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The gene (PTPN13) encoding the protein tyrosine phosphatase PTP-BL/PTP-BAS is located in mouse chromosome region 5E/F and human chromosome region 4q21

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Abstract. Both mouse and human genomic clones were isolated for protein tyrosine phosphatase PTP-BL/PTP-BAS (HGM approved gene symbols *Ptpn13* and PTPN13, respectively). Using these clones as a probe, PTPN13 was assigned to human chromosome region 4q21 and mouse chromosome region 5E/F by fluorescence in situ hybridization (FISH).

Protein tyrosine kinases (PTKs) can exert growth stimulatory responses by transient phosphorylation of cellular protein tyrosine residues. Protein tyrosine phosphatases (PTPases) were, therefore, considered negative regulators of cell growth and differentiation. However, evidence is accumulating that PTPases can also stimulate cell proliferation and maturation and can work in concert with PTKs in signal transduction pathways (Brady-Kalnay and Tonks, 1994). Over 30 PTPase family members have been characterized, displaying different structural characteristics (Walton and Dixon, 1993).

Recently, we obtained mouse partial cDNA clones encoding the PTPase PTP-BL (Accession number Z32740; Hendriks et al., 1995) which has also been cloned by others and termed RIP (Chida et al., 1995). The deduced amino acid sequence of murine PTP-BL displays 80% overall sequence homology with human PTP-BAS (Maekawa et al., 1994), also known as hPTP1E (Banville et al., 1994) or PTPL1 (Saras et al., 1994). Like its human homolog PTP-BAS, PTP-BL shares intriguing sequence homologies with submembranous tumor suppressors. It contains a band 4.1-like motif also present in the tumor suppressors neurofibromatosis type 2 and *expanded* (Boedigheimer et al., 1993). At the carboxy-terminal end the single catalytic phosphatase domain is present. In between are five 80 amino acids repeats, a protein motif that is also found in the Drosophila discs-large tumor suppressor. These discs-large homologous regions (DHRs) have now been identified in a large number of intracellular proteins and are thought to act as subcellular routing signals (Ponting and Phillips, 1995). Recently, it was shown that one of the DHRs of PTP-BAS can associate with the regulatory region of Fas, thereby inhibiting Fas-induced apoptosis (Sato et al., 1995). Since tissue homeostasis is dependent upon balanced cell proliferation and cell death, changes in PTP-BL/PTP-BAS expression levels or activities might have pathological consequences.

Knowledge of the exact chromosome location of PTPN13 will facilitate studies that address the possible involvement of PTP-BL/PTP-BAS in tumorigenesis. Recently, PTP-BL and PTP-BAS genes were assigned to mouse chromosome 5 and human chromosome 4, using interspecific backcross mapping (Chida et al., 1995) or PCR on a panel of somatic cell hybrids (Banville et al., 1994), respectively. We used fluorescence in situ hybridization (FISH) to refine the location of the PTP-BL/PTP-BAS genes to mouse chromosome region 5E/F and human chromosome region 4q21, respectively.

Materials and methods

Isolation of mouse PTP-BL genomic clones

Two *Hin*dIII fragments of 1.1 kb and 1.3 kb were isolated from the partial 6.5-kb mouse PTP-BL cDNA clone mPTP14-2 (Hendriks et al., 1995). These fragments were labeled radioactively and used to screen a mouse strain 129/SvEv genomic cosmid library. Positive clones were purified by subse-

