Visconti
> 1951

CARNEGIE INSTITUTION OF WASHINGTON
DEPARTMENT OF GENETICS
COLD SPRING HARBOR, LONG ISLAND, N. Y.

16 January.

Dear Joshua,

I am sorry I didn't manage to send you in time my manuscript for the ~~APPS~~ meeting. You know how these things are: you are never through until the last day. I hope they will not publish a volume on the Symposium, but in case they did I would like to know what you think about my paper. I also proposed to Wenrich, who organized the Symposium

to split viruses from bacteria, and to ask somebody else to do these last ones. If you happen to have a little time free, you might take a look at the paper and tell me very frankly what you think about it. I am starting work for California where I will be with Delbrück until the end of February.

Very sincerely yours

Niccolò

M. Visconti

In recent years recombination has been discovered in bacteria and viruses.

The two organisms in which recombination was first demonstrated were the colon bacillus and its virus T2. Both organisms are good to work with because they grow fast and they can be counted easily. On the surface of an agar plate a single bacterium develops into a colony. Colonies can be counted if they are not too crowded. If colonies are too crowded they grow in a thin continuous film on the surface of the agar. If a bacterial virus or bacteriophage is present on this surface it will multiply by lysing the bacteria. Bacteriophage, like plant and animal viruses, multiply only in living cells. A colony of phage is called a plaque; it appears like a small hole in the film of bacteria that covers the surface of the agar.

At first sight the life cycle of a bacteriophage particle seems simple. It gets adsorbed to a bacterium and after a characteristic period of time called the latent period, which is 21 minutes for T2, the bacterium bursts open releasing several hundreds of particles identical to the one adsorbed at the beginning. To the scientist interested in the biological problem of self duplication, this example looks very interesting. Where do these particles come from? How are they formed? In order to answer these questions we must see what is going on inside the bacterium during the latent period. It is very hard to believe that a method can be developed to take a peep inside the bacterium without disturbing the process of phage formation. Suppose instead we break the bacterium before the spontaneous lysis. This might

show us something of what is going on. Will phage be found? and in what amount? or will some precursor of the phage be set free? and what will be the properties of this precursor or prophage? Will it be revealed by some serological or biochemical technique?

In 1942 at Vanderbilt University, Dr. Delbrück and Dr. Luria started doing experiments following this line of thinking. The difficult matter was to break the bacterium without damaging the phage or whatever was to be found inside. One agent was known that could do the job: that agent was the phage itself. Suppose a bacterium is infected with two phages T_1 and T_2. T_1 has a latent period of 13', T_2 of 21'. The two phages will start to grow together but after 13' the progeny of T_1 will break the bacterium open. What will happen to the progeny of T_2? Delbrück and Luria did this experiment, and here starts the series of unexpected results that will lead to the discovery of recombination. In the bacteria infected with T_1 and T_2 nothing happened at 13 minutes; at 21' the cells burst, but only T_2 was found in the lysed culture. If instead of a mixture of T_1 and T_2 only T_2 had been used, the result would have been the same. Explanation: T_2 excludes T_1. Both phages are adsorbed to the same bacterium but only one can grow. If instead of infecting simultaneously with T_1 and T_2 we give an advantage of 4' to T_1, many bacteria will yield T_1 but no T_2. The bacterial culture as a whole may yield both phages but each burst coming from one bacterium will consist entirely either or T_1 or of T_2. This effect was called mutual exclusion. At that time Delbrück and Luria made another peculiar observation. If a bacterium was infected with many phage particles all of the same strain, instead of only one, the latent period and the yield did not change. This

looked as if actually only one particle took part in the growth. The principal of mutual exclusion could thus be extended to particles of the same strain. As soon as one particle starts growing in a bacterium an unknown reaction is started that results in the exclusion of all other particles even if they are already adsorbed. Quoting from Delbrück: "The mutual exclusion effect is so novel that its explanation calls for a bold hypothesis. We assume that the first virus which penetrates the cell wall will make the cell wall impermeable to other virus particles just as the fertilization of an egg by one spermatozoon makes the egg membrane impermeable to other spermatozoa."

