

Correspondence Between Immunological and Functional Domains in the Transforming Protein of Fujinami Sarcoma Virus

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Monoclonal antibodies reactive with either *gag* or *fps* portions of the wild-type Fujinami sarcoma virus transforming protein have been used to probe the structure of proteins encoded by mutant genomes constructed in vitro. The pattern of immunoreactivity suggests that the functional domain defined in genetic studies (Stone et al., Cell 37:549-558, 1984) corresponds to a discrete immunological domain in the native, wild-type Fujinami sarcoma virus protein. At least one mutation affecting both the structure and function of the proposed NH₂-terminal *fps*-specific domain encodes a product with high specific activities in kinase assays. Furthermore, a cell line expressing high levels of this mutant protein is only moderately transformed. The striking correspondence between the immunological domain defined here and the functional domain inferred from the results of transfection experiments suggests that this non-kinase-specifying region constitutes a discrete structural as well as functional component of the viral protein.

The transforming proteins of a number of acutely transforming retroviruses exhibit homology with the COOH-terminal half of pp60^{src} and have or are associated with tyrosine-specific kinase activity (1). Each of these acute transforming virus represents a unique recombination event between a nontransforming retrovirus that lacked a kinase-encoding oncogene and one member of a family of related proto-oncogene (*c-onc*) sequences. These *c-onc* genes apparently arose during evolution by a combination of events including gene duplication, recombination, and sequence divergence. In each case, the homologous kinase-encoding *c-onc* segment is found flanked by different cellular coding sequences. Such non-kinase-encoding segments are thought to encode protein components that normally function in concert with the kinase domain to regulate normal cellular growth and metabolism and are more or less represented in the corresponding transduced viral oncogenes.

Whereas there is a little doubt that the kinase-encoding region of each of these transforming proteins is crucial in transformation, evidence is accumulating that non-kinase-specifying regions adjacent in the primary structure also play a role. In pp60^{src} of Rous sarcoma virus, for example, variant proteins affected in either of two regions NH₂ terminal to the COOH-terminal kinase domain have altered transforming or tumor-inducing potential (3, 10, 11).

The transforming protein of one strain of Fujinami avian sarcoma virus (FSV) is a *gag-fps* fusion protein of 130,000 molecular weight with the schematic structure: NH₂-p19-p10-Δp27-fps-COOH (12). P130^{*gag-fps*} is composed of 308 residues of *gag*-derived material followed by 873 *c-fps*-related residues, the COOH-terminal 280 of which exhibit obvious homology with pp60^{src} and probably constitute the kinase-specifying domain (12, 15). To determine whether parts of P130^{*gag-fps*} other than the kinase specifying region are important in transformation, a molecularly cloned FSV genome has been subjected to in-phase insertion mutagenesis, and mutant genomes have been assayed for their ability to transform a normal rat cell line (13). The salient findings of

these studies were as follows: FSV genomes with in-phase insertions in either the NH₂-terminal *fps*-specific region or the COOH terminal *fps*-specific region have reduced transforming potential, whereas one position between these two *fps*-derived regions tolerates insertion of either single or multiple copies of a synthetic hexameric nucleotide sequence without loss of transforming function. The results suggested that the 5'-*fps*-related sequences encode a functionally distinct domain in the FSV transforming protein.

In this communication we describe studies exploring the proposed NH₂-terminal *fps* domain of P130^{*gag-fps*}. Insertion mutant proteins have been probed with monoclonal antibodies reactive with wild-type P130^{*gag-fps*}, and results have been obtained suggesting that mutations altering the NH₂-terminal *fps* domain cause a major conformational change in this region of the protein. A cell line expressing at high levels a representative mutation in this region and a line expressing wild-type FSV information have been studied in detail in terms of their level of transformation and their levels of P130^{*gag-fps*} protein and kinase activities. The results imply that the NH₂-terminal *fps*-specific region constitutes a discrete structural as well as functional domain, and that the function of this region is not intimately related to the phosphotransfer reaction.

