MOLECULAR BIOLOGY OF TUMOR VIRUSES

SECOND EDITION

RNA TUMOR VIRUSES

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Preface

Almost a decade has passed since the publication of the first edition of *The Molecular Biology of Tumor Viruses.* In the interim, so much has happened in the field of tumor virology that the current volume can only dimly be viewed as a revision of the first. In the first edition, RNA tumor viruses commanded about 200 pages of the 700 or so in the entire volume; now RNA tumor viruses (or, more properly, retroviruses) require a massive volume of their own, with almost 1400 pages of text and appendixes. This profusion of information has required an editorial consortium, a large collection of authors and critics, and a major effort by the Cold Spring Harbor Publications staff to assemble a monograph that is almost entirely new, but one, we hope, that preserves the qualities that made the first edition so useful for both new and old members of the tumor virus community. Of the material appearing in the first edition, only a little remains—some historical perspectives to be found in Chapter 1 and a scattering of figures and tables throughout the remainder of the book.

The enthusiasm required to assemble this large book has been fired by remarkable progress during the past several years in efforts to describe the major biochemical and genetic features of the curious viruses we study: the organization of viral RNA and proviral DNA; the strategies for viral replication and gene expression; the nature and origin of viral transforming genes; and the structure and function of endogenous viral genomes. Although retroviruses are dauntingly numerous and some isolates discon-

certingly idiosyncratic, the gratifying theme that has pervaded the work of the past decade is one of unity of design. It is that theme that we have exploited in the construction of this book. Thus, we have emphasized conceptual rather than taxonomic categories in the choice of chapter topics, in the belief that the occasional exceptions to the general rules are more usefully discussed in the context of accounts of how most retroviruses work. The rapid expansion of our information about retroviruses, particularly since the introduction of recombinant DNA and DNA sequencing methods, has been sufficiently great to forbid a graceful blending of all the pertinent facts within the text. To remedy this situation, we have appended compilations of restriction endonuclease maps of viral genomes and of nucleotide and amino acid sequences.

A word should be said about the manner in which we expect this book to be used. We have attempted to create a text that will meet the needs of students learning about retroviruses for the first time and of working scientists—both those who are new to the field and those who have grown up with it. To achieve such general utility, we have had to sacrifice the virtue of brevity. Thus, for the uninitiated, we have provided the historical and experimental perspectives that nurtured the theoretical conclusions and factual detail now available; and, for the expert, we have provided the inclusive descriptions, bibliographies, and information relevant to current experimentation as appropriate for a standard reference work. To diminish the dangers of making this book all things to all people, we have copiously subdivided the chapters and listed the subdivisions at the start of each chapter, abstracted much of the detailed material into tables within chapters and placed the rest in the appendices, and provided a detailed index that should facilitate, in particular, the retrieval of information about individual viruses from chapters organized around conceptual principles. What emerges is not a text to be read from cover to cover, but one that can be readily dissected into components germane to readers with different backgrounds and different demands. For example, each chapter begins with an overview that should serve as an introduction to its central ideas and as a guide to further reading within the chapter or elsewhere. To minimize overlaps, we have made abundant use of cross-references between chapters. Several figures and tables, in addition to the Appendixes, should be helpful through-

out the book. These include Tables 2.3 (a list of retroviruses), 4.1 (viral genes), 6.1 (viral proteins), and 9.1 (*onc* genes); Figures 4.1 (genome structures), 5.1 (the replication cycle), and 6.1 (proteins and virion structure); and the tables following Chapter 7 (a catalog of viral mutants).

We have considered it appropriate to designate by name the individuals chiefly responsible for the composition of each of the chapters (other than Chapter 1, which was derived mainly from Chapter 1 of the first edition). However, we have also attempted to make this book one that reflects the consensus of the community of retrovirologists; to this end, we have relied heavily upon short contributions to, and critical readings of, most of these chapters by many of our colleagues. We hope to have obtained from this wide participation the balanced viewpoints that should characterize the standard reference text in a field as diverse as our own.

Compensation for working on a production such as this is never equal to the task; all we can offer is our sincere gratitude to those who contributed to it in one way or another. Many of the named authors made significant, unattributed contributions to other chapters, and several other colleagues helped with drafts that become obsolete with the passage of time or wrote (or rewrote) short portions of the various chapters that now appear. Among these otherwise unsung contributors are David Baltimore, Karen Beemon, Henry Bose, June East, Ray Erikson, Ashley Haase, Nancy Hogg, Wally Mangel, Jim Neil, Roel Nusse, Gordon Peters, Naomi Rosenberg, David Steffen, George Vande Woude, and Lu-Hai Wang. Detailed editorial assistance was provided by too many colleagues to list, but the special efforts of several (Bob Weinberg, John Wyke, Chuck Sherr, Harriet Robinson, and Craig Cohen) deserve special mention. Even the extraordinary persistence of Steve Hughes in compiling the appendixes would have been insufficient without the cooperation of many colleagues, especially Dennis Schwartz, Chuck Van Beveren, Tom Shinnick, Steve Oroszlan, and others, who provided unpublished and annotated maps and sequences. We are also indebted to Betsy Matthews for assembling the index, to Audrey Simson for typing a large portion of the text, and to Audrey Simons, Gerry Leach, Mike Ockler, and Fran Cefalu for producing the excellent graphics. Last, but far from least, we are grateful to Nancy Ford and

