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Isolation of 16L virus: A rapidly transforming sarcoma virus from an avian leukosis virus-induced sarcoma

(avian retrovirus/recombination/Fujinami sarcoma virus/*onc* genes/transforming proteins)

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ABSTRACT **We have isolated a replication-defective rapidly transforming sarcoma virus (designated 16L virus) from a fibrosarcoma in a chicken infected with td107A, a transformation-defective deletion mutant of subgroup A Schmidt–Ruppin Rous sarcoma virus. 16L virus transforms fibroblasts and causes sarcomas in infected chickens within 2 wk. Its genomic RNA is 6.0 kilobases and contains sequences homologous to the transforming gene (*fps*) of Fujinami sarcoma virus (FSV). RNase T1 oligonucleotide analysis shows that the 5′ and 3′ terminal sequences of 16L virus are indistinguishable from (and presumably derived from) td107A RNA. The central part of 16L viral RNA consists of *fps*-related sequences. These oligonucleotides fall into four classes: (*i*) oligonucleotides common to the putative transforming regions of FSV and another *fps*-containing avian sarcoma virus, UR1; (*ii*) an oligonucleotide also present in FSV but not in UR1; (*iii*) an oligonucleotide also present in UR1 but not in FSV; and (*iv*) an oligonucleotide not present in either FSV, UR1, or td107A. Cells infected with 16L virus synthesize a protein of M_r 142,000 that is immunoprecipitated with anti-gag antiserum. This protein has protein kinase activity. These results suggest that 16L virus arose by recombination between td107A and the cellular *fps* gene.**

Avian leukosis viruses (ALV) are a group of slowly transforming viruses that usually cause B-cell lymphomas arising in the bursa of Fabricius of infected birds after a latent period of 4–12 months. However, occasionally these viruses also induce other neoplasms, such as erythroblastosis, nephroblastoma, and fibrosarcoma (1, 2). Slowly transforming viruses such as ALV lack a specific gene (*onc* gene or transforming gene) whose product is responsible for neoplastic transformation. ALV differ from the rapidly transforming viruses, such as the avian sarcoma viruses and acute leukemia viruses, which cause neoplastic disease rapidly and with high efficiency. In recent years, a wide variety of biochemical and genetic evidence has established that the transforming ability of rapidly transforming viruses resides in *onc* genes that have been transduced from the normal cellular genome. Transformation by these viruses appears to be the consequence of placing this normal cellular gene under viral transcriptional control.

Recently, we have shown that most B-cell lymphomas caused by ALV appear to result from proviral integration adjacent to the cellular counterpart (c-*myc*) of the transforming gene of the avian rapidly transforming virus MC29 (3, 4). This results in activated transcription of the c-*myc* locus, usually with the synthesis of new transcripts containing both viral 5′- and c-*myc*-related information. However, no rapidly transforming virus is produced from these tumors, presumably because the new transcripts lack viral information (3) necessary for viral RNA packaging (5, 6) and replication (7, 8). These findings have been confirmed in other laboratories (9–12).

In the course of our studies of ALV oncogenesis, we encountered one chicken that developed a fibrosarcoma after a long latent period. This tumor contained high levels of RNA related to the transforming gene (*fps*) of Fujinami sarcoma virus (FSV) and expressed a new 6.0-kilobase (kb) virus-specific RNA. We originally thought that oncogenesis in this tumor had resulted from activation of the cellular counterpart (c-*fps*) (13) of the *fps* gene by a mechanism similar to that involved in ALV lymphomagenesis. However, extracts of this tumor contained a rapidly transforming virus (designated 16L virus) that appears to be a recombinant between the infecting ALV and c-*fps*.

MATERIALS AND METHODS

Cells and Viruses. Primary and secondary chicken embryo fibroblast (CEF) cultures were prepared from group-specific antigen-negative, chicken helper factor-negative chicken embryos as described (14). Cell culture conditions and virus titration methods were those of Hanafusa (14). FSV, td107A, and UR1 were obtained from laboratory stocks.

