Rab Proteins and the Compartmentalization of the Endosomal System

Angela Wandinger-Ness[1] and Marino Zerial[2]

[1]Department of Pathology MSC08 4640, University of New Mexico HSC, Albuquerque, New Mexico 87131
[2]Max Planck Institute of Molecular and Cell Biology and Genetics, 01307 Dresden, Germany
Correspondence: wness@unm.edu; zerial@mpi-cbg.de

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Of the approximately 70 human Rab GTPases, nearly three-quarters are involved in endocytic trafficking. Significant plasticity in endosomal membrane transport pathways is closely coupled to receptor signaling and Rab GTPase-regulated scaffolds. Here we review current literature pertaining to endocytic Rab GTPase localizations, functions, and coordination with regulatory proteins and effectors. The roles of Rab GTPases in (1) compartmentalization of the endocytic pathway into early, recycling, late, and lysosomal routes; (2) coordination of individual transport steps from vesicle budding to fusion; (3) effector interactomes; and (4) integration of GTPase and signaling cascades are discussed.

The general working principle of Rab GTPases is that they contribute to the structural and functional identity of intracellular organelles. These functions rely on the versatile GTP/GDP cycle for the assembly of multiprotein machineries on the cytoplasmic surface of intracellular membranes. Rab GTPase protein assemblies are spatially and temporally regulated, can vary quantitatively over time, and are reversible, thus allowing for a change in the membrane composition and intracellular fate of organelles.

Rab proteins belong to the Ras superfamily of small GTPases and share properties characteristic of small GTPases in common with other subfamilies (Rojas et al. 2012). First, Rab GTPases undergo cycles of GTP binding and hydrolysis to GDP, which serves to drive a reversible conformational change that is decoded by interacting proteins (Wittinghofer et al. 1993). Guanine-nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) catalyze the exchange and hydrolysis reactions and, therefore, act as regulators of the GTP–GDP cycle (Yoshimura et al. 2010; Wu et al. 2011; Barr 2013; Guo et al. 2013; Kotting and Gerwert 2013). Second, Rab GTPases behave both as soluble and specifically localized, integral-membrane proteins. Rab GTPases are kept soluble in the cytosol and in the inactive (GDP-bound) conformation through association with Rab GDP-dissociation inhibitors (Rab-GDI) (Ullrich et al. 1993; Gavriljuk et al. 2013). Prenylation—addition of one or two geranyl-geranyl groups—on conserved carboxy-terminal cysteine residues serves together with upstream hypervariable regions in promoting specific and stable membrane association (Sa-

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saki et al. 1990; Chavrier et al. 1991; Seabra et al. 1991; Kinsella and Maltese 1992; Ullrich et al. 1993; Seabra and Wasmeier 2004; Lane and Beese 2006; Wu et al. 2010). Third, in the active, GTP-bound conformation, Rab GTPases bind effector proteins that "implement" their downstream effects. Rab effectors are, however, distinct in that they are specialized for functions related to efficient membrane trafficking between compartments (see Box 1 for full names of Rab effectors). Fourth, similar to other GTPases, Rab GTPases are functionally interconnected with downstream Rab GTPases in cascades, as well as upstream GTPases of distinct subfamilies, most notably the Rho, Arf, and Arl family GTPases (Burguete et al. 2008; Agola et al. 2011; de Curtis and Meldolesi 2012; Hall 2012; Mizuno-Yamasaki et al. 2012; Pfeffer 2013a).

There are, however, some Rab GTPase characteristics that are either unique or that have not yet been recognized for other small GTPases. One such trait is that Rab GTPases may rely on specialized protein cofactors such as PRA-1 for membrane recruitment in the GDP-bound form, entailing displacement of GDI in the absence of nucleotide exchange (Dirac-Svejstrup et al. 1997; Horiuchi et al. 1997; Bucci et al. 2001; Seabra and Wasmeier 2004; Sivars et al. 2005; Bhagatji et al. 2010; Dickison et al. 2012). However, PRA-1 acts promiscuously on early and late endosomal Rab proteins and, therefore, cannot be the main determinant of specific Rab membrane localization (Blumer et al. 2012). In the case of Rab1, the *Legionella* DrrA protein, which acts as a Rab1 GEF, is sufficient to seamlessly catalyze both GDI dissociation and guanine-nucleotide exchange in a single step without requiring another intermediary (Schoebel et al. 2009; Wu et al. 2010; Blumer et al. 2012; Oesterlin et al. 2012). By catalyzing GDP/GTP exchange, GEFs activate Rab proteins and allow effector binding, making them inaccessible to Rab GDI and stabilizing them on the membrane (Barr 2013). Consistent with their role as rate-limiting factors for GTPase activation and membrane localization, if GEFs are artificially localized to a given membrane compartment, they induce the accumulation of their target Rab proteins on that membrane (Blumer et al. 2013). Posttranslational modification of mammalian Rab GTPases through adenylation or phosphocholination by bacterial enzymes can occur on the membrane-bound, inactive Rab and preclude GDI rebinding (Oesterlin et al. 2012). Perhaps this is a regulatory mechanism that occurs also in the absence of infection as an alternative to GDI displacement and membrane stabilization.

Another unique characteristic is the high degree of functional complexity among Rab effectors. The complexity is particularly striking for some Rab GTPases and raises the question of whether all Rab proteins require a complex "interactome" for their function. Effector proteins generate specialized lipid platforms for further protein recruitment of the machinery required for protein sorting, vesicle budding, cytoskeletal translocation, vesicle tethering, and fusion. In addition, Rab effectors can be shared between different Rab proteins to functionally couple one Rab to another in networked cascades (Mizuno-Yamasaki et al. 2012; Pfeffer 2013a). The described complexity reflects the fact that Rab effectors are spatially and temporally regulated to assemble a functional organelle transport machinery that seamlessly completes the sequential steps required for membrane flux from one compartment to the next in the pathway.

Various aspects of the function of Rab GTPases have been summarized and discussed in several excellent review articles (Novick and Zerial 1997; Chavrier and Goud 1999; Zerial and McBride 2001; Pfeffer and Aivazian 2004; Schwartz et al. 2007; Stenmark 2009; Agola et al. 2011; Mizuno-Yamasaki et al. 2012; Stein et al. 2012b; Aloisi and Bucci 2013; Barr 2013; Pfeffer 2013a), and other articles in this collection refer to Rab GTPases and their effectors (Cossart and Helenius 2014; Di Fiore and von Zastrow 2014; Gautreau et al. 2014; Gonzalez Gaitan and Jülicher 2014; Klumperman and Raposo 2014). In this article, we focus on the current state of our understanding of the role of Rab GTPases in the endocytic pathway, their localization, function, and underlying machinery, as well as how the basic Rab transport machinery is modulated by signaling and metabolic pathways in response to physiological needs.

