View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

µ1B, a novel adaptor medium chain expressed in polarized epithelial cells[1]

Hiroshi Ohno[a,*], Takuya Tomemori[c], Fubito Nakatsu[a], Yasushi Okazaki[a], Ruben C. Aguilar[b], Heike Foelsch[d], Ira Mellman[d], Takashi Saito[a], Takuji Shirasawa[c], Juan S. Bonifacino[b,†]

[a] *Department of Molecular Genetics, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan*
[b] *Cell Biology and Metabolism Branch, National Institute of ChildHealth and Human Development, National Institutes of Health, Building 18T, Room 101, 18 Library Dr. MSC 5430, Bethesda, MD 20892-5430, USA*
[c] *Department of Molecular Genetics, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakae-cho, Itabashi-ku, Tokyo 173-0015, Japan*
[d] *Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06520-8002, USA*

Received 16 February 1999; received in revised form 15 March 1999

Abstract The apical and basolateral plasma membrane domains of polarized epithelial cells contain distinct sets of integral membrane proteins. Biosynthetic targeting of proteins to the basolateral plasma membrane is mediated by cytosolic tail determinants, many of which resemble signals involved in the rapid endocytosis or lysosomal targeting. Since these signals are recognized by adaptor proteins, we hypothesized that there could be epithelial-specific adaptors involved in polarized sorting. Here, we report the identification of a novel member of the adaptor medium chain family, named µ1B, which is closely related to the previously described µ1A (79% amino acid sequence identity). Northern blotting and in situ hybridization analyses reveal the specific expression of µ1B mRNA in a subset of polarized epithelial and exocrine cells. Yeast two-hybrid analyses show that µ1B is capable of interacting with generic tyrosine-based sorting signals. These observations suggest that µ1B may be involved in protein sorting events specific to polarized cells.

© 1999 Federation of European Biochemical Societies.

1. Introduction

The plasma membrane of polarized epithelial cells is differentiated into apical and basolateral domains, each of which contains a distinct complement of membrane proteins (reviewed in [1,2,3]). The segregation of membrane proteins into these domains occurs by sorting events that take place at the *trans*-Golgi network (TGN) and/or endosomes [1,2,3]. Sorting to the apical domain is thought to involve incorporation of proteins into sphingolipid- and cholesterol-rich membrane 'rafts' [4] or interaction with lectin-like sorting receptors [5,6]. In contrast, sorting to the basolateral domain is largely determined by specific sorting signals contained within the cytosolic domains of the proteins [1,2]. Basolateral sorting signals are highly degenerate, although many of them resemble tyrosine-based or di-leucine-based motifs involved in rapid endocytosis and lysosomal targeting [1,2]. Other basolateral sorting signals are co-linear with but distinct from these motifs [7,8] and some do not conform to any recognizable canonical sequence [9–12].

The resemblance of many basolateral sorting signals to endocytic and lysosomal targeting signals suggests that they may be recognized by similar components of the sorting machinery. It is now well-established that both tyrosine-based and di-leucine-based signals involved in endocytosis and lysosomal targeting are recognized by the heterotetrameric adaptor complexes AP-1 (γ-β1-µ1-σ1), AP-2 (α-β2-µ2-σ2) and AP-3 (δ-β3-µ3-σ3) (reviewed in [13–17]). The medium subunits µ1, µ2 and µ3 of the respective adaptor complexes are directly responsible for the recognition of tyrosine-based sorting signals [18–22]. Di-leucine-based signals have been shown to interact with µ1 and µ2 [23,24] as well as with β1 [25]. These observations led us to hypothesize that sorting signals that function specifically in polarized epithelial cells might likewise be recognized by adaptor proteins.

Here, we report the identification of a novel member of the adaptor medium chain family, termed µ1B, which is specifically expressed in polarized epithelial cells and some exocrine cells. µ1B is most closely related to the ubiquitously-expressed µ1A subunit of AP-1 (79% identity at the amino acid level). Two-hybrid assays demonstrate that µ1B is capable of interacting with generic tyrosine-based sorting signals, consistent with the possibility that µ1B may be an adaptor protein involved in protein sorting in polarized cells.

2. Materials and methods

2.1. Cloning of human and mouse µ1B cDNAs

A human EST clone from the I.M.A.G.E. Consortium (LLNL, ID 123283, [26]) encoding a portion of a protein closely related to the known mouse µ1 (GenBank #M62419, [27]) was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). A human placenta cDNA library (Clontech Laboratories, Palo Alto, CA, USA) was screened using the insert from the EST clone as a probe in order to obtain a full length cDNA. A mouse clone (ID 372139) highly homologous to human µ1B was also obtained from the ATCC. The missing 5′-part of the encoding region was obtained from mouse kidney RNA by a 5′-RACE PCR, using the SMART PCR cDNA Synthesis kit (Clontech Laboratories).

2.2. In vitro transcription/translation

^{35}S-labelled mouse µ1A, human µ1B and mouse µ2 were prepared

*Corresponding author. Fax: (81) (43) 222 1791.
E-mail: ohno@gene.m.chiba-u.ac.jp
†Second corresponding author. Juan S. Bonifacino,
Fax: (1) (301) 402 0078. E-mail: juan@helix.nih.gov

[1] The nucleotide sequences reported in this paper have been submitted to GenBank with accession numbers AF020797 (human µ1B) and AF067146 (mouse µ1B).

