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Confirmed assignment of a novel human tyrosine kinase gene (JAK1A) to 1p32.3→p31.3 by nonisotopic in situ hybridization

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Abstract. JAK1A is a recently isolated class 3 (nonreceptor) tyrosine kinase that has catalytic domain sequence homology with other kinases and is known to be ubiquitously expressed in all human tissues thus far examined. The gene for this enzyme had previously been localized to chromosome 1 using somatic cell hybrids and linkage analyses. In the present study, fluorescence in situ hybridization was utilized to confirm its localization and regionally assign the gene to chromosome region 1p32.3→p31.3.

Tyrosine phosphorylation has been demonstrated to play a central role in signal transduction from the cell membrane to the nucleus. With the increasing realization that kinases play important roles in every aspect of normal and abnormal cell growth and metabolism, it has become necessary to isolate and characterize the cDNAs and genes for these essential proteins.

Protein kinases are a group of enzymatic proteins that catalyze the phosphorylation of serine, threonine, or tyrosine residues. These kinases can be grouped into three major families, based upon the particular amino acid phosphorylated and whether they are located in the cell membrane as a receptor or in the cytoplasm. The first group includes the receptor kinases, which contain three structural components: a ligand-binding domain, a transmembrane domain, and a tyrosine kinase domain. The second class of tyrosine kinases is composed of proteins that lack the transmembrane domain but do contain a regulatory domain, SH2 or SH3. These are found primarily in the cytosol and are often associated with membrane proteins. The third class contains a group of nonreceptor kinases that have only recently been isolated and are poorly characterized at this time. These large proteins lack both a transmembrane and regulatory domain but do contain a classical tyrosine kinase domain and a degenerate kinase-like domain (Firmbach-Kraft et al., 1990).

Many kinases have been isolated based on catalytic domain homology (Hanks et al., 1988). The first method used low-stringency library screening; more recently, PCR cloning of the conserved catalytic domain has been used. One such kinase, Jak1, has recently been sequenced (Wilks et al., 1991). Jak1 kinase is a member of the recently identified class 3 tyrosine kinases.

Jak1 message has been detected in all human cell types tested to date (Wilks et al., 1991; Howard et al., 1992). The other members of this kinase family (TYK2 and JAK2) have also been detected in all cell types. Kinase activity has not been demonstrated for the full-length Jak1 protein. However, a fusion protein containing glutathione transferase and the classical kinase domain does have phosphorylating activity when expressed in bacterial cells (Wilks et al., 1991). In our studies, the steady-state message level of Jak1 increased in human monocytes in response to γ-interferon. Recently Tyk2 has been linked with the signal transduction pathway of α- and β-inter-

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feron (Velazquez et al., 1992). Thus, Jak1 is a member of a unique kinase family and itself has several unique properties.

We have previously reported the assignment of the gene for Jak1 (JAK1A) to human chromosome 1 by human × rodent somatic cell hybrids and linkage studies using the CEPH database (Howard et al., 1992). Here, we report in situ hybridization results that confirm our initial assignment and sublocalize the gene to region 1p32.3→p31.3.

Materials and methods

A 2-kb fragment of Jak1 cDNA cloned in Bluescript KS⁺ (Stratagene) containing both catalytic and degenerate kinase domains was used as a hybridization probe to metaphase chromosomes. The protocol utilized for fluorescence in situ hydridization is given in Hoehe et al. (1991). Metaphase chromosomes were derived from normal donors and grown in short-term tissue culture for 96 h. The Jak1 cDNA clone was labeled with biotin-11-dUTP following nick translation and included in a 10-µl volume containing 50 ng/µl probe DNA and 300 ng/µl sheared salmon sperm DNA in a solution of 50% formamide, 10% dextran sulfate, and 2 × SSC, pH 7.0. Both the probe and chromosomal DNA were denatured, and hybridization occurred overnight at 37°C. The slides were washed at 40°C in 50% formamide, 2 × SSC and then three times (5 min each) in 2 × SSC alone. Hybridization was detected by immersing the slides in a solution of 4 × SSC, 3% bovine serum albumin containing 5 µg/ml fluorescein isothiocyanate (FITC)-conjugated avidin DCS. Slides were stained in Hoechst 33258 (0.6 µg/ml) for 20 min, followed by actinomycin D (0.5 µg/ml) for 25 min and then left overnight at 4°C. Chromosomes were viewed in an antifade solution of 2% *n*-propylgallate in 50% glycerol, 4 × SSC containing 5 µg/ml propidium iodide.

