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Short sequence-paper

Isolation, molecular characterization, and tissue-specific expression of a novel putative G protein-coupled receptor

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Abstract

We isolated a 40 kDa integral membrane protein (p40) from human erythrocyte ghosts by affinity chromatography, using a C-terminal peptide of stomatin, and obtained partial sequences which enabled us to isolate two full-length cDNAs from human bone marrow and fetal brain cDNA libraries. The cDNA sequences were identical and encoded a novel putative G protein-coupled receptor (399 amino acids). Northern and RNA dot blot analyses demonstrated that the major 4.8 kb-transcript is predominantly expressed in brain. In situ hybridization studies of tissue sections revealed high expression in neurons of the brain and spinal cord, in thymocytes, megakaryocytes, and macrophages. © 1998 Elsevier Science B.V.

Keywords: G protein-coupled receptor; Membrane protein; Erythrocyte; Neuron; Stomatin; In situ hybridization

The human erythrocyte membrane was frequently used for the primary isolation and characterization of widely distributed membrane proteins like the glucose transporter [1], anion transporter [2], water channel [3], complement receptor [4], and Duffy chemokine receptor [5], apart from the major cytoskeletal components. We have been studying the erythrocyte membrane protein 7.2b [6–8], also termed stomatin [9], which is expressed in various tissues [9,10]. Its function is not known, however, because of

its absence in the high Na⁺, low K⁺ red cells of patients with overhydrated hereditary stomatocytosis [9,11,12]. It has been hypothesized that it may act as a regulator of an ion channel [13] in association with the cytoskeleton [14]. Such an association has partially been proven for the *Caenorhabditis elegans* stomatin-like protein MEC-2 [15], which is a necessary component of the mechanosensory apparatus. There is indirect evidence that human stomatin is associated with the cytoskeleton [14,16], but so far an associated ion channel or receptor has not been identified. In the present study we describe the isolation of the membrane protein p40 by stomatin peptide-affinity chromatography, the cloning and characterization of the corresponding cDNA, and the tissue-specific expression.

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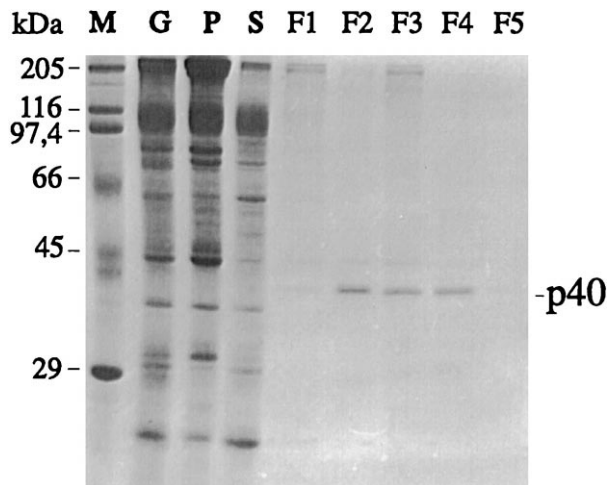


Fig. 1. Isolation of p40 from human erythrocyte membranes. Erythrocyte membrane proteins were solubilized with 0.5% Triton X-100 and subjected to affinity chromatography using a C-terminal peptide of stomatin. Samples from various stages of the isolation were electrophoresed into a 10% SDS-PAGE slab and stained with Coomassie Blue. Marker proteins (M), erythrocyte ghosts (G), Triton X-100 insoluble material (P), Triton X-100 solubilized proteins (S), and affinity chromatography elution fractions (F1–F5) are shown. The p40-containing fractions F2–F4 were pooled for sequence analysis.

In an effort to identify a stomatin-associated membrane protein by various biochemical and molecular biological methods, we also used 13 affinity chromatography columns, containing different peptides (15–24-mers) of putatively surface-exposed regions [17] of stomatin, for the adsorption of solubilized (0.5% Triton X-100) erythrocyte membrane proteins. Only the C-terminal peptide IDMLQGIIGAKHSHL, corresponding to amino acids 272–286 of stomatin [7], yielded a protein after elution at pH 2.5 (Fig. 1), about 20 μ g/100 ml packed red cells. This 40 kDa-membrane protein (p40) is one of the minor erythrocyte membrane proteins with an estimated copy number of 20 000/red cell, as judged by SDS-PAGE [18] and Coomassie Blue-staining. N-terminal amino acid sequence analysis showed that the N-terminus of p40

