Molecular cloning, subcellular location and expression profile of signal transducer and activator of transcription 2 (STAT2) from turbot, \textit{Scophthalmus maximus}

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\textbf{A R T I C L E  I N F O}

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\textbf{A B S T R A C T}

Signal transducer and activator of transcription 2 (STAT2) is an important molecule involved in the type I interferon signalling pathway. To date, little STAT2 homologue is available in fish except Atlantic salmon and goldfish. In this paper, STAT2 was firstly cloned and characterized from turbot, a marine flatfish with high economic value. Briefly, turbot STAT2 cDNA is 3206 bp in length encoding a predicted protein of 793 amino acids. The phylogenetic tree shows that turbot STAT2 protein shared the closest relationship with Atlantic salmon. Analysis of subcellular distribution indicates that STAT2 is mainly present in the cytoplasm of TK cells. \textit{Stat2} mRNA is constitutively expressed in widespread tissues and induced by several folds in turbot tissues and TK cells after stimulation with \textit{Vibrio anguillarum} and lymphocystis disease virus (LCDV). Unlike the higher vertebrate STAT2, turbot STAT2 nuclear export signal (NES) exists not in the C-terminal 79 amino acids but in N-terminal 137 amino acids (STAT\_alpha domain). The nuclear translocation of turbot STAT2 after Poly(I:C) treatment proved its transcription activity in TK cells. All these results suggested that STAT2 may be involved in the immune response in turbot as a transcription factor.

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1. Introduction

The Janus kinase (JAK) and signal transducers and activators of transcription (STAT) signalling pathway play critical roles in the regulation of cell proliferation, growth, haematopoesis, and immune response [1,2]. So far, seven STAT family members including STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6 and four JAK family members including JAK1–4 have been identified in mammals [3]. The binding of extracellular cytokines and growth factors to their receptors firstly triggered JAKs\textsuperscript{a} activation, followed by tyrosine phosphorylation of STATs. Phosphorylated STATs form dimers which enter the nucleus to activate the transcription of their target genes [3–6].

As an exceptional member of the STAT family, STAT2 does not form homo-dimer like other STATs. Instead, in response to interferon (IFN) \textit{z} and \textit{b}, STAT2 forms heterodimer with STAT1 by tyrosine-phosphorylation [7–9]. The STAT2–STAT1 heterodimer subsequently translocates into the nucleus and combines IFN regulatory factor 9 (IRF-9) to form IFN-stimulated gene factor 3 (ISGF3) which activates IFN-stimulated response element driven genes [7–11]. The STAT2 knockout mice exhibit susceptible to viral infection due to their impaired ability to respond to IFN \textit{z}/\textit{b} signalling, likely with the phenotype of STAT1 knockout mice [12–14].

Besides the association with STAT1 and IRF-9, STAT2 has also been shown to interact with Interferon-alpha/beta receptor alpha chain (IFNAR1) [15], Interferon-alpha/beta receptor beta chain (IFNAR2) [16], mediator of RNA polymerase II transcription subunit 14 (MED14) [17], and regulator of calcineurin 1 (RCAN1) [18]. Recent studies have shown that STAT2 plays a pivotal role in blood development, immune response, and myogenic differentiation [19,20]. In addition, in response to the evolutionary struggle between host and pathogen, many viruses such as paramyxovirus, measles virus, lymphocytic choriomeningitis virus, and herpes simplex virus have developed a strategy to bypass the IFN antiviral
system by interacting with STAT2 [21–25]. The understanding of
STAT2 inhibition mechanism is helpful for the design of STAT2-
directed therapeutics for treatment of diseases.

So far, little study about STAT2 in fish is available except Atlantic
salmon [26] and goldfish [27]. In turbot, an important economic
marine fish, lymphocystis disease virus (LCDV) and Vibrio anguill-
larum represent two of the major pathogens causing significantly
economic losses in China. In order to explore the host–pathogen
interaction mechanism, several SEV libraries have been constructed
in turbot and many immune related EST sequences such as IRF-3,
IRF-7, IRF-10 and JAK1 have been identified [28–32].