16L virus was isolated from liver tumors present in chicken no. 16, which was infected with td107A by intravenous injection of virus into an 11-day embryo. The tumor, which had been frozen, was homogenized by grinding in a mortar containing extraction solution (Tris/saline containing 5% fetal calf serum) to give 20% (wt/vol) homogenates. The resulting suspension was centrifuged, and the supernatant was used to inoculate secondary CEF cultures treated with DEAE-dextran. These cultures were overlaid with agar and examined at 1 wk for evidence of transformation. Initial virus titers were 10^3 to 10^4 focus-forming units/ml. Confluently transformed cultures were obtained after several cycles of transfer and agar overlay. Virus produced from such cultures had a titer of about 10^7 focus-forming units/ml and was used for all subsequent experiments.

Tumor RNA Analysis. Tumor RNA was extracted as described (3). cDNA probes for ALV genes and v-*onc* genes and hybridization kinetic analysis were as described (3, 4, 15).

Viral RNA Isolation. CEF cultures confluently transformed by 16L virus were labeled with carrier-free $^{32}P_i$ (1 mCi/ml; 1 Ci = 3.7×10^{10} becquerels) for 12 hr as described (16). The 12-hr supernatant was discarded and culture fluids were then collected at 2-hr intervals for 10 hr. To avoid RNA degradation, cellular debris were removed immediately. Virus was pelleted

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Abbreviations: ALV, avian leukosis virus(es); CEF, chicken embryo fibroblast(s); FSV, Fujinami sarcoma virus; rASV, recovered avian sarcoma virus(es); *fps*, transforming gene of FSV and related viruses; kb, kilobase(s).

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by ultracentrifugation, and 60–70S RNA was extracted as described. This RNA was heat-denatured and poly(A)-selected by oligo(dT)-cellulose chromatography. Poly(A)-RNA was size-fractionated by sucrose density gradient centrifugation. Fractions from the 35S ("helper") and 30S ("16L") regions of the gradient were pooled separately and precipitated with ethanol.

5′-End Selection of Viral RNA. $cDNA_{5'}$ (7, 8) was prepared in large amounts as described (17). Deoxycytidylic acid residues were added to the 3′ end of this cDNA with polynucleotide kinase. After hybridization of ^{32}P-labeled viral RNA to the deoxycytidylic acid-tailed cDNA, RNA molecules containing viral 5′ information were selected by chromatography over oligo(dG)-cellulose as described (17).

RNase T1 Oligonucleotide Mapping. ^{32}P-Labeled and selected 35S and 30S RNAs were analyzed by RNase T1 digestion and oligonucleotide mapping as described (16, 18). The nucleotide composition of selected oligonucleotides was determined by published methods (16, 18, 19).

Protein Analysis. Cell cultures were labeled with [^{35}S]methionine and extracted as described (20). Virus-related proteins were analyzed by immunoprecipitation of the cell extract with anti-gag antiserum, followed by electrophoresis on $NaDodSO_4$/polyacrylamide gels (21) as described (20). Preparation and properties of the antisera have been described (22). Antiserum specific for the *fps* gene product was prepared from rats immunized with FSV-infected rat cells (23). *In vitro* protein kinase assay was performed on immunoprecipitates as described (22).

RESULTS

Biological Properties of 16L Virus. Several embryos were infected with td107A, a deletion mutant of subgroup A Schmidt–Ruppin Rous sarcoma virus (24) that lacks most, if not all, of the *src* gene and does not give rise to recovered avian sarcoma virus (rASV) (25). Instead, chickens infected with td107A usually develop B-cell lymphomas after a long latent period. However, upon sacrifice at 19 wk, one chicken (no. 16) had multiple large nodules in the liver, kidneys, heart, and peritoneal wall, with no bursal involvement. Histopathologic analysis revealed that the tumors were fibrosarcomas.

When a liver nodule was homogenized and an aliquot of the supernatant fluid was plated on CEF, discrete foci of transformed cells were observed (data not shown). Interference assay (data not shown) revealed the presence of a subgroup A retrovirus. Injection of the supernatants into the wing webs of 1–2 day-old chickens led to palpable tumors in 5–7 days and death in 10–14 days (10/10 birds). No FSV strains or other *fps*-containing retroviruses were being injected into laboratory flocks at this time nor were any other unexplained sarcomas noted in other birds.

Size and Genetic Content of 16L RNA. Table 1 shows the results of hybridization kinetic analysis of RNA from a liver tumor (16L) as well as a few other representative tumors induced by td107A and other leukosis viruses. Whereas the vast majority of ALV-induced tumors are lymphomas, which show elevated *myc* expression (see ref. 4), tumor 16L expressed *fps*-related information at about 300 times normal level. A kidney nodule taken from the same bird (animal no. 16) also exhibited increased expression of *fps*. The level of expression of *fps* in normal muscle tissue from bird no. 16 was not elevated.

