Rab GTPases and membrane identity: Causal or inconsequential?

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Rab GTPases are highly conserved components of vesicle trafficking pathways that help to ensure the fusion of a vesicle with a specific target organelle membrane. Specific regulatory pathways promote kinetic proofreading of membrane surfaces by Rab GTPases, and permit accumulation of active Rabs only at the required sites. Emerging evidence indicates that Rab activation and inactivation are under complex feedback control, suggesting that ultrasensitivity and bistability, principles established for other cellular regulatory networks, may also apply to Rab regulation. Such systems can promote the rapid membrane accumulation and removal of Rabs to create time-limited membrane domains with a unique composition, and can explain how Rabs define the identity of vesicle and organelle membranes.

Rab GTPases regulate membrane tethering and vesicle fusion

Eukaryotic cells are defined in part by their complex membrane organelles. This organization permits the coexistence of different chemical environments within the same cell. For example, the endoplasmic reticulum (ER) is a neutral pH, reducing environment containing chaperones conducive to protein folding and the formation of disulfide bonds, whereas the lysosomes are ∼pH 5 and contain catabolic enzymes maximally active at acidic pH. Though valuable, this organization requires some form of active transport machinery for the exchange of material between these compartments because large hydrophilic molecules such as proteins cannot easily cross membranes. This transfer of molecules between compartments is achieved by vesicular transport systems that use cytosolic coat protein complexes to select small regions of membrane and shape these into defined 40–80-nm-diameter transport vesicles (Bonifacino and Glick, 2004; Faini et al., 2013). Vesicle coats contain binding sites for specific transport sequences, and thus only transfer a subset of proteins into the vesicle. Once produced, these vesicles have to identify, tether to, and then fuse with a specific target organelle (Zerial and McBride, 2001). Research over many years has defined small transmembrane proteins (SNAREs) and a set of accessory factors as the minimal machinery for membrane fusion (McNew et al., 2000; Shi et al., 2012). Tethering is a less well-defined event involving the Rab GTPases and effector protein complexes, typically large extended molecules thought to bridge the space between two approaching membranes (Gillingham and Munro, 2003).

Rab GTPases were first linked to vesicle transport by groundbreaking genetic screens for mutants defective in protein secretion (Novick et al., 1980; Salminen and Novick, 1987). Sec4, Rab8 in humans, was found to function in the terminal step of the secretory pathway, delivery of Golgi-derived transport vesicles to the cell surface (Salminen and Novick, 1987; Goud et al., 1988). Ypt1, Rab1 in humans, was then shown to regulate secretion at the Golgi apparatus (Segev et al., 1988; Bacon et al., 1989). These findings led to an influential model for Rab function in which the cycle of GTPase activation and inactivation is coupled to recognition events in vesicle docking (Bourne, 1988). Consistent with the idea that they control vesicle targeting, work in mammalian cells then showed that there is a large family of highly conserved Rab GTPases, each with a specific subcellular localization (Chavrier et al., 1990). A series of seminal studies has since provided direct evidence that Rab1 and Rab5 promote membrane fusion (Gorvel et al., 1991; Segev, 1991) by regulating the activation and engagement of SNAREs (Lian et al., 1994; Søgaard et al., 1994), as a consequence of recruiting tethering factors to membrane surfaces (Segev, 1991; Sapperstein et al., 1996; Cao et al., 1998; Christoforidis et al., 1999; McBride et al., 1999; Allan et al., 2000; Shorter et al., 2002). Similar findings were also made for the Rab Ypt7, which functions in vacuole docking in yeast (Price et al., 2000; Ungermann et al., 2000), a system that allows direct visualization of docked or tethered intermediates due to the large size of the membrane structures (Wang et al., 2002).

The evidence that Rabs function upstream of SNARE protein in vesicle trafficking pathways has led to the notion that Rabs help to define the identity of vesicle and organelle

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Abbreviations used in this paper: GAP, GTPase-activating protein; GDI, guanine nucleotide displacement inhibitor; GEF, guanine nucleotide exchange factor; TRAPP, transport protein particle.

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membranes (Pfeffer, 2001; Zerial and McBride, 2001). This is best exemplified by the early endocytic pathway, where the identity of early and late endosomes is thought to be determined by Rab5 and Rab7, respectively (Rink et al., 2005). However, in most other cases it remains unclear if this is a causal relationship, where the Rab directly defines the identity of the membrane rather than acting as an upstream regulator of vesicle targeting before the SNARE-mediated membrane fusion event. In addition to Rabs, GTPases of the Arf/Arl family and specific phosphoinositide lipids have also been proposed to act in specifying membrane identity (Munro, 2002; Di Paolo and De Camilli, 2006). It therefore seems likely that no single factor can explain how membrane identity is achieved in vesicle transport, and that Rabs, phosphoinositides, and other factors act in concert.

