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Tail-to-Tail Orientation of the Atlantic Salmon Alpha- and Beta-Globin Genes

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Abstract. We report the cloning of a cDNA and two corresponding β-globin genes of the Atlantic salmon (*Salmo salar* L.) as well as two genes for α-globins. Nucleotide sequence analysis of the cDNA shows that the predicted β-globin peptide comprises 148 amino acids with a calculated molecular mass of 16,127 Da and an overall amino acid similarity of 40–50% to higher vertebrates and 60–90% to fish sequences. The study of the genomic organization of α- and β-globin genes shows that, as is the case in *Xenopus*, the salmon genes are adjacent. Two sets of linked α- and β-globin genes were isolated and restriction-enzyme polymorphisms indicate that they belong to two distinct loci, possibly as a result of the salmon tetraploidy. In each locus the α- and β-globin genes are oriented 3′ to 3′ relative to each other with the RNA coding sequences located on opposite DNA strands. This is the first evidence for this type of arrangement found for globin genes. Moreover, while the linkage found in salmon and *Xenopus* supports the hypothesis of an initial tandem duplication of a globin ancestor gene, our results raise the question of the actual original orientation of the duplicated genes.

Key words: α-globin — β-globin — Atlantic salmon — Genomic organization

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Introduction

The study of globin genes in fish has been limited, to date, to the protein level (Wilkins and Iles 1966; Westman 1970; Powers and Edmunson 1972; Iuchi 1973; Brunari 1975; Giles and Randal 1980; Wilkins 1985; Giles and Rystephanuk 1989; Fyhn and Withler 1991). In the Atlantic salmon (*Salmo salar* L.), the relative percentage of certain α- and β-globin-type chains seemed to increase with time (Wilkins 1985). These changes are progressive rather than abrupt as in higher vertebrates. The possible reason for this specific regulation in fish was not understood and it was thought that the solution might be found at the molecular level in the details of the genomic organization of the fish globin genes.

The genomic organization of α- and β-globin genes is remarkably well conserved among the vertebrates. In fact, in all but two of the organisms investigated to date, globin genes are arranged in distinct clusters located on different chromosomes and defined as the α-like and β-like globin gene clusters. In each cluster, the genes are arranged in their temporal order of expression with genes expressed earlier in development located 5′ of the adult genes (Orkin and Kazanian 1984; Karlsson and Nienhuis 1985; Higgs et al. 1989; Orkin 1990; Fyhn and Withler 1991). The first exception to this type of organization was found in chicken, in which an embryonic β-globin gene is located downstream of the adult β-gene (Dodgson et al. 1979). The second exception is in *Xenopus*, where the α- and

β-genes are linked on the same chromosome. In this case, as in higher vertebrates, all of the genes are transcribed from the same strand (Jeffreys et al. 1980; Patient et al. 1980, 1982; Hosbach et al. 1983). In *X. laevis* and *X. borealis* two distinct loci of linked α- and β-globin genes have been identified. As only one such locus was found in *X. tropicalis*, it was suggested that the second locus appeared as a consequence of the tetraploidization event that the two former species underwent, perhaps 40–60 million years ago, but which did not take place in *X. tropicalis* (Jeffreys et al. 1980; Knochel et al. 1986; Stalder et al. 1988). The linkage of the genes in the *Xenopus* species supports the hypothesis of a tandem duplication of a single primordial globin gene about 500–570 million years ago, prior to the subsequent duplication and divergent evolution of the α- and β-globin clusters (Goodman and Moore 1975). Thus, if a single event is believed to be responsible for disrupting the α–β-globin-gene linkage, leading to the organization observed in mammals and birds today, it must have occurred in a common ancestor of both lineages after the Paleozoic divergence of amphibians and reptiles about 350 million years ago but before the appearance of the mammalian line perhaps 300 million years ago. Consequently all the lower vertebrates, including fish, should exhibit linked genes (Colbert 1969, Jeffreys et al. 1980; Knochel et al. 1986).

We have studied the genomic organization of the α-like and β-like globin genes in Atlantic salmon (*Salmo salar* L.) which, as an advanced bony fish and a teleostean, is a member of the most successful modern fish group. Two distinct loci of linked adult α- and β-like globin genes have been isolated. However, in contrast to the relative orientations of the genes in *Xenopus*, the α- and β-like globin sequences are arranged 3′ end to 3′ end in salmon with RNA coding sequences located on opposite DNA strands.

