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Antibodies of predetermined specificity detect two retroviral oncogene products and inhibit their kinase activities

(nucleotide sequences/synthetic peptides/transforming proteins/tyrosine kinases)

STACY SEN*, RICHARD A. HOUGHTEN*, CHARLES J. SHERR†, AND ARUP SEN*‡

*Committee for the Study of Molecular Genetics, Research Institute of Scripps Clinic, La Jolla, California 92037; and †Laboratory of Tumor Virus Genetics, National Cancer Institute, Bethesda, Maryland 20205

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ABSTRACT **Oligopeptides predicted from the nucleotide sequence of the oncogene v-*fes* of feline sarcoma virus (FeSV) were synthesized chemically and used to generate specific antibodies. Antisera against a 12-amino-acid-long oligopeptide (12-mer) located 42 residues from the carboxyl terminus of the v-*fes* coding sequence efficiently recognized the transforming proteins encoded by Snyder–Theilen (ST) and Gardner–Arnstein (GA) strains of FeSV. This 12-mer also contains 10 amino acid residues homologous in order and position to those predicted from the nucleotide sequence of the oncogene v-*fps* of avian Fujinami sarcoma virus (FSV). The anti-12-mer immunoprecipitated the FSV-specific transforming protein molecules from FSV-transformed cells. Binding of these antipeptide antibody molecules to the v-fes and the v-fps gene products inhibited their associated tyrosine-specific protein kinase (EC 2.7.1.37) activities. The ability to generate such site-specific antisera to the products of related oncogenes will be valuable in the molecular characterization of retroviral transforming proteins and their normal cellular homologs.**

Molecular cloning of acutely transforming retroviral genomes and the use of subgenomic DNA fragments to transform cells have allowed the identification of a number of distinct viral oncogenes and their homologs in the normal cellular DNAs (1, 2). At least six of these retroviral oncogenes, including the v-*fes* gene of feline sarcoma virus (FeSV), encode transforming proteins, all of which exhibit a tyrosine-specific protein kinase (EC 2.7.1.37) activity (1). Recently, the nucleic acid sequences of several avian and mammalian retroviral oncogenes have been determined (3–10). The nucleotide sequences of four of these oncogenes (designated v-*src*, v-*fps*, v-*yes*, and v-*fes*) predict that they encode protein products with identifiable domains of homology (7–10). At least one homologous region containing a tyrosine residue serves as a phosphate acceptor site in the products of this group of oncogenes (11, 12). Molecular characterization of these transforming proteins and their normal cellular homologs would be greatly aided by antisera of defined immunological specificities.

Chemically synthesized peptides predicted from nucleotide sequences have been used to raise specific antisera that can immunoprecipitate the native products of several genes, including two retroviral oncogenes (13–18). Synthetic peptides can elicit antibodies of predetermined specificity and also can facilitate the detection of gene products for which conventional antisera are unavailable (19). These antibodies can be directed toward amino acid sequences within evolutionarily conserved structural or functional domains and would be useful in the detection and molecular characterization of products of homologous genes.

Here, we describe studies using antisera directed against synthetic peptides predicted by the nucleotide sequence of a common domain near the carboxyl terminus of the v-*fes* oncogene of Snyder–Theilen (ST) and Gardner–Arnstein (GA) strains of FeSV. This region of v-*fes* was selected because of its predicted homology to the transforming gene products of Fujinami and Rous strains of avian sarcoma viruses (FSV and RSV). Antisera directed against two overlapping peptides of v-*fes* recognized the native viral-transforming proteins. The results reported here have two important features. First, these sera efficiently immunoprecipitate the products of cognate feline (v-*fes*) and avian (v-*fps*) oncogenes from stably transformed cells. Second, one antiserum inhibits the tyrosine-specific protein kinase activities associated with these two transforming proteins.

