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JAK3 Maps to Human Chromosome 19p12 within a Cluster of Proto-oncogenes and Transcription Factors

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JAK3 is the most recently discovered member of a family of cytoplasmic tyrosine kinases (including JAK1, JAK2, and TYK2) of the cytokine receptor signaling system (13). JAK3 has a role in the interleukin (IL)-2 signaling pathway that regulates T and B lymphocyte development and proliferation (6, 18). In contrast to the other members of the Jak family, JAK3 is expressed in only a limited number of tissues, primarily those involved in hematopoesis and lymphocyte development. Mutations of the JAK3 protein have been implicated in both cancer and immune system pathologies, most clearly in cases of severe combined immune deficiency (SCID) caused by a lack of mature T cells (9, 15); Jak3-deficient mice show defective development of both B and T cells (12, 18). There are also indications that splice variants of JAK3 may be implicated in breast cancer (3, 7).

JAK3 was recently localized to the p13.1 band of human chromosome 19 (14). We have localized the gene to a cosmid contig at the p12–p13.1 boundary, revised its placement relative to the genes MEL and ERBAL2, and determined physical distances between the contig and nearby polymorphic markers. It is intriguing that JAK3, an important regulator of cell proliferation, is located in the midst of a cluster of proto-oncogenes and leukemia-associated breakpoints.

The JAK3 gene was originally assigned to human chromosome 19 by PCR amplification from a panel of monochromosomal human–rodent hybrids (from the Coriell Cell Repository, Catalog No. MMP0002 Mini Map). Primers selected from the 3′ end of the Jak3 sequence were JAK-3F (ggtgagggaagattagactc) and JAK3-R2 (tatggagcatcgactgtgtg); amplifications were performed as described in Ref. (2). Amplifications of human placental DNA yielded an 80-bp product, which was labeled with ^{32}P and used to screen an 11X arrayed cosmid library specific for human chromosome 19, as described in Ref. (19). Cosmids identified as JAK3-positive in the screening were digested to completion with *Eco*RI, and the fragments were separated on a gel electrophoresis system and blotted as described in Ref. (5). The 80-bp PCR product was used to probe the Southern blot to confirm JAK3-positive cosmids. Additionally, purified DNA from each cosmid was used as a template for PCR amplification by the same primers; appropriate-sized products were successfully amplified from each of the cosmids.

The results of these analyses have placed the JAK3 gene within a 425-kb cosmid contig. This contig is part of the highly annotated, cosmid-based physical map of chromosome 19 described in Ref. (1). High-resolution interphase FISH mapping (20) of a JAK3-positive cosmid (30092) has placed the contig between the polymorphic markers D19S710 and D19S212, near the p12–p13.1 boundary of human chromosome 19. Another Jak gene family member, TYK2, is located near EPOR on 19p13.2, about 5 Mb distal of JAK3 (1). This region of human chromosome 19 is syntenic to portions of two different murine chromosomes, numbers 8 and 9 (Mouse Genome Database), indicating that there has been at least one rearrangement in mice relative to humans.

Five potentially oncogenic genes are in the 2-Mb region surrounding the JAK3 gene; two are telomeric and three are centromeric of JAK3 (Fig. 1). A transforming oncogene derived from a melanoma cell line, c-MEL (11), is about 1.4 Mb telomeric of JAK3. ERBAL2, which encodes a nuclear

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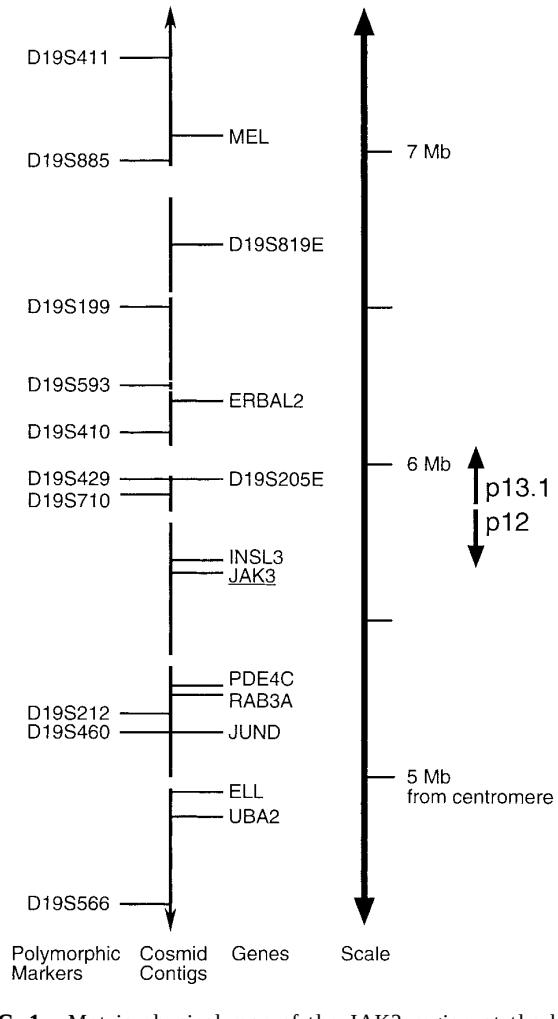

FIG. 1. Metric physical map of the JAK3 region at the human chromosome 19p12–p13.1 boundary. Ten polymorphic markers and 11 genes are localized within the region. JAK3 is underlined.

orphan receptor that is part of the steroid/thyroid hormone receptor family and is highly similar to the c-erbA oncogene (10), is about 400 kb telomeric of JAK3.

