Reverse Transcription: One Year Later

When the tenth International Cancer Congress convened in Houston last May, a young University of Wisconsin professor named Howard M. Temin was on the program. Competition for attention among the thousands of participants was considerable, and when Howard Temin came to the podium to present his paper, he found himself addressing a sparsely settled audience in one of the convention hall's cavernous rooms. Indeed, the congress was so mammoth that few participants could claim to have drawn much notice, with the possible exceptions of Vice President Spiro Agnew who came to speak and tumor virologist Renato Dulbecco who stayed home on that account. A year ago, Howard Temin was not nearly famous enough to compete with such luminaries, but today, because of what he had to say, things have changed.

Quite simply, he told his listeners that RNA can make DNA—that a Rous sarcoma virus with its genetic core of RNA can make a DNA copy of itself. The DNA copy then serves as the template for synthesis of a new virus, one in which the genetic information from the original RNA genome has been transcribed to DNA, which can be integrated into the chromosome of a virally infected host cell. This startling reverse transcription of genetic information, Temin stated, requires an enzyme called RNA-dependent DNA polymerase, and such an enzyme exists within the virion or core of the Rous sarcoma virus.

Temin reported experiments performed with his colleague, Satoshi Mizutani, in which Rous sarcoma virions from infected chicken cells were purified and deoxyribonucleotides, the component parts of DNA, were added to the viral preparation. If RNA were, in fact, acting as a template and if RNA-dependent DNA polymerase were present as postulated, the nucleotides would be assembled, forming strands of DNA. They were. Further indication that RNA was the template came from experiments showing that the reaction could be inhibited by preliminary incubation of the virions with ribonuclease, an enzyme that destroys RNA. These two experiments furnished presumptive evidence of the existence of RNA-dependent DNA polymerase. Within weeks, other laboratories confirmed the findings.

The discovery of this enzyme, which opened to scrutiny another channel for the flow of genetic information, sparked a burst of activity among investigators who will be able to approach some of the central questions in biology with a new perspective. Indeed, the discovery of an RNA-dependent DNA polymerase provides, in theory, (i) a missing measure of plausibility to existing concepts of viruses as being at the root of all cancer, (ii) a new angle for studying immunological memory and the transfer of information from cell to cell, and (iii) the thought that reverse transcription, as the phenomenon came to be called, may be the key to differentiation in embryonic tissue. Conceivably, the implications of reverse transcription could be central to questions of general control mechanisms in mammalian cells and could be an important tool for unraveling the intricacies of cellular regulation.

Nearly all investigators in the field are aware that, in spite of the excitement that has surrounded this discovery and in spite of the volume of data published within the last year, work on RNA-dependent DNA polymerase is not absolutely definitive, and that extrapolation from existing data to sweeping generalizations is perilous.

What Howard Temin said in Houston—that RNA *can* make DNA—was not new. As early as 1964 he linked RNA-dependent DNA polymerase to the malignant transformation of cells. But Temin and the few others who believed in reverse transcription 7 years ago did not have sufficient data to support this somewhat unorthodox notion, and it was generally dismissed. At the Houston meeting, his evidence for RNA-dependent DNA polymerase came from experiments in biology, and there were those who waited for biochemical confirmation of what Temin proposed.

Though it was not generally known at the time of the cancer congress, Temin was not the only investigator who had discovered the enzyme for reverse transcription. Quite independently, David Baltimore of the Massachusetts Institute of Technology had detected RNA-dependent DNA polymerase activity in preparations of Rous sarcoma virus and Rauscher mouse leukemia virus. Unlike Temin, who was deliberately looking for the enzyme, Baltimore came upon it in the course of experiments designed to test the hypothesis that all big, bulky RNA viruses have their own enzyme systems. Both men published their findings in June in *Nature,* and within weeks their results were duplicated and extended by several other teams.

