

Molecular cloning and characterization of *MPL*, the human homolog of the *v-mpl* oncogene: Identification of a member of the hematopoietic growth factor receptor superfamily

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ABSTRACT We have cloned the human homolog of the *v-mpl* oncogene transduced in the myeloproliferative leukemia retrovirus, which presents striking homologies with members of the hematopoietin receptor superfamily. We obtained two types of clones, *MPLP* and *MPLK*, which had the same 5' extremity but differed at their 3' ends. The resulting deduced polypeptides are composed of a common extracellular domain with a putative signal sequence and a common transmembrane domain, but they differ in their cytoplasmic domain after a stretch of 9 common amino acids. The extracellular domain of *MPL* contains the consensus sequences described for the members of the hematopoietin receptor superfamily. In addition, as for murine interleukin 3 and human and murine granulocyte-macrophage colony-stimulating factor type β receptors, this domain can be divided into two subunits. An additional motif specific for *MPL* could be displayed by hydrophobic cluster analysis in the first subdomain. When RNAs from various hematopoietic cell lines were analyzed by Northern blot, *MPL* was detected only in the human erythroleukemia (HEL) cell line as a major 3.7-kilobase (kb) mRNA (*MPLP*) and a minor 2.8-kb mRNA (*MPLK*). However, study of *MPL* expression by PCR analysis indicated that *MPL* is expressed at a low level in a large number of cells of hematopoietic origin and that the two types of mRNAs (P and K) were always found to be coexpressed.

The myeloproliferative leukemia virus (MPLV) is an acute leukemogenic murine retrovirus that displays unique biological features since, upon *in vivo* infection, a broad spectrum of MPLV-infected hematopoietic progenitors immediately acquired factor independence for both proliferation and terminal differentiation (1, 2). Moreover, direct *in vitro* infection of bone marrow cells gave rise to a variety of autonomous cell lines that probably derived from the outgrowth of infected multipotential stem cells (3).

In a previous report (3), we showed that MPLV has transduced in its envelope a cellular oncogene named *v-mpl* that shares striking structural similarities with members of the cytokine receptor superfamily.

The extracellular domains of these growth factor receptors are organized in 200 amino acid modules that display a distinctive conservation of four cysteine residues at their N terminus and a WSXWS box close to the transmembrane domain (4). No consensus sequence for kinase activity can be detected in the cytoplasmic region (5).

In the present paper, we describe the molecular cloning of the human *MPL* protooncogene.[¶] Two families of human cDNA clones were obtained, which share common extracel-

lular and transmembrane domains but differ in their cytoplasmic regions. Sequence analysis indicated that human *MPL* shares overall structural and amino acid homology with members of the cytokine receptor superfamily. Like the murine interleukin 3 (IL-3) receptor (6), the extracellular region of *MPL* can be divided into two subunits. No consensus sequence for tyrosine kinase activity could be detected in the cytoplasmic domain of either clone. The biological significance of these two clones is not known at the moment, but study of the expression of *MPL* by PCR analysis showed that whenever it was expressed, both families of mRNAs were detected.

MATERIALS AND METHODS

RNA Preparation and Northern Blot Analysis. Total RNA was prepared by the guanidium thiocyanate/cesium chloride method (7). Poly(A)⁺ RNA selection and Northern blot analysis were performed as described (3).

Construction of the Human Erythroleukemia (HEL) cDNA Library. cDNA was synthesized from HEL poly(A)⁺ RNA with the Amersham cDNA synthesis kit and was ligated with *EcoRI* adaptors. cDNAs >2.0 kilobases (kb) long were size selected on a 5–20% potassium acetate gradient (8) and cloned into *EcoRI*-digested λ gt10 (Stratagene). After *in vitro* packaging (Gigapack, Stratagene), recombinant phages containing *MPL* were identified by hybridization with *v-mpl*-specific RNA probes as described (3).

DNA Sequencing. DNA sequencing was performed by the dideoxynucleotide chain-termination method (9) modified for the use of the T7 DNA polymerase (Sequenase; United States Biochemical).

Sequence Analysis and Comparison. The sequence of *MPL* and that of the members of the cytokine receptor superfamily (10) were analyzed and compared by the two-dimensional hydrophobic cluster analysis (HCA) method (11, 12). This approach is more sensitive than that of classical one-dimensional methods in the detection of similar three-dimensional folding of protein globular domains (12, 13).

