*Proc. Natl. Acad. Sci. USA*
Vol. 77, No. 4, pp. 2018–2022, April 1980
Biochemistry

# Fujinami sarcoma virus: An avian RNA tumor virus with a unique transforming gene

(sarcoma formation/defectiveness/RNA and protein electrophoresis/mapping specific and group-specific RNA sequences/transforming protein)

WEN-HWA LEE*, KLAUS BISTER*, ANTHONY PAWSON†, TERRY ROBINS*, CARLO MOSCOVICI‡, AND PETER H. DUESBERG*

*Department of Molecular Biology and Virus Laboratory, and †Department of Zoology, University of California, Berkeley, California 94720; and ‡Virus Research Laboratory, Veterans Administration Hospital, Gainesville, Florida 32601

*Communicated by Harry Rubin, February 6, 1980*

**ABSTRACT** **The oncogenic properties and RNA of the Fujinami avian sarcoma virus (FSV) and the protein it encodes were investigated and compared to those of other avian tumor viruses with sarcomagenic properties such as Rous sarcoma virus and the acute leukemia viruses MC29 and erythroblastosis virus. Cloned stocks of FSV caused sarcomas in all chickens inoculated and were found to contain a 4.5-kilobase (kb) and an 8.5-kb RNA species. The 4.5-kb RNA was identified as the genome of defective FSV because it was absent from nondefective FSV-associated helper virus and because the titer of focus-forming units increased with the ratio of 4.5-kb to 8.5-kb RNA in virus preparations. This is, then, the smallest known tumor virus RNA with a transforming function. Comparisons with other viral RNAs, based on oligonucleotide mapping and molecular hybridization, indicated that 4.5-kb FSV RNA contains a 5′ *gag* gene-related sequence of 1 kb, an internal specific sequence of about 3 kb that is unrelated to Rous sarcoma virus, MC29, and erythroblastosis virus, and a 3′-terminal sequence of about 0.5 kb related to the conserved C region of avian tumor viruses. The lack of some or all nucleotide sequences of the essential virion genes, *gag, pol,* and *env,* and the isolation of FSV-transformed nonproducer cell clones indicated that FSV is replication defective. A 140,000-dalton, *gag*-related nonstructural protein was found in FSV-transformed producer and nonproducer cells and was translated *in vitro* from full-length FSV RNA. This protein is expected to have a transforming function both because its intracellular concentration showed a positive correlation with the percentage of transformed cells in a culture and because FSV is unlikely to code for major additional proteins since the genetic complexities of FSV RNA and the FSV protein are almost the same. It is concluded that the transforming *onc* gene of FSV is distinct from that of Rous sarcoma virus and other avian tumor viruses with sarcomagenic properties. Hence, multiple mechanisms exist for sarcomagenic transformation of avian cells.**

There are a number of sarcomagenic viruses in the avian tumor virus group that fall into discrete RNA subgroups, defined here on the basis of helper-virus unrelated, specific RNA sequences. [See below and ref. 1. Both defective and nondefective viruses can be classified consistently by using RNA subgroups (1). In contrast, only nondefective viruses can be classified on the basis of envelope subgroups (2).] The Rous subgroup includes the replication-defective Bryan Rous sarcoma virus (RSV), the nondefective Schmidt–Ruppin strain, and the Prague strain of RSV (2, 3). The most common neoplasm produced by the RSV RNA subgroup of viruses is a sarcoma. The Rous subgroup is defined by a related class of transforming genes of 1.5 kilobases (kb) termed *src* (1, 4–7), which are located near the 3′ end of viral RNA (6) and encode a 60-kilodalton nonstructural protein product thought to have a transforming function (8, 9). The ES4 strain of avian erythroblastosis virus (AEV) (ES for erythroblastosis and sarcoma) and the avian myelocytomatosis virus MC29, in addition to acute leukemias and carcinomas, also cause sarcomas in animals and transform fibroblasts in culture (1, 3, 10, 11). Their RNAs contain specific nucleic acid sequences that, in part, encode nonstructural protein products of about 75–110 kilodaltons with probable transforming function (1, 12–20). Thus, AEV and MC29 define two additional subgroups of avian tumor viruses with sarcomagenic properties (1, 20).

Here we analyze the Fujinami sarcoma virus (FSV) (3, 21) to determine how this virus is related to the known avian tumor viruses with sarcomagenic properties. Sarcoma formation and transformation of cells in culture are the only oncogenic activities that have been reported for this virus (3, 21–25). We have found that FSV has a unique transforming *onc* gene that is unrelated to the *src* gene of RSV and distinct from those of AEV and MC29.

