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GRAB is a binding partner for the Rab11a and Rab11b GTPases

Conor P. Horgan, Sara R. Hanscom, Mary W. McCaffrey *

Molecular Cell Biology Laboratory, School of Biochemistry and Cell Biology, BioSciences Institute, University College Cork, Cork, Ireland

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ABSTRACT

Co-ordination of Rab GTPase function has emerged as a crucial mechanism in the control of intracellular trafficking processes in eukaryotic cells. Here, we show that GRAB/Rab3IL1 [guanine nucleotide exchange factor for Rab3A; RAB3A interacting protein (rabin3)-like 1], a protein that has previously be shown to act as a GEF (guanine nucleotide exchange factor) for Rab3a, Rab8a and Rab8b, is also a binding partner for Rab11a and Rab11b, but not the closely related Rab25 GTPase. We demonstrate that exogenous expression of Rab11a and Rab11b shift GRAB's distribution from the cytoplasm onto membranes. We find that the Rab11a/Rab11b-binding region of GRAB lies within its carboxy-terminus, a region distinct from its GEF domain and Rab3a-binding region. Finally, we describe a GRAB deletion mutant ($GRAB_{\Delta 223-228}$) that is deficient in Rab11-binding ability. These data identify GRAB as a dual Rab-binding protein that could potentially link Rab3 and Rab11 and/or Rab8 and Rab11-mediated intracellular trafficking processes.

1. Introduction

The endomembrane system in eukaryotic cells consists of a diverse and functionally interrelated collection of membrane bound cellular compartments and their transport intermediates. Key amongst the protein machinery that regulates the intracellular trafficking processes within, and between, these organelles are the members of the Rab GTPase family. Sixty-six Rabs exist in humans, with most exhibiting a ubiquitous expression profile [1,2]. Rab proteins are post-translationally modified by prenylation and undergo reversible association with the cytosolic face of intracellular membranes, where they regulate all stages of intracellular trafficking processes [1]. A salient feature of Rab GTPases is their ability to act as molecular switches by undergoing guanine nucleotide-dependent structural changes [1]. Rabs are inactive when GDP-bound and active when GTP-bound. GEFs (guanine nucleotide exchange factors) activate Rab proteins by facilitating nucleotide exchange (GDP for GTP), whereas GAPs (GTPase-activating proteins) stimulate the intrinsic GTPase activity of Rabs and thus lead to their inactivation [3]. When active, Rabs recruit heterogeneous downstream 'effector' proteins through which their biological effects are elicited [1,4]. Recently, the Rabs have been grouped into six evolutionary-related supergroups [2]. Within the group IV assemblage, Rab11a, Rab11b and Rab25 constitute a functionally-related subfamily which share many effector proteins [5,6]. Members of the Rab11 subfamily display prominent localisation to the perinuclear ERC (endosomal-recycling compartment), alternatively known as the recycling endosome, and control intracellular trafficking through this compartment [5–9].

GRAB (guanine nucleotide exchange factor for Rab3A), which is also known as Rab3IL1 [RAB3A interacting protein (rabin3)-like 1], shares a high degree of homology with the murine protein Rabin3 [10], and its human homologue Rabin8/Rab3IP (RAB3A-interacting protein). Each of these proteins possess an amino-terminally positioned GEF domain which is homologous to the GEF domain present in the yeast protein sec2p [10–12]. GRAB binds Rab3a and InsP6K-1 (inositol hexakisphosphate kinase-1) via an amino-terminal region which encompasses a putative coiled-coil domain [10]. GRAB was reported to act as a Rab3a GEF as it stimulates nucleotide exchange activity on this protein and regulates dopamine release from PC12 cells and human growth hormone release from bovine adrenal chromaffin cells [10]. Interestingly, a subsequent independent study also demonstrated that GRAB displays GEF activity towards Rab3a [13]; however, in a more recent study it was reported that GRAB does not have GEF activity towards Rab3a but instead, like its homologue Rabin8/Rab3IP, stimulates nucleotide exchange towards Rab8a and Rab8b [14].

Here, we demonstrate that GRAB is a dual Rab-binding protein by showing that it associates with the Rab11a and Rab11b GTPases via a region distinct from the coiled-coil/GEF region which was previously shown to mediate the interaction with Rab3a.

Abbreviations: ERC, endosomal-recycling compartment; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; GRAB, guanine nucleotide exchange factor for Rab3A; InsP6K-1, inositol hexakisphosphate kinase-1; Rab3IL1, RAB3A interacting protein (rabin3)-like 1; Rab3IP, RAB3A-interacting protein.

* Corresponding author. Fax: +353 21 490 1379.
E-mail addresses: conor.horgan@ucc.ie (C.P. Horgan), s.hanscom@ucc.ie (S.R. Hanscom), m.mccaffrey@ucc.ie (M.W. McCaffrey).

