ACCOUNTS OF CHEMICAL RESEARCH

VOLUME 7 NUMBER 6 JUNE, 1974

Ribonucleic Acid Directed Deoxyribonucleic Acid Synthesis in Viruses and Cells

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Received November 19, 1973

A major goal of present-day biology is to elucidate the rules governing the transmission of information from molecule to molecule in biological systems, and a major goal of present-day biochemistry is to elucidate the molecular mechanisms of this information transfer.

It has been known for about 20 years that the genetic information in all living cells is encoded in molecules of the large polymer, deoxyribonucleic acid (DNA). The genetic information for each organism is written in a four-letter alphabet made up of the four deoxyribonucleosides. In cells, short passages of the genetic message (individual genes) are transcribed by a specific enzyme or polymerase into ribonucleic acid (RNA). A length of RNA, called messenger RNA, is translated into a particular protein. (Other molecules of RNA are part of this translation machinery.) When a cell divides, the information contained in each of the two strands of DNA is replicated, thereby equipping the daughter cells with the full genetic blueprint of the parental cell.

Francis Crick, the discoverer with Jim Watson of the helical structure of DNA, originally proposed that information can be transferred from nucleic acid to nucleic acid and from nucleic acid to protein, but that "once information has passed into protein, it cannot get out again": that is, information cannot be transferred from protein to protein or from protein to nucleic acid. These concepts were simplified into what came to be known as the "central dogma" of molecular biology, which held that information is sequentially transferred from DNA to RNA to protein.

In the flow of information described in "the central dogma," there was only DNA-directed DNA synthesis and DNA-directed RNA synthesis. This scheme had to be enlarged to describe viruses whose genome consisted of RNA.

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Most RNA viruses, such as the viruses that cause poliomyelitis, the common cold, and influenza, replicate by RNA-directed RNA synthesis. However, other RNA viruses, now to be discussed, replicate by RNA-directed DNA synthesis and then by DNA-directed DNA synthesis and DNA-directed RNA synthesis.

In this Account I shall discuss a number of questions related to these viruses: What is RNA-directed DNA synthesis? Where is RNA-directed DNA synthesis found in animal cells and viruses? What is the function of RNA-directed DNA synthesis in animal cells and viruses? Are the RNA-directed DNA syntheses of chicken cells and avian viruses related?

RNA-Directed DNA Synthesis

DNA synthesis was originally believed to involve only DNA without direct involvement of RNA. A DNA template directed the order of the addition of deoxyribonucleoside monophosphates to the 3′-OH end of a DNA primer (Figure 1, top).

About 10 years ago, study of the replication of an RNA tumor virus of chickens, the Rous sarcoma virus, led to the suggestion that RNA-directed DNA synthesis, that is, DNA synthesis in which RNA acts as a template for the order of the newly polymerized deoxyribonucleotides (Figure 1, bottom), existed.

The original evidence for this hypothesis, which was called the DNA provirus hypothesis, came from studies of the infection of chicken embryo fibroblasts in cell culture by Rous sarcoma virus. Inhibition of DNA synthesis with specific inhibitors blocked this infection and inhibition of DNA-directed RNA synthesis with actinomycin D blocked formation of Rous sarcoma virus RNA. More recently this hypothesis has been confirmed by isolation of infectious DNA with Rous sarcoma virus information and by nucleic acid hybridization experiments. These experiments have been recently reviewed.[1-3]

(1) H. M. Temin, *Annu. Rev. Microbiol.*, **25**, 610 (1971).
(2) H. M. Temin in: "Molecular Studies in Viral Neoplasia. 25th M. D. Anderson Symposium," J. Bowen, Ed., University of Texas, Houston, 1974.
(3) H. M. Temin, *Advan. Cancer Res.*, **19**, 47 (1974).

