

April 1, 1976

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Dear Don:

I suppose you are right to be surprised that MMTV is under steroid regulation in HTC cells. We were less surprised, though very pleased, since we knew that two other heterologous, epithelial host cells (cat kidney and mink lung) had behaved similarly when infected by MMTV in Camden (Dan Moore's group). We have not directly measured the rate of degradation of MMTV RNA in these cells, but Gordon has shown that the steady state concentration and rate of synthesis in the presence of dexamethasone are similar to the concentration and rate in dex-treated ER tumor cells. (Unfortunately, we've had trouble doing a good experiment to measure degradation or deinduction rates in GR cells.) Although the HTC cells make less than one virus particle per cell per day, they do contain MMTV protein (by immunofluorescence testing), and the nature of their non-permissiveness is a very interesting mystery. Perhaps it would be a good focus for your proposal. Gordon is, of course, working on the mechanism of steroid regulation of MMTV in HTC cells and that includes at least a partial analysis of the RNA metabolism. (He will, by the way, be joining Keith Yamamoto in the biochemistry department in several months, but we expect to maintain a coordinated and partially collaborative program.) But there is clearly room for more hands in this area, specifically in studying the size and location of viral RNA. Gordon has begun to think about immunoprecipitating virus-specific polysomes from these cells (the method seems to be working in ASV0infected cells), but I don't know if he'll be able to carry this through along with everything else he's doing. (The basic idea is to fractionate transcripts which code for only a portion of the viral proteins, in order to do some provisional map-making.) Alternatively, you might think about working with viral DNA. The infected HTC cells have about 20 integrated and 10 unintegrated copies, of which about 2-3 are closed circular and amenable to purification. Restriction mapping, transfection experiments, or heteroduplex formation between viral DNA molecules from different strains are some of the obvious possibilities. Of course, any of these things would be nicer if there were some genetics in the MMTV system. I've been wondering whether it would be worthwhile to attempt to transfect explants of virus-negative lactating mammary tissues, in hopes that an observable biological change and virus production would occur. (It is not clear why murine cells are resistant to infection in vitro.) Another potentially interesting (? dangerous) possibility would be to infect some human mammary tumor lines developed (and offered by me) by Nat Young at NIH. These lines have been well-characterized and are responsive in different ways to a variety of hormones. The behaviour of MMTV in such cells, particularly if virus were produced, might be worth studying - both from an endocrinological point of view and as an exploration of the idea that an MMTV-like agent is implicated in human mammary carcinoma.

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I realize you don't want to outline anything that will appreciably overlap with Gordon's work, but I don't think it's a major concern (neither does he - I've talked with him about it). It is very hard to predict just what phase of things he will be working on eight months from now. At this point, with Gordon moving upstairs and Vince Morris going to Ontario, it appears that you are the only person committed (more or less) to the MMTV project. Since I have apparently just been awarded a five year grant to work on it, I will probably try to entice at least one other fellow or student into the area and may also have my primary technician, Suzanne Heasley, devote more time to it. (She is currently growing clons of infected HTC cells.)

Let me know if I can be of further help.

Best regards,

Harold E. Varmus, M.D.
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HEV:js