[Frontiers in Bioscience 14, 2115-2129, January 1, 2009]

Rab family small G proteins in regulation of epithelial apical junctions

Noriyuki Nishimura, Takuya Sasaki

Department of Biochemistry, Institute of Health Biosciences, The University of Tokushima Graduate School, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan

TABLE OF CONTENTS

1. ABSTRACT

Tight junctions (TJs) and adherens junctions (AJs) comprise epithelial apical junctions that adhere neighboring epithelial cells and determine tissue organization. They are highly dynamic structures that undergo continuous remodeling during physiological morphogenesis and under pathological conditions. The assembly and disassembly of epithelial apical junctions is regulated by the interplay between a variety of cellular processes, such as the remodeling of actin cytoskeletons and the endocytic recycling of apical junctional proteins, and coordinated by many signaling pathways. Accumulating evidences demonstrate that Rab family small G proteins are crucially involved in the regulation of epithelial apical junctions. Rab proteins localized both at endosomes and apical junctions can influence the assembly and disassembly of epithelial apical junctions. In this review, we summarize how Rab proteins influence epithelial apical junctions and describe the role of Rab8/13-a junctional Rab13-binding protein (JRAB)/molecule interacting with CasL-like 2 (MICAL-L2) complexes in the regulation of epithelial apical junctions.

2. INTRODUCTION

Rab family small G proteins are first identified as evolutionarily conserved, essential regulators of membrane traffic in the 1980s (1-3). They are members of the wider Ras superfamily of small G proteins and appear to control a variety of cellular processes ranging from membrane traffic to membrane-cytoskeleton interactions and signal transduction (4, 5). To date, over 70 Rab and Rablike proteins have been identified in human, and several Rab proteins are implicated in the assembly and/or disassembly of epithelial apical junctions (Figure 1) (6). Epithelial apical junctions defined by tight junctions (TJs) and adherens junctions (AJs) provide important adhesive contacts between neighboring epithelial cells and crucially determine tissue organization both in health and disease (7). They are very dynamic cellular structures that are continuously remodeled and control the cellular morphogenesis and tissue patterning. It is not surprising that many signaling pathways and cellular processes regulate epithelial apical junctions. In this review, we first provide an overview of Rab proteins and epithelial apical junctions. Then we summarize how Rab proteins influence

epithelial apical junctions and describe the role of Rab8/13-a junctional Rab13-binding protein (JRAB)/molecule interacting with CasL-like 2 (MICAL-L2) complexes in the regulation of epithelial apical junctions.

3. RAB FAMILY SMALL G PROTEINS

3.1. Activation/inactivation of Rab proteins

Rab proteins interconvert between active GTP-bound forms and inactive GDP-bound forms, and serve as membrane-associated molecular switches. This switch is controlled by guanine nucleotide exchange factor (GEF), which triggers the binding of GTP, and GTPase-activating protein (GAP), which accelerates hydrolysis of the bound GTP to GDP (8-10). Rab proteins associate tightly with membranes by virtue of their carboxyl terminal single or double geranylgeranylation and undergo a membrane association/dissociation cycle coupled with a GTP/GDP cycle. GDP dissociation inhibitor (GDI) binds to a geranylgeranylated Rab protein in its GDP-bound form, extracting it from the membranes and keeping it in the cytosol (11). The cytosolic Rab-GDI complex carries all of the information that is needed for the correct targeting of Rab proteins to membranes. GDI displacement factor (GDF) dissociates Rab proteins from the Rab-GDI complex and enables membrane attachment of Rab proteins (12-14). Once dissociated from GDI, Rab proteins are converted to their GTP-bound form by their specific GEFs. The active membrane-bound Rab proteins exert their variety of functions by binding to their specific effector proteins. After inactivation by their specific GAPs, the GDP-bound Rab proteins can be extracted from the membrane by GDI and recycled back to the cytosol.

A Rab effector protein responds to a specific Rab protein and mediates at least one element of its downstream effects (15). Rapidly growing list of Rab effector proteins has revealed that each Rab protein appears to signal through a variety of different effector proteins that together act to translate the signal from one Rab protein to several diverse aspects of cellular processes. Rab proteins contribute the specificity in membrane traffic by regulating budding, transport, tethering, and fusion steps in vesicular transport and by establishing membrane domains (16, 17). They also play important regulatory roles in membrane-cytoskeleton interactions by associating with molecular motors and other cytoskeleton-binding proteins (18, 19). In addition, they participate in the regulation of numerous signal transduction pathways (20, 21).

3.2. Rab proteins in membrane traffic

In vesicular transport, Rab proteins can control cargo collection during transport vesicle formation, enable motor proteins to interact with membranes to drive vesicle motility, and mediate the complex events of accurate tethering and fusion of transport vesicles with their target membranes (15). Rab9 effector TIP47 binds to GTP-bound Rab9 and increase its affinity for mannose 6-phosphate receptor (M6PR), facilitating the capture of M6PR into Rab9-positive transport carrier vesicles (22). GTP-bound Rab6 binds to the microtubule motor Rabkinesin-6 and promotes the delivery of vesicles from the Golgi to endoplasmic reticulum (23). A long coiled-coil tethering factor p115 that tethers endoplasmic reticulum-derived vesicles to the Golgi is identified as a Rab1 effector protein (24). GTP-bound Rab5 recruits another long coiled-coil tethering factor EEA1 onto early endosome, and the interaction of EEA1 with the soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) protein Syntaxin13 is required for homotypic early endosome fusion (25).

Rab protein is localized to the distinct subcellular membrane compartment and each compartment has a unique set of Rab proteins, which can serve as markers of the particular compartment (26, 27). For instance, Rab1 is on endoplasmic reticulum, Rab6 is on the Golgi, and Rab3 is on secretory granules and synaptic vesicles. Although the molecular mechanisms controlling Rab localization are not fully understood, the correct targeting of Rab proteins to their specific membranes is intimately linked to their activation. Rab proteins also contribute to establish specific membrane domains, which are well-characterized in the endocytic pathway (16, 17). Early endosomes harbor only Rab5 or a combination of Rab4 and Rab5, whereas recycling endosomes carry distinct domains of Rab4 and Rab11. Rab7 and Rab9 similarly share late endosomes. Rab5 GEF Rabex5 activates Rab5 on early endosomes and activated Rab5 interacts with Rab5 effector Rabaptin5 that in turn binds to Rabex5 and increases the exchange activity of Rabex5 on Rab5 (28-30). This Rabex5-Rab5-Rabaptin5 complex serves as a positive feedback loop to establish a Rab5-domain on early endosomes. Importantly, the Rab5-containing early endosomes can be converted into Rab7-containing late endosomes. This conversion is mediated by the six subunits class C homotypic fusion and vacuole protein sorting (HOPS)/vacuole protein sorting (VPS) complex that contains a Rab5 effector hVps11 and a Rab7 GEF hVps39 (31). The coupling of a downstream Rab GEF with an upstream Rab effector could be a way to achieve specificity in membrane traffic (32).

3.3. Rab proteins in membrane-cytoskeleton interactions

In addition to myosin and kinesin motor proteins, Rab proteins also interact with non-motor cytoskeleton-binding proteins directly or via an intermediary protein, and control membrane-cytoskeleton interactions (18, 19). These non-motor cytoskeleton-binding proteins include alpha-actinin, EB1, and Hook1. Rab3 effector Rabphilin3 binds to actin cytoskeletons in an alpha-actinin-dependent manner and facilitates the vesicle-F-actin network interactions below the plasma membrane (33, 34). Rab27 effector Melanophilin, which links Rab27 to myosin Va on melanosome, also interacts with microtubule plus-end tracking protein EB1 (35). A microtubule-binding protein Hook1 physically associates with endocytic Rab7, Rab9 and Rab11 as well as membranes, and the *Drosophila* homologue of Hook1 regulates the membrane traffic of internalized ligands to late endosomes (36, 37).

3.4. Rab proteins in signal transduction

Rab proteins are increasingly found downstream of signal transduction pathways that direct a variety of cellular processes, including gene expression, cell survival, cell growth, differentiation, proliferation, cell cycle, and apoptosis. Rab4 is phosphorylated by the mitotic Cdk1 kinase and participates in the control of the endosomal compartment during mitosis (38). GTP-bound Rab8 interacts with a member of germinal center kinases (GCKs) that regulate eukaryotic stress responses (39). GTP-bound Rab11 associates with phosphatidylinositol 4-kinase beta (PI4Kbeta) that is implicated in the endocytic recycling and activation of extracellular signal-regulated kinase 1/2 (ERK1/2) (40, 41).

4. EPITHELIAL APICAL JUNCTIONS

TJs and AJs are located at the apical end of the basolateral membrane, and define the organization of epithelial apical junctions (7). Whereas TJs seal the intercellular space and delineate the boundaries between the apical and basolateral membranes, AJs principally initiate and maintain cell-cell contacts. Both TJs and AJs are built according to the same architectural principle as other adhesion complexes. A set of different transmembrane proteins mediates cell-cell adhesion and is linked to cytosolic plaque proteins that anchor the junction to the cytoskeleton. At TJ, the principal transmembrane proteins forming the paracellular diffusion barrier are claudins that comprise claudin family consisting of at least 24 members in mammalian cells (42-44). Other transmembrane proteins identified at TJ include occludin, tricellulin, junction adhesion molecules (JAMs), coxsackievirus and adenovirus receptor (CAR), and Crumb3 (CRB3). Occludin is the first identified transmembrane protein at TJs, whose physiological function remains to be established (45). Tricellulin is recently identified as another TJ component specifically localized to the tricellular junctions (46). JAMs consist of at least 5 JAM family members and the first JAM to be identified, JAM-A, is involved in the accumulation of a cell polarity protein complex, the Par3/Par6/atypical protein kinase C (aPKC) complex, at TJ (47-49). CAR associates with JAM-C and mediates attachment and infection by group B coxsackieviruses (CVB) and adenoviruses (50). CRB3 forms another cell polarity protein complex, the CRB3/PALS1/PATJ complex (51). At AJ, the transmembrane protein, E-cadherin, forms the characteristic structures of AJs (52). E-cadherin is a member of cadherin superfamily that comprises more than 100 members, each of which is expressed in non-epithelial cells as well as in epithelial cells (53). Nectins are identified as additional transmembrane proteins at AJ and involved in the organization of AJ either in cooperation with or independently of E-cadherin (54). These transmembrane proteins are associated with TJ and AJ plaque proteins in the cytosol, which form an organizing platform for a variety of scaffolding, signaling, and membrane traffic proteins, including zonula occludens (ZO) proteins (ZO-1, ZO-2, and ZO-3), membrane-associated guanylate kinase inverted (MAGI) proteins (MAGI-1, MAGI-2, and MAGI-3), catenins, the Par3/Par6/aPKC and CRB3/PALS1/PATJ complexes, Rab3B, Rab8, Rab13, and Rab34 (55-57).

