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The JAK and STAT family members of the mandarin fish *Siniperca chuatsi*: Molecular cloning, tissues distribution and immunobiological activity

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ABSTRACT

The JAK/STAT signal transduction pathway plays a critical role in host defence against viral and bacterial infections. In the present study, we report cDNA cloning and characterization of the JAK family (mJAK1-3 and mTYK2) and STAT family members (mSTAT1, mSTAT3-6) from the mandarin fish *Siniperca chuatsi*. To our knowledge, JAK2, TYK2 and STAT6 genes were cloned from fish for the first time. The mJAK family proteins consist of 1112–1177 residues with a FERM domain, an SH2 domain, a pseudokinase domain, and a tyrosine kinase domain. The mSTAT family members contain 716–786 residues with similar architecture, including an N-terminal domain, a coiled coil domain, a DNA binding domain, a linker domain, an SH2 domain, and a transcription activation domain. Multiple sequence alignments of mJAKs/mSTATs and phylogenetic analysis showed that mJAK1 was closed to mTYK2, and mJAK2 was closed to mJAK3. Quantitative real-time PCR results revealed that mJAK/mSTAT family members were expressed in most tissues examined except muscle. In mandarin fish fry cells, the expressions of IRF-1, Mx, SOCS1 and SOCS3 genes were significantly induced by poly(I:C) stimulation, indicating that the mJAK/mSTAT signal pathway is activated by poly(I:C). Furthermore, expressions of all four mJAKs and four mSTATs were all up-regulated after poly(I:C) stimulation, but expression of mSTAT5 was inhibited by poly(I:C). These results suggest that mandarin fish has the JAK/STAT signal transduction pathways similar to those in mammals, and these signalling pathways may play an important role in regulation of antiviral responses in fish.

1. Introduction

The Janus kinase (JAK) – signal transducer and activator of transcription (STAT) signal transduction pathway is activated by a large number of cytokines and growth factors, including interferon (IFN)-α/β, IFN-γ, interleukin-2 (IL-2), IL-4, IL-5, IL-6, IL-10, growth hormone (GH), and so on [1,2]. In mammals, the JAK/STAT signaling pathway plays a significant role in mediating biological activities such as growth, survival, differentiation, and pathogen resistance [3].

The JAK/STAT pathway is based on two families of proteins: JAKs and STATs. JAK family members are cytosolic protein tyrosine kinases associated with membrane receptors [4]. In mammalian cells, the JAK family contains four members: JAK1, JAK2, JAK3 and TYK2 [5]. The JAK family members share a common architecture: a FERM (Band 4.1, ezrin, radixin, moesin homology) domain associated with cytokine receptors, a conserved Src homology 2 (SH2) domain that lacks affinity for phosphotyrosine-containing proteins, a pseudokinase domain which might regulate activity of the tyrosine kinase domain, and a tyrosine kinase domain [6]. Activated JAKs phosphorylate tyrosine residues of cytokine receptors to create docking sites for specific signaling molecules, including members of the STAT family [7]. STATs are a family of cytoplasmic latent transcription factors that are activated to regulate gene expression in response to a large number of extracellular signals [8]. STAT family contains seven members, including STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6. The STAT family members share conserved domain structure: an N-terminal domain, a coiled coil domain, a DNA binding domain, a linker domain, an Src homology 2 (SH2) domain and a transcription activation domain [9]. STATs become activated after binding to an activated cytokines receptor, thereby phosphorylated by the activated JAKs. The phosphorylated STATs then dissociate from the receptors, and form an activated dimer or tetramer, which translocates to the cell nucleus and bind to specific DNA element to induce transcription of target genes [10–12].