MATERIALS AND METHODS

Cells and Viruses. The GA-FeSV-transformed nonproducer, ST-FeSV-transformed producer of ST-FeSV (FeLV-B), and nontransformed producer of FeLV-B helper were all derived from the mink cell line CCL64 as described (20, 21). FSV- and RSV-transformed rat cell clones were generously provided by H. Hanafusa (The Rockefeller University, New York) and Peter K. Vogt (University of Southern California, Los Angeles), respectively. All cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum.

Peptide Synthesis. Peptides were synthesized by the solid-phase method (22) by using a cysteine resin as described (23). The individual amino acids had their side chains protected as follows: Arg-tosyl; Ser-, Thr-, Glu-, and Asp-*o*-benzyl; Tyr-*o*-bromobenzyloxy carbamyl; Trp-*N*-formyl. The *N*-formyl group on the "Trp" residues was removed after cleavage of the peptide from the resin support as described (24). The efficiency of coupling at each step was monitored with ninhydrin (25) or picric acid (26), or both, and was >99% in all cases. An amino acid analysis of the completed peptide gave the correct values.

Peptide Conjugation, Immunization, and Estimation of Antiserum Titers. Peptides were conjugated to freshly activated keyhole limpet hemocyanin as described (13). Adult male New Zealand White rabbits were each injected with 200 μg of peptide–KLH conjugate in complete Freund's adjuvant on day 1, with 200 μg of the conjugate in incomplete Freund's adjuvant on day 14, and with 100 μg of conjugate in aluminum hydroxide on day 21. Preimmune sera and test bleeds were partially fractionated by adding ammonium sulfate to 40% final saturation and the precipitated proteins were dissolved in phosphate-buffered saline (P_i/NaCl) before use.

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Abbreviations: FeSV, feline sarcoma virus; FSV, Fujinami sarcoma virus; RSV, Rous sarcoma virus; ST and GA strains, Snyder–Theilen and Gardner–Arnstein strains of FeSV, respectively; P_i/NaCl, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; FeLV, feline leukemia virus.

‡To whom correspondence should be addressed.

Antibody titers against the peptides were measured by an enzyme-linked immunosorbent assay (ELISA) by using a glucose oxidase conjugate of affinity-purified goat antiserum to rabbit immunoglobulins as the assay enzyme (27). Absorbance values were plotted against serum dilutions. The dilution giving one-half maximal color at the end of the linear range of the plot was taken as the titer of the antiserum.

Metabolic Labeling, Immunoprecipitations, and Immune Complex Kinase Assays. Cells grown to ≈80% confluency were rinsed once and then were incubated for 2 hr in methionine-free Dulbecco's modified Eagle's medium. Cultures were labeled for 2–4 hr with 50 μCi of [^{35}S]methionine (1,000 Ci/mmol; 1 Ci = 3.7×10^{10} Bq; New England Nuclear) in methionine-free medium. The cell monolayers then were lysed with 0.4 ml of IP buffer [immunoprecipitation buffer that contained 20 mM Tris·HCl (pH 7.2), 1% Nonidet P-40, 0.5% Na deoxycholate, 0.1% $NaDodSO_4$, 2 mM Na azide, 2 mM phenylmethylsulfonyl fluoride, 2% aprotinin, and 1 M NaCl]. Lysates were clarified at 12,000 × *g* for 15–20 min, precleared by incubation for 2 hr at 4°C with preimmune rabbit sera and removal of the immune complexes by incubation with 100 μl of Pansorbin (Calbiochem–Behring, La Jolla, CA). Aliquots of precleared lysates were incubated with 10 μl of test sera at 4°C for 2 hr. Immune complexes were collected by using Pansorbin, washed three times with IP buffer, washed once with 0.1 M Tris·HCl (pH 8.5) containing 0.5 M lithium chloride, and finally washed with P_i/NaCl. The washed immune complexes were dissolved directly in Laemmli sample buffer (28), boiled, and clarified by centrifugation. The supernatants were analyzed on discontinuous acrylamide gels (28). The gels were treated with EN^{3}HANCE (New England Nuclear) and then were dried and exposed to Kodak XRP-5 film.