## RESULTS

**Oncogenic Properties of FSV.** Fig. 1 *A–C* shows the morphology of chicken embryo cells 4–5 days after infection with uncloned FSV (a gift of H. M. Temin), producing $10^2$ focus-forming units (FFU)/ml of FSV, after infection with FSV that was cloned (17) once, producing $10^3$–$10^4$ FFU/ml, and after infection with FSV cloned a second time, producing $10^4$–$10^5$ FFU/ml. Passage of FSV-transformed cells (such as those shown in Fig. 1*C*) at weekly intervals for 4–6 weeks reproducibly resulted in cultures that had almost the morphology of normal fibroblasts (see Fig. 1*A*) and that released virus at a titer of $<10^2$ FFU/ml. Mass cultures and foci of most virus-transformed fibroblasts had a fusiform morphology (Fig. 1). The growth media of transformed cells became viscous at daily or half-daily intervals, presumably due to hyaluronic acid, whose synthesis and secretion are induced by FSV (24, 25). FSV-infected fibroblasts also grew into virus-producing and -nonproducing colonies in agar suspension prepared as described (refs. 13 and 26; see below). Cloned FSV caused tumors in all infected chickens 10–15 days after inoculation, which proved to be fibromyxosarcomas upon histological examination (Fig. 2) as had been observed with the original FSV isolate (21). In different experiments, these sarcomas appeared in the inoculated wing web in nine out of nine or three out of three chickens. The input virus was recovered from cultured cells prepared from these tumors and again induced sarcomas in the wings of six out of six chickens 10 days after inoculation. Examination of the blood failed to show any evidence of leukemia in surviving chickens up to 6 weeks after infection. Under the same conditions, leukemia would have been observed in chickens inoculated with acute leukemia viruses (11). No tumors were observed in

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: FSV, Fujinami sarcoma virus; FAV, FSV-associated helper virus; RSV, Rous sarcoma virus; AEV, avian erythroblastosis virus; kb, kilobase(s); FFU, focus-forming units.

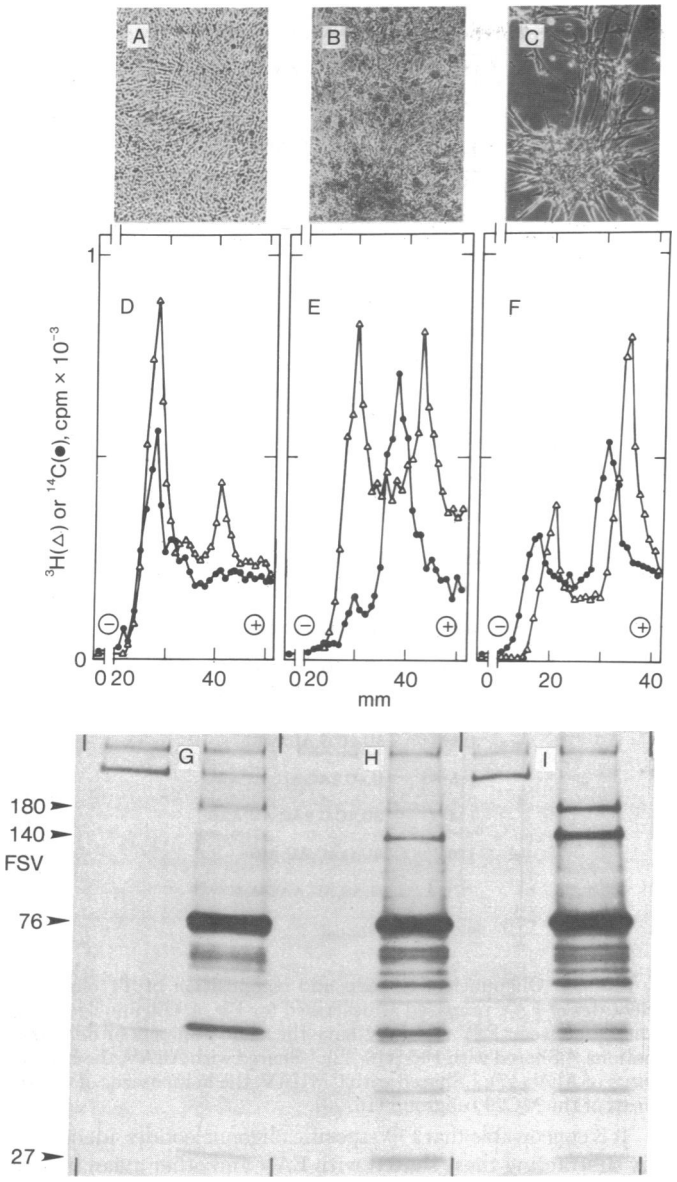


FIG. 1. Morphology, extracellular viral RNA, and intracellular viral proteins of cells infected with FSV(FAV) stocks differing in ratios of FSV to FSV-associated virus (FAV). (*A–C*) Morphology of chicken embryo fibroblasts about 1 week after infection with the uncloned FSV(FAV) stock as obtained from H. Temin (*A*) and with FSV focus-cloned once (*B*) and twice (*C*). (*D–F*) Electrophoresis in 2% polyacrylamide (12, 26) of the [$^3$H]RNA monomers of virus produced by the cells shown in *A*, *B*, and *C*, respectively. RNA standards were 8.5-kb FAV RNA (*D*), 5.7-kb MC29 and 8.5-kb MC29-associated virus RNAs (*E*), and 5.2-kb Moloney sarcoma virus clone 3 and 9.5-kb Moloney leukemia virus RNAs (*F*). △, FSV(FAV); ●, RNA standards. (*G–I*) Fluorographs of FSV(FAV)-specific [$^{35}$S]proteins, immunoprecipitated from lysates of cells shown in *A*, *B*, and *C*, respectively, after electrophoresis in a gradient (6–18%) NaDodSO$_4$/polyacrylamide gel (13, 16). Immunoprecipitation was with normal rabbit serum (left lanes) or antiserum against whole virus (PR RSV-C) (right lanes) (13, 16). Numbers indicate the size in kilodaltons of known viral proteins: Pr180 *gag-pol*, Pr76 *gag*, p27, and the 140-kilodalton FSV protein.