Rab GEFs provide the minimal machinery for targeting and activation

Despite the progress in defining Rab function, the claim that Rab GTPases define organelle identity therefore remains premature due to crucial unanswered questions. In particular, the issue of how Rabs are targeted to specific organelles, or even restricted to subdomains of these organelles, has remained problematic. Initial work using chimeric GTPases suggested that the variable C-terminal region of the different Rabs provided a targeting mechanism (Chavrier et al., 1991). However, subsequent work indicated that this failed to provide a general mechanism to explain specific Rab targeting, and that multiple regions of the Rab including C-terminal prenylation contribute to membrane recruitment (Ali et al., 2004). Emerging evidence based on the improved understanding of the family of Rab guanine nucleotide exchange factors (GEFs) now provides an alternative view for Rab activation at specific membrane surfaces. Mechanistic details of how Rab GEFs activate Rabs have been discussed elsewhere (Barr and Lambright, 2010), and are not directly relevant for this discussion so won't be detailed further. Two studies now show that Rab GEFs can provide the minimal machinery needed to target a Rab to a specific membrane within the cell (Gerondopoulos et al., 2012; Blümer et al., 2013). In both cases, Rab GEFs were fused to mitochondrial outer membrane targeting sequences, and the effects on different Rabs observed. Using this strategy it was possible to specifically target Rab1, Rab5, Rab8, Rab35, and Rab32/38 to mitochondria with biochemically defined cognate GEFs (Gerondopoulos et al., 2012; Blümer et al., 2013). Mutants that either reduced the nucleotide exchange activity of the GEF or the target GTPase gave a correspondingly reduced rate of Rab targeting (Blümer et al., 2013). Alone this does not provide a full explanation for Rab targeting; for this an understanding of the interaction of prenylated Rabs with the chaperone GDI (guanine nucleotide displacement inhibitor) is needed. Structural and biophysical analysis of the Ypt1–GDI complex has revealed two components of this interaction relevant for Rab targeting (Pylypenko et al., 2006). Domain I of GDI interacts with the switch II region of Ypt1 only when this is in the GDP-bound inactive form. The doubly prenylated C terminus of Ypt1 occupies a hydrophobic cavity created by domain II of GDI. Simulation of this system and direct biophysical measurements suggests that in the absence of other factors GDI will rapidly deliver Rabs to and extract them from a lipid bilayer (Pylypenko et al., 2006; Wu et al., 2010). These ideas can be combined into a simple model for Rab activation at specific membrane surfaces (Fig. 1 A). In simple terms this model is a form of molecular speed-dating in which the Rab spends a short time sampling each membrane surface it encounters before finally meeting its cognate GEF partner, triggering a period of longer residence at that site (Fig. 1 A). In this model, GEF-mediated nucleotide exchange renders the Rab resistant to extraction by GDI, and thus drives accumulation of the active GTP-bound form of the Rab. This active Rab can then recruit effector proteins to the membrane surface and promote the desired recognition event. Such a system is analogous to the rapid proofreading of amino-acyl tRNAs during protein synthesis by the ribosome (Ibba and Söll, 1999). All amino-acyl tRNAs can enter the so-called acceptor site, but only if stable codon recognition occurs is the peptidyltransferase reaction initiated, otherwise the tRNA is rejected (Steitz, 2008). The two-stage kinetic proofreading of membrane surfaces by Rabs may similarly increase fidelity at little overall cost to the rate of vesicular traffic.

Although this minimal Rab-targeting system does not require any additional factors, it is important to mention that this does not mean such factors do not exist. A family of membrane proteins with prenylated Rab-binding activity that can promote dissociation of some prenylated Rabs from GDI and favor retention of the GDP-bound form of the Rab downstream of membrane delivery by GDI has been identified (Dirac-Svejstrup et al., 1997; Martincic et al., 1997; Hutt et al., 2000; Sivars et al., 2003). These may therefore favor Rab activation, although recent data has suggested that such factors are not generally essential (Blümer et al., 2013). Intriguingly, other evidence links this family of proteins to factors involved in shaping subdomains of the ER and to the Golgi apparatus (Yang et al., 1998; Calero et al., 2001; Chen et al., 2004; Voeltz et al., 2006), perhaps suggesting that they may play roles in defining at which subdomain of an organelle an active Rab is enriched (Fig. 1 B).

