TUMOR VIRUS RNAs AND TUMOR VIRUS GENES

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Abstract

The RNAs of several classes of avian and murine RNA tumor viruses were compared. The 60–70S RNA complex of cloned nondefective avian sarcoma viruses contains only 30–40S RNA species of class a, which are larger than the 30–40S RNA species of class b found in avian transformation-defective or leukosis viruses. The RNA of a recombinant avian sarcoma virus, carrying a host range marker of a leukosis virus parent, also consists only of class a subunits. This implies that recombination among avian tumor viruses involves crossing-over rather than reassortment. The class a RNA subunits of nondefective avian sarcoma viruses and the class b RNAs of transformation-defective, leukosis viruses of the same subgroup and strain have very similar oligonucleotide-fingerprint patterns and are at least 60 % homologous if compared by RNA-DNA hybridization. It is suggested that the class b RNA of leukosis viruses is a deletion of class a RNA from corresponding nondefective sarcoma viruses and that their structural relationship may be expressed as $a = b + x$. We assume, that x represents genetic information directly or indirectly involved in transformation of fibroblasts.

Passage at high multiplicity of cloned sarcoma viruses, containing only 30–40S RNA of class a, led to the appearance of 30–40S RNA of class b in progeny virus.

Two replication-defective avian sarcoma viruses (Bryan RSV and MC 29) lack 30–40S RNA of class a. They probably contain distinct types af 30–40S RNA resembling class b RNA of leukosis viruses in size but differing in composition. The Kirsten murine sarcoma virus, which appears to be more defectice than Bryan RSV or MC 29, has a 30–40S RNA which is even smaller than of its leukosis helper virus.

These observations suggest that a correlation exists between the size of the viral RNA species and defects in transformation-and/or replication genes of the corresponding viruses. The greater the extent of the defectiveness, the smaller is the size of the viral RNA. Possible mechanisms generating deletions in tumor virus RNA are discussed.

Different classes of RNA tumor viruses:

Two classes of RNA tumor viruses can be readily distinguished on the basis of their pathological effects on cells (1–6): (i) Sarcoma viruses, which transform fibroblasts in tissue culture and cause solid tumors in the animal and (ii) leukosis or leukemia viruses, most of which fail to transform fibroblasts in tissue culture, but cause a lymphatic or myeloblastic leukemia in the animal. A possible exception appear to be

some endogenous leukosis-type viruses; for example, the endogenous avian leukosis virus RAV (O) has so far not been found to cause a disease in chicken (Vogt, P. K., G. Purchase and R. Weiss (1973) personal communication). Endogenous murine leukemia virus of AKR-mice has the same serotype and N-tropism as known leukemogenic virus strains, however it has not yet been tested whether it causes leukemia in mice (Rowe, W. P. (1973) personal communcation).

Since in the avian tumor virus group, sarcoma and leukosis viruses have been found which resemble each other in most biological and biochemical properties except transforming ability for fibroblasts (4, 5), many, and perhaps all avian leukosis viruses may be considered as defective sarcoma viruses which have lost their ability to transform fibroblasts. Consequently those avian leukosis viruses which have been derived in the laboratory (4, 5) from sarcoma viruses have been termed transformation-defective (td) viruses (7).

In addition replication-defective sarcoma viruses exist in the avian (8) and murine tumor virus group (9). These may be considered sarcoma viruses which have lost all or part of the genetic information required for virus-replication and/or virus-structure. All replication-defective RNA tumor viruses rely of necessity on associated helper viruses or helper cells to complement their defective structural or replicative functions (8, 10). Since the known helper viruses do not repair, by genetic recombination, the defectiveness of replication-defective sarcoma viruses, their defectiveness is genetically stable (8, 10).

If we were to set up a hierarchy of decreasing genetic potentials among different classes of RNA tumor viruses we could distinguish the following categories: Firstly, the nondefective sarcoma viruses, which carry all genes essential for replication and cell transformation (examples: Prague Rous sarcoma virus (PR RSV), Schmidt Ruppin (SR) RSV, B77 RSV). From these the category of transformation-defective (td) and leukosis viruses could be formally derived by deletion of genes required for cell transformation (examples: td PR RSV, Rous associated virus (RAV) and murine leukemia virus. Similarly the replication-defective sarcoma viruses may be considered as a viral category which has lost all or part of the genetic potential of nondefective sarcoma viruses required for virus replication (examples: Bryan RSV, Kirsten murine sarcoma virus (MSV), Moloney MSV). In addition sarcoma viruses may exist which are both replication- and transformation-defective and leukosis viruses may exist which are replication-defective (see Fig. 8).

It is the purpose of this report to summarize and extend correlations made (3, 5, 6, 7, 26, 31) between different genetic potentials of RNA tumor viruses and various physically defined classes of viral RNA.

The 60–70S RNA complex of RNA tumor viruses:

RNA tumor viruses contain a 60–70S RNA corresponding to an approximate molecular weight of 1×10^7 (11). This RNA probably represents part or all of the viral genome (11, 12). The 60–70S RNA can be dissociated by heat or organic solvents (formamide, dimethysulfoxide) to yield molecules which sediment at 30–40S and some smaller RNA species (11, 13) and was therefore proposed to have a subunit structure (11).

Some recent observations have lent biological credibility to the possible subunit structure of viral 60–70S RNA. It was found that avian sarcoma viruses harvested a 3 min intervals from cells contain 30–40S instead of 60–70S RNA. Upon incubation of such virus at 40° the 30–40S RNA species is converted to 60–70S RNA (14). Hence the 30–40S subunits appear to be precursors rather than fragments of 60–70S RNA (14).

The 30–40S RNA species of nondefective avian sarcoma viruses has been resolved electrophoretically into two size classes, a and b (3). Electrophoresis in formamide gels indicates that class b RNA is about 12 % smaller than class a RNA (13). Molecular weight estimates of class a RNA in formamide gels are between 2.9 and 3.4 x 10^6 depending on the reference RNA used as marker (13). The presence of class a RNA is well correlated with viral ability to induce focus formation in fibroblast cultures (7). Exceptions to this rule are replication-defective viruses such as the Bryan RSV and avian leukosis virus strain MC 29 which have focus forming ability but contain RNA which migrates with size class b (5, 6). Avian leukosis viruses and transformation-defective derivatives of sarcoma viruses contain only class b RNA (3, 5, 6, 7).

