Biochemical and Biophysical Research Communications 435 (2013) 414–419

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Ubiquitination of the heterotrimeric G protein α subunits Gαi2 and Gαq is prevented by the guanine nucleotide exchange factor Ric-8A

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ARTICLE INFO

Article history:
Received 26 April 2013
Available online 9 May 2013

Keywords:
Ric-8A
Gαq
Gαi
Ubiquitination

ABSTRACT

The cytosolic protein Ric-8A acts as a guanine nucleotide exchange factor for Gα subunits of the Gi, Gq, and G12/13 classes of heterotrimeric G protein *in vitro*, and is also known to increase the amounts of these Gα proteins *in vivo*. The mechanism whereby Ric-8 regulates Gα content, however, has not been fully understood. Here we show that Ric-8 Astabilizes Gαi2 and Gαq by preventing their ubiquitination. Ric-8A interacts with and stabilizes Gαi2, Gαq, Gα12, but not Gαs, when expressed in COS-7 cells. The protein levels of Gαi2 and Gαq appear to be controlled via the ubiquitin–proteasome degradation pathway, because these Gα subunits undergo polyubiquitination and are stabilized with the proteasome inhibitor MG132. The ubiquitination of Gαi2 and Gαq is suppressed by expression of Ric-8A. The suppression likely requires Ric-8A interaction with these Gα proteins; the C-terminal truncation of Gαq and Gαi2 completely abrogates their interaction with Ric-8A, their stabilization by Ric-8A, and Ric-8A-mediated inhibition of Gα ubiquitination.

1. Introduction

Heterotrimeric G proteins transduce signals from cell surface G-protein-coupled receptors (GPCRs) to effector proteins [1]. Agonist binding to a GPCR elicits GDP/GTP exchange on the Gα subunit; thus GPCR in the ligand-bound form functions as a guanine nucleotide exchange factor (GEF) for Gα. The exchange leads to heterotrimer dissociation to Gα–GTP and Gβγ, both of which interact with and activate their respective downstream effectors. The signaling is terminated by GTP hydrolysis via the intrinsic GTPase activity of Gα: the resultant Gα–GDP associates with Gβγ to reform the inactive heterotrimer. Besides their classical functions in GPCR signaling pathways, heterotrimeric G proteins can be activated in a GPCR-independent manner [2,3]. The cytosolic protein Ric-8 (resistance to inhibitors of cholinesterase 8) is an evolutionarily-conserved nonreceptor GEF for Gα, and considered to participate in both GPCR-dependent and -independent signaling pathways [4–7].

Ric-8 has been originally identified as a crucial component for GPCR-mediated signaling via Gαq during synaptic transmission in *Caenorhabditis elegans* [8]. It has subsequently been shown that Ric-8 is essential for asymmetric cell division of the *C. elegans* zygote and that of neuroblasts in *Drosophila melanogaster*, both events of which require heterotrimeric G proteins but not GPCRs [5,6,9–14]. In mammals, there are two distinct genes for Ric-8-related proteins, *i.e.*, Ric-8A and Ric-8B. Although mammalian Gα subunits are grouped into four classes (Gs, Gi, Gq, and G12/13), on the basis of sequence homology and effector protein specificity [1], Ric-8B interacts with the Gs and Gq class Gα proteins [4,15,16]; it is still controversial whether Ric-8B functions as a GEF for these Gα [16,17]. On the other hand, Ric-8A exhibits an *in vitro* GEF activity for the α subunits of three of the four classes except the Gs class [4,7,17], and indeed potentiates Gq signaling [7]. In addition to the role in the GPCR-dependent signaling, Ric-8A participates in G13 signaling downstream of a receptor tyrosine kinase [18], and regulates mitotic spindle orientation in a GPCR-independent manner [19].

Although genetic ablation of Ric-8A or Ric-8B in mice results in embryonic lethality [20,21], the mechanisms by which Ric-8 controls Gα signaling have not been well understood. Ric-8 is required not only for localization of Gα to the plasma membrane [11–14,18] but also for maintenance of Gα protein levels [11,14,16,21]; Ric-8 is currently considered to function as a biosynthetic chaperone for Gα folding [21–23]. On the other hand, how Gα proteins are degraded in animal cells has not been well understood. Among mammalian Gα proteins, Gαs has been shown to be polyubiquitinated for degradation by the proteasomal pathway [16,24,25]. It has been shown that Ric-8B stabilizes Gαs by inhibiting its ubiquitination [16], although the role of Ric-8A in Gα ubiquitination has remained to be elucidated. In addition, a few or no reports have demonstrated ubiquitination of Gαi or Gαq, respectively [26,27].

Abbreviations: GPCR, G-protein-coupled receptor; GEF, guanine nucleotide exchange; CHX, cycloheximide.

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http://dx.doi.org/10.1016/j.bbrc.2013.04.103

In the present study, we show that Gαq as well as Gαi2 is polyubiquitinated and probably degraded via the ubiquitin–proteasome pathway. Ric-8A prevents ubiquitination of Gαq and Gαi2 to stabilize these Gα proteins.