chickens inoculated with FSV-associated helper virus (FAV) (see below).

**The RNA of FSV.** The RNA monomers of the 50–70S FSV RNA complex were analyzed by polyacrylamide gel electrophoresis (26). Two species were resolved (Fig. 1 *D–F*) with estimated sizes of 4.5 and 8.5 kb, based on their electrophoretic mobilities relative to those of 5.7-kb MC29, 5.2-kb Moloney sarcoma clone 3, 9.6-kb Moloney leukemia, and 8.5-kb MC29-associated virus RNA standards (12, 27–29). The size of the 4.5-kb species is similar to that of the 5- to 6-kb RNAs of defective avian acute leukemia viruses (12, 14–17) and of defective murine sarcoma viruses (27–29), whereas the 8.5-kb RNA is typical of nondefective avian leukemia viruses (26, 30). Suggesting that the 4.5-kb RNA species is the genome of FSV.

As shown in Fig. 1, a positive correlation was found between the presence of the 4.5-kb RNA component and both the focus titer of the virus stock from which it was isolated and the number of transformed cells in cultures producing this virus. This implies that the 4.5-kb RNA component carries the transforming function. FSV RNA was directly identified by comparing the RNAs of FSV(FAV) with that of nontransforming FAV. FAV was obtained by diluting the uncloned stock of FSV(FAV) $10^6$-fold, beyond the end point of $10^2$ FFU (31). As shown in Fig. 1, the RNA of FAV was a single electrophoretic species that coelectrophoresed with the 8.5-kb RNA species of the FSV(FAV) stock. We conclude that the 4.5-kb RNA species is the genome of FSV.

**Identification of Specific and Group-Specific Sequences of FSV RNA.** The relationship between 4.5-kb FSV RNA and the RNAs of FAV and other viruses of the avian tumor virus group was determined by RNA·cDNA hybridization. Electrophoretically purified (12, 14–17, 19) FSV RNA was hybridized in separate experiments with cDNAs transcribed from Prague RSV of envelope subgroup B (PR-B) and from a transformation-defective (td), *src* deletion of PR-B (12, 14, 15, 17). At concentrations at which these cDNAs would hybridize over 90% of their RNA templates, 33 ± 5% or FSV RNA was hybridized by each, or by equimolar mixtures of these cDNAs. This indicates that 67%, or 3 kb, of FSV RNA is specific. About 33%, or 1.5 kb, of FSV RNA is related to these viruses of the avian tumor virus group and are therefore termed group-specific, following previous definition (1, 14). Because PR-B cDNA hybridized the same percentage of FSV RNA as td PR-B cDNA, it followed that FSV shares no major specific sequence with the *src* gene of RSV (1, 4–7).

A more stringent, albeit less complete, measure of sequence relationship than is afforded by hybridization is obtained by identifying RNase T1-resistant oligonucleotides shared between FSV and other viruses of the avian tumor virus group. Two-dimensional electrophoresis/chromatography of the T1 oligonucleotides of FSV and FAV RNAs are shown in Fig. 3. The RNase A-resistant sequences of the oligonucleotides numbered in Fig. 3 are reported in Figs. 4 and 5. FSV RNA shares four out of fifteen large oligonucleotides with FAV, which probably represent the group-specific sequences identified above by hybridization. Two of these (i.e., nos. 1 and 5)

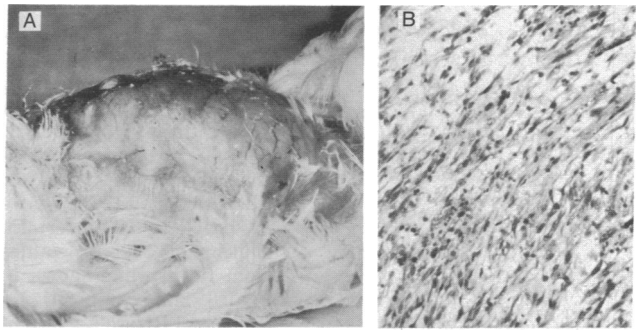


FIG. 2. (*A*) Fibromyxosarcoma in the wing of a 4-week-old chicken 26 days after inoculation with 0.1 ml of filtrate (0.45 μm) of a cloned FSV stock into the wing web. (*B*) Photomicrograph of a sarcoma in the wing web of a chicken. It shows a malignant, spindle cell tumor with areas of collagenization alternating with myxoid change. The histology is characteristic of fibromyxosarcoma. (Hematoxylin and eosin; ×40.)

