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Genomic remnants of α-globin genes in the hemoglobinless antarctic icefishes

(notothenioid fishes/rockcods/dragonfishes/β-globin gene deletion or divergence)

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ABSTRACT **Alone among piscine taxa, the antarctic icefishes (family Channichthyidae, suborder Notothenioidei) have evolved compensatory adaptations that maintain normal metabolic functions in the absence of erythrocytes and the respiratory oxygen transporter hemoglobin. Although the uniquely "colorless" or "white" condition of the blood of icefishes has been recognized since the early 20th century, the status of globin genes in the icefish genomes has, surprisingly, remained unexplored. Using α- and β-globin cDNAs from the antarctic rockcod *Notothenia coriiceps* (family Nototheniidae, suborder Notothenioidei), we have probed the genomes of three white-blooded icefishes and four red-blooded notothenioid relatives (three antarctic, one temperate) for globin-related DNA sequences. We detect specific, high-stringency hybridization of the α-globin probe to genomic DNAs of both white- and red-blooded species, whereas the β-globin cDNA hybridizes only to the genomes of the red-blooded fishes. Our results suggest that icefishes retain inactive genomic remnants of α-globin genes but have lost, either through deletion or through rapid mutation, the gene that encodes β-globin. We propose that the hemoglobinless phenotype of extant icefishes is the result of deletion of the single adult β-globin locus prior to the diversification of the clade.**

In 1954 Ruud (1) published the first systematic analysis of the "white" blood of an antarctic icefish, *Chaenocephalus aceratus*. He reported that fresh blood was nearly transparent, contained leukocytes at 1% by volume, but lacked erythrocytes and the respiratory transport pigment hemoglobin. Furthermore, the oxygen-carrying capacity of *C. aceratus* blood was approximately 10% that of two red-blooded notothenioids. Subsequent investigations extended these observations to other icefish species and revealed that icefish blood contains small numbers of "erythrocyte-like" cells that, nevertheless, are devoid of hemoglobin (2, 3). Thus, limited to oxygen physically dissolved in their blood, the icefishes have evolved compensatory physiological and circulatory adaptations—e.g., modest suppression of metabolic rates, enhanced gas exchange by large, well-perfused gills and cutaneous respiration, and large increases in cardiac output and blood volume—that ensure adequate oxygenation of their tissues (4, 5).

We have initiated studies to determine the status of globin genes in channichthyid genomes and to evaluate potential evolutionary mechanisms leading to the hemoglobinless phenotype. Icefishes evolved from the red-blooded Notothenioidei (6, 7), which, in contrast to temperate fishes, are characterized by a paucity of hemoglobin forms (7–10). Adults of the family Nototheniidae (antarctic rockcods) generally possess a major hemoglobin, Hb 1 (≈95% of the total), and a second, minor hemoglobin, Hb 2, that differ in their α chains (α1 and α2, respectively) (11–13). The more phyletically derived harpagiferids and bathydraconids have a single hemoglobin. The trend toward reduced hemoglobin multiplicity in the notothenioid suborder, which reaches its extreme in the icefishes (the sister group to the bathydraconids), probably results from evolutionary loss or mutation to transcriptional inactivity of globin genes. To investigate these possibilities, we have cloned cDNAs encoding the globin chains (α1 and β) of Hb 1 from the red-blooded rockcod *Notothenia coriiceps*.§ Using these probes, we find that three icefish species, representing both primitive and advanced genera, retain inactive α-globin-related sequences in their genomes but apparently lack the gene for β-globin. Our results suggest that loss of the single adult β-globin gene occurred prior to the diversification of the icefish clade.

MATERIALS AND METHODS

Fishes. Specimens of two antarctic rockcods (*N. coriiceps* and *Gobionotothen gibberifrons*), a dragonfish (*Parachaenichthys charcoti*), and three icefishes (*C. aceratus, Champsocephalus gunnari*, and *Chionodraco rastrospinosus*) were collected by bottom trawling from the R/V *Polar Duke* near Low and Brabant Islands in the Palmer Archipelago. They were transported alive to Palmer Station, Antarctica, where they were maintained in seawater aquaria at −1 to +1°C. A testis from the temperate New Zealand black cod, *Notothenia angustata*, was the gift of A. L. DeVries (University of Illinois, Urbana).

