

# Feline Sarcoma Virus-Coded Polyprotein: Enzymatic Cleavage by a Type C Virus-Coded Structural Protein

ARIFA S. KHAN<sup>1</sup> AND JOHN R. STEPHENSON<sup>2\*</sup>

*Viral Oncology Program, Frederick Cancer Research Center, Frederick, Maryland 21701,<sup>1</sup> and Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland 20014<sup>2</sup>*

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A purified 15,000-molecular-weight (Mr) Prague strain Rous sarcoma virus *gag* gene-coded structural protein, p15, was shown to enzymatically cleave the previously described 130,000 Mr feline sarcoma virus-coded polyprotein, Pr130. Cleavage products included proteins ranging in molecular weight from 12,000 to 110,000. The specificity of this cleavage reactivity was indicated by the fact that, under similar conditions, neither purified type C viral structural proteins nor nonviral proteins such as bovine serum albumin were cleaved to significant extents. Moreover, feline leukemia virus Pr65<sup>agg</sup> was efficiently cleaved, resulting in the generation of proteins of 30,000 (p30), 15,000 (p15), 12,000 (p12), and 10,000 (p10) Mr. Using enzymatically (p15) treated feline sarcoma virus Pr130 as starting material, we were able to purify a major 72,000 Mr cleavage product and to show it to contain the previously described feline sarcoma virus-coded nonstructural component.

In a previous report, we described the isolation and immunological characterization of a 130,000-molecular-weight (Mr) precursor polyprotein encoded by replication-defective feline sarcoma virus (FeSV) (5, 6). This protein, designated FeSV Pr130, was shown to be coded by the amino terminal region of the FeSV genome and to be subject to posttranslational processing, giving rise to feline leukemia virus (FeLV) p15 and p12, as well as to a less well-defined nonstructural component(s). The possibility that FeSV Pr130 may contain a transformation-specific component(s) was suggested by its recognition by sera from select cats with known high-titered antibody directed against the feline oncornavirus-associated cell membrane antigen (15, 17). To analyze FeSV Pr130 further and to better define its nonstructural component(s), we attempted, in the present study, its enzymatic cleavage at specific sites by use of virus-coded structural proteins. This approach was suggested by a previous report in which von der Helm (21) demonstrated enhanced cleavage of a Rous sarcoma virus (RSV)-coded polyprotein in an ascites cell-free *in vitro* translational system subsequent to the addition of a 15,000 Mr avian type C viral structural protein.

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## MATERIALS AND METHODS

**Viruses and chemicals.** Mammalian type C viruses used included an endogenous isolate of *Papio cynocephalus* baboon origin (M7), woolly monkey virus, the Rauscher strain of murine leukemia virus, a horizontally transmitted type C virus isolate of gibbon ape origin, an endogenous feline virus (RD114), and the Theilen strain of FeLV (16). A murine amphotropic type C virus (4070-A) pseudotype of FeSV, designated FeSV(4070-A), has been described (5). Other mammalian retroviruses included the Mason-Pfizer monkey virus, squirrel monkey retrovirus, mouse mammary tumor virus, and bovine leukemia virus (16). Viruses were obtained as sucrose gradient-purified preparations from R. Gilden, Frederick Cancer Research Center, Frederick, Md., and Pfizer, Inc., Maywood, N.J., through the courtesy of J. Gruber, Office of Resources and Logistics, National Cancer Institute, Bethesda, Md. Prague strain RSV (Pr RSV-C), obtained from E. Bernstein (University Laboratories, New Brunswick, N.J.), was purified in our laboratory by sucrose density gradient centrifugation.

Chemicals used, including bovine serum albumin (BSA) (69,000 Mr),  $\beta$ -galactosidase (135,000 Mr), and equine myoglobin (18,000 Mr), were obtained from Sigma Chemical Co., St. Louis, Mo.

**Isolation of Pr RSV-C *gag* and *env* gene-coded structural proteins.** For purification of Pr RSV-C structural proteins, sucrose density gradient-purified virus (20 mg) was sonically disrupted for  $3 \times 30$  s in 0.1 M Tris-hydrochloride (pH 9.0) buffer containing 1.0% Triton X-100, followed by centrifugation at 40,000 rpm for 30 min in a Beckman Ti 50 rotor. The process was repeated, and the pooled supernatants were dialyzed overnight against 200 volumes of 0.01 M N,N-

