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MICAL-1 isoforms, novel rab1 interacting proteins

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Abstract

Rab1 GTPases participate in regulating the vesicular transport of ER-Golgi compartments. Recently, GM130, p115, and Golgin-84 were identified as effectors of the active conformation of rab1. Here, we describe a novel protein, MICAL-1b, a splice variant of the MICAL-1a protein. Using the yeast two-hybrid system, we showed that it specifically interacts with rab1 in a nucleotide-dependent manner. The interaction was confirmed by GST pulldown experiments. Cell fractionation revealed that in contrast to the mainly membrane-associated rab1 effector GM130, MICAL-1 displays a predominantly cytosolic localization. We mapped the rab1 interacting domain to the C-terminus of MICAL-1, which also mediates binding to the intermediate filament vimentin. Therefore, the interaction of MICAL-1 and rab1 might provide a link between the Golgi apparatus and the intermediate filament cytoskeleton. We suggest that MICAL-1 isoforms with their multidomain structure are novel rab1 interacting proteins that function as scaffold proteins connecting different components in the cell.

Keywords: rab1 GTPase; MICAL; Vesicular traffic; ER-Golgi transport

So far more than 60 rab/Ypt proteins have been identified and it has become clear that each of them transmits signals via downstream interacting molecules to regulate various processes like the formation, motility, and docking of vesicles or the remodeling and/or fusion of membranes [1,2].

The rab1 isoforms are localized at the endoplasmic reticulum (ER)-Golgi membranes and are involved in the vesicular traffic between these compartments [3–5]. To elucidate the function of rab1 GTPases, it is essential to identify their interacting proteins. Using an affinity approach, six proteins were found that specifically interact with the activated form of rab1. One of the six proteins was identified as p115 [6]. This protein is the mammalian homologue of the yeast Uso1p protein and was previously characterized as ER-Golgi and *intra* Golgi transport factor and, more recently, as binding partner of GM130 and Giantin [7–10]. Allan et al. showed that rab1 recruits p115 into a *cis* SNARE complex and, therefore programs budding coat protein (COP) II vesicles from the ER for fusion with the Golgi membranes, suggesting that the rab1-dependent recruitment of p115 is an initial step at the donor membranes. In addition, p115 coordinates the sequential tethering and docking of COP I vesicles to Golgi membranes [10,11]. GM130 is a direct effector protein of activated rab1 and Moyer et al. demonstrated that activated rab1 interacts even with a whole GM130 effector complex, containing the Golgi re-assembling protein 65, Grasp65, and several unknown proteins [12–15]. The C-terminus of GM130 binds directly to Grasp65 and both proteins cycle via membranous tubules between the Golgi complex and a subdomain of the intermediate compartment [16,17]. We mapped the rab1 binding site to the third coiled-coil (cc) domain of GM130 and, therefore could show that the rab1/GM130 interaction is independent of the p115 and Grasp65 binding sites of GM130 [15]. In addition, Golgin-84, an integral membrane protein with coiled-coil domains, that functions in the assembly and maintenance of the Golgi ribbon was recently identified as rab1 interacting protein [18,19,32].

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In the present work, we identified a new rab1 interacting protein by using the yeast two-hybrid approach. Sequence analysis revealed that our clone encodes the C-terminal part of MICAL-1 (MICAL-1a + MICAL-1b). This protein associates with CasL, a $p130^{Cas}$ family member, with vimentin, a major component of the intermediate filaments and in neuronal cells with plexin A [20,21]. MICAL proteins contain monooxygenase domains and multiple further domains that are known to be important for protein interactions, e.g., with actin, intermediate filaments, and cytoskeletal-associated adaptor proteins [20,21]. Using yeast two-hybrid co-transformation and in vitro binding assays, we could show that the rab1/MICAL-1 interaction is nucleotide-dependent and so far tested rab1-specific. Furthermore, we mapped the rab1 binding site of MICAL-1 and could demonstrate that MICAL-1 displays some significant differences in comparison to the previously characterized rab1 effectors GM130 and p115.

Methods

Plasmids

Rab1b and rab6A mutants have been described [15,22,23]. Details of further plasmids and corresponding primers of this study are available from Angelika Barnekow.

Yeast two-hybrid methods

The yeast two-hybrid screen and two-hybrid assays were previously described in more detail [15,24].