MATERIALS AND METHODS

The construction and preliminary characterization of in-phase insertion mutations in a molecularly cloned FSV genome have been described previously (13). Each RX mutation was generated by insertion of one (RXm) or more (RXp) copies of a *Xho*I hexameric recognition sequence into an *Rsa*I restriction sequence in pJ2. This plasmid is a pBR322 derivative that allows efficient expression of FSV sequences in rat-2 cells and allows coselection of mutant FSV sequences with a linked *tk* gene from herpes simplex virus type 1. Genetic loci are numbered according to the published sequence (12), with position 1 representing the presumed cap site of the FSV transcript and with restriction sites labeled by their first nucleotide.

Cell culture. The permanent fibroblast line rat-2 (14) was grown in Dulbecco modified Eagle medium supplemented with penicillin and streptomycin and 10% fetal bovine serum

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TABLE 1. Immunoreactivity of wild-type and insertion mutant FSV proteins^a

FSV protein			Monoclonal antibody			
FSV genotype	Locus	Cell line	R254E (anti- <i>gag</i>)	88A6 (anti- <i>fps</i>)	P26C (anti- <i>fps</i>)	No primary antibody
+	-	A49-9	+	+	+	-
-	-	rat-2	-	-	-	-
RX42p	569	N14	+	+	+	-
RX5p	1059	N16-2	+	+	+	-
RX31m	1582	12-3H	+	-	-	-
RX22m	1843	A13bc	+	-	-	-
RX32p	1978	F22	+	-	-	-
RX32m	1978	A22	+	-	-	-
RX8m	2152	28-5H	+	-	-	-
RX18p	2285	F29c	+	+/-	+/-	-
RX18m	2285	A30	+	+	+	-
RX26m	3853	A46	+	+	+	-

^a Immunoreactivity of FSV proteins was judged by the intensity of the P130^{*gag-fps*} band obtained in an in vitro kinase assay as follows: +, strong signal; +/-, weak signal; -, no detectable signal. With lines A49-1, A13bc, and rat-2, similar results were obtained with cells metabolically labeled with [³⁵S]methionine.

at 37°C in a 5% CO₂ atmosphere. Lines expressing FSV genomes were established by isolating foci or *tk*⁺ colonies after transfection with plasmid using a calcium phosphate procedure. Some of the lines have been described previously, and all are listed in Table 1. Selection of *tk*⁺ colonies and other details of cell culture methods were as described previously (13).

Analysis of wild-type and insertion mutant FSV transforming proteins. The labeling of cells with [³⁵S]methionine, the in vitro immune complex autophosphorylation assay, and the analysis of proteins by electrophoresis in 7.5% and 10% sodium dodecyl sulfate-polyacrylamide gels were as described by Weinmaster et al. (15). Details of each experiment are given in the figure legends. Lysates of [³⁵S]methionine-labeled protein were preadsorbed with Formalin-fixed *Staphylococcus aureus* at 2.5% weight per final volume for 1 h on ice to reduce the level of normal cellular proteins in the final immune precipitate. After the adsorbed cellular proteins were removed, the preadsorbed lysate was split into four aliquots, each containing either one of the three anti-P130^{*gag-fps*} monoclonal antibodies, or no primary antibody. The monoclonal reagents R254E (anti-*gag*), 88AG (anti-*fps*), and p26C (anti-*fps*), their precipitation with rabbit anti-mouse immunoglobulin G (IgG) or rabbit anti-rat IgG-coated *S. aureus* cells, and washing procedures were as described by Ingman-Baker et al. (9). To assay FSV kinase activity with an exogenous substrate, rabbit muscle enolase (Boehringer Mannheim Corp.) was acid treated and incorporated in the kinase reaction as described by Cooper et al. (4). Radioactivity in specific gel bands was quantitated by either Cherenkoff counting (³²P) or scintillation counting (³⁵S), and values were corrected by subtracting the amount measured in the corresponding region of the control lane obtained with no primary antibody.

RESULTS

Immunological probing of wild-type and insertion mutant P130^{*gag-fps*} species. The observation that insertion mutations affecting either the NH₂- or COOH-terminal *fps*-specific portions of P130^{*gag-fps*} result in an FSV genome with reduced transforming potential, whereas insertion of multiple copies

of a synthetic hexameric oligonucleotide in one position between these regions has little or no effect, suggests that the NH₂-terminal *fps*-specific region, like the COOH-terminal one, may constitute a more or less autonomous component of the transforming protein. No function demonstrable in vitro can be attributed to this portion of the protein, and no defined structural landmark (e.g., a phosphopeptide) has been unambiguously mapped to this region. Consequently, protease digestion experiments cannot be readily exploited to test this hypothesis. The availability of *fps*-specific monoclonal antisera, however, has permitted a sensitive, if indirect, means of comparing the anatomy of mutant and wild-type FSV proteins.

