J Hum Genet (2002) 47:614–619

SHORT COMMUNICATION

Shoko Yoshida · Haruhito Harada · Hisaki Nagai
Kouichi Fukino · Akira Teramoto · Mitsuru Emi

Head-to-head juxtaposition of Fas-associated phosphatase-1 (*FAP-1*) and c-Jun NH2-terminal kinase 3 (*JNK3*) genes: genomic structure and seven polymorphisms of the *FAP-1* gene

Received: April 5, 2002 / Accepted: July 25, 2002

Abstract When characterizing the 5′ flanking region of the c-Jun NH2-terminal kinase 3 (*JNK3*) gene at 4q21–22, where frequent allelic losses and loss of expression had been detected in patients with brain tumors and hepatocellular carcinomas, we discovered that the Fas-associated phosphatase-1 (*FAP-1*) gene was located only 633 bp upstream from *JNK3* in a head-to-head orientation. A short G/C-rich region between the cap sites of the two genes suggested that they might share a bidirectional promoter region that appeared to contain multiple cis elements, including Sp1, AP-1, AP-2, GATA-1, a GC box, and a CCAAT box. The *FAP-1* gene, consisting of 48 exons, initiates transcription within exon 2 and terminates in exon 48. Exons 2–5, 21–23, 25–28, 29–30, 33–34, and 34–36 encode six Gly-Leu-Gly-Phe repeat domains, and exons 12–17 and 44–88 encode the membrane-binding and catalytic domains, respectively. Seven polymorphisms were identified within functional domains or the putative promoter region, including two with amino acid substitutions, Leu1419Pro and Ile1522Met.

Key words *FAP-1* · *JNK3* · Promoter region · Genomic structure · Single-nucleotide polymorphism

Introduction

Human cancers frequently show allelic loss on the long arm of chromosome 4; we ourselves have defined a 1-cM region at chromosome 4q21–22 that is commonly deleted in hepatocellular carcinomas (Bando et al. 1999). We recently characterized the c-Jun NH_2-terminal Kinase 3 (*JNK3*) gene, a member of the JNK group of mitogen-activated protein kinase (MAP kinase), within this region (Yoshida et al. 2001).

The Fas-associated phosphatase-1 (*FAP-1*) gene, previously assigned to 4q21.3 (Inazawa et al. 1996), regulates Fas-induced apoptosis by interacting with the third Gly-Leu-Gly-Phe (GLGF) domain in the C terminus of the Fas receptor (Sato et al. 1995). Its cDNA has been variously called Fas-associated protein-tyrosine phosphatase nonreceptor-type 13 (PTPN13; Inazawa et al. 1996), PTPL1 (Saras et al. 1994), PTP-BAS (Maekawa et al. 1994), and hPTP1E (Banville et al. 1994). Negative regulation of Fas-mediated apoptosis by FAP-1 in human cancer cells was recently described by Sato and his colleagues (Li et al. 2000).

During our characterization of the 5′ flanking region of the *JNK3* gene (Yoshida et al. 2001) using genome sequencing and 5′ rapid amplification of cDNA ends (5′ RACE) experiments, we discovered that the *FAP-1* gene is located only 633 bp upstream from *JNK3* in a head-to-head orientation. The work reported here characterized the positional relationship of these two genes and determined the genomic structure of *FAP-1*, in which we detected seven single-nucleotide polymorphisms (SNPs) in a 192-chromosome population sample.

Subjects and methods

We cloned the 5′ ends of the *FAP-1* and *JNK3* cDNAs by means of 5′ RACE experiments with three cancer-cell lines, D283Med (brain tumor), MCF7 (breast cancer), and Caki-1 (kidney cancer), using a SMART (Switching Mechanism at 5′ end of RNA transcript) RACE cDNA amplification kit (Clontech, Palo Alto, CA, USA). For amplifying the 5′ end of *JNK3*, we used a gene-specific primer (5′-CACTTCCACACTGTAGAACTGGTTGTCAACTTTG-3′) corresponding to nucleotides 217–250 of the archived partial cDNA (GenBank accession no. HSU34820) and a