Poly(A)-RNA from tumor 16L was analyzed by blot hybridization (26). A new 6.0-kb RNA was found in addition to the expected 8.1- and 3.2-kb td107A mRNAs (15, 27) (data not shown). However, unlike the new RNAs found in ALV-induced lymphomas—which have only 5′-specific viral information—the 6.0-kb RNA hybridized to a probe ($cDNA_{rep}$) representative of the total ALV genome. In addition, this RNA hybridized to a molecularly cloned probe for the v-*fps* gene of FSV (data not shown).

Table 1. Viral and transformation-specific RNAs in ALV-induced tumor tissues

Tissue*	RNA copies/cell†						
	5′	rep	*src*	*erb*	*myb*	*myc*	*fps*
Bursa, uninfected (12)	<1	<1	3	2	3	2	1
Liver, uninfected (31)	<1	<1	2	4	1	2	1
Kidney, lymphoma (7)	100	2	4	6	5	110	1
Bursa, lymphoma (11)	2,500	2,000	3	2	4	180	2
Muscle, normal (16)	ND	ND	ND	ND	ND	2	<1
Liver, sarcoma (16)	2,900	2,700	4	5	2	8	300
Kidney, sarcoma (16)	4,400	5,700	ND	ND	ND	3	150

* Animal numbers are given in parentheses.

† RNA concentrations were determined by liquid hybridization as described (15). cDNA probes used were: $cDNA_{5'}$, corresponding to the 5′-terminal 101 nucleotides of Rous-associated virus 2 RNA (7, 8); $cDNA_{rep}$, containing a relatively uniform distribution of all Rous-associated virus 2 sequences; unique (presumably transformation-specific) sequences of Rous sarcoma virus (*src*), avian erythroblastosis virus (*erb*), avian myeloblastosis virus (*myb*), avian myelocytomatosis virus (*myc*), and Fujinami sarcoma virus (*fps*). ND, not determined.

Oligonucleotide Mapping of 16L RNA. We further characterized the genetic content of 16L viral RNA by oligonucleotide mapping. After poly(A) selection and sucrose density gradient centrifugation, 35S (helper viral RNA) and 30S (16L transforming component) peaks were observed. Preliminary mapping experiments demonstrated that 30S RNA isolated in this fashion was significantly contaminated with fragments of the 35S species (data not shown). To reduce this contamination, cDNA homologous to the 5′ end of ALV RNA was prepared, extended with poly(dC), and hybridized to 30S RNA. The resulting hybrids were selected by oligo(dG)-cellulose chromatography. Approximately 60% of the poly(A)-containing 30S RNA was recovered following this procedure.

Fig. 1 shows the RNase T1 oligonucleotide patterns of 16L 35S and 30S RNAs; the oligonucleotide compositions of spots found in 16L but not in td107A are shown in Table 2. Comparison of these oligonucleotide patterns with patterns of td107A, FSV, and UR1 RNAs [UR1 is a recently characterized independent isolate of avian sarcoma virus that contains the *fps*

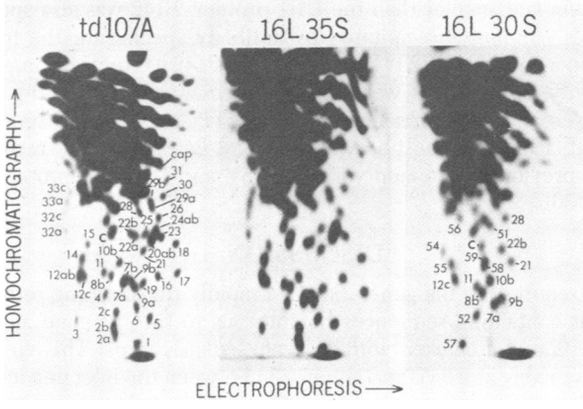

FIG. 1. Chromatographic analysis of RNase T1-resistant oligonucleotides of td107A and 16L viral RNAs. ^{32}P-Labeled viral RNA was purified by sucrose density gradient centrifugation and oligo(dT)-cellulose column chromatography. The 30S component of 16L viral RNA was further purified by hybridization to poly(dC)-tailed $cDNA_{5'}$. Numbering of td107A oligonucleotides followed that in ref. 28.