is blocked, however, internal sequences were obtained by tryptic digestion [19] and analysis of the peptides by automated Edman degradation (ABI model 476A sequencer). Oligonucleotide probes were designed on the basis of the amino acid sequence data and corresponding nucleotide sequence information from the dbEST databank, obtained by a search with the TBLASTN program of Altschul et al. [20]. 5'- and 3'-RACE by nested PCR was performed with the primers and Marathon-Ready cDNA libraries from human bone marrow and human fetal brain (Clontech), using the Advantage cDNA PCR Kit (Clontech). Nine partially overlapping clones were isolated and sequenced [21]. Nucleotide sequence compilation, amino acid sequence analysis, and database searches were accomplished by the use of the GCG (Genetics Computer Group, Madison) software package available on EMBnet. Analysis of the combined sequences revealed an open reading frame of 1304 bp, including 104 bp 5'-UTR (Fig. 2). The putative start codon is located within an appropriate consensus sequence for the initiation of translation [22]. The 3'-UTR contains a polyadenylation signal (GATAAAGATAAA) at position 1561 (relative to the start ATG), which is similar to a double-repeat of the consensus AATAAA. This signal is followed by a poly(A) tail 13 bp downstream. Further 3'-RACE experiments yielded three additional fragments revealing a second polyadenylation signal (AATAAA) in position 4412, followed by a poly(A) tail 16 bp downstream. The composite sequences derived from the clones of the two libraries were identical, except for one exchange at position 2667 (G in fetal brain, A in bone marrow). Searching the dbEST databank with the complete cDNA sequence revealed homologous ESTs from mouse (gb/W54092) and rat (gb/H33811), with about 90% and 85% identity, respectively.

The deduced amino acid sequence of human p40 was confirmed by sequence identity with six peptides

Fig. 2. Nucleotide and deduced amino acid sequence of human p40 cDNA. Nucleotides and amino acid residues are numbered starting from the first ATG in the open reading frame. The seven putative membrane-spanning domains are boxed and labeled TM I–VII. The translation termination codon is indicated by an asterisk. An imperfect and a perfect polyadenylation signal is double-underlined. Amino acid sequences of erythroid peptides are single-underlined. Putative glycosylation sites are marked by diamonds, putative phosphorylation sites by (P). The nucleotide sequence accession number is Y11395 (EMBL).

