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Localization of the 75-kDa inositol polyphosphate-5-phosphatase (INPP5B) to human chromosome band 1p34

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Abstract. The 75-kDa (type II) inositol polyphosphate-5-phosphatase, originally described in platelets, is one of at least three known enzymes capable of dephosphorylating inositol-1,4,5-trisphosphate (IP_3) to inositol-1,4-bisphosphate (IP_2). To further characterize these enzymatic forms, we have mapped the gene (INPP5B) coding for the 75-kDa type II enzyme. Using a combination of human × rodent somatic cell hybrids and fluorescence in situ hybridization, we have determined that this gene maps to human chromosome band 1p34.

The 75-kDa inositol polyphosphate-5-phosphatase is an enzyme that was originally isolated from human platelets (Mitchell et al., 1989) and cloned from human megakaryocytic and placental cDNA libraries (Ross et al., 1991). It is one of at least three enzymes known to catalyze the conversion of inositol-1,4,5-trisphosphate (IP_3) to inositol-1,4-bisphosphate (IP_2). IP_3 is formed by the cleavage of membrane-bound 4,5-phosphatidyl inositol phosphate and acts as a second messenger capable of releasing Ca^{++} from intracellular stores (Berridge, 1993). Inositol-1,3,4,5-tetrakisphosphate (IP_4) is likewise capable of releasing intracellular Ca^{++} (Berridge, 1993) and is also a substrate for one or more of these enzymes.

Three enzymes with this activity have so far been identified, all of them having different properties. A 45-kDa polypeptide (type I) has been isolated from platelets and brain (Connolly et al., 1985). It will hydrolyze both IP_3 and IP_4 but has a higher affinity for IP_4. The 75-kDa enzyme (known as type II or type III, depending on the author; see Irvine, 1992), has a similar K_m for IP_3 as the 45-kDa enzyme, but a lower affinity for IP_4, and it is the only enzyme for which the gene has been cloned (INPP5B). In addition, the 45-kDa enzyme can be phosphorylated by protein kinase C, whereas the 75-kDa enzyme does not undergo this modification (Mitchell et al., 1989). A third enzyme (also called type III or type II; see Irvine, 1992) has a molecular weight of ~ 120 kDa. This enzyme, first purified from bovine brain (Hansen et al., 1987), hydrolyses IP_3 but has a very low affinity for IP_4.

To further characterize the gene for the 75-kDa enzyme and to evaluate whether the different enzyme activities are encoded by different genes, we have used a mapping approach. Here we report the localization of the gene for the 75-kDa enzyme (INPP5B) to human chromosome band 1p34.

Materials and methods

Somatic cell hybrids

The chromosomal assignment of the gene for the 75-kDa enzyme was performed initially by Southern blot and PCR from the following cell lines: GM1416, a 48,XXXX human lymphoblastoid cell line (Coriell Institute, Camden, NJ); RJK88 Chinese hamster lung V79 (Fuscoe et al., 1983); MR 8.2, a hybrid containing chromosome 1 and the X chromosome on an RJK88 background (Ledbetter et al., 1986); NBE-N1, A9/1492, and A9/GM0201, hybrids containing portions of chromosome 1 on a mouse background (Dracopoli et al., 1988); a panel of human monochromosomal hybrids on a rodent background (Coriell Institute). Mouse genomic DNA was made from female 129Sv mouse liver by standard methods (Sambrook et al., 1989).

Ten micrograms of human genomic DNA (GM 1416), mouse genomic DNA (129Sv), and Chinese hamster genomic DNA (RJK88) and 13 µg of hybrid DNAs were digested with *Eco*RI and separated by electrophoresis in a 0.85% agarose gel. The gel was transferred onto a nylon membrane (Zetabind) and hybridized at 42 °C with a 1.0-kb mouse cDNA probe (from the 3'-end of the mouse gene for the 75-kDa enzyme) at low stringency (25%

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(formamide). The mouse cDNA was obtained by screening an NIH3T3 cDNA library (using the human gene [Ross et al., 1991] as a probe) and by sequence comparison was found to be 98% identical at the amino acid level to its human homolog (L. Charnas, unpublished result). It was therefore a suitable probe for this study. The filter was washed at 65 °C in 1 × SSC, 0.1% SDS (30 min) and then in 0.5 × SSC, 0.1% SDS (30 min). The membrane was subsequently exposed to X-ray film at − 70 °C for 72 h (Sambrook et al., 1989).

