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Characterization of a 170,000-Dalton Polyprotein Encoded by the McDonough Strain of Feline Sarcoma Virus

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In this study, we demonstrated the expression of a 170,000-M_r polyprotein in each of several McDonough feline sarcoma virus (FeSV)-transformed mink cell clones and one McDonough FeSV-transformed rat clone. This polyprotein, designated McDonough FeSV P170, contained feline leukemia virus (FeLV) p15, p12, and p30 immunological determinants and shared two of its five [^{35}S]methionine-labeled tryptic peptides with FeLV Pr180$^{gag\text{-}pol}$. Both of these peptides were shown to be specific to the p30 component of Pr180$^{gag\text{-}pol}$. The remaining McDonough FeSV P170 methionine-containing peptides were not represented within either FeLV Pr180$^{gag\text{-}pol}$ or Pr82^{env}. Of interest, of the three peptides specific to the nonstructural component of McDonough FeSV P170, one was also represented in the 115,000-M_r polyproteins encoded by the Gardner and Snyder-Theilen strains of FeSV. These findings raise the possibility that the nonstructural components of polyproteins encoded by each of the three independently derived feline transforming viruses contained both common and unique regions. Moreover, if the sequences encoding these components are involved in transformation, as appears to be the case, our findings establish that the position of their insertion within the *gag-pol* region of the FeLV genome can vary among individual isolates.

Several independent isolates of feline sarcoma virus (FeSV) have been described (4, 10, 17). In the presence of an appropriate type C helper virus, each of these isolates has been shown to induce fibrosarcomas in vivo and morphologically transform embryo fibroblasts in vitro. The Gardner and Snyder-Theilen strains of FeSV encode 115,000-M_r polyproteins and less well defined, highly related proteins of around 72,000 and 80,000 daltons, respectively (6, 7, 11, 13, 17; J. R. Stephenson, W. J. M. Van de Ven, A. S. Khan, and F. H. Reynolds, Jr., Cold Spring Harbor Symp. Quant. Biol., in press). Such polyproteins contain immunological determinants in common with translational products of the 5′ terminus of the feline leukemia virus (FeLV) *gag* gene (p15, p12, and a portion of p30). In addition, nonstructural components lacking either immunological cross-reactivity or structural relatedness to known FeLV translational products and containing probable transforming function have been identified. The latter components are acidic (5), highly phosphorylated (20), and, at least in the case of Gardner FeSV, known to possess an associated protein kinase activity which recognizes P115 as its major substrate (20). The demonstration that three of five [^{35}S]methionine-labeled tryptic peptides specific to Gardner FeSV P115 are also represented in Snyder-Theilen FeSV P115 (19) argues that the number of feline cellular sequences, whose acquisition by FeLV results in the generation of replication-defective transforming virus (3), is relatively limited.

An additional, although less well characterized, isolate of FeSV was originally described by McDonough et al. (10). Cells nonproductively transformed by this virus have been shown to express FeLV p15, p12, and p30 cross-reactive antigenic determinants (11, 19). In the present study, we report the isolation of several new clones of mink cells nonproductively transformed by McDonough FeSV and the analysis of each with respect to expression of virus-specific translational products. A polyprotein of around 170,000 daltons containing FeLV p15, p12, and p30 immunological determinants and a 55,000-M_r possible cleavage product are described. The 170,000-dalton polyprotein was analyzed with respect to phosphorylation, protein kinase activity, and relatedness to Gardner FeSV P115, Snyder-Theilen FeSV P115, and FeLV Pr180$^{gag\text{-}pol}$.

MATERIALS AND METHODS

Cells and viruses. Cells were maintained in the Dulbecco modification of Eagle medium supplemented with 10% calf serum (Colorado Serum Co., Denver, Colo.). A fetal mink lung cell line, CCL64, and a normal rat kidney cell line NRK, have been described (17).

McDonough FeSV-transformed subclones of CCL64 and NRK, designated G2-MINK and G2-NRK, respectively, were generously provided by E. M. Scolnick, National Cancer Institute. A line of mink cells productively infected with an FeLV pseudotype of McDonough FeSV was kindly provided by P. Reddy, National Cancer Institute. A Gardner FeSV nonproductively transformed CCL64 mink subclone, designated 64F3, and a clonal isolate of FeLV subgroup A have been described previously (19).

Competition immunoassays. Competition immunoassays were performed by analysis of unlabeled antigens at serial twofold dilutions for ability to compete with ^{125}I-labeled FeLV subgroup A p30, p15, or p12 for binding limiting amounts of goat antiserum directed against detergent-disrupted FeLV subgroup C. Proteins were purified and labeled with ^{125}I by previously described procedures (6). Competition immunoassay reaction mixtures contained 0.01 M Tris-hydrochloride (pH 7.8), 1.0 mM EDTA, 0.4% Triton X-100, 1% bovine serum albumin, and 0.2 M NaCl in a total volume of 0.2 ml. Antiserum and unlabeled competing antigen were incubated at 37°C for 1 h before the addition of 10,000 cpm of ^{125}I-labeled antigen. After further incubation for 3 h at 37°C and 18 h at 4°C, antigen-antibody complexes were immunoprecipitated as previously described (6).

Immunoprecipitation and SDS-PAGE. Exponentially growing cells were labeled with $^{32}P_i$ (carrier-free; New England Nuclear Corp., Boston, Mass.) or [^{35}S]methionine (1,075 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) as previously described (19). After labeling, cell monolayers were washed twice with serum-free Dulbecco-modified Eagle medium and disrupted at 4°C in PBSTDS lysis buffer (10 mM sodium phosphate [pH 7.2], 0.9% NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate [SDS]). Extracts (1 ml) were incubated at 4°C for 18 h after the addition of a solution containing RNase A (200 μg/ml), RNase T_2 (0.1 μg/ml), DNase I (4.0 μg/ml), normal goat serum (5 μl), and protein A–Sepharose C1-4B (1.0 ml of a 10% [vol/vol] suspension) (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) and clarified by centrifugation for 1 h at 100,000 × *g*. Virus-specific proteins were immunoprecipitated by incubation for 18 h at 4°C in the presence of 5 μl of a specific antiserum, addition of 50 μl of a 10% (vol/vol) protein A–Sepharose C1-4B suspension, and incubation for an additional 2 h at 4°C. Immunocomplexes were collected by centrifugation at 2,000 × *g* for 10 min, washed three times in PBSTDS lysis buffer and analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) on 5 to 20% polyacrylamide gradient slab gels by the method of Laemmli (8). Radioactivity was visualized by scintillation autoradiography as described by Bonner and Laskey (2).