Materials and Methods

Preparation of a Probe Encoding a Partial Atlantic Salmon β-Globin cDNA. Sequence comparisons of the major adult β-like globin cDNAs from the African clawed frog (*Xenopus laevis*), bullfrog (*Rana catesbeiana*), chicken (*Gallus gallus*), rat (*Rattus norvegicus*), and human (*Homo sapiens*) were used to design two degenerate oligonucleotide primers for the PCR amplification of an Atlantic salmon β-globin cDNA fragment. All the sequences were obtained from Genbank and the sequence comparisons were performed by using the multiple alignment program clustalV (Higgins and Sharp 1988).

Primer 1:
5′-TA(C,T)CC(T,C)TGGACCCA(G,A)AG(G,A)T(A,T)(C,T)T-3′
(base 103 to 124 on the upper strand of the human chain starting from the initiation codon)

Primer 2:
5′-AAGTTC(T,A)(C,A)(A,T,G)GG(A,G)TCCAC(A,G)TG-3′
(bases 311 to 292 on the lower strand).

The bases in brackets represent the degenerate sites. PCR amplification of the salmon sequence was performed on an adult fish kidney cDNA library previously made in λGT10 (Wolff and Gannon 1988). PCR amplification was performed in Taq polymerase buffer (10 mM Tris-HCl pH 8.8, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100), using 100 pmol of each degenerate primer, 0.2 mM of each dNTP and 2.5 units of Taq polymerase in a volume of 100 μl. One or 4 cycles at the nonstringent annealing temperature of 37°C for 1 min were followed by 29 or 26 cycles, respectively, at an annealing temperature of either 42°C or 45°C for 1 min. The denaturation was performed for 1 min at 94°C and the elongation for 1 min at 72°C. The inserts of 4.10^6 plaque-forming units (pfu) from the λgt10 kidney cDNA library were first PCR amplified using oligonucleotide primers flanking the insertion site of the vector (λgt10 reverse and forward primers, Promega). The phages were first lysed by a 5-min incubation at 70°C followed by a rapid chilling to 0°C. The reaction was performed at an annealing temperature of 45°C for 1 min for 30 cycles in the same conditions as described before. After the amplification was completed, the DNA was phenol/chloroform extracted, ethanol precipitated, and resuspended in 50 μl of sterile water. Various amounts (1 μl, 2 μl, and 5 μl) of this template were used for the specific β-globin PCR amplification.

Screening of the Salmon Kidney cDNA Library. 1.5×10^5 pfu of the λgt10 kidney cDNA library were screened. Each plate was transferred in duplicate to a nylon membrane (HybondN, Amersham). The filters were baked for 2 h in a 80°C oven and prehybridized for 6 h at 65°C in 6 × SSC, 5 × Denhardt's, 0.5% sodium dodecyl sulfate, and 0.1 mg/ml calf thymus DNA. The hybridization was performed overnight at 65°C after addition of 10^6 cpm/ml of the 210-bp random primed (Brent et al. 1987) β-globin PCR fragment ([α-^{32}P]dCTP, 3,000 Ci/mmol, Amersham) using the Promega random prime kit. Unbound probe was removed by washing at the same temperature in 2 × SSC, 0.1% sodium dodecyl sulfate three times for 30 min. The stringency of the washes was further increased to 0.1 × SSC for 15 min. The two largest were subcloned in the *Eco*RI site of the Bluescript SK^- plasmid (Stratagene) and sequenced. The sequencing reactions were performed with [α-^{35}S]dATP, (6,000 Ci/mmol, Amersham) using the Pharmacia T7 sequencing kit. The cDNA was further subcloned using one internal *Bam*HI site, and bidirectional DNA sequencing was completed by using oligonucleotide primers specific to the sequence (Applied Biosystems). Sequence comparison analyses were performed by using the computer program clustalV (Higgins and Sharp 1988).

