

September 3, 1974

Dr. Luis M. de la Maza
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Dear Dr. de la Maza,

I have just received and read your (our) manuscript and in general I think it makes a nice story. It would seem appropriate to me to submit it as a note to Virology, or, as you suggest, as a short paper to Cell. It could also be a letter to Nature.

I have three criticisms of the paper. (1) I think slightly more experimental detail should be provided in places I have noted in the manuscript. (2) More information about the fractionation is required. A Table along the lines of the one I have suggested (see ms.) would help summarize the data available; in addition, it would be helpful to the reader to know how much of the DNA ended up in each of the fractions (including the intermediate fraction). What was the composition of the intermediate fractions? Was the fractionation done several times? Was the extent of fractionation reproducible? I am troubled by the rather poor fractionation of the heterochromatin in the transformed and revertant *M. agrestis*. Since a substantial portion of these fractions are (presumably, unless you have evidence to the contrary) euchromatin, I don't see why some viral sequences are not detectable in those fractions. (3) I think the conclusions you draw (a) about a "preferential" integration site and (b) about the likelihood of "post-transcriptional" control are completely unwarranted. (a) Viral DNA has been found in euchromatin in the cells descended from only two infected cells - i.e., the 3T3 cells originally infected with B77 and the *M. agrestis* cell originally transformed by B77 (the revertant cells being daughters of this cell). Hence we have looked only at two integration events (unless one presumes that viral DNA can be excised and reintegrated, a hypothesis for which there is no evidence), and it happens that both events occurred in euchromatic fractions. That does not exclude the possibility that the event is random. An answer to that could come from looking at many clones, or at DNA from mass cultures of acutely-infected cells in which a large fraction of the cells have acquired viral DNA. (b) There is no evidence here to substantiate the assumption that the site of integration determines transcription and that therefore virus expression must be regulated at a post-transcriptional level. On the

contrary, in our studies of the revertants derived from RSV-transformed hamster cells (see the enclosed preprint, in press in Virology), the reverted cells have lower concentrations of viral RNA. It may of course be true that in the hamster or the Microtus cells that post-transcriptional factors may be operative. But I don't want to sign my name to the proposal without some evidence for it.

Thanks for sending this to me, and please let me see the final version before it is submitted for publication.

Yours,

Harold E. Varmus, M.D.
Associate Professor of Microbiology

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