2. Materials and methods

2.1. Plasmid construction

The cDNAs encoding human Ric-8A, Gαi2, Gαq, Gα12, and Gαs (the long form of 394 amino acids) were prepared by PCR using Human Multiple Tissue cDNA panels (BD Biosciences). The cDNA for Ric-8A was ligated to the mammalian expression vector pEF-BOS for expression as a FLAG-tagged protein [28]. For expression of Gα proteins with an internal EE-tag (Gαi2–EE, Gαq–EE, Gα12–EE, and Gαs–EE), the cDNA regions for amino acid residues 167–172 (DYIPTQ) in Gαi2, 171–176 (AYLPTQ) in Gαq, 193–198 (NYFPSK) in Gα12, and 189–194 (DYVPSD) in Gαs were replaced with the DNA fragment encoding the peptide EYMPTE by PCR-mediated site-directed mutagenesis. The cDNAs for Gα proteins were ligated to pcDNA3 (Invitrogen). The cDNAs for Gα lacking the C-terminal 9 amino acids, Gαi2–EE-ΔC (amino acid residues 1–346) and Gαq–EE-ΔC (1–350), were prepared by PCR using Gαi2–EE and Gαq–EE as a template, respectively. Mutations leading to the indicated substitution were introduced by PCR-mediated site-directed mutagenesis. The vector pCGN-HA–Ub for expression of human ubiquitin as an HA-tagged protein [29] was generously gifted from Prof. Mitsuyoshi Nakao (Kumamoto University). The cDNA for $(His)_6$-tagged human ubiquitin was prepared by PCR and ligated to pcDNA3 (pcDNA3-His–Ub). All of the constructs were sequenced for confirmation of their identities.

2.2. Antibodies

Anti-FLAG (M2) and anti-β-tubulin (TUB 2.1) mouse monoclonal antibodies were purchased from Sigma–Aldrich; an anti-HA rat monoclonal antibody (3F10) from Roche Applied Science; anti-HA (16B12) and anti-Glu-Glu (EE) mouse monoclonal antibodies from Covance; anti-$(His)_5$ monoclonal antibodies from QIAGEN; and negative control mouse IgG1 from DakoCytomation.

2.3. Cell transfection and immunoblot analysis

COS-7 cells were transfected using LipofectAMINE and Plus Regent (Invitrogen) with indicated cDNAs and cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum. For analysis of the effect of cycloheximide (CHX), transfected cells were treated for 5 or 10 h with 10 μg/ml of CHX (Wako Pure Chemical Industries). For analysis of the effect of MG132, transfected cells were treated for 12 h with 10 μM MG132 (Calbiochem). Cells were lysed at 4 °C with a lysis buffer (150 mM NaCl, 0.4 mM EDTA, 1 mM DTT, 1.0% Triton X-100, 10% glycerol, and 50 mM Tris–Cl, pH 7.5) containing Protease Inhibitor Cocktail (Sigma–Aldrich). The lysates were analyzed by immunoblot with the anti-EE antibody. The blots were developed using ECL-plus (GE Healthcare Biosciences) for visualization of the antibodies.

2.4. Immunoprecipitation assay

The immunoprecipitation assay was performed as previously described [30]. Briefly, proteins were precipitated from the lysate of transfected COS-7 cells with the anti-FLAG antibody or control IgG in the presence of protein G-Sepharose (GE Healthcare Biosciences). The precipitants were analyzed by immunoblot.

2.5. In vivo ubiquitination assays

In vivo ubiquitination assays were performed according to the method of Kuo et al. [31] with minor modifications. COS-7 cells cotransfected with the cDNAs for Gα and Ric-8A, and pcDNA3-His–Ub or pCGN-HA–Ub were cultured for 8.5–12.5 h with 10 μM MG-132. For detection of His-tagged ubiquitinated Gα proteins, cells were suspended in a urea lysis buffer (8 M urea, 10 mM NaH_2PO_4, 10% glycerol, 0.1% Triton X-100, 0.5 M NaCl, 10 mM imidazole, 10 mM 2-mercaptoethanol, and 10 mM Tris–HCl, pH 8.0), followed by sonication. The sonicate was centrifuged for 5 min at 20,400×*g*, and the resultant supernatant was mixed for 4 h with His-Select Nickel Affinity Gel (Sigma–Aldrich). After washing 5 times, proteins were eluted from the gel with an elution buffer (200 mM imidazole, 5% SDS, 30% glycerol, 0.72 M 2-mercaptoethanol, and 150 mM Tris–HCl, pH 6.8), followed by immunoblot analysis. For detection of HA-tagged ubiquitinated Gα proteins, proteins were precipitated with the anti-HA antibody in the presence of protein G-Sepharose, followed by immunoblot analysis.

3. Results

3.1. Ric-8A stabilizes Gαi2, Gαq, and Gα12, but not Gαs

It has been shown that Ric-8A deficiency in mouse ES cells leads to destabilization of Gαi, Gαq, and Gα13, but not Gαs [21]. To confirm the role for Ric-8A in other types of cell, we expressed FLAG-tagged Ric-8A together with EE-tagged Gαi2, Gαq, Gα12, or Gαs in COS-7 cells, and performed immunoblot analysis. As shown in Fig. 1A, expression of Ric-8A resulted in an increased protein level of Gαi2, Gαq, and Gα12, but not Gαs. The selectivity among Gα subunits is consistent with the preference in Ric-8A interaction: Ric-8A directly binds to Gα proteins that belong to the Gi, Gq, and G12/13 classes, but not to the Gαs class [4]. Also in intact cells, Ric-8A interacted with Gαi2, Gαq, and Gα12, but not with Gαs (Fig. 1B). These findings raised the possibility that the amounts of Gαi2, Gαq, and Gα12 are increased by Ric-8A via direct interaction. We next monitored the protein level of Gαi2 and Gαq after treatment with the protein synthesis inhibitor CHX. CHX treatment of control COS-7 cells led to a decrease in Gαi2 and Gαq (Fig. 1C). The degradations of these proteins were both inhibited by expressing Ric-8A in cells (Fig. 1C). Thus Ric-8A likely increases the amounts of Gαi2 and Gαq at the posttranslational level.

