FEBS Letters 566 (2004) 294–300 FEBS 28320

Identification and characterization of a novel Rho GTPase activating protein implicated in receptor-mediated endocytosis

Tomohiro Sakakibara[a,b], Yasuo Nemoto[a,*], Toshihiro Nukiwa[b], Hiroshi Takeshima[a]

[a]*Department of Biochemistry, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8575, Japan*
[b]*Department of Respiratory Oncology and Molecular Medicine, Institute of Development, Aging and Cancer, Tohoku University, 2-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8575, Japan*

Received 14 January 2004; revised 22 March 2004; accepted 22 March 2004

Available online 20 April 2004

Edited by Lukas Huber

Abstract Cbl-interacting protein of 85 kDa (CIN85) is a recently identified adaptor protein involved in the endocytic process of several receptor tyrosine kinases. Here we have identified a novel RhoGAP, CIN85 associated multi-domain containing RhoGAP1 (CAMGAP1) as a binding protein for CIN85. CAMGAP1 is composed of an Src homology 3 (SH3) domain, multiple WW domains, a proline-rich region, a PH domain and a RhoGAP domain, and has the domain architecture similar to ARHGAP9 and ARHGAP12. CAMGAP1 mRNA is widely distributed in murine tissues. Biochemical assays showed its GAP activity toward Rac1 and Cdc42. Protein binding and expression studies indicated that the second SH3 domain of CIN85 binds to a proline-rich region of CAMGAP1. Overexpression of a truncated form of CAMGAP1 interferes with the internalization of transferrin receptors, suggesting that CAMGAP1 may play a role in clathrin-mediated endocytosis.
© 2004 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: CIN85; Clathrin-mediated endocytosis; Rho GTPase; Rho GAP; Actin cytoskeleton

1. Introduction

Cbl-interacting protein of 85 kDa (CIN85) is a recently discovered protein that interacts with Cbl proto-oncogene product and contains three Src homology 3 (SH3) domains, a proline-rich region and a coiled-coil region [1,2]. Recent works have shown that CIN85 is involved in the regulation of clathrin-mediated endocytosis of several plasma membrane receptors [2–4]. Upon ligand binding activated receptor tyrosine kinases such as epidermal growth factor and hepatocyte growth factor receptors associate with and phosphorylate Cbl family of ubiquitin ligases, which in turn ubiquitinates the activated receptors. The phosphorylation of Cbl confers the increased binding to the SH3 domains of CIN85. The proline-rich region of CIN85 is constitutively associated with endophilin, an accessory endocytic protein implicated in invagination of the plasma membrane during the early steps of clathrin-mediated endocytosis and the recruitment of other endocytic proteins including dynamin and synaptojanin. CIN85 thus functions as an adaptor protein that brings the endocytic machinery in the vicinity of membrane receptors in a stimulation-dependent manner.

Growing evidence indicates a functional link between clathrin-mediated endocytosis and actin cytoskeleton in mammalian cells [5,6]. Several components in the endocytic machinery interact with F-actin or with activators of Arp2/3-dependent actin polymerization [6,7]. Perturbations in actin assembly and disassembly by toxin treatment inhibit receptor-mediated endocytosis in some cell lines [8,9]. Although several roles for actin dynamics in receptor-mediated endocytosis are proposed, its precise function in the process remains unclear.

In an attempt to elucidate the mechanism of clathrin-mediated endocytosis of the plasma membrane proteins, we further looked for binding proteins for CIN85 by employing yeast two hybrid screen. We identified a novel member of RhoGAPs, a family of proteins accelerating the GTPase activity of Rho GTPases and thereby regulating their activities.

2. Materials and methods

2.1. Yeast two hybrid screen and isolation of CAMGAP1 cDNA

Molecular cloning of rat CIN85 cDNA as a binding protein for rat endophilin 2 will be described elsewhere. The DNA fragment encoding the amino-terminal SH3 domains of rat CIN85 (amino acids 1–381) was amplified by PCR from the cloned cDNA and subcloned into pBTM116 [10], and the plasmid was used as bait to screen a rat brain cDNA library constructed in pGAD10 vector (Clontech) as described [11]. Among 5×10^6 TRP^+LEU^+ transformants, 89 HIS^+LacZ^+ clones were selected which interacted specifically with the SH3 domains of rat CIN85. By restriction mapping and partial sequence analysis, 42 of them were found to be independent isolates of four overlapping cDNAs. To obtain the full-length cDNA, the insert DNA fragment was radiolabelled with [α-^{32}P]dCTP (Amersham Pharmacia Biotec) by oligolabelling method and used as probe for screening of a rat brain cDNA library constructed in λZAPII (Stratagene) following standard techniques [12].

2.2. Northern blot analysis

Murine Poly(A)$^+$ RNA Northern Blots (OriGene Technologies) were probed for three CAMGAPs under stringent condition [12]. A 612-bp DNA fragment spanning three WW domains of murine CAMGAP1 (amino acids 230–433), a 621-bp fragment corresponding to the N-terminal portion of murine homologue of CAMGAP2/ARHGAP12 (amino acids 3–209) and a 618-bp fragment corresponding to a region between the WW domain and the pleckstrin

* Corresponding author. Fax: +81-22-217-8090.
E-mail address: ynemoto@mail.cc.tohoku.ac.jp (Y. Nemoto).