Library Construction and Screening. Cytoplasmic RNA was purified from erythrocytes of *N. coriiceps* (14, 15), and poly(A)$^+$ RNA was selected by affinity chromatography on oligo(dT)–cellulose (16). $(dT)_{12-18}$-primed, double-stranded cDNA was synthesized from the poly(A)$^+$RNA (17), the cDNA was ligated by means of *Eco*RI adapters into the phagemid pT7T3 18U (Pharmacia), and a library was generated by electroporation-mediated transformation (18) of competent *Escherichia coli* NM522 cells with the recombinant phagemid stock. The library (3×10^5 transformants per μg of cDNA) was screened for clones containing α- or β-globin cDNA inserts by hybridization of replica filters (15) to ^{32}P-labeled (15, 19) adult α- or β-globin cDNAs from *Xenopus laevis* (20–22). Hybridization was performed in 6× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate)/0.05× BLOTTO [1× BLOTTO = 5% (wt/vol) nonfat dry milk/0.02% sodium azide] (15) for 16–18 h at 55°C, after which the membranes were washed to a final stringency of 2× SSC/0.1% SDS at 55°C (15 min). Hybridization-positive colonies were detected autoradiographically (Kodak XAR-5 X-Omat film; exposed at −70°C with intensification).

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§The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U09186 and U09187).

DNA Sequence Analysis. Two overlapping cDNA clones for α1-globin from *N. coriiceps* (NcHbα1-1 and NcHbα1-2) and two for the β-chain (NcHbβ1-1 and NcHbβ1-2) were selected for double-stranded sequencing by the dideoxynucleotide chain-termination method (23) and T4 DNA polymerase (Sequenase II; United States Biochemical). The first member of each cDNA pair—i.e., NcHbα1-1 or NcHbβ1-1—was sequenced in its entirety on both strands by using parental clones and restriction-fragment or deletion (24) subclones, and the second member of each pair was sequenced partially to complete the 5′ (α) or 3′ (β) regions.

Southern Analysis of Genomic DNAs. High-molecular-weight testicular DNA was purified (15) from one male of each species. Aliquots of the DNAs (5 or 10 μg) were subjected to restriction-endonuclease digestion, the restricted DNAs were separated by electrophoresis on horizontal 0.7% agarose slab gels, and DNA fragments were transferred to Nytran membranes (Schleicher & Schuell) by the method of Southern (25). The Southern replicas were probed for globin gene sequences by hybridization to cDNAs (NcHbα1-1 or NcHbβ1-1) that had been labeled with ^{32}P by nick translation or by random priming (15, 19). Prehybridization and hybridization of the membranes were performed in 3× SSC/5× Denhardt's solution (1× Denhardt's is 0.02% each of Ficoll 400, polyvinylpyrrolidone, and bovine serum albumin; ref. 26)/50 μg of sonicated, denatured *E. coli* DNA per ml/0.5% SDS/1 mM EDTA at various temperatures for 1 h and 16–20 h, respectively. The membranes were then washed with increasing stringency and exposed to Kodak XAR-5 X-Omat film (exposed at −70°C with intensification). Hybridization temperatures and final wash stringencies are specified in the legends to Figs. 2 and 3.