-104		CCGAGAAGGGCTTC	-91
-90	AGGACGCGGGAGGCGCACTTGCCTCAAGTCGCGGGCGTGGGAACGGGGTTGCAAAAACGGGGCCTTTTATCCGGGCTTGCCTCCGGCGTC		-1
1	ATGCGCTCAAAGGGCCCTCCCGAATCCTTATGCTGATTATAACAAATCCCTGGCCGAAGGCTACTTTGATGCTGCCGGGAGGCTGACTCCT		90
1	M A Q R A F P N P Y A D Y N K S L A E G Y F D A A G R L T P		30
91	GAGTTCTCAACAACGCTTGACCAATAAGATTGCGGAGCTTCTTCAGCAAATGGAGAGAGGCGCTGAAATCAGCAGACCCCTCGGGATGGCACC		180
31	E F S Q R L T N K I R E L L Q Q M E R G L K S A D P R D G T		60
181	GGTTACACTGGCTGGGACAGTATGCTGTGCTTTACTTACATCTTTATGATGATTTGGGGACCCCTGCCCTACCTACAGTTAGCACATGGC		270
61	G Y T G W A G I A V L Y L H L Y D V F G D P A Y L Q L A H G		90
	TM I		
271	TATGTAAAGCAAAGTCTGCAACTGCTTAAACCAAGCGCTCCATCACCTTCTTTGTGGGGATGCAGGCCCTTGGCAGTGGCCGCTGTGCTA		360
91	Y V K Q S L N C L T K R S I T F L C G D A G P L A V A A V L		120
	(P)	TM II	
361	TATCACAAGATGAACAATGAGAAGCAGGCAAGATTGCATCACACGGCTAATTCACCTAAATAAGATTGATCCTCATGCTCCAAATGAA		450
121	Y H K M N N E K Q A E D C I T R L I H L N K I D P H A P N E		150
451	ATGCTCTATGGGCGAATAGGCTACATCTATGCTCTTCTTTTGTCAATAAGAACTTTGGAGTGGAAAAGATTCTCAAAGCCATATTTCAG		540
151	M L Y G R I G Y I Y A L L F V N K N F G V E K I P Q S H I Q		180
	TM III		
541	CAGATTTGTAAACAATTTTAACTCTGGAGAAAACCTAGCTAGGAAGAGAAACTTACGGCAAAGTCTCCACTGATGATGAATGGTAC		630
181	Q I C E T I L T S G E N L A R K R N F T A K S P L M Y E W Y		210
	(P)	(P)	
631	CAGGAAATTTATGATAGGGGCTGCTCATGGCCTGGCTGGAATTTATTACTACCTGATGCAGCCAGCCTTCAAGTGAGCCAAGGGAAGTTA		720
211	Q E Y Y V G A A H G L A G I Y Y Y L M O P S L Q V S Q G K L		240
	TM IV		
721	CATAGTTTGGTCAAGCCAGTGTAGACTACGTCTGCCAGCTGAAATTCCTTCTGGCAATTACCCTCCATGTATAGGTGATAATCGAGAT		810
241	H S L V K P S V D Y V C Q L K F P S G N Y P P C I G D N R D		270
811	CTGCTTGTCCATTTGGTCCATGGCGCCCTGGGGTAATCTACATGCTCATCCAGGCCATAAAGGTATTGAGAGGAAAAGTATCTCTGT		900
271	L L V H W C H G A P G V I Y M L I O A Y K V F R E E K Y L C		300
	TM V	(P)	
901	GATGCCATCAGTGTCTGATGTGATCTGGCAATATGGGTTGCTGAAGAAGGGATATGGGCTGTGCCACGGTCTGCAGGGAATGCCTAT		990
301	D A C A D V I W Q Y G L L K A G S Y G L C H G S A G N A Y		330
	TM VI		
991	GCCTTCTGACACTCTACAACTCACACAGGACATGAAGTACCTGTATAGGGCCCTGTAAGTTTCTGTAATGGTGTCTTAGAGTGAAGAA		1080
331	A F L T L Y N L T Q D M K Y L Y R A C K F A E W C L E Y G E		360
1081	CATGGATGCAGAACCCAGACACCCTTCTCTCTCTTTGAAGGAATGGCTGGAAACAATATATTTCTGGCTGACCTGCTAGTCCCCACA		1170
361	H G C R T P D T E F S L F E G M A G G T I Y F L A D L L V P T		390
	TM VII		
1171	AAAGCCAGGTTCCCTGCATTTGAACTCTGAAAGGATAGCATGCCACCTGCAACTCATGCGATGACCCCTTCTGTATATTCAAACCCAAGC		1260
391	K A R F P A F E L *		399
1261	TAAGTGCTTCCGTTGCTTTCCAAGGAAACAAAGAGTCAAACCTGTGGACTTGATTTTGTAGCTTTTTTTCAGAAATTTATCTTTCATTGAGT		1350
1351	TCCTTCCATTATCAITTTACTTTTACTTAGAAGTATCCAAAGGAGTCTTTTAACTTTAATTTCCATTTCTTCCFAAAGGGAGAGTGAGTG		1440
1441	ATATGTACAGTGTTTGAGATTGTATACATATATCCAGAATCTGGAGGAAATCTTATTTAAGTTTATGAATATAACCATCTGTTTACTGT		1530
1531	TCTAAAAATGTTTAAAAGAACTCAATACAGATAAAGATAAATATGTGACTATTATGGGTATTACACTTCACITCTCTTTTAAATATTTT		1620
1621	CCTCCAATCGGAGGGCAGACAAATTTCTGACTGCTTTCTCTAGGTGGTTCATTTTGAAGGGGACAGAAAATAACTAAATGCTTCCA		1710