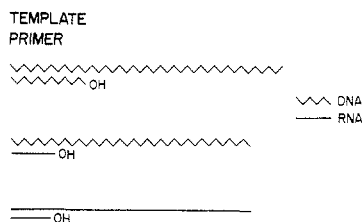

Figure 1. Diagrammatic representation of template-primers for DNA synthesis. In all cases, deoxyribonucleoside monophosphates are added to the 3′-OH of the primer. Top: DNA template and DNA primer; middle: DNA template and RNA primer; bottom: RNA template and RNA primer.

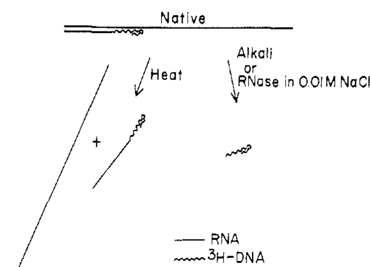

Figure 2. Diagrammatic representation of a complex of RNA and DNA product from RNA-directed DNA synthesis. Usually only the DNA is radioactively labeled.

A third type of DNA synthesis also exists. Recent work with phage M13 and *Escherichia coli* (see ref 4 and 5) has indicated that DNA-directed DNA synthesis may also involve an RNA primer (Figure 1, middle). In addition to the three types of DNA synthesis which are diagrammed in Figure 1, a fourth type, RNA-directed, DNA-primed DNA synthesis, could possibly exist.

In a DNA polymerase reaction involving purified DNA polymerases, templates and primers are added. When this reaction is constructed with purified templates and primers, it may be unambiguously defined as either RNA or DNA directed and either RNA or DNA primed.

Another type of DNA polymerase activity exists in DNA polymerase–nucleic acid complexes isolated from some animal viruses and cells. This DNA polymerase activity is called endogenous. It is undefined with respect to the type of template–primer present. The polymerase plus template–primer, which has endogenous DNA polymerase activity, could be considered a *holoenzyme.* Since no additional templates or primers are added to an endogenous reaction, the DNA synthesis involves an undefined template–primer. It is in the study of endogenous DNA polymerase activity that questions concerning a possible role of RNA have often originated.

Some biochemical criteria have proven useful in distinguishing among the different types of endogenous DNA synthesis (see ref 6 and references therein). It has been especially important to distinguish endogenous RNA-directed DNA synthesis from DNA-directed, RNA-primed DNA synthesis.

The simplest criteria to separate DNA-directed, DNA-primed synthesis from DNA synthesis where RNA is involved as either a template or a primer are the effects of treatments with the enzymes ribonuclease A and deoxyribonuclease I. Endogenous DNA polymerase activity can be completely resistant to pretreatment with deoxyribonuclease I and inhibited over 90% by pretreatment with ribonuclease A. This sensitivity to ribonuclease A clearly shows that RNA is involved in the endogenous DNA synthesis. However, it does not establish whether the RNA acts as a template, as a primer, or as both.

However in the systems so far studied, the antibiotic actinomycin D at high concentrations, 80–100 μg/ml, inhibits DNA-directed DNA synthesis almost completely, while RNA-directed DNA synthesis is about 50% resistant to these concentrations of actinomycin D. (After the initial RNA-directed DNA synthesis, the product DNA can act as a template for DNA-directed DNA synthesis. The actinomycin D blocks this DNA-directed DNA synthesis.) So far endogenous DNA polymerase reactions which have been both sensitive to ribonuclease A and about 50% resistant to actinomycin D have been RNA directed. The endogenous DNA polymerase reactions which have been both sensitive to ribonuclease A and sensitive to actinomycin D have not been RNA directed and apparently are DNA directed and RNA primed.

When RNA acts as a template for DNA synthesis, the product DNA has a nucleic acid sequence which is complementary to that of the RNA template. This complementarity can be tested by nucleic acid hybridization experiments. The existence of nucleic acid hybrids can be established by centrifugation in equilibrium cesium sulfate density gradients, by chromatography on hydroxylapatite columns, or by resistance to a nuclease specific for single-stranded nucleic acids.