When first discovered, it looked as if the RNA-dependent DNA polymerase was the exclusive property of tumor viruses, and that it might, therefore, be central to cancer. Its existence lent credence to the oncogene theory in which National Cancer Institute investigators Robert Huebner and George Todaro postulate that a cancer gene, derived from a C-type RNA virus particle, is present in all cells, transmitted vertically from mother to child and cell to cell, like all other genetic information. Likewise, the existence of the enzyme fits Temin's own cancer theory that a DNA provirus derived from an RNA viral template is essential for the incorporation of malignant information in the host cell genome. The essential difference between the two is that Huebner suggests that a total gene package for cancer is present in every cell, while Temin suggests that only bits of information are present initially and that new gene synthesis occurs as the process of malignant transformation is activated. The thought that cancer control might be achieved through RNA-dependent enzyme inhibition took shape, and several groups of investigators began a series of experiments to test that possibility, after first confirming that the enzyme really did exist.

By July quite a bit of pertinent data had been collected and was published in September in *Nature.* Sol Spiegelman and his colleagues at Columbia University showed that the new DNA

was complementary to the viral RNA —substantial proof that the RNA is actually the template—and that, in addition to having RNA-dependent DNA polymerase activity, six oncogenic animal viruses also have DNA-dependent DNA polymerase activity, an indication that transcription is at least a two-stage process. Maurice Green and his co-workers at St. Louis University School of Medicine also reported confirmation of Temin's and Baltimore's work when they showed, from experiments with mouse sarcoma and leukemia viruses, that viral RNA-DNA hybrids are formed in an intermediate step in the process of viral replication. Hence, the DNA is complementary to the RNA.

From the University of California, at San Francisco, Warren Levinson and co-workers said that they too had confirmed the existence of the enzyme, adding that they had also determined the presence of minute quantities of DNA particles within the Rous sarcoma virion. This observation is considered to be at the heart of one of the major, and unresolved, issues in the field at the present time.

Also reporting on Rous sarcoma studies, G. S. Martin from Berkeley described a temperature-sensitive RNA tumor virus mutant whose properties indicate that the viral RNA genome is required for the transformation and maintenance of a normal cell into a malignant one. Similar findings have been reported by Peter Vogt, of the University of Washington. Again, this was an important observation that is fundamental to yet unanswered questions about the biological role of RNA-dependent DNA polymerase—questions which may rely on further studies of enzyme-deficient mutants for answers.

The majority of molecular biologists have generally been propelled by the "central dogma," as propounded by Watson and Crick, namely that the flow of genetic information is undirectional, from DNA to RNA to protein. Therefore, the notion of information traveling in the opposite direction took some getting used to. Chemically, however, the idea of reverse transcription is not considered to be too revolutionary. After all, other deviations from the standard pattern were quite acceptable: single- and double-stranded RNA's and DNA's, RNA-DNA hybrids, and RNA-RNA polymerases all represent variations on the theme of the DNA double helix.

In August, Crick published a paper in *Nature* in which he explained RNA-dependent DNA polymerase in the framework of the "central dogma." He declared that the "central dogma" allows for all types of information transfer except those initiated by protein. The "dogma," as set down in 1958, he said, postulates three classes of genetic information transfer. The first includes "general transfers," those which can occur in all cells. The obvious cases are

$$\text{DNA} \rightarrow \text{DNA}$$
$$\text{DNA} \rightarrow \text{RNA}$$
$$\text{RNA} \rightarrow \text{protein}$$

Then there are "special transfers" that may occur in special circumstances. Possible candidates are

$$\text{RNA} \rightarrow \text{RNA}$$
$$\text{RNA} \rightarrow \text{DNA}$$
$$\text{DNA} \rightarrow \text{protein}$$

The third class refers to those transfers which the dogma holds can never occur —anything beginning with protein. "Stated this way," Crick observed, "it is clear that the special transfers are those about which there is the most uncertainty. It might indeed have profound implications for molecular biology if any of these special transfers could be shown to be general, or—if not in all cells—at least widely distributed."