Genomic Southern Blot. Genomic DNA was prepared from the blood of a single adult Atlantic salmon by using a modified salting out method (Miller et al. 1988). Prior to the lysis of the nuclear membranes, the cytoplasmic membranes of red blood cells were lysed with a mild detergent solution (10 mM Tris-HCl pH 7.5, 10 mM NaCl, 2 mM $MgCl_2$, 0.5% (v/v) Nonidet-P40). The intact nuclei were recovered by centrifugation and treated as described in the protocol. In addition, a phenol/chloroform extraction step was carried out prior to ethanol precipitation of the DNA; 10 μg of DNA was digested overnight, electrophoresed through a 0.8% agarose gel, transferred bidirectionally onto two nylon membranes (HybondN, Amersham) and cross-linked by a 5-min exposure to UV. The specific Atlantic salmon α-globin probe was labeled by random priming of a 153-bp-long *Hin*fI restriction fragment of the α-globin cDNA (Wolff and Gannon 1988). Similarly, the PCR-amplified β-globin fragment was used as the second probe. Prehybridization, hybridization, and

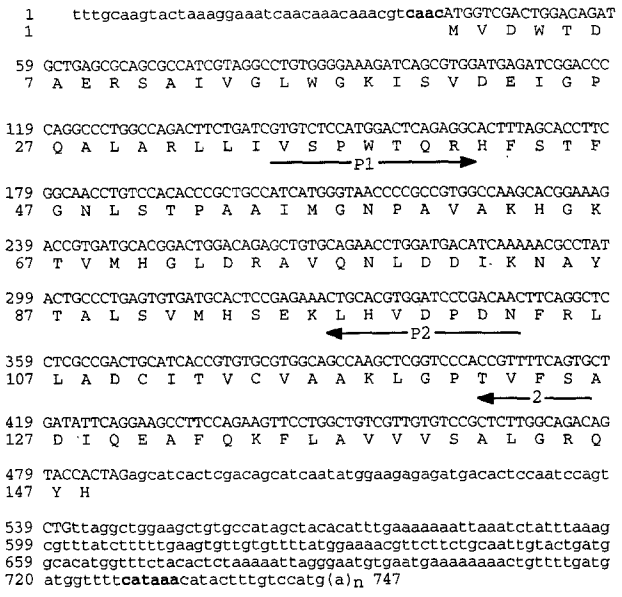

Fig. 1. Nucleotide and predicted amino acid sequences of the cDNA encoding the Atlantic salmon β-globin cDNA. The *upper line* shows the cDNA nucleotide sequence with coding sequence in *uppercase* and 5′ and 3′ untranslated sequences in *lowercase letters*. The deduced amino acid sequence is shown below. Nucleotide positions are *numbered* on the left top line and amino acid residues on the left bottom line. *Bold letters* represent the putative polyadenylation signal and initiator sequences. The positions of the degenerate oligonucleotides (P1, P2) and the PCR primer 2 (Brent et al. 1987) are similarly indicated. The orientation of the *arrows* gives the 5′ to 3′ orientation of the primers.

washes were performed at 50°C in the same conditions as for the cDNA library screening.

Screening of the Atlantic Salmon Liver Genomic Library. Some 1×10^6 pfu of a Sau3A partial digested Atlantic salmon genomic liver DNA constructed in the *Bam*HI site of the λDASH vector (kindly provided by G.A. Bannon) were screened separately with the adult salmon α- and β-like globin full-length cDNAs labeled by random priming. The hybridization conditions were the same as described for the screening of the cDNA library. Positive clones were digested by *Eco*RI, which releases the insert, and after Southern blot analysis the fragments were subcloned in the *Eco*RI site of pBluescript SK^-.

Restriction Mapping of the Genomic Clone Type I and II. Some 1–2 μg of two clones of type I and type II were digested using the restriction enzymes *Eco*RI, *Bam*HI, and *Hin*dIII and then electrophoresed through an 0.8% agarose gel. The same gel was transferred bidirectionally onto two HybondN membranes. The DNA was UV cross-linked for 5 min to the membrane and hybridized with the full-length α-like globin cDNA and β-like globin cDNA labeled by random priming (Wolff and Gannon, 1988). Hybridizations and washes were performed at 65°C as described above.

