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Functional characterization of a BCL10 isoform in the rainbow trout *Oncorhynchus mykiss*

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ABSTRACT

The complexes formed by BCL10, MALT1 and members of the family of CARMA proteins have recently been the focus of much attention because they represent a key mechanism for regulating activation of the transcription factor NF-κB. Here, we report the functional characterization of a novel isoform of BCL10 in the trout *Oncorhynchus mykiss*, which we named tBCL10. tBCL10 dimerizes, binds to components of the CBM complex and forms cytoplasmic filaments. Functionally, tBCL10 activates NF-κB transcription factor and is inhibited by the deubiquitinating enzyme A20. Finally, depletion experiments indicate that tBCL10 can functionally replace the human protein. This work demonstrates the evolutionary conservation of the mechanism of NF-κB activation through the CBM complex, and indicates that the rainbow trout *O. mykiss* can serve as a model organism to study this pathway.

1. Introduction

The NF-κB family of transcription factors is a group of evolutionarily conserved proteins that are important regulators of the immune system function, controlling the expression of numerous proteins involved in innate and adaptive immunity [1,2]. NF-κB also transcribes genes that exert a positive effect on cell survival and proliferation, and disregulation of the mechanisms controlling its activation often results in immunoproliferative, inflammatory and autoimmune phenotypes [1,2].

The human Caspase recruiting domain (CARD)-containing protein BCL10 is a 233 amino acids protein originally identified as a target of translocation in a subset of mucosa-associated lymphoid tissue (MALT) lymphoma cells [3–5]. As a consequence of a translocation, BCL10 is overexpressed, and that results in a constitutive NF-|B activation which is eventually responsible for the neoplastic transformation [3–5]. Gene targeting of the BCL10 locus in murine strains results in immunodeficiency, having $BCL10^{-/-}$ mice severe defects in humoral and cellular immune responses and antigen-induced proliferation, due to impaired NF-|B activation following stimulation in both T and B cells [6]. Thus, BCL10 is indispensable for NF-|B activation following antigen receptor stimulation on B and T lymphocytes [6].

The biological function of BCL10 is explicated through participation at the CBM complex, a molecular complex that includes one of three members of the family of CARMA proteins and MALT1 [7]. The three CARMA proteins, CARMA1, 2 and 3, constitute a family of proteins conserved across many species and are characterized by the presence of different functional domains shared by all members of the family [8]. Functionally, all three CARMA proteins are able to associate BCL10 through an homophilic interaction between the corresponding CARD domains, and to cooperate with it in inducing the transcriptional activity of NF-κB [8].

Compared to mammalian NF-κB, very little is known about piscine regulators of this transcription factor. Recently, extensive analysis of fish genomes have reported the presence of several CARD domain containing proteins encoded by the genome of fish such as zebrafish and the rainbow trout *Oncorhynchus mykiss* [9–12]. In particular, because of multiple whole-genome duplications occurred in salmonid species [13], for the rainbow trout genome have been annotated four different genes encoding for putative proteins that share aminoacidic similarity with human BCL10. However, it is not established whether any of these genes is actually expressed, and no functional data is available regarding any of these proteins. In this work, we report on the functional

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characterization of a rainbow trout *O. mykiss* BCL10 ortholog, herein defined tBCL10.

2. Materials and methods

2.1. Ethics

All the procedures involving animals were conducted as indicated in the Italian National Guidelines (D.L. No. 100/2006, and D.L. No. 116/1992) and in the appropriate European Directives (EEC Council Directive 86/609, 1.12.1987), adhering to the Guide for the Care and Use of Laboratory Animals (United States National Research Council, 1996). All the in vivo experimental activities were approved by the Animal Ethics Committee (CESA) of Biogem (Italy).

2.2. RNA extraction and cloning of tBCL10 full-length cDNA

Total RNA was extracted from trout peripheral blood leukocytes by using Trizol reagent, and 1 μg of total RNA was reverse-transcribed to generate a first-strand cDNA. Primers used to amplify tBCL10 were the following: forward 5′-ATGGACTCCTGG TGTATCACTGAC-3′ and reverse 5′-TCAGACTCTTAAGGTCCCGG GCTC-3′. PCR conditions were as follows: 98 °C for 30 s, 30 cycles

Table 1
Loci encoding for proteins similar to human BCL10 in the rainbow trout genome.

Name	Accession number	Length	Predicted MW	Similarity to human BCL10
BCL10a isoform 1	CAF31504	203	22,567	40%
BCL10a isoform 3	CDQ87110	199	22,126	38%
BCL10b isoform 1	CDQ56929	270	29,611	46%
BCL10b isoform 2	CDQ91425	262	28,612	44%

BCL10-like proteins encoded by the genome of *Oncorhynchus mykiss* and their similarity to the human protein.

Table 2
Loci encoding for proteins similar to human BCL10 in the rainbow trout genome.