For immune complex kinase assays, immunoprecipitates prepared from unlabeled cell lysates were washed once in 10 mM Tris·HCl (pH 7.4) containing 5 mM $MgCl_2$. Each washed pellet then was incubated in 25 μl of the above Tris·HCl/$MgCl_2$ buffer containing 1–2 μCi of [γ-^{32}P]ATP (3,000 Ci/mmol) for 20 min at 22°C. At the end of the incubation, pellets were washed once with P_i/NaCl, dissociated with Laemmli $NaDodSO_4$/polyacrylamide gel electrophoresis sample buffer, and electrophoresed. The gels were dried and exposed to Kodak XRP-5 film.

RESULTS

Antibody Response to the Synthetic Peptides. The following 30-amino-acid-long polypeptide located near the carboxyl terminus of the v-fes product was predicted from the nucleotide sequence of the gene (7):

$$\overset{1}{NH_2}\text{-Ser-Asp-Val-Trp-Ser-Phe-Gly-}\overset{10}{\text{Ile-Leu-Leu-Trp-Glu-Thr-Phe-Ser-}}$$
$$\overset{20}{\text{Leu-Gly-Ala-Ser-Pro-Tyr-Pro-Asn-Leu-Ser-Asn-Gln-Thr-}}\overset{30}{\text{Arg-COOH}}$$

This amino acid sequence has several notable features. First, it is located 27 residues to the carboxyl-terminal side of the single tyrosine residue which serves as a phosphate acceptor within the FeSV polyprotein itself (12). Second, 27 of 30 amino acid residues (except Thr at position 13, Ser at position 19, and Pro at position 22) are homologous in position to amino acids encoded by the v-*fps* gene of FSV (8). Finally, 17 residues are homologous to amino acids specified by the v-*src* gene of RSV and include residues 1–10, 12, 13, 17, 20–22, and 26 (3, 10). We synthesized chemically the entire 30-mer (peptide I) and a 12-mer comprising residues 18–30 at the carboxyl-terminal end (peptide II).

Table 1. Antibody titer of immunized rabbit sera against synthetic peptides

Immunizing antigen	Antibody titer against	
	Peptide I	Peptide II
Peptide I		
Animal 1	1:5,600	1:250
Animal 2*	1:4,000	1:200
Peptide II		
Animal 1	1:2,000	1:10,000
Animal 2	1:1,800	1:10,000

ELISA on immobilized peptide was performed and the results were plotted as described in the *Materials and Methods*. The antiserum dilution required to attain one-half maximal color intensity is expressed as the "titer."

*This animal received KLH–peptide I conjugate only for primary immunization. Subsequent injections used free peptide I.

Antipeptide titers (Table 1) were detectable within 5 weeks after the primary immunization, rose, and were maintained subsequently for 6–8 weeks, even if animals no longer received booster injections. The antibody titer to peptide II was ≥1:10,000 after 7 weeks and crossreacted with peptide I. A lower antibody response was obtained with peptide I (1:5,000) and these antisera crossreacted weakly with peptide II. Once immunized with the KLH-coupled conjugate, animals responding to peptide I mounted a secondary response when they received booster injections with the unconjugated peptide alone.

Antipeptide Sera Immunoprecipitate FeSV Polyproteins from Stably Transformed Cells. The genomes of FeSVs originated by recombination between the genome of replicating feline leukemia virus (FeLV) and the normal feline cellular protooncogenic sequence, c-*fes*. The genomes of both ST and GA strains of FeSV have the gene order 5′-Δgag-fes-Δenv-3′, where parts of the helper-derived *gag* and *env* genes have been deleted (20, 29). In both FeSV genomes *gag* and v-*fes* sequences constitute an open translational reading frame to encode fused gag-fes polyprotein molecules characteristic of each FeSV strain (30, 31). GA-FeSV contains certain sequences derived from the 3′ end of the *gag* gene and the 5′ end of the *fes* gene which are not present in the ST-FeSV genome (7). Hence, the GA-FeSV polyprotein is larger [M_r 108,000 (GA-FeSV P108 gag-fes)] than the ST-FeSV polyprotein [M_r 85,000 (ST-FeSV P85 gag-fes)].

