1981

Hidesaburo Hanafusa, 1980

The Rockefeller University

CELLULAR ORIGIN OF TRANSFORMING GENES OF RNA TUMOR VIRUSES*

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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 carcinogenesis 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 disclosure 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 discussion presented, which will focus on the possible general process 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 Inamoto (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

*Lecture delivered March 20, 1980.

ISBN 0-12-312075-6

TABLE I
AVIAN RNA TUMOR VIRUSES

	Sarcoma virus	Acute leukemia virus	Lymphoid leukosis virus
Virus strains	Rous sarcoma virus (RSV) Bryan strain (BH-RSV) Schmidt–Ruppin strain (SR-RSV) Prague strain (PR-RSV) Bratislava strain 77 (B77) Fujinami sarcoma virus (FSV)	Avian myeloblastosis virus (AMV) Avian erythroblastosis virus (AEV) Avian myelocytomatosis virus (MCV)	Avian lymphomatosis virus RPL12 Rous associated viruses (RAVs) Resistance inducing factor (RIF)
Target cells	Fibroblasts	Bone marrow cells (fibroblasts) (epithelial cells)	Bursa of Fabricius
Latent period (*in vivo*)	5–10 days	1–3 weeks	4–6 months
Virus replication	+ or −	−	+

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 leukosis 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 leukosis 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 39 S 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 leukosis 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 leukosis 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 leukosis 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, *ts*NY68, 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 leukosis 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 leukosis 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 leukosis 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 II
CONTENT OF GENE-SPECIFIC RNAS IN VIRUS-INFECTED CELLS[a]

Region analyzed	RNA content (copies/cell)		
	Uninfected cells	RAV-2-infected cells	SR-RSV-infected cells
gag	0.2	9,800	10,000
pol	ND[b]	9,400	10,200
env	0.5	16,000	20,000
src	5	7	18,000
c	ND	17,000	19,500

[a] From Hayward (1977).
[b] Not determined.

(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 *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 *in vitro* and *in vivo*. In order to rule out the

TABLE III
FORMATION OF SOLID TUMORS BY *td* MUTANTS OF SR-RSV

Experiment	Virus	Number of chickens with solid tumors/total
1	Control	0/5
	td 101	2/5
	td 105	1/5
	td 107	2/4
	td 108	3/4
2	RAV-1	0/8
	td 106	0/7
	td 107	2/7
	td 101 Repurified	4/16
	td 107A Repurified	0/15
	td 108 Repurified	9/16
	td 109 Repurified	4/8

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 *td*106 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 T_1-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

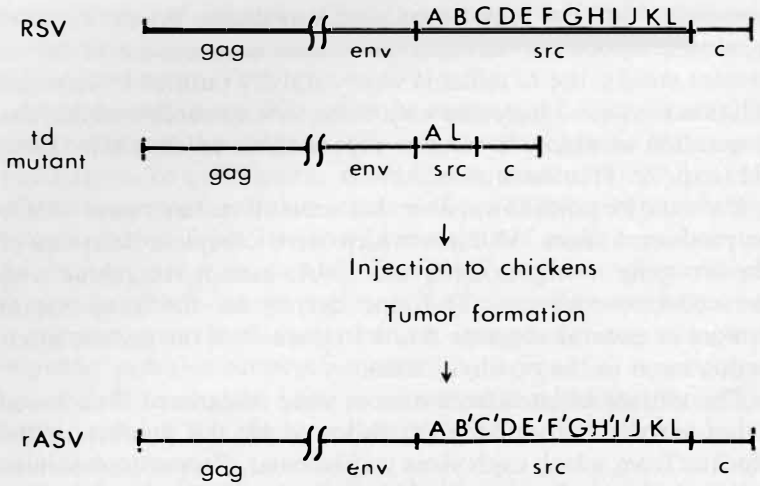

FIG. 1. The generation of rASV. The genetic structures of SR-RSV, its *td* mutant and rASV are schematically shown, based on data obtained from the size of RNAs, hybridization with $cDNA_{src}$ and T_1 oligonucleotide fingerprints.

comparison of fingerprints of chicken-derived rASVs and quail-derived rASVs showed a few distinct differences between these two types of virus RNA, while RNAs of rASVs recovered from the same species of animals were identical to each other (Wang *et al.*, 1979) (Fig. 2). These experiments demonstrated that the sequences regained in recovered ASV *src* gene were indeed derived from cellular sequences present in each species of animals which were closely related but not identical.