Northern Analysis of RNAs. Total RNA, isolated from each tissue by a modification (27) of the acid guanidinium isothiocyanate/phenol/chloroform method (28), was electrophoresed through a 1.2% agarose gel containing 2.2 M formaldehyde (15). RNAs were transferred (15) to MagnaGraph nylon membranes (Micron Separations, Westboro, MA), and the Northern replicas were probed for α- or β-globin mRNA

A

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                                                    -12  GGCCGCAGCAAG

NcHbα1    1  ATGAGTCTCTCCGACAAAGACAAGGCAGCA GTCAAGGCTCTGTGGAGCAAGATCGGCAAG
AA        1  MetSerLeuSerAspLysAspLysAlaAla ValLysAlaLeuTrpSerLysIleGlyLys

NcHbα1   61  TCAGCTGATGCGATTGGAAACGATGCTCTG AGCAGGATGATCGTCGTCTATCCGCAGACC
AA       21  SerAlaAspAlaIleGlyAsnAspAlaLeu SerArgMetIleValValTyrProGlnThr

NcHbα1  121  AAGACCTACTTCTCCCACTGGCCTGACGTG ACCCCCGGCTCTCCTCACATTAAGGCCCAT
AA       41  LysThrTyrPheSerHisTrpProAspVal ThrProGlySerProHisIleLysAlaHis

NcHbα1  181  GGCAAGAAAGTGATGGGTGGAATCGCTCTG GCTGTGTCCAAGATTGACGACCTGAAAGCT
AA       61  GlyLysLysValMetGlyGlyIleAlaLeu AlaValSerLysIleAspAspLeuLysAla

NcHbα1  241  GGTCTGTCTGACCTCAGCGAGCAGCACGCC TACAAGCTGCGAGTGGACCCGGCCAACTTC
AA       81  GlyLeuSerAspLeuSerGluGlnHisAla TyrLysLeuArgValAspProAlaAsnPhe

NcHbα1  301  AAGATCCTGAACCACTGCATCCTGGTGGTG ATCAGCACCATGTTCCCGAAGGACTTCACC
AA      101  LysIleLeuAsnHisCysIleLeuValVal IleSerThrMetPheProLysAspPheThr

NcHbα1  361  CCCGAGGCCCACGTCTCTCTGGATAAATTC CTCTCTGGAGTGGCCCTGGCTCTCGCTGAG
AA      121  ProGluAlaHisValSerLeuAspLysPhe LeuSerGlyValAlaLeuAlaLeuAlaGlu

NcHbα1  421  AGATACCGC TAA ACTGCACGCGGAGGGAAATGCCACATGCAGGCCATCCTTTCACTCAT
AA      141  ArgTyrArg Och

NcHbα1  480  TGTGAACAATTAGAGAATAAACTGCATGCCGCTCAAAATTGGCTTTGTGTTTTTCATGTCT
        541  TCTGAAATGTGTCTTTTAATTCACACAATACTTTATTTCTTGCAAGAGAATAAATGAAGAG
        602  AGCATCTAAAAAA
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B

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                                                    -27  CTAAACACACATTAAACATCAGCCATC

NcHbβ1    1  ATGGTTAATTGGAGCGATTCCGAGCGTGCC ATTATCACCGACATCTTCTCCCACATGGAC
AA        1  MetValAsnTrpSerAspSerGluArgAla IleIleThrAspIlePheSerHisMetAsp

NcHbβ1   61  TATGATGACATCGGACCCAAGGCTCTCAGC AGGTGTCTGATCGTTTATCCCTGGACTCAG
AA       21  TyrAspAspIleGlyProLysAlaLeuSer ArgCysLeuIleValTyrProTrpThrGln

NcHbβ1  121  AGGCACTTCAGCGGCTTTGGAAACCTCTAC AATGCTGAGGCCATCCTCGGCAACGCCAAC
AA       41  ArgHisPheSerGlyPheGlyAsnLeuTyr AsnAlaGluAlaIleLeuGlyAsnAlaAsn

NcHbβ1  181  GTGGCAGCCCACGGCATCAAGGTGCTGCAC GGCCTGGACCGCGGCGTGAAGAACATGGAC
AA       61  ValAlaAlaHisGlyIleLysValLeuHis GlyLeuAspArgGlyValLysAsnMetAsp

NcHbβ1  241  AAAATCGTGGACGCCTACGCCGAGCTGAGC ATGCTGCACTCCGAGAAGCTGCACGTCGAC
AA       81  LysIleValAspAlaTyrAlaGluLeuSer MetLeuHisSerGluLysLeuHisValAsp