In this study, a STAT2 homologue was cloned and characterized
from turbot. Followed by the phylogenetic tree construction and
subcellular location analysis of STAT2, its involvements in immune
response of several tissues and TK cell line to V. anguillarum and
LCDV, were also investigated.

2. Materials and methods

2.1. Fish, cell line and total RNA isolation

Turbots weighing about 100 g were obtained from Haiyang
Aquatic Company of Yantai and every six turbots were raised in a
72 l breeding tank with running seawater at 16 °C. The TK cell line,
derived from turbot kidney [33], was cultured in Eagle’s minimal
essential medium (MEM) (Gibco) supplemented with 10% foetal
bovine serum (FBS) (Gibco) at 24 °C.

Eleven tissues including brain, gill, skin, muscle, fin, heart, liver,
spleen, kidney, head kidney and intestine were collected from three
individuals, separately. And total RNAs of tissues were isolated
using Trizol reagent (Invitrogen) according to the manufacturer’s
instructions.

2.2. Turbot STAT2 cloning

Based on the 987-bp partial sequence of turbot STAT2 cDNA
(DQ848884) identified from turbot spleen cDNA library [30],
primers Stat2_51/52, Stat2_31/32 were designed for the 5′ and 3′
RACE followed as the protocol of the BD SMART™ RACE cDNA
amplification kit (BD Biosciences Clontech).

In brief, the first strand cDNA synthesis for 5′RACE/3′RACE was
performed on liver-derived RNA with primers OligodT, 5′-CDS and
3′-CDS, respectively. To obtain the 5′ fragment, primers Stat2_51/
UPM and Stat2_52/NUP were used for the primary PCR and the
nested PCR respectively. Similarly, the 3′ fragment of STAT2 was
obtained by nested PCR with primers Stat2_31/UPM and Stat2_32/
NUP. These two fragments were ligated to pMD-18T vector and
sequenced by ABI 3730 DNA Analyzer.

Finally, these three fragments were joined into the full-length
STAT2 cDNA, which was further confirmed by sequencing the PCR
product amplified with primers Stat2_1 and Stat2_2 within the 5′
and 3′ UTR, respectively.

2.3. Sequence analysis of turbot STAT2

Turbot STAT2 amino acids sequence deduced by Vector NTI 11.5
software was submitted into BLAST program (http://blast.ncbi.
imn.nih.gov) in search of its counterpart sequences. Multiple
sequences alignment of STATs proteins including STAT1, STAT2,
STAT3, STAT4, STAT5 and STAT6 was carried out with ClustalX
program. Subsequently, an unrooted phylogenetic tree was con-
structed with MEGA 5.0, based on proteins alignment.

The phylogenetic tree was tested for reliability by 1000 bootstrap
replications.

2.4. The subcellular location of STAT2 in TK cell line

The TK cell line was used to analyze the subcellular location of
turbot STAT2. Firstly, primers GFPSS2-U1, GFPSS2-D1, GFPSS2-DN1,
GFPSS2-UN2, GFPSS2-U2, GFPSS2-D2, GFPSS2-U3 and GFPSS2-D3 were
designed to amplify the corresponding cDNA of the full-length CDS
region (1–793 amino acids), N terminal region (1–312 amino
acids), N1 terminal region (1–136 amino acids), N2 terminal region
(137–312 amino acids), C terminal region (313–793 amino acids),
predicted NES (nuclear export signal) region (715–793 amino acids)
and C-NES region (313–714 amino acids) of STAT2. Secondly,
these seven fragments were ligated into the vector pEGFP-N3
(BD Biosciences Clontech) based on HindIII/EcoRI sites to construct
seven GFP vectors pSTAT2-GFP1–793, pSTAT2-N-GFP1–136, pSTAT2-N1-GFP1–136, pSTAT2-N2-GFP137–312, pSTAT2-C-GFP313–793, pSTAT2-NES-GFP715–793 and pSTAT2-C-NES-GFP313–714. Finally,
seven vectors were transfected into 12-well plates with Lipofet-
amine 2000 (3 µl:1 µl) follow as the protocol. For the identification
of NES in turbot STAT2, Leptomycin B (LMB, the Crm1-dependent
NES inhibitor) is introduced at 6 h after transfection with a final
concentration of 10 ng/ml as the previous study [34]. At 36 h after
transfection, the transfected cells were rinsed with PBS for 5 min,
fixed with 4% paraformaldehyde for 15 min, rinsed again with PBS
for 5 min, treated with 0.1% Triton X-100 for 5 min, and stained with
DAPI (100 ng/ml) for 10 min.

