

Human *c-ros-1* Gene Homologous to the *v-ros* Sequence of UR2 Sarcoma Virus Encodes for a Transmembrane Receptorlike Molecule

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We isolated a human gene (designated *c-ros-1*) homologous to the *v-ros* sequence of UR2 sarcoma virus. Ten exons, 1,414 base pairs spanning 26 kilobases, contained a tyrosine kinase domain, a transmembrane domain, and a part of an extracellular domain carrying an N glycosylation site which was not acquired by UR2 sarcoma virus. The predicted structure of *c-ros-1* is unique among the *src* family and clearly distinct from the human insulin receptor.

UR2 sarcoma virus, a recent isolate of acutely transforming retrovirus of chickens (1), encodes for a fusion protein, p68^{gag-ros}, which has tyrosine-specific protein kinase activity (7). Nucleotide sequence analysis of the UR2 genome has revealed that the oncogene *v-ros* of UR2 carries a kinase domain homologous to those present in the oncogenes of the *src* family (12). The *v-ros* gene is considered to be derived from a cellular counterpart, proto-oncogene *c-ros* of chickens (15). Since the predicted chicken *c-ros* gene product, as well as the *v-ros* product, has a hydrophobic short stretch upstream of its kinase domain, it seems likely that the *c-ros* gene encodes for a transmembrane protein similar to the cell surface receptor for cell growth or differentiation factors (11, 12). Furthermore, recent reports have indicated that the deduced amino acid sequence of the kinase domain in the human insulin receptor (HIR) gene is highly homologous to the kinase domain in the *v-ros* sequence (6, 17). However, the phylogenetic conservation of the *c-ros* gene in mammalian species, including humans, and the relationship between the *c-ros* gene and the HIR gene have not yet been examined. In this study, we made an attempt to clarify these points.

High-molecular-weight genomic DNA was extracted from human placenta, mouse thymus, fish testis, *Drosophila melanogaster*, and yeast cells (*Saccharomyces cerevisiae*) and hybridized with a *v-ros*-specific probe (*Eco*RI-*Pvu*II 0.8-kilobase (kb) fragment; probe IV [see Fig. 2]). Under hybridization conditions of very relaxed stringency (20% formamide, 10% dextran sulfate, 1M NaCl; 37°C), we were able to detect clear bands in all DNAs examined, except for yeast DNA (Fig. 1). The *c-ros* sequence appeared to be conserved in vertebrate species from fish to mammals, including humans.

In human placenta DNA digested with the restriction endonuclease *Bam*HI, two discrete bands were detected at 15 and 10 kb (Fig. 1). Because these bands were observed only under very relaxed hybridization conditions, we molecularly cloned these *Bam*HI fragments, which may contain a portion(s) of the *c-ros* gene, before screening a large number of phages in a human genomic DNA library. With Charon 30

as a vector, four independent clones (two clones each from the 15- and 10-kb *Bam*HI fragments) were isolated by the method described by Benton and Davis (3) (Fig. 2). Partial DNA sequencing analysis by the dideoxy method (13) revealed that recombinant phage HYuros8 contained DNA sequences highly homologous to the *v-ros* sequence, whereas phage HYuros4 contained human sequences partially related to *v-ros* (data not shown). Both HYuros5 and HYuros1 were found to contain human sequences incidentally homologous to *v-ros*. Therefore, the human genes