members of her staff (Dorothy Brown, Judy Cuddihy, Joan Ebert, Gail Anderson) for their patience and extraordinary editorial skills, displayed throughout this long and arduous undertaking. Without their conviction that there would one day be a complete product, this volume would probably not exist.

The Editors

RNA TUMOR VIRUSES

Contents

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Origins of Contemporary RNA Tumor Virus Research

INTRODUCTION

Most, if not all, complex eukaryotic organisms are subject to disorders of cell growth and differentiation that result in the appearance of localized or disseminated tumors. Although a large number of physical, chemical, and biological agents have been implicated in the etiology of neoplastic growth (Hiatt et al. 1977), it is generally agreed that some stage of tumorigenesis usually involves specific genetic alterations in individual cells whose progeny constitute the tumor mass (Cairns 1975, 1980). Those genetic alterations may be multiple (Armitage and Doll 1957), they may be inherited (Ponder 1980), or they may be induced or contributed by some of the agents implicated in tumorigenesis. Such a statement implies that the etiology of cancer is complex, a view that is reinforced by the study of the pathology and clinical behavior of tumors arising in the many cell lineages in higher organisms.

The great attraction of oncogenic viruses for experimentalists interested in cancer depends in large part on the apparent simplicity of many of these agents and on the correlative hope that a detailed understanding of the genetic contribution such viruses make to a cell will enlarge our understanding of neoplastic conversion in general. In the happiest of all experimental situations, a virus introduces into a normal cell a single gene whose product is capable of initiating and maintaining the oncogenic state. This state of affairs appears to apply to some viral agents, prompting a number of immediate questions to which at least partial answers are now available: Where do tumor genes come from? How is the gene introduced, reproduced, and expressed? What kind of protein does such a gene make?

What does the protein do, directly or indirectly, to the metabolism of host cells? As knowledge accrues from such simple systems, more complex viruses, as well as nonviral agents that effect genetic change by more elusive means, are becoming accessible targets for experimental work.

Tumor viruses, like other viruses, are conventionally categorized according to the chemical composition, organization, and size of their genomes. In a companion volume in this series (Tooze 1980), the pertinent properties of tumor viruses carrying DNA genomes have been reviewed. Virtually all classes of DNA viruses include members that manifest some property sufficient to implicate them as tumor viruses: induction of tumors in certain hosts, alteration of the morphological and growth properties of cultured cells (transformation), or epidemiological associations with tumors of uncertain etiology. Thus, the field of DNA tumor virology is, in fact, a conglomeration of several virological subspecialties dealing with papovaviruses, adenoviruses, herpesviruses, and even hepatitis viruses.

The RNA tumor viruses, in contrast, include members of only a single taxonomic group of RNA viruses. Although RNA tumor viruses have been isolated from an extraordinarily diverse group of animals (Chapter 2) and can produce a vast array of pathological consequences (Chapter 8) through a variety of mechanisms (Chapter 9), they are unified by the nature of their genomes (Chapter 4), their means of entering cells (Chapter 3), their composition and structure (Chapter 6), and their mode of replication via a DNA-intermediate (Chapter 5). The last property, more than any other, distinguishes this class of viruses from other RNA viruses. As a result, viruses that use virus-coded, RNA-directed DNA polymerases to replicate their RNA genomes are grouped together as "retroviruses" regardless of whether they are capable of producing the biological effects expected of true tumor viruses. Some nontumorigenic retroviruses are mutants, or closely related natural variants of tumor viruses, and some are related to the tumorigenic retroviruses only by the fundamental principles guiding virus replication and morphology. In either case, it is instructive to consider all members of the retrovirus group within a volume that concentrates principally on its oncogenic members.

Retrovirology has now progressed to a point where it is possible to confront the central themes of genetic organization, replication,

and oncogenesis as they apply to the entire class of viruses, rather than to describe what is known about each of the isolated members of the class in turn. This perspective promotes economy and stimulates consideration of design and mechanism, but it does not necessarily provide the uninitiated student with an adequate sense of the slow and ungainly pace at which the threads were drawn together to form the conceptual fabric upon which disciples of this field now rely. The study of RNA tumor viruses, like most other scientific enterprises, has its roots in a number of serendipitous observations, some refractory to study at the time and some promptly and profitably exploited. Some candidate tumor viruses have been ignored out of prejudice or set aside for want of suitable techniques. Others have been much more productive of informative research because of certain inherent biological properties or because they were delivered into the right hands at the right time. Much of the intriguing history of tumor virology has been admirably summarized by Gross (1970), but the ensuing pages recapitulate some of the major moments in the experimental approach to the most useful of RNA tumor viruses.

