50th anniversary of the discovery of reverse transcriptase

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ABSTRACT The simultaneous discovery in 1970 of reverse transcriptase in virions of retroviruses by Howard Temin and David Baltimore was perhaps the most dramatic scientific moment of the second half of the 20th century. Ten years previously, Temin’s observation of cells transformed by Rous Sarcoma virus led him to the conclusion that retroviruses replicate through a DNA intermediate he called the provirus. This heretical hypothesis was greeted with derision by fellow scientists; Temin and Baltimore performed a simple experiment, rapidly reproduced, and convincing to all. Its result was a major paradigm shift—reversal of the central dogma of molecular biology. It immediately grabbed the attention of both the scientific and lay press. It also came at a key time for cancer research, at the start of the “War on Cancer.” As a theoretical base and fundamental molecular tool, it enabled a decade of (largely fruitless) search for human oncogenic retroviruses but laid the foundation for the discovery of HIV 13 years later, leading to the development of effective therapy. I had the good fortune, as a student in Temin’s lab, to witness these events. I am honored to be able to share my recollection on the occasion of their 50th anniversary.

INTRODUCTION

The second half of the 20th century was a golden age of biomedical science. From a starting point of almost complete ignorance of the molecular basis of fundamental biological processes, this period witnessed a large set of remarkable advances in knowledge of the structure and function of genes, the nature of infectious agents, the development of wondrous new techniques for obtaining knowledge, and the application of the knowledge and technology to the understanding and treatment of disease. Striking discoveries that provided the underpinnings for modern biomedicine, almost too numerous to count, include the structure of DNA, the nature of genes and regulation of their expression, discovery of many important infectious viral agents—from adenovirus to Zika—and elucidation of their molecular structure and replication strategies. These discoveries were often enabled by technologies that would have sounded like science fiction to a practicing scientist in 1950—x-ray crystallography, cryoelectron microscopy, molecular cloning, DNA sequencing, PCR, and monoclonal antibodies are but a few. The tree of discovery, well-fertilized by unprecedented public support for basic biology, has borne—and continues to bear—fruit in terms of benefits to human health. We now have vaccines to prevent infection by once deadly diseases, including polio, measles, hepatitis B, and SARS CoV-2; effective drugs to treat HIV and HCV; the means to respond rapidly to pandemic infection; altogether new immunotherapies for cancer and other conditions; and much more. Of all the discoveries of this fruitful period, perhaps none had such an immediate and dramatic impact on biomedical science and science policy as the discovery, in two laboratories simultaneously, of reverse transcriptase. I had the very good fortune to be present in one of the laboratories, and I am pleased to share my recollection of the event and its impact.

When I entered the University of Wisconsin as a graduate student of Howard Temin in the fall of 1967, two things were true: “What’s true for E. coli is true for the elephant,” that is, faith that the unity of molecular biology across all life means that everything you need to know about genes can be learned by studying simple, inexpensive, and easy to manipulate bacterial model systems; and “the Central Dogma,” as enunciated by Francis Crick (Crick 1958), that information encoded in genes flows from DNA through RNA to protein, and never the reverse. (Whether Crick meant only that flow from protein was impossible, but did not mean to exclude RNA–DNA, as he was later to claim, will remain a topic for historians to
debat...
about half of them, according to an informal poll) of the existence of the provirus was evident in his demeanor, especially in the first few days following his return from conferences. Nevertheless, his belief in the provirus remained unshaken. By then, however, he had set provirus studies aside to concentrate on other aspects of RSV biology, including the physiological basis for the differences in growth properties between RSV-transformed and normal cells, uncovering a factor in serum that preferentially stimulated growth of transformed cells (Temin, 1967; Pierson and Temin, 1972). One of my projects at that time was to develop a technique for infection of cells stating with purified RNA, as had been done with other viruses, including polio (Smull and Ludwig, 1965). For this purpose, I decided to use an extraction protocol based on the nonionic detergent Nonidet P40 (NP40), a product of Shell Chemical, from whom it had to specifically requested. Little did I suspect that my experiments could not possibly succeed, but that little bottle of NP40 would play a key role in the events to come.