BOX 1. FULL NAMES OF Rab EFFECTORS

ACAP2	ArfGAP with coiled-coil, ankyrin repeat and PH domains 2
AKAP10	A kinase (PRKA) anchor protein 10
ANKFY1	Ankyrin repeat and FYVE domain containing 1
ANKRD7	Ankyrin repeat domain 7
APBA3	Amyloid β(A4) precursor protein-binding, family A, member 3
APPL1	Adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 1
APPL2	Adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 2
ATG16L1	Autophagy related 16-like 1 (*Saccharomyces cerevisiae*)
BICD1	Bicaudal D homolog 1 (*Drosophila*)
CASP1	Caspase 1
CCDC64	Coiled-coil domain containing 64
CD2AP	CD2-associated protein
CORO1C	Coronin, actin-binding protein, 1C
DENND5A	DENN/MADD domain-containing 5A/Rab6-interacting protein
EEA1	Early endosome antigen 1
EXOC6	Exocyst complex component 6/Sec15 (yeast)
F8A1 (HAP40)	Coagulation factor VIII-associated 1
FSCN1	Fascin homolog 1, actin-bundling protein
FYCO1	FYVE and coiled-coil domain containing 1
GABARAP	GABA(A) receptor-associated protein
GCC2	GRIP and coiled-coil domain containing 2
GOLGA2	Golgin A2
GOLGB1	Golgin B1
GRIPAP1	GRIP1 associated protein 1
HPS4	Hermansky–Pudlak syndrome 4
INPP4A	Inositol polyphosphate-4-phosphatase, type I, 107 kDa
INPP5B	Inositol polyphosphate-5-phosphatase, 75 kDa
ITGA2	Integrin, α2
ITGA11	Integrin, α11
LEPR/OBR	Leptin receptor
LEPROT	Leptin receptor overlapping transcript
KIAA0226	Beclin-1 associated RUN domain-containing protein, Rubicon
KIF16B	Kinesin family member 16B
KIF20A	Kinesin family member 20A
MAP4K2	Mitogen-activated protein kinase kinase kinase kinase 2
MICALL1	MICAL (microtubule associated monooxygenase, calponin, and LIM domain containing)-like 1
MICALL2/ JRAB	MICAL (microtubule associated monooxygenase, calponin, and LIM domain containing)-like 2; junctional Rab13-binding protein
MLPH	Melanophilin
MYH10	Myosin, heavy polypeptide 10, nonmuscle
MYH9	Myosin, heavy chain 9, nonmuscle
MYO5A	Myosin VA
MYO5B	Myosin VB
MYO5C	Myosin VC
MYRIP	Myosin VIIA and Rab-interacting protein
OCRL	Oculocerebrorenal syndrome of Lowe
ODF2	Outer dense fiber of sperm tails 2
OPTN/ RAB11FIP2	Optineurin/RAB11 family-interacting protein 2

Continued

OSBPL1A	Oxysterol-binding protein-like 1A/ORP1L
OTOF	Otoferlin
PI4KB	Phosphatidylinositol-4-kinase, catalytic, beta
PIK3CB	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit beta
PIK3R4	Phosphoinositide-3-kinase, regulatory subunit 4
PLEKHM1	Pleckstrin homology domain containing, family M (with RUN domain) member
PLIN3	Perilipin 3
PRKAR2A	Protein kinase, cAMP-dependent, regulatory, type II, α
PSMA7	Proteasome (Prosome, Macropain) subunit, α-type, 7/XAPC7
PXR1	Peroxisomal biogenesis factor 5/TPR-containing Rab8b-interacting protein (Trip8b)
RAB3IL1	Rab3A-interacting-like 1/ guanine nucleotide exchange factor for Rab3A (GRAB)
RAB3IP	RAB3A-interacting protein/Rabin8
RAB11FIP1	RAB11 family-interacting protein 1 (class I)
RAB11FIP3	RAB11 family-interacting protein 3 (class II)
RAB11FIP4	RAB11 family-interacting protein 4 (class II)
RAB11FIP5	RAB11 family-interacting protein 5 (class I)
RABEP1	Rabaptin, RAB GTPase-binding effector protein 1
RABEP2	Rabaptin, RAB GTPase-binding effector protein 2
RABEPK	Rab9 effector protein with kelch motifs
RABGEF1	Rabaptin-5-associated exchange factor for Rab5 (RABEX5)
RABIF	RAB-interacting factor
REP15	RAB15 effector protein
RHOBTB3	Rho-related BTB domain containing 3
RILP	Rab-interacting lysosomal protein
RIMS1	Regulating synaptic membrane exocytosis 1
RIMS2	Regulating synaptic membrane exocytosis 2
RNF115	Ring finger protein 115
RPH3A	Rabphilin 3A
RPH3AL	Rabphilin 3A-like (without C2 domains)
RUFY1	RUN and FYVE domain containing 1
SGSM2	Small G protein signaling modulator 2
SH3TC2	SH3 domain and tetratricopeptide repeats 2
SYN1	Synapsin I
SYTL1	Synaptotagmin-like 1
SYTL2	Synaptotagmin-like 2
SYTL3	Synaptotagmin-like 3
SYTL4	Synaptotagmin-like 4
SYTL5	Synaptotagmin-like 5
TBC1D14	TBC1 domain family, member 14
TBC1D2B	TBC1 domain family, member 2B
TMF1	TATA element modulatory factor 1
UACA	Uveal autoantigen with coiled-coil domains and ankyrin repeats, apoptosome subunit
UNC13B	unc-13 homolog B (*Caenorhabditis elegans*)
UNC13D	unc-13 homolog D (*C. elegans*)
VPS35	Vacuolar protein sorting 35 homolog (*S. cerevisiae*)
VPS41	Vacuolar protein sorting 41 homolog (*S. cerevisiae*)
VPS45	Vacuolar protein sorting 45 homolog
VPS52	Vacuolar protein sorting 52 homolog (*S. cerevisiae*)
VPS8	Vacuolar protein sorting 8
WDR44	WD repeat domain 44/rabphilin-11, rab11-binding protein
ZFYVE20	Zinc finger, FYVE domain-containing 20
ZFYVE27	Zinc finger, FYVE domain-containing 27

The emerging picture is that Rab GTPases act as master regulators of organelle biogenesis and cellular homeostasis, functions that are well beyond their originally proposed roles in vesicular transport proofreading.

Rab GTPases ON THE ENDOCYTIC PATHWAY COMPARTMENTALIZE ENDOCYTIC FUNCTIONS

The Rab family of small GTPases shows dramatic evolutionary plasticity. Tracing Rab proteome evolution through bioinformatics analyses of the whole eukaryotic phylogeny in combination with modeling approaches reveals that as eukaryotes diverged, they lost or gained Rab proteins—leading to thousands of variants among the 247 analyzed genomes (Diekmann et al. 2011; Klopper et al. 2012; Rojas et al. 2012). The last eukaryotic common ancestor expressed at least 20 Rab proteins that fall into six discrete groups, presumably regulating the trafficking routes that are fundamental to eukaryotes (Klopper et al. 2012). Whereas the budding yeast *Saccharomyces cerevisiae* expresses 11 family members, 66 Rab proteins are encoded in the human genome (Klopper et al. 2012). These include Rab isoforms that can have overlapping yet distinct functions. Among the best characterized endosomal isoforms are Rab4a, b; Rab5a, b, c; Rab7a, b; Rab11a, b; and Rab25/Rab11c (Table 1) (Chavrier et al. 1991; Van Der Sluijs et al. 1991; Feng et al. 1995; Yang et al. 2004; Chen et al. 2009a; Kelly et al. 2012). The large expansion of Rab genes in metazoans correlates with multicellularity and the increasing complexity in cell organization and specialization, including acquisition of a cytoskeleton and endocytic pathways (Diekmann et al. 2011; Klopper et al. 2012; Marijuan et al. 2013; see also Wideman et al. 2014). Most Rab GTPases are ubiquitous, but many display tissue specificity in expression. Notable among endocytic Rab GTPases are Rab27a/b, which regulate multivesicular body-exosome secretion in immune cells and melanocytes, and Rab32/38, which control lysosome-related organelle and granule biogenesis in melanocytes and platelets (Ambrosio et al. 2012; Bultema et al. 2012; Fukuda 2013). New functions required the emergence of new human Rab subfamilies with unique interaction surfaces that likely underlie complex Rab interactomes (Stein et al. 2012a). No other family of proteins of the intracellular transport machinery has shown such a degree of expansion, suggesting that "it is the changes in Rab proteins that primarily underlies the variation in organelles between species and cell types" (Klopper et al. 2012).

First glimpses into the function of Rab proteins came in the 1990s through studies characterizing localization to endocytic pathways, cell-free assays, and expression of dominant-negative mutants in mammalian and yeast systems. The first mammalian Rab proteins localized to the endocytic pathway were Rab5 and Rab7 (Chavrier et al. 1990), which sequentially regulate the essential steps in endocytosis, cargo uptake into early endosomes, and transport to lysosomes (Bucci et al. 1992; Feng et al. 1995; Vitelli et al. 1997). Since then, nearly three-quarters of the known Rab proteins have been found associated with endocytic organelles, where they regulate (1) internalization from the cell surface; (2) recycling of receptors, cell adhesion molecules, and transport machinery; (3) degradation and organelle biogenesis; and (4) cell-type-specific trafficking steps (Table 1).