5. ENDOCYTIC RECYCLING PATHWAYS IN EPITHELIAL CELLS

Endocytosis regulates the entry of small and large extracellular molecules into cells, and is multistep process involving the budding of plasma membrane and the formation of vesicles followed by their delivery and fusion with specific intracellular compartments (58, 59). Endocytosis can be divided into phagocytosis, which is the uptake of particles, and pinocytosis, which is the uptake of fluid. Furthermore, there are four basic mechanisms for pinocytosis: macropinocytosis, clathrin-dependent endocytosis, caveolin-dependent endocytosis, and clathrin- and caveolin-independent endocytosis. Whereas the formation of large actin-coated vacuolae are responsible for macropinocytosis, the polymerization of a specific coat protein clathrin and the invagination of caveolin-containing cholesterol-enriched microdomains drive clathrin-dependent and caveolin-dependent endocytosis, respectively. There are several clathrin- and caveolin-independent endocytosis pathways that can be further classified based on the requirement for dynamin and the involvement of Cdc42, RhoA, and ARF6 (59).

Most cargo molecules internalized from plasma membrane are delivered to early endosome, which consists of two spatially separated populations of apical and basolateral early endosomes (AEE and BEE) in polarized epithelial cells. Whereas some internalized molecules in basolateral early endosome may directly return to basolateral membrane, internalized molecules in apical and basolateral early endosomes eventually merge in a tubulovesicular compartment. This compartment is variously termed apical recycling endosome (ARE), common endosome (CE), or subapical compartment (SAC), and serves as a sorting station that determines the fate of internalized molecules (60). Subsequently, they may enter recycling endosome to return to plasma membrane, or be degraded in late endosome and lysosomes (Figure 1).

Endosomal recycling vesicles containing the internalized cargo molecules are eventually fused with plasma membrane, which is catalyzed by SNARE proteins (61). They have over 30 members resided at distinct subcellular compartments in mammalian cells and functionally can be classified into ‘v-SNARE’ on the vesicle and ‘t-SNARE’ on the target membrane. Specific interaction of v-SNARE with the cognate t-SNARE forms a SNARE complex that drives membrane fusion. In polarized epithelial cells, two major t-SNARE proteins, Syntaxin3 and Syntaxin4, are spatially segregated into different plasma membrane domains with the apical membrane-confined Syntaxin3 and basolateral membrane-confined Syntaxin4 (62). Whereas the apical targeting requires the tetanus neurotoxin (TeNT)-resistant v-SNARE TI-VAMP (VAMP7), the basolateral targeting involves the TeNT-sensitive v-SNARE cellubrevin (VAMP3) (63, 64).

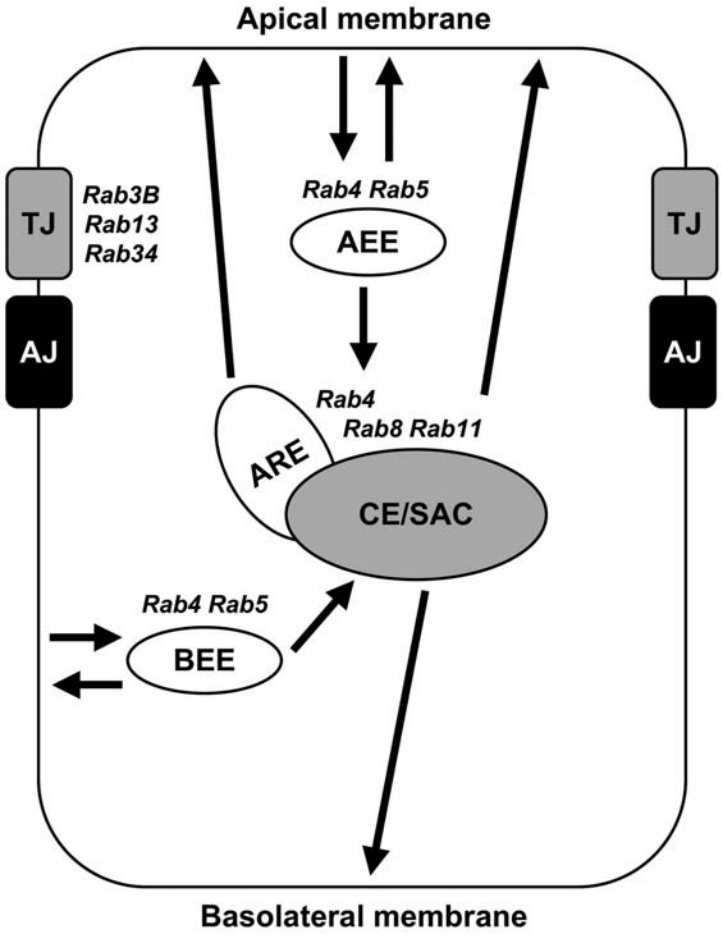

Figure 1. Rab proteins implicated in regulation of epithelial apical junctions. Rab proteins, epithelial apical junctions, and endosomal compartments are shown. AEE, apical early endosome; BEE, basolateral early endosome; CE, common endosome; SAC, subapical compartment; ARE, apical recycling endosome; TJ, tight junctions; AJ, adherens junctions.

Before the SNARE-dependent fusion reaction, endosomal recycling vesicles need to be tethered with plasma membrane. Whereas SNARE proteins on opposing membrane bring the two membranes into very close apposition for membrane fusion, tethering factors physically link the two membranes at some distances with a degree of reversibility. Central to the tethering of vesicles with plasma membrane is Rab proteins and a large octameric complex called the exocyst (65). The exocyst is composed of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84, and associated with the apical junctions and recycling endosome in polarized epithelial cells. It interacts with several known regulators of epithelial apical junctions such as Ral, ARF6, and Rab11, and functions in the endocytic recycling as well as the basolateral membrane transport (66, 67).

6. ASSEMBLY AND DISASSEMBLY OF EPITHELIAL APICAL JUNCTIONS

Epithelial cells are very plastic and remodel intercellular junctions even within apparently stable, confluent cultured monolayers (68, 69). To assemble or disassemble epithelial apical junctions, epithelial cells need to regulate the functions of apical junctional proteins at the cell-surface. The remodeling of actin cytoskeletons and the endocytic recycling of apical junctional proteins provide important molecular mechanisms. Failure in this regulation is manifested in a variety of diseases, such as tissue fibrosis and tumor invasion/metastasis (70).

The role of actin cytoskeleton in the assembly and maintenance of epithelial apical junctions is demonstrated by the fact that actin-disrupting pharmacological agents such as cytochalasin D and latrunculin A rapidly and efficiently disrupt epithelial apical junctions (71, 72). During the assembly of epithelial apical junctions, the formation of E-cadherin-mediated contacts triggers remodeling of actin cytoskeletons, and their maturation is accompanied by the assembly of a circumferential actin belt and TJs. Although the established model of E-cadherin-mediated AJ formation predicts a stable link between the E-cadherin-beta-catenin complex and the actin cytoskeleton that is mediated by alpha-catenin, the recent data shows that alpha-catenin does not stably couple E-cadherin to the circumferential actin belt,

but can directly regulates actin-filament organization by suppressing Arp2/3-mediated actin polymerization (73, 74).

Whereas apical junctional proteins exist predominantly at the cell-surface under basal conditions, their endosomal pools can be detected in a variety of cellular contexts (75-77). For endocytosis of apical junction proteins, four distinct pathways have been revealed. These include macropinocytosis in IFN-gamma-treated human colorectal cancer T84 cells (78) and CVB-exposed human colon epithelial Caco2 cells (57), clathrin-dependent endocytosis in confluent kidney epithelial MDCK cells (79), Ca^{2+}-depleted T84 cells (80), AJ-enriched fraction of rat liver (81), caveolin-dependent endocytosis in Ca^{2+}-depleted SCC12f keratinocytes (82), *Escherichia coli* cytotoxic necrotizing factor-1-treated T84 cells (83), actin-depolymerized MDCK cells (84), and clathrin-independent endocytosis in isolated human breast cancer MCF7 cells (85). Internalized apical junction proteins are also detected in multiple sites including Rab5-positive early endosomes (79), Rab11-positive recycling endosomes (78, 83, 86), Rab7-positive late endosomes (87), Rab13-positive vesicles (88), Syntaxin4-positive compartments (80), and Syntaxin3-positive vacuolar apical compartments (89). Although the endocytosed proteins in Rab7-positive late endosomes are likely targeted to lysosomal degradation, they are recycled from these compartments back to the plasma membrane.