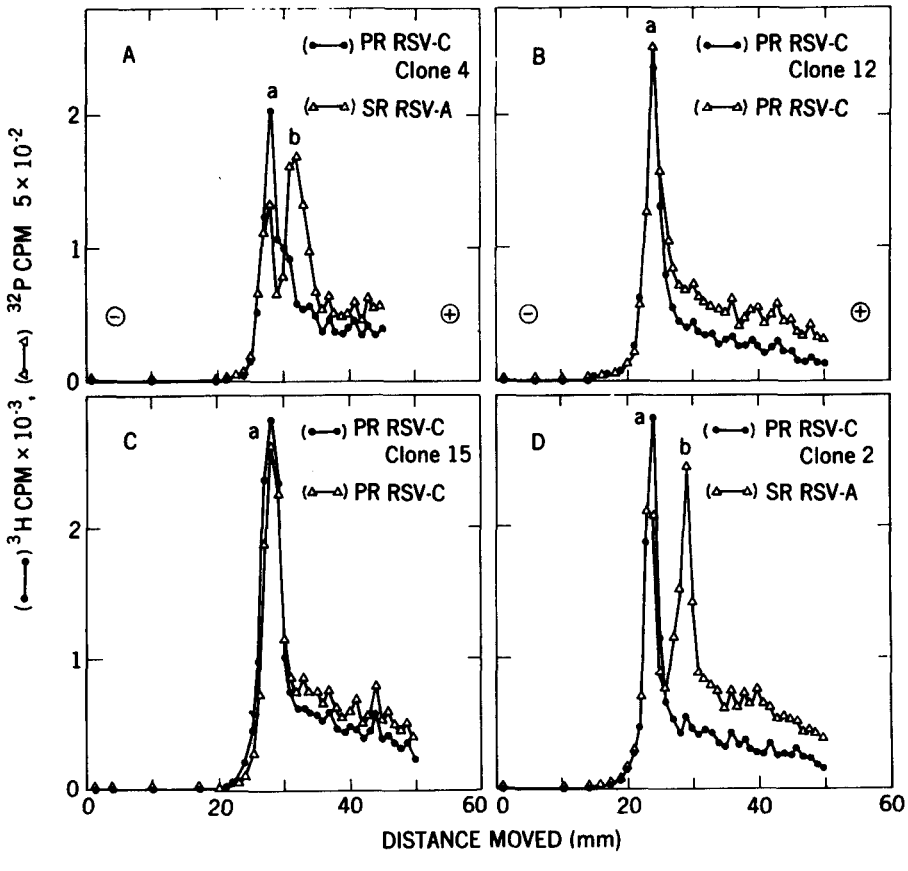


Fig. 1: The 60–70S RNA of 4 clones of PR RSV-C, propagated in cloned colonies of transformed cells, after heat-dissociation an electrophoresis in polyacrylamide. (A) Single colony-derived PR RSV-C clone 4 with a standard of uncloned SR RSV-A containing both class a and class b RNA. (B) Single colony-derived PR RSV-C clone 12 with cloned PR RSV-C as described for (A). (C) Single colony-derived PR RSV-C clone 15 with cloned PR RSV-C as for (A). (D) Single colony-derived PR RSV-C clone 2 with uncloned SR RSV-A as for (A). The data are from Duesberg and Vogt (7).

The 60–70S RNA of cloned avian leukosis and sarcoma viruses contains only one size class of 30–40S RNA.

The ratio of class a and b RNA in conventionally prepared stocks of avian sarcoma viruses is not constant; it varies with the passage history of the virus as well as with the virus strain (3, 5, 7). It has been shown that nondefective avian sarcoma viruses can segregate transformation-defective derivatives which no longer form foci in tissue culture but can replicate and are antigenically indistinguishable form the parental sarcoma virus (4, 5). Such transformation-defectives contain only class b RNA (3, 5, 6, 7). This observation suggested that class b RNA present in conventional stocks of nondefective sarcoma viruses might derive from transformation-defective segregants. Therefore, sarcoma viruses were cloned, both by single focus isolation and from colonies developing in agar after infection at low multiplicity (7). Figures 1. 5A,C and 6A,D show that rigorous cloning eliminates class b RNA from nondefective avian sarcoma viruses. The characteristic RNA pattern showing only class a RNA was seen in cultures derived from individual agar colonies for over two month, the lifetime of the cultures. We conclude that nondefective sarcoma viruses contain only class a RNA (7).

The 70S RNA of a recombinant: Support for crossing-over.

The 60–70S RNA of pure sarcoma viruses thus contains only the larger size class a subunits. The RNA subunits of leukosis viruses are exclusively of the smaller class b. The RNA of a recombinant between leukosis and sarcoma virus should then reveal the mechanism of recombination: if class a und b RNA are genome segments and recombinants arise by reassortment of these segments (15 ,16), the 60–70S RNA of the recombinant should contain both class a and class b subunits. However if recombinants are the result of crossing-over, their 60–70S RNA need not contain both size classes of subunits. Figure 2 shows an electropherogram of the heat-dissociated 60–70S RNA obtained from a recombinant of PR RSV-A and RAV-2. The gel pattern shows one major peak which co-migrates with class a RNA of a standard cloned sarcoma virus PR RSV-B. No distinct peak of class b RNA was discernible. This observation suggests the occurrence of crossing-over between RNA tumor virus genomes. However, the suggestion is based on an idealized interpretation of the electropherograms, which assumes that the heterogeneous material migrating faster than class a RNA conists of breakown products derived from class a RNA. Recently we have obtained direct support for this assumption. Comparing the RNA associated with the peak fractions of class a RNA of this recombinant to the minor heterogenous RNAs of the same virus by oligonucleotide fingerprinting (Duesberg, P. H, & Lai, M. M-C. unpublished, 1973), we found that their oligonucleotide fingerprints were identical. This indicates that the majority of the smaller RNA species are breakdown products of class a RNA rather than distinct subgenomic fragments.

