Abstract Ral-binding protein 1 (RalBP1) is a putative effector protein of Ral and possesses the GTPase-activating activity for Rac1 and CDC42. We examined the roles of the post-translational modifications of Ral and Rac1 for the action of RalBP1. In COS cells, RalG23V, a constitutively active form, was mainly detected in the membrane fraction while most of RalG23V/C203S, a Ral mutant which is not post-translationally modified, was found in the cytosol fraction. When RalBP1 was expressed alone in COS cells, it was found in the cytosol but not in the membrane fraction. When RalBP1 was coexpressed with RalG23V, a part of RalBP1 was found in the membrane fraction. However, when RalBP1 was coexpressed with RalG23V/C203S, all of RalBP1 was recovered in the cytosol fraction. Although Ral bound to RalBP1 at a molar ratio of 1:1, the interaction of Ral with RalBP1 did not affect the GTPase-activating activity of RalBP1 for Rac1. Furthermore, RalBP1 was more active on the post-translationally modified form of Rac1 and CDC42 than the unmodified form. These results suggest that the post-translational modification of Ral is important for the subcellular localization of RalBP1 and that the interaction of Ral with RalBP1 is not essential for the activity of RalBP1 but plays a role in recruiting RalBP1 to the membrane where its substrates, Rac1 and CDC42, reside.

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Key words: Ral; RalBP1; GAP; Subcellular localization; Rac1

1. Introduction

RAL is a member of small G-protein superfamily and consists of RalA and RalB [1]. As well as other small G proteins, Ral has the GDP-bound inactive and the GTP-bound active forms. The GDP-bound form of Ral is converted to the GTP-bound form by RalGDS [2], and inversely the GTP-bound form is changed to the GDP-bound form by RalGAP [3]. We and other groups have found that RalGDS is a putative effector protein of Ras [4—6]. Since RalGDS stimulates the GDP/GTP exchange of Ral [2], it is possible that there is a signaling pathway from Ras to Ral through RalGDS. Indeed, it has been shown that RalGDS stimulates the GDP/GTP exchange of Ral in a Ras-dependent manner in COS cells and that a dominant negative form of Ral blocks a Ras-dependent transformation in NIH3T3 cells [7]. Furthermore, it has been demonstrated that RalGDS constitutes a Ras-signaling pathway distinct from Ras and that RalGDS and Raf synergistically regulate cell proliferation and gene expression [8,9]. Thus, evidence has been accumulated that Ral and RalGDS act downstream of Ras and that Ral is an important small G protein in the intracellular signal transduction system. However, the functions of Ral are not well known.

One possible clue to clarify the functions of Ral is RalBP1 which has been identified as a putative effector protein of Ral [10—12]. RalBP1 contains a Ral-binding domain in its C-terminal region and a RhoGAP homology domain in its central region. RalBP1 exhibits the GAP activity for Rac1 and CDC42 but not for RhoA. Therefore, RalBP1 may link between Ras and Rac1 or CDC42. However, the modes of activation and action of RalBP1 are not clear since the studies using full-length RalBP1 have not yet been done.

The post-translational modifications of small G proteins are critical for their activation and action [13,14]. In a Ras/RalGDS/Ral signaling pathway, we have shown that the post-translational modification of Ras is required for determining the subcellular localization of RalGDS and that the modification of Ral enhances the GDP/GTP exchange activity of RalGDS [15]. These results indicate that the post-translational modifications of Ras and Ral are important for the action of RalGDS. Therefore, we examined here the roles of the modifications of Ral and Rac1 in the action of RalBP1.

2. Materials and methods

2.1. Materials and chemicals

The RalB, and Rac1 and CDC42 cDNAs were provided by Drs. R. Weinberg (Whitehead Institute for Biomedical Research, Cambridge, MA) and K. Kaibuchi (Nara Institute of Science and Technology, Ikoma, Japan), respectively. Baculoviruses expressing GST-RalB, GST-Rac1, and GST-CDC42 were supplied from Dr. Y. Matsaura (National Institute of Health, Tokyo, Japan). The cDNA of RalBP1 was isolated by reverse transcriptase PCR as described [15]. All procedures of passage, infection, and transduction of Sf9 cells and the isolation of baculoviruses were carried out as described [16]. MBP-RalBP1, MBP-RalBP1(364—647), GST-RalB, GST-Rac1, and GST-CDC42 were produced in and purified from Sf9 cells [15]. Small G proteins purified from E. coli were used as the post-translationally unmodified form. The post-translationally modified form of GST-RalB was purified from the membrane fraction of Sf9 cell [15]. The post-translationally modified form of GST-Rac1 and GST-CDC42 were purified from Sf9 cells by the use of Triton X-114 as described [19,20]. Other materials and chemicals were from commercial sources.