Two-dimensional tryptic peptide analysis. Virus-specific proteins were purified by immunoprecipitation and SDS-PAGE. After electrophoresis, gels were washed extensively with 10% trichloroacetic acid, followed by a solution containing 10% acetic acid and 10% methanol, and dried under vacuum. Labeled proteins were localized by autoradiography and cut from the dried gel, and selected gel slices were further washed in 10% methanol and lyophilized. Proteins were digested by incubation of gel slices in tolysulfonyl phenylalanyl chloromethyl ketone-trypsin (Worthington Biochemicals Corp., Freehold, N.J.) (50 μg/ml in 0.05 M ammonium bicarbonate, pH 8.0) for 6 h at 37°C. Supernatant fluids were removed and replaced by a fresh tolylsulfonyl phenylalanyl chloromethyl ketone-trypsin solution for additional digestion at room temperature for 16 h. Supernatant fractions were pooled, filtered, lyophilized, and washed twice. [^{35}S]methionine-labeled digests were incubated for 2 h at 0°C in 0.1 ml of chilled performic acid (30% H_2O_2–90% formic acid [1:9] preincubated for 2 h at room temperature). Samples were subsequently diluted with 2 ml of water, lyophilized, suspended in electrophoresis buffer (acetic acid–formic acid–water [15:5:80]), and spotted on cellulose-coated thin-layer chromatography glass plates (10 by 10 cm; EM Laboratories, Inc., Elmsford, N.Y.). Electrophoresis was performed for 30 min at 500 V, and chromatography was conducted in the second dimension in buffer containing butanol, pyridine, acetic acid, and water (32:25:5:20). Radiolabeled tryptic peptides were visualized by autoradiography with Kodak X-Omat R film. Detection of [^{35}S]methionine-labeled peptides was enhanced by spraying the thin-layer chromatography plates with ether containing 7% 2,5-diphenyloxazole (2).

Assay for protein kinase activity. Tissue culture cells (10^7) were disrupted by repeated aspiration through a 25-gauge needle in 5 ml of a solution of 10 mM Tris-hydrochloride (pH 7.2), 100 mM NaCl, 1 mM EDTA, and 0.1% Triton X-100 and clarified by centrifugation at 2,000 × *g* for 10 min. Virus-specific proteins were immunoprecipitated as described above, and protein kinase activity was assayed by suspension of immunoprecipitates in 20 mM Tris-hydrochloride (pH 7.2)–5 mM $MgCl_2$ buffer containing 1 μCi of [γ-^{32}P]ATP (2,000 Ci/mmol; New England Nuclear Corp., Boston, Mass.) and incubation of reaction mixtures for 10 min at 30°C. Reactions were terminated by addition of 3 ml of ice-cold PBSTDS lysis buffer, and nonprotein-bound ^{32}P-label was removed by repeated centrifugation in excess PBSTDS lysis buffer. After the final wash, immunoprecipitates were suspended in 20 μl of a solution of 0.65 M Tris-hydrochloride (pH 6.7), 1% SDS, 10% glycerol, 2.5% 2-mercaptoethanol, and 0.1% bromophenol blue and heated for 2 min at 90°C. Samples were analyzed by SDS-PAGE with a 5% acrylamide–0.133% bisacrylamide stacking gel and a 9-cm, 5 to 20% linear polyacrylamide separation gel (acrylamide/bisacrylamide ratio; 30:0.8) in a Tris-glycine-SDS buffer system at 20 to 30 mA as described by Laemmli (8). Radioactivity was visualized by autoradiography with Kodak X-Omat R film.

RESULTS

FeLV *gag* gene protein expression in cells nonproductively transformed by the McDonough strain of FeSV. We previously reported the expression of FeLV p15, p12, and p30 in a mink cell clone, G2-MINK, nonproductively transformed by the McDonough strain of FeSV (19). To further determine the specificity and significance of such reactivities, we obtained a cell line chronically infected with the original McDonough FeSV seed stock from an independ-

ent source and, by endpoint cloning on CCL64 mink cells, successfully derived two new nonproductively transformed cell lines. By competition immunoassay, both of these two newly derived cell lines and a McDonough FeSV-transformed rat cell clone, G2-NRK, provided by E. M. Scolnick, were found to express levels of p15, p12, and p30 immunological reactivity comparable to those previously reported for the G2-MINK cell line (data not shown).

For further characterization of these reactivities, extracts of the McDonough FeSV C115-1 and G2-MINK cell clones were subjected to SDS-PAGE analysis after labeling in vivo with [^{35}S]methionine and immunoprecipitation by sera with specificity for individual FeLV structural proteins. As shown in Fig. 1, proteins of around 170,000, 160,000, and 55,000 M_r were immunoprecipitated from McDonough FeSV C115-1 cells by goat antis rum directed against detergent-disrupted FeLV (lane A) and by sera with specificity for purified FeLV structural proteins, including p15, p12, and p30 (lanes B through D), but not by goat anti-FeLV p10 or gp70 (lanes E and F). That these proteins were McDonough FeSV specific was indicated by their absence in immunoprecipitates of normal mink cells. Similar analysis of the G2-MINK cell line indicated the presence of a 170,000-M_r polyprotein immunoprecipitable by antisera directed against FeLV and by sera against FeLV p15, p12, and p30. A second major protein of around 160,000 M_r was precipitated from G2-MINK cells by anti-FeLV and to a lesser extent by antisera directed against individual FeLV structural proteins (lanes H through M).

Comparison of McDonough FeSV P170 to Gardner FeSV P115 by immunoprecipitation and SDS-PAGE analysis. In view of our previous demonstration of structural relatedness between polyproteins of around 115,000 M_r encoded by the Gardner and Snyder-Theilen strains of FeSV, it was of interest to test McDonough FeSV P170 and Gardner FeSV P115 for possible serological cross-reactivity. For this purpose, we utilized an antiserum prepared in goats and previously shown to recognize both the structural (p15 and p12) and nonstructural components of Gardner FeSV P115. This serum, designated anti-Gardner FeSV P115, is of particular interest in that, in addition to FeSV P115, it recognizes a 150,000-M_r cellular phosphoprotein with associated protein kinase activity and binding affinity for FeSV P115 (F. H. Reynolds, Jr., W. J. M. Van de Ven, and J. R. Stephenson, submitted for publication).