S. Yoshida · H. Harada · H. Nagai · K. Fukino · M. Emi (✉)
Department of Molecular Biology, Institute of Gerontology, Nippon Medical School, 1-396 Kosugi-cho, Nakahara-ku, Kawasaki 211-8533, Japan
Tel. +81-44-733-5230; Fax +81-44-733-5192
e-mail: memi@nms.ac.jp

A. Teramoto
Department of Neurosurgery, Nippon Medical School, Kawasaki, Japan

nested gene-specific primer (5′-GGCAATTTTCACATCC AATGTTGGTTCACTGCAG-3′) corresponding to nucleotides 115–148 of the partial cDNA. For amplifying the 5′ end of *FAP-1*, we used a gene-specific primer (5′-AGATGGCAGCAACAGCAGAGAC-3′) corresponding to nucleotides 225–246 of the archived partial cDNA (GenBank; D21209) and a nested gene-specific primer (5′-ATATTACCGGCTGGTCCCGAG-3′) corresponding to nucleotides 45–65 of the partial cDNA. A bacterial artificial chromosome (BAC) containing human *FAP-1* genomic sequence was isolated by three-dimensional polymerase chain reaction (PCR) screening, and the BAC DNA was directly sequenced to determine exon–intron boundaries using primers derived from GenBank cDNA sequence D21209. Partial comparisons were carried out with archived draft sequences (GenBank AF101267, AC022865, and AC007525).

DNA samples were obtained with written informed consent from 96 Japanese volunteers recruited for the study, which was approved by the Institutional Review Board of the Nippon Medical School. PCR-Single-strand conformation polymorphism analysis of each exon of *FAP-1* in these samples was carried out as described previously (Yoshida et al. 2001) using the PCR primer sets shown in Table 1.

Results and discussion

We invoked the 5′ RACE technique to identify an additional 190-bp sequence on the 5′ end of the *JNK3* exon 2, and subjected the result to a basic local alignment search tool (BLAST) search; this entire sequence exactly matched the distal promoter region of the human *FAP-1* gene archived as AF101267 in the GenBank database. This fact revealed that the human *JNK3* and *FAP-1* genes were arranged in close proximity, but in opposite directions, head to head (Fig. 1). The transcription-start sites of the two genes were only 633 bp apart. We examined these sites by means of 5′ RACE assays using primer sequences located in