The monoclonal antibodies R254E (anti-*gag*), 88AG (anti-*fps*), and p26C (anti-*fps*) were each used separately to assay for P130^{*gag-fps*} in lysates prepared from lines expressing insertion mutant FSV genomes. P130^{*gag-fps*} was either labeled metabolically with [³⁵S]methionine or labeled in vitro with [³²P]ATP with the immune complex autophosphorylation reaction.

A line transformed by a wild-type FSV genome, A49-1, expresses a 130,000-molecular-weight protein that precipitates specifically with each antibody (Fig. 1). Normal rat-2 cells contain no such protein (data not shown). The line A13bc is partially transformed and expresses the RX22m insertion mutant FSV genome. The mutation is predicted to

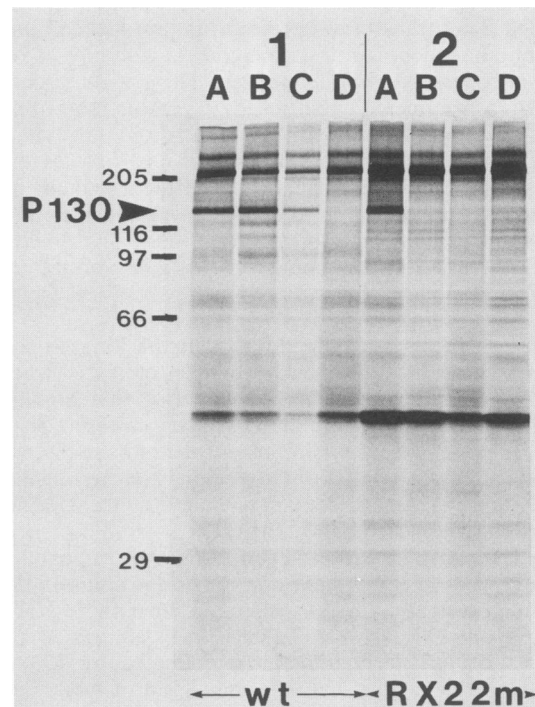


FIG. 1. Analysis of P130^{*gag-fps*} in A49-1 and A13bc cells by immune precipitation and gel electrophoresis of ³⁵S-labeled proteins. A 100-mm plate was seeded with 3 × 10⁶ cells of either type and then incubated for 12 h in 3.0 ml of medium containing 1% fetal bovine serum and 200 μCi of [³⁵S]methionine (1,000 Ci/mmol) as the only source of this amino acid. Cells were lysed, and samples of each lysate were probed with monoclonal antibodies as described in the text. Autoradiographic exposure was for 3 days. Sections: 1, wild-type FSV protein expressed in A49-1 cells; 2, RX22m insertion mutant FSV protein expressed in A13bc cells. Precipitation was with (A) R254E (anti-*gag*), (B) 88AG (anti-*fps*), (C) P26C (anti-*fps*), and (D) no primary antibody.

result in the substitution of tyrosine 489, in the middle of the NH₂-terminal *fps*-specific region, by the tripeptide Ser-Arg-Asp (13). A13bc expresses a protein that is reactive with the anti-*gag* antibody, but nonreactive with either of the anti-*fps* antibodies (Fig. 1). Similar results were obtained with the in vitro kinase assay; since the kinase method is more convenient and sensitive, it was used to test the other lines. Typical results obtained with the kinase assay are shown in Fig. 2, and the findings are summarized in Table 1. Note that proteins encoded by virtually transformation-defective FSV genomes have not been analyzed, since cell lines expressing these species have not yet been isolated. The insertion mutant RX31m was previously reported to be transformation defective in the focus induction assay, but has since been shown to induce a mild degree of morphological transformation after coselection in HAT medium with the linked *tk* gene. The slightly transformed line 12-3H was developed from an RX31m-transfected, *tk*⁺ rat cell colony and shown to express catalytically active P130^{*gag-fps*} (Table 1).