A recent genome-wide RNA interference screen for genes required for endocytic recycling in *Caenorhabditis elegans* provides further evidence that the endocytic recycling is essential for the regulation of epithelial apical junctions. This screen identifies the cell polarity proteins, Par3, Par6, aPKC, and Cdc42, which direct the formation and maturation of apical junctions and cell polarity in epithelial cells. The perturbation of Par6 or Cdc42 function inhibits the endocytic recycling both in *Caenorhabditis elegans* coelomocytes and human HeLa cells (90). Furthermore, the endocytosis of E-cadherin is recently proposed as the driving force to dissociate the stable E-cadherin-E-cadherin interactions and thereby disassemble epithelial apical junctions based on the observations that maneuvers inhibiting E-cadherin endocytosis also prevent the disassembly of E-cadherin-E-cadherin interactions. This contrasts with the current model, in which the circumferential actin belt mediates the clustering and stabilization of the weak E-cadherin-E-cadherin interactions between two opposing plasma membranes and then assembles epithelial apical junctions (81, 91-93). Although these disparate models remain to be resolved, both models emphasize the close functional and mechanistic relationship between the E-cadherin-E-cadherin interactions and the E-cadherin endocytosis. The current model suggests that the free E-cadherin, rather than the bound E-cadherin engaged in adhesion, undergoes endocytosis and the E-cadherin-E-cadherin interactions prevent E-cadherin endocytosis, perhaps by activating Rac1 signaling and remodeling the actin cytoskeleton (81, 93). In contrast, the new model implies that endocytosis targets the bound E-cadherin engaged in adhesion, rather than the free E-cadherin (91, 92).

7. RAB PROTEINS IMPLICATED IN REGULATION OF EPITHELIAL APICAL JUNCTIONS

7.1. Rab3B

Although Rab3 subfamily proteins (Rab3A, Rab3B, Rab3C, and Rab3D) are enriched in neuronal/secretory cells and control the regulated exocytosis through the interaction with Rab3 effector proteins, Rabphilin3, Rim1/2, and Noc2, Rab3B expression is also detected in other cells (4). In epithelial cells, Rab3B is recruited to TJ upon cell-cell contact formation and involved in the transport of polymeric immunoglobulin receptor (94, 95). Rab3B also regulates the reorganization of actin cytoskeleton and the targeting of ZO-1 to the plasma membrane through a process, in which phosphatidylinositol 3-kinase (PI3K) is involved, in neuroendocrine PC12 cells (96).

7.2. Rab4

Rab4 is localized predominantly to early endosome and, to a lesser extent, to recycling endosome and thought to be mainly involved in recycling from early endosome to plasma membrane. In Sertoli cells, Rab4 associates with alpha- and beta-catenins as well as with actin cytoskeletons and is involved in the disassembly of a testis-specific F-actin-based junctional structure, “ectoplasmic specialization”, that shares features of TJ, AJ, and focal adhesion (FA) (97). In fibroblasts, Rab4 also regulates cell-extracellular matrix interactions by controlling the PDGF-dependent recycling of alphavbeta3 integrin through the interaction with Rab4 effector Rabip4 (98, 99).

7.3. Rab5

Rab5 is a key regulator of the transport from plasma membrane to early endosomes and also implicated in the macropinocytosis (100, 101). In CVB-exposed Caco2 cells, Rab5 and its effector Rabankyrin5 regulate the endocytosis of occludin (57). Rab5 activation is involved in the hepatocyte growth factor (HGF)/scatter factor (SF)- or 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced disruption of cell-cell adhesion and subsequent cell migration through co-endocytosis of E-cadherin and c-Met in MDCK cells (102, 103). In HGF/SF-stimulated MDCK cells, Rab5 activation is mediated by the sequential action of c-Met, Ras, and Rab5 GEF RIN2 (104). In v-Src-induced epithelial to mesenchymal transitions (EMT), Rab5 activation also mediates the lysosomal targeting of E-cadherin (105). During zebrafish gastrulation, Rab5 controls the Wnt11-dependent endocytosis of E-cadherin and the cohesion of mesendodermal cells. (106). In *Drosophila* epithelial cells, loss of Rab5 results in the cellular accumulation of a cell polarity protein CRB (107).

7.4. Rab8

Rab8 is localized to the trans-Golgi network (TGN), recycling endosome, cytosolic vesicular structures, membrane protrusions, and primary cilia, and implicated in the polarized membrane traffic to the dendritic membrane, the actin-dependent movement of melanosomes, and the formation of membrane protrusions and primary cilia (108-115). Rab8 associates with MyosinVb (116), Optineurin-

myosin VI (117), Rab8 GEF Rabin8 (118), JRAB/MICAL-L2 (119), Optineurin-huntingtin (120), and cenexin/Odf2 (121), and connected to actin and microtubule cytoskeletons. Recently, Rab8 is linked to two human diseases, microvillus inclusion disease and Bardet-Biedle syndrome, which shows the shortening of microvilli in intestinal epithelial cells and the primary cilia dysfunction, respectively (114, 122).

Rab8 is also involved in cell-cell adhesion during *Dictyostelium discoideum* development, and associates with E-cadherin as well as actin cytoskeletons in Sertoli cells (123, 124). In epithelial cells, Rab8 is shown to mediate the epithelial-specific adaptor protein complex AP-1B-dependent basolateral transport (117, 120, 125). Although E-cadherin is initially recognized as an AP-1B-independent basolateral cargo (126, 127), it is recently linked to AP-1B through the interaction with phosphatidylinositol-4-phosphate 5-kinase gamma (PIP5Kgamma) (128). Consistent with this, Rab8 associates with JRAB/MICAL-L2 and is involved in E-cadherin transport (119).

7.5. Rab11

Rab11 is distributed across a variety of post-Golgi membranes, but serves as the most prominent recycling endosome marker. Rab11 interacts with a component of the exocyst Sec15, and is implicated in regulating the post-Golgi traffic (129). In *Drosophila* epithelial cells, E-cadherin accumulates in Rab11-positive recycling endosomes upon inactivation of components of the exocyst Sec5, Sec6, and Sec15 (130). Rab11 also interacts with the same effector proteins FIP3/arfophilin-1and FIP4/arphophilin-2 as ARF6, a key regulator for the endocytic recycling of E-cadherin, and controls the transport of E-cadherin from the TGN to basolateral membranes via an intermediate compartment, Rab11-positive recycling endosome, in epithelial cells (131, 132).

7.6. Rab13

Whereas Rab13 associates with vesicles throughout the cytosol in fibroblasts, it accumulates at TJ in polarized epithelial cells and is recruited to cell-cell contacts from a cytosolic pool at an early stage of junctional complex assembly (133, 134). Rab13 mediates the endocytic recycling of occludin and is implicated in the assembly of functional TJs in epithelial cells (88, 135). Rab13 also regulates the scattering of MDCK cells in response to TPA, the neurite outgrowth, and the regeneration of neurons (136-138). cGMP phosphodiesterase delta subunit (delta-PDE), protein kinase A (PKA), and JRAB/MICAL-L2 have been identified as Rab13-binding proteins. delta-PDE exhibits two putative carboxyl PDZ binding motifs and regulates the membrane association and disassociation of Rab13 (139). GTP-bound Rab13 interacts directly with PKA and inhibits the PKA-dependent phosphorylation and TJ recruitment of vasodilator-stimulated phosphoprotein (VASP) (140, 141).

7.7. Rab34

Rab34 is localized to the Golgi, membrane ruffles, and macropinosome in fibroblasts (142, 143), and associated with the TJ and cytosolic vesicles containing caveolin in epithelial cells (57). Whereas Rab34 is implicated in the formation of membrane ruffles and macropinocytosis in fibroblasts (143), its activity is required for the CVB-induced endocytosis of occludin in Caco2 cells (57). In contrast to IFN-gamma that triggers macropinocytosis of JAM, occludin, and claudins (78), CVB induces macropinocytosis of occludin without affecting the localization of other TJ membrane proteins (57). In CVB-exposed Caco2 cells, activated Rab34 facilitates the constitutive occludin endocytosis downstream of Ras.

7.8. Rab8/13-JRAB/MICAL-L2 complex

MICAL is originally identified as a novel binding protein of CasL/HEF1/NEDD9 that regulates the scattering of epithelial cells and the progression and metastasis of cancer cells (144, 145). Now it belongs to a MICAL family consisted of five members (MICAL-1, MICAL-2, MICAL-3, MICAL-L1, and JRAB/MICAL-L2) in mammals and two members (D-MICAL and D-MICAL-L) in *Drosophila* (146). MICAL family proteins are large, multidomain, cytosolic proteins expressed in specific neuronal and non-neuronal cells both during development and in adulthood. They contain calponin homology (CH), LIM, and coiled-coil (CC) domains. MICAL-1, MICAL-2, MICAL-3, and D-MICAL also possess a flavin-adenine dinucleotide (FAD)-binding monooxygenase domain. Members of MICAL family proteins are shown to associate with Semaphorin receptor Plexin, Rab1, vimentin, and microtubule, and implicated in the invasive growth (144, 146-149). MICAL-1, MICAL-2, MICAL-3, and D-MICAL function downstream of Semaphorin receptor Plexin in axon guidance (146, 150). MICAL-2 isoforms (PVa and PVb) are involved in the progression of prostate cancer (151). JRAB/MICAL-L2 plays a role in the scattering of MDCK cells in response to TPA (136).

