Fudan Graduate Textbook Series

Key Notes on Medical Molecular Virology

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Philip P Mortimer
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Abstract

This book provides a key for students to open the door to medical molecular virology, and therefore it only introduces the important themes of medical molecular virology. Questions are included between sections in all chapters, but with no "standard" answers. These questions are raised to prompt active and innovative discussion.

The contents of this book are divided into two parts. The first 9 chapters introduce the important aspects of general medical molecular virology. Some chapters ask questions like "What is a virus?" "Are virus and cells interdependent?" "Is persistent virus infection a favorable balance?" "Why are there so many types of interferons?" "Why is virus nomenclature important?" Other chapters describe viral oncogenesis, antiviral development, vaccines and prions. The latter three chapters describe selected individual viruses, which cover the RNA viruses (influenza virus, enteroviruses, Hantaan virus and flaviviruses), the RNA-DNA intermediate viruses (human immunodeficiency virus and hepatitis B virus), and DNA viruses (herpesviruses, human papilloma viruses, adenoviruses and poxviruses).

It is hoped that by reading this book and by participating in discussions, the students will be interested in a further reading, and a list of reading materials is suggested at the end of each chapter.

PREFACE

There are already many excellent textbooks that explain the complexities of traditional virology and the recent progress in molecular virology. However, most of them are not suitable for graduate students who have limited time to study these books. Besides, graduate students who are majoring in, for instance, immunology, pathology, molecular genetics, epidemiology, infectious diseases, etc. may only wish to know the essence of medical molecular virology, and they only need an introduction guide to virology prepared to meet their needs. This slim book presents the essence of medical molecular virology and can serve as a basis for further pursuit of any aspect of this broad field.

In teaching students, I have realized that the important task is not simply to give the students facts and conclusions which have been found and reached previously, but to help the students understand how these important conclusions were reached, and what new fields they could probe and investigate in the future. In this book, therefore, the important themes of medical molecular virology are emphasized and questions raised that will prompt active and innovative discussion. Questions for the students are included between paragraphs. There are no "standard" answers. Students are encouraged to think, to study references and to be provided with a key that will open the door to medical molecular virology, and I therefore have called it "key" notes to medical molecular virology. I hope the book will provide the key points of medical molecular virology. The word "key" has a broad meaning.

Writing this book has already not been an easy task, the rapid

progress in elucidating the pathogenesis of infectious diseases and in the genome-based molecular biology of microbes has made the task more difficult. Though English is not my own native language, I teach students, who speak Chinese or other languages, to use English virological terms correctly. This is important if students are to involve themselves in the international scientific community in their future career.

I want to thank my friend Philip P Mortimer (Centre for Infections, Colindale, London, UK), who has helped and encouraged me to complete this book. Without his support and painstaking editing, it would have been difficult to write this book. I also want to acknowledge the contribution of Jiayou Zhang of University of Kentucky, a former student and now a close colleague working on retroviruses, for writing the chapter on human immunodeficiency virus (HIV).

I hope students will consult the references for further reading listed at the end of each chapter, and the *Field's Virology*, a seminal textbook from which most of the essence of this book was gathered. I would appreciate any frank feedback or comments from students and colleagues, so that I can improve this book in the future.

Yu-Mei Wen

June 2005

Shanghai, China

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Chapter 1

Is a virus just a packet of genes?

The definition of what a virus has been and is still developing as more and more is learnt about the characteristics of this unique form of microorganism.

Infections caused by viruses had been described hundreds, even thousands of years ago before any viruses were identified. A picture of a cripple patient presumably caused by poliomyelitis has been found in an Egyptian tomb and smallpox was described as a contagious disease by Chinese traditional practitioners in the tenth century BC. In 1892, a virus (tobacco mosaic plant virus) was described by a Russia botanist, Ivanovsky. He showed that this pathogen could pass through a Chamberland filter under pressure. Since no bacteria could pass through the filter, he concluded that the "sterile" infectious fluid "contained bacteria of unusually small size". Beijerinck, a Dutch microbiologist, confirmed Ivanovsky's work, and went on to use thick agar diffusion method to separate this pathogen from all other particles, including aerobic, anaerobic bacteria and their spores. With these experiments he conceived of the idea of a "contagium vivum fluidum" or contagious living fluid. This presented for the first time the concept of a pathogen distinct from bacteria. Soon studies revealed that viruses, i. e., "filterable agent" could infect animals (foot and mouth disease), humans (yellow fever) and in fact every kind of living organisms. This included bacteria, fungi, protozoa and mycoplasma. Reviewing the history of virology, one can also see that the recognition of viruses was closely associated with the study of the diseases that they caused in these organisms.

