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Differential Requirements of *gag* and γ-Actin Domains for Transforming Potential of Gardner-Rasheed Feline Sarcoma Virus

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The oncogene of Gardner-Rasheed feline sarcoma virus (GR-FeSV) encodes the 70-kilodalton protein containing *gag*(p15), γ-actin, and *fgr* domains. To determine the role of these domains in the biological activity of P70$^{gag\text{-}actin\text{-}fgr}$, we have constructed in-frame deletion and insertion mutants of GR-FeSV. We found, first, that the γ-actin region could be deleted without affecting the transforming ability of these constructs, although an insertion mutant in the middle of the γ-actin domain (map position 671) was partially defective in transformation and specifically had a reduced level of in vitro autophosphorylation activity. Second, mutations affecting the C-terminal third of the *gag* region appeared to abolish the ability to transform NIH 3T3 cells and autophosphorylation activity. These results suggest that the γ-actin domain is not essentially required for the transforming activity of GR-FeSV but that it may take part in maintaining the conformational integrity of P70$^{gag\text{-}actin\text{-}fgr}$ and that the *gag*(p15) domain might have a critical role in modulating the function of P70$^{gag\text{-}actin\text{-}fgr}$.

The Gardner-Rasheed strain of feline sarcoma virus (GR-FeSV) causes fibrosarcoma in cats and rapidly transforms various cells in culture (16). Its transforming gene, v-*fgr*, has been molecularly cloned, and it appeared to be expressed as a *gag*-linked fusion protein of about 70 kilodaltons (11, 13). It was classified as a nonreceptor type in the tyrosine-specific protein kinase family. Since it has become apparent that *src* and other members of this gene family contain N-terminal domains modulating transforming activity, we were interested in investigating whether these modulating functions are detected in the case of the v-*fgr* oncogene that was generated by recombination of the viral *gag* sequence and two distinct cellular genes, γ-actin and *fgr*-specific sequences (12).

The *gag* component (amino acid residues 1 to 118) derived from helper feline leukemia virus contained only the p15 antigenic determinant that is the N-terminal polypeptide of a precursor protein, p65^{gag}. Although the role of the *gag* component for transformation by the v-*abl* and v-*fps* genes has been studied (3, 14, 20) and the *gag* sequence was reported to contribute to activation of the chicken c-*fps* proto-oncogene (4) and the human c-*fps*/*fes* locus (17), implications of *gag* fusion were not always consistent among the reported observations.

The first portion of cellular origin (residues 141 to 268) is homologous to the first 128 amino acids of γ-actin, which is a major cytoskeletal protein of eucaryotic cells. It is not yet certain whether the conservation of the γ-actin sequence in P70$^{gag\text{-}actin\text{-}fgr}$ indicates that it serves a function in oncogenic transformation. However, the possible involvement of cytoskeletal proteins in the mechanisms used for the expression of the transformed phenotype has long been recognized in several cases. It has been reported that mutations in β- and γ-actins and alterations in their expression may be related to transformation of human fibroblast cells and HL-60 cells, respectively (1, 7, 8). It is also worth noting that the *trk* oncogene, isolated from a human colon carcinoma, has been proven to contain a domain (221 amino acids) of a nonmuscle tropomyosin, another cytoskeletal protein (10). Studies on the γ-actin domain in the v-actin-*fgr* gene might be able to provide important insight into the possible involvement of cytoskeletal proteins in carcinogenesis.

The second cell-derived sequence (residues 269 to 657) is *fgr*-specific and closely related to the tyrosine kinase gene family of oncogenes, and it is supposed to be critical in transformation. The boundary of the meaning of *fgr* should be clearly defined because the oncogene of GR-FeSV was derived from two cellular sequences of different origin. We use the term *fgr* for specifying the second cellular sequence encoding the tyrosine kinase domain. The viral oncogene and its product of GR-FeSV are named v-actin-*fgr* and P70$^{gag\text{-}actin\text{-}fgr}$ in this report, while the cellular counterpart of the tyrosine kinase domain is called c-*fgr*.

To further elucidate the mechanism of GR-FeSV-induced oncogenesis it is necessary to resolve the functional role of each domain of the v-actin-*fgr* protein. Here we report the effect of mutations in *gag*(p15), γ-actin, and *fgr*-specific determinants on transformation of mouse NIH 3T3 cells.