High frequency recombination as observed between various strains of avian and murine RNA tumor viruses (15, 16, 17, 18) can be explained readily with a polyploid nonsegmented genome model (11, 12, 19, 20). The polyploid progeny from a mixed infection would be largely heterozygous. Such heterozygotes would contain different genomes united in the same 60–70S complex, thus increasing the chances for

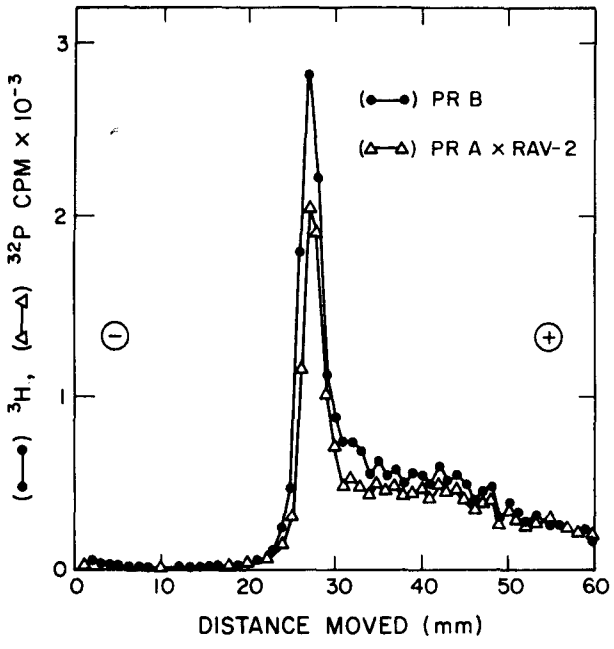


Fig. 2: The 60—70S RNA of cloned nondefective sarcoma virus PR RSV-B and of a recombinant between PR RSV-A and leukosis virus RAV-2 after heat-dissociation and electrophoresis in 2 % polyacrylamide. Conditions were as described for Fig. 1. The data are from Duesberg and Vogt (unpublished 1973).

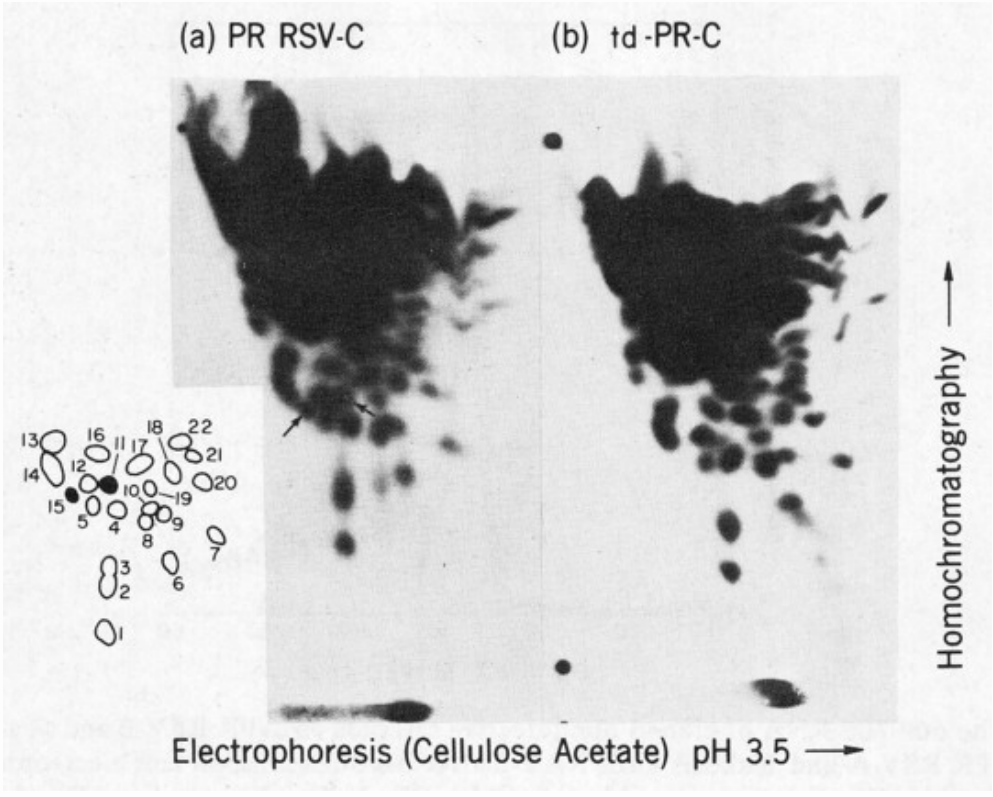
crossing-over in the next cycle of infection. DNA synthesis directed by the heterozygote 70S RNA template seems the most likely step for the occurrence of high-frequency crossing-over (20).

Sequence homology between class a and class b RNA of avian tumor viruses of the same subgroup and strain.

Given sarcoma viruses with only a subunits and transformation-defecitve (td) derivatives with only b subunits (4, 5, 6, 7, 26) we may predict that a subunits and b subunits of viruses of the same subgroup and strain must share homologous sequences. This follows because such corresponding virus strains probably share all replicative and many structural properties and therefore must have genetic information in common. We have tested this prediction by comparative oligonucleotide fingerprint analyses and RNA-DNA hybridizations of a and b subunits of corresponding viruses (21).

It was found by "fingerprinting" that all (about 20—25) large RNase TI-resistant oligonucleotides present in class b subunits of three transformation-defective viruses (td PR RSV-C, td B77 RSV-C and td SR RSV-A have homologous counterparts in the class a subunits of corresponding nondefective sarcoma viruses (PR RSV-C, B77 RSV-C and SR RSV-A (Fig. 3A, B, C)). In addition class a subunits contain a few (one or two) additional oligonucleotides which are not present in class b (Fig. 3 A, a; B, a; C, a, c). Thus the oligonucleotide fingerprints of transforming and td avian tumor viruses of the same subgroup and strain are very similar. By contrast the patterns of the avian tumor viruses of different strains and subgroups shown in Fig. 3 A, B and C were very different (21). This indicates that this method is well suited to detect similarities and differences among related virus strains.

