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Introduction

The question I am going to discuss is, "How is the genome of a normal cell changed into the genome of a cancer cell?" This question is distinct from the questions of how the biochemistry of a cancer cell differs from that of a normal cell or how the genes for cancer are expressed in a cancer cell. The question of the origin of cancer genes can be formalized as in Chart 1.

Before considering this question, I shall discuss some characteristics of ribodeoxyviruses, that is, viruses whose virions contain RNA and a DNA polymerase. I shall particularly deal with the two groups of avian ribodeoxyviruses, the avian leukosis-sarcoma viruses and the reticuloendotheliosis viruses. I shall then present our current ideas about the origin of these ribodeoxyviruses and indicate why I believe that this origin provides a model for the formation in normal cells of the genes for cancer.

Ribodeoxyviruses

Ribodeoxyvirus is the name of a large group of animal viruses (20). Virions of ribodeoxyviruses have a diameter of about 100 nm and consist of an envelope surrounding an internal core with no clearly observable symmetry. The envelope consists of a lipid bilayer and external glycoproteins. The core contains a ribonucleoprotein particle consisting of 60 to 70 S RNA, basic proteins, and a DNA polymerase.

The ribodeoxyvirus group includes viruses such as RSV,2 mouse mammary tumor virus, mouse leukemia virus, and visna virus. As will be discussed below for avian ribodeoxyviruses, the various ribodeoxyviruses differ very much in their effects on infected cells and organisms.

RSV

RSV is the prototype ribodeoxyvirus. It, as well as all of the other avian ribodeoxyviruses (7), have C-type morphology; that is, the central core is not eccentrically located. RSV is a member of the avian leukosis-sarcoma group of viruses as shown by the antigenicity of its core proteins (the group-specific basic proteins and the group-specific DNA polymerase) and by the nucleotide sequence of its RNA. It causes rapid appearance of sarcomas after injection into sensitive birds. In cell culture, it causes rapid cell transformation with alterations in cell morphology and cell multiplication. These alterations occur within 2 or 3 days and can involve all of the cells in a culture. The cell properties converted by infection with RSV include the serum requirement for cell multiplication, the rate

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2 The abbreviations used are: RSV, Rous sarcoma virus; RAV-O, Rous-associated virus-O.
of hyaluronic acid synthesis and the amount of the enzyme hyaluronic acid synthetase, the control of glycolysis, the rate of glucose transport, the concentration of cyclic 3′, 5′-AMP, the production of plasminogen activator, and some surface properties.

Variants of RSV exist that are altered in the type of neoplastic transformation that they cause, the morphology of the transformed cells, the temperature stability of the neoplastic transformation (mutants temperature sensitive for transformation), and the presence or absence of neoplastic transformation (id or NT mutants) (see review in Ref. 20). All of these genetically different viruses appear to have very similar virions. Therefore, the existence of these different virus variants indicates that the RSV genome consists of two parts: one part contains the genes specifying the virion proteins and, therefore, the virion structure; the other part contains the genes for neoplastic transformation (Chart 2).

These genes are RNA in RSV virions. When RSV causes cancer, these genes are transcribed into DNA and integrated with the cell DNA forming new DNA genes for neoplastic transformation, as diagrammed in Chart 3. This assertion, called the DNA provirus hypothesis, is supported by many lines of evidence. The most conclusive is the isolation from cells infected by RSV of infectious DNA containing the information for production of RSV and for neoplastic transformation. The infectious RSV DNA was first reported by Hill and Hillova (8) in 1971. We have confirmed and extended their results (Table 1). The data in this table indicate that DNA purified from either chicken or rat cells infected with RSV can cause the production of new virus and morphological transformation in recipient chicken cells. The amount of DNA required to cause transformation in one-half of the treated cultures was 0.1 μg. The infectious material was shown to be DNA by its sensitivity to treatment with DNase and its resistance to treatment with alkali, RNase, and Pronase.

This description makes it clear that RSV is extremely efficient at causing tumors and forming cancer genes. There is no progression in the sense used in cancer research in RSV-induced transformation of chicken cells. All of the cancer genes appear to be present soon after infection.

However, RSV is not a natural virus. It does not persist in nonlaboratory populations. RSV is a laboratory creature, passaged and preserved by virologists.

