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STRUCTURE AND EXPRESSION OF NOVEL PROTEIN-TYROSINE KINASES, EMB AND EMT, IN HEMATOPOIETIC CELLS

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Two novel tyrosine kinase cDNAs were obtained from murine mast cells. These kinases, Emb and Emt, constitute a novel tyrosine kinase subfamily which may also include Tec, a kinase preferentially expressed in liver, and Dsrc28, a fruit fly kinase. Both lack hydrophobic stretches characteristic of the transmembrane domains found in growth factor receptor tyrosine kinases and carboxyl-terminal, negative regulatory tyrosine residue found in Src family kinases. In addition to the Src homology region 2 (SH2) and SH3 domains characteristic of the Src family kinases and other signaling molecules, Emb and Emt share a similar amino-terminal domain comprised mainly of two repeat segments. The *emb* 2.7-kb transcript was expressed in mast cells, myeloid cells and B lymphocytes while the *emt* 4.6-kb mRNA in mast cells, myeloid cells and T lymphocytes. The evidence for *in vitro* tyrosine kinase activity of Emb and Emt proteins is also provided.

The protein-tyrosine kinases (PTKs) constitute a large family of enzymes which play important roles in proliferation and differentiation of multicellular organisms. A major class of PTKs represented by the receptor for epidermal growth factor possess an extracellular, ligand-binding domain, a hydrophobic transmembrane domain, and an intracytoplasmic, ligand-stimulated kinase domain that initiates signal transduction events (1,2). Members of another major class of PTKs, the Src family, are intracellular proteins associated with the inner surface of the plasma membrane. Some members of this family are implicated in specialized hematopoietic cell functions. For example, p56^{lck}, the protein encoded by the *lck* gene, is specifically associated with two transmembrane proteins, CD4 and CD8, which are implicated in T cell activation (3,4). CD4

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Abbreviations:
BMMC, mouse bone marrow-derived mast cells; PCR, polymerase chain reaction; PTK, protein-tyrosine kinase; SH, Src homology region.

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cross-linking is accompanied by an increase in the kinase activity of p56^{lck} (5). This and other Src family kinases are associated with, and activated by cross-linking of, other cell surface glycoproteins (6-9).

There seems to be many more kinases to be found. Indeed, Partanen *et al.* (10) showed that a single cell expresses more than ten distinct PTK genes. Because of our interest in signal-transducing mechanisms initiated by the high-affinity IgE receptor complex, where PTK(s) plays an essential role in mast cell activation (11), we have searched for new PTK genes in mouse mast cells. In the present report, we show the identification and structural analyses of the two new PTK genes, *emb* and *emt*. The evidence for their limited expression in hematopoietic cells and tyrosine kinase activity is also presented.

MATERIALS AND METHODS

Isolation of cDNAs coding for protein kinases. Bone marrow-derived mouse mast cells (BMMC) were obtained as described (12). Poly(A)$^+$ RNA was purified from 10^8 cells using FastTrack RNA Isolation Kit (Invitrogen, San Diego, CA). The 3' RACE method (13) was applied to isolate the 3' portions of cDNAs encoding protein kinases. cDNAs were synthesized by Superscript RNase H$^-$ reverse transcriptase (GIBCO-BRL, Gaithersburg, MD) according to the manufacturer's protocol, except that the oligonucleotide, 5'-GACTCGAGTCGACATCGATTTTTTTTTTTTTTT, was used as a dT17-adaptor primer. The first round polymerase chain reaction (PCR) used a 5' sense-strand primer, 5'-T(A/T)C(A/G)TCCAC(A/C)GNGA(C/T)(C/T)T, corresponding to the conserved PTK catalytic domain sequence (Y/F)(I/V)HRDL, and the 3' adaptor sequence (5'-GACTCGAGTCGACATCG). 0.5 % of the first PCR products were used as templates for a second nested PCR, using a 5' sense-strand primer, 5'-GCGGATCCGA(C/T)GT(C/G/T)TGG(G/T)CNT(A/T)(C/T)GG, that corresponds to another conserved PTK sequence, DVW(S/A)(F/Y)G, and the 3' adaptor sequence. The second PCR products were size-selected by agarose gel electrophoresis and cloned into the *Bam*HI/*Sal*I-digested pBluescript II KS+ vector (Stratagene, La Jolla, CA). In order to isolate the cDNA portions corresponding to the regions upstream of the cDNAs cloned by the 3' RACE, the latter cDNA fragments were labeled with ^{32}P by nick-translation and used as probes for screening λ phage cDNA libraries derived from BMMC or the mouse mast cell line, PT-18, using Lambda ZAP II vector (Stratagene). Clone pM-1 was obtained by this approach using a PCR-derived clone (2C98) as a hybridization probe (Fig. 1). 5' RACE (13) was also employed for the same purpose. Briefly, a 20-mer oligonucleotide (oligo A), corresponding to the anti-sense strand of a sequence found ~200-300 nucleotides downstream of the 5' end of the previously isolated 3' cDNA clones, was used as a primer for cDNA synthesis with reverse transcriptase and PT-18-derived poly(A)$^+$ RNA. The cDNAs were poly(dA)-tailed at their 3' ends with terminal deoxynucleotidyl transferase (Stratagene), and were then used as templates for PCR between the 5' primer, dT17-adaptor, and the 3' primer, oligo A. PCR products ≥500 base pair (bp)-long were gel-purified and cloned into pCR1000 (Invitrogen). Clones with 500-1,500 bp inserts that hybridized with the 3' cDNA clones were sequenced.