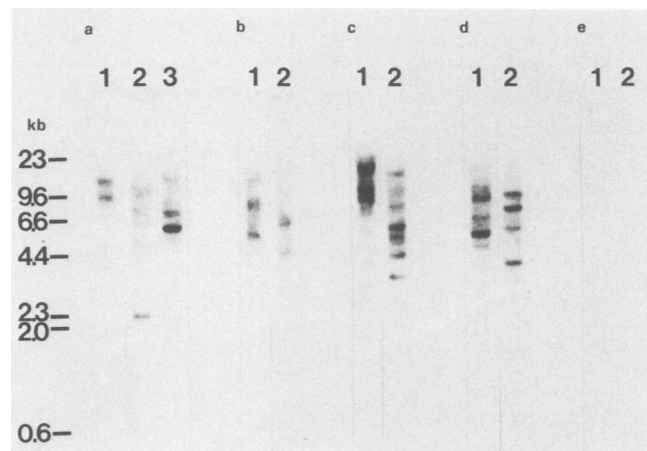


FIG. 1. Hybridization of a *v-ros*-specific probe to genomic DNAs. Cellular DNAs (10 μ g in panels a to c, 3 μ g in panel d, and 2 μ g in panel e) were digested with various endonucleases, electrophoresed on 0.8% agarose gels, and transferred to nitrocellulose filters (16). These filters were hybridized with a *v-ros*-specific probe (probe IV in Fig. 2) under hybridization conditions of low stringency (see text). Panels a to c were exposed to X-ray film at -70°C for 3 days; panels d and e were exposed for 6 days with an intensifying screen. Molecular weight markers were lambda DNAs digested with *Hind*III. Genomic DNAs used in Southern blot analyses were from human placenta (a), mouse thymus (b), fish testis (c), *D. melanogaster* (d), and *S. cerevisiae* (e) cells. These DNAs were digested with the following endonucleases. (a) Lanes: 1, *Bam*HI; 2, *Eco*RI; 3, *Hind*III. (b) Lanes: 1, *Bam*HI; 2, *Eco*RI. (c) Lanes: 1, *Bam*HI; 2, *Hind*III. (d) Lanes: 1, *Eco*RI; 2, *Hind*III. (e) Lanes: 1, *Bam*HI; 2, *Eco*RI.

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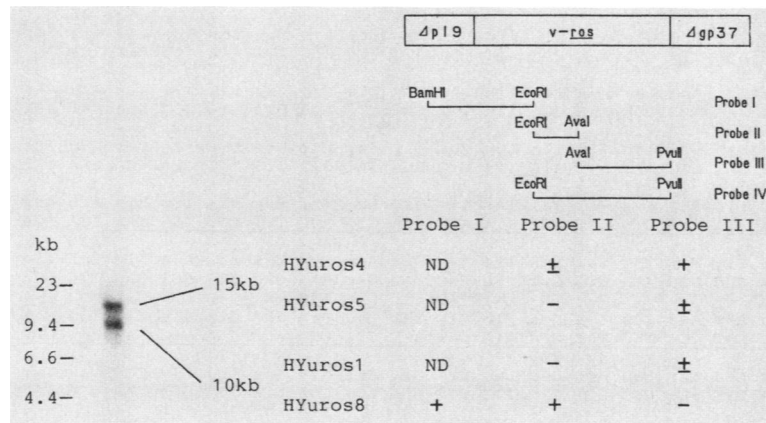


FIG. 2. Molecular cloning of human *c-ros* genes. Four independent clones were isolated. HYuros4 and HYuros5 were derived from phages recombined with 15-kb *Bam*HI fragments; the other two clones were derived from 10-kb *Bam*HI fragments. These four clones were hybridized with various *v-ros* probes under hybridization conditions of low stringency. Symbols: +, hybridization; ±, weak hybridization; -, no detected hybridization. ND, Not determined. Probes used are illustrated at the top of the figure. Molecular size markers (in kilobases) are indicated to the left of the gel.

found in HYuros8 and HYuros4 were designated human *c-ros-1* and human *c-ros-2*, respectively, and the human *c-ros-1* gene was further characterized in this study. The details of the analysis of the human *c-ros-2* gene will be described elsewhere.

We constructed a human genomic DNA library by the method described by Maniatis et al. (9), and gene walking of overlapping *c-ros-1* DNA sequences of about 60 kb was carried out. By restriction endonuclease mapping and by DNA sequencing of DNA fragments which hybridized with various *v-ros* probes, seven exons (exons 4 to 10) were found to encode for the entire kinase domain of this gene (Fig. 3a and 4). However, an approximately 240-base-pair region at the 5' end of the *v-ros* gene, including a possible transmembrane domain, was not detected by cross-hybridization between the human 60-kb DNA sequence and the *v-ros* sequence. We expected that the chicken *c-ros*

