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A look back: moving forward in reverse

DNA can be duplicated or read to produce RNA, which is translated to generate protein. So ran the 'central dogma' of biology, devised by Francis Crick in 1958 to explain the directional flow of information in the cell[1]. Except nothing in nature is ever quite that simple, and discoveries such as prions and microRNAs have substantially reshaped the assertions of the central dogma, demonstrating that DNA, RNA and proteins can transmit information in unexpected ways. But the first major challenge to the dogma came decades ago, not long after its inception.

In the early 1960s, Howard Temin was studying the RNA-based Rous sarcoma virus (RSV) at the University of Wisconsin. Among other observations, Temin had determined that "mutation in a viral gene present in an infected cell often led to change in the morphology of that infected cell, that two different viruses infecting one cell were stably inherited, and that the intracellular viral genomes were probably located at only one or two sites in the cell genome"[2]. These findings gave rise to his 'provirus hypothesis', which suggested that viral genomic information was somehow being converted into DNA, enabling viral gene heritability. He presented his hypothesis to a nonplussed audience at a National Cancer Institute meeting in 1964. "At this meeting and for the next six years," he would later recall, "this hypothesis was essentially ignored"[2]. Nonetheless, Temin stood by his findings and toiled away at experiments intended to prove his theory.

Meanwhile, another young scientist, David Baltimore, was following a parallel path. Baltimore first met Temin as a high school student, at a summer retreat at the Jackson Laboratory. "He was four years older than I was, and so he was kind of a college guru to the high school students," says Baltimore, "and I learned to admire Howard when I was in high school." Baltimore later followed Temin to Swarthmore College, where he was also a 'legend'. Later, as a researcher at MIT in the late 1960s, Baltimore was pursuing studies similar to Temin's, relating to the properties of RNA viruses. Baltimore's work with vesicular stomatitis virus had led to the identification of a viral RNA-dependent RNA polymerase, and he now contemplated other RNA-based viruses—specifically, RNA tumor viruses. "I was aware of Howard's work and Howard's thinking in this, and I had actually taught RNA tumor virology at MIT, so I knew all of the work," says Baltimore. "The truth of the matter was, the first polymerase I looked for was an RNA-dependent RNA polymerase... but I'd done a lot of work on DNA synthesis before as a post-doc, so it was an easy job for me to look for an RNA-dependent *DNA* polymerase—which I did the next day. And there it was."

In 1970, both men independently published *Nature* articles documenting the existence of viral RNA-dependent DNA polymerases[3,4], later dubbed 'reverse transcriptases' (RTs), and the two shared the 1975 Nobel prize in medicine (along with Renato Dulbecco) for their work. Temin's hypothesis had been vindicated, and the seed was now planted for a molecular biology revolution, challenging fundamental assumptions about viruses and the origins of cancer, and enabling powerful new research techniques, wherein scarce and vulnerable RNA could be replaced by hardy and adaptable DNA.

The earliest techniques for the RT-driven synthesis of complementary DNA (cDNA) to an RNA of interest tended to lose sequence from the 5′ end of the mRNA, as a result of the nuclease used to cleave the hairpin loop generated during cDNA synthesis. A turning point came in 1982, with the development of a vector-based synthesis technique by Stanford University researchers Hiroto Okayama and Paul Berg[5]. Their approach was complicated but enabled the sequential synthesis of cDNA strands without the use of nuclease, preserving the quality of 5′ ends.

Nonetheless, it wasn't easy. One investigator who had particular difficulty was Ueli Gubler of Hoffman-LaRoche; his response was to develop a method of his own, one requiring neither nucleases nor vector backbones, but instead using a 'nick translation' approach to generate the second cDNA strand. "I have no problem admitting that the real reason for developing the procedure was my own inability to get the Okayama and Berg method to work successfully," wrote Gubler in 1993, citing the widespread use of his technique[6]. Indeed, as of 2005, the *Gene* article describing his technique[7] has been cited over 4,400 times and remains the foundation for many current gene cloning strategies, including the protocol presented here.

cDNA technology has already played a crucial role in elucidating fundamental biological systems such as splicing; now, with groups worldwide laboring at still-grander analyses of gene structure and expression with libraries and more comprehensive microarrays, it seems certain that this protocol will remain a key component of the molecular biology canon.

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