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2. Materials and methods

2.1. Plasmids

pGADT7-AD/GRAB was generated by PCR (AAAGAATTCATGTG GAGCGGCCCACCCCAG, AAAGAATTCCTAAGCCTCCTGGGGGAAGAA G) with pCMV-SPORT6/Rab3IL1 (GRAB) (Source Bioscience, Image Clone #4390573; Accession Number BC022239) as template and cloned into pGADT7-AD (Clontech). pGADT7-AD/GRAB$_{1-331}$ was generated by the removal of a ~190 bp *Sma*I–*Sma*I fragment from pGADT7-AD/GRAB, and the resultant backbone fragment re-ligated to create GRAB$_{1-331}$. pGADT7-AD/GRAB$_{164-382}$ was generated by PCR (AAAGAATTCTCCACACCAGCCTCTCCCAAC, AAAGGATCCCTAAG CCTCCTGGGGGAAGAA) with pTrcHisB/GRAB as template and cloned into pGADT7-AD. pGADT7-AD/GRAB$_{1-263}$ was generated by the removal of a ~400 bp *Sac*I–*Sac*I fragment from pGADT7-AD/GRAB, and the resultant backbone fragment re-ligated to create GRAB$_{1-263}$. pGADT7-AD/GRAB$_{\Delta 223-228}$ was generated by sub-cloning the *Eco*RI fragment from pEGFP-C2/GRAB$_{\Delta 223-228}$ into pGADT7-AD. pGBKT7/Rab11a, pGBKT7/Rab11b and pGBKT7/Rab25 were previously described [15]. pGBKT7/Rab11a Q70L was generated by subcloning the *Bam*HI–*Pst*I fragment from pLexA/Rab11a Q70L [16] into pGBKT7 (Clontech). pGBKT7/Rab11a S25N was generated by subcloning the *Bam*HI–*Pst*I fragment from pLexA/Rab11a S25N [16] into pGBKT7. pGBKT7/Rab11b Q70L was generated by PCR (GCCGAATTCATGGGGACCCGGGAC, GCGGGAT CCTCACAGGTTCTGCAG) with pEGFP-C2/Rab11b Q70L as template and cloned into pGBKT7. pGBKT7/Rab11b S25N was generated by PCR (GCCGAATTCATGGGGACCCGGGAC, GCGGGATCCTCACAGG TTCTGCAG) with pEGFP-C2/Rab11b S25N as template and cloned into pGBKT7. pGBKT7/Rab25 T26N was generated by subcloning the *Xho*I–*Sal*I fragment from pEGFP-C1/Rab25 T26N and into pGBKT7. pGBKT7/Rab14 Q70L was generated by PCR (CCGGAATTCATGGCAACTGCACCATACAACTACTC, CGCGGATCCC-TAGCCTTCTCTCTGGGG) with pEGFP-C1/Rab14 Q70L as template and cloned into pGBKT7. pGBKT7/Rab14 S25N was generated by PCR (CCGGAATTCATGGCAACTGCACCATACAACTACTC, CGCGGATC CCTAGCCTTCTCTCTGGGG) with pEGFP-C1/Rab14 S25N as template and cloned into pGBKT7. pmCherry-C1/FIP3 was generated by subcloning the *Eco*RI fragment from pEGFP-C1/FIP3 into pmCherry-C1. pcDNA3.1-HisC/GRAB was generated by subcloning the *Eco*RI fragment from pGADT7-AD/GRAB into pcDNA3.1-HisC (Invitrogen). pEGFP-C2/GRAB was generated by PCR (AAAGAA TTCATGTGGAGCGGCCCACCCCAG, AAAGAATTCCTAAGCCTCCTGGG GGAAGAAG) with pCMV-SPORT6/Rab3IL1 as template and cloned into pEGFP-C2. pEGFP-C2/GRAB$_{\Delta 223-228}$ was generated by SDM (site-directed mutagenesis) (GGAGGTGGACACACAGGCCTGGAG GG, CCCTCCAGGCCTGTGTGTCCACCTCC) with pEGFP-C2/GRAB as template. pTrcHisB/GRAB was generated by subcloning the *Eco*RI fragment from pGADT7-AD/GRAB into pTrcHisB (Invitrogen). pmCherry-C1/GRAB$_{1-163}$ was generated by SDM (TGAAGACA CTGGTCATCACGTAGACACCAGCCTCTCCCAACCGC, GCGGTTGGGA-GAGGCTGGTGTCTACGTGATGACCAGTGTCTTCAA) with pmCherry-C1/GRAB as template. pmCherry-C1/GRAB was generated by PCR (AAAGAATTCTATGTGGAGCGGCCCACCCCAG, AAAGGATCCCTAAG CCTCCTGGGGGAAGAAG) with pTrcHisB/GRAB as template and cloned into pmCherry-C1. pmCherry-C1/GRAB$_{164-382}$ was generated by PCR (AAAGAATTCATCCACACCAGCCTCTCCCAAC, AAAG GATCCCTAAGCCTCCTGGGGGAAGAA) with pTrcHisB/GRAB as template and cloned into pmCherry-C1. pmCherry-C1/GRAB$_{73-163}$ was generated by PCR (AAAGAATTCAGAGAAGGGCTCCGAGTTCCTG, AA AGGATCCCTACGTGATGACCAGTGTCTTCAA) with pTrcHisB/GRAB as template and cloned into pmCherry-C1. pEGFP-C3/Rab11a was previously described [17]. pEGFP-C3/Rab11a Q70L was generated by subcloning the *Eco*RI–*Pst*I fragment from the previously described pGEM/Rab11a Q70L [18] into pEGFP-C3. pEGFP-C3/Rab11a S25N was previously described [19]. pEGFP-C2/Rab11b and pEGFP-C1/Rab25 were previously described [15]. pEGFP-C2/Rab11b Q70L was generated by SDM (AGATCTGGGACACCGCTG GCCTGGACCGCTACCGC, GCGGTAGCGCTCCAGGCCAGCGGTGTCC-CAGATCT) with pEGFP-C2/Rab11b as template. pEGFP-C2/Rab11b S25N was generated by SDM (GGGGACTCAGGCGTGGGCAAGA ACAACCTGCTGTCGCGCTTCACC, GGTGAAGCGCGACAGCAGGTTGTT CTTGCCCACGCCTGAGTCCCC) with pEGFP-C2/Rab11b as template. pEGFP-C1/Rab25 T26N was generated by SDM (GCGAATC AGGTGTGGGGAAGAACAATCTACTCTCCCGATTCACG, CGTGAATCGG GAGAGTAGATTGTTCTTCCCCACACCTGATTCGCC) with pEGFP-C1/Rab25 as template.

