Unique Aspects of the Interactions of Retroviruses with Vertebrate Cells: C. P. Rhoads Memorial Lecture

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Viruses have provided a means for scientists to study fundamental cellular mechanisms, such as DNA replication and gene regulation, in much more readily dissectable ways than by studying the much more complex cell. Time and again, this conceptual approach has proven itself. Thus, it was not without precedence that many scientists, in efforts to learn more about how normal cells become malignant, turned their attention over the past 2 decades to the study of tumor viruses. While years ago one could not predict how soon or how great the rewards of this approach would be, we are now seeing its fruits in studies that are ever more directly applicable to man.

The ability to quantitate the effects of viruses in tissue culture systems has been a crucial step in their subsequent analysis. In the late 1960s, we developed some continuous inbred mouse cell lines with properties that made them very useful for studying the actions of tumor viruses. BALB/3T3 and NIH/3T3 cells demonstrated what we then called "contact- or density-dependent" inhibition of growth. When tumor viruses were added to cultures of these cells, the tumor virus-infected cell was not killed but instead continued to proliferate, even when uninfected cells in the population ceased to divide. Virus-transformed colonies could, thus, be readily recognized and easily quantitated against the background of nontransformed cells. Such colonies, when picked and grown up to mass culture, made tumors, whereas the control cells were not malignant. These findings verified that the tissue culture systems provided a valid measure of the most important virus function, malignant transformation.

The viruses which we and others have been studying intensively over the past 10 or more years are called type C retroviruses. As shown in Table 1, they consist of two groups, chronic and acute transforming retroviruses. The chronic viruses, when inoculated in susceptible animals, cause tumors, mostly leukemias, but only after a prolonged latent period of several months. These viruses replicate in the absence of any apparent transforming effect on known assay cells in tissue culture. In contrast, acute transforming viruses induce tumors within a very short period of days to weeks. They cause a variety of tumors, including sarcomas, hematopoietic tumors, and even carcinomas. In tissue culture, these viruses generally induce foci of transformation in appropriate assay cells.

Over the past several years, we have learned a great deal about the structure of chronic leukemia viruses due in large part to advances of recombinant DNA techniques and nucleotide sequencing. The leukemia virus genome contains gag, pol, and env genes, which code for internal structural proteins, reverse transcriptase, and envelope proteins, respectively (6). The proviral genome also contains a repeat sequence of any-

where from 300 to 600 bases at either terminus of the viral genome. These long terminal repeats contain signals for the initiation and termination of transcription and resemble prokaryotic transposable elements (12, 31). The chronic leukemia virus genome does not appear to possess an additional discrete transforming gene. While the mechanism by which these viruses induce leukemia is still uncertain, there are a number of attractive hypotheses which are presently being actively studied. Moreover, Gallo and coworkers (22) have provided recent evidence that one such virus may be etiologically involved in a particular form of adult human T-cell leukemia and lymphoma.

One of the outcomes of the development of continuous mouse cell lines was the finding that viruses closely related to chronic leukemia viruses could be induced from such cells spontaneously (1) or following treatment with certain chemicals (4, 18). Viral antigens could also be detected in non-virus-producing cells, and it was possible to detect multiple copies of sequences related to these viruses in normal cell DNA (for review, see Ref. 3). We know today that endogenous viruses are present in many vertebrate species. With recent advances in DNA technology, there is now even clear evidence that endogenous viral sequences are present within the germ line of humans (8, 19). However, we do not know as yet the functions of these endogenous viruses or the selective pressures that have led to their persistence in the vertebrate cell genome.

I would next like to discuss acute transforming viruses for which the mechanisms of action are somewhat better understood and which may be more immediately relevant to understanding of naturally occurring cancers. As with so many other biological investigations, our ability to learn about these viruses has been immeasurably aided by the development and application of modern molecular biological techniques.

It is apparent from observation of the physical maps (Chart 1) of 3 mouse-derived acute transforming retroviruses molecularly cloned in our laboratory why years ago we initially found that these viruses were capable of transformation but were replication defective (2). In each case, the genome of the acute transforming virus is smaller than that of the chronic leukemia virus. In addition, each has substituted a discrete segment of information. Thus, each of these viruses lacks essential leukemia virus information required for its replication. Of particular importance with respect to cancer research are the discrete segments of these viruses unrelated to the leukemia virus genome. In the viruses shown, each of these segments differs from the other; however, when probes are prepared from these segments, they each detect in normal mouse cell DNA not multiple, but one or at most a few copies of related sequences. Similar findings by a number of laboratories have led to the understanding that acute transforming retroviruses have arisen in nature by recombination of leukemia viruses with cellular genes.
Table 1