Antisera directed to the synthetic peptides were tested for their ability to immunoprecipitate metabolically labeled proteins from extracts of cultured cells (Fig. 1). Normal mink cells (Fig. 1*A*), mink cells productively transformed by ST-FeSV (FeLV-B) (Fig. 1*B*), a nonproducer clone transformed by GA-FeSV (Fig. 1*C*), and an untransformed mink line infected with the FeLV-B helper virus (Fig. 1*D*) were tested. An antiserum to peptide II (5016) precipitated a M_r 85,000 (lane 8) and a M_r 108,000 (lane 13) protein from ST- and GA-FeSV transformants, respectively, but failed to precipitate either protein from the normal (lane 2) or the FeLV-infected (lane 17) cells. An antiserum to peptide I (5014) precipitated the ST-FeSV (lane 7) and not the GA-FeSV (lane 12) polyprotein. An antiserum to the FeLV gag gene product p27 precipitated the ST-FeSV P85 (lane 9) and

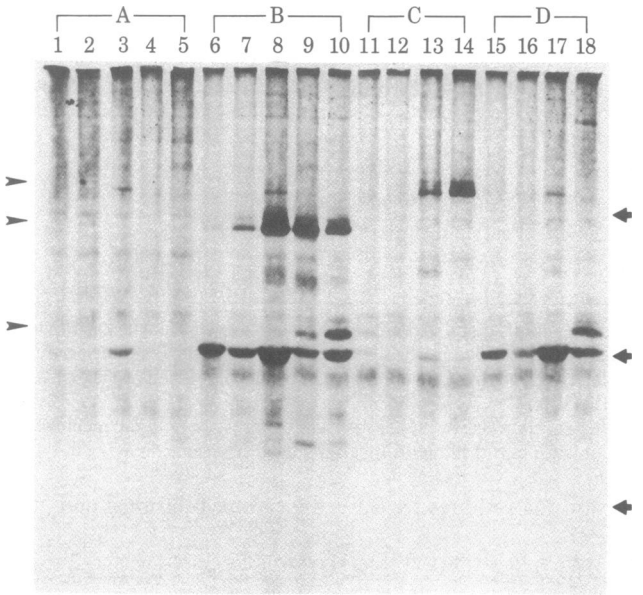

FIG. 1. $NaDodSO_4$/polyacrylamide gel electrophoresis analysis of immunoprecipitates of metabolically labeled FeSV polyproteins from FeSV-transformed cells. Extracts prepared from the following cell lines after labeling for 2 hr with [^{35}S]methionine were precleared with preimmune rabbit serum: (*A*) normal mink cells; (*B*) transformed mink cells producing ST-FeSV (FeLV-B); (*C*) a GA-FeSV-transformed nonproducer mink cell line; and (*D*) mink cells infected with FeLV-B helper alone. Precleared lysates from each cell line were incubated with either preimmune serum (lanes 1, 6, 11, and 15) or antisera against peptide I (lanes 2, 7, 12, and 16), peptide II (lanes 3, 8, 13, and 17), FeLVp15 (lanes 4 and 9), and FeLVp27 (lanes 5, 10, 14, and 18). Immunoprecipitates were analyzed on a denaturing discontinuous 10% acrylamide gel (28). Positions of molecular weight markers (arrows at the right from top to bottom) are: phosphorylase A (M_r 95,000), immunoglobulin heavy chain (M_r 55,000), and ovalbumin (M_r 44,000). Arrowheads at the left indicate the positions of GA-FeSV P108 (top), ST-FeSV P85 (middle), and FeLV Pr65gag (bottom). Only the top 12 cm of the gel is shown; there were no significant labeled protein bands below this portion of the gel. Variable amounts of a labeled protein migrating just ahead of the Pr65gag species are seen in different lanes of the gel; this is possibly a contaminating species that associates nonspecifically at random with immune complexes.