DNA fragment might be useful for isolating the transmembrane domain of the human *c-ros-1* gene more efficiently than could be done with the *v-ros* sequence as a probe. The ³²P-labeled 5' region of chicken *c-ros* DNA (5.2-kb *Eco*RI fragment [11]) was hybridized with the human DNA fragment upstream of the kinase domain (exon 1 in Fig. 3a), and then the nucleotide sequence of this region was determined. Although we did not obtain an exon(s) for a transmembrane domain in this region, to our surprise we found a new exon surrounded by a consensus splice acceptor site and a donor site (Fig. 3b); the predicted amino acid sequence had a potential site of N-linked glycosylation (Asn-Gly-Ser, amino acid residues 19 to 21; Fig. 4). In chicken *c-ros* DNA, an exon highly homologous to this human sequence at the level of predicted amino acids was also observed (Fig. 5). Since the amino acid sequence in this new exon (amino acid residues 1 to 63) was not present in the

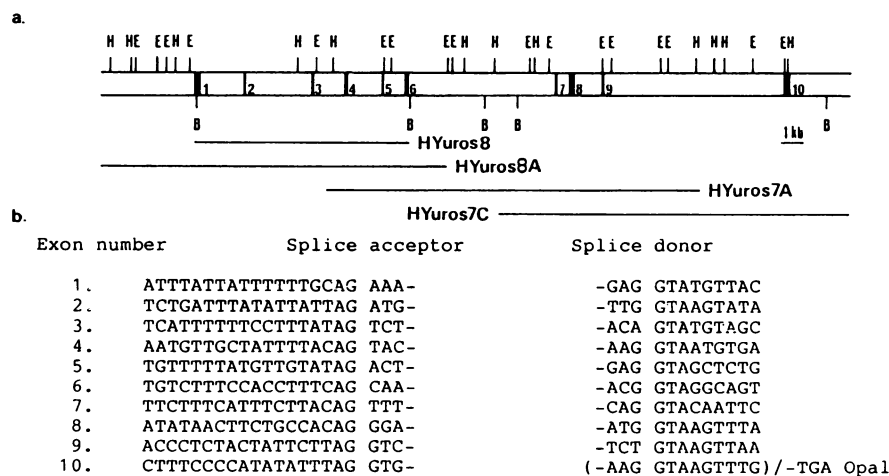


FIG. 3. Restriction map and gene organization of the human *c-ros-1* gene. (a) Four overlapping clones spanning 32-kb of cellular DNA are indicated. HYuros8 was a recombinant phage with Charon 30 *Bam*HI arms; the other clones, HYuros8A, HYuros7A, and HYuros7C, had Charon 4A *Eco*RI arms. The restriction map was determined by double or triple digestion with the following various endonucleases: B, *Bam*HI; E, *Eco*RI; and H, *Hind*III. The positions of exons in the human *c-ros-1* gene were determined by DNA sequencing. Black and white boxes indicate exons and introns, respectively. The numbers to the right of the black boxes indicate exon numbers. (b) Possible splice junctions in the human *c-ros-1* gene.