Another characteristic of endogenous RNA-directed DNA polymerase reactions has been the formation of a complex of RNA and product DNA in the early stages of the DNA polymerase reaction. Such complexes have been formed in the presence of 100 μg/ml of actinomycin D in the DNA polymerase reaction mixture and have been shown to be a hybrid of RNA and the DNA product. In some cases the RNA in the complex has also been shown to be covalently bound to the DNA product, indicating that RNA has acted as a primer for the endogenous DNA synthesis. The properties of an early complex of DNA product and RNA from an endogenous DNA synthesis reaction using RNA as the template and RNA as the primer are shown in Figure 2. The native complex banded at the density of RNA in equilibrium cesium sulfate density gradients and had a sedimentation value in sucrose gradients characteristic of the RNA template. The RNA–DNA complex was destroyed by treatment with alkali or ribonuclease A and was partially destroyed by treatment with heat. These treatments led to a shift in the buoyant density of the newly synthesized DNA, that is, the product DNA banding at the RNA region of an equilibrium cesium sulfate density gradient disappeared. There was also a decrease in the sedimentation value of the early DNA product as a result of the degradation of the RNA template.

When an endogenous RNA-primed DNA polymerase reaction is carried out with α-^{32}P-labeled deoxyribonucleoside triphosphates as substrates, ^{32}P is found on a ribonucleoside monophosphate after alkaline hydrolysis. The α-^{32}P is transferred from the first

(4) D. Brutlag, R. Schekman, and A. Kronberg, *Proc. Nat. Acad. Sci. U. S.*, **68**, 2826 (1971).
(5) A. Sugino, S. Hirose, and R. Okazaki, *Proc. Nat. Acad. Sci. U. S.*, **69**, 1863 (1972).
(6) H. M. Temin and D. Baltimore, *Advan. Virus Res.*, **17**, 129 (1972).

Table I
Empirical Characteristics of DNA Syntheses

Treatment	DNA template, DNA primer	DNA template, RNA primer	RNA template, RNA primer
Ribonuclease A	R[a]	S[b]	S
Deoxyribonuclease I	S	S	R
Actinomycin D (100 μg/ml)	S[d]	S	R/2[c]
Hybridization of product to RNA	No	No	Yes
Density (g/cm^3) of early complex in equilibrium cesium sulfate density gradients			
Native complex	1.42–1.45	1.42–1.48	1.60–1.65
Heat denatured complex	1.42–1.45	1.53–1.56[e]	1.53–1.56[e]

[a] Resistant. [b] Sensitive. [c] 50% resistant. [d] Not rigorously established for single-stranded DNA templates. [e] These values are when there is a low molecular weight primer not covalently bound to the template. If the primer is the looped-back 3′ terminus of a high molecular weight template, the denaturation will not change the density of the product.

deoxyribonucleoside monophosphate added to the RNA primer. This experiment demonstrates chemically that a deoxyribonucleoside monophosphate is added to the 3′-OH of a ribonucleoside monophosphate in this synthesis.

The results of these experiments have characterized endogenous DNA polymerase activities as to whether or not they involved RNA and, when they did involve RNA, whether they were RNA directed, RNA primed, or both. Table I gives a summary of the properties of the three types of DNA synthesis illustrated in Figure 1 as determined from experiments similar to those described above. DNA-directed, DNA-primed DNA synthesis has been resistant to ribonuclease A, sensitive to deoxyribonuclease I, and sensitive to 100 μg/ml of actinomycin D. The product DNA did not hybridize to RNA and was not complexed to RNA. DNA-directed, RNA-primed DNA synthesis has been sensitive to ribonuclease A, sensitive to deosyribonuclease I, and sensitive to 100 μg/ml of actinomycin D. The product DNA did not hybridize to RNA, but an early product was covalently bound to RNA. RNA-directed, RNA-primed DNA synthesis has been sensitive to ribonuclease A, resistant to deoxyribonuclease I, and about 50% resistant to 100 μg/ml of actinomycin D. The product DNA did hybridize to RNA, and an early DNA product–RNA complex containing RNA–DNA hybrid molecules and covalently bound RNA–DNA was isolated. (The hypothetical RNA-directed, DNA-primed DNA synthesis probably would be sensitive to ribonuclease A, sensitive to deoxyribonuclease I, and resistant to actinomycin D. The product DNA would hybridize to RNA, and the early DNA product–RNA complex would not have any DNA covalently linked to RNA.)