Considering the results obtained with R254E (anti-*gag*), all lines expressed a reactive, catalytically intact P130^{*gag-fps*} species, including two lines expressing FSV genomes with inserts in the *gag* region. The RX42p mutation has been shown by DNA sequence analysis to consist of six copies of the hexamer CTCGAG inserted into the *Rsa*I site at nucleotide position 569 (13). This locus is within the p19 encoding sequence. The results presented here show that RX42p does not significantly distort the epitope of R254E, which has been localized to the p19 region of *gag* (9).

The anti-*fps* monoclonal antibodies react with proteins encoded by mutations in the *gag* region, by mutations in the insertion tolerant site (RX18p and RX18m), and by a mutant affecting the COOH-terminal region (RX26m). In the case of line F29c (RX18p), the reactivity of the mutant FSV protein with either of the anti-*fps* antibodies was relatively weak; this pattern was observed in duplicate experiments. Neither of the anti-*fps* monoclonal antibodies reacted significantly with any of the proteins altered in the NH₂-terminal *fps* region of P130^{*gag-fps*}. The results suggest that mutations at each of the four adjacent loci effect a similar alteration in this region of the transforming protein and that some normal structural features in this part of the wild-type protein constitute the epitopes of these two monoclonal antibodies.

Comparison of wild type- and RX22m-encoded P130^{*gag-fps*} kinase activities. To compare transforming proteins defective in the NH₂-terminal *fps* region with wild-type protein in terms of their catalytic properties, attempts were made to compare relative levels of P130^{*gag-fps*} protein, P130^{*gag-fps*} autophosphorylating activity, and P130^{*gag-fps*} enolase-phosphorylating activity in lines A49-1 and A13bc.

Cells of line A49-1 express wild-type FSV protein, are overtly transformed by morphological criteria, and form large colonies when seeded in soft agar (Fig. 3). Cells of line A13bc express the RX22m insertion mutant FSV genome. The cells are only moderately transformed and form small colonies in soft agar. In liquid culture A13bc cells tend to be elongated, as do cells expressing RX32p or RX32m, which map 135 base pairs to the right (13). When grown without subculturing, A13bc cells form a confluent, contact-inhibited monolayer, whereas A49-1 cells grow to high density and then lose viability.

Plates containing an equal number of cells of either line were assayed for P130^{*gag-fps*} protein by immune precipitation of [³⁵S]methionine-labeled material with the anti-*gag* monoclonal antibody, followed by gel electrophoresis and scintillation counting of the P130^{*gag-fps*} band. Duplicate

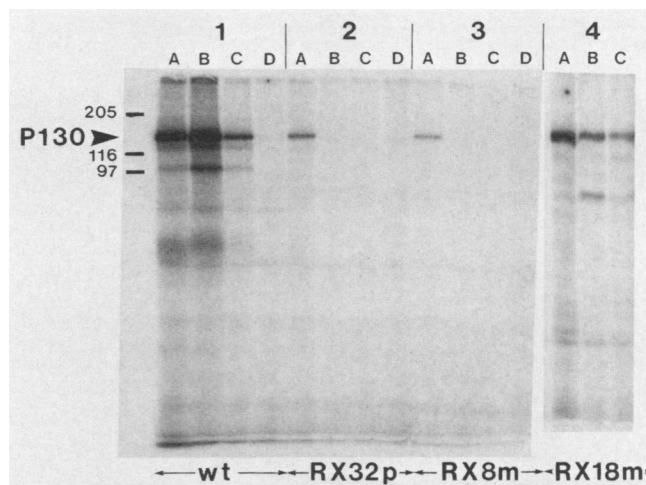


FIG. 2. Analysis of P130^{*gag-fps*} autophosphorylating activities in wild-type and insertion mutant FSV-transformed lines. In each case a 100-mm plate of cells was lysed, and samples were precipitated with different monoclonal antibodies. Immune precipitates were incubated with [γ -³²P]ATP to allow autophosphorylation before electrophoresis through 10% sodium dodecyl sulfate-polyacrylamide gels. Autoradiographic exposure was for 7 days. Sections: 1, line A49-1, expressing wild-type FSV information; 2, line F22, expressing the RX32p insertion mutant genome; 3, line 25-5H, expressing RX8m; 4, line A30, expressing RX18m. Antibodies were (A) R254E (anti-*gag*), (B) 88 AG (anti-*fps*), (C) p26C (anti-*fps*), and (D) no primary antibody.

plates of each were assayed in parallel with an in vitro kinase assay with enolase incorporated to serve as an exogenous protein substrate. Typical results are shown in Fig. 4, and the results of quantitating radioactive bands in three experiments are presented in Table 2.