Preparation of cell extracts and cell fractionation

BHK, HEL, and HEK 293 cells were cultivated as previously described [15]. For preparation of cell extracts or cell fractionations, cells were washed three times with ice-cold PBS and scraped into the required buffer. All buffers contained the proteinase cocktail complete, EDTA-free. For the extracts shown in Fig. 1C, cell lysis buffer E was used (LBE: 50 mM Tris/HCl, pH 7.4, 150 mM NaCl, 2 mM EGTA, 50 mM NaF, 0.1% SDS, 0.1% DOC, 1.0% NP40, and 200 μM sodiumorthovanadate). The lysates were centrifuged (1 h, 16,000*g*, 4 °C) and the supernatant was analyzed by SDS–PAGE and Western blot. Cell extracts for in vitro binding assays were prepared in LB (see below).

Cell fractionation. After two freeze/thaw cycles, cells were homogenized by 10 passes through a 25-gauge needle in 250 mM sucrose, 10 mM Hepes/KOH, pH 7.4, and 1 mM EDTA. The post nuclear supernatant (PNS) was collected (2 min, 1000*g*), underlayered with 10 μl of 50% sucrose, and centrifuged (15 min, 14,000*g*, 4 °C). The supernatant was further centrifuged (1 h, 100,000*g*, 4 °C) to yield the S100 supernatant and P100 pellet. The pellets (P14 and P100) were rinsed in 250 mM sucrose, 10 mM Hepes, 1 mM EDTA and lysed with 2× HKT (20 mM Hepes/KOH, 200 mM KCl, 2 mM EDTA, 2 mM DTT, 1% Triton X-100, and Complete), adjusted to 1× HKT, and clarified by centrifugation (30 min, 16,000*g*, 4 °C). Aliquots of the samples were analyzed by SDS–PAGE and Western blotting.

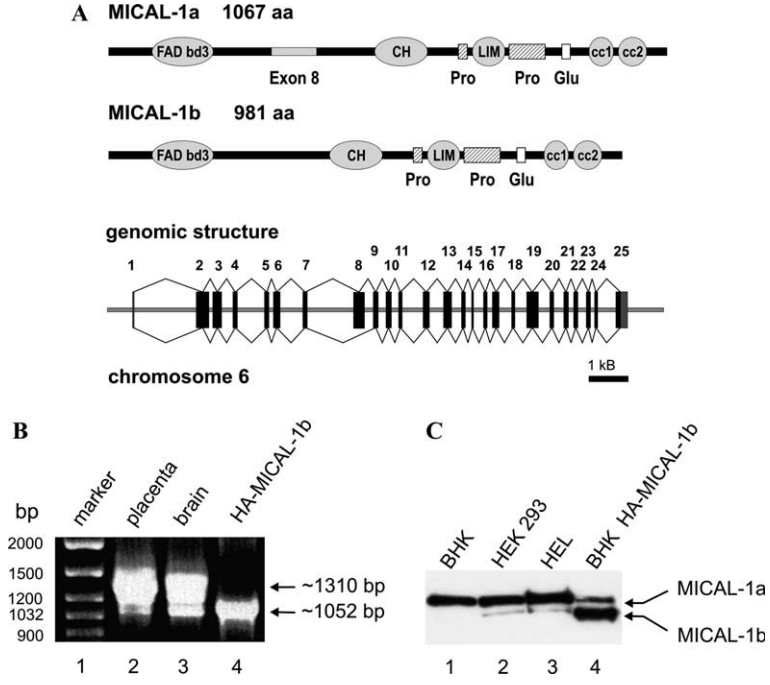

Fig. 1. (A) Schematic overview of the MICAL-1 gene structure and the domain architecture of the MICAL-1a/b proteins. The novel splice variant lacks the amino acids that correspond to exon 8. (B) PCR analysis: lane 1, marker; lanes 2 and 3, PCR analysis of placenta and brain cDNAs, respectively; and lane 4, MICAL-1b positive control (cDNA). (C) Western blot analysis of extracts from non-transfected BHK cells (1) or from transfected BHK cells, overexpressing HA-MICAL-1b (4). Extracts from human HEK 293 (2) and human HEL cells (3) contain both MICAL-1 isoforms. The upper band corresponds to MICAL-1a, the lower band to MICAL-1b. The MICAL-1 isoforms were detected with affinity purified #663 antibody and HRP-anti-rabbit IgG. FAD bd3, monooxygenase domain; CH, calponin homology domain; Pro, proline rich aa stretch; LIM, LIM domain; glutamic acid-rich aa stretch; and cc, coiled-coil domain.