In order to prove the transcription activity of turbot STAT2,
Poly(I:C) was introduced with a final concentration of 10 ng/ml at 6
h after transfection with pSTAT2-GFP1–793. At 24 h after Poly(I:C)
stimulation, the cells were treated as above method for DAPI staining.

All treated samples were observed and captured on a Nikon
ECLIPSE TE 2000-U fluorescence microscope.

2.5. Pathogen challenge in turbot tissues and TK cells

The bacterium V. anguillarum, which has been shown to be
pathogenic in turbot [35], was cultured at 28 °C to mid-logarithmic
growth on 2216E medium and a final concentration of 7 × 106 cfu
(colony forming units) suspended in 0.9% saline (PS) per fish was
intraperitoneally injected in twenty turbots. And the turbots
injected with PS were used as the control. At 6 h, 12 h, 24 h, 48 h,
72 h and 96 h post injection, three individuals from each point were
anaesthetized and tissues including liver, spleen, kidney and head
kidney were collected.

LCDV was used for virus challenge in present study. The iso-
lation, culture and titration determinations were performed as pre-
vious described [36]. 1 × 107 TCID50 (50% tissue culture infective
dose) LCDV per fish was intraperitoneally injected in twenty tur-
bots. And the turbots injected with PS were used as the control. At
6 h, 12 h, 24 h, 48 h, 72 h and 96 h post injection, three individuals
from each point were anaesthetized and tissues including liver,
spleen, kidney and head kidney were collected.

For TK cells infection, cells in six-well plates were treated for 1 h
with 50 CFU V. anguillarum or 50 TCID50 LCDV suspensions per well.
Three wells were collected for RNA extraction at 6 h, 12 h, 24 h post
V. anguillarum infection and 24 h, 48 h, 96 h post LCDV infection.
In addition, the untreated cells were used as the negative control.

Total RNA was extracted from above samples by Trizol reagent
(Invitrogen) according to the manufacturer’s instructions. Reverse
transcription was carried out with ReverTra Ace (Toyobo) in the
presence of random 9-mer primer and dNTPs for cDNA synthesis.

2.6. The quantitative RT-PCR analysis

To reveal the expression pattern of Stat2 under normal and
challenge condition, the quantitative RT-PCR (qRT-PCR) was...
conducted on an Applied Biosystems 7500 Real-Time PCR System with SYBR® Premix Ex Taq™ (Takara). The cDNA of 11 normal tissues and V. anguillarum/LCDV infected tissues or TK cells were chosen for detection of Stat2 expression pattern with primers Stat2_u and Stat2_d (Table 1). For normalization, the expression of β-actin was used as an internal control with primers actin_u and actin_d (Table 1).

All RT-qPCRs were performed in a 20 μl volume containing 1 μl cDNA sample, 10 μl SYBR® Premix Ex Taq™, 0.4 μl ROX Reference Dye II, 0.4 μl PCR forward/reverse primers (10 μM) and 7.8 μl nuclease free water. The PCR conditions were as follows: 95 °C for 30 s, 40 cycles of 95 °C for 5 s and 58 °C for 34 s. Finally a dissociation curve analysis was added to verify the amplification of a single PCR product. The reaction was carried out with three duplicates for each sample.