Determination of the Relative Orientation of the α- and β-Globin Genes. The plasmid primer SK and the universal M13 primer were used together with sets of primers specific for α- and β-globin cDNAs. These primers were designed from the known cDNA sequences for both genes (Wolff and Gannon 1988 and Fig. 1). For each gene, primers of opposite orientation were used together with SK or M13 universal primer: Primer 1: GGCACTTTAGCACCTT (nucleotides 167–183 on the coding strand) and Primer 2: ATACAGCACTGAAAAC (nucleotides 427–412 on the antiparallel strand) for β-like globin cDNA; Primers 3: CAGTCCGACAGCATTAC (nucleotides 273–257 on the antiparallel strand) and Primer 4: ATGGGTGCAATTGGTAAT (nucleotides 244–261 on the coding strand) for α-like globin cDNA. PCR amplification was performed in 100-μl reactions. (1 × Taq buffer, 0.2 mM dNTPs, 100 pmol each primer, 2.5 units Taq polymerase) for 30 cycles, at an annealing temperature of 42°C for primers 1 or 2 plus SK and at 50°C for primers 3 or 4 plus M13 universal primer. (See Fig. 3.) Ten microliters of the PCR reaction was loaded on a 1.5% agarose gel. Gel transfer and hybridization were performed as described above. Negative controls using water instead of template DNA were included in each amplification reaction set.

Results

Isolation and Sequencing of an Atlantic Salmon β-Globin cDNA

Since no fish β-globin cDNA sequences other than carp were available at the start of this project, the PCR amplification of a 210-bp β-globin cDNA fragment was achieved by using two degenerate primers designed from nucleotide sequence comparisons from different species of higher vertebrates. The PCR product was used as a probe to screen the λgt10 adult salmon kidney cDNA library (Wolff and Gannon 1988). From the 1.5×10^5 plaques screened in duplicate, 13 positive clones were isolated. The two largest inserts were subcloned in pBluescript SK^- and sequenced. Both sequences were identical. The sequence of the largest clone is shown in Fig. 1. The cDNA is 745 bp long and encodes a 148-amino-acid peptide (including the initiator Met) with a calculated overall molecular mass of 16,127 Da and a pH_i of 6.12. The results of sequence comparisons at the peptide level are given in Table 1. The Atlantic salmon sequence shows a 96.6% similarity with the β-globin component of rainbow trout hemoglobin IV (HbIV). The similarity is, however, surprisingly lower—59.6%—with the β-globin chain of rainbow trout hemoglobin I (HbI), which is characterized by its lack of Bohr effect (Barra et al. 1983; Petruzzelli et al. 1984). The percentage of similarity decreases when compared to fish less related to salmon and to other species. The length of 5′ untranslated sequence which is well conserved in mammals (between 50 and 56 nucleotides; Collins and Weissman 1984; Orkin and Kazanian 1984) is about 75 bases in the salmon mRNA as determined by primer extension analysis (data not shown). The 3′UTR (260 base pairs) of the isolated salmon cDNA is quite long as compared to other species and does not exhibit a strict consensus polyadenylation signal. One potential polyadenylation signal

Table 1. Comparisons of the salmon β-globin amino acid sequence with the major adult sequences in other vertebrate species (%)[a]

Rainbow trout									
βI	βIV	Goldfish	Carp	Tuna	Shark	*X. laevis*	Chicken	Rat	Human
59.6	96.6	76.7	71.4	62.3	37.1	40	54.8	50.7	48.6

[a] Rainbow trout (*Oncorhynchus mykiss*) (Barra et al. 1983; Petruzzelli et al. 1984) goldfish (*Carassius auratus*) (Rodewald and Braunitzer 1984), carp (*Cyprinus carpio*) (Grujic-Injac et al. 1980), tuna (*Thunnus thynnus*) (Rodewald et al. 1987), shark (*Heterodontus portusjacksoni*) (Fisher et al. 1977), *Xenopus laevis* (Williams et al. 1980), chicken (*Gallus gallus*) (Dolan et al. 1983), rat (*Rattus norvegicus*) (Rodosavlejic and Crkvenjakov 1989 and human (*Homo sapiens*) (Marotta et al. 1977)

(CAUAAA) is located 16 bases upstream of the polyA tail present in the cDNA clone.

Genomic Southern Blot Analysis

To initiate the analysis at the genomic level, DNA isolated from red blood cells was digested with the restriction enzymes *Sac*I, *Hin*dIII, and *Eco*RI. None of these enzymes cut inside the isolated cDNA sequences. After migration, the gel was transferred and the blot was hybridized either with α- or β-globin salmon cDNA fragments. The α-globin probe was a 153-bp-long *Hin*fI restriction digest fragment of the cDNA, and according to the genomic organization in other species, it should contain most of the third exon of the gene. The 210-bp-long probe for β-globin corresponds to most of the second exon of the β-globin gene. The hybridization showed some bands which hybridized with both probes (Fig. 2): a 9 kb common band could be seen in the *Sac*I lane, as well as a 4.4-kb band in the *Hin*dIII and three (7-kb, 5–5.5-kb, and 3.7-kb) bands in the *Eco*RI lanes. Although the common band sizes could be due to different DNA fragments, this result suggests that the Atlantic salmon α- and β-globin genes were adjacent to each other on the chromosome.