3.2. Gαi2 and Gαq is polyubiquitinated, which is inhibited by Ric-8A

The stabilization of Gαi2 and Gαq by Ric-8A may be modulated via the ubiquitin–proteasome pathway, because these Gα proteins were stabilized with the proteasome inhibitor MG132 (Fig. 1D). To test this possibility, we performed an *in vivo* ubiquitination assay: COS-7 cells expressing $(His)_6$-tagged ubiquitin together with Gαi2 or Gαq were lysed under denaturing conditions, and $(His)_6$-tagged ubiquitinated proteins in the lysate were precipitated with nickel-agarose beads, followed by immunoblot analysis. As shown in Fig. 2A, a ladder of ubiquitinated Gαi2 proteins were detected, indicative of polyubiquitination. A similar ladder formation was observed when HA-tagged ubiquitin was expressed instead of His–ubiquitin (Fig. 2B). Intriguingly, Gαi2 ubiquitination was effectively blocked by expression of Ric-8A (Fig. 2A and B). Furthermore, as shown in Fig. 2C, Gαq also underwent polyubiquitination, which was inhibited by Ric-8A (Fig. 2C). Taken together with the role of Ric-8A in Gα stabilization (Fig. 1), Ric-8A appears to stabilize Gαi2 or Gαq by preventing ubiquitination.

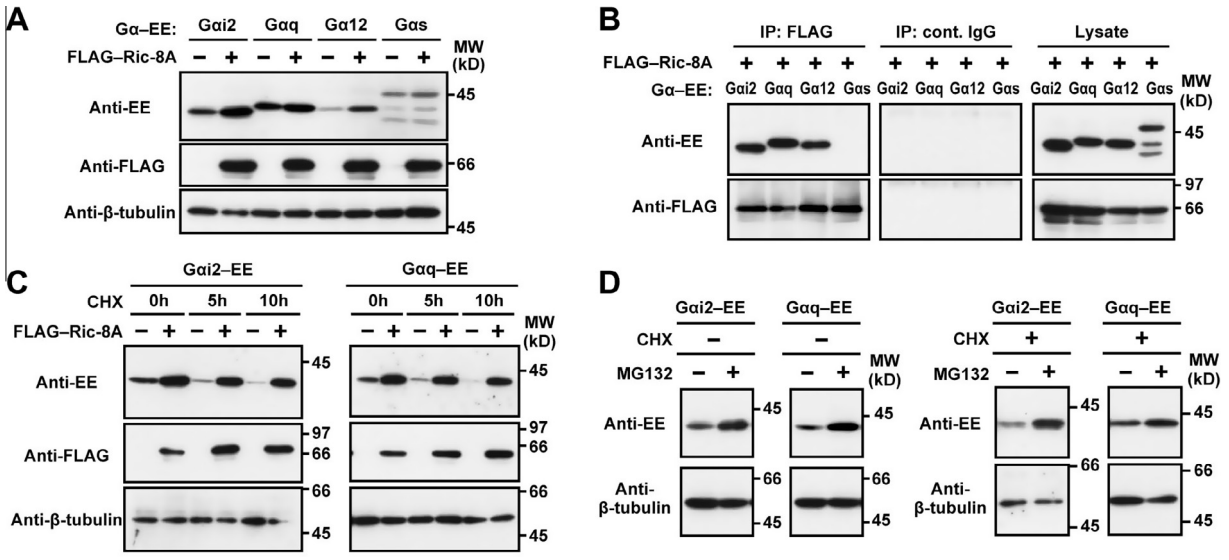

Fig. 1. Stabilization of Gαi2, Gαq, and Gα12 by Ric-8A. COS-7 cells were transfected with pEF-BOS-FLAG–Ric-8A and pcDNA3 encoding the indicated type of Gα as an EE-tagged protein, and cultured in the presence or absence of 10 μg/ml of CHX and/or 10 μM MG132. (A,C,D) Cell lysates were analyzed by immunoblot with the indicated antibody. (B) Proteins in cell lysates were immunoprecipitated (IP) with the anti-FLAG antibody or control IgG, followed by immunoblot analysis with the indicated antibody. Positions for marker proteins are indicated in kDa.

