MEETING REVIEW

Thinking about Howard Temin

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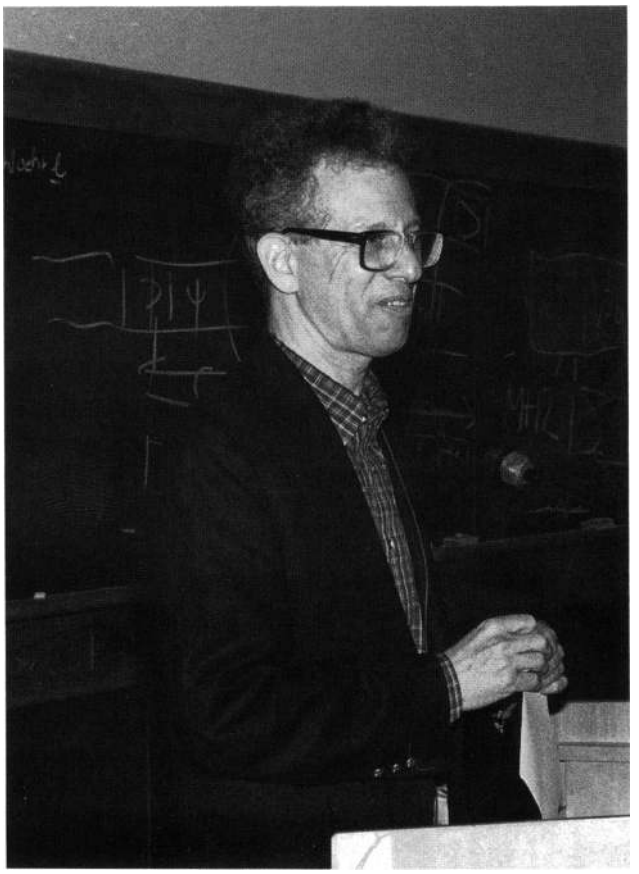
Twenty years after the discovery of oncogenes, we have come to Keystone to assess progress and find new pathways to discovery. To begin this homage to the power of molecular biology, I want to go back to the historic roots of this discipline. Each spring there is a gathering of scientists at Cold Spring Harbor to discuss some aspect of contemporary biology and they are published as the Cold Spring Harbor Symposia on Quantitative Biology. That phrase, "quantitative biology" seems quaint today—who among us could imagine non-quantitative biology? But those symposia are a record of a new and revolutionary influence on 20th century biology, the desire for quantitative measurement in a field that had been dominated by observation.

I got to thinking about the phrase "quantitative biology" as I thought about Howard Temin's influence on cancer biology. He brought quantitation to tumor virology and changed the nature of the field. He died on February 9, 1994 and we lost one of the generative influences on modern cancer biology.

Howard was 59 when he died. From my perspective, he died young. Certainly in spirit, he died young. For those of you who entered oncogene research only recently, let me remind you of his contributions.

Howard went from being an undergraduate at Swarthmore College to a graduate fellowship at Cal Tech in 1955. He quickly set about developing the first quantitative assay for viral transformation. This assay had deep historic roots. When d'Hérelle first described bacterial viruses in 1917, he recognized that they could be quantitated by a plaque assay in which a single virus could initiate production of a clear area in a lawn of bacterial growth (d'Hérelle 1917). Thus was born quantitative virology. It took almost 40 years before such a technique could be used in animal virology because the ability to grow poliovirus in cultured animal cells was only discovered by Enders, Weller, and Robbins in 1949 (Enders et al. 1949).

Cal Tech was the place to be for a virologist in the 1950's. When Howard arrived there his teachers were Delbrück, Dulbecco, and other key members of the phage group, that remarkable collection of mid-20th century scientists who used bacteriophages as tools for understanding genetic principles. Delbrück, as early as 1939, had understood that the simplicity of the plaque assay for bacteriophages, and the simplicity of the viruses themselves, allowed more rapid progress than could be made on other, more complicated genetic systems (Delbrück 1940). Dulbecco—moving from phage to animal viruses—had fused the insights of Delbrück and the Enders group to develop a plaque assay for poliovirus and other animal viruses in the early 1950's (Dulbecco 1952). Thus the stage was set for Howard. Working with Harry Rubin in Dulbecco's laboratory, he showed, in 1958, that cell transformation by Rous sarcoma virus could be quantitated by a focus-forming assay on chicken embryo fibroblasts (Temin and Rubin 1958). This opened up viral cancer research to the application of quantitative methods and led directly to separation of Rous sarcoma virus from its accompanying Rous associated viruses by Hanafusa, Vogt, and their colleagues. That, in turn, provided the background for Stehelin, Var-



Howard Temin. (Photo courtesy of Cold Spring Harbor Laboratory Archives.)

mus, Bishop, and Vogt to show that the *src* oncogene is an entity independent from the rest of the Rous virus that has its origin in cellular genetic information (Stehelin et al. 1976). Then was born the revolution in cancer biology that we celebrate here this week.

