The RNA Tumor Viruses—Background and Foreground

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ABSTRACT The members of the RNA tumor virus (or leukovirus) group of animal viruses replicate via a DNA intermediate and transmit their information stably in cells as DNA. Although some of these viruses are capable of inducing neoplastic transformation, others are not. These viruses may be related to spontaneous neoplasia, but the relationship is that of analogy rather than etiology. The relationship of these viruses to human disease is unknown.

Two models of spontaneous neoplasia that involve either activation of the information of a transforming RNA tumor virus or the creation of information for neoplastic transformation by recombination involving information transfer from RNA to DNA are being tested in model systems.

In this paper, I shall discuss the known properties of RNA tumor viruses and some of the problems requiring further study. RNA tumor virus is the name of a group of animal viruses, also called leukoviruses, that were discovered in 1908 by Ellerman and Bang (who found a virus causing leukemia in chickens) and in 1911 by Peyton Rous (who found a virus causing a sarcoma in chickens). Leukoviruses are widely distributed in nature. They are associated with tumors in various animals, not yet shown to include man, and are also very widely distributed in normal chickens and mice. A virion of a leukovirus is defined as being medium sized (80–100 nm), with an envelope containing a nucleocapsid with no clearly observable symmetry and single-stranded RNA of 60–70S size. Additional characteristics are: the replication of the virus requires early DNA synthesis and is sensitive to actinomycin D, the sedimentation coefficient of the RNA changes to about 35S after a brief treatment with denaturing conditions, and the virion contains a DNA polymerase. [I have recently written a review of these viruses, which contains references to most work published before 1971 (1).] The virions of the RNA tumor viruses are somewhat similar to the virions of myxoviruses. Myxovirus virions are also medium sized, enveloped, and contain single-stranded RNA. However, the nucleocapsids of myxoviruses are clearly of helical symmetry, and the RNA is either about 55S (paramyxoviruses or myxovirus II) or disperse (orthomyxovirus or myxovirus I).

Replication of RNA tumor viruses

The replication of the classical RNA tumor viruses does not lead to cell death, as does the replication of most other animal viruses. Instead, the infected cell divides. After infection by the prototype RNA tumor virus, Rous sarcoma virus, all infected cells appear to become transformed into neoplastic cells. These cells may or may not produce virus. However, both in cells producing virus and in cells not producing virus, the information of the virus is transmitted to daughter cells at mitosis. The structure containing the viral information is called the provirus. In rat cells infected with Rous sarcoma virus, there is no production of virus, and virus production cannot be induced by superinfection with another virus. However, the entire viral genome remains in the infected cell, and infectious virus appears after fusion of the cell with chicken cells. [As will be discussed later, it appears that some abnormality in viral RNA synthesis in the infected rat cells prevents virus production (2)].

Much of the recent excitement involving RNA tumor viruses relates to the novel mode of information transfer that appears to be a part of their replication. A model for this replication is summarized in Fig. 1. Information is transferred from viral RNA to DNA, the provirus. This DNA is then a template for the production of progeny viral RNA. When an infected cell divides, the DNA provirus also divides. This division explains the maintenance of viral information in dividing, infected cells. The evidence for the existence of the DNA provirus is still indirect, and is also summarized in Fig. 1. Inhibitor experiments show that virus production is blocked by actinomycin D, an inhibitor of DNA-directed RNA synthesis, and that infection is prevented by inhibitors of DNA synthesis, but not by inhibitors of protein synthesis. After a provirus has been formed, further DNA synthesis is not required for virus production. The most convincing evidence for the physical existence of the DNA provirus are experiments, reported by Boettiger and Temin (3) and by Balduski and Morgan (4), indicating that in cultures of stationary cells a provirus can be labeled with bromodeoxyuridine and thereby rendered sensitive to inactivation with visible light. Under such conditions, cell DNA is not labeled with the bromodeoxyuridine. Therefore,
irradiation with light does not kill the cells, but it does prevent the infection. In Boettiger’s experiments (3), it was further shown that the shape of the killing curve depended upon the initial multiplicity of infection, indicating that the input virus controls the number of DNA proviruses.

Because of the failure of inhibitors of protein synthesis to block DNA provirus formation, we looked for and found RNA-directed DNA polymerase activity in virions of Rous sarcoma virus (5). Independently, Baltimore found a similar polymerase activity in virions of a murine leukemia virus (6). The DNA polymerase of the leukovirus virion requires for full activity a detergent or some other means of disruption of the virion, a divalent cation (such as magnesium), four deoxyribonucleoside triphosphates, and a buffer. The polymerase activity is sensitive to treatment with ribonuclease, indicating that the template is RNA. The polymerase is located in the virion core (7, 8). The product of the polymerase reaction is DNA, as shown by its chemical and physical behavior. This DNA product is complementary to viral RNA, as shown by nucleic acid hybridization experiments (9, 10). Polynucleotide ligase and other nucleic acid-related enzymes are also present in the virion (11, 12). These results suggest that the virion transfers information from RNA to integrated DNA (13).