Abbreviations: GAP, GTPase activating protein; CIN85, Cbl-interacting protein of 85 kDa; SH3, Src homology 3; PH, pleckstrin homology; CHO, Chinese hamster ovary; GST, glutathione *S*-transferase; MBP, maltose binding protein; PAGE, polyacrylamide gel electrophoresis; HA, hemagglutinin; kb, kilobase(s)

0014-5793/$22.00 © 2004 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2004.03.101

homology (PH) domain of murine CAMGAP3/ARHGAP9 (amino acids 253–458) were obtained by PCR using total RNA isolated from adult mouse brain. The DNA fragments were radiolabelled and used as probes. The hybridized blots were exposed to KODAK X-ray films with intensifying screens at −80 °C for 24 h.

2.3. Fusion protein production, protein binding assays and immunoprecipitation

The PCR-generated fragments encoding various portions of rat CAMGAP1 and the SH3 domains of rat CIN85 (SH3-A, amino acids 1–67; SH3-B, amino acids 96–171; SH3-C, amino acids 306–379) were subcloned into pGEX4T-1 (Amersham Pharmacia Biotec) or pMalC2 vector (New England Biolabs). The glutathione *S*-transferase (GST) fusion proteins were expressed in *E. coli* DH5α and purified by glutathione Sepharose 4B column chromatography following the manufacturer's instructions. The maltose binding protein (MBP) fusion proteins were expressed in *E. coli* DH5α and purified by amylose resin (New England Biolabs). The purity of protein preparations was checked on Coomassie blue-stained SDS–polyacrylamide gel electrophoresis (PAGE). Affinity purification with the immobilized recombinant proteins and immunoprecipitation from Triton extracts of rat brain and Chinese hamster ovary (CHO) and COS-7 cell lysates were performed as described [11]. For blot overlay, protein samples were separated by 10% SDS–PAGE and transferred onto nitrocellulose membranes. The membranes were probed with GST fusion proteins (2 μg/ml) for 2 h at room temperature and bound proteins were detected by rabbit anti-GST antibody as described [13].

2.4. In vitro GTPase activating protein (GAP) activity assay

The procedures essentially followed the methods described previously [14]. Briefly, the GST fusion proteins of rat CAMGAP1 RhoGAP domain (amino acids 668–869), human RhoA, Rac1 and Cdc42 were prepared as described above. The purified GST-RhoA, Rac1 and Cdc42 were incubated at 30 °C for 10 min in 100 μl of buffer containing 50 mM Tris–HCl, pH 7.6, 50 mM NaCl, 5 mM $MgCl_2$, 10 mM EDTA and 5 mM dithiothreitol and 10 μCi of [γ-^{32}P]GTP (Amersham Pharmacia Biotec). The preloaded GST-tagged GTPase (0.1 μg) was incubated with or without GST-GAP protein in GAP assay buffer containing 50 mM Tris–HCl, pH 7.6, 50 mM NaCl, 5 mM $MgCl_2$ and 5 mM dithiothreitol at 25 °C for the indicated time. The reactions were terminated by adding 1 ml of ice-cold GAP assay buffer and filtered through nitrocellulose membranes (Schleicher & Schuell). The radioactivity retained on the membranes was measured by liquid scintillation counting.

