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Structure, Expression, and Chromosomal Location of the Human c-*fgr* Gene

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The nucleotide sequence of seven exons of the human c-*fgr* gene, a cellular homolog of the oncogene of Gardner-Rasheed feline sarcoma virus, was determined. Twenty-six independent genomic clones were obtained from a human gene library with a DNA clone of Y73 avian sarcoma virus oncogene, v-*yes*, as a probe under relaxed hybridization conditions. Restriction mapping and partial sequence analyses revealed that two of these clones were derived from the c-*fgr* gene, distinct from the c-*yes* gene. Interestingly, the splicing points of the c-*fgr* gene were identical with those of the c-*src* gene throughout the seven exons, suggesting that the two proto-oncogenes were generated by gene duplication of an ancestral gene containing intervening sequences. On RNA blot hybridization the major transcript was found to be 2.6 kilobases long. Two additional transcripts of 3.5 and 4.7 kilobases were also detected. Furthermore, karyotype analysis of several human-mouse hybrid cells and Southern blot analyses of DNAs of the hybrids with a human c-*fgr* locus-specific probe showed that this gene is located on chromosome 1.

It is widely accepted that an acutely oncogenic retrovirus contains a viral oncogene that is derived from a cellular counterpart and is responsible for the initiation and maintenance of cellular transformation. In animals, cellular oncogenes have been highly conserved throughout evolution, suggesting that they play important roles in normal cells. However, the normal functions of most cellular oncogenes are still unknown (2). More than 10 oncogenes were found to be structurally related to the most well-characterized oncogene, the *src* gene of Rous sarcoma virus, and so are called the "*src* family." Most members of this gene family have been found to encode products that are associated with tyrosine kinase activity, and it is generally assumed that kinase activity plays a key role in cell transformation (2).

In the *src* family, the *yes* gene, identified as an oncogene of avian Y73 sarcoma virus (15), is the most closely related to the *src* gene (18). The oncogene product of the Y73 virus is $p90^{gag\text{-}yes}$, which shows tyrosine-specific protein kinase activity like the *src* gene product (17). The human cellular *yes*-related sequence, the proto-*yes* gene, was recently assigned to chromosome 18q21 by somatic hybrid cell analysis (33) and by in situ hybridization experiments (40), suggesting its possible involvement in the pathogenesis of follicular lymphoma, which is known frequently to be associated with translocation between chromosomes 14 and 18 (9).

Another oncogene closely related to *src*, the *fgr* gene, has been identified as an oncogene of Gardner-Rasheed feline sarcoma virus (27, 31). The amino acid sequence of the v-*fgr* product, $p70^{gag\text{-}fgr}$ (25), was deduced from nucleotide sequence data. The carboxy-terminal half of the oncogene product showed highest homology (80%) with that of the v-*yes* product (26). Interestingly, the kinase domain and the viral *gag* sequence in the amino-terminal portion were interrupted by an actin-like sequence (26). Previously we reported the isolation of a human c-*fgr* gene clone by using a v-*yes* probe and the nucleotide sequence of the two exons (28). The actin gene-like sequence of the viral *fgr* gene is not present in the c-*fgr* gene, indicating that the v-*fgr* gene is a tripartite gene consisting of the viral *gag* gene and two cellular genes, the actin gene and the c-*fgr* gene (28).

In this report, we present further structural analysis of the human c-*fgr* gene. The exon-intron structure of the c-*fgr* gene was found to be identical to those of the c-*src* genes of chickens and humans, suggesting that the *fgr* and *src* genes originated by duplication of a prototype gene. In addition, using the gene specific probe, we detected the expression and location of the gene on chromosome 1, clearly indicating that the *yes* gene and the *fgr* gene are distinct oncogenes.

