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IN the past decade, RNA tumor virus research has attracted a
great deal of attention, as a model system of viral carcino­
genesis and perhaps in part because of the discovery of reverse
transcriptase in virions which catalyzes the synthesis of DNA
using RNA as a template (Temin and Mizutani, 1970; Baltimore,
1970). Recently there seems to be another surge of interest in
the RNA tumor viruses. One reason for the renewed interest is
the long awaited identification of the viral gene product which
is directly responsible for cell transformation (Brugge and
Erikson, 1977; Purchio et al., 1977). Another factor is the dis­
closure of the unique mechanism with which virus is able to
integrate into host cell DNA and regulate the expression of the
integrated provirus. These two aspects will be parts of the dis­
cussion presented, which will focus on the possible general pro­
cess in the evolution of the oncogenic viruses and its implication
to the understanding of the process of cell transformation.

I. RNA Tumor Virus as a Transforming Agent

The RNA tumor virus systems have developed into a large
family of viruses since isolation of viruses early this century by
Ellermann and Bang (1909), Rous (1911), and Fujinami and In­
amoto (1914). The viruses are classified into three major groups:
sarcoma, acute leukemia, and lymphoid leukosis viruses, on the
basis of their pathogenicity in infected animals (Hanafusa, 1977).
Typical RNA tumor viruses of avian species are listed in Table
I. In addition to Rous sarcoma virus (RSV), two independent

TABLE I
Avian RNA Tumor Viruses

<table>
<thead>
<tr>
<th>Virus strains</th>
<th>Sarcoma virus</th>
<th>Acute leukemia virus</th>
<th>Lymphoid leukemia virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rous sarcoma virus (RSV)</td>
<td>Avian myeloblastosis virus (AMV)</td>
<td>Avian myelocytomatosis virus (MCV)</td>
<td></td>
</tr>
<tr>
<td>Bryan strain (BH-RSV)</td>
<td>Avian erythroblastosis virus (AEV)</td>
<td>Avian lymphomatosis virus RPL12</td>
<td></td>
</tr>
<tr>
<td>Schmidt–Ruppin strain (SR-RSV)</td>
<td>Rous associated viruses (RAVs)</td>
<td>Rous associated viruses (RAVs)</td>
<td></td>
</tr>
<tr>
<td>Prague strain (PR-RSV)</td>
<td>Resistance inducing factor (RIF)</td>
<td>Resistance inducing factor (RIF)</td>
<td></td>
</tr>
<tr>
<td>Bratislava strain 77 (B77)</td>
<td>Fujinami sarcoma virus (FSV)</td>
<td>Fujinami sarcoma virus (FSV)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Target cells</th>
<th>Fibroblasts</th>
<th>Bone marrow cells (fibroblasts) (epithelial cells)</th>
<th>Bursa of Fabricius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latent period (in vivo)</td>
<td>5–10 days</td>
<td>1–3 weeks</td>
<td>4–6 months</td>
</tr>
<tr>
<td>Virus replication</td>
<td>+ or –</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

isolates, Fujinami sarcoma virus (FSV) and Bratislava 77 (Thurzo et al., 1963), are known as sarcoma viruses, and recently more additions have been reported (Carr and Campbell, 1958; Itohara et al., 1978). There are a number of “strains” of RSV which are called by the name of researchers or by the name of the place where the original Rous virus was passaged and acquired some altered properties (Hanafusa, 1977). Acute leukemia viruses and lymphoid leukemia viruses, the second and third groups (Table I), are distinguishable on a number of accounts. First, acute leukemia viruses, as indicated by the name, cause an acute type of leukemia in animals compared to lymphoid leukemia viruses, which need a long latent period of several months. The primary target tissues are different: the bone marrow for acute
leukemia virus and the bursa of Fabricius for leukosis viruses. Perhaps more importantly, all of the acute leukemia viruses are defective in virus replication because of the substitution of their genomes by inserts of unique sequences, presumably derived from host cells. Therefore, as will be further discussed later, our current understanding is that acute leukemia viruses, and also sarcoma viruses, are able to transform host cells because they incorporate their unique transforming sequences into the cells.

