Molecular Cloning of Integrated Gardner-Rasheed Feline Sarcoma Virus: Genetic Structure of Its Cell-Derived Sequence Differs from That of Other Tyrosine Kinase-Coding onc Genes

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Gardner-Rasheed feline sarcoma virus (GR-FeSV) is an acute transforming retrovirus which encodes a gag-onc polypeptide possessing an associated tyrosine kinase activity. The integrated form of this virus, isolated in the Charon 21A strain of bacteriophage λ, demonstrated an ability to transform NIH/3T3 cells at high efficiency upon transfection. Foci induced by GR-FeSV DNA contained rescuable sarcoma virus and expressed GR-P70, the major GR-FeSV translational product. The localization of long-terminal repeats within the DNA clone made it possible to establish the length of the GR-FeSV provirus as 4.6 kilobase pairs. The analysis of heteroduplexes formed between λ feline leukemia virus (FeLV) and λ GR-FeSV DNAs revealed the presence of a 1,700-base-pair FeLV unrelated segment, designated v-fgr, within the GR-FeSV genome. The size of this region was sufficient to encode a protein of approximately 68,000 daltons and was localized immediately downstream of the FeLV gag gene coding sequences present in GR-FeSV. Thus, it is likely that this 1.7-kilobase-pair stretch encodes the onc moiety of GR-P70. Utilizing probes representing v-fgr, we detected homologous sequences in the DNAs of diverse vertebrate species, implying that v-fgr originated from a well-conserved cellular gene. The number of cellular DNA fragments hybridized by v-fgr-derived probes indicated either that proto-fgr is distributed over a very large region of cellular DNA or represents a family of related genes. By molecular hybridization, v-fgr was not directly related to the onc genes of other known retroviruses having associated tyrosine kinase activity.

The study of acute transforming retroviruses has provided a potentially important approach to elucidating mechanisms involved in malignant transformation. Such viruses have arisen in nature by the recombination of replication-competent type-C retroviruses with evolutionarily well-conserved cellular genes. These latter sequences, termed onc genes, have been shown to be required for the induction and maintenance of the viral transformed state (for a review, see references 8 and 11). Certain independent virus isolates of the same, and even different, species have transduced closely related onc genes (2, 4, 6, 14–16, 20). These findings have suggested that vertebrates contain only a limited number of cellular genes that can acquire transforming properties when recombined with retroviral sequences.

Many acute transforming retroviruses, including avian-derived Rous, Fujinami, Y73, UR2, and PRC II sarcoma viruses, as well as the mammalian-derived Abelson murine leukemia virus and Snyder-Theilen feline sarcoma virus (FeSV), code for phosphoproteins that have associated protein kinase activities with specificity for tyrosine residues (3, 12, 18, 19, 24, 27, 30, 32, 34, 45, 47). The results of molecular hybridization studies indicate that most of these onc genes have arisen from different cellular genes (8, 41). Yet, nucleotide sequence analysis has revealed that some of these genes possess a striking degree of homology at the amino acid sequence level (23, 28, 35a, 40). Thus, accumulating evidence indicates an evolutionary relationship among the onc genes of those retroviruses associated with tyrosine kinase activity.

Recently, an acute transforming retrovirus, designated Gardner-Rasheed FeSV (GR-FeSV) was isolated from a naturally occurring fibrosarcoma of a domestic cat (35). GR-FeSV codes for
a 70,000-dalton phosphoprotein (GR-P70) whose associated protein kinase activity is specific for tyrosine residues (31a, 35). To determine the detailed structure of the GR-FeSV genome and to compare it with other acute transforming retroviruses having tyrosine kinase activity, we undertook the molecular cloning of the integrated form of GR-FeSV.

MATERIALS AND METHODS

Cells and virus. NIH/3T3 (26), racoon fibroblast (35), feline embryo fibroblast (37), and QT-6 (31) cell lines have been described elsewhere. Human embryo lung fibroblasts were established in tissue culture from explants. The isolation of a clonal racoon fibroblast line nonproductively transformed by GR-FeSV (292-1) has been previously reported (35). A clonal isolate of mouse amphotropic virus (API29) (21) was also utilized.