***Questions*: Are there non-pathogenic viruses? If yes, how can you**

prove it? What roles would these viruses play in nature? Would viruses be able to attack prions (infectious protein pathogen, see the chapter on prions)? Why yes and why no?

Early studies of the morphology of viruses under the electron-microscope described their shapes as being spherical, rod-like, thread-like or brick-like. Using the bacteriophage model and with purification, crystallization and observation under high magnification electron-microscopy, X-ray diffraction and positive or negative staining of virus particles, the fine structures and chemical composition of viruses were revealed.

The size of viruses ranges approximately from 20 to 400 nm in diameter, while in filamentous viruses the length is usually 300 nm or more. In the *Filoviridae* family (includes the Ebola virus) the length varies significantly but the longest reaches 1 000 nm. The biochemistry of viruses is the biochemistry of protein and nucleic acids. In late 1950s and early 60s, scientists concentrated on studies of the proteins of viruses. The ultrastructure of a virion is very well organized with the genome packaged in a protein shell that is often strictly symmetrical, either with icosahedral or as helical symmetry. Even in the complex structure of poxviruses (vaccinia virus is in this family), symmetry can still be seen in the outer membrane, the inner core and lateral bodies. The numbers of capsomeres on the surface of icosahedral viruses are fixed for different families of viruses. Some viruses are further enveloped in a lipid-containing membrane, with peplomers protruding from the membrane. This envelope is acquired when the virus buds from the surface of host cells and while the protein or glycoprotein components of this membrane are encoded by the viral genome, the lipid of the envelope is derived from host cells. Enveloped viruses, for examples of retrovirus and influenza virus (in family of *Orthomyxoviridae*), can be pleomorphic (either spherical or filamentous), because their envelope is not rigid.

***Questions*: What determines the symmetrical arrangement of viral capsomeres? Why is helical symmetry limited to RNA viruses? Would mutations in genes encoding the capsid proteins result in a change in the number of capsomeres of a viral strain? State the reasons.**

Studies of the nature and the functions of nucleic acids in viruses started with bacteriophage. These are viruses of bacteria. By separate

labeling of the protein and nucleic acid with ^{35}S and ^{32}P, it was possible to trace these two components after phage infection of bacteria. More than 80% of the nucleic acid was found in the phage progeny, while very low amounts of protein were detected. This elegant experiment defined that it was the viral nucleic acid which carried and transferred the genetic information to the host cell, and directed the cells to produce the protein components of the virus progenies. The genome of viruses is highly variable. A given virus may contain either RNA or DNA, which may be single-stranded or double-stranded, linear or circular, positive, negative or ambisense (*Bunyaviridae*, Hanta virus), segmented (Influenza virus, rotavirus) or non-segmented. The viral genome is packaged in the protein capsid. This protects the viral genome from degradation and serves as a vehicle to transfer the genome to appropriate cells.

***Questions*: Try to explain why the genomes of viruses are so variable. Compare the genomes of viruses with bacterial genomes. What approaches can one employ to study the functional genomics of viruses?**

The intracellular and extracellular forms of virions are distinct from each other in their biological activities. In their extracellular forms, virions are biologically inert, but they are converted to active living organisms when they are inside host cells. After attachment to specific receptor(s) on host cells, virions either penetrate or are engulfed by host cells. The genome of the virus has to be released from its protein shell (uncoating) in order to direct synthesis of large numbers of progeny in host cells. This process is similar to making copies from a blueprint, and is therefore referred to as "replication". The essence of virus replication is that specific virus mRNAs are transcribed from viral genomes for the expression and duplication of viral nucleic acids. Based on different structures of viral nucleic acids, viruses make use of different pathways to synthesize their own mRNAs. According to the stage of mRNA synthesis and transcription, the genes of double-stranded (ds) DNA viruses are divided into early and late genes. After successful transcription, viral protein synthesis is accomplished using cellular components, cell enzymes and energies generated in cells. The duplication of the genome of dsDNA viruses uses a semi-conservative replication strategy to synthesize complementary strands of DNA. DNA synthesis of the majority DNA viruses (for example, the parvoviruses, the papovaviruses, the adenoviruses, and the herpes viruses) depends

on the cellular RNA polymerase Ⅱ. Therefore, the signals that control the expression of these DNA viral genes are similar to those of the cellular genes. Proper regulation of the transcription of viral genes requires promoters and enhancers. Regulation of transcription sometimes requires the participation of sequence-specific DNA-binding proteins. These will be discussed further in the chapter on virus-cell interactions. Human single-stranded DNA viruses are rare; and most of ssDNA viruses are bacteriophages which replicate via a rolling circle strategy.