MATERIALS AND METHODS

Screening of a human gene library. A human gene library was constructed from *Alu*I-*Hae*III partial digests of human placenta DNA and the Charon 4A *Eco*RI arm by the method of Maniatis et al. (22). This library was screened by hybridization to v-*yes*-specific pYS-2 (33) insert DNA labeled with ^{32}P by nick translation. Considering the divergence of the v-*yes* gene and human c-*yes* gene, in screening we used relatively relaxed conditions for hybridization (30% formamide–0.6 M NaCl–60 mM sodium citrate–10× Denhardt solution [6]–50 mM sodium HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] [pH 7.0]–0.2 mg of denatured salmon sperm DNA per ml–0.1 mg of yeast RNA per ml at 42°C) and for washing out the probe (0.3 M NaCl–30 mM sodium citrate–0.1% sodium dodecyl sulfate at room temperature twice and 66 mM NaCl–6.6 mM sodium citrate–0.1% sodium dodecyl sulfate at 50°C four times).

Restriction mapping and sequencing. The cloned DNAs were subjected to restriction mapping followed by Southern blot analysis to identify restriction fragments that contained possible exon sequences. The DNA sequence was determined by the dideoxynucleotide method of Messing (23) and of Sakaki et al. (personal communication) with M13mp18, M13mp19, and pUC19 (29) as cloning-sequencing vectors.

Chromosome mapping. To determine the chromosomal location of the human c-*fgr* gene, we needed a human c-*fgr*-specific probe. For this purpose, we used an *Eco*RI-

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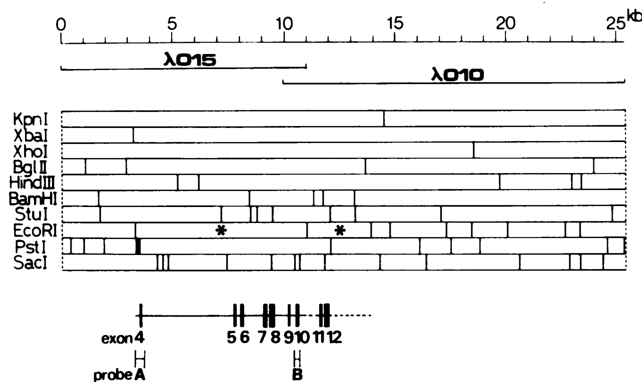

FIG. 1. Restriction endonuclease map of the human c-*fgr* locus. The human c-*fgr* locus was defined by two overlapping clones, λ015 and λ010. Restriction mapping was done by analysis of double-digestion products. Hybridization experiments showed that only two *Eco*RI fragments of approximately 7.7 and 2.9 kb, marked by asterisks, contained the *yes*-related gene sequence, the c-*fgr* sequence. Sequence analysis of the *Eco*RI 7.7-kb fragment showed 7 exons (exon 4 to 10) in this region. The exon numbers correspond to these of the chicken c-*src* gene assigned by Takeya and Hanafusa (37). The positions of the exons are indicated by black boxes. Exons 11 and 12 were defined by Parker et al. (30) to be parts of the c-*src*-2 gene and were partially confirmed by sequence analysis. A 0.4-kb *Eco*RI-*Sca*I fragment (probe A) and a 0.2-kb *Sac*I-*Sac*I fragment (probe B) were used as c-*fgr*-specific probes.

*Sca*I 0.4-kilobase (kb) fragment containing the exon 4 sequence that had been recloned into pUC9 (probe A in Fig. 1). We used the specific probe under stringent conditions of hybridization (50% formamide–0.45 M NaCl–45 mM NaCl–10× Denhardt solution–50 mM sodium HEPES [pH 7.0]–0.2 mg of denatured salmon sperm DNA per ml–0.1 mg of yeast RNA per ml at 42°C] and of washing out (0.3 M NaCl-30 mM sodium citrate–0.1% sodium dodecyl sulfate at room temperature twice and 30 mM NaCl–3 mM sodium citrate–0.1% sodium dodecyl sulfate at 50°C four times). Although the probe contains the exon 4 sequence of the c-*fgr* gene, it could detect the human c-*fgr* locus but could not hybridize to any mouse DNA under the stringent conditions. Somatic cell hybrids were generated by fusion of human embryo fibroblasts with mouse cells (33). DNAs extracted from human placenta, mouse cells, and 12 hybrid clones were digested with *Hin*dIII and subjected to Southern blot analysis with ^{32}P-labeled probe A. The human N-*ras*-specific probes pNP1 and pNP5 (34) were kindly supplied by K. Shimizu.