2.2. Yeast two-hybrid assay

The yeast two-hybrid assay was performed using the Matchmaker Gold Yeast Two-Hybrid System according to the manufacturers' instructions (Clontech). Co-transformation of the bait and prey plasmids (diploids) were determined by the ability of *Saccharomyces cerevisiae* to grow on minimal media lacking Tryptophan (Trp) and Leucine (Leu). Protein:protein interactions were determined by the ability of the co-transformed yeast to grow on minimal media lacking Trp and Leu and activate transcription of one (HIS3; lacks Trp, Leu, His), two [HIS3/ADE2; lacks Trp, Leu, His and Adenine (Ade)] or three [HIS3/ADE2/AUR1-C; lacks Trp, Leu, His and Adenine (Ade) and includes Aurobasisin A (Aba)] reporter genes.

2.3. Cell biology and fluorescence microscopy

The HeLa (human cervical carcinoma) cell line was obtained from the ECACC (European Collection of Cell Cultures) and cultured in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) foetal bovine serum, 2 mM L-glutamine and 25 mM HEPES and grown in 5% CO_2 at 37 °C. Cells were transfected with plasmid constructs using TurboFect (Fermentas) as the transfection reagent. Fluorescence microscopy was performed as previously described [20]. The primary antibody used was mouse anti-Xpress (Invitrogen; R910–25). Images were recorded in a temperature-controlled environment (18 °C) using a Zeiss LSM 510 META confocal microscope fitted with a 63 × 1.4 plan apochromat lens. Images were processed using Zeiss LSM Image Browser or Zeiss ZEN Light Edition software and Adobe Illustrator. All micrographs shown are 3D-projections from the optical sections of the entire Z-stack.

3. Results

3.1. GRAB is a binding partner for Rab11a and Rab11b

A 2005 study that investigated pair-wise interactions among the products of 8100 Gateway-cloned open reading frames detected 2800 protein:protein interactions, among which was an interaction between Rab11 and GRAB [21]. To confirm and further explore this finding, we investigated the ability of GRAB to bind each of the Rab11 subfamily members using the Matchmaker Gold yeast two-hybrid system. We observed an interaction between GRAB and wild-type Rab11a and Rab11b, but not Rab25, when we assayed for expression of one (*HIS3*), two (*HIS3/ADE2*) or three (*HIS3/ADE2/AUR1-C*) protein:protein interaction reporter genes (Fig. 1). Using the yeast two-hybrid system these interactions were found to be nucleotide-independent as the dominant-positive Rab11a (Rab11a Q70L) and Rab11b (Rab11b Q70L) mutants, as well as the dominant-negative Rab11a (Rab11a S25N) and Rab11b (Rab11b S25N) mutants also bound GRAB (Fig. 1). As controls, we

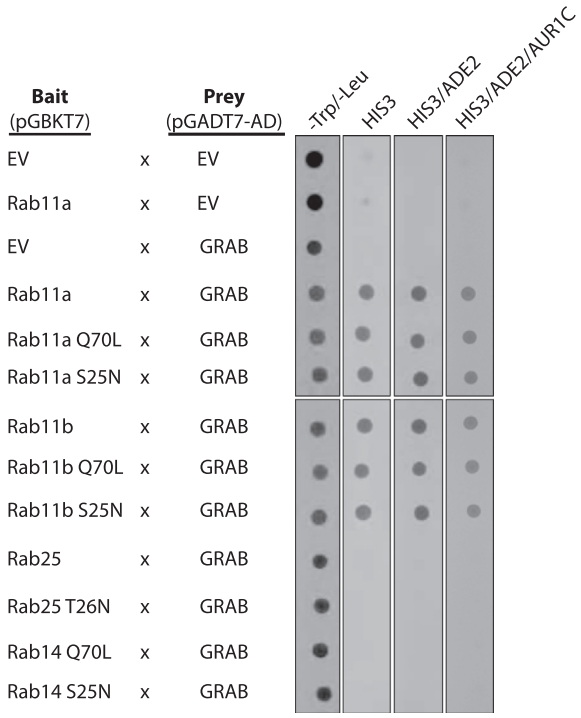

Fig. 1. GRAB binds Rab11a and Rab11b, but not Rab25. Matchmaker Gold yeast two-hybrid analyses of direct protein:protein interactions between GRAB and the indicated Rab proteins and Rab mutants. Co-transformation of the bait and prey plasmids (diploids) were determined by the ability of *S. cerevisiae* to grow on minimal media lacking Trp and Leu (*-Trp/-Leu*). Protein:protein interactions were determined by the ability of the co-transformed yeast to grow on minimal media lacking Trp and Leu and activate transcription of one (*HIS3*; lacks Trp, Leu, His), two [*HIS3/ADE2*; lacks Trp, Leu, His and Adenine (Ade)] or three [*HIS3/ADE2/AUR1-C*; lacks Trp, Leu, His and Adenine (Ade) and includes Aurobasisin A (Aba)] protein:protein interaction reporter genes. *EV*, empty vector.

confirmed that neither Rab11a nor GRAB activated expression of protein:protein interaction reporter genes when co-transformed with the cognate empty vector plasmids, and that GRAB did not bind Rab14 Q70L or Rab14 S25N (Fig. 1).