Table 2. *fps*-related large RNase T1-resistant oligonucleotides present in 16L virus RNA

Spot no.	RNase-resistant fragments
51	1–2 U, 4 C, G, A-C, 2 A-U
52	U, 4 C, G, A-C, 2 A-U, 1.5 A-A-C
54	4 C, G, 2 A-C, A-A-C
55	6 C, 2 A-C, A-U, A-G
56	U, 7 C, G, 0.4 A-C, A-U
57	2 U, 8 C, 4 A-C, A-U, A-G
58 + 59	4 U, 10 C, G, 2 A-C, 3 A-U, A-G

The composition analysis of oligonucleotides was done with spots recovered from the oligonucleotide analysis of purified 30S 16L viral RNA shown in Fig. 1.

gene (29, 30)] shows the following (Figs. I and 2): (*i*) The 35S (helper) component of the 16L virus preparation has an oligonucleotide pattern identical to that of td107A. (*ii*) The 30S component (transforming component) has 5′ and 3′ oligonucleotides indistinguishable from td107A but has a large substitution for sequences in the interior of the td107A genome. (*iii*) The substituted sequence in 16L viral RNA contains four classes of oligonucleotides: (*a*) the majority (5/8) of the oligonucleotides are present in the *fps*-specific sequences of both FSV and UR1; (*b*) one spot, no. 58, is present in FSV but not in UR1; (*c*) another spot, no. 55, is present in UR1 but not in FSV; and (*d*) one spot, no. 59, is not present in td107A, FSV, or UR1 RNAs. (*iv*) The oligonucleotide differences found among 16L, FSV, and UR1 cannot be explained by single base changes (refs. 30 and 31 and Table 2). The simplest interpretation of these data is that the substituted sequence in 16L virus is derived from cellular *fps* (c-*fps*) information. FSV and UR1 presumably also acquired c-*fps* information and subsequently diverged slightly.

Analysis of Virus-Specific Proteins in 16L-Infected Cells. FSV (31) and UR1 (30) encode gag–fps fusion polyproteins that have associated protein kinase activities specific for tyrosine residues and which are the putative transforming proteins of these viruses (30, 32). To analyze 16L virus-specific proteins, CEFs infected with 16L were labeled with [^{35}S]methionine, and virus-specific proteins in the cell lysate were immunoprecipitated and electrophoresed on NaDodSO$_4$/polyacrylamide gels (Fig. 3 *Right*). 16L virus-infected cells synthesized a M_r 142,000 protein (P142) that was precipitated by anti-gag antiserum. Electrophoresis of P142, FSV P140, and UR1 P150 in adjacent lanes indicates that the 16L viral protein is larger than the FSV protein and smaller than the UR1 protein. P142 was also specifically immunoprecipitated by antibody specific for the fps component of P140 (23). To see whether P142 has protein kinase activity, cell extracts were immunoprecipitated with anti-gag antiserum in the presence of [γ-^{32}P]ATP. In such an experiment, P142 was phosphorylated (Fig. 3 *Left*). A similar result was previously obtained with the FSV and UR1 polyproteins (22, 30).

DISCUSSION

We report here the generation of a rapidly transforming retrovirus containing sequences homologous to the *fps* gene after infection of a chicken with an avian leukosis virus. This virus presumably arose via recombination between the infecting leukosis virus and a source of *fps* information.

Several lines of evidence suggest that the *fps* information is of cellular origin: (*i*) The presence of a c-*fps* sequence in normal chicken DNA has been demonstrated (13). (*ii*) The tumor from which the virus was isolated arose at a site distal from virus injection and only after a long latent period. If there were a contaminating acute virus such as FSV or UR1 in the initial virus