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

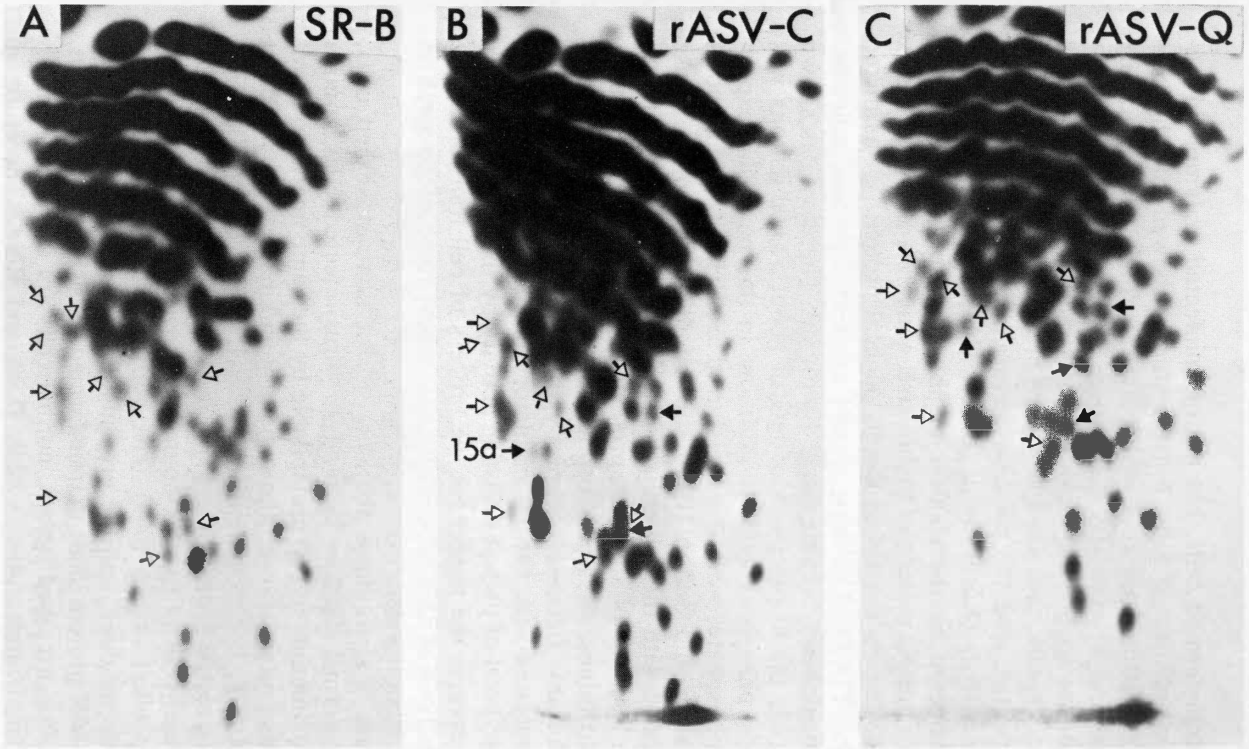

FIG. 2. Fingerprint patterns of RNase T_1-resistant oligonucleotides of viral ^{32}P-labeled RNAs. ^{32}P-labled viral 60 to 70 S RNAs were digested with RNase T_1, and digestion products were separated by electrophoresis and homochromatography. (A) SR-RSV; (B) rASV-C 145 obtained from a chicken tumor; 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 leukosis 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 aceptor protein molecule. In this case, antigen–antibody complex precipitated by the aid of staphylococcal protein A was mixed with [γ-^{32}P]ATP. The most closely associated protein molecule, immunoglobulin became labeled with ^{32}P. 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