Howard Temin, of course, played an even bigger role in this story. Two decades elapsed between his arrival at Cal Tech and the Varmus/Bishop work. Much of that time, there were very few people who were aware of Howard's work. But he was very busy. During his time at Cal Tech he had an insight that seemed obvious to him but that few others took seriously. When he left Cal Tech in 1960 to go to the University of Wisconsin, he was determined to find a way to prove this heretical notion but it took ten years before a way emerged. The heretical notion he developed while at Cal Tech was that Rous sarcoma virus, although an RNA virus, must be carried in an integrated DNA form in the infected cell. I once asked Renato Dulbecco when Howard first formulated this notion and he was certain that it was in the late 1950's because he remembered that at Howard's thesis exam, Max Delbrück was very impressed with everything except Howard's speculation on a possible DNA intermediate in the virus growth.

I want to spend a moment on two issues here: Why was Howard so certain that there was a DNA intermediate and why was this heretical? To answer the first question we need to go back into the history of bacteriophage research. After d'Hérelle discovered phage in 1917, there ensued much confused work on what they were and how they acted. Part of the confusion came from the unrecognized existence of two kinds of phage-host relationships, one lytic and the other lysogenic. The lysogenic state was the hard one for the early workers to understand. Before World War II, key observations were made by Burnet, working in Australia (Burnet and Lush 1936), and by the elder Wollmans, two French scientists who were arrested at the Institut Pasteur and perished in the concentration camps of World War II (Wollman and Wollman 1936). Their son carried on the work after the war, working with François Jacob (Jacob and Wollman 1961). This pre-World War II work, on a phage of *Bacillus megaterium*, showed that bacteria can harbor a phage genome in a noninfectious form.

After the war, André Lwoff picked up the work and by 1950 he had proved that each bacterium of a lysogenic *B. megaterium* strain maintains the phage genetic material as a prophage and he soon showed that the phage could be induced into lytic growth by ultraviolet light (Lwoff et al. 1950). Then phage λ was discovered by Esther Lederberg in *E. coli* K12 (Lederberg 1951) and the field moved to its study because it provided a much richer experimental system. One of the oddities here is that the phage group was largely an American phenomenon and yet the key early work on lysogeny took place in the chaos and poverty of post-war France. Stent has suggested that this was a result of Delbrück's focus on the T-even phages, none of which have a lysogenic phage (Stent 1963). Delbrück had set off a revolution by convincing the phage group to concentrate on the T phages of *E. coli* but to some extent became a victim of that choice and resisted the notion of lysogeny until it was more than evidently true.

By the mid-1950's, lysogeny was well-established as a phenomenon and it was known that the prophage was integrated into host cell DNA. When Howard Temin was looking for a model of a stable host-virus relationship, the obvious one was the lysogenic state. The issue is why did Howard think about this at all. Here we must remember that first and foremost, Howard was an experimentalist. He was always attracted by theoretical notions, and many thought of him as a theorist, but it was the transformed cells in the dish that were talking to Howard and he was listening. What they shouted at him was stability. The transformed state was a permanent one. Every transformed cell gave rise to more transformed cells. Most persuasively, he found that strains of Rous Virus that gave altered morphologies to cells did so stably—this was a key argument for the control of the transformed cell by the viral genome (Temin 1960). It was also important that cancer was an irreversible process of cellular change. To Howard this meant that there had to be a change in the cell's DNA.

Howard had learned the lessons of Avery and Hershey: DNA carries the heredity in cells. There was only one problem with this notion. Rous sarcoma virus was an RNA-containing virus. In the late 1950's, it had just been shown that viral RNA could be infectious (Gierer and Schramm 1956), so there was no difficulty with the concept of Rous sarcoma virus RNA being the carrier of hereditary traits. The difficulty was that RNA was generally considered a transient molecule in cells, easily degraded and with no hiding place from which it could direct cell metabolism indefinitely. Furthermore, there existed the seductive example of bacteriophage lysogeny. But how could the Rous virus RNA integrate into cellular DNA? Therein lay the puzzle.

Howard's solution was chemically simple but without precedent: if the RNA were copied into DNA, then everything would fall into line. The RNA would become DNA, the DNA could integrate just like a lysogenic phage and the integrated genome could be transcribed back into RNA (Temin 1974). Conceptually, a snap—but totally unacceptable to almost everyone then in molecular biology because it ran counter to the guiding dogma, that DNA makes RNA makes protein. There was no place in that dogma for reversing the flow of information and it seemed dangerous to even conceive of such a process because of the evolutionary implications. If RNA could be copied into DNA, then it was possible that experience could feed back on the genome. One could imagine, for instance, that learning could involve RNA molecules and that they might then, as DNA, become part of the genome. In that way, the experience of one generation could be transmitted to the next. Such a mode of inheritance seemed more efficient than the Darwinian random mutation and selection but was ruled out by the central dogma.