RNA isolation and blotting. Total RNAs were isolated from human placenta and several cultured cells by the guanidine isothiocyanate solubilization method (4). Oligo(dT)-cellulose chromatography was used to isolate polyadenylated RNA. RNA was fractionated on 1% agarose–formamide gel (20) and transferred to nitrocellulose. Prehybridization, hybridization, and washing were carried out as described previously (33). For use as a human c-*fgr* specific probe for RNA blotting, we recloned the *Sac*I-*Sac*I 220-base-pair (bp) fragment, which contains a 154-bp exon 10 sequence, into pUC19 (probe B in Fig. 1) and labeled it by nick translation.

RESULTS

Two genomic clones derived from the c-*fgr* locus. We isolated 26 independent genomic clones by using the v-*yes*-specific probe under nonstringent conditions. Restriction maps showed that two of these clones, λ015 and λ010, had an overlap of about 1 kb, indicating that they were derived from the same locus. Figure 1 shows the restriction maps of the inserts of λ015 and λ010. The inserts of these clones were 11.0 and 15.4 kb long, respectively. Southern blot analysis of the cloned DNAs showed that two *Eco*RI fragments (7.7 and 2.9 kb) contained the v-*yes*-related sequence. The 7.7-kb EcoRI fragment was subcloned into plasmid pBR327, and its nucleotide sequence was analyzed by the dideoxy sequencing method.

As a result, seven putative exons were found in the 7.7-kb *Eco*RI fragment. The positions of the exons are shown in Fig. 1. The splicing sites were predicted from the homology of the sequence with that of the v-*yes* gene and by comparison with the consensus splicing site structures. The GT-AG rule (24) was maintained in the splicing sites (Table 1). In addition, the features of the surrounding nucleotide sequences (24) were also well conserved, suggesting that these regions are indeed splicing positions. The total length of the exon sequences is 920 bp, coding 306 amino acid residues. Although these two genomic clones were isolated from a genomic library by sequential screening with the v-*yes* probe, the exon sequence had higher homology to the v-*fgr* gene than to the v-*yes* gene, human c-*src* gene, or other oncogenes (Fig. 2 and 3; Table 2). The 5′ extremity of the sequenced exons had no homology with the v-*fgr* gene corresponding to the actinlike portion (26). Thus, as discussed previously, the v-*fgr* gene product was considered to be a tripartite gene product consisting of the viral *gag* gene and two cellular genes, the actin gene and the c-*fgr* gene (28). The extensive homology with the v-*fgr* gene suggests that these genomic clones were derived from the c-*fgr* gene, which is distinct from the c-*yes* gene.

Splicing positions of c-*fgr* are identical with those of c-*src*. The coding sequence of the chicken c-*src* gene is interrupted

TABLE 1. Nucleotide sequences around the splicing sites of the human c-*fgr* gene[a]

Exon		Intron				Exon no.
No.	Sequence	Sequence	No.	Sequence		
			3	GTTCTGTTCTGTGCCTACAG	TGAAGG	4 (99)
4	TGAAGA	GTAAGTAGGGATTGGGGCAA	4 (4,100)	TTGCCTGCCTTTCCCAACAG	GTGGTA	5 (104)
5	CCAAAG	GTAGGGGTGGTGCCACCCCC	5 (179)	AAAAGTGATCCTCTCCACAG	GTGCCT	6 (150)
6	ACATGG	GTGAGGGCAGGGGCCTCAGA	6 (982)	CTTCATGACCCCTCCCCTAG	AGGTGA	7 (156)
7	GGCTGG	GTACGGAGCTCCCGGGGGCC	7 (74)	ACAAGACAGCCTCCGAGCAG	GCACGT	8 (180)
8	CACACG	GTCAGGAGGCGG—ND—	8 (480)	ACTTTCTGGCTTCTTCCCAG	GCAGCT	9 (77)
9	GCCCAG	GTAACTGGGCCAGCAGCCTT	9 (265)	TCCACACTATGGTCCCCCAG	GTAGCT	10 (154)
10	GCCAAG	GTGCCCTGCTTCACCCCACC	10 (900)			

[a] Numbers in parentheses indicate lengths of exons and introns, in base pairs. Introns have the same number as the exons on their 5′ side. The lengths of introns were estimated by nucleotide sequence determination and restriction mapping. ND, Not determined.

