

November 4, 1977

Dr. Ed Southern  
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Dear Ed:

I am enclosing our first preprint in which we make extensive use of the transfer procedure, but I am writing mainly to inquire about your response to the idea of helping us with a combined bureaucratic-scientific problem. We are now within range, scientifically, of cloning integration sites for the proviruses of both avian sarcoma-leukosis and murine mammary tumor viruses, since we have identified restriction fragments from clones of infected cells containing host sequences linked to the termini of the viral genome. Both systems show evidence of specificity in the integration events, since the recombinational site on the viral DNA seems to be mostly if not always the same (i.e., at the position on the DNA corresponding to the termini of the viral RNA). In the case of the avian viruses, we have defined some sites in the cellular genome that are used preferentially by certain strains of virus, although there are clearly multiple sites available; in the mammary tumor virus system, there are a large number of available sites, but we do not yet know whether the number of available sites is limited or whether some sites are employed preferentially. In either case, it is of obvious interest to clone several fragments containing host and viral sequences to determine whether the cellular and viral sites are homologous and to compare the features of the various cellular sites used by each virus. Our emphasis is upon the fragment which contains the end of the provirus encoding the 5' terminus of the RNA, since the 100 bases at the 5' terminus of avian virus RNA have been sequenced (the same should soon be true for the mammary virus) and since interesting regulatory machinery is likely to be found upstream from the provirus. Clearly, we have a formidable enrichment process to worry about before cloning, but we would expect that a judicious combination of gel electrophoresis (? gene machine), restriction enzymes, reverse phase chromatography, etc. should be adequate to achieve a 100-plus enrichment, enough to make cloning and detection within the realm of reason.

Beyond this, however, lie the bureaucratic hurdles. At present, everything we wish to do is unambiguously P4. We are applying to use a P4 facility, if and when available, but I can't be very optimistic about this approach. If the new guidelines are signed by Fredrickson---an unlikely event for at least six months---we could do some things in polyoma under P3 conditions, but again many things would have to be started in P4 unless an EK3 system were available. One of the things I hope to learn at ICRF is the use of polyoma vector system, with Mike Fried, in case it appears that cloning

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in polyoma is the best way out. Certainly, we'd rather do the work in procaryotes. The last option would seem to be to do some of the work in a country, such as Great Britain, in which the rules appear more flexible, even though they may be cautiously administered. I personally think a good case can be made for cloning the sorts of fragments I am thinking about, since we can trim the viral components down to 50-100 bases with the appropriate restriction enzymes; it would seem very unlikely to me that so few sequences could trigger a cancer epidemic! There is very little interest in these matters among the obvious people at ICRF and I am writing to solicit your views of these experiments in general and to learn your attitude towards a collaborative program between our labs to try to do some. If you were interested, and if simpler solutions do not appear bureaucratically feasible by the time I leave for sabbatical (next July), I would like to consider the possibility of filing a joint application to GMAC. If approved, I would come up from London for whatever time was necessary (I am here remembering your kind invitation to consider doing some experiments in Edinburgh) and would hope that the post-doctoral fellow in our lab most involved in the avian work (Steve Hughes, formerly with Mario Capecchi) would also be able to come over for part of this time.

I will be curious to hear what you think of these ravings. I look forward, in any case, to having some time together next year.

Best regards,

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

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Encls.