Howard Martin Temin – Co-discoverer of Reverse Biological Information Flow

In science, most often, it is very difficult to challenge a closely held theory or hypothesis. When it is done successfully, it is a great contribution in itself, as it expands the horizons of our knowledge and opens up new fields of investigations. One of the closely held 'dogmas' of modern molecular biology was that biological information can be transferred only in a unique direction; DNA to RNA to Protein. One of the scientists who successfully challenged this view was Howard Temin. In a series of elegant experiments, Temin and coworkers showed that at least the first step of the pathway is reversible; i.e., biological information can also flow from RNA to DNA. This happens in a class of viruses that infect birds and animals and cause particularly malignant forms of cancer known as sarcomas. These studies not only helped to overturn a closely held belief about the direction of biological information transfer, but also heralded new insights into the mechanism of cancer. Though the direct role of viruses has not been established in human cancers, these studies ultimately led to the understanding that cancer is caused by molecular militants within. For this major contribution, Temin shared the 1975 Nobel Prize in Medicine and Physiology with David Baltimore and his own former mentor, Renato Dulbecco.

Temin's interest in biology was kindled at a young age when he was a high school student and used to attend summer programmes at the Jackson Laboratory, Maine. His research career started as a graduate student in the laboratory of Dulbecco at the California Institute of Technology, Pasadena. This was an exciting time when the fundamental principles of molecular biology were being worked out, primarily using bacterial systems, including bacterial viruses. The research problem that Temin chose was the biology of a class of tumour viruses that have RNA as genetic material and cause sarcomas in chicken. These viruses, originally studied by Peyton Rous since 1911, are now known as Rous Sarcoma Virus (RSV). Careful studies by Rous had shown that the tumours could be transmitted to other birds by using cell-free filtrates obtained from tumour cells, suggesting a viral agent. Unfortunately, even the fact that nucleic acids are the carriers of genetic information was not known at that time and the work on RSV was stopped because of the absence of basic knowledge about biological systems. Further results had to await major breakthroughs in molecular biology such as understanding the nature and structure of the genetic material.

One of the first observations of Temin, working under the guidance of Harry Rubin, a post doc in Dulbecco's lab, was that when RSV was added to a sparse layer of chicken embryonic cells, infected centers showed up as foci, the cell-culture equivalents of tumours. Even today, this is an important assay for the tumerogenic potential of a virus. Temin also found that the morphology of the foci were different when genetic variants of the virus was used, leading to the important conclusion that transformation is the result of action by genes carried by the virus. Subsequent studies by others established that these are cellular genes 'hijacked' by the virus, whose function has been modified. Thus it was realized that modification of normal cellular genes can lead to cancer.

Temin continued his studies of RSV as an independent investigator when he became an Assistant Professor at the McArdle Laboratory for Cancer Research at the University of Wisconsin, Madison, in 1960. One of the major conclusions

that Temin could make from his studies is that the viral genome is stably integrated as a part of the host genome, reminiscent of the prophage state seen in bacterial viruses. The viral genome could be transmitted to the next generation of the host cells when they divide. Named as the 'provirus hypothesis', this was essentially a genetic hypothesis and did not make any conclusions about the molecular nature of the provirus. This immediately led to a major conceptual problem, because, the viral genome is RNA whereas that of the host is DNA. How is it possible for the RNA viral genome to be part of the host genome made of DNA? Another major experimental result was that, when the compound actinomycin D, an inhibitor of RNA synthesis, was used in virus infected cells, it stopped production of progeny virus, suggesting that the provirus is DNA. This was the first indirect evidence for the conversion of the RNA viral genome into the DNA provirus. This was further supported by the demonstration of viral DNA forms in infected cells. This led to the bold proposal that *the viral RNA genome is used as a template to make a DNA copy that is integrated into the host genome. The DNA provirus is then used as a template that is transcribed to give the viral RNA copies.* In the absence of conclusive proof in the form of an enzyme that actually carries out the step of 'reverse transcription', the scientific community was skeptical about the model.

Despite the unenthusiastic reception to his ideas, Temin was convinced of the model. The search was on to find the enzyme DNA-dependent RNA polymerase or reverse transcriptase. One of the leads he had was the observation that viral infection did not need any new protein synthesis during the early part of the infection, suggesting that the enzyme may be present in the virion itself. Viruses carrying polymerases was not a new idea. RNA viruses were known to carry the RNA-dependent RNA polymerase enzyme that is essential to make additional copies of the genome. A hunt for the virion polymerase was successful. An enzyme activity that used an RNA template and deoxyribonucleotide triphosphates to synthesize DNA was identified independently by Temin and Baltimore. Their results were published in the same issue of the journal *Nature* in 1970. This was the conclusive evidence that the scientific community expected before finally accepting a violation of the central dogma. Today, the enzyme that Temin and Baltimore had isolated, viz. reverse transcriptase, is a favourite enzyme among molecular biologists to make DNA copies from RNA.

Temins's scientific contributions did not end here. As pointed out earlier, one of the major conclusions made following the studies on RSV was that their extreme tumerogenic potential is related to hijacked cellular genes that they carry. This realization prompted an explosive burst of studies in different labs that resulted in the discovery of a large number of cellular genes, that, when modified, caused cancer. Thus Temin's work on RSV led to the discovery of oncogenes that can cause cancer independent of viral influence (see M S S Murthy, *Resonance*, Vol.5, No.1, p.45, 2000). For these major contributions to science, he was awarded many honours, culminating in the Nobel Prize in 1975. Temin died in 1994, after witnessing the important contributions made by his colleagues who followed his leads. Today, RSV also serves as an important paradigm for understanding another deadly retrovirus that was discovered subsequently, the HIV virus that causes AIDS.

S Mahadevan
Department of Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore 560012, India.
Email mahi@mrdg.iisc.ernet.in