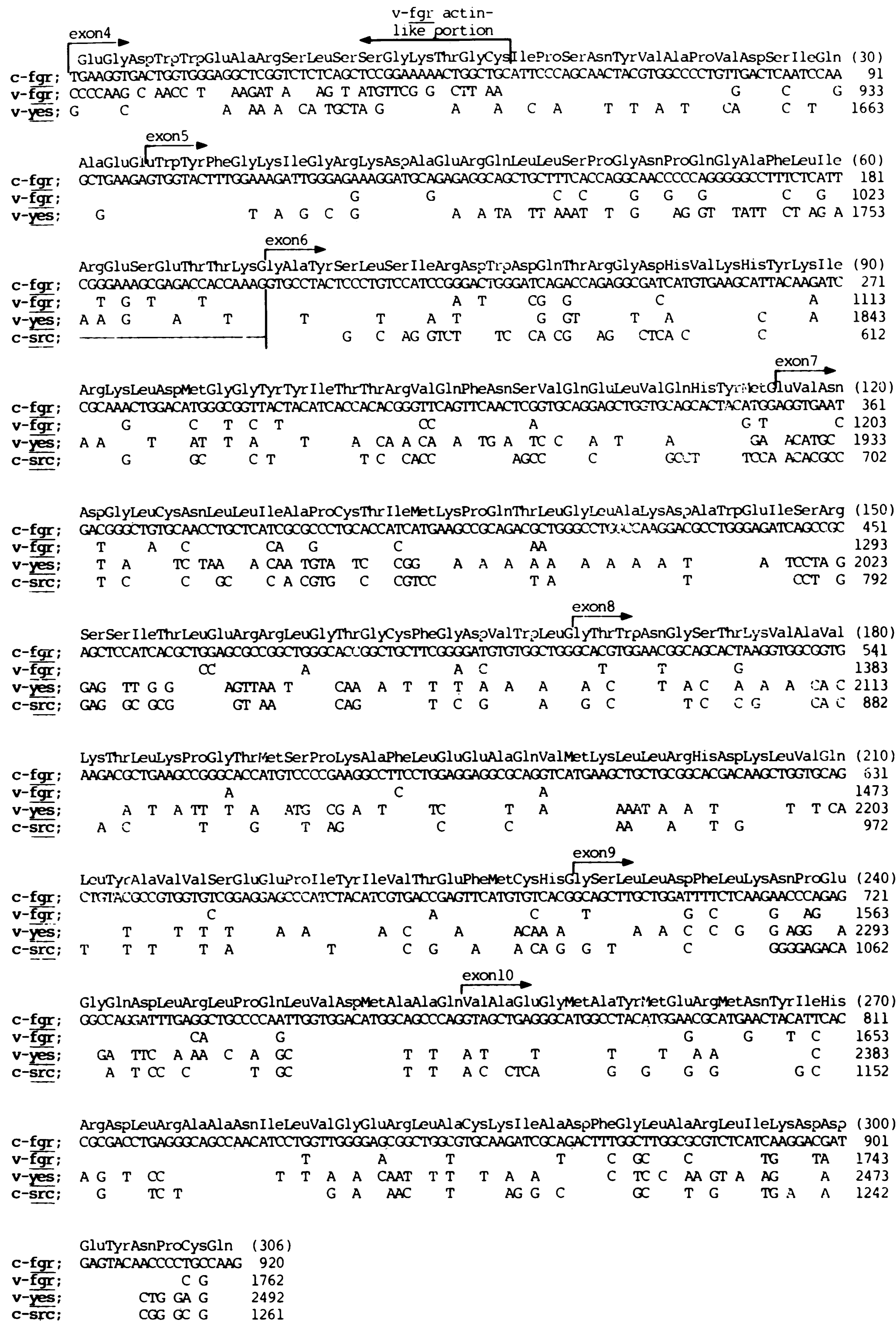

FIG. 2. Nucleotide sequence of the exons of the c-*fgr* gene. The sequences were compared with those of the v-*fgr* (26), v-*yes* (18), and human c-*src* (1) genes. Only nucleotides in the v-*fgr*, v-*yes*, and human c-*src* genes that differ from that of the human c-*fgr* gene are shown. Numbers of nucleotide and amino acids (in parentheses) are given at the end of each line. The c-*fgr* nucleotide sequence is numbered from the first nucleotide of the predicted exon 4. The numbers of other three oncogenes are from each references. The nucleotide sequence of human c-*src* gene was reported about the exons 6 through 12.

```
           v-fgr actin-
        <- like portion
c-fgr; EGDWWEARSL SSGKTGCIPS NYVAPVDSIQ AEEWYFGKIG RKDAERQLLS PGNPQGAFLI  (60)
v-fgr; PKANR KMTQ IMFE FN                                        AR    V (311)
v-yes;          I AT    Y         A             M        L  N    QR I  V (458)

c-fgr; RESETTKGAY SLSIRDWDQT RGDHVKHYKI RKLDMGGYYI TTRVQFNSVQ ELVQHYMEVN (120)
v-fgr;                    EA                T         A             V    (371)
v-yes;                    EV    N           N         A  E L  K  K  R HA (518)
c-src; ————————   C  VS F NA K LN           S  F    S T    L  Q  AY SKHA (234)

c-fgr; DGLCNLLIAP CTIMKPQTLG LAKDAWEISR SSITLERRLG TGCFGDVWLG TWNGSTKVAV (180)
v-fgr;     H  T A   T     M                  Q                M          (431)
v-yes;     HK TTV  PTV    Q          P  E LR  VK   Q    E  M      T    I (578)
c-src;     HR TTV  PTS    Q          P  E LR  VK   Q    E  M        R  I (294)

c-fgr; KTLKPGTMSP KAFLEEAQVM KLLRHDKLVQ LYAVVSEEPI YIVTEFMCHG SLLDFLKNPE (240)
v-fgr;               S     I                  P                   E   DQ  (491)
v-yes;     L   M  E   Q   I  K       P                   TK         EG  (638)
c-src;             E           K   E                     Y SK         GET (354)

c-fgr; GQDLRLPQLV DMAAQVAEGM AYMERMNYIH RDLRAANILV GERLACKIAD FGLARLIKDD (300)
v-fgr;     T                       D                   V             E N (551)
v-yes;  KF K           I D     I                    DN V             E N (698)
c-src;  KY             I S     V     V               N V  V          E N (414)

c-fgr; EYNPCQ (306)
v-fgr;     R  (557)
v-yes;   TAR  (704)
c-src;   TAR  (420)
```