MATERIALS AND METHODS

Cells, viruses, and recombinant DNA. NIH 3T3 cells transformed by GR-FeSV and the plasmid pv-*fgr*1700 were kindly provided by K. C. Robbins (12). High-molecular-weight DNA purified from transformed cells was digested to completion with *Eco*RI restriction endonuclease and fractionated by agarose gel electrophoresis. Fractions of DNA eluted from gel slices enriched for *fgr* sequences were identified by Southern blotting, ligated to *Eco*RI arms of Charon 27, and packaged into bacteriophage lambda particles in vitro. After plaques that hybridized strongly with an *fgr* probe were screened, the cloned *Eco*RI DNA fragments were isolated from purified phage DNA, subcloned into the *Eco*RI sites of pUC13 and pSV2*neo* (18), and subjected to in vitro mutagenesis.

DNA transfection and cell culture. DNA transfection was performed as previously described (22). NIH 3T3 cells were

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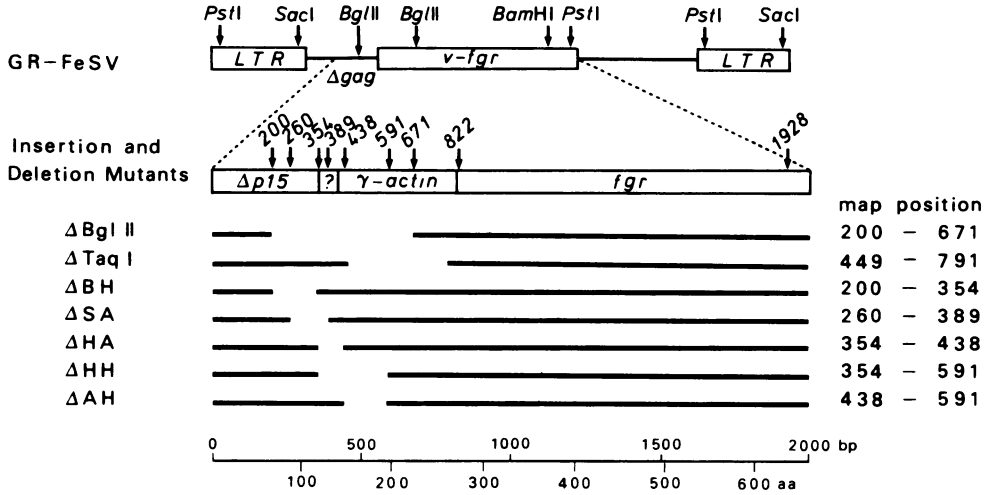

FIG. 1. Construction of insertion and deletion mutants of GR-FeSV. At the top is the structure of the GR-FeSV proviral genome. Beneath are shown those of mutants. The 7.3-kbp *Eco*RI fragment containing the whole proviral genome DNA of GR-FeSV (4.6 kbp) was isolated from the Charon 27 phage gene library and ligated to pSV2*neo* to generate the pSV2*neo*-GR-FeSV plasmid. The 4.0-kbp *Sac*I fragment and the 471-base-pair *Bgl*II fragment of proviral DNA were prepared from the pSV2*neo*-GR-FeSV plasmid, subcloned into pUC13 derivatives lacking the *Eco*RI site and containing a unique *Bgl*II site, subjected to mutagenesis, and ligated stepwise to bring them back to their original structure. Map position 1 corresponds to the first nucleotide of the ATG codon at the amino terminus of the *gag* gene as previously reported (12). Arrows indicate the restriction sites and the positions where insertion of oligonucleotides was made in each insertion mutant. In each case the structure of the individual mutant was determined by restriction enzyme mapping and partial DNA sequencing.

cultured in Dulbecco modified Eagle medium (DMEM) supplemented with 10% calf serum. Neomycin-resistant colonies were selected with DMEM and G418 (400 μg/ml). For tests of anchorage independence, transformed cells or G418-resistant colonies were suspended in DMEM–20% calf serum–0.25% washed agar at 10^3 cells per 60-mm dish as previously described (21). The numbers and sizes of colonies were measured 3 weeks after seeding.

