

Isolation and characterization of β -actin gene of carp (*Cyprinus carpio*)

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A β -actin gene of carp (*Cyprinus carpio*) was isolated from a genomic EMBL3 library. The nucleotide sequence of the gene indicates six exons spanning 3.6 kb. Southern blot hybridization of restriction endonuclease digests of carp genomic DNA indicate that there are two copies of the β -actin isotype and several other species of actin genes. The transcriptional start site is 85 bp and 24 bp downstream respectively from consensus CCAAT and TATA promoter elements. The organization of the carp β -actin gene is identical to that of chicken, human, and rat genes in terms of size, exon/intron locations and junctions and in having a translationally silent first exon. The fish gene is 90% and 99% conserved at the nucleotide and amino acid levels, respectively, with land vertebrate β -actin genes. Northern blot analysis of β -actin gene expression indicated that the gene is highly expressed in brain, less so in muscle, and much less so in liver cells. The putative β -actin proximal promoter of carp, identified by the conservation of known actin regulatory sequences, is transcriptionally active in both mammalian and piscine cells.

Key words: Genomic library, promoter, mapping, tissue-specific expression, evolutionary conservation

Actin is one of the most highly conserved proteins in evolution (Pollard and Cooper, 1986) due to its central role as the basic component of cellular microfilaments. At least six isoforms of actin have been identified in vertebrates. These include two more divergent cytoplasmic actins, β and γ , and four muscle-type α -actins (skeletal, cardiac, aorta-type smooth muscle, and stomach-type smooth muscle actins) (Vandekerckhove and Weber, 1979).

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Muscle-type actins are tissue specific and participate in muscle contractions. Cytoplasmic β - and γ -actins are expressed in many, if not all, tissue types, participate in a variety of cellular functions (Clarke and Spudich, 1977), and are more closely related to the actins found in lower eukaryotes than are the vertebrate muscle actins.

Owing to its role in establishing cell structure, actin proteins and their mRNAs are relatively abundant. This abundance suggests a strong transcriptional promoter (Gunning et al., 1987; Liu et al., 1990). We are interested in using the wide tissue specificity of the β -actin gene promoter in the construction of expression vectors for gene transfer into fish, a very useful model system for studies of gene regulation (Powers, 1989). Transparent fish eggs and embryos which normally develop outside of the female have several advantages for studying the cascade of gene expression at early stages of growth. Since the gene is apparently well conserved, and the promoter elements for several β -actin genes have been partially characterized (Frederickson et al., 1989; Ng et al., 1989) we initiated a search for the β -actin genes in two species of carp for which we had genomic libraries in order to obtain functional enhancer/promoter complexes.

Here we characterize the putative β -actin gene from the common carp (*Cyprinus carpio*) and compare its regulatory components and expression to that of other vertebrate actin genes. Our results indicate a surprising conservation of the regulatory sequences in the 5'-flanking and first-intron sequences to the

homologous sequences of land vertebrates and, as expected, the promoter elements are active in both fish and mammalian tissue culture cell lines. The organization of the gene into exons and the positioning of the protein coding sequences is nearly the same as in land vertebrates and the amino acid sequence of the carp β -actin gene 99% conserved between carp, mouse, humans and birds.

RESULTS AND DISCUSSION

Identification of a carp β -actin gene

A single recombinant clone, CA16, containing a 13.5 kb insert was isolated from a carp liver genomic DNA library by screening with a chicken cDNA probe. Restriction enzyme mapping and Southern-blot hybridization analyses were used to characterize the clone (fig. 1). The isolated clone contains sequences corresponding to the entire β -actin coding region plus 4.5 kb upstream sequence and about 5 kb of downstream sequence. Exons 2–6 were determined by homology comparison with the corresponding exons of the chicken β -actin gene using the Intelligenetics GENALIGN program. Exon 1 and intron 1 were determined by homology comparison of carp sequences with grass carp sequences (Liu et al., 1989) and by S1 nuclease mapping and primer extension (see p. 132).

Hybridization analysis of carp genomic DNA suggests the presence of multiple sequences related to the carp β -actin gene (fig. 2). *EcoRI*, *PstI*, *HindIII*, *BglII*, and *Sall* generates at least eight bands that hybridize with probes specific for the 5'-flanking and protein-coding portions of the gene (see fig. 1). In contrast, the 3'-specific probe hybridizes to only two DNA bands with the same restriction endonucleases. Of the bands shown in fig. 2, those corresponding to the CA16 clone are the 1.1 kb *HindIII*, 4.06 kb *HindIII* and 3.0 kb *PstI* fragments identified with the 5'-probe; the 4.06 kb *HindIII* fragment identified by the internal probe; and the 3.3 kb *HindIII*, 6.7 kb *Sall* and the triplet 4.7/5.6/6.6 kb *PstI* fragments identified by the 3'-probe. Identification of multiple non- β -actin genes with 5' and internal probes, but not with 3'-specific probes, is common for other vertebrate actin genes identified with β -actin probes (Nakajima-Iijima et al., 1985).