In 1944 Luria found the first mutation in phage T_2. Strain B of the colon bacillus, sensitive to T_2, can mutate to B/2, resistant to T_2. But T_2 can mutate to T_2h, which lyses B/2. Therefore we have two types of phage T_2 and T_2h, and two types of bacteria B and B/2. T_2h lyses both bacterial strains, T_2 only B and not B/2. Now suppose we plate on B, T_2 cannot be distinguished from T_2h. If we plate on B/2, only T_2h will give plaques. Now what will happen if a bacterium of strain B is infected with T_2 and T_2h? Will only one type be found in the burst? This was a crucial test for the theory of mutual exclusion. Luria did some experiments by infecting bacteria with a mixture of T2 and T_2h, and the results he got seemed to indicate that every burst yields either T_2 or T_2h. In point of fact Luria's experiment was indecisive on this point, for reasons we need not go into here.

In 1945 another mutant was found by Dr. Hershey at Washington University. This mutant was called r. It gave much larger plaques than the wild type r^+. This mutant presented the advantage that it

could be recognized directly on the plate. Hershey was a little skeptical about the principle of self-interference, so he tried mixed infection with the r^+ and r to analyze the burst of single bacteria. In order to do this, he used a technique which had already been worked out by Delbrück. After infection, he plated the infected bacteria before the burst. In this case each plaque on the plate originates from the phages coming from one bacterium, as the burst takes place in the agar and remains, so to speak, concentrated at one point. If the principle of mutual exclusion for particles of the same strain was correct, all the plaques should have been either r or r^+. Hershey had no idea how mixed plaques would look: what he was planning to do was to sample some plaques at random and analyze the population obtained from each plaque. But the answer came right away just by looking at the first plate. The plaques were neither of the r nor the r^+ type. They were mottled, r and r^+ growing together, give, as you can see, a type of plaque very different from either one. The result was immediately confirmed by showing that the two types were actually present in the same plaque. At first Delbrück was quite surprised at this result, maybe also a little annoyed, but as the lead looked promising he followed it. Mutual exclusion had been demonstrated in a very clear way for morphologically and serologically unrelated strains at T_1 and T_2. But what would happen if similar strains were used, for instance T_2 and T_4, which have many features in common? Delbrück found that T_2 and T_4 could grow together in the same bacterium.

In one experiment Delbrück used instead of T_2 a strain of T_2r. The original experiment of Hershey with mixed infected bacteria could be repeated, not using T_2r and R_2r^+, but by using T_2r and T_4r^+.

But at this stage something quite amazing occurred. The bacteria infected with T_2r and T_4r^+, besides yielding both infecting types, yielded also two new types T_4r and T_2r^+. Recombination in phage had been discovered. Not only could two different phages grow in the same bacterium, but they could also recombine some of their characters. At the C.S.H. Symp. Quant. Biol. of 1946 Delbrück announced this result, but the title of his paper was "Induced Mutations in Bacterial Viruses." The following scheme is given in this paper to represent the new phenomenon.

$$T_4r^+ \longrightarrow T_4r \quad \text{(Under the influence of } T_2r\text{)}.$$

It is also a general principle in biology that a female can generate offspring with some new character under the good influence of the right male. At the end of his paper Delbrück makes the following statement: "perhaps one might dispute the propriety of calling the observed changes induced mutation. In some respects they look more like transfers or even exchanges of genetical materials." At the same symposium, Hershey gave very convincing evidence for the independent occurrence of h and r mutations in phage T_2. The field was finally open for genetic investigation. A two factor cross, hr by h^+r^+, could be attempted. In a private conversation Hershey made the prediction that out of this cross he would obtain 25% of each of the four types (two parental and 2 recombinant). To which Delbrück asked: Do you think that genes are in solution?

"Yes" replied Hershey.

"I bet you a dollar," said Delbrück.

At this time Delbrück moved from Vanderbilt University to his new position at the California Institute of Technology and for some

time he disappeared from the story of recombination. We let him go to California where shortly afterwards he received a dollar for his bet. Actually Hershey lost his bet for some very special reason. What he did was to plate the yield of his cross on B/2 where only the h could grow. In this way he wanted to see if half of the h were r (parental type) and half r^+ (recombinant type). What he found was a large excess of the parental type but this result was not due to lack of mixing, but to a complex phenomenon which he discovered and called later "phenotypic mixing." Had he done the experiment with the correct technique he would have found a ratio very close to the predicted 25%. Together with one dollar, he would have gained the notion of genes in solution.

When we speak of crosses between phages two things should be kept well in mind.