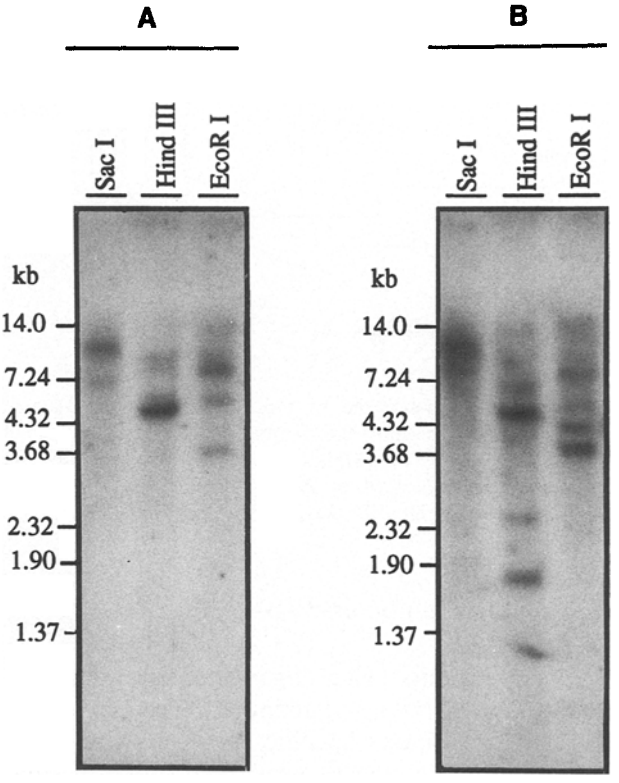

Fig. 2. Southern blot analysis of genomic salmon DNA. Salmon red-blood-cell DNA was digested with the restriction enzymes shown and after electrophoresis blotted bidirectionally onto two nylon filters. One filter was then hybridized to the [α^{32}P]dCTP random primed *Hin*fI fragment of the α-globin cDNA (panel **A**) while the second was hybridized to the PCR-amplified β-globin fragment (panel **B**). Both hybridizations were performed as described in Materials and Methods.

Isolation of the Atlantic Salmon α- and β-Globin Genes

To clone the adult α- and β-globin genes, an Atlantic salmon genomic library was screened in parallel with the corresponding globin cDNAs. Seven different positive clones were isolated. Among these five were positive for the two probes—one hybridized only with the α-globin and one detected only with the β-globin cDNA. After purification and *Eco*RI digestion of lambda clones, the fragments positive for both probes were identified and subcloned into plasmid. Further restriction analysis allowed the division of these subclones in two types different both in length and restriction map. The restriction map of both types for *Bam*HI, *Hin*dIII, and *Hin*cII is represented in Fig. 3. Southern blot analysis using α- and β-globin probes of different length confirmed this result and showed that neither clone contains the first exon of the β-globin gene. According to the approximate size of the two clone types, 4.5 kb and 5.5 kb, respectively, both might correspond to the 5–5.5-kb band on the *Eco*RI digest of the genomic Southern (Fig. 2). The fact that for each clone type the α- and β-globin probes bind to some common bands confirms the close linkage of the two genes on the same chromosome.