A


(a) PR RSV-C
(b) td-PR-C
Homochromatography
Electrophoresis (Cellulose Acetate) pH 3.5

B

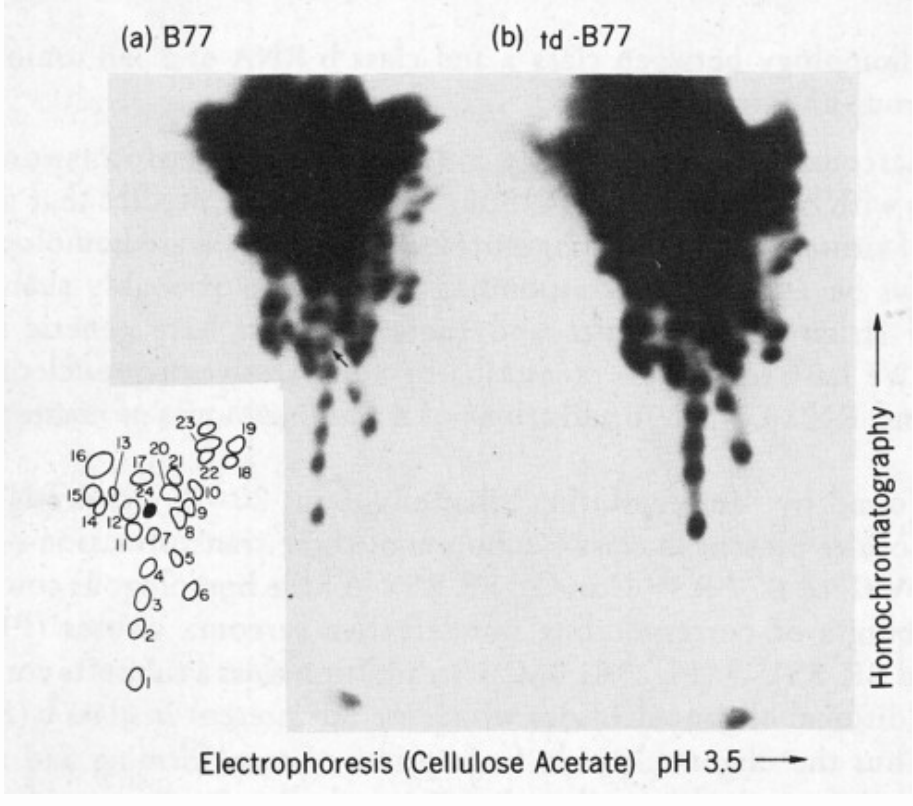
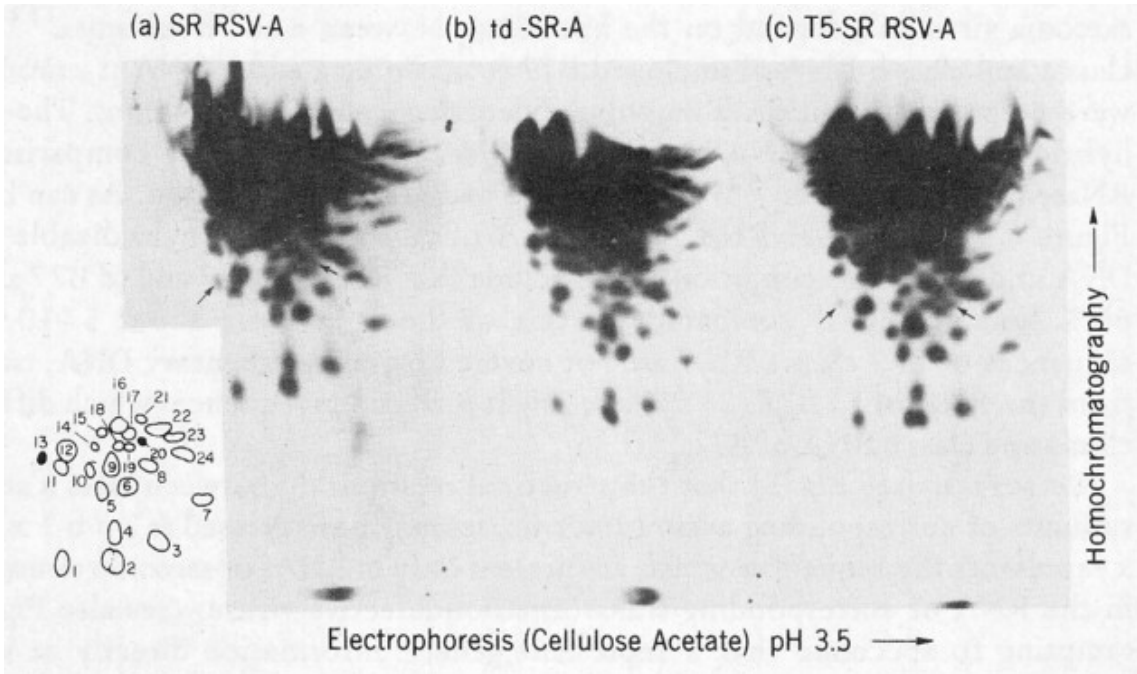

(a) B77
(b) td-B77
Homochromatography
Electrophoresis (Cellulose Acetate) pH 3.5

Fig. 3



C

Fig. 3: Two-dimensional separations of RNase T1 digests of 60–70S ^{32}P-RNAs of three nondefective sarcoma viruses (Aa, Ba, C,a,b) and of their transformation-defective counterparts (A, b, B,b, C,c). The patterns were recorded by autoradiography. The method was a modification of that described by Brownlee and Sanger (36). The oligonucleotide patterns of the following virus strains are shown: PR RSV-C (A,a), transformation-defective PR RSV-C (A,b), B77 RSV-C (B,a), transformation-defective B77 (B,b), SR RSV-A (C,a), a mutant of SR RSV-A which is temperature-sensitive for transforming ability T5 (37) (C,b) and transformation-defective SR RSV-A (C,c). The inserts are tracings of the major large oligonucleotides. The spots traced in black in the inserts and marked by arrows in the autoradiograms denote oligonucleotides found only in sarcoma viruses but not in corresponding transformation-defective viruses. The data are from Lai, Duesberg, Horst and Vogt (21).

However in comparing RNase T1-resistant oligonucleotides we are limited to only 2–3 % of the total viral RNA. The remainder of the RNA is degraded to fragments too small for analysis by "fingerprinting". Moreover, it cannot be said with certainty whether homologous oligonucleotides of a given sarcoma virus and its td derivative have the same sequence (21). Yet, it is plausible that most of the homologous oligonucleotides of a given sarcoma virus and its td derivative have the same or a very similar sequence for two reasons. First it is quite unlikely that two nonidentical RNAs with molecular weights of about 3×10^6 yield two homologous oligonucleotide patterns consisting of 20 large RNase T1-resistant oligonucleotides which have identical electrophoretic and chromatographic properties but have different sequences. Second it may be expected that biologically closely related viruses, such as sarcoma viruses and their td counterparts share many sequences. However we cannot fathom completely the relationship between class a subunits of a given sarcoma virus and the class b subunits of a corresponding td derivative by this method alone.