The post-translational modifications of Ral and Rac1 are important for the action of Ral-binding protein 1, a putative effector protein of Ral

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**Abbreviations: G protein, GDP-binding protein; RalGDS, Ral GDP dissociation stimulator; GAP, GTPase-activating protein; RalBP1, Ral-binding protein 1; PCR, polymerase chain reaction; MBP, maltose-binding protein; GST, glutathione-S-transferase; E. coli, Escherichia coli; HA, hemagglutinin; DTT, dithiothreitol; GTPγS, guanosine 5’-(3-O-thio)triphosphate; BSA, bovine serum albumin; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid


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2.2. Plasmid constructions

Rac1, and pGEKX2T/CDC42 were constructed as described [15,17,18]. To construct pCGN/RalB 
GV (in which Gly-23 was 
changed to Val), pCGN/RalB 
S28N, and pCGN/RalB 
C203S, the 0.6-kb fragments 
encoding RalB 
GV, RalB 
S28N, and RalB 
C203S with XhoI and BamHI sites were synthesized by PCR, and inserted 
into the XhoI and BamHI cut pCGN. To construct pBJ-Myc/RalBPl, 
pUC19/RalBPl [15] was digested with BamHI and the 1.9-kb fragment 
encoding full-length RalBPl was inserted into the BamHI cut pBJ-Myc [18]. To construct pBJ-Myc/RalBPl-(1-415), pUC19/RalBPl was 
digested with BamHI and BglII and the 1.2-kb fragment encoding 
RalBPl was inserted into the BamHI cut pBJ-Myc. To con-
digested with BamHI and the 0.8-kb fragment encoding RalBPl-(364-
647) was inserted into the BamHI cut pBJ-Myc. To construct pMAL/ 
PralBPl, pUC19/RalBPl was digested with BamHI and the 1.9-kb fragment 
encoding full-length RalBPl was inserted into the BamHI cut pMAL-c2.

2.3. Interaction of Ral with RalBPl in COS cells

After COS cells (60-70% confluent on a 10-cm-diameter plate) were 
transfected with pCGN- 
and pB-derivated constructs described above 
by the DEAE-dextran method [21], the cells were lysed as described 
[22]. The lysates expressing Ral with RalBPl were prepared and the proteins of the lysates (0.6 mg) were immunoprecipitated 
with the anti-Myc antibody and probed with the anti-Myc and HA antibodies 
[22,23]. To determine the subcellular localization of Ral and RalBPl, 
COS cells were fractionated into the cytosol and membrane fractions 
[15]. Aliquots (20 μg of protein) of the cytosol and membrane fractions 
were probed with the anti-Myc and HA antibodies.

2.4. Assay for the Ral-binding activity of RalBPl

To make RalBPl or RalBPl-(364-647) immobilized on amylose 
resin, E. coli lysates (100 μg of protein) expressing MBP-RalBPl or 
MBP-RalBPl-(364-647) in lysis buffer (20 mM Tris-HCl (pH 7.5), 200 
mM NaCl, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 20 μg/ml 
aprotinin, and 10 μg/ml leupeptin) were incubated with 200 μl of 
amylose resin for 2 h at 4°C. The resin was precipitated by centrifugation 
and washed with 3 times with 10 mM Tris-HCl (pH 7.5). One 
microtitre of the resin bound 0.6 pmol of MBP-RalBPl and 4.8 
pmol of MBP-RalBPl-(364-647). The [35S]GTPfS- 
or [32P]GDP-bound form of GST-RalB was made as described [15] and incubated 
for 30 min at 4°C with immobilized RalBPl or RalBPl-(364-647) (4 
pmol of each) in 120 μl of reaction mixture (50 mM Tris-HCl (pH 7.5), 
20 mM MgCl2, 10 mM EDTA, 1 mM DTT, and 1 mg/ml BSA). 
After the immobilized RalBPl and RalBPl-(364-647) were precipi-
tated by centrifugation and washed, the remaining radioactivities were 
counted. During the procedures of this assay, neither [35S]GTPfS 
nor [32P]GDP was dissociated from GST-RalB.