Abbreviations: EST, expressed sequence tag; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; TGN, *trans*-Golgi network

0014-5793/99/$20.00 © 1999 Federation of European Biochemical Societies. All rights reserved.
PII: S0014-5793(99)00432-9

A

```
Hmu1B   1  MSASAVFILDVKGKPLISRNYKGDVAMSKIEHFMPLLVQREEEGALAPLLSHGQVHFLWI
Mmu1B   1  MSASAVFILDVKGKPLISRNYKGDVPMTEIDHFMPLLMQREEEGVLAPLLSHGRVHFLWI
Mmu1A   1  MSASAVYVLDLKGKVLICRNYRGDVDMSEVEHFMPILMEKEEEGMLSPILAHGGVRFMWI

Hmu1B  61  KHSNLYLVATTSKNANASLVYSFLYKTIEVFCEYFKELEEESIRDNVVIVYELLDELMDF
Mmu1B  61  KHSNLYLVATTLKNANASLVYSFLYKTVEVFCEYFKELEEESIRDNFVIVYDLLDELMDF
Mmu1A  61  KHNNLYLVATSKKNACVSLVFSFLYKVVQVFSEYFKELEEESIRDNFVIIYELLDELMDF

Hmu1B 121  GFPQTTDSKILQEYITQQSNKLETGKSRVPPTVTNAVSWRSEGIKYKKNEVFIDVIESVN
Mmu1B 121  GFPQTTDSKILQEYITQQGNKLETGKSRVPPTVTNAVSWRSEGIKYKKNEVFIDVIESVN
Mmu1A 121  GYPQTTDSKILQEYITQEGHKLETGAPRPPATVTNAVSWRSEGIKYRKNEVFLDVIEAVN

Hmu1B 181  LLVNANGSVLLSEIVGTIKLKVFLSGMPELRLGLNDRVLFELTGRSKNKSVELEDVKFHQ
Mmu1B 181  LLVNANGSVLLSEIVGTIKLKVFLSGMPELRLGLNDRVLFELTGRSKNKSVELEDVKFHQ
Mmu1A 181  LLVSANGNVLRSEIVGSIKMRVFLSGMPELRLGLNDKVLFDNTGRGKSKSVELEDVKFHQ

Hmu1B 241  CVRLSRFDNDRTISFIPPDGDFELMSYRLSTQVKPLIWIESVIEKFSHSRVEIMVKAKGQ
Mmu1B 241  CVRLSRFDNDRTISFIPPDGDFELMSYRLSTQVKPLIWIESVIEKFSHSRVEIMVKAKGQ
Mmu1A 241  CVRLSRFENDRTISFIPPDGEFELMSYRLNTHVKPLIWIESVIEKHSHSRIEYMVKAKSQ

Hmu1B 301  FKKQSVANGVEISVPVPSDADSPRFKTSVGSAKYVPERNVVIWSIKSFPGGKEYLMRAHF
Mmu1B 301  FKKQSVANGVEISVPVPSDADSPRFKTSVGSAKYVPEKNVVIWSIKSFPGGKEYLMRAHF
Mmu1A 301  FKRRSTANNVEIHIPVPNDADSPKFKTTVGSVKWVPENSEIVWSVKSFPGGKEYLMRAHF

Hmu1B 361  GLPSVEKEEVEGRPPIGVKFEIPYFTVSGIQVRYMKIIEKSGYQALPWVRYITQSGDYQL
Mmu1B 361  GLPSVETEEVEGRPPIGVKFEIPYFTVSGIQVRYMKIIEKSGYQALPWVRYITQSGDYQL
Mmu1A 361  GLPSVEAEDKEGKPPISVKFEIPYFTTSGIQVRYLKIIEKSGYQALPWVRYITQNGDYQL

Hmu1B 421  RTS
Mmu1B 421  RTS
Mmu1A 421  RTQ
```

B

	μ1B (human)		μ1B (mouse)	
	% I	% S	% I	% S
μ1B (human)	100	100	97	98
μ1B (mouse)	97	98	100	100
μ1A (mouse)	79	90	80	91
μ2 (mouse)	38	65	38	65
μ3A (rat)	30	57	29	57
μ3B (rat)	28	55	28	55
μ4 (human)	29	53	28	53

C

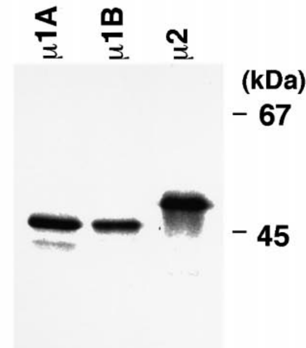


Fig. 1. Sequence analysis and characterization of μ1B. (A) Alignment of human μ1B (AF020797), mouse μ1B (AF067146) and mouse μ1A (W08014) amino acid sequences. Residues that are identical in at least two sequences are boxed. (B) Sequence identity (I) and similarity (S) of different members of the adaptor μ chain family. (C) SDS-PAGE analysis of in vitro transcribed/translated, [^{35}S]methionine-labelled mouse μ1A, human μ1B and mouse μ2. The positions of molecular size markers are indicated on the right.

using the TNT T7 Quick Coupled Transcription/Translation System (Promega, Madison, WI, USA) in the presence of [^{35}S]methionine (Tran ^{35}S-Label, ICN, Costa Mesa, CA, USA). An aliquot of each product was directly mixed with Laemmli sample buffer, separated by SDS-PAGE and analyzed with a Fujix Bio-imaging Analysis System.

