January 2, 1975

Dr. Paul Neiman
Division of Oncology
Providence Hospital
500 17th Avenue
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Dear Paul,

Thanks for sending us your pretty and thought-provoking data; it has given rise to a frenzy of hypothesis-making to account for the obvious discrepancy in our results, but we are still basically at a loss to explain things. We have re-examined the possibility that our probe for the deleted sequences (cDNAx) represents in the main a very small portion of the genome. Our claim that 12% of cloned sarcoma virus RNA anneals to the probe at a 1:1 ration of RNA:DNA and 16% at a 1:2 ratio is of course dependent upon the accuracies of the specific activities of the RNA and DNA. In the experiments in question, the specific activities of RNA and DNA are computed to be about the same (20×10^6/µg); this has been independently verified by annealing the RNA to the unfractionated DNA (from which the specific probe was made) and digesting unannealed nucleic acids with nucleases; the counts remaining in hybrid form were the same for RNA (^{32}P) and DNA (^{3}H), demonstrating the equivalence of specific activities. (This result was independent of whether the hybridization was performed in RNA or DNA excess.) Therefore we feel obliged to conclude that the probe is a virtually uniform transcript of the deleted region.

We have results of the first thermal denaturations of hybrids formed between $cDNA_x$ and avian DNA's/ Stable hybrids are formed with chick and quail DNA's, the Tm's being identical to the Tm's of hybrids formed with unfractionated cDNA and chick cell DNA. The Tm is, however, depressed about 8°C for the duplex formed with duck DNA. This is a pleasing result from the evolutionary standpoint; we are in the process of repeating the experiment and extending it to other birds, transformed cells, etc.

We would be very interested in testing whether our $cDNA_x$ is capable of annealing to your td-PrC RNA, to exclude the remote but still viable possibility that the deletion you are working with is different from the others we have looked at. If, for example, your td virus had a 15% deletion affecting a small region of the transforming gene and a larger region of other non-transforming genes introduced into the cell during infection, there would be an easy reconciliation of our data. Would you be willing to send us about 1 µg of unlabeled RNA for this test? We would like to test your sarcoma virus RNA at the same time, as I mentioned in my last letter.

Let me know if you have any further ideas which might resolve our dilemma. It would be a great step forward in this field if we could try to work out this problem in this way without subjecting journal readers to its confusion and gradual resolution. All of us involved in this project very much appreciate your frank discussion of the issues and your prompt transmission of your data to us. (When Dominique returns from a brief vacation, I will ask him to send you copies of graphs depicting the experi-

ments which bear upon our problem.)

Best regards,

Harold E. Varmus
Associate Professor
Department of Microbiology

HEV/sl

cc: Dom
Mike