In addition to these regulatory factors, covalent modification can also be used to modulate the Rab activation cycle. Phosphorylation of Rab1 and Rab4 in mitosis alters the fraction of these GTPases that can associate with membranes (Bailly et al., 1991; van der Sluijs et al., 1992), although the exact mechanisms remain unclear. Furthermore, emerging evidence indicates that one Rab in yeast, Ypt11, is controlled by a phosphorylation-dependent mechanism regulating its activation and abundance (Lewandowska et al., 2013). A number of bacterial pathogens also encode enzymes that directly modify Rab GTPases and as a consequence alter the Rab regulatory cycle. During *Legionella* infection, Rab1 is modulated by a cycle of adenylylation and de-adenylylation by DrrA and SidA, respectively, and this modification of the conserved tyrosine residue in the switch II renders the protein constitutively active (Müller et al., 2010; Neunuebel et al., 2011; Tan and Luo, 2011). DrrA also has a GEF domain and can therefore directly activate and trap Rab1 in an active form independent of other cellular factors (Schoebel et al., 2009). A second bacterial protein, AnkX, mediates phosphocholination of an adjacent serine within the switch II region (Mukherjee et al., 2011; Campanacci et al., 2013). Pathogens

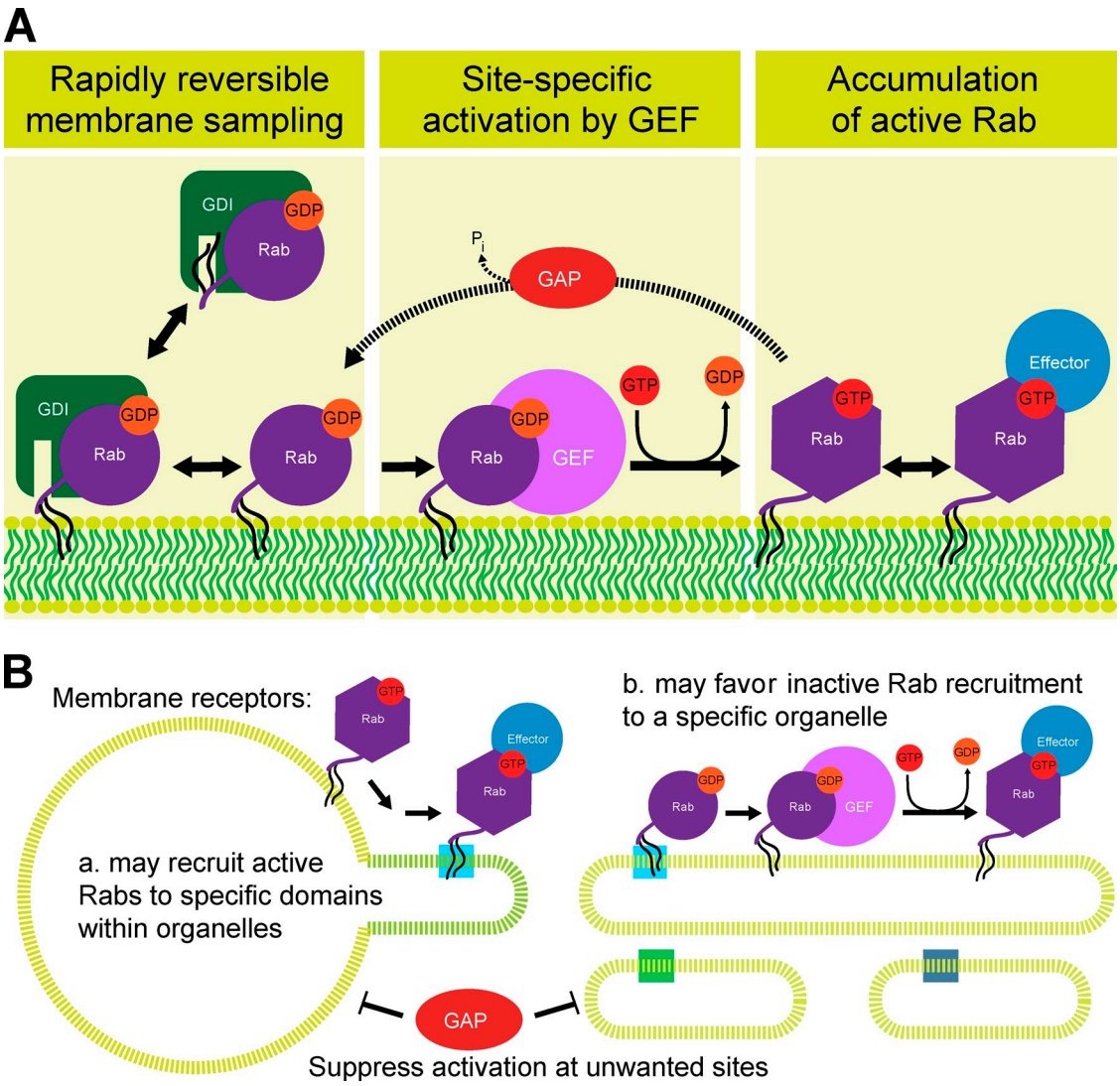

Figure 1. **The Rab activation and inactivation cycle.** (A) Prenylated Rabs (black wavy lines) are bound by the chaperone GDI in the cytosol. Partitioning of the prenylated tail moiety between the hydrophobic pocket in GDI and the membrane bilayer allows Rabs to rapidly and reversibly sample membrane surfaces. When the GDP-bound inactive Rab encounters a cognate GEF nucleotide exchange occurs. This GTP-bound active Rab species does not interact with GDI and can therefore accumulate on the membrane surface, where it may further recruit effector proteins with specific biological functions. This cycle is reset when a GTP-bound Rab encounters a GAP (GTPase-activating protein) and the bound GTP is hydrolyzed to generate GDP and inorganic phosphate. (B) Additional specification