Analysis of RNA by PCR and Analysis of PCR Amplified Products. cDNA was synthesized from 1 μ g of total RNA by using Moloney leukemia virus reverse transcriptase (BRL). One-fourth of the cDNA reaction mixture was subjected to PCR amplification as recommended by the manufacturer (Perkin-Elmer/Cetus). To perform amplifications of sequences corresponding to either clone P or K, we used a 5'

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Abbreviations: MPLV, myeloproliferative leukemia virus; IL-3, interleukin 3; HCA, hydrophobic cluster analysis; Epo, erythropoietin; GM-CSF, granulocyte-macrophage colony-stimulating factor.

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^{||}The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M90102 and M90103).

sense primer common to both clones and 3' antisense primers specific for clones P and K, respectively. Amplified products were analyzed by gel electrophoresis and blotted to nylon membranes for hybridization to ³²P-labeled specific oligonucleotides.

RESULTS

Isolation of Human *MPL* cDNAs. We have previously shown that *MPL* is well conserved among mammals and is mainly expressed in hematopoietic tissues (3). Poly(A)⁺ mRNAs from hematopoietic human cell lines were therefore hybridized with *v-mpl* RNA probes in order to select a human cell line expressing high levels of *MPL* mRNA. Of the 12 lines tested [3 myeloid (HL60, KG1, U937), 3 B lymphoid (Raji, BJAB, Epstein-Barr virus-immortalized B cells), 2 T lymphoid (CCRF CEM, phytohemagglutinin-stimulated T cells), and 4 erythroid (HEL, K562, KU812, Lama) cell lines], only the erythroid HEL cell line (14) was found to express *MPL* as a 3.7-kb mRNA.

A HEL cDNA library was prepared and screened with two *v-mpl*-specific probes, a 300-base-pair (bp) *Sac* I/*Pst* I probe located in the *v-mpl* extracellular and transmembrane domains and a 300-bp *Pst* I/*Pst* I probe that encompasses the *v-mpl* cytoplasmic region. Of 5 × 10⁵ clones, 3 clones (clones 18, 23, and 27) hybridized with both probes, while 3 clones (clones 15, 39, and 41) hybridized only with the *Sac* I/*Pst* I probe. Restriction enzyme mapping of these clones confirmed that they could be divided into two classes. Clones 18, 23, and 27 had a *Pst* I site that did not exist in the 3 other clones (hereafter they are referred to as clones P), and clones 15, 39, and 41 had a *Kpn* I site absent in clones P (referred to as clones K). As shown in Fig. 1, the two types of clones seemed to share the 5' moiety, down to the *Pst* I or *Kpn* I sites, and to differ at their 3' ends. Comparison with the *v-mpl* restriction map and hybridization with the two *v-mpl* probes suggested that clones P could contain the sequence transduced by MPLV. None of the 6 clones had the expected full size of 3.7 kb.

DNA sequence analysis confirmed that clones P and K shared a common sequence at their 5' ends. Since clones P

were incomplete at their 5' ends, they were extended by using a specific oligonucleotide as a primer (oligonucleotide MPL1P; Fig. 1). Five additional clones were obtained. Sequence analysis indicated that one of these, clone 24P, extended 20 bp further than the longest 5' clone K (clone 39K).

Clone 23P contained a poly(A)⁺ signal [ATAAA, 13 bp upstream from a poly(A) tail], while no such signal could be found in the three clones K. The size of clone P cDNA deduced from overlapping clones P was 3.7 kb and corresponded to the size of the major mRNA from HEL cells.

Structure of the Human *MPL* Putative Receptor. The deduced amino acid sequence of clones P and K is shown in Fig. 2. Clones P and K have a long open reading frame potentially encoding proteins of 635 and 572 amino acids, respectively, starting at the methionine codon at position 1. Another potential initiation codon is present at position 8. The nucleotide sequence surrounding this second ATG corresponds better to the consensus sequence for translation initiation sites (15). Hydrophilicity plot analysis of the deduced amino acid sequence predicts two major hydrophobic regions, the first one spanning amino acids 8–25 and corresponding to a signal sequence of 18 amino acids. Therefore, the mature *MPL* protein would in any case start at amino acid position 26. A second hydrophobic region lies at amino acids 492–513 and is a presumed 22-amino acid transmembrane domain.

Both classes of mature proteins are thus composed of a common extracellular region of 463 amino acids and a transmembrane domain of 22 amino acids but differ in their cytoplasmic domain after 9 common amino acids. The protein encoded by clones P has an intracellular portion of 122 amino acids, while the sequence of clones K predicts a cytoplasmic domain of 66 amino acids. The cytoplasmic sequence of clones P has a high content of proline and serine (12% and 11.5%), while only a high content of proline (12%) was found in the cytoplasmic region of clones K. None of these cytoplasmic regions contains sequences typical of protein kinases (16).