As shown in Fig. 2, precipitation of Gardner FeSV P115 and McDonough FeSV P170 from the 64F3 and McDonough FeSV C115-1 cell lines, respectively, was obtained with goat anti-FeLV but not with normal goat serum (lanes A, D, E, and H). Although both polyproteins were

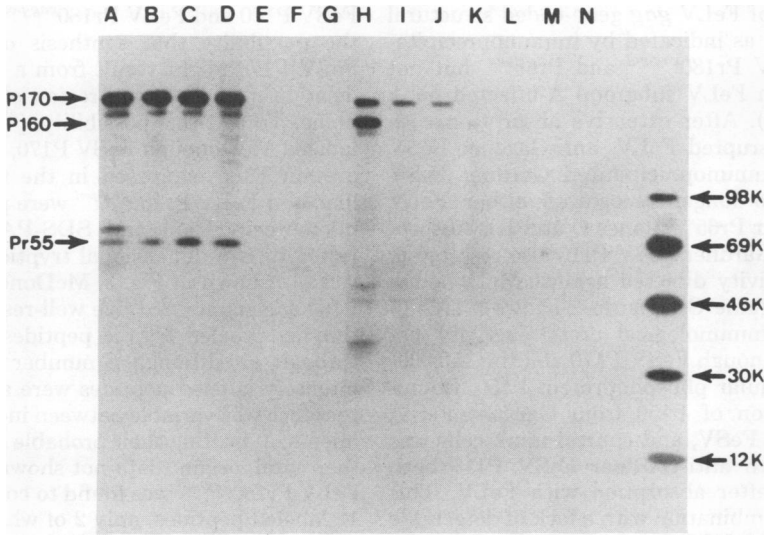

FIG. 1. *Immunoprecipitation and SDS-PAGE analysis of polyproteins encoded by the McDonough strain of FeLV. Cell lines including McDonough C115-1 (A through G), G2-MINK (H through M), and control CCL64 mink (N) were pulse-labeled in [^{35}S]methionine (100 μCi/ml)-containing medium for 30 min, immunoprecipitated with goat antiserum directed against detergent-disrupted FeLV (A, H, and N), with antisera with specificity for FeLV p30 (B and I), FeLV p15 (C and J), FeLV p12 (D and K), Rauscher murine leukemia virus p10 (E and L), and FeLV gp70 (F and M), and with control goat serum (G), and analyzed by SDS-PAGE. Molecular weight standards included ^{14}C-labeled phosphorylase B (98,000), bovine serum albumin (69,000), ovalbumin (46,000), carbonic anhydrase (30,000), and cytochrome c (12,000).*

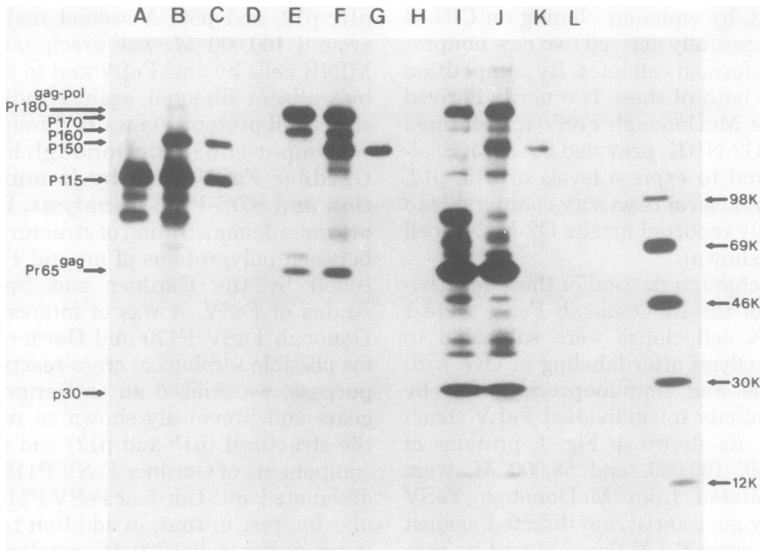

FIG. 2. *Comparison of McDonough FeSV P170 and Gardner FeSV P115 by immunoprecipitation and SDS-PAGE analysis. Cells, including the Gardner FeSV-transformed mink clone 64F3 (A through D), McDonough C115-1 (E through H), and CCL64 mink cells productively infected with FeLV subgroup A (I through L), were pulse-labeled in [^{35}S]methionine (100 μCi/ml)-containing medium for 30 min, immunoprecipitated, and analyzed by SDS-PAGE. Sera included anti-FeLV (A, E, and I), anti-Gardner FeSV P115 (B, F, and J), anti-Gardner FeSV P115 extensively absorbed with FeLV (C, G, and K), and normal goat serum (D, H, and L). Molecular weight markers are as described in the legend to Fig. 1.*

also efficiently precipitated by anti-Gardner FeSV P115 (lanes B and F), this could reflect recognition of FeLV *gag* gene-coded structural components, as indicated by immunoprecipitation of FeLV Pr180$^{gag\text{-}pol}$ and Pr65^{gag}, but not Pr82^{env}, from FeLV subgroup A-infected mink cells (lane J). After extensive absorption with detergent-disrupted FeLV, anti-Gardner FeSV P115 still immunoprecipitated Gardner FeSV P115 but no longer recognized either FeLV Pr180$^{gag\text{-}pol}$ or Pr65^{gag} (lanes C and K). Absorption of anti-Gardner FeSV P115 also resulted in loss of reactivity directed against McDonough FeSV P170 (lane G). Figure 2 shows a lack of detectable immunological cross-reactivity between McDonough FeSV P170 and the 150,000-M_r mink cellular phosphoprotein P150. Immunoprecipitation of P150 from Gardner FeSV, McDonough FeSV, and control mink cells was observed with anti-Gardner FeSV P115 both before and after absorption with FeLV. This finding, in combination with a lack of detectable precipitation of McDonough FeSV P170 by FeLV-absorbed anti-Gardner FeSV P115, argues that, if these two proteins share immunological determinants, they are not recognized by this antiserum.

Comparison of [^{35}S]methionine-labeled tryptic peptide compositions of FeLV Pr180$^{gag\text{-}pol}$ and McDonough FeSV P170. The above results demonstrate significant immunological cross-reactivity between McDonough FeSV P170 and FeLV Pr180$^{gag\text{-}pol}$ and thus raise the possibility that synthesis of McDonough FeSV P170 might result from a small in-phase deletion in the *gag-pol* region of the FeSV genome. To test this possibility, [^{35}S]methionine-labeled McDonough FeSV P170, the 160,000-M_r protein P160 expressed in the G2-MINK cell line, and FeLV Pr180$^{gag\text{-}pol}$ were purified by immunoprecipitation and SDS-PAGE and subjected to two-dimensional tryptic peptide analysis. As shown in Fig. 3, McDonough P170 and P160 each contained five well-resolved [^{35}S]methionine-labeled tryptic peptides, designated a through e. Although a number of minor, less intensely labeled peptides were also seen, their presence was variable between individual experiments, indicating their probable cellular, rather than viral, origin (data not shown). In contrast, FeLV Pr180$^{gag\text{-}pol}$ was found to contain a total of 12 labeled peptides, only 2 of which (peptides 2 and 11) appeared to correspond in map position to peptides represented in McDonough FeSV P170.