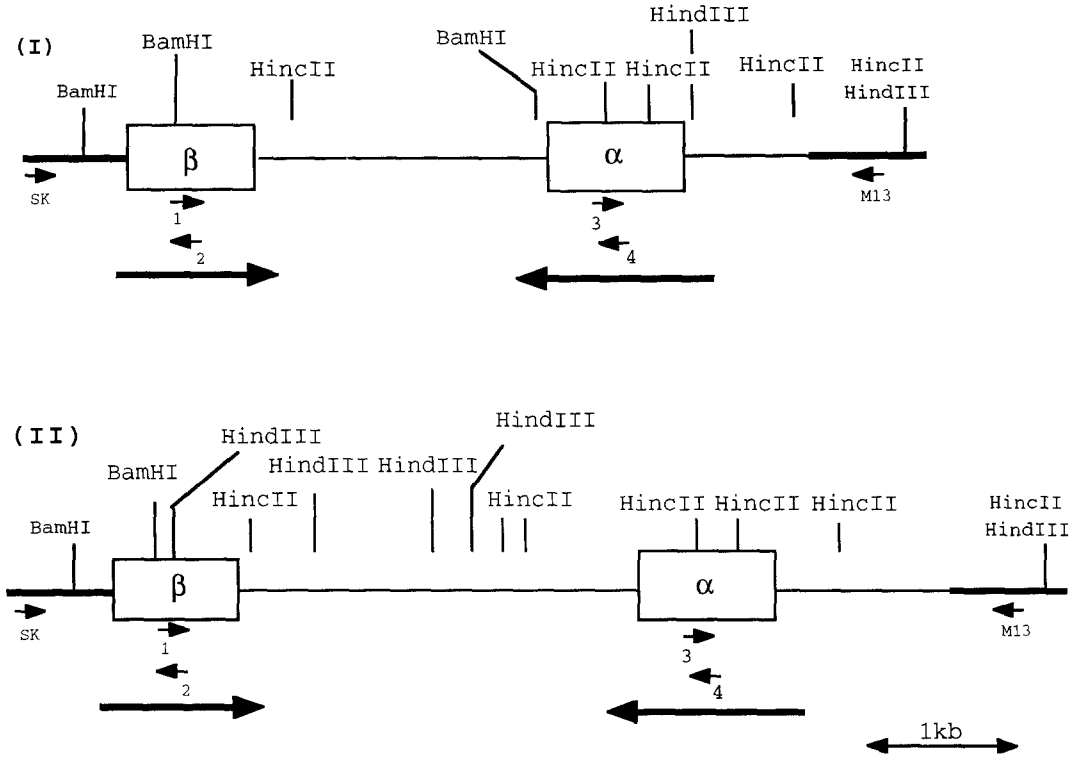

Fig. 3. Organization of the two types of linked α- and β-globin genes. The diagrams show the size and partial restriction map of the two clone types. Type (I) is 4.3 kb in length and contains two *Bam*HI and one *Hin*dIII site. Type (II) is 5.2 kb in length, contains four different *Hin*dIII sites, and lacks one *Hin*dIII and one *Bam*HI site present in type I clones. The vector is represented by a *thick line*. α- and partial β-globin (starting in the first intron) genes are shown as *boxes*. *Small arrows* mark the orientation and approximate location of the oligonucleotide primers used while *large arrows* give the transcription direction.

Relative Orientation of the α- and β-Globin Genes

PCR experiments using oligonucleotide primer sequences in the vector flanking the insert in combination with primers specific to the α- and β-like globin cDNA sequences were performed to determine the relative orientation of the genes in both clone types. (See Fig. 3.) For each gene, oligonucleotide primers of opposite orientation were tested with primers for the flanking plasmid sequences. No amplification was observed with primers 1 and SK or 4 and M13 universal primer. One band of approximately 590 bp was amplified from clone type I when primer 2 was used with SK (Fig. 4A). Using the same primers, a slightly smaller band of 540 bp was observed for clone type II. Relative to the cDNA sequence (see Fig. 1), the sizes of both fragments indicate that the two clone types contain the full second exon of the β-globin gene, and since they do not contain the first exon, they must start inside the first intron. PCR amplification with the primer 3 and the M13 universal primer gave products of about 1.4 kb and 1.8 kb for clone types I and II, respectively. The specificity of the products was verified by hybridization with either adult α- or β-like globin cDNAs as probes (Fig. 4B, C, respectively). Given the orientation of the different primers in the cDNA sequences, it follows that the adult α- and β-like globin genes are linked in a 3′-end-to-3′-end orientation with the RNA coding sequences located on opposite DNA strands for both clone types.

