Rous Sarcoma Virus: A New Role for Transfer RNA

It is not uncommon for viruses to pirate fragments of cellular material for their own purposes. Generally the pirated material is a glycoprotein or glycolipid, substances used to maintain the superstructure of the virus. Certain RNA tumor viruses, for example, adapt glycolipids from the external membrane of a host cell to form an envelope around the viral core. Other viruses make similar use of cellular structural components.

But new evidence presented last month in Atlantic City at the national meeting of the American Chemical Society suggests that at least one virus may utilize cellular materials in an unusual fashion more intimately related to viral function. James E. Dahlberg of the University of Wisconsin Medical Center, Madison, disclosed that the Rous sarcoma virus, a type C RNA virus, and the cause of certain sarcoma-like tumors in chickens, appears to use a cellular transfer RNA as a primer for its own RNA-directed DNA polymerase (reverse transcriptase), an enzyme that is crucial to both replication of the virus and transformation of healthy cells to malignant ones.

The Rous sarcoma virus was the first virus shown to cause tumors in any species and is typical of the many other RNA viruses that have since been shown to be tumorigenic. Reverse transcriptase uses the RNA genome of the virus as a template to produce a DNA copy, called the provirus. The provirus can then serve as a template for the production of more virus particles or it can be integrated into the DNA genome of the host cell. This integration, many scientists now think, is essential if the virus is to transform the host cell. All known RNA viruses that cause tumors contain a reverse transcriptase, but not all RNA viruses that contain reverse transcriptase are capable of causing tumors.

David Baltimore of the Massachusetts Institute of Technology, Cambridge—who discovered reverse transcriptase simultaneously with Howard Temin of the McArdle Laboratory for Cancer Research, Madison—had earlier shown that this enzyme required an RNA primer to begin synthesis of DNA. In in vitro experiments with the enzyme, most investigators have used a synthetic oligonucleotide composed entirely of deoxythymidylic acid [oligo(dT)] as a primer. The priming activity of oligo(dT) was once thought to be specific for reverse transcriptase, and its use as a probe was responsible for the many fallacious reports of reverse transcriptase activity in cells not infected by RNA tumor viruses. The polynucleotide sequenced by Dahlberg is the first naturally occurring primer that has been identified.

The suspected primer is one of several RNA polynucleotides closely associated with the RNA genome of Rous sarcoma virus particles grown in chick embryo cells. In collaboration with J. Michael Bishop of the University of California Medical Center, San Francisco, and Anthony J. Faras of the University of Michigan, Dahlberg showed that it is the last polynucleotide dissociated from the viral genome as the temperature is slowly raised up to 69°C, and that template primer activity is halted when it becomes dissociated. They also found that labeled nucleotides used as substrates for the reverse transcriptase become covalently bonded to the suspected primer. These facts led them to conclude that the polynucleotide is, in fact, the primer, at least for reactions in vitro.

Dahlberg and his associates Robert C. Sawyer, Fumio Harada, and Toshimichi Ikemura then determined the sequence of the 75 nucleotides that make up the primer (Fig. 1). They found that the molecule contains four sets of homologous sequences that are bonded by Watson-Crick base pairing to fold the molecule into the cloverleaf shape characteristic of transfer RNA's, molecules that carry amino acids for protein synthesis. At the appropriate site on the folded polynucleotide, they observed the anticodon C_mCA (2′-methylcytosine-cytosine-adenosine), which is known to be specific for the amino acid tryptophan. They then tested the primer under standard conditions for linking transfer RNA's to amino acids and found that it would indeed accept only tryptophan. (Bishop's group performed the same test and found that the primer would accept only methionine. This difference, which is believed to be due to differences in experimental techniques, has not yet been explained.)

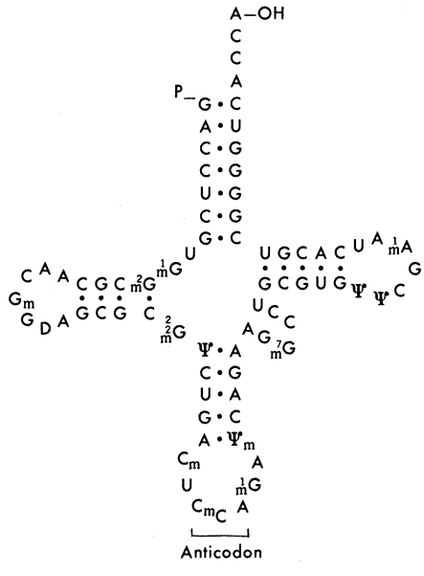

Fig. 1. The sequence of the reverse transcriptase primer as determined by J. E. Dahlberg. [Courtesy of Dr. Dahlberg]

Dahlberg further found that the primer produces a characteristic pattern of oligonucleotides when it is digested with the enzyme ribonuclease T1 and then subjected to two-dimensional paper electrophoresis. He then searched for and found polynucleotides with identical patterns in chicken cells, other avian cells, rat cells, and human cells. It seems likely that these polynucleotides are the tryptophan-specific transfer RNA used by the cells.

Several important questions remain to be answered before the full importance of Dahlberg's discovery can be assessed. The most crucial of these is whether the tryptophan-specific transfer RNA serves as a primer for reverse transcriptase in infected cells because all of the experiments were performed in vitro and it is known that RNA tumor viruses capture many pieces of apparently extraneous cellular RNA when they are encapsulated. Beyond that, there is yet no evidence to suggest how the primer binds to the RNA genome, exactly how it functions, and what its fate is after the synthesis of the provirus. But if it is confirmed that the in vitro primer performs that function in infected cells, Dahlberg's results will provide another strong piece of evidence to support the hypothesis that RNA tumor viruses were originally derived from cellular components.

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