exon 1	Lys Ser Thr Ser Asn Asn Leu Gln Asn	10	Gln Asn Leu Arg Trp Lys Met Thr Phe Asn Gly	*	20	Ser Cys Ser Ser	
	A AAG AGC ACT TCA AAT AAT TTA CAG AAC CAG AAT TTA AGG TGG AAG ATG ACA TTT AAT GGA TCC TGC AGT AGT						73
	Val Cys Thr Trp Lys Ser Lys Asn Leu Lys Gly Ile Phe Gln Phe Arg Val Val Ala Ala Asn Asn Leu Gly Phe	30	40				
	GTT TGC ACA TGG AAG TCC AAA AAC CTG AAA GGA ATA TTT CAG TTC AGA GTA GTA GCT GCA AAT AAT CTA GGG TTT						148
	Gly Glu Tyr Ser Gly Ile Ser Glu Asn Ile Ile Leu Val Gly Asp Phe Trp Ile Pro Glu Thr Ser Phe Ile	50	60	70			
	GGT GAA TAT AGT GGA ATC AGT GAG AAT ATT ATA TTA GTT GGA GAT GAT TTT TGG ATA CCA GAA ACA AGT TTC ATA						223
	Leu Thr Ile Ile Val Gly Ile Phe Leu Val Val Thr Ile Pro Leu Thr Phe Val Trp His Arg Arg Leu Lys Asn	80	90	100			
	CTT ACT ATT ATA GTT GGA ATA TTT CTG GTT GTT ACA ATC CCA CTG ACC TTT GTC TGG CAT AGA AGA TTA AAG AAT						298
	Gln Lys Ser Ala Lys Glu Gly Val Thr Val Leu Ile Asn Glu Asp Lys Glu Leu Ala Glu Leu Arg Gly Leu Ala	110	120	130			
	CAA AAA AGT GCC GAA GAA GGG GTG ACA GTG CTT ATA AAC GAA GAC AAA GAG TTG GCT GAG CTG CGA GGT CTG GCA						375
	Ala Gly Val Gly Leu Ala Asn Ala Cys Tyr Ala Ile His Thr Leu Pro Thr Gln Glu Glu Ile Glu Asn Leu Pro	140	150	160			
	GCC GGA GTA GGC CTG GCT AAT GCC TGC TAT GCA ATA CAT ACT CTT CCA ACC CAA GAG GAG ATT GAA AAT CTT CCT						448
	Ala Phe Pro Arg Glu Lys Leu Thr Leu Arg Leu Leu Leu Gly Ser Gly Ala Phe Gly Glu Val Tyr Glu Gly Thr	170	180	190			
	GCC TTC CCT CGG GAA AAA CTG ACT CTG CGT CTC TTG CTG GGA AGT GGA GCC TTT GGA GAA GTG TAT GAA GGA ACA						523
	Ala Val Asp Ile Leu Gly Val Gly Ser Gly Glu Ile Lys Val Ala Val Lys Thr Leu Lys Lys Gly Ser Thr Asp	200	210	220			
	GCA GTG GAC ATC TTA GGA GTT GGA AGT GGA GAA ATC AAA GTA GCA GTG AAG ACT TTG AAG AAG GGT TCC ACA GAC						598
	Gln Glu Lys Ile Glu Phe Leu Lys Glu Ala His Leu Met Ser Lys Phe Asn His Pro Asn Ile Leu Lys Gln Leu	230	240	250			
	CAG GAG AAG ATT GAA TTC CTG AAG GAG GCA CAT CTG ATG AGC AAA TTT AAT CAT CCC AAC ATT CTG AAG CAG CTT						673
	Gly Val Cys Leu Thr Asn Glu Pro Gln Tyr Ile Ile Leu Glu Leu Met Glu Gly Gly Asp Leu Thr Thr Tyr Leu	260	270	280			
	GGG GAT TGT CTG CTG AAT GAA CCC CAA TAC ATT ATC CTG GAA CTG ATG GAG GGA GGA GAC CTT CTT ACT TAT TTG						748
	Arg Lys Ala Arg Met Ala Thr Phe Tyr Gly Pro Leu Leu Thr Leu Val Asp Leu Val Asp Leu Cys Val Asp Ile	290	300	310			
	CGT AAA GCC CGG ATG GCA ACG TTT TAT GGT CCT TTA CTC ACC TTG GTT GAC CTT GTA GAC CTG TGT GTA GAT ATT						823
	Ser Lys Gly Cys Val Tyr Leu Glu Arg Met His Phe Ile His Arg Asp Leu Ala Ala Arg Asn Cys Leu Val Ser	320	330	340			
	TCA AAA GGC TGT GTC TAC TTG GAA CCG ATG CAT TTC ATT CAC AGG GAT CTG GCA GGT AGA AAT TGC CTT GTT TCC						898
	Val Lys Asp Tyr Thr Ser Pro Arg Ile Val Lys Ile Gly Asp Phe Gly Leu Ala Arg Asp Ile Tyr Lys Asn Asp	350	360	370			
	GTG AAA GAC TAT ACC AGT CCA CGG ATA GTG AAG ATT GGA GAC TTT GGA CTC GCC AGA GAC ATC TAT AAA AAT GAT						973
	Tyr Tyr Arg Lys Arg Gly Glu Gly Leu Leu Pro Val Arg Trp Met Ala Pro Glu Ser Leu Met Asp Gly Ile Phe	380	390	400			
	TAC TAT AGA AAG AGA GGG GAA GGC CTG CTC CCA GTT CCG TGG ATG GCT CCA GAA AGT TTG ATG GAT GGA ATC TTC						1048
	Thr Thr Gln Ser Asp Val Trp Ser Phe Gly Ile Leu Ile Trp Glu Ile Leu Thr Leu Gly His Gln Pro Tyr Pro	410	420	430			
	ACT ACT CA _n TCT GAT GTA TGG TCT TTT GGA ATT CTG ATT TGG GAG ATT TTA ACT CTT GGT CAT CAG CCT TAT CCA						1123
	Ala His Ser Asn Leu Asp Val Leu Asn Tyr Val Gln Thr Gly Gly Arg Leu Glu Pro Pro Arg Asn Cys Pro Asp	440	450	460			
	GCT CAT TCC AAC CTT GAT GTG TTA AAC TAT GTG CAA ACA GGA GGG AGA CTG GAG CCA CCA AGA AAT TGT CCT GAT						1198
	Asp Leu Trp Asn Leu Met Thr Gln Cys Trp Ala Gln Glu Pro Asp Gln Arg Pro Thr Phe His Arg Ile Gln Asp	470	480	490			
	GAT CTG TGG AAT TTA ATG ACC CAG TGC TGG GCT CAA GAA CCC GAC CAA AGA CCT ACT TTT CAT AGA ATT CAG GAC						1273
	Gln Leu Gln Leu Phe Arg Asn Phe Phe Leu Asn Ser Ile Tyr Lys Ser Arg Asp Glu Ala Asn Asn Ser Gly Val	500	510	520			
	CAA CTT CAG TTA TTC AGA AAT TTT TTC TTA AAT AGC ATT TAT AAG TCC AGA GAT GAA GCA AAC AAC AGT GGA GTC						1348
	Ile Asn Glu Ser Phe Glu Gly Lys Phe Asp Ser Ser Glu Phe Ser Phe Arg Cys Thr Val Asn Opal	530	540	550			
	ATA AAT GAA AGC TTT GAA GGT AAG TTT GAT TCT TCA GAA TTT TCT AGT TTT CGC TGC ACT GTG AAC TGA						1417

