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Molecular cloning of rat JAK3, a novel member of the JAK family of protein tyrosine kinases

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Abstract

We have cloned and sequenced a cDNA (JAK3) encoding a novel member of the JAK family of protein tyrosine kinases. JAK3 was identified by RT-PCR of rat mesangial cells using degenerate oligonucleotide primers, and a full-length clone was isolated from a rat spleen cDNA library. The primary structure of JAK3 showed cDNA with an open reading frame of 1,100 amino acids which comprises the PTK catalytic domain and a second kinase-related domain characteristic for JAK kinase. JAK3 was phylogenetically shown to be most closely related to JAK2 among the previously known JAK family members, JAK1, JAK2 and Tyk2. Southern analysis revealed that JAK3 is a single copy gene and well conserved in the vertebral genome. Northern analysis indicated that the 4.0 kb mRNA was transcribed in a variety of tissues including spleen, lung, kidney and intestine.

Key words: JAK3; Protein tyrosine kinase; cDNA cloning; Molecular evolution; RT-PCR

1. Introduction

Protein tyrosine kinases (PTKs) play critical roles in the signal transduction pathways that initiate cellular proliferation or cellular differentiation in response to external stimuli [1]. In general, PTKs can be grouped into two classes based on their cellular location, sequence similarities and structural characteristics. The first class of kinases are ligand-binding receptors such as receptors for PDGF, FGF, NGF and EGF. The receptor tyrosine kinases are membrane proteins that have ligand-binding extracellular domains and cytoplasmic catalytic domains. The second class of kinases are non-receptor PTKs, which include the src subfamily and abl subfamily. This class of PTKs transduces extracellular signals by binding to integral membrane proteins such as the B-cell antigen receptor and the T-cell receptor complex, CD4, or CD8 molecule. Recently, another family of non-receptor PTK designated as JAK has been defined and characterized [2]. To date, three members of the JAK family kinases; i.e. JAK1, JAK2, and Tyk2, have been so far isolated and characterized [2–4]. In the primary structure of all members of JAK family kinases, the characteristic second kinase-related domain locates in the N-terminal region of the tyrosine kinase domain, although the enzymatic specificity of this kinase-like domain has remained an enigma. Quite recently these members of JAK family were proved to play a central role in the signal transduction through IFN receptor [5–7], IL-3 receptor [2], erythropoietin (EPO) receptor [8], growth hormone (GH) receptor [9], interleukin-6 signal transducer gp130 [10], and LIF receptor β [11]. In these systems, JAK kinases function as the most proximal kinases activated in response to growth factors or cytokines and are involved in the phosphorylation of the transcription complex that initiate the factor- or cytokine-induced gene expression [6,10,12,13] (reviewed in [14]). Corresponding to various signals of distinct ligands, a novel kinase was predicted and expected to be a member of JAK family.

In this communication, we report the isolation of a novel member of the JAK protein tyrosine kinases designated as JAK3. We used the reverse transcription-polymerase chain reaction (RT-PCR) using degenerate primers to identify potential kinases in rat mesangial cells. One of the three novel PTK sequences was identified as a new member of JAK kinases according to its sequence similarity. We show here that JAK3 has the characteristic structure and expression of JAK family protein tyrosine kinases.

2. Materials and methods

2.1. Polymerase chain reaction

Degenerate oligonucleotides, 5′-CA(T/C) CGI GA(T/C) (T/C)TI GCI (G/A)CI (C/A)G-3′ and 5′-A(T/C)I CCI (T/A)(G/A)I (G/C)(T/A)C CAI AC(G/A) TC-3′, deduced from the conserved amino acid

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sequences of PTK domain VI (HRDLAAR) and domain IX (DVWSFGV) were used as primers in a PCR reaction with the first strand cDNA reverse-transcribed from mRNA of a rat renal mesangial cell line. The reaction was 48 cycles of denature for 45 s at 96°C, anneal for 4 min at 50°C and extension for 3 min at 72°C. The amplified fragment was blunted and cloned into Bluescript (Stratagene, La Jolla, CA). DNA sequencing was carried out in both directions using double-strand plasmid and Sequenase (US Biochemical Corp.). The deduced amino acid sequences of PCR fragments were compared to known sequences in NBRF database. One of the novel sequences which showed significant homology to JAK family PTK was used as a hybridization probe for screening of the cDNA library.