We next investigated the subcellular distribution of exogenous GRAB and found that when expressed either as an mCherry, Xpress or GFP-fusion that the protein was predominantly distributed to the cytoplasm, with a minor proportion occasionally labelling punctate structures (Fig. 2C). However, when GFP-fusions of wild-type Rab11a or Rab11b were co-expressed with mCherry-GRAB, GRAB was associated with endosomal membranes as evidenced by strong co-localisation with wild-type Rab11a and Rab11b (Fig. 2A and B). Strong co-localisation was also evident with the dominant-positive Rab11a and Rab11b mutants (Fig. 2A and B). In contrast, the dominant-negative Rab11a and Rab11b mutants, which were predominantly cytosolic albeit with a minor proportion on the perinuclear compartment, displayed little or no co-localisation with mCherry-GRAB, and GRAB was found to be largely cytosolic in cells expressing these mutants (Fig. 2A and B). In contrast to wild-type Rab11a and Rab11b, wild-type Rab25 did not co-localise with mCherry-GRAB, nor was it capable of shifting GRAB's distribution onto cellular membranes (Fig. 2D). The dominant-negative Rab25 mutant (Rab25 T26N) also failed to co-localise with mCherry-GRAB (Fig. 2D), nor did a GFP-fusion of wild-type Rab14 (data not shown).

To further explore the association of GRAB to Rab11-positive membranes, we examined the distribution of GRAB in cells exogenously expressing FIP3. FIP3 is a Rab11 effector protein that when overexpressed dramatically alters the morphology of the ERC and condenses ERC marker protein distribution, including Rab11 subfamily members, into the pericentrosomal region of the cell [7,22–25]. Thus, exogenous FIP3 can be used as a useful tool to investigate the association of putative ERC-linked proteins to that compartment. We found in cells co-expressing mCherry-fused FIP3 and GFP-fused GRAB that GRAB's distribution was altered by FIP3 expression whereby it was concentrated on the perinuclear FIP3-positive compartment (Fig. 2E; and compare with Fig. 2C).

3.2. Rab11a and Rab11b bind the carboxy-terminal region of GRAB

As previously reported, Rab3a binds GRAB within the region corresponding to amino acid residues 45–221 ([10] and Fig. 3A). The putative coiled-coil domain present in GRAB, which corresponds to GRAB's GEF domain, also lies within this region (Fig. 3A). To determine if Rab11a/Rab11b bind to the same region of GRAB as Rab3a, we generated a range of GRAB truncation mutants (Fig. 3A) and tested their ability to bind wild-type Rab11a and Rab11b using the yeast two-hybrid system. We found that Rab11a/Rab11b failed to bind the carboxy-terminally truncated $GRAB_{1–263}$ mutant or $GRAB_{73–163}$ (data not shown), and only weak binding was observed with the $GRAB_{1–331}$ mutant, indicating that the Rab11 and Rab3a-binding regions of GRAB are distinct (Fig. 3A and B). In contrast, Rab11a/Rab11b bound the amino-terminally truncated $GRAB_{164–382}$ mutant under the condition of highest stringency using this assay (Fig. 3A and B).

We next analysed the subcellular distribution of a selection of GRAB truncation mutants in HeLa cells transiently expressing either mCherry or GFP-fusions of various GRAB polypeptides. We found that, like the wild-type GRAB protein, each of the GRAB truncation mutants tested ($GRAB_{73–163}$, $GRAB_{1–263}$ and $GRAB_{1–331}$ $GRAB_{164–382}$) were predominantly distributed to the cytoplasm (Fig. 3C), with a minority of cells occasionally displaying a punctate pattern (data not shown). When these GRAB truncation mutants were co-expressed with GFP or mCherry-fused Rab11a, we found that the truncation mutants that failed to bind Rab11a/Rab11b in our yeast two-hybrid experiments ($GRAB_{1–263}$ and $GRAB_{1–331}$) remained predominantly distributed to the cytosol (Fig. 3D; note that GRAB truncations were occasionally distributed to puncta that were negative for Rab11a – as an example, these data are shown in the *GFP-*$GRAB_{1–331}$ panel). The shorter $GRAB_{1–163}$ and $GRAB_{73–163}$ truncation mutants also failed to co-localise with Rab11a (data not shown). In marked contrast, the amino-terminally truncated $GRAB_{164–382}$ polypeptide displayed a punctate pattern which displayed strong co-localisation with Rab11a. These data are consistent with our yeast two-hybrid data which found that $GRAB_{164–382}$ encompasses the Rab11-binding region of GRAB.

It has been previously demonstrated that deletion of a six amino acid motif (amino acids 300–305; *SLYNEF*) in the GRAB-related protein Rabin8 perturbs its interaction with Rab11 [26]. To determine if an equivalent motif may be present in GRAB, we analysed the Rabin8 and GRAB amino acid sequences by *ClustalW* multiple sequence alignment. These analyses revealed a short six amino acid motif present in GRAB (amino acid residues 223–228; *ILFAEF*) that shares 50% amino acid identity with the Rabin8 *SLYNEF* motif (Fig. 3E). Interestingly this short motif is predicted to form part of one of the short α-helices present in the carboxy-terminal Rab11-binding region of GRAB (Fig. 3A). To determine if this region plays a role in the ability of GRAB to bind Rab11a/Rab11b, we generated a GRAB deletion mutant which lacks this short motif ($GRAB_{\Delta223–228}$) and tested it in the yeast two-hybrid system. These data revealed that $GRAB_{\Delta223-228}$ is deficient in Rab11-binding ability (Fig. 3A and B). When we examined the distribution of $GRAB_{\Delta223–228}$ in transiently transfected HeLa cells, it was found to be predominantly distributed to the cytoplasm (Fig. 3C). However, when we co-transfected $GRAB_{\Delta223–228}$ with exogenous Rab11a, unlike the wild-type GRAB protein, $GRAB_{\Delta223–228}$ remains largely cytosolic