Recombinant proteins

BL21 *Escherichia coli* cells were transformed with pGEX plasmids encoding the last 203 aa of MICAL-1b (ΔN778) and full-length Pra1, or pET plasmids encoding rab1b S22N and rab1b Q67R, respectively. After inducing the expression (1 mM IPTG for 3 h, 30 °C) cells were pelleted, washed, and resuspended in PBS with Complete, sonicated (four times for 10 s) and 1% Triton X-100 was added. After 30-min incubation on ice, samples were centrifuged at 16,000*g*. The supernatant was stored at −70 °C. The cleared lysate was incubated with glutathione–Sepharose 4B beads (overnight at 4 °C). Beads were washed two times with PBS and once with LB (10 mM Tris, pH 7.4, 150 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.2% Triton X-100, and Complete, EDTA-free).

In vitro binding assay

The binding assays were carried out with a 10 μl volume of packed glutathione–Sepharose 4B beads coated with ~15 μg of the GST, GST-MICAL1b ΔN778, or GST–Pra1 fusion proteins. The beads were incubated (2.5 h, 4 °C) with purified His-rab1 mutants or with extracts from BHK cells transfected with HA-rab1b wt or the HA-rab1 mutants, adjusted to 300 μl volume with LB (see above), washed three times with LB (containing 0.1% SDS in case of His-tagged proteins), and eluted with sample buffer. Aliquots were analyzed by SDS–PAGE (13%).

Antibodies

A rabbit serum against MICAL-1 (#663) was obtained by using a His-tagged protein corresponding to the last 404 aa of MICAL-1 as antigen (Eurogentech, Belgium). It was affinity purified with NHS-activated Sepharose 4 Fast Flow (Amersham Bioscience, Freiburg, Germany) coupled with GST-MICAL-1b ΔN778. Monoclonal antibodies (mAb) against p115 and calnexin were from BD Biosciences (Heidelberg, Germany), the HA-antibody 16B12 was from BabCO (USA). The mAb 1E7 against rab1b has been described earlier [23]. Secondary antibodies coupled to peroxidase were purchased from Amersham Bioscience.

Results

To identify interacting proteins of rab1b, the GTPase-deficient mutant rab1b Q67R was used as bait in a yeast two-hybrid screen of a human placenta cDNA library. The clones K35 and K39 which contain the same ~2.0 kb DNA insert induced strong β-galactosidase activity when co-expressed with the GTPase-deficient mutants rab1b Q67R, rab1b Q67L, and rab1a Q70L. The mutant rab1b N121I also interacts with K35/39. We did not detect any interaction between K35/39 and rab1a wild-type (wt), rab1b wt, the inactive rab1a S25N and rab1b S22N mutants.

As a control to analyze the rab1 specificity, we also used rab2 and rab6A GTPases, which are localized at membranes of the intermediate compartment and the Golgi apparatus. Rab2 and rab6A and the corresponding mutants (rab2 Q65L, rab6A T27N, rab6A N126I, and rab6A Q72R) failed to interact with the clone K35/39. Moreover, co-transformations with further members of the rab family (rab3A, rab7, and rab9) or HA-ras and rho Q63L (data not shown) did not activate the reporter genes, suggesting that the K35/39-rab1 interaction is nucleotide-dependent and highly rab1-specific (Table 1A).

Next, we wanted to find out, which structural components of rab1 are important for the interaction with K35/39 and, therefore used the recently described rab1b Q67R deletion mutants [15]. The rab1b Q67R deletion mutant without the prenylation site (rab1b Q67R ΔC197) displayed a high activation of the reporter gene. However, the three rab1b Q67R truncation mutants without the N- and/or C-terminal hypervariable regions (rab1b Q67R ΔN9, rab1b Q67R ΔC163, and rab1b Q67R ΔC163–196) completely failed to activate the β-galactosidase, indicating that the N- and C-terminal hypervariable regions are essential for the K35/39 interaction (Table 1A).