After exposure of cells to Rous sarcoma virus, some cellular processes are needed before virus production begins (Fig. 2). If the cells are in the G1-b phase of the cell cycle (14), exposure to virus will not lead to virus production or transformation. If serum is added, so that the cells enter into mitosis, viral antigens appear in the cells, virus production starts, and the cells become transformed. This requirement for processes in the mitotic cell cycle has not been found for replication of most other groups of animal viruses.

One result of the activated infection is to transform infected cells into neoplastic cells, which differ in their morphology and growth properties from normal cells (see ref. 1 for references). This neoplastic transformation appears to be controlled by genes in the virus, as shown by morphological and temperature-sensitive mutants for this conversion (15–18).

One of the most striking aspects of this transformation is the difference in multiplication between transformed and normal cells. This difference in multiplication appears to be related to an increased efficiency of the transformed cells in the utilization of specific multiplication-stimulating factors in serum (19, 20). One of these factors has been purified about 7000-fold from calf serum (21) (Fig. 3).

Many of these features of the replication of Rous sarcoma virus, including such properties of the virion as the presence of a DNA polymerase and a DNA intermediate for replication, are shared by all the other members of the classical RNA tumor virus group. However, other leukoviruses differ from Rous sarcoma virus in the efficiency of transformation. For

![Fig. 2. Activation of Rous sarcoma virus synthesis. Stationary chicken cells were exposed to Schmidt–Ruppin strain of Rous sarcoma virus at a multiplicity of infection of 1 focus-forming unit/cell. At different times after infection, the cells were examined for the presence of avian tumor virus antigens by the use of fluorescent antibodies. At the same times, the medium was assayed for the amounts of virus present. At the times indicated by arrows, serum was added to some cultures to cause the cells to multiply (Humphries and Temin, unpublished).](image)

![Fig. 3. Tracing of gel electrophoregrams of (a) whole serum, (b) partially purified multiplication-stimulating activity, and (c) insulin (from ref. 21).](image)
instance, avian myeloblastosis virus does not usually transform fibroblasts, but can transform precursor cells from the reticuloendothelial system. Avian lymphoid leukemia virus does not transform any cells in culture; in animals, it causes tumors only at low frequencies and after long latent periods. Avian erythroblastosis strain R-associated virus does not seem to cause any tumors in infected animals (22). Therefore, one may speak of strongly transforming, weakly transforming, and nontransforming leukoviruses (13). The induced leukoviruses reported by Weiss et al. (23) and discussed by W. P. Rowe and G. J. Todaro (29, 31) in this symposium are probably other examples of nontransforming leukoviruses.

RNA-directed DNA polymerases

After a DNA polymerase was found in virions of Rous sarcoma virus and Rauscher murine leukemia virus, the virions of many other RNA viruses were examined for the presence of a DNA polymerase. RNA viruses containing a DNA polymerase in their virion can be separated into the classes listed in Table 1. Some of these viruses, like Rous sarcoma virus, are able to induce tumors; others are notable to induce tumors but have been isolated from tumors (many murine C-type viruses). Still others appear not to induce tumors and have not been isolated from tumors. This distribution suggests that the presence of a DNA polymerase in a virion of an RNA virus does not necessarily mean that the virus containing it causes neoplastic transformation. A virion DNA polymerase might have a selective advantage as a means for a nontransforming virus to establish a latent infection (24). The presence of a virion DNA polymerase in an RNA virus does not mean that the virus does not kill cells; both visna and the syncytium-forming viruses kill cells in their normal hosts.

After the finding of DNA polymerases in the virions of RNA tumor viruses, a search was started for related DNA polymerases in infected and in uninfected cells. This search was both for soluble enzymes and for particulate polymerases. The work with soluble enzymes is difficult to interpret because, as first noted by Lee-Huang and Cavaliere (25), all DNA polymerases appear to have the capacity to use RNA as a template. A virus polymerase has been separated from two cellular DNA polymerases in virus-producing cells from mice (26).

The work with particulate enzymes is also somewhat difficult to interpret. RNA-directed DNA polymerase systems that appear to be precursors of virions are found in infected, virus-producing cells (8). In infected cells that do not produce virus, such as rat cells infected by Rous sarcoma virus, an RNA-directed DNA polymerase system can also be isolated. This preparation shares certain properties with precursors of the Rous sarcoma virus virion core, but, by nucleic acid hybridization experiments, does not appear to use virion RNA as a template (2). There is some relationship between the RNA of these particles from rat cells infected with Rous sarcoma virus and RNA from uninfected rat cells, as shown by annealing experiments with the DNA product of the cellular RNA-directed DNA polymerase system. In addition, there is an RNase-sensitive DNA polymerase system in uninfected rat cells. This DNA polymerase system in normal rat cells is similar to a virion DNA polymerase system in its requirement for all four deoxyribonucleoside triphosphates, its sensitivity to ribonuclease, and its synthesis of a DNA product (2).