Toward the end of the decade, attention in the lab returned to the provirus. I think it likely that the precipitating incident was a 1968 study by Jan Swoboda of the University of Prague, on the ability of RSV, a virus of chickens, to induce sarcomas in rats. Unlike infection of chickens, where the transformed cells could produce infectious virus, the transformed rat cells showed no sign of virus, or even virus proteins. However, if the rat tumor cells were fused with chicken cells, production of infectious RSV, identical to the initial virus used to infect the rat, ensued (Swoboda et al., 1968). The conclusion that the "virogenic" rat tumor cells contained RSV information in a stable genetic form (i.e., DNA), as predicted by the provirus hypothesis, was inescapable, and Swoboda's result did not escape Temin's attention. Soon afterward, two new recruits to the lab, David Boettiger, a graduate student, and Satoshi Mizutani, a postdoctoral fellow, initiated provirus-related projects. In a study that was never published, Mizutani found that establishment of RSV infection of cells was insensitive to drugs that block protein synthesis, implying that the enzyme(s) necessary for making the provirus do not need to be made after infection (as is the case for polio and many other viruses), but must already be present in the cell, or, perhaps in the infecting virus particle (virion). Boettiger took advantage of an analogue, 5-bromodeoxyuridine (BrdU), which could substitute effectively for thymidine in DNA with little consequence to cell viability or RSV replication, except that it made DNA much more sensitive to inactivation by visible light. He found that if cultures treated with BrdU for a day immediately after infection were exposed to light, the number of foci observed declined greatly, as compared with untreated cultures or those kept in the dark. Under the conditions of the experiment, the cells themselves were not affected.

The Boettiger study, although it provided the most compelling indirect evidence for the provirus to date, was submitted for publication to Nature in March, 1970 but had little effect on the field, because it did not appear in print until November, long after everyone was already convinced (Boettiger and Temin, 1970). But it probably did have an important effect on one scientist. David Baltimore was in the audience when Temin presented Boettiger's work at a 1969 Gordon Conference.

Baltimore, an Associate Professor at MIT, was already a very well-known and highly regarded virologist, who had devoted most of his career to that point to the study of poliovirus replication and had made numerous fundamental contributions to the subject. He and Temin had uncannily similar career paths. Both attended a summer science camp at the Jackson Laboratory in Maine, where Temin, then a student at Swarthmore, was a counselor, while Baltimore was still in high school. Baltimore then followed Temin to Swarthmore, graduating 4 years later, and, after his PhD (with Richard Franklin) and a postdoctoral period with James Darnell, was also recruited by Dulbecco, as junior faculty in the new Salk Institute. Most recently, thanks to the work of his postdoc (and spouse), Alice Huang, he had turned his attention to another virus, vesicular stomatitis virus (VSV), like polio, a small virus with an RNA genome but a very different strategy of replication. Unlike polio, purified RNA from VSV virions could not initiate infection or even viral RNA synthesis in transfected cells. Reasoning that some factor in the virion in addition to genome RNA must also be necessary, Huang quickly discovered an RNA-directed RNA polymerase (known as transcriptase) necessary for initiating infection, establishing the principle that virions were not just carriers of genomes, but also could contain enzymatic components essential to the replication process. The implication of this discovery was of obvious importance for Baltimore's subsequent work, and perhaps for Temin's, although the VSV paper was not published until June, 1970 (Baltimore et al., 1970), and it is unclear if Temin knew about the finding before then.

Madison, 1970

1970 was a turbulent year on U.S. campuses. Student protests, usually nonviolent, were widespread. I can still remember the chants as the protesters marched down University Avenue, a short distance from the McArdle lab, and the smell of tear gas used by the police, I suppose to ensure that they stayed peaceful. Peaceful wasn't always the case that year: in January, a mob occupied the offices of the President and Chairman at MIT; in May, four student protesters were shot and killed at Kent State University; and in August, a car bomb set by two local terrorists exploded at the Wisconsin physics building, killing a postdoctoral fellow working late at night on his experiments. Against this backdrop, remarkably simple experiments were being performed; experiments whose results would affect the course of biomedical research and policy for decades to follow.

