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SCHOOL OF MEDICINE — SAN FRANCISCO, CALIFORNIA 94122

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Dr. Paul Neiman
Oncology Division
Department of Medicine
University of Washington
Seattle, Washington

Dear Paul:

Thank you for sending your grant proposal. I have read it with interest and have shown it to Mike Bishop and to Chris Hansen, a graduate student working on the same problem.

Needless to say, the results you present appear very promising. One of our concerns is that you may not be using sufficiently high ratios of DNA/RNA to detect maximal hybridization of the RNA. For example, in the "low C_0t" experiments, you use a 20/1 ratio of DNA/RNA (the ratio may be higher in Figure 6 but you don't provide the specific activity of the RNA); assuming the molecular weight of the RSV genome to be 10^7 daltons and of the ~~which~~ chick genome about 8 x 10^{11} daltons, at a 20/1 ratio the concentration of RSV sequences would be equal to the concentration of cell sequences represented 4000 times per genome. Of course, it is likely that only a small fraction of your RNA is actually represented in repetitive sequences, so that the ratio is probably 10-100 fold better. The $C_0t_{1/2}$ of 20 suggests you are detecting a family of about 1000 copies; if 10% of the RNA were homologous to this DNA, you would have a 2.5/1 ratio of DNA/RNA. Since Melli and Bishop indicate that DNA-DNA interactions are ~~formed~~ favored over RNA-DNA interactions and since this is particularly so when your DNA is not adequately sheared, it is conceivable that much less than 10% of the RNA would form hybrids. Our view, therefore, is that you may not have maximized the low C_0t annealing; however, our own experiments have not demonstrated what we would term significant (more than about 5%) annealing of viral RNA or polymerase product DNA at low C_0ts. We are therefore in accord with your answer as a qualitative result.

At high C_0t values, where you incubate 5 ng of RNA with 100 μg of cell DNA, we calculate that you have 8 viral genomes per diploid cell genome, suggesting that the maximal annealing to single copy DNA would be about 20% (if each chromosomal complement had 1 copy). (Again, the results of Melli and Bishop and the problems of incompletely sheared DNA could work to your disadvantage). Your results, of course, indicate that you may soon see more than that, raising the possibilities of multiple copies of viral sequences or of a larger than

expected size of the viral genome. It would be useful to know when the DNA in your experiments is reannealing, mainly as a check on your claim that the cRNA is copied from unique sequence DNA. (We would be interested in learning more of the details of preparation of the cRNA). It would also be useful to know that the depurination procedure, a somewhat unusual approach to DNA shearing, was not affecting the reassociation kinetics of the DNA - of course, the stability of your hybrids suggests that appreciable untoward effects have not occurred. (Again, we would be interested in the details of the depurination procedure and its reliability).

Chris has not gotten as far as you have with this approach. He has been dealing with some of the logistical problems, aiming for viral RNA of very high specific activity (greater than 3×10^5 cpm/μg) and large amounts of unique sequence cell DNA. (It seems to me, by the way, that you might be able to increase your specific activity by using more ^{3}H-uridine, which is relatively cheap).

I have mulled over the differences between your results and mine and at present see no easy reconciliations. If the double-stranded DNA probe were, in fact representative of the whole genome, then I would calculate only 1-2 copies per chick cell. However, then what could I make of the slowly reassociation product which is 4-5 times rune complex than the bulk of the DNA? and how would I account for the discrepancy between our results and classical findings with reassociation kinetics? and how could I explain cell lines from quail or rat which appear to have 4 or 2 copies with current calculations and would then appear to have only fractions of genomes or genomes in only some of the cells? (Gelb et al have this problem with SV-40 sequence detection in 3T3 cells).

Our most interesting recent finding is an apparent absence of RSV sequences in normal 3T3 cells, with about 2 copies per diploid cell in B77 and Schmidt-Ruppin transformed 3T3 lines. The detection is difficult and we're not saying too much about this until we perform some experiments to give us qualitative (YES/NO) results, as well as shifting of $C_o t$ curves.

Again, thanks for the proposal - let's keep in touch. Have you tried normal chick DNA yet?

Yours,

Harold E. Varmus
Department of Microbiology

P.S. We'd like to see reprints of your articles you refer to.

HEV:bb