PCR and YAC library screening

PCR primers were designed from the 3' untranslated region (3'-UTR) of INPP5B (GenBank name: HUMINP5P; GenBank accession No. M74161) (forward primer: 5' GAG TGG CAG CTT ATT GCT TCG 3'; reverse primer: 5' GAG TGT GCT AAC CAA TGG 3'). One microgram of DNA from each hybrid was used for the PCR reaction. PCR reactions contained 100 pmol of each primer, 20 nmol of each of the deoxynucleotides, and 2.5 U of AmpliTaq polymerase (Perkin-Elmer Cetus). The PCR was performed in a Perkin-Elmer thermal cycler for 30 cycles (each cycle was 95 °C for 1 min, 50 °C for 2 min, and 72 °C for 2 min). The products were analyzed on a 2% agarose gel. The PCR primers were then used to screen two yeast artificial chromosome (YAC) libraries (CEPH and St. Louis libraries) using the same PCR conditions as described above. Seven positive YACs (four from the St. Louis library and three from the CEPH library) were obtained from this screening. One YAC, 291B2, from the CEPH library, was used in the in situ hybridization.

Fluorescence in situ hybridization (FISH)

For FISH, 5–10 μg of total DNA, made from the yeast clone carrying YAC 291B2, was labeled with biotin by a nick-translation kit (ONCOR, Gaithersburg, MD). The probe was hybridized to a metaphase spread made from PHA-stimulated lymphocytes, isolated from whole blood of a normal female using standard methods, and to a metaphase made from a lymphoblastoid cell line carrying a chromosome 1 translocation, 46,XY,t(1;2)(p21;p21) (M. Muenke, personal communication). During hybridization, background from human and yeast repetitive sequences was suppressed with 40 μg of denatured human placental DNA and with 10 μg of DNA from a YAC clone carrying a random human DNA insert.

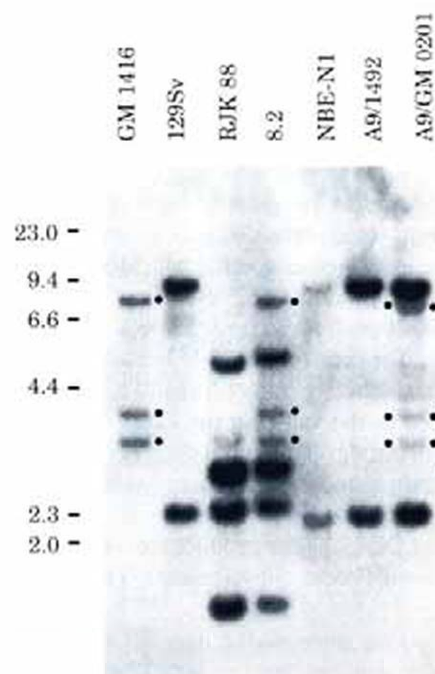

Fig. 1. Southern blot analysis of rodent × human hybrids containing portions of chromosome 1. Hybrid 8.2 is on a Chinese hamster background and contains human chromosomes 1 and X. NBE-N1 contains a fragment from 1p22→qter. A9/1492 contains fragment 1pter→p21, and A9/GM0201 contains fragment 1pter→p32. Three distinct human bands can be seen (marked with dots). These bands can be clearly seen in hybrid 8.2 and in A9/GM0201. They can also be faintly seen in hybrid A9/1492 but are absent from NBE-N1. This blot sublocalizes INPP5B to 1pter→p32.

