikely to be phage lambda which lysogenized both male and female cells(Publ.17). Later studies of a non-lysogenic male obtained from Elie Wollman confirmed this. At this time Zinder and Lederberg had not yet published their work on Salmonella transduction.

In September 1952 I was invited to the 2nd. International Symposium on Microbial Genetics,sponsored by the Rockefeller Foundation and held at Pallanza on the shores of Lake Maggiore, Italy. There I met most of the rather small number of Europeans in the field at that time,as well as some Americans including Jim Watson. I therefore had the opportunity to present my donor-recipient hypothesis to a well informed and critical audience, while Luca Cavalli supported the more orthodox homothallic viewpoint. In his book "The Double Helix"(1968),Jim Watson remarks that "Bill's appearance was the sleeper of the three day gathering; before his talk no one except Cavalli-Sforza knew he existed. As soon as he had finished his unassuming report, however,everyone in the audience knew that a bombshell had exploded in the world of Joshua Lederberg"!

At about this time a London friend of mine,Dr.Clive Spicer

who had worked with the Lederbergs and with whom I had been discussing my hypothesis,told me that he had a pair of parental K12 strains,similar to my A and B parents, which had lost their capacity to produce recombinants on storage. I had been attempting without success to isolate a male strain that had lost its postulated vector by looking for A colonies that were no longer fertile with the B female. Perhaps Spicer had been more successful! He kindly gave me his strains which I crossed with my fertile ones. It turned out that it was indeed the male strain that was defective. The crucial experiment was to see whether its fertility could be infectively restored by contact with a normal male. Accordingly I labelled the defective A(spicer) strain with two independent mutations(resistance to streptomycin and sodium azide) and then grew it overnight in mixed culture with my fertile A strain,sensitive to both agents. Before I knew the result I wrote to Luca Cavalli,who was now collaborating with Joshua and Esther Lederberg in Wisconsin,telling him what I had done and saying that if the experiment worked he would have to accept the fact of infectious transfer. The experiment did work; 25% of colonies of the re-isolated strain A(spicer) were now normally fertile.

However when I got Cavalli's reply to my letter he said that he already knew what the result of my experiment would be since he and the Lederbergs had done basically the same experiment three weeks earlier! Their approach had been quite different from mine. When crossing various K12 mutants with one another they had found that certain strains which were fertile with other so-called F^+ strains were infertile with one another and were therefore called F^-(fertility minus). F^+x F^+ crosses were fertile but about ten times less so than F^+x F^- crosses. The recombinants from F^+x F^- crosses were always F^+. Their experiment was to show that the F^+ character was transferred by a transmissible agent,which they called F or the sex factor,at a frequency some 10,000 times greater than that of recombinant formation. But their interpretation was quite different from mine. Since F^+x F^-,F^+x F^+ and F^-x F^- crosses displayed a heirarchy of decreasing fertility,they held that the sex factor conferred on the parents of a cross what they termed "relative sexuality", but they did not attempt to explain this in mechanistic terms. Thus these two quite different modes of experimentation and thinking converged in the coincidental discovery of the first transmissible plasmid,the F factor(Publ.18).

A limited survey by me showed that F^+ and F^- cells, as defined by the Lederbergs and Cavalli, corresponded unambiguously to donors and recipients respectively. The ability infectively to transform recipients into donors meant that we could now study the genetical effects of "reversal of F polarity" by comparing the outcome of $A.F^+ x\ B.F^-$ and $B.F^+ x\ A.F^-$ crosses. If, as Lederebrg et al. believed, the two parents contributed equally to the zygote, both crosses should give the same result. They did not. First, the recombinants inherited most of their characters from the F^- parent. Second, the characters inherited from the F^+ parent were limited to a few linked ones and were quite different in the two crosses. Everything behaved as if the F^+ donor transferred only part of its genome to the F^- recipient, the particular part being that selected to make good the auxotrophic defect of the F^- recipient which, of course, differed in the two crosses.

Joshua Lederberg's explanation was quite different. He did not accept the donor-recipient hypothesis and proposed instead that complete zygotes are formed but that a fraction of the F^+ genome is then eliminated - the "post-zygotic exclusion" hypothesis for whioh a precedent was known in Cheironomus. It was not until the hypothesis of one-way partial chromosome transfer was proven beyond reasonable doubt by the work of Wollman and Jacob four years later that Lederberg accepted it. I had the great advantage of knowing virtually no genetics while Lederberg knew too much!

Since the Pallanza meeting Jim Watson had begun to take an increasing interest in E.coli genetics and used to visit me when he came to London for discussions with Dr.Maurice Wilkins on X-ray diffraction analysis of DNA. From the results of my genetic analyses he thought that there was good evidence that the E.coli genome comprised three linkage groups. Lederberg et al. in 1951 had already defined three sets of linked genes which showed non-linear interactions whose nature they did not understand. We suggested that the three linkage groups reflected discrete chromosomes,only one of which normally became associated with the transmissible F vector at any one mating event and was transduced to the recipient cell. Occasionally,however,the F factor became associated with two chromosomes which were therefore co-transduced, thus explaining Lederberg's non-linear interactions. This model was submitted as a joint paper to PNAS through Max Delbrück and published early in 1953(Publ.19).