Results and discussion

Examination of 37 metaphase cells indicated specific hybridization in 14 cells (38%) to the region 1p32.3→p31.3. No significant background was observed at any other chromosomal locations (Fig. 1). These results enable assignment of JAK1A to this location.

Recently, the gene for Tyk2, the prototype of this tyrosine kinase family, was assigned to human chromosome band 19p13.2 (Firmbach-Kraft et al., 1990). With the assignment of JAK1A to human chromosome region 1p32.3→p31.3, it is now evident that the genes encoding these unique nonreceptor kinases are not clustered on a single chromosome. With the chromosomal assignment of JAK1A, we can begin to address the neoplasia potentially associated with this gene: There are two reported translocations that give rise to acute lymphoblastic leukemia with a breakpoint in 1p32 (Kaneko et al., 1989; Raimondi et al., 1989). Additionally, two larger translocations or deletions have been identified in the region 1p36→p32. These structural abnormalities are reported to give rise to neuroblastoma or glioma (Franke et al., 1985; Jenkins et al., 1989). The human homolog of the murine myeloproliferative leukemia virus, which causes an acute leukemia, maps nearby at band 1p34 (Le Coniat et al., 1989). Further studies of neoplasia associated with 1p32.3→p31.3 could demonstrate a function for the JAK1 protein. Future studies of the intron/exon arrangement of JAK1 could serve as a model for the genetic arrangement of other members of the class 3 tyrosine kinase family.

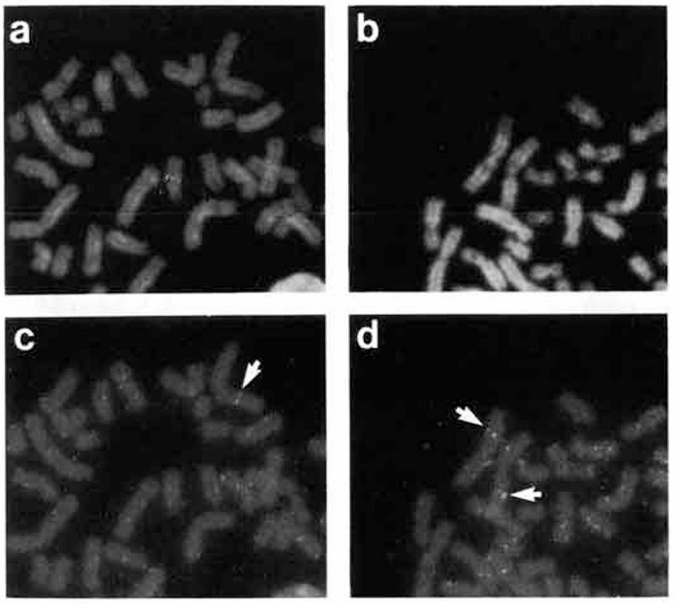

Fig. 1. Partial metaphase cells illustrating simultaneous QFH-banding with Hoechst 33258 (**a**, **b**) and fluorescence in situ hybridization with the Jak1 cDNA clone (**c**, **d**). Arrows indicate paired hybridization signals on the short arm of chromosome 1.F1

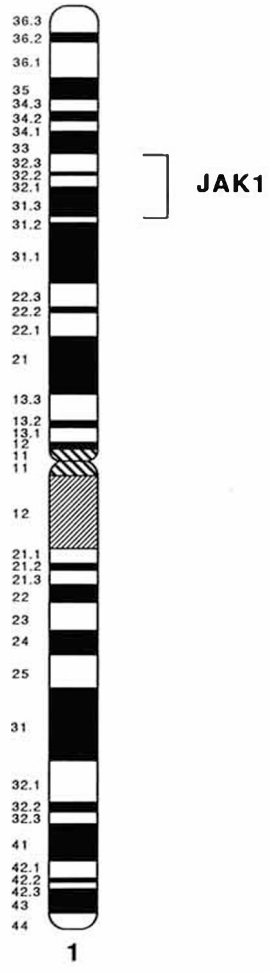

Fig. 2. Idiogram of chromosome 1 showing localization of JAK1A to region 1p32.3→p31.3.

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