bis-(2-hydroxyethyl)-2-aminoethanesulfonic acid (pH 6.5)–0.5 M EDTA–0.1% Triton X-100 (BET). Solubilized proteins were applied to a phosphocellulose column (1.0 by 5.0 cm; Whatman P11; H. Reeve Angel and Co., Clifton, N.J.) pre-equilibrated with the same buffer. The column was washed with 50 ml of BET buffer, and 2-ml fractions of a 0.0 to 0.5 M KCl linear gradient were collected. Pr RSV-C p15, detected by competition radioimmunoassay (10), eluted from the column between 0.2 and 0.4 M KCl. Fractions containing peak p15 reactivity were pooled, dialyzed against 0.01 M Tris-hydrochloride buffer (pH 8.5) containing 0.001 M EDTA and 0.1% Triton X-100, and applied to a DEAE-cellulose column (1.5 by 5 cm; Whatman DE52) previously equilibrated with the same buffer. Fractions containing p15 reactivity (eluting in the column wash) were pooled, dialyzed against 0.01 M Tris-hydrochloride (pH 7.8)–1 mM EDTA–0.1% Triton X-100, concentrated by use of an Amicon filter UM-10 (Amicon Corp., Lexington, Mass.), and applied to an Ultrogel Aca 44 (LKB Products, Rockville, Md.) column (1.5 by 90 cm) equilibrated with Tris-hydrochloride (pH 7.8), 1 mM EDTA, and 0.2 M KCl. Fractions of 1 ml were collected and analyzed for p15 reactivity by competition radioimmunoassay. Fractions containing peak reactivity were pooled, aliquoted, and stored under liquid nitrogen.

Other Pr RSV-C viral structural proteins, including *gag* gene-coded proteins of 27,000 (p27), 19,000 (p19), and 12,000 (p12) Mr and the *env*-coded viral envelope glycoprotein gp85, were isolated according to previously published methods (10). Viral proteins were  $^{125}\text{I}$  labeled, and competition immunoassays were developed for each as previously described (10).

**Purification of FeSV Pr130.** Isolation of FeSV Pr130 was achieved by sequential molecular sizing and ion exchange chromatography. For this purpose, 20 mg of sucrose density gradient-purified FeSV(4070-A) pseudotype virus was pelleted at  $100,000 \times g$  for 30 min. The pellet was suspended in 0.5 ml of 0.5 M Tris-hydrochloride (pH 8.0)–20 mM dithiothreitol–1 mM EDTA buffer containing 8 M guanidine hydrochloride and applied to an agarose A-15m (100–200 mesh) gel filtration column (1.5 by 90 cm; Bio-Rad Products, Richmond, Calif.) in the presence of 6 M guanidine hydrochloride, 0.01 M dithiothreitol, and 0.02 M sodium phosphate buffer (pH 6.5). Fractions of 1 ml were collected, dialyzed against 0.01 M Tris-hydrochloride (pH 7.8)–0.2 mM EDTA, and tested at serial twofold dilutions in a previously described (6) homologous competition immunoassay for FeLV p12. Fractions containing peak FeLV p12 reactivity at a molecular weight of around 130,000 were pooled, dialyzed exhaustively against BET buffer, and applied to a phosphocellulose column (1.0 by 5.0 cm) pre-equilibrated with the same buffer. The column was washed with 50 ml of buffer, and bound proteins were eluted with 100 ml of a 0.0 to 1.0 M NaCl linear gradient. Fractions of 2 ml were collected and tested at serial twofold dilutions in a homologous competition immunoassay for FeLV p12. Fractions containing peak reactivity were located in the column wash. These were pooled, dialyzed against 0.01 M Tris-hydrochloride (pH 7.8), and radiolabeled at high specific activity with  $^{125}\text{I}$  (Amersham/Searle, Arlington Heights, Ill.)

by a previously described modified chloramine T procedure (5).

**Conditions for enzymatic Pr RSV-C p15-mediated cleavage of FeSV Pr130.** Cleavage reactions were carried out in 0.05 ml of 0.01 M Tris-hydrochloride (pH 7.8)–1.0 mM EDTA–0.01 M NaCl, containing 10  $\mu\text{g}$  of Triton X-100 (1%)–disrupted virus or 1  $\mu\text{g}$  of purified avian type C viral structural proteins in combination with appropriate substrates (tracer amounts of  $^{125}\text{I}$ -labeled or 10  $\mu\text{g}$  of unlabeled proteins), at 22 or 37°C and were incubated for various times ranging from 0.5 to 24 h. Reactions were terminated by heating samples for 2 min at 90°C in 20  $\mu\text{l}$  of 0.06 M Tris-hydrochloride (pH 7.8) buffer containing 10% (vol/vol) glycerol, 5% (vol/vol) 2-mercaptoethanol, 3% (wt/vol) sodium dodecyl sulfate (SDS), and 0.0005% (wt/vol) bromophenol blue. Samples were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) as described by Van Zaane et al. (19). Gels were stained for 30 min in 0.1% (wt/vol) Coomassie brilliant blue and destained in 10% (vol/vol) acetic acid with 5% (vol/vol) methanol. Radioactivity was visualized by autoradiography with Kodak X-Ray RP Royal X-mat film according to the method of Bonner and Laskey (2).