In view of the above results indicating immunological cross-reactivity between FeLV *gag* gene-coded proteins and McDonough FeSV P170, we undertook to further characterize the two peptides common to McDonough FeSV

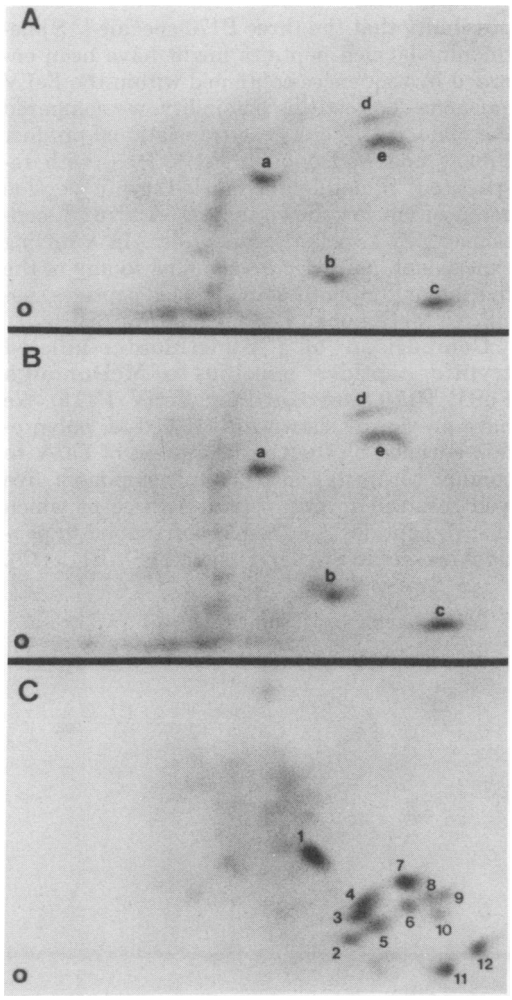

FIG. 3. *Comparison of [^{35}S]methionine-labeled tryptic peptide compositions of McDonough FeSV P170 (A), McDonough FeSV P160 (B), and FeLV Pr180$^{gag\text{-}pol}$ (C). [^{35}S]methionine-labeled P170 and P160 were isolated from G2-MINK cells by immunoprecipitation and SDS-PAGE as described in the legend to Fig. 1. FeLV Pr180$^{gag\text{-}pol}$ was isolated from [^{35}S]methionine-labeled FeLV subgroup A-infected mink cells. Proteins were digested with tolylsulfonyl phenylalanyl choloromethyl ketone-trypsin as described in the text. Tryptic digests (6,000 to 10,000 cpm) were suspended in electrophoresis buffer and spotted on cellulose thin-layer glass plates (o). Separation in the first dimension was by electrophoresis, and that in the second dimension by ascending chromatography. McDonough FeSV [^{35}S]methionine-labeled peptides are designated a through e, and the 12 major [^{35}S]methionine-labeled peptides represented in FeLV Pr180$^{gag\text{-}pol}$ are labeled 1 through 12.*

P170 and FeLV Pr180$^{gag\text{-}pol}$. For this purpose, FeLV Pr65^{gag} and p30 were analyzed with respect to [^{35}S]methionine-labeled tryptic peptide composition. The results demonstrated two methionine-containing peptides specific to p30, both of which were represented in Pr65^{gag} (Fig. 4).

To further test whether the [^{35}S]methionine-labeled tryptic peptides common to FeLV Pr180$^{gag\text{-}pol}$ and FeSV P170 were FeLV p30 specific, we performed additional mixing experiments. In an initial experiment, [^{35}S]methionine-labeled FeLV Pr180$^{gag\text{-}pol}$ and Pr65^{gag} were mixed at a 1:1 ratio before tryptic peptide analysis. The fact that the 2 Pr65^{gag}-specific peptides (peptides 2 and 11) comigrated with 2 of the 12 Pr180$^{gag\text{-}pol}$ peptides confirms that the 180,000-M_r protein examined was, in fact, FeLV Pr180 (Fig. 5A). As shown in Fig. 5B, in a mixing experiment involving FeLV Pr180$^{gag\text{-}pol}$ and FeSV P170, the only comigration was between peptides 2 and 11 and b and c, respectively. Finally, in a mixing experiment between McDonough FeSV P170 and FeLV p30, the two [^{35}S]methionine-labeled FeLV p30 tryptic peptides were shown to correspond in position to McDonough FeSV P170 peptides b and c (Fig. 5C).

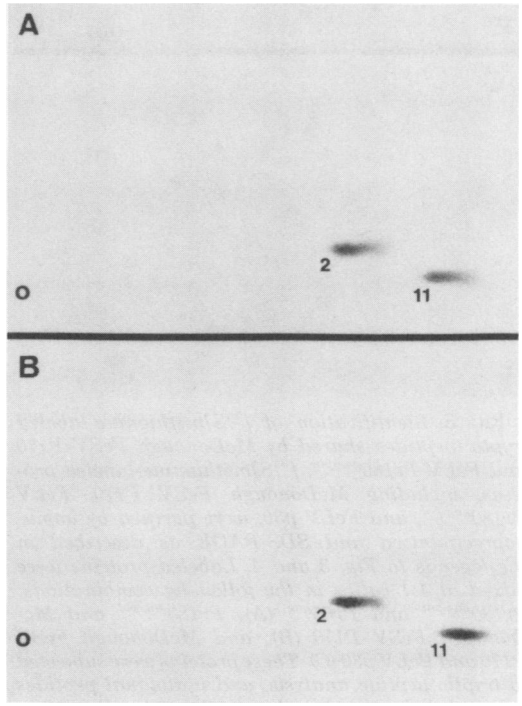

FIG. 4. *Two-dimensional [^{35}S]methionine-labeled tryptic peptide maps of FeLV Pr65^{gag} (A) and FeLV p30 (B). Proteins were immunoprecipitated from FeLV subgroup A-infected mink cells by an antiserum against FeLV p30, further purified by SDS-PAGE, and subjected to two-dimensional tryptic peptide analysis as described in the legend to Fig. 3. Peptides are labeled according to their map positions relative to Pr180$^{gag\text{-}pol}$ peptides shown in Fig. 3.*