I must admit that, when Satoshi Mizutani asked if he could borrow my bottle of NP40, I had no idea what he wanted it for, and I didn't find out until Temin informed the lab that they had found the enzyme responsible for making the provirus. Mizutani had followed up on the finding that the enzyme necessary for RSV DNA synthesis must preexist infection either in the cell or in the virion. He looked first in the virion. Starting from a concentrated virus stock, he added the components of standard assays for DNA polymerase: deoxyribonucleoside triphosphates, one of which was 3H labeled, buffer, a magnesium salt, and, importantly, a bit of my NP40, a nonionic detergent necessary to disrupt the viral membrane and allow access of the reagents to the components inside the virion. The increase of 3H labeled DNA with time was clear evidence of the presence in the virion of both DNA polymerase and template (genome RNA) and primer (later shown to be a host tRNA) necessary for synthesis of DNA. With a few more similarly simple control experiments, to establish the template as RNA, and confirm the necessity of all the reaction components, they were ready to think about publishing. Temin asked around the lab if we thought he and Mizutani should publish now with what they had, or wait for more data. When he asked me, I advised waiting until they could nail down the nature of the DNA—that it was really a copy of the RSV genome.

As it turned out that was terrible advice, and fortunately it was ignored. While the paper was being prepared, Temin traveled to Houston to give a talk at the 10th International Cancer Congress (May 22–29, 1970). In a tumor virus session, coincidentally chaired by Harry Rubin, who also gave the first talk, Temin presented his
newly obtained evidence not only for the provirus, but for the enzyme responsible for its synthesis. I can still see him on his return to Madison, bubbling over at the impression he had made on the senior virologists in attendance, and particularly for his triumph over Rubin, his former colleague and long-time foe, who, not yet knowing what Temin was going to say, had presented a talk full of specious arguments against the provirus hypothesis.

Very soon after his return came a surprise in the form of a phone call from David Baltimore, who informed Temin that he had obtained exactly the same result and, worse, had already submitted his paper to Nature. Given the Baltimore lab’s discovery of RNA polymerase in VSV, and his knowledge of the BrdU experiment, it is not at all surprising that he thought to look for the analogous enzyme in virions of an RNA tumor virus (as retroviruses were called at the time). In his case, the virus was murine leukemia virus, obtained in concentrated form from a government contractor. The experiments and results of Mizutani and Baltimore were essentially identical, except that Baltimore’s assay worked in the absence of detergent, an oddity I have long thought resulted from the fact that the virus grown by the lowest bidder was somewhat leaky due to mishandling in preparation.

The good news was that Nature was willing to hold the Baltimore paper for a short time to wait for Temin’s. He and Mizutani rushed to get theirs finished and air mailed to England, where it arrived June 15, and the two papers appeared back to back June 27 (Baltimore, 1970; Temin and Mizutani, 1970). I believe that 12 d from receipt to publication was a new record for the journal. Indeed, the printed copy arrived on Temin’s desk long before the proofs, leaving some significant errors, including the editorial switch in the order of authors, uncorrected. The papers were accompanied by a breathless News and Views piece (anonymous, but written by John Tooze, Nature’s cell biology correspondent at the time, who later coined “reverse transcriptase”) under the headline “Central Dogma Reversed.” Reverse transcriptase–related articles soon dominated the research pages of Nature, and related headlines appeared over News and Views stories no less than seven more times during the next year (Figure 1).

**FIGURE 1:** Collage of Nature News and Views headlines related to reverse transcriptase 1970–1971.

**Impact of the discovery on science**

The scientific impact of this work was immediate, even before publication of the papers. David Baltimore presented his work at the annual Cold Spring Harbor Symposium, June 4–9. After hearing Baltimore’s talk, Sol Spiegelman, a leading molecular virologist very well known for his phage work, went back to his lab at Columbia that night, and returned to the meeting the next morning to announce that he had repeated the result. Spiegelman, along with numerous other molecular virologists, including Maurice Green, a leader in the study of adenoviruses, rapidly turned significant parts of their lab to reverse transcriptase, publishing numerous papers over the next 6 months (Fujinaga et al., 1970; Green et al., 1970; Rokutanda et al., 1970; Spiegelman et al., 1970a,b,c) characterizing the enzyme and its DNA product. I remember well the annual Cold Spring Harbor meeting on tumor viruses in August of that year, the same meeting where, in previous years, Temin had faced much derision. Temin chaired the session on reverse transcriptase, sitting on the stage with a contented smile, secure in the knowledge that the discovery really belonged to him (Baltimore was not there), while Spiegelman, Green, and others competed over cleaning up the biochemical details.