FIG. 4. Nucleotide sequence and predicted amino acid sequence of the human *c-ros-1* gene. The putative transmembrane domain and kinase domain are underlined and boxed, respectively. A potential site of N-linked glycosylation is indicated by an asterisk. The horizontal arrows above the amino acid sequences indicate the junctions between exons and introns. The nucleotides above the dashed line indicate a possible splice donor site. Ala, Alanine; Cys, cysteine; Asp, aspartic acid; Glu, glutamic acid; Phe, phenylalanine; Gly, glycine; His, histidine; Ile, isoleucine; Lys, lysine; Leu, leucine; Met, methionine; Asn, asparagine; Pro, proline; Gln, glutamine; Arg, arginine; Ser, serine; Thr, threonine; Val, valine; Trp, tryptophan; Tyr, tyrosine.

v-ros sequence of UR2 sarcoma virus, it seemed most likely that this exon belonged to the extracellular domain of the *c-ros* gene not acquired in the viral genome.

By nucleotide sequencing of the entire cellular DNA of about 6 kb in length from exon 1 to exon 4 in Fig. 3a, two exons, exon 2 and exon 3, were detected. The predicted amino acid sequence of exon 2 showed an extremely hydrophobic stretch of 21 amino acids; that of exon 3 carried a sequence 63% homologous to the corresponding exon of the chicken *c-ros* gene (Fig. 4, Table 1). Although the nucleotide sequence of exon 2 greatly diverged from that of the chicken *c-ros* transmembrane domain and the peptide length was 2 amino acids shorter than that in chickens, we consider this hydrophobic stretch to be the transmembrane domain of the human *c-ros-1* gene because of its partial homology to the hydrophobic amino acid sequence of the chicken *c-ros* gene and because its length is sufficient to pass through the lipid bilayer of the cell membrane. The extents of nucleotide

homology of the regions of exon 2 and exon 3 with the corresponding regions of the chicken *c-ros* gene were 52 and 60%, respectively. This weak homology may explain the failure to detect cross-hybridization between these sequences and the *v-ros* sequence by Southern blot analysis. Such a high degree of heterogeneity in the nucleotide sequence outside the kinase domain of an oncogene between avian and human species has also been reported in the case of the *c-src* gene; exon 3 in the human *c-src* gene could not be detected by cross-hybridization using the *v-src* sequence as a probe (8).