Unfortunately, it is not easy to interpret the RNA-directed DNA polymerase systems that are apparently not related to virus and are in uninfected or virus-infected cells. Two types of interpretation can be imagined. One is that these polymerase particles are protoviruses or other precursors of induced leukoviruses (27), the other is that they are some kind of artifact caused by the binding of a cellular DNA polymerase to RNA. The reason the latter hypothesis has been given some credence is that recent work [see Mizutani and Temin (12)] has shown that virions of RNA tumor viruses contain various enzymes, for example hexokinase, that do not appear to be related to viral replication, but may be bound to virion components. A similar binding might occur in cells and result in formation of a particulate RNA-directed DNA polymerase system.

**Etiology of "spontaneous" neoplasia**

How do these experimental results relate to the question of the etiology of human neoplasia? We do not know the etiology of most human neoplasias. A number of hypotheses can be proposed, some related to RNA viruses, and some not. Those hypotheses not related to RNA viruses include classical
genetic hypotheses, epigenetic hypotheses, and hypotheses involving DNA viruses. Those hypotheses related to RNA viruses include hypotheses of vertically transmitted virions requiring a sensitive target cell for neoplastic transformation, and the oncogene and protovirus hypotheses (Fig. 4).

For neoplastic transformation by Rous sarcoma virus, there must be formation in the cell, by means of RNA-directed DNA synthesis, of the genes for viral replication and of the genes for neoplastic transformation. The expression of these two sets of genes can be completely separate, as shown by virus-producing untransformed cells and by cells that do not produce virus. In the oncogene hypothesis, the entire information of a transforming leukovirus, including that for neoplastic transformation, is transmitted in the germ cells. In the protovirus hypothesis, only some information related to the information for neoplastic transformation is transmitted in the germ cells. The oncogene and protovirus hypotheses also differ in the nature of the changes that lead to neoplasia. In the oncogene hypothesis, the change is derepression of pre-existing information. In the protovirus hypothesis, the change is a series of successive RNA to DNA to RNA information transfers that lead to modification of the original information. This modification is usually for physiological processes, but the influence of carcinogenic agents can lead to misexpression and the production of the information for neoplastic transformation. (Other evolutions could lead to formation of leukoviruses.)

How does the work with leukoviruses relate to the testing of these hypotheses? A virus like Rous sarcoma virus seems to contain separate sets of genes for neoplastic transformation and for viral replication. Infection by Rous sarcoma virus leads to neoplastic transformation as a result of the formation in cells of the genes for viral replication and for neoplastic transformation. The processes that virologists know how to study are those related to viral replication. Therefore, most studies of models of the etiology of human neoplasia relate to viral replication and not to neoplastic transformation. However, we must remember that viral replication is not the same as neoplastic transformation. As later talks at this symposium will point out, there are many approaches: looking for viral nucleic acids, for viral-related enzymes, and for the appearance of viruses from normal cells. These approaches are important, but they are looking at viral replication and information for replication, rather than at information for neoplastic transformation.

Our experiments are subject to the same criticism. We have been studying two processes related to the start of viral replication. These involve the induction of virus production from rat cells infected and transformed by Rous sarcoma virus and the production of virus from stationary chicken cells. A rat cell infected with Rous sarcoma virus does not contain virus, nor precursors of the virus, but it does contain small amounts of the virion group-specific antigen (about 2% as much as is present in virus-producing chicken cells) and of virion nucleic acids. Quantitative study of the nucleic acids in these cells by the use of nucleic acid hybridization suggests that viral RNA is produced in much smaller amounts in these cells than in virus-producing chicken cells (2). Virus production after fusion can be explained by the passage from chicken cells to the rat cells of something that enables the rat cells to synthesize this missing class of RNA. In the case of activation of virus production from stationary infected chicken cells, there is some suggestion that there may be changes in transport of viral-specific RNA from the cell nucleus. Study of these two systems suggests that we need to understand more about the control of RNA synthesis in animal cells, especially whether the control of RNA synthesis by a provirus of an RNA tumor virus or by a related element is the same as that of other RNA synthesis. The other approach we have been taking is to search for protoviruses in uninfected cells (2).

It might be worth while to consider briefly the induction phenomenon discussed by Rowe and Todaro (23, 29–31). This phenomenon has several noteworthy features: the cells that are induced may not be immune to the induced virus, the induced cells are killed, and the frequency of induction seems to increase with long passage of cells, i.e., in cell lines. These features are more compatible with a genetic hypothesis of virus induction, like the protovirus hypothesis, than with a derepression hypothesis, like the oncogene hypothesis. However, since the virus induction does not lead to concomitant transformation, this phenomenon is only a model for neoplastic transformation.

We have been discussing the status of the scientific study of RNA tumor viruses. It is clear that much more work can be done and needs to be done before we understand the replication of these viruses and their relationship to human neoplasia. Now, we should also ask the question whether there should be an expansion of cancer research. I think the answer to this question depends upon the source of the money to support this expansion. If this money is taken from the defense budget, fine. An expansion of cancer research which causes a diminution of the money spent on defense would be a double benefit to our society. However, if the money for an expansion of cancer research comes at the expense of the vast domestic social needs that are tearing our society apart, I oppose an expansion of cancer research. Other problems of our society, I feel, are more important at present. Even in the field of biological research, work on the pollution of the biosphere and on population control is more important than an expansion of cancer research.

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