JRAB/MICAL-L2 is originally identified as a Rab13 effector protein that mediates the endocytic recycling of occludin and the formation of functional TJs (152). It is resided in recycling endosome, cytosolic vesicular structures, and plasma membrane, and is also associated with actin cytoskeletons and localized to TJs in epithelial cells and distributed along stress fibers in fibroblasts (152). JRAB/MICAL-L2 also interacts with both Rab8 and Rab13 via its carboxyl-terminal region with CC domain. Is there any difference between the Rab8-JRAB/MICAL-L2 and Rab13-MICAL-L2 complexes? Rab8 and Rab13 compete with each other for the binding to JRAB/MICAL-L2 and form the two distinct JRAB/MICAL-L2 complexes within a cell. Whereas Rab8, Rab13, and JRAB/MICAL-L2 are all localized to recycling endosome, cytosolic vesicular structures, and plasma membrane, JRAB/MICAL-L2 interacts with Rab8 and Rab13 at the distinct sites. JRAB/MICAL-L2 shows a closer relationship with Rab8 at recycling endosome and with Rab13 at plasma membrane, respectively (Figure 2). Whereas JRAB/MICAL-L2 regulates the transport of claudins, occludin, and E-cadherin, Rab13 specifically mediates the transport of claudins and occludin but not E-cadherin,

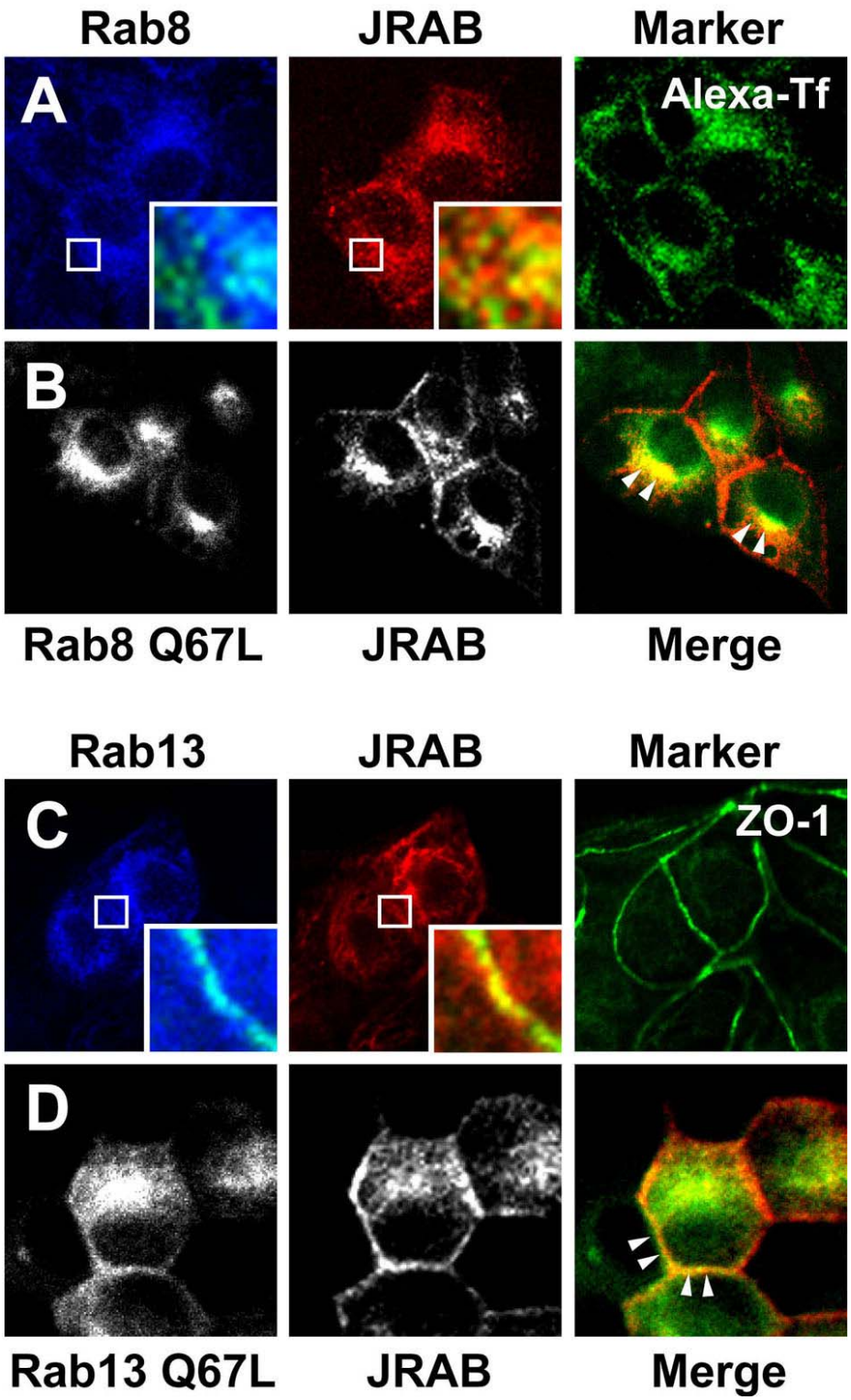

Figure 2. Subcellular localization of Rab8, Rab13, and JRAB/MICAL-L2. MDCK cells co-expressing HA-JRAB/MICAL-L2 with FLAG-Rab8A (A), FLAG-Rab8A Q67L (B), FLAG-Rab13 (C), or FLAG-Rab13 Q67L (D) were labeled with anti-HA antibody, anti-FLAG antibody, and organella marker (Alexa-Tf or anti-ZO-1 antibody). Magnified images in inserts show the notable colocalization with organella markers. Rab8A Q67L (B)/Rab13 Q67L (C) was green and JRAB was red in merged images. Bars, 20 μm. Reproduced with permission from 119.

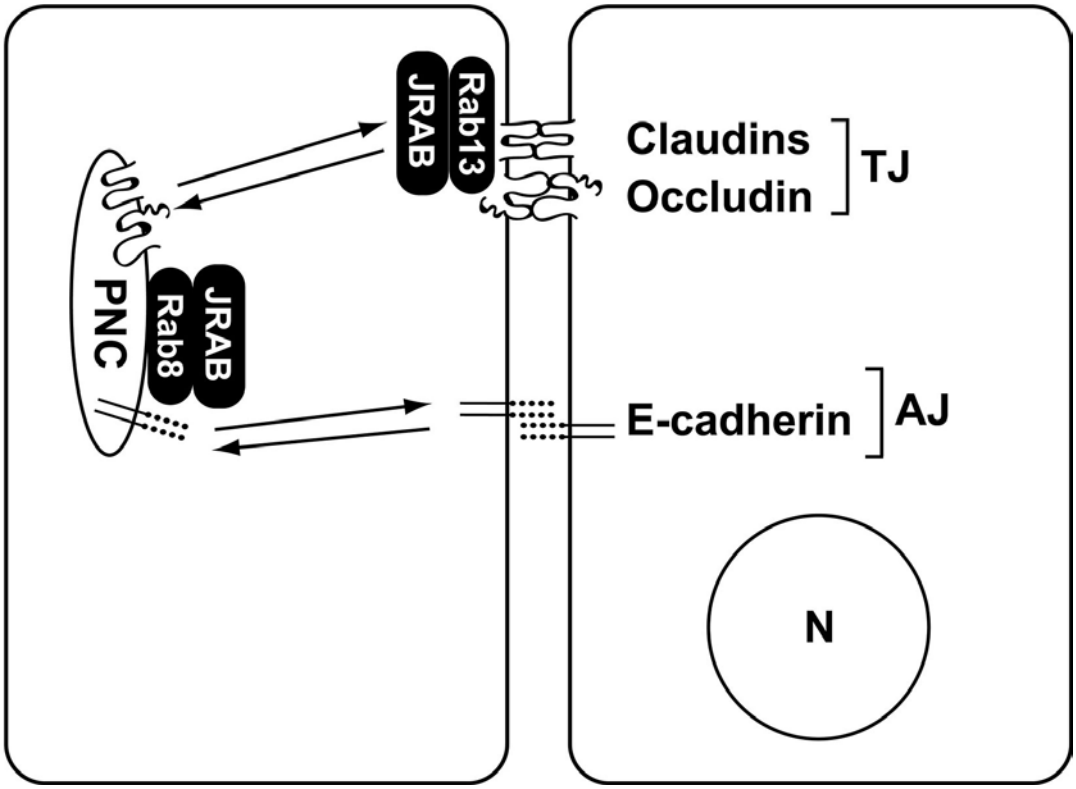

Figure 3. Rab8/13-JRAB/MICAL-L2 complex. A schematic model for the action of Rab8-JRAB/MICAL-L2 and Rab13-JRAB/MICAL-L2 complexes is shown. Whereas the Rab8-JRAB/MICAL-L2 complex resided at the PNC mediates the recycling of E-cadherin to the plasma membrane and the assembly of AJs, the Rab13-JRAB/MICAL-L2 complex resided at the plasma membrane regulates the recycling of claudins and occludin to the plasma membrane and the formation of TJs. N, nucleus. PNC, perinuclear recycling/storage compartments. Reproduced with permission from 119.

and Rab8 controls the Rab13-independent transport of E-cadherin. JRAB/MICAL-L2 regulates the Rab8-dependent E-cadherin transport at perinuclear recycling/storage compartments (PNC) and the Rab13-dependent claudins and occludin transport at plasma membrane, respectively (Figure 3) (119).

Although an increasing number of Rab effector proteins are reported to interact with closely related multiple Rab proteins (153), JRAB/MICAL-L2 is a novel type of Rab effector proteins that associate with multiple Rab proteins forming mutually exclusive complexes. In order to ensure the specificity in membrane traffic, the action of each Rab protein needs to be coordinated with other Rab proteins (32). The Rab coupling is potentially mediated by Rab-binding proteins that can interact with multiple Rab proteins. Three types of these Rab-binding proteins are currently identified. First type functions as an effector protein for one Rab protein and as a GEF for another Rab protein. The identification of Sec2 and the class C-VPS/HOPS complex as this type of Rab-binding proteins leads to a "Rab cascade" concept (31, 154). Second type is a divalent Rab effector protein that binds simultaneously to two Rab proteins associated with compartments in dynamic continuity. Rabaptin5, Rabenosyn5, and Rabip4' are able to interact simultaneously with Rab4 and Rab5, and are likely involved in the coordination of the endocytic recycling pathway as well as the organization of Rab4 and Rab5 domains on endosomal membranes (155-157). Third type is a Rab effector protein that associates with multiple Rab proteins in a mutually exclusive manner. JRAB/MICAL-L2 is a shared Rab effector protein that forms mutually distinct complexes with Rab8 and Rab13 and coordinates the assembly of epithelial apical junctions (119).