Generation of antibodies. A rat tumor serum was obtained from rat tumors induced by GR-FeSV and was provided by K. Owada. An anti-*fgr* antiserum used in this study was raised in mice by injecting a *cro-lac-fgr* (residues 275 to 587) fusion protein expressed in *Escherichia coli*. *E. coli* NF1 and expression vectors (pEX series) (19) were provided by H. Saiga. The pEX2-*fgr* expression plasmid was prepared by ligating the 0.93-kilobase-pair (kbp) *Eco*RI (*Hae*III at map position 822)-*Bam*HI fragment of the GR-FeSV mutant *in*822 into the *Bam*HI site of the pEX2 vector by using a *Bam*HI linker. This plasmid was used to transform *E. coli* NF1, and the fused *cro-lac-fgr* protein was induced by shifting the temperature from 30 to 42°C for 2 h, which inactivated the c*Its*857 repressor of lysogenized phage lambda. The induced protein was purified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electroelution of the specific fused-protein band. This band migrates on the gel as a 160-kilodalton protein, as compared with the band of *cro-lac* protein alone, which migrates as a 117-kilodalton protein. Antisera were prepared by intraperitoneal injection of 200 μg of the protein emulsified in complete Freund adjuvant into BALB/c mice. Control antisera were prepared with the *cro-lac* protein alone.

Immunoprecipitation and in vitro protein kinase assay. Cells were labeled for 4 h with 100 μCi of [^{35}S]methionine per ml in methionine-free DMEM. Conditions for preparing cell extracts, immunoprecipitation, and SDS-PAGE have been described (2). Immunoprecipitates were assayed for autophosphorylation of P70$^{gag\text{-}actin\text{-}fgr}$. Each immune complex was suspended in a final volume of 30 μl containing 50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), pH 7.4; 10 mM $MnCl_2$; 10% glycerol; and 3 μCi of [γ-^{32}P]ATP. Incubation was continued for 15 min at 30°C. Reactions were terminated by boiling in sample buffer and were analyzed by 8.5% SDS-PAGE. Radioactivity in autophosphorylated P70$^{gag\text{-}actin\text{-}fgr}$ was quantified by scintillation counting of bands excised from SDS-polyacrylamide gel, and values were corrected for gel background. To calculate the relative specific activity of the P70$^{gag\text{-}actin\text{-}fgr}$ kinase in each experiment, the count of ^{32}P incorporated into P70$^{gag\text{-}actin\text{-}fgr}$ was divided by the relative amount of P70$^{gag\text{-}actin\text{-}fgr}$ in the parallel [^{35}S]methionine-labeled lysates, determined either by ^{35}S radioactivity of the excised protein band or by densitometric scanning of the autoradiogram.

RESULTS

Construction of GR-FeSV mutants. We have molecularly cloned the 7.3-kbp *Eco*RI fragment containing the whole proviral genome of GR-FeSV from the phage Charon 27 gene library of NIH 3T3 cells transformed by GR-FeSV and subcloned in pUC13 derivatives suitable for in vitro mutagenesis and DNA sequencing. Although it was initially reported that amino acids 141 to 268 of P70$^{gag\text{-}actin\text{-}fgr}$ differ at two positions from the published sequence of mammalian γ-actin (12), we found that these sequences were completely conserved in our isolate of GR-FeSV DNA. Structures and properties of mutations are schematically shown in Fig. 1 and Table 1.

Deletion mutants were generated by partial digestion and self-ligation at the *Bgl*II and *Taq*I sites and were screened by colony hybridization for lack of desired restriction fragments. Insertion mutants were constructed fundamentally by linker insertion mutagenesis as previously described (6, 9). In brief, plasmids were linearized by restriction enzymes with 20 to 100 μg of ethidium bromide per ml and were ligated to the *Eco*RI fragment of the kanamycin-resistant gene via the *Pvu*II or *Bam*HI linker. After kanamycin-

TABLE 1. Properties and transforming potential of insertion and deletion mutants of GR-FeSV in NIH 3T3 cells