Tyrosine kinase activity of Emb and Emt expressed in transfected COS-7 cells. In order to reconstitute the entire coding sequence of *emb*, cDNAs were sythesized from BMMC-derived poly(A)$^+$ RNA using a primer, 5'-TCTATGGAGTCCTCAGCTTC, that corresponds to the anti-sense strand of nucleotides 998-1,017 of the corresponding cDNA. The cDNAs were used as templates for a PCR between the 5' primer, 5'-TCGCTCAGACTGTCCTTCCT (nucleotides 16-35), and the 3' primer, 5'-TACTCCTCGCCCTTTCGCAA (nucleotides 890-909). The 894-bp PCR products were cloned into pCR1000, and the resulting *Eco*RI-*Hind*III fragment with the appropriate orientation was substituted for the shorter (350-bp) *Eco*RI-*Hind*III fragment of pM-1 to generate the entire *emb*-coding sequence (pM-emb). For *emt* reconstitution, PT-18-derived poly(A)$^+$ RNAs were reverse-transcribed using the primer, 5'-ATGGCTGTGTCTGGTCAATG, corresponding to nucleotides 1,967-1,986. PCR was performed on the resulting cDNAs using the

5' primer, 5'-TTGGTCTCCGCTGCTCCTCT (nucleotides 24-43), and the 3' primer, 5'-ATGGCTGTGTCTGGTCAATG. PCR products of the expected size (1,963 bp) were cloned into pCR1000 (pCR-emt). The reconstituted cDNAs were cloned into a eukaryotic expression vector, pME18S, harboring the promoter SRα (14), to obtain pME-emb and pME-emt, respectively. COS-7 cells were transfected by electroporation (15) with the *emb* or *emt* expression vectors. For immunoprecipitation experiments, affinity-purified anti-peptide antibodies raised in rabbits were used; anti-Emt is against the carboxyl-terminal twelve amino acids, LSQLAElAEAGL (residues 608-619), while anti-Emb is a mixture of two anti-peptide antibodies specific for the sequences IHQLKNVIRYNSDLVQKYHP (125-144) and KPGSSHRKTKKPLPPTPEED (176-195). Immunoprecipitation and immune complex kinase assays were done as described (11, 16).

Sequence analyses and other procedures. Nucleotide sequence was determined by a modified Sanger method (17) on both strands of plasmid DNAs. Analysis of nucleotide and amino acid sequences were performed using MacVector Sequence Analysis Software (International Biotechnologies, Inc., New Haven, CT). Unless otherwise specified, standard molecular biological techniques (18) were used. The *emb* and *emt* sequences have been deposited in the database Genbank (accession numbers are L10627 (*emb*) and L10628 (*emt*).

RESULTS AND DISCUSSION

Isolation of the emb and emt cDNAs. cDNAs synthesized from poly(A)$^+$ RNA obtained from BMMC were used as templates in anchored PCRs. The PCRs were designed to amplify the putative 3' fragments of PTK cDNAs between the 3' ends of mRNAs and two degenerate oligonucleotides, corresponding to conserved PTK catalytic domain sequences, used as 5' nested primers. The PCR products were cloned and sequenced. Among seventy clones sequenced, two novel sequences as well as six known kinase sequences, *lyn*, *hck*, *jak1*, *jak2*, IGF-I receptor and B-*raf*, were found.

One of the two new genes (*emb*) is represented by a clone, pM-1, which was obtained by screening a BMMC cDNA library with the PCR-derived cDNA clone, 2C98, as a hybridization probe (Fig. 1). The *emb* upstream sequence (m411) and the coding sequences of the other gene (*emt*) were determined by consecutive 5' RACE procedures using mRNAs derived from the PT-18 murine mast cell line. Sequence analysis of pM-1 and its 5' extended PCR clones revealed an open reading frame with an ATG initiation codon at nucleotides 182-184 (Fig. 2A), whose surrounding sequence (GCCATGG) matches Kozak's rule (19) well. This is most likely the initiation codon, since an in-frame stop codon (TAA) is found fifteen codons upstream (nucleotides 137-139). The putative Emb protein, defined by the above-mentioned start site and a TGA stop codon at nucleotides 2,162-2,164, has a calculated molecular weight of 76,572 daltons.