Ten exons of the human *c-ros-1* gene were identified within this 26-kb human DNA separated by 1- to 6-kb-long introns (Fig. 3a). The entire coding sequence and the predicted amino acid sequence of the human *c-ros-1* gene are shown in Fig. 4. The kinase domain of the human *c-ros-1* gene was found in the sequences from exon 4 to a part of exon 10 (Fig. 4). In these exons, the structure was highly



FIG. 5. Comparison of the amino acid sequences among the human *c-ros-1* gene, the chicken *c-ros* gene, and the HIR gene. Symbols: colon (:), identical amino acid; +, conserved amino acid; -, deletion of amino acid.

homologous to chicken *c-ros* and *v-ros* genes not only in the DNA sequence but also in the predicted amino acid sequence, except for exon 10 (Table 1, Fig. 5). Furthermore, the predicted products of the human *c-ros-1* gene and the chicken *c-ros* gene shared similar inserts of 2 to 5 amino acids (amino acid residues 252 to 254, 260 to 261, and 299 to 303) which were not present in any other members of the *src* family, and the splice junctions in these two genes were completely matched to each other (11). From these results, we conclude that the human *c-ros-1* gene is a cellular DNA homolog of *v-ros* in the human genome. Recent studies on the structure of the HIR gene have shown that the kinase domain of the HIR gene deduced from the cDNA sequence is more homologous to that of *v-ros* than it is to those of other *src* family members. However, by comparison of the

predicted amino acids of the human *c-ros-1* gene and the HIR gene, these two molecules were demonstrated to be clearly different from each other; homology in the level of amino acids in the kinase domain was 48.5% (Fig. 5).

In the overall structure, the human *c-ros-1* gene carried an extracellular domain with a potential site of N-linked glycosylation, a hydrophobic 24-amino acid stretch, and a tyrosine kinase domain. These structural organizations are similar to those of the *c-erbB* (the gene for epidermal growth factor receptor), the *c-fms* (the gene for macrophage colony-stimulating-factor receptor), and the HIR genes (5, 6, 14, 17, 18). These results strongly suggest that the human *c-ros-1* gene encodes for a transmembrane molecule which may function as a receptor for a cell growth or differentiation factor(s). Recently isolated transforming genes, *neulerbB2* and *oncD*, appear to be derived from the same category of receptor-type proto-oncogene (2, 4, 10, 19).

The biological function of the *c-ros* gene product and the significance of the *c-ros* gene in tumorigenicity in animals remain to be elucidated. Expression of the *c-ros* gene in 7- to 14-day-old healthy chickens was strongly repressed in many tissues, but two to three copies per cell of *c-ros* RNA were detected in kidneys by liquid hybridization and Northern blotting methods (11, 15). These results might indicate that the *c-ros* gene has a function in a limited stage of development or in a particular cell population in some tissue such as the kidney. Molecularly cloned human *c-ros-1* DNA may be very useful for examining the expression or abnormalities of this gene in normal or malignant tissues.

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TABLE 1. Homology between the human *c-ros-1* gene and the chicken *c-ros* gene in terms of nucleotide sequence and predicted amino acid sequence

Human <i>c-ros-1</i> exon no.	No. of nucleotides identical to chicken <i>c-ros</i> (n)	% Homology	No. of amino acids identical to chicken <i>c-ros</i> (n)	% Homology
1	121 ^a (191) ^b	63.4 ^a	32 ^a (63) ^b	50.8
2	44 (84)	52.4	9 (28)	32.1
3	81 (136)	59.6	29 (46)	63.0
4	121 (163)	74.2	42 (54)	77.8
5	55 (65)	84.6	19 (22)	86.4
6	102 (130)	78.5	34 (43)	79.1
7	68 (98)	69.4	24 (33)	72.7
8	156 (201)	77.6	57 (67)	85.1
9	96 (135)	71.1	29 (45)	64.4
10	100 (211)	47.4	25 (70)	35.7

^a This possible extracellular domain in the chicken *c-ros* gene is not present in the *c-ros* sequence reported by Neckameyer et al. (11) and has been sequenced in this study.

^b n, Total number of nucleotides or amino acids in each exon.

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