IMPERIAL CANCER RESEARCH FUND LABORATORIES

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Dear Paul,

Many thanks for your long and fascinating letter. The R 1 restriction enzyme is certainly providing a beautiful experimental system for analysing SV 40. I have passed your letter around the lab to most of those who are concerned with this problem.

The growth factor work goes along slowly, though I am not myself doing this now. Probably the nicest thing is a "movement factor" which Bob Burk finds is produced by transformed but not normal fibroblasts. It is now fairly clean and concentrated and he has separated it from the overgrowth factors which are produced indiscriminately by normal and transformed cells. Personally, I have got side-tracted on a funny effect of cytochalasin B which Vitorio Defendi and I have run into. We think that it inhibits DNA synthesis separately from its known effect on cytokinesis. After removal of the drug we get in effect two rounds of chromosome replication for one cell division. It probably tells us something about controls operating during the cycle, and of course it is a way of getting tetraploid cells. Comparing (pseudo)diploid BHK and tetraploid BHK recently made this way the stable transformation frequencies by polyoma are identical.

Now about the stable and abortive clones: It seems clear that the five abortives that you tested are all negative from the Cot $\frac{1}{2}$. Unfortunately, none of these clones are recoverable from our refrigerated stocks here. We would like very much to know if they have T antigen and

grow in agar etc. Presumably Marianne will have some idea of their morphology. Do they still all look normal in this respect? I hope Marianne will be calling in here when she is in Europe and we can ask her about this.

As you know, we have more data on the four additional abortive clones that we sent you recently (MA 1, MA 3, MA 9 and SA 2). In addition the plating efficiences in agar which are as follows: ST 1 - 24%, ST 6 - 24%, MA 1 - 11% MA 3 - 14% MA 9 - 24%, SA 2 - nil. I wish the MA series had been tested at an earlier stage after they were first isolated.

ST 1 remains particularly interesting. Is it possible to find out with more confidence whether there is a deleted genome present? It could either be an odd spontaneous transformant independent of virus infection, or alternatively it has a half genome including the transforming gene but not the T antigen gene.

You discuss, the idea of repeated integration and excision, except for the odd excision defective molecule (Tsa ?) which remains in. There is one consequence of this, namely increasing multiplicities should reduce the efficiency of transformation because of rescue by wild type molecules (assuming these are in excess). In fact something like this does happen. If you look at the old dose response data you will see that it is linear up to a point, but then remains level or even falls with increasing multiplicity. But why is it even linear?

I wonder if one could explain the difference between SV40 and polyoma by supposing that polyoma always expresses if it is integrated, but that SV 40 may be integrated in an expressing or nonexpressing site, on the chromosome. This would explain why we do not find revertants with polyoma genomes.

Jon Warner, who has been here on a sabbatical has been looking for SV 40 genomes in the abortive 3T3 system by fusion with permissive cells. He recovers virus from a fair proportion of the stably

transformed clones, but interestingly has got no infectious virus out of some twenty* normal, (presumed revertant) clones. It would be interesting if it could be shown that some of these had indeed got SV 40 genomes. Unfortunately, he will be returning to the Einstein now and will not be able to do this. Do you know if HeleneSmith or Martin are still set up, or could you handle the SV 40 system?

In the polyoma system, at least, it is clear that a lot more needs to be done on the ups and downs of the phenotypic characters of these supposedly normal cells which now seem to change in behaviour, in passage far more readily than cells that have never been infected. If you find virus genomes in some, but not all of the total abortives that have been already isolated then I suppose there is a hard job correlating the presence of the genome, and perhaps also viral RNA synthesis, with the alterations in the cells over time.

If on the other hand, all the abortive clones are negative for virus genomes, then it seems to me that we have a situation which is interesting in itself, because unlike SV 40.

I certainly think we should get together to go over the data as soon as you have the results on the remainder of the clones. Are these likely to be through by mid-September? Unfortunately, I shall not be over in the States again this year so far as I know at present. However, I shall be here the second half of September and it would be splendid if you could come here. Perhaps it should depend on how quickly the results will come through. If you can come, can you raise any money for the trip or would you like us to pay?

About your request for the original small plaque virus used for the transformations. Lionel has recently replaqued the small plaque stock. He and Bill Folk will consult about ways of providing you when they are back in the lab from leave shortly.

All the best,

Yours,

Tim

* I may have got the actual number wrong