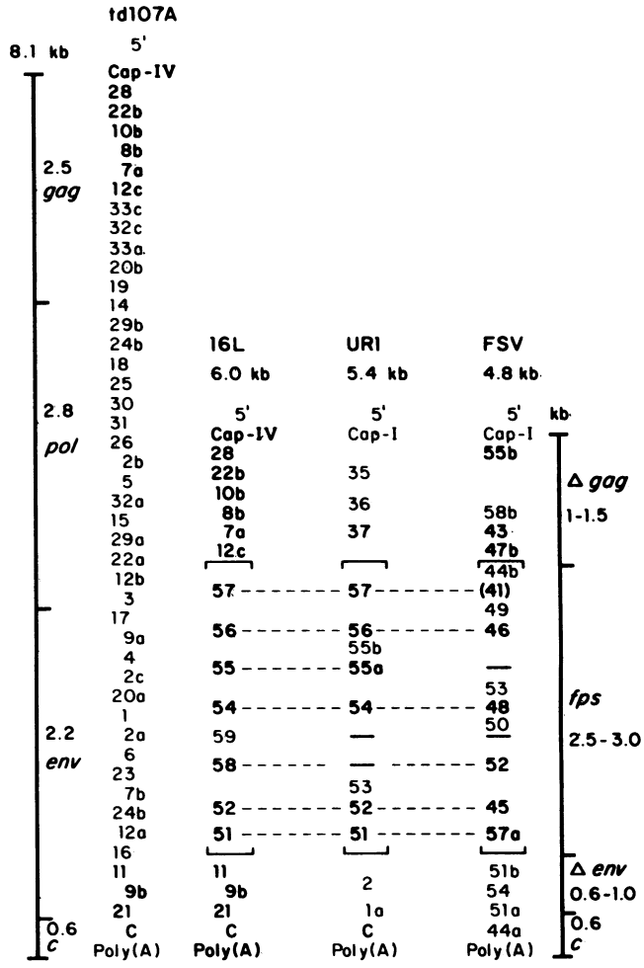

FIG. 2. Oligonucleotide maps of 16L virus and related viruses. The order of RNase T1-resistant oligonucleotides of td107A, UR1, and FSV has been published (28, 30, 31). The oligonucleotide map of 16L 30S RNA was deduced from the analyses shown in Fig. 1. The scales at the left and right represent the genomes of td107A and the three sarcoma viruses. The size of each genetic region is indicated. 16L RNA contains oligonucleotides 9b and 11, which are derived from the gp37 coding region of td107A (unpublished). However, it is not clear whether any of the 3′ oligonucleotides of UR1 or FSV are derived from the *env* gene. 16L virus oligonucleotides are shown in boldface, as are identical oligonucleotides present in td107A, FSV, or UR1. Oligonucleotides within the brackets of each sarcoma viral RNA map are derived from *fps*-related sequences of the corresponding sarcoma virus. In addition to being designated by boldface numbers, shared *fps*-specific oligonucleotides are connected by dashed lines. The base compositions of *fps*-specific oligonucleotides in 16L are shown in Table 2. A — indicates the absence of a particular oligonucleotide at the corresponding position of the map. The precise order of *fps*-related oligonucleotides in 16L viral RNA were not determined in this study. The indicated order of oligonucleotides common to FSV and UR1 is based on the previously determined oligonucleotide maps of these viruses (30, 31). The precise location of oligonucleotide 59 is not certain. Oligonucleotides outside the specific sequence of 16L RNA are identical to the indicated td107A oligonucleotides, and the order of these oligonucleotides shown here is based upon the map of td107A established previously (32). Oligonucleotide 41 has a base composition similar to that of oligonucleotide 57 (Table 2 and ref. 31) except that oligonucleotide 41 is two nucleotides longer. It is not certain that oligonucleotides 41 and 57 represent homologous sequences, so 41 is shown in parentheses. Cap-I and Cap-IV are cap-containing oligonucleotides of different length and composition as described (18).

stock, neoplasia would be expected to develop much more rapidly (2–4 wk) and at the site of injection. (*iii*) No similar neo-

