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# Feline oncornavirus-associated cell membrane antigen: Evidence for an immunologically crossreactive feline sarcoma virus-coded protein

(tumor-specific antigen/feline leukemia virus/*src* gene product/immunosurveillance/type C viral proteins)

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ABSTRACT **The feline oncornavirus-associated cell membrane antigen (FOCMA) acts as a target for natural immunosurveillance against tumor development in the cat. In the present study, mink and rat cells nonproductively transformed by feline sarcoma virus (FeSV) were shown to express FOCMA as well as 5′-terminal feline leukemia virus (FeLV) *gag* gene proteins, p15 and p12. In contrast, such cells lack detectable levels of other FeLV *gag* gene-coded proteins or the *env* gene product, gp70. FOCMA, p15, and p12 antigen expression is initially in the form of an 80,000–100,000 molecular weight precursor which, upon post-translational cleavage, gives rise to a 65,000 molecular weight component that contains FOCMA and a 25,000 molecular weight component containing p15 and p12. Feline lymphoma cells, including those from several tumors that lacked detectable levels of FeLV structural protein expression, were shown to be FOCMA-positive. These findings strongly suggest that FOCMA represents an FeSV-coded transformation specific protein and provide preliminary information regarding the position within the FeSV genome coding for its synthesis.**

In the cat, tumors induced by feline leukemia virus (FeLV) and feline sarcoma virus (FeSV) express a cell surface antigen designated "feline oncornavirus-associated cell membrane antigen" (FOCMA) (1, 2). Analysis of anti-FOCMA titers in sera of virus-exposed cats has suggested that development of antibody directed against FOCMA may constitute an immunosurveillance defense against tumor development (1, 3). Although the nature of FOCMA is unknown, recent findings indicate that it is distinct from both the major 30,000 molecular weight ($M_r$) FeLV structural antigen (p30) (4, 5) and the 70,000 $M_r$ envelope glycoprotein (gp70) (5). The possibility that FOCMA may be distinct from all FeLV-coded structural proteins is suggested by the demonstration that nontransformed FeLV-infected fibroblasts and normal lymphoid cells fail to express FOCMA (6, 7).

Mink cells nonproductively transformed by FeSV express FOCMA in the absence of detectable levels of FeLV p30 or gp70 (6). Analysis of helper leukemia virus antigen expression in such cells has led to the demonstration of a 25,000 $M_r$ precursor containing FeLV p15 and p12 (8). In addition, a higher molecular weight protein (80,000–100,000) containing both antigenic reactivities was demonstrated (8).

The results of the present study indicate that FOCMA is coded for by genetic sequences contained within the FeSV genome. It is distinct from all known FeLV structural proteins but is activated in leukemia and lymphoma cells. In addition to the 80,000–100,000 $M_r$ protein expressed in nonproductively transformed mink cells, which contains FeLV p15 and p12, a lower molecular weight protein (65,000) lacking either p15 or p12 is also present. Both the 80,000–100,000 and 65,000 $M_r$ proteins are shown to exhibit immunologic determinants in common with FOCMA.

## MATERIALS AND METHODS

**Cells and Viruses.** Cells were grown in Dulbecco's modification of Eagle's medium supplemented with 10% calf serum, except that F1-74 was grown in McCoy's 5A with 20% fetal bovine serum. The cells included a normal mink fetal lung cell line, CCl-64 (9), and subclones of CCl-64 nonproductively transformed by either FeSV or the Moloney strain of murine sarcoma virus (M-MSV). The mink lines were generously provided by G. J. Todaro (National Cancer Institute). A normal rat kidney cell line and subclones nonproductively transformed with FeSV or M-MSV have also been described (9, 10). The KT strain of FeLV, propagated in F1-74 cells, was kindly provided by R. V. Gilden (Frederick Cancer Research Center). For immunoprecipitation analysis, cells were labeled with [$^{35}$S]methionine as described (11).

**Double-Antibody Radioimmunoprecipitation and Competition Immunoassays.** Radioimmunoprecipitation and competition immunoassays for FeLV *gag* gene-coded structural proteins of $M_r$ 30,000 (p30), 15,000 (p15), 12,000 (p12), and 10,000 (p10) (8) and the 70,000 $M_r$ *env* gene-coded glycoprotein, gp70 (5), were performed by the double-antibody procedure as described (5, 8).