Another interesting event at this time was my accidental discovery of the Hfr(high frequency of recombinants) strain,HfrH, which arose spontaneously in a stock culture of the A.F^+ donor strain and yielded about 1000 times more recombinants in crosses with B.F^-. This discovery was not original since Cavalli had first described the Hfr state in 1951. However I went on to show that the fertility of HfrH was relatively unaffected by Sm treatment,

i.e. it was its donor ability that was enhanced; that UV irradiation did not increase the frequency of recombinants implying that this was already maximal; that the donor state was no longer transmissible at high frequency; and that only one linkage group was transferred at high frequency although markers on other linkage groups could be selected at low frequency, a proportion of these latter recombinants being Hfr donors like the parent.

HfrH seemed to fit well into the Watson-Hayes model by invoking a mutant F factor that had become stably associated with one of the three chromosomes. This hypothesis, of course, turned out to be basically incorrect although not a bad approximation to the truth; but the main importance of HfrH was that I gave it to Elie Wollman and François Jacob of the Institut Pasteur, Paris, with whom I had already established a close liaison, in whose hands it played a key role in the many experiments of their brilliant series that revealed the true nature of E.coli sexuality.

Thanks to Jim Watson's contacts in the USA, Max Delbrück, whose habit was to believe nothing that has'nt been proven to the hilt and even then to have some doubts, apparently thought that my work was worth promoting. In this he was supported by Elie Wollman who had visited me in London in the spring of 1952 and, shortly afterwards, met Max at a bacteriophage conference at the Abbaye Royaumont, France. The upshot was that I was invited to contribute to the 1953 Cold Spring Harbor Symposium on "Viruses" where I presented all the experiments and hypotheses so far reviewed here(Publ.20). At the meeting, by a curious coincidence, support for the Watson-Hayes model came from an unexpected quarter when E.D.DeLamater independently claimed, from his controversial studies of alleged mitosis in bacteria, that at least three chromosomes could be visualised in E.coli K12; but this claim was later withdrawn. A highlight of the symposium was Watson's account of the structure of DNA (which he delivered with his shirt tails hanging down outside his shorts!) During the meeting Delbrück invited me to spend six months, from the autumn of that year, as a Research Fellow at the California Institute of Technology and I accepted with alacrity.

13. Caltech Interlude. I set sail for New York again in September, accompanied by my wife and three year old son, with the intention of buying a car and driving from Cold Spring Harbor to Pasadena. Since British currency restrictions were then severely limiting,

Jim Watson kindly lent me $500 with which we bought a second-hand car in good condition, the rear of which was convertable into a reasonably comfortable double bed so that, during our three-week trip, we spent most nights on the road.

The most adventurous part of the journey was undoubtedly its beginning since Salvador Luria, who was also leaving Cold Spring Harbor, suggested that we could best find our way across New York to the Pennsylvania Turnpike by following his car. So, with a few hours of experience of driving a strange car on the wrong side of the road, we set off. It seemed to me not only that Salva drove as if intent on winning a Grand Prix, but whenever a traffic light turned amber he invariably accelerated!

A memorable break on our journey was a visit to Seymour and (the late) Dotty Benzer at Lafayette, Indiana. From there I went by train to Madison, Wisconsin to spend a few days with Joshua and Esther Lederberg whom I had not yet met. Joshua displayed a portly figure matched by a powerful personality and an aura of deep intelligence. I was told that his bedtime reading was a treatise on advanced mathematics by Sir Arthur Eddington. He was also a great talker so that his wife Esther's views, for example on the lysogeny of E. coli K12 by phage lambda which she had discovered in 1951, took very much second place. Joshua brought a blackboard into the garden where I gave a very informal talk. Joshua did not accept my hypothesis, and cited his work on a relatively stable diploid strain of E. coli that segregated the parental types but lacked a specific chromosomal segment from one of the parents, to support his doctrine of complete zygote formation followed by post-zygotic elimination. I did not fully understand his arguments and explanations which Watson later described as being of "rabbinical complexity".

I met Joshua on two subsequent occasions, after he had been awarded the Nobel Prize with George Beadle and E.L. Tatum in 1958 and had moved to Stanford University. During an evening at his Stanford home he told me that the Prize had confronted him with a choice, either to continue in active research or to use his prestige to influence the progress of science in broader and more administrative ways, and he thought he would take the latter step but without losing touch with genetical research. At that time he had become involved in exobiology and was, if I remember, on a NASA committee concerned with the detection of life on Mars. He showed me an apparatus he had devised whereby a long articulated arm transferred small samples of soil from his neighbour's

garden to substrates indicating various enzyme activities but which,at that time had merely proclaimed the absence of life on earth! My last meeting with Joshua was in 1960 at a Society for General Microbiology Symposium in London when I at first failed to recognize the slim figure that he had then achieved.