of membrane domains within complex organelles, such as tubular domains of endosomes, or the fenestrated rims and different cisternae of the Golgi apparatus, may involve membrane receptors for Rabs (shown as light blue, dark blue, and green boxes). This could either involve (a) sequestration of the active Rab to a subdomain defined by the membrane receptor, or (b) direction of GDI unloading of an inactive Rab to specific sites on the organelle membrane also defined by a membrane receptor. Accumulation of a Rab at a specific site may be favored by GAPs opposing Rab activation at unwanted sites (Haas et al., 2007).

such as *Legionella* use this covalent modification of Rabs to modulate their localization and activation (Stein et al., 2012). Although cellular enzymes that carry out related modification of Rabs are currently unknown, it would be premature to dismiss the possibility of their existence and use by cells to similarly control Rab activation and inactivation at specific sites.

Evidence for Rab activation on vesicle and target membrane surfaces

Based on the model and discussion so far it seems obvious that Rabs accumulate on the same membrane as their cognate GEF. Indeed, there is evidence that Rab1 may be activated and recruit the p115 tethering factor during the COP II vesicle formation stage of ER-to-Golgi transport (Allan et al., 2000). This would have the advantage that identity would be created at an early stage in vesicle biogenesis, and the vesicle could therefore be tethered to the Golgi before completion of the vesicle, thus increasing targeting efficiency. However, there is also evidence that Rab activation can occur at the target membrane and not only on a vesicle surface. Careful analysis of cell-free ER–Golgi transport assays revealed that Ypt1–Rab1 is not always required on the vesicle fraction, but is essential on the target Golgi membranes (Cao and Barlowe, 2000). Furthermore, a Ypt1 mutant with reduced nucleotide hydrolysis (which prevents its recycling from the Golgi compartment; Richardson et al., 1998), or Golgi membrane-anchored forms of Ypt1 (Cao and Barlowe, 2000)