The 6.7 kb *Sall* fragment identified by the internal probe is very weak, presumably due to substantial methylation at this site. The triplet *PstI* fragments are due to incomplete digestion at the *PstI* site which forms the left-hand border of the internal probe. With the exception of the 6.7 kb *Sall* band, the genomic fragments homologous to the CA16 gene are relatively heavy in the blot shown in fig. 2. Bands which are more intensely labelled than these known bands presumably are composed of more than one hybridizing fragment. We suppose that the less intense bands

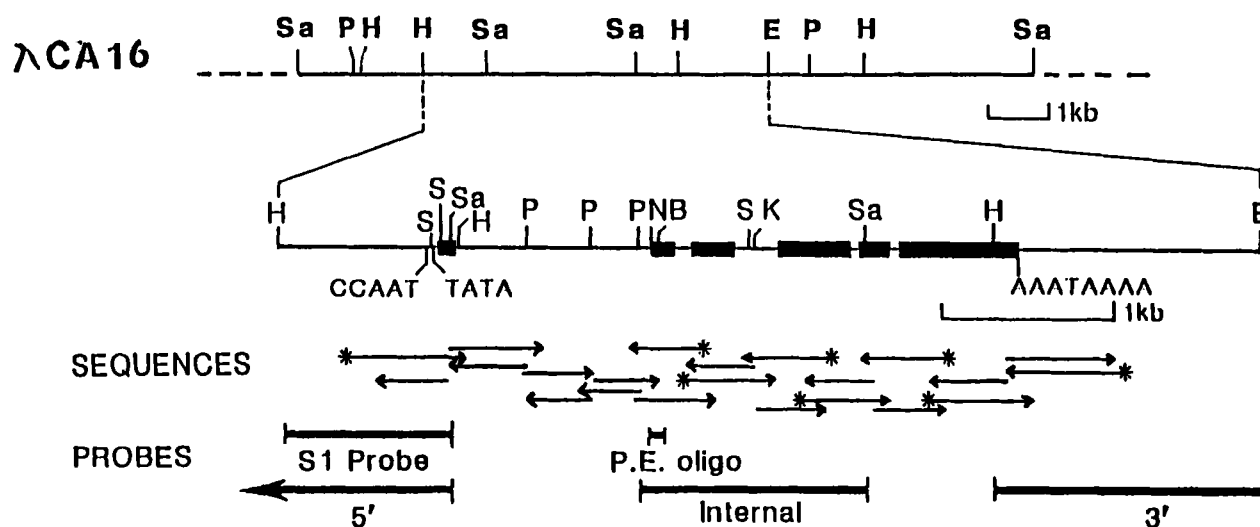


Figure 1 Map of the lambda (λ) CA16 clone of the carp β -actin gene. The top two lines show the restriction enzyme map of the β -actin gene. E, *EcoRI*; H, *HindIII*; K, *KpnI*; P, *PstI*; S, *SstI*; Sa, *Sall*. Positions of the CCAAT, TATA and AATAAAA transcription regulatory regions are indicated on the second line; note the change in scale. The sequencing strategy is indicated in the third set of lines; sequences beginning with *s were derived from clones made by the reverse cloning procedure (Liu and Hackett, 1989). The nuclease S1 (S1) and primer extension (PE oligo) probes used for mapping the transcriptional initiation site (see fig. 4) and the 5'-specific, internal, and 3'-specific probes used to count the number of genomic sequences homologous to the CA16 gene (see fig. 2) are shown at the bottom.