NcHbβ1  301  CCCGACAACTTCAAGTTGCTGTCTGACTGC ATCACCATCGTTGTGGCCGCCAAAATGGGT
AA      101  ProAspAsnPheLysLeuLeuSerAspCys IleThrIleValValAlaAlaLysMetGly

NcHbβ1  361  CACGCTTTCACTCCAGAGATTCAGGGCGCC TTCCAGAAGTTCCTGGCCGTCGTGGTGTCC
AA      121  HisAlaPheThrProGluIleGlnGlyAla PheGlnLysPheLeuAlaValValValSer

NcHbβ1  421  GCCCTGGGAAAGCAGTACCAC TAA ACCAGCAGCTTGTGCTCCTGGTAACCATTAAGGAG
AA      141  AlaLeuGlyLysGlnTyrHis Och

NcHbβ1  480  ATCTGACTTCTGTCAAGAACTCTCCTTAATAAAAAAATAAAGTCTCACTGAAGAAAAAAAA
        541  AAAAAAAAAAAAAAAAAAAAA
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FIG. 1. Nucleotide sequences of globin cDNAs from *N. coriiceps* and primary amino acid sequences of the encoded α- and β-globins. Each nucleotide sequence was established by sequencing two overlapping partial cDNA clones. Presumptive translation initiation (29) and polyadenylylation (AATAAA) (30) signal sequences are underlined, and Och denotes the ochre translation termination codon, TAA. With the exception of the initiator methionines, the α- and β-globin primary sequences predicted by these cDNAs match exactly those derived by automated Edman degradation (12, 13). (*A*) Composite α1-globin cDNA sequence (derived from clones NcHbα1-1 and NcHbα1-2) and deduced amino acid sequence (AA) of the encoded α1-globin. Two consensus sequences for polyadenylylation occur within the 3′-untranslated sequence, the second of which precedes a short poly(A) stretch by 14 nucleotides. (*B*) Composite β-globin sequence (determined from clones NcHbβ1-1 and NcHbβ1-2) and deduced primary amino acid sequence of the encoded β-globin. The poly(A) tail begins 13 nucleotides after the second of two adjacent consensus polyadenylylation signals. The GenBank accession numbers for the α- and β-globin cDNA sequences are U09186 and U09187, respectively.

by hybridization to cDNAs (NcHbα1-1 or NcHbβ1-1) that had been ^{32}P-labeled by random priming (15, 19). Prehybridization and hybridization of the membranes were performed in 6× SSC/0.1% sodium pyrophosphate/0.2% SDS/50 μg of heparin per ml at 65°C for 1 h and 16–20 h, respectively. The membranes were washed (final stringency: 0.1× SSC, 65°C, 10 min), and hybridization to target RNAs was detected by autoradiography.

RESULTS

Globin cDNAs from a Red-Blooded Notothenid. To generate probes for genomic analysis of the antarctic fishes, we have isolated cDNAs that encode the globin chains of Hb 1 from *N. coriiceps*. Fig. 1 shows composite nucleotide sequences and deduced primary sequences for the α1-globin (Fig. 1*A*) and β-globin (Fig. 1*B*) cDNAs. The open reading frames of these cDNAs predict α1- and β-globin precursors (143 and 147 amino acids, respectively) that, like globin precursors from other vertebrates, contain amino-terminal methione residues not found in the corresponding mature polypeptides. The α1- and β-globins of *N. coriiceps* are closely related to the major α-, and β-globin chains of other antarctic fishes, with which they share 88–96% and 80–90% sequence identity, respectively (8, 9, 13).