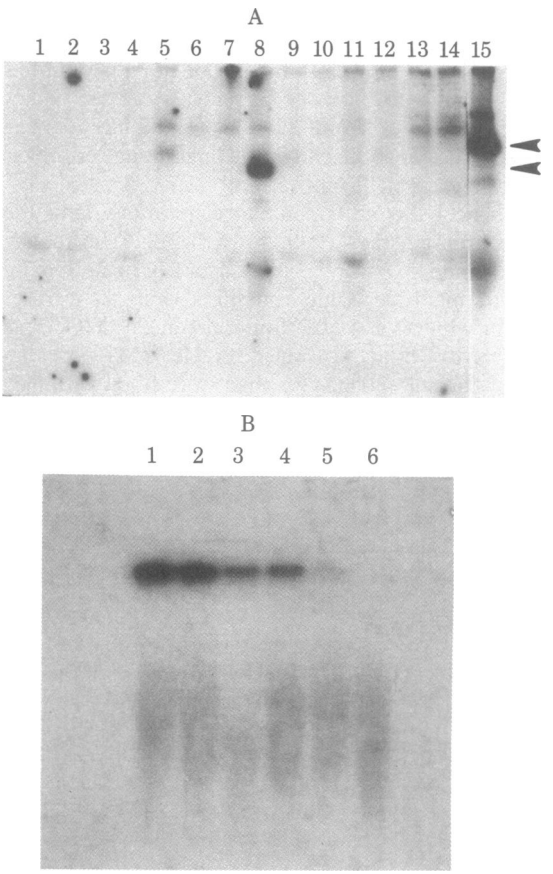

FIG. 2. Analysis and inhibition of protein kinase activity associated with FeSV polyproteins. (*A*) $NaDodSO_4$/polyacrylamide gel electrophoresis analysis of products phosphorylated by incubation of immune complexes with [γ-^{32}P]ATP. Immune complexes were prepared from unlabeled lysates of normal (lanes 1–4), ST-FeSV (FeLV-B) producer-transformed (lanes 5–8), FeLV-B helper-infected (lanes 9–11), and GA-FeSV-transformed nonproducer (lanes 12–15) mink cells. Immune complexes bound to formalinized *Staphylococcus aureus* protein A were incubated with 2 μCi of [γ-^{32}P]ATP (3,000 Ci/mmol) in 10 mM Tris·HCl, pH 7.4/5 mM $MgCl_2$ for 20 min at 22°C. Phosphorylated proteins were subjected to denaturing gel electrophoresis. Immunoprecipitates were prepared with either preimmune rabbit serum (lanes 1, 5, 9, and 12) or antiserum against peptide I (lanes 2, 6, and 13), peptide II (lanes 3, 7, 10, and 14), and FeLVp27 (lanes 4, 8, 11, and 15). Arrowheads at the right indicate the positions of GA-FeSV P108 and ST-FeSV P85. (The immune complex-associated kinase activity in the GA-FeSV nonproducer was much higher than that in the other samples; as such, the autoradiogram of lane 15 was aligned after a shorter exposure.) (*B*) Inhibition of kinase activity by using antiserum (5016) to peptide II. Fifty-microliter aliquots of lysates from ST-FeSV-transformed cells precleared with preimmune serum were incubated with the following serum mixtures: lane 1, 5 μl of 1:25 diluted anti-FeLVp27 and 15 μl of preimmune serum; lane 2, 5 μl of 1:25 diluted anti-FeLVp27, 12.5 μl of preimmune serum, and 2.5 μl of 5016; lane 3, 5 μl of 1:25 diluted anti-FeLVp27, 10.0 μl of preimmune serum, and 5.0 μl of 5016; lane 4, 5 μl of 1:25 diluted anti-FeLVp27, 7.5 μl of preimmune serum, and 7.5 μl of 5016; lane 5, 5 μl of 1:25 diluted anti-FeLVp27, 5.0 μl of preimmune serum, and 10.0 μl of 5016; and lane 6, 10.0 μl of preimmune serum and 10.0 μl of 5016. Immune complexes were washed and incubated with [γ-^{32}P]ATP and the labeled products formed after a 20-min incubation were subjected to electrophoresis.

the GA-FeSV P108 (lane 14); it detected the FeLV gag precursor Pr65gag from both cell lines producing FeLV (lanes 9 and 18). An antiserum directed against the FeLV gag determinant p15 also recognized the ST-FeSV P85 and the FeLV gag precursor from the productively ST-FeSV-transformed cells (lane 10). Neither of the gag sera detected any specific protein from the control mink cells (lanes 4 and 5).