Therfore we have compared class a and class b RNA of B77 RSV-C by hybridization with the DNA transcribed from RNA of td B77 by viral polymerase. It is expected that this DNA will be about 70 % complementary to b subunit (22). However, the extent of hybridization between this "class b" DNA and class a RNA of a corresponding

sarcoma virus will depend on the homology between a and b subunits. ^{32}P-labeled class a and class b RNA of uncloned B77 (containing a and b RNA at a ratio of 1 : 2) were prepared by elution from polyacrylamidegel electropherograms. The extent of hybridization of ^{32}P-RNA with the ^{3}H-DNA was measured by comparison of the RNase-resistance of the ^{32}P-RNA before and after hybridization. As can be seen in Figure 4, 70 % of class b RNA and 60–65 % of class a RNA was hybridizable to td B77 DNA under the same condition. We conclude that RNAs of B77 and td B77 are at least 60 % homologous if compared by this method. Further, about 5–10 % of the sequences of B77 class a RNA are not covered by complementary DNA, transcribed from the RNA of td B77 (21). These might well be the sequences which differentiate class a and class b RNA of B77.

We suggest (see Fig. 5) that the structural relationship between class a and class b subunits of corresponding avian tumor viruses may be expressed as a = b + x, and that x represents the sequences which are present only in RNAs of sarcoma viruses but not in the RNA of corresponding transformationdefective viruses (see also Fig. 8). It is tempting to speculate that x represents genetic information directly or indirectly involved in transformation of fibroblasts.

Reappearance of class b RNA in cloned stocks of nondefective avian sarcoma viruses.

The close chemical relationship between a and b subunits made it likely that leukosis viruses may originate from sarcoma viruses by deletion of a sequence(s) x. This hypothesis was suggested by earlier studies in which we had observed that transformation-defective viruses segregate spontaneously from cloned nondefective sarcoma viruses (4, 5). We are asking now whether extensive or complete transition

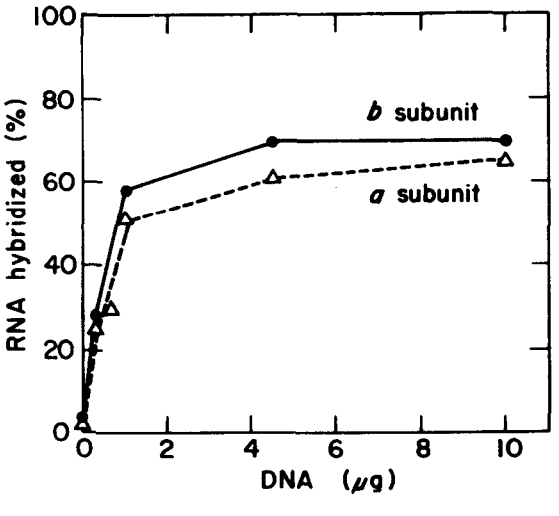


Fig. 4: Hybridization of ^{32}P-labeled class a (△---△) and class b (●——●) RNA of B77 RSV-C with DNA synthesized in vitro (22) from transformation-defective B77 virus. The percentage of RNA hybridized to DNA of transformation-defective B77 was measured by comparison of the RNase-resistance of ^{32}P-RNA before and after hybridization. The data are from Lai, Duesberg, Horst and Vogt (21).