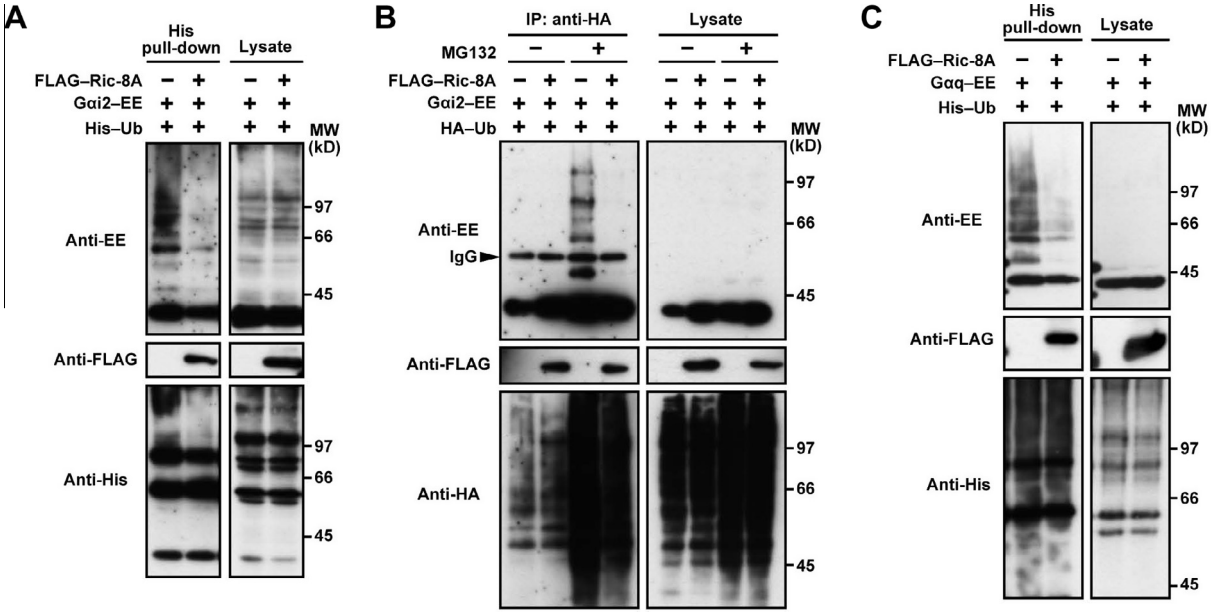

Fig. 2. Ubiquitination of Gαi2 and Gαq. COS-7 cells were transfected with the following plasmids: (A) pcDNA3-Gαi2–EE, pcDNA3-His–Ub, and/or pEF-BOS-FLAG–Ric-8A; (B) pcDNA3-Gαi2–EE, pCGN-HA–Ub, and/or pEF-BOS-FLAG–Ric-8A; or (C) pcDNA3-Gαq–EE, pcDNA3-His–Ub, and/or pEF-BOS-FLAG–Ric-8A. Transfected cells were cultured in the presence of 10 μM MG132, and proteins in cell lysates were precipitated with His-Select Nickel Affinity Gel (A,C); or with the anti-HA antibody in the presence of protein G-Sepharose (B). Precipitants were analyzed by immunoblot with the indicated antibody. Positions for marker proteins are indicated in kDa.

3.3. Lipid modification of Gαi2 and Gαq is dispensable for stabilization by Ric-8A

The N-terminal region of Gα proteins is modified by myristoylation and/or palmitoylation, both of which are crucial for membrane localization [32,33]. We next addressed the question whether these modifications participate in stabilization by Ric-8. The N-terminal glycine residue of the Gi class α subunits is known to be myristoylated, which is required for further palmitoylation of cystein residues in the N-terminal region and for membrane localization [32]. To know the role of fatty acid modification, we expressed a mutant Gαi2 that carries the G2A substitution and tested the effect of Ric-8A. As shown in Fig. 3A, Ric-8A stabilized Gαi2 (G2A) as effectively as wild-type Gαi2. Consistent with this, polyubiquitination of Gαi2 (G2A) was prevented by Ric-8A (Fig. 3B). It is known that the Gq class α subunits undergo palmitoylation at Cys-9 and Cys-10, but not N-terminal myristoylation [33]. A mutant Gq with the C9S/C10S substitution was fully stabilized by expression of Ric-8A (Fig. 3C). These findings indicate that Ric-8-mediated stabilization of Gαi2 and Gαq does not require lipid modifications (and probably subsequent membrane localization).