MPLP and -K contain four potential N-linked glycosylation sites in their extracellular regions. The estimated molecular mass of the two proteins would be 71 kDa for clones P and 65 kDa for clones K.

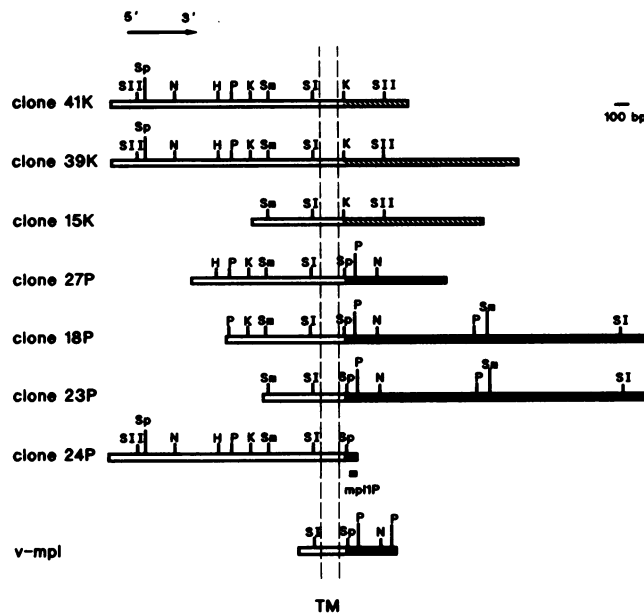


FIG. 1. Restriction map of *MPLP* and *-K*. Open boxes, region common to both clones; hatched boxes, region specific for clones K; solid boxes, region specific for clones P, highly homologous to *v-mpl*. TM, transmembrane domain. B, *Bam*HI; H, *Hind*III; K, *Kpn* I; N, *Nco* I; P, *Pst* I; SI, *Sac* I; SII, *Sac* II; Sm, *Sma* I; Sp, *Sph* I.

H-MPL	-	MPSWALFVYVTSCLLLAPONLAQVSSQDVSLASDSEPLKCFKSRTPEDLTC	-	50
H-MPL	-	PWDEEEAAPSQTYQLLYAYPREKPRACPLSSQSPHFGRVYVCFQDPQEE	-	100
H-MPL	-	VRLFFPLHLWVKNVFLNQTTRQTRVLFVDSVGLPAPPSIIKAMGSSQSGEL	-	150
H-MPL	-	QISWEEPAPEISDFLRYELRYGPRDPKNSGTPTVYQLIATETCCPALQRP	-	200
H-MPL	-	HSASALDQSPCAQPTMPWQDGPKQTSPSREASALTAEGGSCGISGLQPGN	-	250
H-MPL	-	SYWQLRSEPDGISLGGSSNGSWSLPVTVDLPGDAVALGQCFPLDLKNT	-	300
H-MPL	-	CQWQQDHASSQGFYHSRARCPRDRYPIWENCEEEETNPGLQTPQFS	-	350
H-MPL	-	RCHFKNRSDSIHILVEVTTAPGVTHSYLSGSPFWIHQAVRLPTPNLHWRE	-	400
V-MPL	-		LE	- 2
H-MPL	-	ISSGHLELEWQHPSWAAQETCYQLRYTGEHQDWKVLPEPLGARGGTLE	:	450
V-MPL	-	LRPRARYSLQLRRLRNGPTYQGPWSANSPPARVSTGSETAWITLVYVALLL	:	52
H-MPL	-	LRPRARYSLQLRRLRNGPTYQGPWSANSPPARVSTGSETAWITLVYVALLL	:	500
V-MPL	-	YLSGLAVLGLLRLRWQFPFAHYRRLRHALWPSLPDLHRVGLQVLRDSTAALS	:	102
H-MPL P	-	YLSGLAVLGLLRLRWQFPFAHYRRLRHALWPSLPDLHRVGLQVLRDSTAALS	:	550
H-MPL K	-	YLSGLAVLGLLRLRWQFPFAHYRYPKQAGDWRWRWRSRTCKQAFVLRVSVT	:	550
V-MPL	-	PSKATVDSCEEVEPSLLEILPKSSESTPLPLCPSQPMQDYRGLQPC-LR	:	151
H-MPL P	-	PPKATVDSCEEVEPSLLEILPKSSESTPLPLCPSQPMQDYRGLQPC-LR	:	600
H-MPL K	-	PDLRPPVRYTYGFALPARHLWDSRLLTL	:	579
V-MPL	-	TMPLSVCPMAETGSCCTTHIANHSYLPLSYWQ	:	184
H-MPL P	-	TMPLSVCPMAESGSCCTTHIANHSYLPLSYWQQP	:	635

FIG. 2. Deduced amino acid sequence of *MPLP* and *MPLK* and alignment with amino acid sequence of *v-mpl*. Underlined sequences, signal sequence and transmembrane domain (boldface); :, conserved amino acids; stars, potential N-glycosylation sites.