Molecular cloning. The Charon 21A strain of λ phage was propagated in Escherichia coli K-12 DP50supF (13). DNA was purified from CsCl-bandaged phage as described elsewhere (17). High-molecular-weight DNA was extracted from clone 292-1 cells and treated with HindIII. DNA fragments containing integrated GR-FeSV were partially purified by preparative agarose gel electrophoresis, ligated with HindIII-cleaved Charon 21A DNA, and packaged in vitro into phage particles as described elsewhere (44). The plaques were screened by the method of Benton and Davis (7), using labeled λ feline leukemia virus (FeLV) DNA as a probe (39).

DNA transfection. The transfection of NIH/3T3 cells with the molecular clone of GR-FeSV DNA was performed by the calcium-phosphate precipitation technique (22), as modified by Wigler et al. (46). Briefly, 1 ml of 0.25 M CaCl₂ containing various amounts of test DNA and 50 μg of salmon sperm DNA as the carrier was mixed with an equal volume of a 50 mM Hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer (pH 7.1) containing 280 mM NaCl and 1.5 mM sodium phosphate. The calcium-phosphate precipitate was added to a 10-cm petri dish, on which 1 × 10⁵ to 2 × 10⁵ NIH/3T3 cells had been plated 24 h earlier. After overnight incubation, the supernatant was removed, and 10 ml of Dulbecco modified Eagle medium (DMEM) supplemented with 5% calf serum was added. The transformed foci were scored at 14 to 21 days.

For the analysis of GR-FeSV rescue, individual transformed foci induced at limiting DNA concentration were picked by the cloning cylinder technique, grown to mass culture, and superinfected with mouse amphotropic virus. Tissue culture fluids were assayed 2 to 3 weeks later for focus-forming activity on NIH/3T3 cells (1).

Immunoprecipitation. Subconfluent cultures of around 10⁵ cells were labeled for 3 h at 37°C with 4 μCi of [³⁵S]methionine per ml (1,200 Ci/mmol). The radiolabeled cells were disrupted with 1 ml of lysis buffer (10 mM sodium phosphate [pH 7.5], 100 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, and 1 mM phenylmethylsulfonyl fluoride) per petri dish, clarified at 100,000 × g for 30 min, and divided into five equal portions. Samples were incubated with 10 μl of antiserum for 60 min at 4°C. Immunoprecipitates were recovered with the aid of Staphylococcus aureus protein A bound to Sepharose beads (Pharmacia Fine Chemicals, Inc.) and analyzed in sodium dodecyl sulfate-polyacrylamide gels as previously described (5). Antisera against chromatographically purified FeLV p15, FeLV p30, or isopycnically banded FeLV virions were provided by the Office of Resources and Logistics of the National Cancer Institute.

Electron microscopy. For heteroduplex analysis, the DNA's were mixed and denatured in 0.1 M NaOH–50 mM EDTA for 10 min at 22°C. The pH of the sample was adjusted to 8.5 with 2 M Tris-hydrochloride (pH 7.2), diluted with an equal volume of formamide, and incubated for 2 h at 22°C. After hybridization, the samples were spread onto a distilled water hypophase, transferred to Parlodion-coated grids, stained with 0.1 M uranyl acetate in 90% ethanol, and shadowed with platinum-palladium. Nucleic acid contour lengths were measured with the aid of a Tektronix computerized graphic system. Single- and double-stranded Φ X174 DNAs were used as length standards.

DNA analysis. High-molecular-weight cellular DNA prepared by the method of Cemanoni and Aaronson (9) was treated with restriction endonucleases (New England Biolabs) under the conditions recommended by the supplier. Treated DNAs were fractionated by agarose gel electrophoresis and subjected to Southern blotting analysis under previously reported conditions (42). The DNA fragments to be used as probes were labeled by nick-translation (36).