2.2. Cloning of JAK3 cDNA clone and sequence analysis

cDNA libraries used in this study were rat brain cDNA library in λZAP [16], rat lung cDNA library in λgt11 (Clontech, #RL1048a), and rat spleen cDNA library in λgt10 (Clontech, #RL1050a). Partial-length cDNAs, JB-1 (1.8 kb), JL-3 (2.2 kb) and JS-1 (3.6 kb), were cloned from brain, lung, and spleen cDNA library, respectively. The 5′ region of cDNA, JS-44 (0.3 kb), was cloned from spleen cDNA library probing 0.2 kb *Eco*RI–*Acc*I fragment of JS-1. DNA sequencing was carried out in both directions using single-strand M13 phage and Tth polymerase (Pharmacia, Milwaukee, WI). Sequence alignment and a phylogenetic tree were made using the Clustal method contained Lasergene software package (DNASTAR, London, UK).

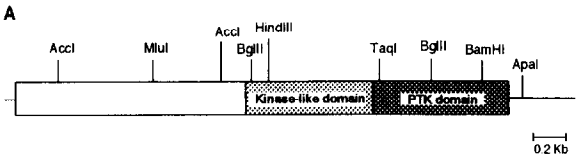
2.3. Southern blot analysis and RNA blot analysis

The filter of zooblot was purchased from BIOSIS (New Haven, CT). High molecular weight DNA was prepared from rat liver as described [15]. Hybridization was performed as described previously [16]. Zooblot filter was washed at 50°C in 2 × SSC and 0.5% SDS. The filter of rat genomic DNA was washed at 65°C in 0.5 × SSC and 0.5% SDS. Washed filters were then exposed to Kodak XAR-5 films with intensifying screens. For RNA blot analysis, tissue blot was purchased from BIOS (New Haven, CT). Washing was performed at 65°C in 0.5 × SSC and 0.5% SDS. The probe used for Southern analysis was a 420 bp *Hin*dIII–*Nar*I (1,766–2,182) fragment. The probe used for RNA blot analysis was a 2,010 bp *Hin*dIII–*Eco*RI (1,766–3,777) fragment.

3. Results and discussion

3.1. Cloning of JAK3, a novel member of the JAK family protein tyrosine kinases (PTK)

The potential tyrosine kinases expressed in rat mesangial cells were identified by RT-PCR using degenerative oligonucleotides corresponding to the consensus motifs of PTK catalytic domain (T. Takahashi, in preparation). One of the three novel PTK sequences was identified as a new member of the JAK family according to its amino acid sequence homology and used as a probe. Screening of a rat spleen cDNA library allowed the isolation of several overlapping clones which covered the entire coding region of the new JAK family member designated as JAK3 (Fig. 1). The cDNA contained an open reading frame of 3,300 bp and the 5′ end had an in-frame stop codon before the first ATG codon (nucleotide sequence is available in the GSDB/DDBJ/EMBL/NCBI databases). The JAK3 open reading frame encodes 1,100 amino acids with a calculated molecular mass of 123 kDa and isoelectric point of 6.5 (Fig. 1). A database search revealed that the rat JAK3 gene is closely related to the murine JAK2 [4], human JAK1 [2], and human Tyk2 genes [3]: 54%, 47% and 52% identity at the nucleotide level in the coding region, and 47%, 38% and 38% identity at the amino acid level, respectively (Fig. 2). In the 580-amino acids C terminus, JAK3 shows PTK catalytic domain and second kinase-related domain (Fig. 1), which are characteristic of JAK kinase (Fig. 2). In the kinase-related domain, several consensus sequences for the kinase domain are leaky; i.e. it shows the replacement of consensus GXGXXG to GXGXXT in subdomain I (amino acids 639–644 in Fig. 2), the replacement of I to L in subdomain IV (amino acid 715), the replacement of consensus HRDLAARN to HGNVCXXNXLLAR in subdomain IV (amino acids 777–789), the replacement of consensus DFG to DPG in subdomain VII (amino acid 806), and the replacement of consensus DVW to DKW in subdomain IX (amino acid 843). All these features suggest a different catalytic specificity for this domain, if it functions, from tyrosine or serine/threonine kinase, although the real function of this putative domain remains still undefined. Alternatively, this domain may function as a regulatory domain in the absence of the SH2 domain as proposed by Wilks [2]. The 520-amino acid N terminus, on the other hand, is less conserved among members and lacks obvious SH2 or SH3 domains which is a characteristic structure in other families of non-receptor PTK.