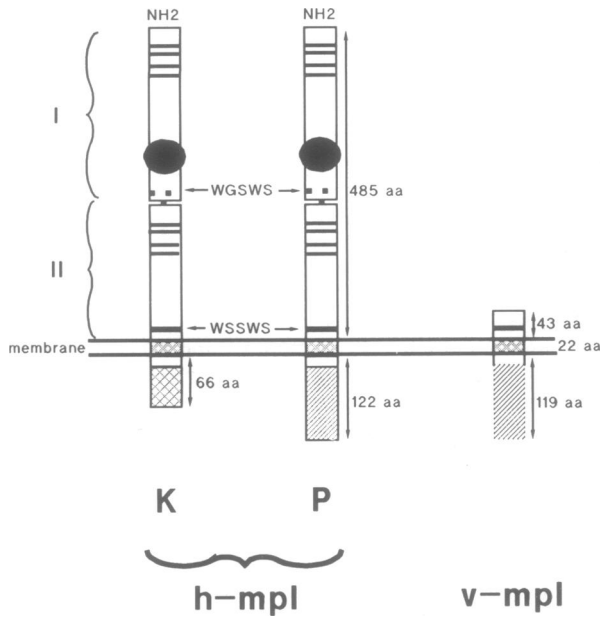


FIG. 3. Schematic drawing of MPLP and MPLK polypeptides compared to v-mpl. Solid bars, cysteine residues; thick solid bars, WSXWS boxes; thick dotted bars, degenerated WSXWS box; solid circle, MPL-specific extra domain. aa, Amino acids.

When the sequence of clones P was aligned with that of v-mpl, 86% homology was found at the amino acid level (Fig. 2). The strongest homology was in the cytoplasmic region (92%), although MPLP had 3 additional amino acids in this domain. The extracellular domain has been largely truncated in v-mpl, since it is composed of only 43 amino acids (Fig. 3). The WSXWS box is different for MPL (WSSWS) and v-mpl (WSAWS).

Comparison of the Putative MPL Receptor with Members of the Hematopoietin Receptor Superfamily. When the sequence of the extracellular domain of MPL was compared with that of members of this superfamily, a number of amino acid homologies could be observed (Fig. 4). The WSXWS box and the conserved cysteine residues were present in MPL. In addition, like the IL-3 receptor, the extracellular domain of MPL could be divided into two subunits, both containing the consensus sequences (Figs. 3 and 4).

The MPL protein sequence was further investigated by hydrophobic cluster analysis (12). The derived two-dimensional representation of the MPL extracellular domain had conserved features described for the other members of

the superfamily (10). The MPL extracellular domain could be subdivided into 4 SD100 subdomains with the consensus S1-S8 structural segments observed in all the receptors (Fig. 5). This analysis also corroborated the duplication of the extracellular domain of MPL, since SD100(1) and -(3), and SD100(2) and -(4) could be aligned. However, in SD100(2) an additional motif not present in SD100(4) or in other receptors could be identified between S4 and S5 (Fig. 5).

Comparison with the other members of the superfamily indicated that MPL was close to the IL-3 and erythropoietin (Epo) receptors. MPL seems to be closely related to murine Epo receptor since MPL SD100(4) and murine Epo receptor SD100(2) have 26% sequence identity with a HCA score of 73%, which indicates a rather good three-dimensional homology (12). In comparison, MPL SD100(4) and murine IL-3 receptor SD100(4) share a median overall value of 18% sequence identity.

Expression of MPL RNA. Among 12 human hematopoietic cell lines, only the HEL cell line was found to express MPL by Northern blot analysis. Although MPL is detectable by this method in murine hematopoietic tissues (3), similar negative results were obtained with most murine erythroid, myeloid, or lymphoid cell lines (data not shown).