Discussion

We report the cloning of a cDNA for a salmon β-globin as well as two sets of genes for α- and β-globins. The 3′UTR of the β-globin cDNA does not contain the consensus polyadenylation signal AAUAAA. Instead the sequence CAUAAA is found 16 bp upstream of the polyadenylation tail. Comparisons of vertebrate cDNA sequences showed that this mutated sequence occurs at a frequency of 2% and yields only 10–20% of the polyadenylation activity of the consensus signal (Sheets et al. 1990; Wickens 1990). In human globin genes, two different point mutations have been described. In the first case, the β-globin gene, the mutated AACAAA sequence leads, in vivo, to a polyadenylation 900 bp downstream of the normal site at the next AAUAAA sequence in the 3′ flanking region

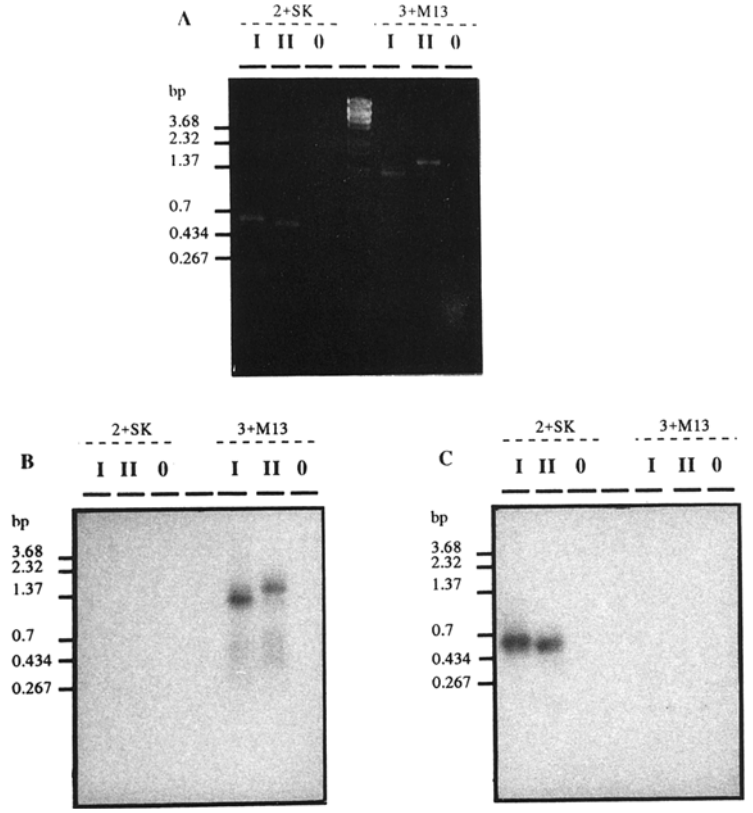

Fig. 4. Definition of the relative orientation of the α- and β-like globin genes in clone types I and II. **A** Ethidium-bromide-colored gel before bidirectional transfer. *Lanes I, II,* and *O* contain, respectively, 10 μl of the PCR amplification reactions performed on DNA from clone type I, type II, and on water using the two sets of oligonucleotide primers 2 + SK and 3 + M13 universal primer. The *middle lane* contains the molecular weight DNA marker. **B** Autoradiogram of the Southern blot probed with the [α-^{32}P]dCTP random-primed full-length salmon α-globin cDNA **C** and β-globin cDNA. Hybridizations were performed under high-stringency conditions as described in Materials and Methods.

(Orkin and Kazanian 1984). The second case, in a human α_2-globin gene from a patient with α-thalassemia, the polyadenylation signal was mutated into AAUAAG and the gene was expressed at reduced level (Higgs et al. 1989). In the salmon β-globin cDNA we isolated, polyadenylation occurred even without any consensus polyadenylation signal; the impact of the variant signal and the generality of its occurrence in β-globin in salmon are unknown.

The sequence comparisons between the salmon and the rainbow trout β-globin components of HbI and HbIV clearly show that the salmon cDNA isolated encodes a peptide belonging to the Bohr-effect-type chains (Barra et al. 1983; Petruzzelli et al. 1984). This observation is further supported by the conservation of the amino acid residues most strongly involved in that effect, which are the amino-terminal histidine (β-147 relative to the human chain when including the initiator Met), tyrosine (b146), and arginine (b31). The glutamic acid in position 95 of the human chain is conservatively changed in salmon to an aspartic acid (Perutz 1970).

