

Career Summary

Like many other physicians who now practice molecular biology, I was a relative late-comer to the laboratory. When I entered college twenty years ago, I assumed, out of filial devotion to my father's profession, that I would ultimately practice medicine. I was, however, soon seduced out of my good intentions into the study of English literature and philosophy; although I managed to complete my pre-medical requirements, I spent most of my time reading Dickens and running the college newspaper, with little time or patience for laboratory science. During my first post-graduate year, as a graduate student in English literature at Harvard, I developed a nagging sense that I was leaving something(s) important behind by failing to go to medical school - a connection with a more exciting world, whatever talent I had for science, a conception of work as an altruistic endeavor. So, with the aim of merging my verbal and scientific interests, presumably in psychiatry, I entered medical school the following year, only to discover that I was as enchanted by the "hard" sciences of the curriculum (especially neuroanatomy and biochemistry) as by the "soft". By the time of my third year, I was feeling quite committed to a career in academic internal medicine, with research interests in endocrinology or hematology, although my laboratory experience was still confined to a couple of rather unsatisfactory summers (e.g., ref. #1).

My attitude was, however, shifted once again shortly after my arrival at the NIH in 1968. I had planned, like many in my medical school generation, to prepare for a clinically-oriented academic career as a Public Health Service trainee at NIH. Before starting my two years as a medical house staff officer at Presbyterian Hospital, I had been happily matched to work with Ira Pastan, presumably on regulation of secretion of thyroid hormones. However, by the time I was ready to move to Bethesda, Pastan and Perlman had discovered that cyclic AMP reverses glucose repression of beta-galactosidase synthesis in E. coli. As a consequence, I inadvertently entered one of the most exciting areas in molecular biology in the late 1960's, the mechanism of positive, pleiotropic regulation of genes by cyclic AMP. This meant that I had to learn a large amount of enzymology, nucleic acid biochemistry, and bacterial genetics rather quickly, an effort rewarded by experiments which showed that the regulation occurred at the level of transcription of DNA into RNA, both in vivo and in a reconstructed system (see refs. 2-12). To my surprise, I loved working in the laboratory, was excited by answers to problems I had known nothing about a year earlier, and found my interest in clinical medicine dwindling rapidly.

Nevertheless, I felt my background and proclivities were more appropriate to the study of animal cells. Stimulated by accounts in courses taken at NIH of the disorderly conception of how RNA tumor viruses replicate, I sought a postdoctoral position in animal virology. Ironically, just as I was moving in the summer of 1970 to join the new group formed by Leon Levintow, Mike Bishop, and Warren Levinson in San Francisco, reverse transcriptase was discovered by Baltimore and Temin. Their discovery, however, not only injected some order into the field, it also infused it with money, competition, and (most importantly) experimental possibilities.

For the past seven years, working principally with the avian sarcoma and

mouse mammary tumor viruses, I have watched these possibilities grow, rather than wither. Although many have tried (generally unsuccessfully (cf ref.38)) to use RNA tumor viruses as models to search for human tumor viruses, I have been more interested in exploiting their utility for the investigation of several fundamental problems in cell biology - DNA synthesis, integration, transcriptional regulation, evolution, the origin of viruses, and even the genetic basis of human disease. A large portion of my work has been devoted to the questions surrounding the synthesis and integration of proviral DNA; we have shown that viral DNA is made in the cytoplasm in infected cells, that it enters a covalently closed form in the nucleus, and that it integrates covalently into the host cell genome. These observations and closely related work with several viruses are recorded primarily in references 14,20,28,36,41, 42,43,49,58,59,66, 71,80,85,88, and 89. I have been interested in the expression of viral genes (refs. 27,61,65,83,84,87,90), particularly in non-permissive cells infected by avian sarcoma virus (refs. 40,43,67) and in the regulation of mouse mammary tumor virus genes by glucocorticoid hormones (refs. 50,54,70, 86). Our efforts to follow the evolution of viral genes endogenous to normal DNA (refs. 14,18,31,56,60,69,72) and to understand the origin and function of viral genes, particularly the transforming gene of avian sarcoma virus (refs. 53,55, 64,75, and 82) have been relevant to the new field of molecular evolution. Some of the technology required for these studies has, in addition, returned me to old hematological interests, and I have collaborated with Y.W. Kan and his colleagues on the analysis of the genetic bases of the thalassemias (refs. 47,51, and 63).