**RAV-O**

RAV-O is another avian leukosis virus. Its virions contain the same group-specific antigens and DNA polymerase as do RSV virions, and its RNA has nucleotide sequences homologous to most of the nucleotide sequences in Rous sarcoma and other avian leukosis virus RNA (13, 14). However, RAV-O causes no disease in infected chickens. Crittenden found no increase in the incidence of leukemia in chickens either naturally or artificially infected with RAV-O (L. B. Crittenden, personal communication).

RAV-O is a natural virus, in contrast to RSV which is a laboratory virus. However, RAV-O is maintained in chicken populations, not as a virus but as a provirus or cellular genes. The existence of RAV-O as cellular genes in chickens is most clearly shown by the experiments of Crittenden et al. (4) diagramed in Chart 4. Crittenden et al. showed that a cross of two chickens that did not produce RAV-O yielded progeny that could produce RAV-O. The two parental chickens did not produce RAV-O for different reasons. One did not carry the gene for RAV-O production (V⁻), while the other was resistant to the spread of RAV-O (tvb²).

While most of the nucleotide sequences of RAV-O RNA are the same as those of RSV RNA, some are different. Some nucleotide sequences in RSV RNA are not present in RAV-O RNA. This is most clearly established by experiments of Neiman et al. (14), who showed by competition hybridization that RSV RNA contained some nucleotide sequences that were not competed by RAV-O RNA.

The genes for RAV-O are present in some chicken cells as DNA. This can be shown by nucleic acid hybridization (10, 13) or more definitively by infectious DNA assay (Table 2). These experiments demonstrate that the RAV-O genome is present in cells as DNA and that the RAV-O genome does not contain genes for transformation, only the genes for virus production. Therefore, the RAV-O genome (Chart 5) is simpler than the RSV genome (Chart 2). The RAV-O genome contains genes for virion proteins, but it does not contain genes for transformation.

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_H. M. Temin_
visions of DNA in infected cells. virus. It was selected by passing virus from myeloblastosis, a myeloid leukemia of chickens. In cell culture, it contains the same core group-specific antigens and DNA embryonic reticuloendothelial and muscle cells (12). There transforming RSV, avian myeloblastosis virus is a laboratory resistance to Subgroup B avian leukosis-sarcoma viruses. avian leukosis-viruses. On injection into susceptible chickens, polyoma virus produces RAV-O;Cli, chicken cells not producing virus. nfect fibroblasts with virus production, but there is no transformation. However, it can cause rapid transformation of embryonic reticuloendothelial and muscle cells (12). Therefore, avian myeloblastosis virus is a cell-dependent, strongly transforming virus. Again, as in the case of the strongly transforming RSV, avian myeloblastosis virus is a laboratory virus. It was selected by passing virus from myeloblastosis in young chickens and selecting virus that caused rapid production of myeloblastosis (2).

Rous-associated virus-A is by the same criteria another avian leukemia virus. In animals, it causes some leukemia (chicken leukemia) with a low frequency. These leukemias appear only after a high rate of virus production for a long time, that is, after a long latent period. In cell culture, Rous-associated virus-A causes no transformation of fibroblasts or of reticuloendothelial cells. It is, therefore, a weakly transforming virus. Rous-associated virus-A is a natural virus. It is passed from infected hens to their eggs by infection by virions (5). The infected hens do not develop leukemia in all cases, and when they do it is after they have laid many infected eggs. Thus, Rous-associated virus-A persists in nonlaboratory populations of chickens.

Reticuloendotheliosis Viruses

Reticuloendotheliosis viruses form another group of avian ribodeoxyviruses. They share with the avian leukosis-sarcoma viruses the general characteristics of ribodeoxyviruses and even C-type morphology. They differ from the avian leukosis-sarcoma viruses in the nature of their envelope glycoproteins, their core basic proteins, and their DNA polymerase and in the nucleotide sequence of their 60 to 70 S RNA.

After injection into young fowl, reticuloendotheliosis viruses may cause rapid death as a result of an acute infection with necrosis of the spleen and liver, or reticuloendotheliosis, which is a proliferation of primitive mesenchymal elements (17). The acute disease is mimicked in cell culture of fibroblasts where these viruses cause rapid appearance of a strong cytopathic effect. A chronic infection without cell death or transformation is then established (23).

Like the avian leukemia viruses, the reticuloendotheliosis viruses replicate through a DNA intermediate. As shown by the data in Table 3, DNA isolated from cells infected with reticuloendotheliosis viruses can cause formation of plaque-forming virus in sensitive cells.