1.) The phage particles are haploid, or if they are diploid they are homozygous. I make this statement for the sake of simplicity because Hershey has shown that 2% of the phage particles originating from a cross are heterozygous for any character for which the two parents are different. In an annual report to the Public Health Service in 1950 Hershey wrote the following: "Experiments on the mechanism of genetic recombination have been temporarily abandoned for lack of ideas." In his 1951 report, after quoting the previous statement, he wrote: "At present we go to the opposite extreme." This new hope was based on some very interesting facts concerning the heterozygotes. But these very interesting facts remain up to now unexplained, and the new hope has again faded away. Anyhow at the present moment we can ignore the heterozygotes and just consider them some abnormal form which appears in the progeny

of crosses with a frequency of 2%. The remaining 98% of the phage particles behave like haploids.

2.) The other fact we must bear in mind is the following. We are not dealing with crosses between two individual particles, but rather between some particles of one strain and some particles of another. What we actually do is to infect a bacterium with something between 10 and 20 particles of which half will be of one type and half of the other one. Following random variations from the 1 : 1 ratio of adsorbed particles, we have in the population of bacteria many individuals which have adsorbed an excess of either one or the other parent. These variations can be calculated.

A cross can be made between more than two parents. Suppose we have three phage strains with the following markers Abc, aBc, abC. If we infect a bacterium with let us say 5 particles of each type, will the type ABC, which has at least one marker from each parent appear in the progeny? This type of cross can be called triparental. Hershey did this experiment and found in the yield recombinants of the type ABC. This means one of two things:

1.) Either the new phage is formed out of some kind of pool of the parental characters.

2.) Or matings are possible between parents and recombinants.

Actually what we are doing is to choose between some completely new type of recombination, probably connected with reproduction of the phage, and the classical mechanism of recombination by mating between pairs. Both hypotheses proposed for the first time by Hershey may look quite appealing to people working in the field of phage. But while the first hypothesis is still rather vague, the second one has been defined in a very elaborate and complete way on

the assumption that phage particles mate at random inside the bacterium and that before the burst several rounds of mating have taken place. The whole theory of mating between phage particles has been recently developed by Delbrück for two factor and three factor, diparental and triparental crosses. The two parameters which are kept constant are the number of rounds of mating taking place inside one bacterium, and the linkage relation between two given markers. What can be changed are the relative ratios of infecting particles per bacterium and the markers entering into the cross. The predictions of the theory fit very well with the experimental results so far obtained. This does not mean that other theories may not give predictions equally good. To explain the implications of the mating theory let us make an analogy. Suppose we have two genetically different strains of Drosophila of which we put ten flies of each type (5 males and 5 females) in a vial. For some unknown reason we cannot open the vial for a long period of time, let us say 2 months, until the flies have used all the food present in the vial. Let us suppose also that at this moment the vial bursts open and the progeny set free can be finally examined and classified genetically. Assuming random mating and absence of selection we can calculate the number of generations from the frequency of recombination for unlinked characters, and knowing the number of generations we can calculate the linkage value per generation for characters situated on the same chromosome. The phage particles can be compared to the flies, the bacterium to the vial, and the latent period of 20 minutes to the hypothetical period of two months. As I said before the calculation of the number of matings is based on the knowledge that some of the characters

are unlinked. This fact had not been clearly demonstrated for phage. Hershey had found three linkage groups each of which showed 40% recombination with the other two. As higher values had not been obtained, these groups were considered to be unlinked. This meant in the terms of the mating theory that since the number of matings was limited we never arrived at the complete breakdown of the parental combinations. To clarify this point Hershey and I started last year to do some experiments which gave a clear demonstration of the validity of Mendel's laws for bacteriophage. Segregation between unlinked markers should be 1 : 1 as a result of the mating between two different particles. Suppose we have the following strains Abc and aBC. Let us choose among the progeny a class of recombinants for A and B, for instance Ab. Every phage particle AB must have mated at least once with the opposite parent. What will then be the ratio of C:c in the class AB? The ratio was found to be 1:1. This finding can be considered proof of unlinked characters. In order to obtain this result the bacteria must be infected with the same number of particles for the two parental types. If this ratio is unequal, the results are quite different. It was for the purpose of analyzing these results that Delbrück started his work on the theory of successive matings.

Mutations can be defined as heritable changes in the action of genes. What we observe are alterations of structure or function in an organism. We refer these changes to genes when mutation or recombination tests show that the change has originated in a unitary structure. We can now say that some characters in bacterial viruses depend on gene action. We have seen in our brief review how this evidence has been imposed by new facts successively disproving all previous hypotheses. Only ten years ago the expression "naked gene" was used for viruses. Now we can say that from the genetical viewpoint T2 is just as complex as any other organism. Moreover, all facts known at present can be explained in the framework of classical genetics.