The ubiquitous Rab GTPases Rab5, Rab4, and Rab11 function on the early endocytic pathway, whereas Rab7 and Rab9 function on the late endocytic pathway (Fig. 1). Rab5 can also be detected on the plasma membrane (Chavrier et al. 1990), positioning it to function in the formation of clathrin-coated vesicles (CCVs), CCV fusion with early endosomes, and in the homotypic fusion between early endosomes (Gorvel et al. 1991; Bucci et al. 1992; McLauchlan et al. 1998). Rab7 acts downstream from Rab5 to regulate transport from early to late endosomes and lysosomes (Feng et al. 1995; Vitelli et al. 1997; Gutierrez et al. 2004; Jager et al. 2004). In addition, Rab7 plays an important role in autophagy (Feng et al. 1995; Vitelli et al. 1997; Gutierrez et al. 2004; Jager et al. 2004). Rab4 and Rab11 regulate the transport along the recycling pathway, from early and recycling endosomes to the cell surface (van der

Table 1. Endocytic Rab GTPase localizations, functions, and effectors

RAB	Main localization	Cellular functions	Main effectors
Early endosomal compartments			
RAB4A,B,C	CCV, early endosomes, recycling endosomes	Endocytic recycling of integrins, receptor tyrosine kinases, G-coupled receptors, and neurotransmitter receptors among other cargo Mitochondrial homeostasis	AKAP10, CD2AP, GRIPAP1, RAB11FIP1, RABEP1, RUFY1, Rabenosyn-5(ZFYVE20)-VPS45
RAB5A,B,C	CCV, early endosomes, phagosomes	Early endosome fusion, early endosome biogenesis, nuclear envelope disassembly in mitosis	ANKFY1, APPL1/2, EEA1, F8A1, INPP4A, INPP5B, PIK3CB, PIK3R4, OCRL, RABEP1, RABEP2, VPS8 (yeast), ZFYVE20-VPS45
RAB13	Early endosomes, tight junctions	Recycling endosome-to-plasma membrane transport, GLUT4 trafficking, epithelial junction development	LEPR/OBR, LEPROT, MICALL1, MICALL2/JRAB
RAB20	Early and late macropinosomes	Macropinosome maturation, hypoxia-induced apoptosis, vacuolar ATPase, and connexin 43 trafficking	N.D.
RAB21	Early endosomes	Integrin endocytosis, cytokinesis	APPL, ITGA2, ITGA11
RAB22A	Early endosomes, TGN	Early endosome-Golgi transport	EEA1, RABGEF1, TBC1D2B
RAB31/ RAB22B	Early endosomes, TGN, phagosomes	TGN-to-early endosome transport, phagosome maturation	OCRL, TBC1D2B
RAB23	Plasma membrane, early endosomes	Phagosome-lysosome fusion, Hedgehog signaling, ciliogenesis	N.D.
RAB35	Plasma membrane, CCV, early endosomes	Endocytic recycling of MHC class I and II and T-cell receptor, phosphoinositide regulation, cytokinesis, actin dynamics	ACAP2, FSCN1, MICALL1, OCRL
Golgi, recycling compartments, and secretory organelles			
RAB3A, B,C,D	Secretory granules, synaptic vesicles	Regulated exocytosis	RAB3IP, RIMS1/2, RPH3A, RPH3AL, SYN1, SYTL4/5
RAB6A,B,C	Golgi	Golgi-to-plasma membrane transport, Golgi–endosome transport, intra-Golgi transport, ER–Golgi transport, cytokinesis	APBA3, BICD1, CCDC64, DENND5A, GCC2, GOLGB1, KIF20A, MYH9, MYH10, OCRL, TMF1, VPS52
RAB8A,B	Recycling endosomes, GLUT4 vesicles	TGN-plasma membrane trafficking, GLUT4 vesicle translocation, cilial transport, adherens junction assembly	MAP4K2, MICALL1, MICALL2, MYO5B, OCRL1, ODF2, OPTN/RAB11FIP2 (8A,8B), OTOF (8B), PXR1, RABEP1RPH3A, SYTL1
RAB10	TGN, GLUT4 vesicles	TGN-to-plasma membrane trafficking, GLUT4 trafficking, ciliogenesis, phagosome maturation	MICAL1, MYO5A/B/C, RIMS1

Continued

Table 1. *Continued*

RAB	Main localization	Cellular functions	Main effectors
RAB11A,B	Recycling endosomes, TGN	Endocytic recycling, recycling endosome-to-plasma membrane transport, cytokinesis, ciliogenesis, autophagy	DENND5A, EXOC6, MYO5B, PI4KB, RAB3IL1, RAB3IP, RAB11FIP1, RAB11FIP2/OPTN, RAB11FIP3, RAB11FIP4, RAB11FIP5, SH3TC2, TBC1D14, WDR44, ZFYVE27
RAB25 (RAB11C)	Recycling endosomes	RE-to-plasma membrane transport, integrin recycling	MYO5B, RAB3IP, RAB11FIP1, RAB11FIP1, RAB11FIP2/OPTN, RAB11FIP3, RAB11FIP4, RAB11FIP5
RAB14	Early endosomes, TGN	Golgi-to-early endosome transport	KIF16B, RUFY1, ZFYVE20
RAB15	Early endosomes, recycling endosomes	Endocytic recycling	RABIF, REP15
RAB17	Recycling endosomes, melanosomes	Endocytic recycling, ciliogenesis, melanosome trafficking	N.D.
RAB26	Secretory granules	Regulated exocytosis	RIMS1
RAB33A,B	Golgi, autophagosome	ER–Golgi and intra-Golgi transport, autophagy	ATG16L1, GOLGA2, RABEP1
RAB37	Golgi, secretory granules	Mast cell degranulation, Wnt signaling, TNF-α secretion	RIMS1, UNC13B
RAB39A, B	Golgi, endosomes, phagosomes	Phagosomal acidification, IL-1 (39A) and myocilin (39B) secretion	CASP1 (39A), MYO5A (39B), UACA (39A)
Late endosomes, lysosomes, lysosome-related organelles			
RAB7A,B	Late endosomes, lysosomes, autophagosome	Early-to-late endosome and late endosome-Golgi transport, lysosome biogenesis, autophagosome maturation	FYCO1, KIAA0226, OSBPL1A, PIK3R4, PLEKHM1, PSMA7, RILP, RNF115, VPS35 (retromer), VPS41 (HOPS complex)
RAB9A,B	Late endosomes	Endosome-to-TGN transport	GCC2, HPS4 (BLOC-3 subunit), PLIN3, RABEPK, RHOBTB3, SGSM2
RAB12	Recycling endosomes, lysosomes	Transport to lysosomes	N.D.
RAB24	Autophagosomes, mitotic spindle	Autophagy, cell division (chromosome segregation and cytokinesis)	GABARAP
RAB27A	Melanosomes, secretory granules	Dynamics of lysosome-related organelles and secretory granules	CORO1C, MLPH, MYO5A, MYRIP, RPH3A, RPH3AL, SYTL1-5, UNC13D
RAB32	Tyrp1-containing organelles, melanosomes, mitochondria, autophagosome	Autophagy, distribution of mitochondria, trafficking of melanogenic enzymes to melanosomes	ANKRD7, PRKAR2A

Continued

Table 1. *Continued*

RAB	Main localization	Cellular functions	Main effectors
RAB34	Golgi, plasma membrane, macropinsomes, phagosomes	Macropinocytosis, phagosome maturation, lysosome morphogenesis and positioning	RILP, UNC13B
RAB36	Golgi	Spatial distribution of late endosomes and lysosomes	RILP
RAB38	Tyrp1-containing organelles, melanosomes	Transport of tyrosinase to immature melanosomes	ANKRD7

Rab GTPases are clustered according to their localizations on (1) early endosomes; (2) Golgi, recycling compartments, and secretory organelles; and (3) late endosomes, lysosome, and lysosome-related organelles (Zerial and McBride 2001; Soldati and Schliwa 2006; Ohya et al. 2009; Agola et al. 2011; Huotari and Helenius 2011; Pfeffer 2011; Mizuno-Yamasaki et al. 2012; Rojas et al. 2012).