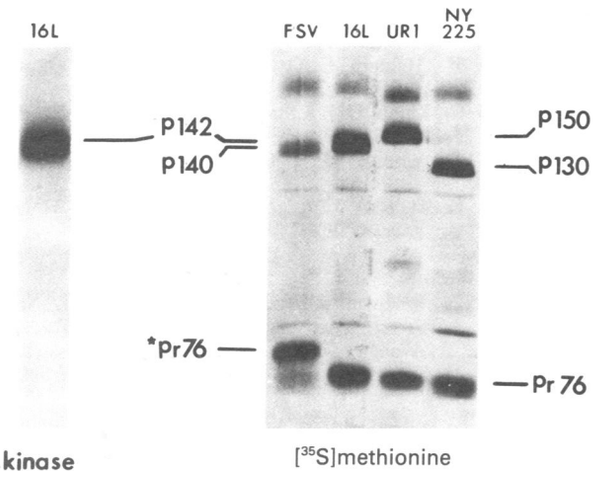

FIG. 3. Identification and properties of 16L P142. (*Left*) A cell lysate was prepared from cell cultures infected with 16L virus. This lysate was immunoprecipitated with anti-gag antiserum, assayed for protein kinase activity, and analyzed by electrophoresis on 5–15% $NaDodSO_4$/polyacrylamide gradient gels. The position of migration of 16L virus P142 is indicated. (*Right*) Cell cultures infected with FSV, 16L, or NY225 (a temperature-sensitive mutant of FSV) were labeled with [^{35}S]methionine. After extraction, cell lysates were immunoprecipitated with anti-gag antiserum and analyzed by polyacrylamide gel electrophoresis as above. The positions of migration of 16L P142, FSV P140, UR1 P150, NY 225 P130, and the gag precursor polyprotein Pr76 are indicated. *Pr76 is the gag precursor polyprotein of the natural helper virus of FSV. This protein migrates more slowly than the gag polyproteins of the other helper viruses used in this study under our electrophoresis conditions.

plasms were noted in other birds infected at the same time with the same virus stock. The virus stock used to infect these birds was cloned several times and was not capable of producing foci on CEF (24). (*iv*) No *fps*-containing virus-infected birds were present in this laboratory at the time of these experiments. (*v*) 16L virus contains some RNase T1 oligonucleotides present in the *fps* sequences of FSV but not UR1 and some present in UR1 but not FSV as well as sequences not present in either of these viruses. Thus, if 16L virus were derived from a viral contaminant, there would have to have been either three contaminating viruses or a new, as yet undescribed, contaminating virus. UR1 was not even available in this laboratory when tumor 16L was obtained. There are some oligonucleotides present in FSV or UR1 or both that are not present in 16L (Figs. 1 and 2). This could represent divergence from the c-*fps* sequence. Alternatively, only part of the c-*fps* gene (or its flanking sequences) may have been transduced into 16L virus. 16L virus appears to be more closely related to UR1 than to FSV. This may reflect the more recent isolation and less extensive passage history of UR1 (29) compared with FSV (33). (*vi*) All of the non-*fps* oligonucleotides in 16L virus that correspond to retroviral structural and replicative genes are found in td107A. These oligonucleotides are distinct from those of the non-*fps* sequences present in either FSV or UR1. If recombination with a viral contaminant had occurred, one might expect 16L virus to contain non-*fps* information from the contaminant as well. (*vii*) No evidence was obtained for the presence in chicken no. 16 of another *fps*-containing virus, which should have its original 5′ and 3′ terminal sequences.

16L virus is a direct example of an avian rapidly transforming virus arising from recombination *in vivo* between a slowly transforming virus and a cellular gene. Earlier, it was shown that viruses (termed rASV) that are recombinants between certain transformation-defective deletion mutants of Rous sarcoma virus and the cellular *src* gene could be isolated after injection of td mutants of Rous sarcoma virus into birds (25, 28, 34, 35). However, rASV were isolated only from mutants that retained a significant part (20–30%) of the viral *src* gene. The genetic structure of the other known avian rapidly transforming viruses (cell-related information inserted between viral 5′ and 3′ sequences) suggests that these viruses also arose via recombination between a slowly transforming virus and a cellular gene. However, in many cases, extensive passage of virus strains *in vivo* and *in vitro* occurred before their biochemical analysis was possible. While this work was in progress, Stavnezer *et al.* (36) reported the isolation of defective rapidly transforming viruses after passage of ALV in CEF derived from a certain line (SPAFAS) of chickens. The reason for the recovery of these viruses at such high efficiency in this particular tissue culture system is unclear.

In the murine system, the Harvey (37) and Kirsten (38) strains of murine sarcoma virus were isolated after serial passage of murine leukemia viruses in rats; Moloney murine sarcoma virus was isolated after leukemia virus passage in mice (39). Similarly, the feline sarcoma viruses (40, 41) were isolated from tumors induced in cats infected with feline leukemia viruses. However, as with the avian rapidly transforming viruses, biochemical analysis of these viruses was not performed at early passage, so no direct comparison could be made with the parent viruses. Recently, recombinant mammalian rapidly transforming viruses have also been obtained in tissue culture (42–45).

