

CALIFORNIA INSTITUTE OF TECHNOLOGY

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KERCKHOFF LABORATORIES
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Dr. J. Lederberg

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Madison 6/Wisc.

January 18, 1952.

Dear Dr. Lederberg:

Thank you very much for your very interesting letter. First of all, however, I must disappoint you with respect to the activity of Hfr; all three lines you sent me did not show any appreciable increase in recombination frequency as compared to 58-161, at least not under the conditions I use (i.e. 2 $\frac{1}{2}$ - 3 hrs. old aerated cultures grown in AC yeast broth to 5×10^8 cells/ml). I, therefore, restricted myself to the cross Y24 (C⁻Pa⁻B⁻) x Y70/1 (T⁻L⁻B⁻Lac⁻V⁺_{1,5}) in B₁-supplemented M9-medium, which, under the conditions mentioned, yields more recombinants than the cross 58-161 x Y70/1.

What regards my work, little progress has been achieved as to the ^{nature of} the mechanism of recombination, involved in K12. All my trials to increase the frequency of recombination considerably in order to make a direct observation of the assumed "sexual fusion" possible have failed. There are, perhaps, two findings, which in connection with your "F⁺-substance" might be of some interest to you. (1) Broth added in a concentration 1:100 to the mixed washed suspensions increases the number of recombinants (2) Usually there is a marked discrepancy between the number of recombinants and the product of the parental concentrations if you compare large dilution steps; i.e. the number of recombinants is disproportionately high if you plate 1-2x 10⁸ cells as compared with 1x 10⁷ cells. It is very likely that this relationship is the expression of the same phenomenon observed by Nelson in his "time" experiments where he observed an induction period at low concentrations. Crowding might be favorable for the production of this "inducing factor" or simply allow of a faster attainment of ~~the~~^{its} effective threshold concentration. Therefore, I was very interested to hear about your F⁺-substance. If your strain Y24 does not show a high recombination frequency, I shall be very glad to send you my line, in case you would like to check for its production of "F⁺-substance".

So far, I did not publish any note on my microscopical observations since I wanted first to run some additional experiments which suggested themselves by the observations. As I already wrote to Mr. Zinder, washed suspensions of the two lines I use show granular masses which, by itself, is not astonishing, since both lines are lysogenic. There is some indication that certain conditions which increase granule formation are also favorable to prototroph formation (aeration in buffer for some time, refrigeration, possibly acting also by sedimentation), spreading on minimal agar if not covered by a coverslip); this correlation has, however, still to be worked out more precisely. In addition, a striking dissimilarity in the

behavior of both lines on minimal medium was observed. Cells of Y70/1 remains dark (in phase contrast) for at least two days. They may eventually swell to very refringent bacteria, preferentially if they are closely packed. In presence of broth 1:100 they will undergo 2-4 divisions and finally again swell to refringent cells (approximately of double normal size, sometimes even to round forms). If more broth is added, the swollen cells start again to divide and form bacteria of normal shape and size. - Strain Y24, on the other hand, will never grow on minimal medium, also not in presence of broth 1:100. The cells will get pale very soon and finally disappear. This type of autolysis appears also to give rise to granules; ~~which it~~ possibly gives rise to free granules and not to the granular masses characteristic of lambda lysis. This point has still to be verified. As you will certainly have guessed, my intention was now to try different methods of lysis on the strain Y24 (mechanical breaking up combined with freezing, penicillin, UV), avoiding filtration methods. I would be very interested to know if you could also confirm Hayes' interesting result that only 58-161 treated with UV will increase recombination; as you see from the findings described above, I had thought of a similar possibility in the case of the cross Y24xY70, i.e. that a deleterious effect on Y 24 (which need not necessarily be related with lambda production) might be a favorable factor for recombination. I had also intended to cross two sensitive strains to exclude the role of lambda; I am glad that ~~you~~^{one} can already exclude this factor due to Mrs. Lederberg's experiments.

In addition to the bacterial extracts-experiments, I shall still continue some microscopical work in combination with a micromanipulation technique in order to see if I can find some indications for cell fusions, be it only between sister cells, although I am not very hopeful in that respect.

These are all the news I can provide you with. As you see, they, unfortunately, so far, do not throw much light upon the mechanism underlying genetic recombination in K12.

With best regards,
yours sincerely

Margaret Vogt