The Biological Role of RNA-Directed DNA Synthesis

RNA-directed DNA polymerase activity was first discovered in virions of RNA tumor viruses. Since that discovery, virions of many groups of viruses have been shown to contain RNA and a DNA polymerase, and a fraction from uninfected chicken cells has been shown to have RNA-directed DNA polymerase activity. Table II lists these groups of viruses and the cell fraction. The first five groups of viruses (1–5) are often classified as RNA tumor viruses, even though many do not cause tumors (see below). The last three groups of viruses (6–8) are all cytopathic in acute infections of cells of their natural hosts, while some members of group 8 also cause tumors in animals. A fraction from uninfected chicken cells also has endogenous RNA-directed DNA polymerase activity. This activity has biochemical features characteristic of RNA-directed, RNA-primed DNA synthesis as discussed in Figure 2 and Table I. The potential for RNA-directed DNA synthesis is, therefore, widely distributed among viruses, and it is also found in uninfected cells.

(To avoid misunderstanding, it should be noted that besides the viruses in Table II there are other groups of animal viruses whose virions contain RNA and an RNA polymerase, and other groups of animal viruses whose virions contain RNA and no polymerase.)

The biological systems listed in Table II have many differences. As listed in Table III, some of the viruses whose virions contain RNA and a DNA polymerase are cell killing, and others are cell transforming. In addition, the chicken fraction is not an infectious virus. Table III illustrates that biological systems with RNA and a DNA polymerase are not necessarily viruses and that viruses with RNA and a DNA polymerase are not necessarily tumor-forming

Table II
Distribution of RNA-Directed DNA Synthesis[a]

A. Virus Groups
1. Avian leukosis-sarcoma viruses
2. Mammalian C-type viruses
   - Mouse
   - Feline
   - Rat
   - Hamster
   - Simian
   - RD-114-like
3. Viper C-type viruses
4. Mouse mammary tumor virus
5. Mason–Pfizer monkey virus
6. Visna viruses
7. Syncytium-forming viruses
8. Reticuloendotheliosis viruses

B. Cell Fraction
1. Uninfected chicken cell fraction

[a] See ref 6 and 10.

Table III
Biological Properties of Some Virus and Cellular DNA Polymerase–RNA Complexes

Name	Infectious	Cell killing	Cell transforming
Rous sarcoma virus	+[a]	0[b]	+
Rous-associated virus-0	+	0	0
Trager duck spleen necrosis virus	+	+[c]	0
Chicken fraction	0	0	0

[a] Present. [b] Absent. [c] In acute infection.[7]

(7) H. M. Temin and V. K. Kassner, *J. Virol,* 13, 291 (1974).

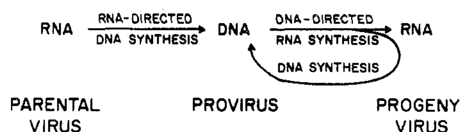

Figure 3. Replication of viruses whose virions contain RNA and a DNA polymerase.