It is interesting that the cellular gene incorporated into 16L virus is c-*fps*. *fps*-related information is found in a number of independently derived rapidly transforming viruses of both avian (FSV, UR1, PRCII, 16L) and mammalian (Snyder–Theilen and Gardner–Arnstein strains of feline sarcoma virus) origin (refs. 30, 31, 46–48; this report). In contrast, most other c-*onc* genes have been identified in relatively few independently isolated viruses. Whether this is merely due to the chance isolation of an abnormally large number of *fps*-containing viruses or, instead, reflects some specific property of the c-*fps* gene or its flanking sequences awaits further analysis of the c-*fps* locus.

Although several transforming viruses contain *fps*, the extent and the precise organization of *fps* information differ in different viruses. FSV, UR1, and 16L virus all appear to have approximately the same amount of *fps*, but PRCII has only about 60% of this (ref. 48; this report). Comparison of the sizes of the *fps*-related inserts in feline sarcoma virus (49) and FSV (50) suggests that the feline sarcoma virus *onc* gene (*fes*) shares only part of *fps* as well, and this portion appears to be sufficient to cause cell transformation. The amount of *gag*-related information in the gag–fps fusion proteins also differs (ref. 51; unpublished). Because different viruses have different gag–fps junctions, it is unlikely that *gag* has specific regions necessary for recombination with cellular sequences.

Avian leukosis viruses, and presumably other slowly transforming viruses, appear to be able to induce oncogenesis via two routes. In either case, the fundamental oncogenic mechanism appears to be placing a cellular gene under viral transcriptional control. More commonly, random proviral integration places an ALV long terminal repeat adjacent to the c-*myc* gene of a lymphoid cell (4). The resultant activation of c-*myc* leads to lymphomagenesis. Much more rarely, the infecting ALV can recombine with a c-*onc* gene—for example, c-*fps*—and generate a new rapidly transforming virus such as 16L virus. The failure of earlier workers to isolate such recombinants from chickens experimentally infected with ALV is probably because the combination of events necessary to generate such a virus occurs only rarely.