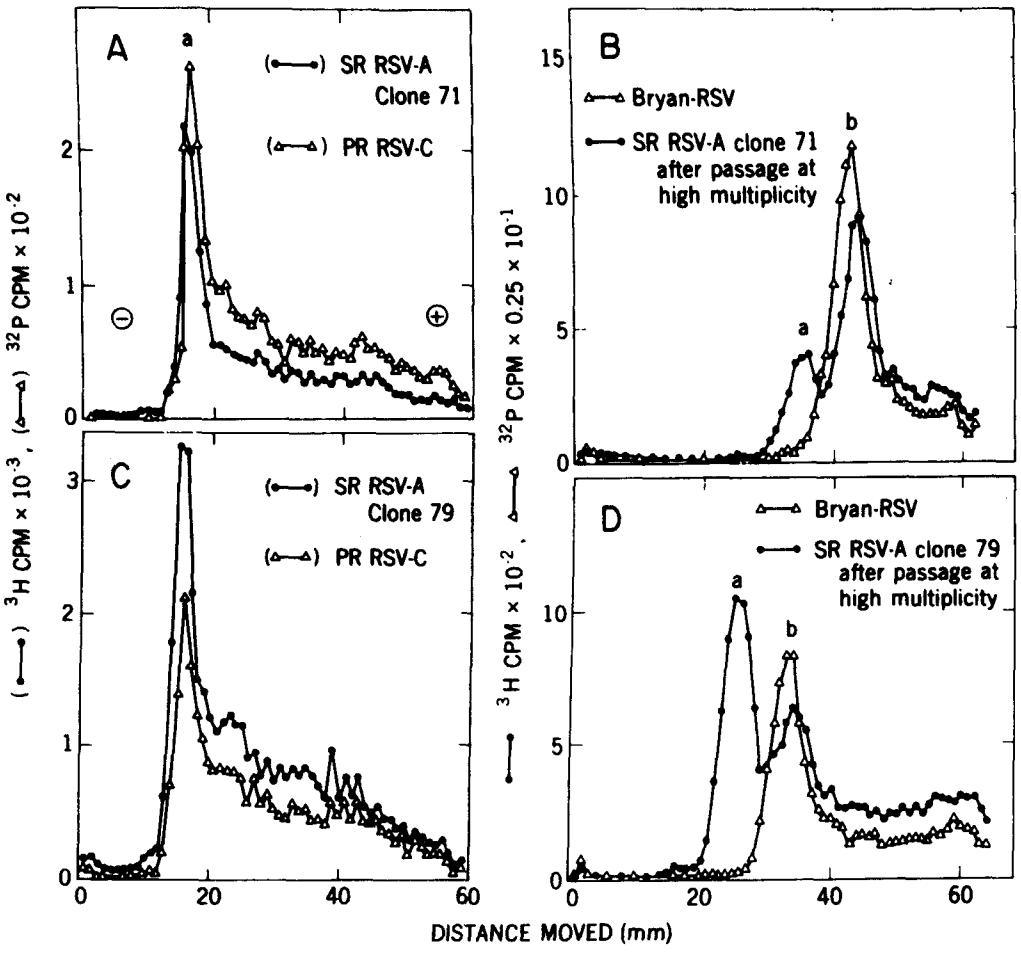


Fig. 5: The 60—70S RNA of 2 clones of SR RSV-A before (A, C) and after (B, D) five passages of the virus at high multiplicity of infection in chick embryo fibroblasts, after heat-dissociation and elektrophoresis in polyacrylamide. (A, C) show the RNA of clones 71 and clone 79 of SR RSV-A with a class A RNA standard of cloned PR RSV-C RNA as described for Fig. 1. (B, D) show the RNA of the two clones of SR RSV-A after passage at high multiplicity with a standard of Bryan RSV(RAV) RNA which contains only 30—40S RNA of class b (5, 6). The data are from Duesberg, Vogt, Maisel, Lai and Canaani (26).

from class a RNA of cloned nondefective sarcoma viruses to class b RNA of td derivatives can be demonstrated within a few viral life cycles. It was shown that the occurrence of deletions leading to incomplete or defective cytocidal viruses is favored by passage of viruses at high multiplicity of infection (23, 24, 25).

Several clonal preparations of nondefective avian sarcoma virus (SR RSV-A and B77) were propagated at high multiplicities (10 FFU per cell) for five passages with harvests at five day intervals (26). At the end of the fifth passage the viral RNA was labeled, extracted and analyzed by gel electrophoresis after heat-dissociation. Figures 5 und 6 show that the avian sarcoma viruses which, at the beginning of this experiment, contained only class a RNA (Figs. 5A, C, 6A, D) show significant amounts of class b RNA (Figs. 5B, D, 6B, C, E, F). In two cases class b even exceeded the level of class a (Figs. 5B, 6E, F) and in one, class a RNA was no longer detectable (Fig. 6B, C). Further the reappearance of class b RNA correlated roughly with a decrease (SR RSV) or an almost complete loss (B77) of the transformed morphology

of the host cell (26). It is likely that the reappearance of class b RNA represents segregation of transformation-defective viruses from the cloned sarcoma virus, and biological tests to examine this hypothesis are in progress. The results summarized in Figs. 5 and 6 suggest that infection at high multiplicity may lead to the appearance of class b RNA in nondefective avian sarcoma virus stocks similar to the von Magnus phenomenon in myxoviruses (23, 27). However, our experiments are still preliminary, and a causal relationship between high multiplicity of infection and reemergence of class b remains to be established (26). Nevertheless it appears that a complete transition from class a to class b RNA can be obtained within a few passages of nondefective avian sarcoma viruses at high multiplicity of infection.

The RNA of replication-defective RNA tumor viruses.

Most RNA sarcoma viruses are defective in replication and depend on a helper virus for the synthesis of infectious progeny. The extent of the defect varies with different virus strains: Bryan RSV is able to produce certain structural proteins which are

