Rab regulation by GEFs and GAPs during membrane traffic

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Rab GTPases and their regulatory proteins play a crucial role in vesicle-mediated membrane trafficking. During vesicle membrane tethering Rab GTPases are activated by GEFs (guanine nucleotide exchange factors) and then inactivated by GAPs (GTPase activating proteins). Recent evidence shows that in addition to activating and inactivating Rab GTPases, both Rab GEFs and GAPs directly contribute to membrane tethering events during vesicle traffic. Other studies have extended the range of processes in which Rabs function, and revealed roles for Rabs and their GAPs in the regulation of autophagy. Here we will discuss these advances and the emerging relationship between the domain architectures of Rab GEFs and vesicle coat protein complexes linked with GTPases of the Sar, ARF and Arl families in animal cells.

Rab GTPases underpin eukaryotic membrane traffic

Soon after their discovery three decades ago, it was appreciated that Rab GTPases localise to specific membrane domains and organelle membranes within eukaryotic cells [1]. This led to the idea that Rab GTPases proofread the interaction between a vesicle and its target membrane prior to the membrane fusion reaction and thus act as key regulators of membrane traffic [2]. Subsequently, this idea has been further developed based on observations that Rabs recruit the components of physical tethers to membrane surfaces during vesicle docking [1]. To fulfil this function, Rabs interact with specific effector proteins and must be activated and inactivated in a manner that is coordinated with the processes of vesicle formation, by coat protein complexes, and vesicle fusion, mediated by the SNARE proteins (Figure 1A and 1B).

As reviewed extensively elsewhere, the required spatial and temporal control is provided by specific GEF and GAPs regulators [3, 4].

Co-evolution of Rab GEFs and vesicle coat complexes

Phylogenetic analysis reconstructing the evolution of Rab GTPases suggests that the last eukaryotic common ancestor possessed 20 such GTPases and had all of the core organelles associated with eukaryotic cells today – endoplasmic reticulum, Golgi, endosomes, recycling endosomes, lysosomes, lysosome-like storage compartments, and cilia [5]. A complement of GEF proteins required to activate these Rabs at specific organelle and vesicle membranes is also necessary. These include the TRAPPI/II Rab1/11 GEFs [6, 7], the VPS9 domain family of Rab5/21 GEFs [8-10], the SEC2 Rab8 GEF [11, 12], the RIC1-RGP1 Rab6 GEF [13] and the MON1-CCZ1/HPS1-HPS4 group of Rab7/32/38 GEFs [14-16]. Metazoans have elaborated this basic complement, and humans have over 60 Rab GTPases [5]. This has been matched by an increase in GEF complexes, notably the DENN family of GEFs which act on diverse Rabs involved in polarised transport and endocytosis [17, 18]. Additional GEFs, unrelated in structure to those in the last eukaryotic common ancestor have arisen for some Rabs. In metazoans, Rab11 has two GEFs, the ancestral TRAPPII complex and the SH3BP5/REI-1 GEF first discovered in *C. elegans* [19, 20]. Exactly why metazoans have two distinct Rab11 GEFs remains unclear, since work in *D. melanogaster* shows that while Rab11 is an essential gene, SH3BP5 and the specific subunits of TRAPPII are not essential and instead they act redundantly [21]. A simple explanation for these findings is that this arrangement increases the diversity of regulatory pathways controlling the Rab and thus reflects underlying cell- or tissue-specific processes. However, what these processes are

remains uncertain and further research in different model organisms will be required to address this question. Slightly more puzzling is the loss of an obvious Rab8 GEF SEC2 homologue but retention of Rab8 in fruit flies. Again, further work is needed to explain this, but the discovery of SH3BP5 shows that unknown GEFs exist and thus may spur attempts to find the missing fly Rab8 GEF.