2.5. Assay for the GAP activity of RalBPl

After the post-translationally modified or unmodified form of GST-
RalC1 and GST-CDC42 (4 pmol of each) were pre-incubated for 4 min 
and 30°C in 20 μl of the pre-incubation mixture (20 mM Tris-HCl (pH 
7.5), 2 μM [γ-32P]GTP (4000-6000 cpm/pmol), 2 mM EDTA, 0.3% 
CHAPS, 1 mM o-dimyristoylphosphatidylcholine, 1 mM DTT, and 
40 μg/ml BSA), 4 μl of mixture (100 mM Tris-HCl (pH 7.5), 100 mM 
MgCl2, and 10 mM DTT) was added. To this pre-incubation mixture, 
16 μl of reaction mixture (17.5 mM Tris-HCl (pH 7.5), 2.5 mM GTP, 
and 1 mM DTT) containing MBP-RalBPl was added, and the second 
icubation was performed with GST-RalC1 for 5 min at 25°C or with 
GST-CDC42 for 10 min at 25°C. Assays were quantified by rapid 
filtration on nitrocellulose filters. When the effect of the interaction 
of Ral with RalBPl on the GAP activity of RalBPl was examined, 
RalBPl immobilized on amylose resin was incubated with 1 μM 
GTPfS-bound post-translationally modified or unmodified form of Ral 
to make immobilized RalBPl interacting with Ral. The amylose 
resin was washed as described above and an aliquot was applied to 
SDS-polyacrylamide gel electrophoresis to estimate the amount of the 
immobilized proteins. The suspension of the amylose resin containing 
immobilized RalBPl or RalBPl interacting with Ral was used instead 
of RalBPl solution in the GAP assay for the post-translationally 
modified form of Rac1.

2.6. Other methods

Protein concentrations were determined by the method of Lowry 
using BSA as a standard [24]. Triton X-114 phase separation assay of 
small G proteins was carried out as described [15,19].

3. Results

3.1. Interaction of Ral with RalBPl in intact cells

RalBPl has been identified as a putative effector protein of Ral, 
and in vitro and yeast two-hybrid experiments have shown 
that the C-terminal region of RalBPl interacts with Ral [10-12]. 
However, the studies in mammalian cells have not yet been done. 
Therefore, we first examined whether full-length RalBPl interacts 
with Ral in mammalian cells. RalBPl was coexpressed with Ral 
GV, a constitutively active form, or RalS28N, a dominant negative 
form, in COS cells (Fig. 1A, lanes 1-4). RalBPl was tagged with the Myc epitope 
at the N-terminus. Ral GV and RalS28N were tagged with the 
HA epitope at the N-terminus. When the lysates coexpressing 
Ral GV and RalBPl were immunoprecipitated with the anti-Myc 
and HA antibodies, both Ral GV and RalBPl were detected in 
the RalBPl immune complex (Fig. 1A, lane 6). When the lysates 
expressing Ral GV alone were immunoprecipitated with the 
anti-Myc antibody, Ral GV was not detected (Fig. 1A, lane 
5). Neither Ral GV nor RalBPl was immunoprecipitated with 
non-immune immunoglobulin from the lysates expressing both 
proteins (data not shown). When RalBPl was coexpressed with 
RalS28N (Fig. 1A, lane 3), RalBPl did not make a complex with RalS28N 
(Fig. 1A, lane 7). These results indicate that RalBPl interacts with 
the GTP-bound form of Ral in intact mammalian cells. To exclude the possibility 
that the interaction of Ral with RalBPl in COS cells is non-specific 
due to overexpression of these proteins, we expressed Ral GV 
with RalBPl-(1-415) or RalBPl-(364-647). These RalBPl de-
etion mutants were tagged with the Myc epitope at the N-
terminus. The expression level of RalBPl-(1-415) was higher 
than that of RalBPl-(364-647) (Fig. 1B, lanes 1 and 2). The 
expression levels of Ral GV 
were similar in these cells. 
Ral GV interacted with RalBPl-(364-647) but not with 
RalBPl-(1-415) (Fig. 1B, lanes 3 and 4). These results indicate that 
the C-terminal region of RalBPl is necessary and sufficient 
for its interaction with Ral in intact cells and that the 
interaction of Ral with RalBPl observed in Fig. 1A is not 
non-specific.

