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Prof. Joshua Lederberg June 1st, 1962

Department of Genetics
Stanford University Medical School
Stanford Medical Center
300 Pasteur Drive, Palo Alto
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Dear Dr. Lederberg :

Thank you very much for your advices which you kindly gave us for our experiment while you were in our University and for your suggestions in your letter to Prof. Y. Takagi dated on May 22, 1962. I am very glad to learn that you finished enjoying trip safely and started to work at Stanford.

We (I and my co-worker, Mr. Kitsuji) keep, of course, to perform the recombination experiments and the results so far obtained are as follows.

1) The first question. "Is Thy gene on E. coli K12 chromosome ?"

The conclusion is that Thy gene is independent with other markers on E. coli K12 chromosome. This was derived from the experiment to see he segregation rates in the crosses between F^+(58-161) and F^-(W-1177 Thy_2^-). In the cases of Hfr X F^- experiments, the Thy gene was found not to be between Lac, TL and Mtol, because Thy gene was transferred after TL and Lac if one used Hfr which injects the chromosome at the order of M,B1, TL----- and after TL and Mtol if one used the Hfr which injects the chromosome at the order of L,T,B1---. The selective markers were one of those markers described above and SM-resistancy,and F^- used was W-1177 Thy_2^-. In spite of the fact that the Thy gene enter into F^- after T,L,B1 and Mtol, about 90 % of Thy^+ recombinants had the F^- unselected markers, $T^-L^-B1^-$ and $Mtol^-$.

On the other hand, Thy^+ gene was incorporated very rapidly into F^- chromosome when F^+(58-161) and F^-(W-1177 Thy_2^-) were used. The rate of incorporation of Thy^+ gene into Thy^- strain at 180 min. after mixing was ca. 2.1×10^{-2}. This rate is much higher in contrast to the low incorporation rate of TL^+ and Lac^+ which was ca. 2×10^{-4} and 3×10^{-4} respectively, and also to the low incorporation rate of Thy^+(2×10^{-5}) from Hfr to F^-. This results seems to suggest that Thy gene behaves like F agent.

B) The second question. "Is the Thy gene is episome ? Are the Thy^- bacteria those free from Thy-episome ?"

This possibility was also neglected, because
a) Thy^- strain mutated back to Thy^+ condition spontaneously,
b) the two Thy^- strains isolated independently recombined to give Thy^+ recombinants, and
c) Thy^- gene was transferred from F^+ to F^-Thy^+ strain at such low frequency as 1/630 TL recombinants (the crosses between F^+_{Thy} and F^-Thy^- strain gave us ca. 10 % of Thy+ recombinants).

Thus it is concluded that the Thy^- strain is not free from Thy episome but containing it.

C) The third question. "Is Thy gene transferred depending on F agent ?"

One experiment with F^-Thy^+ and F^-Thy^- strains gave us no Thy^+ transferring. This indicates that the Thy gene cannot be transferred to F^- strain without F agent.

D) The fourth question. "Is Thy gene on F agent ?"

The results described above seems to show that Thy gene could be on F agent but it was not the case, because of the fact that F^-Thy^+ strain is present. It is, therefore, concluded that Thy gene is present independently with F agent but Thy gene transferring will need F agent. We don't know yet whether the presence of F agent is necessary for Thy gene transferring or transferring of F agent for Thy gene transferring.

In conclusion : the Thy gene is episome-like (or perisome) particle in cytoplasm which transfer very rapidly from F^+ to F^- and independently with chromosome. The presence of transferring of F agent is necessary for Thy gene transferring, so that Thy gene is transferred from F^+ to F^- and not from F^- to F^+(?) and F^- to F^-. The Thy^+ recombinants of Hfr X F^- crosses would be obtained by the cross of F^+ derived from spontaneously, with F^-. I would be very glad if you could give us any suggestions for our experiments.

The experiment you suggested in your letter has been started by Mr. Homma in our laboratory. We wanted to do the same experiment with amethopterine as well as sulfanylamide. The chemical, however, could not be obtained in Japan. I would be very grateful if you have it and could share it to me, and also if you could add my name in your mailing list.

Please give my best wishes to Mrs. Lederberg.

Sincerely Yours

Toshiko Okada

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