## RESULTS

**Analysis of avian and mammalian retroviruses for virion-associated enzymatic cleavage reactivity.** Initial studies were performed to test representative mammalian retroviruses as well as one avian type C isolate, Pr RSV-C, for enzymatic cleavage of FeSV Pr130. For this purpose, highly purified  $^{125}\text{I}$ -labeled FeSV Pr130 was preincubated overnight at room temperature with 10- $\mu\text{g}$  portions of each virus and subsequently analyzed by SDS-PAGE as described in Materials and Methods (Fig. 1). Incubation of  $^{125}\text{I}$ -labeled Pr130 with density gradient-purified Pr RSV-C resulted in cleavage to major proteins of around 72,000 and 35,000 Mr. An additional protein of around 110,000 Mr (Pr110) observed in all samples can be shown by pulse-chase experiments to represent a spontaneous cleavage product of Pr130 and was observed even upon overnight incubation of FeSV Pr130 in the absence of virus (data not shown).

In an effort to determine the generality of the virion-associated cleavage reactivity, a number of type B, C, and D mammalian retroviruses were similarly analyzed for ability to cleave FeSV Pr130. Under conditions in which efficient Pr RSV-C-mediated cleavage was observed, none of the mammalian retroviruses tested exhibited significant cleavage reactivity. In contrast, incubation of FeSV Pr130 with a second type C virus isolate of avian origin, avian myeloblastosis virus, resulted in cleavage comparable to that observed with Pr RSV-C (data not shown).

**Identification of the Pr RSV-C cleavage enzyme as p15.** For identification of the avian

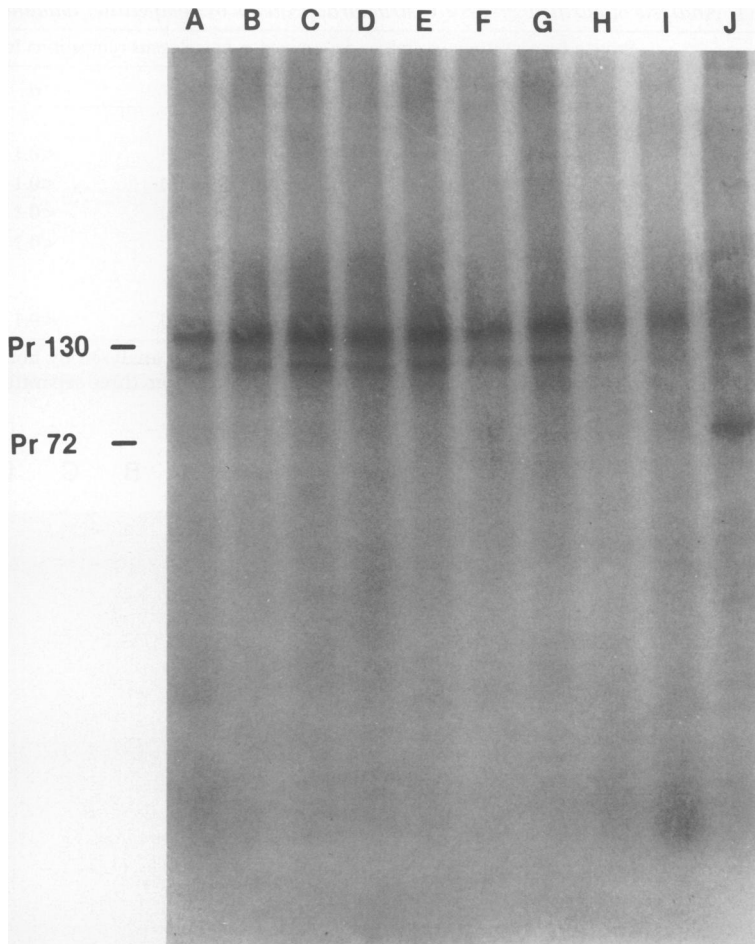


FIG. 1. Analysis of mammalian type B, C, and D and avian type C retroviruses for virion-associated enzymatic cleavage of  $^{125}\text{I}$ -labeled FeSV Pr130.  $^{125}\text{I}$ -labeled FeSV Pr130 (500,000 cpm) was added to 0.05-ml reaction mixtures of 0.01 M Tris-hydrochloride (pH 7.8)-1.0 mM EDTA-0.01 M NaCl containing 10  $\mu\text{g}$  of Triton X-100-disrupted sucrose density gradient-purified (A) FeLV; (B) Rauscher murine leukemia virus; (C) AKR murine leukemia virus; (D) gibbon ape leukemia virus; (E) RD114; (F) M7 endogenous baboon virus; (G) Mason-Pfizer monkey virus; (H) squirrel monkey retrovirus; (I) mouse mammary tumor virus; and (J) Pr RSV-C. After incubation for 18 h at room temperature, reactions were terminated, and samples were subjected to SDS-PAGE analysis as described in the text.