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MAPPSEETPL ISQRSCSLSS SEAGALHVLL PPRGPGPPQR LSFSFGDYLA   50
EDLCVRAAKA CGILPVYHSL FALATEDLSC WFPPSHIFSI EDVDTQVLVY  100
RLRFYFPGWF GLETCHRFGL HKDLTSAILD VHVLEHLFAQ HRSDLVSGRL  150
PVGLSLKDQG EFLSLAVLDL AQMARKQAQR PGELLKSVSY KACLPPSLRD  200
LIQGQSFVTR RRIRRTVVQA LAPCSSLPSR PYALMAKYIL DLERLHPAAT  250
TESFLVGLPG AQEEPGCLRV TGDNGIAWSS KDQELFQTFC DFPEIVDVSI  300
KQAPRVGPAG EHRLVTITRM DGHILEAEFP GLPEALSFVA LVDGYFRLIC  350
DSRHFFCKEV APPRLLEEEA ELCHGPITLD FAIHKLKAAG SLPGSYILRR  400
SPQDYDSFLL TACVQTPLGP DYKGCLIRQD PSGAFSLVGL SQLHRSLQEL  450
LTACWHSGLQ VDGTALNLTS CCVPRPKEKS NLIVVRRGRN PTPAPGHSPS  500
CCALTKLSFH TIPADSLEWH ENLGHGSFTK IFHGHRREVV DGETHDTEVL  550
LKVMDSRHQN CMESFLEAAS LMSQVSYPHL VLLHGVCMAG DSIMVQEFVY  600
LGAIDTYLRK RGHLVPASWK LQVTKQLAYA LNYLEDKGLP HGNVSARKVL  650
LAREGVDGNP PFIKLSDPGV SPTVLSLEML TDRIPWVAPE CLQEAGTLNL  700
EADKWGFGAT TWEVFSGAPM HITSLEPAKK LKFYEDRGQL PALKWTELEG  750
LIAQCMAYDP GRRPSFRAIL RDLNGLITSD YELLSDPTPG IPNPRDELCG  800
GAQLYACQDP AIFEERHLKY ISLLGKGNFG SVELCRYDPL GDNTGPLVAV  850
KQLQHSGPEQ QRDFQREIQI LKALHCDFIV KYRGVSYGPG RQELRLVMEY  900
LPSGCLRDFL QRHRARLHND RLLLFAWQIC KGMEYLGARR CVHRDLAARN  950
ILVESEAHVK IADFGLAKLL PLGKDYYVVR VPGQSPIFWY APESLSDNIF 1000
SRQSDVWSFG VVLYELFTYS DKSCSPSTEF LRMIGPEREG SPLCHLLELL 1050
AEGRRLPPPS TCPTEVQELM QLCWSPNPQD RPAFDTLSPQ LDALWRGSPG 1100
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Fig. 1. Primary structure of rat JAK3. (A) Scheme of the JAK3 cDNA clone. The box indicates the open reading frame. Shaded boxes indicate kinase-related domain and PTK domain, respectively. The relevant restriction enzyme sites are shown. (B) Deduced amino acid sequence of rat JAK3. Amino acid residues are numbered on the right. The nucleotide sequence is available from GSDB/DDBJ/EMBL/NCBI databases under the accession number D28508.

3.2. Molecular evolution of JAK kinase family

To analyze an evolutional relationship among the members of the JAK family, a phylogenetic tree on the