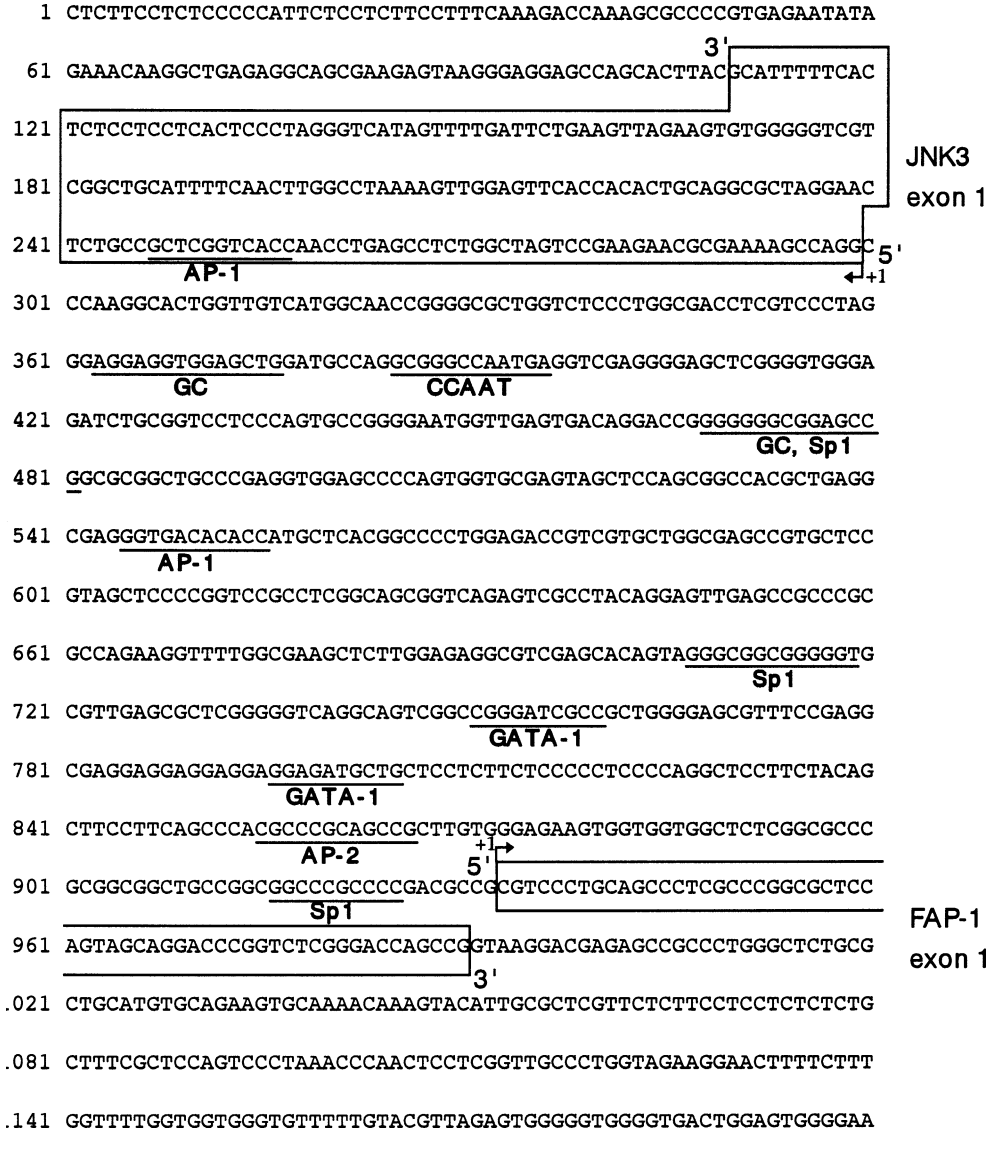

Fig. 1. The putative promoter region shared by *JNK3* and *FAP-1*. Exon 1 for *JNK3* and exon 1 for *FAP-1* are indicated by *boxed-in areas*. A potential CCAAT box, GC boxes, and GATA-1, Sp1, AP-1, and AP-2 sites are delineated by *underlines*