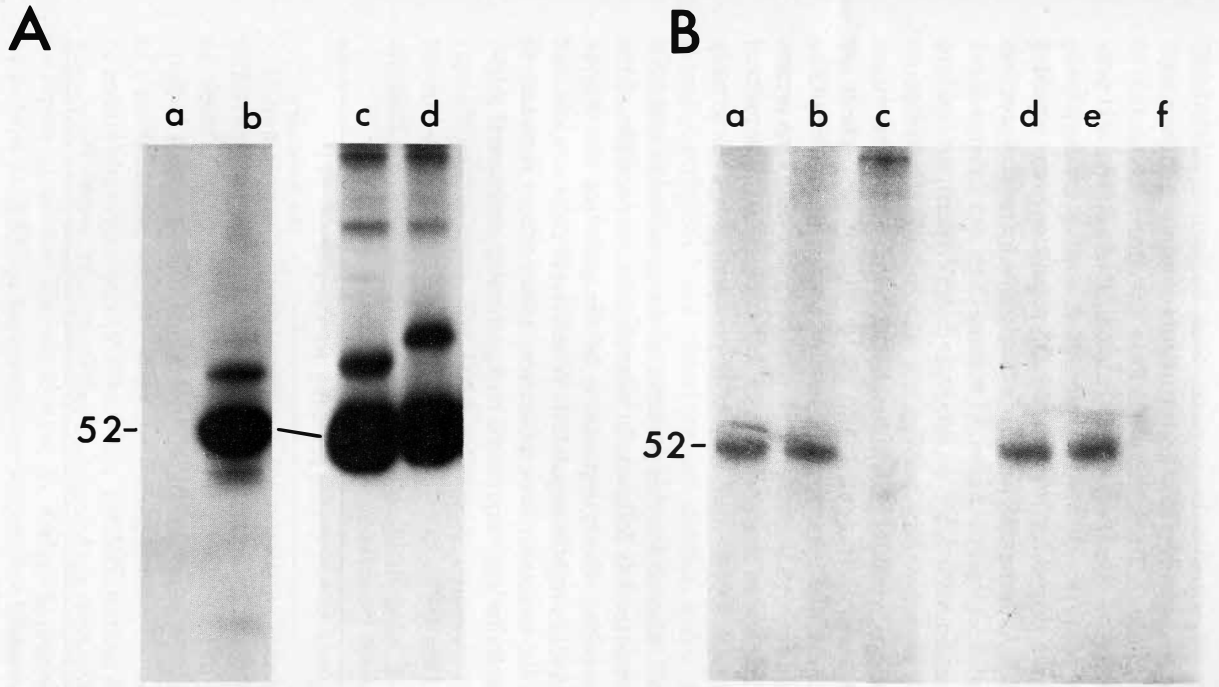

FIG. 3. Autoradiogram of products of protein kinase reaction after electrophoresis in 8.5% polyacrylamide gel. (A) Transformed and (B) nontransformed cells. (A) Kinase activity by immunoprecipitates of (a) SR-RSV-infected cells with nonimmune serum; (b) SR-RSV-infected cells with immune serum; (c) rASV-C 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 *td*108-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 IV

COMPARISON OF TRANSCRIPTIONAL AND TRANSLATIONAL PRODUCTS OF *src*-SPECIFIC GENETIC INFORMATION IN NORMAL AND TRANSFORMED CELLS[a]

Cell type	Copies per cell		p60 molecules per cell
	DNA	RNA	
Uninfected	2	5	200
Infected by			
td 108	2	5	200
SR-RSV	5	3,000	40,000
rASV #165	ND[b]	2,000	ND
rASV #1441	4	ND	60,000

[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

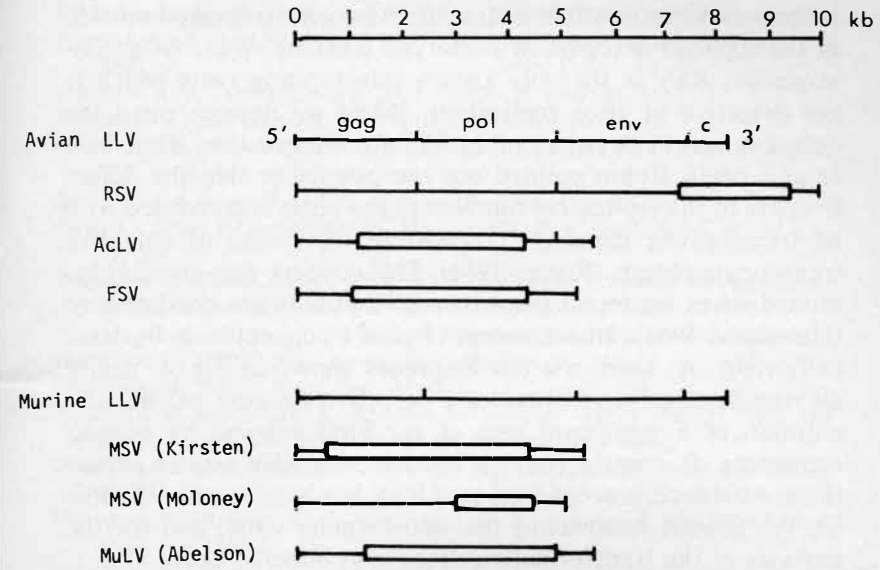

FIG. 4. The genetic structures of some representative retroviruses. The regions of the genomes shown by the open bars are the inserts unique to each virus. Data are obtained from Wang *et al.* (1975), Bister *et al.* (1979), Hanafusa *et al.* (1980), Shih *et al.* (1978), Donoghue *et al.* (1979), and Shields *et al.* (1979).

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.

Acknowledgments

I would like to express my deep gratitude to all of my colleagues who contributed to the work described. In particular, I wish to thank Teruko Hanafusa, for her continued dedicated work. Much of the work has received support from the National Cancer Institute and the American Cancer Society.

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