

June 11, 1974

Dr. David Boettiger
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Dear David:

It was good to see you last week and to get a chance to compare notes. I have read your manuscript and think it is very good; I have not been asked to review it, but I cannot imagine it will encounter any difficulties. I do have a few minor suggestions: on page 1, your account of our experiments might be clearer if you indicated (1.21) that infections were done with B77 rescued from mammalian cells; on page 2, there is a misprint in the third line (R^+), and it would be better to refer to "nucleic acid hybridization studies" in the 6th line from bottom; in Table 5 is there some reason for the 2 log differences in titres? Would it be useful to list the VSV pseudotype data for these strains? (do you have such data for viruses rescued from the R^+T^- cells?) I am not completely content about calling your clones R^+T^- , since I would not be surprised to find that there are infected cells, bearing some or all of the RSV genome, from which virus cannot be rescued. We would then have two sub-classes (or perhaps three) of infected, non-transformed cells (which is what I call the general class): R^+T^- , R^-T^- , and R^-T^- with only a partial genome. Is there some reason why you do not cite Kotler's work? I am sure we agree it is not very satisfying, but it doesn't cost much to avoid his ire. Do you think it would be presumptuous of me to ask whether you would mention in as a personal communication in your discussion or as a postscript the fact that Peter and I have isolated clones of 3T3 cells similar to your NRK clones.

We now have three clones in which about 1 copy of RSV DNA has been shown to be integrated by the network test; in one of these (3T3-B5) the $C_{rt1/2}$ is about $2-3 \times 10^4$, three fold higher than in the transformed B77/3T3, i.e., there is 3 fold less viral RNA. In the other two (B4 and B8), the analysis has not yet been carried to sufficient C_{rt} values to obtain much hybridization, but the $C_{rt1/2}$ is certainly greater than 5×10^4 . We have now rescued virus from B5 and B8 using a semi-quantitative adaptation of your procedure; in both cases, rescue is less efficient than with the transformed cell. Interestingly, B4, from which virus has not been rescued, appears to have less than a full copy of DNA, thus raising in my mind the possibility alluded to earlier; this aspect is very preliminary, and requires further examination of subclones, repeat hybridization and rescue, etc. We have looked at a few subclones, and they breed true. In addition, we have six additional clones that have RSV DNA, but they have not been further examined.

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Let's exchange CSH manuscripts when they are ready. I trust you have not forgotten about sending me your mammaltropic RSV. And think about coming here for a while in the fall. Regards to you and Robin and Steve, etc.

Yours,

Harold E. Varmus, M.D.
Assistant Professor
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HEV:bb