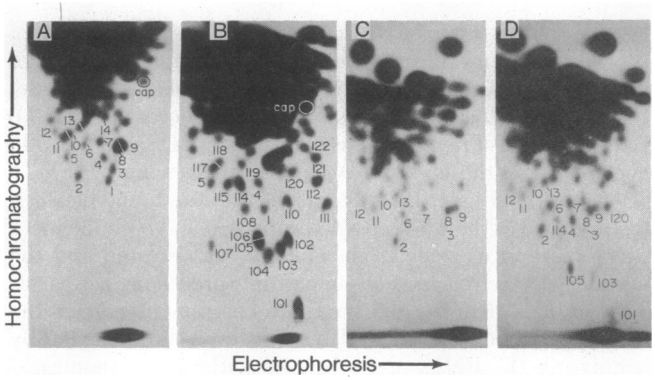


FIG. 3. Autoradiographs of the RNase T1-resistant oligonucleotides of 4.5-kb (*A*) and 8.5-kb (*B*) RNA components of FSV(FAV), and FSV RNA sequences unrelated to FAV RNA (*C*) and to RNAs of other avian tumor viruses (*D*) after two-dimensional chromatography (fingerprinting). (*A* and *B*) The 4.5-kb and 8.5-kb RNA components of FSV(FAV) stocks were prepared electrophoretically and, after digestion with RNase T1, subjected to electrophoretic/homochromatographic fingerprint analysis as described (12, 14–17). (*C*) T1 oligonucleotides of FSV RNA sequences unrelated to FAV were prepared by first hybridizing 1 $\mu$g of FSV(FAV) cDNA to 10 $\mu$g of FAV RNA for 12 hr at 40°C in 10 $\mu$l of 70% (vol/vol) formamide/0.3 M NaCl/30 mM Na citrate/15 mM phosphate at pH 7 (12, 14) and then to 0.25 $\mu$g of FSV(FAV) [$^{32}$P]RNA (specific activity $4 \times 10^6$ cpm/$\mu$g) for 1 hr in 20 $\mu$l under the above conditions. After digestion of unhybridized RNA with RNases A and T1, the hybrid was isolated, melted (14), and fingerprinted. (*D*) T1 oligonucleotides of FSV and FAV RNA sequences unrelated, or poorly related, to Prague RSV-B, MC29, and AEV were prepared as described for *C* except that 1 $\mu$g of FSV(FAV) cDNA was prehybridized with 10 $\mu$g each of the above RNAs prior to hybridization with FSV(FAV) [$^{32}$P]RNA.

are highly conserved sequences probably belonging to the *gag* genes of other nondefective avian tumor viruses (6, 32) as well as of the defective acute leukemia viruses, MC29 and AEV, which carry *gag*-related sequences (Figs. 4 and 5) (12, 14, 16, 17). However, FSV lacks other conserved *gag* oligonucleotides (32). FSV RNA also lacked oligonucleotides that have been diagnosed as conserved markers of the *pol* and *env* genes in nondefective avian tumor viruses, signaling the absence of these genes in FSV. FSV contained none of the conserved oligonucleotides of *src* and none of the specific sequences of MC29 or AEV subgroups of avian acute leukemia viruses (14, 16, 17, 19, 20).

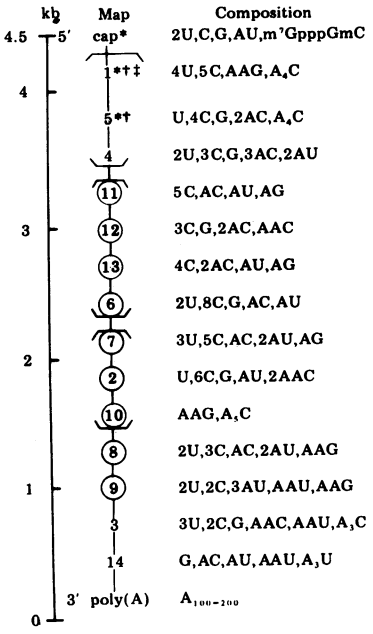


FIG. 4. Oligonucleotide map and composition of T1 oligonucleotides of FSV. Mapping of oligonucleotides in kb units from the 3′ poly(A) terminus of viral RNA has been described (6). The order of oligonucleotides in brackets is uncertain. Elution of oligonucleotides from fingerprints shown in Fig. 3 and compositional analysis of RNase A-resistant fragments followed published procedures (5, 6). FSV-specific oligonucleotides are circled. * Shared with MC29 (12, 19); † Shared with AEV (17); ‡ Shared with PR-B (6, 32).