ROUS SARCOMA VIRUS

Discovery

The first tumor virus to be studied seriously proved ultimately to be the most useful of the RNA tumor viruses and was found in 1911 by Peyton Rous, working in New York at the Rockefeller Institute. For several years he had been studying a spontaneous chicken sarcoma (tumor of connective tissue) that he had been passaging through closely related Plymouth Rock chickens (Rous 1910). With each passage, the tumor acquired heightened transplantability and showed a greater tendency to spread from the original graft site. Rous then tested to see whether cell-free filtrates could also induce a tumor to grow at the inoculation site. Although previous experiments with extracts of transplantable tumors of rats, mice, and dogs failed, Rous immediately succeeded, first using material that passed through ordinary filter paper and then using filters known to hold back bacteria, thereby establishing a virus as the etiological cause of the tumor (Rous 1911). Now the name Rous sarcoma virus (RSV) is given not only to the original virus isolated by Rous, but also to a

number of independently isolated chicken viruses that induce sarcomas by a similar genetic mechanism (Chapters 2 and 9).

Immunological Responses to RSV

In Rous's original experiments, very large numbers of viral particles were injected for every visible tumor produced. Tumors arose most frequently when young chickens were inoculated, and especially large numbers of viral particles had to be injected in order to induce tumors in adult chickens. Moreover, when adult chickens were used as recipients, the tumors frequently regressed; as we now realize, the high frequency of takes in young chickens probably reflected their lesser ability to mount an immunological response. Most likely, for every visible tumor produced, even in very young chickens, thousands of cells were transformed into cancer cells and only a small fraction somehow overcame immunological attack. Already in 1913, Rous could distinguish antibodies against the infectious viral particles from those against the tumor cells, but the immature state of immunology at that time prevented a real understanding of what was happening (Rous 1913; Rous and Murphy 1914).

Host Range of RSVs

The first isolated strains of RSV had restricted host ranges; they would induce tumors in only a few strains of chickens. Continued passage of such RSV, however, led to a much wider host range, and tumors were induced in young turkeys, ducks, guinea fowl, and pigeons. It had been thought that RSV would not induce tumors in mammals, but in 1957 Zilber and Svet-Moldavsky in Moscow showed that infection by the Carr-Zilber RSV strain induced a fatal hemorrhagic disease in adult rats (Svet-Moldavsky 1957; Zilber and Kriukova 1957). The following year, Svet-Moldavsky (1958) observed the formation of sarcomas after inoculating newborn rats with the same RSV strain, although RSV will not establish a productive infection in mammalian cells.

Growth of RSV in Embryonated Eggs

As early as 1911, Rous and Murphy realized that RSV would grow in embryonated chicken eggs. Both the embryo itself and its

surrounding membranes contain cells that can be transformed by the virus. Most important, at this stage, no immunological response exists and tumor regression does not complicate studies of cell transformation. But it was not until 1938 that Keogh in England employed growth of RSV on the chorioallantoic membrane as a quantitative assay for RSV (Keogh 1938). Following direct inoculation on the membrane, small tumors developed, and their number was directly proportional to the concentration of the virus suspension. This result established the very important principle that infection by a single RSV particle can transform a normal cell into a cancer cell.

Transformation of Cultured Cells

Over the past two decades, most work with RSV and retroviruses generally has involved the use of cultured cells. In cultures of chicken fibroblasts, RSV grows to high titers and morphologically altered transformed cells become visible several days after infection (Manaker and Groupe 1956). When chick fibroblasts growing as monolayers on petri dishes are infected with RSV, the transformed sarcoma cells stand out as easily countable foci, the number of foci being directly proportional to the concentration of virus added (Temin and Rubin 1958) (see Chapters 3 and 8).

Host Susceptibility and Virus Interference

Occasionally, fibroblasts prepared from a chicken embryo prove totally resistant to RSV. Such resistant cells frequently contain closely related leukemia viruses that multiply in chicken fibroblasts without causing obvious morphological changes (Rubin 1960, 1961). A number of different strains of such avian leukemia viruses (ALVs) exist, each conferring a distinctive pattern of resistance to infection by RSV (Vogt and Ishizaki 1966). The resistance or sensitivity of chick cells to infection by particular strains of RSV is also controlled by chromosomal genes that are inherited in a simple Mendelian fashion. Sensitivity is dominant and usually reflects the presence at the cell surface of specific sites that allow particular strains of RSV to bind to and penetrate the cells. Both of these mechanisms are considered in Chapter 3.