On the other hand, lymphoid leukosis viruses do not appear to have such specific cellular sequences within their genome. It is considered, therefore, that leukosis viruses cause tumors in lymphoid cells because of replication of the virus for a long period of time, thereby altering the expression of some host cell genes. The mechanism of tumor formation by leukosis viruses is one of the issues actively being pursued by many groups and will not be discussed here. It should be stressed, however, that leukosis viruses are not defective in replication and serve as helper viruses for both defective sarcoma virus and defective acute leukemia viruses.

Cell transformation by avian sarcoma virus is a rapid and quantitative process (Hanafusa, 1969). Upon infection with one or more infectious virus, cells change dramatically in their appearance: cells which we call morphologically transformed become more refractile and assume either a round or spindle shape due to the changes in cell surface. These morphological changes are generally associated with changes in a number of other cellular phenotypes, including the capacity of continuous growth under conditions where normal cells cannot grow (Hanafusa, 1977).

These changes can take place within 24–30 hours following introduction of a single virus into the cell. After infection, viral genome RNA is copied into DNA by reverse transcriptase which is associated with viral RNA within the viral particles. Double-stranded viral DNA thus formed will be integrated into cellular DNA, and thereafter transmitted to daughter cells by cell division (Varmus et al., 1975; Gaianni et al., 1975; Weinberg, 1977). Recently it was discovered that in the process of the synthesis of viral DNA using viral RNA templates, the nucleotide sequences
present at the termini of viral RNA are duplicated and added to both ends of linear DNA in a form of direct repeats (Shank et al., 1978; Hsu et al., 1978). Furthermore, these long terminal repeats (LTR) are preserved in the integrated proviral DNA, and they are flanked by cellular sequences, suggesting that the LTR is directly involved in the process of integration of viral DNA (Hughes et al., 1978; Sabran et al., 1979; Hager et al., 1979; Vande Woude et al., 1979). As the nucleotide sequences of the LTR of various retrovirus have been analyzed, it has become apparent that these terminal sequences contain a set of sequences similar to those found in other eukaryotic DNAs which may serve as a promoter of RNA transcription (Yamamoto et al., 1980; Sutcliffe et al., 1980; Blair et al., 1980). This means that the proviral DNA transcription is not controlled by a cellular promoter but rather by its own promoter signal introduced into the cell chromosome together with the coding sequences of the viral genome.

The primary transcript of RSV provirus appears to be the 39S RNA which is the same size as the genomic RNA found in virions. This RNA contains four defined genes: gag for viral structural proteins, pol for the reverse transcriptase and env for envelope glycoproteins, and src for the transforming protein. The first three genes are essential for making complete virus and are shared by both lymphoid leukemia virus and nondefective strains of RSV. The src gene codes for a 60,000-dalton protein which is considered to be responsible for cell transformation and sarcoma formation (Brugge and Erikson, 1977; Purchio et al., 1977). The four genes described above are mapped on the nondefective RSV genome in the order 5'-gag-pol-env-src-c-poly(A)-3' (Wang, 1978). The sequences known as "c" are commonly present in most avian retroviruses, and a portion of it forms a part of the LTR which includes the promoter sequences. The corresponding genetic structure of lymphoid leukemia virus genome is shown by 5'-gag-pol-env-c-poly(A)-3'.

II. GENERATION OF NEW SARCOMA VIRUSES

Historically, the comparison of RNAs of these two types of viruses led to the recognition of the presence of the src gene.
When nondefective RSV is passaged in tissue cultures, usually a mutant is segregated, which retains replicating capacity but not transforming capacity (Vogt, 1971; Kawai and Hanafusa, 1972; Martin and Duesberg, 1972). The mutants are called transformation defective or td mutants. The RNA of td mutants is smaller than that of RSV and characteristically lacks a specific portion of the RNA sequences near the 3' end (Duesberg and Vogt, 1973; Wang et al., 1975, 1976; Joho et al., 1975; Lai et al., 1973). Therefore, the genetic structure of these td mutants is essentially the same as that of lymphoid leukemia viruses. Because the deletion of this set of RNA sequences always correlates with the lack of cell-transforming capacity of the virus, this region is considered to be responsible for cell transformation and thus defined as the src gene (Wang et al., 1976; Stehelin et al., 1976a; Hayward, 1977; Hanafusa, 1977; Vogt, 1977; Wang, 1978). The extent of the deletion was originally thought to be uniform for various td mutants (Wang et al., 1975).