<table>
<thead>
<tr>
<th>Properties</th>
<th>Chronic leukemia viruses</th>
<th>Acute transforming viruses</th>
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<tr>
<td>I. Induce</td>
<td>Lymphomas</td>
<td>Sarcomas, hematopoietic tumors, or carcinomas</td>
</tr>
<tr>
<td>II. Tumor latent period</td>
<td>Relatively long</td>
<td>Relatively short</td>
</tr>
<tr>
<td>III. Transform in culture</td>
<td>No direct assay</td>
<td>Fibroblasts and/or hematopoietic cells</td>
</tr>
<tr>
<td>IV. Replication competent</td>
<td>Yes</td>
<td>Generally not</td>
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Chart 1. Relationship of 3 murine transforming retroviral genomes to the chronic leukemia virus genome. The onc gene of each retrovirus is indicated by a differently shaded box to denote the different cellular origin of each. Each of the viral genomes was cloned and physically characterized as reported previously (5, 32, 34). M-, Moloney; A-, Abelson; kbp, kilobase pairs; LTR, long terminal repeats.

We know that their cell-derived sequences, designated onc genes, are required for viral transforming functions. Moreover, the cellular genes from which they have arisen are well conserved in evolution, suggesting that these genes may possess important biological functions in normal cells. Of the more than 2 dozen acute transforming viruses thus far studied, independent isolates, in some cases from the same species but also from different species, have been shown to contain highly related onc genes. Thus, acute transforming viruses have incorporated from a potential battery of many thousand cellular genes only a very few. These findings strongly argue that the number of cellular genes that can be altered or activated to become transforming genes when incorporated within the retrovirus genome is not unlimited.

All mammalian acute transforming retroviruses transform mouse fibroblast cells in culture. However, they can exhibit rather extraordinary specificity in their transforming actions for other cells. We have recently shown that BALB and Harvey MSVs, which induce sarcomas and erythroleukemias in mice or rats, also cause transformation of a particular lymphoid precursor cell in vivo (21). Using an assay developed by Rosenberg et al. (27), for study of hematopoietic cells transformed by Abelson MuLV (27), we detected growth of colonies in mouse bone marrow cultures infected with BALB or Harvey MSV (Fig. 1A). Analysis of colonies induced by these viruses revealed that they were composed of lymphoid blast cells (Fig. 1B). These colonies could be propagated as continuous lines in tissue culture and were malignant when inoculated into mice. Unlike Abelson MuLV pre-B-lymphoid cell transformants, these cells lacked Fc receptors or the ability to express immunoglobulin heavy chain, characteristics of pre-B-lymphoid cells.

Addition, BALB and Harvey MSV hematopoietic cell transformants expressed high levels of terminal deoxynucleotidyl transferase, an enzyme associated with cells early in the lymphoid differentiation pathway (7). These markers readily distinguish the BALB or Harvey MSV early lymphoid transformants from those prelymphoid transformants induced by Abelson MuLV under identical conditions (21). These results illustrate the exquisite specificity that different onc genes can have for transformation of cells even within the same hematopoietic lineage.

Retroviral onc genes appear to act by means of their translational products. In some cases, it has been possible to detect such proteins by means of antisera prepared in animals bearing tumors induced by these viruses. With Rous sarcoma virus, the pioneering studies of Erickson and others have shown that the Rous sarcoma virus src gene product is a protein kinase (9, 17) with rather unique specificity for phosphorylating tyrosine residues (15). We have utilized another strategy to obtain antisera capable of recognizing onc gene products. Due to the advances of recombinant DNA and nucleotide sequencing, it is possible to prepare antisera to peptides synthesized on the basis of a known nucleotide sequence. By such an approach, it is hoped that the antiserum will be capable of recognizing the entire translational product of the gene in question.

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Fig. 1. Morphology of BALB-MSV-induced hematopoietic colonies. A, colony 4 days postinfection; B, Wright-Giemsa-stained preparations of cells from BALB-MSV colonies.

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2 The abbreviations used are: MSV, murine sarcoma virus; MuLV, murine leukemia virus; SSV, simian sarcoma virus.
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The SSV sis gene, as well as several other onc genes sequenced by scientists in our laboratory, contain open reading frames that initiate in helper viral sequences within a few bases to the left of their cell-derived onc gene and continue for several hundred nucleotides into the onc genes themselves (11, 25). A peptide composed of 15 amino acids from the NH2-terminal region of the predicted sis protein was used to immunize rabbits. As shown in Fig. 2, this antiserum detected a M, 28,000 protein in SSV-transformed cells that could not be precipitated with preimmune serum or detected by the antiserum in uninfected cells. Moreover, precipitation of this protein could be completely inhibited by preincubation with the peptide (Fig. 2c) (26). This protein corresponds in size to that predicted for the SSV-transforming protein from our sequence studies (11), further indicating that we have indeed identified the SSV-transforming protein in SSV-transformed cells. Hopefully, this approach not only will be useful in characterizing the sis gene product but will also help in identifying and characterizing the onc gene products of other acute transforming retroviruses.