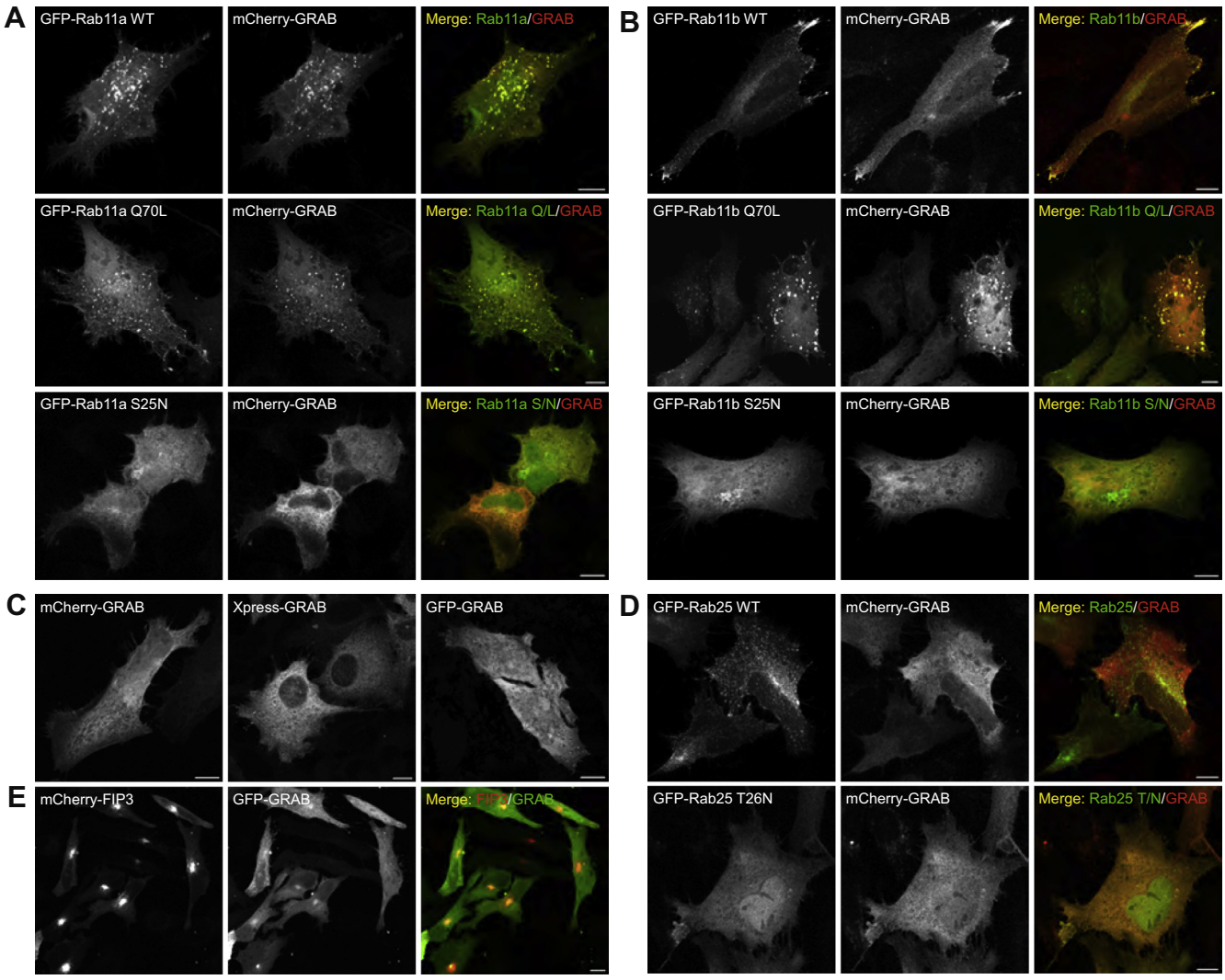

Fig. 2. GRAB co-localises with the Rab11a and Rab11b GTPases. (A–E) HeLa cells were transfected with constructs encoding the indicated proteins. 16–18 h post-transfection, the cells were processed for immunofluorescence microscopy and, where indicated, immunostained with an anti-Xpress antibody. Images were acquired by confocal microscopy and represent z-stacks. Scale bar indicates 10 μm.

and does not co-localise with Rab11a (Fig. 3D). These data are consistent with our yeast two-hybrid data which found that $GRAB_{\Delta 223-228}$ is deficient in Rab11-binding ability.

4. Discussion

In the present study, we have demonstrated that GRAB is a binding partner for the Rab11a and Rab11b GTPases and that exogenous expression of Rab11a or Rab11b shifts GRAB's distribution onto membranes. While our yeast two-hybrid studies indicate that these GTPases bind GRAB in a nucleotide-independent manner, our cell biology data indicate that the nucleotide-bound status of the Rab plays an important role. A preference for the GTP-bound Rab, rather than the GDP-bound form, would be consistent with a role for GRAB as a Rab11 effector protein, as is believed to be the case for the related Rabin8 protein [27].

In our studies, we did not observe an interaction between Rab25 and GRAB. This was somewhat surprising given that Rab25 is closely related to Rab11a and Rab11b and shares other effectors with these proteins [5,6]. This raises interesting questions as to how only two of the three Rab11 subfamily members can bind GRAB. Interestingly, at the protein level, GRAB was previously reported to be expressed in rat brain and testes but found to be largely absent from the other peripheral tissues tested [10]. Given that Rab25 is epithelial-specific [28], it is conceivable that Rab25 and GRAB would not bind one another. Notably, GRAB mRNA was found to be widely distributed in rat tissues [10] so we cannot exclude the possibility that GRAB functions in conjunction with Rab11 GTPases in a wide variety of tissues and that other factors such as the rate of turnover of GRAB in some tissues types accounts for the more confined GRAB expression profile observed at the protein level.