type C viral protein responsible for cleavage of FeSV Pr130, known Pr RSV-C *gag* and *env* gene-coded proteins were purified to apparent homogeneity and individually analyzed for cleavage reactivity. The high degree of purity of these viral proteins is indicated by the fact that each competed efficiently only in its respective homologous competition immunoassay (Table 1). Moreover, after labeling at high specific activity with  $^{125}\text{I}$ , p15 (Fig. 2), p30, p19, p12, and gp85 (data not shown) each migrated by SDS-PAGE as single, well-defined peaks at their respective molecular weights. When analyzed for cleavage of  $^{125}\text{I}$ -labeled FeSV Pr130, Pr RSV-C p15 exhibited enzymatic reactivity similar to

that observed using total virus. In contrast, incubation of Pr130 with other Pr RSV-C proteins including p27, p19, p12, or gp85 failed to cause significant cleavage (Fig. 3). Similarly, avian myeloblastosis virus reverse transcriptase lacked detectable reactivity when analyzed for cleavage of FeSV Pr130 (data not shown).

For determination of the time course of cleavage,  $^{125}\text{I}$ -labeled FeSV Pr130 was incubated with unlabeled Pr RSV-C p15 for various times at either room temperature or 37°C. After 1 or 2 h of incubation at 22°C, approximately equal amounts of Pr130 and Pr72 were observed, whereas by 24 h of incubation over 90% of Pr130 was cleaved (Fig. 4). However, by this time

TABLE 1. Analysis of purified Pr RSV-C structural proteins by competition immunoassay<sup>a</sup>

Viral protein	Protein concentration ( $\mu\text{g/ml}$ ) as determined in homologous competition immunoassays for:					
	p19	p12	p27	p15	rt	gp85
<i>gag</i> coded						
p19	34	<0.1	<0.2	<0.1	<0.1	<0.1
p12	<0.1	22	<0.2	<0.1	<0.1	<0.1
p27	<0.1	<0.1	85	<0.1	<0.1	<0.1
p15	<0.1	<0.1	<0.2	41	<0.1	<0.1
<i>env</i> coded						
gp85	0.1	<0.1	<0.2	<0.1	<0.1	27

<sup>a</sup> Pr RSV-C *gag* and *env* gene-coded structural proteins were purified and analyzed by homologous competition immunoassays as described in the text. Results represent mean values from three separate determinations. Reactivities in homologous assays are indicated in the boxes.

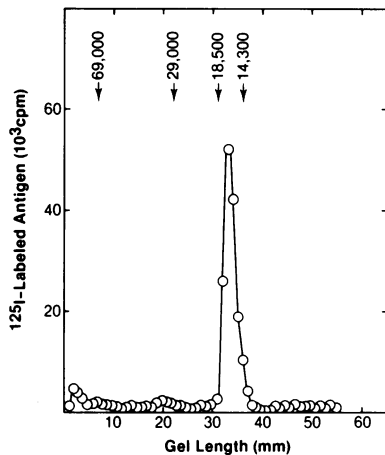


FIG. 2. SDS-PAGE analysis of <sup>125</sup>I-labeled Pr RSV-C p15. Approximately 150,000 cpm of <sup>125</sup>I-labeled Pr RSV-C p15 was applied to a 55-mm SDS-polyacrylamide (12%) gel at 2.5 mA for 2 h (5). Gels were sliced into 1-mm fractions and assayed for radioactivity. Molecular weight standards, subjected to electrophoresis on parallel gels and located by staining with Coomassie blue, included: BSA (69,000), carbonic anhydrase (29,000),  $\beta$ -lactoglobulin (18,500), and lysozyme (14,300).

accumulation of significant amounts of lower-molecular-weight cleavage products was observed, and the actual concentration of FeSV Pr72 was thus somewhat reduced. Similar extents of cleavage occurred when incubation was carried out at 37°C.