CCV, Clathrin-coated vesicles; N.D., no data; TGN, *trans*-Golgi network.

Sluijs et al. 1992; Ullrich et al. 1996). Rab4 regulates the recycling of integrins, receptors, ubiquitin ligases, and other transport machinery (Arjonen et al. 2012; Cheng et al. 2013; Cui and Zhang 2013). Rab11 isoforms and their downstream effectors have critical roles in cell polarity, and defects underlie cancer, gastrointestinal, and degenerative disease (for reviews, see Jing and Prekeris 2009; Kelly et al. 2012; Mitra et al. 2012). Rab9 regulates the recycling of cargo (mannose-6-phosphate receptor) from late endosomes to the Golgi complex (Lombardi et al. 1993; McGourty et al. 2012; Dong et al. 2013). The described GTPases contribute to the functional endosomal compartmentalization and cargo transport to early endosomes, plasma membrane, and Golgi recycling, and transport to degradation.

Rab GTPases are in general functionally connected to each other to regulate cargo flow through intracellular compartments (for review, see Zerial and McBride 2001; Stenmark 2009; Pfeffer 2012, 2013a). Effectors as well as GEFs and GAPs can bind promiscuously and are shared between different Rab proteins (Fukuda 2003). Some bind Rab GTPases via the same binding site, but others can also bind diverse Rab proteins via distinct binding domains. Rabaptin-5 was the first Rab effector shown to bind different Rab proteins, Rab5 and Rab4, via distinct binding sites, thus coupling cargo entry into early endosomes to recycling (Vitale et al. 1998). More divalent or multivalent Rab effectors have been identified, such as Rabenosyn-5 for Rab5 and Rab4 (de Renzis et al. 2002), Rab coupling protein (RCP) for Rab4 and Rab11 (Lindsay et al. 2002), and CORVET/HOPS for Rab5 and Rab7 (Numrich and Ungermann 2013; Solinger and Spang 2013). Studies in the yeast secretory pathway have shown that Rab GTPases can be sequentially activated via GEF/effector complexes (Ortiz et al. 2002). A subset of GEFs could be identified via bioinformatics approaches based on the presence of a conserved DENN domain (Yoshimura et al. 2010; Wu et al. 2011); however, there are GEF family members that are structurally distinct (Esters et al. 2001; Guo et al. 2013). Clearly, more factors such as GEFs, GAPs, and effectors coupling the activities of individual and sequentially acting Rab proteins need to be identified and characterized to fully understand how cargo flows between compartments on the endocytic pathway and endocytosis is coordinately up-regulated to address physiological demands.

Rab GTPases AND LIPID METABOLISM IN THE CONTROL OF TRAFFICKING AND COMPARTMENTALIZATION

A breakthrough in discerning the mechanisms whereby Rab GTPases control intracellular membrane transport came from the discovery

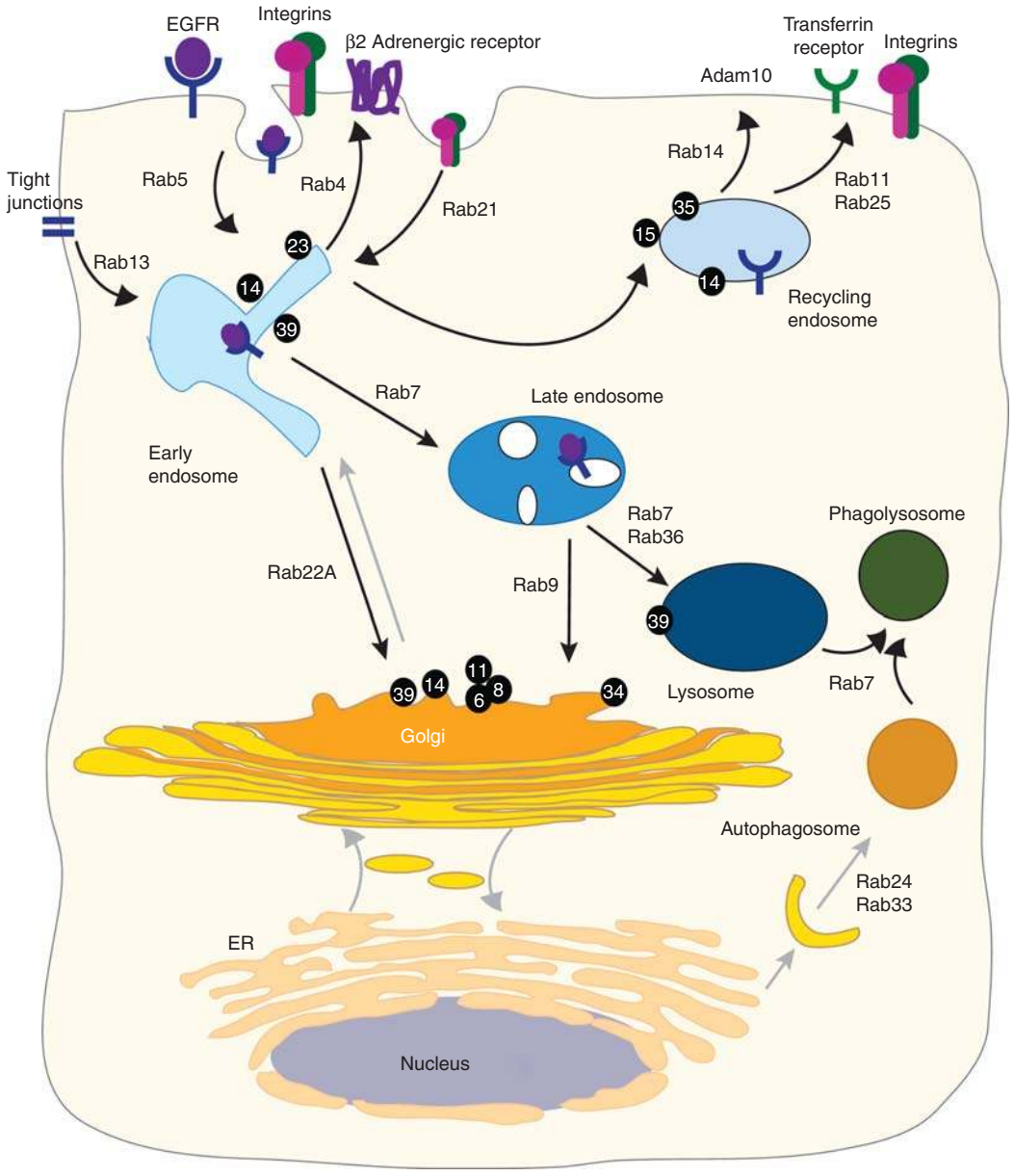

Figure 1. Overview of Rab GTPases on the endocytic pathway. Rab GTPases function in internalization and transport to degradation, as well as recycling to the plasma membrane and the Golgi. For details regarding individual Rab GTPase function, refer to the text and Table 1.