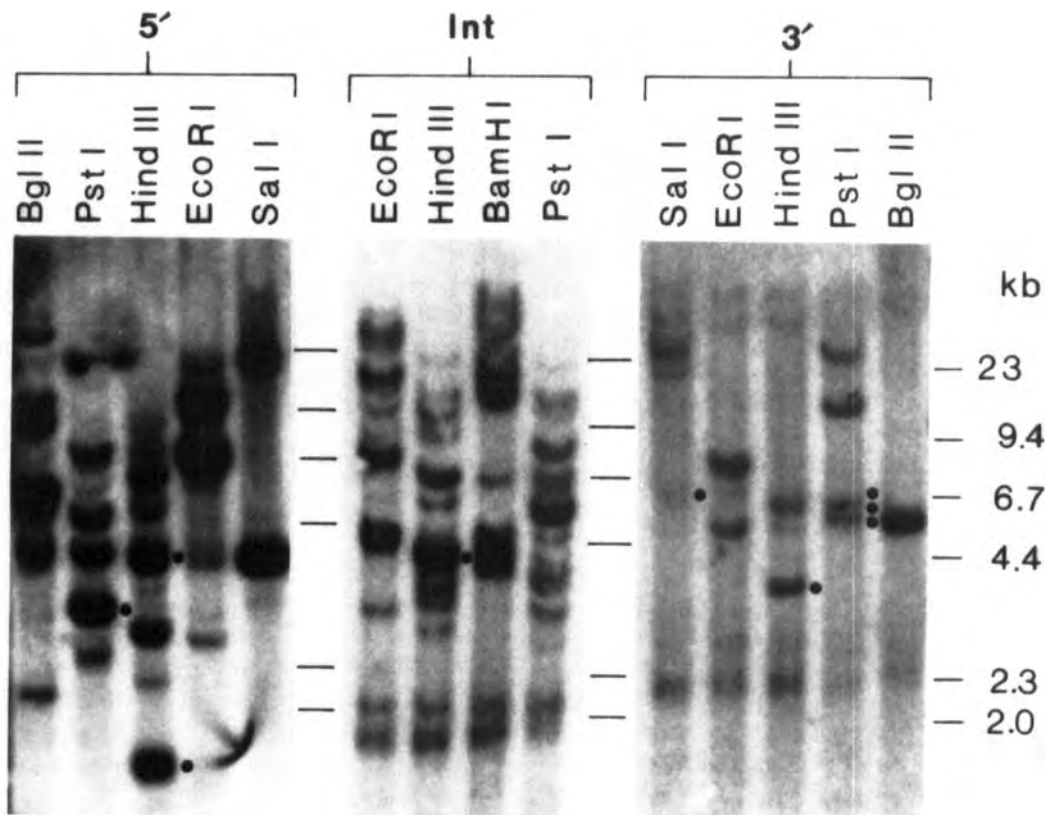


Figure 2 Southern hybridizations of carp β -actin gene sequences to carp genomic DNA cleaved with several restriction enzymes indicated at the top of each lane of the agarose gels. The left-hand gel was probed with the 5'-specific probe, the middle gel with the internal probe and the right-hand gel with the 3'-specific probe (probes are shown in fig. 1). The same gel was used for hybridization with the 5' and 3' probes; the middle gel was run in parallel. Lambda (λ) DNA cleaved with *HindIII* was used for size markers (right margin) which were visualized by ethidium bromide staining. The dots to the right of selected bands correspond to fragments derived from the CA16 β -actin gene clone.

come from fragments with partial length homology or with greater sequence divergence. Multiple copies of actin genes in vertebrates have been noted previously (Minty et al., 1983; Hightower and Meagher, 1986). The 5' and internal β -actin probes often hybridize to other actin genes such as those encoding α -actin and γ -actin (Erba et al., 1988) whereas 3'-actin probes display a greater specificity (Ponte et al., 1983; Yaffe et al., 1985; Kim et al., 1989).

Sequence of a putative carp β -actin gene

The nucleotide sequence of the presumed carp β -actin gene is given in fig. 3. The gene consists of 6 exons with the protein-coding sequence beginning close to the 5' end of exon 2, as is the case with the human, chick, and rat β -actin genes (Kost et al., 1983; Nudel et al., 1983; Nakajima-Iijima et al., 1985; Ng et al., 1985). The boundaries of exons 2–6 were defined by comparison with the chicken β -actin

sequence. Since the first exon for all vertebrate β -actin genes currently known are non-coding, the sequence conservation of exon 1 is relatively low. As shown in Table 1, all intron–exon boundaries are identical to those of chicken β -actin including those which diverge from the usual AG/GT motif in the donor site. At the intron-1/exon-2 junction, the carp sequence is like that of chicken (Kost et al., 1983) and not similar to that in the rat (Nudel et al., 1983). A 1.3 kb first intron divides the 5'-untranslated region and is located 90–91 nt downstream from the transcriptional initiation site, and 2 nt upstream of translational initiation codon AUG. In the chicken, human, and rat β -actin genes, the exon-1/intron-1 boundaries are located 91, 78, 74 nucleotides, respectively, downstream from their transcriptional initiation sites. The first intron of the β -actin gene is large in all species, 0.8 kbp in human, 0.9 kbp in chicken, and 0.8 kbp in rat. Introns 2–5 are all smaller in the carp β -actin gene than in the chicken gene.

Table 1 Sequence of carp β -actin gene organization

Exon	Intron	Size	Intron boundaries		
			5'	...	3'
1		90 nt			
	1	1264 nt	AG/GT	...	AG/CC
2		125 nt			
	2	106 nt	AG/GT	...	AG/GG
3		240 nt			
	3	303 nt	AG/GT	...	AG/AT
4		439 nt			
	4	93 nt	GG/GT	...	AG/GT
5		182 nt			
	5	101 nt	AG/GT	...	AG/AT
6		698 nt			
		778 nt*			

* depending on which AAUAAA polyadenylation signal is used.