8. SUMMARY AND PERSPECTIVES

Epithelial apical junctions control paracellular fluxes and membrane polarity, and encompass a platform for regulatory and signaling proteins that establishes the epithelial phenotype. Their function is determined by, and regulated through, a variety of cellular processes. In this review, we focused the endocytic recycling of apical junctional proteins and highlight the role of Rab proteins. Although it is becoming increasingly clear that several Rab proteins are critically involved in the regulation of epithelial apical junctions, the relation and coordination of each Rab proteins remain elusive. We have described that JRAB/MICAL-L2 coordinated the Rab8-dependent AJ protein traffic and the Rab13-dependent TJ protein traffic. Of course, the Rab8/13-JRAB/MICAL-L2 complexes are not the only processes. Further studies are required to elucidate their interplay with other cellular processes such

as surface clustering of apical junctional proteins, cytoskeletal activity, and cell signaling. As Rab8 is recently linked to two human diseases, microvillus inclusion disease and Bardet-Biedle syndrome (114, 122), studies of the Rab8/13-JRAB/MICAL-L2 complexes will contribute to understand how epithelial cells establish their own phenotypes in physiological and pathological conditions.

9. ACKNOWLEDGEMENTS

We thank past and present members of our laboratory for participating in this study. This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, Culture, and Technology of Japan.

10. REFERENCES

1. Salminen A, P. J. Novick: A ras-like protein is required for a post-Golgi event in yeast secretion. *Cell* 49, 527-538 (1987)

2. Schmitt H. D, P. Wagner, E. Pfaff, D. Gallwitz: The ras-related YPT1 gene product in yeast: a GTP-binding protein that might be involved in microtubule organization. *Cell* 47, 401-412 (1986)

3. Touchot N, P. Chardin, A. Tavitian: Four additional members of the ras gene superfamily isolated by an oligonucleotide strategy: molecular cloning of YPT-related cDNAs from a rat brain library. *Proc Natl Acad Sci USA* 84, 8210-8214 (1987)

4. Takai Y, T. Sasaki, T. Matozaki: Small GTP-binding proteins. *Physiol Rev* 81, 153-208 (2001)

5. Wennerberg K, K. L. Rossman, C. J. Der: The Ras superfamily at a glance. *J Cell Sci* 118, 843-846 (2005)

6. Colicelli J: Human RAS superfamily proteins and related GTPases. *Sci STKE* 2004, RE13 (2004)

7. Farquhar M, G. Palade: Junctional complexes in various epithelia. *J Cell Biol* 17, 375-412 (1963)

8. Wada M, H. Nakanishi, A. Satoh, H. Hirano, H. Obaishi, Y. Matsuura, Y. Takai: Isolation and characterization of a GDP/GTP exchange protein specific for the Rab3 subfamily small G proteins. *J Biol Chem* 272, 3875-3878 (1997)

9. Fukui K, T. Sasaki, K. Imazumi, Y. Matsuura, H. Nakanishi, Y. Takai: Isolation and characterization of a GTPase activating protein specific for the Rab3 subfamily of small G proteins. *J Biol Chem* 272, 4655-4658 (1997)

10. Nagano F, T. Sasaki, K. Fukui, T. Asakura, K. Imazumi, Y. Takai: Molecular cloning and characterization of the noncatalytic subunit of the Rab3 subfamily-specific GTPase-activating protein. *J Biol Chem* 273, 24781-24785 (1998)

11. Sasaki T, A. Kikuchi, S. Araki, Y. Hata, M. Isomura, S. Kuroda, Y. Takai: Purification and characterization from bovine brain cytosol of a protein that inhibits the dissociation of GDP from and the subsequent binding of GTP to smg p25A, a ras p21-like GTP-binding protein. *J Biol Chem* 265, 2333-2337 (1990)

12. Sivars U, D. Aivazian, S. Pfeffer: Yip3 catalyses the dissociation of endosomal Rab-GDI complexes. *Nature* 425, 856-859 (2003)

13. Ingmundson A, A. Delprato, D. G. Lambright, C. R. Roy: Legionella pneumophila proteins that regulate Rab1 membrane cycling. *Nature* 450, 365-369 (2007)

14. Machner M. P, R. R. Isberg: A bifunctional bacterial protein links GDI displacement to Rab1 activation. *Science* 318, 974-977 (2007)

15. Grosshans B, D. Ortiz, P. Novick: Rabs and their effectors: achieving specificity in membrane traffic. *Proc Natl Acad Sci USA* 103, 11821-11827 (2006)

16. Zerial M, H. McBride: Rab proteins as membrane organizers. *Nat Rev Mol Cell Biol* 2, 107-117 (2001)

17. Pfeffer S: Membrane domains in the secretory and endocytic pathways. *Cell* 112, 507-517 (2003)

18. Hammer J, X. Wu: Rabs grab motors: defining the connections between Rab GTPases and motor proteins. *Curr Opin Cell Biol* 14, 69-75 (2002)

19. Jordens I, M. Marsman, C. Kuijl, J. Neefjes: Rab proteins, connecting transport and vesicle fusion. *Traffic* 6, 1070-1077 (2005)

20. Miaczynska M, L. Pelkmans, M. Zerial: Not just a sink: endosomes in control of signal transduction. *Curr Opin Cell Biol* 16, 400-406 (2004)

21. Bucci C, M. Chiariello: Signal transduction gRABs attention. *Cellular Signalling* 18, 1-8 (2006)

22. Carroll K. S, J. Hanna, I. Simon, J. Krise, P. Barbero, S. R. Pfeffer: Role of Rab9 GTPase in facilitating receptor recruitment by TIP47. *Science* 292, 1373-1376 (2001)

23. Echard A, F. Jollivet, O. Martinez, J. Lacapere, A. Rousselet, I. Janoueix-Lerosey, B. Goud: Interaction of a Golgi-associated kinesin-like protein with Rab6. *Science* 279, 580-585 (1998)

24. Allan B, B. Moyer, W. Balch: Rab1 recruitment of p115 into a cis-SNARE complex: programming budding COPII vesicles for fusion. *Science* 289, 444-448 (2000)

25. McBride H, V. Rybin, C. Murphy, A. Giner, R. Teasdale, M. Zerial: Oligomeric complexes link Rab5 effectors with NSF and drive membrane fusion via interactions between EEA1 and syntaxin 13. *Cell* 98, 377-386 (1999)

26. Pfeffer S, D. Aivazian: Targeting Rab GTPases to distinct membrane compartments. *Nat Rev Mol Cell Biol* 5, 886-896 (2004)

27. Seabra M, C. Wasmeier: Controlling the location and activation of Rab GTPases. *Curr Opin Cell Biol* 16, 451-457 (2004)

28. Stenmark H, G. Vitale, O. Ullrich, M. Zerial: Rabaptin-5 is a direct effector of the small GTPase Rab5 in endocytic membrane fusion. *Cell* 83, 423-432 (1995)

29. Rybin V, O. Ullrich, M. Rubino, K. Alexandrov, I. Simon, M. Seabra, R. Goody, M. Zerial: GTPase activity of Rab5 acts as a timer for endocytic membrane fusion. *Nature* 383, 266-269 (1996)

30. Horiuchi H, R. Lippé, H. McBride, M. Rubino, P. Woodman, H. Stenmark, V. Rybin, M. Wilm, K. Ashman, M. Mann, M. Zerial: A novel Rab5 GDP/GTP exchange factor complexed to Rabaptin-5 links nucleotide exchange to effector recruitment and function. *Cell* 90, 1149-1159 (1997)

31. Rink J, E. Ghigo, Y. Kalaidzidis, M. Zerial: Rab conversion as a mechanism of progression from early to late endosomes. *Cell* 122, 735-749 (2005)

32. Markgraf D, K. Peplowska, C. Ungermann: Rab cascades and tethering factors in the endomembrane system. *FEBS Lett* 581, 2125-2130 (2007)

33. Kato M, T. Sasaki, T. Ohya, H. Nakanishi, H. Nishioka, M. Imamura, Y. Takai: Physical and functional interaction of rabphilin-3A with alpha-actinin. *J Biol Chem* 271, 31775-31778 (1996)

34. Baldini G, A. Martelli, G. Tabellini, C. Horn, K. Machaca, P. Narducci, G. Baldini: Rabphilin localizes with the cell actin cytoskeleton and stimulates association of granules with F-actin cross-linked by alpha-actinin. *J Biol Chem* 280, 34974-34984 (2005)

35. Wu X, G. Tsan, J. Hammer: Melanophilin and myosin Va track the microtubule plus end on EB1. *J Cell Biol* 171, 201-207 (2005)

36. Krämer H, M. Phistry: Mutations in the *Drosophila* hook gene inhibit endocytosis of the boss transmembrane ligand into multivesicular bodies. *J Cell Biol* 133, 1205-1215 (1996)

37. Luiro K, K. Yliannala, L. Ahtiainen, H. Maunu, I. Järvelä, A. Kyttälä, A. Jalanko: Interconnections of CLN3, Hook1 and Rab proteins link Batten disease to defects in the endocytic pathway. *Hum Mol Genet* 13, 3017-3027 (2004)

38. van der Sluijs P, M. Hull, L. A. Huber, P. Mâle, B. Goud, I. Mellman: Reversible phosphorylation--dephosphorylation determines the localization of rab4 during the cell cycle. *EMBO J* 11, 4379-4389 (1992)

39. Ren M, J. Zeng, C. De Lemos-Chiarandini, M. Rosenfeld, M. Adesnik, D. Sabatini: In its active form, the GTP-binding protein rab8 interacts with a stress-activated protein kinase. *Proc Natl Acad Sci USA* 93, 5151-5155 (1996)

40. de Graaf P, W. T. Zwart, R. A. van Dijken, M. Deneka, T. K. Schulz, N. Geijsen, P. J. Coffer, B. M. Gadella, A. J. Verkleij, P. van der Sluijs, P. M. van Bergen en Henegouwen: Phosphatidylinositol 4-kinase beta is critical for functional association of rab11 with the Golgi complex. *Mol Biol Cell* 15, 2038-2047 (2004)