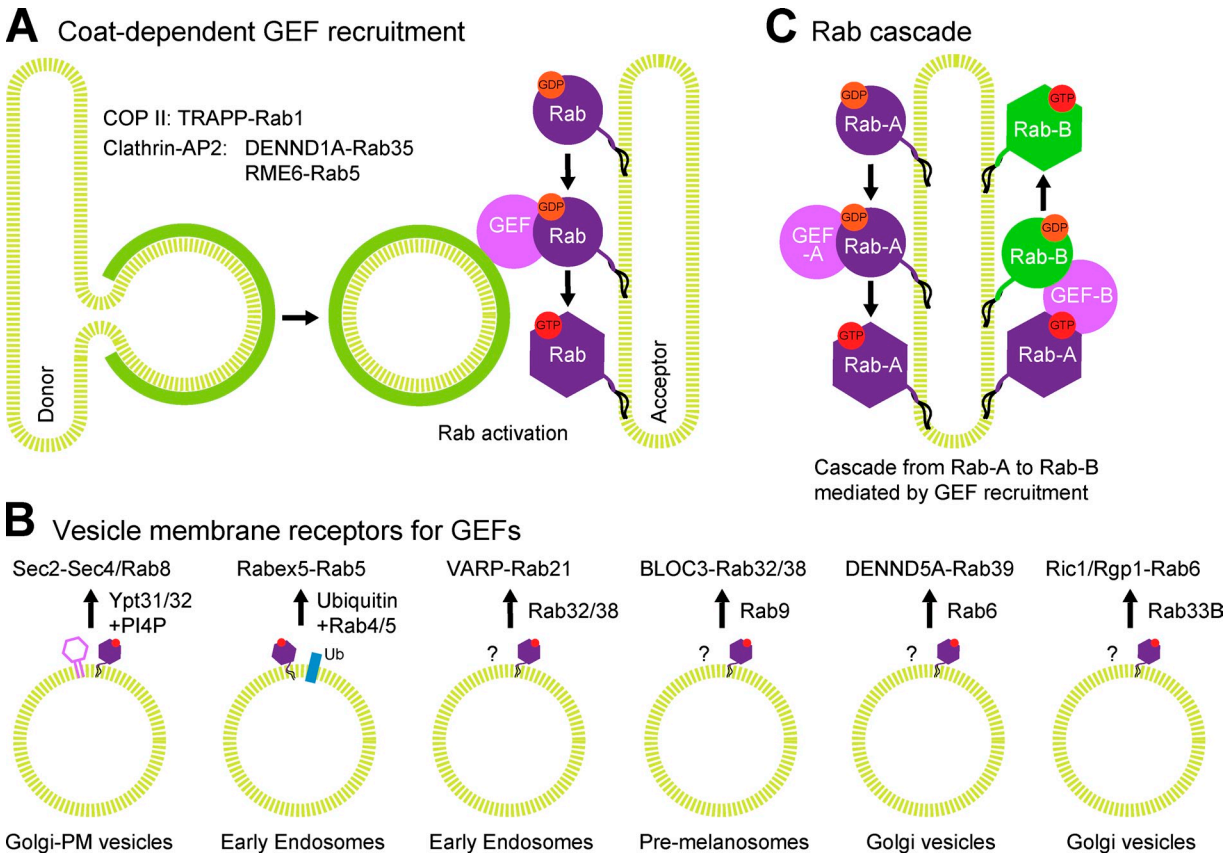

Figure 2. **Recruitment mechanisms for Rab GEFs.** Rab GEFs can be divided into two groups according to the mechanism of membrane recruitment. (A) Discrete coat protein complexes (green) recruit the first group. For example, COP II recruits the Rab1 GEF TRAPP to ER-Golgi vesicles, while clathrin-AP2 recruits DENND1A, the Rab35 GEF, to endocytic sites at the cell surface. In the case of TRAPP, biochemical and genetic data suggest that Rab1 can be activated on the target membrane, before vesicle tethering and SNARE-mediated fusion. (B) The larger second group of Rab GEFs is recruited by Rab GTPases either alone or in combination with a second factor (Rabs/factors listed next to arrow). For example, the GEF Sec2 is recruited to late-Golgi vesicles trafficking to the bud in yeast by the activated Rab Ypt31/32 and phosphatidylinositol 4-phosphate (PI4P), where it activates the Rab Sec4 (Rab8 in humans). The Rabex5–rabaptin complex, which is a Rab5 GEF, interacts with activated Rab4 or Rab5 and ubiquitylated cargo proteins on endocytic vesicles and early endosomes. A number of other GEFs (some additional examples shown) have been found to interact with active Rabs. Whether or not these represent the sole mode of membrane interaction for these GEFs is not defined at this time. PM, plasma membrane. (C) In situations where the GEF for a second Rab in the pathway is an effector for the first, a cascade can develop, where Rab-A promotes the recruitment of GEF-B for this second Rab-B.

both support apparently normal ER–Golgi transport and cell growth. Subsequently, it was found that the COP II coat required to form ER–Golgi transport vesicles is the membrane receptor for the Ypt1–Rab1 GEF TRAPP (transport protein particle; Jones et al., 2000; Wang et al., 2000; Cai et al., 2007), indicating that Rab1 activation may occur on the coated vesicle. This raises questions about how the cytosolic Rab–GDI complex can access the membrane surface of a still-coated vesicle. However, because the COP II coat has an open lattice structure (Faini et al., 2013), it may be possible in this case for Ypt1–Rab1 to approach the membrane and insert. A further possibility is that COP II vesicles recruit TRAPP and promote the activation of Ypt1–Rab1 at the adjacent Golgi membranes to signal that an ER-derived vesicle is in close proximity (Fig. 2 A). This Golgi pool of activated Rab would then recruit effector proteins such as Uso1/p115 that trap and tether the incoming vesicle by directly engaging with vesicle SNAREs (Cao et al., 1998; Shorter et al., 2002).

