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Genomic Sequence, Organization, and Chromosomal Localization of Human *JAK3*

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Members of the Janus (JAK) protein tyrosine kinase family including JAK3 have recently emerged as important components in cytokine signal transduction. Mutations of JAK3 have been found in a number of patients who present with severe combined immunodeficiency. To facilitate the further identification of JAK3–SCID patients and to understand the structure of JAK3 better, we undertook the determination of the genomic sequence, organization, and chromosomal localization of the *JAK3* gene. The *JAK3* gene was found to consist of 19 exons and 18 introns. Interestingly, the organization of the kinase–(JH1) and pseudokinase–(JH2) domains were found to be dissimilar. In addition, the *JAK3* gene was localized to human chromosome 19p13.1. These data should facilitate the identification of patients with this new form of immunodeficiency and will provide insight into the structure of this kinase.

INTRODUCTION

Cytokines and hormones that bind to members of the hematopoietic cytokine receptor superfamily (Bazan, 1990) have critical functions in regulating the growth and differentiation of a variety of cells. Cytokine receptors lack intrinsic kinase domains but nonetheless, ligand binding induces the rapid tyrosine phosphorylation of a variety of intracellular substrates (Taniguchi, 1995; Ihle *et al.*, 1994). Members of the Janus family (JAK) of protein tyrosine kinases (PTKs) have emerged as key elements in the signal transduction mechanism of cytokine receptors (Velazques *et al.*, 1992).

There are four mammalian JAKs. In contrast to JAK1, JAK2, and Tyk2, which have ubiquitous tissue expression, JAK3 is preferentially expressed in hematopoietic cells (Kawamura *et al.*, 1994). It is activated in response to IL-2 and related cytokines (Johnston *et al.*, 1994, Tortolani *et al.*, 1995; Musso *et al.*, 1995, Witthuhn *et al.*, 1994). JAK3 binds to γc, a subunit that is a component of the receptors for a number of cytokines including IL2, IL4, IL7, IL9, and IL15 (Johnston *et al.*, 1995; Russell *et al.*, 1994). Consequently, JAK3 is activated by these cytokines.

Mutations of γc have been shown to result in X-linked severe combined immunodeficiency (X-SCID) (Noguchi *et al.*, 1993; Leonard *et al.*, 1994; Tassara *et al.*, 1995). The intimate association of JAK3 and γc led to the search for patients with immunodeficiency due to mutations of JAK3. The identification of such patients has recently been reported (Macchi *et al.*, 1995; Russell *et al.*, 1995). Like X-SCID patients, patients with JAK3 deficiency also presented with persistent severe infections in early life and were found to lack T and NK cells. Although B cells were present, the patients have hypoglobulinemia. These findings underscore the requirement for JAK3 in the proper development and function of the immune system.

In view of its fundamental role in this form of immunodeficiency and to facilitate the identification of additional patients, it was important to define the genomic sequence and chromosomal localization of JAK3. We report here the complete genomic sequence of the *JAK3* gene and show that it comprises 19 exons and 18 introns. Additionally, we localized the *JAK3* gene to chromosome 19p13.1.

MATERIALS AND METHODS

Genomic cloning. Charon 4A human genomic λ phage library was obtained from Dr. T. Maniatis (Harvard Medical School). Briefly, 5×10^5 PFU of phage were plated and duplicate filters were probed with a ^{32}P-labeled PCR product from the 5′ end of JAK3 cDNA corresponding to the JH3–JH7 domains. Three positive clones were identified. λ phage were grown and purified by standard methods (Sambrook *et al.*, 1989). In addition, P1 plasmids were obtained from Genome Systems (St. Louis, MO) using the following primer pairs corresponding to the JAK3 cDNA: forward primer, 5′CAGCACCCAAGTCCTGCT3′ (JAK3 bases 374–391); reverse primer, 5′TGGGAGAGGAACTCTGACT3′ (JAK3 bases 556–575). Three clones were

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provided. The P1 clones were grown and purified following the protocol recommended by Genome Systems. Cellular genomic DNA was extracted using Easy-DNA Kit (Invitrogen, San Diego, CA).