In addition, the efficiency of primers’ amplification was detected follow as the dilution method [37]. Briefly, the liver cDNA preparation was diluted over a 20-fold range. For each dilution sample, amplification was carried out with primers of Stat2 and β-actin. A plot of the log cDNA dilution versus ΔCT was made. Slop close to zero means the amplification efficiencies of Stat2 and β-actin are the same. Due to that the absolute value of the slop in present study was 0.087, the 2−ΔΔCT method could be used for the analysis of relative quantification.

Finally, the data were submitted to one-way ANOVA (analysis of variance) test followed by an unpaired, two-tailed t-test. p < 0.01 was considered statistically significant.

3. Results

3.1. Cloning and characterization of turbot STAT2 cDNA

Based on a 987 bp turbot STAT2 fragment, the 5′ and 3′ RACE fragments were acquired according to the BD SMART™ RACE cDNA amplification kit with primers Stat2_51/52, Stat2_31/32 and adaptor primers UPM, NUP. Finally these three fragments were joined into turbot STAT2 cDNA (GenBank number: FJ719015) with length of 3206 bp which consisted of a 5′ UTR of 116 bp, an open reading frame of 2379 bp encoding a polypeptide of 793 amino acids, and a 3′ UTR of 708 bp with a poly (A) tail (Fig. 1).

3.2. Amino acid sequence analysis and phylogenetic relationships

SMART (http://smart.embl-heidelberg.de/) analysis indicated the existence of four domains including STAT_int (STAT protein, protein interaction domain) at 2−122 amino acids, STAT_alpha (STAT protein, all-alpha domain) at 137−312 amino acids, STAT_b (STAT protein, DNA binding domain) superfamily at 314−560 amino acids and SH2 domain at 568−678 amino acids (Fig. 2) in turbot STAT2 protein. Similar structures were found in zebrafish (Danio rerio), goldfish (Carassius auratus) and western clawed frog (Xenopus tropicalis) STAT2 proteins. However, STAT_alpha domain is missing in Atlantic salmon (Salmo salar) STAT2 protein. Besides four conserved domains, human (Homo sapiens), bovine (Bos taurus), horse (Equus caballus), Rhesus monkey (Macaca mulatta) and house mouse (Mus musculus) STAT2 proteins also has STAT2_C domain which contains a nuclear export signal (NES) allowing export of STAT2 into the cytoplasm [38].

Analyzed with BLASTp of NCBI website, turbot STAT2 predicted protein exhibited 64% identity to Atlantic salmon STAT2, 38% identity to human STAT2 and 33% identity to murine STAT2. In further, the phylogenetic analysis (Fig. 3) showed that turbot STAT2 shared the closest relationship with Atlantic salmon (S. salar). All the known fish STAT2 including zebrafish (D. rerio), Atlantic salmon (S. salar), turbot (Scophthalmus maximus) and goldfish (C. auratus) form a different group with other species. Two groups are separated with a bootstrap value of 100%, one including STAT1, STAT2, STAT3 and STAT4, the other including STAT5 and STAT6.

3.3. The subcellular location and identification of functional NES of STAT2 in TK cells

To examine the subcellular location of turbot STAT2, pSTAT2-GFP1−793 was constructed with a green fluorescent protein fused with wild type STAT2 (Fig. 4A). The transfection of pSTAT2-GFP1−793 resulted in uniform distribution of fluorescence in the cytoplasm of TK cells (Fig. 4B).