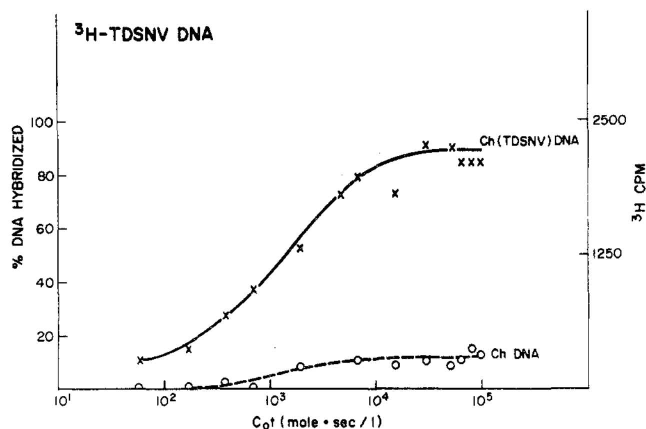

Figure 4. Hybridization of Trager duck spleen necrosis virus DNA and uninfected and virus-infected chicken cell DNA. [^{3}H]DNA complementary to Trager duck spleen necrosis virus (TDSNV) RNA was prepared using purified Rous sarcoma virus–Rous-associated virus-0 DNA polymerase.[10] Annealing was carried out under standard conditions with 3 mg/ml of DNA isolated from chicken cells (Ch) or from chicken cells chronically infected by Trager duck spleen necrosis virus [Ch(TDSNV)]. (Data were obtained from C.-Y. Kang and H. M. Temin, manuscript in preparation.)

viruses. RNA-directed DNA synthesis is apparently a normal mode of information transfer and replication for a variety of biological systems.

The role of virus RNA-directed DNA synthesis is to form a DNA intermediate, the DNA provirus, for virus replication (Figure 3). As discussed above, the DNA provirus hypothesis was proposed about 10 years ago on the basis of virological experiments to explain the replication of RNA tumor viruses. The discovery of a DNA polymerase in the virions of RNA tumor viruses gave great impetus to the acceptance of this hypothesis. The existence of a DNA provirus has been established by experiments using nucleic acid hybridization and by experiments with infectious DNA (see ref 3 and references therein). In fact, even cell-killing viruses, for example, visna virus and Trager duck spleen necrosis virus, replicate through a DNA intermediate. The DNA provirus of these cell-killing viruses has been demonstrated by nucleic acid hybridization of labeled DNA copies of the virion RNA or of labeled virion RNA to DNA extracted from infected sheep or chicken cells (Figure 4).[8]

The virion DNA polymerase apparently synthesizes the DNA provirus. Linial and Mason[9] have described a temperature-sensitive mutant of Rous sarcoma virus whose DNA polymerase activity is also temperature sensitive. At 41°, the nonpermissive temperature, this virus apparently cannot synthesize a DNA provirus because its DNA polymerase activity is not functional. Probably the DNA polymerase is inactivated at 41°.

However, virions of Trager duck spleen necrosis virus, which replicates through a DNA intermediate, have no apparent endogenous RNA-directed DNA polymerase activity.[10,11] This observation indicates the difficulty in determining the biological capability of a DNA polymerase, for example, whether or not it is involved in RNA-directed DNA synthesis, from its behavior in biochemical experiments.

The template–primer preferences of some partially purified DNA polymerases apparently correlate with a role of those DNA polymerases in RNA-directed synthesis (see references in ref 6, 12, and 13). However, these template–primer preferences only provide suggestive evidence about the biological role of a DNA polymerase. For example, Loeb, *et al.*,[14] showed tht under certain conditions *Escherichia coli* DNA polymerase I could use ribosomal RNA as a template for DNA synthesis.

Relationship of Avian Viruses and Chicken Cell Fraction Containing RNA and a DNA Polymerase

The cellular endogenous RNA-directed DNA polymerase activity has not yet been shown to have a definite function. It has been suggested that it is important in differentiation and other normal functions.[15,16] However, no evidence in favor of this hypothesis has yet been presented. A corollary to this hypothesis is that viruses whose virions contain RNA and a DNA polymerase arose from normal cell components. This corollary hypothesis predicts that there are evolutionary relationships between viruses whose virions contain RNA and a DNA polymerase and cell nucleic acids and DNA polymerases. We have been looking for evidences of such a relationship by nucleic acid hybridization experiments and by study of the serological relationships among DNA polymerases.