41. Kapp-Barnea Y, L. Ninio-Many, K. Hirschberg, M. Fukuda, A. Jeromin, R. Sagi-Eisenberg: Neuronal calcium sensor-1 and phosphatidylinositol 4-kinase beta stimulate extracellular signal-regulated kinase 1/2 signaling by accelerating recycling through the endocytic recycling compartment. *Mol Biol Cell* 17, 4130-4141 (2006)

42. Furuse M, K. Fujita, T. Hiiragi, K. Fujimoto, S. Tsukita: Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *J Cell Biol* 141, 1539-1550 (1998)

43. Furuse M, H. Sasaki, K. Fujimoto, S. Tsukita: A single gene product, claudin-1 or -2, reconstitutes tight junction strands and recruits occludin in fibroblasts. *J Cell Biol* 143, 391-401 (1998)

44. Tsukita S, M. Furuse, M. Itoh: Multifunctional strands in tight junctions. *Nat Rev Mol Cell Biol* 2, 285-293 (2001)

45. Furuse M, T. Hirase, M. Itoh, A. Nagafuchi, S. Yonemura, S. Tsukita: Occludin: a novel integral membrane protein localizing at tight junctions. *J Cell Biol* 123, 1777-1788 (1993)

46. Ikenouchi J, M. Furuse, K. Furuse, H. Sasaki, S. Tsukita, S. Tsukita: Tricellulin constitutes a novel barrier at tricellular contacts of epithelial cells. *J Cell Biol* 171, 939-945 (2005)

47. Ebnet K, A. Suzuki, S. Ohno, D. Vestweber: Junctional adhesion molecules (JAMs): more molecules with dual functions? *J Cell Sci* 117, 19-29 (2004)

48. Weber C, L. Fraemohs, E. Dejana: The role of junctional adhesion molecules in vascular inflammation. *Nat Rev Immunol* 7, 467-477 (2007)

49. Suzuki A, S. Ohno: The PAR-aPKC system: lessons in polarity. *J Cell Sci* 119, 979-987 (2006)

50. Coyne C. B, J. M. Bergelson: CAR: a virus receptor within the tight junction. *Adv Drug Deliv Rev* 57, 869-882 (2005)

51. Shin K, V. Fogg, B. Margolis: Tight junctions and cell polarity. *Annu Rev Cell Dev Biol* 22, 207-235 (2006)

52. Takeichi M: Cadherin cell adhesion receptors as a morphogenetic regulator. *Science* 251, 1451-1455 (1991)

53. Halbleib J, W. Nelson: Cadherins in development: cell adhesion, sorting, and tissue morphogenesis. *Genes Dev* 20, 3199-3214 (2006)

54. Takai Y, H. Nakanishi: Nectin and afadin: novel organizers of intercellular junctions. *J Cell Sci* 116, 17-27 (2003)

55. Gonzalez-Mariscal L, A. Betanzos, P. Nava, B. Jaramillo: Tight junction proteins. *Prog Biophys Mol Biol* 81, 1-44 (2003)

56. Mruk D, A. Lau, A. Conway: Crosstalk between Rab GTPases and cell junctions. *Contraception* 72, 280-290 (2005)

57. Coyne C. B, L. Shen, J. R. Turner, J. M. Bergelson: Coxsackievirus entry across epithelial tight junctions requires occludin and the small GTPases Rab34 and Rab5. *Cell Host Microbe* 2, 181-192 (2007)

58. Conner S, S. Schmid: Regulated portals of entry into the cell. *Nature* 422, 37-44 (2003)

59. Mayor S, R. Pagano: Pathways of clathrin-independent endocytosis. *Nat Rev Mol Cell Biol* 8, 603-612 (2007)

60. Hoekstra D, D. Tyteca, S. C. van IJzendoorn: The subapical compartment: a traffic center in membrane polarity development. *J Cell Sci* 117, 2183-2192 (2004)

61. Jahn R, R. Scheller: SNAREs-engines for membrane fusion. *Nat Rev Mol Cell Biol* 7, 631-643 (2006)

62. Low S, S. Chapin, T. Weimbs, L. Kömüves, M. Bennett, K. Mostov: Differential localization of syntaxin isoforms in polarized Madin-Darby canine kidney cells. *Mol Biol Cell* 7, 2007-2018 (1996)

63. Low S, S. Chapin, C. Wimmer, S. Whiteheart, L. Kömüves, K. Mostov, T. Weimbs: The SNARE machinery is involved in apical plasma membrane trafficking in MDCK cells. *J Cell Biol* 141, 1503-1513 (1998)

64. Fields I, E. Shteyn, M. Pypaert, V. Proux-Gillardeaux, R. Kang, T. Galli, H. Fölsch: v-SNARE cellubrevin is required for basolateral sorting of AP-1B-dependent cargo in polarized epithelial cells. *J Cell Biol* 177, 477-488 (2007)

65. Munson M, P. Novick: The exocyst defrocked, a framework of rods revealed. *Nat Struct Mol Biol* 13, 577-581 (2006)

66. Grindstaff K, C. Yeaman, N. Anandasabapathy, S. Hsu, E. Rodriguez-Boulan, R. Scheller, W. Nelson: Sec6/8 complex is recruited to cell-cell contacts and specifies transport vesicle delivery to the basal-lateral membrane in epithelial cells. *Cell* 93, 731-740. (1998)

67. Oztan A, M. Silvis, O. A. Weisz, N. A. Bradbury, S. C. Hsu, J. R. Goldenring, C. Yeaman, G. Apodaca: Exocyst requirement for endocytic traffic directed toward the apical and basolateral poles of polarized MDCK cells. *Mol Biol Cell* 18, 3978-3992 (2007)

68. Nagafuchi A, S. Ishihara, S. Tsukita: The roles of catenins in the cadherin-mediated cell adhesion: functional analysis of E-cadherin-alpha catenin fusion molecules. *J Cell Biol* 127, 235-245 (1994)

69. Bertet C, L. Sulak, T. Lecuit: Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation. *Nature* 429, 667-671 (2004)

70. Thiery J: Epithelial-mesenchymal transitions in development and pathologies. *Curr Opin Cell Biol* 15, 740-746 (2003)

71. Bershadsky A: Magic touch: how does cell-cell adhesion trigger actin assembly? *Trends Cell Biol* 14, 589-593 (2004)

72. Mège R. M, J. Gavard, M. Lambert: Regulation of cell-cell junctions by the cytoskeleton. *Curr Opin Cell Biol* 18, 541-548 (2006)

73. Drees F, S. Pokutta, S. Yamada, W. J. Nelson, W. I. Weis: alpha-catenin is a molecular switch that binds E-cadherin-beta-catenin and regulates actin-filament assembly. *Cell* 123, 903-915 (2005)

74. Yamada S, S. Pokutta, F. Drees, W. I. Weis, W. J. Nelson: Deconstructing the cadherin-catenin-actin complex. *Cell* 123, 889-901 (2005)

75. Bryant D, J. Stow: The ins and outs of E-cadherin trafficking. *Trends Cell Biol* 14, 427-434 (2004)

76. D'Souza-Schorey C: Disassembling adherens junctions: breaking up is hard to do. *Trends Cell Biol* 15, 19-26 (2005)

77. Ivanov A, A. Nusrat, C. Parkos: Endocytosis of the apical junctional complex: mechanisms and possible roles in regulation of epithelial barriers. *Bioessays* 27, 356-365 (2005)

78. Bruewer M, M. Utech, A. Ivanov, A. Hopkins, C. Parkos, A. Nusrat: Interferon-gamma induces internalization of epithelial tight junction proteins via a macropinocytosis-like process. *FASEB J* 19, 923-933 (2005)

79. Le T, A. Yap, J. Stow: Recycling of E-cadherin: a potential mechanism for regulating cadherin dynamics. *J Cell Biol* 146, 219-232 (1999)

80. Ivanov A, A. Nusrat, C. Parkos: Endocytosis of epithelial apical junctional proteins by a clathrin-mediated pathway into a unique storage compartment. *Mol Biol Cell* 15, 176-188 (2004)

81. Izumi G, T. Sakisaka, T. Baba, S. Tanaka, K. Morimoto, Y. Takai: Endocytosis of E-cadherin regulated by Rac and Cdc42 small G proteins through IQGAP1 and actin filaments. *J Cell Biol* 166, 237-248 (2004)

82. Akhtar N, N. Hotchin: RAC1 regulates adherens junctions through endocytosis of E-cadherin. *Mol Biol Cell* 12, 847-862 (2001)

83. Hopkins A, S. Walsh, P. Verkade, P. Boquet, A. Nusrat: Constitutive activation of Rho proteins by CNF-1 influences tight junction structure and epithelial barrier function. *J Cell Sci* 116, 725-742 (2003)

84. Shen L, J. Turner: Actin depolymerization disrupts tight junctions via caveolae-mediated endocytosis. *Mol Biol Cell* 16, 3919-3936 (2005)

85. Paterson A, R. Parton, C. Ferguson, J. Stow, A. Yap: Characterization of E-cadherin endocytosis in isolated MCF-7 and chinese hamster ovary cells: the initial fate of unbound E-cadherin. *J Biol Chem* 278, 21050-21057 (2003)

86. Balzac F, M. Avolio, S. Degani, I. Kaverina, M. Torti, L. Silengo, J. V. Small, S. F. Retta: E-cadherin endocytosis regulates the activity of Rap1: a traffic light GTPase at the crossroads between cadherin and integrin function. *J Cell Sci* 118, 4765-4783 (2005)

87. Matsuda M, A. Kubo, M. Furuse, S. Tsukita: A peculiar internalization of claudins, tight junction-specific adhesion molecules, during the intercellular movement of epithelial cells. *J Cell Sci* 117, 1247-1257 (2004)