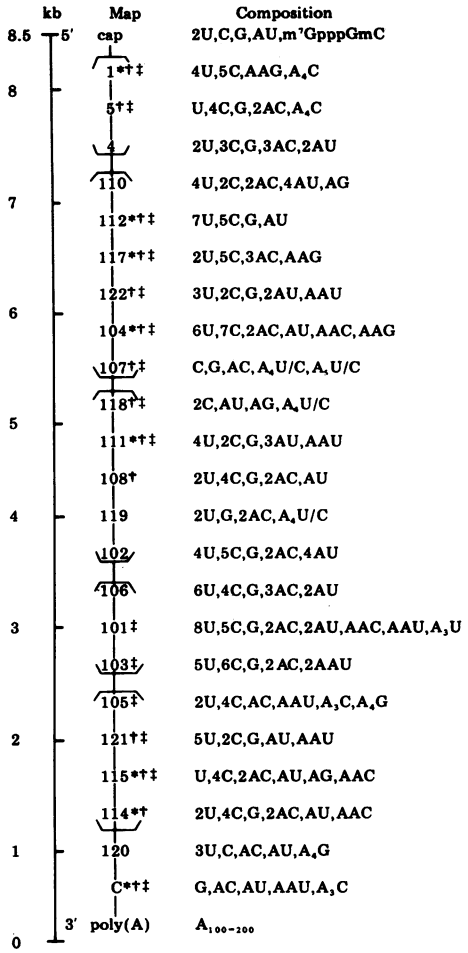


FIG. 5. Oligonucleotide map and composition of T1 oligonucleotides of FAV prepared as described for Fig. 4. Oligonucleotides shared between FSV and FAV have the same numbers or denominations. * Shared with PR-B (6, 32); † Shared with AEAV, the helper virus of AEV (17); ‡ Shared with CMIIAV, the helper virus of CMII virus of the MC29 subgroup (16, 20).

It is conceivable that FSV-specific oligonucleotides, identified by subtracting those shared with FAV and other avian tumor viruses, represent FSV RNA sequences related to but not identical with FAV, PR-B, MC29, or AEV. To test for this possibility, we searched for specific oligonucleotides from FSV RNA sequences that are subject to competition by RNAs from other viruses of the avian tumor virus group during hybridization with FSV cDNA. For this purpose, FSV(FAV) [$^{32}$P]RNA was hybridized with cDNA from FSV and FAV that had been prehybridized with a large excess of unlabeled FAV RNA. Under these conditions, only FSV-specific sequences, unrelated to FAV RNA, can form hybrids. The RNA of such hybrids was found to contain all FSV-specific oligonucleotides defined above by subtraction, except for no. 3 and 14 (Fig. 3*C*; see below). The specific FSV oligonucleotides were also not subject to competition by the RNAs of RSV, MC29, and AEV (Fig. 3*D*). In addition, oligonucleotides no. 3 and 4 of FSV and no. 4, 101, 103, 105, 114, and 120 of FAV were not, or were poorly, competed for by the RNAs of these other viruses. These would be derived from hybrids involving group-specific cDNA sequences of FSV or FAV not closely related to their allelic counterparts in other viruses. We conclude that, of the 11 FSV oligonucleotides not identically shared with FAV, two (no. 3 and 14) have related counterparts in FAV and other viruses and therefore represent group-specific sequences.

**Oligonucleotide Maps of FSV and FAV RNA.** Oligonucleotide maps were prepared to locate particular oligonucleo-

tides in the RNAs of FSV and FAV. The method of ordering oligonucleotides relative to the 3′ poly(A) coordinate of viral RNA by fingerprinting poly(A)-tagged RNA fragments of discrete size classes has been described (6, 32). The maps (Figs. 4 and 5) show the 5′ cap oligonucleotide at the top and the other oligonucleotides in the order of their decreasing distance from the 3′ poly(A) coordinate. Figs. 4 and 5 show that the 5′ 1-kb section of FSV RNA shares four oligonucleotides [i.e., no. 5′ cap, 4, and probable *gag* oligonucleotides (32) no. 1 and 5] with FAV. The 3′ terminus of FSV RNA includes group-specific oligonucleotides no. 3 and 14. No. 14, resolved as a unique oligonucleotide only in short poly(A)-tagged RNA fragments (not shown), is a variant of the C-oligonucleotide conserved in many other avian tumor viruses (6, 32), including FAV (Fig. 5). The specific FSV oligonucleotides (circled in Fig. 4) form a contiguous 3-kb RNA section and so represent helper-unrelated, specific genetic information.

