מכון ויצמן למדע
THE WEIZMANN INSTITUTE OF SCIENCE
REHOVOT · ISRAEL רחובות · ישראל

DEPARTMENT OF GENETICS המחלקה לגנטיקה

MS/id

July 4, 1972

Dr. Daniel Nathans,
Department of Microbiology
Johns Hopkins University
School of Medicine
Baltimore, Maryland 21205
U. S. A.

Dear Dan,

We realize that we should have written long ago and apologize for the delay. During our recent phone conversation, we promised Kathleen Danna to write concerning our results with the H. influenzae restriction endonuclease. The brief outline that follows is really a progress report of work done with Sara Lavi and Shmuel Rozenblatt. All the results were obtained with the enzyme you sent us. Lack of sufficient enzyme, because of our own failure to make a good preparation, has kept us from doing more until now. As Kathleen probably told you, although our enzyme preparation appeared to proceed well (using E. coli DNA as substrate for the viscosity assay) and gave material with no detectable activity against H. influenzae DNA by viscometry, SV40 DNA was degraded to acid soluble products. We are now getting ready to try again using the original procedure rather than the "alternative" procedure utilizing streptomycin sulfate.

The digestion experiments have been done with strain 777. Two separate clonal isolates, grown at low dilution, gave essentially identical patterns which, though similar to your published pattern for 776, differed in some aspects. This finding is presumably consistent with the varying patterns for different strains which you recently published in J. Mol. Biol. Are any of those 777? In confirmation of your data, a plot of log of percent of total counts against mobility gives a line: the bigger fragments fall on the line, the smaller ones scatter some. At least one of the larger fragments is a doublet. We have also investigated virus obtained from undiluted serial passaged of one of the clones. With this preparation, most of the peaks seen with the clone are apparent but in very low amount. Almost all the radioactivity is in only 4 peaks, 2 or possibly 3 of which do not coincide with peaks of the original virus. All these fragments are smaller

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than the largest fragments found with the clone. This late passage virus has a high proportion of sequences homologous to DNA of the BS-C-1 host cells (see Lavi and Winocour). These sequences appear to be concentrated in two of the new peaks. From the hybridization data obtained with the fragments, we are trying to estimate the percentage of BSC sequences in the virion DNA. As a complementary approach, we have prepared filters with the fragments obtained from the cloned preparation and are trying to determine which 'normal' sequences are missing from the late passage material. These results are preliminary, but all suggest that extensive regions of the viral DNA have been replaced by host sequences.

Because we know from independent experiments that serially passaged virus varies with respect to the extent of host substitution depending on the original clone, our results are not necessarily to be expected from any other serially passaged virus.

One further question we have been wanting to ask you is if you ever tried to hybridize any one of the fragments with the others (using a double label)?

It is clear to us that you have developed an extraordinarily powerful technique in the use of restriction enzymes to study DNA structure. Hopefully Maxine will be able to prepare useful enzyme prior to her departure in mid-August, so that the group here can readily continue this line of work. She will certainly be in touch with you upon her return to NIH.

With best regards, and many thanks,

Sincerely yours,

Ernest Winocour Maxine Singer