In 1975, Sadaaki Kawai working in our laboratory at the time, obtained several isolates of this class of spontaneous deletion mutants of Schmidt–Ruppin strain of RSV (SR-RSV). Defined as transformation defective, these mutants do not cause any cell transformation in infected cultures. However, we obtained evidence suggesting that, instead of the complete deletion of the src gene, some of the mutants still retain a small portion of the src gene (Kawai et al., 1977). Previously we isolated a mutant of RSV which is temperature sensitive (ts) in its capacity to transform cells, but not to replicate (Kawai and Hanafusa, 1971). Therefore, the mutation must be in the src gene. When cells are doubly infected with this ts mutant, tsNY68, and one of the td mutant isolates, the products of some combinations were less temperature sensitive, indicating the formation of recombinants which had lost the ts lesion. The results were interpreted as indicating that these td mutants contained a small portion of src which corresponded to the temperature-sensitive lesion of the ts mutant. Indeed, the size of the RNA of these td mutants was shown to be slightly larger than that of other standard leukemia viruses (Kawai et al., 1977).

The first indication that the src gene originated from cellular DNA sequences came from a hybridization study by Stehelin
et al., (1976a,b). These investigators prepared DNA complementary to the src gene of RSV using reverse transcriptase. This cDNA$_{src}$ can hybridize with RNA of RSV but not with RNA of lymphoid leukemia viruses or td mutants. However, DNA of normal chicken cells showed a substantial degree of hybridization with this cDNA$_{src}$. Furthermore, the sequences homologous to the src gene were detectable in DNA of normal cells of many avian species (Stehelin et al., 1976b) and also later in mammalian and fish DNAs when less stringent conditions were used for molecular hybridization (Spector et al., 1978a). This src-related DNA in normal cells is transcribed into RNA (Wang et al., 1977; Spector et al., 1978b), which sediments at approximately 26 S in a sucrose gradient. The amounts of src-related RNA in normal cells were independent of the level of expression of leukemia virus-related sequences present in DNA of chicken cells. Furthermore, the 26 S src-related RNA is apparently not linked to the endogenous viral sequences, and src-related sequences and endogenous viral sequences were shown to reside at different sites on the cell chromosomes (Wang et al., 1977; Padgett et al., 1977; Spector et al., 1978b).

The amounts of such RNA species in various types of cells are shown in Table II. In normal uninfected chicken cells which happen to be the type expressing very little viral RNA, the amount of RNA transcripts of gag or env genes is extremely low.

<table>
<thead>
<tr>
<th>Region analyzed</th>
<th>RNA content (copies/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninfected cells</td>
</tr>
<tr>
<td>gag</td>
<td>0.2</td>
</tr>
<tr>
<td>pol</td>
<td>ND$^b$</td>
</tr>
<tr>
<td>env</td>
<td>0.5</td>
</tr>
<tr>
<td>src</td>
<td>5</td>
</tr>
<tr>
<td>c</td>
<td>ND</td>
</tr>
</tbody>
</table>

$^a$ From Hayward (1977).

$^b$ Not determined.
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(Hayward and Hanafusa, 1973). In cells infected with the lymphoid leukosis virus, RAV-2, the amount of RNA transcripts of these genes is high because of the active synthesis of viral RNA. However, the level of about 5–10 copies of src-related sequences present in uninfected cells remains unchanged in the RAV-2 infected cells, indicating that exogenous virus infection does not alter the extent of expression of endogenous src-related sequences (Hayward, 1977; Wang et al., 1977). Cells infected with RSV obviously contain large amounts of RNA transcripts of all four genes, because all of them are present in the sarcoma virus genome.