A similar analysis of the evolution of vesicle coats has revealed that the subunits of these protein complexes arose from two families that can be linked with specific small GTPases [22, 23]. The COPI family includes the Golgi-associated COPI coat, clathrin cargo-adaptor protein (AP) complexes and an ancestral coat referred to as TSET. ARF-family GTPases are involved in controlling the membrane recruitment of both COPI and the clathrin APs. The COPII family is more diverse in terms of function and includes the ER-Golgi COPII coat as well as the HOPS-CORVET endosomal-vacuole membrane tethering complex and the nuclear pore complex (NPC) required for regulated exchange of proteins and RNA between the cytoplasm and the nucleus. A different GTPase, Sar1, is required for recruitment of COPII to membranes, whereas transport through NPCs requires the GTPase Ran and HOPS-CORVET interact with endocytic Rabs. These two coat families are highly modular in terms of their structures but are formed from a restricted subset of common domains contributing specific features to the coat assembly. The edges of the coat lattice structures are typically α-helical solenoids and the vertices joining these edges formed by 7-bladed β-propellors. In addition, some members of the COPI and COPII families have longin/roadblock domains. Intriguingly, these domains are also present or predicted in many Rab GEFs [24, 25]. X-ray crystallography of Rab-GEF complexes has revealed the presence of heterodimeric longin-domain pairs in the Rab1-binding site of the GEF TRAPPI [6] and the Rab7-binding site of

the MON1-CCZ1 GEF [16]. The TRAPPI active site is formed by a heterodimer of TRAPPC1 and TRAPPC4 [6]. Two other subunits TRAPPC2 and TRAPPC2L also have longin domains [24], but don't appear to play a direct role in GEF activity towards Rab1 [26]. In the case of the dimeric MON1-CCZ1 GEF and the related Rab32/38 GEF HPS1-HPS4, each subunit is thought to contribute a single longin domain [14, 15, 25]. Furthermore, single longin domains are found in a large family of DENN GEFs. The structure of a Rab35-DENND1 GEF complex shows that in this case one longin domain forms part of the active site [27]. While less is known about other Rab GEFs, the RAB3GAP2 subunit of the Rab18 GEF associated with regulation of the ER is predicted to contain a 7-bladed β-propellor but this has not been directly confirmed by a structure. However, disease causing mutations map to this region suggesting it is of functional importance [28].

As previously noted, longin/roadblock domains are found in regulatory systems or proteins interacting with other families of GTPases, and not only Rab GEFs [24, 25]. This provides some support to the view that the diverse GTPase regulated membrane vesicle coats and Rab-regulated tethering systems arose early in eukaryote evolution from a common ancestor. Stronger evidence for this view comes from recent investigation of the *Lokiarcheota* indicating the ancestral COPII and TRAPP pathways, and hallmark domains, were present in a subset of archael lineages prior to emergence of the first eukaryotic cell [29]. Following this line of reasoning, one might imagine that similarities exist between Rab GEFs and other groups of vesicle coat protein complexes. Indeed, the RIC1-RGP1 Rab6 GEF is predicted to contain tandem COPI-like β-propellors in the RIC1 subunit while RGP1 is an arrestin-like protein [30]. Arrestin domains were originally identified because of their role in the sorting of activated G-protein coupled receptors into endocytic

vesicles and have subsequently been found in subunits of the retromer and retromer-related endocytic sorting complexes [31, 32]. Retromer and related complexes form a distinct class of membrane coat to the clathrin adaptor proteins, COPI or COPII [33]. It is therefore intriguing that the RIC1-RGP1 Rab6 GEF complex has features reminiscent of both the COPI/II and retromer family of coats. This may indicate that other members of the wider group of RIC1-RGP1 and arrestin/retromer-like complexes have Rab GEF activity.