2.3. Cell lines

Rat basophilic leukemia cells (clone 2H3) were kindly provided by Dr Henry Metzger (National Institutes of Health, Bethesda, MD, USA). MDCK II (canine kidney epithelial) and Caco-2 (human colon adenocarcinoma) cells were kindly provided by Drs Sachiko and Shoichiro Tsukita (Kyoto University, Kyoto, Japan). These cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin (Sigma Chemical, St. Louis, MO, USA). The following cells were obtained from the ATCC and cultured as recommended by the supplier: HT-29 (human colon adenocarcinoma), HEC-1-A (human endometrial adenocarcinoma), RL95-2 (human endometrial adenosquamous carcinoma), AV-3 (human amnion epithelial), VERO C1008 (African green monkey kidney), LLC-PK1 (porcine kidney epithelial), HeLa (human cervical carcinoma), Jurkat (human acute T-cell leukemia), JY (human B lymphoblastoma), A-549 (human lung carcinoma) and HuTu 80 (human duodenum adenocarcinoma).

2.4. Northern analyses

Total RNA was extracted from cultured cells and various mouse tissues according to Chomczynski and Sacchi [28]. 10 μg of RNA was electrophoresed on 1% agarose gels containing 0.22 M formaldehyde and transferred to Nylon membranes (Hybond N^{+}, Amersham International, Buckinghamshire, UK). Human Multiple Tissue Northern