In order to verify whether turbot STAT2 also contained a NES in the C-terminal similar with human STAT2, NES prediction analysis was conducted according to the NES sequences in NES databases (www.cbs.dtu.dk/ dbs basesP NESbase) [39] and the common

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**Table 1**

<table>
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<tr>
<th>Primers</th>
<th>Sequences</th>
<th>Primer information</th>
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<td>(T)10 VN</td>
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structures of NES: L-x(2,3)-[LIVFM]-x(2,3)-L-x-[LI]. Firstly, turbot C-terminal 79 amino acids sequence (715e793 amino acids) was considered as the predicted NES region. However, the transfection of the corresponding plasmid pSTAT2-NES-GFP715e793 led to a uniform distribution of fluorescence in cytoplasm and nucleus, similar with the pattern of pEGFP-N3 (Fig. 4B).

To identify the functional NES of turbot STAT2, several plasmids were constructed by fusion of GFP with STAT2 truncated motifs. As shown in Fig. 4, transfection of pSTAT2-N-GFP1e312, which expressed a GFP fusion protein with domains STAT_int and STAT_alpha, resulted in strong GFP signals in the cytoplasm with punctate distribution; transfection of pSTAT2-C-GFP313e793 or pSTAT2-C-NES-GFP313e714, which fused GFP protein with STAT_bind, SH2 and other structures, both led to weak GFP signals in the cytoplasm (Fig. 4B). The results showed that the functional NES may be located in the N terminal 1e312 amino acids. So, we constructed two other plasmids pSTAT2-N1-GFP1e136 and pSTAT2-N2-GFP137e312 which contained GFP fusion protein with STAT_int and STAT_alpha, respectively. The transfection assay revealed that pSTAT2-N1-GFP1e136 expressed similar GFP pattern with pEGFP-N3, while pSTAT2-N2-GFP137e312 expressed uniform GFP signals exclusively in the cytoplasm (Fig. 4C), which suggested that N terminal 137e312 amino acids in STAT2 may contained the functional...
Fig. 3. Phylogenetic analysis of STATs protein sequences. A phylogenetic tree was constructed with the neighbour-joining algorithm in MEGA 5.05. The branches were validated by bootstrap analysis from 1000 replications, which were represented by percentage in branch nodes. STATs proteins sequences used in this analysis: Homo sapiens STAT1 (NP_009330), Mus musculus STAT1 (NP_033309), Danio rerio STAT1 (NP_571555), Tetraodon fluviatilis STAT1 (AA09414), Homo sapiens STAT2 (NP_005410), Bos taurus STAT2 (XP_582820), Danio rerio STAT2 (XP_693577), Mus musculus STAT2 (Q9WVL2), Carassius auratus STAT2 (Q0804927), Scophthalmus maximus STAT2 (ACX69848), Sus scrofa STAT2 (BAA20332), Xenopus tropicalis STAT2 (AA67467), Salmo salar STAT2 (NP_001138896), Rattus norvegicus STAT2 (NP_001011905), Pan troglodytes STAT2 (XP_509146), Macaca mulatta STAT2 (XP_001115072), Equus caballus STAT2 (XP_001504891), Homo sapiens STAT3 (NP_644805), Mus musculus STAT3 (AAA19452), Danio rerio STAT3 (AAP58320), Oncorhynchus mykiss STAT3 (NP_001119180), Scophthalmus maximus STAT3 (ACX69847), Homo sapiens STAT4 (NP_003142), Mus musculus STAT4 (NP_035617), Danio rerio STAT4 (NP_035610), Tetraodon fluviatilis STAT4 (AAU9416), Homo sapiens STAT5A (NP_003143), Homo sapiens STAT5B (NP_036598), Mus musculus STAT5A (AAH13274), Mus musculus STAT5B (NP_035619), Danio rerio STAT5.1 (AAE62352), Danio rerio STAT5.2 (AA95392), Oncorhynchus mykiss STAT5 (AAG14946), Takifugu rubripes STAT5 (AA580167), Homo sapiens STAT6 (NP_003144), Mus musculus STAT6 (NP_033310), Tetraodon fluviatilis STAT6 (AAO22057), Danio rerio STAT6 (AA62530).
NES. Also, the introduction of NES inhibitor Leptomycin B (LMB) caused the redistribution of GFP from cytoplasm to nucleus, which further proved that the existence of NES in the N terminal 137–312 amino acids (STAT_alpha domain).

