

This Week's Citation Classic

Kacian D L, Watson K F, Brrny A & Spiegelman S. Purification of the DNA polymerase of avian myeloblastosis virus. *Biochim. Biophys. Acta* **246**:365-83, 1971. [Institute of Cancer Research, College of Physicians & Surgeons, Columbia University, New York, NY]

Purification of reverse transcriptase from avian myeloblastosis virus is described. The enzyme is a complex of two subunits with molecular weights 110,000 and 69,000. The complex possesses the RNA-, ONA-, and RNA:DNA hybrid-directed DNA polymerase activities found in the virion. [The *SCI*® indicates that this paper has been cited in over 305 publications since 1971.]

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"When the discovery of RNA-dependent DNA-polymerase activity in retroviruses was announced, I was a graduate student in Sol Spiegelman's lab at Columbia University, one of the few in his group still working on Q β replicase. The others were studying RNA tumor viruses. Spiegelman hoped these replicated via a template-specific polymerase and that variant viral RNAs could be made that would compete for the enzyme. If human cancers were caused by these viruses, the variant RNAs might be useful as agents for specifically destroying malignant cells. Only in infected cells containing the viral replicase would the variant RNAs multiply exponentially and so destroy them.

"The evidence presented by Baltimore¹ and Temin² that these viruses replicate via a DNA intermediate had profound implications for this approach. Spiegelman was at the meeting where the announcement was made, and he returned early the next day to redirect our

efforts. I am told that the discoveries were received in other labs with equal excitement.

"Temin and Baltimore's work was confirmed and extended by several labs well before it appeared in print. DNA- and RNA: DNA hybrid-dependent DNA polymerase activities were found, indicating that within the virion were all the enzymes needed to make a doublestranded DNA copy of its genome to integrate into the host cell. But were one or several enzymes responsible for the nucleic acid polymerase activities observed and, more importantly, were they specific for their own template RNA? If specificity were found, the hope that variant RNAs could be useful therapeutic agents remained alive. If, however, the enzyme could copy any RNA, an important tool for molecular genetics would clearly be in hand.

"Working out the enzyme isolation, as is often the case, was essentially a lengthy, trial-and-error process. At the end, we had shown that a single enzyme complex with two subunits possessed all the nucleic acid polymerase activities previously found in the virion. More importantly, we found the enzyme was not template-specific and could be used to make DNA copies of a variety of RNAs.³ It would be some years, however, before Jeanne Myers and I^{4,5} were able to achieve another goal I'd set, the in vitro synthesis of full DNA copies of large, polycistronic RNAs.

"The usefulness of DNA transcripts of RNAs for hybridization probes and recombinant DNA studies undoubtedly accounts for the number of citations the paper has received. We deliberately tried to develop a procedure that was simple and gave high yields from the costly starting material. That researchers continue to find it useful is gratifying. The paper also provided information on the subunit structure and template specificities of the enzyme and is often cited in related studies.

"An excellent review of the early work on reverse transcriptase appeared in 1977."⁶

1. **Baltimore D.** RNA-dependent DNA polymerase in virions of RNA tumour viruses. *Nature* **226**:1209-11, 1970. [The *SCI* indicates that this paper has been cited in over 930 publications since 1970.]
2. **Temin H M & Mizutani S.** RNA-dependent DNA polymerase in virions of Rous sarcoma virus. *Nature* **226**:1211-13, 1970. [Citation Classic. *Current Contents* (47):14, 21 November 1977.]
3. **Spiegelman S, Walton K F & Kacian D L.** Synthesis of DNA complements of natural RNAs: a general approach. *Proc. Nat. Acad. Sci. US* **68**:2843-5, 1971.
4. **Kacian D L & Myers J C.** Synthesis of extensive, possibly complete, DNA copies of poliovirus RNA in high yields and at high specific activities. *Proc. Nat. Acad. Sci. US* **73**:2191-5, 1976.
5. **Myers J C, Spiegelman S & Kacian D L.** Synthesis of full-length DNA copies of avian myeloblastosis virus RNA in high yields. *Proc. Nat. Acad. Sci. US* **74**:2840-3, 1977.
6. **Verma I M.** The reverse transcriptase. *Biochim. Biophys. Acta* **473**:1-38, 1977.