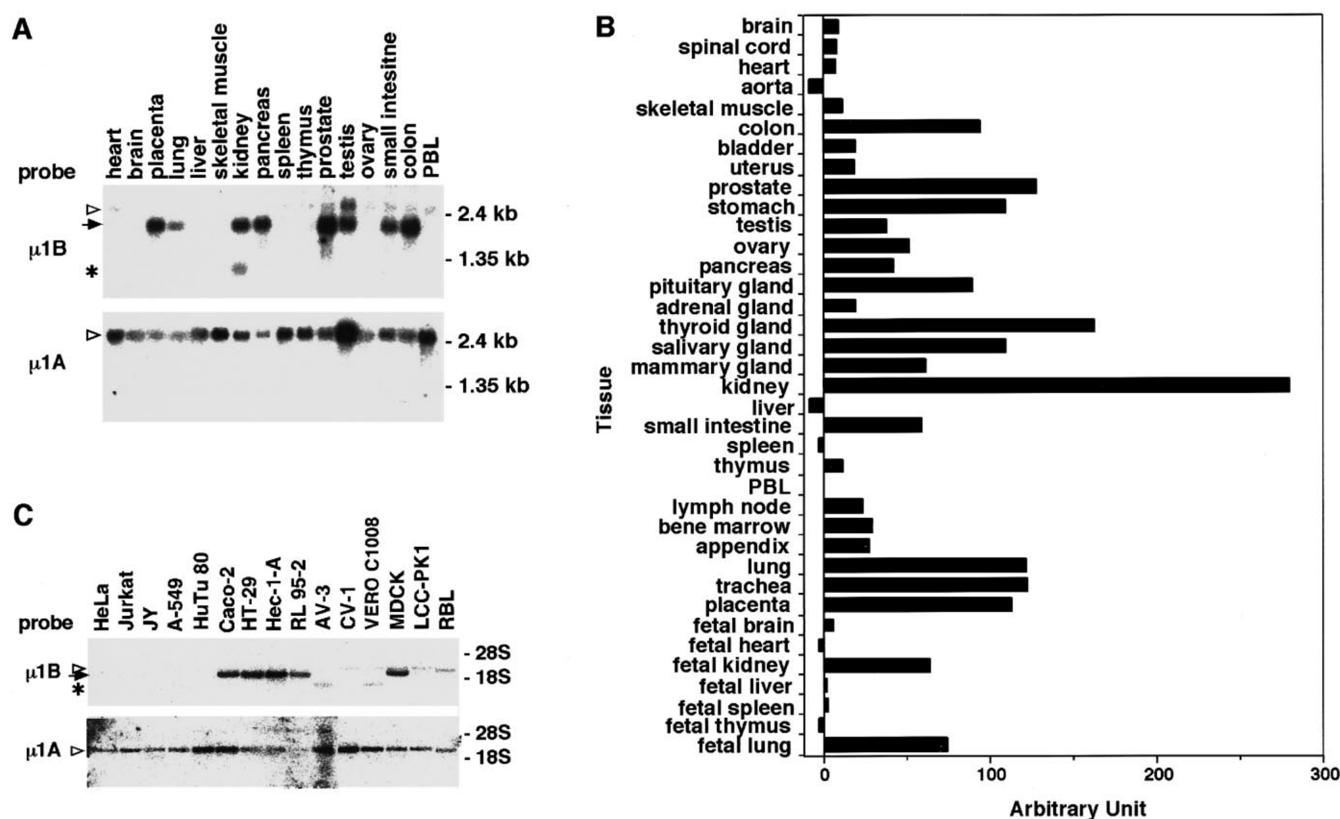


Fig. 2. Analyses of μ1B mRNA expression. (A) Human multiple tissue Northern blots probed with ^{32}P-labelled human μ1B and μ1A cDNAs. Positions of μ1B (arrow) and μ1A (open triangles) transcripts are indicated on the left. The asterisk indicates a faster migrating μ1B RNA species detected in kidney. The positions of RNA size markers are indicated on the right. (B) Quantitation of a human RNA dot blot probed with ^{32}P-labelled human μ1B cDNA. (C) Total RNAs (10 μg) from various cultured cell lines probed with ^{32}P-labelled human μ1B and μ1A cDNAs. The positions of μ1B (arrow) and μ1A (open triangles) transcripts are indicated on the left. The asterisk indicates a faster migrating μ1B RNA species detected in the lanes corresponding to AV-3 and VERO C1008 cells. The positions of the 18S and 28S rRNAs are indicated on the right.

(MTN) Blots I and II, as well as Human RNA Master Blot, were purchased from Clontech. For human RNA samples, Nylon membranes were first probed with ^{32}P-labelled human μ1B cDNA (a PCR fragment corresponding to nucleotides 757–1363 of human μ1B, GenBank AF020797). After stripping off the probe, membranes were re-probed with ^{32}P-labelled human μ1A cDNA (the insert of an EST clone, ID hbc4916, containing a segment of the human μ1A cDNA). Mouse RNA samples were sequentially probed with mouse μ1B (a *Pst* I fragment corresponding to nucleotides 391–1313, GenBank AF067146) and μ1A (a PCR fragment corresponding to nucleotides 73–342, GenBank W08014) cDNA fragments.

2.5. In situ hybridization

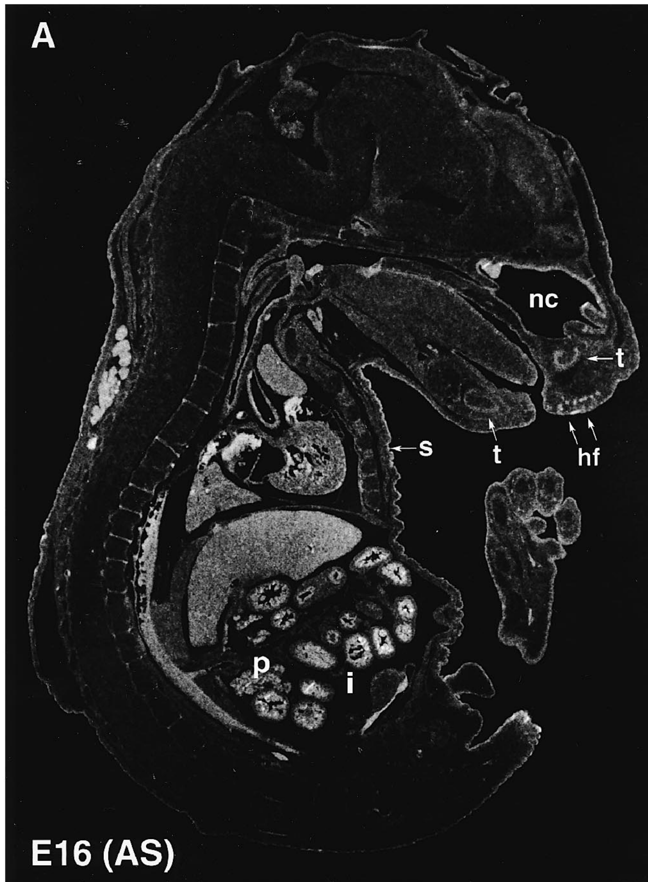
C57BL/6 mouse embryos at day 16 p.c. were fixed in 4% paraformaldehyde in PBS at 4°C overnight, dehydrated with ethanol and embedded in paraffin. Serial sections of 5 μm were cut and mounted on poly-L-lysine coated slides. After removal of paraffin, sections were post-fixed in 4% paraformaldehyde, treated with 0.25% acetic anhydride in 0.1 M triethanolamine and dehydrated again. Procedures for in situ hybridization were essentially as described [29]. For the antisense probe of mouse μ1B, pGEM3Zf(+) plasmid (Promega, Madison, WI, USA) harboring a 922 bp *Pst* I fragment (nucleotides 369–1291) from mouse μ1B cDNA was linearized with *Hin*d III and transcribed with T7 polymerase according to the manufacturer's instructions (Promega, Madison, WI, USA) with [^{35}S]UTP (740 MBq/ml, Amersham). The sense probe was linearized with *Bam* HI and transcribed with SP6 polymerase. Hybridization, washing and exposure were performed as previously described [29]. Sections were counterstained with hematoxylin and eosin (H and E). Dark-field images were superimposed with bright-field images, modified with color filters and photographed.