GR-FeSV proviral genome DNA	Site of mutation	No. of deleted amino acids	Inserted amino acid[a]	Relative transformation efficiency[b]	Colony formation in soft agar[c]	Relative in vitro autophosphorylation activity (mutant/wt, ratio)[d]
Wild type				1.00	+	1.00
*del*BglII	*Bgl*II-*Bgl*II	157		<0.01	−	
*del*TaqI	*Taq*I-*Taq*I	114		0.92	+	0.85
*del*BH	*Bgl*II-*Hae*III	51	IRIP	<0.01	−	
*del*SA	*Sau*3A-*Acc*II	43	SEFQ	<0.01	−	
*del*HA	*Hae*III-*Acc*II	28	AGIP	1.00	+	0.70
*del*HH	*Hae*III-*Hae*III	79	AGIP	0.97	+	0.73
*del*AH	*Acc*II-*Hae*III	51	AGIP	1.01	+	1.05
*in*200	*Bgl*II		IRIR	0.95	+	0.89
*in*260	*Sau*3A		SEFQ	<0.01	−	
*in*354	*Hae*III		AGIP	1.10	+	1.10
*in*389	*Acc*II		LEFQ	1.06	+	0.96
*in*438	*Acc*II		AGIP	1.01	+	1.22
*in*591	*Hae*III		AGIP	0.92	+	1.32
*in*671	*Bgl*II		IRIR	0.15	+/−	0.35
*in*822	*Hae*III		AGIP	1.04	+	1.15
*in*1928	*Hae*III		LEFQ	<0.01	−	

[a] Inserted amino acid residues are I, Ile; R, Arg; S, Ser; E, Glu; F, Phe; Q, Gln; A, Ala; G, Gly; P, Pro; L, Leu.

[b] Transfection with wild-type GR-FeSV produced approximately 2×10^3 foci per μg of DNA, and its transformation efficiency was normalized to 1.00.

[c] Positive cells reached 200 to 500 colonies and their size was greater than 0.2 mm in diameter, whereas negative cells exhibited no colonies greater than 10 cells of pinpoint size. Cells transfected with the insertion mutant *in*671 reached 200 to 500 colonies, but their size was intermediate, i.e., 0.1 to 0.2 mm in diameter as shown above (+/−).

[d] To assess the relative kinase activities of the mutant and wild-type (wt) $P70^{gag\text{-}actin\text{-}fgr}$, protein products were immunoprecipitated from transfected NIH 3T3 cells and were incubated with [γ-^{32}P]ATP in immune complex kinase reactions. The incorporation of ^{32}P into $P70^{gag\text{-}actin\text{-}fgr}$ was corrected for the relative abundance of $P70^{gag\text{-}actin\text{-}fgr}$ by immunoprecipitating $P70^{gag\text{-}actin\text{-}fgr}$ from parallel cultures labeled with [^{35}S]methionine, and specific activities were calculated as described in Materials and Methods. The mean values of three different experiments are presented.

resistant transformants were selected, excision of the kanamycin-resistant fragment by *Eco*RI and self-ligation left the 12-base-pair insertion of CTGGAATTCCAG at the *Hae*III and *Acc*II sites and GATCCGAATTCG at the *Bgl*II and *Sau*3A sites. In addition, we chose two insertion mutants containing a single *Eco*RI site at the mutated sites which are in the same reading frame and manipulated them further to create the in-phase deletions *del*BH, *del*SA, *del*HA, *del*HH, and *del*AH. The integrity and in-phase nature of all recombinant junctions were determined by partial DNA sequence analysis.

Transformation of NIH 3T3 cells by GR-FeSV and mutants. Constructed mutants were ligated to pSV2*neo* and transfected into NIH 3T3 cells by using the calcium phosphate method. Transfectants were assessed in two ways: observation of focus formation against a background of a contact inhibited cell monolayer and selection of colonies for the pSV2*neo* marker. The degrees of morphological transformation were compared periodically at a variety of cell densities. Cells derived from each colony formed in the presence of G418 after the transfection were assayed for growth in soft agar to determine the degree of expression of the transformed phenotype.

As summarized in Table 1, all the mutants with a deletion in the *gag*(p15) region, i.e., *del*BglII, *del*BH, and *del*SA, failed to transform NIH 3T3 cells. Cells derived from G418-resistant colonies after transfection of these deletion mutants did not grow in soft agar. These results were consistent with the findings that transformed cells have not been obtained by transfection of the insertion mutant *in*260. Deletion mapping clearly defined the contributed *gag* region to map position 260 to 354, the C-terminal third of p15, which is known to be clustered by the prolyl residues (5).