We have found here that Rab11a and Rab11b bind the carboxy-terminal region of GRAB. This region encompasses a number of short α-helices that may mediate the Rab11:GRAB interaction (Fig. 3A). Consistent with this, our GRAB deletion mutant ($GRAB_{\Delta 223-228}$) which lacks six amino acids that lie within one of these α-helices was deficient in Rab11-binding ability. As the carboxy-terminally truncated $GRAB_{1-331}$ polypeptide was also deficient in Rab11-binding ability, these data indicate that the region of GRAB between amino acid residues 164 and 331 is not sufficient to mediate a robust interaction between GRAB and Rab11a/Rab11b. This suggests that at least some of the amino acids between residues 332 and 382, a region that is also predicted to have α-helical structure (Fig. 3A), are also involved in these interactions.

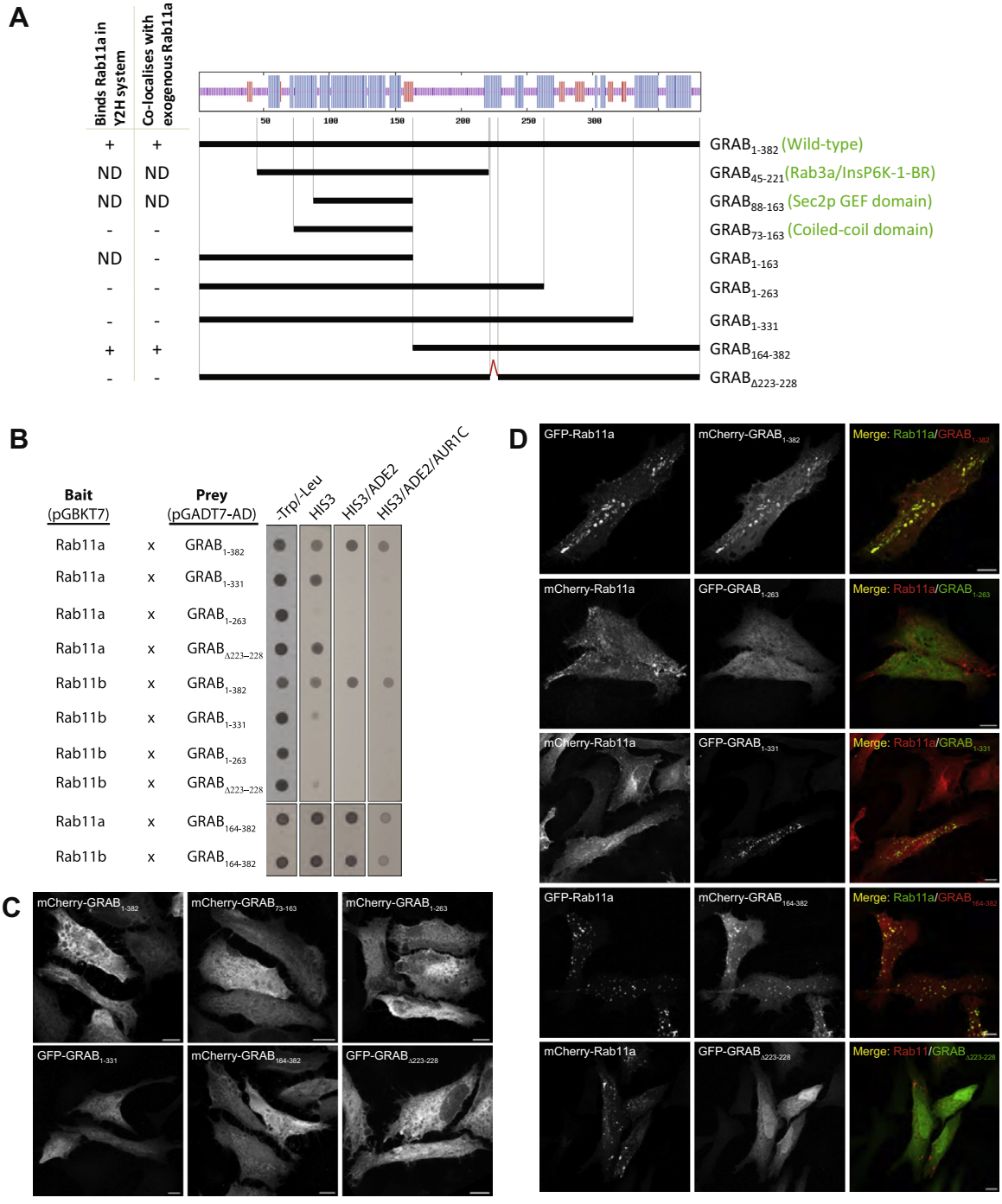

Fig. 3. Rab11a and Rab11b bind the carboxy-terminal region of GRAB. (A) Schematic representation of the domain architecture of GRAB and the GRAB truncation and deletion mutants analysed in this study. Shown at the top of the panel is the secondary structure of GRAB as predicted using the *Hierarchical Neural Network* algorithm. Purple lines represent predicted areas of random coil, red lines correspond to predicted areas of extended strands and red lines correspond to predicted areas of α-helices. *ND*, not done. *BR*, binding-region. (B) Matchmaker Gold yeast two-hybrid analyses of direct protein:protein interactions between Rab11a and Rab11b and the indicated GRAB polypeptides. Co-transformation of the bait and prey plasmids (diploids) were determined by the ability of *S. cerevisiae* to grow on minimal media lacking Trp and Leu (*-Trp/-Leu*). Protein:protein interactions were determined by the ability of the co-transformed yeast to grow on minimal media lacking Trp and Leu and activate transcription of one (*HIS3*; lacks Trp, Leu, His), two [*HIS3/ADE2*; lacks Trp, Leu, His and Adenine (Ade)] or three [*HIS3/ADE2/AUR1-C*; lacks Trp, Leu, His and Adenine (Ade) and includes Aurobasisin A (Aba)] protein:protein interaction reporter genes. *EV*, empty vector. (C and D) HeLa cells were transfected with constructs encoding the indicated proteins. 16–18 h post-transfection, the cells were processed for fluorescence microscopy. Images were acquired by confocal microscopy and represent z-stacks. Scale bar indicates 10 μm. (E) Portion of a *ClustalW* multiple sequence alignment of the Rabin8 and GRAB amino acid sequences. Identities are highlighted in black and similarities are highlighted in grey. The red boxed region indicates the six amino acid motifs in Rabin8 and GRAB that when deleted disrupts their interactions with Rab11a. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Rab3a has been previously demonstrated to bind the amino-terminal, GEF domain-containing, region of GRAB [10]; whereas, here, we have implicated the carboxy-terminal region of GRAB in mediating the Rab11a/Rab11b interactions. This identifies GRAB as a dual Rab-binding protein that could potentially couple the action of Rab GTPases in cells. While we do not know the functional significance of the GRAB:Rab11 interactions it is tempting to speculate that GRAB could function in a Rab cascade in which its activity as an effector for one Rab (Rab11a/Rab11b) could be coupled to its activity as a GEF to another (Rab3a/Rab8a/Rab8b). In this respect, Rabin8 coordinates Rab11 and Rab8 function during primary ciliogenesis whereby Rab11 regulates the trafficking of Rabin8 and then stimulates its GEF activity towards Rab8 [27,29,30]. It remains to be determined if GRAB plays a role in this, or other, Rab11 and Rab8-controlled cellular processes. In a similar way, it is possible that GRAB might function in a Rab cascade involving Rab11 and Rab3. Indeed, Rab11b, which like GRAB and Rab3 is enriched in the brain, co-localises with Rab3 on secretory vesicles in PC12 cells, and on mature synaptic vesicles in brain tissue, and plays a role in calcium-mediated exocytosis [31–34]. Determining if GRAB coordinates Rab function in these and other cellular processes represent exciting challenges for the future.