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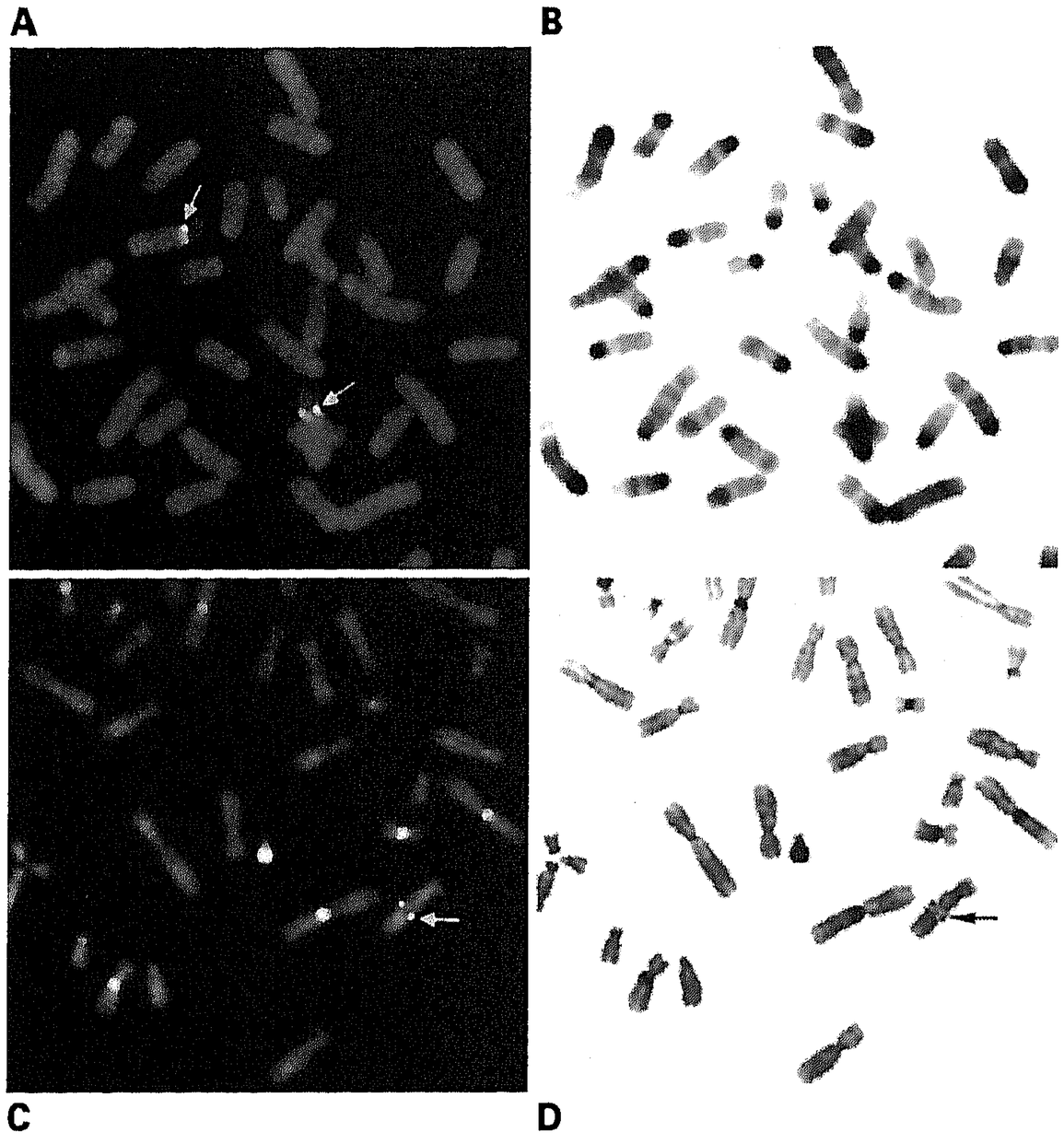

Fig. 1. FISH of PTP-BL/PTP-BAS performed on mouse (**A**, **B**) and human (**C**, **D**) partial metaphase spreads. Hybridization signals of the mouse mCOSPTPBL/2 cosmid probe (arrows) to mouse chromosome region 5E/F (**A**). Hybridization signals of the human hλPTPBAS/1 phage probe (arrow) to human chromosome region 4q21 (**C**). Converted DAPI staining images B and D were used to allow detailed assignment of the mouse and human gene, respectively.

quent rounds of replica screening. The authenticity of the genomic clones was confirmed by subcloning an *Eco*RI fragment of cosmid mCOSPTPBL/2 (subclone mPTPBLE/2.4) and subsequent sequencing by the double-stranded dideoxy chain termination method using primer PTPBL/BASseq (5′-ATCTGAATTTCACTGCC-3′; corresponding to nucleotides 6968–6984 of the mouse PTP-BL sequence (Hendriks et al., 1995)).

Isolation of human PTP-BAS genomic clones

To serve as a probe, we first generated a human PTP-BAS cDNA fragment by reverse transcription PCR, essentially according to Hendriks et al. (1995). Briefly, human keratinocyte total RNA was used as a template for random primed cDNA synthesis. Subsequently, a 969-bp PTP-BAS cDNA fragment was specifically amplified using PTP-BAS specific oligonucleotides PTP-BASF (5′-CAAGAAGCTGAAGTTATCCAGT-3′) and PTP-BASR (5′-AGGCAGTGAAATTCAGATGAG-3′) corresponding to nucleotides 6220–6241 and 7169–7189, respectively, of the human PTP-BAS sequence (Maekawa et al., 1994). The PTP-BAS cDNA fragment was used as a probe to screen a human genomic EMBL3 phage library. Positive phage recombinants were plaque-purified and used for FISH analysis. The identity as being genomic PTP-BAS clones was confirmed by subcloning an *Eco*RI fragment of hλPTPBAS/1 phage (subclone hPTPBASE/1.3) and subsequent sequencing using the previously mentioned primer PTPBL/BASseq (corresponding to nucleotides 7172–7188 of the human PTP-BAS sequence (Maekawa et al., 1994)).