Identification of exon-1 and the transcriptional initiation site

The transcriptional initiation site was determined as described in Materials and Methods (p. 134). A 23 oligonucleotide primer complementary to nts 75–97 of exon 2 (see fig. 3) was hybridized to total RNA from carp brain and muscle and extended with reverse transcriptase (fig. 4A). A 187/188 nt primer extension product was evident in RNA from brain cells (fig. 4A, lane 13) and two products of 187/188 nt and 156 nt were obtained with RNA from muscle cells (fig. 4A, lane 6). Since the 5' end of exon 2 was 74 nt upstream from the primer, the consistent 187/188 nt primer extension product suggests an additional 5'-untranslated exon of 90/91 nt is upstream of exon 2. A search of the 5'-upstream sequences revealed potential CCAAT and TATA promoter proximal elements plus a candidate 5'-exon with a 3'-splice site that corresponded to that seen in other vertebrate β -actin genes. Primer extension for the 187/188 nt product indicated that the transcriptional initiation site was at the CC residues (nts 210/211, fig. 3). The identification of that initiation site was confirmed by S1 nuclease analysis. The 1.1 kbp *HindIII/SalI* fragment, which overlaps the putative start point, was hybridized to carp brain total RNA, digested with nuclease S1, and the products were displayed on a sequencing gel (fig. 4B). The resulting 73 nt product indicated that the 5' end of exon-1 was at the C at position 211 in the sequence shown in fig. 3. A prominent S1 product of 47 nt was not observed with brain RNA, suggesting that the 156 nt primer extension product from muscle RNA was either an artifact, possibly by inhibition of elongation due to secondary structure of the 5' end of the RNA

(McKnight et al., 1982) in this reaction, or heterogeneity in the muscle RNAs (see below, fig. 8). Characteristic promoter elements are present in the 5'-flanking region of the gene (Wasylyk, 1988): a consensus TATAAA sequence is located at 22–27 nt, upstream from the transcriptional initiation site, and a CCAATC sequence was found at 84–89 nt upstream from the start site (see fig. 4). The results of primer extension and S1 mapping, taken together with phylogenetic comparisons of other β -actin genes, strongly indicate that transcription of the carp β -actin gene begins at position 211 on the sequence shown in fig. 3. The gene would direct the synthesis of an hnRNA precursor of 3.6–3.7 kb and an mRNA of about 1.8 or 1.9 kb, depending on which poly(A) site was used and assuming a poly(A) sequence of about 100 nt.

Comparison of carp, chicken, human, and rat β -actin genes

The nucleotide sequences of β -actin genes are very conserved through evolution. The evidence that the carp gene reported above is a true β -actin gene rather than some other actin isoform rests on four conserved homology regions:

- (1) The coding exons of the carp gene are very similar to that of other β -actin genes and less so with other non- β -actin genes (comparisons not shown); the strongest conservation in the coding region was found between carp and chicken; and slightly less conservation between carp and either the human or the rat genes (Table 2).
- (2) The conserved nucleotide sequence of the promoter region is very similar to that for chicken, human, and rat β -actin genes (fig. 5A) (Kost et al., 1983; Nudel et al., 1983; Nakajima-Iijima et al., 1985; Ng et al., 1985). In particular the CC (A/T)₆GG, or CArG sequence (Minty and Kedes, 1986), is found in all actin sequences including the carp gene promoter.
- (3) The 40 nt sequence immediately upstream of the AATAAA poly(A) signal, reportedly involved in the down-regulation of expression of the β -actin gene in mouse during myogenesis (Deponti-Zilli, Seiler-Tuyns and Paterson, 1988), is conserved (fig. 5C).
- (4) Two blocks of sequences of 20 nt and 16 nt are completely conserved in the introns of carp and other β -actin genes (Kawamoto et al., 1988; Ng et al., 1989) with 13 nt of divergent sequence in between (fig. 5B). In carp the conserved intron sequences are located 1055 nt downstream of the 5'-splice junction