Globin-Related Sequences in the Genomes of White- and Red-Blooded Notothenioids. Using ^{32}P-labeled globin cDNAs, we probed Southern blots of genomic DNA from *N. coriiceps* and from the channichthyid *C. aceratus* for complementary polynucleotide sequences (Fig. 2). Under high-stringency conditions and when equivalent amounts of the genomic DNAs were loaded, the *N. coriiceps* α-globin cDNA probe hybridized strongly to single DNA fragments in each restriction enzyme digest of the icefish and rockcod DNAs (Fig. 2*A*). Several weakly hybridizing fragments, which may contain small segments of the α1 gene or reflect cross-hybridization to the adult α2-globin gene, were also observed for the red-blooded fish. The potential homology of the *C. aceratus* fragments to α-globin coding sequences is supported by the following observations: (*i*) their hybridization to a 348-bp probe that encompasses α-globin codons 4–119 (generated from the NcHbα1-1 cDNA by PCR; data not shown) and (*ii*) their failure to hybridize to a myoglobin coding sequence probe from *N. coriiceps*. [The latter probe yields a distinct fragment pattern for this myoglobinless channichthyid (data not shown and B. D. Sidell and M. E. Vayda, personal communication).] By contrast, the β-globin cDNA detected one strongly hybridizing and three or four weakly hybridizing DNA fragments in the rockcod DNA digests at moderate stringency but failed to hybridize to restricted genomic DNA from the icefish (Fig. 2*B*). Reduction of the hybridization stringency (6× SSC, 55°C) produced, in the notothenid DNA digests, specific banding with the β-globin cDNA probe against an elevated, nonspecific background, while hybridization of the β-globin probe to the icefish DNA digests gave only nonspecific binding. Together, our results suggest strongly that *C. aceratus* retains genomic DNA sequences that are closely related to the adult α-globin gene(s) of its red-blooded notothenioid ancestors and contemporaries, whereas its ancestral β-globin gene sequences either have been deleted or have diverged beyond the limits of detection by complementarity to a notothenid β-globin cDNA.

Failure to detect β-globin sequences in the genome of the icefish might result from compromised integrity of the target DNA or from deficiencies in the blotting and hybridization procedures. To control for these possibilities, the β-globin Southern blot (Fig. 2*B*) was stripped and reprobed with a cDNA specific for the gene family encoding β-tubulins, whose members presumably have been maintained in the icefish genome by positive selection pressure. (For comparisons of the neural β-tubulin polypeptides expressed by notothenid and channichthyid fishes, see refs. 32 and 33). As our probe, we used a neural class-II β-tubulin cDNA, Ncnβ1, from *N. coriiceps* (31) that cross-hybridizes to the coding sequences of most, if not all, of the β-tubulin genes of this notothenid (unpublished results). Fig. 2*C* shows that the β-tubulin cDNA detects 10–15 different fragments of similar intensity in the genomes of both fishes, which suggests that the icefish conforms to prior estimates of β-tubulin gene number in the rockcod (31). Thus, the lack of hybridization of the β-globin

