Genomic Organization and Chromosomal Localization of the DUSP2 Gene, Encoding a MAP Kinase Phosphatase, to Human 2p11.2–q11

HUAFANG YI, CYNTHIA C. MORTON,* STANISLAWA WEREMOWICZ,*
O. WESLEY MCBRIDE, AND KATHLEEN KELLY†[,1]

*Laboratory of Biochemistry and †Laboratory of Pathology, National Cancer Institute, National Institutes of Health,
Bethesda, Maryland 20892; and *Department of Pathology, Brigham and Women's Hospital
and Harvard Medical School, Boston, Massachusetts 02115*

The mitogen-induced gene, DUSP2, encodes a nuclear protein, PAC1, that acts as a dual-specific protein phosphatase with stringent substrate specificity for MAP kinase. MAP kinase phosphorylation and consequent enzymatic activation is a central and often obligatory component in signal transduction initiated by growth factor stimulation or resulting from various types of oncogenic transformation. DUSP2 downregulates intracellular signal transduction through the dephosphorylation/inactivation of MAP kinases. To facilitate assessment of the possible role of DUSP2 in growth processes, the genomic structure and chromosomal location of the gene have been determined. DUSP2 has been localized to the pericentromeric region of human chromosome 2 (2p11.2–q11) by analysis of somatic cell hybrids, *in situ* chromosome hybridization, and genetic linkage analysis using a single-strand conformational polymorphism (SSCP) that has been identified in the 3' UTR of the gene. No consistent translocations or deletions at this chromosomal site have been reported in hematopoietic neoplasias or other tumors. © 1995 Academic Press, Inc.

INTRODUCTION

PAC1 is a mitogen-induced, nuclear dual-specific phosphatase that is expressed predominantly in T cells and other hematopoietic cells (Rohan *et al.*, 1993). PAC1 has been shown to function as a physiologically relevant MAP kinase phosphatase (Ward *et al.*, 1994). MAP kinases are activated by combined tyrosine and threonine phosphorylation within a Thr–Glu–Tyr motif catalyzed by MAP kinase kinase (MEK) (Ahn *et al.*, 1992). The majority of phosphorylated MAP kinase

translocates to the nucleus, where it acts to phosphorylate several transduction targets, including transcription factors (Alvarez *et al.*, 1991; Chen *et al.*, 1992; Davis, 1993; Gonzalez *et al.*, 1993).

MAP kinases are thought to play a crucial role in signal transduction induced by various growth factors, tumor promoters, and differentiation factors. In addition, MAP kinase is a downstream target of oncogenic ras and raf (McCormick, 1993; Van Aelst *et al.*, 1993; Vojtek *et al.*, 1993). Recently, it has been shown that introduction of constitutively activated MEK is sufficient to transform 3T3 cells (Mansour *et al.*, 1994). Consistent with the idea that MAP kinase activation is necessary in growth factor stimulation of cells, we have shown that constitutive expression of PAC1 inhibits the growth of 3T3 cells (Kelly and Ward, 1994).

A serum growth factor-inducible phosphatase isolated from fibroblasts, CL100/MKP-1, is structurally similar to PAC1, especially within the carboxy-terminal 15 kDa of PAC1 containing the catalytic site (approximately 80% homology) (Rohan *et al.*, 1993; Emslie *et al.*, 1994). However, it is interesting that PAC1 and CL100 are significantly different within their 15-kDa amino-terminal domains, having only about 30% similarity. The tissue distribution of the two phosphatases is distinct. PAC1 is predominantly in hematopoietic tissues, while CL100 is widely expressed (Charles *et al.*, 1992; Noguchi *et al.*, 1993b; Rohan *et al.*, 1993; Emslie *et al.*, 1994). This suggests that the regulatory domains of PAC1 and CL100/MKP-1 may have been selected for unique functions in different cell types. The extent of a potential family of MAP kinase phosphatases is an important question in considering MAP kinase regulation.

As MAP kinase activation is a downstream target of several oncogenes and constitutive MAP kinase activation is sufficient for oncogenic transformation, DUSP2 is a candidate tumor suppressor gene. As a first step to evaluate this possibility, we have determined the genomic structure and chromosomal location of DUSP2.

