RECOMBINANT DNA RESEARCH AND VIRUSES

Cloning and Expression of Viral Genes

edited by

Yechiel Becker

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The Hebrew University of Jerusalem, Israel
Julia Hadar, Managing Editor

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RECOMBINANT DNA RESEARCH AND VIRUSES

DEVELOPMENTS IN MOLECULAR VIROLOGY

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PREFACE

The development of recombinant DNA technology has made a marked impact on molecular virology. The cleavage of viral DNA genomes with restriction enzymes and the cloning of such DNA fragments in bacterial plasmids has led to the amplification of selected viral DNA fragments for sequencing and gene expression. RNA virus genomes which can be transcribed to their cDNA form were also cloned in bacterial plasmids, facilitating the study of RNA virus genes. With the elucidation in recent years of the promoter sequence of various viral genes and the expression of these genes in bacteria or yeast, the understanding of many viral gene functions has made great progress. Cloning and expression of viral genes in mammalian cells was made possible by the construction of shuttle plasmid vectors which carry the origins of DNA replication from bacteria and/or mammalian viruses. The expression of viral genes in bacteria, yeast and eukaryotic cells gives reason to hope that it will be possible to produce viral antigens in large quantities for use as human or animal vaccines.

The present volume attempts to capture for the reader some of the highlights of recombinant DNA research in the field of animal and plant viruses. The isolation and characterization of genes such as oncogenes, as well as genes coding for viral antigens, are presented, together with strategies for the transfer of viral genes to new hosts (which can be either cells or viruses). The development of approaches for the efficient expression of different viral genes in foreign hosts are described. Current studies on plant viruses and their future use in gene transfer in plants are presented along with developments in gene research of animal viruses.

I wish to thank all authors for their fine contributions and to express my appreciation especially to those who sent their manuscripts on time. My thanks to Mrs. Esther Herskovics for her excellent secretarial help.

Yechiel Becker
Jerusalem, June 1984

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DEVELOPMENTS IN MOLECULAR VIROLOGY: CLONING OF RETROVIRUS DNA IN BACTERIA AND CLONING OF OTHER DNA IN RETROVIRUSES

HOWARD M. TEMIN
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SUMMARY

Retroviruses are natural vectors for the insertion of foreign DNA into the cell genome. With the use of recombinant DNA techniques retrovirus structure has been studied and retroviruses have been made into vectors to introduce cloned DNA into the cell genome.

INTRODUCTION

Retroviruses are a family of RNA-containing animal viruses whose replication is through a DNA intermediate that integrates into the cell genome. Because of this integration, retroviruses are natural vectors for the insertion of foreign DNA into the cell genome. Recent work using recombinant DNA technologies has shown that retroviruses are evolutionarily related to cellular movable genetic elements and that reverse transcription of cellular nucleotide sequences and integration into germ-line DNA has occurred repeatedly (1-3). Thus, studies of retroviruses are relevant to genetics in general. Moreover, some retroviruses rapidly cause some cancers in vertebrates (4). Cellular genes related to genes of these retroviruses have been implicated in non-viral mouse and human cancers (5). Thus, study of retroviruses is relevant to oncology in general.

Although hypotheses were proposed relating to these areas over a decade ago, it was not until the use of recombinant DNA technologies, as well as DNA transfection, that direct evidence was secured supporting these hypotheses. These technologies also made possible construction of retrovirus vectors to introduce cloned DNA into the cell genome.

It is necessary to know a little about the retrovirus life cycle to understand the types of cloning strategies which have been used in the study of retroviruses (4). Retrovirus RNA consists of two identical genomic molecules and associated transfer RNA molecules. (The transfer RNA is used as a primer for viral DNA synthesis.) The viral genomic RNA has a small

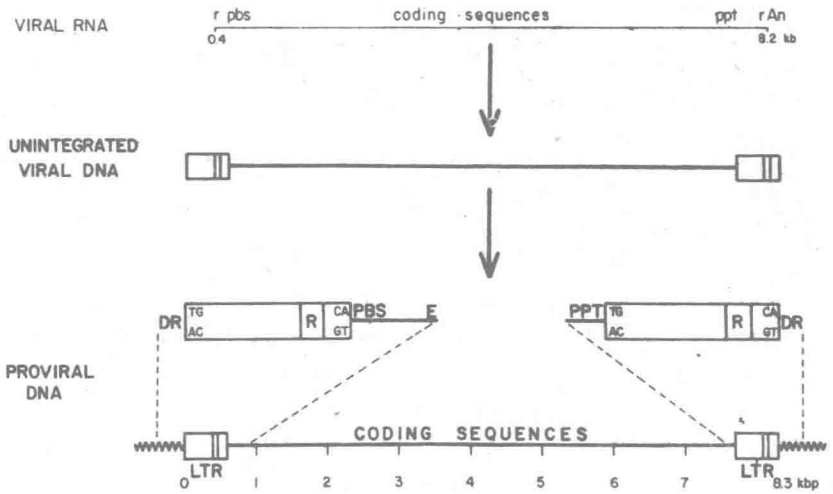

FIGURE 1. Formation of retrovirus provirus. In the virus particles, there are two molecules of viral RNA and a tRNA primer. In the infected cell there is also unintegrated circular viral DNA. r is repeat in viral RNA; pbs and PBS are primer binding site in viral RNA and DNA, respectively; ppt and PPT are polypurine track required for viral DNA synthesis in viral RNA and DNA, respectively; DR is direct repeat of cell DNA around provirus; E is encapsidation sequence; LTR is long terminal repeat; zig-zag line is cell DNA.

terminal direct repeat. After infection, this RNA is reverse transcribed by the viral reverse transcriptase to give double-stranded linear unintegrated viral DNA containing a large terminal repeat (LTR) at both ends (Figure 1). Thus, the molecular weight of unintegrated viral DNA is a little greater than two times the molecular weight of one molecule of viral genomic RNA. Closed circular DNA molecules containing one, two, or even three copies of the LTR are also found in smaller numbers.