In addition to the specific gag-fes polyproteins, the antiserum to peptide II detected another minor protein of M_r 100,000–105,000 in normal (lane 3), transformed (lane 8), and the FeLV-infected (lane 17) mink cells. The latter protein could represent the product of the mink c-*fes* gene, expressed at low levels in all cell lines tested, including the GA-FeSV-transformed line in which the P108 was not well resolved from the P100–105 (lane 13).

Antipeptide II Blocks Phosphotransfer Catalyzed by FeSV Polyproteins *in Vitro*. Immune complexes formed between the ST- and GA-FeSV polyproteins and antibodies to gag determinants catalyze the phosphorylation of a unique tyrosine residue within the viral polyproteins *in vitro* (12, 32, 33). We tested protein kinase activities in immune complexes formed with anti-gag or antipeptide antisera from FeSV-transformed cell lysates (Fig. 2*A*). By using antisera to gag determinants, immune complexes containing either ST-FeSV P85 or GA-FeSV P108 catalyzed phosphotransfer from [γ-^{32}P]ATP to the respective polyproteins (lanes 8 and 15). Parallel immune complexes with antipeptide I sera (lanes 6 and 13) or with antipeptide II sera (lanes 7 and 14) failed to catalyze any phosphorylation, although the amount of antipeptide II sera used in these experiments precipitated metabolically labeled polyproteins in amounts comparable to those obtained with the anti-gag sera. As con-

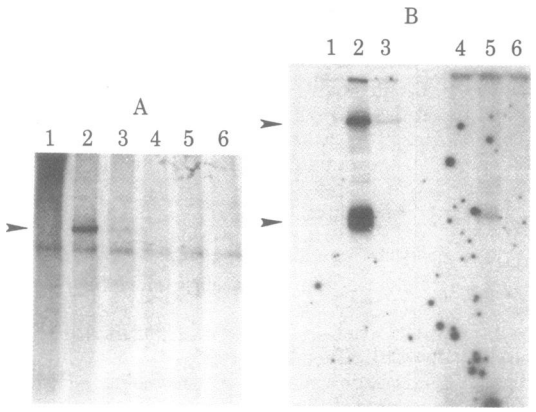

FIG. 3. Immunoprecipitation of FSV-encoded polyprotein and inhibition of associated protein kinase activity. (*A*) $NaDodSO_4$/polyacrylamide gel electrophoresis analysis of immunoprecipitates from metabolically labeled FSV-transformed (lanes 1–3) and untransformed (lanes 4–6) rat cells. [^{35}S]Methionine-labeled cell extracts were precleared with preimmune rabbit serum and then were immunoprecipitated with preimmune serum (lanes 1 and 4) or antiserum against peptide II (lanes 2 and 5) or peptide I (lanes 3 and 6). The immunoprecipitates were denatured and analyzed on a 10% acrylamide gel. The figure shows only the top one-half of the gel. The arrowhead on the left indicates the position of the FSV P130 polyprotein. (*B*) $NaDodSO_4$/polyacrylamide gel electrophoresis analysis of products phosphorylated *in vitro* in immune complexes from normal or FSV-transformed rat cells. Immune complexes were prepared from extracts of FSV-transformed (lanes 1–3) or normal (lanes 4–6) rat cells with preimmune rabbit serum (lanes 1 and 4), a RSV tumor-bearing rabbit serum (a generous gift from Jaon Brugge, State University of New York, Stony Brook) which crossreacts with the FSV gene product (lanes 2 and 5), or antiserum against v-fes peptide II (lanes 3 and 6). Products of incubation with [γ-^{32}P]ATP were analyzed. The arrowheads at the left indicate the positions of FSV P130 and immunoglobulin heavy chains. The portion of the gel containing samples from the normal rat cells was exposed 5 times longer than the other half containing samples from the transformed cells. Upon even longer exposures, a faint band of radioactivity in the immunoglobulin heavy-chain region could be seen in lane 5, possibly reflecting endogenous c-src kinase activity.