Results and discussion

Using a combination of human × rodent somatic cell hybrids, as well as an independent hybrid containing chromosome 1 (8.2; Fig. 1), we localized INPP5B to chromosome 1. Three distinct human bands, of 7.8, 3.5, and 3.0 kb in size, could be visualized in the chromosome 1 hybrids. Digestion with *Eco*RI was found to give the best separation of bands between human, mouse, and Chinese hamster DNA. Using a set of partial chromosome 1 hybrids, NBE-N1 (1p22→qter), A9/1492 (1pter→p21), and A9/GM0201 (1pter→p32), we further localized the gene to the short arm of chromosome 1. The sublocalization is consistent within all three of the partial chromosome 1 hybrids and places the gene in 1pter→1p32. PCR primers from the 3'-UTR were capable of amplifying ~ 200 bp product from human genomic DNA, as well as from only those somatic cell hybrids that showed human bands in the Southern blot (data not shown). Therefore, the presence of the gene in those hybrids showing a positive signal by Southern analysis is supported by the PCR analysis.

As an independent approach, two YAC libraries were screened by PCR. Seven YACs were obtained: CEPH YACs 93D5, 97C2, and 291B2; St. Louis YACs A180A7, A189D2, A245A2, and A245A10. YAC 291B2 was used in the FISH analysis. Thirty-one normal (46,XX) metaphases and 15 trans-

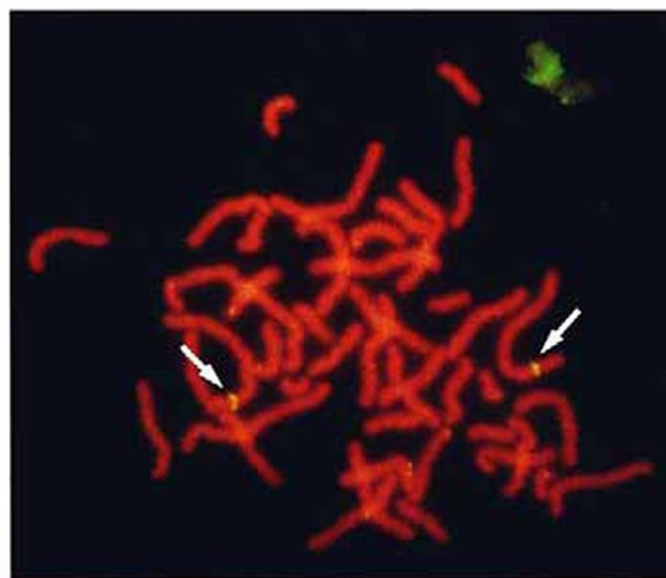

Fig. 2. Human metaphase from a normal female following fluorescence in situ hybridization with YAC 291B2. The arrows indicate the positions of the hybridization signals on chromosome 1 at 1p34. As can be seen, this is the only signal present, thus suggesting that YAC 291B2 is not chimeric.

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location metaphases with a 46,XY,t(1;2)(p21;p21) karyotype were analyzed after hybridization with YAC 291B2. Specific hybridization was seen at 1p34 (Fig. 2). The use of a cell line carrying a chromosome 1 translocation provided additional evidence that INPP5B maps to 1p34. When total yeast was used as a probe, the satellite regions of the acrocentric chromosomes also showed a hybridization signal. This probably represents cross-hybridization between human and yeast rRNA genes. No other specific hybridization signal was observed, suggesting that YAC 291B2 is not chimeric. The result from the FISH analysis is consistent with the hybrid mapping.

The gene for the 75-kDa inositol polyphosphate-5-phosphatase is highly homologous to a gene, OCRL1, recently identified as the locus for the Lowe oculocerebrorenal syndrome, a human developmental disorder affecting the eyes, central nervous system, and kidneys (Attree et al., 1992). This mapping study provides definitive evidence that the 75-kDa protein and the OCRL1 gene product are encoded by two different loci, on two different chromosomes. However, OCRL1 may still encode one of the other previously described inositol polyphosphate-5-phosphatase activities. The function of the OCRL1 gene product, particularly its role in inositol metabolism, remains to be determined.

It is not possible to predict the phenotype that would result from a loss of the 75-kDa inositol polyphosphate-5-phosphatase enzymatic activity. A review of previously mapped genetic diseases near 1p34 provides no obvious candidate diseases. However, a phenotype may be achieved by producing mice with a targeted disruption of the gene for the 75-kDa enzyme as a result of homologous recombination in embryonic stem (ES) cells (Capecchi, 1989; Smithies, 1993).

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