3.2. Translocation of RalBPl by Ral in COS cells

It has been reported that Ral is associated with the membrane 
fractones such as plasma membranes, clathrin-coated vesicles, 
and secretory vesicles [1]. By analogy with other small 
G proteins, the post-translational modification of Ral at its C-
termini could be important for its binding to the 
membrane [25]. Therefore, we examined the role of Ral in 
subcellular localization of RalBPl in intact cells. When Ral GV 
was expressed in COS cells, Ral GV 
appeared in both the cytosol and membrane fractions (Fig. 2A, lanes 3 
and 4). On the other hand, most of Ral GV 
was a dominant negative form, and was not post-translationally 
motivated in Ral GV 
the cytosol fraction (Fig. 2A, lanes 1 and 2). When RalBPl was 
expressed alone in COS cells, it was detected in the cyto-
sol fraction (Fig. 2A, lanes 1 and 2). When RalBPl was coex-
pressed with Ral GV, a part of RalBPl was detected in the 
membrane fraction (Fig. 2A, lanes 3 and 4). Although the
amount of RalBPl in the membrane fraction appeared to be small, this result was reproducible. However, when RalBPl was coexpressed with RalG23V/C203S, RalBPl was found in the cytosol fraction but not in the membrane fraction (Fig. 2A, lanes 5 and 6). These observations were confirmed using RalBPl-(364-647) (Fig. 2B). When RalBPl-(364-647) was expressed alone in COS cells, it was found in the cytosol fraction. When RalBPl-(364-647) was coexpressed with RalG23V, RalBPl-(364-647) was found in both the cytosol and membrane fractions, while when RalBPl-(364-647) was coexpressed with RalC23V/C203S, RalBPl-(364-647) was found in the cytosol fraction. These results suggest that Ral localized on the membrane recruits RalBPl from the cytosol to the membrane.

3.3. Effect of the interaction of Ral with RalBPl on the GAP activity of RalBPl

It has been demonstrated that the Ral-binding domain of RalBPl is not necessary for the GAP activity [10]. Therefore, we examined whether the interaction of Ral with RalBPl affected the GAP activity of RalBPl. Since the Ral-binding activity of RalBPl using full-length RalBPl had not yet been biochemically characterized, we measured its $K_d$ and $B_{max}$. RalBPl interacted with the GTPyS-, but not with the GDP-, bound form of Ral in a dose-dependent manner (Fig. 3A). Scatchard plot analysis revealed that RalBPl bound to Ral at a molar ratio of 1:0.9 with a $K_d$ value of 230 nM. The Ral-binding activity of RalBPl-(364-647) was similar to that of full-length RalBPl, and this deletion mutant bound to Ral at a molar ratio of 1:0.8 with a $K_d$ value of 200 nM (Fig. 3A). Previously we have shown that the post-translational modification of Ral increases its affinity for RalBPl [15]. Under the conditions that the post-translationally modified or unmodified form of Ral bound to RalBPl stochiometrically, we measured the GAP activity of RalBPl for Rac1. In this experiment we used RalBPl immobilized on amylase resin as a source of GAP and the post-translationally modified form of Rac1 as a substrate. The GAP activity of immobilized RalBPl was attenuated as compared with that of soluble RalBPl (compare Fig. 3B with Fig. 4A). RalBPl interacting with the modified or unmodified form of Ral exhibited the same GAP activity as RalBPl itself (Fig. 3B). These results indicate that the interaction of Ral with RalBPl does not affect the GAP activity of RalBPl.