In contrast, RNA viruses employ different strategies for mRNA synthesis and protein expression as well as genome duplication. All RNA viruses except retroviruses encode an RNA-dependent RNA polymerase which is not present in host cells. The evidence for a viral-encoded RNA-dependent RNA polymerase was first discovered in poliovirus-infected cells, following many attempts to purify this enzyme and show that this enzyme can copy viral RNA. This enzyme is currently known as the polioviral RNA polymerase $3D^{pol}$, and its amino acids sequences have been used as a model to compare with other viral RNA dependent RNA polymerases. Since the development of cloning, sequencing and expression technology, other viral RNA-dependent RNA polymerases have been identified, using viral protein motif characterization, e.g., the L proteins of paramyxoviruses, the PB1 protein of influenza viruses and the nsP4 protein of alpha viruses. The RNA-directed RNA synthesis process differs slightly from that of DNA-dependent DNA synthesis, as the former does not need a primer, is catalyzed by virus-coded polymerase and, sometimes requires viral accessory proteins and even host cell proteins. Another group of viruses that replicated neither via the RNA-dependent RNA synthesis process nor via the DNA-dependent DNA polymerase process was revealed by the elegant independent studies of Temin and Baltimore. Temin proposed that retroviral RNA information could be integrated into host DNA. However, at the time he presented this hypothesis it was difficult in the face of skepticism to prove this hypothesis experimentally. Baltimore's involvement in retroviruses had started as an interest in virion-associated polymerases, and he and Temin both began to search for an RNA-dependent DNA polymerase, resulting in the discoveries of retroviral reverse transcriptase (RT). Later, it was found that RT could also be found in other animal viruses, such as the hepadnaviruses and some plant viruses, and these viruses were classified as retroid viruses. Furthermore, RT activity was found in some strains of myx-

obacteria and *E. coli*, and consideration of the origin of this enzyme influenced concepts of the evolution of the living world. It is now widely acknowledged that RT activity was required for DNA to evolve, so that retroid viruses may be viewed as living fossils of very primitive life forms.

***Questions*: Do you agree with the concept that the discovery of RT settled the arguments about the origin of biological evolution? Do you have further comments?**

In summary, a virus is an organized package of genes, only active in cells that can lead to a variety of pathogenic outcomes. Various strategies have been developed by viruses to ensure their host cell based replication.

Further Reading

1. Baltimore D. RNA-dependent DNA polymerase in virions of RNA tumour viruses. Nature. 1970; 226:1209-1211.
2. Domingo E. Viruses at the edge of adaptation. Virology. 2000; 270:251-253.
3. Hu J, Toft DO, Seeger C. Hepadnavirus assembly and reverse transcription require a multi-component chaperone complex which is incorporated into nucleocapsids. EMBO J. 1997; 16: 59-68.
4. Joyce GF. The antiquity of RNA-based evolution. Nature. 2002; 418:214-221.
5. Lillie JW, Green MR. Transcription activation by the adenovirus E1a protein. Nature. 1989; 338:39-44.
6. Lustig A, Levine AJ. One hundred years of virology. J Virol. 1992; 66:4629-4631.
7. Temin HM, Mizutani S. RNA-dependent DNA polymerase in virions of Rous sarcoma virus. Nature. 1970; 226: 1211-1213.
8. Weitzman MD, Kyostio SR, Kotin RM, Owens RA. Adeno-associated virus (AAV) Rep proteins mediate complex formation between AAV DNA and its integration site in human DNA. Proc Natl Acad Sci USA. 1994; 91:5808-5812.

Chapter 2

Viruses and host cells — Are they interdependent?

Viruses are inert particles unless they are inside living host cells. This obligative parasitic characteristic is fundamental and shared by all viruses; whether bacteriophage, plant or animal virus. Even though some viral genes can be expressed by recombinant technology in bacterial or yeast cells other than their natural hosts, the reproduction of viruses is based on a full replication cycle of the virus in susceptible and permissive host cells. Under other circumstances viruses may enter cells that are non-permissive, but the replication cycle is blocked, resulting in either integration of the viral genes in host cell genome, or persistence of the viral genome as episomal DNA. The persistence of viral DNA leads to transformation of the cells, latent infection or chronic infection, depending on how the virus and the host cells interact; but regardless of which interaction results, living cells are indispensable for virus to display its biological functions. Clearly there is a dependent relationship of viruses on cells. However, because of the invasion of viruses, normal cellular physiological functions are disturbed, or even destroyed resulting in cell death due to necrosis or apoptosis.