Each datum in Table 2 represents either the labeled protein or the kinase activities recovered from a plate of cells by a rather extended series of manipulations. Furthermore, the kinase activities represent single endpoint determinations, not reaction rates. Thus the data are semiquantitative. Bearing these considerations in mind, it appears that A13bc (RX22m transformed) expresses slightly more FSV protein, has increased autophosphorylation activity, and has slightly reduced levels of enolase-phosphorylating activity, relative to A49-1 (wild-type FSV transformed). Two-dimensional tryptic phosphopeptide maps of autophosphorylated wild type- and RX22m-encoded proteins were essentially identical (data not shown), indicating that the phosphoacceptor sites of the mutant protein are not disturbed by the peptide insertion.

DISCUSSION

Correspondence between a functional and an immunological domain in the NH₂-terminal *fps*-specific portion of P130^{*gag-fps*}. In previous work with in-phase insertion mutations throughout the FSV genome, four mutations at three loci in the NH₂-*fps* specific region were shown to induce foci infrequently. Since these foci gave rise to moderately transformed lines with detectable FSV kinase activity, we concluded that the mutations were in-phase, nonpolar insertions that encoded transforming proteins defective in some transforming function performed by the NH₂-terminal *fps*-specific region of P130^{*gag-fps*}. This conclusion is supported by a number of other studies demonstrating that the virus PRCII