Efficiency was not the only issue. The Lysenkoists who controlled Soviet science believed in the inheri-

tance of acquired traits because it fit the Communist notions of the ability of the social environment to modify behavior. If environmental events could direct inheritance, they believed that in a few generations the Soviet state could change the fundamental nature of man. I am not exaggerating the political implications of what Howard was thinking: when Howard and I finally proved that reverse transcription occurs, we heard from Soviet and Chinese scientists who believed that we had provided evidence for the inheritance of acquired characteristics.

Howard left Cal Tech in 1960, going to the University of Wisconsin where he spent the rest of his life. It was ten years before Mizutani, a postdoctoral fellow with Howard, began the biochemical experiments that led to their discovery of the reverse transcriptase (Temin and Mizutani 1970). During those ten years, Howard never lost faith in his belief that there was a DNA provirus in infected cells. He tested that notion in various ways but could not find an experiment that would convince others. It was a classic case of technology lagging theory.

One experiment, I think, was fairly convincing—the inhibition of virus growth by actinomycin D (Temin 1963). This drug was shown by my doctoral mentor, Richard Franklin, to inhibit DNA viruses but not RNA viruses (Franklin and Baltimore 1962). There were two exceptions: Rous sarcoma virus and influenza virus. Actinomycin D binds to DNA but not RNA and therefore its inhibition of Rous virus seemed like a strong argument for a DNA intermediate. However, drug inhibition experiments are never wholly satisfying because of uncertainties about specificity and secondary effects. The influenza virus case is a good example—it does not have a DNA intermediate in its growth but it needs host cell DNA-dependent RNA synthesis to provide the caps for its messenger RNA. The possibility that Rous virus inhibition had some similar indirect explanation robbed Howard's experiment of its explanatory power.

In another attempt at proving his point, Howard undertook DNA hybridization experiments to look for the provirus in infected cells (Temin 1964). That sounded like a critical test and would have been except that in his experiments the background was so high that the signal was ambiguous. In retrospect, Howard had actually discovered that chicken cells have endogenous viruses related to Rous sarcoma virus but at the time, the background merely served to obscure the signal and the experiment was not convincing.

In the end, the experiment that finally convinced the world utilized an old technology. It was based on biochemistry that Arthur Kornberg and Severo Ochoa had pioneered in the mid-1950's (Kornberg 1989). What was required was merely to imagine that the DNA-dependent RNA polymerase might be packaged in the virions. That thought occurred to me and to Mizutani and Temin at about the same time (Baltimore 1970; Temin and Mizutani 1970). Once the idea was there, the experiments were straightforward. For me, it was literally a few days between getting the required viral stocks and showing that the enzyme was present there. Imagining that there might be a polymerase in the virion was not revolutionary in 1970. A few years earlier, the first virion polymerases had been discovered; they were RNA polymerases in vaccinia virus and reovirus (Borsa and Graham 1968; Kates and McAuslan 1967; Munyon et al. 1967; Shatkin and Sipe 1968). Earlier in 1970, I had found an RNA polymerase in the virions of vesicular stomatitis virus (Baltimore et al. 1970)—the key result that led me to the reverse transcriptase—but I believe that Howard was not aware of that work.

In summarizing this history, I have tried to illustrate one of the truisms of science, that revolutionary ideas often have deep historic roots and clear precedents. That does not trivialize them: history does not provide an analytic basis for discovery, it provides analogies that may or may not be applicable. It is to Howard's everlasting credit that he saw the appropriate analogies in the lysogeny model and continued that belief for more than ten years while others derided his efforts to convince them.

When Howard conceived of the proviral intermediate in retrovirus replication, he was working in a time when the fundaments of molecular biology were being put into place. It was a time of many revolutionary ideas and experiments: elucidation of the structure of DNA, discovery of messenger RNA, realization of the role of transfer RNA, discovery of gene regulation, to mention only a few. These were biochemical and physiological discoveries but they had their basis in genetics. Howard embodied that perspective. Only genetics had the subtlety and abstraction to occupy and satisfy his analytic mind but his great discovery was one of an unsuspected biochemical reaction.

Of the original phage group, only Dulbecco moved on early to working with animal cells (Kevles 1993). That move was prophetic and had the practical consequence that Rubin and Temin responded and took up the challenge of adapting the Cal Tech way to the cancer problem. The Cal Tech way was to think about genetics and about abstract issues of molecular biology but not be afraid to take the experiments into biochemical and physiological contexts. Temin's housemate at Cal Tech was Matthew Meselson. He too took an abstract idea and made it a biochemical reality in the famous 1958 Meselson-Stahl experiment that showed the semi-conservative nature of DNA replication (Meselson and Stahl 1958).