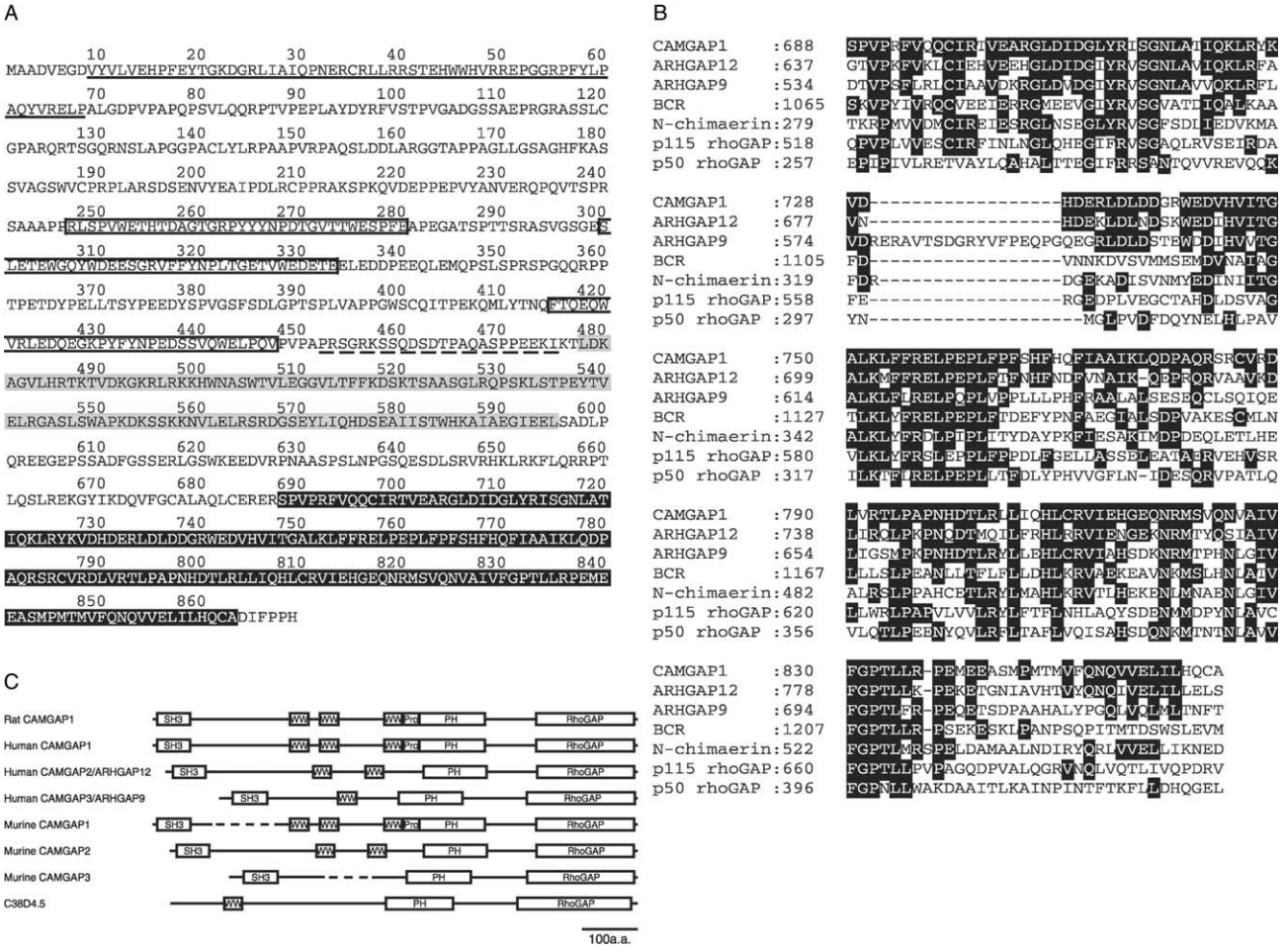

Fig. 1. Primary structure of rat CAMGAP1. (A) The deduced amino acid sequence of rat CAMGAP1. The domains in CAMGAP1 are shown as follows: *single underline*, SH3 domain; *open boxes*, WW domain; *dashed underline*, CIN85 binding proline-rich region; *shadowed box*, PH domain; *black box*, RhoGAP domain. The sequence data are available from Genbank/EMBL/DDBJ under Accession No. AY394725. (B) Alignment of RhoGAP domains from rat CAMGAP1, human ARHGAP12 (AAK52311), human ARHGAP9 (BAB56159), human breakpoint cluster region protein (BCR) (P11274), human N-chimaerin (P15882), human p115 rhoGAP (P98171) and human p50 rhoGAP (Q07960). Identical residues with CAMGAP1 are shown by *black boxes*. Gaps are indicated by *dashes*. Clustal W program was used to create the alignment. (C) Schematic diagrams of the domain structure of rat CAMGAP1 and the homologues. Human CAMGAP1 is compiled from ESTs BQ049142, AI972838, BI906615, BX400801, BX328361, BX279931 and BQ709443. Murine CAMGAP1 is compiled from ESTs BY717416, BI103060, AI786575, BE307041, BG963022 and BU898711, murine CAMGAP2 from BU51689, BU696071, BQ831585, CA751353, BB861743 and CD773071, and murine CAMGAP3 from BI249799, BB651844, BF137452 and BB34371. The amino-terminal region of murine CAMGAP3 was determined by PCR using the primers based on ESTs, AA542262 and BI249799. The unknown regions in murine CAMGAP1 and 3 are indicated by *dashes*. Pro; proline-rich region.

2.5. Construction of expression plasmids

For the cell expression of the various portions of CAMGAP1, the PCR-generated fragments encoding the domains were subcloned in pHA, a modified pcDNA3 vector for production of N-terminal hemagglutinin (HA)-tagged proteins [15]. The DNA fragment encoding full-length N-terminal FLAG-tagged rat CIN85 was subcloned into pTRE (Clontech) to form pTRE-FLAG CIN85, the plasmid producing N-terminal FLAG-tagged CIN85 protein under the control of a tetracycline-regulated promoter.

2.6. Cell culture and generation of tetracycline-inducible CHO cell lines

CHO and COS-7 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin G and 100 μg/ml streptomycin sulfate. CHO cells expressing FLAG-tagged rat CIN85 under the control of a tetracycline-responsive promotor were generated by using Tet-On gene expression system essentially as described by the vendor (Clontech). Briefly, CHO cells were transfected with pTet-On for expression of tetracycline-regulated activator rtTA. The rtTA function in the neomycin-resistant clones was tested by using the reporter plasmid pTRE-luc. The selected CHO clone was transfected with pTRE-FLAG CIN85 and pPUR. After selection in neomycin (200 μg/ml) (Nacalai tesque) and puromycin (5 μg/ml) (Sigma), the protein expression in the isolated clones was induced by addition of 2 μg/ml doxycycline (Clontech) and tested for CIN85 expression by immunoblotting.

2.7. Immunofluorescence microscopy

CHO or COS-7 cells were grown on coverslips and transfected with the expression plasmids using lipofectamine following the manufacturer's instructions (Invitrogen). Coverslips were fixed and processed for indirect immunofluorescence as described [16]. The coverslips were mounted in Vectashield (Vector Laboratories) and examined with an epifluorescent microscope Olympus BX60 in Fig. 5A or a confocal microscope Olympus IX70 in Fig. 6.

2.8. Antibody

A rabbit polyclonal antibody against rat CIN85 was generated against a C-terminal 18-amino acid synthetic peptide (RLRLQMEVNDIKKALQSK) coupled via cysteine at the amino-terminal residue to keyhole limpet hemocyanin following the standard procedure [17]. The antiserum was purified by the peptide covalently coupled to SulfoLink coupling gel (Pierce). Mouse monoclonal anti-FLAG antibody M5 was obtained from Sigma. Rabbit polyclonal anti-MBP antibody N-17 was from Santa Cruz Biotechnology. Mouse monoclonal anti-HA antibody 12CA5 and rabbit polyclonal anti-HA antibody Y-11 were from Babco and Santa Cruz Biotechnology, respectively.