As the scope of my work has grown, so has the size of our laboratory group and the effort involved in sustaining its momentum. Between us, Mike Bishop and I now closely supervise the activities of about 16 postdoctoral fellows and students and 4 technicians. I am the principal investigator for two research grants (from NIH and ACS), and co-investigator for another research grant and two training grants. Beyond these responsibilities, my obligations to the medical campus have involved me in several areas outside my immediate research interests - as a teacher of medical microbiology (bacteriology and immunology, as well as virology) and as a committee member (for lecture series, student research, biosafety, the M.D.-Ph.D. program, curriculum, promotions, and others). My life has been further complicated by extramural functions - as a speaker at (all too frequent) meetings and on many campuses; as an editor of manuscripts (officially for Cell and Virology, unofficially for several other journals); and, most exhausting of all, as a reviewer of grant applications (particularly as a member of the NIH Virology Study Section). When I review my current way of life in this peculiar fashion, I am amazed that I can still read books, fish for trout, play the oboe, talk with my family, and, yes, work at the bench. But sometimes I do.

Proposal

My principal objectives in planning a sabbatical year are not unusual: to work in a new scientific and cultural environment, after eight years in the same one; to shed, even temporarily, an increasing number of distractions from laboratory work; and to learn experimental approaches that will permit at least modest readjustments of my long-range goals in research. Since I do not wish to make fundamental changes in the kind of research I do, and since the experiments I wish to do involve scientific risks, political rules, and pragmatic barriers to the acquisition of reagents, I have decided to plan several kinds of experiments and to affiliate myself with a multi-disciplinary group that could satisfy my needs with any of those experiments. I will therefore be working at the ICRF in London, under the direct sponsorship of Dr. Michael Stoker, with Drs. Mike Fried, Bob Kamen, and/or Alan Smith, depending upon the success I have in preparing for the various experiments.

In the main, I wish to extend our on-going studies of the replication of avian sarcoma and mouse mammary tumor viruses in new directions, using new tools of genetic engineering (creation of deletion mutants and DNA cloning, in particular), and to begin to study the genetic organization and replication of hepatitis B virus. Three projects on which I hope to make some progress during the sabbatical year are described below.

1. Genetic organization of hepatitis B virus (HBV). I have recently decided to commit a portion of my laboratory's efforts to the study of HBV, a medically important virus, as yet very incompletely described, which promises to be amenable to the sorts of techniques we have employed in the study of RNA tumor viruses. My immediate objectives are to prepare radiolabeled HBV DNA and to use it in the analysis of virus-specific nucleic acids in infected liver cells.

Because of logistical, ethical, and biohazard considerations, I am attempting to gather the appropriate reagents for these experiments prior to my departure. This will require preparation of viral DNA from a suitable donor who produces Dane particles (the putative infectious virus); amplification of viral DNA in vitro using a DNA replication system, derived from T4 bacteriophage, which we are using for studies of RNA tumor virus-specific DNA in collaboration with Dr. Bruce Alberts; and extraction of both DNA and RNA from either (a) liver biopsies obtained from experimentally-infected chimpanzees, or (b) livers obtained at autopsy from patients with active hepatitis B. If some or all of these reagents can be assembled, I will proceed during my sabbatical to develop a more satisfactory restriction map of Dane particle DNA; to further define the structure and heterogeneity of the molecule (which now appears to be a gapped double-stranded circle with at least moderate sequence heterogeneity and a weight of about 1.6×10^6 daltons); to measure the amount and strand of origin of the virus-specific RNA in cells; to characterize and translate the RNA synthesized from viral DNA in vitro by various RNA polymerases; to translate RNA from infected cells, using preparative hybridization to enrich for viral RNA and appropriate antisera to test for synthesis of known viral antigens; and to examine cellular DNA for the existence of integrated viral DNA and for the specificity of any integration sites (using restriction endonucleases and the DNA transfer procedure, with DNA labeled in vitro as hybridization reagent). Although I am obviously familiar with several of the technical procedures involved, there are areas (e.g., detailed

restriction mapping, in vitro synthesis of RNA and proteins, immunoprecipitation) in which I expect to be helped a great deal by my new colleagues. Obviously, limitations on materials are likely to restrict the number of questions I can satisfactorily answer in this area; for that reason, I expect to work on the problems discussed below as well.