The deletion (*del*HA and *del*HH) or the insertion (*in*389) of oligonucleotides into the sequence between the *gag* and γ-actin domains (map position 355 to 420) that may be derived from the 5′-flanking region of the feline γ-actin gene did not cause significant effects on their transforming abilities. Results from deletion mutants of γ-actin (*del*TaqI, *del*HH, and *del*AH) demonstrated that the γ-actin sequence was also removed from GR-FeSV DNA without reducing its transforming potential in the presence of the upstream *gag* sequence. However, detailed analysis disclosed that at least map position 671 in the middle of the γ-actin domain was sensitive to tetrapeptide insertion. The insertion mutant *in*671 produced a reduced number of transformed foci and produced colonies of smaller size in soft agar as compared with the wild type. Morphological alterations of cells transformed with *in*671 were more subtle, more flat, and less refractile than those transformed with wild-type GR-FeSV DNA. In addition, they were clearly distinguished from fully transformed cells by the intermediate transformed phenotype in other transformation parameters, such as saturation cell density and the requirement for serum factors (Table 2).

Although the insertion at map position 822 in the N-terminal *fgr*-specific region did not affect transforming ability, the insertion mutant *in*1928 in the C-terminal region produced no foci. These results suggest that the C-terminal region of the *fgr* domain also plays a critical role in transformation, in agreement with the observations that Arg-

TABLE 2. Growth as a function of serum concentration of NIH 3T3 cells transfected with GR-FeSV and mutants

Transfected plasmid	Saturation cell density (10^4 cells per cm^2)	
	In 0.5% calf serum	In 10% calf serum
None	1.9	9.0
*in*260	1.8	10.5
*in*671	3.5	21.5
*del*TaqI	7.9	24.5
GR-FeSV	7.0	25.0

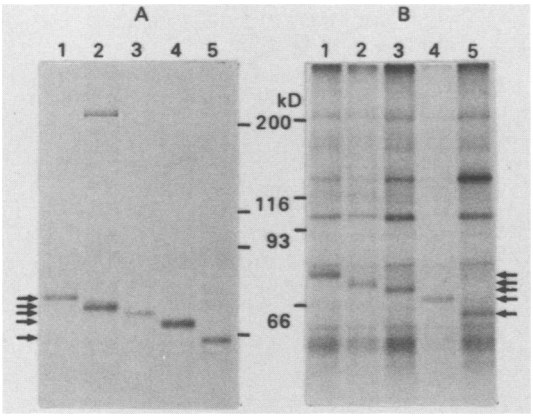

FIG. 2. Proteins generated by wild-type GR-FeSV and mutants deleted the γ-actin region. Cells stably transfected with GR-FeSV and its deletion mutants were radioactively labeled for 4 h with 200 μCi of [^{35}S]methionine (800 Ci/mmol; Amersham) in 2.0 ml of methionine-free DMEM containing 5% dialyzed calf serum. At the end of labeling, cells were washed twice with cold phosphate-buffered saline and were scraped into 2.0 ml of RIPA buffer (1.0% Triton X-100, 1.0% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.05 M Tris hydrochloride [pH 7.2]) with a rubber policeman. The cell extracts were clarified by centrifugation at 27,000 × *g* for 15 min, and portions (200 μg of soluble cell protein) were immunoprecipitated with an antiserum from rat tumors induced by GR-FeSV. Half of the immunoprecipitates was analyzed for P70 by loading onto an 8.5% SDS-polyacrylamide gel and exposing with fluorography (A), and the remaining half was subjected to an in vitro kinase assay (B). Transfected plasmids: Lane 1, Wild-type GR-FeSV; lane 2, *del*HA; lane 3, *del*AH; lane 4, *del*HH; and lane 5, *del*TaqI. Protein markers with molecular sizes in kilodaltons are shown. Arrows indicate the positions of transforming proteins.

Pro-Thr-Phe (residues 643 to 646) around map position 1928 of v-actin-*fgr* were extensively conserved among the tyrosine kinase gene family.

Analysis of the oncogene proteins generated by GR-FeSV and mutants. The levels of oncogene production in cells transfected with GR-FeSV and its mutants were determined by immunoprecipitation with an antiserum from rat tumors induced by GR-FeSV. The [^{35}S]methionine-labeled proteins immunoprecipitated are shown in Fig. 2A and 3A. The wild-type P70$^{gag\text{-}actin\text{-}fgr}$, the mutant proteins with a deletion in the γ-actin region, and all the insertion mutant proteins except for the *in*260 mutant protein were readily identified after immunoprecipitation. The protein products of deletion mutants in the γ-actin domain were smaller than the P70 protein corresponding to each deletion range, whereas those of insertion mutants were almost identical in size to the P70 protein. They were expressed at levels similar to that of the wild-type P70. Therefore, reduction of the transforming activity of the *in*671 and *in*1928 mutants was not due to diminutions in the level of P70 expression. In contrast, oncogene products were not detected in the extracts from cells transfected with *del*BglII, *del*BH, and *del*SA deletion mutants in the *gag* region (data not shown). Similar results were obtained with the insertion mutant *in*260 (Fig. 3A, lane 3), which also has a mutation at the C-terminal third of p15. Thus, further analyses were required for these four mutants affecting the *gag* domain.