Sequencing of the JAK3 gene. Primers were constructed approximately every 200 bases from the published sequence of the JAK3 cDNA (GenBank Accession No. HSU09607). To amplify DNA fragments from a genomic template, 200 ng of template DNA (purified λ phage, P1 plasmid genomic DNA, or human T-cell DNA) was added to 10× PCR buffer (Boehringer-Mannheim, Indianapolis, IN), 2.5 U *Taq* polymerase (Boehringer-Mannheim), 1 m*M* dNTP (Boehringer-Mannheim), 10 pmol each primer, and nuclease-free ddH_2O to make a final volume of 100 μl. The reaction was cycled 30 times. The PCR products were electrophoresed on a horizontal 0.8% agarose gel containing 0.5 μg/ml ethidium bromide.

PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen Inc., Chatsworth, CA) or the QIAquick PCR Purification Kit. Purified PCR fragments (100 ng) and 10 pmol of individual primers were added to the ABI PRISM DyeDeoxy Terminator Cycle Sequencing Ready Reaction Kit (Perkin–Elmer, Foster City, CA). The reaction was subjected to 25 cycles. Products were analyzed on the ABI PRISM 373A DNA Sequencer and compiled using GCG software (Genetics Computer Group, Inc., Madison, WI). The fragments were aligned based upon the cDNA sequence of human JAK3, and intron–exon boundaries followed the AG/GT rule (Mount, 1982). Sequencing of both strands was performed to verify the results. Discrepancies were then resolved by resequencing the region in question using P1 plasmid, λ phage, and human T-cell DNA.

Chromosomal localization. Chromosome preparations were made from BrdU-treated human peripheral blood cultures as described by Hirai and colleagues (Hirai *et al.,* 1994). λ Phage DNA from two *JAK3* genomic clones was labeled with digoxigenin-11–dUTP (Boehringer Mannheim, Germany) by nick-translation (Boehringer Mannheim). This probe was visualized using an anti-digoxigenin labeled with rhodamine. A second probe corresponding to an α-satellite DNA unique to human chromosome 19 was prepared from a human monochromosomal hybrid using PCR to incorporate biotinylated nucleotides (Weier *et al.,* 1991). The chromosome 19 probe was visualized using FITC-labeled avidin. Hybridization was performed and DAPI, a DNA dye, was used as a counterstain as described by Hirai *et al.* (1994). Twenty metaphase spreads were viewed with a Zeiss Axiophot microscope equipped with a triple bandpass filter to detect signals simultaneously. Images were collected and merged using a CCD camera (KAF 1400, Photometrics, Tucson, AZ) and IPLab Spectrum software (Signal Analytics Vienna, VA).

RESULTS

Genomic sequencing. To determine the sequence and structure of the JAK3 gene, clones were isolated from human genomic λ phage (Charon 4A) and P1 plasmid libraries. Primers were derived from the JAK3 cDNA sequence or were synthesized to traverse intronic sequence as required. The *JAK3* genomic sequence comprises approximately 13,600 bp, which corresponds to 19 exons and 18 introns. All of the exon–intron splice junctions agree with the GT/AG rule (Mount, 1982), and Table 1 outlines these junctions. The first 200 bases define a portion of the 5′ untranslated region and the most 3′ exon (exon 19) also contains untranslated sequence. Figure 1 is a schematic representation of the *JAK3* gene and the exon–intron usage as it relates to the encoded protein. The locations of the various JAK homology (JH) domains are shown. The catalytic domain of JAK3 and other Janus kinases is the JH1 domain, which is encoded by exons 16–19. The pseudokinase domain, JH2, is encoded by exons 9–15.

Chromosomal localization. Using fluorescence *in situ* hybridization on PHA-activated peripheral blood leukocytes, a total of 20 metaphase cells were examined after hybridization with two genomic *JAK3* probes. All 20 of the cells revealed paired hybridization signals (JAK3 and the chromosome 19 α-satellite) with a strong signal at chromosome 19p13.1 (Fig. 2A). No significant background was noted at any other chromosomal locations with the probes. The chromosomal localization of *JAK3* was verified by specific amplification of human JAK3 from a panel of rodent/human hybrid cells (Coriell Cell Repositories, Camden, NJ), which contain specific human chromosomes. *JAK3* fragments were amplified, in three separate experiments, from cellular DNA obtained from cells containing human chromosome 19 and not from those cells containing other human chromosomes (data not shown). Rodent *JAK3* was not amplified from these cells with the primer pairs utilized.