3.4. Effect of the post-translational modifications of Rac1 and CDC42 on the GAP activity of RalBPl

Finally we examined the effect of the post-translational modifications of small G proteins on the GAP activity of RalBPl. We confirmed that GST-Rac1 and GST-CDC42 purified from Sf9 cells are post-translationally modified and those from E. coli are post-translationally unmodified by the Triton X-114 phase separation method (data not shown). GTP was hydrolyzed in both the modified and unmodified forms of these small G proteins with the similar efficiency (data not shown). Consistent with previous observations, RalBPl stimulated the GTPase activity of the post-translationally unmodified form of Rac1 and CDC42 (Fig. 4A,B). RalBPl was more active on the post-translationally modified form of Rac1 and CDC42 than the unmodified form (Fig.
Fig. 2. Translocation of RalBPl by Ral. A: Translocation of RalBPl by Ral\textsuperscript{G23V} but not by Ral\textsuperscript{G23V/C203S}. COS cells expressing RalBPl alone (lanes 1 and 2), both Ral\textsuperscript{G23V} and RalBPl (lanes 3 and 4), or both Ral\textsuperscript{G23V/C203S} and RalBPl (lanes 5 and 6) were disrupted and separated into the cytosol (lanes 1, 3, and 5) and membrane (lanes 2, 4, and 6) fractions. Aliquots of each sample were probed with the anti-Myc and HA antibodies. B: Translocation of RalBPl-(364-647) by Ral\textsuperscript{G23V}. COS cells expressing RalBPl-(364-647) alone (lanes 1 and 2), both Ral\textsuperscript{G23V} and RalBPl-(364-647) (lanes 3 and 4), or both Ral\textsuperscript{G23V/C203S} and RalBPl-(364-647) (lanes 5 and 6) were disrupted and separated into the cytosol (lanes 1, 3, and 5) and membrane (lanes 2, 4, and 6) fractions. Aliquots of each sample were probed with the anti-Myc and HA antibodies. C, cytosol fraction; M, membrane fraction; Ab, antibody. Arrowheads indicate the positions of RalBPl and RalBPl-(364-647). Arrows indicate the positions of Ral\textsuperscript{G23V} and Ral\textsuperscript{G23V/C203S}. The results shown are representative of three independent experiments.

Fig. 3. Effect of the interaction of Ral with RalBPl on the GAP activity of RalBPl. A: Interaction of Ral with RalBPl in vitro. RalBPl(○) or RalBPl-(364-647) (■) immobilized on amylose resin (4 pmol of each) was incubated with the indicated concentrations of the [\textsuperscript{35}S]GTP\textsubscript{S-} (—-) or [\textsuperscript{3}H]GDP- (•••••) bound post-translationally unmodified form of GST-RalB. After the samples were precipitated by centrifugation, the remaining radioactivities were counted. B: The GAP activity of RalBPl. The [\textsuperscript{\gamma-32}P]GTP-bound post-translationally modified form of GST-Rac1 (4 pmol) was incubated for 5 min at 25°C with the indicated concentrations of immobilized RalBPl (○) or immobilized RalBPl stoichiometrically interacting with the GTP\textsubscript{S}-bound modified (●) or unmodified (○) form of GST-RalB. The results shown are representative of four independent experiments.
Fig. 4. Effect of the post-translational modifications of small G proteins on the GAP activity of RalBPl. The GAP activities of the indicated concentrations of RalBPl for the post-translationally modified (•) and unmodified (○) forms of GST-Rac1 or GST-CDC42 (4 pmol of each) were assayed. A: Rac1. B: CDC42. The results shown are representative of three independent experiments.

4A,B). These results indicate that the post-translational modifications of Rac1 and CDC42 enhance the GAP activity of RalBPl.