The existence of sequences related to the src gene of RSV in DNA of normal chickens and other vertebrate cells suggests the idea that virus might have picked up these sequences and thus become oncogenic. However, in the past, attempts to obtain sarcoma virus from chickens infected with lymphoid leukosis viruses have failed. On the other hand, there are some observations which imply the process of the generation of murine sarcoma viruses (MSV). In contrast to the isolation of sarcoma viruses from spontaneous tumors in chickens, many strains of MSV now widely used were made in the laboratory. When murine leukemia virus was maintained by repeated passages in mice (Moloney, 1966), or rats (Harvey, 1964; Kirsten and Mayer, 1967), sarcomas were formed in these animals. Viruses isolated from the resulting tumors were defective in replication; these isolates were called murine sarcoma viruses because they induced foci on mouse cells and produced sarcoma in mice. Interesting observations were made when cDNAs homologous to the sarcoma virus and nonhomologous to helper leukemia sequences were employed to examine their homology with cellular and viral nucleic acids (Scolnick et al., 1973, 1975; Scolnick and Parks, 1974; Roy-Burman and Klement, 1975; Anderson and Robbins, 1976; Frankel and Fischinger, 1976, 1977). First, sequences specific to Kirsten sarcoma virus and Moloney sarcoma virus were not the same. Second, cDNA to Kirsten sarcoma virus-specific sequences hybridized well with rat cell DNA, but not with mouse cell DNA. Conversely, cDNA to Moloney sarcoma virus sequences hybridized with DNA of mouse cells but
not with that of rat cells. Therefore, it appeared that each sarcoma virus was generated via recombination with respective host cell sequences.

Because we found that some \(td\) mutants of RSV retained a small portion of the \(src\) gene, we considered that these sequences might serve for homologous recombination with the cellular \(src\)-related sequences, and thus increase the probability of generating sarcoma virus. Table III shows some data of early experiments. \(Td\) mutants were inoculated into the wing-web of newborn chicks. Newborn chicks were used because they were known to support growth of virus without immediate immunological response, and also because animals should provide a larger number of cells compared with \textit{in vitro} tissue cultures; thus, this would increase the chance of recombination between viral and cellular genomes.

By definition, \(td\) mutants do not cause cell transformation, and indeed did not produce tumors at the site of injection. Then, beginning at about 2 months, we noticed the appearance of tumors, generally one, but sometimes in multiple numbers, at various sites distal from the original injection site: back, breast, leg, and various internal organs. From every tumor we were able to recover viruses fully capable of inducing sarcomagenic transformation both \textit{in vitro} and \textit{in vivo}. In order to rule out the

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Virus</th>
<th>Number of chickens with solid tumors/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>(td) 101</td>
<td>2/5</td>
</tr>
<tr>
<td></td>
<td>(td) 105</td>
<td>1/5</td>
</tr>
<tr>
<td></td>
<td>(td) 107</td>
<td>2/4</td>
</tr>
<tr>
<td></td>
<td>(td) 108</td>
<td>3/4</td>
</tr>
<tr>
<td>2</td>
<td>RAV-1</td>
<td>0/8</td>
</tr>
<tr>
<td></td>
<td>(td) 106</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>(td) 107</td>
<td>2/7</td>
</tr>
<tr>
<td></td>
<td>(td) 101 Repurified</td>
<td>4/16</td>
</tr>
<tr>
<td></td>
<td>(td) 107A Repurified</td>
<td>0/15</td>
</tr>
<tr>
<td></td>
<td>(td) 108 Repurified</td>
<td>9/16</td>
</tr>
<tr>
<td></td>
<td>(td) 109 Repurified</td>
<td>4/8</td>
</tr>
</tbody>
</table>
possibility that the tumor formation was due to the presence of a minute amount of sarcoma virus as a contaminant in the td mutant stocks, the td mutants were carefully purified by terminal dilution passages. Injection with these viruses confirmed that the generation of sarcoma virus is reproducible as shown in Table III (exp. 2) (Hanafusa et al., 1977).

It should be pointed out here that some td mutants were unable to produce tumors. Mutants which have complete deletions of the src gene, such as td106 and 107A, cannot recombine with the cellular sequences. The long latency for the formation of tumors in general suggests a low frequency of the genetic interaction even in the positive cases.