Figure 2B illustrates an ideogram of human chromosome 19 depicting the localization of *JAK3* to p13.1. Interestingly, the *JAK3* locus is in proximity to another Janus kinase, Tyk2 (19p13.2) (Frimbach-Kraft *et al.,* 1990). In addition, the erythropoietin receptor and the proto-oncogene Vav (Ropers and Pericak-Vance, 1991) also map to 19p13.2. Interestingly, another PTK, Chk, which is expressed at high levels in NK cells, also maps to chromosome 19 (Authors' unpublished observations).

DISCUSSION

The importance of JAK3 in cytokine signal transduction and in lymphoid development has been emphasized by the recent finding that diverse mutations in this gene result in severe combined immunodeficiency in humans (Russell *et al.,* 1995; Macchi *et al.,* 1995). These patients lack T cells and have greatly reduced B-cell numbers. The complete sequence of the *JAK3* gene should facilitate the identification of new patients with JAK3 deficiency.

The mapping of *JAK3* to human chromosome 19p13.1 is interesting in that the closely related family member Tyk2 is also localized to chromosome 19 at band p13.2 (Frimbach-Kraft *et al.,* 1990). In contrast, the gene encoding human JAK1 has been localized to chromosome 1 (p31.3), and JAK2, the family member with the greatest homology to JAK3, has been provisionally localized to human chromosome 9 (p24) (Pritchard *et al.,* 1992). Although these kinases share considerable structural homology at the protein level, the finding that they reside upon three separate chromosomes suggests that the genes most likely result from distant evolutionary duplication.

Many of the genes that encode PTKs appear to have arisen by repeated gene duplication of a common ancestor. An excellent example of this is c-*src,* and the related family members *fgr, lck, lyn,* and *yes,* which share

TABLE 1

Exon–Intron Boundaries of the *JAK3* Gene

Exon (donor site)	Intron	Exon (acceptor site)
1 GCCAAGGCCAGCG	gtgagtgcatc . . . tccccctgtag	2 GCATCCTGCCTGTG
2 CTGTACAGGATTCG	gttcgacagga . . . tctcctcacag	3 CTTTTACTTCCCCAA
3 ACCTCTTTGCCCAG	gtggggttctg . . . ggcccccccag	4 CACCGCATTGACCT
4 CTGAAGACTGTCAG	gtgagagccac . . . cccctgcag	5 CTACAAGGCTGCCT
5 CCAGGGAGAACAGG	gtgaggacgga . . . acaacccag	6 TCCTCCAGCCCTTC
6 CAACCAGATTTTAG	gtggtgcagg . . . gtccctcgcag	7 GAGGCCGAGTTCCC
7 CTGTCTGTGTCCAG	gtcggtctact . . . actcttcttgcg	8 AACCCCCTTGGTCC
8 CCCCAGACCCAAAG	gtgaggcccc . . . ctctccccatag	9 AAAAGTCCAACCTG
9 ACAGCCTGGAGTGG	gtaagaggcc . . . tcaccattcag	10 CATGAGAACCTGGG
10 AGAACTGCATGGAG	gtgaggtggg . . . accttccccag	11 TCATTCCTGGAAGC
11 TGGCTGGAGACAG	gtgagtgctcc . . . tatgggctgag	12 CACCATGGTGCAGG
12 CCCTCAACTATCTG	gtgagtgctcct . . . ggccccccttag	13 GAGGACAAAGGCCT
13 TGTTAAGCCTGGAG	gtaagttcctgg . . . tcattccctcag	14 ATGCTCACCGACAG
14 TGGATCCTGCTAAG	gtcagagcccc . . . tcaccctcag	15 AAACTCCAATTTTAT
15 CCTCATCTCTTCAG	gtgccccgctgg . . . ccaccccag	16 ACTATGAGCTCCTC
16 ACAGCTGGGCAAG	gtaaggtgggc . . . ctatcctcgag	17 GGCAACTTTGGCAG
17 AGCTATGGCCCGG	gtgagccagct . . . tgccttctccag	18 GCCGGCCAGACCT
18 GCAGATCTGCAAG	gtgagagggg . . . ccccccccag	19 GGCATGGAGTACCT . . . CCTGTCCTTTTCATAG

closely related nucleotide sequences and substantial exon–intron organization (Yasmanashi *et al.*, 1987). When other JAK family genomic sequences are reported, comparisons can be made between the Janus family members as to similarities in exon–intron usage, thus determining whether they share a common ancestry.