Species	Protein	Length	Identities	Positives
Oncorhynchus mykiss	BCL10a isoform 2 (tBCL10)	207	83/207 (40%)	169/207 (79%)
Homo sapiens	BCL10	233		
Oncorhynchus mykiss	tBCL10 CARD (6–116)	111	59/111 (53%)	97/111 (79%)
Homo sapiens	BCL10 CARD (8–115)	108		

Amino acidic similarity between hBCL10 and tBCL10 in the entire protein and in the CARD domains.

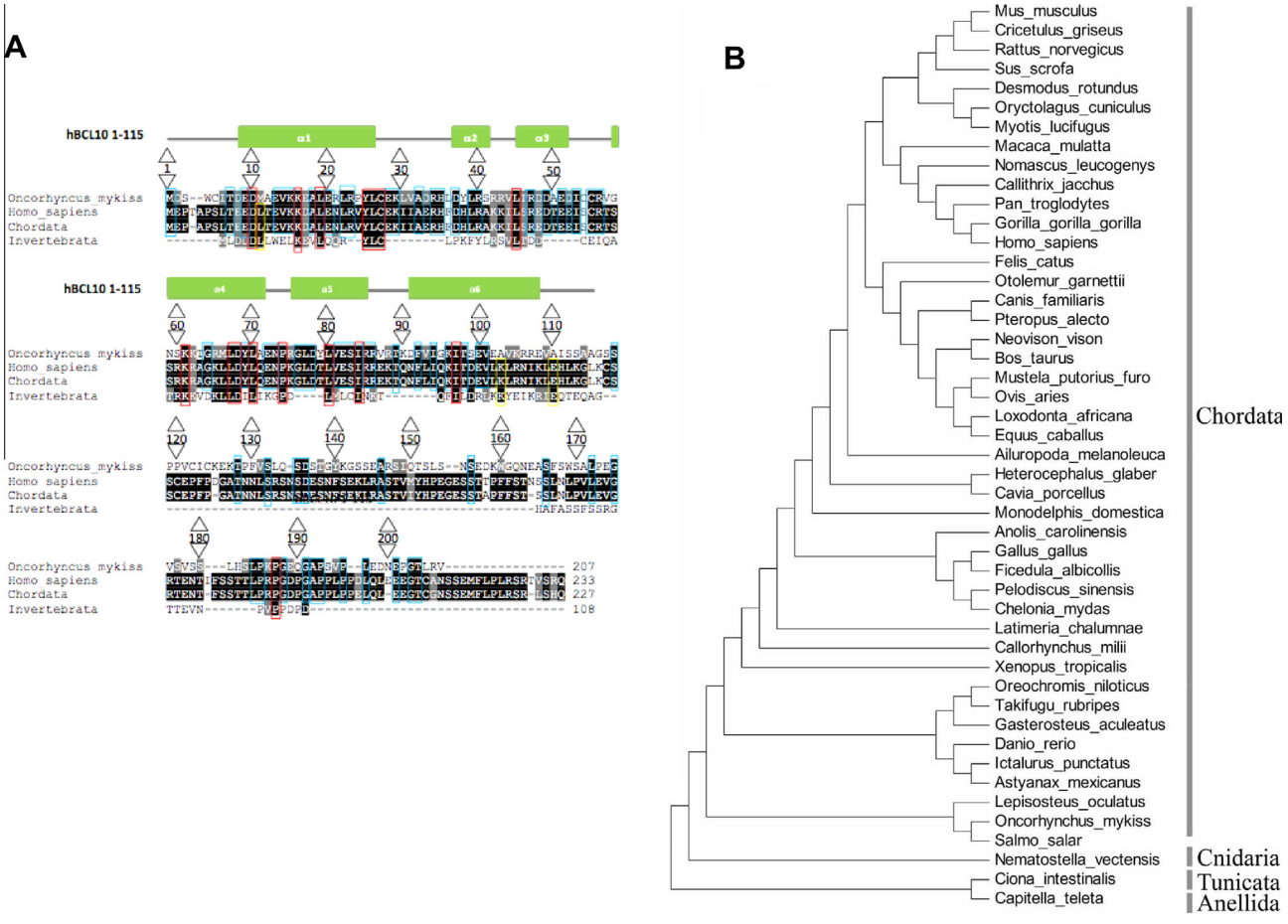

Fig. 1. *Alignment and phylogenetic tree of tBCL10.* (A) Alignment of tBCL10 sequence with the human BCL10 sequence and the consensus sequences generated by aligning the BCL10 sequences of Chordata and the CARD domains of three Invertebrata proteins. At the top of the alignment the six alpha helix regions of the CARD are shown. Amino acid numbering refers to the tBCL10 sequence. The alignment was using ClustalW and the printout from multiple-aligned sequences was done with BOXSHADE. The black background designates identical amino acids, the gray background conservative substitutions. Colored rectangles indicate amino acids conserved among the sequences examined. The sequences used for generation of the consensus are available in Supplementary Material. (B) Phylogenetic tree analysis of BCL10 proteins. The phylogenetic tree was constructed based on the full-length amino acid sequences using the neighbor-joining method within the Mega program. The sequences used for alignment and generation of the consensus are available in Supplementary Material.