To investigate whether a low level of MPL expression could be detected, we applied the PCR technique for amplification of RNA. By this technique, MPL could be detected in most of the samples found negative by Northern blotting, in particular in two erythroid (KU812 and UT7) and one myeloid (KG1) cell lines. In addition, MPL expression could be found in placenta, bone marrow, fetal liver (but not adult liver), fetal blood (34 weeks), cord blood, peripheral blood, and phytohemagglutinin-stimulated lymphocytes. More importantly, when MPL was expressed both clones K and P were detected, which indicated that clones P and K are both physiologically expressed (Fig. 6A).

Finally, we investigated MPLP and -K expression in HEL cells. Two 3' probes specific for each family were used. The P-specific probe detected the 3.7-kb mRNA after 24 hr, while the K-specific probe lit up a faint 2.6- to 2.8-kb mRNA after a 10-day exposure of the autoradiogram (data not shown). This indicated that in HEL cells the K species of mRNA are less abundant and shorter than the P species. Actually, when HEL RNA was hybridized with a 5' probe, the 2.7-kb mRNA species was detected after a 10-day exposure of the autoradiogram (Fig. 6B).

DISCUSSION

We previously reported that the v-mpl oncogene transduced in MPLV could be a truncated form of an additional member

hmp1I	37	P	L	K	C	F	S	R	(5aa)	T	C	F	W	D	(10aa)	Q	L	L	Y	A	Y	(6aa)	A	C	P	L	S	(9aa)	Y	V	C	Q	F	P	D	(55aa)	S	W	E		
hmp1II	288	G	L	Q	C	F	T	L	(5aa)	T	C	Q	W	Q	(8aa)	G	F	F	Y	H	S	(3aa)	C	C	P	R	D	(4aa)	E	N	C	E	E	E	(62aa)	H	W	R			
mILSR	128	N	L	T	C	T	T	H	(16aa)	R	C	T	W	L	(11aa)	F	L	L	Y	R	Q	(5aa)	K	C	Q	E	Y	S	(7aa)	T	A	C	W	F	P	R	(56aa)	Q	W	E	
mIL3RI	36	T	L	E	C	I	N	D	(5aa)	I	C	S	W	A	(11aa)	T	L	L	Y	H	Q	(7aa)	S	C	E	L	S	E	(10aa)	H	R	C	V	-	P	R	(56aa)	E	W	S	
mIL3RII	251	N	L	Q	C	F	F	D	(5aa)	H	C	S	W	E	(11aa)	G	L	H	Y	R	L	(7aa)	K	C	S	P	V	V	(10aa)	Y	R	C	S	L	P	V	(46aa)	H	W	E	
mIL4R	31	E	P	T	C	F	S	D	(5aa)	T	C	E	W	F	(11aa)	C	L	H	Y	R	L	(9aa)	T	C	I	P	R	N	(5aa)	C	V	C	H	H	E	M	(51aa)	T	W	N	
mIL7R	39	S	F	W	C	H	S	Q	(5aa)	S	Q	H	L	L	(12aa)	N	L	E	F	O	I	(7aa)	K	C	L	T	L	N	(9aa)	T	S	E	F	L	I	(55aa)	K	Y	L		
hIL2Rβ	33	Q	F	T	C	F	Y	N	(5aa)	S	C	V	W	S	(11aa)	V	H	A	W	P	D	(6aa)	T	C	E	L	L	P	(5aa)	W	A	C	N	L	I	L	(60aa)	S	W	E	
hIL6R	118	Q	L	S	C	F	R	K	(6aa)	V	C	E	W	G	(11aa)	V	L	L	V	R	K	(11aa)	P	C	Q	-	Y	S	(5aa)	F	S	C	Q	L	A	V	(56aa)	T	W	Q	
mEPOR	49	E	L	L	C	F	T	Q	(5aa)	V	C	F	W	E	(11aa)	S	F	S	Y	Q	L	(6aa)	S	C	S	L	H	Q	(9aa)	F	W	C	S	L	E	T	(53aa)	R	W	L	
hGMCsFR	123	N	F	S	C	F	I	Y	(5aa)	N	C	T	W	A	(11aa)	F	L	L	Y	I	R	N	(7aa)	R	C	P	Y	V	I	(6aa)	V	G	C	H	L	D	N	(53aa)	R	W	K
rPRLR	28	I	H	K	C	R	S	P	(5aa)	T	C	W	W	N	(11aa)	S	L	L	T	V	S	K	(7aa)	E	C	P	D	Y	K	(4aa)	N	S	C	F	F	S	K	(59aa)	K	W	S
hGHR	53	F	T	K	C	R	S	P	(5aa)	S	C	H	W	T	(13aa)	Q	L	F	Y	T	R	(11aa)	E	C	P	D	V	Y	(4aa)	N	S	C	Y	F	N	S	(57aa)	R	W	E	
mGCSFR	104	N	L	S	C	L	N	H	(6aa)	V	C	Q	W	E	(13aa)	D	S	F	R	S	R	(11aa)	K	C	V	A	K	K	(2aa)	N	H	C	S	I	P	R	(61aa)	S	W	K	