MATERIALS AND METHODS

Genomic clone isolation and characterization. A genomic library was prepared as a partial *Mbo*I digest of DNA obtained from the human lung fibroblast WI-38 cloned into the Lambda FIX vector (Stratagene, La Jolla, CA). The library was screened with a full-length PAC1 cDNA probe under stringent hybridization conditions. Stringent hybridization was carried out in 50% formamide, 0.5% SDS, 5× SSC, 5× Denhardt's solution, and 2 mg/ml herring sperm DNA at 42°C for 24 h. Filters were washed in 0.1× SSC–0.5% SDS at 55°C. A positive clone containing an 18-kb insert was partially characterized and subsequently subcloned into Bluescript SK(−) as a 4.0-kb *Sal*I fragment (nucleotide 4 on the 5′ side to a cloning site in the phage arm on the 3′ side), as a 2.5-kb *Sst*I fragment (extending 5′ from the *Sst*I site at nucleotide 387), and as a 1.5-kb *Sst*I fragment (extending between *Sst*I sites 387 and 1373). Numbering is relative to the cDNA sequence (GenBank Accession No. L11329). Double-stranded sequencing of the subcloned DUSP2 gene using DUSP2-specific primers was performed. Intronic sequences have been submitted to GenBank (Accession No. U23853).

Genomic blots. Southern blots of DNA restriction digests of human thymus DNA on nitrocellulose were prepared after (0.8%) agarose gel electrophoresis and hybridized with ³²P-labeled probes. Stringent hybridization conditions were as detailed above. Nonstringent hybridization conditions were identical except that the maximum wash temperature was 45°C.

Cell hybrids and filter hybridization. Isolation and characterization of a panel of human/rodent somatic cell hybrids has been described (McBride *et al.*, 1982). The human chromosome content of each hybrid cell line was determined by standard isoenzyme analyses. Southern blotting using previously localized genes as probes, and cytogenetic analysis. Southern blots of DNA restriction digests of hybrid cells on positively charged nylon membranes were prepared after (0.7%) agarose gel electrophoresis and hybridized at high stringency with ³²P-labeled probes (Olson *et al.*, 1991) under conditions allowing no more than 10% divergence of hybridizing sequences. Blots were reused after removal of probe with alkali and neutralization.

Some hybrid cell DNA was also used as templates for PCR amplification with chromosome-specific oligonucleotide primers (Abbott and Povey, 1991; Theune *et al.*, 1991) to localize chromosomal breakpoints or to confirm the presence or absence of the DUSP2 gene in a cell line. PCR products were evaluated by ethidium staining after electrophoresis in (2%) agarose minigels.

DNA restriction fragment length polymorphism. DNA from 10 unrelated normal individuals was separately digested with 12 different restriction enzymes and examined for RFLPs by Southern blotting with a 1.7-kb PAC1 cDNA probe after agarose gel electrophoresis.

In situ hybridization. In situ hybridization of a 1.5-kb DUSP2 genomic fragment, extending between the *Sst*I sites 387 and 1373 (see Fig. 1), was performed as previously described (Morton *et al.*, 1984). The probe used in the hybridization was radiolabeled to a specific activity of 7.9 × 10⁶ cpm/µg with tritium by nick-translation using all four tritiated nucleotides. Following hybridization and washing, slides were exposed to Kodak NTB2 emulsion for 21 days at 4°C.

Oligonucleotides. All oligonucleotides were made using a MilliGen 8700 DNA synthesizer. DUSP2-specific primers were PAC1A (coding strand) = 5′ AGGGCTGCTACCTCCTCAGAG 3′ and PAC1B (noncoding strand) = 5′ CCAGCGCCAGCACAGGTGTGA 3′; a 410-bp sequence located within the 3′ UTR of DUSP2 was amplified using these primers.

PCR and SSCP analyses. SSCP analyses (Orita *et al.*, 1989) were performed as previously described (Noguchi *et al.*, 1993a). Each PCR was performed in a volume of 15 µl containing 200 ng of genomic DNA, 1× PCR buffer (Promega), primers (0.5 µM each), dATP, dGTP, and dTTP (200 µM each), 25 µM dCTP with 1.0 µCi of [³²P]dCTP, and 0.45 U of *Taq* polymerase (Promega). Initial denaturation was for 5 min at 94°C, followed by 30 cycles of 1 min each at 94, 55, and
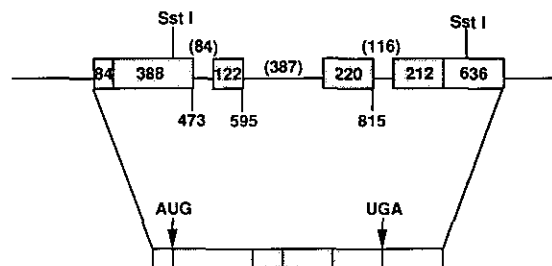

FIG. 1. Genomic structure of the human DUSP2 gene. Protein coding regions are shown as shaded boxes and untranslated regions as unshaded boxes. The numbers within the boxes represent the nucleotide length of exons; the numbers within parentheses represent the nucleotide length of introns. The numbers below the genomic diagram represent the nucleotide number of the exon/intron boundary relative to the cDNA sequence (Accession No. L11329). The *Sst*I sites that comprise the fragment ends used for *in situ* hybridization mapping are shown.