Why did Howard focus his legendary mind on the cancer problem? I have no idea what drove him to choose that direction but it certainly put him well ahead of his time. Matt Meselson said to me that he rarely discussed work with Howard because Howard had chosen such an unusual path in science, one with little obvious intersection with the concerns of his co-students. I can remember my first visit to Cal Tech in about 1962. There I found in the sub-basement the Dulbecco lab and found two students with whom I felt a special kinship. I sensed that Dulbecco was being the pathfinder of my career in science and, thinking back, can see how once Howard decided that cancer would be his preoccupation, Dulbecco's lab was perhaps the only place in the world where he could have realized his ambitions. But Dulbecco was not the kind of person to sell his science—I believe that

Howard must have made his own decisions about his directions and it is hard to know what drove him to cancer. I can make one guess, a simple one but nonetheless likely to be close to the truth. At that time, cancer was to animal cell biology what genetic mutations were to phage and bacteria. In the 1950's one couldn't make mutations in animal cells but the transformation to cancer was the type of aberration that could illuminate normal cell behavior. Virus-induced cancer was the obvious choice for study and Rous sarcoma virus was the obvious virus. That particular choice was made at Cal Tech by Harry Rubin, I believe, who came to the field from a background in veterinary medicine.

To finish, I thought I would share some musings on the present state of biology occasioned by Howard's untimely death. The 1950s to the 1970s were the heroic times in modern biology. The Big Questions were posed and answered. We came out of that era with an understanding of the outlines of molecular processes. How about biology today? Does it offer us Big Questions as targets for investigation? How long will such questions be out there?

There are Big Questions for biology but they are not in what we traditionally consider molecular biology. Molecular biology certainly still holds many more surprises—probably enough to keep my lifetime in science one of continual excitement—but there are few areas of such confusion that we can sense a Big Surprise hiding in the bushes. The most recent area of pregnant controversy, the mystery of the prion, seems to have come to consensus even if the mechanistic aspects remain uncertain. The next years should see this worked out and will probably uncover multiple examples of prion-like behavior, just as happened when RNA catalysis was first demonstrated.

The Big Questions that are easily seen are in neuroscience. Here we have yet to deal effectively with the age-old puzzles of memory and learning, of consciousness and sleep and of how all that wiring gets put together. A remarkable amount is happening in the field, however, and answers to these questions are starting to take shape. Discovery of multiple levels of memory consolidation involving specific enzymes; recognition that a general principle of determining neural connections is the concept that neurons that fire together wire together; realization that a monkey's perception can be altered by focal stimulation of columns of cells in its brain—these and other advances are rapidly taking the mystery out of these Big Questions. Neuroscience has many years ahead of it before a satisfactory picture emerges but even the Big Questions are already losing some of their power.

The Big Question that faced our field 25 years ago, what influence causes cancer cells to grow without control, fell to the power of modern biology over the ensuing quarter-century and today we are comfortable that we have an outline of the answer. In cancer biology, like most other areas of biology, we have moved towards a science of particulars and practicalities, not principles. It's very satisfying to understand how a particular human disease works and to devise an intervention but I am sure that more than one young scientist hankers for the days of the Big Questions, when a recent college graduate could entertain heretical thoughts about cancer-inducing viruses.

Let me end by introducing a field where the Big Questions are as big as ever but where biology will yet have an illuminating entry. It is controversial and many may not even want to think about it, but it is there and desperately needs illumination. I refer to interpersonal relations. We are all ready to believe that our bodies reflect our genes and that we evolved to be what we are. But we are a social species that lives within a complex society. The particulars of that society are certainly learned and inherited as culture, not genes. The principles of the social relations, however, are to a great extent a reflection of genetically programmed capabilities. The mating behavior of animals is a good example. We know, particularly well from studies on birds, how much inheritance has to do with the sexual styles of individual species. Human language is another area where there is a glimmer of understanding of the interaction of cultural particulars with genetic determination.

There are certainly Howard Temins out there thinking radical thoughts about the Big Questions of human behavior and their answers are likely to bring shocking realizations about the biological underpinnings of human life. First in neuroscience but later in sociobiology we will see these principles emerge and then become the basis for investigations of particulars and then rational intervention. It is a world that many fear because of the erosion of perceived free will but it is the world of biological reality and I, at least, believe that by facing it we can become richer, healthier, and more satisfied human beings.

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Genes Dev. 1995, **9:**
Access the most recent version at doi:10.1101/gad.9.11.1303