

REVERSE TRANSCRIPTASE AND cDNA SYNTHESIS

Kunitada Shimotohno

Kyoto University, Japan

Keywords: retrovirus, reverse transcriptase, Rous sarcoma virus, chicken, murine, transposon, mobile genetic element, human immunodeficiency virus, human t-cell leukemia virus, copia, ty-element, cDNA, Actinomycin-D, ribonuclease H, tRNA, primer, template, inhibitor, azidothymidine, LINE, genome, hepatitis B virus, cauliflower mosaic virus, thermus thermophilus, DNA polymerase, polymerase chain reaction, avian myeloblastosis virus, RNase

Contents

1. Introduction
 2. Discovery of Reverse Transcriptase
 3. Characteristic Features of Retroviral Reverse Transcriptases
 - 3.1. Template and Primer Requirements for Reverse Transcriptase
 - 3.2. Substrates for the Enzyme
 - 3.3. Other Factors Required for Reverse Transcription
 - 3.3. Natural Primers for cDNA Synthesis of Retrovirus Genome
 - 3.5. Inhibitors of Reverse Transcriptases
 4. Reverse Transcriptase Activity in Other Organisms
 - 4.1. Reverse Transcriptase Activity in Retroposons
 - 4.2. Reverse Transcriptase Activity in Pararetroviruses
 - 4.2.1. Reverse Transcriptase Activity in Hepatitis B Virus (HBV)
 - 4.2.2. Reverse Transcriptase Activity in Cauliflower Mosaic Virus (CaMV)
 - 4.3. Reverse Transcriptase Activity and Telomerase
 - 4.4. Reverse Transcriptase Activity in Thermus Thermophilus DNA Polymerase
 5. Conserved Amino Acid Residues in Putative Reverse Transcriptase
 6. Structure of Retroviral Reverse Transcriptases
 7. cDNA Synthesis by Reverse Transcription
- Glossary
Bibliography
Biographical Sketch

Summary

This article describes reverse transcriptase that is responsible for transcription of RNA to DNA, from its discovery to application in molecular biology. Genetic information flows from nucleic acid to protein, in particular, from DNA to RNA and to protein. However, the discovery of reverse transcriptase from RNA tumor viruses, now termed retroviruses, led to the general concept that many organisms use this enzyme activity for their genome replication. This article presents an overview of reverse transcriptase from a historical viewpoint and describes how this enzyme is involved in retroviral replication. Retroposons that are transposed through an intermediate RNA are briefly introduced. Finally, the important role of this enzyme in molecular biology is described.

1. Introduction

Two groups working independently in the United States in 1970 discovered reverse transcriptase activity in retroviruses. Howard Temin and Satoshi Mizutani, and David Baltimore respectively found the activity of RNA-dependent DNA synthetase (now called reverse transcriptase) in chicken and murine retroviruses, which are RNA-containing viruses that cause tumor formation upon infection. Almost seven years before the discovery of the enzyme, Temin had conducted an experiment in which he hypothesized the possible involvement of reverse transcription in retroviral replication. He had long been working on chicken retroviruses, including Rous sarcoma virus (RSV), to determine the mechanism of cancer caused by the viruses, and had previously established the focus assay system for transformation by RSV. This work made it possible to titrate an amount of the virus by counting the number of foci. Using this technique, he analyzed replication of the virus and made a series of unexpected observations indicating that the replication of RSV was fundamentally different from that of other RNA-containing viruses. The unexpected observations included the fact that the genetic information determining the morphology of cells transformed by RSV infection was transmitted to daughter cells following cell division, even in the absence of virus replication or of virus genomes in infected cells.

Based on this observation, Temin proposed that the viral genome was present in infected cells in a stably inherited form. He called this inherited form of the virus a “provirus.” Actinomycin-D (Act-D), a metabolic inhibitor of DNA synthesis that inhibits DNA-directed RNA synthesis, was added to the culture medium of RSV-infected cells to analyze whether focus formation by RSV was affected. He found that this drug inhibited virus production. Moreover, this inhibition occurred at the early stage of cell infection by RSV. Thus, it appeared that DNA synthesis was required at an early stage of RSV infection and DNA-directed synthesis was subsequently required for production of progeny virus. Temin proposed the DNA provirus hypothesis on the basis of these observations. It was generally believed at that time that genetic information flows through DNA to RNA and then to protein and not from protein to nucleic acid or even from RNA to DNA, and thus Temin’s radical proposal took a long time to be accepted. Direct confirmation of his hypothesis required evidence of the presence of viral DNA integrated into the host chromosome and demonstration of an enzyme activity responsible for conversion from viral RNA to DNA. Temin tried to demonstrate the presence of viral DNA in RSV-infected cells by RNA-DNA hybridization experiments, but the data were not sufficient to confirm the presence of viral DNA in the chromosome.

2. Discovery of Reverse Transcriptase

Before the discovery of reverse transcriptase in RNA viruses, there was accumulating evidence that viruses such as vaccinia virus, reovirus and vesicular stomatitis virus have enzyme activities to synthesize nucleic acid in their virus particles. Temin and Mizutani, and Baltimore independently, demonstrated the presence of reverse transcriptase activity in virions. RNA tumor viruses are classified into types A, B, C, and D based on structural differences revealed under electron microscopy. After reverse transcriptase in RNA viruses was discovered, it was proposed to call them

“retroviruses.” This term is now generally accepted and is used in this article in the case of C-type RNA tumor viruses such as RSV and murine retroviruses. Treatment of virions with nonionic detergent or organic solvent such as ether or acetone increases the enzyme activity, suggesting that these viruses are coated with lipid. In some cases the enzyme activity can be observed without detergent treatment. However, in such cases it is likely that the virions are partially disrupted during preparation.