88. Morimoto S, N. Nishimura, T. Terai, S. Manabe, Y. Yamamoto, W. Shinahara, H. Miyake, S. Tashiro, M. Shimada, T. Sasaki: Rab13 mediates the continuous endocytic recycling of occludin to the cell surface. *J Biol Chem* 280, 2220-2228 (2005)

89. Utech M, A. Ivanov, S. Samarin, M. Bruewer, J. Turner, R. Mrsny, C. Parkos, A. Nusrat: Mechanism of IFN-gamma-induced endocytosis of tight junction proteins: myosin II-dependent vacuolarization of the apical plasma membrane. *Mol Biol Cell* 16, 5040-5052 (2005)

90. Balklava Z, S. Pant, H. Fares, B. D. Grant: Genome-wide analysis identifies a general requirement for polarity proteins in endocytic traffic. *Nat Cell Biol* 9, 1066-1073 (2007)

91. Troyanovsky R, E. Sokolov, S. Troyanovsky: Endocytosis of cadherin from intracellular junctions is the driving force for cadherin adhesive dimer disassembly. *Mol Biol Cell* 17, 3484-3493 (2006)

92. Troyanovsky R. B, O. Laur, S. M. Troyanovsky: Stable and unstable cadherin dimers: mechanisms of formation and roles in cell adhesion. *Mol Biol Cell* 18, 4343-4352 (2007)

93. Kusumi A, K. Suzuki, K. Koyasako: Mobility and cytoskeletal interactions of cell adhesion receptors. *Curr Opin Cell Biol* 11, 582-590 (1999)

94. Weber E, G. Berta, A. Tousson, P. St John, M. Green, U. Gopalokrishnan, T. Jilling, E. Sorscher, T. Elton, D. Abrahamson, K. Kirk: Expression and polarized targeting of a rab3 isoform in epithelial cells. *J Cell Biol* 125, 583-594 (1994)

95. van IJzendoorn S, M. Tuvim, T. Weimbs, B. Dickey, K. Mostov: Direct interaction between Rab3b and the polymeric immunoglobulin receptor controls ligand-stimulated transcytosis in epithelial cells. *Dev Cell* 2, 219-228 (2002)

96. Sunshine C, S. Francis, K. Kirk: Rab3B regulates ZO-1 targeting and actin organization in PC12 neuroendocrine cells. *Exp Cell Res* 257, 1-10 (2000)

97. Mruk D, A. Lau, O. Sarkar, W. Xia: Rab4A GTPase catenin interactions are involved in cell junction dynamics in the testis. *J Androl* 28, 742-754 (2007)

98. Vukmirica J, P. Monzo, Y. Le Marchand-Brustel, M. Cormont: The Rab4A effector protein Rabip4 is involved in migration of NIH 3T3 fibroblasts. *J Biol Chem* 281, 36360-36368 (2006)

99. Roberts M, S. Barry, A. Woods, P. van der Sluijs, J. Norman: PDGF-regulated rab4-dependent recycling of alphavbeta3 integrin from early endosomes is necessary for cell adhesion and spreading. *Curr Biol* 11, 1392-1402 (2001)

100. Li G, C. D'Souza-Schorey, M. A. Barbieri, J. A. Cooper, P. D. Stahl: Uncoupling of membrane ruffling and pinocytosis during Ras signal transduction. *J Biol Chem* 272, 10337-10340 (1997)

101. Schnatwinkel C, S. Christoforidis, M. R. Lindsay, S. Uttenweiler-Joseph, M. Wilm, R. G. Parton, M. Zerial: The Rab5 effector Rabankyrin-5 regulates and coordinates different endocytic mechanisms. *PLoS Biol* 2, E261 (2004)

102. Imamura H, K. Takaishi, K. Nakano, A. Kodama, H. Oishi, H. Shiozaki, M. Monden, T. Sasaki, Y. Takai: Rho and Rab small G proteins coordinately reorganize stress fibers and focal adhesions in MDCK cells. *Mol Biol Cell* 9, 2561-2575 (1998)

103. Kamei T, T. Matozaki, T. Sakisaka, A. Kodama, S. Yokoyama, Y. Peng, K. Nakano, K. Takaishi, Y. Takai: Coendocytosis of cadherin and c-Met coupled to disruption of cell-cell adhesion in MDCK cells--regulation by Rho, Rac and Rab small G proteins. *Oncogene* 18, 6776-6784 (1999)

104. Kimura T, T. Sakisaka, T. Baba, T. Yamada, Y. Takai: Involvement of the Ras-Ras-activated Rab5 guanine nucleotide exchange factor RIN2-Rab5 pathway in the

hepatocyte growth factor-induced endocytosis of E-cadherin. *J Biol Chem* 281, 10598-10609 (2006)

105. Palacios F, J. Tushir, Y. Fujita, C. D'Souza-Schorey: Lysosomal targeting of E-cadherin: a unique mechanism for the down-regulation of cell-cell adhesion during epithelial to mesenchymal transitions. *Mol Cell Biol* 25, 389-402 (2005)

106. Ulrich F, M. Krieg, E. Schötz, V. Link, I. Castanon, V. Schnabel, A. Taubenberger, D. Mueller, P. Puech, C. Heisenberg: Wnt11 functions in gastrulation by controlling cell cohesion through Rab5c and E-cadherin. *Dev Cell* 9, 555-564 (2005)

107. Lu H, D. Bilder: Endocytic control of epithelial polarity and proliferation in *Drosophila*. *Nat Cell Biol* 7, 1232-1239 (2005)

108. Huber L, S. Pimplikar, R. Parton, H. Virta, M. Zerial, K. Simons: Rab8, a small GTPase involved in vesicular traffic between the TGN and the basolateral plasma membrane. *J Cell Biol* 123, 35-45 (1993)

109. Huber L, M. de Hoop, P. Dupree, M. Zerial, K. Simons, C. Dotti: Protein transport to the dendritic plasma membrane of cultured neurons is regulated by rab8p. *J Cell Biol* 123, 47-55 (1993)

110. Ang A, H. Fölsch, U. Koivisto, M. Pypaert, I. Mellman: The Rab8 GTPase selectively regulates AP-1B-dependent basolateral transport in polarized Madin-Darby canine kidney cells. *J Cell Biol* 163, 339-350 (2003)

111. Ang A, T. Taguchi, S. Francis, H. Fölsch, L. Murrells, M. Pypaert, G. Warren, I. Mellman: Recycling endosomes can serve as intermediates during transport from the Golgi to the plasma membrane of MDCK cells. *J Cell Biol* 167, 531-543 (2004)

112. Chabrillat M, C. Wilhelm, C. Wasmeier, E. Sviderskaya, D. Louvard, E. Coudrier: Rab8 regulates the actin-based movement of melanosomes. *Mol Biol Cell* 16, 1640-1650 (2005)

113. Hattula K, J. Furuhjelm, J. Tikkanen, K. Tanhuanpaa, P. Laakkonen, J. Peranen: Characterization of the Rab8-specific membrane traffic route linked to protrusion formation. *J Cell Sci* 119, 4866-4877 (2006)

114. Nachury M. V, A. V. Loktev, Q. Zhang, C. J. Westlake, J. Peränen, A. Merdes, D. C. Slusarski, R. H. Scheller, J. F. Bazan, V. C. Sheffield, P. K. Jackson: A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. *Cell* 129, 1201-1213 (2007)

115. Yoshimura S, J. Egerer, E. Fuchs, A. Haas, F. Barr: Functional dissection of Rab GTPases involved in primary cilium formation. *J Cell Biol* 178, 363-369 (2007)

116. Roland J, A. Kenworthy, J. Peranen, S. Caplan, J. Goldenring: Myosin Vb interacts with Rab8a on a tubular network containing EHD1 and EHD3. *Mol Biol Cell* 18, 2828-2837 (2007)

117. Au J, C. Puri, G. Ihrke, J. Kendrick-Jones, F. Buss: Myosin VI is required for sorting of AP-1B-dependent cargo to the basolateral domain in polarized MDCK cells. *J Cell Biol* 177, 103-114 (2007)

118. Hattula K, J. Furuhjelm, A. Arffman, J. Peränen: A Rab8-specific GDP/GTP exchange factor is involved in actin remodeling and polarized membrane transport. *Mol Biol Cell* 13, 3268-3280 (2002)

119. Yamamura R, N. Nishimura, H. Nakatsuji, S. Arase, T. Sasaki: The interaction of JRAB/MICAL-L2 with Rab8 and Rab13 coordinates the assembly of tight junctions and adherens junctions. *Mol Biol Cell* 19, 971-983 (2008)

120. Hattula K, J. Peränen: FIP-2, a coiled-coil protein, links Huntingtin to Rab8 and modulates cellular morphogenesis. *Curr Biol* 10, 1603-1606 (2000)

121. Ishikawa H, A. Kubo, S. Tsukita, S. Tsukita: Odf2-deficient mother centrioles lack distal/subdistal appendages and the ability to generate primary cilia. *Nat Cell Biol* 7, 517-524 (2005)

122. Sato T, S. Mushiake, Y. Kato, K. Sato, M. Sato, N. Takeda, K. Ozono, K. Miki, Y. Kubo, A. Tsuji, R. Harada, A. Harada: The Rab8 GTPase regulates apical protein localization in intestinal cells. *Nature* 448, 366-369 (2007)

123. Powell R, L. Temesvari: Involvement of a Rab8-like protein of *Dictyostelium discoideum*, Sas1, in the formation of membrane extensions, secretion and adhesion during development. *Microbiology* 150, 2513-2525 (2004)

124. Lau A, D. Mruk: Rab8B GTPase and junction dynamics in the testis. *Endocrinology* 144, 1549-1563 (2003)

125. Sahlender D, R. Roberts, S. Arden, G. Spudich, M. Taylor, J. Luzio, J. Kendrick-Jones, F. Buss: Optineurin links myosin VI to the Golgi complex and is involved in Golgi organization and exocytosis. *J Cell Biol* 169, 285-295 (2005)

