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Dr. Joshua Lederberg
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Dear Dr. Lederberg:

Your letter of July 28th, with information on the Oak Ridge Symposium volume, was forwarded to me in Europe. Thanks very much indeed. I was able to locate this publication in Geneva and to read a good part of it then with great interest.

As a consequence of writing my paper on the origin of sexuality (which is now in the hands of an editor, who has not informed me of its fate), I have been inspired to speculate on a number of interesting problems. One thing that intrigues me is the question as to whether reciprocal "crossing over" occurs between adjacent nuclear bodies in the multinucleate (usually quadrinucleate) cell of Escherichia coli, when it undergoes ordinary asexual division in the "haploid" state. So far I have found no answer to this question either from the literature or from discussion with friends and colleagues (Adelberg, Garber, Szybalski). Yet it seems quite likely that you have information that could dispose of the question quite readily.

On the off-chance that you have not investigated the problem, I thought to write you, describing a theoretical model that I have derived as a possible means of testing such a phenomenon. As a non-bacteriologist, I do not have the experience and knowledge to suggest much more than certain principles and I realize that an actual test depends upon the availability of suitable genetic systems, which may or may not exist.

The basic requirement for such a test would, as I see it, be that one must be able to distinguish the four types of descendants that would be derived from a single heterokaryotic E. coli cell containing four nuclei of the type AB, Ab, aB, and ab. The only way that I can conceive of deriving such a cell would be to have (induce) a double mutation followed by a "cross-over" one nuclear generation after this mutational event. A scheme of doing this may be proposed as follows:

1. One must have a double auxotrophic mutant ab, requiring substances α and β. Certain necessary, or desirable, properties of AB, Ab, aB, and ab organisms are:

   a) Wild type (AB) must produce enough diffusible α and β to permit both types of single auxotrophs (Ab and aB) and (preferably to a lesser extent) double auxotrophs (ab) to grow more slowly in the presence of AB cells on agar plates,

   b) It would be highly desirable if some easy way of distinguishing the four genotypes (by color; colonial morphology;

reactions to some indicator medium, or media; etc.) were to exist.

2. The mutation rate of $\underline{a} \rightarrow \underline{A}$ and $\underline{b} \rightarrow \underline{B}$ probably would have to be sufficiently high under normal conditions that double mutations within a single cell would occur at, say, about the level of one in 10^6 or 10^7 cells. (I realize that this is very high, but I understand at least one "mutabile" strain of E. coli exists.) Or, to put it a bit differently, the rate of double-back mutations after heavy irradiation (say with 10^4 survivors out of 10^8 cells) should be about one in 10^2 or 10^3 surviving cells.

3. One would then grow ab cells on an appropriate medium (containing α and β) to a density of say 1×10^8 cells/ml., irradiate heavily so that a small percentage survives (1×10^4), and then wash, dilute appropriately, and plate out on minimal medium so as to give between (?)100-500 surviving cells per plate (if all could grow).

4. Presumably only AB cells would grow, plus mutant cells in close enough proximity to receive enough substances α and/or β. Possibly also Ab cells could grow in company with aB if they produced the reciprocally needed factors.

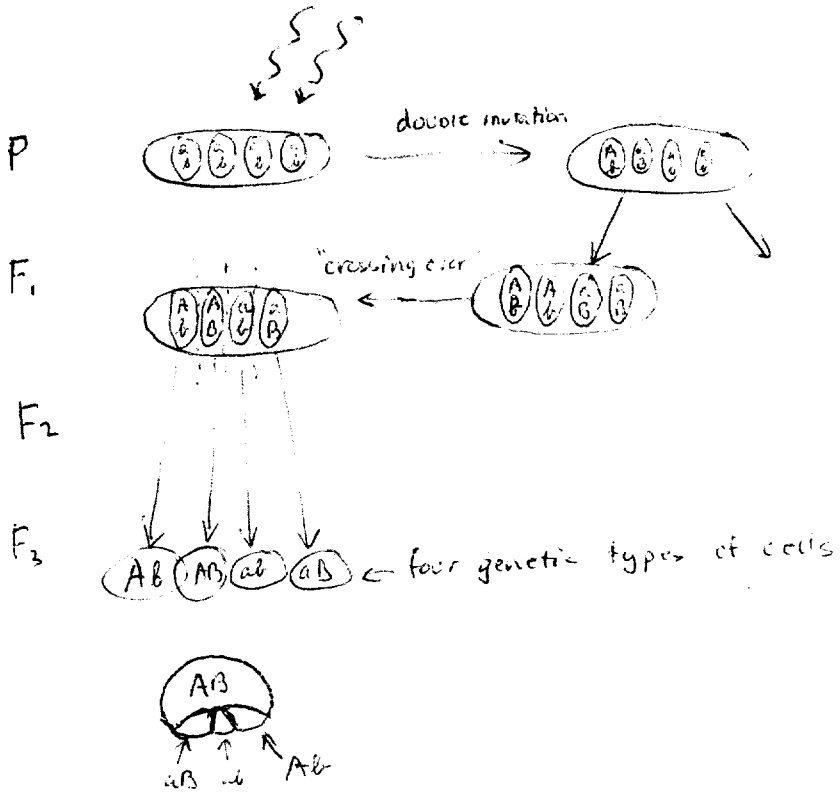
5. The colonies should then be studied for sectoring, which would be evident by irregular colonial shape or (better) by more easily detectable morphological properties. Those colonies with four types of sectors representing AB, Ab, aB, and ab could presumably come only from a cross-over (in accordance with accompanying diagram).

I should be much interested in your comments on the foregoing scheme. There may be fallacies not apparent to me. Also some other (more practical) way of distinguishing four such genetic types deriving from a single cell may well occur to you.

Very sincerely yours,

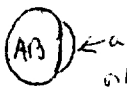
Ellsworth C. Dougherty

Enclosure
ECD:jd



Possible colonial morphology

Other types of mixed colonies might be:



which might result from a unidirectionable transfer ~~change~~ between nuclei after double mutation within cell

which might be expected to occur from segregation of ~~[illegible]~~ separately mutated nuclei from a single cell