2.9. Miscellaneous

Procedures for SDS–PAGE and immunoblotting were as described [15]. Protein concentrations were determined by the method of Bradford [18] using bovine serum albumin as standard. PCR was performed by using Vent polymerase (New England Biolabs). The primer sequences used in this study are available upon request. The integrity of the PCR-derived fragments was verified by nucleotide sequencing with an ABI Prism 310 DNA sequencer before introducing into the appropriate vectors. Transferrin uptake experiment followed the procedure described previously [19].

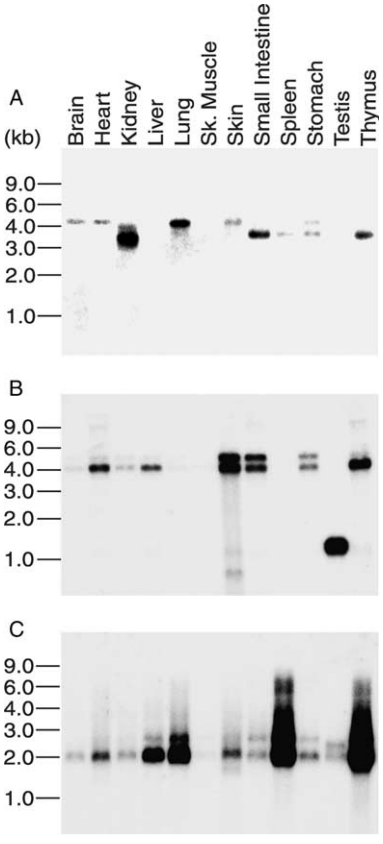

Fig. 2. Northern blot analysis of murine CAMGAPs. A murine multiple tissue Northern blot was hybridized with the specific probes for CAMGAP1 (A), CAMGAP2 (B) and CAMGAP3 (C). *Numbers* on the *left* indicate the positions of the molecular markers in kilobase (kb). The minor 6-kb band in spleen and thymus in (C) is observed only after a long exposure.

3. Results and discussion

By yeast two hybrid screen for a CIN85-binding protein using rat brain cDNA libraries, the cDNA potentially encoding a new RhoGAP domain-containing protein was iso-

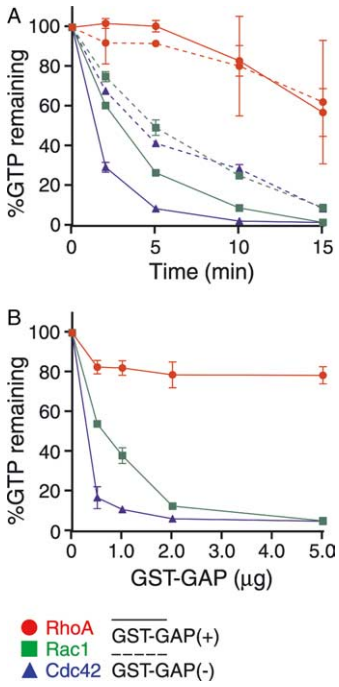

Fig. 3. CAMGAP1 has GTPase activating activity toward Rac1 and Cdc42 in vitro. (A) The GTPase activities of RhoA, Rac1 and Cdc42 were measured in the presence (*solid line*) or absence (*dashed line*) of the GST-RhoGAP domain of CAMGAP1 (0.25 μg). (B) GTPase activities were measured in the presence of various amounts of GST-RhoGAP domain for 5 min. In both panels, the values for RhoA, Rac1 and Cdc42 are indicated by *red*, *green* and *blue* lines, respectively. Results are expressed as means ± S.D. of three independent experiments.

lated. The putative open reading frame of the cDNA encodes a protein of 869 amino acids with a predicted M_r of 96.3 which we termed CIN85 associated multi-domain containing RhoGAP 1 (CAMGAP1) (Fig. 1A). Sequence analysis revealed that the carboxy-terminal region of this novel protein (amino acids 688–863) exhibits a high degree of similarity to several members of mammalian RhoGAP, especially to the GAP domains of two recently found proteins, ARHGAP9 and ARHGAP12 (50% and 62% amino acid identities, respectively) (Fig. 1B) [20,21]. Besides the RhoGAP domain, CAMGAP1 contains several functional domains; from its amino- to carboxy-terminus, an SH3 domain, three WW domains, a proline-rich region and a PH domain (Fig. 1A). Notably, CAMGAP1, ARHGAP9 and ARHGAP12 have the very similar domain structure with the difference being the number of the WW domains (Fig. 1C). BLAST searches indicate the presence of three human and murine CAMGAP homologues. Thus, these three proteins define a novel subfamily of mammalian RhoGAPs. We propose to call ARHGAP12 and ARHGAP9 as human CAMGAP2 and 3, respectively.