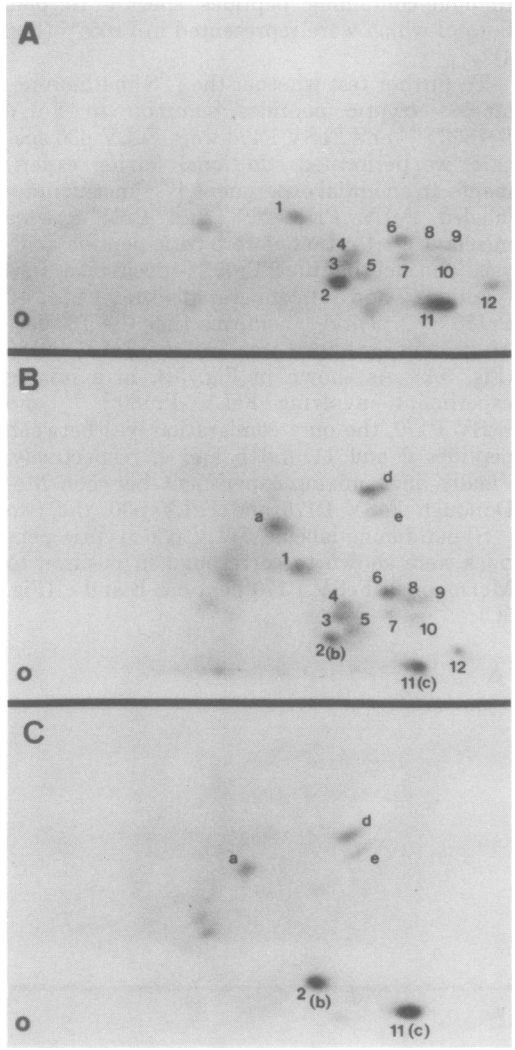

FIG. 5. *Identification of [^{35}S]methionine-labeled tryptic peptides shared by McDonough FeSV P170 and FeLV Pr180$^{gag\text{-}pol}$. [^{35}S]methionine-labeled proteins, including McDonough FeSV P170, FeLV Pr180$^{gag\text{-}pol}$, and FeLV p30, were purified by immunoprecipitation and SDS-PAGE as described in the legends to Fig. 3 and 4. Labeled proteins were mixed at 1:1 ratios in the following combinations: Pr180$^{gag\text{-}pol}$ and Pr65^{gag} (A); Pr180$^{gag\text{-}pol}$ and McDonough FeSV P170 (B); and McDonough FeSV P170 and FeLV p30 (C). These proteins were subjected to tryptic peptide analysis, and individual peptides were labeled as described in the legend to Fig. 3.*

Lack of [^{35}S]methionine-labeled tryptic peptides common to FeLV Pr82^{env} and McDonough FeSV P170. The above results established that only a portion of the coding capacity for McDonough FeSV P170 was represented within the *gag-pol* region of the FeLV genome. These findings, however, left open the possibility that the three P170-specific [^{35}S]methionine-labeled peptides might have been encoded by sequences contained within the FeLV *env* gene. To test this possibility, we compared the major FeLV *env* gene translational product Pr82^{env} and McDonough FeSV P170 with respect to [^{35}S]methionine-labeled tryptic peptide composition. As shown in Fig. 6A, Pr82^{env} contained only two labeled peptides. In a mixing experiment, neither corresponded to any of the five major [^{35}S]methionine-labeled peptides specific to McDonough FeSV P170 (Fig. 6B).

Comparison of [^{35}S]methionine-labeled tryptic peptides specific to McDonough FeSV P170 and Gardner FeSV P115. We have previously shown the 115,000-M_r polyprotein encoded by the Gardner strain of FeSV to contain, within its nonstructural component, five well resolved tryptic peptides, three of which (Fig. 7, peptides 2, 4, and 5) correspond to peptides present in Snyder-Theilen FeSV P115 (19).

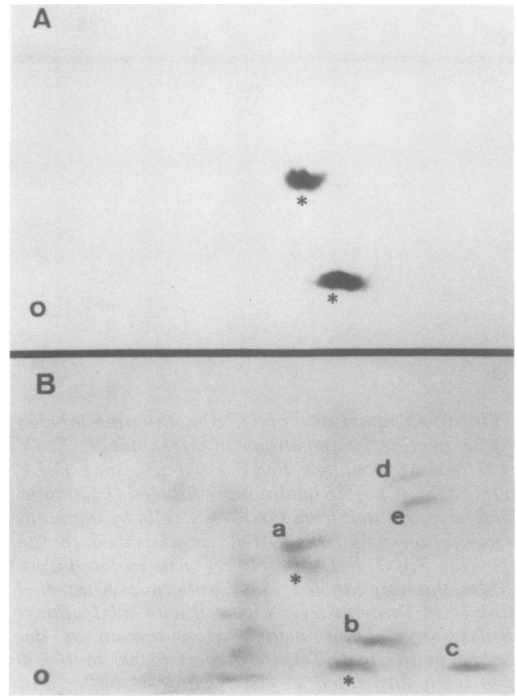

FIG. 6. *Comparison of [^{35}S]methionine-labeled tryptic peptide compositions of FeLV Pr82^{env} and McDonough FeSV P170. [^{35}S]methionine-labeled proteins were purified by immunoprecipitation and SDS-PAGE and subjected to tryptic peptide analysis as described in the legend to Fig. 3. FeLV Pr82^{env} was analyzed either alone (A) or mixed 1:1 with McDonough FeSV P170 (B). The two major Pr82^{env} [^{35}S]methionine-labeled peptides are designated * and McDonough FeSV P170 peptides are designated a through e.*

It was thus of interest to compare the tryptic peptide compositions of McDonough FeSV P170 and Gardner FeSV P115. Figure 7 shows that, although the tryptic peptide compositions of [^{35}S]methionine-labeled isolates of McDonough FeSV P170 from two independent transformed clones, McDonough C115-1 (Fig. 7A) and G2-MINK (Fig. 7B), were indistinguishable, both were distinct from Gardner FeSV P115 (Fig. 7C). In a mixing experiment, however, McDonough FeSV P170 peptide a was shown to correspond in position to Gardner FeSV P115 peptide 2. The remaining four [^{35}S]methionine-labeled peptides, specific to each, exhibited no detectable correspondence in tryptic map position.