1711	GGAGAAAATTTCCAAGATTCAACTCGGACTTCAATCTGAACTTAAATCTATTTTAAATCTTGTATGCCTATTTGGACTAGAGGTAACAT		1800
1801	ACTTTCAGATTGGCCTGTTTTTGTGCGTAAGGCATACAGCCTTCAGAAGCCAACTTTTAAATCAAAAACCTATAAAAACATGATGATCAT		1890
1891	TGTGAAAATTTCTGAGTTGAAGGTTAGTTTAAAGATAAGACTAAACAATGAGTCTGTGTTTTCTTAAAATAATCTGAGTTTTTGGAACTC		1980
1981	TTTTTAAATATGTTGTTTTTTCAGTATTTCAAATAAGATCAGGAAGCCAAITTTCTATGTAATGAAATATGCTTTTACCTAGGATTTTCA		2070
2071	CCACTCTGACTGACTTTCTAACTTTAACTTGGGTTTTTACAGTACTATGCAATGATGCTGACTCTTTGGTATAAGCCATAAAAATATTT		2160
2161	TCCTTCCATTAATTTATCTGAACTTTGGTCTTTTCACTAAATTTGTAAGTATTTCTACTTCTGTTTTAAAAGGGGAGATGAGAAAAGGAA		2250
2251	TACTATCTAAACCAATCTGAACAAAACAATAAGCAATTAAGCAATTAATAGAAAATGCTTTTTATTGAGGAGGTATTATCCAGATTTCTG		2340
2341	CTTAGAACAAATGCATCTTTGCGTATCTAGACTTAAACAATTCATCAGTTTTCTGAGACCACAGAATCAGGTTTTCCGTAGTAGATAAGA		2430
2431	CTCTCTGGTCTTCAAATCTGTTCAAGTGTTTTGACTCATCAGCTTCTACTCTTTCTATTACTGCCTTTGGCCTGGCTGTTTTGTCTCT		2520
2521	TTGCAACTGATTTTGCAAAAAAAATTTGATGCTTTAAAATAACAGGGCTAAAGTATTTAAAATGTGCTTATTTCACAGCTCTCTGGTCA		2610
2611	CAAAAACCTGCTATTTTATTTGGAACCTTCAAACCAATCCCACTGAGTGTGACTGTTTCTGACAGGTAGCAGTCTCCTATTATCTCCT		2700
2701	GTTTAGCACAAAAGAGCTAATATTATTTGAAACTGACCTTTTAAAGGCCACTGGCAGTAGGATTTAAAAGCAGCCCACTGCTCAGTTT		2790
2791	CCAGGATCAGCTTCCCTCTGTGCACTTGTGTAAGTTGGCACTACTTTGTGCTCTCAGATTGCTGAAGTGTCTGCTGGTAAGCATGTGC		2880
2881	ATGCTCTGCTTCTTGTGAAAATTTTCAATCAGCGATATCAGCACTTACAGTAAGAAGTAAAAGTAGTGCAAGCAAGCAATTTGCT		2970
2971	TTTGGCTGGGGTGTTCAGCTTGAAGAATAAAGCTCAITTTGGTTTAGTTAAATGTCTTACTCTACTGTGCTATGCTTTTACCTGCTG		3060
3061	CTAAGCAAGGAAAAATAACAGTTTTCTCTGAGCCAGAGAAGACTTGTACAGTTCTCCAAGCATCGTATAGCAATGCTTAAACCCAGG		3150
3151	AAGATTTCAAGGCAGGGAGAAGAACTTTCAAATAAGATTTCTGTAAACCCATTTATGCTAGTGTCCATTATGGAATGCTAAGCTGT		3240
3241	TGGAGTCAATTTACCTCTACTGCTCAAAGTCAATGGCAAGGCTGATTTTTTCAACAAAATAATGCAACCCCAAGCAATAAGGGTTAG		3330
3331	CTACTGTCATCAGTTAGCAAATTCATCCACAAAACAATTTAGAGTTTGGTTTTTTTTTAAAGCTTTTCAAACCTTACTAACTGGCACA		3420
3421	ATTTTATATGATGCTATTTTGTGATTTTATGCTTAAAGAGCAAAAAGTTTTGATGGGATTTTAAATTCAGCAAAAGCCTACAACGCTGAG		3510
3511	ACAATCCCTAACCAATGTAAGTAACTAAAGAACTTTTACTAGGCTCTTAGTTTAAAAGGAAGTGGCATCAATGTTTCAAGTTCT		3600
3601	AGTTTGTATTTTCTCTCAGATAATTTCTTCTTAAAATCTTTCCAGAAAGTGGTTCCTAGAAAACCTCAATACCATCATCTCTTATC		3690
3691	TCTATACAGGACTAGGTAATAAACCCTCAAAGTTGTCAAAGGTCATCAAGCAGTGTTCATTTATCCGTGCACATGTTTCTGTTTCTA		3780
3781	TAGTAATTTAGAAAATGCAAAATAGTTAACTTTTCACTAGTAAAGAAAGTAAACATTTCCATTTCCATAGATACTTGAAGCGGGTGTG		3870
3871	GCCTGAGTTGTGCTTTTAAATCCTGAGTCAATGGCTCTCTTTTCACTTTGATGTCAGTTCCAATTTTGGCATCAAAAACCTTCA		3960
3961	GGTAGTCAATTTAGGTAAAAGTGGATCTAGGCTTACTTTCTTAAATCAATTTTCTTAAATAACTGAAATGAGACATACTCTGCTA		4050
4051	CTATGCTCAGGTTAAATTTTGTCTGATCTTACGATGCCCTGCCCTTTTACTAGTACTTTAGAAAATAGAAAATGTGAAGAGTACTATT		4140
4141	TACATGTATACTCTTTGGCTGCTAGAACTCATCTGTAGTCTTTATTTTAACTGAAATTTCACTGAAATTTCAAATTTCTCTCCGCTAAGTAA		4230
4231	GAGCACTACTTCCCTGTGTTTTCTCTACTATTGAGCTGTAGACCAACTGTTTTCTCTAAATATAAAGCAAACTTTTGGGATTTACAGGA		4320
4321	AACTACCCCAATGTTATGTTGTCATTTAATGGGAAAGGCTGGGATCATATGATTTTCTATGTTCTGTAAGTATTTGACTTACTAGTTCT		4410
4411	CAATAAAAATTTTATTAGACTATAAAAAA		4440