The tissue distributions of these three CAMGAPs were determined by Northern blot analysis. The CAMGAP1 probe detected major two transcripts at 4.5 and 3.7 kb in a wide range of murine tissues. The signals were especially abundant in kidney, lung, small intestine and thymus (Fig. 2A). The

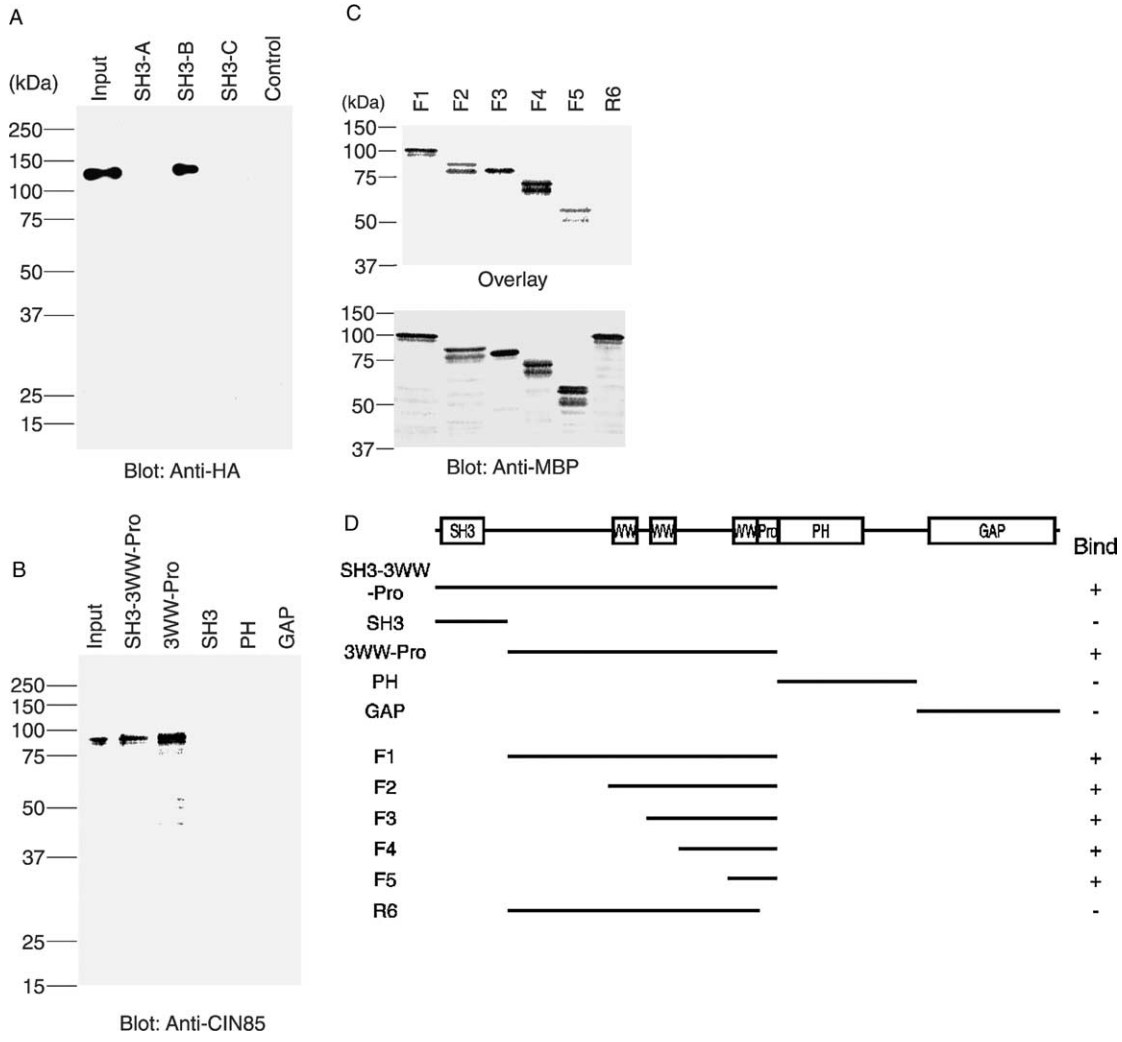

Fig. 4. In vitro binding between the proline-rich region of CAMGAP1 and the SH3-B domain of CIN85. (A) Affinity purification of CAMGAP1. Lysates from COS-7 cells transiently expressing HA-tagged CAMGAP1 were incubated with the immobilized GST-SH3-A (amino acids 1–67), -B (amino acids 96–171) and -C (amino acids 306–379) domains of CIN85 or GST alone (*control*). HA-tagged CAMGAP1 in the lysates (*input*) and the eluates were detected by SDS–PAGE and immunoblotting with anti-HA antibody. (B) Affinity purification of CIN85. The rat brain extract was incubated with the immobilized GST fusion of various CAMGAP1 fragments; SH3-3WW-Pro (amino acids 1–475), 3WW-Pro (amino acids 101–475), SH3 (amino acids 1–78), PH (amino acids 459–698) and GAP (amino acids 668–869). CIN85 in the rat brain extract (*input*) and the eluates were detected by SDS–PAGE and immunoblotting with anti-CIN85 antibody. (C) Mapping of CIN85 binding region in CAMGAP1. *Top panel:* The MBP fusion proteins of the amino acids 101–475 (F1), amino acids 241–475 (F2), amino acids 294–475 (F3), amino acids 340–475 (F4), amino acids 408–475 (F5) and amino acids 101–451 (R6) of CAMGAP1 were subjected to SDS–PAGE and transferred to the nitrocellulose membrane. The membrane was overlaid with GST-CIN85 SH3-B fusion protein and detected by anti-GST antibody. *Bottom panel:* The comparable amount of MBP-fusion protein was loaded in each sample as detected by immunoblotting using anti-MBP antibody. *Numbers* on the *left* indicate the positions of the molecular weight markers in kDa. (D) The summary of binding between the CAMGAP1 regions and CIN85. The binding ability to the SH3-B domain of CIN85 is indicated on the *right*.