The open reading frame of the *emt* cDNA encompassed by three overlapping PCR clones (Fig. 1) starts with a putative initiation codon at nucleotides 99-101. Although the flanking sequence of this codon (ACCATGA) does not match Kozak's rule quite well, it most likely represents the translation initiation site for the putative protein product, Emt, having a calculated molecular weight of 71,448 (Fig. 2A), since an in-frame stop codon is found twelve codons upstream. Comparison with the homologous Emb sequence (see below) also supports this assignment of the initiation codon. The identity of these putative initiation codons in the reconstituted *emb* and *emt* cDNAs was confirmed by *in vitro* translation of the corresponding RNA transcripts in rabbit reticulocyte lysates (data not shown) and transfected COS-7 cells (see below).

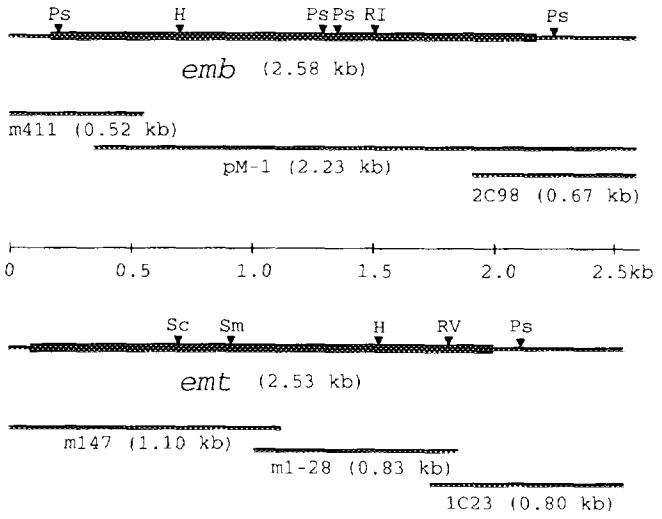

Fig. 1. Physical maps of *emb* and *emt*. Positions of the nested PCR 5' primers, targeted at conserved PTK sequences, are shown above the *emb* sequence with their corresponding amino acid sequences. 2C98 and 1C23 were derived by the initial PCR amplification of the 3' ends of PTK genes. pM-1 was obtained by screening a BMMC library with 2C98 as a probe. The other cDNA segments were cloned by the 5' RACE method based on downstream sequences. The coding regions are indicated by thick lines, and the 5' and 3' untranslated sequences by thin lines. Abbreviations of restriction enzymes used for mapping the clones are: H, *Hind* III; Ps, *Pst* I; RI, *Eco* RI; RV, *Eco* RV; Sc, *Sca* I; Sm, *Sma* I.

Structural features of Emb and Emt. The deduced amino acid sequences of Emb and Emt have salient features characteristic of PTKs. For example, the ATP-binding motif GXGXXG followed by a lysine residue found 16 residues downstream starts at residue 411 (Emb) or 369 (Emt), and the invariant DFG motif is found at residues 540-542 (Emb) or 499-501 (Emt). Two sequences, DLAARN (residues 522-527 and 481-486 in Emb and Emt, respectively) and PV(R/K)W(S/A)(P/S)PE (residues 561-568 and 520-527 in Emb and Emt, respectively), are conserved among tyrosine kinases, but not among serine/threonine kinases.

Fig. 2. A. Predicted amino acid sequences of Emb and Emt. The standard single-letter code for amino acids was employed. Insertions/deletions were introduced in order to optimize sequence homology. Dashes (-) denote the absence of an amino acid in a given position. Dots in the Emt sequence indicates identity with the Emb sequence. The putative autophosphorylation site is marked by an asterisk, and the proline-rich sequence motifs are underlined.
B. Alignment of the SH3 and SH2 domains of Emb and Emt in comparison with the corresponding domains of Dsrc28 (30) and chicken c-Src (40).
C. Homology of kinase domains among cytoplasmic PTKs. The delineation of kinase domains used by Hanks *et al.* (41) was followed. Percent identities at the amino acid level are shown. The Emb/Emt/Tec/Dsrc28 and Src subfamilies are highlighted by boxes.
D. Putative gene duplications in the upstream regions of the Emb, Emt, and Dsrc28 kinases. Identities and conservative changes of all four residues or of three out of four residues in a given position of the Emb and Emt sequences are boxed by heavy or light lines, respectively. Possible gene duplications in the Dsrc28 gene are boxed by light lines.
E. The CD22-related sequence motif in Emb. Identical or similar amino acids are boxed.
F. Schematic domain structure of Emb and Emt.