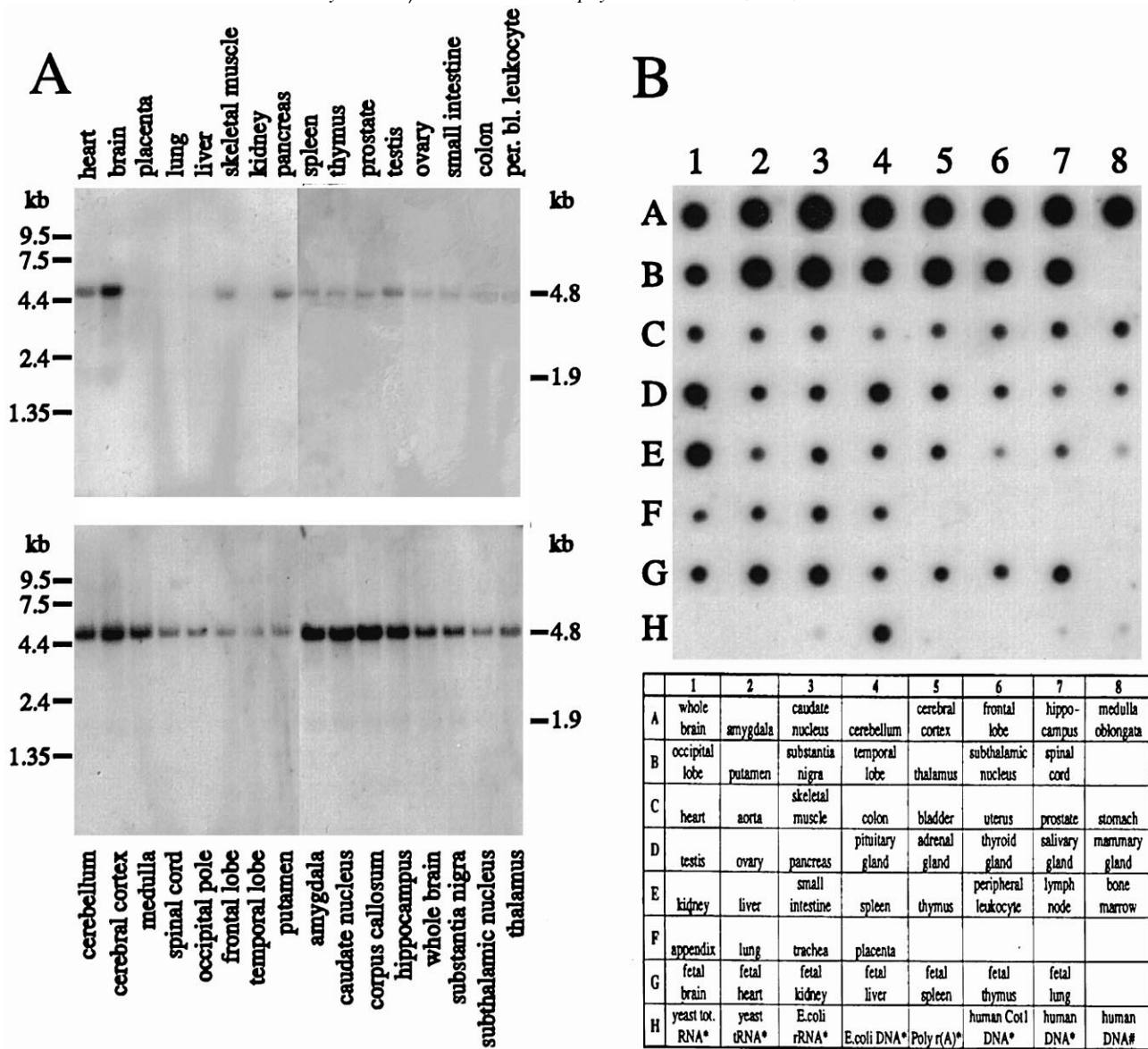
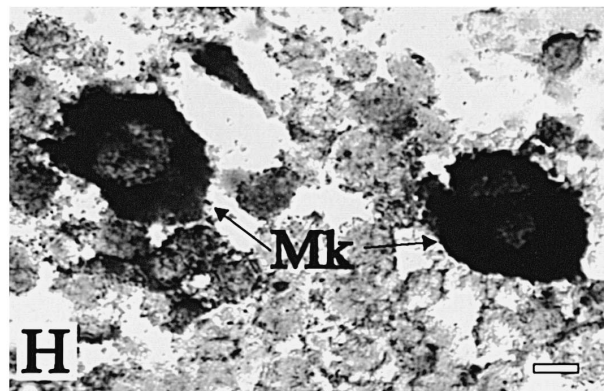
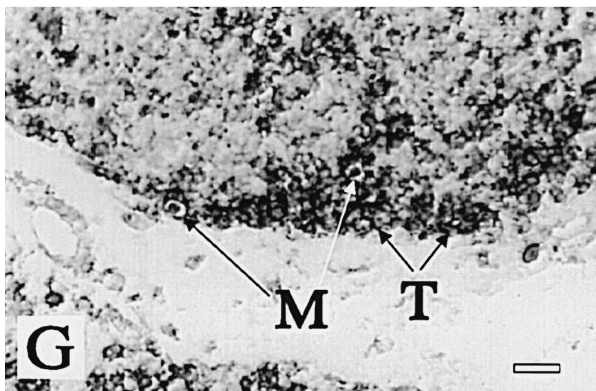
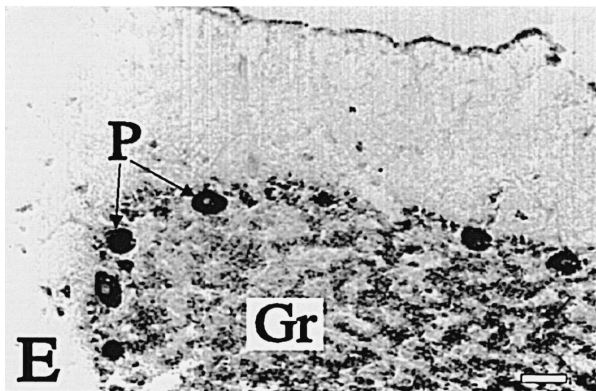
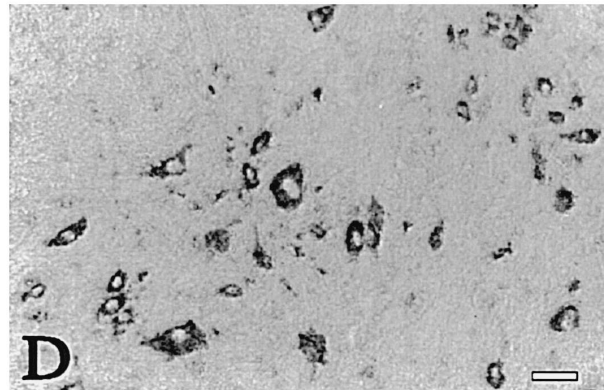
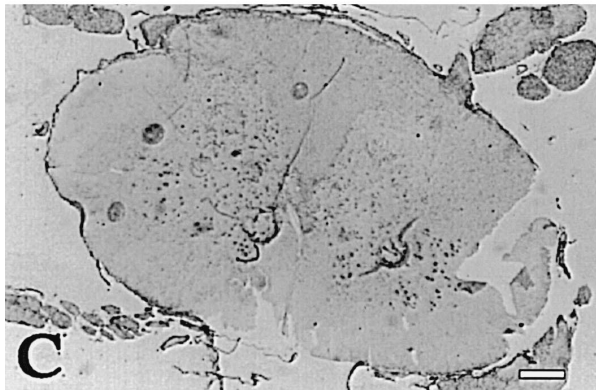
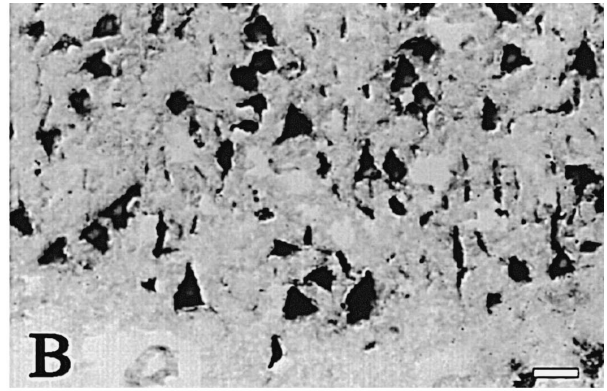
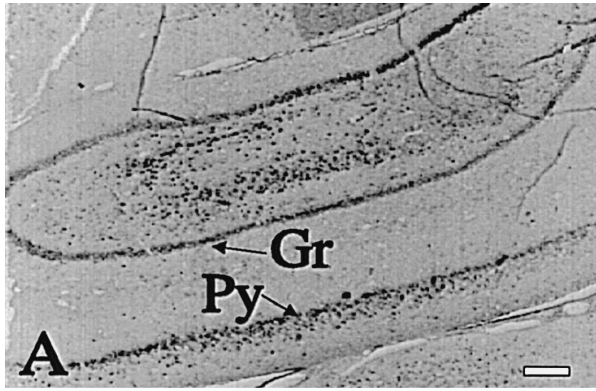