hmp1I	(95aa)	S	Y	W	L	Q	L	R	S	E	P	(5aa)	G	G	S	W	G	S	W	S	L	P	V	T	(9aa)	(22aa)	
hmp1II	(56aa)	R	Y	S	L	Q	L	R	A	R	L	(5aa)	Q	G	P	W	S	S	W	S	D	P	T	T	R	(9aa)	(22aa)
mILSR	(43aa)	T	Y	S	L	Q	V	R	A	A	V	(6aa)	P	G	R	W	G	E	W	S	Q	P	I	Y	(12aa)	(22aa)	
mIL3RI	(53aa)	I	Y	A	A	R	V	R	T	R	L	(6aa)	S	G	R	P	S	R	W	S	-	P	E	Y	(5aa)	(26aa)	
mIL3RII	(46aa)	S	Y	C	A	R	V	R	V	K	P	(4aa)	D	G	I	W	S	E	W	S	-	N	E	Y	(5aa)	(26aa)	
mIL4R	(51aa)	Y	Y	T	A	R	V	R	Y	R	S	(3aa)	T	G	T	W	S	E	W	S	-	P	S	I	(13aa)	(24aa)	
mIL7R	(39aa)	H	Y	E	T	K	V	R	S	I	P	(5aa)	K	G	F	W	S	E	W	S	-	P	S	S	(14aa)	(25aa)	
hIL2Rβ	(49aa)	Q	Y	E	F	Q	V	R	V	K	P	(4aa)	F	T	T	W	S	P	W	S	-	P	L	A	(12aa)	(25aa)	
hIL6R	(47aa)	R	H	V	V	Q	L	R	A	Q	E	(3aa)	Q	G	E	W	S	E	W	S	-	P	E	A	(48aa)	(28aa)	
mEPOR	(47aa)	R	Y	T	F	A	V	R	A	R	N	(5aa)	S	G	F	W	S	A	W	S	E	P	A	S	(9aa)	(22aa)	
hGMCsFR	(48aa)	E	P	R	A	K	H	S	V	K	I	(6aa)	I	L	N	W	S	S	W	S	E	A	I	E	(5aa)	(27aa)	
rPRLR	(48aa)	K	Y	L	V	Q	T	R	C	K	P	-	H	G	Y	N	S	R	W	S	Q	-	L	S	(12aa)	(24aa)	
hGHR	(48aa)	E	Y	E	V	R	V	R	S	K	Q	R	N	-	S	G	N	Y	G	E	F	S	V	L	Y	(16aa)	(24aa)
mGCSFR	(47aa)	V	Y	T	L	Q	R	R	C	I	R	(3aa)	P	G	F	W	S	P	W	S	P	G	L	Q	(299aa)	(24aa)	

FIG. 4. Comparison of amino acid sequence of the extracellular domain of MPL with that of the members of the hematopoietic growth factor receptor superfamily. Conserved amino acid residues are boxed. aa, Amino acids.