The viruses isolated from tumors were designated "recovered avian sarcoma virus" (rASV) followed by the number of the chicken from which each virus was isolated. Foci of transformed cells induced by the recovered viruses were similar to each other, despite the varying origin of tissues where tumors were formed. They were rather distinct, however, from foci induced by standard Schmidt–Ruppin RSV from which the td mutants were derived. These results suggested that we had obtained a new type of sarcoma virus. The isolation of similar rASVs was observed by Vigne et al. (1979).

The scheme for the generation of rASV is essentially as shown in Fig. 1. In most cases the size of RNA of the recovered viruses was similar to that of Schmidt–Ruppin RSV. This was attributed to the restoration of the src gene, because RNA of rASV hybridized with more than 90% of cDNA_{src}, whereas RNA of td mutants used for generation of rASVs contained only 10 to 30% of src-specific sequences (Halpern et al., 1979). Therefore, one can conclude that from 70 to 90% of the src gene of recovered ASV is derived from the host cell sequences.

The relationship of the src-specific sequences between recovered virus and standard RSV was studied by L.-H. Wang and others (Wang et al., 1978) by comparing RNase T1-resistant oligonucleotide fingerprints. The fingerprint patterns were surprisingly similar to each other, but RNA of several isolates of rASV consistently contained a new set of oligonucleotides. Furthermore, we were able to obtain similar recovered viruses from quails infected with the same td mutants used for chickens. The
III. Transformation as a Result of the Expression of Normal Cellular Enzyme

The product of the src gene of RSV was first identified by Erikson and his associates (Brugge and Erikson, 1977; Purchio et al., 1977). They injected Schmidt-Ruppin RSV into newborn rabbits and produced tumors. Sera of rabbits carrying regressing RSV tumors were shown to have a capacity to precipitate a protein of molecular weight of about 60,000 from extracts of

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Fig. 1. The generation of rASV. The genetic structures of SR-RSV, its td mutant and rASV are schematically shown, based on data obtained from the size of RNAs, hybridization with cDNA src and T1 oligonucleotide fingerprints.
FIG. 2. Fingerprint patterns of RNase T₁-resistant oligonucleotides of viral $^{32}$P-labeled RNAs. $^{32}$P-labeled viral 60 to 70 S RNAs were digested with RNase T₁, and digestion products were separated by electrophoresis and homochromatography. (A) SR-RSV; (B) rASV-C 145 obtained from a chicken tumor; and (C) rASV-Q 3042-13 obtained from a quail tumor. Spots indicated by arrows are src-specific oligonucleotides: open arrows are those present in SR-RSV RNA and solid arrows are those not present in RNA of SR-RSV but present in rASV RNAs.
RSV-transformed cells, but not of uninfected or lymphoid leukemia virus-infected cells. Further studies showed that this protein is a phosphoprotein which has a protein kinase activity (Collett and Erikson, 1978; Levinson et al., 1978).

When we applied this technique to extracts of cells transformed by rASVs, 60K proteins (p60) were precipitable by the antisera as expected, and the proteins were associated with a protein kinase activity (Karess et al., 1979). The protein kinase activity was detected by the transfer of phosphate from γ-ATP to an acceptor protein molecule. In this case, antigen–antibody complex precipitated by the aid of staphylococcal protein A was mixed with [γ-32P]ATP. The most closely associated protein molecule, immunoglobulin became labeled with 32P. Therefore, as shown in Fig. 3, the heavy chain of IgG of about 52,000 daltons was strongly phosphorylated. In addition, in this particular experiment phosphates were also transferred to p60 itself. Later studies by Hunter and Sefton (1980) showed that this protein kinase associated with the src protein is unique in phosphorylating tyrosine residues of substrate proteins rather than serine or threonine residues, sites of phosphorylation by known cellular protein kinases. Because phosphorylation of proteins is known to be associated with various regulatory processes in cells, the increased level of phosphorylating activity by the src protein may well serve as the trigger for the process of cell transformation; the analysis of this process is the current topic of research in many laboratories.