Rab GEF targeting mechanisms

Other than sharing a common origin, there are other plausible reasons why Rab GEFs and vesicle coat subunits are formed from related domains and have similar architectures. One such possibility is that Rab GEFs need to be compatible with vesicle coat assemblies so that they can be incorporated into forming vesicles. This would ensure that all vesicles contain the GEF component required to generate an active Rab signal and thus trigger membrane tethering. The best support for this view comes from observations that the Rab1 GEF TRAPPI is a component of COPII vesicles and has been found to interact with the SEC23 component of the vesicle coat [34]. In addition the related TRAPPIII complex interacts with SEC13/31 COPII components via the TRAPPC12 subunit [21] and the Rab35 GEF DENND1 is found in complex with plasma-membrane clathrin AP2 complexes [17, 35]. Whether these are properties shared more generally requires further investigation and understanding of the mechanisms by which Rab GEFs are recruited to vesicle and organelle membranes. While many questions remain unanswered, a number of common themes have emerged. Rab GEFs target through interaction with vesicle coat components, or organelle specific landmarks including phosphatidylinositol (PI)

phosphates and GTPases of the ARF or Rab families. At the medial Golgi, the mammalian form of the Rab6 GEF RIC1-RGP1 has been shown to bind to a Golgi Rab33B and the Rab8 GEF SEC2 interacts with the Golgi lipid PI-4P. On early endosomes, the VPS9 domain Rab5 GEF Rabex is part of a complex interacting with ubiquitinated cargo proteins and PI3-P. The maturation to late endosomes and lysosomes is then triggered by the Rab5 and PI3-P dependent recruitment of the MON1-CCZ1 Rab7 GEF. It is therefore important to understand how these transient organelle specific landmarks are generated if we are to fully understand how Rabs direct membrane transport.

Roles for GEFs and the Rab hypervariable region in membrane targeting

The biochemical properties of Rabs necessitate a GEF activity to promote rapid nucleotide exchange. However, this does not mean that the GEF alone is sufficient to explain Rab targeting. As discussed elsewhere, earlier literature based on domain swap experiments using endocytic Rabs described a role for the hypervariable C-terminal region (HVR) of the Rabs in targeting [36]. More recently using cell and chemical biology approaches the Rab35 HVR was shown to be necessary for plasma membrane targeting due to electrostatic interaction with negatively charged lipids, and the equivalent region of Rab7 to contribute to late-endosome localisation [37]. An elegant chemical biology approach exploiting acryl-bearing GTP and GDP derivatives that can be covalently linked with strategically placed cysteines within the GTPase of interest has been used to study targeting of Rab5 *in vivo* [38]. This approach revealed that “active” Rab5-aGppNHpGG although membrane associated did not show the expected early endosome localization whereas “inactive” Rab5-aGDP-GG targeted to the Golgi [38]. This fits with cell biology showing that inactive

mutants of Rab5 or wild-type Rab5 inactivated by a cognate GAP mis-localise to the Golgi [39]. Thus, the GTPase nucleotide state alone does not confer accurate organelle localisation [38, 40], and therefore there is a missing component, most likely a GEF. Indeed, other evidence shows that GEFs are sufficient, in some cases, to direct Rabs to a specific membrane [40, 41]. A series of studies have examined Rab targeted for well-characterised Rab-GEF pairs, Rab5A-Rabex5, Rab1A-DrrA, Rab8-Rabin 8 (SEC2), Rab35-DENND1 and Rab18-RAB3GAP1/2 [28, 41, 42]. In all of these cases specific targeting of the GEFs to mitochondria resulted in re-direction of the cognate Rabs to the same membrane.

A way to reconcile these apparently different lines of evidence comes from recent work on the TRAPPII GEF showing that the specificity for the Rab11 family GTPases Ypt31/32 comes from the HVR, with the precise length acting as a specific constraint controlling access to the GEF active site [43]. This clearly shows that specific features of the Rab outwith the core GTP-binding domain are necessary for proper targeting, and are in fact crucial for GEF specificity. Thus, the composition and length of the Rab HVR can play a crucial role in determining the membrane-interaction and GEF specificity for Rab11-TRAPPII. Whether or not this is more generally true requires careful investigation of other Rab-GEF systems.