Table 1. Primer sequences used for PCR-SSCP analysis

Function	Amino acid position	cDNA (D21209) position	Product size (bp)	Forward primer (5′–3′)	Reverse primer (5′–3′)
Putative promoter region			185	CTCCTCTTCCTTTCAAAGAC	AGGCCAAGTTGAAAATGCAG
			190	AAGTTAGAAGTGTGGGGGTC	TCGCCAGGGAGACCAGCGCC
			190	GCACTGGTTGTCATGGCAAC	TCGGGCAGCCGCGCCGGCTC
			199	TGGTTGAGTGACAGGACCGG	CTCCTGTAGGCGACTCTGAC
			195	TCCCCGGTCCGCCTCGGCAG	TCTCCTCCTCCTCCTCCTCG
			184	GCTGGGGAGCGTTTCCGAGG	GCTGCAGGGACGCGGCGTCG
			170	CGCGGCGGCTGCCGGCGG	GGAAGAGAACGAGCGCAATG
			161	CGCTGCATGTGCAGAAGTGC	CCCCCACTCTAACGTACAAAAAC
GLGF1	38–121	175–426	213	TTTAAGTACCCAATACTGAAACAAC	GCTGATGCATAGACCATCTG
			328	AAGTGATTGCTGTTTCCTGTG	CATTGTCTTTATTTGACCTATCCC
			221	CAAAATAATCTGTTTTTATAGCACTTGC	GGAAGAGCTATGATATAAGGTG
			261	GCTCACATTTACTCACTGGC	TATGACTATATTTACTATTGTAACTGTAG
Membrane-binding domain	571–881	1774–2706	266	CTTATAATGCTTATTTTAATAAATGCCG	GCTAAATTTCTATACTGTACTCTG
			270	TGCAAGTCTGACCAAAAAGTG	AGCAAAAGAGAAACTCTTGTG
			212	AGGCCAAGATGTCTGTGTG	CCAAACAAAAATGGTCCTGG
			232	CCAATAAAAGGCAAACAATTGCTTATG	CAAAGCTTATCATTTGTAATCTTGG
			249	AAAGTGTATTGCTATGGTATGG	ATATAATGAAAAGAGGTTAAATGGAC
			230	CTTACTATAATTATGAATACCCTGG	GTCATCTGAAATAGTCTGCTTC
GLGF2	1094–1178	3343–3597	195	AACAAGCTGACAGTCTTAATGC	CCAACAATCATAGACCTTCAAG
			196	CTTTGAAAGCAGCAATATGAAAATC	GTCAGAAAATACTTAGAAAACTACC
			204	TTGTTGTTGAAAATACTGGATTGTC	GAAACAGAATACATAAGTAAGAGAC
GLGF3	1368–1452	4165–4419	143	TTCATCACCTCCTAAGCCTG	GACATACAATAGGCTGCTGG
			218	TTTCTAGGCCTGAGATTTGAAAG	TGTTGTAATATTCATACATATAATTCCCAC
			164	TAGGATTTTTATTTATGATTTGAACTGCC	TCTAAGGTACTGTAAAAGGTTGG
			271	ATTAACTATACAAAGTCCTGAAAATGTATC	AGGTGAGATTGTAGTCTCCC
GLGF4	1501–1588	4565–4827	305	TGTTCCTATGTAAACACAGCATG	TGCTATACTCCACATGCTAG
			193	CATTTGTCATTCATGGTACCC	CACAGCTGAATTAGTTAGTTGC
GLGF5	1789–1868	5428–5667	227	AGACAACCTAAAAGTATTACTGC	CACAAATAATCCATTCCTCTTAAATG
			247	ATTGTGATCTTCACATGCCC	AAGAAAATCATATTGTACAAAGTTTGAC
GLGF6	1883–1965	5710–5958	304	TAAGAGTAAGTACTTTTATGACTG	ACACACGCACAATCAAAACTC
			166	AGGTCAAGGCTACAATGTCC	ATTACCGTAGGAGAGTACAG
Catalytic domain	2232–2473	6757–7482	184	TATTACTGAATTACTGCTTATCTCAAC	CTGATCACATATTAAATCTGAAGCC
			225	GCATGATATGGATGGCTCTG	GAGTCATCATGGCTATCACTG
			183	TTACATTGCCTGCCAAGGAC	AGTCGAAGTCTGTTGCTGAC
			174	TCAAATGCCAGCGCTATTGG	GAGATACACCTTCCAACAGG
			158	CATGTCATGATCCACTTATTCTG	CTGATCTGTGGATGTGTCTC
			213	CCAGACCATGATACACCTTC	ATTTCTGAACAAATGCTTACTCAGC
			181	CCCTCTGTGATCCTTTTGAG	GAATCTTTACAAAAATTCTCATTAGGAGG
			220	CATCACTTCCAATAGTGGTATAGC	CAGAGGCTCTTTTCATGTCAC