and functional characterization of their regulators and effectors. Through an affinity chromatography approach, an unexpected molecular complexity of the regulators and effectors downstream from Rab5 was first revealed (Christoforidis et al. 1999). To date, this remains the largest complement of molecular interactors (more than 50 proteins) for a Rab protein or for any small GTPase. An important series of Rab effectors was found to regulate phosphoinositide metabolism. A key effector of both Rab5 and Rab7 and important for endosome

function is the heterodimeric phosphatidylinositol-3-kinase (PI3K) Vps34/Vps15 complex (Christoforidis et al. 1999; Murray et al. 2002; Stein et al. 2003, 2005). Originally discovered in yeast as an essential component for vacuolar protein sorting (Schu et al. 1993), Vps34 catalyzes the phosphorylation of phosphatidylinositol to yield phosphatidylinositol-3-phosphate (PI3P). PI3P is highly enriched on early endosomes, late endosomes, and in the internal vesicles of multivesicular bodies and serves as a recognition motif for binding of FYVE-domain proteins (Stenmark et al. 1996; Gaullier et al. 1998; Gillooly et al. 2000). The FYVE domain is named after four cysteine-rich proteins—Fab 1 (mammalian PIKfyve), YOTB, Vac 1 (vacuolar protein), and EEA1 (early endosome antigen 1) in which this zinc finger domain was first found (Stenmark et al. 1996). Rab5 and Rab7 do not recruit Vps34 onto early and late endosomal membranes but rather spatially and temporally stimulate enzymatic activity following GTPase activation (Christoforidis et al. 1999; Murray et al. 2002; Stein et al. 2003; Shin et al. 2005), thus leading to the localized synthesis of PI3P. Through what has been termed a coincidence detection mechanism (Carlton and Cullen 2005), Rab5 and PI3P cooperate in the recruitment of proteins required for early endosome membrane tethering and fusion, such as EEA1 (Simonsen et al. 1998; Christoforidis et al. 1999; Lawe et al. 2000; Mishra et al. 2010), Rabenosyn-5 (Nielsen et al. 2000), and Rabankyrin-5 (Schnatwinkel et al. 2004). Rab7-stimulated PI3P formation leads to the recruitment of FYVE-domain-containing myotubularin lipid phosphatases onto endosomes, which both degrade the PI3P and directly inactivate the PI3K (Cao et al. 2008). This suggests that the combinatorial activity of Rab5 and Rab7 with PI3K and the myotublarins enables rapid and dynamic control over Rab-regulated PI3P and contributes to the regulation of early and late endocytic trafficking.

In addition to Vps34, Rab5 interacts with the PI3 kinase PI3Kβ and with PI5- and PI4-phosphatases and stimulates their activity (Shin et al. 2005; Hyvola et al. 2006; Erdmann et al. 2007). This series of enzymatic activities can be ordered in a pathway where the synthesis of phosphatidylinositol-3,4,5-triphosphate $PI(3,4,5)P_3$ or $PI(3,4)P_2$ at the plasma membrane (Posor et al. 2013; Schmid and Mettlen 2013) is followed by the sequential dephosphorylation through Rab5-interacting phosphatases, also leading to the synthesis of PI3P. The enzymes modulating phosphoinositides are shared by phagosomes (Bohdanowicz et al. 2012). The PIP turnover regulated by Rab5 at the plasma membrane serves multiple functions, including regulation of actin remodeling, vesicle budding, macropinocytosis, cell motility, and growth factor signaling (McLauchlan et al. 1998; Lanzetti et al. 2004; Palamidessi et al. 2008). Thus, PI3P contributes to spatiotemporal regulation and the compartmentalization of endosomal functions.

An increasing gradient of membrane cholesterol from peripheral early to late endosomes is regulated by Rab GTPases and conversely controls Rab and endosome function and compartmentalization (Holtta-Vuori et al. 2002; Rocha et al. 2009). For example, Rab11-mediated endosomal recycling is central to cholesterol esterification and homeostasis, whereas excess cholesterol accumulation in endosomes abrogates Rab4-dependent recycling, sequesters Rab9, and causes immobilization of Rab7-positive late endosomes and redistribution of internalized cargo (Lebrand et al. 2002; Choudhury et al. 2004; Ganley and Pfeffer 2006; Chen et al. 2008; Rocha et al. 2009). Rab7 endosome motility on microtubules is selectively regulated by protein interactions that are sensitive to cholesterol levels (Chen et al. 2008; Rocha et al. 2009). When cholesterol levels are low, the Rab7 effector oxysterol-binding protein-related protein 1 L (ORP1L) together with two integral membrane proteins (STARD3 and STARD3NL) induces late endosome interactions with the endoplasmic reticulum VAP protein, allowing dissociation of the p150/dynein motor complex (Alpy et al. 2013). Early endosome contacts with the endoplasmic reticulum have also been observed but remain functionally undefined (Friedman et al. 2013). At high cholesterol concentrations, the Rab7–p150–dynein motor complex is stabilized on late endosomes, which

accumulate in the perinuclear region, thus effecting endosome fusion and signaling (Taub et al. 2007; van der Kant et al. 2013). These examples illustrate the interdependence of endosomal cholesterol and Rab-regulated endosome function and transport.

Rab EFFECTORS AND COORDINATION OF SEQUENTIAL MEMBRANE TRAFFICKING EVENTS

Rab proteins may have many different effectors and interacting partners fulfilling discrete functions in the transport process (Fig. 2). For this reason, Rab GTPases can be viewed as the coordinators of all steps in membrane transport, from the sorting of cargo and formation of functional transport vesicles, motility along the cytoskeleton, and, eventually, membrane tethering and fusion (Somsel Rodman and Wandinger-Ness 2000; Park 2013).

Rab GTPases in Vesicle Budding

The mechanisms controlling Rab-regulated vesicle budding are as yet emerging. The most detailed analysis of Rab-regulated cargo sorting derives from studies on the rhodopsin photoreceptor and the polycystins. A short carboxy-terminal signal (VxPx) in the cargo proteins serves to bind Arf4 and recruit a cohort of Rab

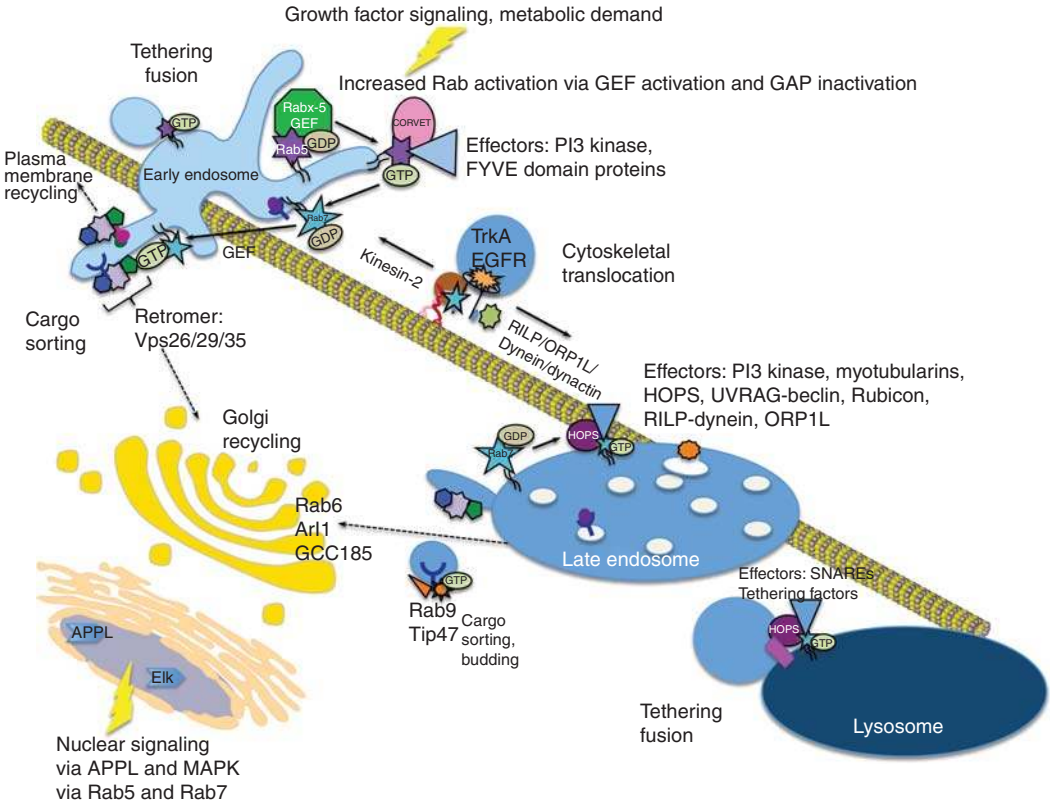

Figure 2. Rab GTPases integrate membrane trafficking events and signaling. Rab GTPases regulate cargo sorting, vesicle budding, membrane tabulation, cytoskeletal translocation, vesicle docking, and fusion. Rab GTPases decode extracellular signals to provide a coordinated response to physiological and metabolic demands. Select pathways are shown; not depicted are Rab-mediated interactions with the endoplasmic reticulum (ER) and other organelles for lipid exchange or interactions with the actin cytoskeleton.