Acknowledgments

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References

[1] E.E. Kelly, C.P. Horgan, B. Goud, M.W. McCaffrey, The Rab family of proteins: 25 years on, Biochem. Soc. Trans. 40 (2012) 1337–1347.
[2] T.H. Klopper, N. Kienle, D. Fasshauer, S. Munro, Untangling the evolution of Rab G proteins: implications of a comprehensive genomic analysis, BMC Biol. 10 (2012) 71.
[3] F. Barr, D.G. Lambright, Rab GEFs and GAPs, Curr. Opin. Cell Biol. 22 (2010) 461–470.
[4] C.P. Horgan, M.W. McCaffrey, Rab GTPases and microtubule motors, Biochem. Soc. Trans. 39 (2011) 1202–1206.
[5] C.P. Horgan, M.W. McCaffrey, The dynamic Rab11-FIPs, Biochem. Soc. Trans. 37 (2009) 1032–1036.
[6] E.E. Kelly, C.P. Horgan, M.W. McCaffrey, Rab11 proteins in health and disease, Biochem. Soc. Trans. 40 (2012) 1360–1367.
[7] C.P. Horgan, S.R. Hanscom, R.S. Jolly, C.E. Futter, M.W. McCaffrey, Rab11-FIP3 links the Rab11 GTPase and cytoplasmic dynein to mediate transport to the endosomal-recycling compartment, J. Cell Sci. 123 (2010) 181–191.
[8] O. Ullrich, S. Reinsch, S. Urbe, M. Zerial, R.G. Parton, Rab11 regulates recycling through the pericentriolar recycling endosome, J. Cell Biol. 135 (1996) 913–924.
[9] C.P. Horgan, M.W. McCaffrey, Endosomal trafficking in animal cytokinesis, Front. Biosci. (Schol Ed) 4 (2012) 547–555.
[10] H.R. Luo, A. Saiardi, E. Nagata, K. Ye, H. Yu, T.S. Jung, X. Luo, S. Jain, A. Sawa, S.H. Snyder, GRAB: a physiologic guanine nucleotide exchange factor for Rab3A, which interacts with inositol hexakisphosphate kinase, Neuron 31 (2001) 439–451.
[11] C. Walch-Solimena, R.N. Collins, P.J. Novick, Sec2p mediates nucleotide exchange on Sec4p and is involved in polarized delivery of post-Golgi vesicles, J. Cell Biol. 137 (1997) 1495–1509.
[12] K. Hattula, J. Furuhjelm, A. Arffman, J. Peranen, A Rab8-specific GDP/GTP exchange factor is involved in actin remodeling and polarized membrane transport, Mol. Biol. Cell 13 (2002) 3268–3280.
[13] A.C. Figueiredo, C. Wasmeier, A.K. Tarafder, J.S. Ramalho, R.A. Baron, M.C. Seabra, Rab3GEP is the non-redundant guanine nucleotide exchange factor for Rab27a in melanocytes, J. Biol. Chem. 283 (2008) 23209–23216.
[14] S. Yoshimura, A. Gerondopoulos, A. Linford, D.J. Rigden, F.A. Barr, Family-wide characterization of the DENN domain Rab GDP–GTP exchange factors, J. Cell Biol. 191 (2010) 367–381.
[15] P. Lall, C.P. Horgan, S. Oda, E. Franklin, A. Sultana, S.R. Hanscom, M.W. McCaffrey, A.R. Khan, Structural and functional analysis of FIP2 binding to the endosome-localised Rab25 GTPase, Biochim. Biophys. Acta (2013).
[16] A.J. Lindsay, A.G. Hendrick, G. Cantalupo, F. Senic-Matuglia, B. Goud, C. Bucci, M.W. McCaffrey, Rab coupling protein (RCP), a novel Rab4 and Rab11 effector protein, J. Biol. Chem. 277 (2002) 12190–12199.
[17] E.E. Kelly, C.P. Horgan, C. Adams, T.M. Patzer, D.M. Ni Shuilleabhain, J.C. Norman, M.W. McCaffrey, Class I Rab11-family interacting proteins are binding targets for the Rab14 GTPase, Biol. Cell 102 (2010) 51–62.
[18] M. Wilcke, L. Johannes, T. Galli, V. Mayau, B. Goud, J. Salamero, Rab11 regulates the compartmentalization of early endosomes required for efficient transport from early endosomes to the trans-golgi network, J. Cell Biol. 151 (2000) 1207–1220.
[19] C.P. Horgan, S.R. Hanscom, E.E. Kelly, M.W. McCaffrey, Tumor susceptibility gene 101 (TSG101) is a novel binding-partner for the class II Rab11-FIPs, PLoS One 7 (2012) e32030.