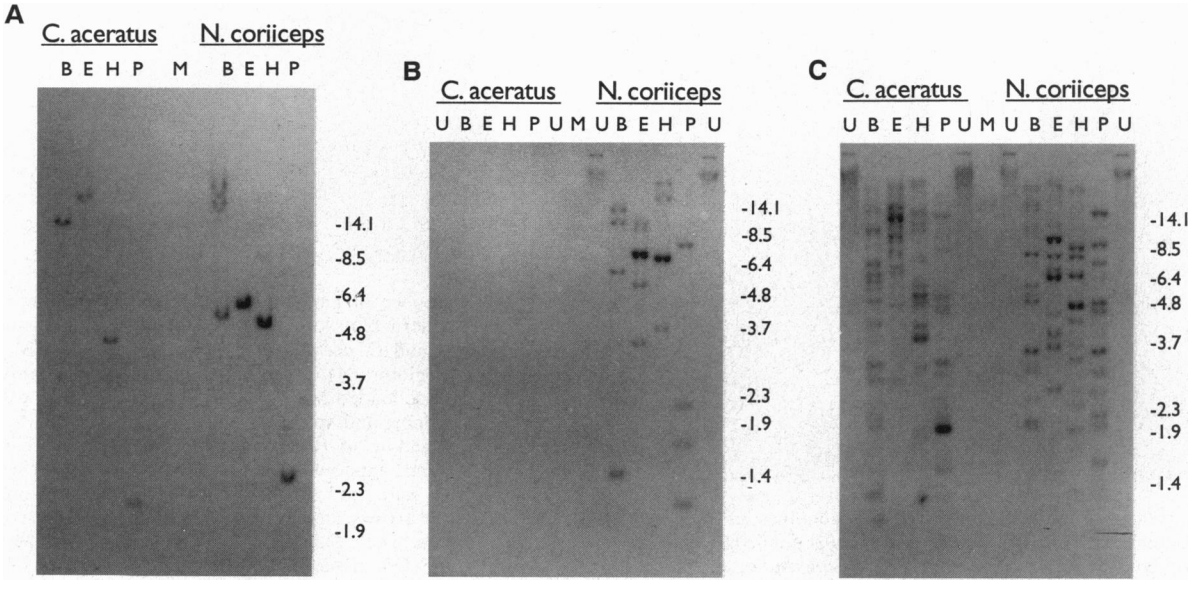

FIG. 2. The genome of the icefish *C. aceratus* contains sequences complementary to α-globin but not β-globin cDNAs from an antarctic notothenid. Southern blots of genomic DNAs from *C. aceratus* and *N. coriiceps* were probed with ^{32}P-labeled notothenid cDNAs for α1-globin (*A*) or β-globin (*B*). The β-globin blot (*B*) was stripped and rehybridized with a β_{II}-tubulin cDNA from *N. coriiceps* (31) to yield blot *C*. Hybridization stringency for *A–C* was 3× SSC at 65°C. Final wash stringencies: (*A*) four 15-min washes of 0.1× SSC at 65°C; (*B*) two 15-min washes of 3× SSC at 65°C; and (*C*) four 15-min washes of 1× SSC at 66–67°C. Lanes B, E, H, and P contain genomic DNA digested with *Bam*HI, *Eco*RI, *Hin*dIII, and *Pst* I, respectively, and lanes marked U contain undigested DNA. Lanes M contain DNA size standards, the sizes (kb) of which are indicated to the right of each autoradiograph.

cDNA to the icefish genome cannot be attributed to experimental artifacts but rather reflects the bona fide absence of sequences homologous to the β-globin genes of red-blooded notothenioids.

The 15 known species of icefishes all share the hemoglobinless condition (2, 3, 7). As a first step toward systematic evaluation of globin gene status in the channichthyids, we compared globin genomic Southern hybridization patterns for three icefish species (*C. aceratus, C. gunnari,* and *C. rastrospinosus*), representing both primitive and advanced genera, with those for several red-blooded notothenioids. Fig. 3 shows that the presence of α-globin-related DNA sequences (Fig. 3*A*) and the apparent absence of β-globin genes (Fig. 3*B*) are features common to these icefish genomes. By contrast, the genomes of three red-blooded antarctic notothenioids [the rockcods *G. gibberifrons* and *N. coriiceps* (family Nototheniidae) and the dragonfish *P. charcoti* (family Bathydraconidae)] and a temperate notothenioid (the New Zealand black cod, *N. angustata*) yielded, as expected, strong hybridization signals for both α- and β-globin cDNA probes (Fig. 3 *A* and *B*). Thus, the icefish survey, although incomplete, suggests that establishment of the hemoglobinless phenotype preceded the evolutionary radiation of the icefish genera and involved, either directly or indirectly, the deletion or rapid divergence of adult β-globin genes.

Transcriptional Status of α-Globin-Related Sequences in the Icefish *C. aceratus*. Retention of α-globin-related sequences in icefish genomes raises the possibility that these genes may have been recruited to serve another function. To determine whether these sequences are transcriptionally active, we assessed steady-state mRNA levels in major tissues and organs, both hematopoietic and nonhematopoietic, of *C. aceratus* and *N. coriiceps*. Neither α-globin nor β-globin transcripts were detected in any icefish tissue (Fig. 4), including the cellular component (largely leukocytes) of blood and the hematopoietic organs [pronephric (head) kidney, opisthonephric (trunk) kidney, and spleen; ref. 34]. By contrast, in the rockcod, high levels of two α-globin mRNAs (Fig. 4*A*) and one β-globin transcript (Fig. 4*B*) were detected in erythrocytes, head kidney, and spleen; smaller quantities of the messages (presumably contributed by contaminating red blood cells) were present in highly vascularized tissues (e.g., heart, brain, gills, and liver), and the transcripts were absent in several other nonhematopoietic tissues [white skeletal muscle and testis (latter not shown)]. Thus, we conclude that the α-globin-related genomic DNA sequences of *C. aceratus*, and by inference of other icefishes, probably represent nonexpressed derivatives of the α-globin genes of red-blooded notothenioids.