**FOCMA Antigen and Antibody Detection.** Antibody directed against FOCMA was quantitated by the indirect membrane immunofluorescence test (6, 12). Fresh lymphoma biopsy tissue was prepared by coarsely mincing with scissors in McCoy's medium, mechanically teasing with forceps, and drawing off free cells with a fine-bore pasteur pipette.

**Immunoabsorptions.** Absorption studies were performed by incubating 1 mg of cellular or viral protein with 0.1 ml of antiserum in reaction mixtures containing 0.01 M Tris·HCl (pH 7.8), 1 mM EDTA, and 0.05 M NaCl in a total volume of 0.2 ml for 3 hr at 37° and then 18 hr at 4°.

**Immunoprecipitation and Polyacrylamide Gel Electrophoresis.** Immunoprecipitation of [$^{35}$S]methionine-labeled viral proteins and analysis by sodium dodecyl sulfate/polyacrylamide gel electrophoresis were performed according to the methods of Van Zaane *et al.* (11). Radioactivity was visualized by scintillation autoradiography (13).

## RESULTS

**Analysis of FOCMA and FeLV Structural Protein Expression in FeSV-Transformed Nonproducer Cells.** Initial

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Abbreviations: FeLV, feline leukemia virus; FeSV, feline sarcoma virus; FOCMA, feline oncornavirus-associated cell membrane antigen; $M_r$, molecular weight; M-MSV, Moloney strain of murine sarcoma virus.

Table 1. Expression of FOCMA and FeLV structural proteins in mammalian sarcoma virus-transformed nonproducer cells

| Cell line | Designation | FeLV-coded antigen expression* | | | | | FOCMA antigen† |
|---|---|---|---|---|---|---|---|
| | | *env*-coded | | *gag*-coded | | | |
| | | gp70 | p30 | p15 | p12 | p10 | |
| Normal mink | Mv1-Lu | <10 | <2 | <4 | <1 | <5 | − |
| FeSV-transformed mink NP | 64F3 | <10 | <2 | 1280 | 1500 | <5 | + |
| Clone of FeSV-transformed mink NP | 64F3 clone 7 | <10 | <2 | 1600 | 1180 | <5 | + |
| M-MSV-transformed mink | 64M1 | <10 | <2 | <4 | <1 | <5 | − |
| FeLV-infected 64F3 clone 7 | 64F3 clone 7(FeLV) | 3200 | 4000 | 2200 | 2000 | 1900 | + |
| FeLV-infected normal mink | Mv1-Lu (FeLV) | 1700 | 2100 | 1900 | 1100 | 1300 | − |
| Normal rat kidney | NRK | <10 | <2 | <4 | <1 | <5 | − |
| FeSV-transformed NRK NP | NRKF1 | <10 | <2 | 400 | 410 | <5 | ± |
| M-MSV-transformed NRK NP | NRKM1 | <10 | <2 | <4 | <1 | <5 | − |

* Cell extracts were analyzed by competition immunoassay. Results are expressed as ng of viral protein per mg of cell protein and represent mean values from three separate determinations.

† Cells were trypsinized, washed, resuspended in rapidly agitated fresh growth medium for 3 hr at 37°, and analyzed for FOCMA by the indirect membrane immunofluorescence test (6). +, Strong reaction; −, no reaction; ±, borderline or weakly positive reaction.

studies were performed to compare FOCMA and FeLV structural protein expression in mink and rat cell lines nonproductively transformed by FeSV. Although both nonproducer lines were negative for FeLV gp70, p30 and p10, the two remaining FeLV structural proteins, p15 and p12, were expressed at readily detectable levels (Table 1). The level of antigenic expression in the transformed mink cell line, however, was much higher than in the FeSV-transformed rat cell line. Similarly, although the FeSV-transformed mink cells were strongly positive for FOCMA antigen, the FeSV-transformed rat cells were weakly positive when standard FOCMA antiserum of cat origin was used. Control cultures, including normal as well as M-MSV-transformed mink and rat cells, were negative for each FeLV structural protein tested and for FOCMA. These results establish FOCMA to be distinct from FeLV gp70, p30, or p10, but the possibility that expression of FeLV p15 and p12 could account for the positive FOCMA reactivity of the two FeSV-transformed cell lines could not be ruled out.