3. Characteristic Features of Retroviral Reverse Transcriptases

3.1. Template and Primer Requirements for Reverse Transcriptase

This enzyme requires RNA as well as DNA as a template. Enzyme assay using partially disrupted retroviruses showed that RNA was required as a template because the activity was abolished by treatment with ribonuclease (RNase). Later, using the purified enzyme, it was shown that this enzyme also catalyzes DNA synthesis using DNA as a template. Retroviruses contain a dimeric RNA genome in each infectious virion and produce viral DNA by reverse transcription. In addition to reverse transcriptase activity, ribonuclease H activity as well as DNA polymerase activity is required for the synthesis of a complete infectious virus DNA *in vivo*. In the completed virus DNA after reverse transcription, additional viral sequences that are not included in genomic RNA as such are recruited to the 5' and 3' end, and thus the DNA forms long terminal repeats (LTR) at both ends of the molecule. In this reaction, the enzyme requires tRNA as a primer as described in detail below. Any RNA, including homopolymers such as poly- (riboinosine), poly- (ribocytidine), and poly- (ribouridine) or mRNA, can also act as a template, although the efficiency of reverse transcription differs with different templates and combinations of template and primer sequences. Due to the low sequence specificity for the template, retroviral reverse transcriptases are now widely used to make cDNA from various RNAs. Moreover, as mentioned above, DNA can also serve as a template due to the DNA-dependent DNA polymerase activity in reverse transcriptase. However, it should be mentioned that complete double-stranded DNA or RNA molecules cannot be transcribed. The reverse transcribed retroviral product is covalently linked with an RNA primer. Moreover, a specific dinucleotide product having a structure of covalently linked of ribo-adenosine and deoxyribo-adenosine was discovered after complete digestion of the reaction product of reverse transcription of the genome of avian myeloblastosis virus, an avian retrovirus, with DNase and RNase. This indicated the involvement of some specific primers in reverse transcription.

3.2. Substrates for the Enzyme

In reverse transcription of viral genomic RNA, deoxyribo nucleoside triphosphates, dATP, dCTP, dGTP, and TTP are required as substrates. Nucleotide sequence analysis showed the synthesized DNA to have a sequence complementary to the template. However, ribonucleoside triphosphates are not incorporated into the reaction product, and show inhibitory effects at high concentrations. Synthetic nucleoside triphosphates, not present as a natural form, can be incorporated in some cases and used as inhibitors for retroviral replication.

3.3. Other Factors Required for Reverse Transcription

Metal ions such as magnesium and manganese ions are essential for the reaction, and polyamines such as spermidine and spermine enhance the activity. The presence of a reducing agent is also essential. The optimal pH range for the reaction is between 8.0 and 8.5. Reaction temperature is 37 °C, but slightly higher temperatures are sometimes used for cDNA synthesis.

-
-
-

TO ACCESS ALL THE 15 PAGES OF THIS CHAPTER,
Visit: <http://www.eolss.net/Eolss-sampleAllChapter.aspx>

Bibliography

- Baltimore D. (1970). RNA-dependent DNA polymerase in virions of RNA tumor viruses. *Nature* **226**, 1209–1211. [This article describes discovery of reverse transcriptase.]
- Cech T.R., Nakamura T.M., Lingner J. (1997). Telomerase is a true reverse transcriptase. *Biochemistry* **62**, 1202–1205. [This article reviews telomerase].
- Fields B.N., Knipe D.M., and Howley P.M. (eds.) (1996). *Fields Virology*. Lippincott-Raven. [This book covers molecular biology, virus replication, pathogenesis and biology.]
- Harada F., Sawyer R.C., Dahlberg J.E. (1975). A primer ribonucleic acid for initiation of *in vitro* Rous sarcoma virus deoxyribonucleic acid synthesis. *J Biol Chem.* **250**, 3487–3497. [This article describes tRNA as a primer for reverse transcriptase.]
- Singer M.F. (1995). Unusual reverse transcriptases. *J Biol Chem.* **270**, 24623–24626. [This article reviews various reverse transcriptases, which are different from those from retroviruses.]
- Temin H.M. and Mizutani S. (1970). RNA-dependent DNA polymerase in virions of Rous sarcoma virus. *Nature* **226**, 1211–1213. [This article describes discovery of reverse transcriptase.]
- Weiss R., Teich N., Varmus H., and Coffin J. (eds.) (1985). *RNA Tumor Viruses: Molecular Biology of Tumor Viruses*. [This book covers molecular biology and pathogenesis of RNA tumor viruses.]

Biographical Sketch

Kunitada Shimotohno gained his B.A. at Tohoku University, Sendai, in 1967, and his Ph.D. from the Graduate School of Hokkaido University in 1972. From 1972 to 1983 he was Research Associate at the Department of Molecular Genetics, National Institute of Genetics; from 1978 to 1980 Research Associate at the McArdle Laboratory for Cancer Research, University of Wisconsin, United States; from 1983 to 1985 Section Head, at the Virology Division, National Cancer Center Research Institute, Tokyo; and from 1985 to 1997 Head of the Virology Division. Since 1997 he has been a professor at the Institute for Virus Research, Kyoto University. His research interests includes the clarification of viral carcinogenesis, and molecular mechanisms of immortalization of cells by human T-cell leukemia virus infection.