We used the in vitro autophosphorylation assay to measure protein kinase activity in immunocomplexes formed by a rat tumor serum (Fig. 2B and 3B; Table 1). Interestingly, the P70 protein of the *in*671 mutant in the γ-actin region specifically displayed a reduction of approximately 40% of autophosphorylation activity compared with the activity of GR-FeSV, although the kinase activities of the other insertion and deletion mutant proteins in the γ-actin region were comparable to the activity of the wild-type P70 protein. The kinase activity of the mutant *in*1928 protein was below the level of detection, as expected. These findings were parallel to those of biological activities observed with these mutants.

Identification of the oncogene products encoded by insertion and deletion mutants in the *gag* region of GR-FeSV. Since insertion and deletion mutants in the *gag* region of GR-FeSV were negative in the focus formation assay and immunoprecipitation with a rat tumor serum failed to disclose the presence of their oncogene products, it was necessary to develop a new approach to examine whether the oncogene products were appropriately translated in the cells stably transfected with these mutants. We considered it most likely that the rat tumor serum reacts mainly with the determinant in the *gag* region, where these mutants have deletions and insertions, and we prepared an anti-*fgr* antiserum by immu-

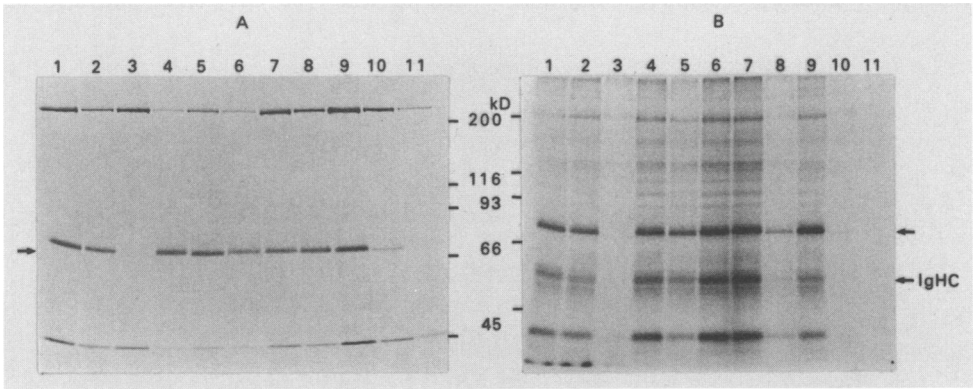

FIG. 3. Proteins generated by GR-FeSV and insertion mutants. Immunoprecipitations were prepared as described in the legend to Fig. 2. Metabolically labeled proteins (A) and in vitro kinase assay (B) are shown. Transfected plasmids: Lane 1, Wild-type GR-FeSV; lane 2, *in*200; lane 3, *in*260; lane 4, *in*354; lane 5, *in*389; lane 6, *in*438; lane 7, *in*591; lane 8, *in*671; lane 9, *in*822; lane 10, *in*1928; and lane 11, mock-transfected NIH 3T3 cell extracts. Arrows indicate the positions of P70$^{gag\text{-}actin\text{-}fgr}$ and immunoglobulin heavy chains. Protein markers with molecular sizes in kilodaltons are shown.