A

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Emb  MAAVI-LESIFLKRSQQKKKTSPLNFKKRLFLLTVHKLSYYEYDFERGRRGSKKGSIDVEKITCVETAIPEKNPPPERQI  79
Emt  •NNF•L••EQLI•K••••RR•••S•••V•F•V••KAS•A•F•••R-H•KKRTL••••ELSR•K•••IVKSD---------  69

Emb  PRRGEESSEMEQISIIERFPYPFQVVYDEGPLYVFSPTEELRKRWIHQLKNVIRY-NSDLVQKYHPCFWIDGQYLCCSQT 158
Emt  ------------•••PCHYK•••••H•NYL••••A•DC•S•Q••VLT••EET•NN•••S••••N••M••RWR••••L 136

Emb  AKNAMGCQILEN-RNGSLKPGSSHRKTKKPLPPTPEEDQIWILKKPLPPEPTAAPISTTELKKVVALYDYMPMNANDLQL 237
Emt  E•P•V••APYDPSK•A----------S•••••••••DNRRSFQE------•E•L----------I•••••QTNDPQE•A• 192

Emb  RKGEEYFILEESNLPWWRARDKNGQEGYIPSNYITEAE-DSIEMYEWYSKHMTRSQAEQLLKQEGKEGGFIVRDSSKAGK 316
Emt  •CD•••YL•DS•EIH•••VQ••••H•••A••S•LV•KSPNNL•T••••N•SIS•DK••K••LDT••••A•M••••RTP•T 272

Emb  YTVSVFAKSTGEPQGVIRHYVVCSTPQS--QYYLAEKHLFSTIPELINYHQHNSAGLISRLKYPVSKQNKNAPSTAGLGY 394
Emt  ••••••T•AIISENPC•K••HIKE•ND•PKR••V•••YV•DS••L••Q•••Y•GG••VT••R•••CSWRQK••V••••R• 352

Emb  GSWEIDPKDLTFLKELGTGQFGVVKYGKWRGQYDVAIKMIREGSMSEDEFIEEAKVMN-LSHEKLVQLYGVCTKQRPIFI 473
Emt  •K•V•Q•SE•••VQ•I•S••••L•HL•Y•LNKDK••••T•Q••A•••ED•••••E••MK•••P•••••••••LE•A••CL 432
                                                                                    *
Emb  ITEYMANGCLLNYLREMRHRFQTQQLLEMCKDVCEAMEYLESKQFLHRDLAARNCLVNDQGVVKVSDFGLSRYVLDDEYT 553
Emt  VF•F•EH•••SD•••SQ•GL•AAET••G••L••••G•A•••KACVI••••••••••GENQ•I••••••MT•F••••Q•• 512

Emb  SSVGSKFPVRWSPPEVLMYSKFSSKSDIWAFGVLMWEIYSLGKMPYERFTNSETAEHIAQGLRLYRPHLASERVYTIMYS 635
Emt  ••T•T••••K•AS•••SSF•RC•••••V•S•••••••VF•E••I•••NRS•••VV•D•ST•F•••K•R•••CH••Q••NH 592

Emb  CWHEKADERPSFKILLSNILDVMDEES                                                      660
Emt  ••K••PED••P•SQ•••QLAEIAEAGL                                                      619
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B SH3

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Emb    223/ALYDYMPMNANDLQLRKGE---EYFILEESNLPWWRARD-KNGQEGYIPSNYI
Emt    178/•••••QTNDPQE•A••CD------YL•DS•EIH•••VQ•-•••H•••A••S•L
Dsrc28 141/•••LGKAIEGG••SV---••KNA••EVIDD•QEH••KVK•-AL•NV•••••••V
Src     87/•••••ESRTET••SFK------RLQ•VNNTEGD••L•HSLTT••T•••••••V
Consensus  •••••       •• F •••       ••       ••        •  • •• ••V
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SH2

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Emb    283/WYSKHMTRSQAEQLLKQ-EGKEGGFIVRDSS-KAGKYTVSVFAKSTGEPQG
Emt    239/••N•SIS•DK••K••LD-T••••A•M••••R-TP•T••••••T•AIISENP
Dsrc28 211/••VGY•S•QR••S•••••GD•••C•V••K•••TK•L••L•LHT•---V••S
Src    147/••FGKI••RES•R••LNP•NPR•T•L••E•ETTK•A•CL••-SDFDNAKGL
Consensus  •• G  S•  D••R •L       •S•L••E••T   •  •SL••     D   G