**Specificity of Pr RSV-C p15-associated cleavage reactivity.** As a measure of the specificity of Pr RSV-C p15-mediated enzymatic cleavage, a number of viral and nonviral <sup>125</sup>I-labeled proteins were exposed to p15 under conditions shown above to result in efficient cleavage of FeSV Pr130. The results indicate a lack of significant cleavage of any of the known FeLV *gag* or *env* gene-coded proteins or of nonviral

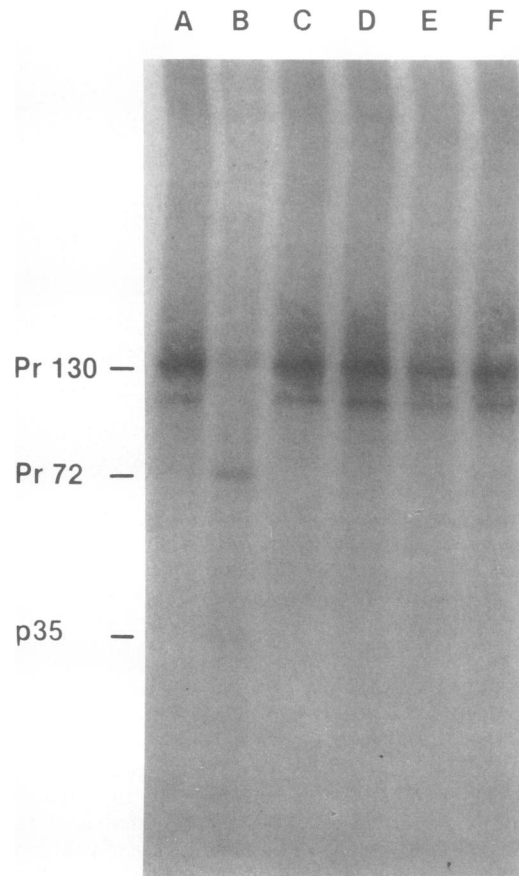


FIG. 3. Analysis of individual Pr RSV-C structural proteins for enzymatic cleavage of FeSV Pr130. Samples of <sup>125</sup>I-labeled FeSV Pr130 (500,000 cpm), either (A) untreated or preincubated for 18 h at room temperature in 0.05-ml reaction mixtures of 0.01 M Tris-hydrochloride (pH 7.8)-1.0 mM EDTA-0.01 M NaCl containing highly purified preparations (10  $\mu\text{g}$ ) of Pr RSV-C (B) p15; (C) p27; (D) p19; (E) p12; and (F) gp85, were subjected to SDS-PAGE analysis as described in the legend to Fig. 1.

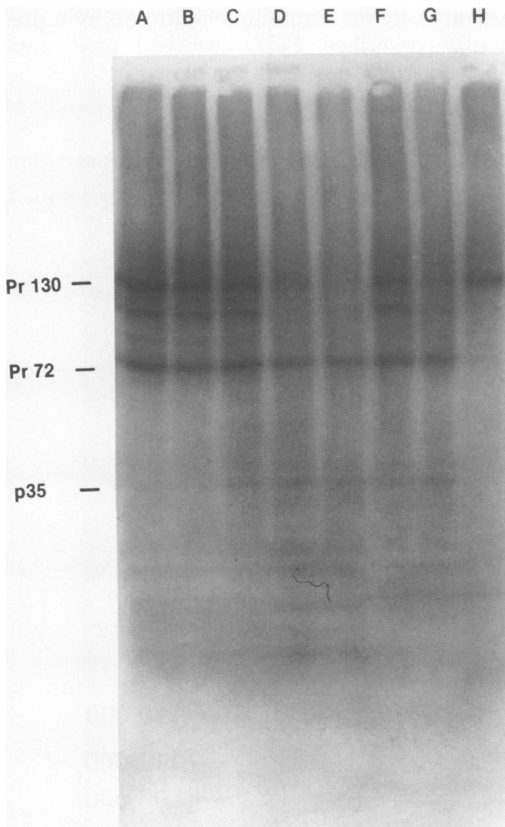


FIG. 4. Time course of RSV p15-mediated enzymatic cleavage of FeSV Pr130. <sup>125</sup>I-labeled FeSV Pr130 (500,000 cpm) was incubated with 1 μg of Pr RSV-C p15 in 0.01 M Tris-hydrochloride (pH 7.8)-1.0 mM EDTA-0.01 M NaCl reaction mixtures (0.05 ml) at room temperature for (A) 0.5, (B) 1, (C) 2, (D) 18, or (E) 24 h; at 37°C for (F) 1 and (G) 18 h; and (H) at room temperature for 24 h in the absence of Pr RSV-C p15. Samples were analyzed for 24 h in the absence of Pr RSV-C p15 by SDS-PAGE as described in the legend to Fig. 1.

proteins tested such as BSA, β-galactosidase, and myoglobin. These findings are summarized in Table 2, and the SDS-PAGE data indicating lack of cleavage of one representative protein, BSA, are shown in Fig. 5. The recognition by Pr RSV-C p15 for specific sites within the FeLV gag gene-coded precursor polyprotein, Pr65<sup>gag</sup>, was indicated by direct cleavage to proteins of 30,000 (p30), 15,000 (p15), 12,000 (p12), and 10,000 (p10) Mr (Table 2).