By now there is preliminary evidence that reverse transcription may, indeed, be "widely distributed," at least in proliferating tissue, but there is no real confirmation of the point because there is no absolute proof that the enzyme which has been detected in tumor viruses is the same as that which has been demonstrated in other cells. At present, largely technical problems appear to stand in the way of a resolution.

Spiegelman demonstrated that a synthetic RNA template is as much as a hundredfold more potent at stimulating an enzyme reaction than are natural templates; a number of experimental results, by Spiegelman and others, are based on evidence of RNA-dependent DNA polymerase activity gained with use of this synthetic polymer. However, one cannot state with certainty that an enzyme detected in such experiments is identical, or even *virtually* identical, to the viral enzyme.

Last November, at the second Lepetit Colloquium at the Pasteur Institute in Paris, Dr. Robert C. Gallo of the National Cancer Institute reported evidence of RNA-dependent DNA polymerase activity in human leukemia cells. His data were derived from analyses with both natural and synthetic RNA templates. Green and Spiegelman had reported similar data. Subsequently, Spiegelman announced detection of an enzyme activity in more than 100 leukemias and solid human tumors, in human and animal embryonic tissue, and in regenerating liver. Because he was using synthetic templates and only crude extracts of tissue, correlations of these findings to the enzyme are risky. Nonetheless, there is evidence of a relation between an RNA-dependent enzyme activity and proliferating tissue.

What remained to be seen was whether the enzyme activity could be found in normal adult cells. In January, George Todaro and his colleagues at the Cancer Institute suggested that it could. Using a synthetic template for the detection of RNA-dependent DNA polymerase activity, they identified activity in normal mouse cells established in tissue culture and human diploid fibroblasts from normal individuals also established in culture. Partially purified polymerases from both sources, they said, have properties that are similar to those of the mouse leukemia virus enzyme though, quantitatively, activity is higher in cancerous than in normal tissue.

Though Todaro's discovery of enzyme activity in normal cells is not as shattering to previous notions as some thought, it is also not as clear-cut as it was initially presumed to be. Apparently, the synthetic template is far less specific than previously believed and in certain circumstances it will pick up activity from polymerases other than the RNA-dependent enzyme. Thus, many molecular biologists believe that the full significance of Todaro's January experiments remains to be determined.

Because the best available data shows high correlations between the presence of RNA-dependent enzyme and cancer cells, considerable effort is being expended in a search for drugs which selectively inhibit its activity. Gallo, Green, Spiegelman, Todaro, Nobel Laureate Melvin Calvin, and others have been screening various rifampicin derivatives (this antibiotic itself is ineffective against the enzyme) and have turned up about a dozen candidates. Those that show significant inhibition without the required high specificity will be turned over to the cancer

chemotherapy program at NIH on the chance that they may prove as useful as some existing therapies. And meanwhile, the search for selective inhibitors will continue, not only among the rifampicins but among other compounds as well. Researchers from Johns Hopkins and the Upjohn Company have reported promising leads from studies of the streptovaricins, structurally similar to the rifampicins.

One argument against the possibility that such agents could be effective against cancer is that once transformation takes place, RNA-dependent enzyme inhibition would be useless. Even if this is true, Gallo postulates a role for enzyme inhibitors in prophylaxis—in preventing what he suspects are second or third malignant transformations in patients who have had cancer but who have been free of disease for a year or more. In effect, he takes issue with the supposition that cancer recurs because the last malignant cell was never destroyed but lived to propagate. While this may sometimes be the case some scientists think that some cancers recur because of some factor inherent in the patient (RNA tumor virus information, for example). If this is the case, inhibition of the process of reverse transcription by rifampicins or other drugs could block the lethal step of cell transformation.

Spiegelman is among those who foresee the use of assays for activity resembling that of RNA-dependent DNA polymerase in the diagnosis of malignant disease and in the prediction of the onset of relapse well in advance of its occurrence. In a double-blind experiment with RNA-DNA hybrids, Spiegelman's group diagnosed 24 of 25 cases of cancer and corrected, in one case, a false diagnosis of leukemia. The ability to predict a relapse by detecting a reappearance of enzyme activity in blood (it is not detectable in persons in remission) offers obvious clinical advantages.