PCR-SSCP, Polymerase chain reaction-single-strand conformation polymorphism

Table 2. Exon–intron boundary sequences of the *FAP-1* gene

Exon number	Exon length (bp)	cDNA (D21209) position	Splice acceptor	Splice donor
1	58	1–58		GGACCAGCCG**gt**aaggacga
2	120	59–178	tgtttccc**ag**GTAATATGCA	TTCAGAAAAG**gt**aagctgct
3	179	179–357	tacaaacc**ag**TAAGCCTAGC	TGTTGAAAAG**gt**aactgtta
4	66	358–423	ctattcct**ag**ATCCACATTT	TCAGAGCCAA**gt**aagttaag
5	186	424–609	ttatattc**ag**CCTATTAAGC	TCTTTCTGGG**gt**aagctaca
6	88	610–697	ctgtgtac**ag**ACAGATCAGC	TTACCAACAG**gt**aagagtat
7	561	698–1258	atcattcc**ag**GAAGAAGCTC	AATGTAGAAG**gt**tagtaatt
8	96	1259–1354	atcctttt**ag**AACCAGTTCG	TTCAGACAAG**gt**aggaggca
9	94	1355–1448	tcataatt**ag**TGAGAAGAAG	AGAGACCGAG**gt**atgtcatg
10	223	1449–1671	ttctatat**ag**CAGACAATAT	AAAACTGAGG**gt**aagttgat
11	75	1672–1746	tgatttgc**ag**AATTTCTTTG	GTCTATTCTT**gt**aagtaata
12	175	1747–1921	tcaattgt**ag**ACTAAGAAAG	ACCCTCAAAG**gt**accaagac
13	154	1922–2075	ttctgttt**ag**ATAATGAATA	GTCTAATACA**gt**gagtacac
14	139	2076–2214	gtttttc**ag**ACATACTCTG	TCAACCAGAG**gt**aggatttg
15	153	2215–2367	ttttatct**ag**GTTCATGGTG	ATTTTTAAAG**gt**aagcatcc
16	183	2368–2550	attccaac**ag**GTCTGCCAAA	ATCTTTTTCT**gt**atgtccat
17	163	2551–2713	aatattgt**ag**AAAAAGAAAA	CAAGATATTG**gt**aaggagaa
18	418	2714–3131	ttccttgt**ag**AGAGAGCTTC	AACTTAATAA**gt**aagaacat
19	98	3132–3229	ccctcttc**ag**TTCAAAGTCT	TATGTTCTAG**gt**cagcaaaa
20	57	3230–3286	tatgccac**ag**GAATGACTAT	AAAGAAAATG**gt**aggtttac
21	90	3287–3376	ttcccaat**ag**ATGTGCTACA	TATGGCTTGG**gt**aagtcacc
22	108	3377–3484	tattttac**ag**GATTTCAAAT	TTGAAGCCAG**gt**actttaca
23	132	3485–3616	atttgtac**ag**GAGACCGTTT	ATATCCAAAG**gt**aatgtgaa
24	464	3617–4080	tgacttttt**ag**TGCCTTCTAC	ACCAAAACAGgcatagttta
25	132	4081–4212	ctttgtta**ag**GAATCTTCCT	AAGTGTCACG**gt**actgtttg
26	94	4213–4306	ttctcttt**ag**GGAGGTGTGA	ATTCACAAAG**gt**atagtgtt
27	86	4307–4392	taacttct**ag**GTGATCGCGT	TACAGGACAG**gt**aacagatc
28	160	4393–4552	acttatcc**ag**GTGGTTCATC	GTCACTGAAG**gt**caggcctt
29	215	4553–4767	ttttctcc**ag**AAAATACATT	ATCTCAGCAG**gt**gagcccct
30	99	4768–4866	gtaccccc**ag**GAAGTCATAT	TGCGCTTTTG**gt**gagactta
31	365	4867–5231	tccattac**ag**ACCCCACTTC	TTGAGGACAG**gt**atcatcaa
32	181	5232–5412	gtctctgt**ag**TAATCCTTCC	CTTTGAACTG**gt**aagttgtt
33	159	5413–5571	tttcctt**ag**GAAGTAGAAC	GCTCATAAAG**gt**gagacatt
34	172	5572–5743	gtttttgt**ag**GTTAATGATA	GAGGAGTTGG**gt**aatgaaaa
35	211	5744–5954	ttatttca**ag**GTTTTTCCTT	AAGCAACAAG**gt**actctgca
36	73	5955–6025	tacaacac**ag**AAATGATCTT	AAAGGCAATG**gt**aaggatat
37	62	6026–6087	gtcctttc**ag**GTTCCTACAG	ATTCTCCACG**gt**aagaaaaa
38	94	6088–6181	tatgcttt**ag**GTTGCTGGGG	CTGCCCAAAG**gt**agttttcc
39	138	6182–6319	atttttca**ag**AATCTTATAT	TGTGGTCCAG**gt**acgtgaac
40	89	6320–6408	cctctctc**ag**GTACATTAAA	TTTTACTGAG**gt**aacaataa
41	56	6409–6464	tttattgt**ag**GCCACCAAAA	AAAGTGAAAG**gt**gagaaaat
42	104	6465–6568	gttttcat**ag**CTTAATTCAG	TCTGATAAAGgcaagaattt
43	149	6569–6717	ttcctatt**ag**ATCATTCCTT	GGAGCTGGAG**gt**aagtggct
44	91	6718–6808	ttgcttac**ag**AATCTTCAAG	ATACTTCCCT**gt**aagttcca
45	338	6809–7146	aaattcac**ag**ATGATGCTAC	AGATATTCAG**gt**aagtgaat
46	216	7147–7362	acaatttc**ag**ACCAGAGAGG	GGATCTTGAT**gt**gagtacaa
47	63	7363–7425	cctctgac**ag**TTTGACATCTC	TCAGACAGAG**gt**gagtcatg
48	694	7426–8119	ttggccat**ag**GATCAATATA	TTAAAACATGaacaagccaa