FIG. 3. Comparison of the amino acid sequences of the c-*fgr*, v-*fgr*, (26), v-*yes* (18), and human c-*src* (1) genes. Only amino acid residues of the compared oncogene products that differ from that of the human c-*fgr* gene product are shown. The amino acid sequence of exons 4 and 5 of human c-*src* has not been reported yet. Numbers of amino acids are given in parentheses at the end of each line. The standard one-letter abbreviation system is used.

by 11 introns (37). Very recently, Anderson et al. (1) reported that the human c-*src* gene also consists of 12 coding exons. The lengths of the exons of chicken and human c-*src* genes are identical, utilizing common splicing positions (1, 37). These results indicated that introns were acquired before the divergence of Aves and Mammals. Interestingly, the predicted splicing site positions around the kinase domain of the human c-*fgr* gene were also identical with those of the chicken and human c-*src* genes (Fig. 2). The predicted exons 4 to 10 of the c-*fgr* were numbered according to these of the corresponding exons of chicken c-*src*. This coincidence seemed to support the predicted splicing positions.

Allocation of the human c-*fgr* gene to chromosome 1. We determined the chromosomal location of the c-*fgr* gene by karyotype analysis of human-mouse hybrid cells and Southern blot analysis of DNAs of the hybrid cells. With a c-*fgr*-specific probe (probe A in Fig. 1), *Hin*dIII-digested DNAs of 12 independent human-mouse hybrid cells were analyzed by Southern blotting (Fig. 4A). From the results of a hybridization experiment and the human chromosome content determination by the differential staining method (39), the human c-*fgr* gene was assigned to chromosome 1 (Table 3). For confirmation of this result, the Southern blot filter was rehybridized with human N-*ras*-specific probes after washing out the c-*fgr*-specific probe (Fig. 4B), since the human N-*ras* gene has been located on chromosome 1 by several groups (5, 12, 32). The DNAs of the hybrid cells that retained the human c-*fgr* sequence were also found to contain the N-*ras*-specific sequence. Furthermore, the relative intensities of the bands detected with the c-*fgr* probe and N-*ras* probes seemed to correlate well. Thus, it was confirmed that the human c-*fgr* gene is located on chromosome 1.