**FeLV Antigen and Antibody Detection in Tissues and Sera of FeLV-Exposed Lymphomatous Cats.** To test whether the FOCMA reactivity exhibited by feline lymphoma cells could be attributed to FeLV p15 or p12 expression, lymphomas of several viremic and nonviremic cats were tested for FeLV structural proteins and for FOCMA. All lymphomas contained large numbers of FOCMA-positive cells (Table 2). However, in contrast to lymphomas of the viremic cats, which were highly positive for all five FeLV structural proteins, tumor cells of the three nonviremic cats were uniformly negative in all assays. In fact, tumors from a total of 19 nonviremic cats tested to date have been found to lack detectable levels of FeLV structural proteins and such tumor cells were always positive for FOCMA (7).

Table 2. Expression of FeLV structural proteins in lymphomas of viremic and nonviremic cats*

| Cat | FeLV antigen expression, ng/mg cell protein | | | | | FOCMA positive cells, % |
|---|---|---|---|---|---|---|
| | *env*-coded | | *gag*-coded | | | |
| | gp70 | p30 | p15 | p12 | p10 | |
| Viremic | | | | | | |
| AV 1 | 960 | 1000 | 460 | 200 | 180 | >90 |
| AV 2 | 4280 | 3000 | 1280 | 1500 | 1200 | >90 |
| AV 3 | 1810 | 1500 | 1300 | 1450 | 1210 | 75 |
| Nonviremic | | | | | | |
| AN 1 | <10 | <2 | <4 | <1 | <5 | 40 |
| AN 2 | <10 | <2 | <4 | <1 | <5 | 75 |
| AN 3 | <10 | <2 | <4 | <1 | <5 | >90 |
| AN 4 | <10 | <2 | <4 | <1 | <5 | >90 |
| AN 5 | <10 | <2 | <4 | <1 | <5 | 65 |

* Cell extracts were prepared and tested by competition immunoassay. FOCMA assays were performed by the indirect membrane immunofluorescence test using a high-titered anti-FOCMA sera preabsorbed with FeLV (7).

Further evidence for the above conclusion was obtained from an analysis of more than 80 sera from viremic and nonviremic cats obtained from a high-leukemia-incidence household. The results indicated that, although sera from certain nonviremic cats contained high titers of antibody to FOCMA as well as to FeLV structural proteins, other anti-FOCMA-positive nonviremic cat sera lacked detectable antibody to any of the structural proteins tested. Moreover, all of the anti-FOCMA-positive sera from viremic cats tested lack detectable antibody to any of the FeLV structural proteins (data not shown).

**Molecular Weight Analysis of FeLV Structural Proteins Expressed in FeSV-Transformed Nonproducer Cells.** Extracts of FeSV-transformed mink and rat nonproducer lines were subjected to molecular size analysis by agarose gel filtration under denaturing conditions, and individual column fractions were assayed for FeLV p15 and p12. In the FeSV-transformed mink cells, major peaks of FeLV p15 and p12 antigenic reactivity were observed at $M_r$ of 25,000 and 80,000–100,000 (Fig. 1). In the transformed rat cells, a major precursor protein containing p15 and p12 antigenic determinants was observed at a $M_r$ of 25,000 with smaller amounts of protein at $M_r$ 15,000 and 12,000 (Fig. 2). A less pronounced peak containing both p15 and p12 at a position corresponding to the major 80,000–100,000 $M_r$ protein present in the mink transformed line was also observed. These findings suggest that FeLV p15 and p12 are initially synthesized in the form of a high precursor that is first cleaved to produce a 25,000 $M_r$ precursor containing only p15 and p12 and finally p15 and p12 at $M_r$ of 15,000 and 12,000, respectively.

**Analysis of Proteins Expressed in FeSV-Transformed Cells for Antigenic Determinants in Common with FOCMA.** Although the above results establish FOCMA to be distinct from known FeLV-coded structural proteins, they do not rule out the possibility that FOCMA may represent a FeSV-induced cellular protein. In an attempt to eliminate this possibility and to tentatively map the position within the FeSV genome coding for FOCMA, the above-described FeLV p15 and p12 con-