trols, immune complexes from normal (lanes 1–4) or FeLV-infected (lanes 9–11) mink cells did not catalyze any phosphorylation.

We tested if the failure to detect phosphotransfer activity in immune complexes formed with antiserum to peptide II was due to antibody inhibition of the phosphotransfer reaction. Equal aliquots of a lysate of cells transformed by ST-FeSV were incubated in parallel with mixtures containing different ratios of anti-gag and antipeptide sera, keeping the total amount of antibody constant, exceeding the amount necessary to quantitatively precipitate the polyprotein. Immune complex kinase assays (Fig. 2*B*) show that as the ratio of antipeptide to anti-gag serum increased (lanes 1–6), the extent of phosphorylation of ST-FeSV P85 was progressively decreased. In a parallel immunoprecipitation incubation, when an excess of free peptide II was added to block the antipeptide antibodies, the resulting immune complexes again catalyzed phosphorylation of the ST-FeSV P85 (data not shown). These results show that binding of antibodies to the gag-encoded amino-terminal domain of gag-fes polyprotein molecules does not affect kinase activity, whereas antipeptide antibodies that bind to the evolutionarily conserved determinants within the v-*fes*-encoded region inhibit this function.

Antisera to v-fes Peptide Detect v-fps Product and Inhibit Associated Protein Kinase Activity. The amino acid sequence of peptide II is similar to a sequence within the avian FSV-transforming protein (P130gag-fps) but lacks strong homology to the v-src product of RSV (7–10) as shown below:

v-*fes*	...F-S-L-G-A-	S-P-Y-P-N-L-S-N-Q-Q-T-R	-E-F-V-E
v-*fps*	...F-S-L-G-A-	V-P-Y-A-N-L-S-N-Q-Q-T-R	-E-A-I-E
v-*src*	...T-T-K-G-R-	V-P-Y-P-G-M-G-N-G-E-V-L	-D-R-V-E

Therefore, we tested the ability of antisera to the v-fes peptides I and II to crossreact with the transforming proteins expressed in FSV-transformed rat cells. Immunoprecipitation studies (Fig. 3*A*) show that antiserum to peptide II reacted with FSV P130 (lane 2), whereas the antipeptide I sera did not (lane 3). Neither of the two antipeptide sera precipitated the pp60src protein from RSV-transformed rat cells (data not shown).

Immune complexes formed between anti-src (serum from rabbits bearing RSV tumor) sera and FSV P130 can catalyze the phosphorylation of the FSV polyprotein as well as the immunoglobulin heavy chains (ref. 34; Fig. 3*B*, lane 2). Immunoprecipitates prepared with antipeptide II serum and FSV-transformed rat cell extracts did not phosphorylate FSV P130 or the immunoglobulin heavy chains (lane 3). No significant kinase activities could be detected in immune complexes prepared from normal rat cells with either serum (lanes 4–6). Thus, antipeptide II inhibits autophosphorylation and substrate phosphorylation catalyzed by FSV P130 in immune complexes. This antiserum then might bind directly to or indirectly affect a related active domain necessary for the protein kinase activity associated with both the FeSV- and the FSV-transforming proteins.