Lower-case letters refer to intronic sequence and upper case to exonic sequence. Boldface type indicates agreement with the GT–AG rule

the most upstream exons (exon 1) in cDNAs from cell lines derived from brain, breast, or kidney cancers, and found extension products of the same length and sequence corresponding to the upstream cap sites in all three lines. Because we examined only the most upstream, or distal, promoter region of *FAP-1* in our 5′ RACE assay, we were unable to detect shorter products that might otherwise have appeared if we had used primers specific for the more proximal promoter sequences that Irie et al. (2001) had described in several cancer-cell lines.

Figure 1 displays the structure of the 5′-flanking sequence shared by *FAP-1* and *JNK3*; no CAAT or TATA boxes were present for either gene. The features noted in Fig. 1 are characteristic of the promoters of housekeeping genes (Dynan 1986). All binding sites for transcription factors found in the shared region were examined with the TRANSFAC program (http://www.motif.genome.ad.jp/), which revealed a G/C-rich region and a CCAAT box, as well as GATA-1, Sp1, AP-1, and AP-2 motifs. The results suggested that the *JNK3* and *FAP-1* genes are likely to share a bidirectional promoter.

When we extended our search for transcription-factor binding sites of the *FAP-1* gene 1 kb farther into the *JNK3* region, we identified an additional AP-1 binding site on exon 1 of the *JNK3* gene (Fig. 1). Functional assays for *FAP-1* promoter activity will be required to more precisely