72°C with final extension for 7 min at 72°C. A 2.5-µl aliquot of the ³²P-labeled PCR product was diluted with 10 µl of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol, denatured 10 min at 95°C, and quenched in an ice bath. Aliquots (4 µl) of the ³²P-labeled single-stranded DNA were applied to the wells of a 30 cm × 40 cm × 0.4 mm gel containing 5% polyacrylamide in 1× TBE buffer (pH 8.0). Electrophoresis was performed at constant power (25 W) at room temperature, with cooling from a fan blowing over a tray of ice in front of the gel, until the bands had migrated to within 10 cm from the bottom of the gel. Gels were transferred to Whatman 3MM paper and dried, and band locations were determined by autoradiography; 30-min exposures were generally sufficient.

RESULTS

To characterize the DUSP2 gene, we isolated human DUSP2 genomic clones and determined the nucleotide sequence of the complete gene. A schematized summary of the data is shown in Fig. 1. The entire gene without the 5′ upstream sequences is contained within 2.3 kb and consists of four exons and three relatively small introns. All four exons encode protein sequence. The first and fourth exons also encode 5′ and 3′ untranslated sequence, respectively. DUSP2 and CL100 have a common intron/exon structure (Noguchi *et al.*, 1993b) indicating that the two genes have evolved from a similarly spliced ancestral gene.

To investigate the size of the DUSP2/CL100 gene family, we performed Southern blots using low-stringency hybridization of probes from the 5′ and 3′ halves of the protein coding region or the 3′ untranslated region (UTR) of DUSP2 (Fig. 2). PAC1 and CL100 are most similar in their carboxy halves, the region containing the catalytic domain (Rohan *et al.*, 1993; Emslie *et al.*, 1994). At low stringency, probes encoding either amino or carboxy halves of the protein hybridize with 10 to 20 genomic fragments, indicating a large gene family. In addition, there appears to be a gene that is highly related but distinct from DUSP2 as shown by its stringent hybridization to coding region probes and no hybridization with a 3′ UTR probe. Absence of hy-
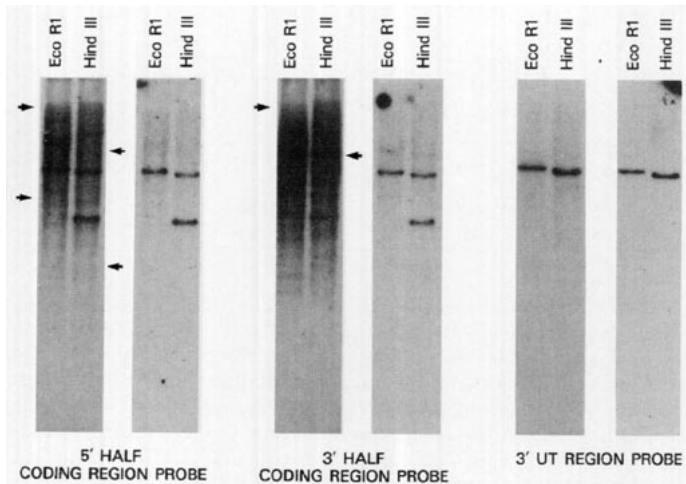
FIG. 2. Several DUSP2-related genes are observed in human genomic DNA. Southern blots of digested human thymus DNA were probed with either 5' (nucleotides 1–609) or 3' (nucleotides 610–1192) coding region probes or a 3' untranslated region probe (1193–1709) derived from a human PAC1 cDNA clone, and the blots were sequentially washed at low stringency (left blots) or high stringency (right blots) as described under Materials and Methods. The arrows indicate the location of the CL100 gene as determined from parallel blots.

FIG. 3. Idiogram of chromosome 2 showing the distribution of grains on chromosome 2 from the *in situ* hybridization experiment. The peak of grains is located in the pericentromeric region of chromosome 2 (2p11.2–q11).

bridization with a 3' UTR probe suggests that the related sequences do not encode a pseudogene of DUSP2.

