CLASSIC EXPERIMENT 4.2

THE DISCOVERY OF REVERSE TRANSCRIPTASE

D. Baltimore, 1970, *Nature* **226:**1209
H. M. Temin and S. Mizutani, 1970, *Nature* **226:**1211

Through a series of experiments conducted in the 1940s, 1950s, and 1960s, the principles by which genetic information is transferred in biological systems were demonstrated. DNA served as the code, which was then transcribed into a type of RNA (mRNA) that carried the message to be translated into proteins. These experiments formed a paradigm so firmly believed it was known as the "central dogma." However, in 1970, work on RNA tumor viruses showed that perhaps the central dogma did not explain the whole picture.

Background

In 1961, Howard Temin began to gather evidence that was inconsistent with the central dogma. Temin, who devoted his life to studying RNA tumor viruses (now known as retroviruses), focused his early work on Rous sarcoma virus (RSV). This RNA virus is capable of transforming normal cells into cancerous cells. Temin felt the best explanation for the virus's behavior was a model whereby the virus remains in a dormant, or proviral, state in the cell. However, since RNA is notoriously unstable, Temin proposed that the RNA genome of RSV is converted into a DNA provirus. With this model in mind, he set out to prove his hypothesis. He amassed data showing that RSV is sensitive to inhibitors of DNA synthesis and suggesting that DNA complementary to the RSV genomic RNA is present in transformed cells. Other researchers, however, remained unconvinced. A definitive experiment would be required to finally prove his model.

Meanwhile, another virologist, David Baltimore, had been studying the replication of viruses. He was taking a biochemical approach, looking directly for RNA and DNA synthesis in the virions themselves. Previously he had isolated an RNA-dependent RNA polymerase activity in virions of vesicular stomatitis virus, a nontumorigenic RNA virus. His attention then turned to the RNA tumor viruses, finally settling on the Rauscher murine leukemia virus (R-MLV). With this organism, he would independently prove Temin's model.

The Experiment

Remarkably, these two scientists traveled separate pathways to the same critical set of experiments. Both began with pure stocks of virus, which they then partially disrupted using nonionic detergents. With a stock of disrupted viruses in hand they could ask the critical question: Can a retrovirus perform DNA synthesis? To answer this question, each group added radiolabeled deoxythymidine triphosphate (dTTP) along with the other three deoxynucleotide triphosphates (dATP, dCTP, dGTP) to the virion preparations, and looked for the incorporation of radioactive dTTP into DNA. Indeed, in each experiment radiolabeled dTTP was incorporated into nucleic acid. When Baltimore added a radiolabeled ribonucleotide triphosphate (rNTP) and the three other ribonucleotide triphosphates to disrupted viruses, he could detect no RNA synthesis. To prove that the product being formed was in fact DNA, they treated it with enzymes that specifically degrade either RNA (ribonuclease, or RNase) or DNA (deoxyribonuclease, or DNase). They found the product to be sensitive only to DNase. The results of these simple experiments, summarized in the Table, showed that an enzyme in the particles could synthesize DNA. However, the question remained . . . What was the template?

Demonstration of RNA-dependent DNA Synthesis

	RADIOACTIVE THYMIDINE INCORPORATED INTO NUCLEIC ACID*	
EXPERIMENTAL TREATMENT	R-MLV	RSV
Standard conditions	3.69 pmol	9110 dpm
Virions pretreated with RNase	0.52 pmol	2650 dpm
Untreated product	1425 dpm	8350 dpm
After product is treated with RNase	1361 dpm	7200 dpm
After product is treated with DNase	125 dpm	1520 dpm

*dpm = disintegrations per minute; pmols = picmoles.
SOURCE: R-MLV data from D. Baltimore, 1970, *Nature* **226:**1209. RSV data from H. M. Temin and S. Mizutani, 1970, *Nature* **226:**1211.

To show once and for all that DNA could be synthesized from an RNA template, Baltimore and Temin both preincubated the virions with RNase, which catalyzes the degradation of RNA into ribonucleotide monophosphates (rNMPs). If RNA was truly the template, then degredation of the template would prevent DNA synthesis by the virion preparations. This in fact was the case. The longer the pretreatment of virions with RNase, the lower the amount of DNA-synthesizing activity, thus proving that an enzyme in the virion could catalyze RNA-dependent DNA synthesis. Because this activity was the reverse of the well-known DNA-dependent RNA synthesis seen in transcription, the enzyme that catalyzed it was soon referred to as reverse transcriptase. Initially, many scientists were unwilling to believe that reverse transcriptase existed because its activity violated the central dogma. Subsequent isolation and characterization of the paradigm-shattering enzyme soon convinced the skeptics.

Discussion

Temin and Baltimore were led independently by different key deductions to the discovery of reverse transcriptase.

Temin firmly believed the activity existed. For him, it was the process of doing biochemical experiments on purified virions, rather than on infected cells, that allowed him to prove to the world what he knew. Baltimore, on the other hand, believed that viruses carried their polymerase activities with them. His key insight was to test for the RNA-dependent DNA polymerization activity that Temin had proposed. Both scientists, however, had to have the conviction to believe and report what they were seeing, despite its being contrary to a seemingly unshakable paradigm.

The discovery of reverse transcriptase has influenced life in and out of science in a myriad of ways. The ability to convert mRNA to DNA permitted creation of cDNA libraries, collections of DNA made up solely of genes expressed in a particular tissue. This has facilitated the cloning and study of genes involved in all facets of biology. The discovery also caused an explosion of research into retroviruses, RNA viruses that replicate via reverse transcription. This groundwork was critical 15 years later when the human innunodeficiency virus (HIV), which causes AIDS, was shown to be a retrovirus. The importance of Temin's and Baltimore's work was quickly recognized, leading to their receiving the Nobel Prize in Physiology or Medicine in 1975 for the discovery of reverse transcriptase.