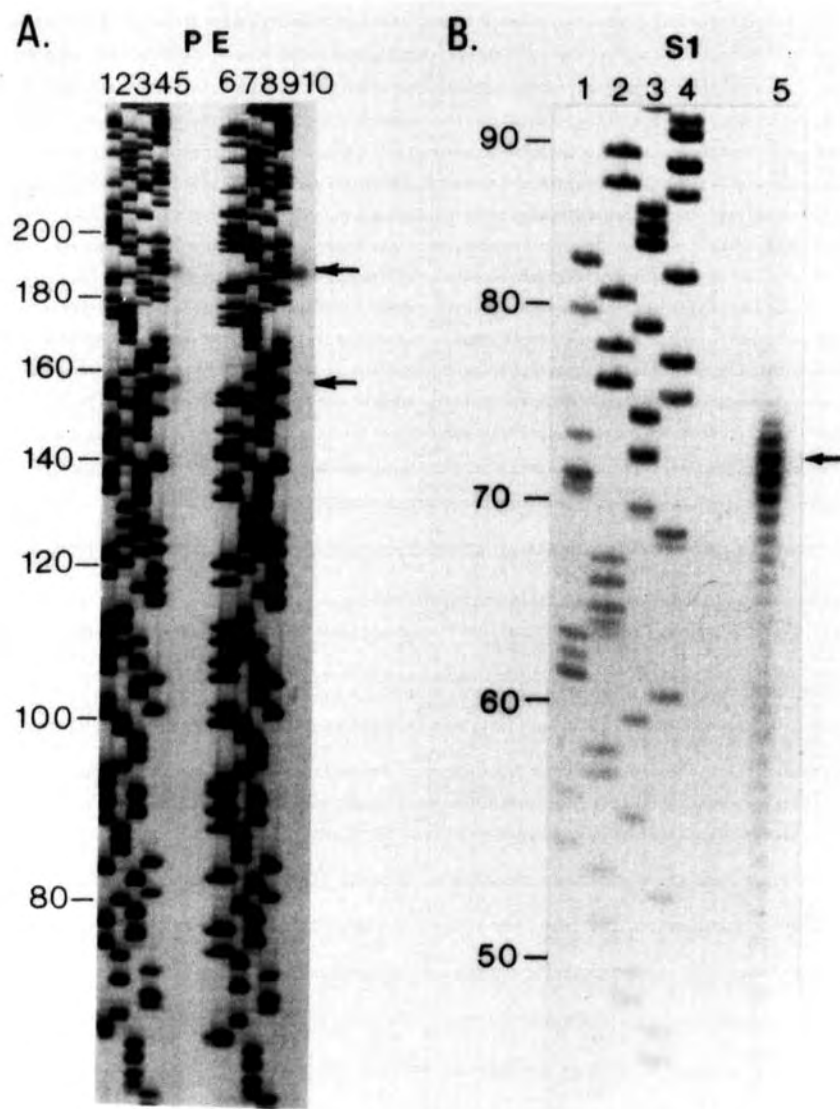


Figure 4 Transcriptional start-site mapping. The PE and S1 probe locations are shown in figs 1 and 3. (A) Primer extension of the 23 nt primer with total RNA from either muscle (lane 5) or brain (lane 10) was done as described in Materials and Methods. Two products were found with muscle RNA of 156 and 186/187 nt, whereas brain RNA gave just a single 186/187 nt product (arrows). Lanes 1–4 and 6–9 are Sanger sequence ladders used for size determination as indicated by the scale. (B) Nuclease S1 mapping with a uniformly-labelled DNA Probe (see Methods and Materials) hybridized to muscle RNA. Lanes 1–4, sequence ladder size markers.

and 161 nt upstream of the 3'-splice junction of intron-1. We have compared all the actin genes in the GENBANK and EMBL data bases and found that the conserved intron sequence only appears in β -actin genes. The considerable conservations of sequences in the 5'-flanking region, first intron, and 3'-untranslated region of the carp and other β -actin

Table 2 Conservation of selected vertebrate β -actin genes

Homology of	with	chicken (%)	human (%)	rat (%)
Carp nt sequences		91.0	88.5	87.9
Carp aa sequences		99.5	99.0	99.0

genes suggested that these sequences may be involved in regulation, perhaps tissue-specific or developmental stage-specific expression of the β -actins.

The carp β -actin gene proximal sequences direct transcription

The homology of the putative carp β -actin sequence with that of land vertebrates, the completeness of the protein coding region for the gene giving a protein 99% similar to those of other vertebrates, the conservation of all splicing junctions, and the identity of the presumed transcriptional control signals in the carp sequence with other, previously identified transcriptional control signals for β -actin genes was persuasive evidence that we had cloned a functional β -actin gene. To further verify our conclusion, we (1) used the derived carp sequence to design two primers for PCR amplification of carp cell mRNA; and (2) used the identified transcriptional control sequences to drive transcription of a reporter gene in transfected mouse and fish tissue culture cells.

As described in Materials and Methods (p. 134), we used a sequence complementary to the 3' end of the carp gene close to the first poly(A) signal and a second extending into the gene from the 5' end identified by S1 mapping and primer extension (see fig. 3) for PCR amplification (Frohman, Dush and Martin, 1988). The results using both 5 and 50 μ g of total RNA are shown in fig. 6. We were not able to collect RNA from the same common carp (Wuhan, China) as the source of the library; so we isolated RNA from common carp cells obtained from Dr Boaz Moav (Tel Aviv, Israel). The 5 μ g sample provided a clean, single band of about 1800 bp in length, the expected sum of the lengths of the exons between the two primers. The 50 μ g RNA samples reproducibly gave a poorer signal of the same size. The cDNA was cloned, sequenced and, to our surprise, showed several third base changes in the coding sequence (data not shown) but in all other ways confirmed our identification of the carp β -actin genes. We presume that the few changes in sequence were due to slight evolutionary drift in sequences between separate isolations of common carp fish in different localities. The PCR amplified sequence was cleaved with six restriction enzymes; *Pst*I and *Eco*RI did not cut the DNA, *Bam*HI, *Bgl*II, and *Hind*III cleaved once and *Sal*I cleaved twice. All fragments were of the expected size (see fig. 6).