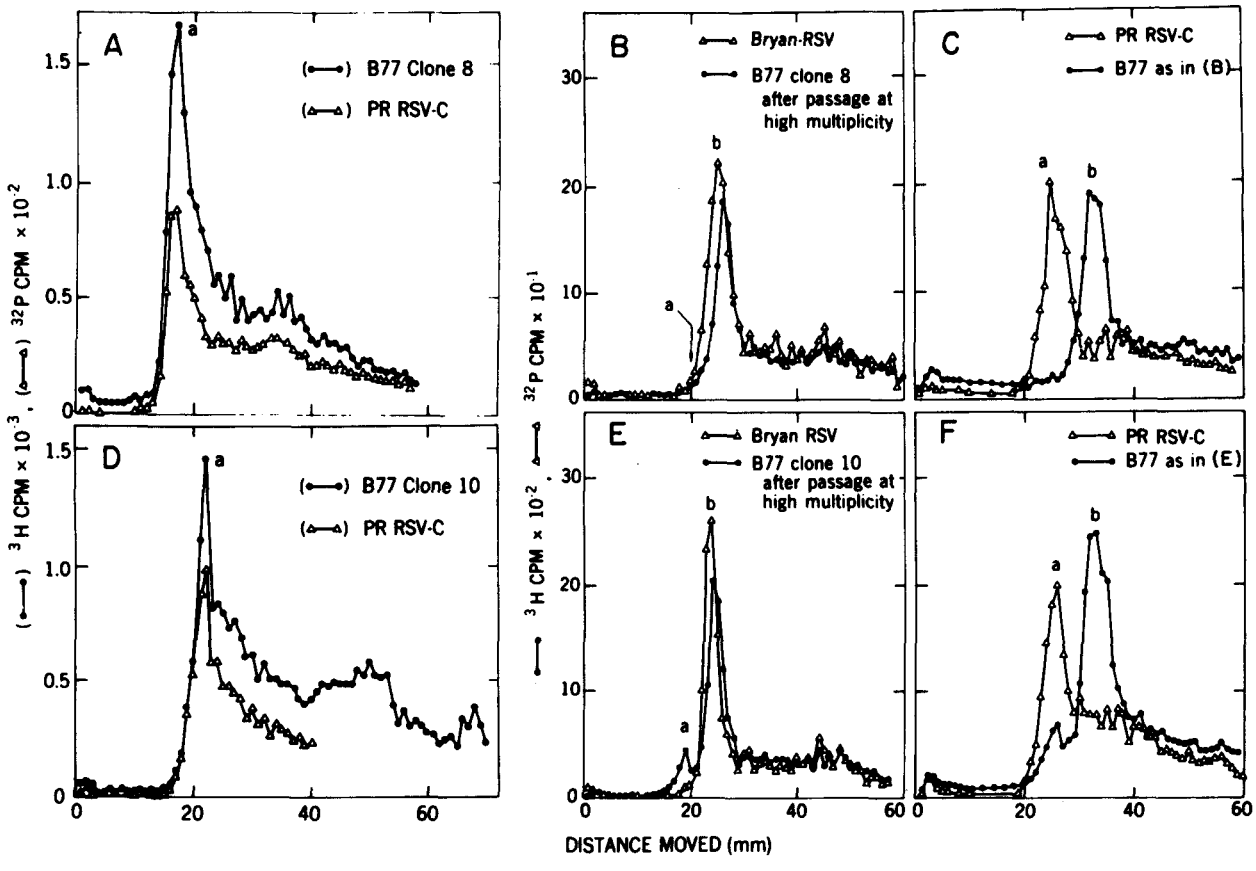


Fig. 6: The 60—70S RNAs of 2 clones of B77 RSV-C before (A, D) and after (B. C and E, F) five passages of the virus at high multiplicity of infection in chick embryo fibroblasts, after heat-dissociation and electrophoresis in polyacrylamide. (A, D) show the RNA of clone 10 and Clone 8 of B77 RSV-C with a class a RNA standard of cloned PR RSV-C as described for Fig. 1. (B, C and E, F) show the RNA of the 2 clones of B77 RSV-C after passage at high multiplicity with a standard of Bryan RSV(RAV) containing only class b RNA (B, E) and of cloned PR RSV-C RNA containing only class b RNA (B, E) and of cloned P RSV-C RNA containing only class a RNA (C, F). The data are from Duesberg, Vogt, Maisel, Lai und Canaani (26).

assembled into noninfectious virus particles (8, 28). Some of the murine sarcoma viruses show a similar degree of defectiveness (S+L-particles, [29, 30]). However in others, for example in Kirsten MSV the defects are more extensive so that no synthesis of viral structural proteins can be detected in the transformed cells (9).

Early analyses in our laboratory of the RNAs of Bryan RSV and MC 29, two defective sarcoma viruses, indicated absence of a subunit (5,6). One excuse to explain the absence of a subunit in these viruses was the following consideration. Since the defective sarcoma viruses studied were associated with a large excess of helper leukosis virus, electrophoretic detection of a subunit could have been obscured by the large preponderance of b subunit from leukosis virus. Alternatively the RNA of a defective sarcoma virus could have been smaller than a, due to deletions of structural and/or replicative functions of the RNA. Such a defective class a subunit may be only detectable if it were electrophoretically significantly different from class b subunit of associated helper virus or if cloned defective sarcoma virus, free of helper virus were available for analysis.

Scheele and Hanafusa have reported that the RNA subunits of RSV(-), presumably the cloned defective sarcoma virus of the Bryan RSV, have the same size as the b subunit of RAV-2, a typical leukosis virus (28). This is compatible with our earlier reports on the RNAs of Bryan RSV (5) and MC 29 (6). Because of the striking biological differences between these focus forming, replication-defective viruses and avian leukosis viruses, we hypothesize that the class b RNA found in Bryan RSV or in MC 29 differs from class b RNA found in leukosis viruses in two ways: (i) The RNA of defective sarcoma virus should contain sequences typical of its transforming ability and not present in leukosis viruses and (ii) it should lack sequences required for replication, which are present in leukosis viruses (compare model in Fig. 8).

Because of its defectiveness Kirsten MSV is of necessity associated with a helper murine leukemia virus (MLV). We have recently shown that when propagated in rat kidney cells Ki MSV is favored about 10–100 fold over its helper Ki MLV (31). Thus the RNA of the mixture of MSV and MLV released from such cells should be predominantly MSV RNA. Given a source of predominantly Kirsten MSV RNA, it was possible to compare Kirsten MSV RNA to that of pure Kirsten MLV RNA by electrophoresis in polyacrylamide gels. Kirsten MSV RNA was found to be smaller than that of its leukosis helper virus (31) (Fig. 7). However, the relationship between the RNAs of Kirsten MSV and Kirsten MLV is not clear at present. Available evidence suggests that the two RNAs share little perhaps no homologous sequences (31). Therefore we cannot say whether the RNA of defective Ki MSV resulted from deletions of an unknown nondefective murine sarcoma virus, which must be largely unrelated to Kirsten MLV, or whether the RNA of Kirsten MSV is perhaps cellular oncogenic information transducible by MLV.

DISCUSSION

Nondefective sarcoma viruses contain the complete information for virus reproduction and for cell transformation. The RNA which corresponds to this complete genetic endowment is size class a. From these sarcoma viruses, transformation-defectives can

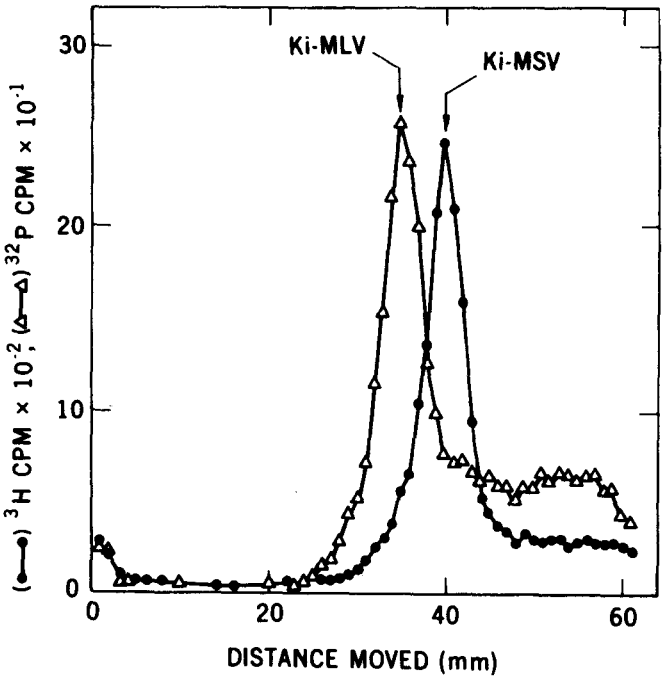


Fig. 7: The 50–70S RNA of Kirsten murine leukemia virus (Ki MLV) and Kirsten murine sarcoma virus (Ki MSV) after heat-dissociation and electrophoresis in polyacrylamide. The data are from Maisel, Klement, Lai and Duesberg (31).