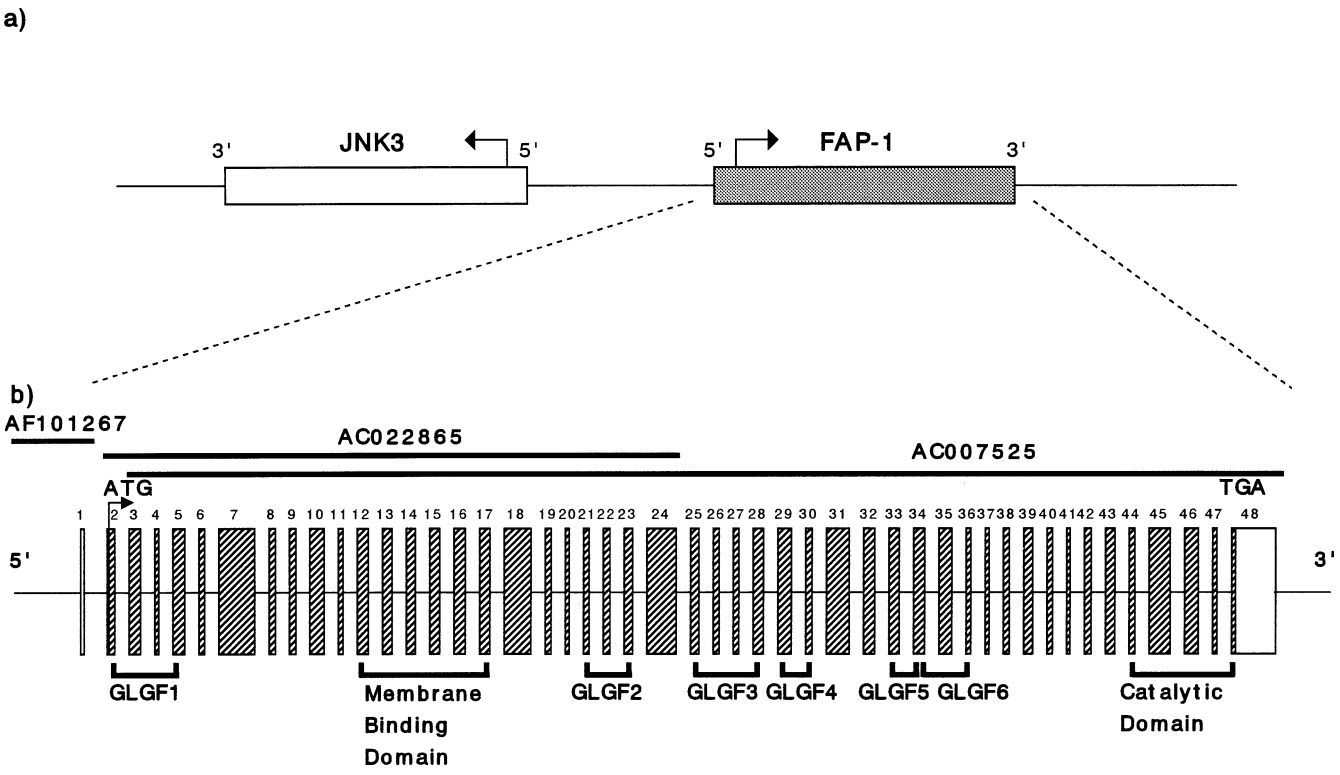

Fig. 2. **a** Schema showing positions of the *FAP-1* and *JNK3* genes, in a head-to-head orientation. **b** Schematic structure of the human *FAP-1* gene. Coding regions are indicated by *hatched boxes*. GenBank sequences that cover the region are indicated *above* the map, with their accession numbers. Locations of functional domains (GLGF 1–6, a membrane-binding domain, and a catalytic domain) are also indicated

Table 3. Single-nucleotide polymorphisms found in the functional domains of *FAP-1*

Function	Nucleotide sequence	Codon position	Nucleotide position	Genotypes (n = 95 or 96)			Allelic frequencies	
Putative promoter region	**A** → **C**		−101	A/A	A/C	C/C	A	C
				60	26	10	0.76	0.24
Putative promoter region	**T** → **A**		−53	T/T	T/A	A/A	T	A
				48	38	10	0.7	0.3
GLGF1 (intron 2)	**C** → **T**		+24	C/C	C/T	T/T	C	T
				94	2	0	0.99	0.01
GLGF2 (intron 21)	**G** → **C**		+11	G/G	G/C	C/C	G	C
				83	12	0	0.94	0.06
GLGF3 (exon 27)	C**T**A → C**C**A	1419	+13	T/T	T/C	C/C	T	C
	(Leu) (Pro)			94	1	0	0.995	0.005
GLGF4 (exon 29)	AT**A** → AT**G**	1522	+77	A/A	A/G	G/G	A	G
	(Ile) (Met)			36	39	20	0.58	0.42
Catalytic domain (exon 46)	AG**A** → AG**G**	2363	+6	A/A	A/G	G/G	A	G
	(Arg) (Arg)			94	1	0	0.995	0.005

Nucleotide position is identified from exon–intron boundary. Boldface letters indicate single-nucleotide polymorphism

define the region in question. However, the issue is too complex to resolve at present in view of the multiplicity of transcription-initiation sites noted for *FAP-1* and because different tissue- and cell-specificities depend on distinct *FAP-1* promoters. We will carry out functional promoter assays after these issues are clarified. We merely note here that these two coordinately controlled genes exert their effects in different pathways of apoptosis: the *JNK3* signaling pathway mediates apoptosis in the nervous system (Yang et al. 1997), whereas the *FAP-1* is a negative regulator of Fas-induced apoptosis. Novel transcription factors that affect both genes in *trans* may bind to this region. Additional, distinct regulatory elements may be present further upstream of each gene.