**A *gag* Gene-Related, 140-Kilodalton Nonstructural Protein Is Coded by FSV.** As shown in Figs. 1 *G–I* and 6, a specific protein estimated at 140 kilodaltons from its electrophoretic mobility relative to those of known viral and other (not shown) marker proteins was immunoprecipitated from lysates of FSV-transformed cells by serum against disrupted Prague RSV. Other proteins shown in Figs. 1 and 6 (which are processing intermediates of Pr76 or incomplete translation products) are not FSV specific because they were not found in FSV-transformed nonproducer cells (Fig. 6*D*). The intracellular ratio of the 140-kilodalton protein to the helper viral *gag* gene product, Pr76, increased in parallel both with the ratio of the 4.5-kb FSV to 8.5-kb FAV RNA in virus released [as observed previously with the MC29 virus complex (19)] and also with the degree of transformation of infected cultures (Fig. 1). The 140-kilodalton protein, but not the helper viral proteins, was also found in FSV-transformed nonproducer cells (Fig. 6*D*) and was absent from FAV-infected cells (not shown). This indicates that the 140-kilodalton protein is coded for by FSV RNA and is involved in transformation. Immunoprecipitation with specific antisera against the *gag* proteins p27 and p19 demonstrated that the 140-kilodalton FSV protein is related to one or both of these *gag* gene products (Fig. 6*B*). However, the 140-kilodalton protein was not found to be a major component in extracellular virus (not shown). Antiserum against glycoprotein, which precipitated the FAV glycoprotein precursor Pr95, and antiserum against DNA polymerase, which precipitated the polymerase precursor Pr180, both failed to precipitate the 140-kilodalton FSV protein (Fig. 6*B*). *In vitro* translation of virion RNA further proved that FSV RNA, but not FAV RNA, is the template for a 140-kilodalton protein (Fig. 6*A*) which is indistinguishable from the 140-kilodalton protein synthesized *in vivo* both on the basis of its electrophoretic (Fig. 6*C*) and immunological (not shown) properties. Translation of discrete size classes of poly(A)-selected FSV(FAV) RNA demonstrated that full-length 4.5-kb (26S) FSV RNA was the template of the 140-kilodalton protein and that full-length 8.5-kb FAV RNA was the template for the Pr76 protein (Fig. 6*A*). Cell-free translation did not reveal any other FSV-specific proteins. We conclude that the 140-kilodalton FSV protein is a *gag*-related, nonstructural protein that is serologically unrelated to the *env* and *pol* gene products of avian tumor viruses.

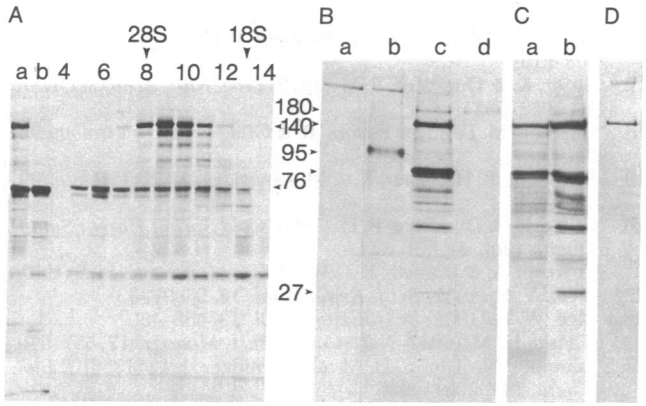


FIG. 6. The 140-kilodalton FSV protein. *In vitro* translation from 4.5-kb FSV RNA (*A*), serological analysis of the *in vivo* protein (*B*), comparison of *in vitro* and *in vivo* proteins (*C*), and detection of the protein in FSV-transformed nonproducer cells (*D*). (*A*) Electrophoresis in a linear 7.5% polyacrylamide gel (14) of [$^{35}$S]proteins translated in a rabbit reticulocyte lysate from heat-dissociated, poly(A)-selected FSV(FAV) RNA (lane a) and FAV RNA (lane b); translation of different size classes of poly(A)-selected FSV(FAV) RNA fractionated by glycerol gradient sedimentation (fractions 4–14) (14). Positions of the 28S and 18S rRNA standards are from a parallel gradient. (*B*) Proteins from lysates of FSV(FAV)-infected quail cells were immunoprecipitated with normal rabbit serum (lane a), antiserum to the envelope glycoprotein of PR RSV-C (lane b), antiserum to the *gag* gene proteins p27 and p19 (lane c), and antiserum to the viral DNA polymerase (a gift of H. Oppermann and J. M. Bishop) preabsorbed with 1 μg of Nonidet P-40-disrupted PR RSV-C per μl of serum (lane d), as described (13, 16, 19). (*C*) Coelectrophoresis as in *B* of proteins translated *in vitro* (lane a) as in *A*, and of proteins precipitated from lysates of FSV(FAV)-infected quail cells with antiserum against whole virus serum (lane b). (*D*) Lysates from FSV-transformed chicken cell nonproducer clone 8 were immunoprecipitated with antiserum to the *gag* gene proteins p27 and p19, as in *B*. Electrophoresis in *B*, *C*, and *D* was as in Fig. 1. The numbers between *A* and *B* indicate the sizes of viral proteins in kilodaltons (see Fig. 1*C*).

## DISCUSSION

**FSV Defines a Distinct Subgroup of Sarcomagenic Avian Tumor Viruses.** The 4.5-kb RNA of FSV is the smallest tumor virus RNA with oncogenic function described to date. The relatively small size of FSV RNA, the lack of some or all sequences of the three essential virion genes (*gag*, *pol*, and *env*), and the ability of the virus to transform cells in the absence of virus production indicate that FSV is replication defective. The genetic structure of FSV RNA resembles that of defective avian acute leukemia viruses (1, 12, 14, 16, 17, 20) and also those of defective mammalian sarcoma viruses (1, 33). Like these viruses, FSV RNA has an internal, specific RNA sequence flanked by terminal group-specific sequences, the 5′-terminal sequence being *gag*-related and the 3′-terminal sequence related to the conserved c region of avian tumor viruses (5). Furthermore, the 140-kilodalton *gag* gene-related, nonstructural FSV protein resembles *gag*-related, nonstructural proteins that are diagnostic of other defective transforming avian (13, 14, 16–20) and mammalian (34, 35) tumor viruses. Nevertheless, the size and specific sequences of RNA and protein of FSV are unique.