be derived which resemble leukosis viruses in tissue culture: they replicate efficiently but do not induce transformation in fibroblasts. These transformation-defectives as well as leukosis viruses proper contain 30–40S RNA subunits of a smaller size, class b. Since transformation-defective derivatives share many properties with the parental sarcoma viruses, class a and b RNA must have genetic information in common. Chemical analyses directly indicate that class b RNA probably contains only sequences which are also represented in class a RNA. However, class a RNA contains some sequences which are not present in class b. Therefore we proposed their relation may be a = b + x, where x represents genetic information necessary for focus formation and b contains all information required for the synthesis of progeny virus particles. Some replication-defective viruses such as Bryan RSV and MC 29 lack class a RNA despite an ability to form foci in tissue culture. In an electrophoretic comparison the RNA of these replication-defectives was indistinguishable from the RNA of transformation-defective viruses and leukosis viruses. Nevertheless, it appears unlikely that 30–40S RNA of size class b obtained from transformation-defective and leukosis viruses contains the same basic information as class b RNA of replication-defective viruses. Both may be derived from class a but, while transformation-defectives have a deletion of focus forming genes resulting in 30–40S RNA of class b the replication-defectives may have a similar size deletion in replicative genes, resulting in defective 30–40S RNA of class a (Fig. 8). This relationship could be expressed by, defective a = a – y wherein a defective a contains all of the genetic information necessary for focus formation and y represents replicative genes deleted from a. More extensive replicative defects correspond to a smaller RNA, as might be indicated by the observations with Kirsten sarcoma virus.

Besides the known defective viruses which contain lesions in either focus forming ability or in replication, other defectives may occur which lack transforming as well as replicative information. These would be replication-defective leukosis, or replication and transformation-defective sarcoma viruses (Fig. 8). Such viruses would be difficult to detect directly but could perhaps be demonstrated by marker rescue or be detected as radioactive defective virions. It has been postulated that defective viruses represent deletions of nondefective sarcoma viruses (4, 5, 6, 10) or are actually incomplete viral intermediates evolving from cellular genes (10), as proposed by Temin's protovirus hypothesis (32). We conclude that the various size classes of tumor virus RNA can be formally derived from class a RNA of nondefective sarcoma viruses by various deletions in replicative or transforming genes (Fig. 8).

We can only speculate as to how defective tumor virus RNAs arise. It could happen during transcription from RNA to DNA or DNA back to RNA; it could also be due to posttranscriptional cleavage or to an event similar to recombination between multiple DNA proviruses in the cell. The phenomenon of defective viral RNAs is not limited to RNA tumor viruses. The first historic example is the von Magnus phenomenon of influenza virus (23, 27). It describes the origin of defective virus after passage at high multiplicity. We have found in 1968, that von Magnus virus contains RNAs smaller than that of standard virus (33). Vescicular stomatitis virus was shown to form defective interfering particles with RNAs shorter than those of complete virions (25) and other RNA and DNA viruses were shown to produce defective and interfering particles containing an incomplete complement of nucleic acid (24, 5). Even soluble

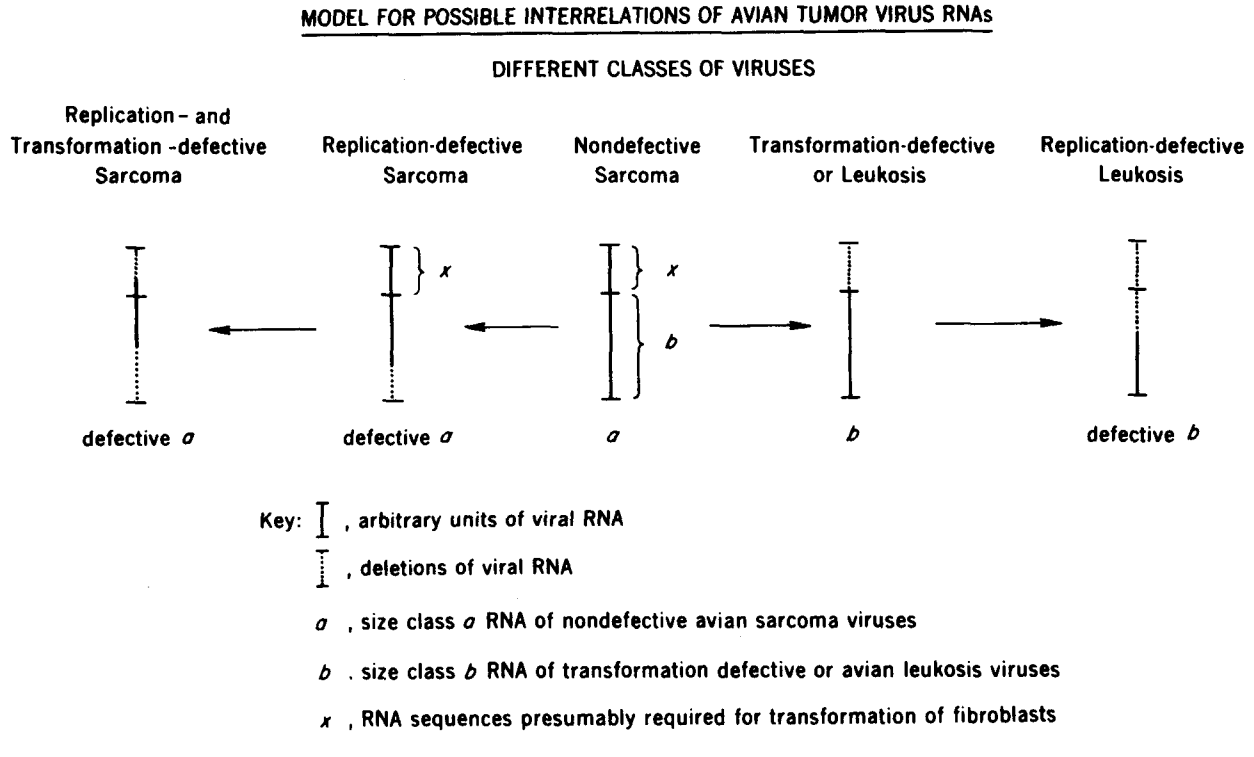


Fig. 8: Models for possible relationships between the RNAs of different classes of RNA tumor viruses.

Qß-phage replicase, given Qß RNA as template, synthesizes under conditions of high selective pressure, little "monster" RNAs which are only 17 % as large as the complete Qß RNA template (34, 5).

The origin of all these defective viruses or RNA molecules has one principal in common: They arise at high multiplicity of infection presumably in highly competitive conditions. These conditions may be more readily established by RNA tumor viruses than by cytocidal viruses, because most RNA tumor viruses do not kill the cell and can virtually saturate the cell with particles competing for replication. So perhaps defective virus particles are not so bad after all, by competing and interfering with their nondefective counterparts, they may function as natural contraceptives for viruses.

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