GTPases (Rab6, Rab8, and Rab11) and regulatory proteins (ASAP1, Rabin8) that act in a concerted cascade to promote the ciliary targeting of their cargo (Mazelova et al. 2009; Ward et al. 2011, 2012). Rab5, Rab7, and Rab9 are implicated in endosomal cargo sorting mediated by retromer—a tripartite complex of Vps26, Vps29, and Vps35—and sorting nexins (Seaman 2012; Vardarajan et al. 2012; Dong et al. 2013; Pfeffer 2013b; Seaman et al. 2013; see also Burd and Cullen 2014). Rab5 activation is only indirectly required, but GTP-bound Rab7 is pivotal in the membrane recruitment of the retromer complex, and TBC1D5, one of two GAPs for Rab7, causes inactivation of the GTPase and retromer release from endosomal membranes. Retromer and discrete sorting nexins are important for differentially sorting cargo (including integrins, G-coupled receptors, mannose-6-phosphate receptor, and a luminal protein required for epithelial tube formation) from endosomes to the Golgi and the plasma membrane. Rab3b directly interacts with the cytoplasmic domain of the polymeric immunoglobulin receptor (pIgR), and this binding stimulates its transcytosis to the apical surface (van IJzendoorn et al. 2002). Evidence suggests that Rab5 may be, directly or indirectly, required for clathrin-mediated endocytosis (McLauchlan et al. 1998), which may reflect a coupling between the targeting and fusion machinery. Other rab proteins may play roles in cargo recruitment, for example, Rab7 interacts with the TrkA neurotrophin receptor cargo, although it remains unclear if directly or indirectly (Saxena et al. 2005; BasuRay et al. 2010). The Rab9 effector TIP47 binds the cytoplasmic domains of mannose-6-phosphate receptors (MPRs) in a Rab9-GTP-stimulated manner and is required for the return of this cargo to the Golgi complex (Carroll et al. 2001). Because TIP47 is also involved in lipid droplet biogenesis under conditions of rapid fat storage, it remains to be determined if TIP47 might be a shared effector that also interacts with one of the myriad of Rab GTPases involved in lipid droplet formation or, alternatively, promotes interorganellar tethering to lipid droplets to allow access to critical lipid regulators of endocytosis (Barbero et al. 2001; Bulankina et al. 2009; Hynson et al. 2012). Indeed, Tip47 and Rab9 are important targets in viral infectivity and have been associated with lipid droplet consumption, suggesting that resolving this open question will be of significant interest (Murray et al. 2005; Chen et al. 2009b; Carvalho et al. 2012; Vogt et al. 2013). Based on the cumulative evidence, it is speculated that Rab-mediated cargo selection and vesicle budding will involve specific lipid recruitment through *cis* or *trans* membrane interactions, signal-dependent cargo binding, cooperativity between Arf and Rab GTPases, and GTPase regulatory factors for spatiotemporal control of protein–protein interactions.

Rab GTPases and Microtubule-Dependent Translocation

Long-range endosome motility depends on microtubules. Rab effectors include regulators of such intracellular motility. Endosome movement is bidirectional and occurs in a stop-and-go fashion because of the alternating activities of plus-end-directed kinesin motors and the minus-end-directed dynein-dynactin motor. Several studies have shown that microtubule-dependent motility of endosomes depends on Rab proteins. Rab5 regulates the motility of early endosomes on microtubules (Nielsen et al. 1999) through the recruitment of the early endosome motor KIF16B that depends on both active Rab5 and the generation of PI3P (Hoepfner et al. 2005). Rab4 regulates KIFC2 activity on early/recycling endosomes, which in conjunction with other microtubule motors regulates the motility as well as the fission of early endosomes (Bananis et al. 2004). Rab9 is present on late endosomes that display bidirectional microtubule-dependent motility (Barbero et al. 2002). On late endosomes and lysosomes, the recruitment of dynein by Rab7 is a complex process that requires the activity of multiple proteins. Rab7 recruits two effectors, RILP (Rab-interacting lysosomal protein) (Cantalupo et al. 2001; Jordens et al. 2001) and ORP1L (Johansson et al. 2007). RILP interacts directly with the dynactin arm p150(Glued)

to recruit dynein. ORP1L is also required for dynein motor activity by binding βIII spectrin, which acts as a receptor for the dynactin complex (Johansson et al. 2007) and triggers the translocation of late endosomes to microtubules and dynein-dependent minus-end motility. Interestingly, RILP also binds the tethering HOPS complex, thus coupling membrane tethering to microtubule minus-end transport (van der Kant et al. 2013). RILP is also an effector of Rab36 that regulates retrograde melanosome transport in melanocytes (Matsui et al. 2012), indicating that effectors can be shared between different Rab proteins localized to distinct intracellular compartments. Rab9 interaction with a Golgi-tethering factor, GCC185, promotes interaction with Rab6, Arl1, and the CLASP microtubule-anchoring protein and is suggested to integrate dynein-mediated delivery with docking of endosome-derived vesicles to the Golgi (Hayes et al. 2009). Additional insights into the regulation of Rab7 and Rab9 endosome motility—cooperativity with Arf-like (Arl) proteins and kinesin-interacting proteins—have in part been gained through analyses of how bacterial pathogens usurp RabGTPase effectors during the establishment of an intracellular niche (Jackson et al. 2008; Garg et al. 2011; Mrakovic et al. 2012; Stein et al. 2012b). What emerges from the composite work is that Rab GTPases interact in a nucleotide-dependent manner with microtubule motor complexes and thereby directly control cytoskeletal translocation.

Rab GTPases and Actin-Dependent Translocation

Many endocytic compartments, endosomes, lysosomes, melanosomes, and phagosomes also move propelled by actin-dependent motors, usually over shorter distances. Microtubule- and actin-based motility of endosomes and lysosome-related organelles is frequently coordinated through Rab-regulated handoffs between motor proteins that enable long-range transport on microtubules and motors that enable shorter-range transit or proteins that mediate membrane docking in preparation for targeted fusion (Agola et al. 2011). Rab11a was originally found to interact with myosin Vb, and this interaction is required for the recycling of cargo to the surface (Lapierre et al. 2001). Rab27a is an important regulator of lysosome-related organelles, including melanosomes in melanocytes and lytic granules in cytotoxic T lymphocytes (Menasche et al. 2000; Wilson et al. 2000; Hume et al. 2001; Matesic et al. 2001; Stinchcombe et al. 2001; Wu et al. 2001). Among the functions of Rab27 is the membrane recruitment of Myosin Va to regulate melanosome motility and membrane docking (Nagashima et al. 2002; Seabra et al. 2002; Strom et al. 2002; Wu et al. 2002a,b). Myosin Va is a member of the unconventional class V myosin family, and mutations in the myosin Va gene cause pigment granule transport defects in human Griscelli syndrome and dilute mice. Rab27a binds Slac2-a (synaptotagmin-like protein homolog lacking C2 domains-a)/melanophilin, and this associates with myosin Va, thus forming a tripartite protein complex (Rab27a–Slac2-a–myosin Va) (Strom et al. 2002; Fukuda 2003). It subsequently became apparent that myosin Va binds multiple Rab GTPases (3A, 8A, 10, 11A, 27A, 3B, 3C, 3D, 6A, 6A′, 6B, 11B, 14, 25, 39B), including three new Rab subfamilies (Rab6, Rab14, Rab39B) (Lindsay et al. 2013). As illustrated by the given examples, different Rab proteins can use the same motor protein to regulate organelle movement. However, the interaction of Rab GTPases with the actin cytoskeleton involves interactions beyond those with the motor proteins. For example, Rab5 binds the effector HAP40, and this recruits huntingtin to the membrane of the early endosomes (Pal et al. 2006). This causes the attachment of early endosomes to the actin cytoskeleton and slows long-range motility (Lindsay et al. 2013). Rab7 via interactions with retromer is associated with locally assembled actin complexes involved in endosome tubulation that are important for cargo sorting (Seaman et al. 2013). In sum, Rab GTPase interactions with actin promote short-range transport, promote vesicle docking, and stabilize specialized endosomal membrane domains involved in sorting and tubulation.