126. Miranda K, T. Khromykh, P. Christy, T. Le, C. Gottardi, A. Yap, J. Stow, R. Teasdale: A dileucine motif targets E-cadherin to the basolateral cell surface in Madin-Darby canine kidney and LLC-PK1 epithelial cells. *J Biol Chem* 276, 22565-22572 (2001)

127. Fölsch H: The building blocks for basolateral vesicles in polarized epithelial cells. *Trends Cell Biol* 15, 222-228 (2005)

128. Ling K, S. Bairstow, C. Carbonara, D. Turbin, D. Huntsman, R. Anderson: Type I gamma phosphatidylinositol phosphate kinase modulates adherens

junction and E-cadherin trafficking via a direct interaction with micro1B adaptin. *J Cell Biol* 176, 343-353 (2007)

129. Zhang X, S. Ellis, A. Sriratana, C. Mitchell, T. Rowe: Sec15 is an effector for the Rab11 GTPase in mammalian cells. *J Biol Chem* 279, 43027-43034 (2004)

130. Langevin J, M. J. Morgan, J. B. Sibarita, S. Aresta, M. Murthy, T. Schwarz, J. Camonis, Y. Bellaïche: *Drosophila* exocyst components Sec5, Sec6, and Sec15 regulate DE-Cadherin trafficking from recycling endosomes to the plasma membrane. *Dev Cell* 9, 355-376 (2005)

131. Hickson G, J. Matheson, B. Riggs, V. Maier, A. Fielding, R. Prekeris, W. Sullivan, F. Barr, G. Gould: Arfophilins are dual Arf/Rab 11 binding proteins that regulate recycling endosome distribution and are related to *Drosophila* nuclear fallout. *Mol Biol Cell* 14, 2908-2920 (2003)

132. Lock J, J. Stow: Rab11 in recycling endosomes regulates the sorting and basolateral transport of E-cadherin. *Mol Biol Cell* 16, 1744-1755 (2005)

133. Zahraoui A, G. Joberty, M. Arpin, J. Fontaine, R. Hellio, A. Tavitian, D. Louvard: A small rab GTPase is distributed in cytoplasmic vesicles in non polarized cells but colocalizes with the tight junction marker ZO-1 in polarized epithelial cells. *J Cell Biol* 124, 101-115 (1994)

134. Sheth B, J. J. Fontaine, E. Ponza, A. McCallum, A. Page, S. Citi, D. Louvard, A. Zahraoui, T. P. Fleming: Differentiation of the epithelial apical junctional complex during mouse preimplantation development: a role for rab13 in the early maturation of the tight junction. *Mech Dev* 97, 93-104 (2000)

135. Marzesco A, I. Dunia, R. Pandjaitan, M. Recouvreur, D. Dauzonne, E. Benedetti, D. Louvard, A. Zahraoui: The small GTPase Rab13 regulates assembly of functional tight junctions in epithelial cells. *Mol Biol Cell* 13, 1819-1831 (2002)

136. Kanda I, N. Nishimura, H. Nakatsuji, R. Yamamura, H. Nakanishi, T. Sasaki: Involvement of Rab13 and JRAB/MICAL-L2 in epithelial cell scattering. *Oncogene* 27, 1687-1695 (2008)

137. Di Giovanni S, A. De Biase, A. Yakovlev, T. Finn, J. Beers, E. Hoffman, A. Faden: *In vivo* and *in vitro* characterization of novel neuronal plasticity factors identified following spinal cord injury. *J Biol Chem* 280, 2084-2091 (2005)

138. Di Giovanni S, C. Knights, M. Rao, A. Yakovlev, J. Beers, J. Catania, M. Avantaggiati, A. Faden: The tumor suppressor protein p53 is required for neurite outgrowth and axon regeneration. *EMBO J* 25, 4084-4096 (2006)

139. Marzesco A, T. Galli, D. Louvard, A. Zahraoui: The rod cGMP phosphodiesterase delta subunit dissociates the small GTPase Rab13 from membranes. *J Biol Chem* 273, 22340-22345 (1998)

140. Lawrence D, K. Comerford, S. Colgan: Role of VASP in reestablishment of epithelial tight junction assembly after Ca^{2+} switch. *Am J Physiol Cell Physiol* 282, C1235-1245 (2002)

141. Köhler K, D. Louvard, A. Zahraoui: Rab13 regulates PKA signaling during tight junction assembly. *J Cell Biol* 165, 175-180 (2004)

142. Wang T, W. Hong: Interorganellar regulation of lysosome positioning by the Golgi apparatus through Rab34 interaction with Rab-interacting lysosomal protein. *Mol Biol Cell* 13, 4317-4332 (2002)

143. Sun P, H. Yamamoto, S. Suetsugu, H. Miki, T. Takenawa, T. Endo: Small GTPase Rah/Rab34 is associated with membrane ruffles and macropinosomes and promotes macropinosome formation. *J Biol Chem* 278, 4063-4071 (2003)

144. Suzuki T, T. Nakamoto, S. Ogawa, S. Seo, T. Matsumura, K. Tachibana, C. Morimoto, H. Hirai: MICAL, a novel CasL interacting molecule, associates with vimentin. *J Biol Chem* 277, 14933-14941 (2002)

145. Kim M, J. Gans, C. Nogueira, A. Wang, J. Paik, B. Feng, C. Brennan, W. Hahn, C. Cordon-Cardo, S. Wagner, T. Flotte, L. Duncan, S. Granter, L. Chin: Comparative oncogenomics identifies NEDD9 as a melanoma metastasis gene. *Cell* 125, 1269-1281 (2006)

146. Terman J, T. Mao, R. Pasterkamp, H. Yu, A. Kolodkin: MICALs, a family of conserved flavoprotein oxidoreductases, function in plexin-mediated axonal repulsion. *Cell* 109, 887-900 (2002)

147. Fischer J, T. Weide, A. Barnekow: The MICAL proteins and rab1: a possible link to the cytoskeleton? *Biochem Biophys Res Commun* 328, 415-423 (2005)

148. Weide T, J. Teuber, M. Bayer, A. Barnekow: MICAL-1 isoforms, novel rab1 interacting proteins. *Biochem Biophys Res Commun* 306, 79-86 (2003)

149. Comoglio P, L. Trusolino: Invasive growth: from development to metastasis. *J Clin Invest* 109, 857-862 (2002)

150. Pasterkamp R, H. Dai, J. Terman, K. Wahlin, B. Kim, B. Bregman, P. Popovich, A. Kolodkin: MICAL flavoprotein monooxygenases: expression during neural development and following spinal cord injuries in the rat. *Mol Cell Neurosci* 31, 52-69 (2006)

151. Ashida S, M. Furihata, T. Katagiri, K. Tamura, Y. Anazawa, H. Yoshioka, T. Miki, T. Fujioka, T. Shuin, Y. Nakamura, H. Nakagawa: Expression of novel molecules, MICAL2-PV (MICAL2 prostate cancer variants), increases

with high Gleason score and prostate cancer progression. *Clin Cancer Res* 12, 2767-2773 (2006)

152. Terai T, N. Nishimura, I. Kanda, N. Yasui, T. Sasaki: JRAB/MICAL-L2 is a junctional Rab13-binding protein mediating the endocytic recycling of occludin. *Mol Biol Cell* 17, 2465-2475 (2006)

153. Fukuda M: Distinct Rab binding specificity of Rim1, Rim2, rabphilin, and Noc2. *J Biol Chem* 278, 15373-15380 (2003)

154. Ortiz D, M. Medkova, C. Walch-Solimena, P. Novick: Ypt32 recruits the Sec4p guanine nucleotide exchange factor, Sec2p, to secretory vesicles: evidence for a Rab cascade in yeast. *J Cell Biol* 157, 1005-1015 (2002)

155. Vitale G, V. Rybin, S. Christoforidis, P. Thornqvist, M. McCaffrey, H. Stenmark, M. Zerial: Distinct Rab-binding domains mediate the interaction of Rabaptin-5 with GTP-bound Rab4 and Rab5. *EMBO J* 17, 1941-1951 (1998)

156. de Renzis S, B. Sönnichsen, M. Zerial: Divalent Rab effectors regulate the sub-compartmental organization and sorting of early endosomes. *Nat Cell Biol* 4, 124-133 (2002)

157. Fouraux M, M. Deneka, V. Ivan, A. van der Heijden, J. Raymackers, D. van Suylekom, W. van Venrooij, P. van der Sluijs, G. Pruijn: Rabip4' is an effector of rab5 and rab4 and regulates transport through early endosomes. *Mol Biol Cell* 15, 611-624 (2004)

Abbreviations: TJ: tight junction, AJ: adherens junction, JRAB: a junctional Rab13-binding protein, MICAL-L2: molecule interacting with CasL-like 2, GEF: guanine nucleotide exchange factor, GAP: GTPase-activating protein, GDI: GDP dissociation inhibitor, GDF: GDI displacement factor, M6PR: mannose 6-phosphate receptor, SNARE: soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor, HOPS: homotypic fusion and vacuole protein sorting, VPS: vacuole protein sorting, JAM: junction adhesion molecule, CAR: coxsackievirus and adenovirus receptor, CRB: Crumb, aPKC: atypical protein kinase C, CVB: group B coxsackieviruses, FA: focal adhesion, HGF: hepatocyte growth factor, SF: scatter factor, TPA: 12-*O*-tetradecanoylphorbol-13-acetate, TGN: trans-Golgi network, delta-PDE: cGMP phosphodiesterase delta subunit, PKA: protein kinase A, CC: coiled-coil.

Key Words: Rab8, Rab13, JRAB, MICAL-L2, Apical Junction, Endocytic Recycling, Review

Send correspondence to: Takuya Sasaki, Department of Biochemistry, Institute of Health Biosciences, The University of Tokushima Graduate School, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan, Tel: 81-88-633-9223, Fax: 81-88-633-9227, E-mail: sasaki@basic.med.tokushima-u.ac.jp