2. Characterization of the sites of integration of RNA tumor virus DNA.

A major goal of my laboratory for the past few years has been a detailed characterization of the mechanism of synthesis and integration of viral DNA in cells infected by RNA tumor viruses. Recently, using restriction endonucleases, hybridization reagents specific for various portions of the viral genomes, and the DNA transfer procedure developed by Southern, we have described the location and orientation of integrated (proviral) DNA in several kinds of cells infected by avian sarcoma virus (ASV) and mouse mammary tumor virus (MMTV); in addition, we have similarly studied the ASV- and MMTV-related DNA endogenous to avian and murine cells. In both systems, we generally find proviral DNA to be colinear with the viral RNA genome, rather than permuted; in some cases (e.g., some strains of ASV in chicken cells) we have found preferred cellular sites for integration, whereas, in other cases, preferred sites, if they exist, have yet to be defined. In all cases, however, we are now able to identify fragments of cellular DNA containing only a few virus-specific nucleotides (corresponding to a terminus of the viral genome) plus the adjacent cellular sequences. For purposes of understanding both the integration mechanism and the regulation of viral gene expression at the transcriptional level, it is necessary to determine the nucleotide sequence of the adjacent cellular DNA. This approach requires the preparation of cellular DNA enriched for the appropriate fragments (using reverse phase chromatography or R-loop formation in conjunction with preparative gel electrophoresis), followed by cloning and detecting the fragment in a suitable vector and host. I hope to develop such clones (though not to sequence the sites) during my sabbatical year. Since the experiments involve the use of recombinant DNA, I am in the process of applying to the committee which governs such research in Britain for permission to do them. A large number of possible combinations will be offered for consideration; in general, the experiments emphasize the inclusion of very small 5' terminal regions of the viral genomes, outside the genetically-defined areas and distant from known transforming genes; the use of defective polyoma DNA as a vector; and the study of endogenous viral sequences. The ICRF is well-equipped with facilities for such experiments (with a containment area more stringently designed than our P3 facilities) and Mike Fried would be an expert guide for me in conducting these studies since he is currently using his polyoma mutants as cloning vehicles. Obviously, however, execution of these experiments is dependent upon favorable action on my applications.

3. Generation of new deletion mutants of avian RNA tumor viruses for dissection of oncogenic functions.

The use of deletion mutants has been a powerful tool in virology, particularly with the recent development of methods to produce small deletions in defined regions of papova virus genomes. Although RNA tumor virus-specific DNA is available in much smaller quantities, we have identified and prepared closed circular viral DNA from ASV-infected cells in amounts suitable for restriction endonuclease mapping and for infectivity studies, and my reading of the literature suggests that creation and isolation of deletion mutants should be feasible. This approach has obvious utility in confirming the genetic map of

ASV and in attempting to identify new genes. Although transformation of cultured fibroblasts (and production of sarcomas) appears to be controlled by a single, well-studied gene (called src), there is thus far no genetic assignment for the capacity of many RNA tumor viruses to cause leukemias. Leukemogenesis could be dependent upon a known gene (e.g., env), upon as yet undiscovered genes, or upon some consequence of the positioning of the leukosis viral DNA in the host cell genome. I would like to begin to investigate this question by preparing a series of deletion mutants of an avian leukosis virus (ALV). A restriction endonuclease map of an ALV strain will have been prepared prior to my departure, as well as an adequate amount of closed circular DNA from cells acutely-infected with the parental strain. To make the mutants, circular DNA will be cleaved with enzymes shown to have a single recognition site in viral DNA (or with other enzymes in the presence of intercalating dyes which prevent more than one scission per molecule); the ends of the resultant linear DNA will be trimmed with an appropriate exonuclease; and the DNA will be used to infect permissive cultures, in the presence and absence of a non-oncogenic helper virus (e.g., Rous associated virus-0). Newly-acquired mutant viruses will be confirmed by restriction mapping and cloned, if necessary. My objective during the sabbatical year will be the development of a series of such mutants, with emphasis upon mutations in the unmapped regions of the genome, particularly the region between the env gene and the 3' terminus. I would then arrange to have these mutants tested for leukemogenic activity by any of several investigators who have helped us with such problems in the past. Any mutants which have lost their leukemogenic potential will be subjected to detailed analysis of their sites of integration in various cell types, and we will attempt to generate mutants with similar phenotypes by making lesions in the same and neighboring sequences. This project could thus lead to new directions in the context of our present research program. There would be several advantages to starting such work at the ICRF: Mike Fried has had remarkable success in producing and describing a wide variety of deletion mutants of polyoma virus; in addition, Robin Weiss, John Wyke and their colleagues would be available for consultation on the genetics and biology of avian leukosis viruses.