Rab GTPases AND MACROMOLECULAR ASSEMBLIES IN MEMBRANE TETHERING AND FUSION

A common function of Rab effectors is their activity as tethering molecules for membranes that have complementary sets of soluble NSF attachment protein (SNAP) receptors (SNAREs) for fusion.

Rab GTPases and Tethering

A combination of in vitro and in vivo studies led to the consensus view that Rab GTPases are part of large molecular complexes that orchestrate the orderly and timely membrane recruitment and activity of docking/tethering factors that bring together membranes compatible for fusion (Zerial and McBride 2001; Grosshans et al. 2006; see also Gautreau et al. 2014). Direct experimental evidence for a role of Rab GTPases and their effectors in membrane docking or tethering has been obtained in vitro using purified or recombinant proteins and organelle preparations obtained by subcellular fractionation (Christoforidis et al. 1999; Wang et al. 2003). The first Rab effector directly shown to tether endosomal membranes in vitro was EEA1 (Christoforidis et al. 1999).

Recent work in yeast and metazoans has uncovered an unexpected modular assembly of tethering complexes in the endocytic pathway that is likely conserved in mammals, where they are as yet incompletely characterized (for review, see Numrich and Ungermann 2013; Solinger and Spang 2013). Many of these proteins were identified in yeast based on the characterization of "class C" vacuolar protein sorting (VPS) mutants. Class C mutants were distinguished phenotypically as lacking vacuoles and accumulating vesicles, multilamellar membranes, and Golgi (Banta et al. 1988). Prevacuolar early endosomal compartments rely on the class C core CORVET complex, which is recruited by the Rab GTPase Ypt51/Vps21 for vacuole/endosome tethering (Peplowska et al. 2007). Subsequent homotypic vacuole fusion requires tethering by the homotypic fusion and vacuole protein sorting (HOPS) complex, which is recruited by the Rab GTPase Ypt7p (Mayer and Wickner 1997; Seals et al. 2000; Ungermann et al. 2000; Numrich and Ungermann 2013). CORVET and HOPS are highly conserved multiprotein complexes consisting of six subunits—four shared in common (Vps11, Vps16, Vps18, and Vps33) and two specific subunits, Vps39p/Vam6p and Vps41p/Vam2p for CORVET/HOPS, respectively (Peplowska et al. 2007; Plemel et al. 2011; Lo et al. 2012). Interestingly, the transition from CORVET to HOPS occurs by exchange of subunits, thus leading to the formation of intermediate complexes. It is unclear why such interconversion is necessary, but it is likely that the two complexes perform specialized functions in the early and late endocytic pathway (Solinger and Spang 2013).

Rab GTPases and SNARE-Mediated Membrane Fusion

Membrane docking and fusion are driven by the formation of *trans*-SNARE complexes, leading to closely apposed docked membranes that can overcome the free-energy barrier to fusion (Weber et al. 1998; McNew et al. 2000; Jahn and Scheller 2006; Zimmerberg and Gawrisch 2006; Wickner and Schekman 2008). Rab GTPases and their tethering effectors act upstream of SNAREs and, therefore, provide the first layer of specificity in the recognition of membranes compatible for fusion. In addition, they contribute key functions and factors required for SNARE-mediated membrane fusion. Rab5 and PI3P recruit an effector complex consisting of Rabenosyn-5 and Vps45, a member of the Sec1/Munc18 protein family, to the early endosomal membrane (Nielsen et al. 2000). Sec1/Munc18 proteins regulate the formation of SNARE complexes but also cooperate with SNAREs to stimulate membrane fusion (Carr and Rizo 2010; Rizo and Sudhof 2012). Vps33p, a member of the Sec1 family of proteins, is also a component of the HOPS complex that regulates SNARE complex assembly in yeast vacuole fusion (Seals et al. 2000). In addition, Rab effectors physically interact with SNAREs, the mechanistic significance of which is unclear at present. For example, the Rab5 effec-

tor EEA1 interacts with the early endosomal SNAREs syntaxin6 and syntaxin13 (McBride et al. 1999; Simonsen et al. 1999). The HOPS complex recruits the soluble SNARE Vam7p onto vacuolar membranes, thus fulfilling a key rate-limiting step in the fusion of yeast vacuoles (Zick and Wickner 2013).

Consistent with a role in determining the structural and functional identity of organelles, Rab GTPases and their effectors have a much narrower compartmental distribution when compared with SNAREs that undergo cycling between donor and acceptor compartments. On endosomes, Rab GTPases are compartmentalized into distinct domains, such as those harboring Rab5, Rab4, or Rab11 on early endosomes (Sonnichsen et al. 2000) or Rab7 or Rab9 on late endosomes (Barbero et al. 2002). In the case of Rab5, such a compartmentalization may be due to the fact that the Rab5 effectors form large oligomeric complexes on the early endosome membrane (McBride et al. 1999). This may be the reason why Rab5 displays a restricted lateral mobility on the early endosome membrane (Pelkmans et al. 2004).

The concept of cooperativity has recently received strong support from in vitro studies in which the Rab and SNARE machineries of early endosomes (Rab5, its effectors, and the SNARE proteins syntaxin 13, syntaxin 6, VTI1A, and VAMP4) (Ohya et al. 2009) and yeast vacuoles (Ypt7 and the vacuolar SNARE proteins Vam3p, Vti1p, Vam7p, and Nyv1p) (Stroupe et al. 2009) were reconstituted in proteoliposomes. These studies have shown that the "synthetic" endosomes could efficiently fuse with each other in vitro, with far higher efficiency than membranes with SNAREs alone, matching the activity of purified native early endosomes. In addition, membrane fusion requires cooperativity between Rab effectors and SNAREs. For example, Ypt7p, the HOPS complex, and a Q-SNARE complex were all required to bring the membranes into proximity before fusion (Stroupe et al. 2009). As illustrated by these examples, both membrane tethering and fusion depend extensively on the cooperativity among Rab effectors and between Rab effectors and SNAREs.

Rab CONVERSION AND Rab CASCADES

The term "Rab conversion" was coined to describe the remodeling of the endosomal membrane during cargo progression from early to late endosomes (Rink et al. 2005; Vonderheit and Helenius 2005). As described above, a fundamental property of Rab GTPases is that their association with the membrane depends on a dynamic equilibrium between nucleotide exchange and hydrolysis, and shuttling between membrane and cytosol regulated by Rab-GDI. In principle, this equilibrium can be tuned to achieve a stable assembly or a dynamic assembly and disassembly. Live-cell imaging studies revealed that the levels of Rab5 are not stable on the endosomal membrane but fluctuate dynamically and can lead to complete disassembly as cargo is transported along the endocytic pathway (Rink et al. 2005; Vonderheit and Helenius 2005; Huotari and Helenius 2011). These studies have shown that, through repetitive fusion and fission events, endocytosed cargo transported into peripheral early endosomes becomes concentrated in fewer and larger endosomes, which progressively move to the perinuclear region. On these endosomes, the levels of Rab5 reach a peak, following which there is the complete loss of Rab5 and its replacement with Rab7. Thus, conversion of Rab5 to Rab7 marks the transition of cargo from early to late endosomes. Note that also the budding of Rab7 domains from the early endosomal membrane has been proposed as an alternative mechanism of cargo transport from early to late endosomes (Rink et al. 2005; Vonderheit and Helenius 2005; Huotari and Helenius 2011). Rab5-to-Rab7 conversion also occurs on phagosomes (Vieira et al. 2003; Henry et al. 2004) and during macropinosome maturation (Kerr et al. 2006).