**Demonstration of additional intermediate and complete cleavage products of FeSV Pr130.** In view of the above findings suggesting specific recognition of natural cleavage sites within FeLV Pr65<sup>gag</sup> by the Pr RSV-C-coded cleavage enzyme, p15, it was of interest to

further analyze p15-generated cleavage products of <sup>125</sup>I-labeled FeSV Pr130. For this purpose incubation of FeSV Pr130 in the presence of p15 was allowed to continue for 48 h in an effort to generate lower-molecular-weight products. The results (Fig. 6) indicate a number of such products ranging in molecular weight from 12,000 to 110,000. The composition and map position of these various cleavage products within FeSV Pr130 have yet to be resolved. However, it should be noted that the 72,000 and 15,000 Mr products are relatively prominent, whereas only a very minor band is located at 12,000 (p12) Mr. This observation suggests that processing of p15 is more complete than that of p12 and thus raises the possibility that p12 may be contained in Pr72.

**The 72,000 Mr cleavage product of FeSV Pr130 contains an FeSV-coded nonstructural component(s).** The above-described enzymatic cleavage of Pr130 to generate a protein of around 72,000 Mr suggested to us the possibility that this might provide an approach for the isolation of the FeSV-coded nonstructural protein in the absence of FeLV structural components. To test this possibility, 200 μg of unla-

TABLE 2. Specificity of Pr RSV-C p15-mediated enzymatic cleavage<sup>a</sup>

Substrates	Mol wt	
	Control	Pr RSV-C treated
<b>FeLV structural proteins</b>		
<i>env</i> -coded		
Gp70	70,000	70,000
<i>gag</i> -coded		
p30	30,000	30,000
p15	15,000	15,000
p12	12,000	12,000
p10	10,000	10,000
<b>FeLV Pr65<sup>gag</sup></b>		
	65,000	30,000
		15,000
		12,000
		10,000
<b>Nonviral proteins</b>		
Myoglobin	18,000	18,000
BSA	69,000	69,000
β-Galactosidase	135,000	135,000

<sup>a</sup> <sup>125</sup>I-labeled viral and nonviral proteins (around 500,000 cpm) were incubated in the presence or absence of Pr RSV-C p15 (1 μg) for 18 h at 22°C in 0.01 M Tris-hydrochloride (pH 7.8)-1.0 mM EDTA-0.01 M NaCl and analyzed by SDS-PAGE as described in the text. [<sup>35</sup>S]methionine-labeled FeLV Pr65<sup>gag</sup> (300,000 cpm) was obtained by immunoprecipitation from [<sup>35</sup>S]methionine 2-h pulse-labeled FeLV subgroup C-infected CCL64 mink cells (17).

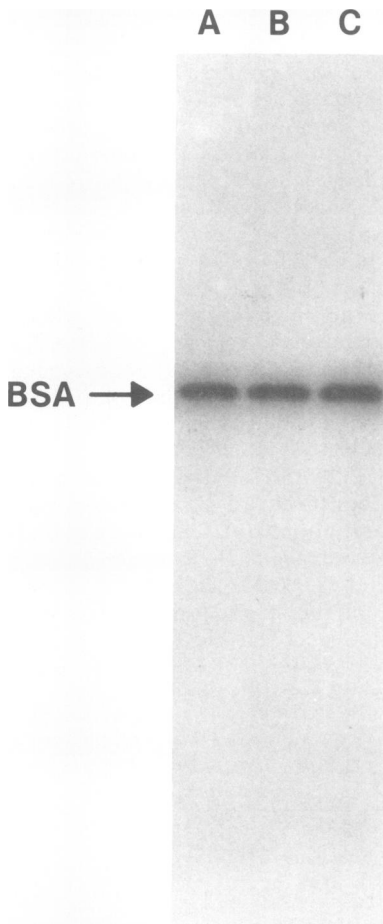


FIG. 5. Lack of detectable enzymatic cleavage of  $^{125}\text{I}$ -labeled BSA (500,000 cpm) either (A) untreated or incubated for 24 h in the presence of RSV p15 at (B)  $4^\circ\text{C}$  or (C)  $37^\circ\text{C}$ . Analyzed as described in the legend to Fig. 4.