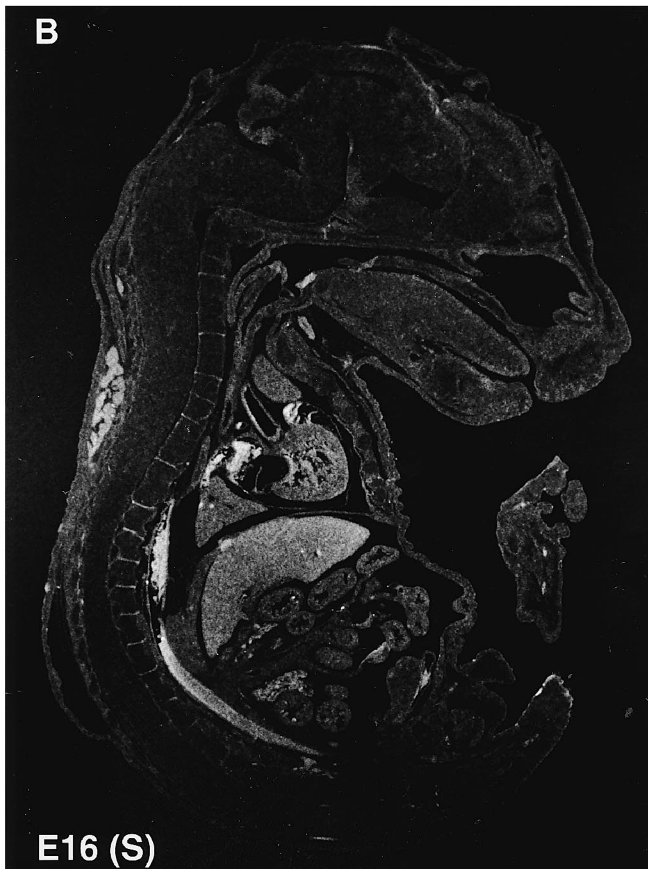
2.6. Yeast two-hybrid analysis

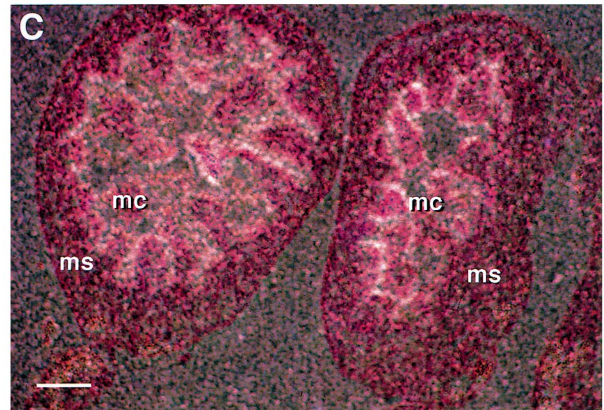
YXXØ signals fused to GAL4bd were derived from a random combinatorial library prepared for analyzing specificity μ chain interactions with tyrosine-based signals [20]. GAL4ad-μ1B was made by ligation of a *Sal* I-*Xho* I fragment from a μ1B cDNA clone into the *Xho* I site of the vector pACT2. Other GAL4ad-μ chain constructs and procedures of the two-hybrid analyses were described previously [18–20].

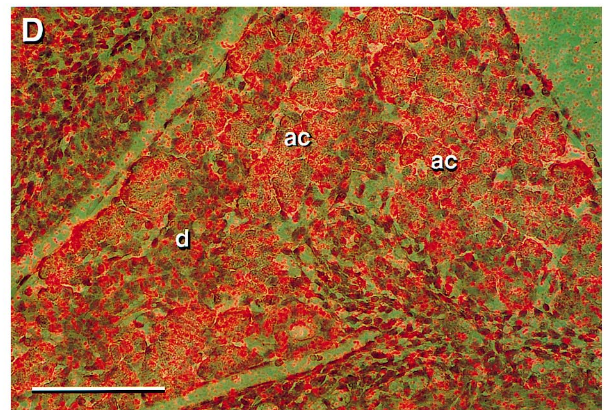
3. Results and discussion

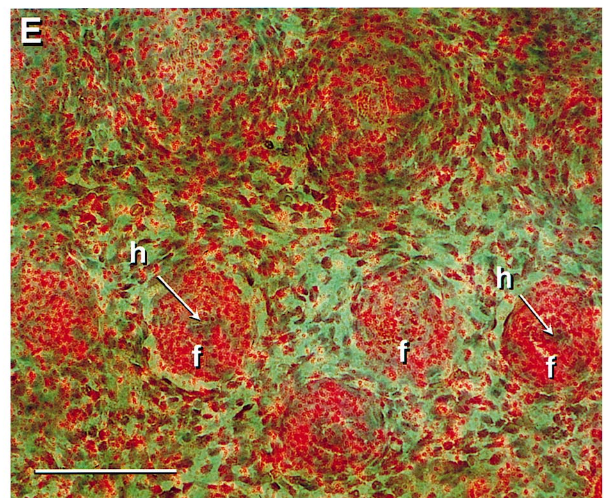
A human cDNA encoding a novel μ chain was cloned based on sequence information found in EST databases. The cDNA encoded a protein of 423 amino acids and a predicted molecular mass of 48 030 Da. The novel protein was most closely related to mouse μ1 (79% identity at the amino acid sequence level) (Fig. 1A) and more distantly related to other members of the adaptor medium chain family (30–40% identity to μ2, μ3A, μ3B and μ-ARP2 (μ4, [30]) and ~20% identity to δ-COP [31]) (Fig. 1B). We also cloned a mouse cDNA encoding the murine homolog of the human protein (97% identity at the amino acid level) (Fig. 1A), thus demonstrating that the novel protein corresponds to a conserved gene product distinct from the previously described μ1. Because μ1 and the novel protein were distinct but closely related, they were designated μ1A and μ1B, respectively. The μ1A and μ1B polypeptides produced by in vitro transcription/