Nucleic acid hybridization is another way to demonstrate the DNA intermediate of reticuloendotheliosis viruses. Sixty S

Table 2
Infectious DNA for RAV-O

<table>
<thead>
<tr>
<th>Donor DNA</th>
<th>Virus Transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch(RSV)</td>
<td>0.1</td>
</tr>
<tr>
<td>Ch(RAV-O)</td>
<td>0.2 &gt;10</td>
</tr>
<tr>
<td>Ch</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

a Ch(RSV), chicken cells producing RSV; Ch(RAV-O), chicken cells producing RAV-O; Ch, chicken cells not producing virus.

Table 3
Infectious DNA for reticuloendotheliosis viruses

<table>
<thead>
<tr>
<th>Donor DNA</th>
<th>50% infectious dose (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch(TDSNV)</td>
<td>3 DAI</td>
</tr>
<tr>
<td>Ch(TDSNV)</td>
<td>14 DAI</td>
</tr>
<tr>
<td>Ch spleen(REV-T)</td>
<td>0.2</td>
</tr>
<tr>
<td>Ch(TDSNV)-DNase</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Ch(TDSNV)-RNase, low salt</td>
<td>0.1</td>
</tr>
<tr>
<td>Ch(TDSNV)-alkali</td>
<td>1.0</td>
</tr>
<tr>
<td>Ch(TDSNV)-1.71 g CsCl/cm</td>
<td>0.05</td>
</tr>
</tbody>
</table>

a Ch(TDSNV), chicken fibroblasts producing Trager duck spleen necrosis virus; DAI, days after infection; Ch spleen(REV-T), chicken spleen cells producing reticuloendotheliosis virus (strain T).
RNA was isolated from purified reticuloendotheliosis virus virions, labeled with $^{125}$I, and annealed to DNA from infected cells (Chart 6). Most of the RNA became RNase resistant, indicating that most of the nucleotide sequences of the RNA were present as DNA in infected cells.

RNA Viruses and Cancer

Our discussion of these avian RNA viruses with different degrees of pathogenesis allows us to formulate criteria for whether an RNA virus can modify a cell genome to cause neoplasia. As can be seen in Table 4, the viruses that cause neoplasia contain cancer genes. The more efficient the viruses are at causing neoplastic transformation, the more cancer genes they contain. The viruses that do not cause transformation, like RAV-O and Trager duck spleen necrosis virus, do not contain genes for cancer. Rous-associated virus-A, which has a low efficiency of causing cancer, apparently has only some of the genes for cancer (11, 14).

However, this formulation provides an answer only to a special case of the question posed in Chart 1. When the strongly transforming RNA viruses cause cancer, they do so as described for RSV in Chart 3, that is, by synthesizing in cells the genes for cancer coded in the viral genome. However, this formulation does not tell us how the genes for cancer are formed when there is no overt evidence of a strongly transforming RNA tumor virus.

Possible Mechanisms of Carcinogenesis

A number of hypotheses can be proposed to explain the creation of the genes for cancer in normal cells (Chart 7). [The infection mechanism discussed above (Chart 3) is the fourth one.] In all the hypotheses the cancer cells contain the genes for neoplastic transformation. The hypotheses differ as to whether or not these genes are present in normal cells and, therefore, the nature of the genetic changes in carcinogenesis.

In a mutation hypothesis, a normal cell does not contain genes for cancer. During carcinogenesis, these appear by mutation.

In a differentiation hypothesis, normal cells contain the genes for cancer in an inactive form. During carcinogenesis, these genes are activated so that their phenotypic expression leads to the formation of a cancer cell. The oncogene hypothesis is a special case of a differentiation hypothesis (6, 9). In the oncogene hypothesis, as in all other differentiation hypotheses, the genes for cancer are present in normal cells in an inactive form and become active during carcinogenesis. The

Chart 6. Hybridization of Trager duck spleen necrosis virus (TDSNV) $^{125}$I-labeled RNA to DNA’s from Trager duck spleen necrosis virus-infected and uninfected cells. TDSNV $^{125}$I-labeled RNA (3000 cpm) was hybridized to DNA from uninfected chickens (Ch DNA), an uninfected Pekin duck (P. Du DNA), and TDSNV-infected chicken cells [Ch/TDSNV DNA]. After different times of hybridization, the extent of hybridization was determined by RNase A and RNase T1 digestion. The RNase-resistant cpm (200 cpm) were subtracted before the calculation of percentage of hybridization. Data from paper of Kang and Temin (10).