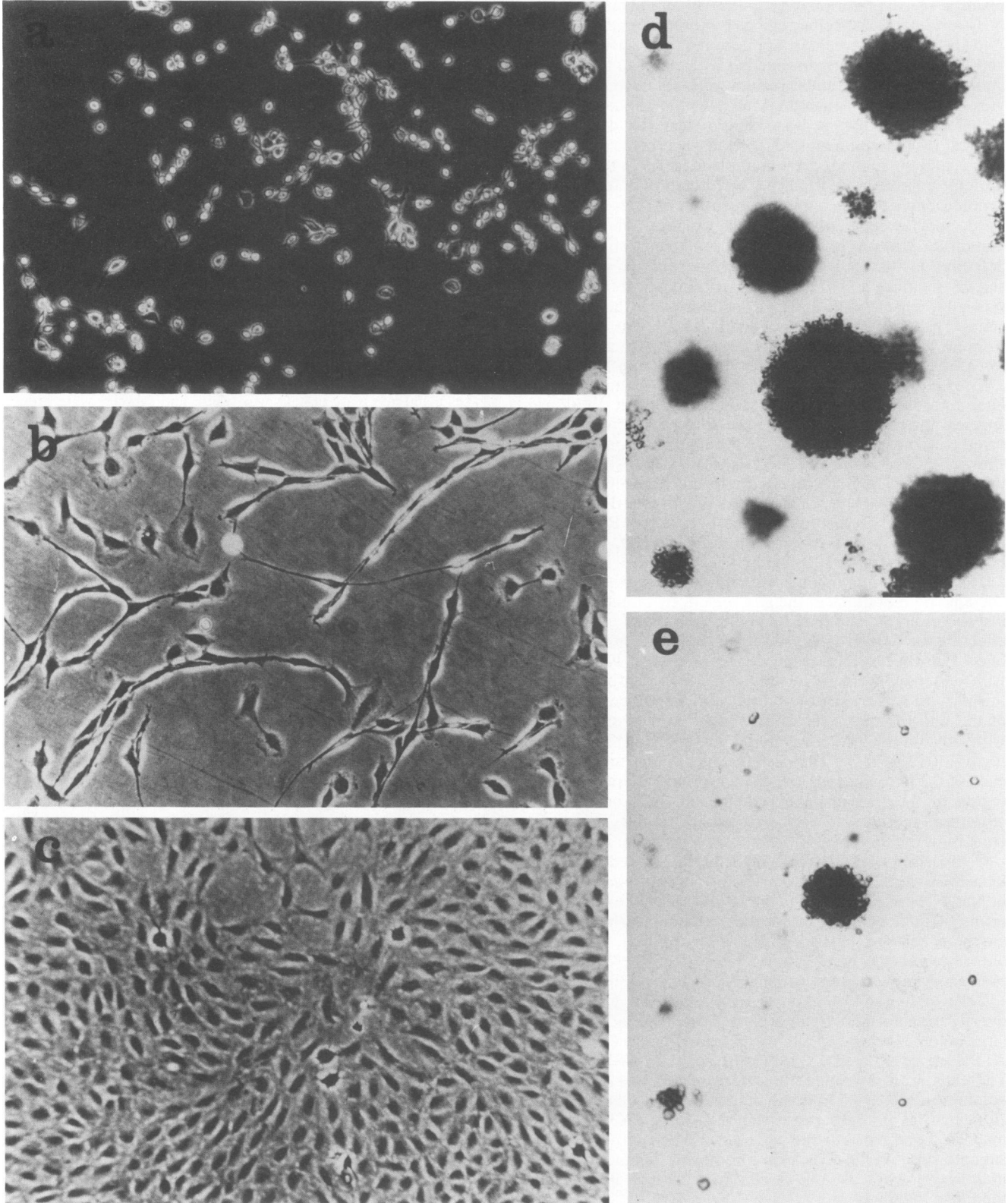


FIG. 3. Properties of A13bc partially transformed line expressing RX32m, an NH₂-terminal *fps*-defective FSV genome. (A) A49-1, an overtly transformed line expressing wild-type FSV information, grown in liquid culture. (B) A13bc, a partially-transformed line expressing RX32m, grown in liquid culture. (C) rat-2, the normal rat fibroblast line used to derive A49-1 and A13bc, grown in liquid culture. (D) A49-1 cells grown for 16 days in soft agar, typical field. (E) A13bc cells grown for 16 days in soft agar, field selected to show most significant growth. rat-2 cells reproducibly fail to grow in soft agar (data not shown).

has both a reduced transforming potential and a deletion of NH₂-terminal *fps* sequences relative to FSV (2, 5, 7). The biological differences between FSV and PRCII, however, may arise from other genetic differences between these viruses (8).

The present studies have shown that RX31m, although totally defective in focus induction, is capable of eliciting a slight degree of morphological transformation after coselection with a linked *tk* gene. Also, this mutant FSV genome encodes a catalytically active protein kinase. Thus RX31m is implicitly in phase, and the region of P130^{*gag-fps*} affected by this insertion might reasonably be considered as part of the hypothesized NH₂-terminal *fps*-specific domain.

Based on the common properties of five kinase-encoding mutations at four loci (13; this study) the NH₂-terminal *fps*-specific functional domain is proposed to extend over an approximately 300-amino-acid residue segment extending from near the *gag-fps* boundary to a position between RX8 and the insertion tolerant site, RX18, or roughly the second quarter of the P130^{*gag-fps*} primary structure (Fig. 5).

The immunological analysis of insertion mutant FSV transforming proteins has revealed a striking correspondence between mutations affecting the epitopes of the two anti-*fps* monoclonal antibodies and those affecting the proposed NH₂-terminal *fps*-specific functional domain.

Two questions arise. (i) What is the nature of the *fps* epitopes recognized by these antibodies? (ii) What structural changes in the NH₂-terminal *fps*-specific mutant proteins account for their failure to react with the anti-*fps* monoclonal antibodies?