A
nc
t
s
t
hf
p
i
E16 (AS)


B
E16 (S)


C
mc
mc
ms
ms


D
ac
ac
d


E
h
h
f
f
f

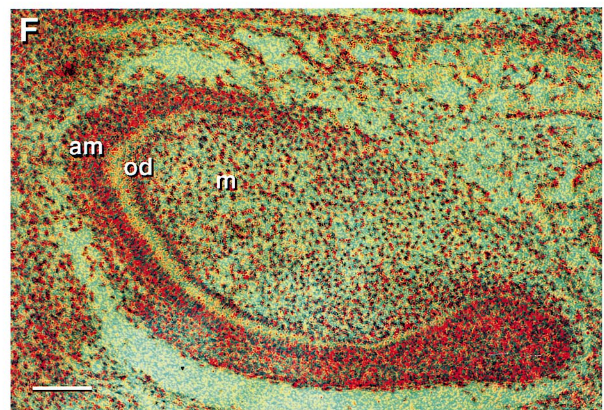

F
am
od
m

Fig. 3. In situ hybridization of μ1B mRNA in mouse embryonal tissues. (A, B) Parasagital sections of E16 mouse embryo, hybridized with antisense (A) or sense (B) RNA probes for μ1B. Intense signals are detected in the intestine (i) whereas moderate signals are found in the pancreas (p), hair follicles (hf), tooth primordia (t) and nasal cavity (nc). (C–F) Sections of fetal intestine (C), fetal pancreas (D), fetal hair follicles (E) and a fetal tooth bud (F), hybridized with antisense RNA probes for μ1B and counterstained with H and E. (C) Scale bar, 200 μm. (D–F) Scale bar, 100 μm.
←

translation co-migrated on SDS-PAGE with an apparent molecular mass of ∼47 kDa (Fig. 1C).

Northern analyses revealed that μ1B mRNA was expressed in only some human tissues, including placenta, lung, kidney, pancreas, prostate, testis, small intestine and colon (Fig. 2A), in contrast to μ1A mRNA which was expressed in all human tissues examined (Fig. 2A). In addition, dot blot analyses demonstrated a high level expression of μ1B mRNA in stomach, pituitary gland, salivary glands and trachea (Fig. 2B). The same pattern of differential expression of μ1A and μ1B mRNAs was observed in mouse tissues (data not shown). A common feature of all the tissues that express μ1B mRNA is the presence of epithelial and/or exocrine cell-types, both of which are polarized. To investigate whether μ1B mRNA was specifically expressed in polarized cell-types, we performed Northern analyses of various cultured cell lines (Fig. 2C). These experiments demonstrated expression of μ1B mRNA in Caco-2, HT-29, Hec-1-A, RL 95-2 and MDCK cells, all epithelial cells that become polarized in culture. Some epithelial cells (e.g. LLC-PK1) as well as non-epithelial cells expressed little or no μ1B mRNA, although they all expressed μ1A mRNA (Fig. 2C).

To analyze the spatial expression of the μ1B gene in mouse tissues, we performed in situ hybridization on mouse embryos. In a parasagital section of day 16 p.c. embryos, the highest levels of μ1B mRNA were observed in the gastrointestinal mucosa (Fig. 3, compare A and B). Moderate levels of μ1B gene expression were detected in the pancreas, hair follicles, tooth buds and nasal cavity (Fig. 3A and B). In the gastrointestinal tract, μ1B mRNA was highly expressed in the intestinal mucosa (mc) but was barely detected in the submucosa and muscle layer (ms) (Fig. 3C). In the pancreas, strong in situ signals were observed in acinar cells (ac) but not in the intercalated duct cells (d) (Fig. 3D). μ1B mRNA was also specifically expressed in epithelial cells of hair follicles (Fig. 3E), as well as in ameloblasts (am) and odontoblasts (od), polarized epithelial cells that secrete enamel and dentin, respectively, in developing teeth (Fig. 3F). These results confirmed that μ1B mRNA is predominantly expressed in polarized epithelial cells and exocrine cells.

Next, we tested whether μ1B was capable of interacting with cytosolic sorting signals using the yeast two-hybrid system [18–20]. Yeast cells were co-transformed with GAL4ad-μ1B and GAL4bd fused to generic YXXØ-type tyrosine-based sorting signals [20]. Like other members of the μ chain family, μ1B was found to interact with a subset of YXXØ-type signals in a tyrosine-dependent manner, as demonstrated by the growth of co-transformed yeast cells on medium lacking histidine (Fig. 4). This interaction is consistent with the fact that some YXXØ-type signals function as basolateral sorting signals [32–36] and suggests that μ1B could play a role in the recognition of YXXØ signals in polarized cells.

