The Symmetrical Arrangement of Carp α - and β -Globin Genes

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The arrangement of carp α - and β -globin genes were examined at the nucleotide sequence level. Then, the nucleotide sequence of 1,249 base pairs (bp) for the 5' untranslated region of the carp β -globin gene was determined. This region showed a high homology with the previously reported carp α -globin gene No. 7. The overall nucleotide sequence homology of these two clones was 96.7%. DNA sequencing analysis indicated that the α -globin gene closely linked the β -globin gene. Additionally, the close linkage of carp α - and β -globin genes was proven by Southern blot hybridization and PCR amplification. The direction of the transcription of the α - and β -globin genes was the opposite. The length between the α - and β -globin gene translation initiation site was 792 bp and the nucleotides of this region was high in of A and T contents. Carp α - and β -globin genes show unique symmetrical arrangement.

Key words: α -globin gene, β -globin gene, carp, globin gene loci, evolution

Comparison of globin genes from mammalian, avian, and amphibian indicate that the gene family evolved from a single ancestral gene by duplication and sequence divergence.¹⁾ α - and β -Globin genes in mammals are arranged in two clusters on separate chromosomes.^{2–5)} The genes of mammals, the arrangements of globin genes for both α and β clusters are such that in the direction of transcription embryonic genes are 5' and adult genes are 3'. *Xenopus laevis* possesses two globin gene clusters, each containing a close linkage of tadpole and adult α - and β -globin genes.^{6–8)} This is very different from that in mammals.^{6–8)} Carp and amphibians diverged from the ancestor several hundred millions years ago. Therefore, we are very interested in the arrangement of α - and β -globin genes of fish which are derived from the same ancestral gene. Knowledge of carp globin sequences provides useful information concerning the evolution of the globin gene family. We have recently reported the cloning and determination of the nucleotide sequences of carp α - and β -globin genes of carp.^{9–12)}

Herein, we cloned a carp globin gene containing a close linkage of α - and β -globin genes.

Materials and Methods

DNA Manipulation

The previously cloned 3.4 kilo base (kb) *Eco*RI DNA fragment which was coded for the β -globin gene¹²⁾ was used in this study. This DNA fragment was subcloned into pUC118 and pUC119 with various restriction enzymes for further analysis. Sequencing was performed by the dideoxy termination method with T7 DNA polymerase.^{13,14)} DNA and predicted amino acid sequences were analyzed using a Genetyx computer program (SDC software, Tokyo).

Southern Blot Hybridization Analysis

Ten μ g genomic DNA of carp were digested to completion with *Eco*RI and *Pst*I, and used for Southern blot hybridization analysis.¹⁵⁾ DNA probes of carp α - and β -globin cDNA*³ were labeled with α -³²P-dCTP using a commercial random primer labeling kit (Takara Shuzo Co., Ltd., Kyoto, Japan). Hybridization proceeded under a highly stringent condition.¹⁶⁾

PCR Amplification

PCR was performed by a standard method using the following oligonucleotides; CBG5U: 5'-GCCTGTGGGG-AAAGCTCAACCCGAT-3' and CBG3U: 5'-TGAGA-ATCTAGTGGTACTGTCT-3' based on the beginning of the 1st exon coding region and ending on the 3rd exon coding region of β -globin. The seven clones, No. 1 to No. 7,^{9,10)} corresponding to the seven fragments of *Eco*RI-digested carp genomic DNA were used as templates for amplification. The reaction conditions were; 30 cycles with denaturation for 1 min at 94°C, annealing for 1 min at 55°C, and extension for 2 min at 72°C using Taq DNA polymerase according to the manufacturer's instructions. PCR products were analyzed by 0.8% agarose gel electrophoresis.

Results

The nucleotide sequences of the 5' upstream of β -globin gene was determined up to 1,249 nucleotides in length (Fig. 1). Figure 1 also shows the nucleotide sequence of the β -globin gene (1,250–2,694 bp), which was previously reported.¹²⁾ The α -globin gene is situated in the 5' upstream region of the β -globin gene. The coding region of exons 1, 2, and 3 was 95, 208, and 129 bp in length, respectively. The first and second intervening sequences were 179 and

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Fig. 1. Comparisons of the nucleotide sequences of the carp α -globin genes and a closely linked β -globin gene. CBG1, carp β -globin gene clone number 1; CAG7, carp α -globin gene clone number 7.

99 bp in length, respectively. When our determined gene was compared with previously reported carp α -globin genes which were from a carp genomic DNA library different from this study, carp α -globin gene No. 7 showed the highest homology. The overall nucleotide sequence homology of

protein coding regions in them was 98.8%. This result suggested that there is genetic polymorphism in the carp globin genes. The consensus sequences of TATA box and polyadenylation sites were observed in the 5'- and 3'-untranslated regions (Fig. 2).