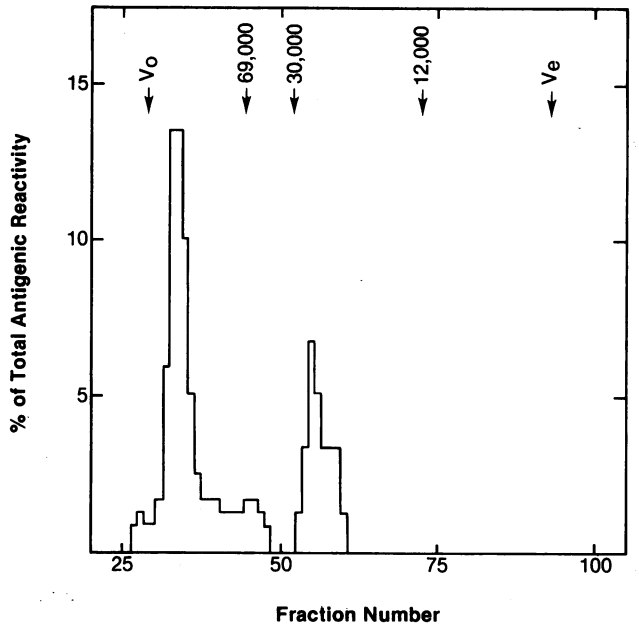


FIG. 1. Molecular size analysis of FeLV *gag* gene-coded proteins expressed in the FeSV-transformed mink nonproducer line, 64F3 clone 7. Cell extract (20 mg) was subjected to agarose gel filtration in the presence of 6 M guanidine·HCl and individual column fractions were tested in a homologous competition immunoassay for FeLV p15. Results are expressed as the percentage of total antigenic reactivity in each column fraction and are based on the degree of displacement of competition curves relative to standards.

taining 80,000–100,000 $M_r$ protein expressed in FeSV-transformed cells was tested for antigenic determinants immunologically crossreactive with FOCMA. For this purpose, absorption studies were performed with a serum from an FeLV-exposed cat containing both anti-FOCMA reactivity and moderate- or high-titered antibody against each of the above FeLV structural proteins. More than 95% of antibody directed against FeLV structural proteins was removed by absorption with density gradient-purified FeLV but the anti-FOCMA titer remained essentially unaltered (Table 3). Absorption of the same serum with an extract of FeSV-transformed mink cells resulted in reduction of anti-FeLV p15 and p12 and anti-FOCMA titers but did not remove antibody to other FeLV structural proteins. In contrast, an extract of the M-MSV-transformed mink cell line, 64M1, failed to absorb any of these reactivities to significant extents. Interestingly, however, although both the 25,000 and 80,000–100,000 $M_r$ precursors partially purified from FeSV-transformed mink cells efficiently adsorbed antibody to FeLV p15 and p12, only the higher $M_r$ precursor adsorbed anti-FOCMA reactivity.

**Immunoprecipitation Analysis of Precursor Proteins Expressed in FeSV-Transformed Mink Cells.** As an independent means of characterizing precursor proteins expressed in FeSV-transformed mink cells, [$^{35}$S]methionine-labeled cells were subjected to immunoprecipitation analysis. The results (Fig. 3) show the presence of an 80,000–100,000 $M_r$ protein immunoprecipitable by goat anti-FeLV but not by normal goat sera. Moreover, this reactivity was absorbed by highly purified FeLV p15 and p12, indicating that the precipitated protein was analogous to the 80,000–100,000 $M_r$ p15–p12 precursor described above. The fact that the same protein could be immunoprecipitated by cat anti-FOCMA sera even after extensive absorption with FeLV p15 and p12 further indicates that it possesses antigenic determinants in common with FOCMA.

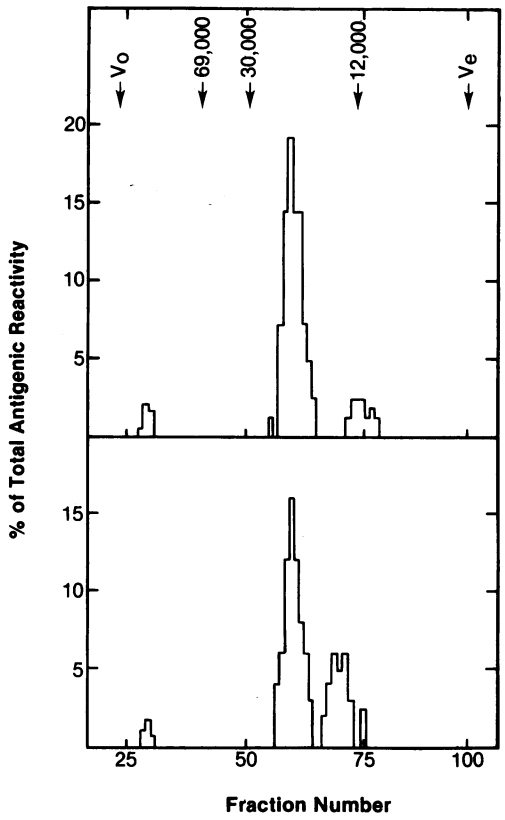


FIG. 2. Molecular size analysis of FeLV *gag* gene-coded proteins expressed in the FeSV-transformed rat nonproducer line, NRKF1. Cell extract (20 mg) was subjected to agarose gel filtration as in Fig. 1 and individual column fractions were tested by competition immunoassay. (*Upper*) FeLV p12; (*Lower*) FeLV p15.