Nucleic Acids. To determine whether there is any relationship between the nucleic acids of viruses and of cell fractions containing RNA and a DNA polymerase, a DNA copy of the viral or cellular RNA templates was prepared and used in nucleic acid hybridization experiments.[10] When hybridization experiments were performed with a labeled DNA copy of RNA of a virus and RNA of other viruses of the same group, all of the viral RNAs annealed in the same fashion, indicating a great deal of nucleic acid sequence homology among the virus RNAs (Figure 5). This type of nucleic acid hybridization experiment appears to be an excellent test to show relationships among viruses. However, when RNA of viruses from two different groups, for example, from the reticuloendotheliosis viruses and the avian leukosis-sarcoma viruses, was annealed to a labeled DNA copy of one of them, little cross-hybridization was seen (Figure 5). The low level of hybridization seen may indicate a very small degree of relationship, perhaps

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(9) M. Linial and W. S. Mason, *Virology*, **53**, 258 (1973).
(10) C.-Y. Kang and H. M. Temin, *Virology*, 12, 1314 (1973).
(11) S. Mizutani, personal communication, 1973.
(12) H. M. Temin and S. Mizutani in *Enzymes, 3rd Ed.*, in press.
(13) P. S. Sarin and R. C. Gallo, *MTP (Med. Tech. Publ. Co.) Int. Rev. Sci.*, in press.
(14) L. A. Loeb, K. D. Tartof, and E. C. Travaglini, *Nature (London), New Biol.*, **242**, 66 (1973).
(15) H. M. Temin, *J. Nat. Cancer Inst.*, 46, III (1971).
(16) H. M. Temin in "RNA Viruses and Host Genomes in Oncogenesis,", P. Emmelot and P. Bentvelzen, Ed., North-Holland, Amsterdam, London, 1972, pp 351–363.

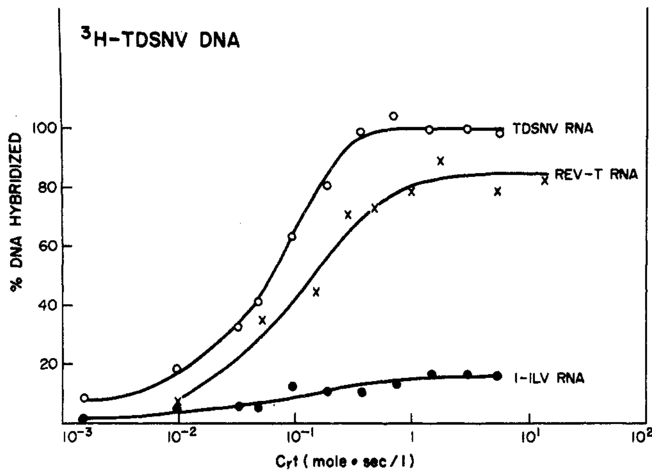

Figure 5. Hybridization of Trager duck spleen necrosis virus DNA with RNA from reticuloendotheliosis and avian leukosis-sarcoma viruses. Hybridization was carried out as described in the legend of Figure 4 with RNA from purified virions of Trager duck spleen necrosis virus (TDSNV), reticuloendotheliosis virus (strain T) (REV-T), and Reaseheath line I-induced leukosis virus (I-ILV). Some of the data are from ref 10.

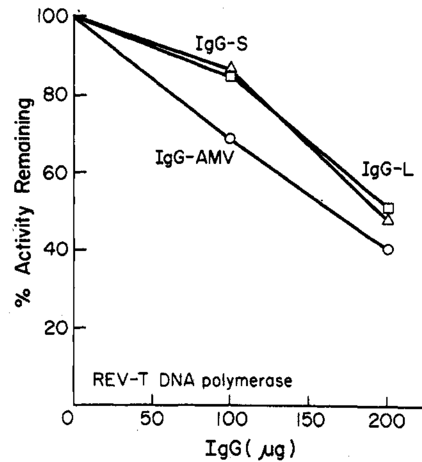

Figure 6. Neutralization of partially purified reticuloendotheliosis virus (strain T) DNA polymerase. Partially purified DNA polymerase from reticuloendotheliosis virus (strain T) was incubated at room temperature in 30 μl with antibodies against avian myeloblastosis DNA polymerase (IgG-AMV), chicken cell large (10S) DNA polymerase (IgG-L), and chicken cell small (3–4S) DNA polymerase (IgG-S).[18] After 30 min, the remaining activity of the reticuloendotheliosis virus (strain T) DNA polymerase was assayed with calf thymus DNA. (Data were obtained from ref 19.)

