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20th.April 1954.

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This letter a bit messy I am afraid. Thanks for what you say re mapping: a lot to be done yet of course. I shall of course be v. glad if we can, when we get it straightened out, write up the cell pedigrees as a joint enterprise.

Dear Josh,

Many thanks for your letter of 28 March, also for the "Progress Report) which arrived about the same time. I have read both several times, and done some cogitation and re-examination of records of earlier micro-manipulation experiments (one or two new ones but no new information), hence delay in answering.

Before getting down to hypotheses to account for trails, there are a few points about your technique I would like to get straight. (i) When you say e.g. "not earlier than 14th.generation" do you mean that you have followed one line of descent, splitting up progeny every generation (or 2 or 3 generations), or is this an estimate based on estimated population size. and/or calculated number of generation times assuming exponential growth ? Or, as in my date, a bit of both ? If the former you must have worked like a slave; I take it your oil chambers are kept at room temperature. (ii) I would be interested to know what method you use for isolating single motile descendent from crowded droplet. I take it you were using real micro-pipettes, not the semi-micro ones you mention in your "single method" note. (I failed to make this work last week, but on re-reading now I see I had misunderstood it. I will try again). (As to re-use of pipettes, I find dipping in boiling water a satisfactory way of re-sterilising a (soft glass) micro-pipette, e.g. after accidentally sucking up a lot of cells). (iii) You say you use lag phase (SW 666) as recipient. Is this a 37° culture grown to saturation, e.g. overnight, and then diluted in fresh broth before, or at the same time as, adding phage ? I am specially interested in trying to get your single motiles giving motile and non-motile clones at 1st.division, which I expected to find but so far without result.

Which (thus)

Now as to hypotheses to account for abortive transduction etc. As to polyteny. I think this too far fetched when it gets to 100-fold or so, and I gather you agree. I think any supplementary hypothesis such as yours on transfer of gene products, or mine expanded below, will account for all the things to account for which we were each inclined to invoke polyteny becomes an unnecessary postulate. Your hypothesis has some attractive features: I am not keen on having the phage transfer two different kinds of material, gene and gene product, but as gene product might be partial gene replicas piled beside gene I suppose there is not much in this. But if it is to explain all abortive motilisation one must postulate transfer of number of products varying from 1 to more than a hundred.

A satisfactory hypothesis must cover the macroscopic observations as well. The long unbranched trails produced by, for instance, SW 553, prove that there are some cells which carry a unit conferring motility (in agar) which is never, or almost never, replicated-and-partitioned. (a)Analogy with the micro-experiments would lead one to expect that most cells producing 1 trail wouldproduce several. But using SW 541 and lysate of TM 2, in parallel macro and micro experiments, one finds at least the great majority of macro-trails arise as single trails, contrary to expectation. (b) I did some counts on numbers of colonies in trails, I forget the strain and have not notes here, but making correction for "end-error" by comparing countable colonies in a trail after, say, 12 and 24 hours, it was clear that number was much greater than number of generation times in 12 hours, hence one must conclude several generations "phenotypic lag". (Ithink the wide crowded trails produced by e.g. SW 545 make this clear without counting). But both my and your experiments give no clear evidence of lag, after the 15th generations or so anyway. The only evidence I had for it were the anomalous droplets, grown from single cell isolated after, say, 10 generations, containing, amongst usual 0 majority, say a dozen motiles, none of which gave anything but 0 progeny. Obviously one can't get positive evidence of phenotypic lag unless one watches all progeny for several generations, to exclude death of gene-bearer etc. as explanation, but lag is certainly much less frequent than one would expect from macro-experiments, if indeed it occurs at all. (c) Comparisons between micro and macro experiments are difficult unless one is comparing same cells. But such few experiments as I have done (and I gather you have had same results) shows that if one picks say, 40 motile cells, puts 20 in droplets and transfers 20 to gel-agar, there is approx. agreement in proportion giving swarms, (5-10%) but gross discrepancy re trails; most cells which give "trail equivalents" in oil chamber, give single colonies in gel-agar at 37°. at 20°

(d) You have had one cell giving motile glone and "semiclones". (I don't get rationale for this term which I take to mean same as my "trail-equivalent"; the latter I agree begs the question but your term I find insufficiently self-explanatory). We have had one example

(macro) of a trail terminating in a swarm; this looked fairly definite.

To account for these observations without postulating totally irregular replication of the "super-numerary gene", I propose the following scheme: Phage transfers gene, which is either incorporated (or replicad) in continuity into chromosome, to give clone; or accepted into some other situation. This might be either as side-branch of chromosome, or in cytoplasm. In the latter case, one must postulate that the gene decays in some way so as to (nearly always) prevent later incorporation. In either case the cell is an "E" cell, capable of forming a trail in agar-gel. (Alternatively the transferred particle which gives an abortive was pre-determined to do so before it entered cell, e.g. was defective in "matching-up" groups, or was a primary gene product, probably partial replica).