To verify the transcription activity of turbot STAT2, Poly(I:C) was introduced in TK cells transfected with pSTAT2-GFP. As a result, the nuclear translocation phenomenon of GFP signal from the cytoplasm to nucleus was observed (Fig. 4C). It indicates that STAT2 acts as a transcriptional activator in TK cells.

3.4. The expression pattern of turbot Stat2

The qRT-PCR was employed to reveal the expression pattern of turbot Stat2 of tissues or TK cells in normal and pathogen-challenged individuals. The mRNA transcripts of Stat2 mRNA were detected in all tested 11 tissues with higher expression levels in liver, spleen, brain, gill and lower expression levels in heart and muscle (Fig. 5).

In _V. anguillarum_ infected turbot, Stat2 relative expression levels in detected tissues displayed different degree of increase tendency compared with the saline injected individuals (Fig. 6). In liver and

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**Fig. 4.** The subcellular location of turbot STAT2 in TK cells. Part A presents a schematic diagram of wild type STAT2-GFP plasmid and various truncations. Part B shows the subcellular location of STAT2-GFP, STAT2-N-GFP, STAT2-C-GFP, STAT2-C-NES-GFP and STAT2-NES-GFP. The pSTAT2-GFP was uniformly expressed in cytoplasm of TK cell line. The pSTAT2-N-GFP was strongly expressed in cytoplasm of TK cell line with punctate distribution. The pSTAT2-C-GFP and pSTAT2-C-NES-GFP transfected cells showed weak signals in the cytoplasm of TK cells. In pSTAT2-NES-GFP transfected cells, no particular but uniform distribution in cytoplasm and nucleus were observed. Part C showed pSTAT2-N1-GFP expressed similar GFP pattern with pEGFP-N3, while pSTAT2-N2-GFP expressed uniform GFP signals exclusively in the cytoplasm. Also, the introduction of NES inhibitor Leptomycin B (LMB) caused the redistribution of GFP from cytoplasm to nucleus in pSTAT2-N2-GFP transfected cells. In the pSTAT2-GFP transfected cells, Poly(I:C) treatment resulted in the redistribution of GFP from cytoplasm to nucleus.

**Fig. 5.** The expression pattern of Stat2 in turbot different tissues detected by the quantitative RT-PCR. Stat2 mRNA was expressed in all tissues detected with higher expression levels the liver, spleen, brain, gill and heart. The β-actin gene was used as an internal control to calibrate the cDNA template for all the samples. Vertical bars represented the mean ± S.D. (n = 3).
kidney, Stat2 expression levels were significantly increased at 6 h \((p < 0.01)\), 12 h \((p < 0.01)\) and 24 h \((p < 0.01)\) after infection, while the expression levels decreased to the control level from 48 h post infection till 96 h. In spleen and head kidney, Stat2 expression was significantly up-regulated only at 6 h \((p < 0.01)\) after infection.

After LCDV infection, Stat2 expression level in liver was significantly up-regulated at 36 h \((p < 0.01)\), 48 h \((p < 0.01)\) and 96 h \((p < 0.01)\) respectively. And in kidney, Stat2 expression level was significantly up-regulated at 12 h \((p < 0.01)\), 48 h \((p < 0.01)\) and 96 h \((p < 0.01)\) respectively.

In TK cells, Stat2 expression level was significantly up-regulated at 12 h \((p < 0.01)\) and 24 h \((p < 0.01)\) after V. anguillarum infection. Stat2 expression level was significantly up-regulated at 24 h \((p < 0.01)\), 48 h \((p < 0.01)\) and 96 h \((p < 0.01)\) after LCDV infection (Fig. 7).