Table 4

<table>
<thead>
<tr>
<th>Virus</th>
<th>Ability to cause neoplasia</th>
<th>Presence of cancer genes in virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Avian myeloblastosis virus</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Rous-associated virus-A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RAV-O</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trager duck spleen necrosis virus</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*a Hypothesized.

b Lai et al. (11); Neiman et al. (14).
oncogene hypothesis differs from other differentiation hypotheses in that the oncogene hypothesis proposes that the genes for cancer are those of a strongly transforming RNA tumor virus. Therefore, in the oncogene hypothesis these genes for cancer are associated with the genes for an RNA tumor virus virion.

The protovirus hypothesis is a special case of a mutation hypothesis (20). In the protovirus hypothesis, normal cells do not have the genes for neoplastic transformation. These genes appear by variational processes during carcinogenesis. The protovirus hypothesis differs from other mutation hypotheses in that the oncogene hypothesis proposes that the genetic variations leading to the formation of the genes for cancer occur in a part of the normal cell genome involved in DNA to RNA to DNA information transfer.

Some data can be secured relating to these hypotheses by asking what genes for cancer are present in normal cells. When DNA was extracted from normal cells, as it was extracted for the experiments described in Tables 1, 2, and 3, and added to other normal chicken cells, we found no transformation or virus production (Table 2). This experiment indicates that normal, nonvirus-producing chicken cells do not contain genes for neoplastic transformation. In addition, it shows that normal, nonvirus-producing chicken cells do not contain genes for a complete provirus.

Therefore, the genes for RAV-O in normal chickens discussed above (Chart 4) are for a defective or incomplete provirus of RAV-O. Some genetic change must occur for the cells to start producing RAV-O. This genetic change is reflected in the ability of the DNA from RAV-O-producing chicken cells to transfer the ability to produce RAV-O and the lack of ability of DNA from chicken cells not producing virus to transfer the ability to produce RAV-O.

These experiments are not consistent with simple differentiation hypotheses, including the oncogene hypothesis. These results, however, do not rule out more complex differentiation hypotheses in which there are several genes for cancer and these genes are present in normal cells in an unlinked form.

The Protovirus Hypothesis

Now, I shall consider the protovirus hypothesis for cancer in more detail. This hypothesis states that genes for neoplastic transformation arise in an organism as a result of misevolution of a normal system of DNA to RNA to DNA information transfer.

At present, there is no evidence directly testing this hypothesis. There is, however, evidence consistent with a protovirus hypothesis for the origin of ribodeoxyviruses. This hypothesis states that ribodeoxyviruses arise as a result of misevolution of normal cellular genes. The evidence comes from experiments involving nucleic acid hybridization and study of DNA polymerase relationships.

When nucleic acid hybridization experiments, like those described in Chart 6, were carried out between RNA's from a reticuloendotheliosis virus or from RAV-O and DNA from a variety of fowl, different extents of hybridization were found (Table 5). The distribution of sequences of RAV-O RNA in DNA paralleled the closeness of the relationship of the fowl to chickens. There was most hybridization to chicken DNA; less to pheasant, quail, and turkey DNA; and none to duck DNA.

Endogenous sequences of reticuloendotheliosis virus RNA in fowl DNA were found to the extent of about 10% in all of the gallinaceous birds, but none were found in duck DNA.

In addition to these nucleotide sequence homologies between virus RNA and cell DNA, there are serological relationships between the DNA polymerases of both reticuloendotheliosis and avian leukosis-sarcoma viruses and the DNA polymerases of normal cells. An example of these relationships is seen in the data in Chart 8, which indicate that reticuloendotheliosis virus DNA polymerases specifically bind antibody made to the chicken large DNA polymerase.

In addition to these relationships, there are a large number of other homologies between the genes of avian ribodeoxyviruses and chicken cells and the genes of mammalian ribodeoxyviruses and mammalian cells. (See Refs. 21 and 22 for a more complete discussion of these relationships.)

These data indicate that the avian ribodeoxyviruses are related to the class Aves, especially to the order Galliformes. The avian leukosis-sarcoma viruses apparently are an offshoot from the genus Gallus. However, it cannot yet be determined from which fowl the reticuloendotheliosis viruses evolved (21, 22).