To further validate the identity of the putative carp β -actin gene, we fused the 5'-flanking sequences of the isolated gene to the CAT reporter gene. The

fusions shown in fig. 7 indicate that the first 68 base pairs of exon 1 plus either 1100 or 193 nt of 5'-flanking sequence could drive CAT gene transcription in either mouse L-cells (see fig. 7) or carp cells (liu, unpublished) in tissue culture. Since the first exon does not have an AUG initiation codon, translation begins at the normal initiation codon for the chloramphenicol acetyltransferase. Cells were harvested 60 h after transfection and assayed for CAT activity (see Materials and Methods (p. 134). An equal amount of extract, determined by the Bradford (1976) procedure, was used in each assay. The assays were repeated five times and the averages for each construct are given. The results were the same in fish and mouse cells. The variation between experiments was

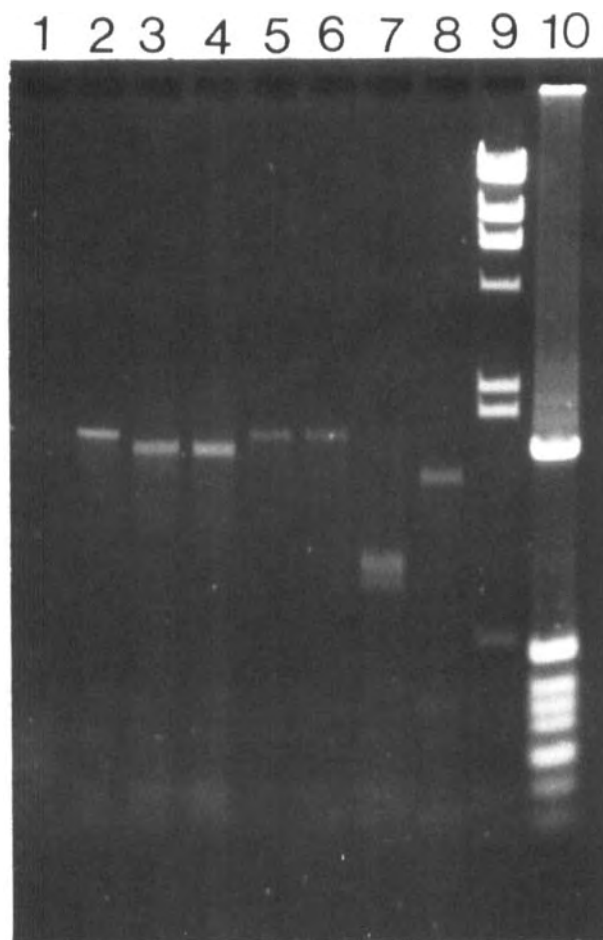


Figure 6 PCR amplification of total carp RNA with carp β -actin specific primers. Approximately 100 ng of isolated amplified DNA was examined by electrophoresis on an agarose gel with pBR322 cleaved with *Hinf*I (lane 10) and lambda (λ) cleaved with *Hind*III (lane 9) size markers. Lane 1, DNA from 50 μ g cytoplasmic RNA; lane 2, DNA from 5 μ g RNA; lanes 3–8, DNA from the 5 μ g RNA sample cleaved respectively with *Bam*HI, *Hind*III, *Pst*I, *Eco*RI, *Sal*I and *Bgl*II.