Expression of human c-*fgr* gene. To examine the expression of the c-*fgr* gene, we carried out RNA blot hybridization. We used a 220-bp *Sac*I-*Sac*I fragment containing a 154-bp sequence of exon 10 as a c-*fgr*-specific probe to detect the transcript (probe B in Fig. 1). Stringent hybridization conditions were adopted to avoid cross-hybridization with the transcripts of the *yes* and *src* genes. The result of RNA blot hybridization is shown in Fig. 5. Weak expression could be detected in placenta after 8 days of exposure. The major transcript was 2.6 kb long, and two additional longer species of 3.5 and 4.7 kb were also detected. The transcript of the c-*yes* gene was 4.8 kb long (33); its expression was found to be lower in placenta cells than in A431 cells (33), in which no expression of the c-*fgr* gene was detectable. The three species of c-*fgr* gene transcripts are probably not the result of cross-hybridization with c-*yes* mRNA.

Relatively higher expression was found in a human leukemia cell line, IM-9, which was considered to be derived from a lymphocyte cell established by Epstein-Barr virus infection (8). However, the expression level of the c-*fgr* gene in IM-9 cells was almost the same as that of the c-*yes* gene observed in various tissues and thus could not be regarded as overexpression. No amplification or rearrangement of the c-*fgr* gene was detected in IM-9 cells (data not shown). The

TABLE 2. Nucleotide and amino acid sequence homology[a]

Oncogene	% Homology	
	Nucleotide sequence	Amino acid sequence
v-*fgr*	90.0	90.7
v-*yes*	68.7	77.5
v-*src*	71.9	73.0
c-*src*	72.9	73.1

[a] Nucleotide and amino acid sequence homology between the c-*fgr* gene and three viral oncogenes, v-*fgr* (26), v-*yes* (18), and v-*src* (36), and human c-*src* gene (1). Since the 5′ extremity of the sequenced region corresponds to the v-*fgr* gene actinlike sequence (26, 28), the 5′ termini of 52 bases (17 amino acid residues) were omitted here in calculation of homologies. The homology between the two human cellular oncogenes c-*fgr* and c-*src* was calculated about the exons 6 through 10.