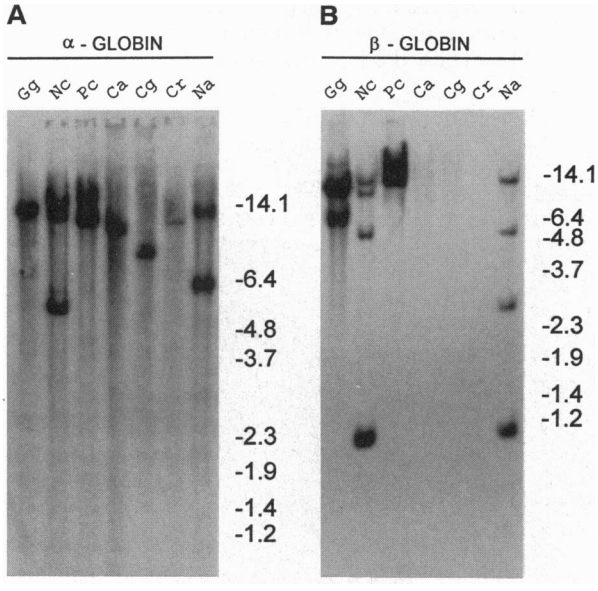

FIG. 3. Globin-related sequences in the genomes of red- and white-blooded antarctic fishes. Southern blots of genomic DNAs from three notothenioids (Gg, *G. gibberifrons*; Na, *N. angustata*; and Nc, *N. coriiceps*), a bathydraconid (Pc, *P. charcoti*), and three channichthyids (Ca, *C. aceratus*; Cg, *C. gunnari*; and Cr, *C. rastrospinosus*) were probed with *N. coriiceps* cDNAs for α-globin (*A*) or for β-globin (*B*). Hybridization stringencies: (*A*) 3× SSC at 65°C and (*B*) 3× SSC at 61°C. Final wash stringencies for *A* and *B* were four 15-min (*A*) or two 15-min (*B*) washes of 0.33× SSC at 65°C. DNAs were digested with *Bam*HI. The sizes (kb) of DNA standards are indicated to the right of each autoradiograph.

DISCUSSION

The coastal waters of Antarctica are both cold (temperature ≈ −1.9 to +2°C) and oxygen rich. Under these conditions, the metabolic demand of the poikilothermic antarctic fishes for oxygen is relatively low, the physical solubility of oxygen in their plasma is high, and the energetic cost associated with circulation of a highly corpuscular blood fluid is large (7–10).