Fig. 3. (A) Northern blot analysis. Blots containing 2 μ g poly(A)⁺ RNA per lane (Clontech) from 16 human tissues (top), and 16 regions from brain and spinal cord (bottom) were hybridized with a ³²P-labeled p40 cDNA probe. A major transcript of 4.8 kb is seen in brain, various brain regions, heart, skeletal muscle, pancreas and testis. Additionally, a minor 1.9 kb transcript can be seen, especially after long exposure (three days). RNA size markers are shown on the left side. (B) RNA dot blot analysis. A Human RNA Master Blot (Clontech) containing normalized loading of 100–500 ng of each poly(A)⁺ mRNA per dot from 50 different human tissues and six different control RNAs and DNAs was hybridized with the p40 cDNA probe. Predominant expression is seen in various parts of the brain and in the spinal cord. Strong signals are also observed in testis, pituitary gland, and kidney. In fetal tissues, expression of p40 is lower in the brain than in the heart and kidney. * – 100 ng (H1–H7); # – 500 ng(H8).

Fig. 4. In situ hybridization analysis. Fixed cryosections of tissues from four days old rhesus monkeys were hybridized with the digoxigenin-labeled p40-antisense RNA probe (A–E, G, H) or control sense RNA probe (F). Strong signals were observed in the pyramidal cells of the hippocampus (A, B), Purkinje cells of the cerebellum (E), the granular layer of the hippocampus (A) and cerebellum (E), and in motor neurons of the spinal cord (C, D). Intensive staining was also seen in cortical T-cells and macrophages of the thymus (G), and megakaryocytes of the bone marrow (H). The negative control (F) corresponds to (B). Gr, granular layer; Py, pyramidal cell layer; P, Purkinje cells; M, macrophages; T, cortical T-cells; Mk, megakaryocytes. Scale bar, 1 mm (A, C), 100 μ m (D, E, G), 50 μ m (B, F), and 20 μ m (H).