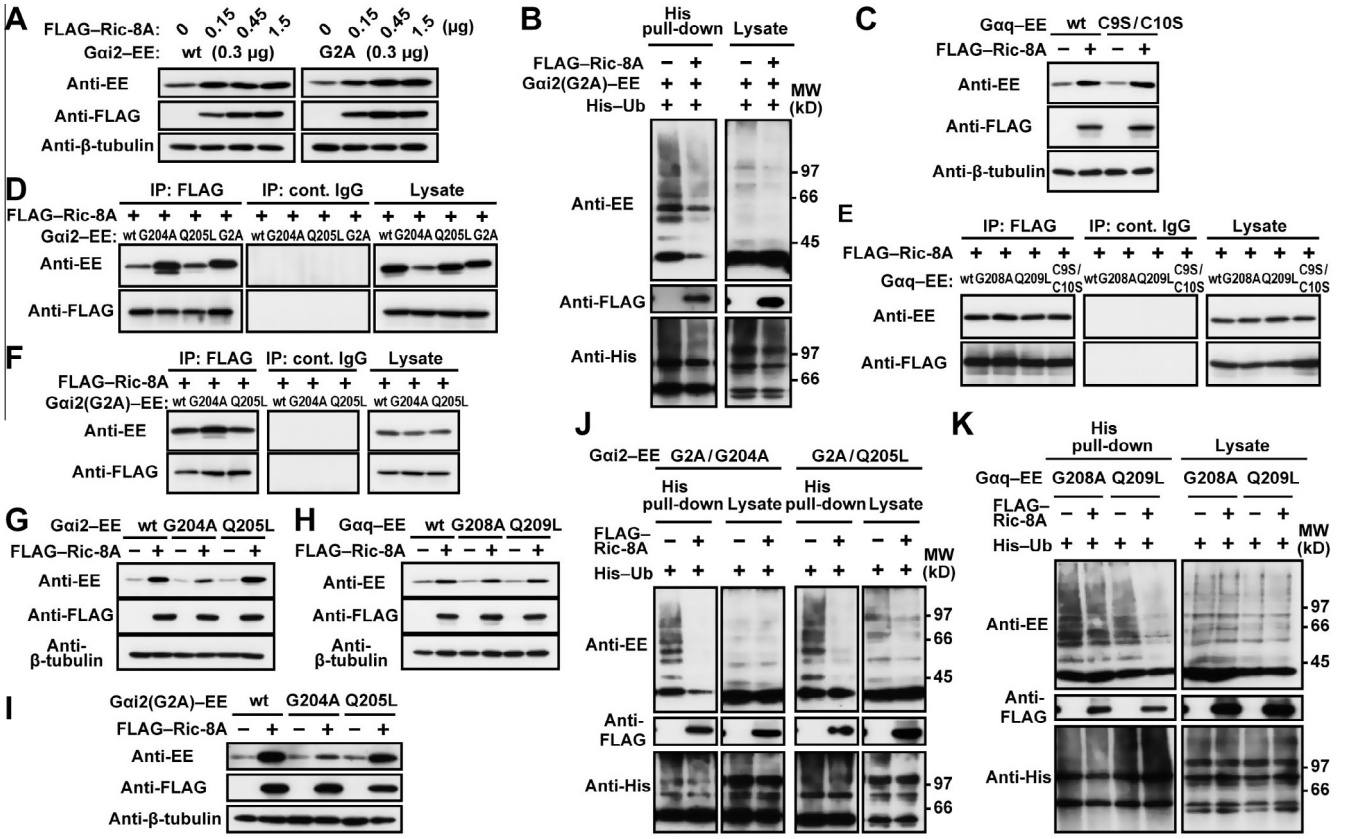

Fig. 3. Effects of lipid modification and nucleotide binding on stabilization and ubiquitination of Gαi2 and Gαq. COS-7 cells were transfected with the indicated plasmids, and cultured as described in "Materials and methods." (A,C,G–I) Cell lysates were directly analyzed by immunoblot with the indicated antibody. (B,J,K) Proteins in cell lysates were precipitated with His-Select Nickel Affinity Gel, followed by immunoblot analysis with the indicated antibody. (D–F) Proteins in cell lysates were immunoprecipitated (IP) with the anti-FLAG antibody or control IgG, followed by immunoblot analysis with the indicated antibody. Positions for marker proteins are indicated in kDa.

3.4. Ric-8A stabilizes both GDP- and GTP-bound forms of Gαi2 and Gαq

It is generally considered that Ric-8A preferentially binds to GDP-bound Gα, based on *in vitro* (cell-free) binding experiments [4]. As expected, the GDP-bound form of Gαi2 (G204A) associated with Ric-8A more effectively than the GTPase-deficient, GTP-bound mutant Gαi2 (Q205L), although the latter mutant was still capable of interacting with Ric-8 (Fig. 3D). On the other hand, both the GDP-bound Gαq (G208A) and GTP-bound Gαq (Q209L) were as effective as the wild-type protein in binding to Ric-8A (Fig. 3E), which agrees with the previous observation that Gαq (Q209L) associates with Ric-8A similarly to wild-type Gαq in the yeast two-hybrid system [4]. Among lipid-free Gαi2 proteins, Gαi2 (G2A/G204A) interacted with Ric-8A to a slightly larger extent than Gαi2 (G2A) and Gαi2 (G2A/Q205L) (Fig. 3F). Thus Ric-8A appears to have a potential to interact with both GDP- and GTP-bound forms of Gαi2 and Gαq in cells. Consistent with this, Ric-8A stabilized GDP- and GTP-bound forms of Gαi2 and Gαq (Fig. 3G–I) and inhibited ubiquitination of both forms of these Gα proteins (Fig. 3J and K).

3.5. The C-terminal region of Gαi2 and Gαq is required for their stabilization by Ric-8A

We finally tested the role of the C-terminal region of Gα proteins in Ric-8A-mediated stabilization. It has been shown that the C-terminus of Gαi is required for direct interaction with Ric-8A, on the basis of assays using purified proteins [22,34]. Here we tested the role of the C-terminal region in the interaction of Ric-8 with Gαi2 and Gαq at the cellular level. As shown in Fig. 4A, a mutant Gαi2 that lacked the C-terminal 9 amino acids (Gαi2-ΔC) did not interact with Ric-8A, when expressed in COS-7 cells, indicating that the C-terminal region of Gαi2 appears to play a crucial role also *in vivo*. Intriguingly, Gαi2-ΔC, in both the membrane-bound and soluble (G2A) forms, was not stabilized by the expression of Ric-8A (Fig. 4B). Similarly, truncation of the extreme C-terminal 9 amino acids in Gαq resulted in a complete loss of both interaction with Ric-8A and stabilization by Ric-8A (Fig. 4C and D). Consistent with this, Ric-8A was incapable of preventing these C-terminally truncated proteins from being ubiquitinated (Fig. 4E and F). Thus stabilization of Gαi2 and Gαq by Ric-8A appears to require direct interaction with Ric-8A via the C-terminal region.