It has been suggested that the JH2 domain arose by duplication of the JH1 domain. If this were the case, it might be expected that the exon structure of the JH2 domain would be roughly equivalent to that of the JH1 domain. Interestingly, this is not the case. This lack of similarity may suggest that the JH1 and JH2 domains arose from different ancestral genes.

Many PTKs have been described as oncogenes that are involved in critical cellular signaling events. During transformation, there can be a dysregulated expression or function of these kinases. It is of interest, therefore, that JAK3 maps in proximity to loci involved in acute myelogenous leukemia and B-cell lymphoma (Ropers and Pericak-Vance, 1991; Mitelman *et al.*, 1991; Kaneko *et al.*, 1989). Interestingly, JAK3 is expressed at high levels in bone marrow aspirates from patients with B-cell ALL and in cell lines derived from patients with B-cell lymphomas (Tortolani *et al.*, 1995). Importantly, it has been demonstrated that mutations of a JAK can result in leukemia in *Drosophila* (Harrison *et al.*, 1995, Binari and Perrimon, 1994). Constitutive activation of the JAK3 pathway has been shown in HTLV1 (Migone *et al.*, 1995) and v-*abl* transformed cells. It will be important to determine if the *JAK3* gene is disrupted in any of these hematologic malignan-

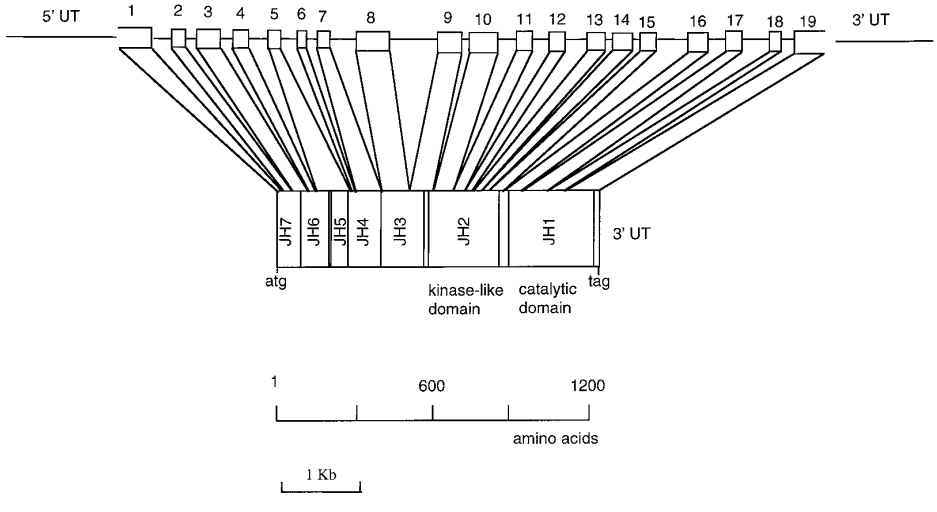

FIG. 1. Genomic organization and comparison to putative domains of JAK3 protein. The exons and introns encoding the various Janus homology (JH) domains are indicated.