In view of the similar sarcomagenic properties of FSV and RSV (see above and refs. (3, 21–25)), it is a particular surprise that neither the primary nor the genetic structure of FSV RNA bears resemblance to the *src* gene of RSV (5, 6, 32) nor that the 140-kilodalton FSV protein resembles the 60-kilodalton *src* gene product (8, 9).

Our analysis does not confirm two earlier reports, one by Stehelin *et al.* (7) and the other, more recent, by Wang *et al.* (36), that FSV contains the *src* gene of RSV. However, the presence of the *src* gene in the presumed FSV stock, which was obtained by Stehelin *et al.* and by us from P. K. Vogt, has been confirmed (unpublished data). The discrepancy must be a consequence of mistaken identity. Defective RSV and FSV may have been easily confused in biological assays measuring only sarcomagenic function, particularly because other diagnostic, biological markers are not available in FSV, as is typical for *gag*-, *pol*-, and *env*-defective transforming viruses (1, 20). It may be argued that the true FSV is indeed like RSV and that

we have identified a previously unidentified virus. However, this appears unlikely in view of the history of the virus described here. Our FSV stock was obtained by H. M. Temin from H. Rubin in 1958, both then at California Institute of Technology. Rubin had received it in February 1958 from C. H. Andrewes in London, who has acknowledged receiving virus from W. E. Gye (ref. 37: C. H. Andrews, personal communication). Gye had received it directly from A. Fujinami (22).

From specific RNA sequences that are unrelated to essential *gag*, *pol*, and *env* virion genes as a basis for subclassification (1), FSV is defined as prototype of a distinct subgroup of oncogenic avian tumor viruses. This subgroup also differs from the RSV subgroup of avian sarcoma viruses and the MC29 and AEV subgroups of acute leukemia viruses in specific, nonstructural proteins. Nevertheless, the oncogenic spectrum of FSV strongly overlaps with that of the RSV RNA subgroup (3, 21–25) and, to a lesser degree, with that of the MC29 and AEV RNA subgroups (10, 11). Further biological testing of FSV is necessary to fathom the extent of overlap among the oncogenic properties of these viruses. The presence of multiple sarcomagenic RNA subgroups in the avian tumor virus group is mirrored by the murine Moloney and the Harvey–Kirsten sarcoma virus RNA subgroups (1) and the two RNA subgroups of feline sarcoma viruses (38). Because all known defective sarcomagenic RNA viruses have a genetic structure similar to that of FSV (1, 20, 33) but unlike that of RSV (1, 4–6), it would appear that RSV, rather than FSV, is an exceptional sarcoma virus.

**The *onc* Gene of FSV.** The transforming *onc* genes of defective transforming tumor viruses have not been defined genetically because *onc* deletion mutants are biologically undetectable and because recombinants are difficult to define for lack of secondary markers (1, 19, 20). Moreover, known gene products of defective transforming viruses typically represent only a fraction of the coding capacity of their RNAs (1, 14, 16–20, 33–35, 39). Although such products have been proposed as having transforming functions (1, 14, 16, 20, 35, 39), the failure to account for all genetic information of a defective virus in terms of protein products leaves open the question as to whether additional, unidentified gene products are also necessary for transformation by these viruses. Because in FSV the genetic complexities of the 4.5-kb FSV RNA and the 140-kilodalton FSV protein approximately coincide, and because the 140-kilodalton FSV protein is a nonstructural protein and also present in nonproducer cells, it appears likely that the *onc* gene of FSV is identical with the viral RNA and that the 140-kilodalton protein is its only product and, therefore, a transforming protein. A more accurate analysis of the complexity of the viral RNA and protein, as well as genetic data, would be necessary to validate this proposal.

The analysis of FSV and previous analyses of the *onc* genes of the RSV, MC29, and AEV RNA subgroups of avian tumor viruses indicate that at least four different classes of *onc* genes that have sarcomagenic potential exist in the avian tumor virus group. Unless the products of each of these *onc* genes are functionally similar it would follow that multiple mechanisms for sarcomagenic transformation exist in avian cells.

**Note Added in Proof.** The 140 kilodalton FSV protein is a phosphoprotein *in vivo* and is also phosphorylated *in vitro* by itself or by an associated kinase. By contrast, the *src* gene product of RSV primarily phosphorylates *src* antibody (9), and the nonstructural, *gag*-related protein of AEV does not phosphorylate itself or its antibody (unpublished data).