On arrival at Caltech I was given a lab/office of my own in the Kerckhoff Laboratory. At that time George Beadle was Chairman of the Division of Biology. Among my associates,apart from Max Delbrück,were Guiseppe Bertani,Renato Dulbecco,Sterling Emerson, Norman Horowitz,Dale Kaiser,Edward Lewis,Robert de Mars,Robert Sinsheimer,George Streisinger,A.H.Sturtevant,Marguerite Vogt, Jean Weigle and Jim Watson. My lab was close to that shared by Weigle and Bertani who were able to perform round-the-clock experiments because of their unusual and complementary living habits. Weigle arose at about 4.30am and worked till about mid-day when he went home to relax. In contrast,Bertani rarely came to the lab before mid-day or later and continued there into the night.

Before I came to Caltech Watson,who was appointed Senior Research Fellow in 1953,had suggested that we might continue working there together on E.coli conjugation. However the discovery of DNA structure aroused his interest in RNA so that he collaborated instead with Alexander Rich on X-ray diffraction studies. At that time he had recently learnt to drive and had bought a car jointly with de Mars,but no one would go on trips with him except my wife and I! So we had several expeditions together up the San Gabriel Mountains where Jim took me for strenuous walks on which I found it hard work to keep pace with him.

Prior to my arrival at Caltech,Marguerite Vogt,at one time a student of Hermann Muller with whom she had collaborated in research,had been working with E.coli K12 F^+x F^- crosses but was about to change her field - to join Dulbecco in polymyelitis research,I think. So I took over her equipment and stocks of culture media reagents,all highly purified,which turned out to have an interesting but initially embarrassing denoument since no recombinants arose when I plated F^+ or Hfr crosses on minimal media prepared from them. On the other hand these media supported the growth of prototrophic recombinants and yielded recombinants when seeded with zygotes preformed in complete medium. Some step in the process of recombination was clearly being prevented on the Caltech medium. I then found that crosses became normally

fertile when aspartate,and to a lesser extent pyruvate,fumarate or succinate were added to the medium. Addition of glutamic or other amino acids had no effect. Apart from prompting a seminar on the value of aspartate as a bacterial aphrodisiac,the direct outcome of these observations was the first analysis of the energetic requirements for conjugation by K.W.Fisher who became my first PhD student in bacterial genetics in London in 1954 (see Publ.40).

Since I now had my Hfr strain I decided to return to my initial project which had had such unforeseen consequences,and to study the kinetics of mating with more hope of success. This time however,instead of streptomycin I used a high multiplicity of the virulent phage T6 to kill the sensitive Hfr donor at intervals after mixing with a resistant recipient in broth. The results were clearcut and reproducible. When untreated samples were plated,recombinants began to appear immediately after mixing the parental cultures. On the other hand the treated samples yielded no recombinants during the first 8-10 minutes; recombinants then began to appear and increased linearly with time until a plateau was reached about 30 minutes later.

I had imagined that the donor genome,then visualized as a discrete "nucleoid",would be transferred en bloc over a very short period. On several occasions,therefore,I scored recombinants from the phage-treated mixture for inheritance of the donor $\underline{lac}^+$ and phage $\underline{T6}^{\underline{s}}$ alleles which were located on the same linkage group as the selected markers($\underline{thr}^+$.$\underline{leu}^+$), only to find them absent. I ascribed this to killing of the $\underline{T6}^{\underline{s}}$ recombinant segregants by the phage and failed to recognize its significance until Wollman and Jacob published their famous interrupted mating experiment in 1956.

The ability to prepare "zygote suspensions" from which the Hfr bacteria had been eliminated by treatment with phage, suggested further experiments on the kinetics of segregation, and on the expression of genes determining resistance to sodium azide and phage T1 which are closely linked to the selective markers on the Hfr chromosome. For example,if a suspension of newly formed zygotes is diluted and incubated in fresh broth and samples then plated at intervals for recombinants,the time at which the number of recombinants begins to increase indicates the commencement of division among the recombinant segregants so that the time of segregation is assessed.

Furthermore, if the diploid zygotes are plated on media containing the inhibitory or lethal substance, only those in which the resistance gene is dominant can segregate resistant recombinants so that a comparison of the kinetics of segregation and expression distinguishes dominant from recessive alleles.

These new methods were initiated at Caltech and the definitlve experiments completed during 1954, but were not published until two years later when Wollman and François Jacob invited me to collaborate with them in a joint paper to the 1956 Cold Spring Harbor Symposium which we all attended (Publs.21,22,23).

In the autumn of 1955 I was invited by (the late) Harriet Ephrussi-Taylor to spend 3-4 months at her laboratory in Paris where I continued my work on conjugation. While there I learnt a little about pneumococcal type transformation on which Harriet had worked initially with Oswald Avery at the Rockefeller Institute, New York, and had later developed as a system of fine structure bacterial genetics; and about "petite" mutations in yeast from Boris Ephrussi. But my stay in Paris was mainly unproductive apart from discussions with Wollman and Jacob and the planning of our Cold Spring Harbor paper.