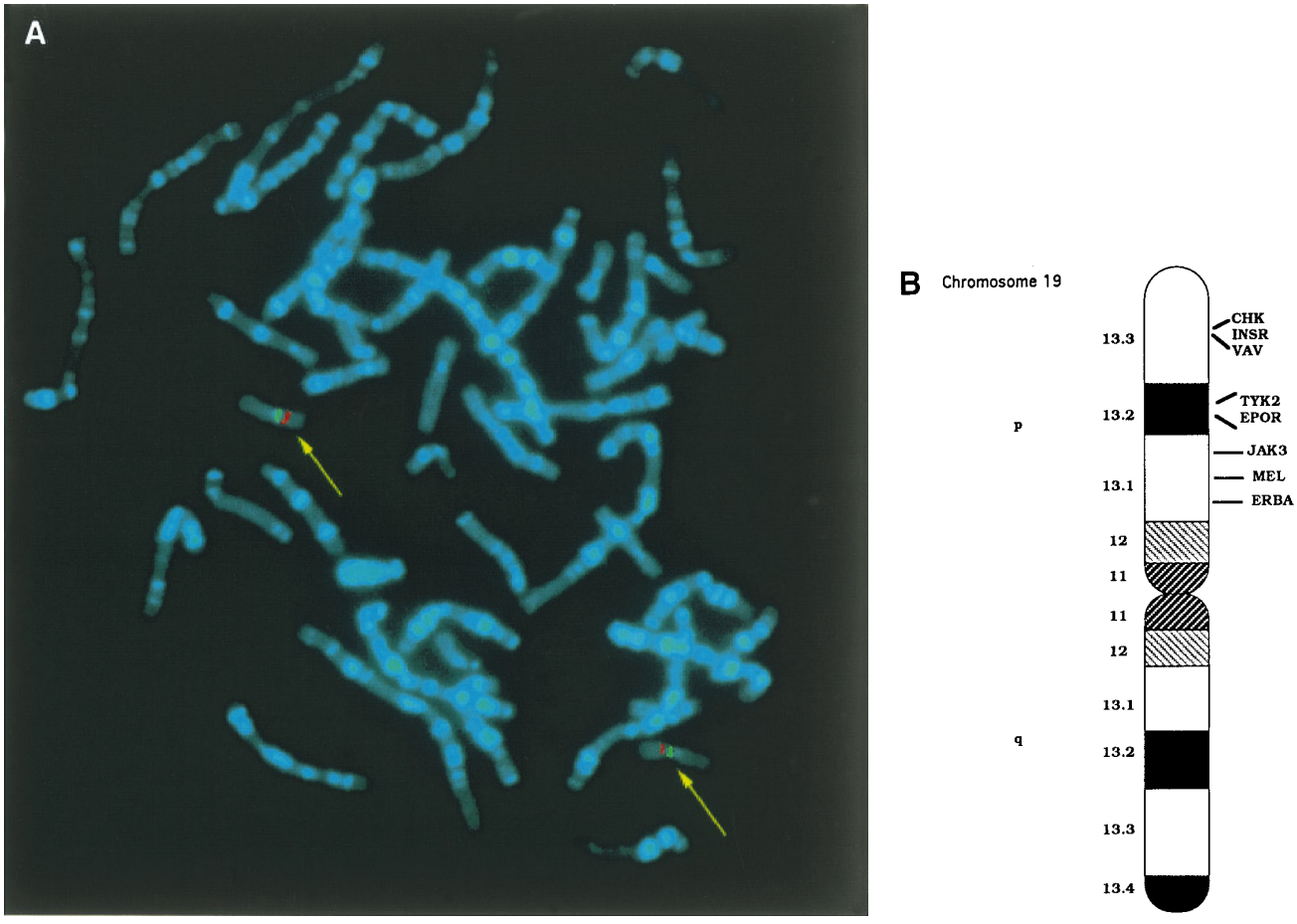

FIG. 2. (A) Human metaphase spread showing the specific site of hybridization to chromosome 19. The arrow pointing to the green band highlights *JAK3* at 19p13.1. Chromosome identification was carried out using an α-satellite probe (red). (B) Idiogram of human chromosome 19 (Ashworth *et al.*, 1995). Localization of other genes within close proximity of JAK3 is illustrated.

cies and to determine if JAK3 contributes to transformation. Additionally, it was reported by Lai and colleagues that there are variant forms of JAK3 found in breast carcinoma cell lines and in a chronic mylogenous leukemic cell line (Lai *et al.*, 1995). Surprisingly, the exon–intron structure of *JAK3* does not explain the genesis of such altered forms of the JAK3 protein.

In summary, this report assigns the locus of JAK3–autosomal SCID to human chromosome 19p13.1. The knowledge of the gene structure will help to identify new patients and to develop therapeutic approaches aimed at this gene.

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Note added in proof. The murine *JAK3* gene has been localized to chromosome 8 (Kono *et al.*, 1996). Other murine homologues of human genes expressed on human chromosome 19p13.1 also map to chromosome 8, suggesting that these regions are indeed syntenic.

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