Determination of relatedness of McDonough FeSV P170 to mink cellular P150. As discussed above, we have previously described a 150,000-dalton mink cellular phosphoprotein with associated protein kinase activity and binding affinity for Gardner FeSV P115 (Reynolds et al., submitted for publication). Although P150 has been shown to lack structural relatedness to Gardner or Snyder-Theilen FeSV P115 (19), the possibility that it might be partially related to McDonough FeSV P170 was not excluded. Tryptic peptide analysis of P150 revealed a total of 11 methionine-containing peptides (Fig. 8A), none of which appeared to correspond to the 5 major [^{35}S]methionine-labeled peptides specific to McDonough FeSV P170 (Fig. 8B). To confirm the apparent lack of correspondence between the tryptic peptide maps of these two proteins, a mixing experiment was performed (Fig. 8C). Again, none of the 11 [^{35}S]methionine-labeled peptides specific to P150, labeled 1 through 11, corresponded in map position to those contained within McDonough FeSV P170 (labeled a through e).

Analysis of McDonough FeSV P170 for labeling in vivo by $^{32}P_i$ and for associated protein kinase activity. High-molecular-weight polyproteins encoded by the Gardner and Snyder-Theilen strains of FeSV have been shown to contain multiple sites of phosphorylation in their nonstructural components (19, 20).

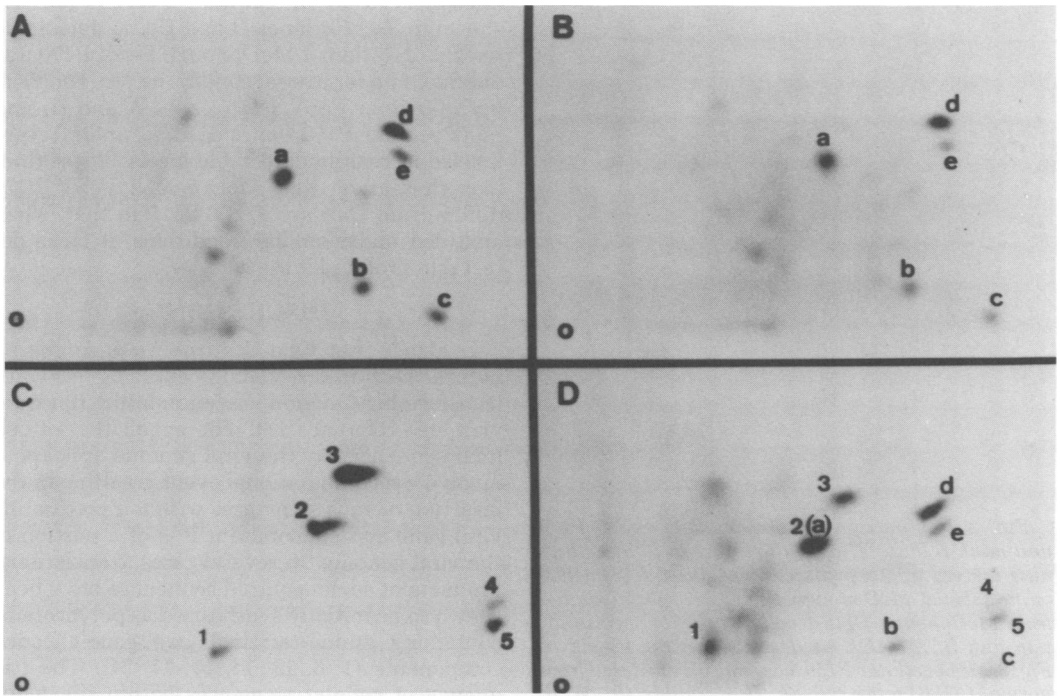

FIG. 7. *Comparison of [^{35}S]methionine-labeled tryptic peptide compositions of McDonough FeSV P170 and Gardner FeSV P115. [^{35}S]methionine-labeled McDonough FeSV P170 and Gardner FeSV P115 were isolated by immunoprecipitation and SDS-PAGE as described in the legend to Fig. 2. Tryptic peptide maps were generated as described in the legend to Fig. 3; polyproteins tested included McDonough FeSV P170 isolated from McDonough C115-1 cells (A), McDonough FeSV P170 isolated from G2-MINK cells (B), Gardner FeSV P115 isolated from the 64F3 cell line (C), and a 1:1 mixture of McDonough FeSV P170 isolated from McDonough C115-1 cells and Gardner FeSV P115 isolated from the 64F3 cell line (D). The major McDonough FeSV P170 and Gardner FeSV P115 tryptic peptides are designated a through e and 1 through 5, respectively.*