Rab GEF targeting and regulation

The mechanism of GEF targeting is of crucial importance for understanding how Rabs are activated at a particular membrane site. At present, two different solutions to the problem depending on the GEF are known. First, as already mentioned, is vesicle coat–dependent GEF targeting (Fig. 2 A). Three examples are known at present: COP II recruitment of the Rab1 GEF TRAPP-I (Cai et al., 2007), and clathrin-adaptor protein complex 2–dependent recruitment of either the Rab35 GEF DENND1A (Allaire et al., 2010; Yoshimura et al., 2010) or the Rab5 GEF RME-6 (Sato et al., 2005; Semerdjieva et al., 2008) during endocytic transport from the plasma membrane. In the latter cases the exact nature of the membrane on which the target Rab is activated is unclear, but it is tempting to speculate that like COP II, the coated vesicle promotes Rab activation on the target organelle to signal the presence of an incoming vesicle to be tethered. The second larger group of GEFs comprises those known to interact with active Rab GTPases (Fig. 2 B). The first of these Rab GEF effectors defined was the Rabex-5–rabaptin complex, which is both a Rab5 exchange factor and effector for Rab4 and Rab5 (Horiuchi et al., 1997). Rabex-5 also binds to ubiquitin via a specific domain and this is important for regulating its recruitment to early endosomes (Lee et al., 2006; Mattera et al., 2006; Mattera and Bonifacino, 2008) where it activates Rab5.

Specific phosphatidylinositols play a key role in defining membrane identity (Di Paolo and De Camilli, 2006)), and this is in part due to a role in recruitment or regulation of Rab exchange factors. Sec2, the exchange factor for Sec4–Rab8, is recruited to post-Golgi vesicles by a combination of the Rab Ypt32 and phosphatidylinositol 4-phosphate generated by Pik1 (Ortiz et al., 2002; Sciorra et al., 2005; Mizuno-Yamasaki et al., 2010). Similarly, in mammalian cells the Rab GEF Sec2–Rabin8 is recruited by the Ypt31/32 orthologue Rab11 (Knödler et al., 2010), and phosphatidylinositol 4-phosphate generated by the Pik1 orthologue Fwd is important for Rab11 regulation in *Drosophila* (Polevoy et al., 2009). Although less is known about the targeting of other Rab GEFs, the clear theme is that many are effectors for a Rab other than the one they activate (Fig. 2 B). The Ric1–Rgp1 complex is a GEF for Rab6 and effector for Rab33B at the Golgi (Pusapati et al., 2012) and the Rab21 GEF VARP is an effector for Rab32/38 (Zhang et al., 2006; Tamura et al., 2009). Additionally, a GEF for Rab32/38 is an effector for Rab9 (Kloer et al., 2010; Gerondopoulos et al., 2012), and the DENND5A Rab39 GEF is an effector for Rab6 (Recacha et al., 2009; Yoshimura et al., 2010). In addition to these canonical trafficking functions there are specialized examples that indicate there is some plasticity to both GEF targeting and specificity. The Ypt1 GEF TRAPP exists in an alternate form (TRAPP-II) with additional subunits that promote late-Golgi targeting and may create additional GEF activity toward Ypt31/32 (Morozova et al., 2006). Interestingly, in higher eukaryotes there is evidence that TRAPP-II may regulate the Ypt31/32 orthologues Rab11 in male meiotic cytokinesis in flies (Robinett et al., 2009) and Rab-A in plant cell polarization and division (Qi et al., 2011), respectively. TRS85 in another alternate TRAPP complex (TRAPP-III) promotes localization to the forming autophagosome and activates Rab1 during autophagy (Lynch-Day et al., 2010).

The counterpart to this interlinked network of Rab activation is an equally complex set of interactions between Rabs and Rab GAPs. The GAP Gyp1 is an effector for Ypt32 and promotes GTP hydrolysis by Ypt1 in budding yeast (Rivera-Molina and Novick, 2009). In the absence of Gyp1, Ypt1 spreads into the later compartments of the secretory pathway that should be occupied by Ypt32 (Rivera-Molina and Novick, 2009). Interestingly, one of the cellular GAPs for Ypt1–Rab1 is a transmembrane protein of the ER that may prevent Rab1 activity from spreading earlier in the pathway to the ER rather than act to terminate Rab1 activity at the Golgi (Haas et al., 2007; Sklan et al., 2007). Similarly, two related proteins, RUTBC1 and RUTBC2, bind to active Rab9 and are GAPs for Rab32 and Rab36, respectively (Nottingham et al., 2011, 2012).

Together, these findings have led to the general idea that the order of trafficking events in a pathway can potentially be defined by a series of Rabs acting as a cascade (Fig. 2 C). In such models one Rab triggers the next in the pathway by recruiting its cognate GEF, and then feedback develops as a GTPase-activating protein (GAP) is recruited to terminate the action of the previous Rab in the series (Mizuno-Yamasaki et al., 2012; Pfeffer, 2013). In part, this simply passes the problem on because we are then left with the question of how the previous Rab in the pathway or a cofactor for recruitment such as phosphatidylinositol 4-phosphate or ubiquitin is localized and generated only when required. In the case of the secretory pathway the ER provides a defined starting point where activation of Rab1–Ypt1 will inevitably result in a defined and correctly timed wave of Rab activation through the secretory pathway. However, a note of caution is needed when considering these ideas because far more support from experimental data looking at the biochemical properties of these systems both in vitro and in vivo is required to come to any definitive conclusions.