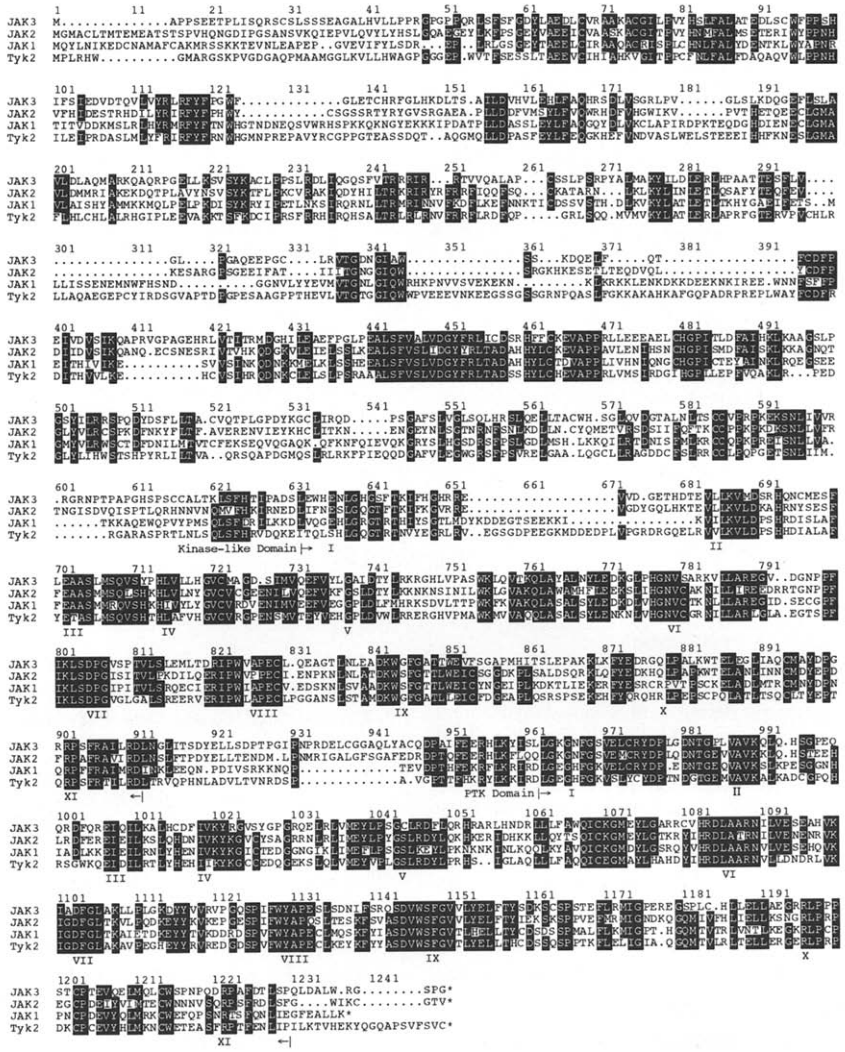

Fig. 2. Amino acid alignment among members of the JAK family. The amino acid sequence (in single-letter code) of rat JAK3 has been aligned with those of human JAK1 [2], mouse JAK2 [4] and human Tyk2 [3] using a computer program contained in Lasergene; dots denote gaps that have been introduced to maximize the alignment. Positions at which at least three of the sequences are identical are shown by black boxes. The proposed kinase-related domain and PTK catalytic domain structure are marked below the sequences according to the subdomain structure of the kinase [18].

basis of amino acid homology was made (Fig. 3). The tree shows that JAK3 is most closely related to JAK2 and that JAK1, JAK2, and JAK3 have evolved from the same ancestor with a relative distance of 32.5 while Tyk2 has evolved from the original ancestor with a relative distance of 46.5. Since these members are all involved in various cytokine signal systems, it is intriguing to find out how they have shared and differentiated their biological role in the signal transduction of various cytokine systems during the molecular evolution. To further investigate the molecular evolution of the JAK3 gene in the invertebral and vertebral genome, Southern blot analysis was carried out (Fig. 4). In rat genome the JAK3 cDNA probe specifically detected 12 kb *Eco*RI, 4.5 kb *Bam*HI, and 20 kb *Hin*dIII fragments (Fig. 4B), indicating that JAK3 is a single-copy gene in rat genome and that the JAK3 probe does not hybridize to the other members of the JAK family. In various organisms, the JAK3 probe detected a homologous locus in the genome of human, mouse, chicken, snake, cow, and frog, but no band in fish, mussel, fruit fly, nematode, and yeast (Fig. 4A), suggesting that JAK3 is evolutionally conserved in the genome of mammals, birds and reptiles, but failed to be detected in the genome of fish, invertebrates and yeast.

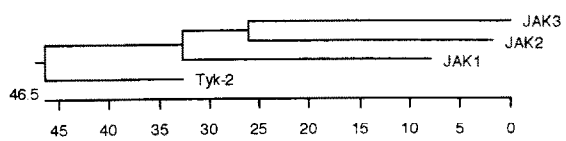

Fig. 3. Phylogenetic tree of JAK kinase. The entire sequence of rat JAK3 was compared to those of mouse JAK2, human JAK1 and human Tyk2. The branch order represents the structural similarity and the branch length represents the sequence divergence. The scale beneath the tree measures the relative distance between sequences.