Figure 3 also shows that extracts of nontransformed cells, either normal chicken cells or td virus-infected cells contain limited amounts of p60 precipitable by the same immune serum, and this protein showed the same protein kinase activity (Collett et al., 1978, 1979; Oppermann et al., 1979; Karess et al., 1979). An src-related protein in normal chicken cells can be found also in normal cells of many mammalian species (Oppermann et al., 1979; Collett et al., 1979). The phosphorylation is specific to tyrosine residues, and the proteins are structurally very similar to the 60K protein made in transformed cells (Hunter and Sefton, 1980). As discussed before, normal cells are known to contain src-related nucleotides in both DNA and RNA. Therefore, it
Fig. 3. Autoradiogram of products of protein kinase reaction after electrophoresis in 8.5% polyacrylamide gel. (A) Transformed and (B) nontransformed cells. (A) Kinase activity by immunoprecipitates of (a) SR-RSV-infected cells with nonimmune serum; (b) SR-RSV-infected cells with immune serum; (c) rASV-C 1441-infected cells with immune serum; (d) rASV-C 157-infected cells with immune serum. (B) Kinase activity immunoprecipitated from uninfected cells (a, b, c) or td108-infected cells (d, e, f): (a, d) immune serum; (b, e) immune serum preabsorbed with virion proteins; (c, f) nonimmune serum.
seems very reasonable to assume that these src-related sequences are responsible for the formation of protein kinase similar to that found in transformed cells.

Table IV shows the level of src-related DNA, RNA, and protein in various types of cells. Uninfected normal chicken cells contain 1 or 2 copies of src-related DNA and express about 5 copies of RNA and relatively small amounts of p60. Infection with lymphoid leukosis virus or td mutant does not change these levels. Infection with RSV or recovered ASV incorporates into the cell one or a few new proviruses which carry the src gene. As noted before, viral RNA is transcribed in these sarcoma virus-infected cells very efficiently, and the levels of src protein are proportionately high. As described above, from 70 to 90% of the src sequences of rASV are derived from normal cells. This means that the efficiency of transcription of the same sequences present in normal cell DNA and in rASV-proviral DNA is greatly different.

Thus, one scheme for the process of the generation of rASV can be summarized as follows. In normal cells, expression of the cellular src gene, which appears to be the gene for the unique protein kinase, is tightly regulated. When these cells are infected with some td mutants, recombination takes place between td virus and cellular src sequences and generates recovered ASV.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>DNA</th>
<th>RNA</th>
<th>p60 molecules per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td>2</td>
<td>5</td>
<td>200</td>
</tr>
<tr>
<td>Infected by</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>td 108</td>
<td>2</td>
<td>5</td>
<td>200</td>
</tr>
<tr>
<td>SR-RSV</td>
<td>5</td>
<td>3,000</td>
<td>40,000</td>
</tr>
<tr>
<td>rASV #165</td>
<td>ND$^b$</td>
<td>2,000</td>
<td>ND</td>
</tr>
<tr>
<td>rASV #1441</td>
<td>4</td>
<td>ND</td>
<td>60,000</td>
</tr>
</tbody>
</table>

$^a$ From Karess et al. (1979).

$^b$ Not determined.
This process itself is essentially similar to the transduction in bacteriophage, although the process of recombination involved in the transduction may not be similar. The cellular protein kinase gene is now linked to the viral genome, which has a strong promoter that allows efficient transcription of the entire viral genome, irrespective of the site of integration, which results in the production of a high level of the kinase.

However, one still cannot dismiss another possibility: that a portion of the src gene retained by the td mutants could have a crucial role. In the retained portion, the sequences of viral src and its cellular counterpart could be different; only p60 made by viral sequences could be effective for cell transformation.

If the first scheme is correct, this means that the mere overproduction of a certain protein (such as protein kinase) could be the cause of cell transformation. At the moment, there is no clear indication whether viral and cellular products are functionally different, and therefore we cannot make a definitive conclusion in the chicken system. However, with Moloney murine sarcoma virus, molecular cloning of the viral transforming sequences and its cellular counterpart was performed (Vande Woude et al., 1979). When cellular counterpart DNA was transfected into normal cells, no cell transformation took place. But when the cellular counterpart DNA was ligated to the terminal repeats of the virus, the DNA efficiently induced cell transformation upon transfection (Oskarsson et al., 1980). These results support the idea described in the first scheme.