In order to confirm these results, we performed co-transformation assays with the recently described rab1b Q67L/rab6A, rab6A Q72L/rab1b, and rab6A Q72R/rab1b chimeras [24]. Interestingly, the rab1b Q67L chimera that contains the rab6A C-terminal hypervariable region leads to a strong activation of the reporter genes, whereas both rab6A chimeras that contain the C-terminal hypervariable region of rab1b failed to show any activity.

Thus, the co-transformation assays with the deletion mutants and chimeras indicate that the K35/39-rab1 interaction mainly depends on the active conformation and probably on the rab subfamily domains 1–3, which are localized within the first 123 amino acids of rab1b (until the G4 box).

Surprisingly, K35/39 is also able to bind Ypt1p, the yeast counterpart of mammalian rab1. A high reporter gene activation was observed when Ypt1p wt or the mutants Ypt1p Q67L or Ypt1p N121I were used as baits, whereas the inactive Ypt1p S22N mutant did not react, leading to the conclusion that the K35/39-rab1 interaction is conserved during evolution (Table 1B). Two-hybrid assays of rab1 with the mammalian suppressor of sec4, Mss4, as an example for a protein with rab guanosine exchange factor (GEF) activity, illustrate that K35/39 behaves more like an effector protein that prefers the activated rab1 conformation (right column in Table 1). It is worth to note, that GM130 (aa 224–990) does not interact with Ypt1p.

Sequence analysis of the prey cDNA insert of K35/39 revealed that it represents the C-terminal half of the recently described MICAL-1 protein [21]. MICAL-1, which is identical to the theoretical protein FLJ11937, consists of some interesting protein domain features, like a flavoprotein monooxygenase domain, a calponin homology (CH) domain, and a LIM domain that is flanked by two proline-rich regions. We identified two putative coiled-coil (cc) domains at the C-terminus of MICAL-1. Furthermore, a highly charged polyglutamic

Table 1
Interaction of rab1 isoforms and other rab proteins with MICAL-1, Mss4, and GM130

Bait	Prey			
	K35/39[a]		Mss4[b]	
	his3	lacZ	his3	lacZ
A				
Control[c]	+/–	–	+/–	–
rab1a wt	+/–	–	+/–	–
rab1a S25N	+/–	–	++	+++
rab1a Q70L	+++	+++	–	–
rab1b wt	+/–	–	+/–	–
rab1b S22N	+/–	–	+++	+++
rab1b N121I	++	++	+++	+++
rab1b Q67L	+++	++	+/–	–
rab1b Q67R	+++	+++	+/–	–
rab1b Q67R ΔC197	+++	+++	n.d.	n.d.
rab1b Q67R ΔC163	+/–	–	n.d.	n.d.
rab1b Q67R ΔC163–196	+/–	–	n.d.	n.d.
rab1b Q67R ΔN9	+/–	–	n.d.	n.d.
rab6A wt	+/–	–	+/–	–
rab6A T27N	+/–	–	+/–	–
rab6A N126I	+/–	–	+/–	–
rab6A Q72R	+/–	–	+/–	–
rab2 wt	+/–	–	+/–	–
rab2 Q65L	+/–	–	n.d.	n.d.
rab3A wt ΔC	+/–	–	+/–	+/–
rab3A wt T36N ΔC	+/–	–	+++	+++
rab7 wt ΔC	+/–	–	+/–	–
rab9 wt ΔC	+/–	–	–	–
Chimeras				
rab1b Q67L rab6A	+++	+++	n.d.	n.d.
rab6A Q72L rab1b	+/–	–	n.d.	n.d.
rab6A Q72R rab1b	+/–	–	n.d.	n.d.

	Prey					
	K35/39[a]		K9/18[d]		Mss4[b]	
	his3	lacZ	his3	lacZ	his3	lacZ
B						
Ypt1 wt	++	++	+/–	–	+/–	–
Ypt1 S22N	+/–	–	+/–	–	++	++
Ypt1 N121I	++	++	+/–	–	++	++
Ypt1 Q67L	++	++	+/–	–	+/–	–

Characterization of the interaction between rab1 isoforms and the clones K35/K39 (MICAL-1b aa 577–981), Mss4, and K9/18 (GM130 aa 224–990). To test for two-hybrid interactions, corresponding strains were cultivated in synthetic media lacking leucine, tryptophan, and histidine, supplemented with 50 mM 3AT. In addition, β-galactosidase reporter gene activity (lacZ) was determined on replica filters using X-gal as substrate.