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JAK and STAT family members have been identified from *Drosophila* to human, and they play a significant role in cytokines signaling [13]. Recently, many cytokines have been identified in a variety of fish species, including IL-1β [14], IL-4 [15], IL-6 [16], IL-8 [17], IL-10 [18], IL-11 [19], IL-15 [20], IL-18 [21], IL-22 [22], IL-26 [23], type I interferon, IFN-γ [24]. Many molecules associated with the JAK/STAT signal transduction pathway have also been reported in fish, such as interferon regulatory factor-1 (IRF-1) [25], IRF-3 and IRF-7 [26], and STAT1 [27]. Moreover, several genes downstream of the JAK/STAT signaling pathway have been cloned and characterized in fish, including Mx [28], SOCS1, SOCS2, and SOCS3 [29,30]. However, the role of JAK and STAT members in fish antiviral immune responses is still not well understood.

The mandarin fish, *Siniperca chuatsi* (Basilewsky), is widely cultured and has a relatively high market value in China [31,32]. However, outbreaks of diseases caused by parasites, bacteria and viruses have greatly threatened the aquaculture industry [33]. Research has been conducted to understand the immune system in mandarin fish [34–38]. In this study, we report cDNA cloning, characterization, and tissue distribution of the JAK/STAT family members from the mandarin fish. JAK2, TYK2 and STAT6 genes were cloned for the first time from fish, and the expression profiles of the JAK/STAT members in mandarin fish fry cells after poly(I:C) stimulation were also investigated.

2. Materials and methods

2.1. Cells and animals

Mandarin fish fry (MFF-1) cell line was constructed in our lab and cultured in Dulbecco's modified Engle's medium (DMEM) (Gibco, USA) supplemented with 10% foetal bovine serum (FBS) (Gibco, USA) at 27 °C under humidified atmosphere containing 5% CO_2 [39]. When the cells reached 5×10^6 density, they were harvested for RNA extraction.

For immune challenged experiments with polyinosinic: polycytidylic acid [poly(I:C), Sigma , USA], MFF-1 cells were cultured in 6-well plates at 2×10^6 cells per well overnight before further treatment. Cell culture supernatants were replaced with DMEM medium supplemented with 100 μg/ml^{-1} poly(I:C) and cells were harvested at various times (2 h, 4 h, 6 h, 8 h, 12 h, 16 h, 24 h, 48 h, 72 h, 96 h and 120 h) after treatment by centrifugation.

Healthy mandarin fish (weight ~250 g) were obtained from a fish farm in Nan Hai (Guangdong Province, China) and maintained for at least two weeks in aquaria at 28 °C. Tissues including gonad, liver, gill, brain, spleen, heart, intestine, hind kidney, head kidney and muscle were dissected from fish for RNA extraction immediately.

2.2. Cloning of partial mJAK/mSTAT cDNAs

To amplify the conserved sequence of mJAK1, two-step PCR was performed using the degenerated primers based on the conserved amino acid sequences of known JAK1. Total RNA was extracted from MFF-1 cells using Trizol reagent (Invitrogen, USA) according to the manufacturer's protocol, and then treated with RNase-free DNase (Promega, USA) to remove contaminating DNA. cDNA was synthesized from 1 μg of total RNA with murine leukaemia virus reverse transcriptase (MLVRTase) (Promega, USA) following the manufacture's instruction using an Oligo $(dT)_{18}$ primer. cDNA from MFF-1 cells was used as template for nested PCR reactions. The initial PCR was performed with degenerated primer JAK1-F1 and -R1 (Table 1) to amplify the conserved sequence of mJAK1. Nested PCR was performed using the product of initial PCR as template with degenerated nested primer JAK1-F2 and JAK1-R2 (Table 1). The two PCR reactions were performed using the same conditions: 1 cycle of denaturation at 94 °C for 2 min, 30 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 3 min, followed by a 10 min extension at 72 °C. The same strategy was applied to amplify the conserved sequences of other mJAKs (mJAK2, mJAK3 and mTYK2) and mSTATs (mSTAT1, mSTAT3, mSTAT4 and mSTAT5) using the corresponding primer pairs listed in Table 1. Finally, the nested PCR products were subcloned into pGEM-T Easy vector (Promega, USA) and sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Perkin–Elmer, USA) on an Applied Biosystems model 3730 automatic DNA sequencer by Invitrogen Inc (Shanghai, China).

Table 1
Primers used for cDNA cloning of mJAKs/mSTATs conserved regions.

