Available online at www.sciencedirect.com
ScienceDirect
Developmental and Comparative Immunology 31 (2007) 708–719
Developmental & Comparative Immunology
www.elsevier.com/locate/devcompimm

Cloning and expression analysis of an Atlantic salmon (*Salmo salar* L.) tapasin gene

Sven Martin Jørgensen[a], Unni Grimholt[b], Tor Gjøen[a,*]

[a]*Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, 0316 Oslo, Norway*
[b]*Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, 0033 Oslo, Norway*

Received 4 April 2006; received in revised form 16 October 2006; accepted 16 October 2006
Available online 20 November 2006

Abstract

Loading of the major histocompatibility complex (MHC) class I molecule with peptide is mediated by the multimeric peptide-loading complex in the ER where the glycoprotein tapasin (TAPBP) is required for stabilization of the complex and for control of peptide loading onto MHC class I. To expand our knowledge on antigen presentation genes in Atlantic salmon, we isolated a full-length salmon tapasin cDNA sequence (*Sasa-TAPBP*). It encoded a 443 bp amino acid sequence with two *N*-glycosylation sites, two conserved mammalian tapasin signature motifs, two Ig superfamily (IgSf) domains, a transmembrane (TM) domain and an ER-retention KK motif at the C-terminal end, indicative of a similar function as mammalian tapasins. We analysed the regulation of *Sasa-TAPBP* under immunostimulatory conditions and found an mRNA-upregulation during early infectious salmon anemia virus (ISAV) infection and poly I:C stimulation in vivo and in vitro, in line with our previous findings for other MHC class I pathway genes.

Keywords: TAPBP; Tapasin; MHC class I; Infectious salmon anemia virus; Interferon; Poly I:C

1. Introduction

The activation of cytotoxic T lymphocytes by recognition of antigenic (Ag) peptides presented on major histocompatibility complex (MHC) class I molecules is a critical process in the adaptive immunity of vertebrates. Optimal antigen presentation is dependent on the interplay of multiple proteins commonly referred to as MHC class I pathway molecules. In the endoplasmic reticulum (ER), the multimeric peptide-loading complex consisting of transporter associated proteins 1 and 2 (TAP1/2), the type I transmembrane glycoprotein tapasin, the lectin chaperone calreticulin and the thiol-oxidoreductase ERp57 ensure proper assembly and peptide loading of MHC class I molecules (reviewed in [1–3]). Tapasin stabilizes this complex in several ways [4–9]. It recruits β_2-microglobulin (β_2m)-associated class I heavy chain into the complex by specifically binding to the α_2 and α_3-domains via its N and C terminus, respectively. Tapasin also controls class I association with TAP and ERp57 by binding these molecules via its C and N terminus, respectively. Although not all aspects of tapasin function are understood, a lack of tapasin leads to reduced stability of MHC I: peptide

*Corresponding author. Tel.: +47 2284 4943; fax: +47 2284 4944.
E-mail address: tor.gjoen@farmasi.uio.no (T. Gjøen).

0145-305X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dci.2006.10.004

complexes at the cell surface, suggesting that it acts as an editor of the total peptide repertoire loaded upon the class I complex [7,10–13]. After binding of high affinity peptide, MHC class I: peptide complex dissociate from tapasin and TAP [14,15] to exit ER for transportation to the cell surface.

During viral infection, interferons (IFNs) are important regulators of a large number of immune genes, and activation of MHC class I pathway genes is believed to increase the efficiency of the cell-mediated immune response [16]. In mammals, IFN-α and IFN-β produced by fibroblasts and other cell types and IFN-γ derived from natural killer (NK) and T cells rapidly induce MHC class I transcription [17]. Activation by type I IFNs is mediated through IFN-stimulated response promoter elements (ISRE), while a gamma activation site (GAS) is found in promoters of IFN-γ inducible genes [16]. The mammalian tapasin promoter contains ISRE and GAS elements and is inducible by both type I and II IFNs [18]. The IFN system of Atlantic salmon was recently characterized and type I IFN is induced by double-stranded RNA (dsRNA) and viral infection (reviewed in [19]).

The infectious salmon anemia virus (ISAV) is an aquatic orthomyxovirus causing a severe disease which continuously affects salmon farms on the northern hemisphere. Vaccines have been produced and tested with promising effects in the USA and Canada [20,21] but is currently forbidden in the EU. Thus, selective breeding is the only alternative for improving disease resistance, and understanding of the host immune responses to ISAV infection is needed. A recent study on ISAV-host interaction showed that a cellular immune response was mainly contributing to virus clearance compared to a humoral response [22]. In addition, ISAV disease resistance has been associated with specific MHC class I and II genotypes [23]. The regulation and structure of several genes involved in the antigen presentation in Atlantic salmon are characterized [24–26], but less is known about the components of the peptide-loading complex. Full-length TAPBP sequences have been described from medaka (*Oryzias latipes*) [27] and zebrafish (*Danio rerio*) [28], and a comprehensive study of TAPBP and TAPBP-related (TAPBP-R) genes was recently published on rainbow trout (*Oncorhynchus mykiss*) [29]. The functional trout TAPBP was mapped to the classical class I region, suggesting coordinated regulation with other MHC class I-resident genes. We have previously reported that several salmon MHC class I pathway genes are rapidly induced in a concerted manner following ISAV infection and dsRNA stimulation in vitro, and that this corresponds to a strong induction of type I and II IFN [30]. In this study we extend our knowledge by including the tapasin gene and present the characterization and immune regulation of the MHC class I-resident TAPBP from Atlantic salmon.

2. Material and methods

2.1. cDNA cloning and sequencing

Full length TAPBP was amplified from cDNA isolated from salmon head kidney (SHK) cells. Cloning primers were designed based on predicted salmon TAPBP ESTs from the GRASP database [31] (found by tblastn using the zebrafish (*Danio rerio*) TAPBP (NP571049)). An unknown 350 bp sequence gap was PCR-amplified (BD Advantage 2, BD Biosciences Clontech, USA) using a 30 bp sense primer (5′-ATGGCCAATATTTCAACAATTTA-CAAGCTA-3′), based on the two clones *ssal.rgb-508.043* (NM_130974) and *ssal.rgb-516.287* (NM_130974) covering the putative ORF start codon and part of the 5′UTR, and a 30 bp antisense primer (5′-TTACTTCACTTTCTTCTCTTGT-GAGTTAGT-3′), based on the EST clone *ssal.hbng-007.032-1* plus the rainbow trout (*O. mykiss*) EST clone *omyk.rtwh-080.353* (AB073377), covering end-terminus of TAPBP. The approximate 1300 bp PCR fragment was separated on a 1.5% agarose-ethidium bromide gel, exiced and purified (GeneClean, Bio101, USA). TOPO-TA cloning (Invitrogen, USA) was performed according to manufacturer's protocol and LB-agar plate colonies were checked for insert by PCR. Plasmid minipreps of ten clones were sequenced (GATC Biotech AG, Konstanz, Germany) and a 1329 bp putative full-length salmon *Sasa-TAPBP* coding region was obtained (DQ451008).

2.2. ISAV infected tissues and cells

Tissue samples from ISAV infected fish at the stage of early viremia originated from an infection trial performed at VESO (Vikan, Norway, http://www.veso.no/?Vikan) with unvaccinated juvenile Atlantic salmon kept in similar isolated tanks at 12 °C under controlled conditions. In one tank, 100 fish (average size 35.3 g at start of experiment, hereafter referred to