Ultrasensitive Rab activation switches

One of the key tenets of the membrane identity hypothesis is that Rabs should rapidly and accurately establish membrane identity and then be lost once the membrane recognition event is over. Although biochemical data on Rab GEFs clearly indicate these molecules generally have sufficiently high specificity to ensure activation of only one Rab or a set of closely related Rabs (Delprato et al., 2004; Yoshimura et al., 2010; Gerondopoulos et al., 2012), how rapid switch-like accumulation is ensured is less obvious. Similar issues exist for termination of the Rab cycle by Rab GAPs. As already mentioned, Rab cascade models give part of the solution to this problem, and provide features that can ensure vectorial flow in a membrane traffic pathway (Mizuno-Yamasaki et al., 2012; Pfeffer, 2013). However, they do not fully explain how switch-like transitions and defined compartmental boundaries are achieved (Del Conte-Zerial et al., 2008). A possible solution to this problem comes from studies on the regulation of other complex biological systems, exemplified by control of cell cycle transitions (Tyson et al., 2001). Rather than displaying the expected Michaelis-Menten kinetics (Fig. 3 A), Rab cycles may yield properties of ultrasensitivity (Goldbeter and Koshland, 1981, 1984). This would appear to be a valid proposal if the Rab cycle is treated as being analogous to a covalent modification (Rab and Rab-modified, for GDP and GTP forms, respectively) and because GEF activity is generally assumed to be limiting (Blümer et al., 2013). In such a situation, inputs activating the GEF, for example membrane recruitment requiring multiple or binding of an activator, would be amplified and give rise to very large changes in the amount of activated Rab (Fig. 3 A). When combined with feedback loops, this can create a bistable switch between two states as shown for cell cycle transitions (Novak and Tyson, 1993; Pomerening et al., 2003). In the case of GTPase regulation, as the input controlling the GEF increases then the system transitions to a Rab-active state that remains stable over a wide range of GEF activity. GAP activation could then trigger exit from this state. This is also useful for providing a potential explanation for the timing properties of a Rab cascade. Ultrasensitivity and bistability are therefore likely to be useful concepts when explaining the behavior of Rabs, especially when considering complex interlinked cycles (Fig. 3 B) because they avoid the futile cycles where GAPs and GEFs fight one another and thus don't do any useful work.

A groundbreaking study in this area has applied these ideas to the conversion of Rab5-positive early endosomes to Rab7-positive late endosomes and lysosomes (Del Conte-Zerial et al., 2008). This analysis has provided strong evidence that