In addition to basic virology, there was also an immediate effect on the U.S. cancer research program. The discovery validated the idea that viruses could lead to cancer by directly altering the genetic makeup of cells, laying obvious groundwork for future research. Moreover, it also lent credence to the hypothesis that similar viruses might be involved in human cancer, as well as a means to look for them, and possible approaches to treatment and prevention. Spiegelman, for example, developed a sensitive, apparently specific, assay for reverse transcriptase in virions (Schlom and Spiegelman, 1971), which he used to find evidence of viruses in breast and other cancers (Schlom et al., 1971). Unfortunately, their published results could not be reproduced, and, to date, only one human cancer is known to be directly caused by a retrovirus (human T-cell lymphotropic virus, discovered by the Gallo lab; Poiesz et al., 1980). Green’s lab focused on discovery of reverse transcriptase
inhibitors as antiviral drugs (Gurgo et al., 1971), even though they would not be expected to be of any value against cancers in which the provirus was already present.

The contract-based Special Virus Cancer Program, initiated by the NCI in 1964, and focused at first on DNA viruses, such as Epstein-Barr virus (a herpes virus), polyomavirus, and adenovirus, turned its attention to RNA tumor viruses and reverse transcriptase, lavishly funding studies to find, characterize, and develop treatments for human retroviruses. The next decade saw many reports of such viruses, all of which turned out to be artifacts based on cellular DNA polymerase masquerading as reverse transcriptase, or on unsuspected contamination with endogenous viruses acquired following transplantation of the cancer in other species, usually mice. So frequent were the irreproducible or rapidly refuted claims of retroviruses associated with human cancer that they came to be known as “human rumor viruses,” as recounted in a comprehensive review by Robin Weiss and colleagues (Voisset et al., 2008).

Despite the failure of most attempts to associate retroviruses with human cancer, recognition of the mechanism of RNA tumor virus replication that followed the Temin and Baltimore discovery led directly to the two discoveries that form the foundation of modern cancer research. First was the observation that src, the RSV gene responsible for the transformed cell phenotype (referred to as a viral oncogene), is derived from a normal cellular gene (Spector et al., 1978). Since then, many more oncogenes and their normal cell precursors (or protooncogenes) have been identified (Rosenberg and Jolicoeur, 1997). Second was the finding that related retroviruses that do not carry such oncogenes can nonetheless cause cancer if their provirus, by chance, integrated close enough to, or within, a protooncogene in a way that could override the normal control of its expression to allow the cell to lose the normal restraints on its growth (Hayward et al., 1981). Proviral integration sites in virus-induced cancers in experimental animals led to the identification of large numbers of protooncogenes, many of which were associated with human cancer, in this case with expression or structure altered by mutation or chromosomal rearrangement rather than proviral integration.

The failed efforts of the 1970s were to bear fruit in the 1980s in more unexpected ways. In 1981, the first cases of the disease that would be called AIDS were reported in the U.S. (Centers for Disease, 1981). Although it was unclear at first that AIDS was caused by an infectious agent, lessons learned from the tumor virus searches enabled the rapid discovery of the virus responsible, and, based on results of assays for reverse transcriptase, its identification as a retrovirus (Barre-Sinoussi et al., 1983), later named HIV (Coffin et al., 1986). Although HIV and the disease it caused were unlike any seen before, the sophisticated understanding of retrovirus biology obtained (the hard way) in the previous decade made possible the rapid development of a blood test for anti-HIV antibodies, followed, not much later, by the discovery and approval for therapeutic use of the nucleoside analogue 3’-Azidothymidine, a potent inhibitor of HIV reverse transcription. Finally, the development of additional inhibitors of reverse transcriptase and other viral enzymes in the 1990s made possible the combination antiretroviral therapy that converted HIV infection from an inevitable death sentence to a chronic condition. By taking one combination pill per day, HIV-infected patients can now live a nearly normal life—thanks to one of the medical miracles of the 20th century. Without Temin’s and Baltimore’s discovery, and the further work built on it, this series of events would have taken much longer to unfold, at a cost of many more lives.

In addition to its relevance to infectious disease, reverse transcriptase turned out to be one of the key tools that enabled modern molecular biology. The ability to make DNA copies of RNA meant that one could readily synthesize radiolabeled DNA copies of cellular mRNA (Verma et al., 1972) for use as a hybridization probe, or, eventually, to enable insertion of the cDNA into a bacterial plasmid to create a molecular clone (Rougon et al., 1975). With the application of more modern technology, including PCR, next generation sequencing, and the like, reverse transcriptase continues to play a central role in biology today. Of particular importance is the use of quantitative reverse transcriptase–based PCR assays to measure viral RNA loads in clinical samples, used extensively for detecting and monitoring HIV, HCV, and other RNA viruses, most recently SARS CoV-2, in infected patients. In 2019 alone, before the current pandemic, the market for molecularly cloned commercial RT for research and clinical applications was approximately $300 million (Anonymous, 2020).