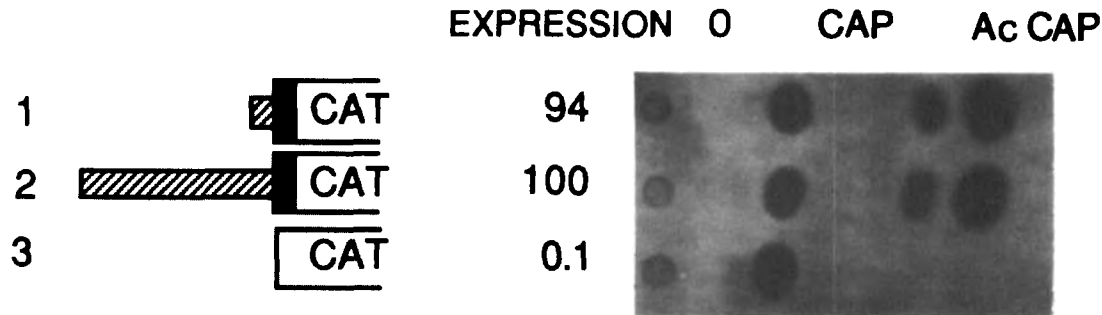


Figure 7 Transcriptional activity of carp β -actin proximal promoter sequences. Either just the CAT gene (construct 3) or two constructs (1 and 2) containing 5'-flanking sequences of the carp β -actin gene were fused at the *Sall* site in exon-1 to the bacterial CAT gene, cloned into the polycloning site of pUC119, transfected into mouse L-cells, and assayed for expression. Construct 1, 193 bp of flanking sequence plus 68 bp of exon-1 fused to CAT; construct 2, 1100 bp of flanking sequence plus 68 bp of exon-1 fused to CAT; construct 3 lacks all carp sequences. O, chromatographic origin; CAP, [14 C] chloramphenicol; AcCAP acetylation at carbon-1 (left spot); and carbon-3 (right spot). The relative expression values are the averages of at least five independent experiments, normalized to the average of construct 2.

less than 20% for each construct. The data indicate the following: (1) The putative carp β -actin gene promoter (constructs 1 and 2) is very active in tissue cultured cells, enhancing transcription 1000-fold above background (construct 3). (2) The first 193 base pairs of 5'-flanking sequence, containing the CCAAT, CArG, and TATA motifs conserved in all β -actin promoters (see fig. 5) are sufficient for dramatic initiation of transcription and are not affected by an additional 1000 bp of distal flanking sequence.

Tissue-preference expression of the carp β -actin gene

To determine relative levels of expression of actin mRNA, we first used the coding region of the carp β -actin gene as a probe (see fig. 1) to determine the expression of this gene. We found a 1.8 kb actin RNA band with liver RNA, one major 1.8 kb band and a minor 1.6 kb band with brain RNA, and a diffuse set of RNAs ranging between 1.3 and 1.8 kb from muscle RNA (fig. 8, left). For greater resolution of β -actin RNA expression we used the 3'-untranslated region (3'-UTR) of the β -actin gene which should be isotype-specific (see fig. 2; Ponte et al., 1983; Yaffe et al., 1985; Erba et al., 1988; Kim et al., 1989) and which should show the steady-state amounts of β -actin mRNA in the three different tissues. Total carp cellular RNA, 10 μ g, from each tissue was electrophoresed through a 1% agarose-formaldehyde gel, transferred to a nitrocellulose filter, and hybridized to the β -actin-specific, 3'-UTR probe. An autoradiograph of the filter is shown in fig. 8, right panel. The results clearly revealed the presence of a single 1.8 kb β -

actin mRNA in all tissues, both muscle and non-muscle (fig. 8, right), which was the upper band detected with the coding region probe (fig. 8, left). The level of β -actin varied between carp tissues. It was expressed highest in brain, intermediate in muscle, and lowest in liver. The autoradiogram was scanned with an optical densitometer. The expression in brain is 2.9-fold higher than in muscle, and 7.3-fold higher than in liver. These results are consistent with the report that the highest expression of β -actin in mouse is in the brain (Erba et al., 1988).

Conclusions

(1) A carp β -actin gene spanning 3.6 kb and containing 6 exons, as in all other vertebrate β -actin genes, has been isolated and sequenced. (2) There appears to be at least eight actin-like gene sequences in carp. (3) The carp β -actin gene shares strong sequence similarity with all other characterized vertebrate β -actin genes, not only in the coding region, but also in the promoter region, a portion of the first intron, and in the 3' non-translated region of exon 6. The 40 nt conserved region in the 3' untranslated sequence may mediate down-regulation of β -actin during myogenesis. The conserved sequences in the promoter region, as well as the first intron (Liu et al., 1990), have transcriptional promoter activity. (4) The carp β -actin gene is preferentially expressed in brain tissues while the expression in muscle and liver tissues is much lower. We are currently testing the use of the identified, conserved sequences in expression vectors for use in studies of gene expression in transgenic fish.

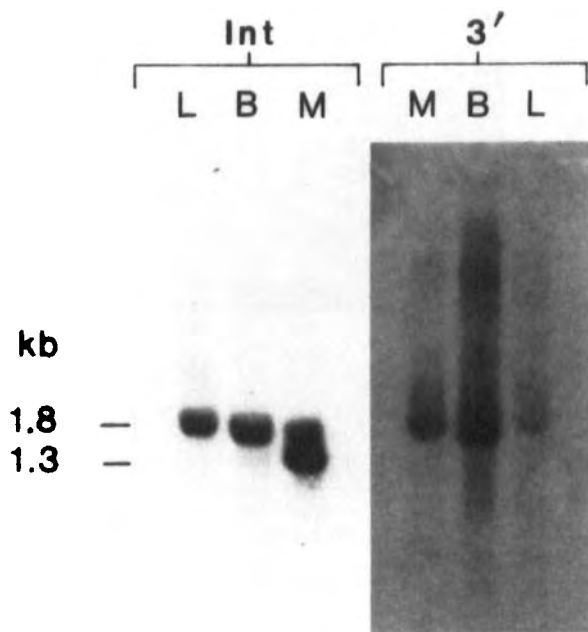


Figure 8 Northern hybridization of carp β -actin probes to total carp RNA from carp liver (L), brain (B) and muscle (M) tissues. The RNAs were probed with the internal (INT) and 3'-specific (3') probes shown in fig. 1, 5-fold more liver RNA was loaded when the internal probe was used in order to have a detectable signal. Sizing of the RNAs was determined using rRNA markers.