Structural analysis revealed that the *FAP-1* gene consists of 48 exons interrupted by 47 introns; its transcription–initiation site is within exon 2 and the termination codon lies in exon 48. Exons 2–5, 21–23, 25–28, 29–30, 33–34, and 34–36 encode GLGF repeat domains 1–6, respectively. Exons 12–17 encode the membrane-binding domain and

exons 44–48 encode the catalytic domain (Fig. 2). Exon–intron boundary sequences compatible with the consensus rule are shown in Table 2.

Among 192 human chromosomes from Japanese volunteers, we found a total of seven sequence polymorphisms within functional domains or the putative promoter region of the *FAP-1* gene (Table 3). Among these SNPs, two were nonsynonymous substitutions, i.e., Leu1419Pro and Ile1522Met; three did not affect amino acid sequence, and the remaining two were in the putative promoter region. The exon–intron boundaries reported here, and the novel polymorphisms, should prove useful for genetic studies seeking to clarify activities of *FAP-1* in diseases involving cell growth and inhibition of apoptosis.

References

Bando K, Nagai H, Matsumoto S, Koyama M, Kawamura N, Onda M, Emi M (1999) Identification of a 1-cM region of common deletion on 4q35 associated with progression of hepatocellular carcinoma. Genes Chromosomes Cancer 25:284–289

Banville D, Ahmad S, Stocco R, Shen SH (1994) A novel protein-tyrosine phosphatase with homology to both the cytoskeletal proteins of the band 4.1 family and junction-associated guanylate kinases. J Biol Chem 269:22320–22327

Dynan WS (1986) Promoters for house-keeping genes. Trends Genet 2:196–197

Inazawa J, Ariyama T, Abe T, Druck T, Ohta M, Huebner K, Yanagisawa J, Reed JC, Sato T (1996) PTPN13, a Fas-associated protein tyrosine phosphatase, is located on the long arm of chromosome 4 at band q21.3. Genomics 31:240–242

Irie S, Li Y, Kanki H, Ohyama T, Deaven LL, Somlo S, Sato T (2001) Identification of two Fas-associated phosphatase-1 (FAP-1) promoters in human cancer cells. DNA Seq 11:519–526

Li Y, Kanki H, Hachiya T, Ohyama T, Irie S, Tang GL, Mukai J, Sato T (2000) Negative regulation of Fas-mediated apoptosis by FAP-1 in human cancer cells. Int J Cancer 87:473–479

Maekawa K, Imagawa N, Nagamatsu M, Harada S (1994) Molecular cloning of a novel protein-tyrosine phosphatase containing a membrane-binding domain and GLGF repeats. FEBS Lett 337:200–206

Saras J, Claesson-WL, Heldin CH, Gonez LJ (1994) Cloning and characterization of PTPL1, a protein tyrosine phosphatase with similarities to cytoskeletal-associated proteins. J Biol Chem 269:24082–24089

Sato T, Irie S, Kitada S, Reed JC (1995) FAP-1: a protein tyrosine phosphatase that associates with Fas. Science 268:411–415

Yang DD, Kuan CY, Whitmarsh AJ, Rincon M, Zheng TS, Davis RJ, Rakic P, Flavell RA (1997) Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the *Jnk3* gene. Nature 389:865–870

Yoshida S, Fukino K, Harada H, Nagai H, Imoto I, Inazawa J, Takahashi H, Teramoto A, Emi M (2001) The c-Jun NH2-terminal kinase3 (*JNK3*) gene: genomic structure, chromosomal assignment, and loss of expression in brain tumors. J Hum Genet 46:182–187