Although the cDNA was isolated from a library constructed from adult fish tissue, it will remain uncertain whether it is a specifically adult-stage expressed mRNA until more analyses at the protein level are performed. In contrast to all other species studied to date, the shifts in hemoglobin type in fish blood are neither abrupt nor restricted to a discrete period of their development; they seem instead to be gradual, with levels of the different hemoglobins rising or diminishing progressively as the fish increases in size (Powers and Edmunson 1972; Giles and Randal 1980; Wilkins 1985; Giles and Rystephanuk 1989; Fyhn and Withler 1991). Some recent studies on the human β-globin cluster have suggested that the relative position of each gene inside the cluster might be involved in the sequential expression of fetal, embryonic, and adult genes. In this context, the tail-to-tail orientation reported here for salmon may be, at least partially, involved in the different kind of developmental regulation of globin gene expression which seems to exist in salmon. In addition, since tissue-specific enhancer sequences have been located in the 3′ region of β-like globin genes in human and chicken, it will be interesting to assess whether this type of sequence is present in salmon, especially if it is shared between the linked α- and β-like globin genes in both clone types (Choi and Engel 1986; Emerson et al. 1987; Kollias et al. 1987; Nickol and Felsenfeld 1988; Purucker et al. 1990).

So far, the only other vertebrate species for which linked α- and β-globin genes have been isolated is *Xenopus* (Jeffreys et al. 1980; Patient et al. 1980). In *X. laevis* and *X. borealis,* which have been through a tetraploidization step, as opposed to *X. tropicalis,* which has not, two such types of clusters

were found (Knochel et al. 1986; Stalder et al. 1988). The facts that only one cluster was found in *X. tropicalis* and that the gene arrangement was almost identical between the two loci in *X. laevis* and *X. borealis* strongly favored the hypothesis of tetraploidization through genome duplication in the latter species. In each cluster, the linked adult α- and β-globin genes are flanked by the corresponding larval genes with the same transcriptional polarity (Hosbach et al. 1983). The intergenic distance between the adult α- and β-globin genes is about 8–9 kb (Jeffreys et al. 1980; Patient et al. 1980; Hosbach et al. 1983). We show in this report that in Atlantic salmon, the distance between the two genes is approximately 2 kb in type I clones and 2.5 kb in type II clones as determined by restriction-enzyme analysis and PCR amplification. Both types of clones show marked restriction-enzyme polymorphisms and significant variations between homologous sequences by partial sequence analysis (result not shown), which suggests that they correspond to different loci—possibly as a consequence of the tetraploidy in salmon.

The size of the α-globin gene as defined by restriction mapping is approximately 1 kb, which is in the size range of human and *Xenopus* α-globin genes (Stalder et al. 1988; Higgs et al. 1989). Southern blot analysis and PCR amplification experiments showed that the two isolated loci contain only 0.8-kb partial β-globin genes starting inside the first intron of the gene. Thus the size of the second intron appears to be small as compared to amphibians, where it ranges from 0.85 kb to 0.97 kb (Patient et al. 1980, 1982; Hosbach et al. 1983).

In terms of globin gene evolution, the fact that the α- and β-like globin genes are linked in salmon and *Xenopus* supports the hypothesis of an initial tandem duplication of the globin ancestor gene about 500–570 million years ago (Goodman and Moore 1975; Jeffreys et al. 1980; Knochel et al. 1986). However, it raises the question of whether or not this corresponds to the original form of the duplicated ancestor gene. Recombination events which gave rise to duplications, insertions, deletions, and even inversions in the globin gene clusters have been well documented in human and appear to be quite common (Orkin and Kazanian 1984; Karlsson and Nienhuis 1985; Higgs et al. 1989). The displacement of the chicken fetal β-globin 3′ of the adult gene might be another example of this kind of rearrangement (Dodgson et al. 1979). So far, however, except for one inversion of an intergenic sequence found in a human β-thalassemia (Jones et al. 1981), the 3′-end-to-3′-end orientation described here for Atlantic salmon is the only example of this kind for globin genes. Another example of change of orientation of genes within gene clusters is given by the difference of histone gene organization between sea urchin and *Drosophila*. In sea urchin the histone genes consist of tandem repeats and are transcribed from the same strand whereas in *Drosophila* three genes are encoded on one strand and two other genes are encoded on the other strand (Maxson et al. 1983; Lifton et al. 1977).

To determine whether the tail-to-tail orientation of the α- and β-globin genes that we report here is restricted to salmon, it will be necessary to characterize the globin gene arrangement in other species such as the cartilaginous fish, which diverged from the bony fish during the Ordovician era, as well as in more recent forms such as coelacanths and lungfish.

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