Direct roles for Rab GEFs and GAPs in membrane tethering

GEFs and GAPs are typically viewed as regulators of the activation state of Rabs [44], which then act in the physical tethering pathway. In some instances, this separation of function is a simplification (Figure 1C and 1D). TRAPPI is recruited to coated COPII vesicles emerging from the ER through interaction with the SEC23 subunit and plays a direct role in their tethering, in addition to its role in Rab1

activation [34]. Thus, in this case the GEF directly initiates the tethering reaction and then further facilitates tethering through Rab activation. Rab GAPs, have like the equivalent factors for Ras, been seen as factors terminating or limiting the time in which the GTPase acts. As already mentioned, this fits with the notion that GTPase activity acts as a timer proofreading the tethering mechanism required for the fidelity of membrane traffic. However, recent evidence paints a somewhat different picture of Rab GAP function. Homozygous mutations in a trans-Golgi localise Rab GAP TBC1D23 contribute to the human developmental disorder pontocerebellar hypoplasia [45]. Other studies then revealed that TBC1D23 is important in a pathway tethering clathrin-AP1 vesicles to the trans-Golgi and the recycling of lysosomal hydrolases by the CI-MPR [46]. What differentiates TBC1D23 from other Rab GAPs is the absence of two key residues required to promote GTP hydrolysis, making it unlikely this role vesicle tethering was due to regulation of a Rab. Instead, TBC1D23 is recruited to the trans-Golgi by the coiled-coil proteins golgin-97 and golgin-245 and promotes rather than terminates vesicle tethering [46]. The catalytically inactive TBC1-domain directly contacts a conserved N-terminal F++L motif in the golgins, whereas the C-terminal domain simultaneously interacts with the FAM21 subunit of the WASH complex found on endosomal vesicles. Additionally, there is a region of TBC1D23 binding to a complex of FAM91A, WDR11 and C17orf75 proposed to capture clathrin-AP1 coated vesicles [47, 48]. By acting as a bridge between these different protein complexes TBC1D23 holds transport vesicles in close proximity to the trans-Golgi.

Do these observations have wider relevance for our understanding of the role of catalytically active TBC1-domain proteins? TBC1D23 arose from a catalytically active ancestor that would have been transiently recruited to promote Rab

inactivation and thus terminate a tethering interaction. Presumably, the TBC1D23-dependent tether is derived from such a system. Alternatively, TBC1-domain Rab GAPs may generally form part of tethering complexes and this is an area that requires further study.

Emerging roles for GEFs and GAPs in autophagy

Rabs are generally associated with vesicle trafficking between membrane compartments during cell growth through regulation of secretion and endocytosis. However, these pathways also contribute to the process of autophagy, where cells selectively encapsulate regions of the cytoplasm and organelle membranes. One of the first links was the finding that Golgi-associated Rab33B binds to the autophagy protein ATG16L and modulates macroautophagy [49]. This was then extended by the finding that the Rab GAP TBC1D25 regulates autophagosome maturation, most likely through regulation of Rab33B [50]. A systematic study of Rab GAPs then found that 14 of these proteins were associated with the small ubiquitin-like modifier ATG8/LC3, which is conjugated to pre-autophagosomal membrane structures (PAS) [51]. One of these TBC1D5, a GAP for Rab7, was found to contain two LC3-interacting regions (LIR). TBC1D5 binds to VPS29 subunit of the endosomal retromer complex and its GAP activity towards Rab7 controls cargo recycling between endosomes and the trans-Golgi [52-54]. In addition, it is crucial for correct sorting of the autophagy related transmembrane protein ATG9 and autophagosome formation [55]. A second Rab7 GAP TBC1D2A has also been found to show LIR-dependent interaction with ATG8/LC3, suggesting Rab7 activity can be modulated by multiple routes [56]. Other evidence shows that Rab7 activation is required for fusion of autophagosomes with endosomes and can also be detected on