The recent resolution of the crystal structure of μ2 [37] makes it possible now to analyze the structural bases for recognition of tyrosine-based sorting signals by members of the adaptor medium chain family. Interestingly, of 11 residues in μ2 shown to be involved in the recognition of YXXØ signals [37], seven are conserved and four are not conserved in μ1B. Among the conserved residues are D^{176}, F^{174}, L^{203}, W^{421}, R^{423} of μ2, all of which are involved in interactions with the critical Y residue of YXXØ signals [37]. In contrast, only two (i.e. V^{401} and V^{422}) of the six μ2 residues involved in interactions with the Ø side chain of YXXØ signals (i.e. L^{173}, L^{175}, V^{401}, L^{404}, K^{420}, V^{422}, [37]) are conserved in μ1B. The substitution of I^{419} in μ2 by L in μ1B could also affect the recognition of residues at the second X position (Y+2). These structural considerations predict that μ1B should be able to interact with a subset of tyrosine-based sorting signals, albeit with an affinity and/or fine specificity distinct from that of μ2. This prediction is fulfilled by the ability of μ1B to bind only some generic sorting signals (Fig. 4), all of which bind to μ2 [20].

The specific expression of μ1B mRNA in polarized epithelial and exocrine cells suggests that this protein might be involved in sorting events necessary for the establishment of cell polarity or for delivery of proteins to different plasma membrane domains. In this regard, it is noteworthy that the porcine kidney epithelial cell line LLC-PK1, which becomes polarized in culture, does not express detectable levels of μ1B mRNA (Fig. 2C). Remarkably, LLC-PK1 cells have been shown to missort two proteins having tyrosine-based signals, the H,K ATPase β subunit and an influenza hemagglutinin construct bearing a tyrosine-based signal, to the apical surface [38]. This is in contrast to MDCK cells, which express μ1B mRNA (Fig. 2C) and sort both the H,K ATPase β subunit and the influenza hemagglutinin construct to the basolateral surface [38]. Since LLC-PK1 cells are otherwise polarized, these observations lend us support to the idea that μ1B may be required for basolateral targeting.

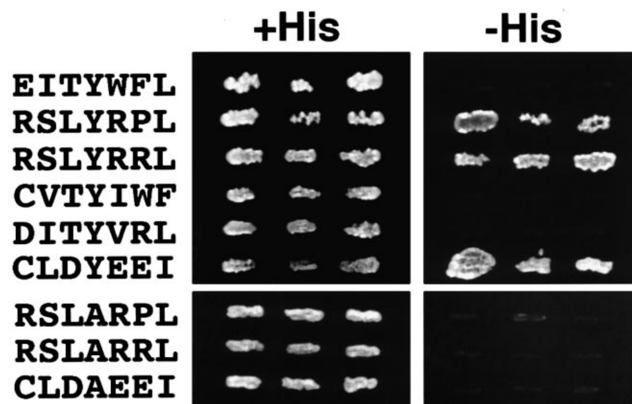


Fig. 4. Interactions of μ1B with sorting signals. Interactions with YXXØ signals. HF7c yeast cells were co-transformed with a plasmid encoding GAL4ad fused to human μ1B and plasmids encoding GAL4bd fused to sequences of various YXXØ signals and their Y to A mutants. Co-transformants were tested for their ability to grow in the presence (+His) or absence (−His) of histidine, as previously described [18].

The similarity of μ1B to μ1A makes it likely that μ1B is an alternative component of the AP-1 clathrin-associated adaptor complex in polarized epithelial cells. We have been unable to test if μ1B is indeed a component of AP-1, as the antibodies that were raised against μ1B did not discriminate μ1B from μ1A (data not shown). Nonetheless, a role for AP-1 and clathrin in the basolateral targeting has been recently proposed by Futter et al. [39], who showed that vesicles bound at the basolateral domain bud from clathrin- and AP-1-coated endosomal tubules. A similar process may take place at the TGN, where AP-1 and clathrin are also located [40]. In this regard, Orzech et al. [41] have recently presented evidence that AP-1 interacts with a basolateral targeting determinant in the cytosolic tail of the polymeric immunoglobulin receptor, an interaction which may mediate sorting of the receptor at the TGN. An intriguing implication of our findings is that other polarized cells such as hepatocytes or neurons, which do not seem to express μ1B, may utilize other adaptor proteins for sorting to the basolateral equivalent of the plasma membrane. This is consistent with the idea that the mechanisms of polarized sorting in hepatocytes, neurons and epithelial cells are related but not identical [2,42].

In conclusion, our results suggest that μ1B may be a component of the protein sorting machinery in polarized epithelial and exocrine cells. The information presented here should now enable further analyses of the role of μ1B in protein sorting in vivo.

Acknowledgements: We thank K. Fujita for the assistance with photography and M.-C. Fournier and M. Sakuma for the excellent technical assistance. This work was supported in part by Grants-in-Aids for Scientific Research from the Ministry of Education, Science, and Culture of Japan (HO and TS), the Naito Foundation (HO) and the Uehara Memorial Foundation (HO).