The discovery of recombination in bacteria follows a different pattern. Recombination was not found, but was looked for. It is also hard to conceive how it could have been found without looking specifically for it. In 1945 Dr. Tatum at Stanford University published a paper on induced mutations in the colon bacillus. The wild type of the colon bacillus can be grown in a synthetic medium containing glucose and inorganic salts. In a population of these bacteria grown in complete medium, for instance broth, some rare individuals may be found which need specific additions in order to grow in synthetic medium. Many of these growth factors turn out to be single well defined chemical substances such as aminoacids or vitamins that the bacterium can normally synthesize. These metabolic changes

are heritable. Tatum found that the frequency of nutritionally exacting cells was increased in populations that had been exposed to X-rays. This suggested that the changes in the colon bacillus might be similar to mutations in higher organisms. Moreover, a variant requiring one growth factor could yield further variants with double requirements, suggesting the independence of the several changes. This was the only kind of evidence, which could be obtained at that time, pointing to the mutational nature of the changed requirement. It was surely an important advance in bacterial genetics, but definite proof for the existence of genes in bacteria could only be given by the recombination test. The classical work of Beadle and Tatum in the biochemical genetics of Neurospora had shown that altered nutritional requirements in this organism resulted from mutations of Mendelian genes. Already at that time Tatum's purpose of going after nutritional variants in bacteria was to test the possibility of recombination. The strain of coli used by Tatum was called K 12. It had been chosen for some previous biochemical work as a good producer of indole. Miss Esther Zimmer helped Dr. Tatum to isolate from K 12 variants with double nutritional requirements. As we will see later both K 12 and Miss Zimmer became very important in the life of a young medical student at Columbia University--Mr. Lederberg. In 1945-46 Lederberg was working for his thesis on selection of prototroph reversions in Neurospora. This gave him the idea of applying the same test to bacteria. Suppose a wild type prototroph strain mutates to A, requiring growth factor A. By a single step back mutation A^- can revert to wild type.

This second mutation can be conveniently studied as the mutated type is selected for by plating a certain amount of A^- cells on synthetic medium. Only the cells mutated to prototrophism will be able to grow. Rare prototrophs can thus be easily detected in a bacterial population. But rare prototrophs, other than by mutation, could also arise by some other rare event as recombination, provided recombination occurred and the right mixture of bacterial strains was used. Suppose we mix two strains of bacteria $A\ B^-$ and A^-B; three different events can lead to the formation of a prototroph: mutation of the first strain to B, mutation of the second strain to A and recombination. The mutation rates of the two strains can be measured by separate plating. Lederberg wrote to Tatum about this idea and when Tatum received this letter from a student suggesting the same idea he had in mind, he thought the best thing to do was to invite this student to work with him on the project. In March 1946 Lederberg went on a fellowship to Yale. After three months, at the C.S.H.S., the same one at which Delbruck gave the first evidence for recombination in phage, Tatum and Lederberg delivered a paper with the title "Novel genotypes in mixed cultures of biochemical mutants of bacteria." By using multiple mutants, requiring at least two growth factors, prototrophs due to back mutation were practically reduced to zero, while when plating the mixture of the two parents in synthetic medium, prototrophs were detected in a proportion of the order of 10^{-7}. But the definite proof for recombination was given later by Lederberg in 1947. By using additional markers full evidence was obtained that what segregates is the locus and not the character.

The classical crosses with characters in coupling and repulsion could be repeated with bacteria. Quoting from Lederberg: "The prototroph provides the putatively sexual progeny which can be surveyed for factor recombination. It must be emphasized that the demonstration of recombination does not rest directly upon mere observation of prototrophs, but upon the occurrence of series of new combinations of unselected markers introduced with the nutritionally differentiated parents."

The choice of K_{12} was an extraordinary lucky one, as very few strains of the colon bacillus proved later to be sexual.

After such a fortunate beginning Lederberg was compelled to abandon medical school and to devote himself to the study of K_{12}. In doing this he was helped by Miss Zimmer, who had been the first to isolate the double mutants, and who became his wife and his closest collaborator.

The story of science is a story of men with some ideas about natural phenomena. Two kinds of emotions are reserved to these men: the surprise of finding something very different from what they thought and the pleasure of confirming their own hypothesis. We have seen how following these two different patterns the general phenomenon of genetical recombination was discovered in two different classes of microorganisms: the viruses and the bacteria.