–, no growth in selection media or no β-galactosidase activity; +/–, background growth or background β-galactosidase activity; ++, strong growth in selection media or strong β-galactosidase activity; +++, very fast growth in selection media or high β-galactosidase activity; n.d., not determined.

[a] Clone K35/K39 in pACT2.
[b] Mss4 in pACT2.
[c] Empty bait vectors pAS2-1 and pGBT9.
[d] ΔN224 fragment of GM130 in pACT2.

acid stretch was found between the second proline-rich region and the first cc domain (Fig. 1A).

An alignment of the MICAL-1 cDNA with the genomic clone RP5-919F19 (AL109947) showed that the putative MICAL-1 gene is ~10 kb in size and localized to chromosome 6. It has 25 exons of which the first exon is a non-coding one (Fig. 1A). Interestingly, an IMAGE clone with the Accession No. BC009972 lacks aa

312–397. These 86 aa correspond exactly to the putative exon 8, arguing for the existence of an alternatively spliced smaller version of human MICAL-1a, MICAL-1b. We performed a PCR analysis (Fig. 1B) with cDNA libraries from placenta and brain to prove this assumption and detected two signals that correspond to two independent MICAL-1 transcripts. Sequencing of the smaller PCR product showed that it lacks the DNA sequence which encodes exon 8 (data not shown). The existence of two MICAL-1 splice variants was also analyzed on the protein level. Using the affinity purified #663 antibody (directed against the last 203 aa of MICAL-1), we were able to detect two proteins in human HEK 293 and HEL cell extracts (Fig. 1C). MICAL-1a seems to be the mainly expressed isoform. But PCR and Western blot analysis demonstrate the existence of an additional smaller MICAL-1 splice variant, MICAL-1b.

To map the rab1 binding site of MICAL-1, we used a set of truncation mutants and tested them in two-hybrid assays (Fig. 2A). Co-transformation with the rab1 GTPase-deficient mutants and prey fusion proteins encoding the C-terminal MICAL-1b truncation mutants ΔN415, ΔN576, ΔN678, ΔN748, and ΔN835 led to a strong reporter gene activation (Fig. 2B), suggesting that the last 145 amino acids determine a putative rab1 interacting domain. A deletion mutant that only encodes the two cc domains (aa 836–942) failed to activate the reporter genes, indicating that the last 39 aa of MICAL-1 are essential for rab1 binding (Fig. 2B). As expected, the mutant that encodes the N-terminal part of MICAL-1b (ΔC524) failed to activate the reporter genes when co-transformed with rab1 and rab6A GTPase-deficient mutants, indicating that MICAL-1 has only one rab1 binding site (Fig. 2B).

Our results were confirmed by an in vitro binding assay. Extracts from BHK cells overexpressing HA-