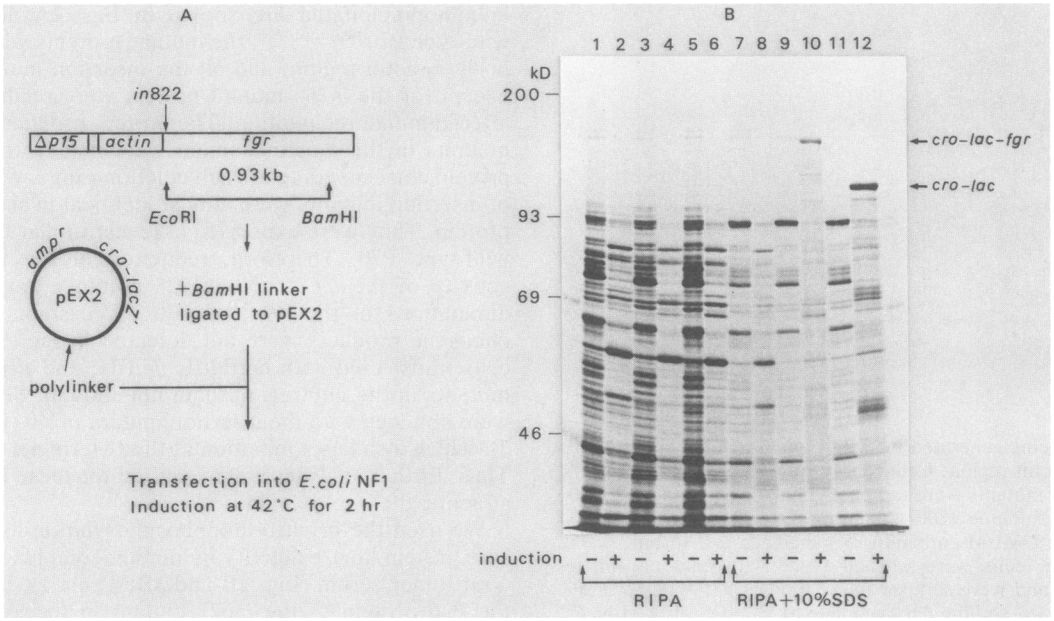

FIG. 4. Generation of the *cro-lac-fgr* hybrid protein in *E. coli*. (A) Strategy for expressing the pEX vector containing the *fgr*-specific sequence. (B) *E. coli* NF1 (lanes 1, 2, 7, and 8), NF1 containing pEX2-*fgr* (lanes 3, 4, 9, and 10), and NF1 containing pEX2 (lanes 5, 6, 11, and 12) were metabolically labeled with [^{35}S]methionine, and lysates were analyzed on 8% SDS-PAGE. Lysates were divided into fractions soluble in RIPA buffer (lanes 1 through 6) and in RIPA buffer containing 10% SDS (lanes 7 through 12). The *cro*-β-galactosidase hybrid proteins, indicated by arrows, were insoluble but were extracted from membrane fractions in the presence of 10% SDS, which facilitated purification of the hybrid proteins. Protein markers with molecular sizes in kilodaltons are shown.

nizing mice with a recombinant *cro-lac-fgr* (residues 275 to 587) hybrid protein generated in *E. coli* as shown in Fig. 4. In the immunoprecipitates produced by incubating the extracts of the mutant-transfected cells with this antiserum, the mutant *del*BH, *del*SA, and *in*260 proteins were identified (Fig. 5A). These bands were not detected by preimmune serum (data not shown). The deletion mutant proteins were smaller than the wild-type and insertion mutant P70 proteins, as expected. But the levels of expression of the mutant proteins were variable and appeared to decrease corresponding to the extent of the deletion range (compared with the level observed with the wild-type P70), i.e., 10, 28, and 30% for *del*BH, *del*SA, and *in*206, respectively. We also measured protein kinase activity in immunocomplexes formed by the anti-*fgr* antiserum because variations in the levels of protein production did not correlate to complete abrogation of transforming activities of all these mutants. We found that these mutant proteins did not show any detectable kinase activity as analyzed by autophosphorylation (Fig. 5B), although phosphorylated bands other than P70$^{gag\text{-actin-}fgr}$ were found. It was unknown whether these background bands were phosphorylated by mutant proteins or by *fgr*-related protein kinases coprecipitated with P70$^{gag\text{-actin-}fgr}$.