Emb       VIRHYVVCSTPQS--QYYLAEKHLFSTIPELINYHQHNSAGLISRLKYPV
Emt       C•K••HIKE•ND•PKR••V•••YV•DS••L••Q•••Y•GG••VT••R•••
Dsrc28    HVK••HIKQ-N-ARCE•••S•••CCE•••D•••••R•••G••AC•••SSP
Src       NVK••KIRK-LD•GG-F•ITSRTQ••SLQQ•VA•YSKHAD••CH••T-N•
Consensus  VK••KI   LD  G  •       •  •L   •V HY      ••  K•
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C

	Emt	Tec	Dsrc28	Csk	Src	Fyn	Lyn	Lck	Abl	Fes
Emb	59.4	63.5	57.8	44.2	41.0	41.0	41.4	40.6	47.4	41.8
Emt	-	64.3	55.1	44.4	43.3	44.0	42.5	41.3	43.7	40.9
Tec		-	61.4	42.9	46.8	46.8	42.9	43.3	45.2	43.7
Dsrc28			-	44.5	48.4	48.8	45.7	45.7	45.7	40.6
Csk				-	46.4	43.5	46.4	44.5	44.8	42.3
Src					-	83.3	67.1	64.7	48.8	42.9
Fyn						-	69.4	66.7	46.8	41.7
Lyn							-	72.3	47.4	38.7
Lck								-	46.4	40.1
Abl									-	42.9

D

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Emb  10 FLKRSQQKKKTSPLNFKKRLFL-LTVHKLSYYEYDFERGRRGSKKGS-IDVEKITCVETAIP  69
Emt  11 LIKKSQQKRRTSPSNFKVRFFV-LTKASLAYFE-DR-HGKKRTLKGS-IELSRIKCVEIVKS  68
Emb 113 VFSPTEELRKRWIHQLKNVIRY-NSDLVQKYHP-CF-WIDGQYLCCSQTAKNAMGCQILEN- 170
Emt  91 VFAPDCESRQRWVLTLKEETRNNNS-LVSKYHP-NF-WMDGRWRCCSQLEKPAVGCAPYDPS 149

Dsrc28  21 IGHEPDQF-QNQRRQRRVLQPRIQRAAVSPNS-STTNSQF  58
Dsrc28 108 FKQSPTLLNGNGNLLDANM-PGGIPTPGTPNSKAKDNSHF 146
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E

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Emb              73 PPPERQIPRRGE-ESSEME  90
CD22            589 SFPEMNIPRTGDAESSEMQ 607
Slime mold PTK1  23 PPPPPQLPVRSE-YEIDFN  40
Frog FGFR       417 IPVRRQVTVSGD-SSSSMN 434
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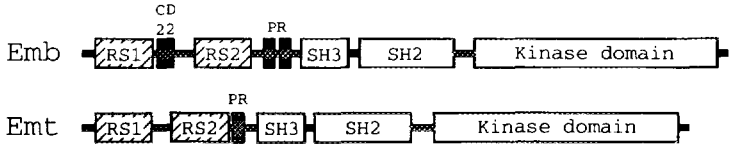
F



Inspection of the overall primary structures of the putative Emb and Emt kinases reveals several notable features. First, they lack hydrophobic amino acid stretches characteristic of the transmembrane domains found in growth factor receptor PTKs. Second, they are similar to the Src family PTKs in displaying SH3 (20), SH2 (21) and a kinase domain that has a consensus autophosphorylation site corresponding to Tyr-416 in $p60^{c\text{-}src}$ (Fig. 2B). Nevertheless, Emb and Emt differ from Src family PTKs in that they lack, first, a glycine residue at the second position that serves as a myristylation and membrane anchoring site (reviewed in ref.22) and, second, a negative regulatory tyrosine residue (corresponding to Tyr-527 in $p60^{c\text{-}src}$) whose phosphorylation suppresses the kinase activity of Src family PTKs (23-26). Although this latter property is shared by the Csk kinase which can phosphorylate the regulatory tyrosine residue of $p60^{c\text{-}src}$ and its homologues (27,28), Csk lacks the autophosphorylation site found in Emb, Emt and Src family kinases. These differences suggest a different mode of regulating the kinase activity of Emb and Emt relative to members of the Src family. Third, the kinase domains (Fig. 2C) reveal relatively high homologies (55-64%) among Emb, Emt, Tec, a putative PTK expressed preferentially in liver (29), and Dsrc28, a Src-related PTK isolated from *Drosophila melanogaster* (30). The homology level between any of these four kinases and other PTKs is significantly lower (<49%).