RESULTS

Molecular cloning of integrated GR-FeSV. In a previous study, the analysis of DNA from cells nonproductively transformed by GR-FeSV had demonstrated that HindIII did not cleave the integrated form of this virus (31a). In an effort to identify the most suitable source of integrated GR-FeSV for molecular cloning, HindIII-cleaved DNAs from several available GR-FeSV-transformed nonproducer clones were analyzed by fractionation in agarose gels, blotting onto nitrocellulose filters, and hybridizing with nick-translated FeLV DNA. Integrated GR-FeSV was localized to a single HindIII fragment of 7.8 kilobase pairs (kb) in DNA of clone 292-1 cells (data not shown). After preparative agarose gel electrophoresis, the 7.8-kbp DNA fragment was ligated to HindIII-treated DNA purified from the Charon 21A strain of λ phage, packaged into phage particles, and plated onto E. coli K-12 LE392. Of 8 × 10⁵ recombinant plaques analyzed for reactivity with FeLV probe, one scored as positive. This phage was plaque purified twice and grown to mass culture. When DNA extracted from the purified phage (designated λ GR-FeSV) was treated with HindIII and analyzed by the Southern procedure, a 7.8-kbp insert was readily detected by hybridization with the FeLV probe (data not shown).

Biological activity of λ GR-FeSV DNA. To determine whether λ GR-FeSV DNA possessed
transforming activity, we analyzed it by the transfection of NIH/3T3 cells. The DNA demonstrated an activity of $8 \times 10^3$ focus-forming units per pmol of DNA insert. Individual transformed foci induced at limiting DNA concentration were selected by the cloning cylinder technique and grown to mass culture for analysis. Upon superinfection with amphotropic mouse type-C virus, focus-forming virus characteristic of GR-FeSV was rescued from each transformant tested.

Previous studies have demonstrated that GR-FeSV-transformed nonproducer cells express a virus-coded gag- onc polypeptide (35), designated GR-P70. A protein of 70,000 daltons was immunoprecipitated from extracts of GR-FeSV DNA-transformed NIH/3T3 cells, using anti-FeLV (Fig. 1, lane a) or anti-FeLV p15 (Fig. 1, lane b) serum, but not with anti-FeLV p30 serum (Fig. 1, lane c). In contrast, neither anti-FeLV (Fig. 1, lane d) nor anti-FeLV p15 (Fig. 1, lane e) serum detected this protein in extracts of uninfected NIH/3T3 cells. These results demonstrated that the recombinant DNA clone contained the entire biologically active GR-FeSV provirus.

Physical map of $\lambda$ GR-FeSV DNA. A physical map of the 7.8-kbp HindIII insert of $\lambda$ GR-FeSV DNA was constructed by analysis of its restriction enzyme digestion products in agarose gels. The relative location of cleavage sites was determined by appropriate double digestions. A summary of these studies is shown in Fig. 2.

The coding sequences of integrated retroviruses are known to be flanked by long-terminal repeats (LTRs) which are several hundred base pairs in length. Examination of the restriction map of $\lambda$ GR-FeSV revealed the presence of two identical constellations of restriction enzyme sites within the cloned DNA fragment. PstI and SacI cleaved the molecule in identical locations with respect to KpnI sites located at 2.5 and 6.4 kbp on the restriction map. Thus, it is likely that such constellations represent GR-FeSV LTRs. PstI and PvuII sites at 1.9 and 6.7 kbp, respectively (Fig. 2), defined the maximum length of the GR-FeSV LTRs as 0.7 ± 0.1 kbp. From the approximate localization of the LTRs, it was possible to calculate the length of integrated GR-FeSV as 4.6 kbp.

Orientation of $\lambda$ GR-FeSV DNA with respect to viral RNA transcription. We utilized the knowledge that the gag gene is localized to the 5' end of the retrovirus genome and that a portion of the FeLV gag gene is expressed in GR-FeSV-transformed nonproducer cells (35) to assign these coding sequences to a region of the $\lambda$ GR-FeSV restriction map. A DNA fragment representing the FeLV gag gene was subcloned into pBR322 and labeled by nick-translation for use as a probe. To confirm that our gag gene-containing fragment represented sequences localized to the 5' region of the FeLV genome, we

![Image of restriction map](https://jvi.asm.org/)

**FIG. 2.** Restriction map of $\lambda$ GR-FeSV DNA. The sites of restriction enzyme cleavage were determined by double-digestion analysis. The positions of the open boxes, which represent LTRs, are approximate.
first analyzed a previously characterized \( \lambda \) FeLV DNA clone (39). This probe hybridized FeLV DNA (Fig. 3, lane a), as well as a 1.6-kbp \( XbaI \) fragment of FeLV DNA which contained gag gene sequences (Fig. 3, lane b) (39). The hybridization of GR-FeSV DNA by this same probe (Fig. 3, lane c) was localized to a 4.7-kbp \( BamHI \) fragment of \( \lambda \) GR-FeSV DNA (Fig. 3, lane d). These results demonstrated that the left portion of the \( \lambda \) GR-FeSV restriction map is 5' with respect to the GR-FeSV genome.

