



Evolutionary history of the ABCB2 genomic region in teleosts[☆]

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Received 10 January 2006; received in revised form 21 July 2006; accepted 23 July 2006

Available online 20 September 2006

Abstract

Gene duplication, silencing and translocation have all been implicated in shaping the unique genomic architecture of the teleost MH regions. Previously, we demonstrated that trout possess five unlinked regions encoding MH genes. One of these regions harbors ABCB2 which in all other vertebrate classes is found in the MHC class II region. In this study, we sequenced a BAC contig for the trout ABCB2 region. Analysis of this region revealed the presence of genes homologous to those located in the human class II (ABCB2, BRD2, ψ DAA), extended class II (RGL2, PHF1, SYGP1) and class III (PBX2, Notch-L) regions. The organization and syntenic relationships of this region were then compared to similar regions in humans, Tetraodon and zebrafish to learn more about the evolutionary history of this region. Our analysis indicates that this region was generated during the teleost-specific duplication event while also providing insight about potential MH paralogous regions in teleosts.

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Keywords: ABCB2; Duplication; MHC; Genomic sequence; Teleost; *Oncorhynchus mykiss*

1. Introduction

Presentation of intracellularly derived peptides by MHC class Ia molecules is an important component of cell-mediated immunity against viral pathogens. Class Ia molecules are sequestered in the endoplasmic reticulum (ER) until they are loaded with appropriate peptide antigen and released to the cell surface for surveillance by CD8⁺ cytotoxic T lymphocytes (CTLs). Endogenous proteins resulting

from intracellular pathogens are processed into small antigenic peptides (8–11 mers) by the immunoproteasome and then transported into the lumen of the ER from the cytosol via the transporters associated with antigen presentation, ABCB2 and ABCB3 (TAP1 and 2) [1]. In mammals, the class I peptide-loading complex consists of a single ABCB2/3 heterodimer, four tapasin (TAPBP) molecules and four class I- β 2m molecules [2], where TAPBP serves as a bridge between the peptide transporters and the class I/b2M complex. The genes encoding the MHC class Ia pathway, together with the MHC class Ib, II and III genes are physically linked forming the major histocompatibility complex in mammals. Similar genomic organizations are observed in all vertebrates studied to date with the exception of bony fish. Bony fish

[☆]The genomic contig described in this paper has been deposited in GenBank under accession number [DQ246664](https://www.ncbi.nlm.nih.gov/nuccore/DQ246664).

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possess MH “regions” [3,4] as the class Ia, Ib and class II genes reside on separate chromosomes. This phenomenon was first described in zebrafish [5] and later in other teleosts including medaka [6], fugu [7] and rainbow trout [4,8].

In recent years, much of the effort in teleost MH genomics has been directed toward comparative studies of the genomic structure of the teleost MHC class I region which identified a high degree of conserved synteny among the teleosts class I region [4,9–12]. Interestingly, most of the genes that are involved directly in the class Ia antigen presentation pathway including PSMB8, PSMB9, PSMB9-like, PSMB10, ABCB3 and TAPBP are linked in the fish MHC class I genomic region forming the class I “core” region, with the exception of ABCB2. From the structural viewpoint, however, there is nothing in common among the MHC class Ia, PSMBs and ABCB2 and 3 molecules. Therefore, the underlining assumption of most comparative studies involving the MHC suggests that the clustering of these genes is the result of selection pressure requiring co-evolution and co-regulation of the MHC-related genes (recently reviewed by [13]).

Previously we demonstrated that the rainbow trout MH class I core region is duplicated, that ABCB2 maps to a separate linkage group (LG) and chromosome and that the class II region is localized to yet another LG and chromosomal region [4]. The extent of the class I duplication event was recently formalized via BAC sequencing of the core regions for the class IA and B regions [12]. More recently we determined that TAPBP is part of the trout class Ia core region and that the trout tapasin-related gene (*Ommy-TAPBP-R.1*) and ABCB2 are co-localized on the short arm of chromosome 2, while *Ommy-TAPBP-R.2* is located on chromosome 3 representing a fifth MH chromosomal region in rainbow trout [14].

Based upon prior studies of the trout MH regions [4,12], we analyzed the ABCB2 locus in trout to determine if genes encoded within this region could lend new insight as to the unique MH architectural arrangements observed in bony fish. In this report, we present the sequence of a 320 kbp chromosomal region (Chr. 2) containing the rainbow trout ABCB2 gene and an initial genomic characterization of the expressed genes in this region to identify conserved synteny with other vertebrates. The identification of BRD, PBX and NOTCH-like genes along with ABCB2 within this region suggests that this region is the likely remnants of the ancestral MHC synteny. Therefore this is the first report describing the

genomic organization of this “lost” MH region in teleosts enabling future studies exploring the possible functional and evolutionary significance of conserved synteny in this region and the potential roles of the encoded genes for fish health.

2. Materials and methods

2.1. BAC library screening and DNA fingerprinting analysis

The Swanson 10X rainbow trout BAC library which was derived from a doubled-haploid homozygous line, was previously screened using radiolabeled probes hybridization to identify clones that harbor the ABCB2 gene [15]. DNA was isolated from 15 positive BACs (Qiagen Corp., Valencia, CA) and digested with HindIII (Promega, Madison, WI). The generated banding patterns were analyzed using Image/FPC software [16] to assemble contigs of overlapping BACs [15].

2.2. BAC sequencing and gene annotation

Three BACs that represent a contig of approximately 320 kbp were processed using the Qiagen Large Construct kit for the construction of BAC DNA shotgun libraries. BAC DNA was sheared into 1- to 3-kbp fragments, subcloned into pBSK⁺, sequenced to 9 × coverage, and assembled using the PHRED-PHAP-CONSED software package [17,18]. Only Phred values of >20 were used for the assembly. Primers for gap filling were derived from the sequence of sub-clones that overlapped sequence gaps. Sequence assembly was confirmed by comparing the actual HindIII restriction digestion pattern of each BAC and the in silico digestion pattern predicted by the PHRAP/CONSED virtual digestion tool. The assembled sequence contig from the three BAC clones was annotated using GeneScan [19] (<http://genes.mit.edu/GENSCAN.html>) including manual adjustments. Full-length sequences of EST cDNA clones displaying high identity (≥98%) to the ORFs (GenBank accession numbers DR782904) were used to refine intron/exon boundaries using Sequencher (Gene Codes Corporation, Ann Arbor, MI). Gene names, abbreviations and locations in the human genome were inferred from Gene Cards (<http://bioinfo.weizmann.ac.il/cards/index.shtml>). Gene locations in the *Tetraodon nigroviridis* and zebrafish genomes were inferred by predicted peptides sequence similarity

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