4. Discussion

RalBPl has been identified as a putative effector protein of Ral and shown to possess the GAP activity for Rac1 and CDC42 [10-12]. However, it has not yet been shown that RalBPl interacts with Ral in intact cells. We have demonstrated for the first time that full-length RalBPl binds to an active form of Ral but not to a dominant negative form of Ral in COS cells. Furthermore, we have shown that RalBPl stoichiometrically binds to the GTPγS-bound form of Ral with a $K_d$ value of 230 nM. These results strongly support the possibility that RalBPl is an effector protein of Ral. We have also demonstrated that the interaction of Ral with RalBPl does not affect the GAP activity of RalBPl for Rac1 under the condition that RalBPl binds to Ral stoichiometrically. Taken together with the previous observation that the Ral-binding domain of RalBPl is not necessary for its GAP activity [10], these results suggest that Ral does not serve as an allosteric regulator of RalBPl and that there is some mechanism by which Ral influences the functions of Rac1 and CDC42 in cells.

Many signal transduction molecules translocate to the membrane. Ras resides on the plasma membrane through its post-translational modification [13,14,26]. Raf is an important Ras effector protein and usually present in the cytosol. When Ras is activated, Raf translocates from the cytosol to the membrane by binding to Ras and is activated to transmit the signal to mitogen activated protein kinase cascade [27]. Raf which has a membrane-localizing motif is constitutively activated without the Ras activation [28,29]. Thus, a role of Ras in the activation of Raf could be to recruit Raf to the membrane. Consistent with these observations, our results have shown that Ral localized on the membrane induces the translocation of RalBPl from the cytosol to the membrane. Ral is present not only in the plasma membrane along with Ras but also in the intracellular vesicles [1]. Rac and CDC42 interact with the membrane and cytoskeleton [30,31] and regulate cell shape and motility [32-34]. Recently Ras has been shown to regulate receptor-mediated endocytosis, especially clathrin-coated vesicle formation [35]. These results suggest that Rac is also present in the intracellular vesicles. Therefore, RalBPl may act as a negative regulator for Rac1 and CDC42 when these small G proteins are colocalized with Ral on the same sites. Inversely, when RalBPl translocates to the membrane where Rac1 and CDC42 are not present, Ral may potentiate the activities of Rac1 and CDC42 by removing RalBPl from a compartment in which it serves as a GAP for Rac1 and CDC42. However, since our experiments have been done by overexpression of RalBPl and/or Ral in COS cells, we cannot exclude the possibility that endogenous RalBPl is localized on the membrane, that overexpressed RalBPl is in the cytosol simply because RalBPl is already saturated on the membrane, and that addition of RalG23V takes this excess of RalBPl to the membrane. Therefore, it is necessary to determine subcellular localization of endogenous Ral and RalBPl by their specific antibodies.

It has been known that the post-translational modifications of small G proteins are critical for their functions and the actions of their GDP/GTP exchange proteins [14,26]. In contrast to GDP/GTP exchange proteins, the post-translational modifications of small G proteins do not affect the activity of RasGAP, RapGAP, or RalGAP [15,36,37]. However, we have shown that the post-translational modifications of Rac1 and CDC42 enhance the GAP activity of RalBPl. Recently Rab3-GAP has been purified from rat brain and found to be active on the post-translationally modified form of Rab3A but not on the unmodified from [38]. Thus, the roles of the post-translational modifications of small G proteins in the GAP action vary depending on GAP types. Taken together with our previous observations that the post-translational modification of Ras is necessary for the translocation of RalGDS and that the modification of Ral enhances the action of RalGDS [15], it is
conceivable that the modifications of Ras, Ra1, and Racl play a role not only for their subcellular localization but also for transmitting the signals effectively on the membranes in the signaling pathway of Ras/RalGDS/Ral/RalBP1/Racl.

The GAP activity of RalBP1 for Racl and CDC42 is rather weak as compared with those of other RhoGAP family members. It is known that Raf, Rabphilin-3A, and phospholipase C-β are the effector proteins of Ras, Rab3A, and Gq, respectively, and that they have the weak GAP activities for these G proteins [39–41]. Furthermore, it has been shown that RalBP1 interacts with Racl in yeast two-hybrid experiment [11]. These results suggest that RalBP1 may act as an effector protein rather than serve as a negative regulator of Racl and CDC42. In addition to the RhoGAP homology domain, RalBP1 has two α-helix regions [10,11]. These regions may associate with other molecules which induce downstream events. Further studies are necessary to understand the whole picture of the physiological roles of the Ral–RalBP1 pathway in the intracellular signal transduction system and cellular functions.

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References