CAMGAP2 probe detected three transcripts; the larger 5.0- and 4.0-kb transcripts in heart, liver, skin, small intestine, stomach and thymus, and smallest 1.3-kb transcript in testis (Fig. 2B). The CAMGAP3 probe detected three transcripts at 2.8, 2.6 and 2.1 kb. The 2.8- and 2.1-kb transcripts were predominantly expressed in thymus and spleen (Fig. 2C). The results suggest that three CAMGAPs are expressed as multiple alternatively spliced forms with different tissue distribution and have distinct cellular functions in the expressed tissues despite the structural similarity.

Each RhoGAP has GTPase activating activity toward the specific member of Rho GTPase family [22]. To determine the biochemical property of the C-terminal RhoGAP domain of CAMGAP1, we measured the effect of the GST fusion protein of the GAP domain of rat CAMGAP1 expressed in *E. coli* on the in vitro GTPase activity of RhoA, Rac1 and Cdc42. When the GTP-bound Rho GTPases were incubated with the recombinant GAP domain of CAMGAP1, the intrinsic GTPase activity of Rac1 and Cdc42, but not of RhoA, was significantly increased (Fig. 3A and B). The result is in line with the previous report for ARHGAP9/CAMGAP3 [20] and indicates that rat CAMGAP1 exhibits GAP activity toward Rac1 and Cdc42 among Rho family GTPases.

We performed biochemical experiments to confirm the binding between CAMGAP1 and CIN85. HA-tagged CAMGAP1 was transiently expressed in COS-7 cells, and its binding ability to GST-CIN85 SH3-A, -B and -C fusions was determined by pull-down experiments. As shown in Fig. 4A, CAMGAP1 binds to the SH3-B domain of CIN85, but not to the SH3-A and -C domains (Fig. 4A).

We generated the GST fusion proteins covering various portions of CAMGAP1 and tested their binding to CIN85 in rat brain. Western blotting with anti-CIN85 antibody revealed that CIN85 binds to the region of CAMGAP1 containing three WW domains and the proline-rich region (amino acids 101–475) (Fig. 4B). To ascertain the CIN85 binding region of CAMGAP1, we made successive deletions of the CAMGAP1 region (amino acids 101–475) and tested the binding to the GST-CIN85 SH3-B protein. The result indicates that a 24-amino acid proline-rich sequence of CAMGAP1 (amino acids 452–475) is necessary for binding to the SH3-B domain of CIN85 (Fig. 4C). Taken together, the biochemical binding between CIN85 and CAMGAP1 is mainly mediated by the SH3-B domain of CIN85 and the proline-rich region of CAMGAP1.

Based on the biochemical experiments, we investigated the intracellular binding between CAMGAP1 and CIN85 and localization of CAMGAP1. Overexpression of CIN85 or the closely related protein CMS/CD2AP has been reported to lead to the formation of multiple punctate structures in COS-7

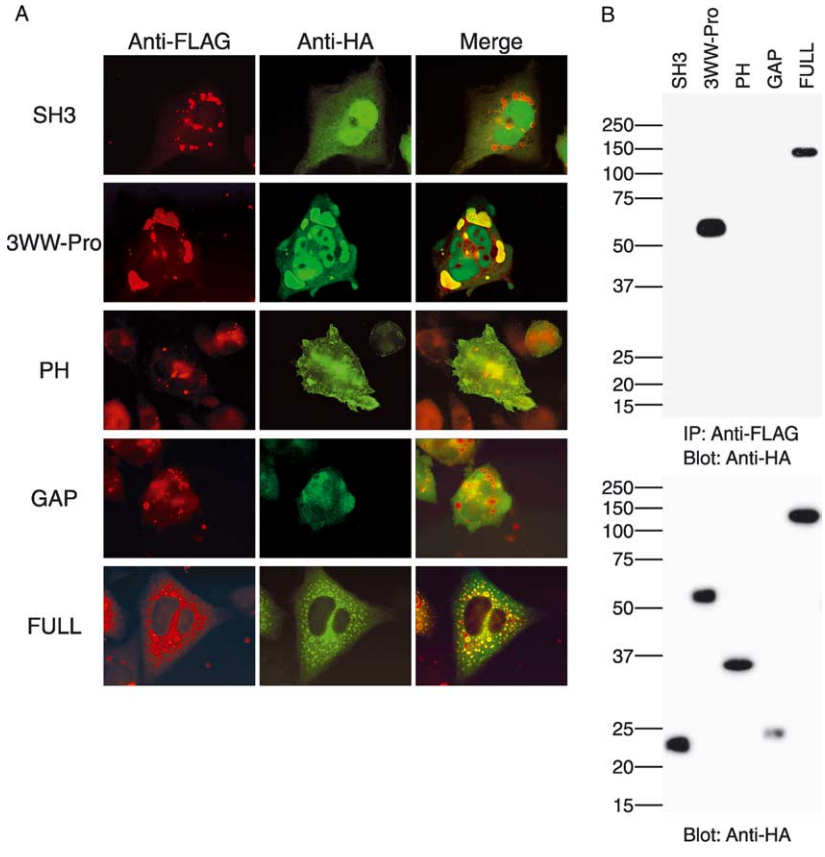

Fig. 5. Colocalization and binding between CAMGAP1 and CIN85 in CHO cells. (A) Double immunofluorescence of CHO cells expressing FLAG-tagged CIN85 and HA-tagged CAMGAP1 constructs. The expression of CIN85 was detected by anti-FLAG antibody (*red*, *left column*). The expression of CAMGAP1 fragment was detected by anti-HA antibody (*green*, *middle column*). The *yellow–orange* color represents colocalization of proteins in the merged images (*right column*). (B) Co-immunoprecipitation of CAMGAP1 and CIN85. *Top panel:* The lysates from CHO cells expressing FLAG-tagged CIN85 and HA-tagged CAMGAP1 constructs were immunoprecipitated with anti-FLAG antibody and protein G Sepharose followed by immunoblotting with anti-HA antibody. *Bottom panel:* The amount of HA-tagged proteins in each cell lysate was detected by immunoblotting using anti-HA antibody. *Numbers* on the *left* indicate the positions of the molecular weight markers in kDa.