beled FeSV Pr130 was cleaved by incubation in the presence of 20  $\mu\text{g}$  of Pr RSV-C, and the major 72,000 Mr cleavage product was subjected to purification by agarose gel filtration in the presence of 6 M guanidine hydrochloride. Fractions in the 70,000 to 75,000 Mr region of the column were pooled, dialyzed exhaustively against 0.01 M Tris-hydrochloride (pH 7.8)-1.0 mM EDTA, concentrated by lyophilization, and resuspended to a volume of 0.5 ml. To determine the extent of purification achieved, FeSV Pr72 was  $^{125}\text{I}$ -labeled at high specific activity by the modified chloramine T procedure (5) and analyzed by SDS-PAGE. The results (Fig. 7) indicate a single homogeneous peak of radioactivity at around 72,000 Mr relative to standards. The possibility that this protein contains the non-structural component of FeSV Pr130 was dem-

onstrated by its immunoprecipitation by a previously described FeLV-absorbed goat anti-FeSV Pr130 serum (data not shown).

## DISCUSSION

### Enzymatic cleavage of a mammalian sarcoma

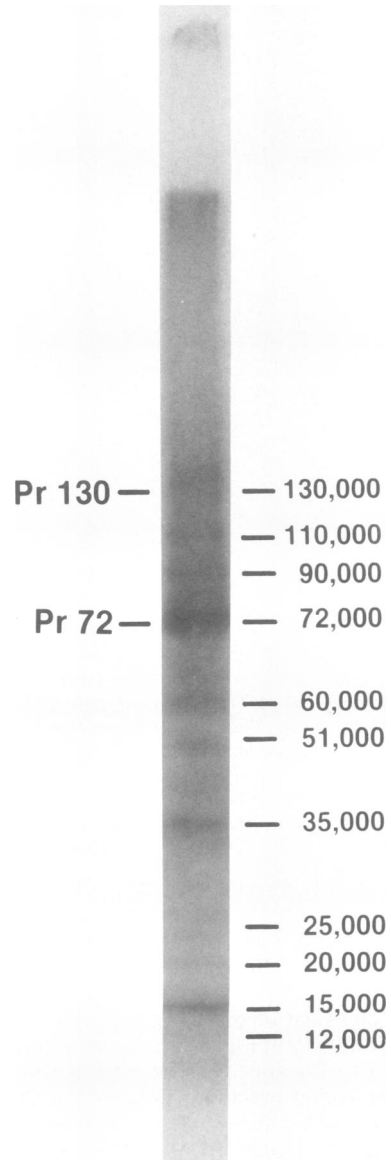


FIG. 6. Identification of lower-molecular-weight intermediate and complete Pr RSV-C p15-generated cleavage products of FeSV Pr130.  $^{125}\text{I}$ -labeled FeSV Pr130 (500,000 cpm) was incubated with 1  $\mu\text{g}$  of Pr RSV-C p15 for 48 h at  $37^\circ\text{C}$  and subsequently analyzed by SDS-PAGE as described in the legend to Fig. 1.

virus-coded polyprotein, FeSV Pr130, by a 15,000 Mr Pr RSV-C structural protein, p15, is demonstrated in the present study. This observation is consistent with the results of a previous report in which addition of RSV p15 to an *in vitro* translation system was shown to result in cleavage of the RSV *gag* gene-coded precursor polyprotein, Pr76 (21). This latter report, however, did not establish whether p15 acted directly as a cleavage enzyme or, alternatively, stimulated or in some way interacted with a cellular cleavage enzyme already present in the reticulocyte extract. In contrast, to demonstrate cleavage in the present study, we used the substrate, FeSV Pr130, in a highly purified form, thus arguing against the possibility that p15 acts to induce cleavage by activation of a cellular enzyme. While the possibility that p15 activates FeSV Pr130 to autocatalytic cleavage cannot be excluded, it seems highly unlikely. These findings thus suggest the recognition by RSV p15 of specific cleavage sites within precursor polyproteins not only of avian but also mammalian type C viral origin. The specificity of RSV p15 cleavage is further indicated by the fact that FeLV structural proteins were not subject to further degradation. Moreover, no significant RSV p15-associated cleavage of  $^{125}\text{I}$ -labeled nonviral proteins such as BSA,  $\beta$ -galactosidase, or myoglobin was observed.