We thank H. M. Temin for providing FSV and for critical discussion and encouragement and G. S. Martin for interest and generous support for one of us (A.P.). We are grateful to R. Alexander (Gainesville, FL) for histological diagnosis of the tumors. Informed of our results, H. Hanafusa told us about two presumed Fujinami virus stocks studied in his laboratory, one similar to RSV (ref. 36) and the other (unpublished) similar to that described here. This work was supported by National Institutes of Health Grants CA 11426, 17542, and 10697, by a Senior Fellowship of the Leukemia Society of America, Inc. to A.P., and by funds from the Veterans Administration.

1. Duesberg, P. H. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **44**, 13–29.
2. Tooze, J. (1973) *The Molecular Biology of Tumour Viruses* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
3. Gross, L. (1970) *Oncogenic Viruses* (Pergamon, Elmsford, NY).
4. Lai, M.-C., Duesberg, P. H., Horst, J. & Vogt, P. K. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 2266–2270.
5. Wang, L.-H., Duesberg, P. H., Kawai, S. & Hanafusa, H. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 447–451.
6. Wang, L.-H., Duesberg, P. H., Beemon, K. & Vogt, P. K. (1975) *J. Virol.* **16**, 1051–1070.
7. Stehelin, D., Guntaka, R. V., Varmus, H. E. & Bishop, J. M. (1976) *J. Mol. Biol.* **101**, 349–365.
8. Brugge, J. S. & Erikson, R. L. (1977) *Nature (London)* **269**, 346–348.
9. Collet, M. S. & Erikson, R. L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2021–2024.
10. Carr, J. G. (1956) *Br. J. Cancer* **10**, 379–383.
11. Beard, J. W., Langlois, A. J. & Beard, D. (1973) in *Unifying Concepts of Leukemia*, Biblioteca Haematologica, eds. Dutcher, R. M. & Cheico-Pianchi, L. (Karger, Basel), Vol. 39, pp. 31–44.
12. Duesberg, P. H., Bister, K. & Vogt, P. K. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4320–4324.
13. Bister, K., Hayman, M. & Vogt, P. K. (1977) *Virology* **82**, 431–448.
14. Mellon, P., Pawson, A., Bister, K., Martin, G. S. & Duesberg, P. H. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5874–5878.
15. Duesberg, P. H. & Vogt, P. K. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1633–1637.
16. Bister, K., Löliger, H.-C. & Duesberg, P. H. (1979) *J. Virol.* **32**, 208–219.
17. Bister, K. & Duesberg, P. H. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5023–5027.
18. Hayman, M. J., Royer-Pokora, B. & Graf, T. (1979) *Virology* **92**, 31–45.
19. Duesberg, P. H., Bister, K. & Moscovici, C. (1979) *Virology* **99**, 121–134.
20. Bister, K. & Duesberg, P. H. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **44**, 801–822.
21. Fujinami, A. & Inamoto, K. (1914) *Z. Krebsforschung* **14**, 94–119.
22. Gye, W. E. (1931) *Br. J. Exp. Pathol.* **12**, 93–97.
23. Gye, W. E. (1932) *Br. J. Exp. Pathol.* **13**, 458–460.
24. Temin, H. M. (1964) *Natl. Cancer Inst. Monogr.* **17**, 557–570.
25. Ishimoto, N., Temin, H. M. & Strominger, J. L. (1966) *J. Biol. Chem.* **241**, 2051–2057.
26. Duesberg, P. H. & Vogt, P. K. (1973) *Virology* **54**, 207–219.
27. Maisel, J., Dina, D. & Duesberg, P. H. (1977) *Virology* **76**, 295–312.
28. Maisel, J., Bender, W., Hu, S., Duesberg, P. H. & Davidson, N. (1978) *J. Virol.* **25**, 384–394.
29. Maisel, J., Klement, V., Lai, M.-C., Ostertag, W. & Duesberg, P. H. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 3536–3540.
30. Duesberg, P. H., Vogt, P. K., Beemon, K. & Lai, M. (1975) *Cold Spring Harbor Symp. Quant. Biol.* **39**, 847–857.
31. Rubin, H. & Vogt, P. K. (1962) *Virology* **17**, 184–194.
32. Wang, L.-H. (1978) *Annu. Rev. Microbiol.* **32**, 561–592.
33. Sherr, C. J., Fedele, L. A., Donner, L. & Turek, L. P. (1979) *J. Virol.* **32**, 860–875.
34. Stephenson, J. R., Khan, A. S., Shski, A. & Essex, M. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5608–5612.
35. Witte, O. M., Rosenberg, N., Paskind, M., Shields, A. & Baltimore, D. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2488–2492.
36. Wang, L.-H., Snyder, P., Hanafusa, T., Moscovici, C. & Hanafusa, H. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **44**, in press.
37. Andrewes, C. H. (1931) *J. Pathol. Bacteriol.* **34**, 91–107.
38. Frankel, A. E., Gilbert, J. H., Porzig, K. J., Scolnick, E. M. & Aaronson, S. A. (1979) *J. Virol.* **30**, 821–827.
39. Scolnick, E. M., Papageorge, A. G. & Shih, T. Y. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5355–5359.