Name	Sequence (5′-3′)
For initial PCR	
mJAK1-F1	ATWGARAACGAGTGYTYGGGHATGGC
mJAK1-R1	CCATGDACCADSTTCTTMTCCTCC
mJAK2-F1	CCGAARCTCCADACRTCTGAVGCCAC
mJAK2-R1	AGRTASTCCATDCCCTTGCAKATCTG
mJAK3-F1	GCSGACCTTTCMTWYTGGTATCCHCC
mJAK3-R1	TGATGTCRCAGTAGGWGAAGAGCTCG
mTYK2-F1	CGCATGAGRTAYTATTTYCRGAAYTGGC
mTYK2-R1	GTBACCCCRAAGGACCARACATCWGAAGC
mSTAT1-F1	GTGGAGAGRCARCCHTGCATGCC
mSTAT1-R1	GTGGARCCSTACACVAAGAAGGAGCT
mSTAT3-F1	ATGGCBCAGTGGAAYCAGYTVCAGC
mSTAT3-R1	GCCAGCTCARCACCTCHGCCAC
mSTAT4-F1	ATYTGTAYGAYGACAACTTYCCHATGG
mSTAT4-R1	GCCTCGTCYTTSGGGATGTC
mSTAT5-F1	ATGGCHGTGTGGATHCARGCC
mSTAT5-R1	TCWGCCAGRGAVCGRATRGAGAAGTC
mSTAT6-F1	GAGAAGCAGCCBCCYCAGGTC
mSTAT6-R1	CACATATGCAATGGTGATGCC
For nested PCR	
mJAK1-F2	TGATGCGDCAGGTBTCYCACAAACAC
mJAK1-R2	GTGTTTGTGRGAVACCTGHCGCATC
mJAK2-F2	AGARTMAGGTWCTATTTYCCYGGCTGGTAC
mJAK2-R2	TCAGGARGARTGYGGTTWTGGC
mJAK3-F2	TGGCAGTGCTGGATYTGTGG
mJAK3-R2	GCARGTACTCCATNCCCTTACAKATCTG
mTYK2-F2	CWGTTTYAARAACGAGAGCCTKGGMATGG
mTYK2-R2	AGGTACKCCATDCCCTCRCAGATYTGCTG
mSTAT1-F2	AAGGRTTTMGNAAGTTYAACATHTTGGG
mSTAT1-R2	CTGAABCVSAGCAGGAASGTYCC
mSTAT3-F2	TTYCCCATGGARCTRCGVCAGTT
mSTAT3-R2	GGRTGRTTSGTSAGCATGTTMTACC
mSTAT4-F2	GCATGCARGAACAGGGDCCDCTGG
mSTAT4-R2	AGCCAYCTBGGAGGRATMACCTTCACCTG
mSTAT5-F2	CACTTYCCCATHGAGGTGCG
mSTAT5-R2	GGYTCDGCAAARGCRTTGTCCC
mSTAT6-F2	AAGTTYTCCACCACWGTRCGC G
mSTAT6-R2	CTKCTKCGCTTCAGTGACTCWGA

R = A/G; Y = C/T; M = A/C; K = G/T; S = C/G; W = A/T; B = C/G/T; D = A/G/T; H = A/C/T; V = A/C/G.

2.3. Rapid application of cDNA ends (RACE)

RACE was performed for mJAKs/mSTATs using the GeneRacer™ Kit (Invitrogen, USA). Total RNA was extracted from the MFF-1 cells as described above. After verifying quantity and purity, total RNA (5 μg) was reverse transcribed into RACE-ready first-strand cDNA following the manufacture's instruction. In order to obtain the full-length cDNAs of mJAKs/mSTATs, two rounds of RACE-PCR were performed using the corresponding primers pairs (Table 2). The first round of 5′ or 3′ RACE was performed using mJAK1_5′-R1/GeneRacer_5′-F1 or mJAK1_3′-F1/GeneRacer_3′-R1, respectively (Table 2). First round PCR was performed using the following conditions: denaturation at 94 °C for 2 min, then 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 2 min, followed by a 10 min extension at