Moreover, after an 18-hr chase of the [$^{35}$S]methionine labeled cells prior to immunoprecipitation, a 65,000 $M_r$ protein was observed that was efficiently precipitated by anti-FOCMA but only to a minor extent by goat anti-FeLV. The specificity of these reactions was established by control studies carried out in parallel with [$^{35}$S]methionine-labeled normal and M-MSV-transformed mink cells.

Table 3. Analysis of expression of FeLV p15 and p12 and FOCMA in FeSV-transformed mink cells*

| Sera | Antibody titer to FeLV structural proteins p30 | p15 | p12 | p10 | Anti-FOCMA titer |
|---|---|---|---|---|---|
| Absorbed with: | | | | | |
| FeLV | 50 | 20 | 2 | 5 | 32–64 |
| 64M1 | 2400 | 900 | 80 | 190 | 16 |
| 64F3 Clone 7 | 1400 | 15 | 4 | 200 | 2–4 |
| Purified precursors† | | | | | |
| 25,000 $M_r$ | 2000 | 18 | 6 | 220 | 32 |
| 80,000–100,000 $M_r$ | 1500 | 10 | 3 | 200 | 4 |
| Unabsorbed | 2500 | 1000 | 64 | 200 | 32–64 |

* Antibody titers to FeLV structural proteins were determined by radioimmunoprecipitation. Results are expressed as the reciprocal of the highest serum dilution at which 20% precipitation of the appropriate $^{125}$I-labeled antigen was achieved.

† The two FeLV p15 and p12 containing precursors expressed in FeSV-transformed mink cells (64F3 Clone 7) were partially purified by sequential phosphocellulose ion exchange chromatography and agarose gel filtration in the presence of 6 M guanidine·HCl as described (14).

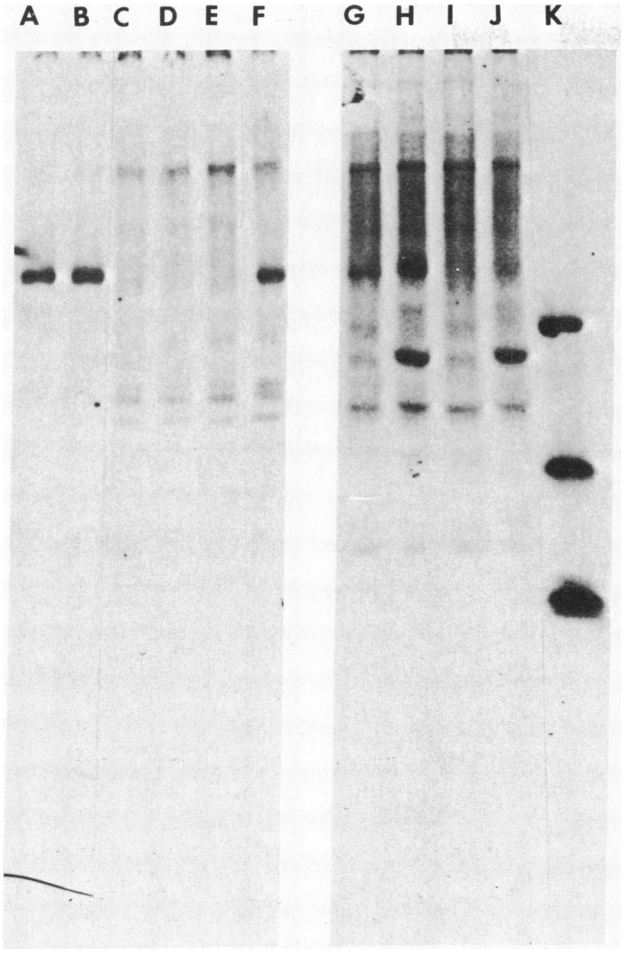


FIG. 3. Immunoprecipitation and sodium dodecyl sulfate/polyacrylamide gel electrophoresis analysis of [$^{35}$S]methionine-labeled viral proteins expressed in FeSV (A–D, F, G, H, J)- and M-MSV (E, I)-transformed mink cells. Cells were incubated with [$^{35}$S]methionine for 2 hr (A–F) followed by an 18-hr chase (G–J). Antisera included goat anti-FeLV (A, E, G, I), goat anti-FeLV absorbed with FeLV p15 and p12 (C), normal goat (D), high-titered feline anti-FOCMA (B, H), and feline anti-FOCMA absorbed with FeLV p15 and p12 (F, J). $M_r$ standards included $^{125}$I-labeled bovine serum albumin (69,000), FeLV p30 (27,000), and FeLV p12 (12,000) (K).