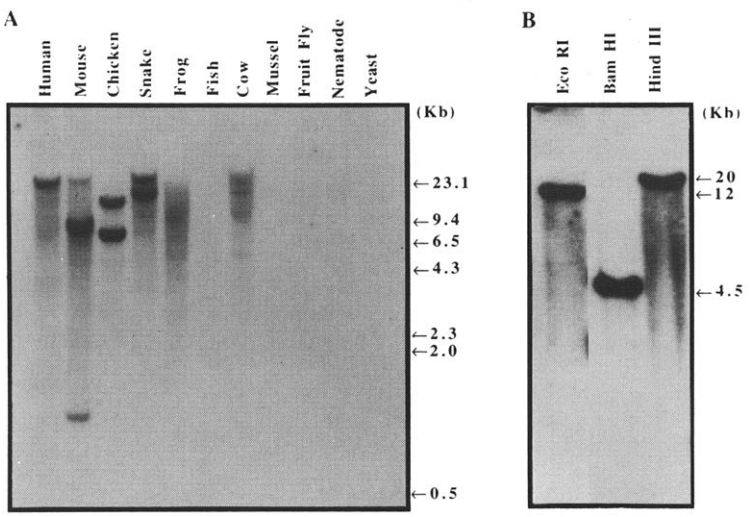

Fig. 4. Southern blot analysis of JAK3. (A) Ten μg of genomic DNA prepared from human, mouse, chicken, snake, frog, fish, cow, mussel, fruit fly, nematode tissues, and yeast were digested with *Eco*RI, electrophoresed, blotted onto a nylon membrane, and hybridized to a ^{32}P-labeled JAK3 cDNA probe. The filter was washed under a mild conditions. (B) Ten μg of rat genomic DNA digested with *Eco*RI, *Bam*HI, or *Hin*dIII were electrophoresed, blotted, and hybridized to a ^{32}P-labeled JAK3 cDNA probe. The filter was washed under stringent conditions.

3.3. Tissue expression of JAK3

To determine the size of JAK3 mRNA and its tissue distribution, we carried out an RNA blot analysis using JAK3 cDNA as a probe. As shown in Fig. 5, a single 4.0 kb mRNA was detected as the major band in various rat tissues. The highest level of expression was observed in spleen, lung and kidney, and lower levels in heart, liver, brain and intestine. Since JAK kinase plays an important role in various cytokines signal transduction systems, it is reasonable that JAK3 is expressed in hematopoietic cells, splenic macrophages, alveolar macrophages and thymic epithelial cells (unpublished observation). Interestingly, JAK3 shows a similar spectrum of expression as JAK1 [2] and JAK2 [17] except in the skeltal muscle and intestine, suggesting that JAK3 may basically function in association with other members of the JAK kinases while in some tissues all members of JAK kinase family may not necessarily be required for the signal transduction of certain system.

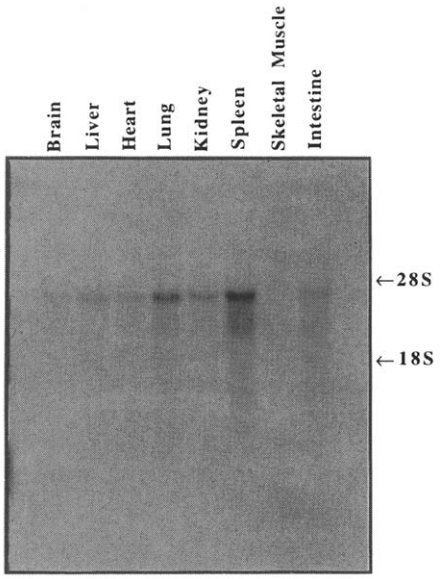

Fig. 5. Expression of JAK3 in rat various tissues. Twenty μg of total RNA prepared from rat brain, liver, heart, lung, kidney, spleen, skeltal muscle and intestine were electrophoresed, blotted onto a nylon membrane, and hybridized to a ^{32}P-labeled JAK3 probe. The filter was exposed for 48 h to Kodak XAR-5 film.

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