MATERIALS AND METHODS

Screening of the carp genomic library

A partial *Mbo*I library of carp liver genomic DNA fragments cloned in lambda (λ) EMBL3 phage (constructed at the Institute of Hydrobiology, Wuhan, China) was screened by filter hybridization to a chicken β -actin cDNA (Kost et al., 1983) probe. A single recombinant clone, CA16, was identified and isolated by repeated plaque purification (Kaiser and Murray, 1984).

Nucleotide sequencing

The restriction endonuclease sites of the 13.5 kb carp DNA insert in the CA16 clone were mapped. Nucleotide sequences were determined by the dideoxyribonucleotide chain-termination method (Sanger, Nicklen and Coulson, 1977) from overlapping restriction fragments as well as from overlapping fragments using the reverse cloning procedure (Liu and Hackett, 1989). Both strands of the 3995 bp sequence containing the gene were completely sequenced. Junctions between overlapping fragments were sequenced using bridge clones generated by reverse cloning (Liu and Hackett, 1989). Only one strand of the cDNA was sequenced since we already had both strands of the genomic DNA.

Determination of the transcriptional initiation site:

A 23 nt synthetic oligonucleotide probe, complementary to a phylogenetically conserved sequence at the end of exon 2,

beginning 70 nt downstream from the presumptive AUG start codon, was used to prime reverse transcription (Kingston, 1987; Williams and Mason, 1985) by AMV reverse transcriptase (Lifesciences Inc.) of total RNA from carp brains and muscles. The labelled cDNA product was subjected to electrophoresis through a 8% polyacrylamide in 8 M urea gel. The size of the extended primer was determined by comparison with a known sequencing ladder. S1 nuclease analysis was performed (Greene, 1987) to confirm the transcriptional initiation sites. A 1.1 kb fragment which overlaps the putative initiation site was continuously labelled (Greene, 1987) and hybridized to 20 μ g total brain RNA, digested with S1 nuclease (BRL) at room temperature or 30 $^{\circ}$ C as specified.

Southern-blot analysis

Carp muscle genomic DNA was prepared using standard procedures (Maniatis, Fritsch and Sambrook, 1982), digested with various restriction enzymes according to manufacturer's instructions, and subjected to electrophoresis on a 0.8% agarose gel. The DNA was transferred to a nitrocellulose filter (Southern, 1975) and hybridized to radiolabelled probes of 3' non-translated region, 5' non-translated region and coding region made by nick translation (Maniatis, Fritsch and Sambrook, 1982).

Northern-blot analysis

Carp RNA was prepared from muscle, brain, and liver by the method of Chomczynski and Sacchi (1987). Total RNA was electrophoresed through a 1% agarose formaldehyde gel and transferred to nitrocellulose filters for hybridization with labelled DNA probes in the presence of 50% formamide (Williams and Mason, 1985).

cDNA cloning and amplification by polymerase chain reaction

Total cellular RNA was isolated from tissue-cultured common carp cells obtained from Dr. Boaz Moav (Tel Aviv, Israel); RNA from the same common carp fish cells as the library was made was not available from Wuhan, China. The carp RNA was prepared as described above, and copied by the RNA-dependent DNA polymerase from avian myeloblastosis virus (Lifesciences, Inc.). Full-length cDNAs were amplified by polymerase chain reaction (PCR) using the 5'-specific (5'-CCTCACTTTGAGCTCCTCC-3') and the 3'-specific (5'-GGATGTCTTACATGTGCACTT-3') oligonucleotides according to the procedure of Frohman, Dush and Martin (1988). The PCR amplified DNAs were cloned into the pUC118 (Vieira and Messing, 1987), and transformed into *E. coli* JM101. The PCR-amplified clone was sequenced as described above.

CAT Analysis

An expression vector consisting of the pUC119 vector, the putative proximal promoter from the isolated β -actin gene, and the bacterial chloramphenicol acetyltransferase (CAT) gene from the pRSV-CAT construct of Gorman et al. (1982) was transfected (Lopata, Cleveland and Sollner-Webb, 1984) into both carp cells and mouse L-cells; 60 h after transfection, cell extracts were prepared for thin layer chromatography analysis of CAT enzymatic activity (Gorman et al., 1982). The radioactivity in the acetylated chloramphenicol spots was determined by cutting the spots off the thin layer plate and counting in a Beckman L1201 scintillation counter.

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