**Localization of the GR-FeSV onc gene.** In an effort to detect and localize onc sequences within \( \lambda \) GR-FeSV DNA, heteroduplexes between \( \lambda \) GR-FeSV and \( \lambda \) FeLV DNAs were analyzed. A representative heteroduplex structure is shown in Fig. 4. Single-stranded terminal regions (Fig. 4, features 1 and 7) defined the cellular sequences flanking the provirus. A large region of nonhomology (Fig. 4, features 3 and 8) and a smaller region of nonhomology (Fig. 4, features 5 and 9) were separated by 0.6 kbp of double-stranded DNA (Fig. 4, feature 4) and flanked by homologous stretches of 1.1 and 0.9 kbp (Fig. 4, features 2 and 6, respectively). These findings demonstrated that two regions located within the transforming virus genome, 1.7 and 0.2 kbp in length, were not related to \( \lambda \) FeLV DNA.

Because of its overall symmetry, the orientation of the heteroduplex structure with respect to GR-FeSV RNA and the restriction map of \( \lambda \) GR-FeSV DNA was not possible. For this purpose, 4.7- and 3.1-kbp \( BamHI \) DNA fragments located 0 to 4.7 and 4.7 to 7.8 kbp, respectively, on the \( \lambda \) GR-FeSV restriction map were sub-cloned in pBR322 and individually annealed with \( \lambda \) FeLV B DNA. The small deletion-substitution loop (Fig. 4, features 5 and 9) was detected only in heteroduplexes formed between \( \lambda \) FeLV DNA and the 3.1-kbp \( BamHI \) fragment of GR-FeSV DNA (data not shown). Thus, the small deletion-substitution loop (Fig. 4, features 5 and 9) was localized to the region of GR-FeSV DNA representing the 3' end of GR-FeSV RNA.

Based upon the results of heteroduplex analysis, a region of the \( \lambda \) GR-FeSV clone located approximately 3.3 to 4.9 kbp on its physical map, designated \( \nu-fgr \) (pronounced "figger"), should contain information unrelated to FeLV. As a test of this possibility, fragments of 400 and 1,000 base pairs spanning 3.2 to 3.6 and 3.8 to 4.8 kbp, respectively, on the \( \lambda \) GR-FeSV restriction map were purified, cloned into pBR322, and used as probes to hybridize Southern blots containing FeLV and GR-FeSV DNA. The hybridization of GR-FeSV DNA, as well as that of FeLV DNA (Fig. 5B), by the \( \nu-fgr400 \) probe, demonstrated that this subclone contained FeLV-related information. In contrast, the \( \nu-fgr1000 \) probe hybridized GR-FeSV but not FeLV DNA (Fig. 5C). These data independently demonstrated that at a minimum the region located 3.8 to 4.8 kbp on the GR-FeSV DNA restriction map was composed of GR-FeSV nucleotide sequences not present in FeLV B DNA.

**Detection of \( \nu-fgr \)-related nucleotide sequences in DNA of normal cells.** It is well established that retrovirus onc genes (\( \nu-oncs \)) are derived from cellular DNA sequences. Those cellular genes related to \( \nu-oncs \), designated proto-\( \nu-oncs \), are represented at low copy number within the cell genome and are well conserved in vertebrate species (8). In an effort to establish the cellular origin of \( \nu-fgr \), DNAs from uninfected quail, mouse, cat, or human cells were treated with

![Image](https://via.placeholder.com/150)
FIG. 4. Heteroduplex analysis of GR-FeSV. Equal amounts (1 μg) of integrated GR-FeSV and unintegrated FeLV DNAs were hybridized for 2 h at 22°C in 50% formamide. The resulting heteroduplexes were spread onto a distilled water hypophase, transferred to Parlodion-coated grids, stained with uranyl acetate, and shadowed with platinum-palladium. (A) Representative heteroduplex. (B) Interpretative sketch. The thick lines represent double strands, and the thin lines represent single strands. Twenty molecules were measured with a Tektronix graphics tablet. Double- and single-stranded Φ X174 DNAs were employed as length standards. (C) Contour lengths (in base pairs): 1 = 2,075 ± 640; 2 = 1,102 ± 150; 3 = 1,716 ± 401; 4 = 606 ± 52; 5 = 217 ± 50; 6 = 900 ± 120; 7 = 1,500 ± 420; 8 = 6,395 ± 476; 9 = 440.8 ± 89. Features 1 to 7 represent λ GR-FeSV, whereas features 2, 4, 6, 8, and 9 represent λ FeLV DNA strands.