The DUSP2 gene was localized to human chromosome 2 by Southern analysis of a panel of *Eco*RI-digested human/rodent somatic cell hybrid DNAs using a full-length, 1.7-kb PAC1 cDNA as a probe. The gene segregated concordantly (100%) with chromosome 2 and discordantly (0.11%) with all other chromosomes in the panel of hybrids (data not shown). Examination of several hybrid cell lines containing only portions of chromosome 2 permitted regional localization of the DUSP2 locus to 2p11–qter (data not shown).

Regional localization of DUSP2 was further refined by *in situ* hybridization (Fig. 3). Analyses of 170 metaphase preparations from peripheral blood lymphocytes from chromosomally normal human males, hybridized with the DUSP2 gene, revealed approximately 8% of metaphase spreads to have silver grain deposition in the pericentromeric region of chromosome 2. Of the 264 silver grains observed to be located on or beside chromosomes, 13 (5%) grains were localized in bands 2p11.2–q12. These 13 grains represented 50% of all grains localized on chromosome 2.

A secondary peak of hybridization was seen at 1p36.3–p36.2, which represents approximately 3% of all grains scored. Whether this secondary peak, which has almost twice the number of grains present at any site other than 2p11.2–q12, represents true homology or a clustering of grains at the telomere remains to be determined. The 1.5-kb *Sst*I insert utilized as a probe consisted of approximately one-third noncontiguous coding sequence and two-thirds noncoding sequence and would not be expected to be very effective at recognizing homologous genes. It seems most likely that the

chromosome 1 peak is a technical artifact, as this finding is not supported by mapping in somatic cell hybrids and some accumulations of grains at telomeres of chromosomes is not an uncommon finding in isotopic *in situ* hybridizations.

Genetic linkage analysis was used as a third method to identify the position of the DUSP2 gene. Southern blots of restriction digests (*Eco*RI, *Hind*III, *Bam*HI, *Xba*I, *Sac*I, *Taq*I, *Msp*I, *Pvu*II, *Pst*I, *Bgl*II, *Eco*RV, and *Kpn*I) of DNAs from 10 unrelated individuals disclosed no RFLPs when probed with the full-length PAC1 cDNA. However, a two-allele SSCP (Fig. 4) was found in the 3' UTR of the gene with allele frequencies of A1:A2 = 0.50:0.50 and PIC = 0.39 among the 80 CEPH parents. All of the CEPH parents were analyzed, and
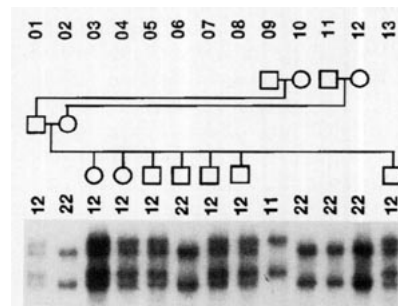

FIG. 4. Mendelian inheritance of the DUSP2 gene in three-generation CEPH family 1340. The pedigree of the family is shown above the lanes, and the alleles in each lane are indicated below the pedigree. SSCP analysis was performed using DUSP2 primers (see Materials and Methods). Due to different rates of migration of the complementary DNA strands, each allele is represented by two bands. The paternal grandfather (134009) is homozygous for allele 1, whereas the mother (134002) is homozygous for allele 2.

TABLE 1

Two-Point Lod Scores for DUSP2 vs Other Loci on Chromosome 2

Loci[a]	θ^b	Z^c	Confidence interval[d]
1. D2S38	(0.017, 0.079)0.046	(20.0)19.5	0.027–0.089
2. D2S25	(0.00, 0.00)0.00	26.2	—
3. D2S43	(0.037, 0.186)0.115	(12.7)12.0	0.065–0.192
4. D2S41	(0.741, 0.194)0.228	(3.8)3.3	0.162–0.337
5. D2S51	(0.00, 0.295)0.247	(2.9)2.1	0.137–0.413
6. D2S54	(0.234, 0.299)0.272	(2.5)2.4	0.172–0.408
7. D2S21	(0.211, 0.369)0.300	(3.7)3.0	—
8. D2S44	(0.257, 0.347)0.307	(3.8)3.5	0.249–0.400

[a] Probe–enzyme combinations for these loci are as follows: D2S38 is CRI-L625/MspI and CRI-L625/TaqI (Donis-Keller *et al.*, 1987) combined as haplotype; D2S25 is CRI-C13B/PstI (Donis-Keller *et al.*, 1987); D2S43 is pYNZ15/TaqI (O'Connell *et al.*, 1989); D2S41 is CRI-L452/BglII (Donis-Keller *et al.*, 1987); D2S51 is pCMM63/MspI (O'Connell *et al.*, 1989); D2S54 is 1pHHH115/MspI and 2pHHH115/MspI (O'Connell *et al.*, 1989) combined as haplotype; D2S21 is CRI-L22/TaqI (Donis-Keller *et al.*, 1987); and D2S44 is pYNH24/MspI (O'Connell *et al.*, 1989). Only the DUSP2 data were generated in this study. The other data are from CEPH database version 5, and the data were provided by the labs of Drs. Ray White and Helen Donis-Keller.