of the isolated erythrocyte membrane protein (Fig. 2). It comprises 399 residues, including the start methionine, and corresponds to 43 kDa. This is somewhat larger than estimated by SDS-PAGE of the isolated protein, however, aberrant electrophoretic mobility is often observed with membrane proteins, due to their hydrophobicity and glycosylation [23]. Hydrophobic regions were identified by the use of the “TMpred”-server [24] and the “predict-protein”-server (PHD) [25] at EMBL Heidelberg, both predicting seven transmembrane domains. This structure suggests that p40 is a member of the large superfamily of seven-transmembrane-domain proteins, which are usually G protein-coupled receptors. There is no significant sequence homology between p40 and the other members of this superfamily, and therefore, p40 may be regarded as an orphan receptor [26]. However, there are several structural features in common: the N-terminal domain, which comprises 58 amino acids contains one putative N-glycosylation site (N-14); a second site (N-337) is located at the beginning of the fourth extracellular region. There are cysteine residues in the extracellular loops, one in the first (C-133), two in the second (C-252 and C-264) and three in the third (C-349, C-355, C-363), which presumably form disulfide bridges [26]. Four additional cysteines are present within the cytoplasmic loops (C-98, C-183, C-300, C-305) and may be palmitoylated, and three cysteines within transmembrane regions (C-108 in TM II, C-276 in TM V, and C-322 in TM VI) may also form thioesters. Furthermore, there are four potential phosphorylation sites [27] within the cytoplasmic loops: two of them are specific for protein kinase C (T-100 / C1-loop and T-200 / C2-loop), one is located in a consensus for casein kinase II (T-188 / C2-loop), and one for a tyrosine kinase (Y-298 / C3-loop), which may be important for desensitization of the receptor [28]. A site at the C-terminal end of TM III with a negative and positive charge (E-172, K-173) is similar to a conserved site (ERY or DRY) that is involved in G protein binding [29]. Analysis of the seven transmembrane domains by the helical wheel projection showed that the helices are typically amphiphilic; especially the charged residues D-110 in TM II, R-155 and K-167 in TM III, E-374 and D-385 in TM VII are located at hydrophilic faces of the helices, probably lining the ligand binding site (data not shown). Inter-