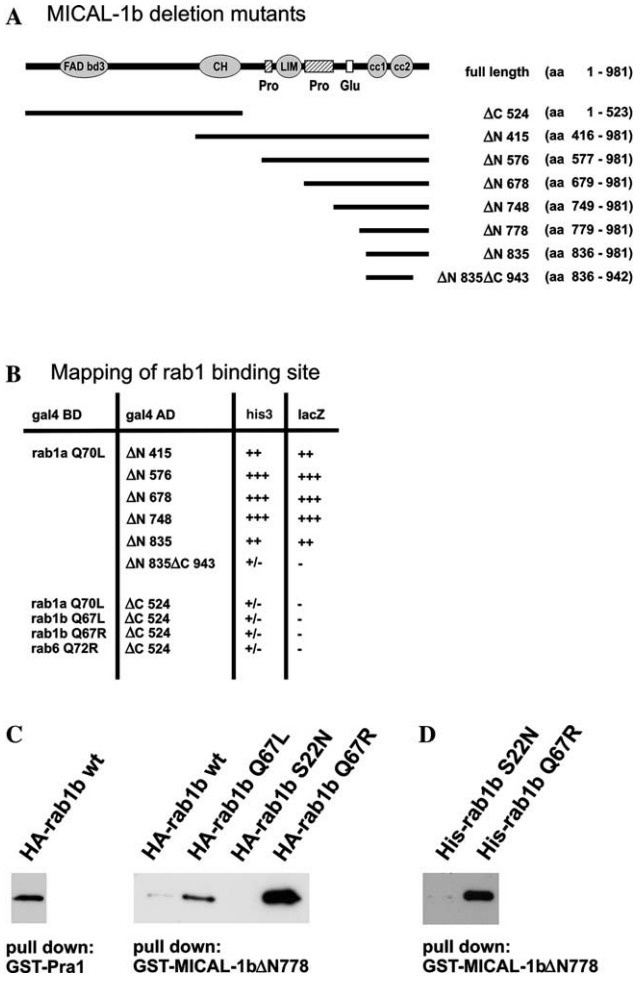

Fig. 2. A set of MICAL-1b truncation mutants (A) was used in two-hybrid assays to characterize the rab1 binding site of MICAL-1 (B). To confirm the yeast two-hybrid data, we performed in vitro binding assays using the GST-MICAL-1b ΔN778 fusion protein to recover overexpressed HA-tagged rab1b wt and HA-tagged active (Q67R/L) or inactive (S22N) rab1b mutants from BHK cell extracts. The GST–Pra1 fusion protein was used to recover rab1b wt as pulldown positive control (C). In order to demonstrate a direct interaction between MICAL-1 and rab1, we performed a pull down experiment with bacterially expressed GST-MICAL-1bΔN778 and His-tagged rab1b S22N and Q67R mutants, respectively (D). The antibody 16B12 was used to detect HA-tagged proteins. Bacterially expressed rab1b was detected by the monoclonal antibody 1E7. As second antibody HRP-anti-mouse antibody was used.

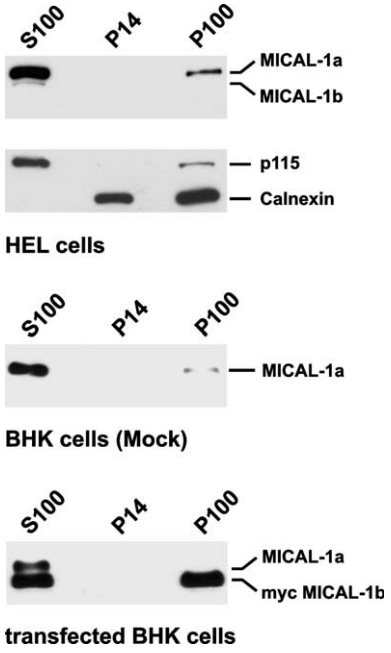

Fig. 3. Cell fractionation of HEL, transfected, and non-transfected BHK cells showed that MICAL-1a is predominantly detectable in the S100 supernatant. However, a small fraction of endogenous MICAL-1a and a significant fraction of overexpressed *myc*-MICAL-1b was found in the P100 pellet. MICAL-1 was detected with affinity purified #663 antibody and HRP-anti-rabbit IgG. As controls, the transmembrane protein calnexin and the membrane-associated p115 protein were detected with anti-p115 and anti-calnexin and HRP-anti-mouse antibody, respectively.

tagged rab1b wt, rab1b S22N, or rab1b Q67R/rab1b Q67L mutants as well as bacterially expressed His-tagged rab1b S22N and Q67R mutants were incubated with GST-MICAL-1b ΔN778 fusion protein.

Only the active rab1b mutants showed a strong interaction with GST-MICAL-1b ΔN778. Use of the rab1b wt and the inactive rab1b S22N mutant (Figs. 2C and D) led to a weak or to no signal at all. GST alone did not bind to rab1 (data not shown). The GST–Pra1 fusion protein was used as a positive control (Fig. 2C).

Next, we performed cell fractionation studies (Fig. 3). For the cell fractionation experiments, we prepared post nuclear supernatants of HEL and BHK cells and separated these extracts into cytosolic (S100) and membrane fractions (P14 and P100), respectively. Antibodies against the ER localized membrane protein calnexin and the membrane-associated p115 protein were used as controls. The affinity-purified #663 anti-serum was used to detect both MICAL-1 isoforms. It is obvious that endogenous MICAL-1a is preferentially localized in the cytosol of the cells, as the most significant signal is found in the S100 fraction. However, a small amount of the endogenous protein can be found in the P100 fraction, corresponding to the distribution of p115 (Fig. 3). These results suggest that in vivo a limited fraction of the MICAL-1 isoforms could be associated with membranes via activated rab1. This is supported by cell fractionation experiments with *myc*-tagged MICAL-1b overexpressing BHK cells, which show significant signals in the S100 and P100 fractions.