The importance of reverse transcription in biology has come into view over the five decades since its discovery. Although the existence of endogenous proviruses in the germline of chickens, mice, and humans (Martin et al., 1981) had been recognized since the 1970s, it was only with the release of the draft human genome sequence in Lander et al. (2001) that the impact of reverse transcription on our DNA was first appreciated. About 8% of our genome is derived from infection of the germ line of our distant ancestors, dating back 100 million years or more and comprising some 80,000 proviruses or proviral fragments, none of which encodes infectious virus. Endogenous proviruses are only the tip of the retroelement iceberg: nearly half of our genome consists of mobile elements (including LINEs, SINEs, and processed pseudogenes) inserted by processes involving reverse transcription (Deininger and Batzer, 2002). Reverse transcriptases have also been found to be involved in the replication of viruses other than retroviruses, including hepatitis B virus (Summers and Mason, 1982), cauliflower mosaic virus (Volovitch et al., 1984), and others.

Temin’s intuitive approach to science did not end with reverse transcriptase, and in 1971, he published a speculative paper proposing that retroviruses evolved from cellular transposable elements possibly involved in normal processes, such as establishment of long-term memory (Temin, 1971). The finding by his lab and others that proviruses are flanked by long terminal repeats (LTRs) with structures resembling that of known transposable elements (Shank et al., 1978; Shimotohno et al., 1980; Ju et al., 1982) was cited in support of this idea. Although this origin idea may yet prove correct, at some level, it is clear that known vertebrate LTR-containing retroelements were derived from retroviruses, not the other way around, and the origin of the viruses is lost in the very distant past. Nevertheless, reverse transcriptase has proven to be an essential part of eukaryotic cell biology, in the form of telomerase, the enzyme essential to the repair of the ends of chromosomal DNA, which are otherwise inexorably shortened through the process of S-phase DNA synthesis (Greider and Blackburn, 1989).

Public impact
The discovery of reverse transcriptase received public attention at a level unparalleled in immediacy and intensity for any basic biomedical discovery up to that time. I think that a number of factors combined to create such a firestorm.

First, 1970 was a propitious year for scientific discovery, especially as it related to cancer. It was a time of heightened public interest, particularly associated with the efforts of Mary Lasker, whose husband, Albert, had died of colon cancer, and who was a tireless activist for public support of cancer research, and whose lobbying the government for greatly increased support of basic research into the root causes of cancer was instrumental in passage of the National...
Cancer Act, firing off the “War on Cancer,” promised by President Nixon in his January 1971 message to the Union Message and signed into law the following December.

Second, the idea that retroviruses replicated their RNA genomes via a DNA intermediate required a paradigm shift of such magnitude that only the strongest evidence could have led to its acception. The simultaneous reports of Temin and Baltimore carried far more weight than would have either one alone. These, combined with the fact that the key experiment was so simple that anyone with some purified virus, a few common reagents, and a scintillation counter could reproduce it in a few hours, and that many scientists in the tumor virus field did exactly that, convinced everyone of the correctness of Temin’s provirus hypothesis literally overnight, leading, as headlined in two Nature News and Views pieces, a deluge of confirmatory reports (Figure 1).

Third, the story line of the lonely scientist working for 10 years in a small lab with an idea he is convinced is correct, but is unable to convince any of his peers, until he comes up with an incredibly simple, “killer” experiment that convinces everyone overnight, was too good a story for a writer to resist.

The second half of 1970 in the Temin lab was memorable for the frequent presence of reporters and photographers wanting a piece of the action, and here is where my second contribution comes in. The February 22, 1971 issue of Newsweek had a story on the war on cancer, with a cover photo of Temin in the lab looking at a small T-flask full of pink cell culture medium. He was the one who filled the flask.

Coda

The reverse transcription story line was also a clear path to a Nobel Prize, shared by Temin, Baltimore, and Dulbecco in 1975.

Tragically, Howard Temin died of cancer in 1994, at the age of 59. Harry Rubin outlived him by more than 25 years, passing away in February 2020 at age 93. At 82, David Baltimore is still alive and still publishing senior authored papers (Frankiw et al., 2020).

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