nal sequence comparison revealed a repeat-structure within the seven helices, with the consensus GX-AGhhY (h being a hydrophobic residue). The relevance of this repeat is not clear. Glycine residues are sometimes found conserved at the interface of densely packed helices, therefore this motif may have a function in the interaction of adjacent helices or possibly with an associated membrane protein. The C-terminal end of p40 is rather short and does not contain cysteines.

One putative G protein-coupled receptor has already been isolated from human erythrocytes, i.e. the Duffy blood group antigen, which shows sequence homology to interleukin-8 receptors and is thought to act as a scavenger for chemokines [5]. The erythroid p40, regardless of its ligand, may play a similar scavenging role in blood circulation. On the other hand, the Duffy blood group/chemokine receptor is also expressed in brain, like several other erythrocyte membrane proteins.

Northern blot analysis of multiple tissues revealed the expression of a major p40-transcript of 4.8 kb (Fig. 3(A)), which is in accordance with the long p40-transcript (Fig. 2). This transcript can be found in all tissues, especially after long exposure of the blots. A second, very faint band of 1.9 kb can also be detected. This size corresponds to the short transcript terminated at the “imperfect” polyadenylation-signal (Fig. 2). Northern blots containing RNA from different brain regions are shown in the lower part of Fig. 3(A). p40-expression was detected throughout the whole brain.

Analysis of an RNA dot blot (Human RNA Master Blot, Clontech) containing mRNA from 50 different human tissues, normalized to the expression levels of eight different “housekeeping” genes (ribosomal protein S9, ubiquitin, 23 kDa highly basic protein, hypoxanthine guanine phosphoribosyl transferase, tubulin, β -actin, phospholipase, and glyceraldehyde-3-phosphate dehydrogenase), revealed insights into the relative expression levels of p40-transcripts (Fig. 3(B)): predominant expression of p40 is seen in various parts of the brain and in the spinal cord, and there are strong signals in testis, pituitary gland, and kidney. Interestingly, the expression level in fetal brain is significantly lower than in adult brain, suggesting a developmental regulation of p40 gene expression. Normalization of the mRNA loading on the

dot blot was verified by hybridization with an ubiquitin probe (data not shown).

In order to detect tissue-specific and cell type-specific p40 expression we hybridized cryosections (6 μ m thick) of rhesus monkey tissues with anti-sense-RNA transcribed from a 712 bp-fragment of the p40 coding region (position 121–832 relative to the start ATG), and with sense-RNA as control. The probes were labeled with digoxigenin and detected by the specific antibody (Boehringer Mannheim) conjugated with alkaline phosphatase (NBT/BCIP-staining). A prominent signal was identified in neurons of the brain and spinal cord. In the tissue sections of hippocampus, the layers of the neural cell bodies gave an intensive staining; observation at higher magnification revealed the presence of p40-RNA in the pyramidal cells (Fig. 4(A) and (B)). There was no signal detectable in the corresponding sense control (Fig. 4(F)). In spinal cord, the motor neurons were intensely stained (Fig. 4(C) and (D)), and in cerebellum, a strong signal in the Purkinje cell layer as well as in the granular layer was observed (Fig. 4(E)). Various other regions of the brain have been investigated, all showing clear expression of p40 in the neurons. High expression of p40 was also observed in haematopoietic cells, like the T-cells and macrophages of the thymic cortex (Fig. 4(G)). The macrophages can be easily recognized by their appearance in the middle of a “hole” in the tissue sections. Alveolar macrophages of the lung (not shown) and megakaryocytes of the bone marrow (Fig. 4(H)) also highly express p40.

In conclusion, we have isolated a novel membrane protein (p40) from human erythrocyte ghosts in a single chromatographic step, based on the specific interaction of this protein with a C-terminal peptide of stomatin. Regardless of the functional significance of this interaction, the described method can be used for the isolation and analysis of p40. On the basis of the structure and specific expression pattern, we postulate that p40 may function as a G protein-coupled receptor for a peptide or neurotransmitter. This receptor is likely to be important for signal transduction in neurons and haematopoietic cells. Further biochemical and functional studies may provide new insight into the nature of the ligand, the associated G protein(s), and will elucidate the possible interaction of p40 with native stomatin.

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