4. Discussion

The present study demonstrates that Gαq as well as Gαi2 is polyubiquitinated and probably degraded via the ubiquitin–proteasome pathway, and that Ric-8A prevents ubiquitination of Gαq and Gαi2 to stabilize these Gα proteins. Although the amounts of mammalian Gαi, Gαo, and Gαs are presumed to be regulated by the proteasome pathway [16,24–26,35,36], solely Gαs [16,24,25] and Gαi2 [26,27] have been shown to be indeed ubiquitinated; ubiquitinated Gαi2 can be degraded either through the proteasomal pathway [26] or in the lysosome [27]. As far as we are aware, the present study represents the first instance in which Gαq is ubiquitinated and ubiquitination routes Gαq for the proteasomal pathway. Membrane recruitment of Ric-8A has been shown to be important for signal enhancement in Gαq- and Gαi2-mediated pathways [7,19], suggesting that Ric-8A functions at the

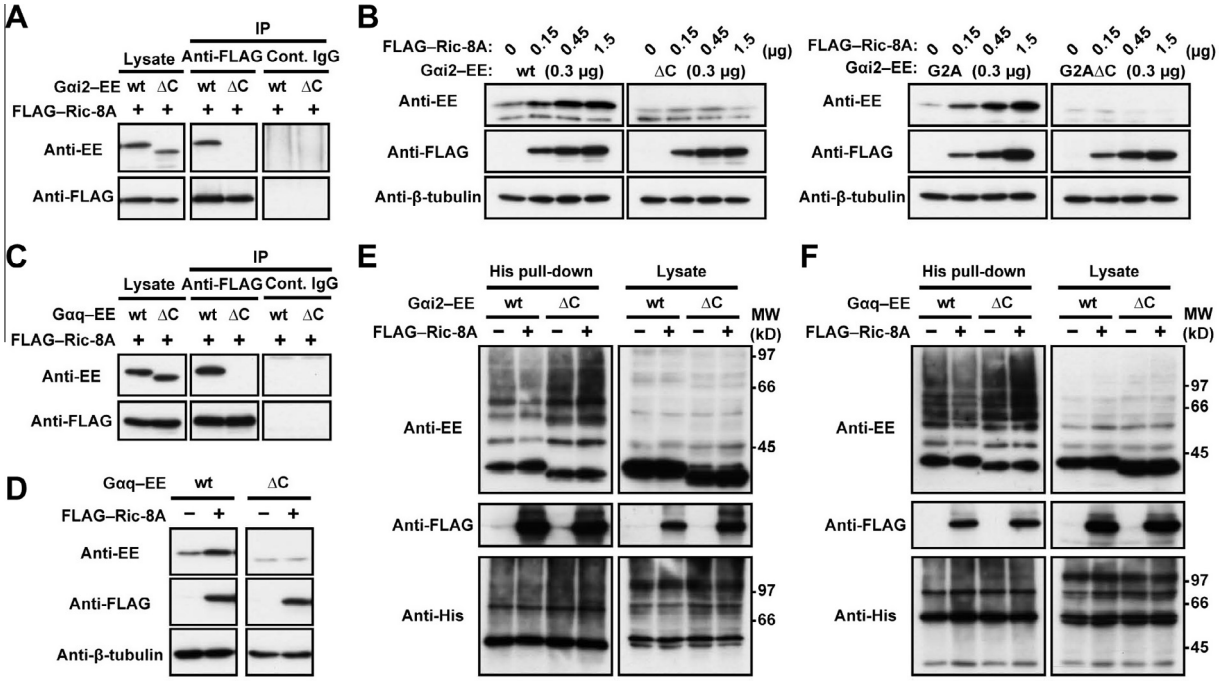

Fig. 4. Role for the C-terminal region of Gαi2 and Gαq in their stabilization and ubiquitination. COS-7 cells were transfected with the indicated plasmids, and cultured as described in "Materials and methods." (A,C) Proteins in cell lysates were immunoprecipitated (IP) with the anti-FLAG antibody or control IgG, followed by immunoblot analysis with the indicated antibody. (B,D) Cell lysates were directly analyzed by immunoblot with the indicated antibody. (E,F) Proteins in cell lysates were precipitated with His-Select Nickel Affinity Gel, followed by immunoblot analysis with the indicated antibody. Positions for marker proteins are indicated in kDa.

membrane. On the other hand, Ric-8A appears to stabilize Gαq and Gαi2 in the cytoplasm, because mutant Gα proteins, defective in lipid modification for membrane anchoring, are stabilized by Ric-8A as effectively as wild-type proteins (Fig. 3).

Here we also show that Ric-8A interaction with Gαq and Gαi2 is required to suppress their ubiquitination. This is because the C-terminal truncation of Gαq and Gαi2 completely abrogates not only their interaction with Ric-8A, but also their stabilization by Ric-8A and Ric-8A-mediated suppression of their ubiquitination (Fig. 4). In addition, Ric-8A is capable of binding to Gαi2, Gαq, and Gα12 and stabilizing these Gα proteins, but it fails to interact with and to stabilize Gαs (Fig. 1). This is consistent with the finding that genetic ablation of Ric-8A in mouse ES cells destabilizes α subunits of three of the four classes of heterotrimeric G proteins except the Gαs class [21]. Finally, various mutant Gα proteins that can interact with Ric-8A are all stabilized by Ric-8A via prevention of ubiquitination (Fig. 3).