Finally, I would like to discuss what we know about the possible involvement of genes related to retroviral onc genes in human cancer. These genes are present within human DNA, and we have now mapped several to specific human chromosomes (23, 33). We have also demonstrated the presence of onc-related transcripts in human cells. In fact, when we analyzed polyadenylated RNAs obtained from cell lines derived from a variety of solid tumors and hematopoietic cancers, it was possible to show rather striking specificity in the detection of transcripts related to certain onc genes such as that of SSV (13, 35). sis-related transcripts were found only in certain fibrosarcoma or glioblastoma cells but not in normal fibroblasts, in other solid tumors, or for the most part in any normal or malignant hematopoietic cells analyzed. In contrast, we found that certain onc genes detected related transcripts not only in tumor cells but also in normal cells (13, 35). Whatever their role in tumors, these genes are likely to be functioning and thus are important in human cells.

The most striking demonstration of the potential involvement of genes related to retroviral onc genes in human cancer has come from very recent studies. Certain human tumor cell DNAs have been shown to possess transforming activity when assayed on NIH/3T3 cells, which are highly susceptible to DNA transfection. The first biologically active human transforming gene has recently been molecularly cloned from human bladder carcinoma cell lines by 3 different investigative groups (14, 24, 30), including one headed by Mariano Barbacid, a senior investigator in our National Cancer Institute laboratory. The T24 bladder tumor-transforming gene possesses focus-forming activity in tissue culture at a high level comparable to that of any retroviral onc gene.

We were very interested in determining whether the T24-transforming gene was related to any of the onc genes that have been incorporated by acute transforming retroviruses. To our surprise and delight, we found that the human bladder tumor gene reciprocally hybridized to the onc gene of BALB MSV. A cloned 6.6-kilobase pair BamHI human DNA fragment harboring the T24 oncogene was readily hybridized with a DNA probe containing 675 base pairs of v-bas, the onc gene of BALB MSV. In reciprocal studies, v-bas DNA was detected with a probe composed of the T24 oncogene (29). Additional studies localized the v-bas related sequences to a 3.0-kilobase pair SacI fragment of the T24 oncogene (Chart 2).

To compare the T24 oncogene with normal human se-

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Fig. 2. In vivo detection of a v-sis translational product by immunoprecipitation analysis. Subconfluent cultures (around 10⁸ cells per 10-cm Petri dish) were labeled for 3 hr at 37° with 4 ml of methionine-fluent cultures (around 10⁸ cells per 10-cm Retri dish) product by immunoprecipitation analysis. Subcon-

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A. Aaronson. manuscript submitted.
sequences related to v-bas [designated c-bas(human)], we isolated c-bas(human) from a library of normal human fetal liver DNA (16). A recombinant λ Charon 4A phage containing a 19-kilobase pair EcoRI insert of human DNA exhibited a 6.4-kilobase pair BamHI segment that specifically hybridized with v-bas. This 6.4-kilobase pair BamHI fragment was subsequently subcloned in pBR322 and a representative plasmid, designated p344, used for restriction enzyme analysis. As shown in Chart 2, the restriction map of this 6.4-kilobase pair BamHI fragment of normal human DNA closely matched that of the T24 oncogene. In fact, the only detectable difference between the 2 molecules was a 200-base pair deletion that mapped between the Sphl and CiaI cleavage sites in c-bas(human) (Chart 2). This deletion maps outside the transforming sequences of the T24 oncogene, as well as outside the sequences related to v-bas (Chart 2). These results established that c-bas(human) is an allele of the T24 bladder carcinoma oncogene.

The T24 oncogene has been shown to transform NIH/3T3 cells efficiently, with a specific activity of $\sim 5 \times 10^4$ focus-forming units/pmol (14, 24, 30). It was of obvious interest to determine whether molecularly cloned c-bas(human) sequences exhibited similar biological activity. As much as 1 μg of c-bas(human) DNA demonstrated no detectable focus-forming activity, whereas in the same experiment NIH/3T3 cells were readily transformed with as little as 1 ng of the T24 oncogene. The above results, taken together, strongly imply that the acquisition of transforming activity by the T24 oncogene must be the result of subtle genetic alterations (29).

Comparative sequence analysis of the T24 oncogene and c-bas(human) will ultimately define not only the nature but also the number of genetic changes that led to the acquisition of transforming activity by c-bas(human) in T24 bladder carcinoma cells. The exact role of this transforming gene in processes leading to malignancy of the T24 bladder tumor will obviously require further investigation. Nonetheless, our own as well as independent, very recent findings of a similar nature (10, 20) suggest that the large fund of knowledge that has been gained from studies of retroviruses over a number of years by many investigators may now be directly applied to studies of naturally occurring tumors of man.

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REFERENCES


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