4. Discussion

STAT2 is an important molecule involved in the type I interferon signalling pathway, which is the key inducer of both innate and adaptive immunity to viruses [40,41]. Although STAT2 gene has been predicted by bio-information analysis from zebra fish genome [42], no STAT2 homologue was cloned from fish [42,43] until the reports in Atlantic salmon [26] and goldfish [27].

In this paper, turbot STAT2 homologue was cloned and identified, which further confirmed the existence of STAT2 in fish. Similar with other species STAT2, the putative 793 amino acids sequences of turbot STAT2 contained four conserved domains: STAT_int2−312, STAT_alpha137−312, STAT_bind314−560 and SH2568−678. In further, the phylogenetic tree analysis clusters turbot STAT2 and the other known fish STAT2 genes as one branch. All these results convinced that the turbot STAT2 was indeed a STAT2 homologue. Similar with Atlantic salmon Stat2 [26], turbot Stat2 transcripts were also detected in various tissues without any stimulation. The up-regulation of Stat2 after pathogen infection in several immune tissues and TK cells suggested that turbot STAT2 plays important roles in immune response. The maximal fold increase of turbot Stat2 was mainly at 6 h in V. anguillarum challenged tissues and at 48 h in LCDV challenged tissues. In pathogen infected TK cell line, the Stat2 response time in virus infection is also later than one of bacterial infection. This interesting phenomenon is similar with previous findings [44,45] which showed that Poly I: C induced Mx responses were more intense and longer lasting than those induced by the bacterial in Atlantic salmon.

The subcellular location revealed that the green fluorescence of pSSTAT2–GFP1−793 was uniformly distributed in the cytoplasm of TK cells, which is similar with the immunofluorescence location of human STAT2 [24,25]. The truncation analysis of human STAT2 demonstrated that deletion of the N-terminal 59 amino acids of human STAT2 completely prevented tyrosine phosphorylation and deletion of the C-terminal 50 amino acids killed all induced transcriptional activity [11]. STAT_alpha domain is also important for the phosphorylation of STAT2 and for the STAT2-independent phosphorylation of STAT2 [16]. STAT_binding domain contains several β-sheets and determines DNA sequence specificity of individual STATs. SH2 domain is a well-known common structural motif, which mediates dimerization via SH2-phosphotyrosyl peptide interactions [46]. In present study, turbot STAT_int2−312 domain and C terminal 68 (715−793) amino acids were both located in the cytoplasm and nucleus with uniform distribution, and STAT_alpha137−312 was exclusively distributed in the cytoplasm, which implied their possible roles in specific regions. Nevertheless, it is worth to investigate whether these domains exhibit similar functions in turbot.

The higher vertebrate STAT2 including human (H. sapiens), mouse (M. musculus), bovine (B. taurus), horse (E. caballus) and Rhesus monkey (M. mulatta) all contain the domain STAT2_C in the C-terminal which contains a NES allowing export of STAT2 into the cytoplasm (Fig. 2). In order to prove whether turbot STAT2 also contained the NES in the C-terminal, turbot putative NES (LLRR-LNN-PN-L) within 715−793 amino acids was screened out.
the common structure of NES sequences. And the translocation of turbort STAT2 from the cytoplasm to nucleus indicates the transcription activity of STAT2 in TK cells. As a transcription factor, turbort STAT2 may also contain nuclear localization signal (NLS) as well as NES for its shuttle function like other proteins [47,48]. The identification and detailed location of these two signals will aid in the understanding the interaction mechanism of turbort STAT2 with other genes and pathogen.

In conclusion, the cloning and identification of turbort STAT2 are described in present study for the first time. It not only further proves the existence of STAT2 homologue in teleosts, but also provides a new target for study the fish JAK–STAT pathway and the control of turbort diseases. In future, the exploration of more pro- teins involved in STAT2 pathway and pathogen—host interaction will help to develop the new strategy for fish diseases treatment.

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