Previous data (9) were consistent with two formal propos-

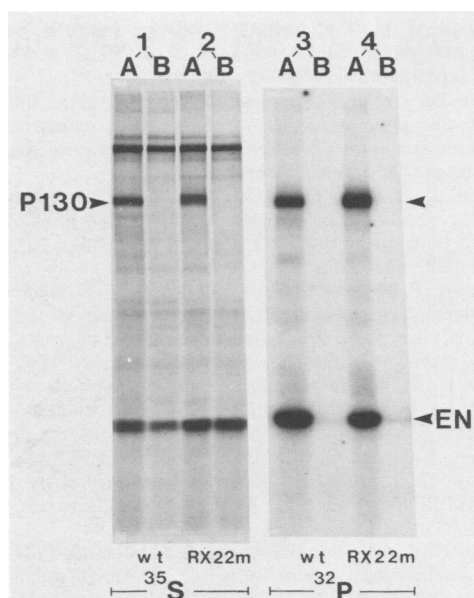


FIG. 4. Comparison of wild-type (wt)- and RX22m-encoded P130^{*gag-fps*} levels in lines A49-1 and A13bc by immune precipitation of [³⁵S]methionine-labeled protein, autophosphorylating activity, and enolase-phosphorylating activity. Sections: 1 and 2, [³⁵S]methionine-labeled P130^{*gag-fps*} from A49-1 (wild type) and AB13bc (RX22m), respectively; 3 and 4, autophosphorylating and enolase-phosphorylating activities in lines A49-1 and A13bc, respectively. Approximately 3 × 10⁶ cells of each genotype were lysed in each case, and half of each lysate was precipitated with either R254E (A) or no primary antibody (B). Autoradiographic exposures were 2.5 days in the case of [³⁵S]methionine-labeled proteins and 1 day in the case of ³²P-labeled proteins. EN, Phosphorylated enolase.

TABLE 2. Comparison of wild-type and RX22m encoded P130^{*gag-fps*} kinase activities^a

Expt	Cell line/FSV genotype	P130 ^{<i>gag-fps</i>} protein		P130 ^{<i>gag-fps</i>} autophosphorylation		Enolase phosphorylation	
		cpm	% FSV ⁺	cpm	% FSV ⁺	cpm	% FSV ⁺
1	A49-1/+	548		463		1,352	
	A13bc/RX22m	944	172	872	188	1,261	93
2	A49-1/+	1,645		1,005		3,607	
	A13bc/RX22m	1,273	77	1,692	168	2,052	57
3	A49-1/+	1,986		1,201		2,994	
	A13bc/RX22m	1,576	79	1,897	158	2,293	76
Avg			109		171		75

^a Numbers given are counts per minute corrected for control values, as described in the text. The labeling protocol in experiment 1 was not the same as in experiments 2 and 3, so absolute numbers are not comparable.

als for the nature of the *fps* epitopes. Either *fps* epitope could be constituted of a contiguous segment of *fps*-encoded polypeptide chain. Alternatively, either epitope could be composed of noncontiguous *fps*-encoded polypeptide segments that are brought into proximity in the native P130^{*gag-fps*} molecule by secondary and tertiary folding. The fact that both antibodies fail to precipitate antigen in the presence of 0.1% sodium dodecyl sulfate suggested that protein conformation was important in antigen recognition. It should also be noted that, although p26C and 88AG are similar in that they fail to recognize the PRCII protein, they differ in their specificity toward the products of other *fps* genes. Thus, the two epitopes are different (9).

The results presented here demonstrate that peptide insertions in four positions within a segment of 190 amino acids in P130^{*gag-fps*} destroy both *fps* epitopes, a result difficult to reconcile with the contiguous peptide model, but readily accommodated by the conformation model for the *fps* epitopes. The results imply that the NH₂-terminal *fps*-specific region of P130^{*gag-fps*} constitutes a discrete structural region, as well as a discrete functional region, and that both of these are disrupted by peptide insertions at each of the four sites within. The ultimate structural consequences of these insertion mutations are unknown, but it may be noteworthy that each of the mutations affecting the NH₂-terminal *fps*-specific domain is predicted to result in the

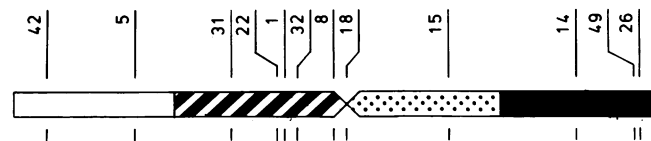


FIG. 5. Schematic diagram of P130^{*gag-fps*} primary structure drawn to scale, showing positions of peptide insertions and boundaries of structural and functional domains. Symbols: open box, *gag* (amino acid residues 1 through 309); hatched box, NH₂-terminal *fps*-specific region (residues 310 through 636); stippled box, region of unknown function (residues 636 through 888); solid box, *src*-homologous, kinase-encoding domain (residues 889 through 1182). The scheme is based in the sequence analysis of Shibuya and Hanafusa (12) and the analysis of insertion mutations (13; this study).

following substitution: Tyr → Ser Arg Asp (or Tyr → Ser [Arg Ala]_{n-1} Arg Asp, where *n* equals the number of hexamer insertions, in the case of the RXp alleles). Possibly the removal of a relatively hydrophobic residue or the insertion of hydrophilic ones (or both) in each case results in the eversion of protein components normally removed from the aqueous environment in a globular domain. In any case, the striking correspondence between the deduced functional and immunological domains in P130^{gag-fps} would tend to validate the use of in-phase insertion mutations for the genetic dissection of complex polypeptides.