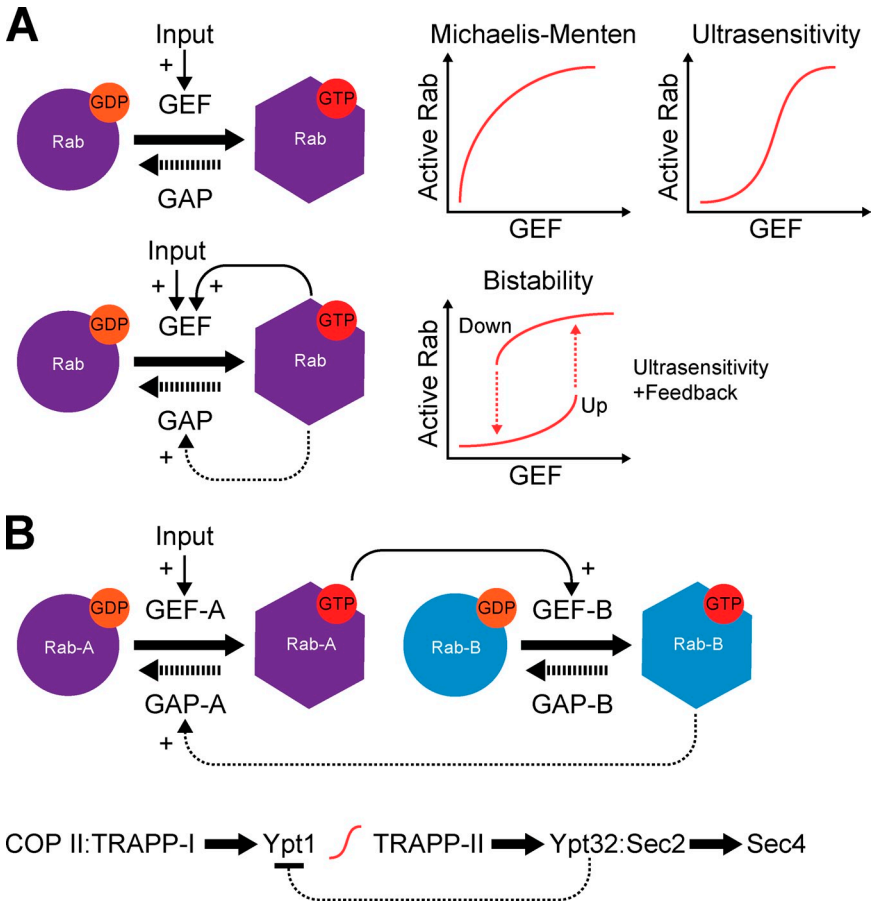

Figure 3. **Ultrasensitivity and bistability in Rab regulatory networks.** (A) A simplified schematic of a Rab activation cycle is shown treating GDP–GTP exchange as equivalent to a covalent modification cycle such as phosphorylation. Because the reaction can only occur at a membrane surface, membrane recruitment factors are treated as activating inputs. Assuming no feedback and normal first-order reaction kinetics, Rab recruitment would be expected to follow Michaelis-Menten behavior. In cases where substrate is saturating and the reaction becomes zero-order, Goldbeter and Koshland (1984) have shown that product formation becomes more sensitive to enzyme concentration. In this case, generation of GTP-bound Rab becomes ultrasensitive to GEF concentration at the membrane surface. If additional positive feedback controls exist as shown in the bottom panel, then bistability may develop. In this case a rapid switch-like transition in Rab activity develops as Rab GEF concentration increases. Once in the active state the system becomes less dependent on continued high GEF activity. (B) A model for an interlinked Rab cascade is shown. The GEF for Rab-B is an effector for activated Rab-A, while the GAP for Rab-A is regulated by Rab-B. An example of this latter situation is provided by the Ypt1–Yp32 system discussed in the main text and shown in the bottom panel, where a Ypt1 GAP Gyp1 is an effector for Ypt32 (Rivera-Molina and Novick, 2009) and inhibits Ypt1. This coupling of the two cycles can result in coupled ultrasensitive switch-like transitions or bistability.

positive and negative feedback loops in this system mediated by Rab GEFs and GAPs result in bistability in the form of a cut-out switch, so that Rab5 accumulation is followed by an abrupt transition at which Rab5 is rapidly lost and Rab7 accumulates (Del Conte-Zerial et al., 2008). Underpinning this is a biochemical network in which the Mon1–Ccz1 Rab7 GEF complex displaces Rabex-5, thus breaking the positive feedback loop to Rab5 activation (Poteryaev et al., 2010) and simultaneously promoting recruitment and activation of Rab7 (Nordmann et al., 2010; Gerondopoulos et al., 2012). Although there are only few studies where these ideas have been considered, they can be experimentally tested and are likely to be of increasing importance in membrane traffic regulation.

Origins of Rab GTPase control systems

One of the most difficult questions in membrane trafficking relates to the origins of complex internal membrane systems in eukaryotes. Analysis of Rab GTPases themselves suggests a pattern of evolution of Rabs consistent with the evolution of a core set of membrane organelles of the endocytic and secretory pathways (Diekmann et al., 2011; Klöpper et al., 2012). Yet, this provides little insight into how membrane organelles initially arose. Recent data on the structure of Rab GTPase regulators and coat protein complexes has identified common features with GTPase regulators in other systems including prokaryotes (Kinch and Grishin, 2006; Zhang et al., 2012; Levine et al., 2013). The conserved Longin–Roadblock fold has emerged as a structural feature of the large family of DENN-domain Rab GEFs in human cells (Yoshimura et al., 2010; Wu et al., 2011; Levine et al., 2013). Intriguingly, related domains are also present in the signal sequence receptor involved in protein translocation into the ER, vesicle coat protein complexes, and the MglA GTPase–MglB bacterial cell polarity regulator (Sun et al., 2007; Miertzschke et al., 2011; Levine et al., 2013). Although far from conclusive, these findings provide important pointers to the development of GTPase control systems, and more generally the early origins of membrane traffic pathways in eukaryotes from membrane-associated GTPases and their effector proteins.

Are Rabs alone capable of triggering the pathways defining membrane identity? Multiple lines of evidence show Rab GTPases are clearly important and far from inconsequential regulators of vesicle traffic; however, further evidence is required before we should conclude that they are causal regulators of vesicle or organelle membrane identity. Neither of the studies using strategies to modulate the cellular localization of Rab GEFs reported that the mitochondria altered their identity or were converted into an endosome or Golgi because of the mistargeted Rabs (Gerondopoulos et al., 2012; Blümer et al., 2013). The picture emerging is therefore one in which Rabs cannot program membrane identity alone and must work in concert with other factors. Defining and reconstituting the systems needed to create membrane identity is therefore a major goal for membrane traffic research.

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