[b] The most likely recombination fractions between DUSP2 and these loci are shown; sex-specific recombination fractions in parentheses (male–female order) and sex average value are also shown.

[c] Lod scores at θ_{max} assuming independent recombination fractions in males and females (parentheses) or sex average recombination fraction.

[d] Confidence intervals for recombination fractions over 10-fold range of likelihood.

one or both parents were heterozygotes in 31 of the 40 families. All members of these families were genotyped at this locus and used for linkage analyses with other loci in the CEPH database. DUSP2 is closely linked to loci in the vicinity of the chromosome 2 centromere (Table 1). The loci in Table 1 are ordered from short arm to long arm based upon published extensive multipoint linkage analyses (Spurr *et al.*, 1992). These loci span a distance of about 37 cM (sex ave.) on the CEPH Consortium map (Spurr *et al.*, 1992), representing about 12% of the entire genetic distance encompassed by chromosome 2 and spanning the region from about 2p12 to 2q21–q22. No recombinants were ob-

served between DUSP2 and D2S25 in almost 90 fully informative meioses, and relatively close linkage was also found between DUSP2 and the two flanking markers (i.e., D2S38 and D2S43). These data suggest that DUSP2 is located very close to the centromere, but the gene cannot be assigned precisely to either the short arm or the long arm because the physical location of these anonymous DNA markers is somewhat uncertain.

DUSP2 was ordered within this group of loci by multipoint linkage analysis (Table 2). DUSP2, D2S38, D2S25, and D2S43 were first ordered using the program CILINK while considering all 12 possible orders. All orders except that depicted in Table 2 were excluded with odds greater than 2.8×10^7, but DUSP2 and D2S25 could not be ordered. The loci D2S41 and D2S51 were then added using CILINK and considering 10 possible orders. The order of loci is identical to that in published results (Spurr *et al.*, 1992) that used the same genotypic data except that for DUSP2. The use of new short tandem repeat markers (Weissenbach *et al.*, 1992) will permit further localization of the gene.

DISCUSSION

The DUSP2 gene has been localized to human chromosome 2p11.2–q11 in close proximity to D2S25. MAP kinase activation appears to be necessary (Pages *et al.*, 1993; Kelly and Ward, 1994) and is probably sufficient for cellular transformation (Mansour *et al.*, 1994). PAC1 is a highly specific MAP kinase phosphatase and has a vital role in modulating signal transduction through down-regulation of MAP kinase activity (Ward *et al.*, 1994). DUSP2 could act as either a recessive tumor suppressor gene or possibly a proto-oncogene in a dominant negative form. Such changes could involve either mutations or translocations and/or rearrangements, and knowledge of the chromosomal location of the gene is important for these considerations. Neither specific chromosomal translocations nor deletions in the 2p11.2–q11 region have been reported to be associated with any neoplasias. However, the potential involvement of DUSP2 in some hematopoietic neoplasias is not excluded, and cytogenetically cryptic mutations of DUSP2 may occur.

TABLE 2

Multipoint Linkage Analysis of DUSP2 with Other Loci (See Table 1) on Chromosome 2

		Odds		
	5.9×10^{12}	8.9×10^{19}	1.8×10^2	2.3×10^7
θ_{male}	0.077	0.075	0.00	0.00
	D2S38 —— (PAC-1, D2S25) —— D2S43 —— D2S41 —— D2S51			
θ_{female}	0.177	0.127	0.053	0.217
θ_{sexave}	0.093	0.100	0.034	0.167

Note. Six loci were ordered using the CILINK program (see Results). The most likely recombination fractions between loci and the odds against reversing the order of adjacent loci are shown.

The MAP kinase phosphatase CL100/MKP-1 has been localized to chromosome 5q34 (Emslie *et al.*, 1994). The existence of a family of genes related to DUSP2 (Fig. 2) and the observation that several PAC1-related gene products are expressed in T cells (Ward *et al.*, 1994; K.K., unpublished) suggest that MAP kinase phosphatase function may be redundant, at least in some cell types. Therefore, dominant negative mutations in DUSP2 may have functional consequences, whereas changes resulting in loss of DUSP2 function may not.

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