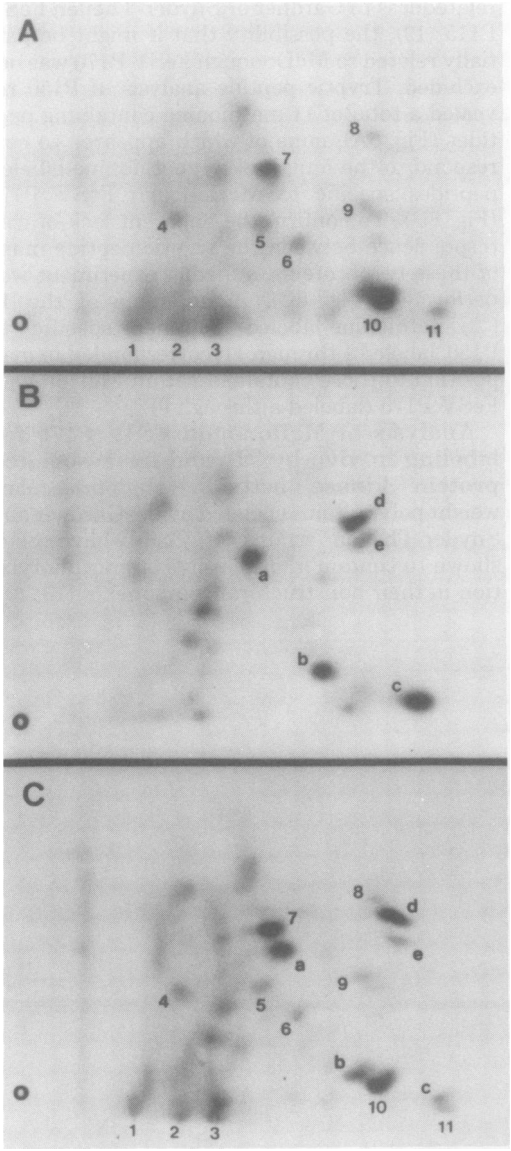

FIG. 8. *Comparison of McDonough FeSV P170 and mink cellular P150 with respect to [^{35}S]methionine-labeled tryptic peptide composition. [^{35}S]methionine-labeled McDonough FeSV P170 was isolated from McDonough C115-1 cells by immunoprecipitation and SDS-PAGE as described in the legend to Fig. 3. Mink cellular P150 was similarly isolated from normal CCL64 mink cells (F. H. Reynolds, Jr., W. J. M. Van de Ven, and J. R. Stephenson, submitted for publication). Tryptic peptide analysis was performed as described in the legend to Fig. 3; proteins tested included mink cellular P150 (A); McDonough FeSV P170 (B); and a 1:1 mixture of mink cellular P150 and McDonough FeSV P170 (C). The major McDonough FeSV P170 and mink cellular P150 tryptic peptides are designated a through e and 1 through 11, respectively.*

To explore the possibility that McDonough FeSV P170 might also be phosphorylated, the McDonough C115-1 cell line was cultured in $^{32}P_i$-containing medium, and labeled proteins were analyzed by immunoprecipitation and SDS-PAGE. As shown in Fig. 9, (lanes A, B, D, and E), P170 was labeled under these conditions, although only to a limited extent. Under similar conditions, 4070-A amphotropic mouse virus Pr65^{gag} (lanes D and E), FeLV Pr65^{gag} (lanes G and H), and mink cellular P150 (lanes B, E, H, J, and K) were each much more efficiently labeled. Thus, in vivo phosphorylation of McDonough FeSV P170 is considerably less than previously reported for polyproteins encoded by the Gardner and Snyder-Theilen strains of FeSV (19, 20).

Several virus-coded transforming proteins, including the nonstructural component of Gardner FeSV P115, have been shown to exhibit protein kinase activity (20). For a further characterization of McDonough FeSV P170, in vitro phosphotransferase activity was assayed under conditions previously shown to result in efficient phosphorylation of Gardner FeSV P115. As shown in Fig. 10 (lanes D and E), no detectable phosphorylation of McDonough FeSV P170 was obtained under these conditions. In contrast, both Gardner FeSV P115 (lanes A and B) and mink cellular P150 (lanes A, B, E, and H) were labeled at readily detectable levels. These findings thus argue that McDonough FeSV P170 differs from Gardner FeSV P115 in that, when analyzed under similar conditions, it lacks detectable protein kinase activity.

DISCUSSION

Evidence that type C retroviruses constitute natural vectors for cloning cellular genes with transforming function is accumulating (for a review, see reference 18). The acquisition of cellular sequences by the viral genome appears to involve a recombinational event resulting in the insertion of such sequences within a preexisting viral gene and concomitant loss of a portion of the viral genome. In several cases, translational products of such acquired sequences have been shown to be initially synthesized as polyproteins containing amino-terminal *gag* gene-encoded components (1, 6, 12, 14, 15, 17, 21). The frequency of cellular sequences within the feline cellular DNA, whose insertion into the type C viral genome confers the transforming potential, appears to be limited. This is indicated by the demonstration that the acquired sequence-encoded translational products of the two FeSV isolates, Gardner and Snyder-Theilen, are immunologically and structurally related (18), and by the results of molecular hybridization studies

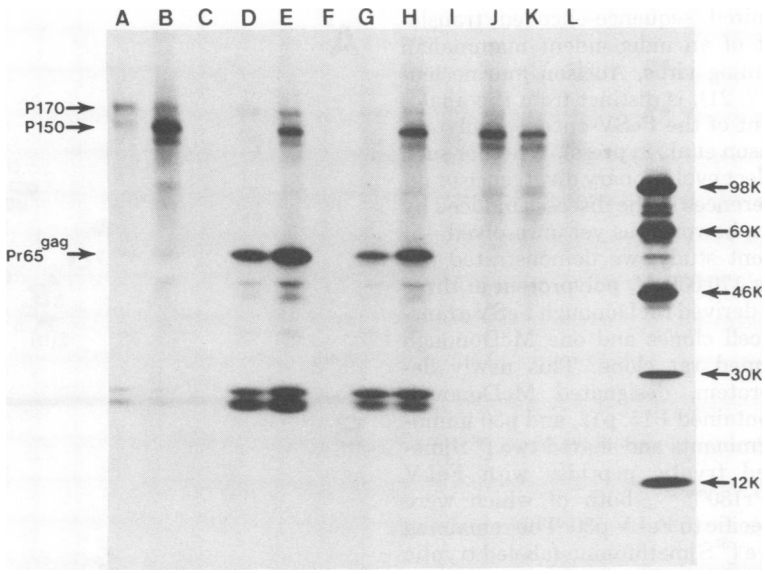

FIG. 9. *Determination of the extent of phosphorylation of McDonough FeSV P170 by immunoprecipitation and SDS-PAGE analysis. Cell lines, including McDonough C115-1 (A through C), McDonough C115-1 superinfected with 4070-A amphotropic wild mouse virus (D through F), McDonough C115-1 superinfected with FeLV subgroup A (G through I), and control CCL64 mink cells (J through L), were pulse-labeled for 2 h in medium containing $^{32}P_i$ (100 μCi/ml) and analyzed by immunoprecipitation and SDS-PAGE. Sera were prepared in goats and included anti-FeLV (A, D, and G), anti-FeSV P115 (B, E, H, J, and K), and normal goat (C, F, I, and L). Molecular weight standards are as described in the legend to Fig. 3.*

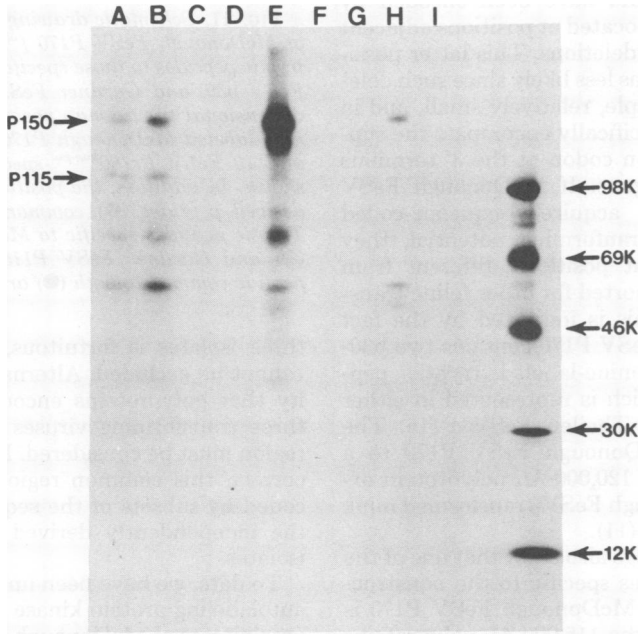

FIG. 10. *Analysis of McDonough FeSV P170 for protein kinase activity. Gardner FeSV-transformed (64F3) (A through C), McDonough FeSV-transformed (McDonough C115-1) (D through F), and uninfected control (G through I) mink cells were disrupted, immunoprecipitated, and analyzed for [γ-^{32}P]ATP phosphotransferase activity as described in the text. Antisera used included anti-FeLV (A, D, and G), anti-FeSV P115 (B, E, and H), and normal goat (C, F, and I). Molecular weight standards are as described in the legend to Fig. 3.*