autophagosomes [57]. This is explained by MON1-CCZ1 recruitment to PAS through the CCZ1 LIR, which results in ATG8-dependent Rab7/Ypt7 activation [58]. This pool of activated Rab7/Ypt7 then recruits the HOPS tethering complex, promoting SNARE-dependent fusion of autophagosomes with the lysosome or vacuole in yeast [59, 60]. Further Rab GEF and GAP regulators have also been implicated in autophagy. TBC1D14 acts together with Rab11 and the autophagy kinase ULK1 on recycling endosomes and promotes autophagosome formation [61]. Moreover, TBC1D14 interacts with the TRAPPC8 subunit of the variant TRAPPIII complex and together they modulate ATG9 traffic between recycling endosomes and the Golgi [62-65]. TRAPPIII has also been implicated in initiation of PAS formation through delivery of endoplasmic reticulum-derived vesicles [66, 67]. Other findings link the Rab12 GEF DENND3 to ULK1-signalling and the transport of autophagosomes towards lysosomes [68].

A selected subset of Rabs and GEF and GAP regulators therefore play a role in redirecting endosome recycling pathways, Golgi and lysosomal traffic to the autophagosome during autophagy. However, the full picture of how these autophagy-associated pathways are regulated and operate alongside the normal vesicle transport processes will only become clear with further investigation.

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Figure

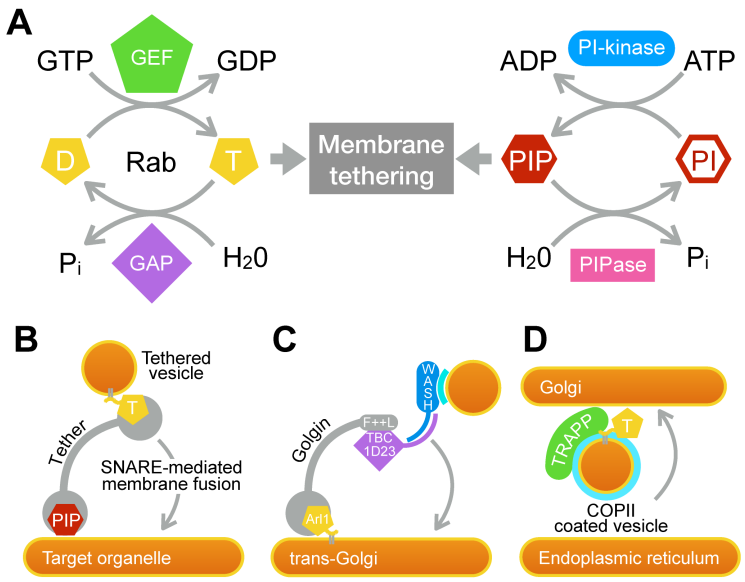

Figure 1. The role of Rab GEFs and GAPs in vesicle tethering

A. Membrane tethering requires transient landmarks or signals on the two membranes that should come together. During this process the generation of an active GTP-bound population (T) of a Rab (yellow) is promoted by a specific GEF (green). This active Rab is returned to the GDP-bound form (D) and inactivated by a GAP (purple). A parallel pathway creates a second landmark, in the example shown a specific phosphatidylinositol phosphate (PIP) by a PI-kinase (blue). This PIP is then removed by a specific PIP phosphatase (PIPase; pink). **B.** A canonical Rab and organelle membrane landmark mediated tethering pathway. In this case a PI-lipid (PIP) is shown, see text for details of other equivalent signals. Tethering complexes contain binding domains for the active Rab and the specific organelle landmark. **C.** Non-canonical golgin-GAP (golgin97/245-TBC1D23) independent of Rab activity and **D.** GEF-coat (TRAPP-COPII) mediated vesicle tethering. The COPII coat is depicted in light blue. TRAPP initiates Rab1 dependent tethering in a subsequent step that is not depicted.

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