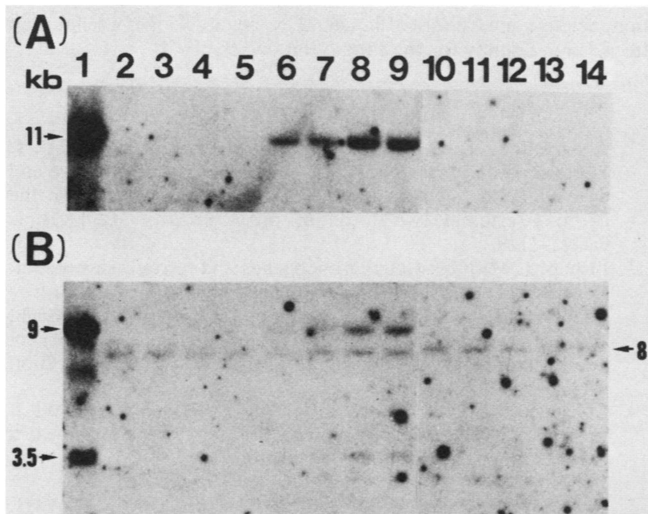

FIG. 4. Identification of human c-*fgr* (A) and N-*ras* (B) genes in DNA of human-mouse somatic hybrid cells. High-molecular-weight DNAs were prepared from human placenta, mouse B82 cells, and human-mouse hybrid cells by proteinase K digestion and phenol extraction. The DNAs were digested with *Hin*dIII restriction endonuclease and subjected to electrophoresis on 1% agarose gel and then Southern blot analysis with the ^{32}P-labeled human c-*fgr* locus-specific probe (A). The c-*fgr*-specific probe detected a fragment of about 11 kb. Subsequently, the filter was rehybridized to human N-*ras*-specific probes (34), which detected two *Hin*dIII fragments of 3.5 and 9 kb (B). The probe also detected an additional 8-kb fragment that was derived from the mouse genome in the DNAs of the hybrid cells and the mouse cells. Lanes; 1 and 14, DNAs from human placenta and mouse B82 cells, respectively; 2 to 13, DNAs from hybrid clones 1A, II-5, III-1, A1, II-6, Bm, 1B1, 3-3, 7-2, 7D4, 6-3, and 3D3, respectively.

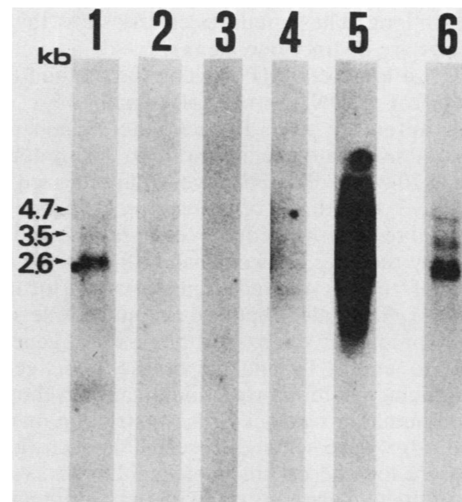

FIG. 5. Expression of the c-*fgr* gene. RNAs were isolated from human placenta (lane 1), human embryo fibroblast cells (lane 2), A431 cells (lane 3), K562 cells (lane 4), and IM-9 cells (lanes 5 and 6). Samples of 2 to 3 μg of polyadenylated RNAs were fractionated by electrophoresis through a 2.2 M formamide–1% agarose gel (20) and transferred directly to a nitrocellulose filter. The filter was hybridized to the ^{32}P-labeled c-*fgr*-specific probe (probe B in Fig. 1) and was exposed for 8 days (lanes 1 to 5) or 15 h (lane 6) after washing out the probe. The sizes of the transcripts were estimated with *Hin*dIII-digested, ^{32}P-labeled λ DNA as a size marker.

significance of the high expression of the c-*fgr* gene in IM-9 cells remains to be investigated.

DISCUSSION

***fgr* and *yes* are distinct oncogenes.** The *fps* gene of Fujinami avian sarcoma virus and the *fes* gene of Gardner feline sarcoma virus are known to correspond to a common cellular counterpart in different animals (11). A similar relation is also accepted for the *raf* gene of a mouse sarcoma virus and the *mil* gene of an avian sarcoma virus (16). In the *src* family, *yes* and *fgr* are closely related. The amino acid sequence of the kinase domain of the v-*fgr* gene shows more than 80% homology with that of the v-*yes* gene (26). The *yes* gene has been identified as an oncogene of an avian sarcoma virus (15). On the other hand, the *fgr* oncogene was found in a feline transforming virus (31). It remained to be determined whether these two genes were derived from distinct cellular loci or from a common proto-oncogene of chickens and cats. In the present study, we isolated a human c-*fgr* clone and obtained specific probes. Using these probes, we found that the c-*fgr* gene was on chromosome 1, and we examined its expression. In previous studies, we located the c-*yes* gene on chromosome 18 and another *yes*-related locus, which is probably a pseudogene, on chromosome 6 (33). Thus, the two oncogenes, the *fgr* gene and the *yes* gene, were proved to be distinct members of the *src* family. The results of RNA blotting also supported this conclusion; the c-*yes* mRNA is 4.8 kb long (33), whereas the major transcript of the c-*fgr*

TABLE 3. Segregation of c-*fgr* gene with human chromosomes in mouse-human cell hybrids[a]

