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Dear Michael:

So often in the last few weeks I've wanted to write to you but an avalanche of deadlines, from which I am only recently recovering, forced delay after delay. How are things at ICRF? Hopefully, science, construction progress on the new labs as well as the many administrative activities you are involved in are going well; Peter Beard's occasional correspondence with people in the lab and Bill Folk's visit brought only incomplete accounts of the happenings in London. Right now I'm wondering how you're faring under strictures of the fuel-electricity shortage; sounds pretty grim! Has it caused hardships in the labs, particularly with being able to maintain necessary services for work and keeping cells viable and alive? I suspect that long ago you had seen to ensuring the laboratories own power supplies and therefore immunity from external exigencies. From what I read in our newspapers I suspect you probably had trouble commuting from Kent.

In the last few months things have gone quite well here in the lab. Let me fill you in on a few of the details.

1) We've mastered the technique of detecting polyoma or SV40 viral DNA sequences by following the kinetics of annealing using S_1, an enzyme which rapidly degrades single-stranded DNA but leaves double-stranded DNA segments intact. In a nutshell, (I assume Bill Folk can relay more specific details of the procedure since he followed our protocols to the letter), we synthesize very highly P^{32}-labeled PY or SV40 DNA using pure E. coli DNA polymerase highly purified unlabeled Form I DNA as template, four very hot d-triphosphates (10-15 mc/μmole) and traces of DN'ase to generate random single-strand nicks in the supercoils. DNA polymerase replicates random segments of the DNA (by "nick-translation") thereby generating highly-labeled viral DNA segments. The labeled DNA which has an average single-strand length of 300-500 bases and a specific activity of 5 - 10 x 10^6 cpm/μg is used as the "probe" for detecting complementary sequences in cellular DNA's. The experiment is quite simple now; e.g. P^{32}-PY DNA is denatured in the presence of either salmon-sperm DNA, BHK DNA or the DNA from cells abortively or stably transformed by PY. Salt is added to the appropriate concentration (0.2-1.5M depending on the rate of annealing we expect) and annealing occurs at 68°. Samples are periodically withdrawn, diluted and frozen. When enough samples have been taken, they are all digested briefly with S_1 and then precipitated with TCA. The amount of annealed DNA is equal to the amount of P^{32}-label precipitated by TCA and retained on the filter. At zero time this 2-4% of the input at the end of the annealing

70-80% of the DNA is insensitive to S_1, (the remainder is probably the fraction of DNA nucleotides which cannot enter into helical structure because of steric-hindrance, or we believe more likely, the non-viral DNA sequences carried by our polyoma stock). The protocol, computer printouts of the data and calculations as well as the photographs show a typical reconstruction experiment when known quantities of cold PY DNA (sheared to same size and denatured) is added to the annealing mixture. Recently, we've simplified this procedure so that samples are taken only during the first few hours of annealing and our computer program computes the second-order rate constant (and thereby Cot 1/2) from the initial points. The second set of computer printout and plots use only the data for the first 24 hours and you can see that the curves are linear (2nd order) even with the points for the first two hours. The sensitivity seems to be good enough to detect 0.5-1 genome equivalent per cell (< 1 part in 10^6) using approximately 1.5 to 2.5 mg of cell DNA.

Using this method we've begun to analyze some of the abortive and stable transformants of BHK we collected when I was in London. Summarizing:

a) P^{32}-PY DNA in the presence of salmon sperm or BHK DNA anneals with an identical Cot 1/2: Martin claims that 3T3 does contain some sequences homologous to SV40 but we shall have to do this many times before one can say definitely that there is or is not a fraction of a PY genome equivalent in normal BHK cells.

b) The abortives MA-8 (methocell) and SA-10 (surface-infected) have no detectable PY sequences. Of the two stables, transformants we've tested, ST-1 and MT-1, only MT-1 contains PY- DNA sequences. (MT-1 3-5 viral equivalents/cell; ST-1 < 1 viral equivalent/cell). Consequently, ST-1 may be a spontaneous transformant but it should be retested. If you have another sample of ST-1 with one that can be screen hopefully, one that could be tested for T-ag, we'd like to retest it.

c) We have just finished growing MA-4, MA-6 and MA-9 and SA-2 as well as one other stable transformant and will do the annealing kinetics with their DNA's shortly.

In view of Smith <u>et al.</u> finding with the SV-3T3 abortives it really is important to determine <u>for sure</u> if PY-BHK abortives really do <u>not</u> have PY-DNA sequences in their DNA. If there is a difference it could be very relevant to the mechanism of integration-excision with each virus. It seems crucial therefore, that we look at as many true abortives as possible to be sure. I have only some of the clones we picked. Do you have more? Or even abortives collected in different experiments over the years? Or do we have to do another experiment to collect a new set (clones grown from microcolonies picked from methocell would seem to be the best since they are most likely abortive). I'd be delighted to come over again to prepare a fresh lot but that's not possible now. Some time in September (Renato has been trying to talk me into coming when he's there in Sept.) would be possible but that seems a long way off; until them is it possible to get any abortively transformed clones, (either ones we collected in 1970 or

any others you have) to look at? Accumulating enough cells for DNA isolation is a limiting factor since our large-scale growing facilities are still primitive. Is it possible for your set-up to produce about 5 gms wet weight of some of the clones? If we could grow some and you could grow others that might speed things up somewhat. Unfortunately, although we are in good shape to do the hybridizations easily enough we are in worse shape to generate and grow well characterized abortives.

2) Our project to insert new segments of DNA into the SV40 DNA molecule has succeeded and now we hope to get to some interesting experiments. Without going into detail here, unique SV40 linear DNA molecules (made by a single double-stranded break with a bacterial restriction enzyme coded for by the drug-resistance transfer factor RTF-1) have been derivatized at their 3'OH ends with either short runs of dA or dT and after annealing to produce non-covalent dimer circles they were covalently joined with DNA polymerase and DNA ligase to produce sealed dimers in 25-35% yield. Using the same kind of SV40 DNA linears and appropriately derivitized linear DNA from λdvgal (mol. wt. 6×10^6; containing about 4×10^6 daltons of λ DNA and about 2×10^6 daltons of E. coli DNA including the entire gal operon), covalently joined hybrid DNA molecules have been made in about 20-25% yield. Still to be completed (this has run into more difficulties than I anticipated) is the insertion of a short piece of synthetic DNA, dI, G:dC (100 base pairs), into the SV40 ring at the R_1 restriction site and ultimately at other sites in the structure. Hopefully, such molecules may give us a way to map the genes of SV40.

There are several other projects moving well enough (Peter Beard's work is coming along quite nicely) but more about these at some future occasion either here, in London, or at least by mail.

I do look forward to your comments on the question of the abortives and of course to hearing something about your own work on the serum factors, as well as of other happenings from ICRF.

My very best wishes and regards for the coming year to your family, and colleagues at ICRF. Please remember me to all.

Sincerely,

PB/l