DISCUSSION

Chemically synthesized peptides predicted from the nucleotide sequences of ST- and GA-FeSV oncogenes were found to elicit antisera that efficiently immunoprecipitated FeSV gag-fes polyproteins—namely, ST-FeSV P85 and GA-FeSV P108 from mink cells transformed by ST- and GA-FeSV, respectively. Immunoprecipitation of these FeSV polyproteins of characteristic molecular weights with antisera directed to the amino-terminal gag determinants and also with site-specific antisera directed to the carboxyl-terminal domain of the v-*fes* gene unequivocally confirms that *gag* and v-*fes* genes are cotranslated into polyproteins in FeSV-transformed cells (30, 31, 35). Although the longer peptide (peptide I) included the shorter dodecamer (peptide II) at its carboxyl-terminal end, antisera to peptide II were more active in immunoprecipitating the FeSV-encoded proteins. It is possible that the hydrophobic amino-terminal domain influenced the folding of the carboxyl-terminal domain in peptide I. Thus, the resulting antiserum appears to be type-specific in that it recognized the ST-FeSV P85 but not the GA-FeSV P108. Antiserum to peptide II also reacted more strongly with ST-FeSV P85 than with GA-FeSV P108 even though both polyproteins were predicted to contain the same amino acid target sequence (7). Thus, when compared to immunoprecipitates with anti-gag sera, antipeptide II precipitated significantly lower levels of GA-FeSV P108 than ST-FeSV P85. We conclude that at least some antigenic determinants containing the target amino acid sequence are displayed differently within the native ST-FeSV- and GA-FeSV-encoded polyproteins.

The v-fes and v-fps gene products show $\approx$70% overall amino acid sequence homology and are presumed to be derived from cognate c-*onc* loci of cats and chickens (7, 8), with 10 homologous residues in peptide II domain. The antipeptide II sera efficiently immunoprecipitated both FeSV and FSV polyproteins. Although the carboxyl-terminal half of the v-*src* gene of RSV shares $\approx$45% of its residues with those of v-*fes* and v-*fps*, only a tripeptide is homologous within peptide II (3, 7, 10). The antipeptide II indeed failed to immunoprecipitate the pp60src protein of RSV. Thus, it might be possible to raise antisera of

predetermined crossreactivity (e.g., between v-*fes* and v-*fps*) and specificity (e.g., between v-*fes* and v-*src*) toward retroviral-transforming proteins.

In at least certain cases, antisera to v-onc proteins detect homologous c-onc-encoded products in normal cells. In the case of *fes* and *fps* genes, the normal cellular products, detected at low levels in feline and avian cells, appear to have a M_r of 95,000 (33, 36). The ability of the antiserum to peptide II to recognize a product of similar molecular weight, M_r 100,000–105,000, in all mink cells indicates that this dodecamer sequence might be conserved within the c-fes or c-fps proteins. Antisera directed against such conserved domains in v-*fes* or v-*fps* may well detect products of these cognate c-*onc* genes in different species.

The availability of high titer antisera to peptide II has allowed additional molecular characterization of FeSV- and FSV-encoded transforming proteins. Under the conditions used in our studies, ST-FeSV P85 and GA-FeSV P108 catalyzed autophosphorylation of the polyproteins themselves in immune complexes formed with anti-gag sera (12, 37). Binding of the antipeptide antibody inhibited this autophosphorylation reaction. In our experiments, FSV P130 catalyzed both the autophosphorylation and substrate immunoglobulin phosphorylation in immune complexes made with anti-src antisera (11, 34). Immune complexing of FSV P130 with the antipeptide sera inhibited both phosphorylation reactions. At present, it is not possible to distinguish whether the antipeptide antibodies bind directly to sequences within the putative active sites near the tyrosine acceptor residues in these putative enzymes or perturb indirectly their function by altering their tertiary structure. Our results indicate that antibodies directed to appropriately chosen sites on such enzymatically active transforming proteins would be useful in structure–function studies of retroviral oncogene products.

Antisera to the synthetic dodecamer used in the present studies have detected homology between products of cognate mammalian and avian v-*onc* genes. The conservation of amino acid sequences among functionally related products of homologous genes might now allow us to produce antiserum that will recognize the products of all of the members of the tyrosine kinase gene family. If these antibodies could be directed toward the active sites of functionally related tyrosine kinases (38–40), they may prove valuable in studying cellular enzymes implicated in growth control.

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