Fluorescence in situ hybridization

Nonradioactive in situ hybridization was performed on normal human lymphocyte metaphase spreads essentially according to De Leeuw et al. (1993), or on cultured embryonal stem (ES) cells of mouse strain 129/Ola, essentially according to Steeghs et al. (1993). In brief, human hλPTPBAS/1 phage DNA (1 μg) or mouse mCOSPTPBL/2 cosmid DNA (1 μg) was labeled with biotin-14-dATP (Life Technologies) by nick-translation, purified, and ethanol precipitated together with 50-fold excess of Cot1-DNA (Life Technologies) or a 200-fold excess of sonicated total mouse genomic DNA, respectively. Subsequently, 250 ng of DNA probe was dissolved in 12 μl of hybridization mixture (consisting of 2 × SSC, 10 % dextran sulphate, 1 % Tween-20, and 50 % formamide). The hybridization mixture was heat-denatured and then incubated overnight at 37 °C to heat-denatured chromosome spreads enclosed under a cover slip. Immunocytochemical detection of the hybridizing probes was achieved using fluorescein isothiocyanate (FITC)-conjugated avidin followed by two amplification steps with rabbit-antiavidin-FITC and mouse anti-rabbit-FITC. Images were obtained using a high-performance cooled CCD camera (Photometrics, Tucson, USA) and analyzed using the Oncor-Image™ F.I.S.H. software package (Oncor-Imaging, Gaithersburg MD, USA).

Results and discussion

To further refine the chromosomal localization of the PTP-BL/PTP-BAS gene we set out to clone mouse and human genomic clones to enable FISH analysis. A human 969-bp PTP-BAS cDNA fragment was generated by reverse transcription PCR using a pair of PTP-BAS specific oligonucleotides.

Using the mouse and human PTP-BL/PTP-BAS cDNA probes three independent but overlapping mouse cosmid clones (mCOSPTPBL/1-mCOSPTPBL/3) and three human PTP-BAS phage clones (hλPTPBAS/1-hλPTPBAS/3) were isolated. To establish the identity of the clones as being PTP-BL and PTP-BAS genomic fragments, appropriate *Eco*RI subclones were partially sequenced using a primer recognizing both the mouse and human PTPase. Sequences revealed a 100 % homology over a 147-bp region between codons 2350 and 2399 of the mouse PTP-BL cDNA sequence or between codons 2384 and 2433 of the human PTP-BAS cDNA sequence, respectively. In addition, a conserved exon-intron boundary was observed in the PTP-BL and PTP-BAS genes after codons 2399 and 2433, respectively.

To determine the regional chromosomal location of the PTP-BL/PTP-BAS gene, FISH analysis was performed using either mouse cosmid clone mCOSPTPBL/2 or human phage clone hλPTPBAS/1 as probes on mouse and human metaphase spreads, respectively. For the mouse PTP-BL gene assignment, 35 metaphase spreads were analyzed. In 31 spreads hybridization signals were found on both homologs in region E/F of mouse chromosome 5 (Fig. 1A). A total of 23 human metaphase spreads were analyzed and in 12 spreads the hybridizing signal of the PTP-BAS probe was observed at 4q21 on both homologs. Figure 1C shows single positive hybridization signals of the human hλPTPBAS/1 clone to chromosome region 4q21 on both chromatids. The FISH results confirm earlier localization data (Banville et al., 1994; Chida et al., 1995) and are in accordance with comparative mapping data of human and mouse genomes that have revealed conservation between human chromosome region 4q21 and mouse chromosome region 5E/F (Searle et al., 1994).

Various cytogenetic abnormalities in human tumors have been reported involving this region of chromosome 4. For instance, loss of region 4q11→q32 has been observed in approximately half of the hepatocellular carcinomas (Seizinger et al., 1991). Also, gain of chromosome 4p16→q35 in acute myeloid leukemia has been reported (Mittelman et al., 1993).

Until now, no obvious candidate disease loci have been identified in mouse in the chromosomal region of PTP-BL.

Further studies on PTP-BL/PTP-BAS are required to elucidate its role in normal or pathological cases of growth, differentiation and apoptosis.

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