Fig. 2. Schematic diagram of a symmetrical arrangement of carp α - and β -globin genes.

Restriction sites are indicated by vertical lines and letters: B, *Bam*HI; E, *Eco*RI; H, *Hind*III; S, *Sph*I. Transcriptional direction is indicated by arrows.

Fig. 3. Autoradiograph of hybridization of carp genomic DNA with α - and β -globin genes of carp.

Lanes 1 to 4 were hybridized with the carp α -globin cDNA. Lanes 5 to 8 were hybridized with the carp β -globin cDNA. Lanes 1 and 5: *Eco*RI-digested wild type carp genomic DNA. Lanes 2 and 6: *Eco*RI-digested German carp genomic DNA. Lanes 3 and 7: *Pst*I-digested wild type carp genomic DNA. Lanes 4 and 8: *Pst*I-digested German carp genomic DNA.

The continuous DNA sequence of a 2.7 kb region encompassed the linked α - and β -globin. The direction of transcription of the α - and β -globin genes were opposite. The promoter regions of the α - and β -globin gene contained a high ratio of contents A and T (68.17%).

The hybridization pattern of genomic DNA digested with *Eco*RI, with a carp α -globin cDNA probe was similar to that of hybridization with a carp β -globin cDNA probe (Fig. 3). The same molecular size DNA fragments from *Pst*I digested chromosomal DNA hybridized with each probe of carp α - and β -globin cDNA were observed (Fig. 3). These findings indicate the close linkage of the carp α - and β -globin genes.

The expected approximate 500 bp genomic DNA fragment encoding the bridge region between the 1st and 3rd exon of β -globin was amplified from all α -globin clone

Fig. 4. Agarose gel electrophoresis of PCR-amplified products from seven α -globin clones.

Lane M: Standard molecular sizes of λ -phage DNA digested with *Hind*III. Lane 1: α -globin clone No. 1, Lane 2: α -globin clone No. 2, Lane 3: α -globin clone No. 3, Lane 4: α -globin clone No. 4, Lane 5: α -globin clone No. 5, Lane 6: α -globin clone No. 6, Lane 7: α -globin clone No. 7, Lane 8: β -globin cDNA.

DNAs from No. 1 to No. 7 by PCR with oligomers CBG5U and CBG3U (Fig. 4). α -Globin genes of carp are found to be linked closely to a β -globin gene.

Discussion

Our determined region of the 5' upstream of the β -globin gene showed a higher degree of homology with the α -globin gene No. 7 among the previously reported carp α -globin genes No. 1 to No. 7.¹¹ The deduced polypeptide of the α -globin contains 142 amino acids. The orientation analysis of the β -globin gene revealed that this gene possessed only one intragenic *Bam*HI cleavage site, which was not found in the α -globin gene No. 7 (Fig. 2), whereas, the α -globin gene No. 7 containing an intragenic *Sph*I cleavage site was not present in the β -globin gene.

We cloned the α - and β -globin genes from the carp genomic library previously. However, the arrangement of these globin genes were not resolved. The α - and β -globin genes of the frog are closely linked to one another in the chromosome.^{6,7} *Xenopus laevis* possesses two globin clusters and the globin genes in the cluster appear to be oriented in the same direction relative to transcription. We have demonstrated a close physical linkage of carp α - and β -globin genes by genomic cloning. Hybridization of α - and β -globin cDNA probes to carp genomic DNA showed evidence of linkage of α - and β -globin genes (Fig. 3). They correspond to the same molecular size, as those isolated from different carp genomic DNA. Moreover, the linkage of carp α - β -globin genes was proved by PCR amplification (Fig. 4). The PCR products corresponding to β -globin genes were amplified from the previously described 7 α -globin gene clones.^{9,10} Recently, Atlantic salmon α - and β -globin genes were isolated by Wagner *et al.*¹⁷ Atlantic salmon α - and β -globin genes were also closely linked, and these were oriented tail to tail relative to each other with the RNA coding sequences. By contrast, carp α - and β -globin genes are oriented head to head relative to each other (Figs. 1 and 2). Wagner *et al.* cloned the minimum length of globin genes and analyzed it by PCR and DNA-DNA hybridization, but did not determined the nucleotide sequences of these genes. Our results were based completely on the nucleotide sequences, Southern blot

analysis, and PCR.

The close linkage arrangement and head to head orientation of the carp α - and β -globin genes are very interesting concerning the evolutionary relationship to vertebrate globin genes. The symmetrical arrangement of α - and β -globin genes in the carp provides evidence that vertebrate α - and β -globin genes evolved by tandem duplication of a single primordial globin gene. The present findings will facilitate not only the understanding of the evolution of the globin gene in vertebrates but also the analysis of the regulation of fish globin gene expression.

In conclusion, the symmetrical arrangement of carp α - and β -globin genes is fundamentally different from amphibian, avian, and mammalian globin gene organization.

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