## DISCUSSION

The present findings provide insight into the nature of FOCMA and have implications regarding the organization of the FeSV genome. That FOCMA is coded for by FeSV but is distinct from known FeLV structural proteins is indicated by the demonstration of FOCMA expression in natural feline lymphoid tumors lacking detectable levels of known FeLV structural proteins. The fact that sera from viremic cats exhibiting high titers of anti-FOCMA reactivity lacked detectable antibody to the FeLV envelope glycoprotein [gp70 (5)], the major nonglycosylated internal antigen [p30 (4, 5)], or any of the lower $M_r$ FeLV-coded structural proteins further argues against the possibility that FOCMA reactivity may be attributable to an FeLV-coded structural gene product. Although none of these findings rules out the possibility that FOCMA is cellular- rather than viral-coded, the demonstration of its expression in FeSV-nonproducer cells of diverse species such as mink and rat favors the latter model.

In each of two FeSV-transformed nonproducer cell lines examined, FeLV p15 and p12 expression was shown to be associated with proteins of $M_r$ 25,000 and 80,000–100,000. In addition to FeLV p15 and p12, the higher $M_r$ protein was shown to exhibit antigenic determinants in common with FOCMA. A third protein, of about 65,000 $M_r$, exhibited only FOCMA determinants. These findings indicate that the 80,000–100,000 $M_r$ protein represents a precursor containing p15, p12, and FOCMA and is subject to post-translational cleavage giving rise to a 25,000 $M_r$ protein containing p15 and p12 and a second protein containing FOCMA antigenic determinants. The demonstration of a 65,000 $M_r$ FeSV-coded protein is of interest in view of a recent report describing a 60,000 $M_r$ avian sarcoma virus-coded transformation-specific protein (15).

Recent findings have established the FeLV *gag* gene sequence as 5′-p15-p12-p30-p10-3′ (8, 14). Thus, in view of the present findings, the possibility that in the FeSV genome, *gag* gene sequences coding for p30 and p10 have been replaced by host sequences coding for FOCMA antigenic determinants must be considered. The fact that the p15-p12-FOCMA precursor is subject to post-translational cleavage is consistent with the possibility that sequences coding for a small portion of p30, and thus the original p12-p30 cleavage site, are present within the FeSV genome. These findings provide direct evidence that FOCMA is coded for by FeSV and tentatively establish the position of sequences within the FeSV genome coding for FOCMA.

Cells nonproductively transformed by a number of different mammalian sarcoma viruses, as well as by other replication-defective transforming oncornaviruses, have been shown to express differing numbers of type C viral *gag* gene-coded proteins (16). Moreover, such expression invariably occurs in a progressive manner from the 5′ to the 3′ terminus of the *gag* gene (8, 17, 18). Thus, approaches analogous to those used in the present study involving analysis of precursors containing viral structural proteins may provide a means of identifying transforming proteins of other RNA tumor viruses.

Although the present results demonstrate that FOCMA antigenic reactivity can at least in part be attributed to an FeSV-coded protein, the question of whether this protein corresponds to the putative transforming or "*src*" gene-coded protein itself is not resolved. For instance, the possibility that FOCMA may be coded by FeSV-acquired cellular sequences located adjacent to, and expressed in conjunction with, *src* cannot be excluded. However, the demonstration of FOCMA expression in naturally occurring feline tumors, even in the absence of FeLV structural proteins, and the results of previous studies indicating antibody directed against FOCMA to provide a natural immunosurveillance defense against feline tumors (1, 3) strongly favor the possibility that FOCMA represents a transformation-specific protein.

**Note Added in Proof.** Sherr *et al.* (19) recently found a phosphorylated polyprotein of approximately 85,000 daltons in pseudotype feline sarcoma virus virions that appears to be analogous to the 85,000–100,000 dalton precursor described in the present study.

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