HindIII and analyzed by the Southern procedure, using v-fgr-derived probes. The pv-fgr1000 probe detected several bands in the DNAs of all four species tested (Fig. 6A).

To determine whether cellular DNA fragments detected by the pv-fgr1000 probe also contained sequences homologous to another region of the GR-FeSV onc sequence, the same HindIII-cleaved cellular DNAs were analyzed with a labeled pv-fgr400 probe. This clone contained a small portion of the GR-FeSV gag gene sequence in addition to approximately 300 base pairs of v-fgr, as determined by heteroduplex and restriction enzyme analysis. Nick-translated FeLV DNA was used in parallel as a probe to determine whether cellular DNA fragments de-
GR-FeSV present in transformed nonproducer cells (31a). These results demonstrated that v-fgr was not identical to fes, fms, abl, or src. The availability of v-fgr DNA clones provided an opportunity to establish conditions of the greatest possible sensitivity for assessing whether even limited homology could be detected between v-fgr and other onc genes whose products possess an associated tyrosine kinase activity. The onc genes of avian Fujinami sarcoma virus and Snyder-Thenlen FeSV have been shown to be distantly related by molecular hybridization (41). Under conditions in which a v-fes-specific probe, pST-fes800 (31a), was able to detect 1 ng of the fes sequence of λ Snyder-Thenlen FeSV BALB DNA, the same probe required 100 ng of the fps sequence present in pFL-5 (29) to achieve a similar signal intensity. In contrast, this probe was not able to hybridize 100 ng of abl, src, or yes (data not shown). Under these same conditions, the pv-fgr1000 probe readily detected 1 ng of the v-fgr sequence present in λ GR-FeSV DNA, but did not hybridize a 100-fold-greater amount of abl-, fps-, fes-, ros-, src-, or yes-specific sequences (Table 1). Thus, no sequence homology could be established between v-fgr and other retrovirus onc genes.

FIG. 5. Localization of v-fgr nucleotide sequences on restriction map of λ GR-FeSV DNA. DNA products resulting from the treatment of λ FeLV DNA with EcoRI (lanes a) or λ GR-FeSV DNA with SacI (lanes b) were visualized by ethidium bromide staining (A) after agarose gel electrophoresis. The same DNA fragments were also blotted onto nitrocellulose filters and hybridized with nick-translated pv-fgr400 (B) or pv-fgr1000 (C) DNAs. M indicates a marker lane which contained HindIII-digested λ and HaeIII-digested Φ X174 replicative-form DNAs.

FIG. 6. Detection of v-fgr-related nucleotide sequences in normal cellular DNAs. Cellular DNAs were treated with HindIII, fractionated by agarose gel electrophoresis, and subjected to Southern blotting analysis using nick-translated pv-fgr1000 (A), pv-fgr400 (B), or λ FeLV (C) DNAs as probes. The DNAs analyzed were purified from QT-6 (lanes a), NIH/3T3 (lanes b), feline embryo fibroblasts (lanes c), and human embry lung fibroblasts (lanes d). The migration of co-electrophoresed HindIII-digested λ DNA is indicated in kbp.
whose products possess associated tyrosine kinase activity.

**DISCUSSION**

The present report describes the molecular cloning of the integrated GR-FeSV genome in its intact, biologically active form. The GR-FeSV DNA clone demonstrated a specific transforming activity rivaling that of the most potent retrovirus transforming genes. Previous studies have shown that GR-FeSV codes for a 70,000-dalton phosphoprotein (GR-P70) (31a, 35), the amino terminus of which contains FeLV p15 and p12 antigenic determinants. By analogy with known retroviruses, whose transforming gene products are synthesized as gag-onc hybrid proteins (8, 11), the remaining 40,000 to 45,000 daltons of GR-P70 were presumed to be derived from its onc sequence. In the present studies, structural analysis of the 4.6-kbp GR-FeSV DNA clone led to the detection and localization of an onc sequence, v-fgr, to a 1.7-kbp region in the center of the genome (Fig. 7). FeLV sequences upstream of v-fgr contained the viral LTR and sufficient FeLV coding information only for FeLV p15 and p12 gag gene products. Since v-fgr contains information sufficient to code for a 68,000-dalton protein, our results suggest that the 70,000-dalton gag-fgr hybrid protein extends into, but probably terminates within this cell-derived sequence.