References

[1] Mellman, I. (1996) Annu. Rev. Cell Dev. Biol. 12, 575–625.
[2] Keller, P. (1997) J. Cell Sci. 110, 3001–3009.
[3] Traub, L.M. (1997) Curr. Opin. Cell Biol. 9, 527–533.
[4] Simons, K. (1997) Nature 387, 569–572.
[5] Scheiffele, P. and Peränen, J. (1995) Nature 378, 96–98.
[6] Gut, A., Kappeler, F., Hyka, N., Balda, M.S., Hauri, H.P. and Matter, K. (1998) EMBO J. 17, 1919–1929.
[7] Prill, V., Lehmann, L., von Figura, K. and Peters, C. (1993) EMBO J. 12, 2181–2193.
[8] Lin, S. and Naim, H.Y. (1997) J. Biol. Chem. 272, 26300–26305.
[9] Matter, K. and Yamamoto, E.M. (1994) J. Cell Biol. 126, 991–1004.
[10] Reich, V. and Mostov, K. (1996) J. Cell Sci. 109, 2133–2139.
[11] Odorizzi, G. (1997) J. Cell Biol. 137, 1255–1264.
[12] Simonsen, A., Stang, E., Bremnes, B., Roe, M. and Prydz, K. (1997) J. Cell Sci. 110, 597–609.
[13] Marks, M.S., Ohno, H., Kirchhausen, T. and Bonifacino, J.S. (1997) Trends Cell Biol. 7, 124–128.
[14] Kirchhausen, T. and Bonifacino, J.S. (1997) Curr. Opin. Cell Biol. 9, 488–495.
[15] Robinson, M.S. (1997) Trends Cell Biol. 7, 99–102.
[16] LeBorgne, R. (1998) Curr. Opin. Cell Biol. 10, 499–503.
[17] Hirst, J. (1998) Biochim. Biophys. Acta 1404, 173–193.
[18] Ohno, H. et al. (1995) Science 269, 1872–1875.
[19] Ohno, H., Fournier, M.C. and Poy, G. (1996) J. Biol. Chem. 271, 29009–29015.
[20] Ohno, H., Aguilar, R.C., Yeh, D., Taura, D. and Saito, T. (1998) J. Biol. Chem. 273, 25915–25921.
[21] Boll, W., Ohno, H., Songyang, Z., Rapoport, I., Cantley, L.C., Bonifacino, J.S. and Kirchhausen, T. (1996) EMBO J. 15, 5789–5795.
[22] Dell'Angelica, E.C., Ohno, H., Ooi, C.E., Rabinovich, E., Roche, K.W. and Bonifacino, J.S. (1997) EMBO J. 16, 917–928.
[23] Rodionov, D.G. (1998) J. Biol. Chem. 273, 6005–6008.
[24] Bremnes, T., Lauvrak, V. and Lindqvist, B. (1998) J. Biol. Chem. 273, 8638–8645.
[25] Rapoport, I., Chen, Y.C., Cupers, P., Shoelson, S.E. and Kirchhausen, T. (1998) EMBO J. 17, 2148–2155.
[26] Lennon, G., Auffray, C., Polymeropoulous, M. and Soares, M.B. (1996), 33, 151–152.
[27] Nakayama, Y., Goebl, M., O'BrineGreco, B., Lemmon, S. and Pingchang Chow, E. (1991) Eur. J. Biochem. 202, 569–574.
[28] Chomczynski, P. (1987) Anal. Biochem. 162, 156–159.
[29] Shirasawa, T., Akashi, T., Sakamoto, K., Takahashi, H. and Maruyama, N. (1993) Dev. Dyn. 198, 1–13.
[30] Wang, X. (1997) FEBS Lett. 402, 57–61.
[31] Faulstich, D. et al. (1996) J. Cell Biol. 135, 53–61.
[32] Geffen, I., Fuhrer, C., Leitinger, B., Weiss, M., Huggel, K. and Griffiths, G. (1993) J. Biol. Chem. 268, 20772–20777.
[33] Naim, H.Y. (1994) J. Biol. Chem. 269, 3928–3933.
[34] Höning, S. (1995) J. Cell Biol. 128, 321–332.
[35] Rajasekaran, A.K., Humphrey, J.S., Wagner, M., Miesenböck, G., LeBivic, A. and Bonifacino, J.S. (1994) Mol. Biol. Cell 5, 1093–1103.
[36] Lodge, R., Lalonde, J.P., Lemay, G. and Cohen, E.A. (1997) EMBO J. 16, 695–705.
[37] Owen, D.J. (1998) Science 282, 1327–1332.
[38] Roush, D.L., Gottardi, C.J., Naim, H.Y. and Roth, M.G. (1998) J. Biol. Chem. 273, 26862–26869.
[39] Futter, C.E. et al. (1998) J. Cell Biol. 141, 611–623.
[40] Robinson, M.S. (1986) J. Cell Biol. 102, 48–54.
[41] Orzech, E., Schlessinger, K., Weiss, A. and Okamoto, C.T. (1999) J. Biol. Chem. 274, 2201–2215.
[42] Matter, K. (1994) Curr. Opin. Cell Biol. 6, 545–554.