Fourth, and more interestingly, Emb and Emt share a unique, extensively homologous amino-terminal region (Fig. 2A). Eighty eight of the 222 amino-terminal residues of Emb are also found within the first 177 residues of Emt. Basic amino acids are abundant in this region, *i.e.*, 41/222 residues in Emb and 33/177 residues in Emt. Within this region, a 21-amino acid sequence (residues 71-91) found in Emb is missing in Emt. Comparison of the amino acid sequences separated by this sequence revealed a significant homology over a stretch of ~54 residues (Fig. 2D), suggesting duplication of a gene segment of about 160 nucleotides in both *emb* and *emt*. A similar duplication event is found in the closely related kinase gene, Dsrc28, albeit at a different segment within its amino-terminal region (Fig. 2D). These duplicated sequences, termed repeat segments 1 and 2 (RS1 and RS2), may be important for the biological function of Emb and Emt (see below), assuming that they form similar secondary structures.

Fifth, the Emb-specific sequence, PERQIPRRGEESSEME, which resides between RS1 and RS2, has a high degree of homology (11 identities and 3 conserved substitutions over the 16-residue stretch) with a portion of the cytoplasmic domain of CD22 (31,32), also known as B lymphocyte cell adhesion molecule (Fig. 2E). Similar sequences with lower homologies were also found in slime mold PTK1 (33) and frog fibroblast growth factor receptor (34). This suggests that the same or similar protein(s) might interact with CD22 and Emb through this hydrophilic sequence motif.

Finally, Emb and Emt share a unique proline-rich motif [(T/S)KKPLPPTPE(E/D)], found between the RS2 and SH3 domains, that has some homology with sequences in microtubule-associated proteins 2 and 4. Emb has another proline-rich motif [KKPLPPEP] seven residues downstream. Based on secondary structure analysis, this motif is predicted to be exposed on the surface of the proteins. Thus, the first threonine (or serine) or second threonine residues of the motif could be subject to phosphorylation by protein kinase C or Erk (MAP II) kinases,

respectively (35). Based on the above considerations, Emb, Emt, Tec, and probably Dsrc28, appear to constitute a novel subfamily of PTKs that may perform similar functions, and share unique modes of regulation.

mRNA expression of emb and emt genes. As a first step towards understanding the functions of these new PTKs, expression of the corresponding genes was investigated in various tissues and cell lines. Northern blot analysis indicated that 2.7-kb *emb* mRNA transcripts were abundant in spleen but very low, if any, in other tissues. Low-level expression of the 4.6-kb *emt* mRNA was also observed in spleen, but only trace amounts were found in heart and lung after a longer exposure (data not shown). Among the cell lines tested (Fig. 3), *emb* was expressed in mast cells, myeloid cells, and B lineage cells, while *emt* was expressed in mast cells, myeloid cells, and T lymphocytes. The nomenclature of these genes is based accordingly on their expression pattern in various hematopoietic cell lines. Neither *emb* nor *emt* transcripts were detected in fibroblasts. Although we cannot exclude the possible expression of these genes in other cell types, it is conceivable that the low hybridization signals obtained with RNAs from non-hematopietic tissues were due to contaminating hematopoietic cells.

Emb and Emt proteins phosphorylate tyrosine residues in vitro. We next expressed Emb and Emt proteins transiently in COS-7 cells. Immune complexes precipitated from detergent lysates of

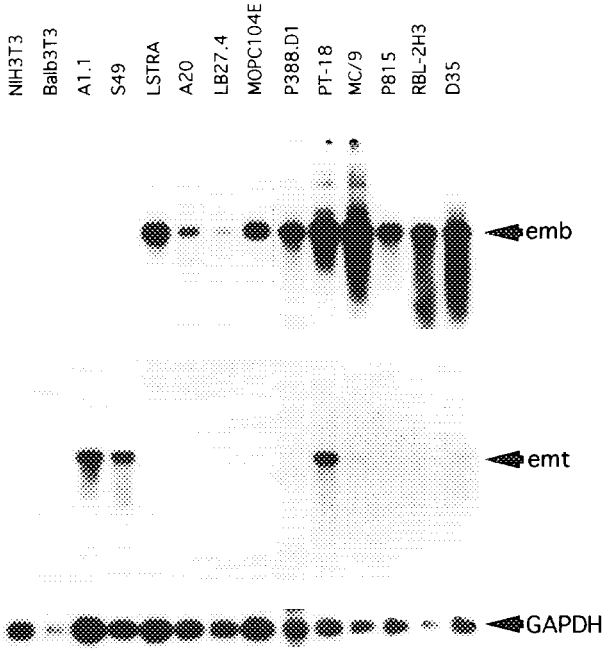


Fig. 3. Northern blot analysis of *emb* and *emt* sequences in various cell lines. Poly(A)$^+$ RNAs were purified from cultured cells and used to prepare two identical blots which were hybridized with *emb* or *emt* cDNAs separately. The *emb* blots were re-hybridized with a glutaraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA probe as a control.