Possible functions of the NH₂-terminal *fps* domain. The drastically reduced number of foci obtained with NH₂-terminal *fps*-affected FSV genomes (13) implies that this region of P130^{gag-fps} plays some role in the initiation of the transformed state. The failure to establish fully transformed lines from these infrequent foci additionally suggests that this region of the FSC protein is important in determining the level of transformation maintained. Previous data, however, did not rule out the possibility that the moderate transformation levels were merely the result of low levels of FSV expression.

From the comparison of FSV protein and kinase levels in lines A49-1 and A13bc, it is clear that the reduced level of transformation exhibited by A13bc is not a simple consequence of low levels of FSV gene expression. Rather, RX22m-encoded protein is defective in some function performed by the NH₂-terminal *fps*-specific region of P130^{gag-fps}. Furthermore, although the apparent increase in autophosphorylation and decrease in enolase phosphorylation activities observed with RX22m-encoded protein *in vitro* may mirror changes in the mutant kinase activity *in vivo*, as opposed to reflecting simple experimental variability, it is also clear that the NH₂-terminal *fps*-specific functional region of P130^{gag-fps} is not directly involved in catalysis. The evidence presented here supports the scheme for at least two *fps*-encoded functional regions previously proposed (13), but sheds little light on what role the NH₂-terminal *fps*-specific region plays in transformation. The nonkinase domain could directly affect substrate-P130^{gag-fps} interaction, or it could facilitate localization of the transforming protein into a particular compartment, in proximity with key cellular targets. Unfortunately, little evidence bearing on either possibility exists. Amino-terminal *fps*-defective insertion mutant proteins and COOH-terminal tryptic fragments of wild-type FSV protein phosphorylate enolase *in vitro*; in the latter case the site of enolase phosphorylation is the same as that observed in transformed cells (4). Thus, an intact NH₂-terminal *fps*-specific region is not necessary for accurate recognition of this exogenous substrate *in vitro*. Further analysis of lines expressing high levels of functionally altered FSV protein in combination with physical approaches to the structural changes documented here should provide further insight into the NH₂-terminal *fps*-specific domain of P130^{gag-fps} and ultimately into the function of *c-fps* and the mode of transformation by FSV.

Domain structure of P130^{gag-fps}. Based on the nucleotide sequence of FSV (12) and the analysis of in-phase insertion mutations (13; this study), a quadrupartite model for P130^{gag-fps} origin, structure, and transforming function is proposed (Fig. 5).

Indisputably, the NH₂-terminal 26% of the FSV polypeptide is related to the *gag* polyprotein of the parental virus that transduced the *c-fps* sequence. The function of *gag* in transformation is less clear, but it seems that this segment is

not crucial for the biological activity of the transforming protein (6, 13).

The COOH terminal 24% of the P130^{gag-fps} sequence exhibits obvious homology to *src*, specifies the kinase activity, and, no doubt, plays a fundamental role in transformation.

NH₂ terminal to the *src* homologous domain is a region of unknown significance in transformation (Fig. 5, stippled segment). This portion of P130^{gag-fps} contains several short segments of peptide exhibiting sequence-identity with pp60^{src} (M. Green, personal communication). The corresponding region of pp60^{src} has been implicated in transformation (3, 10), but is distinct from the region involved in pp60^{src} kinase activity. Further analysis of RX15 and other insertion mutations mapping in this region should clarify its functional significance.

The NH₂-terminal *fps* domain explored here is apparently unique to *fps*-encoded proteins (Fig. 5, hatched segment). The region spans the sites affected by mutants at loci RX31 to RX8, based on the phenotypic similarities between mutants at these sites, but may include the entire segment from the *gag-fps* boundary to near RX18 (Fig. 5).

The insertion-tolerant site defined by the mutants RX18p and RX18m (Fig. 5, constriction) may reflect a natural hinge region in the *fps* proteins that arose when ancestral gene segments were recombined to create *c-fps*, although this remains speculative.

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