To explain these relationships between ribodeoxyviruses and cells, I have postulated an evolution, described in Chart 9, in which successive DNA to RNA to DNA information

### Table 5

<table>
<thead>
<tr>
<th>Source of DNA</th>
<th>Chicken</th>
<th>Pheasant</th>
<th>Quail</th>
<th>Turkey</th>
<th>Duck</th>
<th>Calf thymus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trager duck spleen necrosis virus</td>
<td>10²</td>
<td>10</td>
<td>10</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td></td>
</tr>
<tr>
<td>RAV-O</td>
<td>55</td>
<td>20</td>
<td>15</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* RNA-²³⁸I hybridized at a Cₜ of 8 x 10⁴ moles·sec/liter. Only the saturation value is given.
H. M. Temin

Transfer system is involved in differentiation and in evolution (19, 21).

Summary and Conclusions

There are two groups of avian ribodeoxyviruses, the avian leukosis-sarcoma viruses and the reticuloendotheliosis viruses. Virions of these viruses contain RNA, and the viruses replicate through a DNA intermediate. There is wide variation from none to great among these viruses in their ability to cause

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Chart 9. The protovirus hypothesis for the origin of ribodeoxyviruses. Zig-zag line, DNA; straight line, RNA. The increasing thickness and curvature indicate the evolution of viral genes.

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Chart 10. The protovirus hypothesis for the origin of the genes for cancer. Zig-zag line, DNA; straight line, RNA. The increasing thickness and curvature indicate the evolution of cancer genes. Oval, cancer genes.

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Transfers lead to changes in the cell genome. These changes result in the accumulation and alteration of nucleic acid sequences until the genome of an infectious virus is produced. The virus then exists apart from the cell and can infect other organisms.

I postulate a similar process for the formation of the genes for cancer. As a result of misevolution or variation in successive DNA to RNA to DNA information transfers, the genes for cancer are produced (Chart 10).

Strongly transforming viruses, like RSV, arise as a result of recombination between the new genetic elements described in Charts 9 and 10 (1, 18). This recombination creates a virus genome containing genes for virions and for neoplastic transformation. The cancer genes exist before the cancer virus. The formation of Kirsten murine sarcoma virus may be an example of this process (16).

The cellular system of DNA to RNA to DNA information transfer does not exist to cause formation of ribodeoxyviruses or the genes for cancer. I have postulated that this information transfer system is involved in differentiation and in evolution (19, 21).

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Chart 8. Antibody-blocking activity of DNA polymerases for antibody against chicken large DNA polymerase (IgG-L). Antibody against chicken large DNA polymerase (4 μg) was incubated in 25 μl with the indicated amounts of reticuloendotheliosis virus (strain T) (REV-T), chicken embryo large (CH-L), Trager duck spleen necrosis virus (TDSNV), chicken embryo small (CH-S), rat liver large (RL-L), RSV-RAV-O, and Escherichia coli DNA polymerases. After 20 min, the mixtures were heated to inactivate the DNA polymerases. (The E. coli DNA polymerase had been previously inactivated.) Partially purified chicken embryo large DNA polymerase was added to the incubation mixtures. After 30 min of further incubation, the DNA polymerase activity remaining was assayed with activated calf thymus DNA as a template-primer. The DNA polymerase activity remaining in the sample incubated without antibody was set at 100%. Eighty % of the chicken embryo large DNA polymerase was neutralized by the 4 μg of antibody against chicken embryo large DNA polymerase and was called 0% blocking. The percentage of increase in activity after preincubation with the indicated DNA polymerases. Data from work of Mizutani and Temin (15). The apparent better blocking by reticuloendotheliosis virus (strain T) DNA polymerase than by the homologous chicken embryo large DNA polymerase is the result of the impurity of the chicken embryo large DNA polymerase preparation.

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Chart 10. The protovirus hypothesis for the origin of the genes for cancer. Zig-zag line, DNA; straight line, RNA. The increasing thickness and curvature indicate the evolution of cancer genes. Oval, cancer genes.
cancer. The viruses that cause cancer seem to have genes for cancer. Carcinogenesis by these strongly transforming viruses involves the formation in infected cells of genes for cancer copied from the viral genome. Normal chicken cells do not contain infectious DNA for transformation or for virus production. Therefore, there is not an inactive provirus of a strongly transforming virus in normal chicken cells.

The protovirus hypothesis for cancer states that the genes for neoplastic transformation arise in an organism as a result of misevolution of a normal system of DNA to RNA to DNA information transfer. The protovirus hypothesis for the origin of ribodeoxyviruses states that ribodeoxyviruses arise as a result of misevolution of normal cellular genes. There is evidence from experiments involving nucleic acid hybridization and study of DNA polymerase relationships for the protovirus hypothesis for the origin of ribodeoxyviruses.

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