Integration of viral DNA into the cell genome is a normal part of the viral life cycle. Viral DNA integrated into the host chromosomal DNA is co-linear with unintegrated linear viral DNA with the exception of two base pairs lost from each end. The integrated viral DNA or provirus is the template for synthesis of viral mRNAs and progeny RNA (Figure 2), although unintegrated viral DNA can also be transcribed at lower efficiency (6).

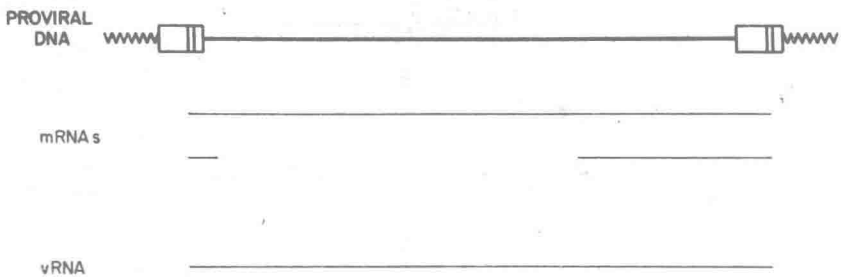

FIGURE 2. Synthesis of viral RNAs. A provirus of a non-defective retrovirus is shown. The mRNAs are for gag and gag-pol (full length) and for env (subgenomic). There may be different mRNAs for gag and gag-pol.

Cloning of Proviruses

Enzymes that do not digest viral DNA. The earliest cloning of retrovirus proviruses took advantage of the fact that the commonly used restriction endonuclease EcoRI does not cut DNA of murine leukemia virus or spleen necrosis virus. Since phage vectors capable of cloning EcoRI cut DNA fragments of 10 to 20 kbp and methods for screening for unique molecules of vertebrate cell DNA had been developed, it was relatively easy to clone provirus DNA (once legal restrictions were removed) (7,8). The integrity of the cloned DNA was validated by recovery of infectious virus after transfection of permissive vertebrate cells by the cloned DNA.

Cloned provirus DNA was recovered at frequencies expected for a small number of copies per cell. Non-infectious molecules were recovered at a frequency similar to that of infectious molecules. These molecules are probably not the result of an artefact of the cloning process, since non-infectious proviruses can be demonstrated in vertebrate cells and retroviruses are known to have a high frequency of genetic variation.

Two kinds of genetic variant do appear during the growth of the proviral clones in bacteria (Figure 3) (7,9). Both probably are the result of homologous recombination of the viral LTR. When the molecule containing the provirus is large, near the carrying capacity of the vector, the provirus frequently is deleted leaving only one LTR and surrounding cellular DNA. Alternatively, when the molecule containing the provirus is small, the provirus frequently duplicates resulting in the structure, vector DNA - cell

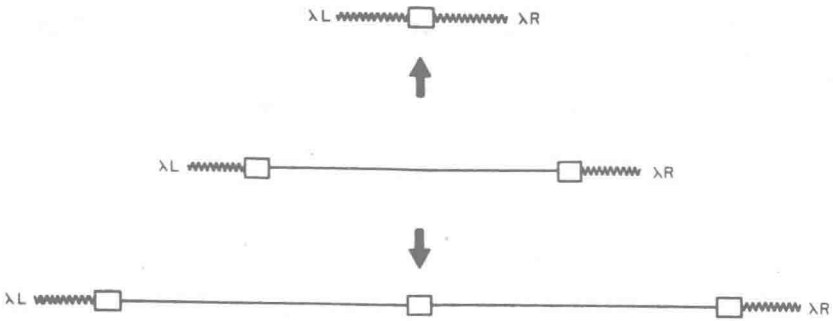

FIGURE 3. Deletion and duplication in provirus clones grown in bacteria. The original clone is in the center. The deleted clone containing cell DNA and one LTR is shown at the top. The duplicated clone containing three LTRs and two coding sequences is at the bottom.

DNA - LTR - viral coding sequences - LTR - viral coding sequences - LTR -cell DNA - vector DNA.

The latter class of molecules is a convenient source of viral molecules free of cell DNA to clone in plasmids. Digestion with a restriction endonuclease that cuts once in viral coding sequences yields a permuted molecule of viral DNA with one LTR. Such molecules are easily subcloned in plasmid vectors. Upon digestion of these subclones with the same enzyme and ligation, infectious viral DNA molecules are recovered in a concatemer.

Enzymes that digest viral DNA. Sometimes it has been necessary to use an enzyme that digests viral DNA. This requirement can be the result of the unavailability of a suitable enzyme that does not digest viral DNA or of a desire to clone partial molecules, for example to avoid "poison" sequences in mouse mammary tumor virus DNA (10,11).

We have cloned reticuloendotheliosis virus strain T (Rev-T) using EcoRI and a strategy similar to that described above even though there are two EcoRI cleavage sites in Rev-T DNA (11). After partial digestion with EcoRI, DNA molecules of 10 to 30 kbp were selected and cloned in a phage vector. Several complete proviral clones were secured, even though later restriction enzyme analysis revealed the presence of internal EcoRI cleavage sites.