Though the discovery of RNA-dependent DNA polymerase is but a year old this month, it has precipitated considerable activity in that time. The biology of the 1950's and 1960's was inspired by molecular biology and rooted firmly in careful exploration of bacterial systems, on the theory that "what is true of the bacterium is true of the elephant." For more than a decade, unraveling the structure and alphabet of genetic molecules has been among the foremost of scientific pursuits. Indeed, the language of life was established with the cracking of the genetic code, and experiments with *Escherichia coli* have been of inestimable value in revealing that language. Now, however, there is increasing interest in exploring the differences between bacterial cells and complex mammalian cells. Reverse transcription nows offers an approach to problems of control and regulation in mammalian cells. For example, RNA to DNA transcription *could* be more than a "special transfer"; it may function generally in cellular control.

Other Applications

Work with viral systems has revealed new complexities of the process. Not only do RNA tumor viruses, until now thought to consist only of a core of nucleic acid enveloped by a protein coat, contain RNA-dependent DNA polymerase, but they also contain other enzymes. Spiegelman, Temin, and others have demonstrated a DNA-dependent DNA polymerase activity; and Temin has evidence of an endonuclease and a ligase or joining enzyme in preparations of RNA tumor virus. Hence, it appears that there is not just an enzyme in the virion but an enzyme system. In the mid-1960's vaccinia and reo-viruses were also shown to contain certain types of polymerases.

Hypotheses being drawn from reverse transcription are based not only on enzyme activity in tumor viruses and malignant cells, but also in embryonic tissue; and there is speculation that the bursts of DNA synthesis observed in developing embryo cells at certain stages of growth may correlate with peaks of activity of reverse transcription enzymes, which somehow may be the key to cellular control and the cancer problem. Malignant tissue, like embryonic, is rapidly proliferating. Immunologically, ties between the two are presumed from evidence that certain antigens are common to both but absent in normal adult cells. Cancer cells may, therefore, be derepressed, they may have reverted to what amounts to an embryonic state, and RNA-dependent DNA polymerase activity may be central to both.

Technically speaking, one must say that this special enzyme, RNA-dependent DNA polymerase, has not been found at all—there is merely evidence of its presence through its biological activity. The enzyme itself still has to be characterized with precision. Comparative analyses—biological, chemical, and physical—of the enzyme from tumor viruses, mouse cells, human cancers, and other types of tissue will have to be performed to support judgments based on presumed identity—or, at least, strong similarity. Also, investigators want to find evidence of the enzyme's activity in vivo.

Central to many questions concerning the enzyme is the controversy that surrounds viral transcription. Spiegelman maintains that the viral enzyme can function with only a single-stranded RNA, but that the cellular enzyme functions much more efficiently with an RNA-DNA hybrid and may be unable to use single-stranded RNA at all. However, Gallo and others point out that cellular enzymes just may not have been sufficiently purified to show such a reaction. (Even trace amounts of ribonuclease, when they contaminate a polymerase preparation from human leukemia cells, would quickly destroy any single-stranded RNA molecules in the neighborhood, and it is more difficult to eliminate ribonuclease from cellular enzyme than from viral enzymes. Also central to the on-going debate is whether the viral RNA-dependent DNA polymerase (or the cellular, for that matter) can initiate DNA synthesis in the process of reverse transcription without the aid of a primer. Known polymerases (enzymes that catalyze nucleic acid synthesis) need just a bit of DNA—not more than a few molecules—to get the reaction going. In fact, some think that RNA-dependent DNA polymerase cannot function without a primer and is, therefore, no different from any other polymerase. As J. Hurwitz of Albert Einstein Medical School puts it, "I've seen no evidence that RNA-dependent DNA polymerase is not a typical enzyme. Biologically, it is obviously very important because it tells you that there are systems which require RNA as an intermediate in nucleic acid synthesis."

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