January 3, 1948

Dr. G. Pontecorvo
Department of Genetics
The University
Glasgow, Scotland

Dear Guido:

First, let me thank you for the picture, rather belatedly since it arrived some time ago. I am sending under separate cover the recent reprints which have come out of our laboratory.

I think you will be interested in learning about some of our more recent efforts. The experiments started out fundamentally with the following point of view. It seemed likely that a method of testing the plasmagene hypothesis would be obtained if the following situation were to be true; that there was a gene which duplicated its plasmagene at an extremely low rate as compared with the self-duplicating capacities of the plasmagene. Under such conditions the inheritance of the corresponding character would for the most part be determined by the cytoplasmic components. Now, in view of the haphazard transmission of such tytoplasmic elements, it would be expected that such a character would be much more highly unstable than one in which the transmission was under the direct influence of the gene. On this basis, it was decided to treat haploid cells with mutagenic agents and to isolate mutants, concentrating on the selection of those which were highly unstable. For ease of detection and study of stability, color mutants were looked for.

A set of such characters were found and it is almost certain, from the point of view of the phenotypic characteristics, that it is the same variant isolated by Tatum and used by Lindegren in his recently published paper. As you will note, his interpretation of his segregation results are not cytoplasmic. We found that we could, with the aid of heat-treatments at temperatures and durations which did not lead to a decrease in the viable count, convert 1 out of 10 individuals from the pink phenotype to the white. Furthermore, we found that we could suppress the appearance of the whites by providing an excess of methionine. Here again the experiments were controlled so that selection is not involved.

Essentially, what was done was to get a known number of cells and plate them on excess methionine plates and normal plates. The same number of clones appear in each type of plate, but the number of whites in the presence of methionine is less than one-tenth of 1% as compared with 25% in the plates with low methionine content. These whites we obtained by plating in the absence of methionine or by heat-treatment of pinks which were then plated on methionine, and required about 200 cell generations on methionine plates to revert to the pink phenotype. We have here then what corresponds to a Dauer modification induced either by heat-treatment or by the absence of a particular substrate in the environment. Except for the ultimate reversion in the presence of substrate, we have then a situation which is analogous to the melibiose situation.

Once this had been established, it was decided to see if we could obtain a purely cytoplasmic type of phenomena by obtaining a situation in which one of the normal plasmagenes mutated in the cytoplasm. This was done by irradiating pinks grown in the presence of methionine under which conditions it would be presumed to have an excess of the pink plasmagene. A red variant was found which was highly unstable and reverted to pink. This red variant was also stabilized by the presence of methionine and in its absence would revert to the extent of 99% of the individuals plated. These red variants, when subjected to the heat-treatment under the same conditions as used for pink to white transformations, reverted to the extent of 70% of the individuals to the pink variety. These pinks derived from the reds have not shown any tendency to revert to the pink. It would appear therefore that we have in the red variant a purely cytoplasmic mutation which can be irreversibly lost by either the absence of substrate or by heat-treatment.

We have also found that the response of this component to temperature is entirely different from that of the cells; that is to say, conditions can be arranged in which the mutation rate from red to pink is a function of the rate of cell division, quite analogous to the experiment with the Kappa. Insofar as the role of substrate in stabilizing the character is concerned, the experiments with the red variants represent a complete analogy with the melibiose case.

I believe that with this material we can not only confirm our earlier experiments, but in addition push the analysis of the gene-plasmagene relation considerably further. Thus, for example, we should be able to estimate the rate of plasmagene duplication as well as the rate of generation from the gene.

I noticed in your recent paper in Nature you made some comments about a reversed mutation which looked like a plasmagene effect. I wonder whether you have any more data on that problem. It is suggested by our experiments that you might be able to tackle this by examining the relation between the division rate and the number of generations which is required for the exhaustion of the character. Also, sub-lethal heat treatment would certainly provide interesting information on that score.

Sincerely yours,

ss/b

S. Spiegelman