After this manuscript was submitted for publication, Dittmar and Moelling (3) reported enzymatic cleavage of Rous-associated virus polyprotein Pr76, as well as certain nonviral proteins such as BSA, ovalbumin, concanavalin A, and casein, in the presence of avian myeloblastosis virus p15. Cleavage of nonviral proteins by p15, however, had a pH optimum of 5.7 and occurred only after boiling of substrates in 0.6% SDS. In contrast, Pr RSV-C p15-mediated enzymatic cleavage reactivity demonstrated in the present study appears to involve specific recognition of cleavage sites within high-molecular-weight virus-coded precursor polyproteins.

The previous demonstration in our laboratory of temperature-sensitive replication mutants of the Rauscher strain of murine leukemia virus characterized by conditional lethal defects in posttranslational processing of the *gag* gene-coded polyprotein Pr65 (18) raises the possibility that mammalian type C virus-coded cleavage enzymes also exist. The identification of such an enzyme(s) will be important in view of the detailed information available concerning the nature of cleavage sites within the *gag* gene-coded precursor polypeptides of these viruses (8). In the present study, however, analysis of a number of mammalian retroviruses indicated a lack of

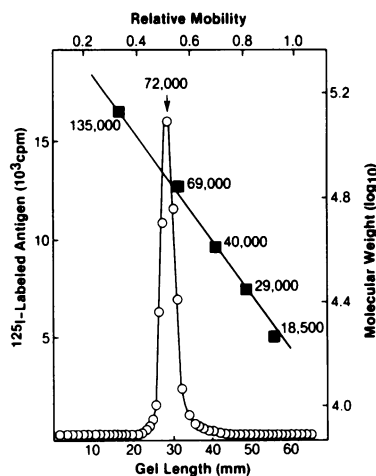


FIG. 7. SDS-PAGE analysis of  $^{125}\text{I}$ -labeled FeSV Pr72. Around 50,000 cpm of  $^{125}\text{I}$ -labeled FeSV Pr72, purified by agarose gel filtration column chromatography from enzymatically cleaved FeSV Pr130 as described in the text, was subjected to electrophoresis on a 55-mm SDS-polyacrylamide (8.0%) gel at 2.5 mA for 2 h (7). After electrophoresis, gels were either stained with Coomassie blue or sliced into 1-mm fractions and analyzed for radioactivity. Molecular weight standards used for calibration included  $\beta$ -galactosidase (135,000), BSA, (69,000), alcohol dehydrogenase (40,000), carbonic anhydrase (29,000), and  $\beta$ -lactoglobulin (18,500). Mobilities were calculated relative to the tracking dye, and a plot of  $\log_{10}$  molecular weight against relative mobility was determined by the method of least squares.

detectable virion-associated cleavage reactivity. It thus appears that whereas the mammalian type C viral genome may code for a cleavage enzyme, analogous to Pr RSV-C p15, it is either synthesized at much lower levels than Pr RSV-C p15 or not efficiently packaged into mature virions.

The intracistronic arrangement of prototype murine (1, 12) and feline (6) type C virus *gag* gene-coded polyproteins has been established as  $\text{NH}_2$ -p15-p12-p30-p10-COOH. In the case of avian type C viruses, p19 has been shown to be located at the amino terminus and p15 at the carboxyl terminus of the *gag* gene-coded precursor polyprotein (4, 10, 20). Information concerning the relative positions of p27 and p12 is indirect and primarily based on analogy to mammalian type C viruses (10). RSV p12 is known to share sequence homology with Rauscher murine leukemia virus p10 (Oroszlan et al., personal communication), whereas RSV p27 closely resembles mammalian type C viral p30 (10). The possibility must be considered that in both avian and mammalian type C viruses the viral-coded

cleavage enzyme may be located adjacent to the carboxyl terminus of the ribonucleoprotein (murine leukemia virus p10 or RSV p12) and that only in the case of avian viruses does this protein constitute a component of the *gag* gene-coded polyprotein. Assuming this model to be correct, the mammalian type C viral cleavage enzyme may be separated from the carboxyl terminal *gag* gene protein by a suppressible termination codon (9), thus resulting in its synthesis only at low level.

An increasing number of mammalian type C transforming viruses in addition to FeSV have been shown to code for 110,000 to 130,000 Mr polyproteins with both structural and nonstructural components (11, 13, 14, 22). Such virus-encoded polyproteins are of interest in view of the possible involvement of their nonstructural components in transformation. Although further studies are required to fully characterize polyprotein cleavage products such as those generated in the present study, p15-mediated enzymatic cleavage appears to provide a method of obtaining nonstructural components of mammalian transforming virus-coded polyproteins free of their structural components. Moreover, by tryptic peptide analysis of individual intermediate and final cleavage products, fine-structure mapping of such transforming virus-encoded polyproteins should be possible.

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