cells, which contain several markers of the endosomal compartment [2,23–25]. Similar multiple punctate structures were observed in CHO cells upon overexpression of CIN85 (Fig. 5A, left column). When CAMGAP1 was expressed in CHO cells together with CIN85, the region of CAMGAP1 containing the proline-rich sequence (amino acids 452–475) colocalized at the multiple punctate structures (Fig. 5A). Moreover, immunoprecipitation of epitope-tagged CIN85 showed that the region of CAMGAP1 containing the proline-rich region is associated with CIN85 (Fig. 5B). The results are consistent with those of pull-down and overlay assays (Fig. 4) and indicate the intracellular binding between CAMGAP1 and CIN85.

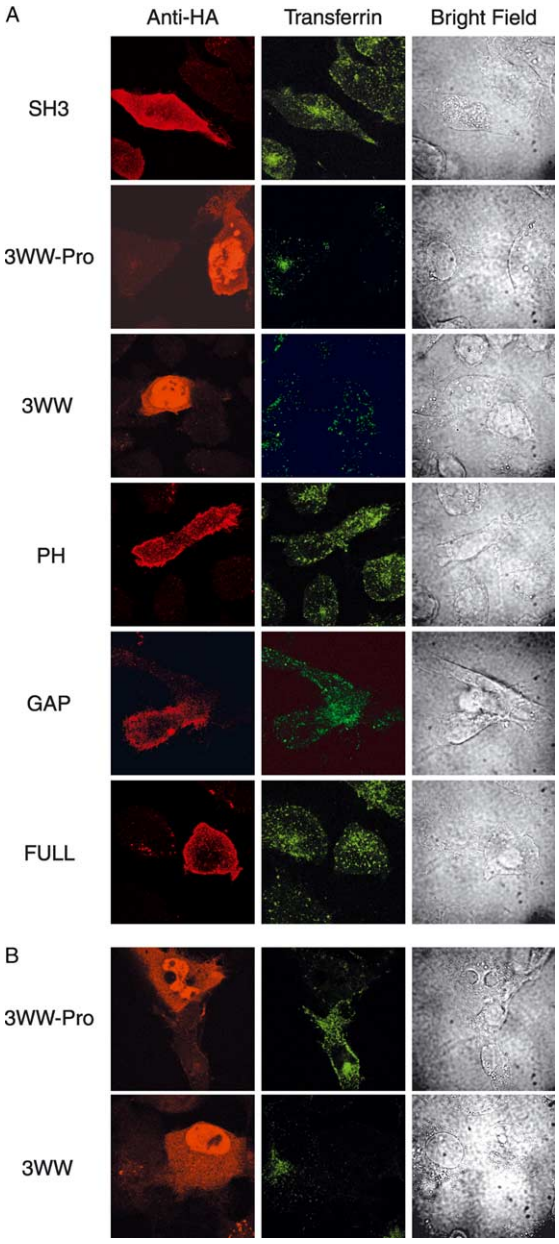

Fig. 6. Transferrin uptake inhibition in CHO and COS-7 cells transfected with truncated CAMGAP1. CHO (A) or COS-7 (B) cells were transiently transfected with HA-tagged CAMGAP1 constructs and incubated with Oregon green-conjugated transferrin. The expression of CAMGAP1 fragment was detected by anti-HA antibody (*red*, *left column*). The intracellular small spots indicate the uptake of transferrin (*green*, *middle column*). The bright field images are shown in *right column*. Shown are the images observed in more than 50% of the 50 transfected cells. The construct of three WW domains contains the region covering amino acids 101–451, the same region expressed in the R6 construct in Fig. 4C.

Recent works have shown that CIN85 is a scaffolding protein involved in regulation of clathrin-mediated endocytosis of several plasma membrane receptors [2–4], raising the possibility that CAMGAP1 may be implicated in the endocytic process as well. To address the possible role of CAMGAP1, we tested the effect of expression of various portions of CAMGAP1 on the receptor-mediated endocytosis of transferrin. Transferrin uptake was inhibited by expression of the three WW domains of CAMGAP1 and not by expression of the other portions of CAMGAP1 or of the full-length CAMGAP1 (Fig. 6A). The same effect was also observed in COS-7 cells (Fig. 6B). The result suggests the involvement of CAMGAP1 in transferrin receptor endocytosis.