As for the mutant *del*BglII whose protein product was not detected in a series of our experiments, there remained a possibility that a cloning artifact might have been responsible for the lack of transforming ability and protein product. However, this possibility was eliminated because inserting the deleted *Bgl*II fragment back into the corresponding position of the mutant *del*BglII to generate a revertant in vitro restored its transforming ability to the same level as that of wild-type GR-FeSV, and also because the RNA

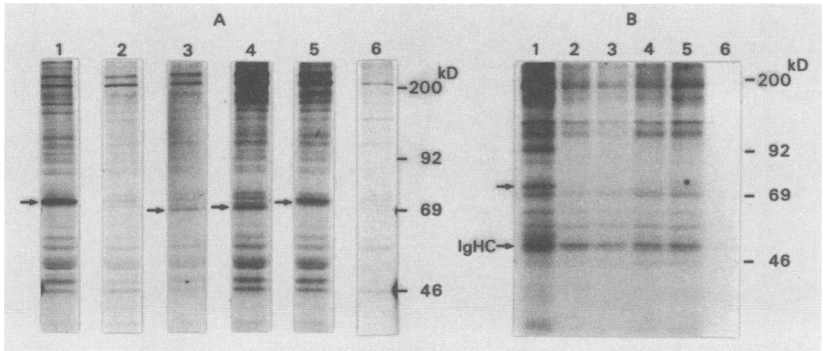

FIG. 5. Proteins generated by GR-FeSV and mutants affecting the *gag* region. Immunoprecipitates were prepared with an anti-*fgr* antiserum. Metabolically labeled proteins (A) and in vitro kinase assay (B) are shown. Transfected plasmids: Lane 1, Wild-type GR-FeSV; lane 2, *del*BglII; lane 3, *del*BH; lane 4, *del*SA; lane 5, *in*260; and lane 6, mock-transfected NIH 3T3 cell extracts. Protein markers with molecular sizes in kilodaltons are shown.

transcript of the mutant *del*BglII was readily detected in Northern (RNA) blot analysis (data not shown).

DISCUSSION

The results obtained from deletion mutants demonstrate that the absence of the γ-actin region apparently has no effect on the transforming potential and the intrinsic kinase activity of P70$^{gag\text{-}actin\text{-}fgr}$. However, insertion mutagenesis showed that the *in*671 mutant in the middle portion of the γ-actin region was partially defective in transformation and autophosphorylation activity, while other insertion mutants within the γ-actin region, *in*438 and *in*591, were fully active. One simple explanation for this discrepancy is that the γ-actin sequence is not actually important and impairment for these functions could be due to a conformational alteration. Another possibility is that the γ-actin domain has a function(s) for regulating kinase activity, maintaining the native structure of the catalytic domain, or forming part of a substrate recognition site which may be impaired by the *in*671 insertion. Unfortunately, our results disclosed that it is difficult to assess directly the putative role of the γ-actin domain in the absence of the *gag* domain, because transformation and kinase activities of P70$^{gag\text{-}actin\text{-}fgr}$ appear to depend primarily not on the γ-actin domain but on the *gag* domain. Although the three-dimensional structure of P70$^{gag\text{-}actin\text{-}fgr}$ is unknown, it is probable that a large deletion in the γ-actin domain and subsequent fusion of the *gag* and *fgr* domains might generate a new protein structurally and functionally different from wild-type P70 except for common properties in transformation and kinase activity. Differences between them are not readily detected, and our unpublished data showing that wild-type P70 and mutant proteins with a deletion in the γ-actin region behave as cytosolic proteins during subcellular fractionation eliminate the involvement of the γ-actin domain in ensuring correct subcellular localization. Therefore, we have to develop a new approach for investigating the role of the γ-actin domain and analyzing the mechanism decreasing kinase function of the *in*671 mutant.

We have also demonstrated that the C-terminal third of the viral *gag*(p15) gene sequence is indispensable for fibroblast transformation by transfection of GR-FeSV DNA. It is notable that even the local disruption of P70$^{gag\text{-}actin\text{-}fgr}$ by tetrapeptide insertion in the specified *gag* region (map position 260) abolished its transforming ability. These findings indicate that fusion to the viral *gag* sequence may be critical for transduction of the proto-oncogene c-*fgr*. Protein stabilization was suggested to explain the lack of transformation by *gag*-deleted mutants of the v-*abl* gene in lymphoid cells (15). Such a mechanism may explain some, but probably not all, of our observations that the amount of oncogene products of *del*BglII, *del*BH, *del*SA, and *in*260 mutants was reduced as compared with that of wild-type GR-FeSV. Since protein stabilization alone could not explain why these mutant proteins were not autophosphorylated, we speculate that an additional mechanism increasing autophosphorylation efficiency may be involved in the *gag*-related mutagenic effect activating the transforming potential of GR-FeSV.

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LITERATURE CITED

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