The present findings are compatible with the recent proposal that Ric-8A is required to fold Gα subunits during biosynthesis [23]. The Ric-8A-dependent increase in Gα content (Fig. 1) can be considered as secondary manifestations of the folding function, because misfolded Gα subunits are expected to be readily degraded. The present observation that both GDP- and GTP-bound forms of Gα are stabilized by Ric-8A (Fig. 3) can be explained by the assumption that Ric-8A may facilitate the transition of Gα from a prefolded globular state to its native state by promoting the first guanine-nucleotide-binding event. On the other hand, Ric-8A binds to Gαi2 (Q205L), a GTPase-deficient mutant that is fixed to the GTP-bound form, to a lesser extent than Gαi2 (G204A), a mutant fixed to the GDP-bound form, in intact cells (Fig. 3D), which agrees with the observation that Ric-8A associates with GDP-bound Gαi1 but not with GTP-bound Gαi1 *in vitro* [4]. If Ric-8A functions solely as a biosynthetic chaperone, Ric-8A may be expected to stabilize the high affinity mutant Gαi2 (G204A) more efficiently than the low affinity mutant Gαi2 (Q205L). However, Gαi2 (Q205L) is more strongly stabilized than Gαi2 (G204A) (Fig. 3G and I), suggesting that the GTP-bound form is more stable—*i.e.*, more resistant to ubiquitination—in the presence of Ric-8A. Thus, it is tempting to postulate that, in addition to the function as a chaperone, Ric-8 serves as a GEF to exchange GDP for GTP on the correctly folded Gα, which may prevent ubiquitination of Gα.

Acknowledgments

We thank Prof. Mitsuyoshi Nakao (Institute of Molecular Embryology and Genetics, Kumamoto University) for pCGN-HA–Ub; Namiko Kubo (Kyushu University) and Yohko Kage (Kyushu University) for technical assistance, and to Minako Nishino (Kyushu University) for secretarial assistance. This work was supported in part by MEXT (the Ministry of Education, Culture, Sports, Science and Technology) KAKENHI Grant Numbers 20117002 and 24113718, and by JSPS (Japan Society for the Promotion of Science) KAKENHI Grant Number 24590385.

References

[1] W.M. Oldham, H.E. Hamm, Heterotrimeric G protein activation by G-protein-coupled receptors, Nat. Rev. Mol. Cell Biol. 9 (2008) 60–71.
[2] F.S. Willard, R.J. Kimple, D.P. Siderovski, Return of the GDI. The GoLoco motif in cell division, Annu. Rev. Biochem. 73 (2004) 925–951.
[3] M. Sato, J.B. Blumer, V. Simon, S.M. Lanier, Accessory proteins for G proteins. Partners in signaling, Annu. Rev. Pharmacol. Toxicol. 46 (2006) 151–187.
[4] G.G. Tall, A.M. Krumins, A.G. Gilman, Mammalian Ric-8A (synembryn) is a heterotrimeric Gα protein guanine nucleotide exchange factor, J. Biol. Chem. 278 (2003) 8356–8362.
[5] H.A. Hess, J.C. Röper, S.W. Grill, M.R. Koelle, RGS-7 completes a receptor-independent heterotrimeric G protein cycle to asymmetrically regulate mitotic spindle positioning in *C. elegans*, Cell 119 (2004) 209–218.
[6] K. Afshar, F.S. Willard, K. Colombo, et al., RIC-8 is required for GPR-1/2-dependent Gα function during asymmetric division of *C. elegans* embryos, Cell 119 (2004) 219–230.
[7] A. Nishimura, M. Okamoto, Y. Sugawara, et al., Ric-8A potentiates Gq-mediated signal transduction by acting downstream of G protein-coupled receptor in intact cells, Genes Cells 11 (2006) 487–498.