In conclusion, we here describe the biochemical properties of a novel Rho GAP, CAMGAP1. One of the most prominent functions of Rho GTPases is the regulation of actin cytoskeletal rearrangement [26–28]. Actin dynamics plays a role in clathrin-mediated endocytosis in mammalian cells and several reports suggest that Rho GTPases are involved in the endocytic process [6,29]. The previous report has shown that endophilin binds to the proline-rich domain of CIN85 [4] and our results indicate that CAMGAP1 associates with the SH3-B domain of CIN85. It is possible that CAMGAP1 and endophilin might simultaneously interact with CIN85 and co-operate functionally in the same cellular process. Our results also suggest that CAMGAP1 might be implicated in transferrin-receptor-mediated endocytosis by binding to unknown proteins through its three WW domains, as overexpression of these domains of CAMGAP1 produced an inhibitory effect on transferrin uptake.Taken together with the result of the in vitro studies that CAMGAP1 has GAP activity toward Rac1/Cac42, we speculate that CAMGAP1 may play a role in the regulation of actin cytoskeletal organization during clathrin-mediated endocytosis. It is worth noting that CMS/CD2AP, a protein closely related to CIN85, interacts with Arp2/3 complex via the binding to cortactin, suggesting a functional link between the CIN85 family of adaptor proteins and actin organization [30]. Further investigation is needed to obtain more direct evidence to understand the physiological function of CAMGAP.

Acknowledgements: We thank Dr. S. Narumiya (Kyoto University, Japan) for advice on the measurement of GAP activity. This research is supported in part by Grant-in-Aid for Scientific Research (B) from Japan Society for the Promotion of Science and Grant-in-Aid for Scientific Research on Priority Areas (B) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, to YN.

References

[1] Take, H., Watanabe, S., Takeda, K., Yu, Z.X., Iwata, N. and Kajigaya, S. (2000) Biochem. Biophys. Res. Commun. 268, 321–328.
[2] Dikic, I. (2002) FEBS Lett. 529, 110–115.
[3] Soubeyran, P., Kowanetz, K., Szymkiewicz, I., Langdon, W.Y. and Dikic, I. (2002) Nature 416, 183–187.
[4] Petrelli, A., Gilestro, G.F., Lanzardo, S., Comoglio, P.M., Migone, N. and Giordano, S. (2002) Nature 416, 187–190.

[5] Qualmann, B., Kessels, M.M. and Kelly, R.B. (2000) J. Cell Biol. 150, F111–F115.
[6] Jeng, R.L. and Welch, M.D. (2001) Curr. Biol. 11, R691–R694.
[7] Schafer, D.A. (2002) Curr. Opin. Cell Biol. 14, 76–81.
[8] Lamaze, C., Fujimoto, L.M., Yin, H.L. and Schmid, S.L. (1997) J. Biol. Chem. 272, 20332–20335.
[9] Fujimoto, L.M., Roth, R., Heuser, J.E. and Schmid, S.L. (2000) Traffic 1, 161–171.
[10] Vojtek, A.B., Hollenberg, S.M. and Cooper, J.A. (1993) Cell 74, 205–214.
[11] Ringstad, N., Nemoto, Y. and De Camilli, P. (1997) Proc. Natl. Acad. Sci. USA 94, 8569–8574.
[12] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[13] McPherson, P.S., Czernik, A.J., Chilcote, T.J., Onofri, F., Benfenati, F., Greengard, P., Schlessinger, J. and De Camilli, P. (1994) Proc. Natl. Acad. Sci. USA 91, 6486–6490.
[14] Self, A.J. and Hall, A. (1995) Methods Enzymol. 256, 67–76.
[15] Nemoto, Y. and De Camilli, P. (1999) EMBO J. 18, 2991–3006.
[16] Cameron, P.L., Sudhof, T.C., Jahn, R. and De Camilli, P. (1991) J. Cell Biol. 115, 151–164.
[17] Harlow, E. and Lane, D. (1988) Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[18] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[19] Slepnev, V.I., Ochoa, G.C., Butler, M.H., Grabs, D. and De Camilli, P. (1998) Science 281, 821–824.
[20] Furukawa, Y., Kawasoe, T., Daigo, Y., Nishiwaki, T., Ishiguro, H., Takahashi, M., Kitayama, J. and Nakamura, Y. (2001) Biochem. Biophys. Res. Commun. 284, 643–649.
[21] Zhang, Z., Wu, C.Q., Wang, S., Huang, W., Zhou, Z.X., Ying, K., Xie, Y. and Mao, Y.M. (2002) Int. J. Biochem. Cell Biol. 34, 325–331.
[22] Moon, S.Y. and Zheng, Y. (2003) Trends Cell Biol. 13, 13–22.
[23] Dustin, M.L., Olszowy, M.W., Holdorf, A.D., Li, J., Bromley, S., Desai, N., Widder, P., Rosenberger, F., van der Merwe, P.A., Allen, P.M. and Shaw, A.S. (1998) Cell 94, 667–677.
[24] Watanabe, S., Take, H., Takeda, K., Yu, Z.X., Iwata, N. and Kajigaya, S. (2000) Biochem. Biophys. Res. Commun. 278, 167–174.
[25] Kirsch, K.H., Georgescu, M.M., Ishimaru, S. and Hanafusa, H. (1999) Proc. Natl. Acad. Sci. USA 96, 6211–6216.
[26] Ridley, A.J. and Hall, A. (1992) Cell 70, 389–399.
[27] Ridley, A.J., Paterson, H.F., Johnston, C.L., Diekmann, D. and Hall, A. (1992) Cell 70, 401–410.
[28] Nobes, C.D. and Hall, A. (1995) Cell 81, 53–62.
[29] Qualmann, B. and Mellor, H. (2003) Biochem. J. 371, 233–241.
[30] Lynch, D.K., Winata, S.C., Lyons, R.J., Hughes, W.E., Lehrbach, G.M., Wasinger, V., Corthals, G., Cordwell, S. and Daly, R.J. (2003) J. Biol. Chem. 278, 21805–21813.