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1. Purchase, H. G., Okazaki, W. G., Vogt, P. K., Hanafusa, H. & Burmester, B. R. (1977) *Infect. Immun.* **15,** 423–428.
2. Purchase, H. G. & Burmester, B. R. (1978) in *Diseases of Poultry,* eds. Hofstad, M. S., Calnek, B. W., Helmboldt, C. F., Reid, W. M. & Yoder, H. W. (Iowa State University, Ames, Iowa), pp. 418–468.
3. Neel, B. G., Hayward, W. S., Robinson, H. L., Fang, J. & Astrin, S. M. (1981) *Cell* **23,** 323–334.
4. Hayward, W. S., Neel, B. G. & Astrin, S. M. (1981) *Nature (London)* **290,** 475–480.
5. Linial, M., Medeiros, E. & Hayward, W. S. (1978) *Cell* **15,** 1371–1381.
6. Shank, P. R. & Linial, M. (1980) *J. Virol.* **36,** 450–456.
7. Haseltine, W., Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 989–993.
8. Shine, J., Czernilofsky, A. D., Friedrich, R., Bishop, J. M. & Goodman, H. M. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1473–1477.
9. Payne, G. S., Courtneidge, S. A., Crittenden, L. B., Fadley, A. M., Bishop, J. M. & Varmus, H. E. (1981) *Cell* **23,** 311–322.
10. Payne, G., Bishop, J. M. & Varmus, H. E. (1982) *Nature (London)* **295,** 209–214.
11. Fung, Y.-K., Fadley, A. M., Crittenden, L. B. & Kung, H.-J. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3418–3422.
12. Cooper, G. M. & Neiman, P. E. (1981) *Nature (London)* **292,** 857–858.
13. Shibuya, M., Hanafusa, H. & Balduzzi, P. C. (1982) *J. Virol.* **42,** 143–152.
14. Hanafusa, H. (1969) *Proc. Natl. Acad. Sci. USA* **63,** 318–325.
15. Hayward, W. S. (1977) *J. Virol.* **24,** 47–63.
16. Wang, L.-H., Duesberg, P. H., Beemon, K. & Vogt, P. K. (1975) *J. Virol.* **16,** 1051–1070.
17. Wang, L.-H., Hanafusa, H., Notter, M. F. D. & Balduzzi, P. C. (1982) *J. Virol.* **41,** 833–841.
18. Wang, L.-H., Duesberg, P. H., Robins, T., Yokota, H. & Vogt, P. K. (1977) *Virology* **82,** 472–492.
19. Beemon, K., Duesberg, P. & Vogt, P. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4254–4258.
20. Hanafusa, T., Mathey-Prevot, B., Feldman, R. A. & Hanafusa, H. (1981) *J. Virol.* **38,** 347–355.
21. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
22. Feldman, R. A., Hanafusa, T. & Hanafusa, H. (1980) *Cell* **22,** 757–765.
23. Mathey-Prevot, B., Hanafusa, H. & Kawai, S. (1982) *Cell* **28,** 897–906.
24. Kawai, S., Duesberg, P. H. & Hanafusa, H. (1977) *J. Virol.* **24,** 910–914.
25. Hanafusa, H., Halpern, C. C., Buchhagen, D. L. & Kawai, S. (1977) *J. Exp. Med.* **146,** 1735–1747.
26. Alwine, J. C., Kemp, D. J. & Stark, G. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5350–5354.
27. Weiss, S. R., Varmus, H. E. & Bishop, J. M. (1977) *Cell* **12,** 983–992.
28. Wang, L.-H., Halpern, C. C., Nadel, M. & Hanafusa, H. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5812–5816.
29. Balduzzi, P. C., Notter, M. F. D., Morgan, H. R. & Shibuya, M. (1981) *J. Virol.* **40,** 268–275.
30. Wang, L.-H., Feldman, R., Shibuya, M., Hanafusa, H., Notter, M. F. D. & Balduzzi, P. C. (1981) *J. Virol.* **40,** 258–267.
31. Hanafusa, T., Wang, L.-H., Anderson, S. M., Karess, R. E., Hayward, W. S. & Hanafusa, H. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3009–3013.
32. Pawson, T., Guyden, J., Kung, T.-H., Radke, K., Gilmore, T. & Martin, G. S. (1980) *Cell* **22,** 767–775.
33. Fujinami, A. & Inamoto, K. (1914) *Z. Krebsforsch.* **14,** 94–119.
34. Halpern, C. C., Hayward, W. S. & Hanafusa, H. (1979) *J. Virol.* **29,** 91–101.
35. Karess, R. E., Hayward, W. S. & Hanafusa, H. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3154–3158.
36. Stavnezer, E., Gerhard, D. S., Binari, R. C. & Balazs, I. (1981) *J. Virol.* **39,** 920–934.
37. Harvey, J. J. (1964) *Nature (London)* **204,** 1104–1105.
38. Kirsten, W. H. & Mayer, L. A. (1967) *J. Natl. Cancer Inst.* **39,** 311–335.
39. Moloney, J. B. (1966) *Natl. Cancer Inst. Monogr.* **22,** 139–142.
40. Snyder, S. P. & Theilen, G. H. (1969) *Nature (London)* **221,** 1074–1075.
41. Gardner, M. B., Rongey, R. W., Arnstein, P., Estes, J. D., Sarma, P., Huebner, R. J. & Rickard, L. G. (1970) *Nature (London)* **226,** 807–809.
42. Rasheed, S., Gardner, M. B. & Huebner, R. J. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2972–2976.
43. Rapp, U. R. & Todaro, G. J. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 624–628.
44. Goldfarb, M. P. & Weinberg, R. A. (1981) *J. Virol.* **38,** 125–135.
45. Goldfarb, M. P. & Weinberg, R. A. (1981) *J. Virol.* **38,** 136–150.
46. Lee, W. H., Bister, K., Pawson, A., Robins, T., Moscovici, C. & Duesberg, P. H. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2018–2022.
47. Neil, J. C., Breitman, M. L. & Vogt, P. K. (1980) *Virology* **108,** 98–110.
48. Shibuya, M., Hanafusa, T., Hanafusa, H. & Stephenson, J. R. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 6536–6540.
49. Sherr, C. J., Fedele, L. A., Oskarsson, M., Maizel, J. & Vande Woude, G. F. (1980) *J. Virol.* **34,** 200–212.
50. Shibuya, M., Wang, L.-H. & Hanafusa, H. (1982) *J. Virol.* **42,** 143–152.
51. Ghysdael, J., Neil, J. C. & Vogt, P. K. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 5847–5851.