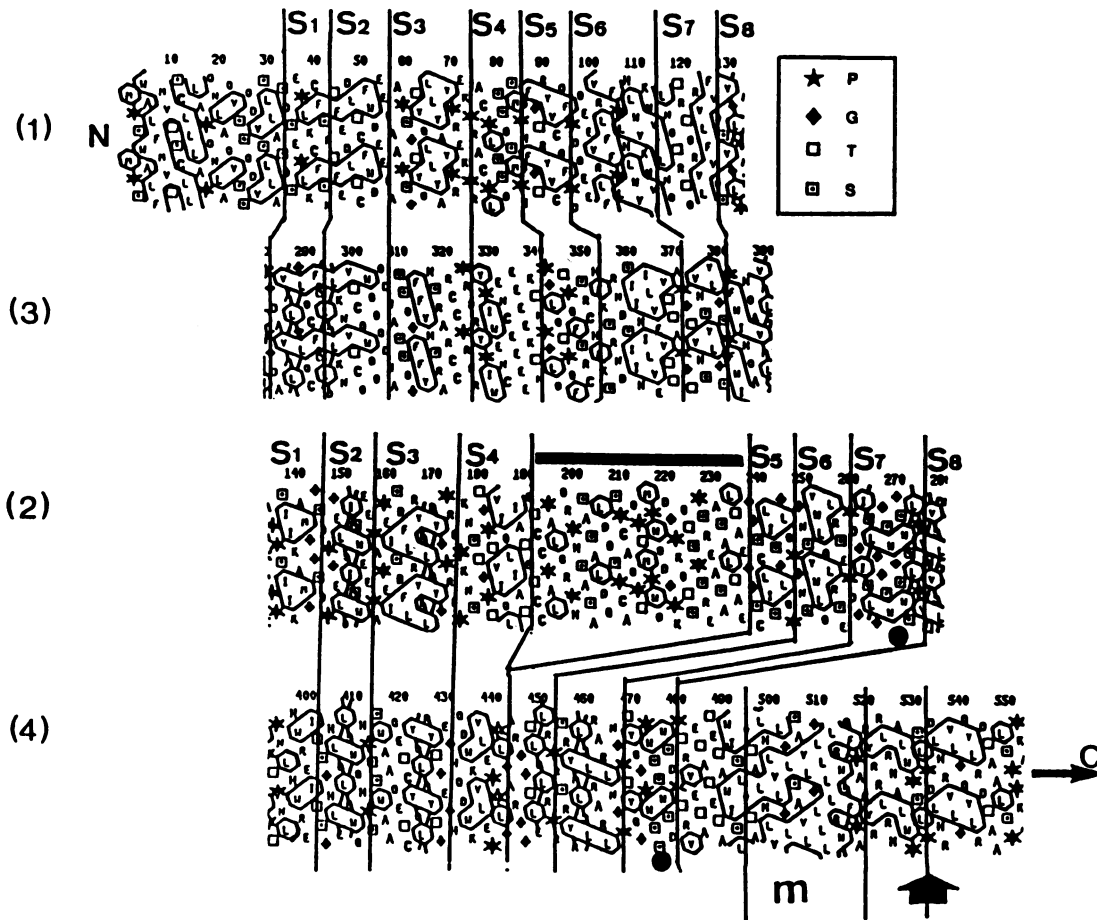


FIG. 5. HCA analysis of MPL. S1–S8, structural segments; N, N-terminal region. Conserved cysteines typical of the family are present in segments S1, S2, S4, and S5 of SD100(1) or -(3) subdomains. The long hydrophilic insertion of the MPL SD100(2) subdomain is indicated by a solid bar and the WSXWS box is noted with solid dots. MPL SD100(2) and -(4) subdomains share 26% sequence identity, while MPL SD100(1) and -(3) share only 14% identity. These values are currently observed among the family (10). After the transmembrane area (m), within the cytoplasmic domain, a limited conserved region between MPLP, murine Epo receptor, and IL-3 receptor, is noted by a solid arrow. Cytoplasmic domain has been truncated for clarity.

of the hematopoietin receptor family (3). Since *MPL* was expressed in hematopoietic tissues, human hematopoietic cell lines were tested with *MPL*-specific probes. A major *MPL* 3.7-kb transcript was detected in the HEL cell line.

In the present paper, we report the molecular cloning of the human homolog of the *v-mpl* from a HEL cDNA library. We obtained two types of clones, *MPLP* and *MPLK*. Sequence analysis indicated that clones P and K differed only at their 3' ends. The resulting deduced polypeptides are composed of a common extracellular domain of 484 amino acids with a putative signal sequence of 18 amino acids and a common transmembrane domain of 22 amino acids, but they differ in their cytoplasmic domain after a stretch of 9 common amino acids. The cytoplasmic domain of *MPLP* is composed of 122 amino acids, while that of *MPLK* contains 66 amino acids. The cytoplasmic region of *MPLP* presented 92% homology with *v-mpl*, while that of *MPLK* diverges completely.

The extracellular domain of *MPL* shares overall structural and amino acid homology with other members of the cytokine receptor superfamily, especially the two pairs of cysteines and the WSXWS box. Furthermore, as for the IL-3 receptor, the β chain of the human granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor (17), and a mouse IL-3-like receptor (18), this domain can be divided into two subunits, both containing the consensus sequences.

Bidimensional HCA analysis corroborated the relationship of *MPL* to the superfamily of receptors and the subdivision of the extracellular domain into two subdomains. In addition,

it displayed an extra domain from amino acids 191–241 in the first subdomain of *MPL* (Fig. 4). This extra domain is probably devoid of classical secondary structures (amphipathic α -helices and β -strands) present in globular domains. Such extra domains have been described for other cytokine receptors such as murine IL-5 receptor (19), human GM-CSF receptor (20), and human IL-6 receptor (21). Interestingly, in all these receptors these domains are located outside the cytokine receptor-specific region, while the *MPL* extra domain lies in the middle of the first subdomain.