(3). The acquired sequence-encoded translational product of an independent mammalian RNA transforming virus, Abelson murine leukemia virus (12, 21), is distinct from the analogous component of the FeSV-encoded polyproteins (Stephenson et al., in press). Whether such differences reflect evolutionary divergence or relate to the differences in the diseases induced by these viruses is, however, as yet unresolved.

In the present study, we demonstrated the expression of a 170,000-M_r polyprotein in three independently derived McDonough FeSV-transformed mink cell clones and one McDonough FeSV-transformed rat clone. This newly described polyprotein, designated McDonough FeSV P170, contained P15, p12, and p30 immunological determinants and shared two [^{35}S]methionine-labeled tryptic peptides with FeLV subgroup A Pr180$^{gag\text{-}pol}$, both of which were shown to be specific to FeLV p30. The remaining three of the five [^{35}S]methionine-labeled tryptic peptides contained within McDonough FeSV were not represented within FeLV Pr180$^{gag\text{-}pol}$ (Fig. 11). These could have corresponded to acquired cellular sequences responsible for the McDonough FeSV transforming function. Alternatively, McDonough FeSV P170 could have arisen as a result of deletions within FeLV Pr180$^{gag\text{-}pol}$, and the peptides unique to McDonough FeSV could have been encoded by genomic sequences located at positions adjacent to the sites of such deletions. This latter possibility, however, seems less likely since such deletions must be multiple, relatively small, and in phase and must specifically encompass the suppressible termination codon at the 3′ terminus of the FeLV *gag* gene. If McDonough FeSV P170 does contain acquired sequence-coded components with tranforming potential, they must be situated at positions different from those previously reported for other feline transforming viruses. This is indicated by the fact that McDonough FeSV P170 contains two p30-specific [^{35}S]methionine-labeled tryptic peptides, neither of which is represented in either Gardner or Snyder-Theilen FeSV P115. The relationship of McDonough FeSV P170 to a previously reported 120,000-M_r polyprotein expressed in McDonough FeSV-transformed mink cells is not resolved (11).

The present findings also show that one of the three tryptic peptides specific to the nonstructural component of McDonough FeSV P170 is also represented in the 115,000-M_r polyproteins encoded by the Gardner and Snyder-Theilen strains of FeSV (Fig. 11). The possibility that comigration of this single methionine-containing tryptic peptide in two-dimensional electrophoretic and chromatographic separations of all

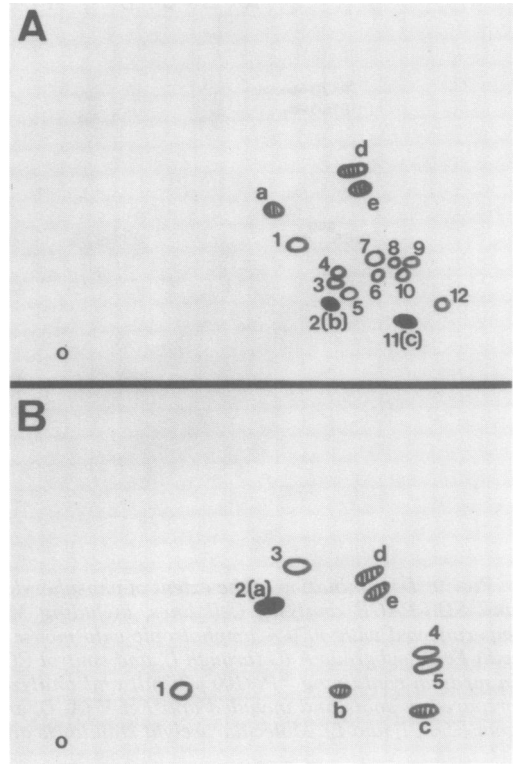

FIG. 11. *Schematic drawing depicting relatedness of McDonough FeSV P170 [^{35}S]methionine-labeled tryptic peptides to those specific to FeLV Pr180$^{gag\text{-}pol}$, FeLV p30, and Gardner FeSV P115. (A), the two-dimensional tryptic map positions of 3 [^{35}S]methionine-labeled McDonough P170-specific peptides (⊘) and 10 FeLV Pr180$^{gag\text{-}pol}$-specific peptides (○) are shown. In addition, the positions of two FeLV p30-derived peptides (●) common to both are shown. Tryptic peptides specific to McDonough FeSV P170 (⊘) and Gardner FeSV P115 (○) and the single peptide common to both (●) are shown in (B).*

three isolates is fortuitous, although unlikely, cannot be excluded. Alternatively, the possibility that polyproteins encoded by each of the three transforming viruses contain a common region must be considered. If the latter model is correct, this common region is apparently encoded by subsets of the sequences acquired by the independently derived transforming virus isolates.

To date, we have been unable to demonstrate autolabeling protein kinase activity in immunoprecipitates of McDonough FeSV P170, indicating that P170 lacks an associated or intrinsic protein kinase for which it is an active substrate. Moreover, McDonough FeSV P170 is not an apparent substrate for mink cellular phosphoprotein P150, which also has associated protein

kinase activity and binding affinity for Gardner FeSV P115 (Reynolds et al., submitted for publication). McDonough FeSV P170 thus differs from the P115 polyprotein encoded by Gardner FeSV, which exhibits an associated protein kinase activity recognizing P115 as a major substrate in specific immunoprecipitates (20). The possibility that McDonough P170 possesses protein kinase activity but lacks necessary acceptor sites to act as substrate cannot, however, be excluded. It is also not possible to exclude the possibility that the nonstructural component of P170 has protein kinase activity but that the enzyme is relatively inactive in an uncleaved polyprotein form.

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