***Questions*: Are necrosis or apoptosis likely to be unfavorable to virus survival? When viruses infect cells, can you envisage cellular changes that might be beneficial both to the virus and to host cells?**

To initiate replication in a host cell, the first step is adherence of the virus to specific cellular receptor(s). Theoretically, the identification of these receptors would seem not too difficult. In cell cultures, different permissive cells for a specific virus can be identified, and the development and application of monoclonal antibodies has proved a

useful tool for the identification of receptors. In practice, after immunizing mice with intact cells, hybridoma cell lines that secrete monoclonal antibodies to cell surface proteins can be obtained. Then, once it is shown that secreted monoclonal antibody blocks the attachment of the virus, the antibody can be used to purify the receptor protein by immunoprecipitation or affinity chromatography. However, to identify the receptors, further experiments will be necessary by using recombinant DNA technology to isolate the receptor DNA and introducing receptor DNA from susceptible cells to non-susceptible cells, so that specific virus is bound. Besides, many viral receptors have been isolated by transfection of a cDNA library of a sensitive cell line into a cell line without the specific viral receptor, and a cloning of the receptor gene is followed.

To date, fewer than 100 viral receptors and co-receptors have been identified — a small number compared with the more than 4,000 known infectious viruses. Though in poliovirus, rhino virus and influenza virus, one type of receptor is sufficient for binding, and there are more and more reports stating that one type of receptor allows virus attachment while another permits viral entry. For human immunodeficiency virus (HIV), for example, the CD4 receptor mediates attachment, while the CCR5 functions as the co-receptor for viral entry. In fact, many viral receptors are functional membrane proteins on cells, such as the integrin receptors for adenoviruses, the Ig-like protein receptors for poliovirus and the complement-regulating protein receptor for measles virus. There are others, such as the sialic acid receptor for Sendai virus, and the heparin sulfate receptor for sindbis virus, etc. Viewing the receptors from the cellular aspect, it is surprising that only small subsets of the large number of cellular membrane components function as viral receptors. Several viruses may use the same cellular molecules as receptors to initiate replication.

***Questions*: Could these cellular membrane molecules have been changed or adapted during evolution to accommodate virus infection? Or, on the other hand, could other components of the cell membrane have been altered during the course of evolution to evade virus infections and thus would not function as virus receptors? What kind of studies could be done to test the former or the latter speculation?**

After viral entry, the viral nucleocapsid has to be transported to

the appropriate location in cells, where, after uncoating, the viral genome can be released and start the replication of the viral genome. For most DNA viruses, retroviruses, influenza virus and Borna-disease virus, virus genomes begin replication in the cell nucleus. The process of transportation of viral genomes into the nucleus is accomplished via the cellular pathway for importation of protein into the nucleus. This transportation involves the recognition of a nucleus localizing signal (NLS) which is usually around 20 basic amino acids located on the virus capsid, and NLS can be recognized by cellular proteins called importins. However, there are still unanswered questions about how each virus passes through the nucleus pore in cells. For RNA viruses, which replicate in the cytoplasm, the site of their replication can either be at the cytoplasmic face of internal membranes, or on the cytoskeleton. During the replication of viral genomes, they take over the cellular transcription machinery and generate specific mechanisms to promote transcription of viral DNA.

For DNA viruses, it is necessary to promote efficient transcription of viral DNA immediately after infection. For example, herpes simplex virus (HSV) carries a viral trans-activator in its virion, and this trans-activator binds to a cellular protein, which in turn binds to specific DNA sequences located in immediate-early gene promoters. This stimulates the transcription of HSV immediate-early viral gene promoters. In some DNA viral genomes there are enhancer sequences located upstream of certain genes. These enhancer sequences interact with cellular transcription factors in the transcription of viral genes, and by their means, high-level transcription of the nearby genes can occur immediately after infection. In fact, the adenovirus E1A transcriptional unit, the human cytomegalovirus IE1/IE2 transcriptional unit and other DNA virus transcriptional units all contain enhancers. It should be emphasized that all these regulatory studies were done in infected cells, or by molecular biological methods using reporter genes. It is more difficult to analyze the molecular functions and mechanisms *in vivo*; moreover, the regulatory proteins may have multiple functions, which make the analysis of these regulatory controls even more difficult. The regulatory control of the transcription of different types of viruses varies, and because the majority of viruses rely on synthesis of mRNA from a DNA template, involving the cellular RNA polymerase Ⅱ transcription system and scientists have employed the virus-cell system as a model to study the molecular biology of transcription and its control in eukaryo-