About 380 kb centromeric of JAK3 is RAB3A, part of a ras-related family of GTP-binding proteins that mediate exocytosis; RAB3A shows high expression in neural tube-related tumors (4). JunD, a transcription factor from the c-Jun family of proto-oncogenes (8) that is critical to the regulation of cell proliferation, is about 500 kb centromeric of JAK3. ELL (11–19 lysine-rich leukemia gene), an RNA polymerase elongation factor that is a breakpoint for acute myeloid leukemia (16, 17), is about 680 kb centromeric of JAK3. In addition to these genes, Fig. 1 indicates 10 polymorphic markers spanning this region that are useful for either linkage or deletion mapping.

Collectively, these proto-oncogenes and breakpoints make up a significant fraction of the known cancer-related sites on human chromosome 19. It is therefore interesting that they are clustered in a relatively small region of the chromosome, which at this time is known to contain only a few other genes. The clustering trend is maintained by JAK3, an important regulator of cell development and proliferation that is potentially of oncogenic significance, which is now also positioned within this limited span of the chromosome.

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Localization of the Rab Escort Protein-2 (REP2) and Inositol 1,4,5-Trisphosphate 3-Kinase (ITPKB) Genes to Mouse Chromosome 1 by *in Situ* Hybridization and Precision of the Syntenic Regions between Mouse and Human 1q42–q44

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A short genomic region of homology has been identified between the telomeric end of human chromosome 1 and the proximal end of mouse chromosome 13. This region was first described by the genetic localization in human and mouse of a gene encoding for the structural protein of basement membrane called nidogen (entactin), respectively to chromosome 1q43 (21) and chromosome 13 (18). A second gene, the ryanodine receptor (RYR2), was mapped to human chromosome 1q42.1–q43 (22) and to mouse chromosome 13A1–A2 (19), further extending the region of homology. Recently, the gene involved in a human immunodeficiency disease, the Chediak–Higashi syndrome, was also mapped to the 1q42–q43 region (6, 14), whereas its mouse homologue, the beige mutation, is tightly linked to nidogen, on mouse chromosome 13 (18). All these data allow the definition of a segment of homology between the human 1q42–q43 region and the proximal end of mouse chromosome 13. The other genes so far described with the same cytogenetic location on human chromosome 1 map elsewhere on the mouse genome (1, 2, 5, 15–17, 23), suggesting that this syntenic region should be restricted in length (Table 1). To define the extent of this region better, we mapped two other genes to the murine genome, the rab escort protein-2 gene (REP2) and the inositol 1,4,5-trisphosphate 3-kinase gene [($InsP_3$) 3-kinase], respectively localized to human to 1q42–qter (9) and 1q41–q43 (13). Both genes were mapped using *in situ* hybridization.

REP2 is involved in the prenylation of Rab proteins by the rab geranylgeranyl transferase and in the control of the orientation of these proteins to their target membranes (3, 12). This gene is also called the choroideremia-like gene because of its similarity in sequence (87%) with the gene REP1 involved in choroideremia (11). Sequencing of REP2 in 19 patients with Usher syndrome type 2, the locus of which lies on distal 1q, has excluded this gene as a candidate for this particular disease (9).

($InsP_3$) 3-kinase plays a crucial role in the phosphoinositides signaling pathway (7, 8). This enzyme catalyzes the formation of inositol 1,3,4,5-tetrakisphosphate ($InsP_4$) by phosphorylation of $InsP_3$, both of which are main effectors of this pathway. $InsP_3$ is a very important second-messenger involved in the regulation of many cellular processes. It is produced, together with diacylglycerol, after cleavage by phospholipase C of the phosphatidylinositol 4,5-bisphosphate. Like $InsP_3$, $InsP_4$ seems to be involved in the fine regulation of intracellular calcium levels (7, 8). Two isoforms of the ($InsP_3$) 3-kinase, ITPKA and ITPKB, have been identified by the screening of a human hippocampus cDNA library (25, 26). These two isoenzymes share sequence identity particularly in two regions. ITPKA and ITPKB have been previously localized on the human genome, respectively to chromosome regions 15q14–q21 and 1q41–q43 by *in situ* hybridization (13). This study reports the murine localization of the isoenzyme ITPKB.

In situ hybridization experiments were carried out using metaphase spreads from a WMP male mouse (10), in which all the autosomes except 19 were in the form of metacentric robertsonian translocations. Concanavalin A-stimulated lymphocytes were cultured at 37°C for 72 h with 5-bromodeoxyuridine added for the final 6 h of culture (60 μg/ml of medium), to ensure a chromosomal R-banding of good quality.

A probe was prepared for each gene tested by *in situ* hybridization. The REP2 clone (1000 bp of the 5′ end of the cDNA) used for this experiment was obtained by PCR amplification of a human cDNA using the specific primers 550, 5′-CACCTCATTTCTTTCATCAG-3′, and 553, 5′-GTCATTGCAATTGAGTGCAG-3′ (9). Similarly, ITPKB probe (557 bp from the 3′ end of the cDNA) was obtained using the primers 5′-GACTCGCCCTGTGTGATGGA-3′ and 5′-GCCCCGAGAGGTAGCCATCC-

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