Like other members of this family, no consensus sequence for kinase activity was found in the cytoplasmic domain of human *MPLP* or *MPLK*. The cytoplasmic domain of human *MPLP* is proline and serine rich, as it has been described for most members of the family (22–24), while that of human *MPLK* is only proline rich, like the cytoplasmic region of the murine IL-5 receptor (19).

Sequence comparison indicated that in *MPLV*, *MPL* was largely truncated since its extracellular domain only retained 43 amino acids. The cytoplasmic region of *v-mpl* has 92% homology with human *MPLP*. Because of this high degree of homology, it is likely that the cytoplasmic region of human *MPLP* contains the sequences required for signal transduction. Such functional domains have been defined for the IL-2 β chain (25) and the Epo receptors (26, 27) and have been shown to be very similar in sequence (26). However, when the cytoplasmic domain of *MPLP* was aligned with these sequences, no significant homology could be detected. The

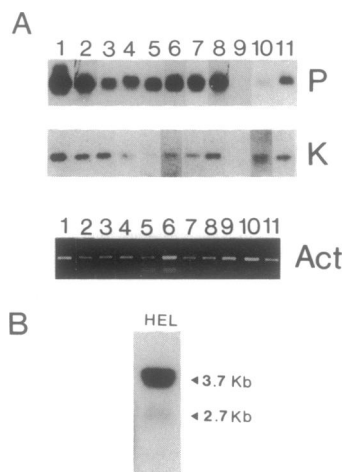


FIG. 6. Detection of *MPLP* and *-K* transcripts. (A) PCR analysis of total RNA from human cell lines and normal fresh tissues. One microgram of total RNA was reverse transcribed and subjected to PCR as described. Reverse transcription was tested by amplification of 1/5th of the reaction mixture with actin-specific oligonucleotides. In that case, products of amplification could be directly visualized after ethidium bromide staining. P, *MPLP* amplification; K, *MPLK* amplification; A, actin amplification. Lanes: 1, HEL; 2, KU812; 3, UT7; 4, KG1; 5, phytohemagglutinin-stimulated lymphocytes; 6, peripheral blood; 7, fetal blood; 8, fetal liver; 9, adult liver; 10, bone marrow; 11, placenta. (B) Northern blot analysis of HEL RNA. Ten micrograms of HEL total RNA was hybridized with a probe specific for 5' *MPL*. Autoradiogram corresponds to a 10-day exposure.

functional role of the *MPLK* cytoplasmic region could be explored by exchanging it with the *v-mpl* cytoplasmic domain in the viral genome. We have isolated two kinds of human *MPL* clones, *MPLP* and *MPLK*. In the same way, two prolactin receptors differing in their cytoplasmic domains have also been described in rats (28).

When HEL DNA digested with *EcoRI*, *HindIII*, *Sac I*, and *EcoRV* was subjected to Southern blot analysis, the same bands hybridized with a P-specific probe or with a K-specific probe (data not shown), which indicates that *MPLP* and *MPLK* probably derive from a unique *MPL* gene by alternative splicing. Alternative splicing has been described for other receptors (17, 29, 30). Often it gives rise to soluble forms, and it has been proposed that these forms could play a regulatory role in hematopoiesis and immune response by rapidly eliminating secreted cytokines (19, 22, 24). We did not isolate soluble forms of human *MPL*. Since the *v-mpl* probes we used to screen the HEL cDNA library mainly contain the transmembrane and cytoplasmic regions, we may not have used the best conditions for the detection of such clones. Nevertheless, human *MPLK* could play a regulatory role, as suggested for the soluble forms of receptors.

When RNAs from various hematopoietic cell lines were analyzed by Northern blot, *MPL* was found to be expressed only in the HEL cell line as a major 3.7-kb (*MPLP*) and a minor 2.8-kb (*MPLK*) mRNA. However, the study of *MPL* expression by PCR analysis indicated that *MPL* is probably expressed at a low level in a large number of cells of hematopoietic origin, since it was detected in various hematopoietic cell lines in which no signal was detected by Northern analysis, and that the two types of mRNAs P and K were always coexpressed.

At the moment, we do not know the nature of the *MPL* ligand, nor do we know whether *MPL* represents an α chain of the receptor or a β chain unable to bind the ligand. Like the β chain of the murine and human GM-CSF receptor, isolated by homology with the IL-3 receptor gene (17, 31), *MPL* could

be a component of one or several cytokine receptor complexes.

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