

# IMPERIAL CANCER RESEARCH FUND LABORATORIES

Registered Charity No. 209631

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Dear Mike, Art, Herman, Nancy---

Enclosed are copies of the information Chris Marshall originally provided me about his hybrid lines. I would still suggest looking at one parent (1447 with wt provirus, B77) and two hybrids derived from it (1577 and 1578). At this point, the isolation of "retransformants" from the hybrid population is the only evidence for retention of the provirus which originally transformed the cell---in view of the evidence that some of my rat-1 revertants are DNA-minus, it might be necessary to map proviruses in parental and hybrid cells if you folds ~~don't~~ don't find viral RNA or protein. (The ~~parental cells could have multiple proviruses, losing the active one during selection of hybrids and retaining silent ones that might be reactivated later. Although unlikely, this possibility is testable and, if indicated, I will do so when I return.~~)

I have discussed our recent results with the revertants of ASV-rat-1 cells (clone 31) with John Wyke. (1) The transformed clone was originally picked as an agar colony and then subjected to single cell cloning. (Hohn does this by picking single cells with a micropipette under the microscope, using a dilute solution of trypsinized cells; the single cells are then grown in a microtitre dish.) This means that it is virtually impossible for the original clone to have been contaminated with uninfected cells, implying that the three DNA-minus revertants have truly lost the ~~their~~ viral genome. We will attempt to get karyotyping done on them here; if the karyotype is unrevealing, I will consider cloning the Clone 31 provirus when I return, in order to make a probe for the adjacent sequences to see whether one copy of them is disordered in the DNA-minus cells as a consequence of excision. (2) Since no examples of revertants I and N have been tested ~~in~~ biochemically for gene expression, it would be nice to test them for kinase and labeled proteins. RNA testing would seem unnecessary at this stage. Obviously there is little point in testing the DNA-minus cells further (G, H, and O). (3) Three revertants (B, I, and J) have been tested for rescue of polymerase ~~containing~~ particles in the absence of transforming activity; they are all positive, but interestingly the titres of stock ~~from~~ from J are ~~significantly~~ higher than stocks from B and I. This seems not to correlate with Herman's observations ~~with~~ about pr76 levels, but ~~these~~ such comparisons are probably premature. (4) John will

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recover T+ virus from revertant E and will prepare clones of retransformed cells from E also. It would be of obvious interest to test these materials for kinase activity and levels of pp60. For comparison, he will rescue nontransforming virus from a few other clones: J (good pp60 band, ? no kinase) and B, F, or M (low pp60, ? no kinase). In addition, he ~~will~~ has a stock of virus rescued from L; this stock is transforming but makes fusiform foci (as you recall, L is kinase +). When these things are ready, we will send viruses in chick cells.

John was, of course, surprised by the observation that his double revertant had only a portion of a single provirus, whereas the putative parent (GE 11, also picked as a single cell) appeared to have three proviruses. I will try to do a little more mapping with these, to see if the partial provirus in the double revertant ~~is~~ in the parent and to get some idea of the organization of this peculiarly deleted provirus (Steve is about to reanneal the first filter with cDNA ~~3, 4~~; please share this letter with him). Although John is certain that the double revertant was isolated from GE11, some of his other lineages are clearly confused (based upon mapping data with revertants of one of two active proviruses), and he is now redoing the experiment from the beginning, making double transformants (avoiding the helper virus and defectives used in the previous protocol) and then selecting both single revertants (of the ~~WE~~ provirus) and double revertants (at 35°). If any of these <sup>latter</sup> appear from the mapping data and from retransformation experiments to be candidates for cellular mutants you will be hearing from us.

John and (needless to say) I appreciate the time and effort that you have put into the clone 31 revertants. When some of the items discussed above are completed, I will put together a brief description of these revertants for publication.

It is nice to be back here, decelerating to the ~~the~~ ICRF rhythm.

Cheers,