the transfected cells with the respective affinity-purified anti-peptide antibodies were incubated with [γ-^{32}P]ATP. As shown in Fig. 4A, a phosphorylated protein of 77 kDa was specifically observed in the immune complex from the *emb*-transfected COS-7 cell lysates while a 72-kDa protein was phosphorylated in the immune complex from the *emt*-transfected cells. These phosphorylated proteins contained phosphotyrosine as revealed by phosphoamino acid analysis (Fig. 4B). We did not try to identify other phosphorylated proteins detected in the immune complex precipitated from the mock- and *emt*-transfected cells with anti-Emt antibody (*i.e.*, proteins of 70 kDa, 62 kDa, 56 kDa and 53 kDa). Since all of these proteins detected in the mock transfetants contained only phosphoserine and phosphothreonine (data not shown), tyrosine phosphorylation of the 72-kDa protein in the *emt*-transfected cells seems to be due to tyrosine kinase activity specifically expressed in the *emt* transfectants. Therefore, we conclude that these 77-kDa and 72-kDa phosphoproteins are autophosphorylated Emb and Emt proteins, respectively.

Several structural features and expression of Emb and Emt in limited hematopoietic cell types suggest that these kinases play important and novel roles in hematopoietic cell signal transduction pathways. After completion of this study, genetic defects of the *emb* gene was shown in X-linked agammaglobulinemia patients (36,37). We have learned that *itk* (38) and *tsk* (39) described very recently as a T cell specific kinase gene are identical to *emt*.

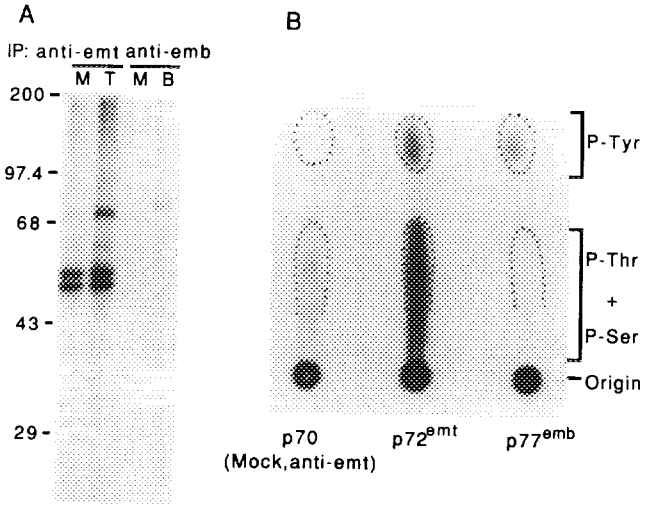


Fig. 4. *In vitro* tyrosine kinase activity of Emb and Emt proteins expressed in COS-7 transfectants.
A. Immune complexes were precipitated from 1% NP-40 lysates of mock (M)-, *emb* (B)-, or *emt* (T)-transfected cells with anti-Emb or anti-Emt antibodies, and incubated with [γ-^{32}P]ATP. Phosphorylated materials were resolved on SDS-PAGE and detected by autoradiography. Positions of molecular weight standards (GIBO BRL) are indicated in kilodaltons.
B. Phosphorylated bands blotted onto Immobilon-P membranes were excised, acid-hydrolyzed, and subjected to ascending chromatography (42). Positions of origin and phosphoamino acids are indicated.