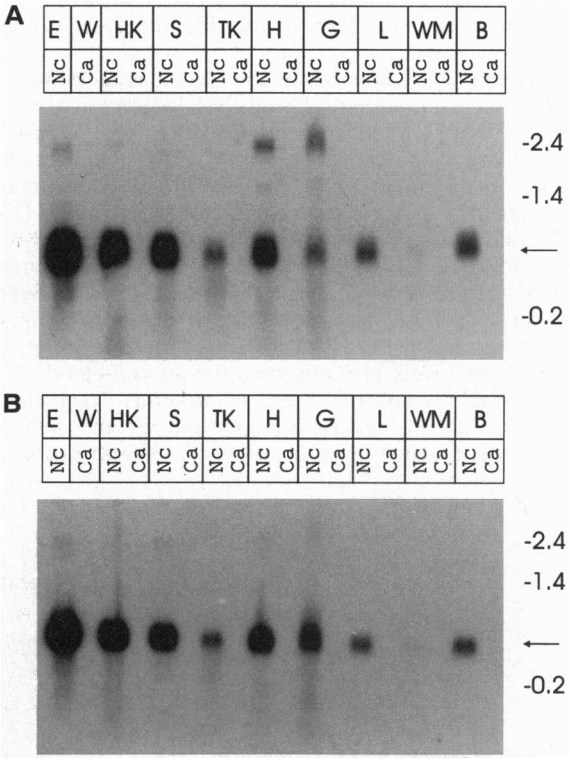

FIG. 4. Absence of globin mRNA expression by the icefish *C. aceratus*. Northern blots of total RNA from various tissues of *N. coriiceps* (Nc) and *C. aceratus* (Ca) were probed with *N. coriiceps* cDNAs for α1-globin (*A*) or β-globin (*B*). Five micrograms of each RNA sample was loaded onto the gel with the exception of erythrocytes, head kidney, and spleen (2 μg of RNA each). Hybridization bands corresponding to two α-globin mRNAs (major band ≈850 nucleotides, minor band ≈950 nucleotides; two bands not resolved in this exposure) and one β-globin mRNA (≈700 nucleotides) are indicated by the arrows, and the molecular weights of RNA standards are given in kb. The ≈2.5-kb RNAs that cross-hybridize with the α-globin probe (Nc tissues E, HK, S, H, and G) may represent transcripts initiated at a distal promoter. Control hybridizations to the *N. coriiceps* β-tubulin cDNA Ncnβ1 (33) demonstrated the presence of intact β-tubulin mRNAs in both icefish and rockcod total RNA samples from tissues in which tubulins are abundantly expressed (brain, gill, testis, and trunk kidney). E, erythrocytes; W, white blood cells; HK, head (pronephric) kidney; S, spleen; TK, trunk (opisthonephric) kidney; H, heart; G, gills; L, liver; WM, white skeletal muscle; B, brain.

With the selective pressure for red blood cells and oxygen-transporting pigments relaxed and with red blood cells posing a rheological disadvantage by increasing blood viscosity, it is not surprising that the cold-living fishes of the notothenioid suborder have evolved reduced hematocrits, low cellular hemoglobin concentrations, and hemoglobins of low multiplicity and reduced oxygen affinity (7–10). As the most phyletically derived of the notothenioid fishes (6, 7), the icefishes might be considered to have attained the extreme of this trend. Alternatively, loss of hemoglobin production in the icefishes may have occurred independently of the trend toward hemoglobin reduction in the other notothenioids (7) and, in the oxygen-rich waters of the Antarctic, may have constituted a nonadaptive, yet nonlethal mutation (7, 35). Irrespective of evolutionary paradigm, the three icefishes examined here, including primitive and advanced genera (6, 7), share retention of α-globin-related DNA sequences in their genomes and apparent loss or rapid mutation of β-globin genes. This common pattern suggests that loss of globin-gene expression is a primitive character for the family and that it was established in the ancestral channichthyid approximately 25 million years ago (7), prior to diversification within the clade.

What mechanisms might explain the appearance of the hemoglobinless condition and the differential divergence of the α- and β-globin genes? Complete extinction of the erythroid lineage appears unlikely, given the presence of erythrocyte-like, albeit hemoglobinless, blood cells in the channichthyids. We propose instead that the primary event leading to the hemoglobinless phenotype was deletion of the single adult β-globin locus of the ancestral channichthyid. The α-globin gene(s), no longer under selective pressure for expression, would then have accumulated mutations in regulatory and coding regions, leading to loss of gene function without, as yet, complete loss of sequence information. Although recruitment of α-globin gene sequences to alternative functions remains a possibility, the present results strongly suggest that these sequences are not expressed in icefishes. If true, these α-globin genetic remnants should prove useful as tools for development of a molecular phylogeny of icefishes and for calibration of a vertebrate mutational clock free of selective constraints. Furthermore, mechanistic analysis of the loss of α-globin gene function in icefishes may also contribute to an improved understanding of the molecular defects that underlie human thalassemias.

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