Discussion

Here, we report that MICAL-1 directly interacts with rab1 GTPases. Data from the two-hybrid and in vitro binding assays demonstrate that the rab1/MICAL-1 interaction is nucleotide-dependent and rab1-specific. Furthermore, the co-transformation assays using truncation mutants indicate that the rab1b interaction with MICAL-1 depends on the C- and N-terminal hypervariable regions of rab1. Additional "two-hybrid" experiments with rab1b/rab6A chimeras suggest that the presence of the C-terminus is necessary, but it does not seem to influence the specificity of the interaction as the chimera containing the N-terminus of rab1b and the C-terminus of rab6A also binds to MICAL-1.

It is worth to note that not only the rab1b binding domain of MICAL-1 but also the rab1b interacting domains of GM130 and Golgin-84 contain coiled-coil regions [12,14,15,19,32]. The p115 protein contains regions that are sufficient for a proper ER intermediate compartment localization which is independent of the GM130 interaction site of p115. However, the rab1b binding domain of p115 has not been characterized so far [6,25].

Interestingly, MICAL-1 is also able to bind to Ypt1p, the yeast counterpart of mammalian rab1 GTPases. This observation supports the authenticity of the rab1/MICAL-1 interaction and leads to the conclusion that it is conserved during evolution.

MICAL-1 isoforms display some interesting features that are different from that of the other rab1 interacting proteins as GM130, p115, and the recently found Golgin-84. All these proteins are membrane-associated effector proteins that play a role in tethering processes between donor and acceptor membranes at ER-Golgi compartments [6,12,25–27]. In contrast to this, MICAL-1 has been characterized as protein that plays a role in the intermediate filament-based cytoskeleton, signal transduction processes, as well as in axon guidance of neurons dependent on monooxygenase activity in *Drosophila*. So far it is known that it directly interacts with the SH3 domain of CasL, a member of $p130^{Cas}$ family, by a PPKPP (aa 830–835) motif and with vimentin and plexin A by its C-terminus [20,21]. The present study shows that the rab1 binding site is also localized at the C-terminus, suggesting that at least three proteins compete for the same binding region. This implies the interesting possibility that the MICAL-1/plexin A interaction in neuronal cells as well as the formation of MICAL-1/vimentin complexes is influenced by the GTP-bound conformation of rab1 isoforms. MICAL-1 isoforms are proteins with a predominantly cytosolic localization that is not limited to ER-Golgi compartments [21]. Nonetheless, a small amount of endogenous MICAL-1a is found in the P100 fraction suggesting that endogenous MICAL-1 could be weakly associated with membranes probably due to its binding to active, membrane-associated rab1. In accordance to this, we found a considerable portion of overexpressed MICAL-1b in the membrane fraction of cells. Thus, the rab1/MICAL-1 interaction might have a transient feature and could be restricted to a certain period of time. Therefore, we cannot exclude the possibility that MICAL-1 acts in concert with the p115 or GM130-Grasp65 complexes at the ER-Golgi membranes [6,12]. As the C-terminus of MICAL-1 (last 404 aa), that mediates the rab1 binding, displays neither a GAP nor GEF activity (data not shown), we propose that MICAL-1 represents a novel rab1 binding protein that prefers the GTP-bound confirmation.

Interestingly, there is increasing evidence that rab proteins play a role in linking membrane dynamics to the intracellular cytoskeleton [28,29]. Recent studies revealed that especially rab6 has an important function in microtubule-based transport by recruiting motor proteins as rab6–KIFL or complexes like the dynactin-complex to Golgi membranes [24,30,31]. For rab11 and rab27 GTPases interactions with unconventional myosins (class V) have been described, suggesting that also

actin-based motor proteins are regulated by rab GTPases in a GTP-dependent manner [28,29].

MICAL-1 isoforms are the first rab interacting molecules that also interact with vimentin, a component of the intermediate filament cytoskeleton [21]. Thus, MICAL-1 proteins could link rab1-dependent membrane traffic to the intermediate filament cytoskeleton. The domain architecture of the protein/protein interaction modules in MICAL-1, like a CH domain, an LIM domain, two proline-rich regions, and cc domains at the C-terminus are hints that MICAL-1 serves as an adaptor/docking protein for further interacting proteins. Thus, future work is necessary to elucidate the exact role of the rab1/MICAL-1 interaction in regulating membrane dynamics at the ER-Golgi compartments.

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