5% or less, between the nucleic acid sequences of some of the members of these two groups of viruses.

No relationship was found between the DNA product of the chicken endogenous RNA-directed DNA polymerase activity and the RNAs of avian leukosis-sarcoma viruses or reticuloendotheliosis viruses—the two groups of avian viruses whose virions contain RNA and a DNA polymerase (Table IV). However, some annealing was found between the DNA copies of both avian leukosis-sarcoma virus RNA and reticuloendotheliosis virus RNA and DNA of uninfected chicken cells (Figure 4 and Table IV). This annealing indicates that some nucleic acid sequences found in these viruses are also found in uninfected cell DNA. Such sequences are called endogenous virus-related genes (see ref 3 and references therein). Normal chicken cells contain the nucleic acid sequences of most of the RNA of a nontransforming avian leukosis virus.[17] These sequences could represent virus precursors or remnants of proviruses introduced by infection with viruses whose virions contain RNA and a DNA polymerase.

DNA Polymerases. To look for relationships among the DNA polymerases of the avian viruses and the chicken fraction containing RNA and a DNA polymerase, antibodies were prepared against a number of partially purified DNA polymerases.[18,19] These included two DNA polymerases from uninfected chicken cells, one 10 S and another 3–4 S, the DNA polymerase of avian myeloblastosis virus, and the DNA polymerase of Trager duck spleen necrosis virus. All of these antibodies neutralized the activity of the homologous DNA polymerase, that is, the DNA polymerase used to prepare the antibody, at antibody concentrations of less than 20 μg per 30 μl of reaction mixture.

In experiments with disrupted virions, no serological cross-reactions were found between the avian-leukosis sarcoma and the reticuloendotheliosis virus DNA polymerases or between these viruses and the cellular DNA polymerases. Moreover, for virions

(17) P. E. Neiman, *Virology*, 53, 196 (1973).
(18) S. Mizutani and H. M. Temin, *J. Virol.*, 12, 440 (1973).
(19) S. Mizutani and H. M. Temin, *J. Virol.*, 13, 1020 (1974).

Table IV
Per Cent Hybridization of Labeled DNA Copies of RNA to Different Viral and Cellular Nucleic Acids

	[³H]DNA		
Nucleic acids	Avian leukosis-sarcoma virus	Reticuloendotheliosis virus	Chicken fraction
Avian leukosis-sarcoma virus RNA	90	0–5	0
Reticuloendotheliosis virus RNA	0	90	0
Chicken DNA	5	0–5	NT[a]

[a] Not tested.

from either group of viruses, all of the virus DNA polymerases were neutralized with the same kinetics by antibody to the DNA polymerase of a virus in that group. For example, it was found that the antibody to the avian myeloblastosis virus DNA polymerase neutralized the activity of the DNA polymerases of all the viruses in the avian leukosis-sarcoma virus group, including avian leukosis viruses which were produced from chicken cells without experimental infection, for example, Rous associated virus-0.

The chicken endogenous RNA-directed DNA polymerase activity was not neutralized by antibody to avian myeloblastosis virus DNA polymerase or by antibody to the chicken 10S DNA polymerase. It was neutralized by antibody to the chicken 3–4S DNA polymerase.