[8] K.G. Miller, M.D. Emerson, J.R. McManus, J.B. Rand, RIC-8 (Synembryn). A novel conserved protein is required for $G_q\alpha$ signaling in the *C. elegans* nervous system, Neuron 27 (2000) 289–299.
[9] K.G. Miller, J.B. Rand, A role for RIC-8 (Synembryn) and GOA-1 ($G_o\alpha$) in regulating a subset of centrosome movements during early embryogenesis in *Caenorhabditis elegans*, Genetics 156 (2000) 1649–1660.
[10] C. Couwenbergs, A.C. Spilker, M. Gotta, Control of embryonic spindle positioning and Gα activity by *C. elegans* RIC-8, Curr. Biol. 14 (2004) 1871–1876.
[11] K. Afshar, F.S. Willard, K. Colombo, et al., Cortical localization of the Gα protein GPA-16 requires RIC-8 function during *C. elegans* asymmetric cell division, Development 132 (2005) 4449–4459.
[12] N.B. David, C.A. Martin, M. Segalen, et al., *Drosophila* Ric-8 regulates Gαi cortical localization to promote Gαi-dependent planar orientation of the mitotic spindle during asymmetric cell division, Nat. Cell Biol. 7 (2005) 1083–1090.
[13] H. Wang, K.H. Ng, H. Qian, et al., Ric-8 controls *Drosophila* neural progenitor asymmetric division by regulating heterotrimeric G proteins, Nat. Cell Biol. 7 (2005) 1091–1098.
[14] B. Hampoelz, O. Hoeller, S.K. Bowman, et al., *Drosophila* Ric-8 is essential for plasma-membrane localization of heterotrimeric G proteins, Nat. Cell Biol. 7 (2005) 1099–1105.
[15] L.E. Von Dannecker, A.F. Mercadante, B. Malnic, Ric-8B, an olfactory putative GTP exchange factor, amplifies signal transduction through the olfactory-specific G-protein Gαolf, J. Neurosci. 25 (2005) 3793–3800.
[16] Y. Nagai, A. Nishimura, K. Tago, et al., Ric-8B stabilizes the α subunit of stimulatory G protein by inhibiting its ubiquitination, J. Biol. Chem. 285 (2010) 11114–11120.
[17] P. Chan, M. Gabay, F.A. Wright, G.G. Tall, Ric-8B is a GTP-dependent G protein α_s guanine nucleotide exchange factor, J. Biol. Chem. 286 (2011) 19932–19942.
[18] L. Wang, D. Guo, B. Xing, et al., Huang, Resistance to inhibitors of cholinesterase-8A (Ric-8A) is critical for growth factor receptor-induced actin cytoskeletal reorganization, J. Biol. Chem. 286 (2011) 31055–31061.
[19] G.E. Woodard, N.-N. Huang, H. Cho, et al., Ric-8A and Giα recruit LGN, NuMA, and dynein to the cell cortex to help orient the mitotic spindle, Mol. Cell. Biol. 30 (2010) 3519–3530.
[20] T. Tõnissoo, S. Lulla, R. Meier, M. Saare, et al., Nucleotide exchange factor Ric-8 is indispensable in mammalian early development, Dev. Dyn. 239 (2010) 3404–3415.
[21] M. Gabay, M.E. Pinter, F.A. Wright, G.G. Tall, et al., Ric-8 proteins are molecular chaperones that direct nascent G protein á subunit membrane association, Sci. Signal. 4 (2011) ra79.
[22] C.J. Thomas, K. Briknarová, J.K. Hilmer, et al., The nucleotide exchange factor Ric-8A is a chaperone for the conformationally dynamic nucleotide-free state of Gαi1, PLoS One 6 (2011) e23197.
[23] P. Chan, C.J. Thomas, S.R. Sprang, G.G. Tall, Molecular chaperoning function of Ric-8A is to fold nascent heterotrimeric G protein α subunits, Proc. Natl. Acad. Sci. USA 110 (2013) 3794–3799.
[24] S. Naviglio, M. Pagano, M. Romano, et al., Adenylate cyclase regulation via proteasome-mediated modulation of $G\alpha_s$ levels, Cell. Signal. 16 (2004) 1229–1237.
[25] T. Tang, M.H. Gao, A. Miyanohara, H.K. Hammond, Gαq reduces cAMP production by decreasing Gαs protein abundance, Biochem. Biophys. Res. Commun. 377 (2008) 679–684.
[26] J. Ogasawara, T. Sakurai, N. Rahman, et al., Acute exercise alters Gαi2 protein expressions through the ubiquitin–proteasome proteolysis pathway in rat adipocytes, Biochem. Biophys. Res. Commun. 323 (2004) 1109–1115.
[27] P. Chandrasekaran, M. Buckley, V. Moore, S. Venkatesan, et al., HIV-1 Nef impairs heterotrimeric G-protein signaling by targeting $G\alpha_{i2}$ for degradation through ubiquitination, J. Biol. Chem. 287 (2012) 41481–41498.
[28] S. Yuzawa, S. Kamakura, Y. Iwakiri, J. Hayase, H. Sumimoto, Structural basis for interaction between the conserved cell polarity proteins Inscuteable and Leu-Gly-Asn repeat-enriched protein (LGN), Proc. Natl. Acad. Sci. USA 108 (2011) 19210–19215.
[29] Y. Nagata, T. Anan, T. Yoshida, et al., The stabilization mechanism of mutant-type p53 by impaired ubiquitination. The loss of wild-type p53 function and the hsp90 association, Oncogene 18 (1999) 6037–6049.
[30] J. Hayase, S. Kamakura, Y. Iwakiri, Y. Yamaguchi, T. Izaki, T. Ito, H. Sumimoto, The WD40 protein Morg1 facilitates Par6–aPKC binding to Crb3 for apical identity in epithelial cells, J. Cell Biol. 200 (2013) 635–650.
[31] M.-L. Kuo, W. den Besten, D. Bertwistle, et al., N-terminal polyubiquitination and degradation of the Arf tumor suppressor, Genes Dev. 18 (2004) 1862–1874.
[32] F. Galbiati, F. Guzzi, A.I. Magee, et al., N-terminal fatty acylation of the α-subunit of the G-protein G_i1. Only the myristoylated protein is a substrate for palmitoylation, Biochem. J. 303 (1994) 697–700.
[33] T.E. Hughes, H. Zhang, D.E. Logothetis, C.H. Berlot, Visualization of a functional $G\alpha_q$-green fluorescent protein fusion in living cells. Association with the plasma membrane is disrupted by mutational activation and by elimination of palmitoylation sites, but not by activation mediated by receptors or ALF_4^-, J. Biol. Chem. 276 (2001) 4227–4235.
[34] C.P. Vellano, F. Shu, S. Ramineni, C.K. Yates, G.G. Tall, J.R. Hepler, Activation of the receptor of G protein signaling 14–Gαi1-GDP signaling complex is regulated by resistance to inhibitors of cholinesterase-8A, Biochemistry 50 (2011) 752–762.
[35] L. Busconi, J. Guan, B.M. Denker, Degradation of heterotrimeric $G\alpha_o$ subunits via the proteosome pathway is induced by the hsp90-specific compound geldanamycin, J. Biol. Chem. 275 (2000) 1565–1569.
[36] T. Fischer, L. De Vries, T. Meerloo, M.G. Farquhar, Promotion of Gαi3 subunit down-regulation by GIPN, a putative E3 ubiquitin ligase that interacts with RGS-GAIP, Proc. Natl. Acad. Sci. USA 100 (2003) 8270–8275.