The E cell, i.e. cell containing Fla+ gene in abnormal situation, manufactures flagella via a series presumably including "primary gene products". E cell presumably contains wild-type amount of these products, including flagella, hence can spread in semi-solid at 37°. If any of the products are "particulate", effective in dose of 1 particle, and not consumed in producing their effects, and are divided at random when the cell divides, then when an E cell divides it will produce an E cell, and a "T" cell, which will produce n T cells in its progeny, where 2 n is the number of particles present in the E cell. Possible candidates for the role of non-replicating weak-motility-producing "particles" would be the primary product, e.g. partial replica, of the Fla+ gene; and the flagella, or their "basal granules" themselves. On this hypothesis, the macro trail marks the path of the E cell, which owing to a ""dose-effect" of the particles is highly motile through semi-solid medium and keeps going till it dies. The super numerary colonies of trail, at first attributed to phenotypic lag, are really "second-order" trails ending because single particle though it confers broth motility does not suffice (for long anyway) in semi-solid. This accounts for failure of macro-trails to split or arise in groups, despite micro findings. The trail to clone examples (your micro, my macro) indicate that the particle which makes a cell an E cell does very rarely later become incorporated in chromosome, i.e. it is a gene or partial gene, which particle of T cell need not be. When T particle is reduced to one there might be no carry-over effect (of necessity if the T particle is a flagellum. Hence absence (in general anyway) of lag in later generations in micro experiments. The failure of most single motile cells (hand picked) to produce trails in semi-solid would indicate they were mostly T cells, not E; in the only experiments I have done, the time of isolation was so late that this might well have been so. (but was early enough that T cells picked could be brothers or cousins of E cell, hence contain >1 T particle.)

This theory makes on prediction which can be checked on existing data. Droplets inoculated with 1 cell which contain many motile cells must have been inoculated with an E cell; therefore if

being defined as "more than n", where 2N is number of T



the clone produced by the original picked cell has been sub-divided into its components at, say, the 8 cell stage, no more than one of the sub-clones should contain many motiles, (many particles per mature E cell). If n may be as large as 7, then my data fit; but this is rather a high value and anyway there are not enough data to test the idea. I shall be interested to hear whether your data contain any exceptions.

As to further experiments, the following seem worth doing, if possible (a). Further tests to see why micro and macro experiments disagree as to early branching etc. Temperature may be relevant, but one macro experiment on semi-solid agar (no gelatin) at 23° give same kind of result as at 37°, both as to singleness of trails, and failure of most hand-picked motiles to initiate trails. As to the latter, it would be nice to compare trail counts on gel-agar and number ofmotiles produced under microscope, but I don't see how to measure latter. (b) If the T particle is a flagellum or basal granule then T cells should differ in electron microscope from wild-type. (They don't seem to show the excessive wobbling motion one gets with a vibrio, but a peritrichous organism with 1 flagellum would be a new unpredictable object). I have grids and arrangements to get them looked at, all I need to do is to find how to deposit single cells on them. (C) Further pedigrees, if possible splitting up abortive clone at each cell division up to 16 or 32 cell stage, to get max. value for n on my theory. (d) re-examine some long trail producers, counting colony increase per generation time, for an independent estimate of n (or of mean phenotypic lag). Nothing else occurs to me at the moment. I suppose we had better each work away at what presents itself, and compare results (and theories)from time to time. The thing has proved surprisingly more complex than seemed likely when I left Madison.

One further point; most of my micro-manipulation experiments were done with SW 541, and lysate of TM 2; a few with lysogenic derivative of SW 541 gave similar results.

Thats all on abortives. We are pressing on with attempt to map; if the argument is sound, the order must be (544) - (28) - (543)- H1- (966) - (553), but 544 is an obstinate devil anyway, and 553 not much better at times. I agree quantitative data needed.

Yours sincerely,

Bruce

love to Esther. regards to others in lab. Am taking week off next week. trip to Paris.

B.Stocker.

P.S. You say in your "Progress Report" that trail ~~production~~ by $H_1$-linked Fla⁻ recipient is suppressed by either serum. Is this a misprint? I find number not obviously affected by presence of serum against donor's H antigen, in most cases at least. Also on rare occasions, trails even in presence of anti-gp serum plate I gave 0 growth which was still SW553 as is retained $H_1$ gp and Fla 553. Trying to get more but usually unsuccessful