Because of a change in sedimentation pattern with different salt concentrations of some partially purified DNA polymerases from reticuloendotheliosis viruses, the antibodies were tested against partially purified DNA polymerases in cross-neutralization experiments. It was found that the partially purified DNA polymerase of reticuloendotheliosis virus (strain T) was partially neutralized by antibodies to avian myeloblastosis virus DNA polymerase, the chicken cell 10S DNA polymerase, and the chicken cell 3–4S DNA polymerase (Figure 6). Similar results were found with the partially purified Trager

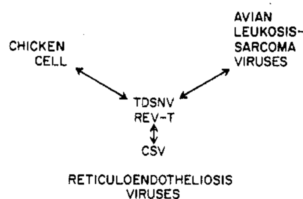

Figure 7. Diagram of serological relationships of viral and cellular DNA polymerases.[19] TDSNV, Trager duck spleen necrosis virus; REV-T, reticuloendotheliosis virus (strain T); CSV, chick syncytial virus.

duck spleen necrosis virus DNA polymerase. The specificity of these neutralization reactions was shown by the ability of the reticuloendotheliosis virus (strain T) DNA polymerase and the Trager duck spleen necrosis virus DNA polymerase to block the activity of the antibodies used in Figure 6 against their homologous DNA polymerases.

Further evidence for serological relationships among the DNA polymerases of the reticuloendotheliosis viruses, the avian leukosis-sarcoma viruses, and the normal chicken cell was the ability of the chicken cell 10S DNA polymerase and the Rous sarcoma virus–Rous associated virus-0 DNA polymerase to block the neutralizing activity of antibody to Trager duck spleen necrosis virus DNA polymerase against the Trager duck spleen necrosis virus DNA polymerase.

These serological relationships were about 100-fold weaker than those of viruses in the same group. These weak serological relationships only appeared after partial purification of the DNA polymerases, perhaps as a result of conformational changes of the DNA polymerases during purification.

These serological relationships are diagrammed in Figure 7. There are serological relationships between the DNA polymerases of chicken cells and reticuloendotheliosis viruses, and there are serological relationships between the DNA polymerases of the reticuloendotheliosis viruses and the avian leukosis-sarcoma viruses. Serological relationships were also found between the DNA polymerases of the avian leukosis-sarcoma viruses and the chicken cell.[19]

These serological relationships among the DNA polymerases of the normal chicken cell and of these avian RNA viruses indicate that these DNA polymerases probably have amino acid sequence homology. This homology indicates a common evolutionary origin from an ancestor DNA polymerase. This homology supports the hypothesis that these viruses originated from normal cell components. Some of the amino acid sequences of the original DNA polymerases may have been conserved in the evolution leading to the formation of these RNA viruses, and these sequences might have given rise to the observed serological relationships.[20]

Summary

In this Account, I have described some biochemical characteristics of RNA-directed DNA polymerase activity and its distribution and possible role in some biological systems. In addition, I have presented evidence supporting the hypothesis that the viruses whose virions contain RNA and a DNA polymerase evolved from normal cell components.

The research in my laboratory is supported by a U. S. Public Health Service Research Grant, CA-07175, from the National Cancer Institute and Research Grant VC-7 from The American Cancer Society. I hold Research Career Development Award CA-08182 from the National Cancer Institute.

(20) H. M. Temin, *Annu. Rev. Genetics*, in press.

On the Aggregation of Dissolved Alkane Chain Molecules

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Received November 26, 1973

The aggregation of chain-like molecules is a fascinating phenomenon; it is of importance in such areas as liquid crystals[1,2] and micelle formation in soaps and detergents,[3-5] and in biological membrane phenomena.[6] From an X-ray diffraction viewpoint, with the exception of the soap studies, no concerted attack on the problem has been made, primarily because of the difficulty in measuring the low intensities characteristic of liquid scattering. With respect to micelles we note the early work of Harkins and collaborators[4] and Philipoff[5] and the culmination of this effort in the classic work of Luzzatti[3] where the basic concepts of micelle formation were clarified.

As far as simple-chain alkanes are concerned, measurements were made many years ago by Stewart and coworkers,[7] and some interpretive work on

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