Since the initial observation that the Rous sarcoma virus src gene product p60 oncoprotein possesses protein kinase activity (12) with specificity for tyrosine residues (25), a number of acute transforming retrovirus onc gene products have been demonstrated to possess similar activities. Avian Fujinami, Y73, UR2, and PRC II viruses, as well as mammalian Abelson murine leukemia virus and the Snyder-Theilen and Gardner-Arnstein strains of FeSV, all possess tyrosine kinase activity (3, 18, 19, 24, 27, 32, 45, 47). Snyder-Theilen and Gardner-Arnstein FeSV onc genes arose from the same cat proto-oncogene (20), whereas Fujinami sarcoma virus apparently incorporated this sequence from avian cells (41). Other tyrosine kinase-associated onc genes appear to have arisen from different proto-oncogenes; however, nucleotide sequence analysis has indicated a striking homology within their coding regions at the amino acid sequence level (23, 28, 35a, 40). These studies have implied that the functional similarities of the onc gene products also reflect evolutionary relatedness among this family of onc genes.

The tyrosine kinase associated with GR-FeSV has raised the possibility that its onc gene may have arisen in the cat from a previously identified proto-oncogene. Recent findings that cloned onc genes of several tyrosine kinase-associated transforming viruses failed to detect integrated GR-FeSV DNA in transformed nonproducer cells have argued against this possibility (31a). Our cloning of v-fgr made it possible to assess with greater sensitivity whether any sequence homology could be detected between it and other onc genes whose products possess an associated tyrosine kinase activity. Under hybridization conditions in which homology between fps and fes was readily demonstrated, no detectable relationship of v-fgr with Rous src, Abelson murine leukemia virus abl, Y73 yes.

**TABLE 1. Lack of homology between v-fgr and onc genes of other retroviruses with associated tyrosine kinase activities**

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<td>W. S. Neckameyer and L.-H. Wang. personal communication</td>
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*Cloned viral DNAs, excised from their λ vectors with the appropriate restriction enzymes, were fractionated on a 0.7% agarose gel, blotted onto nitrocellulose, and hybridized with the nick-translated pv-fgr 1000 probe. The amount of DNA analyzed contained approximately 100 ng of each onc-specific sequence. For pFL-5, plasmid DNA was linearized with EcoRI and analyzed as above, except that the DNA probe was purified from λ GR-FeSV DNA. Hybridization was determined by the exposure of hybridized filters to Kodak XAR-5 film.
Fujinami fps, UR2 ros, or Snyder-Theilen FeSV fes was observed. Thus, whether fgr can be evolutionarily linked to this family of onc genes must await primary nucleotide sequence analysis of this newly isolated onc gene.

By the use of v-fgr as a molecular probe, related sequences were detected in normal cellular DNAs of a wide variety of species, suggesting the well-conserved nature of the v-fgr proto-oncogene among vertebrates. The distinct patterns of cellular DNA fragments detected with probes derived from different regions of v-fgr further indicate that the structure of its proto-oncogene(s) contains intervening sequences not found within the viral genome. Similar conclusions have been obtained from the analysis of a number of other retrovirus onc genes (8, 11).

A striking feature of the normal cellular sequences related to v-fgr was the large number of fragments demonstrated in each species analyzed. In mouse cellular DNA, for example, the sum of individual v-fgr-related DNA fragments would correspond to a single gene of at least 125 kbp. Since the largest previously characterized cellular gene is around 40 kbp (10, 33, 48), it is likely that proto-fgr is less complex, but is present in multiple copies within the normal cell genome. Thus, if v-fgr can be evolutionarily linked to other tyrosine kinase-associated onc genes by nucleotide sequence studies, the size of this family may be significantly increased.

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LITERATURE CITED