The Rab5/Rab7 switch shares similarities with the toggle switch described for the cell cycle but is more similar to a cut-out switch, for example, an electrical safety-breaker (Del Conte-Zerial et al. 2008). Briefly, as the levels of Rab5 on the endosome membrane increase, they also trigger more recruitment of Rab7 (via the CORVET/HOPS complexes). When a threshold level is reached, Rab7 then represses

Rab5 leading to its disassembly. Studies in *Caenorhabditis elegans* identified SAND-1/Mon1 as a critical element of such repression. The conversion results when, first, the SAND-1/Mon1 displaces the Rab5 GEF (RABGEF1/RABEX5), and second, recruits Rab7 to the membrane through an interaction with the CORVET/HOPS complex, which contains the Rab7 GEF. These observations gave rise to the current model in which Rab GTPases that function in sequence are activated and inactivated in a "cascade-like" manner. The recruitment of one GTPase leads to the recruitment of the next GTPase together with simultaneous inactivation of the upstream GTPase via a specific GAP and activation of the downstream GTPase via its GEF (Hutagalung and Novick 2011). The interconnection of Rab GTPases in cascades may incorporate entire circuits involved in specific physiological responses, connecting exocytosis to cargo return via endocytosis. This is particularly evident in specialized pathways such as the coordinate function of exocytic and endocytic Rab GTPases in transport to the primary cilium, insulin-stimulated GLUT4 vesicle secretion, and recycling, as well as responses to metabolic stress that trigger autophagy or lipid droplet formation (Sano et al. 2008; Longatti and Tooze 2009; Murphy et al. 2009; Ward et al. 2011; Westlake et al. 2011; Chen et al. 2012; Longatti et al. 2012; Reed et al. 2013).

ENDOCYTIC Rab GTPases IN THE COORDINATION OF SIGNALING AND PHYSIOLOGICAL RESPONSES

Deficits in endocytosis cause genetic and sporadic human diseases underscoring the pathway's importance in normal cell physiology (Agola et al. 2011; Stein et al. 2012b). As articulated in sections above, the proper regulation of endocytic transport relies on spatiotemporal regulation of Rab function, GTPase cascades, and coordinated assemblies of macromolecular protein complexes. GTPase functions are, in turn, closely coupled to receptor signaling (Fig. 2) (Barbieri et al. 2000; Di Fiore and De Camilli 2001; Wiley 2003; Platta and Stenmark 2011; Cocucci et al. 2012; Numrich and Ungermann 2013). Receptor-mediated control over endocytic trafficking is a pivotal determinant of individual receptor fates, even though a common core endocytosis machinery is used by multiple receptors (Pfeffer 2013b). Key to this control is the regulation of Rab GTPases that control sorting and transport along these routes. Select endosomal GTPases have been shown to be transiently activated following growth factor receptor activation (Rab4, Rab5, and Rab7). Mechanistically, the acute GTPase activation has been partially detailed for Rab5 in response to EGF. Activation is attributed to increased GTP loading via a Rab5-specific guanine-nucleotide exchange factor (Rin1 GEF) and inhibition of GTP hydrolysis by inactivation of the GTPase-activating protein (RN-Tre GAP, which also acts on Rab41 and Rab43) (Lanzetti et al. 2000; Di Fiore and De Camilli 2001; Tall et al. 2001; Haas et al. 2005, 2007). Modulation of PI3P platforms on membranes is also involved (Zoncu et al. 2009). In addition, a population of early endosomes harbors the Rab5 effectors APPL1 and 2 (Miaczynska et al. 2004), which interact with a specific set of receptors and modulate various signaling pathways, such as adiponectin (Mao et al. 2006) and EGF signaling (Zoncu et al. 2009). Rab4 activity like that of Rab5 appears subject to growth factor signaling through the activation of regulatory proteins (Hellberg et al. 2009; Goueli et al. 2012). Rab7 is activated by virus infection and modulated by EGFR signaling—although the mechanisms are incompletely clarified (Buranda et al. 2013; Rush and Ceresa 2013). Analyses of four single-point mutants of Rab7, which are associated with a peripheral neuropathy CMT2B, revealed profound deficits in receptor trafficking in conjunction with defects in cytosolic and nuclear signaling (BasuRay et al. 2010, 2013; McCray et al. 2010). Such signal-dependent regulation of Rab function is expected to include a broad array of GTPases, including those involved in providing access to key metabolites and lipids (Murphy et al. 2009; Rasineni et al. 2013), as well as those involved in specialized pathways (Stockli et al. 2008; Babbey et al. 2010; Chen and Lippincott-

Schwartz 2013). It is speculated that most Rab-regulated trafficking will prove to be tightly coupled to physiological demand through temporal and spatial regulation of Rab GTPase activation, localization, and interorganellar interactions. Evidence for importance at the systems level is exemplified by studies in liver where in vivo depletion of all three Rab5 isoforms is sufficient to cause a dramatic reduction in the number of early endosomes, late endosomes, and lysosomes (Zeigerer et al. 2012). A block of receptor-mediated endocytosis accompanied endosome loss. Furthermore, the fact that ubiquitous Rab GTPases such as Rab5, Rab7, and Rab9 have key roles in neurologic diseases further highlights their importance in the integration of trafficking of specific cargo and signaling, thereby making neurons particularly vulnerable to deficits (Panzeri et al. 2006; McCray et al. 2010; BasuRay et al. 2012; Freeman et al. 2013; Pfeffer 2013b). Thus, gaining a complete picture of Rab compartmentalization will require detailed studies of how receptor signaling and other environmental cues impact Rab regulatory protein activities, Rab protein assemblies, and GTPase cascades.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The findings outlined support the tenet that Rab GTPases provide structural and functional identity to endosomal subcompartments and to cellular organelles. Rab machineries assembled on membranes are highly dynamic, reversible, and subject to cellular and tissue demand. Current data support a model wherein the sequential assembly and disassembly of Rab GTPases and their effectors on endosomal membranes serve as central integrators of endosome biogenesis, cargo transport, and signaling. The processes are fundamental to metabolic and physiological demand and pivotal to organ function and organismal viability.

Important aspects for further study include deciphering the molecular mechanisms whereby Rab GTPases are targeted to specific membranes and how their activation cycle is regulated to control downstream functions. The mechanisms of Rab targeting to a specific compartment are unclear, and the localization of GDF and GEF alone does not provide the solution to this important problem. It is essential to identify the GEFs and GAPs for most Rab GTPases, determine where and when they function, and how they are regulated with respect to metabolic and signaling needs. GTPase regulatory proteins are intimately interconnected to the recruitment of downstream effectors, and distinct regulatory proteins may regulate specific cargo-dependent pathways. In addition, even for effectors that have been identified to have highly conserved functions in tethering and fusion, greater mechanistic detail is needed. For example, we know that Rab effectors physically interact with SNAREs, yet the precise mechanism of how membrane tethering leads to membrane fusion remains an unsolved problem. Addressing the open questions will require the application and development of sophisticated morphological, biochemical, and biophysical approaches, beyond the current state-of-the-art.

The data so far only begin to elucidate the roles of Rab proteins in the organization and functional properties of eukaryotic cells. Clearly, the function of Rab proteins is not limited to the regulation of organelle structure and ubiquitous functions in all cells. The spectacular expansion of Rab proteins throughout evolution points to new functions that remain to be discovered. These functions include cell- and tissue-specific activities, cargo-specific trafficking, interorganellar interactions, metabolic responses, and signaling. Therefore, such exploration will have to be conducted on different cell and animal model systems, because the more-traditional cell culture models are not always capable of recapitulating specialized processes in response to physiological demands.

It is not clear at present whether the complexity of the Rab5 interactome is an exception or Rab proteins in general require a large set of effector molecules and regulators for their function. If we assume an average of 30 interacting molecules per Rab GTPase, the entire human Rab interactome would account for more than 2000 proteins, ~10% of the human proteome. For some of these molecules, their identity can

be predicted bioinformatically. However, this is true only in some cases and is not applicable to the broad array of interacting molecules. Although these numbers are purely suggestive at present and may be overestimated, they give an idea of the central role that the Rab machinery plays in the organization and function of cells and tissues. Once these molecules are identified, the challenge will be not only to understand how they function with respect to an individual Rab protein but how the diverse Rab machineries are integrated. This is a formidable task that requires coordinated efforts and systems-level approaches.

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Angela Wandinger-Ness and Marino Zerial

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