Hybrid	Presence of chromosome:																								Hybridization to c-*fgr*
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	
1a	−	−	+	+	−	+	−	+	−	−	+	+	+	+	+	+	+	−	−	+	+	+	+	+	−
II-5	−	+	+	+	+	+	+	−	−	+	−	+	−	+	+	−	+	+	−	+	+	+	+	−	−
III-1	−	−	+	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−
A1	−	−	+	+	+	+	−	+	−	−	+	−	+	+	+	−	+	+	+	+	+	+	+	+	−
II-6	+	+	−	+	+	+	−	+	−	−	+	−	+	+	+	−	+	+	−	+	+	+	−	−	+
Bm	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
1B1	+	−	+	−	−	−	+	−	−	−	−	−	+	+	−	−	+	−	+	+	−	−	−	−	+
3-3	+	+	−	+	+	−	−	−	−	−	−	−	−	−	+	−	+	−	+	−	+	−	−	−	+
7-2	−	−	+	−	+	−	−	−	−	−	−	+	+	+	−	−	+	−	−	+	+	−	−	−	−
7D4	−	−	−	+	+	−	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−
6-3	−	−	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+	−	+	+	+	+	−	−	−
3D3	−	−	−	−	−	−	−	+	−	−	+	−	−	+	+	−	−	−	−	+	+	−	+	−	−

[a] The human chromosome content was determined by the method of differential staining of human and mouse chromosomes (39).

gene is 2.6 kb long. These findings clearly show that the *yes* and *fgr* genes are distinct oncogenes.

Recently, Le Beau et al. (19), using the method of in situ hybridization of a DNA probe of human c-*src* genomic sequence, detected two distinct loci, 1p34-p36 and 20q12-q13, as *src*-related loci. The c-*src* locus located on chromosome 20 was isolated and well characterized recently (1, 10). In the 3′ two-thirds of the coding sequence, 98% of the amino acid residues are conserved in human and chicken c-*src* (1). Very recently, Parker et al. (30) reported isolation of a clone of the *src*-related gene, c-*src*-2, localized on chromosome 1. They also reported the nucleotide sequence of the two predicted exons of the c-*src*-2 gene, which correspond to exons 11 and 12 of the c-*src* gene. The predicted splicing positions were identical with those of the chicken and human c-*src* genes. The restriction maps of the c-*src*-2 and c-*fgr* were seemed to resemble each other, and both genes are located on chromosome 1. The exon-intron structures of the two are identical with that of the c-*src* gene, at least in their sequenced regions. In addition, the sequence of the c-*src*-2 gene shows extensive homology with that of v-*fgr* (94% nucleotide sequence homology and 95% amino acid sequence homology). Furthermore, our preliminary sequence analysis confirmed the presence of the sequence of exon 12 reported by Parker et al. (30). All of the results of our analyses support the idea that the c-*src*-2 gene is the c-*fgr* gene.

Generation of the c-*fgr* gene. The exon-intron structure of the c-*fgr* gene was found to be identical with those of the chicken (37) and human (1) c-*src* genes. We also found that the same exon-intron structure in the human c-*yes* gene. The lengths of exons 7, 8, and 9 of the c-*yes* genes are 156, 180, and 77 bp, respectively, which exactly coinicide with those of the c-*src* gene and the c-*fgr* gene (unpublished result). These results suggest that the three proto-oncogenes were generated by gene duplications of an ancestral gene harboring the exon-intron structure, as in the case of the generation of the globin gene family (7, 21). Three *src*-related genes were also identified by hybridization experiments in the genome of *Drosophila melanogaster* (14, 35). Partial nucleotide sequence analysis of the most closely *src*-related gene, names D*src*, has been reported (13). The predicted amino acid sequence of the gene showed definite homology with the v-*src* product (54% in amino acid sequence), and the gene was found to contain one intron in the sequenced region. However, the position of the intron of the D*src* is distinct from those of the c-*src* genes of chickens (37) and humans (1). In addition, recent studies showed that the splicing points of cellular counterparts of other members of the *src* gene family, mouse c-*abl* gene (38) and human c-*raf*/*mil* gene (3), were different from those of the chicken c-*src* gene. Accordingly, it seems likely that the *src*, *fgr*, and *yes* genes were formed in the most recent split within the *src* gene family in evolution. Thus, it is likely that the unidentified amino-terminal structure and the normal function of the c-*fgr* gene product have some analogy to those of the c-*src* gene product. Analysis of the structure of the amino-terminal portion of the *fgr* gene product will be helpful in defining the function of the cellular oncogenes.

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LITERATURE CITED

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