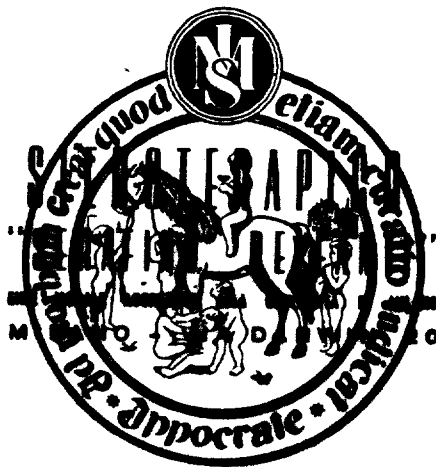
Oct 29,1952

Dear Joshua,

I am answering your letter of Sept.18 with more than one month delay. I have been busy at work on one strain,and hoped to get more information out of it before writing you,as this might also involve a change in symbolism. However,the change is quite trivial and you may approve it even if the evidence is not final,or reject it altogether.

You may remember that in our JGM paper a strain is mentioned,which is a BM-F- I isolated early in Cambridge.It has the curious property of crossing with a frequency 25x smaller to F+ than other F- strains.It does not cross to F-. When I tried to infect it with F+ I did not succeed,even in conditions in which an ordinary F- strain, in the same mixture,was infected in 100% of the cases. When crossed to F+, there was a segregation into F+ and F- in the progeny,and,what is funnier, a 1:1 segregation in 3 crosses to 3 independent F+ auxotrophs. Analysis of one of these showed no linkage with any of 7 markers. I do not yet know enough of the properties of the progeny,in respect of F+infectability,segregation pattern and F+:F- segregation on recrossing to make a precise statement,but I have another experiment (still fairly small) showing that this F- strain is a strain resistant to the virus,and which I am therefore provisionally calling $F^r$ . This experiment can be called one testing absorption of F+,although I still have not F+ in cell-free condition. A suspension of F+ cells is incubated with an excess of F- cells for 45',in aeration, then a small amount of F- cells of a distinguishable type are added and incubation continued for 30'. Finally,the two types of F- are separated,and the second added is tested for F+ . When,in such an experiment, the first F- added is an ordinary one, all the cells of the F- added as second are not infected,although they would be infected in controls. The first F- has absorbed all the available virus which is only slowly formed. When,however, $F^r$ cells are used as the first F- addition,and ordinary F- for the second,the latter are infected,as if $F^r$ had not absorbed the virus. Incidentally, Hfr behaves in this experiment as $F^r$.

I have no good explanation for the low frequency of recombination, except that there is also a change in segregation pattern(chromosome mutation decreasing exchange in TL-M region?).As to the 1:1 segregation, perhaps the easiest explanation is that we have here a locus for maintenance of the virus,and that this locus is on a chromosome not containing loci TL,Lac,Gal,Xyl,Mal,S,$B_1$,Ara,M. I am now testing new mutants for linkage. If this is true,then we have a situation similar to kappa,sigma,lamda etc. It might then be better to call $\varphi$ the virus





and F,f the alleles permitting and inhibting growth/of the virus respectively. The genotypes of ordinary F+ would then be F + φ ,/of ordinary F- would be F + 0 , and of new $F^r$ would be f (+0) . I am now crossing $F^r$ with non-lysogenic and especially with Hfr to see what happens. What do you think of altering F into φ in the joint paper on JGM I agree that the question is not an important one, but it would help I take it, to use the new terminology.

The above experiments have also some bearing on kinetics of F transfer. I am not planning detailed experiments on the physical aspects of this problem, because I hope you and Nelson will be carrying them out. I have only ascertained that dilution reduces infection above a certain point ($10^6$ cells/ml is the smallest amount still giving almost full transduction). I am still confident that the ratio of F+:F- cells is the most important factor. I may continue kinetics under another viewpoint, i.e. that of the physiology of the virus. The findings reported above show that the F+ cell which has infected remains uninfective for some time, as earlier suspected. I have some other data showing that when a mixture of F+ and F- is incubated, and the F+ are a minority, there is no tendency to generalization of th F+ type, as would be expected if infection chains were effective enough to do it. This, by the way, suggests that experiments of filtration may be insufficiently sensitive for this aim. I have used in the past small amounts of F- cells added to F+ filtrates, but even if there were enough F+ particles in the filtrate to infect them, dilution may have made the transduction ineffective or inapparent.

Concerning corrections to the JGM paper : I am preparing a list of them, including all yours, plus some that I have been ripening in the meanwhile, and shall send a copy of it . The most important refer to p.18 last PAR , and to p.21 line 2 ff. , which regard change of text.

You are quite right about the circular flavor of the argument at page 18. I should propose the following alteration, which perhaps obtains the same result as the one you suggest, without too serious alteration :

"Hfr forms an apparent exception to the rule that, in K-12, the presence of F (i.e. of the F+ state) in one of the mates is essential for recombination to occur. This rule would mean etc." All the rest unchanged.

Concerning p. 21, I am not satisfied about the hypothesis of the different degree of ploidy. It seems very difficult to test numerically; a very rough test indicates that it does not work, so that it may be preferable to dispose of it altogether, and remain content with the first. Perhaps one way would be to alter the text as follows : " At least one hypothesis, based on Mendelian theory, can be put forward to account for them: the elimination of a specific segment of the chromosome contributed by the F+ parent may take place regularly at every fertilization. There is at present no definite evidence to suggest whether such elimination might occur during formation of the F+ gametic cell, during fertilisation, or at the ensuing reduction." The paper might perhaps end thus, and we could delete all the rest. My objection to your interpretation of elimination after fertilisation is that homozygous diploids at one locus may originate through somatic c.o. in an unreduced diploid.

Instead of: Two hypothesis etc.

If you approve these corrections I shall incorporate them directly in the proofs when they come.

Finally, I quite agree about your suggestion, of not giving Hfr to other workers for the time being. However, I find myself in some embarrassment on a specific case. Last summer I saw Delbrück at Royaumont, and asked him about results M. Vogt had obtained with Hfr, which I had sent to her from Cambridge two years ago. He said he and she found it quite normal in recombination behaviour, and seemed to doubt the whole story. I therefore promised I would send the strain again. I have not sent it, and think I prefer to wait, in view of what you tell me. We might perhaps agree that I send it later on, at a date convenient for both of us, when the major facts we are interested in at the moment will be known, and reserving perhaps some special use of the strain if necessary. By the way, I have no progress on cytology. Have you ?

One technical point: I find a method of crossing which I call SAT1 rather useful. It is the usual streptomycin azide reinforced

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by $T_1$ in coupling with azide in view of the close linkage. $S^r$ x x $Az^r T_1^r$ are incubated together overnight in aereation, then plated (centrifugation may be useful for enrichment) on $StAzT_1$. The yield is about $10^{-7}$. Controls are clear, which is the only advantage in respect of S Az crossings. I find this useful when testing transductions, as it is much more expedite than any other method. I have prepared sera against F+ and F- to see if the difference could be detected with antisera but have not yet started absorptions.

Yours

Luca.