[20] C.P. Horgan, S.R. Hanscom, M.W. McCaffrey, Dynein LIC1 localizes to the mitotic spindle and midbody and LIC2 localizes to spindle poles during cell division, Cell Biol. Int. 35 (2011) 171–178.
[21] J.F. Rual, K. Venkatesan, T. Hao, T. Hirozane-Kishikawa, A. Dricot, N. Li, G.F. Berriz, F.D. Gibbons, M. Dreze, N. Ayivi-Guedehoussou, N. Klitgord, C. Simon, M. Boxem, S. Milstein, J. Rosenberg, D.S. Goldberg, L.V. Zhang, S.L. Wong, G. Franklin, S. Li, J.S. Albala, J. Lim, C. Fraughton, E. Llamosas, S. Cevik, C. Bex, P. Lamesch, R.S. Sikorski, J. Vandenhaute, H.Y. Zoghbi, A. Smolyar, S. Bosak, R. Sequerra, L. Doucette-Stamm, M.E. Cusick, D.E. Hill, F.P. Roth, M. Vidal, Towards a proteome-scale map of the human protein–protein interaction network, Nature 437 (2005) 1173–1178.
[22] C.P. Horgan, S.R. Hanscom, R.S. Jolly, C.E. Futter, M.W. McCaffrey, Rab11-FIP3 binds dynein light intermediate chain 2 and its overexpression fragments the Golgi complex, Biochem. Biophys. Res. Commun. 394 (2010) 387–392.
[23] C.P. Horgan, A. Oleksy, A.V. Zhdanov, P.Y. Lall, I.J. White, A.R. Khan, C.E. Futter, J.G. McCaffrey, M.W. McCaffrey, Rab11-FIP3 is critical for the structural integrity of the endosomal recycling compartment, Traffic 8 (2007) 414–430.
[24] C.P. Horgan, M. Walsh, T.H. Zurawski, M.W. McCaffrey, Rab11-FIP3 localises to a Rab11-positive pericentrosomal compartment during interphase and to the cleavage furrow during cytokinesis, Biochem. Biophys. Res. Commun. 319 (2004) 83–94.
[25] C.P. Horgan, T.H. Zurawski, M.W. McCaffrey, Purification and functional properties of Rab11-FIP3, Methods Enzymol. 403 (2005) 499–512.
[26] S. Feng, A. Knodler, J. Ren, J. Zhang, X. Zhang, Y. Hong, S. Huang, J. Peranen, W. Guo, A Rab8 guanine nucleotide exchange factor-effector interaction network regulates primary ciliogenesis, J. Biol. Chem. 287 (2012) 15602–15609.
[27] A. Knodler, S. Feng, J. Zhang, X. Zhang, A. Das, J. Peranen, W. Guo, Coordination of Rab8 and Rab11 in primary ciliogenesis, Proc. Natl. Acad. Sci. USA 107 (2010) 6346–6351.
[28] J.R. Goldenring, K.R. Shen, H.D. Vaughan, I.M. Modlin, Identification of a small GTP-binding protein, Rab25, expressed in the gastrointestinal mucosa, kidney, and lung, J. Biol. Chem. 268 (1993) 18419–18422.
[29] S. Chiba, Y. Amagai, Y. Homma, M. Fukuda, K. Mizuno, NDR2-mediated Rabin8 phosphorylation is crucial for ciliogenesis by switching binding specificity from phosphatidylserine to Sec15, EMBO J. 32 (2013) 874–885.
[30] C.J. Westlake, L.M. Baye, M.V. Nachury, K.J. Wright, K.E. Ervin, L. Phu, C. Chalouni, J.S. Beck, D.S. Kirkpatrick, D.C. Slusarski, V.C. Sheffield, R.H. Scheller, P.K. Jackson, Primary cilia membrane assembly is initiated by Rab11 and transport protein particle II (TRAPPII) complex-dependent trafficking of Rabin8 to the centrosome, Proc. Natl. Acad. Sci. USA 108 (2011) 2759–2764.
[31] M.V. Khvotchev, M. Ren, S. Takamori, R. Jahn, T.C. Sudhof, Divergent functions of neuronal Rab11b in Ca^{2+}-regulated versus constitutive exocytosis, J. Neurosci. 23 (2003) 10531–10539.
[32] N.J. Pavlos, M. Gronborg, D. Riedel, J.J. Chua, J. Boyken, T.H. Kloepper, H. Urlaub, S.O. Rizzoli, R. Jahn, Quantitative analysis of synaptic vesicle Rabs uncovers distinct yet overlapping roles for Rab3a and Rab27b in Ca^{2+}-triggered exocytosis, J. Neurosci. 30 (2010) 13441–13453.
[33] N.J. Pavlos, R. Jahn, Distinct yet overlapping roles of Rab GTPases on synaptic vesicles, Small GTPases 2 (2011) 77–81.
[34] M. Geppert, Y. Goda, C.F. Stevens, T.C. Sudhof, The small GTP-binding protein Rab3A regulates a late step in synaptic vesicle fusion, Nature 387 (1997) 810–814.