IV. Existence of Many Transforming Genes

It has become more apparent by now that there are many different transforming genes. The src gene of RSV is only one of them. Recently T. Hanafusa and others in our laboratory studied the genetic structure of Fujinami sarcoma virus as part of a comparative analysis of transforming genes of various strains of avian sarcoma virus (Wang et al., 1980; Hanafusa et al., 1980; Lee et al., 1980). This Fujinami sarcoma virus (FSV) was isolated in Japan at almost the same time as RSV was isolated, and is as potent as RSV in sarcomagenic action in chickens (Fujinami
and Inamoto, 1914). To our surprise, the genome of FSV does not contain the src sequences of RSV. The virus is very defective in replication, and the viral RNA is only 28 S or about 5000 nucleotides. The FSV RNA contains viral sequences at both the 5' and 3' termini, but the middle of the genome is substituted by a set of unique sequences. The viral RNA does not hybridize to cDNA src and contains no oligonucleotide specific to src. A large protein of molecular weight about 140,000 can be found in FSV-transformed cells by immunoprecipitation with antisera against the gag gene product (structural protein) of lymphoid leukosis viruses. This protein of 140,000 daltons (p140) seems to be the only viral product found in FSV-transformed non-virus-producing cells and thus is likely to be a transforming protein. The p140 of FSV also appears to be associated with a protein kinase activity which phosphorylates specifically tyrosine residues of substrate proteins (Feldman et al., 1980). Two other avian sarcoma viruses recently characterized, PRCII and Y73, appear to be similar to FSV rather than RSV, in their overall genetic structures (Vogt et al., 1980; Kawai et al., 1980).

The genetic structures of some representative RNA tumor viruses are shown in Fig. 4, which illustrates the diversity of the genetic structure of the transforming retroviruses. The portions shown by the open bar are the inserts of unique sequences into the genomes of lymphoid leukosis virus. The viral sequences are probably essential in providing the large terminal repeats which ensure the integration and efficient transcription, and perhaps 5'-terminal sequences required for packaging of viral RNA into virions. In many cases, the unique sequences have been shown to have homologous counterparts in DNA of normal vertebrate cells. In general, the inserts of all of these viruses are not common to each other, suggesting that many different sets of cellular sequences can act as transforming genes. Recent studies with FSV showed that the unique sequence of FSV is homologous to the genome of feline sarcoma virus (Shibuya et al., 1980). This means that the sarcomagenic sequences of a chicken virus and a feline virus have a common origin; probably the cognate genes in normal chicken and feline cell DNA were picked up independently by avian and feline retroviruses. Perhaps it is also
worth mentioning that the gene products of some acute leukemia viruses such as avian erythroblastosis virus appear to be specifically involved in the differentiation process of blood cells (Graf and Beug, 1978; Graf et al., 1980).

It seems likely, therefore, that the sequences we call transforming genes are the genes of cellular enzymes possibly involved in various regulatory processes. Linking these genes with virus genomes could be considered as natural cloning of cellular genes, with retrovirus serving as cloning vehicles. Such a recombination may also occur with other cellular genes, but unless some overt changes such as cell transformation are induced, such recombinants would not be recognized and selected for.

The comparison shown in Fig. 4 illustrates that the structure of RSV is unique among these transforming viruses because it
is the only virus in which its transforming gene is located outside of the replicative region of prototype leukosis virus. As a consequence, RSV is the only known transforming virus which is not defective in virus replication. When we demonstrated the defectiveness of Bryan strain of RSV for the first time (Hanafusa et al., 1963), Rubin pointed out the possibility that the defectiveness of the replicative function of the virus is correlated with its transforming capacity, considering the model of defective transducing phages (Rubin, 1964). This concept was quickly dismissed when we found that other RSV strains are nondefective (Hanafusa, 1964). In retrospect, Rubin's concept may be basically right. As seen in a few examples shown in Fig. 4, nearly all transforming retroviruses are defective because of the substitution of a significant part of the viral genome by cellular sequences. It is intriguing that nondefective RSV was an exceptional existence, since this class of RSV has been most invaluable for the genetic analysis of the transforming virus, and for the analysis of the transformation process in infected cells.

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