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REFERENCES

1. Aaronson, S.A. (1991) Science 254, 1146-1153.
2. Ullrich, A. and Schlessinger, J. (1990) Cell 61, 203-212.
3. Rudd, C.E., Trevillyan, J.M. Dasgupta, J.D., Wong, L.L. and Schlossman, S.F. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 5190-5194.
4. Veillette, A., Bookman, M.A., Horak, E.M. and Bolen, J.B. (1988) Cell 55, 301-308.
5. Veillette, A., Bookman, M.A., Horak, E.M., Samelson, L.E. and Bolen, J.B. (1989) Nature 338, 257-259.
6. Burkhardt, A.L., Brunswick, M., Bolen, J.B. and Mond, J.J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 7410-7414.
7. Eiseman, E. and Bolen, J.B. (1992) Nature 355, 78-80.
8. Samelson, L.E., Phillips, A.F., Luong, E.T. and Klausner, R.D. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4358-4362.
9. Yamanashi, Y., Kakiuchi, T., Mizuguchi, T., Yamamoto, T. and Toyoshima, K. (1991) Science 251, 192-194.
10. Partanen, J., Makela, T.P., Alitalo, R., Lehvaslaiho, H. and Alitalo, K. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 8913-8917.
11. Kawakami, T., Inagaki, N., Takei, M., Fukamachi, H., Coggeshall, K.M., Ishizaka, K., and Ishizaka, T. (1992) J. Immunol. 148, 3513-3519.
12. Saito, H., Okajima, F., Molski, T.F.P., Sha'afi, R.I., Ui, M. and Ishizaka, T. (1987) J. Immunol. 138, 3927-3934.
13. Frohman, M.A., Dush, M.K. and Martin, G.R. (1988) Proc. Natl. Acad. Sci. USA 85, 8998-9002.
14. Takebe, Y., Seiki, M., Fujisawa, J., Hoy, P., Yokota, K., Arai, K., Yoshida, M. and Arai, N. (1988) Mol. Cell. Biol. 8, 466-472.
15. Yao, L., Nakauchi, H., Honjo, T. and Kawakami, T. (1990) Immunol. Lett. 24, 267-272.
16. Fukamachi, H., Kawakami, Y., Takei, M., Ishizaka, T., Ishizaka, K. and Kawakami, T. (1992) Proc. Natl. Acad. Sci. USA 89, 9524-9528.
17. Schuurman R. and Keulen, W. (1991) BioTechniques 10, 185.
18. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular cloning. A labaratory manual. 2nd ed., Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY.
19. Kozak, M. (1991) J. Biol. Chem. 266, 19867-19870.
20. Rodaway, A.R.F., Sternberg, M.J.E. and Bentley, D.L. (1989) Nature 342, 624.
21. Sadowski, I., Stone, J.C. and Pawson, T. (1986) Mol. Cell. Biol. 6, 4396-4408.
22. Jove, R. and Hanafusa, H. (1987) Annu. Rev. Cell Biol. 3, 31-56.
23. Cooper, J.A., Gould, K.L., Cartwright, C.A. and Hunter, T. (1986) Science 231,1431-1434.
24. Kmiecik, T.E. and Shalloway, D. (1987) Cell 49, 65-73.
25. Piwnica-Worms, H., Saunders, K.B., Roberts, T.M., Smith, A.E. and Cheng, S.H. (1987) Cell 49, 75-82.
26. Cartwright, C.A., Eckhart, W., Simon, S. and Kaplan, P.L. (1987) Cell 49, 83-91.
27. Nada, S., Okada, M., MacAuley, A., Cooper, J.A. and Nakagawa, H. (1991) Nature 351, 69-72.
28. Bergman, M., Mustelin, T., Oetken, C., Flint, N.A., Amrein, K.E., Autero, M., Burn, P. and Alitalo, K. (1992) EMBO J. 11, 2919-2924.
29. Mano, H., Ishikawa, F., Nishida, J., Hirai, H. and Takaku, F. (1990) Oncogene 5, 1781-1786.
30. Gregory, R.J., Kammermeyer, K.L., Vincent III, W.S. and Wadsworth, S.G. (1987) Mol. Cell. Biol. 7, 2119-2127.
31. Stamenkovic, I. and Seed, B. (1990) Nature 345, 74-77.

32. Wilson, G.L., Fox, C.H., Fauci, A.S. and Kehrl, J.H. (1991) J. Exp. Med. 173, 137-146.
33. Tan, J.L. and Spudich, J.A. (1990) Mol. Cell. Biol. 10, 3578-3583.
34. Musci, T.J., Amaya, E. and Kirschner, M.W. (1990) Proc. Natl. Acad. Sci. USA 87, 8365-8369.
35. Pearson, R.B. and Kemp, B.E. (1991) Methods Enzymol. 200, 62-81.
36. Vetrie, D., Vorechovsky, I., Sideras, P., Holland, J., Davies, A., Flintre, F., Hammarstrom, L., Kinnon, C., Levinsky, R., Bobrow, M., Smith, C.I.E. and Bentley, D.R. (1993) Nature 361, 226-233.
37. Tsukada, S., Saffran, D.C., Rawlings, D.J., Parolini, O., Allen, R.C., Klisak, I., Sparkes, R.S., Kubagawa, H., Mohandas, T., Quan, S., Belmont, J.W., Cooper, M.D., Conley, M.E. and Witte, O.N. (1993) Cell 72, 279-290.
38. Siliciano, J.D., Morrow, T.A. and Desiderio, S.V. (1992) Proc. Natl. Acad. Sci. USA 89, 11194-11198.
39. Heyeck, S.D. and Berg, L.J. (1993) Proc. Natl. Acad. Sci. USA 90, 669-673.
40. Takeya, T. and Hanafusa, H. (1983) Cell 32, 881-890.
41. Hanks, S.K., Quinn, A.M. and Hunter, T. (1988) Science 241, 42-52.
42. Munoz, G. and Marshall, S.H. (1990) Anal. Biochem. 190, 233-237.